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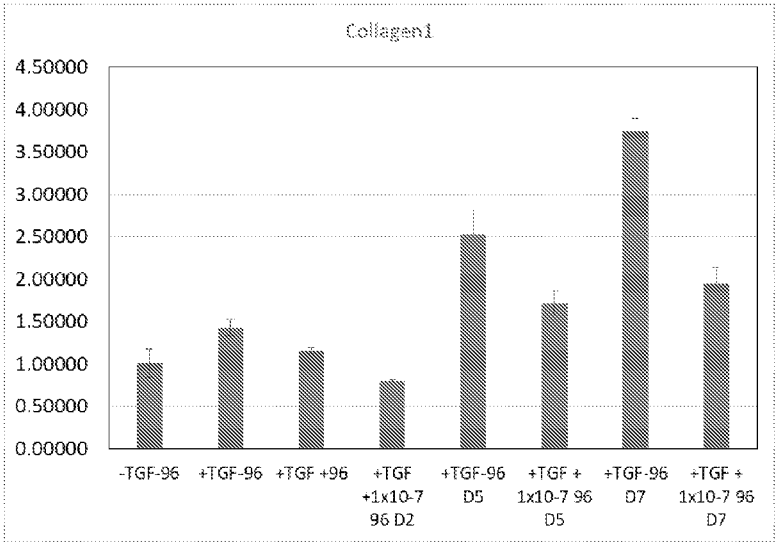

Figure 20

(57) Abstract: The present invention relates to a treatment for diseases associated with eye-related scarring and post-surgical scarring of glaucoma patients. More particularly, the invention relates to compositions and methods of treating eye-related scarring and fibrosis by inhibiting the active focal adhesion kinase using a small molecule inhibitor.

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FOCAL ADHESION KINASE TARGETED THERAPEUTICS FOR THE TREATMENT OF GLAUCOMA AND FIBROSIS

FIELD OF THE INVENTION

The present invention relates to a treatment for diseases associated with eye-related scarring and post-surgical scarring of glaucoma patients. More particularly, the invention relates to compositions and methods of treating eye-related scarring and fibrosis by inhibiting the active focal adhesion kinase using a small molecule inhibitor.

BACKGROUND

Glaucoma is a leading cause of irreversible blindness in Singapore as well as worldwide with an estimated 70 million people affected, of whom over 6 million suffer from bilateral blindness. Despite having successful surgical procedures, current surgical outcomes are limited due to long-term graft failure and rejection, resulting in increased morbidity. Ocular fibrosis and scarring are responsible for the pathogenesis or failure of treatment of almost all the major blinding diseases worldwide. The existing methods to prevent fibrosis after glaucoma surgery use the antimetabolites, Mitomycin C (MMC) and 5-fluorouracil (5-FU). Although these antimetabolites improve the surgical outcome of glaucoma filtration surgery, they have non-specific cytotoxicity which potentially leads to blinding complications like tissue damage, breakdown and infection. In addition, not all patients are responsive to this antimetabolite therapy.

The mechanisms underlying the pathophysiology of fibrosis in glaucoma are poorly understood. It is known that a fibrotic response is usually triggered by a dysregulated normal tissue repair process which leads to accumulation of extracellular matrix and compromised organ function. There is evidence that focal adhesion kinase (FAK) and upregulation of pro-fibrotic factors such as transforming growth factor β (TGFβ), collagen1α1 (COL1A1), and α-smooth muscle actin (αSMA) are involved in the development of fibrotic disorders [McDonnell, *Journal of Ophthalmology* 2014: Article ID 750459, 13 pages (2014); Lagares and Kapoor, *BioDrugs* 27: 15–23 (2013)].

Focal adhesion kinase (FAK) is a 125-kDa non-receptor cytoplasmic tyrosine kinase, which is known to play a role in many physiological processes such as regulating cell migration, proliferation and survival of a variety of cell types.

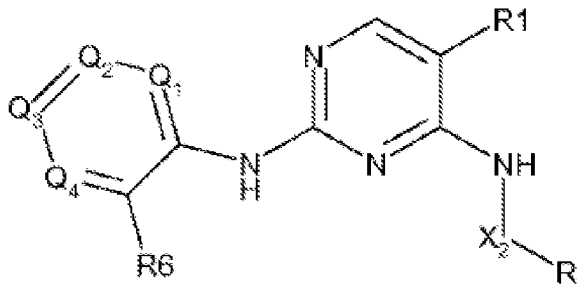
There is a need to develop new anti-fibrotic therapeutics with more specific physiological actions and less cytotoxicity to inhibit the fibrosis and scarring observed following surgery for glaucoma.

SUMMARY OF THE INVENTION

The present invention described herein involves the use of inhibitors of the active focal adhesion kinase (FAK) to reduce post-surgery fibrosis in subjects treated for glaucoma. According to the invention an example of one such inhibitor is the small molecule Aminopyrimidine Pyrimidine, Compound 96; diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate. This small molecule was shown to inhibit the active focal adhesion kinase (Phospho-FAK (Tyr397 and Tyr925)) and reduce the expression of the pro-fibrotic markers collagen I, collagen 3, α smooth muscle actin (αSMA) and SPARC (secreted protein, acidic, rich in cysteine).

According to a first aspect, the present invention provides a composition comprising at least one FAK inhibitor for inhibiting scarring and fibrosis of the eye of a subject.

Suitable FAK inhibitors according to any aspect of the present invention include those disclosed in WO2010/141406, the contents of which is incorporated herein by reference. Such compounds have the general formula 1:



1

wherein R is an optionally substituted aryl or heteroaryl;

R1 is halogen, CF_3, CCH, or other suitable substituent;

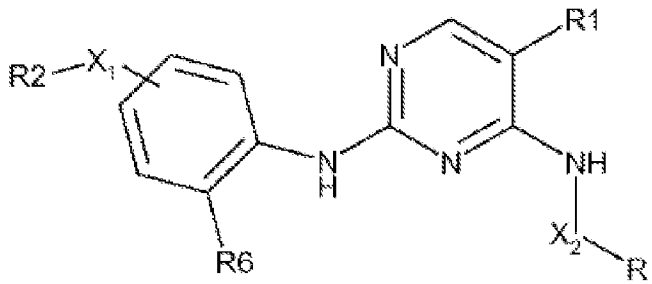
X_2 is $–(CR^7R^8)_{0-2}$-;

each R^7 and R^8 is independently halogen, C_{0-3} aliphatic, or $–OC_{0-3}$ aliphatic, either of which is optionally halogen substituted, except that in the case of X_2, R^7 and R^8 are not halogen or $–OC_{0-3}$ aliphatic;

R6 is halogen, $-OC_{0-3}$ aliphatic, or C_{0-3} aliphatic, either optionally substituted by one or more halogen or by $–OCF_3$;

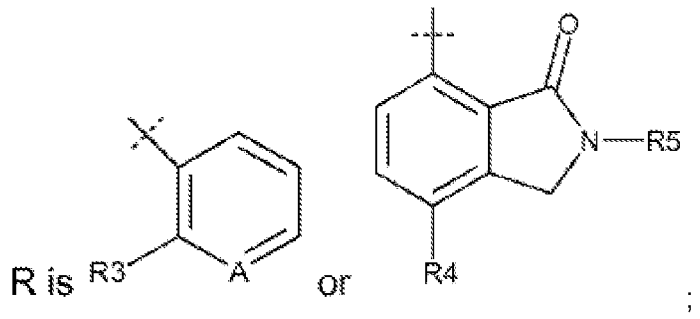
Q_1 to Q_4 are independently >CH, >CF, >N, or >N-oxide; except that at least one of Q_2 to Q_4 includes a substituent that includes a phosphinate, phosphonate, or phosphine oxide.

In some embodiments of formula 1 the compound has formula 1a:



wherein:

X_1 and X_2 are independently $–(CR^7R^8)_{0-1}$-;



;

A is CH or N;

R1 is halogen, $-CF_3$, or –CCH;

R2 is $–P(O)R^9R^{10}$;

R3 is $–S(O)_2R^{11}$, $-S(O)_2NR^{11}R^{12}$, $-C(O)NR^{11}R^{12}$, $-C(O)OR^{11}$, $-NR^{11}S(O)_2R^{12}$, or $-NR^{11}R^{12}$;

R4 is –x-y-z, wherein:

x is $_{4-6}$cyclic; y is absent or $_{4-6}$heterocyclic; and z is absent or C_{1-3}alkyl optionally substituted by 1-2 hydroxy or C_{1-6}alkoxy groups, or z is hydroxy or $–C(O)O-C_{0-3}$alkyl; or

-x-y-z is C_{0-6}alkoxy, C_{2-6}alkenyl, C_{2-6}alkynyl, or C_{0-6}alkyl, each optionally substituted by 1-2 hydroxy groups;

R5 is C_{0-2}alkyl optionally substituted by 1-3 independent hydroxy or halogen groups;

R6 is halo, $-C_{0-3}$alkoxy, $-C_{0-3}$alkyl optionally substituted by halo, or $–OCF_3$;

each R^7 and R^8 is independently H or –CH3;

R^9 and R^{10} are independently hydroxy, C_{1-6}alkoxy, or C_{0-6}alkyl, any of which can be taken together at any of their atoms to form a ring, or aryl, -O-aryl, or $_{4-6}$heterocyclic, wherein any of the foregoing can be further substituted by $_{3-6}$cyclic;

R^{11} and R^{12} are independently C_{0-6}alkyl, which can be taken together at any of their atoms to form a ring containing 1-3 heteroatoms; R^{11} or R^{12} can independently be taken at any of their atoms with the ring to which R3 is attached to form a ring.

In some embodiments, the composition of any aspect of the invention comprises pharmaceutically acceptable salts or solvates, or pharmaceutically functional derivatives of said at least one FAK inhibitor.

In other embodiments, the at least one FAK inhibitor is selected from any one of Examples 1-329 disclosed in WO2010/141406, or a pharmaceutically acceptable salt or solvate thereof.

A suitable FAK inhibitor exemplified herein includes Compound 96; diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate;

N-methyl-4-(4-((3-(N-methylmethan-3-ylsulfonamido)pyrazin-2-yl)methylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)benzamide;

N-methyl-N-(3-((2-(2-oxoindolin-5-ylamino)-5-(trifluoromethyl)pyrimidin-4-ylamino)methyl)pyridin-2-yl)methanesulfonamide benzenesulfonate;

Benzamide, 2-[[5-chloro-2-[[3-methyl-1-(1-methylethyl)-1H-pyrazol-5-yl]amino]-4-pyridinyl]amino]-N-methoxy-;

Benzoic acid, 2,4-dimethyl-, [4-[(1S)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, hydrochloride (1:2);

N-Methyl-N-{3-[({2-[(2-oxo-2,3-dihydro-1H-indol-5-yl)amino]-5-(trifluoromethyl)-4-pyrimidinyl}amino)methyl]-2-pyridinyl}methanesulfonamide hydrochloride (1:1);

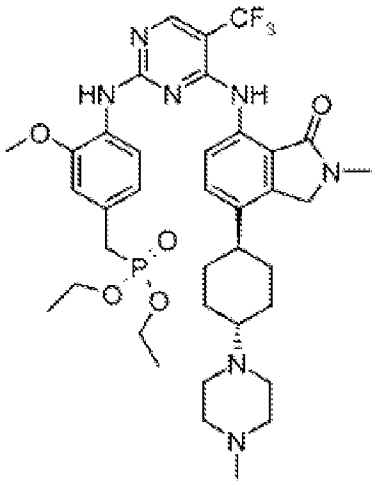
2-(2-(2-methoxy-4-morpholinophenylamino)-5-(trifluoromethyl)pyridin-4-ylamino)-N-methylbenzamide;

Benzamide, 2-[[5-chloro-2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]amino]-N-methyl-;

or a pharmaceutically acceptable salt or solvate thereof for inhibiting scarring and fibrosis of the eye of a subject.

More preferably, the compound is Compound 96; diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate or a pharmaceutically acceptable salt or solvate thereof for inhibiting scarring and fibrosis of the eye of a subject.

The structure of compound 96 is represented by the following formula:



In preferred embodiments, the scarring and fibrosis occurs post-surgery for glaucoma in the subject.

In preferred embodiments, the composition inhibits focal adhesion kinase (FAK) and/or the expression of at least one pro-fibrotic factor.

In preferred embodiments, the composition inhibits the phosphorylation of FAK to form FAK pTyr925 and/or FAK pTyr397.

In preferred embodiments, the at least one pro-fibrotic factor is selected from the group comprising the pro-fibrotic markers collagen1α1 (COL1A1), α smooth muscle actin (αSMA) and SPARC (secreted protein, acidic, rich in cysteine).

In preferred embodiments, the composition further comprises at least one anti-metabolite such as mitomycin C (MMC) and/or 5-fluorouracil (5-FU).

In preferred embodiments, the composition further comprises one or more pharmaceutically acceptable carriers, excipients or diluents.

According to a second aspect, the present invention provides the use of at least one FAK inhibitor or variant thereof for the manufacture of a medicament for inhibiting scarring and fibrosis of the eye of a subject.

In preferred embodiments, the present invention provides the use of compounds of formula 1, preferably of formula 1a, or pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for inhibiting scarring and fibrosis of the eye of a subject.

In preferred embodiments, the present invention provides the use of a compound selected from the group comprising Compound 96; diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate;

N-methyl-4-(4-((3-(N-methylmethan-3-ylsulfonamido)pyrazin-2-yl)methylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)benzamide;

N-methyl-N-(3-((2-(2-oxoindolin-5-ylamino)-5-(trifluoromethyl)pyrimidin-4-ylamino)methyl)pyridin-2-yl)methanesulfonamide benzenesulfonate;

Benzamide, 2-[[5-chloro-2-[[3-methyl-1-(1-methylethyl)-1H-pyrazol-5-yl]amino]-4-pyridinyl]amino]-N-methoxy-;

Benzoic acid, 2,4-dimethyl-, [4-[(1S)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, hydrochloride (1:2);

N-Methyl-N-{3-[({2-[(2-oxo-2,3-dihydro-1H-indol-5-yl)amino]-5-(trifluoromethyl)-4-pyrimidinyl}amino)methyl]-2-pyridinyl}methanesulfonamide hydrochloride (1:1);

2-(2-(2-methoxy-4-morpholinophenylamino)-5-(trifluoromethyl)pyridin-4-ylamino)-N-methylbenzamide;

Benzamide, 2-[[5-chloro-2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]amino]-N-methyl-;

or pharmaceutically acceptable salt or solvate thereof for the manufacture of a medicament for inhibiting scarring and fibrosis of the eye of a subject.

More preferably, the compound is Compound 96; diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate or pharmaceutically acceptable salt or solvate thereof.

In preferred embodiments, the medicament is for inhibiting the scarring and fibrosis that occurs post-surgery for glaucoma in a subject.

In preferred embodiments, the medicament is formulated for subconjunctival or intravenous delivery.

In preferred embodiments, the medicament inhibits focal adhesion kinase (FAK) and/or the expression of at least one pro-fibrotic factor.

In preferred embodiments, the medicament inhibits phosphorylation of FAK to form FAK pTyr925 and/or FAK pTyr397.

In preferred embodiments, the at least one pro-fibrotic factor is selected from the group comprising collagen1α1 (COL1A1), α smooth muscle actin (αSMA) and SPARC (secreted protein, acidic, rich in cysteine).

In preferred embodiments, the medicament is formulated for administration below the conjunctiva or intravenously.

According to a third aspect, the present invention provides a method of prophylaxis or treatment of eye related scarring and fibrosis, comprising administering to a subject in need thereof an effective amount of a composition according to any embodiment of the first aspect.

In some embodiments of the method, the administration is before, during or after surgery for treating glaucoma in the subject.

In preferred embodiments of the method, the composition is administered below the conjunctiva or intravenously.

In preferred embodiments of the method, the composition is administered to reduce fibrosis caused by an implanted drainage device. The drainage device may be valved or non-valved. It would be understood that the composition could be administered separately from the device or be incorporated with the device for slow release.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the toxicity of Compound 96 on 3D cultured rabbit Tenon's fibroblast cells (RTFs). The anti-proliferation effect of various concentrations from 10^{-11} to 10^{-6} M Compound 96 was tested on 3D cultured RTF by measuring cell number with the CellTiter-Glo® Assay (Promega). RLU is relative luciferase units.

Figure 2 shows dose-response of Compound 96 inhibition of pFAK [Y397]. Human Tenon's fibroblast cells (HTFs) were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) (Cell signaling technology #3283), the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 3 shows dose-response of Compound 96 inhibition of Collagen. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using anti-Collagen 1 NB600-408 antibody (Novus Biologicals), the loading control was done using the anti-FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 4 shows dose-response of Compound 96 inhibition of Collagen 1 mRNA expression. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment total RNA was extracted and Collagen 1 mRNA level was determined using quantitative real-time PCR.

Figure 5 shows dose-response of Compound 96 inhibition of αSMA mRNA expression. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment total RNA was extracted and αSMA mRNA level was determined using quantitative real-time PCR.

Figure 6 shows dose-response of Compound 96 inhibition of SPARC mRNA expression. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment total RNA was extracted and SPARC mRNA level was determined using quantitative real-time PCR.

Figure 7 shows dose-response of Compound 96 inhibition of pFAK [Y397]. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-7} M. Post 48 hr of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) (Cell signaling technology #3283), the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 8 shows dose-response of Compound 96 inhibition of Collagen 1. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-7} M. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using anti-Collagen 1 NB600-408 antibody (Novus Biologicals), the loading control was done using the anti-beta Actin antibody AC-15 (Santa Cruz Biotechnology).

Figure 9 shows dose-response of Compound 96 inhibition of αSMA mRNA expression. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-7} M. After 48 hr of treatment total RNA was extracted and αSMA mRNA level was determined using quantitative real-time PCR.

Figure 10 shows dose-response of Compound 96 inhibition of Collagen 1 mRNA expression. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Cells were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis and Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-7} M. After 48 hr of treatment total RNA was extracted and Collagen 1 mRNA level was determined using quantitative real-time PCR.

Figure 11 shows the effect of Compound 96 when added at 0 and 12 to 48 hr before inducing fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at various

concentrations from 10^{-9} to 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. Blots were probed using the Phospho-FAK (Tyr397 and Tyr925) antibodies (Cell signaling technology #3283 and #3284), an anti-Collagen 1 NB600-408 antibody (Novus Biologicals), and the loading control was tested using the FAK SC-557 A-17 antibody and GAPDH BF7 (Santa Cruz Biotechnology). W: wash out; i.e. treated sample was rinsed with buffer and harvested 24 hr post-treatment. W lane shows that the effect of C96 is reversible.

Figure 12 shows the effect on Collagen 1 mRNA expression of Compound 96 when added at 0 and 12 to 48 hr before attempting to induce fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment total RNA was extracted and Collagen 1 mRNA level was determined using quantitative real-time PCR.

Figure 13 shows the effect on Collagen 3 mRNA expression of Compound 96 when added at 0 and 12 to 48 hr before attempting to induce fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment total RNA was extracted and Collagen 3 mRNA level was determined using quantitative real-time PCR.

Figure 14 shows the effect on αSMA mRNA expression of Compound 96 when added at 0 and 12 to 48 hr before attempting to induce fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment total RNA was extracted and αSMA mRNA level was determined using quantitative real-time PCR.

Figure 15 shows the effect of Compound 96 on the expression of fibrosis proteins pFAK(Y397) and collagen 1a when added at 0 and 12 to 48 hr before inducing fibrosis. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before inducing fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397 and Tyr925) antibodies (Cell signaling technology #3283 and #3284) and anti-Collagen 1 NB600-408 antibody (Novus Biologicals). The loading

control was tested using the FAK SC-557 A-17 antibody and GAPDH BF7 (Santa Cruz Biotechnology).

Figure 16 shows the effect on αSMA mRNA expression of Compound 96 when added 12 to 48 hr before inducing fibrosis. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012) and Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment total RNA was extracted and αSMA mRNA level was determined using quantitative real-time PCR.

Figure 17 shows the effect of Compound 96 on the expression of fibrosis markers after TGF-beta-induced fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397 and Tyr925) antibodies (Cell signaling technology #3283 and #3284), the anti-Collagen 1 NB600-408 antibody (Novus Biologicals) and the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 18 shows the effect of Compound 96 on pro-fibrotic Collagen 1 mRNA expression, after TGF-beta-induced fibrosis. RTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment total RNA was extracted and Collagen1 mRNA level was determined using quantitative real-time PCR.

Figure 19 shows the effect of Compound 96 on fibrosis after TGF-beta-induced fibrosis. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment cell lysates were prepared and subjected to western analysis. Lane 3 is used as control where Compound 96 is added together with TGF-beta and HTFs were harvested 48 hr after treatment, while in lane 4 Compound 96 was added 24 hr post-fibrosis and HTFs were harvested 48 hr post-fibrosis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), anti-Collagen 1 NB600-408 antibody (Novus Biologicals) and the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 20 shows the effect of Compound 96 on pro-fibrotic Collagen 1 gene expression, after TGF-beta-induced fibrosis. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment total RNA was extracted and Collagen 1 mRNA level was determined using quantitative real-time PCR.

Figure 21 shows the effect of Compound 96 on pro-fibrotic Collagen 3 gene expression, after TGF-beta-induced fibrosis. HTFs were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012). Compound 96 was added to the cells at 10^{-7} M before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment total RNA was extracted and Collagen 3 mRNA level was determined using quantitative real-time PCR.

Figure 22 shows the effect of FAK inhibitors on preventing the on-set of fibrosis in TGF-beta-treated HTFs. HTFs were cultured as described in Example 2. Compound 96, PF-045 (abbreviated as P04 or P045), PF-00562271 (abbreviated as P005) or GSK2256098 (abbreviated as GSK or GSK2) were added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 24 hr and 48 hr of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 23 shows that other FAK inhibitors are able to inhibit pFAK(Y397) and Collagen in Human embryonic kidney 293 (HEK293) cells. HEK 293 cells were cultured under the same conditions as described in Example 2. Cells were cultured in 10 cm tissue culture plates, upon reaching 70% confluency. Cells were treated simultaneously with 2 ng/ml recombinant TGF-beta2, to induce fibrosis, and FAK inhibitors at various concentrations. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. A first set of blots were probed using the Phospho-FAK (Tyr397) antibody (Cell10 signaling technology #3283); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology).

Figure 24 shows the plasma concentration-time curve of Compound 96 in rabbits following intravenous injection of 2 mg/mL of Compound 96.

Figure 25 shows *in vivo* confocal microscopy images (Heidelberg Rentina Tomography HRT3, Heidelberg Engineering GmbH, Germany) of treated and control eyes at 22 and 29 days post-treatment. Filtering bleb stroma with loose collagen like meshwork and large cystic spaces (yellow stars) were seen in FAKi treated eyes.

Condensed, hyper-reflective and blurred stroma with less microcysts were observed in controls (larger darker spaces observed here are mostly blood vessels, indicated in red circles).

Figure 26 shows that Compound 96 reduced collagen deposition at the surgical site in rabbits in which glaucoma surgery was performed. Hematoxylin and eosin (H&E) staining showed a flattened conjunctiva at the day 28 control operated site (Y7, bottom panels, arrows) whereas the Compound 96-treated operated site subconjunctival space was expanded with an almost clear matrix (Y2, top panels, arrows) at day 30.

Figure 27 shows that Picrosirius red staining of the deposition of scar tissue and densely packed collagen fibers in the subconjunctival space, marked with **, in the vehicle-treated eyes (Y7 bottom panels) was significantly greater than in the Compound 96-treated eyes (Y2 top panels) at day 30.

