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(54) HETEROARYL COMPOUNDS AND USES THEREOF

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(52) **U.S. Cl.**

CPC ***A61K 31/505*** (2013.01); ***A61K 31/5377*** (2013.01); ***A61K 45/06*** (2013.01)

(57) **ABSTRACT**

The present invention provides compounds, pharmaceutically acceptable compositions thereof, and methods of using the same.

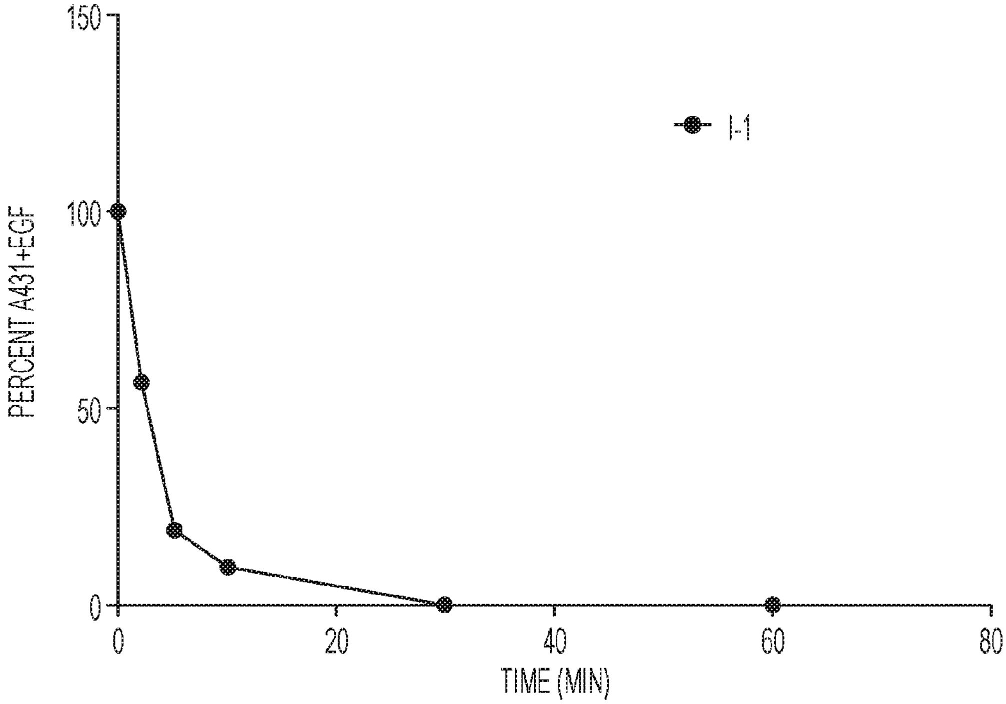

150
100
50
0
PERCENT A431+EGF
I-1
0
20
40
60
80
TIME (MIN)

FIG. 1

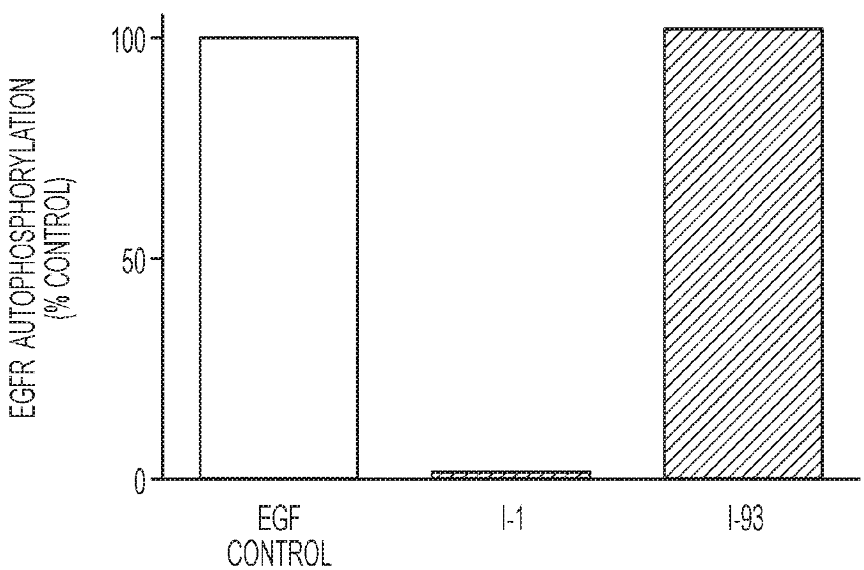

100
50
0
EGFR AUTOPHOSPHORYLATION (% CONTROL)
EGF CONTROL
I-1
I-93

FIG. 2

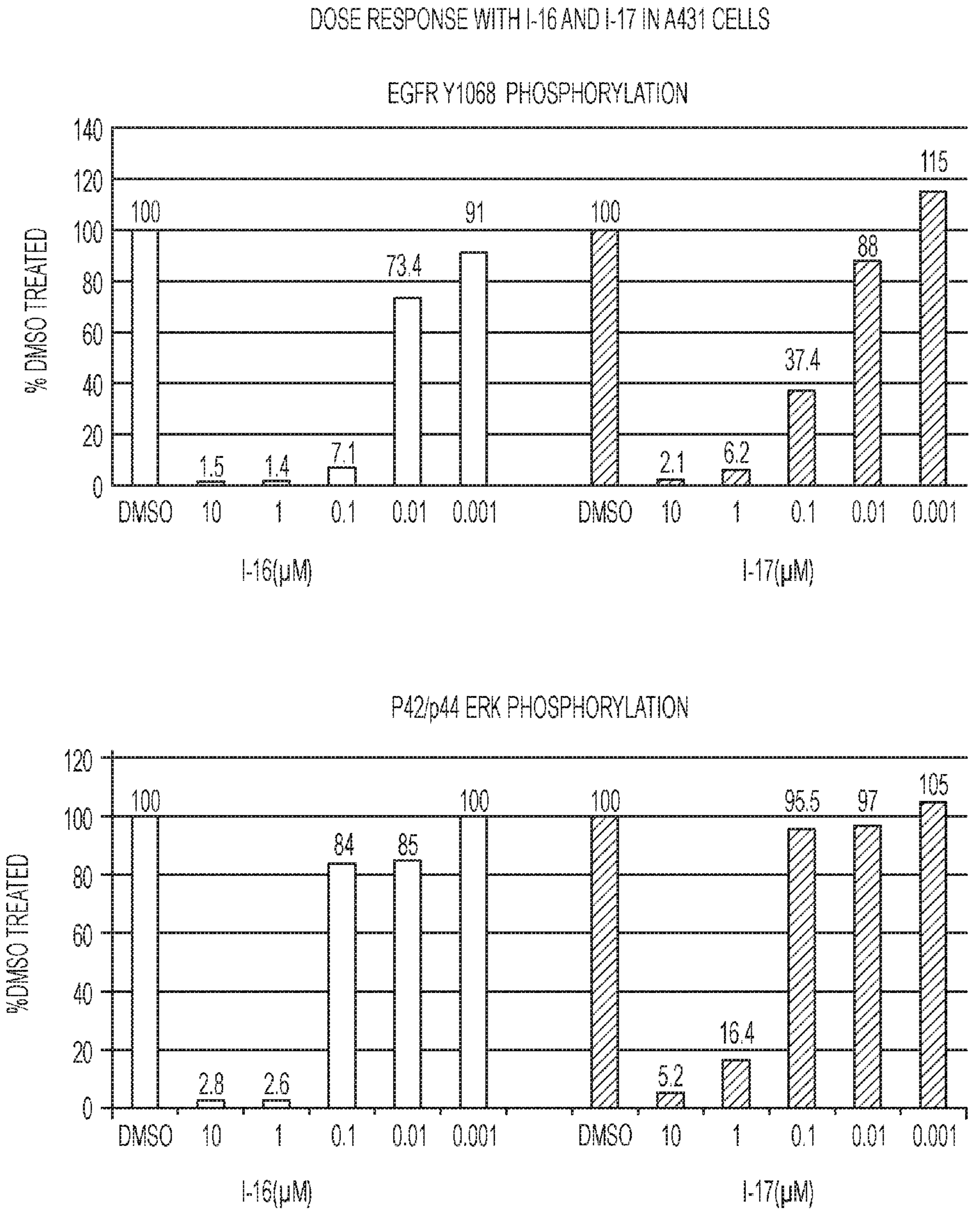

DOSE RESPONSE WITH I-16 AND I-17 IN A431 CELLS
EGFR Y1068 PHOSPHORYLATION
% DMSO TREATED
140
120
100
80
60
40
20
0
100
1.5
1.4
7.1
73.4
91
100
2.1
6.2
37.4
88
115
DMSO
10
1
0.1
0.01
0.001
I-16(μM)
I-17(μM)
P42/p44 ERK PHOSPHORYLATION
%DMSO TREATED
100
2.8
2.6
84
85
100
100
5.2
16.4
95.5
97
105

FIG. 3

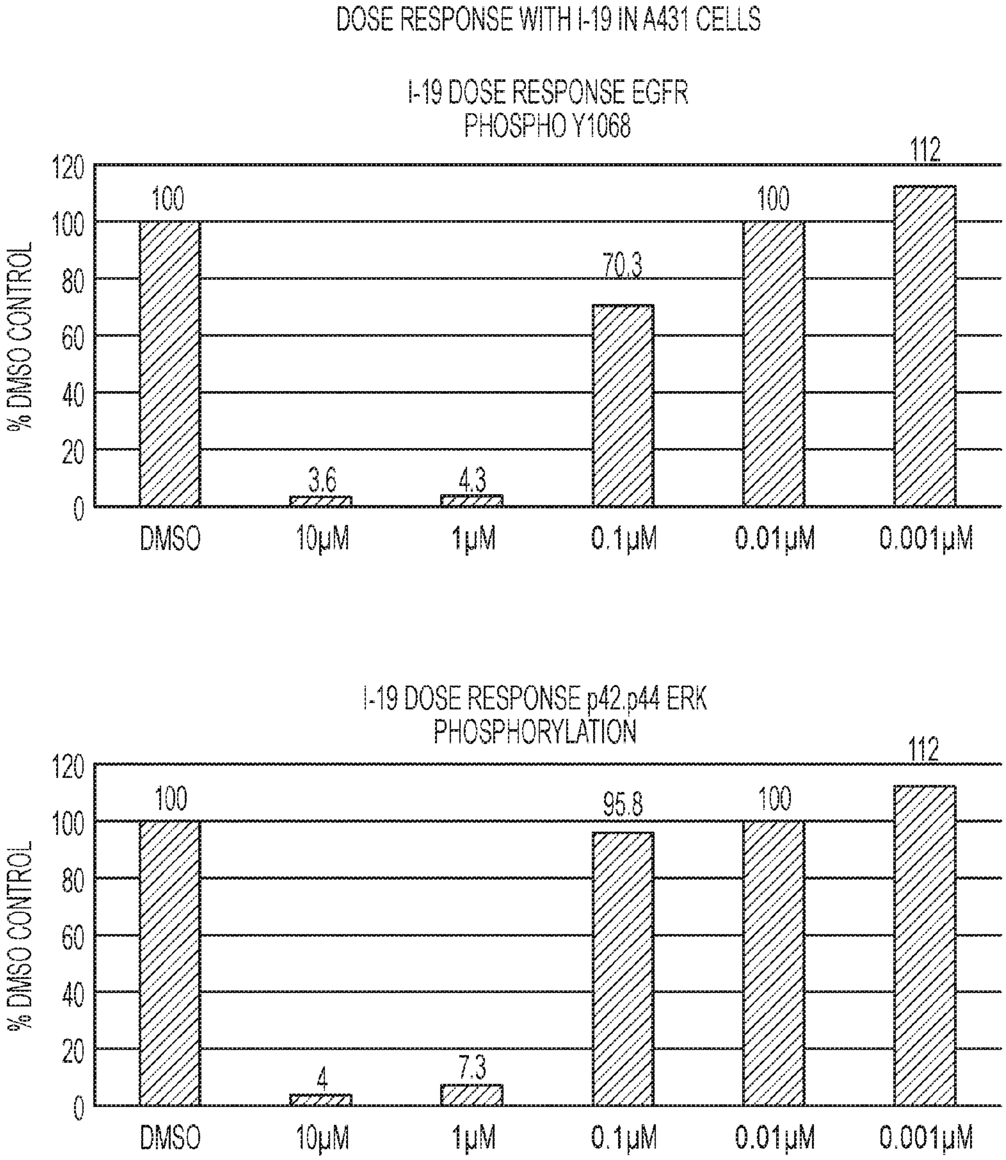
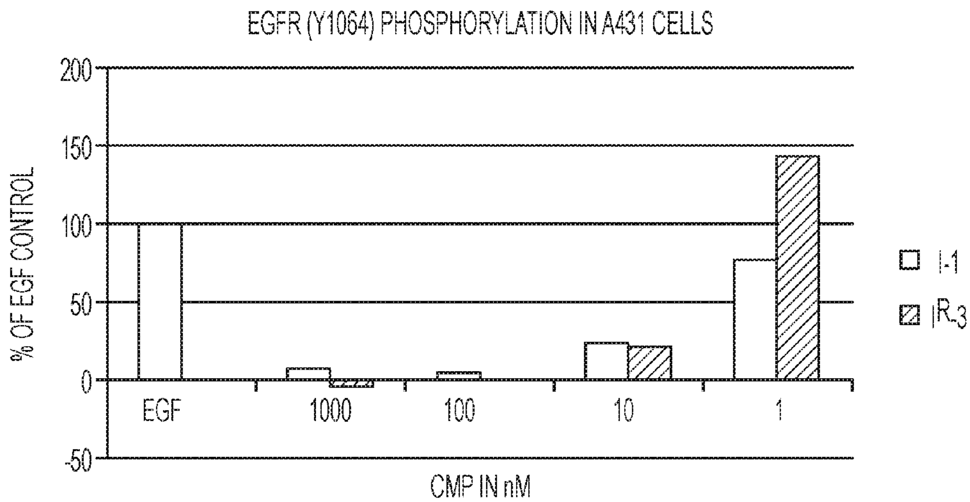
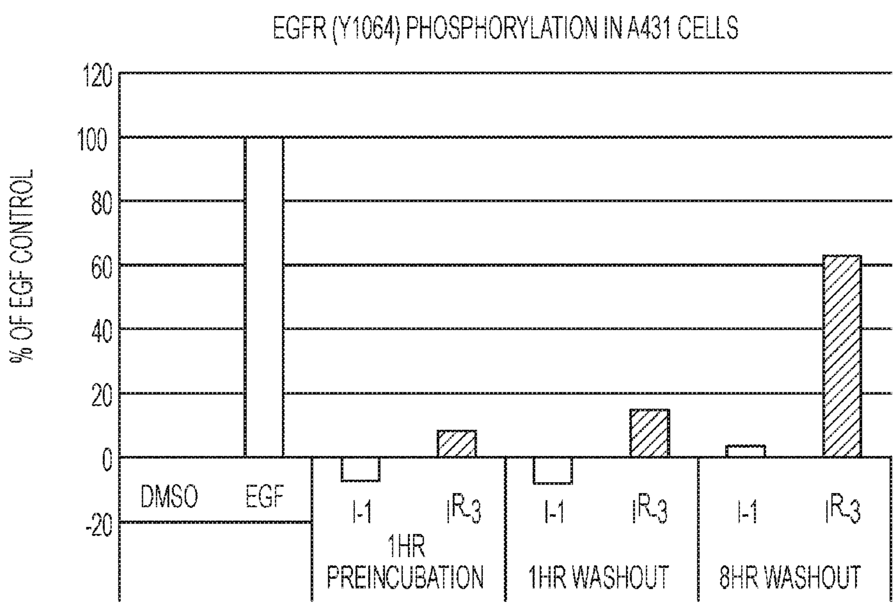

DOSE RESPONSE WITH I-19 IN A431 CELLS
I-19 DOSE RESPONSE EGFR
PHOSPHO Y1068
% DMSO CONTROL
120
100
80
60
40
20
0
100
3.6
4.3
70.3
100
112
DMSO
10μM
1μM
0.1μM
0.01μM
0.001μM
I-19 DOSE RESPONSE p42.p44 ERK
PHOSPHORYLATION
% DMSO CONTROL
120
100
80
60
40
20
0
100
4
7.3
95.8
100
112
DMSO
10μM
1μM
0.1μM
0.01μM
0.001μM

FIG. 4



EGFR (Y1064) PHOSPHORYLATION IN A431 CELLS
200
150
100
50
0
-50
% OF EGF CONTROL
EGF
1000
100
10
1
CMP IN nM
I-1
IR-3

FIG. 5



EGFR (Y1064) PHOSPHORYLATION IN A431 CELLS
120
100
80
60
40
20
0
-20
% OF EGF CONTROL
DMSO
EGF
I-1
IR-3
1HR PREINCUBATION
1HR WASHOUT
8HR WASHOUT

FIG. 6

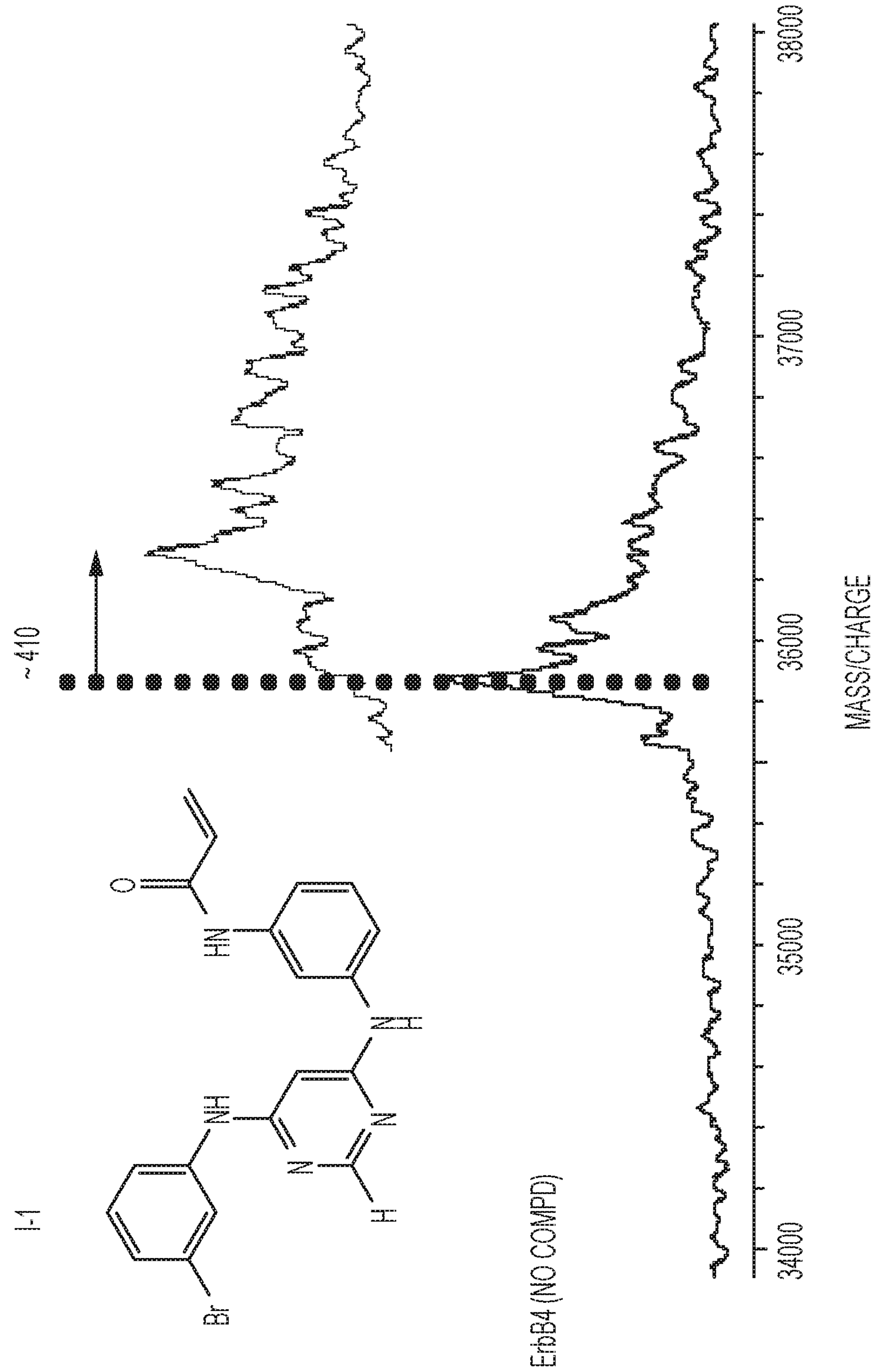

I-1
ErbB4 (NO COMPD)
~410
34000
35000
36000
37000
38000
MASS/CHARGE

FIG. 7

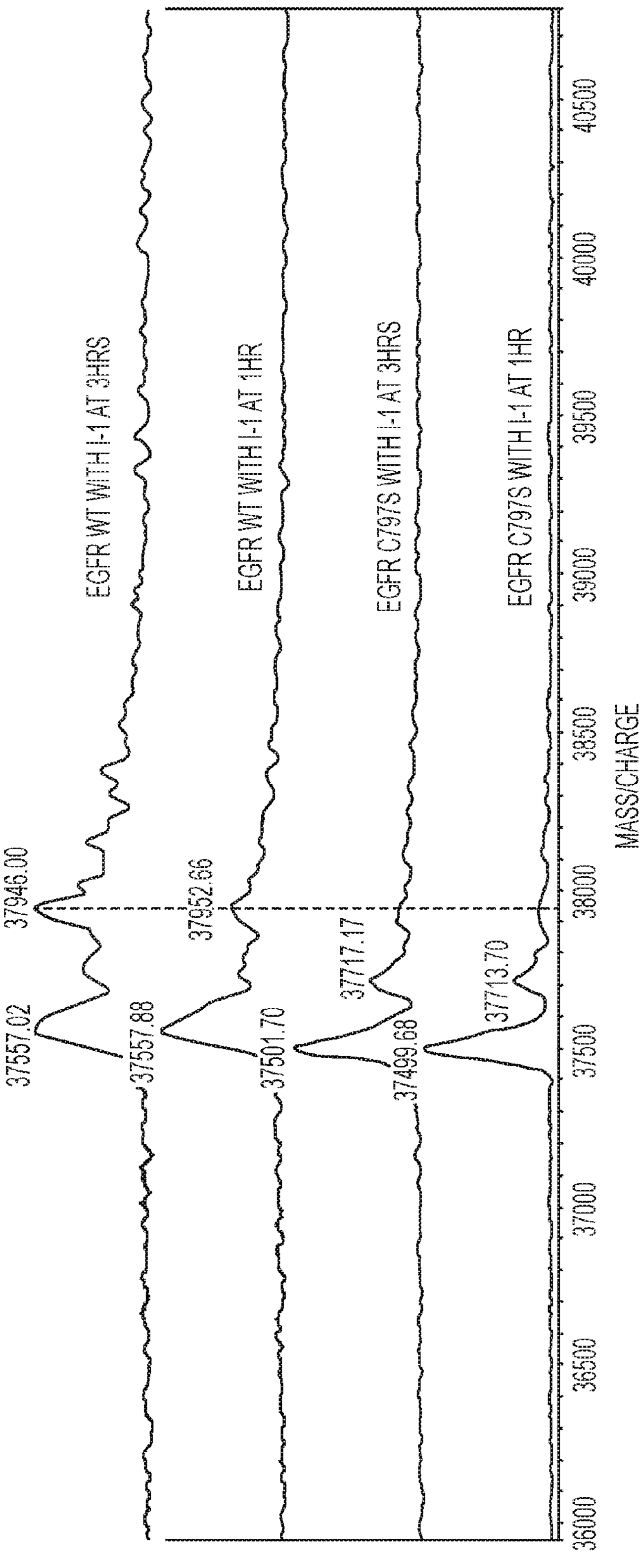

EGFR WT WITH I-1 AT 3HRS
EGFR WT WITH I-1 AT 1HR
EGFR C797S WITH I-1 AT 3HRS
EGFR C797S WITH I-1 AT 1HR
37946.00
37952.66
37557.02
37557.88
37501.70
37499.68
37717.17
37713.70
36000
36500
37000
37500
38000
38500
39000
39500
40000
40500
MASS/CHARGE

FIG. 8

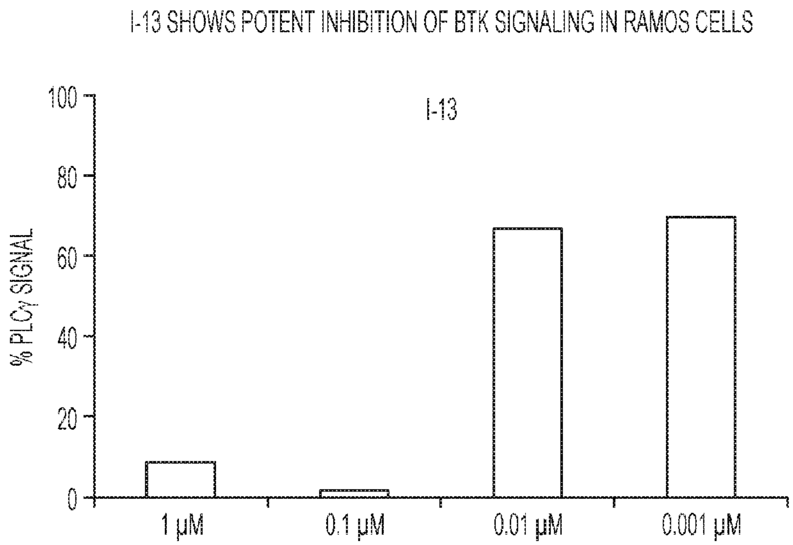

I-13 SHOWS POTENT INHIBITION OF BTK SIGNALING IN RAMOS CELLS
I-13
% PLCγ SIGNAL
100
80
60
40
20
0
1 μM
0.1 μM
0.01 μM
0.001 μM

FIG. 9

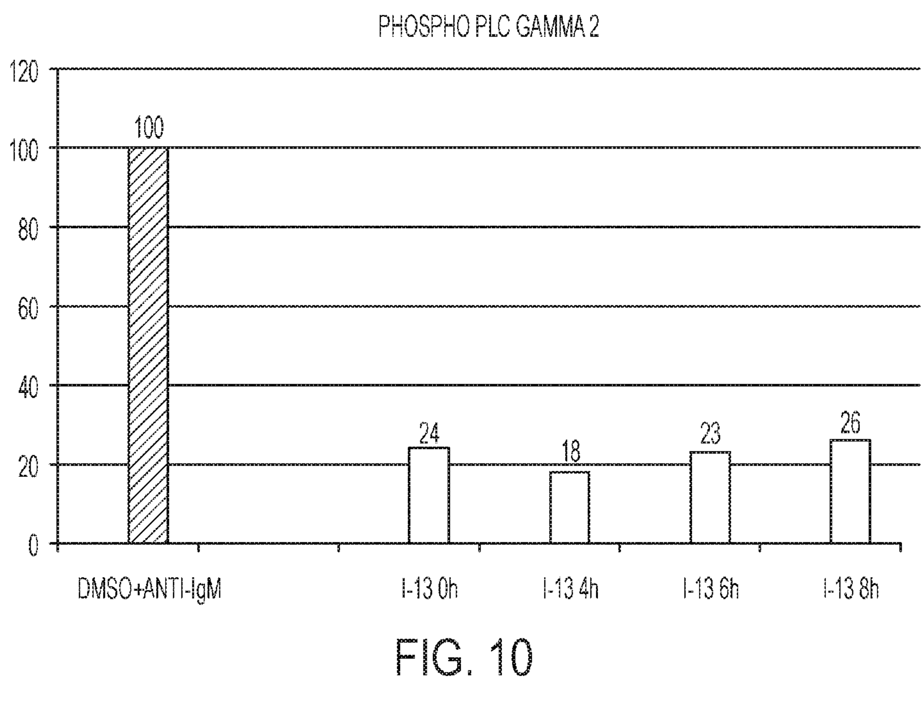

PHOSPHO PLC GAMMA 2
120
100
80
60
40
20
0
100
24
18
23
26
DMSO+ANTI-IgM
I-13 0h
I-13 4h
I-13 6h
I-13 8h

FIG. 10

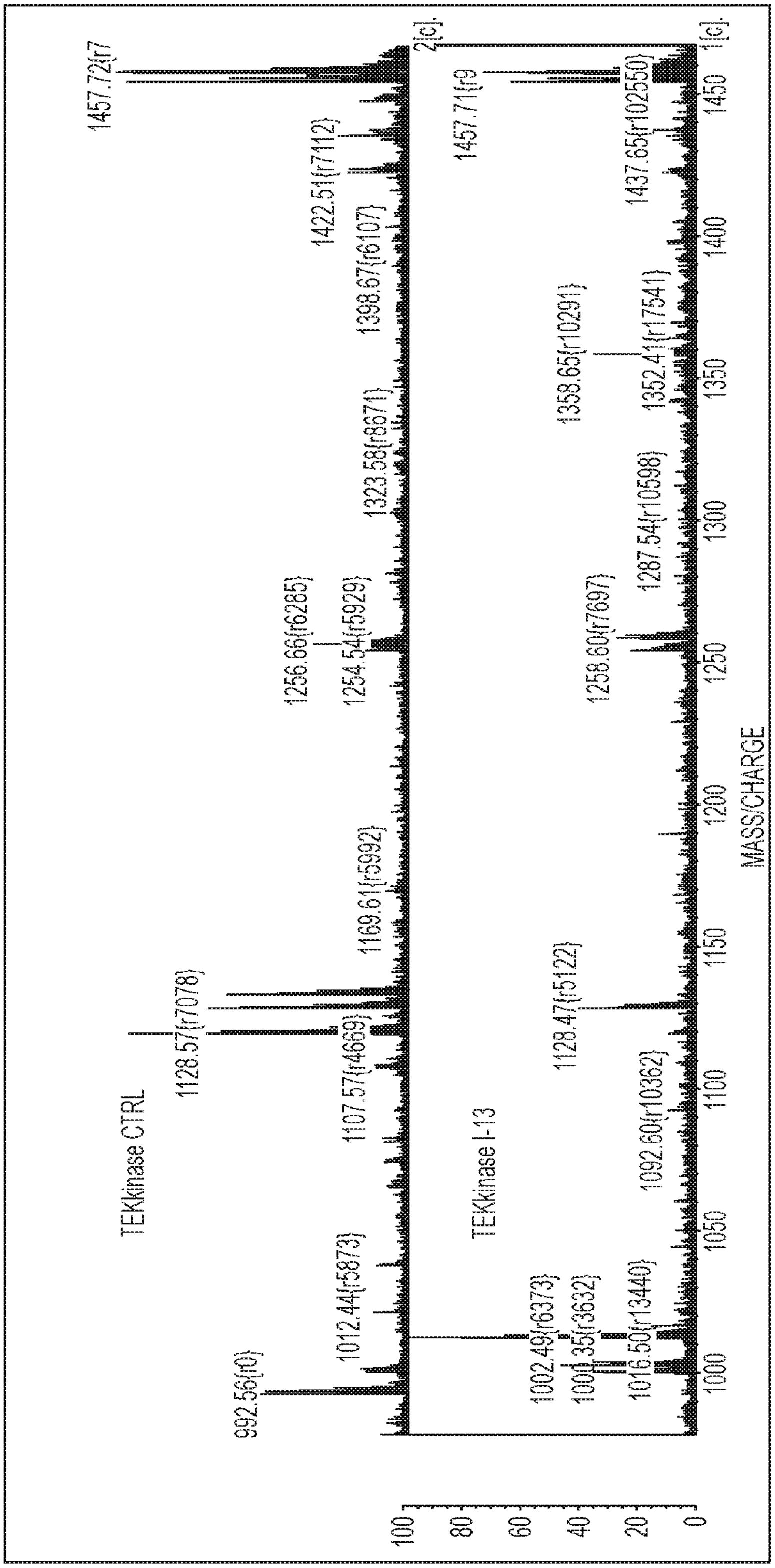
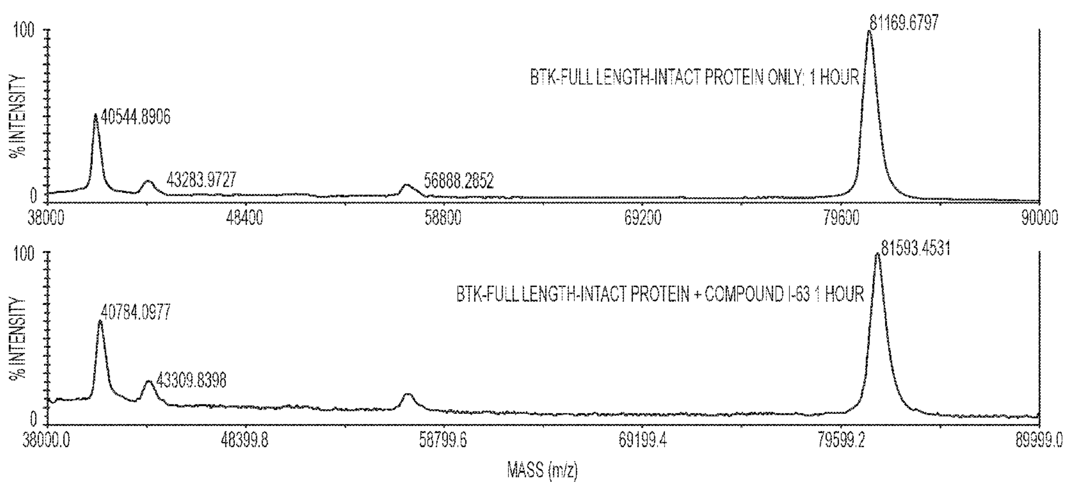

TEC KINASE (I-13) TRYPTIC DIGEST RESULTS
TEKkinase CTRL
TEKkinase I-13
992.56{r0}
1012.44{r5873}
1107.57{r4669}
1128.57{r7078}
1169.61{r5992}
1256.66{r6285}
1254.54{r5929}
1323.58{r8671}
1398.67{r6107}
1422.51{r7112}
1457.72{r7
1002.49{r6373}
1016.50{r13440}
1092.60{r10362}
1128.47{r5122}
1258.60{r7697}
1287.54{r10598}
1358.65{r10291}
1352.41{r17541}
1437.65{r10250}
1457.71{r9
100
80
60
40
20
0
1000
1050
1100
1150
1200
1250
1300
1350
1400
1450
MASS/CHARGE

FIG. 11



100
% INTENSITY
0
81169.6797
BTK-FULL LENGTH-INTACT PROTEIN ONLY; 1 HOUR
40544.8906
43283.9727
56868.2852
38000
48400
58800
69200
79600
90000
100
% INTENSITY
0
81593.4531
BTK-FULL LENGTH-INTACT PROTEIN + COMPOUND I-63 1 HOUR
40784.0977
43309.8398
38000.0
48399.8
58799.6
69199.4
79599.2
89999.0
MASS (m/z)

FIG. 12

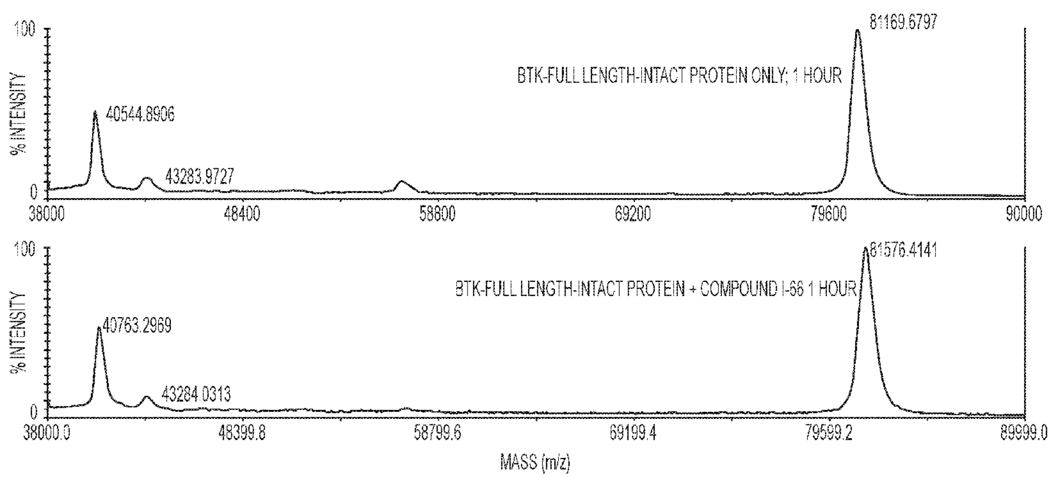

100
0
% INTENSITY
40544.8906
43283.9727
81169.6797
BTK-FULL LENGTH-INTACT PROTEIN ONLY; 1 HOUR
38000
48400
58600
69200
79600
90000
100
0
% INTENSITY
40763.2969
43284.0313
81576.4141
BTK-FULL LENGTH-INTACT PROTEIN + COMPOUND I-66 1 HOUR
38000.0
48399.8
58799.6
69199.4
79599.2
89999.0
MASS (m/z)

FIG. 13

SEQ_ID_1: FULL LENGTH BTK PROTEIN:

MAAVILESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEK
ITCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEEL
RKRWIHQLKNVIRYNSDLVQKYHPCFWIDGQYLCCSQTAKNAMGCQILENRNGSLKPG
SSHRKTKKPLPPTPEEDQILKKPLPPEPAAAPVSTSELKKVVALYDYMPMNANDLQLRK
GDEYFILEESNLPWWRARDKNGQEGYIPSNYVTEAEDSIEMYEWYSKHMTRSQAEQLL
KQEGKEGGFIVRDSSKAGKYTVSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKHLFST
IPELINYHQHNSAGLISRLKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFG
VVKYGKWRGQYDVAIKMIKEGSMSEDEFIEEAKVMMNLSHEKLVQLYGVCTKQRPIFII
TEYMANGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQFLHRDLAARNCLVN
DQGVVKVSDF

Cys=481

Figure 14

SEQ ID 2: FULL LENGTH TEC PROTEIN NP_003206 631 aa

MNFNTILEEILIKRSQQKKKTSPLNYKERLFVLTKSMLTYYEGRAEKKYRKGFIDVSKIK
CVEIVKNDDGVIPCQNKYPFQVVHDANTLYIFAPSPQSRDLWVKKLKEEIKNNNNIMIK
YHPKFWTDGSYQCCRQTEKLAPGCEKYNLFESSIRKALPPAPETKKRRPPPPIPLEEEDNS
EEIVVAMYDFQAAEGHDLRLERGQEYLILEKNDVHWWRARDKYGNEGYIPSNYVTGK
KSNNLDQYEWYCRNMNRSKAEQLLRSEDKEGGFMVRDSSQPGLYTVSLYTKFGGEGS
SGFRHYHIKETTTSPKKYYLAEKHAFGSIPEIIEYHKHNAAGLVTRLRYPVSVKGK
NAPTTAGFSYEKWEINPSELTFMRELGSGLFGVVRLGKWRAQYKVAIKAIREGAMCEE
DFIEEAKVMMKLTHPKLVQLYGVCTQQKPIYIVTEFMERGCLLNFLRQRQGHFSRDVLL
SMCQDVCEGMEYLERNSFIHRDLAARNCLVSEAGVVKVSDFGMARYVLDDQYTSSSG
AKFPVKWCPPEVFNYSRFSSKSDVWSFGVLMWEVFTEGRMPFEKYTNYEVVTMVTRG
HRLYQPKLASNYVYEVMLRCWQEKPEGRPSFEDLLRTIDELVECEETFGR

Cys=449

Figure 15

SEQ ID 3: FULL LENGTH ITK PROTEIN NP 005537 620aa

MNNFILLEEQLIKKSQQKRRTSPSNFKVRFFVLTKASLAYFEDRHGKKRTLKGSIELSRIKCVE
IVKSDISIPCHYKYPFQVVHDNYLLYVFAPDRESRQRWVLALKEETRNNNSLVPKYHPNFWMDG
KWRCCSQLEKLATGCAQYDPTKNASKKPLPPTPEDNRRPLWEPEETVVIALYDYQTNDPQELAL
RRNEEYCLLDSSEIHWWRVQDRNGHEGYVPSSYLVEKSPNNLETYEWYNKSISRDKAEKLLLDT
GKEGAFMVRDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETNDNPKRYYVAEKYVFDSIPLLI
NYHQHNGGGLVTRLRYPVCFGRQKAPVTAGLRYGKWVIDPSELTFVQEIGSGQFGLVHLGYWLN
KDKVAIKTIREGAMSEEDFIEEAEVMMKLSHPKLVQLYGVCLEQAPICLVFEFMEHG**C**LSDYLR
TQRGLFAAETLLGMCLDVCEGMAYLEEACVIHRDLAARNCLVGENQVIKVSDFGMTRFVLDDQY
TSSTGTKFPVKWASPEVFSFSRYSSKSDVWSFGVLMWEVFSEGKIPYENRSNSEVVEDISTGFR
LYKPRLASTHVYQIMNHCWKERPEDRPAFSRLLRQLAEIAESGL

Cys=442

Figure 16

SEQ ID 4: FULL LENGTH BMX PROTEIN NP_001712_675aa

MDTKSILEELLLKRSQQKKKMSPNNYKERLFVLTKTNLSYYEYDKMKRGSRKGSIEIKK
IRCVEKVNLEEQTPVERQYPFQIVYKDGLLYVYASNEESRSQWLKALQKEIRGNPHLLV
KYHSGFFVDGKFLCCQQSCKAAPGCTLWEAYANLHTAVNEEKHRVPTFPDRVLKIPRA
VPVLKMDAPSSSTTLAQYDNESKKNYGSQPPSSSTSLAQYDSNSKKIYGSQPNFNMQYIP
REDFPDWWQVRKLKSSSSSEDVASSNQKERNVNHTTSKISWEFPESSSSEEEENLDDYD
WFAGNISRSQSEQLLRQKGKEGAFMVRNSSQVGMYTVSLFSKAVNDKKGTVKHYH
VHTNAENKLYLAENYCFDSIPKLIHYHQHNSAGMITRLRHPVSTKANKVPDSVSLGNGI
WELKREEITLLKELGSGQFGVVQLGKWKGQYDVAVKMIKEGSMSEDEFFQEAQTMMK
LSHPKLVKFYGVCSKEYPIYIVTEYISNGCLLNYLRSHGKGLEPSQLLEMCYDVCEGMA
FLESHQFIHRDLAARNCLVDRDLCVKVSDFGMTRYVLDDQYVSSVGTKFPVKWSAPEV
FHYFKYSSKSDVWAFGILMWEVFSLGKQPYDLYDNSQVVLKVSQGHRLYRPHLASDTI
YQIMYSCWHELPEKRPTFQQLLSSIEPLREKDKH

Cys=496

Figure 17

SEQ ID 5: FULL LENGTH JAK3 PROTEIN NP 000206 1124 aa

MAPPSEETPLIPQRSCSLLSTEAGALHVLLPARGPGPPQRLSFSFGDHLAEDLCVQAAKASGIL
PVYHSLFALATEDLSCWFPPSHIFSVEDASTQVLLYRIRFYFPNWFGLEKCHRFGLRKDLASAI
LDLPVLEHLFAQHRSDLVSGRLPVGLSLKEQGECLSLAVLDLARMAREQAQRPGELLKTVSYKA
CLPPSLRDLIQGLSFVTRRRIRRTVRRALRRVAACQADRHSLMAKYIMDLERLDPAGAAETFHV
GLPGALGGHDGLGLLRVAGDGGIAWTQGEQEVLQPFCDFPEIVDISIKQAPRVGPAGEHRLVTV
TRTDNQILEAEFPGLPEALSFVALVDGYFRLTTDSQHFFCKEVAPPRLLEEVAEQCHGPITLDF
AINKLKTGGSRPGSYVLRRSPQDFDSFLLTVCVQNPLGPDYKGCLIRRSPTGTFLLVGLSRPHS
SLRELLATCWDGGLHVDGVAVTLTSCCIPRPKEKSNLIVVQRGHSPPTSSLVQPQSQYQLSQMT
FHKIPADSLEWHENLGHGSFTKIYRGCRHEVVDGEARKTEVLLKVMDAKHKNCMESFLEAASLM
SQVSYRHLVLLHGVCMAGDSTMVQEFVHLGAIDMYLRKRGHLVPASWKLQVVKQLAYALNYLED
KGLPHGNVSARKVLLAREGADGSPPFIKLSDPGVSPAVLSLEMLTDRIPWVAPECLREAQTLSL
EADKWGFGATVWEVFSGVTMPISALDPAKKLQFYEDRQQLPAPKWTELALLIQQCMAYEPVQRP
SFRAVIRDLNSLISSDYELLSDPTPGALAPRDGLWNGAQLYACQDPTIFEERHLKYISQLGKGN
FGSVELCRYDPLGDNTGALVAVKQLQHSGPDQQRDFQREIQILKALHSDFIVKYRGVSYGPGRQ
SLRLVMEYLPSG**C**LRDFLQRHRARLDASRLLLYSSQICKGMEYLGSRRCVHRDLAARNILVESE
AHVKIADFGLAKLLPLDKDYYVVREPGQSPIFWYAPESLSDNIFSRQSDVWSFGVVLYELFTYC
DKSCSPSAEFLRMMGCERDVPALCRLLELLEEGQRLPAPPACPAEVHELMKLCWAPSPQDRPSF
SALGPQLDMLWSGSRGCETHAFTAHPEGKHHSLSFS

Cys=909

Figure 18

SEQ ID 6: FULL LENGTH TXK PROTEIN NP_003319 527 aa

MILSSYNTIQSVFCCCCCCSVQKRQMRTQISLSTDEELPEKYTQRRRPWLSQLSNKKQSN
TGRVQPSKRKPLPPLPPSEVAEEKIQVKALYDFLPREPCNLALRRAEEYLILEKYNPHWW
KARDRLGNEGLIPSNYVTENKITNLEIYEWYHRNITRNQAEHLLRQESKEGAFIVRDSRH
LGSYTISVFMGARRSTEAAIKHYQIKKNDSGQWYVAERHAFQSIPELIWYHQHNAAGL
MTRLRYPVGLMGSCLPATAGFSYEKWEIDPSELAFIKEIGSGQFGVVHLGEWRSHIQVAI
KAINEGSMSEEDFIEEAKVMMKLSHSKLVQLYGVCIQRKPLYIVTEFMENG**C**LLNYLRE
NKGKLRKEMLLSVCQDICEGMEYLERNGYIHRDLAARNCLVSSTCIVKISDFGMTRYVL
DDEYVSSFGAKFPIKWSPPEVFLFNKYSSKSDVWSFGVLMWEVFTEGKMPFENKSNLQ
VVEAISEGFRLYRPHLAPMSIYEVMYSCWHEKPEGRPTFAELLRAVTEIAETW

Cys 350

Figure 19

HETEROARYL COMPOUNDS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of co-pending U.S. application Ser. No. 12/426,495, filed Apr. 20, 2009, which is a continuation-in-part of U.S. application Ser. No. 12/253,424, filed Oct. 17, 2008, which claims priority to U.S. provisional application Ser. No. 60/981,432, filed Oct. 19, 2007, and U.S. provisional application Ser. No. 61/052,002, filed May 9, 2008, the entirety of each of which is hereby incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

[0002] The present invention relates to compounds useful as inhibitors of protein kinases. The invention also provides pharmaceutically acceptable compositions comprising compounds of the present invention and methods of using said compositions in the treatment of various disorders.

BACKGROUND OF THE INVENTION

[0003] The search for new therapeutic agents has been greatly aided in recent years by a better understanding of the structure of enzymes and other biomolecules associated with diseases. One important class of enzymes that has been the subject of extensive study is protein kinases.

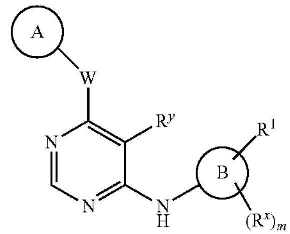
[0004] Protein kinases constitute a large family of structurally related enzymes that are responsible for the control of a variety of signal transduction processes within the cell. Protein kinases are thought to have evolved from a common ancestral gene due to the conservation of their structure and catalytic function. Almost all kinases contain a similar 250-300 amino acid catalytic domain. The kinases may be categorized into families by the substrates they phosphorylate (e.g., protein-tyrosine, protein-serine/threonine, lipids, etc.).

[0005] In general, protein kinases mediate intracellular signaling by effecting a phosphoryl transfer from a nucleoside triphosphate to a protein acceptor that is involved in a signaling pathway. These phosphorylation events act as molecular on/off switches that can modulate or regulate the target protein biological function. These phosphorylation events are ultimately triggered in response to a variety of extracellular and other stimuli. Examples of such stimuli include environmental and chemical stress signals (e.g., osmotic shock, heat shock, ultraviolet radiation, bacterial endotoxin, and H_2O_2), cytokines (e.g., interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α)), and growth factors (e.g., granulocyte macrophage-colony-stimulating factor (GM-CSF), and fibroblast growth factor (FGF)). An extracellular stimulus may affect one or more cellular responses related to cell growth, migration, differentiation, secretion of hormones, activation of transcription factors, muscle contraction, glucose metabolism, control of protein synthesis, and regulation of the cell cycle.

[0006] Many diseases are associated with abnormal cellular responses triggered by protein kinase-mediated events as described above. These diseases include, but are not limited to, autoimmune diseases, inflammatory diseases, bone diseases, metabolic diseases, neurological and neurodegenerative diseases, cancer, cardiovascular diseases, allergies and asthma, Alzheimer's disease, and hormone-related diseases. Accordingly, there remains a need to find protein kinase inhibitors useful as therapeutic agents.

SUMMARY OF THE INVENTION

[0007] It has now been found that compounds of this invention, and pharmaceutically acceptable compositions thereof, are effective as inhibitors of one or more protein kinases. Such compounds have the general formula I:



I

or a pharmaceutically acceptable salt thereof, wherein Ring A, Ring B, m, R^x, R^y, W, and R^1 are as defined herein.

[0008] Compounds of the present invention, and pharmaceutically acceptable compositions thereof, are useful for treating a variety of diseases, disorders or conditions, associated with abnormal cellular responses triggered by protein kinase-mediated events. Such diseases, disorders, or conditions include those described herein.

[0009] Compounds provided by this invention are also useful for the study of kinases in biological and pathological phenomena; the study of intracellular signal transduction pathways mediated by such kinases; and the comparative evaluation of new kinase inhibitors.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. **1** depicts the EGF inhibiting activity of compound I-1.

[0011] FIG. **2** depicts the results of compound I-1 in a "washout" experiment as compared with compound I-93.

[0012] FIG. **3** depicts dose response inhibition of EGFR phosphorylation and p42/p44 Erk phosphorylation with compounds I-16 and I-17 in A431 cells.

[0013] FIG. **4** depicts dose response inhibition of EGFR phosphorylation and p42/p44 Erk phosphorylation with compound I-19 in A431 cells.

[0014] FIG. **5** depicts dose response inhibition of EGFR phosphorylation with compound I-1 in A431 cells as compared with its "reversible control" compound (I^R-3).

[0015] FIG. **6** depicts the results of compound I-1 in a "washout" experiment as compared with its "reversible control" compound (I^R-3).

[0016] FIG. **7** depicts MS analysis confirming covalent modification of ErbB4 by compound I-1.

[0017] FIG. **8** depicts MS analysis confirming covalent modification of ErbB1 at Cys797 by compound I-1.

[0018] FIG. **9** depicts the inhibition of BTK signaling in Ramos cells by compound I-13.

[0019] FIG. **10** depicts the results of compound I-13 in a "washout" experiment with BTK in Ramos cells.

[0020] FIG. **11** depicts MS analysis of the tryptic digests confirming covalent modification of TEC kinase by compound I-13.

[0021] FIG. **12** depicts MS analysis confirming covalent modification of BTK by compound I-63.

[0022] FIG. **13** depicts MS analysis confirming covalent modification of BTK by compound I-66.

[0023] FIG. **14** depicts an amino acid sequence for BTK (SEQ ID 1).

[0024] FIG. **15** depicts an amino acid sequence for TEC (SEQ ID 2).

[0025] FIG. **16** depicts an amino acid sequence for ITK (SEQ ID 3).

[0026] FIG. **17** depicts an amino acid sequence for BMX (SEQ ID 4).

[0027] FIG. **18** depicts an amino acid sequence for JAK3 (SEQ ID 5).

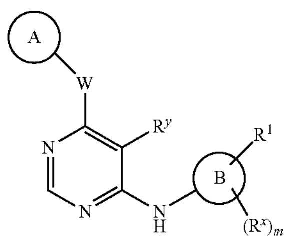
[0028] FIG. **19** depicts an amino acid sequence for TXK (SEQ ID 6).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

I. General Description of Compounds of the Invention

[0029] In certain embodiments, the present invention provides a compound of formula I:

I



[0030] or a pharmaceutically acceptable salt thereof, wherein:

[0031] Ring A is an optionally substituted group selected from phenyl, an 8-10 membered bicyclic partially unsaturated or aryl ring, a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaryl ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

[0032] Ring B is phenyl, a 5-6 membered heteroaryl ring having 1-3 heteroatoms independently selected from N, O or S, a 5-6 membered saturated heterocyclic ring having 1-2 heteroatoms independently selected from N, O or S, or an 8-10 membered bicyclic partially unsaturated or aryl ring having 1-3 heteroatoms independently selected from N, O or S;

[0033] R^1 is a warhead group;

[0034] R^y is hydrogen, halogen, CN, lower alkyl, or lower haloalkyl;

[0035] W is a bivalent C_{1-3} alkylene chain wherein one methylene unit of W is optionally replaced by —NR^2—, —$N(R^2)C(O)$—, —$C(O)N(R^2)$—, —$N(R^2)SO_2$—, —$SO_2N(R^2)$—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO— or —SO_2—;

[0036] R^2 is hydrogen or optionally substituted C_{1-6} aliphatic, or:

[0037] R^2 and a substituent on Ring A are taken together with their intervening atoms to form a 4-6 membered saturated ring, or:

[0038] R^2 and R^y are taken together with their intervening atoms to form a 4-7 membered carbocyclic ring;

[0039] m is 0-4;

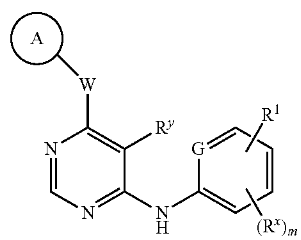
[0040] each R^x is independently selected from —R, halogen, —OR, —CN, —NO_2, —SO_2R, —SOR, —C(O)R, —CO_2R, —$C(O)N(R)_2$, —NRC(O)R, —$NRC(O)NR_2$, —$NRSO_2R$, or —$N(R)_2$; or:

[0041] R^x and R^1 are taken together with their intervening atoms to form a 5-7 membered saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with a warhead group and 0-3 groups independently selected from oxo, halogen, CN, or C_{1-6} aliphatic; and

[0042] each R group is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered heterocylic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0043] In certain embodiments, the present invention provides a compound of formula II:

II



[0044] or a pharmaceutically acceptable salt thereof, wherein:

[0045] Ring A is an optionally substituted group selected from phenyl, an 8-10 membered bicyclic partially unsaturated or aryl ring, a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaryl ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

[0046] R^1 is a warhead group;

[0047] R^y is hydrogen, halogen, CN, lower alkyl, or lower haloalkyl;

[0048] G is CH, or N;

[0049] W is —NR^2—, —S—, or —O—;

[0050] R^2 is hydrogen or optionally substituted C_{1-6} aliphatic, or:

[0051] R^2 and a substituent on Ring A are taken together with their intervening atoms to form a 4-6 membered saturated ring;

[0052] m is 0-4;

[0053] each R^x is independently selected from —R, halogen, —OR, —CN, —NO_2, —SO_2R, —SOR, —C(O)R, —CO_2R, —$C(O)N(R)_2$, —NRC(O)R, —$NRC(O)NR_2$, —$NRSO_2R$, or —$N(R)_2$; or:

[0054] R^x and R^1 are taken together with their intervening atoms to form a 5-7 membered saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with a warhead group and 0-3 groups independently selected from oxo, halogen, CN, or C_{1-6} aliphatic; and

[0055] each R group is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered heterocylic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

2. Compounds and Definitions

[0056] Compounds of this invention include those described generally above, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75^{th} Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5^{th} Ed., Ed.: Smith, M. B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0057] The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle" "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. Unless otherwise specified, aliphatic groups contain 1-6 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-4 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-3 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1-2 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C_3-C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0058] The term "lower alkyl" refers to a C_{1-4} straight or branched alkyl group. Exemplary lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[0059] The term "lower haloalkyl" refers to a C_{1-4} straight or branched alkyl group that is substituted with one or more halogen atoms.

[0060] The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon (including, any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

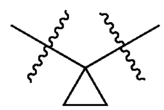
[0061] The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[0062] As used herein, the term "bivalent C_{1-8} (or C_{1-6}) saturated or unsaturated, straight or branched, hydrocarbon chain", refers to bivalent alkylene, alkenylene, and alkynylene chains that are straight or branched as defined herein.

[0063] The term "alkylene" refers to a bivalent alkyl group. An "alkylene chain" is a polymethylene group, i.e., $-(CH_2)_n-$, wherein n is a positive integer, preferably from 1 to 6, from 1 to 4, from 1 to 3, from 1 to 2, or from 2 to 3. A substituted alkylene chain is a polymethylene group in which one or more methylene hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0064] The term "alkenylene" refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one double bond in which one or more hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0065] As used herein, the term "cyclopropylenyl" refers to a bivalent cyclopropyl group of the following structure:



[0066] The term "halogen" means F, Cl, Br, or I.

[0067] The term "aryl" used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to monocyclic and bicyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term "aryl" may be used interchangeably with the term "aryl ring". In certain embodiments of the present invention, "aryl" refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl", as it is used herein, is a group in which an aromatic ring is fused to one or more non-aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[0068] The terms "heteroaryl" and "heteroar-", used alone or as part of a larger moiety, e.g., "heteroaralkyl", or "heteroaralkoxy", refer to groups having 5 to 10 ring atoms, preferably 5, 6, or 9 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. The term "heteroatom" refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. The terms "heteroaryl" and "heteroar-", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3-b]-1,4-oxazin-3(4H)-one. A heteroaryl group may be mono- or bicyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring", "heteroaryl group", or "heteroaromatic", any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0069] As used herein, the terms "heterocycle", "heterocyclyl", "heterocyclic radical", and "heterocyclic ring" are used interchangeably and refer to a stable 5- to 7-membered monocyclic or 7-10-membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl), or ^{+}NR (as in N-substituted pyrrolidinyl).

[0070] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothiophenyl pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle", "heterocyclyl", "heterocyclyl ring", "heterocyclic group", "heterocyclic moiety", and "heterocyclic radical", are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3H-indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl, where the radical or point of attachment is on the heterocyclyl ring. A heterocyclyl group may be mono- or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[0071] As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[0072] As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[0073] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $—(CH_2)_{0-4}R^\circ$; $—(CH_2)_{0-4}OR^\circ$; $—O(CH_2)_{0-4}R^\circ$, $—O—(CH_2)_{0-4}C(O)OR^\circ$; $—(CH_2)_{0-4}CH(OR^\circ)_2$; $—(CH_2)_{0-4}SR^\circ$; $—(CH_2)_{0-4}Ph$, which may be substituted with R°; $—(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $—CH{=}CHPh$, which may be substituted with R°; $—(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $—NO_2$; —CN; $—N_3$; $—(CH_2)_{0-4}N(R^\circ)_2$; $—(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $—N(R^\circ)C(S)R^\circ$; $—(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $—N(R^\circ)C(S)NR^\circ_2$; $—(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $—N(R^\circ)N(R^\circ)C(O)R^\circ$; $—N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $—N(R^\circ)N(R^\circ)C(O)OR^\circ$; $—(CH_2)_{0-4}C(O)R^\circ$; $—C(S)R^\circ$; $—(CH_2)_{0-4}C(O)OR^\circ$; $—(CH_2)_{0-4}C(O)SR^\circ$; $—(CH_2)_{0-4}C(O)OSiR^\circ_3$; $—(CH_2)_{0-4}OC(O)R^\circ$; $—OC(O)(CH_2)_{0-4}SR^\circ$, $SC(S)SR^\circ$; $—(CH_2)_{0-4}SC(O)R^\circ$; $—(CH_2)_{0-4}C(O)NR^\circ_2$; $—C(S)NR^\circ_2$; $—C(S)SR^\circ$; $—SC(S)SR^\circ$, $—(CH_2)_{0-4}OC(O)NR^\circ_2$; $—C(O)N(OR^\circ)R^\circ$; $—C(O)C(O)R^\circ$; $—C(O)CH_2C(O)R^\circ$; $—C(NOR^\circ)R^\circ$; $—(CH_2)_{0-4}SSR^\circ$; $—(CH_2)_{0-4}S(O)_2R^\circ$; $—(CH_2)_{0-4}S(O)_2OR^\circ$; $—(CH_2)_{0-4}OS(O)_2R^\circ$; $—S(O)_2NR^\circ_2$; $—(CH_2)_{0-4}S(O)R^\circ$; $—N(R^\circ)S(O)_2NR^\circ_2$; $—N(R^\circ)S(O)_2R^\circ$; $—N(OR^\circ)R^\circ$; $—C(NH)NR^\circ_2$; $—P(O)_2R^\circ$; $—P(O)R^\circ_2$; $—OP(O)R^\circ_2$; $—OP(O)(OR^\circ)_2$; SiR°_3; $—(C_{1-4}$ straight or branched)alkylene)$O—N(R^\circ)_2$; or $—(C_{1-4}$ straight or branched)alkylene)$C(O)O—N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $—CH_2Ph$, $—O(CH_2)_{0-1}Ph$, $—CH_2$-(5-6 membered heteroaryl ring), or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected froth nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[0074] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $—(CH_2)_{0-2}R^\bullet$, -(halo$R^\bullet$), $—(CH_2)_{0-2}OH$, $—(CH_2)_{0-2}OR^\bullet$, $—(CH_2)_{0-2}CH(OR^\bullet)_2$; —O(halo$R^\bullet$), —CN, $—N_3$, $—(CH_2)_{0-2}C(O)R^\bullet$, $—(CH_2)_{0-2}C(O)OH$, $—(CH_2)_{0-2}C(O)OR^\bullet$, $—(CH_2)_{0-2}SR^\bullet$, $—(CH_2)_{0-2}SH$, $—(CH_2)_{0-2}NH_2$, $—(CH_2)_{0-2}NHR^\bullet$, $—(CH_2)_{0-2}NR^\bullet_2$, $—NO_2$, $—SiR^\bullet_3$, $—OSiR^\bullet_3$, $—C(O)SR^\bullet$, $—(C_{1-4}$ straight or branched alkylene)$C(O)OR^\bullet$, or $—SSR^\bullet$ wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $—CH_2Ph$, $—O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include ═O and ═S.

[0075] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, =NNR*$_2$, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)$_2$R*, =NR*, =NOR*, —O(C(R*$_2$))$_{2-3}$O—, or —S(C(R*$_2$))$_{2-3}$S—, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: —O(CR*$_2$)$_{2-3}$O—, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0076] Suitable substituents on the aliphatic group of R* include halogen, —R$^{\bullet}$, -(haloR$^{\bullet}$), —OH, —OR$^{\bullet}$, —O(haloR$^{\bullet}$), —CN, —C(O)OH, —C(O)OR$^{\bullet}$, —NH$_2$, —NHR$^{\bullet}$, —NR$^{\bullet}_2$, or —NO$_2$, wherein each R$^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, —CH$_2$Ph, —O(CH$_2$)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0077] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include —R†, —NR$^{\dagger}_2$, —C(O)R†, —C(O)OR†, —C(O)C(O)R†, —C(O)CH$_2$C(O)R†, —S(O)$_2$R†, —S(O)$_2$NR$^{\dagger}_2$, —C(S)NR$^{\dagger}_2$, —C(NH)NR$^{\dagger}_2$, or —N(R†)S(O)$_2$R†; wherein each R† is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted —OPh, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R†, taken together with their intervening atom(s) form an unsubstituted 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0078] Suitable substituents on the aliphatic group of R† are independently halogen, —R$^{\bullet}$, -(haloR$^{\bullet}$), —OH, —OR$^{\bullet}$, —O(haloR$^{\bullet}$), —CN, —C(O)OH, —C(O)OR$^{\bullet}$, —NH$_2$, —NHR$^{\bullet}$, —NR$^{\bullet}_2$, or —NO$_2$, wherein each R$^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, —CH$_2$Ph, —O(CH$_2$)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0079] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1-19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

[0080] Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

[0081] Unless otherwise stated, structures depicted herein are also meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, and Z and E conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, all tautomeric forms of the compounds of the invention are within the scope of the invention. Additionally, unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures including the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention. Such compounds are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present invention. In some embodiments, the R^1 group of formula I-a and I-b comprises one or more deuterium atoms.

[0082] As used herein, the term "irreversible" or "irreversible inhibitor" refers to an inhibitor (i.e. a compound) that is able to be covalently bonded to a target protein kinase in a substantially non-reversible manner. That is, whereas a reversible inhibitor is able to bind to (but is generally unable to form a covalent bond) the target protein kinase, and therefore can become dissociated from the target protein kinase, an irreversible inhibitor will remain substantially bound to the target protein kinase once covalent bond formation has occurred. Irreversible inhibitors usually display time dependency, whereby the degree of inhibition increases with the time with which the inhibitor is in contact with the enzyme. Methods for identifying if a compound is acting as an irreversible inhibitor are known to one of ordinary skill in the art. Such methods include, but are not limited to, enzyme kinetic analysis of the inhibition profile of the compound with the protein kinase target, the use of mass spectrometry of the protein drug target modified in the presence of the inhibitor compound, discontinuous exposure, also known as "washout," experiments, and the use of labeling, such as radiolabelled inhibitor, to show covalent modification of the enzyme, as well as other methods known to one of skill in the art.

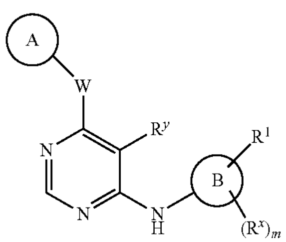
[0083] One of ordinary skill in the art will recognize that certain reactive functional groups can act as "warheads." As used herein, the term "warhead" or "warhead group" refers to a functional group present on a compound of the present invention wherein that functional group is capable of covalently binding to an amino acid residue (such as cysteine, lysine, histidine, or other residues capable of being covalently modified) present in the binding pocket of the target protein, thereby irreversibly inhibiting the protein. It will be appreciated that the -L-Y group, as defined and described herein, provides such warhead groups for covalently, and irreversibly, inhibiting the protein.

[0084] As used herein, the term "inhibitor" is defined as a compound that binds to and for inhibits the target protein kinase with measurable affinity. In certain embodiments, an inhibitor has an IC_{50} and/or binding constant of less about 50 μM, less than about 1 μM, less than about 500 nM, less than about 100 nM, or less than about 10 nM.

[0085] The terms "measurable affinity" and "measurably inhibit," as used herein, means a measurable change in at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3 activity between a sample comprising a compound of the present invention, or composition thereof, and at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, and an equivalent sample comprising at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, in the absence of said compound, or composition thereof.

3. Description of Exemplary Compounds

[0086] According to one aspect, the present invention provides a compound of formula I,



I

[0087] or a pharmaceutically acceptable salt thereof, wherein:

[0088] Ring A is an optionally substituted group selected from phenyl, an 8-10 membered bicyclic partially unsaturated or aryl ring, a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaryl ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

[0089] Ring B is phenyl, a 5-6 membered heteroaryl ring having 1-3 heteroatoms independently selected from N, O or S, a 5-6 membered saturated heterocyclic ring having 1-2 heteroatoms independently selected from N, O or S, or an 8-10 membered bicyclic partially unsaturated or aryl ring having 1-3 heteroatoms independently selected from N, O or S;

[0090] R^1 is -L-Y, wherein:

[0091] L is a covalent bond or a bivalent C_{1-8} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one, two, or three methylene units of L are optionally and independently replaced by cyclopropylene, —NR—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, —SO_2N(R)—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO—, —SO_2—, —C(═S)—, —C(═NR)—, —N═N—, or —C(═N_2)—;

[0092] Y is hydrogen, C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, or a 3-10 membered monocyclic or bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and wherein said ring is substituted with 1-4 R^e groups; and

[0093] each R^e is independently selected from -Q-Z, oxo, NO_2, halogen, CN, a suitable leaving group, or a C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, wherein:

[0094] Q is a covalent bond or a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —N(R)—, —S—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —SO—, or —SO_2—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, or —SO_2N(R)—; and

[0095] Z is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN;

[0096] R^y is hydrogen, halogen, CN, lower alkyl, or lower haloalkyl;

[0097] W is a bivalent C_{1-3} alkylene chain wherein one methylene unit of W is optionally replaced by —NR^2—, —N(R^2)C(O)—, —C(O)N(R^2)—, —N(R^2)SO_2—, —SO_2N(R^2)—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO— or —SO_2—;

[0098] R^2 is hydrogen or optionally substituted C_{1-6} aliphatic, or:

[0099] R^2 and a substituent on Ring A are taken together with their intervening atoms to form a 4-6 membered saturated ring, or:

[0100] R^2 and R^y are taken together with their intervening atoms to form a 4-7 membered carbocyclic ring;

[0101] m is 0-4;

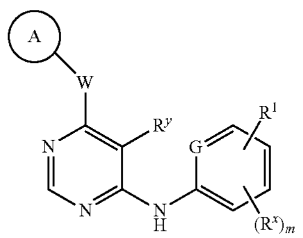
[0102] each R^x is independently selected from —R, halogen, —OR, —CN, —NO_2, —SO_2R, —SOR, —C(O)R, —CO_2R, —C(O)N$(R)_2$, —NRC(O)R, —NRC(O)NR_2, —NRSO_2R, or —N$(R)_2$; or:

[0103] R^x and R^1 are taken together with their intervening atoms to form a 5-7 membered saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with a warhead group and 0-3 groups independently selected from oxo, halogen, CN, or C_{1-6} aliphatic; and

[0104] each R group is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic; phenyl, a 4-7 membered heterocylic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0105] In certain embodiments, the present invention provides a compound of formula II:

II



[0106] or a pharmaceutically acceptable salt thereof, wherein:

[0107] Ring A is an optionally substituted group selected from phenyl, an 8-10 membered bicyclic partially unsaturated or aryl ring, a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaryl ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

[0108] R^1 is -L-Y, wherein:

[0109] L is a covalent bond or a bivalent C_{1-8} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one, two, or three methylene units of L are optionally and independently replaced by cyclopropylene, —NR—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, —SO_2N(R)—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO—, —SO_2—, —C(═S)—, —C(═NR)—, —N═N—, or —C(═N_2)—;

[0110] Y is hydrogen, C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, or a 3-10 membered monocyclic or bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and wherein said ring is substituted with 1-4 R^e groups; and

[0111] each R^e is independently selected from -Q-Z, oxo, NO_2, halogen, CN, a suitable leaving group, or a C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, wherein:

[0112] Q is a covalent bond or a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —N(R)—, —S—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —SO—, or —SO_2—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, or —SO_2N(R)—; and

[0113] Z is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN;

[0114] R^y is hydrogen, halogen, CN, lower alkyl, or lower haloalkyl;

[0115] G is CH, or N;

[0116] W is —NR^2—, —S—, or —O—;

[0117] R^2 is hydrogen or optionally substituted C_{1-6} aliphatic, or:

[0118] R^2 and a substituent on Ring A are taken together with their intervening atoms to form a 4-6 membered saturated ring;

[0119] m is 0-4;

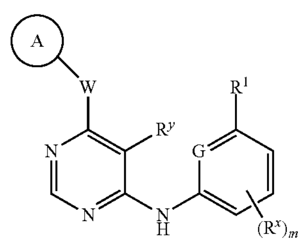
[0120] each R^x is independently selected from —R, halogen, —OR, —CN, —NO_2, —SO_2R, —SOR, —C(O)R, —CO_2R, —C(O)N$(R)_2$, —NRC(O)R, —NRC(O)NR_2, —NRSO_2R, or —N$(R)_2$; or:

[0121] R^x and R^1 are taken together with their intervening atoms to form a 5-7 membered saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with a warhead group and 0-3 groups independently selected from oxo, halogen, CN, or C_{1-6} aliphatic; and

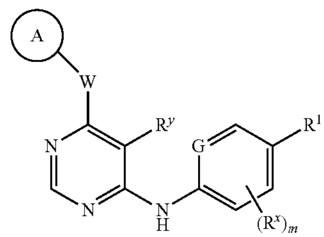
[0122] each R group is independently hydrogen or an optionally substituted group selected from C_{1-6} aliphatic, phenyl, a 4-7 membered heterocylic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0123] According to one aspect, the present invention provides a compound of formula II-a or II-b:

II-a



II-b



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, W, R^1, G, R^y, R^x and m are as defined above for formula II and as described herein.

[0124] In certain embodiments, the present invention provides a compound of formula II-b where in said compound is other than N^6-m-tolyl-N^4-p-tolylpyrimidine-4,6-diamine.

[0125] In certain embodiments, the present invention provides a compound of formula II-a wherein said compound is other than N^4-(3-aminophenyl)-N^6-(3-bromophenyl)pyrimidine-4,6-diamine, N-(3-(6-(3-(trifluoromethyl)phenylamino)pyrimidin-4-ylamino)phenyl)cyclopropane-carboxamide, N-(3-(6-(3-bromophenylamino)pyrimidin-4-ylamino)phenyl)propionamide, N^4-(3-aminophenyl)-N^6-m-tolylpyrimidine-4,6-diamine, or N^4-(3-aminophenyl)-N^6-methyl-N6-phenylpyrimidine-4,6-diamine.

[0126] As defined generally above, the Ring A group of formulae I and II is an optionally substituted group selected from phenyl, an 8-10 membered bicyclic partially unsaturated or aryl ring, a 5-6 membered monocyclic heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an 8-10 membered bicyclic heteroaryl ring having 1-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, Ring A is an optionally substituted phenyl group. In some embodiments, Ring A is an optionally substituted naphthyl ring or a bicyclic 8-10 membered heteroaryl ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, Ring A is an optionally substituted diphenyl ether. In some embodiments, Ring A is an optionally substituted phenyl benzyl ether. In other embodiments, Ring A is an optionally substituted pyridine methoxy phenyl group.

[0127] In certain embodiments, the Ring A group of formulae I and II is substituted as defined herein. In some embodiments, Ring A is substituted with one, two, or three groups independently selected from halogen, R°, or —$(CH_2)_{0-4}$OR°, or —$O(CH_2)_{0-4}$R°, wherein each R° is as defined herein. Exemplary substituents on Ring A include Br, I, Cl, methyl, —CF_3, —C≡CH, —OCH_2phenyl, —OCH_2(fluorophenyl), or —OCH_2pyridyl.

[0128] Exemplary Ring A groups of formulae I and II are set forth in Table 1.

TABLE 1

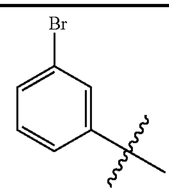
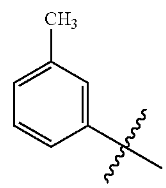
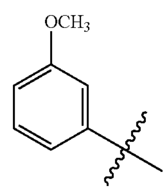
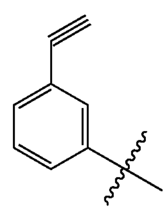
Exemplary Ring A Groups	
	i
	ii
	iii
	iv

TABLE 1-continued

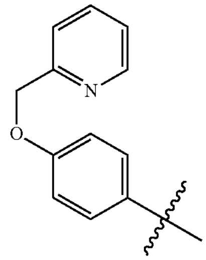
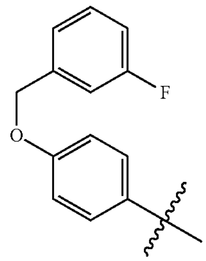
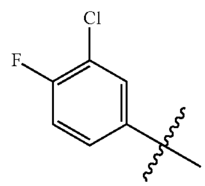
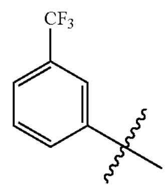
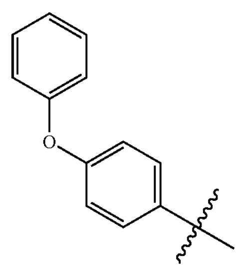
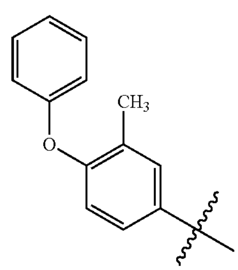
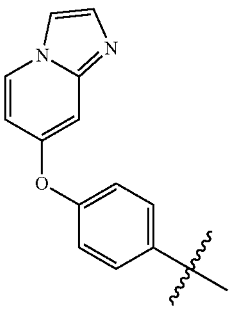
Exemplary Ring A Groups	
	v
	vi
	vii
	viii
	ix
	x

TABLE 1-continued

Exemplary Ring A Groups



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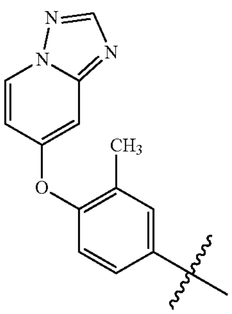

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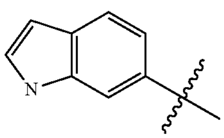

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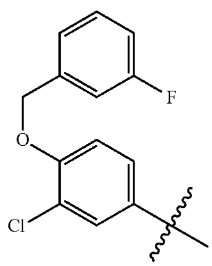

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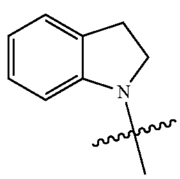

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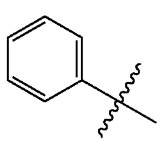

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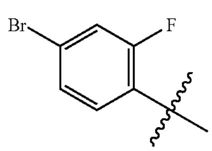

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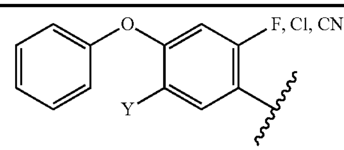
TABLE 1-continued

Exemplary Ring A Groups



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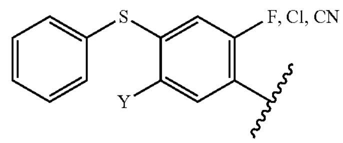

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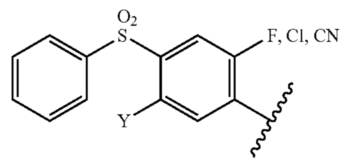

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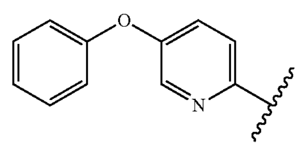

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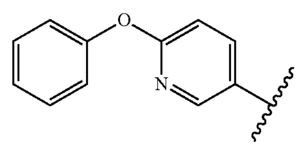

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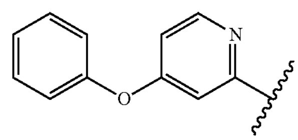

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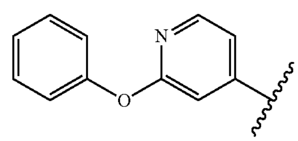

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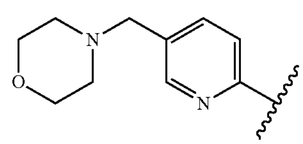

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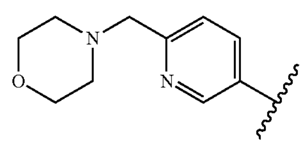

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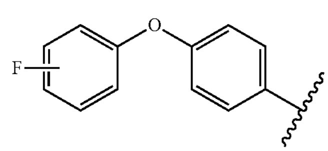

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TABLE 1-continued

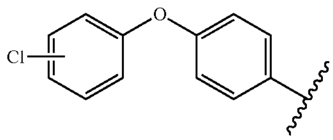
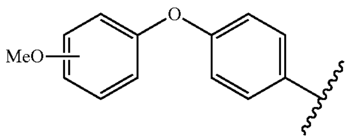
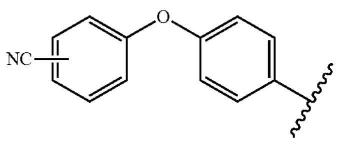
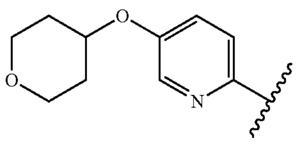
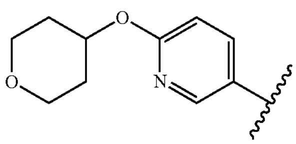
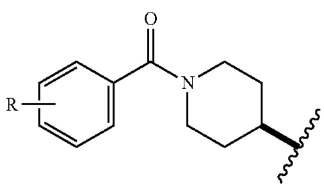
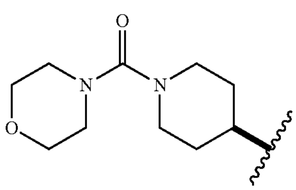
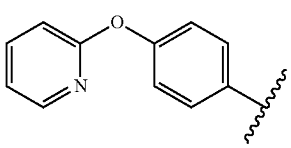
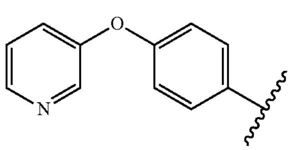
Exemplary Ring A Groups	
	xxviii
	xxix
	xxx
	xxxi
	xxxii
	xxxiii
	xxxiv
	xxxv
	xxxvi

TABLE 1-continued

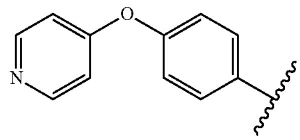
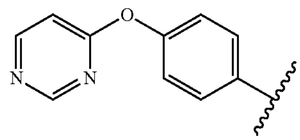
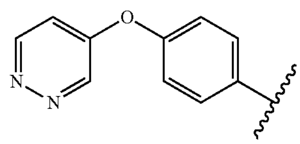
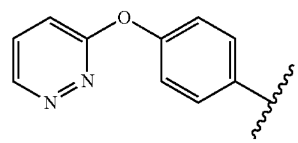
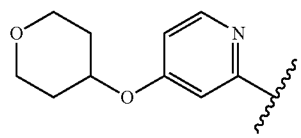
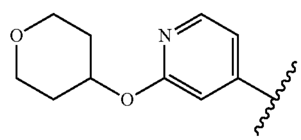
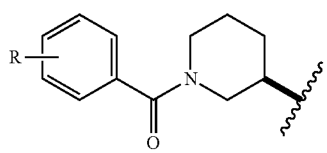
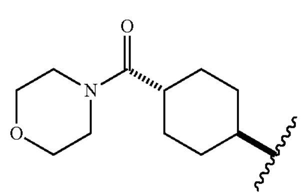
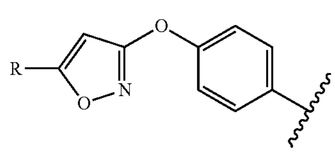
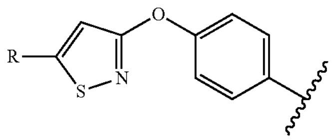
Exemplary Ring A Groups	
	xxxvii
	xxxviii
	xxxix
	xl
	xli
	xlii
	xliii
	xliv
	xlv

TABLE 1-continued

Exemplary Ring A Groups

xlvi



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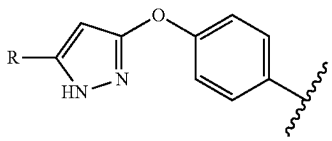

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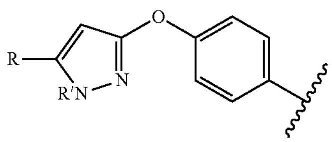

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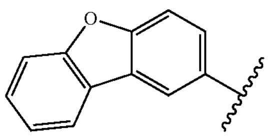

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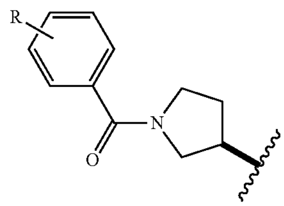

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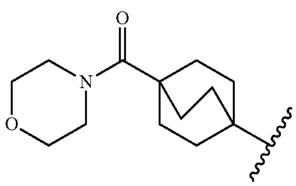

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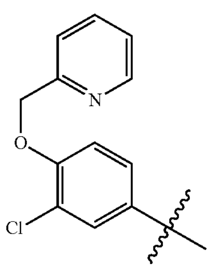

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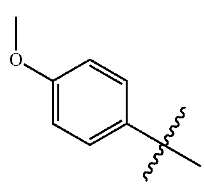
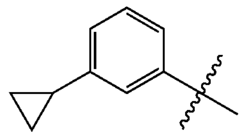


TABLE 1-continued

Exemplary Ring A Groups

liv



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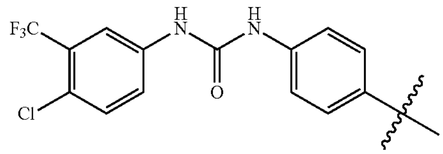

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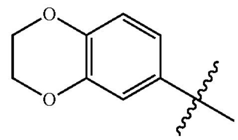


[0129] As defined generally above, the Ring B group of formula I is phenyl, a 5-6 membered heteroaryl ring having 1-3 heteroatoms independently selected from N, O or S, a 5-6 membered saturated heterocyclic ring having 1-2 heteroatoms independently selected from N, O or S, or an 8-10 membered bicyclic partially unsaturated or aryl ring having 1-3 heteroatoms independently selected from N, O or S.

