SUPPLEMENTARY INFORMATION

Development of potent, selective SRPK1 inhibitors as potential topical therapeutics for neovascular eye disease

*Jennifer Batson[a,b], *Hamish D. Toop[a,c], *Clara Redondo[d], *Roya Babaebi-Jadidi[b], Apirat Chaikuad[d,g], Stephen F. Wearmouth[c], Brian Gibbons[a], Claire Allen[b], Cynthia Tallant[d], Jingxue Zhang[c], Chunyun Du[e], Jules Hancox[e], Tom Hawtrey[c], Joana Da Rocha[c], Renate Griffith[f], Stefan Knapp[d,g], David O Bates[a,b+], Jonathan C. Morris[c+]

TABLE OF CONTENTS

SUPPLEMENTARY METHODS

1. Chemistry

General Experimental

Melting points were obtained on OptiMelt Automated Melting Point System with Digital Image Processing Technology and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded at the Nuclear Magnetic Resonance Facility within the Mark Wainwright Analytical Centre at The University of New South Wales on a Bruker Avance III 300 (300 MHz), Bruker DPX 300 (300 MHz), Bruker Avance III 400 (400 MHz), Bruker Avance III 500 (500 MHz) or Bruker Avance III 600 (600 MHz), with data acquired and processed using TopSpin 3.0 software. Chemical shifts are expressed in parts per million (ppm) on the δ scale. Chemical shifts in $CDCl_3$ were referenced relative to C*H*Cl_3 (7.26 ppm) for ^{1}H NMR and *C*HCl_3 (77.16 ppm) for ^{13}C NMR.[1] Infrared

spectra were obtained on a ThermoNicolet Avatar 370 FT–IR spectrometer and are reported in wavenumbers (cm^{-1}). Spectra were recorded from thin films using NaCl plates. HRMS were performed at the Bioanalytical Mass Spectrometry Facility within the Mark Wainwright Analytical Centre at The University of New South Wales on an Orbitrap LTQ XL (Thermo Fisher Scientific, San Jose, Ca, USA) ion trap mass spectrometer using a nanospray (nano-electrospray) ionization source to generate ions from the analyte in solution. The instrument was calibrated with a standard calibration solution (as outlined in the instrument manual) on the day of analysis using direct infusion with the nanospray source. The instrument conditions were optimized for sensitivity on each compound of interest using LC tune software. The analysis was carried out in positive ion mode using the orbitrap FTMS analyser at a resolution of 100000. Samples, 5 μL, (1 μg/mL in methanol or acetonitrile), were injected into a glass needle and inserted into the nanospray source. Ions generated were measured over the mass range 150 to 2000. Data was acquired in full scan mode over 60 seconds. Data was analyzed using the Qual Browser feature in Xcaliber 2.1 (Thermo Fisher Scientific, San Jose, Ca, USA). LCMS was routinely performed using a Shimadzu Prominence High Performance LCMS 2010EV (Phenomenex C18 column, 5 μm, 150 × 2 mm) connected to an autosampler, photo diode array (PDA) lamp detection (190-600 nm) and Shimadzu LCMS 2010EV mass spectrometer. The mobile phase consisted of MilliQ water with 0.1 % (v/v) TFA (Solvent A) and HPLC grade acetonitrile with 0.1 % (v/v) TFA (Solvent B) at a flow rate of 0.2 mL/min using the gradient in the following table.

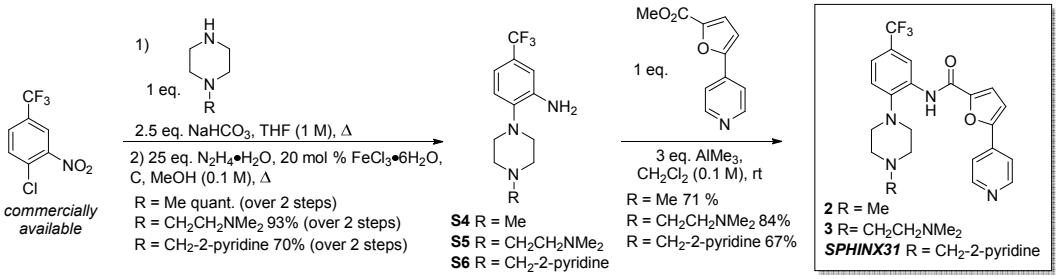
Time (min)	Solvent A (%)	Solvent B (%)
0	95	5
2	95	5
20	0	100
22	0	100
22	95	5
30	95	5

Unless otherwise stated all reactions were performed in flame dried glassware under an atmosphere of dry argon. Reaction temperatures refer to the external bath temperature. Concentration of solvents was performed under reduced pressure on a rotary evaporator after which, residual solvent was removed under high vacuum (~0.1 mm/Hg).

Reagents and solvents were purchased from commercial sources and used without further purification, unless stated below. Reagents and solvents used in reactions were purified according to well established procedures.[1,2] In particular, tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone under an inert atmosphere of argon. *N,N*-Dimethylformamide (DMF) was dried sequentially over three batches of 4Å molecular sieves (3 × 24 h), before finally being stored over a fourth batch of 4Å molecular sieves, under argon. To remove residual *N,N*-dimethylamine from DMF, the solvent was evacuated (~0.1 mm/Hg) for at least 30 min prior to use. Methanol was distilled from magnesium and stored over 3Å molecular sieves, under argon. Dichloromethane was distilled from calcium hydride and stored over 4Å molecular sieves. Petroleum spirit used for chromatography was of the fraction 40-60°C.

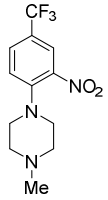
Analytical thin layer chromatography was conducted on Merck, aluminium-backed silica plates 60 F_{254} or silica gel 60 RP-C_{18} F_{254} plates and visualised using UV light and stained with a dip of either a potassium permanganate, vanillin or phosphomolybdic acid. Flash chromatography was routinely performed using Grace Davison Discovery Sciences, Davisil LC60A 40 – 63 micron silica gel, following published guidelines.[3] Solvent was eluted using a Thomson SINGLE StEP pump at the flow rate recommended by the manufacturer (Thomson Instrument Company, Oceanside, Ca, USA). Deactivated silica gel was prepared by washing a column packed with silica gel with neat triethylamine (5 column volumes). After drying, the column was washed with petroleum spirit to remove any residual triethylamine.

Synthesis and Characterisation



General Procedures for the Synthesis of S4, S5 and S6

(1) A solution of piperazine (1 equiv.), benzotrifluoride (1 equiv.) and sodium bicarbonate (2.5 equiv.) in THF (1 M) was heated at reflux for 16 hours. The solution was allowed to cool to room temperature and the reaction solution was filtered through a short pad of Celite, eluting with ethyl acetate. The solvent was removed under reduced pressure to afford the product, which was of sufficient purity to use in the next step. On occasion when the crude product was impure it could be purified by flash chromatography on deactivated silica gel to afford the product.



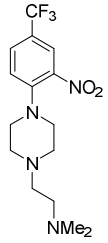
1-Methyl-4-(2-nitro-4-(trifluoromethyl)phenyl)piperazine (S1)[4]

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (s, 3H), 2.56-2.59 (m, 4H), 3.16-3.19 (m, 4H), 7.16 (d, *J* = 8.7 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 8.04 (s, 1H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 46.1, 50.9, 54.6, 120.6, 122.7 (q, $J_{C\text{-}F}$ = 34.5 Hz), 123.6 (q, $J_{C\text{-}F}$ = 270.0 Hz), 124.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 130.2 (q, $J_{C\text{-}F}$ = 3.8 Hz), 140.7, 148.1

IR (NaCl, neat): 1536 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{12}H_{15}F_3N_3O_2$ $(M+H)^+$ 290.1116, found 290.1111.



