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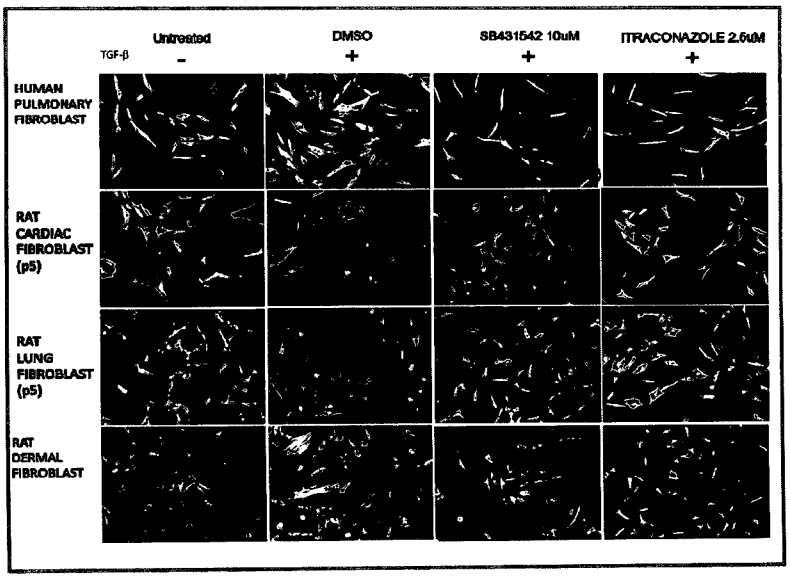
[Continued on next page]

(54) Title: SMALL MOLECULE INHIBITORS OF FIBROSIS

Figure 2



(57) Abstract: Described herein are compounds and compositions for the treatment of a fibrotic disease.

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SMALL MOLECULE INHIBITORS OF FIBROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Patent Application No. 61/832,768, filed June 7, 2013, which is hereby incorporated by reference in its entirety.

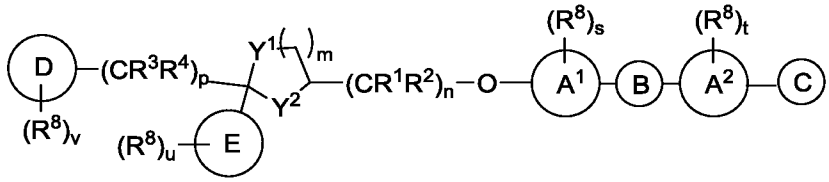
BACKGROUND OF THE INVENTION

[0002] Fibrosis, generally defined as the production of excessive amounts of connective tissue, develops as a consequence of diverse underlying diseases. Chronic inflammation or tissue damage/remodeling are typical fibrosis inducing events. Specific disease examples include idiopathic pulmonary fibrosis (IPF), liver fibrosis associated with the later stages of alcoholic and nonalcoholic liver cirrhosis, kidney fibrosis, cardiac fibrosis, and keloid formation resulting from abnormal wound healing [Wynn, T. A. (2004) Nature Reviews Immunology. 4: 583-594; Friedman, S.L. (2013) Science Translation Medicine. 5(167):1-17]. Additionally, fibrosis is a key pathological feature associated with chronic autoimmune diseases, including rheumatoid arthritis, Crohn's disease, systemic lupus erythematosus, and scleroderma. Diseases representing a dire unmet medical need include idiopathic pulmonary fibrosis (IPF), scleroderma and nonalcoholic steatohepatitis (NASH) related liver fibrosis. The increased incidence of NASH related liver fibrosis is expected to directly parallel those of type 2 diabetes and obesity.

[0003] Scleroderma is a rare chronic autoimmune disease characterized by the replacement of normal tissue with dense, thick fibrous tissue. While the exact underlying cause of scleroderma is unknown, the disease generally involves immune cell mediated activation of dermal myofibroblasts leading to the deposition of excessive amounts of extracellular matrix proteins (e.g., type I collagen) that causes a thickening of the skin and in some cases the hardening and eventual failure of multiple organs. At present, there is no cure for scleroderma. Treatment is limited to attempts to manage symptoms and typically requires a combination of approaches. While scleroderma localized to the skin is typically not life threatening, systemic scleroderma affecting multiple internal organs can be a life-threatening disease.

SUMMARY OF THE INVENTION

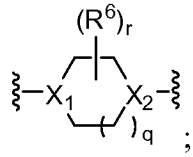
[0004] In one aspect, provided herein is a method to treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, the method comprising administering a composition comprising a therapeutically effective amount of a compound of Formula (I), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (I)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is  ;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

each R8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl, and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

u is 0, 1, 2, 3, 4 or 5; and

v is 0, 1, 2, 3, or 4.

[0005] In some embodiments described above or below of a compound of Formula (I), X_1 and X_2 are N

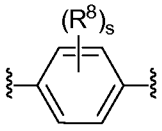
[0006] In some embodiments described above or below of a compound of Formula (I), X_1 is CR^5 and X_2 is N.

[0007] In some embodiments described above or below of a compound of Formula (I), X_1 is N and X_2 is CR^5.

[0008] In some embodiments described above or below of a compound of Formula (I), q is 1 and r is 0.

[0009] In some embodiments described above or below of a compound of Formula (I), A^1 is aryl.

[0010] In some embodiments described above or below of a compound of Formula (I), A^1 is



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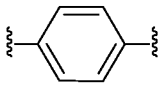
[0011] In some embodiments described above or below of a compound of Formula (I), A^1 is



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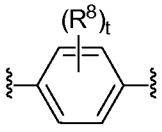
[0012] In some embodiments described above or below of a compound of Formula (I), A^1 is heteroaryl.

[0013] In some embodiments described above or below of a compound of Formula (I), A^2 is aryl.

[0014] In some embodiments described above or below of a compound of Formula (I), A^2 is



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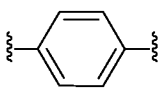
[0015] In some embodiments described above or below of a compound of Formula (I), A^2 is



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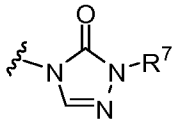
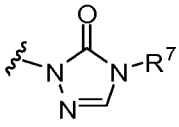
[0016] In some embodiments described above or below of a compound of Formula (I), A^2 is heteroaryl.

[0017] In some embodiments described above or below of a compound of Formula (I), A^2 is pyridine, pyrazine, pyrimidine, pyridazine, or triazine.

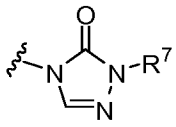
[0018] In some embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heteroaryl. In other embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heterocyclyl.

[0019] In some embodiments described above or below of a compound of Formula (I), C is

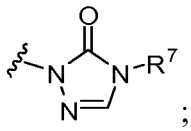
 or  ; and

R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

[0020] In some embodiments described above or below of a compound of Formula (I), C is

 ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

[0021] In some embodiments described above or below of a compound of Formula (I), C is

 ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

[0022] In some embodiments described above or below of a compound of Formula (I), E is alkyl.

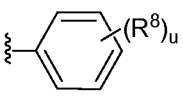
[0023] In some embodiments described above or below of a compound of Formula (I), E is cycloalkyl.

[0024] In some embodiments described above or below of a compound of Formula (I), E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

[0025] In some embodiments described above or below of a compound of Formula (I), E is heterocyclyl.

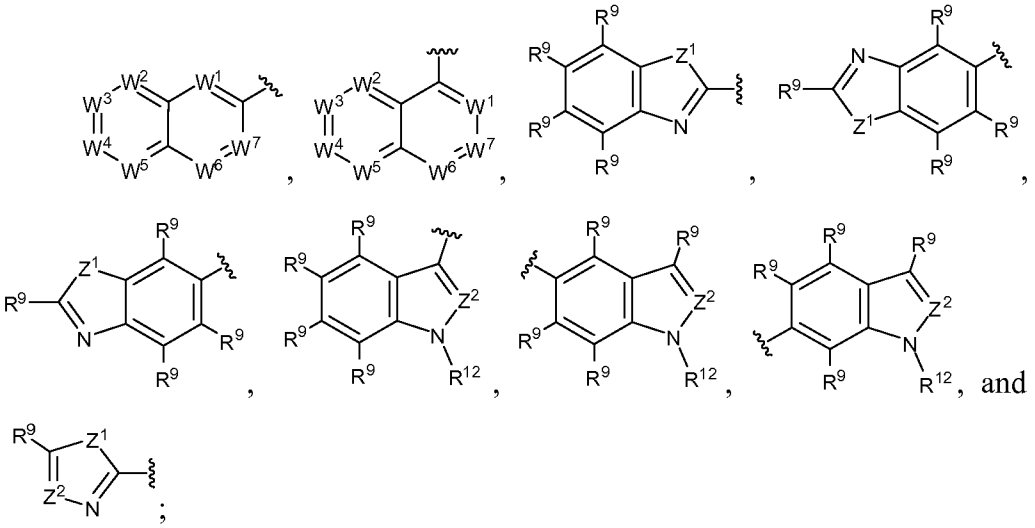
[0026] In some embodiments described above or below of a compound of Formula (I), E is aryl.

[0027] In some embodiments described above or below of a compound of Formula (I), E is

 and u is 0, 1, 2, 3, 4, or 5.

[0028] In some embodiments described above or below of a compound of Formula (I), E is heteroaryl.

[0029] In some embodiments described above or below of a compound of Formula (I), E is selected from:



;

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

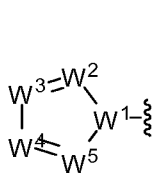
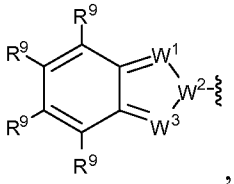
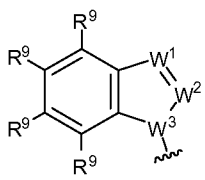
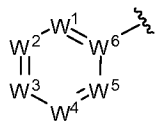
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

[0030] In some embodiments described above or below of a compound of Formula (I), D is aryl.

[0031] In some embodiments described above or below of a compound of Formula (I), E is heteroaryl.

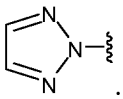
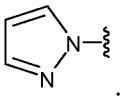
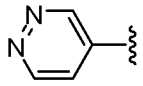
[0032] In some embodiments described above or below of a compound of Formula (I), D is selected from:

 ,  ,  , and  ;

W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

[0033] In certain embodiments described above or below of a compound of Formula (I), D is  . In certain embodiments described above or below of a compound of Formula (I), D is  . In certain embodiments described above or below of a compound of Formula (I), D is  .

[0034] In some embodiments described above or below of a compound of Formula (I), Y^1 and Y^2 are O.

[0035] In some embodiments described above or below of a compound of Formula (I), m is 1.

[0036] In some embodiments described above or below of a compound of Formula (I), p is 1, 2, or 3.

[0037] In some embodiments described above or below of a compound of Formula (I), p is 1.

[0038] In some embodiments described above or below of a compound of Formula (I), R^1, R^2, R^3, and R^4 are hydrogen.

[0039] Further provided herein is a method to treat fibrosis using a compound of Formula (I) wherein the fibrosis is liver fibrosis, idiopathic pulmonary fibrosis, kidney fibrosis, or cardiac fibrosis.

[0040] Further provided herein is a method to treat liver fibrosis using a compound of Formula (I) wherein the liver fibrosis is associated with the later stages of alcoholic or nonalcoholic liver cirrhosis.

[0041] Further provided herein is a method to treat fibrosis using a compound of Formula (I) wherein the fibrosis is idiopathic pulmonary fibrosis.

[0042] Further provided herein is a method to treat a disease using a compound of Formula (I) wherein the disease or disorder characterized by fibrosis is a chronic autoimmune disease.

[0043] Further provided herein is a method to treat chronic autoimmune disease using a compound of Formula (I) wherein the chronic autoimmune disease is rheumatoid arthritis, scleroderma, Crohn's disease or systemic lupus erythematosus.

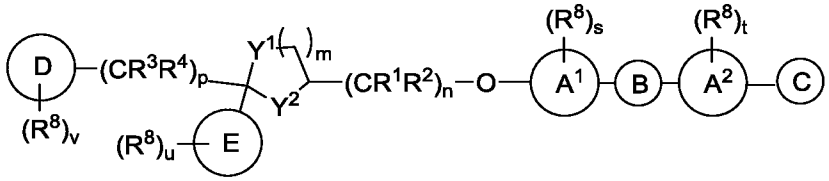
[0044] Further provided herein is a method to treat chronic autoimmune disease using a compound of Formula (I) wherein the chronic autoimmune disease is scleroderma.

[0045] Further provided herein is a method to treat fibrosis using a compound of Formula (I) wherein the fibrosis is keloid formation resulting from abnormal wound healing.

[0046] Further provided herein is a method to treat fibrosis using a compound of Formula (I) wherein the fibrosis occurs after organ transplantation.

[0047] Also provided herein is a method to treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, the method comprising administering a composition comprising a therapeutically effective amount of a compound described herein in combination with one or more pharmaceutical agents. In certain embodiments described above, the one or more pharmaceutical agents are antifibrotic agents. In certain embodiments described above, the one or more pharmaceutical agents are antifungal agents.

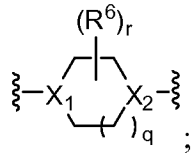
[0048] In one aspect, provided herein are compounds of Formula (II), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (II)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is  ;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl);

each R8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl, and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

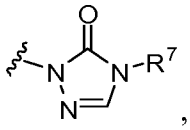
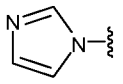
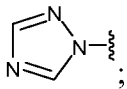
s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

u is 0, 1, 2, 3, 4 or 5;

v is 0, 1, 2, 3, or 4;

provided that:

if X_1 and X_2 are N, r is 0, q is 1, A^1 and A^2 are phenyl, Y^1 and Y^2 are O, m and n are 1, R^1, R^2, R^3, and R^4 are hydrogen, and C is  , then D is not  or  ; and

the compound is not 4-(4-(4-(4-((2-((1H-pyrazol-1-yl)methyl)-2-(2,4-difluorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-1H-1,2,4-triazol-5(4H)-one.

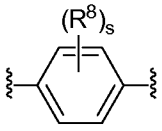
[0049] In some embodiments described above or below of a compound of Formula (II), X_1 and X_2 are N.

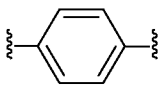
[0050] In some embodiments described above or below of a compound of Formula (II), X_1 is CR^5 and X_2 is N.

[0051] In some embodiments described above or below of a compound of Formula (II), X_1 is N and X_2 is CR^5.

[0052] In some embodiments described above or below of a compound of Formula (II), q is 1 and r is 0.

[0053] In some embodiments described above or below of a compound of Formula (II), A^1 is aryl.

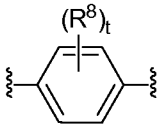
[0054] In some embodiments described above or below of a compound of Formula (II), A^1 is  .

[0055] In some embodiments described above or below of a compound of Formula (II), A^1 is  .

[0056] In some embodiments described above or below of a compound of Formula (II), A^1 is heteroaryl.

[0057] In some embodiments described above or below of a compound of Formula (II), A^2 is aryl.

[0058] In some embodiments described above or below of a compound of Formula (II), A^2 is



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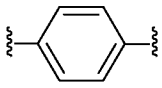
[0059] In some embodiments described above or below of a compound of Formula (II), A^2 is



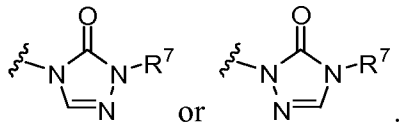
.

[0060] In some embodiments described above or below of a compound of Formula (II), A^2 is heteroaryl.

[0061] In some embodiments described above or below of a compound of Formula (II), A^2 is pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, or triazinyl.

[0062] In some embodiments described above or below of a compound of Formula (II), C is optionally substituted 5- or 6-membered heteroaryl. In other embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heterocyclyl.

[0063] In some embodiments described above or below of a compound of Formula (II), C is



or

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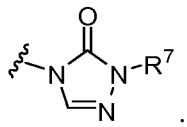
[0064] In some embodiments described above or below of a compound of Formula (II), C is



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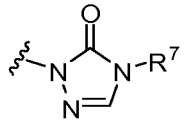
[0065] In some embodiments described above or below of a compound of Formula (II), C is



.

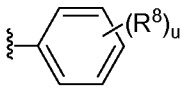
[0066] In some embodiments described above or below of a compound of Formula (II), E is alkyl.

[0067] In some embodiments described above or below of a compound of Formula (II), E is cycloalkyl.

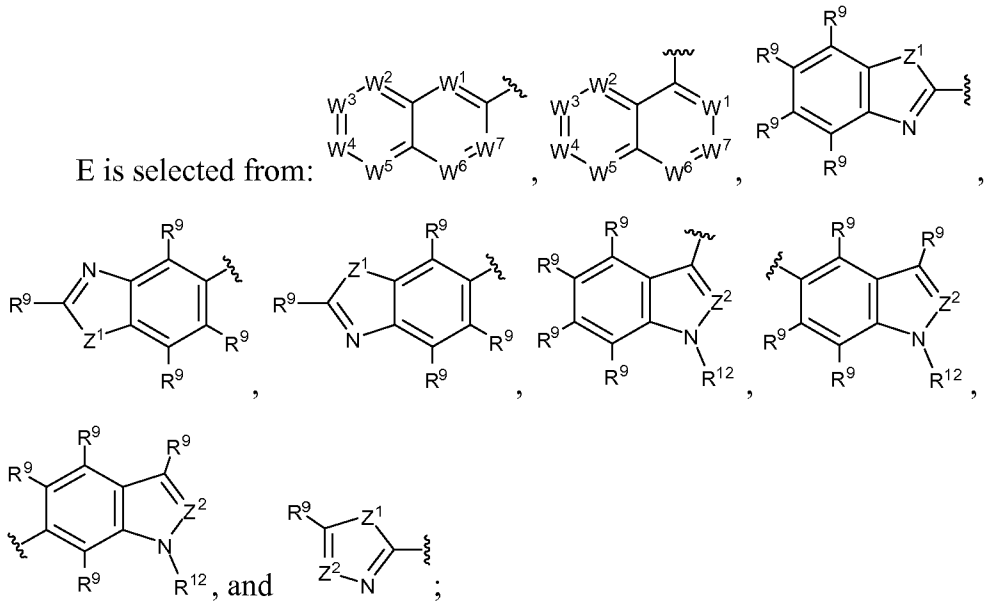
[0068] In some embodiments described above or below of a compound of Formula (II), E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

[0069] In some embodiments described above or below of a compound of Formula (II), E is heterocyclyl.

[0070] In some embodiments described above or below of a compound of Formula (II), E is aryl.

[0071] In some embodiments described above or below of a compound of Formula (II), E is  and u is 0, 1, 2, 3, 4, or 5.

[0072] In some embodiments described above or below of a compound of Formula (II), E is heteroaryl.

[0073] In some embodiments described above or below of a compound of Formula (II), E is selected from: 

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), $-NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

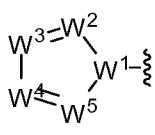
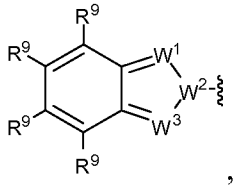
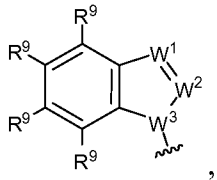
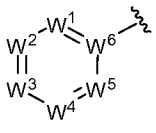
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

[0074] In some embodiments described above or below of a compound of Formula (II), D is aryl.

[0075] In some embodiments described above or below of a compound of Formula (II), D is heteroaryl.

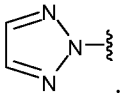
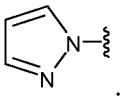
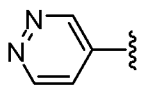
[0076] In some embodiments described above or below of a compound of Formula (II),

D is selected from  ,  ,  , and  ;

W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

[0077] In certain embodiments described above or below of a compound of Formula (II), D is  . In certain embodiments described above or below of a compound of Formula (II), D is  . In certain embodiments described above or below of a compound of Formula (II), D is  .

[0078] In some embodiments described above or below of a compound of Formula (II), Y^1 and Y^2 are O.

[0079] In some embodiments described above or below of a compound of Formula (II), m is 1.

[0080] In some embodiments described above or below of a compound of Formula (II), p is 1, 2, or 3.

[0081] In some embodiments described above or below of a compound of Formula (II), p is 1.

[0082] In some embodiments described above or below of a compound of Formula (II), R^1, R^2, R^3, and R^4 are hydrogen.

[0083] Also provided herein is a pharmaceutical composition comprising a compound of Formula (II) or as described above and below, or a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof, and a pharmaceutically acceptable excipient.

[0084] Further disclosed herein, in some embodiments, are an image-based systems for identifying inhibitors of fibrosis. In some embodiments, the system comprises (a) one or more fibroblasts; and (b) a cell imaging device for producing one or more images of the one or more fibroblasts. In some embodiments, the cell imaging device comprises a fluorescent microscope. In some embodiments, the cell imaging device comprises CCD camera technology. In some embodiments, the cell imaging device is automated. In some embodiments, the cell imaging device is manually operated. In some embodiments, the cell imaging device is thermoelectrically cooled.

[0085] In some embodiments, the system further comprises a light source. In some embodiments, the light source is an LED.

[0086] In some embodiments, the system further comprises a scanner.

[0087] In some embodiments, the system further comprises a computer.

[0088] In some embodiments, the system further comprises one or more memory locations for storing and/or receiving the one or more images. In some embodiments, the system further comprises one or more memory locations for storing and/or receiving one or more instructions for producing the one or more images.

[0089] In some embodiments, the system further comprises one or more processors for analyzing the one or more images of the one or more fibroblasts. In some embodiments, the system further comprises one or more processors for processing the one or more images of the one or more fibroblasts. In some embodiments, the system further comprises one or more processors for transmitting the one or more images of the one or more fibroblasts.

[0090] In some embodiments, the system further comprises one or more software programs for capturing, producing, analyzing, scanning, storing, and/or trasmitting the one or more images.

[0091] In some embodiments, the system further comprises one or more barcode readers for reading one or more barcodes on one or more samples comprising the one or more cells.

[0092] In some embodiments, the system further comprises one or more robots for handling one or more samples comprising the one or more cells. In some embodiments, the system further

comprises one or more robots for treating one or more samples comprising the one or more cells with one or more agents.

[0093] In some embodiments, the one or more agents comprise TGF-beta. In some embodiments, the one or more agents comprise one or more test agents.

[0094] In some embodiments, the system further comprises one or more processors for identifying the one or more test agents as inhibitors of fibrosis. In some embodiments, the system further comprises one or more processors for ranking the inhibitors of fibrosis.

[0095] In some embodiments, the system further comprises one or more algorithms. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts contacted with the one or more agents. In some embodiments, the one or more algorithms analyze an intensity of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze an fluorescence intensity of the one or more fibroblasts.

[0096] In some embodiments, the cell imaging device comprises a CellInsight NXT High Content Screening (HCS) platform.

[0097] In some embodiments, the one or more fibroblasts are hepatic stellate cells (HSCs).

[0098] Further disclosed herein, are methods of identifying inhibitors of fibrosis. In some embodiments, the method comprises (a) contacting a first sample comprising one or more fibroblasts with a cellular growth agent; (b) contacting a second sample comprising one or more fibroblasts with the cellular growth agent and a first test agent; (c) producing one or more images of the one or more fibroblasts of the first sample and one or more images of the one or more fibroblasts of the second sample; and (d) determining whether the first test agent is an inhibitor of fibrosis based on an analysis of the one or more images of the first sample and the one or more images of the second sample.

[0099] In some embodiments, the cellular growth agent is a growth factor. In some embodiments, the cellular growth agent is transforming growth factor beta (TGF-b).

[00100] In some embodiments, the first test agent is a small molecule. In some embodiments, the first test agent is a bioactive small molecule.

[00101] In some embodiments, the second sample is contacted with the cellular growth agent and the first test agent simultaneously. In some embodiments, the second sample is contacted with the cellular growth agent and the first test agent sequentially. In some embodiments, the second sample is contacted with the cellular growth agent prior to contact with the first test agent. In some embodiments, the second sample is contacted with the first test agent prior to contact with the cellular growth agent.

[00102] In some embodiments, the method further comprises one or more additional samples comprising one or more fibroblasts. In some embodiments, the first sample, second sample, and/or one or more additional samples are from the same source. In some embodiments, the first sample, second sample, and/or one or more additional samples are from two or more different sources.

[00103] In some embodiments, the method further comprises contacting the one or more additional samples with the cellular growth agent and one or more additional test agents.

[00104] In some embodiments, the one or more images of the first sample and the one or more images of the second sample are captured simultaneously. In some embodiments, the one or more images of the first sample and the one or more images of the second sample are captured sequentially.

[00105] In some embodiments, the one or more fibroblasts of the first sample are cultured in one or more wells on a first culture plate. In some embodiments, the one or more fibroblasts of the second sample are cultured on one or more wells on a second culture plate. In some embodiments, the one or more fibroblasts of the one or more additional samples are cultured on one or more wells on one or more additional culture plates.

[00106] In some embodiments, the first culture plate and the second culture plate are different. In some embodiments, the first culture plate, the second culture plate, and/or the one or more additional culture plates are different.

[00107] In some embodiments, the first culture plate and the second culture plate are the same. In some embodiments, the first culture plate, the second culture plate, and/or the one or more additional culture plates are the same.

[00108] In some embodiments, the method further comprises contacting the one or more fibroblasts of the first sample and/or the one or more fibroblasts of the second sample with a third agent. In some embodiments, the method further comprises contacting the one or more fibroblasts of the one or more additional samples with a third agent.

[00109] In some embodiments, the third agent is an antibody. In some embodiments, the third agent is an anti-smooth muscle actin (SMA) antibody.

[00110] In some embodiments, the one or more images of the first sample and/or the one or more images of the second sample are based on the images of the one or more fibroblasts contacted with the third agent. In some embodiments, the one or more images of the one or more additional samples are based on the images of the one or more fibroblasts contacted with the third agent.

[00111] In some embodiments, producing the one or more images comprises the use of one or more cell imaging devices. In some embodiments, the cell imaging device comprises a CellInsight NXT High Content Screening (HCS) platform.

[00112] In some embodiments, the method further comprises one or more algorithms. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts contacted with the one or more agents. In some embodiments, the one or more algorithms analyze an intensity of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze an fluorescence intensity of the one or more fibroblasts.

[00113] In some embodiments, the method further comprises detecting transdifferentation of the one or more fibroblasts. In some embodiments, transdifferentiation of the one or more fibroblasts comprises transdifferentiation into one or more myofibroblasts.

[00114] In some embodiments, determining whether the first test agent is identified as an inhibitor of fibrosis is based on a comparison of the myofibroblasts composition in the first sample to the myofibroblasts composition in the second sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the second sample is less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the second sample is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the second sample is at least about 1.5-, 2-, 2.5-, 3-, 3.5-, 4-, 4.5-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, or 15-fold less than the myofibroblasts composition in the first sample.

[00115] In some embodiments, the method further comprises determining whether the one or more additional test agents are inhibitors of fibrosis. In some embodiments, determining whether the one or more additional test agents is identified as an inhibitor of fibrosis is based on a comparison of the myofibroblasts composition in the first sample to the myofibroblasts composition in the one or more additional samples. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is

identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is at least about 1.5-, 2-, 2.5-, 3-, 3.5-, 4-, 4.5-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, or 15-fold less than the myofibroblasts composition in the first sample.

INCORPORATION BY REFERENCE

[00116] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE FIGURES

[00117] **Figure 1** describes a high content imaging assay, based on α-SMA staining and cell morphological changes associated with fibroblast to myofibroblast transdifferentiation that has been established using primary human lung fibroblasts and primary rodent HSCs. Conditions, involving serum starvation and subsequent TGF-β treatment, have been identified that facilitate robust *in vitro* transdifferentiation in a miniaturized (384 well plate) format that is amenable to high throughput small molecule screening. A selective ALK-5 TGF-β1 receptor inhibitor (SB-431542) is used as a positive control.

[00118] **Figure 2** describes Itraconazole inhibiting Myofibroblast differentiation in multiple cell types in vitro. Preparations of primary cardiac, pulmonary, or dermal fibroblasts and HSCs were tested for responsiveness to TGFβ. Cells were analyzed for myofibroblast morphology by staining for smooth muscle actin. ALK5 inhibitor (SB-431542) used as a positive control. Itraconazole (Itra, 2.5 μM) inhibits myofibroblast differentiation in fibroblasts derived from lung, heart, skin or liver (HSCs).

[00119] **Figure 3** describes Itraconazole inhibiting Myofibroblast differentiation in multiple cell types in vitro. Preparations of primary cardiac, pulmonary, or dermal fibroblasts and HSCs were tested for responsiveness to TGFβ. qRT-PCR profiling and western blot analysis of multiple fibrosis-related genes used to confirm in-vitro activity. Pharmacological studies support dual inhibition MOA (VEGF, Hh signaling).

[00120] **Figure 4** shows the efficacy of Itraconazole in rodent PoC studies in Liver (CCl4) and lung (Bleomycin) models. Using pirfenidone and AM-152 as benchmarks, itraconazole was demonstrated to have equal or superior efficacy in carbon tetrachloride-induce liver fibrosis and bleomycin-induced lung and skin fibrosis mouse models.

[00121] **Figure 5** shows data obtained from a myofibroblast activation imaging-based assay: (a) analysis of cells for mean cellular area of SMA staining; (b) analysis of cells for mean fluorescence intensity of SMA staining.

[00122] **Figure 6** shows Western blot analyses of fibrosis-related proteins in cells exposed to TGFβ and itraconazole.

[00123] **Figure 7** shows gene expression analysis of human lung fibroblasts treated with itraconazole: (a) data analysis expressed as fold regulation relative to a sample not treated with TGF-β1; (b) raw data from the fibrosis-focused RT2 Profiler PCR Array.

[00124] **Figure 8** shows qPCR analysis of Hedgehog related genes in rat hepatic stellate cells treated with itraconazole: (a) relative levels of PTCH1 mRNA; (b) relative levels of GLI1 mRNA; (c) raw data for both qPCR experiments.

[00125] **Figure 9** shows western blot analyses of COL1-GFP HSCs after knockdown of Smoothened.

[00126] **Figure 10** shows western blot analysis of VEGFR2 migration pattern after treatment with itraconazole.

[00127] **Figure 11** shows western blot analysis of rat hepatic stellate cells treated with combinations of VEGFR and Hedgehog inhibiting compounds.

[00128] **Figure 12** shows activity of itraconazole and compound 42 in a hedgehog reporter assay: (a) relative GLI-LUC activity of TM3-GLI-LUC cells exposed to 10nM SAG and indicated doses of inhibitor; (b) relative GLI-LUC activtity of TM3-GLI-LUC cells exposed to 400nM SAG and indicated doses of inhibitor.

[00129] **Figure 13** shows western blot analyses of LX2 human hepatic stellate cells after knockdown of VEFGR1, VEGFR2, or SMO.

[00130] **Figure 14** shows the bleomycin-induced lung fibrosis model study design for the evaluation of compound 42 and itraconazole.

[00131] **Figure 15** shows evaluation of compound 42 and itraconazole in the bleomycin-induced lung fibrosis model: (a) mean Ashcroft scores of mice exposed to bleomycin and the indicated doses of drug; (b) graphical representation of model used to evaluate anti-fibrotic drugs; (c) representative images of Masson's trichrome stained lung from indicated treatment groups.

[00132] **Figure 16** shows characterization of the modified Ashcroft scoring system.

[00133] **Figure 17** shows quantification of histology data from evaluation of compound 42 and itraconazole in the bleomycin-induced lung fibrosis model: (a) mean Ashcroft scores; (b) mean percent stained area values.

[00134] **Figure 18** shows the carbon tetrachloride-induced liver fibrosis model study design for the evaluation of compound 42 and itraconazole.

[00135] **Figure 19** shows evaluation of compound 42 and itraconazole in the carbon tetrachloride-induced liver fibrosis model: (a) total percent area positive for Sirius Red staining; (b) numerical data of the image analysis of the CCl_4-induced liver fibrosis model; (c)

representative images of Sirius Red stained liver sections of the CCl_4-induced liver fibrosis model; (d) western blot analysis.

[00136] **Figure 20** shows rodent wound healing model study design for the evaluation of compound 42 and itraconazole.

[00137] **Figure 21** shows study results from the evaluation of compound 42 and itraconazole in the rodent wound healing model.

DETAILED DESCRIPTION OF THE INVENTION

[00138] Fibrosis represents a critically important yet surprisingly neglected health problem. Nearly 45% of all natural deaths in the Western world are attributed to chronic fibroproliferative diseases. However, there is currently only one clinically approved drug (Pirfenidone) that specifically targets the pathogenesis of fibrosis and is directly indicated for the treatment of a fibrotic disease. Fibrosis affects nearly every tissue in the body and, when highly progressive, can lead to organ malfunction and death. Clearly, the identification of novel anti-fibrotic drugs represents an unmet medical need that would have significant beneficial impact on patients in multiple disease populations. In addition, at present there is no cure for scleroderma and treatment is limited to symptom management.

[00139] Fibrosis, generally defined as the production of excessive amounts of connective tissue, develops as a consequence of diverse underlying diseases. Chronic inflammation or tissue damage/remodeling are typical fibrosis inducing events. Fibrosis affects nearly every tissue in the body and, when highly progressive, can lead to organ malfunction and death. Specific disease examples include idiopathic pulmonary fibrosis (IPF); liver fibrosis associated with the later stages of alcoholic and nonalcoholic liver cirrhosis; kidney fibrosis; cardiac fibrosis; and keloid formation resulting from abnormal wound healing. Additionally, fibrosis is a key pathological feature associated with chronic autoimmune diseases, including rheumatoid arthritis, scleroderma, Crohn's disease and systemic lupus erythematosus. As such, fibrosis represents a critically important yet surprisingly neglected health problem. Indeed, nearly 45% of all natural deaths in the Western world are attributed to chronic fibroproliferative diseases. However, at present, there is only one clinically approved drug (Pirfenidone, approved for the treatment of IPF in Europe only) that specifically targets the pathogenesis of fibrosis and is directly indicated for the treatment of a fibrotic disease. Unfortunately, Pirfenidone has significant liver and GI side effects, and patients treated with Pirfenidone are advised to avoid direct sunlight exposure, as it is known to cause photosensitivity reactions leading to rash, dry skin or pruritus. Recently, lysophosphatidic acid 1 (LPA1) antagonists (e.g., AM-152) have been demonstrated to be efficacious in preclinical models of IPF. However, clinical efficacy remains to be demonstrated

for AM-152. Clearly, the identification of novel anti-fibrotic drugs represents a major unmet medical need that would have significant beneficial impact on patients in multiple disease populations.

[00140] Despite the diversity of diseases and triggers that can initiate a fibrotic process in a given tissue or organ, common biochemical and cellular mechanisms occur in all instances studied to date. Following injury or inflammatory insult, resident fibroblasts (in some cases recruited bone marrow-derived circulating fibrocytes or epithelial cells that have undergone an epithelial-to-mesenchymal transition) are activated and "transdifferentiate" into α-smooth muscle actin (α-SMA) expressing myofibroblasts that secrete the extracellular matrix (ECM) components required for wound repair. In the case of liver fibrosis, a resident pericyte population termed a quiescent hepatic stellate cell (HSC) "transdifferentiates" into a type I collagen producing α-SMA expressing fibrogenic "activated" HSC. Transforming growth factor-β1 (TGF-β1) mediated Smad 3/4 signaling commonly drives the transdifferentiation of resident fibroblasts or HSCs to myofibroblasts or activated HSCs and stimulates production of ECM components in the latter populations. Platelet-derived growth factor (PDGF) also serves as a common pro-fibrotic cytokine that drives cell activation and proliferation.

[00141] One therapeutic approach for the treatment of progressive fibrosis diseases is to target one of the multitudes of complex causative immunological processes. This approach is limited by a lack of mechanistic clarity and potential exacerbation of the underlying disease. An attractive alternative approach for the treatment of diverse fibrotic diseases is to directly target the transdifferentiation pathway responsible for interconversion of quiescent fibroblasts and activated pro-fibrotic myofibroblasts. Drugs capable of blocking the conversion of fibroblasts to activated myofibroblasts could be administered prophylactically following injury or insult (e.g., myocardial infaction) or therapeutically in the early stages of disease in organs capable of repair (e.g., liver fibrosis, IPF or scleroderma). Direct suppressors of TGF-β1 production (e.g., Pirfenidone) are not ideal candidates for chronic dosing and are especially undesirable for the treatment of autoimmune diseases, as they have the potential to exacerbate autoimmune responses. Alternatively, drugs capable of inducing the reversion of existing myofibroblasts to a quiescent cell fate would have broad applicability for the treatment of fibrosis in multiple tissue types and could potentially be efficacious at later stages of disease.

[00142] High content imaging assays have been established, using primary human lung fibroblasts and primary rodent HSCs, that enable the identification of small molecules that either inhibit myofibroblast formation/activation or induce the reversion of activated myofibroblasts to a quiescent fibroblast state. Conditions, involving serum starvation and subsequent TGF-β treatment, have been identified that facilitate robust *in vitro* transdifferentiation in a miniaturized

(384 well plate) format that is amenable to high throughput small molecule screening. The inhibition assay is based on α-SMA immunofluorescent staining and cell morphology changes associated with fibroblast to myofibroblast transdifferentiation, Using the selective ALK-5 TGF-β1 receptor inhibitor (SB-43154) as a positive control, the inhibition assay has been used to initiate a screen of a collection of ~100,000 small molecules that consists of bioactive small molecules and a diversity set that has been assembled based on 2D and 3D structural diversity and inherent "drug-likeness" properties. Preliminary screening led to the identification of multiple previously identified anti-fibrotic molecules. The majority of these have limited clinical utility, as a result of known off target toxicity issues or demonstrated lack of *in vivo* efficacy. However, in addition to these, the triazole antifungal agent Itraconazole was identified as a highly efficacious inhibitor of myofibroblast formation (at doses well below those associated with toxicity in fibroblasts or other control cell types).

[00143] The activity of Itraconazole was confirmed *in vitro* by analyzing expression changes in multiple genes associated with fibroblast to myofibroblast transdifferentiation using biochemical methods (i.e., Western blot and RT-PCR). Using pharmacological methods, the mechanistic basis for the anti-fibrotic activity of this molecule has been established as dual inhibition of hedgehog signaling and vascular endothelial growth factor (VEFG) receptor glycosylation/trafficking (neither activity alone is sufficient). Encouragingly, it was established that the activity of Itraconazole translates to both human and rodent cell types, as well as to cells derived from multiple tissue types (e.g., lung, liver, skin, heart). Using Pirfenidone and AM-152 as benchmark control compounds, Itraconazole was demonstrated to have efficacy in both bleomycin-induced lung and carbon tetrachloride-induce liver fibrosis mouse models. As such, the FDA approved drug Itraconazole has been identified as a novel lead for the development of a new class of drugs for the treatment of multiple fibrosis related diseases.

[00144] A potential limitation to the use of this drug as an anti-fibrotic, especially for the treatment of liver fibrosis, is a known liver toxicity profile that is associated with coordination of heme iron by N4 of the 1,2,4 triazole moiety, leading to inhibition of P450 enzymes (most notably Cyp3A4), an activity distinct from the VEGF and Hgh activity of itraconazole. As such, medicinal chemistry efforts aimed at identifying optimized Itraconazole analogues with favorable liver toxicity profiles and further improvements in anti-fibrotic efficacy have been initiated. From an initial panel of ~30 Itraconazole analogues, in which the *pKa* of N4 is reduced or N4 nitrogen is substituted with carbon, several candidate leads have been identified wherein Cyp inhibition activity is eliminated and in vitro anti-fibrotic activity is retained. Notably, the pharmacokinetic properties of these compounds are comparable to those of the parent compound. Based on observed *in vitro* activity, *in vivo* efficacy, rodent serum exposure and human exposure

data, it is anticipated that a 5-fold improvement in efficacy and/or exposure is desirable for the final clinical candidate. A preliminary structure activity relationship study has revealed that potency enhancement can be achieved by making modifications at sites distal to the triazole substituent. Chemistry efforts are ongoing and will be used to optimize efficacy, exposure (C_{max} >5-fold *in vitro* EC_{50}) and toxicity profiles (based on human off-target panel profiling, hERG and AMES *in vitro* toxicity assays, and Cyp induction/inhibition assays). Reproducible disease modifying activity, i.e., efficacy equal to or greater than that of existing (pirfenidone) or potential future (AM-152) standards of care, will be demonstrated for an optimized Itraconazole analogue, using both lung and liver rodent fibrosis models.

Definitions

[00145] In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the invention may be practiced without these details. In other instances, well-known structures have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments. Unless the context requires otherwise, throughout the specification and claims which follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is, as "including, but not limited to." Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed invention.

[00146] Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment. Thus, the appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. Also, as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. It should also be noted that the term "or" is generally employed in its sense including "and/or" unless the content clearly dictates otherwise.

[00147] The terms below, as used herein, have the following meanings, unless indicated otherwise:

[00148] "Amino" refers to the $-NH_2$ radical.

[00149] "Cyano" or "nitrile" refers to the -CN radical.

[00150] "Hydroxy" or "hydroxyl" refers to the -OH radical.

[00151] "Nitro" refers to the $-NO_2$ radical.

[00152] "Oxo" refers to the =O substituent.

[00153] "Oxime" refers to the =N-OH substituent.

[00154] "Thioxo" refers to the =S substituent.

[00155] "Alkyl" refers to a straight or branched hydrocarbon chain radical, has from one to thirty carbon atoms, and is attached to the rest of the molecule by a single bond. Alkyls comprising any number of carbon atoms from 1 to 30 are included. An alkyl comprising up to 30 carbon atoms is referred to as a C_1-C_{30} alkyl, likewise, for example, an alkyl comprising up to 12 carbon atoms is a C_1-C_{12} alkyl. Alkyls (and other moieties defined herein) comprising other numbers of carbon atoms are represented similarly. Alkyl groups include, but are not limited to, C_1-C_{30} alkyl, C_1-C_{20} alkyl, C_1-C_{15} alkyl, C_1-C_{10} alkyl, C_1-C_8 alkyl, C_1-C_6 alkyl, C_1-C_4 alkyl, C_1-C_3 alkyl, C_1-C_2 alkyl, C_2-C_8 alkyl, C_3-C_8 alkyl and C_4-C_8 alkyl. Representative alkyl groups include, but are not limited to, methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *i*-butyl, *s*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), 3-methylhexyl, 2-methylhexyl, vinyl, allyl, propynyl, and the like. Alkyl comprising unsaturations include alkenyl and alkynyl groups. Unless stated otherwise specifically in the specification, an alkyl group may be optionally substituted as described below.

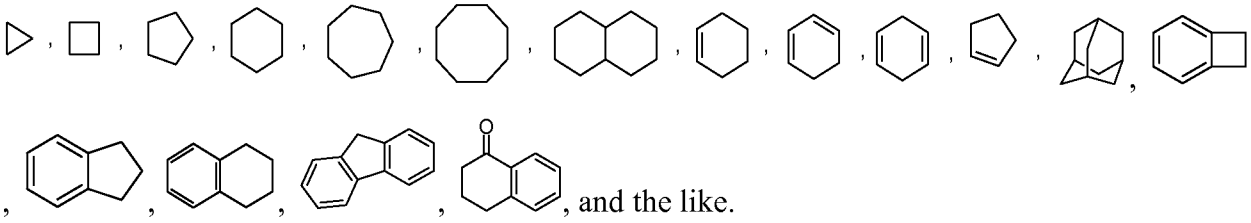
[00156] "Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain, as described for alkyl above. Unless stated otherwise specifically in the specification, an alkylene group may be optionally substituted as described below.

[00157] "Alkoxy" refers to a radical of the formula $-OR_a$ where R_a is an alkyl radical as defined. Unless stated otherwise specifically in the specification, an alkoxy group may be optionally substituted as described below.

[00158] "Aryl" refers to a radical derived from a hydrocarbon ring system comprising hydrogen, 6 to 30 carbon atoms and at least one aromatic ring. The aryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. Aryl radicals include, but are not limited to, aryl radicals derived from the hydrocarbon ring systems of aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, pleiadene, pyrene, and triphenylene. Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-" (such as in "aralkyl") is meant to include aryl radicals that are optionally substituted.

[00159] "Cycloalkyl" or "carbocycle" refers to a stable, non-aromatic, monocyclic or polycyclic carbocyclic ring, which may include fused or bridged ring systems, which is saturated or unsaturated. Representative cycloalkyls or carbocycles include, but are not limited to,

cycloalkyls having from three to fifteen carbon atoms, from three to ten carbon atoms, from three to eight carbon atoms, from three to six carbon atoms, from three to five carbon atoms, or three to four carbon atoms. Monocyclic cycloalkyls or carbocycles include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic cycloalkyls or carbocycles include, for example, adamantyl, norbornyl, decalinyl, bicyclo[3.3.0]octane, bicyclo[4.3.0]nonane, cis-decalin, trans-decalin, bicyclo[2.1.1]hexane, bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, bicyclo[3.2.2]nonane, and bicyclo[3.3.2]decane, and 7,7-dimethyl-bicyclo[2.2.1]heptanyl. Unless otherwise stated specifically in the specification, a cycloalkyl or carbocycle group may be optionally substituted. Illustrative examples of cycloalkyl groups include, but are not limited to, the following moieties:



[00160] "Fused" refers to any ring structure described herein which is fused to an existing ring structure. When the fused ring is a heterocyclyl ring or a heteroaryl ring, any carbon atom on the existing ring structure which becomes part of the fused heterocyclyl ring or the fused heteroaryl ring may be replaced with a nitrogen atom.

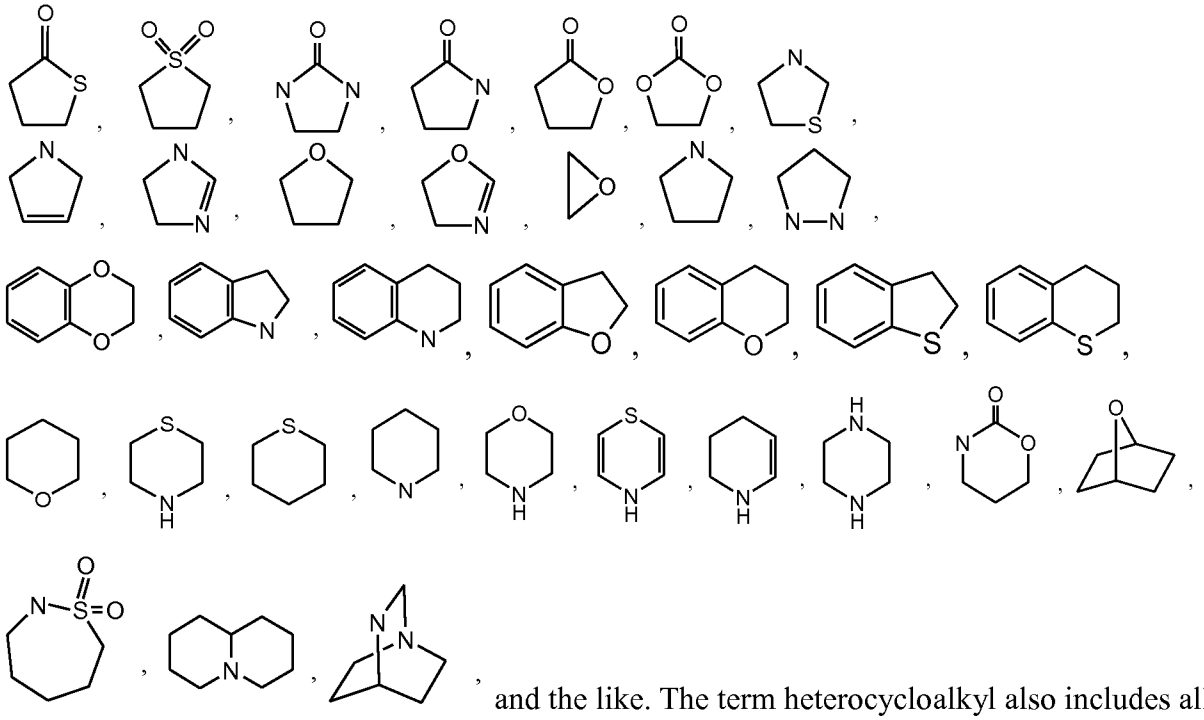
[00161] "Halo" or "halogen" refers to bromo, chloro, fluoro or iodo.

[00162] "Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, *e.g.*, trifluoromethyl, difluoromethyl, fluoromethyl, trichloromethyl, 2,2,2-trifluoroethyl, 1,2-difluoroethyl, 3-bromo-2-fluoropropyl, 1,2-dibromoethyl, and the like. Unless stated otherwise specifically in the specification, a haloalkyl group may be optionally substituted.

[00163] "Haloalkoxy" similarly refers to a radical of the formula $-OR_a$ where R_a is a haloalkyl radical as defined. Unless stated otherwise specifically in the specification, a haloalkoxy group may be optionally substituted as described below.

[00164] "Heterocycloalkyl" or "heterocyclyl" or "heterocyclic ring" or "heterocycle" refers to a stable 3- to 24-membered non-aromatic ring radical comprising 2 to 23 carbon atoms and from one to 8 heteroatoms selected from the group consisting of nitrogen, oxygen, phosphorous and sulfur. Unless stated otherwise specifically in the specification, the heterocyclyl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized; and the heterocyclyl

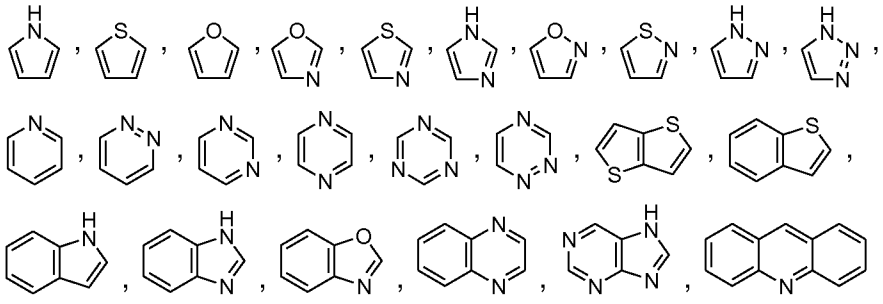
radical may be partially or fully saturated. Examples of such heterocyclyl radicals include, but are not limited to, azetidinyl, dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, 1,1-dioxo-thiomorpholinyl, 12-crown-4, 15-crown-5, 18-crown-6, 21-crown-7, aza-18-crown-6, diaza-18-crown-6, aza-21-crown-7, and diaza-21-crown-7. Unless stated otherwise specifically in the specification, a heterocyclyl group may be optionally substituted. Illustrative examples of heterocycloalkyl groups, also referred to as non-aromatic heterocycles, include:



and the like. The term heterocycloalkyl also includes all ring forms of the carbohydrates, including but not limited to the monosaccharides, the disaccharides and the oligosaccharides. Unless otherwise noted, heterocycloalkyls have from 2 to 10 carbons in the ring. It is understood that when referring to the number of carbon atoms in a heterocycloalkyl, the number of carbon atoms in the heterocycloalkyl is not the same as the total number of atoms (including the heteroatoms) that make up the heterocycloalkyl (i.e. skeletal atoms of the heterocycloalkyl ring). Unless stated otherwise specifically in the specification, a heterocycloalkyl group may be optionally substituted.

[00165] The term "heteroaryl" as used herein, alone or in combination, refers to optionally substituted aromatic monoradicals containing from about five to about twenty skeletal ring atoms, where one or more of the ring atoms is a heteroatom independently selected from among

oxygen, nitrogen, sulfur, phosphorous, silicon, selenium and tin but not limited to these atoms and with the proviso that the ring of said group does not contain two adjacent O or S atoms. In embodiments in which two or more heteroatoms are present in the ring, the two or more heteroatoms can be the same as each another, or some or all of the two or more heteroatoms can each be different from the others. The term heteroaryl includes optionally substituted fused and non-fused heteroaryl radicals having at least one heteroatom. The term heteroaryl also includes fused and non-fused heteroaryls having from five to about twelve skeletal ring atoms, as well as those having from five to about ten skeletal ring atoms. Bonding to a heteroaryl group can be via a carbon atom or a heteroatom. Thus, as a non-limiting example, an imidiazole group may be attached to a parent molecule via any of its carbon atoms (imidazol-2-yl, imidazol-4-yl or imidazol-5-yl), or its nitrogen atoms (imidazol-1-yl or imidazol-3-yl). Likewise, a heteroaryl group may be further substituted via any or all of its carbon atoms, and/or any or all of its heteroatoms. A fused heteroaryl radical may contain from two to four fused rings where the ring of attachment is a heteroaromatic ring and the other individual rings may be alicyclic, heterocyclic, aromatic, heteroaromatic or any combination thereof. A non-limiting example of a single ring heteroaryl group includes pyridyl; fused ring heteroaryl groups include benzimidazolyl, quinolinyl, acridinyl; and a non-fused bi-heteroaryl group includes bipyridinyl. Further examples of heteroaryls include, without limitation, furanyl, thienyl, oxazolyl, acridinyl, phenazinyl, benzimidazolyl, benzofuranyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, benzothiophenyl, benzoxadiazolyl, benzotriazolyl, imidazolyl, indolyl, isoxazolyl, isoquinolinyl, indolizinyl, isothiazolyl, isoindolyloxadiazolyl, indazolyl, pyridyl, pyridazyl, pyrimidyl, pyrazinyl, pyrrolyl, pyrazinyl, pyrazolyl, purinyl, phthalazinyl, pteridinyl, quinolinyl, quinazolinyl, quinoxalinyl, triazolyl, tetrazolyl, thiazolyl, triazinyl, thiadiazolyl and the like, and their oxides, such as for example pyridyl-N-oxide. Illustrative examples of heteroaryl groups include the following moieties:



and the like.

[00166] The heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heteroaryl radical may be optionally oxidized; the nitrogen atom may be optionally quaternized. Examples include, but are not limited to, azepinyl, acridinyl, benzimidazolyl,

benzothiazolyl, benzindolyl, benzodioxolyl, benzofuranyl, benzooxazolyl, benzothiazolyl, benzothiadiazolyl, benzo[*b*][1,4]dioxepinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, isoquinolyl, indolizinyl, isoxazolyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 1-oxidopyridinyl, 1-oxidopyrimidinyl, 1-oxidopyrazinyl, 1-oxidopyridazinyl, 1-phenyl-1*H*-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinazolinyl, quinoxalinyl, quinolinyl, quinuclidinyl, isoquinolinyl, tetrahydroquinolinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, and thiophenyl (*i.e.*, thienyl).

[00167] All the above groups may be either substituted or unsubstituted. The term "substituted" as used herein means any of the above groups (*e.g,* alkyl, alkylene, alkoxy, aryl, cycloalkyl, haloalkyl, heterocyclyl and/or heteroaryl) may be further functionalized wherein at least one hydrogen atom is replaced by a bond to a non-hydrogen atom substituent. Unless stated specifically in the specification, a substituted group may include one or more substituents selected from: oxo, amino, $-CO_2H$, nitrile, nitro, hydroxyl, thiooxy, alkyl, alkylene, alkoxy, aryl, cycloalkyl, heterocyclyl, heteroaryl, dialkylamines, arylamines, alkylarylamines, diarylamines, trialkylammonium ($-N^+R_3$), N-oxides, imides, and enamines; a silicon atom in groups such as trialkylsilyl groups, dialkylarylsilyl groups, alkyldiarylsilyl groups, triarylsilyl groups, perfluoroalkyl or perfluoroalkoxy, for example, trifluoromethyl or trifluoromethoxy. "Substituted" also means any of the above groups in which one or more hydrogen atoms are replaced by a higher-order bond (*e.g.*, a double- or triple-bond) to a heteroatom such as oxygen in oxo, carbonyl, carboxyl, and ester groups; and nitrogen in groups such as imines, oximes, hydrazones, and nitriles. For example, "substituted" includes any of the above groups in which one or more hydrogen atoms are replaced with $-NH_2$, $-NR_gC(=O)NR_gR_h$, $-NR_gC(=O)OR_h$, $-NR_gSO_2R_h$, $-OC(=O)NR_gR_h$, $-OR_g$, $-SR_g$, $-SOR_g$, $-SO_2R_g$, $-OSO_2R_g$, $-SO_2OR_g$, $=NSO_2R_g$, and $-SO_2NR_gR_h$. In the foregoing, R_g and R_h are the same or different and independently hydrogen, alkyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkylalkyl, haloalkyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl. In addition, each of the foregoing substituents may also be optionally substituted with one or more of the above substituents. Furthermore, any of the above groups may be substituted to include one or more internal oxygen, sulfur, or nitrogen atoms. For example, an alkyl group may be substituted with one or more internal oxygen atoms to form an ether or polyether group.

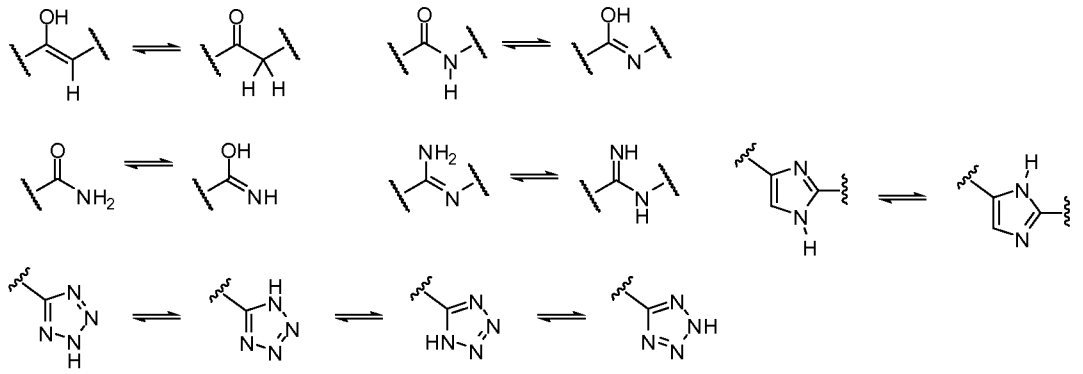
Similarily, an alkyl group may be substituted with one or more internal sulfur atoms to form a thioether, disulfide, etc.

[00168] The term "optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted alkyl" means either "alkyl" or "substituted alkyl" as defined above. Further, an optionally substituted group may be un-substituted (e.g., $-CH_2CH_3$), fully substituted (e.g., $-CF_2CF_3$), mono-substituted (e.g., $-CH_2CH_2F$) or substituted at a level anywhere in-between fully substituted and mono-substituted (e.g., $-CH_2CHF_2$, $-CH_2CF_3$, $-CF_2CH_3$, $-CFHCHF_2$, etc). It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns (e.g., substituted alkyl includes optionally substituted cycloalkyl groups, which in turn are defined as including optionally substituted alkyl groups, potentially ad infinitum) that are sterically impractical and/or synthetically non-feasible. Thus, any substituents described should generally be understood as having a maximum molecular weight of about 1,000 daltons, and more typically, up to about 500 daltons.

[00169] An "effective amount" or "therapeutically effective amount" refers to an amount of a compound administered to a mammalian subject, either as a single dose or as part of a series of doses, which is effective to produce a desired therapeutic effect.

[00170] "Treatment" of an individual (e.g. a mammal, such as a human) or a cell is any type of intervention used in an attempt to alter the natural course of the individual or cell. In some embodiments, treatment includes administration of a pharmaceutical composition, subsequent to the initiation of a pathologic event or contact with an etiologic agent and includes stabilization of the condition (e.g., condition does not worsen) or alleviation of the condition. In other embodiments, treatment also includes prophylactic treatment (e.g., administration of a composition described herein when an individual is suspected to be suffering from a bacterial infection).

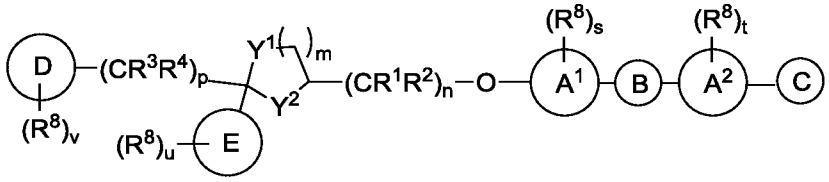
[00171] A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule. The compounds presented herein may exist as tautomers. Tautomers are compounds that are interconvertible by migration of a hydrogen atom, accompanied by a switch of a single bond and adjacent double bond. In bonding arrangements where tautomerization is possible, a chemical equilibrium of the tautomers will exist. All tautomeric forms of the compounds disclosed herein are contemplated. The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Some examples of tautomeric interconversions include:



[00172] A "metabolite" of a compound disclosed herein is a derivative of that compound that is formed when the compound is metabolized. The term "active metabolite" refers to a biologically active derivative of a compound that is formed when the compound is metabolized. The term "metabolized," as used herein, refers to the sum of the processes (including, but not limited to, hydrolysis reactions and reactions catalyzed by enzymes, such as, oxidation reactions) by which a particular substance is changed by an organism. Thus, enzymes may produce specific structural alterations to a compound. For example, cytochrome P450 catalyzes a variety of oxidative and reductive reactions while uridine diphosphate glucuronyl transferases catalyze the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols, carboxylic acids, amines and free sulfhydryl groups. Further information on metabolism may be obtained from The Pharmacological Basis of Therapeutics, 9th Edition, McGraw-Hill (1996). Metabolites of the compounds disclosed herein can be identified either by administration of compounds to a host and analysis of tissue samples from the host, or by incubation of compounds with hepatic cells *in vitro* and analysis of the resulting compounds. Both methods are well known in the art. In some embodiments, metabolites of a compound are formed by oxidative processes and correspond to the corresponding hydroxy-containing compound. In some embodiments, a compound is metabolized to pharmacologically active metabolites.

Compounds

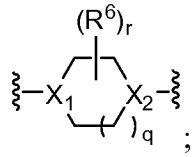
[00173] In one aspect, provided herein are compounds of Formula (II), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (II)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is  ;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl);

each R8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl, and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

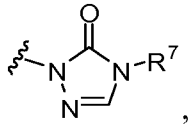
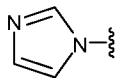
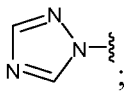
s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

u is 0, 1, 2, 3, 4 or 5;

v is 0, 1, 2, 3, or 4;

provided that:

if X_1 and X_2 are N; r is 0; q is 1; A^1 and A^2 are phenyl; Y^1 and Y^2 are O; m and n are 1; R^1, R^2, R^3, and R^4 are hydrogen; and C is  , then D is not  or  ; and

the compound is not 4-(4-(4-(4-((2-((1H-pyrazol-1-yl)methyl)-2-(2,4-difluorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-1H-1,2,4-triazol-5(4H)-one.

[00174] In some embodiments described above or below of a compound of Formula (II), X_1 and X_2 are N.

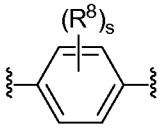
[00175] In some embodiments described above or below of a compound of Formula (II), X_1 is CR^5 and X_2 is N.

[00176] In some embodiments described above or below of a compound of Formula (II), X_1 is N and X_2 is CR^5.

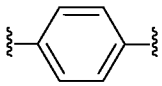
[00177] In some embodiments described above or below of a compound of Formula (II), q is 1 and r is 0.

[00178] In some embodiments described above or below of a compound of Formula (II), A^1 is aryl.

[00179] In some embodiments described above or below of a compound of Formula (II), A^1 is



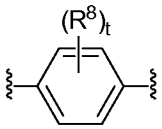
[00180] In some embodiments described above or below of a compound of Formula (II), A^1 is

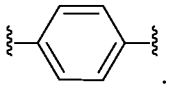
 .

[00181] In some embodiments described above or below of a compound of Formula (II), A^1 is heteroaryl.

[00182] In some embodiments described above or below of a compound of Formula (II), A^2 is aryl.

[00183] In some embodiments described above or below of a compound of Formula (II), A^2 is

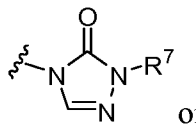
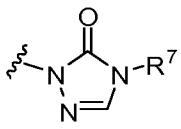
 .

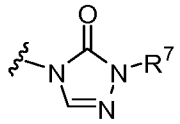
[00184] In some embodiments described above or below of a compound of Formula (II), A^2 is  .

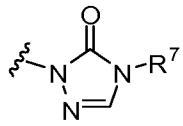
[00185] In some embodiments described above or below of a compound of Formula (II), A^2 is heteroaryl.

[00186] In some embodiments described above or below of a compound of Formula (II), A^2 is pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, or triazinyl.

[00187] In some embodiments described above or below of a compound of Formula (II), C is optionally substituted 5- or 6-membered heteroaryl. In other embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heterocyclyl.

[00188] In some embodiments described above or below of a compound of Formula (II), C is  or  .

[00189] In some embodiments described above or below of a compound of Formula (II), C is  .

[00190] In some embodiments described above or below of a compound of Formula (II), C is  .

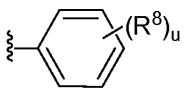
[00191] In some embodiments described above or below of a compound of Formula (II), E is alkyl.

[00192] In some embodiments described above or below of a compound of Formula (II), E is cycloalkyl.

[00193] In some embodiments described above or below of a compound of Formula (II), E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

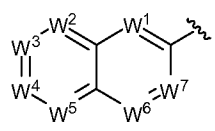
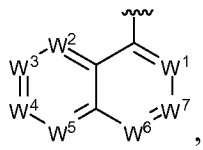
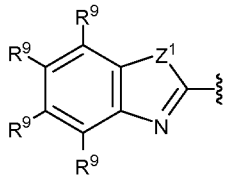
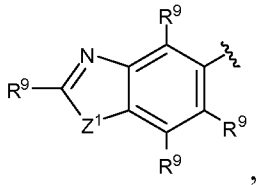
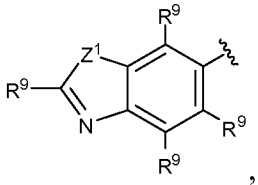
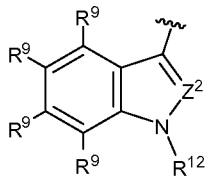
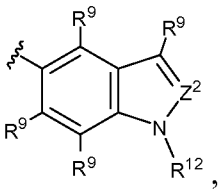
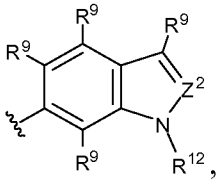
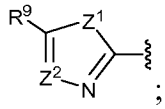
[00194] In some embodiments described above or below of a compound of Formula (II), E is heterocyclyl.

[00195] In some embodiments described above or below of a compound of Formula (II), E is aryl.

[00196] In some embodiments described above or below of a compound of Formula (II), E is  and u is 0, 1, 2, 3, 4, or 5.

[00197] In some embodiments described above or below of a compound of Formula (II), E is heteroaryl.

[00198] In some embodiments described above or below of a compound of Formula (II),

E is selected from:  ,  ,  ,  ,  ,  ,  ,  , and  ;

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

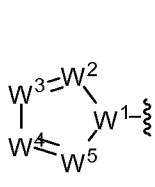
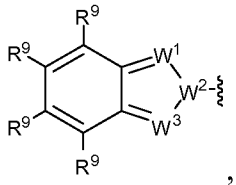
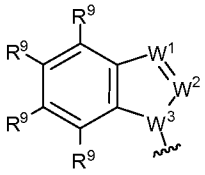
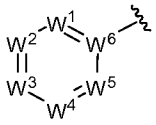
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

[00199] In some embodiments described above or below of a compound of Formula (II), D is aryl.

[00200] In some embodiments described above or below of a compound of Formula (II), D is heteroaryl.

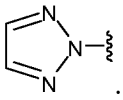
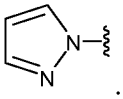
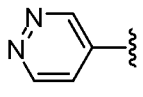
[00201] In some embodiments described above or below of a compound of Formula (II),

D is selected from  ,  ,  , and  ;

W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

[00202] In certain embodiments described above or below of a compound of Formula (II), D is  . In certain embodiments described above or below of a compound of Formula (II), D is  . In certain embodiments described above or below of a compound of Formula (II), D is  .

[00203] In some embodiments described above or below of a compound of Formula (II), Y^1 and Y^2 are O.

[00204] In some embodiments described above or below of a compound of Formula (II), m is 1.

[00205] In some embodiments described above or below of a compound of Formula (II), p is 1, 2, or 3.

[00206] In some embodiments described above or below of a compound of Formula (II), p is 1.

[00207] In some embodiments described above or below of a compound of Formula (II), R^1, R^2, R^3, and R^4 are hydrogen.

[00208] Further provided here is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject one or more compounds identified herein. The disease or condition may be a fibrosis. The fibrosis may be liver fibrosis. The liver fibrosis may be idiopathic pulmonary fibrosis.

[00209] Further provided here is a method of treating a disease or condition in a subject in need thereof, the method comprising administering to the subject one or more compounds identified here. The disease or condition may be a fibrosis. The fibrosis may be a chronic autoimmune disease. The chronic autoimmune disease may be rheumatoid arthritis, scleroderma, Crohn's disease, or systemic lupus erythematosus.

Preparation of Compounds

[00210] Described herein are compounds that treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, and processes for their preparation. Also described herein are pharmaceutically acceptable salts, pharmaceutically acceptable solvates, pharmaceutically active metabolites, and pharmaceutically acceptable prodrugs of such compounds. Pharmaceutical compositions comprising at least one such compound or a pharmaceutically acceptable salt, pharmaceutically acceptable solvate, pharmaceutically active metabolite or pharmaceutically acceptable prodrug of such compound, and a pharmaceutically acceptable excipient are also provided.

[00211] Compounds of of Formula (I) or Formula (I)I may be synthesized using standard synthetic reactions known to those of skill in the art or using methods known in the art. The reactions can be employed in a linear sequence to provide the compounds or they may be used to synthesize fragments which are subsequently joined by the methods known in the art.

[00212] The starting material used for the synthesis of the compounds described herein may be synthesized or can be obtained from commercial sources, such as, but not limited to, Aldrich Chemical Co. (Milwaukee, Wisconsin), Bachem (Torrance, California), or Sigma Chemical Co. (St. Louis, Mo.). The compounds described herein, and other related compounds having different substituents can be synthesized using techniques and materials known to those of skill in the art, such as described, for example, in March, ADVANCED ORGANIC CHEMISTRY 4th Ed., (Wiley 1992); Carey and Sundberg, ADVANCED ORGANIC CHEMISTRY 4th Ed., Vols. A and B (Plenum 2000, 2001); Green and Wuts, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS 3rd Ed., (Wiley 1999); Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier

Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989). (all of which are incorporated by reference in their entirety). Other methods for the synthesis of compounds described herein may be found in International Patent Publication No. WO 01/01982901, Arnold *et al. Bioorganic & Medicinal Chemistry Letters* 10 (2000) 2167-2170; Burchat *et al. Bioorganic & Medicinal Chemistry Letters* 12 (2002) 1687-1690. General methods for the preparation of compound as disclosed herein may be derived from known reactions in the field, and the reactions may be modified by the use of appropriate reagents and conditions, as would be recognized by the skilled person, for the introduction of the various moieties found in the formulae as provided herein.

[00213] The products of the reactions may be isolated and purified, if desired, using conventional techniques, including, but not limited to, filtration, distillation, crystallization, chromatography and the like. Such materials may be characterized using conventional means, including physical constants and spectral data.

[00214] Compounds described herein may be prepared as a single isomer or a mixture of isomers.

Further Forms of Compounds Disclosed Herein

Isomers

[00215] Furthermore, in some embodiments, the compounds described herein exist as geometric isomers. In some embodiments, the compounds described herein possess one or more double bonds. The compounds presented herein include all cis, trans, syn, anti, entgegen (E), and zusammen (Z) isomers as well as the corresponding mixtures thereof. In some situations, compounds exist as tautomers. The compounds described herein include all possible tautomers within the formulas described herein. In some situations, the compounds described herein possess one or more chiral centers and each center exists in the R configuration, or S confirguration. The compounds described herein include all diastereomeric, enantiomeric, and epimeric forms as well as the corresponding mixtures thereof. In additional embodiments of the compounds and methods provided herein, mixtures of enantiomers and/or diastereoisomers, resulting from a single preparative step, combination, or interconversion are useful for the applications described herein. In some embodiments, the compounds described herein are prepared as their individual stereoisomers by reacting a racemic mixture of the compound with an optically active resolving agent to form a pair of diastereoisomeric compounds, separating the diastereomers and recovering the optically pure enantiomers. In some embodiments, dissociable complexes are preferred (e.g., crystalline diastereomeric salts). In some embodiments, the diastereomers have

distinct physical properties (e.g., melting points, boiling points, solubilities, reactivity, etc.) and are separated by taking advantage of these dissimilarities. In some embodiments, the diastereomers are separated by chiral chromatography, or preferably, by separation/resolution techniques based upon differences in solubility. In some embodiments, the optically pure enantiomer is then recovered, along with the resolving agent, by any practical means that would not result in racemization.

Labeled compounds

[00216] In some embodiments, the compounds described herein exist in their isotopically-labeled forms. In some embodiments, the methods disclosed herein include methods of treating diseases by administering such isotopically-labeled compounds. In some embodiments, th emethods disclosed herein include methods of treating diseases by administering such isotopically-labeled compounds as pharmaceutical compositions. Thus, in some embodiments, the compounds disclosed herein include isotopically-labeled compounds, which are identical to those recited herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine and chloride, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, $^{18}0$, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. Compounds described herein, and the metabolites, pharmaceutically acceptable salts, esters, prodrugs, solvate, hydrates or derivatives thereof which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this invention. Certain isotopically-labeled compounds, for example those into which radioactive isotopes such as ^{3}H and ^{14}C are incorporated, are useful in drug and/or substrate tissue distribution assays. Tritiated, i. e., ^{3}H and carbon-14, i. e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavy isotopes such as deuterium, *i.e.*, ^{2}H, produces certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements. In some embodiments, the isotopically labeled compounds, pharmaceutically acceptable salt, ester, prodrug, solvate, hydrate or derivative thereof is prepared by any suitable method.

[00217] In some embodiments, the compounds described herein are labeled by other means, including, but not limited to, the use of chromophores or fluorescent moieties, bioluminescent labels, or chemiluminescent labels.

Pharmaceutically acceptable salts

[00218] In some embodiments, the compounds described herein exist as their pharmaceutically acceptable salts. In some embodiments, the methods disclosed herein include methods of treating diseases by administering such pharmaceutically acceptable salts. In some embodiments, the methods disclosed herein include methods of treating diseases by administering such pharmaceutically acceptable salts as pharmaceutical compositions.

[00219] In some embodiments, the compounds described herein possess acidic or basic groups and therefore react with any of a number of inorganic or organic bases, and inorganic and organic acids, to form a pharmaceutically acceptable salt. In some embodiments, these salts are prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound in its free form with a suitable acid or base, and isolating the salt thus formed.

[00220] Examples of pharmaceutically acceptable salts include those salts prepared by reaction of the compounds described herein with a mineral, organic acid or inorganic base, such salts including, acetate, acrylate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, bisulfite, bromide, butyrate, butyn-1,4-dioate, camphorate, camphorsulfonate, caproate, caprylate, chlorobenzoate, chloride, citrate, cyclopentanepropionate, decanoate, digluconate, dihydrogenphosphate, dinitrobenzoate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hexyne-1,6-dioate, hydroxybenzoate, γ-hydroxybutyrate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, iodide, isobutyrate, lactate, maleate, malonate, methanesulfonate, mandelate metaphosphate, methanesulfonate, methoxybenzoate, methylbenzoate, monohydrogenphosphate, 1-napthalenesulfonate, 2-napthalenesulfonate, nicotinate, nitrate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, pyrosulfate, pyrophosphate, propiolate, phthalate, phenylacetate, phenylbutyrate, propanesulfonate, salicylate, succinate, sulfate, sulfite, succinate, suberate, sebacate, sulfonate, tartrate, thiocyanate, tosylate undeconate and xylenesulfonate.

[00221] Further, the compounds described herein can be prepared as pharmaceutically acceptable salts formed by reacting the free base form of the compound with a pharmaceutically acceptable inorganic or organic acid, including, but not limited to, inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid metaphosphoric acid, and the like; and organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, p-toluenesulfonic acid, tartaric acid, trifluoroacetic acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid,

arylsulfonic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 4-methylbicyclo-[2.2.2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1 -carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid and muconic acid. In some embodiments, other acids, such as oxalic, while not in themselves pharmaceutically acceptable, are employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

[00222] In some embodiments, those compounds described herein which comprise a free acid group react with a suitable base, such as the hydroxide, carbonate, bicarbonate, sulfate, of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary, tertiary, or quaternary amine. Representative salts include the alkali or alkaline earth salts, like lithium, sodium, potassium, calcium, and magnesium, and aluminum salts and the like. Illustrative examples of bases include sodium hydroxide, potassium hydroxide, choline hydroxide, sodium carbonate, $N^+(C_{1-4}\ alkyl)_4$, and the like.

[00223] Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. It should be understood that the compounds described herein also include the quaternization of any basic nitrogen-containing groups they contain. In some embodiments, water or oil-soluble or dispersible products are obtained by such quaternization.

Solvates

[00224] In some embodiments, the compounds described herein exist as solvates. The invention provides for methods of treating diseases by administering such solvates. The invention further provides for methods of treating diseases by administering such solvates as pharmaceutical compositions.

[00225] Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and, in some embodiments, are formed during the process of crystallization with pharmaceutically acceptable solvents such as water, ethanol, and the like. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Solvates of the compounds described herein can be conveniently prepared or formed during the processes described herein. By way of example only, hydrates of the compounds described herein can be conveniently prepared by recrystallization from an aqueous/organic solvent mixture, using organic solvents including, but not limited to, dioxane, tetrahydrofuran or methanol. In addition, the compounds

provided herein can exist in unsolvated as well as solvated forms. In general, the solvated forms are considered equivalent to the unsolvated forms for the purposes of the compounds and methods provided herein.

Polymorphs

[00226] In some embodiments, the compounds described herein exist as polymorphs. The invention provides for methods of treating diseases by administering such polymorphs. The invention further provides for methods of treating diseases by administering such polymorphs as pharmaceutical compositions.

[00227] Thus, the compounds described herein include all their crystalline forms, known as polymorphs. Polymorphs include the different crystal packing arrangements of the same elemental composition of a compound. In certain instances, polymorphs have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. In certain instances, various factors such as the recrystallization solvent, rate of crystallization, and storage temperature cause a single crystal form to dominate.

Prodrugs

[00228] In some embodiments, the compounds described herein exist in prodrug form. The invention provides for methods of treating diseases by administering such prodrugs. The invention further provides for methods of treating diseases by administering such prodrugs as pharmaceutical compositions.

[00229] Prodrugs are generally drug precursors that, following administration to an individual and subsequent absorption, are converted to an active, or a more active species via some process, such as conversion by a metabolic pathway. Some prodrugs have a chemical group present on the prodrug that renders it less active and/or confers solubility or some other property to the drug. Once the chemical group has been cleaved and/or modified from the prodrug the active drug is generated. Prodrugs are often useful because, in some situations, they are easier to administer than the parent drug. They are, for instance, bioavailable by oral administration whereas the parent is not. In certain insatnces, the prodrug also has improved solubility in pharmaceutical compositions over the parent drug. An example, without limitation, of a prodrug would be a compound as described herein which is administered as an ester (the "prodrug") to facilitate transmittal across a cell membrane where water solubility is detrimental to mobility but which then is metabolically hydrolyzed to the carboxylic acid, the active entity, once inside the cell where water-solubility is beneficial. A further example of a prodrug might be a short peptide (polyamino acid) bonded to an acid group where the peptide is metabolized to reveal the active

moiety. (See for example Bundgaard, "Design and Application of Prodrugs" in *A Textbook of Drug Design and Development*, Krosgaard-Larsen and Bundgaard, Ed., 1991, Chapter 5, 113-191, which is incorporated herein by reference).

[00230] In some embodiments, prodrugs are designed as reversible drug derivatives, for use as modifiers to enhance drug transport to site-specific tissues. The design of prodrugs to date has been to increase the effective water solubility of the therapeutic compound for targeting to regions where water is the principal solvent.

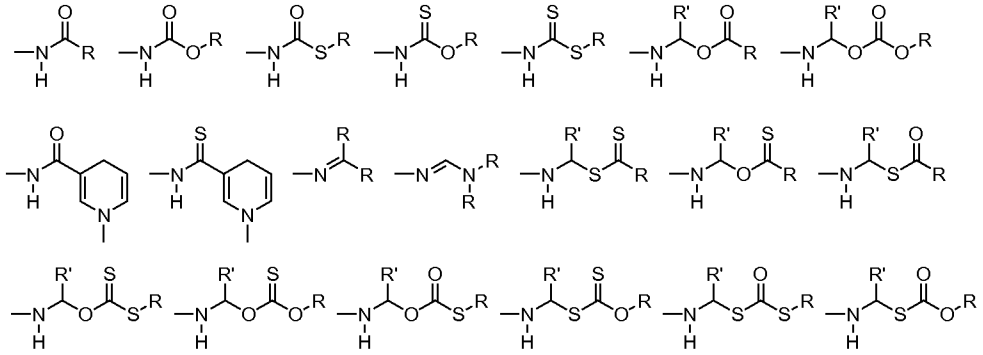
[00231] Additionally, prodrug derivatives of compounds described herein can be prepared by methods described herein are otherwise known in the art (for further details see Saulnier *et al.*, *Bioorganic and Medicinal Chemistry Letters*, **1994**, *4*, 1985). By way of example only, appropriate prodrugs can be prepared by reacting a non-derivatized compound with a suitable carbamylating agent, such as, but not limited to, 1,1-acyloxyalkylcarbanochloridate, *para*-nitrophenyl carbonate, or the like. Prodrug forms of the herein described compounds, wherein the prodrug is metabolized *in vivo* to produce a derivative as set forth herein are included within the scope of the claims. Indeed, some of the herein-described compounds are prodrugs for another derivative or active compound.

[00232] In some embodiments, prodrugs include compounds wherein an amino acid residue, or a polypeptide chain of two or more (e. g., two, three or four) amino acid residues is covalently joined through an amide or ester bond to a free amino, hydroxy or carboxylic acid group of compounds of the present invention. The amino acid residues include but are not limited to the 20 naturally occurring amino acids and also includes 4-hydroxyproline, hydroxylysine, demosine, isodemosine, 3-methylhistidine, norvaline, beta-alanine, gamma-aminobutyric acid, cirtulline, homocysteine, homoserine, ornithine and methionine sulfone. In other embodiments, prodrugs include compounds wherein a nucleic acid residue, or an oligonucleotide of two or more (e. g., two, three or four) nucleic acid residues is covalently joined to a compound of the present invention.

[00233] Pharmaceutically acceptable prodrugs of the compounds described herein also include, but are not limited to, esters, carbonates, thiocarbonates, N-acyl derivatives, N-acyloxyalkyl derivatives, quaternary derivatives of tertiary amines, N-Mannich bases, Schiff bases, amino acid conjugates, phosphate esters, metal salts and sulfonate esters. Compounds having free amino, amido, hydroxy or carboxylic groups can be converted into prodrugs. For instance, free carboxyl groups can be derivatized as amides or alkyl esters. In certain instances, all of these prodrug moieties incorporate groups including but not limited to ether, amine and carboxylic acid functionalities.

[00234] Hydroxy prodrugs include esters, such as though not limited to, acyloxyalkyl (e.g. acyloxymethyl, acyloxyethyl) esters, alkoxycarbonyloxyalkyl esters, alkyl esters, aryl esters, phosphate esters, sulfonate esters, sulfate esters and disulfide containing esters; ethers, amides, carbamates, hemisuccinates, dimethylaminoacetates and phosphoryloxymethyloxycarbonyls, as outlined in *Advanced Drug Delivery Reviews* **1996**, *19*, 115.

[00235] Amine derived prodrugs include, but are not limited to the following groups and combinations of groups:



as well as sulfonamides and phosphonamides.

[00236] In certain instances, sites on any aromatic ring portions are susceptible to various metabolic reactions, therefore incorporation of appropriate substituents on the aromatic ring structures, can reduce, minimize or eliminate this metabolic pathway.

Metabolites

[00237] In some embodiments, compounds of Formula (I) or Formula (II) are susceptible to various metabolic reactions. Therefore, in some embodiments, incorporation of appropriate substituents into the structure will reduce, minimize, or eliminate a metabolic pathway. In specific embodiments, the appropriate substituent to decrease or eliminate the susceptibility of an aromatic ring to metabolic reactions is, by way of example only, a halogen, or an alkyl group.

[00238] In additional or further embodiments, the compounds of Formula (I) or Formula (II) described herein are metabolized upon administration to an organism in need to produce a metabolite that is then used to produce a desired effect, including a desired therapeutic effect.

Pharmaceutical Compositions/Formulations

[00239] In another aspect, provided herein are pharmaceutical composition comprising a compound of Formula (I) or Formula (II) as described herein, or a pharmaceutically acceptable salt, polymorph, solvate, prodrug, N-oxide, stereoisomer, or isomer thereof, and a pharmaceutically acceptable excipient.

[00240] In some embodiments, the compounds described herein are formulated into pharmaceutical compositions. Pharmaceutical compositions are formulated in a conventional

manner using one or more pharmaceutically acceptable inactive ingredients that facilitate processing of the active compounds into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions described herein can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999), herein incorporated by reference for such disclosure.

[00241] Provided herein are pharmaceutical compositions that include a compound of Formula (I) or Formula (II) and at least one pharmaceutically acceptable inactive ingredient. In some embodiments, the compounds described herein are administered as pharmaceutical compositions in which a compound of Formula (I) or Formula (II) is mixed with other active ingredients, as in combination therapy. In other embodiments, the pharmaceutical compositions include other medicinal or pharmaceutical agents, carriers, adjuvants, preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure, and/or buffers. In yet other embodiments, the pharmaceutical compositions include other therapeutically valuable substances.

[00242] A pharmaceutical composition, as used herein, refers to a mixture of a compound of Formula (I) or Formula (II) with other chemical components (i.e. pharmaceutically acceptable inactive ingredients), such as carriers, excipients, binders, filling agents, suspending agents, flavoring agents, sweetening agents, disintegrating agents, dispersing agents, surfactants, lubricants, colorants, diluents, solubilizers, moistening agents, plasticizers, stabilizers, penetration enhancers, wetting agents, anti-foaming agents, antioxidants, preservatives, or one or more combination thereof. The pharmaceutical composition facilitates administration of the compound to an organism. In practicing the methods of treatment or use provided herein, therapeutically effective amounts of compounds described herein are administered in a pharmaceutical composition to a mammal having a disease, disorder, or condition to be treated. In some embodiments, the mammal is a human. A therapeutically effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compound used and other factors. The compounds can be used singly or in combination with one or more therapeutic agents as components of mixtures.

[00243] The pharmaceutical formulations described herein are administered to a subject by appropriate administration routes, including but not limited to, oral, parenteral (e.g., intravenous, subcutaneous, intramuscular), intranasal, buccal, topical, rectal, or transdermal administration

routes. The pharmaceutical formulations described herein include, but are not limited to, aqueous liquid dispersions, liquids, gels, syrups, elixirs, slurries, suspensions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid oral dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, powders, dragees, effervescent formulations, lyophilized formulations, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate and controlled release formulations.

[00244] Pharmaceutical compositions including a compound of Formula (I) or Formula (II) are manufactured in a conventional manner, such as, by way of example only, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or compression processes.

[00245] The pharmaceutical compositions will include at least one compound of Formula (I) or Formula (II) as an active ingredient in free-acid or free-base form, or in a pharmaceutically acceptable salt form. In addition, the methods and pharmaceutical compositions described herein include the use of *N*-oxides (if appropriate), crystalline forms, amorphous phases, as well as active metabolites of these compounds having the same type of activity. In some embodiments, compounds described herein exist in unsolvated form or in solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. The solvated forms of the compounds presented herein are also considered to be disclosed herein.

[00246] Pharmaceutical preparations for oral use are obtained by mixing one or more solid excipient with one or more of the compounds described herein, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, for example, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methylcellulose, microcrystalline cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose; or others such as: polyvinylpyrrolidone (PVP or povidone) or calcium phosphate. If desired, disintegrating agents are added, such as the cross-linked croscarmellose sodium, polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. In some embodiments, dyestuffs or pigments are added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[00247] Pharmaceutical preparations that are administered orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and,

optionally, stabilizers. In soft capsules, the active compounds are dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In some embodiments, stabilizers are added.

[00248] In certain embodiments, delivery systems for pharmaceutical compounds may be employed, such as, for example, liposomes and emulsions. In certain embodiments, compositions provided herein can also include an mucoadhesive polymer, selected from among, for example, carboxymethylcellulose, carbomer (acrylic acid polymer), poly(methylmethacrylate), polyacrylamide, polycarbophil, acrylic acid/butyl acrylate copolymer, sodium alginate and dextran.

Combination Treatment

[00249] The compounds according to Formula (I) or Formula (II) may be used in combination with one or more additional antifibrotic agents. The antifibrotic agent may be a lysophophatidic acid 1 (LPA1) antagonist. The antifibrotic agent may be selected from pirfenidone , nintedanib, thalidomide, carlumab, FG-3019, fresolimumab, interferon alpha, lecithinized superoxide dismutase, simtuzumab, tanzisertib, tralokinumab, hu3G9, huTBTl3_2_1, 2126458, AM-152, IFN-gamma-1b, IW-001, PRM-151, PXS-25, pentoxifylline/N-acetyl-cysteine, pentoxifylline/vitamin E, salbutamol sulfate, [Sar9,Met(O2)11]-Substance P, pentoxifylline, mercaptamine bitartrate, obeticholic acid, aramchol, GFT-505, icosapent ethyl ester, metformin hydrochloride, metreleptin, muromonab-CD3, oltipraz, IMM-124-E, MK-4074, PX-102, and RO-5093151.

[00250] The compounds according to Formula (I) or Formula (II) may be used in combination with one or more additional azole antifungal agents. The azole antifungal agent may be selected from an imidazole antifungal, a triazole antifungal, or a thiazole antifungal. Examples of such antifungal agents include, but are not limited to, Imidazole derivatives like miconazole, ketoconazole, clotrimazole, clomidazole, croconazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, miconazole, neticonazole, oxiconazole, sertaconazole, sulconazole, tioconazole; Triazole derivatives like fluconazole, fosfluconazole, hexaconazole, itraconazole, isavuconazole, posaconazole, voriconzaole, terconazole, albaconazole; and Thiazole derivatives like abafungin.

Administration of Pharmaceutical Composition

[00251] Suitable routes of administration include, but are not limited to, oral, intravenous, rectal, aerosol, parenteral, ophthalmic, pulmonary, transmucosal, transdermal, vaginal, otic, nasal, and topical administration. In addition, by way of example only, parenteral delivery includes

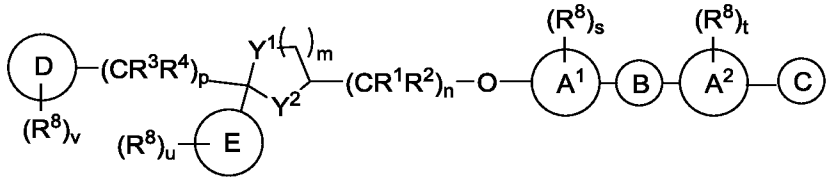
intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intralymphatic, and intranasal injections.

[00252] In some embodiments, compounds of Formula (I) or Formula (II) and compositions thereof are administered in any suitable manner. The manner of administration can be chosen based on, for example, whether local or systemic treatment is desired, and on the area to be treated. For example, the compositions can be administered orally, parenterally (e.g., intravenous, subcutaneous, intraperitoneal, or intramuscular injection), by inhalation, extracorporeally, topically (including transdermally, ophthalmically, vaginally, rectally, intranasally) or the like.

[00253] Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained.

Methods

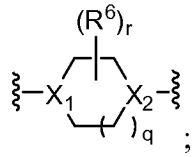
[00254] In one aspect, provided herein is a method to treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, the method comprising administering a composition comprising a therapeutically effective amount of a compound of Formula (I), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (I)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is 

;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

each R8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl, and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

u is 0, 1, 2, 3, 4 or 5; and

v is 0, 1, 2, 3, or 4.

[00255] In some embodiments described above or below of a compound of Formula (I), X_1 and X_2 are N

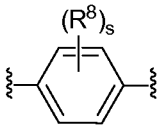
[00256] In some embodiments described above or below of a compound of Formula (I), X_1 is CR^5 and X_2 is N.

[00257] In some embodiments described above or below of a compound of Formula (I), X_1 is N and X_2 is CR^5.

[00258] In some embodiments described above or below of a compound of Formula (I), q is 1 and r is 0.

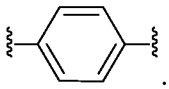
[00259] In some embodiments described above or below of a compound of Formula (I), A^1 is aryl.

[00260] In some embodiments described above or below of a compound of Formula (I), A^1 is



.

[00261] In some embodiments described above or below of a compound of Formula (I), A^1 is

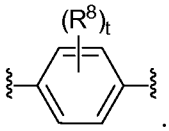


.

[00262] In some embodiments described above or below of a compound of Formula (I), A^1 is heteroaryl.

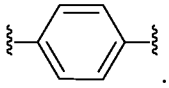
[00263] In some embodiments described above or below of a compound of Formula (I), A^2 is aryl.

[00264] In some embodiments described above or below of a compound of Formula (I), A^2 is



.

[00265] In some embodiments described above or below of a compound of Formula (I), A^2 is



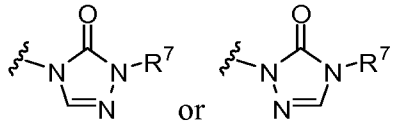
.

[00266] In some embodiments described above or below of a compound of Formula (I), A^2 is heteroaryl.

[00267] In some embodiments described above or below of a compound of Formula (I), A^2 is pyridine, pyrazine, pyrimidine, pyridazine, or triazine.

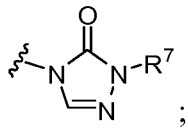
[00268] In some embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heteroaryl. In other embodiments described above or below of a compound of Formula (I), C is optionally substituted 5- or 6-membered heterocyclyl.

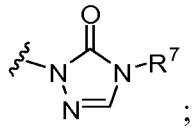
[00269] In some embodiments described above or below of a compound of Formula (I), C is



; and

R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

[00270] In some embodiments described above or below of a compound of Formula (I), C is  ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

[00271] In some embodiments described above or below of a compound of Formula (I), C is  ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

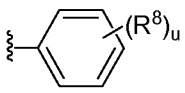
[00272] In some embodiments described above or below of a compound of Formula (I), E is alkyl.

[00273] In some embodiments described above or below of a compound of Formula (I), E is cycloalkyl.

[00274] In some embodiments described above or below of a compound of Formula (I), E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

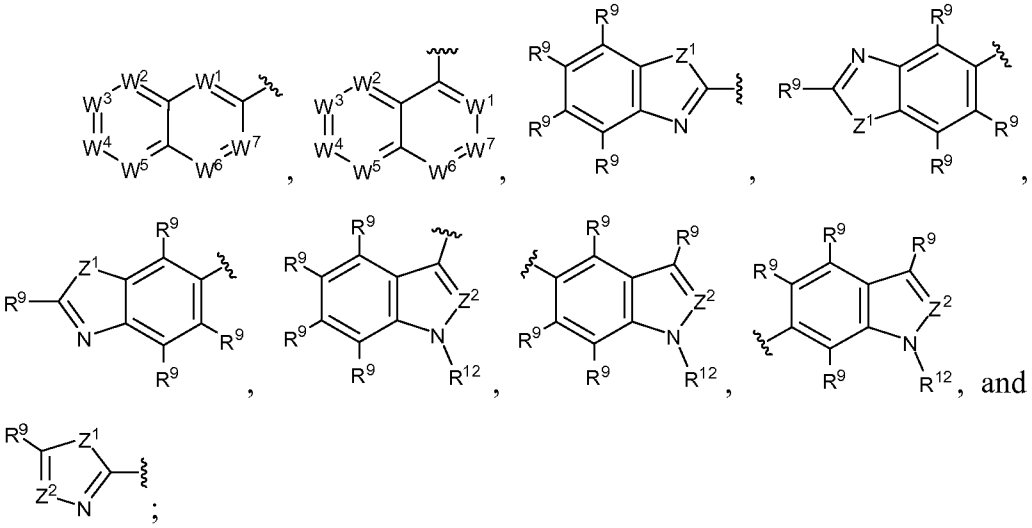
[00275] In some embodiments described above or below of a compound of Formula (I), E is heterocyclyl.

[00276] In some embodiments described above or below of a compound of Formula (I), E is aryl.

[00277] In some embodiments described above or below of a compound of Formula (I), E is  and u is 0, 1, 2, 3, 4, or 5.

[00278] In some embodiments described above or below of a compound of Formula (I), E is heteroaryl.

[00279] In some embodiments described above or below of a compound of Formula (I), E is selected from:



;

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

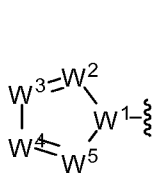
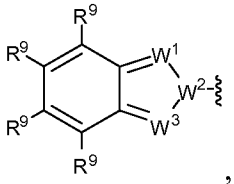
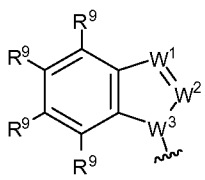
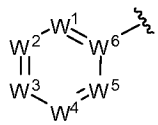
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

[00280] In some embodiments described above or below of a compound of Formula (I), D is aryl.

[00281] In some embodiments described above or below of a compound of Formula (I), E is heteroaryl.

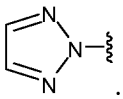
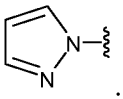
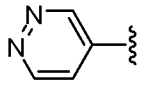
[00282] In some embodiments described above or below of a compound of Formula (I), D is selected from:

W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

[00283] In certain embodiments described above or below of a compound of Formula (I), D is  . In certain embodiments described above or below of a compound of Formula (I), D is  . In certain embodiments described above or below of a compound of Formula (I), D is  .

[00284] In some embodiments described above or below of a compound of Formula (I), Y^1 and Y^2 are O.

[00285] In some embodiments described above or below of a compound of Formula (I), m is 1.

[00286] In some embodiments described above or below of a compound of Formula (I), p is 1, 2, or 3.

[00287] In some embodiments described above or below of a compound of Formula (I), p is 1.

[00288] In some embodiments described above or below of a compound of Formula (I), R^1, R^2, R^3, and R^4 are hydrogen.

[00289] Also provided herein is a pharmaceutical composition comprising a compound of Formula (II) or as described above and below, or a pharmaceutically acceptable salt, solvate,

polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof, and a pharmaceutically acceptable excipient.

[00290] Further provided herein is a method to treat fibrosis using a compound described herein wherein the fibrosis is liver fibrosis, idiopathic pulmonary fibrosis, kidney fibrosis, or cardiac fibrosis.

[00291] Further provided herein is a method to treat liver fibrosis using a compound described herein wherein the liver fibrosis is associated with the later stages of alcoholic or nonalcoholic liver cirrhosis.

[00292] Further provided herein is a method to treat fibrosis using a compound described herein wherein the fibrosis is idiopathic pulmonary fibrosis.

[00293] Further provided herein is a method to treat a disease using a compound described herein wherein the disease or disorder characterized by fibrosis is a chronic autoimmune disease.

[00294] Further provided herein is a method to treat chronic autoimmune disease using a compound described herein wherein the chronic autoimmune disease is rheumatoid arthritis, scleroderma, Crohn's disease or systemic lupus erythematosus.

[00295] Further provided herein is a method to treat chronic autoimmune disease using a compound described herein wherein the chronic autoimmune disease is scleroderma.

[00296] Further provided herein is a method to treat fibrosis using a compound described herein wherein the fibrosis is keloid formation resulting from abnormal wound healing.

[00297] Further provided herein is a method to treat fibrosis using a compound described herein wherein the fibrosis occurs after organ transplantation.

[00298] Also provided herein is a method to treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, the method comprising administering a composition comprising a therapeutically effective amount of a compound described herein in combination with one or more pharmaceutical agents. In certain embodiments described above, the one or more pharmaceutical agents are antifibrotic agents. In certain embodiments described above, the one or more pharmaceutical agents are antifungal agents.

[00299] Further disclosed herein are image-based systems for identifying inhibitors of fibrosis. In some embodiments, the system comprises (a) one or more fibroblasts; and (b) a cell imaging device for producing one or more images of the one or more fibroblasts. In some embodiments, the cell imaging device comprises a fluorescent microscope. In some embodiments, the cell imaging device comprises CCD camera technology. In some embodiments, the cell imaging device is automated. In some embodiments, the cell imaging device is manually operated. In some embodiments, the cell imaging device is thermoelectrically cooled.

[00300] In some embodiments, the system further comprises a light source. In some embodiments, the light source is an LED.

[00301] In some embodiments, the system further comprises a scanner.

[00302] In some embodiments, the system further comprises a computer.

[00303] In some embodiments, the system further comprises one or more memory locations for storing and/or receiving the one or more images. In some embodiments, the system further comprises one or more memory locations for storing and/or receiving one or more instructions for producing the one or more images.

[00304] In some embodiments, the system further comprises one or more processors for analyzing the one or more images of the one or more fibroblasts. In some embodiments, the system further comprises one or more processors for processing the one or more images of the one or more fibroblasts. In some embodiments, the system further comprises one or more processors for transmitting the one or more images of the one or more fibroblasts.

[00305] In some embodiments, the system further comprises one or more software programs for capturing, producing, analyzing, scanning, storing, and/or trasmitting the one or more images.

[00306] In some embodiments, the system further comprises one or more barcode readers for reading one or more barcodes on one or more samples comprising the one or more cells.

[00307] In some embodiments, the system further comprises one or more robots for handling one or more samples comprising the one or more cells. In some embodiments, the system further comprises one or more robots for treating one or more samples comprising the one or more cells with one or more agents.

[00308] In some embodiments, the one or more agents comprise TGF-beta. In some embodiments, the one or more agents comprise one or more test agents.

[00309] In some embodiments, the system further comprises one or more processors for identifying the one or more test agents as inhibitors of fibrosis. In some embodiments, the system further comprises one or more processors for ranking the inhibitors of fibrosis.

[00310] In some embodiments, the system further comprises one or more algorithms. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts contacted with the one or more agents. In some embodiments, the one or more algorithms analyze an intensity of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze an fluorescence intensity of the one or more fibroblasts.

[00311] In some embodiments, the cell imaging device comprises a CellInsight NXT High Content Screening (HCS) platform.

[00312] In some embodiments, the one or more fibroblasts are hepatic stellate cells (HSCs).

[00313] Further disclosed herein, are methods of identifying inhibitors of fibrosis. In some embodiments, the method comprises (a) contacting a first sample comprising one or more fibroblasts with a cellular growth agent; (b) contacting a second sample comprising one or more fibroblasts with the cellular growth agent and a first test agent; (c) producing one or more images of the one or more fibroblasts of the first sample and one or more images of the one or more fibroblasts of the second sample; and (d) determining whether the first test agent is an inhibitor of fibrosis based on an analysis of the one or more images of the first sample and the one or more images of the second sample.

[00314] In some embodiments, the cellular growth agent is a growth factor. In some embodiments, the cellular growth agent is transforming growth factor beta (TGF-b).

[00315] In some embodiments, the first test agent is a small molecule. In some embodiments, the first test agent is a bioactive small molecule.

[00316] In some embodiments, the second sample is contacted with the cellular growth agent and the first test agent simultaneously. In some embodiments, the second sample is contacted with the cellular growth agent and the first test agent sequentially. In some embodiments, the second sample is contacted with the cellular growth agent prior to contact with the first test agent. In some embodiments, the second sample is contacted with the first test agent prior to contact with the cellular growth agent.

[00317] In some embodiments, the method further comprises one or more additional samples comprising one or more fibroblasts. In some embodiments, the first sample, second sample, and/or one or more additional samples are from the same source. In some embodiments, the first sample, second sample, and/or one or more additional samples are from two or more different sources.

[00318] In some embodiments, the method further comprises contacting the one or more additional samples with the cellular growth agent and one or more additional test agents.

[00319] In some embodiments, the one or more images of the first sample and the one or more images of the second sample are captured simultaneously. In some embodiments, the one or more images of the first sample and the one or more images of the second sample are captured sequentially.

[00320] In some embodiments, the one or more fibroblasts of the first sample are cultured in one or more wells on a first culture plate. In some embodiments, the one or more fibroblasts of the second sample are cultured on one or more wells on a second culture plate. In some embodiments, the one or more fibroblasts of the one or more additional samples are cultured on one or more wells on one or more additional culture plates.

[00321] In some embodiments, the first culture plate and the second culture plate are different. In some embodiments, the first culture plate, the second culture plate, and/or the one or more additional culture plates are different.

[00322] In some embodiments, the first culture plate and the second culture plate are the same. In some embodiments, the first culture plate, the second culture plate, and/or the one or more additional culture plates are the same.

[00323] In some embodiments, the method further comprises contacting the one or more fibroblasts of the first sample and/or the one or more fibroblasts of the second sample with a third agent. In some embodiments, the method further comprises contacting the one or more fibroblasts of the one or more additional samples with a third agent.

[00324] In some embodiments, the third agent is an antibody. In some embodiments, the third agent is an anti-smooth muscle actin (SMA) antibody.

[00325] In some embodiments, the one or more images of the first sample and/or the one or more images of the second sample are based on the images of the one or more fibroblasts contacted with the third agent. In some embodiments, the one or more images of the one or more additional samples are based on the images of the one or more fibroblasts contacted with the third agent.

[00326] In some embodiments, producing the one or more images comprises the use of one or more cell imaging devices. In some embodiments, the cell imaging device comprises a CellInsight NXT High Content Screening (HCS) platform.

[00327] In some embodiments, the method further comprises one or more algorithms. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze a morphology of the one or more fibroblasts contacted with the one or more agents. In some embodiments, the one or more algorithms analyze an intensity of the one or more fibroblasts. In some embodiments, the one or more algorithms analyze an fluorescence intensity of the one or more fibroblasts.

[00328] In some embodiments, the method further comprises detecting transdifferentation of the one or more fibroblasts. In some embodiments, transdifferentiation of the one or more fibroblasts comprises transdifferentiation into one or more myofibroblasts.

[00329] In some embodiments, determining whether the first test agent is identified as an inhibitor of fibrosis is based on a comparison of the myofibroblasts composition in the first sample to the myofibroblasts composition in the second sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the second sample is less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the

second sample is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the second sample is at least about 1.5-, 2-, 2.5-, 3-, 3.5-, 4-, 4.5-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, or 15-fold less than the myofibroblasts composition in the first sample.

[00330] In some embodiments, the method further comprises determining whether the one or more additional test agents are inhibitors of fibrosis. In some embodiments, determining whether the one or more additional test agents is identified as an inhibitor of fibrosis is based on a comparison of the myofibroblasts composition in the first sample to the myofibroblasts composition in the one or more additional samples. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or 90% less than the myofibroblasts composition in the first sample. In some embodiments, the first test agent is identified as an inhibitor of fibrosis if the myofibroblasts composition in the one or more additional samples is at least about 1.5-, 2-, 2.5-, 3-, 3.5-, 4-, 4.5-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, or 15-fold less than the myofibroblasts composition in the first sample.

EXAMPLES

List of abbreviations

[00331] As used above, and throughout the description of the invention, the following abbreviations, unless otherwise indicated, shall be understood to have the following meanings:

ACN	
Bn	benzyl
BOC or Boc	*tert*-butyl carbamate
BOP	benzotriazol-1-yl-oxytris (dimethylamino) phosphonium
t-Bu	tert-butyl
Cbz	benzyl carbamate
Cy	Cyclohexyl
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene

DCC	dicyclohexylcarbodiimide
DCM	dichloromethane (CH_2CI_2)
DIC	1,3-diisopropylcarbodiimide
DEAD	diethyl azodicarboxylate
DIAD	diisopropyl azodicarboxylate
DIEA	diisopropylethylamine
DMAP	4-(N,N-dimethylamino)pyridine
DMP reagent	Dess-Martin Periodinane reagent
DMF	dimethylformamide
DMA	N,N-Dimethylacetamide
DME	1,2-Dimethoxy-ethane
DMSO	dimethylsulfoxide
Dppf	1,1'-Bis(diphenylphosphino)ferrocene
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl
eq	equivalent(s)
Et	ethyl
Et_2O	diethyl ether
EtOH	ethanol
EtOAc	ethyl acetate
HOAt	1-hydroxy-7-azabenzotriazole
HOBT	1-hydroxybenztriazole
HOSu	N-hydroxysuccinamide
HPLC	high performance liquid chromatography
LAH	lithium aluminum anhydride
Me	methyl
MeI	methyliodide
MeOH	methanol
MOMCl	methoxymethylchloride
MOM	methoxymethyl
MS	mass spectroscopy
NMP	*N*-methyl-pyrrolidin-2-one
NMR	nuclear magnetic resonance
PyBOP	benzotriazole-1-yl-oxytris-pyrrolidino-phosphonium Hexafluorophosphate

SPHOS	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
TBD	1,5,7-triazabicyclo[4.4.0]-dec-5-ene
RP-HPLC	reverse phase-high pressure liquid chromatography
TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-butyldimethylsilyl chloride
TBTU	O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium
TEOC	2-Trimethylsilylethyl Carbamate
TFA	trifluoroacetic acid
Tf_2O	triflate anhydride
TMG	1,1,3,3-Tetramethylguanidine
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
XPHOS	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

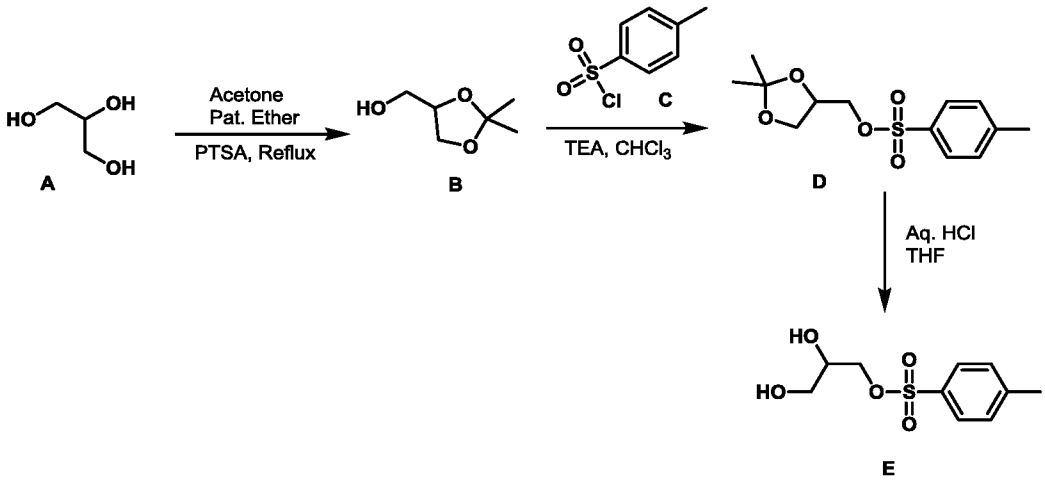
General Examples for the Preparation of Compounds of the Invention

[00332] The starting materials and intermediates for the compounds of this invention may be prepared by the application or adaptation of the methods described below, their obvious chemical equivalents, or, for example, as described in literature such as The Science of Synthesis, Volumes 1-8. Editors E. M. Carreira et al. Thieme publishers (2001-2008). Details of reagent and reaction options are also available by structure and reaction searches using commercial computer search engines such as Scifinder (www.cas.org) or Reaxys (www.reaxys.com).

Part A:

[00333] The following reaction schemes A, B, and C detail the synthesis of starting materials involved in the final formation of compound 46 (scheme D).

Scheme A: Synthesis of Racemic Diol (E):



[00334] To a 250ml three neck round bottom flask equipped with condenser and a Dean-stark was added Intermediate-A (25.0g), acetone (75.0 ml), PTSA.H_2O (0.75 g) and petroleum ether (75.0 ml). Mixture was stirred at reflux temperature for 12 h and monitored by TLC (Hexane: Ethyl acetate (5:5)). The reaction mixture was cooled to room temperature and 0.75 g sodium acetate was added. The mixture was stirred at room temperature for 30 minutes. The organic layer was decanted and concentrated under reduced pressure to give crude liquid, Intermediate-**B** (27.0g, 75.2%).

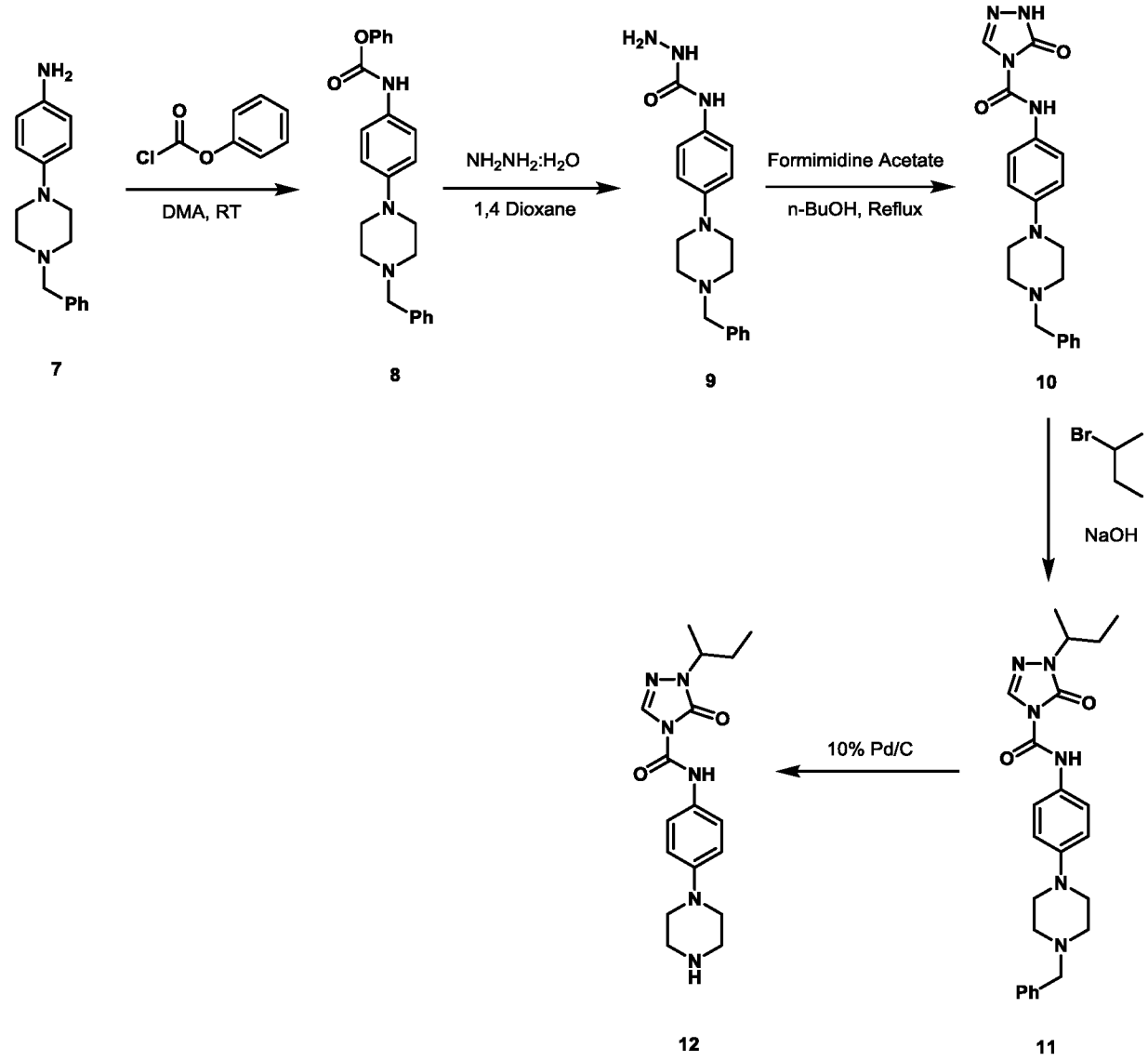
[00335] To a 500ml three neck round bottom flask equipped with calcium chloride guard tube was added Intermediate-B (25.0 g) in chloroform (185 ml). TEA (79.0 ml) was added and reaction cooled to 0°C. Intermediate-**C** (47.0 g) was charged lot wise to the mixture and allowed to stir at room temperature for 5 h. Reaction monitored by TLC (Hexane: Ethyl acetate (5:5)). The reaction mixture was washed with water (200 ml) and the aqueous layer back extracted with $CHCl_3$ (50ml x 2). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give crude oil. Oil was purified by column chromatography (6% ethyl acetate in hexanes). Final product Intermediate-**D** (21.0g, 38.6%).

[00336] To a 250 ml three neck round bottom flask equipped with magnetic stirrer was added Intermediate-D (21.0 g) in THF (100 ml). Solution was cooled to 0°C, mixed with 6N HCl (25.0 ml) and stirred at room temperature for 6 h. Reaction monitored by TLC (Hexane: Ethyl acetate (5:5)). The reaction mixture was diluted with water (100 ml) and neutralized with saturated sodium bicarbonate solution. Product was extracted with $CHCl_3$ (50ml*2). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give thick oil, Intermediate-**E** (16.0g, 88.8%). Intermediate-**E** was used in the next stage of synthesis without further purification.

Intermediate Spectral Data:

Intermediate	Characterization Data (NMR/LCMS)
B	^{1}HNMR (400MHz, $CDCl_3$): 1.23 (s, 3H), 1.3 (s, 3H), 3.44-3.55 (m, 3H), 3.61-3.64(m, 1H), 3.89-3.92 (m, 1H), 4.05-4.10 (m, 1H).
D	LCMS: 98.1 %; m/z: 304 (M + H_20).
E	^{1}HNMR (400MHz, $CDCl_3$): 2.46 (s, 3H), 2.85 (s, 1H), 3.4 (s, 1H), 3.57-3.61 (m, 1H), 3.66-3.70(m, 1H), 3.93-3.96 (m, 1H), 4.04-4.10 (m, 2H), 7.36-7.38 (dd, *J*=8.0 Hz, 2H), 7.79-7.81 (dd, *J*=8.0 Hz, 2H).

Scheme B: Synthesis of Intermediate-12:



[00337] To a 100 ml three neck round bottom flask equipped with calcium chloride tube was added Intermediate-7 (5.0 g) in DMA (25.0 ml). To it phenyl chloroformate (2.8 ml) was added

drop wise and the reaction mixture stirred at RT for half an hour. Reaction monitored by TLC (Hexane: Ethyl acetate (5:5)). Reaction mixture was poured into ice water. Resulting precipitate was collected by filtration, washed with water and dried under vacuum to obtain pure product, Intermediate-**8** (4.4g, 61.1%).

[00338] To a 100 ml three neck round bottom flask equipped with calcium chloride tube was added charged Intermediate-8 (4.4 g) in 1,4 Dioxane (20.0 ml). Hydrazine hydrate (1.37 ml) was added drop wise and the reaction mixture was stirred at RT for 24 h. Reaction monitored by TLC (Hexane: Ethyl acetate (5:5)). Mixture was poured into ice water. Precipitate was collected by filtration, washed with water and dried under vacuum to obtain pure product, Intermediate-**9** (2.80g, 53.8%).

[00339] To a 100 ml three neck round bottom flask equipped with condenser was added Intermediate-9 (2.80 g) in n-BuOH (25.0 ml). Formimidine acetate (4.47 g) was added and the reaction mixture was stirred at 90°C for 4 h. Reaction monitored by TLC (EtOAc). Mixture was cooled to room temperature and the precipitate collected by filtration, washed with ethyl acetate and dried to get pure product, Intermediate-**10** (2.5g, 86.8%).

[00340] To a 100 ml three neck round bottom flask equipped with condenser was added Intermediate-10 (2.50 g) in DMF (30.0 ml). NaOH (1.50 g) and sec- bromo butane (5.11 g) were added and the reaction mixture stirred at 90°C for 8 h. Reaction monitored by TLC (EtOAc). Reaction mixture was poured into ice water. The precipitates were collected by filtration, washed with water and dried under vacuum to obtain pure product, Intermediate-**11** (1.45g, 49.8%).

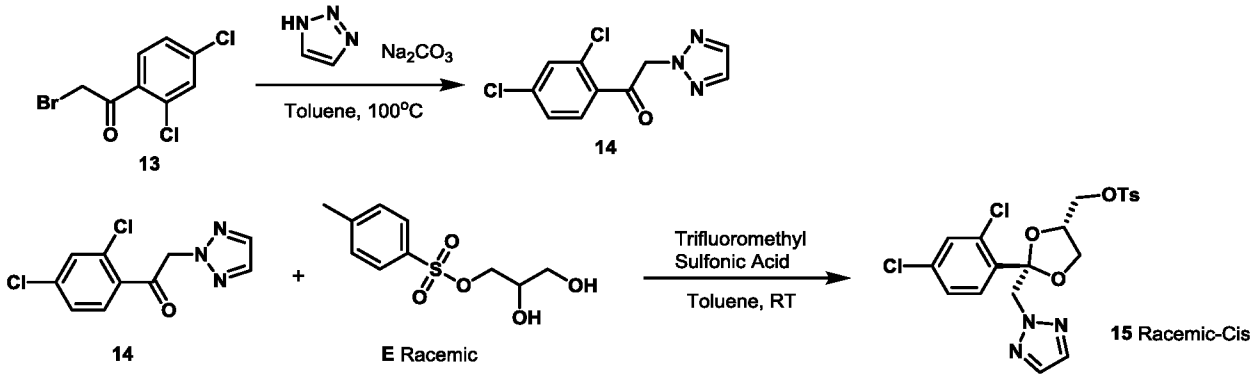
[00341] To a 100 ml Hydrogenator vessel was added Intermediate-11 (1.45 g) in MeOH (25.0 ml). 10% Pd/C (0.15 g) was added and the reaction mixture was hydrogenated at RT under 10 kg of H_2 pressure overnight. Reaction monitored by TLC (EtOAc). After completion of the reaction, catalyst was removed by filtration and solvent was evaporated to give thick oil. The crude product was purified by column chromatography (5% MeOH in MDC). Final product Intermediate-**12** (0.75g, 67.5%).

Intermediate Spectral Data:

Intermediate #	Characterization Data (NMR/LCMS)
8	LCMS: 99.16 % @ 256 nm; m/z: 388 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 2.94-3.05 (m, 2H), 3.13-3.20 (m, 2H), 3.72-3.75 (d, 2H), 4.39-4.40 (d, 2H), 6.95-6.97(d, *J*=8.0 Hz, 2H), 7.19-7.27 (m, 3H), 7.38-7.44 (m, 4H), 7.49-7.50 (m, 3H), 7.59-7.60 (d, *J*=4.0 Hz, 2H).
9	LCMS: 97.61 % @ 254 nm; m/z: 326 (M+H).

Intermediate #	Characterization Data (NMR/LCMS)
10	LCMS: 98.34% @ 256 nm; m/z: 336 (M+H).
11	LCMS: 96.95 % @ 258 nm; m/z: 392 (M+H).
12	LCMS: 95.78 % @ 257 nm; m/z: 302 (M+H).

Scheme C: Synthesis of Intermediate-15



[00342] To a stirred solution of Intermediate-13 (2.0 g) in toluene (30.0 ml) was charged 1H-1,2,3 Triazole (1.96 g) and Na_2CO_3 (3.01 g) at RT. The reaction mixture was stirred at 100°C for 3 h. The completion of reaction was monitored via TLC (Hexane: Ethyl acetate (5:5)). After reaction completion, mixture was cooled to RT and diluted with ethyl acetate (50 ml). The obtained organic layer was washed with water (50 ml x 2). Organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography. (20% ethyl acetate in hexanes). Intermediate-**14** (0.70g, 19.2%).

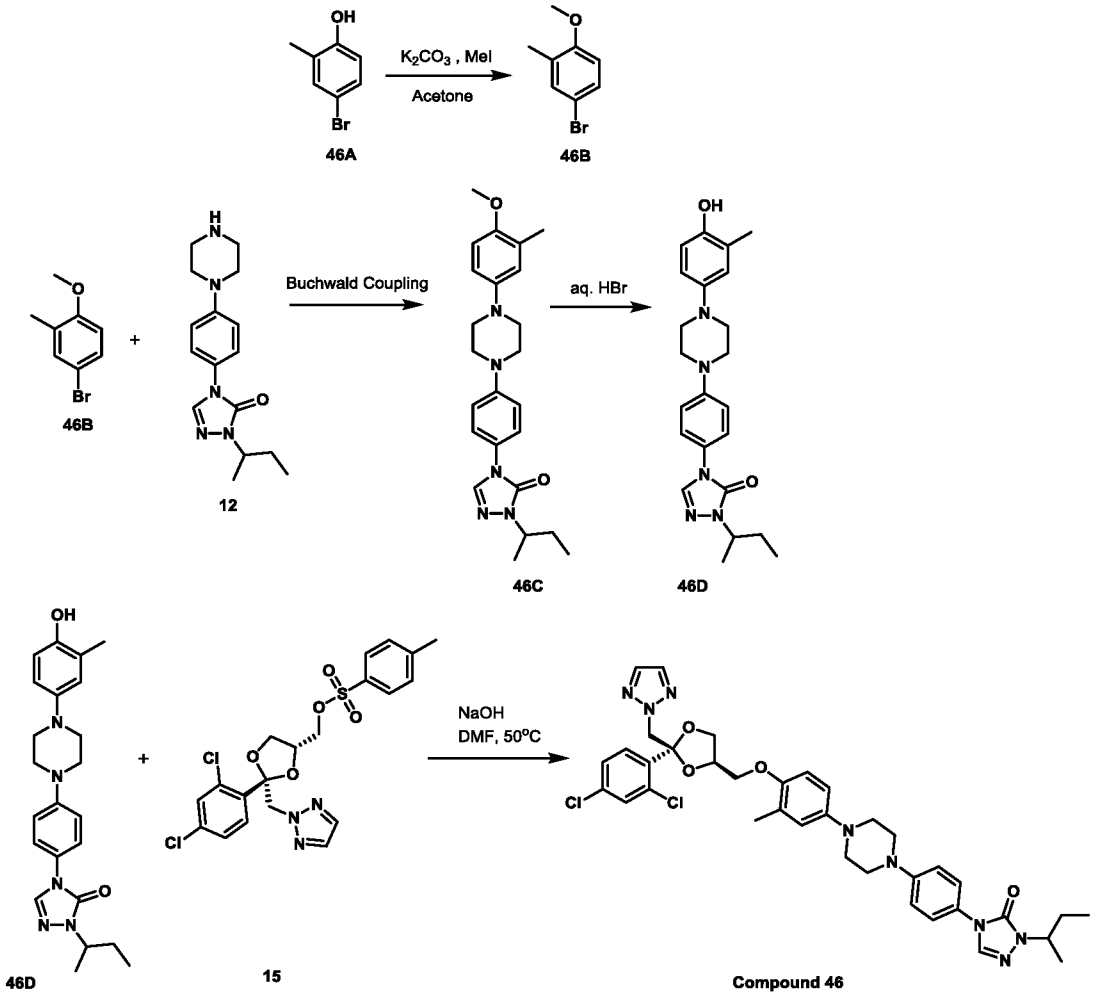
[00343] To a stirred solution of Intermediate-14 (1.30 g) in toluene (15.0 ml) was charged Intermediate-E (1.50 g) at RT under argon atmosphere. The resulting mixture was cooled to 0°C and to it was added drop-wise Triflic acid (1.80 ml). Mixture was warmed to RT and stirred for 60 h. The completion of reaction was monitored via TLC (Hexane: Ethyl acetate (5:5)). After reaction completion, resulting mixture was poured into water (25 ml) and neutralized with saturated sodium bicarbonate solution. Aqueous layer was extracted with ethyl acetate (25 ml x 2). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure to give thick oil. The crude product was purified by column chromatography (10% ethyl acetate in hexanes). Intermediate-**15** (0.32g, 24.6%).

Intermediate Spectral Data:

Intermediate #	Characterization Data (NMR/LCMS)
14	LCMS: 96.62 % @ 252 nm; m/z 258 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 5.91 (s, 2H), 7.36-7.39 (dd, J_1=12.0 Hz and

	J_2=4.0 Hz, 1H), 7.51-7.52 (d, J=4.0 Hz, 1H), 7.64-7.66 (d, J=8.0 Hz, 1H), 7.73(s, 2H).
15	LCMS: 100 % @ 225 nm; m/z 486 (M+2). ^{1}HNMR (400MHz, $CDCl_3$): 2.49 (s, 3H), 3.41-3.45 (m, 1H), 3.75-3.82 (m, 1H), 3.84-3.89 (m, 2H), 4.23-4.26(m, 1H), 4.94-4.98 (d, J=14.4 Hz, 1H), 5.06-5.09 (d , J=14.0 Hz, 1H), 7.18-7.21 (dd, J_1=10.4 Hz and J_2=2.0 Hz, 1H), 7.38-7.40(d, J_1=12.0 Hz and J_2=8.0 Hz, 2H),7.44-7.46 (m, 2H), 7.55 (s, 2H), 7.77-7.79(d, J_1=12.0 Hz and J_2=8.4 Hz, 2H).

Scheme D: Synthesis of Compound 46:



[00344] To a stirred solution of Intermediate-46A (0.50 g) in acetone (7.0 ml) was charged K_2CO_3 (0.44 g) at RT under argon atmosphere. Methyl iodide (0.20 ml) was added drop wise and the mixture warmed to RT and stirred for 24 h. The completion of reaction was monitored via TLC (Hexane: Ethyl acetate (5:5)). After reaction completion, mixture was poured into water (25 ml). Aqueous layer was extracted with ethyl acetate (25 ml x 2). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give crude mass, Intermediate-**46-B** (0.53g, 100%).

[00345] To a stirred solution of Intermediate-46B (0.125 g) in toluene (3.0 ml) was charged Intermediate-12 (0.188 g), Sodium tert butoxide (0.089 g), Ru(Phos) (0.028 g) and $Pd_2(dba)_3$ (0.055 g) at RT under argon atmosphere. Resulting mixture was purged with argon balloon for 10 minutes and stirred at 120°C for 15 h. The completion of reaction was monitored via TLC (Hexane: Ethyl acetate (5:5)). After reaction completion, mixture was poured into water (10 ml). Aqueous layer was extracted with ethyl acetate (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (25% ethyl acetate in hexanes). Intermediate-**46-C** (0.08g, 30.7%).

[00346] To a 5 ml tube was charged Intermediate-46C (0.080 g) in 48% aq. HBr (0.80 ml). Resulting mixture was stirred at reflux temperature overnight. The completion of reaction was monitored via TLC (Ethyl acetate). The reaction mixture was diluted with water (5.0 ml) and neutralized with saturated sodium bicarbonate solution. Resulting precipitate was collected by filtration, washed with water and dried under vacuum to obtain pure Intermediate-**46-D** (0.050g, 64.90%).

[00347] To a stirred solution of Intermediate-46D (0.050 g) in DMF (1.0 ml) was charged Intermediate-15 (0.050 g) and NaOH (0.020 g) at RT under argon atmosphere. The resulting mixture was stirred at 50°C overnight. The completion of reaction was monitored via TLC (Hexane: Ethyl acetate (5:5)). After reaction completion, resulting mixture was poured into water (10 ml) and aqueous layer extracted with ethyl acetate (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by preparative HPLC (eluted in ACN:Water containing 0.1% NH_3 by reverse phase preparative purification). Product fractions were lyophilized to obtain pure product, **46** (0.012g, 11.32%).

Intermediate and Final Compound Spectral Data:

Intermediate/ Final Compound #	**Characterization Data (NMR/LCMS)**
46B	^{1}HNMR (400MHz, $CDCl_3$): 2.19-2.20 (d, *J*=4.0 Hz, 3H), 3.82 (s, 3H), 6.69-6.70 (d, *J*=4.0 Hz, 1H), 7.26-7.28 (d, *J*=8.0 Hz, 2H).
46C	LCMS: 98.3 % @ 261 nm; m/z 422 (M+1).
46D	LCMS: 81.9 % @ 258 nm; m/z 408 (M+1).
46	LCMS: 100% @ 262 nm; m/z 719.51 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.2 Hz, 3H) ,1.40-1.42 (d, *J*=6.8 Hz, 3H) ,1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H),2.16-2.19 (s, 3H),3.25 (s, 4H), 3.38 (s, 4H),3.48-3.52 (q, 1H), 3.91-4.00 (m, 3H),4.29-

Intermediate/ Final Compound #	Characterization Data (NMR/LCMS)
	4.34(m, 1H),4.39-4.41 (m, 1H),5.06-5.09 (d, *J*=14.0 Hz, 1H),5.17-5.20 (d, *J*=14.0 Hz, 1H),6.69-6.71 (d, *J*=8.0 Hz, 1H),6.78-6.84 (m, 2H),7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz and J_2=2.0 Hz, 1H),7.44-7.48 (m, 3H),7.52-7.54 (d, *J*=8.4 Hz 1H),7.60-7.64 (m, 3H).

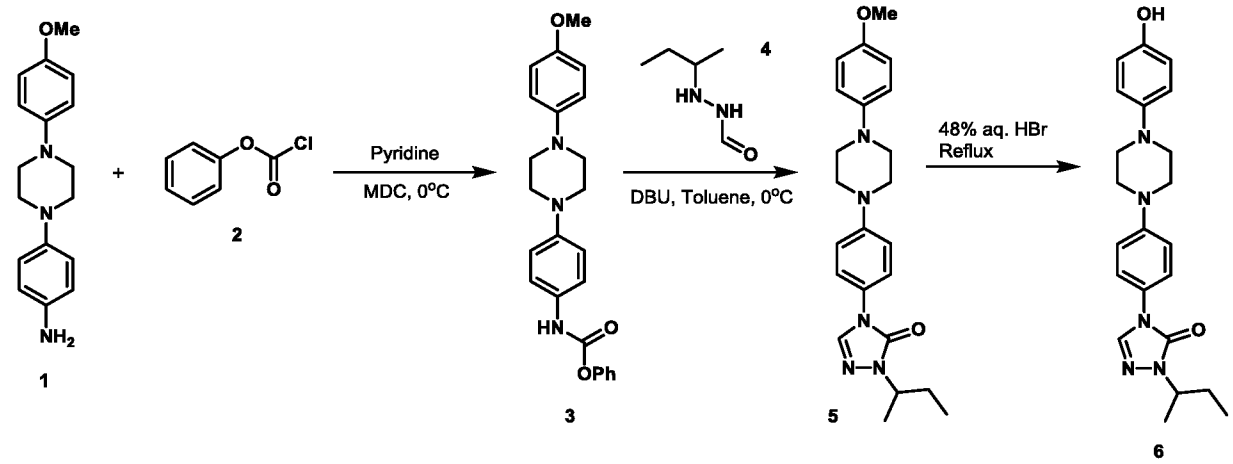
Additional synthetic information:

[00348] Compounds 47-54 were prepared using the identical reaction scheme with their corresponding bromophenols (Intermediate-46A).