[0130] In some embodiments, the Ring B group of formula I is phenyl. In some embodiments, Ring B is a 6-membered heteroaryl ring having 1-3 nitrogens. In some embodiments, Ring B is a 5-membered heteroaryl ring having 1 or 2 or 3 heteroatoms independently selected from N, O or S.

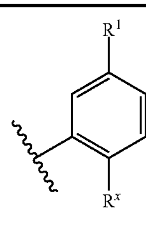
[0131] In some embodiments, the Ring B group of formula I is a 5-6 membered saturated heterocyclic ring having 1 nitrogen. In some embodiments, Ring B is a 9-10 membered bicyclic partially saturated heteroaryl ring having 1-3 nitrogens. In some embodiments, Ring B is a 9-10 membered bicyclic partially saturated heteroaryl ring having 1 nitrogen.

[0132] Exemplary Ring B groups are set forth in Table 2.

TABLE 2

Ring B Groups

i



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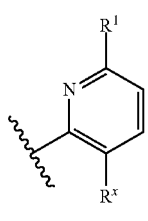


TABLE 2-continued

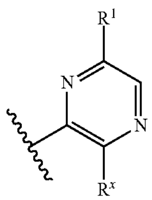
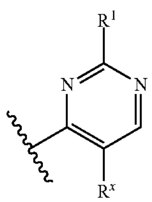
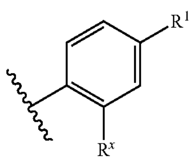
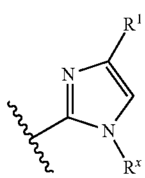
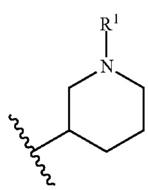
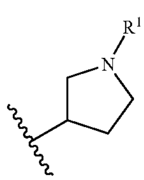
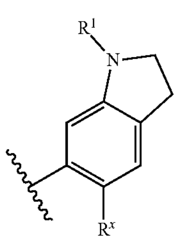
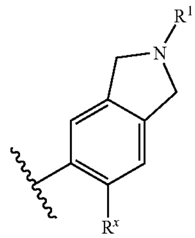
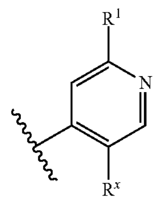
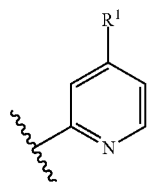
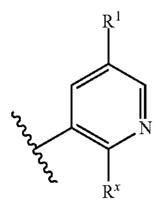
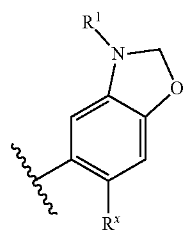
Ring B Groups	
	iii
	iv
	v
	vi
	vii
	viii
	ix

TABLE 2-continued

Ring B Groups	
	x
	xi
	xii
	xiii
	xiv

[0133] In some embodiments, the m moiety of formula I, II, IIa, or IIb is 1, 2, 3 or 4. In some embodiments, m is 1. In other embodiments, m is 0.

[0134] As defined generally above, each R^x group of formula I or II is independently selected from —R, halogen, —OR, —CN, —NO_2, —SO_2R, —SOR, —C(O)R, —CO_2R, —C(O)N(R)$_2$, —NRC(O)R, —NRC(O)NR$_2$, —NRSO$_2$R, or —N(R)$_2$, or

[0135] R^x and R^1 are taken together with their intervening atoms to form a 5-7 membered saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with a warhead group, wherein the warhead group is -Q-Z, and said ring is further substituted with 0-3 groups independently selected from oxo, halogen, CN, or C_{1-6} aliphatic.

[0136] In some embodiments, each instance of R^x is independently selected from —R, —OR or halogen. In certain embodiments, R^x is lower alkyl, lower alkoxy, or halogen.

Exemplary R^x groups include methyl, methoxy, and chloro. In some embodiments, R^x is hydrogen.

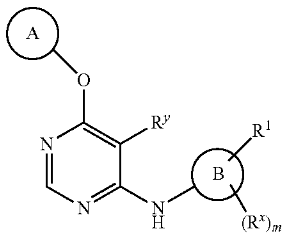
[0137] In some embodiments, the G group of any of formula II, II-a, or II-b is CH. In other embodiments, the G group of any of formula II, II-a, or II-b is N.

[0138] As defined generally above, the W group of formula I is a bivalent C_{1-3} alkylene chain wherein one methylene unit of W is optionally replaced by —NR^2—, —$N(R^2)C(O)$—, —$C(O)N(R^2)$—, —$N(R^2)SO_2$—, —$SO_2N(R^2)$—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO— or —SO_2—.

[0139] In certain embodiments, the W group of formula I is —NH—, —S—, or —O—. In some embodiments, the W group of formula I is —CH_2O—, —CH_2S—, or —CH_2NH—. In some aspects, W is —OCH_2—, —$NHCH_2$—, or —CH_2CH_2—.

[0140] In some embodiments, the W group of formula I is —O— thus forming a compound of formula I-i:

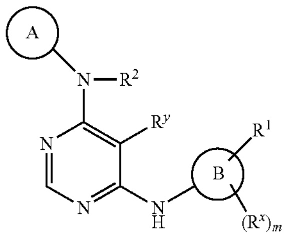
I-*i*



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0141] In some embodiments, W is —NR^2— thus forming a compound of formula I-ii:

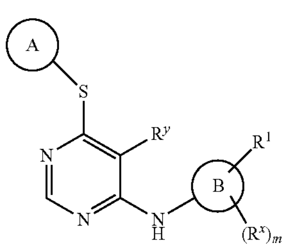
I-*ii*



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^2, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0142] In some embodiments, W is —S— thus forming a compound of formula I-iii:

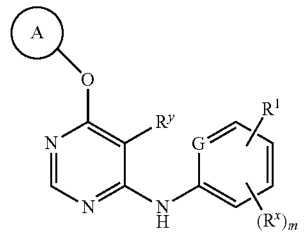
I-*iii*



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0143] In some embodiments, the W group of formula II is —O— thus forming a compound of formula II-i:

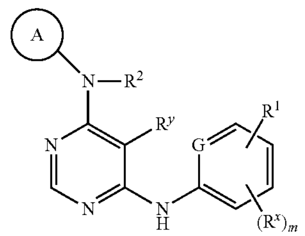
II-*i*



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0144] In some embodiments, the W group of formula II is —NR^2— thus forming a compound of formula II-ii:

II-*ii*

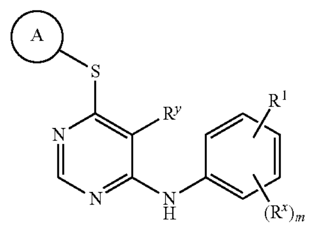


or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0145] In certain embodiments, R^2 is hydrogen. In some embodiments, R^2 is methyl. In still other embodiments, R^2 is lower alkyl.

[0146] In some embodiments, the W group of formula II is —S— thus forming a compound of formula II-iii:

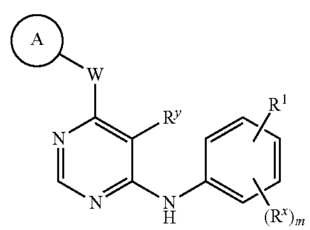
II-iii



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0147] In certain embodiments, the G group of formula II is CH, thus forming a compound of formula III:

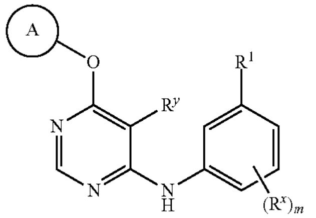
III



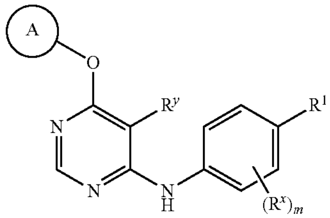
or a pharmaceutically acceptable salt thereof, wherein each of Ring A, W, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0148] In certain embodiments, the compound of formula III is of formula III-a-i, III-b-i, III-a-ii, III-b-ii, III-a-iii, or III-b-iii:

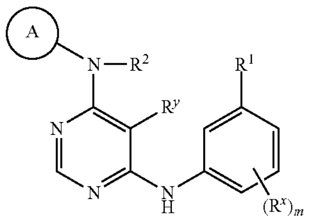
III-a-i



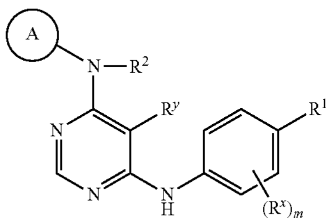
III-b-i



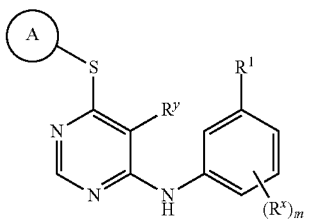
III-a-ii



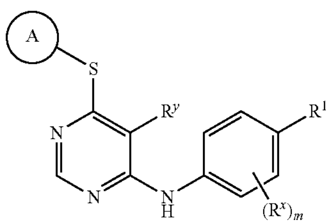
III-b-ii



III-a-iii



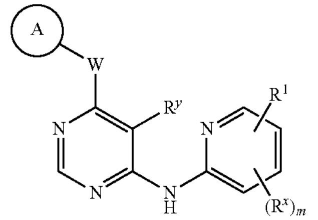
III-b-iii



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^2, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0149] In certain embodiments, the G group of formula II is N, thus forming a compound of formula IV:

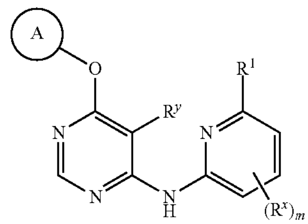
IV



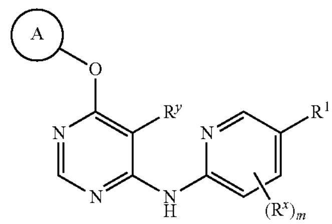
or a pharmaceutically acceptable salt thereof, wherein each of Ring A, W, R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0150] In certain embodiments, the Compound of formula IV is of formula IV-a-i, IV-b-i, IV-a-ii, IV-b-ii, IV-a-iii, or IV-b-iii:

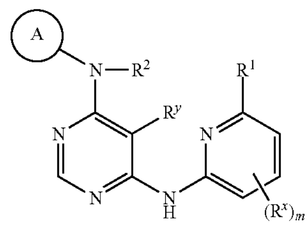
IV-a-i



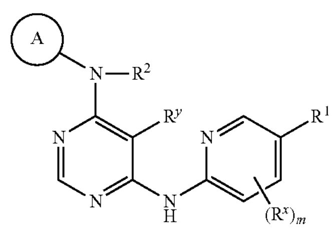
IV-b-i



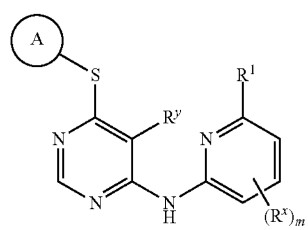
IV-a-ii



IV-b-ii



IV-a-iii



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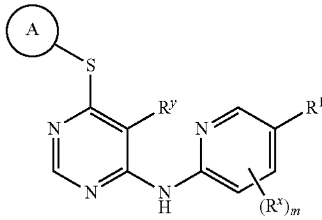
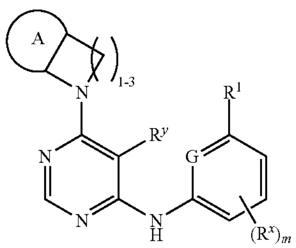
IV-b-iii



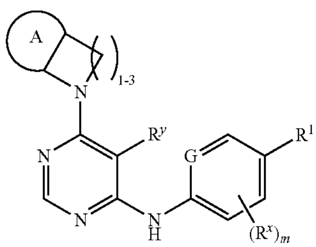
or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^2, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0151] According to some aspects, R^2 and a substituent on Ring A are taken together with their intervening atoms to form a 4-7 membered saturated or partially unsaturated ring, thus forming a compound of formula II-a-iv or II-b-iv:

II-a-iv



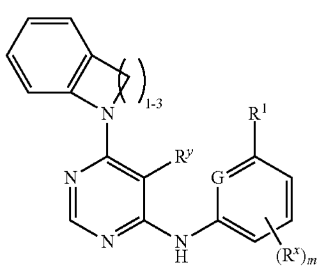
II-b-iv



or a pharmaceutically acceptable salt thereof, wherein each of Ring A, R^1, R^x, and m are as defined above and described in classes and subclasses above and herein.

[0152] In certain embodiments, the present invention provides a compound of formula II-a-v or II-b-v wherein Ring A is phenyl and said compound is of formula II-a-v or II-b-v:

II-a-v



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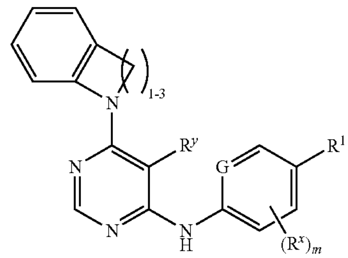
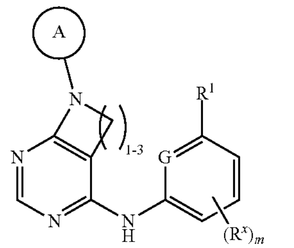
II-b-v



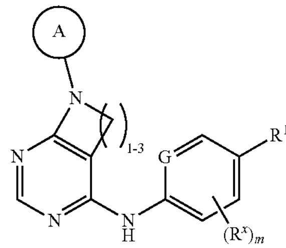
or a pharmaceutically acceptable salt thereof, wherein the Ring A phenyl moiety is optionally substituted and each of R^1, R^x, R^y, and m are as defined above and described in classes and subclasses above and herein.

[0153] In some embodiments, R^2 is hydrogen. In other embodiments, R^2 and R^y are taken together thereby forming a compound of formula II-a-vi or II-b-vi:

II-a-vi



II-b-vi



[0154] As defined generally above, the R^1 group of formulae I and II is -L-Y, wherein:

[0155] L is a covalent bond or a bivalent C_{1-8} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one, two, or three methylene units of L are optionally and independently replaced by cyclopropylene, —NR—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, —SO_2N(R)—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —S—, —SO—, —SO_2—, —C(=S)—, —C(=NR)—, —N=N—, or —C(=N_2)—;

[0156] Y is hydrogen, C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, or a 3-10 membered monocyclic or bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and wherein said ring is substituted with 1-4 R^e groups; and

[0157] each R^e is independently selected from -Q-Z, oxo, NO_2, halogen, CN, a suitable leaving group, or a C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, wherein:

[0158] Q is a covalent bond or a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —N(R)—, —S—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —SO—, or —SO_2—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, or —SO_2N(R)—; and

[0159] Z is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN.

[0160] In certain embodiments, L is a covalent bond.

[0161] In certain embodiments, L is a bivalent C_{1-8} saturated or unsaturated, straight or branched, hydrocarbon chain. In certain embodiments, L is —CH_2—.

[0162] In certain embodiments, L is a covalent bond, —CH_2—, —NH—, —CH_2NH—, —NHCH_2—, —NHC(O)—, —NHC(O)CH_2OC(O)—, —CH_2NHC(O)—, —NHSO_2—, —NH$SO_2$$CH_2$—, —NHC(O)$CH_2$OC(O)—, or —$SO_2$NH—.

[0163] In some embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and one or two additional methylene units of L are optionally and independently replaced by —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, —C(O)O—, cyclopropylene, —O—, —N(R)—, or —C(O)—.

[0164] In certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—, —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—.

[0165] In some embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—.

[0166] As described above, in certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond. One of ordinary skill in the art will recognize that such a double bond may exist within the hydrocarbon chain backbone or may be "exo" to the backbone chain and thus forming an alkylidene group. By way of example, such an L group having an alkylidene branched chain includes —CH_2C(═CH_2)CH_2—. Thus, in some embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one alkylidenyl double bond. Exemplary L groups include —NHC(O)C(═CH_2)CH_2—.

[0167] In certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—. In certain embodiments, L is —C(O)CH═CH(CH_3)—, —C(O)CH═CHCH_2NH(CH_3)—, —C(O)CH═CH(CH_3)—, —C(O)CH═CH—, —CH_2C(O)CH═CH—, —CH_2C(O)CH═CH(CH_3)—, —$CH_2$$CH_2$C(O)CH═CH—, —$CH_2$$CH_2$C(O)CH═CH$CH_2$—, —$CH_2$$CH_2$C(O)CH═CH$CH_2$NH($CH_3$)—, or —$CH_2$$CH_2$C(O)CH═CH($CH_3$)—, or —CH($CH_3$)OC(O)CH═CH—.

[0168] In certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —OC(O)—.

[0169] In some embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—. In some embodiments, L is —CH_2OC(O)CH═CHCH_2—, —CH_2—OC(O)CH═CH—, or —CH(CH═CH_2)OC(O)CH═CH—.

[0170] In certain embodiments, L is —NRC(O)CH═CH—, —NRC(O)CH═CHCH_2N(CH_3)—, —NRC(O)CH═CHCH_2O—, —CH_2NRC(O)CH═CH—, —NRSO_2CH═CH—, —NRSO_2CH═CHCH_2—, —NRC(O)(C═N_2)C(O)—, —NRC(O)CH═CHCH_2N(CH_3)—, —NRSO_2CH═CH—, —NRSO_2CH═CHCH_2—, —NRC(O)CH═CHCH_2O—, —NRC(O)C(═CH_2)CH_2—, —CH_2NRC(O)—, —CH_2NRC(O)CH═CH—, —$CH_2$$CH_2$NRC(O)—, or —$CH_2$NRC(O)cyclopropylene-, wherein each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

[0171] In certain embodiments, L is —NHC(O)CH═CH—, —NHC(O)CH═CHCH_2N(CH_3)—, —NHC(O)CH═CHCH_2O—, —CH_2NHC(O)CH═CH—, —NHSO_2CH═CH—, —NHSO_2CH═CHCH_2—, —NHC(O)(C═N_2)C(O)—, —NHC(O)CH═CHCH_2N(CH_3)—, —NHSO_2CH═CH—, —NHSO_2CH═CHCH_2—, —NHC(O)CH═CHCH_2O—, —NHC(O)C(═CH_2)CH_2—, —CH_2NHC(O)—, —CH_2NHC(O)CH═CH—, —$CH_2$$CH_2$NHC(O)—, or —$CH_2$NHC(O)cyclopropylene-.

[0172] In some embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one triple bond. In certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one triple bond and one or two additional methylene units of L are optionally and independently replaced by —NRC(O)—, —C(O)NR—, —S—, —S(O)—, —SO_2—, —C(═S)—, —C(═NR)—, —O—, —N(R)—, or —C(O)—. In some embodiments, L has at least one triple bond and at least one methylene unit of L is replaced by —N(R)—, —N(R)C(O)—, —C(O)—, —C(O)O—, or —OC(O)—, or —O—.

[0173] Exemplary L groups include —C≡C—, —C≡CCH_2N(isopropyl)-, —NHC(O)C≡C$CH_2$$CH_2$—, —$CH_2$—C≡C—$CH_2$—, —C≡C$CH_2$O—, —$CH_2$C(O)C≡C—, —C(O)C≡C—, or —CH_2OC(═O)C≡C—.

[0174] In certain embodiments, L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein one methylene unit of L is replaced by cyclopropylene and one or two additional methylene units of L are independently replaced by —C(O)—, —NRC(O)—, —C(O)NR—, —N(R)SO_2—, or —SO_2N(R)—. Exemplary L groups include —NHC(O)-cyclopropylene-SO_2— and —NHC(O)— cyclopropylene-.

[0175] As defined generally above, Y is hydrogen, C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, or a 3-10 membered monocyclic or bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and wherein said ring is substituted with at 1-4 R^e groups, each R^e is independently selected from -Q-Z, oxo, NO_2, halogen, CN, a suitable leaving group, or C_{1-6} aliphatic, wherein Q is a covalent bond or a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —N(R)—, —S—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —SO—, or —SO_2—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, or —SO_2N(R)—; and, Z is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN.

[0176] In certain embodiments, Y is hydrogen.

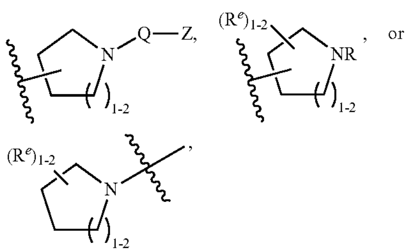
[0177] In certain embodiments, Y is C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN. In some embodiments, Y is C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN. In other embodiments, Y is C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN. In some embodiments, Y is C_{2-6} alkenyl. In other embodiments, Y is C_{2-4} alkynyl.

[0178] In other embodiments, Y is C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN. Such Y groups include —CH_2F, —CH_2Cl, —CH_2CN, and —CH_2NO_2.

[0179] In certain embodiments, Y is a saturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein Y is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein.

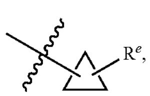
[0180] In some embodiments, Y is a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein. Exemplary such rings are epoxide and oxetane rings, wherein each ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein.

[0181] In other embodiments, Y is a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. Such rings include piperidine and pyrrolidine, wherein each ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In certain embodiments, Y is



wherein each R, Q, Z, and R^e is as defined above and described herein.

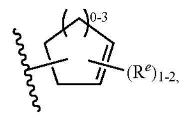
[0182] In some embodiments, Y is a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In certain embodiments, Y is cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, wherein each ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In certain embodiments, Y is



wherein R^e is as defined above and described herein. In certain embodiments, Y is cyclopropyl optionally substituted with halogen, CN or NO_2.

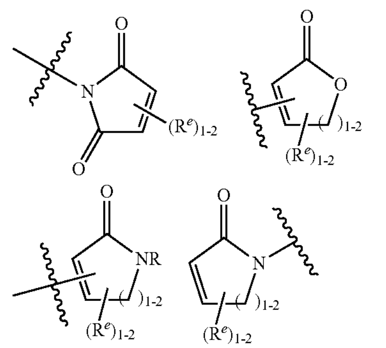
[0183] In certain embodiments, Y is a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein.

[0184] In some embodiments, Y is a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In some embodiments, Y is cyclopropenyl, cyclobutenyl, cyclopentenyl, or cyclohexenyl wherein each ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In certain embodiments, Y is



wherein each R^e is as defined above and described herein.

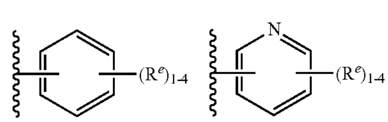
[0185] In certain embodiments, Y is a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein. In certain embodiments, Y is selected from:



wherein each R and R^e is as defined above and described herein.

[0186] In certain embodiments, Y is a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein. In certain embodiments, Y is phenyl, pyridyl, or pyrimidinyl, wherein each ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein.

[0187] In some embodiments, Y is selected from:



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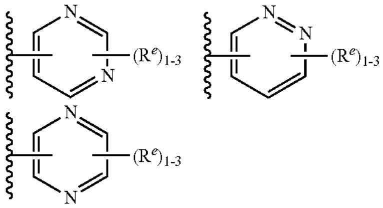
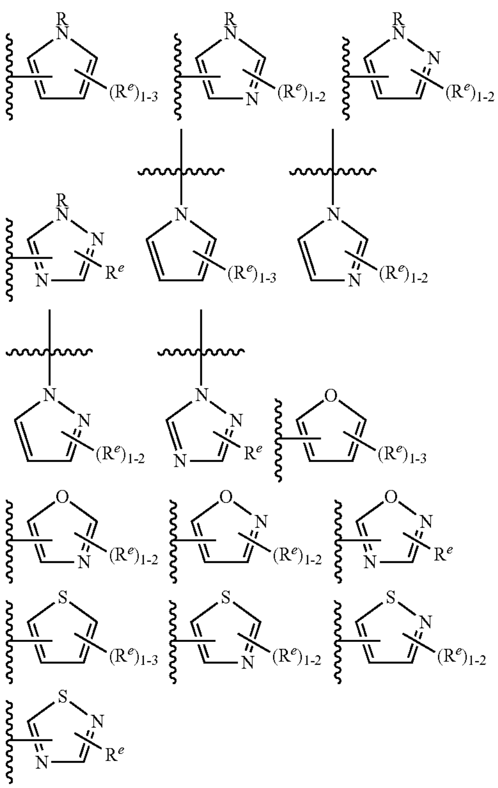


wherein each R^e is as defined above and described herein.

[0188] In other embodiments, Y is a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein. In some embodiments, Y is a 5 membered partially unsaturated or aryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein. Exemplary such rings are isoxazolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, pyrrolyl, furanyl, thienyl, triazole, thiadiazole, and oxadiazole, wherein each ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein. In certain embodiments, Y is selected from:



wherein each R and R^e is as defined above and described herein.

[0189] In certain embodiments, Y is an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein. According to another aspect, Y is a 9-10 membered bicyclic, partially unsaturated, or aryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein. Exemplary such bicyclic rings include 2,3-dihydrobenzo[d]isothiazole, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein.

[0190] As defined generally above, each R^e group is independently selected from -Q-Z, oxo, NO_2, halogen, CN, a suitable leaving group, or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN, wherein Q is a covalent bond or a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —N(R)—, —S—, —O—, —C(O)—, —OC(O)—, —C(O)O—, —SO—, or —SO_2—, —N(R)C(O)—, —C(O)N(R)—, —N(R)SO_2—, or —SO_2N(R)—; and Z is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN.

[0191] In certain embodiments, R^e is C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN. In other embodiments, R^e is oxo, NO_2, halogen, or CN.

[0192] In some embodiments, R^e is -Q-Z, wherein Q is a covalent bond and Z is hydrogen (i.e., R^e is hydrogen). In other embodiments, R^e is -Q-Z, wherein Q is a bivalent C_{1-6} saturated or unsaturated, straight or branched, hydrocarbon chain, wherein one or two methylene units of Q are optionally and independently replaced by —NR—, —NRC(O)—, —C(O)NR—, —S—, —O—, —C(O)—, —SO—, or —SO_2—. In other embodiments, Q is a bivalent C_{2-6} straight or branched, hydrocarbon chain having at least one double bond, wherein one or two methylene units of Q are optionally and independently replaced by —NR—, —NRC(O)—, —C(O)NR—, —S—, —O—, —C(O)—, —SO—, or —SO_2—. In certain embodiments, the Z moiety of the R^e group is hydrogen. In some embodiments, -Q-Z is —NHC(O)CH═CH_2 or —C(O)CH═CH_2.

[0193] In certain embodiments, each R^e is independently selected from oxo, NO_2, CN, fluoro, chloro, —NHC(O)CH═CH_2, —C(O)CH═CH_2, —CH_2CH═CH_2, —C≡CH, —C(O)OCH_2Cl, —C(O)OCH_2F, —C(O)OCH_2CN, —C(O)CH_2Cl, —C(O)CH_2F, —C(O)CH_2CN, or —CH_2C(O)CH_3.

[0194] In certain embodiments, R^e is a suitable leaving group, ie a group that is subject to nucleophilic displacement. A "suitable leaving" is a chemical group that is readily displaced by a desired incoming chemical moiety such as the thiol moiety of a cysteine of interest. Suitable leaving groups are well known in the art, e.g., see, "Advanced Organic Chemistry," Jerry March, 5th Ed., pp. 351-357, John Wiley and Sons, N.Y. Such leaving groups include, but are not limited to, halogen, alkoxy, sulphonyloxy, optionally substituted alkylsulphonyloxy, optionally substituted alkenylsulfonyloxy, optionally substituted arylsulfonyloxy, acyl, and diazonium moieties. Examples of suitable leaving groups include chloro, iodo, bromo, fluoro, acetoxy, methanesulfonyloxy (mesyloxy), tosyloxy, triflyloxy, nitro-phenylsulfonyloxy (nosyloxy), and bromo-phenylsulfonyloxy (brosyloxy).

[0195] In certain embodiments, the following embodiments and combinations of -L-Y apply:

[0196] (a) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and one or two additional methylene units of L are optionally and independently replaced by —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, —C(O)O—, cyclopropylene, —O—, —N(R)—, or —C(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0197] (b) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—, —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0198] (c) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0199] (d) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —C(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0200] (e) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one double bond and at least one methylene unit of L is replaced by —OC(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0201] (f) L is —NRC(O)CH═CH—, —NRC(O)CH═CHCH_2N(CH_3)—, —NRC(O)CH═CHCH_2O—, —CH_2NRC(O)CH═CH—, —NRSO_2CH═CH—, —NRSO_2CH═CHCH_2—, —NRC(O)(C═N_2)—, —NRC(O)(C═N_2)C(O)—, —NRC(O)CH═CHCH_2N(CH_3)—, —NRSO_2CH═CH—, —NRSO_2CH═CHCH_2—, —NRC(O)CH═CHCH_2O—, —NRC(O)C(═CH_2)CH_2—, —CH_2NRC(O)—, —CH_2NRC(O)CH═CH—, —CH_2CH_2NRC(O)—, or —CH_2NRC(O)cyclopropylene-; wherein R is H or optionally substituted C_{1-6} aliphatic; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0202] (g) L is —NHC(O)CH═CH—, —NHC(O)CH═CHCH_2N(CH_3)—, —NHC(O)CH═CHCH_2O—, —CH_2NHC(O)CH═CH—, —NHSO_2CH═CH—, —NHSO_2CH═CHCH_2—, —NHC(O)(C═N_2)—, —NHC(O)(C═N_2)C(O)—, —NHC(O)CH═CHCH_2N(CH_3)—, —NHSO_2CH═CH—, —NHSO_2CH═CHCH_2—, —NHC(O)CH═CHCH_2O—, —NHC(O)C(═CH_2)CH_2—, —CH_2NHC(O)—, —CH_2NHC(O)CH═CH—, —CH_2CH_2NHC(O)—, or —CH_2NHC(O)cyclopropylene-; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0203] (h) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one alkylidenyl double bond and at least one methylene unit of L is replaced by —C(O)—, —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—, and one or two additional methylene units of L are optionally and independently replaced by cyclopropylene, —O—, —N(R)—, or —C(O)—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0204] (i) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein L has at least one triple bond and one or two additional methylene units of L are optionally and independently replaced by —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—, and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0205] (j) L is —C≡C—, —C≡CCH_2N(isopropyl)-, —NHC(O)C≡CCH_2CH_2—, —CH_2—C≡C—CH_2—, —C≡CCH_2O—, —CH_2C(O)C≡C—, —C(O)C≡C—, or —CH_2OC(═O)C≡C—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0206] (k) L is a bivalent C_{2-8} straight or branched, hydrocarbon chain wherein one methylene unit of L is replaced by cyclopropylene and one or two additional methylene units of L are independently replaced by —NRC(O)—, —C(O)NR—, —N(R)SO_2—, —SO_2N(R)—, —S—, —S(O)—, —SO_2—, —OC(O)—, or —C(O)O—; and Y is hydrogen or C_{1-6} aliphatic optionally substituted with oxo, halogen, NO_2, or CN; or

[0207] (l) L is a covalent bond and Y is selected from:

[0208] (i) C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN;

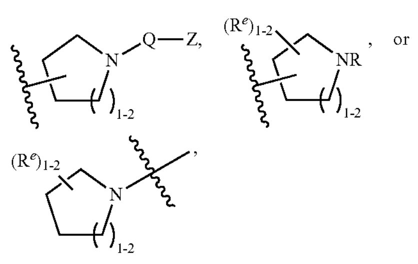
[0209] (ii) C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0210] (iii) C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0211] (iv) a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein; or

[0212] (v) a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0213] (vi)



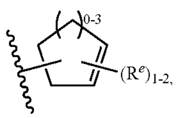
wherein each R, Q, Z, and R^e is as defined above and described herein; or

[0214] (vii) a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0215] (viii) a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0216] (ix) a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

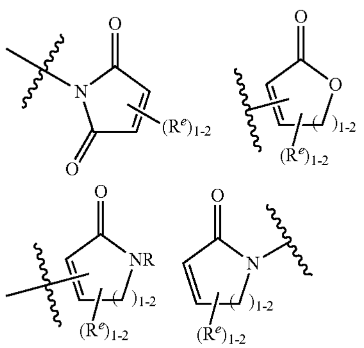
[0217] (x)



wherein each R^e is as defined above and described herein; or

[0218] (xi) a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

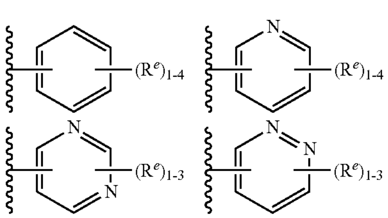
[0219] (xii)



wherein each R and R^e is as defined above and described herein; or

[0220] (xiii) a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein; or

[0221] (xiv)



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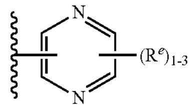
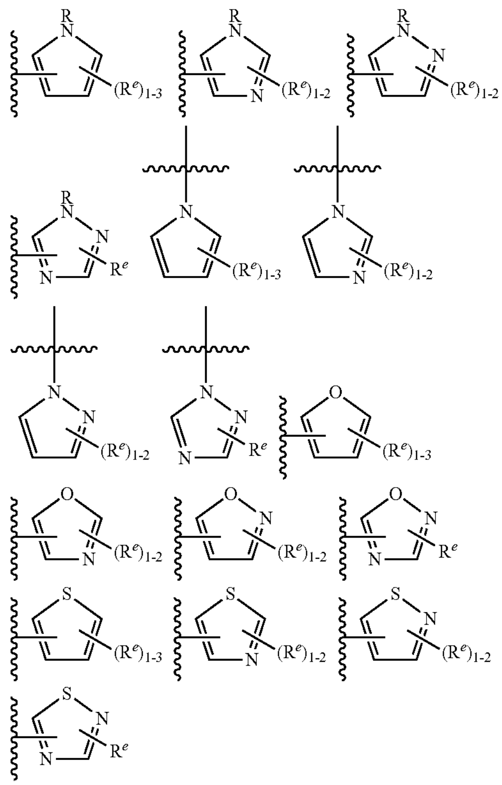


wherein each R^e is as defined above and described herein; or

[0222] (xv) a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein; or

[0223] (xvi)



wherein each R and R^e is as defined above and described herein; or

[0224] (xvii) an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein;

[0225] (m) L is —C(O)— and Y is selected from:

[0226] (i) C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN; or

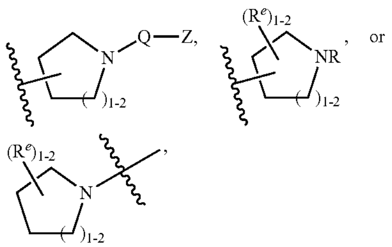
[0227] (ii) C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0228] (iii) C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0229] (iv) a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein; or

[0230] (v) a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0231] (vi)



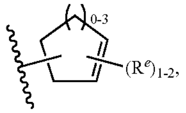
wherein each R, Q, Z, and R^e is as defined above and described herein; or

[0232] (vii) a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0233] (viii) a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0234] (ix) a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

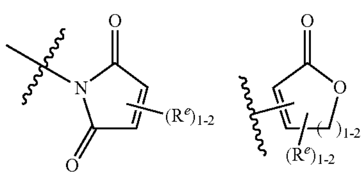
[0235] (x)



wherein each R^e is as defined above and described herein; or

[0236] (xi) a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0237] (xii)



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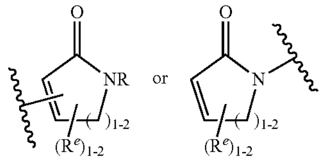
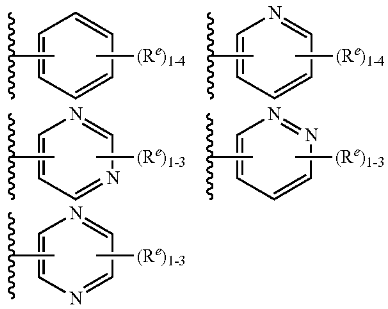


wherein each R and R^e is as defined above and described herein; or

[0238] (xiii) a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein; or

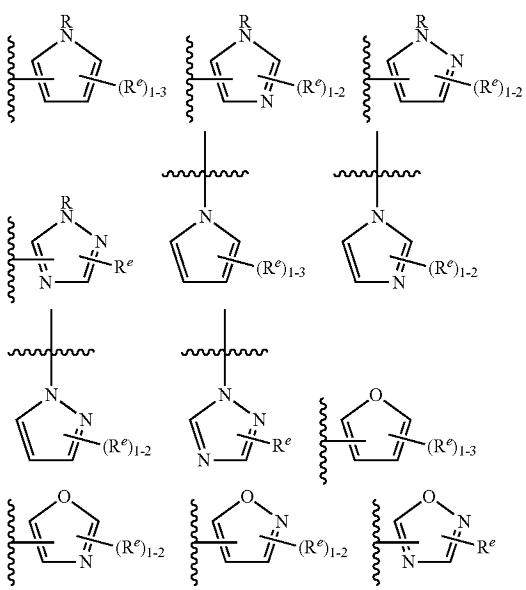
[0239] (xiv)



wherein each R^e is as defined above and described herein; or

[0240] (xv) a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein; or

[0241] (xvi)



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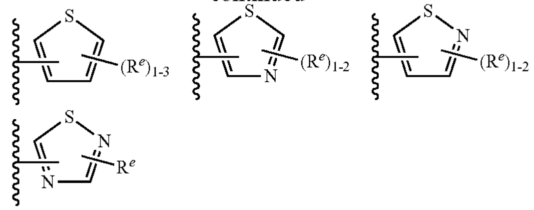


[0242] wherein each R and R^e is as defined above and described herein; or

[0243] (xvii) an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein;

[0244] (n) L is —N(R)C(O)— and Y is selected from:

[0245] (i) C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN; or

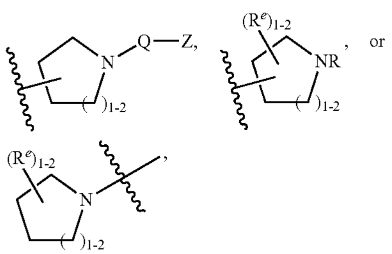
[0246] (ii) C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0247] (iii) C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0248] (iv) a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein; or

[0249] (v) a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0250] (vi)



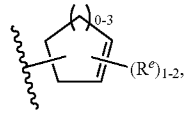
wherein each R, Q, Z, and R^e is as defined above and described herein; or

[0251] (vii) a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0252] (viii) a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0253] (ix) a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

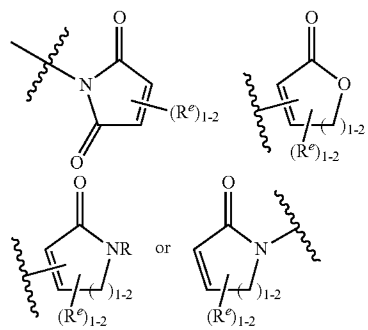
[0254] (x)



wherein each R^e is as defined above and described herein; or

[0255] (xi) a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

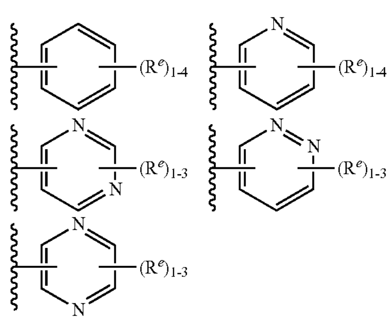
[0256] (xii)



wherein each R and R^e is as defined above and described herein; or

[0257] (xiii) a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein; or

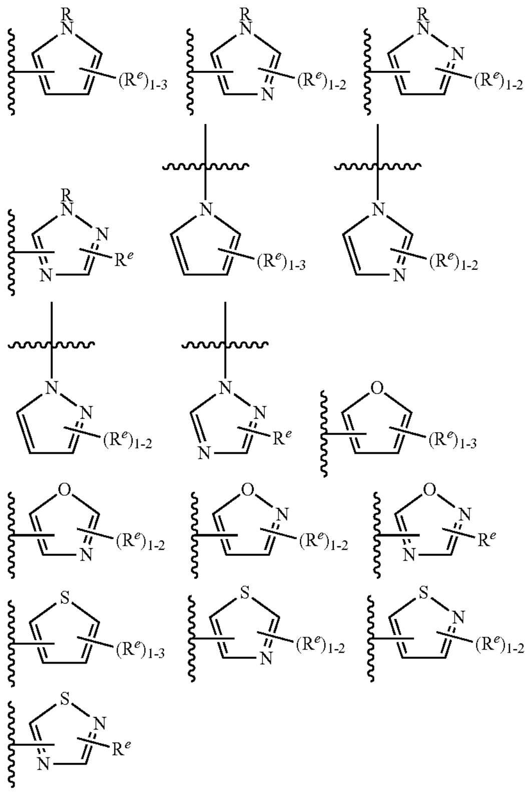
[0258] (xiv)



wherein each R^e is as defined above and described herein; or

[0259] (xv) a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein; or

[0260] (xvi)



[0261] wherein each R and R^e is as defined above and described herein; or

[0262] (xvii) an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein;

[0263] (o) L is a bivalent C_{1-8} saturated or unsaturated, straight or branched, hydrocarbon chain; and Y is selected from:

[0264] (i) C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN;

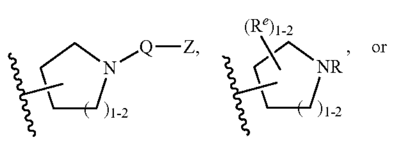
[0265] (ii) C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0266] (iii) C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0267] (iv) a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein; or

[0268] (v) a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0269] (vi)



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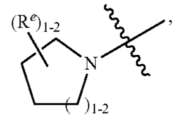


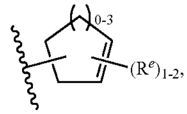
wherein each R, Q, Z, and R^e is as defined above and described herein; or

[0270] (vii) a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0271] (viii) a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0272] (ix) a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

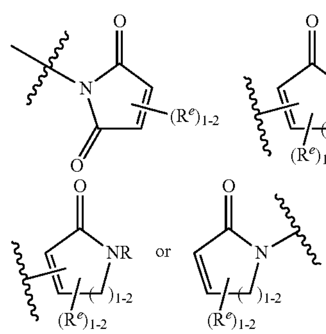
[0273] (x)



wherein each R^e is as defined above and described herein; or

[0274] (xi) a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

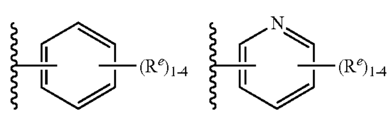
[0275] (xii)



wherein each R and R^e is as defined above and described herein; or

[0276] (xiii) a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 R^e groups, wherein each R^e group is as defined above and described herein; or

[0277] (xiv)



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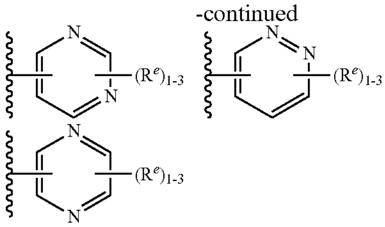
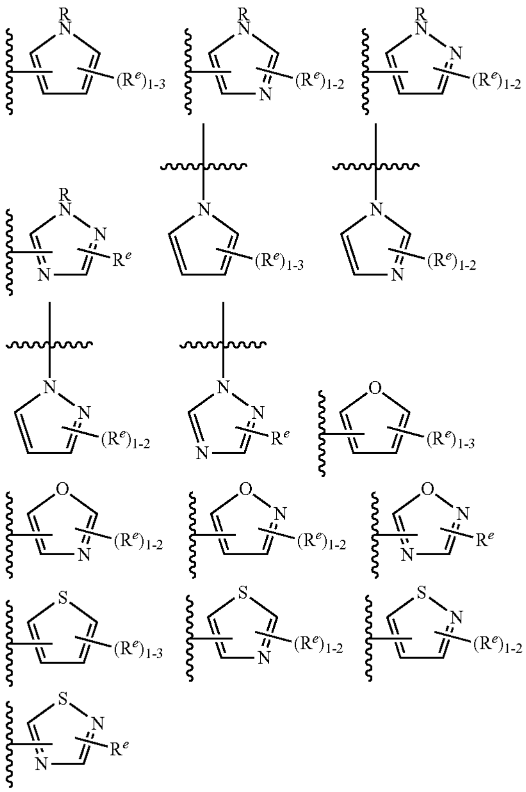


wherein each R^e is as defined above and described herein; or

[0278] (xv) a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein; or

[0279] (xvi)



[0280] wherein each R and R^e is as defined above and described herein; or

[0281] (xvii) an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein;

[0282] (p) L is a covalent bond, —CH_2—, —NH—, —C(O)—, —CH_2NH—, —$NHCH_2$—, —NHC(O)—, —NHC(O)CH_2OC(O)—, —CH_2NHC(O)—, —$NHSO_2$—, —$NHSO_2CH_2$—, —NHC(O)CH_2OC(O)—, or —SO_2NH—; and Y is selected from:

[0283] (i) C_{1-6} alkyl substituted with oxo, halogen, NO_2, or CN; or

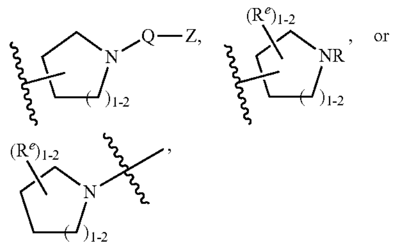
[0284] (ii) C_{2-6} alkenyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0285] (iii) C_{2-6} alkynyl optionally substituted with oxo, halogen, NO_2, or CN; or

[0286] (iv) a saturated 3-4 membered heterocyclic ring having 1 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-2 R^e groups, wherein each R^e is as defined above and described herein; or

[0287] (v) a saturated 5-6 membered heterocyclic ring having 1-2 heteroatom selected from oxygen or nitrogen wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0288] (vi)



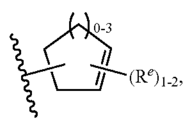
wherein each R, Q, Z, and R^e is as defined above and described herein; or

[0289] (vii) a saturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0290] (viii) a partially unsaturated 3-6 membered monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0291] (ix) a partially unsaturated 3-6 membered carbocyclic ring, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

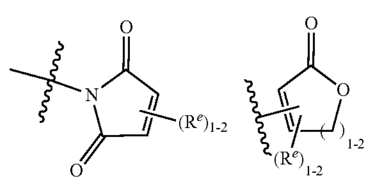
[0292] (x)



wherein each R^e is as defined above and described herein; or

[0293] (xi) a partially unsaturated 4-6 membered heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein each R^e is as defined above and described herein; or

[0294] (xii)



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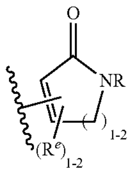
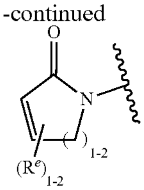
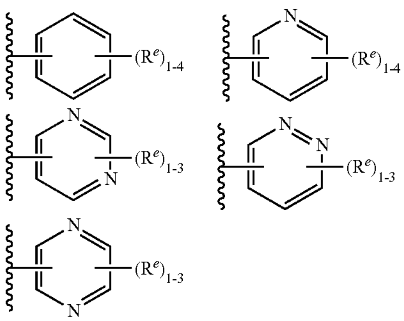
 or 

wherein each R and R^e is as defined above and described herein; or

[0295] (xiii) a 6-membered aromatic ring having 0-2 nitrogens wherein said ring is substituted with 1-4 Re groups, wherein each R^e group is as defined above and described herein; or

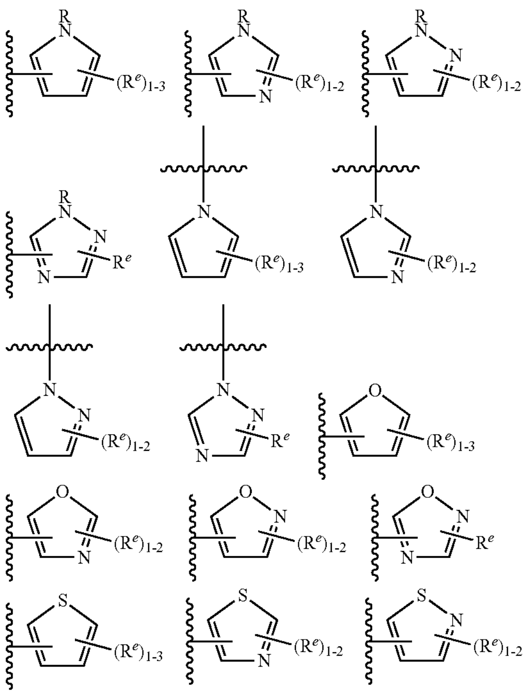
[0296] (xiv)



wherein each R^e is as defined above and described herein; or

[0297] (xv) a 5-membered heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-3 R^e groups, wherein each R^e group is as defined above and described herein; or

[0298] (xvi)



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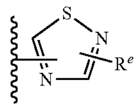
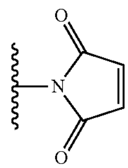
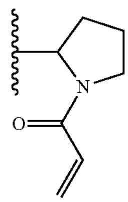
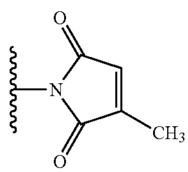
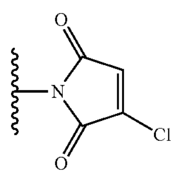
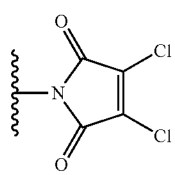
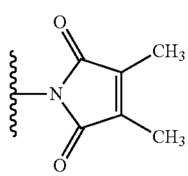


[0299] wherein each R and R^e is as defined above and described herein; or

[0300] (xvii) an 8-10 membered bicyclic, saturated, partially unsaturated, or aryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur, wherein said ring is substituted with 1-4 R^e groups, wherein R^e is as defined above and described herein.

[0301] In certain embodiments, the Y group of formula I is selected from those set forth in Table 3, below, wherein each wavy line indicates the point of attachment to the rest of the molecule.

TABLE 3

Exemplary Y groups:	
	a
	b
	c
	d
	e
	f

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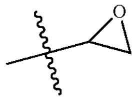
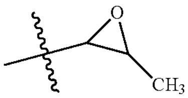
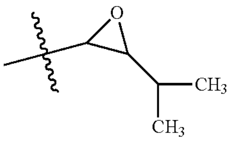
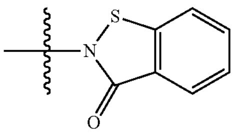
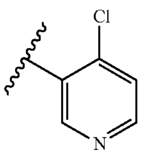
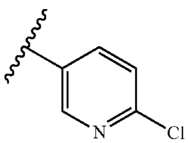
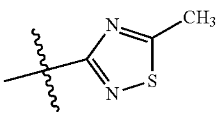
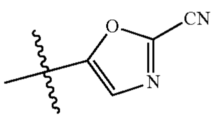
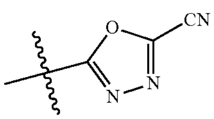
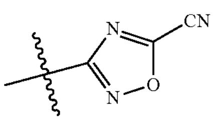
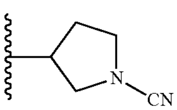
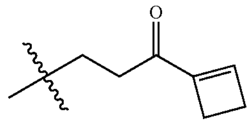
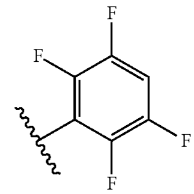
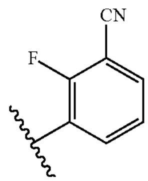
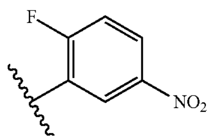
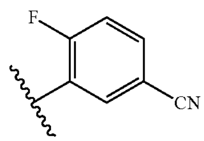
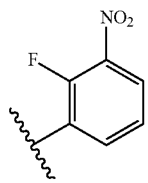
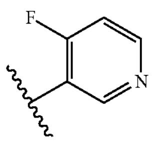
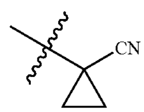
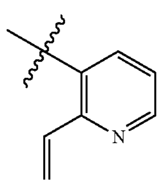
	g
	h
	i
	j
	k
	l
	m
	n
	o
	p
	q
	r
	s
	t
	u
	v
	w
	x
	y
	z

TABLE 3-continued

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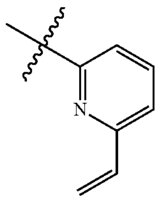
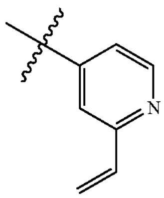
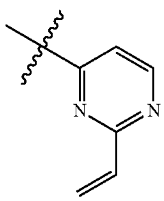
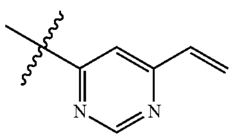
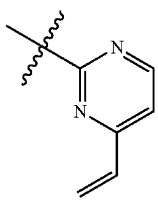
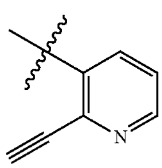
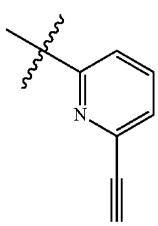
	aa
	bb
	cc
	dd
	ee
	ff
	gg

TABLE 3-continued

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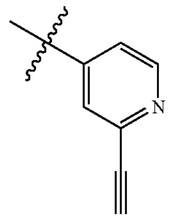
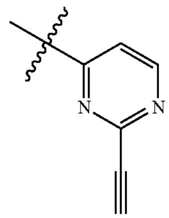
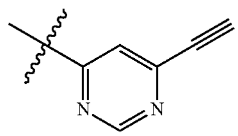
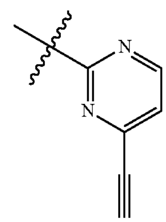
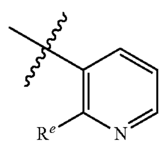
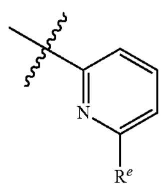
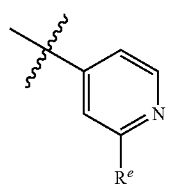
	hh
	ii
	jj
	kk
	ll
	mm
	nn

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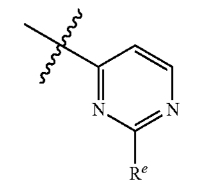
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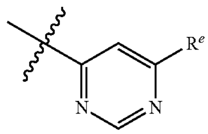
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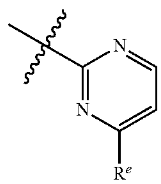
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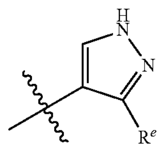
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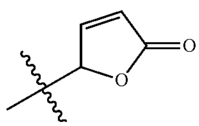
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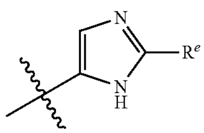
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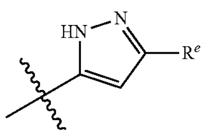
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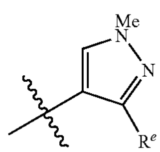
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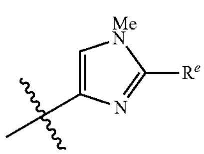
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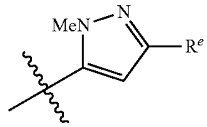
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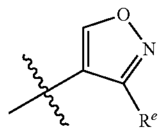
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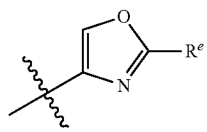
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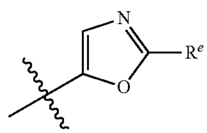
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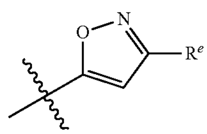
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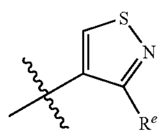
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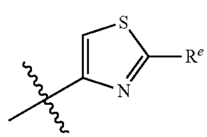
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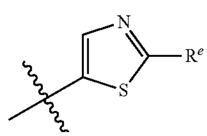
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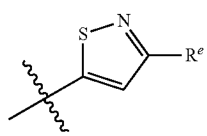
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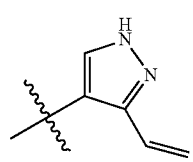
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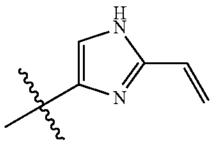
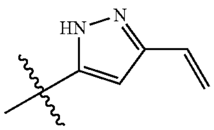
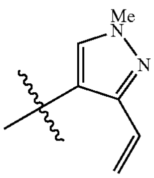
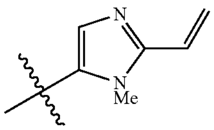
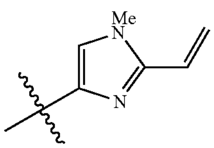
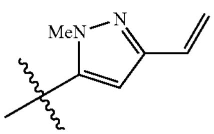
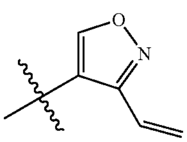
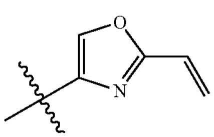
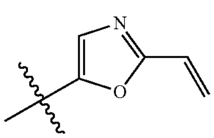
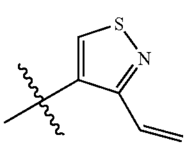
	hhh
	iii
	jjj
	kkk
	lll
	mmm
	nnn
	ooo
	ppp
	qqq

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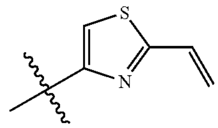
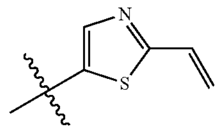
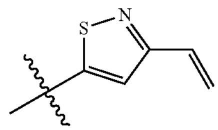
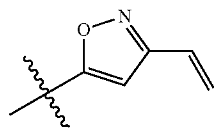
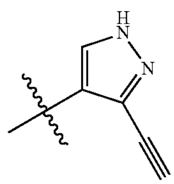
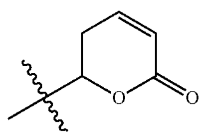
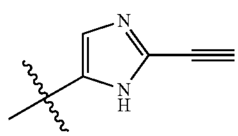
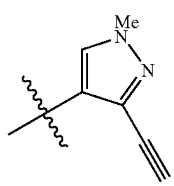
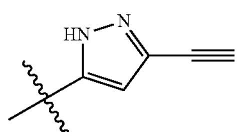
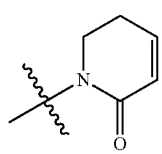
	rrr
	sss
	ttt
	uuu
	vvv
	qqq
	www
	xxx
	yyy
	zzz

TABLE 3-continued

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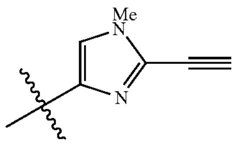
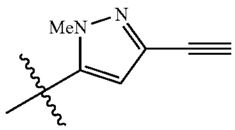
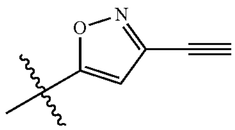
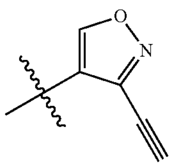
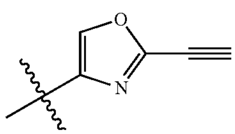
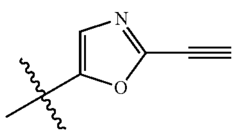
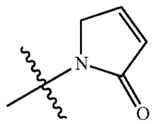
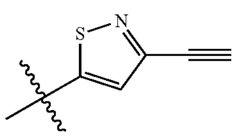
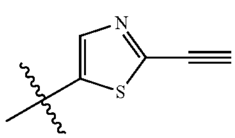
	aaaa
	bbbb
	cccc
	dddd
	eeee
	ffff
	gggg
	hhhh
	iiii

TABLE 3-continued

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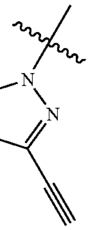
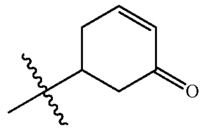
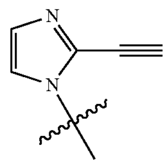
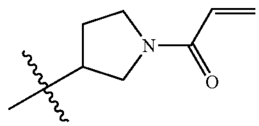
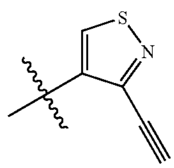
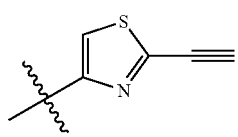
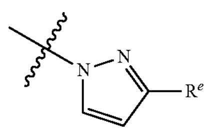
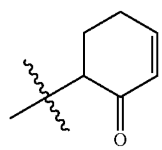
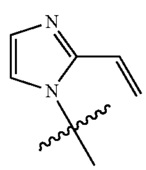
	jjjj
	kkkk
	llll
	mmmm
	nnnn
	oooo
	pppp
	qqqq
	rrrr

TABLE 3-continued

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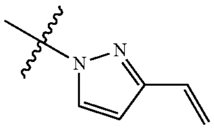
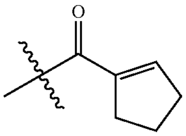
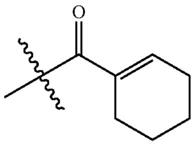
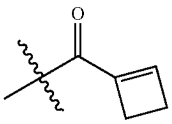
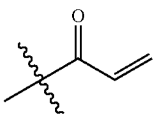
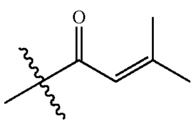
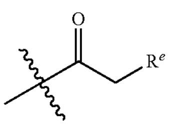
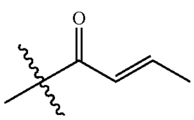
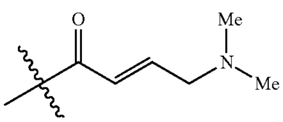
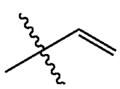
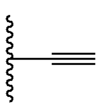
	ssss
	tttt
	uuuu
	vvvv
	wwww
	xxxx
	yyyy
	zzzz
	aaaaa
	bbbbb
	ccccc

wherein each R^e is independently a suitable leaving group, NO_2, CN, or oxo.

[0302] In certain embodiments, R^1 is —C≡CH, —C≡CCH$_2$NH(isopropyl), —NHC(O)C≡CCH$_2$CH$_3$, —CH$_2$—C≡C—CH$_3$, —C≡CCH$_2$OH, —CH$_2$C(O)C≡CH, —C(O)C≡CH, or —CH$_2$OC(═O)C≡CH. In some embodiments, R^1 is selected from —NHC(O)CH═CH$_2$, —NHC(O)CH═CHCH$_2$N(CH$_3$)$_2$, or —CH$_2$NHC(O)CH═CH$_2$.

[0303] In certain embodiments, R^1 is selected from those set forth in Table 4, below, wherein each wavy line indicates the point of attachment to the rest of the molecule.

TABLE 4

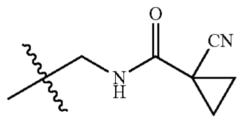
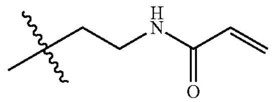
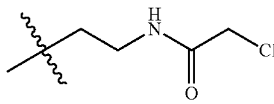
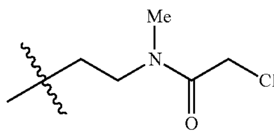
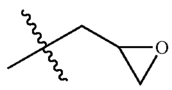
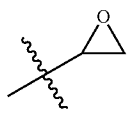
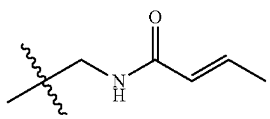
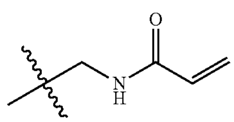
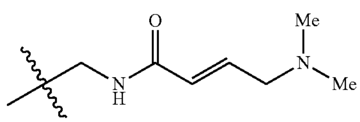
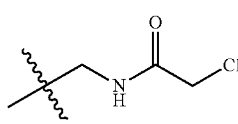
Exemplary R^1 Groups	
	a
	b
	c
	d
	e
	f
	g
	h
	i
	j

TABLE 4-continued

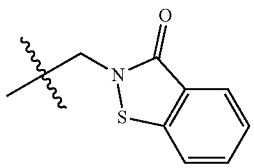
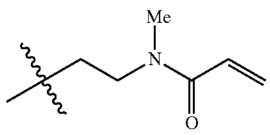
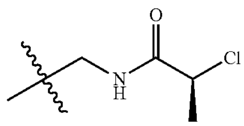
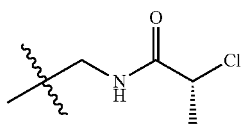
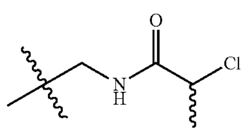
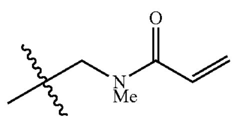
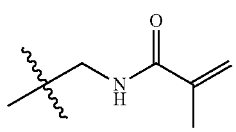
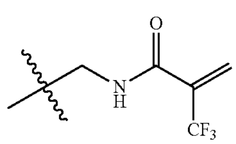
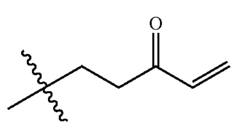
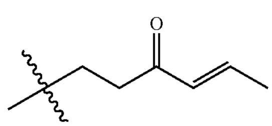
Exemplary R^1 Groups	
	k
	l
	m
	n
	o
	p
	q
	r
	s
	t

TABLE 4-continued

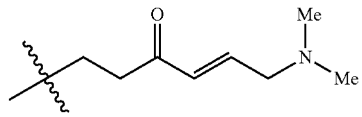
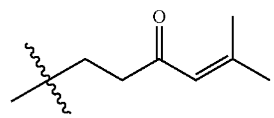
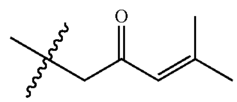
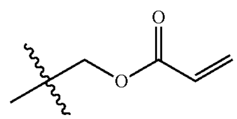
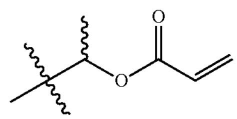
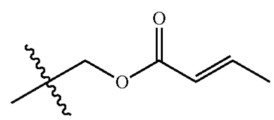
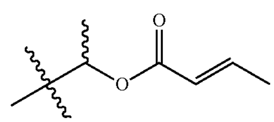
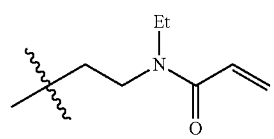
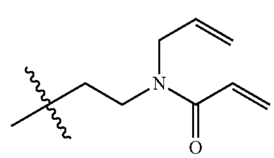
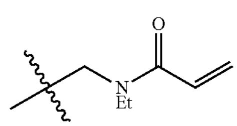
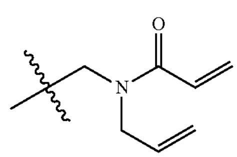
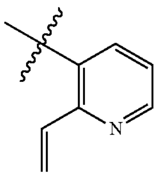
Exemplary R^1 Groups	
	u
	v
	w
	x
	y
	z
	aa
	bb
	cc
	dd
	ee

TABLE 4-continued

Exemplary R^1 Groups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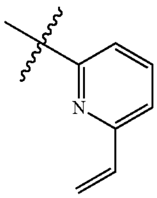
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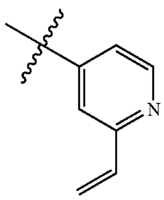
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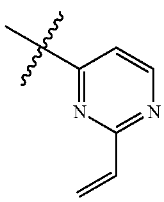
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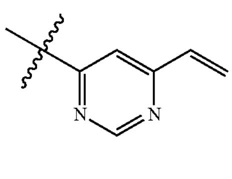
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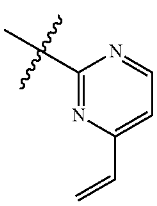
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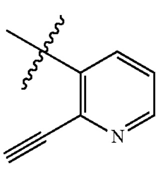
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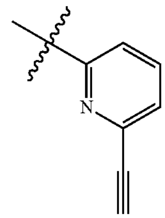
TABLE 4-continued

Exemplary R^1 Groups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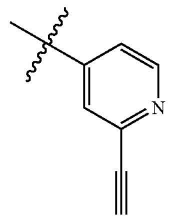
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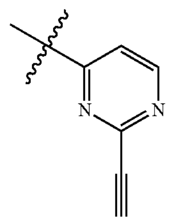
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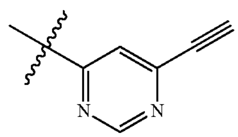
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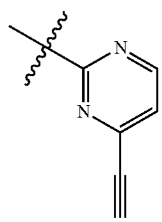
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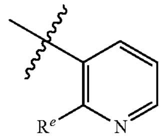
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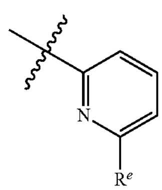
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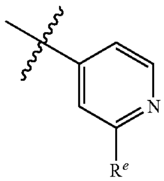
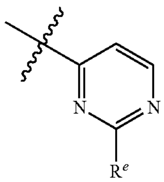
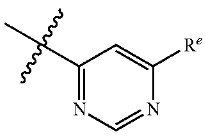
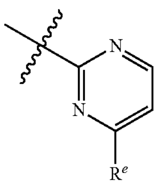
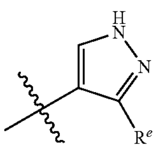
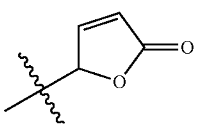
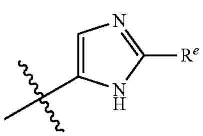
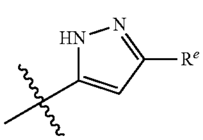
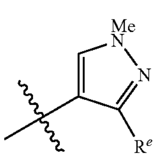
TABLE 4-continued

Exemplary R^1 Groups	
	tt
	uu
	vv
	ww
	xx
	yy
	zz
	aaa
	bbb

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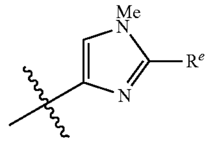
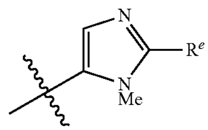
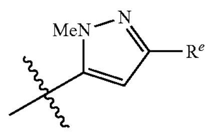
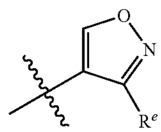
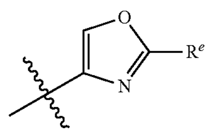
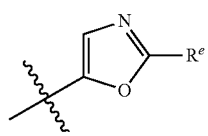
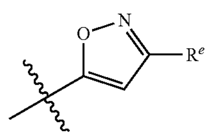
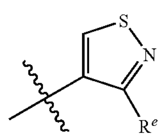
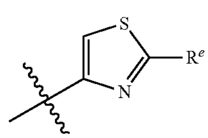
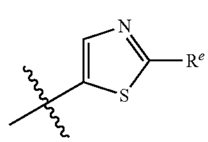
	ccc
	ddd
	eee
	fff
	ggg
	hhh
	iii
	jjj
	kkk
	lll

TABLE 4-continued

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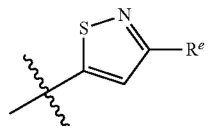
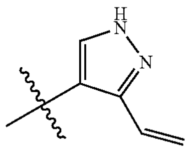
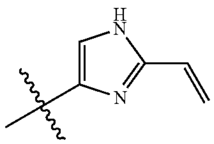
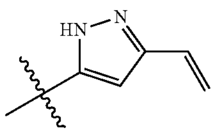
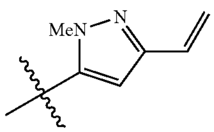
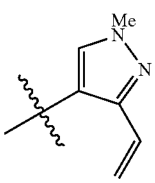
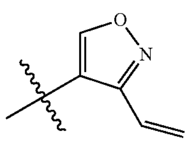
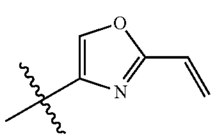
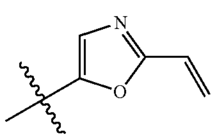
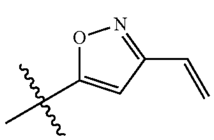
	mmm
	nnn
	ooo
	ppp
	
	qqq
	rrr
	sss
	ttt
	uuu

TABLE 4-continued

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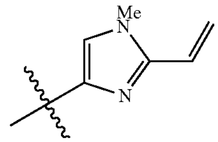
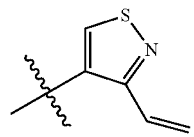
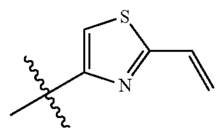
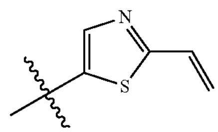
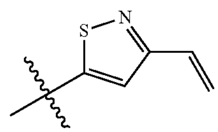
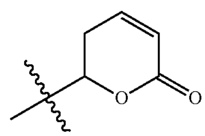
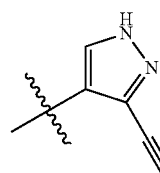
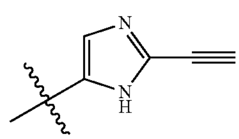
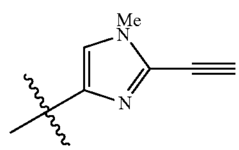
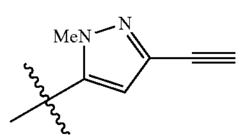
	vvv
	www
	xxx
	yyy
	zzz
	aaaa
	bbbb
	cccc
	dddd
	eeee

TABLE 4-continued

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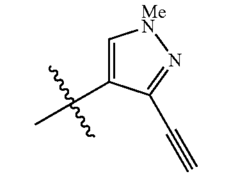
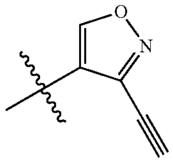
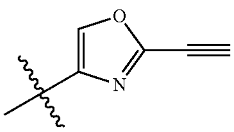
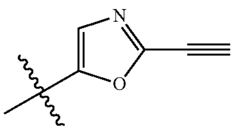
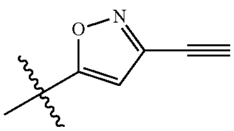
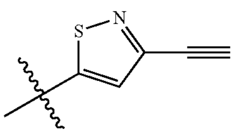
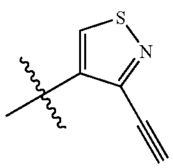
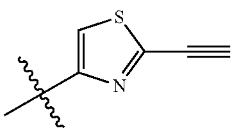
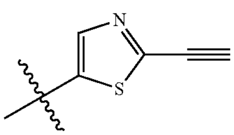
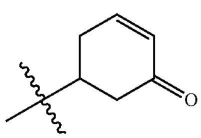
	ffff
	gggg
	hhhh
	iiii
	jjjj
	kkkk
	llll
	mmmm
	nnnn
	oooo

TABLE 4-continued

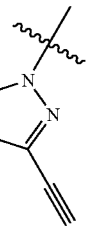
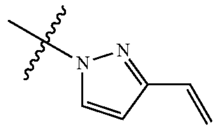
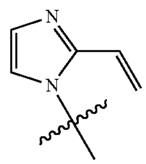
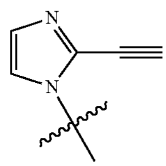
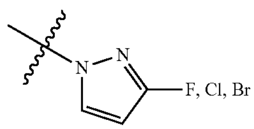
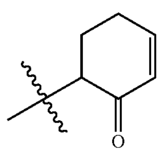
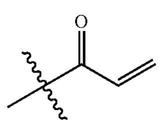
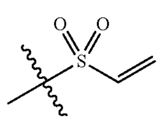
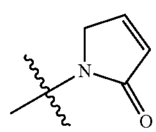
Exemplary R^1 Groups	
	pppp
	qqqq
	rrrr
	ssss
	tttt
	uuuu
	vvvv
	wwww
	xxxx

TABLE 4-continued

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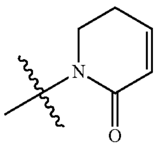
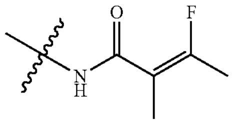
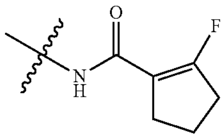
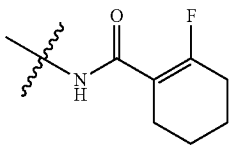
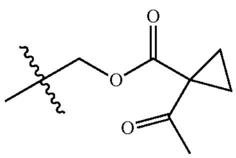
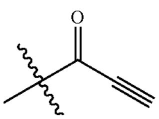
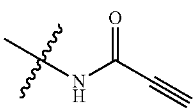
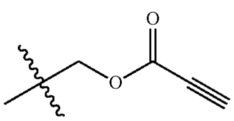
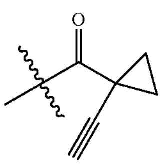
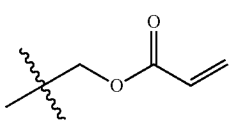
	yyyy
	zzzz
	aaaaa
	bbbbb
	ccccc
	ddddd
	eeeee
	fffff
	ggggg
	hhhhh

TABLE 4-continued

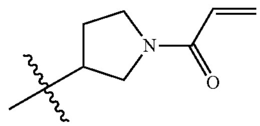
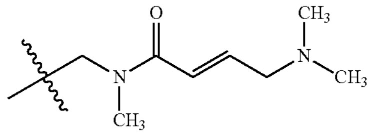
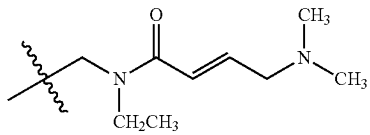
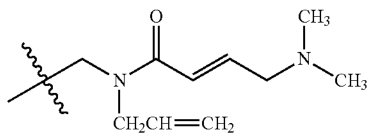
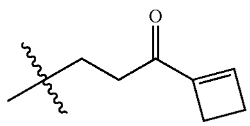
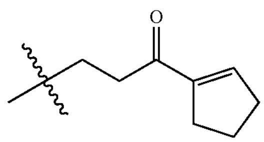
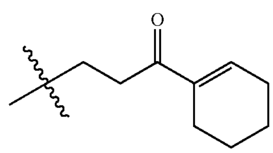
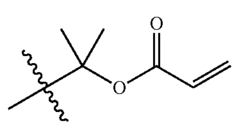
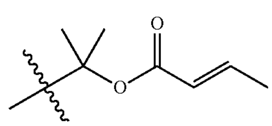
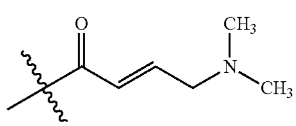
Exemplary R^1 Groups	
	iiiii
	jjjjj
	kkkkk
	lllll
	mmmmm
	nnnnn
	ooooo
	ppppp
	qqqqq
	rrrrr

TABLE 4-continued

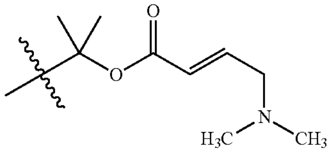
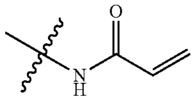
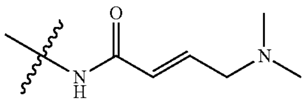
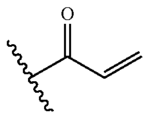
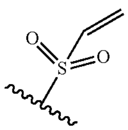
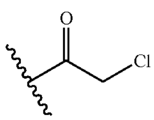
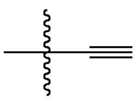
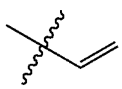
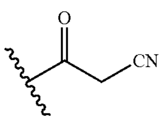
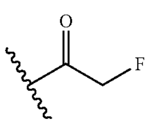
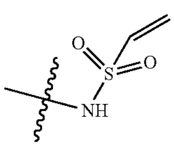
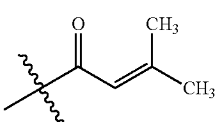
Exemplary R^1 Groups	
	sssss
	ttttt
	uuuuu
	vvvvv
	wwwww
	xxxxx
	yyyyy
	zzzzz
	aaaaaa
	bbbbbb
	cccccc
	dddddd

TABLE 4-continued

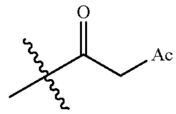
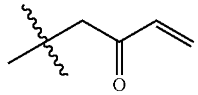
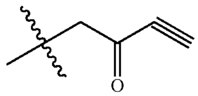
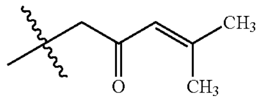
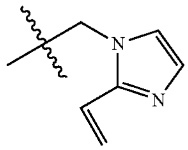
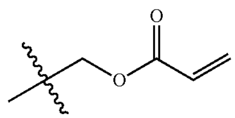
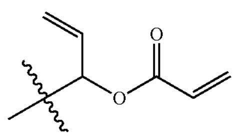
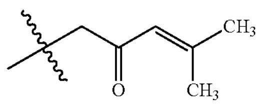
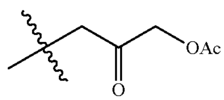
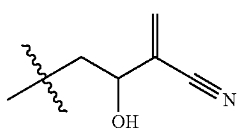
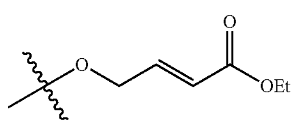
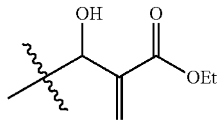
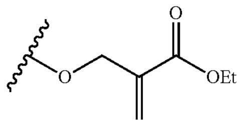
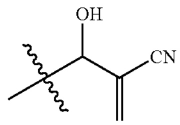
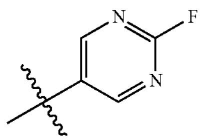
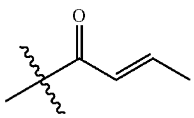
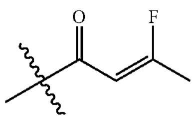
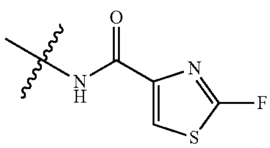
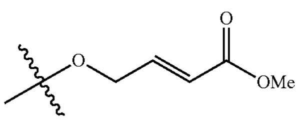
Exemplary R^1 Groups	
	eeeeee
	ffffff
	gggggg
	hhhhhh
	iiiiii
	jjjjjj
	kkkkkk
	llllll
	mmmmmm
	nnnnnn
	oooooo

TABLE 4-continued

Exemplary R^1 Groups	
	pppppp
	qqqqqq
	rrrrrr
	ssssss
	tttttt
	uuuuuu
	vvvvvv
	wwwwww
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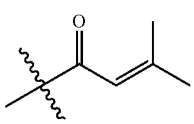
	xxxxxx

wherein each R^e is independently a suitable leaving group, NO_2, CN, or oxo.

[0304] As defined generally above, R^1 is a warhead group, or, when R^1 and R^x form a ring, then -Q-Z is a warhead group. Without wishing to be bound by any particular theory, it is believed that such R^1 groups, i.e. warhead groups, are particularly suitable for covalently binding to a key cysteine residue in the binding domain of certain protein kinases. Protein kinases having a cysteine residue in the binding domain are known to one of ordinary skill in the art and include ErbB1, ErbB2, and ErbB4, or a mutant thereof. In certain embodiments, compounds of the present invention have a warhead group characterized in that inventive compounds target one or more of the following cysteine residues:

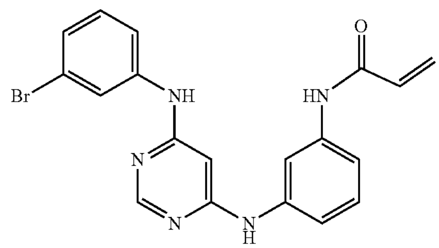
```
ERBB1
SEQ ID 7:
ITQLMPFGCLLDYVREH

ERBB2
SEQ ID 8:
VTQLMPYGCLLDHVREN

ERBB4
SEQ ID 9:
VTQLMPHGCLLEYVHEH
```

[0305] Thus, in some embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue thereby irreversibly inhibiting the enzyme. In certain embodiments, the cysteine residue is Cys797 of ErbB1, Cys805 of ErbB2 and Cys803 of ErbB4, or a mutant thereof, where the provided residue numbering is in accordance with Uniprot (code POO533 for ErbB1; code PO4626 for ErbB2, and Q15303 for ErbB4). It will be understood that the Cys of ErbB1 (EGFR) is variably called 773 or 797 depending on whether the parent sequence contains the signal peptide or not. Thus, in accordance with the present invention, the relevant cysteine residue of ErbB1 may be described as Cys 773 or Cys 797 and these terms are used interchangeably.

[0306] One of ordinary skill in the art will recognize that a variety of warhead groups, as defined herein, are suitable for such covalent bonding. Such R^1 groups include, but are not limited to, those described herein and depicted in Table 4, infra. One of ordinary skill in the art will recognize that ErbB3 has no corresponding residue and, as recognized in the relevant art, is not catalytically active.

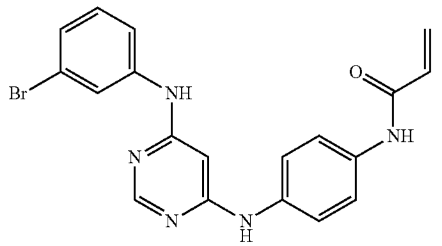
[0307] As depicted in Formula I supra, the R^1 warhead group can be in an ortho-, meta-, or para-position. In certain embodiments, the R^1 warhead group is in a meta-position of the phenyl ring relative to the rest of the molecule. Without wishing to be bound by any particular theory, it is believed that when R^1 is in such a meta-position, the warhead group is better positioned for covalent modification of the cysteine residue thus effecting irreversible inhibition of the enzyme. Indeed, it has been surprisingly found that a compound having a warhead group at a meta-position (compound I-1) irreversibly binds to ErbB1 whereas a compound having a warhead group at a para-position (compound I-93) reversibly binds to ErbB1. These compounds have the following structures:

I-1



-continued

I-93



[0308] This phenomenon was determined by performing a washout experiment using the protocol described in detail in Example 42, infra. The results of this experiment are depicted in FIG. **2** where it is shown that compound I-1 maintains enzyme inhibition after "washout" whereas compound I-93 was washed away in the experiment thereby resulting in reactivated enzyme activity.

[0309] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of TEC, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 449.

[0310] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of BTK, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 481.

[0311] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of ITK, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 442.

[0312] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of BMX, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 496.

[0313] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of JAK3, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 909.

