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(71) Applicant(s)
EverBrilliant Pharma Pty Ltd

(72) Inventor(s)
Wang, Bing Hui;Krum, Henry;Scammells, Peter;Vinh, Natalie;Simpson, Jamie;Chalmers, David

(74) Agent / Attorney
FPA Patent Attorneys Pty Ltd, Level 43 101 Collins Street, Melbourne, VIC, 3000, AU

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(72) Inventor; and
(71) Applicant : **WANG, Bing, Hui** [AU/AU]; 3 Drynmawr Road, Camberwell, Victoria 3124 (AU).

(72) **Inventors**: **KRUM, Henry**; 11 Moorakyne Avenue, Malvern, Victoria 3144 (AU). **SCAMMELLS, Peter**; 31 Duggan Street, North Balwyn, Victoria 3104 (AU). **VINH, Natalie**; 233 Hawthorn Road, Vermont South, Victoria 3133 (AU). **SIMPSON, Jamie**; 73 Cocoa Jackson Lane, Brunswick, Victoria 3056 (AU). **CHALMERS, David**; Wellington Road, Clayton, Victoria 3800 (AU).

(74) **Agent**: **FISHER ADAMS KELLY PTY LTD**; Level 6, 175 Eagle Street, Brisbane, Queensland 4000 (AU).

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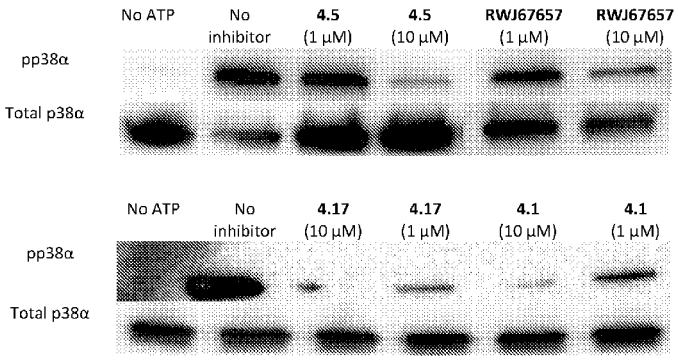

FIG 1

(57) **Abstract**: The present invention relates to certain novel substituted thiophene compounds and the finding that they display useful efficacy in the inhibition of the p38α MAPK enzyme. This provides for use of the compounds in various treatment methodologies related to MAPK inhibition, including the treatment of inflammation.

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MAPK INHIBITORS

FIELD OF THE INVENTION

[0001] The invention relates to the field of medical treatment. More particularly, this invention relates to novel thiophene compounds and their use in treating a disease or condition responsive to mitogen-activated protein kinase (MAPK) inhibition.

BACKGROUND TO THE INVENTION

[0002] Any reference to background art herein is not to be construed as an admission that such art constitutes common general knowledge in Australia or elsewhere.

[0003] A number of drugs are commonly used to treat inflammation and include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and anti-cytokine biologics. NSAIDs exert their anti-inflammatory effect by inhibiting the cyclooxygenase enzymes (COX-1 and COX-2) which are responsible for the synthesis of prostanoids. These drugs however, are relatively non-selective and are often associated with gastrointestinal side effects. Although selective COX-2 inhibitors were developed and found to reduce these side effects, many were withdrawn from the market over safety concerns associated with cardiovascular and thrombotic adverse effects.

[0004] Among the most widely used anti-inflammatory drugs are the corticosteroids which act at multiple stages of the inflammatory cascade, including the up-regulation of anti-inflammatory genes and suppression of pro-inflammatory genes. However, prolonged use of corticosteroids can be associated with adverse effects including growth retardation in children, immunosuppression, hypertension, impairment of wound healing, osteoporosis and metabolic disturbances. Resistance has also been reported and as a consequence there are variations in patient response and in many cases a reduced efficacy with disease progression.

[0005] Aside from the more traditional anti-inflammatory therapies, anti-cytokine biologics developed using recombinant DNA and monoclonal antibody technology have become available for the treatment of several chronic inflammatory

conditions. Despite their effectiveness as anti-inflammatory agents, these biologics have drawbacks that limit their use. Poor cellular penetration and activity, low oral bioavailability resulting in subcutaneous or intravenous administration, short half-life, rapid metabolism and a high cost of manufacture render them less desirable drugs. Given the limitations of existing anti-inflammatory drug therapies, there remains a need to identify alternative drug targets for the treatment of chronic inflammatory diseases.

[0006] With a greater understanding of the molecular basis of the inflammatory response and the mechanisms involved, a number of molecular targets for the treatment of inflammation have been identified. One signalling network found to play an important role in inflammation is the p38α mitogen-activated protein kinase (MAPK) pathway.

[0007] p38α MAPK (also named p38, reactivating kinase (RK) and p40) is a serine/threonine kinase that becomes phosphorylated and activated in response to various stimuli including endotoxin, hyperosmolarity, sodium arsenite, heat shock, and interleukin-1 (IL-1).

[0008] The p38α MAPK isoform plays a central role in the immune and inflammatory process. Its function is critical for the production of the inflammatory response of a number of proteins. Of particular importance, p38α MAPK is responsible for the biosynthesis of inflammatory cytokines through transcription-dependent mechanisms and post-transcriptional regulation. In addition to regulating cytokine biosynthesis, p38α MAPK is known to act downstream of cytokines. Therefore, p38α MAPK inhibition can not only stop production of cytokines but also reduce the deleterious effects of any cytokines that may still be produced which would provide greater efficacy in disease than inhibitors that act on mediators alone.

[0009] Pharmacological inhibition of p38α MAPK has shown anti-inflammatory activity in a number of experimental disease models including models for arthritis, inflammatory bowel disease, asthma and psoriasis. Further links have been established in myocardial injury, cardiac remodelling and renal fibrosis, stroke, cancer, Alzheimer's disease and human immunodeficiency virus.

[0010] p38α MAPK inhibitors to date suffer from a number of drawbacks including sub-optimal efficacy, poor physicochemical characteristics and undesirable

side effects. There is, therefore, a need for alternative compounds for the treatment of diseases responsive to MAPK inhibition generally and, particularly, p38α MAPK inhibition.

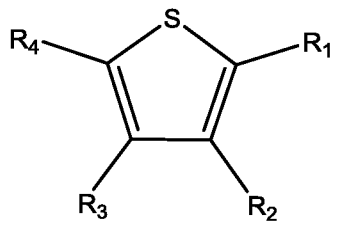
OBJECT OF THE INVENTION

[0011] It is an aim of this invention to provide for a heterocyclic compound suitable for treating a disease responsive to MAPK inhibition which overcomes or ameliorates one or more of the disadvantages or problems described above, or which at least provides a useful alternative.

[0012] Other preferred objects of the present invention will become apparent from the following description.

SUMMARY OF INVENTION

[0013] According to a first aspect of the invention, there is provided a compound of formula (I), or a pharmaceutically acceptable salt thereof:



Formula (I)

wherein, R_1 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, alkanoyl, carboalkoxy, acyloxy, aryl, aroyl, heteroaryl, heteroaroyl, heterocyclyl, heterocycloyl, cycloalkyl, O-alkyl and O-aryl, O-heteroaryl, amino and amido, all of which groups may be substituted or unsubstituted;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, alkylaryl, heterocyclyl and heteroaryl, all of which may be substituted or unsubstituted; and

R_3 and R_4 are independently selected from the group consisting of aryl, heteroaryl, heterocyclyl and cycloalkyl, all of which groups may be substituted or unsubstituted.

[0014] In one embodiment, the compound of the first aspect is a non-naturally occurring compound.

[0015] According to a second aspect of the invention there is provided a pharmaceutical composition comprising an effective amount of a compound of the first aspect, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent and/or excipient.

[0016] Suitably, the pharmaceutical composition is for the treatment or prophylaxis of a disease, disorder or condition responsive to MAPK inhibition, preferably p38 MAPK inhibition, more preferably p38α MAPK inhibition.

[0017] A third aspect of the invention resides in a method of treating a patient suffering from a disease, disorder or condition responsive to MAPK inhibition including the step of administering an effective amount of a compound of the first aspect, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of the second aspect to the patient.

[0018] A fourth aspect of the invention provides for a compound of the first aspect, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of the second aspect for use in the treatment of a disease, disorder or condition responsive to MAPK inhibition.

[0019] A fifth aspect of the invention provides for use of a compound of the first aspect, or a pharmaceutically effective salt thereof, in the manufacture of a medicament for the treatment of a disease, disorder or condition responsive to MAPK inhibition.

[0020] A sixth aspect of the invention provides for a complex of a compound of the first aspect, or a pharmaceutically effective salt thereof, with a p38 MAPK enzyme.

[0021] The various features and embodiments of the present invention, referred to in individual sections above apply, as appropriate, to other sections, *mutatis mutandis*. Consequently features specified in one section may be combined with features specified in other sections as appropriate.

[0022] Further features and advantages of the present invention will become apparent from the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] In order that the invention may be readily understood and put into practical effect, preferred embodiments will now be described by way of example with reference to the accompanying figures wherein:

[0024] FIG 1 is a series of western blots showing the effects of the tested compounds on p38α MAPK activation (phosphorylation);

[0025] FIG 2 is a graphical representation of the effects of a number of compounds of the present invention on monocytic cells (THP-1) TNF-α gene expression (** $p < 0.01$ vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$ vs. stimulated control (LPS));

[0026] FIG 3 is a graphical representation of the effects of a number of compounds of the present invention on monocytic cells (THP-1) IL-6 gene expression (** $p < 0.01$ vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. stimulated control (LPS));

[0027] FIG 4 is a graphical representation of the effects of a number of compounds of the present invention on NCM hypertrophy stimulated by AngII (* $p < 0.05$ vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. stimulated control (AngII));

[0028] FIG 5 is a graphical representation of the effects of a number of compounds of the present invention on NCM hypertrophy stimulated by TNF-α (** $p < 0.01$ vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. stimulated control (TNF-α));

[0029] FIG 6 is a graphical representation of the effects of a number of compounds of the present invention on NCF collagen synthesis stimulated by AngII (*** $p < 0.001$ vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. stimulated control (AngII));

[0030] FIG 7 is a graphical representation of the blood results from an acute toxicity study for compound 4.5.;

[0031] FIG 8 is a graphical representation of the effect of **4.5** on RMC collagen synthesis stimulated by AngII, IS, PCS or MCS (*$p<0.05$, **$P<0.01$, **** $p < 0.0001$

vs. unstimulated control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, ####$p<0.0001$ vs. stimulated control (AngII, IS, PCS or MCS));

[0032] FIG 9 is a graphical representation of the effects of analogues 4.1 and 4.5 on NCF viability;

[0033] FIG 10 (a) and (b) is a series of graphical representations showing (a) Individual body weight gain (rats 1-4 were administered compound 4.5 and rats 5-8 represent the vehicle group and (b) Difference in the body weight on average to the vehicle group;

[0034] FIG 11 is a graphical representation of the average mass of tissues relative to the vehicle group for testing of compound 4.5;

[0035] FIG 12 is a diagrammatic representation of the SAR for a trisubstituted thiophene series;

[0036] FIG 13 is a series of western blots indicating the effects of select compounds on p38α MAPK activation (phosphorylation); and

[0037] FIG 14 is a graphical representation of the competitive binding of certain compounds of the invention with SB203580-fluorescein ligand to inactive p38α MAPK.