Part B:

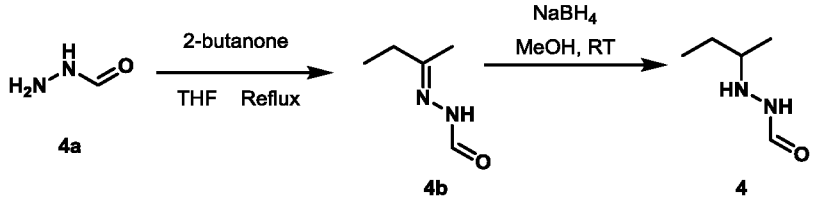
[00349] The following reaction schemes E and F detail the synthesis of starting materials involved in the final formation of compound 42 (scheme G). The same racemic diol produced in Scheme A (Part A) was also used in the synthesis that follows.

Scheme E: Synthesis of Intermediate-6:



[00350] To a 100ml three neck round bottom flask equipped with calcium chloride tube were charged Intermediate-1 (5.13 g), pyridine (12.0 ml) and MDC (50.0 ml). Reaction mixture was cooled to 0°C and to it phenyl chloroformate (5.0 ml) was added. The reaction was stirred at 4°C overnight. Reaction completion was confirmed by TLC (Hexane: Ethyl acetate (5:5)). To the mixture was added water (100 ml) and solution stirred at RT for 30 minutes. The precipitate was filtered, washed with dichloromethane (10 ml x 2) and dried under vacuum to obtain pure product, Intermediate-**3** (5.5g, 75.1%).

Synthesis of Intermediate-4:



[00351] To a 250 ml three neck round bottom flask equipped with condenser and a Dean-Stark trap were charged Intermediate-4a (20.0 g), 2-butanone (29.8 ml) and anhydrous sodium sulfate in THF (150 mg). The mixture was stirred at reflux temperature for 2 h. The completion of the reaction was confirmed by TLC (Ethyl acetate). The reaction was cooled to RT and solvent was evaporated under reduced pressure to give white solid, Intermediate-**4b** (32.0g, 84.2%).

[00352] To a 500 ml three neck round bottom flask equipped with magnetic stirrer were charged Intermediate-4b (32.0 g) in MeOH (350 ml). Resulting mixture was cooled to 0°C followed by addition of $NaBH_4$ (9.01 g). Reaction was stirred at RT for 5 h. The completion of the reaction was confirmed by TLC using (Hexane: Ethyl acetate (5:5)). After completion, solvent was removed by distillation and residue was dissolved in water (100 ml). Product was extracted with $CHCl_3$ (100ml x 3).The organic layers were combined, dried over Na_2SO_4 and concentrated under reduced pressure to give thick oil. The crude product was purified by column chromatography (10% ethyl acetate in hexanes.) Intermediate-**4** (22.0 g, 67.6%).

[00353] To a 100 ml three neck round bottom flask equipped with condenser and calcium chloride tube were charged Intermediate-3 (5.0 g), Intermediate-4 (1.51 g) and DBU (0.022 g) in toluene (50.0 ml). The resulting reaction mixture was stirred at 100°C for 15 h. Reaction completion was confirmed by TLC (Ethyl acetate). Mixture was concentrated under reduced pressure and methanol was added. Solution was stirred at 4°C for 2 h. Resulting precipitate was collected by filtration and dried to get pure Intermediate-**5** (4.35g, 85.9%).

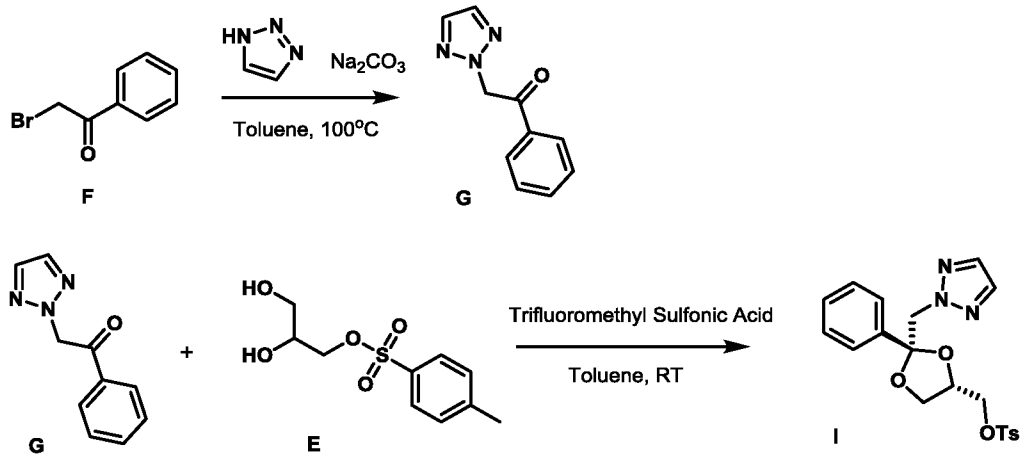
[00354] To a 100 ml three neck round bottom flask equipped with condenser were charged Intermediate-5 (4.35 g) in toluene (43.5 ml). The mixture was stirred at reflux temperature overnight. The completion of the reaction was confirmed by TLC (Ethyl acetate). Reaction mixture was cooled to RT and neutralized with saturated sodium bicarbonate solution. Resulting precipitate was collected by filtration, washed with water and dried to get pure Intermediate-**6** (3.30g, 78.3%).

Intermediate Spectral Data:

Intermediate #	Characterization Data (NMR/LCMS)
3	LCMS: 57.55 % @ 202 nm and 100 % @ 259 nm; m/z: 404.95 (M+H).

4	^{1}HNMR (400MHz, $CDCl_3$): 0.81-0.86 (m, 6H), 0.89-0.93 (m, 6H), 1.12-1.22 (m, 2H), 1.37-1.46 (m, 2H), 2.61-2.66(m, 1H), 2.73-2.74 (d, *J*=4.0 Hz, 1H), 4.79-4.86 (d, *J*=28.0 Hz, 2H), 7.92-8.01 (m, 2H), 8.73-8.76 (d, *J*=12.0 Hz, 1H), 9.31-9.32 (d, *J*=4.0 Hz,1H).
5	LCMS: 77.46 % @ 260 nm; m/z: 409.2 (M+H).
6	LCMS: 72.11 % @ 202 nm and 99.12 @ 250 nm; m/z: 394.6 (M+H).

Scheme F: Synthesis of Intermediate-I:



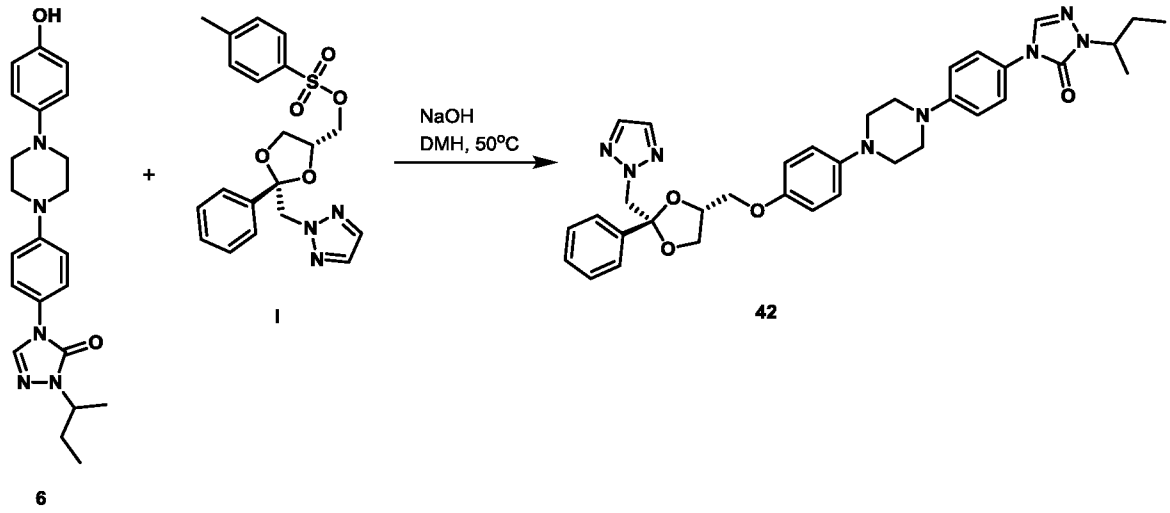
[00355] To a stirred solution of Intermediate-F (2.0 g) in toluene (15.0 ml) was charged 1H-1,2,3 Triazole (1.78 g) and Na_2CO_3 (2.74 g) at RT. The reaction mixture was stirred at 100°C for 3 h. The completion of reaction was monitored on TLC (Hexane: Ethyl acetate (5:5)). After completion, mixture was cooled to RT and diluted with ethyl acetate (25 ml). Solution was washed with water (25 ml*2). Organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure to give crude mass. The crude product was purified by column chromatography (15% ethyl acetate in hexanes). Intermediate-**G** (0.65g, 26.7%).

[00356] To a stirred solution of Intermediate-G (0.25 g) in Toluene (3.5 ml) was charged Intermediate-E (0.39 g) at RT under argon atmosphere. The mixture was cooled to 0°C and to it Triflic acid (0.48 ml) was added drop wise. Solution was then warmed to RT and stirred for 60 h. The completion of reaction was monitored on TLC (Hexane: Ethyl acetate (5:5)). After completion, resulting mixture was poured into water (10 ml) and neutralized with saturated sodium bicarbonate solution. Aqueous layer was extracted with ethyl acetate (10 ml*2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give thick oil. The crude product was purified by column chromatography (6% ethyl acetate in hexanes). Intermediate-**I** (0.12g, 21.8%).

Intermediate Spectral Data:

Intermediate	Characterization Data (NMR/LCMS)
G	LCMS: 100 % @ 245 nm; m/z 182.82 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 5.95 (s, 2H), 7.52-7.56 (m, 2H), 7.65-7.69 (m, 2H), 7.76 (s, 2H), 7.98-8.00(m, 2H)
I	LCMS: 100 % @ 223 nm; m/z 416.2 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 2.19 (s, 2H), 2.47 (s, 2H), 3.58-3.61 (m, 1H), 2.72-2.76 (m, 1H), 3.83-3.87(m, 1H), 3.93-3.97 (m, 1H), 4.09-4.12 (m , 1H), 4.74(s, 2H), 7.28-7.35 (m, 5H), 7.39-7.41(m, 2H),7.62 (s, 2H), 7.66-7.68(d, *J*=8.4 Hz, 2H).

Scheme G: Synthesis of Compound 42:



[00357] To a stirred solution of Intermediate-6 (0.060 g) in DMF (1.0 ml) was charged Intermediate-I (0.070 g) and NaOH (0.024 g) at RT under argon atmosphere. The resulting mixture was stirred at 50°C overnight. The completion of reaction was monitored on TLC (hexane: ethyl acetate (5:5)). After completion, reaction was poured into water (10 ml). Aqueous layer was extracted with ethyl acetate (10 ml*2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give crude mass. The crude product was purified by preparative HPLC (ACN:Water containing 0.1% NH_3 by reverse phase preparative purification). The product fractions were lyophilized to obtain pure product, **42** (0.016g, 16%).

Intermediate Spectral Data:

Final Compound #	Characterization Data (NMR/LCMS)
42	LCMS: 100 % @ 261 nm; m/z 637.45 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.42 (d, *J*=6.8 Hz,

	3H) ,1.72-1.77 (m, 1H), 1.86-1.89 (m, 1H), 3.30-3.43 (m, 9H), 3.73-3.78 (m, 1H), 3.83-3.86(m, 1H), 3.89-3.93 (m, 1H), 4.30-4.33 (m , 1H), 4.37-4.40(m, 1H), 4.87(s, 2H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 7.02-7.07(m, 4H), 7.38-7.42 (m, 3H), 7.44-7.47(d, *J*=8.8 Hz, 2H), 7.56-7.59(m, 2H), 7.64(d, *J*=2.8 Hz,3H)

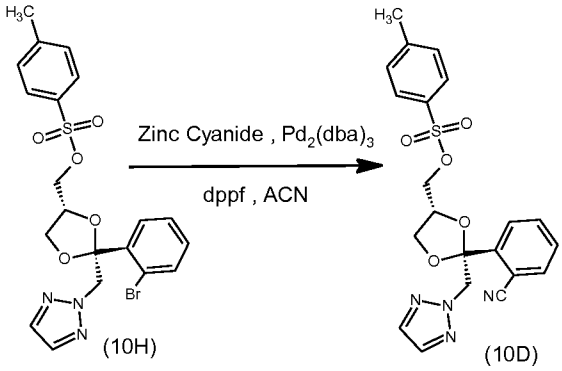
Additional synthetic information:

[00358] Compounds 30-45 were prepared using the identical reaction scheme with their corresponding phenacyl bromides/chlorides (Intermediate-F).

Part C: Synthesis of Compound 74

[00359] Compound 74 was prepared using analogous procedures as those used to prepare Compound 46, substituting Intermediate-10D for Intermediate-15 (Scheme D). Intermediate-10H, the precursor of Intermediate-10D, was prepared using analogous procedures as those used to prepare Intermediate-15.

Scheme H: Synthesis of Intermediate-10D:



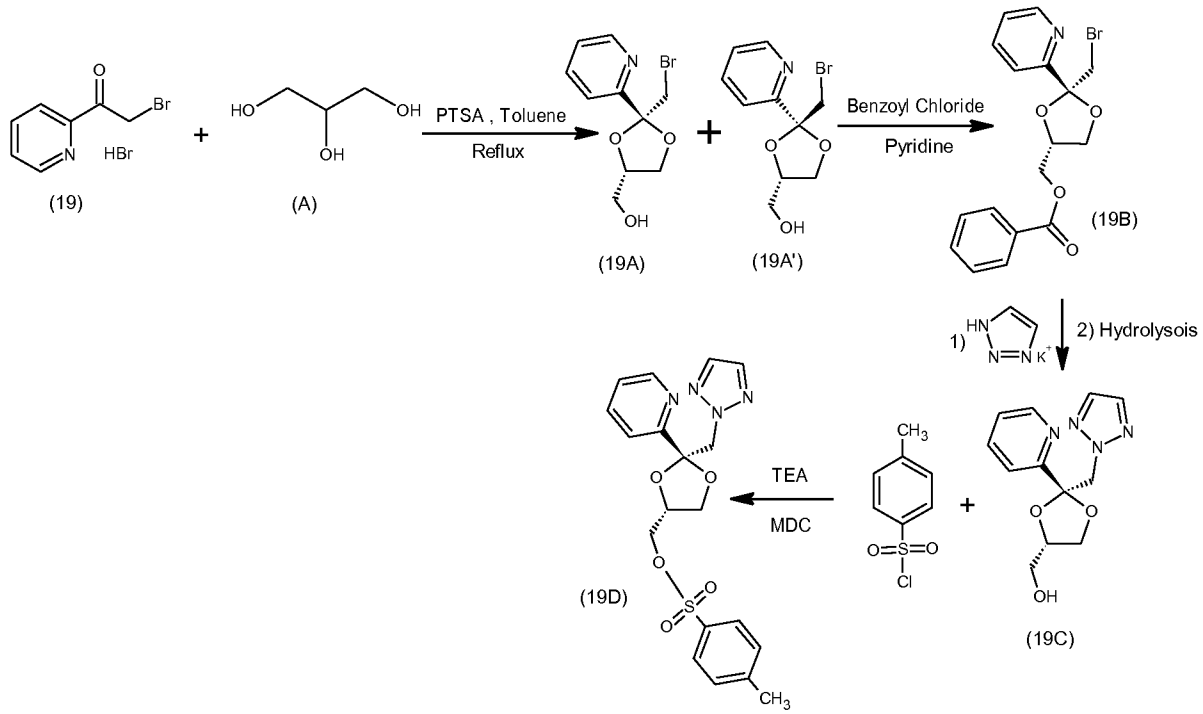
[00360] To a stirred solution of Intermediate-10H (0.1 g) in ACN (7.0 ml) was charged $Zn(CN)_2$ (0.47 g), dppf (0.017 g) and $Pd_2(dba)_3$ (0.027 g) at RT under argon atmosphere. Resulting mixture was purged with argon for 10 min and stirred at 120°C for 15 h. The completion of reaction was monitored by TLC (hexane: ethyl acetate (5:5)). After completion of the reaction, reaction mixture was poured into water (10 ml). Aqueous layer was extracted with ethyl acetate (10 ml x 2). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure to give crude mass which was purified by column chromatography (25% ethyl acetate in hexanes). Intermediate-**10D** (0.013g, 13.4%).

Intermediate Compound #	**Characterization Data (NMR/LCMS)**
10D	LCMS: 95.23 % @ 225 nm; m/z 440.9 (M+H).

Part D: Synthesis of Compound 75

[00361] Compound 75 was prepared using analogous procedures as those used to prepare Compound 42, substituting Intermediate-19D for Intermediate-I (Scheme G). Intermediate-19D was prepared as shown in Scheme I (below), inverting the steps in Schemes A and C such that bromomethyl ketone 19 was reacted with Intermediate A prior to reaction with 1H-1,2,3-triazole.

Scheme I: Synthesis of Intermediate-19D



[00362] To a stirred solution of Intermediate-19 (0.75 g – prepared by analogy to the bromoketone precursor for compound 88) in toluene (6.0 ml) was added Intermediate-A (1.53 g), PTSA (0.076 g) and molecular sieves (0.5 g) at RT. The reaction mixture was stirred at reflux temperature for 24 h. The completion of reaction was monitored by TLC using hexane: ethyl acetate (5:5) as a mobile phase. After completion of the reaction, the resulting mixture was cooled to RT and poured into water (10 ml) and neutralized with saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum to give crude mixture of Intermediates **19A** and **19A'** which was used in the next step without further purification. Weight of mixture of Intermediate-**19A** and Intermediate-**19A'**: 1.0 g (crude). LCMS: 33.84 %; m/z: 276 (M + 2).

[00363] To a stirred solution of Intermediate-19A & -19A' (1.0 g) in MDC (10.0 ml) was added pyridine (0.58 ml) at RT under argon atmosphere. Into the resulting mixture, benzoyl chloride (0.43 ml) was added drop-wise before the mixture was allowed to stir at RT overnight. The

completion of reaction was monitored by TLC using hexane: ethyl acetate (5:5) as a mobile phase. After completion of the reaction, the resulting mixture was poured into water (10 ml). The aqueous layer was extracted with MDC (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum to give thick oil which was further purified by preparative HPLC (ACN:water containing 0.1% formic acid). The product fractions were lyophilized to obtain Intermediate-**19B**. Weight of Intermediate-19B: 0.20 g (Yield: 14.5%). ^{1}H NMR (400 MHz, $CDCl_3$ δ ppm): 3.89-3.97 (m, 2H), 4.15-4.22 (m, 2H), 4.54-4.61 (m, 3H), 7.29-7.33 (m, 1H), 7.46-7.50(m, 2H), 7.58-7.62 (m, 1H), 7.65-7.67 (m, 1H), 7.75-7.79 (m, 1H), 8.09-8.11 (m, 2H), 8.69-8.70 (d, 1H). LCMS: 100% purity; m/z: 380 (M + 2).

[00364] To a stirred solution of Intermediate-19B (0.20 g) in DMF (3.0 ml) was added triazole potassium salt (0.14 g) at RT under argon atmosphere. The resulting mixture was heated with stirring at 130 °C for 24 h. The completion of reaction was monitored by TLC using hexane: ethyl acetate (5:5) as a mobile phase. After completion of the reaction, the resulting mixture was cooled to RT and DMF was removed under vacuum. Residue was dissolved in THF (4.0 ml) before the addition of 32% NaOH aqueous solution (4.0 ml). The resulting mixture was stirred at reflux temperature for 3 h. The completion of hydrolysis was monitored by TLC using MDC: MeOH (9:1) as a mobile phase. After completion of the reaction, the resulting mixture was cool to RT and poured into water (10 ml). The aqueous layer was extracted with ethyl acetate (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum to give a thick oil which was further purified by column chromatography (60% ethyl acetate in hexanes) to provide Intermediate-**19C**. Weight of Intermediate-**19C**: 0.045 g (Yield: 32.6%). ^{1}H-NMR (400 MHz, $CDCl_3$ δ ppm): 2.19 (s, 1H), 3.33-3.37 (d, J=3.2 Hz, 1H), 3.84-3.94 (m, 2H), 4.05-4.08 (m, 1H), 4.32(s, 1H), 4.99-5.03 (d, J=14.0 Hz, 1H), 5.25-5.29 (d, J=14.0 Hz, 1H), 7.35-7.36 (m, 1H), 7.57-7.59 (d, J=9.2 Hz, 1H), 7.66 (s, 2H), 7.75-7.78 (m, 1H), 8.76 (s, 1H). LCMS: 99.27% purity by UV; m/z: 263 (M + 1).

[00365] To a stirred solution of Intermediate-19C (0.045 g) in dichloromethane (1.5 ml) was charged triethylamine (0.04 ml) at room temperature under argon atmosphere. The resulting mixture was cooled to 0°C and to it *p*-TSCl (0.04 g) was added and the mixture was warmed to room temperature and stirred for 3 hrs at RT. The completion of reaction was monitored on TLC using hexane: ethyl acetate (5:5) as a mobile phase. After completion of the reaction, the resulting mixture was poured into water (10 ml) and neutralized with saturated sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate (10 ml x 2). The organic layers were combined, dried over Na_2SO_4 and evaporated under vacuum to give Intermediate-**19D** as a thick oil. Weight of Intermediate-**19D**: 0.075 g (crude). ^{1}H NMR (400 MHz, $CDCl_3$ δ ppm) 2.49 (s, 3H), 3.22-3.28 (m, 1H), 3.69-3.73 (m, 1H), 3.85-3.89 (m, 1H), 3.98-4.01 (m, 1H),

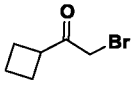
4.42-4.43 (d, J=4.4 Hz, 1H), 5.01-5.12 (m, 2H), 7.19-7.21 (d, J=8.0 Hz, 1H), 7.39-7.44 (m, 2H), 7.44-7.48 (m, 3H), 7.78-7.83 (m, 3H), 7.94-7.96 (d, J=8.4 Hz, 1H), 8.84-8.85(d, J=4.0 Hz, 1H). LCMS: 88.74% purity by UV; m/z: 417 (M + 1).

[00366] Compound 78 was prepared by analogy to Compound 75 except that 2-acetylthiazole was used instead of 2-acetylpyridine as the initial starting material. Bromination can be carried out by analogy to the synthesis of the bromoketone precursor of compound 88.

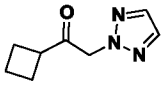
Part E: Synthesis of Compound 88

[00367] Compound 88 is prepared using analogous procedures as those used to prepare Compound 42, substituting cyclobutyl-(N2)triazolyl ketone for Intermediate-14 (Scheme C).

Synthesis of cyclobutyl-(N2)triazolyl ketone



[00368] To a cooled solution of cyclobutyl-methyl ketone (1 equiv) in MeOH was added bromine (1 equiv) dropwise at 0 °C for 30 min. The resulting reaction mixture was stirred at the same temperature for about 2 h, and then at RT for another 30 min. The unreacted bromine was then quenched carefully with the 10 % aqueous solution of $Na_2S_2O_3$. The mixture was then extracted with ether, and washed with $NaHCO_3$ and brine. The combined organic extract was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the desired bromoketone.



[00369] To the solution of the bromoketone (1.0 equiv) and triazole (1.2 equiv) in ACN was added DIPEA (1.2 equiv). The resulting reaction mixture was then heated to reflux for 1 h. The reaction mixture was concentrated and purified by flash column chromatography to afford the desired cyclobutyl-triazolyl ketone as a pale yellow oil which solidified slowly with time.

[00370] Additional compounds appearing in Table 1 were prepared using procedures analogous to the preceding procedures.

Biological Examples

EXAMPLE I: Biological Assays

[00371] A high content imaging assay, based on α-SMA staining and cell morphological changes associated with fibroblast to myofibroblast transdifferentiation, was established using primary human lung fibroblasts and primary rodent HSCs. Conditions, involving serum starvation and subsequent TGF-β treatment, were identified that facilitate robust *in vitro* transdifferentiation in a miniaturized (384 well plate) format that is amenable to high throughput small molecule screening. A selective ALK-5 TGF-β1 receptor inhibitor (SB-431542) was used

as a positive control. The 4 day long assay was amenable to screens on the order of 100,000 wells and facilitated the identification of compounds that selectively inhibit fibroblast to myofibroblast transdifferentiation. This assay was used to identify a hit compound (itraconazole) and was used to evaluate focused panels of itraconazole analogues generated from medicinal chemistry efforts.

[00372] The activity of Itraconazole and its analogues was confirmed by analyzing changes in gene expression of multiple genes associated with fibroblast to myofibroblast transdifferentiation using biochemical methods (i.e., Western blot and RT-PCR). These 4-day assays were used to evaluate sets of 6 compounds at a time in 4-point dose response fashion.

[00373] α-SMA assays

[00374] Quiescent rat hepatic stellate cells were seeded in 384 well plates (350 cells per well) coated with Poly-D-lysine in stellate cell medium (Sciencell). After 24 hour incubation, cells were treated with the indicated doses of itraconazole for 48 hours in the presence of 10ng/mL of TGF-β1 in hepatic stellate medium. Following fixation and staining with an anti smooth muscle actin antibody, cell morphology was analyzed with a Cellomics cell insight imaging reader (Figure 1 for representative images). Cells were analyzed for mean cellular area of a-SMA staining (Figure 5a) or mean fluorescence intensity of SMA staining (Figure 5b). In this assay itraconazole reproducibly induced dose dependent decreases in both mean cellular area and alpha smooth muscle actin staining, indicating the inhibition of myofibroblast transdifferentiation and activation.

[00375] Western blot analyses of fibrosis-related proteins in cells exposed to TGFβ and itraconazole (Itra)

[00376] Human lung fibroblasts were seeded at 10^5 cells per well in 6 well dishes in assay medium (2% fetal bovine serum, DMEM). After 24 incubation, medium was changed to assay medium containing TGF-β1 (10ng/mL) and simultaneously treated with itraconazole (10 μM), SB431542 (10μM) or vehicle control (Figure 6). After incubation for 48 hours, cells were harvested by brief trypsinization and centrifugation. Cells were lysed in Cell Lytic M (Sigma) and lysate concentrations normalized via absorption readings at 260nm. Samples were boiled in 2X sample buffer and 10% beta-mercaptoethanol. Three micrograms of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary antibodies. Blots were incubated with HRP- conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce). In this assay itraconazole reproducibly induced

dose dependent decreases in levels of induced alpha smooth muscle actin protein levels, indicating the inhibition of myofibroblast transdifferentiation and activation.

[00377] Gene expression analysis of human lung fibroblasts treated with itraconazole

[00378] Human lung fibroblasts were seeded at 10^5 cells per well in 6 well dishes in assay medium (2% fetal bovine serum in DMEM). After 24 hours, medium was changed to assay medium containing TGF-β1 (10ng/mL) and itraconazole (500nM). After 48 hours of incubation at 37C, cells were harvested by brief trypsinization and centrifugation. RNA was extracted using RNeasy kit (Qiagen) and cDNA amplified using SuperScript III First-Strand Synthesis kit (Life Technologies). qPCRs reactions were then performed using a fibrosis focused RT2 Profiler PCR Array kit with supplied plate and reagents. Data was analyzed using N=1 per reaction per treatment condition. Data was expressed as fold regulation relative to a sample not treated with TGF-β1 (Figure 7a). Raw data from the fibrosis focused RT2 Profiler PCR Array is shown in Figure 7b. In this assay itraconazole reproducibly induced dose dependent changes in multiple fibrosis-related genes, indicating the inhibition of myofibroblast transdifferentiation and activation.

[00379] Anti-fibrotic activity of itraconazole and analogs thereof did not result from P450 inhibition associated with anti-fungal activity. Itraconazole inhibited VEGF and Hedgehog pro-fibrotic signaling pathways.

[00380] qPCR analysis of Hedgehog related genes in rat hepatic stellate cells treated with itraconazole

[00381] Rat hepatic stellate cells were seeded at 10^5 cells per well in 6 well dishes coated with poly-D-lysine in stellate cell medium (Sciencell) and allowed to proliferate to full confluence (~2 weeks). Medium was then switched to DMEM and 0.5% fetal bovine serum with 1 ug/mL SHH-N (R&D systems). Cells were treated with SHH-N and compounds (cyclopamine 5μM (positive control), itraconazole 1μM) for 24 hours. Cells were harvested by brief trypsinzation and centrifugation. RNA was extracted using RNeasy kit (Qiagen) and cDNA amplified using SuperScript III First-Strand Synthesis kit (Life Technologies). qPCR was performed using a SYBR green mastermix (Takara). Relative levels of PTCH1 (protein patched homolog 1) and GLI1 (GLI family zinc finger 1) mRNA (see Figures 8a and 8b; raw data for both qPCR experiments is shown in Figure 8c) indicated that itraconazole inhibits Hedgehog signaling in rat hepatic stellate cells.

[00382] Western blot analyses of COL1-GFP HSCs after knockdown of Smoothened

[00383] COL1-GFP HSCs (an immortalized mouse hepatic stellate cell line with GFP knocked into the collagen locus) were plated at 7.5^5 cells per well in assay medium (10% fetal bovine serum , DMEM). After 24 hours, cells were transduced with lentiviral particles. After 24 hours of

incubation with viral particles, cells were switched to fresh assay medium and incubated for 48 hours. Cells were lysed in Cell Lytic M (Sigma) and lysate concentrations normalized via absorption readings at 260nm. Samples were boiled in 2X sample buffer and 10% beta-mercaptoethanol. Equal amounts of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary antibodies. Blots were incubated with HRP-conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce); see Figure 9. Constructs for lentivirally delivered shRNAs were obtained from Sigma as MISSION glycerol stocks. Lentiviruses were packaged using 293T cells and packaging vectors pMD2.G and pSPAX2. Clones 71, 12, and 95 correspond to shRNAs to SMO and pLKO is plasmid SCH002 which encodes a non-targeting shRNA. shRNA mediated knockdown of SMO and the resultant inhibition of Hedgehog signaling in rat hepatic stellate cells partially recapitulated the anti-fibrotic activity of itraconazole.

[00384] Western blot analysis of VEGFR2 migration pattern after treatment with itraconazole

[00385] Quiescent rat hepatic stellate cells were seeded at 10^5 cells per well in 6 well dishes coated with Poly-D-Lysine in stellate cell medium (Sciencell). After 24 hour incubation, cells were then treated with various doses of itraconazole for 24 hours. Cells were harvested by brief trypsinization and centrifugation. Cells were lysed in Cell Lytic M (Sigma) and lysate concentrations normalized via absorption readings at 260nm. Samples were boiled in 2X sample buffer and 10% beta-mercaptoethanol. Equal amounts of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary antibodies. Blots were incubated with HRP- conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce); see Figure 10. Itraconazole inhibited VEGFR2 glycosylation and trafficking, thereby causing an inhibition of pro-fibrotic VEGF signaling in rat hepatic stellate cells.

[00386] Western blot analysis of rat hepatic stellate cells treated with combinations of VEGFR and Hedgehog inhibiting compounds

[00387] Quiescent rat hepatic stellate cells were seeded at 10^5 cells per well in 6 well dishes coated with Poly-D-Lysine in stellate cell medium (Sciencell). After 24 hour incubation, cells were switched to stellate cell medium containing 10ng/mL TGF-β1 and the indicated combinations of compounds. After 48 hours of treatment, cells were harvested by brief

trypsinization and centrifugation. Cells were lysed in Cell Lytic M (Sigma) and lysate concentrations normalized via absorption readings at 260nm. Samples were boiled in 2X sample buffer and 10% beta-mercaptoethanol. Three micrograms of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary antibodies. Blots were incubated with HRP- conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce); see Figure 11. Pharmacological inhibition of either VEGF or Hedgehog signaling in rat hepatic stellate cells partially recapitulated the anti-fibrotic activity of itraconazole or analogs thereof. Dual pharmacological inhibition of VEGF and Hedgehog signaling in rat hepatic stellate cells recapitulated the anti-fibrotic activity of itraconazole.

[00388] Activity of itraconazole and compound 42 in a hedgehog reporter assay

[00389] TM3-GLI-LUC cells (stable clone of mouse TM3 cells expressing Gli-Luc reporter) were harvested and then seeded at 5,000 cells per well in 384 well plates in 40uL of assay medium (DMEM:F12 and 2% fetal bovine serum). Compounds were dissolved in DMSO in serial dilutions and transferred to the assay plates using a 100nL pintool head on a Biomek automated workstation. After transfer of compounds, 10uL of assay medium containing the small molecule hedgehog agonist SAG was added such that the final assay concentration of SAG was 10nM or 400nM. Assay plates were incubated for 48 hours before using Bright-Glo reagents to determine luminance signals on an Envision plate reader. Relative GLI-LUC activity of TM3-GLI-LUC cells exposed to 10nM SAG and indicated doses of inhibitor are shown in Figure 12a. Relative GLI-LUC activtity of TM3-GLI-LUC cells exposed to 400nM SAG and indicated doses of inhibitor are shown in Figure 12b. N=3 per dose. Itraconazole and analogs thereof inhibited pro-fibrotic Hedgehog signaling in rat hepatic stellate cells.

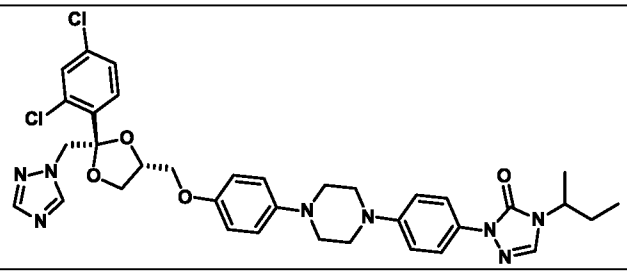
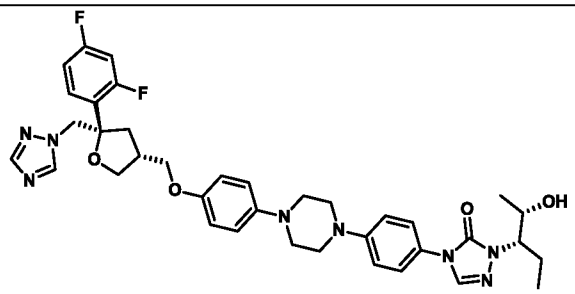
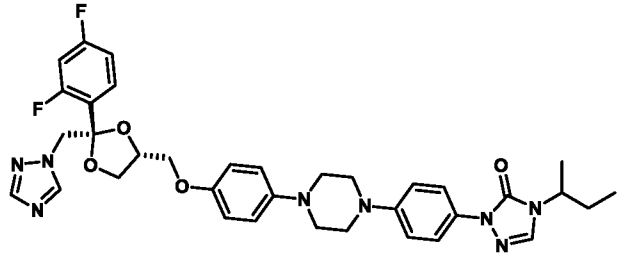
[00390] Western blot analyses of LX2 human hepatic stellate cells after knockdown of VEFGR1, VEGFR2, or SMO.

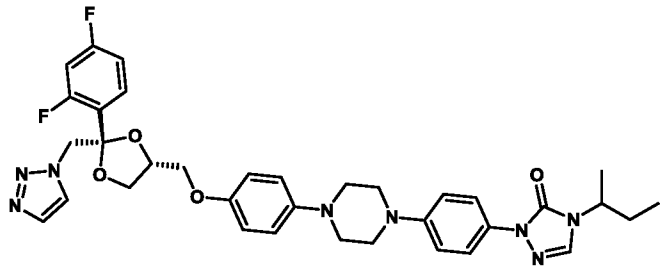
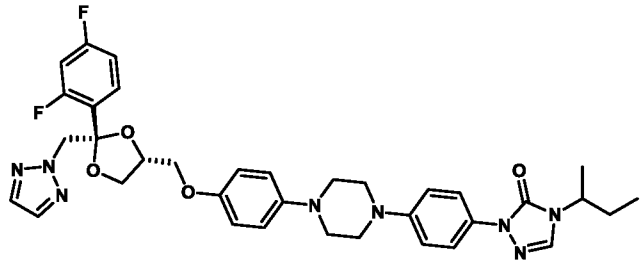
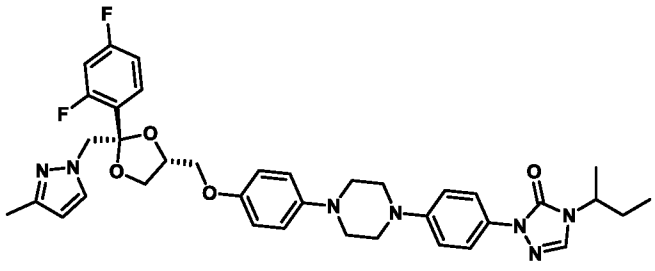
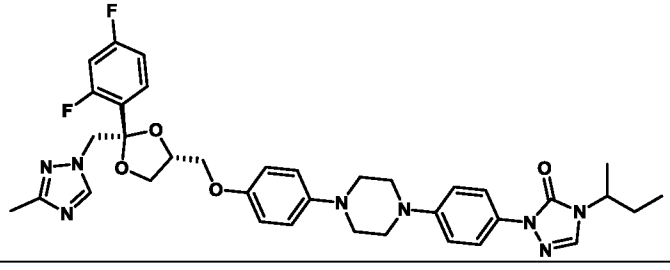
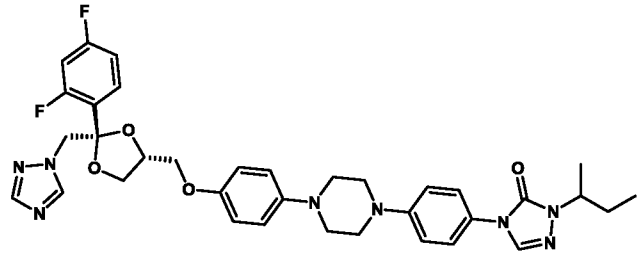
[00391] LX2 cells (an immortalized human hepatic stellate cells line, Scot Friedman lab) were plated at 10^5 cells per well in assay medium (10% fetal bovine serum , DMEM). After 24 hours, cells were transduced with lentiviral particles. After 24 hours of incubation with viral particles, cells were switched to fresh assay medium and incubated for 48 hours. Cells were lysed in Cell Lytic M (Sigma) and lysate concentrations normalized via absorption readings at 260nm. Samples were boiled in 2X sample buffer and 10% beta-mercaptoethanol. Equal amounts of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary

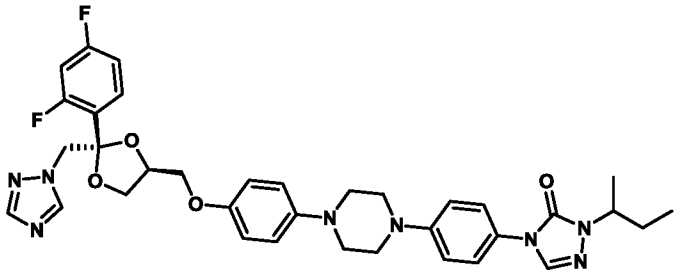
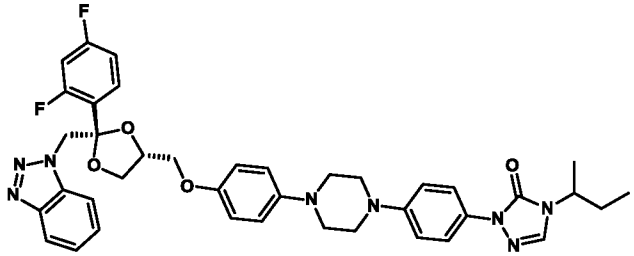
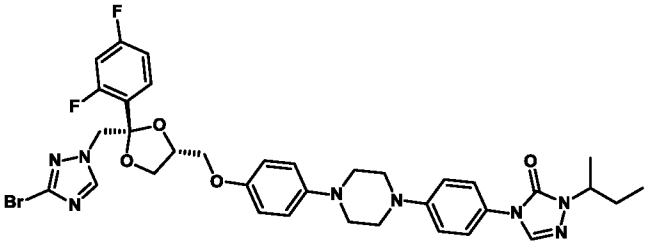
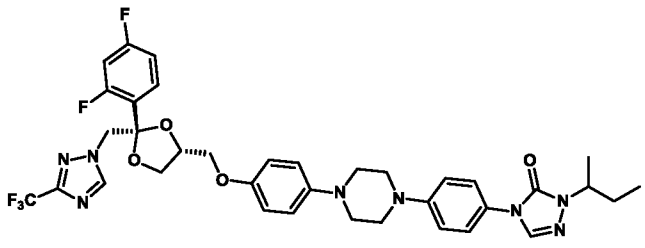
antibodies. Blots were incubated with HRP- conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce); see Figure 13. Constructs for lentivirally delivered shRNAs were obtained from Sigma as MISSION glycerol stocks. Lentiviruses were packaged using 293T cells and packaging vectors pMD2.G and pSPAX2. Clone 65 corresponds to a shRNA targeting SMO, 31 and 32 correspond to a shRNA targeting VEGFR1, clones 86 and 87 correspond to a shRNA targeting VEGFR2, and pLKO is plasmid SCH002 which encodes a non-targeting shRNA. Genetic knockdown of VEGF and Hedgehog signaling in human stellates cells inhibited pro-fibrotic myofibroblast activation and partially recapitulated the anti-fibrotic activity of itraconazole and analogs thereof.

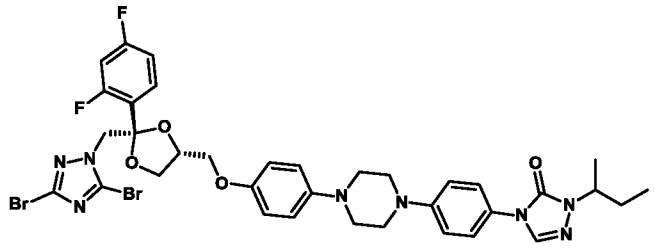
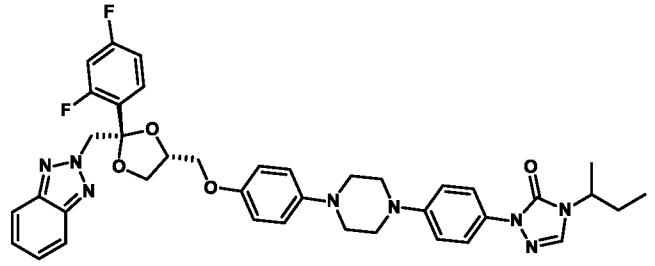
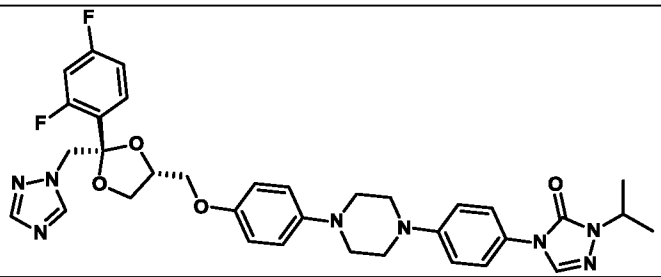
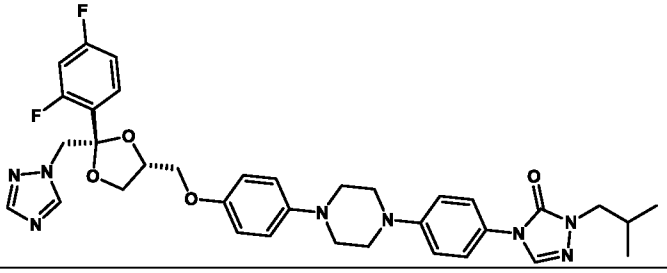
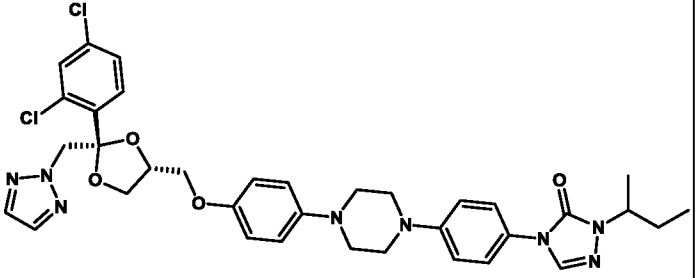
[00392] Table 1 below shows compound analytical data (NMR and MS) as well their biological activity. The EC_{50} for (imaging and western based) activity is graded as: +++ = <500 nM; ++ = 500 nM to 5 μM; + = 5 to 30 μM).

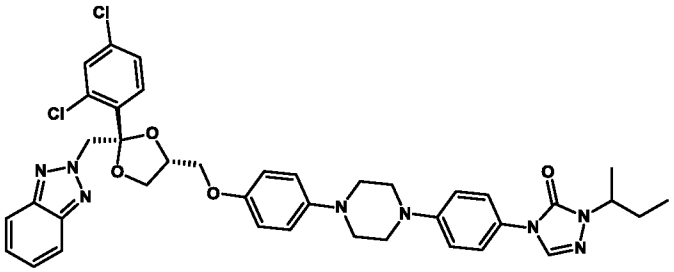
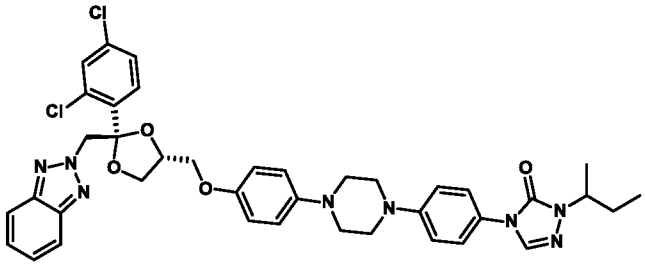
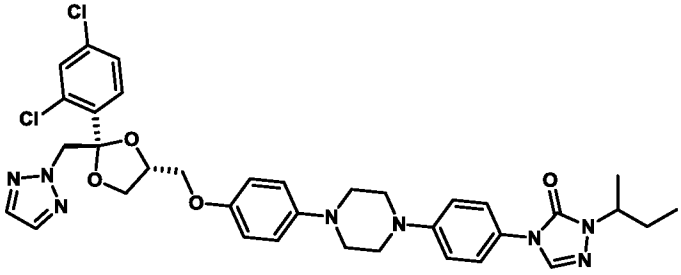
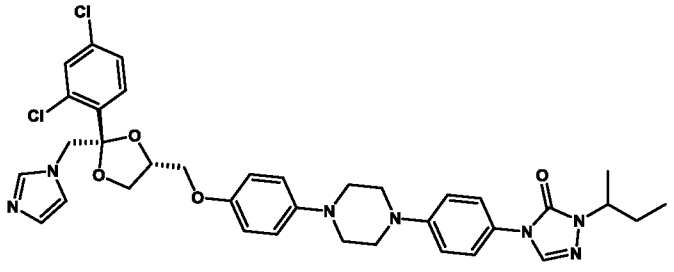
Table 1

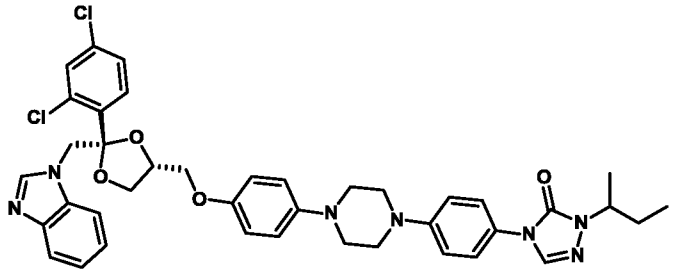
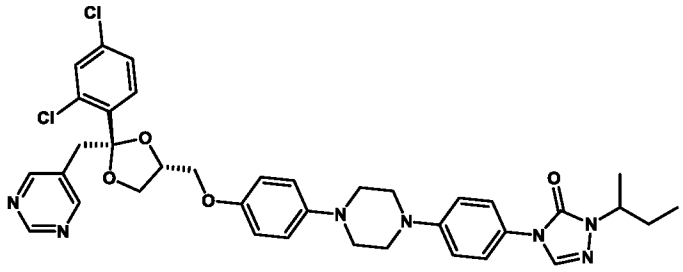
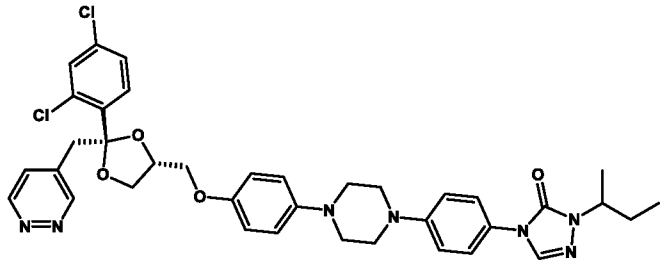
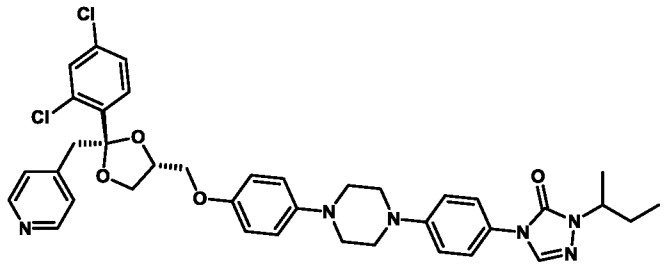
Cpd #	Structure	Characterization Data (NMR and LCMS)	Activity
ITZ		Cis racemate	+++
POS		Cis racemate	+
1		Cis racemate LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.89 (s, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.6 Hz, 2H), 6.94 (d, J = 7.2 Hz, 2H), 6.88 (m, 2H), 6.79 (d, J = 5.2 Hz, 2H), 4.70 (dd, J = 16.4 Hz, 11.6 Hz, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.83-3.77 (m, 2H), 3.49 (dd, J = 8.0 Hz, 5.2 Hz, 1H), 3.37-3.35 (m, 4H), 3.24-3.22 (m, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	++

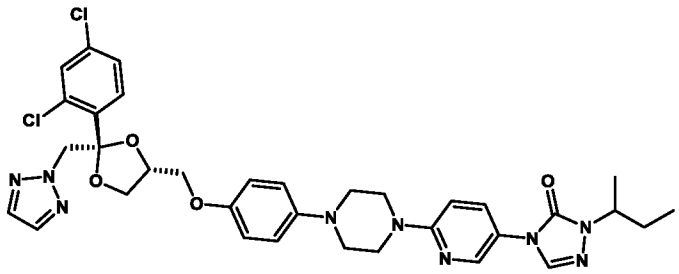
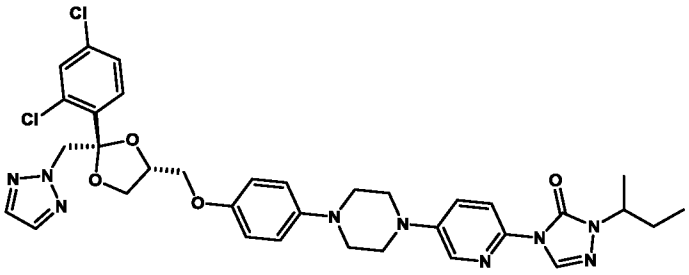
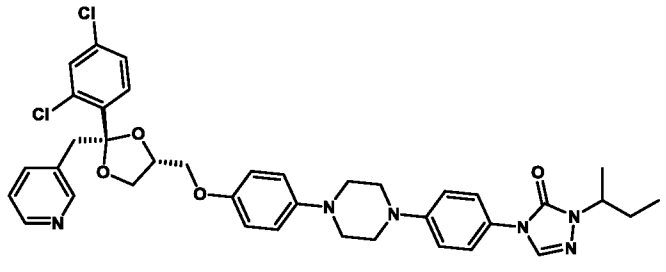
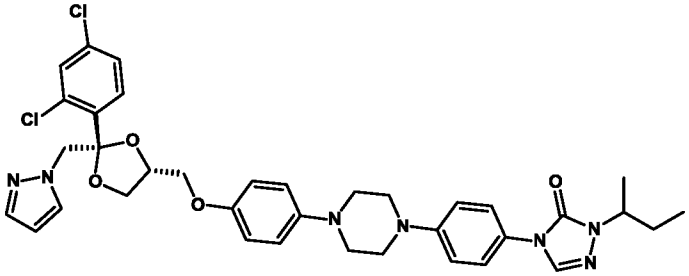
2		LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.73 (d, J = 0.8 Hz, 1H), 7.65 (d, J = 0.8 Hz, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.43 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 6.93 (d, J = 7.6 Hz, 2H), 6.87 (m, 2H), 6.77 (d, J = 7.2 Hz, 2H), 4.90 (s, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.96 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.81 (dd, J = 9.6 Hz, 4 Hz, 1H), 3.75 (dd, J = 7.6 Hz, 3.6 Hz, 1H), 3.38-3.35 (m, 5H), 3.24-3.22 (m, 4H), 1.85 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	++
3		LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.58 (s, 1H), 7.57 (s, 1H), 7.46-7.42 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.00 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
4		LC-MS: m/z 686.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.55 (m, 1H), 7.44-7.30 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.76 (m, 2H), 6.01 (m, 1H), 4.55 (dd, J = 28.8 Hz, 12.0 Hz, 2H), 4.38 (m, 1H), 4.29 (m, 1H), 3.92 (m, 1H), 3.82-3.71 (m, 3H), 3.37 (br s, 4H), 3.25 (m, 4H), 2.25 (s,3H), 1.86 (m, 1H), 1.73 (m, 1H), 1.39 (d, J = 6.4 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
5		LC-MS: m/z 687.0 (M+H)	+
6		Cis chiral LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.89 (s, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.6 Hz, 2H), 6.94 (d, J = 7.2 Hz, 2H), 6.88 (m, 2H), 6.79 (d, J = 5.2 Hz, 2H), 4.70 (dd, J = 16.4 Hz, 11.6 Hz, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.2 Hz, 1H),	++

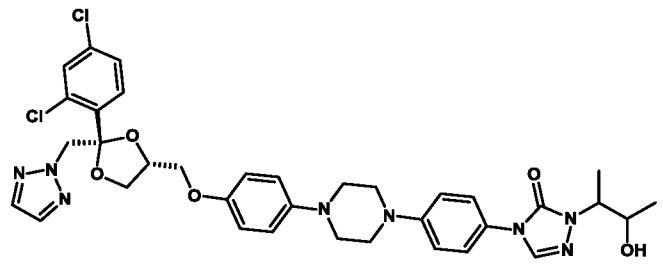
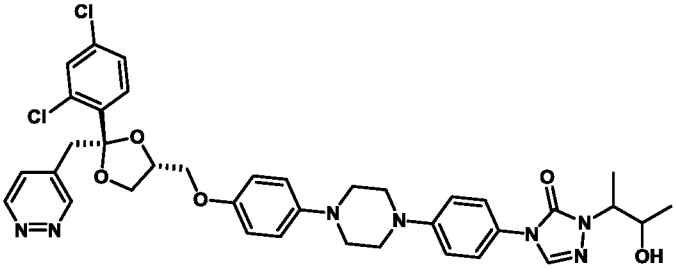
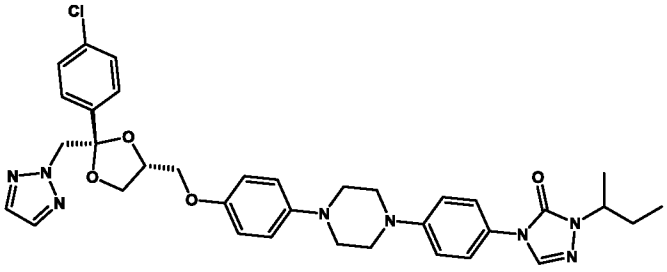
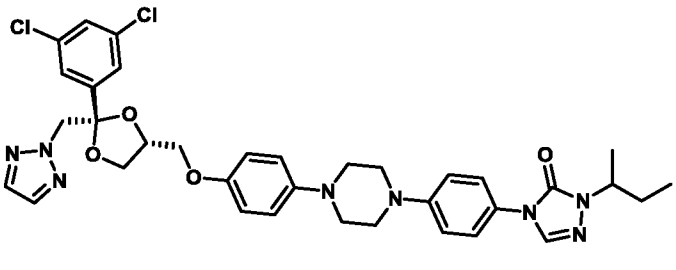
		3.83-3.77 (m, 2H), 3.49 (dd, J = 8.0 Hz, 5.2 Hz, 1H), 3.40 (br s, 4H), 3.28 (br s, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	
7		Trans racemate LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.20 (s, 1H), 7.92 (s, 1H), 7.62 (s, 1H), 7.52 (m, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.04 (d, J = 5.6 Hz, 2H), 6.88-6.80 (m, 4H), 6.72 (m, 2H), 4.66 (dd, J = 16.8 Hz, 11.6 Hz, 2H), 4.29 (m, 1H), 4.18 (m, 1H), 4.01 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.93 (dd, J= 7.2 Hz, 3.6 Hz, 1H), 3.85 (m, 2H), 3.50-3.11 (m, 8H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.6 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
8		LC-MS: m/z 723.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.02 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 6.8 Hz, 1H), 7.62 (s, 1H), 7.54 (m, 1H), 7.47-7.42 (m, 3H), 7.34 (m, 1H), 7.03 (d, J = 7.2 Hz, 2H), 6.95-6.86 (m, 4H), 6.53 (d, J = 7.2 Hz, 2H), 5.15 (dd, J = 20.4 Hz, 12.0 Hz, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 3.88 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.62 (dd, J = 6.8 Hz, 3.6 Hz, 1H), 3.41-3.37 (m, 5H), 3.22 (br s, 4H), 2.88 (m,1H), 1.86 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	++
9		LC-MS: m/z 750.9 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.12 (s, 1H), 7.62 (s, 1H), 7.51 (m, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 6.95-6.88 (m, 4H), 6.84 (m, 2H), 4.65 (dd, J = 15.6 Hz, 12.0 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.86 (dd, J = 6.8 Hz, 4.0 Hz, 1H), 3.82 (dd, J = 7.6 Hz, 3.6 Hz, 1H), 3.52 (dd, J = 7.6 Hz, 4.8 Hz, 1H), 3.37 (br s, 4H), 3.25 (br s, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 5.6 Hz, 3H).	+
10		LC-MS: m/z 741.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.33 (s, 1H), 7.63 (s, 1H), 7.53 (m, 1H), 7.44 (d, J = 6.4 Hz, 2H), 7.04 (d, J = 6.4 Hz, 2H), 6.93-6.89 (m, 4H), 6.82 (br s, 2H), 4.74 (dd, J = 14.0 Hz, 11.6 Hz, 2H), 4.43 (m, 1H), 4.30 (m, 1H), 3.99 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.83 (dd, J = 6.8 Hz, 4.4Hz, 1H),	+

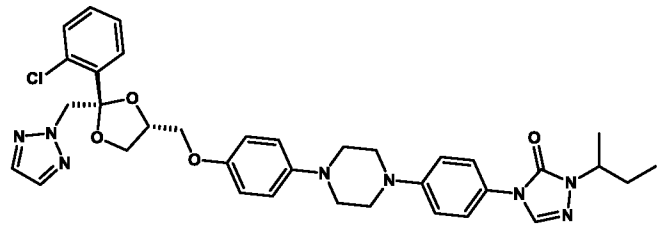
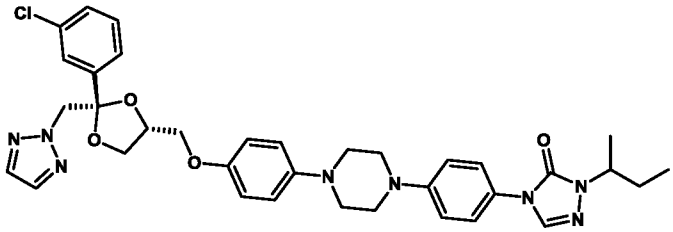
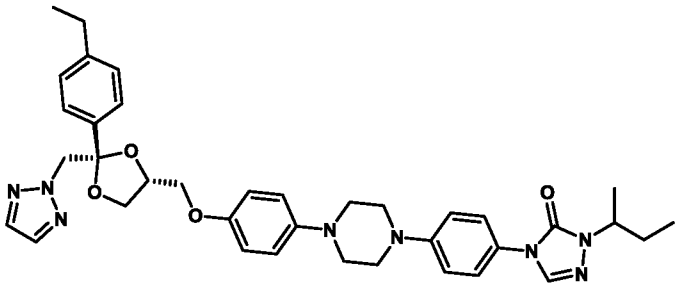
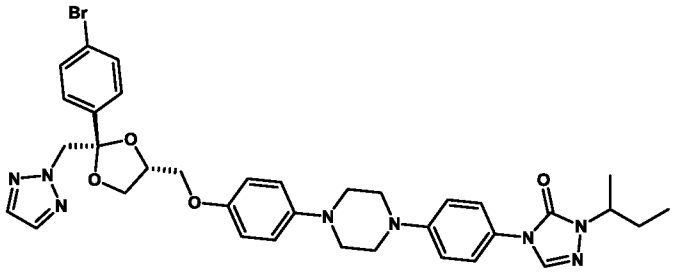
		3.79 (m, 1H), 3.51 (m, 1H), 3.38 (br s, 4H), 3.25 (br s, 4H), 1.85 (m, 1H), 1.73 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 5.6 Hz, 3H).	
11		LC-MS: m/z 829.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.54 (m, 1H), 7.42 (d, J = 6.8 Hz, 2H), 7.03 (d, J = 6.8 Hz, 2H), 6.96-6.88 (m, 4H), 6.83 (m, 2H), 4.67 (dd, J = 24.0 Hz, 11.6 Hz, 2H), 4.43 (m, 1H), 4.29 (m, 1H), 3.97 (m, 1H), 3.90 (dd, J = 7.2 Hz, 4.0Hz, 1H), 3.85 (dd, J = 7.6 Hz, 4.0 Hz, 1H), 3.50 (m, 1H), 3.37 (br s, 4H), 3.26 (br s, 4H), 1.87 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	+
12		LC-MS: m/z 723.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.88-7.87 (m, 2H), 7.62 (s, 1H), 7.54 (m, 1H), 7.43 (m, 4H), 7.04 (d, J = 7.2 Hz, 2H), 6.94-6.83 (m, 4H), 6.41 (d, J = 7.2 Hz, 2H), 5.30 (dd, J = 37.6 Hz, 9.6 Hz, 2H), 4.42 (m, 1H), 4.27 (m, 1H), 3.94 (dd, J = 7.2 Hz, 4.8 Hz, 1H), 3.89 (dd, J = 6.8 Hz, 3.2 Hz, 1H), 3.53 (br s, 1H), 3.36 (br s, 4H), 3.22 (br s, 4H), 2.99 (m,1H), 1.85 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	+
13		LC-MS: m/z 659.0 (M+H)	+
14		LC-MS: m/z 673.0 (M+H)	+
15		LC-MS: m/z 705.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 0.7 Hz, 1H), 7.61 (s, 2H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.51 – 7.40 (m, 3H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.09 – 7.03 (m, 2H), 6.98 – 6.91 (m, 2H), 6.84 – 6.77 (m, 2H), 5.25 – 5.03 (m, 3H), 4.46 – 4.36 (m, 1H), 4.36 – 4.27 (m, 1H), 3.98 – 3.84 (m,	++

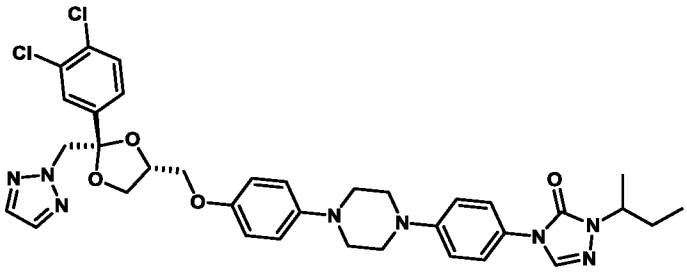
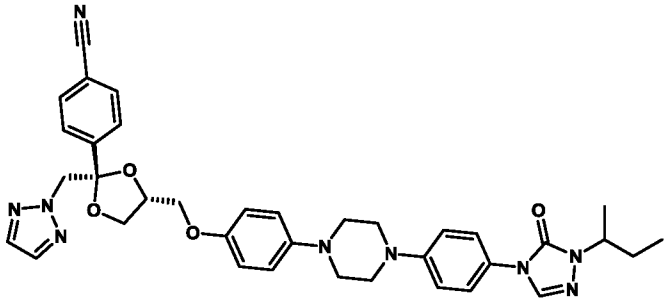
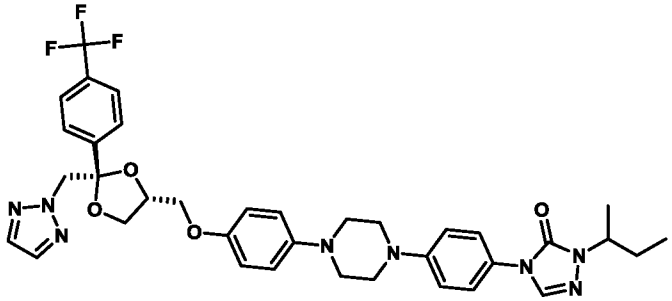
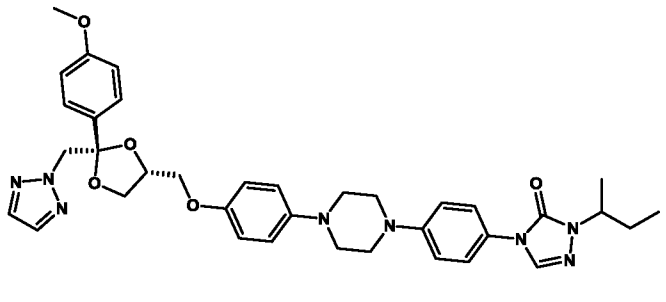
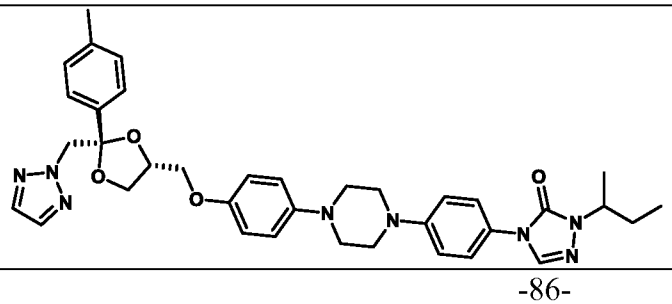
		3H), 3.48 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.39 (m, 4H), 3.26 (m, 4H), 1.89 (m, 2H), 1.74 (m, 1H), 1.42 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	
16		LC-MS: m/z 755.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.12 (d, *J* = 0.6 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.48 – 7.36 (m, 4H), 7.33 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.27 – 7.20 (m, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 5.54 – 5.29 (m, 2H), 4.56 – 4.37 (m, 1H), 4.34 – 4.18 (m, 1H), 4.04 – 3.84 (m, 2H), 3.68 (m, 8H), 3.61 – 3.44 (m, 2H), 1.88 (m, 1H), 1.76 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	+
17		Trans racemic LC-MS: m/z 755.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.86 (m, 2H), 7.63 (dd, *J* = 4.6, 3.9 Hz, 2H), 7.49 – 7.37 (m, 5H), 7.18 (m, 1H), 7.08 – 7.01 (m, 2H), 6.92 – 6.84 (m, 2H), 6.72 – 6.61 (m, 2H), 5.42 – 5.27 (m, 3H), 4.38 – 4.22 (m, 2H), 4.01 – 3.90 (m, 2H), 3.89 – 3.76 (m, 2H), 3.36 (m, 4H), 3.22 (m, 4H), 1.90 (m, 1H), 1.74 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H).	+
18		Trans racemic LC-MS: m/z 705.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 6.7 Hz, 3H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.49 – 7.39 (m, 3H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.08 – 6.99 (m, 2H), 6.96 – 6.84 (m, 2H), 6.77 – 6.63 (m, 2H), 5.19 – 4.96 (m, 2H), 4.32 (m, 2H), 4.03 (m, 1H), 3.95 (m, 1H), 3.86 (m, 1H), 3.75 (m, 1H), 3.30 – 3.15 (m, 4H), 1.88 (m, 1H), 1.81 – 1.64 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).	+
19		LC-MS: m/z 704.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 0.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.54 (s, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.47 – 7.41 (m, 2H), 7.31 – 7.25 (m, 1H), 7.09 – 7.01 (m, 3H), 6.99 – 6.91 (m, 3H), 6.87 – 6.77 (m, 2H), 4.62 – 4.40 (m, 2H), 4.39 – 4.26 (m, 2H), 3.89 (dd, J = 8.4, 6.5 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.41 – 3.34 (m, 4H), 3.31 (dd, J = 9.6,	++

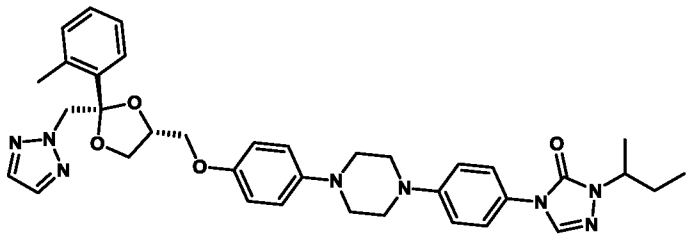
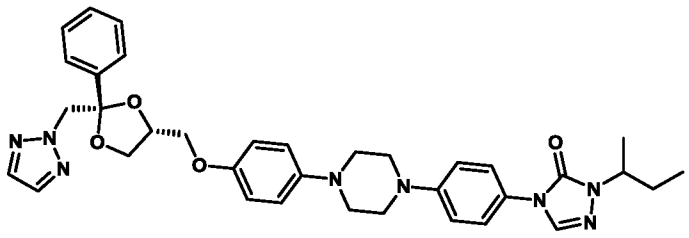
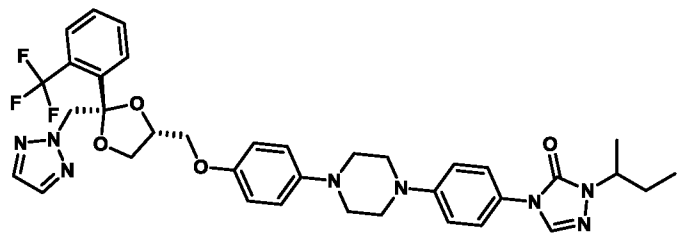
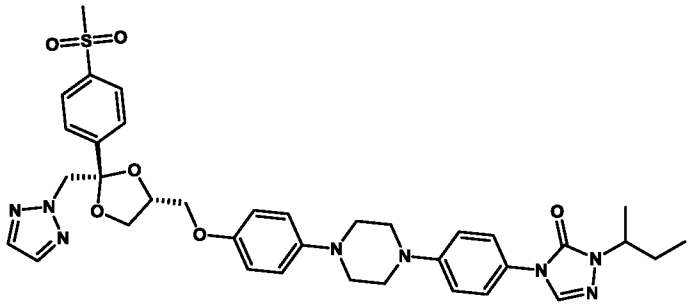
		6.8 Hz, 1H), 3.29 – 3.18 (m, 4H), 1.95 – 1.81 (m, 1H), 1.79 – 1.66 (m, 1H), 1.41 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H).	
20		LC-MS: m/z 754.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 9.43 (s, 1H), 8.11 (d, *J* = 0.6 Hz, 1H), 8.08 – 7.99 (m, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.67 (m, 2H), 7.62 (m, 1H), 7.58 – 7.46 (m, 4H), 7.21 (m, 3H), 6.57 (d, *J* = 8.9 Hz, 2H), 5.33 – 5.05 (m, 3H), 4.51 – 4.36 (m, 2H), 4.33 – 4.17 (m, 1H), 3.89 (dd, *J* = 8.5, 7.4 Hz, 1H), 3.79 (dd, *J* = 8.5, 4.9 Hz, 1H), 3.71 (dd, *J* = 10.6, 3.1 Hz, 1H), 3.60 – 3.46 (m, 9H), 1.89 (m, 1H), 1.82 – 1.72 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	++
21		LC-MS: m/z 716.2 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.98 (s, 1H), 8.65 (s, 2H), 8.10 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.36 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.20 (m, 4H), 6.87 (d, *J* = 9.0 Hz, 2H), 4.35 (m, 1H), 4.25 (m, 2H), 3.96 – 3.66 (m, 4H), 3.57 – 3.38 (m, 8H), 1.89 (m, 1H), 1.75 (m, 1H), 1.40 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	++
22		LC-MS: m/z 716.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (dd, *J* = 2.2, 1.2 Hz, 1H), 9.03 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.42 (m, 2H), 7.36 (dd, *J* = 5.2, 2.3 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.08 – 7.04 (m, 2H), 6.99 – 6.93 (m, 2H), 6.79 – 6.73 (m, 2H), 4.36 – 4.28 (m, 2H), 3.85 (dd, *J* = 8.4, 6.8 Hz, 1H), 3.79 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.74 (dd, *J* = 9.7, 5.0 Hz, 1H), 3.56 – 3.51 (m, 1H), 3.48 (d, *J* = 13.8 Hz, 1H), 3.44 – 3.35 (m, 5H), 3.26 (dd, *J* = 6.5, 3.6 Hz, 4H), 1.87 (m, 1H), 1.80 – 1.71 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+++
23		LC-MS: m/z 715.2 (M+H) ^{1}H NMR (400 MHz, CDCl3) δ 8.51 – 8.44 (m, 2H), 7.64 (m, 1H), 7.51 – 7.42 (m, 4H), 7.24 – 7.15 (m, 3H), 7.10 – 7.03 (m, 2H), 7.00 – 6.91 (m, 2H), 6.80 – 6.71 (m, 2H), 4.39 – 4.27 (m, 2H), 3.90 – 3.79 (m, 2H), 3.59 – 3.45 (m, 3H), 3.45 – 3.33 (m,	+++

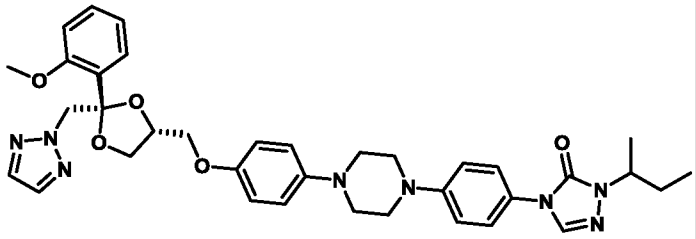
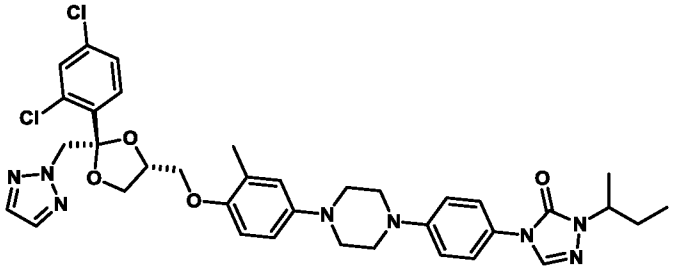
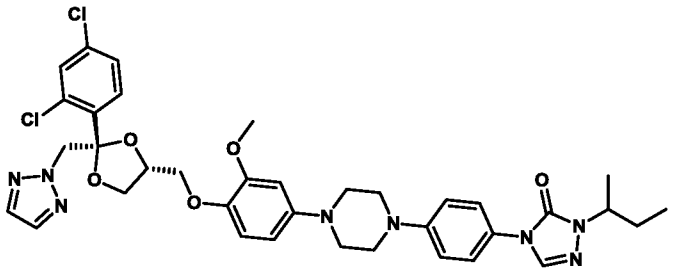
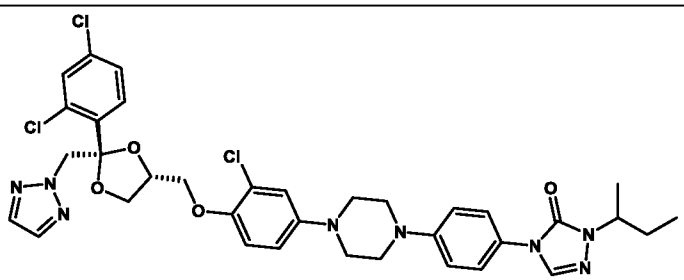
		5H), 3.32 – 3.19 (m, 4H), 1.87 (m, 1H), 1.78 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	
24		LC-MS: m/z 706.3(M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.75 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 3H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.84 – 6.76 (m, 3H), 5.26 – 4.97 (m, 2H), 4.46 – 4.36 (m, 1H), 4.34 – 4.24 (m, 1H), 4.01 – 3.83 (m, 3H), 3.83 – 3.73 (m, 4H), 3.46 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (s, 2H), 0.94 (d, *J* = 7.4 Hz, 3H).	++
25		LC-MS: m/z 706.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.75 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 3H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.84 – 6.76 (m, 3H), 5.26 – 4.97 (m, 2H), 4.46 – 4.36 (m, 1H), 4.34 – 4.24 (m, 1H), 4.01 – 3.83 (m, 3H), 3.83 – 3.73 (m, 4H), 3.46 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 7.4 Hz, 3H).	+
26		LC-MS: m/z 715.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (m, 2H), 7.64 (m, 1H), 7.55 (m, 1H), 7.46 (m, 4H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.16 (m, 1H), 7.05 (m, 2H), 6.94 (m, 2H), 6.75 (m, 2H), 4.31 (m, 2H), 3.81 (m, 2H), 3.72 (m, 1H), 3.49-3.34 (m, 7H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 7.4 Hz, 3H).	++
27		LC-MS: m/z 704.2 (M+H) ^{1}H NMR (400 MHz, CDCl3) δ 7.64 (d, J = 0.6 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.51 (td, J = 2.3, 0.7 Hz, 2H), 7.49 – 7.41 (m, 3H), 7.25 (dd, J = 8.4, 2.1 Hz, 1H), 7.08 – 7.02 (m, 2H), 6.99 – 6.90 (m, 2H), 6.83 – 6.74 (m, 2H), 6.24 (t, J = 2.1 Hz, 1H), 4.91 – 4.63 (m, 2H), 4.45 – 4.24 (m, 2H), 3.94 – 3.74 (m, 3H), 3.43 – 3.30 (m, 5H), 3.29 – 3.15 (m, 4H), 1.94 – 1.85 (m, 1H), 1.76 (m, 1H), 1.42 (d, J = 6.4 Hz, 3H),	+

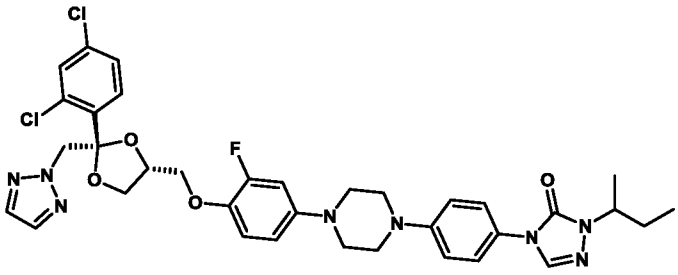
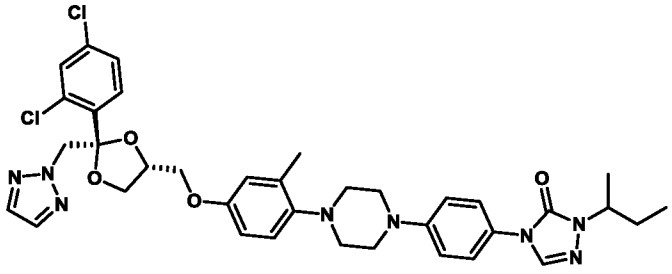
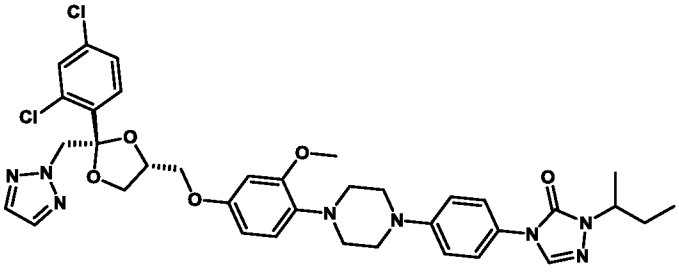
		0.94 (d, J = 7.4 Hz, 3H).	
28		LC-MS: m/z 721.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.09 (s, 1H), 7.57 – 7.50 (m, 5H), 7.43 (d, *J* = 8.9 Hz, 2H), 7.30 (dd, *J* = 8.6, 2.1 Hz, 2H), 7.24 – 7.17 (m, 2H), 7.12 – 7.02 (m, 2H), 5.11 (m, 2H), 4.49 – 4.38 (m, 1H), 4.17 (m, 1H), 3.99 (m, 3H), 3.93 – 3.76 (m, 2H), 3.70 – 3.50 (m, 8H), 1.40 (d, *J* = 6.9 Hz, 3H), 1.28 (d, *J* = 6.4 Hz, 3H).	++
29		LC-MS: m/z 732.2 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 9.16 (s, 1H), 9.04 (s, 1H), 8.10 (d, *J* = 7.5 Hz, 1H), 7.72 (d, *J* = 4.0 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 2.1 Hz, 1H), 7.54 (dd, *J* = 9.1, 2.7 Hz, 2H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.29 – 7.17 (m, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 4.42 – 4.33 (m, 1H), 4.16 (h, *J* = 7.1 Hz, 1H), 4.04 – 3.95 (m, 1H), 3.92 – 3.79 (m, 3H), 3.72 (s, 4H), 3.64 (q, *J* = 6.8, 5.1 Hz, 4H), 3.61 – 3.50 (m, 2H), 1.48 (d, *J* = 6.8 Hz, 3H), 1.15 (d, *J* = 6.3 Hz, 3H).	+++
30		LCMS: 100 % @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H) ,1.40-1.42 (d, *J*=6.4 Hz, 3H) ,1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17-3.36 (m, 4H), 3.41-3.43 (m, 5H), 3.77-3.80 (m, 1H), 3.86-3.90 (m, 2H), 4.29-4.34 (m, 1H), 4.37-4.38 (m, 1H), 4.84 (s, 2H), 6.82 (s, 2H), 6.90-6.91 (m, 2H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.35-7.37 (d, *J*=8.8 Hz, 2H), 7.46-7.54 (d, *J*=8.4 Hz, 4H), 7.63-7.65 (m, 3H).	++
31		LCMS: 100 % @ 262 nm; m/z 705.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.4 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.27-3.39 (s, 4H), 3.39-3.47 (s, 4H), 3.48-3.51 (m, 1H), 3.80-3.84 (m, 1H), 3.89-3.95 (m , 2H), 4.29-4.34 (m, 1H), 4.38-4.42 (m, 1H), 4.83 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H), 6.961 (s, 2H), 7.04-7.07 (d, *J*=9.2 Hz, 2H), 7.36-7.40 (m, 3H), 7.44-7.46 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=2.8 Hz, 3H).	++

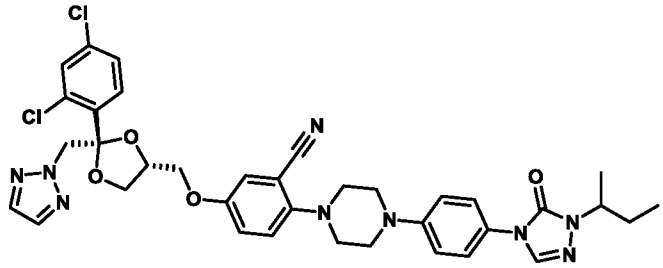
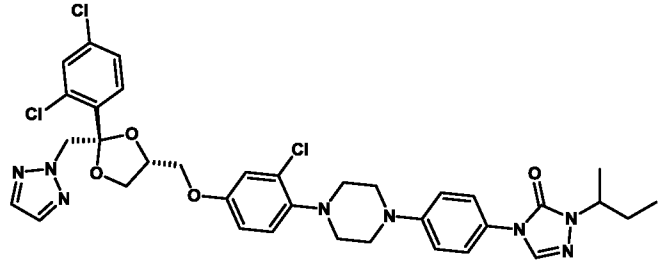
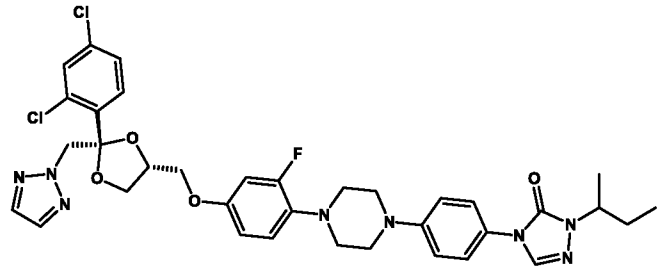
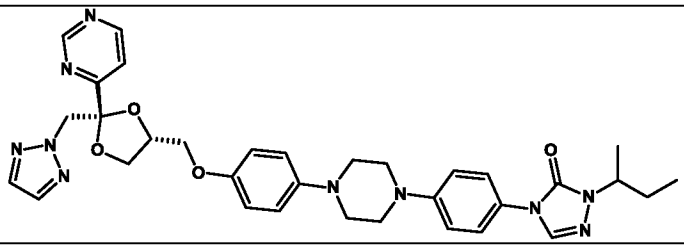
32		LCMS: 100 % @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.43 (d, *J*=4.4 Hz, 3H) ,1.69-1.79 (m, 1H), 1.85-1.92 (m, 1H), 3.26-3.51 (m, 9H), 3.82-3.88 (m, 2H), 3.93-3.96 (m, 1H), 4.29-4.34 (m, 1H), 4.37-4.43 (m, 1H), 5.08-5.11 (d, *J*=14.4 Hz, 1H), 5.22-5.25 (d, *J*=14.4 Hz, 1H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 6.91-6.95 (m, 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.24-7.28 (m, 1H), 7.31-7.35 (m, 1H), 7.44-7.48 (m, 3H), 7.63-7.68 (m, 4H).	+
33		LCMS: 99.39% @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.43 (d, *J*=6.8 Hz, 3H), 1.69-1.79 (m, 1H), 1.83-1.92 (m, 1H), 3.27-3.44 (m, 9H), 3.77-3.81 (m, 1H), 3.86-3.94 (m, 2H), 4.29-4.34 (m, 1H), 4.36-4.42 (m, 1H),4.84 (s, 2H), 6.79-6.81 (d, *J*=8.4 Hz, 2H), 6.95 (s, 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.30-7.37 (m, 2H), 7.41-7.46 (m, 3H), 7.53-7.54 (d, *J*=1.6 Hz, 1H), 7.63-7.65 (m, 3H).	++
34		LCMS: 100% @ 262 nm; m/z 682.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.25-1.28 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.68-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.66-2.71 (q, 2H), 3.29-3.33 (m, 5H), 3.42 (s, 4H), 3.72-3.75 (m, 1H), 3.80-3.83 (m, 1H), 3.89-3.93 (t, *J*=8.4 Hz, 1H), 4.29-4.39 (m, 2H), 4.85 (s, 2H), 6.78-6.80 (d, *J*=8.8 Hz, 2H), 7.02-7.07 (m, 4H), 7.23-7.25 (d, *J*=8.4 Hz, 2H), 7.44-7.50 (q, 4H), 7.64 (s, 3H).	++
35		LCMS: 99.39% @ 262 nm; m/z 717.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.43 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.28 (s, 4H), 3.41-3.45 (m, 5H), 3.77-3.81 (m, 1H), 3.86-3.92 (m, 2H), 4.29-4.39 (m, 2H), 4.84 (s, 2H), 6.80-6.82 (d, *J*=6.8 Hz, 2H), 6.95-6.96 (s, 2H), 7.05-7.07 (d, *J*=9.2 Hz, 2H), 7.40-7.47 (m, 4H), 7.51-7.53 (d, *J*=8.4 Hz, 2H), 7.62-7.64 (d, *J*=7.2 Hz, 3H).	++

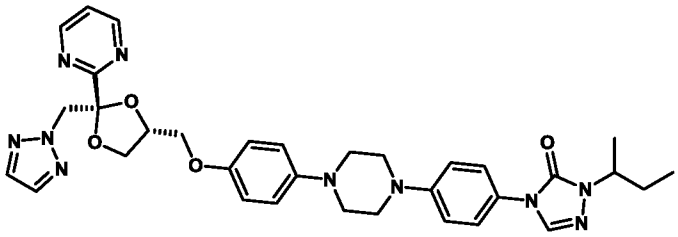
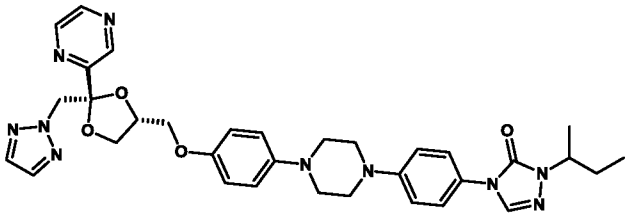
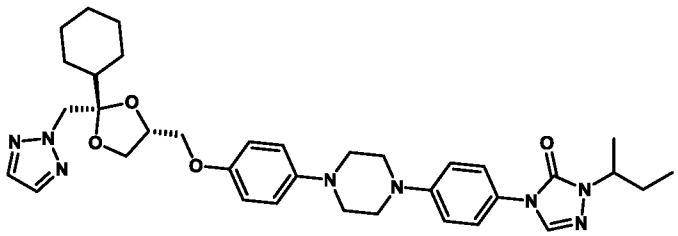
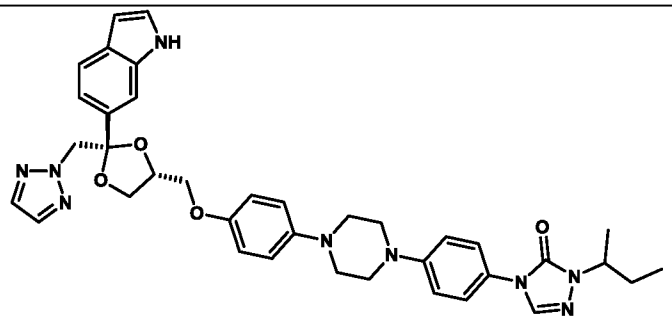
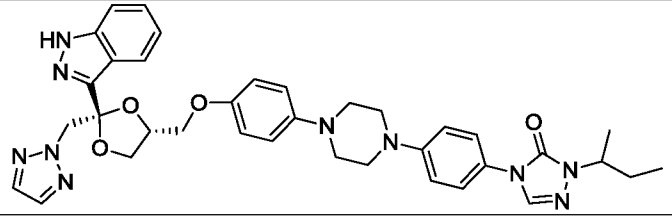
36		LCMS: 100% @ 262 nm; m/z 705.36 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.75 (m, 1H), 1.84-1.88 (m, 1H), 3.26 (s, 4H), 3.39 (s, 4H), 3.46-3.50 (q, 1H), 3.79-3.83 (m, 1H), 3.91-3.92 (d, *J*=5.2 Hz, 2H), 4.30-4.41 (m, 2H), 4.84 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H), 6.95 (s, 2H), 7.04-7.06 (d, *J*=8.8 Hz, 2H), 7.32-7.35 (dd, J_1=10.4 Hz and J_2=2.0 Hz, 3H), 7.44-7.46 (m, 3H), 7.60-7.64 (m, 4H).	+
37		LCMS: 99.81% @ 262 nm; m/z 662.55 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz,3H) ,1.40-1.43 (d, *J*=4.8 Hz,3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.28 (s, 4H), 3.40 (s, 4H), 3.51-3.58 (q, 1H), 3.84-3.89 (m, 1H), 3.92-3.96 (m, 2H), 4.29-4.34 (m, 1H), 4.37-4.40 (m, 1H), 4.86 (s, 2H), 6.81-6.83 (d, *J*=8.8 Hz,2H), 6.97 (s, 2H), 7.02-7.04 (d, 2H), 7.44-7.46 (d, *J*=8.8 Hz,2H), 7.61-7.64 (m, 5H),7.66-7.69 (m, 2H).	++
38		LCMS: 100% @ 262 nm; m/z 705.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17 (s, 1H), 3.27 (s, 2H), 3.41-3.48 (m, 6H), 3.78-3.82 (m, 1H), 3.89-3.92 (m, 2H), 4.29-4.41 (m, 2H), 4.87 (s, 2H), 6.83-6.92 (m, 4H), 7.06-7.08 (d, *J*=8.8 Hz, 2H), 7.46-7.48 (d, *J*=6.0 Hz, 2H), 7.63-7.67 (m, 6H), 7.78-7.98 (m, 1H).	++
39		LCMS: 96.30% @ 261 nm; m/z 667.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H) ,1.40-1.42 (d, *J*=6.4 Hz,3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.26-3.39 (m, 9H), 3.73-3.76 (m, 1H), 3.82-3.92 (m, 5H), 4.29-4.39 (m, 2H), 4.84 (s,2H), 6.80 (s, 2H), 6.90- 6.92 (m, 4H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.45-7.49 (m, 4H), 7.63-7.64 (d *J*=4.8 Hz, 3H).	++
40		LCMS: 100% @ 262 nm; m/z 651.45 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz,3H) , 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.38 (s, 3H), 3.16- 3.39 (m,8H), 3.72-3.76 (m, 2H), 3.82-3.85 (m, 1H),	++

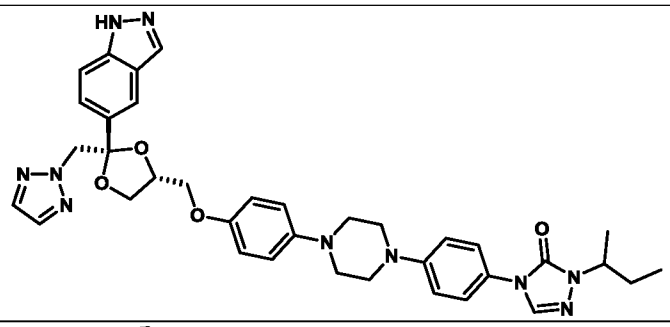
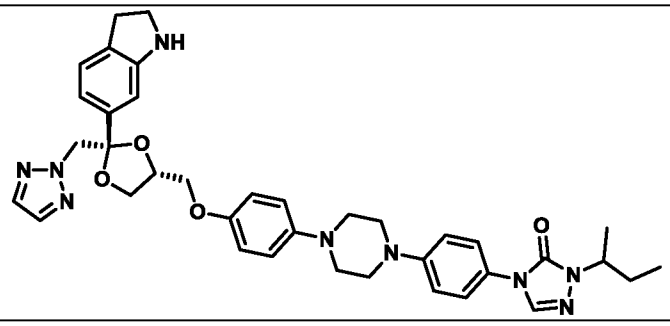
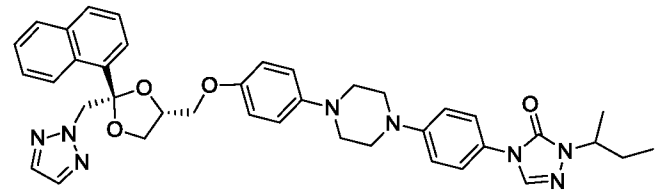
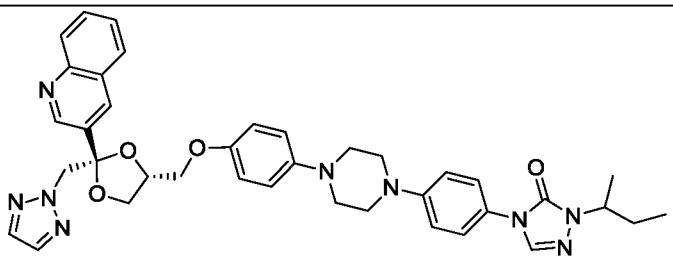
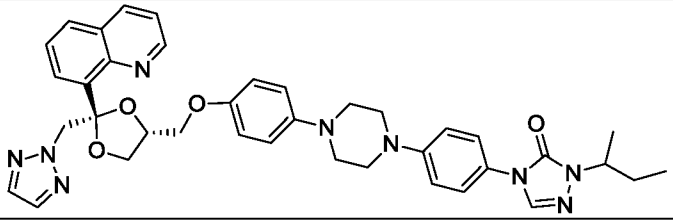
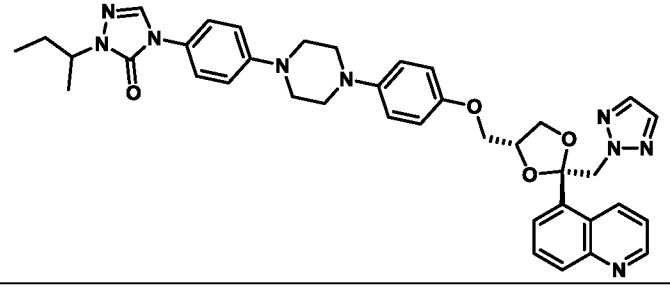
		3.88-3.92 (m, 1H), 4.29-4.38 (m, 2H), 4.85 (s, 2H), 6.80 (s, 2H), 6.89- 6.94 (m, 2H), 7.02-7.07 (d, *J*=8.0 Hz, 2H), 7.20-7.22 (d, *J*=8.0 Hz, 2H), 7.44-7.46 (d, *J*=8.0 Hz, 4H), 7.64-7.65 (m, 3H).	
41		LCMS: 100% @ 261 nm; m/z 651.55 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.65 (s, 3H), 3.29- 3.33 (m, 5H), 3.41 (s, 4H), 3.76-3.81 (m, 2H), 3.88-3.92 (m, 1H), 4.30-4.36 (m, 2H), 4.87-4.95 (q, 2H),6.78-6.81 (d, *J*=8.4 Hz, 2H), 6.97-7.00 (m , 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.21-7.24 (m, 2H), 7.45-7.47 (d, *J*=8.8 Hz,3H), 7.63-7.67 (m, 4H).	++
42		LCMS: 100 % @ 261 nm; m/z 637.45 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.92 (t, *J*=7.6 Hz, 3H), 1.41 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.86-1.89 (m, 1H), 3.30-3.43 (m, 9H), 3.73-3.78 (m, 1H), 3.83-3.86(m, 1H), 3.89-3.93 (m, 1H), 4.30-4.33 (m , 1H), 4.37-4.40(m, 1H), 4.87 (s, 2H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 7.02-7.07 (m, 4H), 7.38-7.42 (m, 3H), 7.44-7.47(d, *J*=8.8 Hz, 2H), 7.56-7.59(m, 2H), 7.64 (m, 3H)	+++
43		LCMS: 100% @ 262 nm; m/z 705.61 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d *J*=6.4 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.26 (s, 4H), 3.37- 3.41 (m, 4H), 3.82-3.87 (m, 2H), 3.90-3.94 (m, 1H), 4.29-4.34 (m, 3H), 4.94-5.04.(q, 2H), 6.78-6.80 (d, *J*=7.6 Hz, 2H), 6.95 (s, 2H), 7.04-7.07 (d, *J*=8.8 Hz,2H), 7.44-7.46 (d, *J*=8.4 Hz, 2H), 7.50-7.55 (m, 2H), 7.63-7.64 (m, 3H), 7.74-7.75 (d, *J*=6.8 Hz,1H), 7.80-7.83 (m, 1H).	+
44		LCMS: 99.84% @ 261 nm; m/z 715.31 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz,3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.08-3.09 (s, 3H), 3.25- 3.27 (m, 4H), 3.37-3.40 (m, 4H), 3.51-3.55 (m, 1H), 3.83-3.85 (m, 1H), 3.92-3.94 (m, 2H), 4.30-4.33 (m, 1H), 4.38-4.40 (m, 1H), 4.88 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H), 6.95-6.97 (d , *J*=8.8 Hz, 2H), 7.04-7.06 (d,	+

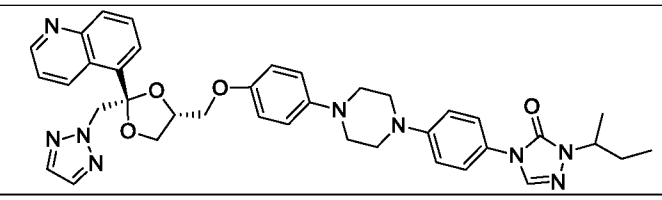
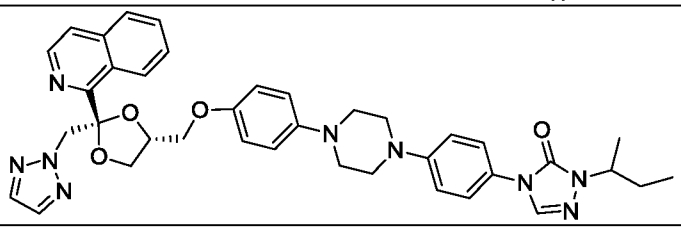
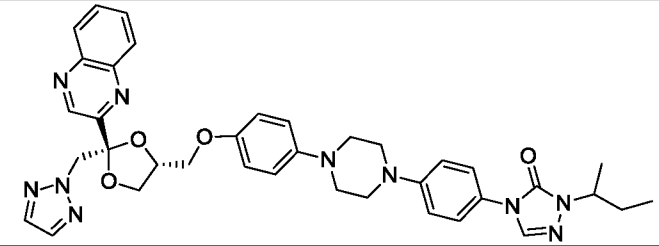
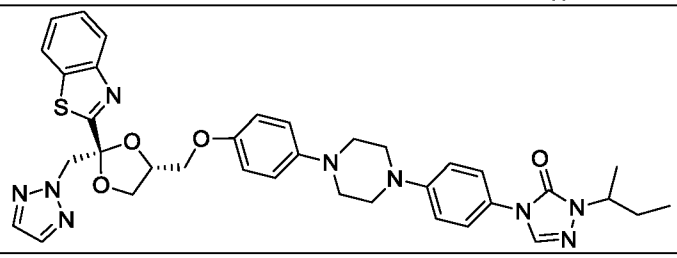
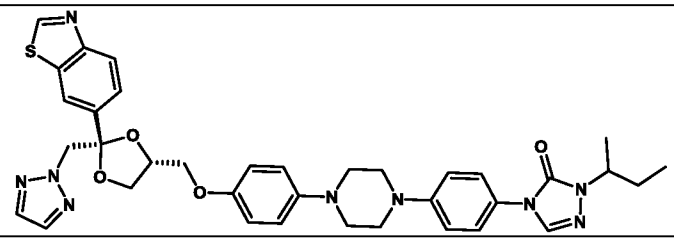
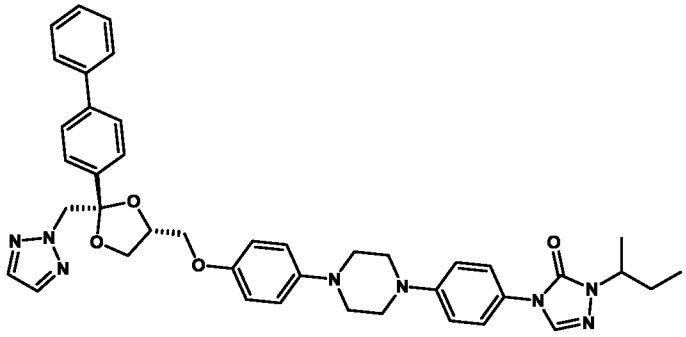
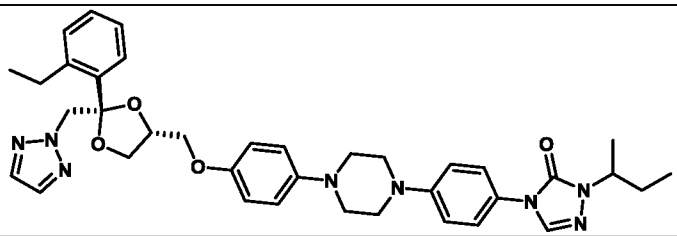
		J=9.2 Hz, 2H), 7.44-7.46 (d, *J*=8.8 Hz, 2H), 7.62-7.64 (d, *J*=8.0 Hz, 3H), 7.72-7.75 (d, *J*=8.8 Hz, 2H), 7.95-7.97 (d, *J*=8.8 Hz, 2H).	
45		LCMS: 100% @ 262 nm; m/z 667.36 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz,3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.26(s, 4H), 3.34-3.39 (m, 5H), 3.74-3.77 (m, 1H), 3.82-3.85 (m, 3H), 3.89-3.91(m, 2H), 4.31-4.38 (m, 2H), 4.84(s, 2H), 6.77-6.81 (s, 2H), 6.90-6.93 (d , *J*=8.8 Hz, 4H), 7.02-7.07 (d, *J*=9.2 Hz, 2H), 7.47-7.54 (m, 4H), 7.63-7.65 (m, 3H).	+
46		LCMS: 100% @ 262 nm; m/z 719.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.16-2.19 (s, 3H), 3.25 (s, 4H), 3.38 (s, 4H), 3.48-3.52 (q, 1H), 3.91-4.00 (m, 3H), 4.29-4.34(m, 1H), 4.39-4.41 (m, 1H), 5.06-5.09 (d, *J*=14.0 Hz, 1H), 5.17-5.20 (d, *J*=14.0 Hz, 1H), 6.69-6.71 (d, *J*=8.0 Hz, 1H), 6.78-6.84 (m, 2H), 7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz and J_2=2.0 Hz, 1H), 7.44-7.48 (m, 3H), 7.52-7.54 (d, *J*=8.4 Hz 1H), 7.60-7.64 (m, 3H).	+
47		LCMS: 100% @ 262 nm; m/z 752.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.69-1.77 (m, 1H), 1.83-1.92 (m, 1H), 3.34 (s, 4H), 3.45-3.52 (m, 5H), 3.85 (s, 3H), 3.94-3.98 (m, 3H), 4.29-4.34 (q, 1H), 4.41-4.44 (q, 1H), 5.06-5.10 (d, *J*=14.4 Hz, 1H), 5.14-5.21 (d, *J*=14.4 Hz, 1H), 6.60 (s, 1H), 6.73 (s, 1H), 6.82-6.84 (d, *J*=8.8 Hz, 1H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.19-7.22 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.44-7.47 (m, 3H), 7.51-7.53 (d, *J*=8.4 Hz, 1H), 7.60-7.65 (m, 3H).	+
48		LCMS: 100% @ 262 nm; m/z 758.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.27-3.28 (s, 4H), 3.37-3.38 (s, 4H), 3.50-3.54 (q, 1H), 3.96-4.03 (m, 3H), 4.29-4.34 (q, 1H), 4.40-4.41	++

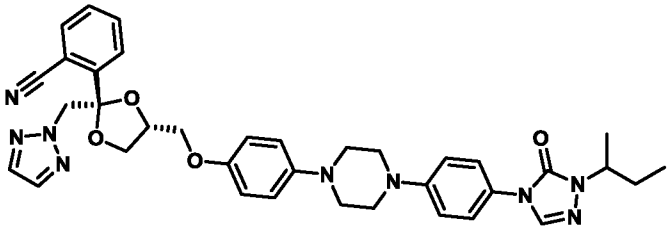
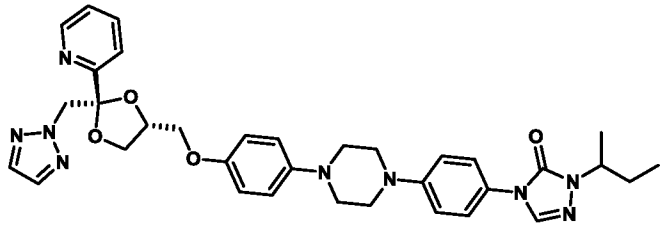
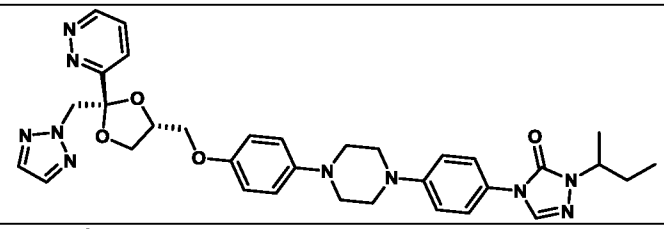
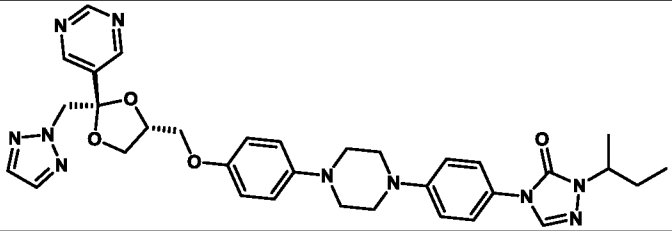
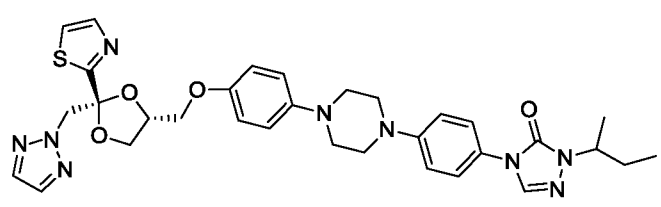
		(q, 1H), 5.07-5.10 (d, *J*=14.4 Hz, 1H), 5.16-5.20 (d, *J*=14.4 Hz, 1H), 6.86 (s, 2H), 7.02-7.06 (m, 3H), 7.20-7.22 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.44 (s, 1H), 7.46-7.47 (m, 2H), 7.52-7.54 (d, *J*=8.8 Hz, 1H), 7.60 (s, 2H), 7.64 (s,1H).	
49		LCMS: 100% @ 263 nm; m/z 723.81 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.84-1.92 (m, 1H), 3.25-3.28 (m, 4H), 3.36-3.38 (m, 4H), 3.50-3.55 (q, 1H), 3.92-3.95 (m, 3H), 4.29-4.34 (q, 1H), 4.37-4.42 (q, 1H), 5.04-5.08 (d, *J*=14.4 Hz, 1H), 5.16-5.20 (d, *J*=14.4 Hz, 1H), 6.65-6.66 (m, 1H), 6.67-6.76 (dd, J_1=16.8 Hz and J_2=3.2 Hz 1H), 6.86-6.90 (t, *J*=9.2 Hz, 1H), 7.03-7.06 (d, *J*=8.8 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.43-7.47 (m, 3H), 7.52-7.55 (d, *J*=8.4 Hz,1H), 7.58-7.64 (m, 3H).	++
50		LCMS: 100% @ 265 nm; m/z 719.61 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, , *J*=7.2 Hz 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 2.34 (s, 3H), 3.05 (s, 4H), 3.38-3.47 (m, 5H), 3.86-3.96 (m, 3H), 4.29-4.41 (m, 2H), 5.05-5.09 (d, , *J*=14.4 Hz 1H), 5.17-5.21 (d, , *J*=14.4 Hz 1H), 6.64-6.73 (m, 2H), 7.02-7.04 (m, 3H), 7.21-7.24 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.44-7.48 (m, 3H), 7.54-7.57 (d, *J*=6.8 Hz, 1H), 7.62-7.65 (m, 3H).	++
51		LCMS: 100% @ 262 nm; m/z 735.31 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.16-3.18 (s, 4H), 3.40-3.42 (m, 4H), 3.47-3.50 (m, 1H), 3.84-3.97 (m, 5H), 4.29-4.34 (q, 1H), 4.39-4.41 (q, 1H), 5.05-5.09 (d, *J*=14.4 Hz, 1H), 5.18-5.22 (d, *J*=14.4 Hz, 1H), 6.34-6.37 (dd, J_1=11.2 Hz and J_2=2.8 Hz 1H 1H), 6.47-6.48 (d, *J*=2.8 Hz, 1H), 6.89-6.91 (d, *J*=8.8 Hz, 1H), 7.04-7.06 (d, *J*=8.8 Hz, 2H), 7.22-7.24 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.42-7.48 (m, 3H), 7.55-7.64 (m, 4H).	++

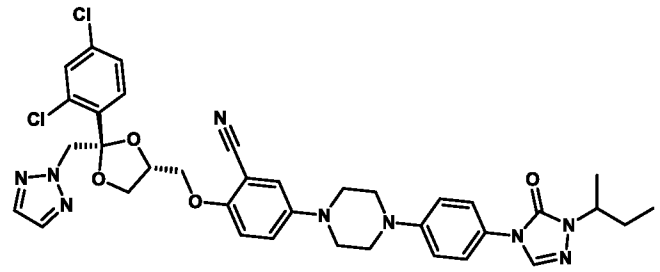
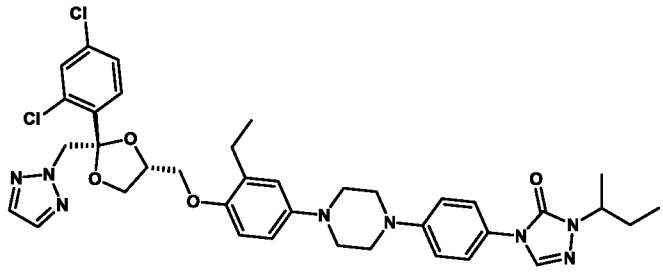
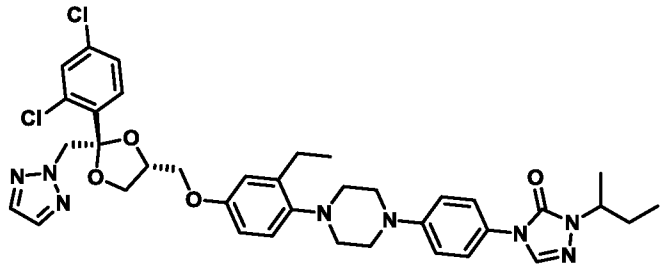
52		LCMS: 99.78% @ 265 nm; m/z 730.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.41-1.42 (d, *J*=6.8 Hz, 3H), 1.71-1.78 (m, 1H), 1.85-1.89 (m, 1H), 3.30-3.37 (m, 5H), 3.47-3.51 (m, 4H), 3.80-3.83(m, 1H), 3.86-3.89 (m, 1H), 3.93-3.97 (m, 1H), 4.31-4.33 (q, 1H), 4.39-4.42 (q, 1H), 5.05-5.09 (d, *J*=14.4 Hz, 1H), 5.17-5.20 (d, *J*=14.4 Hz, 1H), 7.02-7.10 (m, 3H), 7.20-7.26 (m, 3H), 7.46-7.59 (m, 4H), 7.64-7.67 (m, 3H).	++
53		LCMS: 100% @ 263 nm; m/z 741.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.71-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17 (s, 4H), 3.41-3.46 (m, 4H), 3.51 (s, 1H), 3.81-3.84 (m, 1H), 3.87-3.96 (m, 2H), 4.29-4.36 (q, 1H), 4.37-4.41 (q, 1H), 5.05-5.08 (d, *J*=14.4 Hz, 1H), 5.17-5.20 (d, *J*=14.4 Hz,1H), 6.73-6.76 (dd, J_1=11.6 Hz and J_2=2.8 Hz 1H), 6.92-6.93 (d, *J*=302 Hz, 1H), 7.02-7.07 (m, 3H), 7.22-7.25 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.44-7.49 (m, 3H), 7.54-7.57 (m, 1H), 7.63-7.64 (m, 3H).	++
54		LCMS: 100% @ 263 nm; m/z 723.81 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.17-3.20 (m, 4H), 3.38-3.46 (m, 5H), 3.80-3.83 (m, 1H), 3.86-3.96 (m, 2H), 4.29-4.36 (q, 1H), 4.37-4.40 (q, 1H), 5.04-5.08 (d, *J*=14.4 Hz, 1H), 5.17-5.21 (d, *J*=14.4 Hz, 1H), 6.56-6.64 (m, 2H), 6.92-6.97 (t, 1H), 7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.22-7.25 (dd, J_1=10.4 Hz and J_2=2.0 Hz 1H), 7.43-7.48 (m, 3H), 7.55-7.59 (m, 1H), 7.62-7.64 (m, 3H).	+++
55			

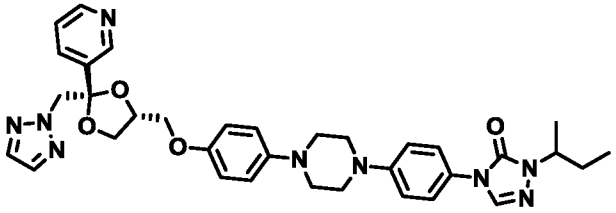
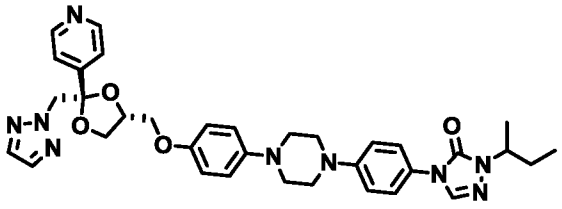
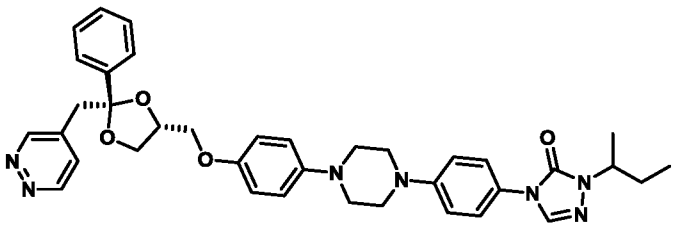
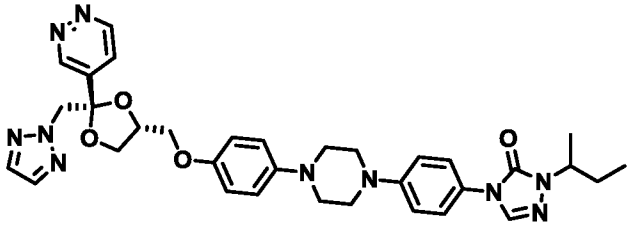
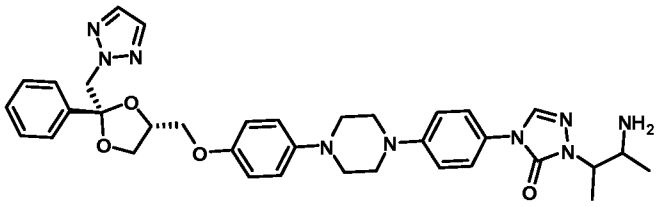
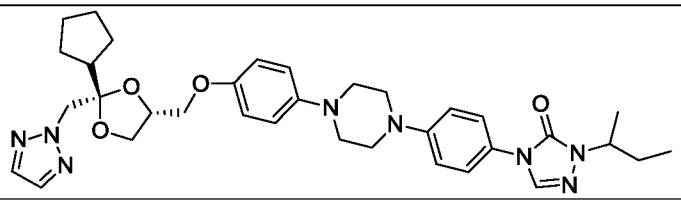
56		LC-MS: m/z 639.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.77 (d, J = 8.63 Hz, 1H), 7.61 (s, 1H), 7.59 (s, 2H), 7.47 (m, 1H), 7.26 (m, 1H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.27 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
57		LC-MS: m/z 639.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 1.5 Hz, 1H), 8.66 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.61 (d, *J* = 2.5 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.62 (s, 2H), 7.48 – 7.40 (m, 2H), 7.08 – 7.01 (m, 2H), 6.94 – 6.86 (m, 2H), 6.79 – 6.69 (m, 2H), 5.22 – 5.05 (m, 2H), 4.42 (m, 1H), 4.37 – 4.22 (m, 1H), 4.15 (m, 3H), 4.09 – 3.93 (m, 4H), 3.37 (m, 4H), 3.23 (m, 4H), 1.98 – 1.83 (m, 1H), 1.82 – 1.72 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).	+
58		LC-MS: m/z 643.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 0.6 Hz, 2H), 7.64 (t, *J* = 0.7 Hz, 1H), 7.49 – 7.42 (m, 2H), 7.11 – 7.03 (m, 2H), 6.97 (m, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.70 (s, 2H), 4.44 – 4.37 (m, 1H), 4.32 (dt, *J* = 8.6, 6.4 Hz, 1H), 4.18 (dd, *J* = 8.3, 6.3 Hz, 1H), 3.93 (dd, *J* = 9.7, 4.8 Hz, 1H), 3.60 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.50 – 3.12 (m, 9H), 2.08 – 1.65 (m, 7H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.22 (m, 6H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
59			
60			

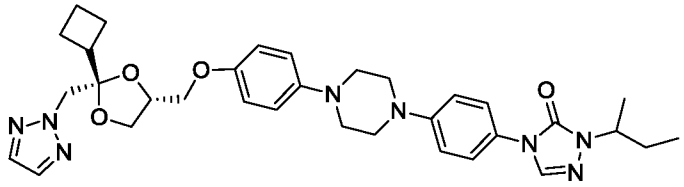
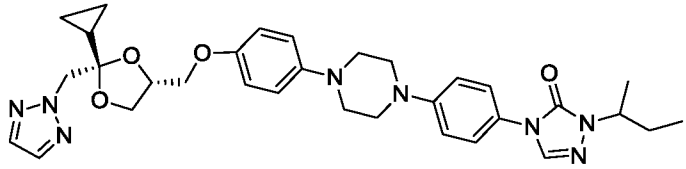
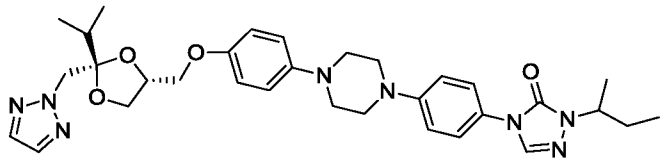
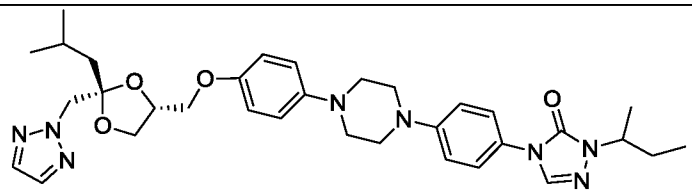
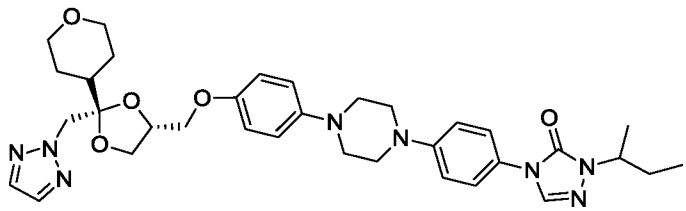
61			
62			
63		LC-MS: m/z 687.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, *J* = 8.6 Hz, 1H), 7.92 (m, 3H), 7.71-7.63 (m, 4H), 7.58 – 7.48 (m, 4H), 7.09-6.71 (m, 6H), 5.24 – 5.05 (m, 2H), 4.44 (d, *J* = 7.6 Hz, 1H), 4.40 – 4.27 (m, 1H), 3.99 (dd, *J* = 8.5, 6.3 Hz, 1H), 3.82 (td, *J* = 10.6, 9.8, 4.8 Hz, 2H), 3.73 – 3.04 (m, 9H), 1.89 (m, 1H), 1.76 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
64			
65			
66			

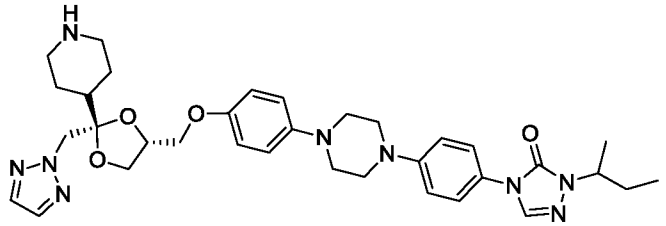
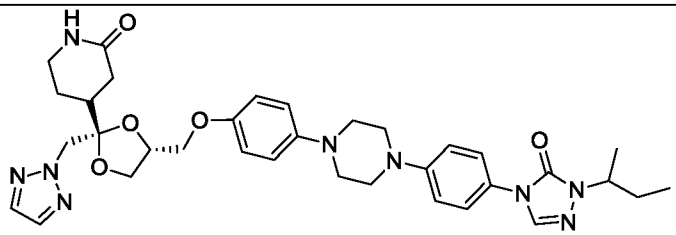
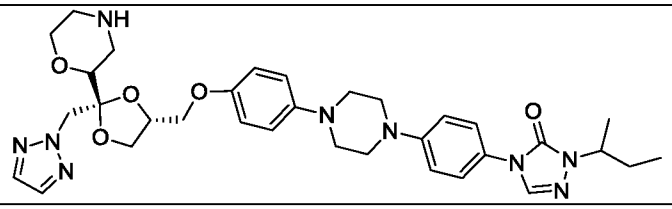
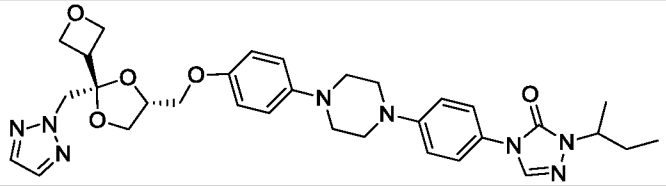
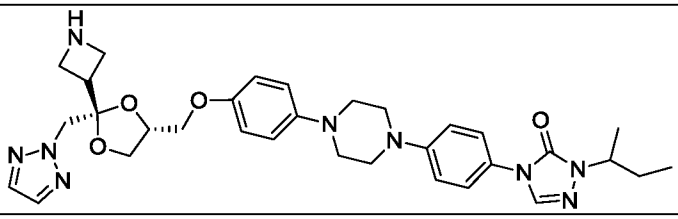
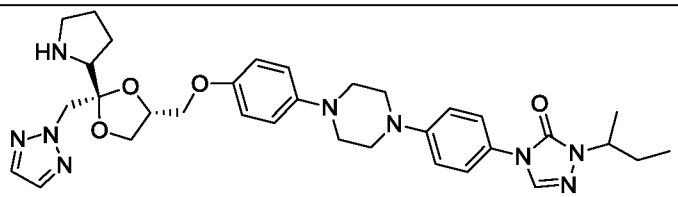
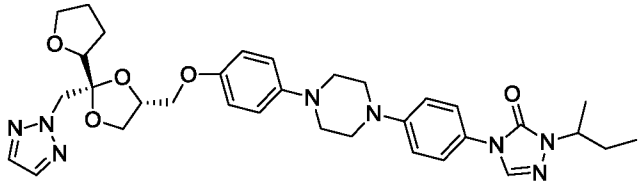
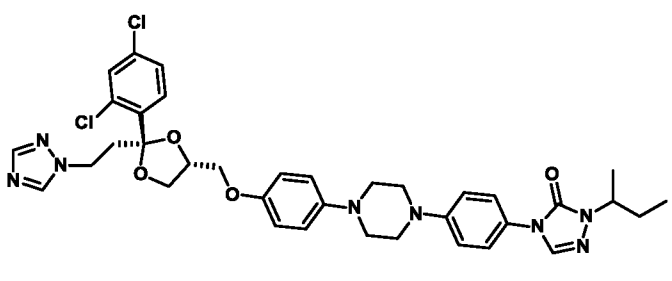
67			
68			
69			
70			
71			
72		LC-MS: m/z 713.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.56 (m, 9H), 7.53 – 7.43 (m, 4H), 7.43 – 7.35 (m, 1H), 7.06 (d, *J* = 9.0 Hz, 2H), 6.97 (m, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 4.91 (s, 2H), 4.43 (ddd, *J* = 9.4, 6.8, 4.6 Hz, 1H), 4.37 – 4.26 (m, 1H), 3.97 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.87 (dd, *J* = 8.5, 4.3 Hz, 1H), 3.79 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.52 – 3.07 (m, 9H), 1.89 (m, 1H), 1.74 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H).	+
73			

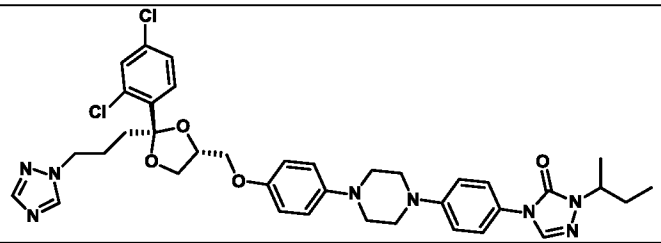
74		LC-MS: m/z 662.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.64 (d, *J* = 0.7 Hz, 1H), 7.62 – 7.48 (m, 5H), 7.45 (dd, *J* = 9.1, 2.2 Hz, 2H), 7.10 – 7.03 (m, 2H), 7.01 – 6.93 (m, 2H), 6.89 – 6.81 (m, 2H), 5.17 – 5.01 (m, 2H), 4.46 (tt, *J* = 6.8, 4.6 Hz, 1H), 4.39 – 4.26 (m, 1H), 4.09 (dd, *J* = 8.6, 4.7 Hz, 1H), 4.07 – 3.95 (m, 2H), 3.70 (dd, *J* = 9.6, 7.0 Hz, 1H), 3.39 (m, 4H), 3.26 (m, 4H), 1.89 (m Hz, 1H), 1.70 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	++
75		LC-MS: m/z 638.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (ddt, *J* = 4.9, 1.7, 0.8 Hz, 1H), 7.76 – 7.68 (m, 1H), 7.64 (d, *J* = 0.7 Hz, 1H), 7.60 (m, 2H), 7.54 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.30 (m, 1H), 7.13 – 7.03 (m, 2H), 7.02 – 6.92 (m, 2H), 6.88 – 6.70 (m, 2H), 5.15 (m, 2H), 4.49 (ddd, *J* = 11.6, 6.7, 4.9 Hz, 1H), 4.42 – 4.26 (m, 1H), 4.07 (dd, *J* = 8.5, 6.4 Hz, 1H), 3.92 (td, *J* = 9.0, 8.4, 4.8 Hz, 2H), 3.56 (dd, *J* = 9.5, 7.2 Hz, 1H), 3.47 – 3.36 (m, 4H), 3.26 (m, 4H), 1.92 (m, 1H), 1.74 (m, 1H), 1.42 (d, *J* = 6.7, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).	+
76			
77			
78		LC-MS: m/z 644.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 6.5, 3.2 Hz, 1H), 7.68-7.64 (m, 3H), 7.47 – 7.41 (m, 2H), 7.39 (t, J = 3.3 Hz, 1H), 7.05 (dd, J = 9.0, 3.7 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.85 – 6.76 (m, 2H), 5.31 – 5.12 (m, 2H), 4.62 (dq, J = 6.8, 5.4 Hz, 1H), 4.39 – 4.25 (m, 1H), 4.21 (dd, J = 8.6, 6.3 Hz, 1H), 3.88 (ddd, J =	+

		10.5, 9.1, 5.1 Hz, 2H), 3.52 (dd, J = 9.6, 7.0 Hz, 1H), 3.45 – 3.32 (m, 4H), 3.30 – 3.14 (m, 4H), 1.98 – 1.83 (m, 1H), 1.82 – 1.72 (m, 1H), 1.42 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H).	
79		LC-MS: m/z 730.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 3H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 2H), 7.15 (d, *J* = 3.0 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 9.2 Hz, 2H), 5.21 – 5.07 (m, 2H), 4.46 (m, 1H), 4.33 (m, 1H), 3.99 (dtd, *J* = 18.1, 8.9, 4.6 Hz, 3H), 3.52 (dd, *J* = 9.5, 7.6 Hz, 1H), 3.41 (m, 4H), 3.31 (m, 4H), 1.96 – 1.88 (m, 1H), 1.79 – 1.72 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	++
80		LC-MS: m/z 733.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.64 (s, 1H), 7.61 (s, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.42 (m, 2H), 7.21 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.87 (d, *J* = 2.8 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 5.25 – 4.97 (m, 2H), 4.44 – 4.37 (m, 1H), 4.37 – 4.27 (m, 1H), 4.05 – 3.86 (m, 3H), 3.50 (dd, *J* = 9.4, 7.4 Hz, 1H), 3.39 (m, 4H), 3.32 – 3.25 (m, 4H), 2.58 (q, *J* = 7.5 Hz, 2H), 1.88 (m, 1H), 1.81 – 1.72 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.17 (t, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H).	+
81		LC-MS: m/z 733.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.62 (s, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.11 – 7.01 (m, 3H), 6.75 (d, *J* = 2.9 Hz, 1H), 6.65 (dd, *J* = 8.6, 3.0 Hz, 1H), 5.27 – 5.00 (m, 2H), 4.40 (m, 1H), 4.32 (dt, *J* = 8.7, 6.4 Hz, 1H), 4.02 – 3.83 (m, 3H), 3.44 (dd, *J* = 9.4, 7.4 Hz, 1H), 3.41 – 3.28 (m, 4H), 3.09 – 2.91 (m, 4H), 2.74 (q, *J* = 7.5 Hz, 2H), 1.98 – 1.85 (m, 1H), 1.81 – 1.70 (m, 1H), 1.43 (d, *J* = 6.7 Hz, 3H), 1.17 (t, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H).	+

82		LC-MS: m/z 638.1 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.10 (s, 1H), 7.67 (s, 2H), 7.54 – 7.38 (m, 3H), 7.34 (m, 2H), 7.27 (m, 1H), 7.24-7.09 (m, 4H), 6.94 (d, *J* = 7.6 Hz, 2H), 4.44 (m, 1H), 4.24 (m, 2H), 4.06 – 3.71 (m, 5H), 3.54-3.43 (m, 8H), 1.96 – 1.85 (m, 2H), 1.31, (d, *J* = 7.0 Hz, 3H), 0.90 (t, *J* = 7.5 Hz, 3H).	+
83		LC-MS: m/z 638.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.62 (d, J = 8.63 Hz, 2H), 7.61 (s, 1H), 7.59 (s, 2H), 7.46-7.42 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.00 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
84		LC-MS: m/z 648.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (dd, *J* = 2.4, 1.3 Hz, 1H), 9.01 (dd, *J* = 5.2, 1.3 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.50 – 7.30 (m, 8H), 7.09 – 7.02 (m, 2H), 6.99 – 6.93 (m, 2H), 6.81 – 6.71 (m, 2H), 4.32 (m, 1H), 3.93 – 3.67 (m, 5H), 3.61 – 3.50 (m, 2H), 3.39 (m, 4H), 3.27 (m, 4H), 3.19 (m, 2H), 1.89 (m, 1H), 1.77 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H).	++
85			
86			
87			

88		LC-MS: m/z 615.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 3H), 7.47 (m, 2H), 7.07-6.88 (m, 6H), 4.59 – 4.47 (m, 2H), 4.26 (m, 2H), 4.15 – 3.88 (m, 2H), 3.82 – 3.72 (m, 1H), 3.60 (t, *J* = 8.1 Hz, 1H), 3.41-3.22 (m, 8H), 2.67 (m, 1H), 2.12-1.70 (m, 8H), 1.40 (m, 3H), 0.97 (m, 3H).	++
89			
90		LC-MS: m/z 603.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 1.0 Hz, 1H), 7.69 (d, *J* = 1.0 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.09 – 7.02 (m, 2H), 7.01 – 6.92 (m, 2H), 6.88 – 6.78 (m, 2H), 4.73 – 4.56 (m, 2H), 4.51 – 4.43 (m, 1H), 4.32 (dp, *J* = 8.6, 6.6 Hz, 1H), 4.19 (dd, *J* = 8.3, 6.5 Hz, 1H), 3.82 (dd, *J* = 10.0, 4.4 Hz, 1H), 3.61 (dd, *J* = 9.9, 5.2 Hz, 1H), 3.55 (t, *J* = 8.0 Hz, 1H), 3.44 – 3.35 (m, 4H), 3.31 – 3.20 (m, 4H), 1.95 – 1.80 (m, 2H), 1.74 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 1.07 (dd, *J* = 11.5, 6.9 Hz, 6H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
91			
92		LC-MS: m/z 645.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.67 (s, 1H), 7.64 (s, 1H), 7.48 – 7.41 (m, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.91 – 6.81 (m, 2H), 4.74 – 4.59 (m, 2H), 4.50 – 4.38 (m, 1H), 4.38 – 4.14 (m, 2H), 4.12 – 3.84 (m, 4H), 3.78 (dd, *J* = 9.9, 5.5 Hz, 1H), 3.60 (t, *J* = 8.3 Hz, 1H), 3.45 – 3.29 (m, 5H), 3.30 – 3.18 (m, 4H), 1.97 – 1.81 (m, 3H), 1.80 – 1.70 (m, 3H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+

93		LC-MS: m/z 644.1 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.11 (d, *J* = 1.6 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 2H), 7.50 (dd, *J* = 9.2, 2.7 Hz, 2H), 7.31 (dd, *J* = 16.7, 9.0 Hz, 2H), 7.19 (dd, *J* = 9.2, 3.1 Hz, 2H), 7.09 – 6.96 (m, 2H), 4.78 – 4.67 (m, 2H), 4.35 – 4.20 (m, 2H), 4.19 – 3.95 (m, 3H), 3.88 (m, 1H), 3.51 (m, 10H), 2.95 (m, 2H), 2.31 – 1.64 (m, 7H), 1.40 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	+
94			
95			
96			
97			
98			
99			
100		LC-MS: m/z 719.2(M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.92 (s, 1H), 7.64 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.42 (m, 3H), 7.28 – 7.24 (m, 1H), 7.10 – 7.03 (m, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 6.96 – 6.87 (m, 2H), 4.53 – 4.21 (m, 4H), 4.19 – 4.03 (m, 3H), 3.90 (t, *J* = 7.8 Hz, 1H), 3.40 (m, 4H),	+++

		3.27 (m, 4H), 2.82 – 2.64 (m, 2H), 1.98 – 1.84 (m, 1H), 1.83 – 1.64 (m, 1H), 1.41 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H).	
101		LC-MS: m/z 733.2 (M+H)	+

Cyp reversible inhibition assay

[00393] The purpose of this assay was to determine reversible inhibition of Cyp enzyme with a given compound using a substrate probe turnover as the surrogate for inhibition. This was done with human liver microsomes rather an isolated enzyme to account for metabolites of parent drug leading to a potential drug-drug interaction through Cyp inhibition. The substrate turnover (for example conversion of midazolam to hydroxyl midazolam) was monitored by LCMSMS (Q1/Q2 = 342.2/203.2). Test compounds were tested in a 7-point dose response curve starting from 50 μM to 50 nM (~3-fold dilution series). For Cyp 3A4, the positive control compound was ketoconazole with an IC_{50} ~ 25 nM.