[0314] In certain embodiments, R^1 is characterized in that the -L-Y moiety is capable of covalently binding to a cysteine residue of TXK, thereby irreversibly inhibiting the enzyme. In some embodiments, the cysteine residue is Cys 350.

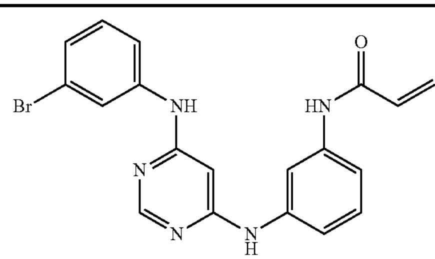
[0315] One of ordinary skill in the art will recognize that a variety of warhead groups, as defined herein, are suitable for such covalent bonding. Such R^1 groups include, but are not limited to, those described herein and depicted in Table 3, infra.

[0316] Exemplary compounds of formula I are set forth in Table 5 below.

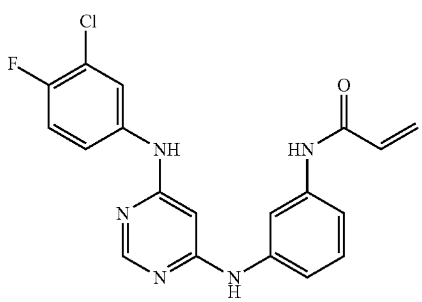
TABLE 5

Exemplary Compounds of Formula I

I-1



I-2



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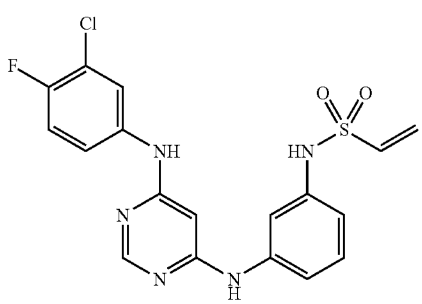
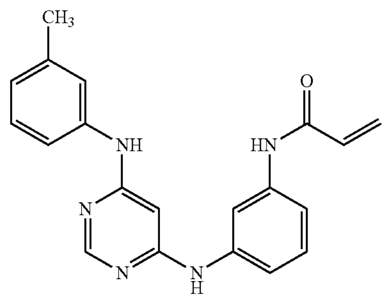


TABLE 5-continued

Exemplary Compounds of Formula I



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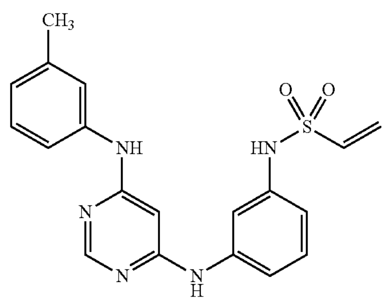
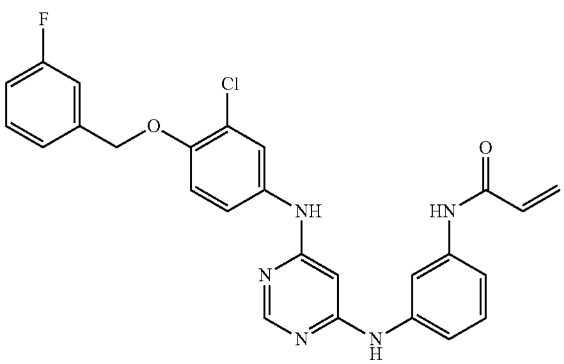
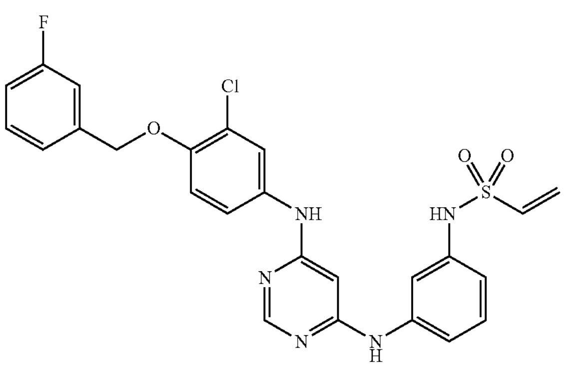


I-5



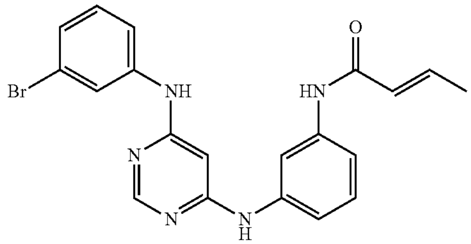
I-6



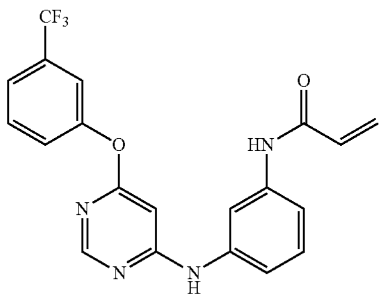
I-7

TABLE 5-continued

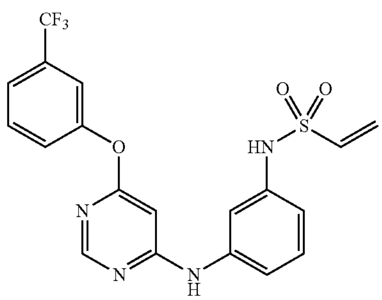
Exemplary Compounds of Formula I



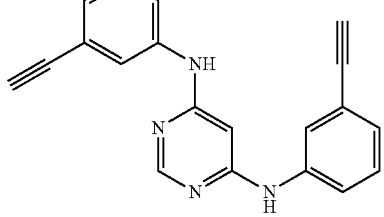
I-8



I-9



I-10



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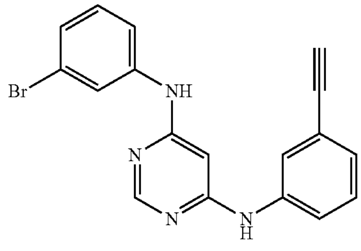


I-12

TABLE 5-continued

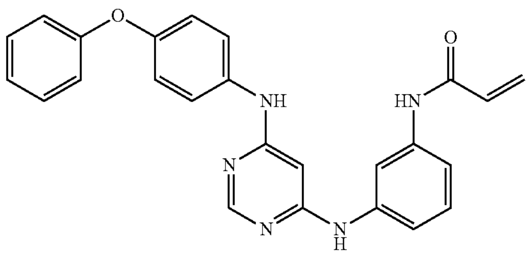
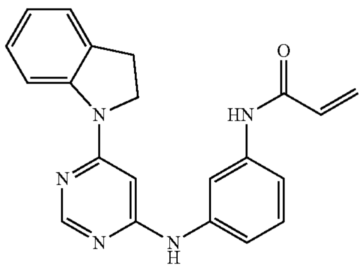
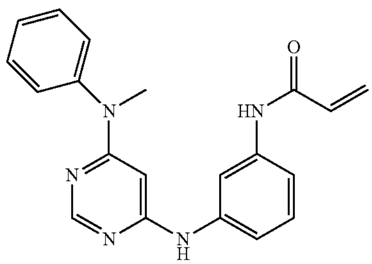
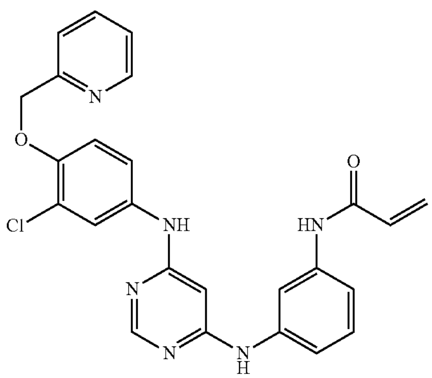
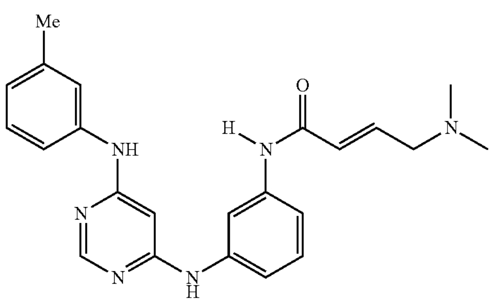
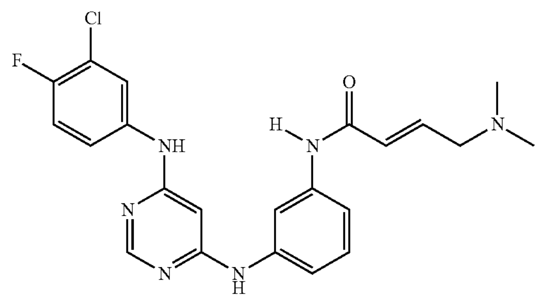
Exemplary Compounds of Formula I	
	I-13
	I-14
	I-15
	I-16
	I-17

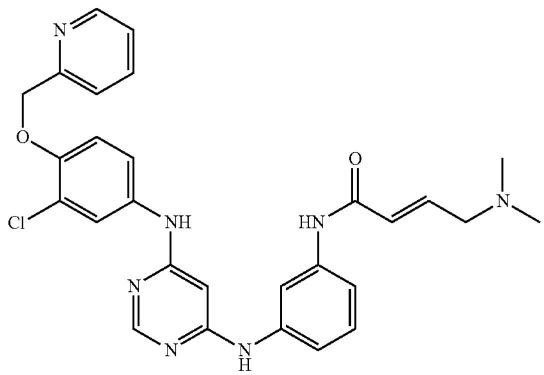
TABLE 5-continued

Exemplary Compounds of Formula I

I-18



I-19



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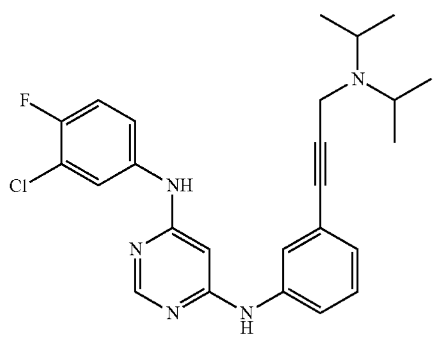

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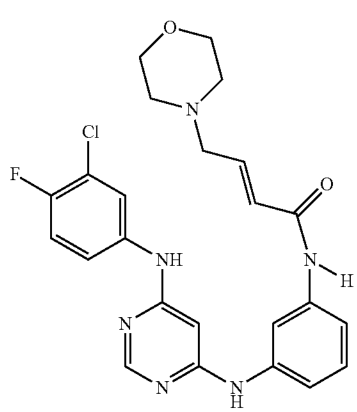
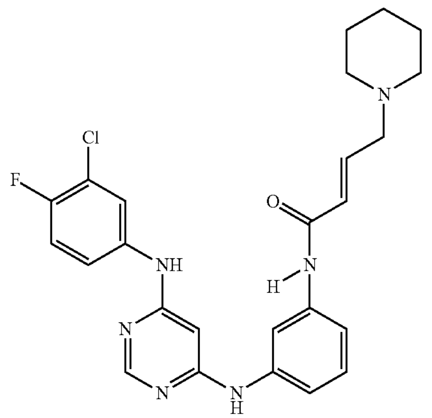


TABLE 5-continued

Exemplary Compounds of Formula I

I-22



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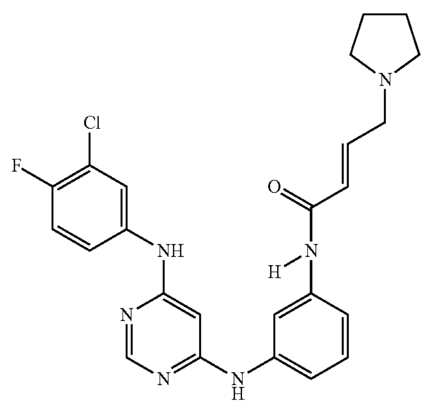

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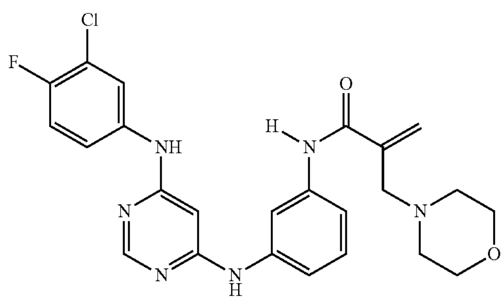

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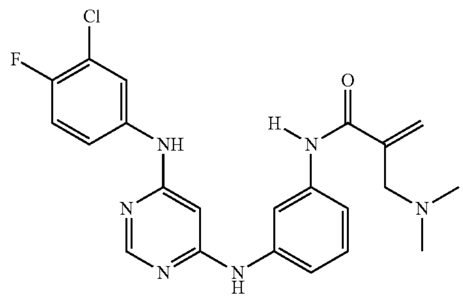
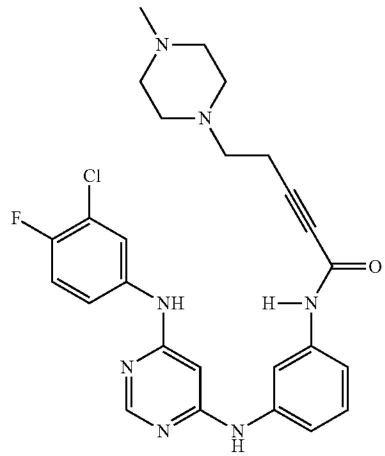


TABLE 5-continued

Exemplary Compounds of Formula I



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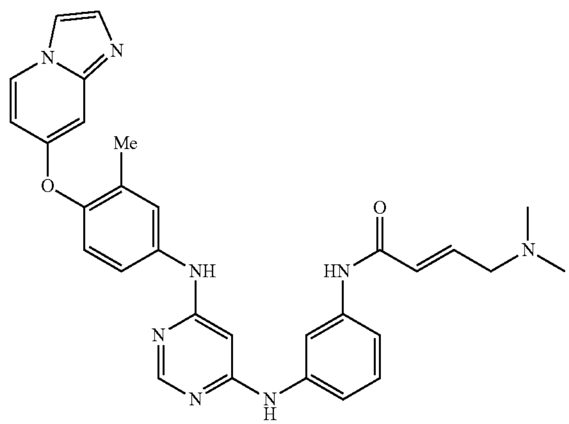

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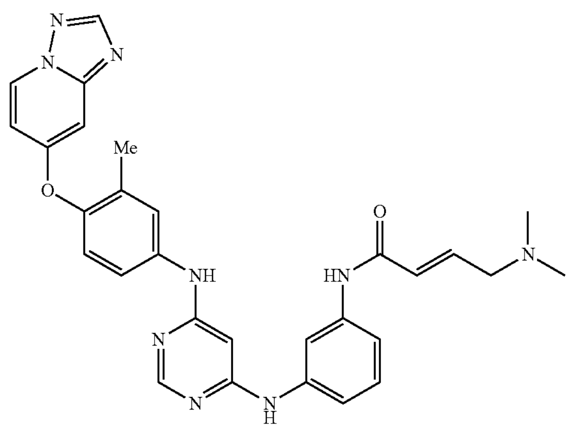

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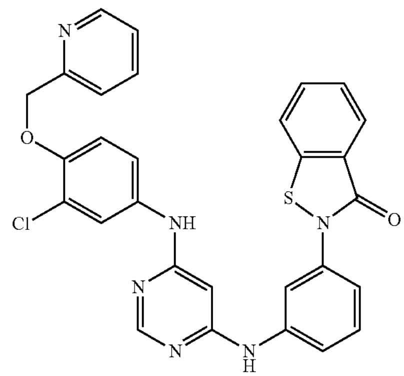
TABLE 5-continued

Exemplary Compounds of Formula I



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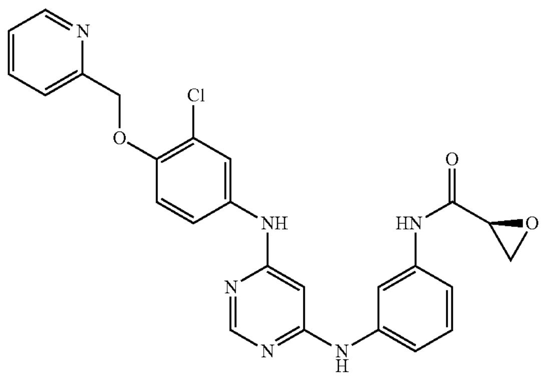

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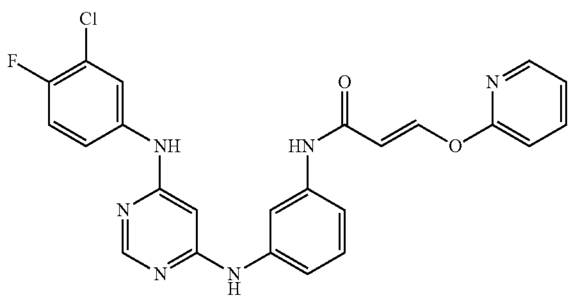

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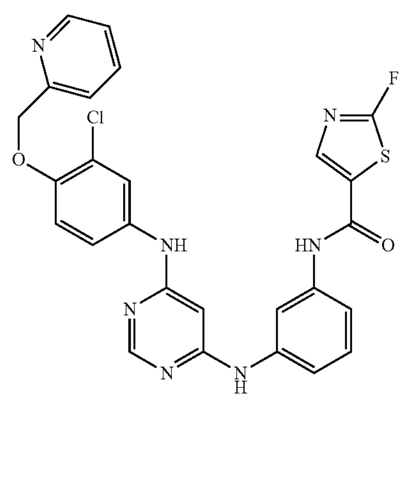

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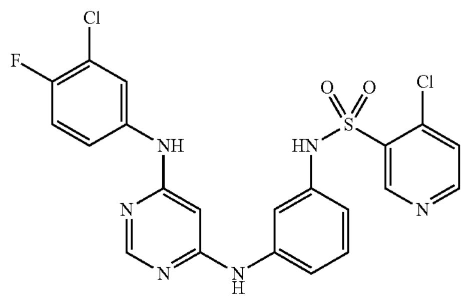
TABLE 5-continued

Exemplary Compounds of Formula I



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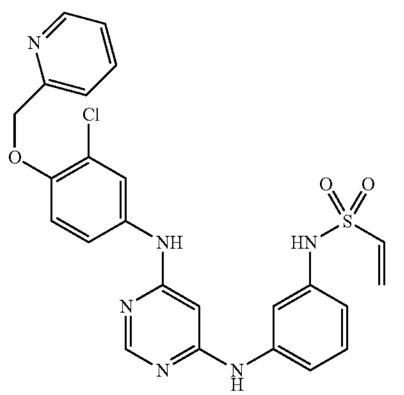

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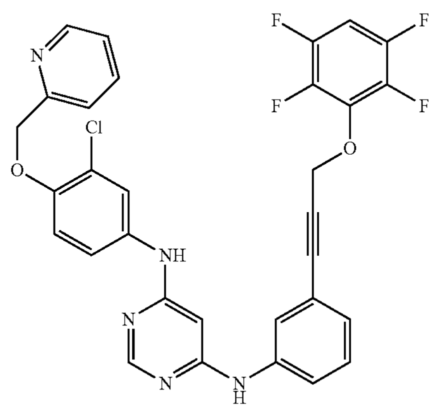

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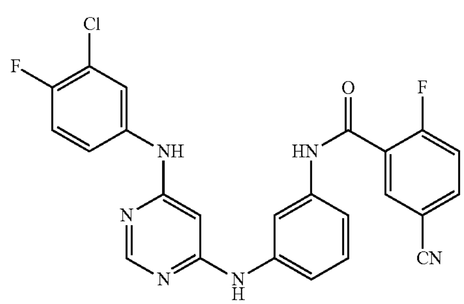

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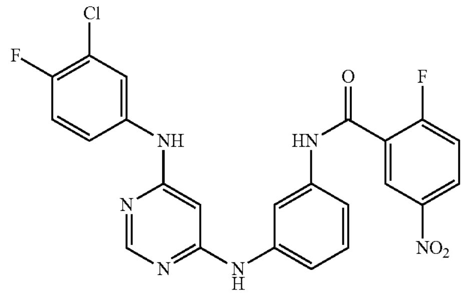
TABLE 5-continued

Exemplary Compounds of Formula I



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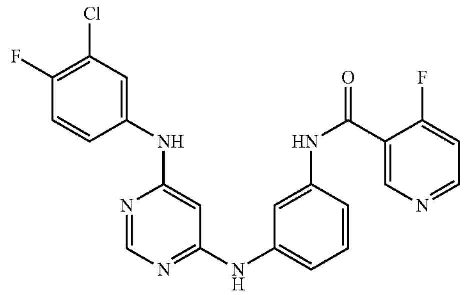

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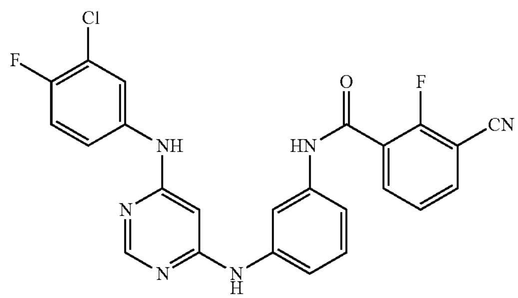

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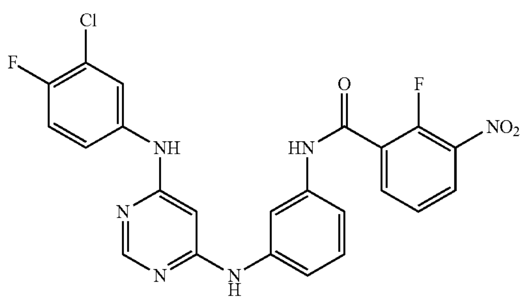

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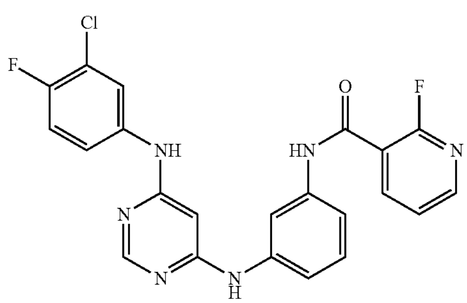

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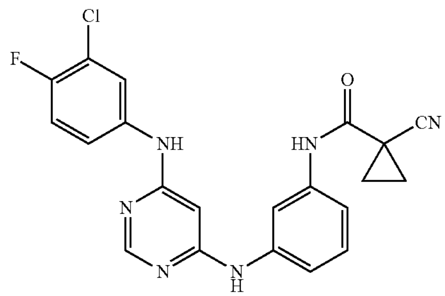
TABLE 5-continued

Exemplary Compounds of Formula I



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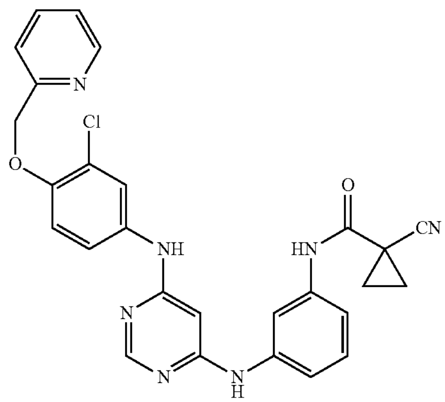

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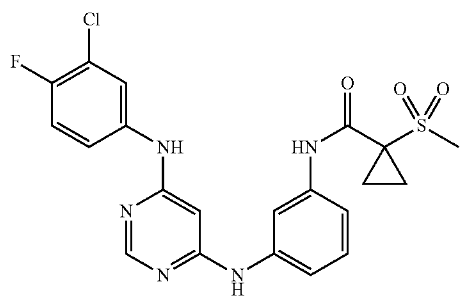

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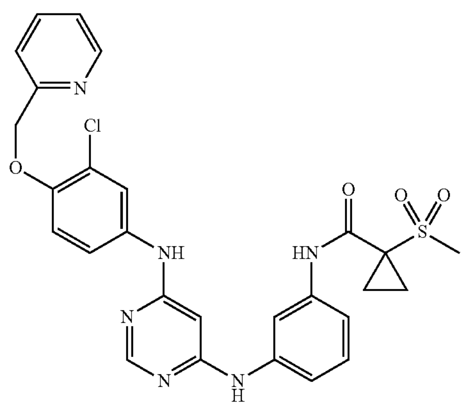

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TABLE 5-continued

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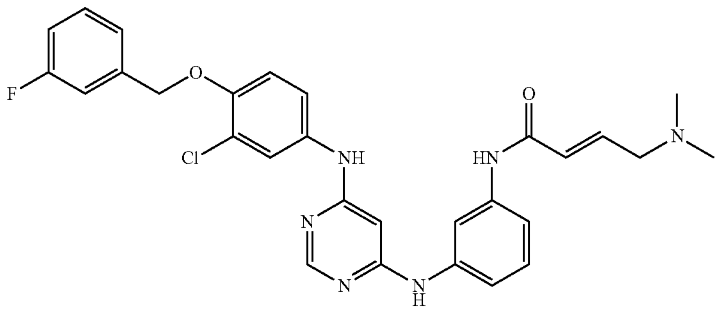
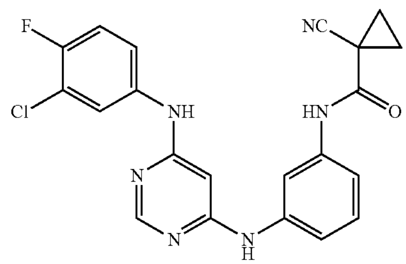
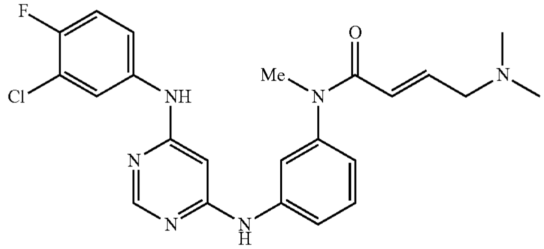
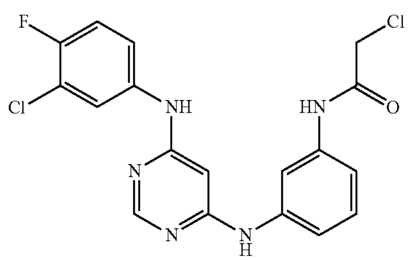
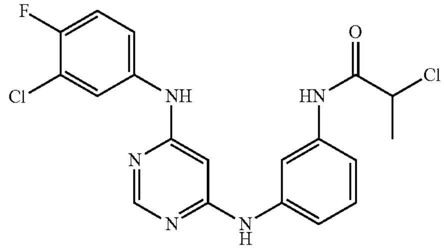
 I-46
 I-47
 I-48
 I-49
 I-50

TABLE 5-continued

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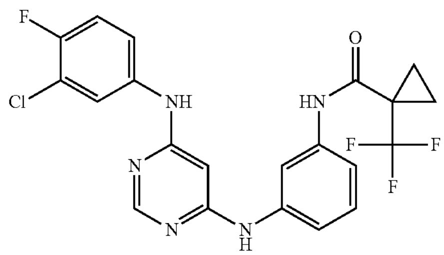

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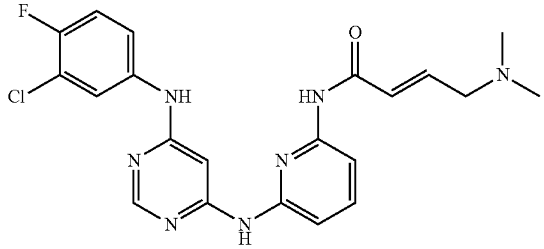

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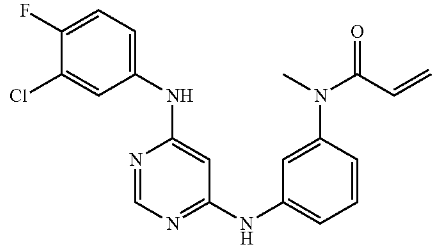

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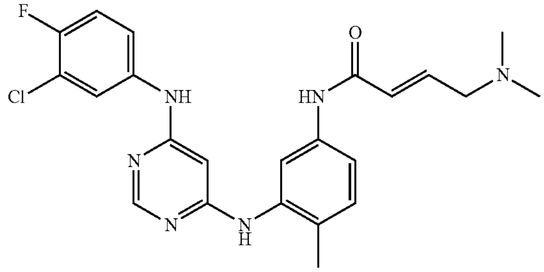

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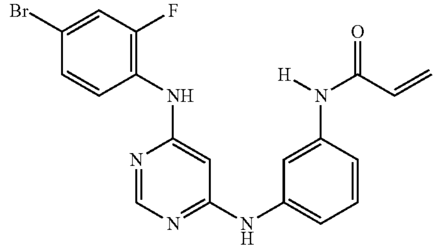

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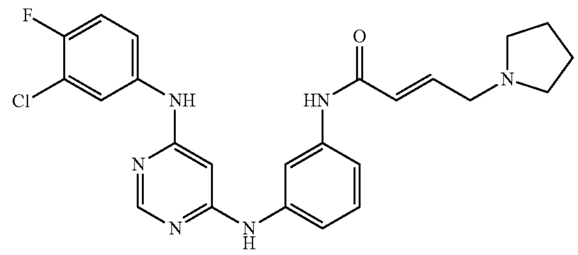
TABLE 5-continued

Exemplary Compounds of Formula I



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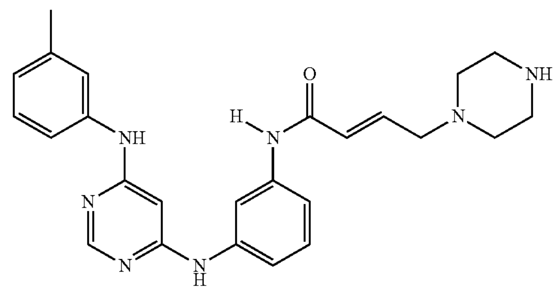

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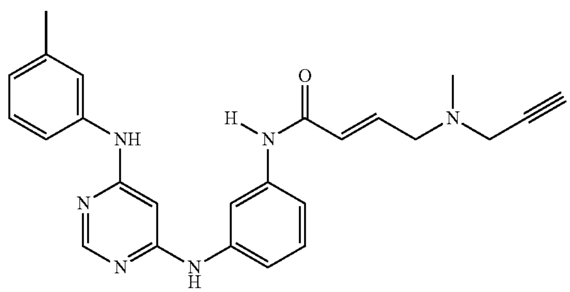

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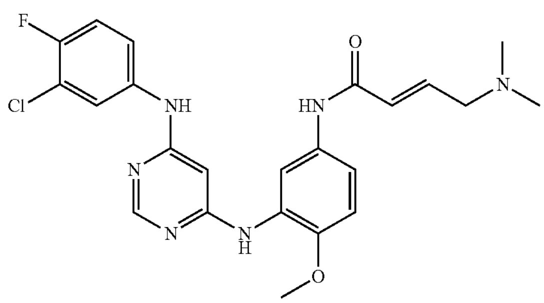

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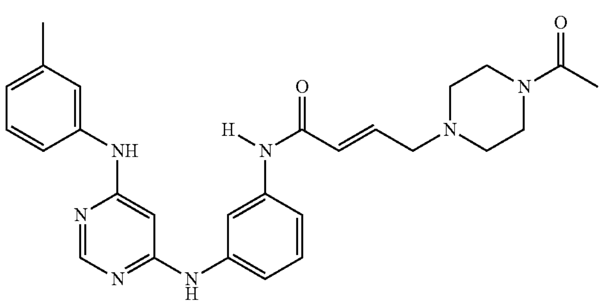

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TABLE 5-continued

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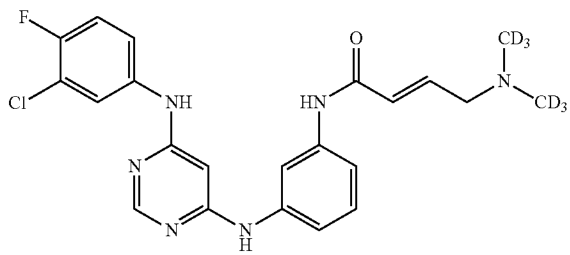

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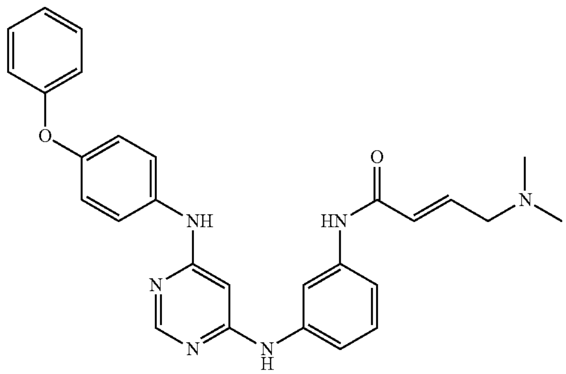

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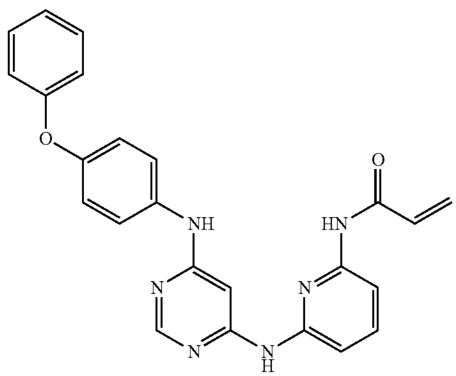

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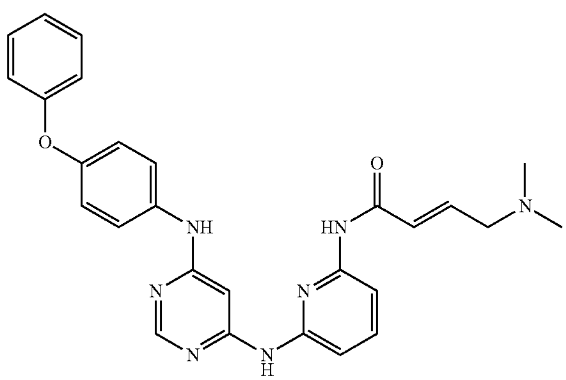

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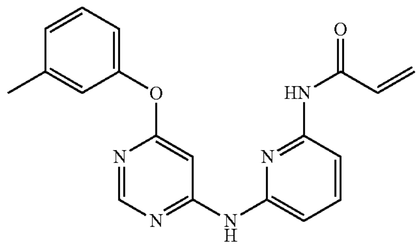
TABLE 5-continued

Exemplary Compounds of Formula I



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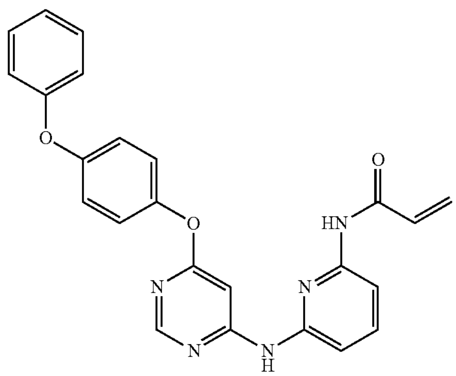

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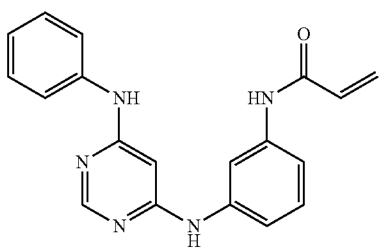

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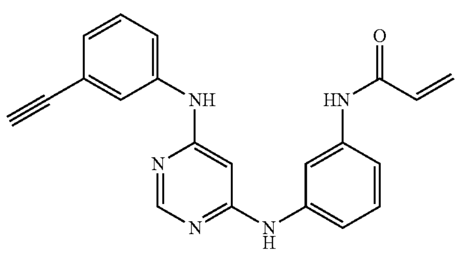

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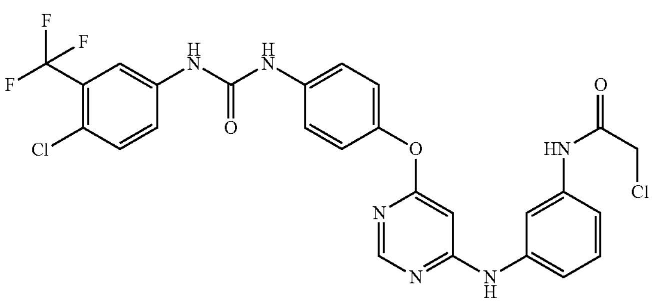

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TABLE 5-continued

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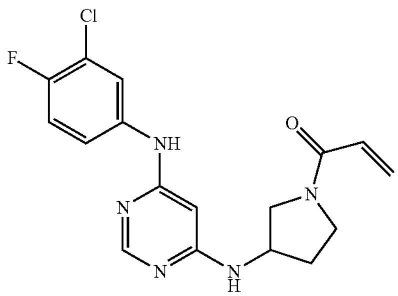
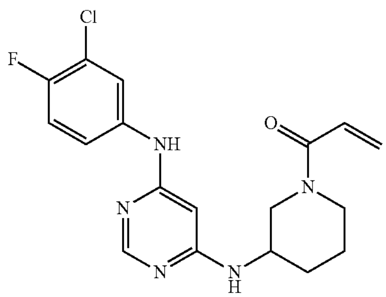
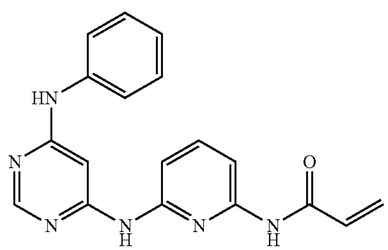
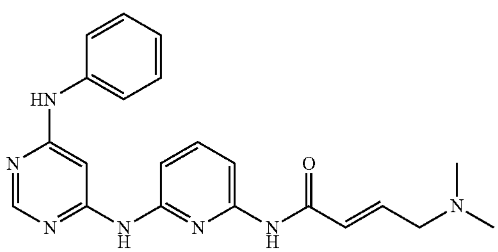
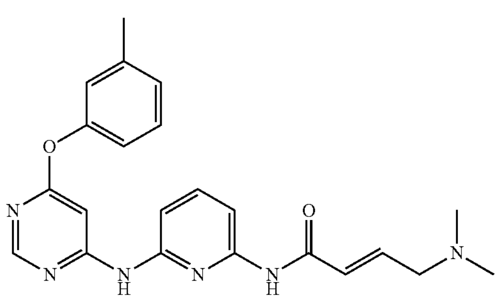
	I-70
	I-71
	I-72
	I-73
	I-74

TABLE 5-continued

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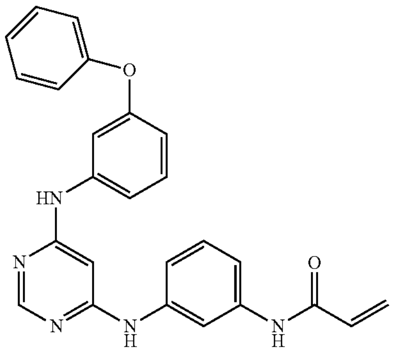

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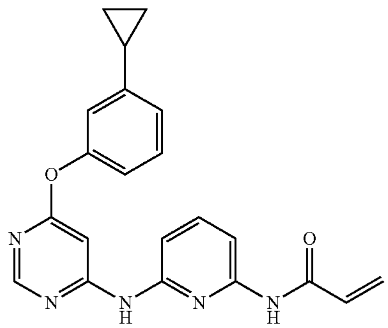

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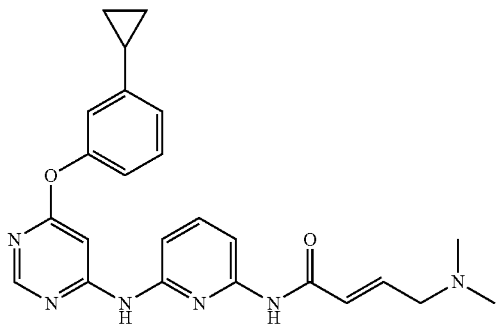

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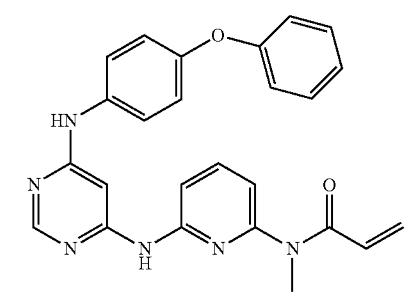

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TABLE 5-continued

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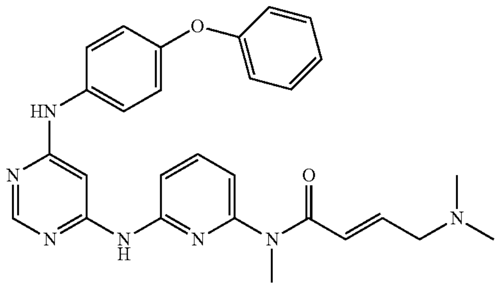

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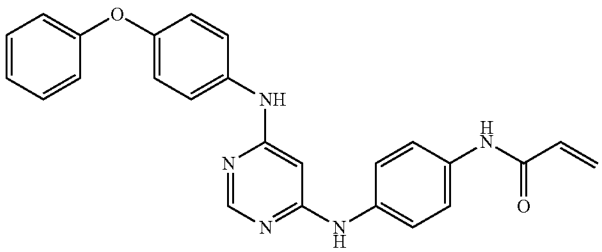

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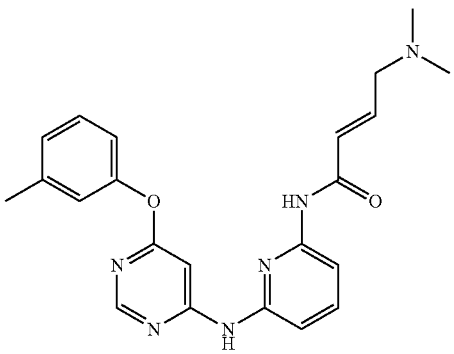

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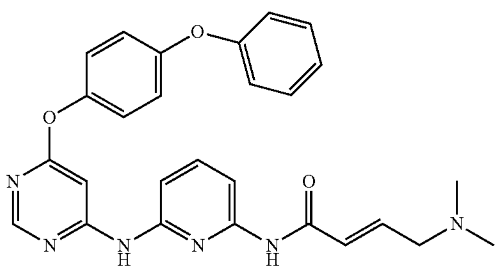

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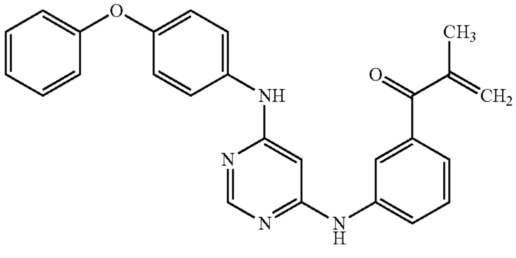


I-83

TABLE 5-continued

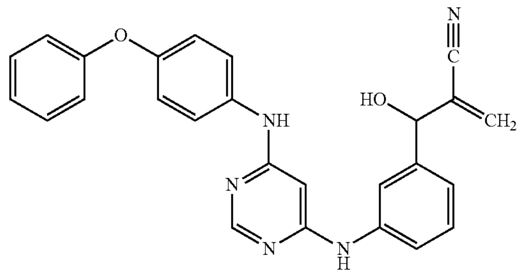
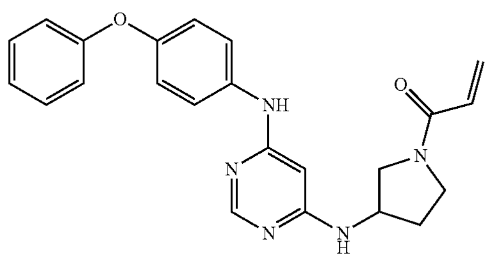
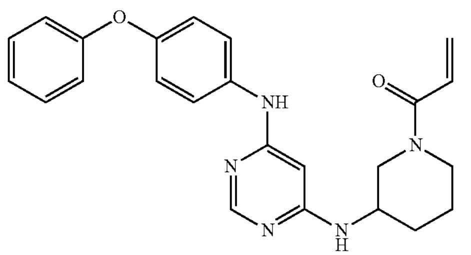
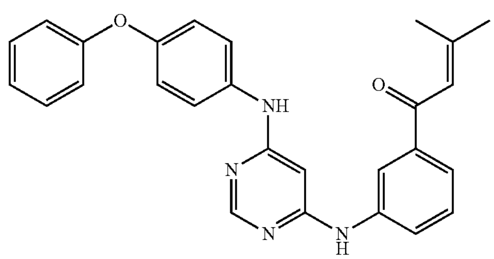
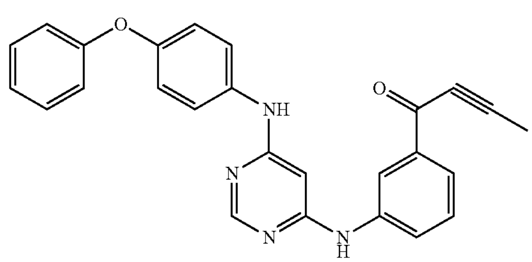
Exemplary Compounds of Formula I	
	I-84
	I-85
	I-86
	I-87
	I-88

TABLE 5-continued

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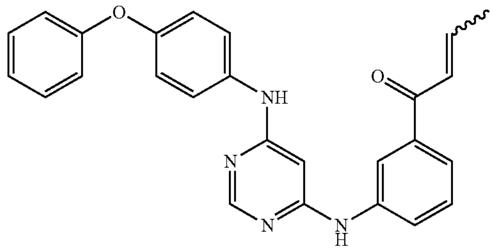
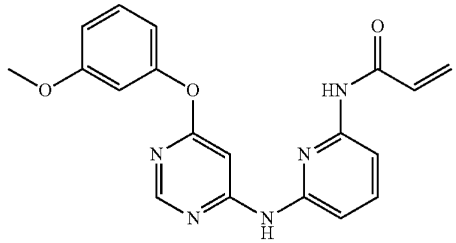
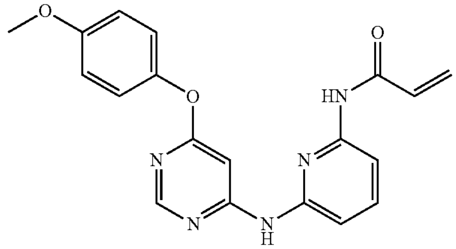


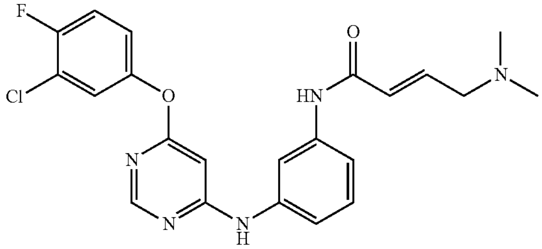
I-89



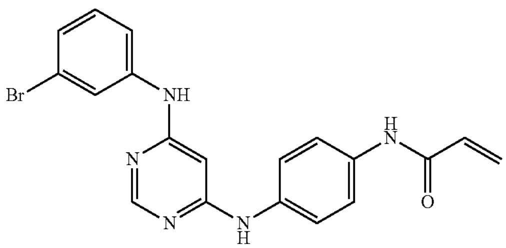
I-90



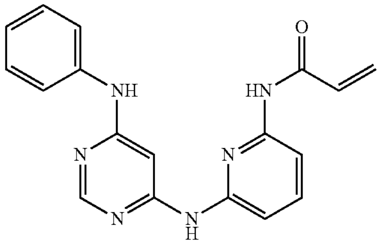
I-91



I-92



I-93



I-94

TABLE 5-continued

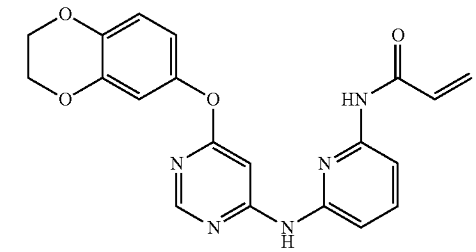
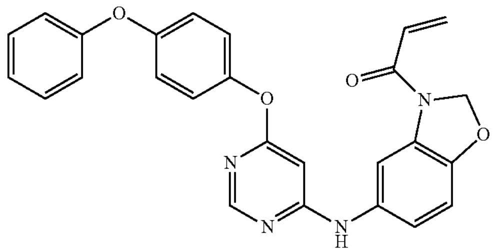
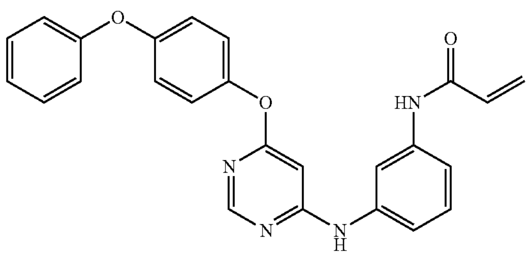
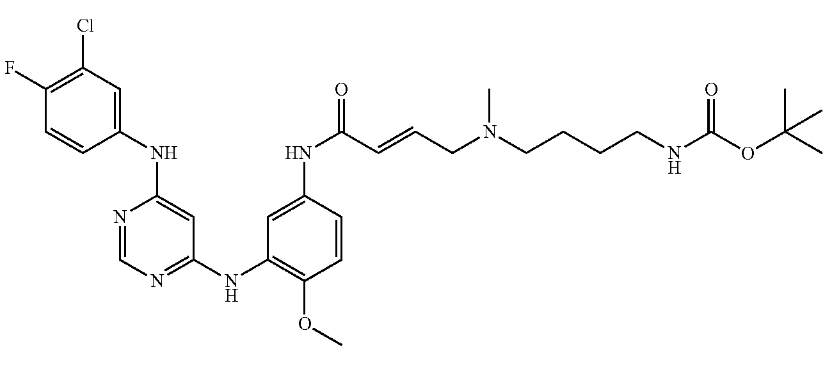
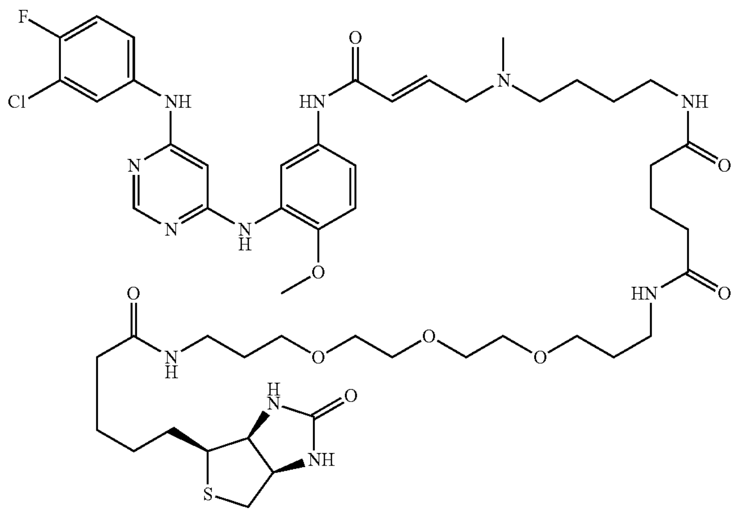
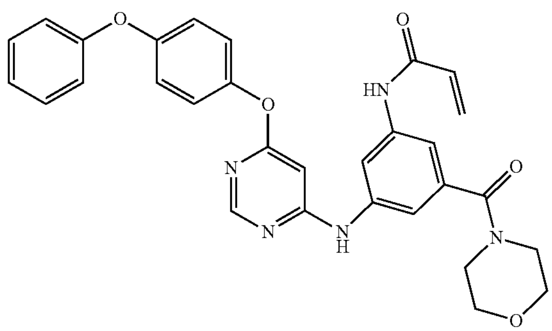
Exemplary Compounds of Formula I	
	I-95
	I-96
	I-97
	I-98

TABLE 5-continued

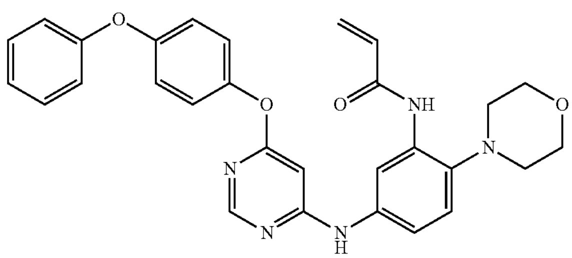
Exemplary Compounds of Formula I



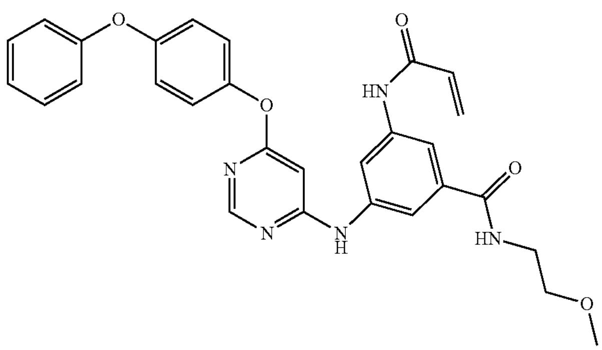
I-99



I-100



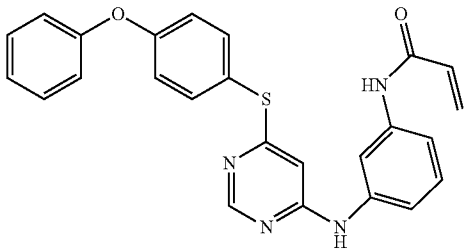
I-101



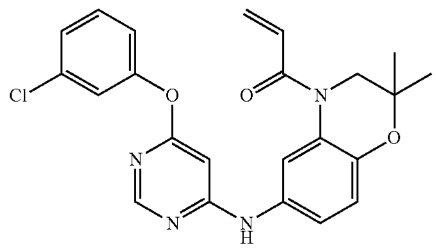
I-102

TABLE 5-continued

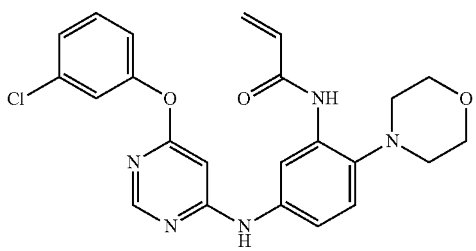
Exemplary Compounds of Formula I



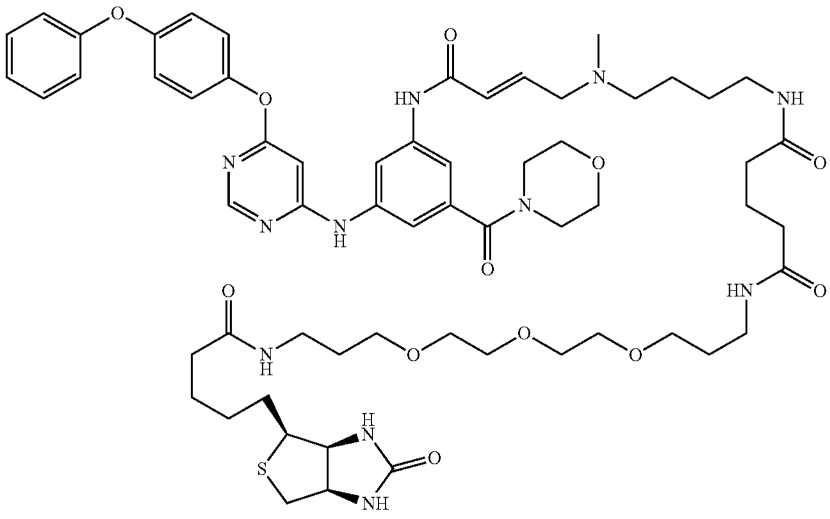
I-103



I-104



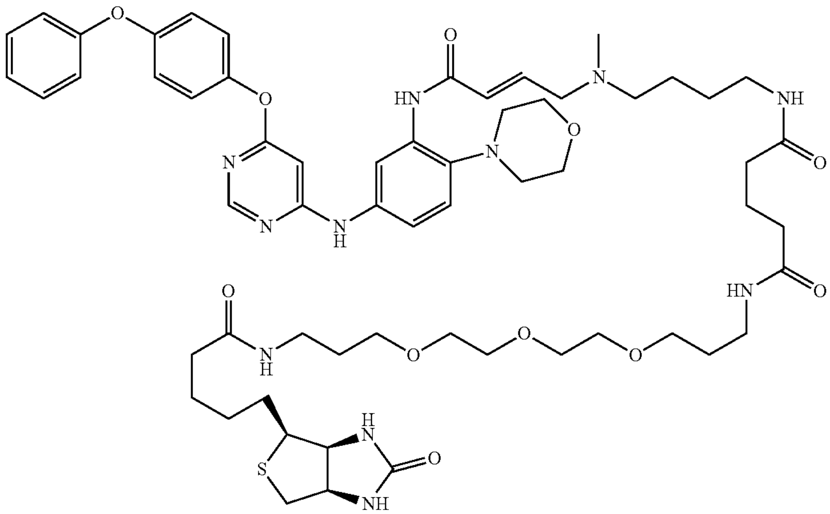
I-105



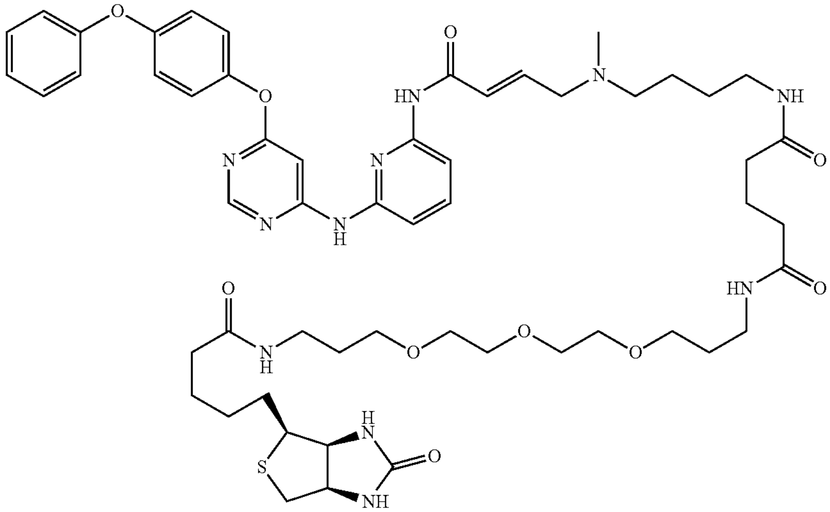
I-106

TABLE 5-continued

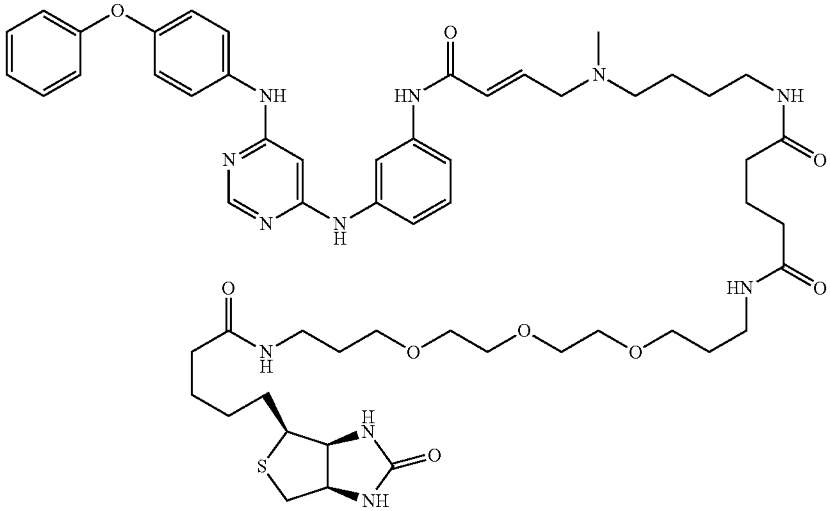
Exemplary Compounds of Formula I



I-107



I-108

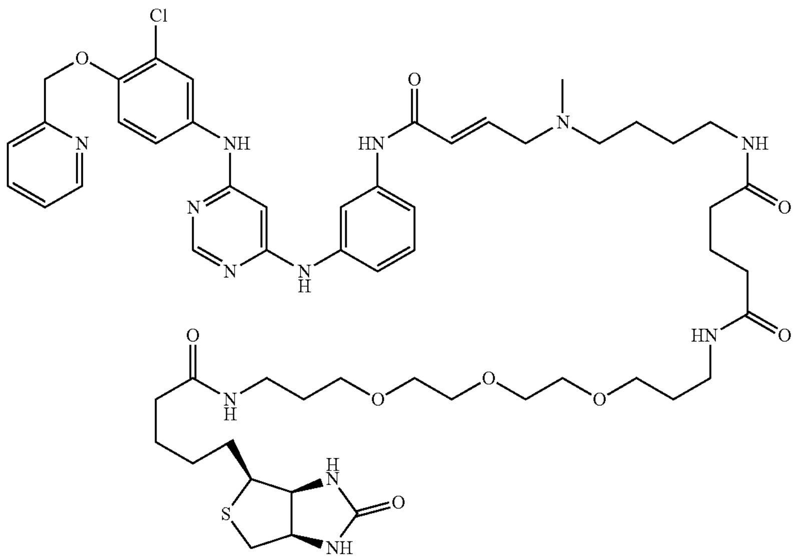


I-109

TABLE 5-continued

Exemplary Compounds of Formula I

I-110



I-111

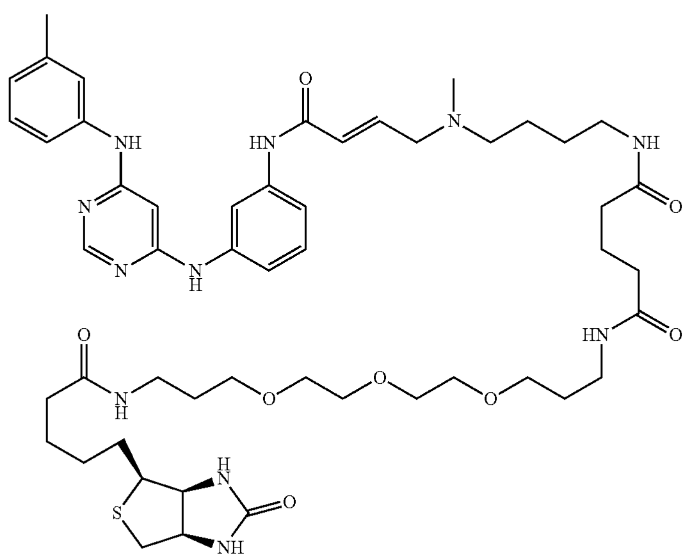
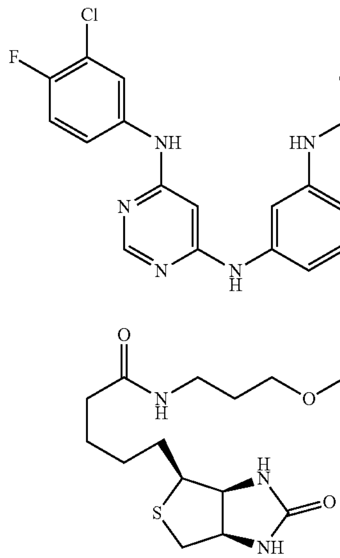


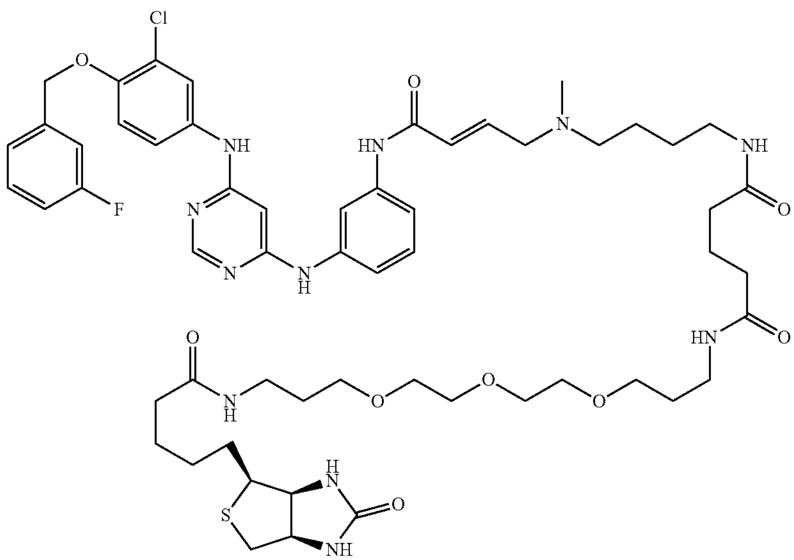
TABLE 5-continued

Exemplary Compounds of Formula I

I-112



I-113



I-114

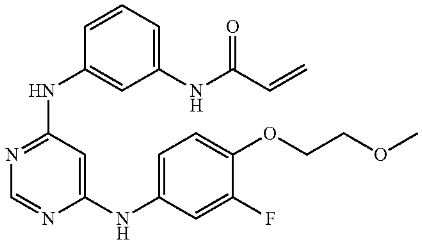
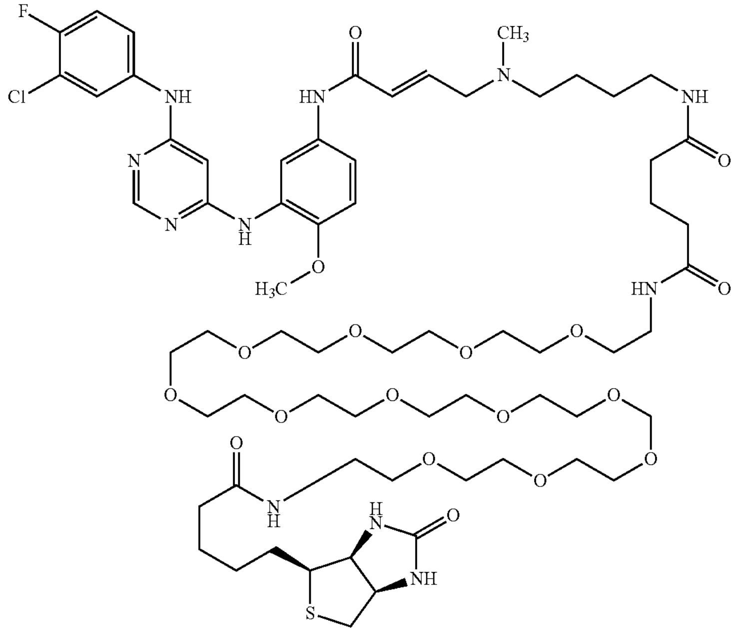


TABLE 5-continued

Exemplary Compounds of Formula I

I-115



I-116

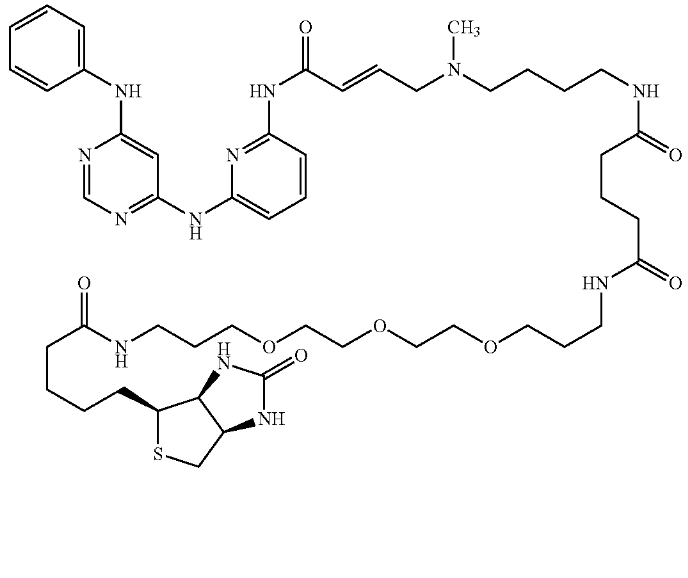
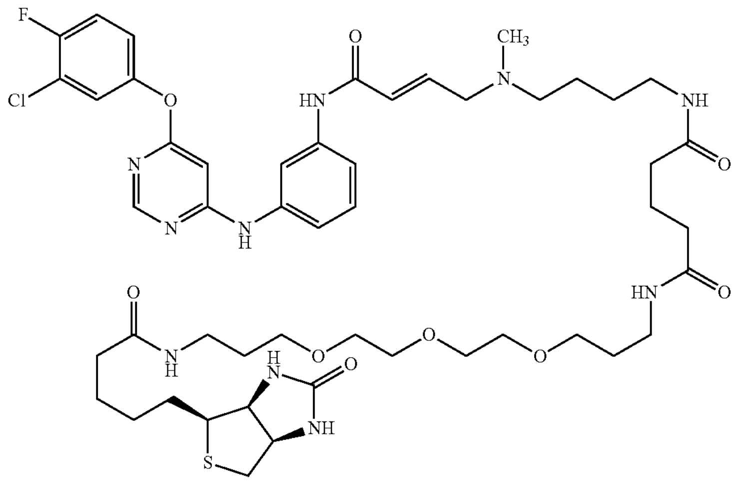
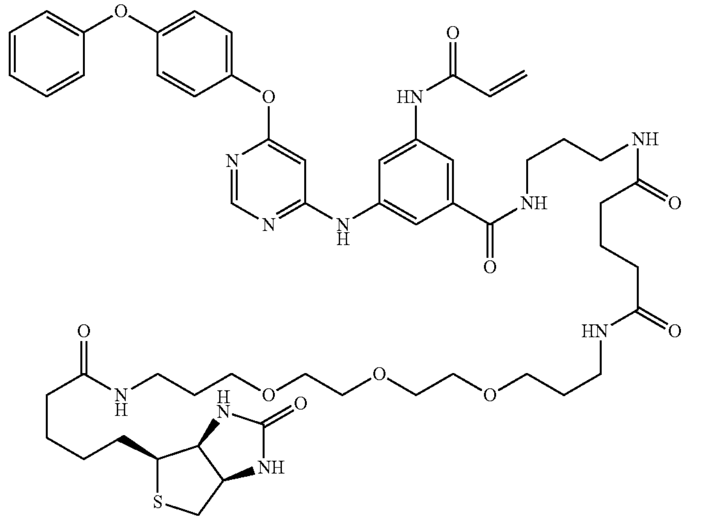


TABLE 5-continued

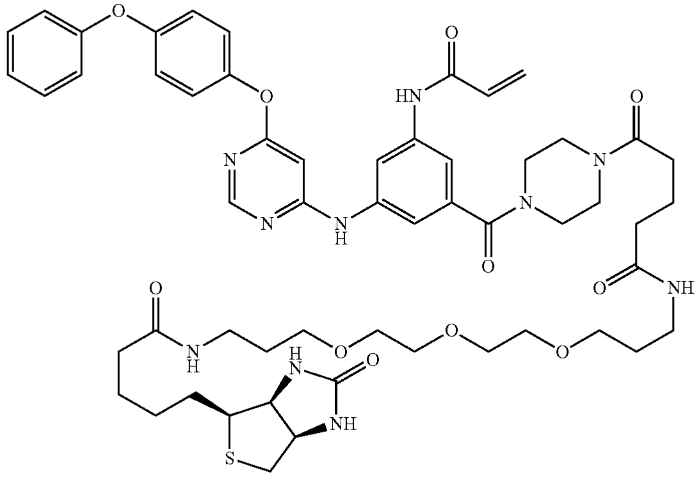
Exemplary Compounds of Formula I



I-117



I-118

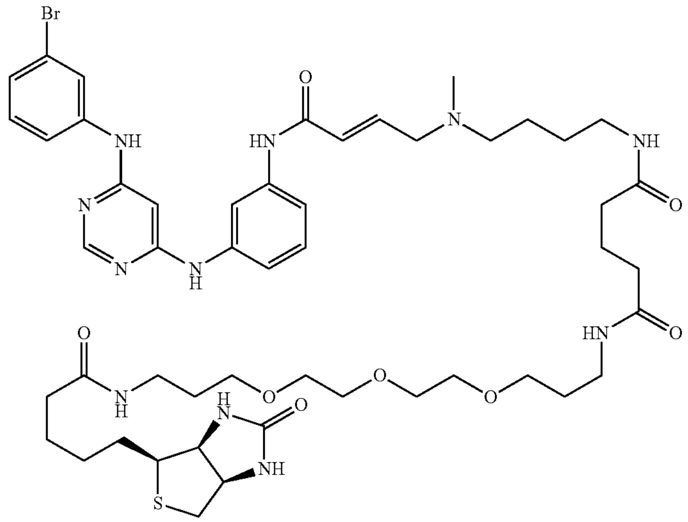


I-119

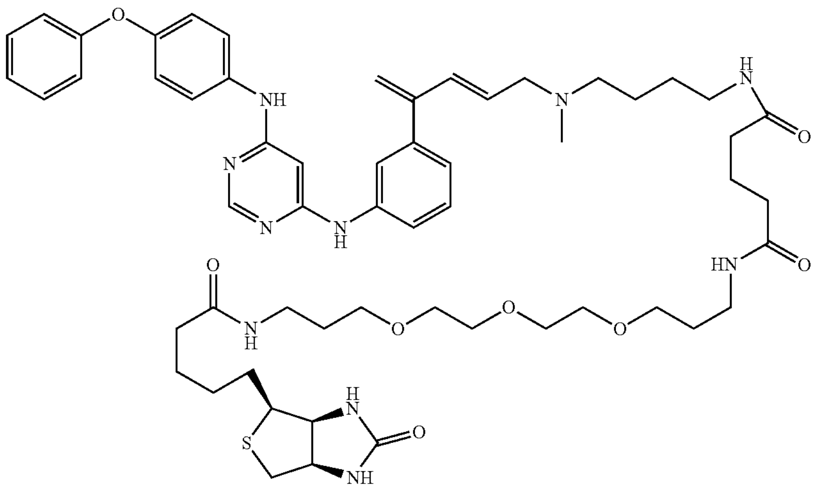
TABLE 5-continued

Exemplary Compounds of Formula I

I-120



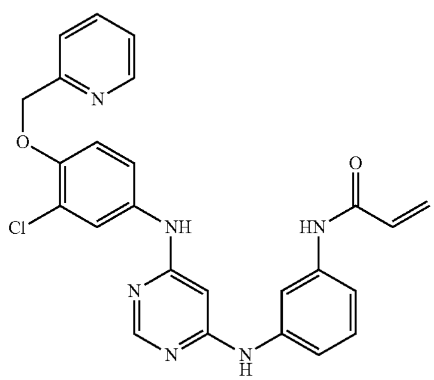
I-121



[0317] In certain embodiments, the present invention provides any compound depicted in Table 5, above, or a pharmaceutically acceptable salt thereof.

[0318] In certain embodiments, the present invention provides a compound selected from:

I-16



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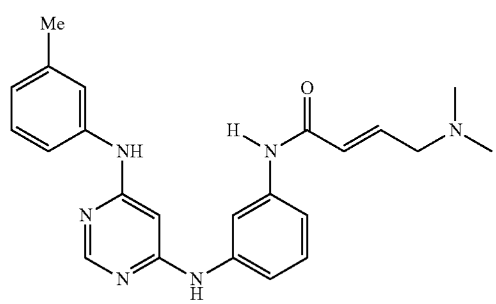
I-17



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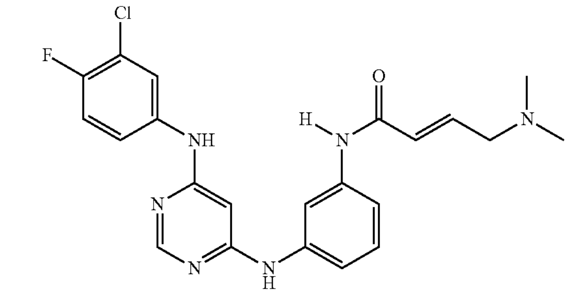
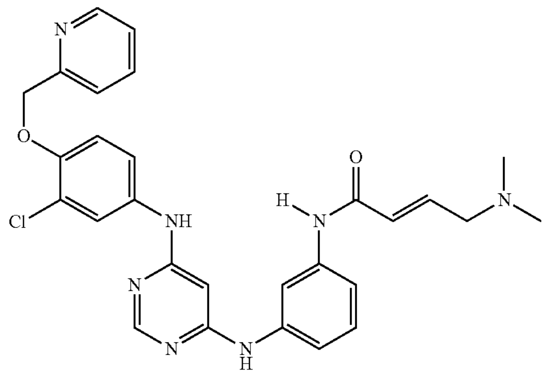
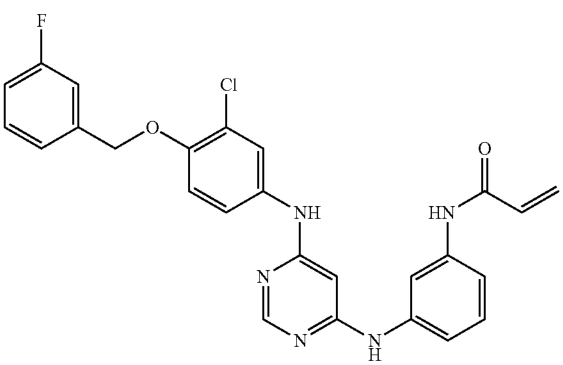
I-18



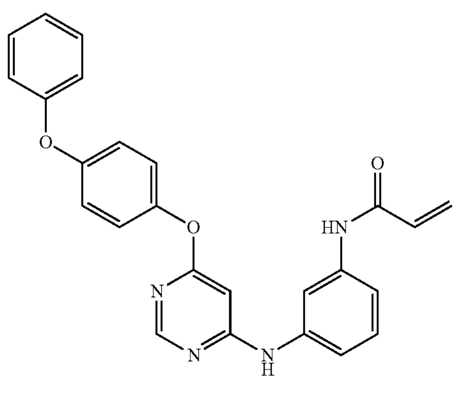
I-19



I-6

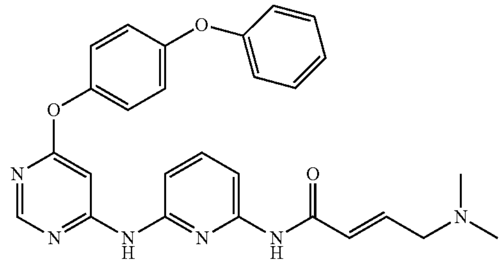


I-66

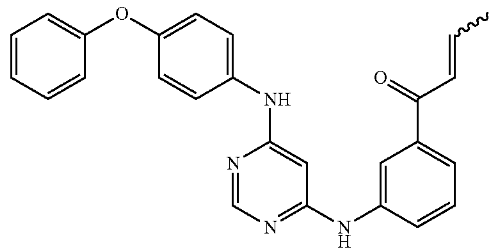


-continued

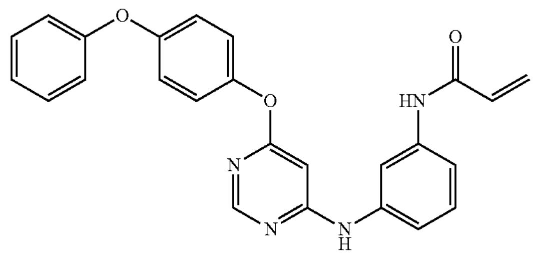
I-82



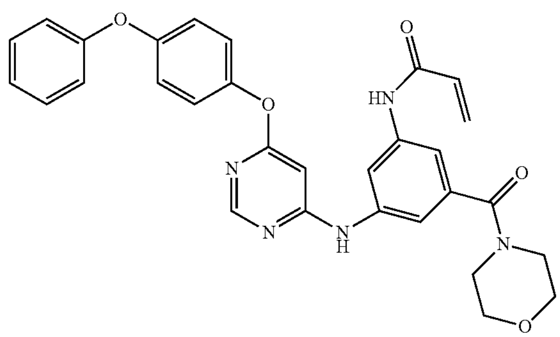
I-89



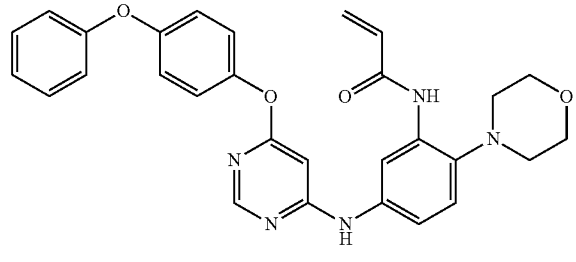
I-97



I-100

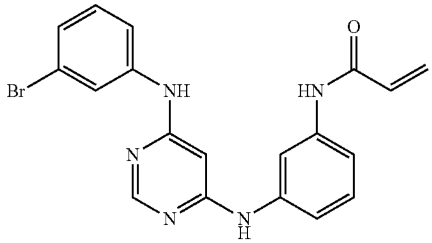


1-101



-continued

I-1



or a pharmaceutically acceptable salt thereof.

[0319] As described herein, compounds of the present invention are irreversible inhibitors of at least one of ErbB1, ErbB2, ErbB3 and ErbB4, or a mutant thereof. In some embodiments, provided compounds are irreversible inhibitors of a TEC-kinase (e.g. BTK) and JAK3. One of ordinary skill in the art will recognize that certain compounds of the present invention are reversible inhibitors. In certain embodiments, such compounds are useful as assay comparator compounds. In other embodiments, such reversible compounds are useful as inhibitors of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, and therefore useful for treating one or disorders as described herein. Exemplary reversible compounds of the present invention are set forth in Table 6, below.

TABLE 6

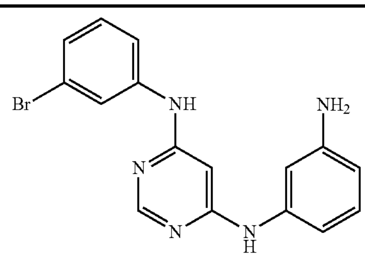
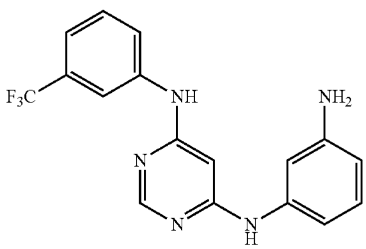
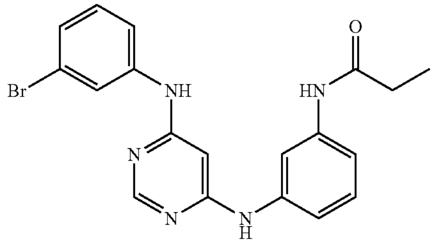
Reversible Inhibitors
I^R-1 
I^R-2 
I^R-3 

TABLE 6-continued

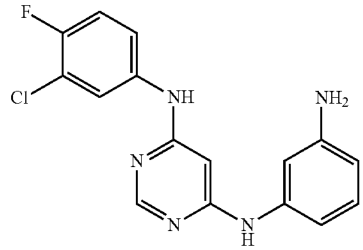
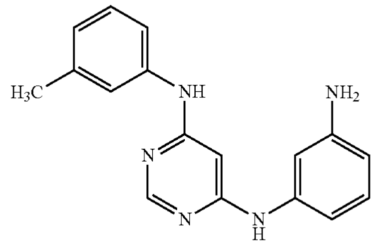
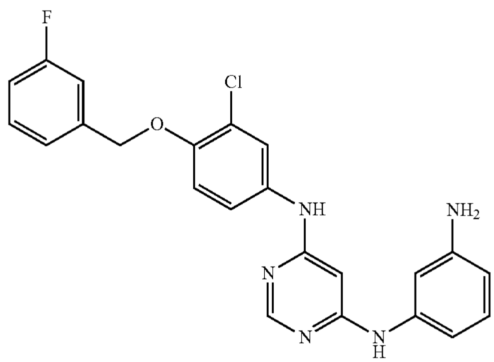
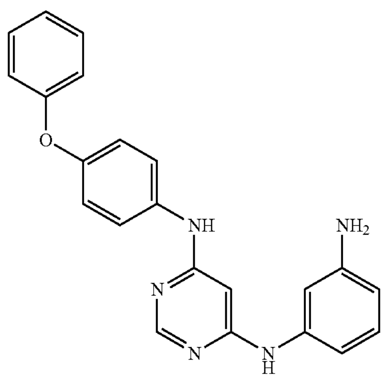
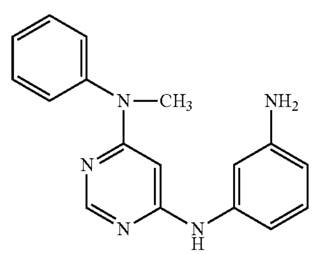
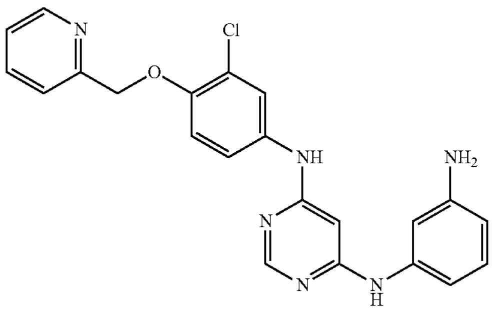
Reversible Inhibitors
I^R-4 
I^R-5 
I^R-6 
I^R-7 
I^R-8 

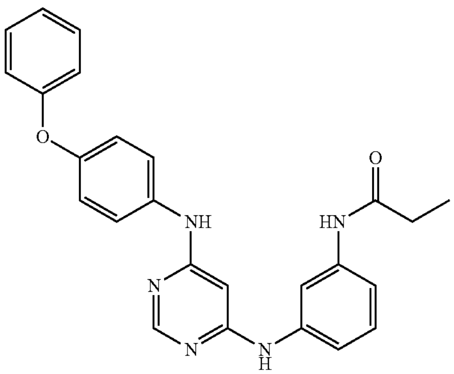
TABLE 6-continued

Reversible Inhibitors

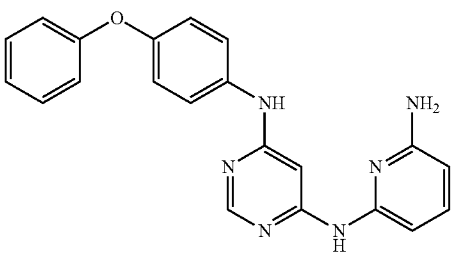
I^{R}-9



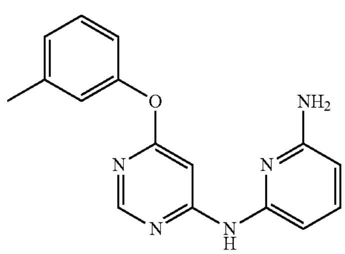
I^{R}-10



I^{R}-11



I^{R}-12



I^{R}-13

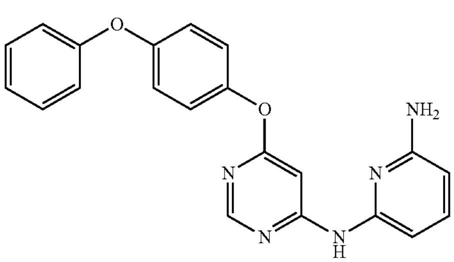
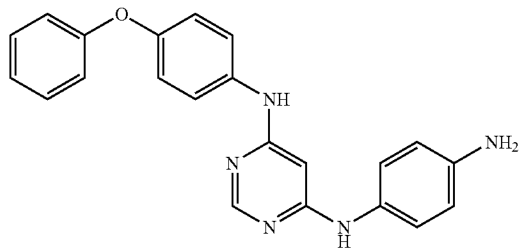


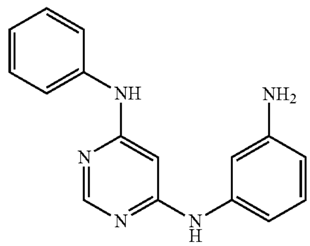
TABLE 6-continued

Reversible Inhibitors

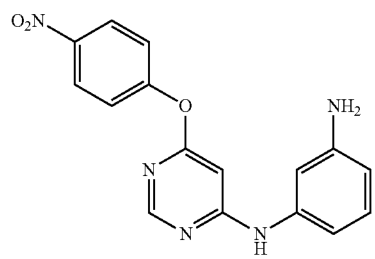
I^{R}-14



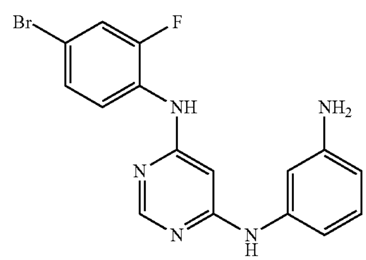
I^{R}-15



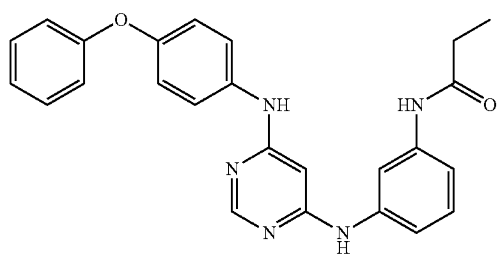
I^{R}-16



I^{R}-17



I^{R}-18



or a pharmaceutically acceptable salt thereof.