***N,N*-Dimethyl-2-(4-(2-nitro-4-(trifluoromethyl)phenyl)piperazin-1-yl)ethan-1-amine (S2)**

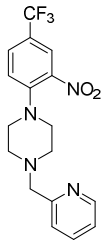
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.29 (s, 6H), 2.47 – 2.52 (m, 2H), 2.56 – 2.61 (m, 2H), 2.63 – 2.66 (m, 4H), 3.17 – 3.20 (m, 4H), 7.15 (d, *J* = 8.7 Hz, 1H), 7.65 (dd, *J* = 8.7, 1.8 Hz, 1H), 8.04 (d, *J* = 1.8 Hz, 1H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 46.0, 50.9, 53.2, 56.6, 56.9, 120.6, 122.2 (q, $J_{C\text{-}F}$ = 33.8 Hz), 123.4 (q, $J_{C\text{-}F}$ = 270 Hz), 124.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 130.2 (q, $J_{C\text{-}F}$ = 3.8 Hz), 140.7, 148.1

IR (NaCl, neat): 1537 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{15}H_{22}F_3N_4O_2$ (M^++H) 347.1695, found 347.1689

1-(2-Nitro-4-(trifluoromethyl)phenyl)-4-(pyridin-2-ylmethyl)piperazine (S3)



^{1}H NMR (500 MHz, $CDCl_3$) δ 2.67 – 2.69 (m, 4H), 3.20 – 3.22 (m, 4H), 3.73 (s, 2H), 7.15 (d, *J* = 8.8 Hz, 1H), 7.17 – 7.20 (m, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.63 – 7.68 (m, 2H), 8.03 (br s, 1H), 8.59 (d, *J* = 4.8 Hz, 1H)

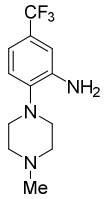
^{13}C NMR (75 MHz, $CDCl_3$) δ 50.9, 52.9, 64.5, 120.6, 122.1 (q, $J_{C\text{-}F}$ = 34.7 Hz), 122.4, 123.4 (q, $J_{C\text{-}F}$ = 270.8 Hz), 123.5, 124.3 (q, $J_{C\text{-}F}$ = 3.9 Hz), 130.2 (q, $J_{C\text{-}F}$ = 3.5 Hz), 136.6, 140.6, 148.1, 149.6, 158.0

IR (NaCl, neat): 1625 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{17}H_{17}F_3N_4O_2Na$ (M^++Na) 389.1201, found 389.1185

(2) Hydrazine hydrate (25 equiv.) was added dropwise to a suspension of nitrocompound **S1**, **S2** or **S3** (1 equiv.), iron(III) chloride hexahydrate (20 mol %) and activated carbon (20 % wt./wt.) in methanol (0.1 M) at room temperature. The solution was heated at reflux for two hours. The solution was allowed to cool to room temperature then filtered through a short pad of Celite, eluting with ethyl acetate. The solvent was removed under reduced pressure. The residue was diluted with water and extracted with ethyl acetate (× 3). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the product as a white solid, which was of sufficient purity to use in the next step.

2-(4-Methylpiperazin-1-yl)-5-(trifluoromethyl)aniline (S4)[4]



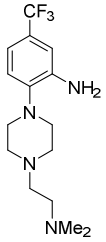
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.52-2.65 (m, 4H), 2.99-3.02 (m, 4H), 4.05 (br s, 2H), 6.93-7.03 (m, 3H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 46.3, 50.7, 55.8, 111.6 (q, $J_{C\text{-}F}$ = 3.8 Hz), 115.5 (q, $J_{C\text{-}F}$ = 3.8 Hz), 119.7, 124.5 (q, $J_{C\text{-}F}$ = 270.1 Hz), 126.1 (q, $J_{C\text{-}F}$ = 33.4 Hz), 141.6, 142.1

IR (NaCl, neat): 3465 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{12}H_{17}F_3N_3$ $(M+H)^+$ 260.1374, found 260.1369

2-(4-(2-(Dimethylamino)ethyl)piperazin-1-yl)-5-(trifluoromethyl)aniline S5



Mp: 107 – 108°C

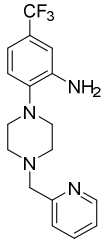
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (s, 1H), 2.46 – 2.49 (m, 2H), 2.53 – 2.58 (m, 2H), 2.64 (br s, 4H), 2.95 – 2.98 (m, 4H), 4.06 (br s, 2H), 6.92 – 7.03 (m, 3H)

^{13}C NMR (75 Hz, $CDCl_3$) δ 46.1, 50.6, 54.3, 56.9, 57.1, 111.5 (q, $J_{C\text{-}F}$ = 3.8 Hz), 115.5 (q, $J_{C\text{-}F}$ = 3.8 Hz), 119.7, 124.5 (q, $J_{C\text{-}F}$ = 270 Hz), 126.5 (q, $J_{C\text{-}F}$ = 33 Hz), 141.64, 142.14

IR (NaCl, neat): 3458 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{15}H_{24}F_3N_4$ (M^++H) 317.1953, found 317.1948

2-(4-(Pyridin-2-ylmethyl)piperazin-1-yl)-5-(trifluoromethyl)aniline S6



Mp 126 – 127°C

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 – 2.69 (m, 4H), 2.97 – 3.00 (m, 4H), 3.74 (s, 2H), 4.07 (br s, 2H), 6.92 – 7.04 (m, 3H), 7.16 – 7.20 (m, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.64 – 7.70 (m, 1H), 8.57 – 8.60 (m, 1H)

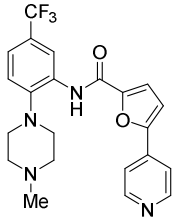
^{13}C NMR (75 MHz, $CDCl_3$) δ 50.7, 54.0, 64.8, 111.6 (q, *J* = 3.9 Hz), 115.5 (q, *J* = 4.1 Hz), 119.7, 122.3, 123.4, 124.6 (q, $J_{C\text{-}F}$ = 271.2 Hz), 126.5 (q, $J_{C\text{-}F}$ = 32.2 Hz), 136.6, 141.7, 142.2, 149.5, 158.5

IR (NaCl, neat): 3187, 3283 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{17}H_{20}F_3N_4$ (M^++Na) 337.1640, found 337.1594

General Procedures for the Synthesis of 2, 3 and SPHINX31

A solution of trimethylaluminium in toluene (2 M, 3 equiv.) was added dropwise to a solution of aniline **S4, S5** or **S6** (1 equiv.) in dichloromethane (0.5 M) at room temperature. The solution was stirred at room temperature for 1 hour after which, a solution of methyl 5-(pyridin-4-yl)furan-2-carboxylate[5] (1 equiv.) in dichloromethane (1 M) was added dropwise at room temperature. The reaction solution stirred at room temperature for an additional 16 hours. To quench the reaction saturated aqueous Rochelle's salt solution was added dropwise at room temperature and the solution allowed to stir at room temperature for a further 15 minutes. The mixture was diluted with saturated aqueous sodium bicarbonate solution and extracted with dichloromethane (× 3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on deactivated silica gel to afford the product.



2

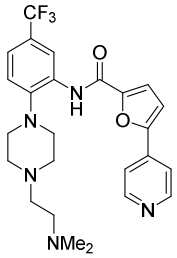
Mp: 146-147°C

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H), 2.66-2.76 (m, 4H), 2.99-3.02 (m, 3H), 7.04 (d, *J* = 3.6 Hz, 1H), 7.29-7.38 (m, 3H), 7.67-7.69 (m, 1H), 8.70-8.72 (m, 2H), 8.85 (s, 1H), 9.63 (br s, 1H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 46.4, 52.2, 56.1, 111.3, 116.5(q, $J_{C\text{-}F}$ = 3.8 Hz), 117.7, 118.5, 121.1, 121.2 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.1 (q, $J_{C\text{-}F}$ = 270.1 Hz), 127.8 (q, $J_{C\text{-}F}$ = 33.4 Hz), 133.4, 136.4, 144.3, 148.5, 150.6, 152.9, 155.5

IR (NaCl, neat): 3306, 1682 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{22}H_{22}F_3N_4O_2$ $(M+H)^+$ 431.1694, found 431.1689.



3

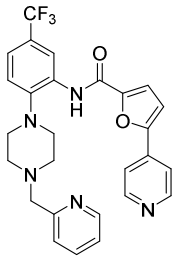
Mp: 143 – 144°C

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 6H), 2.52 – 2.54 (m, 2H), 2.61 – 2.64 (m, 2H), 2.80 (br s, 4H), 3.02 – 3.05 (m, 4H), 7.05 (d, *J* = 3.6 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.38 – 7.40 (m, 2H), 7.69 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.72 (dd, *J* = 4.5, 1.5 Hz, 2H), 8.86 (d, *J* = 1.5 Hz, 1H), 9.65 (br s, 1H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 45.9, 52.2, 54.7, 56.7, 56.9, 111.3, 116.6 (q, $J_{C\text{-}F}$ = 4.5 Hz), 117.7, 118.5, 121.1, 121.2 (q, $J_{C\text{-}F}$ = 4.5 Hz), 124.1 (q, $J_{C\text{-}F}$ = 270 Hz), 127.5 (q, $J_{C\text{-}F}$ = 33 Hz), 33.4, 136.4, 144.4, 148.6, 150.7, 152.9, 155.6

IR (NaCl, neat): 3306, 1673 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{25}H_{29}F_3N_5O_2$ $(M^+ + H)$ 488.2273, found 488.2268