[00394] The detailed protocol was as follows:

- Prepared test compounds and standard inhibitors working solution (100×).
- Pulled microsomes out of the –80°C freezer to thaw on ice, labeled with the date and put back to freezer immediately after using.
- Added 20 μL of the substrate solutions to corresponding wells.
- Added 20 μL PB to Blank wells.
- Added 2 μL of the test compounds and positive control working solution to corresponding wells.
- Added 2 μL MeOH to No Inhibitor wells and Blank wells.
- Prepared HLM working solution.
- Added 158 μL of the HLM working solution to all wells of incubation plate.
- Pre-warmed the plate for about 10 min at 37°C water bath.
- Prepared NADPH cofactor solution.
- Added 20 μL NADPH cofactor to all incubation wells.
- Mixed and incubated for 10 minutes at 37°C water bath.
- At the time point, terminated the reaction by adding 400 μL cold stop solution (200 ng/mL Tolbutamide in ACN).
- Centrifuged the samples at 4000 rpm for 20 minutes to precipitate protein.

- Transferred 200 µL supernatant to 200 µL HPLC water and shook for 10 min.
- Analyzed samples by LC/MS/MS.

[00395] Compound data obtained by this assay are shown in Table 2.

Table 2

Compound #	Cyp 3A4 midazolam LCMS IC_{50} (µM)	Cyp 3A4 testosterone LCMS IC_{50} (µM)
1		4.5
2		>30
15	>50; >50	>50
19	0.0031	0.0069
21	0.144	0.12
22	>50, >50	>50, >50
23	0.321	
24		>10, >30
26	0.18	0.283
27	>50, >50	>50, >50
30	>50	>50
32	>50	>50
36	>50	>50
41	>50, >50	>50
42	>50, >50	>50, >50
49	>50	>50
54	>50	>50
74	>50	>50
79	>50	>50
84	2.55, 2.35	1.77
100	0.372	0.545
ITZ	0.0447, 0.0546	0.0127

Cyp time-dependent inhibition (TDI) assay

[00396] The purpose of this assay was to ascertain the ability for compounds to form irreversibly inhibited cytochrome P450 adducts (also known as mechanism-based inhibition (MBI)) in human liver microsomes. Compared to competitive CYP inhibition, it has been recognized that MBI is a much greater concern in drug discovery and development, because

inactivation of CYPs can lead to non-linear pharmacokinetics and underestimate drug-drug interaction potential. The data from this assay was used in conjunction to the reversible inhibition assay. The TDI assay was generally performed in liver microsomes to assess TDI potential of parent drug as well as metabolites. The readout was an IC_{50} shift with NADPH (the inactivation/TDI set) to allow for compound to be converted to a reactive species and without NADPH (control set to correct for protein degradation during pre-incubation of 20 minutes). Then both incubation sets were diluted with fresh assay buffer containing NADPH Cyp specific substrates (midazolam for Cyp3A4) and inhibition of midazolam hydroxylation was measured by LC-MS/MS (Q1/Q2 = m/z 342.2/203.2). An IC_{50} shift > 1.5-fold was considered to be positive for time dependent inhibition where the 20 min pre-incubation leads to an increase in potency. Troleandomycin was used as a positive control compound, exhibiting a TDI IC_{50} shift of >20. Test compounds were tested in a 7-point dose response curve starting from 50 μM to 50 nM (~3-fold dilution series).

[00397] The detailed protocol was as follows:

- Prepared test compounds and positive control working solution (100×) in 1:1 DMSO/MeOH
- Pulled microsomes out of the –80°C freezer to thaw.
- Prepared incubation mix and added 147.5 μL to all wells of incubation plate.
- Prepared cofactor solution and substrate dilution solution.
    - Added 2.5 μL of the test compounds and positive control working solution to corresponding wells. Final compound concentrations in 7-point dose response from 50 μM to 50 nM
    - Added 2.5 μL 1:1 DMSO/MeOH to NIC wells
- Pre-warmed the plate for about 10 min at 37°C.
- Added 50 μL cofactor to pre-incubation wells
- Added 50 μL substrate dilution solution to incubation wells.
- Mixed and pre-incubated for 20 minutes at 37°C water bath.
- Added 50 μL cofactor to incubation wells;
    - Added 50 μL substrate dilution solution to pre-incubation wells.
- Mixed and incubated for 5 minutes at 37°C water bath.
- At the time point, terminated the reaction by adding cold 250 μL IS-fortified stop solution to all wells.
- Centrifuged the incubation plate at 4000 rpm for 20 min.
- Transferred 200 μL supernatant into 200 μL HPLC water and shook for 10 min.

- Analyzed samples by LCMS.

[00398] Compound data obtained from this assay are shown in Table 3.

Table 3

Compd #	3A4-Midazolam IC_{50} (-) NADPH	3A4-Midazolam IC_{50} (+) NADPH	TDI ratio: (-) NADPH/ (+) NADPH
15	>50 μM	41 μM	~1.2 (no TDI)
42	>50 μM	>50 μM	~1 (no TDI)
22	>50 μM	>50 μM	~1 (no TDI)

PXR (Cyp 3A4) activation assay

[00399] CYP3A4 metabolism was evaluated by using P450--Glo™ CYP3A4 Assay with Luciferin--IPA as the substrate of CYP3A4 and represented as RLU (Relative Luminescence Units). PXR activation was evaluated by using a luciferase detection reagent, ONE--Glo™ and represented as RLU. The luminescence light intensity was directly proportional to the extent of PXR activation and accompanying gene transcription in the DPX2 cells. Compounds were tested at 10, 1, and 0.1 μM in the assay. Fold of induction = (RLU/RFU of compound treated sample)/ (RLU/RFU of vehicle treated sample), RFU was the signal of cell viability. RLU was the signal of CYP3A metabolism and PXR activation. 0.1% DMSO was used as vehicle. Cell viability was detected by using CellTiter--FluorTM and represented as RFU (Relative Fluorescence Units).

[00400] Activation potency was defined as negative, weak, moderate and strong. Negative, weak, moderate and strong activators are those that give < 15%, <40%, <69% and >70%, respectively, of the response produced by 10 μM RIF at 10 μM.

[00401] Compound data obtained from this assay are shown in Table 4.

Table 4

Compd #	PXR activation (fold induction compared to Rifampicin)
15	~1x (no induction)
42	~1.5x (no induction)
22	~1.1x (no induction)

Candida albicans anti-fungal MIC potency determination

[00402] The wild type *Candida albicans* (ATCC 10231) was used and Fluconazole, amphotericin B, itraconazole and terbinafine were purchased from Sigma and used as positive controls.

[00403] The highest assay concentration for all test compounds and fluconazole was 100 μM. Fluconazole was also tested at the highest concentration of 64 μg/ml, and amphotercin B and terbinafine at 16 and 64 μg/ml. The test compounds were in DMSO stock solution at a concentration of 10 mM. Two stock solutions in DMSO were prepared for fluconazole at 10 mM and 6.4 mg/ml. Amphotericin B and terbinafine stock solutions in DMSO were prepared at 1.6 and 6.4 mg/ml.

[00404] Serial dilution of 100X stock solutions: 4 μl of stock solution was added into 196 μl of RPMI1640 (MOPS buffered and free of HEPES and sodium bicarbonate) in the first well of a row of a sterile u-bottom 96-well plate. The rest of the wells was filled with 100 μl RPMI1640. The 2-fold serial dilution was made sequentially by transferring 100 μl solution to the next well and mixing by pipetting, until 11th well. The extra 100 μl in 11th well was discarded. Therefore, the compound wells contained 100 μl of 2× of testing concentrations of drugs in RPMI1640. The 12th well was filled with only 100 μl RPMI1640.

[00405] *C. albicans* 3147 (ATCC 10231) glycerol frozen stock was streaked on Sabouraud dextrose agar (SDA). The plate was incubated at 35°C ambient atmosphere for 20 h. Single colonies were suspended into sterile saline until turbidity reached 0.1 (1-5×106 CFU/ml) using a Siemens turbidity meter. This suspension was diluted 50× in RPMI1640 in a 15 ml conical, and then it was further diluted 20× in RPMI1640 in a 50 ml conical. This gave 1-5×103 CFU/ml suspension and was used as inoculum. The cell density in inoculum was plate-counted to be 4.94×103 CFU/ml.

[00406] MIC determination: Within 15 min, 100 μl of prepared bacterial inoculum was added into each well of compound/RPMI1640-containing plate. The plates were incubated at 35°C in ambient atmosphere. The photos were taken at 24 h and 48 h. The MIC end points for fluconazole and amphotericin B were read according to the M27-A3 protocol (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition, M27-A3 standard published 01/01/2008 by Clinical and Laboratory Standards Institute). The MIC for compounds for test cmpds and terbinafine (not mentioned in M27-A3 protcol) were read as if they were azoles.

[00407] Data for compounds tested in this assay are shown in Table 5.

Table 5

Compound #	*C. Albicans* MIC90 at 24 h (μM)	*C. Albicans* MIC90 at 48 h (μM)
15	>100	>100
19	0.195	0.195
21	>100	>100

Compound #	*C. Albicans* MIC90 at 24 h (μM)	*C. Albicans* MIC90 at 48 h (μM)
22	>100	>100
23	>100	>100
26	>100	>100
27	>100	>100
30	>100	>100
32	>100	>100
36	>100	>100
41	>100	>100
42	>100	>100
49	>100	>100
54	>100	>100
79	>100	>100
84	>100	>100
100	0.781	1.563
101	>100	>100

Mouse pharmacokinetic (PK) studies

[00408] Compounds with suitable *in vitro* ADME properties were progressed to *in vivo* PK studies. A snapshot format in mice was used to quickly assess the potential for lead candidates to be orally bioavailable. The snapshot format involved a single dose (typically 20 mg/kg) given orally in a standard vehicle (5% PEG/DMSO in water for example). Compounds were infused directly into MS analysis to determine unique MRM (multiple reaction monitoring) signal that is concentration dependent in either ESI positive or negative modes. HPLC analysis using various solvent gradients was performed from organic and aqueous solution to ensure good peak shape of desired Q1/Q2 masses. Formulations included 45% Cyclodextrin in water (as is used clinically for Itraconazole). Other formulations that may used are solutol, Eudragit, MC/Tween, etc. Standard curves were generated using plasma spiked with compound at various concentrations (10 ng/mL to 2000 ng/mL) to determine linear range of Q1/Q2 mass signal to compound concentration using female mouse heparinized plasma. Mice were dosed orally with compound, plasma samples collected by retro-orbital bleeds. Five time points were collected (30 min, 1 h, 4 h, 8 h, 24h) into heparinized collection tubes. Protein precipitation was performed and compound extracted in cold acetonitrile and analyzed by MS. Nonwinlin was used for PK modeling as needed.

[00409] Study results are presented in Table 6.

Table 6

Compound #	PK study results
42	20 mg/kg in 45% cyclodextrin with water: $AUC_{0\text{-}24h}$ = 42.67 ± 4.90 μM*h C_{max} = 3.16 ± 0.4 μM
22	20 mg/kg in 0.5% MC/0.5% TW80 suspension vehicle: $AUC_{0\text{-}24h}$ = 2.21 ± 0.02 μM*h C_{max} = 0.17 ± 0.03 μM
22	20 mg/kg in 45% cyclodextrin with water: $AUC_{0\text{-}24h}$ = 8.08 ± 1.72 μM*h C_{max} = 1.71 ± 0.44 μM

EXAMPLE II: Animal Models

[00410] To demonstrate *in vivo* anti-fibrotic activity, carbon tetrachloride (CCl_4) induced liver and bleomycin induced lung and skin rodent fibrosis models were established. These *in vivo* assays took 6-8 weeks to complete (including full histological analysis). Typical experiments consisted of groups of 6-8 animals treated with itraconazole (2-4 doses), 1 or 2 itraconazole analogues (2-4 doses), vehicle, and benchmark treatments (pirfenidone and AM-152). AM-152 (Amira Pharmaceuticals) is an LPA1 (Lysophosphatidic acid 1) receptor antagonist. This target has been described as relevant to exacerbation of IPF systems in a mouse bleomycin model (British Journal of Pharmacology (2010), 160, 1699–1713). AM-152 is an advanced compound on this target as has been previously described in more detail in WO 2012/078805 and was used as a positive control and comparator to the compound disclosed in this application.

[00411] Bleomycin-induced lung fibrosis model

[00412] Nine-week old B6 male mice (Taconic farms) were surgically implanted with osmotic pumps delivering 25mg/kg bleomycin for seven days. 17 days after surgery mice were treated with drug for 2 weeks. Drug treatments were administered as described in study design (Figure 14). Previously described anti-fibrotic drugs AM-152 and pirfenidone were used as positive control drugs. Lung sections were stained with Masson's trichrome and following scanning, eight random fields were taken per animal for analysis. Data are represented as mean and s.e.m. (Figure 15a). A graphical representation of model used to evaluate anti-fibrotic drugs is shown in Figure 15b. Representative images of Masson's trichrome stained lung from indicated treatment groups are shown in Figure 15c. Stained lung sections were analyzed according to a modified

Ashcroft scoring system (Figure 16) and using an unbiased automated image analysis method. Total Masson's trichrome stained area was generated using an automated image analysis macro in ImageJ. Each image was then converted to an RGB stack, and the threshold for stained area was set such that only lung tissue was included in the analysis while empty spaces such as respiratory ducts and alveoli were excluded. Total stained area was then determined by using the area measurement function within ImageJ. Mean Ashcroft scores and mean percent stained area values indicated that itraconazole and analogs thereof dose dependently decreased lung fibrosis disease severity in this model (Figures 17a and 17b). At a dose of 10 mg/kg (SID), compound 42 decreased lung fibrosis disease severity with efficacy that was as good or better when compared to control compounds AM-152 (30 mg/kg BID) and pirfenidone (400 mg/kg, BID).

[00413] Carbon tetrachloride-induced liver fibrosis model

[00414] Drug treatments were administered as described in study design (Figure 18). Liver sections were stained with Sirius Red solution. After scanning, 5 random images per animal were generated. Total percent area positive for Sirius Red staining was generated using an automated image macro in Image J. Briefly, each image was converted to a RGB stack, and the threshold for stained area was set such that no nuclei were included in the analysis. Total red area was quantitatively determined by using the area measurement function within ImageJ (Figure 19a). Numerical data of the image analysis of the CCl_4-induced liver fibrosis model is shown in Figure 19b. Representative images of Sirius Red stained liver sections of the CCl_4-induced liver fibrosis model are shown in Figure 19c. Levels in alpha smooth muscle actin (αSMA) livers were analyzed by western blot (Figure 19d). Pieces of liver from the CCl_4 -induced liver fibrosis model were homogenized in PBS with a homogenizer and steel balls. Debris was discarded by centrifugation and lysate concentrated determined by Nanodrop absorbance measurements at 260nm. Equal amounts of lysate were loaded into each gel lane and then separated by SDS-PAGE on 10% Bis-Tris gels and then transferred via semi dry transfer to PVDF membranes. After blocking in 5% milk in TRIS buffered saline with Tween-20 (0.1%), membranes were exposed to appropriate primary antibodies. Blots were incubated with HRP-conjugated secondary antibodies and visualized using film and SuperSignal West Dura Chemiluminescent Substrate (Pierce). Siruis red staining and western blot analysis indicated that itraconazole dose dependently decreased liver fibrosis disease severity.

[00415] Rodent wound healing model

[00416] The effect of itraconazole and analogs thereof on normal wound healing was evaluated using a standard rodent wound healing model (study design shown in Figure 20; results in Figure 21). Five days prior to the initiation of the model, mice were anesthetized and the back skin hair was removed using Nair. On day 1, mice were weighed and one sterile biopsy punch (5 mm

diameter) was made by punching through the full thickness of the folded back skin (2 x 5 mm dial holes in total). The size of the wounds was measured daily using calipers. Body weight was monitored twice a week thoughout the study. On day 1and until the end of the study, drug treatment (25 mg/kg, SID of itraconazole, 25 mg/kg SID compound 42 or vehicle) was administered daily using 5ml/kg dosing volume. On days 7, 11 and 14, animals were euthanized from each group and wounds were collected for histological analysis. When collecting the wounds, wounds were cut out including a few mm of the surrounding skin. For each animal, two pieces of wound were collected and fixed for histology analysis. Itraconazole and compound 42 had no effect on tissue architecture or rates of normal wound healing compared to vehicle controls, based on histological analysis and daily caliper measurements.

EXAMPLE III: Phase II Clinical Study for scleroderma

[00417] A phase II placebo controlled randomized double blind clinical trial demonstrates Proof of Concept for an optimized anti-fibrotic itraconazole analogue in patients with diffuse cutaneous scleroderma or systemic scleroderma with diffuse cutaneous involvement. Patients that satisfy the enrollment criteria make up two equal groups of 30. The experimental arm is given a single high dose of drug every day for 6 months and the placebo comparator arm will be given placebo every day for 6 months. The single high dose of drug is determined based on preclinical efficacy, target engagement in a preclinical cynomolgus toxicology study and a phase I safety study. The primary outcome measure is a comparison of the efficacy of drug vs placebo based on percent variation in m-RSS (score 0-51, 17 site) between inclusion and monthly visits. Secondary outcome measures are a comparison of efficacy of drug vs placebo based on variation in m-RSS between inclusion and follow-up time points (1, 3 and 6 months); assessment of target engagement as determined by expression profiling of hedgehog and VEGFR target genes using skin biopsies obtained at inclusion and at 6 months; assessment of skin thickness at inclusion and 6 months using skin biopsies; assessment of treatment of non-cutaneous symptoms in systemic scleroderma patients; assessment of quality of life using a health assessment questionnaire and the Dermatology Quality of Life Index; and assessment of tolerance of treatment using clinical and laboratory monitoring of side effects (including assessment of signs of negative inotropic effects using cardiac ultrasound). Successful Proof of Concept will be established if >40% of patients have m-RSS improvement (defined as decline in m-RSS of ≥5.3 units between baseline and last study visit at 6 months).

[00418] The precise target patient population consists of patients diagnosed with diffuse (or severe) cutaneous scleroderma (modified Rodnan skin score, m-RSS ≥16/51). Patients diagnosed with localized diffuse cutaneous scleroderma or systemic scleroderma with diffuse

cutaneous involvement, as defined by the American College of Rheumatology, are the known patient subsets.

[00419] Inclusion and exclusion criteria for patient recruitment of a phase II proof of concept study are as follows. Patients will be 18 years of age or older with documented diagnosis of cutaneous or systemic scleroderma. A baseline m-RSS of ≥16/51 indicative of diffuse cutaneous scleroderma is required. A pre-inclusion cardiac ultrasound ejection fraction score of more than 55% (i.e., normal) is required for inclusion. Patients will be excluded if they have been treated with a drug (e.g., methotrexate, corticosteroids, cyclophosphamide, bosentan) that has the potential to interfere with the course of disease within a 3 month period prior to the start of the trial. Patients suffering from severe organ failure, chronic liver disease (e.g., liver cirrhosis, chronic hepatitis), cancer, chronic illness (e.g., rheumatoid arthritis, systemic lupus erythematosus, diabetes, HIV) or having anomalous blood chemistry will be excluded. Patients having had major surgery less than 4 weeks before inclusion will be excluded. Patients contraindicated to itraconazole, as specified in product specifications, will be excluded. Specifically, patients with evidence of ventricular dysfunction (e.g., congestive heart failure, CHF), at risk of CHF or having been treated with inotropic drugs will be excluded. Patients having suffered myocardial infarction less than 6 months prior to inclusion will be excluded.

[00420] Patients included in a phase II proof of concept study are identified and selected for treatment in collaboration with dermatology clinicians at multiple sites within the United States. A coordinating investigator that is a member of the Scleroderma Clinical Trials Consortium oversees patient recruitment and serves as the study chair. Patients are diagnosed with localized diffuse cutaneous scleroderma or systemic scleroderma with diffuse cutaneous involvement. Patients are stratified based on scleroderma diagnosis (i.e., localized or systemic), sex and age to ensure equal distribution within treatment populations. Additionally, patients are stratified based on the severity of cutaneous involvement to ensure an equal number of patients with severe cutaneous involvement (m-RSS ≥ 20/51) within treatment populations. This stratification requires an initial diagnostic test involving cutaneous induration scale and skin biopsy. Patients are required to undergo a cardiac ultrasound diagnostic test prior to inclusion to ensure normal heart function (ejection fraction score >55%).

[00421] As there is currently no treatment indicated directly to inhibit fibrosis in scleroderma patients, there is no comparator agent to be used in studies of patients. The new agent is dosed alone. Patients taking any medication are monitored to ensure that the new agent does not alter the pharmacokinetic and metabolism properties of the medication being taken.

[00422] The modified Rodnan skin score (m-RSS) is an identified, measurable and validated biomarker used to evaluate clinical efficacy in scleroderma patients. Briefly, total skin surface is

arbitrarily divided into 17 sites. In each area, manual palpitation is used to asses a skin score. The skin score varies from 0-3 based on degree of skin thickening (0, uninvolved; 1, mild; 2, moderate; 3, severe). The total skin score is the of sum scores from each of the 17 areas (maximum score of 51). Patients having a score of between 16 and 19 are classified as diffuse and those having a score of ≥20 are classified as severe. This skin scoring system has been demonstrated to correlate very well with the extent of dermal fibrosis and also correlates well with the extent of fibrosis/dysfunction in internal organs (in systemic scleroderma patients). Target engagement in skin can be assessed by monitoring changes in expression of hedgehog and VEGFR target genes using skin biopsies.

EXAMPLE IV: Phase II Clinical Study for Idiopathic Pulmonary Fibrosis

[00423] The purpose of this study is to determine the safety and efficacy of compound of formula (I) and formula (II) for the treatment of idiopathic pulmonary fibrosis, compared with placebo. The clinical trial is interventional. The allocation of the clinical study participants is randomized; the intervention model is parallel assignment; and there is a double blind masking of the study (subject, caregiver, investigator). The present clinical study primarily measures the rate of change of forced vital capacity, and secondarily measures the safety based on AEs, vital signs and clinical laboratory tests.

[00424] The inclusion criteria for patient recruitment are as follows:

- Both genders between the ages of 40 and 80 years, inclusive, at randomization.
- Have clinical symptoms consistent with Idiopathic Pulmonary Fibrosis (IPF).
- Have first received a diagnosis of IPF at least 6 months and no more than 48 months before randomization. The date of diagnosis is defined as the date of the first available HRCT or surgical lung biopsy consistent with IPF/UIP.
- Have a diagnosis of usual interstitial pulmonary fibrosis (UIP) or IPF by high resolution computed tomography (HRCT) or surgical lung biopsy (SLB)
- Extent of fibrotic changes (honeycombing, reticular changes) greater than the extent of emphysema on HRCT scan.
- Have no features supporting an alternative diagnosis on transbronchial biopsy,BAL, or SLB, if performed.
- Have percent predicted post-bronchodilator FVC between 50% and 80%, inclusive, at screening.

- Have a change in post-bronchodilator FVC (measured in liters) between screening and day 1 that is less than a 10% relative difference, calculated as: the absolute value of 100% (screening FVC (L) - day 1 FVC (L)) / screening FVC(L).
- Have carbon monoxide diffusing capacity (DLCO) between 30% and 80% (adjusted for hemoglobin and altitude, inclusive, at screening.
- Have no evidence of improvement in measures of IPF disease severity over the preceding year, in the investigator's opinion.
- Be able to walk 150 meters or more during the 6 minute walk test (6MWT) at screening.
- Demonstrate a decrease in oxygen saturation of 2 percentage points or greater during the 6MWT at screening (may be performed with supplemental oxygen titrating to keep oxygen saturation levels >88%).
- Are able to understand and sign a written informed consent form.
- Are able to understand the importance of adherence to study treatment and the study protocol and are willing to comply with all study requirements, including the concomitant medication restrictions, throughout the study.
- Women of childbearing potential (WOCBP) and men who are sexually active with WOCBP must use acceptable method(s) of contraception.

[00425] The exclusion criteria for patient recruitment are as follows:

i) Target Disease Exclusions

(1) Has significant clinical worsening of IPF between screening and day 1 (during the screening process), in the opinion of the investigator.

(2) Has forced expiratory volume in 1 second (FEV1)/FVC ratio less than 0.8 after administration of bronchodilator at screening.

(3) Has bronchodilator response, defined by an absolute increase of 12% or greater and an increase of 200 mL in FEV1 or FVC or both after bronchodilator use compared with the values before bronchodilator use at screening.

ii) Medical History and Concurrent Diseases

(1) Has a history of clinically significant environmental exposure known to cause pulmonary fibrosis.

(2) Has a known explanation for interstitial lung disease.

(3) Has a clinical diagnosis of any connective tissue disease.

(4) Currently has clinically significant asthma or chronic obstructive pulmonary disease.

(5) Has clinical evidence of active infection.

(6) Has any history of malignancy likely to result in significant disability or likely to require significant medical or surgical intervention within the next 2 years. This does not include minor surgical procedures for localized cancer (e.g., basal cell carcinoma).

(7) Has any condition other than IPF that, in the opinion of the investigator, is likely to result in the death of the subject within the next 2 years.

(8) Has a history of end-stage liver disease.

(9) Has a history of end-stage renal disease requiring dialysis.

(10) Has a history of unstable or deteriorating cardiac or pulmonary disease (other than IPF) within the previous 6 months.

(11) Has a history of alcohol or substance abuse in the past 2 years.

(12) Has a family or personal history of long QT syndrome and/or Torsades de Pointes (polymorphic ventricular tachycardia).

(13) Has used any of the following specific therapies within 7 days before screening:

(a) Investigational therapy, defined as any drug that has not been approved for marketing for any indication in the country of the participating site.

(b) Any cytotoxic, immunosuppressive, cytokine-modulating, or receptor-antagonist agent, including, but not limited to, azathioprine, bosetan, ambrisentan, cyclophosphamide, cyclosporine, etanercept, iloprost, infliximab, leukotriene antagonists, methotrexate, mycophenolate mofetil, tacrolimus, montelukast, tetrathiomolybdate, tumor necrosis factor alpha inhibitors, NAC, imatinib mesylate, interferon gamma-1b, pirfenidone, and tyrosine kinase inhibitors.

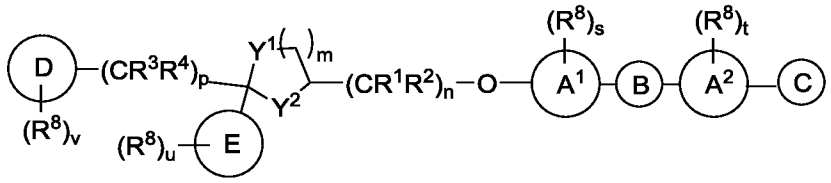
(c) Colchicine, heparin, and warfarin. Sildenafil (daily use) may be used if given for a non-IPF indication if there is no clinically acceptable alternate therapy for the same indication; intermittent use for erectile dysfunction is allowed.

(d) Intermittent use of corticosteroids is allowed for acute respiratory worsening.

(e) Ketoconazole, cyclosporine and steroids for topical and ophthalmic use is permitted.

CLAIMS

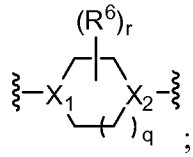
1. A method to treat fibrosis, a disorder characterized by fibrosis, or a disease characterized by fibrosis, the method comprising administering a composition comprising a therapeutically effective amount of a compound of Formula (I), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (I)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is  ;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

each R^8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl,

and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

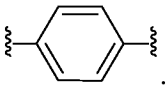
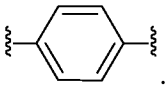
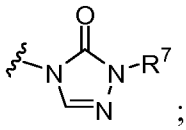
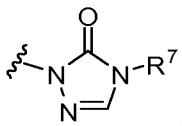
r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

u is 0, 1, 2, 3, 4 or 5; and

v is 0, 1, 2, 3, or 4.

2. The method of claim 1, wherein X_1 and X_2 are N.
3. The method of claim 1, wherein X_1 is CR^5 and X_2 is N.
4. The method of claim 1, wherein X_1 is N and X_2 is CR^5.
5. The method of any one of claims 2-4, wherein q is 1 and r is 0.
6. The method of any of one of claims 1-5, wherein A^1 is aryl.
7. The method of claim 6 wherein A^1 is  .
8. The method of any of one of claims 1-5, wherein A^1 is heteroaryl.
9. The method of any of one of claims 1-8, wherein A^2 is aryl.
10. The method of claim 9, wherein A^2 is  .
11. The method of any of one of claims 1-8, wherein A^2 is heteroaryl.
12. The method of claim 11, wherein A^2 is pyridine, pyrazine, pyrimidine, pyridazine, or triazine.
13. The method of any one of claims 1-12, wherein C is  ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).
14. The method of any one of claims 1-12, wherein C is  ; and R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl).

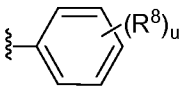
15. The method of any one of claims 1-14, wherein E is alkyl.

16. The method of any one of claims 1-14, wherein E is cycloalkyl.

17. The method of claim 16, wherein E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

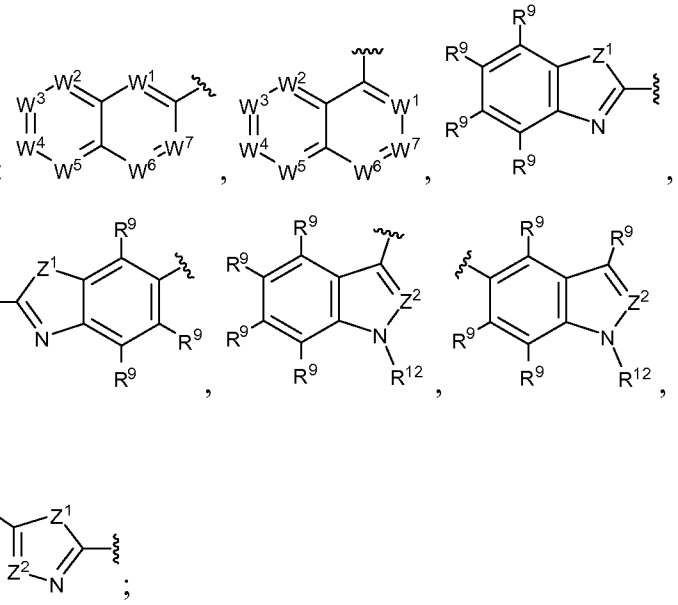
18. The method of any one of claims 1-14, wherein E is heterocyclyl.

19. The method of any one of claims 1-14, wherein E is aryl.

20. The method of claim 19, wherein E is  and u is 0, 1, 2, 3, 4, or 5.

21. The method of any one of claims 1-14, wherein E is heteroaryl.

22. The method of claim 21, wherein

E is selected from: 

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$,

$-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

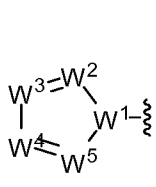
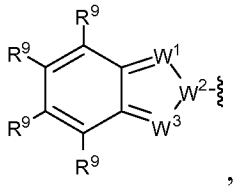
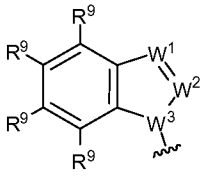
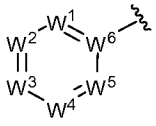
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

23. The method of any one of claims 1-22, wherein D is aryl.

24. The method of any one of claims 1-22, wherein D is heteroaryl.

25. The method of claim 24, wherein

D is selected from  ,  ,  , and  ;

W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

26. The method of any one of claims 1-25 wherein Y^1 and Y^2 are O.

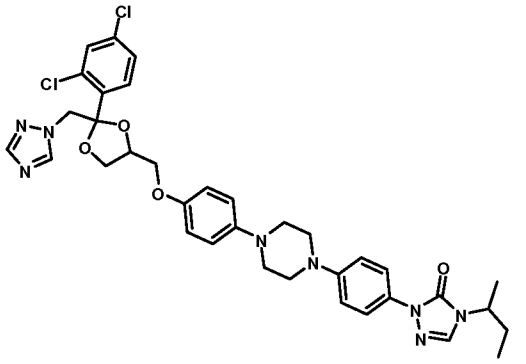
27. The method of claim 26 wherein m is 1.

28. The method of any one of claims 1-27 wherein p is 1, 2, or 3.

29. The method of claim 28 wherein p is 1.

30. The method of any one of claims 1-29 wherein R^1, R^2, R^3, and R^4 are hydrogen.

31. The method of claim 1 wherein the compound is selected from: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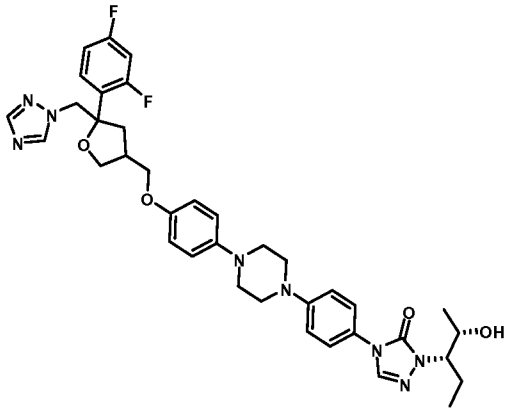
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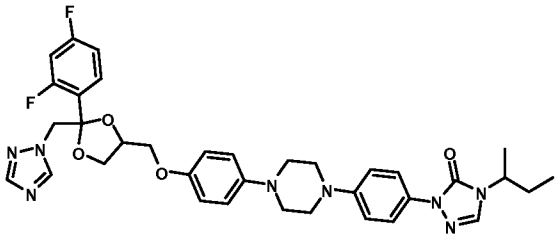
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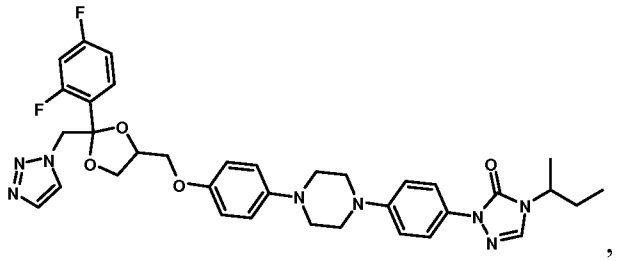
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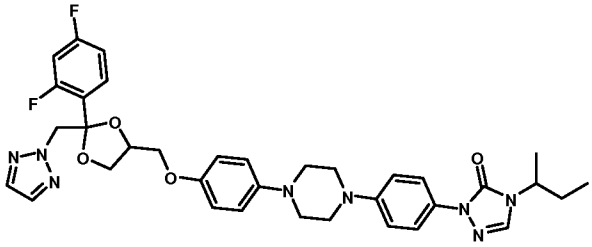
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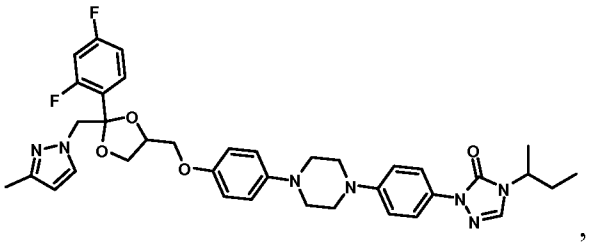
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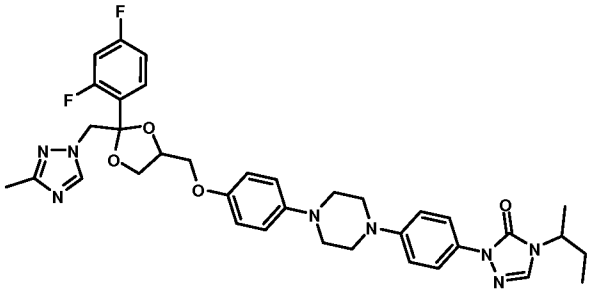
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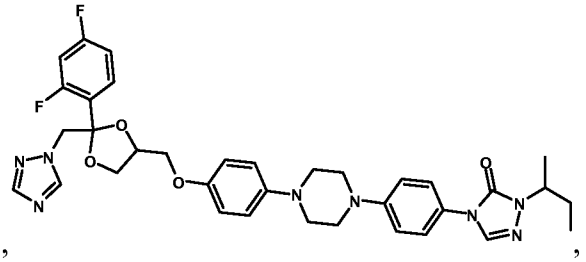
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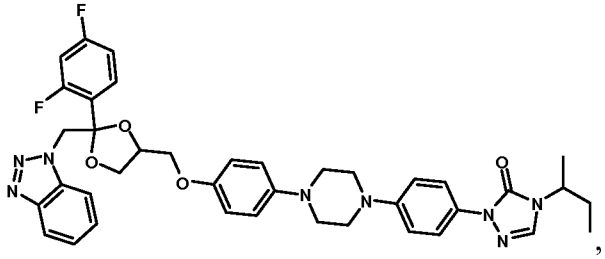
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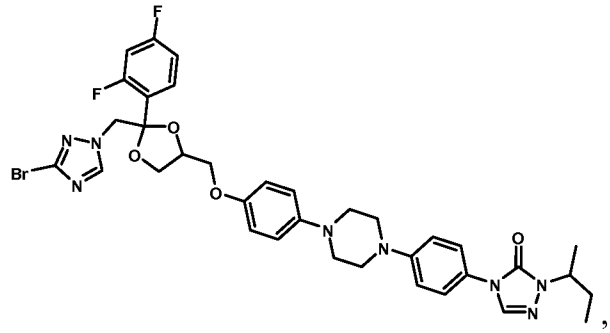
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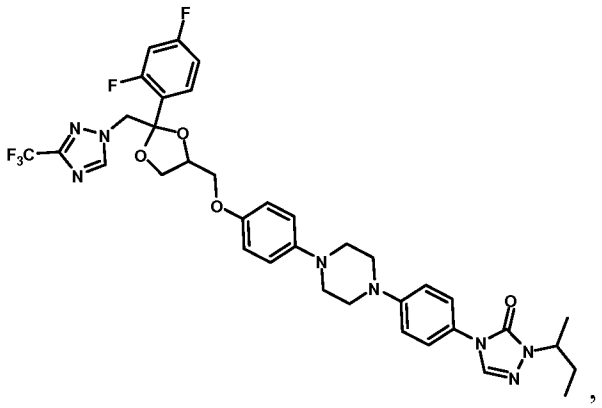

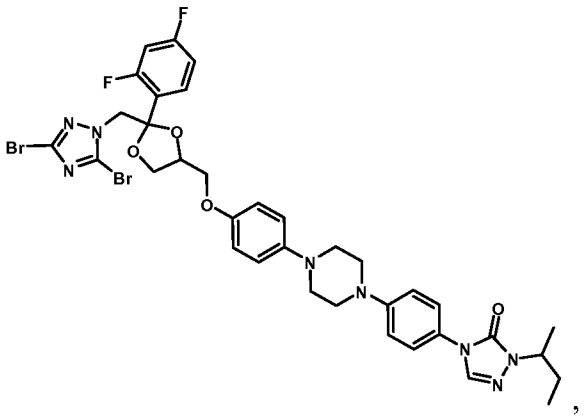

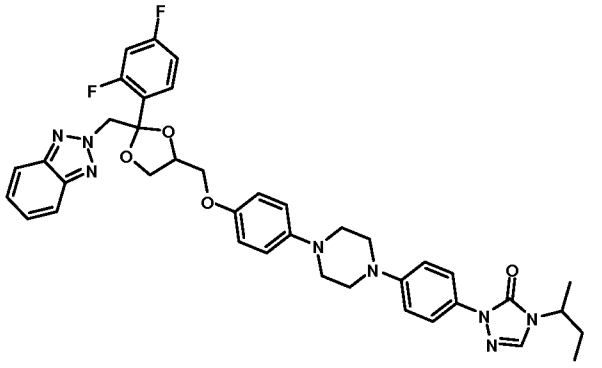

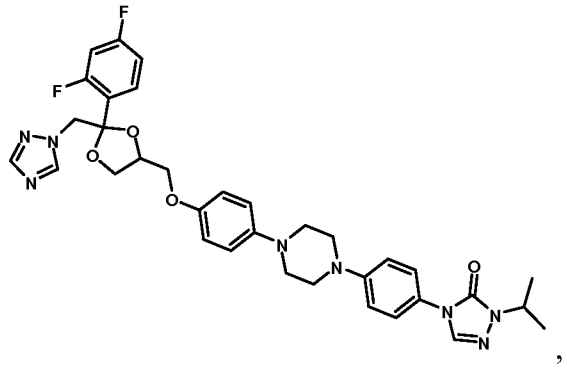

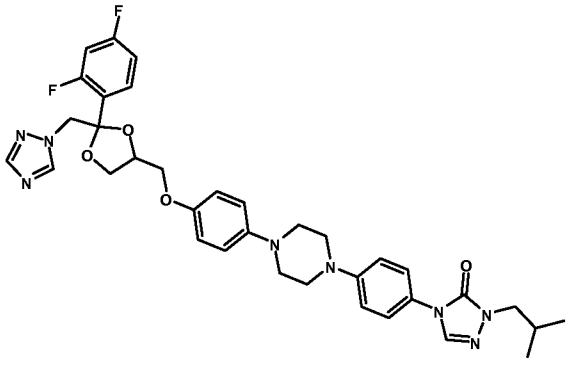

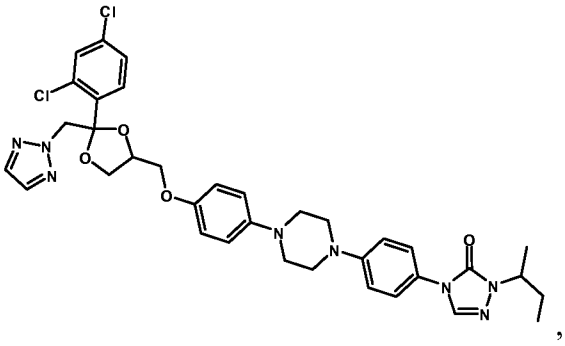

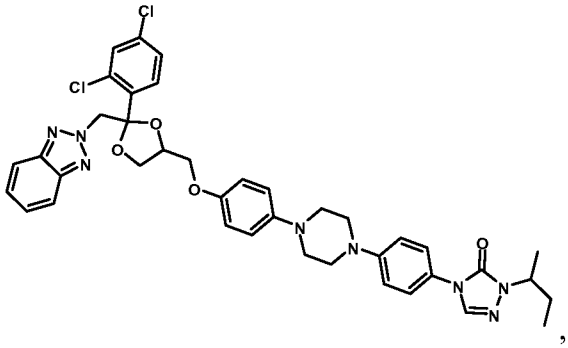

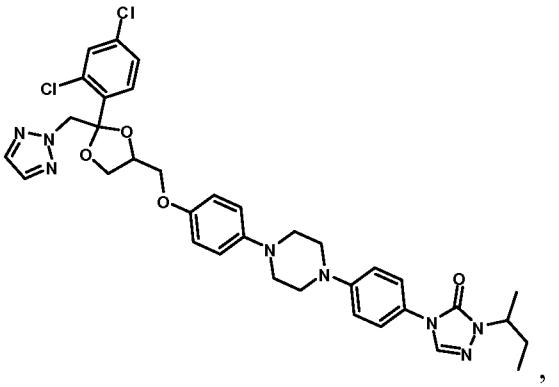

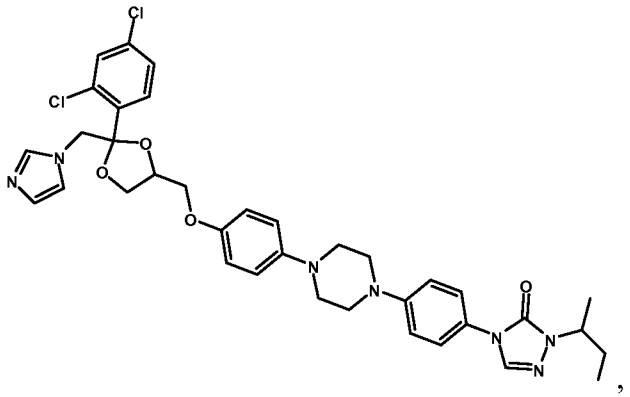

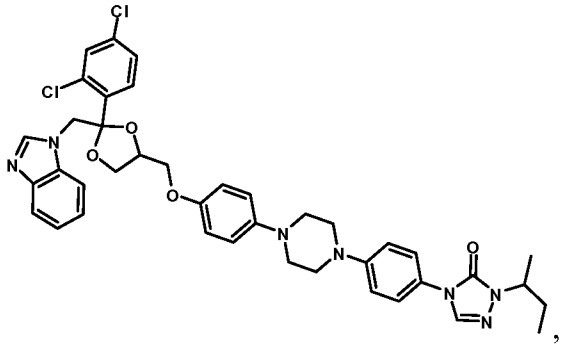

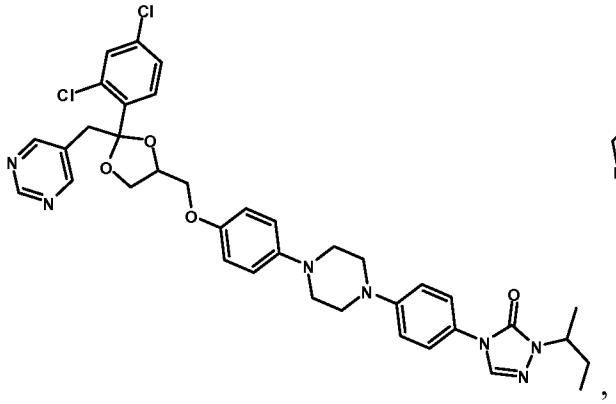

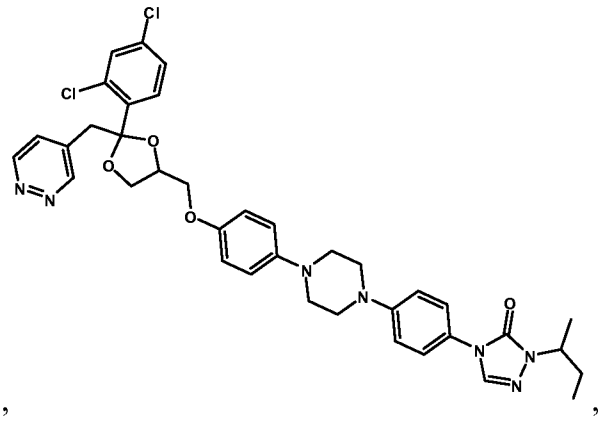

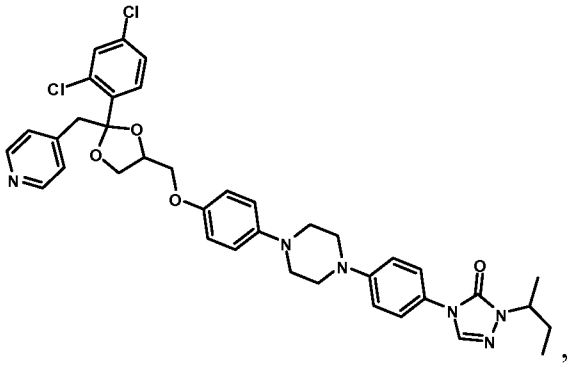

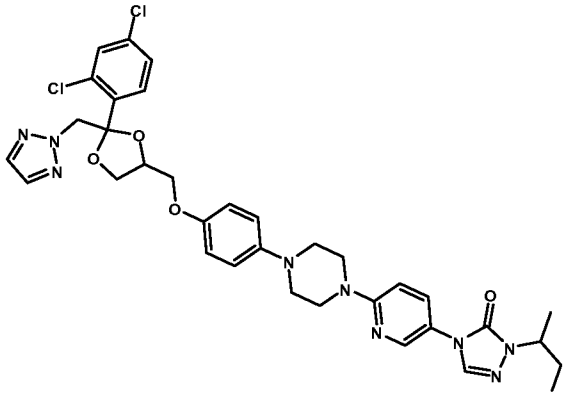

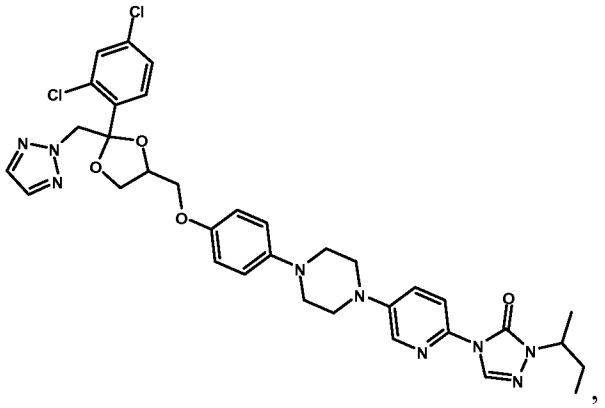

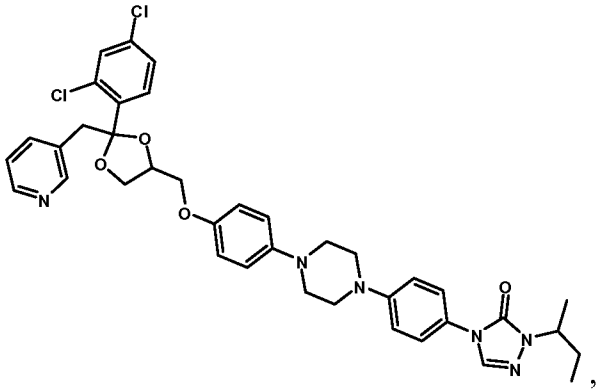

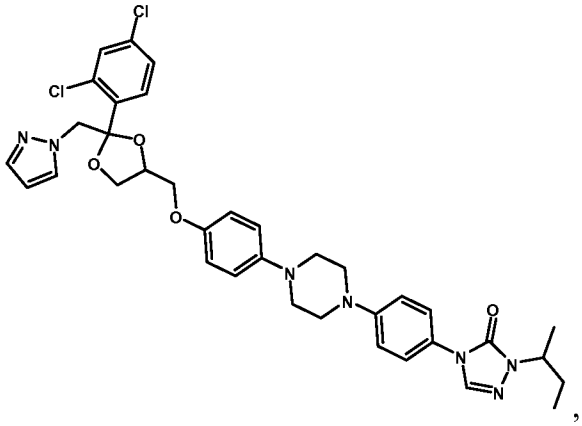
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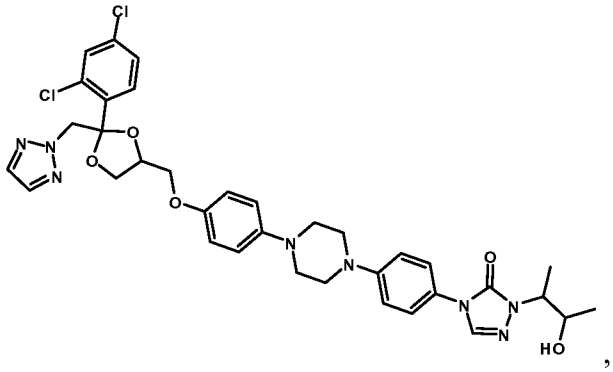
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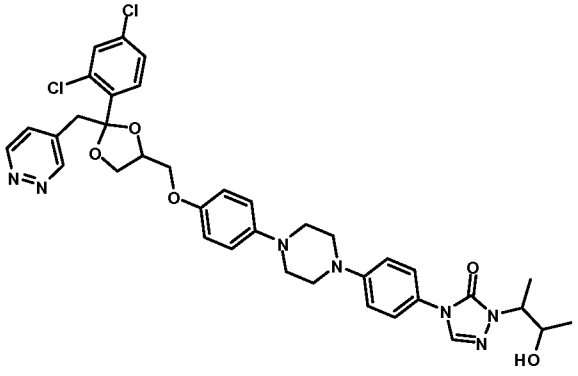
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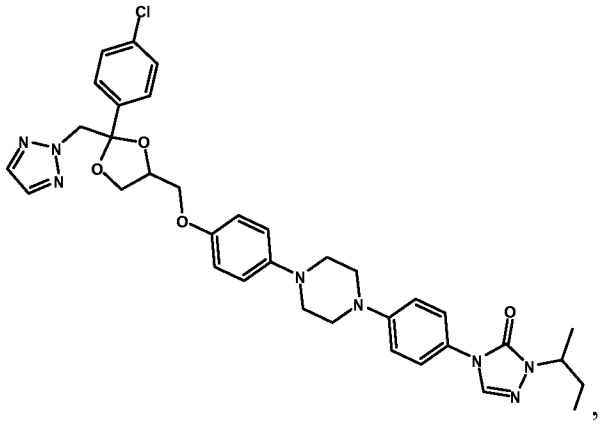
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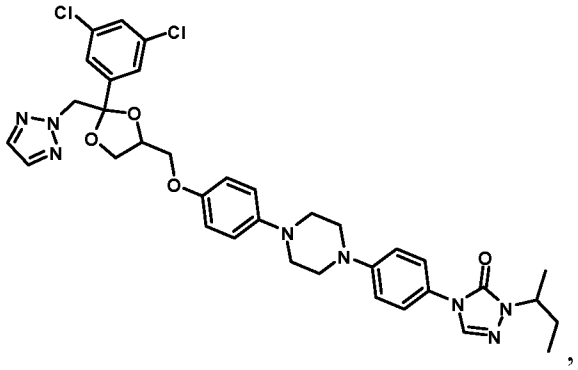
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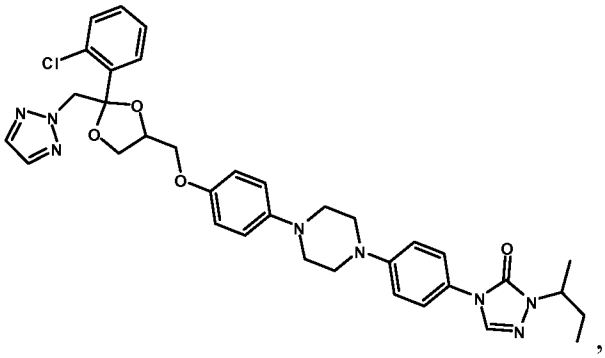
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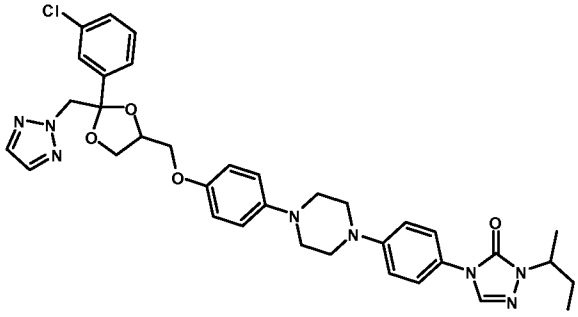
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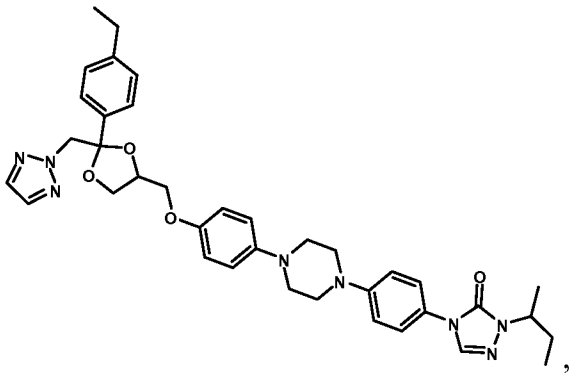
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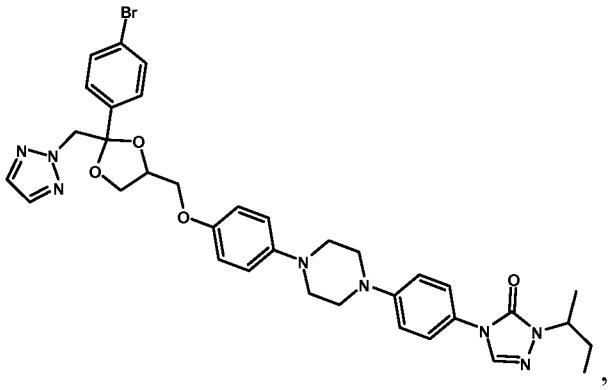

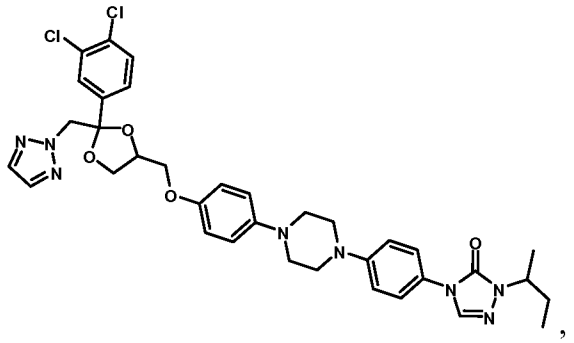

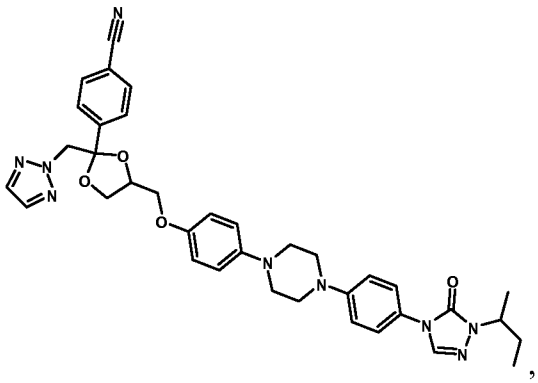

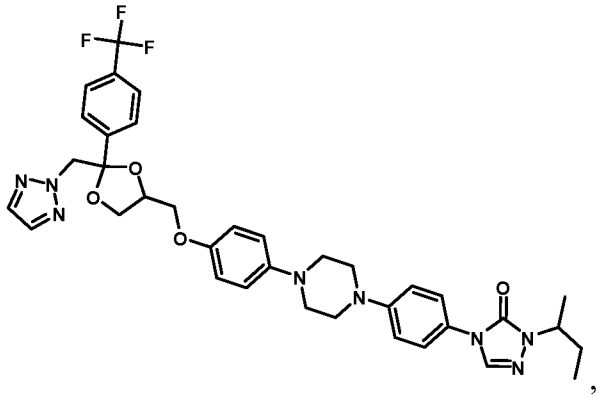

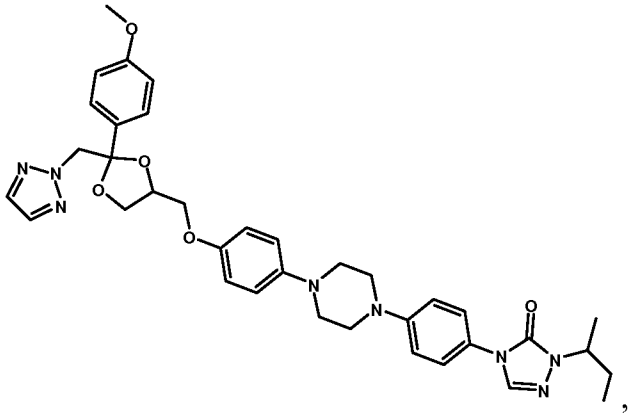

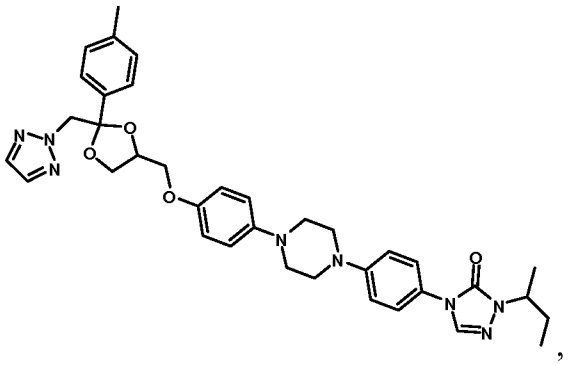

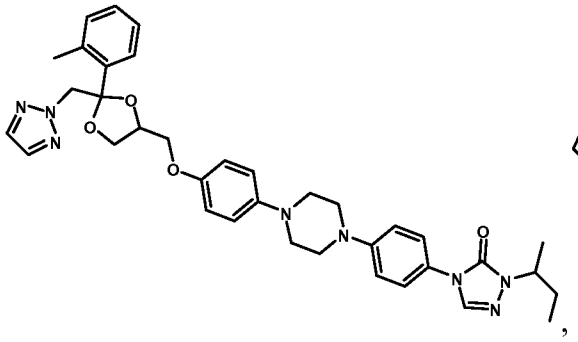

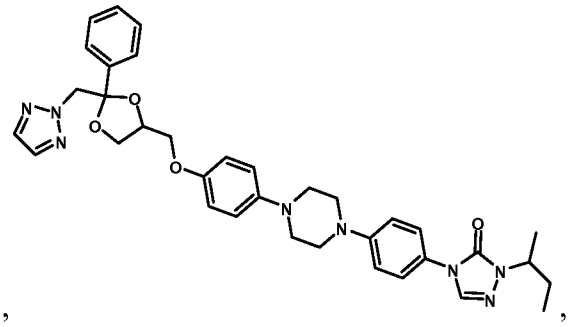

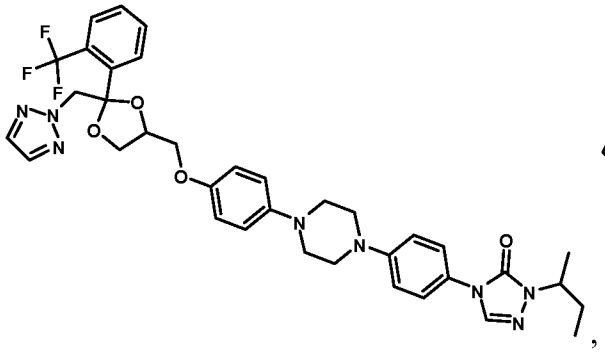

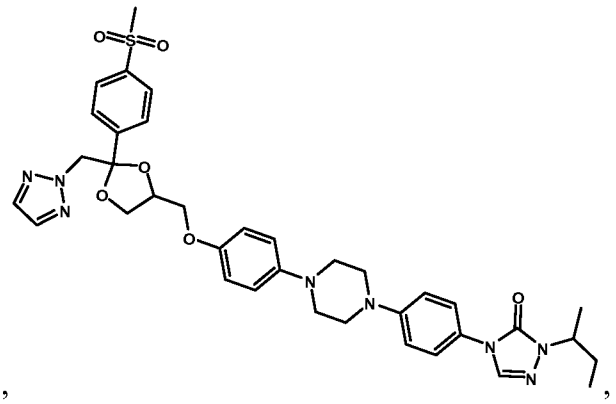

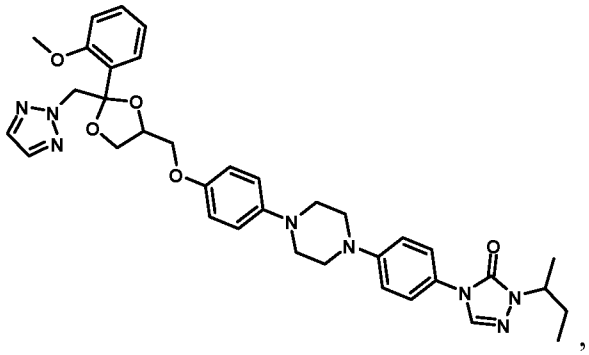

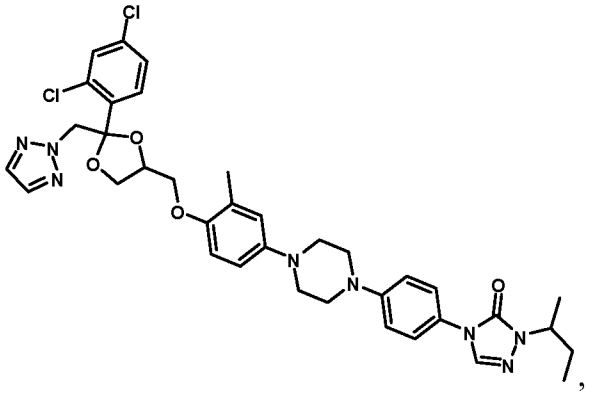

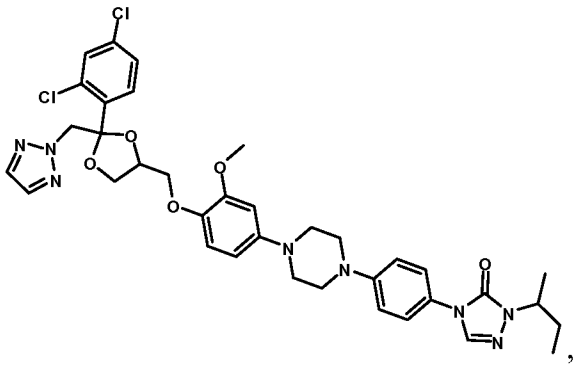

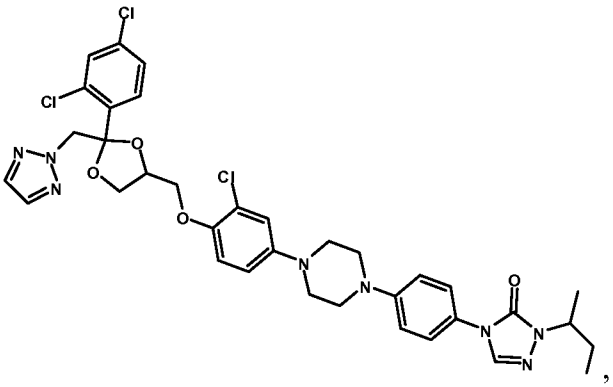

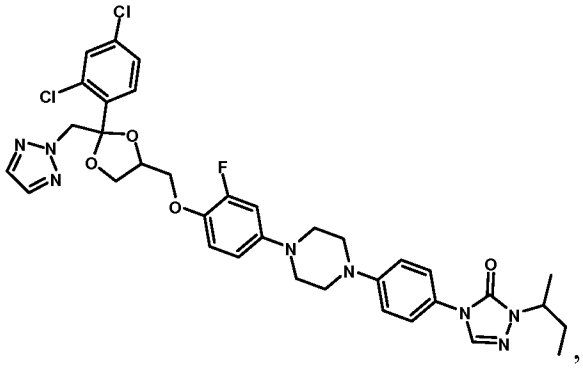

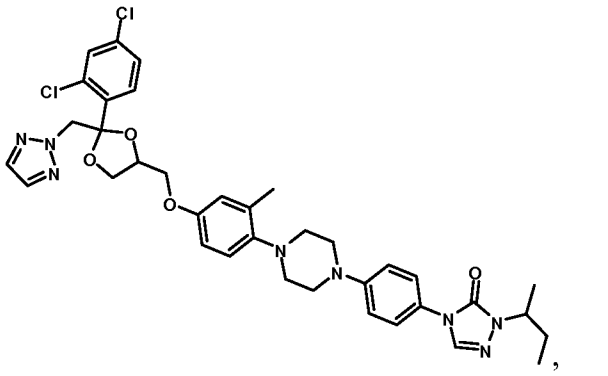

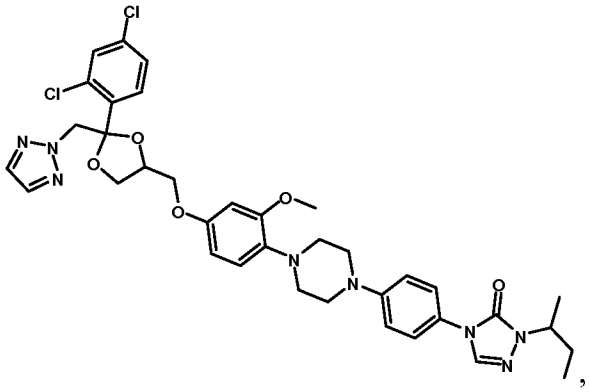

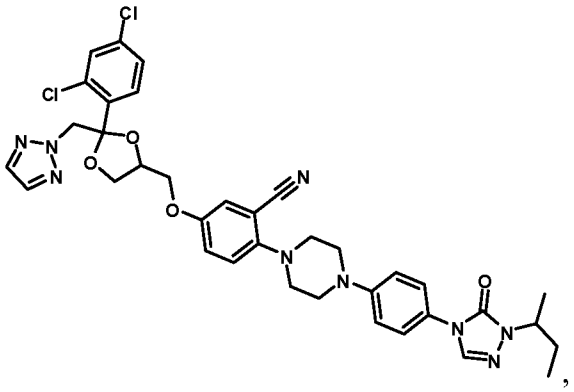

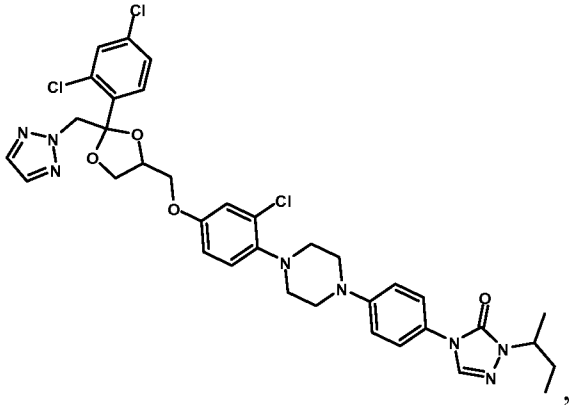

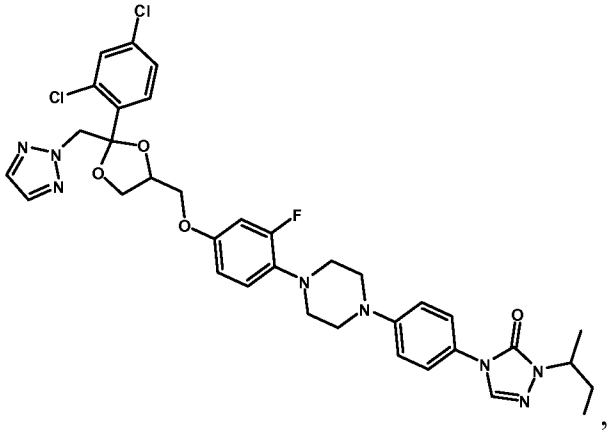

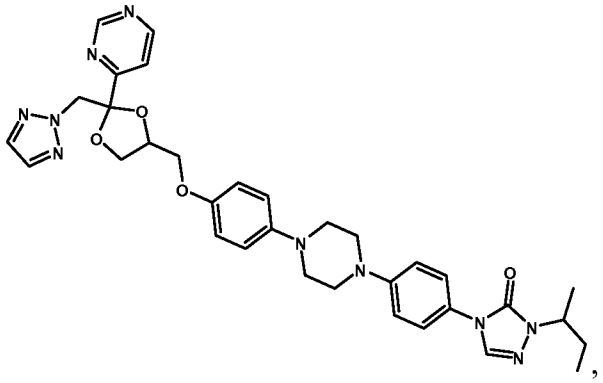

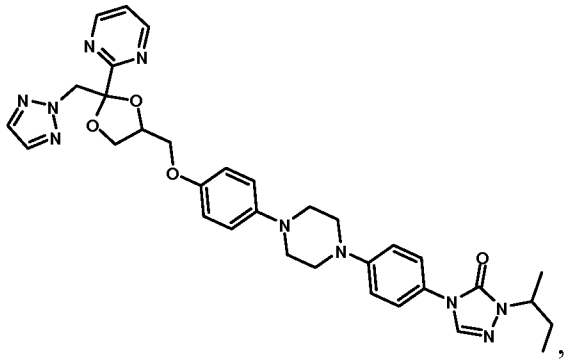

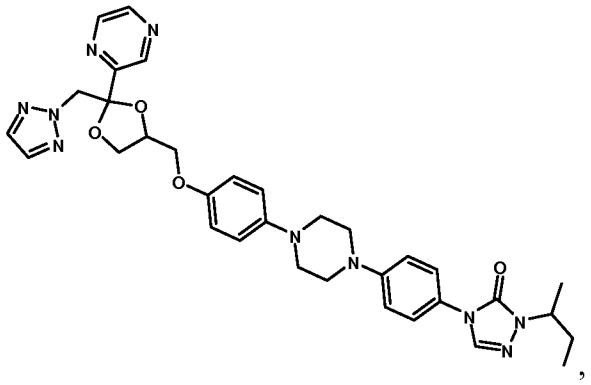

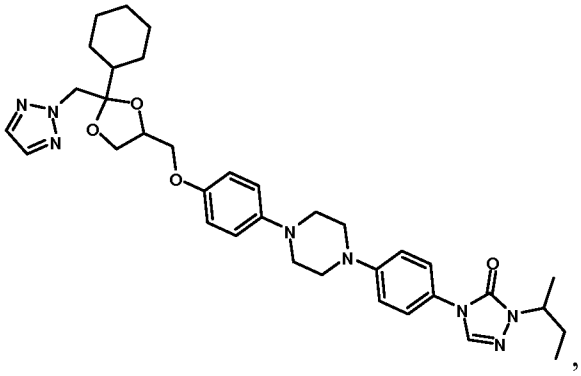

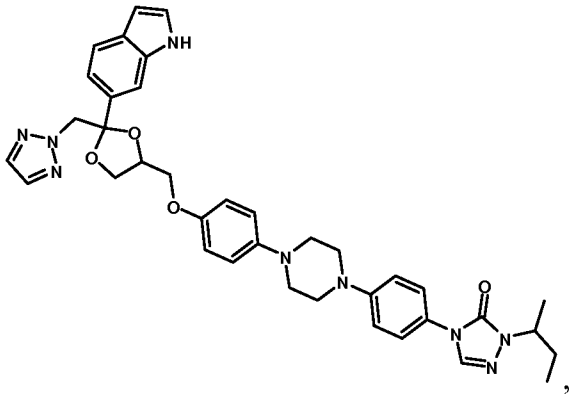

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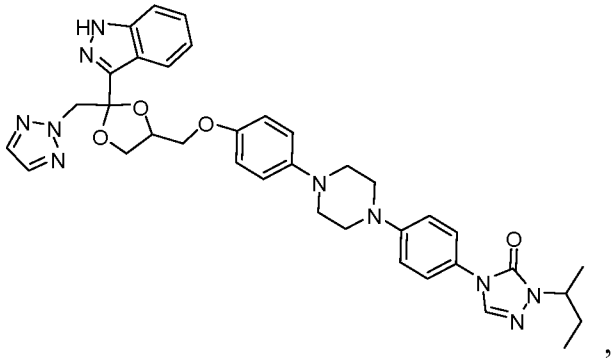

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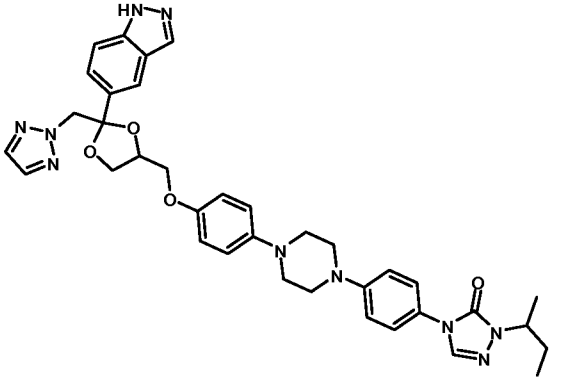

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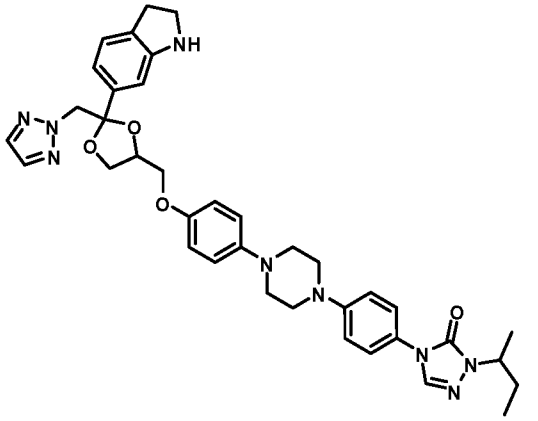

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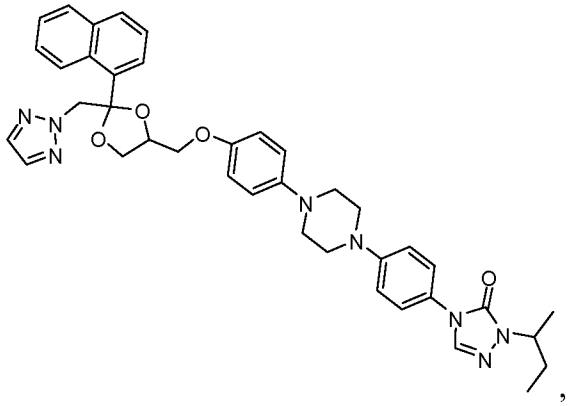

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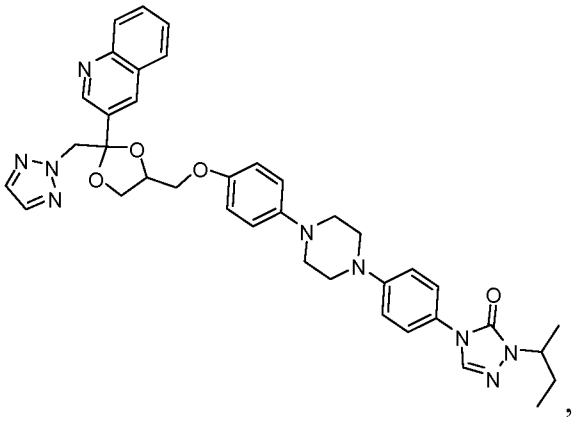

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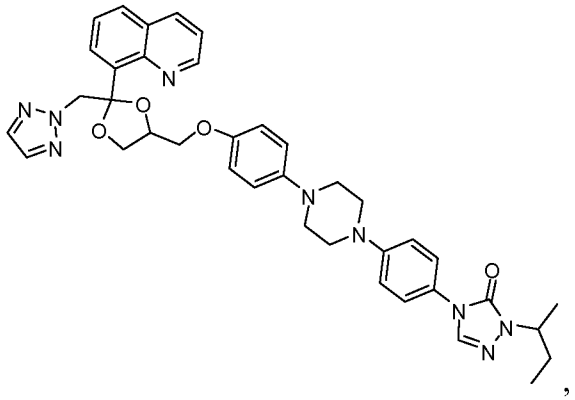

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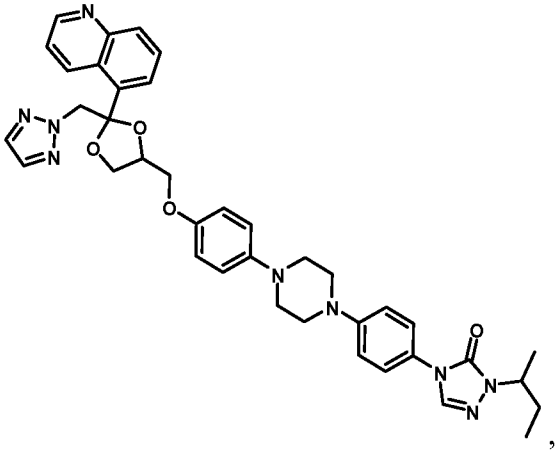

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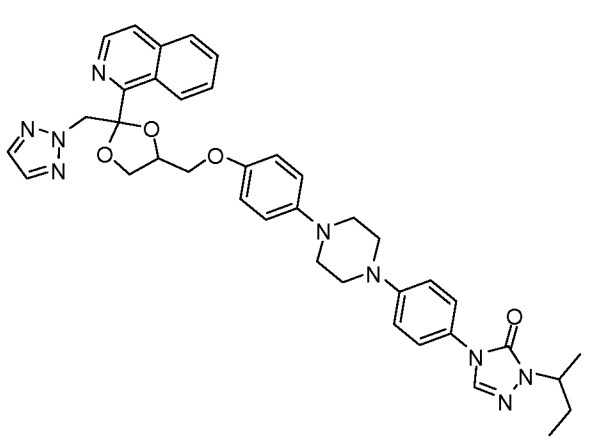

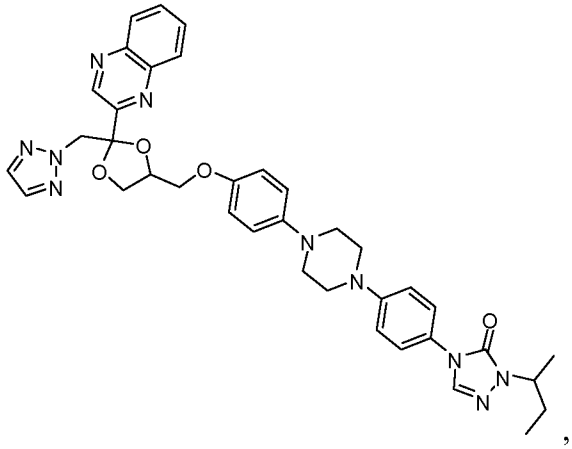

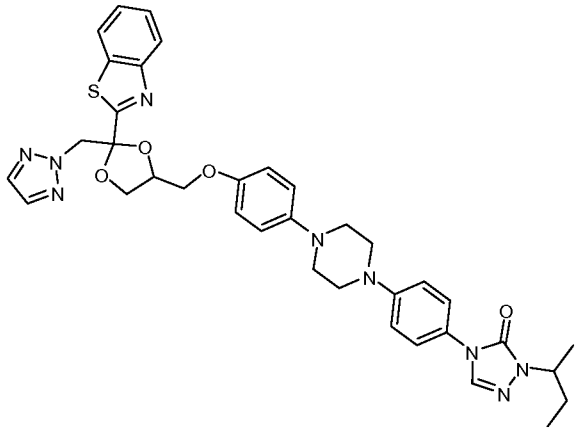

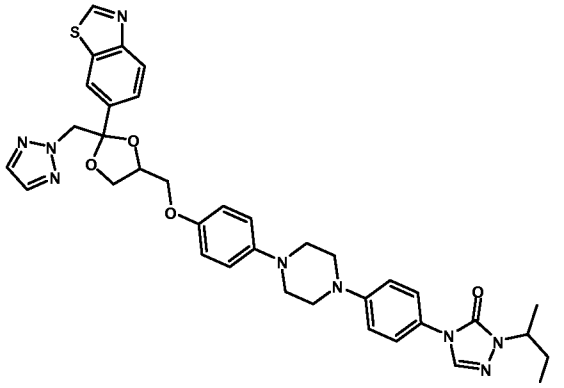

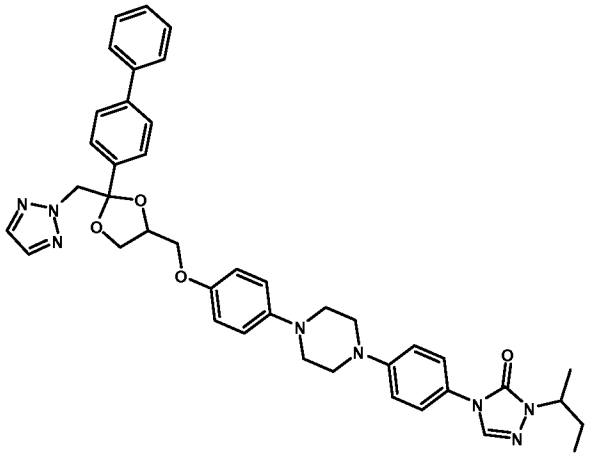

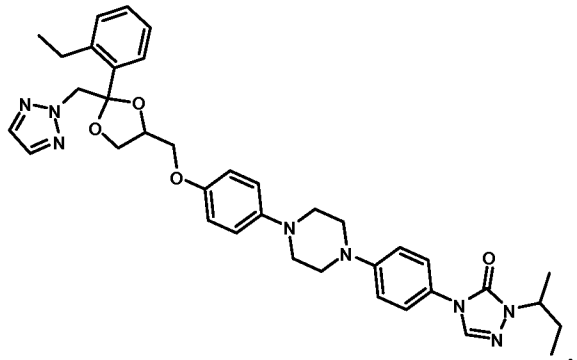

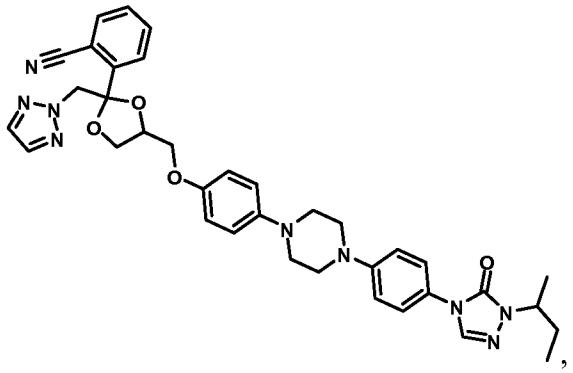

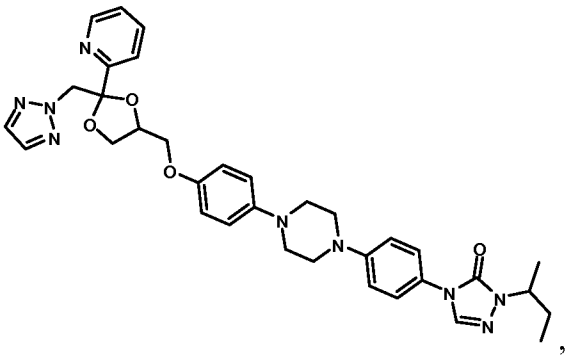

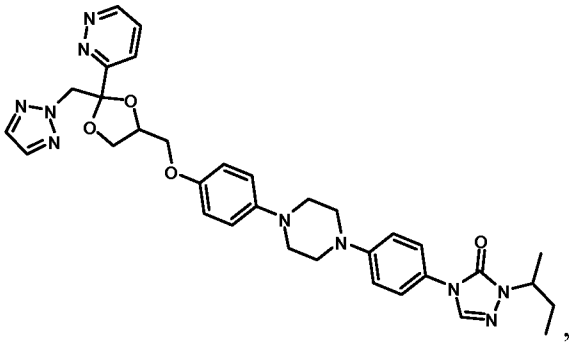

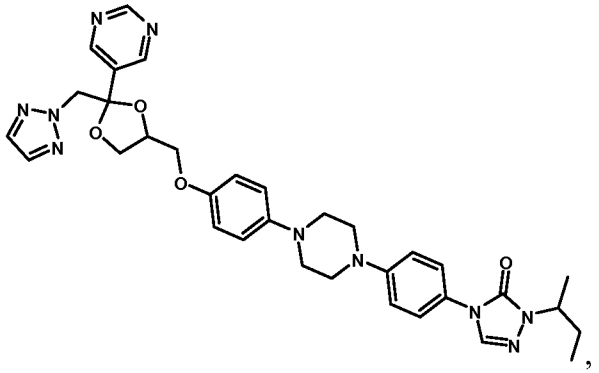

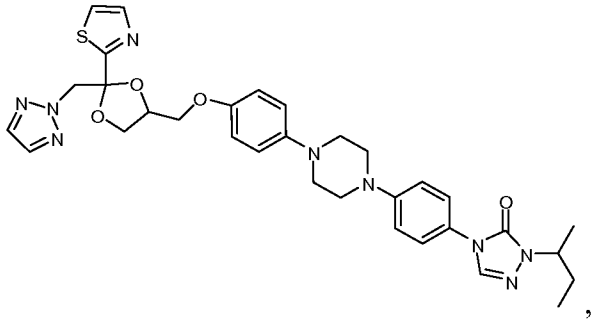

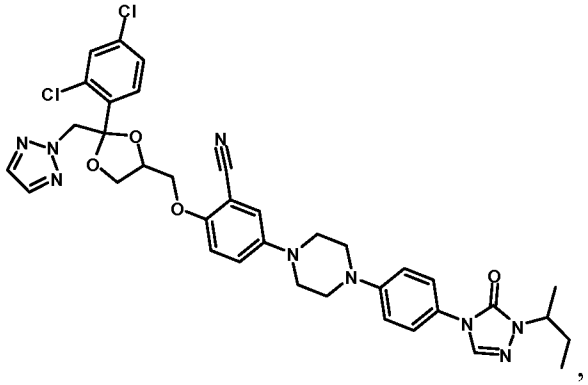

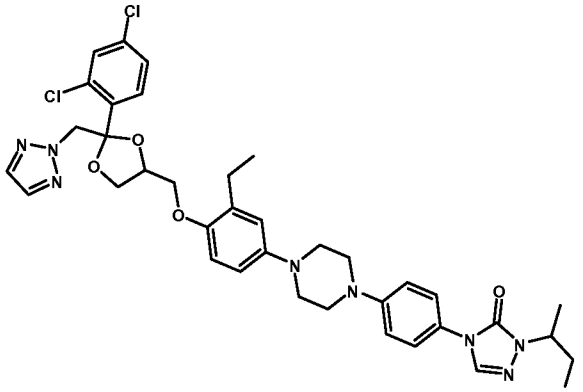

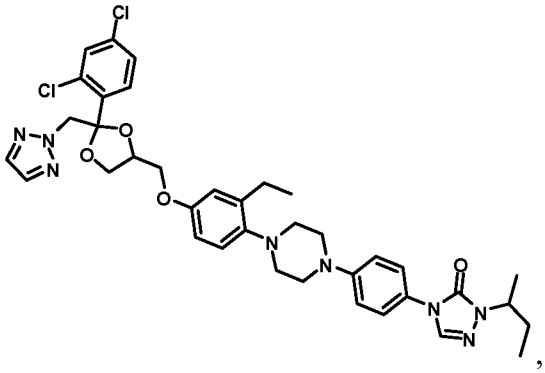

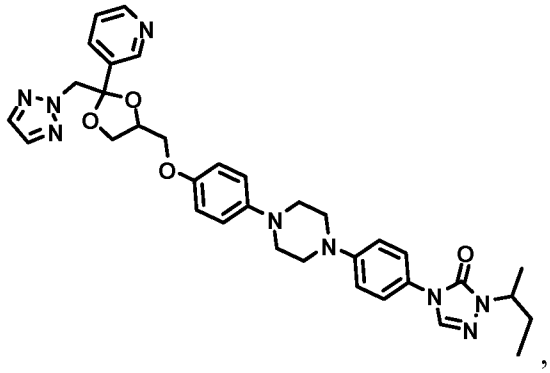

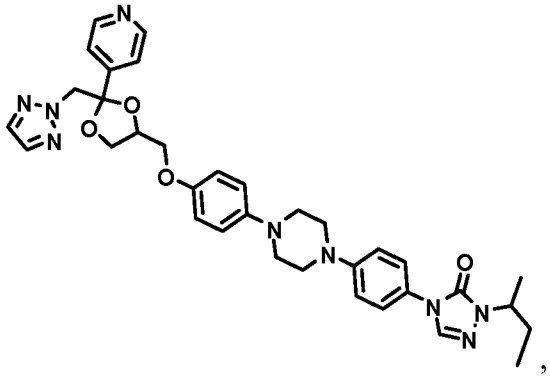

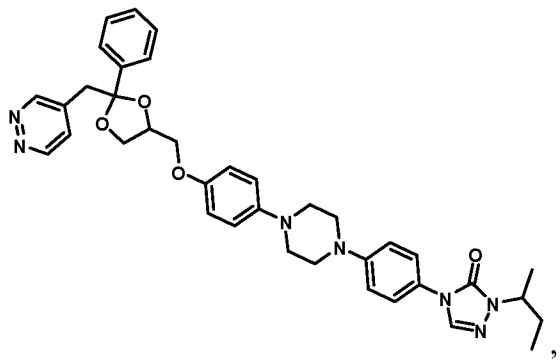
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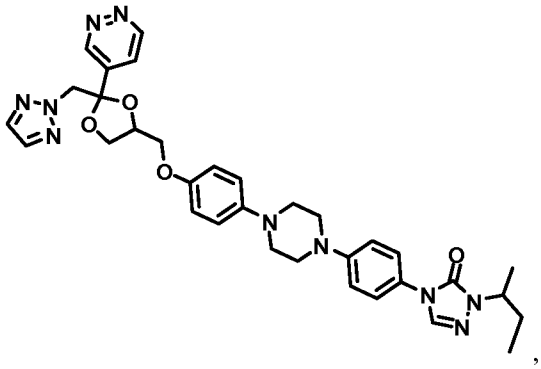
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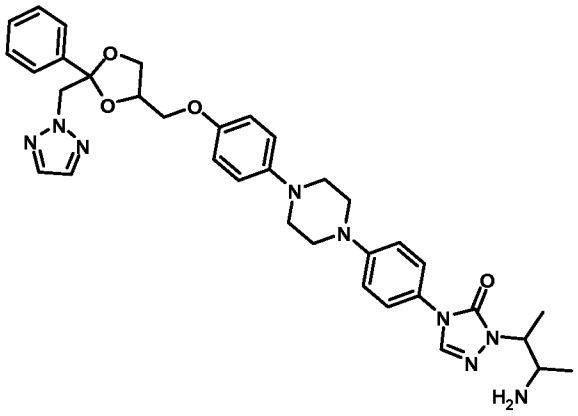
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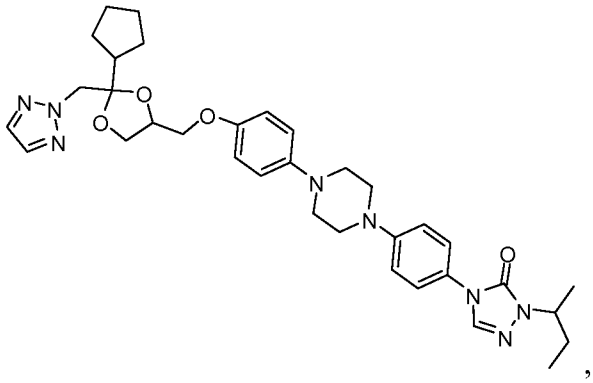
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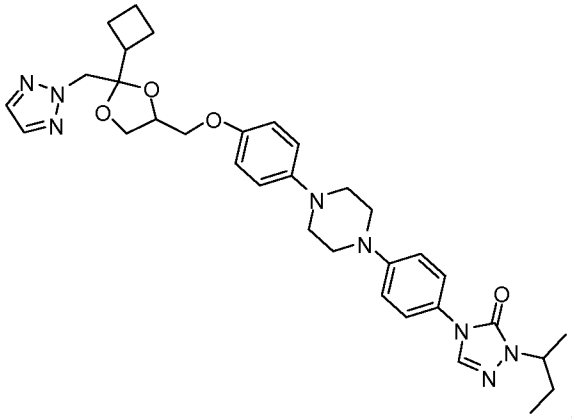
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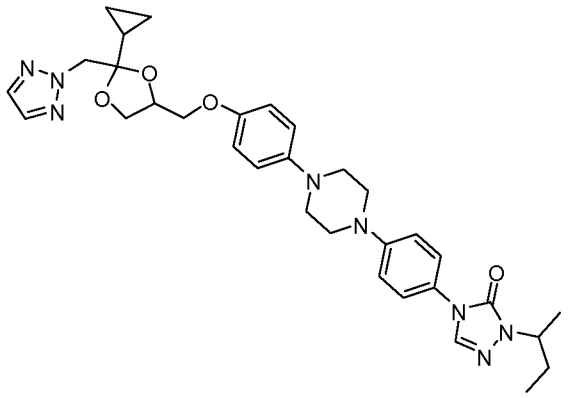
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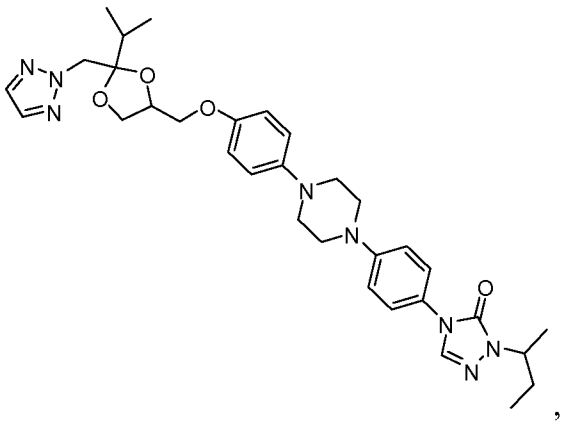
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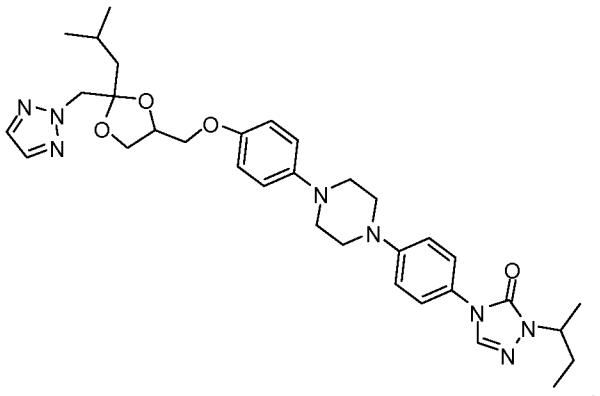
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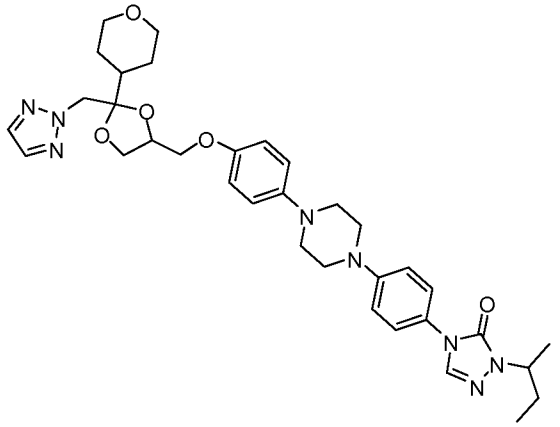
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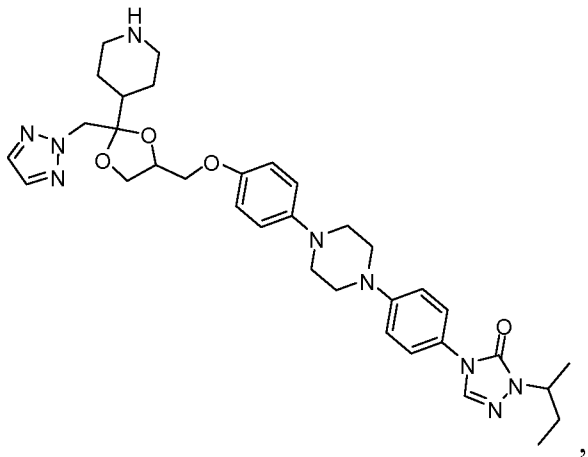
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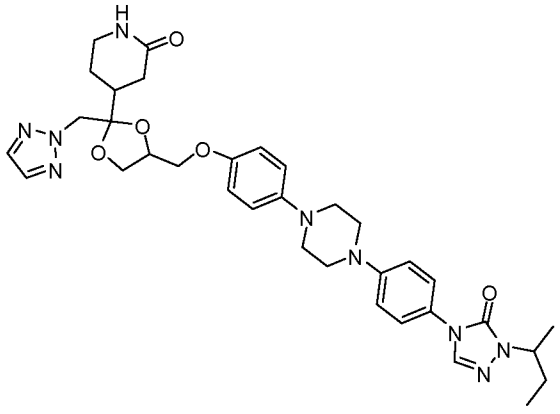
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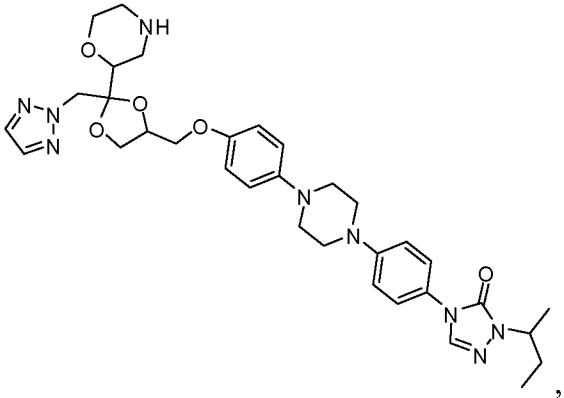
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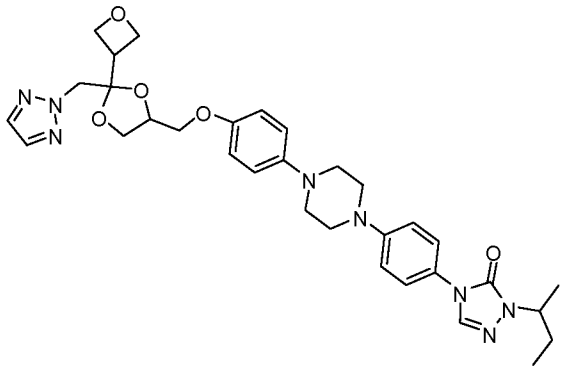
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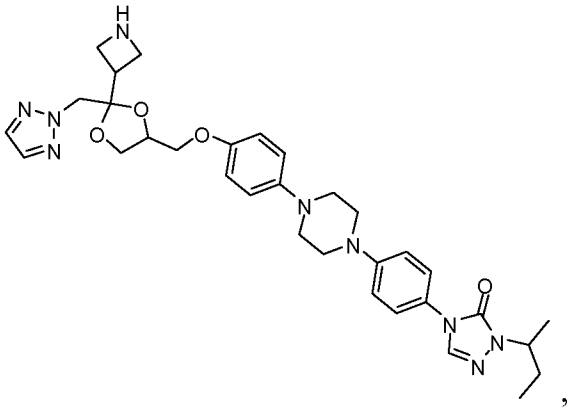
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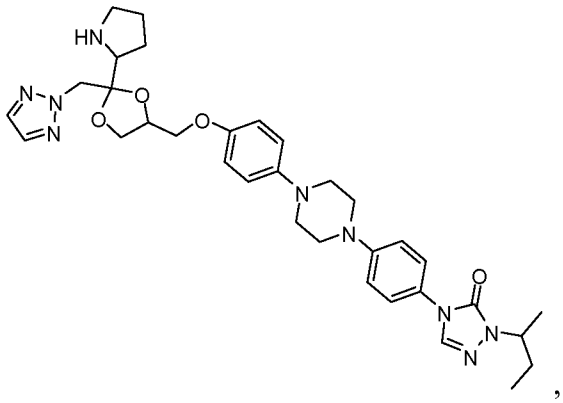
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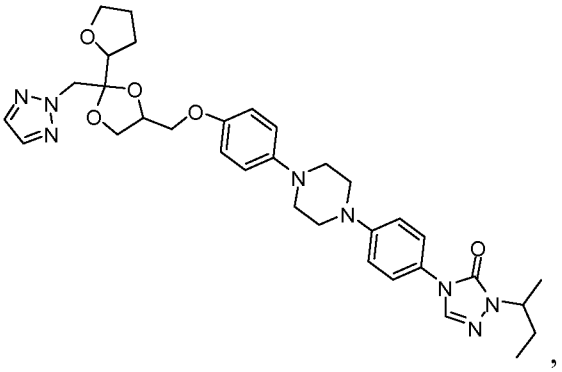
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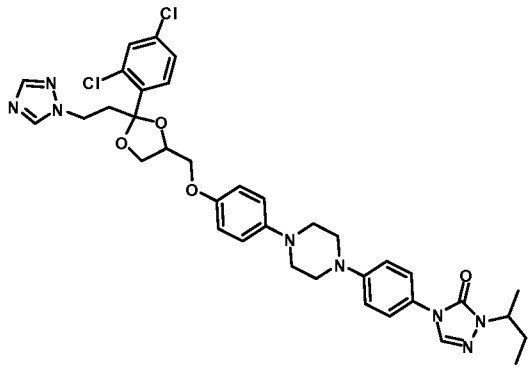
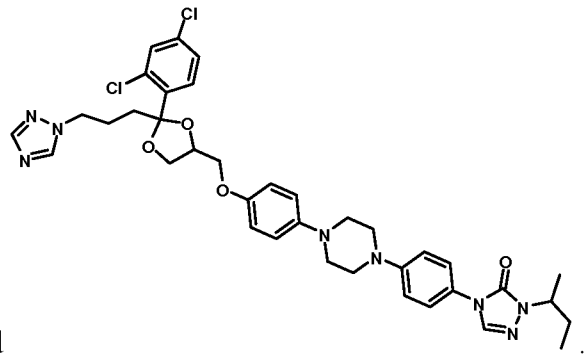
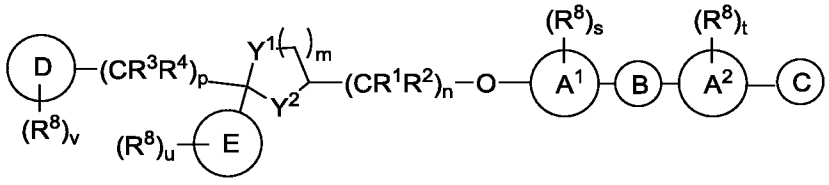




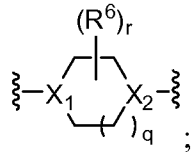
32. The method of any one of claims 1-31 wherein the fibrosis is liver fibrosis, idiopathic pulmonary fibrosis, kidney fibrosis, or cardiac fibrosis.
33. The method of claim 32 wherein the liver fibrosis is associated with the later stages of alcoholic or nonalcoholic liver cirrhosis.
34. The method of claim 32 wherein the fibrosis is idiopathic pulmonary fibrosis.
35. The method of any one of claims 1-31 wherein the disease or disorder characterized by fibrosis is a chronic autoimmune disease.
36. The method of claim 35 wherein the chronic autoimmune disease is rheumatoid arthritis, scleroderma, Crohn's disease or systemic lupus erythematosus.
37. The method of claim 36 wherein the chronic autoimmune disease is scleroderma.
38. The method of any one of claims 1-31 wherein the fibrosis is keloid formation resulting from abnormal wound healing.
39. The method of any one of claims 1-31 wherein the fibrosis occurs after organ transplantation.
40. A compound of Formula (II), a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof:



Formula (II)

wherein:

A^1 and A^2 are independently selected from aryl or heteroaryl;

B is  ;

C is optionally substituted 5- or 6-membered heterocyclyl or optionally substituted 5- or 6-membered heteroaryl, wherein the heterocyclyl or the heteroaryl contains 1 to 4 nitrogen atoms;

D is aryl or heteroaryl;

E is aryl, heteroaryl, carbocyclyl, hetercyclyl, or alkyl;

each R^1, R^2, R^3, and R^4 is independently selected from H, alkyl, haloalkyl, or alkoxy;

X_1 and X_2 are independently selected from N and CR^5;

R^5 is H, OH, alkyl, or alkoxy;

each R^6 is independently alkyl, haloalkyl, halo, alkoxy, -alkylene($NR^{13}R^{14}$), or aryl;

R^7 is alkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), cycloalkyl, heterocyclyl, -alkylene(cycloalkyl), or –alkylene(heterocyclyl);

each R8 is independently selected from alkyl, cycloalkyl, heterocyclyl, halo, hydroxy, nitrile, azido, nitro, alkoxy, haloalkoxy, haloalkyl, hydroxyalkyl, alkoxyalkyl, -alkylene($NR^{13}R^{14}$), -alkylene(cycloalkyl), –alkylene(heterocyclyl), aryl, heteroaryl, -SR^{13}, -SOR^{13}, -SO_2R^{13}, -$SO_2NR^{13}R^{14}$, -$NR^{13}R^{14}$, -$NR^{13}SO_2R^{14}$, -$NR^{13}C(O)R^{14}$, -$NR^{13}C(O)OR^{14}$, -$NR^{13}C(O)NR^{13}R^{14}$, -$C(O)R^{14}$, -$C(O)OR^{14}$, and -$C(O)NR^{13}R^{14}$; or two adjacent R^8 form a heterocyclyl ring;

each R^{13} and R^{14} is independently selected from H, alkyl, cycloalkyl, heterocyclylalkyl, haloalkyl, hydroxyalkyl, alkoxyalkyl, arylalkyl, heteroarylalkyl, aryl, and heteroaryl; or R^{13} and R^{14} taken together form a heterocycle with the atoms to which they are attached;

Y^1 and Y^2 are independently selected from O, CH_2, NH, and NR^{13};

n is 1, 2, or 3;

m is 1 or 2;

p is 1, 2, 3, or 4;

q is 1, 2, or 3;

r is 0, 1, 2, 3, 4, 5, 6, 7, or 8;

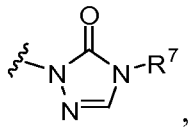
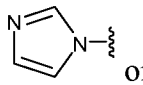
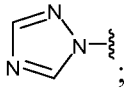
s is 0, 1, 2, 3, or 4;

t is 0, 1, 2, 3, or 4;

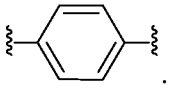
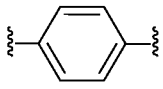
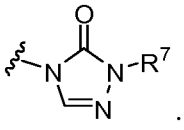
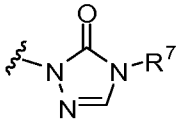
u is 0, 1, 2, 3, 4 or 5; and

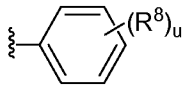
v is 0, 1, 2, 3, or 4;

provided that:

if X_1 and X_2 are N; r is 0; q is 1; A^1 and A^2 are phenyl; Y^1 and Y^2 are O; m and n are 1; R^1, R^2, R^3, and R^4 are hydrogen; and C is  , then D is not  or  ; and

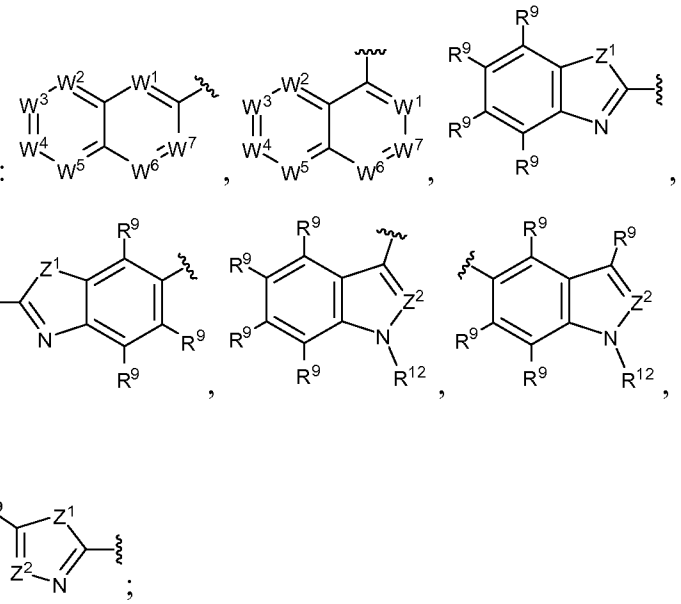
the compound is not 4-(4-(4-(4-((2-((1H-pyrazol-1-yl)methyl)-2-(2,4-difluorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-1H-1,2,4-triazol-5(4H)-one.

41. The compound of claim 40, wherein X_1 and X_2 are N.
42. The compound of claim 40, wherein X_1 is CR^5 and X_2 is N.
43. The compound of claim 40, wherein X_1 is N and X_2 is CR^5.
44. The compound of any one of claims 40-43, wherein q is 1 and r is 0.
45. The compound of any of one of claims 40-44, wherein A^1 is aryl.
46. The compound of claim 45 wherein A^1 is  .
47. The compound of any of one of claims 40-44, wherein A^1 is heteroaryl.
48. The compound of any of one of claims 40-47, wherein A^2 is aryl.
49. The compound of claim 48, wherein A^2 is  .
50. The compound of any of one of claims 40-47, wherein A^2 is heteroaryl.
51. The compound of claim 50, wherein A^2 is pyridine, pyrazine, pyrimidine, pyridazine, or triazine.
52. The compound of any one of claims 40-51, wherein C is  .
53. The compound of any one of claims 40-51, wherein C is  .
54. The compound of any one of claims 40-53, wherein E is alkyl.
55. The compound of any one of claims 40-53, wherein E is cycloalkyl.
56. The compound of claim 55, wherein E is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.
57. The compound of any one of claims 40-53, wherein E is heterocyclyl.
58. The compound of any one of claims 40-53, wherein E is aryl.

59. The compound of claim 58, wherein E is  and u is 0, 1, 2, 3, 4, or 5.

60. The compound of any one of claims 40-53, wherein E is heteroaryl.

61. The compound of claim 60, wherein

E is selected from: 

W^1, W^2, W^3, W^4, W^5, W^6, and W^7 are independently selected from N and CR^9;

Z^1 is NR^{12}, S, or O;

Z^2 is N or CR^9;

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl;

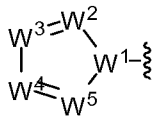
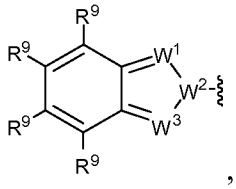
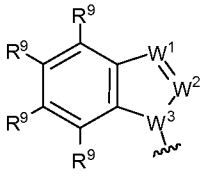
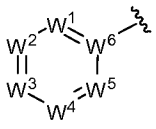
each R^{10} and R^{11} is independently selected from H and alkyl; or R^{10} and R^{11} taken together form a heterocycle with the nitrogen to which they are attached; and

R^{12} is H, alkyl or haloalkyl.

62. The compound of any one of claims 40-61, wherein D is aryl.

63. The compound of any one of claims 40-62, wherein D is heteroaryl.

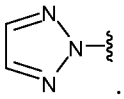
64. The compound of claim 63, wherein

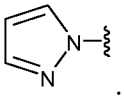
D is selected from  ,  ,  , and  ;

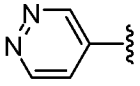
W^1, W^2, W^3, W^4, and W^5 are independently selected from N and CR^9;

W^6 is N or C; and

each R^9 is independently selected from H, halogen, CN, NO_2, alkyl, $-SR^{10}$, $-OR^{10}$, $-NR^{10}R^{11}$, $NR^{10}C(O)$(alkyl), - $NR^{10}C(O)$(cycloalkyl), $-NR^{10}C(O)$(heterocycloalkyl), $-NR^{10}C(O)$(aryl), $-NR^{10}C(O)$(heteroaryl), $-C(O)NR^{10}R^{11}$, $-C(O)NR^{10}$(cycloalkyl), $-C(O)NR^{10}$(heterocycloalkyl), $-C(O)NR^{10}$(aryl), $-C(O)NR^{10}$(heteroaryl), $-NR^{10}C(O)NR^{10}R^{11}$, $-NR^{10}C(O)NR^{11}$(cycloalkyl), $-NR^{10}C(O)NR^{11}$(heterocycloalkyl), $-NR^{10}C(O)NR^{11}$(aryl), $-NR^{10}C(O)NR^{11}$(heteroaryl), $-NR^{10}C(O)O$(alkyl), $-NR^{10}C(O)O$(cycloalkyl), $-NR^{10}C(O)O$(heterocycloalkyl), $-NR^{10}C(O)O$(aryl), $-NR^{10}C(O)O$(heteroaryl), $-NR^{10}SO_2$(alkyl), $-NR^{10}SO_2$(cycloalkyl), $-NR^{10}SO_2$(heterocycloalkyl), $-NR^{10}SO_2$(aryl), $-NR^{10}SO_2$(heteroaryl), $-SO_2NR^{10}R^{11}$, $-SO_2NR^{10}$(cycloalkyl), $-SO_2NR^{10}$(heterocycloalkyl), $-SO_2NR^{10}$(aryl), $-SO_2NR^{10}$(heteroaryl), haloalkyl, aryl, and heteroaryl.

65. The compound of claim 64, wherein D is  .

66. The compound of claim 64, wherein D is  .

67. The compound of claim 64, wherein D is  .

68. The compound of any one of claims 40-67 wherein Y^1 and Y^2 are O.

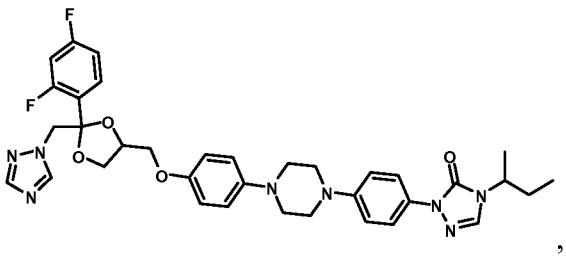
69. The compound of claim 68 wherein m is 1.