4. Uses, Formulation and Administration

[0320] Pharmaceutically Acceptable Compositions

[0321] According to another embodiment, the invention provides a composition comprising a compound of this invention or a pharmaceutically acceptable derivative thereof and a pharmaceutically acceptable carrier, adjuvant, or vehicle. The amount of compound in compositions of this invention is such that is effective to measurably inhibit a protein kinase, particularly at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, in a biological sample or in a patient. In certain embodiments, the amount of compound in compositions of this invention is such that is effective to measurably inhibit at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, in a biological sample or in a patient. In certain embodiments, a composition of this invention is formulated for administration to a patient in need of such composition. In some embodiments, a composition of this invention is formulated for oral administration to a patient.

[0322] The term "patient", as used herein, means an animal, preferably a mammal, and most preferably a human.

[0323] The term "pharmaceutically acceptable carrier, adjuvant, or vehicle" refers to a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0324] A "pharmaceutically acceptable derivative" means any non-toxic salt, ester, salt of an ester or other derivative of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an inhibitorily active metabolite or residue thereof.

[0325] As used herein, the term "inhibitorily active metabolite or residue thereof" means that a metabolite or residue thereof is also an inhibitor of at least one of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof.

[0326] Compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally, intraperitoneally or intravenously. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[0327] For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0328] Pharmaceutically acceptable compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0329] Alternatively, pharmaceutically acceptable compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[0330] Pharmaceutically acceptable compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0331] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[0332] For topical applications, provided pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, provided pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0333] For ophthalmic use, provided pharmaceutically acceptable compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

[0334] Pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0335] Most preferably, pharmaceutically acceptable compositions of this invention are formulated for oral administration.

[0336] The amount of compounds of the present invention that may be combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, provided compositions should be formulated so that a dosage of between 0.01-100 mg/kg body weight/day of the inhibitor can be administered to a patient receiving these compositions.

[0337] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a compound of the present invention in the composition will also depend upon the particular compound in the composition.

[0338] Uses of Compounds and Pharmaceutically Acceptable Compositions

[0339] Compounds and compositions described herein are generally useful for the inhibition of protein kinase activity of one or more enzymes.

[0340] Drug resistance is emerging as a significant challenge for targeted therapies. For example, drug resistance has been reported for Gleevec® and Iressa®, as well as several other kinase inhibitors in development. In addition, drug resistance has been reported for the cKit and PDGFR receptors. It has been reported that irreversible inhibitors may be effective against drug resistant forms of protein kinases (Kwak, E. L., R. Sordella, et al. (2005). "Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib." *PNAS* 102(21): 7665-7670.) Without wishing to be bound by any particular theory, it is believed that compounds of the present invention may be effective inhibitors of drug resistant forms of protein kinases.

[0341] As used herein, the term "clinical drug resistance" refers to the loss of susceptibility of a drug target to drug treatment as a consequence of mutations in the drug target.

[0342] As used herein, the term "resistance" refers to changes in the wild-type nucleic acid sequence coding a target protein, and/or the protein sequence of the target, which change, decrease or abolish the inhibitory effect of the inhibitor on the target protein.

[0343] Examples of kinases that are inhibited by the compounds and compositions described herein and against which the methods described herein are useful include ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof.

[0344] The activity of a compound utilized in this invention as an inhibitor of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, may be assayed in vitro, in vivo or in a cell line. In vitro assays include assays that determine inhibition of either the phosphorylation activity and/or the subsequent functional consequences, or ATPase activity of activated ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof. Alternate in vitro assays quantitate the ability of the inhibitor to bind to ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3. Inhibitor binding may be measured by radiolabeling the inhibitor prior to binding, isolating the inhibitor/ErbB1, inhibitor/ErbB2, inhibitor/ErbB3, inhibitor/ErbB4, inhibitor/TEC-kinase, or inhibitor/JAK3 complex and determining the amount of radiolabel bound. Alternatively, inhibitor binding may be determined by running a competition experiment where new inhibitors are incubated with ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3 bound to known radioligands. Detailed conditions for assaying a compound utilized in this invention as an inhibitor of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, are set forth in the Examples below.

[0345] Protein tyrosine kinases are a class of enzymes that catalyze the transfer of a phosphate group from ATP or GTP to a tyrosine residue located on a protein substrate. Receptor tyrosine kinases act to transmit signals from the outside of a cell to the inside by activating secondary messaging effectors via a phosphorylation event. A variety of cellular processes are promoted by these signals, including proliferation, carbohydrate utilization, protein synthesis, angiogenesis, cell growth, and cell survival.

[0346] ErbB receptors, a major family of receptor tyrosine kinases, are composed of an extracellular ligand binding domain, a single transmembrane domain, and an intracellular domain with tyrosine kinase activity. The ErbB family comprises ErbB1 (commonly known as EGFR), ErbB2 (commonly known as HER2 or neu), ErbB3 (commonly known as HER3), and ErbB4 (commonly known as HER4). More than 10 ligands (including EGF, TGFα, AR, BTC, EPR, HB-EGF, NRG-1, NRG-2, NRG-3, NRG-4) have been identified for the various receptor family members. Upon ligand binding the extracellular domain undergoes conformational change, allowing the formation of homodimers or heterodimers with other members of the ErbB family. Dimerization induces tyrosine phosphorylation of specific residues in the intracellular domain that serve as docking sites for adaptor proteins and downstream effectors. In some contexts, activation of phosphatidyl-inositol 3-kinase (PI3K) and mitogen-activated protein kinase pathways occur, leading to cell proliferation and survival (Lin, N. U.; Winer, E. P., Breast Cancer Res 6: 204-210, 2004).

[0347] Interaction between family members is necessitated by deficiencies in ErbB2, which has no known ligand, and ErbB3, which is kinase dead. EGFR, ErbB3, and ErbB4 bind ligand to induce ErbB receptor homodimerization or heterodimerization, whereas ErbB2 functions as the preferred dimerization partner. The composition of the pairwise combinations is important for signal diversification, as dimer identity determines which downstream pathways are activated. Representative downstream gene products in the ErbB signal transduction pathway include Shc, Grb2, SOS1, Ras, Raf1, Mek, ERK1, ERK2, ERα, Akt, mTOR, FKHR, p27, Cyclin D1, FasL, GSK-3, Bad, and STAT3.

[0348] There is strong precedent for involvement of the EGFR and other members of the ErbB family in human cancer because over 60% of all solid tumors overexpress at least one of these proteins or their ligands. Constitutively active, tumorigenic EGFR vIII, a mutant possessing a truncated extracellular domain, has been reported to be present in up to 78% of breast carcinomas and has also been found in glioblastomas. Overexpression of EGFR is commonly found in breast, lung, head and neck, bladder tumors, while ErbB2 expression is frequently elevated in human tumors of epithelial origin. Activating mutations in the tyrosine kinase domain have been identified in patients with non-small cell lung cancer (Lin, N. U.; Winer, E. P., Breast Cancer Res 6: 204-210, 2004). ErbB1 and/or ErbB2 amplification has also been implicated in squamous cell carcinomas, salivary gland carcinomas, ovarian carcinomas, and pancreatic cancers (Cooper, G. C. Oncogenes. 2nd ed. Sudbury: Jones and Barlett, 1995; Zhang, Y., et al., Cancer Res 66: 1025-32, 2006). Overexpression of ErbB2 has potent transforming activity, likely due to its ability to cooperate with other ErbB receptors (Sherman, L., et al., Oncogene 18: 6692-99, 1999). In fact, some human cancers that overexpress both EGFR and ErbB2 have a poorer prognosis than cancers that overexpress either receptor alone.

[0349] The ErbB signaling network is often a key component in the pathogenesis of breast cancer. Amplification of ErbB2 is associated with an aggressive tumor phenotype that is characterized by relatively rapid tumor growth, metastatic spread to visceral sites, and drug resistance. ErbB2 has been shown to be amplified in 20% of axillary node-negative ("ANN") breast cancer cases, and this amplification has been identified as an independent prognostic factor for risk of recurrence in ANN breast cancer. (Andrulis, I. L., et al., J Clin Oncol 16: 1340-9, 1998).

[0350] Targeted blockade of ErbB signaling with trastuzumab (Herceptin), a monoclonal antibody directed at ErbB2, has been shown to improve survival in women with ErbB2-positive, advanced breast cancer. Other monoclonal antibodies directed against ErbB receptors include cetuximab (Erbitux) and panitumumab (Vectibix).

[0351] Several small molecule tyrosine kinase inhibitors (TKIs) have been found to act selectively upon ErbB family members. Notable examples include gefitinib (Iressa) and erlotinib (Tarceva), both of which target the EGFR. These small molecules compete with ATP for binding to the kinase domain of the receptor. Compared to monoclonal antibodies, TKIs have several advantages in that they are orally bioavailable, well-tolerated, and appear to be active against truncated forms of ErbB2 and EGFR receptors (e.g., EGFR vIII) in vitro. In addition, the small size of small molecule TKIs may allow them to penetrate sanctuary sites such as the central nervous system. Finally, the homology between kinase domains of ErbB receptors allows for development of TKIs that target more than one member of the ErbB family simultaneously, the advantages of which are described herein.

[0352] Although certain malignancies have been linked to the overexpression of individual receptors, efficient signal transduction relies on the coexpression of ErbB receptor family members. This cooperation of ErbB receptor family members in signal transduction and malignant transformation may limit the success of agents that target individual receptors in the treatment of cancer; a potential mechanism of resistance to agents targeting a single ErbB receptor is upregulation of other members of the receptor family (Britten, C. D., Mol Cancer Ther 3: 1335-42, 2004).

[0353] Agents that target two or more ErbB receptors are called pan-ErbB regulators. ERRP is a pan-ErbB negative regulator that is expressed in most benign pancreatic ductal epithelium and islet cells. Tumors have been found to experience a progressive loss in ERRP expression. Pan-ErbB regulators may be more successful in treating tumors than compounds that only target one ErbB receptor. Erbitux and Herceptin show success in a limited patient base (tumors having increased expression of EGFR or ErbB2), which could be partly due to lack of pan-ErbB activity.

[0354] In both in vitro and in vivo models, strategies that employ a dual ErbB approach seem to have greater antitumor activity than agents targeting a single ErbB receptor. Thus, agents that target multiple members of the ErbB family are likely to provide therapeutic benefit to a broader patient population (Zhang, Y., et al., Cancer Res 66: 1025-32, 2006). In certain embodiments, provided compounds inhibit one or more of ErbB1, ErbB2, ErbB3, and ErbB4. In some embodiments, provided compounds inhibit two or more of ErbB1, ErbB2, ErbB3, and ErbB4, or a mutant thereof, and are therefore pan-ErbB inhibitors.

[0355] Clearly, there is growing evidence to support the concurrent inhibition of two or more ErbB (i.e., pan-ErbB) receptors in cancer therapy. Possible pan-ErbB approaches with small molecules include using combinations of agents that target individual ErbB receptors, using single agents that target multiple ErbB receptors, or using agents that interfere with ErbB receptor interactions (e.g., dimerization). Additional strategies include therapies utilizing a small molecule in combination with antibodies, or chemoprevention therapies (Lin, N. U.; Winer, E. P., Breast Cancer Res 6: 204-210, 2004).

[0356] An example of small molecule pan-ErbB inhibition is CI-1033, an irreversible pan-ErbB inhibitor that covalently binds to the ATP binding site of the intracellular kinase domain. Another irreversible pan-ErbB receptor tyrosine kinase inhibitor is HKI-272, which inhibits the growth of tumor cells that express ErbB-1 (EGFR) and ErbB-2 (HER-2) in culture and xenografts, and has antitumor activity in HER-2-positive breast cancer (Andrulis, I. L., et al., J Clin Oncol 16: 1340-9, 1998). Irreversible inhibitors have demonstrated superior antitumor activity in comparison with reversible inhibitors.

[0357] Neurofibromatosis type I (NF1) is a dominantly inherited human disease affecting one in 2500-3500 individuals. Several organ systems are affected, including bones, skin, iris, and the central nervous system, as manifested in learning disabilities and gliomas. A hallmark of NF1 is the development of benign tumors of the peripheral nervous system (neurofibromas), which vary greatly in both number and size among patients. Neurofibromas are heterogeneous tumors composed of Schwann cells, neurons, fibroblasts and other cells, with Schwann cells being the major (60-80%) cell type.

[0358] Abberant expression of the EGFR is associated with tumor development in NF1 and in animal models of NF1, suggesting a role in pathogenesis and representing a novel potential therapeutic target. EGFR expression affects the growth of tumor cell lines derived from NF1 patients under conditions where EGF is not the primary factor driving growth of the cells. These data suggest that EGFR may play an important role in NF1 tumorigenesis and Schwann cell transformation (DeClue, J. E., et al., J Clin Invest 105: 1233-41, 2000).

[0359] Patients with NF1 develop aggressive Schwann cell neoplasms known as malignant peripheral nerve sheath tumors (MPNSTs). Schwann cells are the major supportive cell population in the peripheral nervous system. Neoplastic Schwann cells within these neoplasms variably express the ErbB tyrosine kinases mediating NRG-1 responses (ErbB2, ErbB3, ErbB4). Neuregulin-1 (NRG-1) proteins promote the differentiation, survival, and/or proliferation of many cell types in the developing nervous system, and overexpression of NRG-1 in myelinating Schwann cells induces the formation of malignant peripheral nerve sheath tumors (MPNSTs) (Fallon, K. B., et al., J Neuro Oncol 66: 273-84, 2004).

[0360] Deregulation of Schwann cell growth is a primary defect driving the development of both benign neurofibromas and MPNST in neurofibromatosis type I (NF1) patients. Growth of MPNSTs and transformed mouse Schwann cells in vitro is highly EGF-dependent and can be blocked by EGFR inhibitors under conditions where EGF is the primary growth factor. Some human MPNST cell lines have been found to demonstrate constitutive ErbB phosphorylation. While treatment with ErbB inhibitors abolishes ErbB phosphorylation and reduces DNA synthesis in these lines, effective chemotherapeutic regimens for MPNST remain elusive (Stonecypher, M. S., et al., Oncogene 24: 5589-5605, 2005).

[0361] Schwannomas are peripheral nerve tumors comprised almost entirely of Schwann-like cells, and typically have mutations in the neurofibromatosis type II (NF2) tumor suppressor gene. Ninety percent of NF2 patients develop bilateral vestibular schwannomas and/or spinal schwannomas. Enlarging schwannomas can compress adjacent structures, resulting in deafness and other neurologic problems. Surgical removal of these tumors is difficult, often resulting in increased patient morbidity.

[0362] Both normal human Schwann cells and schwannoma cells express neuregulin receptors (i.e., ErbB receptors), and schwannoma cells proliferate in response to neuregulin. It is possible that aberrant neuregulin production or response contributes to aberrant schwannoma cell proliferation (Pelton, P. D., et al., Oncogene 17: 2195-2209, 1998).

[0363] The NF2 tumor suppressor, Merlin, is a membrane/cytoskeleton-associated protein implicated in the regulation of tyrosine kinase activity. Genetic interactions between a Merlin mutation and EGFR pathway mutations have been documented in *Drosophila* (LaJeunesse, D. R., et al., Genetics 158: 667-79, 2001). Other evidence suggests Merlin can inhibit EGFR internalization and signaling upon cell-cell contact by restraining the EGFR into a membrane compartment from which it can neither signal nor be internalized (McClatchey, A. I., et al., Genes and Development 19: 2265-77, 2005; Curto, M. C., et al., J Cell Biol 177: 893-903, 2007).

[0364] As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

[0365] Provided compounds are inhibitors of one of more of ErbB1, ErbB2, ErbB3, and ErbB4 and are therefore useful for treating one or more disorders associated with activity of one of more of ErbB1, ErbB2, ErbB3, and ErbB4. Thus, in certain embodiments, the present invention provides a method for treating an ErbB1-mediated, an ErbB2-mediated, an ErbB3-mediated, and/or ErbB4-mediated disorder comprising the step of administering to a patient in need thereof a compound of the present invention, or pharmaceutically acceptable composition thereof.

[0366] As used herein, the terms "ErbB1-mediated", "ErbB2-mediated," "ErbB3-mediated," and/or "ErbB4-mediated" disorders or conditions as used herein means any disease or other deleterious condition in which one or more of ErbB1, ErbB2, ErbB3, and/or ErbB4, or a mutant thereof, are known to play a role. Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which one or more of ErbB1, ErbB2, ErbB3, and/or ErbB4, or a mutant thereof, are known to play a role. Specifically, the present invention relates to a method of treating or lessening the severity of a disease or condition selected from a proliferative disorder, wherein said method comprises administering to a patient in need thereof a compound or composition according to the present invention.

[0367] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more disorders selected from a cancer. In some embodiments, the cancer is associated with a solid tumor. In certain embodiments, the cancer is breast cancer, glioblastoma, lung cancer, cancer of the head and neck, colorectal cancer, bladder cancer, or non-small cell lung cancer. In some embodiments, the present invention provides a method for treating or lessening the severity of one or more disorders selected from squamous cell carcinoma, salivary gland carcinoma, ovarian carcinoma, or pancreatic cancer.

[0368] In certain embodiments, the present invention provides a method for treating or lessening the severity of neurofibromatosis type I (NF1), neurofibromatosis type II (NF2) Schwann cell neoplasms (e.g. MPNST's), or Schwannomas.

[0369] The TEC family of non-receptor tyrosine kinases, referred to herein as "TEC-kinases," plays a central role in signaling through antigen-receptors such as the TCR, BCR and Fee receptors (reviewed in Miller A, et al. Current Opinion in Immunology 14; 331-340 (2002). TEC-kinases are essential for T cell activation. Three members of the family, Itk, Rlk and, are activated downstream of antigen receptor engagement in T cells and transmit signals to downstream effectors, including PLC-g. Combined deletion of Itk and Rlk in mice leads to a profound inhibition of TCR responses including proliferation, cytokine production and immune responses to an intracellular parasite (*Toxoplasma* *gondii*) (Schaeffer et al, Science 284; 638-641 (1999)). Intracellular signalling following TCR engagement is effected in ITK/RLK deficient T cells; inositol triphosphate production, calcium mobilization and MAP kinase activation are all reduced. Tec-kinases are also essential for B cell development and activation.

[0370] TEC-kinases include five family members, which are expressed primarily in hematopoietic cells: TEC, BTK, ITK (also known as TSK and EMT), RLK (also known as TXK), and BMX (also known as ETK). Additional related TEC-kinases have been found in *Drosophila melanogaster*, zebrafish (*Danio rerio*), skate (*Raja eglanteria*), and sea urchin (*Anthocidaris crassispina*).

[0371] Provided compounds are inhibitors of one of more TEC-kinases and are therefore useful for treating one or more disorders associated with activity of one or more TEC-kinases. Thus, in certain embodiments, the present invention provides a method for treating a TEC-mediated disorder comprising the step of administering to a patient in need thereof a compound of the present invention, or pharmaceutically acceptable composition thereof.

[0372] The term "TEC-mediated condition", as used herein means any disease or other deleterious condition in which TEC-kinases are known to play a role. Such conditions include those described herein and in Melcher, M et al., "The Role of TEC Family Kinases in Inflammatory Processes", *Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry,* Vol. 6, No. 1, pp. 61-69 (February 2007). Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which TEC-kinases are known to play a role. Specifically, the present invention relates to a method of treating or lessening the severity of a disease or condition selected from autoimmune, inflammatory, proliferative, and hyperproliferative diseases and immunologically-mediated diseases including rejection of transplanted organs or tissues and Acquired Immunodeficiency Syndrome (AIDS), wherein said method comprises administering to a patient in need thereof a composition of the present invention.

[0373] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including diseases of the respiratory tract including, without limitation, reversible obstructive airways diseases including asthma, such as bronchial, allergic, intrinsic, extrinsic and dust asthma, particularly chronic or inveterate asthma (e. g. late asthma airways hyper-responsiveness) and bronchitis. In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including those conditions characterized by inflammation of the nasal mucus membrane, including acute rhinitis, allergic, atrophic thinitis and chronic rhinitis including rhinitis caseosa, hypertrophic rhinitis, rhinitis purulenta, rhinitis sicca and rhinitis medicamentosa; membranous rhinitis including croupous, fibrinous and pseudomembranous rhinitis and scrofoulous rhinitis, seasonal rhinitis including rhinitis nervosa (hay fever) and vasomotor rhinitis, sarcoidosis, farmer's lung and related diseases, fibroid lung, and idiopathic interstitial pneumonia.

[0374] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including diseases of the bone and joints including, without limitation, rheumatoid arthritis, seronegative spondyloarthropathic (including ankylosing spondylitis, psoriatic arthritis and Reiter's disease), Behcet's disease, Sjogren's syndrome, systemic sclerosis, osteoporosis, bone cancer, and bone metastasis.

[0375] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including diseases and disorders of the skin, including, without limitation, psoriasis, systemic sclerosis, atopical dermatitis, contact dermatitis and other eczematous dermatitis, seborrhoetic dermatitis, lichen planus, pemphigus, bullous pemphigus, epidermolysis bullosa, urticaria, angiodermas, vasculitides, erythemas, cutaneous eosinophilias, uveitis, Alopecia, areata and vernal conjunctivitis.

[0376] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including diseases and disorders of the gastrointestinal tract, including, without limitation, celiac disease, proctitis, eosinophilic gastro-enteritis, mastocytosis, pancreatitis, Crohn's disease, ulcerative colitis, food-related allergies which have effects remote from the gut, e.g., migraine, rhinitis and eczema.

[0377] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including those diseases and disorders of other tissues and systemic disease, including, without limitation, multiple sclerosis, artherosclerosis, lupus erythematosus, systemic lupus erythematosus, Hashimoto's thyroiditis, myasthenia gravis, type I diabetes, nephrotic syndrome, eosinophilia fascitis, hyper IgE syndrome, lepromatous leprosy, sezary syndrome and idiopathic thrombocytopenia purpura, restenosis following angioplasty, tumours (for example leukemia, lymphomas including prostate cancers), and artherosclerosis.

[0378] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with TEC-kinases including allograft rejection including, without limitation, acute and chronic allograft rejection following for example transplantation of kidney, heart, liver, lung, bone marrow, skin and cornea; and chronic graft versus host disease.

[0379] In some embodiments, the present invention relates to a method of treating or lessening the severity of one or more of the diseases or conditions associated with TEC-kinases, as recited above, wherein said method comprises administering to a patient in need thereof a compound or composition according to the present invention.

[0380] Bruton's tyrosine kinase ("BTK"), a member of TEC-kinases, is a key signaling enzyme expressed in all hematopoietic cells types except T lymphocytes and natural killer cells. BTK plays an essential role in the B-cell signaling pathway linking cell surface B-cell receptor (BCR) stimulation to downstream intracellular responses.

[0381] BTK is a key regulator of B-cell development, activation, signaling, and survival (Kurosaki, Curr Op Imm, 2000, 276-281; Schaeffer and Schwartzberg, Curr Op Imm 2000, 282-288). In addition, BTK plays a role in a number of other hematopoietic cell signaling pathways, e.g., Toll like receptor (TLR) and cytokine receptor-mediated TNF-α production in macrophages, IgE receptor (Fc epsilon RI) signaling in mast cells, inhibition of Fas/APO-1 apoptotic signaling in B-lineage lymphoid cells, and collagen-stimulated platelet aggregation. See, e.g., C. A. Jeffries, et al., (2003), Journal of Biological Chemistry 278:26258-26264; N. J. Horwood, et al., (2003), The Journal of Experimental Medicine 197: 1603-1611; Iwaki et al. (2005), Journal of Biological Chemistry 280(48):40261-40270; Vassilev et al. (1999), Journal of Biological Chemistry 274(3): 1646-1656, and Quek et al. (1998), Current Biology 8(20): 1137-1140.

[0382] Patients with mutations in BTK have a profound block in B cell development, resulting in the almost complete absence of mature B lymphocytes and plasma cells, severely reduced Ig levels and a profound inhibition of humoral response to recall antigens (reviewed in Vihinen et al Frontiers in Bioscience 5: d917-928). Mice deficient in BTK also have a reduced number of peripheral B cells and greatly decreased serum levels of IgM and IgG3. BTK deletion in mice has a profound effect on B cell proliferation induced by anti-IgM, and inhibits immune responses to thymus-independent type II antigens (Ellmeier et al, J Exp Med 192: 1611-1623 (2000)). BTK also plays a crucial role in mast cell activation through the high-affinity IgE receptor (Fc_epsilon_RI). BTK deficient murine mast cells have reduced degranulation and decreased production of proinflammatory cytokines following Fc_epsilon_RI cross-linking (Kawakami et al. Journal of Leukocyte Biology 65: 286-290).

[0383] Provided compounds are inhibitors of BTK and are therefore useful for treating one or more disorders associated with activity of BTK. Thus, in some embodiments, the present invention provides a method for treating a BTK-mediated disorder comprising the step of administering to a patient in need thereof a compound of the present invention, or pharmaceutically acceptable composition thereof.

[0384] As used herein, the term "BTK-mediated" disorders or conditions as used herein means any disease or other deleterious condition in which BTK, or a mutant thereof, is known to play a role. Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which BTK, or a mutant thereof, is known to play a role. Specifically, the present invention relates to a method of treating or lessening the severity of a disease or condition selected from a proliferative disorder or an autoimmune disorder, wherein said method comprises administering to a patient in need thereof a compound or composition according to the present invention.

[0385] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK. In some embodiments, the disease or condition is an autoimmune disease, e.g., inflammatory bowel disease, arthritis, lupus, rheumatoid arthritis, psoriatic arthritis, osteoarthritis, Still's disease, juvenile arthritis, diabetes, myasthenia gravis, Hashimoto's thyroiditis, Ord's thyroiditis, Graves' disease, Sjogren's syndrome, multiple sclerosis, Guillain-Barre syndrome, acute disseminated encephalomyelitis, Addison's disease, opsoclonus-myoclonus syndrome, ankylosing spondylosis, antiphospholipid antibody syndrome, aplastic anemia, autoimmune hepatitis, celiac disease, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, optic neuritis, scleroderma, primary biliary cirrhosis, Reiter's syndrome, Takayasu's arteritis, temporal arteritis, warm autoimmune hemolytic anemia, Wegener's granulomatosis, psoriasis, alopecia universalis, Behcet's disease, chronic fatigue, dysautonomia, endometriosis, interstitial cystitis, neuromyotonia, scleroderma, or vulvodynia. In some embodiments, the disease or condition is a hyperproliferative disease or immunologically-mediated diseases including rejection of transplanted organs or tissues and Acquired Immunodeficiency Syndrome (AIDS, also known as HIV).

[0386] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, wherein the disease or condition is selected from heteroimmune conditions or diseases, which include, but are not limited to graft versus host disease, transplantation, transfusion, anaphylaxis, allergies (e.g., allergies to plant pollens, latex, drugs, foods, insect poisons, animal hair, animal dander, dust mites, or cockroach calyx), type I hypersensitivity, allergic conjunctivitis, allergic rhinitis, and atopic dermatitis.

[0387] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, wherein the disease or condition is selected from an inflammatory disease, e.g., asthma, appendicitis, blepharitis, bronchiolitis, bronchitis, bursitis, cervicitis, cholangitis, cholecystitis, colitis, conjunctivitis, cystitis, dacryoadenitis, dermatitis, dermatomyositis, encephalitis, endocarditis, endometritis, enteritis, enterocolitis, epicondylitis, epididymitis, fasciitis, fibrositis, gastritis, gastroenteritis, hepatitis, hidradenitis suppurativa, laryngitis, mastitis, meningitis, myelitis myocarditis, myositis, nephritis, oophoritis, orchitis, osteitis, otitis, pancreatitis, parotitis, pericarditis, peritonitis, pharyngitis, pleuritis, phlebitis, pneumonitis, pneumonia, proctitis, prostatitis, pyelonephritis, rhinitis, salpingitis, sinusitis, stomatitis, synovitis, tendonitis, tonsillitis, uveitis, vaginitis, vasculitis, or vulvitis.

[0388] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, wherein the disease or condition is selected from a cancer. In one embodiment, the cancer is a B-cell proliferative disorder, e.g., diffuse large B cell lymphoma, follicular lymphoma, chronic lymphocytic lymphoma, chronic lymphocytic leukemia, acute lymphatic leukemia, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, multiple myeloma (also known as plasma cell myeloma), plasmacytoma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, mantle cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, Burkitt lymphoma/leukemia, or lymphomatoid granulomatosis. In some embodiments, the cancer is breast cancer or prostate cancer, or cancer of the mast cells (e.g., mastocytoma, mast cell leukemia, mast cell sarcoma, systemic mastocytosis). In one embodiment, the cancer is bone cancer. In another embodiment, the cancer is of other primary origin and has metastasized to the bone.

[0389] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK including diseases of the bone and joints including, without limitation, rheumatoid arthritis, seronegative spondyloarthropathis (including ankylosing spondylitis, psoriatic arthritis and Reiter's disease), Behcet's disease, Sjogren's syndrome, systemic sclerosis, osteoporosis, bone cancer, and bone metastasis.

[0390] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, wherein the disease or condition is selected from a thromboembolic disorder, e.g., myocardial infarct, angina pectoris, reocclusion after angioplasty, restenosis after angioplasty, reocclusion after aortocoronary bypass, restenosis after aortocoronary bypass, stroke, transitory ischemia, a peripheral arterial occlusive disorder, pulmonary embolism, or deep venous thrombosis.

[0391] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, including infectious and noninfectious inflammatory events and autoimmune and other inflammatory diseases. These autoimmune and inflammatory diseases, disorders, and syndromes include inflammatory pelvic disease, urethritis, skin sunburn, sinusitis, pneumonitis, encephalitis, meningitis, myocarditis, nephritis, osteomyelitis, myositis, hepatitis, gastritis, enteritis, dermatitis, gingivitis, appendictitis, pancreatitis, cholocystitus, agammaglobulinemia, psoriasis, allergy, Crohn's disease, irritable bowel syndrome, ulcerative colitis, Sjogren's disease, tissue graft rejection, hyperacute rejection of transplanted organs, asthma, allergic rhinitis, chronic obstructive pulmonary disease (COPD), autoimmune polyglandular disease (also known as autoimmune polyglandular syndrome), autoimmune alopecia, pernicious anemia, glomerulonephritis, dermatomyositis, multiple sclerosis, scleroderma, vasculitis, autoimmune hemolytic and thrombocytopenic states, Goodpasture's syndrome, atherosclerosis, Addison's disease, Parkinson's disease, Alzheimer's disease, Type I diabetes, septic shock, systemic lupus erythematosus (SLE), rheumatoid arthritis, psoriatic arthritis, juvenile arthritis, osteoarthritis, chronic idiopathic thrombocytopenic purpura, Waldenstrom macroglobulinemia, myasthenia gravis, Hashimoto's thyroiditis, atopic dermatitis, degenerative joint disease, vitiligo, autoimmune hypopituitarism, Guillain-Barre syndrome, Behcet's disease, scleracierma, mycosis fungoides, acute inflammatory responses (such as acute respiratory distress syndrome and ischemia/reperfusion injury), and Graves' disease.

[0392] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with BTK, selected from rheumatoid arthritis, multiple sclerosis, B-cell chromic lymphocytic leukemia, acute lymphocytic leukemia, hairy cell leukemia, non-Hodgkin's lymphoma, irritable bowel syndrome, Crohn's Disease, lupus and renal transplant.

[0393] Interleukin-2 inducible T-cell kinase ("ITK") is expressed in T cells, mast cells and natural killer cells. It is activated in T cells upon stimulation of the T cell receptor (TCR), and in mast cells upon activation of the high affinity IgE receptor. Following receptor stimulation in T cells, Lck, a Src tyrosine kinase family member, phosphorylates Y511 in the kinase domain activation loop of ITK (S. D. Heyeck et al., 1997, J. Biol. Chem, 272, 25401-25408). Activated ITK, together with Zap-70 is required for phosphorylation and activation of PLC-gamma (S. C. Bunnell et al., 2000, J. Biol. Chem., 275, 2219-2230). PLC-gamma catalyzes the formation of inositol 1,4,5-triphosphate and diacylglycerol, leading to calcium mobilization and PKC activation, respectively. These events activate numerous downstream pathways and lead ultimately to degranulation (mast cells) and cytokine gene expression (T cells) (Y. Kawakami et al., 1999, J. Leukocyte Biol., 65, 286-290).

[0394] The role of ITK in T cell activation has been confirmed in ITK knockout mice. $CD4^+$ T cells from ITK knockout mice have a diminished proliferative response in a mixed lymphocyte reaction or upon Con A or anti-CD3 stimulation. (X. C. Liao and D. R. Littman, 1995, Immunity, 3, 757-769). Also, T cells from ITK knockout mice produced little IL-2 upon TCR stimulation resulting in reduced proliferation of these cells. In another study, ITK deficient $CD4^+$ T cells produced reduced levels of cytokines including IL-4, IL-5 and IL-13 upon stimulation of the TCR, even after priming with inducing conditions. (D. J. Fowell, 1999, Immunity, 11, 399-409).

[0395] The role of ITK in PLC-gamma activation and in calcium mobilization was also confirmed in the T cells of these knockout mice, which had severely impaired IP_3 generation and no extracellular calcium influx upon TCR stimulation (K. Liu et al., 1998, J. Exp. Med. 187, 1721-1727). Such studies support a key role for ITK in activation of T cells and mast cells. Thus an inhibitor of ITK would be of therapeutic benefit in diseases mediated by inappropriate activation of these cells.

[0396] It has been well established that T cells play an important role in regulating the immune response (Powrie and Coffman, 1993, Immunology Today, 14, 270-274). Indeed, activation of T cells is often the initiating event in immunological disorders. Following activation of the TCR, there is an influx of calcium that is required for T cell activation. Upon activation, T cells produce cytokines, including IL-2, 4, 5, 9, 10, and 13 leading to T cell proliferation, differentiation, and effector function. Clinical studies with inhibitors of IL-2 have shown that interference with T cell activation and proliferation effectively suppresses immune response in vivo (Waldmann, 1993, Immunology Today, 14, 264-270). Accordingly, agents that inhibit T lymphocyte activation and subsequent cytokine production, are therapeutically useful for selectively suppressing the immune response in a patient in need of such immunosuppression.

[0397] Mast cells play a critical roll in asthma and allergic disorders by releasing proinflammatory mediators and cytokines. Antigen-mediated aggregation of Fc.epsilon.RI, the high-affinity receptor for IgE results in activation of mast cells (D. B. Corry et al., 1999, Nature, 402, B 18-23). This triggers a series of signaling events resulting in the release of mediators, including histamine, proteases, leukotrienes and cytokines (J. R. Gordon et al., 1990, Immunology Today, 11, 458-464.) These mediators cause increased vascular permeability, mucus production, bronchoconstriction, tissue degradation and inflammation thus playing key roles in the etiology and symptoms of asthma and allergic disorders.

[0398] Published data using ITK knockout mice suggests that in the absence of ITK function, increased numbers of memory T cells are generated (A. T. Miller et al., 2002 The Journal of Immunology, 168, 2163-2172). One strategy to improve vaccination methods is to increase the number of memory T cells generated (S. M. Kaech et al., Nature Reviews Immunology, 2, 251-262). In addition, deletion of

ITK in mice results in reduced T cell receptor (TCR)-induced proliferation and secretion of the cytokines IL-2, IL-4, IL-5, IL-10 and IFN-y (Schaeffer et al, Science 284; 638-641 (1999), Fowell et al, Immunity 11, 399-409 (1999), Schaeffer et al, Nature Immunology 2 (12): 1183-1188 (2001)). The immunological symptoms of allergic asthma are attenuated in ITK−/−mice. Lung inflammation, eosinophil infiltration and mucus production are drastically reduced in ITK−/−mice in response to challenge with the allergen OVA (Mueller et al, Journal of Immunology 170: 5056-5063 (2003)). ITK has also been implicated in atopic dermatitis. This gene has been reported to be more highly expressed in peripheral blood T cells from patients with moderate and/or severe atopic dermatitis than in controls or patients with mild atopic dermatitis (Matsumoto et al, International Archives of Allergy and Immunology 129: 327-340 (2002)).

[0399] Splenocytes from RLK−/−mice secrete half the IL-2 produced by wild type animals in response to TCR engagement (Schaeffer et al, Science 284: 638-641 (1999)), while combined deletion of ITK and RLK in mice leads to a profound inhibition of TCR-induced responses including proliferation and production of the cytokines IL-2, IL-4, IL-5 and IFN-y (Schaeffer et al, Nature Immunology 2 (12): 1183-1188 (2001), Schaeffer et al, Science 284: 638-641 (1999)). Intracellular signalling following TCR engagement is effected in ITK/RLK deficient T cells; inositol triphosphate production, calcium mobilization, MAP kinase activation, and activation of the transcription factors NFAT and AP-1 are all reduced (Schaeffer et al, Science 284: 638-641 (1999), Schaeffer et al, Nature Immunology 2 (12): 1183-1188 (2001)).

[0400] Provided compounds are inhibitors of ITK and are therefore useful for treating one or more disorders associated with activity of ITK. Thus, in some embodiments, the present invention provides a method for treating an ITK-mediated disorder comprising the step of administering to a patient in need thereof a compound of the present invention, or pharmaceutically acceptable composition thereof.

[0401] As used herein, the term "ITK-mediated" disorders or conditions as used herein means any disease or other deleterious condition in which ITK, or a mutant thereof, is known to play a role. Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which ITK, or a mutant thereof, is known to play a role. Specifically, the present invention relates to a method of treating or lessening the severity of a disease or condition selected from a mast cell-mediated condition, a basophil-mediated disorder, an immune or allergic disorder, wherein said method comprises administering to a patient in need thereof a compound or composition according to the present invention.

[0402] In some embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with ITK, wherein the disease or condition is an immune disorder, including inflammatory diseases, autoimmune diseases, organ and bone marrow transplant rejection and other disorders associated with T cell-mediated immune response or mast cell-mediated immune response.

[0403] In certain embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with ITK, wherein the disease or condition is acute or chronic inflammation, an allergy, contact dermatitis, psoriasis, rheumatoid arthritis, multiple sclerosis, type 1 diabetes, inflammatory bowel disease, Guillain-Barre syndrome, Crohn's disease, ulcerative colitis, cancer, graft versus host disease (and other forms of organ or bone marrow transplant rejection) or lupus erythematosus.

[0404] In certain embodiments, the present invention provides a method for treating or lessening the severity of one or more diseases and conditions associated with ITK, wherein the disease or condition is a mast cell driven conditions, a basophil-mediated disorder, reversible obstructive airway disease, asthma, rhinitis, chronic obstructive, pulmonary disease (COPD), peripheral T-cell lymphomas or HIV [also known as Acquired Immunodeficiency Syndrome (AIDS)]. Such conditions include those described in Readinger, et al., PNAS 105: 6684-6689 (2008).

[0405] The Janus kinases (JAK) are a family of tyrosine kinases consisting of JAK1, JAK2, JAK3 and TYK2. The JAKs play a critical role in cytokine signaling. The downstream substrates of the JAK family of kinases include the signal transducer and activator of transcription (STAT) proteins. JAK/STAT signaling has been implicated in the mediation of many abnormal immune responses such as allergies, asthma, autoimmune diseases such as transplant rejection, rheumatoid arthritis, amyotrophic lateral sclerosis and multiple sclerosis as well as in solid and hematologic malignancies such as leukemias and lymphomas. The pharmaceutical intervention in the JAK/STAT pathway has been reviewed [Frank Mol. Med. 5: 432-456 (1999) & Seidel, et al, Oncogene 19: 2645-2656 (2000)].

[0406] JAK1, JAK2, and TYK2 are ubiquitously expressed, while JAK3 is predominantly expressed in hematopoietic cells. JAK3 binds exclusively to the common cytokine receptor gamma chain (yc) and is activated by IL-2, IL-4, IL-7, IL-9, and IL-15.

[0407] The proliferation and survival of murine mast cells induced by IL-4 and IL-9 have, in fact, been shown to be dependent on JAK3- and yc-signaling [Suzuki et al, Blood 96: 2172-2180 (2000)].

[0408] Cross-linking of the high-affinity immunoglobulin (Ig) E receptors of sensitized mast cells leads to a release of proinflammatory mediators, including a number of vasoactive cytokines resulting in acute allergic, or immediate (type I) hypersensitivity reactions [Gordon et al, Nature 346: 274-276 (1990) & Galli, N. Engl. J. Med., 328: 257-265 (1993)]. A crucial role for JAK3 in IgE receptor-mediated mast cell responses in vitro and in vivo has been established [Malaviya, et al, Biochem. Biophys. Res. Commun. 257: 807-813 (1999)]. In addition, the prevention of type I hypersensitivity reactions, including anaphylaxis, mediated by mast cell-activation through inhibition of JAK3 has also been reported [Malaviya et al, J. Biol. Chem. 274: 27028-27038 (1999)]. Targeting mast cells with JAK3 inhibitors modulated mast cell degranulation in vitro and prevented IgE receptor/antigen-mediated anaphylactic reactions in vivo.

[0409] A recent study described the successful targeting of JAK3 for immune suppression and allograft acceptance. The study demonstrated a dose-dependent survival of buffalo heart allograft in Wistar Furth recipients upon administration of inhibitors of JAK3 indicating the possibility of regulating unwanted immune responses in graft versus host disease [Kirken, Transpl. Proc. 33: 3268-3270 (2001)].

[0410] IL-4-mediated STAT-phosphorylation has been implicated as the mechanism involved in early and late stages of rheumatoid arthritis (RA). Up-regulation of proinflammatory cytokines in RA synovium and synovial fluid is a characteristic of the disease. It has been demonstrated that IL-4-mediated activation of IL-4/STAT pathway is mediated through the Janus kinases (JAK 1 & 3) and that IL-4-associated JAK kinases are expressed in the RA synovium [Muller-Ladner, et al, J. Immunol. 164: 3894-3901 (2000)].

[0411] Familial amyotrophic lateral sclerosis (FALS) is a fatal neurodegenerative disorder affecting about 10% of ALS patients. The survival rates of FALS mice were increased upon treatment with a JAK3 specific inhibitor. This confirmed that JAK3 plays a role in FALS [Trieu, et al, Biochem. Biophys. Res. Commun. 267: 22-25 (2000)].

[0412] Signal transducer and activator of transcription (STAT) proteins are activated by, among others, the JAK family kinases. Results form a recent study suggested the possibility of intervention in the JAK/STAT signaling pathway by targeting JAK family kinases with specific inhibitors for the treatment of leukemia [Sudbeck, et al, Clin. Cancer Res. 5: 1569-1582 (1999)]. JAK3 specific compounds were shown to inhibit the clonogenic growth of JAK3-expressing cell lines DAUDI, RAMOS, LC1; 19, NALM-6, MOLT-3 and HL-60. Inhibition of JAK3 and TYK 2 abrogated tyrosine phosphorylation of STAT3, and inhibited cell growth of mycosis fungoides, a form of cutaneous T cell lymphoma.

[0413] According to another embodiment, the invention provides a method for treating or lessening the severity of a JAK3-mediated disease or condition in a patient comprising the step of administering to said patient a composition according to the present invention.

[0414] The term "JAK3-mediated disease", as used herein means any disease or other deleterious condition in which a JAK3 kinase is known to play a role. Accordingly, another embodiment of the present invention relates to treating or lessening the severity of one or more diseases in which JAK3 is known to play a role. Specifically, the present invention relates to a method of treating or lessening the severity of a disease or condition selected from immune responses such as allergic or type I hypersensitivity reactions, asthma, autoimmune diseases such as transplant rejection, graft versus host disease, rheumatoid arthritis, amyotrophic lateral sclerosis, and multiple sclerosis, neurodegenerative disorders such as familial amyotrophic lateral sclerosis (FALS), as well as in solid and hematologic malignancies such as leukemias and lymphomas, wherein said method comprises administering to a patient in need thereof a composition according to the present invention.

[0415] The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for treating or lessening the severity of cancer, an autoimmune disorder, a neurodegenerative or neurological disorder, schizophrenia, a bone-related disorder, liver disease, or a cardiac disorder. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular agent, its mode of administration, and the like. Compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed, and like factors well known in the medical arts. The term "patient", as used herein, means an animal, preferably a mammal, and most preferably a human.

[0416] Pharmaceutically acceptable compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[0417] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0418] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[0419] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0420] In order to prolong the effect of a compound of the present invention, it is often desirable to slow the absorption of the compound from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the compound then depends upon its rate of dissolution that, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered compound form is accomplished by dissolving or suspending the compound in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the compound in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of compound to polymer and the nature of the particular polymer employed, the rate of compound release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly (anhydrides). Depot injectable formulations are also prepared by entrapping the compound in liposomes or microemulsions that are compatible with body tissues.

[0421] Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable nonirritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0422] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0423] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[0424] The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[0425] Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

[0426] According to one embodiment, the invention relates to a method of inhibiting protein kinase activity in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound.

[0427] According to another embodiment, the invention relates to a method of inhibiting ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, activity in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound. In certain embodiments, the invention relates to a method of irreversibly inhibiting ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, activity in a biological sample comprising the step of contacting said biological sample with a compound of this invention, or a composition comprising said compound.

[0428] The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof.

[0429] Inhibition of protein kinase, or a protein kinase selected from ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, activity in a biological sample is useful for a variety of purposes that are known to one of skill in the art. Examples of such purposes include, but are not limited to, blood transfusion, organ-transplantation, biological specimen storage, and biological assays.

[0430] Another embodiment of the present invention relates to a method of inhibiting protein kinase activity in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound.

[0431] According to another embodiment, the invention relates to a method of inhibiting one or more of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, activity in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound. According to certain embodiments, the invention relates to a method of irreversibly inhibiting one or more of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, activity in a patient comprising the step of administering to said patient a compound of the present invention, or a composition comprising said compound. In other embodiments, the present invention provides a method for treating a disorder mediated by one or more of ErbB1, ErbB2, ErbB3, ErbB4, a TEC-kinase, and/or JAK3, or a mutant thereof, in a patient in need thereof, comprising the step of administering to said patient a compound according to the present invention or pharmaceutically acceptable composition thereof. Such disorders are described in detail herein.

[0432] Depending upon the particular condition, or disease, to be treated, additional therapeutic agents, which are normally administered to treat that condition, may also be present in the compositions of this invention. As used herein, additional therapeutic agents that are normally administered to treat a particular disease, or condition, are known as "appropriate for the disease, or condition, being treated."

[0433] For example, compounds of the present invention, or a pharmaceutically acceptable composition thereof, are administered in combination with chemotherapeutic agents to treat proliferative diseases and cancer. Examples of known chemotherapeutic agents include, but are not limited to, Adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, platinum derivatives, taxane (e.g., paclitaxel), vinca alkaloids (e.g., vinblastine), anthracyclines (e.g., doxorubicin), epipodophyllotoxins (e.g., etoposide), cisplatin, an mTOR inhibitor (e.g., a rapamycin), methotrexate, actinomycin D, dolastatin 10, colchicine, emetine, trimetrexate, metoprine, cyclosporine, daunorubicin, teniposide, amphotericin, alkylating agents (e.g., chlorambucil), 5-fluorouracil, campthothecin, cisplatin, metronidazole, and Gleevec™, among others. In other embodiments, a compound of the present invention is administered in combination with a biologic agent, such as Avastin or VECTIBIX.

[0434] In certain embodiments, compounds of the present invention, or a pharmaceutically acceptable composition thereof, are administered in combination with an antiproliferative or chemotherapeutic agent selected from any one or more of Abarelix, aldesleukin, Aldesleukin, Alemtuzumab, Alitretinoin, Allopurinol, Altretamine, Amifostine, Anastrozole, Arsenic trioxide, Asparaginase, Azacitidine, BCG Live, Bevacuzimab, Fluorouracil, Bexarotene, Bleomycin, Bortezomib, Busulfan, Calusterone, Capecitabine, Camptothecin, Carboplatin, Carmustine, Celecoxib, Cetuximab, Chlorambucil, Cladribine, Clofarabine, Cyclophosphamide, Cytarabine, Dactinomycin, Darbepoetin alfa, Daunorubicin, Denileukin, Dexrazoxane, Docetaxel, Doxorubicin (neutral), Doxorubicin hydrochloride, Dromostanolone Propionate, Epirubicin, Epoetin alfa, Erlotinib, Estramustine, Etoposide Phosphate, Etoposide, Exemestane, Filgrastim, floxuridine fludarabine, Fulvestrant, Gefitinib, Gemcitabine, Gemtuzumab, Goserelin Acetate, Histrelin Acetate, Hydroxyurea, Ibritumomab, Idarubicin, Ifosfamide, Imatinib Mesylate, Interferon Alfa-2a, Interferon Alfa-2b, Irinotecan, Lenalidomide, Letrozole, Leucovorin, Leuprolide Acetate, Levamisole, Lomustine, Megestrol Acetate, Melphalan, Mercaptopurine, 6-MP, Mesna, Methotrexate, Methoxsalen, Mitomycin C, Mitotane, Mitoxantrone, Nandrolone, Nelarabine, Nofetumomab, Oprelvekin, Oxaliplatin, Paclitaxel, Palifermin, Pamidronate, Pegademase, Pegaspargase, Pegfilgrastim, Pemetrexed Disodium, Pentostatin, Pipobroman, Plicamycin, Porfimer Sodium, Procarbazine, Quinacrine, Rasburicase, Rituximab, Sargramostim, Sorafenib, Streptozocin, Sunitinib Maleate, Talc, Tamoxifen, Temozolomide, Teniposide, VM-26, Testolactone, Thioguanine, 6-TG, Thiotepa, Topotecan, Toremifene, Tositumomab, Trastuzumab, Tretinoin, ATRA, Uracil Mustard, Valrubicin, Vinblastine, Vincristine, Vinorelbine, Zoledronate, or Zoledronic acid.

[0435] Other examples of agents the inhibitors of this invention may also be combined with include, without limitation: treatments for Alzheimer's Disease such as Aricept® and Excelon®; treatments for Parkinson's Disease such as L-DOPA/carbidopa, entacapone, ropinrole, pramipexole, bromocriptine, pergolide, trihexephendyl, and amantadine; agents for treating Multiple Sclerosis (MS) such as beta interferon (e.g., Avonex® and Rebif®), Copaxone®, and mitoxantrone; treatments for asthma such as albuterol and Singulair®; agents for treating schizophrenia such as zyprexa, risperdal, seroquel, and haloperidol; anti-inflammatory agents such as corticosteroids, TNF blockers, IL-1 RA, azathioprine, cyclophosphamide, and sulfasalazine; immunomodulatory and immunosuppressive agents such as cyclosporin, tacrolimus, rapamycin, mycophenolate mofetil, interferons, corticosteroids, cyclophophamide, azathioprine, and sulfasalazine; neurotrophic factors such as acetylcholinesterase inhibitors, MAO inhibitors, interferons, anti-convulsants, ion channel blockers, riluzole, and anti-Parkinsonian agents; agents for treating cardiovascular disease such as beta-blockers, ACE inhibitors, diuretics, nitrates, calcium channel blockers, and statins; agents for treating liver disease such as corticosteroids, cholestyramine, interferons, and anti-viral agents; agents for treating blood disorders such as corticosteroids, anti-leukemic agents, and growth factors; and agents for treating immunodeficiency disorders such as gamma globulin.

[0436] In certain embodiments, compounds of the present invention, or a pharmaceutically acceptable composition thereof, are administered in combination with a monoclonal antibody or an siRNA therapeutic.

[0437] Those additional agents may be administered separately from an inventive compound-containing composition, as part of a multiple dosage regimen. Alternatively, those agents may be part of a single dosage form, mixed together with a compound of this invention in a single composition. If administered as part of a multiple dosage regime, the two active agents may be submitted simultaneously, sequentially or within a period of time from one another normally within five hours from one another.

[0438] As used herein, the term "combination," "combined," and related terms refers to the simultaneous or sequential administration of therapeutic agents in accordance with this invention. For example, a compound of the present invention may be administered with another therapeutic agent simultaneously or sequentially in separate unit dosage forms or together in a single unit dosage form. Accordingly, the present invention provides a single unit dosage form comprising a compound of formula I, an additional therapeutic agent, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

[0439] The amount of both, an inventive compound and additional therapeutic agent (in those compositions which comprise an additional therapeutic agent as described above)) that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. Preferably, compositions of this invention should be formulated so that a dosage of between 0.01-100 mg/kg body weight/day of an inventive can be administered.

[0440] In those compositions which comprise an additional therapeutic agent, that additional therapeutic agent and the compound of this invention may act synergistically. Therefore, the amount of additional therapeutic agent in such compositions will be less than that required in a monotherapy utilizing only that therapeutic agent. In such compositions a dosage of between 0.01-1,000 μg/kg body weight/day of the additional therapeutic agent can be administered.

[0441] The amount of additional therapeutic agent present in the compositions of this invention will be no more than the amount that would normally be administered in a composition comprising that therapeutic agent as the only active agent. Preferably the amount of additional therapeutic agent in the presently disclosed compositions will range from about 50% to 100% of the amount normally present in a composition comprising that agent as the only therapeutically active agent.

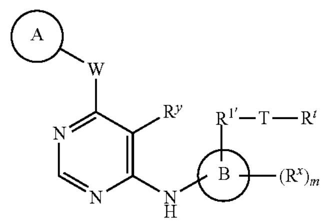
[0442] The compounds of this invention, or pharmaceutical compositions thereof, may also be incorporated into compositions for coating an implantable medical device, such as prostheses, artificial valves, vascular grafts, stents and catheters. Vascular stents, for example, have been used to overcome restenosis (re-narrowing of the vessel wall after injury). However, patients using stents or other implantable devices risk clot formation or platelet activation. These unwanted effects may be prevented or mitigated by precoating the device with a pharmaceutically acceptable composition comprising a kinase inhibitor. Implantable devices coated with a compound of this invention are another embodiment of the present invention.

5. Probe Compounds

[0443] In certain aspects, a compound of the present invention may be tethered to a detectable moiety to form a probe compound. In one aspect, a probe compound of the invention comprises an irreversible protein kinase inhibitor of formula I, as described herein, a detectable moiety, and a tethering moiety that attaches the inhibitor to the detectable moiety.

[0444] In some embodiments, such probe compounds of the present invention comprise a provided compound of formula I tethered to a detectable moiety, R^t, by a bivalent tethering moiety, -T-. The tethering moiety may be attached to a compound of formula I via Ring A, Ring B, or R^1. One of ordinary skill in the art will appreciate that when a tethering moiety is attached to R^1, R^1 is a bivalent warhead group denoted as $R^{1'}$. In certain embodiments, a provided probe compound is selected from any of formula V, VI, or VII:

V



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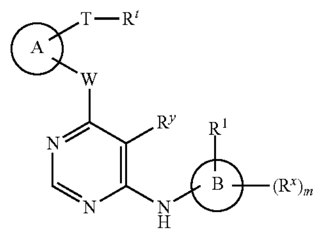

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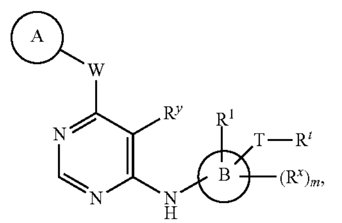


wherein each of Ring A, Ring B, R^1, m, p, R^x, R^y, R^v, W^1, and W^2 is as defined above with respect to formula I, and described in classes and subclasses herein, $R^{1'}$ is a bivalent warhead group, T is a bivalent tethering moiety; and IV is a detectable moiety.

[0445] In some embodiments, R^t is a detectable moiety selected from a primary label or a secondary label. In certain embodiments, R^t is a detectable moiety selected from a fluorescent label (e.g., a fluorescent dye or a fluorophore), a mass-tag, a chemiluminescent group, a chromophore, an electron dense group, or an energy transfer agent.

[0446] As used herein, the term "detectable moiety" is used interchangeably with the term "label" and "reporter" and relates to any moiety capable of being detected, e.g., primary labels and secondary labels. A presence of a detectable moiety can be measured using methods for quantifying (in absolute, approximate or relative terms) the detectable moiety in a system under study. In some embodiments, such methods are well known to one of ordinary skill in the art and include any methods that quantify a reporter moiety (e.g., a label, a dye, a photocrosslinker, a cytotoxic compound, a drug, an affinity label, a photoaffinity label, a reactive compound, an antibody or antibody fragment, a biomaterial, a nanoparticle, a spin label, a fluorophore, a metal-containing moiety, a radioactive moiety, quantum dot(s), a novel functional group, a group that covalently or noncovalently interacts with other molecules, a photocaged moiety, an actinic radiation excitable moiety, a ligand, a photoisomerizable moiety, biotin, a biotin analog (e.g., biotin sulfoxide), a moiety incorporating a heavy atom, a chemically cleavable group, a photocleavable group, a redox-active agent, an isotopically labeled moiety, a biophysical probe, a phosphorescent group, a chemiluminescent group, an electron dense group, a magnetic group, an intercalating group, a chromophore, an energy transfer agent, a biologically active agent, a detectable label, and any combination of the above).

[0447] Primary labels, such as radioisotopes (e.g., tritium, ^{32}P, ^{33}P, ^{35}S, ^{14}C, ^{123}I, ^{124}I, ^{125}I, or ^{131}I), mass-tags including, but not limited to, stable isotopes (e.g., ^{13}C, ^{2}H, ^{17}O, ^{18}O, ^{15}N, ^{19}F, and ^{127}I), positron emitting isotopes (e.g., ^{11}C, ^{18}F, ^{13}N, ^{124}I, and ^{15}O), and fluorescent labels are signal generating reporter groups which can be detected without further modifications. Detectable moities may be analyzed by methods including, but not limited to fluorescence, positron emission tomography, SPECT medical imaging, chemiluminescence, electron-spin resonance, ultraviolet/visible absorbance spectroscopy, mass spectrometry, nuclear magnetic resonance, magnetic resonance, flow cytometry, autoradiography, scintillation counting, phosphoimaging, and electrochemical methods.

[0448] The term "secondary label" as used herein refers to moieties such as biotin and various protein antigens that require the presence of a second intermediate for production of a detectable signal. For biotin, the secondary intermediate may include streptavidin-enzyme conjugates. For antigen labels, secondary intermediates may include antibody-enzyme conjugates. Some fluorescent groups act as secondary labels because they transfer energy to another group in the process of nonradiative fluorescent resonance energy transfer (FRET), and the second group produces the detected signal.

[0449] The terms "fluorescent label", "fluorescent dye", and "fluorophore" as used herein refer to moieties that absorb light energy at a defined excitation wavelength and emit light energy at a different wavelength. Examples of fluorescent labels include, but are not limited to: Alexa Fluor dyes (Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660 and Alexa Fluor 680), AMCA, AMCA-S, BODIPY dyes (BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665), Carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), Cascade Blue, Cascade Yellow, Coumarin 343, Cyanine dyes (Cy3, Cy5, Cy3.5, Cy5.5), Dansyl, Dapoxyl, Dialkylaminocoumarin, 4',5'-Dichloro-2',7'-dimethoxy-fluorescein, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IRDyes (IRD40, IRD 700, IRD 800), JOE, Lissamine rhodamine B, Marina Blue, Methoxycoumarin, Naphthofluorescein, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, PyMPO, Pyrene, Rhodamine B, Rhodamine 6G, Rhodamine Green, Rhodamine Red, Rhodol Green, 2',4',5',7'-Tetra-bromosulfone-fluorescein, Tetramethyl-rhodamine (TMR), Carboxytetramethylrhodamine (TAMRA), Texas Red, Texas Red-X, 5(6)-Carboxyfluorescein, 2,7-Dichlorofluorescein, N,N-Bis(2,4,6-trimethylphenyl)-3,4:9,10-perylenebis(dicarboximide, HPTS, Ethyl Eosin, DY-490XL MegaStokes, DY-485XL MegaStokes, Adirondack Green 520, ATTO 465, ATTO 488, ATTO 495, YOYO-1,5-FAM, BCECF, dichlorofluorescein, rhodamine 110, rhodamine 123, YO-PRO-1, SYTOX Green, Sodium Green, SYBR Green I, Alexa Fluor 500, FITC, Fluo-3, Fluo-4, fluoro-emerald, YoYo-1 ssDNA, YoYo-1 dsDNA, YoYo-1, SYTO RNASelect, Diversa Green-FP, Dragon Green, EvaGreen, Surf Green EX, Spectrum Green, NeuroTrace 500525, NBD-X, MitoTracker Green FM, LysoTracker Green DND-26, CBQCA, PA-GFP (post-activation), WEGFP (post-activation), FLASH-CCXXCC, Azami Green monomeric, Azami Green, green fluorescent protein (GFP), EGFP (Campbell Tsien 2003), EGFP (Patterson 2001), Kaede Green, 7-Benzylamino-4-Nitrobenz-2-Oxa-1,3-Diazole, Bex1, Doxorubicin, Lumio Green, and SuperGlo GFP.

[0450] The term "mass-tag" as used herein refers to any moiety that is capable of being uniquely detected by virtue of its mass using mass spectrometry (MS) detection techniques. Examples of mass-tags include electrophore release tags such as N-[3-[4'-[(p-Methoxytetrafluorobenzyl)oxy]phenyl]-3-methylglyceronyl]isonipecotic Acid, 4'-[2,3,5,6-Tetrafluoro-4-(pentafluorophenoxyl)]methyl acetophenone, and their derivatives. The synthesis and utility of these mass-tags is described in U.S. Pat. Nos. 4,650,750, 4,709,016, 5,360,8191, 5,516,931, 5,602,273, 5,604,104, 5,610,020, and 5,650,270. Other examples of mass-tags include, but are not limited to, nucleotides, dideoxynucleotides, oligonucleotides of varying length and base composition, oligopeptides, oligosaccharides, and other synthetic polymers of varying length and monomer composition. A large variety of organic molecules, both neutral and charged (biomolecules or synthetic compounds) of an appropriate mass range (100-2000 Daltons) may also be used as mass-tags. Stable isotopes (e.g., ^{13}C, ^{2}H, ^{17}O, ^{18}O, and ^{15}N) may also be used as mass-tags.

[0451] The term "chemiluminescent group," as used herein, refers to a group which emits light as a result of a chemical reaction without the addition of heat. By way of example, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) reacts with oxidants like hydrogen peroxide (H_2O_2) in the presence of a base and a metal catalyst to produce an excited state product (3-aminophthalate, 3-APA).

[0452] The term "chromophore," as used herein, refers to a molecule which absorbs light of visible wavelengths, UV wavelengths or IR wavelengths.

[0453] The term "dye," as used herein, refers to a soluble, coloring substance which contains a chromophore.

[0454] The term "electron dense group," as used herein, refers to a group which scatters electrons when irradiated with an electron beam. Such groups include, but are not limited to, ammonium molybdate, bismuth subnitrate, cadmium iodide, carbohydrazide, ferric chloride hexahydrate, hexamethylene tetramine, indium trichloride anhydrous, lanthanum nitrate, lead acetate trihydrate, lead citrate trihydrate, lead nitrate, periodic acid, phosphomolybdic acid, phosphotungstic acid, potassium ferricyanide, potassium ferrocyanide, ruthenium red, silver nitrate, silver proteinate (Ag Assay: 8.0-8.5%) "Strong", silver tetraphenylporphine (S-TPPS), sodium chloroaurate, sodium tungstate, thallium nitrate, thiosemicarbazide (TSC), uranyl acetate, uranyl nitrate, and vanadyl sulfate.

[0455] The term "energy transfer agent," as used herein, refers to a molecule which either donates or accepts energy from another molecule. By way of example only, fluorescence resonance energy transfer (FRET) is a dipole-dipole coupling process by which the excited-state energy of a fluorescence donor molecule is non-radiatively transferred to an unexcited acceptor molecule which then fluorescently emits the donated energy at a longer wavelength.

[0456] The term "moiety incorporating a heavy atom," as used herein, refers to a group which incorporates an ion of atom which is usually heavier than carbon. In some embodiments, such ions or atoms include, but are not limited to, silicon, tungsten, gold, lead, and uranium.

[0457] The term "photoaffinity label," as used herein, refers to a label with a group, which, upon exposure to light, forms a linkage with a molecule for which the label has an affinity.

[0458] The term "photocaged moiety," as used herein, refers to a group which, upon illumination at certain wavelengths, covalently or non-covalently binds other ions or molecules.

[0459] The term "photoisomerizable moiety," as used herein, refers to a group wherein upon illumination with light changes from one isomeric form to another.

[0460] The term "radioactive moiety," as used herein, refers to a group whose nuclei spontaneously give off nuclear radiation, such as alpha, beta, or gamma particles; wherein, alpha particles are helium nuclei, beta particles are electrons, and gamma particles are high energy photons.

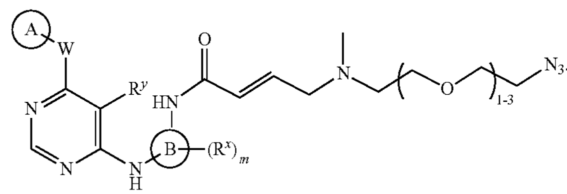
[0461] The term "spin label," as used herein, refers to molecules which contain an atom or a group of atoms exhibiting an unpaired electron spin (i.e. a stable paramagnetic group) that in some embodiments are detected by electron spin resonance spectroscopy and in other embodiments are attached to another molecule. Such spin-label molecules include, but are not limited to, nitryl radicals and nitroxides, and in some embodiments are single spin-labels or double spin-labels.

[0462] The term "quantum dots," as used herein, refers to colloidal semiconductor nanocrystals that in some embodiments are detected in the near-infrared and have extremely high quantum yields (i.e., very bright upon modest illumination).

[0463] One of ordinary skill in the art will recognize that a detectable moiety may be attached to a provided compound via a suitable substituent. As used herein, the term "suitable substituent" refers to a moiety that is capable of covalent attachment to a detectable moiety. Such moieties are well known to one of ordinary skill in the art and include groups containing, e.g., a carboxylate moiety, an amino moiety, a thiol moiety, or a hydroxyl moiety, to name but a few. It will be appreciated that such moieties may be directly attached to a provided compound or via a tethering moiety, such as a bivalent saturated or unsaturated hydrocarbon chain.

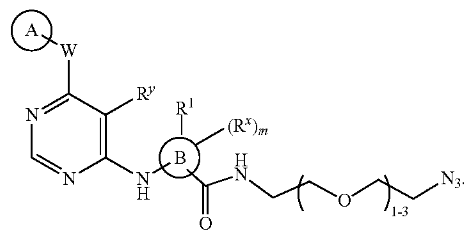
[0464] In some embodiments, such detectable moieties are attached to a provided compound via click chemistry. In some embodiments, such moieties are attached via a 1,3-cycloaddition of an azide with an alkyne, optionally in the presence of a copper catalyst. Methods of using click chemistry are known in the art and include those described by Rostovtsev et al., Angew. Chem. Int. Ed. 2002, 41, 2596-99 and Sun et al., Bioconjugate Chem., 2006, 17, 52-57. In some embodiments, a click ready inhibitor moiety is provided and reacted with a click ready -T-R^t moiety. As used herein, "click ready" refers to a moiety containing an azide or alkyne for use in a click chemistry reaction. In some embodiments, the click ready inhibitor moiety comprises an azide. In certain embodiments, the click ready -T-R^t moiety comprises a strained cyclooctyne for use in a copper-free click chemistry reaction (for example, using methods described in Baskin et al., Proc. Natl. Acad. Sci. USA 2007, 104, 16793-16797).

[0465] In certain embodiments, the click ready inhibitor moiety is of formula:



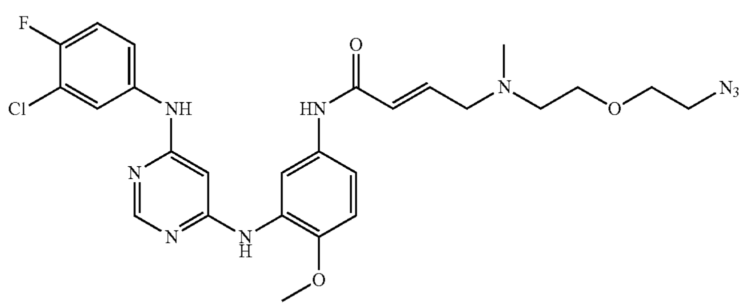
wherein Ring A, Ring B, W, R^y, R^x, and m are as defined above with respect to Formula I and described herein.

[0466] In other embodiments, the click ready inhibitor moiety is of formula:



wherein Ring A, Ring B, W, R^y, R^x, m and R^1 are as defined above with respect to Formula I and described herein.

[0467] Exemplary click ready inhibitors include:



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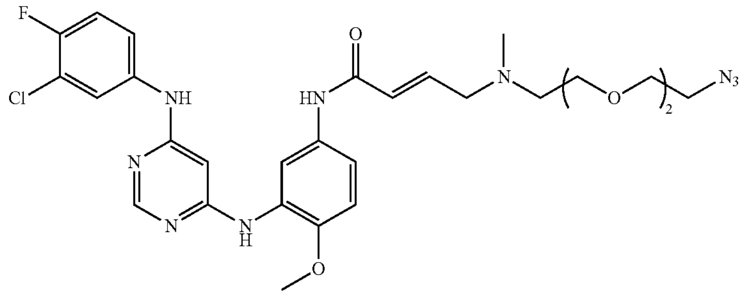
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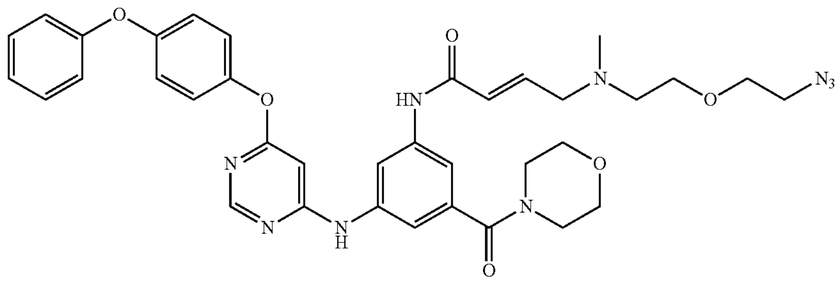
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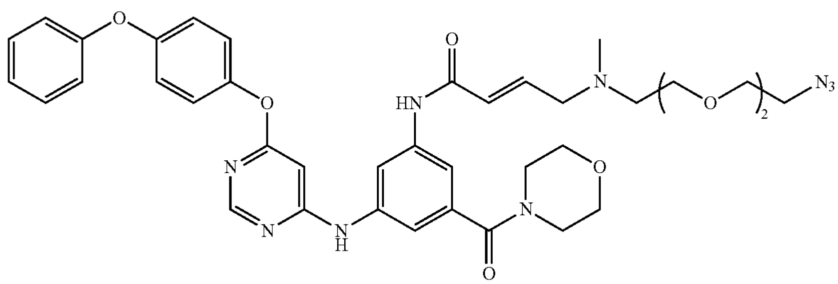
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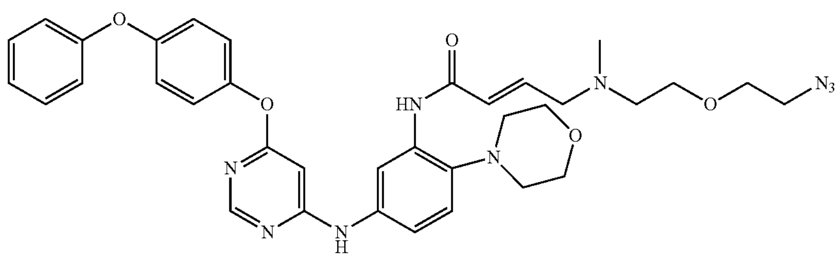
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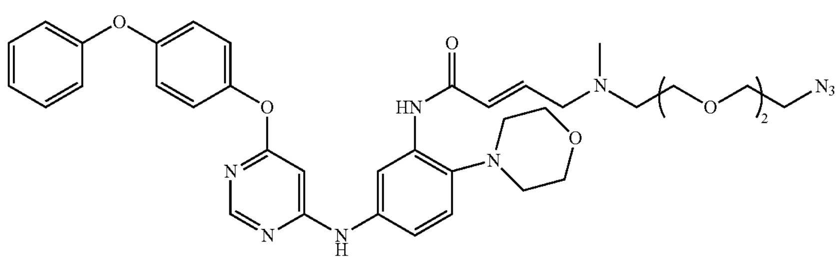
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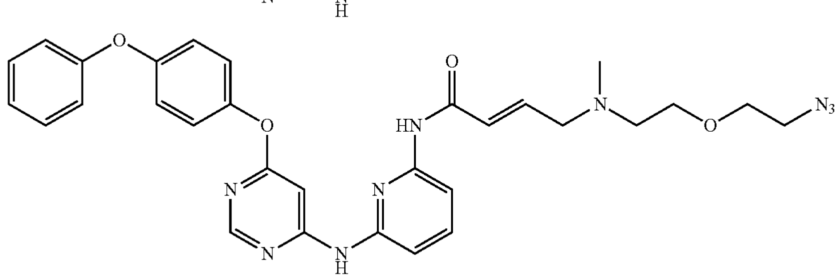
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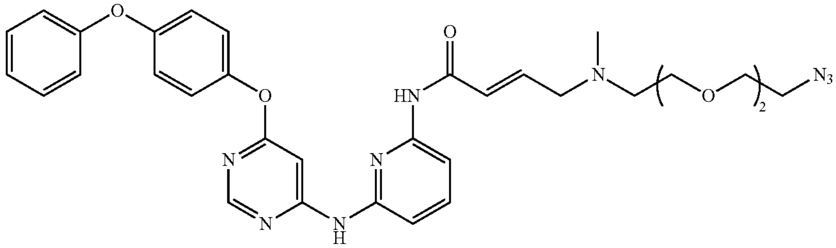
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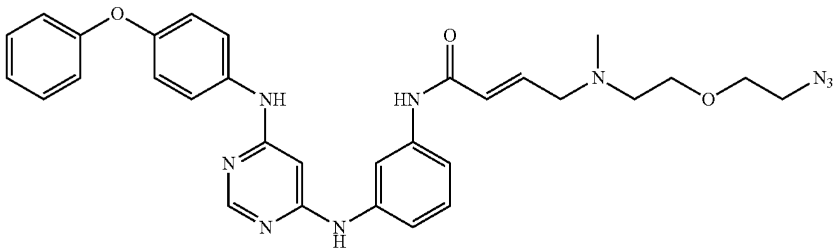
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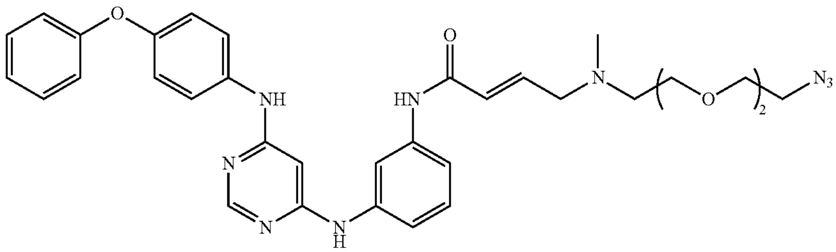
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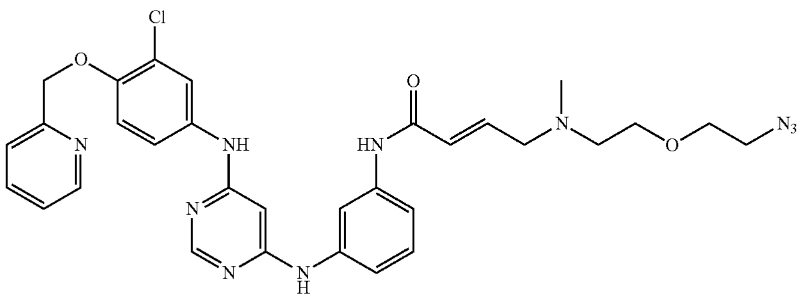
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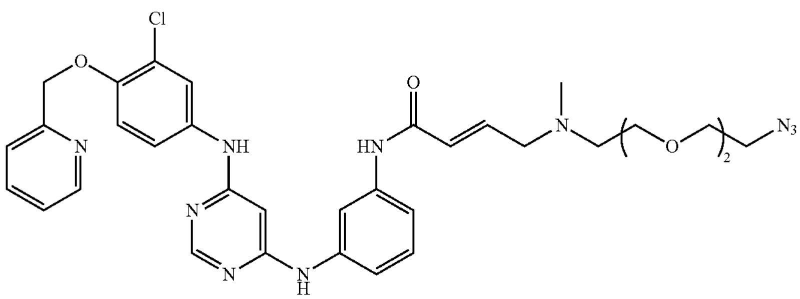
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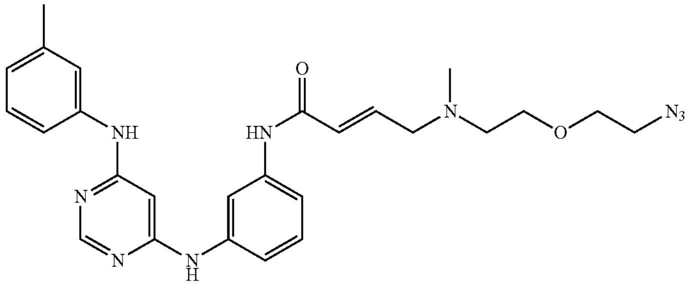
2
N3
N
N
N
H


NH
O
HN
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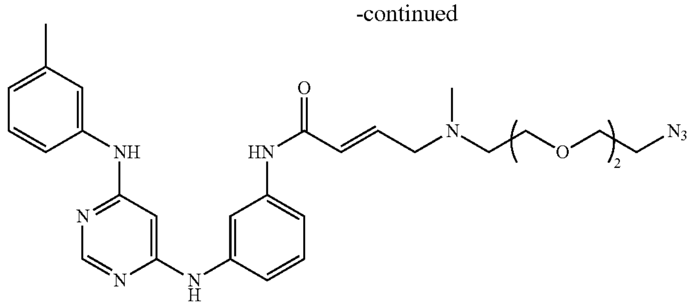
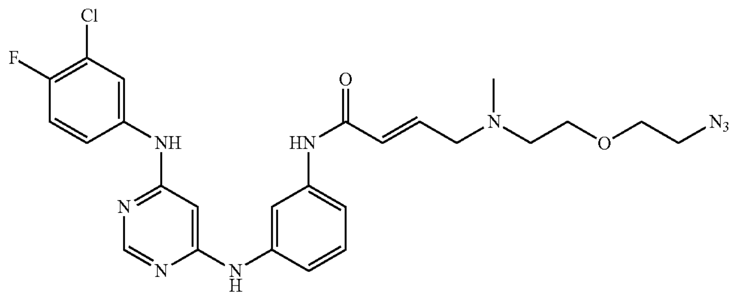
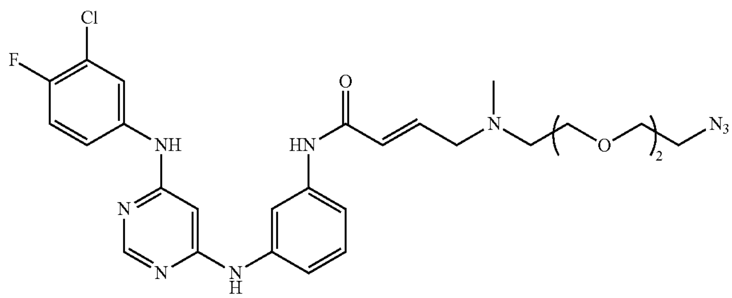
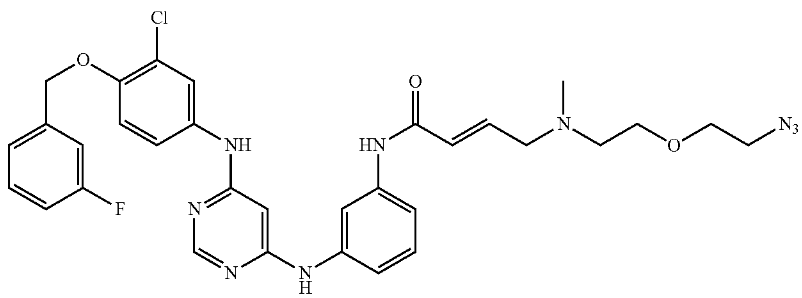
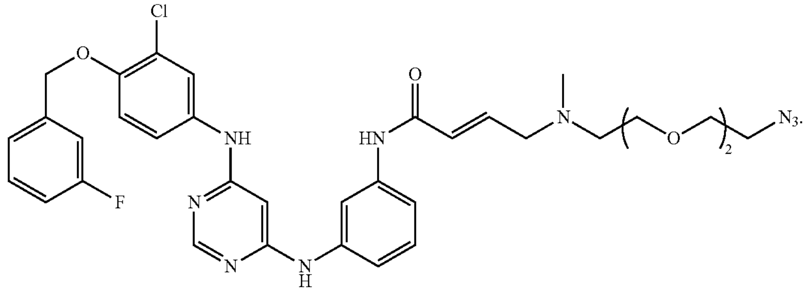


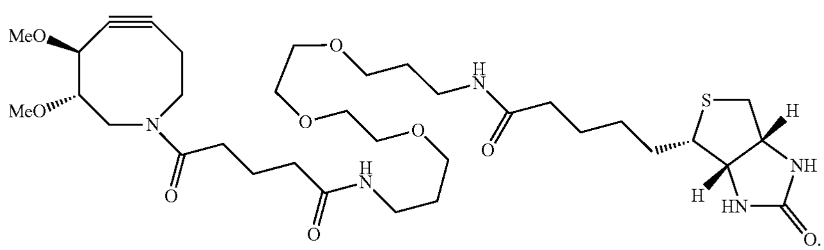




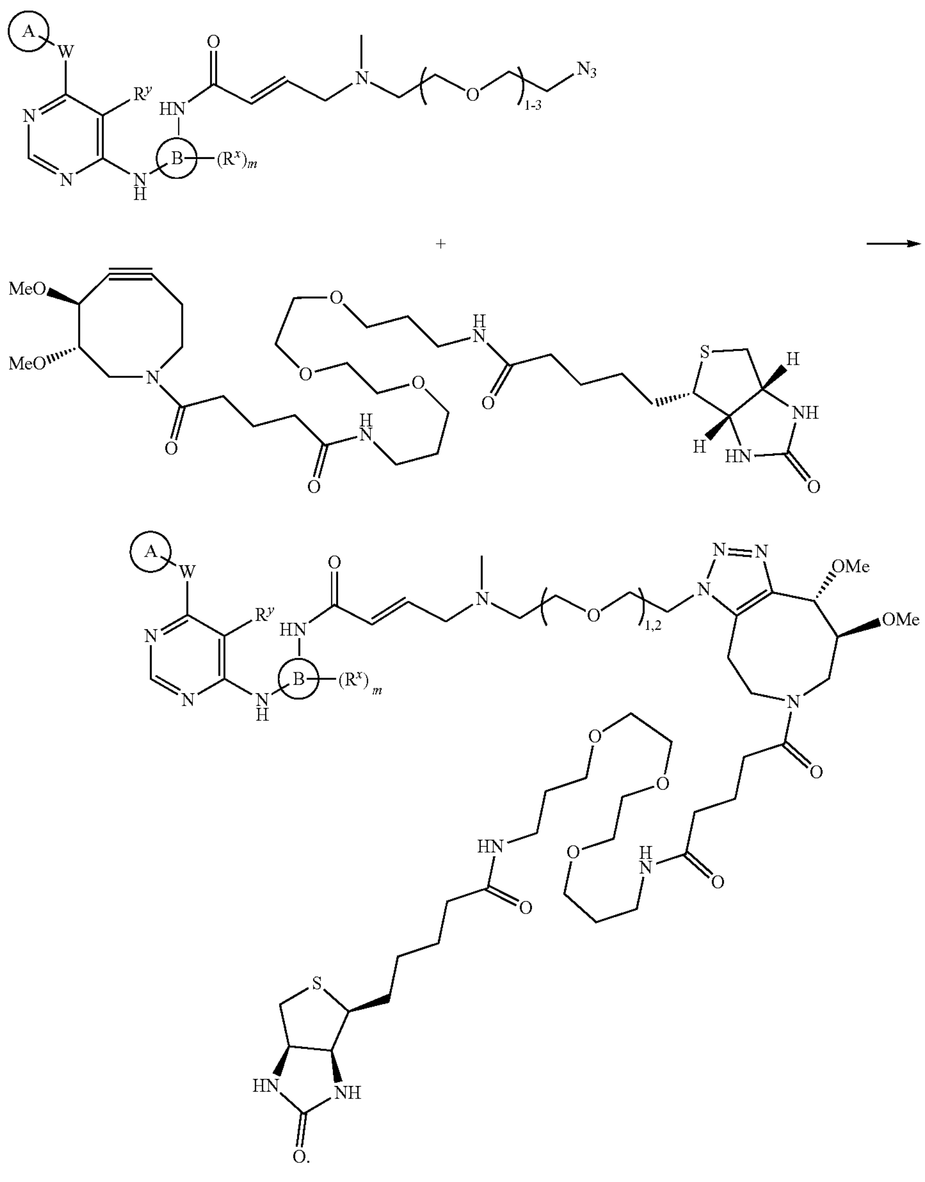




[0468] In some embodiments, the click ready -T-R^t moiety is:



[0469] An exemplary reaction in which a click ready inhibitor moiety and a click ready -T-R^t moiety are joined through a [2+3]-cycloaddition is as follows:



[0470] In some embodiments, the detectable moiety, R^t, is selected from a label, a dye, a photocrosslinker, a cytotoxic compound, a drug, an affinity label, a photoaffinity label, a reactive compound, an antibody or antibody fragment, a biomaterial, a nanoparticle, a spin label, a fluorophore, a metal-containing moiety, a radioactive moiety, quantum dot(s), a novel functional group, a group that covalently or noncovalently interacts with other molecules, a photocaged moiety, an actinic radiation excitable moiety, a ligand, a photoisomerizable moiety, biotin, a biotin analog (e.g., biotin sulfoxide), a moiety incorporating a heavy atom, a chemically cleavable group, a photocleavable group, a redox-active agent, an isotopically labeled moiety, a biophysical probe, a phosphorescent group, a chemiluminescent group, an electron dense group, a magnetic group, an intercalating group, a chromophore, an energy transfer agent, a biologically active agent, a detectable label, or a combination thereof.