DETAILED DESCRIPTION

[0038] The present invention is predicated, at least in part, on the finding that certain substituted thiophene compounds display useful efficacy in the treatment of inflammation through inhibition of the p38α MAPK enzyme.

Definitions

[0039] In this patent specification, the terms 'comprises', 'comprising', 'includes', 'including', or similar terms are intended to mean a non-exclusive inclusion, such that a method or composition that comprises a list of elements does not include those elements solely, but may well include other elements not listed.

[0040] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as would be commonly understood by those of ordinary skill in the art to which this invention belongs.

[0041] As used herein, “effective amount” refers to the administration of an amount of the relevant active agent sufficient to prevent the occurrence of symptoms of the condition being treated, or to bring about a halt in the worsening of symptoms or to treat and alleviate or at least reduce the severity of the symptoms. The effective amount will vary in a manner which would be understood by a person of skill in the art with patient age, sex, weight etc. An appropriate dosage or dosage regime can be ascertained through routine trial.

[0042] The term "pharmaceutically acceptable salt", as used herein, refers to salts which are toxicologically safe for systemic or localised administration such as salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic or organic acids. The pharmaceutically acceptable salts may be selected from the group including alkali and alkali earth, ammonium, aluminium, iron, amine, glucosamine, chloride, sulphate, sulphonate, bisulphate, nitrate, citrate, tartrate, bitartrate, phosphate, carbonate, bicarbonate, malate, maleate, napsylate, fumarate, succinate, acetate, benzoate, terephthalate, palmoate, piperazine, pectinate and S-methyl methionine salts and the like.

[0043] The term "alkyl" refers to a straight-chain or branched alkyl substituent containing from, for example, 1 to about 12 carbon atoms, preferably 1 to about 9 carbon atoms, more preferably 1 to about 6 carbon atoms, even more preferably from 1 to about 4 carbon atoms. Examples of such substituents include methyl, ethyl, propyl, isopropyl, *n*-butyl, *sec*-butyl, isobutyl, *tert*-butyl, pentyl, isoamyl, 2-methylbutyl, 3-methylbutyl, hexyl, heptyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-ethylbutyl, 3-ethylbutyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The number of carbons relates to the carbon backbone and carbon branching but does not include carbon atoms belonging to any substituents, for example the carbon atoms of an alkoxy substituent branching off the main carbon chain.

[0044] The term "alkenyl" refers to optionally substituted unsaturated linear or branched hydrocarbon groups, having 2 to 12 carbon atoms, preferably 2 to 9 carbon atoms, more preferably 2 to 6 carbon atoms and having at least one carbon-carbon double bond. Where appropriate, the alkenyl group may have a specified number of carbon atoms, for example, C_2-C_6 alkenyl which includes alkenyl groups having 2, 3, 4, 5 or 6 carbon atoms in linear or branched arrangements. Non-limiting examples of

alkenyl groups include, ethenyl, propenyl, isopropenyl, butenyl, s- and t-butenyl, pentenyl, hexenyl, hept-l,3-diene, hex-l,3-diene, non-l,3,5-triene and the like.

[0045] The term "alkynyl" as used herein means alkyl moieties wherein at least one saturated C–C bond is replaced by a triple bond. In particular embodiments, alkynyl refers to groups comprising 2 to 12 carbon atoms (C_2-C_{12} alkynyl). In further embodiments, alkynyl refers to groups comprising 2 to 6 carbon atoms (C_2-C_6 alkynyl). In specific embodiments, alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, l-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl.

[0046] The term "carboalkoxy" refers to an alkyl ester of a carboxylic acid, wherein alkyl has the same definition as found above. Examples include carbomethoxy, carboethoxy, carboisopropoxy and the like.

[0047] The term "cycloalkyl" refers to optionally substituted saturated mono-cyclic, bicyclic or tricyclic carbon groups. Where appropriate, the cycloalkyl group may have a specified number of carbon atoms, for example, C_3-C_6 cycloalkyl is a carbocyclic group having 3, 4, 5 or 6 carbon atoms. Non-limiting examples may include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl and the like.

[0048] The term "aryl" refers to an unsubstituted or substituted aromatic carbocyclic substituent, as commonly understood in the art. It is understood that the term aryl applies to cyclic substituents that are planar and comprise 4n+2 π electrons, according to Hückel's Rule.

[0049] The term "heteroaryl" refers to an aryl group containing from one or more (particularly one to four) non-carbon atom(s) (particularly N, O or S) or a combination thereof, which heteroaryl group is optionally substituted at one or more carbon or nitrogen atom(s). Heteroaryl rings may also be fused with one or more cyclic hydrocarbon, heterocyclic, aryl, or heteroaryl rings. Heteroaryl includes, but is not limited to, 5-membered heteroaryls having one heteroatom (e.g., thiophenes, pyrroles, furans); 5 membered heteroaryls having two heteroatoms in 1,2 or 1,3 positions (e.g., oxazoles, pyrazoles, imidazoles, thiazoles, purines); 5-membered heteroaryls having three heteroatoms (e.g., triazoles, thiadiazoles); 6-membered

heteroaryls with one heteroatom (e.g., pyridine, quinoline, isoquinoline, benzoquinoline, acridine); 6-membered heteroaryls with two heteroatoms (e.g., pyridazines, cinnolines, phthalazines, pyrazines, pyrimidines, quinazolines); 6-membered heretoaryls with three heteroatoms (e.g., 1,3,5- triazine); and 6-membered heteroaryls with four heteroatoms. "Substituted heteroaryl" means a heteroaryl having one or more non-interfering groups as substituents.

[0050] "Heterocyclyl" as used herein specifically in relation to certain 'R' groups refers to a non-aromatic ring having 5 to 7 atoms in the ring and of those atoms 1 to 4 are heteroatoms, said ring being isolated or fused to a second ring wherein said heteroatoms are independently selected from O, N and S. Heterocyclic includes partially and fully saturated heterocyclic groups. Heterocyclic systems may be attached to another moiety via any number of carbon atoms or heteroatoms and may be both saturated and unsaturated. Non-limiting examples of heterocyclic include pyrrolidinyl, pyrrolinyl, pyranyl, piperidinyl, piperazinyl, morpholinyl, tetrahydrofuranyl, tetrahydrothiophenyl, pyrazolinyl, dithiolyl, oxathiolyl, dioxanyl, dioxinyl, oxazinyl, azepinyl, diazepinyl, thiazepinyl, oxepinyl and thiapinyl, imidazolinyl, thiomorpholinyl, and the like.

[0051] "Aroyl", "heteroaroyl" and "heterocycloyl" as used herein relate to aryl, heteroaryl and heterocyclyl groups, as described above, when attached to a carbonyl group which is also attached to the thiophene ring.

[0052] The term "halo" or "halogen" as used herein means fluorine, chlorine, bromine, or iodine.

[0053] The term "arylalkyl" as used herein refers to an aryl group, as defined above, linked to the thiophene ring or other moiety through an alkyl group as defined above.

[0054] The term "amino" as used herein means a moiety represented by the structure NR_{10}, and includes primary amines, and secondary and tertiary amines substituted by alkyl (*i.e.*, alkylamino). Thus, R_{10} may represent, for example, two hydrogen atoms, two alkyl moieties, or one hydrogen atom and one alkyl moiety.

[0055] The term "alkanoyl" or "acyl" as used herein means a group formed by removing the hydroxyl group from a carboxylic acid, in which the non-carbonyl moiety

of the group is selected from straight, branched, or cyclic alkyl or lower alkyl; alkoxyalkyl including methoxymethyl; aralkyl including benzyl; aryloxyalkyl such as phenoxymethyl; aryl including phenyl optionally substituted, C1-6 alkyl or C1-6 alkoxy; and substituted benzyl.

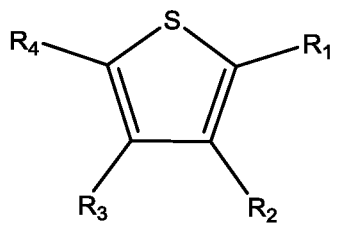
[0056] "Substituted" as used herein in reference to a substituent group refers to substituent groups which may be substituted with one or more moieties, for example, those selected from the group consisting of optionally substituted C1-8 alkyl (*e.g.*, optionally substituted C1-6 alkyl); optionally substituted C1-8 alkoxy (*e.g.*, optionally substituted C1-6 alkoxy); optionally substituted C2-8 alkenyl; optionally substituted C2-8 alkynyl; optionally substituted C5-6 aryl; aryloxy; optionally substituted heteroaryl; optionally substituted heterocycle; halo (*e.g.*, Cl, F, Br, and I); hydroxyl; halogenated alkyl (*e.g.*, CF_3, 2-Br-ethyl, CH_2F, CH_2CF_3, and CF_2CF_3); amino (e.g., NH_2, $NR_{10}H$, and $NR_{10}R_{10}$); alkylamino; arylamino; acyl; amido; CN; NO_2; N_3; CH_2OH; $CONH_2$; $CONR_{10}R_{10}$; CO_2R_{10}; CH_2OR_{10}; $NHCOR_{10}$; $NHCO_2R_{10}$; CF_3S; and CF_3SO_2; and each R_{10} is independently selected from H or optionally substituted C1-6 alkyl