70. The compound of any one of claims 40-69 wherein p is 1, 2, or 3.

71. The compound of claim 70 wherein p is 1.

72. The compound of any one of claims 40-71 wherein R^1, R^2, R^3, and R^4 are hydrogen.

73. The compound of claim 40 wherein the compound is selected from: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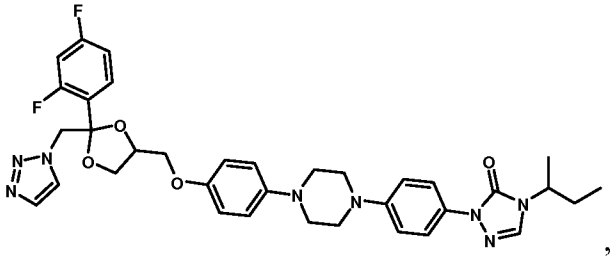
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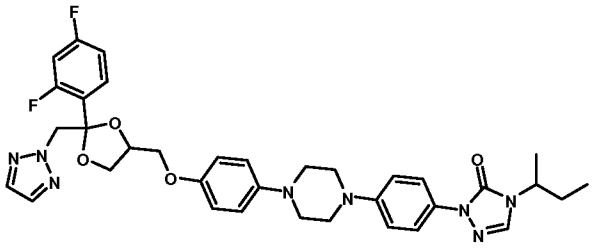
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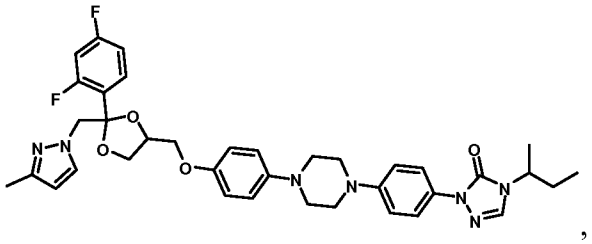
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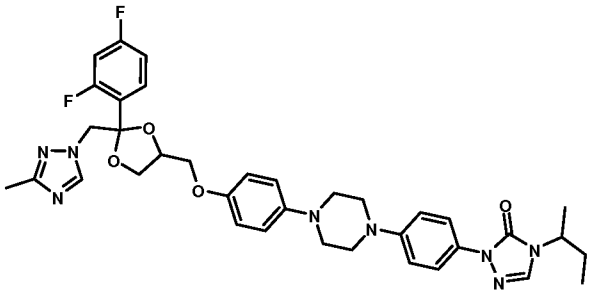
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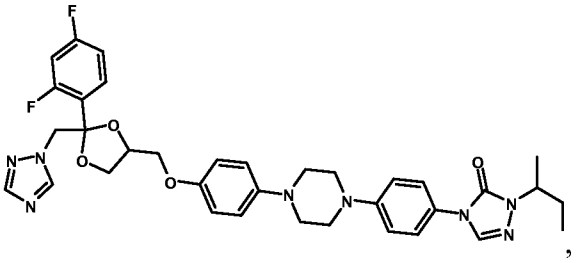
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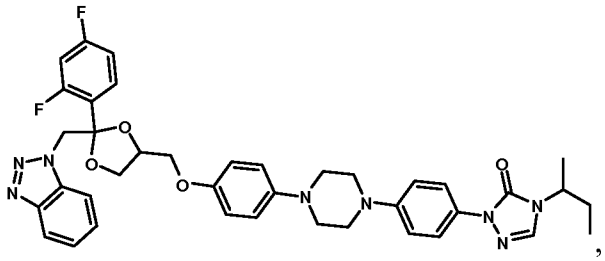
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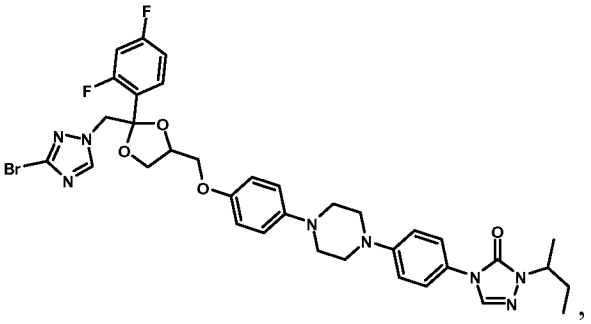
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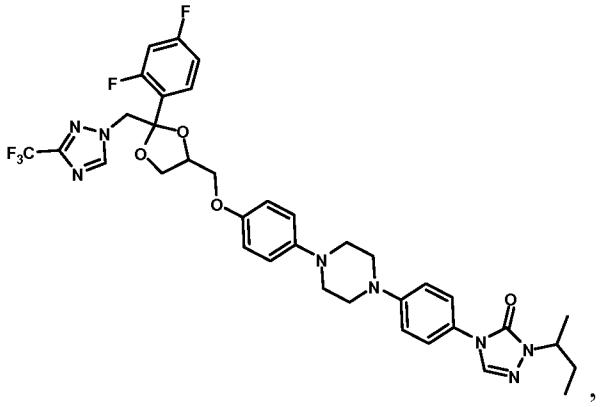
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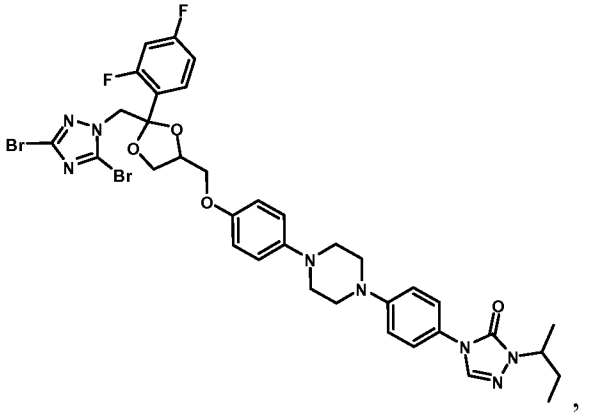
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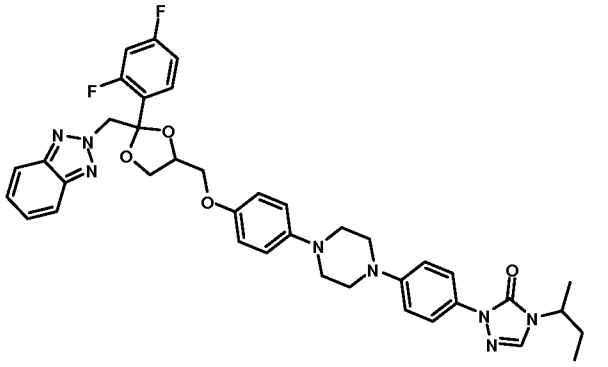
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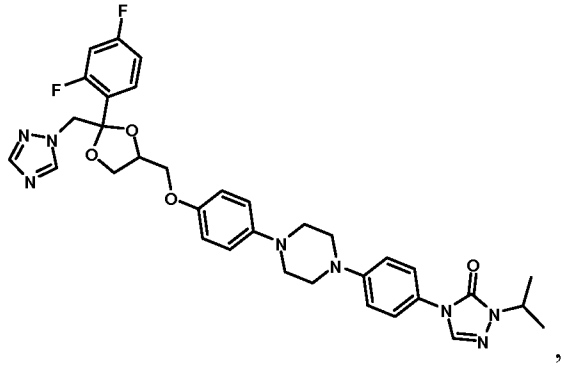
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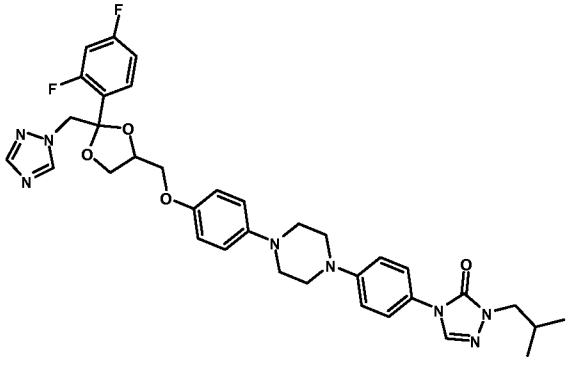
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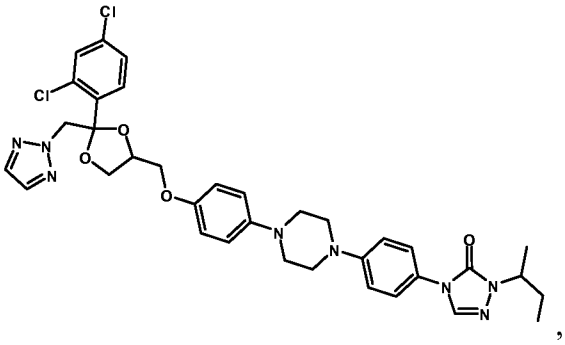
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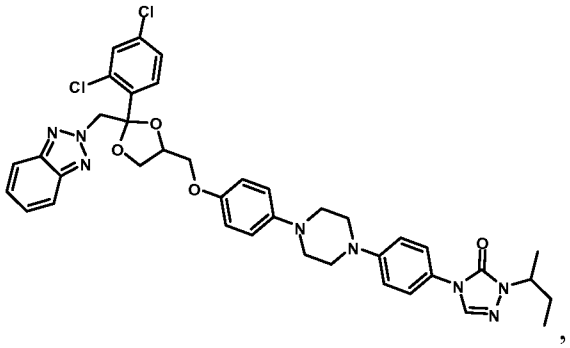
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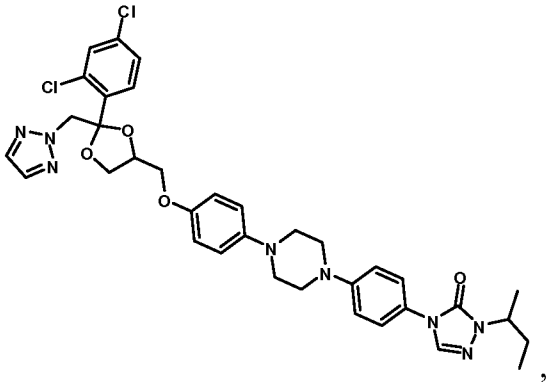
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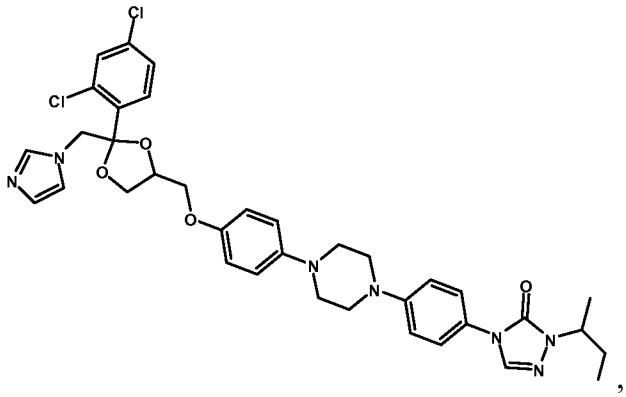
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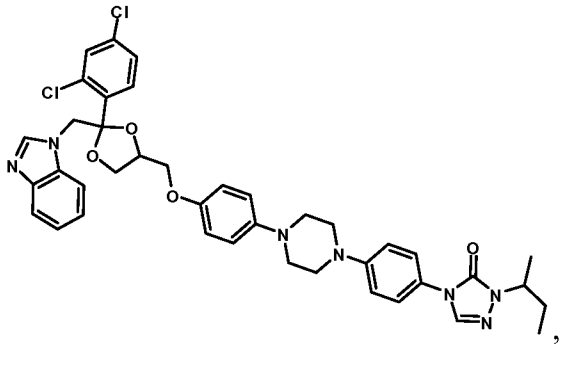
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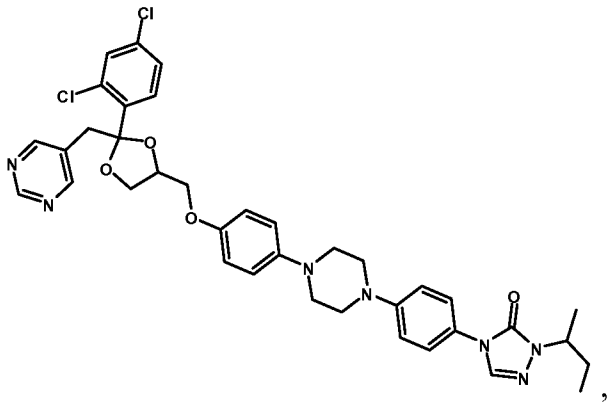

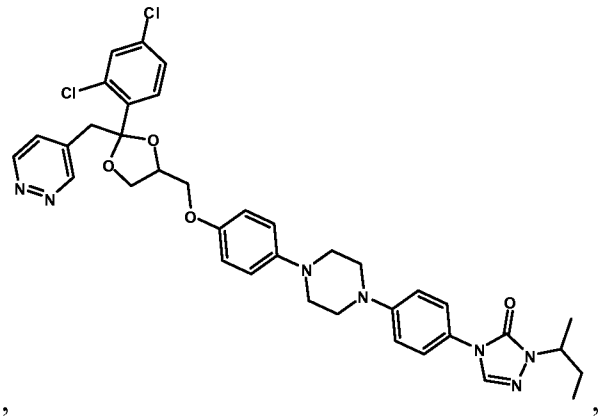

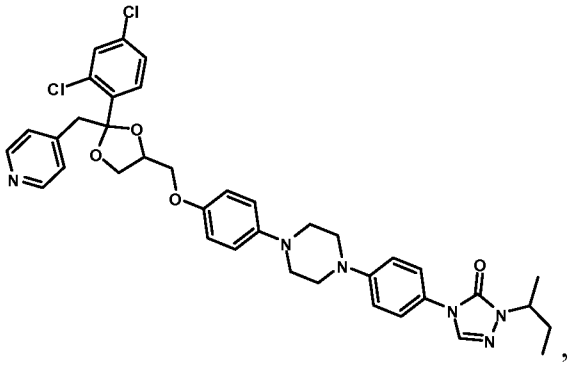

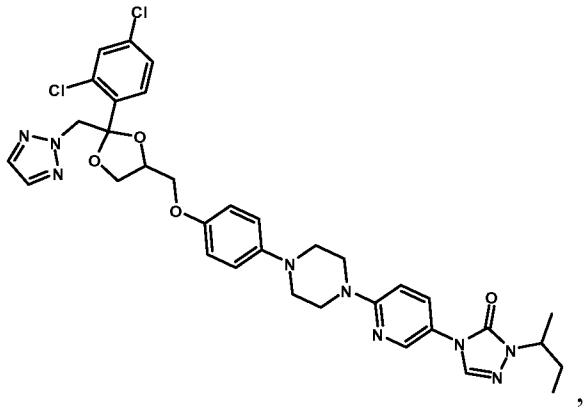

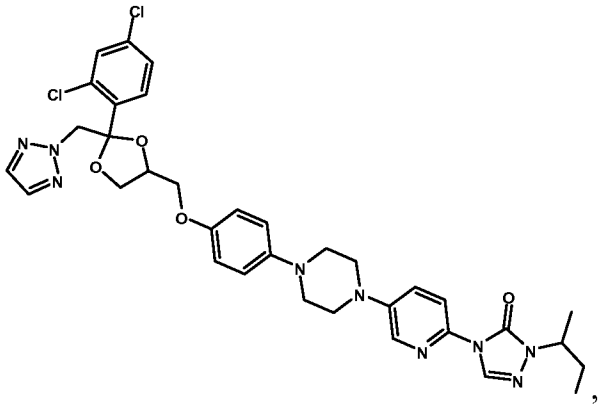

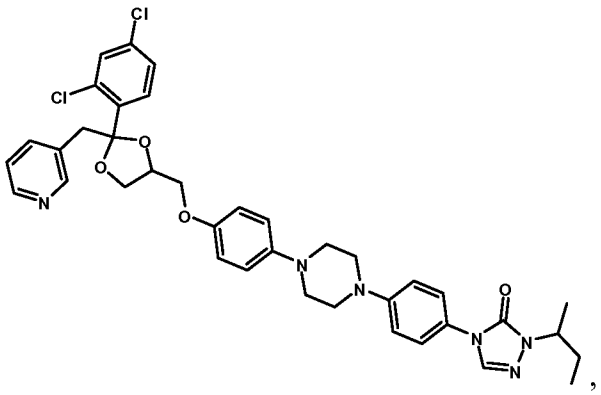

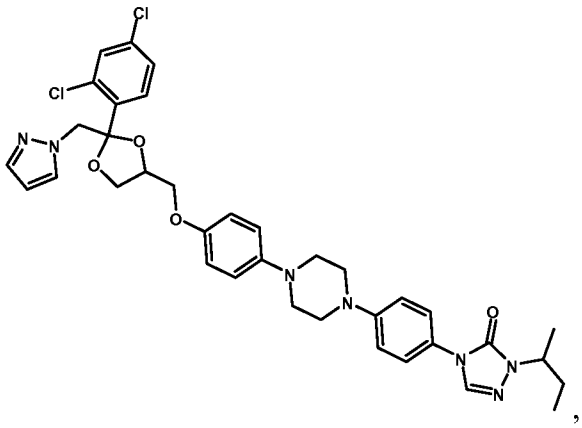

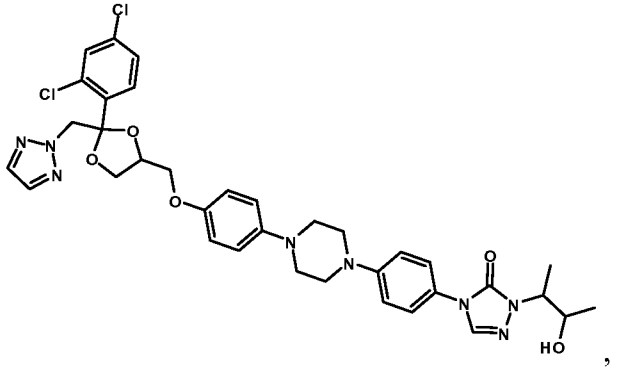

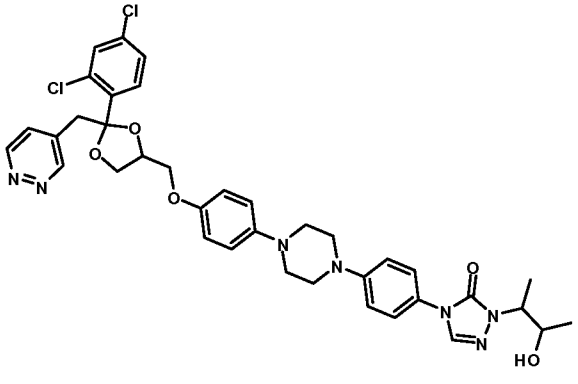

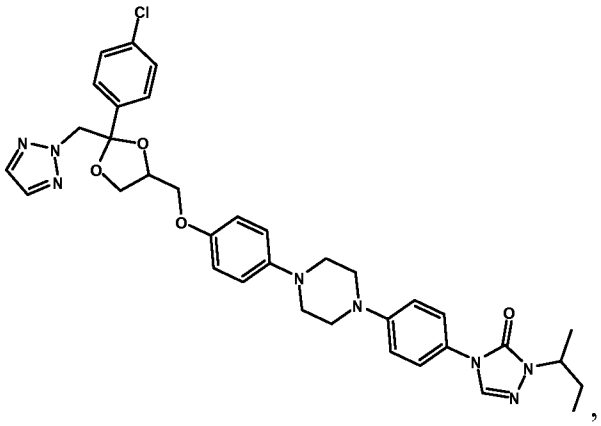

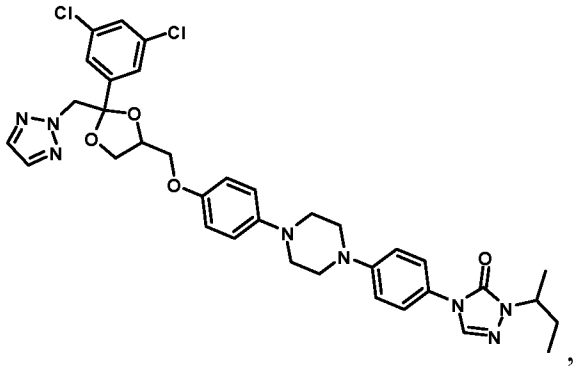

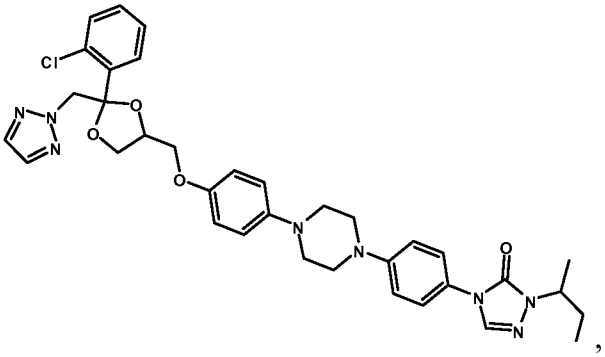

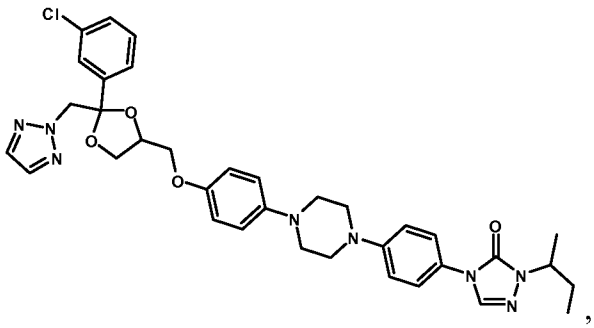

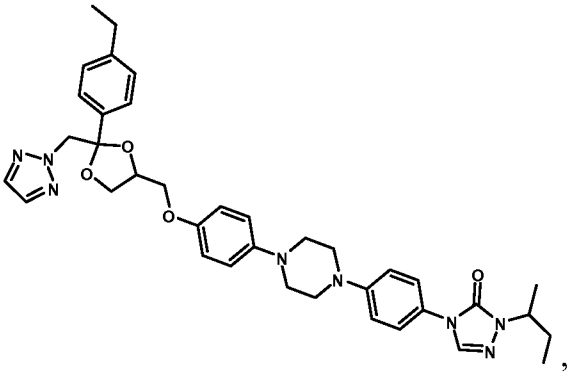

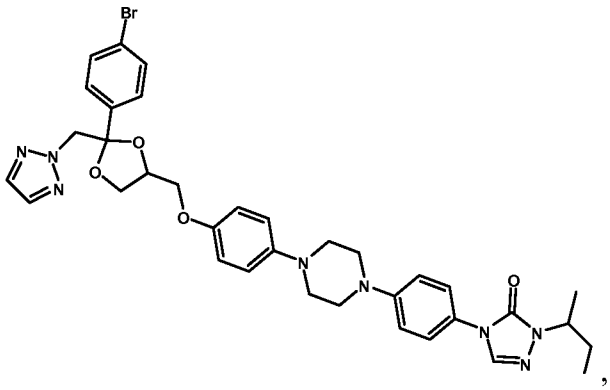

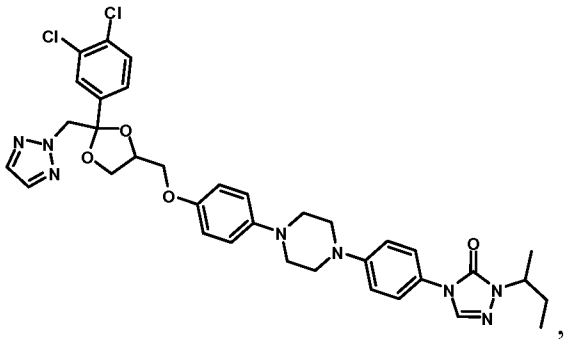

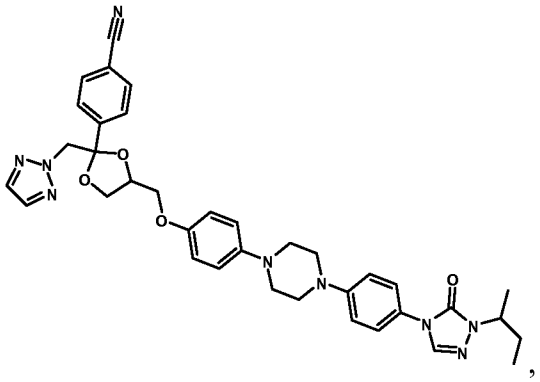

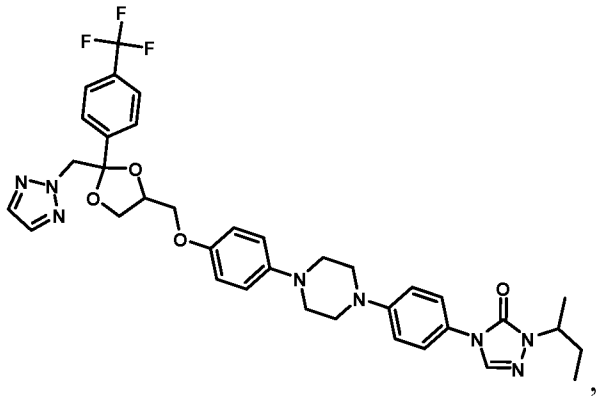

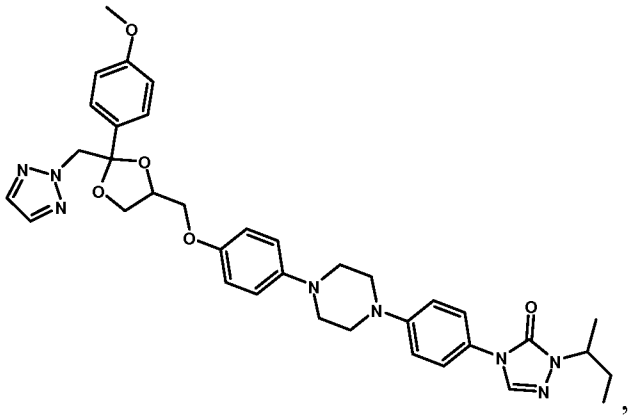

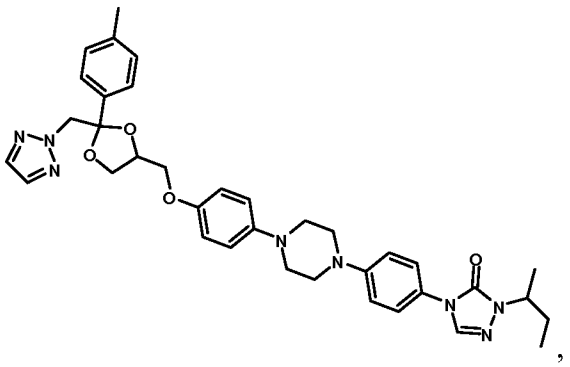

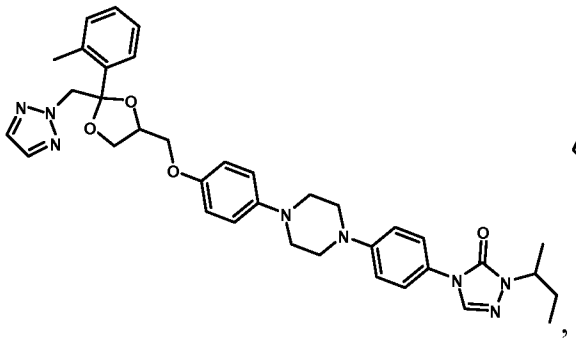

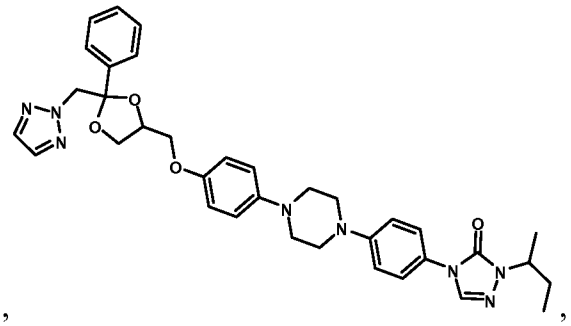

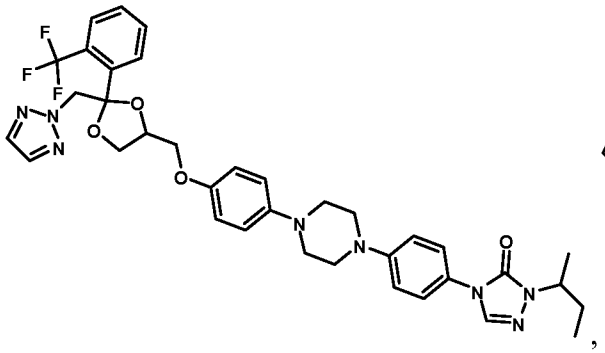

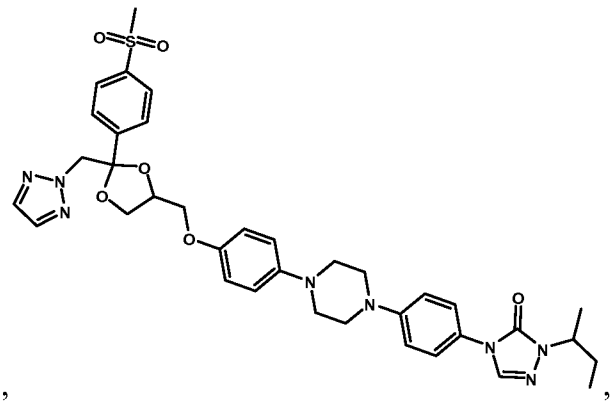

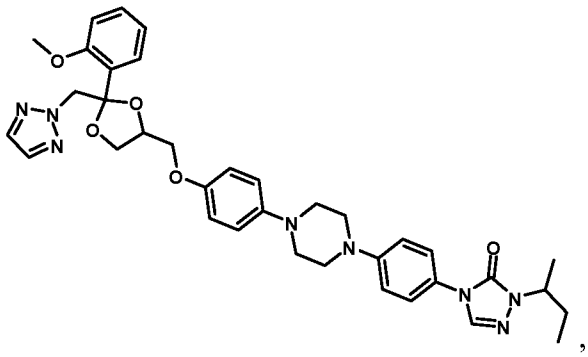

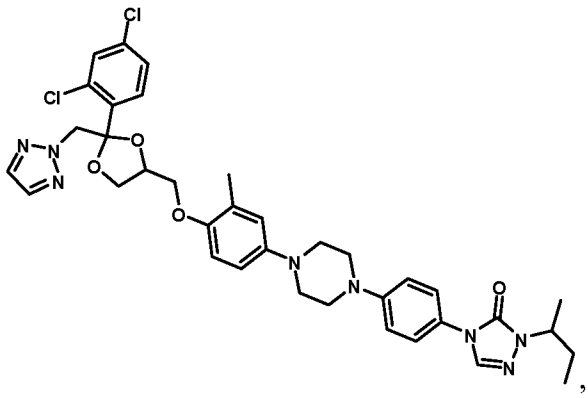

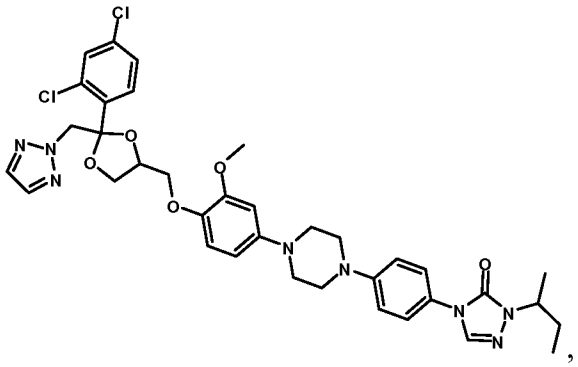

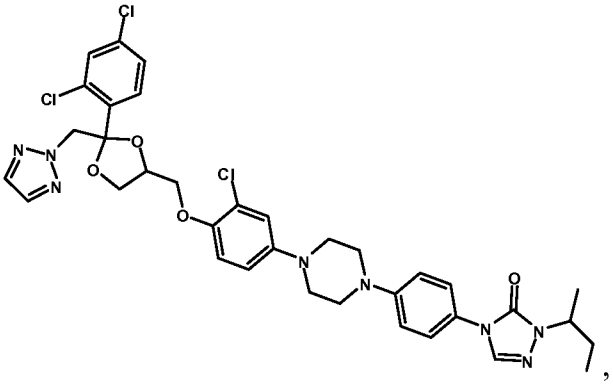

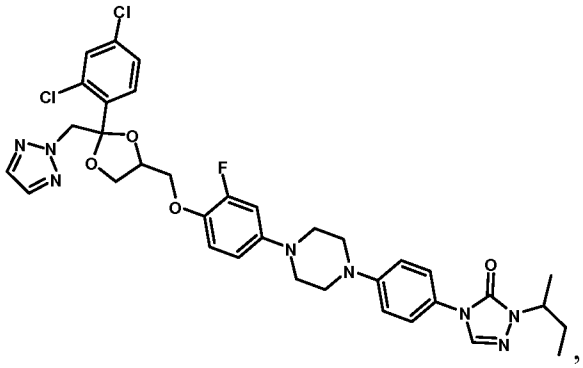

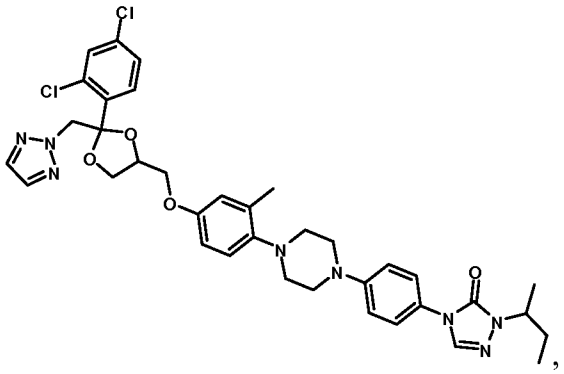

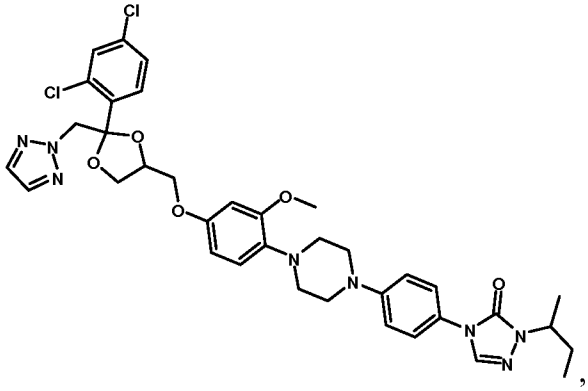

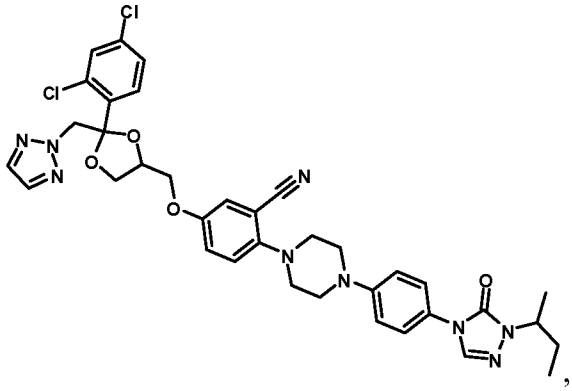

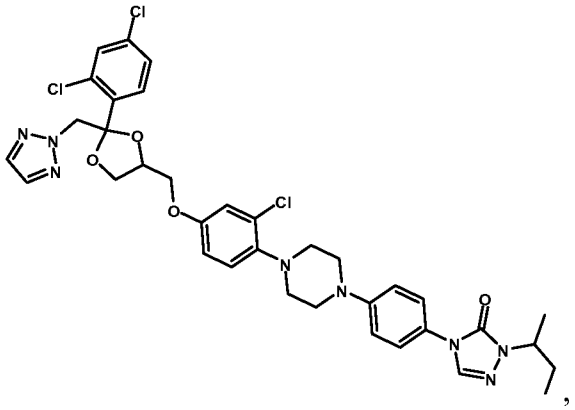

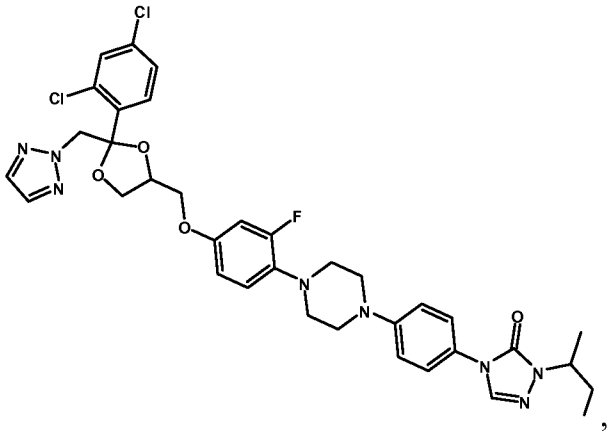

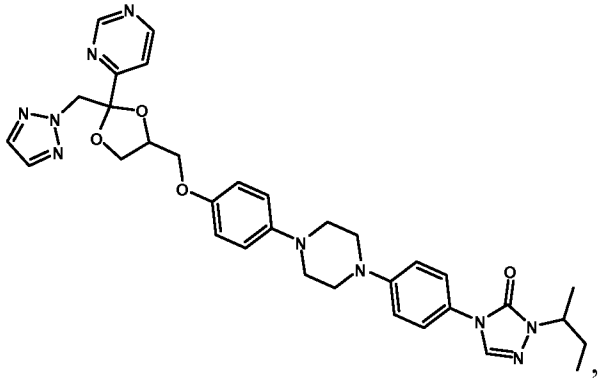

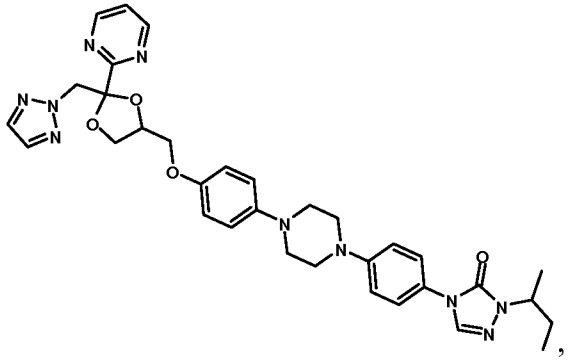

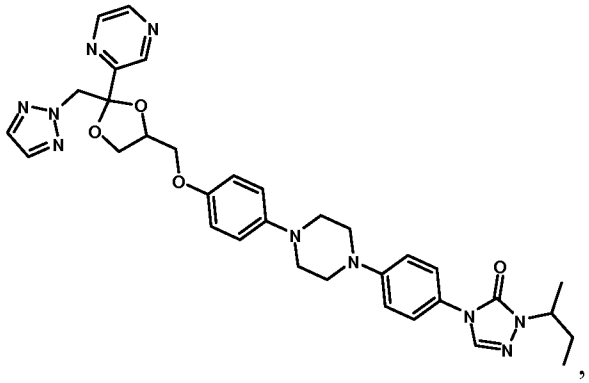

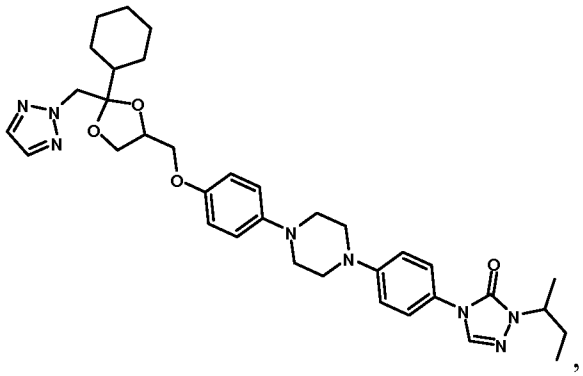

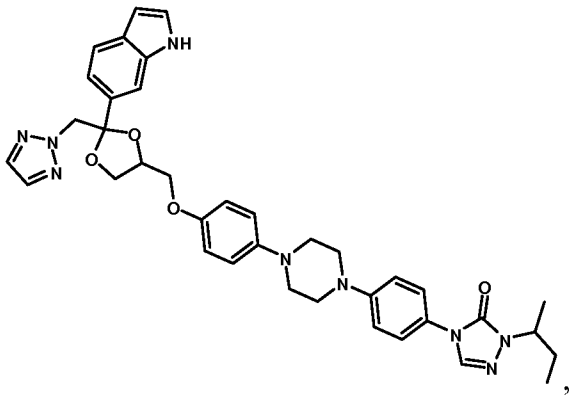

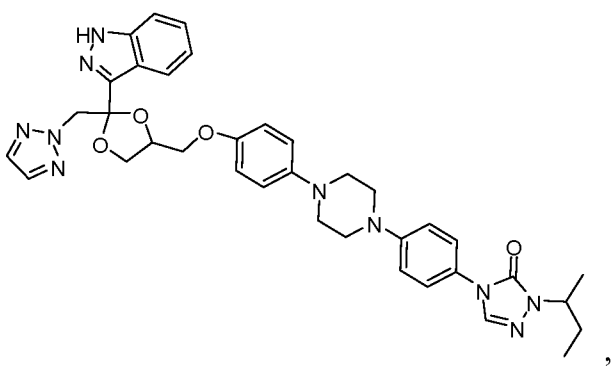

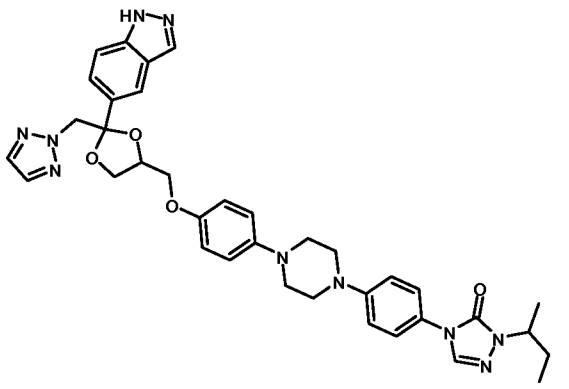
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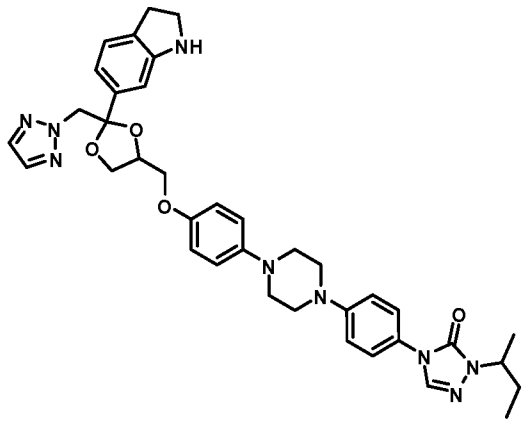
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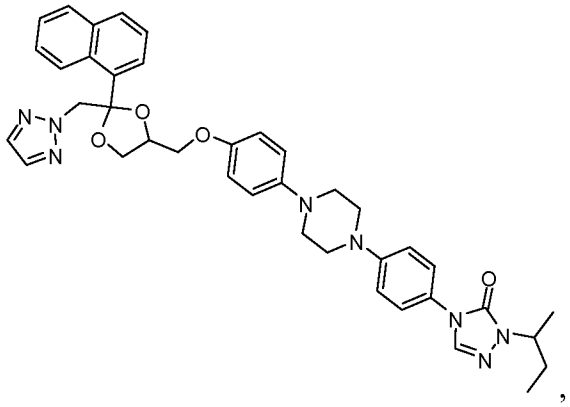
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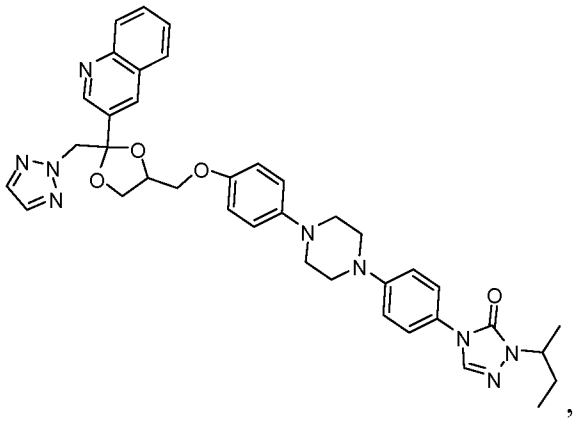
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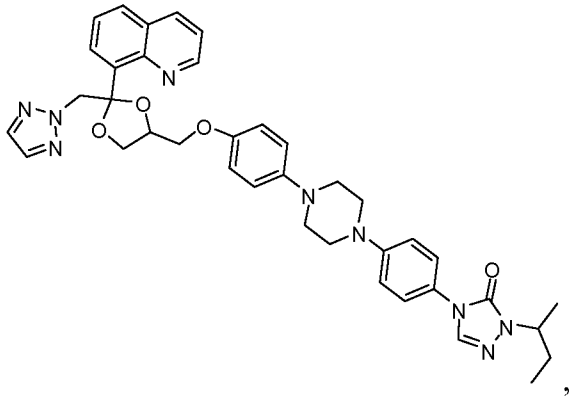
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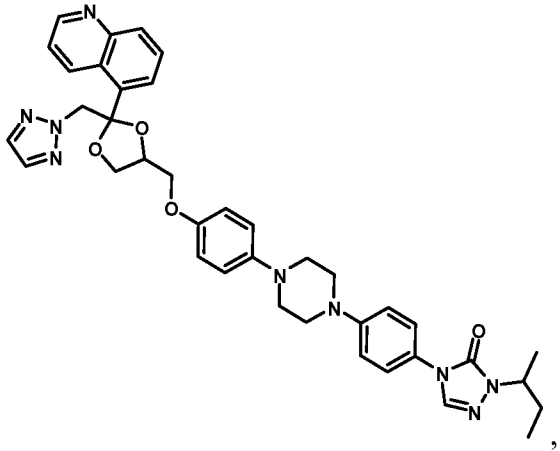
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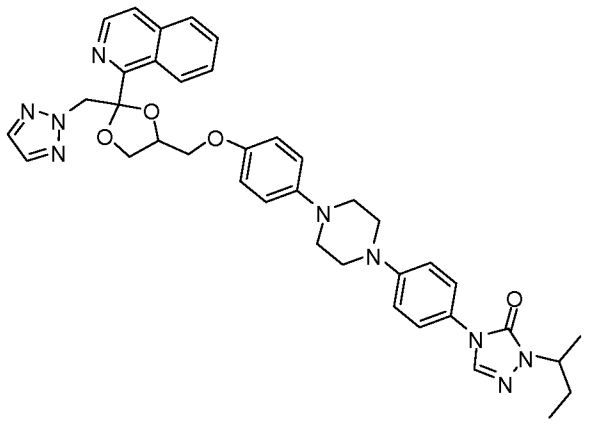
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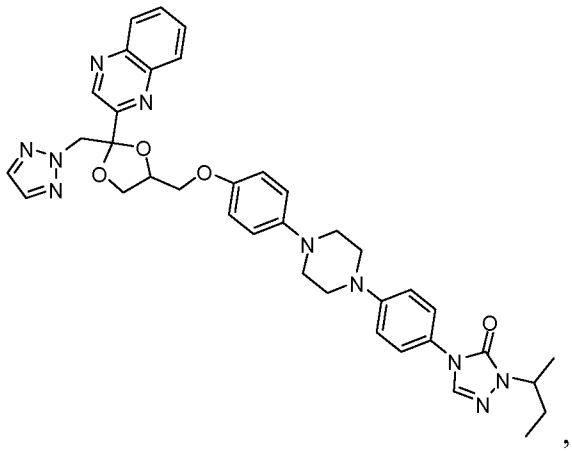
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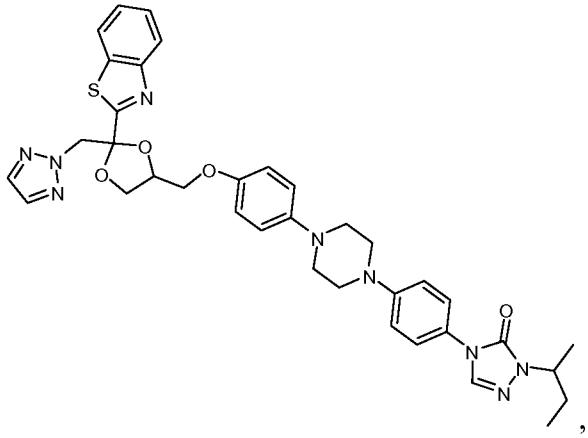

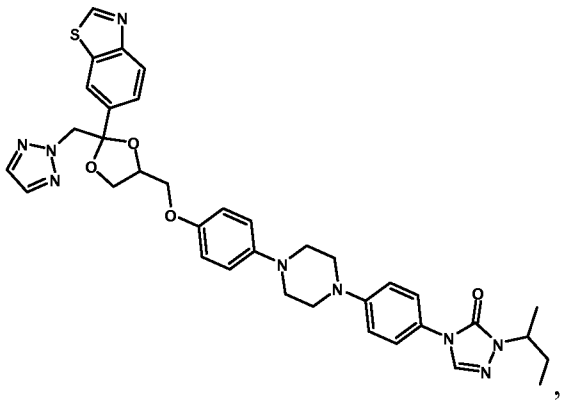

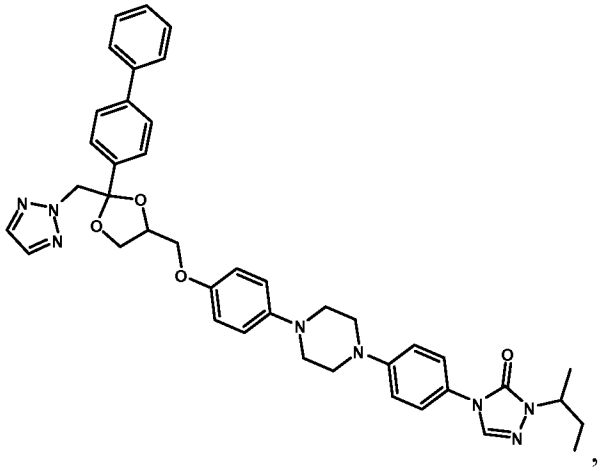

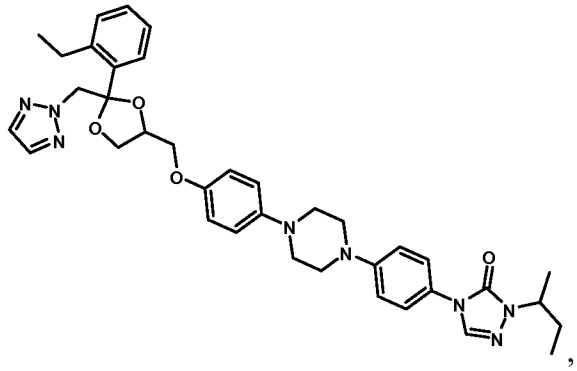

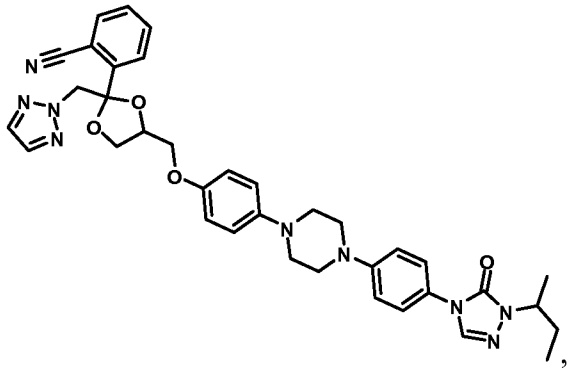

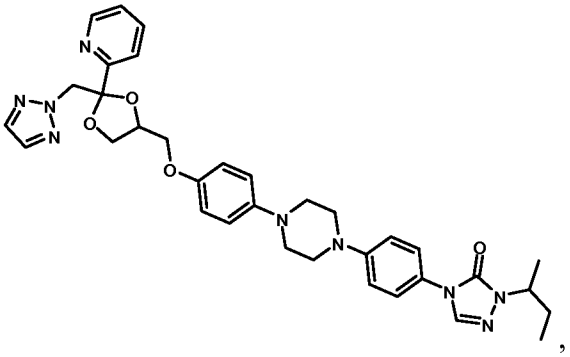

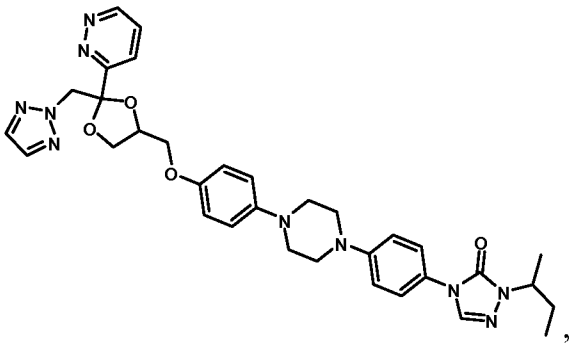

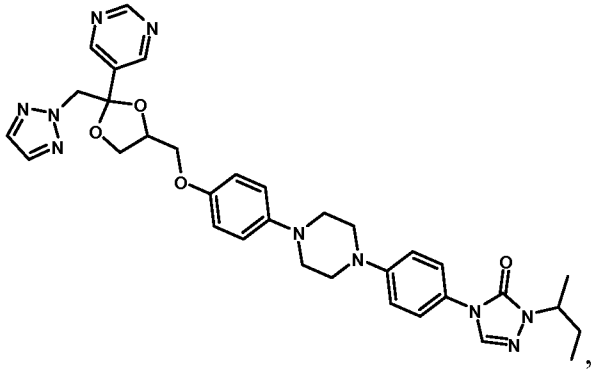

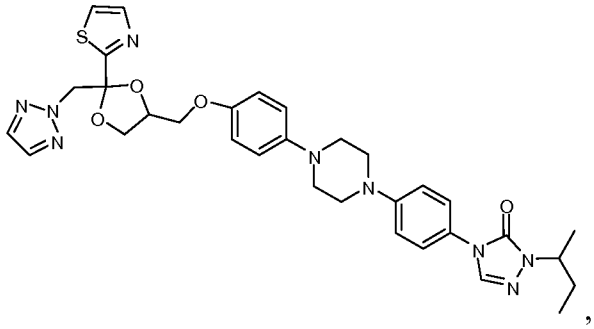

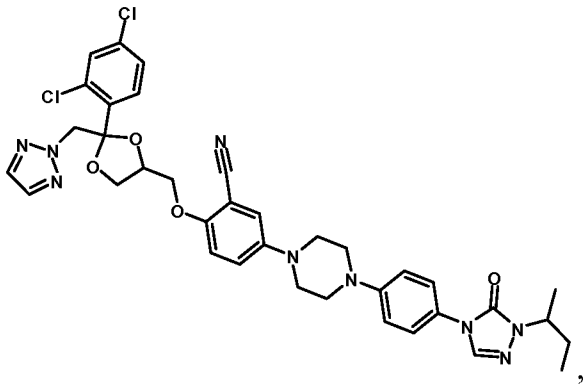

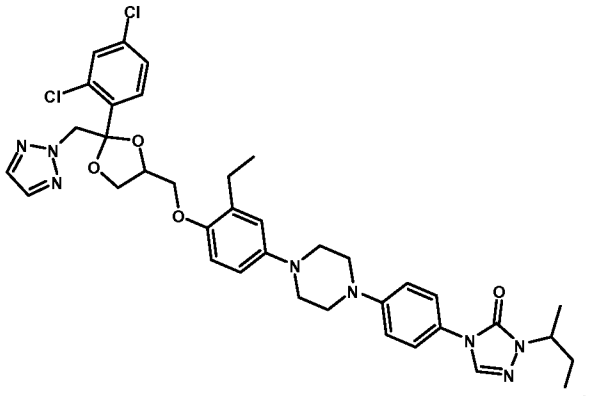

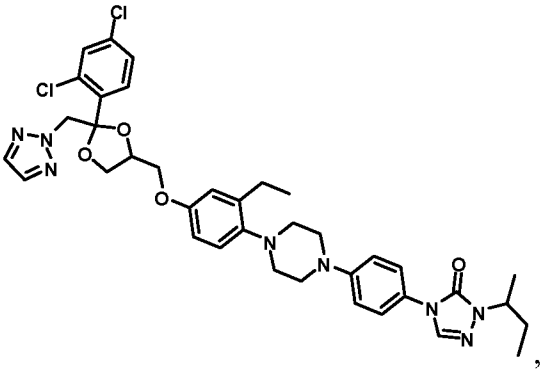

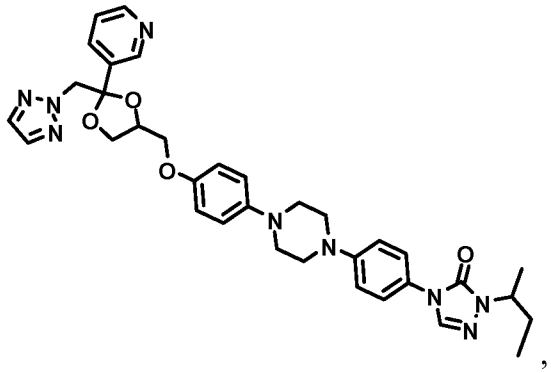

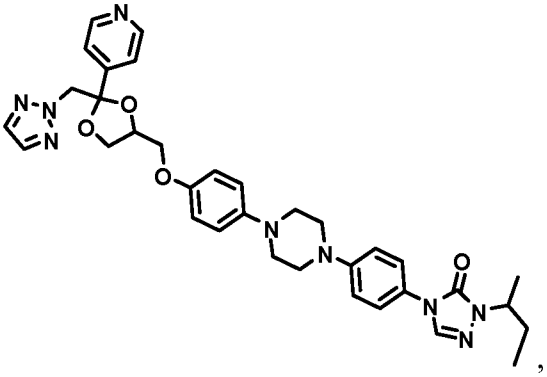

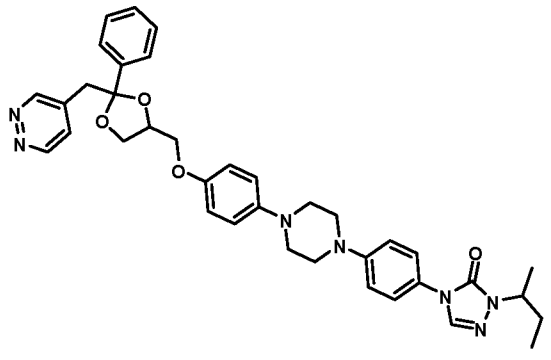

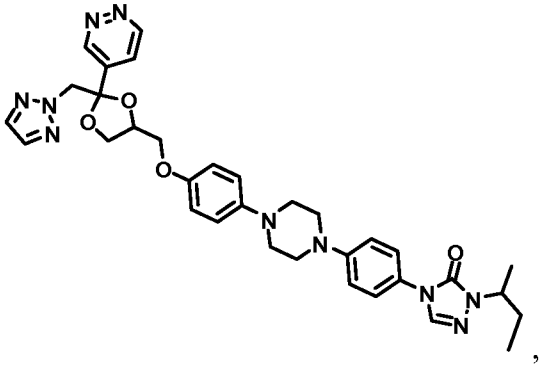

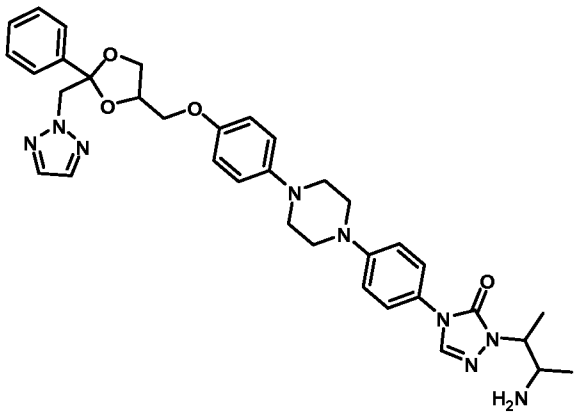
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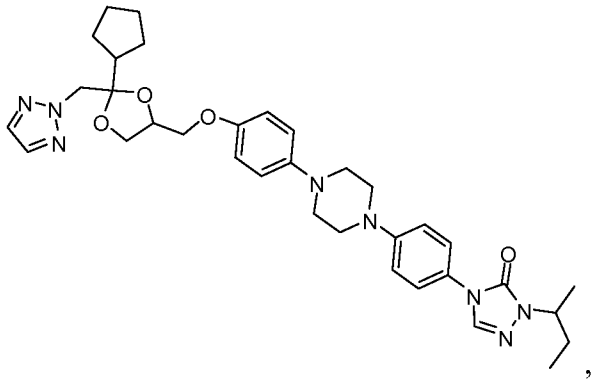
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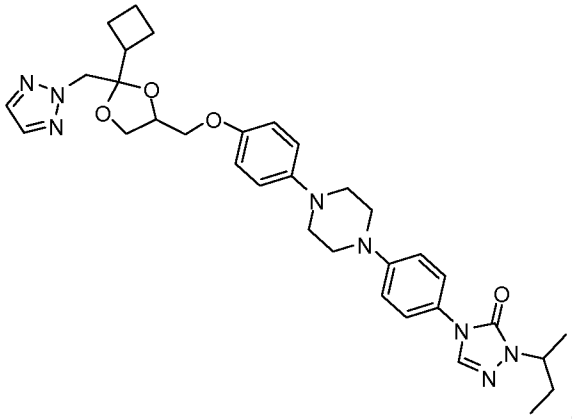
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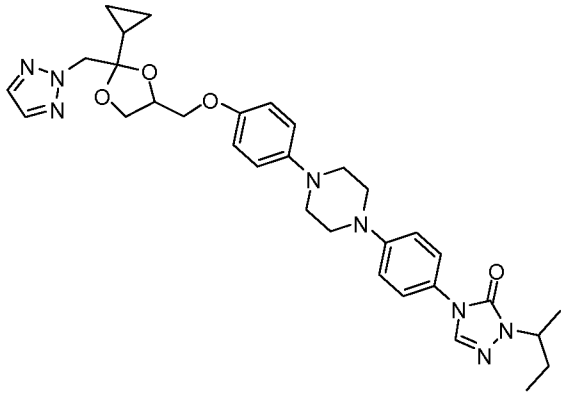
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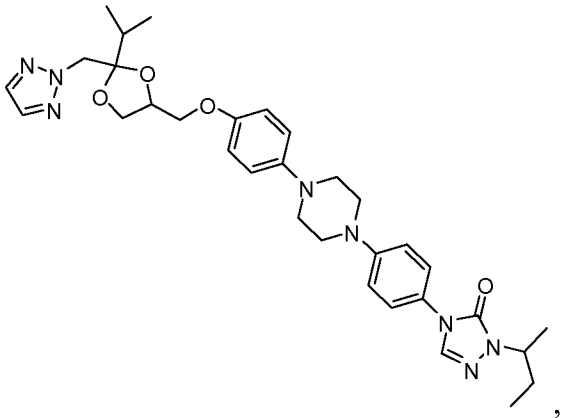
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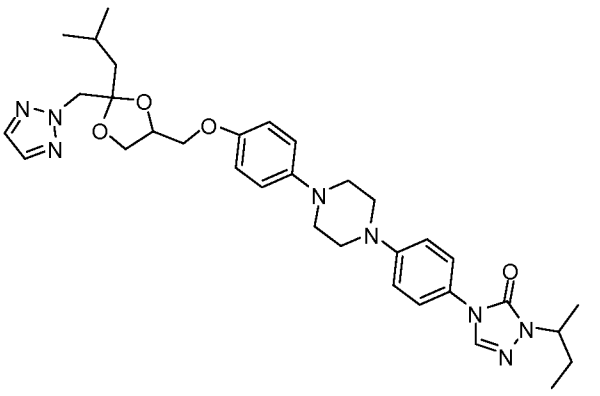
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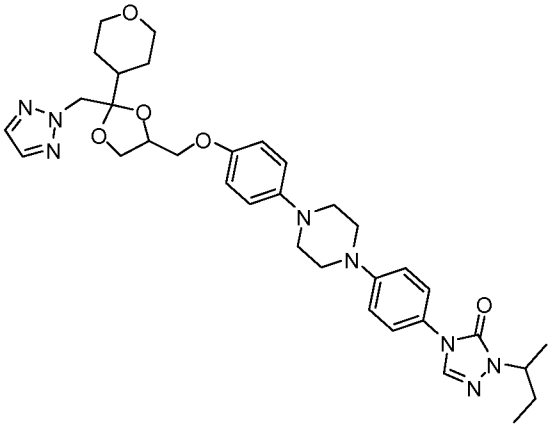
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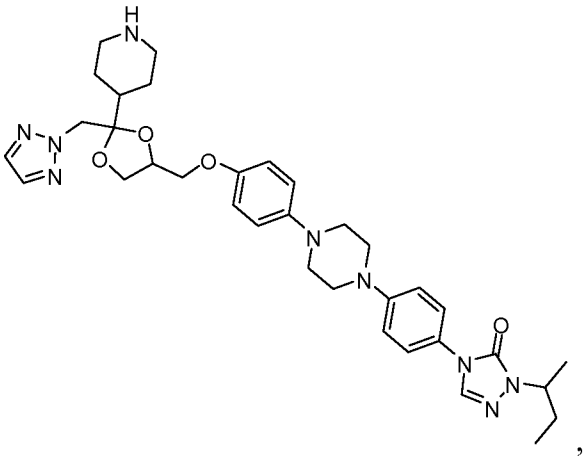
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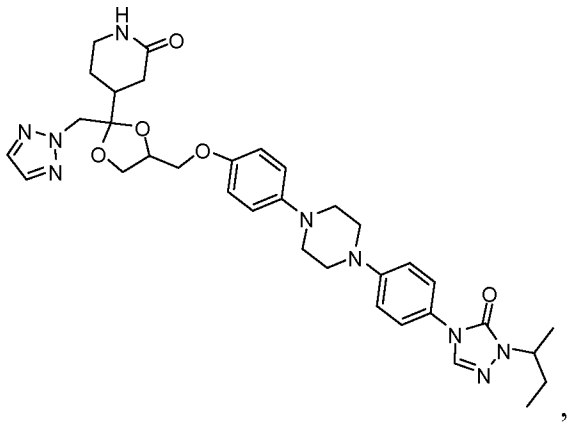
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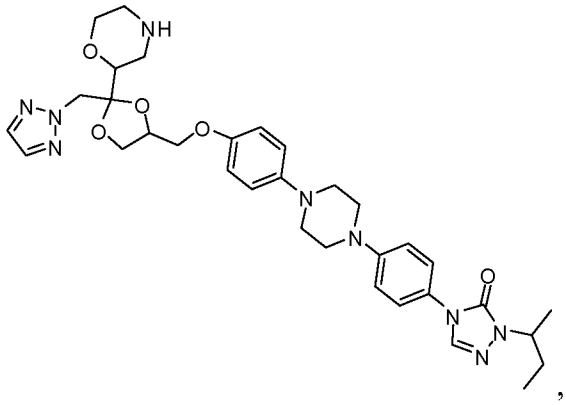
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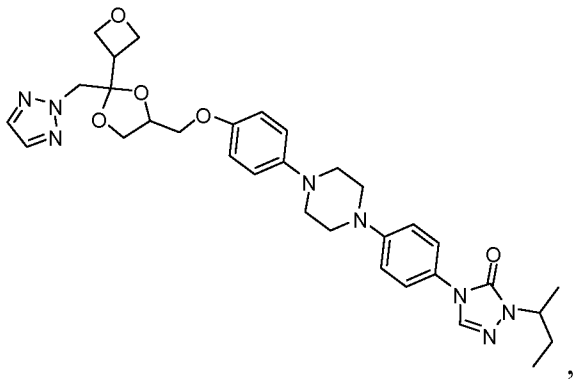
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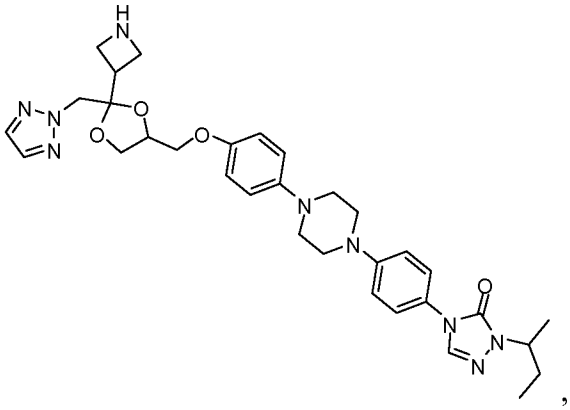
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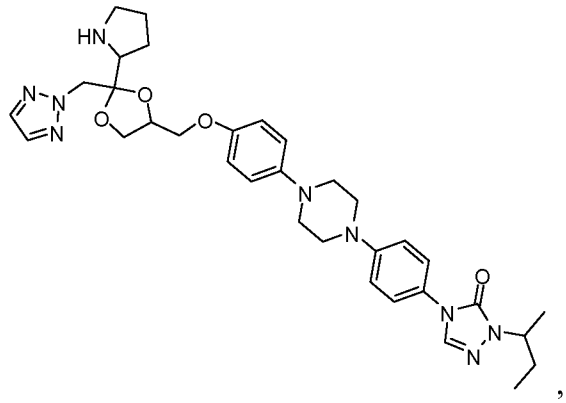
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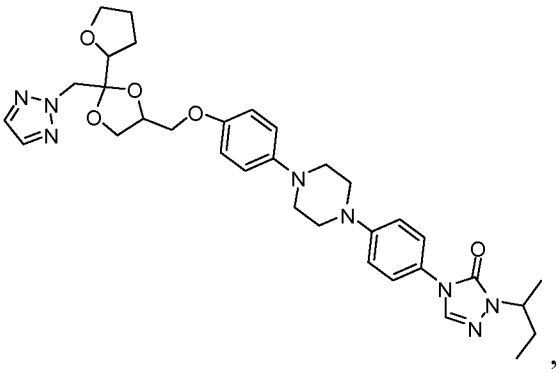
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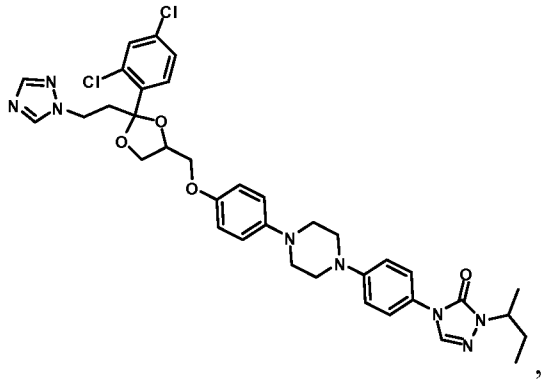
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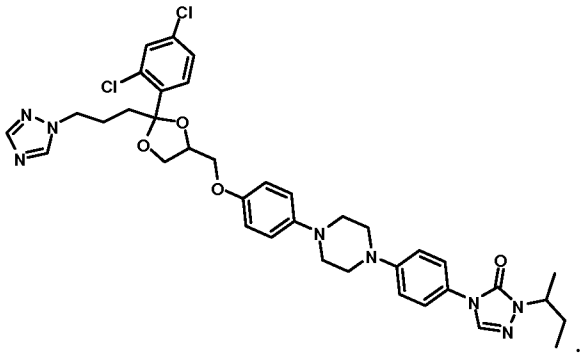

.

74. A pharmaceutical composition comprising a compound of any one of claims 40-73, or a pharmaceutically acceptable salt, solvate, polymorph, prodrug, metabolite, N-oxide, stereoisomer, or isomer thereof, and a pharmaceutically acceptable excipient.

Figure 1


Non-activated
αSMA
Hoechst
TGF-β (5 ng/mL)
TGFβ (5 ng/mL)
SB 431542 (10 μM)

Figure 2

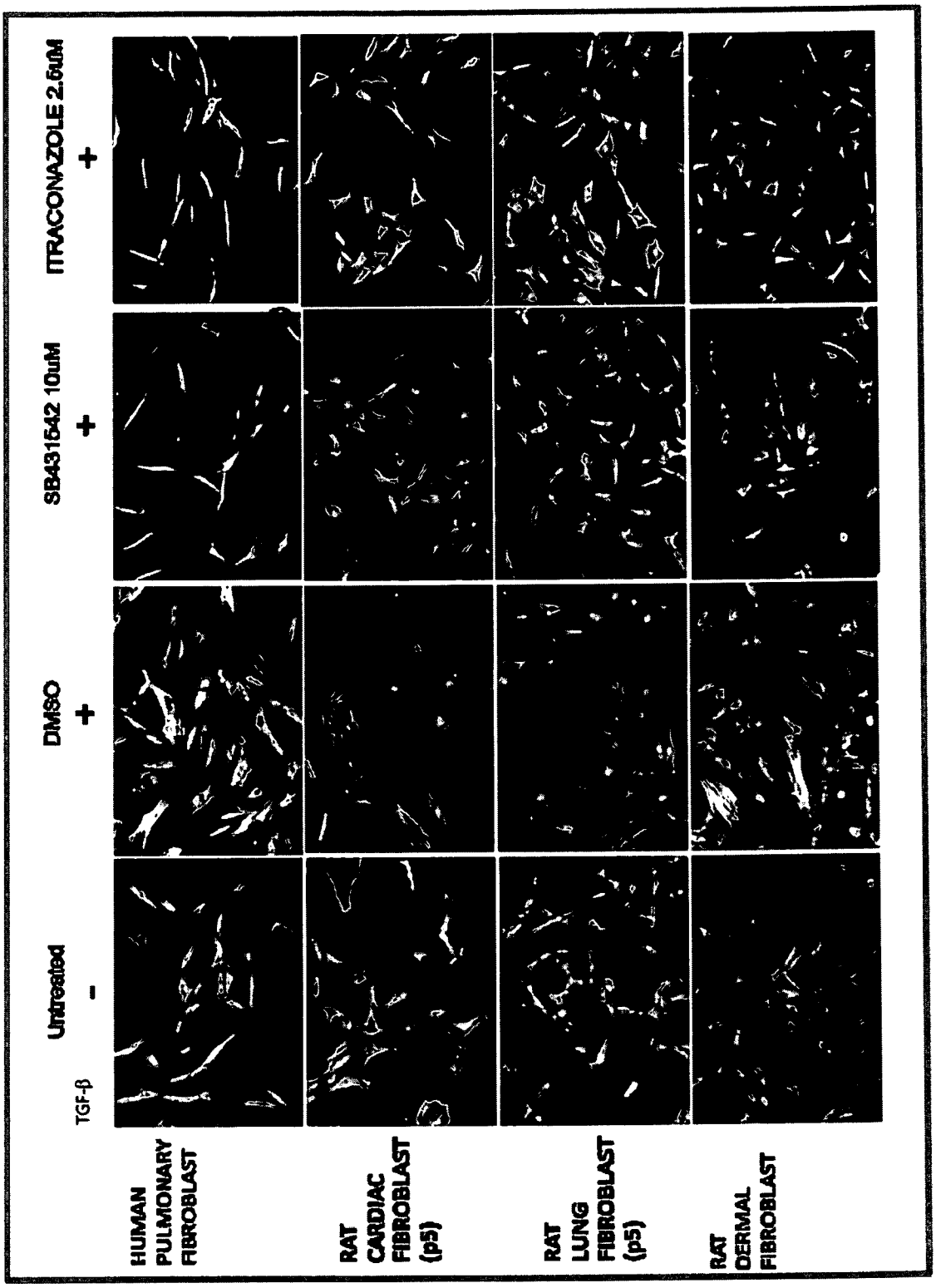

TGF-β
Untreated
-
DMSO
+
SB431542 10uM
+
ITRACONAZOLE 2.6uM
+
HUMAN PULMONARY FIBROBLAST
RAT CARDIAC FIBROBLAST (p5)
RAT LUNG FIBROBLAST (p5)
RAT DERMAL FIBROBLAST

Figure 3

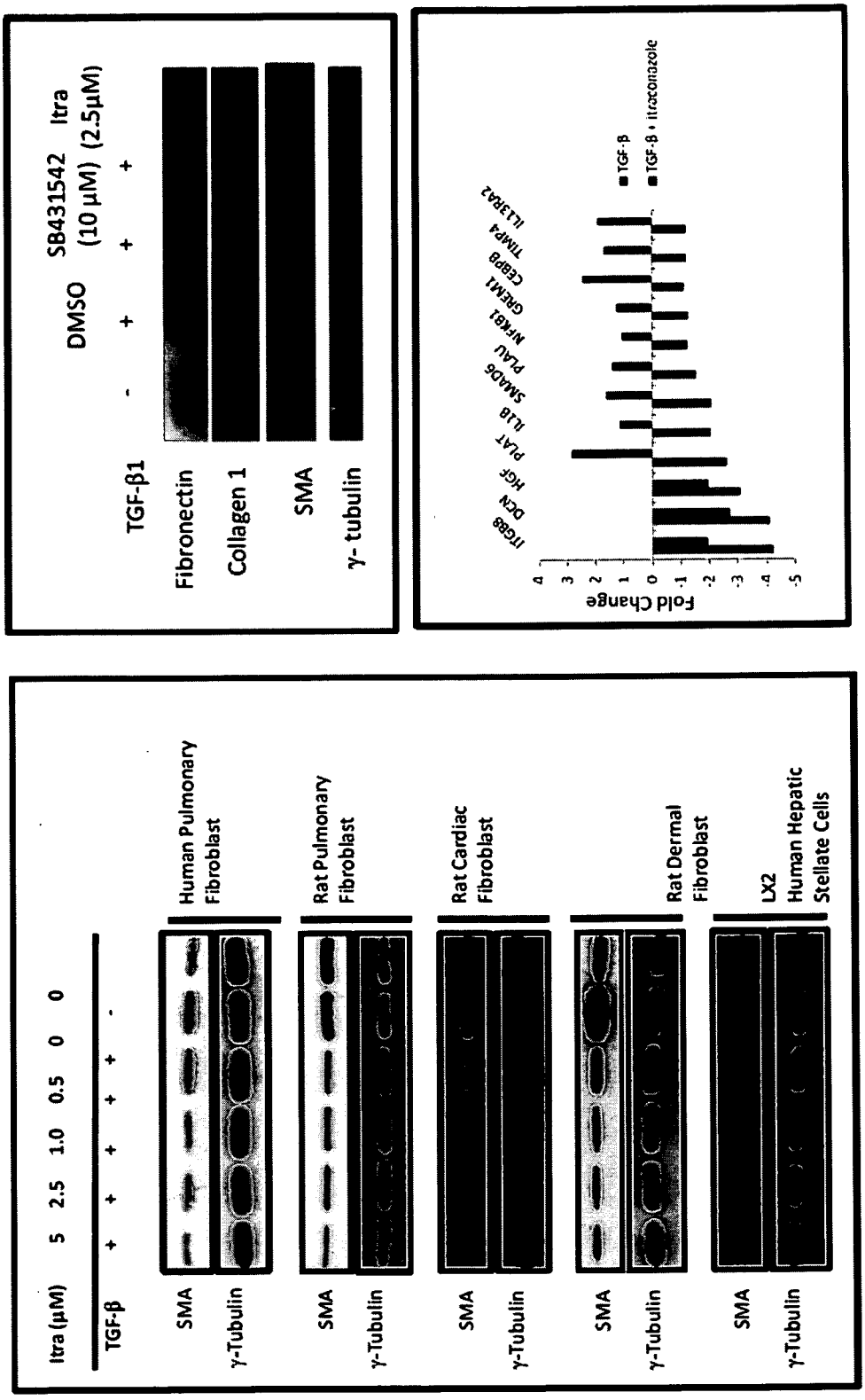

TGF-β1
DMSO
SB431542 (10 μM)
Itra (2.5μM)
Fibronectin
Collagen 1
SMA
γ- tubulin
Fold Change
TGF-β
TGF-β + itraconazole
ITGB8
DCN
HGF
PLAT
IL1B
SMAD6
PLAU
NFKBI
GREM1
CEBPB
TIMP4
IL13RA2
Itra (μM)
5
2.5
1.0
0.5
0
0
TGF-β
SMA
γ-Tubulin
Human Pulmonary Fibroblast
Rat Pulmonary Fibroblast
Rat Cardiac Fibroblast
Rat Dermal Fibroblast
LX2 Human Hepatic Stellate Cells

Figure 4a

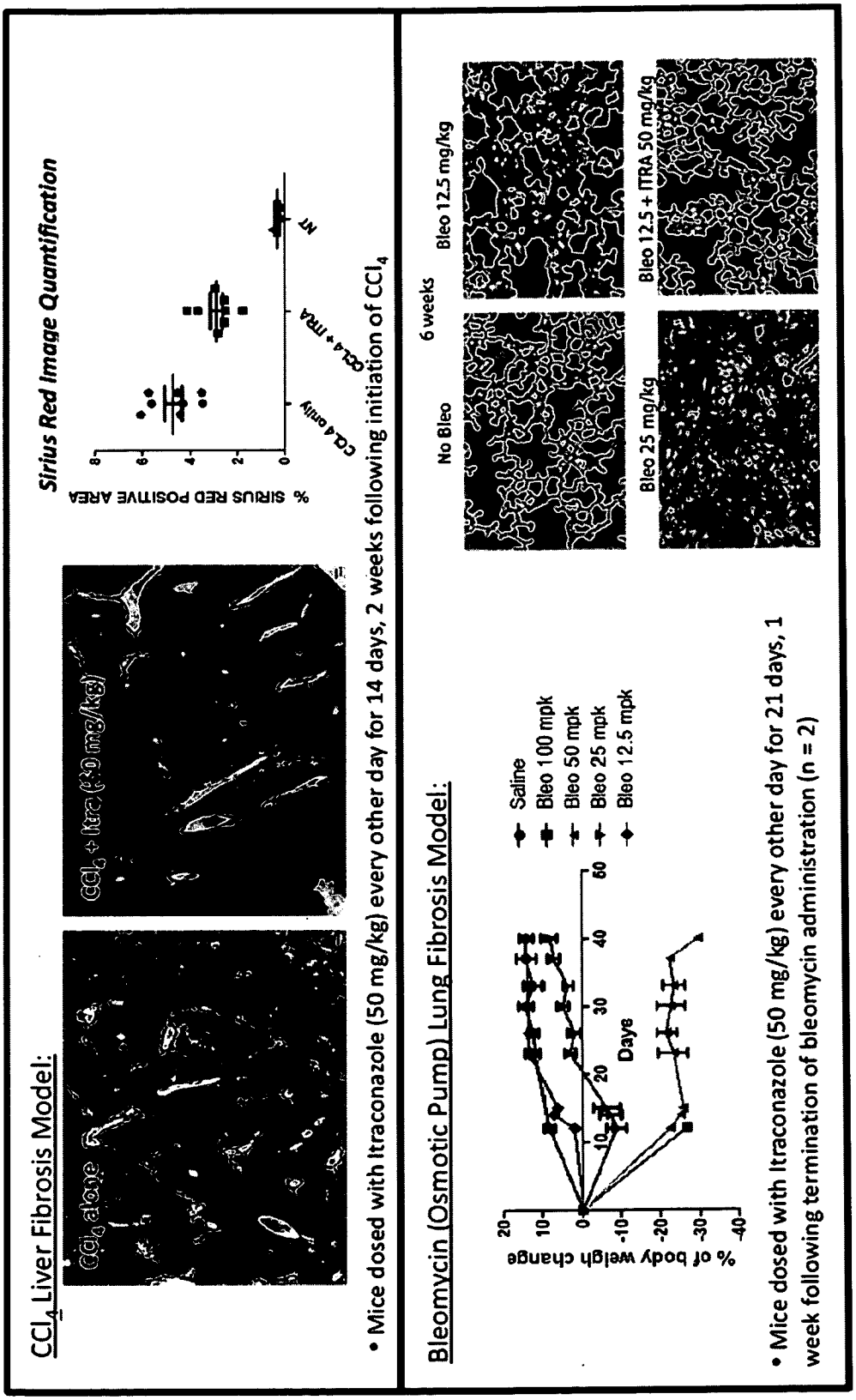
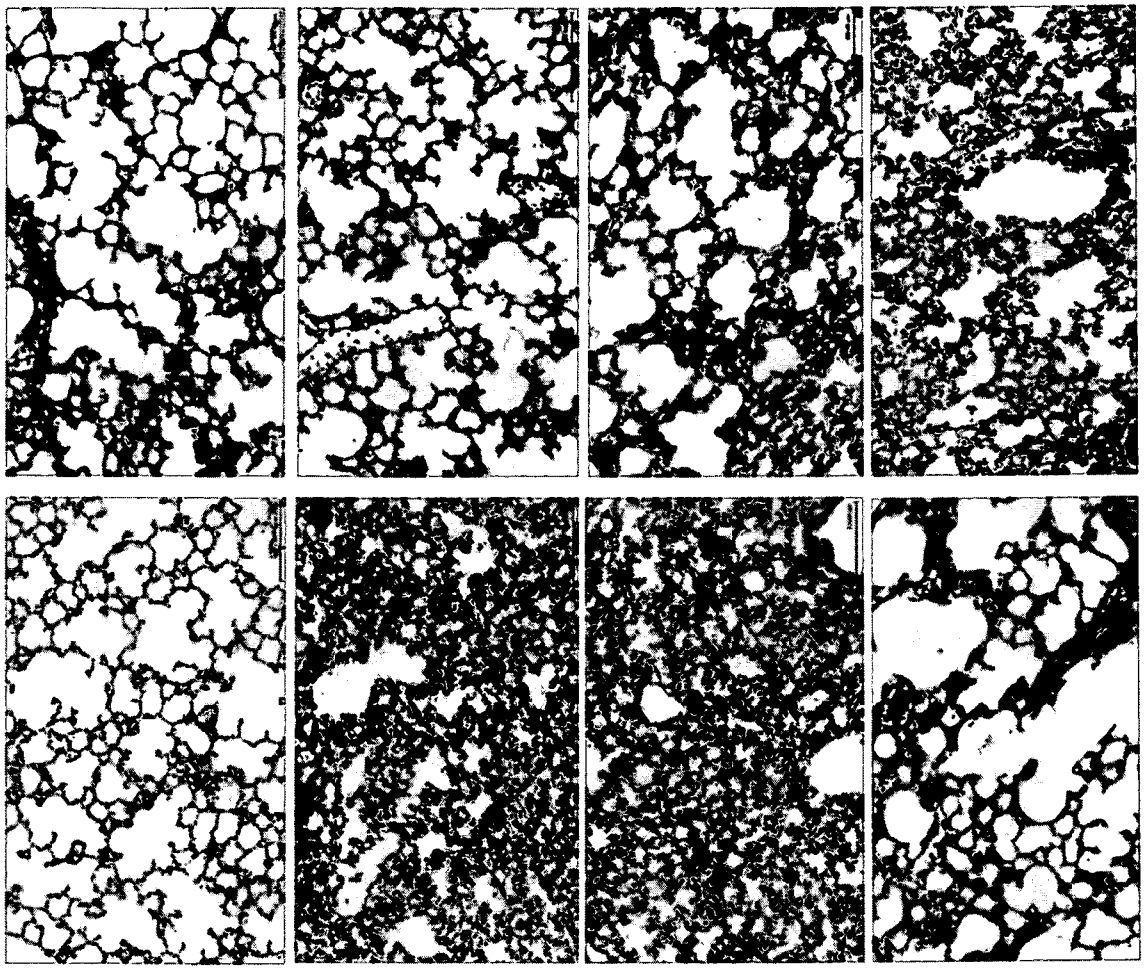

CCl4 Liver Fibrosis Model:
CCl4 alone
CCl4 + Itra (50 mg/kg)
Sirius Red Image Quantification
% SIRIUS RED POSITIVE AREA
CCl4 only
CCl4 + ITRA
NT
• Mice dosed with Itraconazole (50 mg/kg) every other day for 14 days, 2 weeks following initiation of CCl4
Bleomycin (Osmotic Pump) Lung Fibrosis Model:
% of body weigh change
Days
Saline
Bleo 100 mpk
Bleo 50 mpk
Bleo 25 mpk
Bleo 12.5 mpk
No Bleo
6 weeks
Bleo 12.5 mg/kg
Bleo 25 mg/kg
Bleo 12.5 + ITRA 50 mg/kg
• Mice dosed with Itraconazole (50 mg/kg) every other day for 21 days, 1 week following termination of bleomycin administration (n = 2)

Figure 4b


PUMP ONLY
BLEO + VEHICLE
BLEO + DEX (1mg/kg)
BLEO + PIRFENDIONE (400mg/kg BID)
BLEO + AM152 (30mg/kg BID)
BLEO + ITRACONAZOLE (100mg/kg BID)
BLEO + ITRACONAZOLE (50mg/kg BID)
BLEO + ITRACONAZOLE (25mg/kg BID)

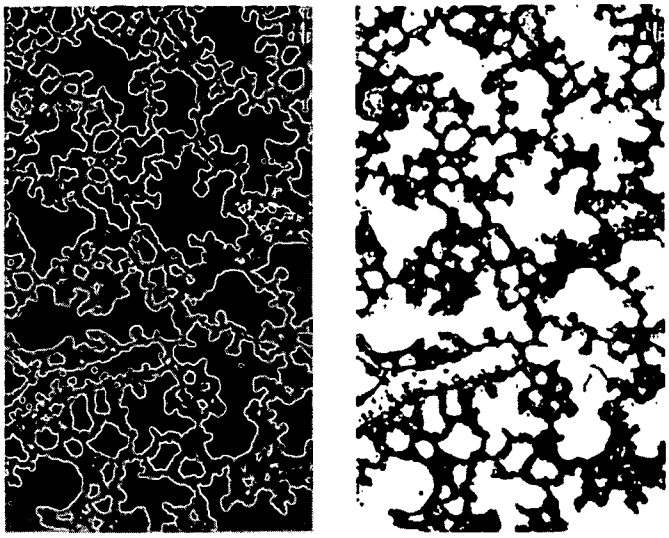


Figure 4c

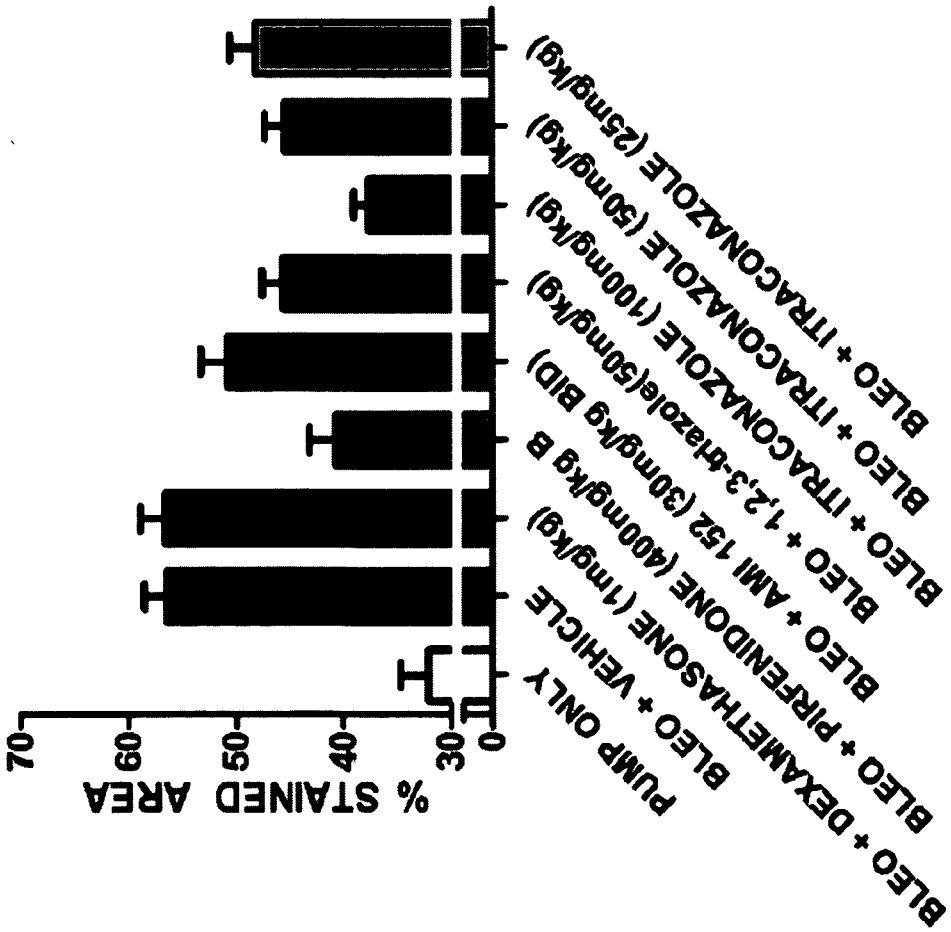

% STAINED AREA
0
30
40
50
60
70
PUMP ONLY
BLEO + VEHICLE
BLEO + DEXAMETHASONE (1mg/kg)
BLEO + PIRFENIDONE (400mg/kg B
BLEO + AMI 152 (30mg/kg BID)
BLEO + 1,2,3-triazole(50mg/kg)
BLEO + ITRACONAZOLE (100mg/kg)
BLEO + ITRACONAZOLE (50mg/kg)
BLEO + ITRACONAZOLE (25mg/kg)

Figure 5

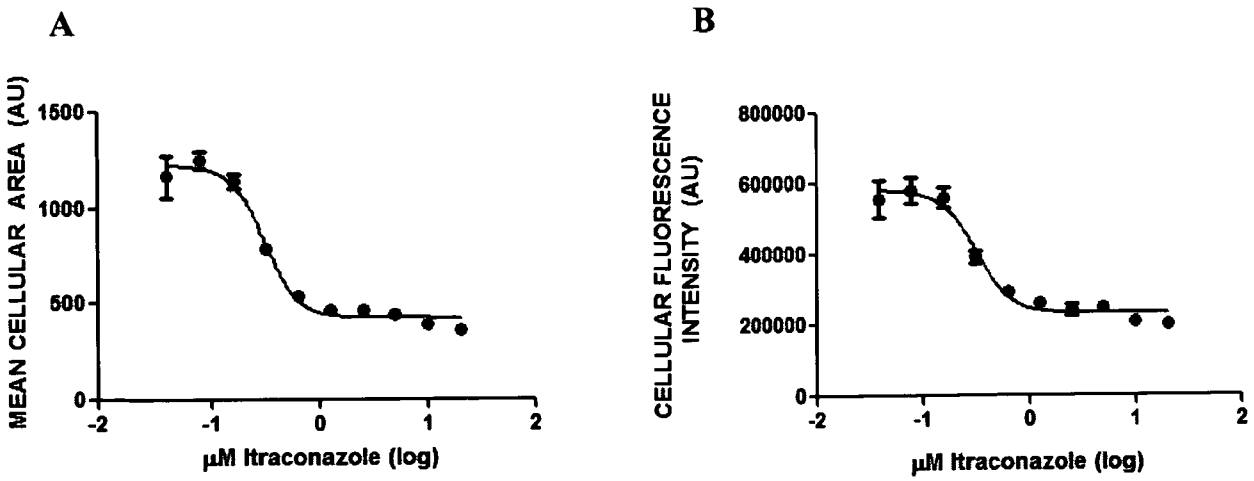


Figure 6

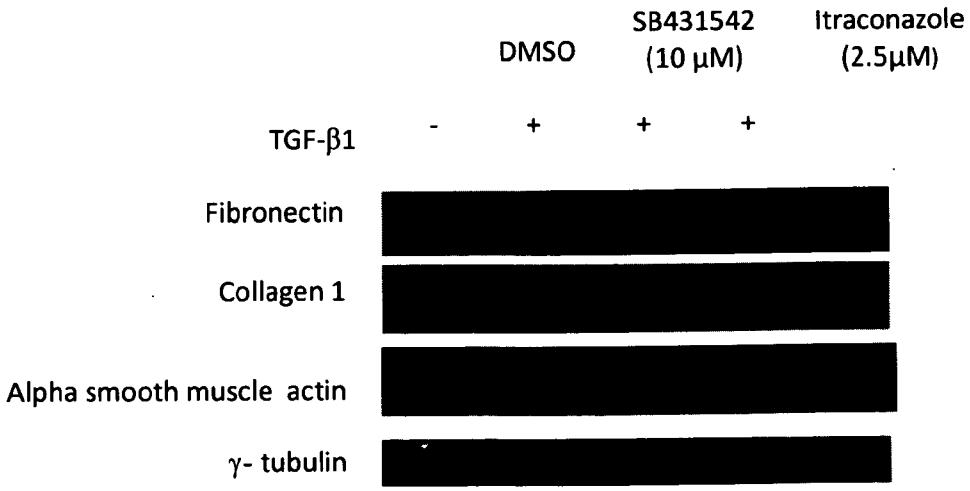
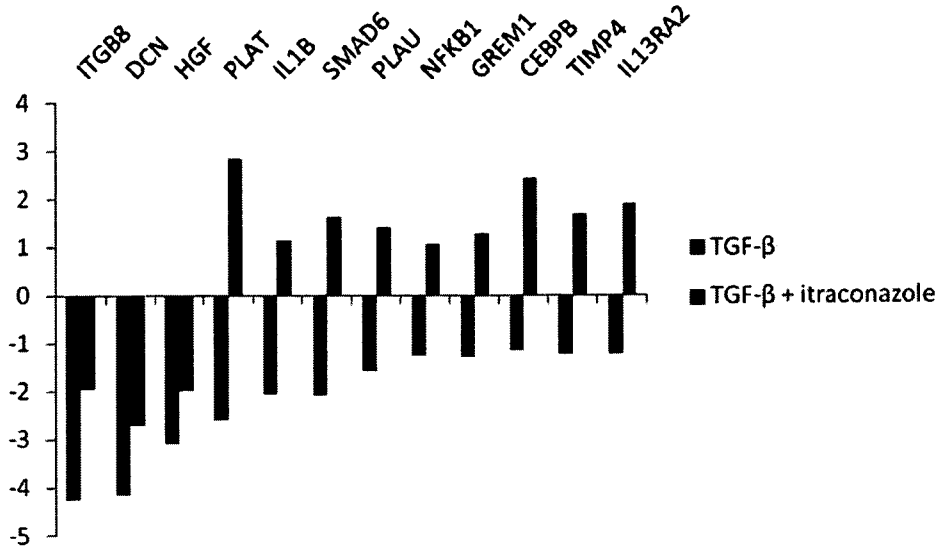


Figure 7

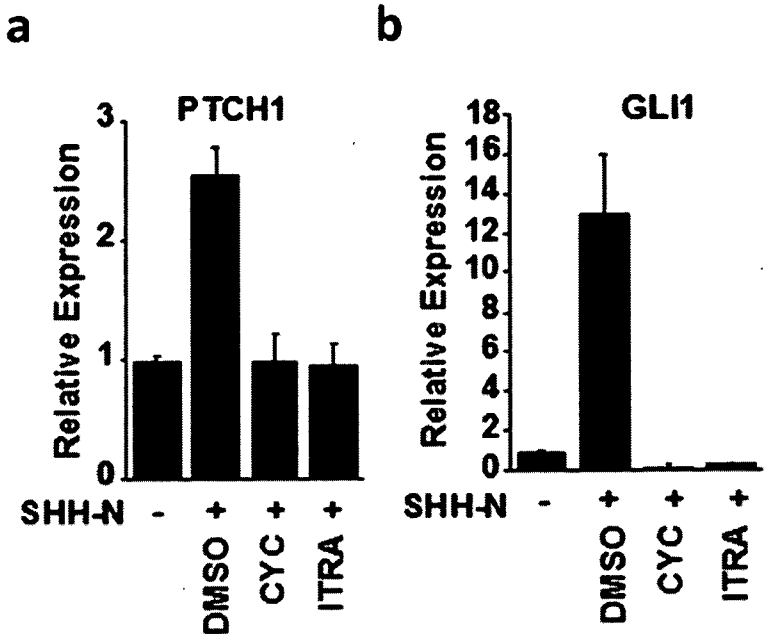
a



b

	TGF-β	TGF-β + CBR-034-1
ITGB8	-4.26	-1.96
DCN	-4.14	-2.72
HGF	-3.09	-2.00
PLAT	-2.61	2.84
IL1B	-2.06	1.15
SMAD6	-2.11	1.61
PLAU	-1.58	1.41
NFKB1	-1.26	1.05
GREM1	-1.28	1.28
CEBPB	-1.16	2.44
TIMP4	-1.25	1.67
IL13RA2	-1.25	1.90

Figure 8



c

PTCH1 gene expression				
Hh Induction Stimulus	**Inhibitor**	**Average RLQ**	**Standard Deviation**	**N**
Untreated	Untreated	1.00	0.03	3
Shh-N (1mg/mL)	DMSO	2.55	0.24	3
Shh-N (1mg/mL)	5μM Cyclopamine	1.00	0.22	3
Shh-N (1mg/mL)	1μM Itraconazole	0.96	0.18	3

GLI1 gene expression				
Hh Induction Stimulus	**Inhibitor**	**Average RLQ**	**Standard Deviation**	**N**
Untreated	Untreated	1.00	0.04	3
Shh-N (1mg/mL)	DMSO	13.00	2.92	3
Shh-N (1mg/mL)	5μM Cyclopamine	0.21	0.15	3
Shh-N (1mg/mL)	1μM Itraconazole	0.32	0.02	3

Figure 9

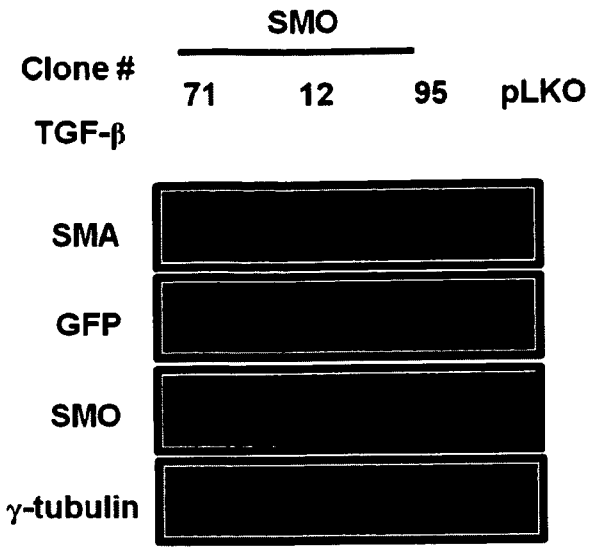


Figure 10

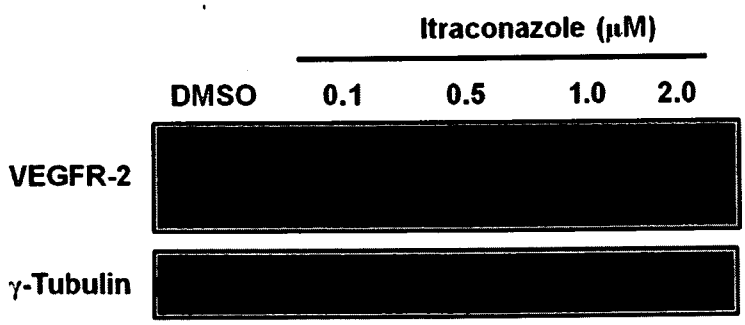
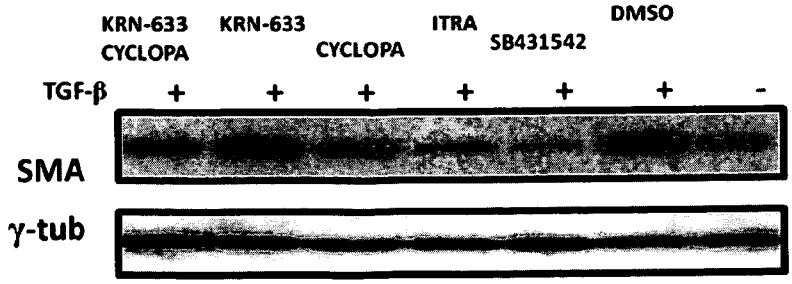


Figure 11



KRN-633 (0.5 μM) pan-VEGFR; CYCLOPA (5 μM);
ITRA (1 μM); SB431542 (10 μM)

Figure 12

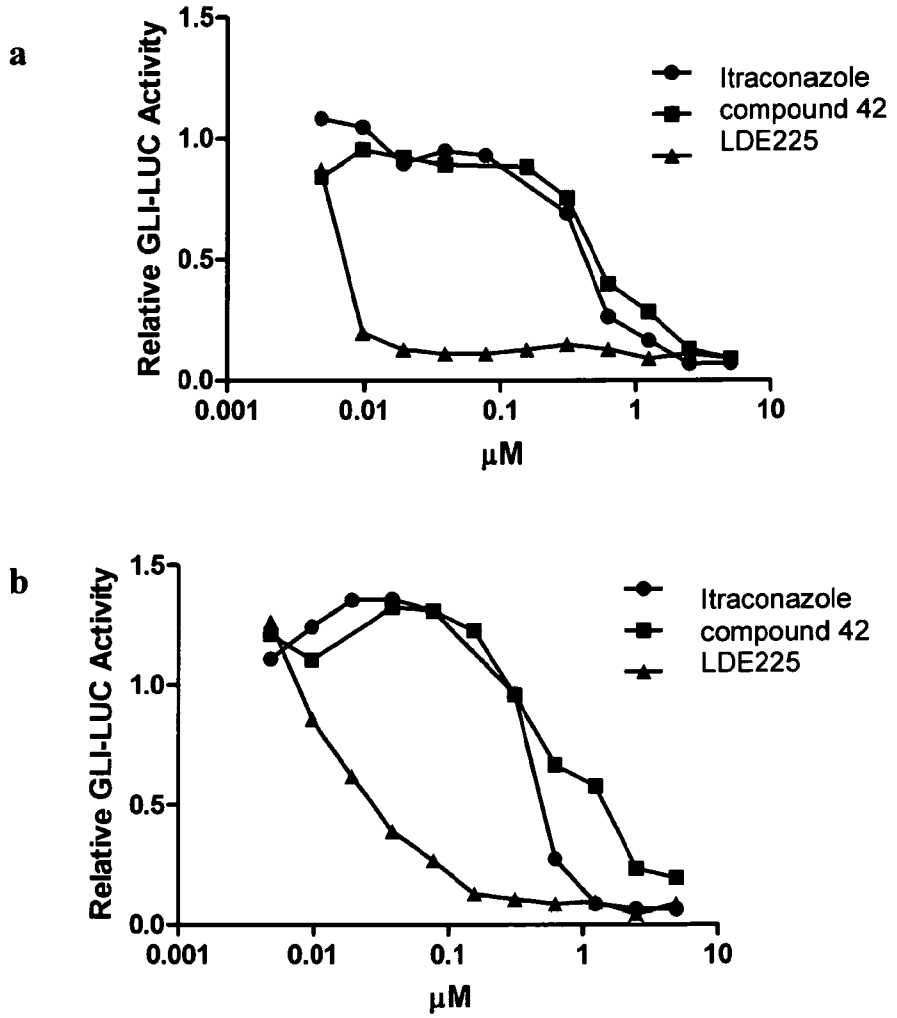


Figure 13

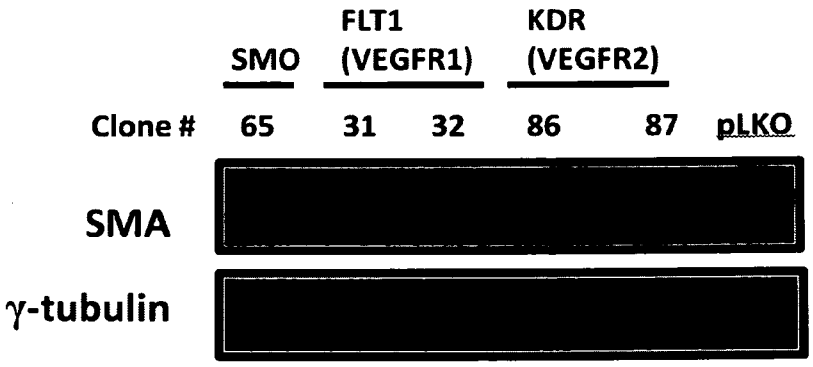


Figure 14

Experimental groups:

- **A- Vehicle implantation**
- **B-25 mg/kg Bleo + Vehicle 1 (45% beta-cycledextrin) SID**
- **C-25 mg/kg Bleo + Vehicle 2 (0.5% MC/0.5% Tween-80) BID**
- **D-25 mg/kg Bleo + Itra 5 mg/kg SID**
- **E-25 mg/kg Bleo + Itra 10 mg/kg SID**
- **F-25 mg/kg Bleo + Itra 25 mg/kg SID**
- **G-25 mg/kg Bleo + Itra 50 mg/kg SID**
- **H-25 mg/kg Bleo + Cmpd #42 @ 5 mg/kg SID**
- **I-25 mg/kg Bleo + Cmpd #42 @ 10 mg/kg SID**
- **J-25 mg/kg Bleo + Cmpd #42 @ 25 mg/kg SID**
- **K-25 mg/kg Bleo + Cmpd #42 @ 50 mg/kg SID**
- **L-25 mg/kg Bleo + Pifenidone 400 mg/kg BID**
- **M-25 mg/kg Bleo + AMI 152 30 mg/kg BID**

Experimental design:

- 104 - 9wk old B6 male mice (Taconic Farms); n=8/ group
- 2 days prior: weigh & single house male B6 mice, 104 total, 8 per group.
- Day 1- Weigh, fill 25 mg/kg bleo into 200ul 7 days Alzet Osmotic pump, prime the filled pumps
- Day 1-2: Surgically implant pumps, 8 mice with saline, 96 mice with bleo.
- Day 1-10: Post surgery monitoring/weigh mice 3 times per week
- Day 17-31: Drug treatment
- Day 30: Bleed at 1, 4, 8, 24 hours after the second last dosing.
- Day 31: Euthanize animals and collect multiple organs. Half of the lungs will be fixed for histology study, half of the lungs will be collected on liquid N2 and stored in -80 for biochemical study. One piece of skin will be fixed for histological study and another piece of skin will be collected on liquid N2 and stored in -80 for biochemical study.

SUBSTITUTE SHEET (RULE 26)

Figure 15

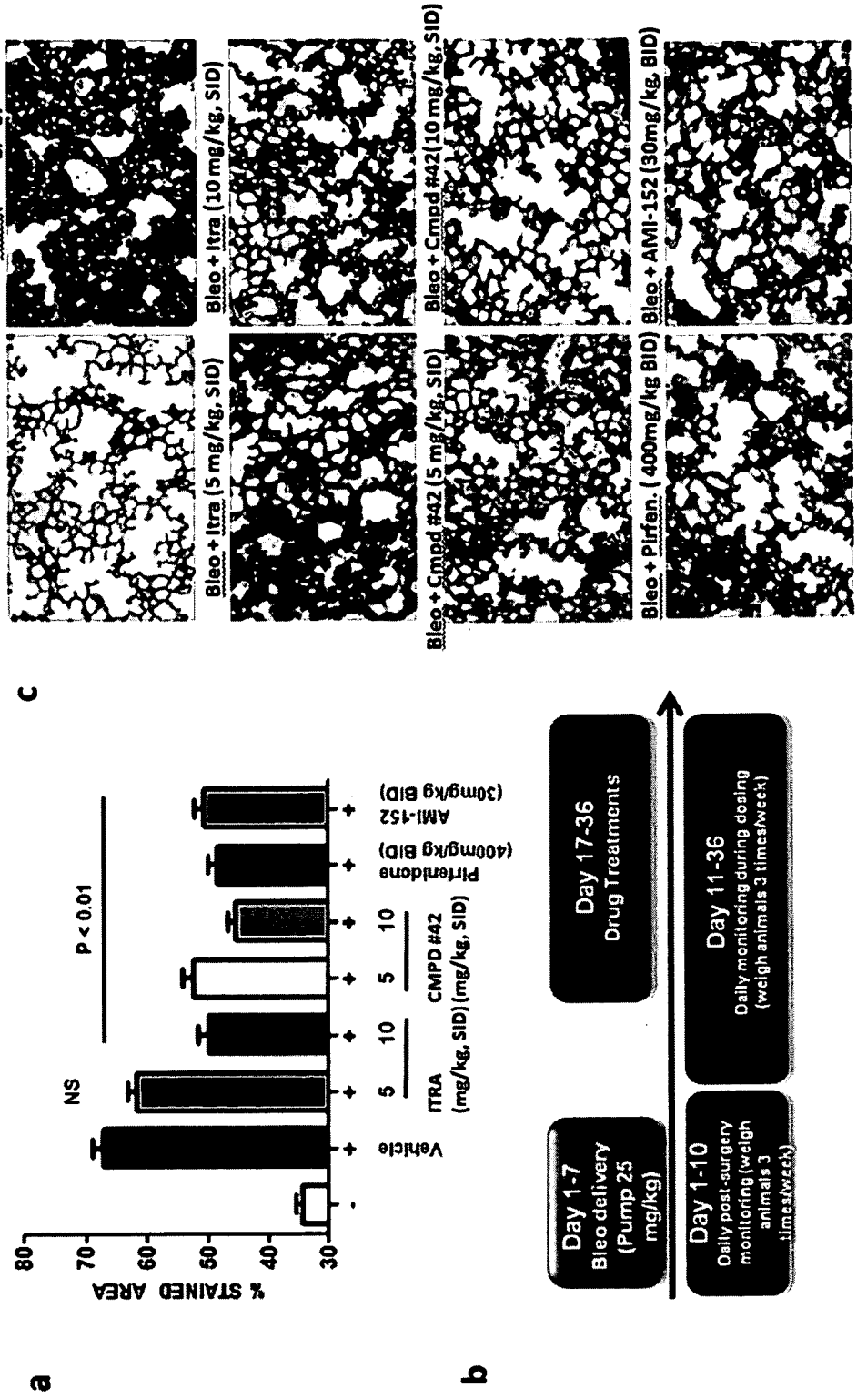

a
% STAINED AREA
80
70
60
50
40
30
P < 0.01
NS
-
Vehicle
ITRA (mg/kg, SID)
5
10
CMPD #42 (mg/kg, SID)
5
10
Pirfenidone (400mg/kg BID)
AMI-152 (30mg/kg BID)
b
Day 1-7
Bleo delivery (Pump 25 mg/kg)
Day 1-10
Daily post-surgery monitoring (weigh animals 3 times/week)
Day 17-36
Drug Treatments
Day 11-36
Daily monitoring during dosing (weigh animals 3 times/week)
c
Sham
Bleo (25 mg/kg)
Bleo + Itra (5 mg/kg, SID)
Bleo + Itra (10 mg/kg, SID)
Bleo + Cmpd #42 (5 mg/kg, SID)
Bleo + Cmpd #42 (10 mg/kg, SID)
Bleo + Pirfen. (400mg/kg BID)
Bleo + AMI-152 (30mg/kg, BID)

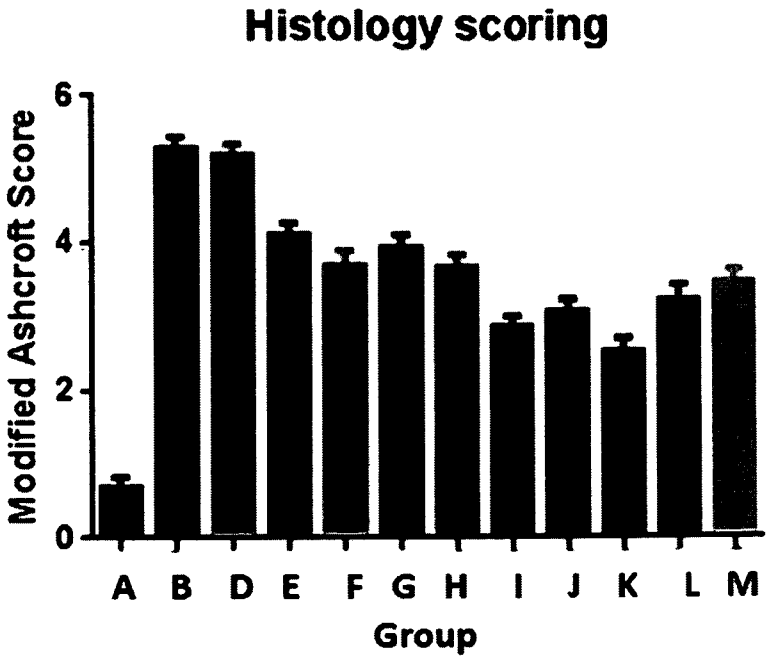
Figure 16

Characterization of the Modified Ashcroft Scale

Grade of fibrosis	Modified Ashcroft Scale
0	**Alveolar septa:** No fibrotic burden at the most flimsy small fibers in some alveolar walls **Lung structure:** Normal lung
1	**Alveolar septa:** Isolated gentle fibrotic changes (septum ≤ 3x thicker than normal) **Lung structure:** Alveoli partly enlarged and rarefied, but no fibrotic masses present
2	**Alveolar septa:** Clearly fibrotic changes (septum > 3x thicker than normal) with knot-like formation but not connected to each other **Lung structure:** Alveoli partly enlarged and rarefied, but no fibrotic masses
3	**Alveolar septa:** Contiguous fibrotic walls (septum > 3x thicker than normal) predominantly in whole microscopic field **Lung structure:** Alveoli partly enlarged and rarefied, but no fibrotic masses
4	**Alveolar septa:** Variable Lung structure: Single fibrotic masses (≤ 10% of microscopic field)
5	Alveolar septa: Variable Lung structure: Confluent fibrotic masses (> 10% and ≤ 50 % of microscopic field). Lung structure severely damaged but still preserved
6	Alveolar septa: Variable, mostly not existent Lung structure: Large contiguous fibrotic masses (> 50 % of microscopic field). Lung architecture mostly not preserved
7	Alveolar septa: Non-existent Lung structure: Alveoli nearly obliterated with fibrous masses but still up to five air bubbles
8	Alveolar septa: Non-existent Lung structure: Microscopic filed with complete obliteration with fibrotic masses

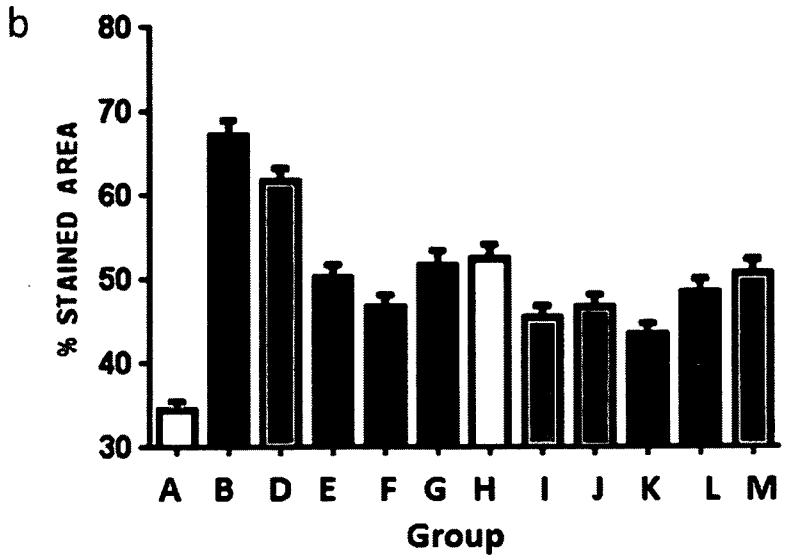
Figure 17a

a



A- Vehicle implantation
B-25 mg/kg Bleo + Vehicle 1 (45% beta-cycledextrin) SID
D-25 mg/kg Bleo + Itraconazole 5 mg/kg SID
E-25 mg/kg Bleo + Itraconazole 10 mg/kg SID
F-25 mg/kg Bleo + Itraconazole 25 mg/kg SID
G-25 mg/kg Bleo + Itraconazole 50 mg/kg SID
H-25 mg/kg Bleo + Cmpd #42 @ 5 mg/kg SID
I-25 mg/kg Bleo + Cmpd #42 @ 10 mg/kg SID
J-25 mg/kg Bleo + Cmpd #42 @ 25 mg/kg SID
K-25 mg/kg Bleo + Cmd #42 @ 50 mg/kg SID
L-25 mg/kg Bleo + Pifenidone 400 mg/kg BID
M-25 mg/kg Bleo + AMI 152 30 mg/kg BID

Figure 17b



A- Vehicle implantation
B-25 mg/kg Bleo + Vehicle 1 (45% beta-cycledextrin) SID
D-25 mg/kg Bleo + Itraconazole 5 mg/kg SID
E-25 mg/kg Bleo + Itraconazole 10 mg/kg SID
F-25 mg/kg Bleo + Itraconazole 25 mg/kg SID
G-25 mg/kg Bleo + Itraconazole 50 mg/kg SID
H-25 mg/kg Bleo + #42 @ 5 mg/kg SID
I-25 mg/kg Bleo + Cmpd #42 @ 10 mg/kg SID
J-25 mg/kg Bleo + Cmpd #42 @ 25 mg/kg SID
K-25 mg/kg Bleo + Cmpd #42 @ 50 mg/kg SID
L-25 mg/kg Bleo + Pifenidone 400 mg/kg BID
M-25 mg/kg Bleo + AMI 152 30 mg/kg BID

Figure 18

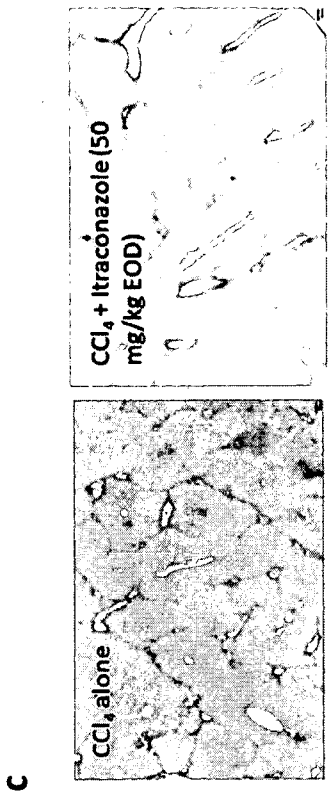
Experimental groups:

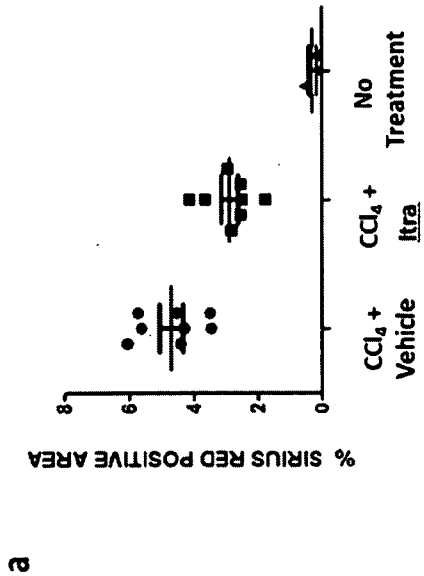
- A-0.4 ml/kg CCl4 + non treatment, n=8
- B-0.4 ml/kg CCl4 + Itraconazole, 10 mg/kg, SID, n=8
- C-0.4 ml/kg CCl4 + Itraconazole, 20 mg/kg, SID, n=8
- D-0.4 ml/kg CCl4 + Itraconazole, 50 mg/kg, SID, n=8

Experimental design:

- 32 - 9wk old B6 male mice (Taconic Farms); n=8/group
- 2 days prior: weigh & sort male B6 mice, 32Ototal, 8 per group
- Day 1 - 35: 0.4 ml/kg CCl4 diluted in olive oil or olive oil alone will be given i.p. at 5ml/kg twice a week for 5 weeks.
- Day 15-35: Drug treatment
- Day 36: Euthanize animals and collect livers. Half of the livers will be fixed for histology study, another half will be collected on liquid N2 and stored in -80 for biochemical study.

Figure 19





b

	Mean Stained Area	Standard Deviation	N (animals)
Vehicle + CCL4	4.73	1.00	8
Itraconazole (50mpk) + CCL4	2.89	0.74	8
Not Treated	0.34	0.21	3

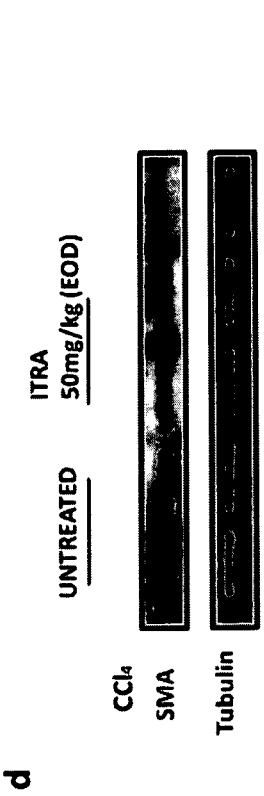


Figure 20

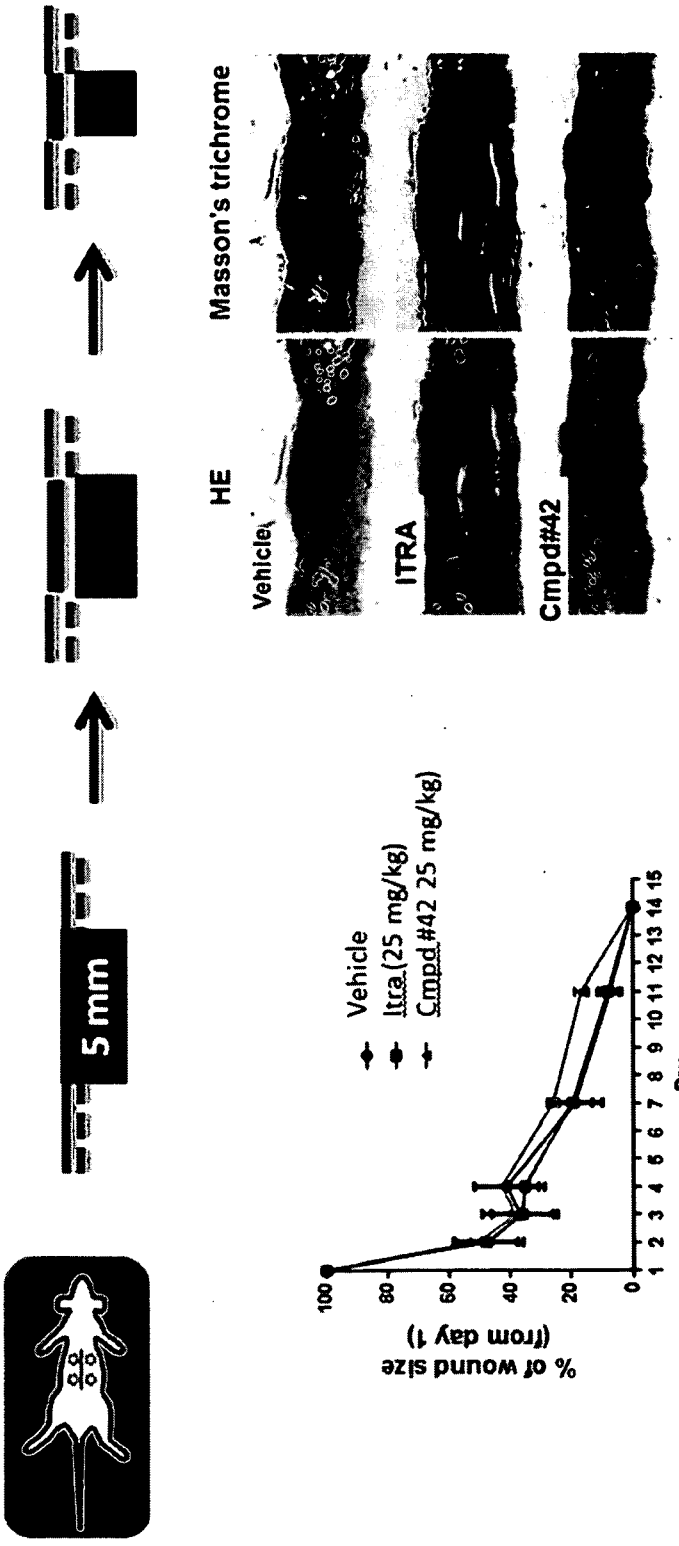
Experimental groups:

- A. Vehicle (40% beta-cyclodextrin), SID, n=6
- B. Itraconazole 25 mg/kg, SID, n=6
- C. Cmpd #42 @ 25 mg/kg, SID, n=6

Experimental design:

- 5 days prior, mice will be anesthetized and the back skin hair will be removed using Nair
- Day 1- Weigh. One sterile biopsy punch (5 mm diameter) will be made by punching through the full thickness of the folded back skin (2 x 5 mm dial holes in total).
- The size of the wounds will be measured daily using calipers.
- Body weight will be monitored twice a week.
- Day 1- end: Drug treatment daily using 5ml/kg dosing volume.
- Day 7: PK bleed all animals at 1 hour after the dosing of that day. Euthanize 2 animals from each group and collect wounds.
- Day 11: PK bleed all animals at 1 hour after the dosing of that day. Euthanize 2 animals from each group and collect wounds.
- Day 14: PK bleed all animals at 1 hour after the dosing of that day. Euthanize 2 animals from each group and collect wounds.
- When collecting wounds, cut out the wounds including a few mm of the surrounding skin. For each animal, two pieces of wound will be collected and fixed for histology analysis.

Figure 21



		Day 2	Day 3	Day 4	Day 7	Day 11	Day 14
Vehicle	Mean % of original wound size	47.12	36.91	41.29	18.57	7.20	0.00
	± SEM	3.44	3.76	3.18	2.64	1.32	0.00
	N	12	12	12	12	8	4
Itra	Mean % of original wound size	47.95	36.11	35.25	19.51	7.88	0.00
	± SEM	3.10	3.06	2.00	1.99	1.27	0.00
	N	12	12	12	12	8	4
Cmpd #42	Mean % of original wound size	50.53	38.07	43.00	25.84	16.53	0.00
	± SEM	2.74	1.86	1.69	2.17	2.40	0.00
	N	12	12	12	12	8	4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/41174

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/40 (2014.01)
CPC - C07D 405/12; C07D 409/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/336, 546/269.7, 548/544, 548/183, 514/369
IPC: A01N 43/40 (2014.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C07D 405/12; C07D 409/12 (See Search Words Below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PATBASE: Full-text = AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO
Google: Scholar/Patents:itraconazole analogs fibrosis disorders tetrazole pyrazole dioxolane phenyl hedgehog inhibitors 1,2,4-tetrazole 1,3,4-tetrazole piperidine piperazine difluorophenyl

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WO2013/036866 A1 (LIU et al.) 14 March 2013 (14.03.2013) para [0060]-[0061];[0065]; pg 41, Table III, Compound 1a	40-41 ---------- 1-5, 31, 42-44, 73
Y	US 2011/0183948 A1 (LEVINE et al.) 28 July 2011 (28.07.2011) para [0005];[0009]	1-5, 31
Y	US 2010/0286114 A1 (THOMAS et al.) 11 November 2010 (11.10.2010) para [0049]; pg 42, Compound 69	3-4, 42-44
Y	US 2004/0019211 A1 (REMENAR et al.) 29 January 2004 (29.01.2004) para [0101];[0107];[0120];[0121]; pg 3, formula III	31, 73

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2014 (30.08.2014)	09 SEP 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/41174

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-30; 32-39; 45-72; 74
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105392364 A
(43)申请公布日 2016.03.09

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(86)PCT国际申请的申请数据
PCT/US2014/041174 2014.06.05
(87)PCT国际申请的公布数据
WO2014/197738 EN 2014.12.11
(71)申请人 加州生物医学研究所
地址 美国加利福尼亚州
申请人 斯克利普斯研究所
(72)发明人 卢克·莱尔森 迈克尔·博尔龙
彼得·G·舒尔茨
阿纳布·K·查特吉 百元·杨
普内特·库马尔 卡维利·乌尔卡兰
(74)专利代理机构 北京安信方达知识产权代理有限公司 11262
代理人 郑霞
(51)Int.Cl.
A01N 43/40(2006.01)

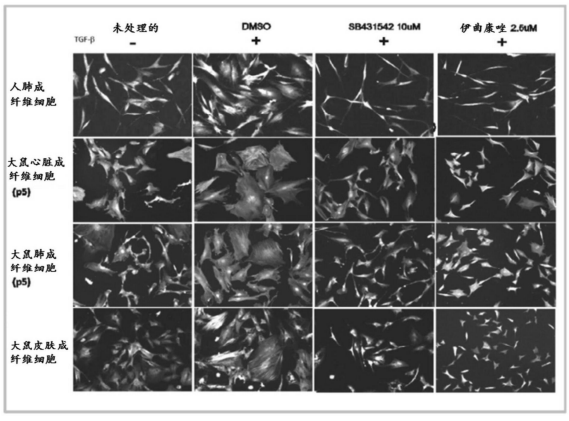
权利要求书32页 说明书91页 附图20页

(54)发明名称

纤维化的小分子抑制剂

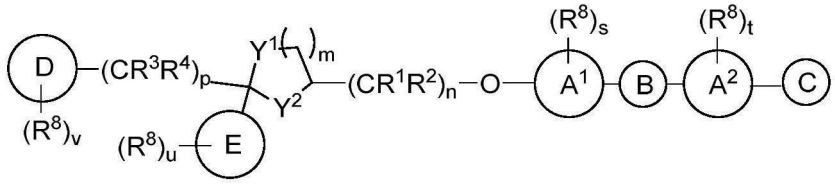
(57)摘要

本文公开了用于治疗纤维化疾病的化合物和组合物。



CN 105392364 A

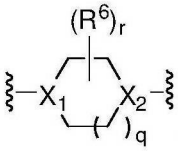
1.一种治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病的方法，该方法包括施用包含治疗有效量的式（I）化合物、其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N-氧化物、立体异构体或异构体的组合物：



式(I)

其中：

A^1和A^2独立地选自芳基或杂芳基；

B为  ；

C为任选取代的5或6元杂环基或任选取代的5或6元杂芳基，其中该杂环基或该杂芳基含有1至4个氮原子；

D为芳基或杂芳基；

E为芳基、杂芳基、碳环基、杂环基或烷基；

每个R^1、R^2、R^3和R^4独立地选自H、烷基、卤代烷基或烷氧基；

X_1和X_2独立地选自N和CR^5；

R^5为H、OH、烷基或烷氧基；

每个R^6独立地为烷基、卤代烷基、卤代、烷氧基、-亚烷基($NR^{13}R^{14}$)或芳基；

每个R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、-亚烷基(环烷基)、-亚烷基(杂环基)、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和$-C(O)NR^{13}R^{14}$；或者两个相邻的$R^8$形成杂环基环；

每个R^{13}和R^{14}独立地选自H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者R^{13}和R^{14}与它们所连接至的原子一起形成杂环；

Y^1和Y^2独立地选自O、CH_2、NH和NR^{13}；

n为1、2或3；

m为1或2；

p为1、2、3或4；

q为1、2或3；

r为0、1、2、3、4、5、6、7或8；

s为0、1、2、3或4；

t为0、1、2、3或4；

u 为 0、1、2、3、4 或 5；且

v 为 0、1、2、3 或 4。

2. 如权利要求 1 所述的方法，其中 X_1 和 X_2 为 N。

3. 如权利要求 1 所述的方法，其中 X_1 为 CR^5 且 X_2 为 N。

4. 如权利要求 1 所述的方法，其中 X_1 为 N 且 X_2 为 CR^5。

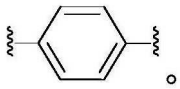
5. 如权利要求 2-4 中任一项所述的方法，其中 q 为 1 且 r 为 0。

6. 如权利要求 1-5 中任一项所述的方法，其中 A^1 为芳基。

7. 如权利要求 6 所述的方法，其中 A^1 为  。

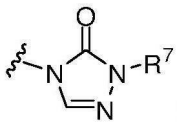
8. 如权利要求 1-5 中任一项所述的方法，其中 A^1 为杂芳基。

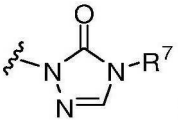
9. 如权利要求 1-8 中任一项所述的方法，其中 A^2 为芳基。

10. 如权利要求 9 所述的方法，其中 A^2 为  。

11. 如权利要求 1-8 中任一项所述的方法，其中 A^2 为杂芳基。

12. 如权利要求 11 所述的方法，其中 A^2 为吡啶、吡嗪、嘧啶、哒嗪或三嗪。

13. 如权利要求 1-12 中任一项所述的方法，其中 C 为  且 R^7 为烷基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、环烷基、杂环基、- 亚烷基（环烷基）或 - 亚烷基（杂环基）。

14. 如权利要求 1-12 中任一项所述的方法，其中 C 为  且 R^7 为烷基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、环烷基、杂环基、- 亚烷基（环烷基）或 - 亚烷基（杂环基）。

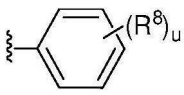
15. 如权利要求 1-14 中任一项所述的方法，其中 E 为烷基。

16. 如权利要求 1-14 中任一项所述的方法，其中 E 为环烷基。

17. 如权利要求 16 所述的方法，其中 E 为环丙基、环丁基、环戊基或环己基。

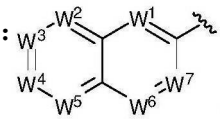
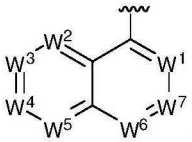
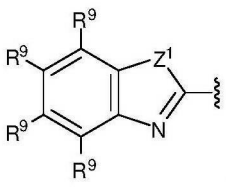
18. 如权利要求 1-14 中任一项所述的方法，其中 E 为杂环基。

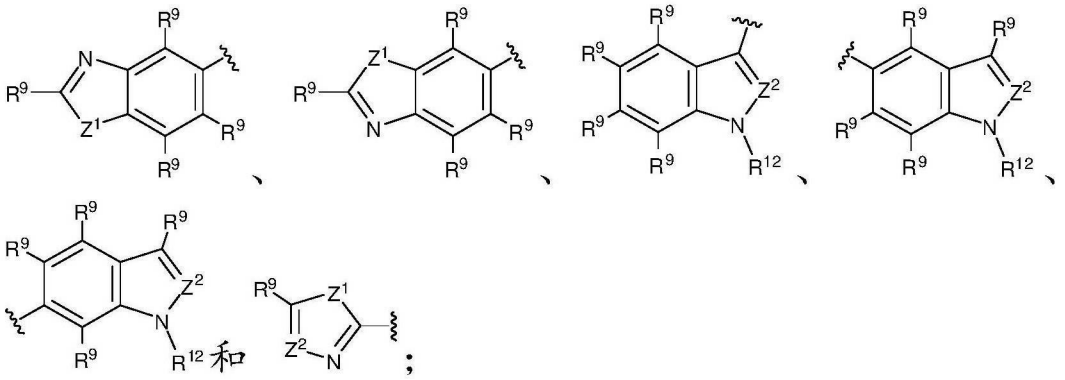
19. 如权利要求 1-14 中任一项所述的方法，其中 E 为芳基。

20. 如权利要求 19 所述的方法，其中 E 为  且 u 为 0、1、2、3、4 或 5。

21. 如权利要求 1-14 中任一项所述的方法，其中 E 为杂芳基。

22. 如权利要求 21 所述的方法，其中

E 选自：  、  、  、



W^1、W^2、W^3、W^4、W^5、W^6和 W^7独立地选自 N 和 CR^9；

Z^1为 NR^{12}、S 或 O；

Z^2为 N 或 CR^9；

每个 R^9独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基；

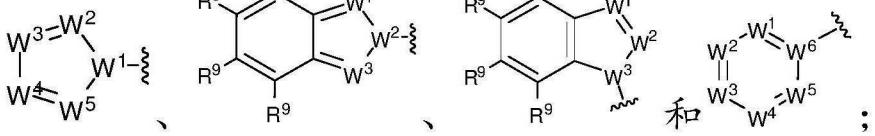
每个 R^{10}和 R^{11}独立地选自 H 和烷基；或者 R^{10}和 R^{11}与它们所连接至的氮一起形成杂环；且

R^{12}为 H、烷基或卤代烷基。

23. 如权利要求 1-22 中任一项所述的方法，其中 D 为芳基。

24. 如权利要求 1-22 中任一项所述的方法，其中 D 为杂芳基。

25. 如权利要求 24 所述的方法，其中

D 选自 

W^1、W^2、W^3、W^4和 W^5独立地选自 N 和 CR^9；

W^6为 N 或 C；且

每个 R^9独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷

基）、$-NR^{10}SO_2$（环烷基）、$-NR^{10}SO_2$（杂环烷基）、$-NR^{10}SO_2$（芳基）、$-NR^{10}SO_2$（杂芳基）、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$（环烷基）、$-SO_2NR^{10}$（杂环烷基）、$-SO_2NR^{10}$（芳基）、$-SO_2NR^{10}$（杂芳基）、卤代烷基、芳基和杂芳基。

26. 如权利要求 1-25 中任一项所述的方法，其中 Y^1 和 Y^2 为 O。

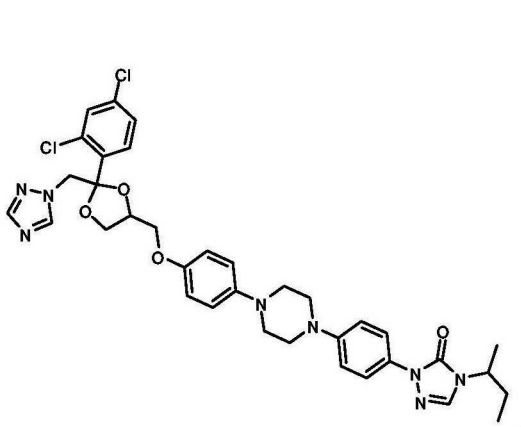
27. 如权利要求 26 所述的方法，其中 m 为 1。

28. 如权利要求 1-27 中任一项所述的方法，其中 p 为 1、2 或 3。

29. 如权利要求 28 所述的方法，其中 p 为 1。

30. 如权利要求 1-29 中任一项所述的方法，其中 R^1、R^2、R^3 和 R^4 为氢。

31. 如权利要求 1 所述的方法，其中所述化合物选自：



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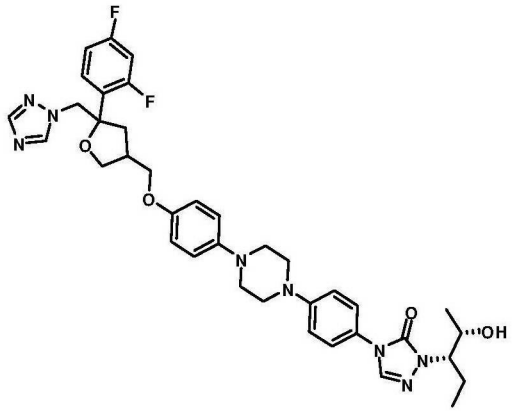

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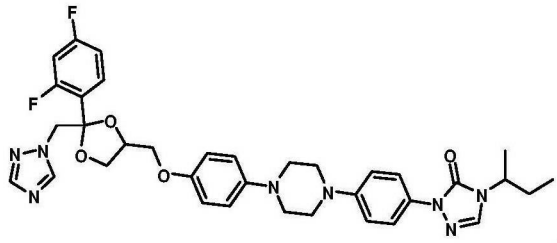

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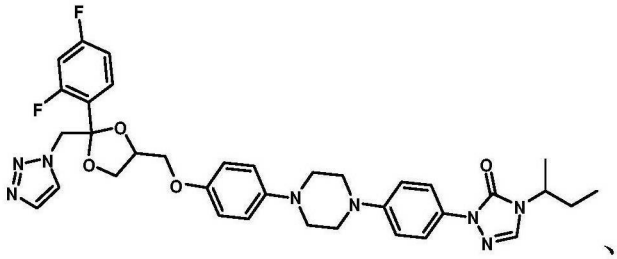

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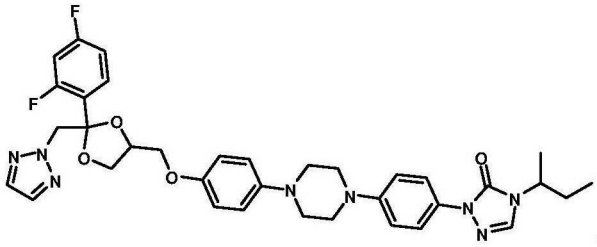

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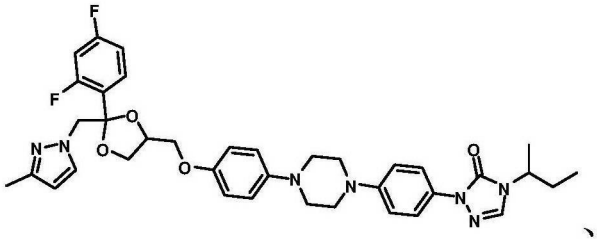

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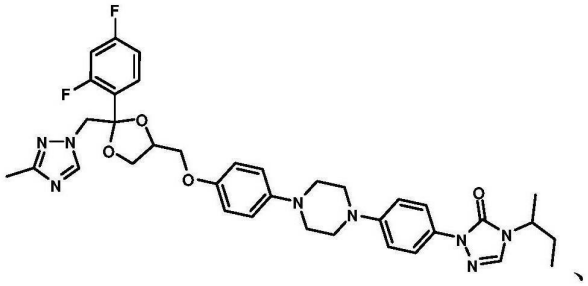

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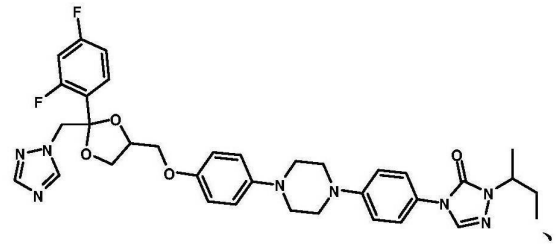

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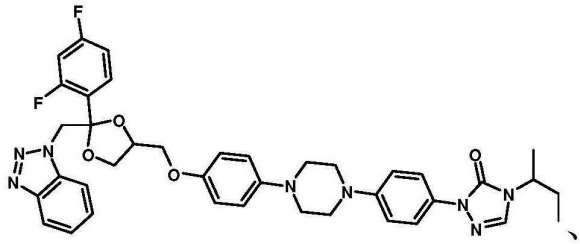

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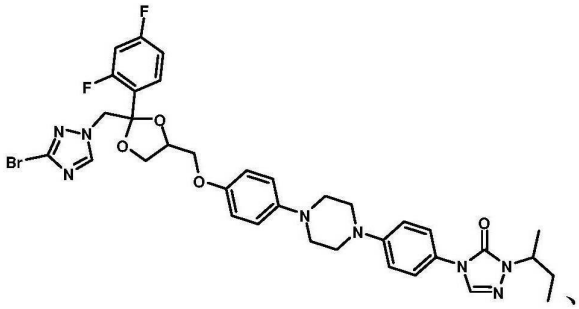

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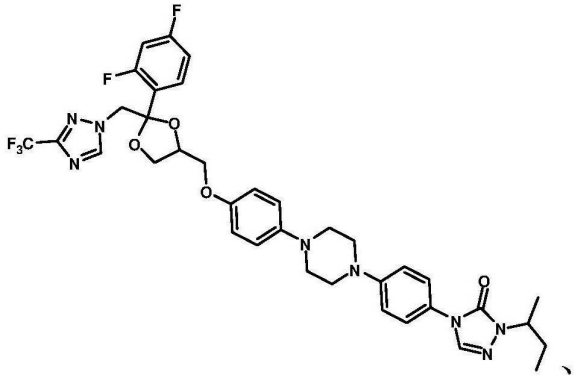

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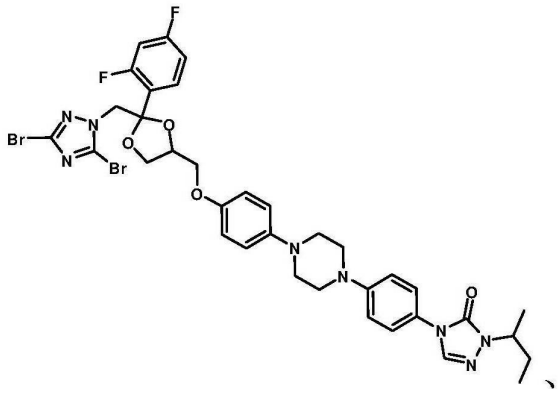

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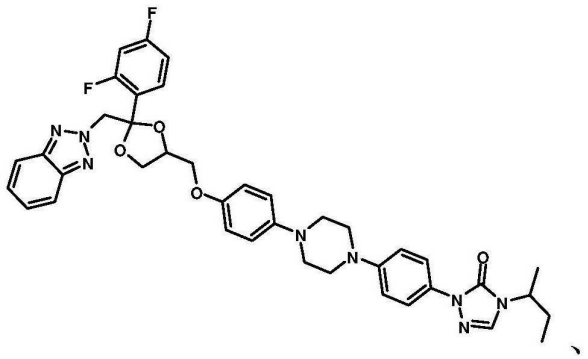

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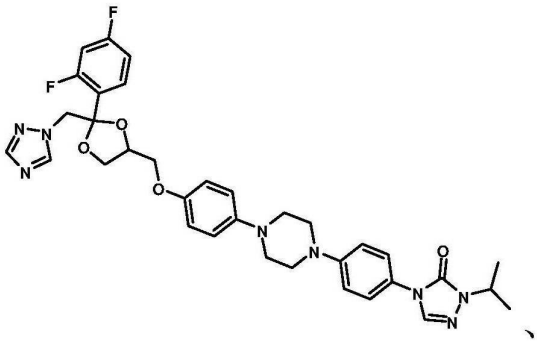