[0471] In some embodiments, R^t is biotin or an analog thereof. In certain embodiments, R^t is biotin. In some embodiments, R^t is biotin sulfoxide.

[0472] In another embodiment, R^t is a fluorophore. In a further embodiment, the fluorophore is selected from Alexa Fluor dyes (Alexa Fluor 350, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660 and Alexa Fluor 680), AMCA, AMCA-S, BODIPY dyes (BODIPY FL, BODIPY R6G, BODIPY TMR, BODIPY TR, BODIPY 493/503, BODIPY 530/550, BODIPY 558/568, BODIPY 564/570, BODIPY 576/589, BODIPY 581/591, BODIPY 630/650, BODIPY 650/665), Carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), Cascade Blue, Cascade Yellow, Coumarin 343, Cyanine dyes (Cy3, Cy5, Cy3.5, Cy5.5), Dansyl, Dapoxyl, Dialkylaminocoumarin, 4',5'-Dichloro-2',7'-dimethoxy-fluorescein, DM-NERF, Eosin, Erythrosin, Fluorescein, FAM, Hydroxycoumarin, IRDyes (IRD40, IRD 700, IRD 800), JOE, Lissamine rhodamine B, Marina Blue, Methoxycoumarin, Naphthofluorescein, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, PyMPO, Pyrene, Rhodamine B, Rhodamine 6G, Rhodamine Green, Rhodamine Red, Rhodol Green, 2',4',5',7'-Tetra-bromosulfone-fluorescein, Tetramethyl-rhodamine (TMR), Carboxytetramethylrhodamine (TAMRA), Texas Red, Texas Red-X, 5(6)-Carboxyfluorescein, 2,7-Dichlorofluorescein, N,N-Bis(2,4,6-trimethylphenyl)-3,4:9,10-perylenebis(dicarboximide, HPTS, Ethyl Eosin, DY-490XL MegaStokes, DY-485XL MegaStokes, Adirondack Green 520, ATTO 465, ATTO 488, ATTO 495, YOYO-1,5-FAM, BCECF, dichlorofluorescein, rhodamine 110, rhodamine 123, YO-PRO-1, SYTOX Green, Sodium Green, SYBR Green I, Alexa Fluor 500, FITC, Fluo-3, Fluo-4, fluoro-emerald, YoYo-1 ssDNA, YoYo-1 dsDNA, YoYo-1, SYTO RNASelect, Diversa Green-FP, Dragon Green, EvaGreen, Surf Green EX, Spectrum Green, NeuroTrace 500525, NBD-X, MitoTracker Green FM, LysoTracker Green DND-26, CBQCA, PA-GFP (post-activation), WEGFP (post-activation), FLASH-CCXXCC, Azami Green monomeric, Azami Green, green fluorescent protein (GFP), EGFP (Campbell Tsien 2003), EGFP (Patterson 2001), Kaede Green, 7-Benzylamino-4-Nitrobenz-2-Oxa-1,3-Diazole, Bex1, Doxorubicin, Lumio Green, or SuperGlo GFP.

[0473] As described generally above, a provided probe compound comprises a tethering moiety, -T-, that attaches the irreversible inhibitor to the detectable moiety. As used herein, the term "tether" or "tethering moiety" refers to any bivalent chemical spacer including, but not limited to, a covalent bond, a polymer, a water soluble polymer, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted heterocycloalkyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted heterocycloalkylalkyl, optionally substituted heterocycloalkylalkenyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted heterocycloalkylalkenylalkyl, an optionally substituted amide moiety, an ether moiety, an ketone moiety, an ester moiety, an optionally substituted carbamate moiety, an optionally substituted hydrazone moiety, an optionally substituted hydrazine moiety, an optionally substituted oxime moiety, a disulfide moiety, an optionally substituted imine moiety, an optionally substituted sulfonamide moiety, a sulfone moiety, a sulfoxide moiety, a thioether moiety, or any combination thereof.

[0474] In some embodiments, the tethering moiety, -T-, is selected from a covalent bond, a polymer, a water soluble polymer, optionally substituted alkyl, optionally substituted heteroalkyl, optionally substituted heterocycloalkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkylalkyl, optionally substituted heterocycloalkylalkenyl, optionally substituted aryl, optionally substituted heteroaryl, and optionally substituted heterocycloalkylalkenylalkyl. In some embodiments, the tethering moiety is an optionally substituted heterocycle. In other embodiments, the heterocycle is selected from aziridine, oxirane, episulfide, azetidine, oxetane, pyrroline, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, pyrazole, pyrrole, imidazole, triazole, tetrazole, oxazole, isoxazole, oxirene, thiazole, isothiazole, dithiolane, furan, thiophene, piperidine, tetrahydropyran, thiane, pyridine, pyran, thiopyrane, pyridazine, pyrimidine, pyrazine, piperazine, oxazine, thiazine, dithiane, and dioxane. In some embodiments, the heterocycle is piperazine. In further embodiments, the tethering moiety is optionally substituted with halogen, —CN, —OH, $—NO_2$, alkyl, S(O), and $S(O)_2$. In other embodiments, the water soluble polymer is a PEG group.

[0475] In other embodiments, the tethering moiety provides sufficient spatial separation between the detectable moiety and the protein kinase inhibitor moiety. In further embodiments, the tethering moiety is stable. In yet a further embodiment, the tethering moiety does not substantially affect the response of the detectable moiety. In other embodiments, the tethering moiety provides chemical stability to the probe compound. In further embodiments, the tethering moiety provides sufficient solubility to the probe compound.

[0476] In some embodiments, a tethering moiety, -T-, such as a water soluble polymer is coupled at one end to a provided irreversible inhibitor and to a detectable moiety, le, at the other end. In other embodiments, a water soluble polymer is coupled via a functional group or substituent of the provided irreversible inhibitor. In further embodiments, a water soluble polymer is coupled via a functional group or substituent of the reporter moiety.

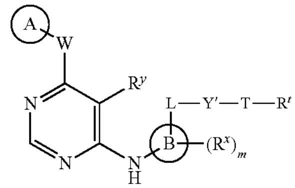
[0477] In some embodiments, examples of hydrophilic polymers, for use in tethering moiety -T-, include, but are not limited to: polyalkyl ethers and alkoxy-capped analogs thereof (e.g., polyoxyethylene glycol, polyoxyethylene/propylene glycol, and methoxy or ethoxy-capped analogs thereof, polyoxyethylene glycol, the latter is also known as polyethylene glycol or PEG); polyvinylpyrrolidones; polyvinylalkyl ethers; polyoxazolines, polyalkyl oxazolines and polyhydroxyalkyl oxazolines; polyacrylamides, polyalkyl acrylamides, and polyhydroxyalkyl acrylamides (e.g., polyhydroxypropylmethacrylamide and derivatives thereof); polyhydroxyalkyl acrylates; polysialic acids and analogs thereof, hydrophilic peptide sequences; polysaccharides and their derivatives, including dextran and dextran derivatives, e.g., carboxymethyldextran, dextran sulfates, aminodextran; cellulose and its derivatives, e.g., carboxymethyl cellulose, hydroxyalkyl celluloses; chitin and its derivatives, e.g., chitosan, succinyl chitosan, carboxymethylchitin, carboxymethylchitosan; hyaluronic acid and its derivatives; starches; alginates; chondroitin sulfate; albumin; pullulan and carboxymethyl pullulan; polyaminoacids and derivatives thereof, e.g., polyglutamic acids, polylysines, polyaspartic acids, polyaspartamides; maleic anhydride copolymers such as: styrene maleic anhydride copolymer, divinylmethyl ether maleic anhydride copolymer; polyvinyl alcohols; copolymers thereof, terpolymers thereof, mixtures thereof, and derivatives of the foregoing. In other embodiments, a water soluble polymer is any structural form including but not limited to linear, forked or branched. In further embodiments, multifunctional polymer derivatives include, but are not limited to, linear polymers having two termini, each terminus being bonded to a functional group which is the same or different.

[0478] In some embodiments, a water polymer comprises a poly(ethylene glycol) moiety. In further embodiments, the molecular weight of the polymer is of a wide range, including but not limited to, between about 100 Da and about 100,000 Da or more. In yet further embodiments, the molecular weight of the polymer is between about 100 Da and about 100,000 Da, including but not limited to, about 100,000 Da, about 95,000 Da, about 90,000 Da, about 85,000 Da, about 80,000 Da, about 75,000 Da, about 70,000 Da, about 65,000 Da, about 60,000 Da, about 55,000 Da, about 50,000 Da, about 45,000 Da, about 40,000 Da, about 35,000 Da, 30,000 Da, about 25,000 Da, about 20,000 Da, about 15,000 Da, about 10,000 Da, about 9,000 Da, about 8,000 Da, about 7,000 Da, about 6,000 Da, about 5,000 Da, about 4,000 Da, about 3,000 Da, about 2,000 Da, about 1,000 Da, about 900 Da, about 800 Da, about 700 Da, about 600 Da, about 500 Da, about 400 Da, about 300 Da, about 200 Da, and about 100 Da. In some embodiments, the molecular weight of the polymer is between about 100 Da and 50,000 Da. In some embodiments, the molecular weight of the polymer is between about 100 Da and 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 1,000 Da and 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 5,000 Da and 40,000 Da. In some embodiments, the molecular weight of the polymer is between about 10,000 Da and 40,000 Da. In some embodiments, the poly(ethylene glycol) molecule is a branched polymer. In further embodiments, the molecular weight of the branched chain PEG is between about 1,000 Da and about 100,000 Da, including but not limited to, about 100,000 Da, about 95,000 Da, about 90,000 Da, about 85,000 Da, about 80,000 Da, about 75,000 Da, about 70,000 Da, about 65,000 Da, about 60,000 Da, about 55,000 Da, about 50,000 Da, about 45,000 Da, about 40,000 Da, about 35,000 Da, about 30,000 Da, about 25,000 Da, about 20,000 Da, about 15,000 Da, about 10,000 Da, about 9,000 Da, about 8,000 Da, about 7,000 Da, about 6,000 Da, about 5,000 Da, about 4,000 Da, about 3,000 Da, about 2,000 Da, and about 1,000 Da. In some embodiments, the molecular weight of a branched chain PEG is between about 1,000 Da and about 50,000 Da. In some embodiments, the molecular weight of a branched chain PEG is between about 1,000 Da and about 40,000 Da. In some embodiments, the molecular weight of a branched chain PEG is between about 5,000 Da and about 40,000 Da. In some embodiments, the molecular weight of a branched chain PEG is between about 5,000 Da and about 20,000 Da. The foregoing list for substantially water soluble backbones is by no means exhaustive and is merely illustrative, and in some embodiments, polymeric materials having the qualities described above are suitable for use in methods and compositions described herein.

[0479] One of ordinary skill in the art will appreciate that when -T-R^t is attached to a compound of formula I-a or I-b via the R^1 warhead group, then the resulting tethering moiety comprises the R^1 warhead group. As used herein, the phrase "comprises a warhead group" means that the tethering moiety formed by —$R^{1'}$-T- of formula V-a or V-b is either substituted with a warhead group or has such a warhead group incorporated within the tethering moiety. For example, the tethering moiety formed by —$R^{1'}$-T- may be substituted with an -L-Y warhead group, wherein such groups are as described herein. Alternatively, the tethering moiety formed by —$R^{1'}$-T- has the appropriate features of a warhead group incorporated within the tethering moiety. For example, the tethering moiety formed by —$R^{1'}$-T- may include one or more units of unsaturation and optional substituents and/or heteroatoms which, in combination, result in a moiety that is capable of covalently modifying a protein kinase in accordance with the present invention. Such —R^1-T- tethering moiety are depicted below.

[0480] In some embodiments, a methylene unit of an —$R^{1'}$-T-tethering moiety is replaced by a bivalent -L-Y'— moiety to provide a compound of formula V-c:

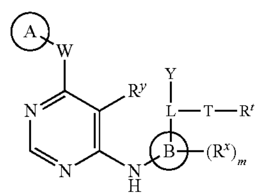
V-c



wherein each of Ring A, Ring B, m, p, R^x, R^y, R^v, W^1, W^2, T, L, Y', and R^t is as defined above and described in classes and subclasses herein and Y' is a bivalent version of the Y group defined above and described in classes and subclasses herein.

[0481] In some embodiments, a methylene unit of an —$R^{1'}$-T- tethering moiety is replaced by an -L(Y)— moiety to provide a compound of formula V-d:

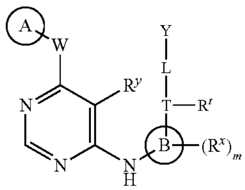
V-d



wherein each of Ring A, Ring B, m, p, R^x, R^y, R^v, W^1, W^2, T, L, Y, and R^t is as defined above and described in classes and subclasses herein.

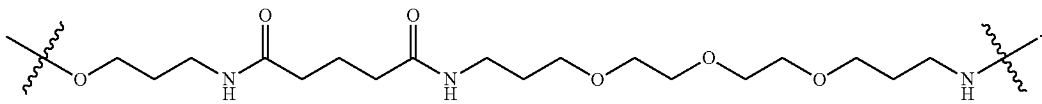
[0482] In some embodiments, a tethering moiety is substituted with an L-Y moiety to provide a compound of formula V-e:

V-e

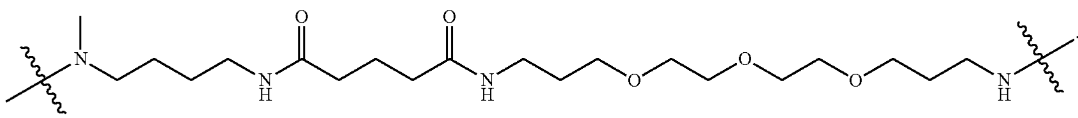


wherein each of Ring A, Ring B, m, p, R^x, R^y, R^v, W^1, W^2, T, L, Y, and R^t is as defined above and described in classes and subclasses herein.

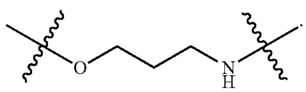
[0483] In certain embodiments, the tethering moiety, -T-, has the following structure:



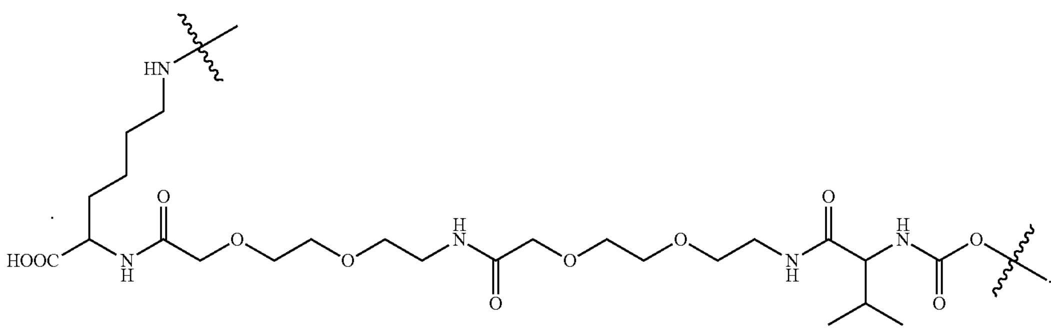
[0484] In some embodiments, the tethering moiety, -T-, has the following structure:



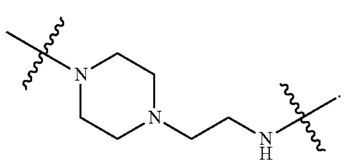
[0485] In other embodiments, the tethering moiety, -T-, has the following structure:



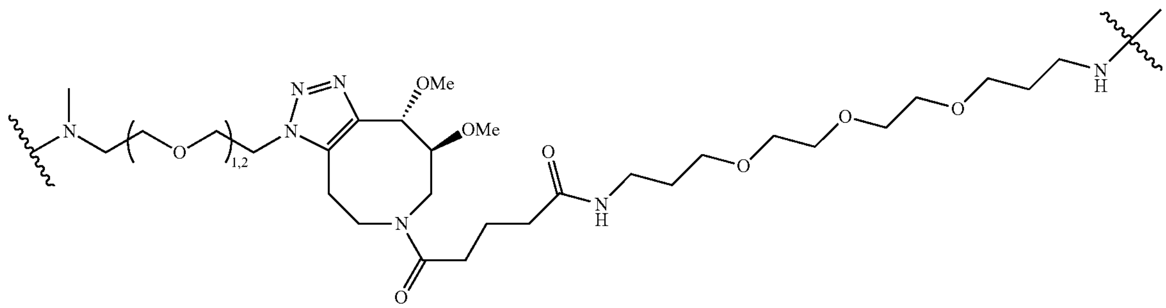
[0486] In certain other embodiments, the tethering moiety, -T-, has the following structure:



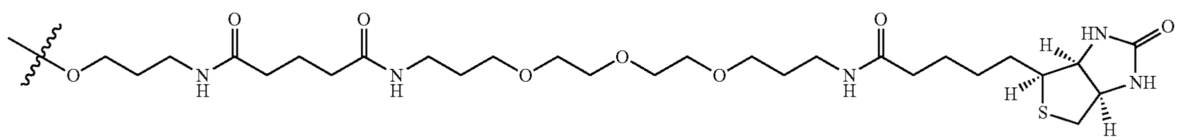
[0487] In yet other embodiments, the tethering moiety, -T-, has the following structure:



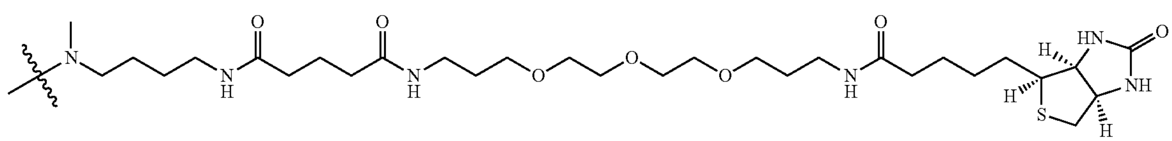
[0488] In some embodiments, the tethering moiety, -T-, has the following structure:



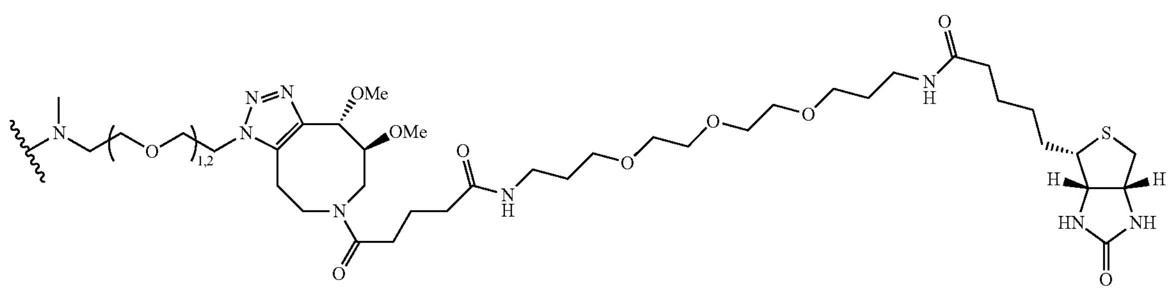
[0489] In some embodiments, -T-R' is of the following structure:



[0490] In other embodiments, -T-R' is of the following structure:



[0491] In certain embodiments, -T-R' is of the following structure:



[0492] In some embodiments, a probe compound of formula V, VI, or VII is derived from any compound of Table 5.

[0493] In certain embodiments, the probe compound is one of the following structures:

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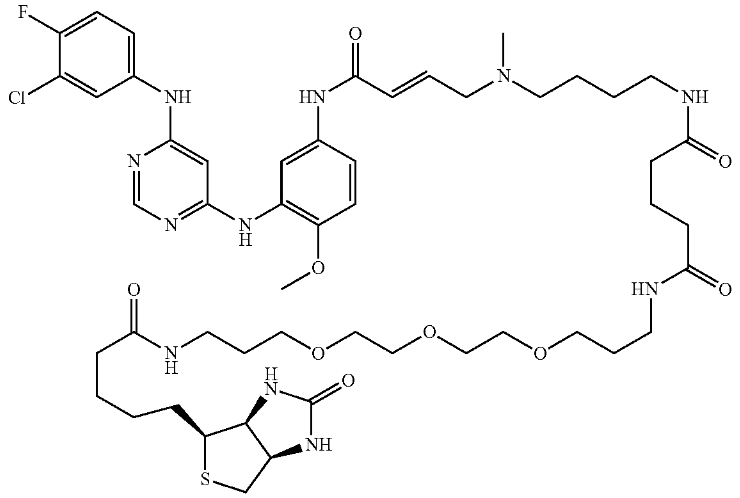

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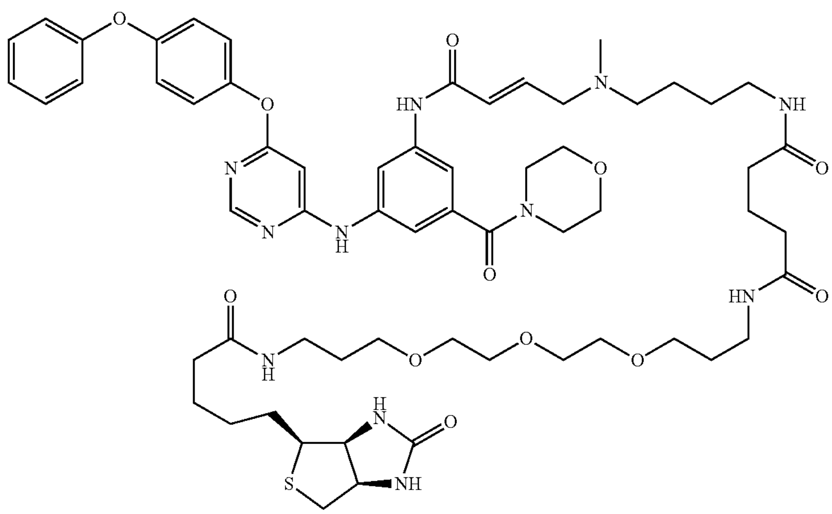

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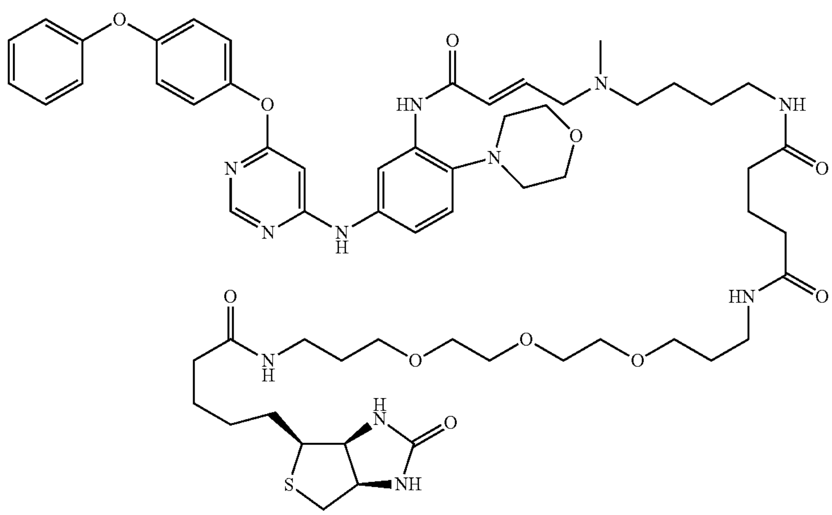

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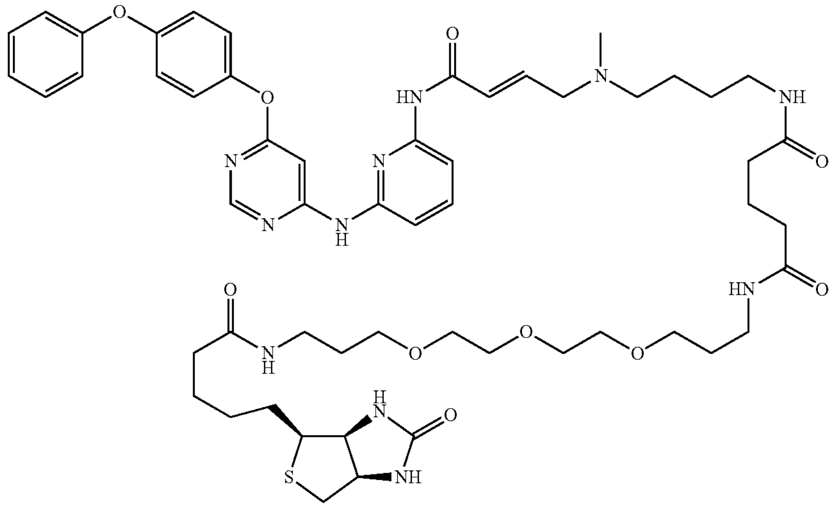

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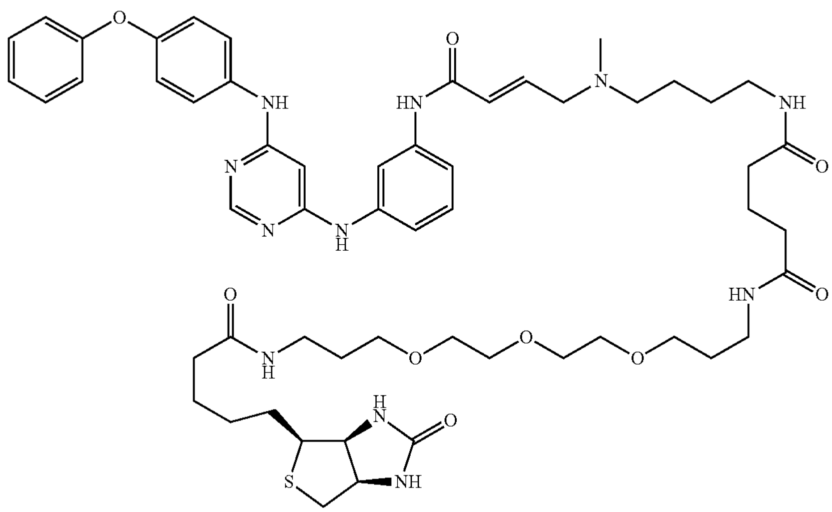

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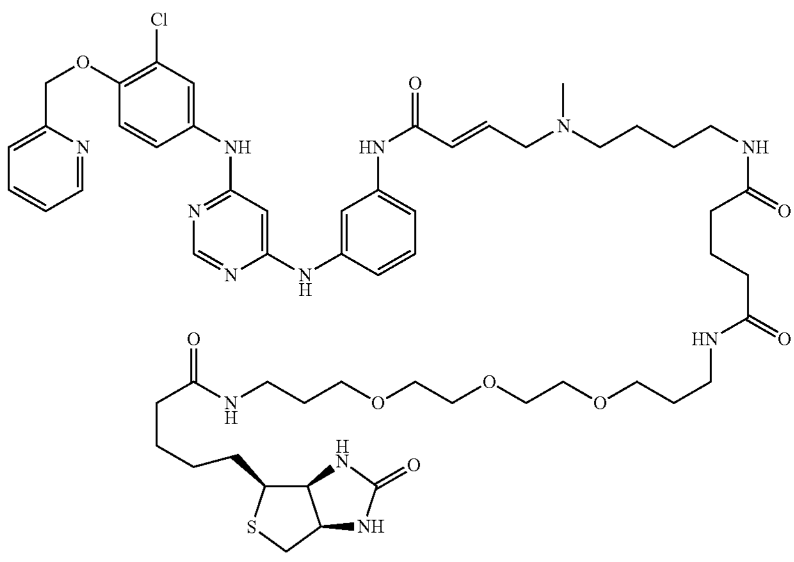

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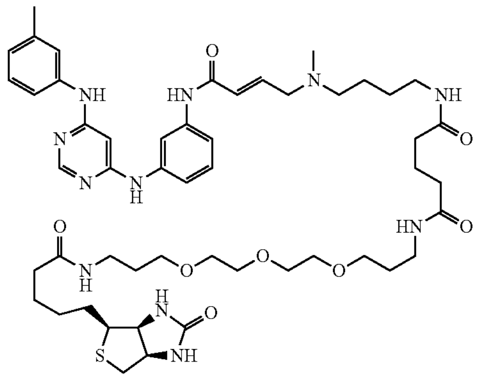

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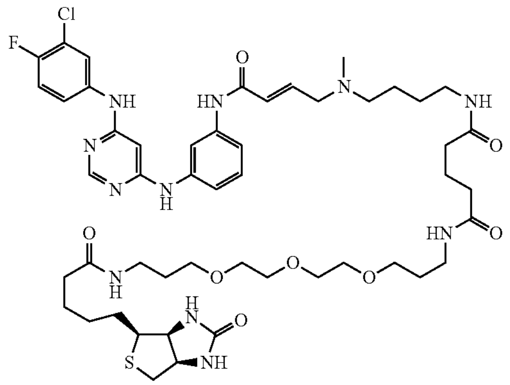

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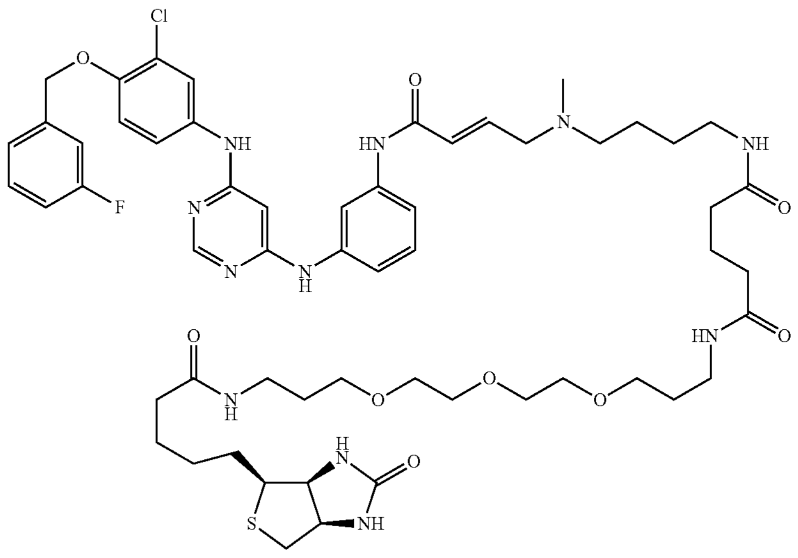

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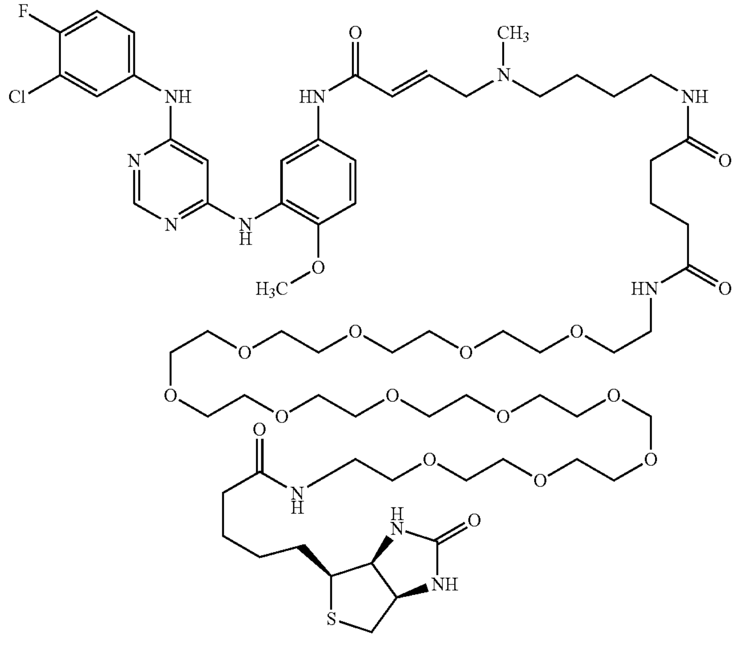

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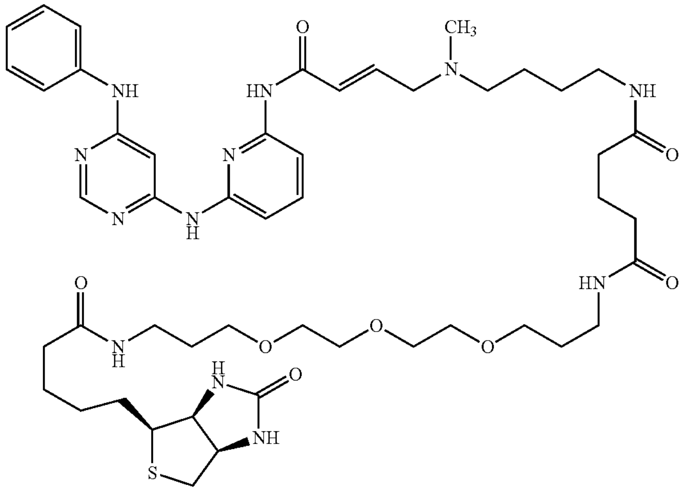

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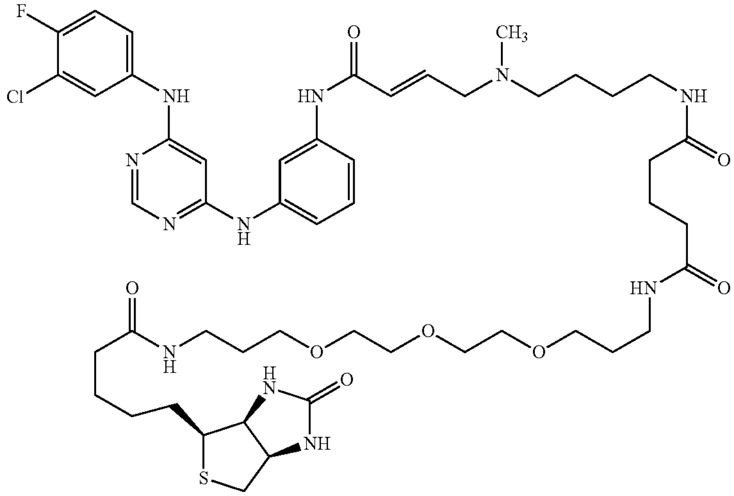

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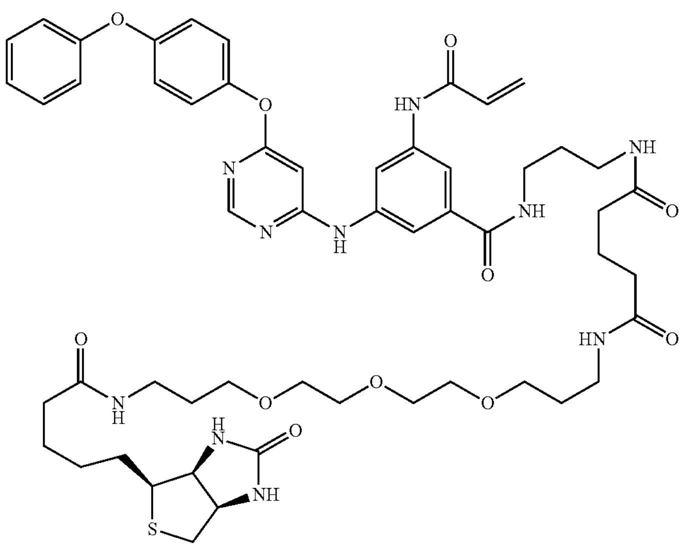

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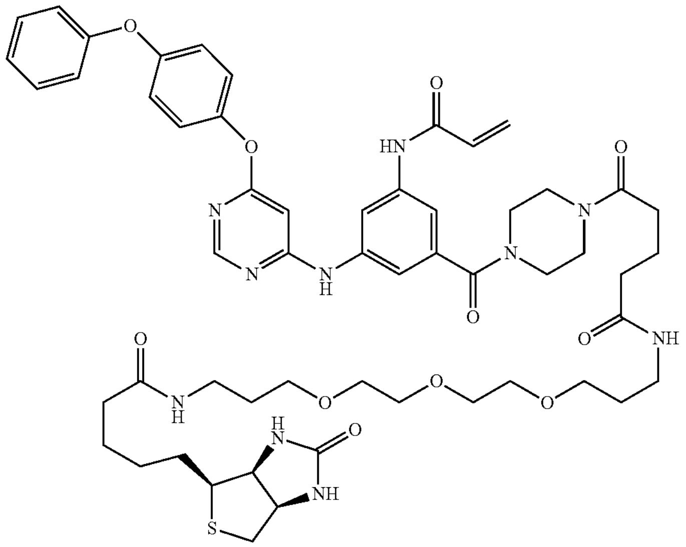

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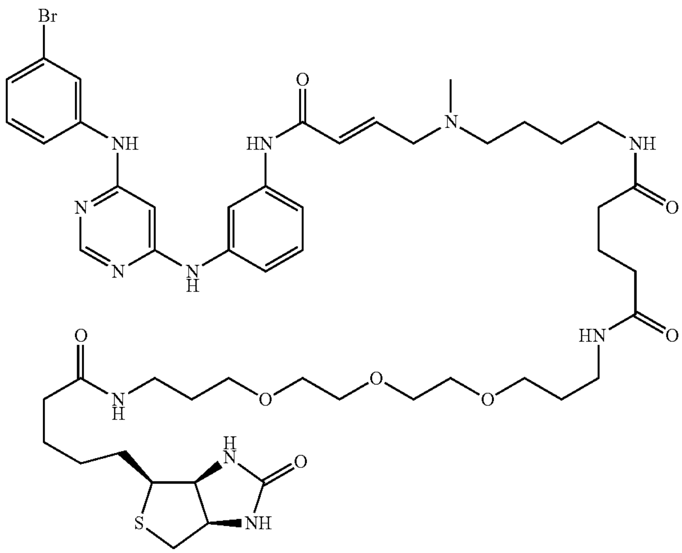

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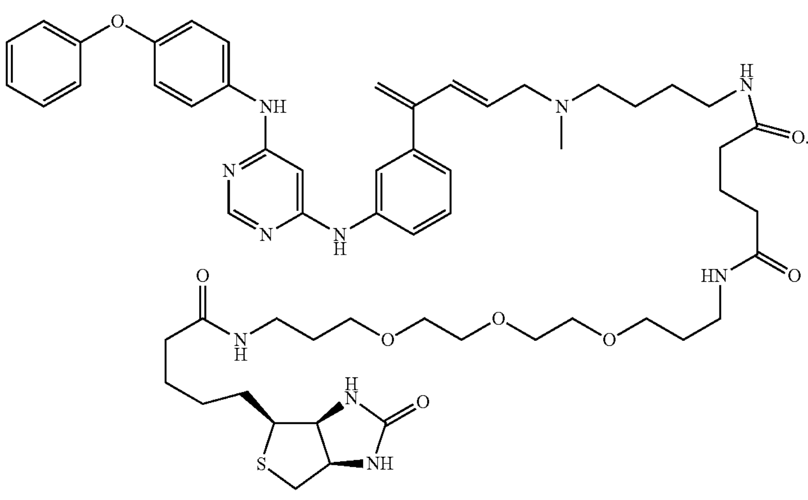


[0494] It will be appreciated that many -T-R^t reagents are commercially available. For example, numerous biotinylating reagents are available from, e.g., Thermo Scientific having varying tether lengths. Such reagents include NHS-PEG_4-Biotin and NHS-PEG_{12}-Biotin.

[0495] In some embodiments, analogous probe structures to the ones exemplified above are prepared using click-ready inhibitor moieties and click-ready -T-R^t moieties, as described herein.

[0496] In some embodiments, a provided probe compound covalently modifies a phosphorylated conformation of a protein kinase. In one aspect, the phosphorylated conformation of the protein kinase is either an active or inactive form of the protein kinase. In certain embodiments, the phosphorylated conformation of the protein kinase is an active form of said kinase. In certain embodiments, the probe compound is cell permeable.

[0497] In some embodiments, the present invention provides a method for determining occupancy of a protein kinase by a provided irreversible inhibitor (i.e., a compound of formula I) in a patient, comprising providing one or more tissues, cell types, or a lysate thereof, obtained from a patient administered at least one dose of a compound of said irreversible inhibitor, contacting said tissue, cell type or lysate thereof with a probe compound (i.e., a compound of formula V, VI, or VII) to covalent modify at least one protein kinase present in said lysate, and measuring the amount of said protein kinase covalently modified by the probe compound to determine occupancy of said protein kinase by said compound of formula I as compared to occupancy of said protein kinase by said probe compound. In certain embodiments, the method further comprises the step of adjusting the dose of the compound of formula I to increase occupancy of the protein kinase. In certain other embodiments, the method further comprises the step of adjusting the dose of the compound of formula I to decrease occupancy of the protein kinase.

[0498] As used herein, the terms "occupancy" or "occupy" refer to the extent to which a protein kinase is modified by a provided covalent inhibitor compound. One of ordinary skill in the art would appreciate that it is desirable to administer the lowest dose possible to achieve the desired efficacious occupancy of the protein kinase.

[0499] In some embodiments, the protein kinase to be modified is BTK. In other embodiments, the protein kinase to be modified is EGFR. In certain embodiments, the protein kinase is JAK. In certain other embodiments, the protein kinase is one or more of ErbB1, ErbB2, ErbB3, or ErbB4. In yet other embodiments, the protein kinase is TEK, ITK, or BMX.

[0500] In some embodiments, the probe compound comprises the irreversible inhibitor for which occupancy is being determined.

[0501] In some embodiments, the present invention provides a method for assessing the efficacy of a provided irreversible inhibitor in a mammal, comprising administering a provided irreversible inhibitor to the mammal, administering a provided probe compound to tissues or cells isolated from the mammal, or a lysate thereof, measuring the activity of the detectable moiety of the probe compound, and comparing the activity of the detectable moiety to a standard.

[0502] In other embodiments, the present invention provides a method for assessing the pharmacodynamics of a provided irreversible inhibitor in a mammal, comprising administering a provided irreversible inhibitor to the mammal, administering a probe compound presented herein to one or more cell types, or a lysate thereof, isolated from the mammal, and measuring the activity of the detectable moiety of the probe compound at different time points following the administration of the inhibitor.

[0503] In yet other embodiments, the present invention provides a method for in vitro labeling of a protein kinase comprising contacting said protein kinase with a probe compound described herein. In one embodiment, the contacting step comprises incubating the protein kinase with a probe compound presented herein.

[0504] In certain embodiments, the present invention provides a method for in vitro labeling of a protein kinase comprising contacting one or more cells or tissues, or a lysate thereof, expressing the protein kinase with a probe compound described herein.

[0505] In certain other embodiments, the present invention provides a method for detecting a labeled protein kinase comprising separating proteins, the proteins comprising a protein kinase labeled by probe compound described herein, by electrophoresis and detecting the probe compound by fluorescence.

[0506] In some embodiments, the present invention provides a method for assessing the pharmacodynamics of a provided irreversible inhibitor in vitro, comprising incubating the provided irreversible inhibitor with the target protein kinase, adding the probe compound presented herein to the target protein kinase, and determining the amount of target modified by the probe compound.

[0507] In certain embodiments, the probe compound is detected by binding to avidin, streptavidin, neutravidin, or captavidin.

[0508] In some embodiments, the probe is detected by Western blot. In other embodiments, the probe is detected by ELISA. In certain embodiments, the probe is detected by flow cytometry.

[0509] In other embodiments, the present invention provides a method for probing the kinome with irreversible inhibitors comprising incubating one or more cell types, or a lysate thereof, with a biotinylated probe compound to generate proteins modified with a biotin moiety, digesting the proteins, capturing with avidin or an analog thereof, and performing multi-dimensional LC-MS-MS to identify protein kinases modified by the probe compound and the adduction sites of said kinases.

[0510] In certain embodiments, the present invention provides a method for measuring protein synthesis in cells comprising incubating cells with an irreversible inhibitor of the target protein, forming lysates of the cells at specific time points, and incubating said cell lysates with an inventive probe compound to measure the appearance of free protein over an extended period of time.

[0511] In other embodiments, the present invention provides a method for determining a dosing schedule in a mammal for maximizing occupancy of a target protein kinase comprising assaying a one or more cell types, or a lysate thereof, isolated from the mammal, (derived from, e.g., splenocytes, peripheral B cells, whole blood, lymph nodes, intestinal tissue, or other tissues) from a mammal administered a provided irreversible inhibitor of formula I, wherein the assaying step comprises contacting said one or more tissues, cell types, or a lysate thereof, with a provided probe compound and measuring the amount of protein kinase covalently modified by the probe compound.

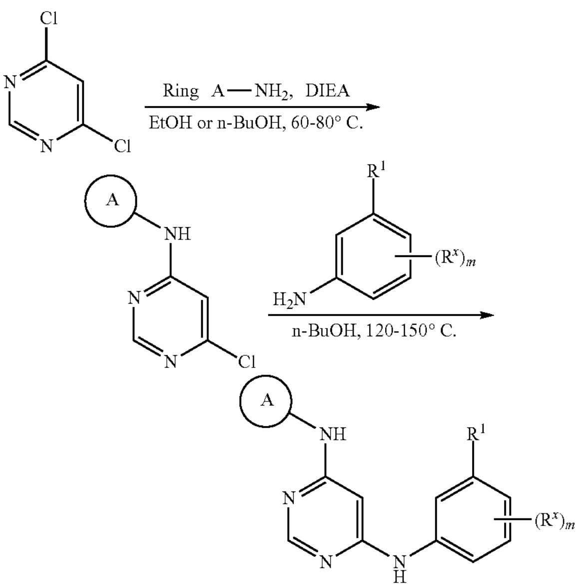
EXEMPLIFICATION

[0512] As depicted in the Examples below, in certain exemplary embodiments, compounds are prepared according to the following general procedures. It will be appreciated that, although the general methods depict the synthesis of certain compounds of the present invention, the following general methods, and other methods known to one of ordinary skill in the art, can be applied to all compounds and subclasses and species of each of these compounds, as described herein.

Example 1

[0513]

SCHEME 1a Synthesis of Compounds of Formula II-a



Synthesis of (6-chloro-pyrimidin-4-yl)-(3-bromo-phenyl)-amine

[0514] A solution of 4,6-dichloropyrimidine (5 g, 33.6 mmol), 3-bromoaniline (5.8 g, 33.7 mmol) and N,N-diisopropylethylamine (DIEA) (5.2 g, 40.2 mmol) in ethanol (40 mL) was heated at 80° C. for 16 hr. The reaction mixture was cooled to ambient temperature, diethylether (35 mL) was added while the mixture was being stirred. The product was precipitated, filtered, washed with water and dried to afford 5.9 g (62% yield) of a light colored solid. MS (m/z): MH^+=284, 286, 288.

Synthesis of 3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenylamine

[0515] A mixture of (6-chloro-pyrimidin-4-yl)-(3-bromo-phenyl)-amine (300 mg, 1.1 mmol) and benzene-1,3-diamine (300 mg, 2.75 mmol) in 3 mL of n-BuOH was heated in a sealed tube to 150° C. for 16 hr. Solvent was removed by vacuum evaporation and the crude product was purified by flash chromatography on silica gel with EtOAc/DCM solvent system to afford 255 mg (65% yield) of the title compound as a yellow solid. MS (m/z): MH^+=356, 358.

[0516] The following compounds were prepared in a manner substantially similar to that described in Scheme 1a and the Examples above:

[0517] (a) 3-Bromoaniline and benzene-1,4-diamine gave 4-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]phenylamine: MS (m/z): MH^+=356, 358.

[0518] (b) 3-Chloro-4-fluoroaniline and benzene-1,3-diamine gave 3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4). MS (m/z): MH^+=330, 332.

[0519] (c) 3-Methylaniline and benzene-1,3-diamine gave 3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-5). MS (m/z): MH^+=292.

[0520] (d) 3-Chloro-4-(3-fluorophenyl)methoxyaniline and benzene-1,3-diamine gave 3-{6-[3-chloro-4-(3-fluorophenyl)methoxyphenylamino]-pyrimidin-4-ylamino}phenylamine (I^R-6). MS (m/z): MH^+=436, 438.

[0521] (e) 3-Bromoaniline and 3-ethynylaniline gave N^4-(3-Bromophenyl)-N^6-(3-ethynylphenyl)pyrimidine-4,6-diamine (I-12). MS ($M+H^+$) 365, 367; ^1H NMR (400 MHz, d^6-DMSO) δ 9.73 (s, 1H), 9.60 (s, 1H), 8.67 (s, 1H), 7.95 (t, 1H), 7.76 (s, 1H), 7.50 (d, 1H), 7.46 (d, 1H), 7.55 (t, 1H), 7.40 (t, 1H), 7.20 (d, 1H), 7.10 (d, 1H), 6.30 (s, 1H), 4.25 (s, 1H) ppm.

[0522] (f) 3-Ethynylaniline gave N^4,N^6-bis(3-ethynylphenyl)pyrimidine-4,6-diamine (I-11). MS ($M+H^+$) 311, 312; ^1H NMR (400 MHz, d^6-DMSO) δ 9.24 (s, 2H), 8.32 (s, 1H), 7.78 (s, 2H), 7.52 (d, 2H), 7.25 (t, 2H), 7.02 (d, 2H), 6.13 (s, 1H), 4.12 (s, 2H) ppm.

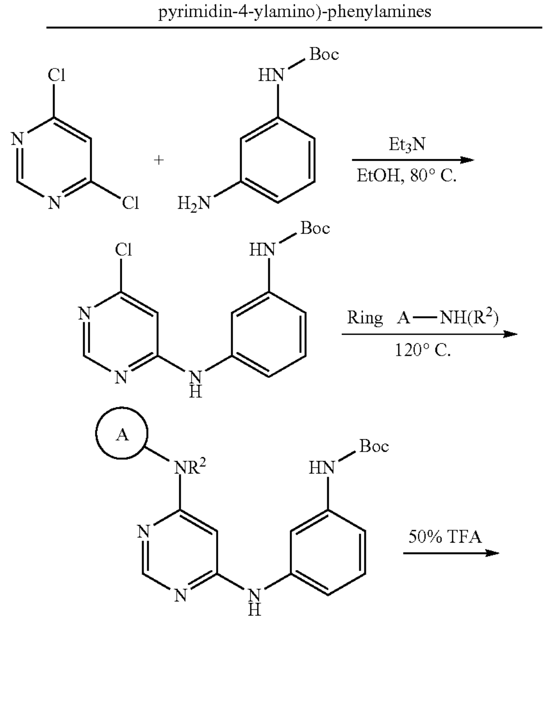
[0523] (g) 4-Phenoxyaniline and 2,6-diaminopyridine gave 2-[6-(4-phenoxyphenyl)-aminopyrimidin-4-yl]amino-6-aminopyridine (I^R-11) MS (m/z): MH^+=372, 371.

[0524] (h) 4-Phenoxyaniline and 1,4-diaminobenzene gave 4-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl]amino-aminobenzene (I^R-14) MS (m/z): MH^+=370

Example 2

[0525]

SCHEME 2a
Sequence A for Synthesis of 3-(6-mono- or disubstituted-amino) pyrimidin-4-ylamino)-phenylamines



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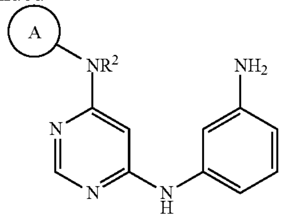


[0526] Although a BOC protecting group is depicted in Scheme 2a above and the ensuing schemes below, one of ordinary skill in the art will recognize that other amine protecting groups are amenable for use in preparing compounds of the present invention. Accordingly, a variety of amine protecting groups is contemplated.

Synthesis of tert-butyl 3-(6-chloropyrimidin-4-ylamino)phenylcarbamate

[0527] 4,6-Dichloropyrimidine (1.5 g, 10 mmol), tert-butyl 3-aminophenylcarbamate (2.1 g, 10 mmol) and triethylamine (2.2 g, 20 mmol) were mixed in ethanol (20 mL), heated at 80° C. for 16 hr. The reaction mixture was cooled to RT and solvent was removed in vacuo. The gummy crude product was mixed with 20 mL of DCM and was stirred at RT to give an off-white solid, which was filtered and dried under vacuum (1.6 g, 5 mmol). MS (m/z): MH^+=321, 323.

Synthesis of tert-butyl 3-(6-[N-methyl-N-phenylamino]pyrimidin-4-ylamino)phenyl-carbamate

[0528] A mixture of tert-butyl 3-(6-chloropyrimidin-4-ylamino)phenylcarbamate (1.6 g, 5 mmol) and N-methylaniline (1.07 g, 10 mmol) was heated at 120° C. in a sealed tube for 2 hr. The reaction mixture was cooled to RT, mixed with 1 mL of 1N NaOH and 5 mL of DCM, stirred for 30 min, the product was filtered and dried under vacuum to give a solid product. MS (m/z): MH^+=392.

Synthesis of 3-[6-(N-methyl-N-phenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-8)

[0529] A mixture of tert-butyl 3-(6-[N-methyl-N-phenylamino]pyrimidin-4-ylamino)phenylcarbamate (1.17 g, 3 mmol) and TFA (50% in DCM, 10 mL) was stirred at RT for 4 hr. The reaction solvent was removed under vacuum. The crude product was mixed with 1 mL of 2N NaOH and 5 mL of EtOAc, was stirred for 30 min, was filtered, and was dried under vacuum to give 0.65 g (75%) of the title compound, an off-white solid. (I^R-8) MS (m/z): MH^+=292.

[0530] The following compounds were prepared in a manner substantially similar to that described in Scheme 2a:

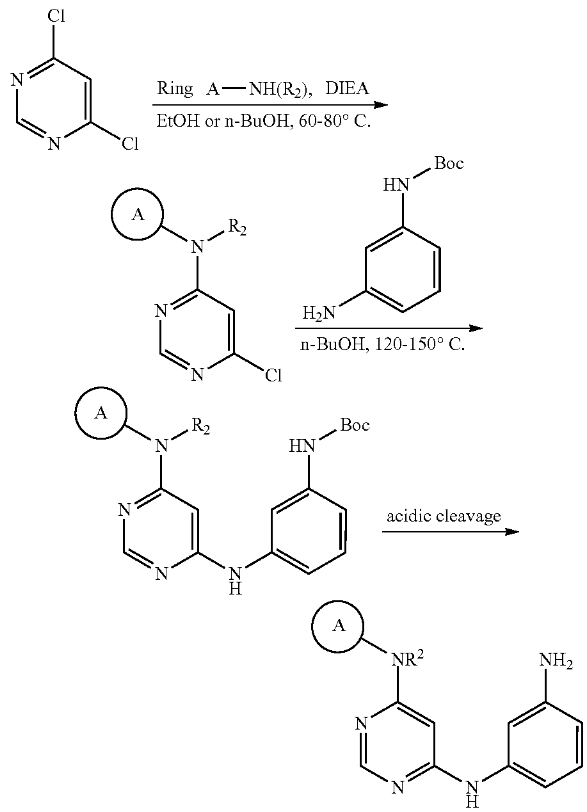
[0531] (a) 4-Phenoxyphenyl amine gave 3-[6-(4-phenoxyphenylamino)-pyrimidin-4-ylamino]phenylamine. (I^R-7) MS (m/z): MH^+=370.

[0532] (b) 3-Chloro-4-(2-pyridyl)methoxyaniline gave 3-{[6-(3-chloro-4-(2-pyridyl)methoxyphenylamino)-pyrimidin-4-ylamino]phenylamine. (I^R-9) MS (m/z): MH^+=419, 421.

[0533] (c) 3-Chloro-4-(3-fluorobenzyloxy)aniline gave 3-[6-(3-chloro-4-{3-fluorobenzyloxy}phenylamino)-pyrimidin-4-ylamino]-phenyl amine. (I^R-6) MS (m/z): M^+=436, 438.

[0534] (d) Aniline gave 3-[6-(phenylamino)-pyrimidin-4-yl]aminophenylamine. (I^R-15) MS (m/z): MH^+=278.

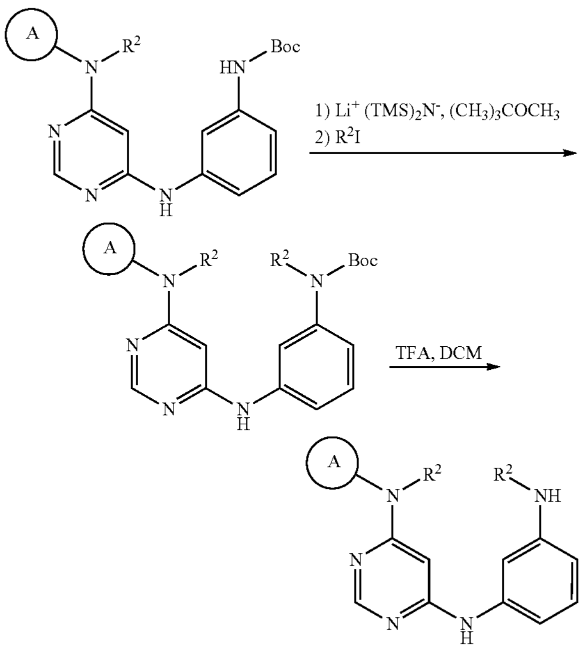
SCHEME 2b
Sequence B for Synthesis of 3-(6-mono- or disubstituted-aminopyrimidin-4-yl)amino-phenylamines



Synthesis of 3-[6-(4-bromo-2-fluorophenylamino)-pyrimidin-4-yl-amino]-phenylamine (I^R-17)

[0535] A solution of 4-bromo-2-fluoroaniline (701 mg, 3.69 mmol), diisopropylethylamine (0.70 mL, 4.03 mmol), and 4,6-dichloropyrimidine (500 mg, 3.36 mmol) in EtOH (10 mL) was heated in an 85° C. oil bath for 5 d. Flash chromatography (2% MeOH/$CHCl_3$) of the residue gave 380 mg (37%) of N-(4-bromo-2-fluorophenyl)-6-chloropyrimidin-4-amine as a pale yellow solid. MS (m/z): 304, 302. A suspension of N-(4-bromo-2-fluorophenyl)-6-chloropyrimidin-4-amine (0.37 g, 1.22 mmol) and tert-butyl 3-aminophenylcarbamate (0.28 g, 1.35 mmol) in n-BuOH (4 mL) was heated in an oil bath at 120-130° C. for 6 h. The reaction mixture was cooled and concentrated to give 0.68 g of a brown foam. Flash chromatography gave 0.16 g (28%) tert-butyl 3-(6-[4-bromo-2-fluorophenyl]aminopyrimidin-4-yl)aminophenylcarbamate as a white solid. MS (m/z): (M+H) 476, 474. tert-Butyl 3-(6-[4-bromo-2-fluorophenyl]aminopyrimidin-4-yl)aminophenylcarbamate (0.15 g, 0.32 mmol) was taken up in 4M HCl in dioxane (5 mL). After several minutes a white solid began to precipitate. The mixture was allowed to stand for 3 h and was concentrated via rotary evaporation. Saturated aqueous sodium bicarbonate (5 mL) was added and the mixture was sonicated for several minutes. The solid was collected by filtration, was washed with water (5 mL), and was dried overnight under vacuum to give 0.12 g (100%) of 3-[6-(4-bromo-2-fluorophenylamino)-pyrimidin-4-yl-amino]-phenylamine (I^R-17) as a white powder. MS (m/z): (M+H) 376, 374.

SCHEME 2c
Synthesis of 3-(6-mono or disubstituted-aminopyrimidin-4-yl) amino-N-substituted phenylamines



Synthesis of tert-butyl N-3-(6-chloropyrimidin-4-ylamino)phenyl-N-methylcarbamate

[0536] To a stirring solution of tert-butyl 3-(6-chloropyrimidin-4-ylamino)phenyl carbamate (2.000 g, 6.235 mmol) in 10 mL of THF at 0° C. under N_2 was added drop-wise a solution of 1.0 M lithium bis(trimethylsilyl)amide in tert-butyl methyl ether (6.23 mL, 6.23 mmol). The light yellow solution was stirred at 0° C. for 30 min then iodomethane (0.43 mL, 6.892 mmol, 1.1 eq) was added. The solution was allowed to slowly warm to room temperature overnight, was concentrated, and was then partitioned between EtOAc and saturated KH_2PO_4 solution. The organic extract was washed with brine solution, dried ($MgSO_4$), was filtered, was concentrated in vacuo and was chromatographed (silica gel, 2% MeOH in CH_2Cl_2) to give 0.904 g of tert-butyl N-3-(6-chloropyrimidin-4-ylamino)phenyl-N-methylcarbamate as an off-white solid. MS (m/z) M+1=335/337 (100/44%), ^{1}H NMR ($CDCl_3$) δ 8.48 (s, 1H), 7.49 (bs, 1H), 7.38 (m, 1H), 7.24 (m, 1H), 6.91 (m, 1H), 6.71 (bs, 1H), 6.30 (s, 1H), 3.48 (s, 3H), 1.53 (s, 9H).

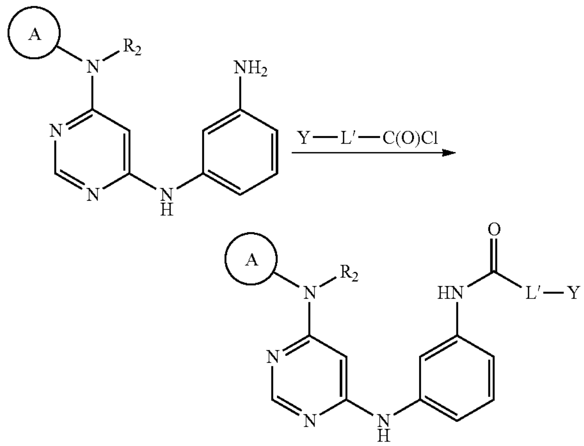
Synthesis of N-3-[6-(3-Chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methylamine (Int-C)

[0537] A solution of tert-butyl N-3-(6-chloropyrimidin-4-ylamino)phenyl-N-methylcarbamate (0.486 g, 1.095 mmol) in 25 mL of CH_2Cl_2 was treated with trifluoroacetic acid (5 mL). The solution was stirred at room temperature under N_2 for 2 h, was concentrated and was partitioned between $CHCl_3$ and 10% aq. NH_4OH solution. The organic extract was washed with brine solution, was dried ($MgSO_4$), was filtered and was concentrated to give 0.630 g of N-3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methylamine (Int-C) as yellow oil. MS (m/z): 344/346 (M+1, 100/68%). TLC (SiO_2, 10% MeOH in $CHCl_3$): R_f 0.44.

Example 3

[0538]

SCHEME 3a
Sequence B for Synthesis of 3-(6-mono- or disubstituted-aminopyrimidin-4-yl)amino-phenylamines



wherein L' is a subset of L, as defined herein, such that Y-L'C(O)Cl results in formation of provided compounds wherein R^1 is -L-Y wherein a terminal methylene unit of L is replaced with —NHC(O)—.

Synthesis of N-{3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenyl}-2-propenamide (I-1)

[0539] A solution of 3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]phenylamine (250 mg, 0.7 mmol) and triethylamine (180 mg, 1.75 mmol) in 5 mL of THF was stirred at RT. Acryloyl chloride (80 mg, 0.9 mmol) was added into the reaction mixture and it was stirred at RT for 1 h. The solvent was removed by vacuum evaporation and the crude product was purified by flash chromatography on silica gel with EtOAc/DCM solvent system to afford 115 mg (40% yield) of the title compound as a light colored solid. MS (m/z): MH^+=410, 412. ^{1}H NMR (DMSO): 10.15 (s, 1H), 9.36 (s, 1H), 9.28 (s, 1H), 8.34 (s, 1H), 8.02 (s, 1H), 8.00 (s, 1H), 7.51-7.11 (m, 5H), 6.47 (dd, 1H, J_1=10.1 Hz, J_2=17.0 Hz), 6.27 (dd, 1H, J_1=1.9 Hz, J_2=17.0 Hz), 6.20 (s, 1H), 5.76 (dd, 1H, J_1=10.1 Hz, J_2=1.9 Hz) ppm.

[0540] The following compounds were prepared in a manner substantially similar to those described in Schemes 3a:

[0541] (a) 4-[6-(3-Bromophenylamino)-pyrimidin-4-ylamino]phenylamine and acryoyl chloride gave N-{4-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]phenyl}-2-propenamide (I-93). MS (m/z): =410, 412. ^{1}H NMR (DMSO): 10.10 (s, 1H), 9.33 (s, 1H), 9.17 (s, 1H), 8.30 (s, 1H), 8.02 (s, 1H), 7.62 (m, 2H), 7.46 (m, 3H), 7.22 (t, 1H, J=8.0 Hz), 7.10 (d, 1H, J=8.0 Hz), 6.43 (dd, 1H, J_1=10.0 Hz, J_2=17.0 Hz), 6.24 (d, 1H, J=17.0 Hz), 6.13 (s, 1H), 5.73 (d, 1H, J=10.0 Hz) ppm.

[0542] (b) 3-[6-(3-Bromophenylamino)-pyrimidin-4-ylamino]phenylamine and propionyl chloride gave N-{3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]phenyl}-propionamide (I^R-3). MS (m/z): MH^+=412, 414. ^1H NMR (DMSO): 9.78 (s, 1H), 9.28 (s, 1H), 9.16 (s, 1H), 8.26 (s, 1H), 7.96 (s, 1H), 7.76 (s, 1H), 7.43 (d, 1H, J=8.2 Hz), 7.20 (m, 2H), 7.05 (m, 3H), 6.12 (s, 1H), 2.27 (q, 2H, 0.1=7.6 Hz), 1.03 (t, 3H, J=7.6 Hz) ppm.

[0543] (c) 3-[6-(3-Bromophenylamino)-pyrimidin-4-ylamino]phenylamine and (E)-2-butenoylchloride gave (E)-N-{3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenyl}-2-butenamide (I-8). MS (m/z): $M+H^+$=424, 426. ^1H NMR (DMSO): 9.87 (s, 1H), 9.29 (s, 1H), 9.18 (s, 1H), 8.27 (s, 1H), 7.96 (s, 1H), 7.82 (s, 1H), 7.44 (d, 1H, J=8.2 Hz) 7.20 (m, 4H), 7.05 (dd, 1H, J, =1.0 Hz, J_2=7.8 Hz), 6.75 (m, 1H), 6.15 (m, 2H), 1.81 (d, 3H, J=7.8 Hz) ppm.

[0544] (d) 3-[6-(3-Chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4) and acryoyl chloride gave N-{3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl}-2-propenamide (I-2). MS (m/z): MH^+=384, 386. ^1H NMR (DMSO): 9.29 (s, 1H), 9.20 (s, 1H), 8.27 (s, 1H), 7.93 (m, 1H), 7.86 (s, 1H), 7.41 (m, 1H), 7.25 (m, 5H), 6.42 (dd, 1H, J, =10.1 Hz, J_2=17.0 Hz), 6.22 (dd, 1H, J, =1.9 Hz, J_2=17.0 Hz), 6.09 (d, 1H, J, =0.7 Hz), 5.69 (dd, 1H, J, =10.1 Hz, J_2=1.9 Hz) ppm.

[0545] (e) 3-[6-(3-Methylphenylamino)-pyrimidin-4-ylamino]phenylamine and acryloyl chloride gave N-{3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenyl}-2-propenamide (I-4). MS (m/z): $M+H^+$=346. ^1H NMR (DMSO): 9.11 (s, 1H), 9.00 (s, 1H), 8.21 (s, 1H), 7.86 (d, 1H, J=1.7 Hz), 7.31-7.05 (m, 7H), 6.74 (d, 1H, J=7.6 Hz), 6.41 (dd, 1H, J, =10.0 Hz, J_2=17.0 Hz), 6.20 (dd, 1H, J, =2.0 Hz, J_2=17.0 Hz), 6.14 (s, 1H), 5.69 (dd, 1H, J, =10.1 Hz, J_2=2.0 Hz), 2.23 (s, 3H) ppm.

[0546] (f) 3-{6-[3-Chloro-4-(3-fluorophenyl)methoxy-phenylamino]-pyrimidin-4-ylamino}phenylamine (I^R-6) and acryoyl chloride gave N-{3-[6-(3-chloro-4-(3-fluoro-phenyl)methoxyphenylamino)-pyrimidin-4-ylamino]phenyl}-2-propenamide (II-6). MS (m/z): $M+H^+$=490, 492. ^1H NMR (DMSO): 9.13 (s, 1H), 9.07 (s, 1H), 8.22 (s, 1H), 7.85 (d, 1H, J=1.5 Hz), 7.40-7.10 (m, 10H), 6.41 (dd, 1H, J, =10.0 Hz, J_2=17.0 Hz), 6.20 (dd, 1H, J, =2.0 Hz, J_2=17.0 Hz), 6.05 (s, 1H), 5.70 (dd, 1H, J, =10.1 Hz, J_2=2.0 Hz), 5.14 (s, 2H) ppm.

[0547] (g) 4-Phenoxyphenyl amine gave 3-[6-(4-phenoxy-phenylamino)-pyrimidin-4-ylamino]phenylamine. (I^R-7) and acryloyl chloride gave N-{3-[6-(4-phenoxyphenylamino)-pyrimidin-4-ylamino]phenyl}-2 propenamide (I-13). MS (m/z): MH^+=424. ^1H NMR (DMSO): 9.14 (s, 1H), 9.10 (s, 1H), 8.22 (s, 1H), 7.89 (s, 1H), 7.52 (d, 2H, J=9.0 Hz), 7.35-6.92 (m, 11H), 6.42 (dd, 1H, J, =10.1 Hz, J_2=16.9 Hz), 6.22 (dd, 1H, J, =1.9 Hz, J_2=16.9 Hz), 6.12 (s, 1H), 5.70 (dd, 1H, J, =1.9 Hz, J_2=10.1 Hz) ppm.

[0548] (h) 3-[6-(N-methyl-N-phenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-8) and acryoyl chloride gave N-{3-[6-(N-methyl-N-phenylamino)-pyrimidin-4-ylamino] phenyl}-2 propenamide (I-15): an off-white solid; 70 mg; 20%; MS (m/z): MH^+=346. ^1H NMR (DMSO): 10.02 (s, 1H), 9.00 (s, 1H), 8.25 (s, 1H), 7.85 (s, 1H), 7.45-7.12 (m, 6H), 6.45 (dd, 1H), 6.20 (d, 1H), 5.70 (m, 1H), 3.35 (s, 3H) ppm.

[0549] (i) 3-{[6-(3-Chloro-4-(2-pyridyl)methoxyphenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-9) and acryloyl chloride gave N-(3-(6-(3-chloro-4-(pyridin-2-ylmethoxy)phenylamino)pyrimidin-4-ylamino)phenyl)-2-propenamide (I-16). MS (m/z): MH^+=473, 475 (3:1). NMR (DMSO): 10.10 (s, 1H), 9.17 (s, 1H), 9.08 (s, 1H), 8.55 (m, 1H), 8.24 (s, 1H), 7.92-7.73 (m, 4H), 7.57 (d, 1H), 7.38-7.06 (m, 5H), 6.45 (dd, 1H), 6.23 (dd, 1H), 6.08 (s, 1H), 5.72 (dd, 1H), 5.20 (s, 2H) ppm.

[0550] (j) 3-[6-(3-Chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4) and 1-cyanocyclopropanecarbonyl chloride gave N-[3-(6-{3-chloro-4-fluorophenylamino}pyrimidin-4-yl)amino]phenyl-1-cyanocyclopropanecarboxamide (I-47). MS (m/z): M+1=423/425, H NMR (DMSO-d_6) δ 10.04 (s, 1H), 9.18 (s, 1H), 9.12 (s, 1H), 8.27 (d, 1H), 7.88 (s, 1H), 7.8 (s, 1H), 7.47-7.16 (m, 9H), 6.74 (b, 1H), 6.28 (d, 1H), 6.1 (s, 1H), 5.19 (s, 2H), 3.05 (s, 2H), 2.18 (s, 6H).

[0551] (k) 3-[6-(3-Chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4) and chloroacetyl chloride gave 2-chloro-N-{3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]-phenyl}-acetamide (I-49). MS (ES^+): $(M+1)^+$=406 (100%), $(M+3)^+$=408 (75%). ^1H-NMR (DMSO-d_6, δ 10.31 (s, 1H), 9.37 (s, 1H), 9.30 (s, 1H), 8.33 (s, 1H), 8.00 (m, 1H), 7.87 (s, 1H), 7.47-7.24 (m, 5H), 6:15 (s, 1H), 4.26 (s, 2H).

[0552] (l) 3-[6-(3-Chloro-4-fluorophenyl)aminopyrimidin-4-yl]aminophenylamine (I^R-4) and 2-chloropropionyl chloride gave 2-chloro-N-[3-(6-{3-chloro-4-fluorophenyl}aminopyrimidin-4-yl)aminophenyl]propionamide (I-50). MS (ES^+): $(M+1)^+$=420 (100%), $(M+3)^+$=422 (75%). ^1H-NMR (DMSO-d_6, δ 10.32 (s, 1H), 9.37 (s, 1H), 9.28 (s, 1H), 8.33 (s, 1H), 8.00-7.98 (m, 1H), 7.87 (s, 1H), 7.47-7.26 (m, 5H), 6.14 (s, 1H), 4.69 (quartet, 1H), 1.61 (d, 3H).

[0553] (m) 3-[6-(3-Chloro-4-fluorophenylamino)pyrimidin-4-ylamino]phenylamine (I^R-4) and 1-trifluoromethylcyclopropanecarbonyl chloride gave N-[3-(6-{3-chloro-4-fluorophenyl}aminopyrimidin-4-yl)aminophenyl]-1-trifluoromethylcyclopropanecarboxamide (I-51). MS (ES) (m/z): 466/468 [M+1, 100/45%]. NMR (DMSO-d_6) δ 9.61 (m, 1H), 9.07-9.18 (m, 2H), 8.14 (m, 1H), 7.63-7.8 (m, 2H), 7.03-7.21 (m, 5H), 5.94 (bs, 1H), 1.12-1.27 (m, 4H).

[0554] (n) N-3-[6-(3-Chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methylamine (Int-D) and acryloyl chloride gave N-3-[6-(3-chloro-4-fluorophenylamino) pyrimidin-4-yl]aminophenyl-N-methyl-2-propenamide (I-53). MS (m/z): 398/400 (M+1, 100/63%). ^1H NMR (DMSO-d_6) δ 10.32 (s, 1H), 9.21 (s, 1H), 8.33 (s, 1H), 7.99 (bs, 1H), 7.25-7.68 (m, 5H), 7.06 (bs, 1H), 6.41-6.47 (m, 1H), 6.26-6.29 (m, 1H), 5.71-5.80 (m, 2H).

[0555] (o) 3-[6-(4-Bromo-2-fluorophenyl)aminopyrimidin-4-yl]aminophenyl amine (I^R-17) and acryloyl chloride gave N-3-[6-(4-bromo-2-fluorophenylamino)pyrimidin-4-yl]aminophenyl]-2-propenamide (I-55). MS ($M+H^+$) 430, 428. ^1H NMR (d^6-DMSO) δ 10.13 (s, 1H), 9.25 (s, 1H), 9.02 (s, 1H), 8.26 (s, 1H), 7.91 (m, 2H), 7.57 (d, J=11 Hz, 1H), 7.45-7.15 (m, 4H), 6.46 (dd, J=12 and 10 Hz, 1H), 6.26 (d, J=12 Hz, 1H), 6.23 (s, 1H), 5.75 (d, J=10 Hz, 1H).

[0556] (p) 2-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl] amino-6-aminopyridine (I^R-11) and acryloyl chloride gave N-6-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl)]aminopyridin-2-ylpropanamide (I-63) NMR (DMSO-d_6) δ ppm: 5.75 (d, J=10.20 Hz, 1H), 6.29 (d, J=17.04 Hz, 1H), 6.62

(dd, J=10.08 & 16.92 Hz, 1H), 6.96-7.00 (m, 4H), 7.07-7.11 (m, 1H), 7.18 (bd, J=3.28 Hz, 1H), 7.33-7.38 (m, 3H), 7.62-7.69 (m, 4H), 8.29 (s, 1H), 9.13 (s, 1H), 9.66 (s, 1H), 10.15 (s, 1H); MS: m/z 425.3 (M+1).

[0557] (q) 2-[6-(3-methylphenoxy)-pyrimidin-4-yl]amino-6-aminopyridine (I^R-12) and acryloyl chloride gave N-6-[6-(3-methylphenoxy)-pyrimidin-4-yl)]aminopyridin-2-ylpropanamide (I-65) NMR ($CDCl_3$) δ ppm: 2.40 (s, 3H), 5.86 (d, J=9.44 Hz, 1H), 6.49-6.61 (m, 2H), 6.83 (bs, 1H), 6.95-7.05 (m, 3H), 7.15 (d, J=7.48 Hz, 1H), 7.34 (t, J=15.08 Hz, 2H), 7.54 (bd, J=5.56 Hz, 1H), 8.78 (s, 1H), 10.78 (s, 1H); LCMS: m/z 348.8 (M+1).

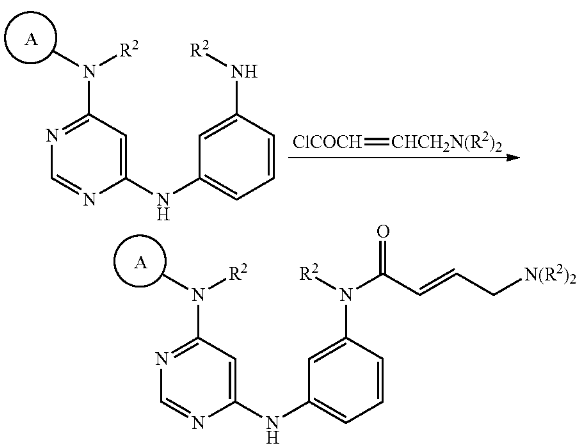
[0558] (r) 2-[6-(4-phenoxyphenoxy)-pyrimidin-4-yl]amino-6-aminopyridine (I^R-13) and acryloyl chloride gave N-6-[6-(4-phenoxyphenoxy)-pyrimidin-4-yl)]aminopyridin-2-yl}propenamide (I-66) NMR (MeOD) δ ppm: 5.92 (dd, J=11.60, Hz, 1H), 6.50-6.54 (m, 2H), 6.75-7.28 (m, 10H), 7.37-7.41 (m, 2H), 7.91 (t, J=16.08 Hz, 1H), 8.63 (s, 1H); LCMS: m/z 426 (M+1).

[0559] (s) 3-[6-(phenylamino)-pyrimidin-4-ylamino]phenylamine. (I^R-15) acryloyl chloride gave N-3-[6-(phenylamino)-pyrimidin-4-yl]aminophenylpropenamide (I-67) ^{1}H NMR (DMSO-d_6) δ ppm: 5.72 (dd, J=2.04 & 10.04 Hz, 1H), 6.19 (s, 1H), 6.25 (dd, J=2 & 16.92 Hz, 1H), 6.46 (dd, J=10.04 & 16.88 Hz, 1H), 6.96 (t, J=7.36 Hz, 1H), 7.19-7.31 (m, 4H), 7.54 (d, J=7.68 Hz, 2H), 7.91 (s, 1H), 8.26 (s, 1H), 9.13 (s, 1H), 9.18 (s, 1H), 10.11 (s, 1H); MS: m/z 332.8 (M+1).

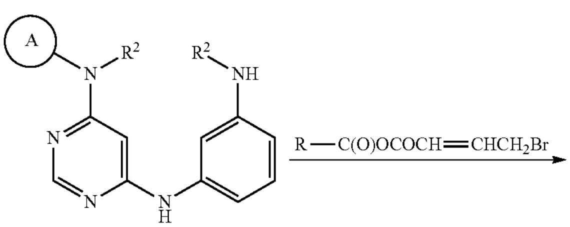
Example 4

[0560]

SCHEME 4a
Sequence A for Synthesis of N-3-(6-mono or disubstituted-aminopyrimidin-4-yl)amino-N-mono- or unsubstituted-phenyl-4-amino-substituted-2-butenamides



SCHEME 4b
Sequence B for Synthesis of N-3-(6-mono or disubstituted-aminopyrimidin-4-yl)amino-N-mono- or unsubstituted-phenyl-4-amino-substituted-2-butenamides



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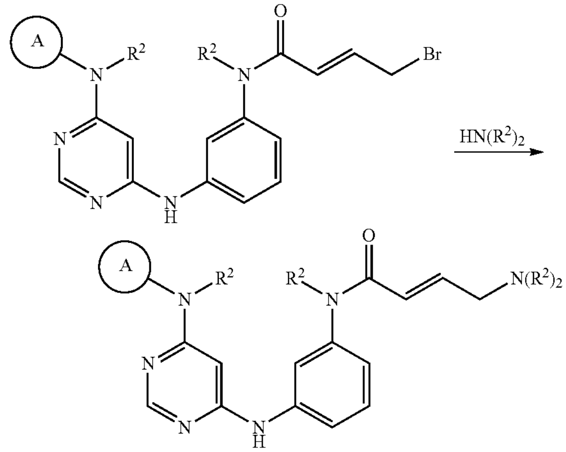


Synthesis of (E)-N-(3-(6-(3-chloro-4-(pyridin-2-ylmethoxy)phenylamino)-pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (I-19)

[0561] 3-{[6-(3-chloro-4-(2-pyridyl)methoxyphenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-9) was dissolved in N-methylpyrrolidinone (1.2 mL) and added dropwise over 10 minutes to the ice-cold solution of (E)-4-(dimethylamino)but-2-enoyl chloride hydrochloride in acetonitrile. The reaction was stirred in an ice bath for 2 hr. To the mixture was added sodium bicarbonate to pH greater than 9. The oil that formed was extracted with EtOAc (3×25 mL). A portion of the material was insoluble and set aside. The organic layer was dried ($MgSO_4$) filtered and evaporated to a dark red oil. Both oils contained substantial amount of product as shown by TLC: SiO_2CHCl_3:MeOH/NH_4OH (8:1) 19:1. The oils were combined and purified by flash column chromatography silica gel (25×300 mm) eluted first with 5% methanol/ammonium hydroxide 8/1 to remove non-polar impurities followed by 10% methanol/ammonium hydroxide 8/1 to elute 59 mg of product. The sample was further purified by a second flash column chromatography silica gel (25×250 mm) eluted with 10% methanol/ammonium hydroxide 8/1 to give the title compound (16.3 mg, 0.03 mmol, 6.4% yield). MS (ES+) 530 (M+): 552, (M+Na); ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 10.04 (s, 1H), 9.19 (s, 1H), 9.14 (s, 1H), 8.60 (s, 1H), 8.27 (s, 1H), 7.88 (s, 2H), 7.57 (s, 1H), 7.36 (d, 1H, J=7.4 Hz), 7.29 (d, 2H, J=7.8 Hz), 7.22 (d, 2H, J=7.9 Hz), 7.18 (m, 2H), 6.74 (m, 1H), 6.30 (d, 1H, J=15 Hz) 6.10 (s, 1H), 5.24 (s, 2H), 3.06 (s, 2H), 2.18 (s, 6H) ppm; HPLC: t_R=5.15 min, 97.5% (YMC-Pack ODS-A 4.6×100 mm, 80% water/20% acetonitrile to 5% water/95% acetonitrile over 5.5 min, hold to 9 min.).