Figure 28 shows reduced collagen, keratin and erythrocyte accumulation in operated rabbit eyes treated with Compound 96. Compound 96-treated eyes (Y4, top panels) were noted to have less collagen deposition with Masson Trichrome stain compared to vehicle-treated eyes (Y8, bottom panels) as well as reduced keratin, erythrocytes and muscle fibers at day 30.

Figure 29 shows that Compound 96 reduced expression of pro-fibrotic marker at the surgical site in operated rabbit eyes. Immunofluorescence analysis of sections from the same eyes used above (Y2 compound 96-treated and Y7 vehicle-treated) with alpha-smooth muscle actin-specific antibody showing reduced staining at day 30 in eyes treated with Compound 96.

Figure 30 shows that C96 when dissolved in SRP80 formulation and tested *in vivo* doesn't cause any irritation, swelling or redness. This was also the case when DMSO was used as excipient. No redness or increased vascularization was seen in all the experimental samples at day 7, 14, 21 and 30 (black arrows). Data for day 14 is shown.

Figure 31 shows Hematoxylin and Eosin-stained sections of sub-conjunctival tissue at day 30 of treatment with DMSO, SRP80 and combinations of DMSO or SRP80 with Compound 96. The presence of goblet cells in the superficial epithelium (black arrows) is a critical parameter that reflects the overall health of the ocular surface. The results show that SRP80 on its own or as an excipient for C96 doesn't adversely affect the goblet cell number.

Figure 32 shows Masson's trichrome stained sections of sub-conjunctival tissue at day 30 of treatment with DMSO, SRP80 and combinations of DMSO or SRP80 with

Compound 96. Images show selective staining of connective tissue (white arrows) which is the collagen stroma. A rabbit treated with 100 μM of the compound in DMSO shows disrupted expression of collagenous stroma (top right image; marked with asterisks *). The drug preparation with the SRP80 shows regular arranged collagenous stroma and a rich layer of conjunctival goblet (red staining with black arrows) cells which are required for protection from external factors. The higher dose of the compound in DMSO shows decreased density of conjunctival goblet cells which may have been the result from chronic irritation produced with this formulation.

Figure 33 shows immunofluorescence staining for a-SMA on sections of sub-conjunctival tissue at day 30 of treatment with DMSO, SRP80 and combinations of DMSO or SRP80 with Compound 96. Staining shows a significantly lower proportion of cells than would normally be seen with scarring were found to express alpha-SMA in all the samples indicating that addition of the drug with either DMSO or SRP80 does not result in trans-differentiation of the epithelial cells to myofibroblasts.

Figure 34 shows (A) XEN® gel stent; (B) preloaded injector and correct handling.

Definitions

Certain terms employed in the specification, examples and appended claims are collected here for convenience.

As used herein, the term “comprising” or “including” is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. However, in context with the present disclosure, the term “comprising” or “including” also includes “consisting of”. The variations of the word “comprising”, such as “comprise” and “comprises”, and “including”, such as “include” and “includes”, have correspondingly varied meanings.

The terms ‘Compound 96’ and ‘C96’ are used herein interchangeably and refer to diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate.

References herein (in any aspect or embodiment of the invention) to said FAK inhibitor compounds includes references to such compounds *per se*, to tautomers of such compounds, as well as to pharmaceutically acceptable salts or solvates, or pharmaceutically functional derivatives of such compounds.

Pharmaceutically acceptable salts that may be mentioned include acid addition salts and base addition salts. Such salts may be formed by conventional means, for example by reaction of a free acid or a free base form of a compound of formula 1 with one or more equivalents of an appropriate acid or base, optionally in a solvent, or in a medium in which the salt is insoluble, followed by removal of said solvent, or said medium, using standard techniques (e.g. *in vacuo*, by freeze-drying or by filtration). Salts may also be prepared by exchanging a counter-ion of a serotonergic compound in the form of a salt with another counter-ion, for example using a suitable ion exchange resin.

Examples of pharmaceutically acceptable salts include acid addition salts derived from mineral acids and organic acids, and salts derived from metals such as sodium, magnesium, or preferably, potassium and calcium.

Examples of acid addition salts include acid addition salts formed with acetic, 2,2-dichloroacetic, adipic, alginic, aryl sulphonic acids (e.g. benzenesulphonic, naphthalene-2-sulphonic, naphthalene-1,5-disulphonic and *p*-toluenesulphonic), ascorbic (e.g. L-ascorbic), L-aspartic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulphonic, (+)-(1*S*)-camphor-10-sulphonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulphuric, ethane-1,2-disulphonic, ethanesulphonic, 2-hydroxyethanesulphonic, formic, fumaric, galactaric, gentisic, glucoheptonic, gluconic (e.g. D-gluconic), glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α-oxoglutaric, glycolic, hippuric, hydrobromic, hydrochloric, hydriodic, isethionic, lactic (e.g. (+)-L-lactic and (±)-DL-lactic), lactobionic, maleic, malic (e.g. (-)-L-malic), malonic, (±)-DL-mandelic, metaphosphoric, methanesulphonic, 1-hydroxy-2-naphthoic, nicotinic, nitric, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, L-pyroglutamic, salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulphuric, tannic, tartaric (e.g.(+)-L-tartaric), thiocyanic, undecylenic and valeric acids.

Particular examples of salts are salts derived from mineral acids such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids; from organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, arylsulphonic acids; and from metals such as sodium, magnesium, or preferably, potassium and calcium.

As mentioned above, also encompassed by FAK inhibitor compounds are any solvates of the compounds and their salts. Preferred solvates are solvates formed by the incorporation into the solid state structure (e.g. crystal structure) of the compounds of the invention of molecules of a non-toxic pharmaceutically acceptable solvent (referred to below as the solvating solvent). Examples of such solvents include water, alcohols (such as ethanol, isopropanol and butanol) and dimethylsulphoxide. Solvates can be prepared by recrystallizing the compounds of the invention with a solvent or mixture of solvents containing the solvating solvent. Whether or not a solvate has been formed in any given instance can be determined by subjecting crystals of the compound to analysis using well known and standard techniques such as thermogravimetric analysis (TGE), differential scanning calorimetry (DSC) and X-ray crystallography.

The solvates can be stoichiometric or non-stoichiometric solvates. Particularly preferred solvates are hydrates, and examples of hydrates include hemihydrates, monohydrates and dihydrates.

The term "antagonist", or “inhibitor” as it is used herein, refers to a molecule which decreases the amount or the duration of the effect of the biological activity of FAK. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or small molecules which decrease the effect of FAK.

Compounds of the present invention will generally be administered as a pharmaceutical formulation in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, which may be selected with due regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutically acceptable carriers may be chemically inert to the active compounds and may have no detrimental side effects or toxicity under the conditions of use. Suitable pharmaceutical formulations may be found in, for example, Remington The Science and Practice of Pharmacy, 19th ed., Mack Printing Company, Easton, Pennsylvania (1995). For parenteral administration, a parenterally acceptable aqueous solution may be employed, which is pyrogen free and has requisite pH, isotonicity, and stability. Suitable solutions will be well known to the skilled person, with numerous methods being described in the literature. A brief review of methods of drug delivery may also be found in e.g. Langer, *Science* 249, 1527-1533 (1990).

Otherwise, the preparation of suitable formulations may be achieved routinely by the skilled person using routine techniques and/or in accordance with standard and/or accepted pharmaceutical practice.

The amount of a compound in any pharmaceutical formulation used in accordance with the present invention will depend on various factors, such as the severity of the condition to be treated, the particular patient to be treated, as well as the compound(s) which is/are employed. In any event, the amount of a compound in the formulation may be determined routinely by the skilled person.

For example, a solid oral composition such as a tablet or capsule may contain from 1 to 99% (w/w) active ingredient; from 0 to 99% (w/w) diluent or filler; from 0 to 20% (w/w) of a disintegrant; from 0 to 5% (w/w) of a lubricant; from 0 to 5% (w/w) of a flow aid; from 0 to 50% (w/w) of a granulating agent or binder; from 0 to 5% (w/w) of an antioxidant; and from 0 to 5% (w/w) of a pigment. A controlled release tablet may in addition contain from 0 to 90% (w/w) of a release-controlling polymer.

A parenteral formulation (such as a solution or suspension for injection or a solution for infusion) may contain from 1 to 50% (w/w) active ingredient; and from 50% (w/w) to 99% (w/w) of a liquid or semisolid carrier or vehicle (e.g. a solvent such as water); and 0-20% (w/w) of one or more other excipients such as buffering agents, antioxidants, suspension stabilisers, tonicity adjusting agents and preservatives.

Depending on the disorder, and the patient, to be treated, as well as the route of administration, compounds may be administered at varying therapeutically effective doses to a patient in need thereof.

However, the dose administered to a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the mammal over a reasonable timeframe. One skilled in the art will recognize that the selection of the exact dose and composition and the most appropriate delivery regimen will also be influenced by inter alia the pharmacological properties of the formulation, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient, as well as the potency of the specific compound, the age, condition, body weight, sex and response of the patient to be treated.

The term ‘treatment’, as used in the context of the invention refers to prophylactic, ameliorating, therapeutic or curative treatment.

DETAILED DESCRIPTION OF THE INVENTION

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

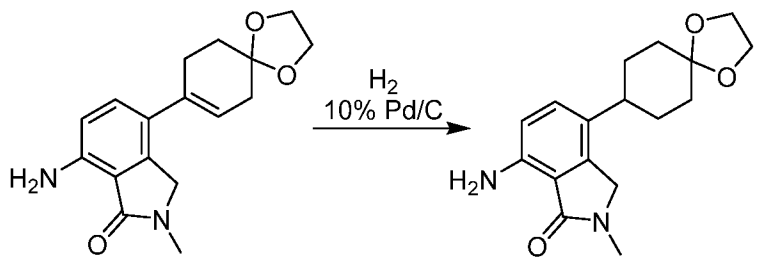
Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (2001).

EXAMPLE 1

Materials and Methods

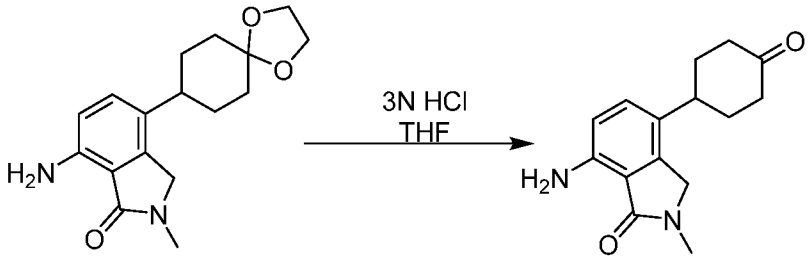
FAK inhibitors

1. Diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate, designated as Compound 96, is a small molecule inhibitor produced by Duke Small Molecule Synthesis Facility laboratory, Department of Chemistry, Durham, North Carolina. A method of synthesis is disclosed in WO2010/141406, the contents of which is incorporated herein by reference and briefly described as follows:

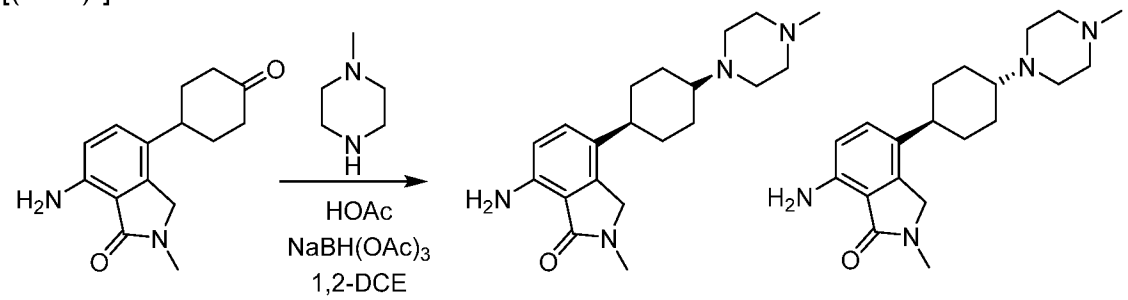


A suspension of the alkene (3.9 g, 13 mmol) and 10% Pd/C (700 mg) in EtOH (80 mL) was stirred overnight under a balloon of H_2 after which time analysis of the

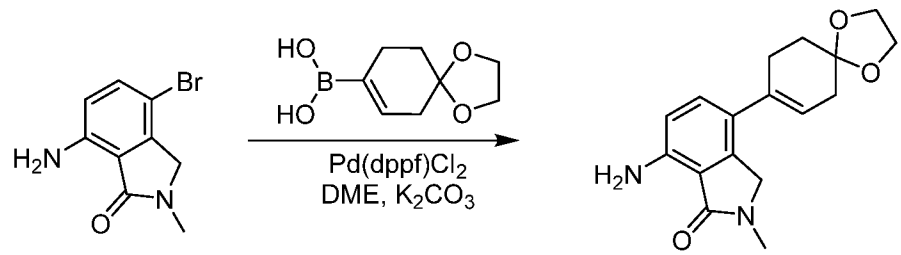
reaction mixture by LCMS indicated complete consumption of starting material and conversion to the desired product (ESI m/z = 303 [(M+H)$^+$]. The mixture was filtered through a pad of Celite. The pad was washed with EtOH (2x100 mL) and the filtrate was concentrated to dryness under reduced pressure giving the product as a pale yellow solid (3.5 g, 89%).



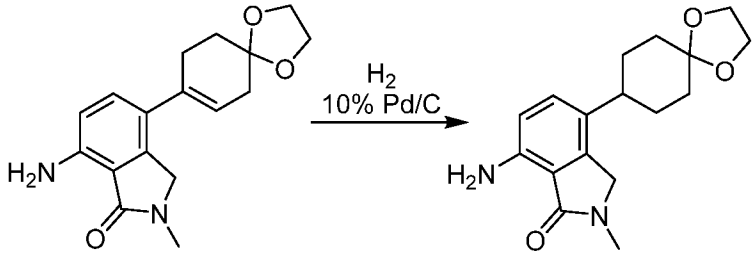
Aqueous 3M HCl (25 mL, 75 mmol, 4.5 eq.) was added in one portion to a room temperature solution of 7-amino-4-(1,4-dioxaspiro[4.5]dec-8-yl)-2-methyl-2,3-dihydro-1H-isoindol-1-one (5 g, 16.5 mmol) in THF (180 mL). A thick suspension resulted. Stirring was continued overnight. A clear amber solution was observed the following morning. Some conversion to the desired ketone was observed by LCMS. Additional water (45 mL) was added and stirring was continued for 4d after which time no acetal remained as determined by LCMS. The resulting mixture was concentrated by one half and diluted with 1M Na_2CO_3 (100 mL). The resulting suspension was extracted with EtOAc (6 x 200 mL). The combined extracts were added to silica gel (~8g) and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f SiO_2 (80 g), 100% CH_2Cl_2 → 50% EtOAc in CH_2Cl_2) gave the product as a light yellow solid (2.4 g, 57%). ESIMS; *m/z* = 259 [(M+H)$^+$].



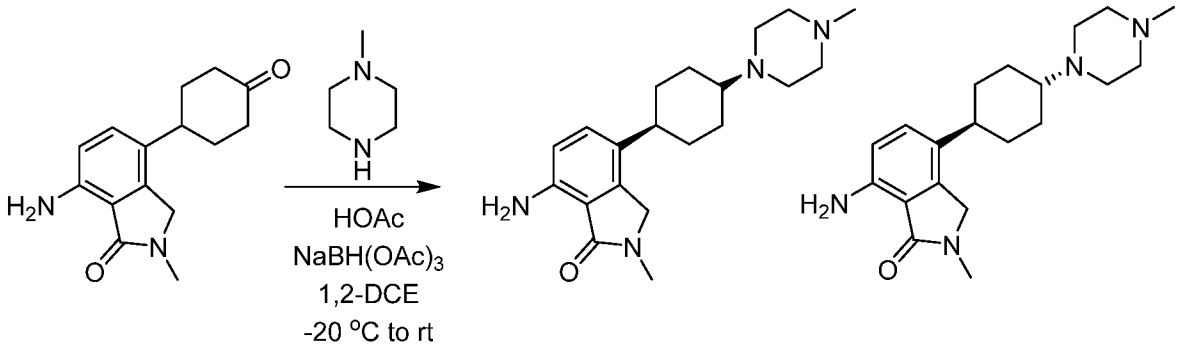
A solution of the ketone (1.2 g, 4.7 mmol) in 1,2-DCE (8 mL) was cooled in an ice-NaCl bath. N-methylpiperazine (0.52 mL, 4.7 mmol), and 17M HOAc (0.27 mL, 4.7 mmol) were added in sequence. After 5-10 minutes, solid $NaBH(OAc)_3$ (1.4 g, 6.5 mmol, 1.4 eq.) was added. The reaction mixture was allowed to warm to room temperature as the cooling bath melted. Stirring was continued overnight. The reaction was quenched with sat. aq. Na_2CO_3 (10 mL) and extracted with EtOAc (3 x 10 mL). Silica gel (~2g) was added to the combined extracts and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f GOLD SiO_2 (12 g), 100% CH_2Cl_2 → 10% MeOH/NH_3 in CH_2Cl_2) gave the *cis*-product as a yellow solid (0.792 g) and the *trans*-product as a light yellow solid (0.38 g). cis:trans (2.1:1) overall yield 73%. ESIMS; *m/z* = 301 [(M+H)$^+$].



A suspension of the bromide (5.12 g, 21.2 mmol) and the boronic acid (5.7 g, 21.2 mmol) in 1,2-DME (150 mL) and 2M K_2CO_3 (52 mL) was subjected to three evacuation/argon backfill cycles. Solid $Pd(dppf)Cl_2$ (1.7 g, 2.1 mmol, 10 mol%) was added. Following three additional evacuation/argon backfill cycles, the mixture was heated to 80 °C (oil bath temperature) for 16h. Analysis of the reaction mixture by TLC (100% EtOAc) and LCMS showed complete consumption of the starting bromide and conversion to the desired cross-coupled product. The reaction mixture cooled to room temperature then slowly poured into ice cold brine (1.2 L). The resulting suspension was stirred until the ice melted and insolubles were removed at the vacuum. Flash column chromatography (RediSepR_f SiO_2 (80 g), 100% hexanes → 100% EtOAc) gave the product as a light brown solid (5.3 g, 83%). ESIMS; *m/z* = 301 [$(M+H)^+$].

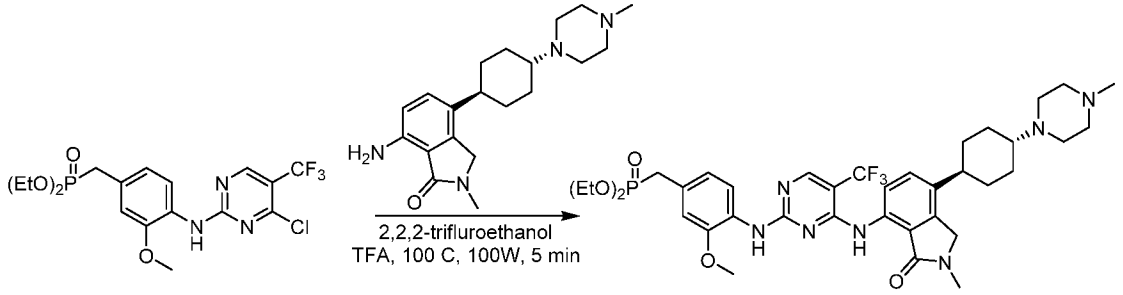


A suspension of the alkene (5.3 g, 17.7 mmol) and 10% Pd/C (1.7 g) in EtOH (180 mL) was stirred overnight under a balloon of H_2 after which time analysis of the reaction mixture by LCMS indicated complete consumption of starting material and conversion to the desired product (ESI m/z = 303 [$(M+H)^+$]. The mixture was filtered through a pad of Celite. The pad was washed with EtOH (2x100 mL) and the filtrate was concentrated to dryness under reduced pressure giving the product as a pale yellow solid (5.0 g, 94%).

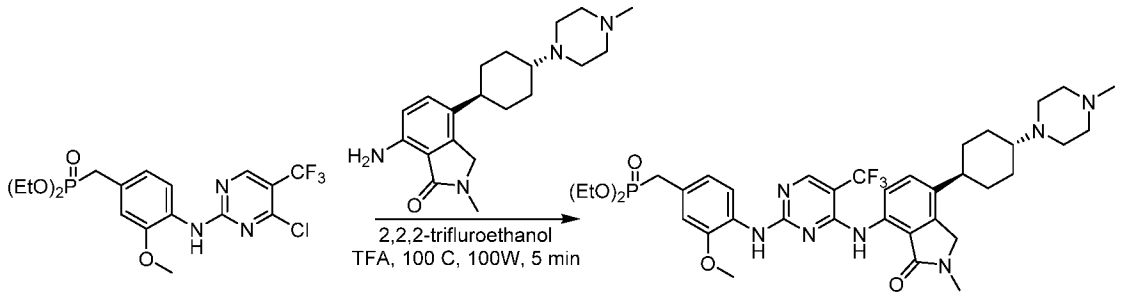


A solution of the ketone (2.4 g, 9.4 mmol) in 1,2-DCE (20 mL) was cooled in an ice-NaCl bath. N-methylpiperazine (1.05 mL, 9.4 mmol), and 17M HOAc (0.55 mL, 9.4 mmol) were added in sequence. After 5-10 minutes, solid $NaBH(OAc)_3$ (2.8 g, 13 mmol, 1.4 eq.) was added. The reaction mixture was allowed to warm to room temperature as the cooling bath melted. Stirring was continued overnight. The reaction was quenched with sat. aq. Na_2CO_3 (10 mL) and extracted with EtOAc (3 x 10

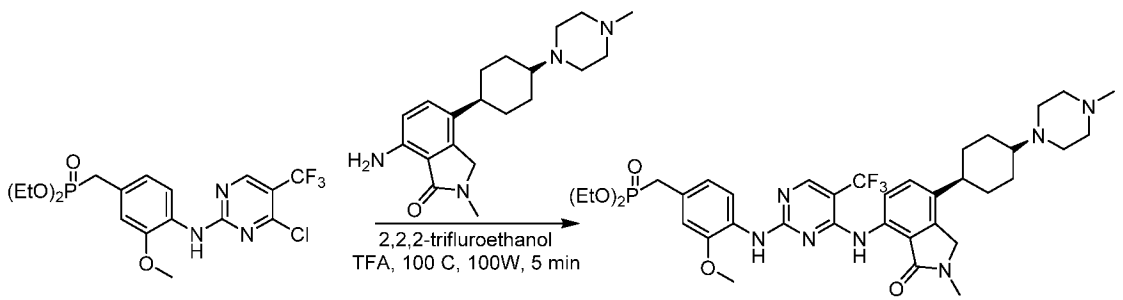
mL). Silica gel (~5g) was added to the combined extracts and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f GOLD SiO_2 (40 g), 100% CH_2Cl_2 → 10% MeOH/NH_3 in CH_2Cl_2) gave the *cis*-product as a yellow solid (1.6 g) and the *trans*-product as a light yellow solid (0.77 g). cis:trans (2.1:1) overall yield 73%. ESIMS; *m/z* = 301 [(M+H)$^+$].



A mixture the pyrimidine (1.02 g, 2.3 mmol), the aniline (0.77g, 2.3 mmol), TFE (5 mL) and TFA (570 µL, 7.6 mmol, 3.3 eq.) was heated in a 35 mL microwave vessel (CEM) at 100 °C (initial power setting of 100W) for 15 min. The mixture was cooled to room temp and quenched by careful addition of 1M Na_2CO_3 (100 mL). The resulting suspension was extracted with EtOAc (3 x 50 mL). The combined extracts were added to silica gel (~5g) and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f GOLD SiO_2 (40 g), 100% CH_2Cl_2 → 10% MeOH/NH_3 in CH_2Cl_2) gave the final product as a pale yellow solid (1.0 g, 60%) ESIMS; *m/z* = 760 [(M+H)$^+$].