[0057] Whenever a range of the number of atoms in a structure is indicated (e.g., a C_1-C_{12}, C_1-C_{10}, C_1-C_9, C_1-C_6, C_1-C_4, or C_2-C_{20}, C_2-C_{12}, C_2-C_{10}, C_2-C_9, C_2-C_8, C_2-C_6, C_2-C_4 alkyl, alkenyl, etc.), it is specifically contemplated that any sub-range or individual number of carbon atoms falling within the indicated range also can be used. Thus, for instance, the recitation of a range of 1-12 carbon atoms (e.g., C_1-C_{12}), 1-9 carbon atoms (e.g., C_1-C_9), 1-6 carbon atoms (e.g., C_1-C_6), 1-4 carbon atoms (e.g., C_1-C_4), 1-3 carbon atoms (e.g., C_1-C_3), or 2-8 carbon atoms (e.g., C_2-C_8) as used with respect to any chemical group (e.g., alkyl, etc.) referenced herein encompasses and specifically describes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and/or 12 carbon atoms, as appropriate, as well as any sub-range thereof (e.g., 1-2 carbon atoms, 1-3 carbon atoms, 1-4 carbon atoms, 1-5 carbon atoms, 1-6 carbon atoms, 1-7 carbon atoms, 1-8 carbon atoms, 1-9 carbon atoms, 1-10 carbon atoms, 1-11 carbon atoms, 1-12 carbon atoms, 2-3 carbon atoms, 2-4 carbon atoms, 2-5 carbon atoms, 2-6 carbon atoms, 2-7 carbon atoms, 2-8 carbon atoms, 2-9 carbon atoms, 2-10 carbon atoms, 2-11 carbon atoms, 2-12 carbon atoms, 3-4 carbon atoms, 3-5 carbon atoms, 3-6 carbon atoms, 3-7 carbon atoms, 3-8 carbon atoms, 3-9 carbon atoms, 3-10 carbon atoms, 3-11 carbon atoms, 3-12 carbon atoms, 4-5 carbon

atoms, 4-6 carbon atoms, 4-7 carbon atoms, 4-8 carbon atoms, 4-9 carbon atoms, 4-10 carbon atoms, 4-11 carbon atoms, and/or 4-12 carbon atoms, etc., as appropriate).

[0058] As used herein, the terms "subject" or "individual" or "patient" may refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy is desired. Suitable vertebrate animals include, but are not restricted to, primates, avians, livestock animals (e.g., sheep, cows, horses, donkeys, pigs), laboratory test animals (e.g., rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g., cats, dogs) and captive wild animals (e.g., foxes, deer, dingoes). A preferred subject is a human in need of treatment for a disease or condition caused by or related to inflammation. However, it will be understood that the aforementioned terms do not imply that symptoms are necessarily present.

[0059] According to a first aspect of the invention, there is provided a compound of formula (I), or a pharmaceutically acceptable salt thereof:



Formula (I)

wherein, R_1 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, alkanoyl, carboalkoxy, acyloxy, aryl, aroyl, heteroaryl, heteroaroyl, heterocyclyl, heterocycloyl, cycloalkyl, O-alkyl and O-aryl, O-heteroaryl, amino and amido, all of which groups may be substituted or unsubstituted;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, alkylaryl, heterocyclyl and heteroaryl, all of which may be substituted or unsubstituted; and

R_3 and R_4 are independently selected from the group consisting of aryl, heteroaryl, heterocyclyl and cycloalkyl, all of which groups may be substituted or unsubstituted.

[0060] In one embodiment, R_1 is selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_2-C_{12} alkanoyl, C_5-C_7 aryl, C_5-C_7 aroyl, C_5-C_7 heteroaryl, C_5-C_7 heteroaroyl, C_5-C_7 heterocyclyl, C_5-C_7 heterocycloyl and C_5-C_7 cycloalkyl, all of which groups may be substituted or unsubstituted;

R_2 is selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_5-C_7 aryl and alkyl-C_5-C_7 aryl, all of which may be substituted or unsubstituted;

R_3 is selected from the group consisting of C_5-C_7 heteroaryl and C_5-C_7 heterocyclyl, each of which groups may be substituted or unsubstituted; and

R_4 is substituted or unsubstituted C_5-C_7 aryl or C_5-C_7 heteroaryl.

[0061] In one embodiment, R_1 is selected from the group consisting of C_2-C_6 alkynyl, C_2-C_6 alkanoyl, C_5-C_6 aryl, C_5-C_6 aroyl, C_5-C_6 heteroaryl, C_5-C_6 heteroaroyl, C_5-C_6 heterocyclyl and C_5-C_6 heterocycloyl, all of which groups may be substituted or unsubstituted;

R_2 is hydrogen;

R_3 is selected from the group consisting of C_6 nitrogen heteroaryl and C_6 nitrogen heterocycyl, each of which groups may be substituted or unsubstituted; and

R_4 is substituted or unsubstituted phenyl.

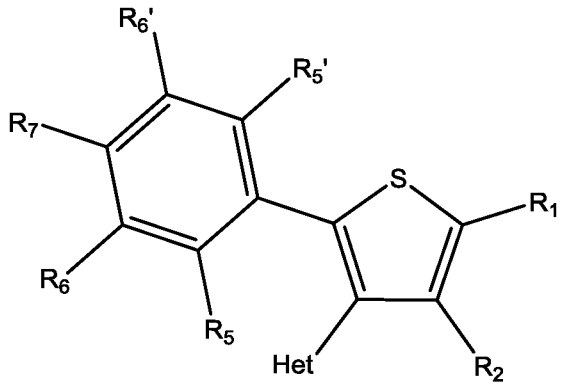
[0062] In one embodiment, R_1 is selected from the group consisting of C_2-C_6 alkynyl, C_2-C_6 alkanoyl, C_5-C_6 aryl, C_5-C_6 aroyl, C_5-C_6 heteroaryl, C_5-C_6 heteroaroyl, C_5-C_6 heterocyclyl and C_5-C_6 heterocycloyl, all of which groups may be substituted or unsubstituted;

R_2 is hydrogen;

R_3 is selected from the group consisting of pyridyl, piperidyl, pyrazyl, pyrimidyl and pyridazyl, each of which groups may be substituted or unsubstituted; and

R_4 is phenyl substituted with a substituent selected from the group consisting of halo, haloalkyl, hydroxy and nitro.

[0063] In one embodiment, there is provided a compound of formula (II), or a pharmaceutically acceptable salt thereof:



Formula (II)

wherein R_1 and R_2 are as described in any one or more of the above embodiments;

Het is selected from the group consisting of C_5-C_7 heteroaryl and C_5-C_7 heterocyclyl, each of which groups may be substituted or unsubstituted; and

R_5, R_5', R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo, haloalkyl, hydroxy and nitro.

[0064] Preferably, R_1 is selected from the group consisting of C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_2-C_{12} alkanoyl, C_5-C_7 aryl, C_5-C_7 aroyl, C_5-C_7 heteroaryl, C_5-C_7 heteroaroyl, C_5-C_7 heterocyclyl, C_5-C_7 heterocycloyl and C_5-C_7 cycloalkyl, all of which groups may be substituted or unsubstituted;

R_2 is hydrogen;

Het is selected from the group consisting of pyridyl, piperidyl, pyrazyl, pyrimidyl and pyridazyl, each of which groups may be substituted or unsubstituted; and

R_5, R_5', R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo and haloalkyl.

[0065] Preferably, R_1 is selected from the group consisting of C_2-C_6 alkynyl, C_2-C_6 alkanoyl, C_5-C_6 aryl, C_5-C_6 aroyl, C_5-C_6 heteroaryl, C_5-C_6 heteroaroyl, C_5-C_6 heterocyclyl and C_5-C_6 heterocycloyl, all of which groups may be substituted or unsubstituted;

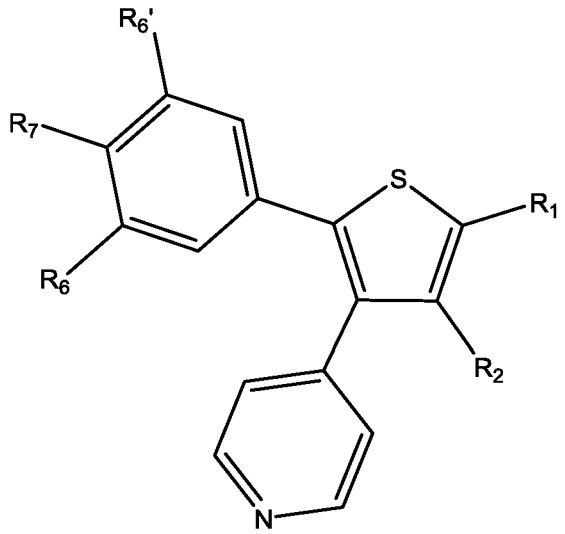
R_2 is hydrogen;

Het is selected from the group consisting of pyridyl, piperidyl and pyrimidyl, each of which groups may be substituted or unsubstituted;

R_5, R_5', R_6 and R_6' are hydrogen; and

R_7 is selected from the group consisting of halo and haloalkyl.

[0066] In one embodiment, there is provided a compound of formula (III), or a pharmaceutically acceptable salt thereof:



Formula (III)

wherein R_1, R_2, R_6, R_6' and R_7 are as described in any one or more of the above embodiments for formula (I) or formula (II).

[0067] Suitably, in one embodiment of the compound of formula (III):

R_1 is selected from the group consisting of C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_2-C_{12} alkanoyl, C_5-C_7 aryl, C_5-C_7 aroyl, C_5-C_7 heteroaryl, C_5-C_7 heteroaroyl, C_5-C_7 heterocyclyl, C_5-C_7 heterocycloyl and C_5-C_7 cycloalkyl, all of which groups may be substituted or unsubstituted;

R_2 is hydrogen; and

R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo, haloalkyl, hydroxy and nitro.

[0068] In one embodiment, wherein R_1 is selected from the group consisting of C_2-C_6 alkynyl, C_2-C_6 alkanoyl, C_5-C_6 aryl, C_5-C_6 aroyl, C_5-C_6 heteroaryl, C_5-C_6 heteroaroyl, C_5-C_6 heterocyclyl and C_5-C_6 heterocycloyl, all of which groups may be substituted or unsubstituted;

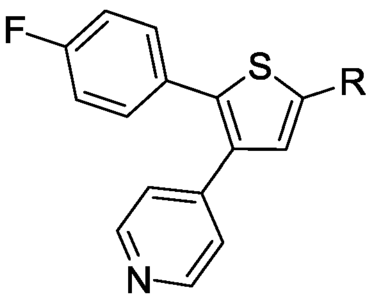
R_2 is hydrogen;

R_6 and R_6' are hydrogen; and

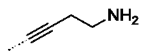
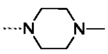
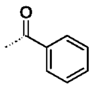
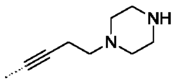
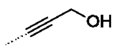
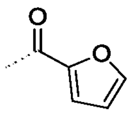
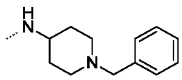
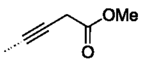
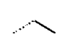
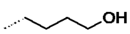
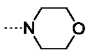
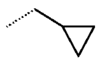
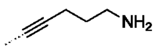
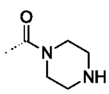
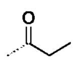
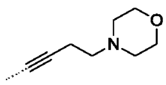
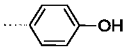
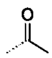
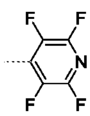
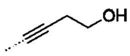
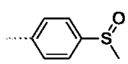
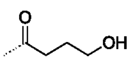
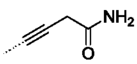
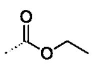
R_7 is selected from the group consisting of halo and haloalkyl.