Synthesis of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-bromo-2-butenamide (Int-D)

[0562] To a stirring solution of 4-bromo-but-2-enoic acid (0.72 g) at 0° C. under nitrogen atmosphere and triethylamine (0.61 mL, 4.38 mmol) in 5 mL of THF was added iso-butyl chloroformate (0.56 mL, 4.32 mmol). The mixture was stirred for 15 min followed by dropwise addition of a solution of N-(3-amino-phenyl)-N'-3-methylphenylamino-pyrimidine-4,6-diamine (1.015 g, 3.483 mmol) in 50 mL of

THF. The reaction was allowed to warm to room temperature overnight. The sample was concentrated then partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, dried ($MgSO_4$), filtered and was concentrated. The resultant brown foamy solid was washed with diethyl ether and vacuum dried to give 0.960 g of crude (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-bromo-2-butenamide (Int-D) as brick-brown solid. MS (m/z): (M+1) 438/440 (71/75%).

Synthesis of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-(methyl-prop-2-ynyl) amino-2-butenamide (I-58)

[0563] To a stirring solution at 0° C. under N_2 of N-[3-(6-{3-methylphenyl}amino-pyrimidin-4-ylamino)-4-bromo-2-butenamide (Int-D) (0.492 g, 1.122 mmol) and triethylamine (0.20 mL, 1.44 mmol) in 10 mL of THF was added (via syringe) N-methyl-propargylamine (0.11 mL, 1.173 mmol). The solution was allowed to warm to room temperature overnight, was concentrated and was then partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, was dried ($MgSO_4$), was filtered and was concentrated. The residue was chromatographed (silica gel, 10% MeOH in $CHCl_3$) to give 0.110 g of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-(methyl-prop-2-ynyl)amino-2-butenamide (I-58). MS (APCI) m/z 427 (M+1, 100%). ^1H NMR (DMSO-d_6) δ 10.06 (s, 1H), 9.07 (s, 1H), 9.17 (s, 1H), 8.27 (s, 1H), 7.90 (s, 1H), 7.16-7.36 (m, 6H), 6.70-6.73 (m, 1H), 6.79 (d, 1H), 6.20 (s, 1H), 6.32 (d, 1H), 3.30-3.49 (m), 3.14-3.27 (m, 3H), 2.18-2.39 (m, 7H which contain singlets at δ 2.25 [3H] and δ 2.29 [3H]).

Synthesis of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-piperazinyl-2-butenamide (I-57)

[0564] To a stirring solution at 0° C. under N_2 of N-[3-(6-{3-methylphenyl}amino-pyrimidin-4-ylamino)-4-bromo-2-butenamide (Int-D) (2.15 g, 4.91 mmol) and triethylamine (0.86 mL, 6.17 mmol) in 10 mL of THF was added dropwise a solution of 1-Boc-piperazine (0.92 g, 4.91 mmol) in 10 mL of THF. The solution was allowed to warm to room temperature overnight, was concentrated and was then partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, was dried ($MgSO_4$), was filtered and was concentrated to give 2.76 g of 4-{3-[3-(6-{3-methylphenyl}amino-pyrimidin-4-ylamino)-phenylcarbamoyl]-allyl}-piperazine-1-carboxylic acid tert-butyl ester as gummy brown solid. This material was dissolved in 100 mL of CH_2Cl_2. 20 mL of trifluoroacetic acid was added and the mixture was stirred at room temperature under N_2 for 2 h. The mixture was concentrated in vacuo, was basified with sat. $NaHCO_3$ solution and was extracted with EtOAc (2×100 ml). The combined organic extract was dried ($MgSO_4$), was filtered and was concentrated in vacuo. The residue was chromatographed (silica gel, 5% MeOH in $CHCl_3$ [500 mL] then 1% NH_4OH-10% MeOH in $CHCl_3$) to give 0.404 g of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-piperazinyl-2-butenamide. (I-57) MS (m/z): 444 (M+1, 100%). ^1H NMR (DMSO-d_6) δ 10.03 (s, 1H), 9.17 (s, 1H), 9.07 (s, 1H), 8.27 (s, 1H), 7.90 (s, 1H), 7.17-7.36 (m, 6H), 6.79-6.80 (d, 1H), 6.72-6.75 (m, 1H), 6.29 (d, 1H), 6.20 (s, 1H), 3.08-3.39 (m, containing water and ~3H), 2.70-2.72 (m, 4H), 2.30-2.42 (m, 4H), 2.29 (s, 3H.

[0565] The following compounds were prepared in a manner substantially similar to those described in Scheme 4a, above:

[0566] (a) 3-(6-[3-chloro-4-{3-fluorobenzyloxy}]phenylamino-pyrimidin-4-yl)aminophenylamine (I^R-6) and 4-dimethylamino-2-butenoyl chloride gave (E)-N-3-([6-(3-chloro-4-{3-fluorobenzyloxy}phenylamino)-pyrimidin-4-yl)aminophenyl-4-(dimethylamino)but-2-enamide (I-46). MS (m/z): =547, 549 (3:1), H-NMR (DMSO-d_6) δ 10.04 (s, 1H), 9.18 (s, 1H), 9.12 (s, 1H), 8.27 (d, 1H), 7.88 (s, 1H), 7.8 (s, 1H), 7.47-7.16 (m, 9H), 6.74 (b, 1H), 6.28 (d, 1H), 6.1 (s, 1H), 5.19 (s, 2H), 3.05 (s, 2H), 2.18 (s, 6H).

[0567] (b) 3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-5) and 4-(dimethylamino)-2-butenoyl chloride gave (E)-N-{3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenyl}-4-dimethylamino-2-butenamide (I-17). MS (m/z): MH^+=403.

[0568] (c) 3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4) and 4-(dimethylamino)-2-butenoyl chloride gave (E)-N-{3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl}-4-dimethylamino-2-butenamide (I-18). MS ($M+H^+$) 441, 443; ^1H NMR (400 MHz, d^6-DMSO) δ 10.01 (s, 1H), 9.30 (s, 1H), 9.20 (s, 1H), 8.28 (s, 1H), 7.95 (dd, J=7 and 3 Hz, 1H), 7.86 (s, 1H), 7.41 (m, 1H), 7.35-7.10 (m, 3H), 6.69 (dt, J=15 and 5 Hz, 1H), 6.26 (d, J=15 Hz, 1H), 6.11 (s, 1H), 3.02 (d, J=5 Hz, 2H), 2.14 (s, 6H) ppm.

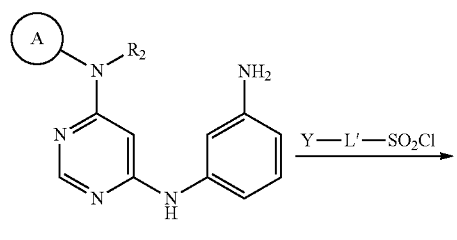
[0569] (d) N-3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methylamine (Int-C) and 4-dimethylamino-2-butenoyl chloride gave (E)-N-(3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methyl-4-(dimethylamino)but-2-enamide (I-48) MS (ES) (m/z): 455/457 (M+1, 37/13%) and 228 (100%). ^1H NMR (DMSO-d_6) δ 10.22 (s, 1H), 9.20 (s, 1H), 8.33 (s, 1H), 7.99 (bs, 1H), 7.25-7.68 (m, 5H), 7.05 (bs, 1H), 6.73-6.76 (m, 1H), 6.26-6.29 (m, 1H), 5.71 (s, 1H), 3.06 (bs, 2H), 1.99 (s, 6H).

[0570] (e) 3-[6-(4-phenoxyphenylamino)-pyrimidin-4-yl] aminophenylamine (I^R-7) and 4-dimethylamino-2-butenoyl chloride gave (E)-N-3-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl]aminophenyl-4-(dimethylamino)but-2-enamide (I-62)^1H NMR (DMSO-d_6) δ ppm: 2.19 (s, 6H), 3.07 (d, J=5.36 Hz, 2H), 6.16 (s, 1H), 6.29 (d, J=15.4 Hz, 1H), 6.68-6.75 (m, 1H), 6.95-6.98 (m, 4H), 7.06-7.10 (m, 1H), 7.20 (dd, J=7.88 & 8.12 Hz, 1H), 7.24 (d, J=8.32 Hz, 2H), 7.36 (dd, J=7.52 & 7.84 Hz, 2H), 7.55 (d, J=8.76 Hz, 2H), 7.90 (s, 1H), 8.24 (s, 1H), 9.15 (d, J=8.84 Hz, 2H), 10.04 (s, 1H); LCMS: m/z 481 (M+1).

Example 5

[0571]

SCHEME 5a
Synthesis of N-{3-[6-(arylamino)-pyrimidin-4-ylamino]-phenyl}-ethensulfonamides



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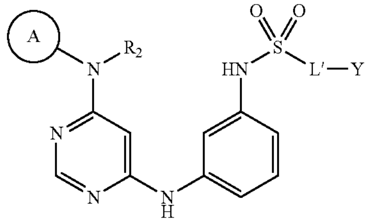


[0572] wherein L' is a subset of L, as defined herein, such that Y-L'SO_2Cl results in formation of provided compounds wherein R^1 is -L-Y wherein a terminal methylene unit of L is replaced with —$NHSO_2$—.

Synthesis of N-{3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl}-ethenesulfonamide (I-3)

[0573] A solution of 3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-4) (300 mg, 0.9 mmol) and triethylamine (500 mg, 5 mmol) in 10 mL of THF was stirred at RT. 2-Chloroethanesulfonyl chloride (360 mg, 2.25 mmol) was added into the reaction mixture and stirring was continued at RT for 1 hr. The crude product was purified by flash chromatography on silica gel with EtOAc/heptane solvent system to afford 35 mg (9%) of the title compound, a brown colored solid. MS (m/z): MH^+=420, 422. ^{1}H NMR (DMSO): 9.92 (s, 1H), 9.29 (s, 1H), 9.21 (s, 1H), 8.26 (s, 1H), 7.92 (m, 1H), 7.40-7.22 (m, 4H), 7.14 (m, 1H), 6.20 (m, 2H), 6.05 (m, 3H) ppm.

[0574] The following compounds were prepared in a manner substantially similar to Schemes 5a:

[0575] (a) 3-{6-[3-chloro-4-(3-fluorophenyl)methoxyphenylamino]-pyrimidin-4-ylamino}phenylamine (I^R-6) gave N-{3-[6-(3-chloro-4-(3-fluorophenyl)methoxy-phenylamino)-pyrimidin-4-ylamino]phenyl}-ethenesulfonamide (I-7). MS (m/z): M+H^+=526, 528 (2:1). MS (m/z): M+H^+=490, 492 (2:1). ^{1}H NMR (DMSO): 9.91 (s, 1H), 9.16 (s, 1H), 9.07 (s, 1H), 8.22 (s, 1H), 7.73 (s, 1H), 7.40-7.10 (m, 9H), 6.70 (m, 2H), 6.12 (d, 1H, J=17.0 Hz), 6.02 (m, 2H), 5.14 (s, 2H) ppm.

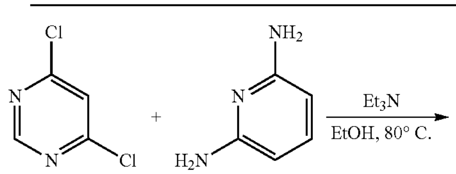
[0576] (b) 3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenylamine (I^R-5) gave N-{3-[6-(3-methylphenylamino)-pyrimidin-4-ylamino]phenyl}-ethenesulfonamide (II-5). MS (m/z): M+H^+=382. ^{1}H NMR (DMSO): 9.90 (s, 1H), 9.12 (s, 1H), 9.00 (s, 1H), 8.21 (s, 1H), 7.37 (d, 1H, J=1.7 Hz), 7.26 (m, 3H), 7.13 (m, 2H), 6.70 (m, 3H), 6.13 (m, 2H), 6.00 (d, 1H, J=10.0 Hz), 2.24 (s, 3H) ppm.

Example 6

[0577]

SCHEME 6a

Synthesis of N^2-acylated 6-(6-mono or disubstituted-aminopyrimidin-4-yl)amino-2-aminopyridines



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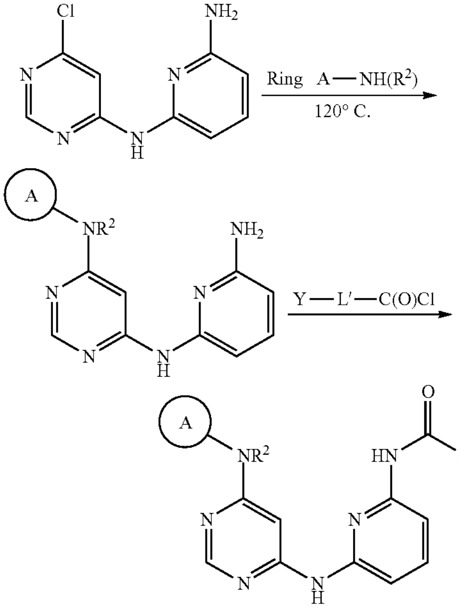


Synthesis of N-(6-chloro-pyrimidin-4-yl)-pyridine-2,6-diamine

[0578] A mixture of 2,6-diaminopyridine (1.530 g, 14.020 mmol) and 4,6-dichloropyrimidine (2.610 g, 17.519 mmol) in 15 mL of n-butanol in a sealed vial was heated at 100° C. for 72 h. The dark brown sample was cooled, was concentrated to remove most of the n-butanol, and was then partitioned between EtOAc and sat. $NaHCO_3$ solution. An emulsion formed, the sample was filtered through a pad of Celite and the layers were separated. The organic extract was washed with sat. KH_2PO_4 and brine solutions, was dried ($MgSO_4$), was filtered and was concentrated to brown oily-solid. The sample was suspended into 50 mL of CH_2Cl_2, was cooled and was filtered to give 1.017 g of N-(6-chloro-pyrimidin-4-yl)-pyridine-2,6-diamine as yellow-orange solid. MS (ES) (m/z) 222/224 (M+1, 100/63%). TLC (SiO_2, 50% EtOAc in hexanes): R_f 0.33.

Synthesis of N-(6-amino-pyridin-2-yl)-N'-(3-chloro-4-fluorophenyl)-pyrimidine-4,6-diamine (Int-E)

[0579] A mixture of N-(6-chloro-pyrimidin-4-yl)-pyridine-2,6-diamine (1.000 g, 4.512 mmol) and 3-chloro-4-fluoroaniline (1.380 mmol) in 10 mL of n-butanol in a sealed vial was heated at 120° C. for 24 h. The sample was cooled, was concentrated to remove most of the n-butanol, and was then diluted with EtOAc and sat. $NaHCO_3$ solution. The sample was stirred at room temperature for 30 min and was filtered. The solid was washed with fresh EtOAc and water and vacuum dried to give 1.063 g of N-(6-amino-pyridin-2-yl)-N'-(3-chloro-4-fluorophenyl)-pyrimidine-4,6-diamine (Int-E) as tan solid. MS (ES) (m/z) 331/333 (M+1, 100/65%). TLC (SiO_2, 10% MeOH in $CHCl_3$): R_f 0.25.

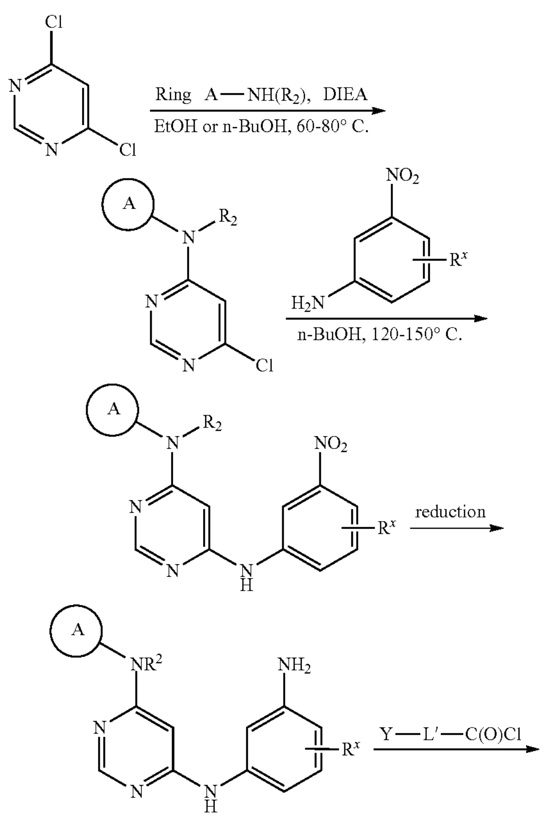
Synthesis of (E)-N-[6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylaminopyridin-2-yl]-4-(dimethylamino)but-2-enamide (I-52)

[0580] To a stirring suspension at 0° C. under N_2 of 4-dimethylamino-but-2-enoic acid, hydrochloride (0.500 g, 3.019 mmol) in 15 mL of THF containing 5 drops of DMF was added drop-wise (via syringe) oxalyl chloride (0.28 mL, 3.210 mmol). Gas formation started immediately. The sample was stirred at 0° C. for ~30 min, room temperature for ~2 h, re-cooled to 0° C., then treated with drop-wise addition of a solution of N-(6-amino-pyridin-2-yl)-N'-(3-chloro-4-fluoro-phenyl)-pyrimidine-4,6-diamine (Int-E) (0.500 g, 1.512 mmol) in 15 mL of THF and 3 mL of NMP. The ice-bath was removed, the sample was stirred at room temperature for 2 h then was partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, dried ($MgSO_4$), filtered and concentrated to a yellow solid. The solid was suspended into EtOAc (~25 mL), stirred at room temperature for ~12 h, filtered and vacuum dried to give 0.459 g (69%) of (E)-N-[6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylaminopyridin-2-yl]-4-(dimethylamino)but-2-enamide (I-52) as light yellow solid. MS (m/z): 442/444 (M+1, 100/37%). ^{1}H NMR (DMSO-d_6) δ 10.19 (s, 1H), 9.78 (s, 1H), 9.43 (s, 1H), 8.36 (s, 1H), 8.05-8.07 (m, 1H), 7.54-7.72 (m, 4H), 7.32-7.35 (m, 1H), 7.10 (bs, 1H), 6.79-6.82 (m, 1H), 6.54-6.57 (m, 1H), 3.14 (bs, 2H), 2.23 (bs, 6H).

Example 7

[0581]

SCHEME 7a
Synthesis of N-acylated 3-(6-mono or disubstituted-aminopyrimidin-4-yl)amino-mono-substituted-phenylamines



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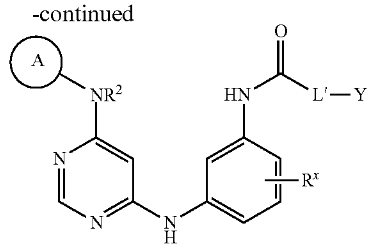


Synthesis of N-(5-Amino-2-methylphenyl)-N'-(3-chloro-4-fluorophenyl)-pyrimidine-4,6-diamine (Int-F)

[0582] A mixture of 4-(3-chloro-4-fluorophenyl)-6-chloropyrimidin-4-ylamine (I^R-4) (1.2 g, 4.6 mmol), 2-methyl-5-nitroaniline (0.85 g, 5.5 mmol) and 1 mL of concentrated HCl in 10 mL of n-butanol was heated at 120° C. for 16 h. With stirring, 5 mL of EtOAc was added to the reaction mixture. The light yellow colored product that precipitated was filtered and dried under vacuum to give N-(3-chloro-4-fluorophenyl)-N-(2-methyl-5-nitrophenyl)pyrimidine-4,6-diamine. MS (APCI) (m/z): 374/376 (M+1). A mixture of N-(3-chloro-4-fluorophenyl)-N'-(2-methyl-5-nitrophenyl)pyrimidine-4,6-diamine (0.55 g, 1.5 mmol) and iron powder (35 mesh, 0.5 g, 5 eq) in 5 mL of HOAc and 2 mL of MeOH was heated at reflux for 2 h. The solvent was removed in vacuo and the dark colored residue was mixed with 150 mL of CH_2Cl_2 and 15 mL of sat. K_2CO_3 solution and was stirred at room temperature for 30 min. The organic layer was dried ($MgSO_4$), was concentrated, and was then purified by flash chromatography (silica gel, $MeOH/NH_4OH/CH_2Cl_2$) to afford 0.115 g of N-(5-amino-2-methylphenyl)-N'-(3-chloro-4-fluorophenyl)pyrimidine-4,6-diamine as a light colored solid. (Int-F) MS (m/z): 344/346 (M+1).

[0583] In a manner substantially similar to that described above 4-(3-chloro-4-fluorophenylamino)-6-chloropyrimidine and 2-methoxy-5-nitroaniline gave N-(5-amino-2-methoxyphenyl)-N'-(3-chloro-4-fluorophenyl)pyrimidine-4,6-diamine. (Int-G) MS (m/z): 360/362 (M+1).

Synthesis of (E)-N-[3-(6-{3-Chloro-4-fluorophenyl}amino-pyrimidin-4-ylamino)-4-methylphenyl]-4-(dimethylamino)but-2-enamide (I-54)

[0584] N-(5-amino-2-methylphenyl)-N'-(3-chloro-4-fluorophenyl)pyrimidine-4,6-diamine (Int-F) and 4-dimethylamino-2-butenoyl chloride were combined in a manner similar to that described in Scheme 4a to give (E)-N-[3-(6-{3-chloro-4-fluorophenyl}amino-pyrimidin-4-ylamino)-4-methylphenyl]-4-(dimethylamino)but-2-enamide (I-54). MS (m/z): 455/457 (M+1, 100/39%). ^{1}H NMR (DMSO-d_6) δ 11.02 (bs, 1H), 10.66 (bs, 1H), 9.92 (bs, 1H), 9.39 (bs, 1H), 7.92-7.94 (m, 1H), 7.75 (bs, 1H), 7.27-7.56 (m, 4H), 6.80 (m, 1H), 6.54 (d, 1H), 5.89 (bs, 1H), 3.92 (bs, 2H), 2.75 (bs, 6H), 2.17 (bs, 3H).

[0585] In a manner substantially similar to that described for the synthesis of (I-54)N-(5-Amino-2-methoxyphenyl)-N'-(3-chloro-4-fluorophenyl)pyrimidine-4,6-diamine (Int-G) and 4-dimethylamino-2-butenoyl chloride gave (E)-N-[3-(6-[3-chloro-4-fluorophenyl)-aminopyrimidin-4-ylamino-4-methoxyphenyl]-4-(dimethylamino)but-2-enamide (I-59). MS (m/z): 471/473 (M+1, 100/41%). ^{1}H

NMR (DMSO-d_6) δ 9.97 (s, 1H), 9.28 (s, 1H), 8.46 (s, 1H), 8.26 (s, 1H), 7.93-7.98 (m, 2H), 7.44-7.51 (m, 2H), 7.30-7.33 (m, 1H), 7.02 (d, 1H), 6.69-6.72 (m, 1H), 6.26 (d, 1H), 6.03 (s, 1H), 3.80 (s, 3H), 3.05 (m, 2H), 2.17 (s, 6H).

Example 8

Synthesis of 2-[6-(3-methylphenoxy)-pyrimidin-4-yl]amino-6-aminopyridine (I^R-12)

[0586] A mixture of 2,6-diaminopyridine (1.530 g, 14.020 mmol) and 4,6-dichloropyrimidine (2.610 g, 17.519 mmol) in 15 mL of n-butanol in a sealed vial was heated at 100° C. for 72 h. The dark brown sample was cooled and was concentrated at reduced pressure to remove most of the n-butanol. The residue was then partitioned between EtOAc and saturated. NaHCO3 solution. An emulsion formed, the sample was filtered through a pad of Celite and the layers were separated. The organic extract was washed with sat. KH_2PO_4 and brine solutions, dried ($MgSO_4$), filtered and concentrated to brown oily-solid. The sample was suspended into ~50 mL of CH_2Cl_2, cooled and filtered to give 1.017 g (36%) of N-(6-chloro-pyrimidin-4-yl)-pyridine-2,6-diamine as yellow-orange solid. MS: m/z 222/224 (M+1, 100/63%). To a solution of N-(6-chloro-pyrimidin-4-yl)-pyridine-2,6-diamine (100 mg, 0.45 mmol) in DMF (1 mL) was added 3-methylphenol (88 mg, 0.8 mmol) and anhydrous K_2CO_3 (93 mg, 0.6 mmol). The reaction mixture was heated at 145° C. for 16 h. It was then cooled and DMF was removed under reduced pressure to get a yellow gummy residue. The residue was taken in EtOAc (20 mL), was washed sequentially with water (5 mL) and brine (5 mL) and was dried over Na_2SO_4. Filtration followed by concentration under reduced pressure afforded a yellow gum which was further purified by column chromatography (SiO_2, 60-120, EtOAc/hexane, 5/5) to give 100 mg of 2-[6-(3-methylphenoxy)-pyrimidin-4-yl]amino-6-aminopyridine (I^R-12) as a pale yellow solid. MS: m/z 294 (M+H)

[0587] The following compounds were prepared in a manner substantially similar to the procedure described above:

[0588] (a) 4-Phenoxyphenol and 2,6-diaminopyridine gave 2-[6-(4-phenoxyphenoxy)-pyrimidin-4-yl]amino-6-aminopyridine (I^R-13) MS (m/z): MH^+=372.

[0589] (b) 4-nitrophenol and 1,3-diaminobenzene gave 3-[6-(4-nitrophenoxy)-pyrimidin-4-yl]amino-aminobenzene (I^R-16) MS (m/z): MH^+=324

Example 9

Synthesis of N-3-[6-(3-ethynylphenylamino)-pyrimidin-4-yl]aminophenyl-2-propenamide (I-68)

[0590] To a stirred solution of I^R-1 (150 mg, 0.42 mmol) in dry DMF (4.0 mL) under N_2 was added $Pd(PPh_3)_2Cl_2$ (14.7 mg, 0.021 mmol), CuI (3.9 mg, 0.021 mmol), PPh_3 (22.07 mg, 0.08 mmol), and diethylamine (94.8 mg, 6.3 mmol). The reaction mixture was purged with N_2 for additional 10 min, trimethylsilylacetylene (45.5 mg, 0.46 mmol) was added, and it was then subjected to microwave irradiation at 120° C. for 30 min. The mixture was cooled, was diluted with 5 mL water, was filtered through Celite® and was extracted with EtOAc (2×10 mL). The combined EtOAc extract was washed with water, then with brine and was dried over Na_2SO_4. Concentration under reduced pressure gave a crude product that was purified by column chromatography (SiO_2, MeOH/$CHCl_3$, 1/99) to give 100 mg of a brown solid. A solution of this material in 2 mL of dry methanol under nitrogen atmosphere containing anhydrous K_2CO_3 (73.9 mg, 0.53 mmol) was stirred at rt for 16 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a residue, which was further purified by column chromatography (SiO_2, 230-400, MeOH/$CHCl_3$, 1/99) to give 70 mg of a light brown solid. To a stirred solution of this material in NMP (1.0 mL) at 0° C. was added acryloyl chloride (105 mg, 1.16 mmol), and the reaction mixture was stirred at 0° C. for 1 h. The reaction mixture was then quenched with water, was basified with 10% $NaHCO_3$ sol and was extracted with EtOAc. The combined EtOAc extract was washed sequentially with water and brine, was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue obtained was further purified by preparative HPLC to give 8 mg of N-3-[6-(3-ethynylphenyl)aminopyrimidin-4-yl]amino-phenyl-2-propenamide (I-68) as an off white solid. NMR (DMSO-d_6) δ ppm: 4.15 (s, 1H), 5.73-5.76 (m, 1H), 6.19 (s, 1H), 6.25 (dd, J=1.96 & 17.00 Hz, 2H), 6.46 (dd, J=10.2 & 16.96 Hz, 1H), 7.05 (d, J=7.64 Hz, 1H), 7.21-7.33 (m, 3H), 7.54 (d, J=7.96 Hz, 1H), 7.81 (s, 1H), 7.91 (s, 1H), 8.32 (s, 1H), 9.28 (d, J=11.08 Hz, 2H), 10.13 (s, 1H); MS: m/z 356.8 (M+1).

Example 10

Synthesis of N-3-[6-(4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]amino]-carbonyl]amino)phenoxy pyrimidin-4-yl]aminophenylchloroacetamide (I-69)

[0591] Step 1:

[0592] To a solution of 3-[6-(4-nitrophenoxy)-pyrimidin-4-yl]amino-aminobenzene (I^R-16) (650 mg, 2.01 mmol) in THF (10 mL) was added Et_3N (305 mg, 3.01 mmol) and $(Boc)_2O$ (525 mg, 2.4 mmol) under N_2 atmosphere. The reaction mixture was further heated at 60° C. for 16 h. A residue was obtained after cooling to room temperature and removal of solvent under vacuum. The residue was dissolved in EtOAc (10 mL). The EtOAc extract was washed sequentially with water (5 mL) and brine (2 mL), was dried over Na_2SO_4. Concentration under reduced pressure followed by purification by column chromatography (SiO_2, 60-120, CHCl3/MeOH, 9/1) gave 400 mg of the Boc derivative as a yellow solid.

[0593] Step 2:

[0594] The material from Step 1 was dissolved in MeOH (8 mL) and 10% Pd/C (40 mg) was added under N_2 atmosphere. The reaction mixture was hydrogenated in a Parr apparatus (H_2, 3 Kg, rt, 16 h). The reaction mixture was filtered through Celite® and the solvent was removed under reduced pressure to give 250 mg, of the amine as a yellow solid.

[0595] Step 3:

[0596] To the material from Step 2 was added a toluene solution of 4-chloro-3-trifluoromethylphenylisocyanate prepared by reacting under nitrogen atmosphere at 0° C. 24 mg of 4-chloro-3-trifluoromethylaniline in 10 mL toluene with 0.08 mL of a 20%-solution of phosgene in toluene followed by addition of Et_3N (0.07 mL) and at 110° C. for 16 h. The reaction mixture of the amine and the isocyanate was further heated at 110° C. for 4 h and then was quenched with water (1 mL) and was extracted with EtOAc (2×20 mL). The EtOAc extract was washed with water (5 mL), brine (2 mL) and dried over Na_2SO_4. Filtration followed by concentration under vacuum offered a residue which was purified by a column chromatography (SiO_2, 230-400, hexane/EtOAc, 9/1) to 20 mg of the Boc/urea intermediate as a yellow solid.

[0597] Step 4:

[0598] To a solution of 45 mg of this intermediate in CH_2Cl_2 (2 mL) at 0° C. was added TFA (0.01 mL) under N_2 atmosphere. The reaction mixture stirred at room temperature for 4 h, was washed sequentially with 10% $NaHCO_3$ solution (1 mL) and brine (1 mL) and was dried over Na_2SO_4. Concentration under reduced pressure gave 25 mg of the amine/urea intermediate as a brownish solid.

[0599] Step 5:

[0600] To a solution of the amine/urea intermediate from Step 4 (45 mg, 0.05 mmol), in THF (2 mL) and Et_3N (10 mg, 0.1 mmol) at 0° C. under N_2 atmosphere was added chloroacetyl chloride (55 mg, 0.1 mmol). The reaction mixture was allowed to come to rt and with stirring for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between EtOAc (4 mL) and water (1 mL). The EtOAc layer was separated, was washed with brine (2 mL) and was dried over Na_2SO_4. Filtration followed by concentration under reduced pressure gave a residue which was further purified by column chromatography (SiO_2, 230-400, $CHCl_3$/MeOH, 9/1) to give N-3-[6-(4-[4-[[[[4-chloro-3-(trifluoromethyl)phenyl]amino]carbonyl]amino]phenoxy]phenyl]amino-pyrimidin-4-yl] aminophenylchloroacetamide (I-69) as a pale yellow solid. ^{1}H NMR (MeOD) δ ppm: 4.19 (s, 2H), 6.61 (s, 1H), 7.13 (d, J=6.92 Hz, 2H), 7.14-7.23 (m, 3H), 7.50-7.55 (m, 3H), 7.63-7.66 (m, 1H), 7.95 (s, 1H), 8.00 (s, 1H), 8.30 (s, 1H); MS: m/z 593 (M+1).

Example 11

Synthesis of (E)-N-3-(6-[3-methylphenylamino]-pyrimidin-4-yl)aminophenyl-4-(4-acetylpiperazin-1yl)-2-butenamide (I-60)

[0601] To a stirring solution at 0° C. under N_2 of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-piperazinyl-2-butenamide (I-57) (0.291 g, 0.655 mmol) and triethylamine (0.14 mL, 1.004 mmol) in 10 mL of THF was added (via syringe) acetyl chloride (0.05 mL, 0.70 mmol). The sample was allowed to warm to room temperature overnight, was concentrated and was then partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, was dried ($MgSO_4$), was filtered, was concentrated and was chromatographed (silica gel, 1% NH_4OH-10% MeOH in $CHCl_3$) to give 0.0705 g of (E)-N-3-(6-[3-methylphenyl]aminopyrimidin-4-yl)aminophenyl-4-(4-acetylpiperazin-1yl)-2-butenamide (I-60), a white solid. ^{1}H NMR (DMSO-d_6 δ 2.00 (s, 3H), 2.29 (s, 3H), 2.35-2.41 (m, 4H), 3.15-3.16 (m, 2H), 3.31-3.46 (m, containing water and ~4H), 6.19 (s, 1H), 6.32 (d, 1H), 6.73-6.80 (m, 2H), 7.16-7.35 (m, 6H), 7.90 (s, 1H), 8.27 (s, 1H), 9.07 (s, 1H), 9.17 (s, 1H) and 10.05 (s, 1H); MS: m/z 486 (M+1, 100%).

Example 12

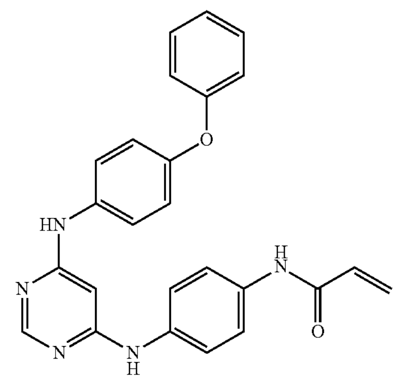
Synthesis of (E)-N-(3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-ylamino]phenyl-N-methyl-4-(dimethyl-d_6-amino)but-2-enamide (I-61)

[0602] To a stirring solution of 4-bromo-but-2-enoic acid (0.28 g) at 0° C. under nitrogen atmosphere and triethylamine (0.25 mL) in 3 mL of THF was added iso-butyl chloroformate (0.22 mL). The mixture was stirred for 15 min followed by dropwise addition of a solution of 3-[6-(3-chloro-4-fluorophenyl)aminopyrimidine-4-yl]aminophenylamine (I^R-4) (0.46 g) in 50 mL of THF. The reaction was allowed to warm to room temperature overnight. The sample was concentrated then partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, dried ($MgSO_4$), filtered and was concentrated. The resultant brown foamy solid was washed with diethyl ether and vacuum dried to give 0.378 g of (E)-N-3-(6-[3-chloro-4-fluorophenylamino]pyrimidin-4-ylamino-phenyl-4-bromo-2-butenamide (Int-E). MS (m/z): 480, 478, 476 (25/100/80%). To a stirring solution at 0° C. under N_2 of Int-E (0.378 g, 0.794 mmol) and triethylamine (0.28 mL, 2.01 mmol) in 10 mL of THF was added in one portion dimethyl-d_6-amine hydrochloride (0.070 g, 0.799 mmol). The sample was allowed to warm to room temperature overnight and then was partitioned between EtOAc and sat. $NaHCO_3$ solution. The organic extract was washed with brine solution, was dried ($MgSO_4$), was filtered and was concentrated. The residue was chromatographed (silica gel, 1% NH_4OH-10% MeOH in $CHCl_3$) to give 0.0582 g (16%) of (E)-N-(3-[6-(3-chloro-4-fluorophenylamino)-pyrimidin-4-yl]aminophenyl-4-(dimethyl-d_6-amino)but-2-enamide (I-61), a tan solid. ^{1}H NMR (DMSO-d_6) δ 3.05-3.07 (m, 2H), 6.16 (s, 1H), 6.29-6.32 (m, 1H), 6.72-6.75 (m, 1H), 7.21-7.47 (m, 5H), 7.90 (s, 1H), 7.92-8.01 (m, 1H), 8.32 (s, 1H), 9.26 (s, 1H), 9.36 (s, 1H) and 10.06 (s, 1H); MS: m/z 447/449 (M+1, 100/49%).

Example 13

[0603] I-80 may be prepared in a manner substantially similar to that described in Scheme 4a, above:

I-80

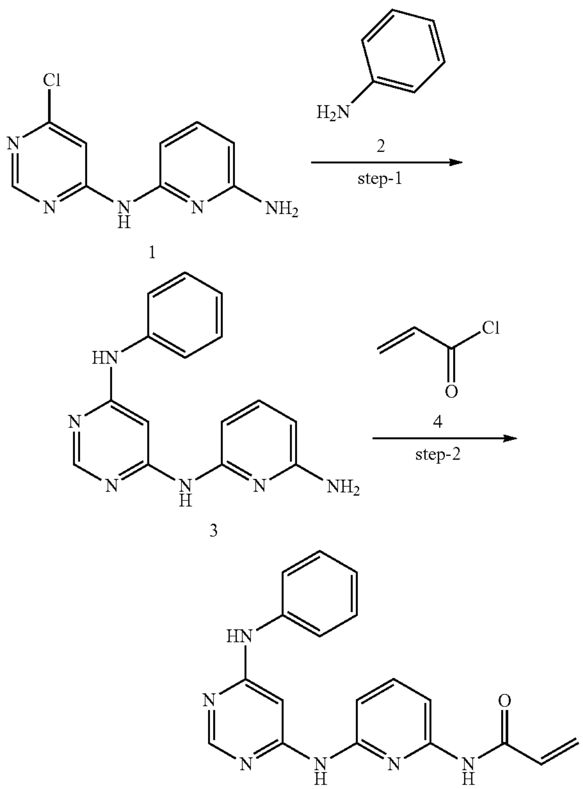


[0604] 4-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl] amino-aminobenzene (I^R-14) and acryloyl chloride gave N-4-[6-(4-phenoxyphenyl)amino-pyrimidin-4-yl]amino-phenylpropenamide (I-80), an off white solid. ^{1}H NMR (DMSO-d_6) δ ppm: 5.73 (dd, J=1.6 & 10.0 Hz, 1H), 6.08 (d, J=5.6 Hz, 1H), 6.24 (dd, J=2 & 16.8 Hz, 1H), 6.43 (dd, J=10 & 16.8 Hz, 1H), 6.95-6.70 (m, 4H), 7.09 (t, J=7.6 Hz, 1H), 7.35-7.39 (m, 2H), 7.45-7.49 (m, 2H), 7.55-7.61 (m, 4H), 8.23 (s, 1H), 9.08 (s, 1H), 9.1 (s, 1H), 10.1 (s, 1H); LCMS: m/e 423.8 (M+).

Example 14

[0605]

SCHEME 14a



I-72

Synthesis of N-6-(6-(phenylamino)pyrimidin-4-ylamino)pyridine-2-yl)acrylamide (I-72)

Step-1

[0606] A solution of N^2-(6-chloropyrimidin-4-yl)pyridine-2,6-diamine (1) (0.25 g, 1.1 mmol) and aniline (2) (0.16 g, 1.7 mmol) in n-BuOH (25 mL) was heated at 120° C. for 12 h in a pressure tube. The reaction mixture was cooled, was dissolved in methanol (10 mL) and was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (35 mL) and was washed successively with 10% sodium bicarbonate solution (20 mL), water (20 mL), and saturated brine (20 mL). The ethyl acetate extract was dried over Na_2SO_4 and was concentrated under reduced pressure to give a residue, which was triturated with diethyl ether to give (260 mg, 83%) N^4-(6-aminopyridin-2-yl)-N^6-phenylpyrimidine-4,6-diamine (3) as an off-white solid.

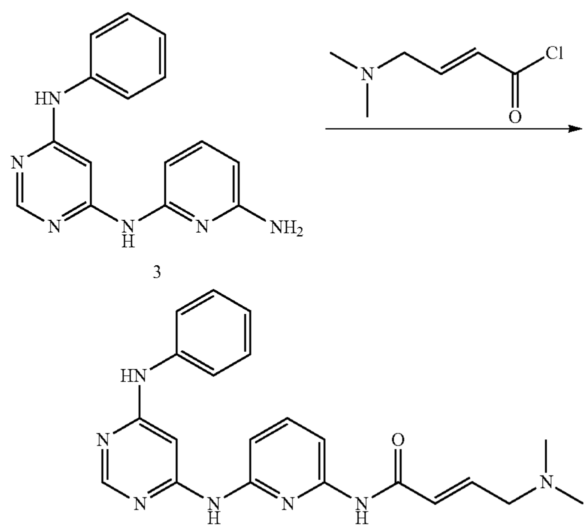
Step-2

[0607] To a stirred solution of 3 (0.2 g, 0.7 mmol) in NMP (10 mL) was added acryloyl chloride (0.097 g, 1 mmol), drop wise at 0° C. The reaction mixture was allowed to stir at the same temperature for 20 min and then warmed to rt for 1.5 h. It was quenched with 10% sodium bicarbonate solution (4 mL) and was extracted with ethyl acetate (2×35 mL). The combined ethyl acetate layer was washed with water (20 mL), saturated brine (20 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, Petroleum ether/EtOAc: 90/10) to give N-6-(6-(phenylamino)pyrimidin-4-ylamino)pyridine-2-yl)acrylamide (I-72) as a brown solid. ^{1}H NMR (DMSO-d_6) δ ppm: 5.80 (dd, J=1.84 & 10.12 Hz, 1H), 6.31 (dd, J=1.8 & 16.96 Hz, 1H), 6.64 (dd, J=10.08 & 16.88 Hz, 1H), 6.96 (t, J=7.32 Hz, 1H), 7.15-7.20 (m, 1H), 7.28 (t, J=7.52 Hz, 2H), 7.34 (s, 1H), 7.60-7.70 (m, 4H), 8.30 (s, 1H), 9.08 (s, 1H), 9.66 (s, 1H), 10.06 (s, 1H); LCMS: m/e 332.6 (M+).

Example 15

[0608]

SCHEME 15a



I-73

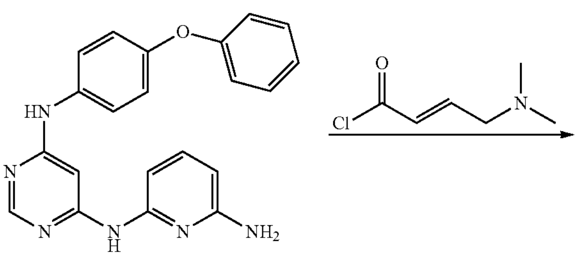
Synthesis of (E)-4-(dimethylamino)-N-(6-(6-(phenylamino)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-73)

[0609] To a stirred solution of acetonitrile (20 mL) and DMF (0.05 mL) under N_2 was added N,N-dimethylamino crotonic acid hydrochloride (0.47 g, 2.8 mmol). After 10 min this solution was cooled to 0-5° C. Oxalyl chloride (0.44 g, 3.5 mmol) was added and the reaction mixture was maintained at 0-5° C. for 30 min. It was allowed to warm to rt and stirring was continued for 2 h. It was then heated to 40° C. for 5 min and again brought to rt and stirred for 10 min to get a light greenish colored solution of dimethylaminocrotonyl chloride that was used as such for next step. To a stirred solution of N^4-(6-aminopyridin-2-yl)-N^6-phenylpyrimidine-4,6-diamine (0.2 g, 0.7 mmol) in NMP (10 mL) was added dropwise under N_2 atmosphere at 0° C. the solution of dimethylaminocrotonyl chloride. The reaction mixture was maintained at this temperature for 30 min and was warmed to rt and stirred for 2 h. The mixture was quenched with sodium bicarbonate solution (1 mL) and was extracted with EtOAc (2×35 mL). The combined ethyl acetate extract was washed with water (20 mL) and brine (20 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 60-120, product was eluted at 4-6% methanol in chloroform) to give (E)-4-(dimethylamino)-N-(6-(6-(phenylamino)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-73) as a yellowish solid. ^{1}H NMR (DMSO-d_6) δ ppm: 2.24 (s, 6H), 3.13 (d, J=5.76 Hz, 2H), 6.56 (d, J=15.52 Hz, 1H), 6.80 (d, J=15.36 Hz, 1H), 6.95 (t, J=7.36 Hz, 1H), 7.11 (t, J=1.16 Hz, 1H), 7.29 (t, J=7.56 Hz, 2H), 7.58 (s, 1H), 7.65-7.7 (m, 4H), 8.31 (d, J=2.44 Hz, 1H), 9.23 (s, 1H), 9.68 (s, 1H), 10.16 (s, 1H); LCMS: m/e 390.3 (M+1).

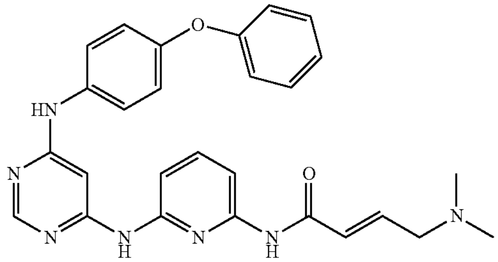
Example 16

[0610]

SCHEME 16a



I^R-11



I-64

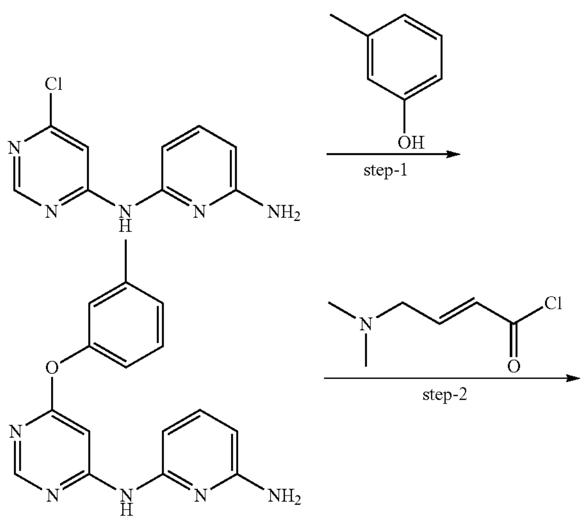
Synthesis of (E)-4-(dimethylamino)-N-(6-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-64)

[0611] A solution of dimethylaminocrotonyl chloride was prepared by reaction of dimethylaminocrotonic acid hydrochloride (0.36 g, 2.16 mmol) in CH_3CN (4 mL) containing DMF (1 drop) with oxalyl chloride (0.34 g, 2.70 mmol) according to the procedure in Example 15a. This acid chloride was added drop wise at 0° C. in to a stirred solution of N^4-(6-aminopyridin-2-yl)-N^6-4-phenoxyphenylpyrimidine-4,6-diamine (I^R-11) (0.2 g, 0.54 mmol) in NMP (8 mL). The reaction was stirred at 0° C. for 1 h, was diluted with EtOAc (5 mL), and was washed with 10% $NaHCO_3$ (2 mL), water (2 mL) and brine (2 mL). Drying over Na_2SO_4 followed by concentration under reduced pressure offered a residue which was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give (E)-4-(dimethylamino)-N-(6-(6-(4-phenoxyphenylamino) pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-64) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm: 2.18 (s, 6H), 3.05 (d, J=5.2 Hz, 2H), 6.48 (d, J=15.2 Hz, 1H), 6.79 (d, J=6 & 15.6 Hz, 1H), 6.96-6.99 (m, 4H), 7.07-7.15 (m, 2H), 7.36 (t, J=7.6 Hz, 2H), 7.42 (s, 1H), 7.64-7.7 (m, 4H), 8.30 (s, 1H), 9.12 (s, 1H), 9.69 (s, 1H), 10.07 (s, 1H); LCMS: m/e 482.4 (M+1).

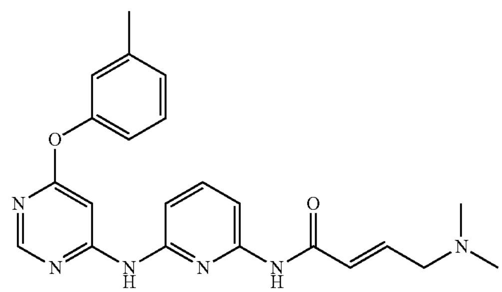
Example 17

[0612]

SCHEME 17a



I^R-12



I-74

Synthesis of (E)-4-(dimethylamino)-N-(6-(6-(3-methylphenoxy)pyrimidin-4-ylamino)pyridine-2-yl) but-2-enamide (I-74)

Step 1

[0613] To a solution of N^2-(6-chloropyrimidin-4-yl)pyridine-2,6-diamine (100 mg, 0.45 mmol) in DMF (1 mL) was added 3-methylphenol (88 mg, 0.8 mmol) and anhydrous K_2CO_3 (93 mg, 0.6 mmol). The reaction mixture was heated at 145° C. for 16 h. It was then cooled and DMF was removed under reduced pressure to get a yellow gummy residue. The residue was taken in EtOAc (20 mL) and washed with water (5 mL), brine (5 mL) and dried over Na_2SO_4. Filtration followed by concentration under reduced pressure afforded a yellow gum which was further purified by column chromatography (SiO_2, 60-120, EtOAc/hexane, 5/5) to give N^2-(6-(3-methylphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (I^R-12) (100 mg, 75%) as a pale yellow solid.

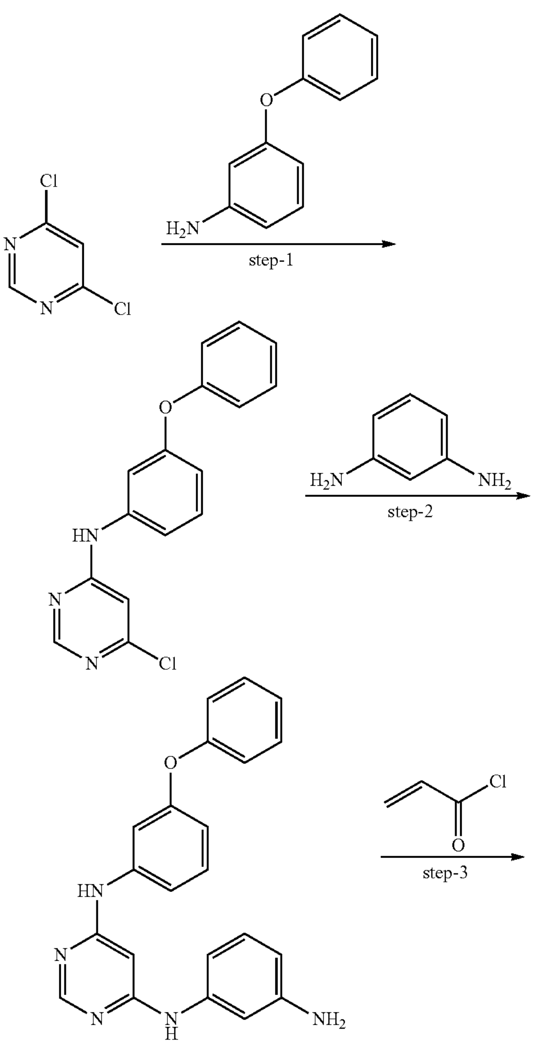
Step 2

[0614] To a solution of I^R-12 (75 mg, 0.25 mmol) in NMP (2 mL) was added dimethylaminocrotonyl chloride (168 mg, 1.02 mmol) at 0° C. The reaction mixture was stirred at room temperature for 1 h and was then quenched with $NaHCO_3$ solution (2 mL). The mixture was extracted with EtOAc (3×5 mL) and the combined EtOAc extract was washed with water (5 mL), brine (5 mL) and was dried over Na_2SO_4. Concentration under reduced pressure afforded a yellow oil, which was further purified by column chromatography (SiO_2, 60-120, Chloroform/Methanol, 9/1) to give (E)-4-(dimethylamino)-N-(6-(6-(3-methylphenoxy)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-74) as a light brown solid. ^1H NMR (CD_3OD) δ ppm: 2.35 (s, 6H), 2.38 (s, 3H), 3.25 (dd, J=1.2 & 6.6 Hz, 2H), 6.40 (d, J=13.16 Hz, 1H), 6.92-7.01 (m, 3H), 7.12 (t, J=8.12 Hz, 2H), 7.34 (t, J=7.84 Hz, 1H), 7.54 (s, 1H), 7.69 (t, J=6.24 Hz, 1H), 7.79 (d, J=8 Hz, 1H), 8.30 (s, 1H); LCMS: m/e 404.8 (M+).

Example 18

[0615]

SCHEME 18a



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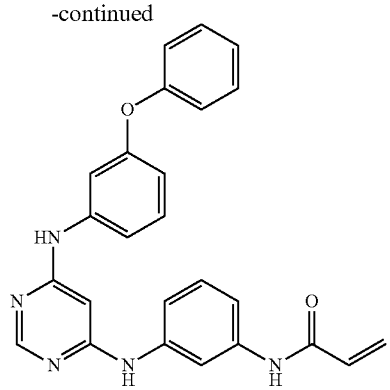


I-75

Synthesis of N-(3-(6-(3-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)acrylamide (I-75)

Step 1

[0616] A solution of 4,6-dichloropyrimidine (0.5 g, 3.3 mmol), 3-phenoxyaniline (0.75 g, 4 mmol) and DIPEA (0.65 g, 5 mmol) in n-butanol (5 mL) was subjected to microwave irradiation (110° C., 30 min). The reaction mixture was cooled, concentrated under reduced pressure and the residue was dissolved in EtOAc (10 mL). This solution was washed with water (5 mL) and brine (5 mL), and was dried over Na_2SO_4. Concentrated under reduced pressure gave a residue that was purified by column chromatography (SiO_2, 60-120, hexane/ethylacetate, 8/2) to give 6-chloro-N-(3-phenoxyphenyl)pyrimidine-4-amine (0.56 g, 56%) as an off-white solid.

Step 2

[0617] A solution of 6-chloro-N-(3-phenoxyphenyl)pyrimidine-4-amine (0.25 g, 0.8 mmol), 1,3-diaminobenzene (0.36 g, 3.3 mmol), n-butanol (10 mL) and conc. HCl (61 mg, 1.6 mmol) was subjected to microwave irradiation (160° C., 15 min). The reaction mixture was cooled, was concentrated under reduced pressure and the residue was taken in EtOAc (10 mL). The EtOAc solution was washed with water (5 mL) and brine (5 mL), and was dried over Na_2SO_4. Concentration under reduced pressure gave a residue that was purified by column chromatography (SiO_2, 60-120, hexane/ethyl acetate, 6/4) to give N^4-(3-aminophenyl)-N^6-(3-phenoxyphenyl)pyrimidine-4,6-diamine (0.1 g, 32%) as a brown solid.

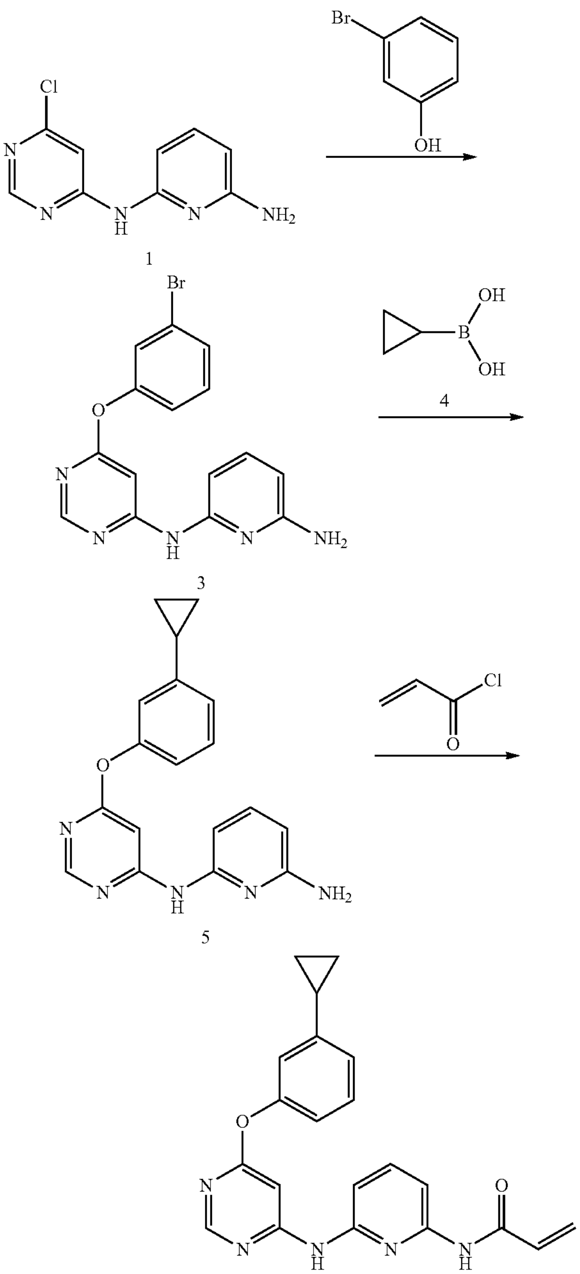
Step 3

[0618] To a stirred solution of N^4-(3-aminophenyl)-N^6-(3-phenoxyphenyl)pyrimidine-4,6-diamine (40 mg, 0.1 mmol), Et_3N (0.03 mL, 0.2 mmol) and NMP (0.4 mL) in CH_2Cl_2 (2 mL), at 0° C., was added acryloyl chloride (6) (29 mg, 0.3 mmol). The reaction mixture was allowed to come to rt and stirred at this temperature for 3 h. It was washed with 10% $NaHCO_3$ solution (2 mL), water (2 mL), brine (2 mL) and dried over Na_2SO_4. Filtration followed by concentration under reduced pressure offered a residue which was purified by column chromatography (SiO_2, 230-400, chloroform/methanol, 9/1) to gave N-(3-(6-(3-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)acrylamide (I-75) as a light brown solid. ^{1}H NMR (DMSO-d_6) δ ppm: 5.73 (dd, J=1.72 & 10 Hz, 1H), 6.18 (s, 1H), 6.24 (dd, J=1.92 & 17 Hz, 1H), 6.45 (dd, J=10.04 & 16.88 Hz, 1H), 6.54-6.57 (m, 1H), 7.01-7.05 (m, 2H), 7.11-7.15 (dd, J=0.88 & 7.48 Hz, 1H), 7.19-7.32 (s, 4H), 7.35-7.41 (m, 4H), 7.89 (s, 1H), 8.26 (s, 1H), 9.2 (s, 1H), 9.25 (s, 1H), 10.26 (s, 1H); LCMS: m/e 423.8 (M+1).

Example 19

[0619]

SCHEME 19a



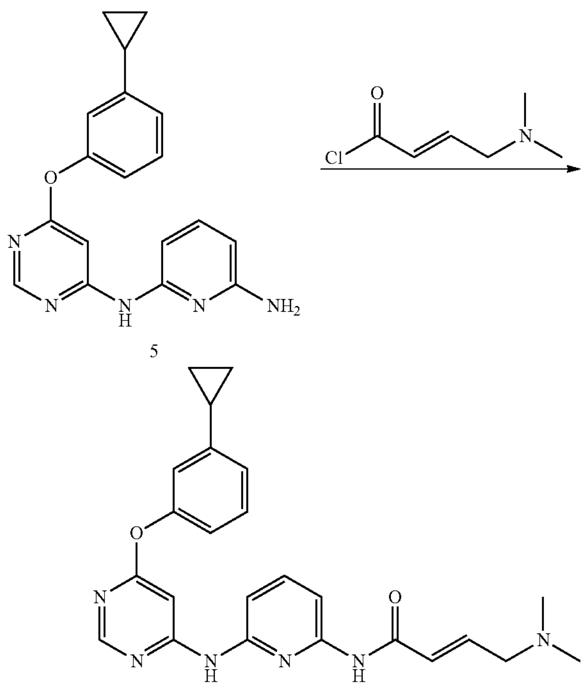
II-76

[0620] Compound I-76 can be prepared according to Scheme 19a by coupling intermediate 1 with 3-bromophenol, followed by boronic acid coupling of intermediates 3 and 4 to give intermediate 5. Intermediate 5 can then be treated with acryloyl chloride using a protocol similar to that in Example 6 to give compound I-76.

Example 20

[0621]

SCHEME 20a



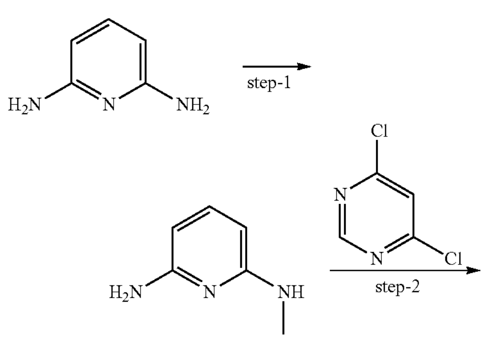
I-77

[0622] Compound I-77 can be prepared according to Scheme 20a by treatment of intermediate 5 with (E)-4-(dimethylamino)but-2-enoyl chloride using a protocol similar to that in Example 6.

Example 21

[0623]

SCHEME 21a



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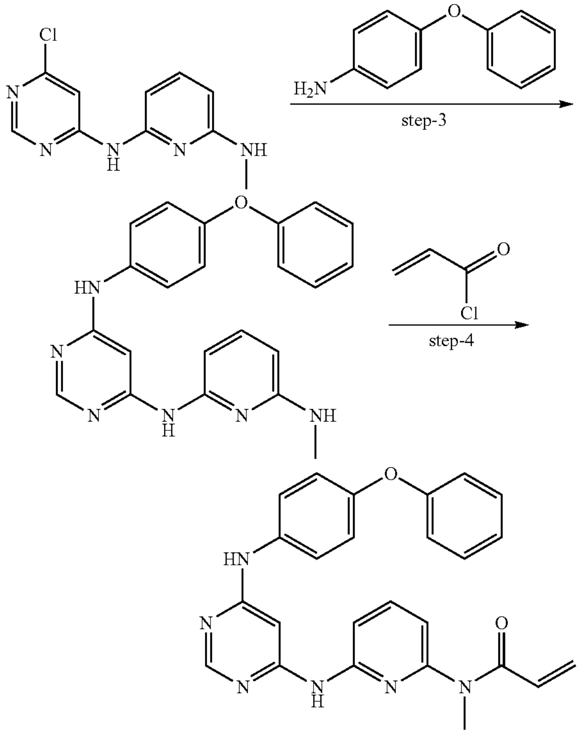


I-78

Synthesis of N-methyl-N-(6-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyridin-2-yl)acrylamide (I-78)

Step 1

[0624] To a solution of 2,6-diaminopyridine (10 g, 91.63 mmol) in dry THF (100 mL) was added K_2CO_3 (18.8 g, 136.23 mmol) and CH_3I (13 g, 91.63 mmol) and the reaction mixture was stirred at room temperature for 16 h. Water was added (10 mL) and the mixture was extracted with EtOAc (100 mL). The EtOAc layer was dried and was concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, chloroform) to give 2-methylamino-6-aminopyridine (1.1 g, 10%) as a brown solid.

Step 2

[0625] A mixture of 2-methylamino-6-aminopyridine (0.5 g, 4.04 mmol), 4,6-dichloropyrimidine (1.51 g, 10.13 mmol), DIPEA (1.5 g, 12.17 mmol) in n-butanol (5 mL) was heated at 120° C. for 16 h. The reaction mixture was cooled, was concentrated under reduced pressure and the residue was taken in dichloromethane (25 mL). The dichloromethane solution was washed with $NaHCO_3$ solution (2 mL), water (2 mL) and brine (2 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 60-120, petroleum ether/ethyl acetate, 6/4) to give N^2-(6-chloropyrimidin-4-yl)-N^6-methylpyridine-2,6,diamine (0.3 g, 33%) as a yellow solid.

Step 3

[0626] A solution of N^2-(6-chloropyrimidin-4-yl)-N^6-methylpyridine-2,6,diamine (0.3 g 1.27 mmol), 4-phenoxy aniline (0.28 g, 1.52 mmol) and conc. HCl (2 drops) in n-butanol (2 mL) was subjected to microwave irradiation (120° C., 1 h). The reaction mixture was cooled, was concentrated under reduced pressure, and the residue was diluted with CH_2Cl_2 (5 mL). The dichloromethane solution was washed with $NaHCO_3$ (2 mL), water (2 mL), and brine (2 mL), and was dried over Na_2SO_4. Filtration followed by concentration under reduced pressure gave a residue which was purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give N^4-(6-(methylamino) pyridine-2-yl)-N^6-(4-phenoxyphenyl)pyrimidine-4,6-diamine (0.2 g, 41%) as a light brown solid.

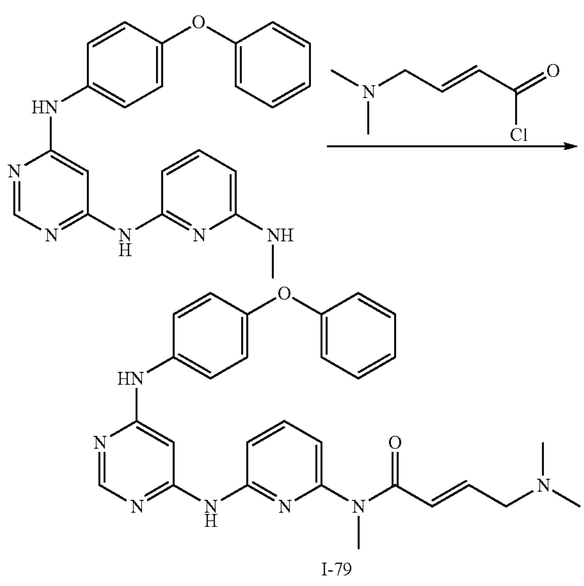
Step 4

[0627] To a solution of N^4-(6-(methylamino)pyridine-2-yl)-N^6-(4-phenoxyphenyl)pyrimidine-4,6-diamine (0.07 g, 0.18 mmol) in NMP (1 mL) was added acryloyl chloride (0.032 g, 0.36 mmol) at 0° C. and the reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with dichloromethane (2 mL), was washed with $NaHCO_3$ (1 mL), water (1 mL), and brine (1 mL). The dichloromethane solution was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give N-methyl-N-(6-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyridin-2-yl)acrylamide (I-78) as a yellow solid. ^1H NMR (DMSO-d_6) δ ppm: 3.22 (s, 3H), 5.60 (dd, J=2.56 & 9.76 Hz, 1H), 6.12-6.16 (m, 2H), 6.79 (d, J=7.56 Hz, 1H), 6.96 (d, J=8.64 Hz, 5H), 7.09 (t, J=7.32 Hz, 1H), 7.23 (s, 1H), 7.33-7.37 (m, 4H), 7.45 (d, J=8.2 Hz, 2H), 7.32 (t, J=7.8 Hz, 1H), 8.24 (s, 1H); LCMS: m/e 439.3 (M+1).

Example 22

[0628]

SCHEME 22a



I-79

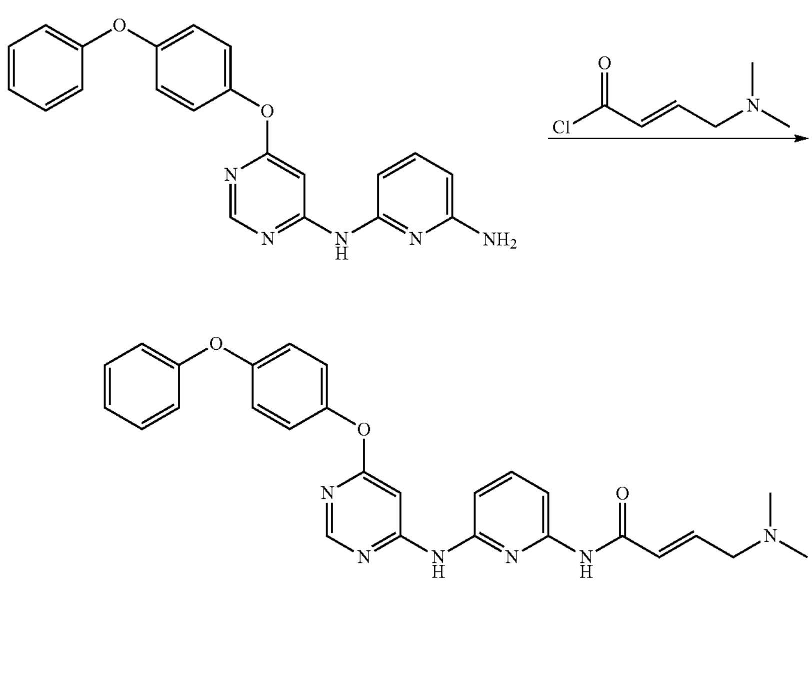
Synthesis of (E)-4-(dimethylamino)-N-methyl-N-(6-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-79)

[0629] To a solution of dimethylaminocrotonic acid hydrochloride (0.120 g, 0.72 mmol) in CH_3CN (1.4 mL) was added DMF (1 drop) followed by oxalyl chloride (0.07 mL, 0.91 mmol) at 0° C. under nitrogen atmosphere. The reaction was allowed to stir at this temperature for 30 min and then at rt for 2 h. This acid chloride was added, drop wise, at 0° C. in to a stirred solution of N^4-(6-(methylamino)pyridine-2-yl)-N^6-(4-phenoxyphenyl)pyrimidine-4,6-diamine (0.07 g, 0.18 mmol) in NMP (2.8 mL). The reaction was allowed to stir at 0° C. for 1 h, diluted with EtOAc (5 mL), and washed with 10% $NaHCO_3$ (2 mL), water (2 mL) and brine (2 mL). drying over Na_2SO_4 followed by concentration under reduced pressure gave a residue which was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give (E)-4-(dimethylamino)-N-methyl-N-(6-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyridine-2-yl)but-2-enamide (I-79) as a yellow solid. ^1H NMR (DMSO-d_6) δ ppm: 2.05 (s, 6H), 2.91 (d, J=6 Hz, 2H), 3.27 (s, 3H), 6.08 (d, J=15.2 Hz, 1H), 6.65 (dd, J=5.6 & 14.8 Hz, 1H), 6.82 (d, J=7.6 Hz, 1H), 6.97-7.00 (m, 4H), 7.10 (t, J=7.2 Hz, 1H), 7.20 (s, 1H), 7.35-7.39 (m, 2H), 7.46 (d, J=8 Hz, 1H), 7.53 (d, J=8.8 Hz, 2H), 7.75 (t, J=8 Hz, 1H), 8.30 (s, 1H), 9.30 (s, 1H), 9.95 (s, 1H); LCMS: m/e 496 (M+1).

Example 23

[0630]

Scheme 23a



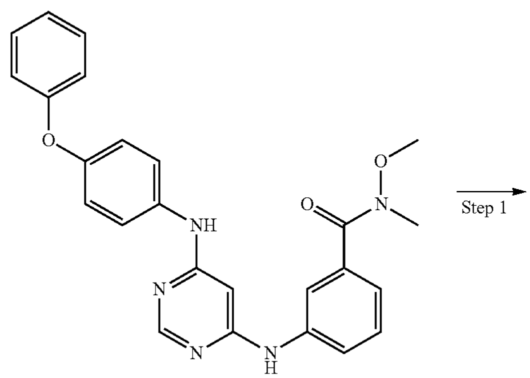
Synthesis of (E)-4-dimethylamino)-N-(6-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)pyridin-2-yl)but-2-enamide (I-82)

[0631] To a stirred solution of N^2-(6-(4-phenoxyphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (0.65 g, 1.75 mmol) in NMP (10 mL) was added dimethylaminocrotonyl chloride (1.026 g, 7 mmol) at 0° C. The reaction mixture was allowed to come to room temperature and kept at it for 1 h. It was diluted with dichloromethane (10 mL), was washed with $NaHCO_3$ solution (2 mL) and water (2 mL), and dried over Na_2SO_4. The dichloromethane solution was filtered and was concentrated under reduced pressure to give a residue that was purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give (E)-4-dimethylamino)-N-(6-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)pyridin-2-yl)but-2-enamide (I-82) as an off white solid. ^1H NMR (DMSO-d_6) δ ppm: 2.19 (s, 6H), 3.08 (d, J=5.52 Hz, 2H), 6.50 (d, J=15.4 Hz, 1H), 6.77 (td, J=5.92 & 15.4 Hz, 1H), 7.00-7.07 (m, 5H), 7.15 (t, J=7.36 Hz, 1H), 7.21 (dd, J=2.2 & 8.92 Hz, 2H), 7.41 (t, J=7.52 Hz, 2H), 7.68 (t, J=7.96 Hz, 1H), 7.77 (d, J=7.96 Hz, 1H), 7.95 (s, 1H), 8.35 (s, 1H), 10.20 (s, 1H), 10.40 (s, 1H); LCMS: m/e 483 (M+1).

Example 24

[0632]

Scheme 24a



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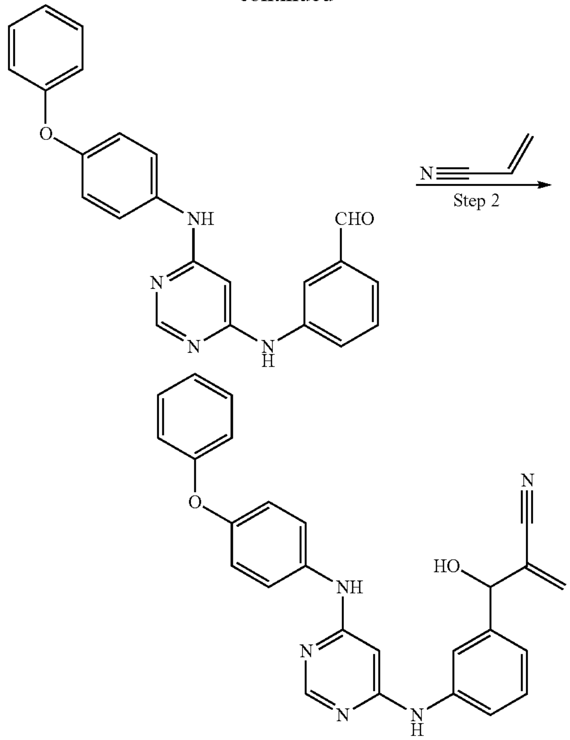


I-84

Synthesis of 2-(hydroxy(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)methyl)acrylamide (I-84)

Step 1

[0633] To a stirred solution of N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (0.75 g, 1.7 mmol) in THF (10 mL) was added LAH (3.4 mL, 3.4 mmol, 1 M solution in THF) at −60° C. The reaction mixture was stirred at −60° C. for 1 h, was quenched with Na_2SO_4 solution (2 mL) and was extracted with ethyl acetate (10 mL). The organic layer was separated and washed with water (2 mL) and with brine solution (2 mL) and was dried over anhydrous Na_2SO_4. Filtration followed by concentration under reduced pressure gave a residue that was purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzaldehyde (0.6 g, 92%) as an off yellow solid.

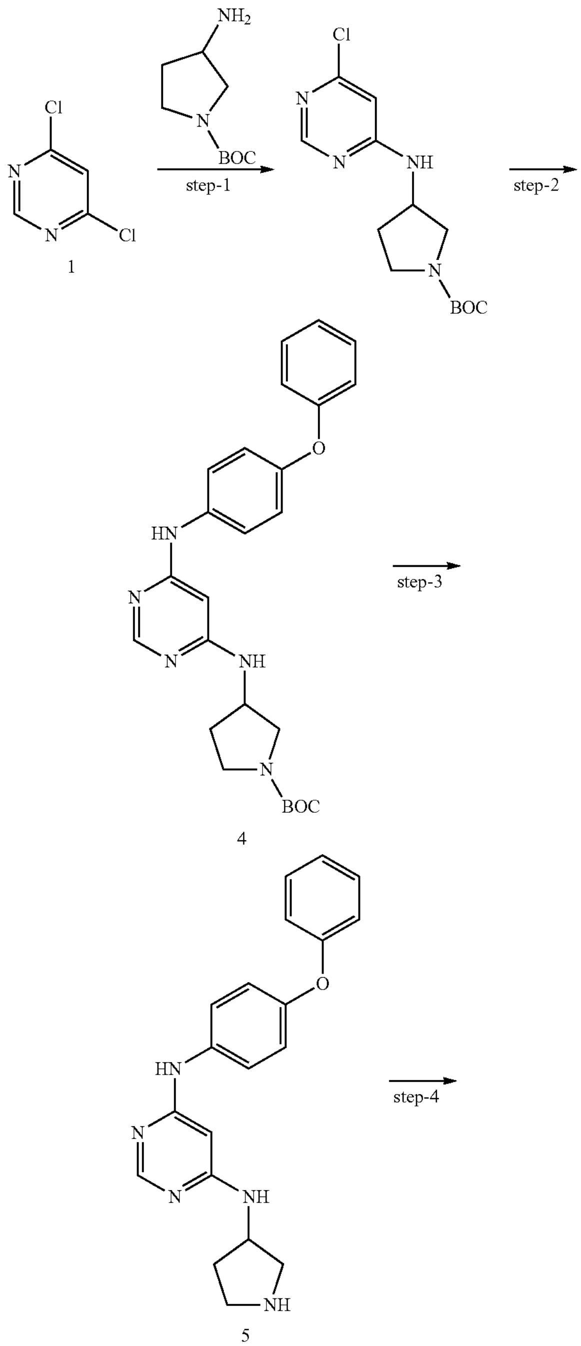
Step 2

[0634] To a stirred solution of 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzaldehyde (100 mg, 0.26 mmol) and acrylonitrile (36 mg, 0.52 mmol) in 1,4-dioxane/H_2O (0.5 mL/0.5 mL) was added DABCO (29 mg, 0.26 mmol) at rt. Stirring was continued at room temperature for 48 h after which time the reaction mixture was concentrated under reduced pressure. The residue obtained was further purified by column chromatography (SiO_2, 60-120, pet ether/ethyl acetate, 6/4) to give 2-(hydroxy(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)methyl)acrylamide (I-84) as a white solid. ^1H NMR (DMSO-d_6) δ ppm: 5.33 (s, 1H), 6.06 (s, 1H), 6.20 (s, 1H), 6.25 (s, 1H), 6.80 (s, 1H), 6.88 (s, 1H), 6.95-7.05 (m, 4H), 7.09-7.13 (m, 2H), 7.16 (bd, J=7.92 Hz, 1H), 7.32-7.39 (m, 5H), 7.47 (s, 1H), 8.31 (s, 1H); LCMS: m/e 436 (M+1).

Example 25

[0635]

Scheme 25a



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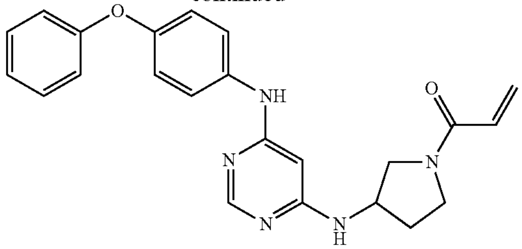


I-85

Synthesis of 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyrrolidin-1-yl)prop-2-en-1-one (I-85)

Step 1

[0636] A solution of 4,5-dichloropyrimidine (0.6 g, 4.02 mmol), 3-amino-Boc-pyrrolidine (0.5 g, 2.6 mmol) and DIPEA (1.73 g, 13.3 mmol) in n-butanol (5.0 mL) was heated in a pressure tube (120° C., 12 h). It was cooled, quenched with water (10 mL), and was extracted with EtOAc (2×25 mL). The combined EtOAc extract was washed with water (5 mL), brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford tert-butyl 3-(6-chloropyrimidin-4-ylamino)pyrrolidine-1-carboxylate (0.4 g, 50%) as a yellow solid.

Step 2

[0637] To a stirring solution of tert-butyl 3-(6-chloropyrimidin-4-ylamino)pyrrolidin-1-carboxylate (0.5 g, 1.6 mmol) and 4-phenoxy aniline (0.309 g, 1.6 mmol) in ethanol (4 mL) was added acetic acid (0.1 mL) and the reaction mixture was heated at 100° C. for 36 h. The reaction mixture was cooled, ethanol was removed under reduced pressure and the residue was taken in ethyl acetate (10 mL). It was washed with $NaHCO_3$ solution (2 mL), brine (2 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to yield tert-butyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyrrolidine-1-carboxylate (0.3 g, 42.8%) as a white solid.

Step 3

[0638] To a stirred solution of tert-butyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyrrolidin-1-carboxylate (0.1 g, 0.2 mmol) in dry CH_2Cl_2 (2.0 mL) at 0° C. was added CF_3COOH (2 mL, 20 vol.) and the reaction mixture was kept at this temperature for 30 min. It was allowed to come to rt and stir at this temperature for 3 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with water (2 mL), basified with $NaHCO_3$ solution, and was extracted with ethyl acetate (2×8 mL). The combined ethyl acetate extract was washed with water (2 mL) and brine (2 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure to give N^4-(4-phenoxyphenyl)-N^6-(pyrrolidin-3-yl)pyrimidine-4,6-diamine (0.025 g, 32.4%) as a light a brown solid.

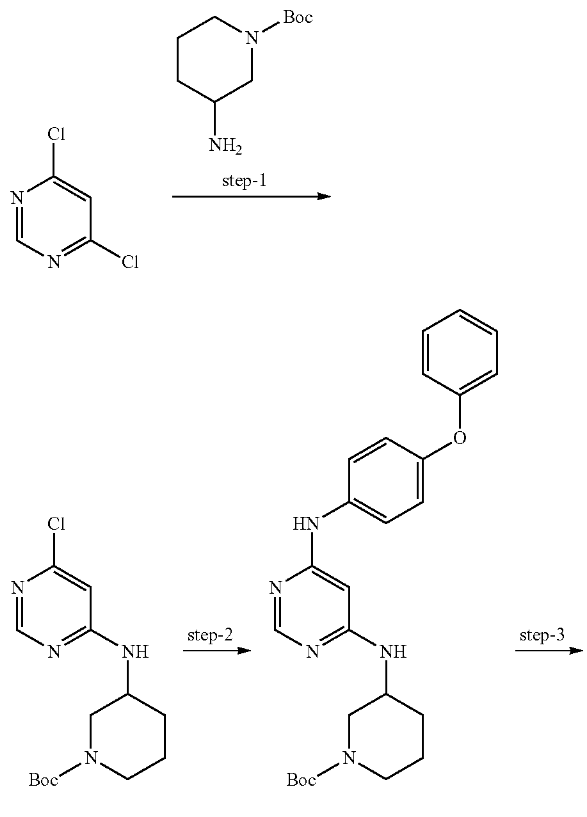
Step 4

[0639] To a stirred solution of N^4-(4-phenoxyphenyl)-N^6-(pyrrolidin-3-yl)pyrimidine-4,6-diamine (0.13 g, 0.3 mmol) in THF (1.5 mL) at −60° C. were added DIPEA (0.07 g, 0.5 mmol) and acryloyl chloride (1 M solution in THF, 0.3 mL, 0.3 mmol), and the reaction mixture was stirred at −60° C. for 5 min. The reaction mixture was quenched by adding water and it was extracted with EtOAc (2×5 mL). The combined EtOAc extract was washed with brine (3 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue obtained was purified by column chromatography (SiO_2, 230-400, chloroform/methanol: 98/2) to give 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)pyrrolidin-1-yl)prop-2-en-1-one (I-85) as a light green solid. NMR (DMSO-d_6) δ ppm: 1.83-1.86 & 1.90-1.94 (m, 1H), 2.07-3.0 & 2.16-2.20 (m, 1H), 3.38-3.86 (m, 4H), 4.2-4.75 & 4.35-4.5 (bs, 1H), 5.65 (dt, J=2 & 10 Hz, 1H), 5.78 (d, J=3.6 Hz, 1H), 6.11 & 6.15 (dd, J=2.4 & 7.0 Hz & dd, J=2.4 & 7.2 Hz, 1H), 6.51-6.64 (m, together 1H), 6.93-7.00 (m, 4H), 7.07-7.18 (m, 2H), 7.36 (t, J=8 Hz, 2H), 7.51 (d, J=8 Hz, 2H), 8.12 (s, 1H), 8.93 (s, 1H); LCMS: m/e 401.8 (M+1).