SPHINX31

Mp: 157 – 159°C

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.85 (br s, 4H), 3.04 (br s, 4H), 3.78 (s, 2H), 7.06 (d, *J* = 3.7 Hz, 1H), 7.20 (m, 1H), 7.31 – 7.41 (m, 4H), 7.65 – 7.72 (m, 3H), 8.60 (d, *J* = 4.5 Hz, 1H), 8.80 – 8.87 (m, 3H), 9.65 (br s, 1H)

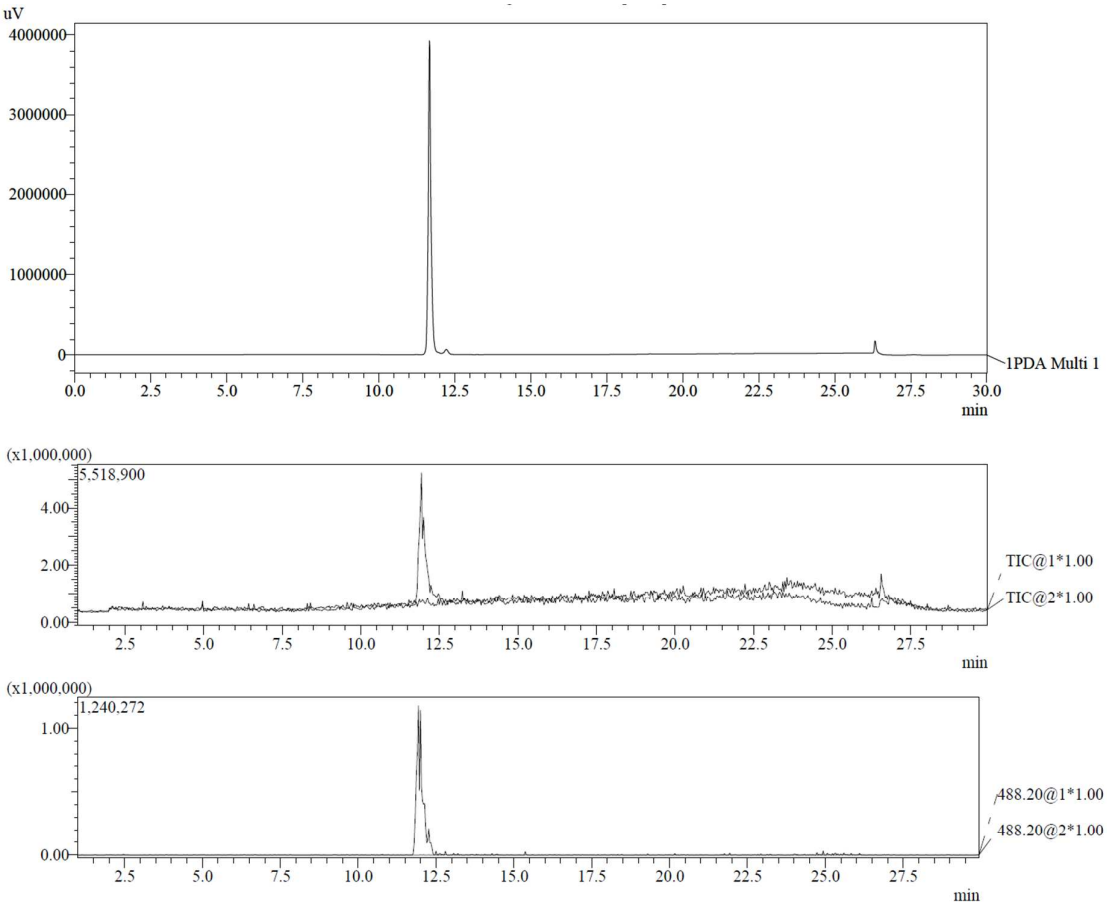
^{13}C NMR (100 MHz, $CDCl_3$) δ 52.2, 54.5, 65.0, 111.3, 116.7 (q, $J_{C\text{-}F}$ = 4.5 Hz), 117.7, 118.5, 121.1, 121.3 (q, $J_{C\text{-}F}$ = 4.5 Hz), 122.5, 123.5, 124.1 (q, $J_{C\text{-}F}$ = 272 Hz), 128.0 (q, $J_{C\text{-}F}$ = 34 Hz), 133.5, 136.7, 148.6, 149.7, 150.8, 153.2, 155.7, 157.9

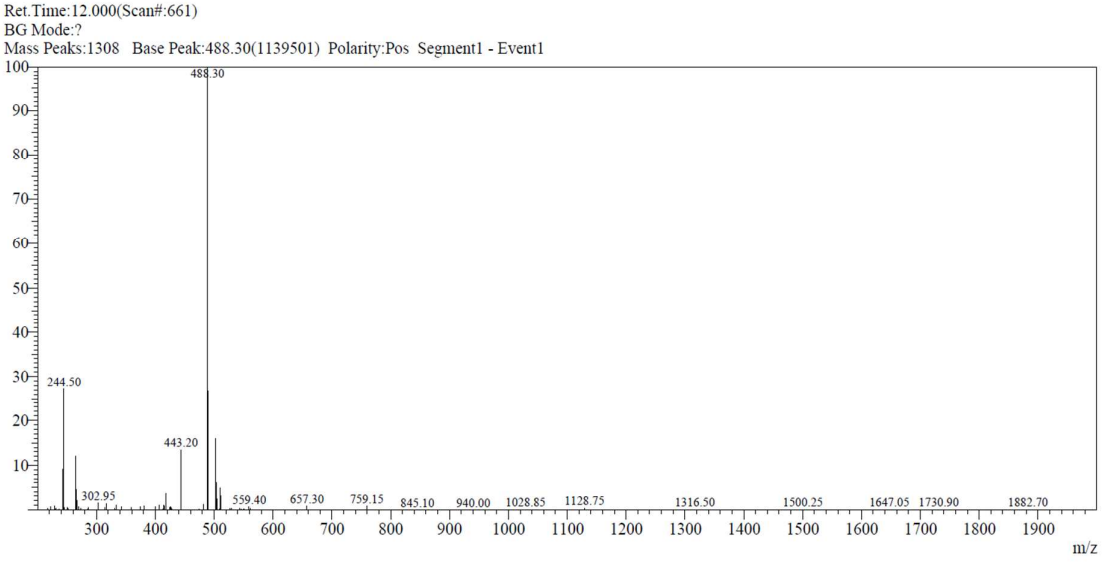
IR (NaCl, neat): 1669, 3332 cm^{-1}

HRMS (ESI-MS): *m/z* calcd for $C_{27}H_{24}F_3N_5O_2$ $(M^+ + H)$ 508.1960, found 508.1932

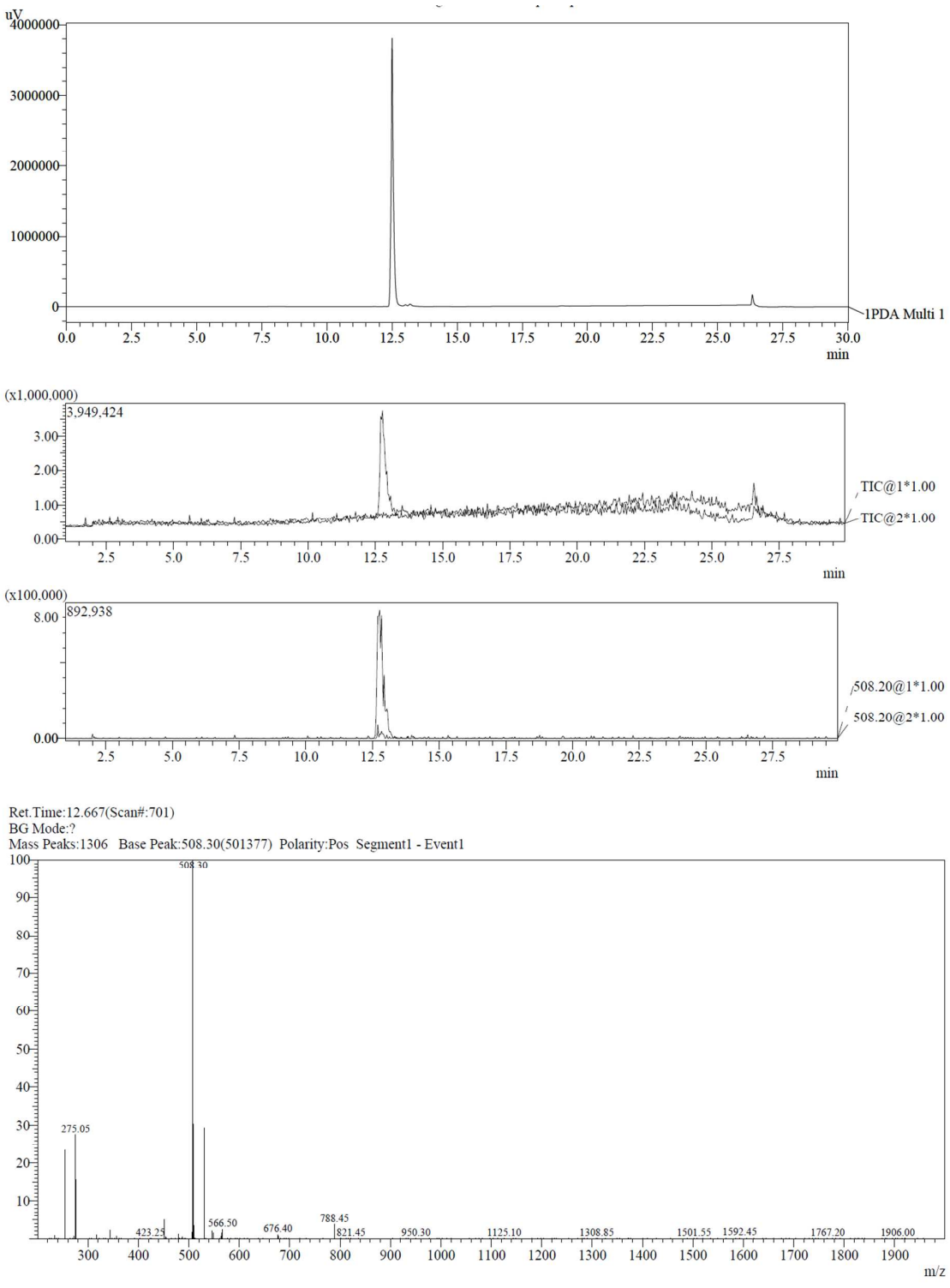
LCMS traces for Compounds 3 and SPHINX31