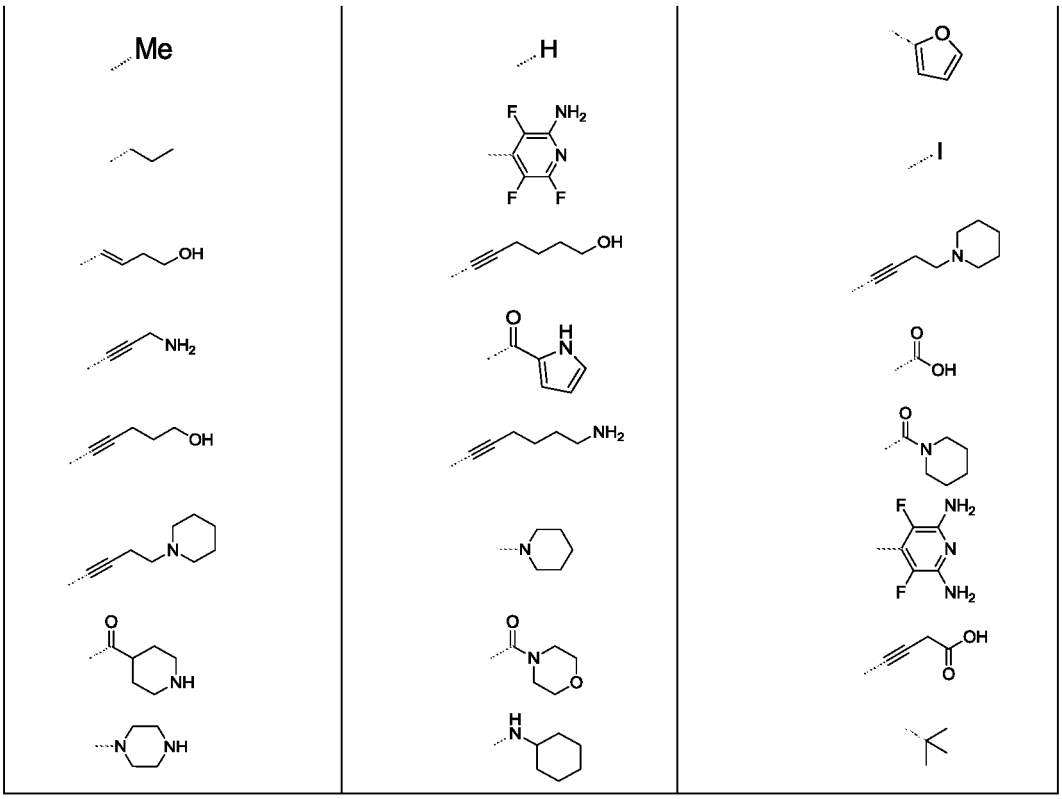
[0069] Compounds of formula (III) wherein R_2 is hydrogen have proven to be particularly efficacious as inhibitors of the p38 α MAPK enzyme and are therefore useful in reducing inflammation in a subject.

[0070] In one embodiment of formula (III) the compound is a compound of the below formula, or a pharmaceutically acceptable salt thereof:



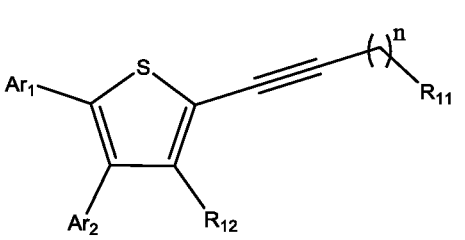
wherein R is selected from the groups shown in the below table

R*	R*	R*
		
		
		
		
		
		
		
		



* Hashed line depicts the bond that is formed.

[0071] In one preferred embodiment, there is provided a compound of formula (I) to formula (III), or a pharmaceutically acceptable salt thereof, selected from the group consisting of:



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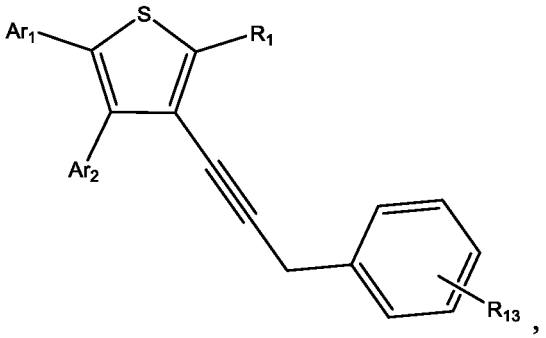

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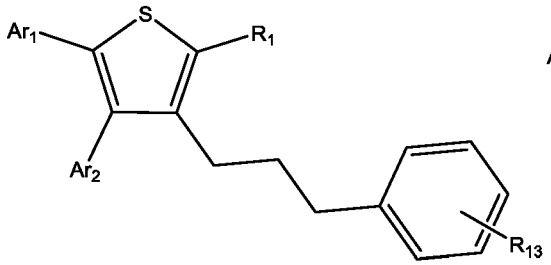

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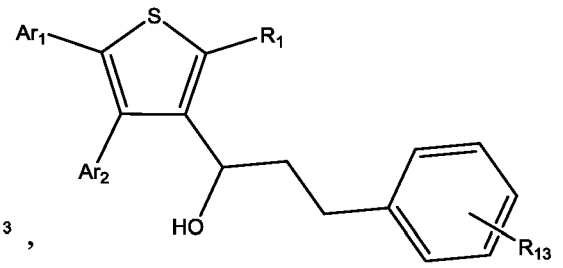

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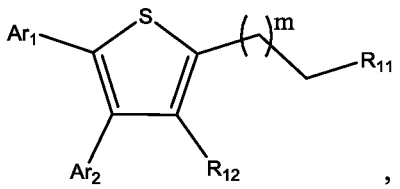

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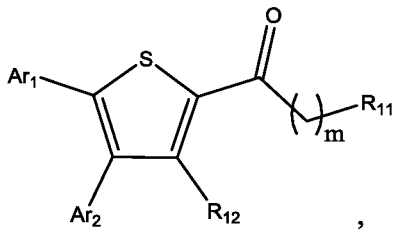

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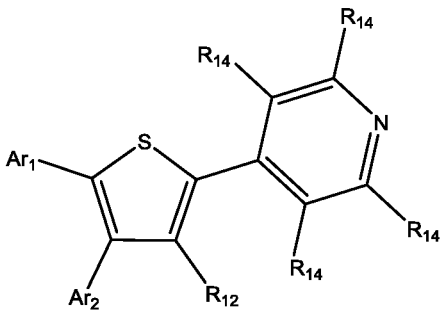

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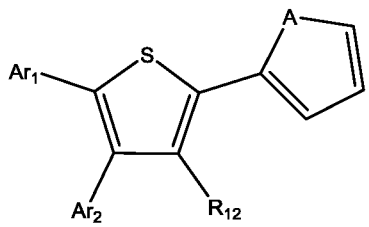


wherein, Ar_1 and Ar_2 are independently substituted or unsubstituted aryl or heteroaryl, A is selected from oxygen, sulphur or nitrogen, n is 1 or 2, m is 0 to 6, R_1 is as described in any one of the embodiments for formula (I) to (III),

R_{11} is selected from the group consisting of hydroxy, amino, C_1-C_6 alkyl, phenyl, furan, morpholine, piperazine and *N*-phthalimide;

R_{12} is selected from the group consisting of hydrogen, alkylphenyl and hydroxyalkyl phenyl wherein the phenyl ring may be substituted with R_{13};

R_{13}, when present, is selected from the group consisting of halo, amino, hydroxy, haloalkyl, C_1-C_6 alkyl and C_1-C_6 alkanoyl; and

each incidence of R_{14} is independently selected from the group consisting of hydrogen, fluoro, chloro, bromo, iodo, amino and aminoalkyl.

[0072] In one embodiment, Ar_1 is substituted or unsubstituted phenyl.

[0073] In any one of the preceding embodiments, Ar_2 is substituted or unsubstituted pyridyl.

[0074] In any one of the preceding embodiments A is preferably oxygen.

[0075] In any one of the preceding embodiments R_{12} is preferably hydrogen.

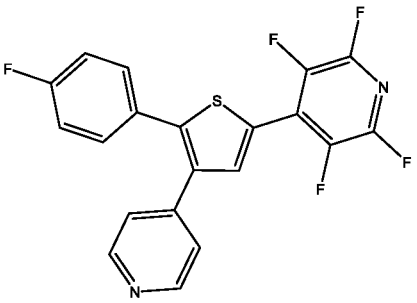
[0076] In any one of the preceding embodiments it is preferred if R_{13} is not present.

[0077] In any one of the preceding embodiments each incidence of R_{14} is independently selected from fluoro or amino.

[0078] In any one of the preceding embodiments, Ar_1 is 4-fluorophenyl.

[0079] In any one of the preceding embodiments, Ar_2 is 4-pyridyl.