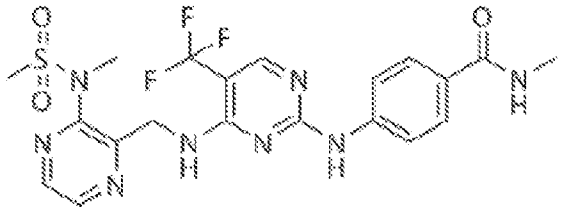


A mixture the pyrimidine (0.43 g, 0.95 mmol), the aniline (0.38g, 1.1 mmol), TFE (1.9 mL) and TFA (250 µL, 3.3 mmol, 3 eq.) was heated in a 10 mL microwave vessel (CEM) at 100 °C (initial power setting of 100W) for 15 min. The mixture was cooled to room temp and quenched by careful addition of 1M Na_2CO_3 (20 mL). The resulting suspension was extracted with EtOAc (3 x 10 mL). The combined extracts were added to silica gel (~1g) and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f GOLD SiO_2 (12 g), 100% CH_2Cl_2 → 10% MeOH/NH_3 in CH_2Cl_2) gave the final product as a pale yellow solid (0.37 g, 51%) ESIMS; *m/z* = 760 [(M+H)$^+$].

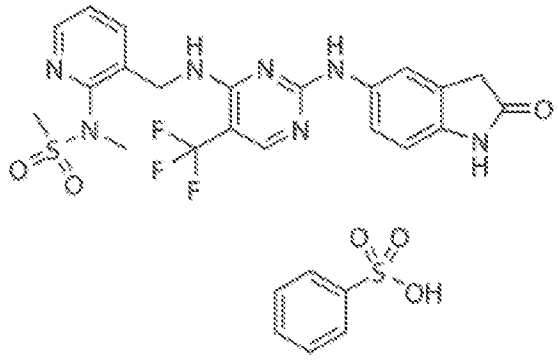


A mixture the pyrimidine (93 mg, 0.23 mmol), the aniline (83 mg), TFE (0.4 mL) and TFA (56 µL, 3 eq.) was heated in a 10 mL microwave vessel (CEM) at 100 °C (initial power setting of 100W) for 15 min. The mixture was cooled to room temp and quenched by careful addition of 1M Na_2CO_3 (20 mL). The resulting suspension was extracted with EtOAc (3 x 10 mL). The combined extracts were added to silica gel (~1g) and the mixture was concentrated to dryness under reduced pressure. Flash column chromatography (RediSepR_f GOLD SiO_2 (12 g), 100% CH_2Cl_2 → 10% MeOH/NH_3 in CH_2Cl_2) gave the final product as a pale yellow solid (88 mg, 51%). ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.25 - 1.30 (m, 6 H), 1.40 - 1.55 (m, 2 H), 1.62 (q, J = 12.80 Hz, 2 H), 1.97 (d, J = 12.63 Hz, 2 H), 2.13 (d, J = 11.87 Hz, 2 H), 2.37 (s, 3 H), 2.50 (d, J = 12.13 Hz, 2 H), 2.61 (br. s., 2 H), 2.77 (br. s., 3 H), 3.14 - 3.20 (m, 2 H), 3.22 (s, 3 H), 3.93 (s, 3 H), 4.00 - 4.10 (m, 4 H), 4.38 (s, 2 H), 6.89 (dt, J = 8.15, 2.24 Hz, 1 H), 6.94 (t, J = 2.02 Hz, 1 H), 7.34 (d, J = 8.59 Hz, 1 H), 7.63 (s, 1 H), 8.29 (d, J = 8.59 Hz, 1 H), 8.39 (s, 1 H), 8.63 (d, J = 8.59 Hz, 1 H), 10.50 (s, 1 H). ESIMS; *m/z* = 760 [$(M+H)^+$].

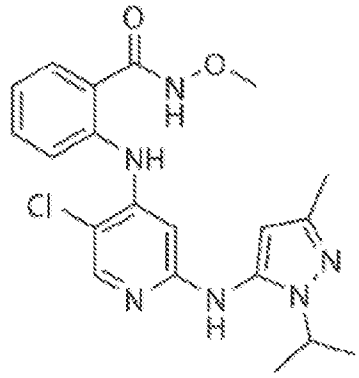
2. PF- 045: Defactinib (VS-6063, PF-04554878) [N-methyl-4-(4-((3-(N-methylmethan-3-ylsulfonamido)pyrazin-2-yl)methylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)benzamide] is a selective, and orally active FAK inhibitor, purchased from Selleck Chemicals LCC.



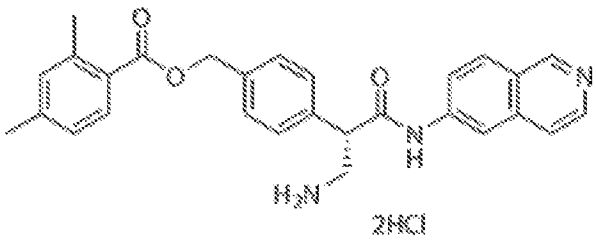
3. PF-00562271 [N-methyl-N-(3-((2-(2-oxoindolin-5-ylamino)-5-(trifluoromethyl)pyrimidin-4-ylamino)methyl)pyridin-2-yl)methanesulfonamide benzenesulfonate] is the benzenesulfonate salt of PF-562271, purchased from Selleck Chemicals LCC



4. GSK2256098 [Benzamide, 2-[[5-chloro-2-[[3-methyl-1-(1-methylethyl)-1H-pyrazol-5-yl]amino]-4-pyridinyl]amino]-N-methoxy-], was purchased from Selleck Chemicals LCC.



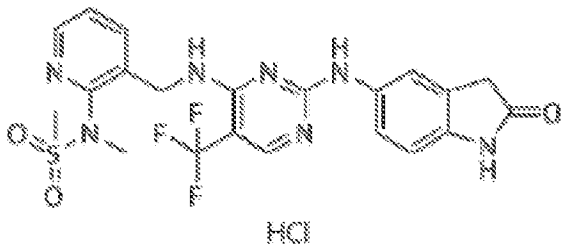
5. Netarsudil (AR-13324) 2HCl [Benzoic acid, 2,4-dimethyl-, [4-[(1S)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, hydrochloride (1:2)] was purchased from Selleck Chemicals LCC



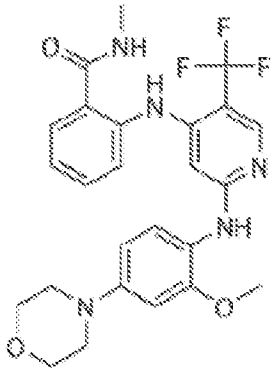
Netarsudil (trade name Rhopressa) is a drug for the treatment of glaucoma. In the United States, the Food and Drug Administrationhas approved a 0.02% ophthalmic solution for the lowering of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension. Netarsudil primarily targets cells in the conventional outflow tract, efficiently decreasing IOP in both human and non-human primate eyes. In addition, netarsudil has been shown to increase outflow facility in non-human primate eyes and to decrease episcleral venous pressure in rabbit eyes [Li G et al 2016 Eur J Pharmacol Sept 15, 787: 20-31].

Netarsudil ophthalmic solution 0.02% is supplied as a sterile, isotonic, buffered aqueous solution of netarsudil dimesylate with a pH of approximately 5 and an osmolality of approximately 295 mOsmol/kg. It is intended for topical application in the eye. Each mL of netarsudil contains 0.2 mg of netarsudil (equivalent to 0.28 mg of netarsudil dimesylate). Benzalkonium chloride, 0.015%, is added as a preservative. The inactive ingredients are: boric acid, mannitol, sodium hydroxide to adjust pH, and water for injection. 0.2 mg/ml = 0.38 mM (380 µM).

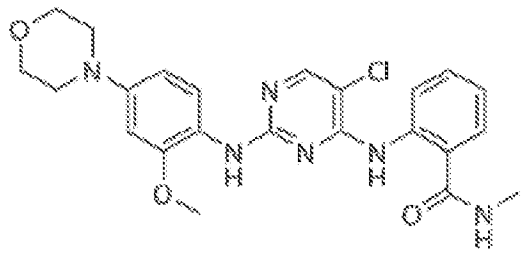
6. PF-562271 HCl [N-Methyl-N-{3-[({2-[(2-oxo-2,3-dihydro-1H-indol-5-yl)amino]-5-(trifluoromethyl)-4-pyrimidinyl}amino)methyl]-2-pyridinyl}methanesulfonamide hydrochloride (1:1)] is the hydrochloride salt of PF-562271 and was purchased from Selleck Chemicals LCC



7. PND-1186 (VS-4718) [2-(2-(2-methoxy-4-morpholinophenylamino)-5-(trifluoromethyl)pyridin-4-ylamino)-N-methylbenzamide] was purchased from Selleck Chemicals LCC



8. TAE226 (NVP-TAE226) [Benzamide, 2-[[5-chloro-2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]amino]-N-methyl-], was purchased from Selleck Chemicals LCC.



Isolation of Tenon fibroblasts conjunctival tissues

1. Harvest conjunctival tissues & transfer into a 1.5 ml micro tube containing approximately 1ml serum-free DMEM (done in animal facility)
2. Transfer tissues to a new 1.5 ml micro tube containing approximately 1 ml of sterile 1XPBS. Pipette up & down or vortex gently to briefly wash the tissues (to remove any serum if D10 media (contains serum) was used in step 1)
3. Transfer tissues to a 24-well plate & add sufficient volume of 20 mg/ml dispase II (reconstituted in D10 media & filtered), to cover the tissues entirely
4. Incubate at 37□C for 1hr
5. Neutralize dispase II activity with 1XPBS. Pipette the tissues up & down (to remove epithelial cells). (Note: After dispase II digestion, tissues are still quite whole but appear softer)
6. Remove most of the PBS-dispase II solution (without disturbing the tissue clumps) from the 24-well plate
7. Add sufficient volume of 2 mg/ml collagenase IV (reconstituted in D10 media & filtered) into the well to cover the tissues entirely

8. Incubate at 37□C for 1hr or until the tissues are digested (i.e. no visible tissue clumps)
9. Transfer all contents to a 15 ml conical tube. Rinse the well with D10 media & transfer to the same tube
10. Centrifuge at 800 rpm for 5 min
11. Discard supernatant & add 2 ml of fresh D10 media to wash the cells
12. Centrifuge at 800 rpm for 5 min
13. Discard supernatant & add 2 ml of fresh D10 media to wash the cells again
14. Centrifuge at 800 rpm for 5 min
15. Add 2 ml of fresh D10 media & transfer the contents to 6-well plate, T75 or T175 flasks. Top up with D10 media
16. Cells were further harvested and kept as frozen stocks 48 hr post-culture.

Reagents

Dispase II (20 mg/ml) Roche

0.02 g in 1 ml DMEM

Collagenase D (2 mg/ml) Roche

0.002 g in 1 ml DMEM

Cell culture and protein lysate preparation

Cells were cultured as described by Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012); described briefly as follows:

Frozen aliquots of the Tenon fibroblast cells were cultured in DMEM with 10% FBS, 37°C, 5% CO_2 using standard tissue culture plates. Cells were plated onto 10 cm tissue culture plates at $1X10^6$ cells/plate and cells were allowed to grow up to 70% confluency before being treated with TGF-β and test compounds, including C96, PF-04554878, PF-00562271, GSK2256098, AR-13324, PF-562271 HCl, PND-1186 or TAE226. Fibrosis was induced using TGF-β at 2ng/ml for 48hr and test compound was added simultaneously during induction of fibrosis. Non-treated cells and TGF-β treated alone were used as controls for each experiment. Cells treated for 48hr were the baseline for pre-fibrosis and post-fibrosis experiments. At the end of the treatment, cells were harvested in 20 mM HEPES (pH 7.4), 137 mM sodium chloride, 1.5 mM magnesium chloride, 1 mM EGTA, 10% (v/v) glycerol, 1% Triton X-100, a mixture of protease inhibitors (Roche Molecular Biochemicals), and 0.2 mM sodium orthovanadate. Cell lysates were precleared by centrifugation at 16000xg for 15 min.

Cleared lysates were separated on SDS-PAGE and immunoblotted with various antibodies. All Western blot data shown are representative of at least three separate individual experiments (Seet et al., *J Cell Mol Med.* 16(6): 1245-59 (2012).

RNA Isolation and Expression

The mRNA levels of pro-fibrotic genes were determined *via* quantitative real-time PCR (qPCR) to assess the effect of Compound 96 or other FAK inhibitors on COL1, aSMA and SPARC expression in RTFs and HTFS. After the experimental treatment was performed, total RNA was isolated using Rneasy MiniKit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized with 1 µg total RNA extract with iScript™ cDNA synthesis kit (Bio-rad) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed in a total volume of 10 µl in 96-well microtiter plates with SsoFast™ EvaGreen® Supermix (Bio-rad) according to the manufacturer's instructions (Table 1) and primers indicated in Tables 2 and 3. The resulting amplification and melt curves were analyzed to ensure the identity of the specific PCR product. Threshold cycle values were used to calculate the fold change in the transcript levels by using the $2^{-\Delta\Delta Ct}$ method. The relative mRNA expression levels were normalized to the beta-actin gene.

Table 1: qPCR conditions

Step	**Temperature**	**Time**
A	95□C	30 s
B	95□C	10 s
C	60□C	30 s
D	Plate read, repeat B & C for 39 cycles	
E	95□C	30 s
F	55□C	1 min
G	55□C to 95□C increment 0.5□C for every 10 s	
H	Plate read & End	

Table 2: Human primers

β-actin	SEQ ID NO: 1	Forward 5'-3'	CCAACCGCGAGAAGATGA
	SEQ ID NO: 2	Reverse 5'-3'	CCAGAGGCGTACAGGGATAG
Collagen1	SEQ ID NO: 3	Forward 5'-3'	CAGCCGCTTCACCTACAGC
	SEQ ID NO: 4	Reverse 5'-3'	TTTTGTATTCAATCACTGTCTTGCC
aSMA	SEQ ID NO: 5	Forward 5'-3'	CCGACCGAATGCAGAAGGA
	SEQ ID NO: 6	Reverse 5'-3'	ACAGAGTATTTGCGCTCCGAA
Fibronectin	SEQ ID NO: 7	Forward 5'-3'	GCTCATCATCTGGCCATTTT
	SEQ ID NO: 8	Reverse 5'-3'	ACCAACCTACGGATGACTCG
Collagen3	SEQ ID NO: 9	Forward 5'-3'	AGGGGAGCTGGCTACTTCTC
	SEQ ID NO: 10	Reverse 5'-3'	AGGACTGACCAAGATGGGAA
Sparc	SEQ ID NO: 11	Forward 5'-3'	GTGCAGAGGAAACCGAAGAG
	SEQ ID NO: 12	Reverse 5'-3'	TGTTTGCAGTGGTGGTTCTG

Table 3: Rabbit primers

rGAPDH	SEQ ID NO: 13	Forward 5'-3'	ACTTTGTGAAGCTCATTTCCTGGTA
	SEQ ID NO: 14	Reverse 5'-3'	GTGGTTTGAGGGCTCTTACTCCTT
rCollagen1	SEQ ID NO: 15	Forward 5'-3'	CTGACTGGAAGAGCGGAGAGTAC
	SEQ ID NO: 16	Reverse 5'-3'	CCATGTCGCAGAAGACCTTGA
raSMA	SEQ ID NO: 17	Forward 5'-3'	GAGCGCAAATACTCCGTCTG
	SEQ ID NO: 18	Reverse 5'-3'	TGTGGGCTAGAAACAGAGCA
rFibronectin	SEQ ID NO: 19	Forward 5'-3'	TCGATCCCTACACGGTTACC
	SEQ ID NO: 20	Reverse 5'-3'	TTTCTCCCTGACGATCCCAC
rCollagen3	SEQ ID NO: 21	Forward 5'-3'	CCTGAAGCCCCAGCAGAA
	SEQ ID NO: 22	Reverse 5'-3'	AACAGAAATTTAGTTGGTCACTTGT ACTG

In Vivo Animal Experiment.

In total, 10 animals were used with two groups. Four were set as experimental, four were used as control and two were used for Pharmacokinetics analysis. This set-up using a minimum number of animal complies with the 3Rs principle of NACLAR Guidelines.

Treatment Regimen

Eight rabbits underwent filtration surgery to the left eye only, with random allocation to one of two treatment groups: subconjunctival injection of 0.1 ml of either 100 µM Compound 96 or PBS as control, 1 day before surgery and immediately after surgery and on each of days 1 through 7 after surgery. The rationale for this dose regimen is to attempt to achieve local availability of the inhibitor at the critical time of pre- and post-operative wound repair and remodeling, as observed in the in *vitro* data and other studies.

The animals were examined before receiving the injections at set intervals for 28 days after surgery. Clinically, bleb appearance, size, height, and surrounding vascularity were recorded in addition to the intraocular pressure (IOP), on days 1, 7, 14, 21, 28. The animals were killed on day 28. Both eyes were enucleated and histologically analyzed. The non-surgical right eyes served as the normal control for histologic comparison.

Two animals were used for pharmacokinetics/ pharmacodynamics analysis to quantify drug efficacy. One was given an intravenous dose of the compound and venous blood was collected at specified time points. The other was given a sub-conjunctival injection of the compound and venous blood was collected at specified time points.

Example 2: Non-toxic effect of Compound 96 on 3D cultured Rabbit Tenon's Fibroblast cells (RTFs).

The anti-proliferation effect of various concentrations from 10^{-11} to 10^{-6} M of Compound 96 was tested on 3D cultured RTF cells by measuring cell number with the CellTiter-Glo® Assay (Promega). This data shows that Compound 96 was found to be non-toxic to the growth of RTFs at all the doses used for this study (no significant difference between control and all the other doses tested, $p >> 0.05\%$, p values were from 0.0692 to 0.9535) (Figure 1).

Example 3: Dose-response of Compound 96 inhibition of pFAK(Y397) and Collagen in Human Tenon's Fibroblast cells (HTFs)

HTFs were cultured as described above.

Cells were cultured in 10 cm tissue culture plates, upon reaching 70% confluency. Cells were treated simultaneously with 2 ng/ml recombinant TGF-beta2, to

induce fibrosis, and Compound 96 at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment cell lysates were prepared and subjected to western analysis and quantitative polymerase chain reaction (qPCR).

A first set of blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology). As the dose of Compound 96 increased, the level of phosphorylation of FAK decreased compared to untreated (Figure 2; lanes 3-8 cf lane 2). The lower figure shows the quantitated relative levels of phosphorylated FAK to control. The data shows that the effective dose to inhibit pFAK close to basal level is between 10^{-7} and 10^{-6} M. A second set of blots were probed using anti-Collagen 1 NB600-408 antibody (Novus Biologicals); the loading control was quantified using the anti-FAK SC-557 A-17 antibody (Santa Cruz Biotechnology) (Figure 3). In the absence of TGF beta and/or serum, the expression of collagen 1 was low (lanes 1-2), whereas increasing concentrations of Compound 96 (lanes 4-9) reduced the expression of TGF beta-stimulated collagen 1 level (lane 3).

Example 4: Compound 96 inhibits Collagen 1, αSMA and SPARC mRNA expression in HTFs

HTFs were cultured as described in Example 3 and were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis. Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-6} M. After 48 hr of treatment, total RNA was extracted and Collagen 1 mRNA level (Figure 4), αSMA mRNA level (Figure 5) and SPARC mRNA level (Figure 6) were determined using quantitative real-time PCR. It can be seen that TGF beta induced collagen 1 expression by over 4-fold and Compound 96 at 10^{-6} M reduced the TGF beta-induced collagen 1 expression by more than 50% (Figure 4). Similarly, TGF beta induced αSMA mRNA expression by 14-fold and Compound 96 had a dose-dependent inhibiting effect on αSMA mRNA levels in TGF beta-treated cells (Figure 5, lanes 3-8 cf lane 2). SPARC mRNA expression was increased 5-fold by TGF beta, which was reduced by over 60% by 10^{-6} M Compound 96 (Figure 6).

Example 5: Dose-response of Compound 96 inhibition of pFAK and Collagen in RTFs

RTFs were cultured as described in Example 1 and were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis. Compound 96 was added to the cells at various

concentrations from 10^{-11} to 10^{-7} M. After 48 hr of treatment cell lysates were prepared and subjected to western analysis.

Blots were probed using an anti-human Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology) (Figure 7).

Blots were probed using anti-Collagen 1 NB600-408 antibody (Novus Biologicals), the loading control was done using the anti-beta Actin antibody AC-15 (Santa Cruz Biotechnology) and the anti-FAK SC-557 A-17 antibody (Santa Cruz Biotechnology) (Figure 8). The data shows that C96 is able to inhibit collagen synthesis in a dose-dependent manner; the strongest inhibition is seen when C96 is added at 10^{-7} M 48 hours post-fibrosis. Inhibition of collagen is a key target to reduce excessive formation of fibrotic tissue.

Example 6: FAK inhibitors prevent the on-set of fibrosis in TGF-beta-treated HTFs

HTFs were cultured as described in Example 3. Compound 96 or other FAK inhibitors were added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 24 hr and 48 hr of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology). Figure 22 shows that PF-04554878, PF-00562271 and GSK2256098 are effective in the inhibition of FAK phosphorylation at Tyr397. The levels of inhibition of phosphorylation by these compounds increased with increasing times of incubation with the compounds.

The inhibitors were purchased from Selleck Chemicals LCC. The IC_{50} of each tested compound is shown in Table 4.

Table 4: IC_{50} of each tested compound on HTF cells.

Inhibitor	IC50
Aminopyrimidine Pyrimidine – (C96)	0.67 nM (DE)
PF-04554878	0.6 nM
PF-00562271	1.5 nM

GSK2256098	0.4 nM
PF-562271 HCl	1.5 nM
PND-1186 (VS-4718)	1.5 nM
TAE226 (NVP-TAE226)	5.5 nM
Netarsudil (AR-13324) 2HCl [ROCK] inhibitor	2.0 nM

Example 7: Compound 96 inhibits αSMA and Collagen 1 mRNA expression in RTFs

RTFs were cultured as described in Example 1 and were treated with 2 ng/ml of TGF-beta 2 to induce fibrosis. Compound 96 was added to the cells at various concentrations from 10^{-11} to 10^{-7} M.

After 48 hr of treatment, total RNA was extracted and α-SMA mRNA level (Figure 9) and Collagen 1 mRNA level (Figure 10) were determined using quantitative real-time PCR. Collagen 1 and α-SMA are expressed in fibrotic tissues and the expression level of these fibrosis-related genes was reduced in samples treated with C96.

Example 8: Effect of Compound 96 on fibrosis in RTFs when added 12 to 48 hr before inducing fibrosis

RTFs were cultured as described in Example 1. Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M before inducing fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), anti-Collagen 1 NB600-408 antibody (Novus Biologicals); the loading control was tested using the FAK SC-557 A-17 antibody and GAPDH BF7 antibody (Santa Cruz Biotechnology) (Figure 11). The data shows that C96 is able to inhibit activation of FAK and collagen synthesis when added to the cells 12 hr to 48 hr before attempting to induce fibrosis with TGF-beta 2. The inhibition is shown in a dose-dependent manner, the strongest inhibition being seen when C96 is added at 10^{-7} M 12 to 48 hours pre-fibrosis. This data supports the notion that C96 can be given pre-operatively as a treatment to prevent fibrosis. The last lane to the right of this Figure marked W is the wash-out sample where the treated sample was rinsed with buffer and harvested 24 hr post-treatment. It shows that the inhibition

effect of the collagen and FAK activation is lost in this sample. Hence, the effect of C96 is reversible, which is an important factor when developing drugs for safe use.