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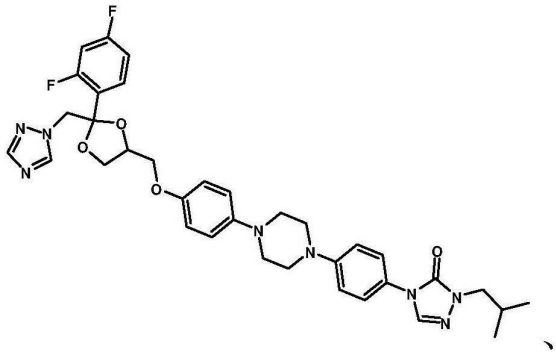

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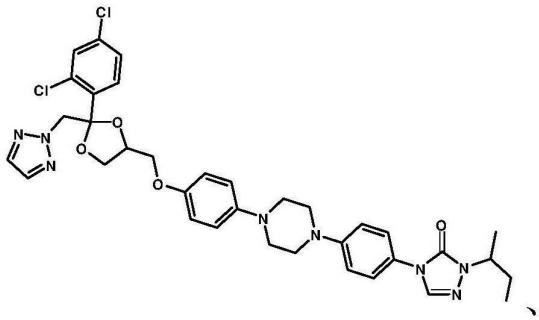

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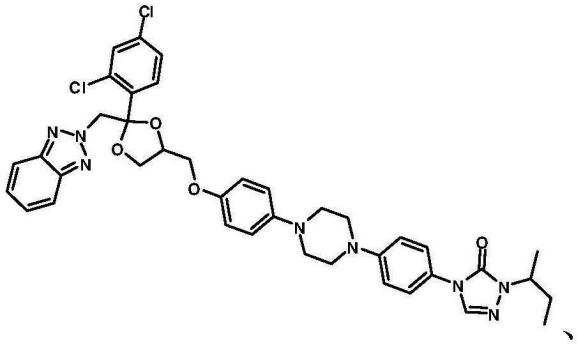

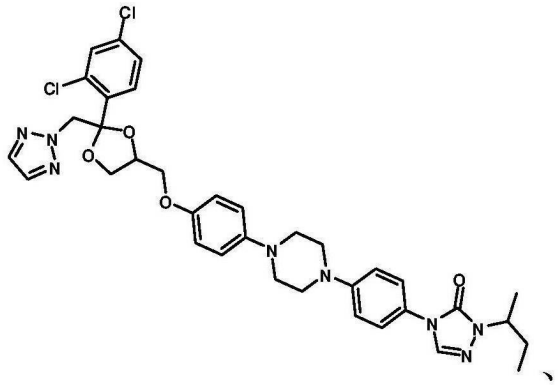

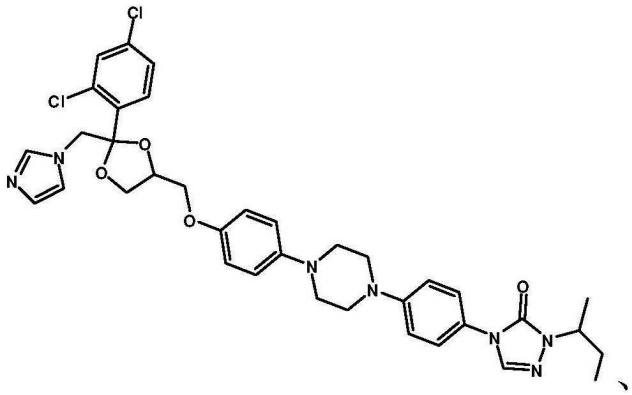

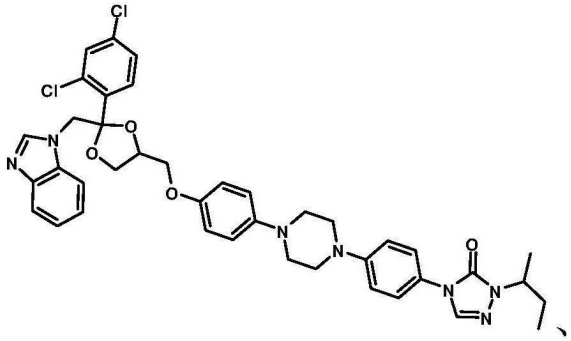

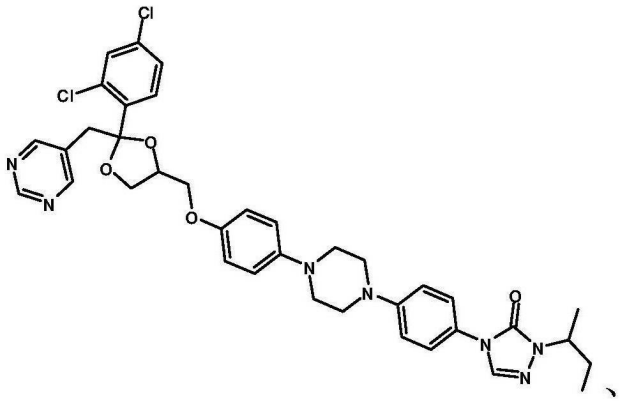

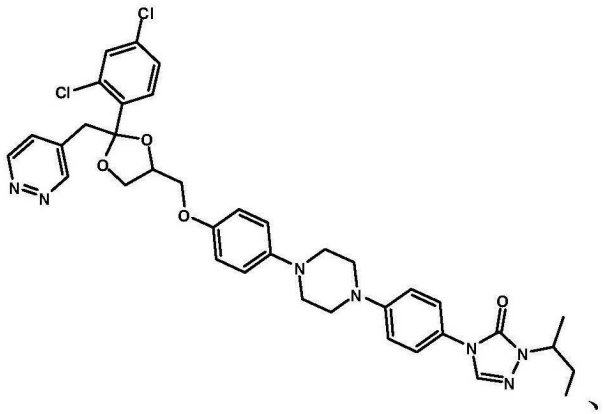

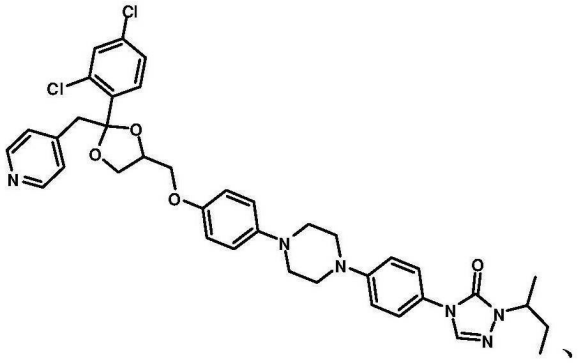

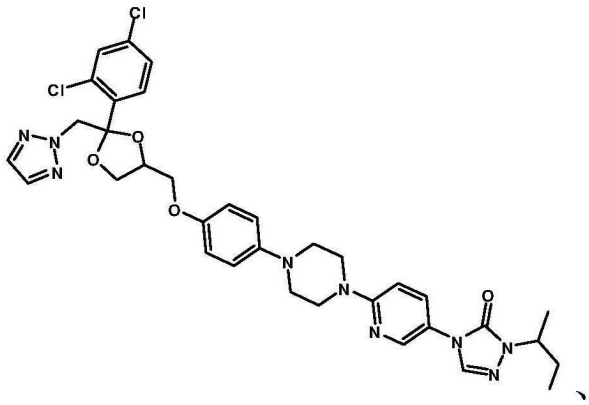

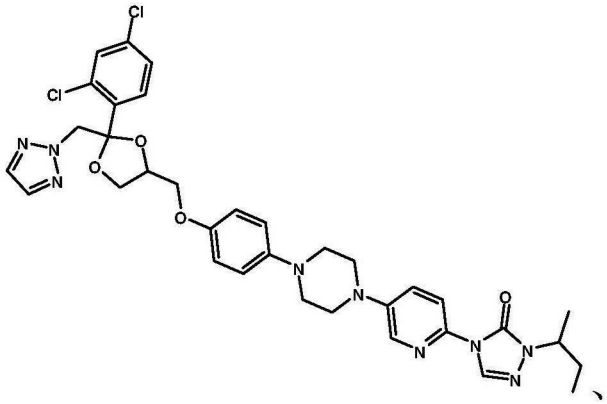

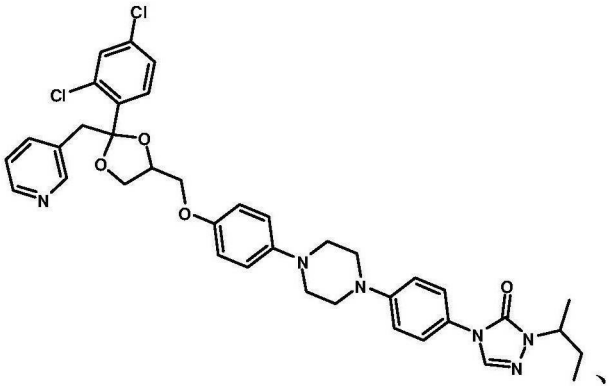

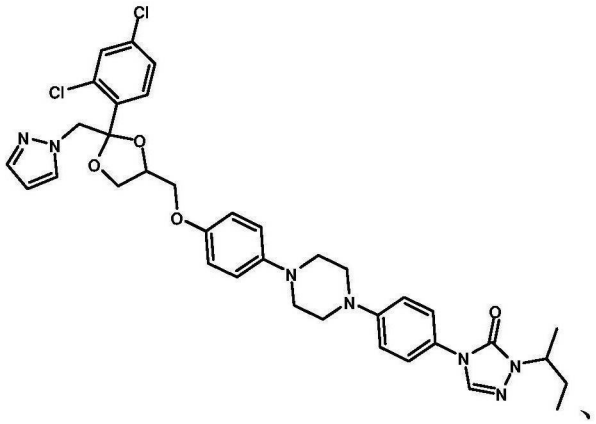

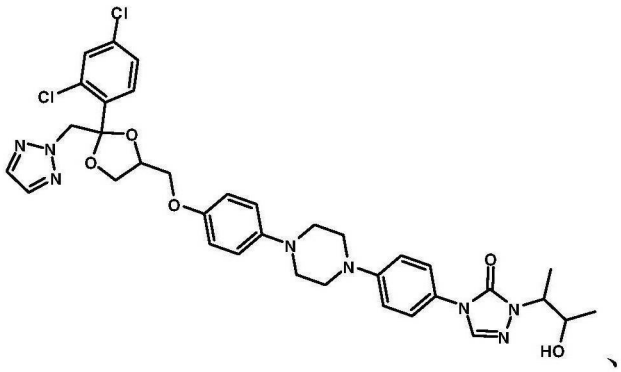

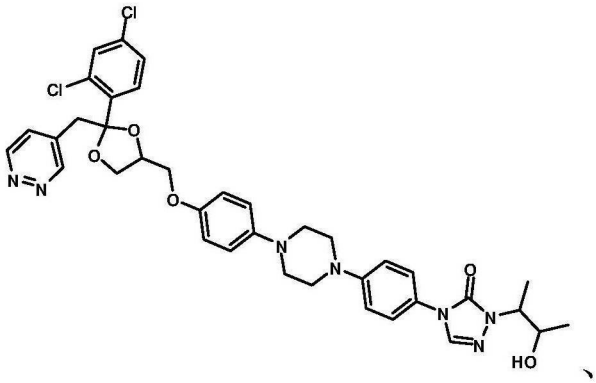

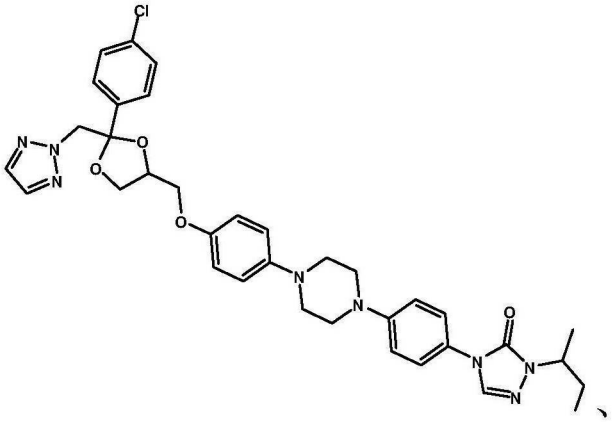

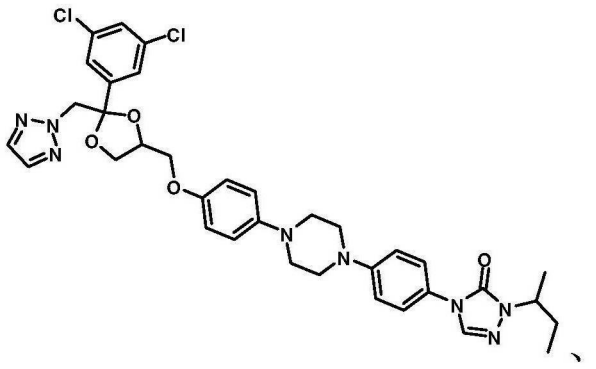

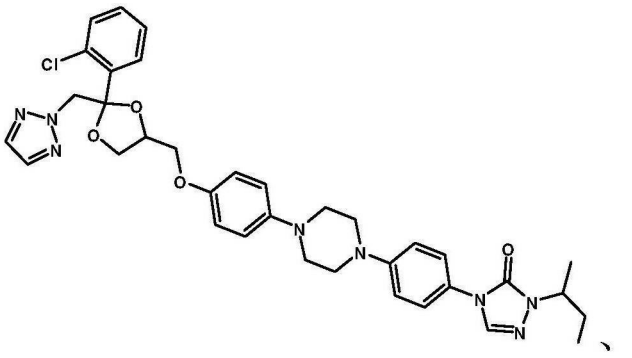

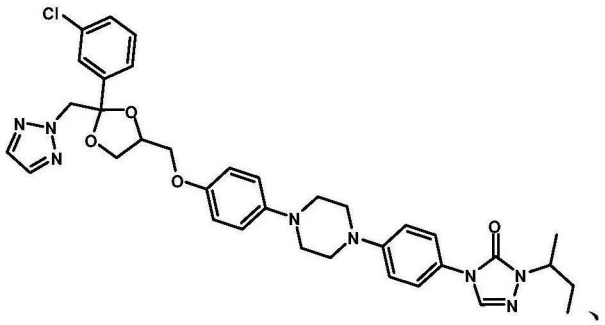

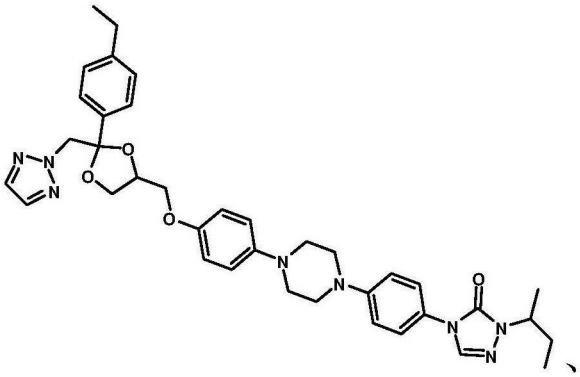

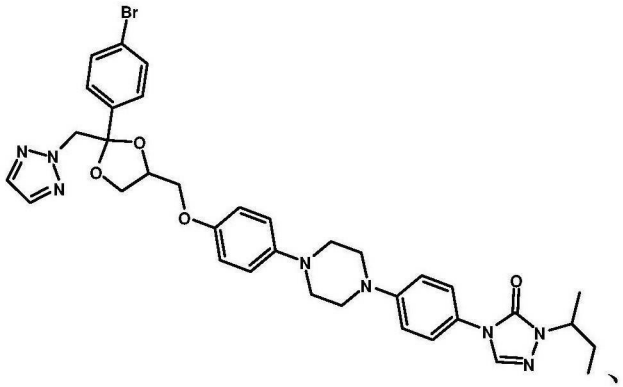

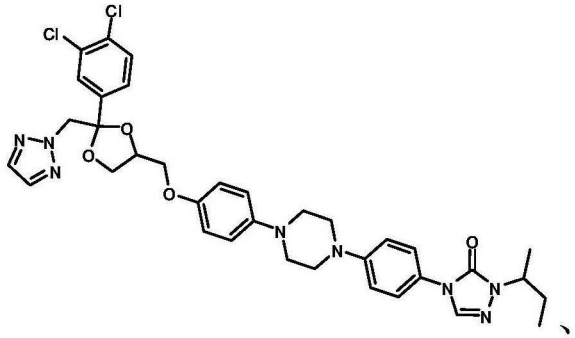

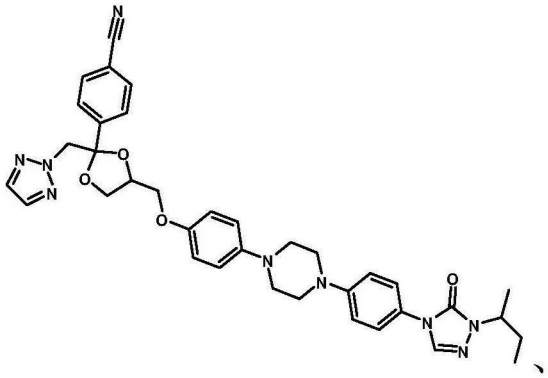

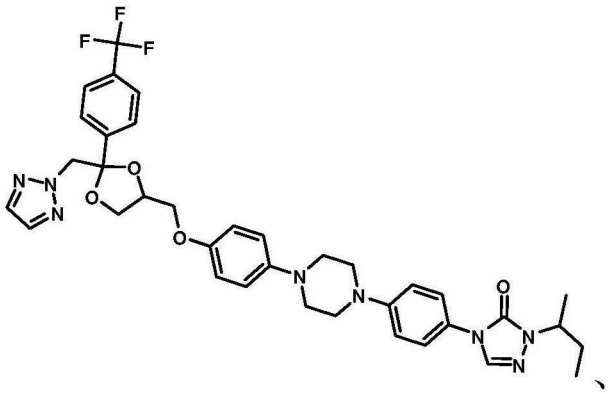

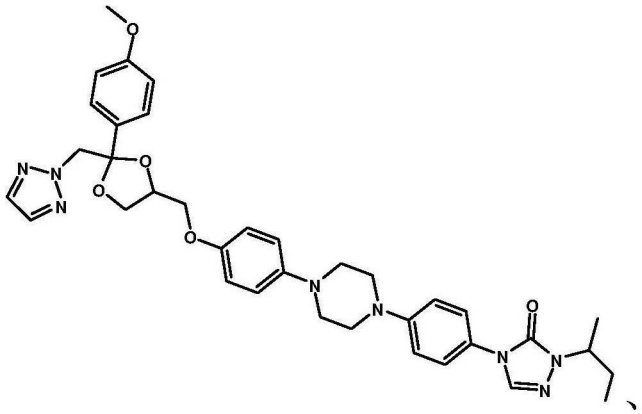

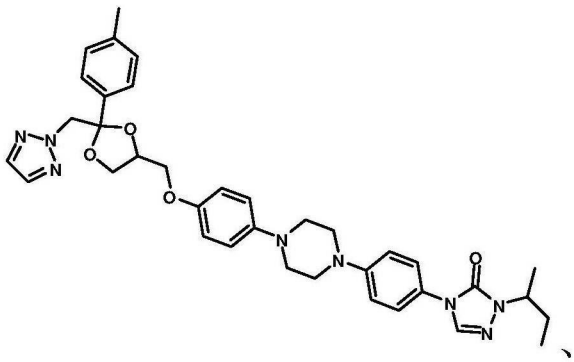

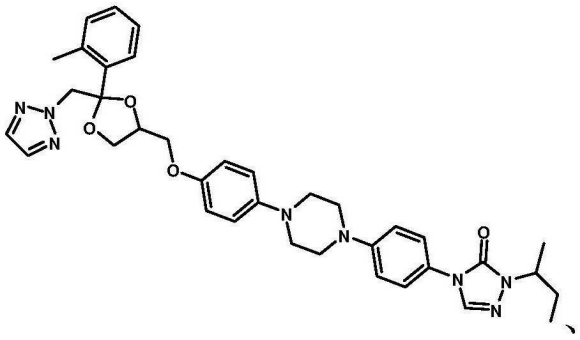

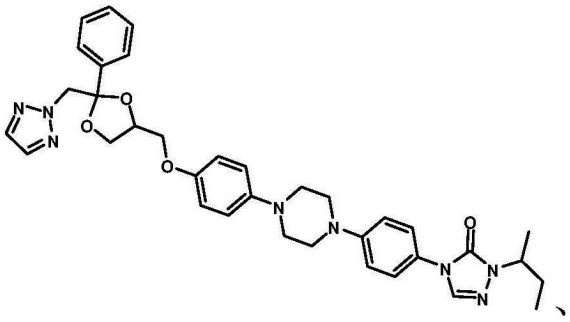

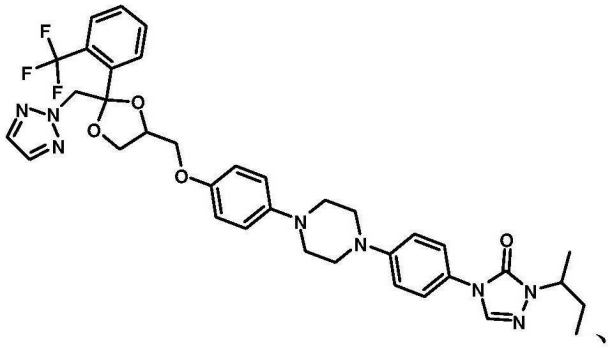

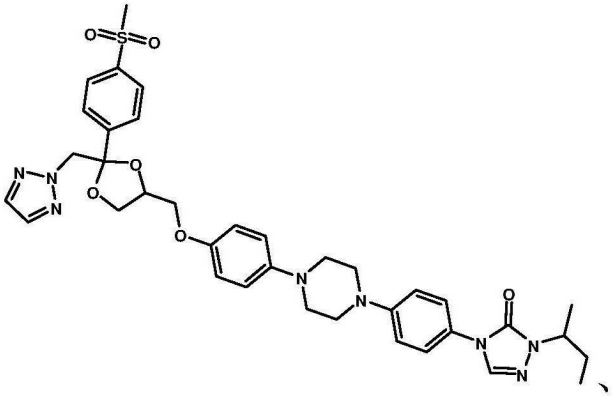

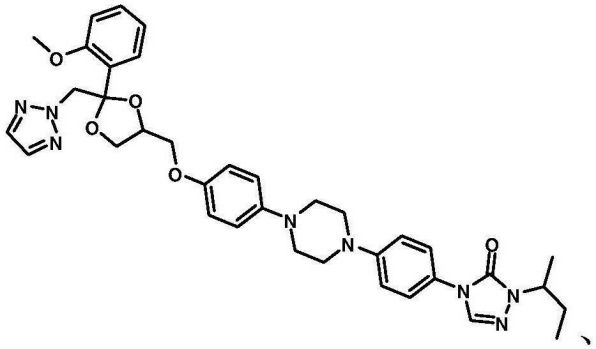

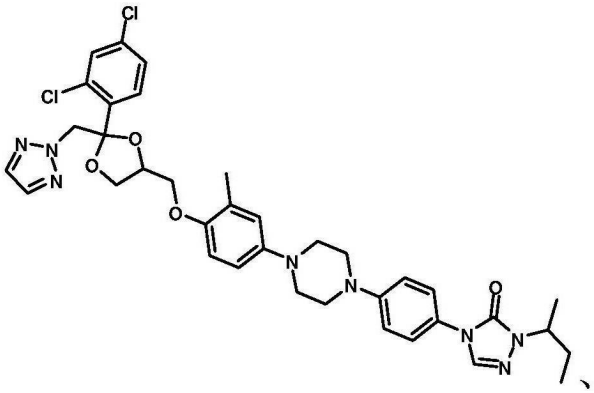

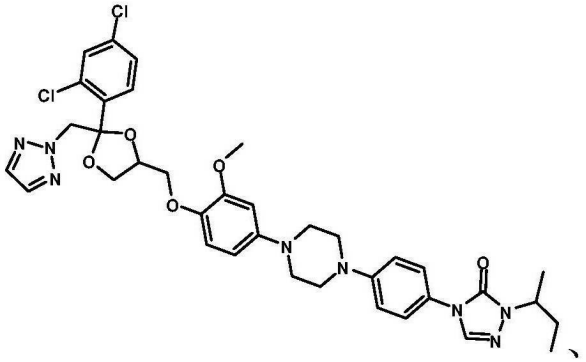

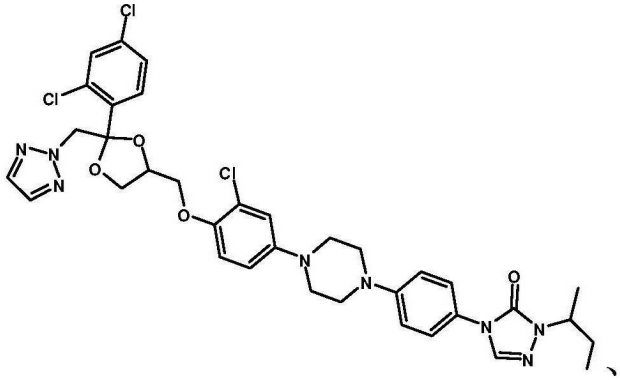

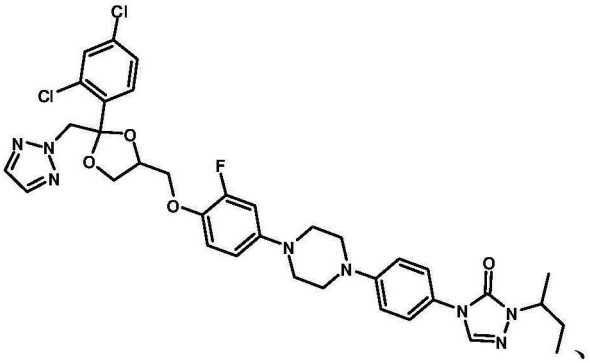

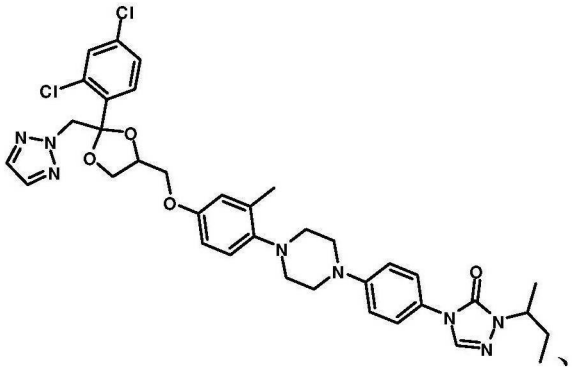

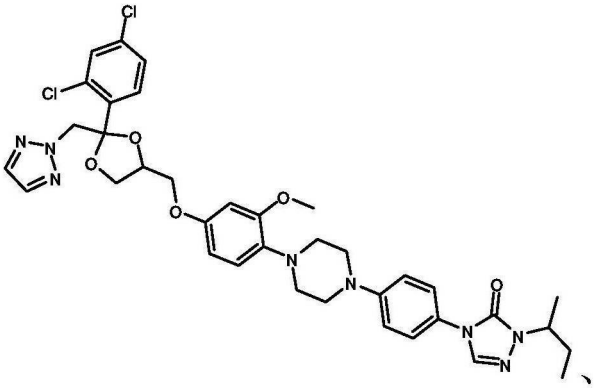

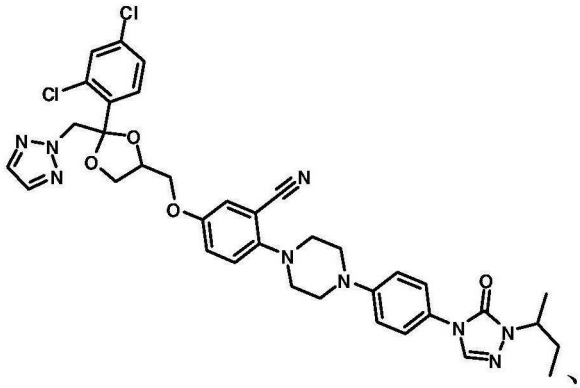

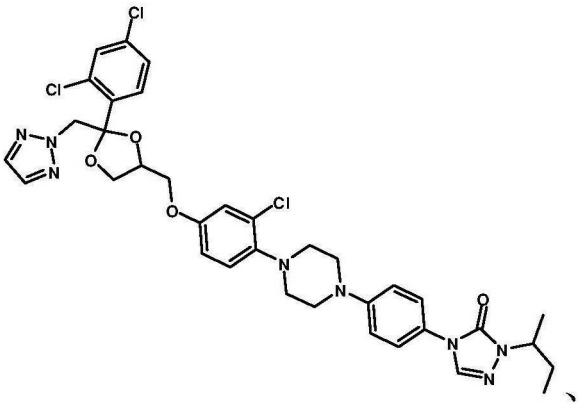

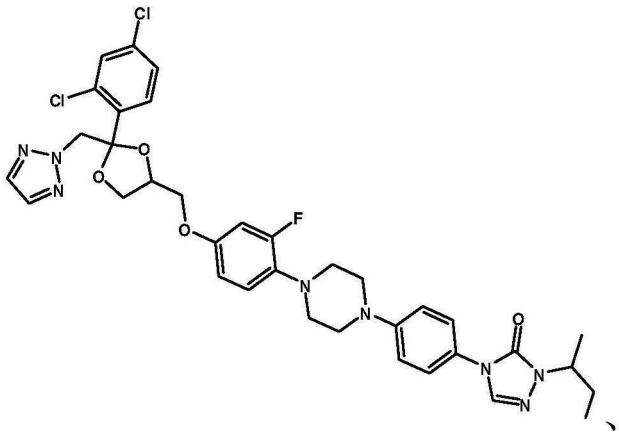

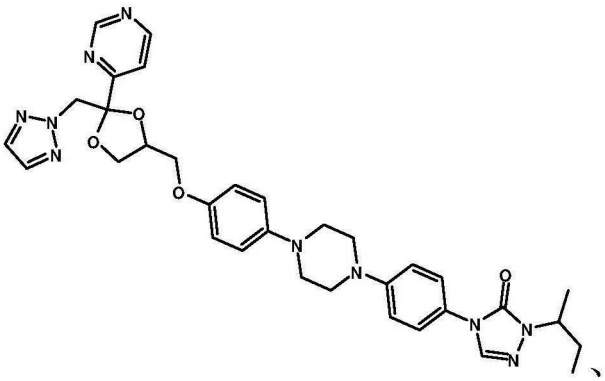

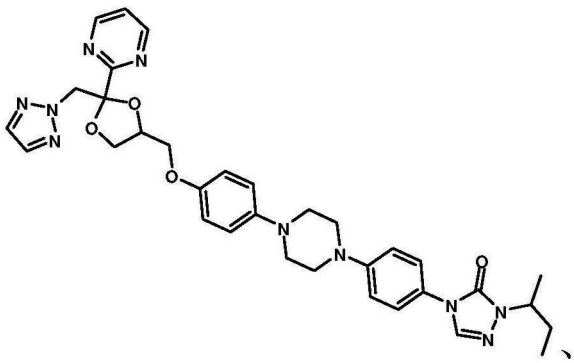

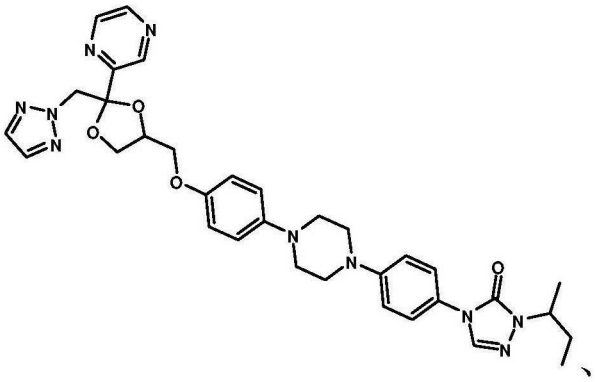

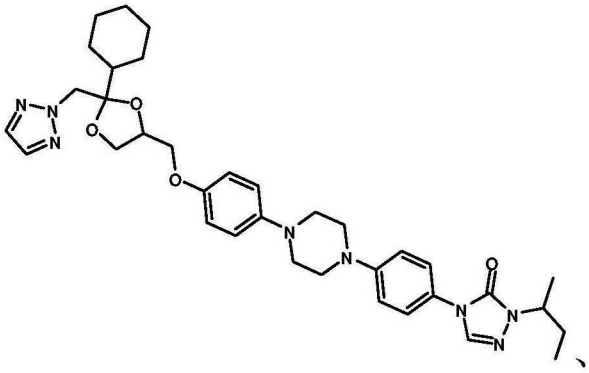

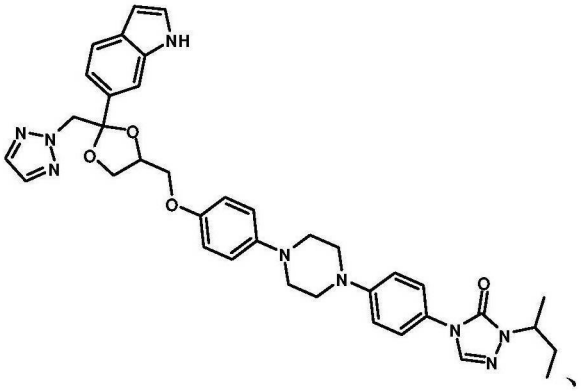

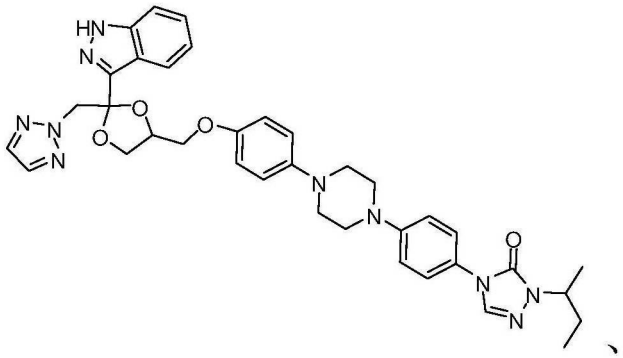

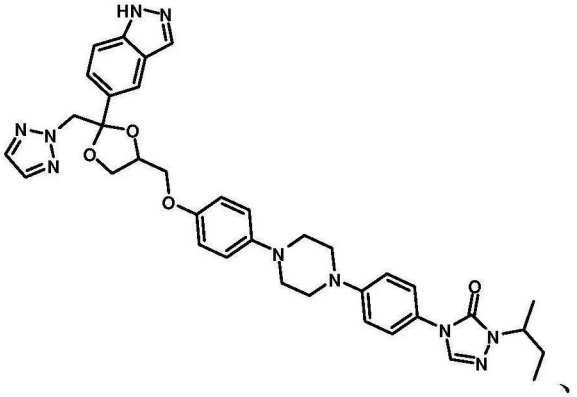

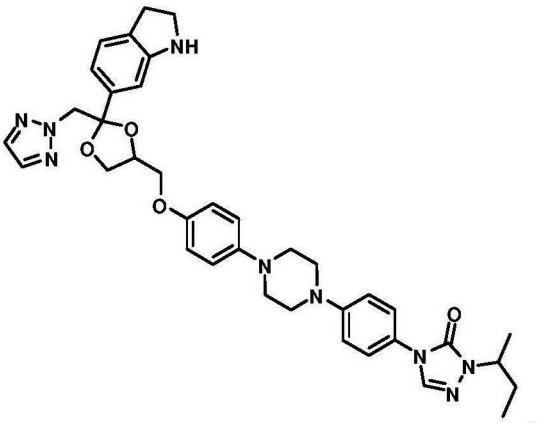

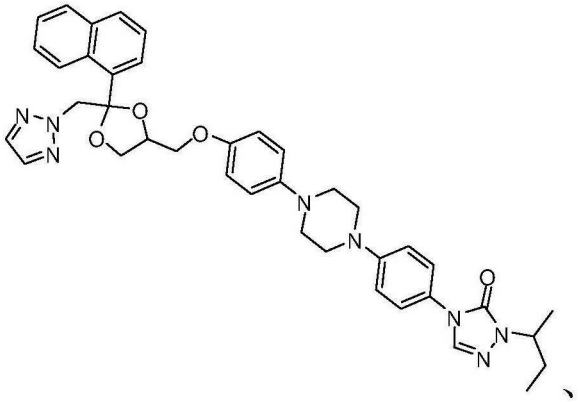

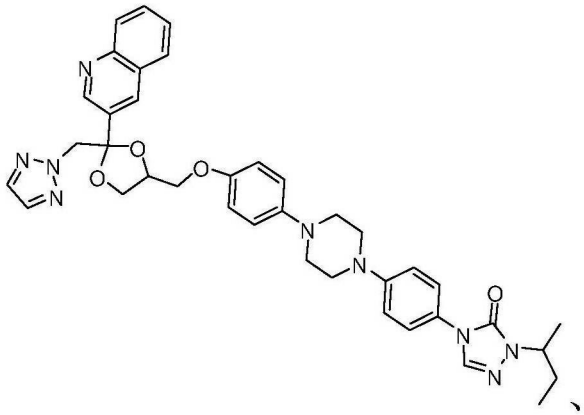

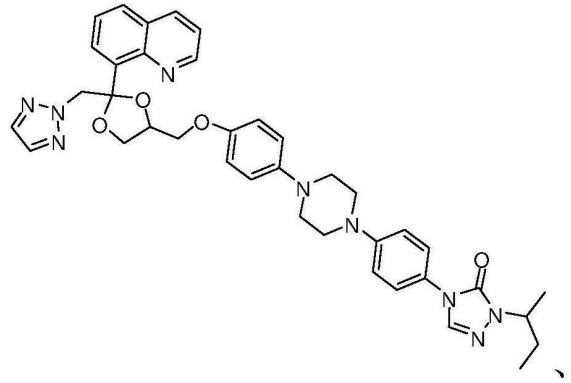

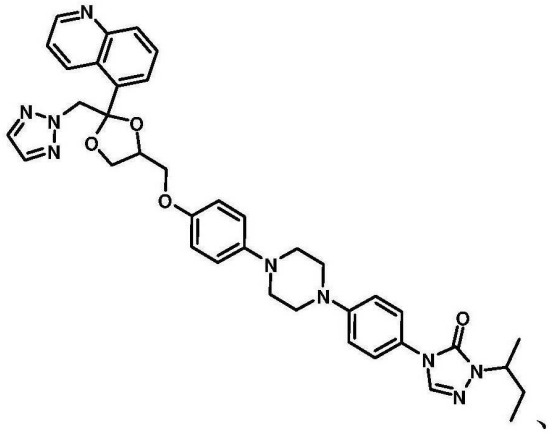

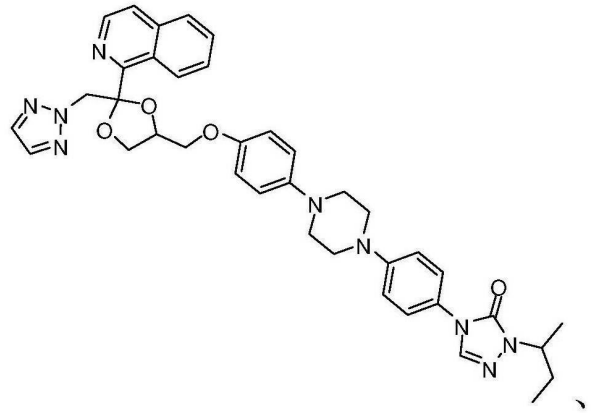

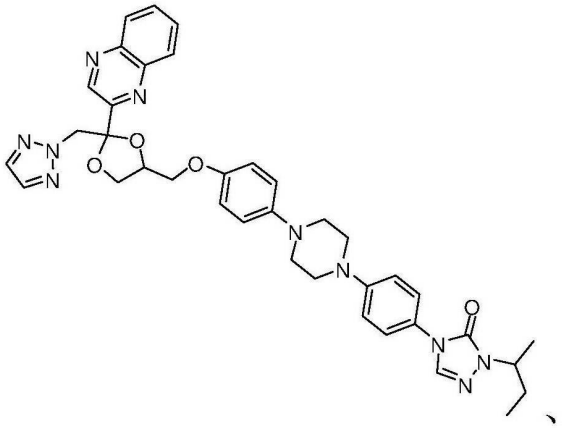

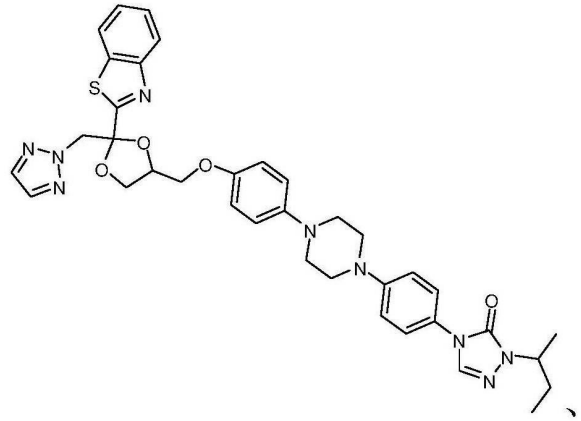

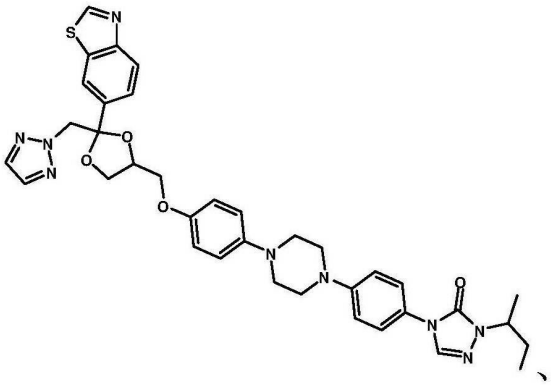

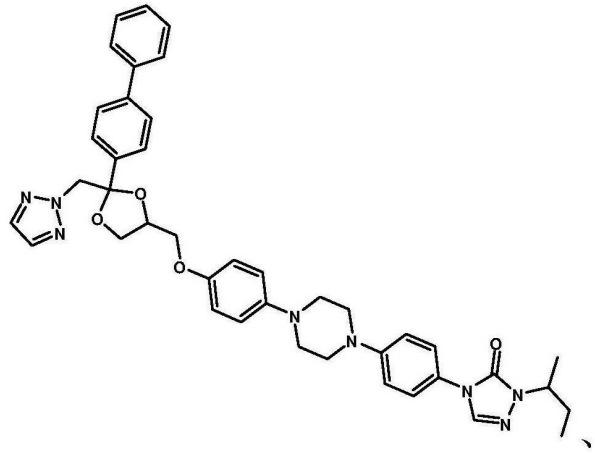

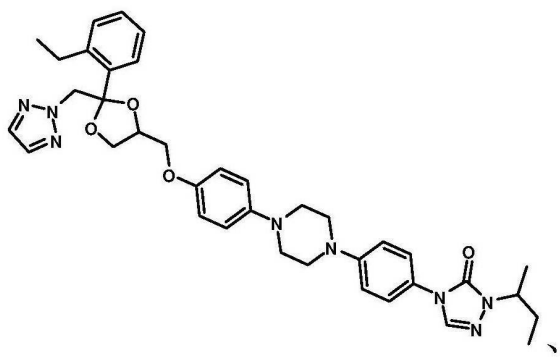

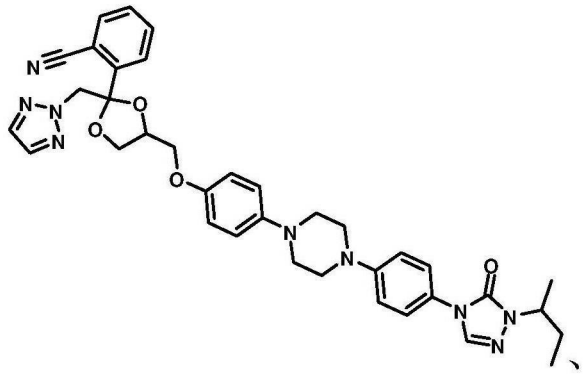

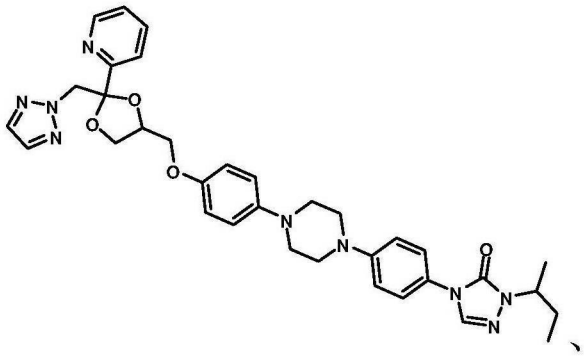

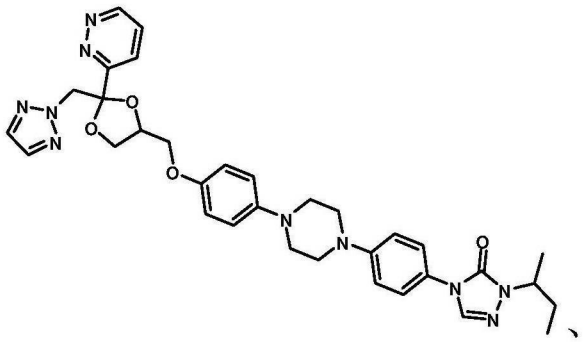

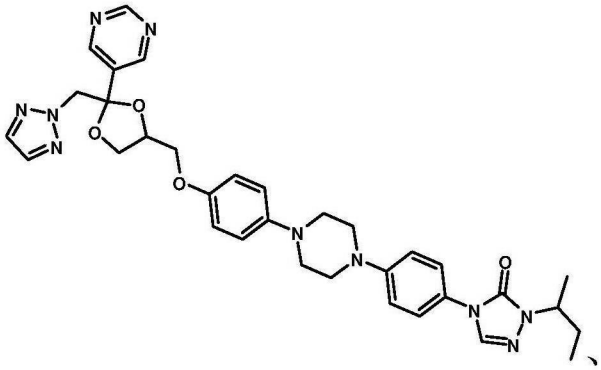

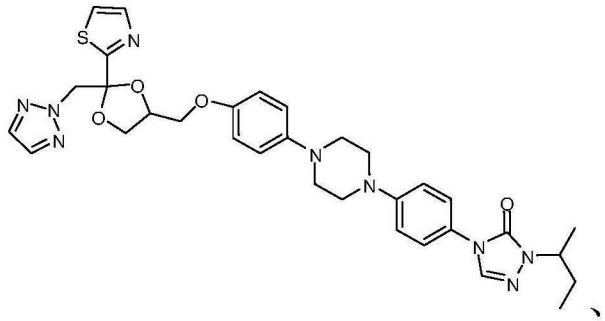

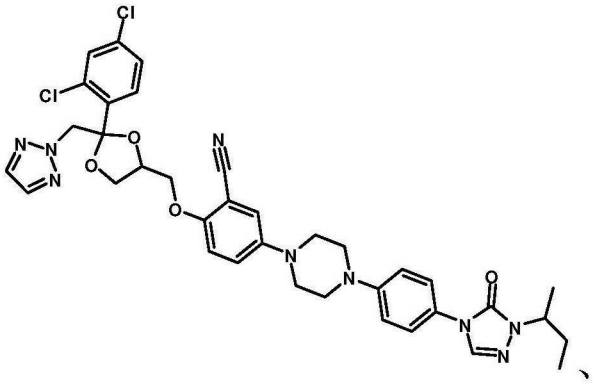

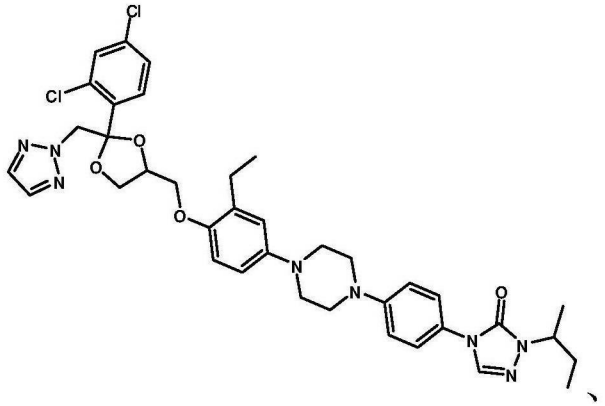

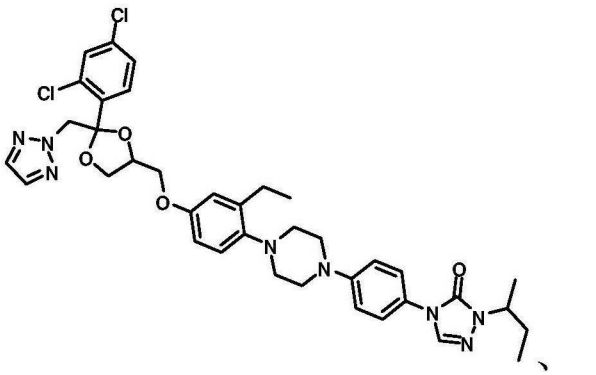

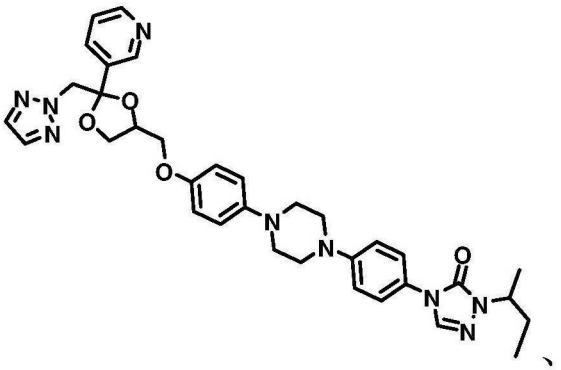

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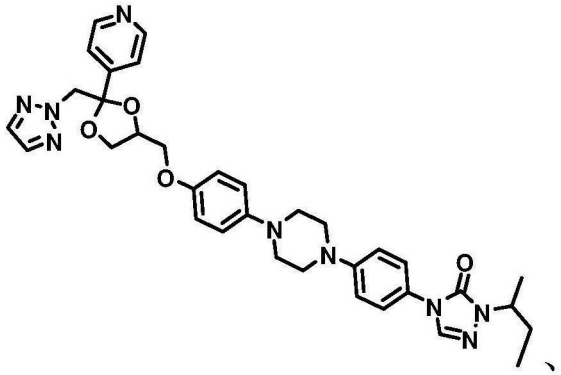

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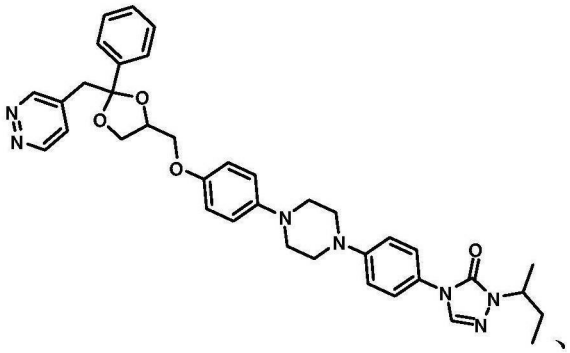

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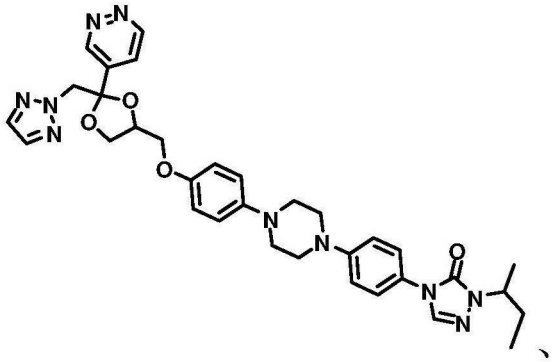

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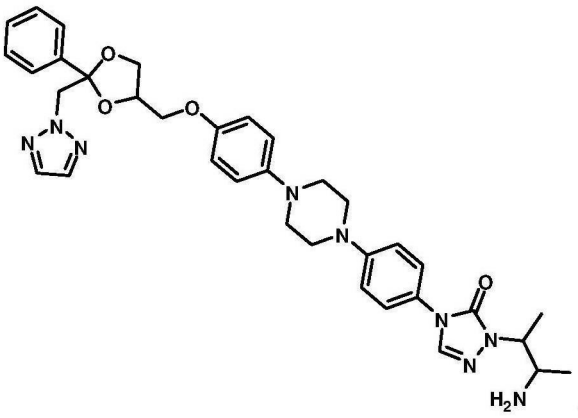

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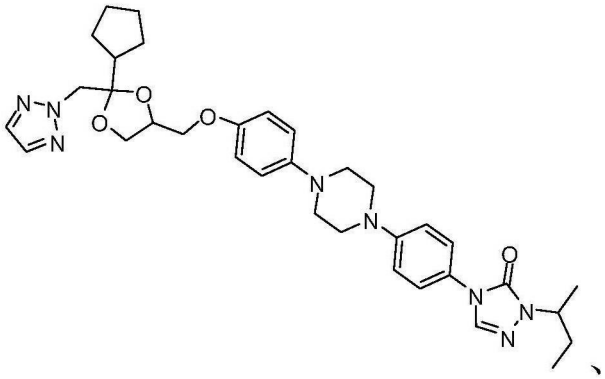

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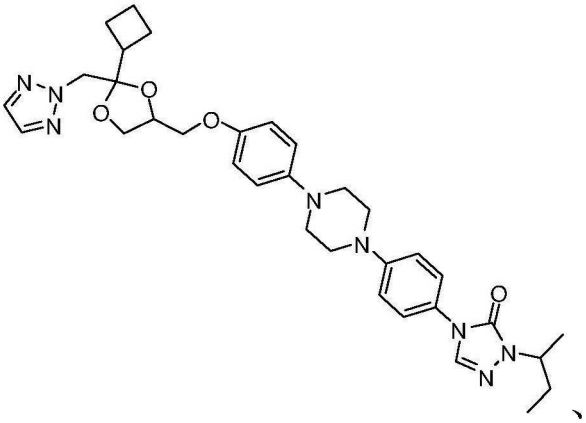

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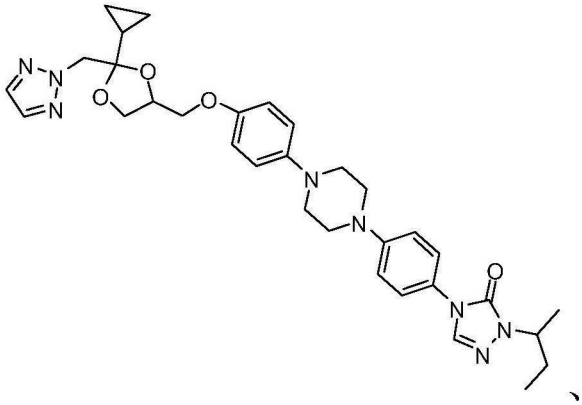

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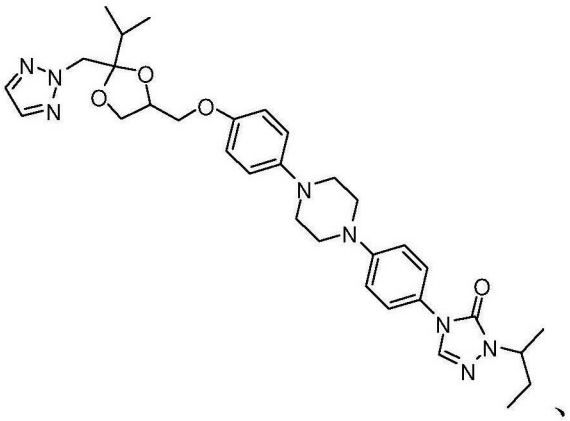

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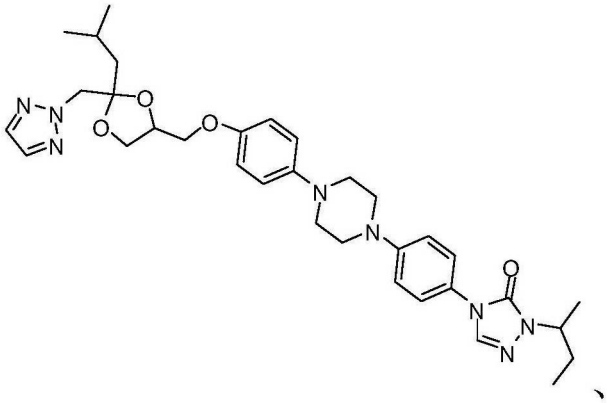

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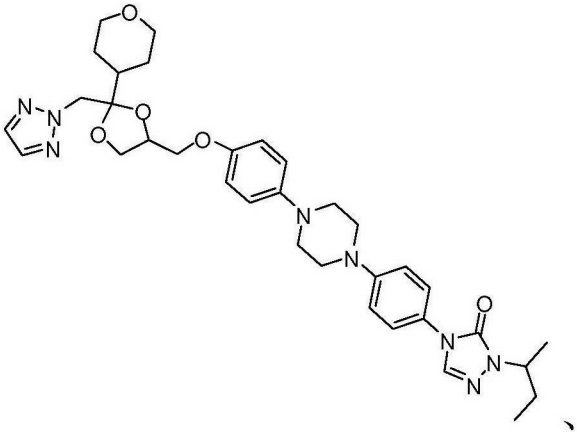

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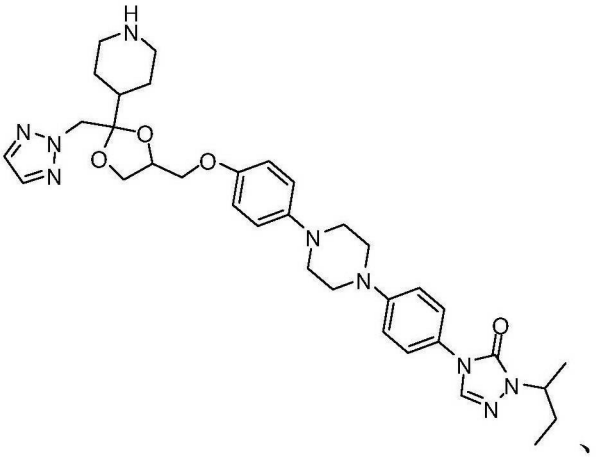

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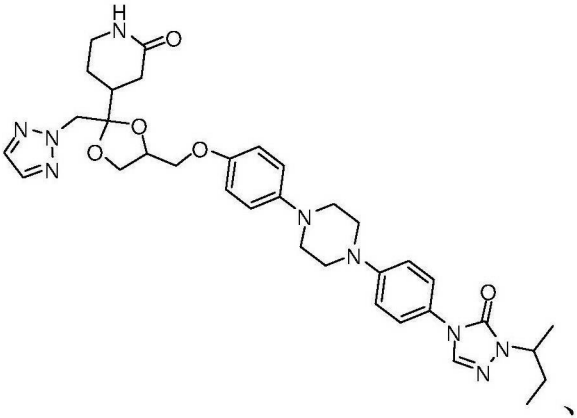

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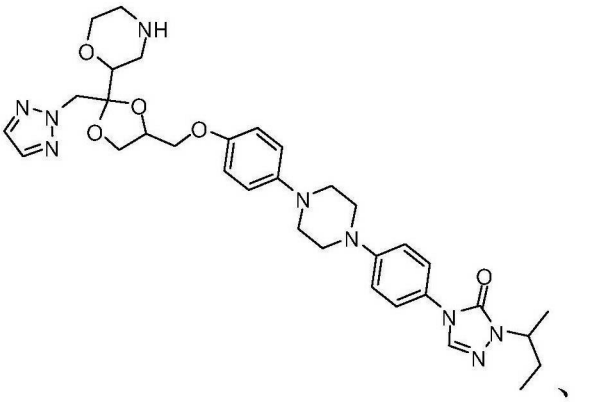

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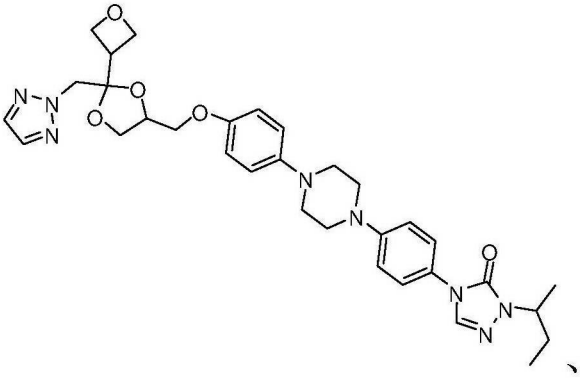

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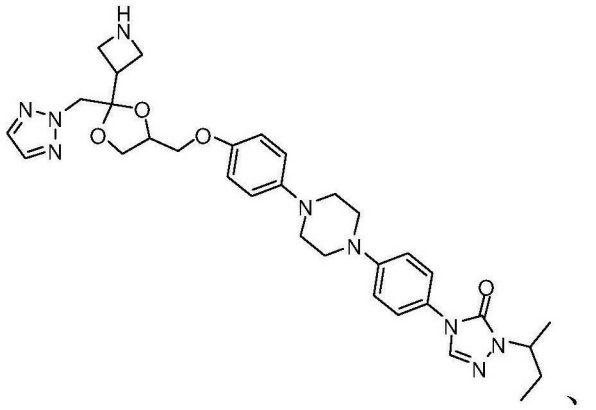

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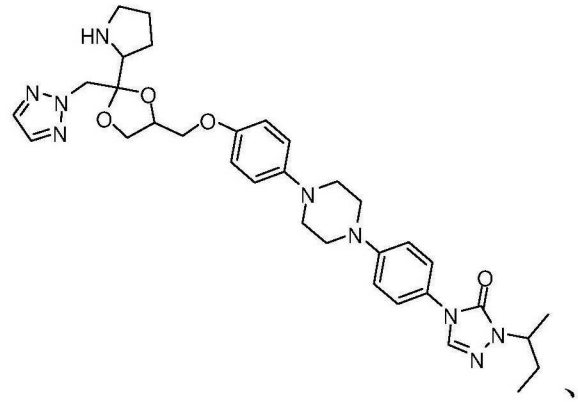
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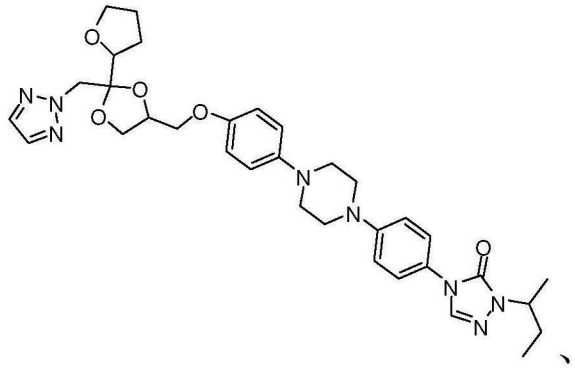
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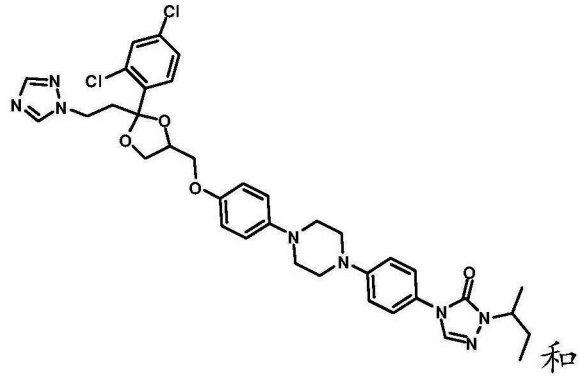
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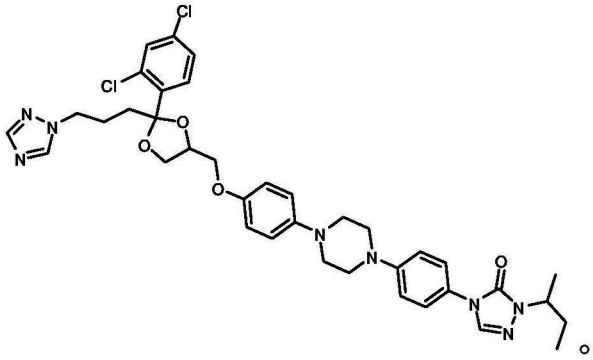

。

32. 如权利要求 1-31 中任一项所述的方法，其中所述纤维化为肝纤维化、特发性肺纤维化、肾纤维化或心脏纤维化。

33. 如权利要求 32 所述的方法，其中所述肝纤维化与酒精性或非酒精性肝硬化的后期相关。

34. 如权利要求 32 所述的方法，其中所述纤维化是特发性肺纤维化。

35. 如权利要求 1-31 中任一项所述的方法，其中所述以纤维化为特征的疾病或病症是慢性自身免疫病。

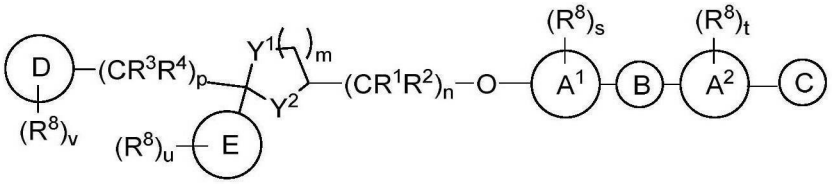
36. 如权利要求 35 所述的方法，其中所述慢性自身免疫病是类风湿性关节炎、硬皮病、克罗恩病或系统性红斑狼疮。

37. 如权利要求 36 所述的方法，其中所述慢性自身免疫病是硬皮病。

38. 如权利要求 1-31 中任一项所述的方法，其中所述纤维化是由异常创伤愈合导致的瘢痕疙瘩形成。

39. 如权利要求 1-31 中任一项所述的方法，其中所述纤维化在器官移植后发生。

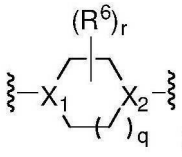
40. 式 (II) 的化合物，其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体：



式(II)

其中：

A^1和 A^2独立地选自芳基或杂芳基；

B 为  ；

C 为任选取代的 5 或 6 元杂环基或任选取代的 5 或 6 元杂芳基，其中该杂环基或该杂芳基含有 1 至 4 个氮原子；

D 为芳基或杂芳基；

E 为芳基、杂芳基、碳环基、杂环基或烷基；

每个 R^1、R^2、R^3和 R^4独立地选自 H、烷基、卤代烷基或烷氧基；

X_1和 X_2独立地选自 N 和 CR^5；

R^5为 H、OH、烷基或烷氧基；

每个 R^6独立地为烷基、卤代烷基、卤代、烷氧基、- 亚烷基 ($NR^{13}R^{14}$) 或芳基；

R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、环烷基、杂环基、- 亚烷基（环烷基）或 - 亚烷基（杂环基）；

每个 R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、- 亚烷基（环烷基）、- 亚烷基（杂环基）、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和 $-C(O)NR^{13}R^{14}$；或者两个相邻的 R^8形成杂环基环；

每个 R^{13}和 R^{14}独立地选自 H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者 R^{13}和 R^{14}与它们所连接至的原子一起形成杂环；

Y^1和 Y^2独立地选自 O、CH_2、NH 和 NR^{13}；

n 为 1、2 或 3；

m 为 1 或 2；

p 为 1、2、3 或 4；

q 为 1、2 或 3；

r 为 0、1、2、3、4、5、6、7 或 8；

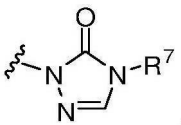
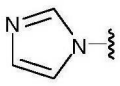
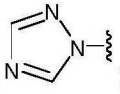
s 为 0、1、2、3 或 4；

t 为 0、1、2、3 或 4；

u 为 0、1、2、3、4 或 5；且

v 为 0、1、2、3 或 4；

条件是：

如果 X_1和 X_2为 N；r 为 0；q 为 1；A^1和 A^2为苯基；Y^1和 Y^2为 O；m 和 n 为 1；R^1、R^2、R^3和 R^4为氢；且 C 为  ，则 D 不是  或  ；且

该化合物不是 4-(4-(4-(4-((2-((1H- 吡唑 -1- 基）甲基）-2-(2,4- 二氟苯

基)-1,3-二氧杂环戊-4-基)甲氧基)苯基)哌嗪-1-基)苯基)-1-异丙基-1H-1,2,4-三唑-5(4H)-酮。

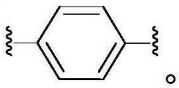
41. 如权利要求 40 所述的化合物,其中 X_1和 X_2为 N。

42. 如权利要求 40 所述的化合物,其中 X_1为 CR^5且 X_2为 N。

43. 如权利要求 40 所述的化合物,其中 X_1为 N 且 X_2为 CR^5。

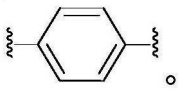
44. 如权利要求 40-43 中任一项所述的化合物,其中 q 为 1 且 r 为 0。

45. 如权利要求 40-44 中任一项所述的化合物,其中 A^1为芳基。

46. 如权利要求 45 所述的化合物,其中 A^1为  。

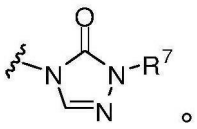
47. 如权利要求 40-44 中任一项所述的化合物,其中 A^1为杂芳基。

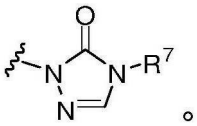
48. 如权利要求 40-47 中任一项所述的化合物,其中 A^2为芳基。

49. 如权利要求 48 所述的化合物,其中 A^2为  。

50. 如权利要求 40-47 中任一项所述的化合物,其中 A^2为杂芳基。

51. 如权利要求 50 所述的化合物,其中 A^2为吡啶、吡嗪、嘧啶、哒嗪或三嗪。

52. 如权利要求 40-51 中任一项所述的化合物,其中 C 为  。

53. 如权利要求 40-51 中任一项所述的化合物,其中 C 为  。

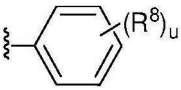
54. 如权利要求 40-53 中任一项所述的化合物,其中 E 为烷基。

55. 如权利要求 40-53 中任一项所述的化合物,其中 E 为环烷基。

56. 如权利要求 55 所述的化合物,其中 E 为环丙基、环丁基、环戊基或环己基。

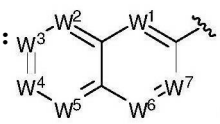
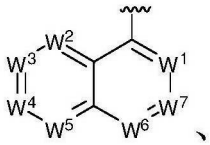
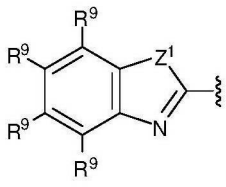
57. 如权利要求 40-53 中任一项所述的化合物,其中 E 为杂环基。

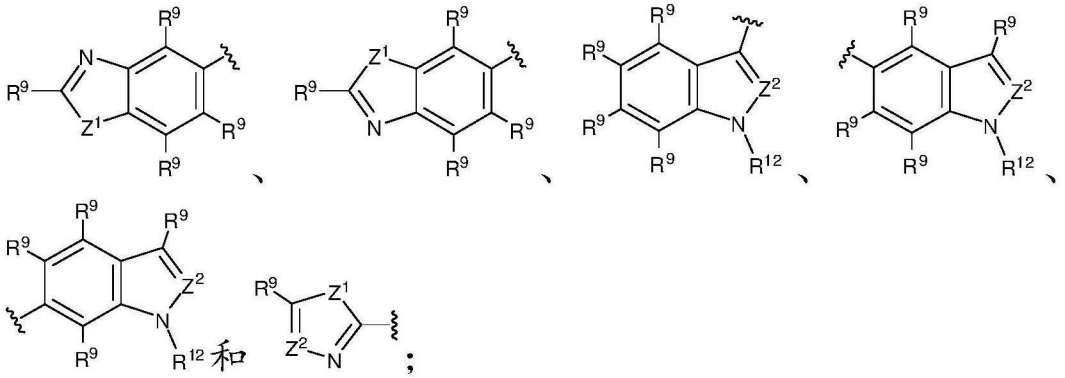
58. 如权利要求 40-53 中任一项所述的化合物,其中 E 为芳基。

59. 如权利要求 58 所述的化合物,其中 E 为  且 u 为 0、1、2、3、4 或 5。

60. 如权利要求 40-53 中任一项所述的化合物,其中 E 为杂芳基。

61. 如权利要求 60 所述的化合物,其中

E 选自:  、  、  、



W^1、W^2、W^3、W^4、W^5、W^6和 W^7独立地选自 N 和 CR^9；

Z^1为 NR^{12}、S 或 O；

Z^2为 N 或 CR^9；

每个 R^9独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基；

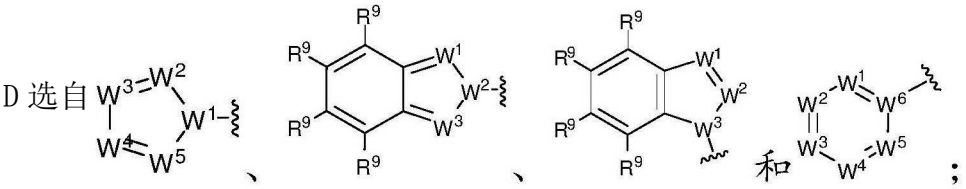
每个 R^{10}和 R^{11}独立地选自 H 和烷基；或者 R^{10}和 R^{11}与它们所连接至的氮一起形成杂环；且

R^{12}为 H、烷基或卤代烷基。

62. 如权利要求 40-61 中任一项所述的化合物，其中 D 为芳基。

63. 如权利要求 40-62 中任一项所述的化合物，其中 D 为杂芳基。

64. 如权利要求 63 所述的化合物，其中

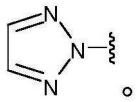


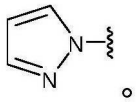
W^1、W^2、W^3、W^4和 W^5独立地选自 N 和 CR^9；

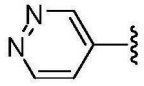
W^6为 N 或 C；且

每个 R^9独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷

基）、$-NR^{10}SO_2$（环烷基）、$-NR^{10}SO_2$（杂环烷基）、$-NR^{10}SO_2$（芳基）、$-NR^{10}SO_2$（杂芳基）、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$（环烷基）、$-SO_2NR^{10}$（杂环烷基）、$-SO_2NR^{10}$（芳基）、$-SO_2NR^{10}$（杂芳基）、卤代烷基、芳基和杂芳基。

65. 如权利要求 64 所述的化合物，其中 D 为  。

66. 如权利要求 64 所述的化合物，其中 D 为  。

67. 如权利要求 64 所述的化合物，其中 D 为  。

68. 如权利要求 40-67 中任一项所述的化合物，其中 Y^1 和 Y^2 为 O。

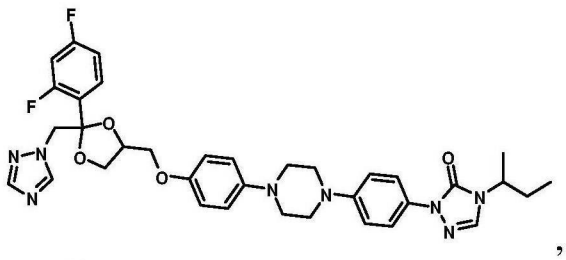
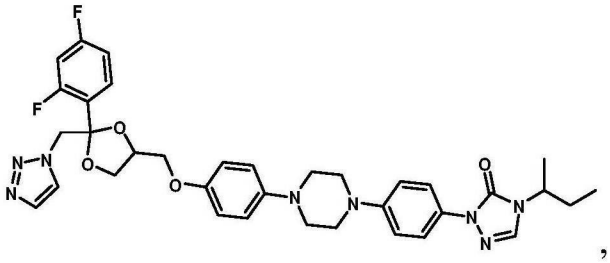
69. 如权利要求 68 所述的化合物，其中 m 为 1。

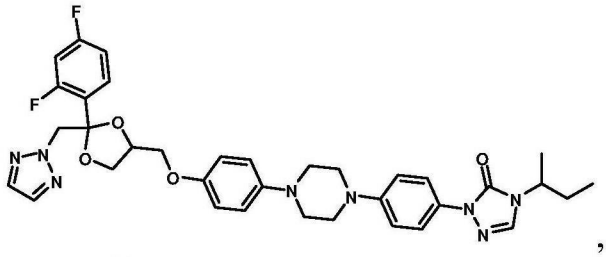
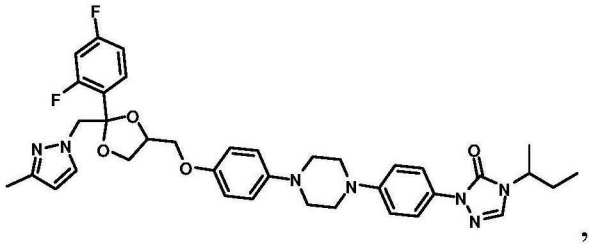
70. 如权利要求 40-69 中任一项所述的化合物，其中 p 为 1、2 或 3。

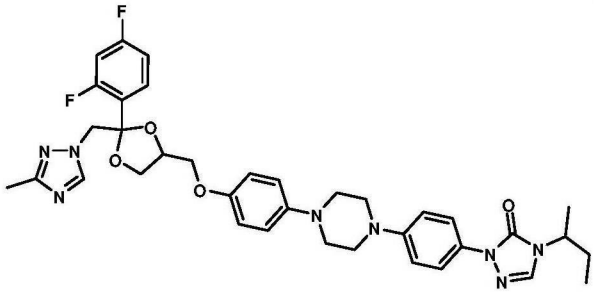
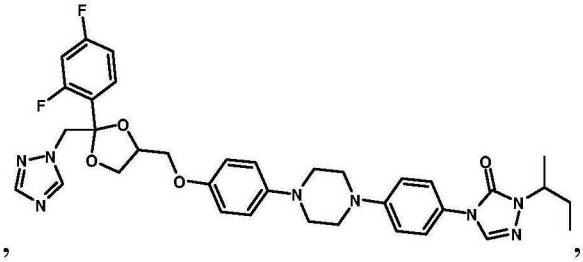
71. 如权利要求 70 所述的化合物，其中 p 为 1。

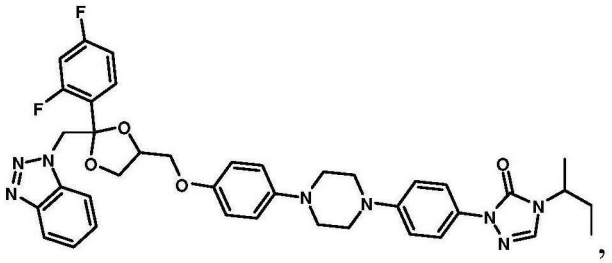
72. 如权利要求 40-71 中任一项所述的化合物，其中 R^1、R^2、R^3 和 R^4 为氢。

73. 如权利要求 40 所述的化合物，其中该化合物选自：

 ，  ，

 ，  ，

 ，  ，



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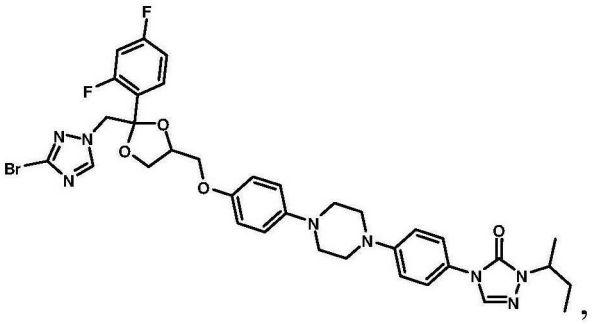

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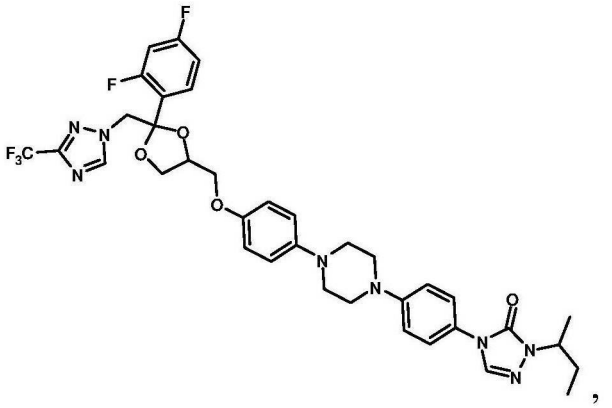

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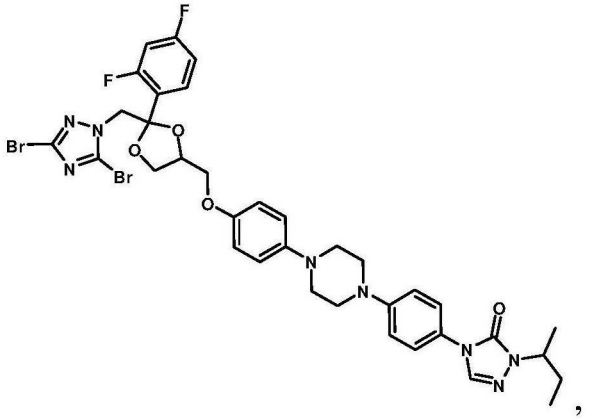

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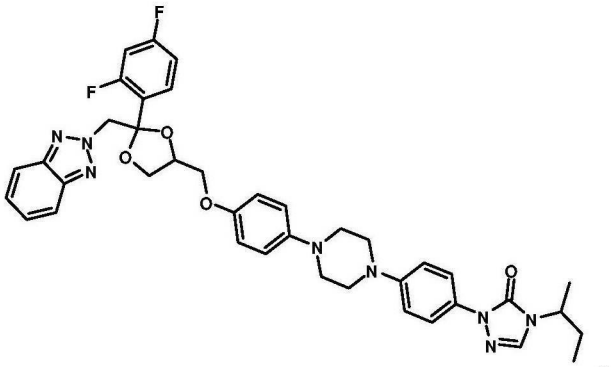

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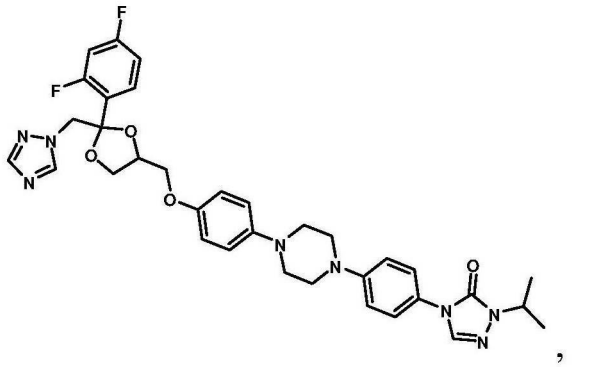

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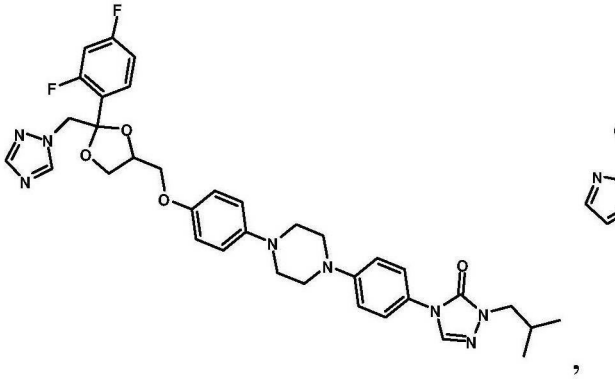

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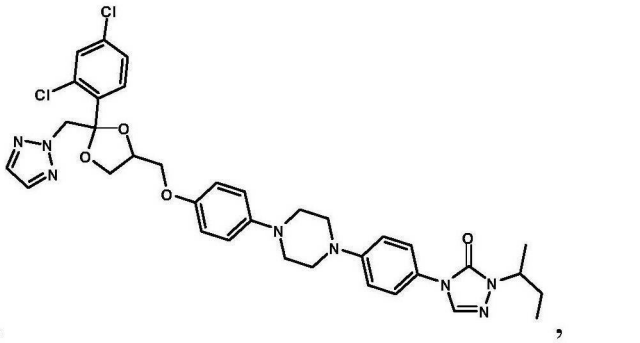

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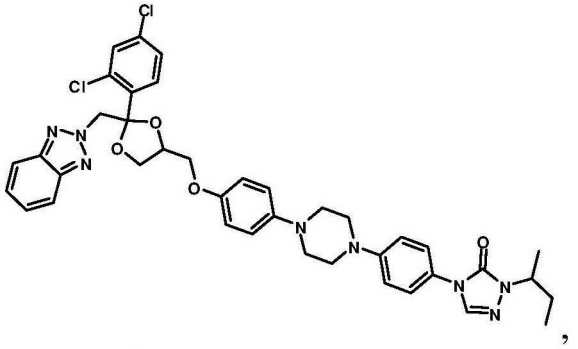

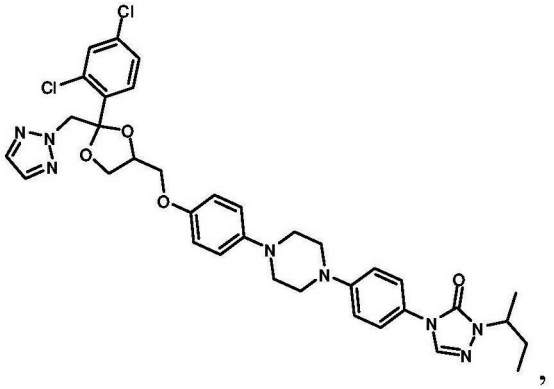

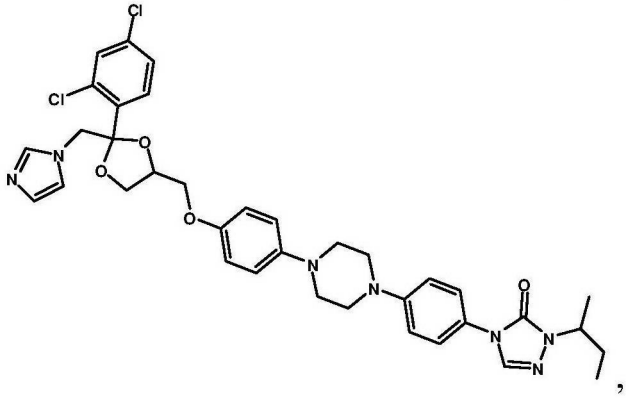

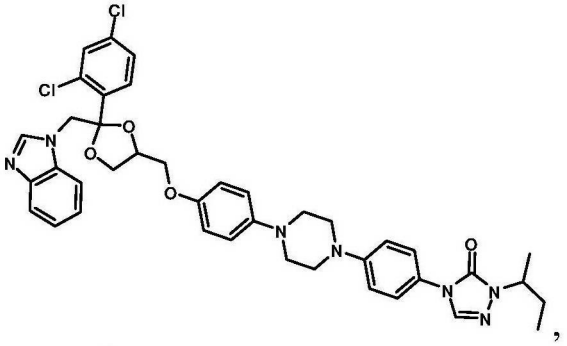

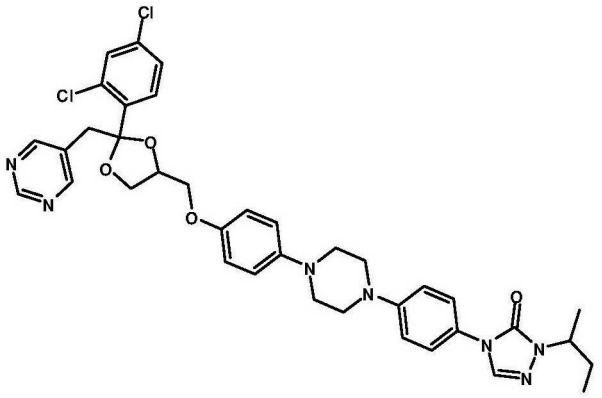

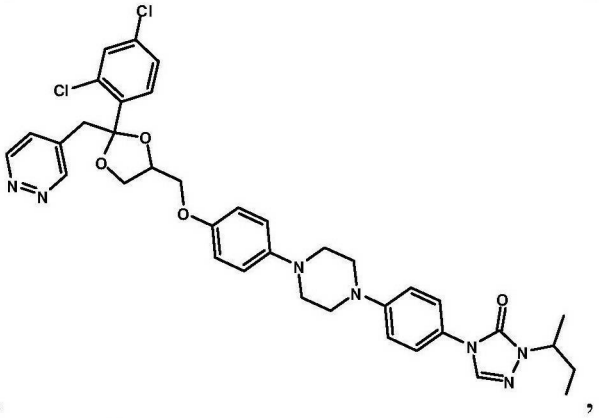

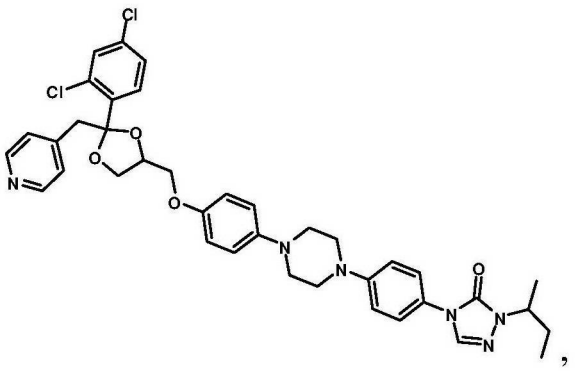

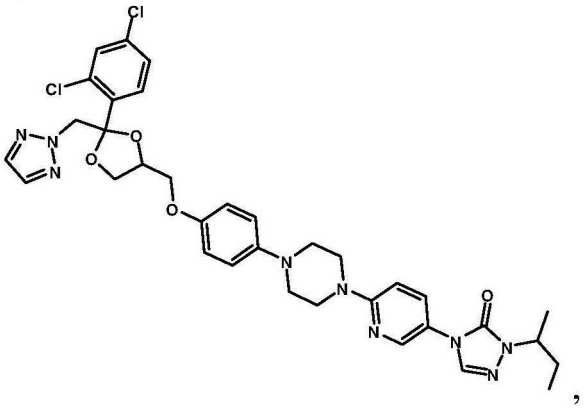

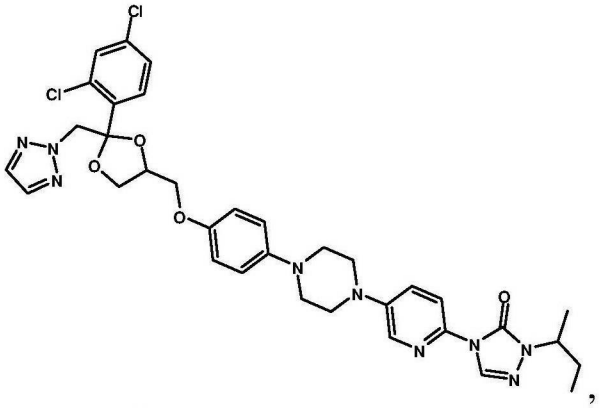

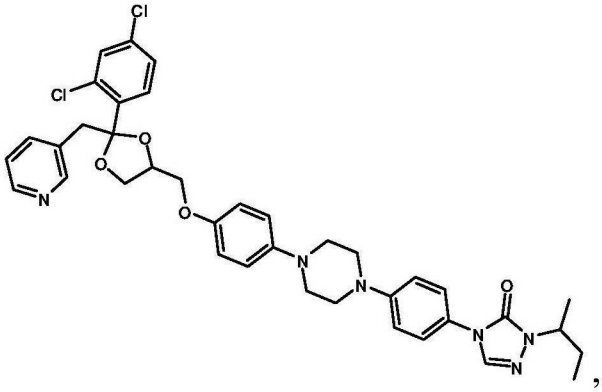

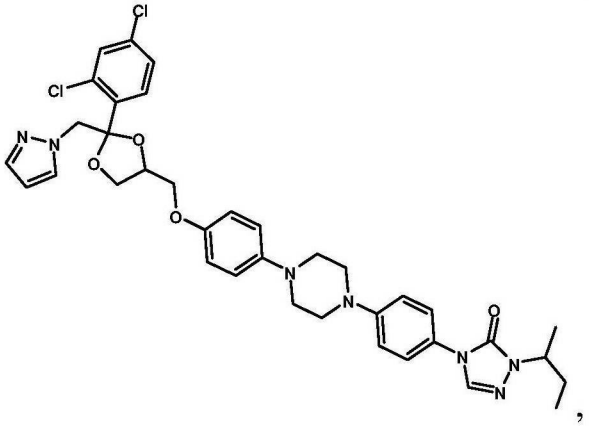

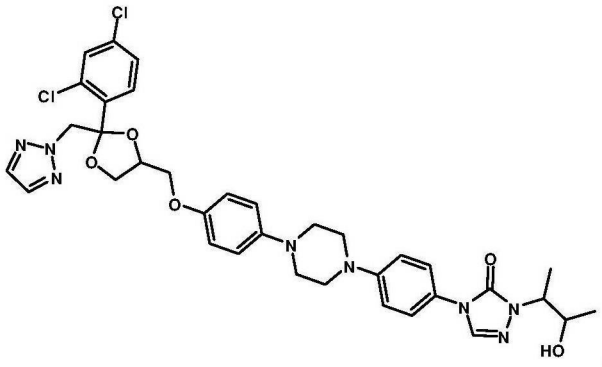

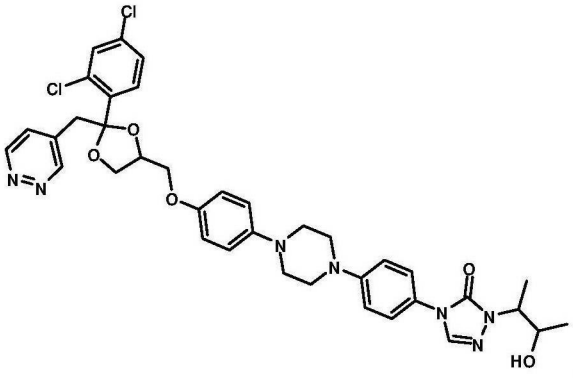

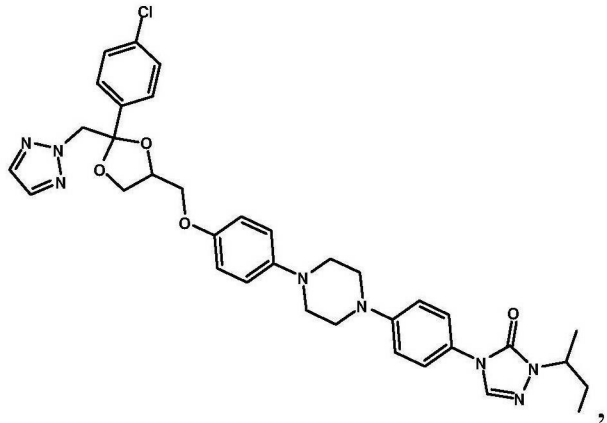

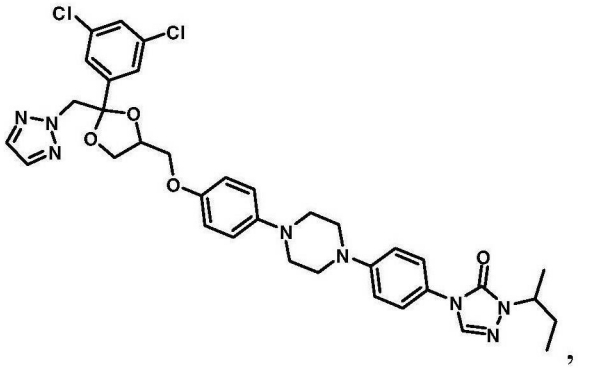

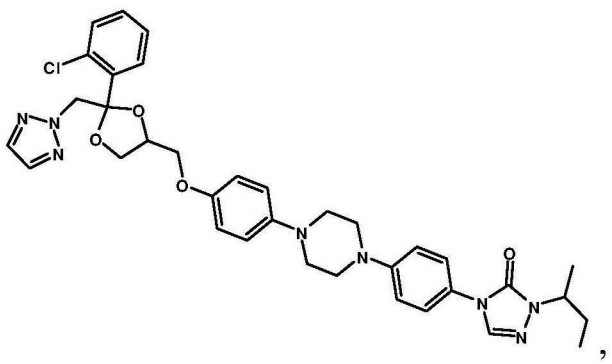

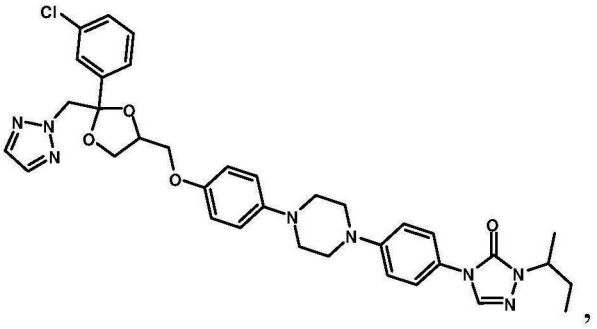

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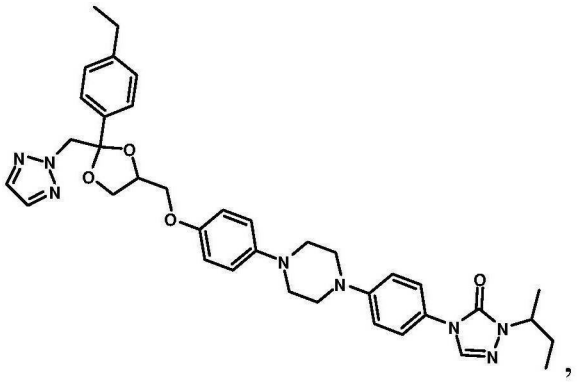

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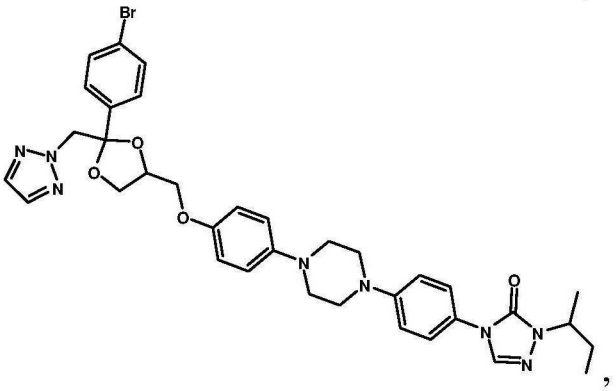

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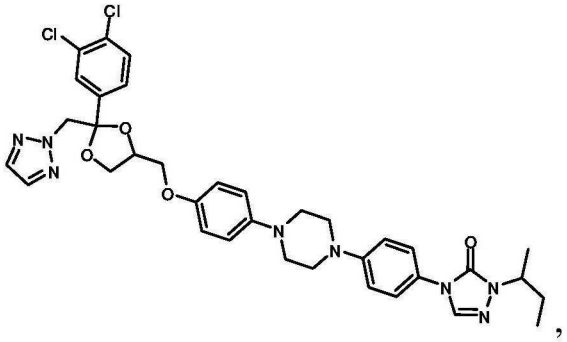

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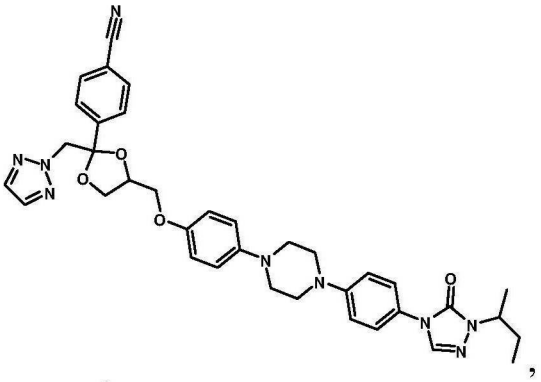

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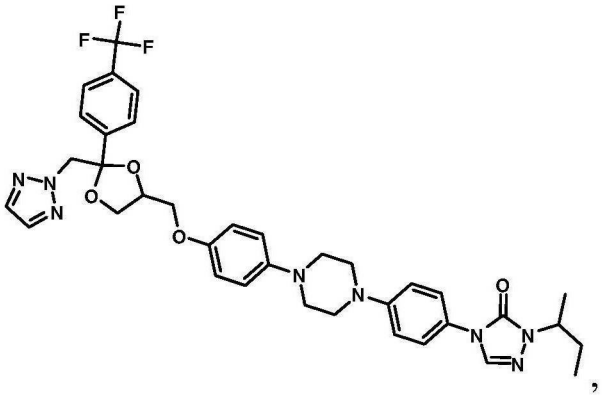

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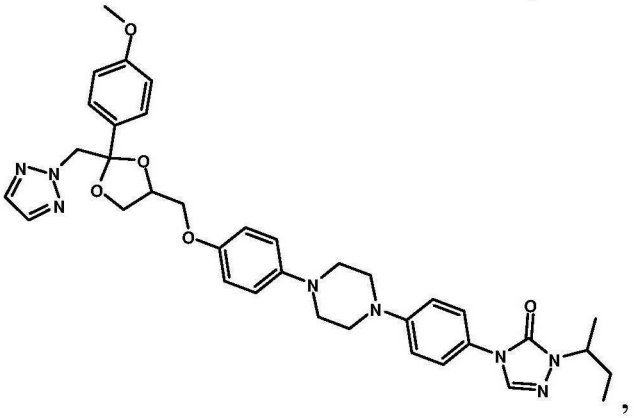

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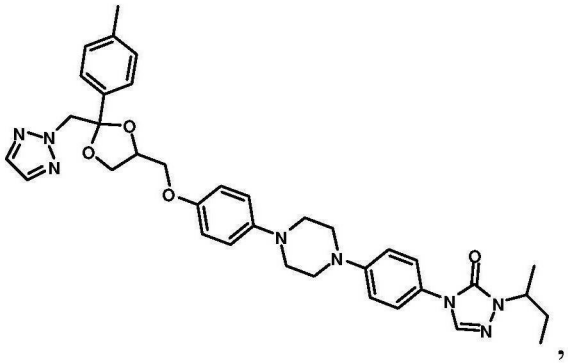

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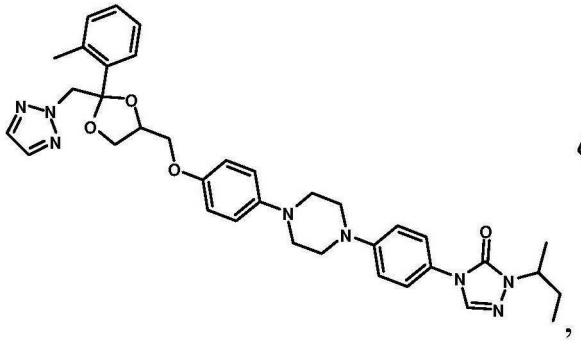

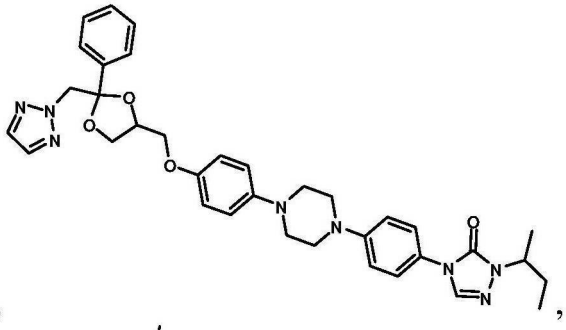

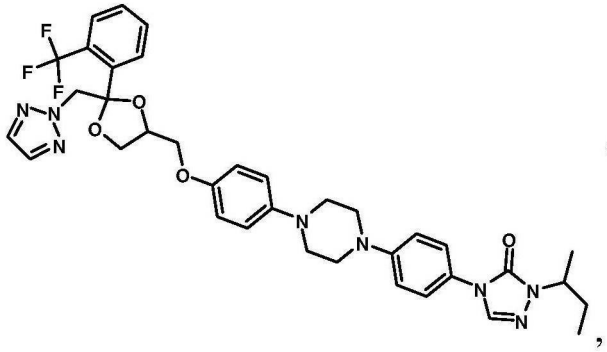

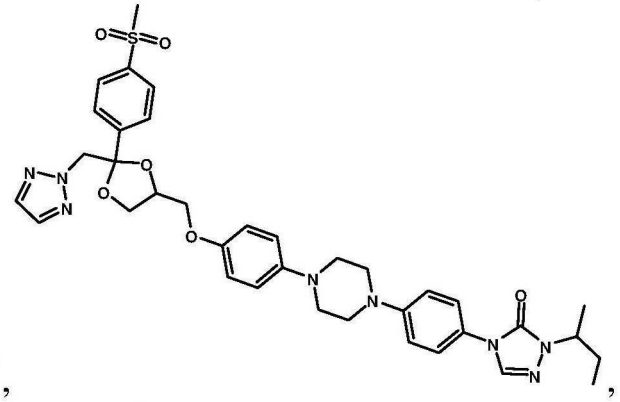

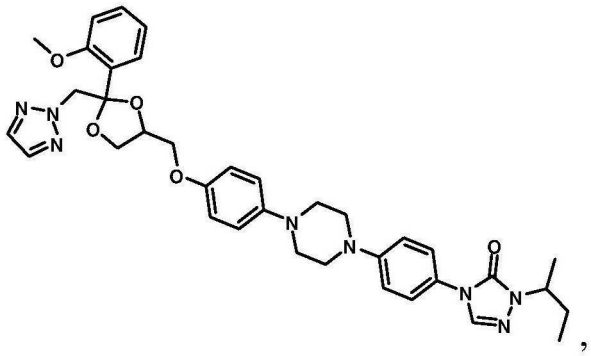

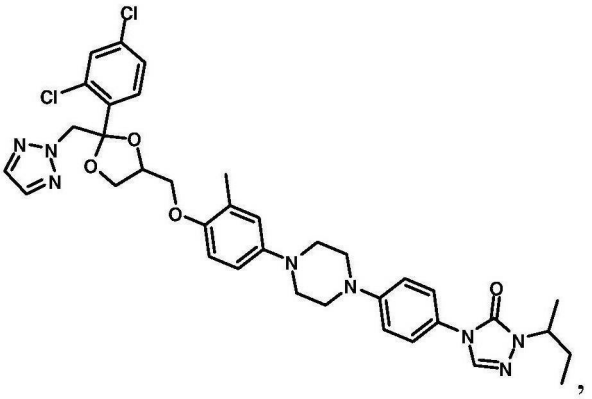

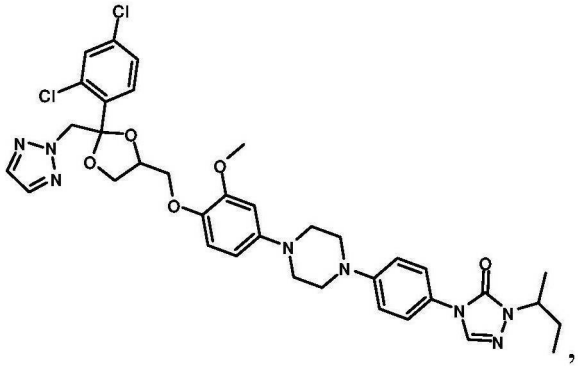

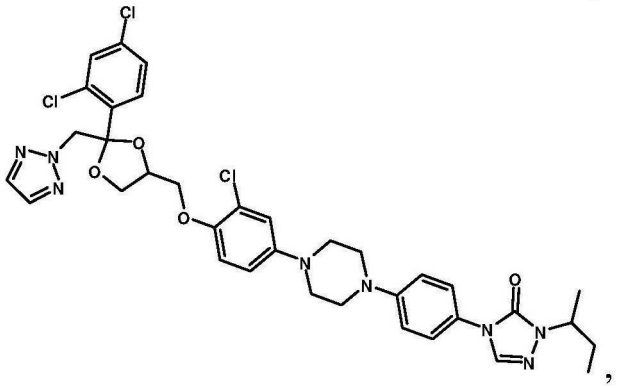

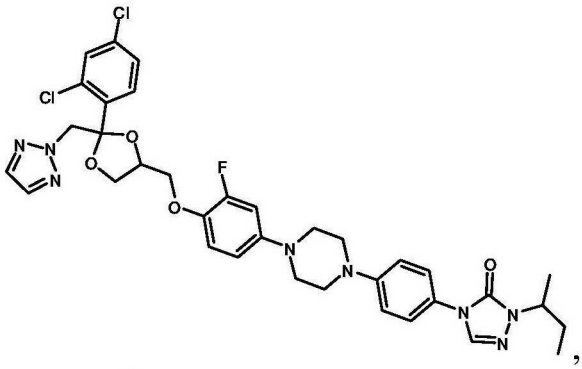

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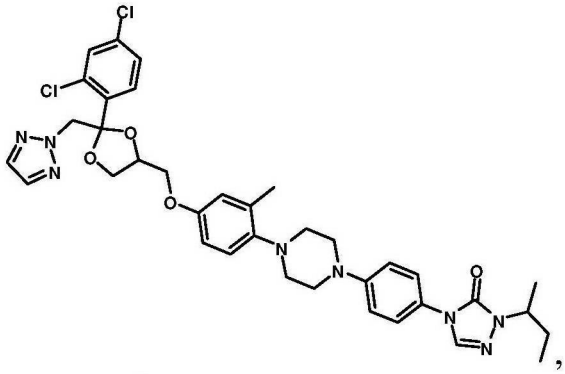

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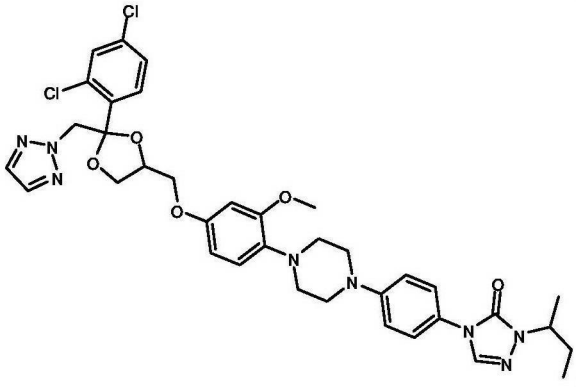

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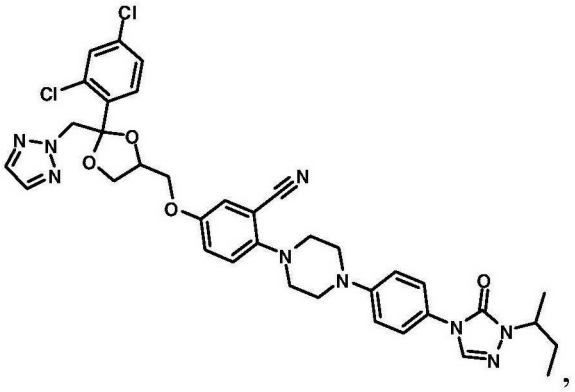

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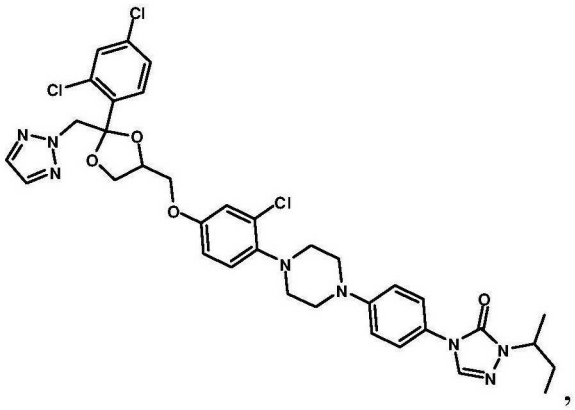

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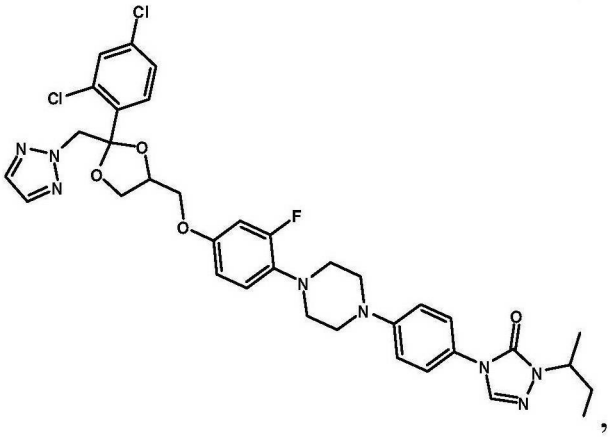

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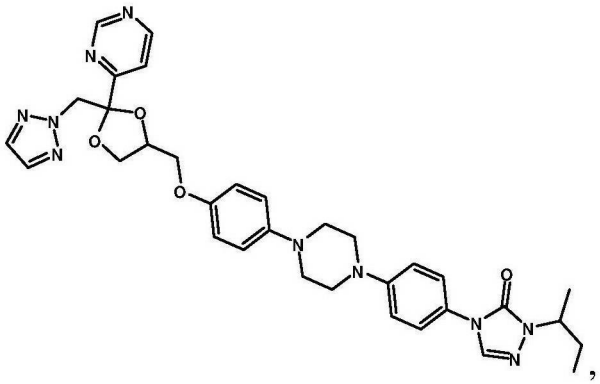

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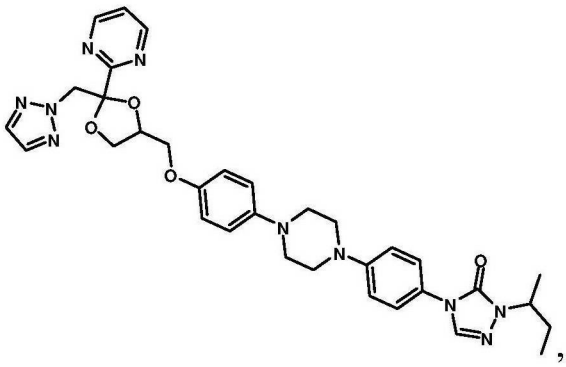

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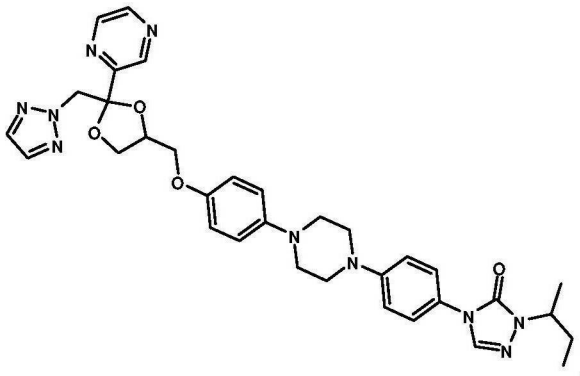

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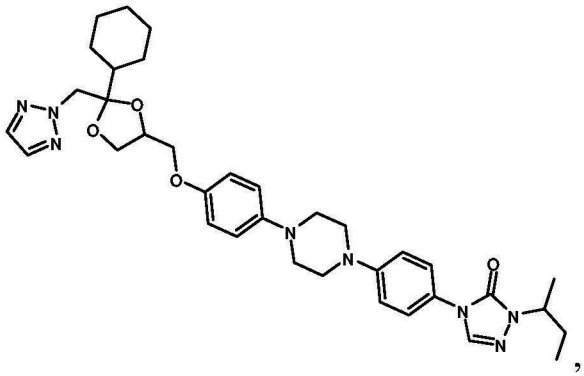

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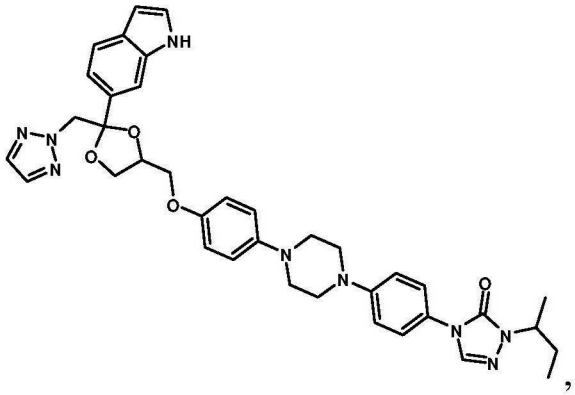

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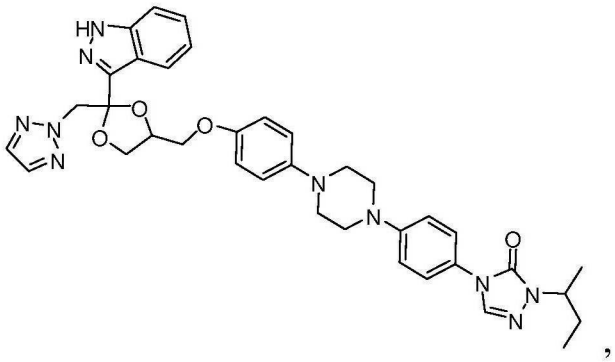

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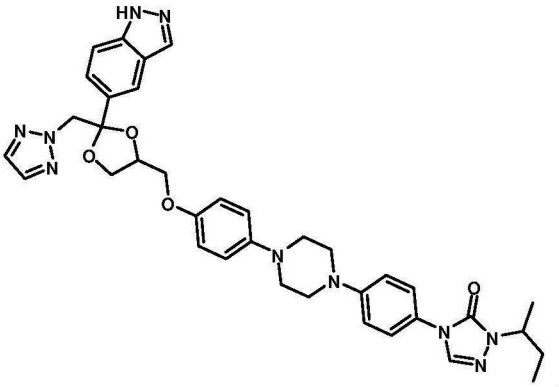

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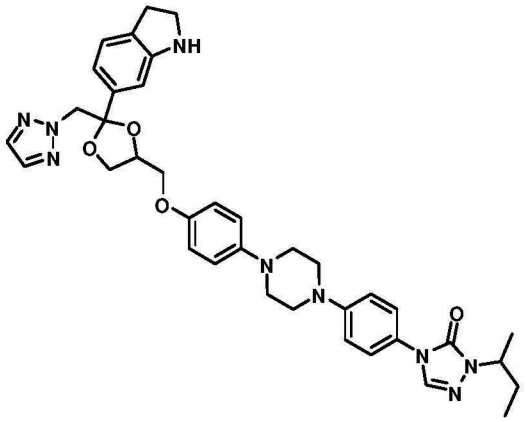

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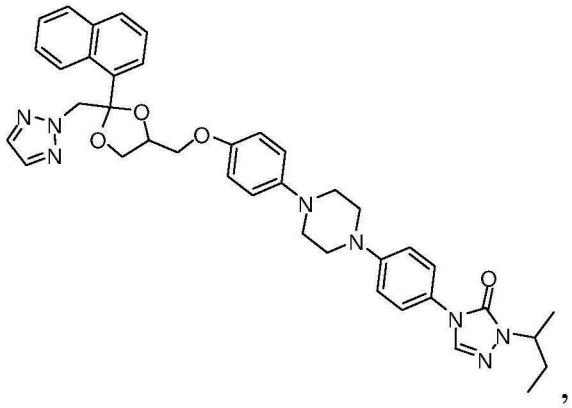

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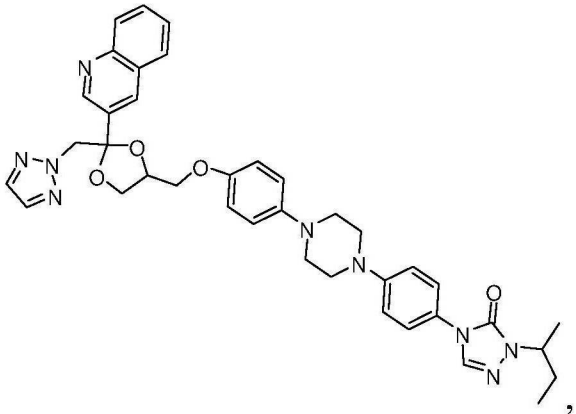

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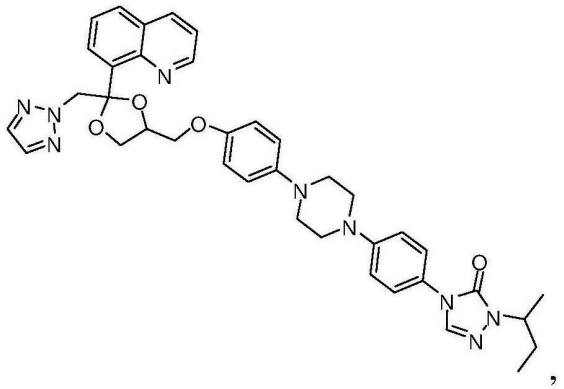

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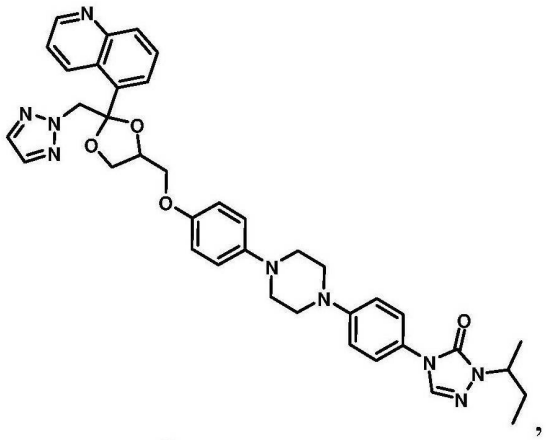

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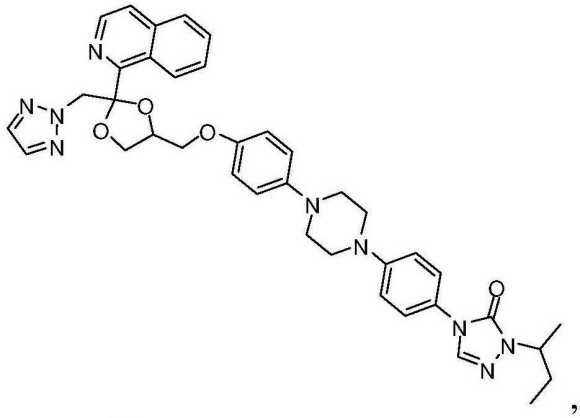

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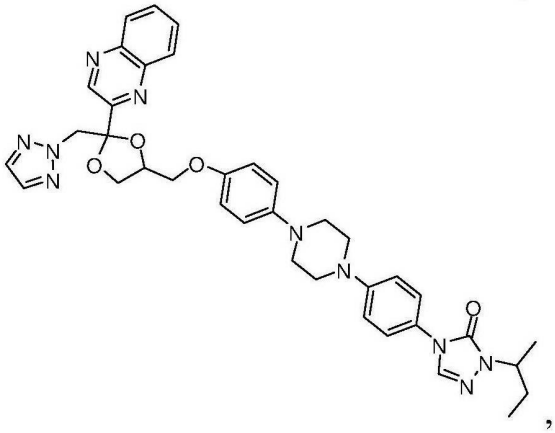

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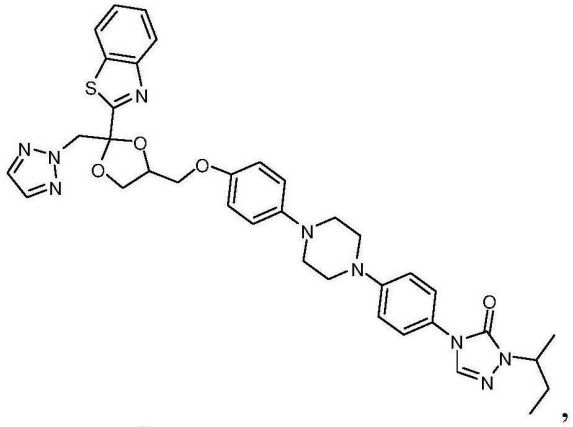

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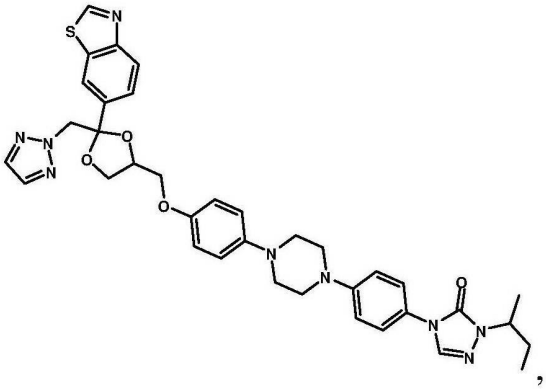

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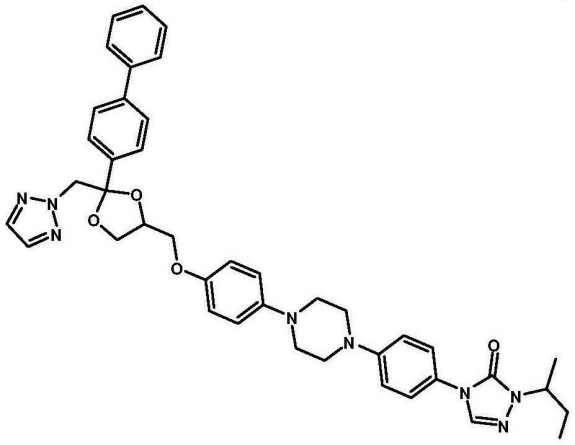

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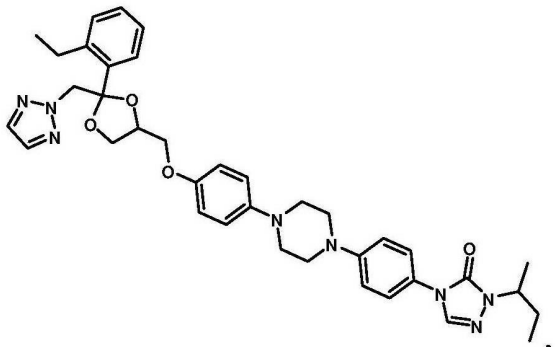

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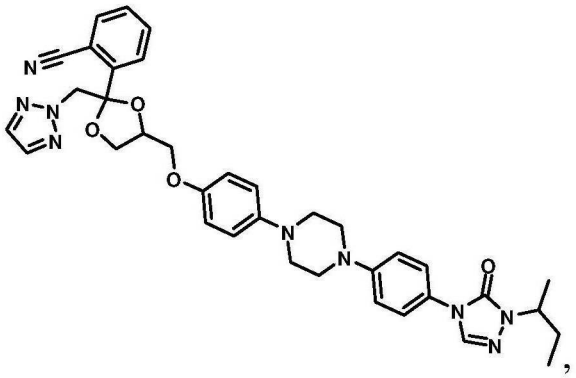

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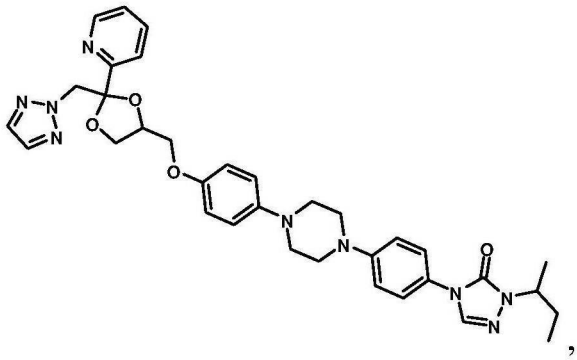

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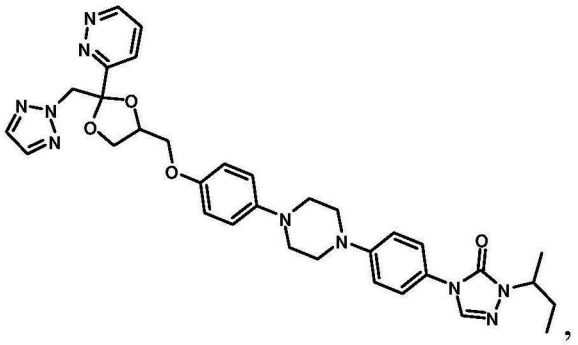

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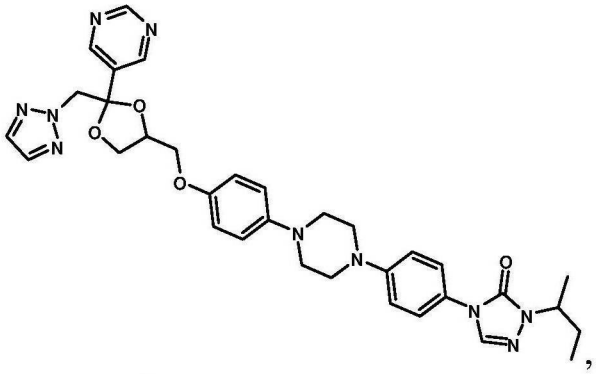

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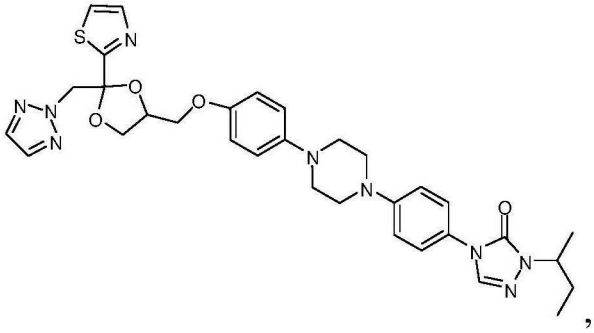

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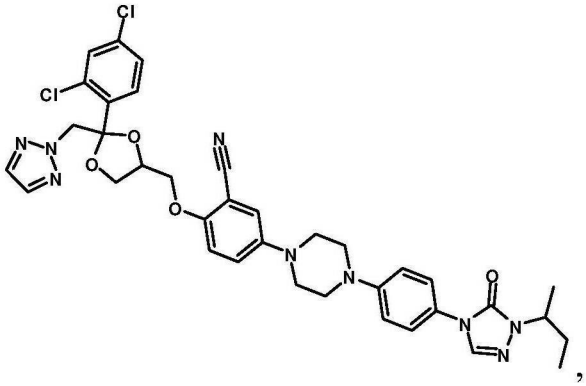

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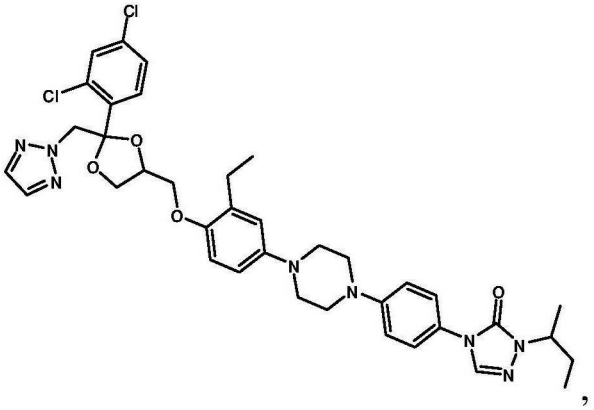

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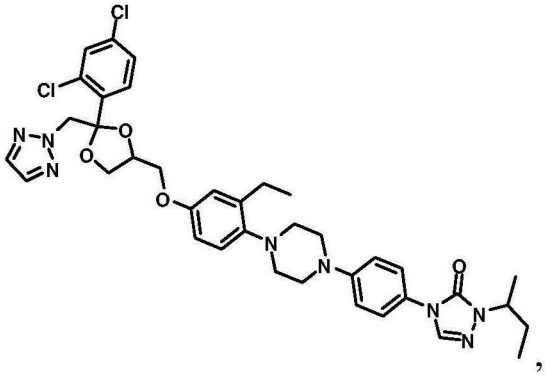

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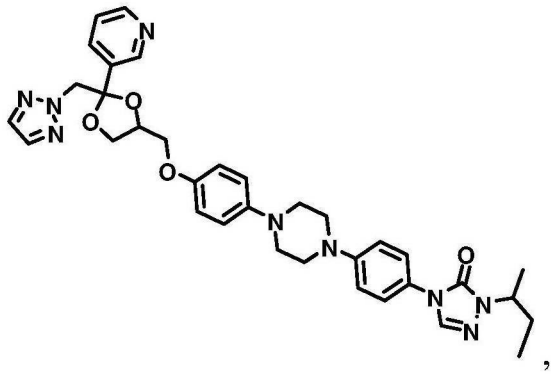

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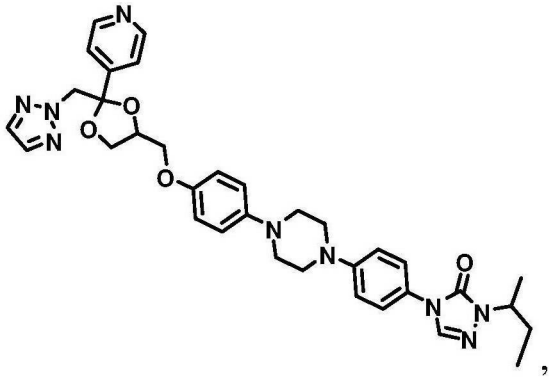

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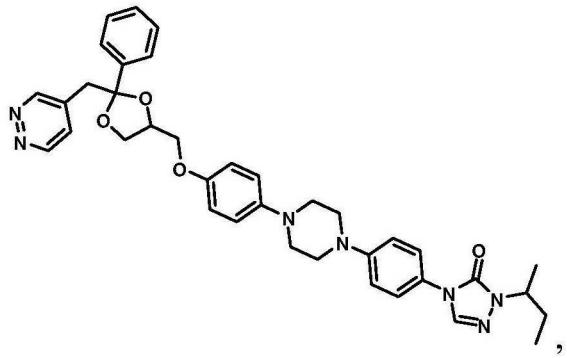

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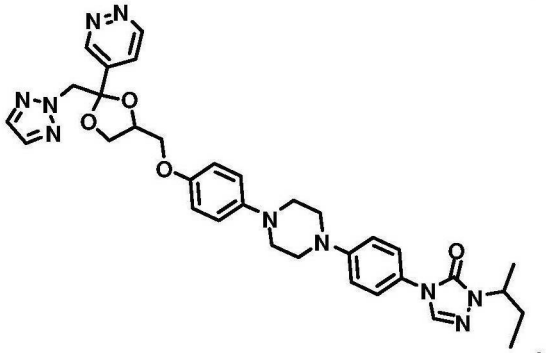

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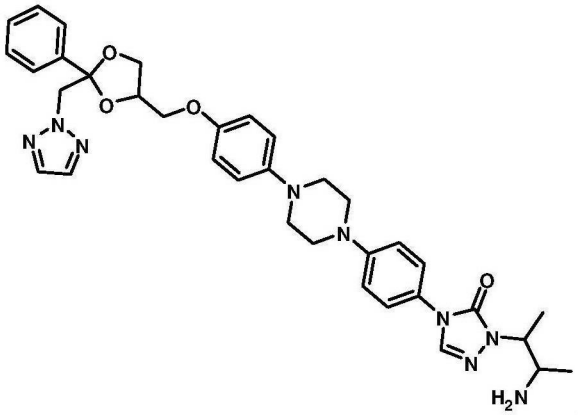