Example 26

[0640]

Scheme 26a



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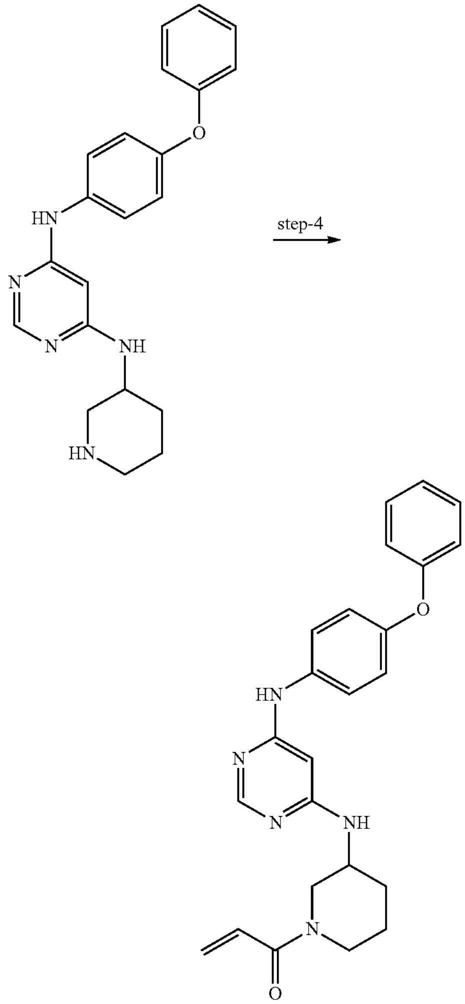


I-86

Synthesis of 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)piperidin-1-yl)prop-2-en-1one (I-86)

Step 1

[0641] A solution of 4,6-dichloropyrimidine (0.1 g, 0.671 mmol), 3-amino-Boc-piperidine (0.16 g, 0.80 mmol) and DIPEA (0.086 g, 6.71 mmol) in n-butanol (5.0 mL) was heated in a pressure tube (120° C., 12 h). The solution was cooled, was quenched with water (2 mL) and was extracted with EtOAc (2×15 mL). The combined EtOAc extract was washed with water (5 mL), brine (5 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure to give tert-butyl 3-(6-chloropyrimidin-4-ylamino)piperidine-1-carboxylate, which was dried under high vacuum and was used as such for next step without further purification.

Step 2

[0642] A solution of tert-butyl 3-(6-chloropyrimidin-4-ylamino)piperidine-1-carboxylate (0.15 g, 0.48 mmol), 4-phenoxyaniline (0.089 g, 0.48 mmol), $Pd(OAc)_2$ (0.010 g, 0.048 mmol), BINAP (0.014 g, 0.024 mmol) and Cs_2CO_3 (0.39 g, 1.2 mmol) in degassed toluene (toluene was purged with N_2 for 15 min) was heated for 12 h at 100° C. under N_2 atmosphere. The reaction mixture was cooled, was diluted with EtOAc (20 mL) and was washed with water (4 mL) and brine (2 mL) and was dried over Na_2SO_4. The crude product obtained was purified by column chromatography (SiO_2, 230-400, chloroform/methanol: 99/1) to give tert-butyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)piperidine-1-carboxylate (90 mg, 40.9%) as a light yellow solid.

Step 3

[0643] To a stirred solution of tert-butyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)piperidine-1-carboxylate (110 mg, 0.238 mmol) in dry CH_2Cl_2 (1.0 mL) at 0° C. was added CF_3COOH (0.5 mL, 5 vol) and the reaction mixture was kept at this temperature for 30 min. It was allowed to come to rt and to stir at this temperature for 3 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with water (2 mL), was basified with $NaHCO_3$ solution, and was extracted with ethyl acetate (2×8 mL). The combined ethyl acetate extract was washed with water (2 mL), brine (2 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give N^4-(4-phenoxyphenyl)-N^6-(piperidin-3-yl)pyrimidine-4,6-diamine (0.07 g, 81%) as a light yellow solid.

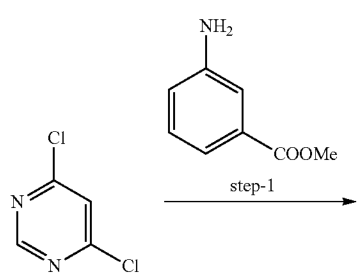
Step 4

[0644] To a stirred solution of N^4-(4-phenoxyphenyl)-N^6-(piperidin-3-yl)pyrimidine-4,6-diamine (0.025 g, 0.069 mmol) in NMP (0.5 mL) at 0° C. was added acryloyl chloride (0.007 g, 0.083 mmol), and the reaction mixture was stirred at 0° C. for 5 min. The reaction mixture was quenched by adding 10% $NaHCO_3$ solution and it was extracted with EtOAc (2×5 mL). The combined EtOAc extract was washed with water (3 mL) and brine (3 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue obtained was purified by column chromatography (SiO_2, 230-400, chloroform/methanol: 98/2) to give 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)piperidin-1-yl)prop-2-en-1one (I-86) as an off white solid. ^1H NMR (MeOD) δ ppm: 1.5-1.75 (m, 2H), 1.80-2.00 (m, 1H), 2.0-2.20 (m, 1H), 2.70-2.90 (m, 1H), 2.90-3.05 (m, 1H), 3.80-4.00 (m, 2H), 4.30-4.45 (m, 1H), 5.65 & 5.75 (d, J=10.8 Hz & d, J=10.8 Hz respectively, together 1H), 5.81 (d, J=10.8 Hz, 1H), 6.14 & 6.18 (d, J=17.2 Hz & d, J=19.2 Hz respectively, together 1H), 6.60-6.70 & 6.70-6.85 (m, together 1H), 6.97-7.00 (m, 4H), 7.04 (t, J=7.6 Hz, 1H), 7.32-7.38 (m, 4H), 8.04 & 8.07 (s, together 1H); LCMS: m/e 416.1 (M+1).

Example 27

[0645]

SCHEME 27a



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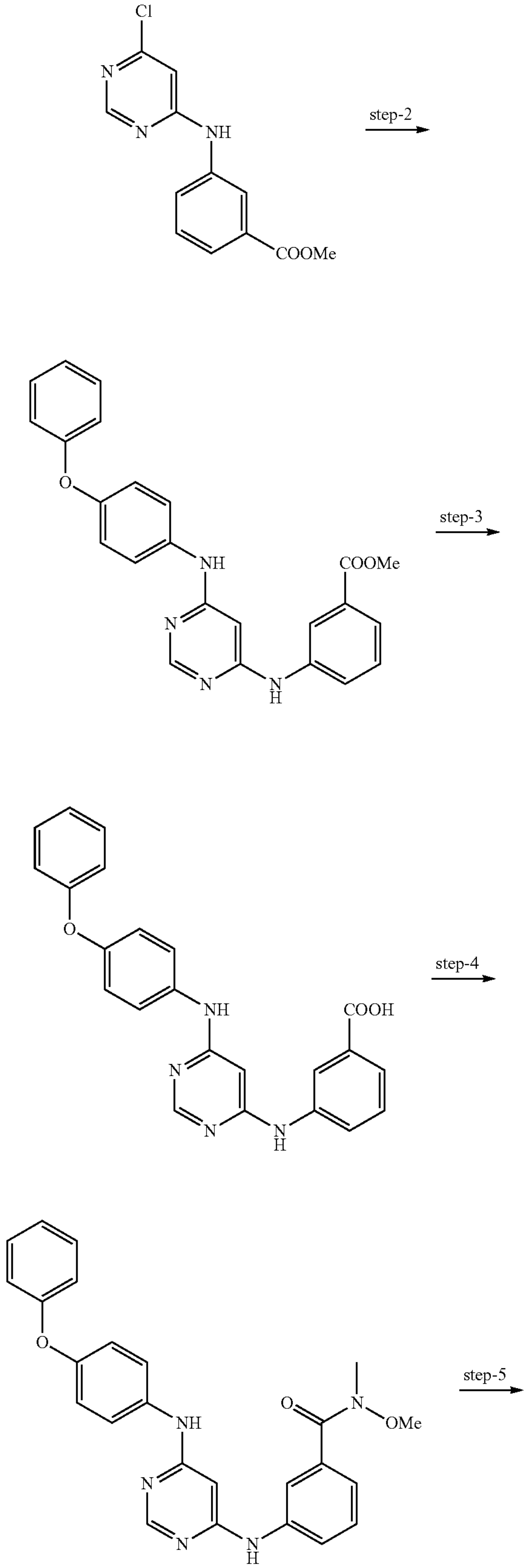

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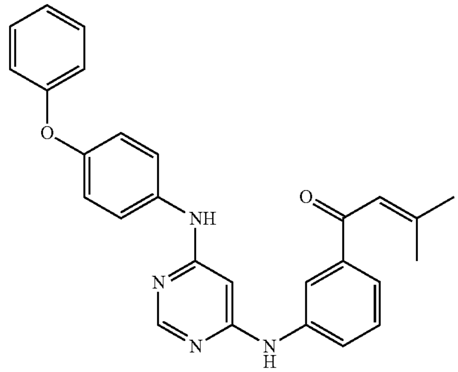


I-87

Synthesis of 3-methyl-1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)but-2-en-1-one (I-87)

Step 1

[0646] To a stirred solution of 4,6-dichloropyrimidine (0.5 g, 3.7 mmol) in n-butanol (10 mL) was added methyl 3-aminobenzoate (0.498 g, 3.7 mmol) and DIPEA (0.65 g, 5.0 mmol) and the reaction mixture was heated at 110° C. for 12 h. It was cooled and excess n-butanol was removed under reduced pressure. The residue was extracted with EtOAc (2×30 mL) and the combined EtOAc extract was washed with water (5 mL), brine (2.5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was stirred with pet ether (30 mL) for 30 min, the pet ether removed by decantation and the solid obtained was dried under high vacuum to give methyl 3-(6-chloropyrimidin-4-ylamino)benzoate (0.3 g, 34%) as light brown solid.

Step 2

[0647] To a stirring solution of methyl 3-(6-chloropyrimidin-4-ylamino)benzoate (1.0 g, 3.8 mmol) and 4-phenoxy aniline (0.703 g, 3.8 mmol) in ethanol (5 mL) was added acetic acid (0.22 mL) and the reaction mixture was heated at 100° C. for 48 h. The reaction mixture was cooled, ethanol was removed under reduced pressure and the residue was dissolved in ethyl acetate (50 mL). It was washed with $NaHCO_3$ solution (5 mL) and brine (5 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to yield methyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzoate (1 g, 66%) as an off-white solid.

Step 3

[0648] To a stirred solution of methyl 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzoate (0.3 g, 0.72 mmol) in methanol/THF (2/2, 4 mL) was added LiOH (0.122 g, 2.9 mmol) in H_2O (4 mL) and the reaction mixture was stirred at rt for 2 h. It was concentrated under reduced pressure the residue was diluted with water (2 mL) and was extracted with dichloromethane (5 mL). The aqueous layer was separated and was acidified with 1.5 N HCl (pH ~5-6) to get a white precipitate, which was collected by filtration and dried under vacuum to give 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzoic acid (0.2 g 69%) as a white solid.

Step 4

[0649] To a stirred solution of 3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzoic acid (0.05 g, 0.12 mmol) in DMF (2 mL) were added MeNH-OMe.HCl (0.0084 g, 0.12 mmol), EDCI.HCl (0.0361 g, 0.18 mmol), HOBT (0.0084 g, 0.062 mmol) and DIPEA (0.023 g, 0.18 mmol). The reaction mixture was stirred at room temperature for 1 h and was quenched with water. A white solid was isolated by filtration and dried under vacuum to give N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (0.025 g, 44.5%) as a white solid.

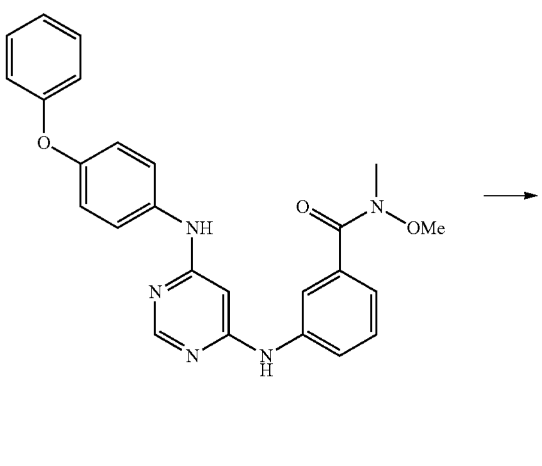
Step 5

[0650] To a stirred solution of N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (50 mg, 0.11 mmol) in THF (0.5 mL) at 0° C. was added 2-methylpropenylmagnesium bromide (1.1 mL, 0.55 mmol, 0.5 M in THF). The reaction mixture was allowed to stir at room temperature for 30 min. It was quenched with sat. NH_4Cl solution (0.5 mL) and was extracted with EtOAc (3×2 mL). The combined organic layer was washed with brine, was dried over anhydrous Na_2SO_4, was filtered and was concentrated under reduced pressure to give a white solid, which was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give 3-methyl-1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)but-2-en-1-one (I-87) as an off white solid. ^1H NMR ($CDCl_3$) δ ppm: 2.01 (s, 3H), 2.20 (s, 3H), 6.14 (s, 1H), 6.71 (s, 1H), 6.95 (s, 1H), 6.99-7.02 (m, 5H), 7.11 (t, J=7.36 Hz, 1H), 7.26-7.28 (m, 2H), 7.34 (t, J=7.56 Hz, 2H), 7.40-7.53 (m, 2H), 7.65 (d, J=7 Hz, 1H), 7.86 (s, 1H), 8.32 (s, 1H); LCMS: m/e 437.2 (M+1).

Example 28

[0651]

Scheme 28a



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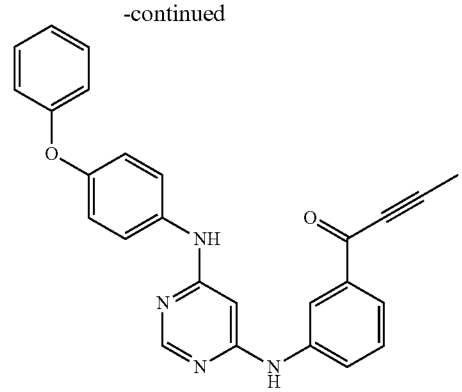


I-88

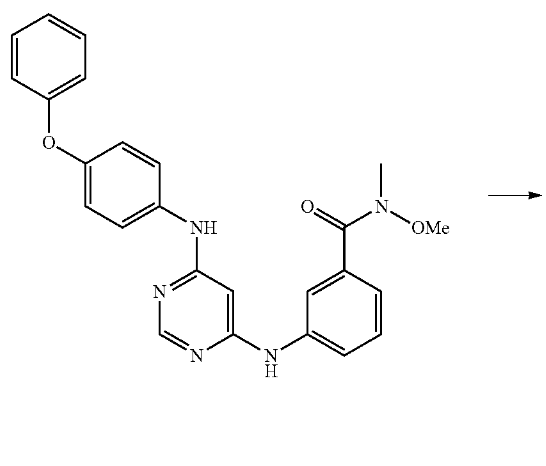
Synthesis of 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)but-2-yn-1-one (I-88)

[0652] To a stirred solution of N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (50 mg, 0.11 mmol) in THF (0.5 mL) at 0° C. was added a THF solution of 1-butynylmagnesium bromide (1.1 mL, 1.1 mmol). The reaction mixture was allowed to come to rt and was stirred at rt for 30 min. The reaction mixture was quenched with saturated NH_4Cl solution (0.5 mL) and was extracted with EtOAc (2×3 mL). The combined EtOAc layer was washed with brine, was dried over anhydrous Na_2SO_4, was filtered and was concentrated under reduced pressure to give a white solid, which h was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give 1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)but-2-yn-1-one (I-88) as an off-yellow solid. ^1H NMR (DMSO-d_6) δ ppm: 2.22 (s, 3H), 6.16 (s, 1H), 6.97 (d, J=8.6 Hz, 2H), 7.01 (d, J=8.8 Hz, 2H), 7.1 (t, J=7.2 Hz, 1H), 7.35-7.39 (m, 2H), 7.48 (t, J=8 Hz, 1H), 7.56 (d, J=8.8 Hz, 2H), 7.66 (d, J=7.2 Hz, 1H), 7.95 (d, J=7.6 Hz, 1H), 8.32 (s, 1H), 8.39 (s, 1H), 9.22 (s, 1H), 9.47 (s, 1H); LCMS: m/e 421.1 (M+1).

Example 29

[0653]

Scheme 29a



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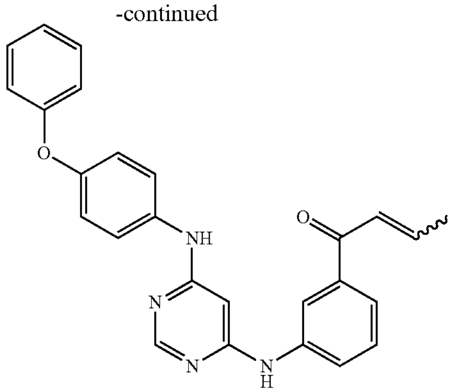


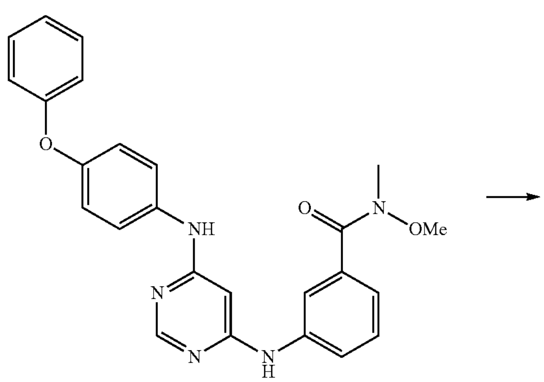
I-89

Synthesis of (E,Z)-1-(3-(6-(4-phenoxyphenylamino) pyrimidin-4-ylamino)phenyl)but-2-en-1-one (I-89)

[0654] To a stirred solution of N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (50 mg, 0.11 mmol) in THF (0.5 mL) at 0° C. was added a THF solution of propenylmagnesium bromide (2.2 mL, 1.1 mL, 0.5 M soln. in THF). The reaction mixture was allowed to stir at room temperature for 30 min. It was quenched with saturated NH_4Cl solution (0.5 mL) and was extracted with EtOAc (2×3 mL). The combined organic layer was washed with brine, was dried over anhydrous Na_2SO_4, was filtered and was concentrated under reduced pressure to get a white solid, which was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give (E,Z)-1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)but-2-en-1-one (I-89) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm: 1.98 (dd, J=1.6 & 6.8 Hz, 3H) & 2.13 (dd, J=1.6 & 7.2 Hz, 3H), 6.10-6.13 (m, 1H), 6.75-6.90 (m, 1H), 6.90-7.13 (m, 7H), 7.25-7.27 (m, 1H), 7.32-7.34 (m, 2H), 7.34-7.50 (m, 2H), 7.63-7.65 (m, 1H), 7.86-7.88 (m, 1H), 8.32 (s, 1H); LCMS: m/e 423 (M+1).

Example 30

[0655]



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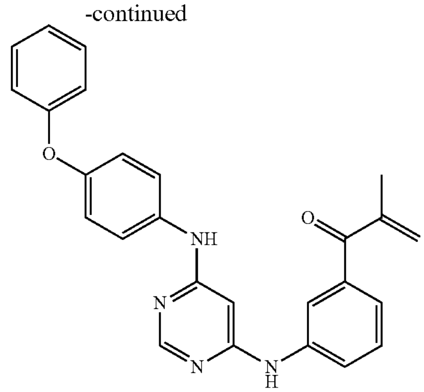


I-83

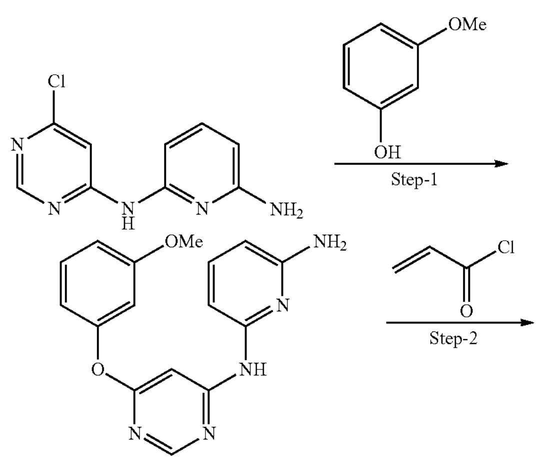
Synthesis of 2-methyl-1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)prop-2-en-1-one (I-83)

[0656] To a N-methoxy-N-methyl-3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)benzamide (0.150 g, 0.340 mmol) at 0° C. was added 2-methylpropenylmagnesium bromide (6.8 mL, 3.4 mmol, 0.5 M solution in THF). The reaction mixture was allowed to stir at room temperature for 30 min. It was quenched with saturated NH_4Cl solution (0.5 mL) and was extracted with EtOAc (2×3 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to get a white solid, which was further purified by column chromatography (SiO_2, 60-120, product getting eluted in methanol/chloroform: 2/98) to give 2-methyl-1-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)prop-2-en-1-one (I-83) as a white solid. ^{1}H NMR (DMSO-d_6) δ ppm: 1.99 (s, 3H), 5.64 (s, 1H), 6.03 (s, 1H), 6.14 (s, 1H), 6.96-7.02 (m, 4H), 7.10 (t, J=7.6 Hz, 1H), 7.25 (d, J=7.6 Hz, 1H), 7.35-7.43 (m, 3H), 7.56 (d, J=8.8 Hz, 2H), 7.88 (d, J=8 Hz, 1H), 7.92 (s, 1H), 8.29 (s, 1H), 9.21 (s, 1H), 9.38 (s, 1H); LCMS: 423 We (M+1).

Example 31

[0657]

Scheme 31a



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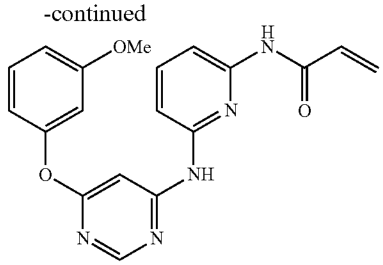


I-90

Synthesis of N-(6-(6-(3-methoxyphenoxy)pyrimidin-4-ylamino)pyridine-2-yl)acrylamide (I-90)

Step 1

[0658] To a solution of N^2-(6-chloropyrimidin-4-yl)pyridine-2,6-diamine (200 mg, 0.90 mmol) in dry DMF (2 mL) was added 3-methoxyphenol (112 mg, 0.90 mmol) and anhydrous K_2CO_3 (186 mg, 1.353 mmol). The reaction mixture was heated at 100° C. for 16 h under N_2 atmosphere. It was then cooled and DMF removed under reduced pressure to give a yellowish gummy residue that was taken in EtOAc (10 mL). It was washed with water (5 mL), brine (5 mL), dried over Na_2SO_4 and then concentrated under reduced pressure to give a crude product. This was further purified by column chromatography (SiO_2, 60-120, hexane/EtOAc, 5/5) to give N^2-(6-(3-methoxylphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (110 mg, 40.7%) as a pale yellow solid.

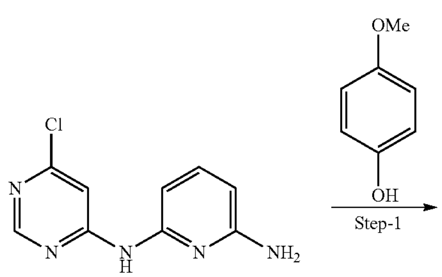
Step 2

[0659] To a stirred solution of N^2-(6-(3-methoxylphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (100 mg, 0.323 mmol) in THF/NMP (1 mL/0.5 mL) was added acryloyl chloride (0.029 g, 0.3 mmol) under N_2 atmosphere at −10° C. The stirring was continued at the same temperature for 2 h and the reaction mixture was concentrated under reduced pressure to give a residue that was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol) to give N-(6-(6-(3-methoxyphenoxy)pyrimidin-4-ylamino)pyridine-2-yl)acrylamide (I-90) as a pale yellow solid. ^1H NMR (DMSO-d_6) δ ppm: 3.75 (s, 3H), 5.79 (dd, J=1.8 & 10.08 Hz, 1H), 6.30 (dd, J=1.8 & 16.96 Hz, 1H), 6.65 (dd, J=10.12 & 16.96 Hz, 1H), 6.74-6.76 (m, 2H), 6.82 (td, J=1.52 & 9.24 Hz, 1H), 7.04 (d, J=7.92 Hz, 1H), 7.33 (t, J=8.28 Hz, 1H), 7.70 (t, J=7.96 Hz, 1H), 7.76 (d, J=8 Hz, 1H), 7.90 (s, 1H), 8.34 (s, 1H), 10.20 (s, 1H), 10.48 (s, 1H); LCMS: m/e 364 (M+1).

Example 32

[0660]

Scheme 32a



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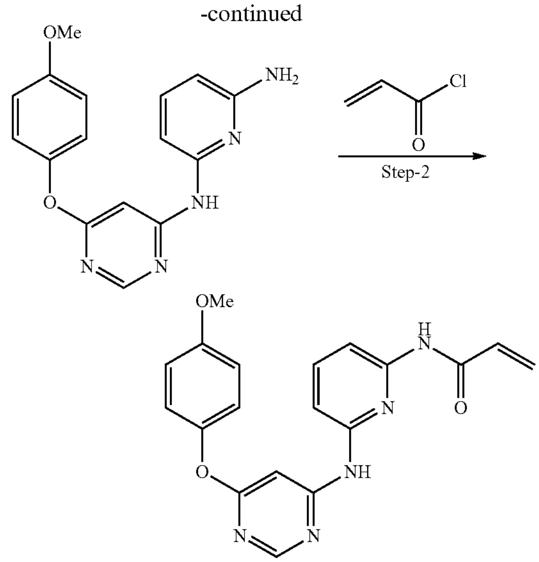


I-91

Synthesis of N-(6-(6-(4-methoxyphenoxy)pyrimidin-4-ylamino)pyridine-2-yl)acrylamide (I-91)

Step 1

[0661] To a solution of N^2-(6-chloropyrimidin-4-yl)pyridine-2,6-diamine (200 mg, 0.90 mmol) in dry DMF (2 mL) was added 4-methoxyphenol (168 mg, 1.3 mmol) and anhydrous K_2CO_3 (179 mg, 1.3 mmol). The reaction mixture was heated at 100° C. for 16 h under N_2 atmosphere. It was then cooled and DMF removed under reduced pressure to give a yellowish gummy residue that was taken in EtOAc (10 mL). The solution was washed with water (5 mL) and brine (5 mL), was dried over Na_2SO_4 and was then concentrated under reduced pressure to get crude product. This was further purified by column chromatography (SiO_2, 60-120, hexane/EtOAc, 5/5) to give N^2-(6-(4-methoxylphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (160 mg, 59.2%) as a pale yellow solid.

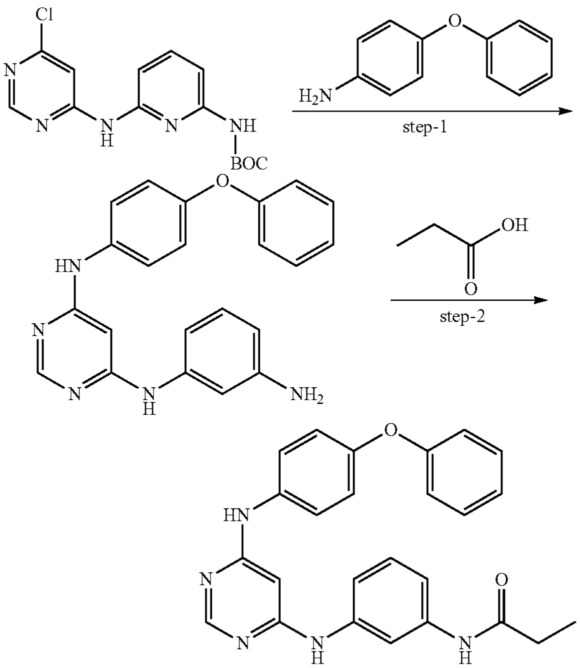
Step 2

[0662] To a stirred solution of N^2-(6-(4-methoxylphenoxy)pyrimidin-4-yl)pyridine-2,6-diamine (150 mg, 0.474 mmol) in THF/NMP (1 mL/0.5 mL) was added acryloyl chloride (64 mg, 0.712 mmol) at −10° C. under N_2 atmosphere. After stirring at this temperature for 30 min, the reaction was stopped and the reaction mixture was slowly added to $NaHCO_3$ solution (10 mL). A white solid was precipitated which was isolated by filtration and was dissolved in a mixture of ethyl acetate (5 mL) and Et_3N (0.5 mL). The solution was washed with water (2 mL) and brine (2 mL). Drying over Na_2SO_4 followed by filtration and concentration under reduced pressure afforded a yellow solid. It was further purified by column chromatography (SiO_2, 60-120, chloroform/methanol, 9/1) to give N-(6-(6-(4-methoxyphenoxy)pyrimidin-4-ylamino)pyridine-2-yl) acrylamide (I-91) as an off white solid. ^1H NMR (DMSO-d_6) δ ppm: 3.76 (s, 3H), 5.79 (dd, J=1.84 & 10.16 Hz, 1H), 6.30 (dd, J=1.84 & 17 Hz, 1H), 6.65 (dd, J=10.12 & 16.92 Hz, 1H), 6.96-6.99 (m, 2H), 7.02 (d, J=7.88 Hz, 1H), 7.09-7.13 (m, 2H), 7.69 (t, J=7.96 Hz, 1H), 7.76 (d, J=7.44 Hz, 1H), 7.86 (s, 1H), 8.30 (d, J=0.88 Hz, 1H), 10.17 (s, 1H), 10.17 (s, 1H); LCMS: m/e 364 (M+1).

Example 33

[0663]

Scheme 33a



I^R10

Synthesis of N-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)propionamide (I^R-10)

Step 1

[0664] A solution of tert-butyl 3-(6-chloropyrimidin-4-ylamino)phenylcarbamate (200 mg, 0.6 mmol), 4-phenoxyaniline (346 mg, 1.8 mmol) and concentrated HCl (45 mg, 1.2 mmol) in n-butanol (8 mL) was subjected to microwave irradiation (160° C., 20 min). The reaction mixture was quenched with $NaHCO_3$ solution (2 mL) and extracted with EtOAc (2×10 mL). The combined EtOAc extract was washed with water (5 mL), brine (5 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, Chloroform/methanol, 9/1) to give N^4-(3-aminophenyl)-N^6-(4-phenoxyphenyl)pyrimidine-4,6-diamine (87 mg, 37.8%) as a brown solid.

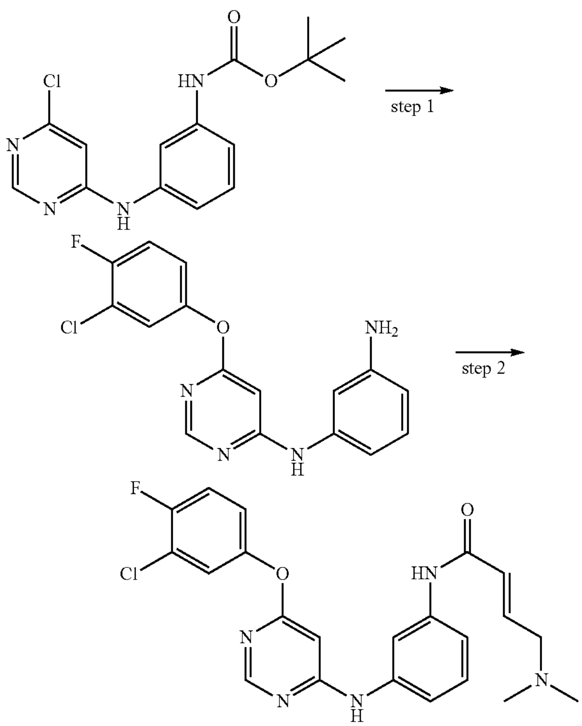
Step 2

[0665] To a solution of propionic acid (12 mg, 0.1 mmol) in DMF (0.6 mL) was added HATU (92 mg, 0.2 mmol) and the reaction mixture was stirred at room temperature for 30 min. To it was added N^4-(3-aminophenyl)-N^6-(4-phenoxyphenyl)pyrimidine-4,6-diamine (60 mg, 0.1 mmol) followed by DIPEA (41 mg, 0.3 mmol) and the reaction mixture was stirred at this temperature for 16 h. It was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (5 mL) and was washed with $NaHCO_3$ solution (2 mL), water (2 mL), and brine (2 mL). Drying over Na_2SO_4 followed by concentration under reduced pressure offered a residue that was purified by column chromatography (SiO_2, 230-400, chloroform/methanol: 9/1) to give N-(3-(6-(4-phenoxyphenylamino)pyrimidin-4-ylamino)phenyl)propionamide (I^R-10) as a brown solid. ^1H NMR (DMSO-d_6) δ ppm: 1.06 (t, J=7.56 Hz, 3H), 2.30 (q, J=7.48 Hz, 2H), 6.13 (s, 1H), 6.94-6.99 (m, 4H), 7.08 (t, J=7.36 Hz, 1H), 7.16-7.24 (m, 3H), 7.36 (t, J=7.44 Hz, 2H), 7.54 (d, J=8.88 Hz, 2H), 7.81 (s, 1H), 8.23 (s, 1H), 9.12 (s, 2H), 9.81 (s, 1H); LCMS: m/e 426.3 (M+1).

Example 34

[0666]

Scheme 34a



Synthesis of (E)-N-(3-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (I-92)

Step 1

[0667] A solution of tert-butyl 3-(6-chloropyrimidin-4-ylamino)phenylcarbamate (1.6 g, 5 mmol), 3-chloro-4-fluorophenol (1.4 g, 10 mmol) and potassium carbonate (1.4 g, 10 mmol) in 15 mL of DMF was heated to 120° C. for 16 h. The reaction mixture was mixed in 15 mL of water, the crude product was precipitated, filtered, purified by flash chromatography on silica gel with MeOH/DCM solvent system to afford 750 mg (45% yield) of N^1-(6-(3-chloro-4-

fluorophenoxy)pyrimidin-4-yl)benzene-1,3-diamine as an off-white solid. MS (m/z): MH^+=331.

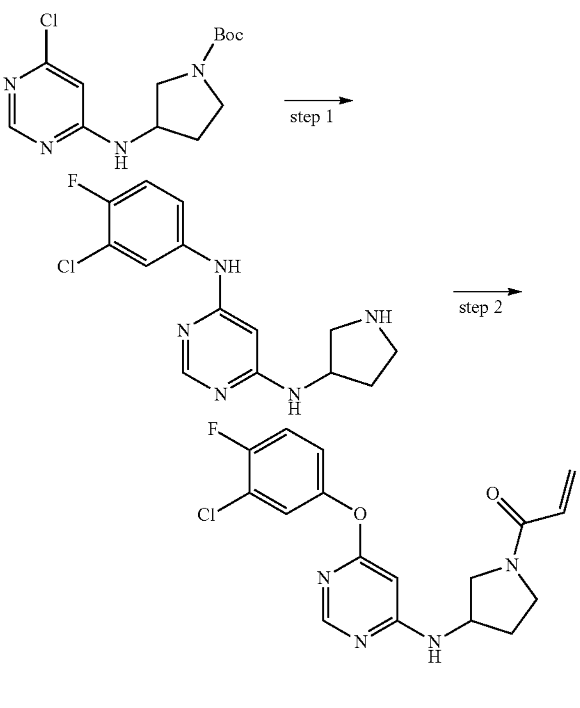
Step 2

[0668] Oxalyl chloride (155 mg, 1.2 mmol) was added dropwise to a mixture of 4-N,N-dimethyl aminocrotonic acid HCl salt (200 mg, 1.2 mmol) in 5 mL of THF at 0° C. To this mixture was added 3 drops of DMF/THF solution (made from 5 drops of DMF in 1 mL of THF). The reaction mixture was stirred at RT for 2 h, then was cooled to 0° C. ice bath. A solution of N^1-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-yl)benzene-1,3-diamine (200 mg, 0.6 mmol) 2 mL of NMP) was added to the dimethylaminocrotonyl chloride solution and the resulting mixture was stirred for 3 h at 0° C. The reaction was quenched with 3 mL of 1N NaOH, and was extracted with EtOAc (2×25 mL). The crude product mixture was purified by flash chromatography on silica gel with MeOH/NH_4OH/DCM solvent system to afford (E)-N-(3-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-ylamino)phenyl)-4-(dimethylamino)but-2-enamide (I-92) as a light colored solid. MS (m/z): MH^+=442, 444 (3:1), H-NMR (DMSO) δ 10.08 (s, 1H), 9.67 (s, 1H), 8.36 (s, 1H), 7.98 (s, 1H), 7.59 (d, 1H), 7.57 (t, 1H), 7.53-7.24 (m, 4H), 6.29 (b, 1H), 6.23 (s, 1H), 3.51 (d, 2H), 2.21 (s, 6H).

Example 35

[0669]

Scheme 35a



Synthesis of 1-(3-(6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylamino)pyrrolidin-1-yl)prop-2-en-1-one (I-70)

Step 1

[0670] A neat mixture of tert-butyl 3-(6-chloropyrimidin-4-ylamino)pyrrolidin-1-carboxylate (354 mg, 1.18 mmol) and 3-chloro-4-fluoroaniline (3.03 g, 20.8 mmol) was heated at 140° C. for 21 hours. Upon cooling to ambient temperature, the melt was diluted with EtOAc and the mixture was stirred for 1 hour. A beige, amorphous precipitate was collected, washed with water and dried in vacuo at 50-60° C. giving 292 mg (80%) of N^4-(3-chloro-4-fluorophenyl)-N^6-(pyrrolidin-3-yl)pyrimidine-4,6-diamine. MS (APCI): (M+1)=308, (M−1)=306.

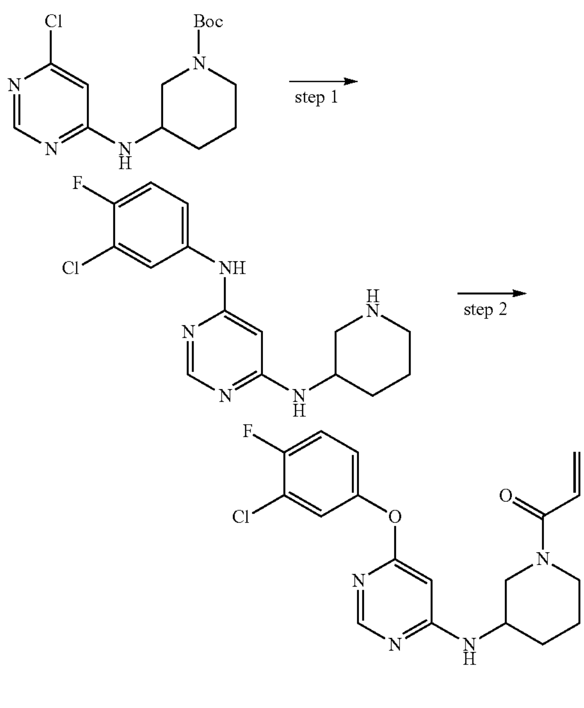
Step 2

[0671] To a solution of N^4-(3-chloro-4-fluorophenyl)-N^6-(pyrrolidin-3-yl)pyrimidine-4,6-diamine (287 mg, 0.93 mmol) and triethylamine (0.32 ml, 2.33 mmol) in anhydrous THF (5 ml) under nitrogen was added acryloyl chloride (91 μl, 1.12 mmol). The reaction mixture was stirred at room temperature for 1 hour and was concentrated under reduced pressure. The residue was eluted through a flash column (silica gel 60, 230-400 mesh, 5% MeOH in EtOAc to 10% MeOH in EtOAc) to give two products. The less polar product (R_f=0.24 in 1:9 MeOH:EtOAc) was found to be a diacrylated analog. The more polar product (R_f=0.14 in 1:9 MeOH:EtOAc) was 1-(3-(6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylamino)pyrrolidin-1-yl)prop-2-en-1-one (I-70). MS (APCI) $(M+1)^+$=362, $(M-1)^+$=360: ^{1}H-NMR DMSO-d_6) δ 9.11 (s, 1H), 8.14 (s, 1H), 7.92 (d, 1H), 7.38-7.23 (m, 3H), 6.58-6.48 (m, 1H), 6.13-6.08 (m, 1H), 5.75 (d, 1H) 5.63-5.59 (m, 1H), 3.64-3.59 (m, 2H), 2.17-1.81 (m, 6H).

Example 36

[0672]

Scheme 36a

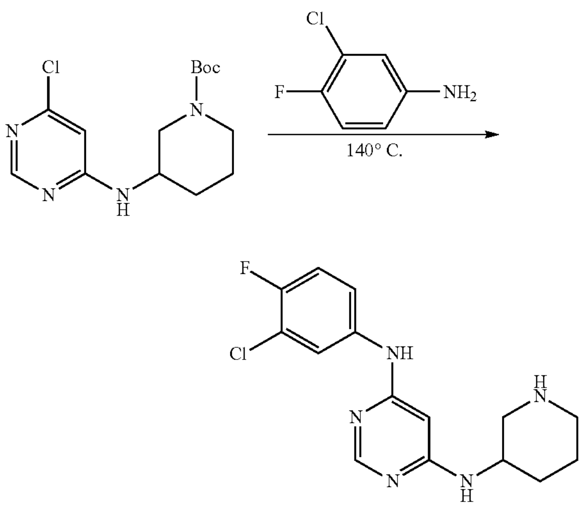


Synthesis of 1-(3-(6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylamino)piperidin-1-yl)prop-2-en-1-one (I-71)

Step 1

N-(3-Chloro-4-fluorophenyl)-N'-piperidin-3-yl pyrimidine-4,6-diamine

[0673]



[0674] A mixture of tert-butyl 3-(6-chloropyrimidin-4-ylamino)piperidine-1-carboxylate (295 mg, 0.94 mmol) and 3-chloro-4-fluoroaniline (2.83 g, 19.4 mmol) was heated neat at 140° C. for 19 hours. Upon cooling, the melt was stirred in EtOAc and allowed to stand at room temperature for 1 hour. The precipitate was collected, washed with EtOAc and dried to give 256 mg (85%) of N^4-(3-chloro-4-fluorophenyl)-N^6-(piperidin-3-yl)pyrimidine-4,6-diamine, a grayish-violet amorphous solid. MS (APCI): $(M+1)^+$=322.

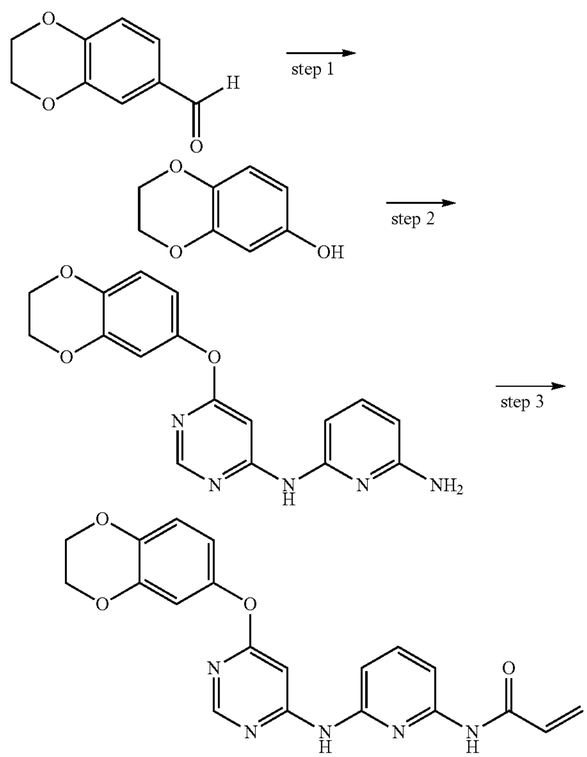
Step 2

[0675] To a solution of N^4-(3-chloro-4-fluorophenyl)-N^6-(piperidin-3-yl)pyrimidine-4,6-diamine (251 mg, 0.78 mmol) and triethylamine (0.27 ml, 1.95 mmol) in anhydrous THF (7 ml) under nitrogen was added acryloyl chloride (76 μl, 0.94 mmol). The reaction mixture was stirred at room temperature for 1 hour and was concentrated under reduced pressure. The residue was eluted through a flash column (silica gel 60, 230-400 mesh with 5% MeOH in EtOAc to give two products. The less polar product (R_f=0.33 in 1:9 MeOH:EtOAc) was found to be a diacrylated analog. The more polar product (R_f=0.24 in 1:9 MeOH:EtOAc) was 1-(3-(6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylamino)piperidin-1-yl)prop-2-en-1-one (I-71). MS (APCI) $(M+1)^+$=376, $(M-1)^+$=374: ^{1}H-NMR DMSO-d_6, δ 9.15 (br s, 1H), 8.18 (d, 1H), 7.95 (br s, 1H), 7.43-7.20 (m, 2H), 7.03-6.48 (m, 3H), 6.26-5.97 (m, 2H), 5.86-5.51 (m, 3H), 3.90-3.66 (m, 2H), 1.98-1.66 (m, 2H), 1.59-1.12 (m, 2H).

Example 37

[0676]

Scheme 37a



Synthesis of N-(6-(6-(2,3-dihydrobenzo[b][1,4]dioxin-6-yloxy)pyrimidin-4-ylamino)pyridin-2-yl) acrylamide (I-95)

Step 1

[0677] To a stirred solution of 2,3-dihydrobenzo[b][1,4] dioxine-6-carbaldehyde (1 g, 6.09 mmol) in CH_2Cl_2 (16 mL) was added m-CPBA (4.204 g, 24.36 mmol). The suspension was heated at 50° C. for 2 days, was cooled to rt, was quenched with saturated $NaHCO_3$ soln. and was extracted with CH_2Cl_2 (3×10 mL). The combined extract was concentrated under reduced pressure, and then was dissolved in MeOH containing NaOH. The solution was stirred at rt for 2 h, was acidified with HCl and was extracted with ethyl acetate (3×10 mL). The combined extract was washed with saturated $NaHCO_3$ solution and brine, was dried over anhydrous Na_2SO_4, was filtered and was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and was filtered. The DCM solution was dried over anhydrous Na_2SO_4, was filtered and was concentrated under reduced pressure to give 2,3-dihydrobenzo[b][1,4]dioxin-6-ol (0.591 g, 63%) as a reddish brown oily liquid.

Step 2

[0678] A stirred mixture of 2,3-dihydrobenzo[b][1,4]dioxin-6-ol (0.137 g, 0.90 mmol), Cs_2CO_3 (0.734 g, 2.25 mmol), CuI (0.02 g, 10% w/w), and N^2-(6-chloropyrimidin-4-yl)pyridine-2,6-diamine (0.29 g, 0.90 mmol) in NMP (1 mL) was heated at 100° C. for 16 h. The reaction mixture was cooled to rt and slowly added to demineralised water. The solid that precipitated was collected by filtration and was further purified by column chromatography (SiO_2, 60-120, pet ether/ethyl acetate, 7/3) to give N^2-(6-(2,3-dihydrobenzo[b][1,4]dioxin-6-yloxy)pyrimidin-4-yl)pyridine-2,6-diamine (0.088 g, 29%) as yellow solid.

Step 3

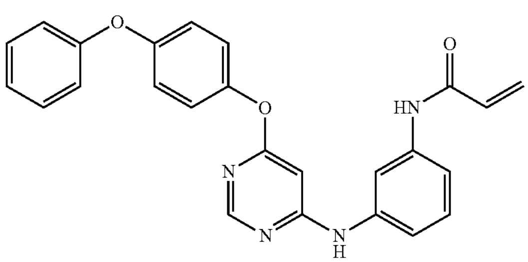
[0679] To a stirred solution of N^2-(6-(2,3-dihydrobenzo[b][1,4]dioxin-6-yloxy)pyrimidin-4-yl)pyridine-2,6-diamine (0.080 g, 0.23 mmol) and potassium carbonate (0.065 g, 0.47 mmol) in NMP (0.8 mL) at 0° C. was added acryloyl chloride (0.026 g, 0.29 mmol) and the reaction mixture was stirred at 0° C. for 30 min. The reaction mixture was added dropwise to a cold, stirring solution of 10% $NaHCO_3$ and was stirred at 0° C. for 30 min. A white solid was isolated by filtration. The solid was washed with cold water and hexane and dissolved in a 2 mL of methanol/dichloromethane (1/1). This solution was concentrated under reduced pressure. The residue obtained was suspended in cold water (5 mL), Et_3N was added to it and it was extracted with ethyl acetate (2×5 mL). The combined ethyl acetate extract was washed with water (2 mL) and brine (2 mL), was dried over Na_2SO_4 and was concentrated under reduced pressure to obtain N-(6-(6-(2,3-dihydrobenzo[b][1,4]dioxin-6-yloxy)pyrimidin-4-ylamino)pyridin-2-yl)acrylamide (I-95) as a light yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm: 4.25 (s, 4H), 5.79 (dd, J=1.84 & 10.08 Hz, 1H), 6.30 (dd, J=1.84 & 16.96 Hz, 1H), 6.62-6.72 (m, 3H), 6.88 (d, J=8.72 Hz, 1H), 7.03 (d, J=7.84 Hz, 1H), 7.69 (t, J=7.96 Hz, 1H), 7.70 (d, J=7.84 Hz, 1H), 7.85 (s, 1H), 8.32 (d, J=0.4 Hz, 1H), 10.16 (s, 1H), 10.47 (s, 1H); LCMS: m/e 392.

Example 38

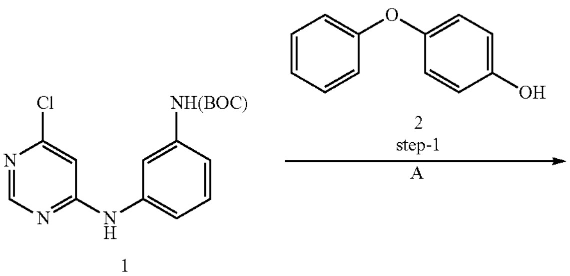
Synthesis of N-(3-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)phenyl)acrylamide (I-97)

[0680]

I-97



[0681] The title compound was prepared according to the schemes, steps and intermediates described below.



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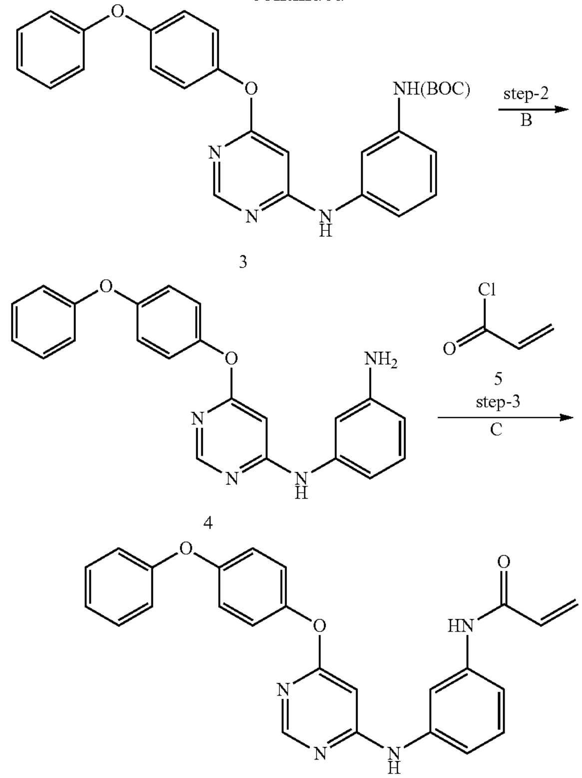


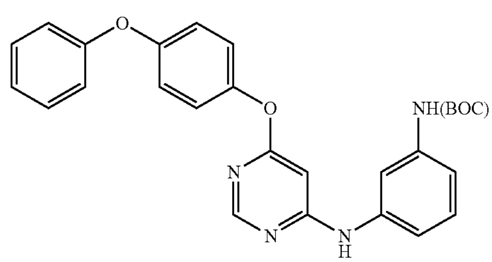
I-97

A) Cs_2CO_3, DMF, 80° C., 16 h; B) TFA, CH_2Cl_2, rt, 16 h; C) K_2CO_3, NMP, 0° C., 60 min.

Step 1

[0682]

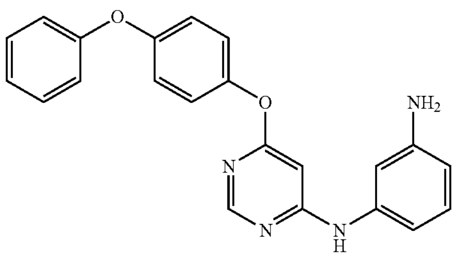
3



[0683] To a stirred solution of 2 (4.35 g, 23.38 mmol) and Cs_2CO_3 (15.26 g, 46.76 mmol) in dry DMF (50 mL) was added 1 (5.0 g, 15.58 mmol) at rt and the reaction was stirred at 80° C. for 16 h under nitrogen atmosphere. The reaction mixture was cooled, concentrated under reduced pressure and the residue was diluted with ethyl acetate (50 mL). It was washed with water (2×10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, pet ether/ethyl acetate, 5/5) to give 3 (1.6 g, 23.3%) as brown solid.

Step 2

[0684]



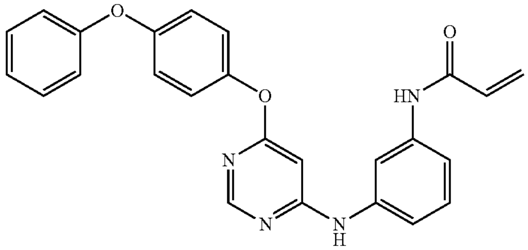
4

[0685] To a stirred solution of 3 (1.6 g, 3.9 mmol) in dry CH_2Cl_2 (8 mL) at 0° C. was added CF_3COOH (4.8 mL) and the reaction mixture was stirred at 0° C. for 30 min. The reaction was allowed to come to rt and stirred for 12 h. It was concentrated under reduced pressure and the residue was quenched With $NaHCO_3$ solution (15 mL). The contents were extracted with ethyl acetate (3×15 mL) and the combined extracts were washed with water (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 4 (1.2 g, 99%) as a light yellow solid.

Step 3

[0686]

I-97



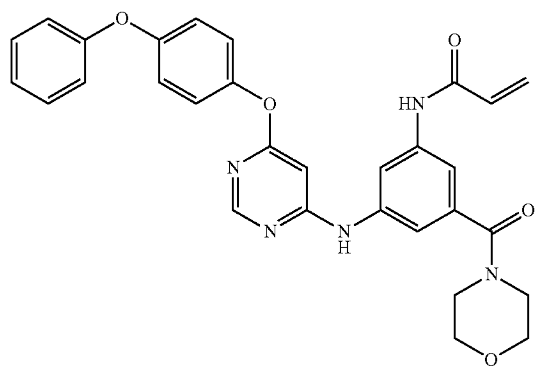
[0687] To a stirred solution of 4 (1.2 g, 3.23 mmol) and potassium carbonate (2.237 g, 16.19 mmol) in NMP (12 mL) at 0° C. was added acryloyl chloride (0.322 g, 3.56 mmol) and the reaction mixture was stirred at 0° C. for 60 min The reaction mixture was added dropwise to a cold, stirring solution of 10% $NaHCO_3$ and stirred at the same temperature (0° C.) for 30 min. A solid precipitated out and was isolated by filtration through a Buchner funnel. It was washed with cold water and hexane, dissolved in a mixture of methanol/dichloromethane (50:50, 5 mL), and concentrated under reduced pressure. The residue obtained was suspended in cold water (10 mL), Et_3N was added to it, and it was extracted with ethyl acetate (2×10 mL). The combined ethyl acetate extract was washed with water (5 mL), brine (5 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was further purified by column chromatography (SiO_2, 60-120, methanol/chloroform: 10/90) to give the title compound (0.83 g, 60.3%) as a light green solid. ^1H NMR (DMSO-d_6) δ ppm: 5.74 (dd, J=1.92 & 10.04 Hz, 1H), 6.16 (s, 1H), 6.24 (dd, J=1.92 & 16.92 Hz, 1H), 6.45 (dd, J=10.08 & 16.96 Hz, 1H), 7.03-7.09 (m, 4H), 7.16 (t, J=7.30 Hz, 1H), 7.20-7.26 (m, 3H), 7.30-7.35 (m, 2H), 7.39-7.44 (m, 2H), 7.98 (s, 11-1), 8.36 (s, 1H), 9.62 (s, 1H), 10.15 (s, 1H); LCMS: m/e 425.2 (M+1).

Example 39

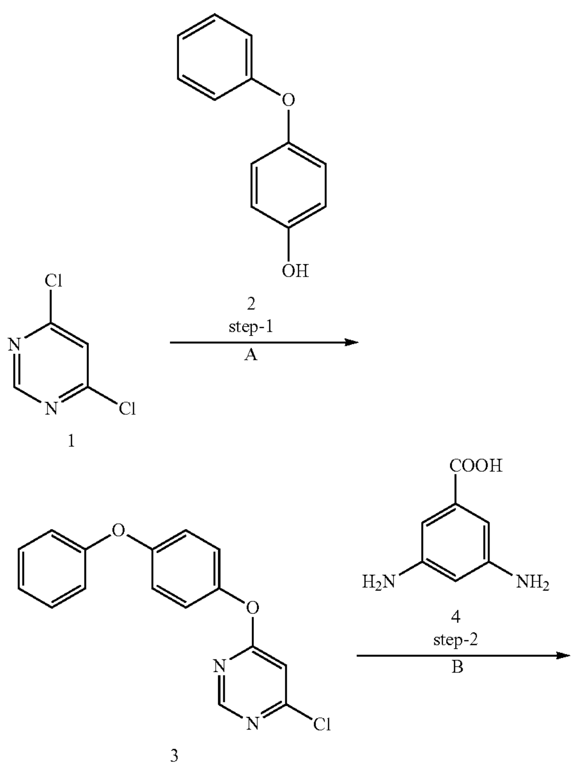
Synthesis of N-(3-(morpholine-4-carbonyl)-5-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)phenyl)acrylamide (I-100)

[0688]

I-100



[0689] The title compound was prepared according to the schemes, steps and intermediates described below.



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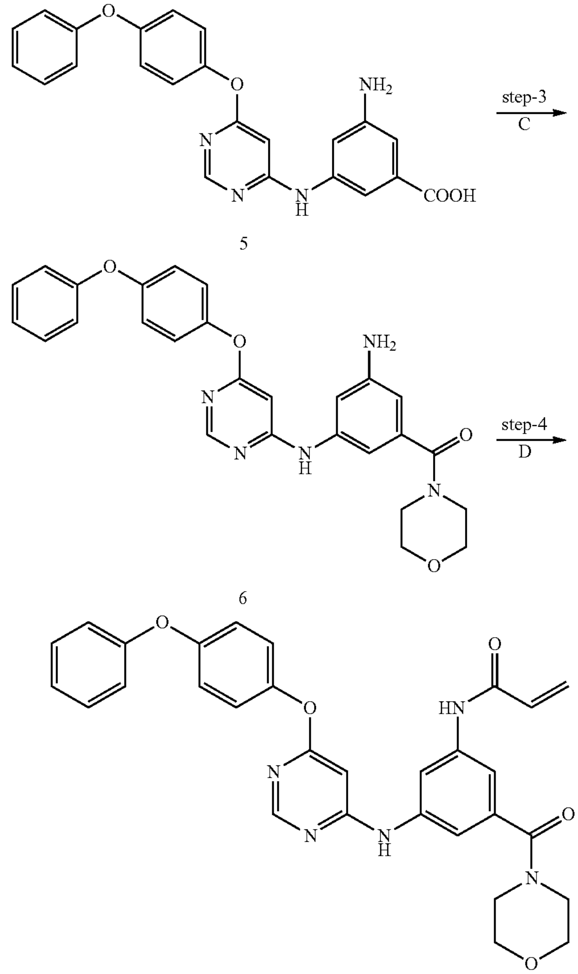


5

6

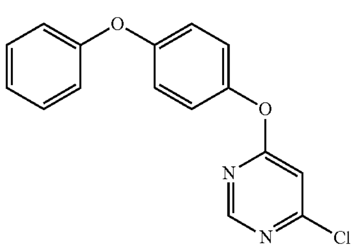
I-100

A) K_2CO_3, DMF, rt, 16 h; B) conc. HCl, EtOH, 90° C., 10 h, pressure tube; C) EDCI•HCl, HOBT, DIPEA, DMF, rt, 18 h; D) K_2CO_3, NMP, 0° C., 30 min.

Step 1

[0690]

3

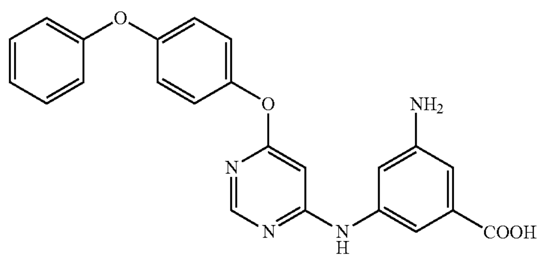


[0691] To a stirring solution of 1 (2.0 g, 10.75 mmol) and K_2CO_3 (2.96 g, 21.5 mmol) in dry DMF (20 mL) was added 2 (1.59 g, 10.75 mmol) and the reaction mixture was stirred at rt for 16 h under nitrogen atmosphere. It was cooled, quenched with water (100 mL) and extracted with EtOAc (2×50 mL). The combined EtOAc extract was washed with 10% $NaHCO_3$ soln. (50 mL), water (3×50 mL), brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give 3 (1.92 g, 60.0%) as a light yellow solid, which was used in the next step without further purifications.

Step 2

[0692]

5

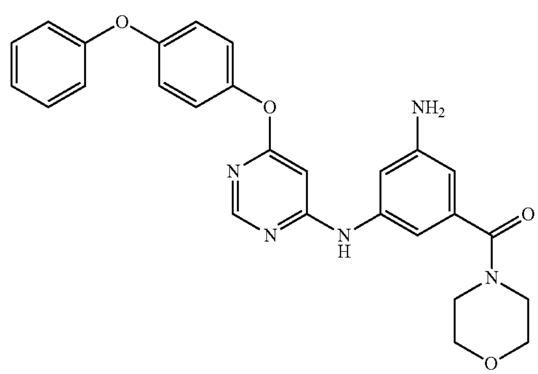


[0693] To a solution of 3 (0.5 g, 1.678 mmol) in ethanol (10 mL) was added conc. HCl (0.172 g, 1.678 mol) and 4 (1.27 g, 8.34 mol). The reaction mixture was stirred in a sealed pressure tube for 10 h at 90° C. The reaction was cooled, quenched with water (50 mL) and extracted with EtOAc (2×25 mL). The combined extracts were washed with 5% citric acid solution (25 mL), water (25 mL), brine (25 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get 5 (0.4 g, 57.53%) as a brown solid.

Step 3

[0694]

6

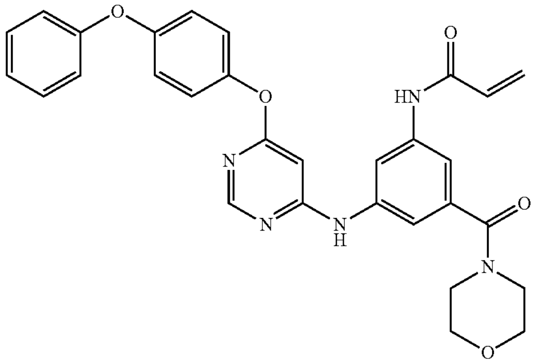


[0695] To a stirred solution of 5 (0.15 g, 0.362 mmol) in DMF (6 mL) were added morpholine (0.095 g, 1.085 mmol), EDCI.HCl (0.104 g, 0.542 mmol), HOBT (0.0049 g, 0.036 mmol) and DIPEA (0.07 g, 0.543 mmol). The reaction mixture was stirred at room temperature for 18 h under nitrogen atmosphere. It was diluted with cold water (30.0 mL) and extracted with EtOAc (2×25 mL). The combined extracts were washed with water (2×15 mL), brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give crude 6. The crude residue was further purified by column chromatography (SiO_2, MeOH/Chloroform: 2/98) to get 6 (0.075 g, 42.87%) as a brown solid.

Step 4

[0696]

I-100



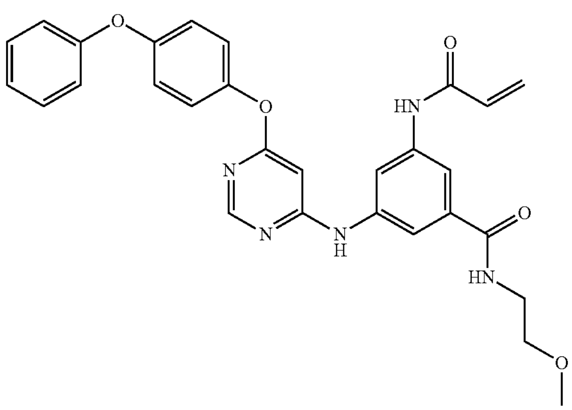
[0697] To a stirred solution of 6 (0.09 g, 0.186 mmol) and potassium carbonate (0.128 g, 0.93 mmol) in NMP (1.5 mL) at 0° C. was added 7 (0.021 g, 0.232 mmol) and the reaction mixture was stirred at 0° C. for 30 min The reaction mixture was added drop wise to a cold, stirring solution of 10% $NaHCO_3$ and stirred at the same temperature (0° C.) for 5 min. A solid precipitated out which was isolated by filtration through a Buchner funnel. The solid was washed with cold water, hexane and dissolved in a mixture of methanol/dichloromethane (50:50, 5 mL) and concentrated under reduced pressure. The residue obtained was suspended in cold water (5 mL), Et_3N was added, and it was extracted with ethyl acetate (2×15 mL). The combined extracts were washed with water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give the title compound (0.08 g, 79.95%) as a pale brown solid. ^{1}H NMR (DMSO-d_6) δ ppm: 3.62 (bs, 8H), 5.79 (dd, J=1.6 & 10.4 Hz, 1H), 6.18 (s, 1H), 6.28 (dd, J=1.6 & 16.8 Hz, 1H), 6.41-6.50 (m, 1H), 7.05-7.10 (m, 4H), 7.17 (t, J=7.2 Hz, 1H), 7.24 (d, J=8.8 Hz, 2H), 7.39 (s, 1H), 7.43 (t, J=8.4 Hz, 2H), 7.51 (s, 1H), 8.0 (s, 1H), 8.41 (s, 1H), 9.79 (s, 1H), 10.31 (s, 1H); LCMS: m/e 538.2 (M+1).

Example 40

Synthesis of 3-acrylamido-N-(2-methoxyethyl)-5-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)benzamide (I-102)

[0698]

I-102



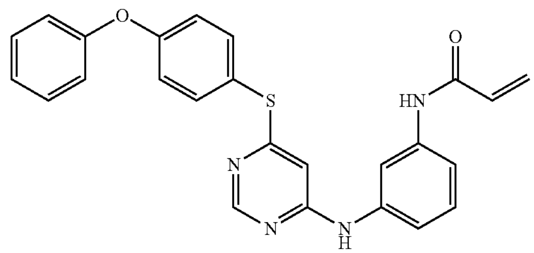
[0699] The title compound was prepared according to the schemes, steps and intermediates described in Example 39, by using 2-methoxyethylamine in step-3. ^{1}H NMR (DMSO-d_6) δ ppm: 3.25 (s, 3H), 3.30-3.50 (m, 4H), 5.76 (dd, J=1.84 & 10.08 Hz, 1H), 6.17 (s, 1H), 6.27 (dd, J=1.88 & 16.96 Hz, 1H), 6.46 (dd, J=10.12 & 16.92 Hz, 1H), 7.03-7.09 (m, 4H), 7.16 (dt, J=0.88 & 7.4 Hz, 1H), 7.22 (td, J=3.28 & 9.96 Hz, 2H), 7.41 (dt, J=1.92 & 7.6 Hz, 2H), 7.70 (d, J=1.64 Hz, 2H), 8.17 (s, 1H), 8.38 (s, 1H), 8.42 (t, J=5.28 Hz, 1H), 9.75 (S, 1H), 10.30 (s, 1H); LCMS: m/e 526.2 (M+1).

Example 41

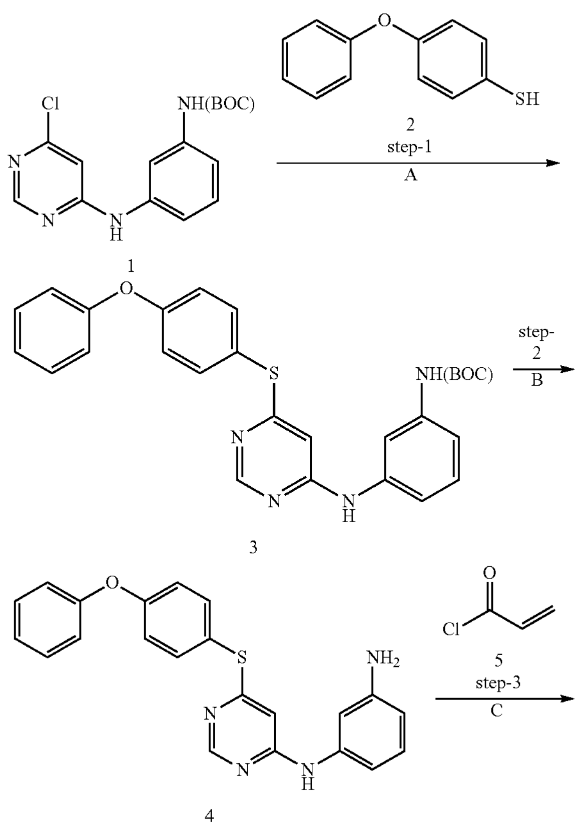
Synthesis of N-(3-(6-(4-phenoxyphenylthio)pyrimidin-4-ylamino)phenyl)acrylamide (I-103)

[0700]

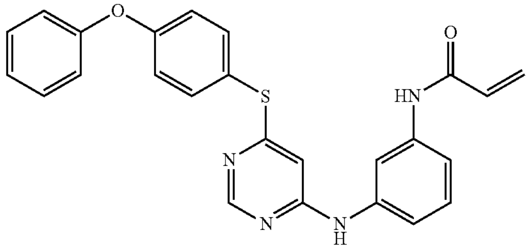
I-103



[0701] The title compound was prepared according to the schemes, steps and intermediates described below.



-continued



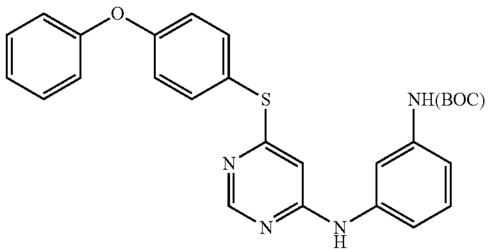
I-103

A) K_2CO_3, DMF, 110° C., 16 h; B) TFA, CH_2Cl_2, rt, 16 h; C) 5, K_2CO_3, NMP, 45 min, rt.

Step 1

[0702]

3

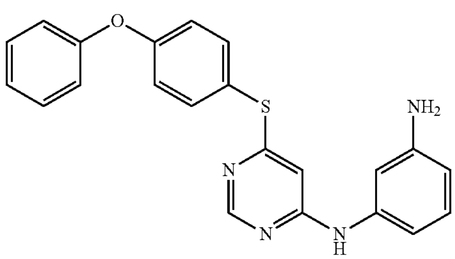


[0703] To a stirring solution of 2 (0.25 g, 1.235 mmol) and K_2CO_3 (0.512 g, 3.705 mmol) in dry DMF (2.5 mL) was added 1 (0.320 g, 1.235 mmol) at rt, and the reaction was stirred at 110° C. for 16 h under nitrogen atmosphere. The reaction mixture was quenched with water and extracted with ethyl acetate (3×5 mL). The combined extracts were washed with water (2×25 mL), brine (25 mL), dried over Na_2SO_4 and concentrated under reduced pressure to give 3 (0.130 g, 21%) as a white solid, which was used in the next step without purification.

Step 2

[0704]

4

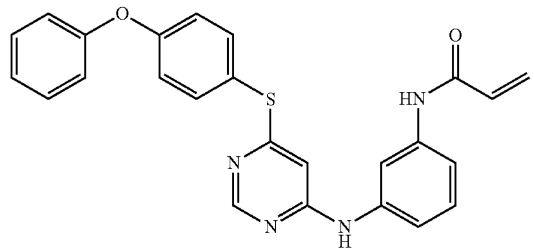


[0705] To a stirred solution of 3 (0.170 g, 0.349 mmol) in dry CH_2Cl_2 (2 mL) at 0° C. was added CF_3COOH (1 mL) and the reaction mixture was stirred at 0° C. for 30 min. The reaction was allowed to come to rt and stirred at it for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with $NaHCO_3$ solution (8 mL). The contents were extracted with ethyl acetate (3×5 mL) and the combined extracts were washed with water (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, 60-120, product getting eluted in 3% methanol/chloroform) to give 4 (0.100 g, 74%).

Step 3

[0706]

I-103



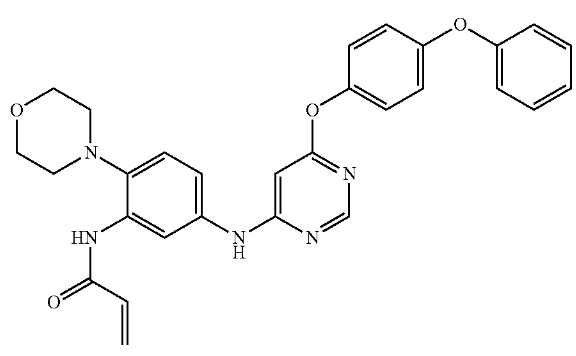
[0707] To a stirred solution of 6 (0.095 g, 0.24 mmol) and potassium carbonate (0.169 g, 1.22 mmol) in NMP (0.95 mL) at 0° C. was added 7 (0.024 g, 0.27 mmol), and the reaction mixture was stirred at 0° C. for 30 min. The reaction mixture was added dropwise to a cold, stirring solution of 10% $NaHCO_3$ and stirred at the same temperature (0° C.) for 5 min. A solid precipitated out and was isolated by filtration through a Buchner funnel. The solid was washed with cold water and hexane, dissolved in a mixture of methanol/dichloromethane (50:50, 5 mL), and concentrated under reduced pressure. The residue obtained was suspended in cold water (5 mL), Et_3N was added, and it was extracted with ethyl acetate (2×15 mL). The combined ethyl acetate extract was washed with water (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to get further to give the title compound (61 mg, 56%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm: 5.75 (dd, J=1.96 & 10.04 Hz, 1H), 6.19 (d, J=0.84 Hz, 1H), 6.25 (dd, J=2 & 16.92 Hz, 1H), 6.45 (dd, J=10.08 & 16.96 Hz, 1H), 7.11-7.15 (m, 4H), 7.20-7.30 (m, 4H), 7.48 (dt, J=1.88 & 6.56 Hz, 2H), 7.65 (dd, J=2.04 & 6.64 Hz, 2H), 7.97 (s, 1H), 8.41 (d, J=0.56 Hz, 1H), 9.55 (s, 1H), 10.15 (s, 1H); LCMS: m/e 441 (M+1).

Example 42

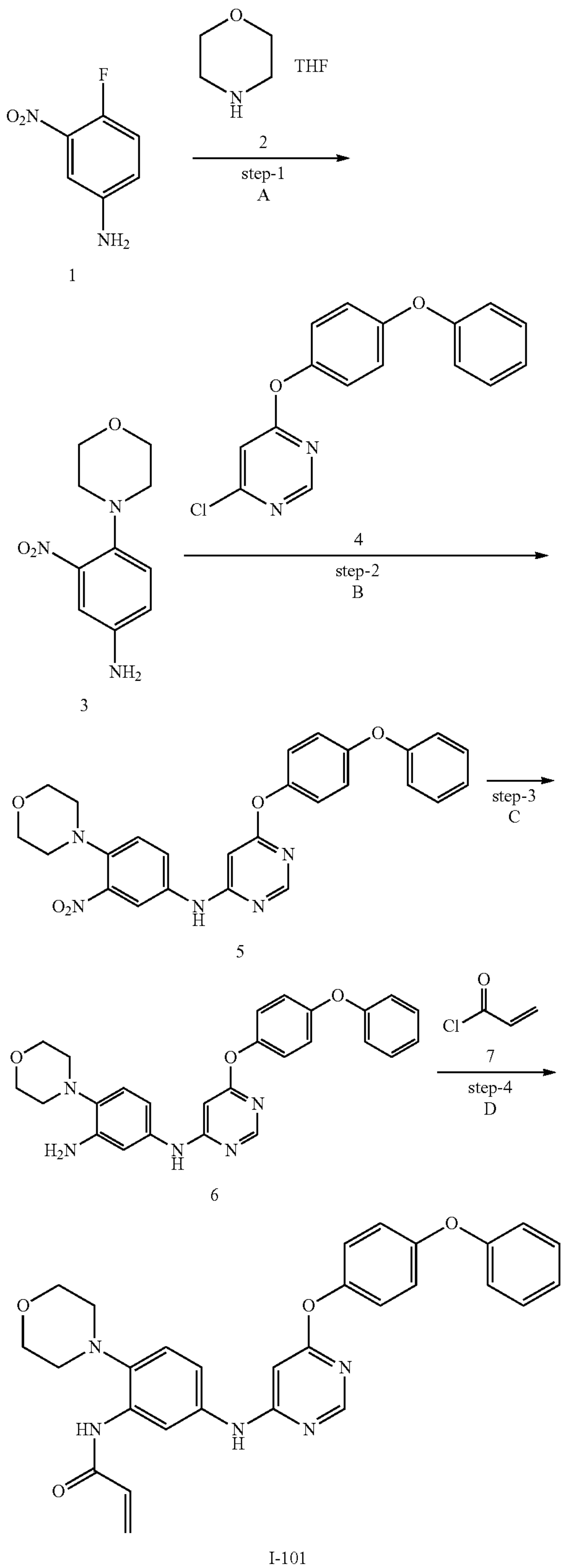
Synthesis of N-(2-morpholino-5-(6-(4-phenoxyphenoxy)pyrimidin-4-ylamino)phenyl)acrylamide (I-101)

[0708]

I-101



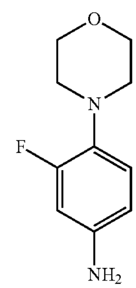
[0709] The title compound was prepared according to the schemes, steps and intermediates described below.



A) THF, reflux, 18 hr; B) Pd(OAc)2, X-Phos, CsCO3, dioxane, reflux, 12 hr; C) Pd/C, H_2, rt, 12 hr; D) DEIA, THF, -10° C.

Step-1

[0710]

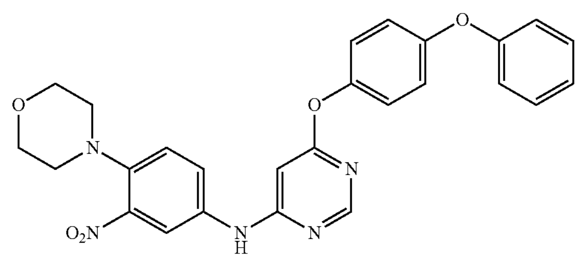


3

[0711] 4-Fluoro-3-nitroaniline (1, 1 g, 1 equiv.) and morpholine (1.67 g, 3 equiv.) were dissolved in THF (10 mL). The reaction mixture was heated at reflux overnight. After cooling, water/brine (10 mL) was added, the mixture was agitated, and the layers were separated. The organic phase was dried over sodium sulfate, and the solvent was removed via rotary evaporation. The material was purified by flash chromatography using 0-40% gradient of heptane/EtOAc to give 800 mg of 3 as an orange-red solid.

Step-2

[0712]



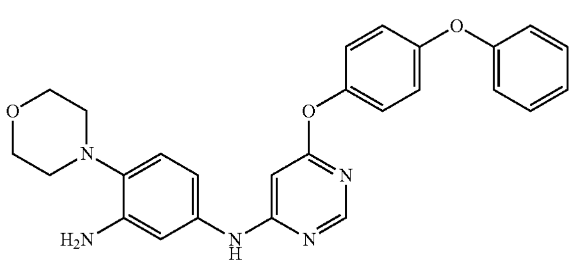
5

[0713] 4-Chloro-6-(4-phenoxyphenoxy)pyrimidine (4, 200 mg, 1 equiv.) and 3-nitro-4-morpholinoaniline (164 mg, 1.1 equiv.) were dissolved in dioxane (5 mL). The solution was degassed for 1 min. Palladium acetate (22 mg, 5 mol %), X-Phos ligand (39 mg, 10 mol %) and $CsCO_3$ (436 mg, 2 equiv.) were added in that order. The suspension was degassed for 1 min and placed under an argon atmosphere, and the mixture was heated to reflux for 12 h. After cooling, solvent was removed via rotary evaporation. The dark oil was partitioned between water/brine and EtOAc (5 mL each), agitated, filtered off precipitate, and separated layers of the filtrate. The organic phase was dried over sodium sulfate. The solvent was removed via rotary evaporation to give a dark oil. The oil was purified by flash chromatography using 0-30% gradient of heptane/EtOAc to afford 100 mg of 5 as an orange-red solid.

Step-3

[0714]

6

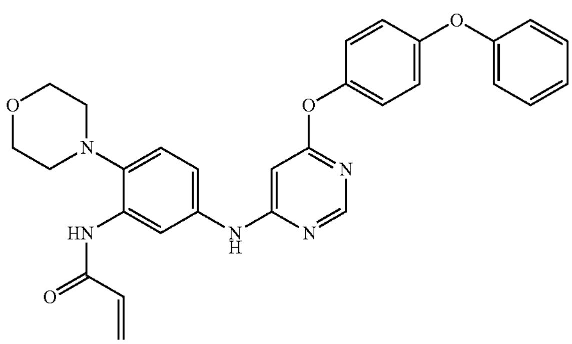


[0715] A catalytic amount of 10% Pd/C was added to a solution of 5 (100 mg) in EtOH (3 mL). Using a balloon, hydrogen was introduced, and the reaction was stirred at rt for 12 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated via rotary evaporation to give 6 as a dark purple film.

Step-4

[0716]

I-101



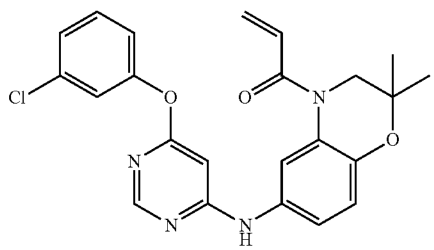
[0717] A solution of 6 (100 mg, 1 equiv.) in THF (4 mL) was cooled in water/ice-MeOH bath (−10° C. Acryloyl chloride was added to the solution (18.6 μL, 1.05 equiv.) and stirred for 10 min, then Hunig's base was added (31.7 μL, 1.05 equiv.) and stirred for 10 min. Water/brine (5 mL) was added, agitated and the layers were separated. The organic phase was dried over sodium sulfate. The solvent was removed via rotary evaporation afford a dark oil. The oil was purified by flash chromatography using 30-80% heptane/EtOAc gradient to give the title compound as a red film. LC/MS (RT=3.029/(M+H)) 510.2

Example 43

Synthesis of 1-(6-(6-(3-chlorophenoxy)pyrimidin-4-ylamino)-2,2-dimethyl-2H-benzo[b][1,4]oxazin-4(3H)-yl)prop-2-en-1-one (I-104)

[0718]

I-104



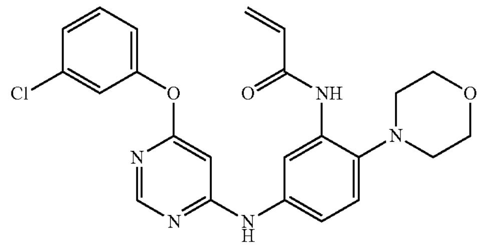
[0719] The title compound was prepared according to the schemes, steps and intermediates described in Example 42, by omitting step-1, using 4-tert-butylcarboxy-6-amino-2,2-dimethyl-2H-benzo[b][1,4]oxazine in step-2 and using TFA in CH_2Cl_2 in lieu of hydrogenation in step-3. LC/MS (RT=3.035/(M+H)) 437.1

Example 44

Synthesis of N-(2-morpholino-5-(6-(3-chlorophenoxy)pyrimidin-4-ylamino)phenyl)acrylamide (I-105)

[0720]

I-105



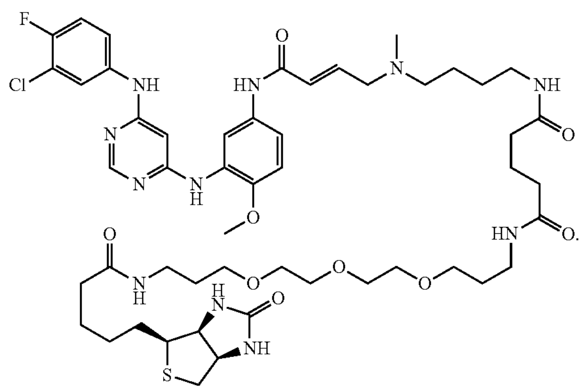
[0721] The title compound was prepared according to the schemes, steps and intermediates described in Example 42 by using 4-chloro-6-(3-chlorophenoxy)pyrimidine in place of 4 in step-2. LC/MS (RT=2.957/(M+H)) 452.1.