Example 9: Effect of Compound 96 on Collagen 1 mRNA, Collagen 3 mRNA and αSMA mRNA when added 12 to 48 hr before inducing fibrosis

RTFs were cultured as described in Example 1. Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M either simultaneously or 12, 24 or 48 hours before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment, total RNA was extracted and Collagen 1 mRNA level (Figure 12), Collagen 3 mRNA level (Figure 13) and αSMA mRNA level (Figure 14) were determined using quantitative real-time PCR. The data from Figs. 12 , 13 and 14 show that C96 is able to inhibit expression of fibrotic markers when added to the cells 12 hr to 48 hr before fibrosis. The inhibition is shown in a dose-dependent manner, the strongest inhibition being seen when C96 is added at 10^{-7} M 12 to 48 hours pre-fibrosis. This data also supports the notion that C96 may be used pre-operative treatment to prevent fibrosis.

Example 10: Effect of Compound 96 on fibrosis in HTFs when added 12 to 48 hrs before inducing fibrosis

HTFs were cultured as described in Example 3. Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M either simultaneously, 12, 24 or 48 hours before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), anti-Collagen 1 NB600-408 antibody (Novus Biologicals); the loading control was tested using the FAK SC-557 A-17 antibody and GAPDH BF7 antibody (Santa Cruz Biotechnology) (Figure 15). The data shows that C96 is able to inhibit activation of FAK and collagen synthesis when added to the cells 12 hr to 48 hr before attempting to induce fibrosis with TGF-beta 2. The inhibition is shown in a dose-dependent manner, the strongest inhibition being seen when C96 is added at 10^{-7} M 12 to 48 hours pre-fibrosis. This data supports the notion that C96 can be given pre-operatively as a treatment to prevent fibrosis.

Example 11: Effect of Compound 96 on αSMA mRNA when added 12 to 48 hrs before inducing fibrosis

HTFs were cultured as described in Example 3, Compound 96 was added to the cells at various concentrations from 10^{-9} to 10^{-7} M either simultaneously or 12, 24 or 48 hours before attempting to induce fibrosis with 2 ng/ml of TGF-beta 2. After 48 hr of treatment, total RNA was extracted and αSMA mRNA level was determined using quantitative real-time PCR (Figure 16). The data shows that C96 is able to inhibit activation of FAK and collagen synthesis when added to the cells 12 hr to 48 hr before fibrosis. The inhibition is shown in a dose-dependent manner and the inhibition effect is seen even up to 2 days treatment with C96 before inducing fibrosis. This data supports the notion that C96 can be given as a pre-operative treatment to prevent fibrosis.

Example 12: Compound 96 prevents on-set of fibrosis in TGF-beta-treated RTFs

RTFs were cultured as described in Example 1. Compound 96 was added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), anti-Collagen 1 NB600-408 antibody (Novus Biologicals); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology) (Figure 17). The data shows that C96 is able to inhibit activation of FAK and collagen synthesis in the fibrosis-induced cells even up to 7 days without new addition of drug.

Example 13: Compound 96 reduces Collagen 1 gene expression, in TGF-beta-treated RTFs

RTFs were cultured as described in Example 1. Compound 96 was added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment, total RNA was extracted and Collagen 1 mRNA level was determined using quantitative real-time PCR (Figure 18). The data shows that C96 is able to inhibit expression of Collagen 1 mRNA in the fibrosis-induced cells even up to 7 days of fibrosis without addition of new drug.

Example 14: Compound 96 reduces indicators of fibrosis in TGF-beta-treated HTFs

HTFs were cultured as described in Example 3, Compound 96 was added to the cells at 10^{-7} M together with 2ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days

of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), anti-Collagen 1a NB600-408 antibody (Novus Biologicals); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology) (Figure 19). The data shows that C96 is able to inhibit activation of FAK and collagen synthesis in the fibrosis-induced HTF cells even up to 7 days of fibrosis without addition of new C96.

Example 15: Compound 96 reduces pro-fibrotic gene Collagen 1 mRNA expression, in TGF-beta-treated HTFs

HTFs were cultured as described in Example 3, Compound 96 was added to the cells at 10^{-7} M together with 2ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment, total RNA was extracted and Collagen1 mRNA level was determined using the quantitative real-time PCR (Figure 20). The data shows that C96 is able to inhibit expression of Collagen 1 mRNA in the fibrosis-induced HTFs cells even up to 7 days of fibrosis without addition of new drug.

Example 16: Effect of Compound 96 on pro-fibrotic genes Collagen 3, in TGF-beta-treated HTFs

HTFs were cultured as described in Example 3. Compound 96 was added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 2 days, 5 days and 7 days of treatment, total RNA was extracted and Collagen 3 mRNA level was determined using the quantitative real-time PCR (Figure 21). The data shows that C96 is able to inhibit expression of Collagen 3 mRNA in the fibrosis-induced HTFs cells even up to 7 days of fibrosis without addition of new drug.

Example 17: Post-fibrosis effect of Small molecule inhibitors on Focal Adhesion Kinase

HTFs were cultured as described in Example 3. Compound 96 or other FAK inhibitors described in the materials and methods were added to the cells at 10^{-7} M together with 2 ng/ml of TGF-beta 2. After 24 hr and 48 hr of treatment, cell lysates were prepared and subjected to western analysis. Blots were probed using the Phospho-FAK (Tyr397) antibody (Cell signaling technology #3283), the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology). The data (Figure 22) shows that the FAK inhibitors are not as effective as the C96 at inhibiting

the pFAK on the first day of fibrosis (0 hr). However, as the time of contact increases, these other FAK inhibitors were all able to inhibit the pFAK level back to the basal level.

Example 18: Other FAK inhibitors are able to inhibit pFAK(Y397) and Collagen in HEK293 cells

Hek 293 were cultured as described previously for RTF cells. Cells were cultured in 10 cm tissue culture plates, upon reaching 70% confluency. Cells were treated simultaneously with 2 ng/ml recombinant TGF-beta2, to induce fibrosis, and FAK inhibitors at various concentrations. After 48 hr of treatment cell lysates were prepared and subjected to western analysis. A first set of blots were probed using the Phospho-FAK (Tyr397) antibody (Cell10 signaling technology #3283); the loading control was tested using the FAK SC-557 A-17 antibody (Santa Cruz Biotechnology). The data (Figure 23) shows that the PF-04554878 Defactinib (VS-6063) [lanes 7 and 8], PF-00562271 -benzenesulfonate salt of PF-562271 [lanes 10 and 11], and GSK2256098 [lanes 13 and 14] are able to inhibit pFAK at 10^{-7} M as well as at 10^{-6}M.

Example 19: *In vivo* animal data

The plasma half-life of Compound 96 in rabbit when dosed intravenously and by subconjunctival injection at specific time points was determined.

Two New Zealand white rabbits were used for Pharmacokinetics/pharmaco-dynamics analysis to quantify drug efficacy. The compound prepared in 0.1% DMSO/PBS pH 7.2 was injected locally. One rabbit was given an intravenous dose of the compound and venous blood was collected at specified time points. The other was given a subconjunctival injection of the compound and venous blood was collected at specified time points.

Venous blood was collected before injection and at 5 min, 15 min, 30 min, 1 hr, 3 hr, 6 hr and 24 hr time points. Plasma was prepared from the blood samples and the drug concentration was determined using the HPLC/MS analysis.

The calibration curve was linear over the concentration range from 2 ng to 500 ng, with the equation y = 0.00152x + 0.00196 (r = 0.9962, 1/(x*x) weighting). The accuracy ranged from 92.4% to 110.0%. The measured plasma levels of C96 at 2 mg/kg after subconjunctival and intravenous injections are shown in Table 5.

Table 5: Plasma concentration (ng/ml) of C96 in New Zealand white rabbits over time following subconjunctival and intravenous injection of 2 mg/ml C96

Time (h)	subconjunctival injection Y9-rabbit		intravenous injection Y5-rabbit	
0	< 0		< 0	
0.083	0.536		162.5	(231 nM)
0.25	0.701	(0.935 nM)	85	(111 nM)
0.5	0.311		75	(98 nM)
1	0.25		17.6	(23 nM)
3	< 0		6.31	(8 nM)
6	< 0		3.61	(4.7 nM)
24	< 0		0.377	
	(2 ng/ml - 2.6 nM)			

Pharmacokinetics study of Compound 96 in Rabbits (Figure 24)

Summary of Results:

At 100 µM injection of C96 subconjunctival and intravenous injections - The plasma levels of C96 was undetectable.

At 2 mg/kg after subconjunctival and intravenous injections:

In the subconjunctival injection, the plasma concentrations of C96 were all below 2 ng (undetectable).

In the intravenous injection, the C96 is successfully quantified till 6 hours after intravenous injection.

Surgical Procedure:

Surgery was performed as described by Wong TTL et al., *Investigative Ophthalmology & Visual Science,* 44: 1097-1103 (2003). The animals were anesthetized with a combination of ketamine (Ketaset; Fort Dodge Animal Health, Southampton, UK) and medetomidine HCl (Domitor; Pfizer Animal Health, Sandwich, UK). Briefly, a partial thickness 8-0 silk corneal traction suture (Ethicon, Edinburgh, Scotland, UK) was placed superiorly, and the eye pulled down. A fornix- based conjunctival flap was raised, after which a blunt dissection of the subconjunctival space was performed of approximately 5 mm along the limbus and 8 mm posteriorly. A

microvitreoretinal (MVR) blade was used to make a partial-thickness scleral incision 3 to 4 mm behind the limbus, and a scleral tunnel to the corneal stroma was fashioned. A 22-gauge, 25-mm intravenous cannula (Venflon 2; Beckton Dickinson, Oxford, UK) was passed through a scleral tunnel anteriorly until the cannula needle was visible in the clear cornea. Entry into the anterior chamber was made with a cannula needle, which was then withdrawn as the cannula was advanced to the midpupillary area. The cannula was trimmed and beveled at its scleral end so that it protruded 1 mm from the insertion point, and a 10-0 nylon suture was placed to fix the tube to the scleral surface.

The conjunctival incision was closed with two interrupted sutures and a central, mattress-type 10-0 nylon suture attached to a needle (B/V 100-4; Ethicon) to give a water-tight closure. One drop each of guttae chloramphenicol and Betnesol-N (Glaxo Wellcome, Uxbridge, UK) ointment was instilled at the end of surgery. Only the left eye was operated on, and the surgical procedure was performed at the same site, superiorly and temporally, in each animal.

Example 20: Loose sub-epithelial connective tissue spaces associated with bleb filtration are seen in Compound 96-treated eyes but not control eyes

Eight rabbits underwent surgery as described in Example 19, and treatment as follows:

Rabbits underwent filtration surgery to the left eye only with random allocation to one of two treatment groups: subconjunctival injection of 0.1 ml of either 100 μM Compound 96 or PBS as control, 1 day before surgery and immediately after surgery and on each of days 1 through 7 after surgery. The rationale for this dose regimen is to attempt to achieve local availability of the inhibitor at the critical time of pre- and post-operative wound repair and remodeling, as observed in other studies.

The animals were examined before receiving the injections at set intervals for 28 days after surgery. Clinically, the intraocular pressure (IOP) was recorded on days 1, 7, 14, 21 and 28. The animals were sacrificed on day 28. Both eyes were enucleated and histologically analyzed. The non-surgical right eyes served as the normal control for histologic comparison.

In vivo confocal images show that loose sub-epithelial connective tissue spaces associated with bleb filtration are seen in treated eyes but not control eyes, at day 22 and day 29 (Figure 25).

Example 21: Tissue Sections from mock glaucoma-operated rabbit eyes

1. Hematoxylin and Eosin (H&E) stain – Hematoxylin – deep blue stains nucleic acids, Eosin pink staining of cytoplasm and extracellular matrix. Hematoxylin and eosin (H&E) staining showed a flattened conjunctiva at the day-28 control operated site (Y7, bottom panels) whereas the Compound 96-treated operated site subconjunctival space was expanded with an almost clear matrix (Y2, top panels) at day 30 (Figure 26).
2. Collagen – Picrosirius red staining (scar collagen – green thin fibers, matured collagen – yellow to orange). Picrosirius red staining revealed that the deposition of scar tissue and densely packed collagen fibers in the subconjunctival space, stained green-yellow, in the vehicle-treated eyes (Y7 bottom panels) were significantly greater in the vehicle-treated (bottom panels) than in the Compound 96-treated eyes (Y2 top panels) at day 30 (Figure 27).
3. Masson Trichrome staining – Red – keratin, erythrocytes and muscle fibres, Blue or green – collagen and bone, light red/pink cytoplasm and dark brown to black cell nuclei. Compound 96-treated eyes (Y4, top panel) were noted to have less collagen deposition with Masson Trichrome stain compared to vehicle-treated eyes (Y8, bottom panel) as well as reduced keratin, erythrocytes and muscle fibers at day 30 (Figure 28).
4. Alpha-Smooth muscle actin (αSMA) - Immunofluorescence analysis of sections from the same eyes used above (Y2 Compound 96-treated and Y7 vehicle-treated) with alpha-smooth muscle actin specific antibody showed reduced staining in eyes treated with Compound 96 at day 30 (Figure 29).

   Colour images of Figures 26-29 can be provided.

Example 22: Formulations

Compound C96 was tested in eye formulations from a commercial source (CRODA).

Polysorbates are hydrophilic, nonionic surfactants widely used as emulsifying agents in the preparation of stable oil-in-water pharmaceutical emulsions. They are also excellent solubilisers, stabilisers, wetting and dispersing agents. However, like other excipients, standard pharmaceutical polysorbates possess impurities that cause formulation challenges especially when working with oxidatively unstable Active Pharmaceutical Ingredients (APIs). As with the Super Refined PEGs, CRODA has

Super Refined a range of polysorbates, removing or reducing polar and oxidative impurities. The formula tested is called Super Refined Polysorbate 80 (SRP80).

Animal Study – White New Zealand rabbit

3 rabbits were given 1 week to acclimatize

Day 0 – control

Check IOP and perform imaging - Slit lamp biomicroscopy of rabbit cornea (DAY = 0 data)

Day 1 to Day 30 eye drop – twice daily

Rabbit # 5 – Control rabbit

Left eye – previous buffer (DMSO 0.01%)

Right eye – SRP80 formula (0.01% v/v)

Rabbit # 11 - Drug + 0.01% (v/v) SRP80 formulation

Left eye – 50 µM C96

Right eye – 100 µM C96

Rabbit # 12 - Drug + 0.01% DMSO

Left eye – 50 µM C96

Right eye – 100 µM C96

Check IOP and perform imaging - Slit lamp biomicroscopy of rabbit cornea at day 7, day 14, day 21 and day 30.

The results for each time point were similar, so only the results for day 14 are shown in Figure 30. The data shows that C96 when dissolved in SRP80 formulation tested *in vivo* doesn't cause any irritation, swelling or redness. This was also seen when DMSO was used as excipient. No redness or increased vascularization was seen in any of the experimental samples (⟶).

To further study the effect of the formulation, sub-conjunctival tissue at day 30 was dissected and histology/immunohistochemistry was performed on tissue sections.

Sub-conjunctival tissue sections at Day 30 were stained for:

(1) H&E - general cell morphology;

(2) Collagen/picrosirius Red;

(3) Masson Trichrome staining -assess collagen deposition and remodeling (reorganizing); and

(4) smooth muscle actin (α-SMA).

The presence of goblet cells in the superficial epithelium is a critical parameter that reflects the overall health of the ocular surface. Hematoxylin and Eosin-stained sections of sub-conjunctival tissue at day 30 of (Figure 31) shows treatment with DMSO, SRP80 and combinations of DMSO or SRP80 with Compound 96 did not significantly affect the goblet cells (⟶).

Picrosirius red-stained sections (data not shown) showed that the deposition of total scar tissue and densely packed collagen fibers in the subconjunctival space were significantly greater in the DMSO-treated eyes and in the drug dissolved in DMSO-treated eyes than in the SRP80-treated eyes and the drug dissolved in SRP80 treated eye at day 30. The green-yellow staining seen in the DMSO formulation-treated eyes is indicative of damaged tissue undergoing scarring. Even though the DMSO combination did not produce any redness in the rabbit eye, the internal morphology of the conjunctival tissue appeared to be going through some pathophysiological changes not observed with the SRP80 formulation.

Masson's trichrome stained sections (Figure 32) shows selective staining of connective tissue blue in the original colour images (white arrows) which is the collagen stroma. Rabbits treated with higher dose of the compound in DMSO show disrupted expression of collagenous stroma (top right image; marked with asterisks *). The drug preparation with the SRP80 showed regular arranged collagenous stroma and a rich layer of conjunctival goblet cells (red staining in original image; black arrows) which are required for protection from external factors. The higher dose of the compound in DMSO showed a decreased density of conjunctival goblet cells which may have been the result from chronic irritation produced with this formulation.

Immunofluorescence staining for α-SMA on sections of sub-conjunctival tissue (Figure 33) showed that in all the samples, a significantly lower proportion of cells was found to express alpha-SMA than would normally be seen with the scarring, indicating that addition of the drug with either DMSO or SRP80 does not result in trans-differentiation of the epithelial cells to myofibroblasts.

The results from the study in Example 22 showed that C96 was soluble in SRP80 at 10^{-6} M and was non-toxic when tested in the rabbit eyes.

Example 23: Adjunct use of FAK inhibitors to reduce implanted drainage device-induced fibrosis

Drainage device implants have shown great promise in reducing IOP as well as having an increased safety profile. Similar to conventional glaucoma filtration surgeries, Adjunct Mitomycin C is commonly used to reduce device-induced fibrosis, with some ophthalmologists using the cytotoxin even post-surgery for needlings. As such, it is important that we find an alternative medication that reduces the fibrosis while being less toxic to the ocular structures.The FAK inhibitors detailed in this invention can serve as non-cytotoxic alternatives to Mitomycin C.

Two such devices are the XEN® GEL Implant (AqueSys Inc., Aliso Viejo, CA, USA) and the InnFocus Microshunt® (InnFocus Inc, Miami, FL, USA) which both drain into the subconjunctival space.

The XEN® gel stent is a hydrophilic tube made of a porcine gelatin cross-linked with glutaraldehyde to achieve permanence in tissue. This material is used for a variety of medical applications because of its well-established biocompatibility, and it does not cause a foreign-body reaction (Figure 34A). The XEN® 45 is the only one currently available and has an inside diameter of ~45 µm, an outside diameter of 150 µm, and is 6 mm long. The implant is hard when dry and becomes soft within 1–2 min when hydrated, adapting to the tissue shape, thus avoiding migration and potential erosion. It has been demonstrated that the gel stent is ~100 times more flexible than the silicon tubing used in traditional tube–shunt surgery [Saheb H and Ahmed II, *Curr Opin Ophthalmol.* 23(2): 96–104 (2012)]. The implant is housed in a disposable preloaded handheld inserter designed specifically for an ab interno surgical implantation (Figure 34B).

The procedure can be performed under local or topical anesthesia. A FAK inhibitor of the invention is injected with a 30-gauge needle in the subconjunctival supero-temporal quadrant space to obtain a bubble that is gently rolled toward the supero-nasal quadrant. This induces a hydroexpansion, which reduces tissue resistance, preparing the space for the implant and supporting the bleb formation. Blood vessels should be avoided during the introduction of the needle into the subconjunctival space as bleeding may compromise visibility required for the stent implantation. The surgical procedure for stent placement is described in De Gregorio A, et al., *Clin Ophthalmol,*12: 773-782 (2018).

The InnFocus Microshunt (InnFocus Inc, Miami, FL, USA), formerly known as the MIDI Arrow, is an aqueous drainage shunt designed to be implanted ab externo. It

is implanted after making a fornix based conjunctival flap, creating a scleral pocket and applying mitomycin-c. This approach creates a bleb space in preparation for the fluid that will be directed from the anterior chamber through the InnFocus MicroShunt® to the bleb.

During development, however, it was found that the fins designed to prevent tube migration could erode through the conjunctiva—hence, the need for a scleral pocket. Since it has been shown that Mitomycin C is cytotoxic, FAK inhibitors of the invention provide a safer alternative.

Summary

A small molecule inhibitor, Compound 96 [diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate] that targets the Y397 and Y925 sites of FAK was synthesized and the effect of this specific FAK inhibitor in eye-related scarring and fibrosis was investigated. In this study using the Human Tenon's Fibroblasts (HTFs) and Rabbit Tenon's Fibroblasts (RTFs) Compound 96 inhibited pY925 and pY-397 FAK phosphorylation as well as reducing the expression levels of pro-fibrotic markers. The anti-scarring effect of this compound is profound; it remains stable and effective prior to induction of fibrosis and active post-induction up to 7 days in *in vitro* studies. Animals treated with the compound showed reduced fibrosis compared to the control animals. Inhibition of fibrosis significantly improved surgical outcome by reducing the amount of scar tissue produced. By targeting the accumulation of the fibrosis, a more controlled and physiological method of modulating scarring may be achieved. The SRP80 excipient for Compound 96 showed a good level of tolerance in the in vivo study done on rabbit eyes at both concentration of the drug formulation.

Additional FAK inhibitors were tested and,from the data obtained, there is great potential for PF-04554878 Defactinib (VS-6063,), PF-00562271 -benzenesulfonate salt of PF-562271, PF-562271 HCl is the hydrochloride salt of PF-562271, GSK2256098 and TAE226 (NVP-TAE226) to be developed as anti-fibrotic drugs.

These FAK inhibitors can be used alone or as an adjunct when drainage devices are implanted in patients with glaucoma.

It would be understood that there are alternatives to inhibit focal adhesive kinase and/or pro-fibrotic factors. For example, antibodies, RNAi, gene therapy and use of nanoparticles. However, such alternatives have inherent shortcomings.

Bevacizumab is a recombinant humanized monoclonal antibody against VEGF. However, Bevacizumab is only effective in combinations with other anti-scarring agents and potentially could be immunogenic. In addition, RNAi can have off-target effects and is immunogenic; gene therapy presents a challenge with the delivery of the gene into the target cell and could be oncogenic; nanoparticles have moderate specificity while potentially being cytotoxic and genotoxic; and small molecule inhibitors such as Rho-kinase (ROCK) inhibitors have the disadvantage of causing ocular redness (conjunctival hyperemia).

References

Any listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that such document is part of the state of the art or is common general knowledge.

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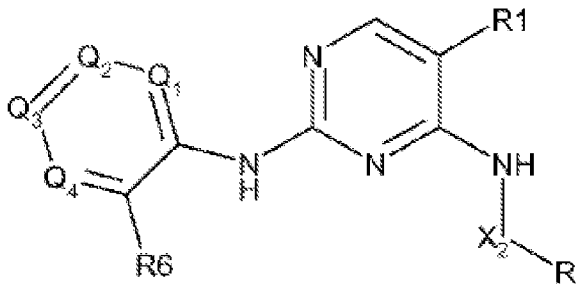
Claims

1. A composition comprising at least one FAK inhibitor or variant thereof for inhibiting scarring and fibrosis of the eye of a subject.

2. The composition of claim 1, wherein the at least one FAK inhibitor has the general formula 1:



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wherein R is an optionally substituted aryl or heteroaryl;

R1 is halogen, CF_3, CCH, or other suitable substituent;

X_2 is $–(CR^7R^8)_{0-2}$-;

each R^7 and R^8 is independently halogen, C_{0-3} aliphatic, or $–OC_{0-3}$ aliphatic, either of which is optionally halogen substituted, except that in the case of X_2, R^7 and R^8 are not halogen or $–OC_{0-3}$ aliphatic;

R6 is halogen, $-OC_{0-3}$ aliphatic, or C_{0-3} aliphatic, either optionally substituted by one or more halogen or by $–OCF_3$;

Q_1 to Q_4 are independently >CH, >CF, >N, or >N-oxide; except that at least one of Q_2 to Q_4 includes a substituent that includes a phosphinate, phosphonate, or phosphine oxide.