[0080] In any one of the preceding embodiments, the compound of the first aspect is selected from the group consisting of: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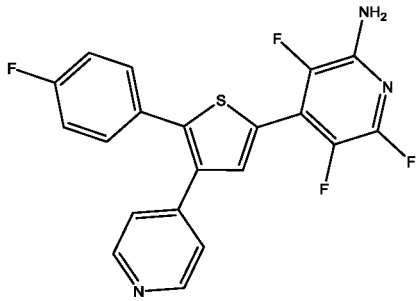
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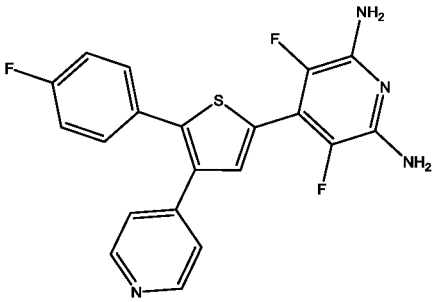
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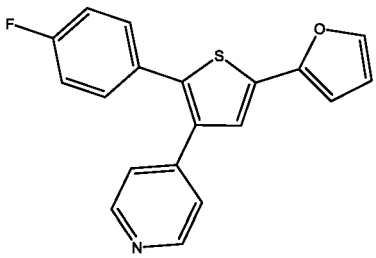
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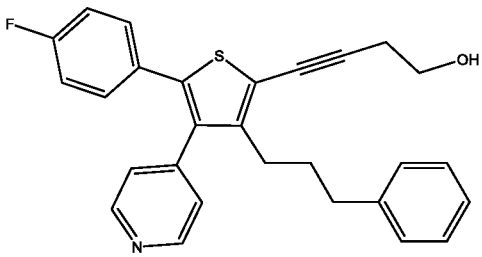
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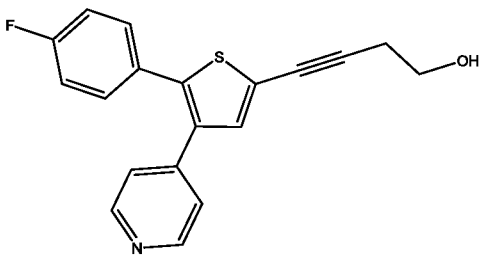
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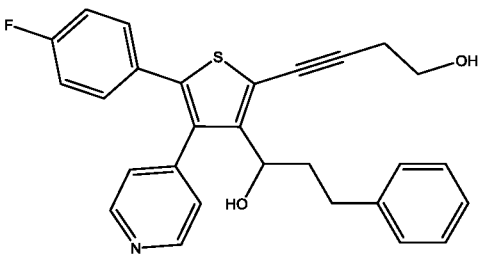
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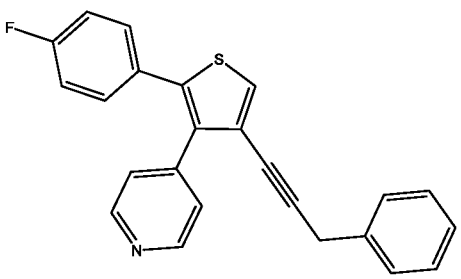
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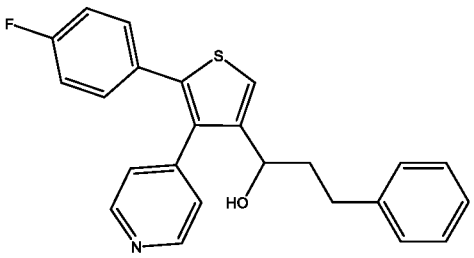
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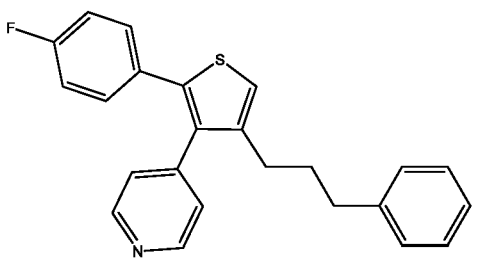
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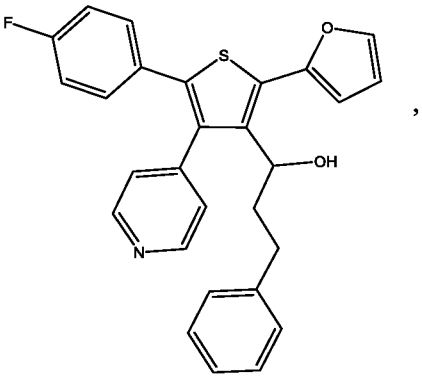
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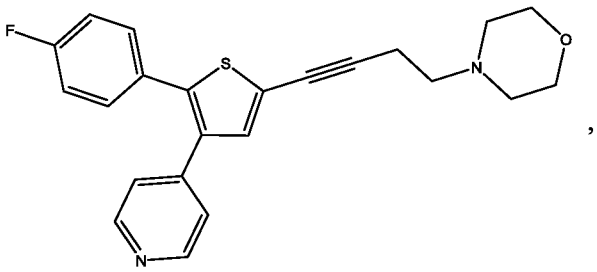
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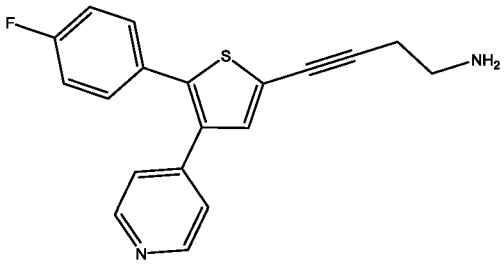
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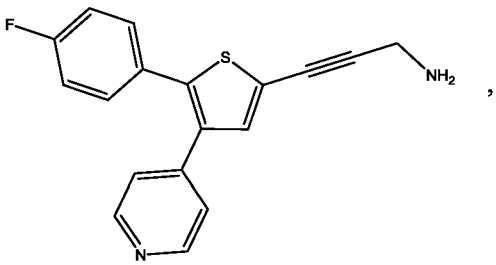
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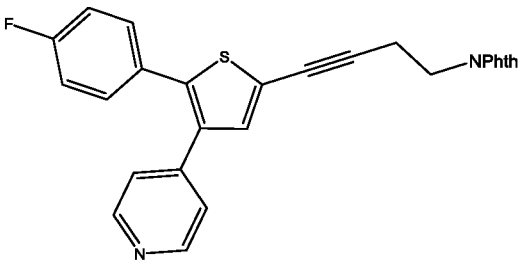
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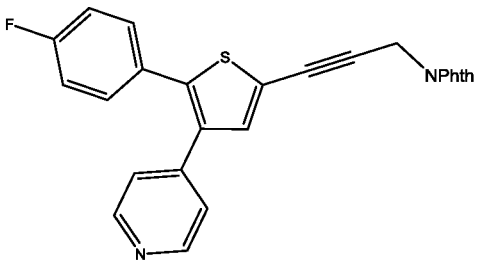
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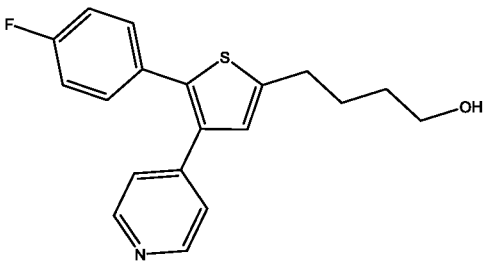
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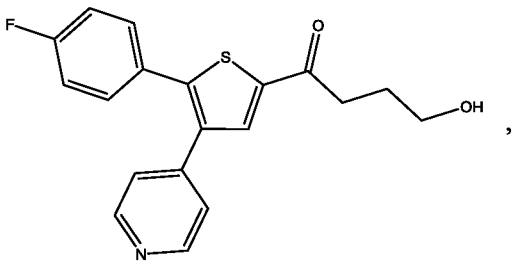
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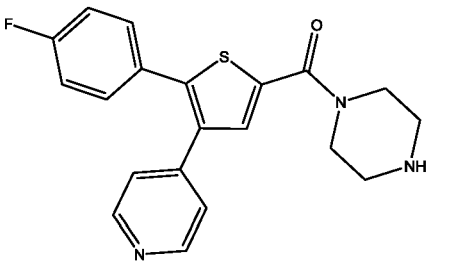
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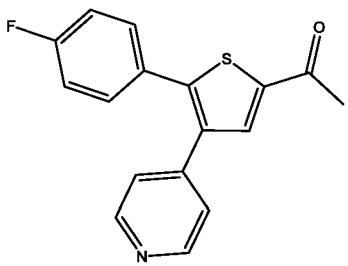
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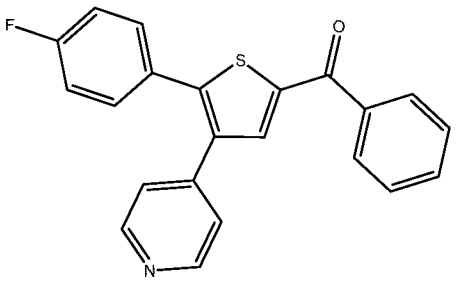

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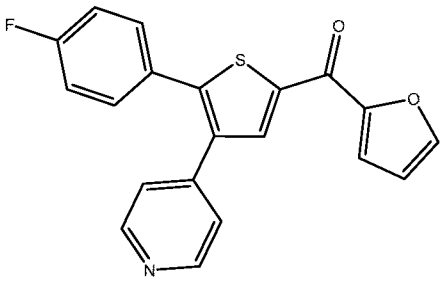

or a pharmaceutically acceptable salt thereof.

[0081] The compounds may be synthesised by a number of pathways which are outlined in the experimental section.

[0082] It will be recognised that certain compounds of the invention may possess asymmetric centres and would therefore be capable of existing in more than one stereoisomeric form. The invention thus also relates to compounds in substantially pure isomeric form at one or more asymmetric centres e.g., greater than about 90% ee, such as about 95% or 97% ee or greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be obtained by asymmetric synthesis, for example using chiral intermediates, or by chiral resolution. The compounds of the invention may, in some examples, exist as geometrical isomers. The invention also relates to compounds in substantially pure cis (Z) or trans (E) forms or mixtures thereof.