Compound 3





SPHINX31



Crystallization, Data collection and Structure determination

Two different recombinant SRPK1 proteins (aa 42-655,Δ256-473,I210T or 58-655,Δ256-473) buffer exchanged into 50 mM HEPES, pH 7.5, 300 mM KCl, 0.5 mM TCEP and 5% glycerol were incubated with compound **1** and SPHINX31 at 1 mM concentration, prior to

setting up the sitting-drop crystallization at 4 ˚C. For SRPK1-compound **1** complex, the crystals were obtained using 20% PEG-6000, 13% Ethylene glycol, 0.1M Tris pH 9.5 and 0.1M Calcium chloride, while the co-crystals of SRPK1-SPHINX31 complex grew in 22% broad-molecular-weight PEG smears and 0.1 M citrate, pH 5.5.[6] For SRPK2, the recombinant protein was purified using the similar procedure as that used for purification of SRPK1. The pure SRPK2 protein in 50 mM HEPES, pH 7.5, 500 mM NaCl and 5% glycerol was mixed with 1 mM compound **1**, and the complex was crystallized at 4 ˚C using the reservoir solution containing 1 M ammonium sulfate and 0.1 M acetate, pH 5.25. All crystals were cryo-protected with the mother liquor supplemented with 20% ethylene glycol before flash-frozen in liquid nitrogen. Diffraction data collected at Diamond Light Source using X-ray at 0.9795 Å wavelength were processed and scaled with XDS[7] and Scala[8] or Aimless[9], respectively. Initial structure solutions were obtained with molecular replacement method using Phaser and the coordinates of SRPK1 (pdb id: 1WAK)[10] or SRPK2 (pdb id: 2X7G). Iterative cycles of model building alternated with structure refinement were performed in COOT[11] and REFMAC[12], respectively. The geometry of the final model was verified by MOLPROBITY.[13] Data collection and refinement statistics are summarized in Supplementary Table 2.

Coordinates and accession codes:

Coordinates have been deposited with the Protein Data Bank with the designation 'for immediate release upon publication'. The Accession Codes are as follows:

SRPK1.**1** pdb id = 5MXX

SRPK1.SPHINX31 pdb id = 5MY8

SRPK2.**1** pdb id = 5MYV

2. Biology

In Vitro Kinase Screening Assay

Candidate compounds were tested for SRPK1 inhibition using a Kinase-Glo assay (Promega, Southampton, UK32). A reaction buffer containing 200 mM Tris pH 7.5, 100 mM $MgCl_2$ and 0.1 mg/ml BSA was added to 43 μM SRSF1Arg-Ser (RS) peptide (NH_2-RSPSYGRSRSRSRSRSRSRSRSNSRSRSY-OH) and 0.1 μg of purified SRPK1 (ProQinase, Freiburg, Germany). Candidate compounds were serially diluted from 10 μM to 0.001 nM and added to the reaction mixture, wells with omitted SRPK1 kinase and omitted compounds were also added as controls. All wells contained 1% DMSO (Fisher Scientific, Loughborough, UK). 1 μM ATP was added, wells minus ATP were used as

background controls. The plate was then incubated at 30°C for 10 minutes. An equal volume of Kinase-Glo (25 μL; Promega) was added to each well and the plate read for luminescence using a Fluostar Optima (BMG Labtech). Radioactive kinase assays were carried out by the MRC Dundee Kinase Centre. Kinase binding assay was carried out by Kinomescan, Discoverex, at 1 μM. Lack of interference with binding to the SRPK1 substrate was carried out using a dose response curve from 0.5-30 μM.

Protein studies – immunoblotting and immunoprecipitation.

Whole cell lysate (nuclear and cytoplasmic) protein extracts were prepared as described.[14] The extracts were then immunoblotted using either mouse anti-SRPK1 (anti-SRPK1; BD 611072; 1:1000), rabbit anti-panVEGF (Santa Cruz A20 sc-152; 1:500), mouse anti-$VEGF_{xxx}b$ (MAB3045; R&D; 1:500), rabbit anti-VEGF-A_x (a kind gift from Paul Fox, Cleveland Clinic Lerner Institute, goat anti-SRSF1 (SC10255; 1:500), mouse anti-SRSF1 (AK96) (Santa Cruz SC-33562), rabbit anti-SRSF1 (Abcam, Ab-129108 1:2000), goat-anti actin (Santa Cruz SC-1615 1:500) or rabbit anti-GAPDH (Sigma G9545, 1:2000). For immunoprecipitation phospho-SRSF1 studies, cell lysates were pre-cleared by incubation with Protein A Dynabeads (Invitrogen) for 1 h at 4 ºC then incubated with a mix of 1 μg mouse anti-SRSF1 (Santa Cruz SC-33562) or IgG control (Mouse IgG2b kappa monoclonal (MPC-11) isotype control Ab18457 Abcam) and Protein A Dynabeads (Invitrogen). To detect phosphorylated SRSF1, the eluent was immunoblotted with either anti-SRSF1 or the anti-Pan-phospho-SR antibody (Millipore MABE50 clone 1H4 1:500).

HERG assay

Whole cell patch clamp recordings of hERG current (I_{hERG}) were made at 37°C from human embryonic kidney cells (HEK 293) cells stably expressing hERG (generously donated by Dr Craig January).[15] Cells were maintained in culture and prepared for electrophysiological recording as described previously.[16,17] Cells were superfused with a normal Tyrode's solution containing (in mM) 140 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Glucose and 5 HEPES (titrated to pH 7.45 with NaOH). Patch-pipettes were filled with a K^+-based dialysate containing (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP and 10 HEPES (titrated to pH 7.2 with KOH). Pipette resistance typically ranged from 1.5–3.5 MΩ and ~70-80% series resistance could be compensated. Data were acquired using an Axopatch 1D amplifier and a CV-4 1/100 headstage and digitised using a Digidata 1200B interface (Axon Instruments, USA). Voltage commands were generated and data acquired using Clampex 8 (Axon Instruments, USA) and data were stored on the hard-disk of a

Viglen computer for off-line analysis. Data were digitized at 10kHz with an appropriate bandwidth set on the amplifier. Compounds were applied using a home-built rapid solution exchange device capable of changing superfusate in <1 second. Large scale screening was undertaken by Essen Biosciences.

T_m-Shift assay

Thermal melting experiments were carried out using a Stratagene Mx3005p Real Time PCR machine (Agilent Technologies). Proteins at 2 μM concentration buffered in 10 mM HEPES, pH 7.5, 500 mM NaCl Were mixed with compounds at a final concentration of 12.5 μM (final DMSO concentration was 0.02%). Experiments were performed in triplicate and data were analysed as previously described.[18]

Isothermal Titration Calorimetry (ITC)

The ITC experiment was performed at 30 ˚C using Nano-ITC (TA instrument). The protein in 50mM HEPES, pH 7.5, 500mM NaCl, 0.5mM TCEP, 5% glycerol at 50 μM was titrated into 6 μM SPHINX31. The heated of binding were analyzed, and the data were fitted to an independent binding model using NanoAnalyze program (TA instrument), from which the thermodynamic parameters were calculated ($\Delta G = \Delta H - T\Delta S = -RTlnKB$, where ΔG, ΔH and ΔS are the changes in free energy, enthalpy and entropy of binding, respectively). Thermodynamic parameters and binding constants are given in Supplemental Table 2.