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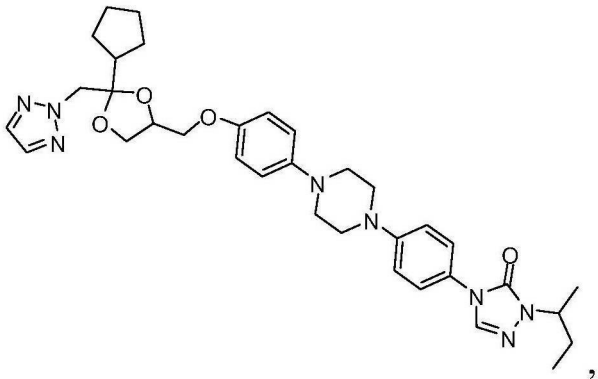

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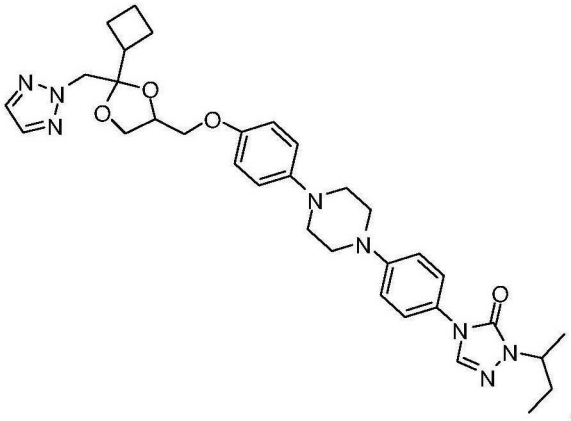

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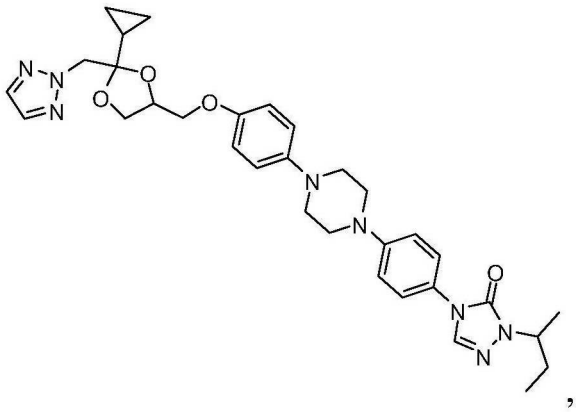

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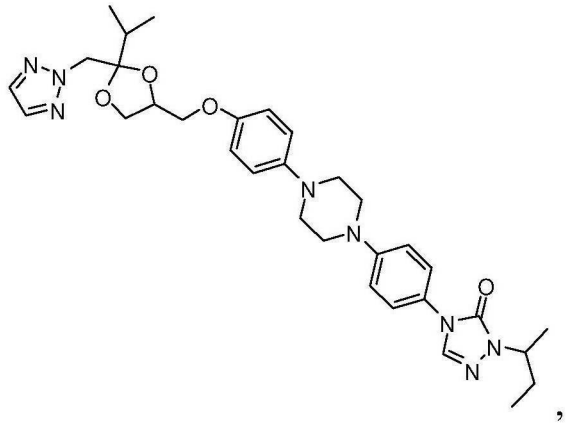
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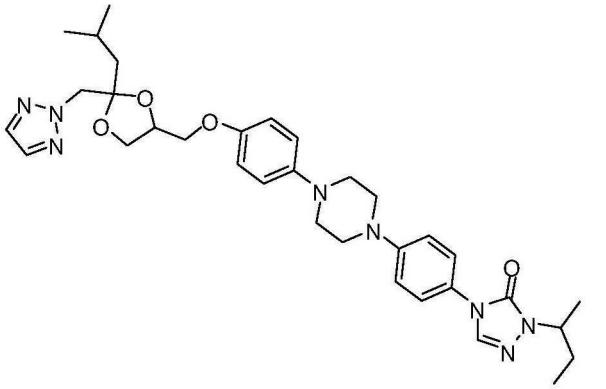
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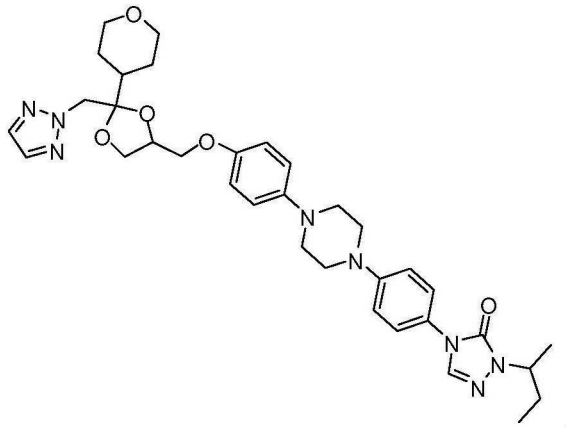
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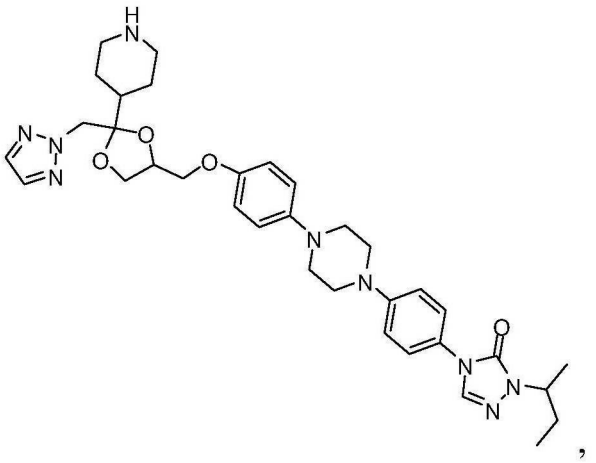
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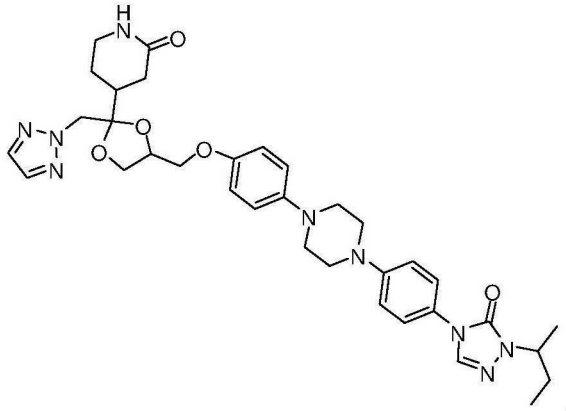
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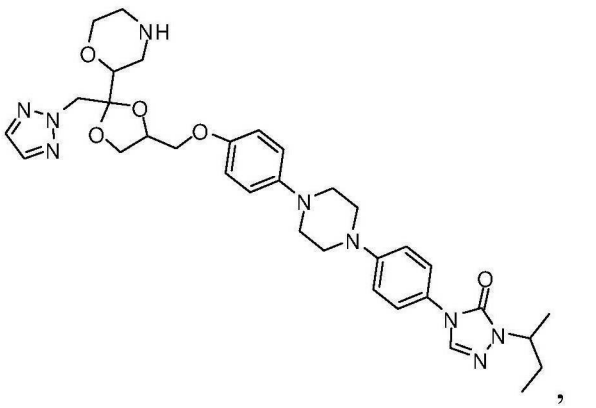
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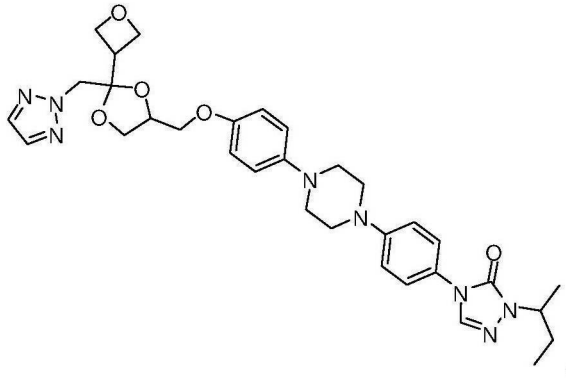
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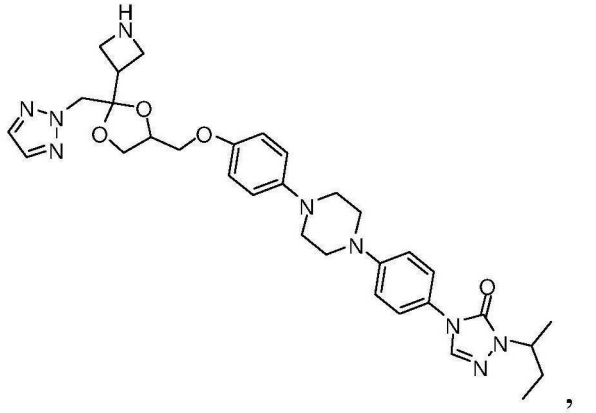
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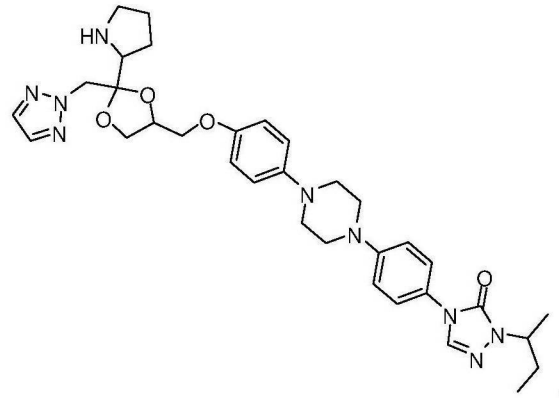
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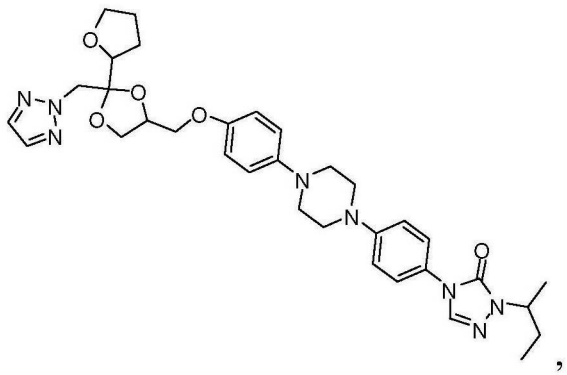
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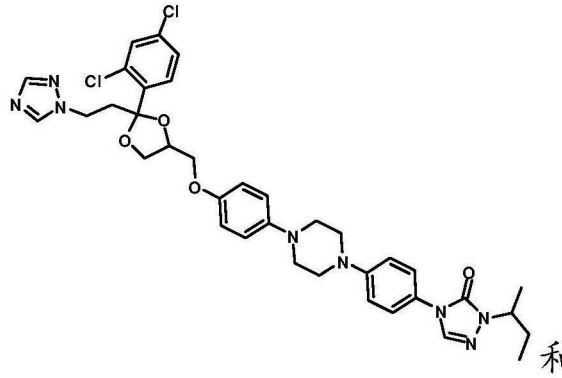
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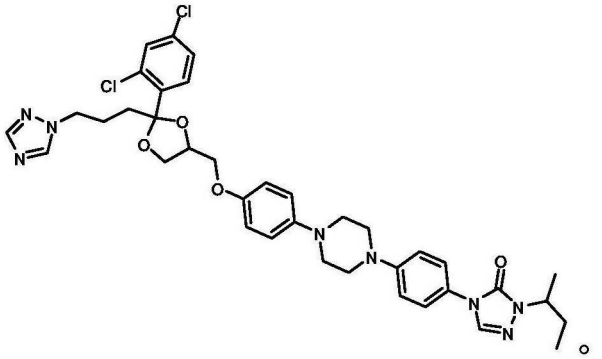

。

74. 一种药物组合物，其包含如权利要求 40-73 中任一项所述的化合物，或其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体，和药学上可接受的赋形剂。

纤维化的小分子抑制剂

相关申请的交叉引用

[0001] 本申请要求 2013 年 6 月 7 日提交的美国专利申请号 61/832,768 的权益，其通过引用全文并入本文。

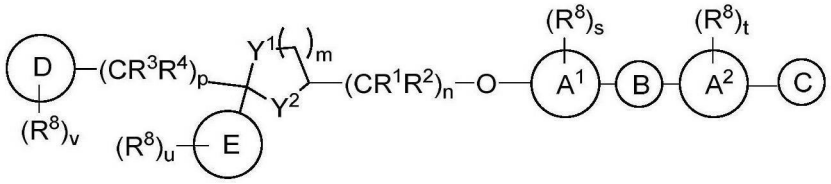
背景技术

[0002] 纤维化，通常被定义为过量结缔组织的产生，是由于多种潜在疾病而发生的。慢性炎症或组织损伤 / 重塑是典型的诱发纤维化的事件。具体的疾病实例包括特发性肺纤维化 (IPF)、与酒精性和非酒精性肝硬化的后期相关的肝纤维化、肾纤维化、心脏纤维化以及由异常创伤愈合导致的瘢痕疙瘩形成 [Wynn, T. A. (2004) Nature Reviews Immunology. 4:583-594 ; Friedman, S. L. (2013) Science Translation Medicine. 5(167):1-17]。另外，纤维化是与慢性自身免疫病相关的关键病理学特征，该慢性自身免疫病包括类风湿性关节炎、克罗恩病、系统性红斑狼疮和硬皮病。代表迫切未满足的医疗需求的疾病包括特发性肺纤维化 (IPF)、硬皮病和非酒精性脂肪性肝炎 (NASH) 相关的肝纤维化。预计 NASH 相关肝纤维化的增加的发病率直接与 2 型糖尿病和肥胖症的发病率并列。

[0003] 硬皮病是一种罕见的慢性自身免疫病，其特征在于正常组织被致密的厚纤维组织替代。尽管硬皮病的确切根本原因未知，但该疾病通常涉及免疫细胞介导的皮肤肌成纤维细胞的活化，从而导致过量细胞外基质蛋白 (例如，I 型胶原蛋白) 的沉积，这导致皮肤变厚，并且在一些情况下导致多个器官的硬化和最终衰竭。目前，硬皮病尚没有治愈性方法。其治疗仅限于尝试控制症状，并且通常需要方法的联合。尽管局限于皮肤的硬皮病通常不危及生命，但影响多个内脏器官的系统性硬皮病可能是威胁生命的疾病。

发明内容

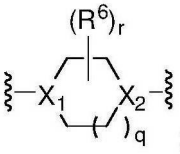
[0004] 在一个方面，本文提供了一种治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病的方法，该方法包括施用包含治疗有效量的式 (I) 化合物、其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体的组合物 ：



式(I)

其中 ：

A^1 和 A^2 独立地选自芳基或杂芳基 ；

B 为  ；

C 为任选取代的 5 或 6 元杂环基或任选取代的 5 或 6 元杂芳基，其中该杂环基或该杂芳基含有 1 至 4 个氮原子；

D 为芳基或杂芳基；

E 为芳基、杂芳基、碳环基、杂环基或烷基；

每个 R^1、R^2、R^3和 R^4独立地选自 H、烷基、卤代烷基或烷氧基；

X_1和 X_2独立地选自 N 和 CR^5；

R^5为 H、OH、烷基或烷氧基；

每个 R^6独立地为烷基、卤代烷基、卤代、烷氧基、- 亚烷基 ($NR^{13}R^{14}$) 或芳基；

每个 R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、- 亚烷基 (环烷基)、- 亚烷基 (杂环基)、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和 $-C(O)NR^{13}R^{14}$；或者两个相邻的 R^8形成杂环基环；

每个 R^{13}和 R^{14}独立地选自 H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者 R^{13}和 R^{14}与它们所连接至的原子一起形成杂环；

Y^1和 Y^2独立地选自 O、CH_2、NH 和 NR^{13}；

n 为 1、2 或 3；

m 为 1 或 2；

p 为 1、2、3 或 4；

q 为 1、2 或 3；

r 为 0、1、2、3、4、5、6、7 或 8；

s 为 0、1、2、3 或 4；

t 为 0、1、2、3 或 4；

u 为 0、1、2、3、4 或 5；且

v 为 0、1、2、3 或 4。

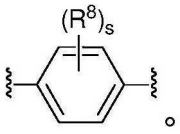
[0005] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1和 X_2为 N。

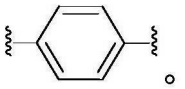
[0006] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1为 CR^5且 X_2为 N。

[0007] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1为 N 且 X_2为 CR^5。

[0008] 在式 (I) 化合物的以上或以下描述的一些实施方案中，q 为 1 且 r 为 0。

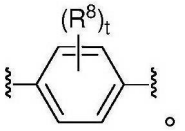
[0009] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1为芳基。

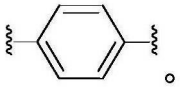
[0010] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1为  。

[0011] 在式(I)化合物的以上或以下描述的一些实施方案中，A^1为  。

[0012] 在式(I)化合物的以上或以下描述的一些实施方案中，A^1为杂芳基。

[0013] 在式(I)化合物的以上或以下描述的一些实施方案中，A^2为芳基。

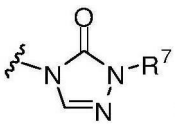
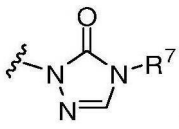
[0014] 在式(I)化合物的以上或以下描述的一些实施方案中，A^2为  。

[0015] 在式(I)化合物的以上或以下描述的一些实施方案中，A^2为  。

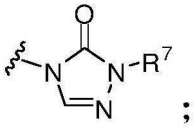
[0016] 在式(I)化合物的以上或以下描述的一些实施方案中，A^2为杂芳基。

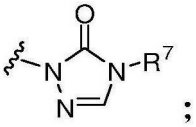
[0017] 在式(I)化合物的以上或以下描述的一些实施方案中，A^2为吡啶、吡嗪、嘧啶、哒嗪或三嗪。

[0018] 在式(I)化合物的以上或以下描述的一些实施方案中，C为任选取代的5或6元杂芳基。在式(I)化合物的以上或以下描述的其他实施方案中，C为任选取代的5或6元杂环基。

[0019] 在式(I)化合物的以上或以下描述的一些实施方案中，C为  或  ；且

R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

[0020] 在式(I)化合物的以上或以下描述的一些实施方案中，C为  ；且R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

[0021] 在式(I)化合物的以上或以下描述的一些实施方案中，C为  ；且R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

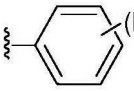
[0022] 在式(I)化合物的以上或以下描述的一些实施方案中，E为烷基。

[0023] 在式(I)化合物的以上或以下描述的一些实施方案中，E为环烷基。

[0024] 在式(I)化合物的以上或以下描述的一些实施方案中，E为环丙基、环丁基、环戊基或环己基。

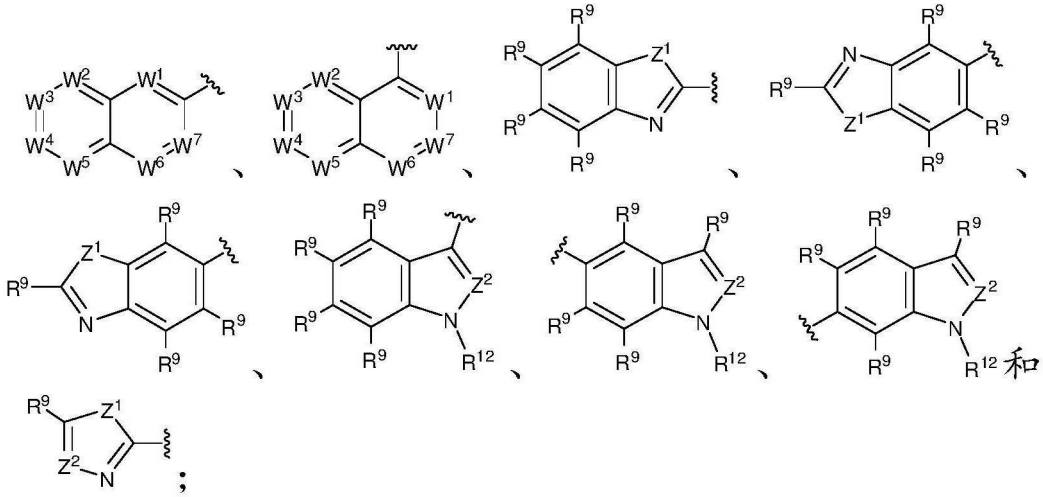
[0025] 在式(I)化合物的以上或以下描述的一些实施方案中，E为杂环基。

[0026] 在式(I)化合物的以上或以下描述的一些实施方案中，E为芳基。

[0027] 在式(I)化合物的以上或以下描述的一些实施方案中,E为  且u为0、1、2、3、4或5。

[0028] 在式(I)化合物的以上或以下描述的一些实施方案中,E为杂芳基。

[0029] 在式(I)化合物的以上或以下描述的一些实施方案中,E选自:



;

W^1、W^2、W^3、W^4、W^5、W^6和W^7独立地选自N和CR^9;

Z^1为NR^{12}、S或O;

Z^2为N或CR^9;

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基;

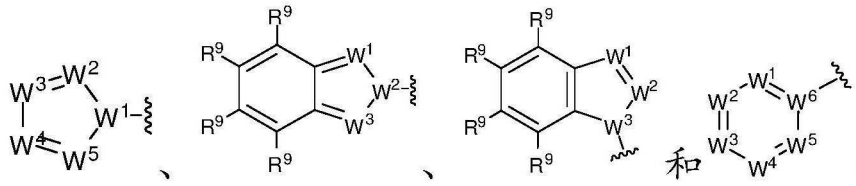
每个R^{10}和R^{11}独立地选自H和烷基;或者R^{10}和R^{11}与它们所连接至的氮一起形成杂环;且

R^{12}为H、烷基或卤代烷基。

[0030] 在式(I)化合物的以上或以下描述的一些实施方案中,D为芳基。

[0031] 在式(I)化合物的以上或以下描述的一些实施方案中,E为杂芳基。

[0032] 在式(I)化合物的以上或以下描述的一些实施方案中,D选自:

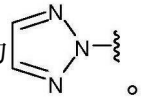
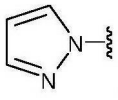
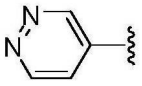


;

W^1、W^2、W^3、W^4和W^5独立地选自N和CR^9;

W^6为N或C；且

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基。

[0033] 在式(I)化合物的以上或以下描述的某些实施方案中，D为  。在式(I)化合物的以上或以下描述的某些实施方案中，D为  。在式(I)化合物的以上或以下描述的某些实施方案中，D为  。

[0034] 在式(I)化合物的以上或以下描述的一些实施方案中，Y^1和Y^2为O。

[0035] 在式(I)化合物的以上或以下描述的一些实施方案中，m为1。

[0036] 在式(I)化合物的以上或以下描述的一些实施方案中，p为1、2或3。

[0037] 在式(I)化合物的以上或以下描述的一些实施方案中，p为1。

[0038] 在式(I)化合物的以上或以下描述的一些实施方案中，R^1、R^2、R^3和R^4为氢。

[0039] 本文进一步提供了一种使用式(I)化合物治疗纤维化的方法，其中该纤维化是肝纤维化、特发性肺纤维化、肾纤维化或心脏纤维化。

[0040] 本文进一步提供了一种使用式(I)化合物治疗肝纤维化的方法，其中该肝纤维化与酒精性或非酒精性肝硬化的后期相关。

[0041] 本文进一步提供了一种使用式(I)化合物治疗纤维化的方法，其中该纤维化是特发性肺纤维化。

[0042] 本文进一步提供了一种使用式(I)化合物治疗疾病的方法，其中以纤维化为特征的疾病或病症是慢性自身免疫病。

[0043] 本文进一步提供了一种使用式(I)化合物治疗慢性自身免疫病的方法，其中该慢性自身免疫病是类风湿性关节炎、硬皮病、克罗恩病或系统性红斑狼疮。

[0044] 本文进一步提供了一种使用式(I)化合物治疗慢性自身免疫病的方法，其中该慢性自身免疫病是硬皮病。

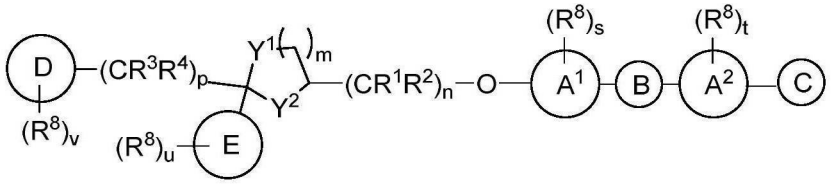
[0045] 本文进一步提供了一种使用式(I)化合物治疗纤维化的方法，其中该纤维化是由异常创伤愈合导致的瘢痕疙瘩形成。

[0046] 本文进一步提供了一种使用式(I)化合物治疗纤维化的方法，其中该纤维化在器官移植后发生。

[0047] 本文还提供了一种治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病

的方法，该方法包括施用包含治疗有效量的本文所述的化合物联合一种或多种药物剂的组合物。在以上描述的某些实施方案中，所述一种或多种药物剂是抗纤维化剂。在以上描述的某些实施方案中，所述一种或多种药物剂是抗真菌剂。

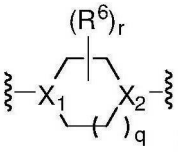
[0048] 在一个方面，本文提供了式（II）的化合物，其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体：



式(II)

其中：

A^1和 A^2独立地选自芳基或杂芳基；

B 为 

；

C 为任选取代的 5 或 6 元杂环基或任选取代的 5 或 6 元杂芳基，其中该杂环基或该杂芳基含有 1 至 4 个氮原子；

D 为芳基或杂芳基；

E 为芳基、杂芳基、碳环基、杂环基或烷基；

每个 R^1、R^2、R^3和 R^4独立地选自 H、烷基、卤代烷基或烷氧基；

X_1和 X_2独立地选自 N 和 CR^5；

R^5为 H、OH、烷基或烷氧基；

每个 R^6独立地为烷基、卤代烷基、卤代、烷氧基、- 亚烷基 $(NR^{13}R^{14})$ 或芳基；

R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 $(NR^{13}R^{14})$、环烷基、杂环基、- 亚烷基（环烷基）或 - 亚烷基（杂环基）；

每个 R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 $(NR^{13}R^{14})$、- 亚烷基（环烷基）、- 亚烷基（杂环基）、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和 $-C(O)NR^{13}R^{14}$；或者两个相邻的 R^8形成杂环基环；

每个 R^{13}和 R^{14}独立地选自 H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者 R^{13}和 R^{14}与它们所连接至的原子一起形成杂环；

Y^1和 Y^2独立地选自 O、CH_2、NH 和 NR^{13}；

n 为 1、2 或 3；

m 为 1 或 2；

p 为 1、2、3 或 4；

q 为 1、2 或 3；

r 为 0、1、2、3、4、5、6、7 或 8；

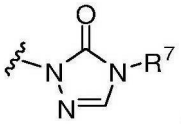
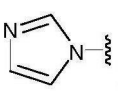
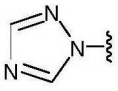
s 为 0、1、2、3 或 4；

t 为 0、1、2、3 或 4；

u 为 0、1、2、3、4 或 5；

v 为 0、1、2、3 或 4；

条件是：

如果 X_1 和 X_2 为 N，r 为 0，q 为 1，A^1 和 A^2 为苯基，Y^1 和 Y^2 为 O，m 和 n 为 1，R^1、R^2、R^3 和 R^4 为氢，且 C 为  ，则 D 不是  或  ；且

该化合物不是 4-(4-(4-(4-((2-((1H-吡唑-1-基)甲基)-2-(2,4-二氟苯基)-1,3-二氧杂环戊-4-基)甲氧基)苯基)哌嗪-1-基)苯基)-1-异丙基-1H-1,2,4-三唑-5(4H)-酮。

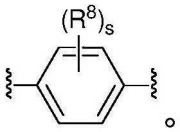
[0049] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 和 X_2 为 N。

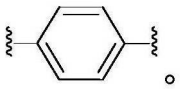
[0050] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 为 CR^5 且 X_2 为 N。

[0051] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 为 N 且 X_2 为 CR^5。

[0052] 在式 (II) 化合物的以上或以下描述的一些实施方案中，q 为 1 且 r 为 0。

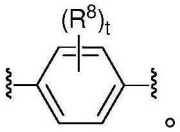
[0053] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为芳基。

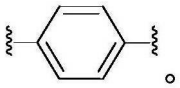
[0054] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为  。

[0055] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为  。

[0056] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为杂芳基。

[0057] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为芳基。

[0058] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

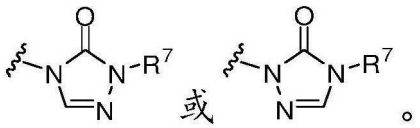
[0059] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

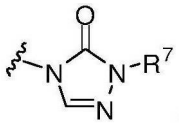
[0060] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为杂芳基。

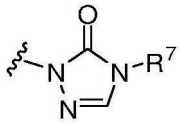
[0061] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为吡啶基、吡嗪基、嘧啶基、哒嗪基或三嗪基。

[0062] 在式 (II) 化合物的以上或以下描述的一些实施方案中，C 为任选取代的 5 或 6 元杂芳基。在式 (II) 化合物的以上或以下描述的其他实施方案中，C 为任选取代的 5 或 6 元杂环基。

[0063] 在式 (II) 化合物的以上或以下描述的一些实施方案中，C 为



[0064] 在式(II)化合物的以上或以下描述的一些实施方案中，C为  。

[0065] 在式(II)化合物的以上或以下描述的一些实施方案中，C为  。

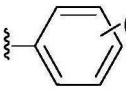
[0066] 在式(II)化合物的以上或以下描述的一些实施方案中，E为烷基。

[0067] 在式(II)化合物的以上或以下描述的一些实施方案中，E为环烷基。

[0068] 在式(II)化合物的以上或以下描述的一些实施方案中，E为环丙基、环丁基、环戊基或环己基。

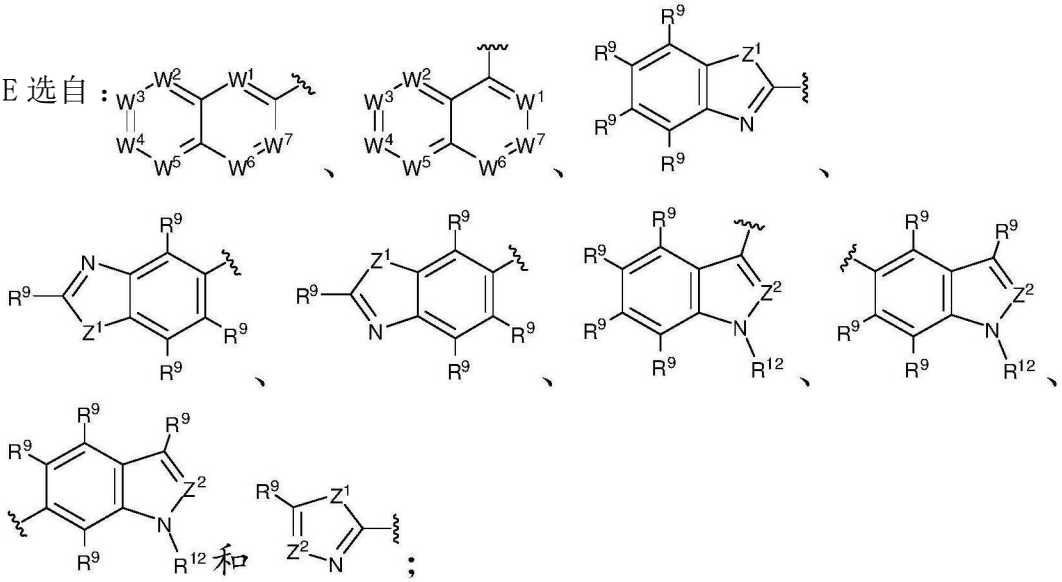
[0069] 在式(II)化合物的以上或以下描述的一些实施方案中，E为杂环基。

[0070] 在式(II)化合物的以上或以下描述的一些实施方案中，E为芳基。

[0071] 在式(II)化合物的以上或以下描述的一些实施方案中，E为  且u为0、1、2、3、4或5。

[0072] 在式(II)化合物的以上或以下描述的一些实施方案中，E为杂芳基。

[0073] 在式(II)化合物的以上或以下描述的一些实施方案中，

E选自：  ；

W^1、W^2、W^3、W^4、W^5、W^6和W^7独立地选自N和CR^9；

Z^1为NR^{12}、S或O；

Z^2为N或CR^9；

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)$

NR^{10}(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基;

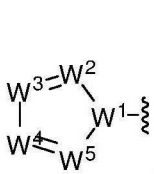
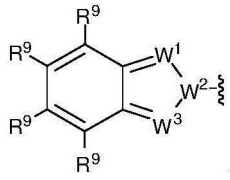
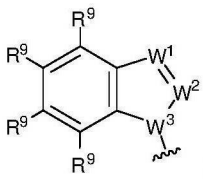
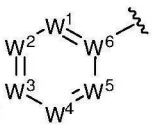
每个R^{10}和R^{11}独立地选自H和烷基;或者R^{10}和R^{11}与它们所连接至的氮一起形成杂环;且

R^{12}为H、烷基或卤代烷基。

[0074] 在式(II)化合物的以上或以下描述的一些实施方案中,D为芳基。

[0075] 在式(II)化合物的以上或以下描述的一些实施方案中,D为杂芳基。

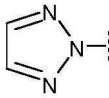
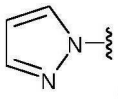
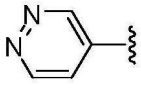
[0076] 在式(II)化合物的以上或以下描述的一些实施方案中,

D选自  、  、  和  ;

W^1、W^2、W^3、W^4和W^5独立地选自N和CR^9;

W^6为N或C;且

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基。

[0077] 在式(II)化合物的以上或以下描述的某些实施方案中,D为  。在式(II)化合物的以上或以下描述的某些实施方案中,D为  。在式(II)化合物的以上或以下描述的某些实施方案中,D为  。

[0078] 在式(II)化合物的以上或以下描述的一些实施方案中,Y^1和Y^2为O。

[0079] 在式(II)化合物的以上或以下描述的一些实施方案中,m为1。

[0080] 在式(II)化合物的以上或以下描述的一些实施方案中,p为1、2或3。

[0081] 在式(II)化合物的以上或以下描述的一些实施方案中,p为1。

[0082] 在式(II)化合物的以上或以下描述的一些实施方案中,R^1、R^2、R^3和R^4为氢。

[0083] 本文还提供了一种药物组合物，其包含式 (II) 或如以上及以下描述的化合物或其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体，和药学上可接受的赋形剂。

[0084] 在一些实施方案中，本文进一步公开了用于鉴定纤维化的抑制剂的基于图像的系统。在一些实施方案中，该系统包括 (a) 一种或多种成纤维细胞 ；和 (b) 用于生成一种或多种成纤维细胞的一幅或多幅图像的细胞成像装置。在一些实施方案中，该细胞成像装置包括荧光显微镜。在一些实施方案中，该细胞成像装置包括 CCD 相机技术。在一些实施方案中，该细胞成像装置是自动化的。在一些实施方案中，该细胞成像装置是手动操作的。在一些实施方案中，该细胞成像装置是热电冷却的。

[0085] 在一些实施方案中，该系统进一步包括光源。在一些实施方案中，该光源是 LED。

[0086] 在一些实施方案中，该系统进一步包括扫描仪。

[0087] 在一些实施方案中，该系统进一步包括计算机。

[0088] 在一些实施方案中，该系统进一步包括一个或多个用于存储和 / 或接收一幅或多幅图像的存储单元。在一些实施方案中，该系统进一步包括一个或多个用于存储和 / 或接收关于生成一幅或多幅图像的一个或多个指令的存储单元。

[0089] 在一些实施方案中，该系统进一步包括一个或多个用于分析一种或多种成纤维细胞的一幅或多幅图像的处理器。在一些实施方案中，该系统进一步包括一个或多个用于处理一种或多种成纤维细胞的一幅或多幅图像的处理器。在一些实施方案中，该系统进一步包括一个或多个用于传送一种或多种成纤维细胞的一幅或多幅图像的处理器。

[0090] 在一些实施方案中，该系统进一步包括用于捕捉、生成、分析、扫描、存储和 / 或传送一幅或多幅图像的一个或多个软件程序。

[0091] 在一些实施方案中，该系统进一步包括一个或多个条形码阅读器，该条形码阅读器用于阅读包含一种或多种细胞的一个或多个样品上的一个或多个条形码。

[0092] 在一些实施方案中，该系统进一步包括用于处理包含一种或多种细胞的一个或多个样品的一个或多个机器人。在一些实施方案中，该系统进一步包括用一种或多种药剂处理包含一种或多种细胞的一个或多个样品的一个或多个机器人。

[0093] 在一些实施方案中，所述一种或多种药剂包括 TGF- β 。在一些实施方案中，所述一种或多种药剂包括一种或多种测试剂。

[0094] 在一些实施方案中，该系统进一步包括用于将一种或多种测试剂鉴定为纤维化的抑制剂的一个或多个处理器。在一些实施方案中，该系统进一步包括用于对纤维化的抑制剂进行排序的一个或多个处理器。

[0095] 在一些实施方案中，该系统进一步包括一种或多种算法。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析与一种或多种药剂接触的一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的强度。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的荧光强度。

[0096] 在一些实施方案中，所述细胞成像装置包括 CellInsight NXT High Content Screening(HCS) 平台。

[0097] 在一些实施方案中，所述一种或多种成纤维细胞是肝星形细胞 (HSC)。

[0098] 本文进一步公开了鉴定纤维化的抑制剂的方法。在一些实施方案中，该方法包括(a)使包含一种或多种成纤维细胞的第一样品与细胞生长剂接触；(b)使包含一种或多种成纤维细胞的第二样品与该细胞生长剂和第一测试剂接触；(c)生成第一样品的一种或多种成纤维细胞的一幅或多幅图像和第二样品的一种或多种成纤维细胞的一幅或多幅图像；以及(d)基于对第一样品的一幅或多幅图像和第二样品的一幅或多幅图像的分析，确定第一测试剂是否是纤维化的抑制剂。

[0099] 在一些实施方案中，所述细胞生长剂是生长因子。在一些实施方案中，所述细胞生长剂是转化生长因子β(TGF-b)。

[0100] 在一些实施方案中，第一测试剂是小分子。在一些实施方案中，第一测试剂是生物活性小分子。

[0101] 在一些实施方案中，第二样品同时与细胞生长剂和第一测试剂接触。在一些实施方案中，第二样品顺序地与细胞生长剂和第一测试剂接触。在一些实施方案中，第二样品在与第一测试剂接触之前与细胞生长剂接触。在一些实施方案中，第二样品在与细胞生长剂接触之前与第一测试剂接触。

[0102] 在一些实施方案中，该方法进一步包括包含一种或多种成纤维细胞的一个或多个额外的样品。在一些实施方案中，第一样品、第二样品和/或所述一个或多个额外的样品来自相同的来源。在一些实施方案中，第一样品、第二样品和/或所述一个或多个额外的样品来自两个或更多个不同的来源。

[0103] 在一些实施方案中，该方法进一步包括使所述一个或多个额外的样品与细胞生长剂和一种或多种额外的测试剂接触。

[0104] 在一些实施方案中，同时捕捉第一样品的一幅或多幅图像和第二样品的一幅或多幅图像。在一些实施方案中，顺序地捕捉第一样品的一幅或多幅图像和第二样品的一幅或多幅图像。

[0105] 在一些实施方案中，第一样品的一种或多种成纤维细胞在第一培养板上的一个或多个孔中培养。在一些实施方案中，第二样品的一种或多种成纤维细胞在第二培养板的一个或多个孔上培养。在一些实施方案中，所述一个或多个额外的样品的一种或多种成纤维细胞在一个或多个额外的培养板的一个或多个孔上培养。

[0106] 在一些实施方案中，第一培养板和第二培养板是不同的。在一些实施方案中，第一培养板、第二培养板和/或所述一个或多个额外的培养板是不同的。

[0107] 在一些实施方案中，第一培养板和第二培养板是相同的。在一些实施方案中，第一培养板、第二培养板和/或所述一个或多个额外的培养板是相同的。

[0108] 在一些实施方案中，该方法进一步包括使第一样品的一种或多种成纤维细胞和/或第二样品的一种或多种成纤维细胞与第三药剂接触。在一些实施方案中，该方法进一步包括使所述一个或多个额外的样品的一种或多种成纤维细胞与第三药剂接触。

[0109] 在一些实施方案中，第三药剂是抗体。在一些实施方案中，第三药剂是抗平滑肌肌动蛋白(SMA)抗体。

[0110] 在一些实施方案中，第一样品的一幅或多幅图像和/或第二样品的一幅或多幅图像基于与第三药剂接触的一种或多种成纤维细胞的图像。在一些实施方案中，所述一个或多个额外的样品的一幅或多幅图像基于与第三药剂接触的一种或多种成纤维细胞的图像。

[0111] 在一些实施方案中，生成一幅或多幅图像包括使用一个或多个细胞成像装置。在一些实施方案中，该细胞成像装置包括 CellInsight NXT High Content Screening(HCS)平台。

[0112] 在一些实施方案中，该方法进一步包括一种或多种算法。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析与一种或多种药剂接触的一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的强度。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的荧光强度。

[0113] 在一些实施方案中，该方法进一步包括检测一种或多种成纤维细胞的转分化。在一些实施方案中，一种或多种成纤维细胞的转分化包括转分化为一种或多种肌成纤维细胞。

[0114] 在一些实施方案中，确定第一测试剂是否被鉴定为纤维化的抑制剂基于第一样品中的肌成纤维细胞组成与第二样品中的肌成纤维细胞组成的比较。在一些实施方案中，如果第二样品中的肌成纤维细胞组成低于第一样品中的肌成纤维细胞组成，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果第二样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或 90％，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果第二样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 1.5、2、2.5、3、3.5、4、4.5、5、6、7、8、9、10、11、12、13、14 或 15 倍，则将第一测试剂鉴定为纤维化的抑制剂。

[0115] 在一些实施方案中，该方法进一步包括确定一种或多种额外的测试剂是否是纤维化的抑制剂。在一些实施方案中，确定一种或多种额外的测试剂是否被鉴定为纤维化的抑制剂基于第一样品中的肌成纤维细胞组成与所述一个或多个额外的样品中的肌成纤维细胞组成的比较。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成低于第一样品中的肌成纤维细胞组成，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或 90％，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 1.5、2、2.5、3、3.5、4、4.5、5、6、7、8、9、10、11、12、13、14 或 15 倍，则将第一测试剂鉴定为纤维化的抑制剂。

援引并入

[0116] 本说明书中所提到的所有出版物、专利和专利申请均通过引用并入本文，其程度如同特别且单独地指出每个单独的出版物、专利或专利申请通过引用而并入。

附图说明

[0117] 图 1 描述了高含量成像试验，其基于与成纤维细胞向肌成纤维细胞的转分化相关的 α-SMA 染色和细胞形态学变化，该转分化已经使用初级人肺成纤维细胞和初级啮齿动物 HSC 确定。已经确定了在适用于高通量小分子筛选的小型化（384 孔板）形式中促进稳

健的体外转分化的条件（包括血清饥饿和后续 TGF-β 处理）。使用选择性 ALK-5TGF-β1 受体抑制剂 (SB-431542) 作为阳性对照。

[0118] 图 2 描述了伊曲康唑在体外多种细胞类型中抑制肌成纤维细胞分化。测试了初级心脏、肺或皮肤成纤维细胞和 HSC 的制备物对 TGFβ 的响应性。通过平滑肌肌动蛋白染色对细胞分析了肌成纤维细胞形态学。使用 ALK5 抑制剂 (SB-431542) 作为阳性对照。伊曲康唑 (Itra,2.5μM) 抑制来源于肺、心脏、皮肤或肝 (HSC) 的成纤维细胞中的肌成纤维细胞分化。

[0119] 图 3 描述了伊曲康唑在体外多种细胞类型中抑制肌成纤维细胞分化。测试了初级心脏、肺或皮肤成纤维细胞和 HSC 的制备物对 TGFβ 的响应性。使用多个纤维化相关基因的 qRT-PCR 谱分析和 Western 印迹分析来确认体外活性。药理学研究支持双重抑制 MOA(VEGF，Hh 信号传导）。

[0120] 图 4 示出了伊曲康唑在肝 (CCl_4) 和肺（博来霉素）模型的啮齿动物 PoC 研究中的效力。使用吡非尼酮和 AM-152 作为基准，证明了伊曲康唑在四氯化碳诱发的肝纤维化和博来霉素诱发的肺及皮肤纤维化小鼠模型中具有同等或更高的效力。

[0121] 图 5 示出了从基于肌成纤维细胞活化成像的试验获得的数据：(a) 针对 SMA 染色的平均细胞面积的细胞分析；(b) 针对 SMA 染色的平均荧光强度的细胞分析。

[0122] 图 6 示出了暴露于 TGFβ 和伊曲康唑的细胞中纤维化相关蛋白质的 Western 印迹分析。

[0123] 图 7 示出了用伊曲康唑处理的人肺成纤维细胞的基因表达分析：(a) 表示为相对于未用 TGF-β1 处理的样品的调节倍数的数据分析；(b) 来自集中于纤维化的 RT2Profiler PCR 阵列的原始数据。

[0124] 图 8 示出了用伊曲康唑处理的大鼠肝星形细胞中 Hedgehog 相关基因的 qPCR 分析：(a)PTCH1mRNA 的相对水平；(b)GLI1mRNA 的相对水平；(c) 两个 qPCR 实验的原始数据。

[0125] 图 9 示出了 Smoothened 敲减后 COL1-GFP HSC 的 Western 印迹分析。

[0126] 图 10 示出了用伊曲康唑处理后 VEGFR2 迁移模式的 Western 印迹分析。

[0127] 图 11 示出了用 VEGFR 和 Hedgehog 抑制化合物联合处理的大鼠肝星形细胞的 Western 印迹分析。

[0128] 图 12 示出了伊曲康唑和化合物 42 在 Hedgehog 报告子试验中的活性：(a) 暴露于 10nM SAG 和指定剂量抑制剂的 TM3-GLI-LUC 细胞的相对 GLI-LUC 活性；(b) 暴露于 400nM SAG 和指定剂量抑制剂的 TM3-GLI-LUC 细胞的相对 GLI-LUC 活性。

[0129] 图 13 示出了 VEFGR1、VEGFR2 或 SMO 敲减后，LX2 人肝星形细胞的 Western 印迹分析。

[0130] 图 14 示出了用于评价化合物 42 和伊曲康唑的博来霉素诱发的肺纤维化模型研究设计。

[0131] 图 15 示出了在博来霉素诱发的肺纤维化模型中对化合物 42 和伊曲康唑的评价：(a) 暴露于博来霉素和指定剂量药物的小鼠的平均 Ashcroft 得分；(b) 用于评价抗纤维化药物的模型的图示；(c) 来自指定治疗组的 Masson 三色染色的肺的代表性图像。

[0132] 图 16 示出了改进的 Ashcroft 评分系统的表征。

[0133] 图 17 示出了来自在博来霉素诱发的肺纤维化模型中对化合物 42 和伊曲康唑的评

价的组织学数据定量：(a) 平均 Ashcroft 得分；(b) 平均百分比染色面积值。

[0134] 图 18 示出了用于评价化合物 42 和伊曲康唑的四氯化碳诱发的肝纤维化模型研究设计。

[0135] 图 19 示出了在四氯化碳诱发的肝纤维化模型中对化合物 42 和伊曲康唑的评价：(a) 天狼星红 (Sirius Red) 染色阳性的总面积百分比；(b)CCl_4诱发的肝纤维化模型的图像分析的数值数据；(c)CCl_4诱发的肝纤维化模型的天狼星红染色肝切片的代表性图像；(d)Western 印迹分析。

[0136] 图 20 示出了用于评价化合物 42 和伊曲康唑的啮齿动物创伤愈合模型研究设计。

[0137] 图 21 示出了来自在啮齿动物创伤愈合模型中对化合物 42 和伊曲康唑的评价的研究结果。

具体实施方式

[0138] 纤维化是一个极为重要却令人惊讶地被忽视的健康问题。在西方国家，所有自然死亡中的近 45％归因于慢性纤维增生性疾病。然而，目前只有一种在临床上批准的药物（吡非尼酮）专门针对纤维化的发病机理并且直接表明用于纤维化疾病的治疗。纤维化影响身体几乎每一个组织，并且当其高度进展时，可以导致器官功能失常和死亡。显然，对新型抗纤维化药物的鉴定是尚未得到满足的医疗需求，其将对多种疾病人群中的患者具有显著的有益影响。此外，目前硬皮病尚没有治愈性方法，而治疗仅限于症状控制。

[0139] 纤维化，通常被定义为过量结缔组织的产生，是由于多种潜在疾病而发生的。慢性炎症或组织损伤 / 重塑是典型的诱发纤维化的事件。纤维化影响身体几乎每一个组织，并且当其高度进展时，可以导致器官功能失常和死亡。具体的疾病实例包括特发性肺纤维化 (IPF)；与酒精性和非酒精性肝硬化的后期相关的肝纤维化；肾纤维化；心脏纤维化；和由异常创伤愈合导致的瘢痕疙瘩形成。另外，纤维化是与慢性自身免疫病相关的关键病理学特征，该慢性自身免疫病包括类风湿性关节炎、硬皮病、克罗恩病和系统性红斑狼疮。就此而言，纤维化是一个极为重要却令人惊讶地被忽视的健康问题。实际上，在西方国家，所有自然死亡中的近 45％归因于慢性纤维增生性疾病。然而，目前只有一种在临床上批准的药物（吡非尼酮，仅批准在欧洲用于治疗 IPF）专门针对纤维化的发病机理并且直接表明用于纤维化疾病的治疗。遗憾的是，吡非尼酮具有显著的肝和胃肠副作用，因此建议用吡非尼酮治疗的患者避免直接暴露于阳光，因为已知这会引起光敏反应，从而导致皮疹、皮肤干燥或瘙痒。最近，溶血磷脂酸 1(LPA1) 拮抗剂（例如，AM-152）已被证明在 IPF 的临床前模型中是有效的。然而，AM-152 的临床效力仍有待证明。显然，对新型抗纤维化药物的鉴定是一个重要的尚未得到满足的医疗需求，其将对多种疾病人群中的患者具有显著的有益影响。

[0140] 尽管可以在给定组织或器官中引起纤维化过程的疾病和诱因具有多样性，但在迄今研究的所有情况下存在共同的生化和细胞机理。在损害或炎性损伤后，固有的成纤维细胞（在某些情况下，募集的、已经经历上皮向间质转变的来源于骨髓的循环纤维细胞或上皮细胞）被激活并“转分化”为表达 α－平滑肌肌动蛋白（α-SMA）的肌成纤维细胞，其分泌创伤修复所需的细胞外基质 (ECM) 成分。在肝纤维化的情况下，被称为静息肝星形细胞 (HSC) 的固有周细胞群体“转分化”成产生 I 型胶原蛋白的、表达 α-SMA 的、纤维发生的、“活化的”HSC。转化生长因子 β1(TGF-β1) 介导的 Smad 3/4 信号传导通常驱使固有的成

纤维细胞或 HSC 转分化为肌成纤维细胞或活化的 HSC，并在后者群体中刺激 ECM 成分的产生。血小板衍生生长因子 (PDGF) 也充当驱使细胞活化和增殖的共同促纤维化细胞因子。

[0141] 用于治疗进行性纤维化疾病的一种治疗性方法是针对于众多的复杂病因免疫过程中的一种。此方法受到机理明确性的缺乏和基础疾病的潜在恶化的限制。用于治疗不同纤维化疾病的一种有吸引力的替代方法是直接针对于转分化途径，该途径负责静息成纤维细胞与活化的促纤维化肌成纤维细胞的相互转化。能够阻断成纤维细胞向活化肌成纤维细胞转化的药物可以在受伤或损伤（例如，心肌梗死）后预防性地施用，或在能够修复的器官疾病的早期（例如，肝纤维化、IPF 或硬皮病）治疗性地施用。TGF-β1 产生的直接抑制剂（例如，吡非尼酮）不是慢性给药的理想候选物，并且对自身免疫病的治疗而言是特别不可取的，因为它们有恶化自身免疫反应的可能。可替代地，能够诱导存在的肌成纤维细胞向静息细胞命运逆转的药物将在多种组织类型中的纤维化治疗中具有广泛的应用性，并可能潜在地在疾病后期有效。

[0142] 已经使用初级人肺成纤维细胞和初级啮齿动物 HSC 建立了高含量成像试验，其使得能够鉴定抑制肌成纤维细胞形成 / 活化或诱导活化肌成纤维细胞向静息成纤维细胞状态逆转的小分子。已经确定了在适用于高通量小分子筛选的小型化 (384 孔板）形式中促进稳健的体外转分化的条件（包括血清饥饿和后续 TGF-β 处理）。抑制试验基于与成纤维细胞向肌成纤维细胞转分化相关的 α-SMA 免疫荧光染色和细胞形态学变化。使用选择性 ALK-5TGF-β1 受体抑制剂 (SB-43154) 为阳性对照，该抑制试验已被用于开始筛选一批约 100,000 种小分子，这批小分子由生物活性小分子和基于 2D 和 3D 结构多样性以及内在“类药性”性质汇集的多样性集合组成。初步筛选导致鉴定出多个先前确定的抗纤维化分子。由于已知的脱靶毒性问题或证实的体内效力缺乏，这些分子中的大多数具有有限的临床实用性。然而，除这些外，三唑抗真菌剂伊曲康唑也被鉴定为肌成纤维细胞形成的高度有效的抑制剂（在远低于与成纤维细胞或其他对照细胞类型中的毒性相关的剂量的剂量下）。

[0143] 通过使用生化方法（即，Western 印迹和 RT-PCR) 分析与成纤维细胞向肌成纤维细胞的转分化相关的多个基因的表达变化，在体外证实了伊曲康唑的活性。使用药理学方法，此分子的抗纤维化活性的机理基础已经被确定为 hedgehog 信号传导和血管内皮生长因子 (VEFG) 受体糖基化 / 运输的双重抑制（单独的任一活性均不是足够的）。令人鼓舞地，确定了伊曲康唑的活性适用于人和啮齿动物细胞类型，以及来源于多种组织类型（例如，肺、肝、皮肤、心脏）的细胞。使用吡非尼酮和 AM-152 作为基准对照化合物，证明伊曲康唑在博来霉素诱发的肺和四氯化碳诱发的肝纤维化小鼠模型中都具有效力。就此而言，FDA 批准的药物伊曲康唑已经被确定为开发用于治疗多种纤维化相关疾病的新的一类药物的新型先导物。

[0144] 此药物作为抗纤维化药物，特别是在治疗肝纤维化中的应用的潜在限制是已知的肝毒性特征，该特征与 1,2,4 三唑部分的 N4 对血红素铁的配位相关，其导致 P450 酶（最显著的是 Cyp3A4) 的抑制，这是与伊曲康唑的 VEGF 和 Hgh 活性不同的活性。就此而言，旨在鉴定具有有利的肝毒性特征并且抗纤维化效力进一步改善的最优化伊曲康唑类似物的药物化学工作已经开始。从最初的一组约 30 种伊曲康唑类似物（其中 N4 的 pKa 降低或 N4 氮被碳取代）中，已经鉴定了几种候选先导物，其中 Cyp 抑制活性消除而体外抗纤维化活性得到保留。值得注意的是，这些化合物的药代动力学性质与母体化合物的药代动力学性质

是可比的。基于观察到的体外活性、体内效力、啮齿动物血清暴露和人暴露数据，预期对于最终临床候选物，效力和 / 或暴露的 5 倍改善是所期望的。初步构效关系研究显示，通过在三唑取代基远侧的位点处进行修饰，可以实现效力增强。化学工作正在进行并且将被用于优化效力、暴露 (C_{max}>5 倍体外 EC_{50}) 和毒性特征（基于人脱靶组概况分析、hERG 和 AMES 体外毒性试验以及 Cyp 诱导 / 抑制试验）。将用肺和肝啮齿动物纤维化模型对最优化的伊曲康唑类似物证明可重现的疾病改善活性（即，效力等于或大于现有的（吡非尼酮）或未来可能的 (AM-152) 医疗标准的效力）。

定义

[0145] 在以下描述中，对某些具体的细节进行了阐述，以便透彻地理解各实施方案。然而，本领域技术人员将理解，本发明可以在没有这些细节的情况下实施。在其他情形下，没有详细示出或描述公知的结构，以避免对实施方案的不必要的模糊描述。除非上下文另有要求，在整个说明书及其后的权利要求书中，词语“包含”及其变体，诸如“包括”和“含有”应当解释为开放式、包括性含义，即作为“包括但不限于”。此外，本文提供的标题仅为了方便，并不解释所请求保护的发明的范围或含义。

[0146] 在整个说明书中提到“一个实施方案”或“实施方案”意指与该实施方案结合描述的特定的特征、结构或特性包含在至少一个实施方案中。因此，在整个说明书的各个位置出现的短语“在一个实施方案中”或“在实施方案中”并不必要均指相同的实施方案。此外，特定的特征、结构或特性可在一个或多个实施方案中以任何合适的方式进行组合。另外，如在本说明书和所附的权利要求书中所使用的，除非内容另有明确说明，单数形式的“一”、“一个”及“该（所述）”包括复数对象。还应指出的是，除非内容另有明确说明，术语“或（或者）”通常以其包括“和 / 或”的含义使用。

[0147] 除非另有说明，如本文所使用的下列术语具有以下含义：

[0148] “氨基”指 $-NH_2$基团。

[0149] “氰基”或“腈”指 -CN 基团。

[0150] “羟基 (Hydroxy 或 hydroxyl)”指 -OH 基团。

[0151] “硝基”指 $-NO_2$基团。

[0152] “氧代”指＝ O 取代基。

[0153] “肟”指＝ N-OH 取代基。

[0154] “硫代”指＝ S 取代基。

[0155] “烷基”指直链或支链的烃链基团，具有 1 至 30 个碳原子，并且通过单键与分子的其余部分连接。涵盖包含 1 至 30 个中的任何数目的碳原子的烷基。包含最高达 30 个碳原子的烷基被称为 C_1-C_{30}烷基，同样地，例如，包含最高达 12 个碳原子的烷基为 C_1-C_{12}烷基。包含其他数目的碳原子的烷基（和本文所定义的其他部分）以相似的方式表示。烷基包括但不限于，C_1-C_{30}烷基、C_1-C_{20}烷基、C_1-C_{15}烷基、C_1-C_{10}烷基、C_1-C_8烷基、C_1-C_6烷基、C_1-C_4烷基、C_1-C_3烷基、C_1-C_2烷基、C_2-C_8烷基、C_3-C_8烷基和 C_4-C_8烷基。代表性的烷基包括但不限于，甲基、乙基、正丙基、1- 甲基乙基（异丙基）、正丁基、异丁基、仲丁基、正戊基、1,1- 二甲基乙基（叔丁基）、3- 甲基己基、2- 甲基己基、乙烯基、烯丙基、丙炔基等等。包含不饱和键的烷基包括烯基和炔基。除非在说明书中另外具体说明，烷基可任选地如下所述被取代。

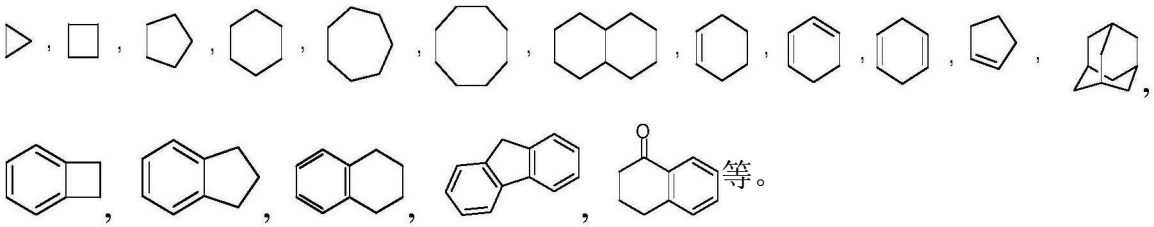
[0156] “亚烷基”或“亚烷基链”指如以上针对烷基所述的直链或支链的二价烃链。除非

在说明书中另外具体说明，亚烷基可任选地如下所述被取代。

[0157] “烷氧基”指式 $-OR_a$ 的基团，其中 R_a 为如上定义的烷基。除非在说明书中另外具体说明，烷氧基可任选地如下所述被取代。

[0158] “芳基”指由包含氢、6 至 30 个碳原子和至少一个芳香环的烃环体系衍生的基团。该芳基可为单环、双环、三环或四环环系，可包括稠合或桥接的环系。芳基包括但不限于由醋蒽烯、苊、醋菲烯、蒽、蒽、苯、䓛、荧蒽、芴、不对称引达省、对称引达省、茚满、茚、萘、非那烯、菲、七曜烯、苉和苯并菲的烃环体系衍生的芳基。除非在说明书中另外具体说明，术语“芳基”或前缀“芳”（如在“芳烷基”中）意在包括任选取代的芳基。

[0159] “环烷基”或“碳环”指稳定的、非芳族的单环或多环碳环状环，其可包括稠合或桥接的环系，其为饱和或不饱和的。代表性的环烷基或碳环包括但不限于，具有 3 至 15 个碳原子、3 至 10 个碳原子、3 至 8 个碳原子、3 至 6 个碳原子、3 至 5 个碳原子或 3 至 4 个碳原子的环烷基。单环环烷基或碳环包括，例如，环丙基、环丁基、环戊基、环己基、环庚基和环辛基。多环基团环烷基或碳环包括，例如，金刚烷基、降冰片基、十氢化萘基、双环 [3.3.0] 辛烷、双环 [4.3.0] 壬烷、顺式十氢化萘、反式十氢化萘、双环 [2.1.1] 己烷、双环 [2.2.1] 庚烷、双环 [2.2.2] 辛烷、双环 [3.2.2] 壬烷和双环 [3.3.2] 癸烷和 7,7- 二甲基 - 双环 [2.2.1] 庚烷基。除非在说明书中另外具体说明，环烷基或碳环可任选地被取代。环烷基的说明性实例包括但不限于下列部分：



[0160] “稠合的”指与存在的环结构稠合的本文所述的任何环结构。当稠环为杂环基环或杂芳基环时，在成为稠合的杂环基环或稠合的杂芳基环的一部分的该存在的环结构上的任何碳原子可被氮原子所替代。

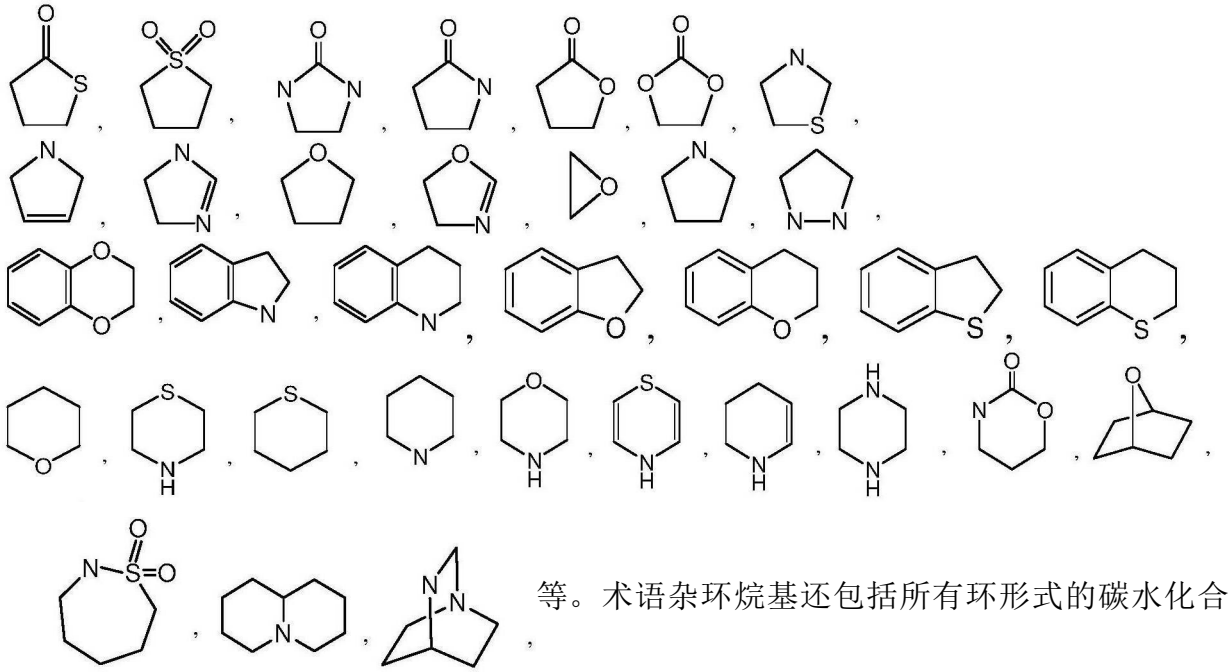
[0161] “卤代”或“卤素”指溴、氯、氟或碘。

[0162] “卤代烷基”指被如上所定义的一个或多个卤素基团取代的如上所定义的烷基，例如，三氟甲基、二氟甲基、氟甲基、三氯甲基、2,2,2- 三氟乙基、1,2- 二氟乙基、3- 溴 -2- 氟丙基、1,2- 二溴乙基等。除非在说明书中另外具体说明，卤代烷基可任选地被取代。

[0163] 类似地，“卤代烷氧基”指式 $-OR_a$ 的基团，其中 R_a 为如上所定义的卤代烷基。除非在说明书中另外具体说明，卤代烷氧基可任选地如下所述被取代。

[0164] “杂环烷基”或“杂环基”或“杂环状环”或“杂环”指包含 2 至 23 个碳原子和 1 至 8 个选自氮、氧、磷和硫的杂原子的稳定的 3 至 24 元非芳香族环基团。除非在说明书中另外具体说明，所述杂环基可为单环、双环、三环或四环环系，可包括稠合或桥接的环系；并且杂环基中的氮、碳或硫原子可任选地被氧化；氮原子可任选地被季铵化；且杂环基可以是部分或完全饱和的。这样的杂环基的实例包括但不限于，氮杂环丁基、二氧戊环基、噻吩基 [1,3] 二噻烷基、十氢异喹啉基、咪唑啉基、咪唑烷基、异噻唑烷基、异噁唑烷基、吗啉基、八氢吲哚基、八氢异吲哚基、2- 氧代哌嗪基、2- 氧代哌啶基、2- 氧代吡咯烷基、噁唑烷基、哌

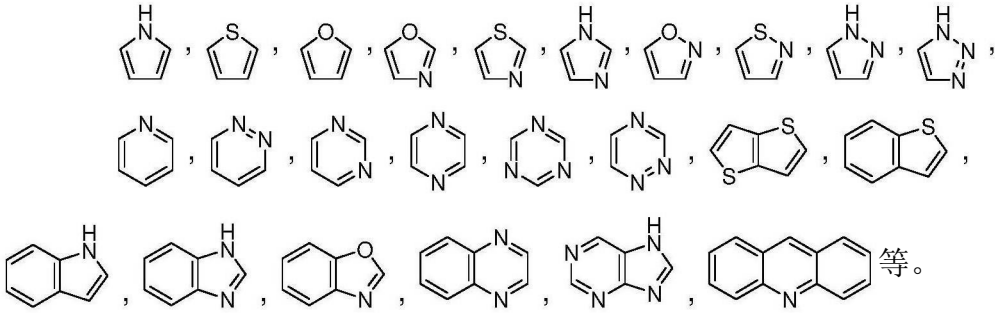
啶基、哌嗪基、4- 哌啶酮基 (4-piperidonyl)、吡咯烷基、吡唑烷基、奎宁环基、噻唑烷基、四氢呋喃基、三噻烷基 (trithianyl)、四氢吡喃基、硫代吗啉基 (thiomorpholinyl)、硫杂吗啉基 (thiamorpholinyl)、1- 氧代 - 硫代吗啉基、1,1- 二氧代 - 硫代吗啉基、12- 冠醚 -4、15- 冠醚 -5、18- 冠醚 -6、21- 冠醚 -7、氮杂 -18- 冠醚 -6、二氮杂 -18- 冠醚 -6、氮杂 -21- 冠醚 -7 和二氮杂 -21- 冠醚 -7。除非在说明书中另外具体说明，杂环基可任选地被取代。杂环烷基的说明性实例也称为非芳香族杂环，包括 ：



等。术语杂环烷基还包括所有环形式的碳水化合物，包括但不限于单糖、二糖和寡糖。除非另有说明，杂环烷基在环中具有 2 至 10 个碳。应理解，当提到杂环烷基中的碳原子的数目时，在该杂环烷基中的碳原子的数目不同于构成杂环烷基（即杂环烷基环的骨架原子）的原子（包括杂原子）的总数。除非在说明书中另外具体说明，杂环烷基可任选地被取代。

[0165] 如本文使用的术语“杂芳基”单独地或组合地是指含有约五个至约十二个骨架环原子的任选取代的芳香族单价基团，其中一个或多个环原子是独立地选自氧、氮、硫、磷、硅、硒和锡的杂原子，但不限于这些原子，并且条件是所述基团的环不含两个相邻的 O 或 S 原子。在环中存在两个或更多个杂原子的实施方案中，这两个或更多个杂原子可以彼此相同，或者这两个或更多个杂原子中的一些或全部可以各自与其他杂原子不同。术语杂芳基包括具有至少一个杂原子的任选取代的稠合和非稠合的杂芳基基团。术语杂芳基还包括具有五个至约十二个骨架环原子的稠合和非稠合的杂芳基，以及具有五个至约十个骨架环原子的稠合和非稠合的杂芳基。可以通过碳原子或杂原子与杂芳基键合。因此，作为非限制性的实例，咪唑 (imidiazole) 基团可以通过任何其碳原子（咪唑 -2- 基、咪唑 -4- 基或咪唑 -5- 基）或其氮原子（咪唑 -1- 基或咪唑 -3- 基）连接至母体分子。同样，杂芳基可以进一步通过任何或全部其碳原子和 / 或任何或全部其杂原子被取代。稠合的杂芳基基团可以含有两个至四个稠环，其中连接的环是杂芳环并且其他各个环可以是脂环的、杂环的、芳族的、杂芳族的或其任意组合。单环杂芳基的非限制性实例包括吡啶基 ；稠环杂芳基包括苯并咪唑基、喹啉基、吖啶基 ；非稠合的二杂芳基包括联吡啶基。杂芳基的其他实例包括但不限于呋喃基、噻吩基、噁唑基、吖啶基、吩嗪基、苯并咪唑基、苯并呋喃基、苯并噁唑基、苯

并噻唑基、苯并噻二唑基、苯并噻吩基、苯并噁二唑基、苯并三唑基、咪唑基、吲哚基、异噁唑基、异喹啉基、吲哚啉基、异噻唑基、异吲哚基噁二唑基、吲唑基、吡啶基、哒嗪基、嘧啶基、吡嗪基、吡咯基、吡嗪基、吡唑基、嘌呤基、酞嗪基、蝶啶基、喹啉基、喹唑啉基、喹喔啉基、三唑基、四唑基、噻唑基、三嗪基、噻二唑基等，及其氧化物，例如吡啶基 -N- 氧化物。杂芳基的说明性实例包括以下部分：



[0166] 杂芳基可以是单环、双环、三环或四环环系，可包括稠合或桥接的环系；且在杂芳基中的氮、碳或硫原子可任选地被氧化；氮原子可任选地被季铵化。实例包括但不限于氮杂萘基、吖啶基、苯并咪唑基、苯并噻唑基、苯并吲哚基 (benzindolyl)、苯并二氧戊环基 (benzodioxolyl)、苯并呋喃基、苯并噁唑基、苯并噻唑基、苯并噻二唑基、苯并 [b][1,4] 二氧杂萘基、1,4- 苯并二噁烷基、苯并萘并呋喃基 (benzonaphthofuranyl)、苯并噁唑基、苯并二氧戊环基、苯并二噁烯基 (benzodioxinyl)、苯并吡喃基、苯并吡喃酮基、苯并呋喃基、苯并呋喃酮基、苯并噻吩基 (benzothienyl 或 benzothiophenyl)、苯并三唑基、苯并 [4,6] 咪唑并 [1,2-a] 吡啶基、咔唑基、噌啉基、二苯并呋喃基、二苯并噻吩基、呋喃基、呋喃酮基、异噻唑基、咪唑基、吲唑基、吲哚基、吲唑基、异吲哚基、吲哚啉基、异吲哚啉基、异喹啉基、吲嗪基、异噁唑基、萘啶基、噁二唑基、2- 氧代氮杂萘基、噁唑基、环氧乙烷基、1- 氧代吡啶基、1- 氧代嘧啶基、1- 氧代吡嗪基、1- 氧代哒嗪基、1- 苯基 -1H- 吡咯基、吩嗪基、吩噻嗪基、吩噁嗪基、酞嗪基、蝶啶基、嘌呤基、吡咯基、吡唑基、吡啶基、吡嗪基、嘧啶基、哒嗪基、喹唑啉基、喹喔啉基、喹啉基、奎宁环基、异喹啉基、四氢喹啉基、噻唑基、噻二唑基、三唑基、四唑基、三嗪基和噻吩基 (thiophenyl)(即，噻吩基 (thienyl))。

[0167] 上述所有基团可以是取代的或未取代的。如本文所使用的术语“取代的”指任何上述基团 (例如，烷基、亚烷基、烷氧基、芳基、环烷基、卤代烷基、杂环基和 / 或杂芳基) 可进一步被官能化，其中至少一个氢原子被连接非氢原子取代基的键所替代。除非在说明书中具体说明，被取代的基团可包括选自以下的一个或多个取代基：氧代、氨基、$-CO_2H$、腈、硝基、羟基、硫代氧基 (thiooxy)、烷基、亚烷基、烷氧基、芳基、环烷基、杂环基、杂芳基、二烷基胺、芳基胺、烷基芳基胺、二芳基胺、三烷基铵 ($-N^+R_3$)、N- 氧化物、酰亚胺和烯胺；在诸如三烷基甲硅烷基、二烷基芳基甲硅烷基、烷基二芳基甲硅烷基、三芳基甲硅烷基的基团中的硅原子，全氟烷基或全氟烷氧基，例如三氟甲基或三氟甲氧基。“取代的”还指其中一个或多个氢原子被连接杂原子的更高级的键 (例如，双键或三键) 所替代的任何上述基团，所述杂原子例如为在氧代、羰基、羧基和酯基团中的氧，和在诸如亚胺、肟、腙和腈的基团中的氮。例如，“取代的”包括其中一个或多个氢原子被 $-NH_2$、$-NR_gC(=O)NR_gR_h$、$-NR_gC(=O)OR_h$、$-NR_gSO_2R_h$、$-OC(=O)NR_gR_h$、$-OR_g$、$-SR_g$、$-SOR_g$、$-SO_2R_g$、$-OSO_2R_g$、$-SO_2OR_g$、$=NSO_2R_g$

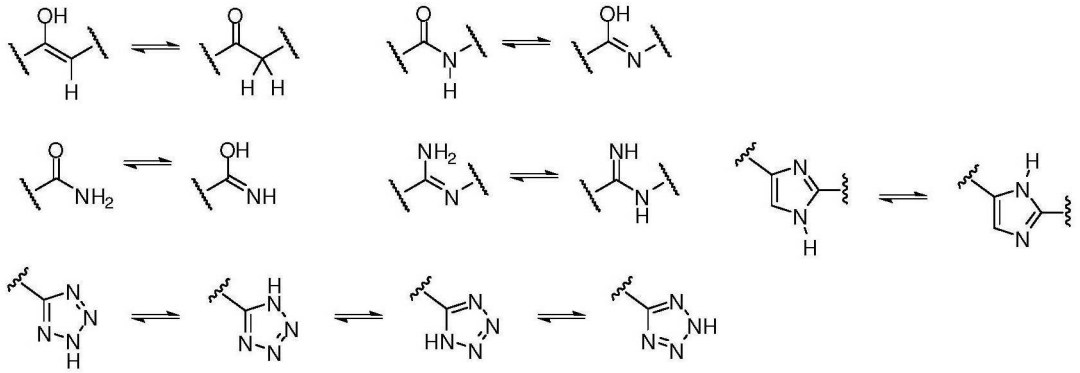
和 $-SO_2NR_gR_h$ 所替代的上述任何基团。在上文中，R_g 和 R_h 相同或不同，并且独立地为氢、烷基、烷氧基、烷基氨基、硫代烷基、芳基、芳烷基、环烷基、环烷基烷基、卤代烷基、杂环基、N- 杂环基、杂环基烷基、杂芳基、N- 杂芳基和 / 或杂芳基烷基。此外，上述取代基中的每一个还可任选地被一个或多个上述取代基所取代。此外，任何上述基团均可被取代，以包括一个或多个内部氧、硫或氮原子。例如，烷基可被一个或多个内部氧原子所取代，以形成醚或聚醚基团。相似地，烷基可被一个或多个内部硫原子所取代，以形成硫醚、二硫化物等。

[0168] 术语“任选的”或“任选地”意指随后描述的事件或情况可能发生或可能不发生，并且该描述包括其中所述事件或情况发生的情形和所述事件或情况不发生的情形。例如，“任选取代的烷基”意指如上所定义的“烷基”或“被取代的烷基”。此外，任选取代的基团可以是未取代的（例如，$-CH_2CH_3$）、完全取代的（例如，$-CF_2CF_3$）、单取代的（例如，$-CH_2CH_2F$），或以在完全取代和单取代之间的任一水平被取代的（例如，$-CH_2CHF_2$、$-CH_2CF_3$、$-CF_2CH_3$、$-CFHCHF_2$ 等）。本领域技术人员将会理解，对于含有一个或多个取代基的任何基团，这些基团并不旨在引入在空间上不能实现的和 / 或在合成上不可行的任何取代或取代模式（例如，被取代的烷基包括任选取代的环烷基，而该环烷基反过来又被定义为包括任选取代的烷基，如此可能无限循环）。因此，所述的任何取代基一般应理解为具有约 1,000 道尔顿，并且更典型地，高达约 500 道尔顿的最大分子量。

[0169] “有效量”或“治疗有效量”指作为单剂量或作为系列剂量的一部分施用于哺乳动物受试者并有效地产生所需治疗效果的化合物的量。

[0170] 对个体（例如，哺乳动物，如人）或细胞的“治疗（处理）”是在试图改变个体或细胞的自然进程中使用的任何类型的干预。在一些实施方案中，治疗包括在病理性事件或与病原体接触开始后施用药物组合物，并包括病况的稳定化（例如，病况不恶化）或病况的缓解。在其他实施方案中，治疗还包括预防性处理（例如当个体被怀疑为患有细菌感染时，施用本文所述的组合物）。

[0171] “互变异构体”指从分子的一个原子到同一分子的另一个原子的质子转移。本文提供的化合物可作为互变异构体存在。互变异构体为通过氢原子的迁移（伴随单键和相邻双键的转换）可相互转化的化合物。在可能发生互变异构的键合排列中，将存在互变异构体的化学平衡。考虑了本文所公开的化合物的所有互变异构形式。互变异构体的确切比例取决于若干因素，包括温度、溶剂和 pH。互变异构体相互转化的一些实例包括：

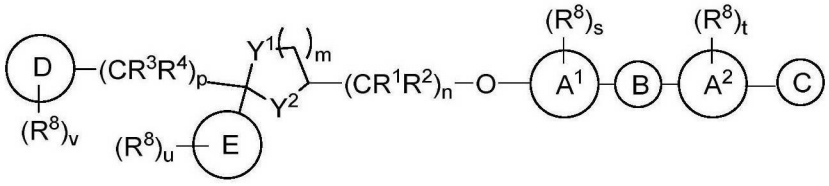


[0172] 本文所公开的化合物的“代谢物”为当化合物被代谢时所形成的该化合物的衍生物。术语“活性代谢物”指化合物被代谢时所形成的该化合物的生物活性衍生物。如本文所使用的，术语“代谢”指通过其使特定物质被生物体所改变的过程（包括但不限于，水解

反应和由酶催化的反应，如氧化反应）的总和。因此，酶可产生化合物的特定结构改变。例如，细胞色素 P450 催化多种氧化和还原反应，而尿苷二磷酸葡糖醛酸基转移酶催化活化的葡糖醛酸分子转变为芳香醇、脂肪族醇、羧酸、胺和游离巯基。关于代谢的进一步信息可从 The Pharmacological Basis of Therapeutics, 第九版，McGraw-Hill(1996) 中获得。本文所公开的化合物的代谢物可通过以下方法来鉴定：将化合物施用于宿主并对来自该宿主的组织样品进行分析，或将化合物与肝细胞在体外温育并对所得化合物进行分析。这两种方法都是本领域公知的。在一些实施方案中，化合物的代谢物通过氧化过程形成，并对应于相应的含羟基化合物。在一些实施方案中，化合物被代谢成药理活性代谢物。

化合物

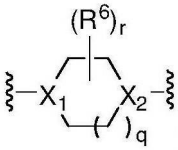
[0173] 在一个方面，本文提供了式 (II) 的化合物，其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体：



式(II)

其中：

A^1和 A^2独立地选自芳基或杂芳基；

B 为 

；

C 为任选取代的 5 或 6 元杂环基或任选取代的 5 或 6 元杂芳基，其中该杂环基或该杂芳基含有 1 至 4 个氮原子；

D 为芳基或杂芳基；

E 为芳基、杂芳基、碳环基、杂环基或烷基；

每个 R^1、R^2、R^3和 R^4独立地选自 H、烷基、卤代烷基或烷氧基；

X_1和 X_2独立地选自 N 和 CR^5；

R^5为 H、OH、烷基或烷氧基；

每个 R^6独立地为烷基、卤代烷基、卤代、烷氧基、- 亚烷基 ($NR^{13}R^{14}$) 或芳基；

R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、环烷基、杂环基、- 亚烷基（环烷基）或 - 亚烷基（杂环基）；

每个 R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、- 亚烷基（环烷基）、- 亚烷基（杂环基）、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和 $-C(O)NR^{13}R^{14}$；或者两个相邻的 R^8形成杂环基环；

每个 R^{13}和 R^{14}独立地选自 H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷

基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者 R^{13} 和 R^{14} 与它们所连接至的原子一起形成杂环；

Y^1 和 Y^2 独立地选自 O、CH_2、NH 和 NR^{13}；

n 为 1、2 或 3；

m 为 1 或 2；

p 为 1、2、3 或 4；

q 为 1、2 或 3；

r 为 0、1、2、3、4、5、6、7 或 8；

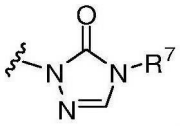
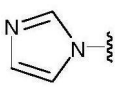
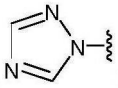
s 为 0、1、2、3 或 4；

t 为 0、1、2、3 或 4；

u 为 0、1、2、3、4 或 5；

v 为 0、1、2、3 或 4；

条件是：

如果 X_1 和 X_2 为 N；r 为 0；q 为 1；A^1 和 A^2 为苯基；Y^1 和 Y^2 为 O；m 和 n 为 1；R^1、R^2、R^3 和 R^4 为氢；且 C 为  ，则 D 不是  或  ；且

该化合物不是 4-(4-(4-(4-((2-((1H-吡唑-1-基)甲基)-2-(2,4-二氟苯基)-1,3-二氧杂环戊-4-基)甲氧基)苯基)哌嗪-1-基)苯基)-1-异丙基-1H-1,2,4-三唑-5(4H)-酮。

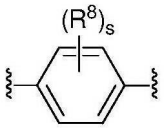
[0174] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 和 X_2 为 N。

[0175] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 为 CR^5 且 X_2 为 N。

[0176] 在式 (II) 化合物的以上或以下描述的一些实施方案中，X_1 为 N 且 X_2 为 CR^5。

[0177] 在式 (II) 化合物的以上或以下描述的一些实施方案中，q 为 1 且 r 为 0。

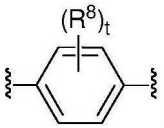
[0178] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为芳基。

[0179] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为 

[0180] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为  。

[0181] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^1 为杂芳基。

[0182] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为芳基。

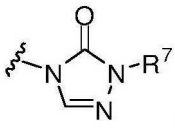
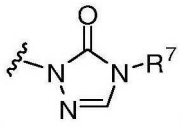
[0183] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

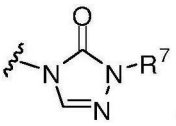
[0184] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

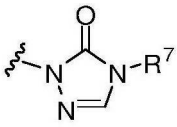
[0185] 在式 (II) 化合物的以上或以下描述的一些实施方案中，A^2 为杂芳基。

[0186] 在式(II)化合物的以上或以下描述的一些实施方案中，A^2为吡啶基、吡嗪基、嘧啶基、哒嗪基或三嗪基。

[0187] 在式(II)化合物的以上或以下描述的一些实施方案中，C为任选取代的5或6元杂芳基。在式(II)化合物的以上或以下描述的其他实施方案中，C为任选取代的5或6元杂环基。

[0188] 在式(II)化合物的以上或以下描述的一些实施方案中，C为  或  。

[0189] 在式(II)化合物的以上或以下描述的一些实施方案中，C为  。

[0190] 在式(II)化合物的以上或以下描述的一些实施方案中，C为  。

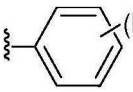
[0191] 在式(II)化合物的以上或以下描述的一些实施方案中，E为烷基。

[0192] 在式(II)化合物的以上或以下描述的一些实施方案中，E为环烷基。

[0193] 在式(II)化合物的以上或以下描述的一些实施方案中，E为环丙基、环丁基、环戊基或环己基。

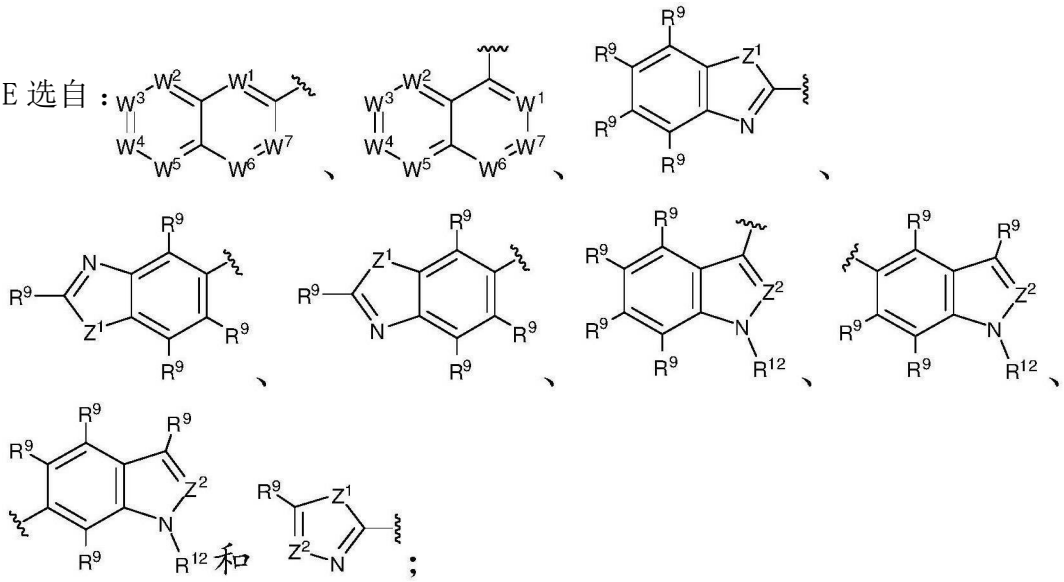
[0194] 在式(II)化合物的以上或以下描述的一些实施方案中，E为杂环基。

[0195] 在式(II)化合物的以上或以下描述的一些实施方案中，E为芳基。

[0196] 在式(II)化合物的以上或以下描述的一些实施方案中，E为  且u为0、1、2、3、4或5。

[0197] 在式(II)化合物的以上或以下描述的一些实施方案中，E为杂芳基。

[0198] 在式(II)化合物的以上或以下描述的一些实施方案中，

E选自：  ；

W^1、W^2、W^3、W^4、W^5、W^6和W^7独立地选自N和CR^9；

Z^1为NR^{12}、S或O；

Z^2为N或CR^9；

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基；

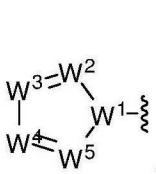
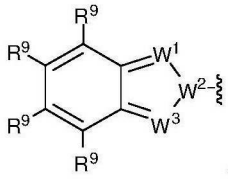
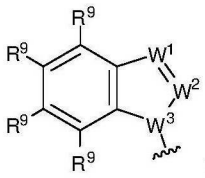
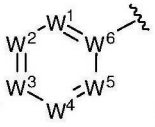
每个R^{10}和R^{11}独立地选自H和烷基；或者R^{10}和R^{11}与它们所连接至的氮一起形成杂环；且

R^{12}为H、烷基或卤代烷基。

[0199] 在式(II)化合物的以上或以下描述的一些实施方案中，D为芳基。

[0200] 在式(II)化合物的以上或以下描述的一些实施方案中，D为杂芳基。

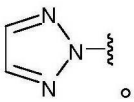
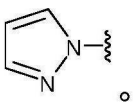
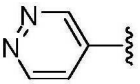
[0201] 在式(II)化合物的以上或以下描述的一些实施方案中，

D选自  、  、  和  ；

W^1、W^2、W^3、W^4和W^5独立地选自N和CR^9；

W^6为N或C；且

每个R^9独立地选自H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基。

[0202] 在式(II)化合物的以上或以下描述的某些实施方案中，D为  。在式(II)化合物的以上或以下描述的某些实施方案中，D为  。在式(II)化合物的以上或以下描述的某些实施方案中，D为  。

[0203] 在式 (II) 化合物的以上或以下描述的一些实施方案中，Y^1和 Y^2为 O。

[0204] 在式 (II) 化合物的以上或以下描述的一些实施方案中，m 为 1。

[0205] 在式 (II) 化合物的以上或以下描述的一些实施方案中，p 为 1、2 或 3。

[0206] 在式 (II) 化合物的以上或以下描述的一些实施方案中，p 为 1。

[0207] 在式 (II) 化合物的以上或以下描述的一些实施方案中，R^1、R^2、R^3和 R^4为氢。

[0208] 此处进一步提供了一种治疗有需要的受试者的疾病或病况的方法，该方法包括向该受试者施用一种或多种此处鉴定的化合物。该疾病或病况可以是纤维化。该纤维化可以是肝纤维化。该肝纤维化可以是特发性肺纤维化。

[0209] 此处进一步提供了一种治疗有需要的受试者的疾病或病况的方法，该方法包括向该受试者施用一种或多种此处鉴定的化合物。该疾病或病况可以是纤维化。该纤维化可以是慢性自身免疫病。该慢性自身免疫病可以是类风湿性关节炎、硬皮病、克罗恩病或系统性红斑狼疮。

化合物的制备

[0210] 本文描述了治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病的化合物，及其制备方法。本文还描述了这些化合物的药学上可接受的盐、药学上可接受的溶剂化物、药学活性代谢物以及药学上可接受的前药。还提供了包含至少一种这样的化合物或该化合物的药学上可接受的盐、药学上可接受的溶剂化物、药学活性代谢物或药学上可接受的前药以及药学上可接受的赋形剂的药物组合物。

[0211] 式 (I) 或式 (II) 的化合物可使用本领域技术人员已知的标准合成反应或使用本领域已知的方法来合成。可以以线性顺序采用反应来提供所述化合物，或者可使用这些反应来合成片段，随后通过本领域已知的方法进行连接。

[0212] 用于合成本文所述的化合物的起始材料可以合成或可从商业来源获得，该商业来源例如是但不限于 Aldrich Chemical Co.(Milwaukee,Wisconsin)、Bachem(Torrance,California) 或 Sigma Chemical Co.(St.Louis,Mo.)。本文所述的化合物及其他具有不同取代基的相关化合物可使用本领域技术人员已知的技术和材料来合成，例如使用描述于诸如以下的技术和材料 :March,ADVANCED ORGANIC CHEMISTRY, 第 4 版 .,(Wiley 1992) ;Carey 和 Sundberg,ADVANCED ORGANIC CHEMISTRY, 第 4 版 .,Vols.A 和 B(Plenum 2000,2001) ;Green 和 Wuts,PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, 第 3 版 .,(Wiley 1999) ;Fieser and Fieser's Reagents for Organic Synthesis,Volumes 1-17(John Wiley and Sons,1991) ;Rodd's Chemistry of Carbon Compounds,Volumes 1-5and Supplementals(Elsevier Science Publishers,1989) ; Organic Reactions,Volumes 1-40(John Wiley and Sons,1991) ; 以及 Larock's Comprehensive Organic Transformations(VCH Publishers Inc.,1989)。(所有这些均通过引用整体并入本文)。用于合成本文所述的化合物的其他方法可见于国际专利公开号 WO 01/01982901,Arnold 等 ,Bioorganic&Medicinal Chemistry Letters 10(2000)2167-2170 ;Burchat 等 ,Bioorganic&Medicinal Chemistry Letters 12(2002)1687-1690。用于制备本文所公开的化合物的一般方法可从本领域已知的反应得到，并且为了引入在如本文所提供的通式中所见的各个部分，如本领域技术人员所公认的，可通过使用适当的试剂和条件对该反应进行修改。

[0213] 如果需要，可使用包括但不限于过滤、蒸馏、结晶及色谱法等常规技术来分离和纯化反应产物。这类材料可使用包括物理常数和波谱数据在内的常规手段来表征。

[0214] 本文所述的化合物可制备成单一异构体或异构体的混合物。

本文公开的化合物的其他形式

异构体

[0215] 此外，在一些实施方案中，本文所述的化合物以几何异构体形式存在。在一些实施方案中，本文所述的化合物具有一个或多个双键。本文提供的化合物包括所有顺式、反式、同侧、对侧、E 型 (E) 和 Z 型 (Z) 异构体以及其相应混合物。在一些情况下，化合物以互变异构体形式存在。本文所述的化合物包括在本文所述的通式内的所有可能的互变异构体。在一些情况下，本文所述的化合物具有一个或多个手性中心且各中心以 R 构型或 S 构型存在。本文所述的化合物包括所有非对映异构、对映异构和差向异构形式以及其相应混合物。在本文提供的化合物和方法的其他实施方案中，由单一制备步骤、组合或相互转化得到的对映异构体和 / 或非对映异构体的混合物可用于本文所述的应用。在一些实施方案中，通过使化合物的外消旋混合物与旋光拆分剂反应形成一对非对映异构化合物、分离非对映异构体并回收光学纯的对映异构体，将本文所述的化合物制备为其单独的立体异构体。在一些实施方案中，优选可分离的复合物 (例如结晶的非对映异构盐)。在一些实施方案中，非对映异构体具有不同的物理性质 (例如熔点、沸点、溶解度、反应性等)，并且利用这些差异进行分离。在一些实施方案中，通过手性色谱法或优选地通过基于溶解度差异的分离 / 拆分技术来分离非对映异构体。在一些实施方案中，然后通过不会引起外消旋的任何实用方式回收光学纯的对映异构体以及拆分剂。

标记的化合物

[0216] 在一些实施方案中，本文所述的化合物以其同位素标记的形式存在。在一些实施方案中，本文公开的方法包括通过施用此类同位素标记的化合物来治疗疾病的方法。在一些实施方案中，本文公开的方法包括通过以药物组合物形式施用此类同位素标记的化合物来治疗疾病的方法。因此，在一些实施方案中，本文公开的化合物包括同位素标记的化合物，除了其中一个或多个原子被替换成具有与在自然界中通常发现的原子质量或质量数不同的原子质量或质量数的原子的事实以外，该同位素标记的化合物与本文所述化合物相同。可引入本发明化合物的同位素的实例包括氢、碳、氮、氧、磷、硫、氟和氯的同位素，分别如 ^{2}H、^{3}H、^{13}C、^{14}C、^{15}N、^{18}O、^{17}O、^{31}P、^{32}P、^{35}S、^{18}F 和 ^{36}Cl。含有上述同位素和 / 或其他原子的其他同位素的本文所述的化合物及其代谢物、药学上可接受的盐、酯、前药、溶剂化物、水合物或衍生物在本发明的范围内。某些同位素标记的化合物，例如其中引入了放射性同位素如 ^{3}H 和 ^{14}C 的那些化合物，可用于药物和 / 或基质组织分布分析。氚标记 (即 ^{3}H) 和碳 -14(即 ^{14}C) 同位素由于其容易制备和可检测性而尤其优选。此外，用重同位素如氘 (即 ^{2}H) 取代产生了由较高代谢稳定性引起的某些治疗优势，例如体内半衰期的延长或剂量需求的减少。在一些实施方案中，同位素标记的化合物、其药学上可接受的盐、酯、前药、溶剂化物、水合物或衍生物通过任何合适的方法制备。

[0217] 在一些实施方案中，本文所述的化合物通过其他方式标记，包括但不限于使用发色团或荧光部分、生物发光标记或化学发光标记。

药学上可接受的盐

[0218] 在一些实施方案中，本文所述的化合物以其药学上可接受的盐的形式存在。在一些实施方案中，本文公开的方法包括通过施用此类药学上可接受的盐来治疗疾病的方法。在一些实施方案中，本文公开的方法包括通过以药物组合物形式施用此类药学上可接受的盐来治疗疾病的方法。

[0219] 在一些实施方案中，本文所述的化合物具有酸性或碱性基团，并因此与多种无机或有机碱和无机与有机酸中的任一种反应形成药学上可接受的盐。在一些实施方案中，这些盐在本发明化合物的最终分离和纯化期间原位制备，或通过使处于游离形式的经纯化的化合物分别与合适的酸或碱反应并分离由此形成的盐来制备。

[0220] 药学上可接受的盐的实例包括通过使本文所述的化合物与无机酸、有机酸或无机碱反应制备的那些盐，此类盐包括乙酸盐、丙烯酸盐、己二酸盐、藻酸盐、天冬氨酸盐、苯甲酸盐、苯磺酸盐、硫酸氢盐、亚硫酸氢盐、溴化物、丁酸盐、丁炔 -1,4- 二酸盐、樟脑酸盐、樟脑磺酸盐、己酸盐、辛酸盐、氯苯甲酸盐、氯化物、柠檬酸盐、环戊烷丙酸盐、癸酸盐、二葡糖酸盐、磷酸二氢盐、二硝基苯甲酸盐、十二烷基硫酸盐、乙烷磺酸盐、甲酸盐、富马酸盐、葡糖庚酸盐、甘油磷酸盐、乙醇酸盐、半硫酸盐、庚酸盐、己酸盐、己炔 -1,6- 二酸盐、羟基苯甲酸盐、γ- 羟基丁酸盐、盐酸盐、氢溴酸盐、氢碘酸盐、2- 羟基乙烷磺酸盐、碘化物、异丁酸盐、乳酸盐、马来酸盐、丙二酸盐、甲烷磺酸盐、扁桃酸盐、偏磷酸盐、甲烷磺酸盐、甲氧基苯甲酸盐、甲基苯甲酸盐、磷酸单氢盐、1- 萘磺酸盐、2- 萘磺酸盐、烟酸盐、硝酸盐、双羟萘酸盐、果胶酸盐、过硫酸盐、3- 苯基丙酸盐、磷酸盐、苦味酸盐、三甲基乙酸盐、丙酸盐、焦硫酸盐、焦磷酸盐、丙炔酸盐、邻苯二甲酸盐、苯基乙酸盐、苯基丁酸盐、丙烷磺酸盐、水杨酸盐、丁二酸盐、硫酸盐、亚硫酸盐、丁二酸盐、辛二酸盐、癸二酸盐、磺酸盐、酒石酸盐、硫氰酸盐、甲苯磺酸盐、十一烷酸盐和二甲苯磺酸盐。

[0221] 此外，本文所述的化合物可制备为通过化合物的游离碱形式与药学上可接受的无机或有机酸反应而形成的药学上可接受的盐，此类药学上可接受的无机或有机酸包括但不限于无机酸，诸如盐酸、氢溴酸、硫酸、硝酸、磷酸、偏磷酸等；和有机酸，诸如乙酸、丙酸、己酸、环戊烷丙酸、乙醇酸、丙酮酸、乳酸、丙二酸、丁二酸、苹果酸、马来酸、富马酸、对甲苯磺酸、酒石酸、三氟乙酸、柠檬酸、苯甲酸、3-(4- 羟基苯甲酰基）苯甲酸、肉桂酸、扁桃酸、芳基磺酸、甲烷磺酸、乙烷磺酸、1,2- 乙烷二磺酸、2- 羟基乙烷磺酸、苯磺酸、2- 萘磺酸、4- 甲基双环 -[2.2.2] 辛 -2- 烯 -1- 甲酸、葡糖庚酸、4,4'- 亚甲基双 -(3- 羟基 -2- 烯 -1- 甲酸）、3- 苯基丙酸、三甲基乙酸、叔丁基乙酸、月桂基硫酸、葡糖酸、谷氨酸、羟基萘甲酸、水杨酸、硬脂酸和粘康酸。在一些实施方案中，诸如草酸的其他酸虽然本身并非药学上可接受的，但用于制备可用作获得本发明化合物及其药学上可接受的酸加成盐中的中间体的盐。

[0222] 在一些实施方案中，包含游离酸基团的本文所述的那些化合物与以下化合物反应：合适的碱，诸如药学上可接受的金属阳离子的氢氧化物、碳酸盐、碳酸氢盐、硫酸盐，氨，或药学上可接受的有机伯胺、仲胺、叔胺或季胺。代表性的盐包括碱金属盐或碱土金属盐，如锂盐、钠盐、钾盐、钙盐和镁盐和铝盐等。碱的说明性实例包括氢氧化钠、氢氧化钾、胆碱氢氧化物、碳酸钠、$N^{+}(C_{1-4}$烷基$)_4$等。

[0223] 可用于形成碱加成盐的代表性有机胺包括乙胺、二乙胺、乙二胺、乙醇胺、二乙醇胺、哌嗪等。应当理解，本文所述的化合物也包括其所含有的任何碱性含氮基团的季铵化。在一些实施方案中，通过此类季铵化获得水溶性或油溶性或可分散性产物。

溶剂化物

[0224] 在一些实施方案中，本文所述的化合物以溶剂化物的形式存在。本发明提供通过施用此类溶剂化物来治疗疾病的方法。本发明进一步提供通过以药物组合物形式施用此类溶剂化物来治疗疾病的方法。

[0225] 溶剂化物含有化学计量或非化学计量的量的溶剂，并且在一些实施方案中，在与药学上可接受的溶剂如水、乙醇等结晶的过程中形成。当溶剂是水时形成水合物，或者当溶剂是醇时形成醇化物。本文所述化合物的溶剂化物可方便地在本文所述的过程中制备或形成。仅举例来说，可方便地使用包括但不限于二氧杂环己烷、四氢呋喃或甲醇的有机溶剂，通过从水性 / 有机溶剂混合物中再结晶来制备本文所述化合物的水合物。此外，本文提供的化合物可以以非溶剂化以及溶剂化的形式存在。一般而言，对于本文提供的化合物和方法而言，溶剂化形式被视为与非溶剂化形式等同。

多晶型物

[0226] 在一些实施方案中，本文所述的化合物以多晶型物的形式存在。本发明提供通过施用此类多晶型物来治疗疾病的方法。本发明进一步提供通过以药物组合物形式施用此类多晶型物来治疗疾病的方法。

[0227] 因此，本文所述的化合物包括其所有结晶形式，称为多晶型物。多晶型物包括化合物的具有相同元素组成的不同晶体堆积排列。在某些情况下，多晶型物具有不同的 X- 射线衍射图案、红外光谱、熔点、密度、硬度、晶体形状、光学和电学性质、稳定性和溶解度。在某些情况下，诸如再结晶溶剂、结晶速率和储存温度等各种因素使单一晶体形式占优势。

前药

[0228] 在一些实施方案中，本文所述的化合物以前药的形式存在。本发明提供通过施用此类前药来治疗疾病的方法。本发明进一步提供以药物组合物形式施用此类前药来治疗疾病的方法。

[0229] 前药一般为药物前体，其在施用于个体且随后吸收后经由诸如通过代谢途径转化的某一过程转化为具有活性或活性更强的物质。一些前药在前药上具有使其活性较低和 / 或赋予药物溶解性或一些其他性质的化学基团。一旦化学基团从前药上裂解和 / 或修饰，即产生活性药物。因为在一些情况下前药比母体药物更易于施用，前药通常为可用的。例如，它们可通过口服施用而可生物利用，而母体药物则不能。在某些情况下，前药在药物组合物中也具有比母体药物改善的溶解度。前药的一个实例是但不限于如本文所述的化合物，其以酯的形式（“前药”）施用以促进跨过其中水溶性对移动性不利的细胞膜输送，但其随后一旦在水溶性有利的细胞内部则代谢水解成羧酸（活性实体）。前药的另一实例可以是与酸基团键合的短肽（聚氨基酸），其中该肽经代谢以显露活性部分。（参见例如 Bundgaard, “Design and Application of Prodrugs”, A Textbook of Drug Design and Development, Krosgaard-Larsen 和 Bundgaard 编，1991, 第 5 章，113-191，其通过引用并入本文）。

[0230] 在一些实施方案中，前药被设计成可逆性药物衍生物，以用作提高药物向位点特异性组织输送的调节剂。迄今为止，前药的设计是为了提高靶向以水为主要溶剂的区域的治疗性化合物的有效水溶性。

[0231] 另外，本文所述化合物的前药衍生物可通过本文所述或本领域已知的方法制

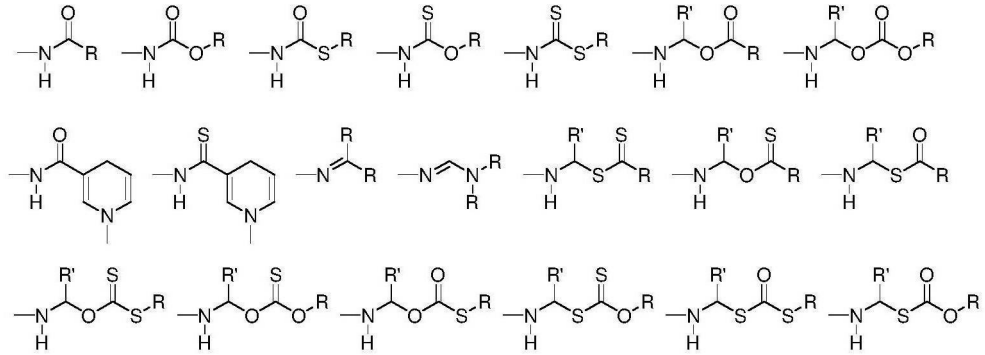
备（关于更多细节，请参见 Saulnier 等人，Bioorganic and Medicinal Chemistry Letters,1994,4,1985）。仅举例而言，可通过使未衍生化的化合物与诸如但不限于氯甲酸 1,1- 酰氧基烷基酯、碳酸对硝基苯基酯等合适的氨基甲酰化试剂反应来制备适当的前药。本文所述化合物的前药形式（其中前药在体内代谢产生如本文所述的衍生物）包括在权利要求书的范围内。实际上，一些本文所述的化合物为另一衍生物或活性化合物的前药。

[0232] 在一些实施方案中，前药包括其中氨基酸残基或具有两个或两个以上（例如 2、3 或 4 个）氨基酸残基的多肽链经由酰胺键或酯键共价连接至本发明化合物的游离氨基、羟基或羧酸基团的化合物。氨基酸残基包括但不限于 20 种天然存在的氨基酸，并且也包括 4- 羟基脯氨酸、羟赖氨酸、锁链赖氨酸 (demosine)、异锁链赖氨酸、3- 甲基组氨酸、正缬氨酸、β - 丙氨酸、γ - 氨基丁酸、瓜氨酸、高半胱氨酸、高丝氨酸、鸟氨酸和甲硫氨酸砜。在其他实施方案中，前药包括其中核酸残基或具有两个或两个以上（例如 2、3 或 4 个）核酸残基的寡核苷酸共价连接至本发明化合物的化合物。

[0233] 本文所述化合物的药学上可接受的前药也包括但不限于酯、碳酸酯、硫代碳酸酯、N- 酰基衍生物、N- 酰氧基烷基衍生物、叔胺的季铵化衍生物、N- 曼尼希碱、席夫碱、氨基酸偶联物、磷酸酯、金属盐和磺酸酯。具有游离氨基、酰胺基、羟基或羧基的化合物可转化为前药。例如，游离羧基可衍生为酰胺或烷基酯。在某些情况下，所有这些前药部分中引入包括但不限于醚、胺和羧酸官能团的基团。

[0234] 羟基前药包括酯，诸如但不限于酰氧基烷基（例如酰氧基甲基、酰氧基乙基）酯、烷氧基羰氧基烷基酯、烷基酯、芳基酯、磷酸酯、磺酸酯、硫酸酯和含有二硫化物的酯；醚、酰胺、氨基甲酸酯、半琥珀酸酯、二甲基氨基乙酸酯和磷酰基氧基甲氧基羰基，如 Advanced Drug Delivery Reviews 1996,19,115 中所概述的。

[0235] 胺衍生的前药包括但不限于以下基团和基团的组合：



以及磺酰胺和磷酰胺。

[0236] 在某些情形下，在任何芳香环部分上的位点易发生各种代谢反应，因此在芳香环结构上并入合适的取代基可减少、最小化或消除该代谢途径。

代谢物

[0237] 在一些实施方案中，式 (I) 或式 (II) 的化合物易发生各种代谢反应。因此在一些实施方案中，将合适的取代基并入结构中将减少、最小化或消除代谢途径。在具体的实施方案中，仅举例而言，减少或消除芳香环对代谢反应的敏感性的合适的取代基为卤素或烷基。

[0238] 在另外的或进一步的实施方案中，式 (I) 或式 (II) 的化合物在施用于有需要的生物体后被代谢以产生代谢物，该代谢物随后用于产生所期望的效果，包括所期望的治疗效

果。

药物组合物 / 制剂

[0239] 在另一方面，本文提供了包含如本文描述的式 (I) 或式 (II) 化合物或其药学上可接受的盐、多晶型物、溶剂化物、前药、N- 氧化物、立体异构体或异构体以及药学上可接受的赋形剂的药物组合物。

[0240] 在一些实施方案中，将本文所述的化合物配制为药物组合物。药物组合物以常规方式使用一种或多种药学上可接受的非活性成分进行配制，该非活性成分便于将活性化合物加工成可在药学上使用的制剂。适当的制剂取决于所选择的给药途径。本文所述的药物组合物的概述可见于，例如，Remington:The Science and Practice of Pharmacy, 第十九版 (Easton,Pa.:Mack Publishing Company,1995) ;Hoover,John E.,Remington's Pharmaceutical Sciences,Mack Publishing Co.,Easton,Pennsylvania 1975 ; Liberman,H.A. 和 Lachman,L. 编著 ,Pharmaceutical Dosage Forms,Marcel Decker,New York,N.Y.,1980 ;以及Pharmaceutical Dosage Forms and Drug Delivery Systems, 第七版 (Lippincott Williams&Wilkins 1999)，这些公开文献通过引用并入本文。

[0241] 本文提供了包含式 (I) 或式 (II) 的化合物以及至少一种药学上可接受的非活性成分的药物组合物。在一些实施方案中，本文所述的化合物作为药物组合物施用，在该药物组合物中式 (I) 或式 (II) 的化合物与其他活性成分混合，如在联合治疗中。在其他实施方案中，药物组合物包含其他医学或药学制剂、载体、佐剂、防腐剂、稳定剂、润湿剂或乳化剂、溶解促进剂 (solution promoter)、用于调节渗透压的盐和 / 或缓冲液。在又一些实施方案中，药物组合物包含其他有治疗价值的物质。

[0242] 本文所使用的药物组合物指式 (I) 或式 (II) 的化合物与其他化学组分 (即，药学上可接受的非活性成分) 的混合物，所述其他化学组分例如是载体、赋形剂、粘合剂、填充剂、悬浮剂、矫味剂、甜味剂、崩解剂、分散剂、表面活性剂、润滑剂、着色剂、稀释剂、增溶剂、湿润剂、增塑剂、稳定剂、渗透促进剂、润湿剂、消泡剂、抗氧化剂、防腐剂或它们的一种或多种组合。药物组合物有利于将化合物施用于生物体。在实施本文提供的治疗方法或应用的过程中，将治疗有效量的本文所述的化合物以药物组合物的形式施用于待治疗的患有疾病、病症或病况的哺乳动物。在一些实施方案中，该哺乳动物是人。治疗有效量可根据疾病的严重程度、受试者的年龄和相对健康、所用化合物的效力和其他因素而发生较大变化。化合物可以单独使用或作为混合物的组分与一种或多种治疗剂组合使用。

[0243] 本文所述的药物制剂通过适当的给药途径施用于受试者，该给药途径包括但不限于，口服、肠胃外 (例如，静脉内、皮下、肌肉内)、鼻内、颊部、局部、直肠或经皮给药途径。本文所述的药物制剂包括但不限于 :水性液体分散剂、液体、凝胶剂、糖浆、酏剂、浆液、悬浮液、自乳化分散剂、固溶体、脂质体分散剂、气雾剂、固体口服剂型、粉剂、立即释放制剂、控制释放制剂、快速熔解制剂 (fast melt formulation)、片剂、胶囊、丸剂、粉剂、锭剂、泡腾制剂、冻干制剂、延迟释放制剂、延长释放制剂、脉冲释放制剂、多颗粒制剂以及立即和控制释放混合制剂。

[0244] 包含式 (I) 或式 (II) 的化合物的药物组合物以常规方法进行制备，诸如，仅举例而言，通过常规的混合、溶解、制粒、制锭、磨细、乳化、包封、包埋或压制方法。

[0245] 所述药物组合物将包含以游离酸或游离碱的形式或以药学上可接受的盐的形式

作为活性成分的至少一种式(I)或式(II)的化合物。此外,本文所述的方法和药物组合物包括使用具有相同类型活性的这些化合物的N-氧化物(如果合适的话)、结晶形式、无定形相以及活性代谢物。在一些实施方案中,本文所述的化合物以非溶剂化形式存在或与药学上可接受的溶剂诸如水、乙醇等以溶剂化形式存在。本文提供的化合物的溶剂化形式也被认为在本文中公开。

[0246] 用于口服使用的药物制剂通过以下方法获得:将一种或多种固体赋形剂与本文所述的一种或多种化合物混合,任选地研磨所得混合物,并在加入合适的助剂(如果需要)后对颗粒混合物进行加工,以得到片剂或锭剂核芯。合适的赋形剂包括,例如,填充剂,诸如糖,包括乳糖、蔗糖、甘露醇或山梨糖醇;纤维素制剂,例如,玉米淀粉、小麦淀粉、大米淀粉、马铃薯淀粉、明胶、黄蓍胶、甲基纤维素、微晶纤维素、羟丙基甲基纤维素、羧甲基纤维素钠;或其他赋形剂,诸如:聚乙烯吡咯烷酮(PVP或聚维酮)或磷酸钙。如果需要,加入崩解剂,诸如交联羧甲基纤维素钠、聚乙烯吡咯烷酮、琼脂或海藻酸或其盐如海藻酸钠。在一些实施方案中,将染料或色素加入到片剂或锭剂包衣中,用于辨识或表征活性化合物剂量的不同组合。

[0247] 口服给药的药物制剂包括由明胶制成的推入配合式(push-fit)胶囊以及由明胶和增塑剂(诸如甘油或山梨糖醇)制成的软密封胶囊。推入配合式胶囊含有与填充剂诸如乳糖、粘合剂诸如淀粉和/或润滑剂诸如滑石或硬脂酸镁以及任选的稳定剂混合的活性成分。在软胶囊中,活性化合物溶解或悬浮于合适的液体如脂肪油、液体石蜡或液体聚乙二醇中。在一些实施方案中,加入稳定剂。

[0248] 在某些实施方案中,可采用药物化合物的递送系统,例如,脂质体和乳剂。在某些实施方案中,本文提供的组合物还可包括选自例如羧甲基纤维素、卡波姆(丙烯酸聚合物)、聚(甲基丙烯酸甲酯)、聚丙烯酰胺、聚卡波非、丙烯酸/丙烯酸丁酯共聚物、海藻酸钠和葡聚糖的粘膜粘着聚合物。

联合治疗

[0249] 根据式(I)或式(II)的化合物可以与一种或多种额外的抗纤维化剂联合使用。该抗纤维化剂可以是溶血磷脂酸1(LPA1)拮抗剂。该抗纤维化剂可以选自吡非尼酮、尼达尼布、沙立度胺、carlumab、FG-3019、夫苏木单抗、干扰素α、卵磷脂化的超氧化物歧化酶、西妥珠单抗(simtuzumab)、tanzisertib、tralokinumab、hu3G9、huTBT13_2_1、2126458、AM-152、IFN-γ-1b、IW-001、PRM-151、PXS-25、己酮可可碱/N-乙酰基-半胱氨酸、己酮可可碱/维生素E、硫酸沙丁胺醇、[Sar9,Met(O2)11]-物质P、己酮可可碱、巯乙胺酒石酸氢盐、奥贝胆酸、aramchol、GFT-505、二十碳五烯酸乙酯、盐酸二甲双胍、美曲普汀、莫罗单抗-CD3、奥替普拉、IMM-124-E、MK-4074、PX-102和RO-5093151。

[0250] 根据式(I)或式(II)的化合物可以与一种或多种额外的唑类抗真菌剂联合使用。唑类抗真菌剂可以选自咪唑抗真菌剂、三唑抗真菌剂或噻唑抗真菌剂。这类抗真菌剂的实例包括但不限于咪唑衍生物如咪康唑、酮康唑、克霉唑、氯苄甲咪唑(clomidazole)、氯康唑、益康唑、奥莫康唑、联苯苄唑、布康唑、芬替康唑、异康唑、咪康唑、奈康唑、奥昔康唑、舍他康唑、硫康唑、噻康唑;三唑衍生物如氟康唑、膦氟康唑、己唑醇、伊曲康唑、艾沙康唑、泊沙康唑、伏立康唑(voriconzaole)、特康唑、阿巴康唑;和噻唑衍生物如阿巴芬净。

药物组合物的给药

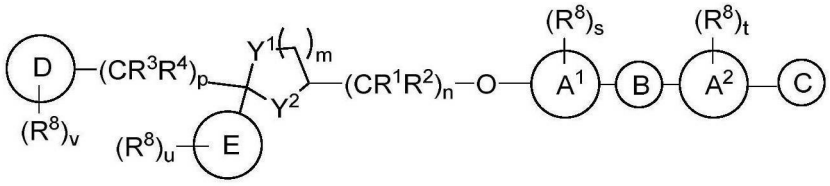
[0251] 合适的给药途径包括但不限于，口服、静脉内、直肠、喷雾、肠胃外、眼、肺、经粘膜、经皮、阴道、耳、鼻和局部给药。此外，仅举例而言，肠胃外递送包括肌肉内、皮下、静脉内、髓内注射，以及鞘内、直接心室内、腹膜内、淋巴管内和鼻内注射。

[0252] 在一些实施方案中，式 (I) 或式 (II) 的化合物及其组合物以任何合适的方式进行给药。给药方式可基于例如是期望进行局部治疗还是全身治疗以及待治疗的区域来进行选择。例如，所述组合物可以口服、肠胃外 (例如，静脉内、皮下、腹膜内或肌肉内注射)、通过吸入、体外、局部 (包括经皮、眼、阴道、直肠、鼻内) 等进行施用。

[0253] 如果使用的话，组合物的肠胃外施用通常以注射为特征。注射剂可以制备为常规形式，作为液体溶液或者悬浮液，适合于在注射前在液体中溶解或悬浮的固体形式，或作为乳剂。用于胃肠外施用的最近修订的方法包括使用缓慢释放或延迟释放系统以保持恒定剂量。

方法

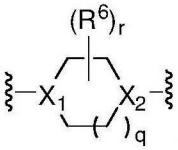
[0254] 在一个方面，本文提供了一种治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病的方法，该方法包括施用包含治疗有效量的式 (I) 化合物、其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体的组合物 ：



式(I)

其中 ：

A^1和 A^2独立地选自芳基或杂芳基 ；

B 为  ；

C 为任选取代的 5 或 6 元杂环基或任选取代的 5 或 6 元杂芳基，其中该杂环基或该杂芳基含有 1 至 4 个氮原子 ；

D 为芳基或杂芳基 ；

E 为芳基、杂芳基、碳环基、杂环基或烷基 ；

每个 R^1、R^2、R^3和 R^4独立地选自 H、烷基、卤代烷基或烷氧基 ；

X_1和 X_2独立地选自 N 和 CR^5；

R^5为 H、OH、烷基或烷氧基 ；

每个 R^6独立地为烷基、卤代烷基、卤代、烷氧基、- 亚烷基 ($NR^{13}R^{14}$) 或芳基 ；

每个 R^8独立地选自烷基、环烷基、杂环基、卤代、羟基、腈、叠氮基、硝基、烷氧基、卤代烷氧基、卤代烷基、羟基烷基、烷氧基烷基、- 亚烷基 ($NR^{13}R^{14}$)、- 亚烷基 (环烷基)、- 亚烷基 (杂环基)、芳基、杂芳基、$-SR^{13}$、$-SOR^{13}$、$-SO_2R^{13}$、$-SO_2NR^{13}R^{14}$、$-NR^{13}R^{14}$、$-NR^{13}SO_2R^{14}$、$-NR^{13}C(O)R^{14}$、$-NR^{13}C(O)OR^{14}$、$-NR^{13}C(O)NR^{13}R^{14}$、$-C(O)R^{14}$、$-C(O)OR^{14}$和 $-C(O)NR^{13}R^{14}$；或者两个相邻的 R^8

形成杂环基环；

每个 R^{13} 和 R^{14} 独立地选自 H、烷基、环烷基、杂环基烷基、卤代烷基、羟基烷基、烷氧基烷基、芳基烷基、杂芳基烷基、芳基和杂芳基；或者 R^{13} 和 R^{14} 与它们所连接至的原子一起形成杂环；

Y^1 和 Y^2 独立地选自 O、CH_2、NH 和 NR^{13}；

n 为 1、2 或 3；

m 为 1 或 2；

p 为 1、2、3 或 4；

q 为 1、2 或 3；

r 为 0、1、2、3、4、5、6、7 或 8；

s 为 0、1、2、3 或 4；

t 为 0、1、2、3 或 4；

u 为 0、1、2、3、4 或 5；且

v 为 0、1、2、3 或 4。

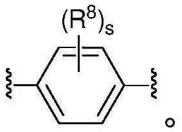
[0255] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1 和 X_2 为 N。

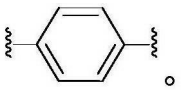
[0256] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1 为 CR^5 且 X_2 为 N。

[0257] 在式 (I) 化合物的以上或以下描述的一些实施方案中，X_1 为 N 且 X_2 为 CR^5。

[0258] 在式 (I) 化合物的以上或以下描述的一些实施方案中，q 为 1 且 r 为 0。

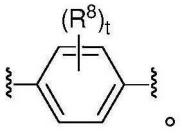
[0259] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1 为芳基。

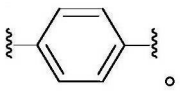
[0260] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1 为  。

[0261] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1 为  。

[0262] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^1 为杂芳基。

[0263] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^2 为芳基。

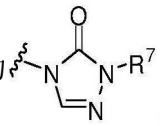
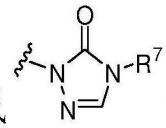
[0264] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

[0265] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^2 为  。

[0266] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^2 为杂芳基。

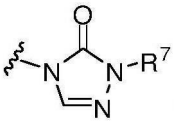
[0267] 在式 (I) 化合物的以上或以下描述的一些实施方案中，A^2 为吡啶、吡嗪、嘧啶、哒嗪或三嗪。

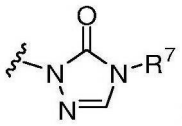
[0268] 在式 (I) 化合物的以上或以下描述的一些实施方案中，C 为任选取代的 5 或 6 元杂芳基。在式 (I) 化合物的以上或以下描述的其他实施方案中，C 为任选取代的 5 或 6 元杂环基。

[0269] 在式(I)化合物的以上或以下描述的一些实施方案中,C为  或  ;

且

R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

[0270] 在式(I)化合物的以上或以下描述的一些实施方案中,C为  且R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

[0271] 在式(I)化合物的以上或以下描述的一些实施方案中,C为  且R^7为烷基、卤代烷基、羟基烷基、烷氧基烷基、-亚烷基($NR^{13}R^{14}$)、环烷基、杂环基、-亚烷基(环烷基)或-亚烷基(杂环基)。

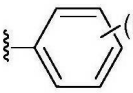
[0272] 在式(I)化合物的以上或以下描述的一些实施方案中,E为烷基。

[0273] 在式(I)化合物的以上或以下描述的一些实施方案中,E为环烷基。

[0274] 在式(I)化合物的以上或以下描述的一些实施方案中,E为环丙基、环丁基、环戊基或环己基。

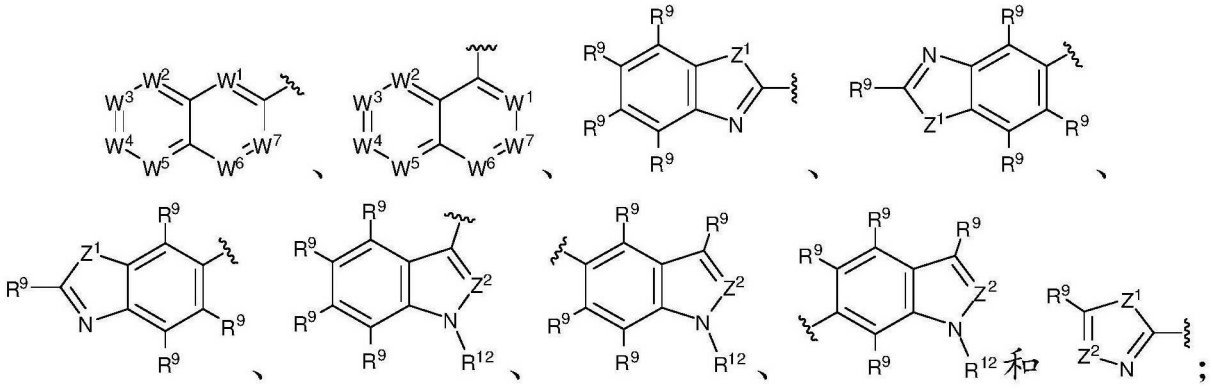
[0275] 在式(I)化合物的以上或以下描述的一些实施方案中,E为杂环基。

[0276] 在式(I)化合物的以上或以下描述的一些实施方案中,E为芳基。

[0277] 在式(I)化合物的以上或以下描述的一些实施方案中,E为  且u为0、1、2、3、4或5。

[0278] 在式(I)化合物的以上或以下描述的一些实施方案中,E为杂芳基。

[0279] 在式(I)化合物的以上或以下描述的一些实施方案中,E选自:



W^1、W^2、W^3、W^4、W^5、W^6和W^7独立地选自N和CR^9;

Z^1为NR^{12}、S或O;

Z^2为N或CR^9;

每个 R^9 独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基；

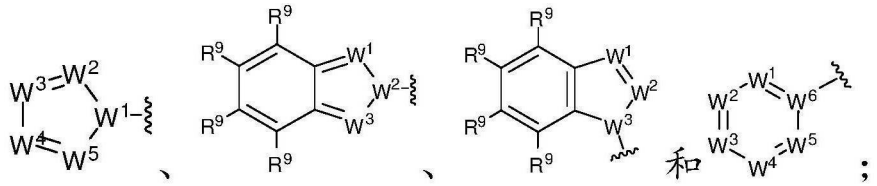
每个 R^{10} 和 R^{11} 独立地选自 H 和烷基；或者 R^{10} 和 R^{11} 与它们所连接至的氮一起形成杂环；且

R^{12} 为 H、烷基或卤代烷基。

[0280] 在式(I)化合物的以上或以下描述的一些实施方案中，D 为芳基。

[0281] 在式(I)化合物的以上或以下描述的一些实施方案中，E 为杂芳基。

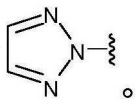
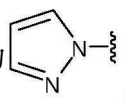
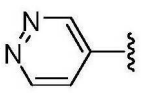
[0282] 在式(I)化合物的以上或以下描述的一些实施方案中，D 选自：



W^1、W^2、W^3、W^4 和 W^5 独立地选自 N 和 CR^9；

W^6 为 N 或 C；且

每个 R^9 独立地选自 H、卤素、CN、NO_2、烷基、$-SR^{10}$、$-OR^{10}$、$-NR^{10}R^{11}$、$NR^{10}C(O)$(烷基)、$-NR^{10}C(O)$(环烷基)、$-NR^{10}C(O)$(杂环烷基)、$-NR^{10}C(O)$(芳基)、$-NR^{10}C(O)$(杂芳基)、$-C(O)NR^{10}R^{11}$、$-C(O)NR^{10}$(环烷基)、$-C(O)NR^{10}$(杂环烷基)、$-C(O)NR^{10}$(芳基)、$-C(O)NR^{10}$(杂芳基)、$-NR^{10}C(O)NR^{10}R^{11}$、$-NR^{10}C(O)NR^{11}$(环烷基)、$-NR^{10}C(O)NR^{11}$(杂环烷基)、$-NR^{10}C(O)NR^{11}$(芳基)、$-NR^{10}C(O)NR^{11}$(杂芳基)、$-NR^{10}C(O)O$(烷基)、$-NR^{10}C(O)O$(环烷基)、$-NR^{10}C(O)O$(杂环烷基)、$-NR^{10}C(O)O$(芳基)、$-NR^{10}C(O)O$(杂芳基)、$-NR^{10}SO_2$(烷基)、$-NR^{10}SO_2$(环烷基)、$-NR^{10}SO_2$(杂环烷基)、$-NR^{10}SO_2$(芳基)、$-NR^{10}SO_2$(杂芳基)、$-SO_2NR^{10}R^{11}$、$-SO_2NR^{10}$(环烷基)、$-SO_2NR^{10}$(杂环烷基)、$-SO_2NR^{10}$(芳基)、$-SO_2NR^{10}$(杂芳基)、卤代烷基、芳基和杂芳基。

[0283] 在式(I)化合物的以上或以下描述的某些实施方案中，D 为  。在式(I)化合物的以上或以下描述的某些实施方案中，D 为  。在式(I)化合物的以上或以下描述的某些实施方案中，D 为  。

[0284] 在式(I)化合物的以上或以下描述的一些实施方案中，Y^1 和 Y^2 为 O。

[0285] 在式(I)化合物的以上或以下描述的一些实施方案中，m 为 1。

[0286] 在式 (I) 化合物的以上或以下描述的一些实施方案中，p 为 1、2 或 3。

[0287] 在式 (I) 化合物的以上或以下描述的一些实施方案中，p 为 1。

[0288] 在式 (I) 化合物的以上或以下描述的一些实施方案中，R^1、R^2、R^3和 R^4为氢。

[0289] 本文还提供了一种药物组合物，其包含式 (II) 或如以上及以下描述的化合物或其药学上可接受的盐、溶剂化物、多晶型物、前药、代谢物、N- 氧化物、立体异构体或异构体和药学上可接受的赋形剂。

[0290] 本文进一步提供了一种使用本文所述化合物治疗纤维化的方法，其中该纤维化是肝纤维化、特发性肺纤维化、肾纤维化或心脏纤维化。

[0291] 本文进一步提供了一种使用本文所述化合物治疗肝纤维化的方法，其中该肝纤维化与酒精性或非酒精性肝硬化的后期相关。

[0292] 本文进一步提供了一种使用本文所述化合物治疗纤维化的方法，其中该纤维化是特发性肺纤维化。

[0293] 本文进一步提供了一种使用本文所述化合物治疗疾病的方法，其中以纤维化为特征的疾病或病症是慢性自身免疫病。

[0294] 本文进一步提供了一种使用本文所述化合物治疗慢性自身免疫病的方法，其中该慢性自身免疫病是类风湿性关节炎、硬皮病、克罗恩病或系统性红斑狼疮。

[0295] 本文进一步提供了一种使用本文所述化合物治疗慢性自身免疫病的方法，其中该慢性自身免疫病是硬皮病。

[0296] 本文进一步提供了一种使用本文所述化合物治疗纤维化的方法，其中该纤维化是由异常创伤愈合导致的瘢痕疙瘩形成。

[0297] 本文进一步提供了一种使用本文所述化合物治疗纤维化的方法，其中该纤维化在器官移植后发生。

[0298] 本文还提供了一种治疗纤维化、以纤维化为特征的病症或以纤维化为特征的疾病的方法，该方法包括施用包含治疗有效量的本文所述化合物联合一种或多种药物剂的组合物。在以上描述的某些实施方案中，所述一种或多种药物剂是抗纤维化剂。在以上描述的某些实施方案中，所述一种或多种药物剂是抗真菌剂。

[0299] 本文进一步公开了用于鉴定纤维化的抑制剂的基于图像的系统。在一些实施方案中，该系统包括 (a) 一种或多种成纤维细胞；和 (b) 用于生成一种或多种成纤维细胞的一幅或多幅图像的细胞成像装置。在一些实施方案中，该细胞成像装置包括荧光显微镜。在一些实施方案中，该细胞成像装置包括 CCD 相机技术。在一些实施方案中，该细胞成像装置是自动化的。在一些实施方案中，该细胞成像装置是手动操作的。在一些实施方案中，该细胞成像装置是热电冷却的。

[0300] 在一些实施方案中，该系统进一步包括光源。在一些实施方案中，该光源是 LED。

[0301] 在一些实施方案中，该系统进一步包括扫描仪。

[0302] 在一些实施方案中，该系统进一步包括计算机。

[0303] 在一些实施方案中，该系统进一步包括一个或多个用于存储和 / 或接收一幅或多幅图像的存储单元。在一些实施方案中，该系统进一步包括一个或多个用于存储和 / 或接收关于生成一幅或多幅图像的一个或多个指令的存储单元。

[0304] 在一些实施方案中，该系统进一步包括一个或多个用于分析一种或多种成纤维细

胞的一幅或多幅图像的处理器。在一些实施方案中，该系统进一步包括一个或多个用于处理一种或多种成纤维细胞的一幅或多幅图像的处理器。在一些实施方案中，该系统进一步包括一个或多个用于传送一种或多种成纤维细胞的一幅或多幅图像的处理器。

[0305] 在一些实施方案中，该系统进一步包括用于捕捉、生成、分析、扫描、存储和 / 或传送一幅或多幅图像的一个或多个软件程序。

[0306] 在一些实施方案中，该系统进一步包括一个或多个条形码阅读器，该条形码阅读器用于阅读包含一种或多种细胞的一个或多个样品上的一种或多种条形码。

[0307] 在一些实施方案中，该系统进一步包括用于处理包含一种或多种细胞的一个或多个样品的一个或多个机器人。在一些实施方案中，该系统进一步包括用一种或多种药剂处理包含一种或多种细胞的一个或多个样品的一个或多个机器人。

[0308] 在一些实施方案中，所述一种或多种药剂包括 TGF-β。在一些实施方案中，所述一种或多种药剂包括一种或多种测试剂。

[0309] 在一些实施方案中，该系统进一步包括用于将一种或多种测试剂鉴定为纤维化的抑制剂的一个或多个处理器。在一些实施方案中，该系统进一步包括用于对纤维化的抑制剂进行排序的一个或多个处理器。

[0310] 在一些实施方案中，该系统进一步包括一种或多种算法。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析与一种或多种药剂接触的一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的强度。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的荧光强度。

[0311] 在一些实施方案中，所述细胞成像装置包括 CellInsight NXT High Content Screening(HCS) 平台。

[0312] 在一些实施方案中，所述一种或多种成纤维细胞是肝星形细胞 (HSC)。

[0313] 本文进一步公开了鉴定纤维化的抑制剂的方法。在一些实施方案中，该方法包括 (a) 使包含一种或多种成纤维细胞的第一样品与细胞生长剂接触；(b) 使包含一种或多种成纤维细胞的第二样品与该细胞生长剂和第一测试剂接触；(c) 生成第一样品的一种或多种成纤维细胞的一幅或多幅图像和第二样品的一种或多种成纤维细胞的一幅或多幅图像；以及 (d) 基于对第一样品的一幅或多幅图像和第二样品的一幅或多幅图像的分析，确定第一测试剂是否是纤维化的抑制剂。

[0314] 在一些实施方案中，所述细胞生长剂是生长因子。在一些实施方案中，所述细胞生长剂是转化生长因子 β (TGF-b)。

[0315] 在一些实施方案中，第一测试剂是小分子。在一些实施方案中，第一测试剂是生物活性小分子。

[0316] 在一些实施方案中，第二样品同时与细胞生长剂和第一测试剂接触。在一些实施方案中，第二样品顺序地与细胞生长剂和第一测试剂。在一些实施方案中，第二样品在与第一测试剂接触之前与细胞生长剂接触。在一些实施方案中，第二样品在与细胞生长剂接触之前与第一测试剂接触。

[0317] 在一些实施方案中，该方法进一步包括包含一种或多种成纤维细胞的一个或多个额外的样品。在一些实施方案中，第一样品、第二样品和 / 或所述一个或多个额外的样品来

自相同的来源。在一些实施方案中，第一样品、第二样品和 / 或所述一个或多个额外的样品来自两个或更多个不同的来源。

[0318] 在一些实施方案中，该方法进一步包括使所述一个或多个额外的样品与细胞生长剂和一种或多种额外的测试剂接触。

[0319] 在一些实施方案中，同时捕捉第一样品的一幅或多幅图像和第二样品的一幅或多幅图像。在一些实施方案中，顺序地捕捉第一样品的一幅或多幅图像和第二样品的一幅或多幅图像。

[0320] 在一些实施方案中，第一样品的一种或多种成纤维细胞在第一培养板上的一个或多个孔中培养。在一些实施方案中，第二样品的一种或多种成纤维细胞在第二培养板的一个或多个孔上培养。在一些实施方案中，所述一个或多个额外的样品的一种或多种成纤维细胞在一个或多个额外的培养板的一个或多个孔上培养。

[0321] 在一些实施方案中，第一培养板和第二培养板是不同的。在一些实施方案中，第一培养板、第二培养板和 / 或所述一个或多个额外的培养板是不同的。

[0322] 在一些实施方案中，第一培养板和第二培养板是相同的。在一些实施方案中，第一培养板、第二培养板和 / 或所述一个或多个额外的培养板是相同的。

[0323] 在一些实施方案中，该方法进一步包括使第一样品的一种或多种成纤维细胞和 / 或第二样品的一种或多种成纤维细胞与第三药剂接触。在一些实施方案中，该方法进一步包括使所述一个或多个额外的样品的一种或多种成纤维细胞与第三药剂接触。

[0324] 在一些实施方案中，第三药剂是抗体。在一些实施方案中，第三药剂是抗平滑肌肌动蛋白 (SMA) 抗体。

[0325] 在一些实施方案中，第一样品的一幅或多幅图像和 / 或第二样品的一幅或多幅图像基于与第三药剂接触的一种或多种成纤维细胞的图像。在一些实施方案中，所述一个或多个额外的样品的一幅或多幅图像基于与第三药剂接触的一种或多种成纤维细胞的图像。

[0326] 在一些实施方案中，生成一幅或多幅图像包括使用一个或多个细胞成像装置。在一些实施方案中，该细胞成像装置包括 CellInsight NXT High Content Screening(HCS) 平台。

[0327] 在一些实施方案中，该方法进一步包括一种或多种算法。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析与一种或多种药剂接触的一种或多种成纤维细胞的形态学。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的强度。在一些实施方案中，所述一种或多种算法分析一种或多种成纤维细胞的荧光强度。

[0328] 在一些实施方案中，该方法进一步包括检测一种或多种成纤维细胞的转分化。在一些实施方案中，所述一种或多种成纤维细胞的转分化包括转分化为一种或多种肌成纤维细胞。

[0329] 在一些实施方案中，确定第一测试剂是否被鉴定为纤维化的抑制剂基于第一样品中的肌成纤维细胞组成与第二样品中的肌成纤维细胞组成的比较。在一些实施方案中，如果第二样品中的肌成纤维细胞组成低于第一样品中的肌成纤维细胞组成，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果第二样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 5％、10％、15％、20％、25％、30％、35％、40％、45％、

50％、55％、60％、65％、70％、75％、80％、85％或 90％，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果第二样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 1.5、2、2.5、3、3.5、4、4.5、5、6、7、8、9、10、11、12、13、14 或 15 倍，则将第一测试剂鉴定为纤维化的抑制剂。

[0330] 在一些实施方案中，该方法进一步包括确定一种或多种额外的测试剂是否是纤维化的抑制剂。在一些实施方案中，确定一种或多种额外的测试剂是否被鉴定为纤维化的抑制剂基于第一样品中的肌成纤维细胞组成与所述一个或多个额外的样品中的肌成纤维细胞组成的比较。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成低于第一样品中的肌成纤维细胞组成，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％或 90％，则将第一测试剂鉴定为纤维化的抑制剂。在一些实施方案中，如果所述一个或多个额外的样品中的肌成纤维细胞组成比第一样品中的肌成纤维细胞组成低至少约 1.5、2、2.5、3、3.5、4、4.5、5、6、7、8、9、10、11、12、13、14 或 15 倍，则将第一测试剂鉴定为纤维化的抑制剂。

实施例

缩写列表

[0331] 如上所使用的，并且在本发明的整篇说明书中，除非另有说明，否则下列缩写应理解为具有以下含义：

ACN	乙腈
Bn	苯基
BOC 或 Boc	氨基甲酸叔丁酯
BOP	苯并三唑 -1- 基 - 氧基三（二甲基氨基）鏻
t-Bu	叔丁基
Cbz	氨基甲酸苄酯
Cy	环己基
DBU	1,8- 二氮杂双环 [5.4.0] 十一碳 -7- 烯
DCC	二环己基碳二亚胺
DCM	二氯甲烷 (CH_2CI_2)
DIC	1,3- 二异丙基碳二亚胺
DEAD	偶氮二甲酸二乙酯
DIAD	偶氮二甲酸二异丙酯
DIEA	二异丙基乙胺
DMAP	4-(N,N- 二甲基氨基）吡啶
DMP 试剂	戴斯 - 马丁氧化剂 (Dess-Martin Periodinane reagent)
DMF	二甲基甲酰胺
DMA	N,N- 二甲基乙酰胺
DME	1,2- 二甲氧基 - 乙烷
DMSO	二甲基亚砜

Dppf	1,1'- 双（二苯基膦基）二茂铁
EDCI	1- 乙基 -3-(3- 二甲基氨基丙基）碳二亚胺 HCl
eq	当量
Et	乙基
Et_2O	二乙醚
EtOH	乙醇
EtOAc	乙酸乙酯
HOAt	1- 羟基 -7- 氮杂苯并三唑
HOBT	1- 羟基苯并三唑
HOSu	N- 羟基琥珀酰胺
HPLC	高效液相色谱法
LAH	酸酐锂铝
Me	甲基
MeI	碘甲烷
MeOH	甲醇
MOMCl	甲氧基甲基氯
MOM	甲氧基甲基
MS	质谱法
NMP	N- 甲基 - 吡咯烷 -2- 酮
NMR	核磁共振
PyBOP	苯并三唑 -1- 基 - 氧基三 - 吡咯烷基 - 鏻六氟磷酸盐
SPHOS	2- 二环已基膦基 -2',6'- 二甲氧基联苯基
TBD	1,5,7- 三氮杂双环 [4.4.0]- 癸 -5- 烯
RP-HPLC	反相高压液相色谱法
TBS	叔丁基二甲基硅烷基
TBSCl	叔丁基二甲基硅烷基氯化物
TBTU	O-（苯并三唑 -1- 基）-N,N,N',N'- 四甲基鎓
TEOC	2- 三甲基硅烷基乙基氨基甲酸酯
TFA	三氟乙酸
Tf_2O	三氟甲磺酸酐
TMG	1,1,3,3- 四甲基胍
THF	四氢呋喃
THP	四氢吡喃
TLC	薄层色谱法
XPHOS	2- 二环已基膦基 -2′ ,4′ ,6′ - 三异丙基联苯基

用于制备本发明化合物的通用实施例

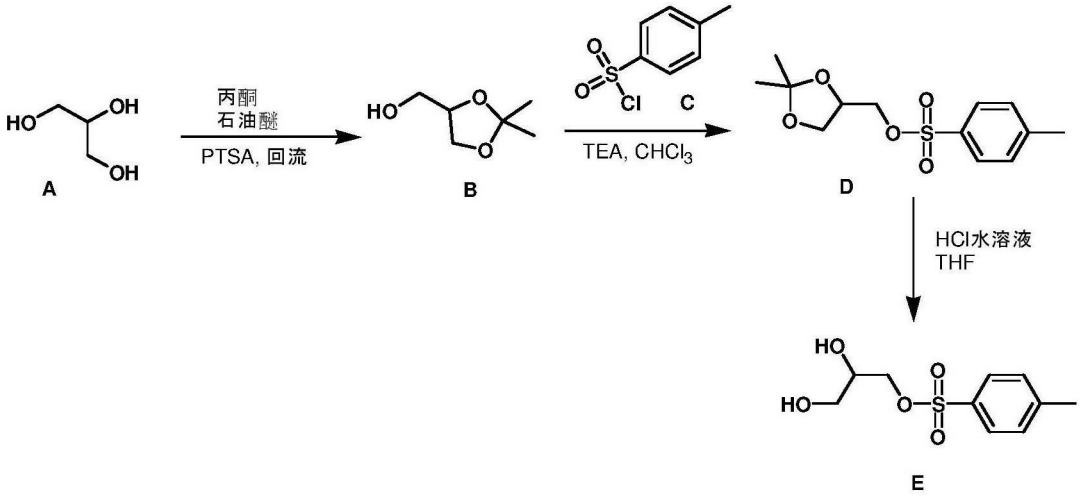
[0332] 用于本发明化合物的起始材料和中间体可以通过应用或修改以下描述的方法，其明显的化学等同物，或者，例如，如以下文献中所述来制备 :The Science of Synthesis, 第 1-8 卷 .E.M.Carreira 等人编 .Thiemepublishers(2001-2008)。试剂和反应选项的细节

也可以通过使用商业计算机搜索引擎如 Scifinder(www.cas.org) 或 Reaxys(www.reaxys.com) 进行结构和反应搜索而获得。

部分 A:

[0333] 以下反应流程 A、B 和 C 详述了与化合物 46 的最终形成 (流程 D) 有关的起始材料的合成。

流程 A :外消旋二醇 (E) 的合成 :



[0334] 向配备有冷凝器和 Dean-stark 的 250ml 三颈圆底烧瓶中加入中间体 -A(25.0g)、丙酮 (75.0ml)、PTSA.H_2O(0.75g) 和石油醚 (75.0ml)。将混合物在回流温度下搅拌 12h 并通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测。将反应混合物冷却至室温并加入 0.75g 乙酸钠。将混合物在室温下搅拌 30 分钟。滗析有机层并减压浓缩,得到粗液体——中间体 -B(27.0g,75.2%)。

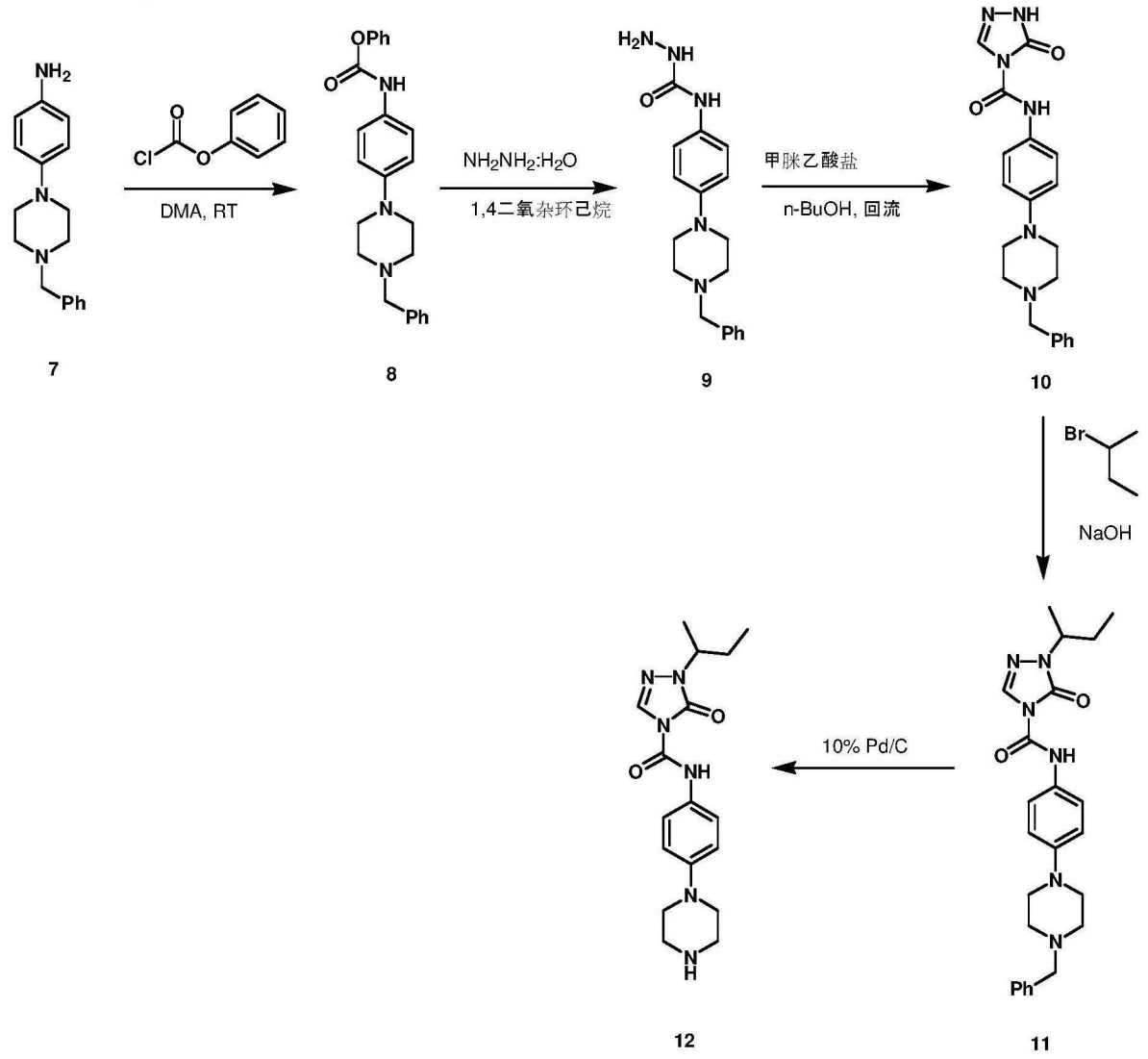
[0335] 向配备有氯化钙防护管的 500ml 三颈圆底烧瓶中加入在氯仿 (185ml) 中的中间体 -B(25.0g)。加入 TEA(79.0ml) 并将反应冷却至 0℃。将中间体 -C(47.0g) 逐批充入到混合物中,并将之在室温下搅拌 5h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应。用水 (200ml) 洗涤反应混合物,并用 $CHCl_3$(50ml x 2) 反萃取水层。将有机层合并,经 Na_2SO_4干燥,并减压浓缩,得到粗油。将油通过柱色谱法 (含 6% 乙酸乙酯的己烷) 纯化。终产物中间体 -D(21.0g,38.6%)。

[0336] 向配备有磁力搅拌器的 250ml 三颈圆底烧瓶中加入在 THF(100ml) 中的中间体 -D(21.0g)。将溶液冷却至 0℃,与 6N HCl(25.0ml) 混合,并在室温下搅拌 6h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应。反应混合物用水 (100ml) 稀释并用饱和碳酸氢钠溶液中和。用 $CHCl_3$(50ml*2) 萃取产物。将有机层合并,经 Na_2SO_4干燥,并减压浓缩,得到稠油——中间体 -E(16.0g,88.8%)。中间体 -E 不经进一步纯化而在合成的下一阶段使用。

中间体光谱数据 :

中间体	表征数据(NMR/LCMS)
B	^{1}HNMR (400MHz, $CDCl_3$): 1.23 (s, 3H), 1.3 (s, 3H), 3.44-3.55 (m, 3H), 3.61-3.64(m, 1H), 3.89-3.92 (m, 1H), 4.05-4.10 (m, 1H).
D	LCMS: 98.1 %； m/z: 304 (M + H_20).
E	^{1}HNMR (400MHz, $CDCl_3$): 2.46 (s, 3H), 2.85 (s, 1H), 3.4 (s, 1H), 3.57-3.61 (m, 1H), 3.66-3.70(m, 1H), 3.93-3.96 (m, 1H), 4.04-4.10 (m, 2H), 7.36-7.38 (dd, *J*=8.0 Hz, 2H), 7.79-7.81 (dd, *J*=8.0 Hz, 2H).