[0082a] In some embodiments, the invention provides a compound selected from the group consisting of:

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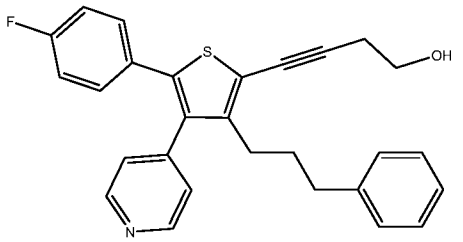

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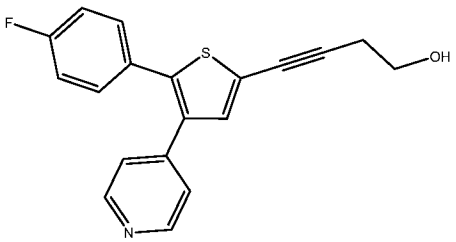

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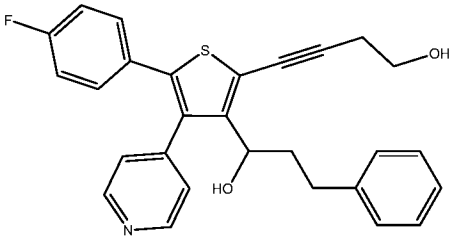

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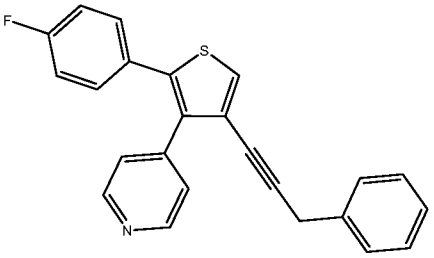

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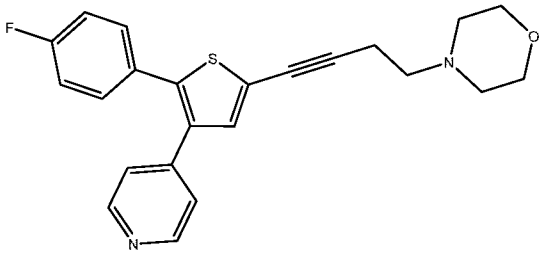

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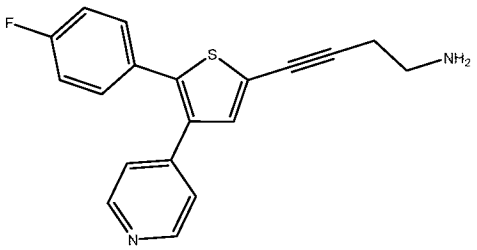

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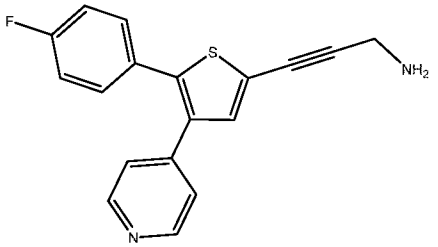

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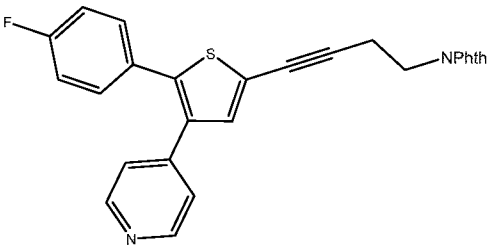

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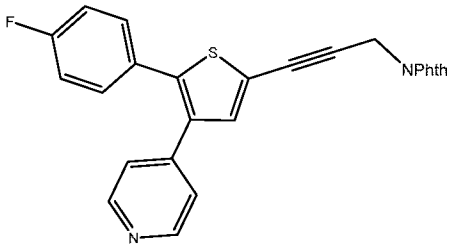

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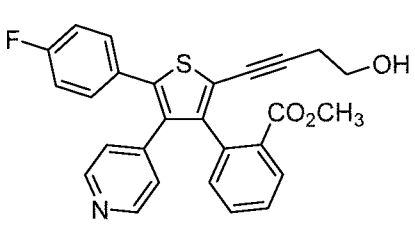
F
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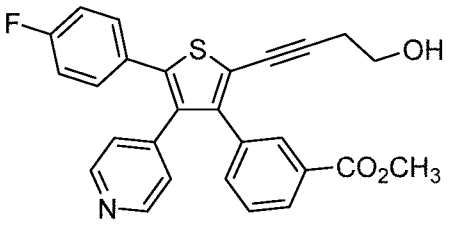
F
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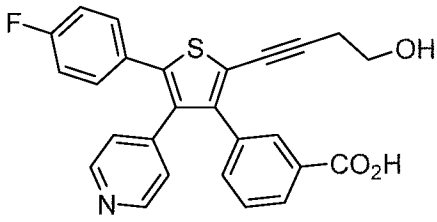
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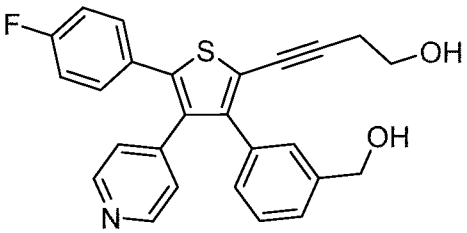
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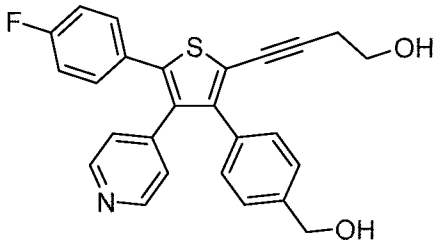
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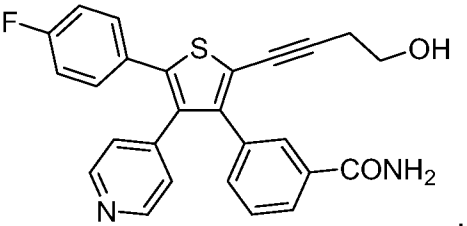
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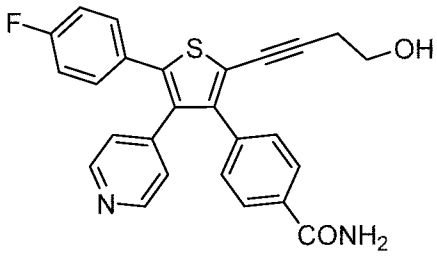
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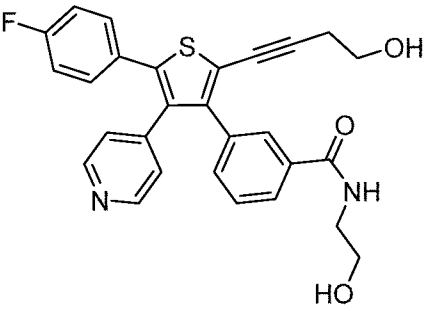
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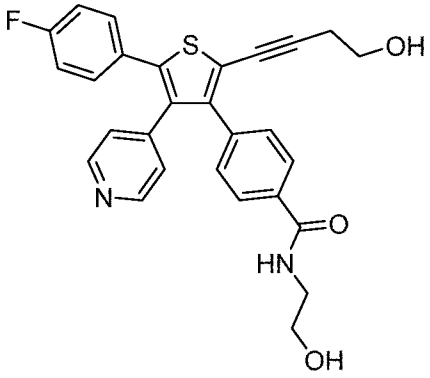
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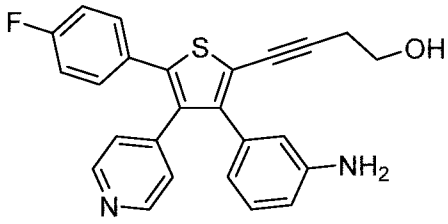
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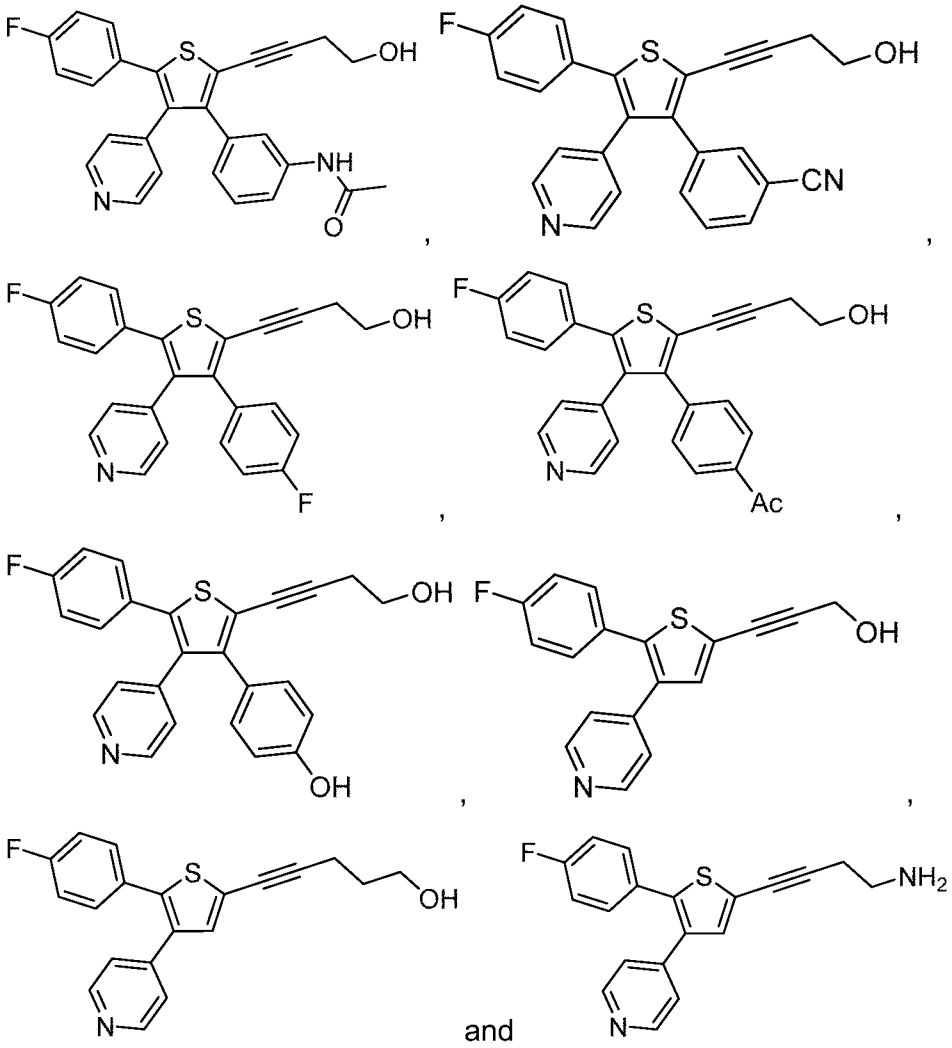


and

or a pharmaceutically acceptable salt thereof.

[0083] According to a second aspect of the invention there is provided a pharmaceutical composition comprising an effective amount of a compound of the first aspect, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent and/or excipient.

[0084] Suitably, the pharmaceutical composition is for the treatment or prophylaxis of a disease, disorder or condition responsive to MAPK inhibition, preferably p38 MAPK inhibition, more preferably p38α MAPK inhibition.

[0085] The pharmaceutical composition may include more than one compound of the first aspect. When the composition includes more than one compound then the compounds may be in any ratio. The composition may further comprise known co-actives, delivery vehicles or adjuvants.