Primary human retinal epithelial cell culture

Primary human RPE isolations were performed on human donor globes obtained within 24 hours post-mortem from the Bristol Eye bank (Bristol Eye Hospital (BEH)). Retinas with choroid-RPE sheets were removed to a petri dish, finely chopped and digested in Dulbecco's Modified Eagle Medium (DMEM):F12(1:1)+GlutaMax (Gibco) supplemented with 0.3mg/ml collagenase for 15 minutes at 37°C. Digested choroid-RPE sheets were suspended in media (DMEM:F12+GlutaMax) supplemented with 10% fetal bovine serum (FBS), 0.5% PenStrep (Invitrogen) and spun at 1500 rpm (251g) for 10 minutes to pellet cells. Pellets were resuspended in media supplemented with 25% FBS (Gibco), grown in cell culture flasks (Greiner) and split at 80% confluence.

Pharmacological inhibitor treatments.

Cells at ~70% confluence were serum starved for 24 h and treated with inhibitors at concentration noted. To determine pSRSF1 levels, cells were pre-treated with SPHINX31

for 1 hr then treated with TNF α (50 ng/ml) for 30 mins. For VEGF immunoblots in PC-3 cells, cells were treated with inhibitors for 24 h, washed off and lysed 24 h after washing.

Enzyme-linked immunosorbent assay (ELISA).

10 µg/ml $VEGF_{165}b$ capture antibody (Duoset VEGF ELISA DY-3045; R&D systems) or 0.25 µg/ml $VEGF_{165}a$ was incubated overnight at room temperature. The plates were blocked (1% BSA in PBS) and serial dilutions of recombinant human (rh)$VEGF_{165}$ or rh$VEGF_{165}b$ standards (ranging from 500 pg/ml to 1.95 pg/ml) were added, incubated alongside sample lysates, typically 200 µg in 100 µl per well in NP40 lysis buffer. The plate was incubated for 2 h at room temperature with shaking, washed and incubated with 100 µl/well of biotinylated goat anti-human VEGF (0.1 µg/ml; R & D systems) for 2 further hours at room temperature. After washing, 100 µl/well of Horseradish Peroxidase (HRP)-conjugated streptavidin (1:200; R&D Systems) was added and plates were left at room temperature for 20 minutes.

The plates were washed and colour change induced with substrate A and B (DY-999; R&D Systems) for 1 hour under light protection. The reaction was stopped by addition of 100 µl/well of 1M HCl and the absorbance was read immediately in an ELISA plate reader (Dynex Technologies Opsys MR system plate reader) at 450 nm with a control reading at 620 nm. A standard curve was calculated from mean absorbance values of standards enabling the estimation of VEGF concentration for each sample.

Laser lesion induction protocol

Six to eight week-old female C57/B6 mice (Charles River) were anaesthetized with an intraperitoneal injection of a mixture of 50mg/kg ketamine and 0.5mg/kg medetomidine. The pupils were dilated with 5% phenylephrine hydrochloride and 1% tropicamide. Four photocoagulation lesions were delivered with a Micron IV laser photocoagulation instrument (Phoenix Instruments) (450mW, 130ms, 75 µm) between the retinal vessels in a peripapillary distribution at a distance of 1-2 disc-diameters from the optic nerve in each eye. Only laser lesions with a sub-retinal bubble at the time of treatment were included in the study. Immediately following laser photocoagulation the animals received topical eye drops twice daily (10 µl) as previously described.[14] On day 7 and 14 animals were anaesthetized and pupils dilated as above, and received 100 µl of 10 mg/ml sodium fluorescein in PBS i.p. and retinae imaged using the Micron IV. Animals were culled on either day 7 (protein extraction) or day 14 (FFA) and eyes were either unfixed for retinal dissection and protein extraction, or fixed and enucleated and choroids stained and

examined. Choroidal lesions were imaged and areas traced by a masked observer, lesions area was quantified using Image J.

Mass Spectrometry

Compounds were serially diluted in water (initial stock dissolved in DMSO) from 5 µg/ml to 0 µg/ml, spiked with an internal standard 1 µg/ml compound **3** to account for any loss of samples during preparation and analysed. The chromatograms produced clear peaks at the expected molecular weights. The area under the peaks were integrated and plotted against concentration to confirm a linear response was observed. After a single topical administration mice were killed at time points 1, 4, 8, and 24 hours. Samples, the analyte and control treated samples, were homogenized and proteins were precipitated out of the samples with an equal volume (300 µl) acetonitrile. An internal standard of 1 µg/ml compound **3** was added to samples to account for any loss of samples during preparation. The solutions of 50% sample and 50% acetonitrile and 1 µg/ml **3** were centrifuged for 15 minutes at 4°C to pellet the proteins and the supernatant taken for analysis. Solutions were evaporated at 37°C for eight hours and resuspended in 100 µl acetonitrile ready for analysis by LCMS using a Varian Triple Quad system. Detection was achieved by positive ion electrospray (ESI+) mass spectrometry using a Varain Triple Quad spectrometer in multiple ion monitoring (MRM) mode. For SPHINX31 Q1 = 508, Q2 =172. For **3** Q1 = 488 and Q3 = 443. Mass spectrometer settings were as follows: column temperature 60 ºC; capillary voltage: 5 kV; Spray Shield 525 V; Gas Temp: 400 ºC, Nebuliser psi: 60; Analyser Temp 42 ºC. Chromatography (flow rate 0.8 $ml \cdot min^{-1}$) was achieved using a Phenomenex Kinetex column (EVO C18 5 µM, 2.1 x 50) equipped with a Phenomenex Security Guard precolumn (Security Guard Ultra UHPLC EVO C18). Solvents were: **A**, HPLC grade H_2O containing 20% CH_3CN + 45 mM ammonium formate pH 8.5; **B**, HPLC grade CH_3CN containing 45 mM ammonium formate pH 8.5. Gradient elution was performed as follows: 0 min, 90 % **A**, 5 min, 30 % **A**, 5.01 min, 100% **B**; 6 min, 100% **B**; 6.06 min, 90% **A**, 15 min 90% **A.** SPHINX31 eluted at 3 min and **3** at 1.9 min. Peaks occurring at these times in the SIM chromatograms for each compound were integrated using Varian Integration software and used to quantify SPHINX31.

For quantification of SPHINX31 in mouse plasma stability, metabolite and liver microsome stability assays, mass spectrometry was performed at O2H, India, using an Agilent 6410 series Triple Quad LC-MS with a Waters X Bridge C18 HPLC column (4.6 x 50 mm, 5 µM). Detection was achieved by positive ion electrospray (ESI+) mass spectrometry using a Agilent 6410 series Triple Quad LC-MS spectrometer in multiple ion monitoring (MRM).

For SPHINX31 Q1 = 508.6, Q2 = 420-520. Mass spectrometer settings were as follows: column temperature 24 ºC; capillary voltage: 4 kV; Gas Temp: 350 ºC, Gas flow 13 l/min Nebuliser psi: 50. Solvents were: **A**, HPLC grade H_2O containing 0.1% Formic acid; **B**, HPLC grade 100% Acetonitrile. Gradient elution was performed as follows: 0 min, 90% **A**, 6 min, 5 % **A**, 8.5 min, 5% **A**; 8.6 min, 10% **B**; 10 min, 10% **B**. SPHINX31 eluted at 3.9 min.

Electroretinography

ERG recordings were taken according to ISCEV guidelines using the Phoenix Ganzfeld ERG system. 8 week old female C57/Bl6 mice were treated with a single topical application of 0.2 µg compound 12, 24 h before ERG recordings and dark adapted for at least 12 h and maintained in complete darkness before ERG. Mice were anaesthetized with an intraperitoneal injection of a mixture of 50 mg/kg ketamine and 0.5 mg/kg medetomidine. The pupils were dilated with 5% phenylephrine hydrochloride and 1% tropicamide and kept hydrated with viscotears. Reference electrodes were placed by the tail and scalp and the eye was positioned in contact with the Ganzfeld corneal contact electrode using Labscribe2 ERG and Ueye Cockpit software. Scotopic ERG recordings were taken at 0, 0.02, 0.12, 3.76, 30, 120 and 1920 $cd.m.s^{-2}$ and photopic recordings at 120 and 1920 $cd.m.s^{-2}$ alternating in order between right and left eyes to control for any differences.