3. The composition of claim 1 or 2, wherein the at least one FAK inhibitor is selected from the group comprising diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate;

N-methyl-4-(4-((3-(N-methylmethan-3-ylsulfonamido)pyrazin-2-yl)methylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)benzamide;

N-methyl-N-(3-((2-(2-oxoindolin-5-ylamino)-5-(trifluoromethyl)pyrimidin-4-ylamino)methyl)pyridin-2-yl)methanesulfonamide benzenesulfonate;

Benzamide, 2-[[5-chloro-2-[[3-methyl-1-(1-methylethyl)-1H-pyrazol-5-yl]amino]-4-pyridinyl]amino]-N-methoxy-;

Benzoic acid, 2,4-dimethyl-, [4-[(1S)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, hydrochloride (1:2);

N-Methyl-N-{3-[({2-[(2-oxo-2,3-dihydro-1H-indol-5-yl)amino]-5-(trifluoromethyl)-4-pyrimidinyl}amino)methyl]-2-pyridinyl}methanesulfonamide hydrochloride (1:1);

2-(2-(2-methoxy-4-morpholinophenylamino)-5-(trifluoromethyl)pyridin-4-ylamino)-N-methylbenzamide; and

Benzamide, 2-[[5-chloro-2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]amino]-N-methyl-.

4. The composition of any one of claims 1 to 3, wherein the at least one FAK inhibitor is selected from the group comprising diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate.

5. The composition of any one of the previous claims, comprising a pharmaceutically acceptable salt or solvate of said at least one FAK inhibitor.

6. The composition of any one of claims 1 to 5, wherein the composition further comprises at least one anti-metabolite such as Mitomycin C (MMC) and/or 5-fluorouracil (5-FU).

7. The composition of any one of claims 1 to 6, wherein the scarring and fibrosis occurs post-surgery for glaucoma in the subject.

8. The composition of any one of claims 1 to 7, wherein the composition inhibits focal adhesion kinase (FAK) and/or the expression of at least one pro-fibrotic factor.

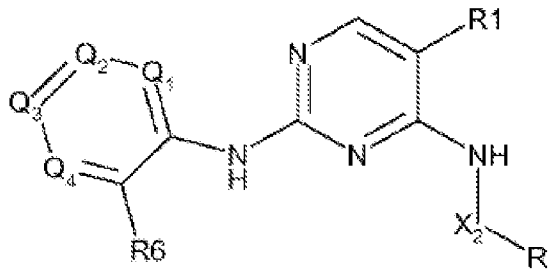
9. The composition of claim 8, wherein phosphorylation of FAK to form FAK pTyr925 and/or FAK pTyr397 is inhibited.

10. The composition of claim 8 or 9, wherein the at least one pro-fibrotic factor is selected from the group comprising the pro-fibrotic markers collagen1α1 (COL1A1), α smooth muscle actin (αSMA) and SPARC (secreted protein, acidic, rich in cysteine).

11. The composition of any one of the previous claims, further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.

12. Use of at least one FAK inhibitor or variant, or pharmaceutically acceptable salt or solvate thereof, for the manufacture of a medicament for inhibiting scarring and fibrosis of the eye of a subject.

13. The use of claim 12, wherein the at least one FAK inhibitor has the general formula 1:



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wherein R is an optionally substituted aryl or heteroaryl;

R1 is halogen, CF_3, CCH, or other suitable substituent;

X_2 is $–(CR^7R^8)_{0-2}$-;

each R^7 and R^8 is independently halogen, C_{0-3} aliphatic, or $–OC_{0-3}$ aliphatic, either of which is optionally halogen substituted, except that in the case of X_2, R^7 and R^8 are not halogen or $–OC_{0-3}$ aliphatic;

R6 is halogen, $-OC_{0-3}$ aliphatic, or C_{0-3} aliphatic, either optionally substituted by one or more halogen or by $–OCF_3$;

Q_1 to Q_4 are independently >CH, >CF, >N, or >N-oxide; except that at least one of Q_2 to Q_4 includes a substituent that includes a phosphinate, phosphonate, or phosphine oxide.

14. The use of claim 13, wherein the at least one FAK inhibitor is selected from the group comprising diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate;

N-methyl-4-(4-((3-(N-methylmethan-3-ylsulfonamido)pyrazin-2-yl)methylamino)-5-(trifluoromethyl)pyrimidin-2-ylamino)benzamide;

N-methyl-N-(3-((2-(2-oxoindolin-5-ylamino)-5-(trifluoromethyl)pyrimidin-4-ylamino)methyl)pyridin-2-yl)methanesulfonamide benzenesulfonate;

Benzamide, 2-[[5-chloro-2-[[3-methyl-1-(1-methylethyl)-1H-pyrazol-5-yl]amino]-4-pyridinyl]amino]-N-methoxy-;

Benzoic acid, 2,4-dimethyl-, [4-[(1S)-1-(aminomethyl)-2-(6-isoquinolinylamino)-2-oxoethyl]phenyl]methyl ester, hydrochloride (1:2);

N-Methyl-N-{3-[({2-[(2-oxo-2,3-dihydro-1H-indol-5-yl)amino]-5-(trifluoromethyl)-4-pyrimidinyl}amino)methyl]-2-pyridinyl}methanesulfonamide hydrochloride (1:1);

2-(2-(2-methoxy-4-morpholinophenylamino)-5-(trifluoromethyl)pyridin-4-ylamino)-N-methylbenzamide; and

Benzamide, 2-[[5-chloro-2-[[2-methoxy-4-(4-morpholinyl)phenyl]amino]-4-pyrimidinyl]amino]-N-methyl-;

or pharmaceutically acceptable salt or solvate thereof.

15. The use of claim 14, wherein the at least one FAK inhibitor is diethyl (3-methoxy-4-{[4-({2-methyl-7-[*trans*-4-(4-methylpiperazin-1-yl)cyclohexyl]-3-oxo-2,3-dihydro-1H-isoindol-4-yl}amino)-5-(trifluoromethyl)pyrimidin-2-yl]amino}benzyl)phosphonate or pharmaceutically acceptable salt or solvate thereof.

16. The use of any one of claims 12 to 15, wherein the medicament further comprises at least one anti-metabolite such as Mitomycin C (MMC) and/or 5-fluorouracil (5-FU).

17. The use of any one of claims 12 to 16, wherein the scarring and fibrosis occurs post-surgery for glaucoma in the subject.

18. The use of any one of claims 12 to 17, wherein the medicament is formulated for subconjunctival or intravenous delivery.

19. The use of any one of claims 12 to 18, wherein the medicament inhibits focal adhesion kinase (FAK) and/or the expression of at least one pro-fibrotic factor.

20. The use of claim 19, wherein phosphorylation of FAK to form FAK pTyr925 and/or FAK pTyr397 is inhibited.

21. The use of claim 19 or 20, wherein the at least one pro-fibrotic factor is selected from the group comprising the pro-fibrotic markers collagen1α1 (COL1A1), α smooth muscle actin (αSMA) and SPARC (secreted protein, acidic, rich in cysteine).

22. The use of any one of claims 12 to 21, wherein the medicament is formulated for administration below the conjunctiva or intravenously.

23. A method of prophylaxis or treatment of eye related scarring and fibrosis, comprising administering to a subject in need thereof an effective amount of a composition of any one of claims 1 to 11.

24. The method of claim 23, wherein the administration is before, during or post-surgery for treating glaucoma in the subject.

25. The method of claim 23 or 24, wherein the composition is administered below the conjunctiva or intravenously.

26. The method of any one of claims 23 to 25, wherein the composition is administered to reduce fibrosis caused by an implanted drainage device.

3D culture Rabbit TFs

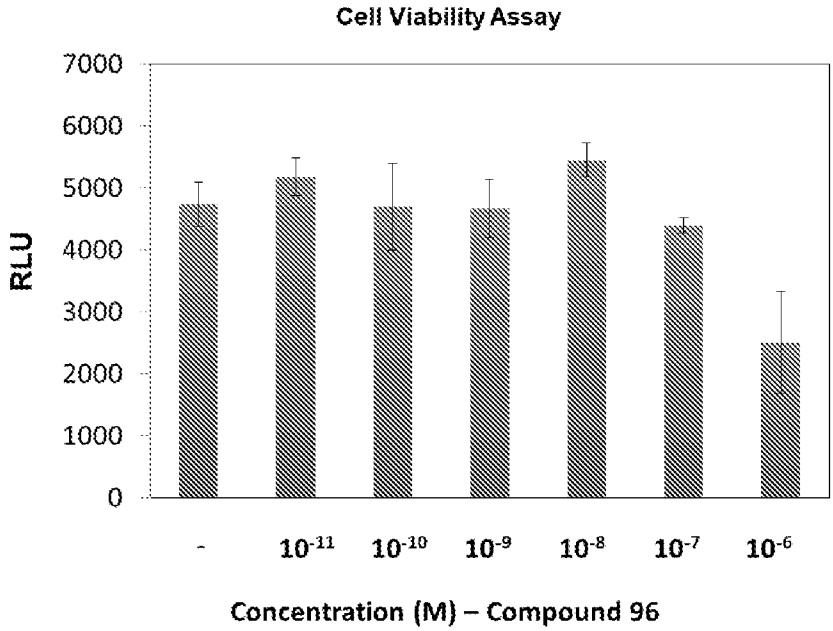

Figure 1

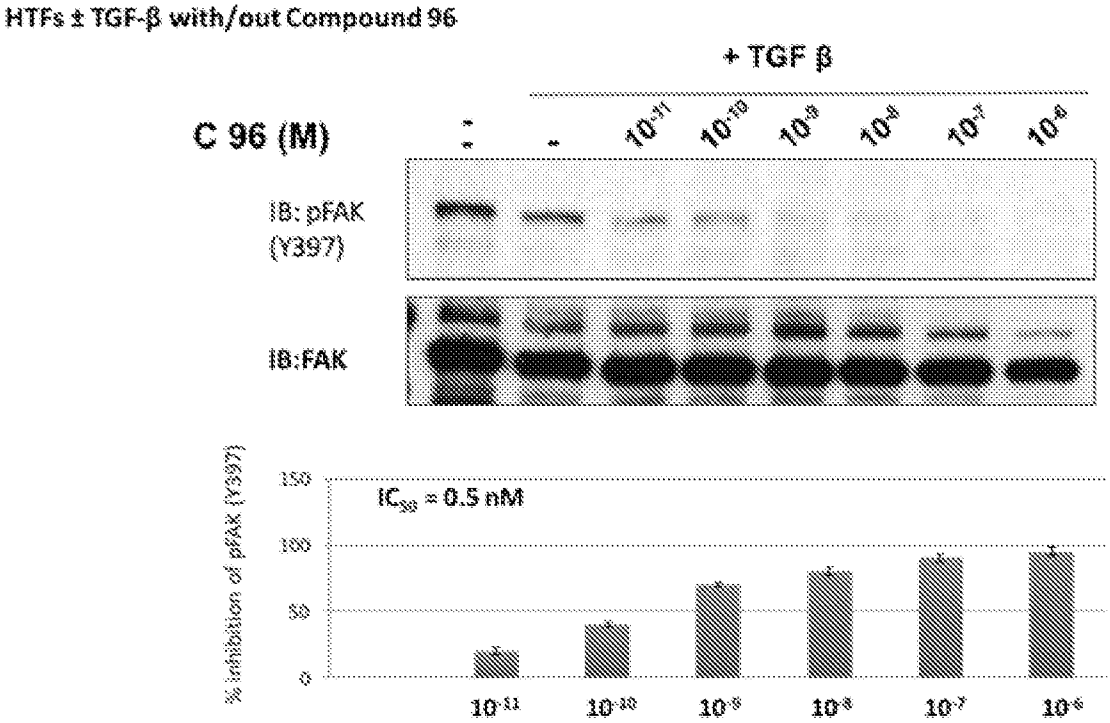

Figure 2

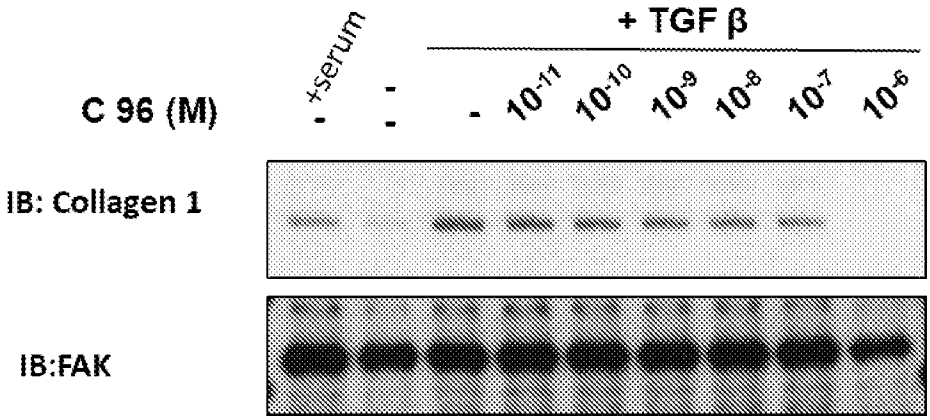

Figure 3

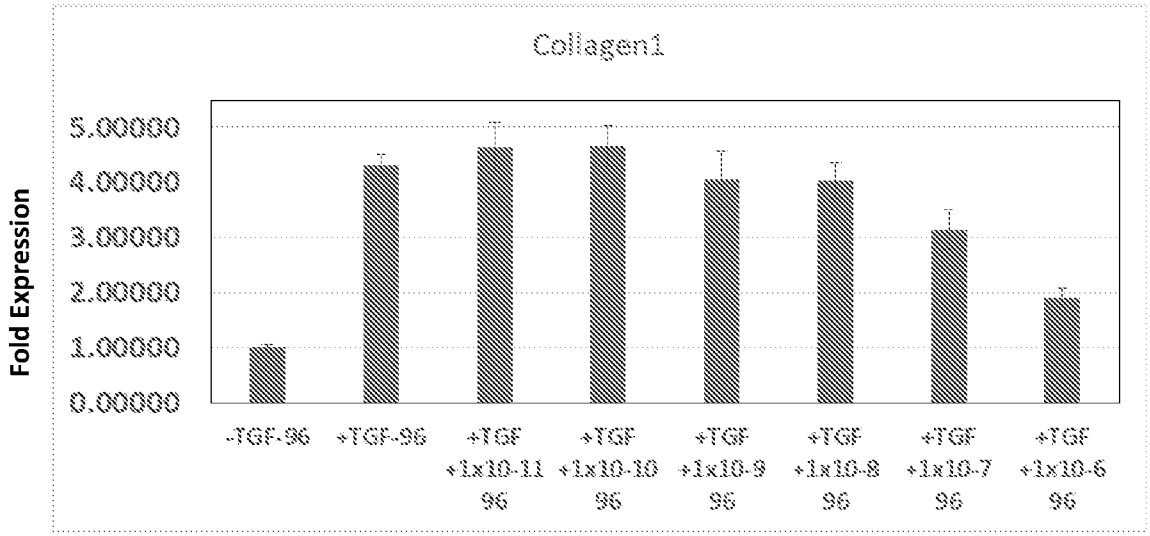

Figure 4

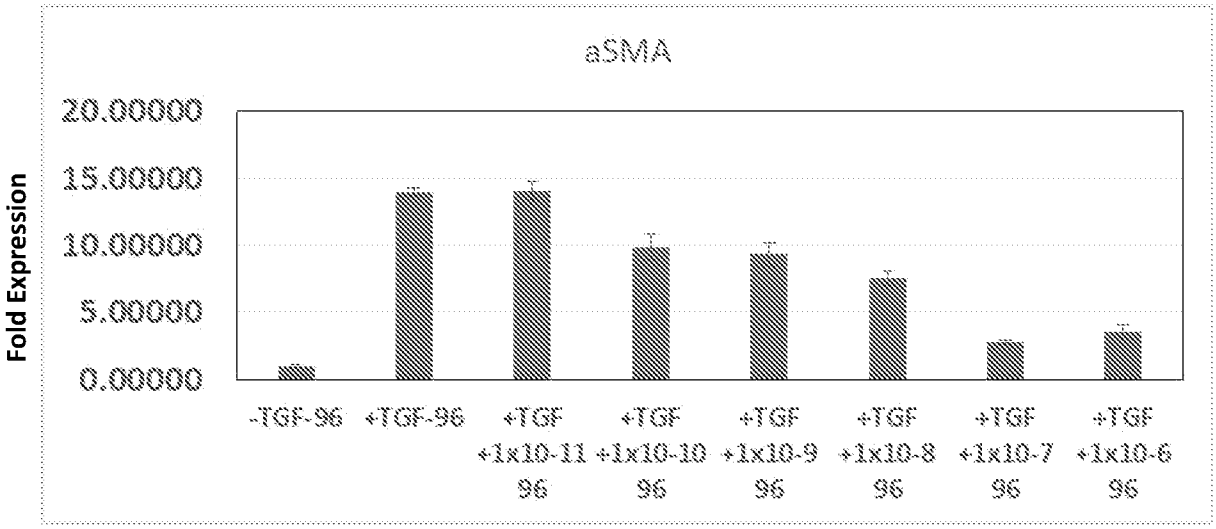

Figure 5

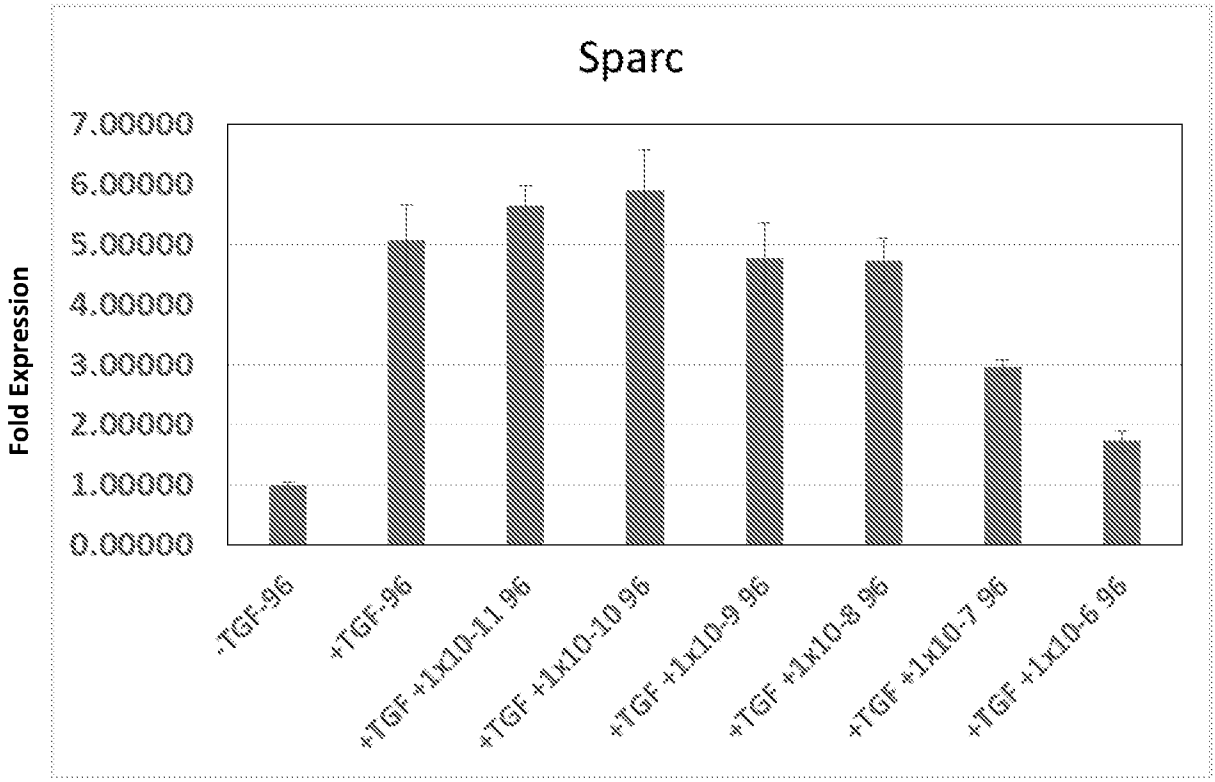
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Figure 6