[0086] The compound of the first aspect is present in the pharmaceutical composition in an amount sufficient to inhibit or ameliorate the disease, disorder or condition which is the subject of treatment. Suitable dosage forms and rates of the compounds and the pharmaceutical compositions containing such may be readily determined by those skilled in the art.

[0087] Dosage forms may include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting devices designed specifically for, or modified to, controlled release of the pharmaceutical composition. Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivates such as hydroxypropylmethyl cellulose. In addition, the controlled release may be affected by using other polymer matrices, liposomes and/or microspheres. Pharmaceutically acceptable carriers for systemic administration may also be incorporated into the compositions of this invention.

[0088] Suitably, the pharmaceutical composition comprises a pharmaceutically-acceptable excipient. By "pharmaceutically-acceptable excipient" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration. Depending upon the particular route of administration, a variety of carriers, well known in the art may be used. These carriers or excipients may be selected from a group including sugars, starches, cellulose and its derivates, malt, gelatine, talc, calcium sulphate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline, and pyrogen-free water.

[0089] Any suitable route of administration may be employed for providing a patient with the pharmaceutical composition of the invention. For example, oral, rectal, parenteral, sublingual, buccal, intravenous, intraarticular, intra-muscular, intra-dermal, subcutaneous, inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed.

[0090] Pharmaceutical compositions of the present invention suitable for administration may be presented in discrete units such as vials, capsules, sachets or tablets each containing a predetermined amount of one or more pharmaceutically

active compounds of the invention, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil emulsion. Such compositions may be prepared by any of the method of pharmacy but all methods include the step of bringing into association one or more pharmaceutically active compounds of the invention with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the agents of the invention with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product in to the desired presentation.

[0091] A third aspect of the invention resides in a method of treating a patient suffering from a disease, disorder or condition responsive to MAPK inhibition including the step of administering an effective amount of a compound of the first aspect, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of the second aspect to the patient.

[0092] A fourth aspect of the invention provides for a compound of the first aspect, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of the second aspect for use in the treatment of a disease, disorder or condition responsive to MAPK inhibition.

[0093] A fifth aspect of the invention provides for use of a compound of the first aspect, or a pharmaceutically effective salt thereof, in the manufacture of a medicament for the treatment of a disease, disorder or condition responsive to MAPK inhibition.

[0094] Suitably, the disease, disorder or condition of the third, fourth and fifth aspect is responsive to p38 MAPK inhibition.

[0095] Preferably, the disease, disorder or condition of the third, fourth and fifth aspect is responsive to p38α MAPK inhibition.

[0096] The method of the third aspect and use of the fourth and fifth aspects may be a method of reducing inflammation, or use in treating inflammation, in a patient by inhibiting MAPK, particularly by inhibiting p38 MAPK, more particularly by inhibiting p38α MAPK.

[0097] The disease, disorder or condition of the third, fourth and fifth aspects may be one or more of arthritis, inflammatory bowel disease, asthma, psoriasis, myocardial injury, cardiac remodelling, renal fibrosis, stroke, cancer, Alzheimer's disease, HIV, COPD, multiple myeloma, myelodysplastic syndrome, acute respiratory distress syndrome, coronary heart disease, acute coronary syndrome, major depressive disorder, dental pain, artherosclerosis, neuropathic pain and inflammation associated with any one or more of these aforementioned diseases, disorders or conditions.

[0098] Preferably, the patient is a domestic or livestock animal or a human. Most preferably, the patient or subject is a human in need of a treatment to reduce inflammation.

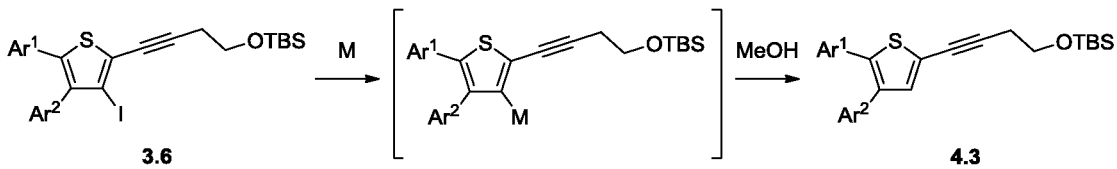
[0099] A sixth aspect of the invention provides for a complex of a compound of the first aspect, or a pharmaceutically effective salt thereof, with a p38 MAPK enzyme.

[00100] In one embodiment, the p38 MAPK enzyme is a p38α MAPK enzyme.

[00101] The following experimental section describes in more detail the characterisation of certain compounds of the invention and their binding to p38α MAPK. The intention is to illustrate certain specific embodiments of the compounds of the invention and their efficacy without limiting the invention in any way.

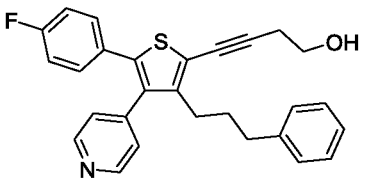
SYNTHETIC APPROACHES AND RESULTS

[00102] A synthetically accessible 3-iodothiophene **3.6** was employed as the starting material nucleophile in a metal-halogen exchange reaction. 3-Iodothiophene **3.6** was treated with isopropylmagnesium chloride lithium chloride complex at -78 °C in tetrahydrofuran. The organomagnesiate of thiophene **3.6** provides a key intermediate that could be used in a variety of organometallic reactions.



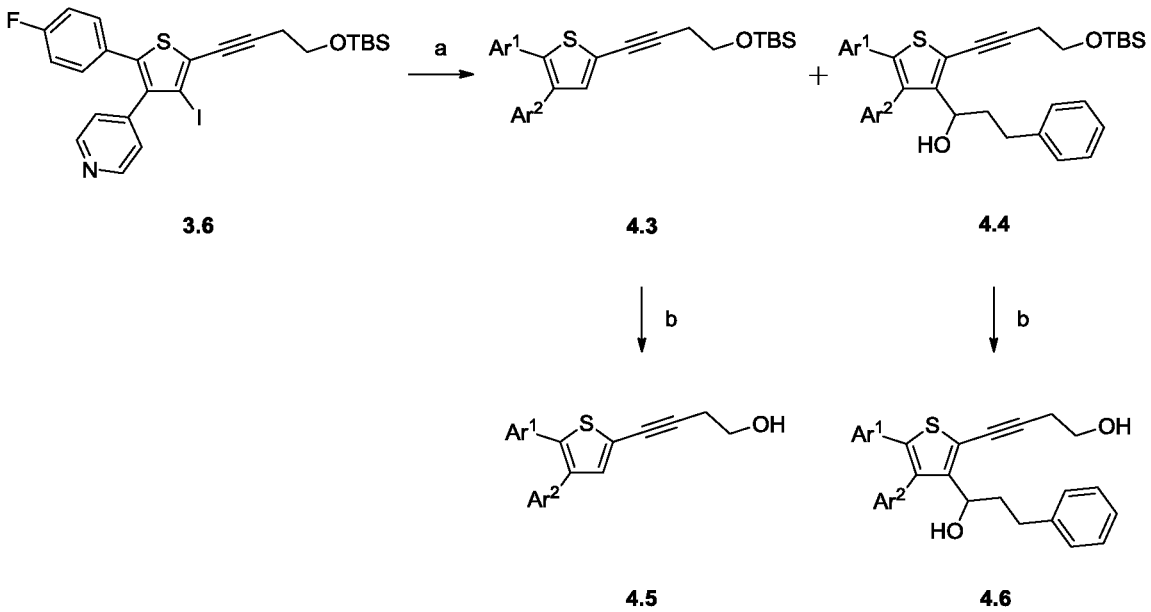
3.6 **4.3**

[00103] Synthesis of thiophene compounds **4.3** and **4.6**:



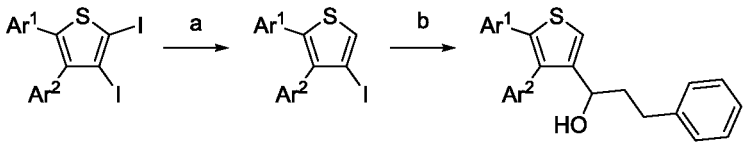
4.6

Reaction of the organomagnesium compound with hydrocinnamaldehyde gave hydroxylated analogue **4.4** in 64% yield as well as the reduced by-product **4.3**. TBS deprotection of compounds **4.3** and **4.4** with ammonium fluoride, as shown in the scheme below, gave compounds **4.5** and **4.6**.



Synthesis of hydroxylated analogue **4.6** (Ar^1 = 4-F-Ph; Ar^2 = Pyr). Reagents and conditions: (a) *i*PrMgCl.LiCl, THF, -78 °C, 30 min, hydrocinnamaldehyde, 0 °C, 1 h, **4.3**: 36%, **4.4**: 64%; (b) NH_4F, MeOH, reflux, 16 h, **4.5**: 95%, **4.6**: 99%.

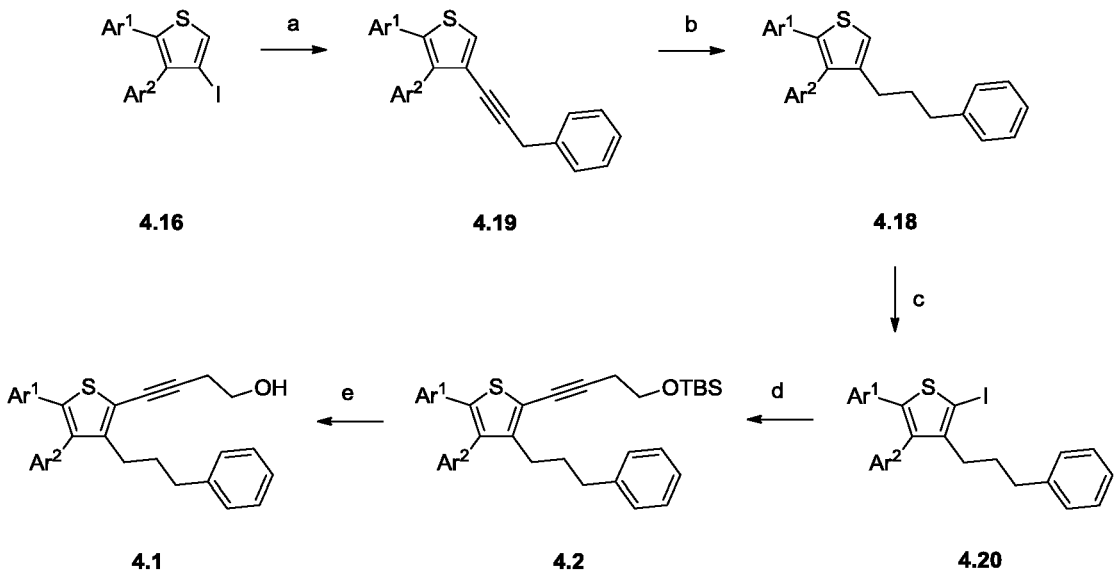
[00104] Compound **4.17** was synthesised in two steps from the di-iodinated thiophene **3.3** (Scheme below). First, a de-halogenation reaction at the α-position of the thiophene was conducted to avoid substitution at this position. Compound **3.3** was treated with isopropylmagnesium chloride lithium chloride complex at -78 °C and subsequently quenched with methanol to give the mono-iodinated compound **4.16** in 95% yield. Compound **4.16** was again metallated and reacted with hydrocinnamaldehyde to form the hydroxylated analogue **4.17** in 63% yield.