Statistical Analyses

If not indicated otherwise, data are shown as mean ± SEM. All data, graphs and statistical analyses were calculated with Microsoft Excel (Microsoft Office Software), GraphPad Prism (GraphPad Software Inc.) and Image J. All results were considered statistically significant at $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

Constructs and protein expression and purification

cDNAs encoding several human splicing kinases [SRPK1-2, MSSK1; CLK1-4; DYRK1A-2, PRPF4B; PIM1-2] were obtained from synthetic sources and used as templates to amplify kinase domain-containing sequences and further sub-cloned into different expression vectors, using ligation independent cloning.[19]

Expression in Escherichia coli

SRPK1 (aa 42-655), SRPK2 (aa 62-699), CLK2 (aa 135-496), CLK3 (aa 134-484),

DYRK1A (aa 127-485), DYRK2 (aa 73-479), MSSK1 (aa 43-533) and PIM2 (aa 1-311) were cloned into pNIC28-Bsa4 [pET expression vector with His6 tag in a 22 aa-N-terminal fusion peptide, with TEV protease cleavage site, (Kan+)] and co-expressed with λ-phosphatase in Escherichia coli BL21 (DE3)-R3 cells. CLK1 (aa 148-484) and PIM1 (aa 1-312) were cloned into PLIC-SGC1 [pET expression vector with His6 tag in a 23 aa-N-terminal fusion peptide, with TEV protease cleavage site, (Amp+)] and co-expressed with λ-phosphatase in Escherichia coli BL21 (DE3)-R3 cells. Transformed cells were initially cultured (from an overnight pre-culture) in either terrific broth (TB) medium (supplemented with 50 µg/mL of appropriate antibiotic) to OD600 of ~1.6 at 37 °C, followed by additional growth while cooling to 18 °C to an OD600 of ~3 before induction with 0.5 mM IPTG overnight, or cultured in Luria-Bertami (LB) medium to OD600 of ~0.4 at 37 °C, followed by additional growth while cooling to 18 °C to an OD600 of ~0.7 before induction with 0.5 mM IPTG overnight. Cells were harvested by centrifugation (JLA 8,100 rotor Beckman Coulter, Avanti J-20 XP centrifuge) and were frozen at -20 ^{0}C. Cells expressing His6-tagged proteins were re-suspended in lysis buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 10 mM Imidazole, 5% glycerol and 0.5 mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride) in the presence of protease inhibitors cocktail (1 µl/ml) and lysed by sonication using a 750 W Sonics Vibra-Cell sonicator, with amplitude set to 35 %, with bursts of 5 sec on-10 sec off, for 5 minutes, on ice. PEI (polyethyleneimine) was added to a final concentration of 0.15 % and lysates were transferred to centrifuge tubes and centrifuged at 53,000×g using a JA-25.50 rotor, for at least 45 minutes, at 4°C. After centrifugation, the clarified supernatant was passed through a gravity column of 5 ml Ni-Sepharose resin, IMAC (GE Healthcare), previously equilibrated in lysis buffer. The resin was first washed with 50 ml of lysis buffer containing 1 M NaCl and 30 mM imidazole, then with 25 ml of Lysis Buffer containing 100 mM imidazole and finally the protein was eluted with 25 ml of Lysis Buffer containing 300 mM imidazole [in the case of CLK1-3, 50 mM L-Arg/L-Glu was added to all buffers thereafter]. The eluted proteins were collected and treated overnight with TEV (Tobacco Etch Virus) protease at 40C to remove the N-terminal tag. Digested proteins were loaded onto a nickel column again to remove the cleaved hexa-histidine expression tag protease used. The flow-through containing the cleaved proteins was collected and concentrated to a 5 ml volume using concentrators (Amicon) and injected onto a Superdex 75 or 200 (16/60) gel filtration column on an AKTA system (GE Healthcare) pre-equilibrated into GF Buffer (50 mM HEPES pH7.5, 300 mM NaCl, 5% glycerol, and 0.5 mM TCEP). The resulting pure protein was stored at −80 °C in 50 mM HEPES, pH 7.5, 300 mM NaCl, 0.5 mM TCEP and 5% glycerol.

Expression in Spodoptera frugiperda (Sf9)

CLK4 (aa 126-476) and PRPF4B (aa 665-1004) were cloned into PFB-LIC-Bse [Baculovirus transfer vector with His6 tag in a 22 aa-N-terminal fusion peptide, with TEV protease cleavage site, (Amp+)] and constructs' DNA was transformed into the DH10Bac Escherichia coli strain (Invitrogen). After transposition the recombinant bacmid DNA was then purified and used directly to transfect insect cells (Sf9), using the baculovirus expression vector system. Recombinant baculoviruses were produced following an established protocol[20] based on the Bac-to-Bac ® system (Invitrogen). Sf9 cells were routinely grown as a suspension in Sf-900™ II SFM (1×) (Invitrogen) at 27°C, with shaking set at 100 rpm. For large scale expression, cells were infected at a density of 2×106/ml with recombinant baculovirus (5 ml of virus stock/1L of cultured cells). Seventy-two hours after infection, the cultures were collected and centrifuged at 900×g for 20 minutes, 4°C, using a JLA 8.1000 rotor on an Avanti J-20XP. The cell pellets were resuspended in cold lysis buffer (25ml/pellet from 1L of culture) consisting of 50 mM HEPES [pH 7.5], 500 mM NaCl, 5 mM imidazole, 5% glycerol, 0.5 mM TCEP tris(2-carboxyethyl)phosphine) and a protease inhibitor cocktail III (1:1000 dilution, Calbiochem). Cell suspensions were lysed and proteins were purified following the same methods described above. The correct mass and purity for all protein constructs was confirmed by an Agilent 1100 Series LC/MSD TOF (Agilent Technologies Inc. – Palo Alto, CA).

3. References

1. G.R. Fulmer, A.J.M. Miller, N.H. Sherden, H.E. Gottlieb, A. Nudelman, B.M. Stoltz, J.E. Bercaw, K.I. Goldberg, *Organometallics* **2010**, *29*, 2176-2179.
2. D.D. Perrin, W.L.F. Armarego, *Purification of Laboratory Chemicals*, 3rd Edn. ed., Pergamon Press Ltd., Great Britain, **1988**.
3. W.C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, *43*, 2923-2925.
4. V. J. Cee, B. K. Albrecht, S. Geuns-Meyer, P. Hughes, S. Bellon, J. Bready, S. Caenepeel, S. C. Chaffee, A. Coxon, M. Emery, J. Fretland, P. Gallant, Y. Gu, B. L. Hodus, D. Hoffman, R. E. Johnson, R. Kendall, J. L. Kim, A. M. Long, D. McGowan, M. Morrison, P. R. Olivieri, V. F. Patel, A. Polverino, D. Powers, P. Rose, L. Wang, H. Zhao, *J. Med. Chem.*, **2007**, 50, 627-640.
5. H.Y. Fu, H, Doucet, *Eur. J. Org. Chem.,* **2011**, *35*, 7163-7173.
6. A. Chaikuad, S. Knapp, F. von Delft, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2015,** *71*, 1627-1639.

7. W. Kabsch, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2010,** *66*, 125-132.
8. P.R. Evans, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2011**, *67*, 282-292.
9. P.R. Evans, G.N. Murshudov, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2013,** *69*, 1204-1214.
10. J.C. Ngo, J. Gullingsrud, K. Giang, M.J. Yeh, X.D. Fu, J.A. Adams, J.A. McCammon, G. Ghosh,*Structure* **2007,** *15*, 123-133.
11. P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2010,** *66*, 486-501.
12. G.N. Murshudov, P. Skubák, A.A. Lebedev, N.S. Pannu, R.A. Steiner, R.A. Nicholls, M.D. Winn, F. Long and A.A. Vagin, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2011,** *67*, 355-367
13. V.B. Chen, W.B. Arendall III, J.J. Headd, D.A. Keedy, R.M. Immormino, G.J. Kapral, L.W. Murray, J.S. Richardson, D.C. Richardson, *Acta Crystallogr. Sect. D-Biol. Crystallogr.* **2010,** *66*, 12-21.
14. M.V. Gammons, O. Fedorov, D. Ivison, C. Du, T. Clark, C. Hopkins, M. Hagiwara, A.D. Dick, R. Cox, S.J. Harper, J.C. Hancox, S. Knapp, D.O. Bates, *Invest. Ophthalmol. Vis. Sci.* **2013**, *54*, 6052-6062.
15. Z. Zhou, Q. Gong, B. Ye, Z. Fan, J.C. Makielski, G.A. Robertson, C.T. January, *Biophys. J.* **1998,** *74*, 230-241.
16. H. Cheng, Y. Zhang, C. Du, C.E. Dempsey, J.C. Hancox, *Brit. J. Pharmacol.* **2012**, *165*, 2260-2273.
17. C.Y. Du, A. El Harchi, Y.H. Zhang, C.H. Orchard, J.C. Hancox, *J. Cardiovasc. Electrophysiol.* **2011,** *22*, 1163-1170.
18. O. Federov, F. H. Niesen, S. Knapp, in Kinase Inhibitors: Methods and Protocols *Methods Mol. Biol.* **2011**, Vol. 795*,* 109-118.
19. C. Strain-Damerell, P. Mahajan, O. Gileadi, N.A. Burgess-Brown, *Methods Mol. Biol*. **2014**, *1091*, 55-72.
20. P. Mahajan, C. Strain-Damerell, O. Gileadi, N.A. Burgess-Brown, *Methods Mol. Biol.* **2014**,*1091*, 95-121.