流程 B :中间体 -12 的合成 :



[0337] 向配备有氯化钙管的 100ml 三颈圆底烧瓶中加入在 DMA(25.0ml) 中的中间

体 -7(5.0g)。向其中逐滴加入氯甲酸苯酯 (2.8ml)，并将反应混合物在室温下搅拌半小时。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应。将反应混合物倒入冰水中。所得的沉淀物通过过滤收集，用水洗涤，并在真空下干燥以获得纯产物——中间体 -8(4.4g，61.1％)。

[0338] 向配备有氯化钙管的 100ml 三颈圆底烧瓶中加入在 1,4- 二氧杂环己烷 (20.0ml) 中的中间体 -8(4.4g)。逐滴加入水合肼 (1.37ml)，并将反应混合物在室温下搅拌 24h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应。将混合物倒入冰水中。通过过滤收集沉淀物，用水洗涤，并在真空下干燥，获得纯产物——中间体 -9(2.80g，53.8％)。

[0339] 向配备有冷凝器的 100ml 三颈圆底烧瓶中加入在 n-BuOH(25.0ml) 中的中间体 -9(2.80g)。加入甲脒 (Formimidine) 乙酸盐 (4.47g) 并将反应混合物在 90℃下搅拌 4h。通过 TLC 监测反应 (EtOAc)。将混合物冷却至室温并通过过滤收集沉淀物，用乙酸乙酯洗涤，并干燥，以得到纯产物——中间体 -10(2.5g，86.8％)。

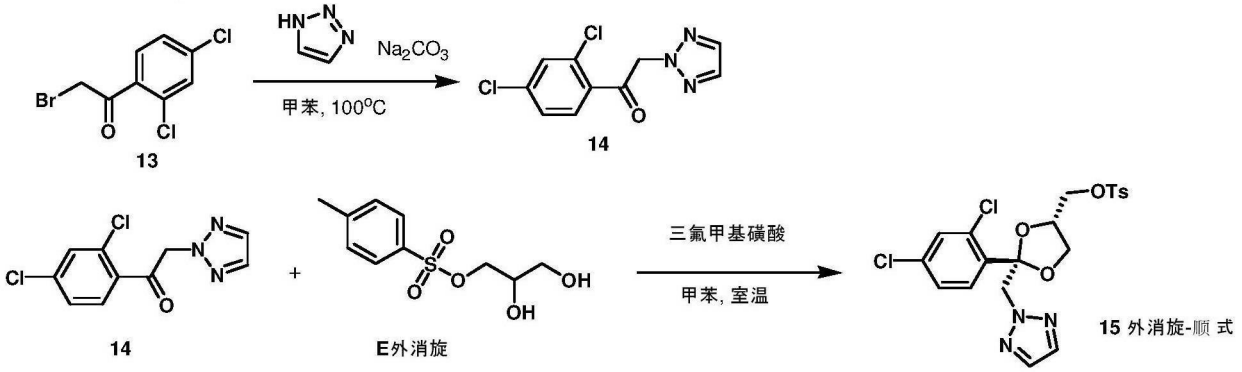
[0340] 向配备有冷凝器的 100ml 三颈圆底烧瓶中加入在 DMF(30.0ml) 中的中间体 -10(2.50g)。加入 NaOH(1.50g) 和仲溴丁烷 (5.11g) 并将反应混合物在 90℃搅拌 8h。通过 TLC(EtOAc) 监测反应。将反应混合物倒入冰水中。通过过滤收集沉淀物，用水洗涤并在真空下干燥，获得纯产物——中间体 -11(1.45g，49.8％)。

[0341] 向 100ml 氢化器容器中加入在 MeOH(25.0ml) 中的中间体 -11(1.45g)。加入 10％ Pd/C(0.15g) 并将反应混合物在室温下在 10kg 的 H_2 压下氢化过夜。通过 TLC(EtOAc) 监测反应。反应完成后，通过过滤除去催化剂，并蒸发溶剂以得到稠油。通过柱色谱法 (含 5％ MeOH 的 MDC) 纯化粗产物。终产物中间体 -12(0.75g，67.5％)。

中间体光谱数据 :

中间体编号	表征数据(NMR/LCMS)
8	LCMS: 99.16 % @ 256 nm; m/z: 388 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 2.94-3.05 (m, 2H), 3.13-3.20 (m, 2H), 3.72-3.75 (d, 2H), 4.39-4.40 (d, 2H), 6.95-6.97(d, *J*=8.0 Hz, 2H), 7.19-7.27 (m, 3H), 7.38-7.44 (m, 4H), 7.49-7.50 (m, 3H), 7.59-7.60 (d, *J*=4.0 Hz, 2H).
9	LCMS: 97.61 % @ 254 nm; m/z: 326 (M+H).
10	LCMS: 98.34% @ 256 nm; m/z: 336 (M+H).
11	LCMS: 96.95 % @ 258 nm; m/z: 392 (M+H).
12	LCMS: 95.78 % @ 257 nm; m/z: 302 (M+H).

流程 C :中间体 -15 的合成



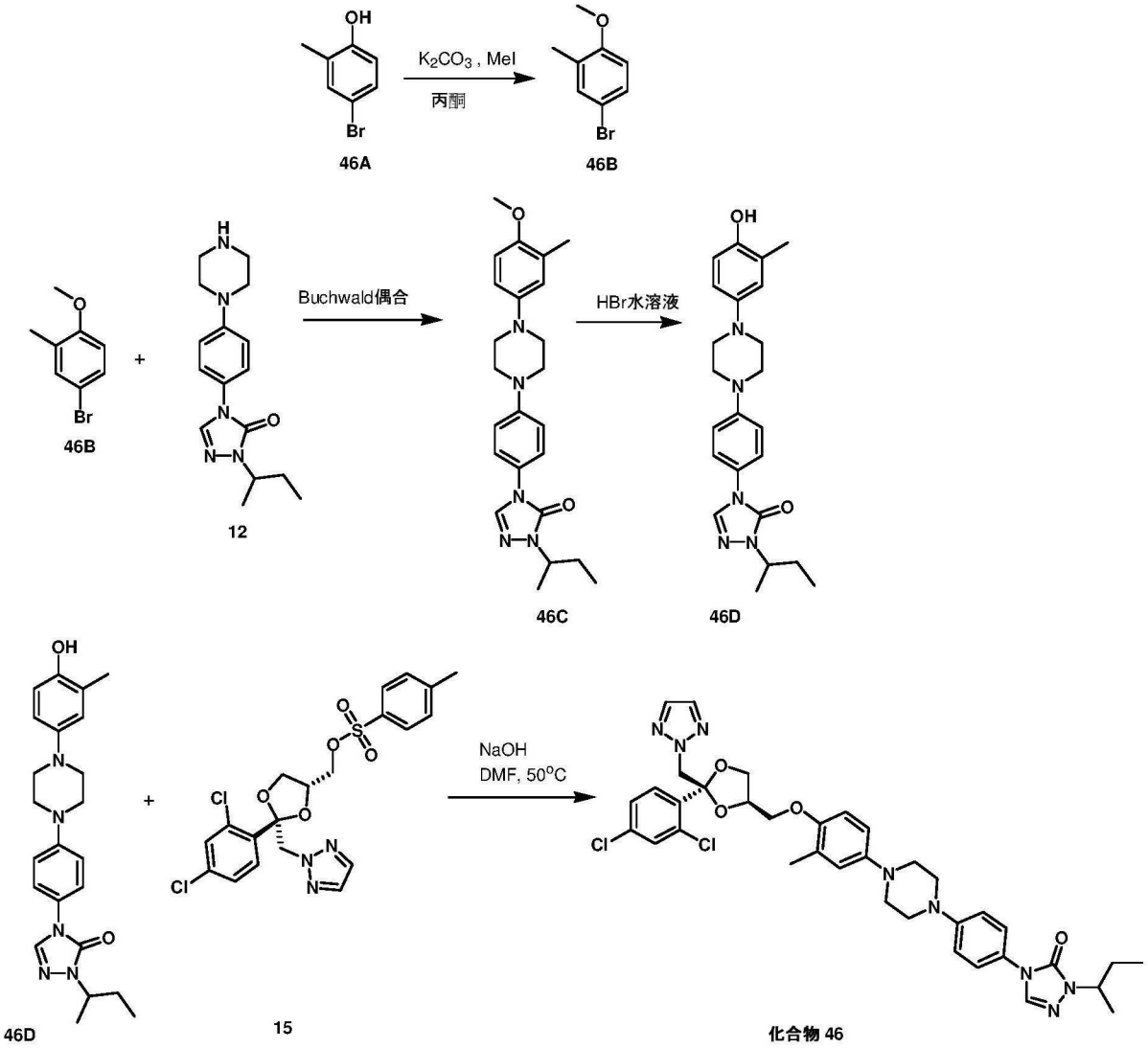
[0342] 在室温下向搅拌的中间体 -13(2.0g) 在甲苯 (30.0ml) 中的溶液中充入 1H-1,2,3 三唑 (1.96g) 和 Na_2CO_3(3.01g)。将反应混合物在 100℃下搅拌 3h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应的完成。反应完成后将混合物冷却至室温并用乙酸乙酯 (50ml) 稀释。获得的有机层用水 (50ml x2) 洗涤。将有机层分离,经 Na_2SO_4干燥并减压浓缩。通过柱色谱法 (含 20%乙酸乙酯的己烷) 纯化粗产物。中间体 -14(0.70g,19.2%)。

[0343] 在室温、氩气氛下向搅拌的中间体 -14(1.30g) 在甲苯 (15.0ml) 中的溶液中充入中间体 -E(1.50g)。将所得混合物冷却至 0℃并向其中逐滴加入三氟甲磺酸 (1.80ml)。使混合物升温至室温并搅拌 60h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应的完成。反应完成后将所得混合物倒入水 (25ml) 中并用饱和碳酸氢钠溶液中和。用乙酸乙酯 (25ml x 2) 萃取水层。将有机层合并,经 Na_2SO_4干燥并减压浓缩以得到稠油。通过柱色谱法 (含 10% 乙酸乙酯的己烷) 纯化粗产物。中间体 -15(0.32g,24.6%)。

中间体光谱数据 :

中间体编号	表征数据(NMR/LCMS)
14	LCMS: 96.62 % @ 252 nm; m/z 258 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 5.91 (s, 2H), 7.36-7.39 (dd, J_1=12.0 Hz 和 J_2=4.0 Hz, 1H), 7.51-7.52 (d, J=4.0 Hz, 1H), 7.64-7.66 (d, J=8.0 Hz, 1H), 7.73(s, 2H).
15	LCMS: 100 % @ 225 nm; m/z 486 (M+2). ^{1}HNMR (400MHz, $CDCl_3$): 2.49 (s, 3H), 3.41-3.45 (m, 1H), 3.75-3.82 (m, 1H), 3.84-3.89 (m, 2H), 4.23-4.26(m,
	1H), 4.94-4.98 (d, J=14.4 Hz, 1H), 5.06-5.09 (d , J=14.0 Hz, 1H), 7.18-7.21 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz, 1H), 7.38-7.40(d, J_1=12.0 Hz 和 J_2=8.0 Hz, 2H),7.44-7.46 (m, 2H), 7.55 (s, 2H), 7.77-7.79(d, J_1=12.0 Hz 和 J_2=8.4 Hz, 2H).

流程 D :化合物 46 的合成 :



[0344] 在室温、氩气氛下向搅拌的中间体 -46A(0.50g) 在丙酮 (7.0ml) 中的溶液中充入 K_2CO_3(0.44g)。逐滴加入甲基碘 (0.20ml) 并使混合物升温至室温并搅拌 24h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应的完成。反应完成后将混合物倒入水 (25ml) 中。用乙酸乙酯 (25ml x 2) 萃取水层。将有机层合并,经 Na_2SO_4干燥并减压浓缩,以得到粗物质——中间体 -46-B(0.53g,100%)。

[0345] 在室温、氩气氛下向搅拌的中间体 -46B(0.125g) 在甲苯 (3.0ml) 中的溶液中充入中间体 -12(0.188g)、叔丁醇钠 (0.089g)、Ru(Phos)(0.028g) 和 $Pd_2(dba)_3$(0.055g)。所得混合物用氩气球吹扫 10 分钟并在 120℃下搅拌 15h。通过 TLC(己烷 : 乙酸乙酯 (5:5)) 监测反应的完成。反应完成后将混合物倒入水 (10ml) 中。用乙酸乙酯 (10ml x 2) 萃取水层。将有机层合并,经 Na_2SO_4干燥并减压浓缩。粗产物通过柱色谱法 (含 25% 乙酸乙酯的己烷) 纯化。中间体 -46-C(0.08g,30.7%)。

[0346] 向 5ml 管中充入在 48% HBr 水溶液 (0.80ml) 中的中间体 -46C(0.080g)。将所得混合物在回流温度下搅拌过夜。通过 TLC(乙酸乙酯) 监测反应的完成。反应混合物用水 (5.0ml) 稀释并用饱和碳酸氢钠溶液中和。所得沉淀物通过过滤收集,用水洗涤并在真空下干燥以获得纯中间体 -46-D(0.050g,64.90%)。

[0347] 在室温、氩气氛下向搅拌的中间体 -46D(0.050g) 在 DMF(1.0ml) 中的溶液中充入

中间体-15(0.050g)和NaOH(0.020g)。将所得混合物在50℃下搅拌过夜。通过TLC(己烷:乙酸乙酯(5:5))监测反应的完成。反应完成后将所得混合物倒入水(10ml)中并用乙酸乙酯(10ml x 2)萃取水层。将有机层合并,经Na_2SO_4干燥并减压浓缩。通过制备型HPLC(通过反相制备型纯化在含有0.1%NH_3的ACN:水中洗脱)纯化粗产物。将产物级分冻干以获得纯产物,46(0.012g,11.32%)。

中间体和最终化合物光谱数据:

中间体/最终化合物编号	表征数据(NMR/LCMS)
46B	^{1}HNMR (400MHz, $CDCl_3$): 2.19-2.20 (d, *J*=4.0 Hz, 3H), 3.82 (s, 3H), 6.69-6.70 (d, *J*=4.0 Hz, 1H), 7.26-7.28 (d, *J*=8.0 Hz, 2H).
46C	LCMS: 98.3 % @ 261 nm; m/z 422 (M+1).
46D	LCMS: 81.9 % @ 258 nm; m/z 408 (M+1).
46	LCMS: 100% @ 262 nm; m/z 719.51 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.2 Hz,

中间体/最终化合物编号	表征数据(NMR/LCMS)
	3H) ,1.40-1.42 (d, *J*=6.8 Hz, 3H) ,1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H),2.16-2.19 (s, 3H),3.25 (s, 4H), 3.38 (s, 4H),3.48-3.52 (q, 1H), 3.91-4.00 (m, 3H),4.29-4.34(m, 1H),4.39-4.41 (m, 1H),5.06-5.09 (d, *J*=14.0 Hz, 1H),5.17-5.20 (d, *J*=14.0 Hz, 1H),6.69-6.71 (d, *J*=8.0 Hz, 1H),6.78-6.84 (m, 2H),7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz, 1H),7.44-7.48 (m, 3H),7.52-7.54 (d, *J*=8.4 Hz 1H),7.60-7.64 (m, 3H).

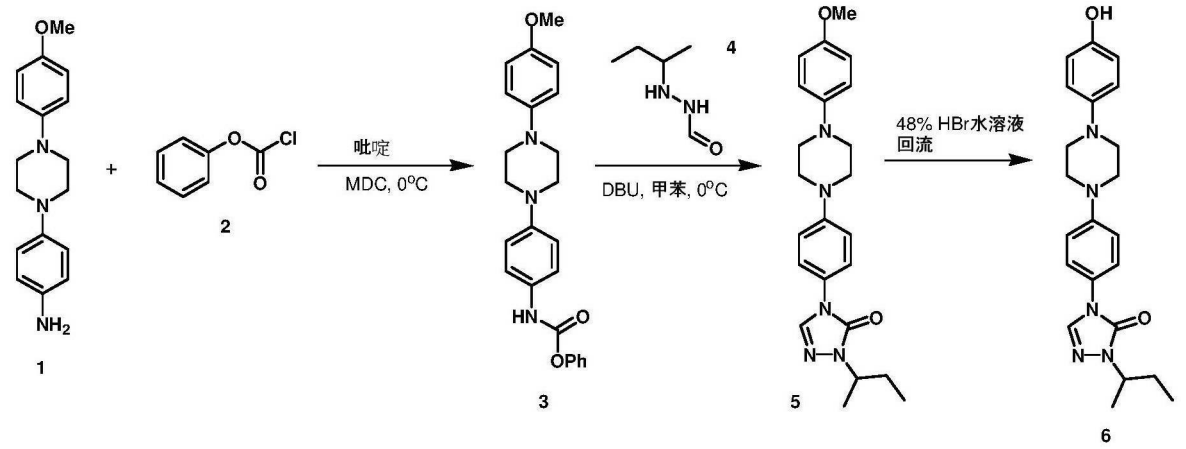
额外的合成信息:

[0348] 化合物47-54使用相同的反应流程用其相应的溴苯酚(中间体-46A)制备。

部分B:

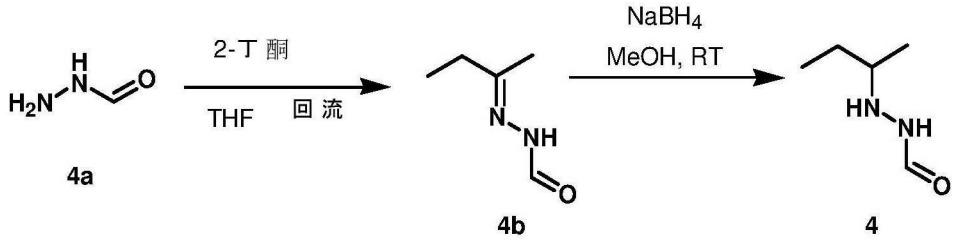
[0349] 以下反应流程E和F详述了与化合物42的最终形成(流程G)有关的起始材料的合成。在流程A(部分A)中产生的相同的外消旋二醇也在下面紧接的合成中使用。

流程E:中间体-6的合成:



[0350] 向配备有氯化钙管的100ml三颈圆底烧瓶中充入中间体-1(5.13g)、吡啶(12.0ml)和MDC(50.0ml)。将反应混合物冷却至0℃并向其中加入氯甲酸苯酯(5.0ml)。将反应在4℃下搅拌过夜。通过TLC(己烷:乙酸乙酯(5:5))监测反应完成。向混合物中加水(100ml)并将溶液在室温下搅拌30分钟。将沉淀物过滤,用二氯甲烷(10ml x 2)洗涤,并在真空下干燥,获得纯产物——中间体-3(5.5g,75.1%)。

中间体-4的合成:



[0351] 向配备有冷凝器和Dean-Stark分水器的250ml三颈圆底烧瓶中充入中间体-4a(20.0g)、2-丁酮(29.8ml)和在THF中的无水硫酸钠(150mg)。混合物在回流温度下搅拌2h。通过TLC(乙酸乙酯)确认反应的完成。将反应冷却至室温并减压蒸发溶剂以得到白色固体,中间体-4b(32.0g,84.2%)。

[0352] 向配备有磁力搅拌器的500ml三颈圆底烧瓶中充入在MeOH(350ml)中的中间体-4b(32.0g)。将所得混合物冷却至0℃,随后加入$NaBH_4$(9.01g)。将反应在室温下搅拌5h。通过使用(己烷:乙酸乙酯(5:5))的TLC确认反应的完成。在完成后,通过蒸馏除去溶剂,并将残余物溶解在水(100ml)中。用$CHCl_3$(100ml x 3)萃取产物。将有机层合并,经Na_2SO_4干燥并减压浓缩以得到稠油。通过柱色谱法(含有10%乙酸乙酯的己烷)纯化粗产物。中间体-4(22.0g,67.6%)。

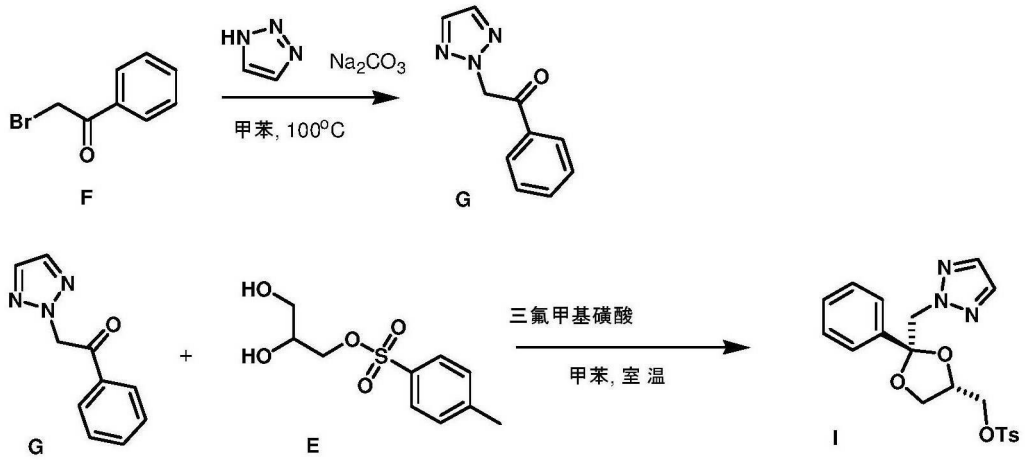
[0353] 向配备有冷凝器和氯化钙管的100ml三颈圆底烧瓶中充入在甲苯(50.0ml)中的中间体-3(5.0g)、中间体-4(1.51g)和DBU(0.022g)。所得反应混合物在100℃下搅拌15h。通过TLC(乙酸乙酯)确认反应完成。减压浓缩混合物并加入甲醇。将溶液在4℃下搅拌2h。所得沉淀物通过过滤收集并干燥以得到纯中间体-5(4.35g,85.9%)。

[0354] 向配备有冷凝器的100ml三颈圆底烧瓶中充入在甲苯(43.5ml)中的中间体-5(4.35g)。将混合物在回流温度下搅拌过夜。通过TLC(乙酸乙酯)确认反应的完成。将反应混合物冷却至室温并用饱和碳酸氢钠溶液中和。所得沉淀物通过过滤收集,用水洗涤并干燥以得到纯中间体-6(3.30g,78.3%)。

中间体光谱数据：

中间体编号	表征数据(NMR/LCMS)
3	LCMS: 57.55 % @ 202 nm 和 100 % @ 259 nm; m/z: 404.95 (M+H).
4	^{1}HNMR (400MHz, $CDCl_3$): 0.81-0.86 (m, 6H), 0.89-0.93 (m, 6H), 1.12-1.22 (m, 2H), 1.37-1.46 (m, 2H), 2.61-2.66(m, 1H), 2.73-2.74 (d, *J*=4.0 Hz, 1H), 4.79-4.86 (d, *J*=28.0 Hz, 2H), 7.92-8.01 (m, 2H), 8.73-8.76 (d, *J*=12.0 Hz, 1H), 9.31-9.32 (d, *J*=4.0 Hz,1H).
5	LCMS: 77.46 % @ 260 nm; m/z: 409.2 (M+H).
6	LCMS: 72.11 % @ 202 nm 和 99.12 @ 250 nm; m/z: 394.6 (M+H).

流程 F：中间体 -I 的合成：



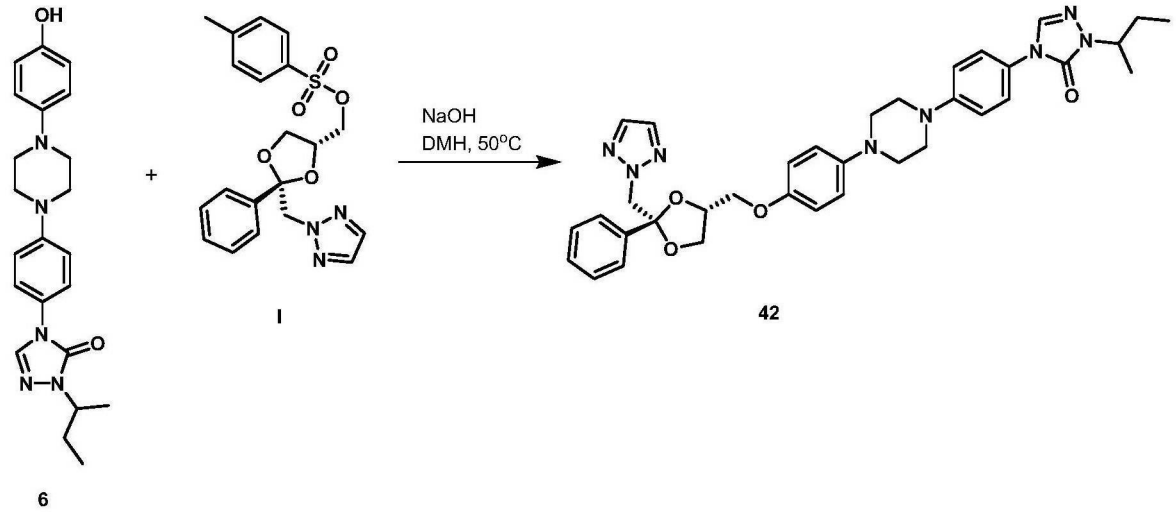
[0355] 在室温下向搅拌的中间体 -F(2.0g) 在甲苯 (15.0ml) 中的溶液中充入 1H-1,2,3 三唑 (1.78g) 和 Na_2CO_3(2.74g)。将反应混合物在 100℃下搅拌 3h。通过 TLC(己烷：乙酸乙酯 (5:5)) 监测反应的完成。完成后，将混合物冷却至室温并用乙酸乙酯 (25ml) 稀释。用水 (25ml*2) 洗涤溶液。将有机层分离，经 Na_2SO_4干燥并减压浓缩以得到粗物质。通过柱色谱法 (含有 15％乙酸乙酯的己烷) 纯化粗产物。中间体 -G(0.65g,26.7％)。

[0356] 在室温、氩气氛下向搅拌的中间体 -G(0.25g) 在甲苯 (3.5ml) 中的溶液中加入中间体 -E(0.39g)。将混合物冷却至 0℃并向其中逐滴加入三氟甲磺酸 (0.48ml)。然后使溶液升温至室温并搅拌 60h。在 TLC(己烷：乙酸乙酯 (5:5)) 上监测反应的完成。完成后，将所得混合物倒入水 (10ml) 中并用饱和碳酸氢钠溶液中和。用乙酸乙酯 (10ml*2) 萃取水层。有机层经 Na_2SO_4干燥并减压浓缩以得到稠油。通过柱色谱法 (含有 6％乙酸乙酯的己烷) 纯化粗产物。中间体 -I(0.12g,21.8％)。

中间体光谱数据：

中间体	表征数据(NMR/LCMS)
G	LCMS: 100 % @ 245 nm; m/z 182.82 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 5.95 (s, 2H), 7.52-7.56 (m, 2H), 7.65-7.69 (m, 2H), 7.76 (s, 2H), 7.98-8.00(m, 2H)
I	LCMS: 100 % @ 223 nm; m/z 416.2 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 2.19 (s, 2H), 2.47 (s, 2H), 3.58-3.61 (m, 1H), 2.72-2.76 (m, 1H), 3.83-3.87(m, 1H), 3.93-3.97 (m, 1H), 4.09-4.12 (m , 1H), 4.74(s, 2H), 7.28-7.35 (m, 5H), 7.39-7.41(m, 2H),7.62 (s, 2H), 7.66-7.68(d, *J*=8.4 Hz, 2H).

流程 G :化合物 42 的合成 :



6 I 42

[0357] 在室温、氩气氛下向搅拌的中间体 -6(0.060g) 在 DMF(1.0ml) 中的溶液中充入中间体 -I(0.070g) 和 NaOH(0.024g)。将所得混合物在 50℃下搅拌过夜。在 TLC(己烷 : 乙酸乙酯 (5:5)) 上监测反应的完成。完成后,将反应液倒入水 (10ml) 中。用乙酸乙酯 (10ml*2) 萃取水层。有机层经 Na_2SO_4 干燥并减压浓缩以得到粗物质。通过制备型 HPLC(通过反相制备型纯化,含有 0.1% NH_3 的 ACN: 水) 纯化粗产物。将产物级分冻干以获得纯产物,42(0.016g, 16%)。

中间体光谱数据 :

最终化合物编号	表征数据(NMR/LCMS)
42	LCMS: 100 % @ 261 nm; m/z 637.45 (M+H). ^{1}HNMR (400MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.42 (d, *J*=6.8 Hz, 3H) ,1.72-1.77 (m, 1H), 1.86-1.89 (m, 1H), 3.30-3.43 (m, 9H), 3.73-3.78 (m, 1H), 3.83-3.86(m, 1H), 3.89-3.93 (m, 1H), 4.30-4.33 (m , 1H), 4.37-4.40(m, 1H), 4.87(s, 2H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 7.02-7.07(m, 4H), 7.38-7.42 (m, 3H), 7.44-7.47(d, *J*=8.8 Hz, 2H), 7.56-7.59(m, 2H), 7.64(d, *J*=2.8 Hz,3H)

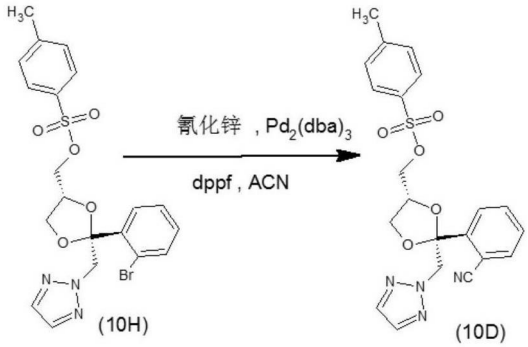
额外的合成信息：

[0358] 化合物 30-45 使用相同的反应流程用其相应的苯酰基溴化物 / 氯化物（中间体 -F）制备。

部分 C：化合物 74 的合成

[0359] 化合物 74 使用与用于制备化合物 46 的程序类似的程序，用中间体 -10D 代替中间体 -15（流程 D）来制备。中间体 -10D 的前体中间体 -10H，使用与用来制备中间体 -15 的程序类似的程序来制备。

流程 H：中间体 -10D 的合成：



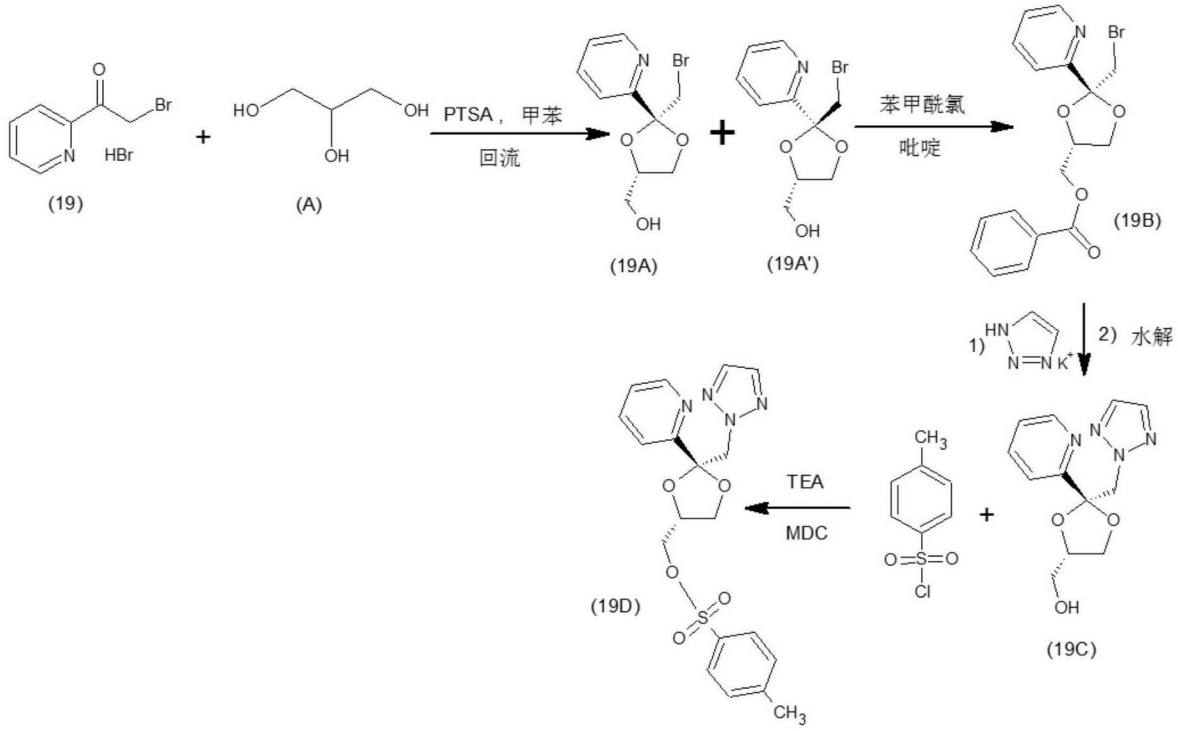
[0360] 在室温、氩气氛下向搅拌的中间体 -10H(0.1g) 在 ACN(7.0ml) 中的溶液中充入 $Zn(CN)_2$(0.47g)、dppf(0.017g) 和 $Pd_2(dba)_3$(0.027g)。所得混合物用氩气吹扫 10min 并在 120℃下搅拌 15h。通过 TLC（己烷：乙酸乙酯 (5:5)）监测反应的完成。反应完成后，将反应混合物倒入水 (10ml) 中。用乙酸乙酯 (10ml x 2) 萃取水层。将有机层合并，经 Na_2SO_4 干燥并减压浓缩以得到粗物质，该粗物质通过柱色谱法（含有 25％乙酸乙酯的己烷）纯化。中间体 -10D(0.013g，13.4％）。

中间体 化合物编号	表征数据(NMR/LCMS)
10D	LCMS: 95.23 % @ 225 nm; m/z 440.9 (M+H).

部分 D :化合物 75 的合成

[0361] 化合物 75 使用与用于制备化合物 42 的程序类似的程序,用中间体 -19D 代替中间体 -I(流程 G) 来制备。中间体 -19D 如流程 I(以下) 所示制备,其将流程 A 和 C 中的步骤颠倒,使得溴甲基酮 19 在与 1H-1,2,3- 三唑反应之前与中间体 A 反应。

流程 I :中间体 -19D 的合成



[0362] 在室温下向搅拌的中间体 -19(0.75g - 与用于化合物 88 的溴酮前体类似地制备) 在甲苯 (6.0ml) 中的溶液中加入中间体 -A(1.53g)、PTSA(0.076g) 和分子筛 (0.5g)。将反应混合物在回流温度下搅拌 24h。使用己烷 : 乙酸乙酯 (5:5) 作为流动相,通过 TLC 监测反应的完成。反应完成后,将所得混合物冷却至室温并倒入水 (10ml) 中并用饱和碳酸氢钠溶液中和。用乙酸乙酯 (10ml x 2) 萃取水层。将有机层合并,经 Na_2SO_4 干燥并在真空下蒸发以得到中间体 19A 和 19A' 的粗混合物,其不经过进一步的纯化而在下一步中使用。中间体 -19A 和中间体 -19A' 的混合物的分子量 :1.0g(粗品)。LCMS:33.84% ;m/z:276(M+2)。

[0363] 在室温、氩气氛下向搅拌的中间体 -19A 和 -19A'(1.0g) 在 MDC(10.0ml) 中的溶液中加入吡啶 (0.58ml)。向所得混合物中逐滴加入苯甲酰氯 (0.43ml),之后将混合物在室温下搅拌过夜。使用己烷 : 乙酸乙酯 (5:5) 作为流动相通过 TLC 监测反应的完成。反应完成后,将所得混合物倒入水 (10ml) 中。用 MDC(10ml x 2) 萃取水层。将有机层合并,经 Na_2SO_4 干燥并在真空下蒸发以得到稠油,将其通过制备型 HPLC(含有 0.1% 甲酸的 ACN: 水) 进一步纯化。将产物级分冻干以获得中间体 -19B。中间体 -19B 的分子量 :0.20g(产率 :14.5%)。^1H NMR(400MHz, $CDCl_3$ δ ppm) :3.89-3.97(m, 2H), 4.15-4.22(m, 2H), 4.54-4.61(m, 3H), 7.29

-7.33(m,1H),7.46-7.50(m,2H),7.58-7.62(m,1H),7.65-7.67(m,1H),7.75-7.79(m,1H),8.09-8.11(m,2H),8.69-8.70(d,1H)。LCMS:100%纯度;m/z:380(M+2)。

[0364] 在室温、氩气氛下向搅拌的中间体-19B(0.20g)在DMF(3.0ml)中的溶液中加入三唑钾盐(0.14g)。将所得混合物在搅拌下于130℃加热24h。使用己烷:乙酸乙酯(5:5)作为流动相通过TLC监测反应的完成。反应完成后,将所得混合物冷却至室温并在真空下除去DMF。将残余物溶解在THF(4.0ml)中,之后加入32%NaOH水溶液(4.0ml)。将所得混合物在回流温度下搅拌3h。使用MDC:MeOH(9:1)作为流动相通过TLC监测水解的完成。反应完成后,将所得混合物冷却至室温并倒入水(10ml)中。用乙酸乙酯(10ml x 2)萃取水层。将有机层合并,经Na_2SO_4干燥并在真空下蒸发以得到稠油,将其通过柱色谱法(含有60%乙酸乙酯的己烷)进一步纯化,以得到中间体-19C。中间体-19C的分子量:0.045g(产率:32.6%)。^{1}H-NMR(400MHz,$CDCl_3$ δ ppm):2.19(s,1H),3.33-3.37(d,J = 3.2Hz,1H),3.84-3.94(m,2H),4.05-4.08(m,1H),4.32(s,1H),4.99-5.03(d,J = 14.0Hz,1H),5.25-5.29(d,J = 14.0Hz,1H),7.35-7.36(m,1H),7.57-7.59(d,J = 9.2Hz,1H),7.66(s,2H),7.75-7.78(m,1H),8.76(s,1H)。LCMS:根据UV测得纯度为99.27%;m/z:263(M+1)。

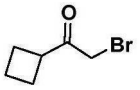
[0365] 在室温、氩气氛下向搅拌的中间体-19C(0.045g)在二氯甲烷(1.5ml)中的溶液中充入三乙胺(0.04ml)。将所得混合物冷却至0℃并向其中加入p-TSCl(0.04g),使混合物升温至室温并在室温下搅拌3h。使用己烷:乙酸乙酯(5:5)作为流动相在TLC上监测反应的完成。反应完成后,将所得混合物倒入水(10ml)中并用饱和碳酸氢钠溶液中和。用乙酸乙酯(10ml x 2)萃取水层。将有机层合并,经Na_2SO_4干燥并在真空下蒸发以得到作为稠油的中间体-19D。中间体-19D的分子量:0.075g(粗品)。^{1}H NMR(400MHz,$CDCl_3$ δ ppm)2.49(s,3H),3.22-3.28(m,1H),3.69-3.73(m,1H),3.85-3.89(m,1H),3.98-4.01(m,1H),4.42-4.43(d,J = 4.4Hz,1H),5.01-5.12(m,2H),7.19-7.21(d,J = 8.0Hz,1H),7.39-7.44(m,2H),7.44-7.48(m,3H),7.78-7.83(m,3H),7.94-7.96(d,J = 8.4Hz,1H),8.84-8.85(d,J = 4.0Hz,1H)。LCMS:根据UV测得纯度为88.74%;m/z:417(M+1)。

[0366] 化合物78与化合物75类似地制备,不同之处在于使用2-乙酰基噻唑代替2-乙酰基吡啶作为初始起始材料。溴化可以与化合物88的溴酮前体的合成类似地进行。

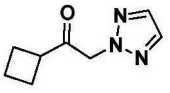
部分E:化合物88的合成

[0367] 化合物88使用与用于制备化合物42的程序类似的程序,用环丁基-(N2)三唑基酮代替中间体-14(流程C)来制备。

环丁基-(N2)三唑基酮的合成



[0368] 在0℃下向冷却的环丁基-甲基酮(1当量)在MeOH中的溶液中逐滴加入溴(1当量)30min。所得反应混合物在相同温度下搅拌约2h,然后在室温下再搅拌30min。然后用10%$Na_2S_2O_3$水溶液小心猝灭未反应的溴。然后将混合物用醚萃取,用$NaHCO_3$和盐水洗涤。合并的有机萃取物经无水$MgSO_4$干燥,过滤,并浓缩,以得到所需的溴酮。



[0369] 向溴酮(1.0当量)和三唑(1.2当量)在CAN中的溶液中加入DIPEA(1.2当量)。

然后将所得反应混合物加热至回流 1h。将反应混合物浓缩并通过快速柱色谱法纯化，以得到呈淡黄色油的所需的环丁基 - 三唑基酮，其随时间缓慢固化。

[0370] 在表 1 中出现的其他化合物使用与前述程序类似的程序制备。

生物学实施例

实施例 I :生物学试验

[0371] 使用初级人肺成纤维细胞和初级啮齿动物 HSC 建立了基于与成纤维细胞向肌成纤维细胞转分化相关的 α -SMA 染色和细胞形态学变化的高含量成像试验。确定了在适用于高通量小分子筛选的小型化 (384 孔板) 形式中促进稳健的体外转分化的条件 (包括血清饥饿和后续 TGF-β 处理)。使用选择性 ALK-5TGF-β 1 受体抑制剂 (SB-431542) 作为阳性对照。为期 4 天的试验适用于对数量级为 100,000 的孔进行筛选，并且有利于鉴定选择性抑制成纤维细胞向肌成纤维细胞转分化的化合物。该试验用于鉴定命中化合物 (伊曲康唑) 并用于评价从药物化学工作产生的集中的各组伊曲康唑类似物。

[0372] 通过使用生化方法 (即，Western 印迹和 RT-PCR) 分析与成纤维细胞向肌成纤维细胞转分化相关的多个基因的基因表达的变化，证实了伊曲康唑及其类似物的活性。使用这些为期 4 天的试验以 4 点剂量响应的方式一次评价 6 种化合物的组。

[0373] α -SMA 试验

[0374] 将静息大鼠肝星形细胞接种在用聚 -D- 赖氨酸包被的 384 孔板 (每个孔 350 个细胞) 中的星形细胞培养基 (Sciencell) 中。24 小时温育后，在肝星形细胞培养基中存在 10ng/mL 的 TGF-β 1 的情况下，用指定剂量的伊曲康唑处理细胞 48 小时。在固定并用抗平滑肌肌动蛋白抗体染色后，用 Cellomics 细胞透视成像读取仪分析细胞形态学 (图 1 为代表性图像)。然后分析细胞的 a-SMA 染色的平均细胞面积 (图 5a) 或 SMA 染色的平均荧光强度 (图 5b)。在该试验中，伊曲康唑可重现地诱导了平均细胞面积和 α 平滑肌肌动蛋白染色两者的剂量依赖性减少，表明对肌成纤维细胞转分化和活化的抑制。

[0375] 暴露于 TGFβ 和伊曲康唑 (Itra) 的细胞中纤维化相关蛋白质的 Western 印迹分析

[0376] 将人肺成纤维细胞以 10^5 个细胞 / 孔接种于 6 孔皿中的测定培养基 (2％胎牛血清，DMEM) 中。24 小时温育后，将培养基更换为含有 TGF-β 1(10ng/mL) 的测定培养基，并同时用伊曲康唑 (10μM)、SB431542(10μM) 或载体对照处理 (图 6)。温育 48 小时后，通过短暂的胰蛋白酶化和离心收获细胞。将细胞在 Cell Lytic M(Sigma) 中裂解，并且通过在 260nm 处的吸光度读数对裂解物浓度进行归一化。将样品在 2X 样品缓冲液和 10％ β - 巯基乙醇中煮沸。将三微克裂解物加载到每个凝胶泳道中，然后通过 SDS-PAGE 在 10％ Bis-Tris 凝胶上分离，然后通过半干式转印仪转移到 PVDF 膜上。在含 Tween-20(0.1％) 的 TRIS 缓冲盐水中的 5％乳中封闭后，将膜暴露于合适的第一抗体。将印迹与 HRP 偶联的第二抗体温育，并采用薄膜和 SuperSignal West Dura 化学发光底物 (Pierce) 使其可视化。在该试验中，伊曲康唑可再现地诱导了诱导的 α 平滑肌肌动蛋白水平的剂量依赖性水平降低，表明对肌成纤维细胞转分化和活化的抑制。

[0377] 用伊曲康唑处理的人肺成纤维细胞的基因表达分析

[0378] 将人肺成纤维细胞以 10^5 个细胞 / 孔接种于 6 孔皿中的测定培养基 (在 DMEM 中的 2％胎牛血清) 中。24 小时后，将培养基更换为含有 TGF-β 1(10ng/mL) 和伊曲康唑

(500nM) 的测定培养基。37℃温育 48 小时后,通过短暂的胰蛋白酶化和离心收获细胞。使用 RNeasy 试剂盒 (Qiagen) 提取 RNA,并使用 SuperScript III 第一链合成试剂盒 (Life Technologies) 扩增 cDNA。然后使用提供有板和试剂的集中于纤维化的 RT2Profiler PCR 阵列试剂盒进行 qPCR 反应。每个处理条件每个反应使用 N = 1 分析数据。数据表示为相对于未用 TGF-β1 处理的样品的调节倍数 (图 7a)。来自集中于纤维化的 RT2Profiler PCR 阵列的原始数据在图 7b 中示出。在该试验中,伊曲康唑可再现地诱导了多个纤维化相关基因的剂量依赖性变化,表明对肌成纤维细胞转分化和活化的抑制。

[0379] 伊曲康唑及其类似物的抗纤维化活性不是由与抗真菌活性相关的 P450 抑制造成的。伊曲康唑抑制 VEGF 和 Hedgehog 促纤维化信号途径。

[0380] 用伊曲康唑处理的大鼠肝星形细胞中 Hedgehog 相关基因的 qPCR 分析

[0381] 将大鼠肝星形细胞以 10^5个细胞 / 孔接种在用聚 -D- 赖氨酸包被的 6 孔皿中的星形细胞培养基 (Sciencell) 中,并使其增殖至完全汇合 (约 2 周)。然后将培养基更换为含 1μg/mL SHH-N(R&D systems) 的 DMEM 和 0.5%胎牛血清。用 SHH-N 和化合物 (环杷明 5μM(阳性对照)、伊曲康唑 1μM) 处理细胞 24 小时。通过短暂的胰蛋白酶化和离心收获细胞。使用 RNeasy 试剂盒 (Qiagen) 提取 RNA,并使用 SuperScript III 第一链合成试剂盒 (Life Technologies) 扩增 cDNA。使用 SYBR 绿主混合物 (Takara) 进行 qPCR。PTCH1(蛋白质修补 (patch) 同系物 1) 和 GLI1(GLI 家族锌指 1)mRNA 的相对水平 (见图 8a 和 8b ;qPCR 实验的原始数据均在图 8c 中示出) 表明,伊曲康唑抑制大鼠肝星形细胞中的 Hedgehog 信号传导。

[0382] Smoothened 敲减后 COL1-GFP HSC 的 Western 印迹分析

[0383] 将 COL1-GFP HSC(GFP 敲入胶原蛋白基因座的无限增殖化小鼠肝星形细胞系) 以 7.5^5个细胞 / 孔接种到测定培养基 (10%胎牛血清,DMEM) 中。24 小时后,用慢病毒颗粒转导细胞。与病毒颗粒温育 24 小时后,将细胞切换至新鲜的测定培养基中并温育 48 小时。将细胞在 Cell Lytic M(Sigma) 中裂解,并且通过在 260nm 处的吸光度读数对裂解物浓度进行归一化。将样品在 2X 样品缓冲液和 10% β- 巯基乙醇中煮沸。将等量的裂解物加载到每个凝胶泳道中,然后通过 SDS-PAGE 在 10% Bis-Tris 凝胶上分离,然后通过半干式转印仪转移到 PVDF 膜上。在含 Tween-20(0.1%) 的 TRIS 缓冲盐水中的 5%乳中封闭后,将膜暴露于合适的第一抗体。将印迹与 HRP 偶联的第二抗体温育,并采用薄膜和 SuperSignal West Dura 化学发光底物 (Pierce) 使其可视化 ;见图 9。用于慢病毒递送的 shRNA 的构建体以 MISSION 甘油储备物的形式从 Sigma 获得。采用 293T 细胞和包装载体 pMD2.G 和 pSPAX2 包装慢病毒。克隆 71、12 和 95 对应于针对 SMO 的 shRNA,pLKO 是编码非靶向 shRNA 的质粒 SCH002。shRNA 介导的 SMO 敲减和所导致的大鼠肝星形细胞中 Hedgehog 信号传导的抑制部分地说明 (recapitulated) 了伊曲康唑的抗纤维化活性。

[0384] 用伊曲康唑处理后 VEGFR2 迁移模式的 Western 印迹分析

[0385] 将静息大鼠肝星形细胞以 10^5个细胞 / 孔接种在用聚 -D- 赖氨酸包被的 6 孔皿中的星形细胞培养基 (Sciencell) 中。温育 24 小时后,用不同剂量的伊曲康唑处理细胞 24 小时。通过短暂的胰蛋白酶化和离心收获细胞。将细胞在 Cell Lytic M(Sigma) 中裂解,并且通过在 260nm 处的吸光度读数对裂解物浓度进行归一化。将样品在 2X 样品缓冲液和 10% β- 巯基乙醇中煮沸。将等量的裂解物加载到每个凝胶泳道中,然后通过 SDS-PAGE 在 10%

Bis-Tris 凝胶上分离，然后通过半干式转印仪转移到 PVDF 膜上。在含 Tween-20(0.1％）的 TRIS 缓冲盐水中的 5％乳中封闭后，将膜暴露于合适的第一抗体。将印迹与 HRP 偶联的第二抗体温育，并采用薄膜和 SuperSignal West Dura 化学发光底物 (Pierce) 使其可视化；见图 10。伊曲康唑抑制了 VEGFR2 糖基化和运输，由此导致大鼠肝星形细胞中的促纤维化 VEGF 信号传导的抑制。

[0386] 用 VEGFR 和 Hedgehog 抑制化合物联合处理的大鼠肝星形细胞的 Western 印迹分析

[0387] 将静息大鼠肝星形细胞以 10^5个细胞 / 孔接种在用聚 -D- 赖氨酸包被的 6 孔皿中的星形细胞培养基 (Sciencell) 中。24 小时温育后，将细胞切换至含 10ng/mL TGF-β1 和指定化合物组合的星形细胞培养基。48 小时的处理后，通过短暂的胰蛋白酶化和离心收获细胞。将细胞在 Cell Lytic M(Sigma) 中裂解，并且通过在 260nm 处的吸光度读数对裂解物浓度进行归一化。将样品在 2X 样品缓冲液和 10％ β- 巯基乙醇中煮沸。将三微克裂解物加载到每个凝胶泳道中，然后通过 SDS-PAGE 在 10％ Bis-Tris 凝胶上分离，然后通过半干式转印仪转移到 PVDF 膜上。在含 Tween-20(0.1％）的 TRIS 缓冲盐水中的 5％乳中封闭后，将膜暴露于合适的第一抗体。将印迹与 HRP 偶联的第二抗体温育，并采用薄膜和 SuperSignal West Dura 化学发光底物 (Pierce) 使其可视化；见图 11。大鼠肝星形细胞中 VEGF 或 Hedgehog 信号传导的药理学抑制部分地说明了伊曲康唑或其类似物的抗纤维化活性。大鼠肝星形细胞中 VEGF 和 Hedgehog 信号传导的双重药理学抑制说明了伊曲康唑的抗纤维化活性。

[0388] 伊曲康唑和化合物 42 在 Hedgehog 报告子试验中的活性

[0389] 收获 TM3-GLI-LUC 细胞（表达 Gli-Luc 报告子的小鼠 TM3 细胞的稳定克隆），然后以 5,000 个细胞 / 孔接种到 384 孔板中的 40uL 测定培养基 (DMEM:F12 和 2％胎牛血清）中。将化合物以系列稀释度溶解在 DMSO 中并在 Biomek 自动化工作站上使用 100nL 针销头 (pintool head) 转移至测定板。化合物转移后，加入含有小分子 hedgehog 激动剂 SAG 的 10uL 测定培养基，使得 SAG 的最终测定浓度为 10nM 或 400nM。将测定板温育 48 小时，之后使用 Bright-Glo 试剂在 Envision 读板仪上确定亮度信号。暴露于 10nM SAG 和指定剂量抑制剂的 TM3-GLI-LUC 细胞的相对 GLI-LUC 活性在图 12a 中示出。暴露于 400nM SAG 和指定剂量抑制剂的 TM3-GLI-LUC 细胞的相对 GLI-LUC 活性在图 12b 中示出。每个剂量 N ＝ 3。伊曲康唑及其类似物抑制了大鼠肝星形细胞中的促纤维化 Hedgehog 信号传导。

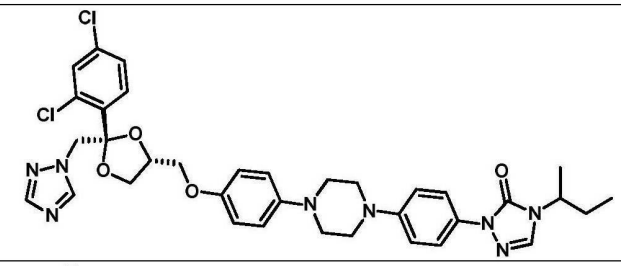
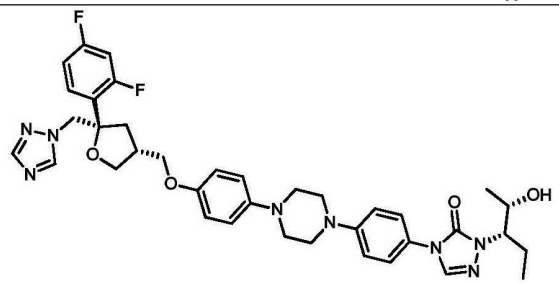
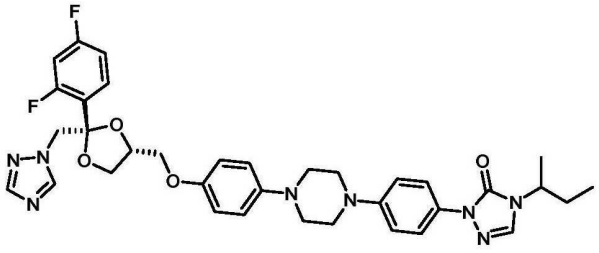
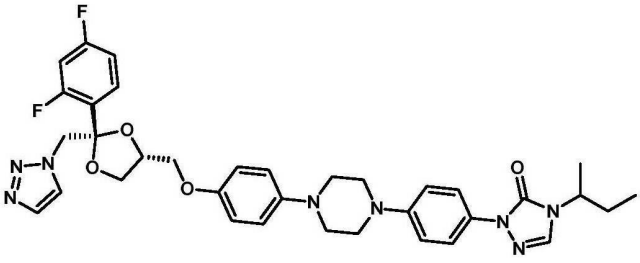
[0390] VEFGR1、VEGFR2 或 SMO 敲减后 LX2 人肝星形细胞的 Western 印迹分析

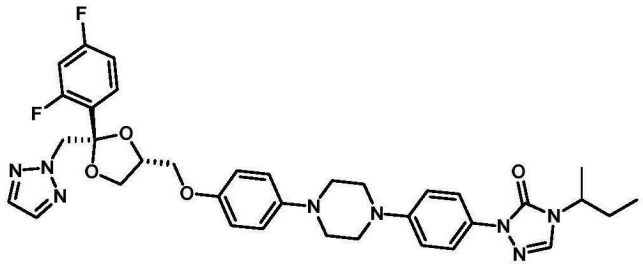
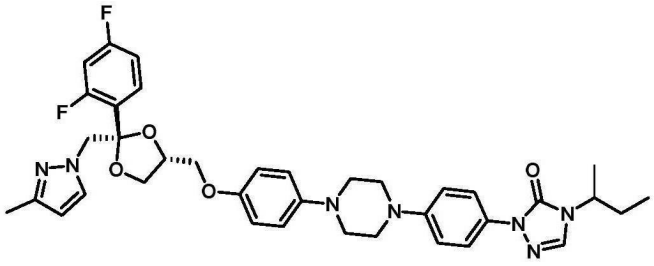
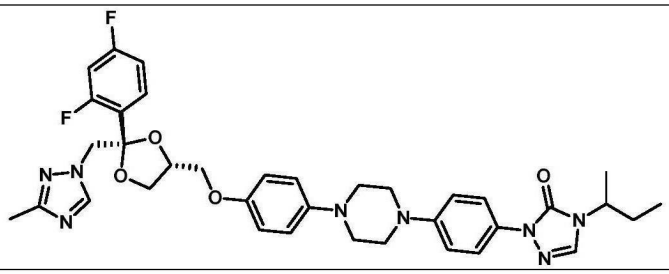
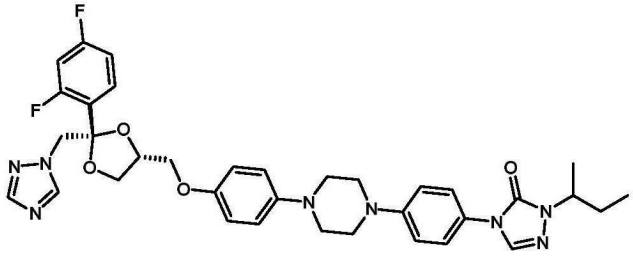
[0391] 将 LX2 细胞（无限增殖化的人肝星形细胞系，Scot Friedman 实验室）以 10^5个细胞 / 孔接种到测定培养基 (10％胎牛血清，DMEM) 中。24 小时后，用慢病毒颗粒转导细胞。与病毒颗粒温育 24 小时后，将细胞切换至新鲜的测定培养基并温育 48 小时。将细胞在 Cell Lytic M(Sigma) 中裂解，并且通过在 260nm 处的吸光度读数对裂解物浓度进行归一化。将样品在 2X 样品缓冲液和 10％ β- 巯基乙醇中煮沸。将等量的裂解物加载到每个凝胶泳道中，然后通过 SDS-PAGE 在 10％ Bis-Tris 凝胶上分离，然后通过半干式转印仪转移到 PVDF 膜上。在含 Tween-20(0.1％）的 TRIS 缓冲盐水中的 5％乳中封闭后，将膜暴露于合适的第一抗体。将印迹与 HRP 偶联的第二抗体温育，并采用薄膜和 SuperSignal West Dura 化学发光底物 (Pierce) 使其可视化；见图 13。用于慢病毒递送的 shRNA 的构建体以 MISSION

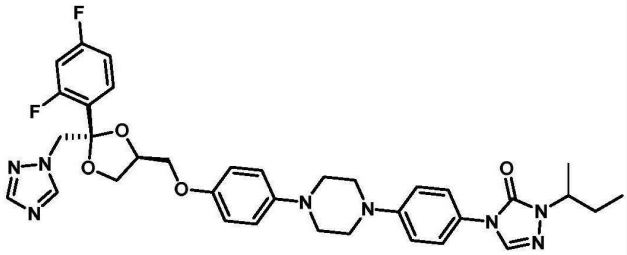
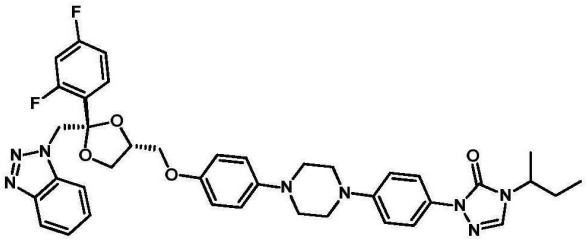
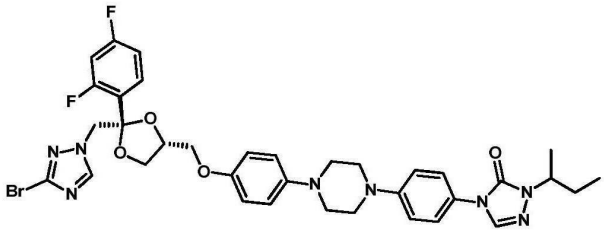
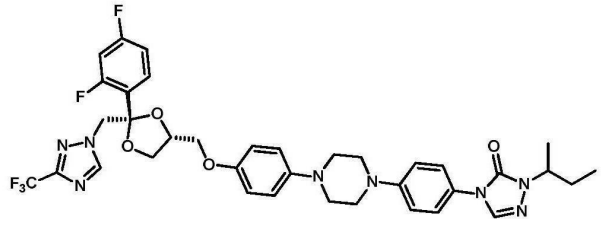
甘油储备物的形式从 Sigma 获得。采用 293T 细胞和包装载体 pMD2.G 和 pSPAX2 包装慢病毒。克隆 65 对应于靶向 SMO 的 shRNA,31 和 32 对应于靶向 VEGFR1 的 shRNA,克隆 86 和 87 对应于靶向 VEGFR2 的 shRNA,而 pLKO 是编码非靶向 shRNA 的质粒 SCH002。人星形细胞中 VEGF 和 Hedgehog 信号传导的基因敲减抑制了促纤维化的肌成纤维细胞活化,并且部分地说明了伊曲康唑及其类似物的抗纤维化活性。

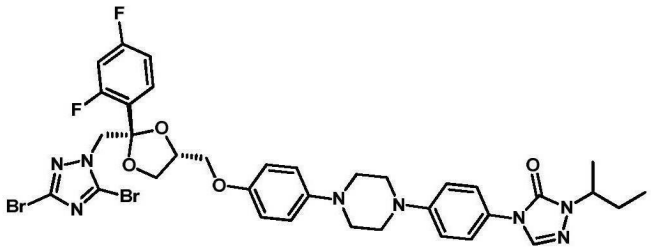
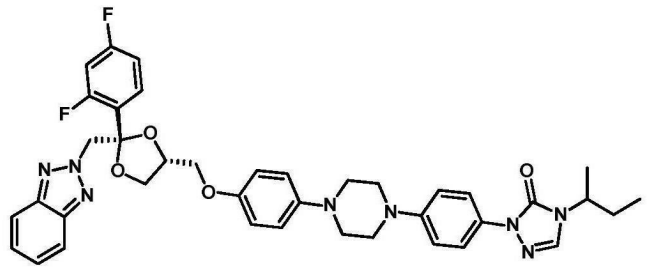
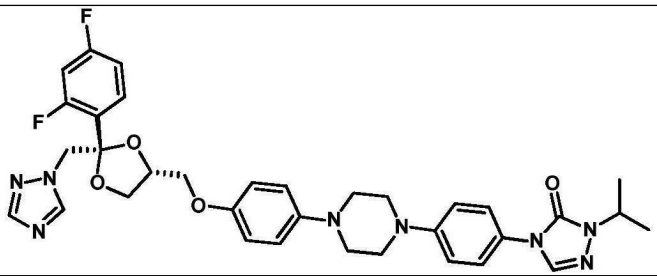
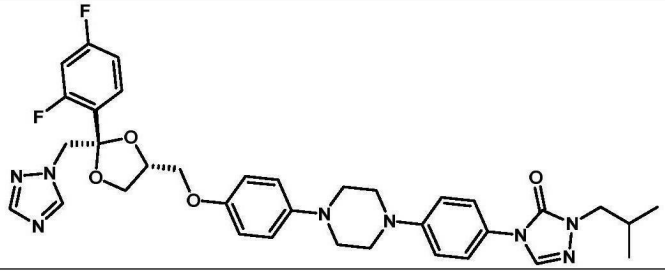
[0392] 以下表 1 示出了化合物分析数据 (NMR 和 MS) 及其生物活性。将 (基于成像和 Western 的) 活性的 EC_{50}分级为 :+++ = <500nM ;++ = 500nM 至 5μM ;+ = 5 至 30μM)。

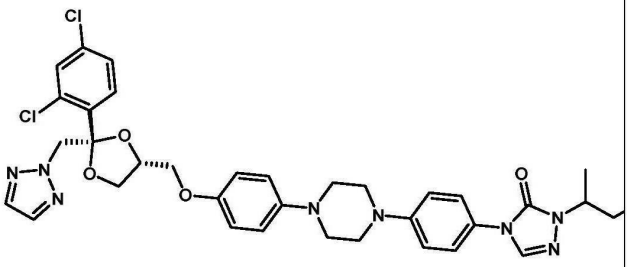
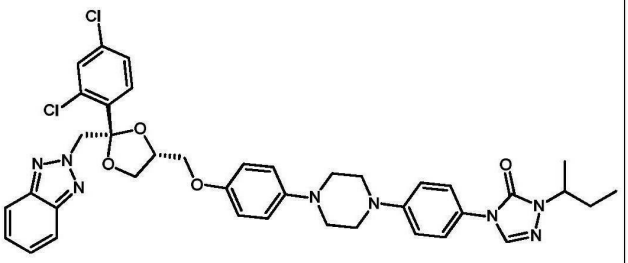
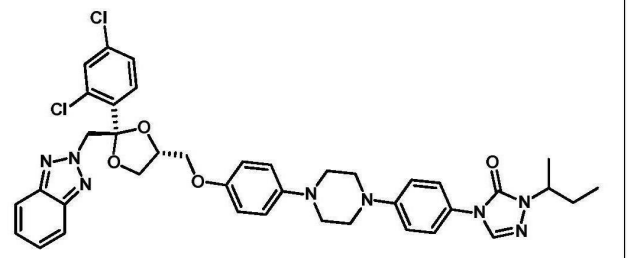
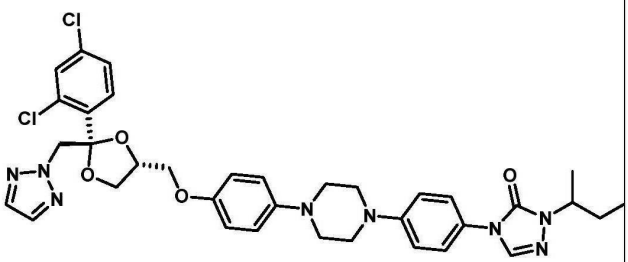
表 1

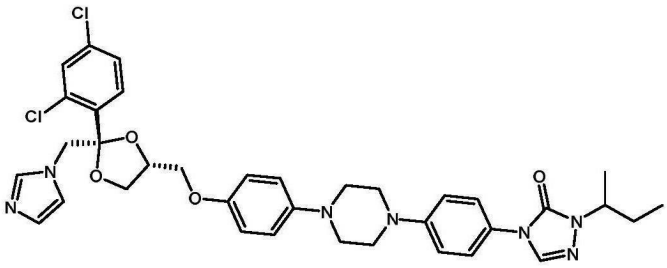
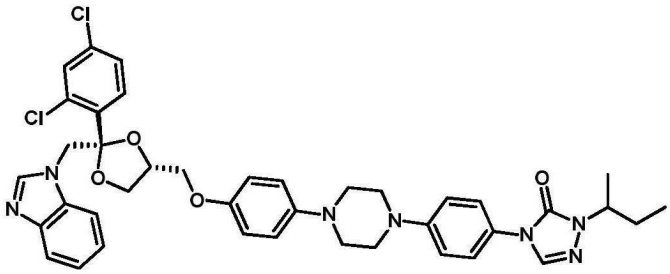
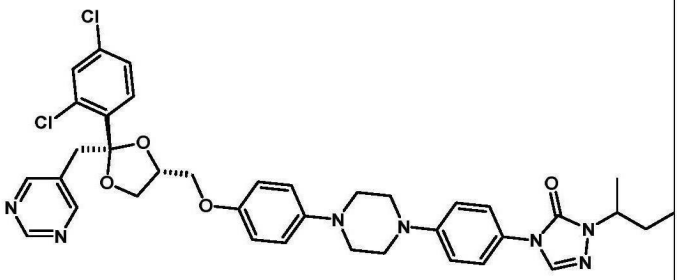
化合物编号	结构	表征数据(NMR 和 LCMS)	活性
ITZ		顺式外消旋物	+++
POS		顺式外消旋物	+
1		顺式外消旋物 LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.89 (s, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.6 Hz, 2H), 6.94 (d, J = 7.2 Hz, 2H), 6.88 (m, 2H), 6.79 (d, J = 5.2 Hz, 2H), 4.70 (dd, J = 16.4 Hz, 11.6 Hz, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.83-3.77 (m, 2H), 3.49 (dd, J = 8.0 Hz, 5.2 Hz, 1H), 3.37-3.35 (m, 4H), 3.24-3.22 (m, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	++
2		LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.73 (d, J = 0.8 Hz, 1H), 7.65 (d, J = 0.8 Hz, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.43 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 6.93 (d, J = 7.6 Hz, 2H), 6.87 (m, 2H), 6.77 (d, J = 7.2 Hz, 2H), 4.90 (s, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.96 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.81 (dd, J = 9.6 Hz, 4 Hz, 1H), 3.75 (dd, J = 7.6 Hz, 3.6 Hz, 1H), 3.38-3.35 (m, 5H), 3.24-3.22 (m, 4H), 1.85 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	++

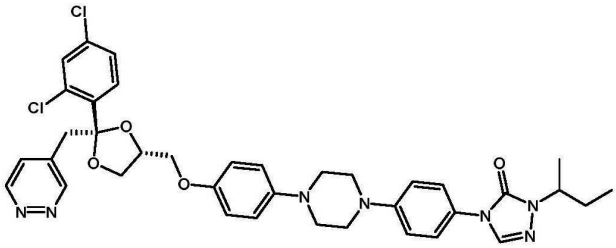
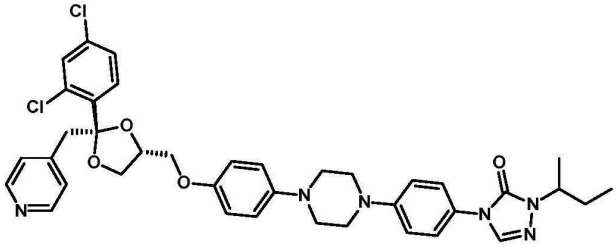
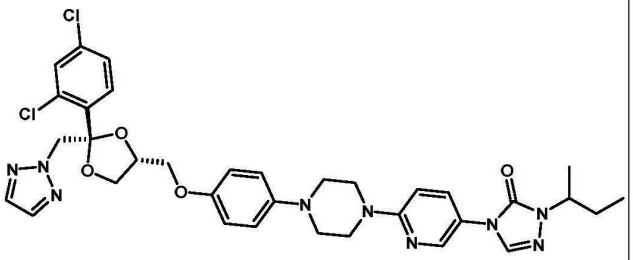
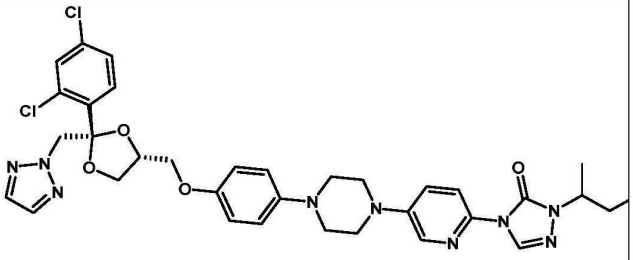
3		LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.58 (s, 1H), 7.57 (s, 1H), 7.46-7.42 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.00 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
4		LC-MS: m/z 686.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.55 (m, 1H), 7.44-7.30 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.76 (m, 2H), 6.01 (m, 1H), 4.55 (dd, J = 28.8 Hz, 12.0 Hz, 2H), 4.38 (m, 1H), 4.29 (m, 1H), 3.92 (m, 1H), 3.82-3.71 (m, 3H), 3.37 (br s, 4H), 3.25 (m, 4H), 2.25 (s,3H), 1.86 (m, 1H), 1.73 (m, 1H), 1.39 (d, J = 6.4 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
5		LC-MS: m/z 687.0 (M+H)	+
6		顺式手性 LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.21 (s, 1H), 7.89 (s, 1H), 7.62 (s, 1H), 7.49 (m, 1H), 7.42 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.6 Hz, 2H), 6.94 (d, J = 7.2 Hz, 2H), 6.88 (m, 2H), 6.79 (d, J = 5.2 Hz, 2H), 4.70 (dd, J = 16.4 Hz, 11.6 Hz, 2H), 4.41 (m, 1H), 4.29 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.83-3.77 (m, 2H), 3.49 (dd, J = 8.0 Hz, 5.2 Hz, 1H), 3.40 (br s, 4H), 3.28 (br s, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	++

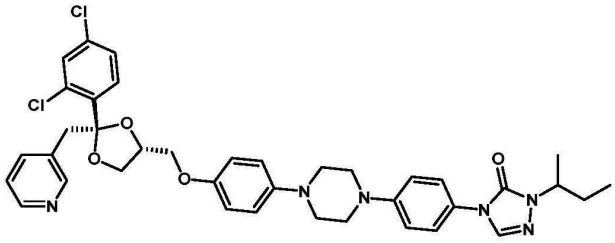
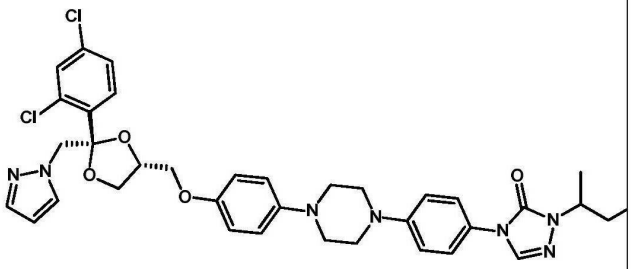
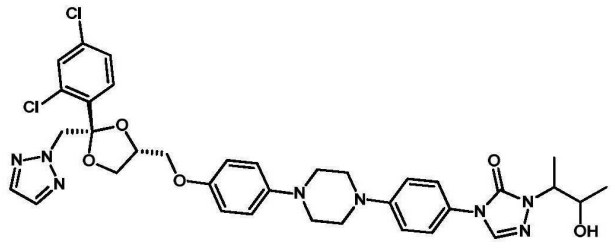
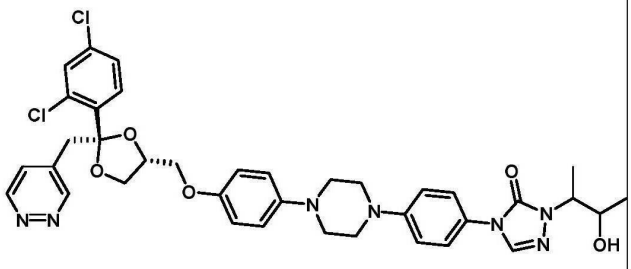
7		反式外消旋物 LC-MS: m/z 673.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.20 (s, 1H), 7.92 (s, 1H), 7.62 (s, 1H), 7.52 (m, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.04 (d, J = 5.6 Hz, 2H), 6.88-6.80 (m, 4H), 6.72 (m, 2H), 4.66 (dd, J = 16.8 Hz, 11.6 Hz, 2H), 4.29 (m, 1H), 4.18 (m, 1H), 4.01 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.93 (dd, J= 7.2 Hz, 3.6 Hz, 1H), 3.85 (m, 2H), 3.50-3.11 (m, 8H), 1.86 (m, 1H), 1.72 (m, 1H), 1.38 (d, J = 5.6 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
8		LC-MS: m/z 723.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.02 (d, J = 8.0 Hz, 1H), 7.74 (d, J = 6.8 Hz, 1H), 7.62 (s, 1H), 7.54 (m, 1H), 7.47-7.42 (m, 3H), 7.34 (m, 1H), 7.03 (d, J = 7.2 Hz, 2H), 6.95-6.86 (m, 4H), 6.53 (d, J = 7.2 Hz, 2H), 5.15 (dd, J = 20.4 Hz, 12.0 Hz, 2H), 4.35 (m, 1H), 4.29 (m, 1H), 3.88 (dd, J = 6.8 Hz, 5.2 Hz, 1H), 3.62 (dd, J = 6.8 Hz, 3.6 Hz, 1H), 3.41-3.37 (m, 5H), 3.22 (br s, 4H), 2.88 (m,1H), 1.86 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	++
9		LC-MS: m/z 750.9 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.12 (s, 1H), 7.62 (s, 1H), 7.51 (m, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.03 (d, J = 7.2 Hz, 2H), 6.95-6.88 (m, 4H), 6.84 (m, 2H), 4.65 (dd, J = 15.6 Hz, 12.0 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.98 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.86 (dd, J = 6.8 Hz, 4.0 Hz, 1H), 3.82 (dd, J = 7.6 Hz, 3.6 Hz, 1H), 3.52 (dd, J = 7.6 Hz, 4.8 Hz, 1H), 3.37 (br s, 4H), 3.25 (br s, 4H), 1.86 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 5.6 Hz, 3H).	+
10		LC-MS: m/z 741.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.33 (s, 1H), 7.63 (s, 1H), 7.53 (m, 1H), 7.44 (d, J = 6.4 Hz, 2H), 7.04 (d, J = 6.4 Hz, 2H), 6.93-6.89 (m, 4H), 6.82 (br s, 2H), 4.74 (dd, J = 14.0 Hz, 11.6 Hz, 2H), 4.43 (m, 1H), 4.30 (m, 1H), 3.99 (dd, J = 6.8 Hz, 5.6 Hz, 1H), 3.83 (dd, J =	+

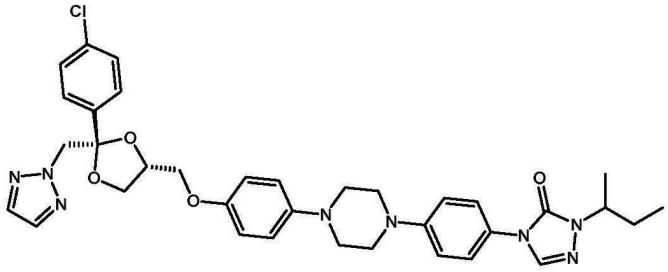
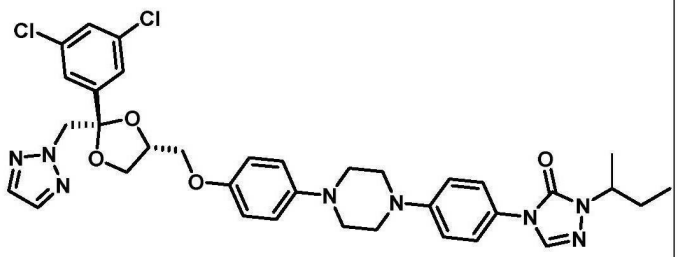
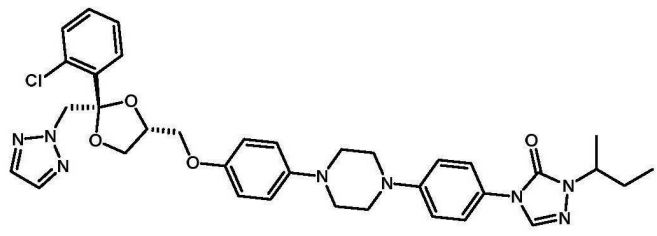
		6.8 Hz, 4.4Hz, 1H), 3.79 (m, 1H), 3.51 (m, 1H), 3.38 (br s, 4H), 3.25 (br s, 4H), 1.85 (m, 1H), 1.73 (m, 1H), 1.39 (d, J = 5.6 Hz, 3H), 0.91 (t, J = 5.6 Hz, 3H).	
11		LC-MS: m/z 829.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.62 (s, 1H), 7.54 (m, 1H), 7.42 (d, J = 6.8 Hz, 2H), 7.03 (d, J = 6.8 Hz, 2H), 6.96-6.88 (m, 4H), 6.83 (m, 2H), 4.67 (dd, J = 24.0 Hz, 11.6 Hz, 2H), 4.43 (m, 1H), 4.29 (m, 1H), 3.97 (m, 1H), 3.90 (dd, J = 7.2 Hz, 4.0Hz, 1H), 3.85 (dd, J = 7.6 Hz, 4.0 Hz, 1H), 3.50 (m, 1H), 3.37 (br s, 4H), 3.26 (br s, 4H), 1.87 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	+
12		LC-MS: m/z 723.0 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 7.88-7.87 (m, 2H), 7.62 (s, 1H), 7.54 (m, 1H), 7.43 (m, 4H), 7.04 (d, J = 7.2 Hz, 2H), 6.94-6.83 (m, 4H), 6.41 (d, J = 7.2 Hz, 2H), 5.30 (dd, J = 37.6 Hz, 9.6 Hz, 2H), 4.42 (m, 1H), 4.27 (m, 1H), 3.94 (dd, J = 7.2 Hz, 4.8 Hz, 1H), 3.89 (dd, J = 6.8 Hz, 3.2 Hz, 1H), 3.53 (br s, 1H), 3.36 (br s, 4H), 3.22 (br s, 4H), 2.99 (m,1H), 1.85 (m, 1H), 1.72 (m, 1H), 1.39 (d, J = 5.2 Hz, 3H), 0.91 (t, J = 6.0 Hz, 3H).	+
13		LC-MS: m/z 659.0 (M+H)	+
14		LC-MS: m/z 673.0 (M+H)	+

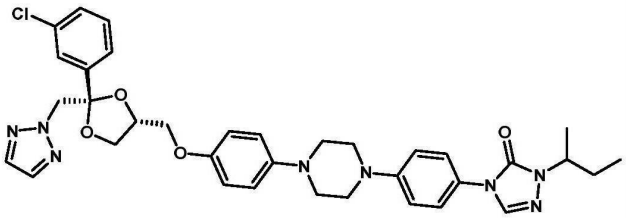
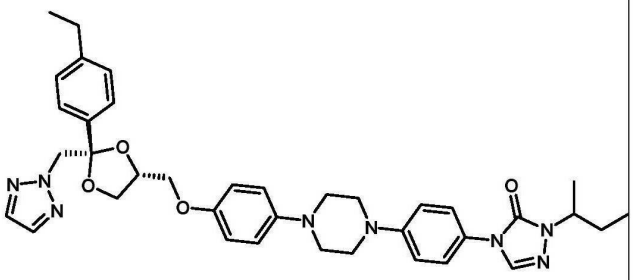
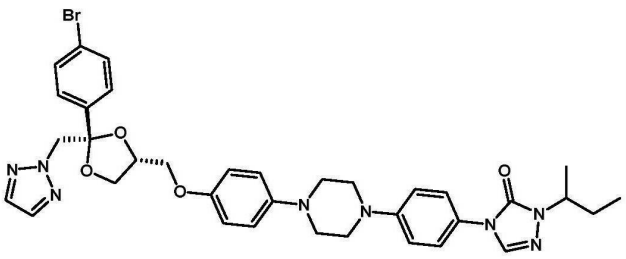
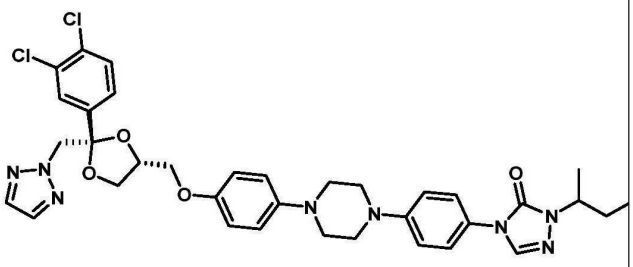
15		LC-MS: m/z 705.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 0.7 Hz, 1H), 7.61 (s, 2H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.51 – 7.40 (m, 3H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.09 – 7.03 (m, 2H), 6.98 – 6.91 (m, 2H), 6.84 – 6.77 (m, 2H), 5.25 – 5.03 (m, 3H), 4.46 – 4.36 (m, 1H), 4.36 – 4.27 (m, 1H), 3.98 – 3.84 (m, 3H), 3.48 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.39 (m, 4H), 3.26 (m, 4H), 1.89 (m, 2H), 1.74 (m, 1H), 1.42 (d, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	++
16		LC-MS: m/z 755.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.12 (d, *J* = 0.6 Hz, 1H), 7.86 – 7.77 (m, 2H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.60 (d, *J* = 2.1 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.48 – 7.36 (m, 4H), 7.33 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.27 – 7.20 (m, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 5.54 – 5.29 (m, 2H), 4.56 – 4.37 (m, 1H), 4.34 – 4.18 (m, 1H), 4.04 – 3.84 (m, 2H), 3.68 (m, 8H), 3.61 – 3.44 (m, 2H), 1.88 (m, 1H), 1.76 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	+
17		反式外消旋 LC-MS: m/z 755.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 – 7.86 (m, 2H), 7.63 (dd, *J* = 4.6, 3.9 Hz, 2H), 7.49 – 7.37 (m, 5H), 7.18 (m, 1H), 7.08 – 7.01 (m, 2H), 6.92 – 6.84 (m, 2H), 6.72 – 6.61 (m, 2H), 5.42 – 5.27 (m, 3H), 4.38 – 4.22 (m, 2H), 4.01 – 3.90 (m, 2H), 3.89 – 3.76 (m, 2H), 3.36 (m, 4H), 3.22 (m, 4H), 1.90 (m, 1H), 1.74 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H).	+
18		反式外消旋 LC-MS: m/z 705.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 6.7 Hz, 3H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.49 – 7.39 (m, 3H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.08 – 6.99 (m, 2H), 6.96 – 6.84 (m, 2H), 6.77 – 6.63 (m, 2H), 5.19 – 4.96 (m, 2H), 4.32 (m, 2H), 4.03 (m, 1H), 3.95 (m, 1H), 3.86 (m, 1H), 3.75 (m, 1H), 3.30 – 3.15 (m, 4H), 1.88 (m,	+

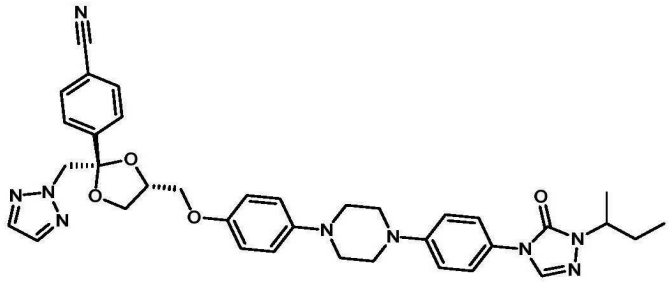
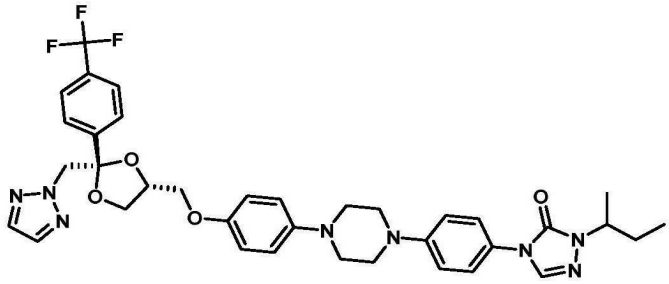
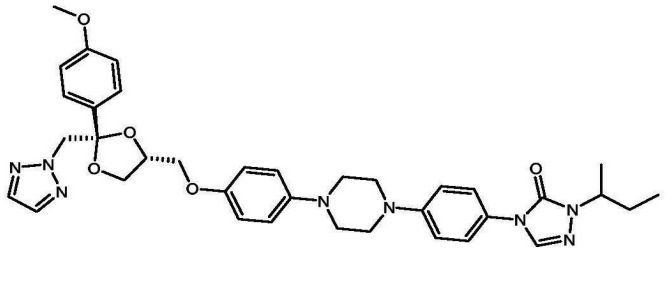
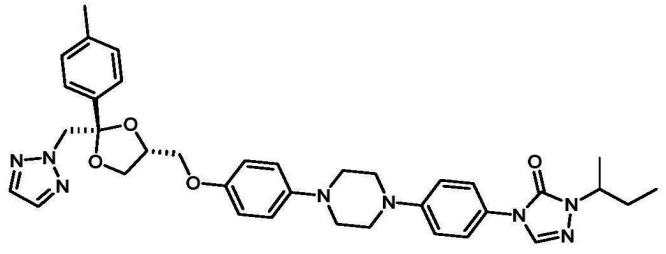
		1H), 1.81 – 1.64 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).	
19		LC-MS: m/z 704.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 0.6 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.54 (s, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.47 – 7.41 (m, 2H), 7.31 – 7.25 (m, 1H), 7.09 – 7.01 (m, 3H), 6.99 – 6.91 (m, 3H), 6.87 – 6.77 (m, 2H), 4.62 – 4.40 (m, 2H), 4.39 – 4.26 (m, 2H), 3.89 (dd, J = 8.4, 6.5 Hz, 1H), 3.82 – 3.71 (m, 2H), 3.41 – 3.34 (m, 4H), 3.31 (dd, J = 9.6, 6.8 Hz, 1H), 3.29 – 3.18 (m, 4H), 1.95 – 1.81 (m, 1H), 1.79 – 1.66 (m, 1H), 1.41 (d, J = 6.7 Hz, 3H), 0.92 (t, J = 7.4 Hz, 3H).	++
20		LC-MS: m/z 754.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 9.43 (s, 1H), 8.11 (d, *J* = 0.6 Hz, 1H), 8.08 – 7.99 (m, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.73 – 7.67 (m, 2H), 7.62 (m, 1H), 7.58 – 7.46 (m, 4H), 7.21 (m, 3H), 6.57 (d, *J* = 8.9 Hz, 2H), 5.33 – 5.05 (m, 3H), 4.51 – 4.36 (m, 2H), 4.33 – 4.17 (m, 1H), 3.89 (dd, *J* = 8.5, 7.4 Hz, 1H), 3.79 (dd, *J* = 8.5, 4.9 Hz, 1H), 3.71 (dd, *J* = 10.6, 3.1 Hz, 1H), 3.60 – 3.46 (m, 9H), 1.89 (m, 1H), 1.82 – 1.72 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	++
21		LC-MS: m/z 716.2 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.98 (s, 1H), 8.65 (s, 2H), 8.10 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.58 (d, *J* = 2.1 Hz, 1H), 7.54 – 7.46 (m, 2H), 7.36 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.20 (m, 4H), 6.87 (d, *J* = 9.0 Hz, 2H), 4.35 (m, 1H), 4.25 (m, 2H), 3.96 – 3.66 (m, 4H), 3.57 – 3.38 (m, 8H), 1.89 (m, 1H), 1.75 (m, 1H), 1.40 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	++

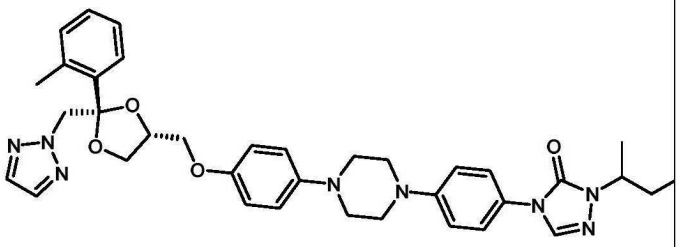
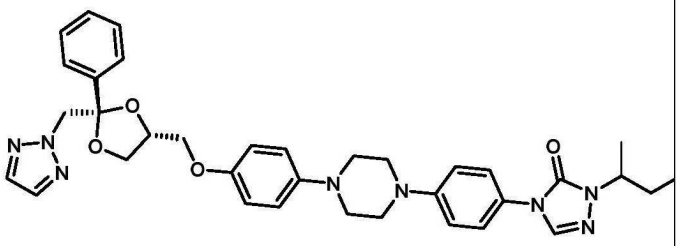
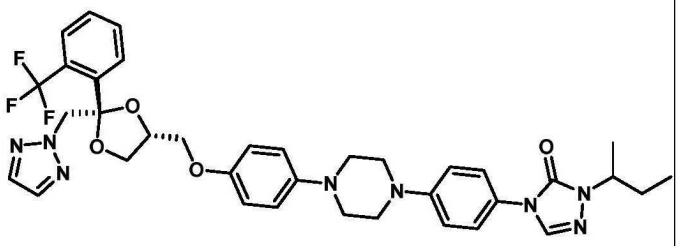
22		LC-MS: m/z 716.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (dd, *J* = 2.2, 1.2 Hz, 1H), 9.03 (dd, *J* = 5.2, 1.2 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.48 – 7.42 (m, 2H), 7.36 (dd, *J* = 5.2, 2.3 Hz, 1H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.08 – 7.04 (m, 2H), 6.99 – 6.93 (m, 2H), 6.79 – 6.73 (m, 2H), 4.36 – 4.28 (m, 2H), 3.85 (dd, *J* = 8.4, 6.8 Hz, 1H), 3.79 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.74 (dd, *J* = 9.7, 5.0 Hz, 1H), 3.56 – 3.51 (m, 1H), 3.48 (d, *J* = 13.8 Hz, 1H), 3.44 – 3.35 (m, 5H), 3.26 (dd, *J* = 6.5, 3.6 Hz, 4H), 1.87 (m, 1H), 1.80 – 1.71 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+++
23		LC-MS: m/z 715.2 (M+H) ^{1}H NMR (400 MHz, CDCl3) δ 8.51 – 8.44 (m, 2H), 7.64 (m, 1H), 7.51 – 7.42 (m, 4H), 7.24 – 7.15 (m, 3H), 7.10 – 7.03 (m, 2H), 7.00 – 6.91 (m, 2H), 6.80 – 6.71 (m, 2H), 4.39 – 4.27 (m, 2H), 3.90 – 3.79 (m, 2H), 3.59 – 3.45 (m, 3H), 3.45 – 3.33 (m, 5H), 3.32 – 3.19 (m, 4H), 1.87 (m, 1H), 1.78 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+++
24		LC-MS: m/z 706.3(M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.75 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 3H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.84 – 6.76 (m, 3H), 5.26 – 4.97 (m, 2H), 4.46 – 4.36 (m, 1H), 4.34 – 4.24 (m, 1H), 4.01 – 3.83 (m, 3H), 3.83 – 3.73 (m, 4H), 3.46 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (s, 2H), 0.94 (d, *J* = 7.4 Hz, 3H).	++
25		LC-MS: m/z 706.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 2.8, 0.7 Hz, 1H), 7.75 (dd, *J* = 9.1, 2.8 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 3H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 – 6.91 (m, 2H), 6.84 – 6.76 (m, 3H), 5.26 – 4.97 (m, 2H), 4.46 –	+

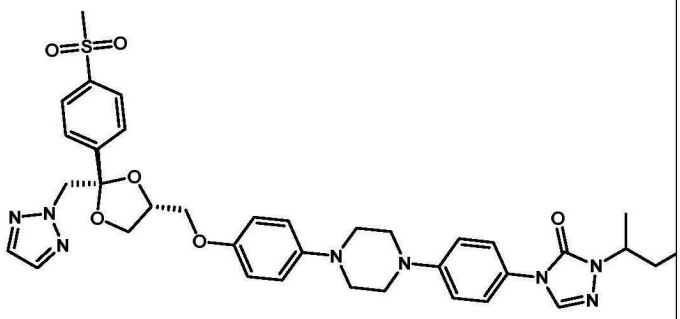
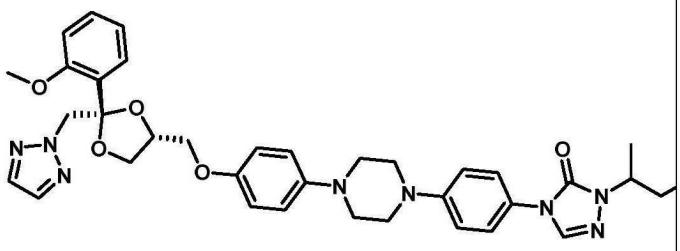
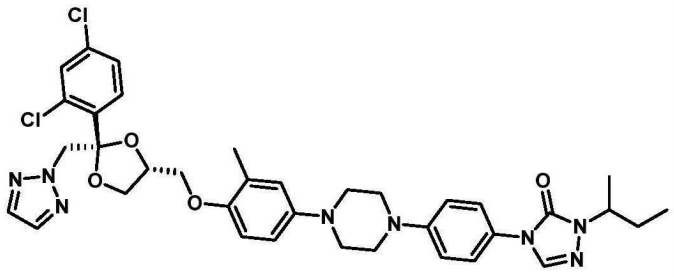
		4.36 (m, 1H), 4.34 – 4.24 (m, 1H), 4.01 – 3.83 (m, 3H), 3.83 – 3.73 (m, 4H), 3.46 (dd, *J* = 9.5, 7.1 Hz, 1H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 7.4 Hz, 3H).	
26		LC-MS: m/z 715.3 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (m, 2H), 7.64 (m, 1H), 7.55 (m, 1H), 7.46 (m, 4H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.16 (m, 1H), 7.05 (m, 2H), 6.94 (m, 2H), 6.75 (m, 2H), 4.31 (m, 2H), 3.81 (m, 2H), 3.72 (m, 1H), 3.49-3.34 (m, 7H), 3.27 – 3.14 (m, 4H), 1.89 (m, 1H), 1.80 – 1.72 (m, 1H), 1.42 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 7.4 Hz, 3H).	++
27		LC-MS: m/z 704.2 (M+H) ^{1}H NMR (400 MHz, CDCl3) δ 7.64 (d, J = 0.6 Hz, 1H), 7.60 (d, J = 8.5 Hz, 1H), 7.51 (td, J = 2.3, 0.7 Hz, 2H), 7.49 – 7.41 (m, 3H), 7.25 (dd, J = 8.4, 2.1 Hz, 1H), 7.08 – 7.02 (m, 2H), 6.99 – 6.90 (m, 2H), 6.83 – 6.74 (m, 2H), 6.24 (t, J = 2.1 Hz, 1H), 4.91 – 4.63 (m, 2H), 4.45 – 4.24 (m, 2H), 3.94 – 3.74 (m, 3H), 3.43 – 3.30 (m, 5H), 3.29 – 3.15 (m, 4H), 1.94 – 1.85 (m, 1H), 1.76 (m, 1H), 1.42 (d, J = 6.4 Hz, 3H), 0.94 (d, J = 7.4 Hz, 3H).	+
28		LC-MS: m/z 721.3 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.09 (s, 1H), 7.57 – 7.50 (m, 5H), 7.43 (d, *J* = 8.9 Hz, 2H), 7.30 (dd, *J* = 8.6, 2.1 Hz, 2H), 7.24 – 7.17 (m, 2H), 7.12 – 7.02 (m, 2H), 5.11 (m, 2H), 4.49 – 4.38 (m, 1H), 4.17 (m, 1H), 3.99 (m, 3H), 3.93 – 3.76 (m, 2H), 3.70 – 3.50 (m, 8H), 1.40 (d, *J* = 6.9 Hz, 3H), 1.28 (d, *J* = 6.4 Hz, 3H).	++
29		LC-MS: m/z 732.2 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 9.16 (s, 1H), 9.04 (s, 1H), 8.10 (d, *J* = 7.5 Hz, 1H), 7.72 (d, *J* = 4.0 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 2.1 Hz, 1H), 7.54 (dd, *J* = 9.1, 2.7 Hz, 2H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.29 – 7.17 (m, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 4.42 – 4.33 (m, 1H), 4.16 (h, *J* = 7.1 Hz, 1H),	+++

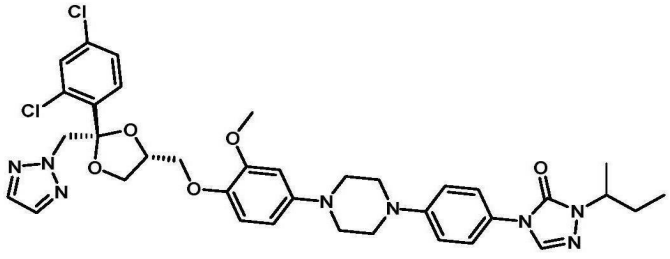
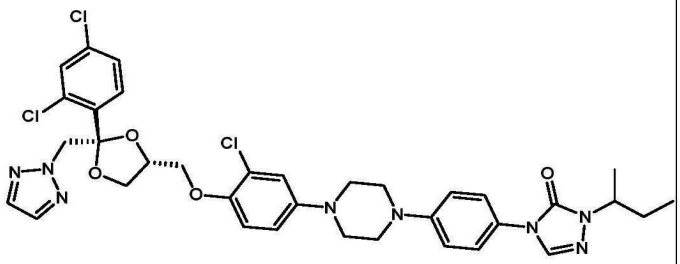
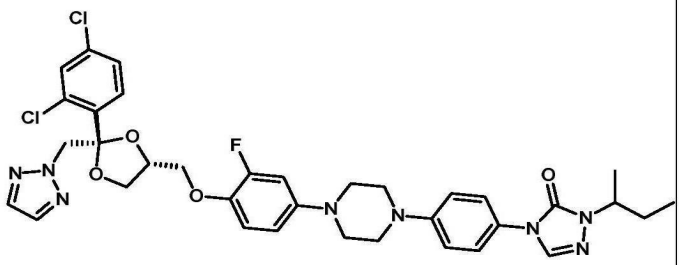
		4.04 – 3.95 (m, 1H), 3.92 – 3.79 (m, 3H), 3.72 (s, 4H), 3.64 (q, *J* = 6.8, 5.1 Hz, 4H), 3.61 – 3.50 (m, 2H), 1.48 (d, *J* = 6.8 Hz, 3H), 1.15 (d, *J* = 6.3 Hz, 3H).	
30		LCMS: 100 % @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H) ,1.40-1.42 (d, *J*=6.4 Hz, 3H) ,1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17-3.36 (m, 4H), 3.41-3.43 (m, 5H), 3.77-3.80 (m, 1H), 3.86-3.90 (m, 2H), 4.29-4.34 (m, 1H), 4.37-4.38 (m, 1H), 4.84 (s, 2H), 6.82 (s, 2H), 6.90-6.91 (m, 2H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.35-7.37 (d, *J*=8.8 Hz, 2H), 7.46-7.54 (d, *J*=8.4 Hz, 4H), 7.63-7.65 (m, 3H).	++
31		LCMS: 100 % @ 262 nm; m/z 705.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.4 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.27-3.39 (s, 4H), 3.39-3.47 (s, 4H), 3.48-3.51 (m, 1H), 3.80-3.84 (m, 1H), 3.89-3.95 (m , 2H), 4.29-4.34 (m, 1H), 4.38-4.42 (m, 1H), 4.83 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H), 6.961 (s, 2H), 7.04-7.07 (d, *J*=9.2 Hz, 2H), 7.36-7.40 (m, 3H), 7.44-7.46 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=2.8 Hz, 3H).	++
32		LCMS: 100 % @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.43 (d, *J*=4.4 Hz, 3H) ,1.69-1.79 (m, 1H), 1.85-1.92 (m, 1H), 3.26-3.51 (m, 9H), 3.82-3.88 (m, 2H), 3.93-3.96 (m, 1H), 4.29-4.34 (m, 1H), 4.37-4.43 (m, 1H), 5.08-5.11 (d, *J*=14.4 Hz, 1H), 5.22-5.25 (d, *J*=14.4 Hz, 1H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 6.91-6.95 (m, 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.24-7.28 (m, 1H), 7.31-7.35 (m, 1H), 7.44-7.48 (m, 3H), 7.63-7.68 (m, 4H).	+

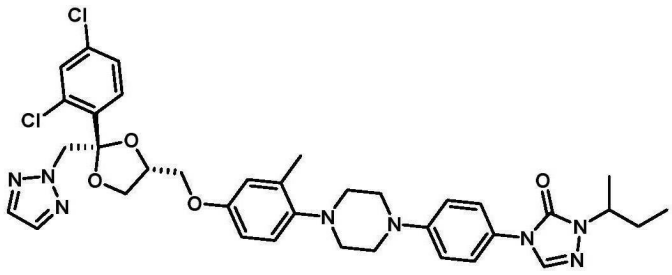
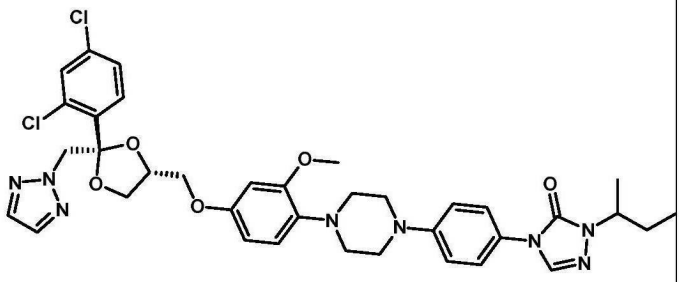
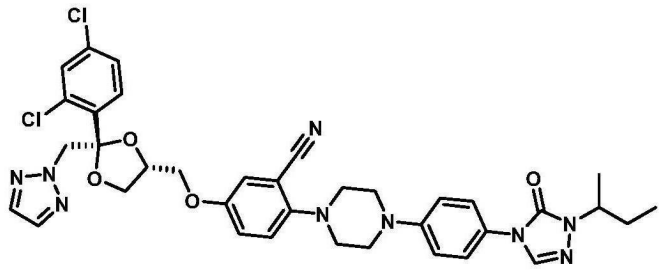
33		LCMS: 99.39% @ 262 nm; m/z 671.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.43 (d, *J*=6.8 Hz, 3H), 1.69-1.79 (m, 1H), 1.83-1.92 (m, 1H), 3.27-3.44 (m, 9H), 3.77-3.81 (m, 1H), 3.86-3.94 (m, 2H), 4.29-4.34 (m, 1H), 4.36-4.42 (m, 1H),4.84 (s, 2H), 6.79-6.81 (d, *J*=8.4 Hz, 2H), 6.95 (s, 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.30-7.37 (m, 2H), 7.41-7.46 (m, 3H), 7.53-7.54 (d, *J*=1.6 Hz, 1H), 7.63-7.65 (m, 3H).	++
34		LCMS: 100% @ 262 nm; m/z 682.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.25-1.28 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.68-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.66-2.71 (q, 2H), 3.29-3.33 (m, 5H), 3.42 (s, 4H), 3.72-3.75 (m, 1H), 3.80-3.83 (m, 1H), 3.89-3.93 (t, *J*=8.4 Hz, 1H), 4.29-4.39 (m, 2H), 4.85 (s, 2H), 6.78-6.80 (d, *J*=8.8 Hz, 2H), 7.02-7.07 (m, 4H), 7.23-7.25 (d, *J*=8.4 Hz, 2H), 7.44-7.50 (q, 4H), 7.64 (s, 3H).	++
35		LCMS: 99.39% @ 262 nm; m/z 717.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.43 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.28 (s, 4H), 3.41-3.45 (m, 5H), 3.77-3.81 (m, 1H), 3.86-3.92 (m, 2H), 4.29-4.39 (m, 2H), 4.84 (s, 2H), 6.80-6.82 (d, *J*=6.8 Hz, 2H), 6.95-6.96 (s, 2H), 7.05-7.07 (d, *J*=9.2 Hz, 2H), 7.40-7.47 (m, 4H), 7.51-7.53 (d, *J*=8.4 Hz, 2H), 7.62-7.64 (d, *J*=7.2 Hz, 3H).	++
36		LCMS: 100% @ 262 nm; m/z 705.36 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.75 (m, 1H), 1.84-1.88 (m, 1H), 3.26 (s, 4H), 3.39 (s, 4H), 3.46-3.50 (q, 1H), 3.79-3.83 (m, 1H), 3.91-3.92 (d, *J*=5.2 Hz, 2H), 4.30-4.41 (m, 2H), 4.84 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H),	+

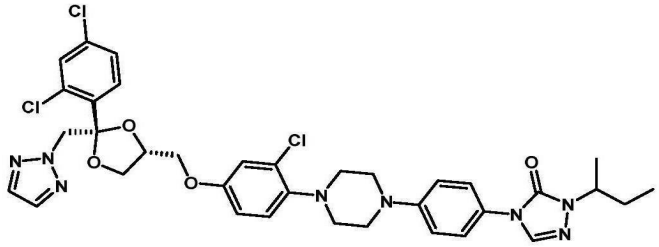
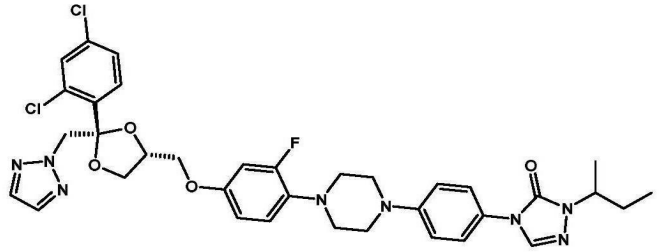
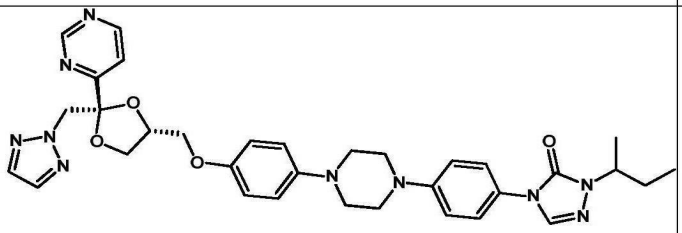
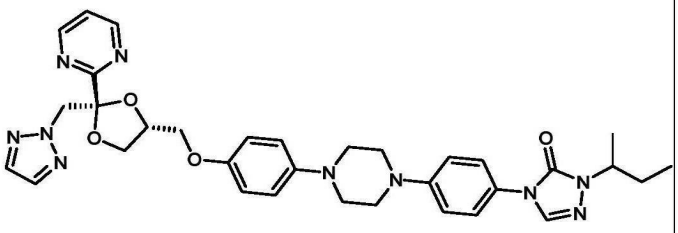
		6.95 (s, 2H), 7.04-7.06 (d, *J*=8.8 Hz, 2H), 7.32-7.35 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz, 3H), 7.44-7.46 (m, 3H), 7.60-7.64 (m, 4H).	
37		LCMS: 99.81% @ 262 nm; m/z 662.55 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz,3H) ,1.40-1.43 (d, *J*=4.8 Hz,3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.28 (s, 4H), 3.40 (s, 4H), 3.51-3.58 (q, 1H), 3.84-3.89 (m, 1H), 3.92-3.96 (m, 2H), 4.29-4.34 (m, 1H), 4.37-4.40 (m, 1H), 4.86 (s, 2H), 6.81-6.83 (d, *J*=8.8 Hz,2H), 6.97 (s, 2H), 7.02-7.04 (d, 2H), 7.44-7.46 (d, *J*=8.8 Hz,2H), 7.61-7.64 (m, 5H),7.66-7.69 (m, 2H).	++
38		LCMS: 100% @ 262 nm; m/z 705.41 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17 (s, 1H), 3.27 (s, 2H), 3.41-3.48 (m, 6H), 3.78-3.82 (m, 1H), 3.89-3.92 (m, 2H), 4.29-4.41 (m, 2H), 4.87 (s, 2H), 6.83-6.92 (m, 4H), 7.06-7.08 (d, *J*=8.8 Hz, 2H), 7.46-7.48 (d, *J*=6.0 Hz, 2H), 7.63-7.67 (m, 6H), 7.78-7.98 (m, 1H).	++
39		LCMS: 96.30% @ 261 nm; m/z 667.46 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H) ,1.40-1.42 (d, *J*=6.4 Hz,3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.26- 3.39 (m, 9H), 3.73-3.76 (m, 1H), 3.82-3.92 (m, 5H), 4.29-4.39 (m, 2H), 4.84 (s,2H), 6.80 (s, 2H), 6.90- 6.92 (m, 4H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.45-7.49 (m, 4H), 7.63-7.64 (d *J*=4.8 Hz, 3H).	++
40		LCMS: 100% @ 262 nm; m/z 651.45 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz,3H) , 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.38 (s, 3H), 3.16-3.39 (m,8H), 3.72-3.76 (m, 2H), 3.82-3.85 (m, 1H), 3.88-3.92 (m, 1H), 4.29-4.38 (m, 2H), 4.85 (s, 2H), 6.80 (s,	++

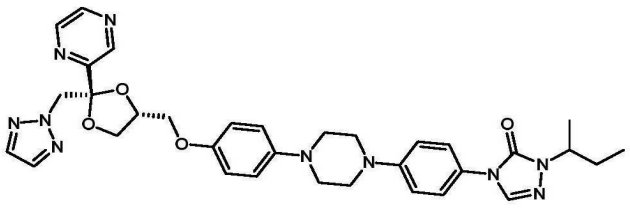
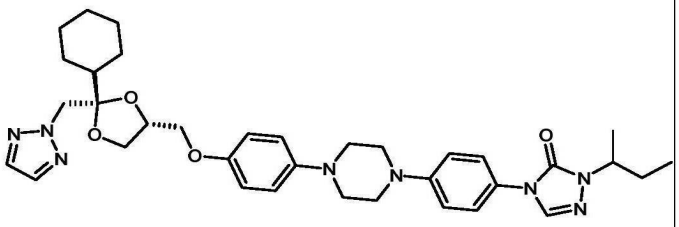
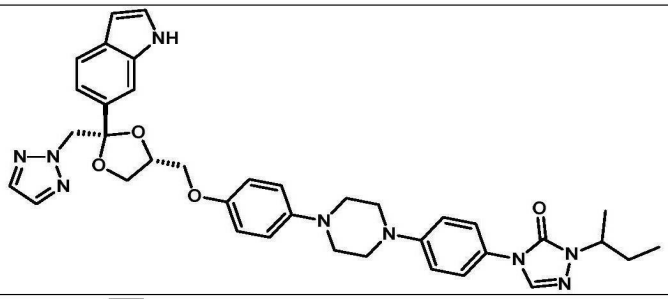
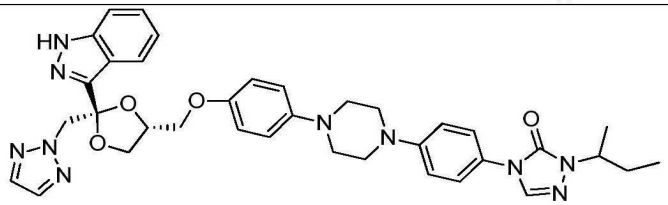
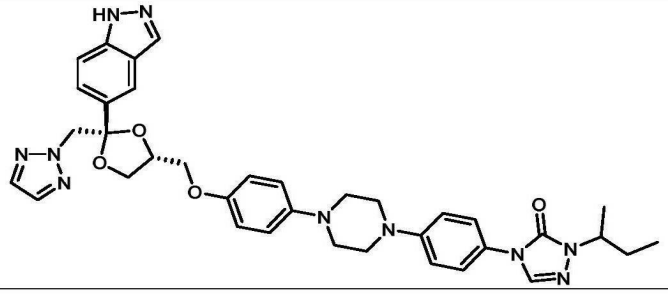
		2H), 6.89- 6.94 (m, 2H), 7.02-7.07 (d, *J*=8.0 Hz, 2H), 7.20-7.22 (d, *J*=8.0 Hz, 2H), 7.44-7.46 (d, *J*=8.0 Hz, 4H), 7.64-7.65 (m, 3H).	
41		LCMS: 100% @ 261 nm; m/z 651.55 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H) ,1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.65 (s, 3H), 3.29- 3.33 (m, 5H), 3.41 (s, 4H), 3.76-3.81 (m, 2H), 3.88-3.92 (m, 1H), 4.30-4.36 (m, 2H), 4.87-4.95 (q, 2H),6.78-6.81 (d, *J*=8.4 Hz, 2H), 6.97-7.00 (m , 2H), 7.02-7.07 (d, *J*=8.8 Hz, 2H), 7.21-7.24 (m, 2H), 7.45-7.47 (d, *J*=8.8 Hz,3H), 7.63-7.67 (m, 4H).	++
42		LCMS: 100 % @ 261 nm; m/z 637.45 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.92 (t, *J*=7.6 Hz, 3H), 1.41 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.86-1.89 (m, 1H), 3.30-3.43 (m, 9H), 3.73-3.78 (m, 1H), 3.83-3.86(m, 1H), 3.89-3.93 (m, 1H), 4.30-4.33 (m , 1H), 4.37-4.40(m, 1H), 4.87 (s, 2H), 6.79-6.81 (d, *J*=8.0 Hz, 2H), 7.02-7.07 (m, 4H), 7.38-7.42 (m, 3H), 7.44-7.47(d, *J*=8.8 Hz, 2H), 7.56-7.59(m, 2H), 7.64 (m, 3H)	+++
43		LCMS: 100% @ 262 nm; m/z 705.61 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d *J*=6.4 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.26 (s, 4H), 3.37-3.41 (m, 4H), 3.82-3.87 (m, 2H), 3.90-3.94 (m, 1H), 4.29-4.34 (m, 3H), 4.94-5.04.(q, 2H), 6.78-6.80 (d, *J*=7.6 Hz, 2H), 6.95 (s, 2H), 7.04-7.07 (d, *J*=8.8 Hz,2H), 7.44-7.46 (d, *J*=8.4 Hz, 2H), 7.50-7.55 (m, 2H), 7.63-7.64 (m, 3H), 7.74-7.75 (d, *J*=6.8 Hz,1H), 7.80-7.83 (m, 1H).	+