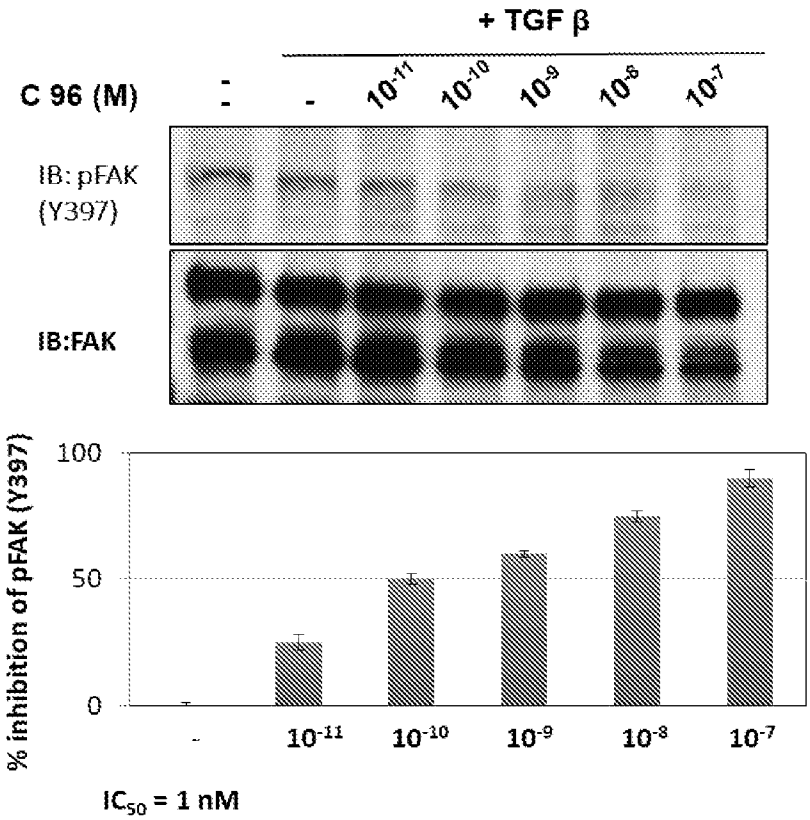


Figure 7

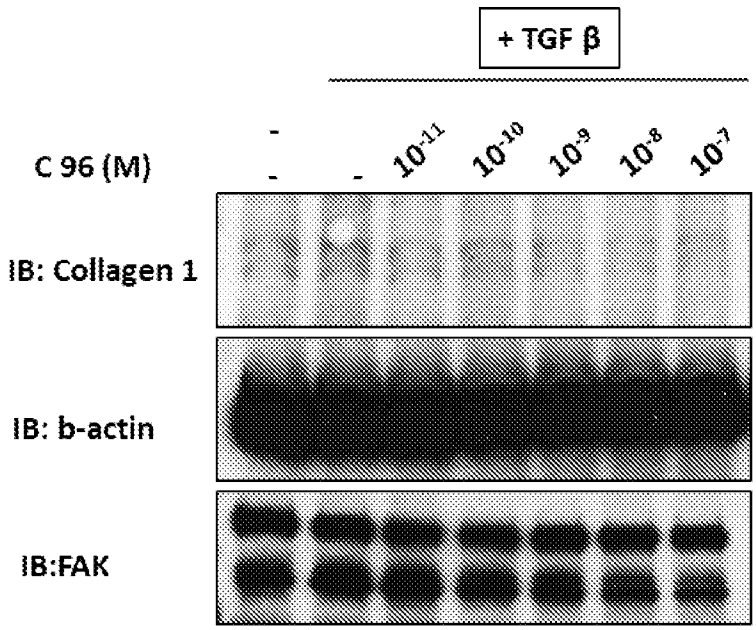


Figure 8

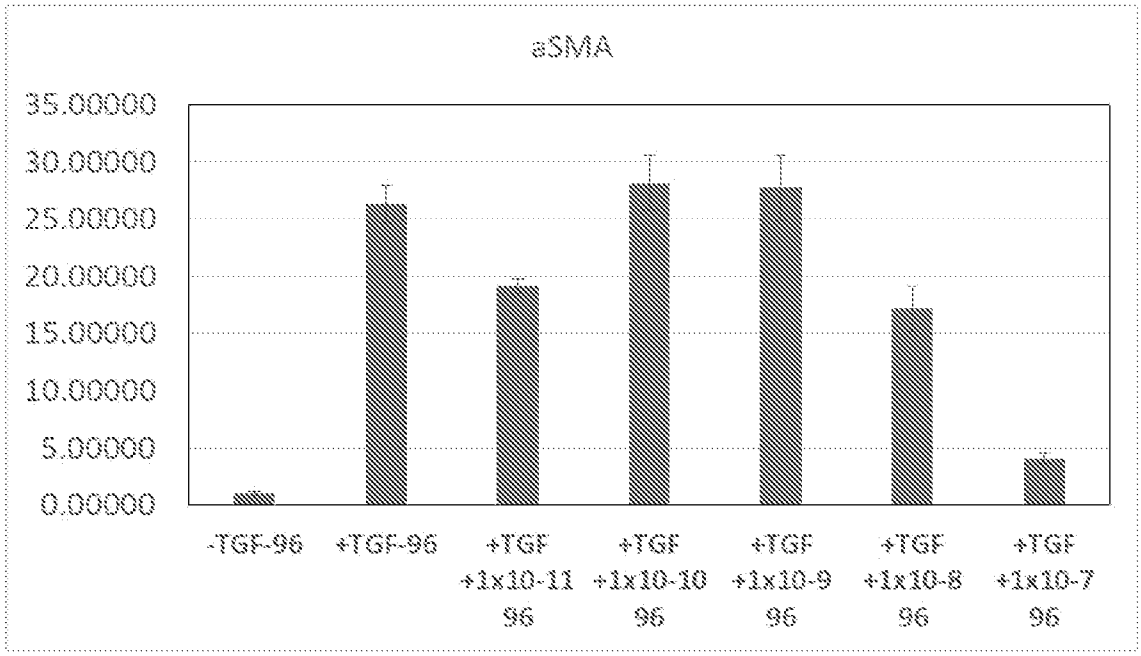


Figure 9

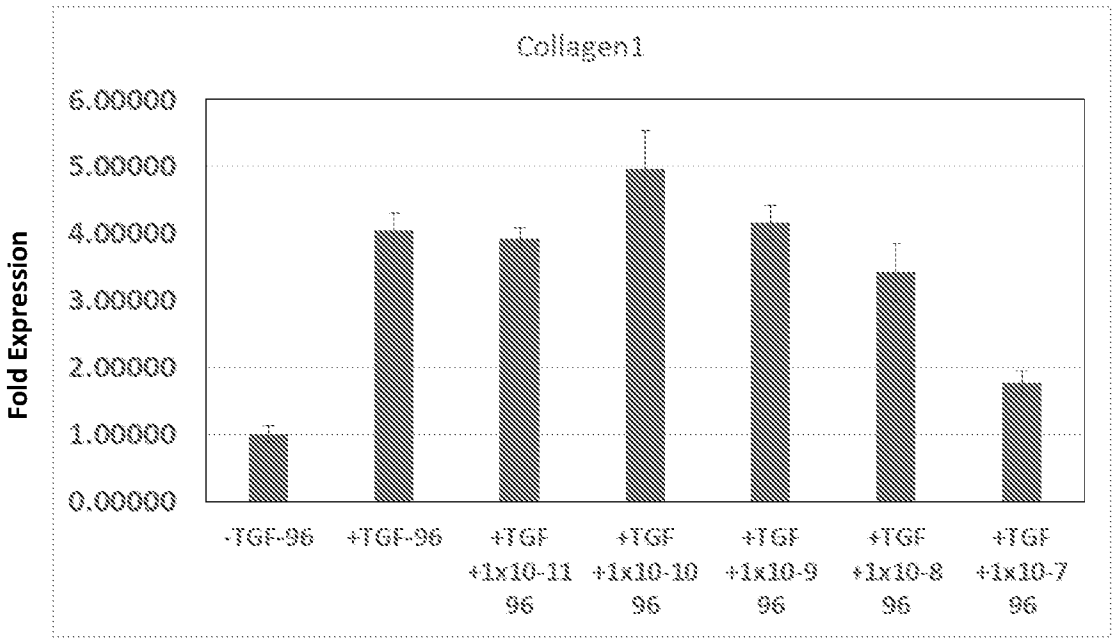


Figure 10

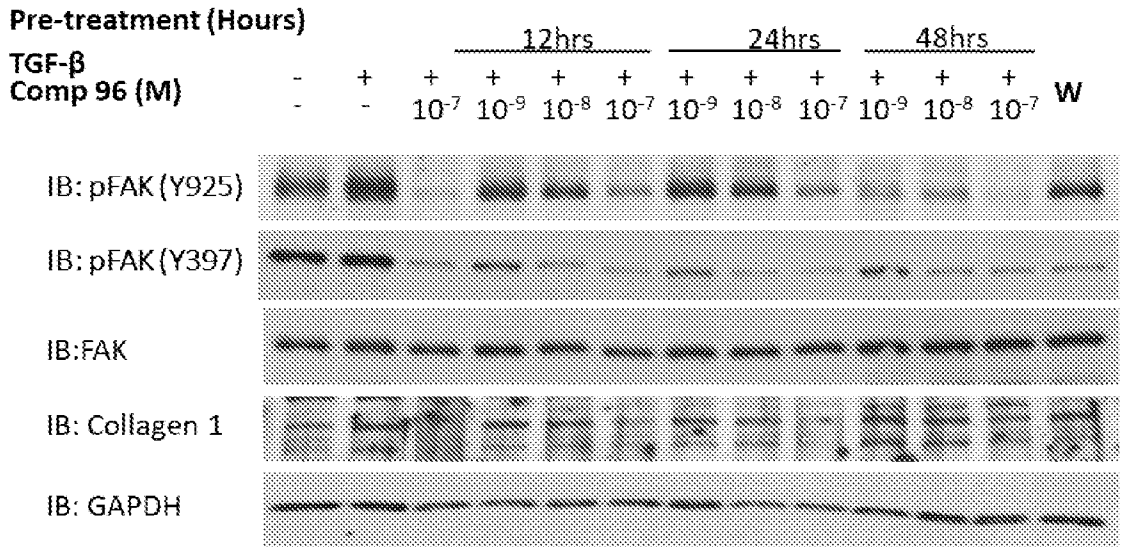


Figure 11

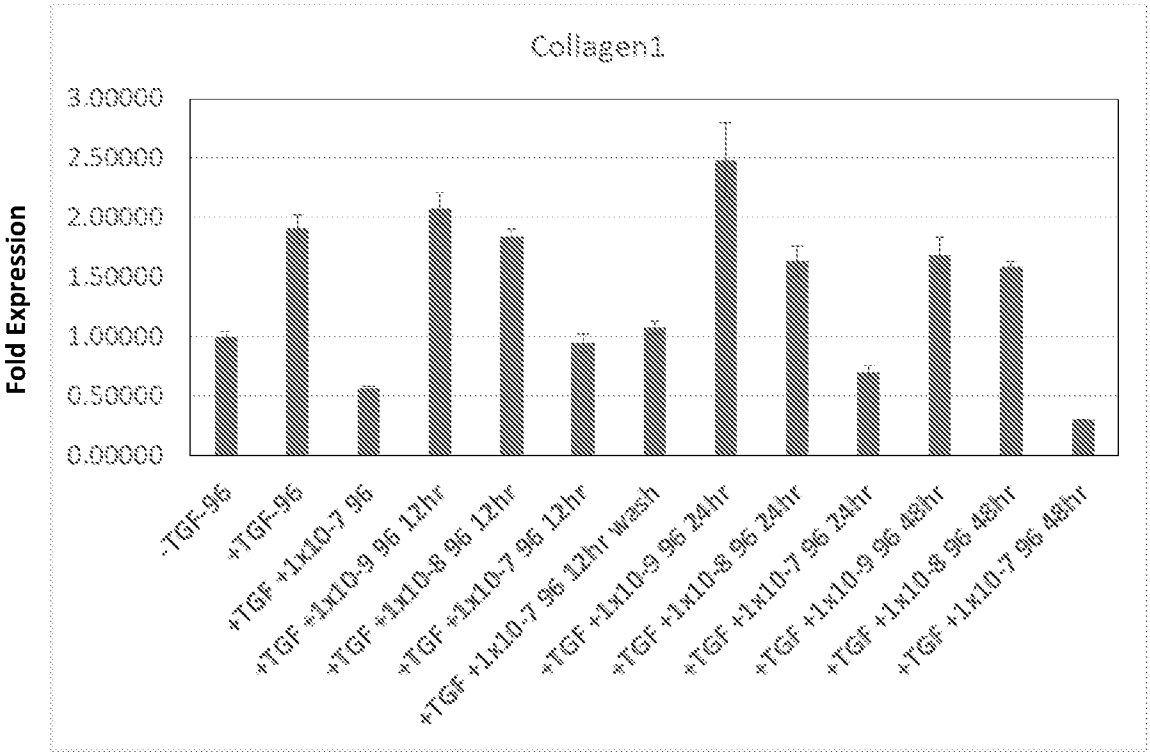


Figure 12

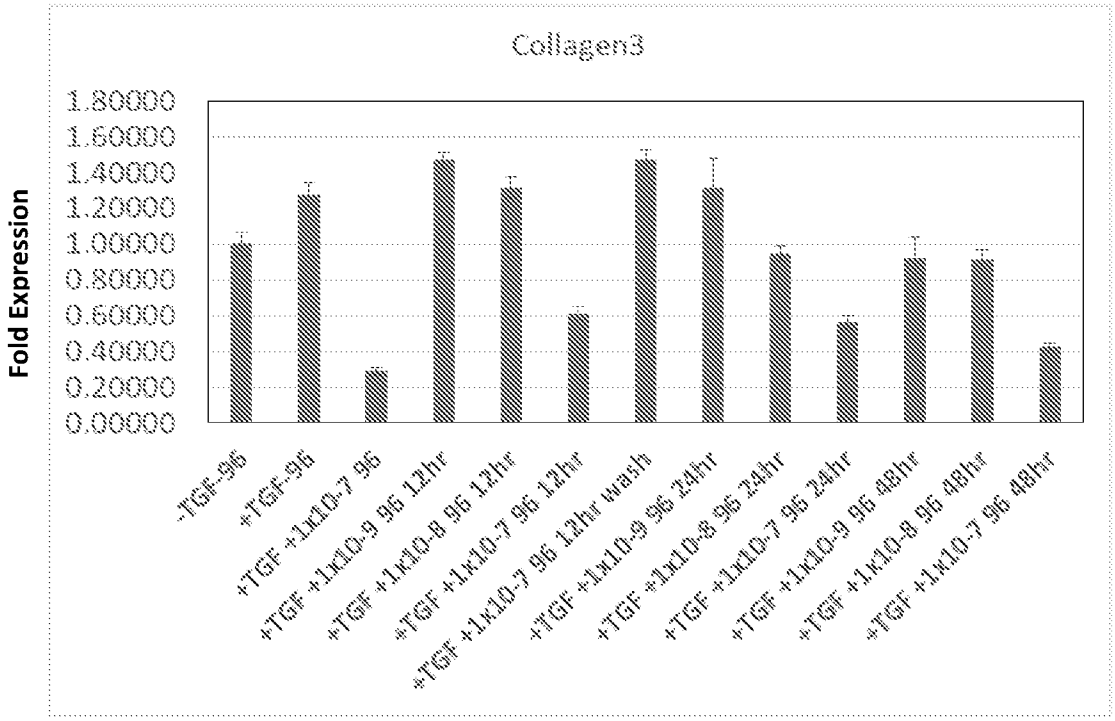


Figure 13

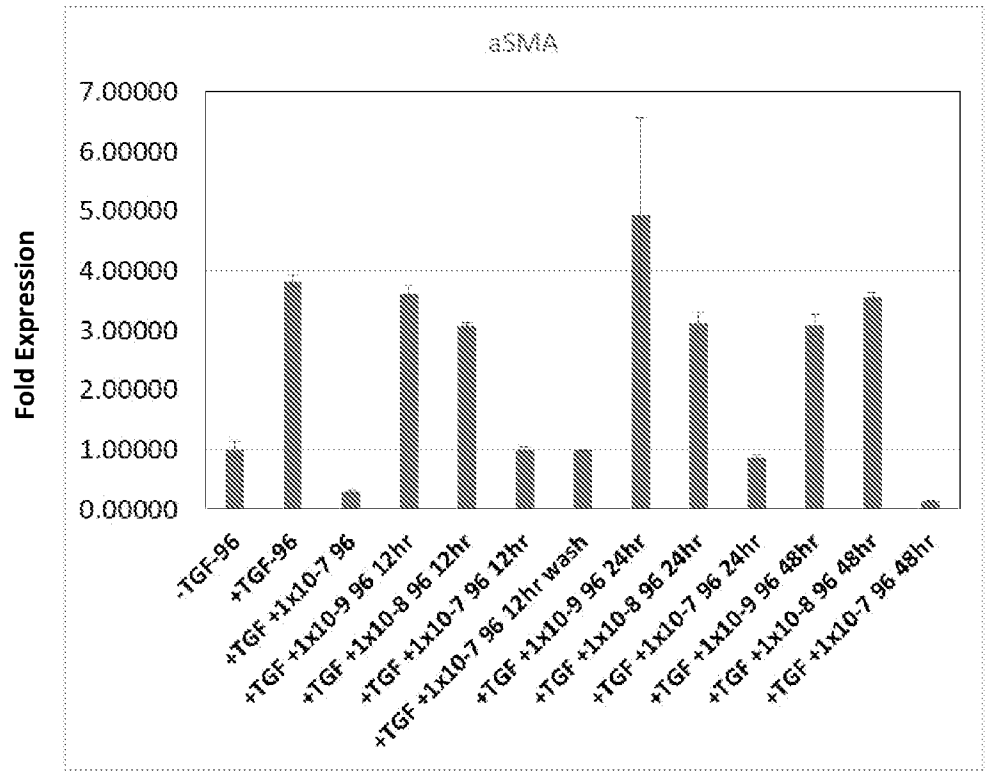


Figure 14

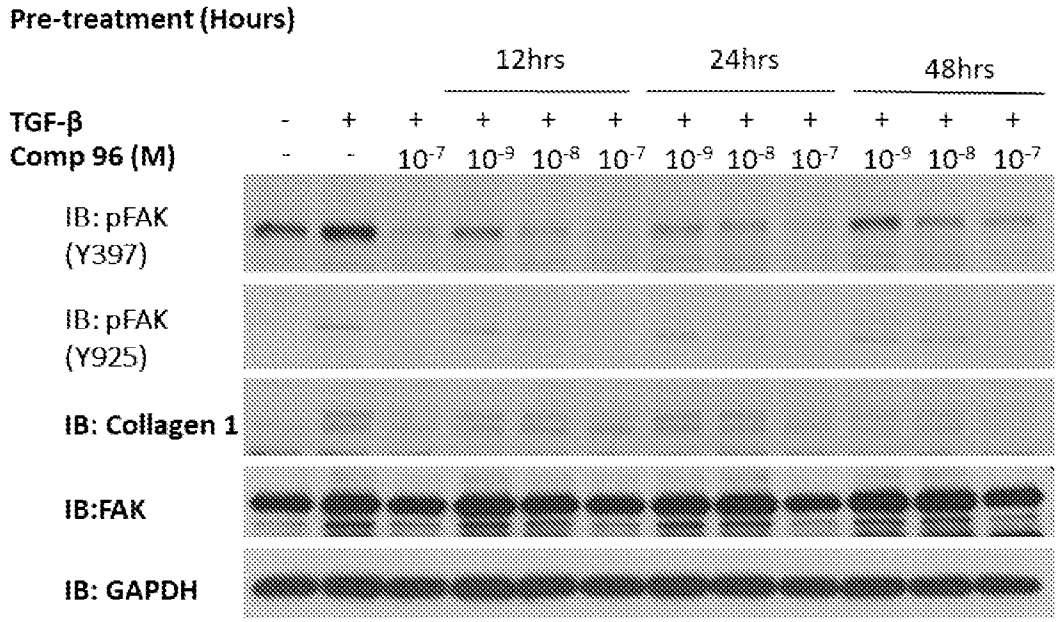


Figure 15

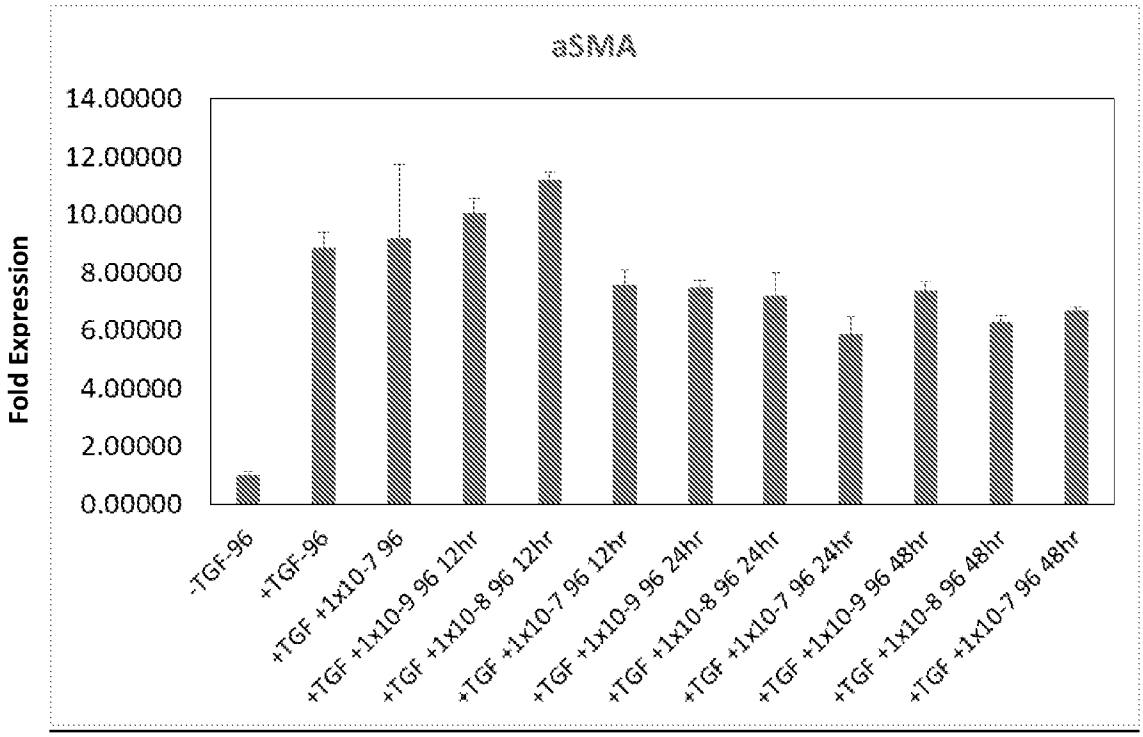


Figure 16

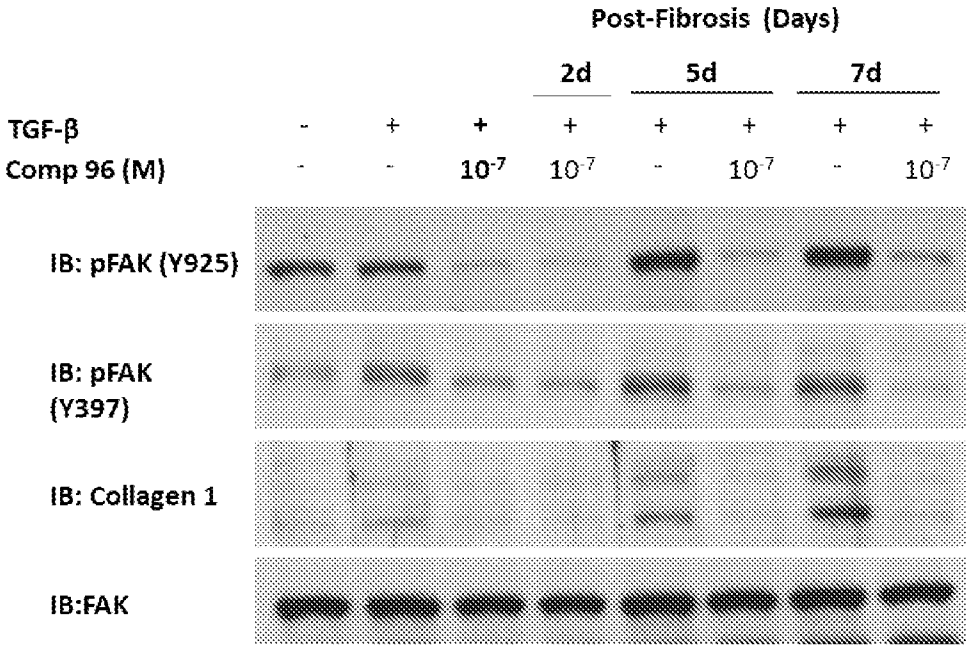


Figure 17

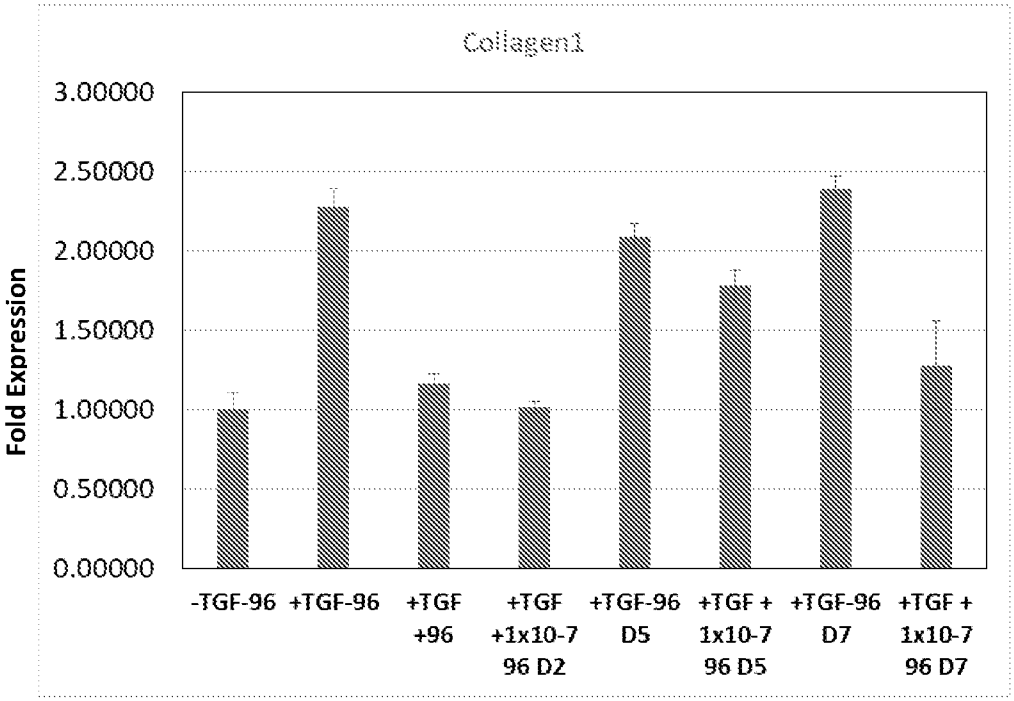


Figure 18

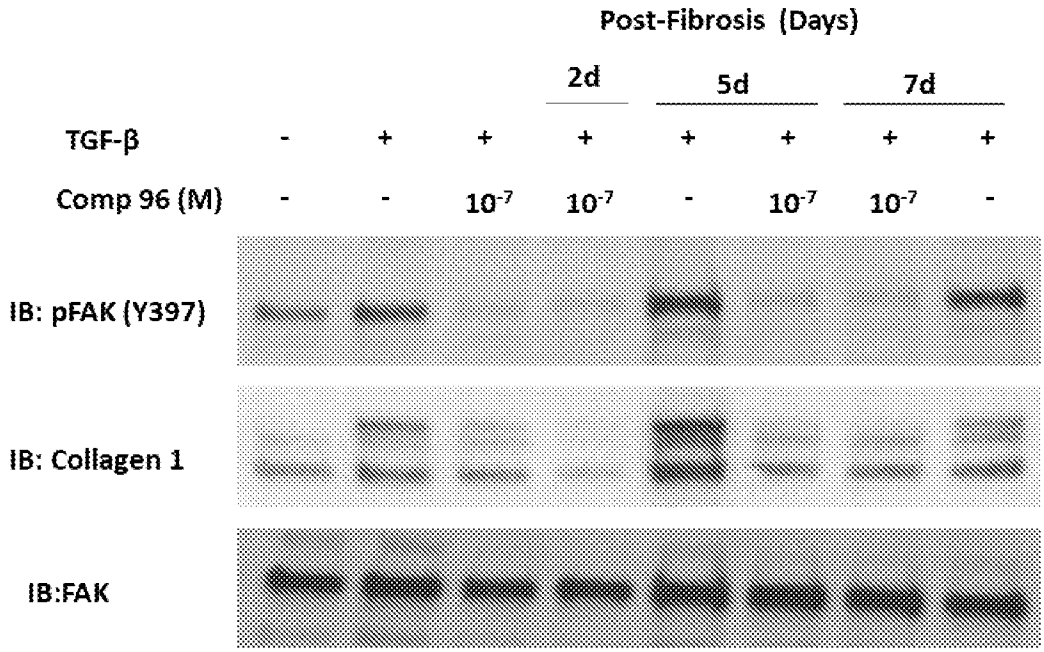