Supplementary Table 1. Tm data characterizing compound 1

Kinase	ΔTm (°C)	Protein Family
ACVR1A	-0.245	TKL
ACVR2A	-0.34	TKL
ADRBK2A	-0.27	AGC
AKT3A	0.37	AGC
BMXA	0.01	Kinase-TK
CAMKK2B	0.4	Other
CDC123A	0.19	AGC
CDK2A	0.06	CMGC
CDK8A	-0.21	CMGC
CDKL1A	-0.31	CMGC
CHEK2A	0.43	CAMK
CLK1A	0.67	CMGC
CLK2A	1.41	CMGC
CLK3A	-0.46	CMGC
CSNK1G1A	-0.055	Other
CSNK1G3A	0.36	Other
CSNK2A1A	-0.1	Other
CSNK2A2A	0.62	Other
FESA	-0.06	TK
GPRK5A	-0.28	AGC
GRK1A	0.01	AGC
GSG2A	0.1	Other
GUCY2DA	0.39	Other
LOC340156A	0.26	CAMK
MAP2K2A	0.15	STE
MAPK13A	0.15	CMGC
MAPK3A	-0.6	CMGC
MAPK8B	0.33	CMGC
MAPK9A	0.32	CMGC
MSSK1A	8.49	CMGC
MST4A	0.1	STE
MYLK2A	0.7	CAMK
OSR1A	-0.11	STE
PAK4B	0.68	STE
PCTK1A	0.23	CMGC
PIM1A	0.59	CAMK
PIM1Z	1.235	CAMK
PIM3A	2.46	CAMK
PLK4A	0.76	Other
PRKCL1A	-0.4	AGC
PRKD2A	-0.09	AGC
RPS6KA1A	-0.1	AGC
RPS6KA6A	-0.22	AGC
SLKA	0.34	STE
SRPK1A	8.54	CMGC
SRPK2A	4	CMGC
STK10A	0.35	STE
STK16A	0.87	Other
STK17AA	-0.23	CAMK
STK17BA	0.53	CAMK
STK24A	-0.1	STE
STK33A	0.66	CAMK
STK38LA	0.56	AGC
TNIKA	0.13	STE
YANK1A	-0.39	AGC

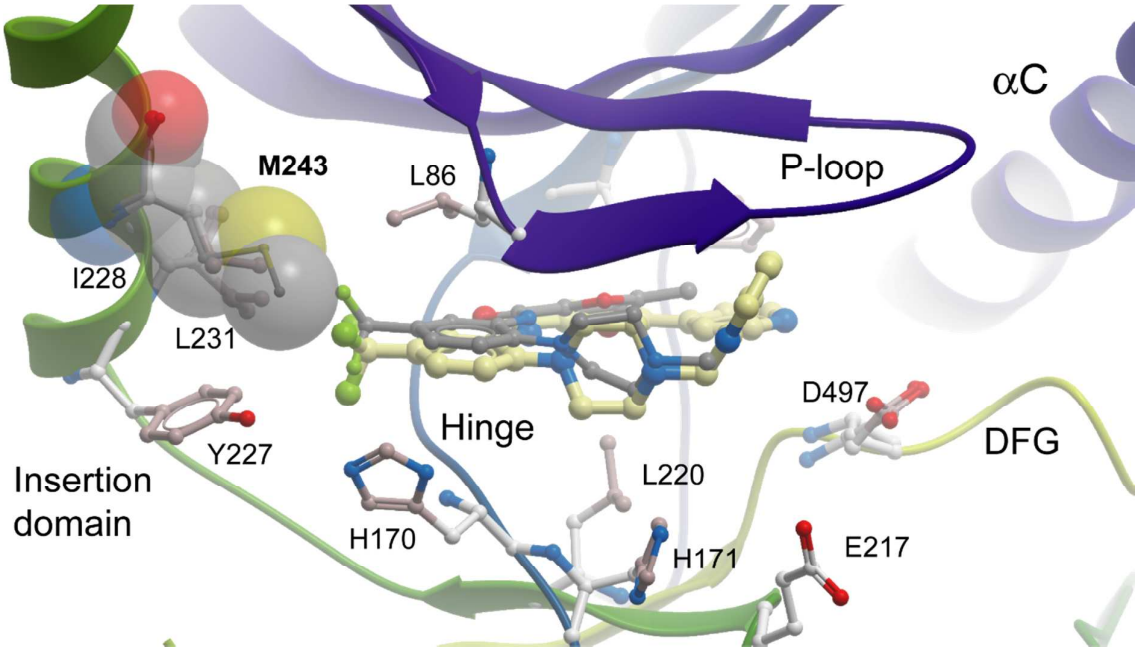
Supplemental Table 2: Crystallographic data and refinement

	SRPK1-**1**	SRPK1-**SPHINX31**	SRPK2-**1**
Data collection			
Space group	$P\,4_3\,2_1\,2$	$P\,6_522$	$C\,222_1$
Cell dimensions			
a, *b*, *c* (Å)	82.6 82.6 133.8	74.9, 74.9, 310.1	188.8, 249.0, 146.7
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 90.0
Resolution (Å)	29.19-1.75 (1.75-1.78)	28.74-1.70 (1.70-1.79)	29.87-2.90 (3.06-2.90)
R_{merge}	0.059 (0.826)	0.078 (0.799)	0.138 (0.957)
$I/\sigma I$	28.4 (3.7)	14.2 (2.7)	9.5 (1.6)
Completeness (%)	99.8 (96.0)	99.2 (99.0)	99.3 (99.8)
Redundancy	14.2 (13.8)	7.6 (7.9)	5.8 (6.1)
Refinement			
Resolution (Å)	29.19-1.75	28.74-1.70	29.87-2.90
No. reflections	47,639 (2,468)	57,614 (8,123)	75,969 (11,061)
R_{work} / R_{free}	0.173/ 0.205	0.164/ 0.200	0.199/ 0.217
No. atoms			
Protein	2,937	2,971	11,373
ligand	26	37	104
Water and solvents	276	503	303
B factors (Å^2)			
Protein	29	30	78
ligand	26	27	88
Water and solvents	38	45	101
r.m.s. deviations			
Bond lengths (Å)	0.013	0.016	0.011
Bond angles (°)	1.5	1.6	1.2

Values in brackets are for highest resolution shells

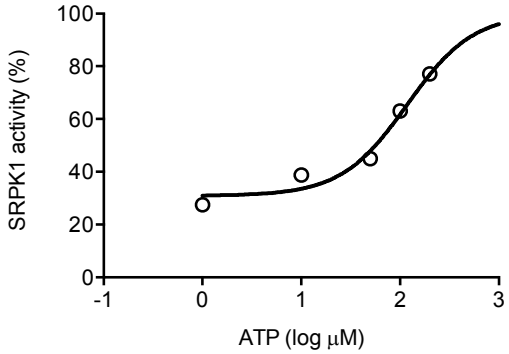
Supplemental Figure 1. Comparison of the co-crystal structures of SPHINX31.SRPK1 with 1.SRPK2.

The main interacting residues are labelled. SPHINX31 carbon atoms are coloured in yellow and 1 in grey. The main differences between the two ATP sites is due to residue changes in the insertion domain helix, in particular the exchange of L231 in SPRK1 with M243 (shown as a CPK model and labelled in bold) in SRPK2. The bulkier methionine results in re-orientation of the inhibitor and less favourable contacts. The main interacting residues are shown in ball and stick representation and are labelled (SRPK1). For all SRPK1 residues shown, an essentially similar side chain orientation was observed in SRPK2. The SRPK2 residues are therefore not shown or labelled for clarity. Main structural elements are labelled as well.