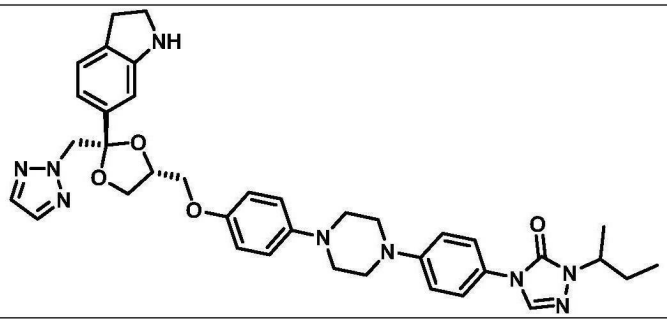
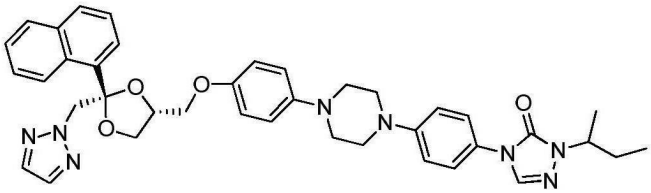
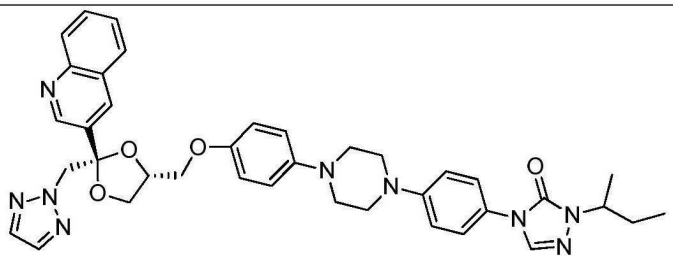
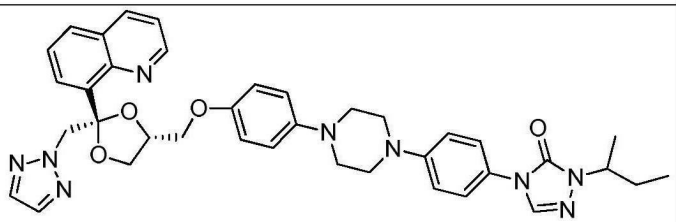
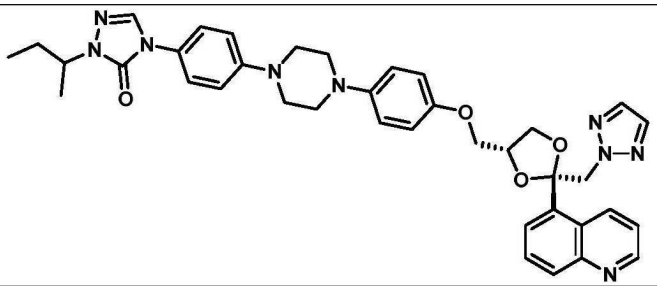
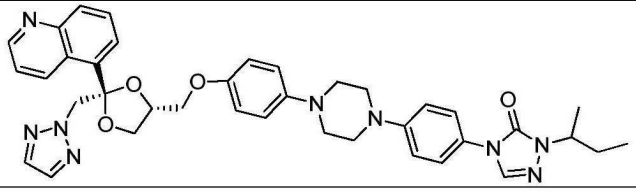
44		LCMS: 99.84% @ 261 nm; m/z 715.31 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz,3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.08-3.09 (s, 3H), 3.25- 3.27 (m, 4H), 3.37-3.40 (m, 4H), 3.51-3.55 (m, 1H), 3.83-3.85 (m, 1H), 3.92-3.94 (m, 2H), 4.30-4.33 (m, 1H), 4.38-4.40 (m, 1H), 4.88 (s, 2H), 6.80-6.82 (d, *J*=8.8 Hz, 2H), 6.95-6.97 (d , *J*=8.8 Hz, 2H), 7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.44-7.46 (d, *J*=8.8 Hz, 2H), 7.62-7.64 (d, *J*=8.0 Hz, 3H), 7.72-7.75 (d, *J*=8.8 Hz, 2H), 7.95-7.97 (d, *J*=8.8 Hz, 2H).	+
45		LCMS: 100% @ 262 nm; m/z 667.36 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz,3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.26(s, 4H), 3.34-3.39 (m, 5H), 3.74-3.77 (m, 1H), 3.82-3.85 (m, 3H), 3.89-3.91(m, 2H), 4.31-4.38 (m, 2H), 4.84(s, 2H), 6.77-6.81 (s, 2H), 6.90-6.93 (d , *J*=8.8 Hz, 4H), 7.02-7.07 (d, *J*=9.2 Hz, 2H), 7.47-7.54 (m, 4H), 7.63-7.65 (m, 3H).	+
46		LCMS: 100% @ 262 nm; m/z 719.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 2.16-2.19 (s, 3H), 3.25 (s, 4H), 3.38 (s, 4H), 3.48-3.52 (q, 1H), 3.91-4.00 (m, 3H), 4.29-4.34(m, 1H), 4.39-4.41 (m, 1H), 5.06-5.09 (d, *J*=14.0 Hz, 1H), 5.17-5.20 (d, *J*=14.0 Hz, 1H), 6.69-6.71 (d, *J*=8.0 Hz, 1H), 6.78-6.84 (m, 2H), 7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz, 1H), 7.44-7.48 (m, 3H), 7.52-7.54 (d, *J*=8.4 Hz 1H), 7.60-7.64 (m, 3H).	+

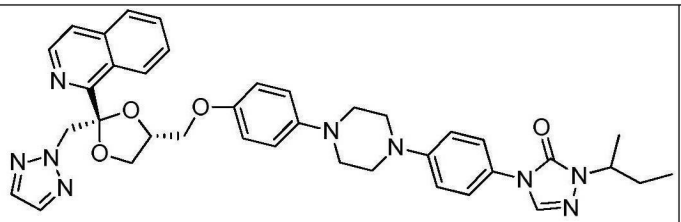
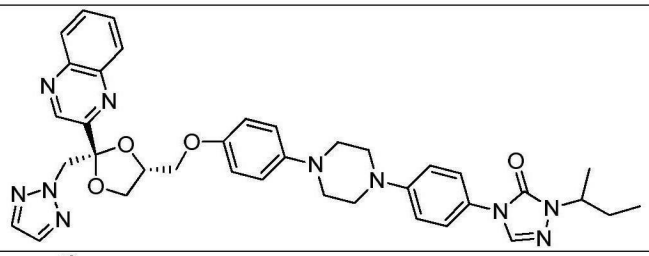
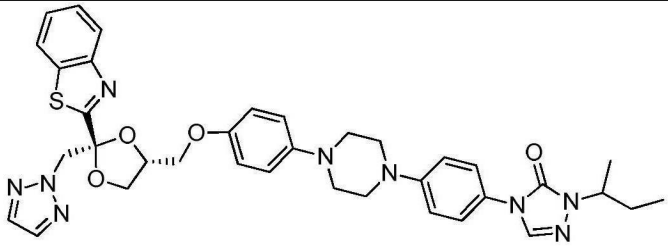
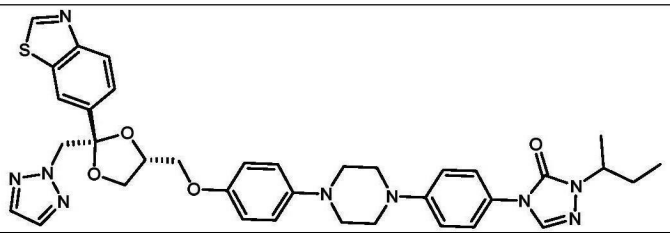
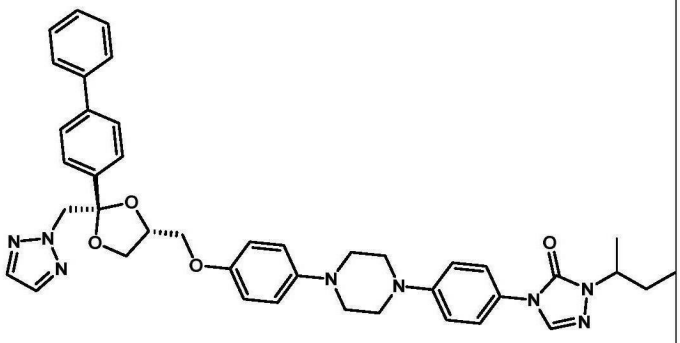
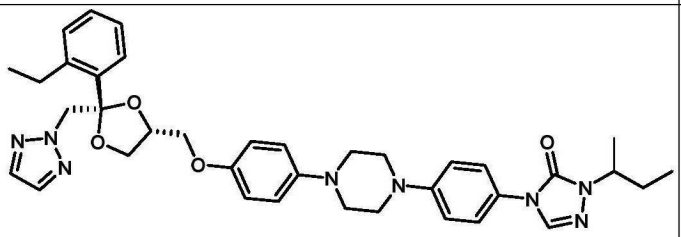
47		LCMS: 100% @ 262 nm; m/z 752.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.69-1.77 (m, 1H), 1.83-1.92 (m, 1H), 3.34 (s, 4H), 3.45-3.52 (m, 5H), 3.85 (s, 3H), 3.94-3.98 (m, 3H), 4.29-4.34 (q, 1H), 4.41-4.44 (q, 1H), 5.06-5.10 (d, *J*=14.4 Hz, 1H), 5.14-5.21 (d, *J*=14.4 Hz, 1H), 6.60 (s, 1H), 6.73 (s, 1H), 6.82-6.84 (d, *J*=8.8 Hz, 1H), 7.05-7.07 (d, *J*=8.8 Hz, 2H), 7.19-7.22 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.44-7.47 (m, 3H), 7.51-7.53 (d, *J*=8.4 Hz, 1H), 7.60-7.65 (m, 3H).	+
48		LCMS: 100% @ 262 nm; m/z 758.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.27-3.28 (s, 4H), 3.37-3.38 (s, 4H), 3.50-3.54 (q, 1H), 3.96-4.03 (m, 3H), 4.29-4.34 (q, 1H), 4.40-4.41 (q, 1H), 5.07-5.10 (d, *J*=14.4 Hz, 1H), 5.16-5.20 (d, *J*=14.4 Hz, 1H), 6.86 (s, 2H), 7.02-7.06 (m, 3H), 7.20-7.22 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.44 (s, 1H), 7.46-7.47 (m, 2H), 7.52-7.54 (d, *J*=8.8 Hz, 1H), 7.60 (s, 2H), 7.64 (s,1H).	++
49		LCMS: 100% @ 263 nm; m/z 723.81 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.84-1.92 (m, 1H), 3.25-3.28 (m, 4H), 3.36-3.38 (m, 4H), 3.50-3.55 (q, 1H), 3.92-3.95 (m, 3H), 4.29-4.34 (q, 1H), 4.37-4.42 (q, 1H), 5.04-5.08 (d, *J*=14.4 Hz, 1H), 5.16-5.20 (d, *J*=14.4 Hz, 1H), 6.65-6.66 (m, 1H), 6.67-6.76 (dd, J_1=16.8 Hz 和 J_2=3.2 Hz 1H), 6.86-6.90 (t, *J*=9.2 Hz, 1H), 7.03-7.06 (d, *J*=8.8 Hz, 2H), 7.20-7.23 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.43-7.47 (m, 3H), 7.52-7.55 (d, *J*=8.4 Hz,1H), 7.58-7.64 (m, 3H).	++

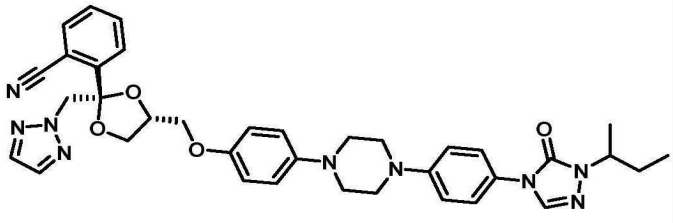
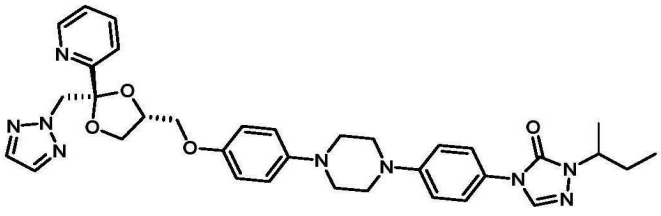
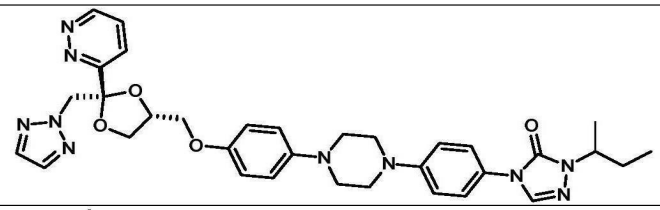
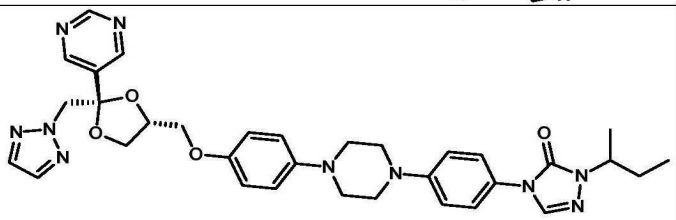
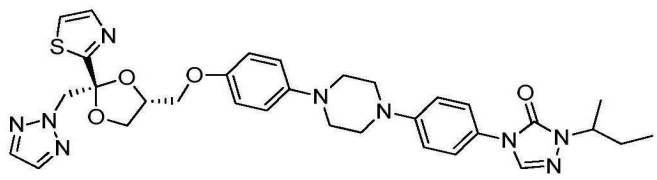
50		LCMS: 100% @ 265 nm; m/z 719.61 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, , *J*=7.2 Hz 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H) ,1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 2.34 (s, 3H), 3.05 (s, 4H), 3.38-3.47 (m, 5H), 3.86-3.96 (m, 3H), 4.29-4.41 (m, 2H), 5.05-5.09 (d, , *J*=14.4 Hz 1H), 5.17-5.21 (d, , *J*=14.4 Hz 1H), 6.64-6.73 (m, 2H), 7.02-7.04 (m, 3H), 7.21-7.24 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.44-7.48 (m, 3H), 7.54-7.57 (d, *J*=6.8 Hz, 1H), 7.62-7.65 (m, 3H).	++
51		LCMS: 100% @ 262 nm; m/z 735.31 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.72-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.16-3.18 (s, 4H), 3.40-3.42 (m, 4H), 3.47-3.50 (m, 1H), 3.84-3.97 (m, 5H), 4.29-4.34 (q, 1H), 4.39-4.41 (q, 1H), 5.05-5.09 (d, *J*=14.4 Hz, 1H), 5.18-5.22 (d, *J*=14.4 Hz, 1H), 6.34-6.37 (dd, J_1=11.2 Hz 和 J_2=2.8 Hz 1H 1H), 6.47-6.48 (d, *J*=2.8 Hz, 1H), 6.89-6.91 (d, *J*=8.8 Hz, 1H), 7.04-7.06 (d, *J*=8.8 Hz, 2H), 7.22-7.24 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.42-7.48 (m, 3H), 7.55-7.64 (m, 4H).	++
52		LCMS: 99.78% @ 265 nm; m/z 730.51 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.41-1.42 (d, *J*=6.8 Hz, 3H), 1.71-1.78 (m, 1H), 1.85-1.89 (m, 1H), 3.30-3.37 (m, 5H), 3.47-3.51 (m, 4H), 3.80-3.83(m, 1H), 3.86-3.89 (m, 1H), 3.93-3.97 (m, 1H), 4.31-4.33 (q, 1H), 4.39-4.42 (q, 1H), 5.05-5.09 (d, *J*=14.4 Hz, 1H), 5.17-5.20 (d, *J*=14.4 Hz, 1H), 7.02-7.10 (m, 3H), 7.20-7.26 (m, 3H), 7.46-7.59 (m, 4H), 7.64-7.67 (m, 3H).	++

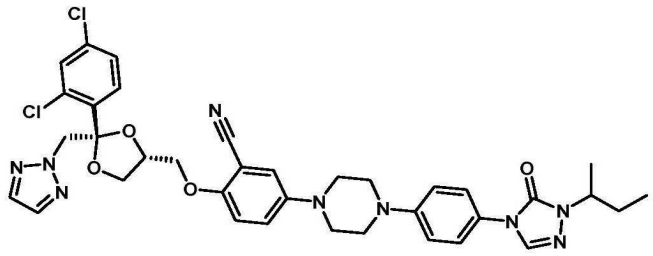
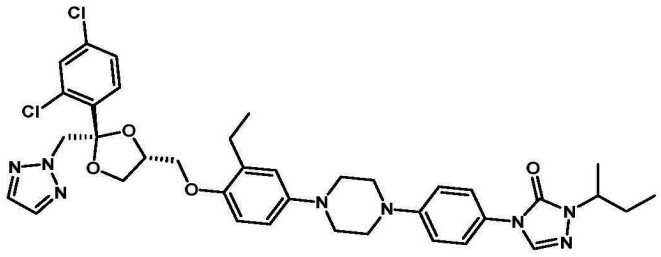
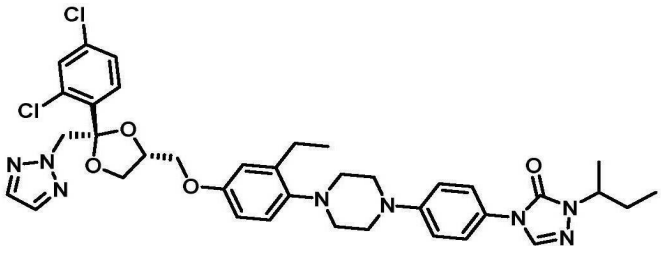
53		LCMS: 100% @ 263 nm; m/z 741.56 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.91-0.94 (t, *J*=7.6 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.71-1.77 (m, 1H), 1.85-1.90 (m, 1H), 3.17 (s, 4H), 3.41-3.46 (m, 4H), 3.51 (s, 1H), 3.81-3.84 (m, 1H), 3.87-3.96 (m, 2H), 4.29-4.36 (q, 1H), 4.37-4.41 (q, 1H), 5.05-5.08 (d, *J*=14.4 Hz, 1H), 5.17-5.20 (d, *J*=14.4 Hz,1H), 6.73-6.76 (dd, J_1=11.6 Hz 和 J_2=2.8 Hz 1H), 6.92-6.93 (d, *J*=302 Hz, 1H), 7.02-7.07 (m, 3H), 7.22-7.25 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.44-7.49 (m, 3H), 7.54-7.57 (m, 1H), 7.63-7.64 (m, 3H).	++
54		LCMS: 100% @ 263 nm; m/z 723.81 (M+H). ^{1}HNMR (400 MHz, $CDCl_3$): 0.90-0.94 (t, *J*=7.2 Hz, 3H), 1.40-1.42 (d, *J*=6.8 Hz, 3H), 1.70-1.77 (m, 1H), 1.85-1.92 (m, 1H), 3.17-3.20 (m, 4H), 3.38-3.46 (m, 5H), 3.80-3.83 (m, 1H), 3.86-3.96 (m, 2H), 4.29-4.36 (q, 1H), 4.37-4.40 (q, 1H), 5.04-5.08 (d, *J*=14.4 Hz, 1H), 5.17-5.21 (d, *J*=14.4 Hz, 1H), 6.56-6.64 (m, 2H), 6.92-6.97 (t, 1H), 7.04-7.06 (d, *J*=9.2 Hz, 2H), 7.22-7.25 (dd, J_1=10.4 Hz 和 J_2=2.0 Hz 1H), 7.43-7.48 (m, 3H), 7.55-7.59 (m, 1H), 7.62-7.64 (m, 3H).	+++
55			
56		LC-MS: m/z 639.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.77 (d, J = 8.63 Hz, 1H), 7.61 (s, 1H), 7.59 (s, 2H), 7.47 (m, 1H), 7.26 (m, 1H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.27 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38	+

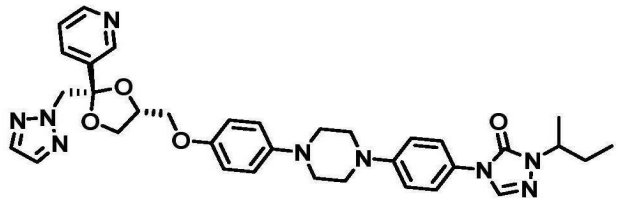
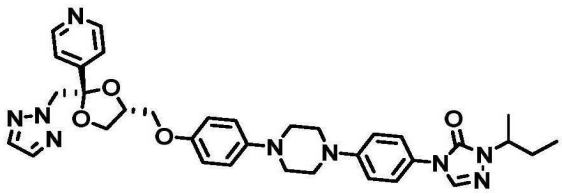
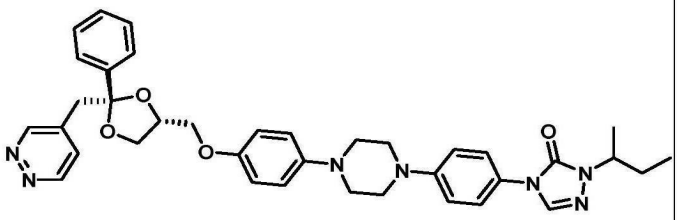
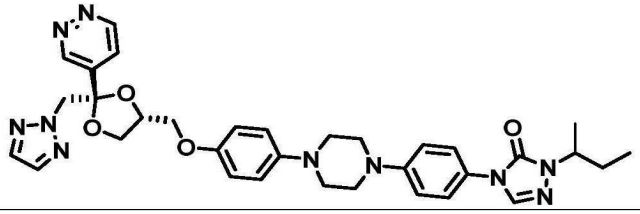
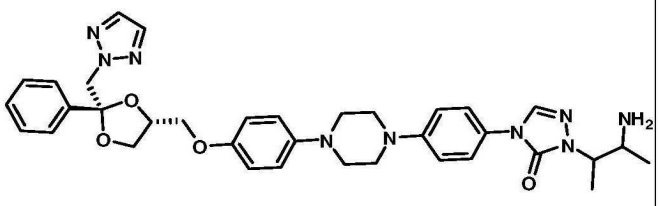
		(d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	
57		LC-MS: m/z 639.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 1.5 Hz, 1H), 8.66 (dd, *J* = 2.5, 1.5 Hz, 1H), 8.61 (d, *J* = 2.5 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.62 (s, 2H), 7.48 – 7.40 (m, 2H), 7.08 – 7.01 (m, 2H), 6.94 – 6.86 (m, 2H), 6.79 – 6.69 (m, 2H), 5.22 – 5.05 (m, 2H), 4.42 (m, 1H), 4.37 – 4.22 (m, 1H), 4.15 (m, 3H), 4.09 – 3.93 (m, 4H), 3.37 (m, 4H), 3.23 (m, 4H), 1.98 – 1.83 (m, 1H), 1.82 – 1.72 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).	+
58		LC-MS: m/z 643.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 0.6 Hz, 2H), 7.64 (t, *J* = 0.7 Hz, 1H), 7.49 – 7.42 (m, 2H), 7.11 – 7.03 (m, 2H), 6.97 (m, 2H), 6.85 (d, *J* = 8.4 Hz, 2H), 4.70 (s, 2H), 4.44 – 4.37 (m, 1H), 4.32 (dt, *J* = 8.6, 6.4 Hz, 1H), 4.18 (dd, *J* = 8.3, 6.3 Hz, 1H), 3.93 (dd, *J* = 9.7, 4.8 Hz, 1H), 3.60 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.50 – 3.12 (m, 9H), 2.08 – 1.65 (m, 7H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.22 (m, 6H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
59			
60			
61			

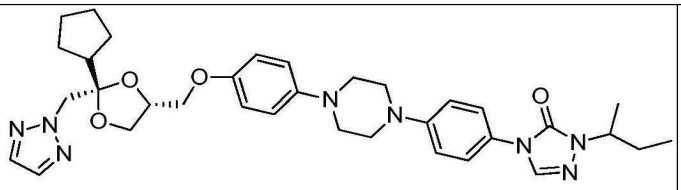
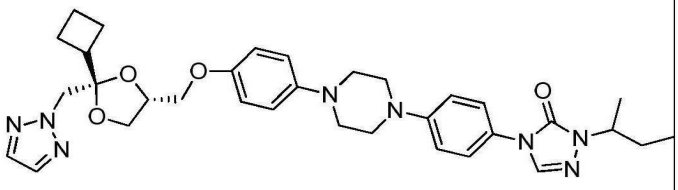
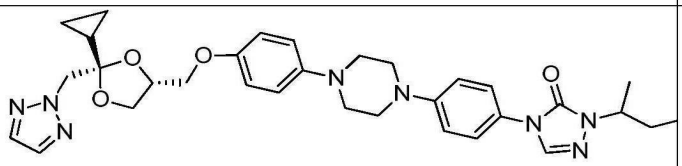
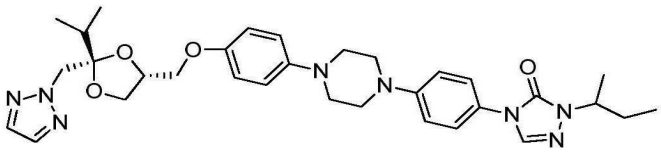
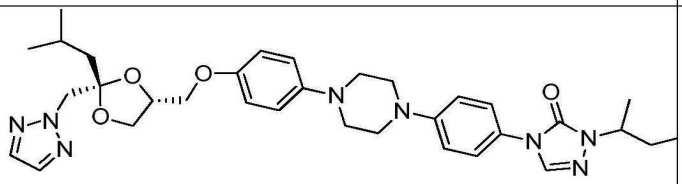
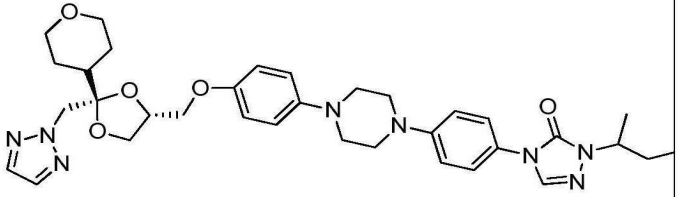
62			
63		LC-MS: m/z 687.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, *J* = 8.6 Hz, 1H), 7.92 (m, 3H), 7.71-7.63 (m, 4H), 7.58 – 7.48 (m, 4H), 7.09-6.71 (m, 6H), 5.24 – 5.05 (m, 2H), 4.44 (d, *J* = 7.6 Hz, 1H), 4.40 – 4.27 (m, 1H), 3.99 (dd, *J* = 8.5, 6.3 Hz, 1H), 3.82 (td, *J* = 10.6, 9.8, 4.8 Hz, 2H), 3.73 – 3.04 (m, 9H), 1.89 (m, 1H), 1.76 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
64			
65			
66			
67			

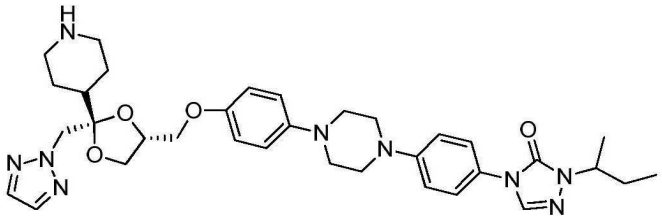
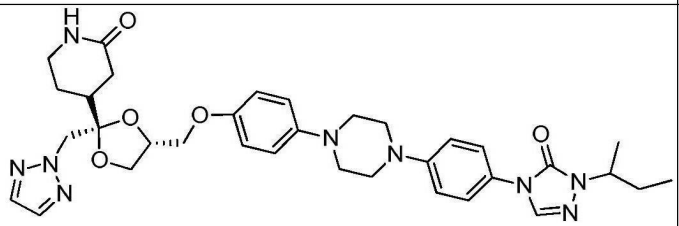
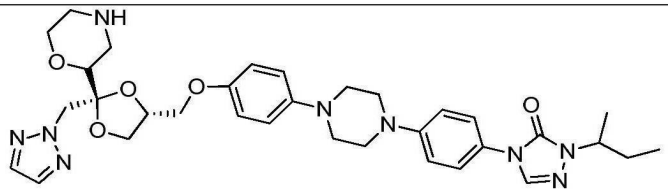
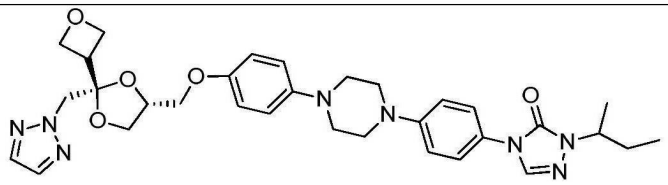
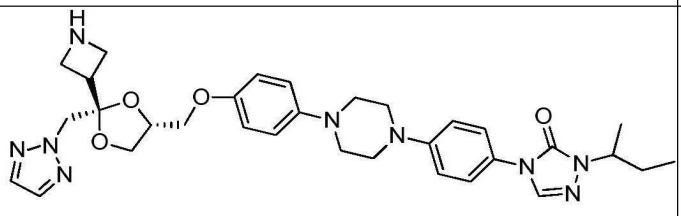
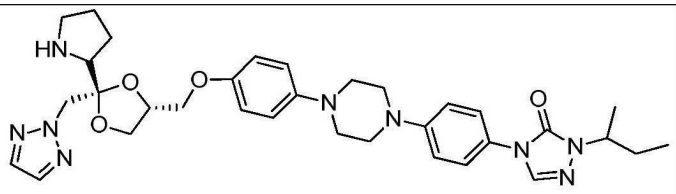
68			
69			
70			
71			
72		LC-MS: m/z 713.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.56 (m, 9H), 7.53 – 7.43 (m, 4H), 7.43 – 7.35 (m, 1H), 7.06 (d, *J* = 9.0 Hz, 2H), 6.97 (m, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 4.91 (s, 2H), 4.43 (ddd, *J* = 9.4, 6.8, 4.6 Hz, 1H), 4.37 – 4.26 (m, 1H), 3.97 (dd, *J* = 8.5, 6.5 Hz, 1H), 3.87 (dd, *J* = 8.5, 4.3 Hz, 1H), 3.79 (dd, *J* = 9.4, 5.1 Hz, 1H), 3.52 – 3.07 (m, 9H), 1.89 (m, 1H), 1.74 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H).	+
73			

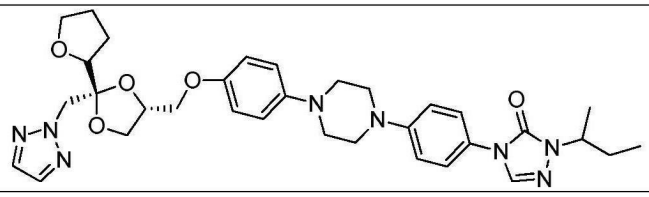
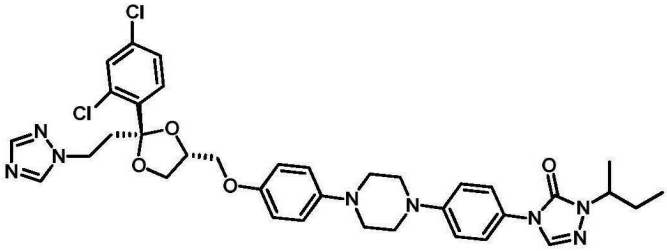
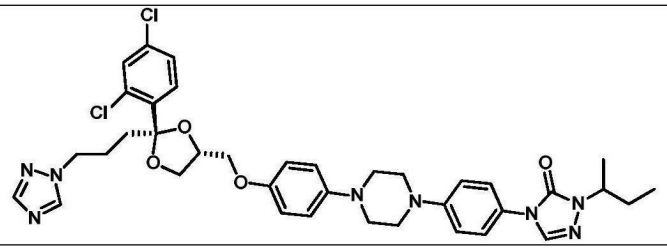
74		LC-MS: m/z 662.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.64 (d, *J* = 0.7 Hz, 1H), 7.62 – 7.48 (m, 5H), 7.45 (dd, *J* = 9.1, 2.2 Hz, 2H), 7.10 – 7.03 (m, 2H), 7.01 – 6.93 (m, 2H), 6.89 – 6.81 (m, 2H), 5.17 – 5.01 (m, 2H), 4.46 (tt, *J* = 6.8, 4.6 Hz, 1H), 4.39 – 4.26 (m, 1H), 4.09 (dd, *J* = 8.6, 4.7 Hz, 1H), 4.07 – 3.95 (m, 2H), 3.70 (dd, *J* = 9.6, 7.0 Hz, 1H), 3.39 (m, 4H), 3.26 (m, 4H), 1.89 (m Hz, 1H), 1.70 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	++
75		LC-MS: m/z 638.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (ddt, *J* = 4.9, 1.7, 0.8 Hz, 1H), 7.76 – 7.68 (m, 1H), 7.64 (d, *J* = 0.7 Hz, 1H), 7.60 (m, 2H), 7.54 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.30 (m, 1H), 7.13 – 7.03 (m, 2H), 7.02 – 6.92 (m, 2H), 6.88 – 6.70 (m, 2H), 5.15 (m, 2H), 4.49 (ddd, *J* = 11.6, 6.7, 4.9 Hz, 1H), 4.42 – 4.26 (m, 1H), 4.07 (dd, *J* = 8.5, 6.4 Hz, 1H), 3.92 (td, *J* = 9.0, 8.4, 4.8 Hz, 2H), 3.56 (dd, *J* = 9.5, 7.2 Hz, 1H), 3.47 – 3.36 (m, 4H), 3.26 (m, 4H), 1.92 (m, 1H), 1.74 (m, 1H), 1.42 (d, *J* = 6.7, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).	+
76			
77			
78		LC-MS: m/z 644.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 6.5, 3.2 Hz, 1H), 7.68-7.64 (m, 3H), 7.47 – 7.41 (m, 2H), 7.39 (t, J = 3.3 Hz, 1H), 7.05 (dd, J = 9.0, 3.7 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.85 – 6.76 (m, 2H), 5.31 – 5.12	+

		(m, 2H), 4.62 (dq, J = 6.8, 5.4 Hz, 1H), 4.39 – 4.25 (m, 1H), 4.21 (dd, J = 8.6, 6.3 Hz, 1H), 3.88 (ddd, J = 10.5, 9.1, 5.1 Hz, 2H), 3.52 (dd, J = 9.6, 7.0 Hz, 1H), 3.45 – 3.32 (m, 4H), 3.30 – 3.14 (m, 4H), 1.98 – 1.83 (m, 1H), 1.82 – 1.72 (m, 1H), 1.42 (d, J = 6.8 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H).	
79		LC-MS: m/z 730.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 3H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.24 (dd, *J* = 8.4, 2.1 Hz, 2H), 7.15 (d, *J* = 3.0 Hz, 1H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 9.2 Hz, 2H), 5.21 – 5.07 (m, 2H), 4.46 (m, 1H), 4.33 (m, 1H), 3.99 (dtd, *J* = 18.1, 8.9, 4.6 Hz, 3H), 3.52 (dd, *J* = 9.5, 7.6 Hz, 1H), 3.41 (m, 4H), 3.31 (m, 4H), 1.96 – 1.88 (m, 1H), 1.79 – 1.72 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	++
80		LC-MS: m/z 733.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.64 (s, 1H), 7.61 (s, 2H), 7.54 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.42 (m, 2H), 7.21 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.87 (d, *J* = 2.8 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 5.25 – 4.97 (m, 2H), 4.44 – 4.37 (m, 1H), 4.37 – 4.27 (m, 1H), 4.05 – 3.86 (m, 3H), 3.50 (dd, *J* = 9.4, 7.4 Hz, 1H), 3.39 (m, 4H), 3.32 – 3.25 (m, 4H), 2.58 (q, *J* = 7.5 Hz, 2H), 1.88 (m, 1H), 1.81 – 1.72 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.17 (t, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H).	+
81		LC-MS: m/z 733.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.62 (s, 2H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 2.1 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.23 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.11 – 7.01 (m, 3H), 6.75 (d, *J* = 2.9 Hz, 1H), 6.65 (dd, *J* = 8.6, 3.0 Hz, 1H), 5.27 – 5.00 (m, 2H), 4.40 (m, 1H), 4.32 (dt, *J* = 8.7, 6.4 Hz, 1H), 4.02 – 3.83 (m, 3H), 3.44 (dd, *J* = 9.4, 7.4 Hz, 1H), 3.41 – 3.28 (m, 4H), 3.09 – 2.91 (m, 4H),	+

		2.74 (q, *J* = 7.5 Hz, 2H), 1.98 – 1.85 (m, 1H), 1.81 – 1.70 (m, 1H), 1.43 (d, *J* = 6.7 Hz, 3H), 1.17 (t, *J* = 8.0 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H).	
82		LC-MS: m/z 638.1 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.10 (s, 1H), 7.67 (s, 2H), 7.54 – 7.38 (m, 3H), 7.34 (m, 2H), 7.27 (m, 1H), 7.24-7.09 (m, 4H), 6.94 (d, *J* = 7.6 Hz, 2H), 4.44 (m, 1H), 4.24 (m, 2H), 4.06 – 3.71 (m, 5H), 3.54-3.43 (m, 8H), 1.96 – 1.85 (m, 2H), 1.31, (d, *J* = 7.0 Hz, 3H), 0.90 (t, *J* = 7.5 Hz, 3H).	+
83		LC-MS: m/z 638.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$): 8.62 (d, J = 8.63 Hz, 2H), 7.61 (s, 1H), 7.59 (s, 2H), 7.46-7.42 (m, 3H), 7.03 (m, 2H), 6.95 (m, 2H), 6.87 (m, 2H), 6.81 (m, 2H), 5.00 (dd, J = 24.4 Hz, 11.6 Hz, 2H), 4.42 (m, 1H), 4.30 (m, 1H), 3.99 (m, 1H), 3.97-3.81 (m, 2H), 3.50 (dd, J = 7.6 Hz, 5.6 Hz, 1H), 3.37 (m, 4H), 3.25 (m, 4H), 1.87 (m, 1H), 1.70 (m, 1H), 1.38 (d, J = 5.2 Hz, 3H), 0.90 (t, J = 6.0 Hz, 3H).	+
84		LC-MS: m/z 648.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (dd, *J* = 2.4, 1.3 Hz, 1H), 9.01 (dd, *J* = 5.2, 1.3 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.50 – 7.30 (m, 8H), 7.09 – 7.02 (m, 2H), 6.99 – 6.93 (m, 2H), 6.81 – 6.71 (m, 2H), 4.32 (m, 1H), 3.93 – 3.67 (m, 5H), 3.61 – 3.50 (m, 2H), 3.39 (m, 4H), 3.27 (m, 4H), 3.19 (m, 2H), 1.89 (m, 1H), 1.77 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.5 Hz, 3H).	++
85			
86			

87			
88		LC-MS: m/z 615.2 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (m, 3H), 7.47 (m, 2H), 7.07-6.88 (m, 6H), 4.59 – 4.47 (m, 2H), 4.26 (m, 2H), 4.15 – 3.88 (m, 2H), 3.82 – 3.72 (m, 1H), 3.60 (t, *J* = 8.1 Hz, 1H), 3.41-3.22 (m, 8H), 2.67 (m, 1H), 2.12-1.70 (m, 8H), 1.40 (m, 3H), 0.97 (m, 3H).	++
89			
90		LC-MS: m/z 603.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 1.0 Hz, 1H), 7.69 (d, *J* = 1.0 Hz, 1H), 7.64 (d, *J* = 0.6 Hz, 1H), 7.49 – 7.41 (m, 2H), 7.09 – 7.02 (m, 2H), 7.01 – 6.92 (m, 2H), 6.88 – 6.78 (m, 2H), 4.73 – 4.56 (m, 2H), 4.51 – 4.43 (m, 1H), 4.32 (dp, *J* = 8.6, 6.6 Hz, 1H), 4.19 (dd, *J* = 8.3, 6.5 Hz, 1H), 3.82 (dd, *J* = 10.0, 4.4 Hz, 1H), 3.61 (dd, *J* = 9.9, 5.2 Hz, 1H), 3.55 (t, *J* = 8.0 Hz, 1H), 3.44 – 3.35 (m, 4H), 3.31 – 3.20 (m, 4H), 1.95 – 1.80 (m, 2H), 1.74 (m, 1H), 1.41 (d, *J* = 6.8 Hz, 3H), 1.07 (dd, *J* = 11.5, 6.9 Hz, 6H), 0.93 (t, *J* = 7.4 Hz, 3H).	+
91			
92		LC-MS: m/z 645.1 (M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.67 (s, 1H), 7.64 (s, 1H), 7.48 – 7.41 (m, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.00 – 6.93 (m, 2H), 6.91 – 6.81 (m, 2H), 4.74 – 4.59 (m, 2H), 4.50 – 4.38 (m, 1H), 4.38 – 4.14 (m, 2H), 4.12 – 3.84 (m,	+

		4H), 3.78 (dd, *J* = 9.9, 5.5 Hz, 1H), 3.60 (t, *J* = 8.3 Hz, 1H), 3.45 – 3.29 (m, 5H), 3.30 – 3.18 (m, 4H), 1.97 – 1.81 (m, 3H), 1.80 – 1.70 (m, 3H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H).	
93		LC-MS: m/z 644.1 (M+H) ^{1}H NMR (400 MHz, MeOD) δ 8.11 (d, *J* = 1.6 Hz, 1H), 7.77 (d, *J* = 7.2 Hz, 2H), 7.50 (dd, *J* = 9.2, 2.7 Hz, 2H), 7.31 (dd, *J* = 16.7, 9.0 Hz, 2H), 7.19 (dd, *J* = 9.2, 3.1 Hz, 2H), 7.09 – 6.96 (m, 2H), 4.78 – 4.67 (m, 2H), 4.35 – 4.20 (m, 2H), 4.19 – 3.95 (m, 3H), 3.88 (m, 1H), 3.51 (m, 10H), 2.95 (m, 2H), 2.31 – 1.64 (m, 7H), 1.40 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.4 Hz, 3H).	+
94			
95			
96			
97			
98			

99			
100		LC-MS: m/z 719.2(M+H) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.92 (s, 1H), 7.64 (s, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.51 – 7.42 (m, 3H), 7.28 – 7.24 (m, 1H), 7.10 – 7.03 (m, 2H), 7.00 (d, *J* = 8.7 Hz, 2H), 6.96 – 6.87 (m, 2H), 4.53 – 4.21 (m, 4H), 4.19 – 4.03 (m, 3H), 3.90 (t, *J* = 7.8 Hz, 1H), 3.40 (m, 4H), 3.27 (m, 4H), 2.82 – 2.64 (m, 2H), 1.98 – 1.84 (m, 1H), 1.83 – 1.64 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H).	+++
101		LC-MS: m/z 733.2 (M+H)	+

Cyp 可逆抑制试验

[0393] 本试验的目的是使用底物探针转换 (turnover) 作为抑制的替代物,用给定化合物确定 Cyp 酶的可逆抑制。这使用人肝微粒体而不是分离的酶进行,以解释母体药物的代谢物通过 Cyp 抑制导致潜在的药物 - 药物相互作用。通过 LCMSMS(Q1/Q2 = 342.2/203.2)监测底物转换 (例如,咪达唑仑向羟基咪达唑仑的转化)。在从 50μM 开始至 50nM(约 3 倍稀释系列) 的 7 点剂量响应曲线中对测试化合物进行测试。对于 Cyp 3A4,阳性对照化合物是 IC_{50}约为 25nM 的酮康唑。

[0394] 详细方案如下 :

●制备测试化合物和标准抑制剂工作溶液 (100×)。

●将微粒体从 -80℃冰箱中取出以在冰上融化,标以日期并在使用后立即放回冰箱。

●向对应的孔添加 20μL 的底物溶液。

●向空白孔添加 20μL PB。

●向对应的孔添加 2μL 的测试化合物和阳性对照工作溶液。

●向无抑制剂孔和空白孔添加 2μL MeOH。

●制备 HLM 工作溶液。

●向温育板的所有孔添加 158μL 的 HLM 工作溶液。

●在 37℃水浴下预热该板约 10min。

●制备 NADPH 辅因子溶液。

●向所有温育孔添加 20μL NADPH 辅因子。

●在 37℃水浴下混合并温育 10 分钟。

●在该时间点，通过添加 400 μL 冷终止溶液（ACN 中的 200ng/mL 甲苯磺丁脲）终止反应。

●将样品以 4000rpm 离心 20 分钟以使蛋白质沉淀。

●将 200 μL 上清液转移至 200 μL HPLC 水中并摇动 10min。

●通过 LC/MS/MS 分析样品。

[0395] 通过该试验获得的化合物数据在表 2 中示出。

表 2

化合物编号	Cyp 3A4 咪达唑仑 LCMS IC_{50} (μM)	Cyp 3A4 睾酮 LCMS IC_{50} (μM)
1		4.5
2		>30
15	>50; >50	>50
19	0.0031	0.0069
21	0.144	0.12
22	>50, >50	>50, >50
23	0.321	
24		>10, >30
26	0.18	0.283
27	>50, >50	>50, >50
30	>50	>50
32	>50	>50

化合物编号	Cyp 3A4 咪达唑仑 LCMS IC_{50} (μM)	Cyp 3A4 睾酮 LCMS IC_{50} (μM)
36	>50	>50
41	>50, >50	>50
42	>50, >50	>50, >50
49	>50	>50
54	>50	>50
74	>50	>50
79	>50	>50
84	2.55, 2.35	1.77
100	0.372	0.545
ITZ	0.0447, 0.0546	0.0127

Cyp 时间依赖性抑制 (TDI) 试验

[0396] 本试验的目的是确定化合物在人肝微粒体中形成不可逆抑制的细胞色素 P450 加合物 (adduct)(也称为基于机理的抑制 (MBI)) 的能力。相比于竞争性的 CYP 抑制，已经认识到 MBI 在药物发现和开发中受到更大的关注，因为 CYP 的灭活可以导致非线性药代动力学并低估药物 - 药物相互作用潜力。来自该试验的数据与可逆抑制试验结合使用。TDI 试验通常在肝微粒体中进行，以评价母体药物以及代谢物的 PDI 潜力。读出值是存在允许将化合物转化为反应性物质的 NADPH(灭活 /TDI 组) 和不存在 NADPH(用于校正 20 分钟预温育期间蛋白质降解的对照组) 时的 IC_{50}变化。然后这两个温育组均用含有 NADPH Cyp 特异性底物 (咪达唑仑用于 Cyp3A4) 的新鲜测定缓冲液稀释，并通过 LC-MS/MS(Q1/Q2 = m/z 342.2/203.2) 测量咪达唑仑羟基化的抑制。IC_{50}变化 >1.5 倍被认为对时间依赖性抑制是阳性的，其中 20min 预温育导致效力增加。使用醋竹桃霉素作为阳性对照化合物，展现出 TDI IC_{50}变化 >20。在从 50 μM 开始至 50nM(约 3 倍稀释系列) 的 7 点剂量响应曲线中对测试化合物进行测试。

[0397] 详细方案如下：

●在 1:1DMSO/MeOH 中制备测试化合物和阳性对照工作溶液 (100×)。

●将微粒体从 -80℃冰箱中取出以融化。

●制备温育混合物并向温育板的所有孔添加 147.5 μL。

●制备辅因子溶液和底物稀释溶液。

○向对应的孔添加 2.5 μL 的测试化合物和阳性对照工作溶液。最终化合物浓度在 50 μM 至 50nM 的 7 点剂量响应中。

○向 NIC 孔添加 2.5 μL 1:1DMSO/MeOH。

●将该板在 37℃下预热约 10min。

●向预温育孔添加 50μL 辅因子。

●向温育孔添加 50μL 的底物稀释溶液。

●在 37℃水浴下混合并预温育 20 分钟。

●向温育孔添加 50μL 辅因子；

○向预温育孔添加 50μL 的底物稀释溶液。

●在 37℃水浴下混合并温育 5 分钟。

●在该时间点，通过向所有孔添加 250μL IS 强化的终止溶液终止反应。

●将温育板以 4000rpm 离心 20 分钟。

●将 200μL 上清液转移至 200μL HPLC 水中并摇动 10min。

●通过 LCMS 分析样品。

[0398] 从该试验获得的化合物数据在表 3 中示出。

表 3

化合物编号	3A4-咪达唑仑 IC50 (-) NADPH	3A4-咪达唑仑 IC50 (+) NADPH	TDI 比：(-) NADPH/ (+) NADPH
15	>50 μM	41 μM	~1.2 (无 TDI)
42	>50 μM	>50 μM	~1 (无 TDI)
22	>50 μM	>50 μM	~1 (无 TDI)

PXR(Cyp 3A4) 活化试验

[0399] 通过采用 P450--Glo™ CYP3A4 试验，用萤光素—IPA 作为 CYP3A4 的底物，评价了 CYP3A4 代谢，并表示为 RLU（相对发光单位）。使用萤光素酶检测试剂 ONE--Glo™评价 PXR 活化并表示为 RLU。发光的光强度与 DPX2 细胞中 PXR 活化和伴随基因转录的程度成正比。在该试验中在 10、1 和 0.1μM 下测试化合物。诱导倍数＝（化合物处理的样品的 RLU/RFU)/（载体处理的样品的 RLU/RFU)，RFU 是细胞活力的信号。RLU 是 CYP3A 代谢和 PXR 活化的信号。使用 0.1％ DMSO 作为载体。通过使用 CellTiter--Fluor™检测细胞活力，并表示为 RFU（相对荧光单位）。

[0400] 活化效力被确定为阴性、弱、适中和强。阴性、弱、适中和强活化剂分别是达到 10μM RIF 在 10μM 下产生的响应的 <15％、<40％、<69％和 >70％的那些活化剂。

[0401] 从该试验获得的化合物数据在表 4 中示出。

表 4

化合物编号	PXR 活化（相比于利福平的诱导倍数）
15	～ 1x（无诱导）
42	～ 1.5x（无诱导）
22	～ 1.1x（无诱导）

白色假丝酵母抗真菌 MIC 效力测定

[0402] 采用野生型白色假丝酵母 (ATCC 10231)，氟康唑、两性霉素 B、伊曲康唑和特比萘芬从 Sigma 购买并用作阳性对照。

[0403] 所有测试化合物和氟康唑的最高测定浓度为 100μM。氟康唑还在 64μg/ml 的最高浓度下进行了测试，而两性霉素 B 和特比萘芬在 16 和 64μg/ml 下进行测试。测试化合物以 10mM 的浓度存在于 DMSO 储备溶液中。以 10mM 和 6.4mg/ml 制备氟康唑在 DMSO 中的两种储备溶液。以 1.6mg/ml 和 6.4mg/ml 制备两性霉素 B 和特比萘芬在 DMSO 中的储备溶液。

[0404] 100X 储备溶液的系列稀释：将 4μl 储备溶液添加至无菌 u 形底 96 孔板的行的第一孔中的 196μl RPMI1640(MOPS 缓冲的，并且没有 HEPES 和碳酸氢钠）中。其他孔用 100μl RPMI1640 填充。通过将 100μl 溶液转移至下一孔并通过移液混合，直至第 11 孔，连续制得 2 倍系列稀释液。将第 11 孔中额外的 100μl 丢弃。因此，化合物孔含有 100μl 在 RPMI1640 中的 2× 测试浓度的药物。第 12 孔仅用 100μl RPMI1640 填充。

[0405] 将白色假丝酵母 3147(ATCC 10231) 甘油冷冻储备物在 Sabouraud 右旋糖琼脂 (SDA) 上划线。将板在 35℃环境气氛下温育 20h。将单菌落悬浮在无菌盐水中，直至使用 Siemens 浊度仪测得浊度达到 0.1(1-5×10^6CFU/ml)。该悬浮液在 15ml 锥形管中的 RPMI1640 中稀释 50×，然后进一步在 50ml 锥形管中的 RPMI1640 中稀释 20×。这产生了 1-5×10^3CFU/ml 的悬浮液并将其用作接种物。对接种物中的细胞密度进行平板计数，为 4.94×10^3CFU/ml。

[0406] MIC 测定：在 15min 内，将 100μl 制备的细菌接种物添加至含化合物 /RPMI1640 的板的每个孔中。将板在 35℃和环境气氛下温育。在 24h 和 48h 时拍摄照片。根据 M27-A3 方案（临床和实验室标准化学会 (Clinical and Laboratory Standards Institute) 于 2008 年 1 月 1 日发表的 Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts；Approved Standard- 第三版，M27-A3 标准）读取氟康唑和两性霉素 B 的 MIC 终点。测试化合物和特比萘芬的化合物的 MIC（未在 M27-A3 方案中提及）按其为唑类读取。

[0407] 在该测定中测得的化合物数据在表 5 中示出。

表 5

化合物编号	24 h 时白色假丝酵母 MIC90 (μM)	48 h 时白色假丝酵母 MIC90 (μM)
15	>100	>100
19	0.195	0.195
21	>100	>100
22	>100	>100
23	>100	>100

化合物编号	24 h 时白色假丝酵母 MIC90 (μM)	48 h 时白色假丝酵母 MIC90 (μM)
26	>100	>100
27	>100	>100
30	>100	>100
32	>100	>100
36	>100	>100
41	>100	>100
42	>100	>100
49	>100	>100
54	>100	>100
79	>100	>100
84	>100	>100
100	0.781	1.563
101	>100	>100

小鼠药代动力学 (PK) 研究

[0408] 具有合适的体外 ADME 性质的化合物进一步进行体内 PK 研究。使用在小鼠中的快照 (snapshot) 形式快速评价先导候选物的口服可利用的潜力。快照形式涉及在标准载体 (例如,5% PEG/DMSO 水溶液) 中口服给予的单剂量 (通常为 20mg/kg)。将化合物直接注入 MS 分析中,以确定独特的 MRM(多反应监测) 信号,该信号以 ESI 阳性或阴性模式是浓度依赖性的。使用从有机溶液至水溶液的多种溶剂梯度进行 HPLC 分析,以确保期望的 Q1/Q2 质量的良好峰形。制剂包括 45%环糊精的水溶液 (如临床上针对伊曲康唑使用的)。可以使用的其他制剂为 solutol、Eudragit、MC/Tween 等。使用雌性小鼠肝素化的血浆,使用掺加不同浓度 (10ng/mL 至 2000ng/mL) 的化合物的血浆生成标准曲线,以确定 Q1/Q2 质量信号相对于化合物浓度的线性范围。对小鼠进行化合物的口服给药,通过眼眶后取血收集血浆样品。在 5 个时间点 (30min、1h、4h、8h、24h) 收集至肝素化的收集管中。进行蛋白质沉淀,化合物在冷乙腈中萃取并通过 MS 进行分析。如需要,将 Nonwinlin 用于 PK 建模。

[0409] 研究结果在表 6 中呈现。

表 6

化合物编号	PK 研究结果
42	在含水的 45%环糊精中 20 mg/kg: AUC_{0-24h} = 42.67 ± 4.90 μM*h C_{max} = 3.16 ± 0.4 μM
22	在 0.5% MC/0.5% TW80 悬浮液载体中 20 mg/kg: AUC0-24h = 2.21 ± 0.02 μM*h C_{max} = 0.17 ± 0.03 μM
22	在含水的 45%环糊精中 20 mg/kg: AUC0-24h = 8.08 ± 1.72 μM*h C_{max} = 1.71 ± 0.44 μM

实施例 II :动物模型

[0410] 为证明体内抗纤维化活性,建立了四氯化碳 (CCl_4) 诱发的肝及博来霉素诱发的肺和皮肤啮齿动物纤维化模型。这些体内试验耗时 6-8 周完成 (包括完整的组织学分析)。典型的实验由包括 6-8 只动物的组组成,这些动物用伊曲康唑 (2-4 个剂量)、1 或 2 种伊曲康唑类似物 (2-4 个剂量)、载体和基准治疗 (吡非尼酮和 AM-152) 来处理。AM-152(Amira Pharmaceuticals) 是 LPA1(溶血磷脂酸 1) 受体拮抗剂。该目标已经被描述为与小鼠博来霉素模型中 IPF 系统的恶化相关 (British Journal of Pharmacology(2010),160,1699 - 1713)。如先前已经在 WO 2012/078805 中更详细描述的,AM-152 在该目标上是在先的 (advanced) 化合物,因此将其用作阳性对照和本申请中公开的化合物的比较剂。

[0411] 博来霉素诱发的肺纤维化模型

[0412] 向九周龄的 B6 雄性小鼠 (Taconic farms) 中手术植入持续 7 天递送 25mg/kg 博来霉素的渗透泵。手术后 17 天,用药物治疗小鼠两周。如研究设计中描述的施用药物治疗 (图 14)。先前描述的抗纤维化药物 AM-152 和吡非尼酮被用作阳性对照药物。用 Masson 三色染色对肺切片进行染色,并且在扫描后,每个动物取八个随机区域进行分析。数据表示为平均值和标准差 (s.e.m.)(图 15a)。用于评价抗纤维化药物的模型的图示在图 15b 中示出。来自指定治疗组的 Masson 三色染色的肺的代表性图像在图 15c 中示出。根据改良的 Ashcroft 评分系统 (图 16),使用无偏差的自动化图像分析方法分析染色的肺切片。使用 ImageJ 中的自动化图像分析宏生成 Masson 三色染色总面积。然后将每个图像转化为 RGB 堆栈 (stack),并且设置染色面积的阈值,使得仅肺组织被包含在分析中而空的间隙如呼吸导管和肺泡被排除在外。然后使用 ImageJ 内的面积测量功能确定总染色面积。平均 Ashcroft 得分和平均染色面积百分比值表明,伊曲康唑及其类似物以剂量依赖性的方式减轻了该模型中的肺纤维化疾病严重程度 (图 17a 和图 17b)。在 10mg/kg(SID) 的剂量下,化合物 42 减轻了肺纤维化疾病严重程度,其效力与对照化合物 AM-152(30mg/kg BID) 和吡非尼酮 (400mg/kg,BID) 相比同样好或更好。

[0413] 四氯化碳诱发的肝纤维化模型

[0414] 如研究设计中描述的施用药物治疗(图18)。用天狼星红溶液对肝切片进行染色。扫描后,每只动物生成5幅随机图像。使用Image J中的自动化图像宏产生天狼星红染色阳性的总面积百分比。简而言之,每幅图像均转化为RGB堆栈,并且设置染色面积的阈值,使得分析中不包含细胞核。使用ImageJ内的面积测量功能定量确定总红色面积(图19a)。CCl_4诱发的肝纤维化模型的图像分析的数值数据在图19b中示出。CCl_4诱发的肝纤维化模型的天狼星红染色肝切片的代表性图像在图19c中示出。通过Western印迹分析肝中α平滑肌肌动蛋白(αSMA)的水平(图19d)。使用匀浆器和钢球将来自CCl_4诱发肝纤维化模型的肝的小片在PBS中匀浆化。通过离心丢弃残渣,并通过在260nm处的Nanodrop吸光度测定确定浓缩的裂解物。将等量的裂解物加载到每个凝胶泳道中,然后通过SDS-PAGE在10%Bis-Tris凝胶上分离,然后通过半干式转印仪转移到PVDF膜上。在含Tween-20(0.1%)的TRIS缓冲盐水中的5%乳中封闭后,将膜暴露于合适的第一抗体。将印迹与HRP偶联的第二抗体温育,并采用薄膜和SuperSignal West Dura化学发光底物(Pierce)使其可视化。天狼星红染色和Western印迹分析表明,伊曲康唑以剂量依赖的方式减轻肝纤维化疾病严重程度。

[0415] 啮齿动物创伤愈合模型

[0416] 使用标准啮齿动物创伤愈合模型,评价了伊曲康唑及其类似物对正常创伤愈合的影响(研究设计示于图20;结果在图21中)。该模型开始前五天,将小鼠麻醉并用Nair去除背部皮肤毛发。在第1天,将小鼠称重,并通过刺穿折叠的背部皮肤的整个厚度进行了一个无菌活检穿孔(5mm直径)(总共2x 5mm转盘(dial)孔)。使用卡尺每天测量创伤的大小。在整个研究期间每周监测体重两次。第1天直至研究结束,使用5ml/kg的给药体积每天施用药物治疗(伊曲康唑为25mg/kg,SID,化合物42或载体为25mg/kg SID)。在第7、11和14天,使来自每组的动物安乐死,并收集创伤用于组织学分析。收集创伤时,将创伤连同几mm的周围皮肤一起切出。对于每只动物,收集并固定两片创伤用于组织学分析。基于组织学分析和每天卡尺测量,相比于载体对照,伊曲康唑和化合物42对组织结构或正常创伤愈合速率没有影响。

实施例III:硬皮病的II期临床研究

[0417] 安慰剂对照的、随机化的双盲II期临床试验显示了优化的抗纤维化伊曲康唑类似物在弥漫性皮肤硬皮病或系统性硬皮病伴弥漫性皮肤累及的患者中的概念验证。满足入选标准的患者组成两个各30人的同等组。实验组每天被给予单次高剂量的药物持续6个月,而安慰剂比较组将每天给予安慰剂持续6个月。药物的单次高剂量根据临床前效力、临床前食蟹猴毒理学研究中的目标契合(engagement)和I期安全性研究来确定。主要结果评价是基于纳入时与每月访视之间的m-RSS(分数0-51,17个部位)的变化百分比,药物与安慰剂的效力比较。次要结果评价是基于纳入时与随访时间点(1、3和6个月)之间的m-RSS变化,药物与安慰剂的效力比较;目标契合的评价,其使用在纳入时和6个月时获得的皮肤活检物,通过hedgehog和VEGFR靶基因的表达谱分析来确定;使用皮肤活检物对纳入时和6个月时的皮肤厚度的评估;在系统性硬皮病患者中对非皮肤症状的治疗的评价;使用健康评价问卷和皮肤病学生活质量指数(Dermatology Quality of Life Index)对生活质量的评价;以及使用副作用的临床和实验室监测对治疗耐受性的评价(包括使用心脏

超声对阴性变力性效应的指征的评价）。如果 >40％的患者有 m-RSS 改善（定义为基线与 6 个月时的最后研究访视之间 m-RSS 下降≥ 5.3 单位），则将确定概念验证成功。

[0418] 精确的目标患者群体由诊断为弥漫性（或重度）皮肤硬皮病（改良的 Rodnan 皮肤得分 m-RSS ≥ 16/51）的患者组成。诊断为局部弥漫性皮肤硬皮病或系统性硬皮病伴弥漫性皮肤累及的患者（由美国风湿病学会 (American College of Rheumatology) 确定的）是已知的患者亚组。

[0419] II 期概念验证研究的患者招募的入选和排除标准如下。患者为 18 岁或以上，有皮肤或系统性硬皮病的诊断记录。需要指示弥漫性皮肤硬皮病的≥ 16/51 的基线 m-RSS。入选需要入选前的心脏超声射血分数得分大于 55％（即正常）。如果患者在试验开始前的 3 个月内已接受有可能干扰病程的药物（例如，甲氨蝶呤、皮质类固醇、环磷酰胺、波生坦）的治疗，则患者将被排除。患有严重器官衰竭、慢性肝病（例如，肝硬化、慢性肝炎）、癌症、慢性疾病（例如，类风湿性关节炎、系统性红斑狼疮、糖尿病、HIV）或具有异常血液化学的患者将被排除。在入选前不到 4 周内有过重大手术的患者将被排除。如产品说明书中说明的，禁忌伊曲康唑的患者将被排除。具体而言，有心室功能障碍（例如，充血性心力衰竭，CHF）的迹象、有 CHF 风险或已经接受变力性药物治疗的患者将被排除。在入选前不到 6 个月内发生心肌梗死的患者将被排除。

[0420] 确定并选择了包含在 II 期概念验证研究中的患者，以在美国的多个地点与皮肤科临床医生合作治疗。作为硬皮病临床试验团体 (Scleroderma Clinical Trials Consortium) 成员的协调研究者监督患者的招募并担任研究的主席。患者被诊断为局部弥漫性皮肤硬皮病或系统性硬皮病伴弥漫性皮肤累及。基于硬皮病诊断（即，局部的或系统性的）、性别和年龄对患者进行分层，以确保治疗群体内的同等分布。另外，基于皮肤累及的严重程度对患者进行分层，以确保治疗群体内有相等人数的患者具有严重的皮肤累及 (m-RSS ≥ 20/51)。该分层需要包括皮肤硬化度量和皮肤活检的初始诊断试验。患者需在入选前经历心脏超声诊断试验，以确保心脏功能正常（射血分数得分 >55％）。

[0421] 由于目前没有直接指明抑制硬皮病患者中的纤维化的疗法，因此在患者的研究中没有使用比较剂。新药剂单独给药。监测施用任何药物的患者，以确保新药剂不改变所用药物的药代动力学和代谢性质。

[0422] 确定改良 Rodnan 皮肤得分 (m-RSS)，使用可测量的和经验证的生物标志物来评价在硬皮病患者中的临床效力。简而言之，将总皮肤表面任意地分成 17 个部位。在每一区域中，采用手动触诊来估计皮肤得分。皮肤得分基于皮肤增厚程度从 0 至 3 变化 (1，未累及；2，轻度；2，中度；3，重度）。总皮肤得分为 17 个区域中的每一个的得分之和（最大得分为 51)。得分在 16 到 19 之间的患者被分类为弥漫性的，而得分≥ 20 的患者被分类为重度的。该皮肤评分系统已经被证明与真皮纤维化的程度非常好地相关，并且与内脏器官的纤维化 / 功能障碍的程度（在系统性硬皮病患者红）也很好地相关。皮肤中的目标契合可以通过使用皮肤活检监测 hedgehog 和 VEGFR 靶基因的表达变化而评估。

实施例 IV：特发性肺纤维化的 II 期临床研究

[0423] 本研究的目的是确定式 (I) 和式 (II) 化合物相比于安慰剂，在治疗特发性肺纤维化中的安全性和有效性。该临床试验是干预性的。该临床研究参与者的分配是随机化的；干预模型为平行指定；并且该研究为双盲遮掩（受试者、照护者、研究者）。本临床研究主

要测量用力肺活量的变化率，而次要地基于不良事件、生命体征和临床实验室检测评价安全性。

[0424] 患者招募的入选标准如下：

●随机化时，性别不限，年龄为 40 至 80 岁（含）。

●具有与特发性肺纤维化（IPF）吻合的临床症状。

●在随机化前至少 6 个月并且不超过 48 个月内，已经第一次接受了 IPF 的诊断。诊断日期被定义为第一次可获得的与 IPF/UIP 吻合的 HRCT 或外科肺活检的日期。

●通过高分辨率计算机断层扫描（HRCT）或外科肺活检（SLB）诊断为普通型间质性肺纤维化（UIP）或 IPF。

●在 HRCT 扫描上，纤维化变化（蜂窝状、网状变化）的程度大于肺气肿的程度。

●如果进行，在支气管活检、BAL 或 SLB 上没有支持替代诊断的特征。

●筛选时，预测的支气管扩张剂后 FVC 百分比为 50％至 80％（含）。

●在筛选时与第一天之间，支气管扩张剂后 FVC（以升测量）有变化，该变化小于 10％相对差异，计算为：100％绝对值（筛选 FVC(L)- 第 1 天 FVC(L))/ 筛选时 FVC(L)。

●筛选时，一氧化碳弥散量（DLCO）为 30％至 80％（含）（针对血红蛋白和高度调整）。

●在研究者看来，在之前的一年中，IPF 疾病严重程度的测量值没有改善的迹象。

●筛选时，在 6 分钟行走测试（6MWT）中能够行走 150 米或更远。

●筛选时，显示出在 6MWT 过程中氧饱和度降低 2 个百分点或更多（可以用补充氧气滴定法进行，以保持氧饱和度水平 >88％）。

●能够理解并签署书面知情同意书。

●能理解坚持研究治疗和研究方案的重要性，并愿意在整个研究过程中遵守所有的研究要求，包括伴随用药限制。

●有生育能力的女性（WOCBP）和与 WOCBP 具有活跃性行为的男性必须使用可接受的避孕方法。

[0425] 患者招募的排除标准如下：

i）目标疾病排除

(1) 在研究者看来，在筛选时与第一天之间（在筛选过程中）IPF 有显著的临床恶化。

(2) 筛选时，施用支气管扩张剂后 1 秒内用力呼气量（FEV1)/FVC 比小于 0.8。

(3) 筛选时，具有支气管扩张剂响应，其被定义为相比于使用支气管扩张剂前的值，在使用支气管扩张剂后 FEV1 或 FVC 或两者有 12％或更多的绝对增加，以及 200mL 的增加。

ii）医疗史及并发疾病

(1) 具有已知会导致肺纤维化的临床显著的环境暴露史。

(2) 具有已知的对间质性肺病的解释。

(3) 有任何结缔组织疾病的临床诊断。

(4) 当前具有临床显著的哮喘或慢性阻塞性肺疾病。

(5) 有活动性感染的临床证据。

(6) 有任何可能导致显著失能或可能在未来 2 年内需要显著医疗或手术干预的恶性肿瘤史。这不包括针对局部癌症（例如，基底细胞癌）的小手术过程。

(7) 在研究者看来，有除 IPF 外的，可能导致受试者在未来 2 年内死亡的任何病状。

(8) 有终末期肝病史。

(9) 有需要透析的终末期肾病史。

(10) 在之前的 6 个月内,有不稳定或恶化的心脏或肺疾病(除 IPF 外)史。

(11) 在过去两年内,有酒精或药物滥用史。

(12) 有长 QT 综合征和 / 或扭转性室速(多形性室性心动过速)的家族史或个人史。

(13) 筛选前 7 日内已经使用了任何以下具体疗法 :

(a) 研究性疗法,定义为在参与地点的国家中尚未批准上市用于任何适应症的任何药物。

(b) 任何细胞毒性的、免疫抑制的、细胞因子调节的或受体 - 拮抗剂药剂,包括但不限于硫唑嘌呤、波生坦 (bosetan)、安立生坦、环磷酰胺、环孢菌素、依那西普、伊洛前列素、英夫利昔单抗、白三烯拮抗剂、甲氨喋呤、吗替麦考酚酯、他罗利姆、孟鲁司特、四硫钼酸盐、肿瘤坏死因子 α 抑制剂、NAC、甲磺酸伊马替尼、干扰素 γ-1b、吡非尼酮和酪氨酸激酶抑制剂。

(c) 秋水仙素、肝素和华法林。如果对于同一适应症没有临床上可接受的替代疗法,如果是针对非 IPF 适应症给予,则可以使用西地那非(每日使用);针对勃起功能障碍的间歇使用是允许的。

(d) 针对急性呼吸系统恶化间歇使用皮质类固醇是允许的。

(e) 局部和眼科使用的酮康唑、环孢菌素和类固醇是允许的。

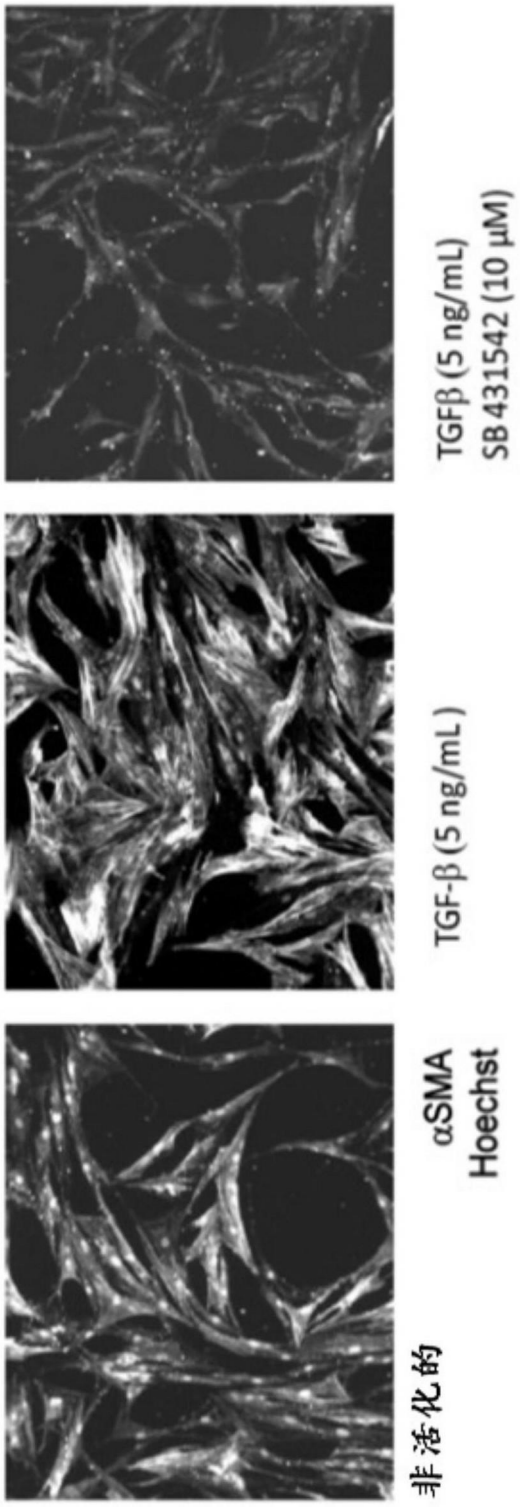


图 1

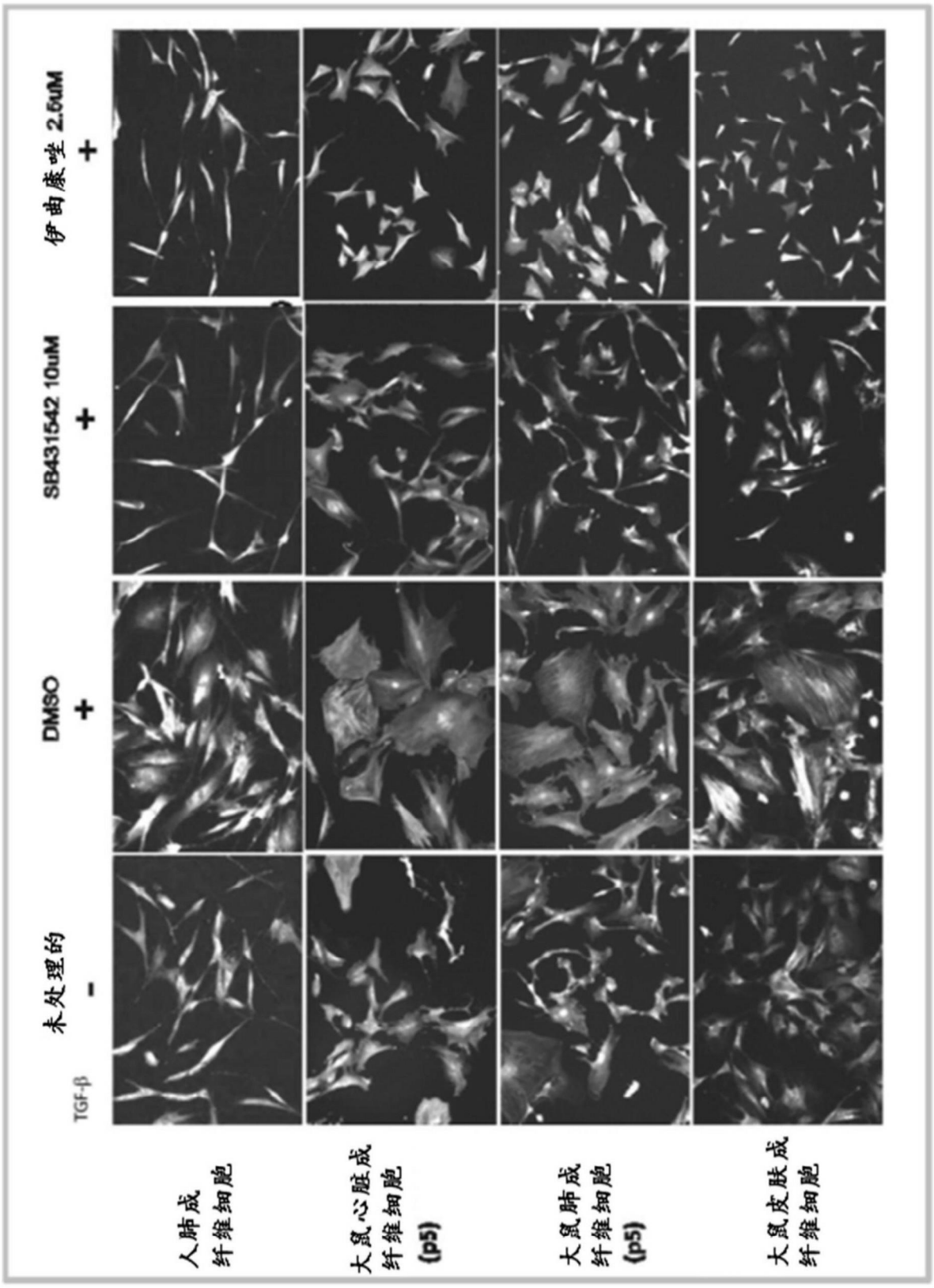

未处理的
DMSO
SB431542 10uM
伊曲康唑 2.5uM
TGF-β
-
+
+
+
人肺成纤维细胞
大鼠心脏成纤维细胞(p5)
大鼠肺成纤维细胞(p5)
大鼠皮肤成纤维细胞

图 2

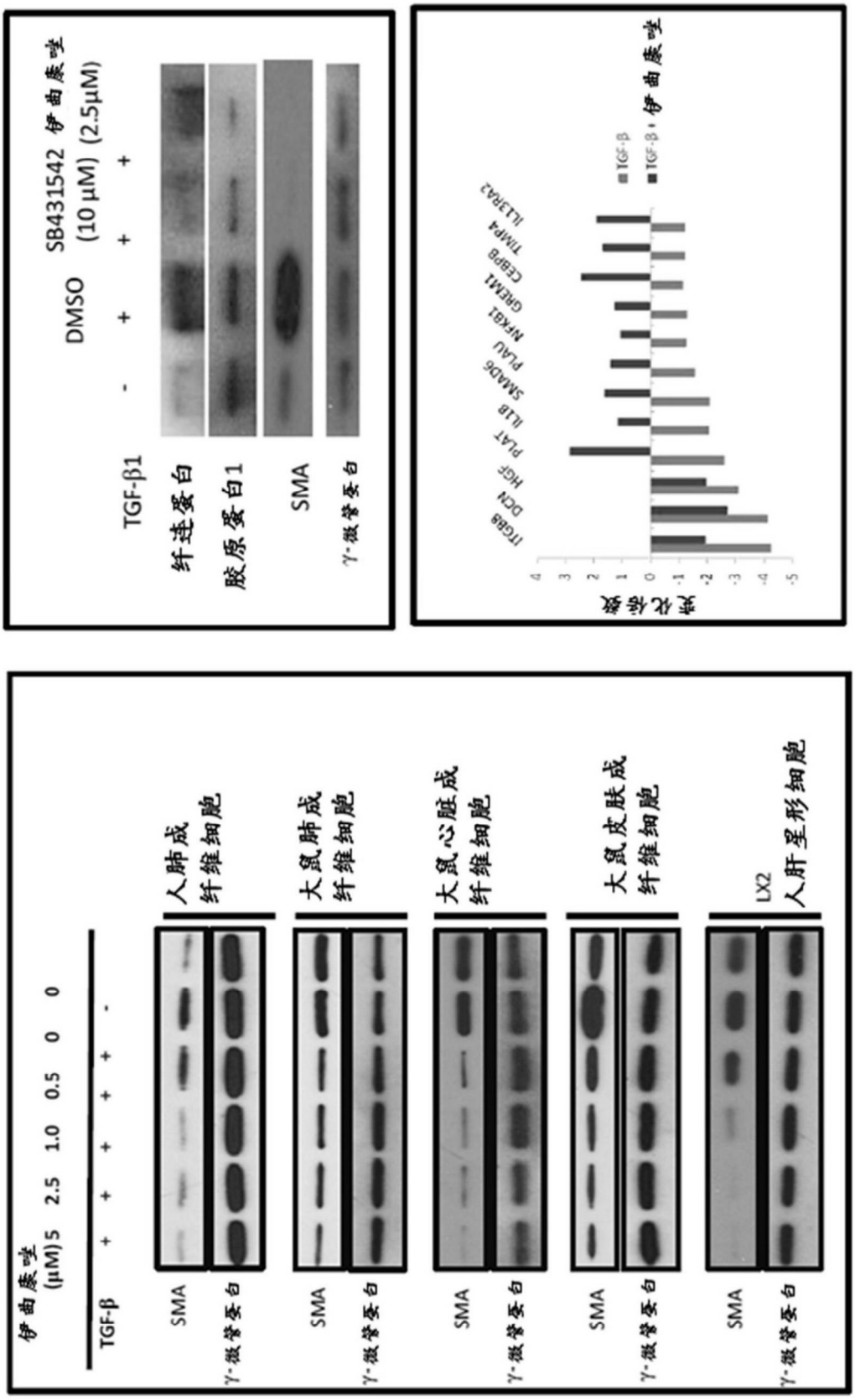

伊曲康唑 (μM) 5 2.5 1.0 0.5 0 0
TGF-β + + + + + -
SMA
γ-微管蛋白
人肺成纤维细胞
大鼠肺成纤维细胞
大鼠心脏成纤维细胞
大鼠皮肤成纤维细胞
LX2
人肝星形细胞
DMSO SB431542 (10 μM) 伊曲康唑 (2.5μM)
TGF-β1 - + + +
纤连蛋白
胶原蛋白1
SMA
γ-微管蛋白
TGF-β
TGF-β，伊曲康唑
ITGB8
DCN
HGF
PLAT
IL1B
SMAD6
PLAU
NFKBI
GREM1
CEBPB
TIMP4
IL13RA2

图 3

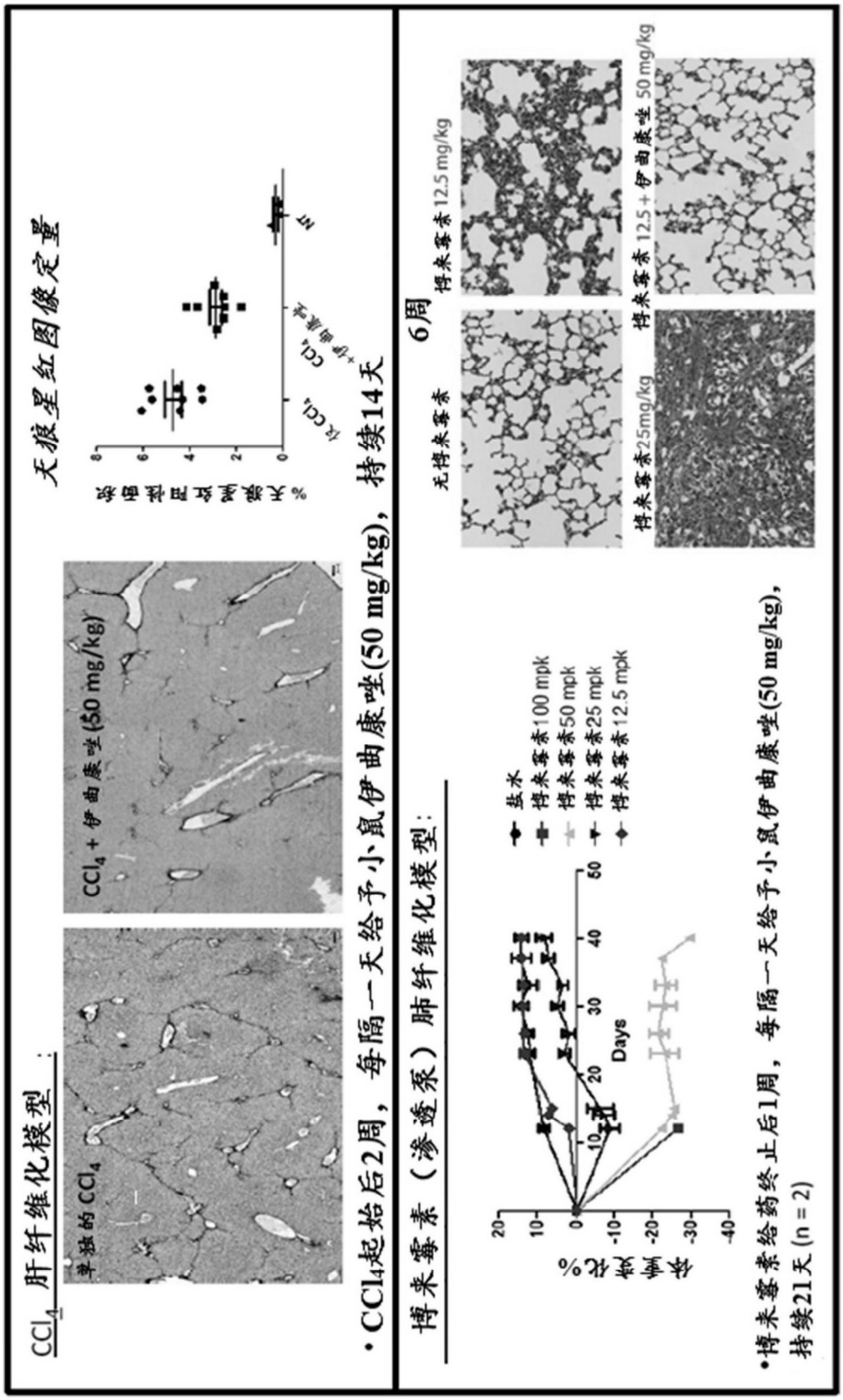

CCl_4 肝纤维化模型：
单独的 CCl_4
CCl_4 + 伊曲康唑(50 mg/kg)
天狼星红图像定量
• CCl_4起始后2周，每隔一天给予小鼠伊曲康唑(50 mg/kg)，持续14天
博来霉素（渗透泵）肺纤维化模型：
盐水
博来霉素100 mpk
博来霉素50 mpk
博来霉素25 mpk
博来霉素12.5 mpk
Days
6周
无博来霉素
博来霉素12.5 mg/kg
博来霉素25mg/kg
博来霉素12.5 + 伊曲康唑 50 mg/kg
•博来霉素给药终止后1周，每隔一天给予小鼠伊曲康唑(50 mg/kg)，持续21天 (n = 2)

图 4a

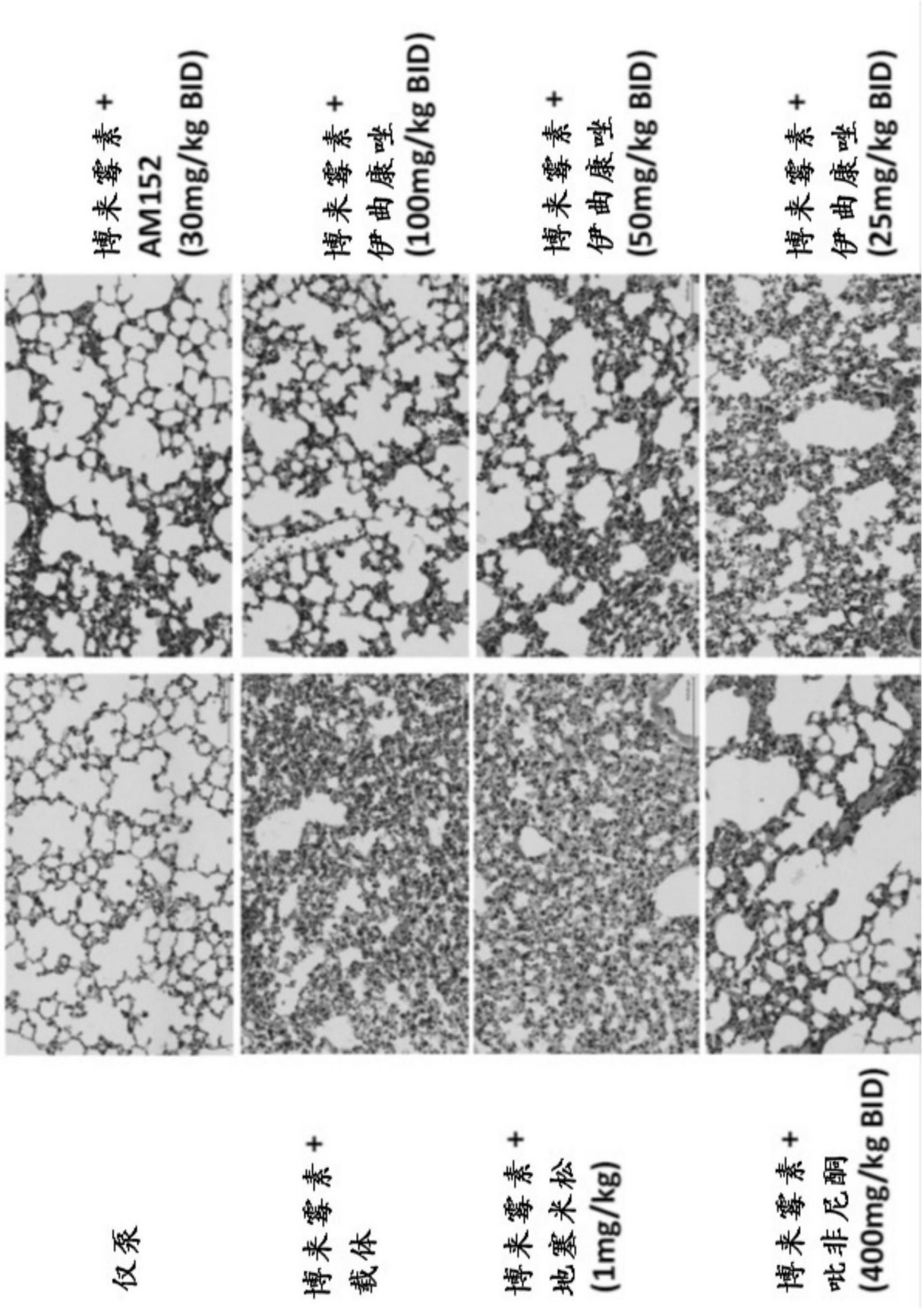
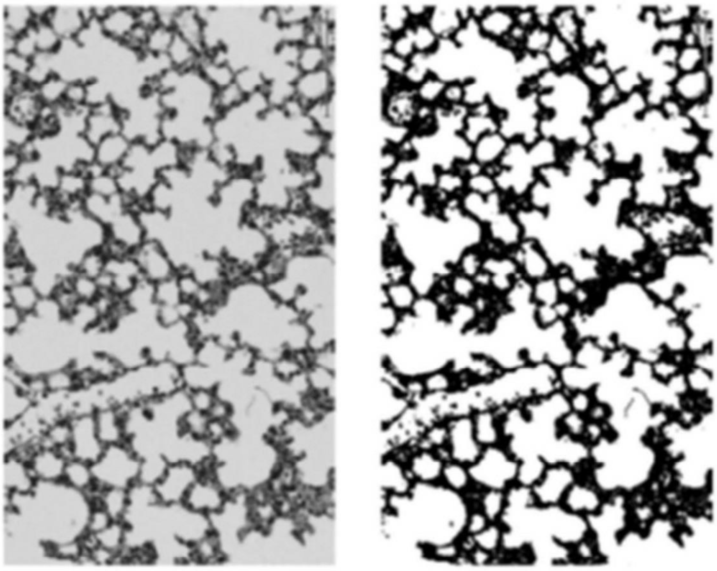

博来霉素 + AM152 (30mg/kg BID)
博来霉素 + 伊曲康唑 (100mg/kg BID)
博来霉素 + 伊曲康唑 (50mg/kg BID)
博来霉素 + 伊曲康唑 (25mg/kg BID)
仅泵
博来霉素 + 载体
博来霉素 + 地塞米松 (1mg/kg)
博来霉素 + 吡非尼酮 (400mg/kg BID)

图 4b



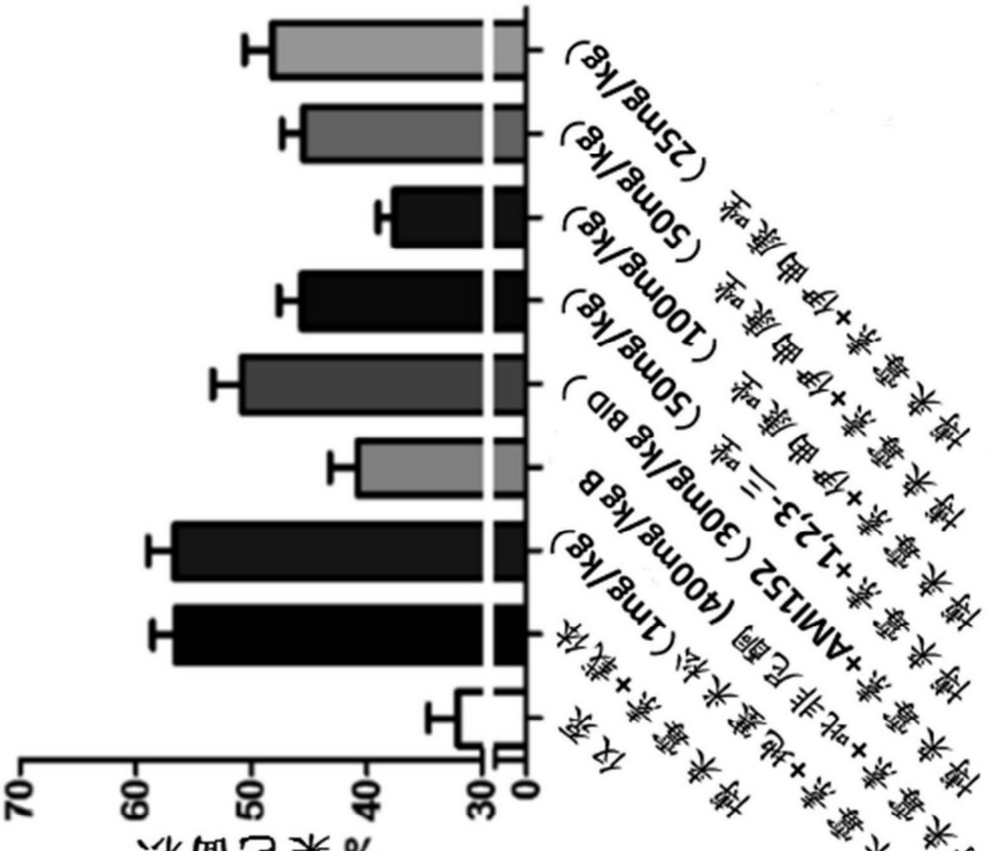


图 4c

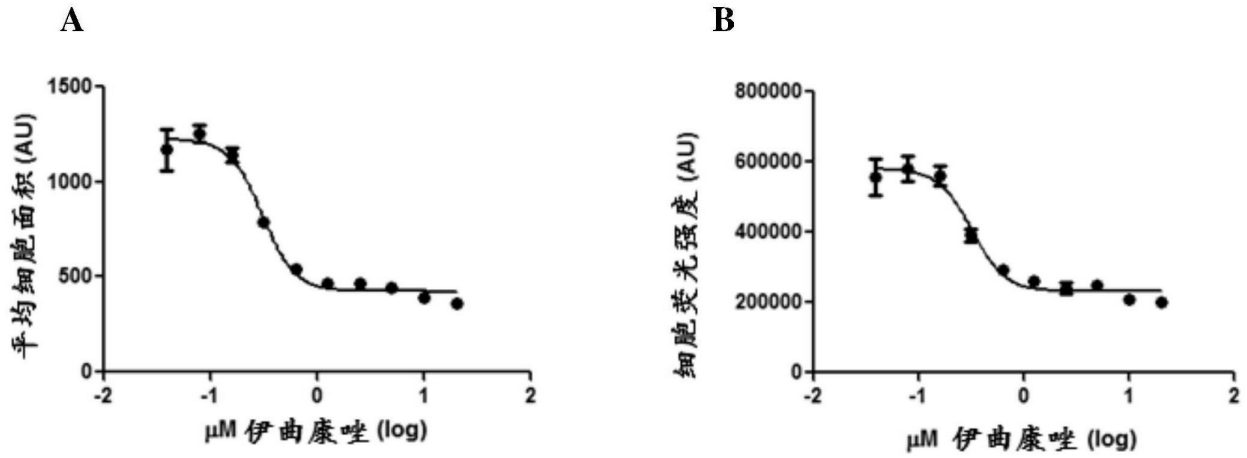

A
B
平均细胞面积 (AU)
1500
1000
500
0
-2
-1
0
1
2
μM 伊曲康唑 (log)
细胞荧光强度 (AU)
800000
600000
400000
200000
0
-2
-1
0
1
2
μM 伊曲康唑 (log)

图 5

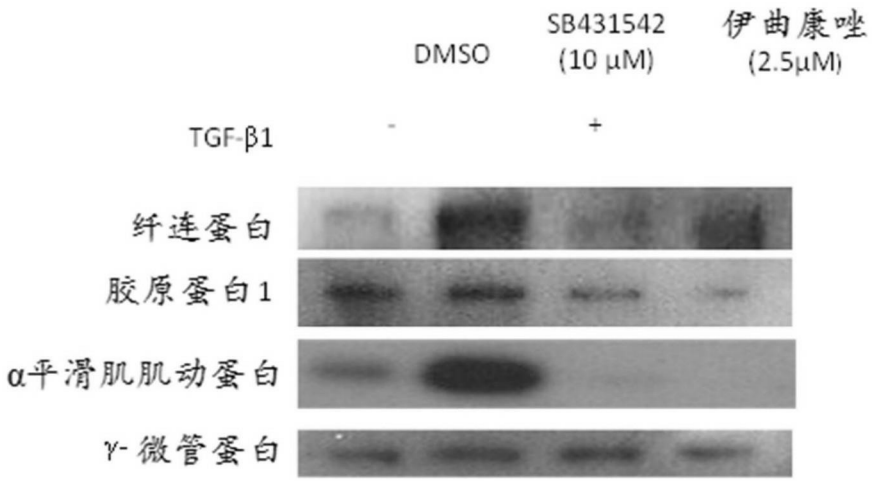
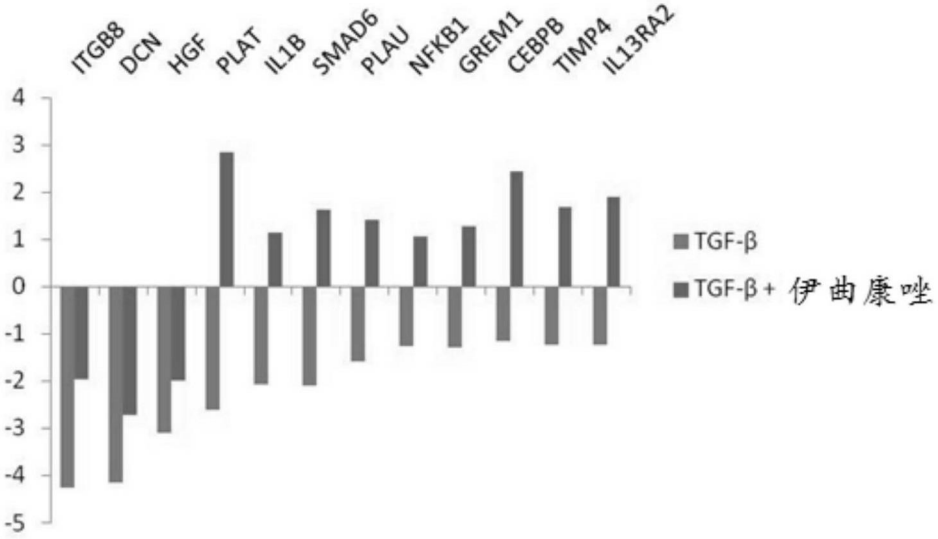

DMSO
SB431542
(10 μM)
伊曲康唑
(2.5μM)
TGF-β1
-
+
纤连蛋白
胶原蛋白1
α平滑肌肌动蛋白
γ-微管蛋白

图 6

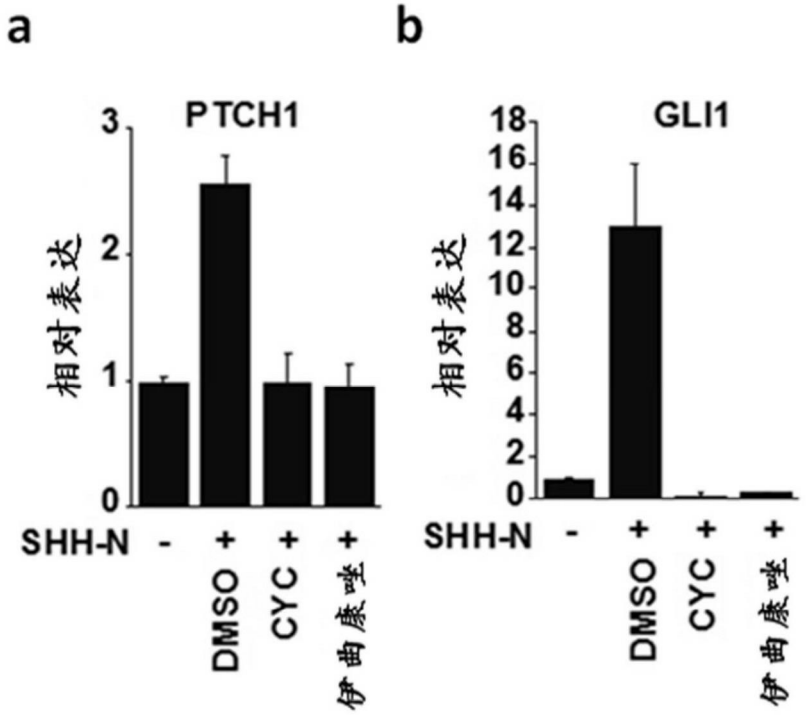
a



b

	TGF-β	TGF-β + CBR-034-1
ITGB8	-4.26	-1.96
DCN	-4.14	-2.72
HGF	-3.09	-2.00
PLAT	-2.61	2.84
IL1B	-2.06	1.15
SMAD6	-2.11	1.61
PLAU	-1.58	1.41
NFKB1	-1.26	1.05
GREM1	-1.28	1.28
CEBPB	-1.16	2.44
TIMP4	-1.25	1.67
IL13RA2	-1.25	1.90

图 7



c

PTCH1 基因表达				
Hh 诱导刺激物	抑制剂	平均RLQ	标准偏差	N
未处理的	未处理的	1.00	0.03	3
Shh-N (1mg/mL)	DMSO	2.55	0.24	3
Shh-N (1mg/mL)	5μM 环杷明	1.00	0.22	3
Shh-N (1mg/mL)	1μM 伊曲康唑	0.96	0.18	3

GLI1 基因表达				
Hh 诱导刺激物	抑制剂	平均RLQ	标准偏差	N
未处理的	未处理的	1.00	0.04	3
Shh-N (1mg/mL)	DMSO	13.00	2.92	3
Shh-N (1mg/mL)	5μM 环杷明	0.21	0.15	3
Shh-N (1mg/mL)	1μM 伊曲康唑	0.32	0.02	3

图 8

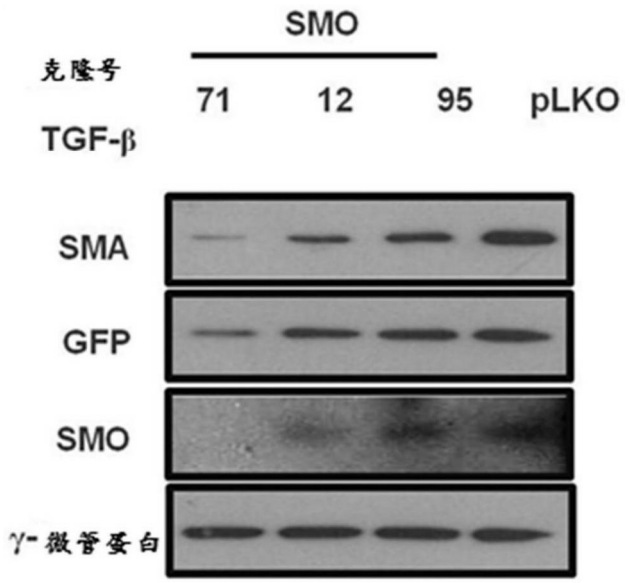

SMO
克隆号
71
12
95
pLKO
TGF-β
SMA
GFP
SMO
γ-微管蛋白

图 9

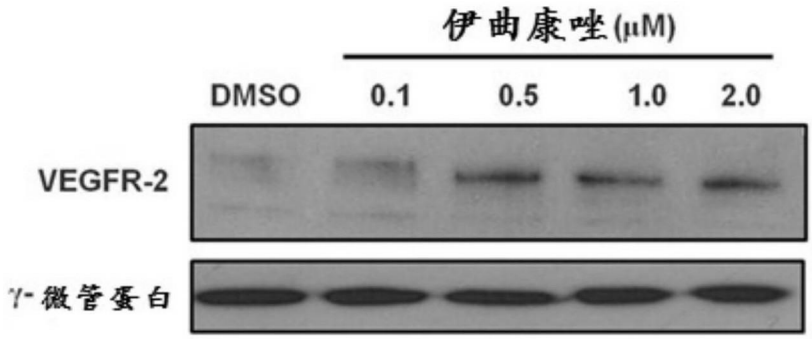

伊曲康唑(μM)
DMSO
0.1
0.5
1.0
2.0
VEGFR-2
γ-微管蛋白

图 10

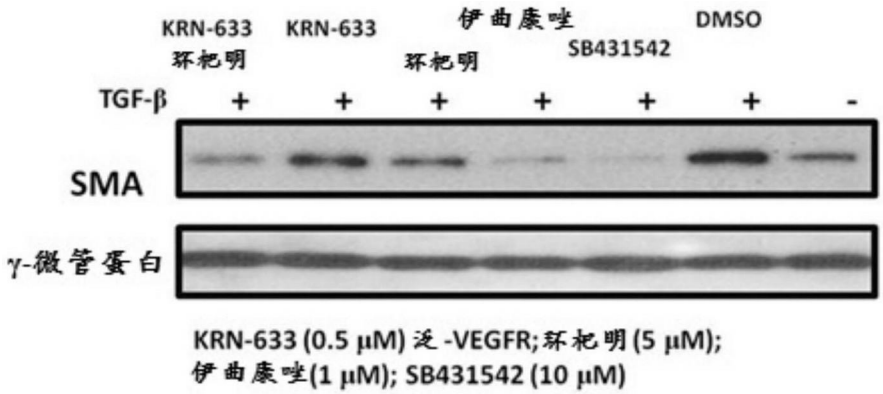

KRN-633
环杷明
KRN-633
伊曲康唑
环杷明
SB431542
DMSO
TGF-β
+
+
+
+
+
+
-
SMA
γ-微管蛋白
KRN-633 (0.5 μM) 泛 -VEGFR;环杷明 (5 μM);
伊曲康唑(1 μM); SB431542 (10 μM)

图 11

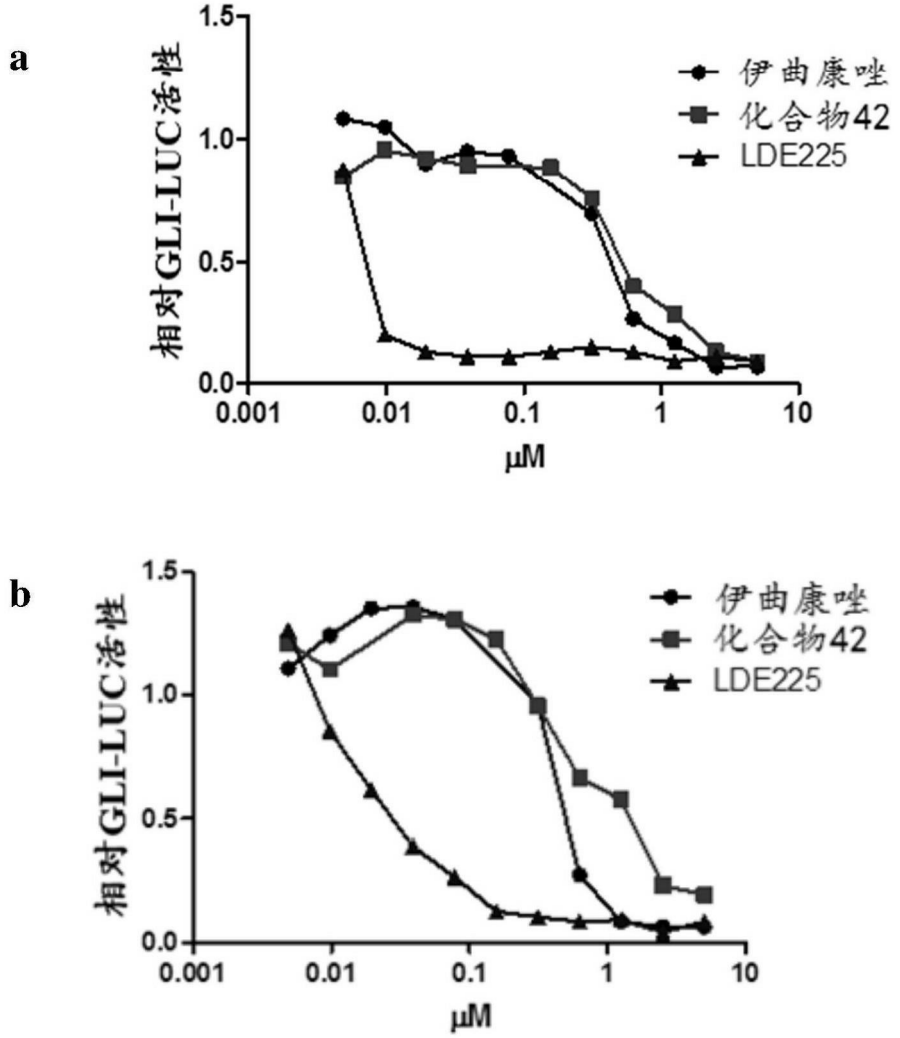

a
相对GLI-LUC活性
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
μM
伊曲康唑
化合物42
LDE225
b
相对GLI-LUC活性
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
μM
伊曲康唑
化合物42
LDE225

图 12

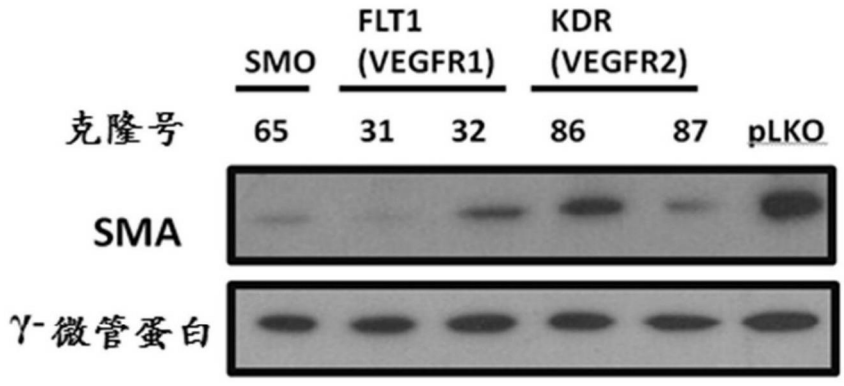

SMO
FLT1 (VEGFR1)
KDR (VEGFR2)
克隆号
65
31
32
86
87
pLKO
SMA
γ-微管蛋白

图 13

实验组：

- A-载体植入
- B-25 mg/kg 博来霉素+载体 1 (45% -环糊精) SID
- C-25 mg/kg 博来霉素+载体 2 (0.5% MC/0.5% Tween-80) BID
- D-25 mg/kg 博来霉素+伊曲康唑 5 mg/kg SID
- E-25 mg/kg 博来霉素+伊曲康唑 10 mg/kg SID
- F-25 mg/kg 博来霉素+伊曲康唑 25 mg/kg SID
- G-25 mg/kg 博来霉素+伊曲康唑 50 mg/kg SID
- H-25 mg/kg 博来霉素+化合物 42 @ 5 mg/kg SID
- I-25 mg/kg 博来霉素+化合物 42 @ 10 mg/kg SID
- J-25 mg/kg 博来霉素+化合物 42 @ 25 mg/kg SID
- K-25 mg/kg 博来霉素+化合物 42 @ 50 mg/kg SID
- L-25 mg/kg 博来霉素+吡非尼酮 400 mg/kg BID
- M-25 mg/kg 博来霉素+ AMI 152 30 mg/kg BID

实验设计：

- 104 - 9 周龄 B6 雄性小鼠(Taconic Farms)；n=8 只/组
- 之前 2 天：称重并单独笼养雄性 B6 小鼠，总共 104 只，每组 8 只
- 第 1 天：称重，将 25 mg/kg 博来霉素填充至 200ul 7 天 Alzet 渗透泵中，准备好经填充的泵
- 第 1-2 天：手术植入泵，8 只小鼠使用盐水，96 只小鼠使用博来霉素
- 第 1-10 天：术后监测/称重小鼠，每周 3 次
- 第 17-31 天：药物治疗
- 第 30 天：在倒数第二次给药后 1、4、8、24 小时取血
- 第 31 天：使动物安乐死并收集多个器官。肺的一半将被固定用于组织学研究，另一半将被收集在液 N2 上并储存在-80℃用于生化研究。一片皮肤将被固定用于组织学分析而另一片皮肤将收集在液 N2 上并储存在-80℃用于生化研究。

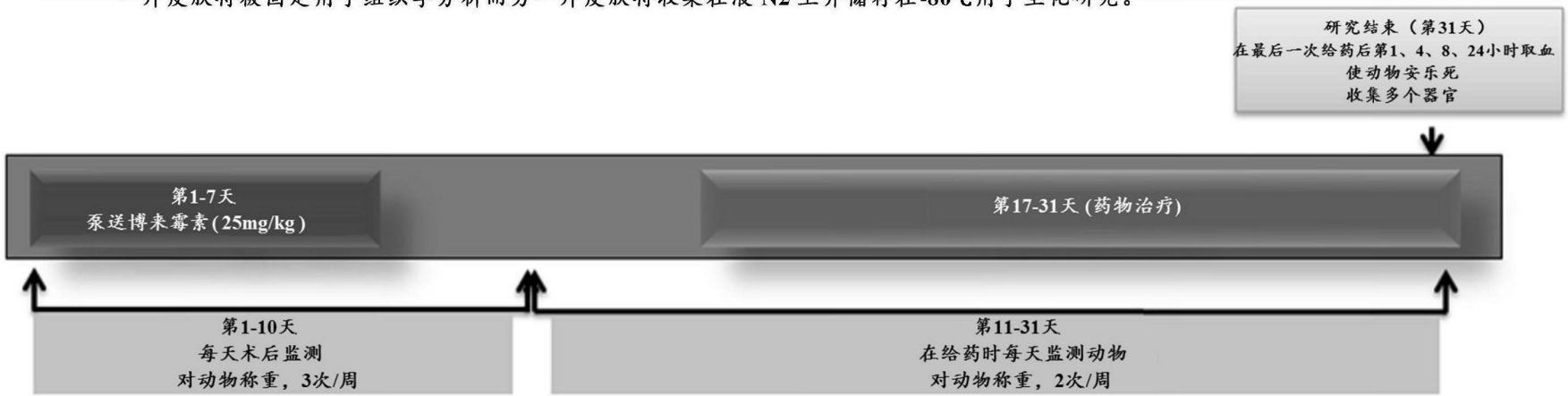


图 14

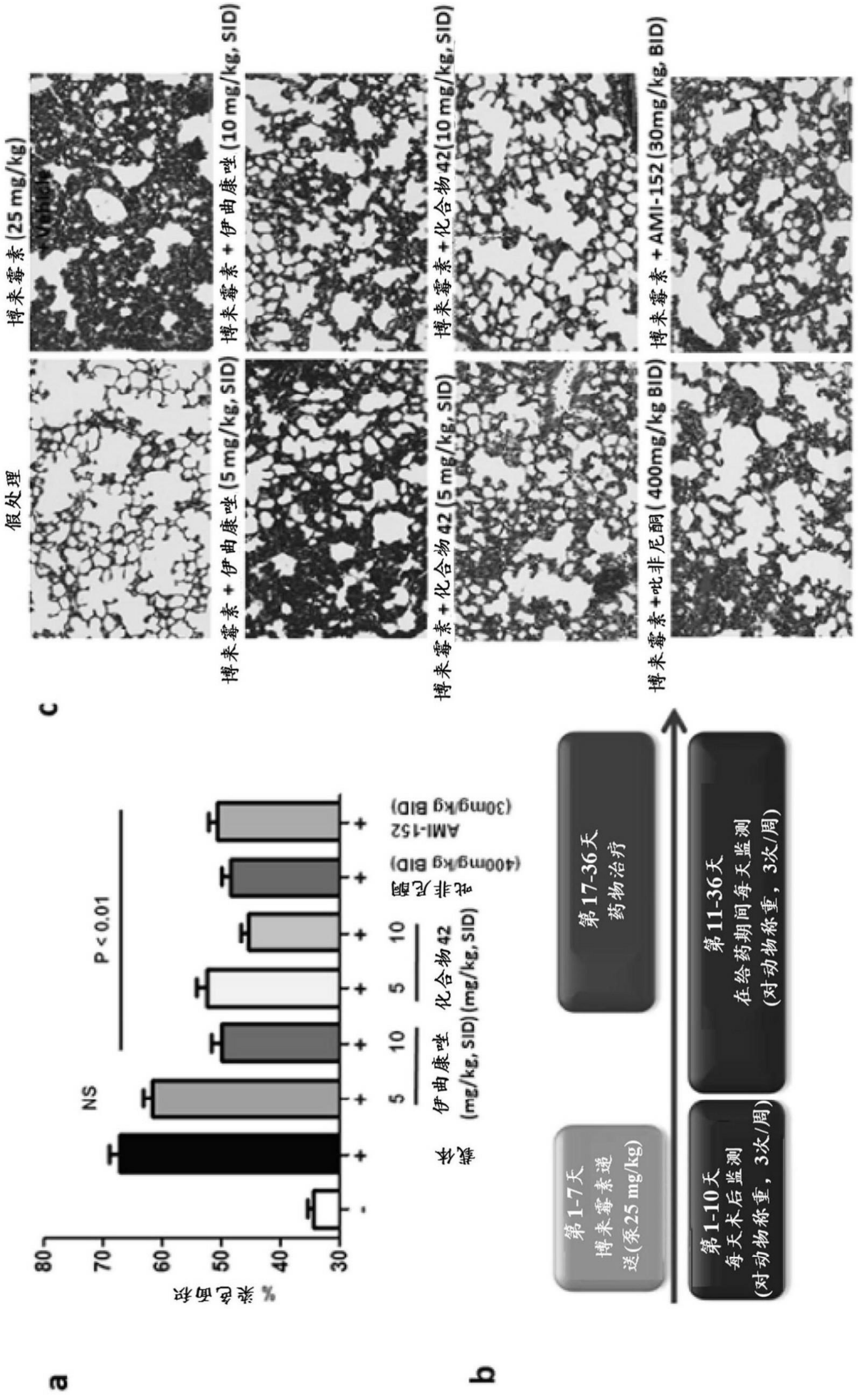

a
b
c
% 染色面积
80
70
60
50
40
30
NS
P < 0.01
伊曲康唑 (mg/kg, SID)
化合物42 (mg/kg, SID)
吡非尼酮 (400mg/kg BID)
AMI-152 (30mg/kg BID)
第1-7天 博来霉素递送(泵25 mg/kg)
第1-10天 每天术后监测 (对动物称重，3次/周)
第17-36天 药物治疗
第11-36天 在给药期间每天监测 (对动物称重，3次/周)
假处理
博来霉素 (25 mg/kg) + Vehicle
博来霉素 + 伊曲康唑 (5 mg/kg, SID)
博来霉素 + 伊曲康唑 (10 mg/kg, SID)
博来霉素 + 化合物42 (5 mg/kg, SID)
博来霉素 + 化合物42 (10 mg/kg, SID)
博来霉素 + 吡非尼酮 (400mg/kg BID)
博来霉素 + AMI-152 (30mg/kg, BID)

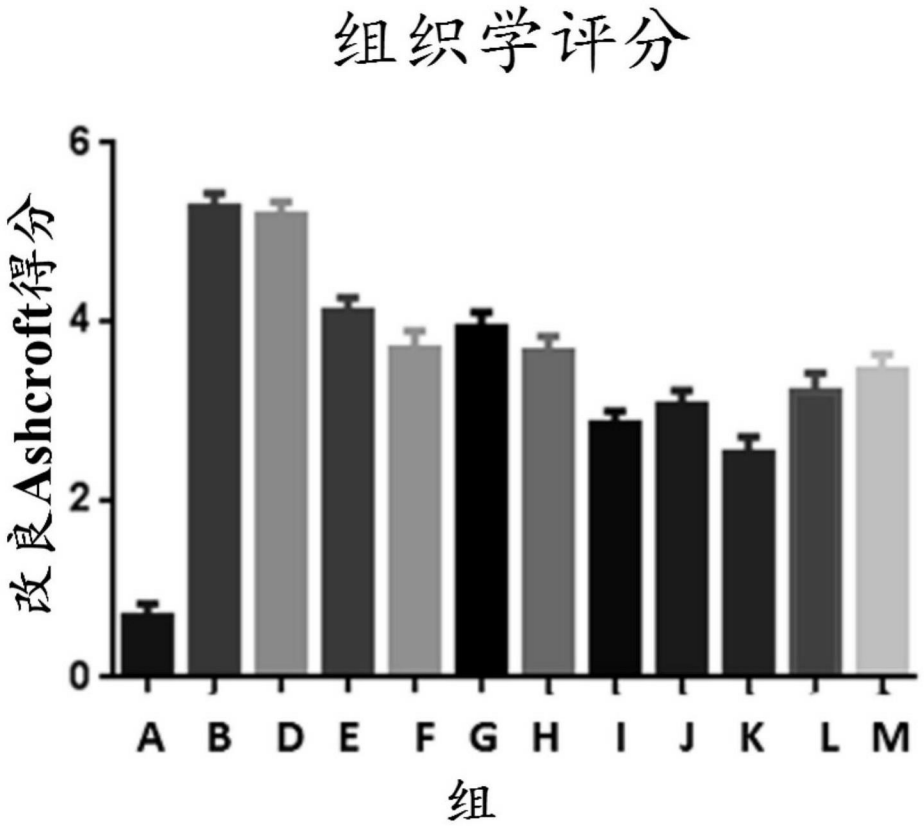
图 15

改良的Ashcroft量表的表征

纤维化等级	改良的Ashcroft量表
0	**肺泡隔：** 在一些肺泡壁中最脆弱的小纤维处无纤维化负荷 **肺结构：** 正常肺
1	**肺泡隔：** 孤立的轻度纤维化变化（隔膜比正常的厚≤ 3X），有结状形成但彼此不相连 **肺结构：** 肺泡部分增大和稀薄，但没有纤维化团块
2	**肺泡隔：** 孤立的轻度纤维化变化（隔膜比正常的厚≤ 3X），有结状形成但彼此不相连 **肺结构：** 肺泡部分增大和稀薄，但没有纤维化团块
3	**肺泡隔：** 整个显微镜视野中主要是连续的纤维化壁（隔膜比正常的厚>3X） **肺结构：** 肺泡部分增大和稀薄，但没有纤维化团块
4	**肺泡隔：** 可变的 **肺结构：** 单个纤维化团块（≤显微镜视野的10%）
5	**肺泡隔：** 可变的 **肺结构：** 汇合的纤维化团块（>显微镜视野的10%但< 50%）。肺结构严重受损但仍保持
6	**肺泡隔：** 可变的，大部分不存在 **肺结构：** 大的汇合的纤维化团块（>显微镜视野的10%但< 50%）。肺结构大部分没有保持
7	**肺泡隔：** 不存在 **肺结构：** 肺泡几乎被纤维化团块彻底破坏，但仍有至多五个气泡
8	**肺泡隔：** 不存在 **肺结构：** 显微镜下遍布被纤维化团块完全破坏的结构

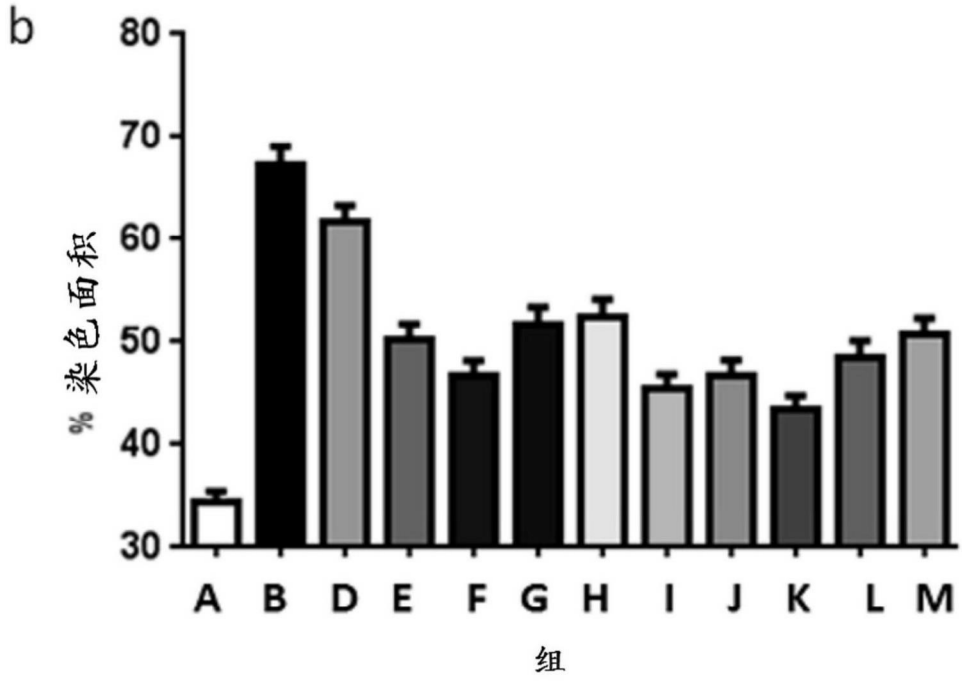
图 16

a



A- 载体植入
B-25 mg/kg 博来霉素 + 载体1 (45% β-环糊精) SID
D-25 mg/kg 博来霉素 + 伊曲康唑 5 mg/kg SID
E-25 mg/kg 博来霉素 + 伊曲康唑 10 mg/kg SID
F-25 mg/kg 博来霉素 + 伊曲康唑 25 mg/kg SID
G-25 mg/kg 博来霉素 + 伊曲康唑 50 mg/kg SID
H-25 mg/kg 博来霉素 + 化合物42 @ 5 mg/kg SID
I-25 mg/kg 博来霉素 + 化合物42 @ 10 mg/kg SID
J-25 mg/kg 博来霉素 + 化合物42 @ 25 mg/kg SID
K-25 mg/kg 博来霉素 + 化合物42 @ 50 mg/kg SID
L-25 mg/kg 博来霉素 + 吡非尼酮 400 mg/kg BID
M-25 mg/kg 博来霉素 + AMI 152 30 mg/kg BID

图 17a



A- 载体植入
B-25 mg/kg 博来霉素 + 载体 1 (45% β-环糊精) SID
D-25 mg/kg 博来霉素 + 伊曲康唑 5 mg/kg SID
E-25 mg/kg 博来霉素 + 伊曲康唑 10 mg/kg SID
F-25 mg/kg 博来霉素 + 伊曲康唑 25 mg/kg SID
G-25 mg/kg 博来霉素 + 伊曲康唑 50 mg/kg SID
H-25 mg/kg 博来霉素 + 化合物 42 @ 5 mg/kg SID
I-25 mg/kg 博来霉素 + 化合物 42 @ 10 mg/kg SID
J-25 mg/kg 博来霉素 + 化合物 42 @ 25 mg/kg SID
K-25 mg/kg 博来霉素 + 化合物 42 @ 50 mg/kg SID
L-25 mg/kg 博来霉素 + 吡非尼酮 400 mg/kg BID
M-25 mg/kg 博来霉素 + AMI 152 30 mg/kg BID

图 17b

实验组:
A-0.4 ml/kg CCl4 + 无处理,n=8
B-0.4 ml/kg CCl4 + 伏立康唑,10 mg/kg, SID, n=8
C-0.4 ml/kg CCl4 + 伏立康唑,20 mg/kg, SID, n=8
D-0.4 ml/kg CCl4 + 伏立康唑,50 mg/kg, SID, n=8
实验设计:
32 - 9周龄B6雄性小鼠(Taconic Farms): n=8只/组
之前2天: 称重并分类雄性B6小鼠, 总共320只, 每组8只
第1-35天, 在橄榄油中稀释的0.4 ml/kg CCl4或单独的橄榄油将以5ml/kg腹膜内给予, 每周两次, 持续5周。
第15-35天: 药物治疗
第36天: 使动物安乐死并收集肝脏。肝脏的一半将被固定用于组织学研究, 另一半将被收集在液N2上并储存在-80℃用于生化研究。
研究结束(第35天)
使动物安乐死
收集肝脏
第1-35天
腹腔内注射(0.4 ml/kg CCl4或橄榄油)或体重监测器, 每周两次
第15-35天(药物治疗)

图 18

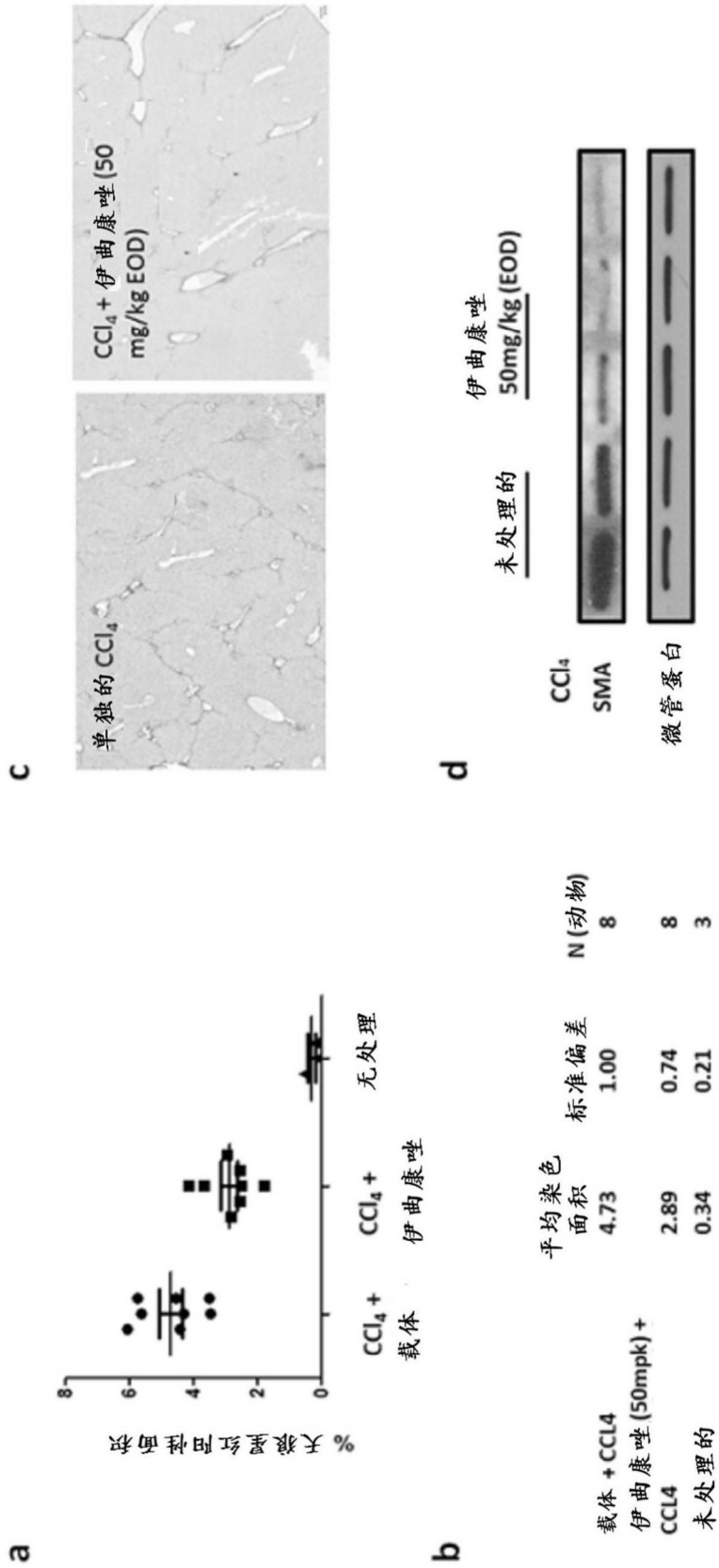

a
% 天狼星红阳性面积
8
6
4
2
0
CCl4 + 载体
CCl4 + 伊曲康唑
无处理
b
平均染色面积 标准偏差 N (动物)
载体 + CCl4 4.73 1.00 8
伊曲康唑 (50mpk) + CCl4 2.89 0.74 8
未处理的 0.34 0.21 3
c
单独的 CCl4
CCl4 + 伊曲康唑 (50 mg/kg EOD)
d
未处理的
伊曲康唑 50mg/kg (EOD)
CCl4
SMA
微管蛋白

图 19

实验组：

- A. 载体(40% β-环糊精), SID, n=6
- B. 伊曲康唑 25 mg/kg, SID, n=6
- C. 化合物 42 @ 25 mg/kg, SID, n=6

实验设计：

- 之前五天，将小鼠麻醉并使用Nair去除背部皮肤毛发。
- 第1天 - 称重。通过刺穿折叠的背部皮肤的整个厚度进行一个无菌活检穿孔（5 mm直径）（总共2 x 5 mm转盘孔）。
- 将使用卡尺每天测量创伤的大小。
- 在整个研究期间将每周监测体重两次。
- 第1天 - 结束：使用5 ml/kg给药体积每天进行药物治疗
- 第7天：在当天给药后1小时对所有动物进行PK取血。使每组2只动物安乐死并收集创伤。
- 第11天：在当天给药后1小时对所有动物进行PK取血。使每组2只动物安乐死并收集创伤。
- 第14天：在当天给药后1小时对所有动物进行PK取血。使每组2只动物安乐死并收集创伤。
- 收集创伤时，将创伤连同几mm的周围皮肤一起切出。对于每只动物，将收集并固定两片创伤用于组织学分析。

图 20

相对于初始创伤大小的%

		第2天	第3天	第4天	第7天	第11天	第14天
载体	相对于初始创伤大小的平均%	47.12	36.91	41.29	18.57	7.20	0.00
	± SEM	3.44	3.76	3.18	2.64	1.32	0.00
	N	12	12	12	12	8	4
伊曲康唑	相对于初始创伤大小的平均%	47.95	36.11	35.25	19.51	7.88	0.00
	± SEM	3.10	3.06	2.00	1.99	1.27	0.00
	N	12	12	12	12	8	4
化合物 42	相对于初始创伤大小的平均%	50.53	38.07	43.00	25.84	16.53	0.00
	± SEM	2.74	1.86	1.69	2.17	2.40	0.00
	N	12	12	12	12	8	4

图 21