Figure 19

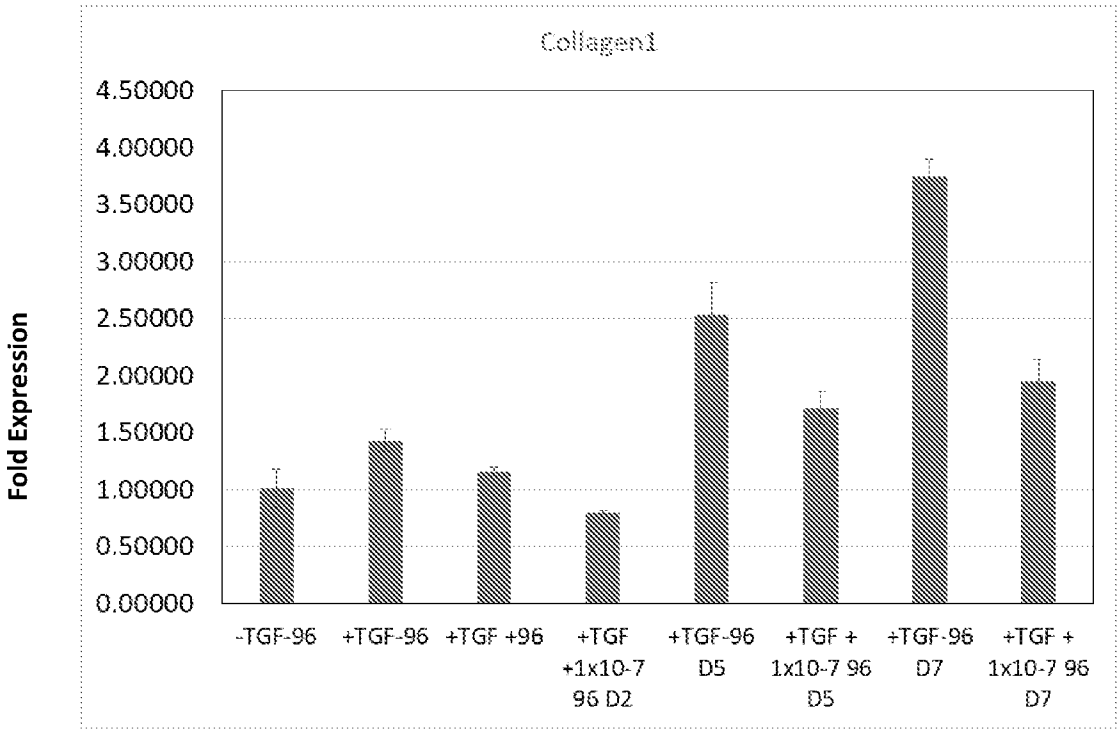


Figure 20

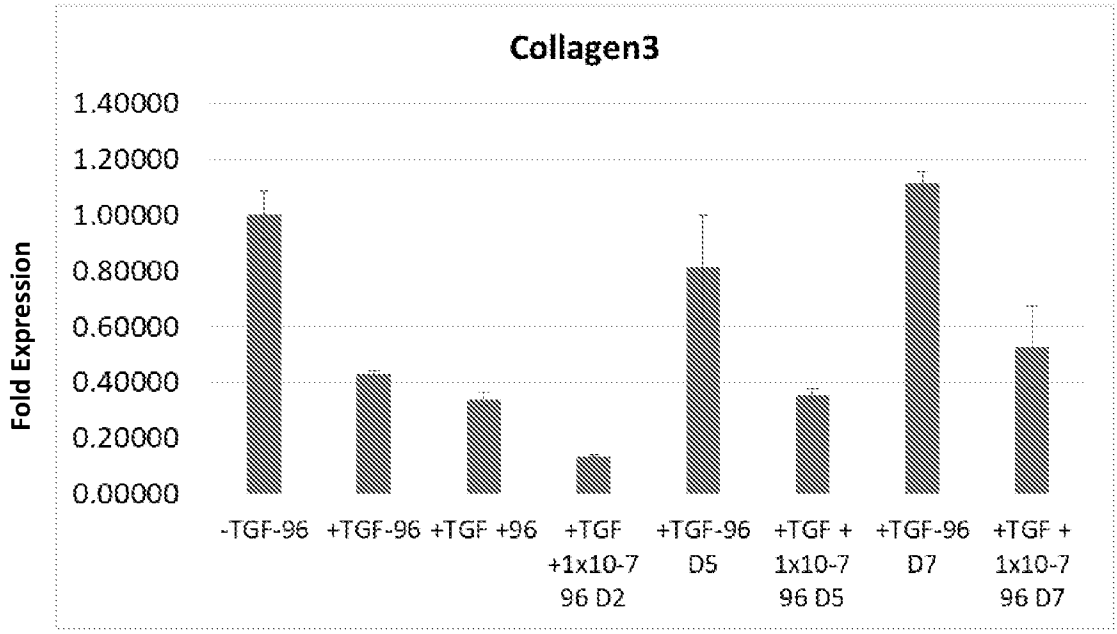

Collagen3
Fold Expression
1.40000
1.20000
1.00000
0.80000
0.60000
0.40000
0.20000
0.00000
-TGF-96
+TGF-96
+TGF +96
+TGF +1x10-7 96 D2
+TGF-96 D5
+TGF + 1x10-7 96 D5
+TGF-96 D7
+TGF + 1x10-7 96 D7

Figure 21

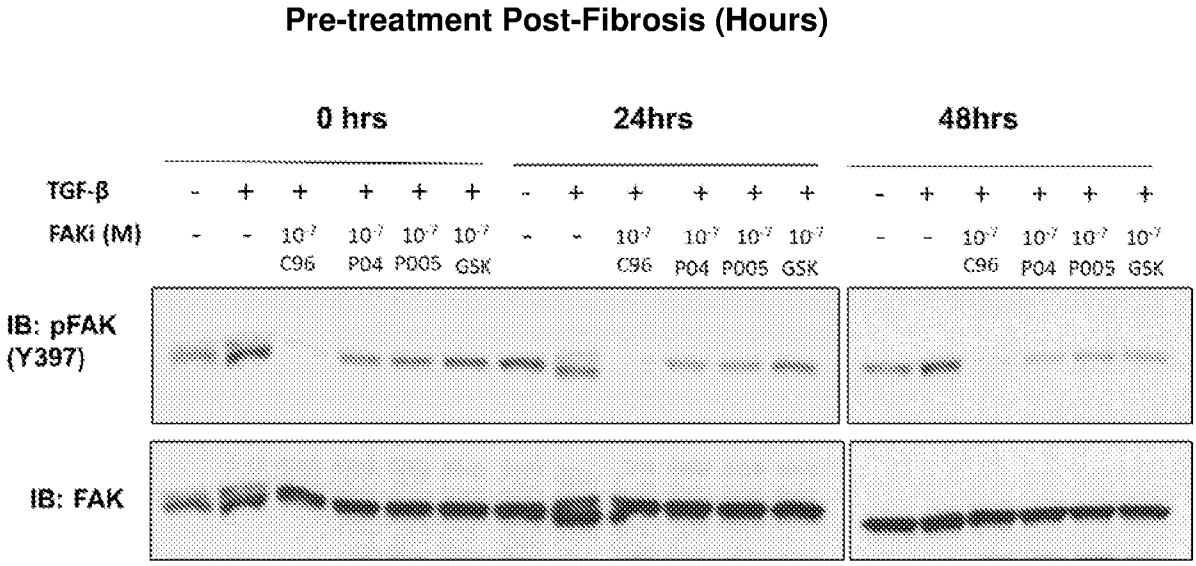


Figure 22

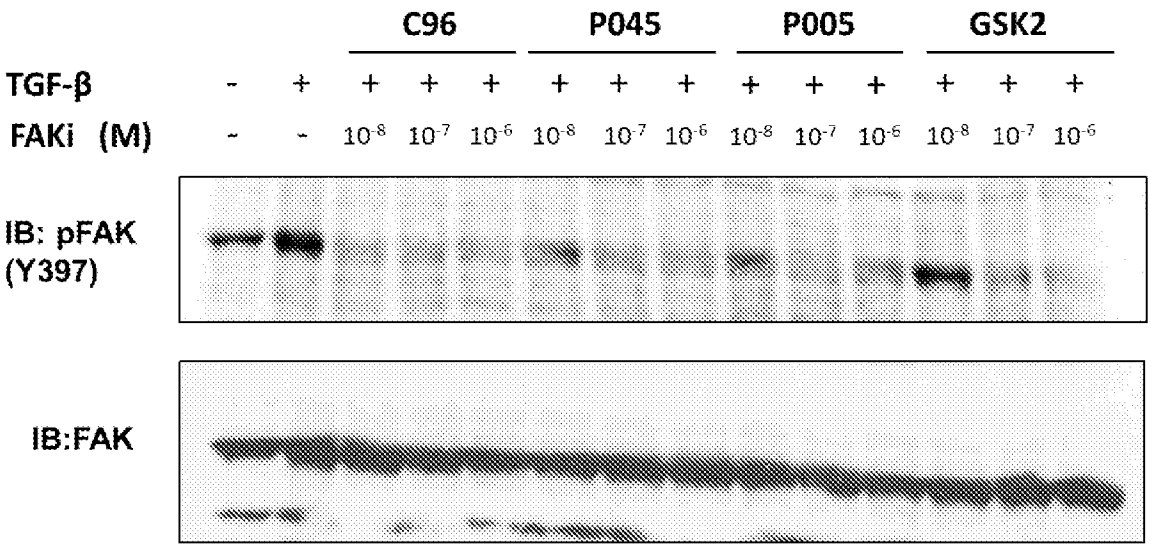


Figure 23

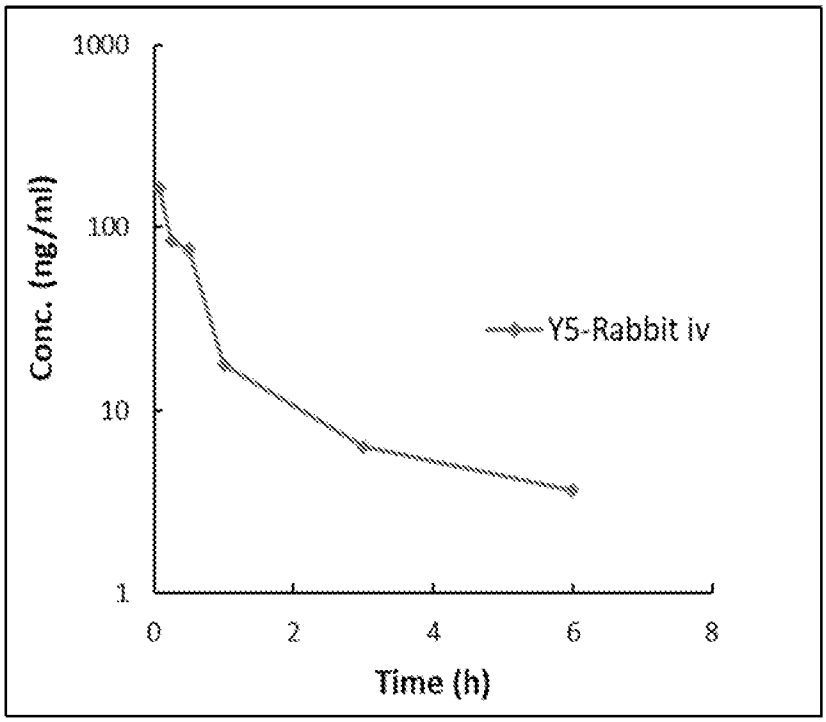


Figure 24

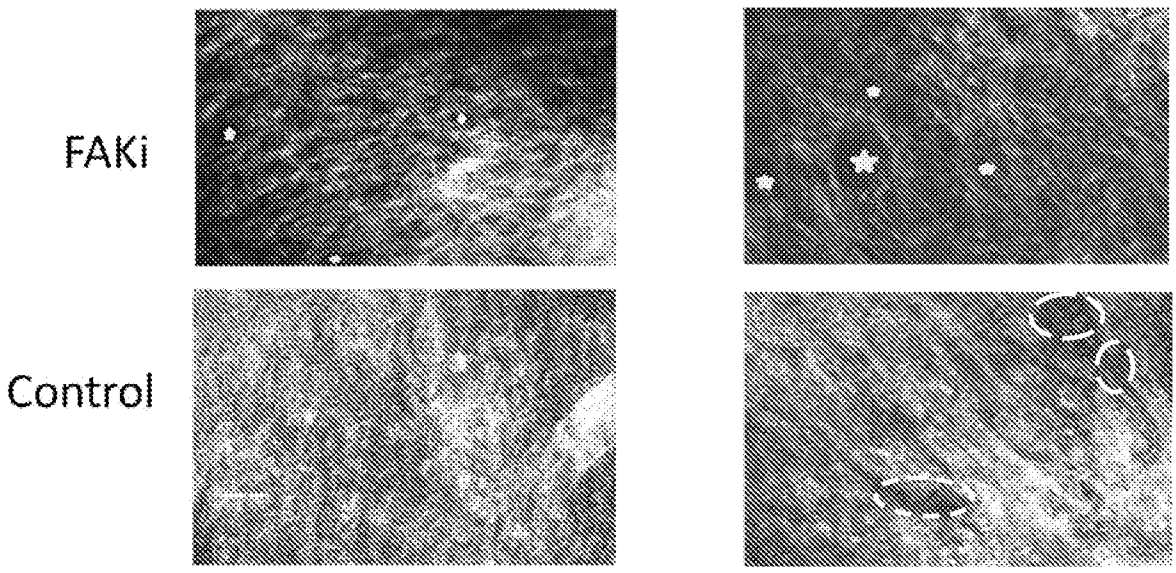


Figure 25

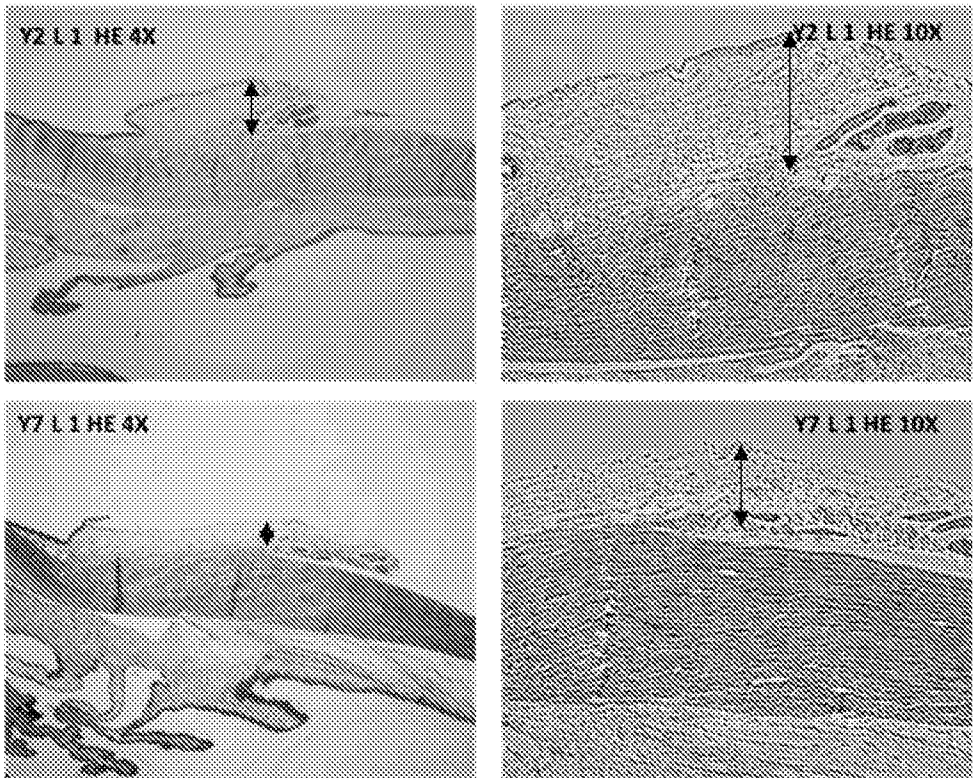
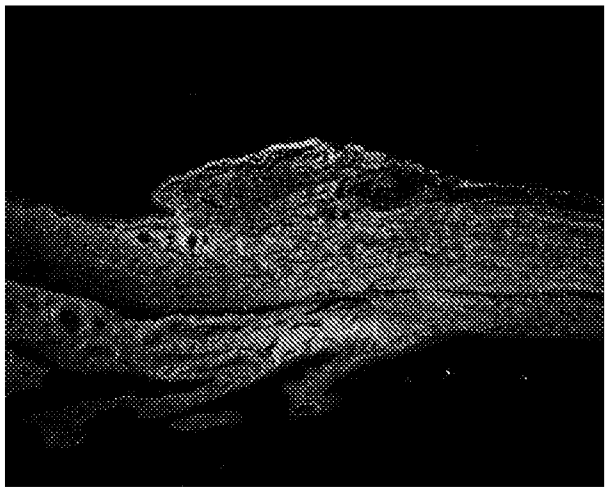
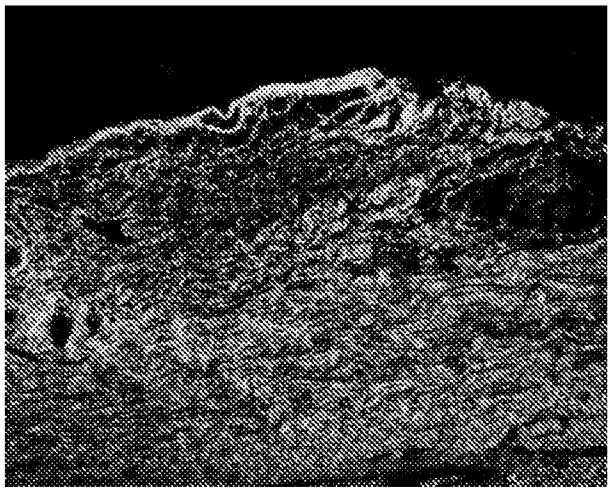