Example 45

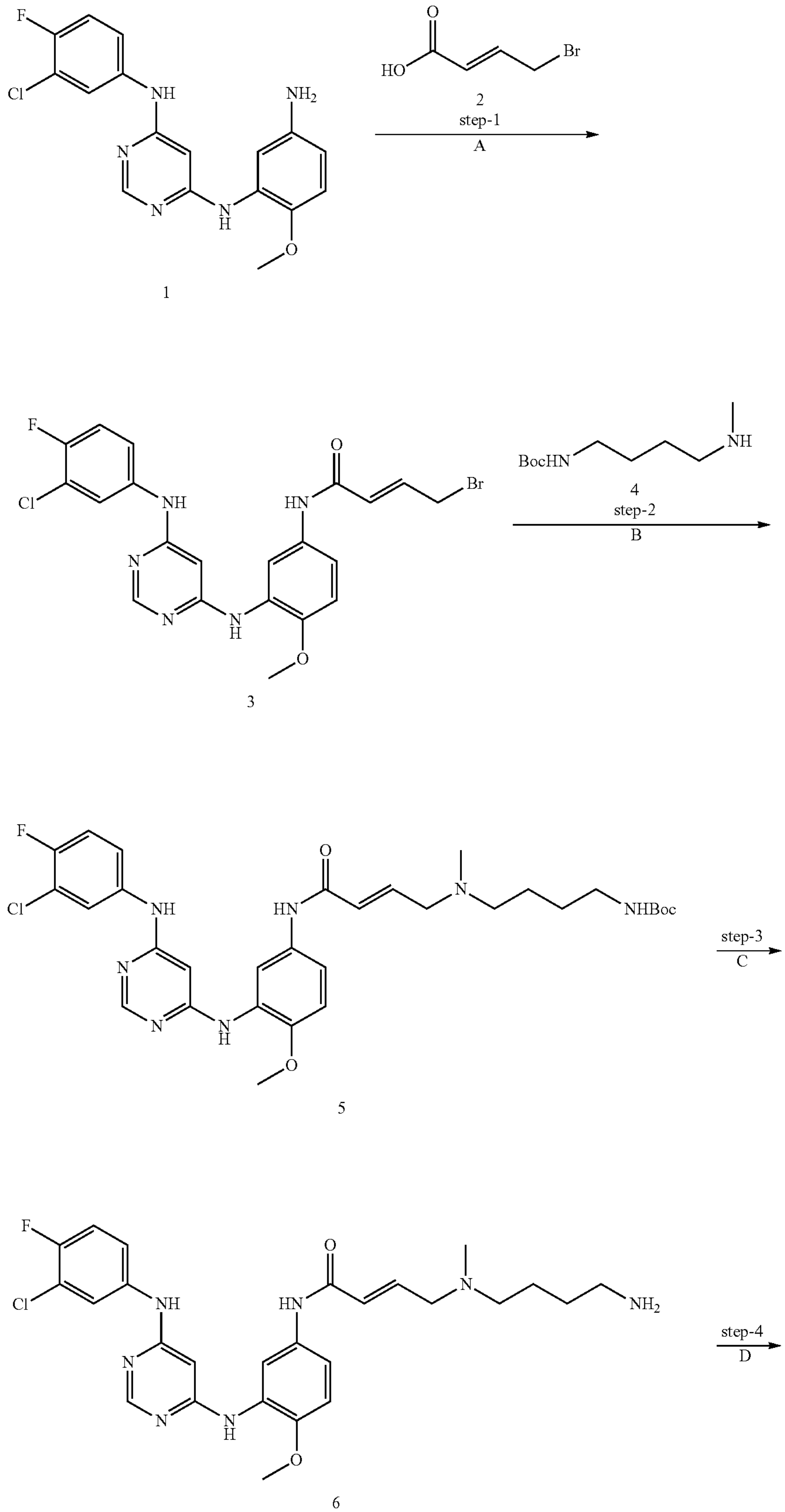
Synthesis of N^1-(4-(((E)-4-(3-(6-(3-chloro-4-fluorophenylamino)pyrimidin-4-ylamino)-4-methoxyphenylamino)-4-oxobut-2-enyl)(methyl)amino)butyl)-N^5-(15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)glutaramide (I-99)

[0722]

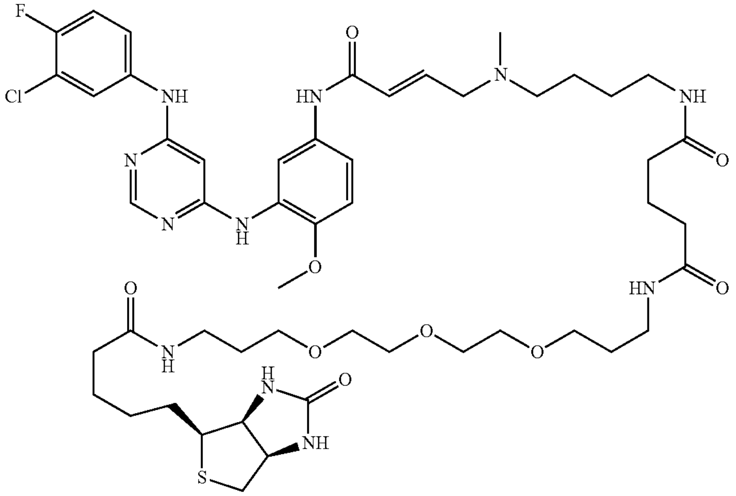
I-99



[0723] The title compound was prepared according to the schemes, steps, and intermediates described below.


F
Cl
NH
NH2
N
N
H
O
1
O
HO
Br
2
step-1
A
F
Cl
NH
O
HN
Br
N
N
H
O
3
BocHN
NH
4
step-2
B
F
Cl
NH
O
N
HN
NHBoc
N
N
H
O
5
step-3
C
F
Cl
NH
O
N
HN
NH2
N
N
H
O
6
step-4
D

-continued



I-99

A) 2, HATU, DIPEA, DMF; B) 4, K2CO3, acetone; C) TFA; DCM; D) N-biotinyl-NH-PEG$_2$-COOH•DIPEA, EDCl, HOBt, NMM, DMI

[0724] Step-1

[0725] The preparation of N^4-(5-amino-2-methoxyphenyl)-N^6-(3-chloro-4-fluorophenyl)pyrimidine-4,6-diamine (1) is described in Example 7 (Int-G). To a stirred solution of 1 (150 mg, 0.41 mmol) in DMF (8 mL) was added 2 (137 mg, 0.83 mmol), HATU (317 mg, 0.83 mmol) and DIPEA (215 mg, 1.67 mmol) at 0° C. The reaction mixture was stirred for 18 h at RT. After the completion of reaction (monitored by TLC), the crude mixture was diluted with water and extracted with EtOAc (4×100 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuo to afford 3 (100 mg, 47.3%) as off-white solid, which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.35 (s, 1H), 8.12 (s, 1H), 7.48-7.43 (m, 1H), 7.24 (s, 1H), 7.16-7.10 (m, 2H), 7.03 (s, 1H), 6.84 (t, J=9.0 Hz, 2H), 6.49 (s, 1H), 6.15 (s, 1H), 5.29 (s, 1H), 3.85 (s, 3H), 3.33 (d, J=5.0 Hz, 2H), 2.80 (s, 3H). Mass: m/z 506 (M$^+$+1).

[0726] Step-2

[0727] To a stirred solution of 3 (300 mg, 0.59 mmol) and 4 (179 mg, 0.89 mmol) in acetone (25 mL) was added K_2CO_3 (163 mg, 1.88 mmol) at RT, and the reaction mixture was stirred for 18 h at RT. After the completion of reaction (monitored by TLC), the reaction mixture was diluted with water (200 mL) and extracted with EtOAc (7×100 mL). The organic layers were washed with water (4×100 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuo. The crude compound was purified by column chromatography to afford 5 (205 mg, 55.1%) as off-white solid. NMR (DMSO-d$_6$, 500 MHz): δ 9.91 (s, 1H), 9.24 (s, 1H), 8.41 (s, 1H), 8.23 (s, 1H), 7.95 (dd, J=2.5, 6.5 Hz, 1H), 7.90 (s, 1H), 7.47 (dd, J=2.5, 9.0 Hz, 1H), 7.47-7.41 (m, 1H), 7.30 (t, J=9.0 Hz, 1H), 7.00 (d, J=9.0 Hz, 1H), 6.80-6.76 (m, 1H), (dt, J=5.5, 16.0 Hz, 1H), 6.23 (d, J=6.0 Hz, 1H), 6.00 (s, 1H), 3.77 (s, 3H), 3.09 (d, J=5.5 Hz, 2H), 2.93-2.87 (m, 2H), 2.29 (t, J=6.5 Hz, 2H), 2.13 (s, 3H), 1.42-1.32 (m, 13H). Mass: m/z 628 (M$^+$+1).

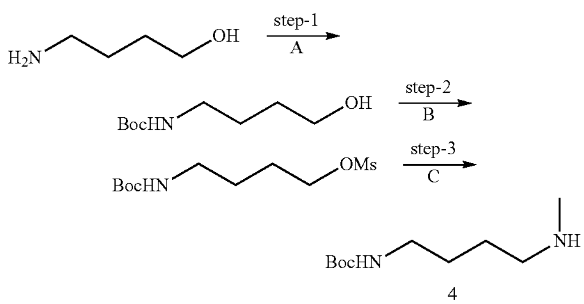
[0728] Step-3

[0729] TFA (0.5 mL) was added to a solution of 5 in dry DCM (1 mL). After stirring 2 h at room temperature, the mixture was concentrated under reduced pressure to give 6. The residue was used in the next step without purification.

[0730] Step-4

[0731] The residue 6 from Step-3 was diluted in dry DMF (1 mL), and HOBt (10.2 mg), EDCI (14.5 mg), N-biotinyl-NH-PEG$_2$-COOH.DIPEA (52.1 mg), and N-methyl morpholine (23 uL) were added. The resulting mixture was stirred overnight at room temperature. The crude product was directly injected to semi-prep HPLC (TFA modifier) and gave a white solid as a TFA salt. ^{1}H NMR (DMSO-d$_6$) δ ppm: 10.2 (s, 1H), 9.66 (s, 1H), 9.60 (br, 1H), 9.08 (br, 1H), 8.25 (s, 1H), 7.78 (m, 3H), 7.68 (dd, J=5.5 & 11.4 Hz, 2H), 7.43 (dd, J=2.3 & 8.7 Hz, 1H), 7.30 (m, 2H), 7.00 (d, J=9.2 Hz, 1H), 6.64 (m, 1H), 6.36 (d, J=15.1 Hz, 1H), 6.28 (m, 1H), 5.93 (s, 1H), 4.21 (dd, J=4.6 & 7.8 Hz, 1H), 4.03 (dd, J=4.6 & 7.8 Hz, 1H), 3.89 (m, 1H), 3.71 (s, 3H), 3.38 (m, 9H), 3.28 (t, J=6.4 Hz, 4H), 2.97 (m, 9H), 2.71 (dd, J=5.0 & 12.4 Hz, 1H), 2.66 (d, J=4.1 Hz, 3H), 2.47 (d, J=12.4 Hz, 1H), 1.95 (m, 6H), 1.1-1.6 (m, 13H); LCMS: m/e 1070.4 (M+1).

[0732] tert-Butyl 4-(methylamino)butylcarbamate (4) was prepared by the scheme shown below.



A) $(Boc)_2O$, TEA, DCM; B) MsCl, TEA, DCM; C) $MeNH_2$, EtOH.

[0733] Step-1

[0734] To a stirred solution of 4-aminobutanol (5 g, 56.0 mmol) in DCM (100 mL) was added Boc anhydride (12.2 g, 56.0 mmol) and TEA (7.7 mL, 56.0 mmol) at 0° C. and the reaction mixture was stirred at RT for 8 h. After the completion of reaction (monitored by TLC), the reaction mixture was diluted with water and extracted with DCM (3×25 mL). The organic layers were dried over anhydrous

Na_2SO_4 and concentrated under vacuo. The crude compound was purified by column chromatography to afford tert-butyl 4-hydroxybutylcarbamate (6.0 g, 56.6%) as colorless viscous liquid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.63 (bs, 1H), 3.72-3.60 (m, 2H), 3.23-3.08 (m, 2H), 1.99-1.50 (m, 4H), 1.43 (s, 9H).

[0735] Step-2

[0736] To a stirred solution of tert-butyl 4-hydroxybutylcarbamate (7 g, 37.0 mmol) in DCM (100 mL) was added TEA (9.35 mg, 92.5 mmol) followed by methanesulfonyl chloride (5.09 g, 44.4 mmol) over a period of 20 minutes at 0° C. The reaction mixture was further stirred for 18 h at RT. After the completion of reaction (monitored by TLC), the reaction mixture was diluted with water (100 mL) and extracted with DCM (3×30 mL). The organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuo. The crude compound was purified by column chromatography to afford (4-tert-butyloxycarbonylamino)-butyl methanesulfonate (5 g, 52.2%) as light yellow viscous liquid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 4.62 (bs, 2H), 4.25 (t, J=6.0 Hz, 2H), 3.14 (q, J=6.6 Hz, 2H), 3.01 (s, 3H), 1.85-1.70 (m, 2H), 1.78-1.54 (m, 2H), 1.44 (s, 9H). Mass: m/z 168 (M^++1-Boc).

[0737] Step-3

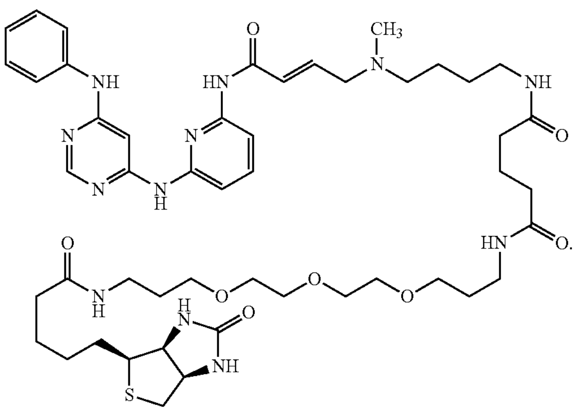
[0738] To a stirred solution of (4-tert-butyloxycarbonylamino)-butyl methanesulfonate (5 g, 18.7 mmol) in ethanol (25 mL) was added methylamine (25 mL) at 0° C. The reaction mixture was stirred for 18 h at RT. After the completion of reaction (monitored by TLC), the volatiles were removed under reduced pressure to afford tert-butyl 4-(methylamino)butylcarbamate (4) (3.4 g, 89.9%) as colorless viscous liquid. The crude compound was used in the next step without further purification. NMR ($CDCl_3$, 200 MHz): δ 5.15 (bs, 2H), 3.22-3.10 (m, 2H), 2.95 (t, J=7.0 Hz, 2H), 2.70 (s, 3H), 1.90-1.70 (m, 2H), 1.88-1.50 (m, 2H), 1.43 (s, 9H).

Example 46

Preparation of N^1-(4-(methyl((E)-4-oxo-4-(6-(6-(phenylamino)pyrimidin-4-ylamino)pyridin-2-ylamino)but-2-enyl)amino)butyl)-N^5-(15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)glutaramide (I-116)

[0739]

I-116



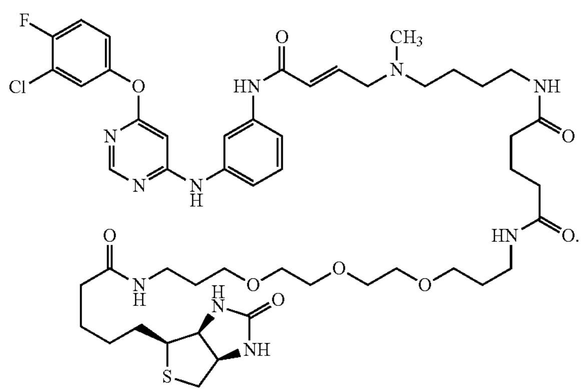
[0740] The title compound can be prepared according to the schemes, steps, and intermediates described in Example 45 by using N^4-(6-aminopyridin-2-yl)-N^6-phenylpyrimidine-4,6-diamine in place of 1 in Step-1. The synthesis of N^4-(6-aminopyridin-2-yl)-N^6-phenylpyrimidine-4,6-diamine is described in Example 14, and N^4-(6-aminopyridin-2-yl)-N^6-phenylpyrimidine-4,6-diamine is described as an intermediate in the preparation of I-73 in Example 15.

Example 47

Preparation of N^1-(4-(((E)-4-(3-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-ylamino)phenylamino)-4-oxobut-2-enyl)(methyl)amino)butyl)-N5-(15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)glutaramide (I-117)

[0741]

I-117



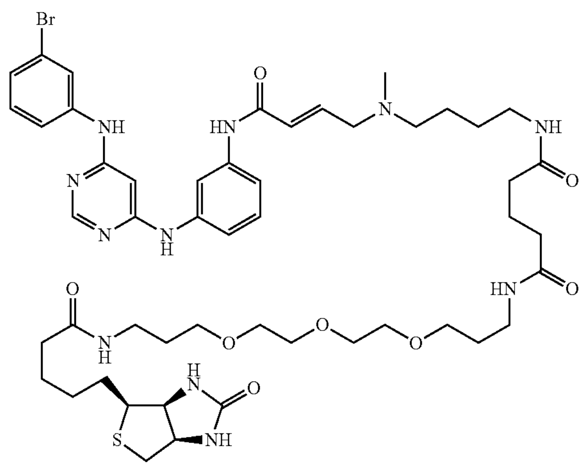
[0742] The title compound can be prepared according to the schemes, steps, and intermediates described in Example 45 by using N^1-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-yl)benzene-1,3-diamine in place of 1 in Step-1. The synthesis of N^1-(6-(3-chloro-4-fluorophenoxy)pyrimidin-4-yl)benzene-1,3-diamine is described in Example 34 in the preparation of I-92.

Example 48

Preparation of N^1-(4-(((E)-4-(3-(6-(3-bromophenylamino)pyrimidin-4-ylamino)phenylamino)-4-oxobut-2-enyl)(methyl)amino)butyl)-N5-(15-oxo-19-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10-trioxa-14-azanonadecyl)glutaramide (I-120)

[0743]

I-120



[0744] The title compound can be prepared according to the schemes, steps, and intermediates described in Example 45 by using 3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenylamine in place of 1 in Step-1. The synthesis of 3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenylamine is described in Example 1, and 3-[6-(3-bromophenylamino)-pyrimidin-4-ylamino]-phenylamine is described as an intermediate in the preparation of I-1 in Example 3.

[0745] Described below are assays used to measure the biological activity of provided compounds as inhibitors of ErbB1 (EGFR), ErbB2, ErbB4, TEC, BTK, ITK, BMX, and JAK3.

Example 49

Cloning, Expression and Purification of EGFR-WT and EGFR C797S Mutant Using Baculovirus and Insect Cells

(i) Subcloning of EGFR-WT and Mutant Kinase Domains

[0746] Amino acids 696 to 1022 of the EGFR-WT kinase domain (NM_005228, NP_005219.2) was subcloned into the NcoI and HindIII sites of the pFastHTa vector (Invitrogen, Carlsbad, Calif.). To make the EGFR-mutant protein, the cysteine at position 797 was changed to a serine using the Stratagene QuikChange kit (Stratagene, Cedar Creek, Tex.), according to manufacturer's instructions.

(ii) Expression

[0747] PI baculovirus stocks were generated in SF9 cells via Blue Sky Biotech's suspension transfection protocol (Worcester, Mass.). Expression analysis was conducted in 125 ml culture of SF21 insect cells ((grown in SF900I SFM (Invitrogen cat #10902-088), supplemented with 10 mg/L gentamicin (Invitrogen, Carlsbad, Calif., cat#15710-064)) using a viral load of 0.1 ml of virus per 100 ml of cell suspension. Expression was optimized using Blue Sky Biotech's Infection Kinetics Monitoring system (Worcester, Mass.).

(iii) Purification

[0748] Infected insect cells were pelleted. Cell pellets were resuspended in Blue Sky Biotech's lysis buffer (Worcester, Mass., 1×WX; solubilization buffer, containing a protease inhibitor cocktail of leupeptin, pepstatin, PMSF, aprotinin and EDTA) at a ratio of 10 ml per gram of wet cell paste. Cells were lysed by sonication and the lysate was clarified by centrifugation at 9,000 RPM for 30 minutes in a GSA rotor. 500 μl bed volume of NiNTA resin (Qiagen, Valencia, Calif.) was added to the supernatants and batch bound for two hours with constant agitation. The material was transferred by gravity into an empty 2 ml column. The column was washed with 2 ml of wash buffer (Blue Sky Biotech, Worcester, Mass., 1×WX, 25 mM imidazole). The protein was eluted with 1×WX+imidazole at varying concentrations: Elution 1: 75 mM imidazole (2 fractions, 1 column volume); Elution 2: 150 mM imidazole (2 fractions, 1 column volume); Elution 3: 300 mM imidazole (2 fractions, 1 column volume). All the elution fractions were analyzed by SDS page followed by Coomassie staining and Western Blotting using anti-penta-his antibody (Qiagen, Valencia, Calif.). The carboxy-terminal six-histidine "tag" was removed from some of the purified protein using AcTEV Protease kit (Invitrogen, Carlsbad, Calif., Cat#12575-015), following manufacturer's instructions. All the samples (pre- and post-Tev cut) were analyzed by SDS page followed by Coomassie staining and Western Blotting, as described above.

Example 50

Mass Spectrometry for EGFR

[0749] EGFR wild type and EGFR mutant (C797S) was incubated with 10-fold excess of compound I-1 for 1 hr and 3 hrs. 1 ul aliquots of the samples (total volume 5-8 ul) were diluted with 10 ul of 0.1% TFA prior to micro C4 ZipTipping directly onto the MALDI target using Sinapinic acid as the desorption matrix (10 mg/ml in 0.1% TFA:Acetonitrile 50:50). Intact mass measurement reveals that the wild type has a nominal mass of about 37557 and the mutant slightly lower at 37500. Reactivity was only observed for the wild type EGFR with a new peak appearing at a mass consistent with a single site covalent modification with compound I-1 which has a mass of 410 Da. (See FIG. **8**). The mutant EGFR (C797S) showed no significant reactivity even after 3 hrs, confirming modification of the cysteine of interest, Cys797.

Example 51

Omnia Assay Protocol for Potency Assessment Against EGFR (WT) and EGFR (T790M/L858R) Active Enzymes

[0750] The protocol below describes continuous-read kinase assays to measure inherent potency of compounds against active forms of EGFR (WT) and EGFR (T790M/L858R) enzymes. The mechanics of the assay platform are best described by the vendor (Invitrogen, Carlsbad, Calif.) on their website at the following URL: http://www.invitrogen.com/content.cfm?pageid=11338 or http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/Target-and-Lead-Identification-and-Validation/KinaseBiology/KB-Misc/Biochemical-Assays/Omnia-Kinase-Assays.html.

[0751] Briefly, 10× stocks of EGFR-WT (PV3872) from Invitrogen and EGFR-T790M/L858R (40350) from BPS Bioscience, San Diego, Calif., 1.13×ATP (AS001A) and appropriate Tyr-Sox conjugated peptide substrates (KCZ1001) were prepared in 1× kinase reaction buffer consisting of 20 mM Tris, pH 7.5, 5 mM $MgCl_2$, 1 mM EGTA, 5 mM β-glycerophosphate, 5% glycerol (10× stock, KB002A) and 0.2 mM DTT (DS001A). 5 μL of each enzyme were pre-incubated in a Corning (#3574) 384-well, white, non-binding surface microtiter plate (Corning, N.Y.) for 30 min. at 27° C. with a 0.5 μL volume of 50% DMSO and serially diluted compounds prepared in 50% DMSO. Kinase reactions were started with the addition of 45 μL of the ATP/Tyr-Sox peptide substrate mix and monitored every 30-90 seconds for 60 minutes at $\lambda_{ex}360/\lambda_{em}485$ in a Synergy[4] plate reader from BioTek (Winooski, Vt.). At the conclusion of each assay, progress curves from each well were examined for linear reaction kinetics and fit statistics (R^2, 95% confidence interval, absolute sum of squares). Initial velocity (0 minutes to ~30 minutes) from each reaction was determined from the slope of a plot of relative fluorescence units vs time (minutes) and then plotted against inhibitor concentration to estimate IC_{50} from log [Inhibitor] vs Response, Variable Slope model in GraphPad Prism from GraphPad Software (San Diego, Calif.).

[0752] The EGFR-WT- and EGFR T790M/L858R-modified optimized reagent conditions are:

[0753] [EGFR-WT]=5 nM, [ATP]=15 mM, [Y12-Sox]=5 mM (ATP KMapp ~12 mM); and [EGFR-T790M/L858R]=3 nM, [ATP]=50 mM, [Y12-Sox]=5 mM (ATP KMapp ~45 mM).

Example 52

[0754] Table 7 shows the activity of selected compounds of this invention in the EGFR inhibition assay. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an $IC_{50}\leq 10$ nM; compounds having an activity designated as "B" provided an IC_{50} 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of 1000-10,000 nM; and compounds having an activity designated as "E" provided an $IC_{50}\geq 10,000$ nM.

TABLE 7

EGFR Wild Type and EGFR (mutant C797S) Inhibition Data

Compound #	EGFR inhibtion	EGFR (T790M/L858R) inhibiton
I-1	A	A
I-2	A	A
I-3	A	A
I-4	A	B
I-5	A	A
I-6	B	B
I-7	A	B
I-8	A	D
I-9	A	C
I-10	A	C
I-11	B	E
I-12	B	E
I-13	A	B
I-14	A	B
I-15	A	B
I-16	A	B
I-17	A	B
I-18	A	A
I-19	A	A
I-46	A	A
I-47	A	E
I-48	C	C
I-49	A	A
I-50	A	B
I-51	A	D
I-52	A	A
I-53	B	C
I-54	A	A
I-55	A	A
I-56	A	A
I-57	—	C
I-58	—	C
I-59	A	A
I-60	D	C
I-61	—	A
I-63	A	A
I-65	A	B
I-66	A	C
I-67	A	A
I-68	A	—
I-69	B	—
I-70	A	—
I-71	A	—
I-73	A	—
I-75	A	—

TABLE 7-continued

EGFR Wild Type and EGFR (mutant C797S) Inhibition Data

Compound #	EGFR inhibtion	EGFR (T790M/L858R) inhibiton
I-78	A	—
I-79	A	—
I-80	C	—
I-81	A	—
I-82	A	—
I-83	A	—
I-84	B	—
I-85	B	—
I-86	B	—
I-87	B	—
I-88	A	—
I-89	A	—
I-90	A	—
I-91	A	—
I-92	A	—
I-93	A	—
I-94	A	A
I-95	A	—
I-97	A	—
I-98	A	—
I-99	A	—
I-100	A	—
I-101	A	—
I-102	A	—
I-103	A	—
I-104	A	—
I-105	A	—

Example 53

Cellular Assays for EGFR Activity

[0755] Compounds were assayed in A431 human epidermoid carcinoma cells using a method substantially similar to that described in Fry, et al., *Proc. Natl. Acad. Sci. USA* Vol 95, pp 12022-12027, 1998. Specifically, A431 human epidermoid carcinoma cells were grown in 6-well plates to 90% confluence and then incubated in serum-free media for 18 hr. Duplicate sets of cells were treated with 1 μM designated compound for 2, 5, 10, 30, or 60 min. Cells were washed free of the compound with warmed serum-free medium, incubated for 2 hr, washed again, incubated another 2 hr, washed again, and then incubated another 2 hr washed again and incubated for additional 2 hr and then stimulated with 100 ng/ml EGF for 5 min. Extracts were made as described Fry, et al. FIG. **1** depicts the EGFR inhibiting activity of compound I-1.

[0756] Compounds were assayed in A431 human epidermoid carcinoma cells using a method substantially similar to that described in Fry, et al. Specifically, A431 human epidermoid carcinoma cells were grown in 6-well plates to 90% confluence and then incubated in serum-free media for 18 hr. Cells were then treated with 10, 1, 0.1, 0.01, or 0.001 μM test compound for 1 hr. Cells were then stimulated with 100 ng/ml EGF for 5 min, and extracts were made as described in Fry, et al. 20 ug total protein from lysates were loaded on gel and blots were probed for either EGFR phosphorylation or p42/p44 Erk phosphorylation.

[0757] Dose response inhibition of EGFR phosphorylation and p42/p44 Erk phosphorylation with compound I-16 and I-17 in A431 cells is depicted in FIG. **3**. Dose response inhibition of EGFR phosphorylation and p42/p44 Erk phosphorylation with compound I-19 in A431 cells is depicted in FIG. **4**. Dose response inhibition of EGFR phosphorylation with compound I-1 in A431 cells, as compared with its "reversible control" compound (I^R-3), is depicted in FIG. **5**.

Example 54

Washout Experiment for EGFR Activity

[0758] A431 human epidermoid carcinoma cells were grown in 6-well plates to 90% confluence and then incubated in serum-free media for 18 hr. Duplicate sets of cells were treated with 1 μM designated compound for 1 hr. One set of cells was then stimulated with 100 ng/ml EGF for 5 min, and extracts were made as described. The other set of cells was washed free of the compound with warmed compound-free medium, incubated for 2 hr, washed again, incubated another 2 hr, washed again, and then incubated another 2 hr washed again and incubated for additional 2 hr and then stimulated with EGF. The results of this experiment are depicted in FIG. **6** where it is shown that compound I-1 maintains enzyme inhibition after "washout" whereas its "reversible control" compound (I^R-3) was washed away in the experiment thereby resulting in reactivated enzyme activity.

Example 55

Mass Spectrometry for ErbB4

[0759] ErbB4 kinase domain (Upstate) was incubated with compound for 90 minutes at 10-fold excess of compound I-1 to protein. 1 ul aliquots of the samples (total volume of 4.24 ul) were diluted with 10 ul of 0.1% TFA prior to micro C4 ZipTipping directly onto the MALDI target using Sinapinic acid as the desorption matrix (10 mg/ml in 0.1% TFA: Acetonitrile 50:50). For intact protein mass measurement the instrument was set in Linear mode using a pulsed extraction setting of 16,952 for the myoglobin standard used to calibrate the instrument (Shimadzu Axima TOF^2).

[0760] Intact ErbB4 protein occurs at MH+ of 35850 with corresponding sinapinic (matrix) adducts occurring about 200 Da higher. A stoichiometric incorporation of the compound I-1 (Mw of 410 Da) produced a new mass peak which is approximately 410 Da higher (MH+ of 36260). This is consistent with covalent modification of ErbB4 with compound I-1 as depicted in FIG. **7**.

Example 56

ErbB1, ErbB2 and/or ErbB4 Kinase Inhibition

[0761] Compounds of the present invention were assayed as inhibitors of one or more of ErbB1, ErbB2, and/or ErbB4 in a manner substantially similar to the method described by Invitrogen Corp (Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, Calif., Calif.; http://www.invitrogen.com/downloads/Z-LYTE_Brochure_1205. pdf) using the Z'-LYTE™ biochemical assay procedure or similar biochemical assay. The Z'-LYTE™ biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage.

Example 57

[0762] Table 8 shows the activity of selected compounds of this invention in the ErbB inhibition assay. The compound numbers correspond to the compound numbers in Table 5.

TABLE 8

ErbB1, ErbB2, and/or ErbB4 Inhibition Data			
Compound #	ErbB1 % Inhibition	ErbB2 % Inhibition	ErbB4 % Inhibition
I-1	99% @ 10 nM	76% @ 1 μM 61% @ 100 nM	86% @ 1 μM 64% @ 100 nM
I-2	98% @ 100 nM 75% @ 10 nM	96% @ 1 μM 39% @ 100 nM	75% @ 1 μM
I-3	96% @ 100 nM 56% @ 10 nM	89% @ 1 μM	95% @ 1 μM
I-4	100% @ 10 nM	86% @ 1 μM	78% @ 1 μM
I-5	100% @ 10 nM	86% @ 1 μM	95% @ 1 μM
I-6	100% @ 100 nM 49% @ 10 nM	84% @ 1 μM	97% @ 1 μM
I-7	100% @ 100 nM 53% @ 10 nM	89% @ 1 μM	100% @ 1 μM
I-8	83% @ 10 nM	—	57% @ 1 μM
I-9	100% @ 1 μM	—	75% @ 1 μM
I-10	96% @ 1 μM	—	—
I-11	79% @ 1 μM	—	—
I-12	82% @ 1 μM	—	—
I-13	92% @ 1 μM	98% @ 1 μM	—
I-14	96% @ 1 μM	—	—
I-15	98% @ 1 μM	—	—
I-16	98% @ 1 μM	87% @ 100 nM 24% @ 10 nM	93% @ 100 nM 26% @ 10 nM
I-17	95% @ 1 μM	89% @ 1 μM	94% @ 1 μM
I-18	—	91% @ 1 μM	94% @ 1 μM
I-19	96% @ 1 μM	98% @ 1 μM	97% @ 1 μM

Example 58

Mass Spectrometry for TEC Kinase

[0763] TEC kinase (45 pmols; Invitrogen) was incubated with (I-13) (450 pmols) for 3 hrs at 10× access prior to tryptic digestion. Iodoacetamide was used as the alkylating agent after compound incubation. A control sample (45 pmols) was also prepared which did not have the addition of (I-13). For tryptic digests a 5 ul aliquot (7.5 pmols) was diluted with 15 ul of 0.1% TFA prior to micro C18 Zip Tipping directly onto the MALDI target using alpha cyano-4-hydroxy cinnamic acid as the matrix (5 mg/ml in 0.1% TFA:Acetonitrile 50:50).

[0764] As depicted in FIG. **11**, the expected peptide (G CLLNFLR) to be modified was immediately evident at MH+ of 1358.65. This is the mass to be expected when compound I-13, with an adduct mass of 423.17, is added to the peptide mass of 935.51. The peptide was also quite evident in the control sample as modified by Iodacetamide at MH+ of 992.56. Interestingly the Iodoacetamide modified peptide was not evident in the digest reacted with compound I-13 indicating that the reaction was complete. There was no evidence of any other modified peptides.

[0765] Evidence of compound I-13 was observed at MH+ of 424.20 in the low mass range of the spectra. The fragmentation spectra of the 424.20 peak did show many diagnostic fragments that were apparent in the PSD spectra of the modified peptide at 1358.65 (see FIG. **11**).

[0766] To further verify the presence of the modified peptide with compound I-13, the peptide at MH+ of 1358.65 was subjected to PSD (MS/MS) analysis. A correlational analysis with the homosapien database identified the correct peptide modified by I-13.

Instrumental:

[0767] For tryptic digests the instrument was set in Reflectron mode with a pulsed extraction setting of 2200. Calibration was done using the Laser Biolabs Pep Mix standard

(1046.54, 1296.69, 1672.92, 2093.09, 2465.20). For CID/PSD analysis the peptide was selected using cursors to set ion gate timing and fragmentation occurred at a laser power about 20% higher and He was used as the collision gas for CID. Calibration for fragments was done using the P14R fragmentation calibration for the Curved field Reflectron.

Example 59

Omnia Assay Protocol for Potency Assessment Against BTK

[0768] The Omnia Assay Protocol for potency assessment against BTK is performed in a substantially similar manner as that described in Example 25 above except that the modified BTK-optimized reagent conditions are:

[0769] [BTK]=5 nM, [ATP]=40 mM, [Y5-Sox]=10 mM (ATP KMapp ~36 mM).

Example 60

[0770] Table 9 shows the activity of selected compounds of this invention in the BTK inhibition assay. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an IC_{50}≤10 nM; compounds having an activity designated as "B" provided an IC_{50} 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of 1000-10,000 nM; and compounds having an activity designated as "E" provided an IC_{50}≥10,000 nM.

TABLE 9

BTK Inhibition Data

Compound #	BTK Inhibition
I-1	B
I-2	B
I-3	B
I-4	A
I-5	A
I-6	C
I-7	B
I-8	E
I-9	C
I-10	C
I-11	E
I-12	E
I-13	A
I-14	C
I-15	B
I-16	B
I-17	A
I-18	B
I-19	B
I-46	C
I-47	E
I-48	B
I-49	A
I-50	D
I-51	E
I-52	A
I-53	B
I-54	C
I-55	B
I-56	B
I-57	D
I-58	D
I-59	C
I-60	D
I-61	B
I-62	A
I-63	A

TABLE 9-continued

BTK Inhibition Data

Compound #	BTK Inhibition
I-64	A
I-65	A
I-66	A
I-67	B
I-68	B
I-69	C
I-70	C
I-71	D
I-73	A
I-75	A
I-78	A
I-79	A
I-80	D
I-81	A
I-82	A
I-83	A
I-84	D
I-85	B
I-86	B
I-87	E
I-88	A
I-89	A
I-90	A
I-91	A
I-92	B
I-93	D
I-94	A
I-95	A
I-97	C
I-98	C
I-99	A
I-100	A
I-101	A
I-102	B
I-103	C
I-104	A
I-105	B

Example 61

BTK Ramos Cellular Assay

[0771] Compounds I-13 and (I-52) were assayed in Ramos human Burkitt lymphoma cells. Ramos cells were grown in suspension in T225 flasks, spun down, resuspended in 50 mls serum-free media and incubated for 1 hour. Compound was added to Ramos cells in serum free media to a final concentration of 1, 0.1, 0.01, or 0.001 μM. Ramos cells were incubated with compound for 1 hour, washed again and resuspended in 100 ul serum-free media. Cells were then stimulated with 1 μg of goat F(ab')2 Anti-Human IgM and incubated on ice for 10 minutes to activate B cell receptor signaling pathways. After 10 minutes, the cells were washed once with PBS and then lysed on ice with Invitrogen Cell Extraction buffer. 16 μg total protein from lysates was loaded on gels and blots were probed for phosphorylation of the BTK substrate PLCγ2. At 1 μM I-13 showed 85% inhibition and (I-52) showed 50% inhibition of BTK signaling in Ramos cells. Additional dose response inhibition of BTK signaling with I-13 is depicted in FIG. **9**.

[0772] Table 10 provides inhibition data for selected compounds in Ramos cells. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an IC_{50}≤10 nM; compounds having an activity designated as "B" provided an IC_{50} of 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of ≥1000 nM.

TABLE 10

BTK Ramos Cellular Inhibition Data	
Compound #	BTK Inhibition (nM)
I-5	C
I-13	B
I-17	D
I-63	A
I-64	B
I-65	A
I-66	A
I-78	A
I-79	A
I-81	A
I-82	A
I-95	A
I-99	A
I-100	B

Example 62

Washout Experiment with Ramos Cells for BTK Activity

[0773] Ramos cells were serum starved for one hour in RPMI media+1% glutamine at 37° C. After starvation, Ramos cells were treated with 100 nM compound diluted in serum free RPMI media for 1 hour. After compound treatment, the media was removed and cells were washed with compound-free media. Subsequently, Ramos cells were washed every 2 hours and resuspended in fresh compound-free media. Cells were collected at specified timepoints, treated with 1 ug anti-human IgM (Southern Biotech cat #2022-01) for 10 minutes on ice to induce BCR signaling and then washed in PBS. Ramos cells were then lysed in Cell Extraction Buffer (Invitrogen FNN0011) supplemented with Roche complete protease inhibitor tablets (Roche 11697498001) and phosphatase inhibitors (Roche 04 906 837 001) and 18 ug total protein lysate was loaded in each lane. Inhibition of BTK kinase activity was assayed by measuring its substrate (PLCγ2) phosphorylation by western blot with phospho-specific antibodies from Cell Signaling Technologies cat#3871. FIG. **10** depicts the results of compound I-13 in the washout experiment at 0 hour, 4 hour, 6 hour, and 8 hour time points. As shown in FIG. **10**, compound I-13 maintains inhibition of BTK for 8 hours.

[0774] Table 11 provides data for selected compounds in the Ramos washout assay.

TABLE 11

BTK Washout Data	
Compound #	BTK Inhibition Type
I-13	irreversible
I-63	irreversible
I-64	irreversible
I-65	reversible
I-66	irreversible
I-82	irreversible

Example 63

Mass Spectrometry for BTK

[0775] Intact BTK was incubated for 1 hr at a 10× fold excess of compound I-63 or I-66 to protein. Aliquots (2 ul) of the samples were diluted with 10 ul of 0.1% TFA prior to micro C4 ZipTipping directly onto the MALDI target using Sinapinic acid as the desorption matrix (10 mg/ml in 0.1% TFA:Acetonitrile 20:80). Mass spectrometry traces are shown in FIG. **12** and FIG. **13**. The top panels of FIGS. **12** and **13** show the mass spec trace of the intact BTK protein (m/z 81,169 Da). The bottom panels show the mass spec trace when BTK was incubated with compound I-63 (mw 424.5) or compound I-66 (mw 425.5) in FIGS. **12** and **13**, respectively. The centroid mass (m/z 81,593 kDa) in the bottom panel of FIG. **12** shows a positive shift of about 424 Da, indicating complete modification of BTK by compound I-63. The centroid mass (m/z 81,593 kDa) in the bottom panel of FIG. **13** shows a positive shift of about 407 Da, indicating complete modification of BTK by compound I-66.

Example 64

Omnia Assay Protocol for Potency Assessment Against Active Forms of ITK Kinase

[0776] This example describes continuous-read kinase assays to measure inherent potency of compound against active forms of ITK enzymes as described in Example 10 above except that the modified ITK-optimized reagent conditions are:

[0777] [ITK]=10 nM, [ATP]=25 μM, [Y6-Sox]=10 μM (ATP K_{Mapp}=33 μM).

Example 65

[0778] Table 12 shows the activity of selected compounds of this invention in the ITK inhibition assay. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an IC_{50}≤10 nM; compounds having an activity designated as "B" provided an IC_{50} 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of 1000-10,000 nM; and compounds having an activity designated as "E" provided an IC_{50}≥10,000 nM.

TABLE 12

ITK Inhibition Data	
Compound #	ITK inhibition
I-10	E
I-13	D
I-14	D
I-15	D
I-63	D
I-64	B
I-65	B
I-66	D
I-78	B
I-79	A
I-81	B
I-88	B
I-89	C
I-90	C

TABLE 12-continued

ITK Inhibition Data	
Compound #	ITK inhibition
I-94	B
I-95	E
I-98	E
I-100	E
I-101	C

Example 66

Omnia Assay Protocol for Potency Assessment Against Active Forms of BMX Kinase

[0779] This example describes continuous-read kinase assays to measure inherent potency of compound against active forms of BMX enzymes as described in Example 10 above except that the modified BMX-optimized reagent conditions are:

[0780] [BMX]=2.5 nM, [ATP]=100 μM, [Y5-Sox]=7.5 μM (ATP K_{Mapp}=107 μM).

Example 67

[0781] Table 13 shows the activity of selected compounds of this invention in the BMX inhibition assay. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an IC_{50}≤10 nM; compounds having an activity designated as "B" provided an IC_{50} 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of 1000-10,000 nM; and compounds having an activity designated as "E" provided an IC_{50}≥10,000 nM.

TABLE 13

BMX Inhibition Data	
Compound #	BMX inhibition
I-10	—
I-13	A
I-14	—
I-15	—
I-63	A
I-64	B
I-65	A
I-66	A
I-78	B
I-79	A
I-81	A
I-94	A

Example 68

Omnia Assay Protocol for Potency Assessment Against the Active Form of Janus-3 Kinase (JAK3)

[0782] The Omnia Assay Protocol for potency assessment against JAK3 is performed in a substantially similar manner as that described in Example 25 above except that the modified JAK3-optimized reagent conditions are:

[0783] [JAK3]=5 nM, [ATP]=5 μM, [Y12-Sox]=5 μM (ATP KMapp ~5 μM).

Example 69

[0784] Table 14 shows the activity of selected compounds of this invention in the JAK3 inhibition assay. The compound numbers correspond to the compound numbers in Table 5. Compounds having an activity designated as "A" provided an IC_{50}≤10 nM; compounds having an activity designated as "B" provided an IC_{50} 10-100 nM; compounds having an activity designated as "C" provided an IC_{50} of 100-1000 nM; compounds having an activity designated as "D" provided an IC_{50} of 1000-10,000 nM; and compounds having an activity designated as "E" provided an IC_{50}≥10,000 nM.

TABLE 14

JAK3 Inhibition Data	
Compound #	JAK3 Inhibition
I-1	A
I-2	A
I-3	A
I-4	A
I-5	A
I-6	D
I-7	C
I-8	D
I-9	C
I-10	C
I-11	E
I-12	E
I-13	C
I-14	A
I-15	B
I-16	B
I-17	B
I-18	B
I-19	C
I-46	D
I-47	E
I-48	E
I-49	A
I-50	C
I-51	E
I-52	A
I-53	C
I-54	D
I-55	B
I-56	B
I-57	E
I-58	C
I-59	D
I-60	D
I-61	B
I-62	C
I-63	B
I-64	A
I-65	A
I-66	C
I-67	A
I-68	A
I-69	C
I-70	C
I-71	D
I-73	A
I-75	C
I-78	A
I-79	A
I-80	E
I-81	A
I-82	B
I-84	E
I-85	D
I-86	E
I-92	B

TABLE 14-continued

JAK3 Inhibition Data	
Compound #	JAK3 Inhibition
I-93	E
I-94	A

Example 70

Measuring ErbB Occupancy

[0785] Protein samples from ErbB expressing cells (e.g., A431 human epidermoid carcinoma lysates or SKOV3 ovarian cancer tumor cells grown in vivo) that are or are not previously treated with a covalent inhibitor will be incubated with a covalent probe compound for 1 h. The target-covalent probe complex will be captured using streptavidin beads or using an antibody that recognizes the ErbB target. The captured proteins will be separated by SDS-PAGE and analyzed by Western blot using the complementary approach, i.e. an anti-ErbB antibody for the complexes captured with streptavidin or streptavidin for the complexes captured using the anti-ErbB antibody. The amount of target that is bound to the covalent probe will be quantitated and compared to the amount of total ErbB protein to determine the percentage of target that is occupied by the covalent drug.

[0786] While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example.

SEQUENCE LISTING

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<211> LENGTH: 540
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

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Lys Lys Lys Thr Ser Pro Leu Asn Phe Lys Lys Arg Leu Phe Leu Leu
            20                  25                  30

Thr Val His Lys Leu Ser Tyr Tyr Glu Tyr Asp Phe Glu Arg Gly Arg
        35                  40                  45

Arg Gly Ser Lys Lys Gly Ser Ile Asp Val Glu Lys Ile Thr Cys Val
    50                  55                  60

Glu Thr Val Val Pro Glu Lys Asn Pro Pro Pro Glu Arg Gln Ile Pro
65                  70                  75                  80

Arg Arg Gly Glu Glu Ser Ser Glu Met Glu Gln Ile Ser Ile Ile Glu
                85                  90                  95

Arg Phe Pro Tyr Pro Phe Gln Val Val Tyr Asp Glu Gly Pro Leu Tyr
            100                 105                 110

Val Phe Ser Pro Thr Glu Glu Leu Arg Lys Arg Trp Ile His Gln Leu
        115                 120                 125

Lys Asn Val Ile Arg Tyr Asn Ser Asp Leu Val Gln Lys Tyr His Pro
    130                 135                 140

Cys Phe Trp Ile Asp Gly Gln Tyr Leu Cys Cys Ser Gln Thr Ala Lys
145                 150                 155                 160

Asn Ala Met Gly Cys Gln Ile Leu Glu Asn Arg Asn Gly Ser Leu Lys
                165                 170                 175

Pro Gly Ser Ser His Arg Lys Thr Lys Lys Pro Leu Pro Pro Thr Pro
            180                 185                 190

Glu Glu Asp Gln Ile Leu Lys Lys Pro Leu Pro Pro Glu Pro Ala Ala
        195                 200                 205

Ala Pro Val Ser Thr Ser Glu Leu Lys Lys Val Val Ala Leu Tyr Asp
    210                 215                 220
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Tyr Met Pro Met Asn Ala Asn Asp Leu Gln Leu Arg Lys Gly Asp Glu
225 230 235 240

Tyr Phe Ile Leu Glu Glu Ser Asn Leu Pro Trp Trp Arg Ala Arg Asp
245 250 255

Lys Asn Gly Gln Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Glu Ala
260 265 270

Glu Asp Ser Ile Glu Met Tyr Glu Trp Tyr Ser Lys His Met Thr Arg
275 280 285

Ser Gln Ala Glu Gln Leu Leu Lys Gln Glu Gly Lys Glu Gly Gly Phe
290 295 300

Ile Val Arg Asp Ser Ser Lys Ala Gly Lys Tyr Thr Val Ser Val Phe
305 310 315 320

Ala Lys Ser Thr Gly Asp Pro Gln Gly Val Ile Arg His Tyr Val Val
325 330 335

Cys Ser Thr Pro Gln Ser Gln Tyr Tyr Leu Ala Glu Lys His Leu Phe
340 345 350

Ser Thr Ile Pro Glu Leu Ile Asn Tyr His Gln His Asn Ser Ala Gly
355 360 365

Leu Ile Ser Arg Leu Lys Tyr Pro Val Ser Gln Gln Asn Lys Asn Ala
370 375 380

Pro Ser Thr Ala Gly Leu Gly Tyr Gly Ser Trp Glu Ile Asp Pro Lys
385 390 395 400

Asp Leu Thr Phe Leu Lys Glu Leu Gly Thr Gly Gln Phe Gly Val Val
405 410 415

Lys Tyr Gly Lys Trp Arg Gly Gln Tyr Asp Val Ala Ile Lys Met Ile
420 425 430

Lys Glu Gly Ser Met Ser Glu Asp Glu Phe Ile Glu Glu Ala Lys Val
435 440 445

Met Met Asn Leu Ser His Glu Lys Leu Val Gln Leu Tyr Gly Val Cys
450 455 460

Thr Lys Gln Arg Pro Ile Phe Ile Ile Thr Glu Tyr Met Ala Asn Gly
465 470 475 480

Cys Leu Leu Asn Tyr Leu Arg Glu Met Arg His Arg Phe Gln Thr Gln
485 490 495

Gln Leu Leu Glu Met Cys Lys Asp Val Cys Glu Ala Met Glu Tyr Leu
500 505 510

Glu Ser Lys Gln Phe Leu His Arg Asp Leu Ala Ala Arg Asn Cys Leu
515 520 525

Val Asn Asp Gln Gly Val Val Lys Val Ser Asp Phe
530 535 540

<210> SEQ ID NO 2
<211> LENGTH: 631
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met Asn Phe Asn Thr Ile Leu Glu Glu Ile Leu Ile Lys Arg Ser Gln
1 5 10 15

Gln Lys Lys Lys Thr Ser Pro Leu Asn Tyr Lys Glu Arg Leu Phe Val
20 25 30

Leu Thr Lys Ser Met Leu Thr Tyr Tyr Glu Gly Arg Ala Glu Lys Lys
35 40 45

-continued

```
Tyr Arg Lys Gly Phe Ile Asp Val Ser Lys Ile Lys Cys Val Glu Ile
    50                  55                  60

Val Lys Asn Asp Asp Gly Val Ile Pro Cys Gln Asn Lys Tyr Pro Phe
65                  70                  75                  80

Gln Val Val His Asp Ala Asn Thr Leu Tyr Ile Phe Ala Pro Ser Pro
                85                  90                  95

Gln Ser Arg Asp Leu Trp Val Lys Lys Leu Lys Glu Glu Ile Lys Asn
            100                 105                 110

Asn Asn Asn Ile Met Ile Lys Tyr His Pro Lys Phe Trp Thr Asp Gly
        115                 120                 125

Ser Tyr Gln Cys Cys Arg Gln Thr Glu Lys Leu Ala Pro Gly Cys Glu
    130                 135                 140

Lys Tyr Asn Leu Phe Glu Ser Ser Ile Arg Lys Ala Leu Pro Pro Ala
145                 150                 155                 160

Pro Glu Thr Lys Lys Arg Arg Pro Pro Pro Pro Ile Pro Leu Glu Glu
                165                 170                 175

Glu Asp Asn Ser Glu Glu Ile Val Val Ala Met Tyr Asp Phe Gln Ala
            180                 185                 190

Ala Glu Gly His Asp Leu Arg Leu Glu Arg Gly Gln Glu Tyr Leu Ile
        195                 200                 205

Leu Glu Lys Asn Asp Val His Trp Trp Arg Ala Arg Asp Lys Tyr Gly
    210                 215                 220

Asn Glu Gly Tyr Ile Pro Ser Asn Tyr Val Thr Gly Lys Lys Ser Asn
225                 230                 235                 240

Asn Leu Asp Gln Tyr Glu Trp Tyr Cys Arg Asn Met Asn Arg Ser Lys
                245                 250                 255

Ala Glu Gln Leu Leu Arg Ser Glu Asp Lys Glu Gly Gly Phe Met Val
            260                 265                 270

Arg Asp Ser Ser Gln Pro Gly Leu Tyr Thr Val Ser Leu Tyr Thr Lys
        275                 280                 285

Phe Gly Gly Glu Gly Ser Ser Gly Phe Arg His Tyr His Ile Lys Glu
    290                 295                 300

Thr Thr Thr Ser Pro Lys Lys Tyr Tyr Leu Ala Glu Lys His Ala Phe
305                 310                 315                 320

Gly Ser Ile Pro Glu Ile Ile Glu Tyr His Lys His Asn Ala Ala Gly
                325                 330                 335

Leu Val Thr Arg Leu Arg Tyr Pro Val Ser Val Lys Gly Lys Asn Ala
            340                 345                 350

Pro Thr Thr Ala Gly Phe Ser Tyr Glu Lys Trp Glu Ile Asn Pro Ser
        355                 360                 365

Glu Leu Thr Phe Met Arg Glu Leu Gly Ser Gly Leu Phe Gly Val Val
    370                 375                 380

Arg Leu Gly Lys Trp Arg Ala Gln Tyr Lys Val Ala Ile Lys Ala Ile
385                 390                 395                 400

Arg Glu Gly Ala Met Cys Glu Glu Asp Phe Ile Glu Glu Ala Lys Val
                405                 410                 415

Met Met Lys Leu Thr His Pro Lys Leu Val Gln Leu Tyr Gly Val Cys
            420                 425                 430

Thr Gln Gln Lys Pro Ile Tyr Ile Val Thr Glu Phe Met Glu Arg Gly
        435                 440                 445
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Cys Leu Leu Asn Phe Leu Arg Gln Arg Gln Gly His Phe Ser Arg Asp
450 455 460

Val Leu Leu Ser Met Cys Gln Asp Val Cys Glu Gly Met Glu Tyr Leu
465 470 475 480

Glu Arg Asn Ser Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu
485 490 495

Val Ser Glu Ala Gly Val Val Lys Val Ser Asp Phe Gly Met Ala Arg
500 505 510

Tyr Val Leu Asp Asp Gln Tyr Thr Ser Ser Ser Gly Ala Lys Phe Pro
515 520 525

Val Lys Trp Cys Pro Pro Glu Val Phe Asn Tyr Ser Arg Phe Ser Ser
530 535 540

Lys Ser Asp Val Trp Ser Phe Gly Val Leu Met Trp Glu Val Phe Thr
545 550 555 560

Glu Gly Arg Met Pro Phe Glu Lys Tyr Thr Asn Tyr Glu Val Val Thr
565 570 575

Met Val Thr Arg Gly His Arg Leu Tyr Gln Pro Lys Leu Ala Ser Asn
580 585 590

Tyr Val Tyr Glu Val Met Leu Arg Cys Trp Gln Glu Lys Pro Glu Gly
595 600 605

Arg Pro Ser Phe Glu Asp Leu Leu Arg Thr Ile Asp Glu Leu Val Glu
610 615 620

Cys Glu Glu Thr Phe Gly Arg
625 630

<210> SEQ ID NO 3
<211> LENGTH: 620
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Met Asn Asn Phe Ile Leu Leu Glu Glu Gln Leu Ile Lys Lys Ser Gln
1 5 10 15

Gln Lys Arg Arg Thr Ser Pro Ser Asn Phe Lys Val Arg Phe Phe Val
20 25 30

Leu Thr Lys Ala Ser Leu Ala Tyr Phe Glu Asp Arg His Gly Lys Lys
35 40 45

Arg Thr Leu Lys Gly Ser Ile Glu Leu Ser Arg Ile Lys Cys Val Glu
50 55 60

Ile Val Lys Ser Asp Ile Ser Ile Pro Cys His Tyr Lys Tyr Pro Phe
65 70 75 80

Gln Val Val His Asp Asn Tyr Leu Leu Tyr Val Phe Ala Pro Asp Arg
85 90 95

Glu Ser Arg Gln Arg Trp Val Leu Ala Leu Lys Glu Glu Thr Arg Asn
100 105 110

Asn Asn Ser Leu Val Pro Lys Tyr His Pro Asn Phe Trp Met Asp Gly
115 120 125

Lys Trp Arg Cys Cys Ser Gln Leu Glu Lys Leu Ala Thr Gly Cys Ala
130 135 140

Gln Tyr Asp Pro Thr Lys Asn Ala Ser Lys Lys Pro Leu Pro Pro Thr
145 150 155 160

Pro Glu Asp Asn Arg Arg Pro Leu Trp Glu Pro Glu Glu Thr Val Val
165 170 175

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Ile Ala Leu Tyr Asp Tyr Gln Thr Asn Asp Pro Gln Glu Leu Ala Leu
            180                 185                 190

Arg Arg Asn Glu Glu Tyr Cys Leu Leu Asp Ser Ser Glu Ile His Trp
        195                 200                 205

Trp Arg Val Gln Asp Arg Asn Gly His Glu Gly Tyr Val Pro Ser Ser
    210                 215                 220

Tyr Leu Val Glu Lys Ser Pro Asn Asn Leu Glu Thr Tyr Glu Trp Tyr
225                 230                 235                 240

Asn Lys Ser Ile Ser Arg Asp Lys Ala Glu Lys Leu Leu Leu Asp Thr
                245                 250                 255

Gly Lys Glu Gly Ala Phe Met Val Arg Asp Ser Arg Thr Ala Gly Thr
            260                 265                 270

Tyr Thr Val Ser Val Phe Thr Lys Ala Val Val Ser Glu Asn Asn Pro
        275                 280                 285

Cys Ile Lys His Tyr His Ile Lys Glu Thr Asn Asp Asn Pro Lys Arg
    290                 295                 300

Tyr Tyr Val Ala Glu Lys Tyr Val Phe Asp Ser Ile Pro Leu Leu Ile
305                 310                 315                 320

Asn Tyr His Gln His Asn Gly Gly Gly Leu Val Thr Arg Leu Arg Tyr
                325                 330                 335

Pro Val Cys Phe Gly Arg Gln Lys Ala Pro Val Thr Ala Gly Leu Arg
            340                 345                 350

Tyr Gly Lys Trp Val Ile Asp Pro Ser Glu Leu Thr Phe Val Gln Glu
        355                 360                 365

Ile Gly Ser Gly Gln Phe Gly Leu Val His Leu Gly Tyr Trp Leu Asn
    370                 375                 380

Lys Asp Lys Val Ala Ile Lys Thr Ile Arg Glu Gly Ala Met Ser Glu
385                 390                 395                 400

Glu Asp Phe Ile Glu Glu Ala Glu Val Met Met Lys Leu Ser His Pro
                405                 410                 415

Lys Leu Val Gln Leu Tyr Gly Val Cys Leu Glu Gln Ala Pro Ile Cys
            420                 425                 430

Leu Val Phe Glu Phe Met Glu His Gly Cys Leu Ser Asp Tyr Leu Arg
        435                 440                 445

Thr Gln Arg Gly Leu Phe Ala Ala Glu Thr Leu Leu Gly Met Cys Leu
    450                 455                 460

Asp Val Cys Glu Gly Met Ala Tyr Leu Glu Glu Ala Cys Val Ile His
465                 470                 475                 480

Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Gly Glu Asn Gln Val Ile
                485                 490                 495

Lys Val Ser Asp Phe Gly Met Thr Arg Phe Val Leu Asp Asp Gln Tyr
            500                 505                 510

Thr Ser Ser Thr Gly Thr Lys Phe Pro Val Lys Trp Ala Ser Pro Glu
        515                 520                 525

Val Phe Ser Phe Ser Arg Tyr Ser Ser Lys Ser Asp Val Trp Ser Phe
    530                 535                 540

Gly Val Leu Met Trp Glu Val Phe Ser Glu Gly Lys Ile Pro Tyr Glu
545                 550                 555                 560

Asn Arg Ser Asn Ser Glu Val Val Glu Asp Ile Ser Thr Gly Phe Arg
                565                 570                 575
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-continued

Leu Tyr Lys Pro Arg Leu Ala Ser Thr His Val Tyr Gln Ile Met Asn
580 585 590

His Cys Trp Lys Glu Arg Pro Glu Asp Arg Pro Ala Phe Ser Arg Leu
595 600 605

Leu Arg Gln Leu Ala Glu Ile Ala Glu Ser Gly Leu
610 615 620

<210> SEQ ID NO 4
<211> LENGTH: 675
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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Gln Lys Lys Lys Met Ser Pro Asn Asn Tyr Lys Glu Arg Leu Phe Val
20 25 30

Leu Thr Lys Thr Asn Leu Ser Tyr Tyr Glu Tyr Asp Lys Met Lys Arg
35 40 45

Gly Ser Arg Lys Gly Ser Ile Glu Ile Lys Lys Ile Arg Cys Val Glu
50 55 60

Lys Val Asn Leu Glu Glu Gln Thr Pro Val Glu Arg Gln Tyr Pro Phe
65 70 75 80

Gln Ile Val Tyr Lys Asp Gly Leu Leu Tyr Val Tyr Ala Ser Asn Glu
85 90 95

Glu Ser Arg Ser Gln Trp Leu Lys Ala Leu Gln Lys Glu Ile Arg Gly
100 105 110

Asn Pro His Leu Leu Val Lys Tyr His Ser Gly Phe Phe Val Asp Gly
115 120 125

Lys Phe Leu Cys Cys Gln Gln Ser Cys Lys Ala Ala Pro Gly Cys Thr
130 135 140

Leu Trp Glu Ala Tyr Ala Asn Leu His Thr Ala Val Asn Glu Glu Lys
145 150 155 160

His Arg Val Pro Thr Phe Pro Asp Arg Val Leu Lys Ile Pro Arg Ala
165 170 175

Val Pro Val Leu Lys Met Asp Ala Pro Ser Ser Ser Thr Thr Leu Ala
180 185 190

Gln Tyr Asp Asn Glu Ser Lys Lys Asn Tyr Gly Ser Gln Pro Pro Ser
195 200 205

Ser Ser Thr Ser Leu Ala Gln Tyr Asp Ser Asn Ser Lys Lys Ile Tyr
210 215 220

Gly Ser Gln Pro Asn Phe Asn Met Gln Tyr Ile Pro Arg Glu Asp Phe
225 230 235 240

Pro Asp Trp Trp Gln Val Arg Lys Leu Lys Ser Ser Ser Ser Ser Glu
245 250 255

Asp Val Ala Ser Ser Asn Gln Lys Glu Arg Asn Val Asn His Thr Thr
260 265 270

Ser Lys Ile Ser Trp Glu Phe Pro Glu Ser Ser Ser Ser Glu Glu Glu
275 280 285

Glu Asn Leu Asp Asp Tyr Asp Trp Phe Ala Gly Asn Ile Ser Arg Ser
290 295 300

Gln Ser Glu Gln Leu Leu Arg Gln Lys Gly Lys Glu Gly Ala Phe Met
305 310 315 320

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Val Arg Asn Ser Ser Gln Val Gly Met Tyr Thr Val Ser Leu Phe Ser
                325                 330                 335

Lys Ala Val Asn Asp Lys Lys Gly Thr Val Lys His Tyr His Val His
            340                 345                 350

Thr Asn Ala Glu Asn Lys Leu Tyr Leu Ala Glu Asn Tyr Cys Phe Asp
        355                 360                 365

Ser Ile Pro Lys Leu Ile His Tyr His Gln His Asn Ser Ala Gly Met
    370                 375                 380

Ile Thr Arg Leu Arg His Pro Val Ser Thr Lys Ala Asn Lys Val Pro
385                 390                 395                 400

Asp Ser Val Ser Leu Gly Asn Gly Ile Trp Glu Leu Lys Arg Glu Glu
                405                 410                 415

Ile Thr Leu Leu Lys Glu Leu Gly Ser Gly Gln Phe Gly Val Val Gln
            420                 425                 430

Leu Gly Lys Trp Lys Gly Gln Tyr Asp Val Ala Val Lys Met Ile Lys
        435                 440                 445

Glu Gly Ser Met Ser Glu Asp Glu Phe Phe Gln Glu Ala Gln Thr Met
    450                 455                 460

Met Lys Leu Ser His Pro Lys Leu Val Lys Phe Tyr Gly Val Cys Ser
465                 470                 475                 480

Lys Glu Tyr Pro Ile Tyr Ile Val Thr Glu Tyr Ile Ser Asn Gly Cys
                485                 490                 495

Leu Leu Asn Tyr Leu Arg Ser His Gly Lys Gly Leu Glu Pro Ser Gln
            500                 505                 510

Leu Leu Glu Met Cys Tyr Asp Val Cys Glu Gly Met Ala Phe Leu Glu
        515                 520                 525

Ser His Gln Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val
    530                 535                 540

Asp Arg Asp Leu Cys Val Lys Val Ser Asp Phe Gly Met Thr Arg Tyr
545                 550                 555                 560

Val Leu Asp Asp Gln Tyr Val Ser Ser Val Gly Thr Lys Phe Pro Val
                565                 570                 575

Lys Trp Ser Ala Pro Glu Val Phe His Tyr Phe Lys Tyr Ser Ser Lys
            580                 585                 590

Ser Asp Val Trp Ala Phe Gly Ile Leu Met Trp Glu Val Phe Ser Leu
        595                 600                 605

Gly Lys Gln Pro Tyr Asp Leu Tyr Asp Asn Ser Gln Val Val Leu Lys
    610                 615                 620

Val Ser Gln Gly His Arg Leu Tyr Arg Pro His Leu Ala Ser Asp Thr
625                 630                 635                 640

Ile Tyr Gln Ile Met Tyr Ser Cys Trp His Glu Leu Pro Glu Lys Arg
                645                 650                 655

Pro Thr Phe Gln Gln Leu Leu Ser Ser Ile Glu Pro Leu Arg Glu Lys
            660                 665                 670

Asp Lys His
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<212> TYPE: PRT
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<400> SEQUENCE: 5

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Ser Leu Leu Ser Thr Glu Ala Gly Ala Leu His Val Leu Leu Pro Ala
            20                  25                  30

Arg Gly Pro Gly Pro Pro Gln Arg Leu Ser Phe Ser Phe Gly Asp His
        35                  40                  45

Leu Ala Glu Asp Leu Cys Val Gln Ala Ala Lys Ala Ser Gly Ile Leu
    50                  55                  60

Pro Val Tyr His Ser Leu Phe Ala Leu Ala Thr Glu Asp Leu Ser Cys
65                  70                  75                  80

Trp Phe Pro Pro Ser His Ile Phe Ser Val Glu Asp Ala Ser Thr Gln
                85                  90                  95

Val Leu Leu Tyr Arg Ile Arg Phe Tyr Phe Pro Asn Trp Phe Gly Leu
            100                 105                 110

Glu Lys Cys His Arg Phe Gly Leu Arg Lys Asp Leu Ala Ser Ala Ile
        115                 120                 125

Leu Asp Leu Pro Val Leu Glu His Leu Phe Ala Gln His Arg Ser Asp
    130                 135                 140

Leu Val Ser Gly Arg Leu Pro Val Gly Leu Ser Leu Lys Glu Gln Gly
145                 150                 155                 160

Glu Cys Leu Ser Leu Ala Val Leu Asp Leu Ala Arg Met Ala Arg Glu
                165                 170                 175

Gln Ala Gln Arg Pro Gly Glu Leu Leu Lys Thr Val Ser Tyr Lys Ala
            180                 185                 190

Cys Leu Pro Pro Ser Leu Arg Asp Leu Ile Gln Gly Leu Ser Phe Val
        195                 200                 205

Thr Arg Arg Arg Ile Arg Arg Thr Val Arg Arg Ala Leu Arg Arg Val
    210                 215                 220

Ala Ala Cys Gln Ala Asp Arg His Ser Leu Met Ala Lys Tyr Ile Met
225                 230                 235                 240

Asp Leu Glu Arg Leu Asp Pro Ala Gly Ala Ala Glu Thr Phe His Val
                245                 250                 255

Gly Leu Pro Gly Ala Leu Gly Gly His Asp Gly Leu Gly Leu Leu Arg
            260                 265                 270

Val Ala Gly Asp Gly Gly Ile Ala Trp Thr Gln Gly Glu Gln Glu Val
        275                 280                 285

Leu Gln Pro Phe Cys Asp Phe Pro Glu Ile Val Asp Ile Ser Ile Lys
    290                 295                 300

Gln Ala Pro Arg Val Gly Pro Ala Gly Glu His Arg Leu Val Thr Val
305                 310                 315                 320

Thr Arg Thr Asp Asn Gln Ile Leu Glu Ala Glu Phe Pro Gly Leu Pro
                325                 330                 335

Glu Ala Leu Ser Phe Val Ala Leu Val Asp Gly Tyr Phe Arg Leu Thr
            340                 345                 350

Thr Asp Ser Gln His Phe Phe Cys Lys Glu Val Ala Pro Pro Arg Leu
        355                 360                 365

Leu Glu Glu Val Ala Glu Gln Cys His Gly Pro Ile Thr Leu Asp Phe
    370                 375                 380

Ala Ile Asn Lys Leu Lys Thr Gly Gly Ser Arg Pro Gly Ser Tyr Val
385                 390                 395                 400
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Leu Arg Arg Ser Pro Gln Asp Phe Asp Ser Phe Leu Leu Thr Val Cys
                405                 410                 415

Val Gln Asn Pro Leu Gly Pro Asp Tyr Lys Gly Cys Leu Ile Arg Arg
            420                 425                 430

Ser Pro Thr Gly Thr Phe Leu Leu Val Gly Leu Ser Arg Pro His Ser
        435                 440                 445

Ser Leu Arg Glu Leu Leu Ala Thr Cys Trp Asp Gly Gly Leu His Val
    450                 455                 460

Asp Gly Val Ala Val Thr Leu Thr Ser Cys Cys Ile Pro Arg Pro Lys
465                 470                 475                 480

Glu Lys Ser Asn Leu Ile Val Val Gln Arg Gly His Ser Pro Pro Thr
                485                 490                 495

Ser Ser Leu Val Gln Pro Gln Ser Gln Tyr Gln Leu Ser Gln Met Thr
            500                 505                 510

Phe His Lys Ile Pro Ala Asp Ser Leu Glu Trp His Glu Asn Leu Gly
        515                 520                 525

His Gly Ser Phe Thr Lys Ile Tyr Arg Gly Cys Arg His Glu Val Val
    530                 535                 540

Asp Gly Glu Ala Arg Lys Thr Glu Val Leu Leu Lys Val Met Asp Ala
545                 550                 555                 560

Lys His Lys Asn Cys Met Glu Ser Phe Leu Glu Ala Ala Ser Leu Met
                565                 570                 575

Ser Gln Val Ser Tyr Arg His Leu Val Leu Leu His Gly Val Cys Met
            580                 585                 590

Ala Gly Asp Ser Thr Met Val Gln Glu Phe Val His Leu Gly Ala Ile
        595                 600                 605

Asp Met Tyr Leu Arg Lys Arg Gly His Leu Val Pro Ala Ser Trp Lys
    610                 615                 620

Leu Gln Val Val Lys Gln Leu Ala Tyr Ala Leu Asn Tyr Leu Glu Asp
625                 630                 635                 640

Lys Gly Leu Pro His Gly Asn Val Ser Ala Arg Lys Val Leu Leu Ala
                645                 650                 655

Arg Glu Gly Ala Asp Gly Ser Pro Pro Phe Ile Lys Leu Ser Asp Pro
            660                 665                 670

Gly Val Ser Pro Ala Val Leu Ser Leu Glu Met Leu Thr Asp Arg Ile
        675                 680                 685

Pro Trp Val Ala Pro Glu Cys Leu Arg Glu Ala Gln Thr Leu Ser Leu
    690                 695                 700

Glu Ala Asp Lys Trp Gly Phe Gly Ala Thr Val Trp Glu Val Phe Ser
705                 710                 715                 720

Gly Val Thr Met Pro Ile Ser Ala Leu Asp Pro Ala Lys Lys Leu Gln
                725                 730                 735

Phe Tyr Glu Asp Arg Gln Gln Leu Pro Ala Pro Lys Trp Thr Glu Leu
            740                 745                 750

Ala Leu Leu Ile Gln Gln Cys Met Ala Tyr Glu Pro Val Gln Arg Pro
        755                 760                 765

Ser Phe Arg Ala Val Ile Arg Asp Leu Asn Ser Leu Ile Ser Ser Asp
    770                 775                 780

Tyr Glu Leu Leu Ser Asp Pro Thr Pro Gly Ala Leu Ala Pro Arg Asp
785                 790                 795                 800
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Gly Leu Trp Asn Gly Ala Gln Leu Tyr Ala Cys Gln Asp Pro Thr Ile
                805                 810                 815

Phe Glu Glu Arg His Leu Lys Tyr Ile Ser Gln Leu Gly Lys Gly Asn
            820                 825                 830

Phe Gly Ser Val Glu Leu Cys Arg Tyr Asp Pro Leu Gly Asp Asn Thr
        835                 840                 845

Gly Ala Leu Val Ala Val Lys Gln Leu Gln His Ser Gly Pro Asp Gln
    850                 855                 860

Gln Arg Asp Phe Gln Arg Glu Ile Gln Ile Leu Lys Ala Leu His Ser
865                 870                 875                 880

Asp Phe Ile Val Lys Tyr Arg Gly Val Ser Tyr Gly Pro Gly Arg Gln
                885                 890                 895

Ser Leu Arg Leu Val Met Glu Tyr Leu Pro Ser Gly Cys Leu Arg Asp
            900                 905                 910

Phe Leu Gln Arg His Arg Ala Arg Leu Asp Ala Ser Arg Leu Leu Leu
        915                 920                 925

Tyr Ser Ser Gln Ile Cys Lys Gly Met Glu Tyr Leu Gly Ser Arg Arg
    930                 935                 940

Cys Val His Arg Asp Leu Ala Ala Arg Asn Ile Leu Val Glu Ser Glu
945                 950                 955                 960

Ala His Val Lys Ile Ala Asp Phe Gly Leu Ala Lys Leu Leu Pro Leu
                965                 970                 975

Asp Lys Asp Tyr Tyr Val Val Arg Glu Pro Gly Gln Ser Pro Ile Phe
            980                 985                 990

Trp Tyr Ala Pro Glu Ser Leu Ser  Asp Asn Ile Phe Ser  Arg Gln Ser
        995                 1000                1005

Asp Val  Trp Ser Phe Gly Val  Val Leu Tyr Glu Leu  Phe Thr Tyr
    1010                1015                1020

Cys Asp  Lys Ser Cys Ser Pro  Ser Ala Glu Phe Leu  Arg Met Met
    1025                1030                1035

Gly Cys  Glu Arg Asp Val Pro  Ala Leu Cys Arg Leu  Leu Glu Leu
    1040                1045                1050

Leu Glu  Glu Gly Gln Arg Leu  Pro Ala Pro Pro Ala  Cys Pro Ala
    1055                1060                1065

Glu Val  His Glu Leu Met Lys  Leu Cys Trp Ala Pro  Ser Pro Gln
    1070                1075                1080

Asp Arg  Pro Ser Phe Ser Ala  Leu Gly Pro Gln Leu  Asp Met Leu
    1085                1090                1095

Trp Ser  Gly Ser Arg Gly Cys  Glu Thr His Ala Phe  Thr Ala His
    1100                1105                1110

Pro Glu  Gly Lys His His Ser  Leu Ser Phe Ser
    1115                1120
```

<210> SEQ ID NO 6
<211> LENGTH: 527
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

```
Met Ile Leu Ser Ser Tyr Asn Thr Ile Gln Ser Val Phe Cys Cys Cys
1               5                   10                  15

Cys Cys Cys Ser Val Gln Lys Arg Gln Met Arg Thr Gln Ile Ser Leu
            20                  25                  30
```

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```
Ser Thr Asp Glu Glu Leu Pro Glu Lys Tyr Thr Gln Arg Arg Arg Pro
        35                  40                  45

Trp Leu Ser Gln Leu Ser Asn Lys Lys Gln Ser Asn Thr Gly Arg Val
    50                  55                  60

Gln Pro Ser Lys Arg Lys Pro Leu Pro Pro Leu Pro Pro Ser Glu Val
65                  70                  75                  80

Ala Glu Glu Lys Ile Gln Val Lys Ala Leu Tyr Asp Phe Leu Pro Arg
                85                  90                  95

Glu Pro Cys Asn Leu Ala Leu Arg Arg Ala Glu Glu Tyr Leu Ile Leu
            100                 105                 110

Glu Lys Tyr Asn Pro His Trp Trp Lys Ala Arg Asp Arg Leu Gly Asn
        115                 120                 125

Glu Gly Leu Ile Pro Ser Asn Tyr Val Thr Glu Asn Lys Ile Thr Asn
    130                 135                 140

Leu Glu Ile Tyr Glu Trp Tyr His Arg Asn Ile Thr Arg Asn Gln Ala
145                 150                 155                 160

Glu His Leu Leu Arg Gln Glu Ser Lys Glu Gly Ala Phe Ile Val Arg
                165                 170                 175

Asp Ser Arg His Leu Gly Ser Tyr Thr Ile Ser Val Phe Met Gly Ala
            180                 185                 190

Arg Arg Ser Thr Glu Ala Ala Ile Lys His Tyr Gln Ile Lys Lys Asn
        195                 200                 205

Asp Ser Gly Gln Trp Tyr Val Ala Glu Arg His Ala Phe Gln Ser Ile
    210                 215                 220

Pro Glu Leu Ile Trp Tyr His Gln His Asn Ala Ala Gly Leu Met Thr
225                 230                 235                 240

Arg Leu Arg Tyr Pro Val Gly Leu Met Gly Ser Cys Leu Pro Ala Thr
                245                 250                 255

Ala Gly Phe Ser Tyr Glu Lys Trp Glu Ile Asp Pro Ser Glu Leu Ala
            260                 265                 270

Phe Ile Lys Glu Ile Gly Ser Gly Gln Phe Gly Val Val His Leu Gly
        275                 280                 285

Glu Trp Arg Ser His Ile Gln Val Ala Ile Lys Ala Ile Asn Glu Gly
    290                 295                 300

Ser Met Ser Glu Glu Asp Phe Ile Glu Glu Ala Lys Val Met Met Lys
305                 310                 315                 320

Leu Ser His Ser Lys Leu Val Gln Leu Tyr Gly Val Cys Ile Gln Arg
                325                 330                 335

Lys Pro Leu Tyr Ile Val Thr Glu Phe Met Glu Asn Gly Cys Leu Leu
            340                 345                 350

Asn Tyr Leu Arg Glu Asn Lys Gly Lys Leu Arg Lys Glu Met Leu Leu
        355                 360                 365

Ser Val Cys Gln Asp Ile Cys Glu Gly Met Glu Tyr Leu Glu Arg Asn
    370                 375                 380

Gly Tyr Ile His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Ser Ser
385                 390                 395                 400

Thr Cys Ile Val Lys Ile Ser Asp Phe Gly Met Thr Arg Tyr Val Leu
                405                 410                 415

Asp Asp Glu Tyr Val Ser Ser Phe Gly Ala Lys Phe Pro Ile Lys Trp
            420                 425                 430
```

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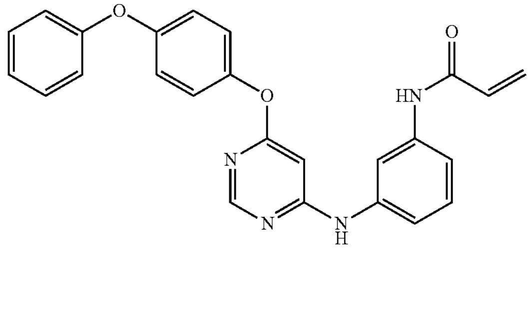
```
Ser Pro Pro Glu Val Phe Leu Phe Asn Lys Tyr Ser Ser Lys Ser Asp
        435                 440                 445

Val Trp Ser Phe Gly Val Leu Met Trp Glu Val Phe Thr Glu Gly Lys
    450                 455                 460

Met Pro Phe Glu Asn Lys Ser Asn Leu Gln Val Val Glu Ala Ile Ser
465                 470                 475                 480

Glu Gly Phe Arg Leu Tyr Arg Pro His Leu Ala Pro Met Ser Ile Tyr
                485                 490                 495

Glu Val Met Tyr Ser Cys Trp His Glu Lys Pro Glu Gly Arg Pro Thr
            500                 505                 510

Phe Ala Glu Leu Leu Arg Ala Val Thr Glu Ile Ala Glu Thr Trp
        515                 520                 525


<210> SEQ ID NO 7
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Ile Thr Gln Leu Met Pro Phe Gly Cys Leu Leu Asp Tyr Val Arg Glu
1               5                   10                  15

His


<210> SEQ ID NO 8
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Val Thr Gln Leu Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu
1               5                   10                  15

Asn


<210> SEQ ID NO 9
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr Val His Glu
1               5                   10                  15

His
```

1-2. (canceled)

3. A combination comprising:

(i) a composition comprising a compound selected from the group consisting of:

I-97



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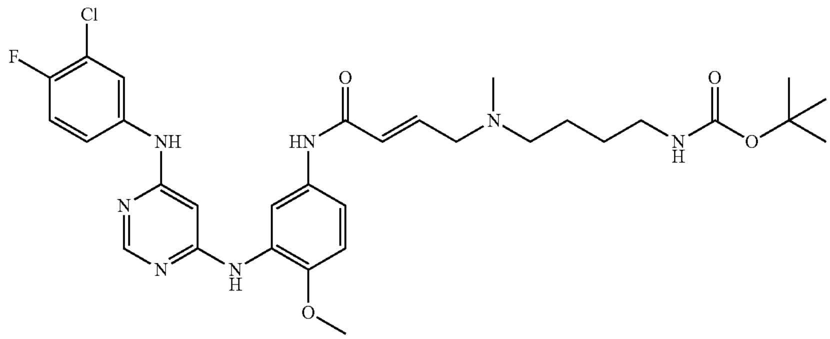
I-98



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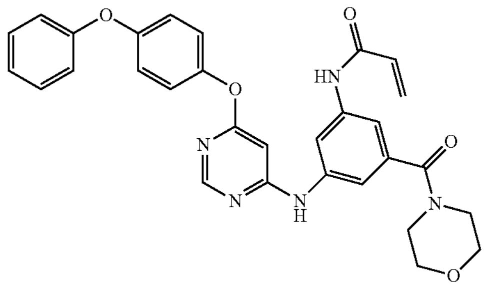
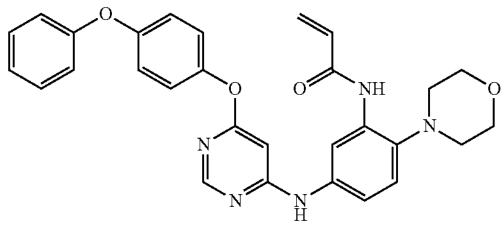


I-101



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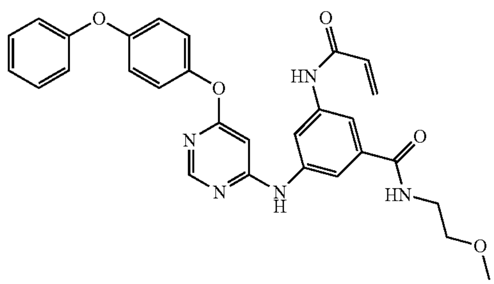

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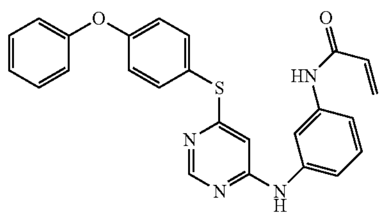

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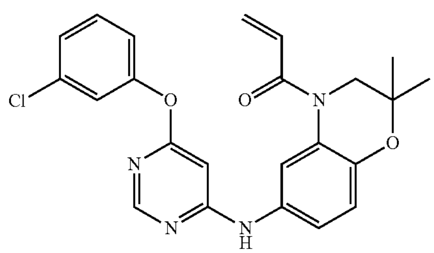

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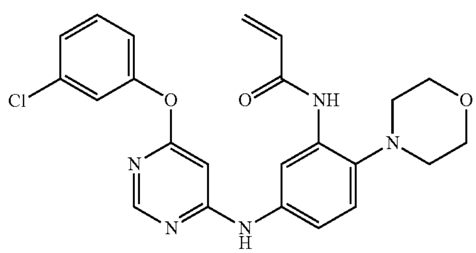
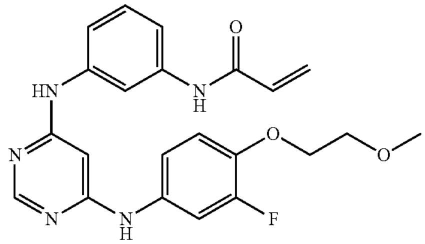


and

I-114



,

or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable adjuvant, carrier, or vehicle, and

(ii) an additional therapeutic agent.

4. The combination according to claim **3**, wherein the additional therapeutic agent is a chemotherapeutic agent.

5-39. (canceled)

40. The combination according to claim **3**, wherein the composition and the additional therapeutic agent are combined in a single unit dosage form.

41. The combination according to claim **3**, wherein the composition and the additional therapeutic agent are in separate unit dosage forms.

42. The combination according to claim **40**, wherein the additional therapeutic agent is a chemotherapeutic agent.

43. The combination according to claim **41**, wherein the additional therapeutic agent is a chemotherapeutic agent.

* * * * *