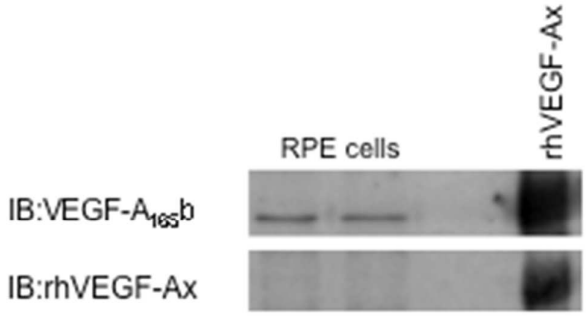


Supplemental Figure 2: SPHINX31 is an ATP competitive inhibitor.

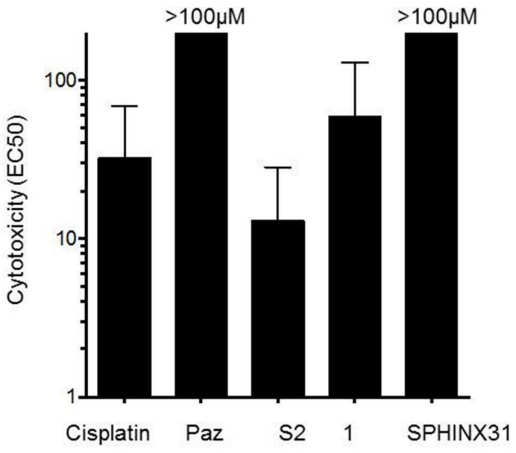
Kinase activity of SRPK1 in the presence of 10 nM compound SPHINX31 and increasing concentration of ATP.



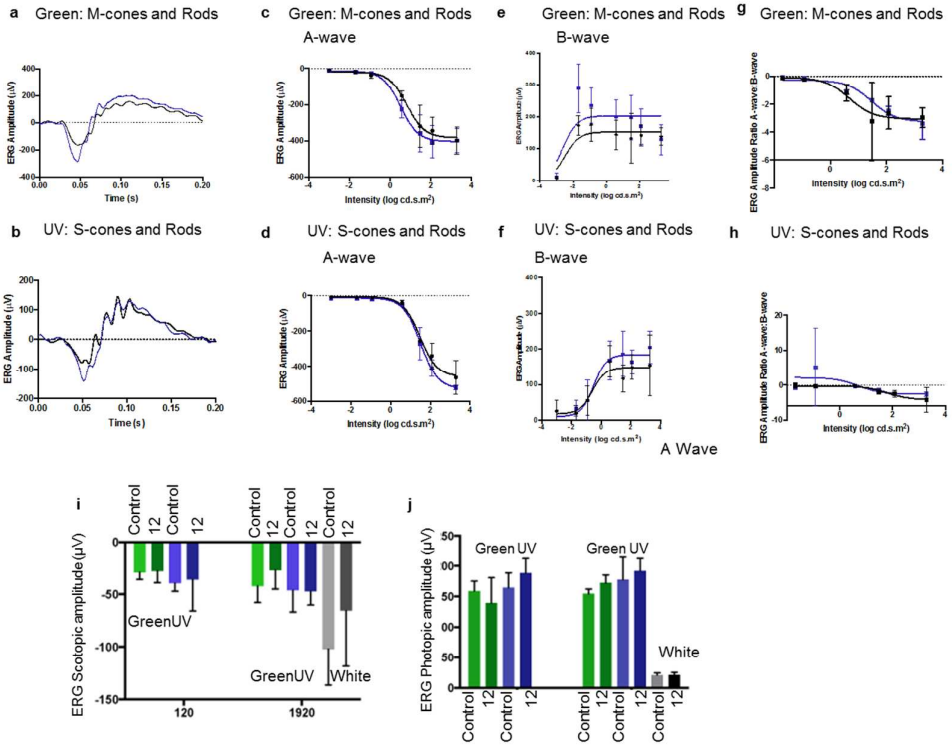
Supplemental Figure 3: Detection of VEGF-A_{165}b and VEGF-Ax. VEGF-A_{165}b but not VEGF-Ax was detected in untreated RPE cells.



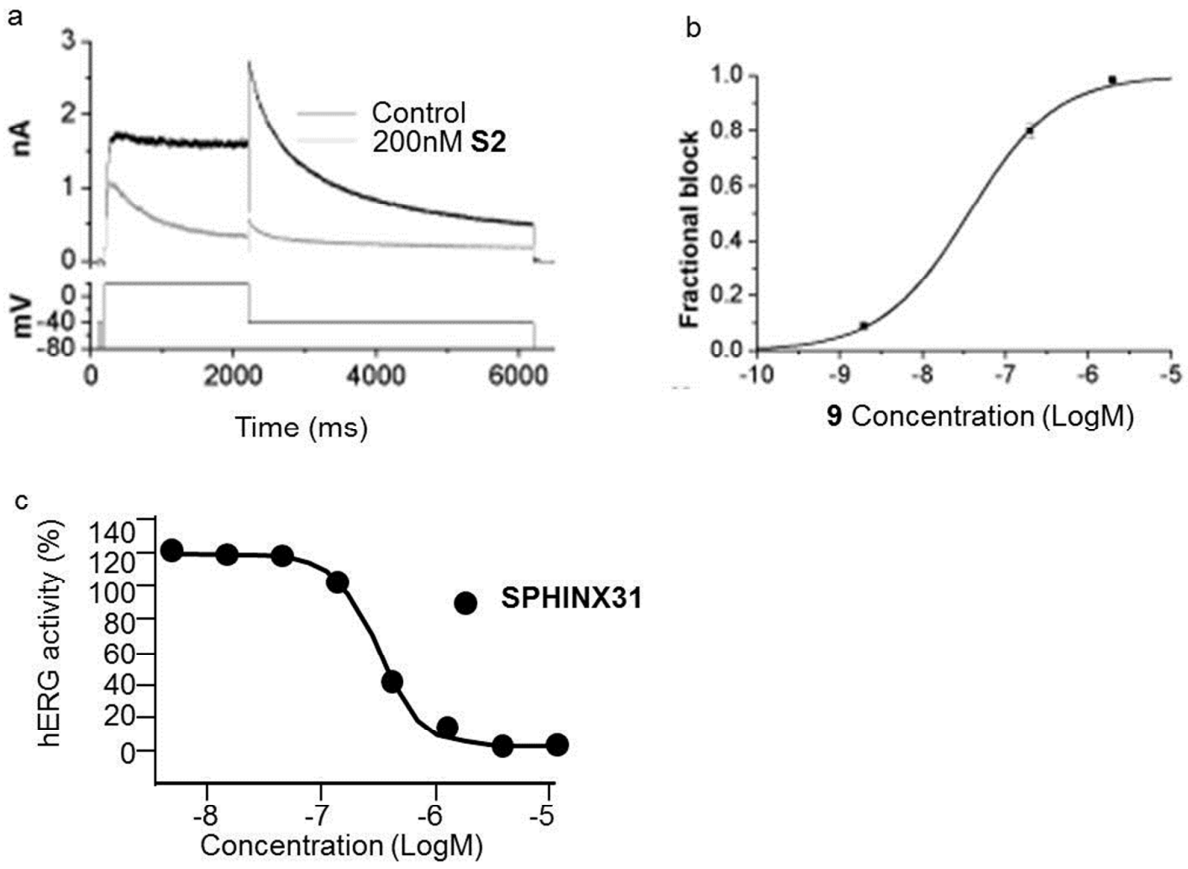
Supplemental Figure 4. SRPK inhibitors as a class are not toxic. RPE cells were treated with cisplatin, pazopanib, or different SRPK1 inhibitors and cytotoxicity measured using an MTT assay.



Supplemental Figure 5. Dosing with SPHINX31 does not inhibit retinal function. Mice (N=3) were dosed six times a day for 14 days, and retinal function measured by electroretinography. No changes were seen compared with vehicle control treatment.



Supplemental Figure 6. SPHINX31 inhibits hERG with an IC_{50} of 300 nM. hERG activity (tail current, **a.** of compounds **S2** and SPHINX31 were measured. **b.** Fractional block was determined (example for compound **S2** shown). Compound **S2** had an IC_{50} of 36 nM. **c.** SPHINX31 had an IC_{50} of 300 nM.



Supplemental Figure 7. VEGF-A_{165}b, but not VEGF-Ax, was expressed in mouse retina after treatment with SPHINX31. Six C57/Bl6 mice were subjected to laser CNV and killed after 4 days. Three retinae were pooled and protein extracted and subjected to **a.** immunoblotting for VEGF-A_{165}b and VEGF-Ax. No VEGF-Ax was seen in mouse retina or human RPE cells, but was seen in mouse colon spleen and kidney. **b-c.** Densitometric quantification of western blots. ELISA for **d.** VEGF-A_{165}a and **e.** VEGF-A_{165}b demonstrated that **f.** a switch in isoform expression had occurred.