Figure 26

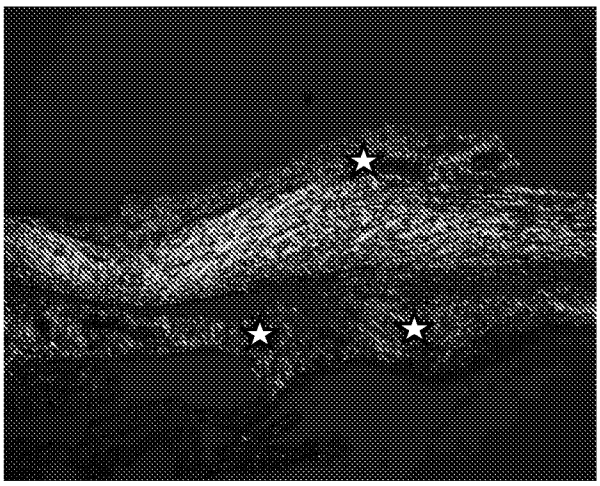
Y2 L 2 PSR 4X



Y2 L 2 PSR 10X



Y7 L 8 PSR 4X



Y7 L 8 PSR 10X

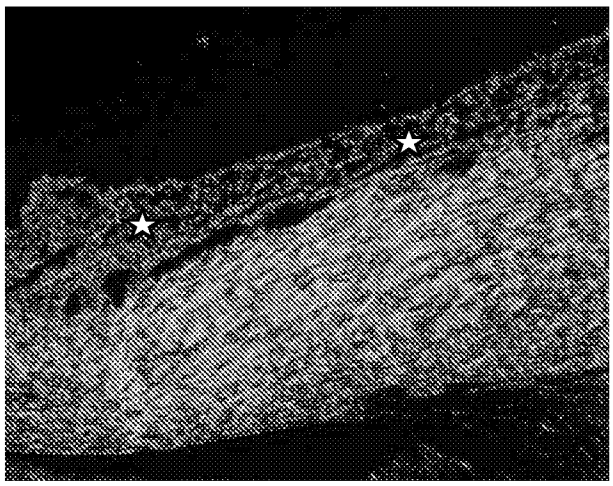


Figure 27

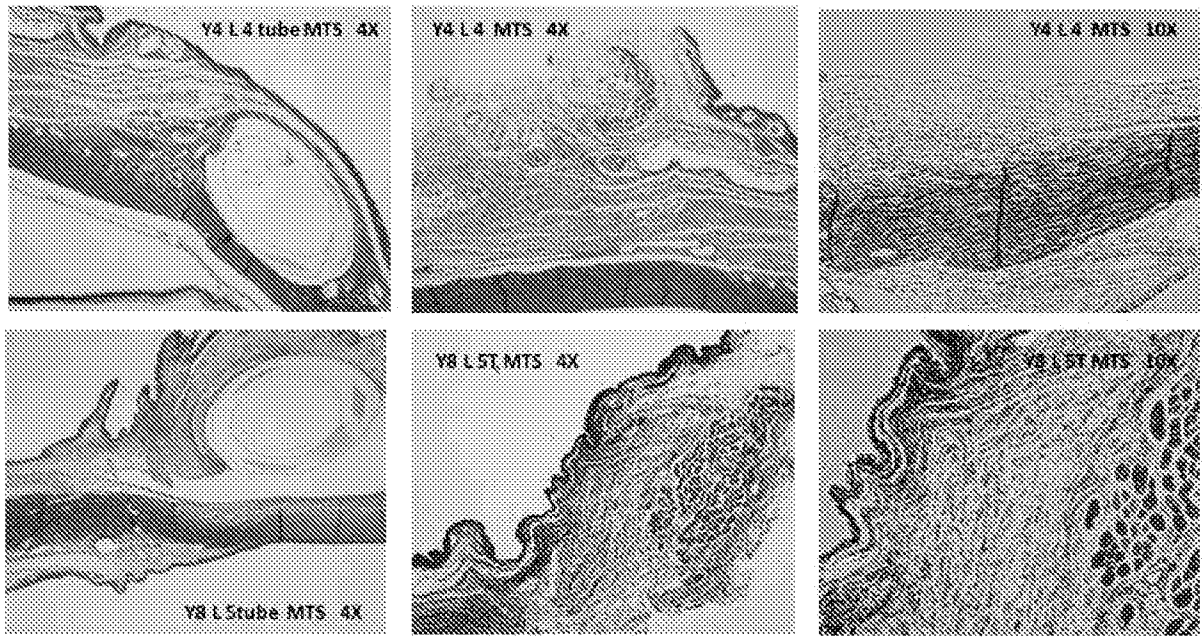


Figure 28

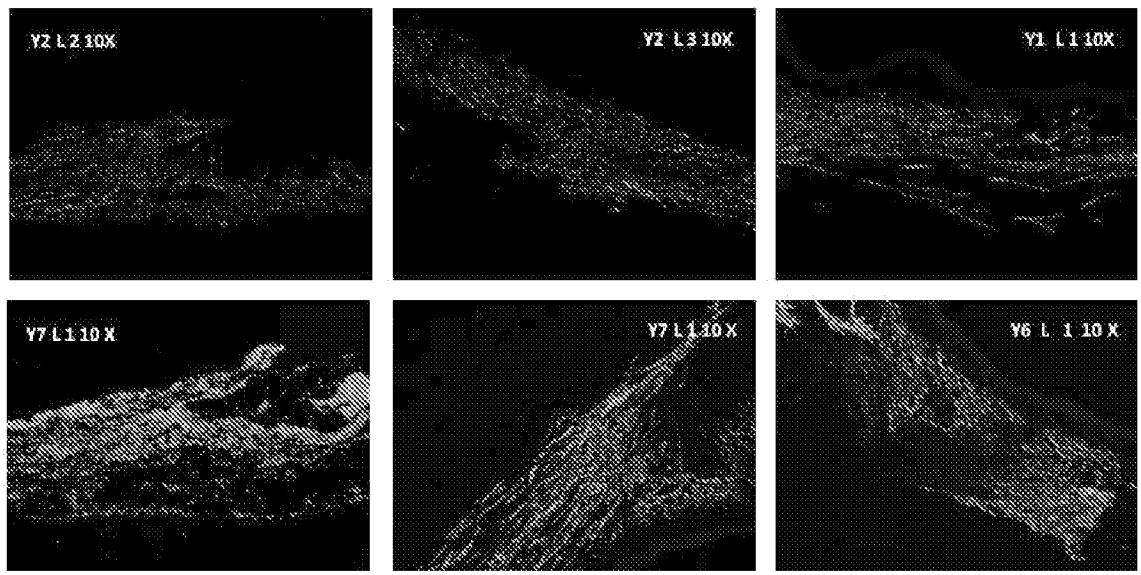


Figure 29

5

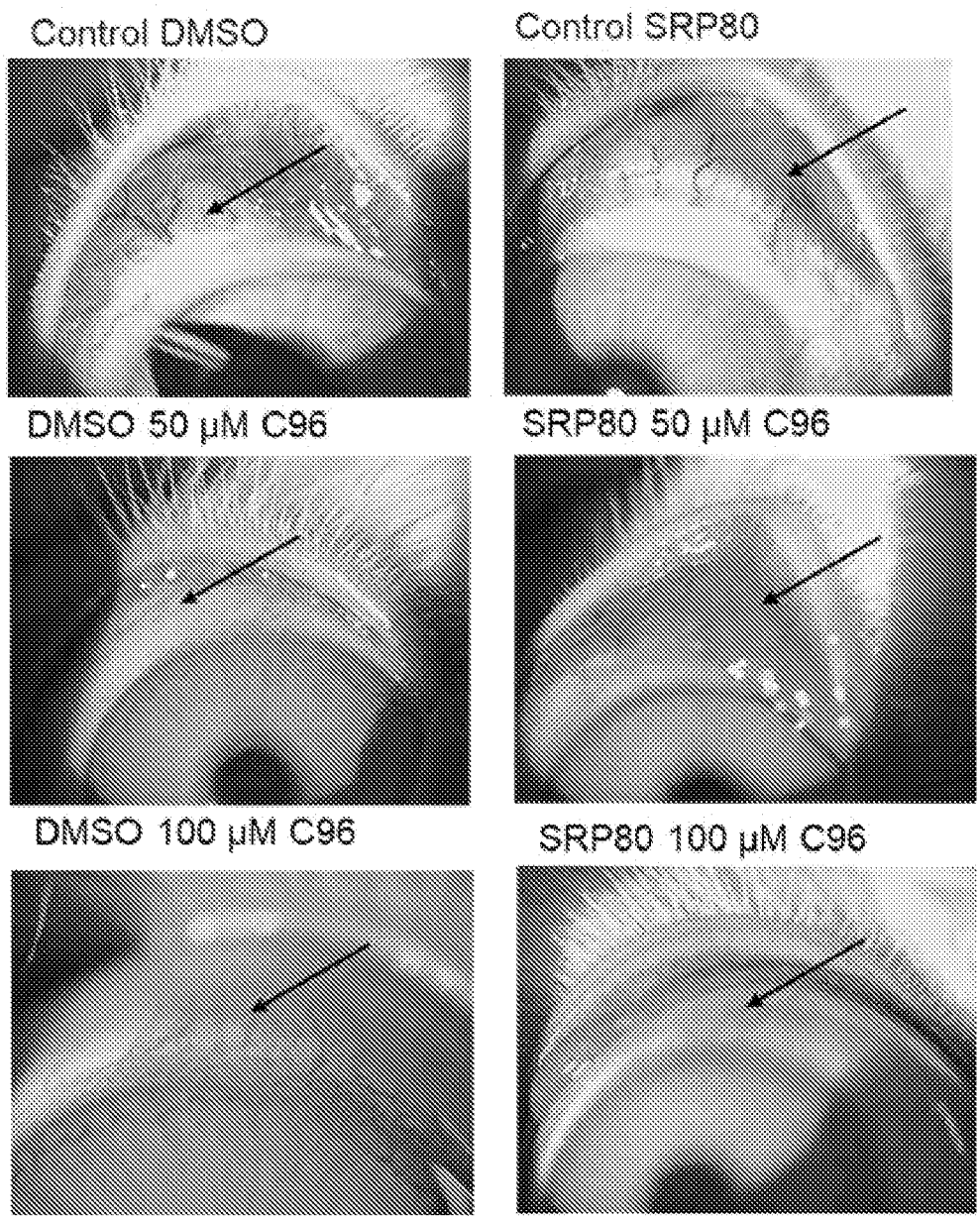

Control DMSO
Control SRP80
DMSO 50 µM C96
SRP80 50 µM C96
DMSO 100 µM C96
SRP80 100 µM C96

Figure 30

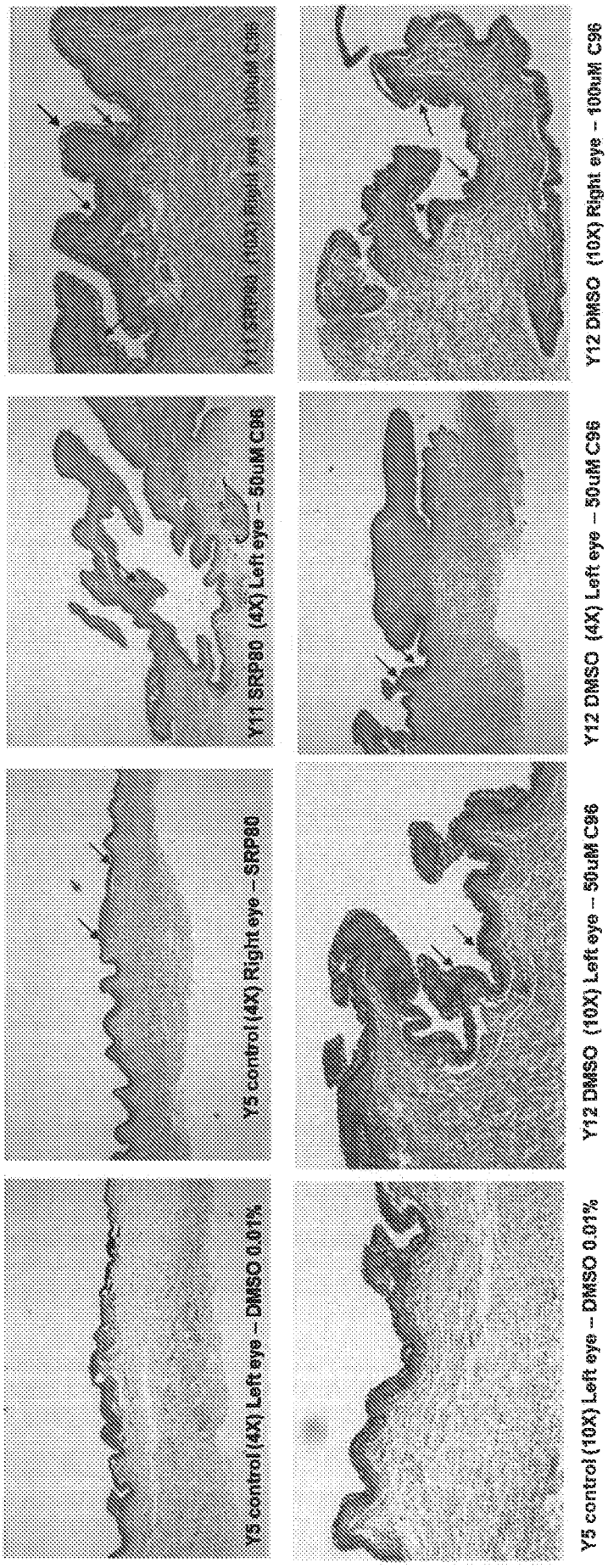

Y5 control (4X) Left eye – DMSO 0.01%
Y5 control (4X) Right eye – SRP80
Y11 SRP80 (4X) Left eye – 50uM C96
Y12 DMSO (4X) Left eye – 50uM C96
Y5 control (10X) Left eye – DMSO 0.01%
Y12 DMSO (10X) Left eye – 50uM C96
Y12 DMSO (10X) Right eye – 100uM C96

Figure 31

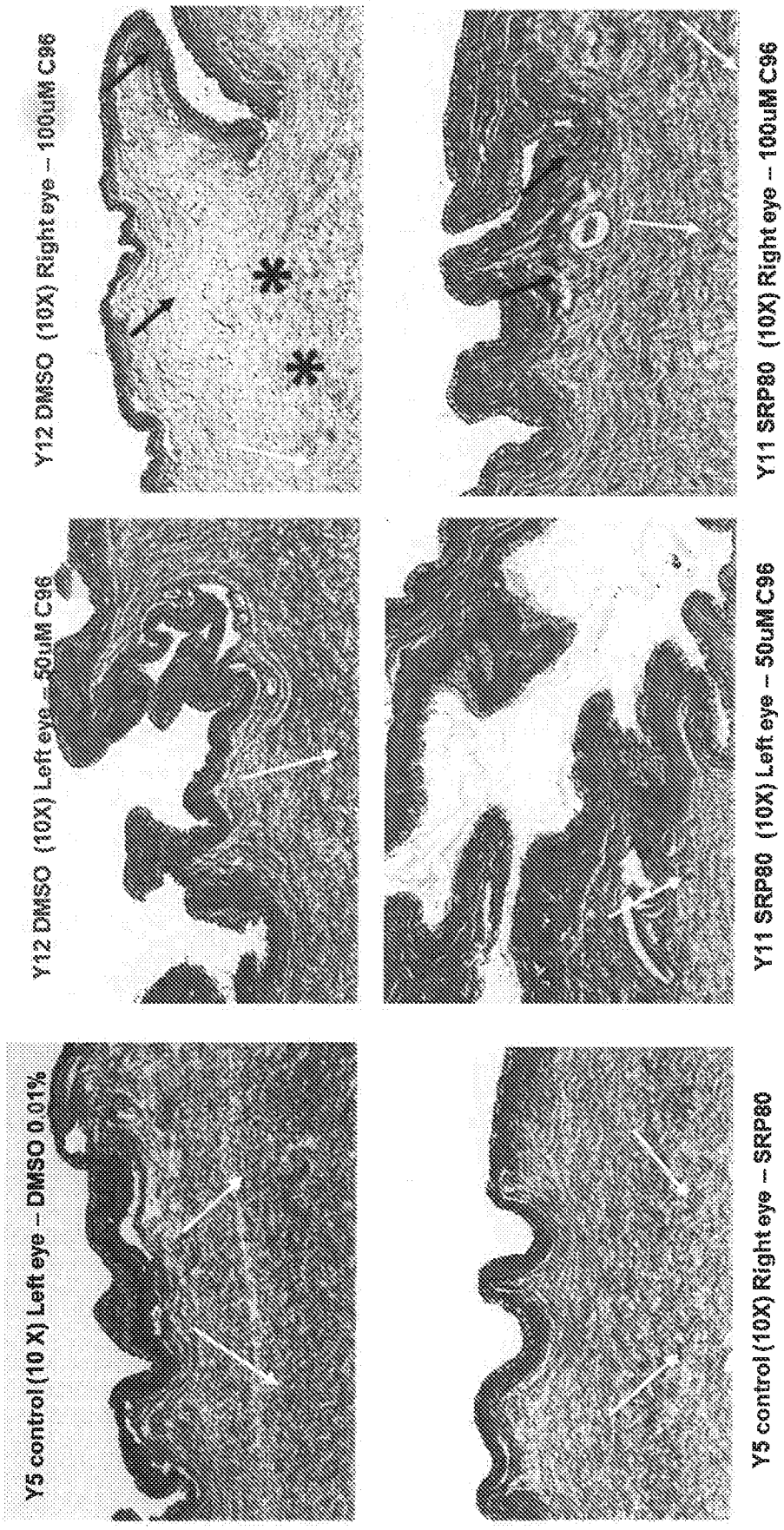

Y5 control (10 X) Left eye – DMSO 0.01%
Y12 DMSO (10X) Left eye – 50uM C96
Y12 DMSO (10X) Right eye – 100uM C96
Y5 control (10X) Right eye – SRP80
Y11 SRP80 (10X) Left eye – 50uM C96
Y11 SRP80 (10X) Right eye – 100uM C96

Figure 32

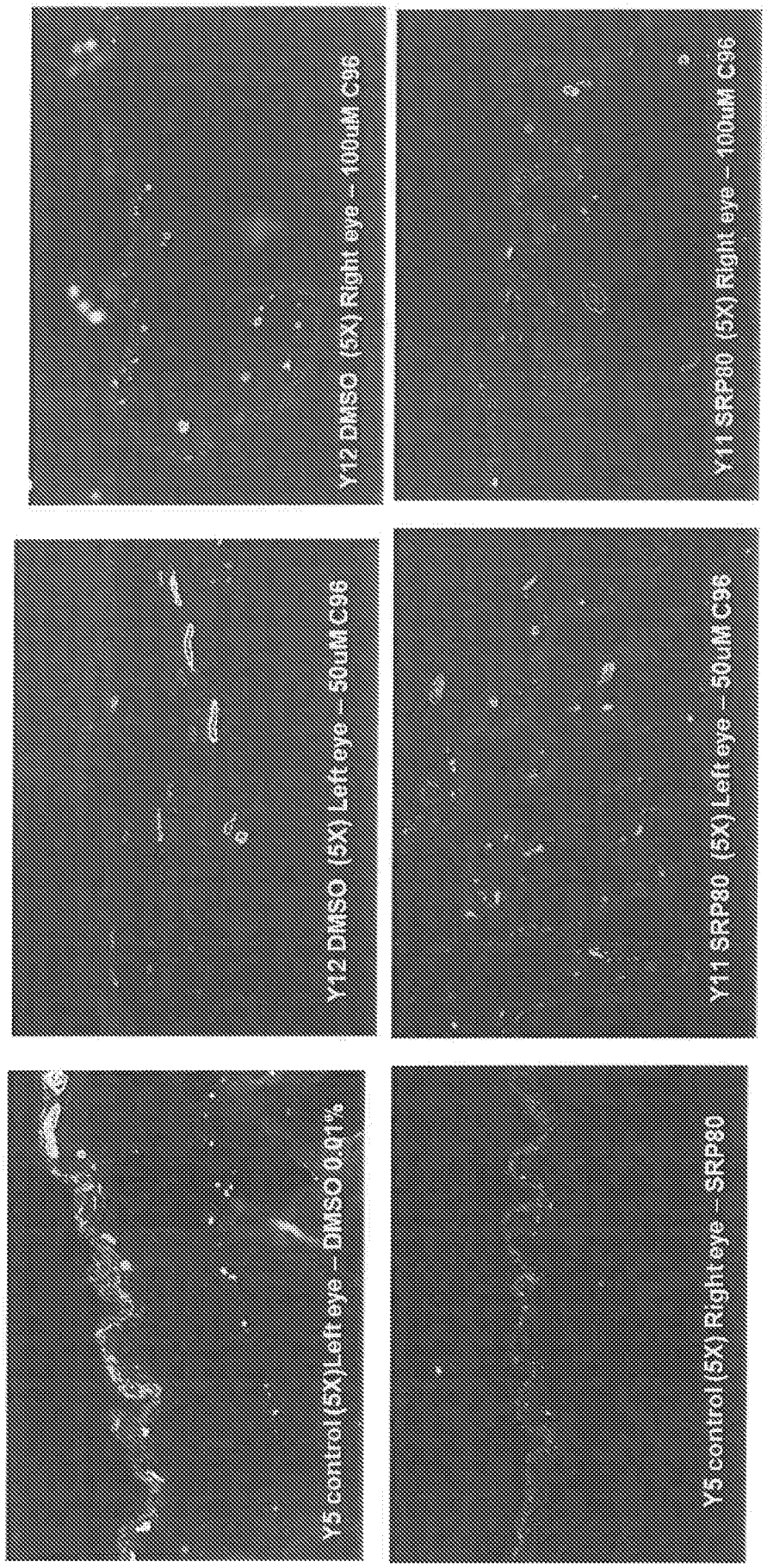

Y5 control (5X) Left eye – DMSO 0.01%
Y5 control (5X) Right eye – SRP80
Y12 DMSO (5X) Left eye – 50uM C96
Y11 SRP80 (5X) Left eye – 50uM C96
Y12 DMSO (5X) Right eye – 100uM C96
Y11 SRP80 (5X) Right eye – 100uM C96

Figure 33

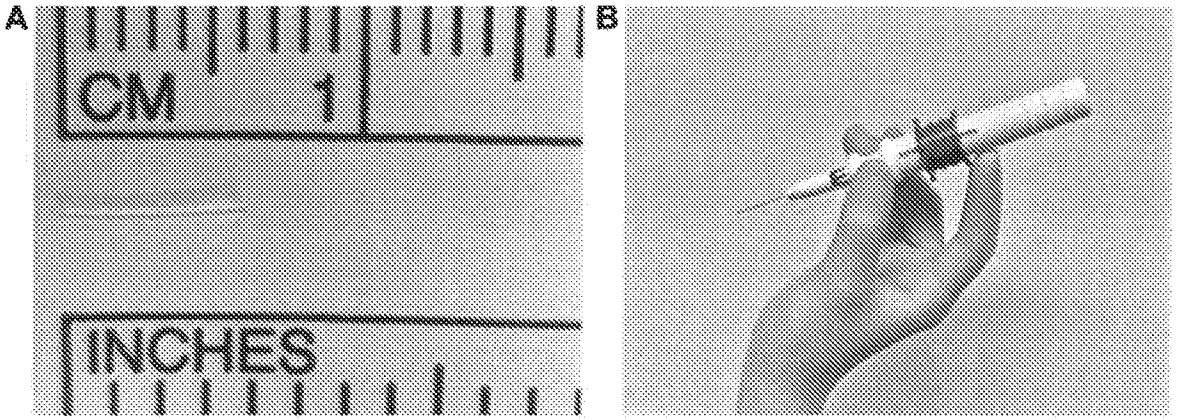


Figure 34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050612

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/496 (2006.01) ***A61K 31/4439 (2006.01)*** ***A61P 27/02 (2006.01)***

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61P , A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

FAMPAT, CAPLUS, REGISTRY, EMBASE, BIOSIS, MEDLINE, PubChem: structure search, 1073154-85-4, 939791-38-5, 1224887-10-8, 1253952-02-1, 717907-75-0, 1061353-68-1, 761437-28-9, FAK, PTK2, eye, retina, cornea, ophthalmic, fibrosis, scarring and related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TSUKAHARA, R. ET AL., Focal adhesion kinase family is involved in matrix contraction by transdifferentiated Müller cells. *Experimental Eye Research*, 15 August 2017, Vol. 164, pages 90-94 [Retrieved on 2019-02-15] <DOI: 10.1016/J.EXER.2017.08.010> abstract, page 91 Section 3.2, page 93 left col 1st para, right col 2nd para	1-26
X	NAKAMURA, K. WT AL., Inhibitor of FAK blocks activation of corneal fibroblasts induced by TGFb. *IOVS*, 1 May 2005, Vol. 46, page 2142 [Retrieved on 2019-02-15] <DOI: NOT AVAILABLE> abstract	1-26
X	US 2013/0158005 A1 (HEINRICH, T. ET AL.) 20 June 2013 [0024], [0139]-[0140], [0153]-[0154], [0170], [0279]	1, 5-11

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15/02/2019 *(day/month/year)*	*26/02/2019* *(day/month/year)*

Name and mailing address of the ISA/SG

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050612

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AOPT 2015- Association for Ocular Pharmacology and Therapeutics, Poster title: Effects of Rho kinase inhibitor AR-13324 on the actin cytoskeleton and on TGFβ2- and CTGF-induced fibrogenic activity in Human Trabecular Meshwork Cells. 26 February 2015 [Retrieved on 2019-02-15 from https://www.aopt.org/twelfth-meeting] Poster abstract, page 130	1, 5, 7-11
X	US 8394826 B2 (DELONG, M. A. ET AL.) 12 March 2013 col 3 2nd para, col 47 compound E146, col 113 examples 457	1, 3, 5, 7-11
X A	US 7741336 B2 (KATH, J. C. ET AL.) 22 June 2010 col 40 line 34-36, col 50 line 12-14, col 65 line 16	1, 3, 5-11 2, 4, 12-26
X A	WO 2010/141406 A2 (OSI PHARMACEUTICALS, INC.) 9 December 2010 example 96	1-5, 7-11 6, 12-26
X A	US 7893074 B2 (GARCIA-ECHEVERRIA, C. ET AL.) 22 February 2011 col 83 line 50-60	1, 3, 5-11 2, 4, 12-26
X A	US 8440822 B2 (LUZZIO, M. J. ET AL.) 14 May 2013 col 55 line 31, example 15A	1, 3, 5-11 2, 4, 12-26
X A	US 9505719 B2 (LEI, Y. ET AL.) 29 November 2016 col 1 line 25-40	1, 3, 5-11 2, 4, 12-26
X A	WO 2010/062578 A1 (GLAXOSMITHKLINE LLC) 3 June 2010 example 41a	1, 3, 5-11 2, 4, 12-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2018/050612

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   Although a sequence listing has been filed or furnished, it was not used for the purposes of this search.

   Since only one version or copy of a sequence listing has been filed or furnished, the statements under item 2 are not required.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG2018/050612

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		RS 54882 B1	31/10/2016
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG2018/050612

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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