3.3 **4.16** **4.17**

Synthesis of the analogue **4.17** (Ar^1 = 4-F-Ph, Ar^2 = Pyr). Reagents and conditions: (a) *i*PrMgCl.LiCl, THF, -78 °C, 30 min, 95%; (b) *i*PrMgCl.LiCl, THF, -78 °C, 30 min, hydrocinnamaldehyde, 0 °C, 1 h, 63%.

[00105] To synthesise 3-phenylpropylthiophene **4.18** a Sonogashira cross coupling reaction with the 3-iodothiophene intermediate **4.16** was conducted followed by hydrogenation. Reaction of 3-iodothiophene **4.16**, 3-phenyl-1-propyne and the bis(triphenylphosphine)palladium(II) dichloride catalyst under basic conditions afforded alkyne **4.19** in 28% yield (Scheme shown below). Reduction of the triple bond was carried out using standard hydrogenation conditions to give 3-phenylpropylthiophene **4.18** quantitatively. Iodination of the thiophene at the α-position using silver nitrate and potassium iodide formed 2-iodothiophene **4.20** quantitatively. Synthesis of 2-iodothiophene **4.20** allowed a Sonogashira reaction with the TBS protected but-3-yn-1-ol to give the protected thiophene **4.2** in 58% yield. Reaction of compound **4.2** with ammonium fluoride in methanol at reflux gave the final product **4.1** in 96% yield.



4.16 **4.19** **4.18**

4.1 **4.2** **4.20**

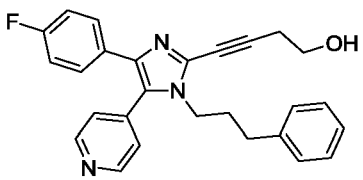
Synthesis of **4.1** (Ar^1 = 4-F-Ph, Ar^2 = Pyr). Reagents and conditions: (a) 3-phenyl-1-propyne, CuI, PPh_3, $PdCl_2(PPh_3)_2$, Et_3N, THF, 120 °C, 2 h, 28%; (b) H_2, Pd/C, EtOH, rt, 3 d, quant.; (c) $AgNO_3$, I_2, MeCN, rt,

1.5 h, quant.; (d) (but-3-yn-1-yloxy)(*tert*-butyl)dimethylsilane **3.5**, CuI, PPh_3, $PdCl_2(PPh_3)_2$, Et_3N, THF, 120 °C, 2 h, 58%; (e) NH_4F, MeOH, reflux, 16 h, 96%.

Biological evaluation of Compound 4.1 and related thiophene analogues

[00106] The objective of the biological studies on the compound **4.1** thiophene and analogues was to determine the binding affinities to both the inactive and active forms of p38α MAPK and subsequently their inhibitory activity. Certain compounds were also selected for study in an *in vitro* activation assay to determine whether they also inhibit the phosphorylation of p38α MAPK. Further evaluation in cellular assays was carried out to establish whether the compounds suppress production of pro-inflammatory cytokines TNF-α and IL-6. These inflammatory mediators are known to play a role in cardiac remodelling and heart failure progression and therefore cellular assays on cardiac myocytes and fibroblasts were conducted to investigate the effects of the compounds on cardiac hypertrophy and fibrosis. An important factor is to determine the metabolic and toxicity profiles of the synthesised compounds.

[00107] The binding affinity of **4.1** and intermediates and related compounds were determined using a fluorescence polarisation (FP) binding assay using both the inactive non-phosphorylated and the active phosphorylated forms of p38α MAPK. A number of compounds were also assessed for the inhibition of p38α MAPK activity (www.kinase-screen.mrc.ac.uk) in which a radioactive (^{33}P-labelled ATP) filter binding assay was used to directly measure phosphate incorporation. Table 1 summarises the binding affinities of the synthesised compounds to both forms of the enzyme as well as the inhibitory activities against p38α MAPK. By way of enabling a comparison with a non-thiophene compound the biological testing was also carried out for known inhibitor RWJ67657, shown in table 1.

Compound	K_i (μM, mean ± SEM) inactive p38α	K_i (μM, mean ± SEM) active p38α	IC_{50} (μM, mean)
 RWJ67657 (**1.49**)	0.21 ± 0.04	0.013 ± 0.006	lit. 0.03 ± 0.003

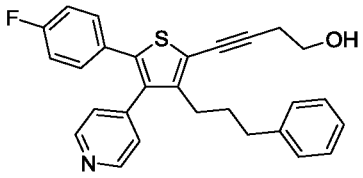
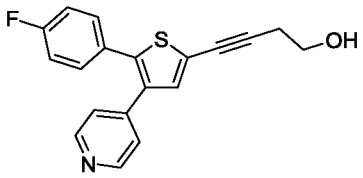
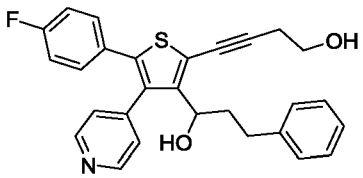
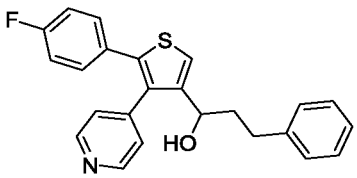
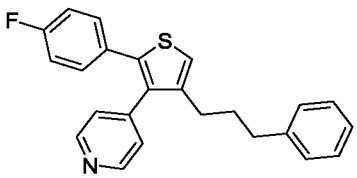
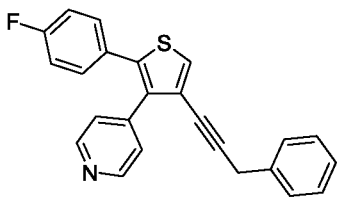
 **4.1**	2.3 ± 0.2	0.6 ± 0.1	0.18
 **4.5**	2.0 ± 0.2	0.56 ± 0.06	0.16
 **4.6**	> 10.0	3.85	4.72
 **4.17**	1.9 ± 0.3	0.99 ± 0.09	0.26
 **4.18**	1.5 ± 0.5	0.72 ± 0.03	0.27
 **4.19**	> 10.0	9.44	n.d.

Table 1: n.d. not determined. For compounds with K_i values below 3 μM; binding assay was carried out in triplicate with n = 3 experiments using inactive p38α MAPK and in duplicate with n = 2 - 3 experiments using active p38α MAPK. IC_{50} values were determined by taking the average of two experiments.

[00108] It was observed that the binding affinities of the thiophene compounds were often two- to four-fold stronger for the phosphorylated form of the enzyme relative to the non-phosphorylated form. Some structure activity relationships can be deduced from the data. Addition of a benzylic hydroxyl group resulted in some loss of

activity with compound **4.6** having an IC_{50} value of 4.72 μM. Interestingly, the removal of the alkyne substituent in compound **4.17**, compared to the tetra-substituted compound **4.6**, significantly improves the activity giving an IC_{50} of 0.26 μM. Compound **4.18** was equipotent with an IC_{50} of 0.27 μM. Having an alkyne in the 3-position of the thiophene in compound **4.19** results in the complete loss of binding, suggesting that the rigidity of the alkyne at this position may be detrimental to activity. However, removing the substituent at the 3-position while keeping the butynol moiety resulted in good binding with compound **4.5** having a K_i value of 0.56 μM to active p38α MAPK and moderate inhibitory activity with an IC_{50} of 0.16 μM. In summary, the similarity in the inhibitory activity values of the two best compounds, **4.1** and **4.5**, suggest that the addition of a fourth substituent to the thiophene core is not necessary for activity. Compounds **4.17** and **4.18**, which have moderate IC_{50} values, also indicate that three substituents around the thiophene core are preferred for binding and inhibition.

In Vitro Activation Assay

[00109] The most potent compounds were also assessed in an *in vitro* activation assay using a published method. The assay was analysed by immunoblotting using antibodies against pan p38 MAPK and activated (phosphorylated) p38α MAPK. The assay evaluates whether compounds bind to the inactive non-phosphorylated p38α enzyme and prevent its activation by upstream MKK6. The protocol involves pre-incubating inactive non-phosphorylated p38α MAPK with the test compounds at 10 and 1 μM concentrations for 30 minutes. The reaction is initiated by the addition of ATP and MKK6 and proceeds for 15 minutes. Ethylenediaminetetracetic acid (EDTA) was used to stop the reaction and subsequent western blot analysis was carried out to qualitatively determine the extent of inhibition of p38α MAPK activation (phosphorylation). FIG 1 shows the western blots of the activation assays. All compounds were found to inhibit phosphorylation of p38α MAPK at 10 μM concentration. Initial ligand binding experiments showed these compounds bind weakly to the inactive non-phosphorylated form of p38α MAPK (Table 1). This would suggest that the compounds to some extent occupy the active site of the non-phosphorylated enzyme causing conformational changes that prevent its activation.

Cellular Assays

[00110] Assays were conducted to determine the anti-inflammatory effects of the synthesised thiophene **4.1** and its analogues by inhibition of cytokine gene expression. Further downstream effects were also investigated. Pro-inflammatory cytokines are known to adversely affect cardiac function by stimulation of cardiac remodelling including hypertrophy and collagen synthesis (fibrosis). Therefore the effects of the synthesised compounds on cardiac remodelling were also determined.

Anti-inflammatory Effects of **4.1** and Analogues

[00111] Certain of the compounds were tested in a cellular assay for suppression of TNF-α and IL-6 gene expression in monocytic (THP-1) cells and the assay results are depicted in FIGs 2 and 3. A published method was used whereby treating THP-1 cells with LPS induces TNF-α and IL-6 gene expression. FIGs 2 and 3 include the cytokine suppression by the two most potent compounds synthesised, compound **4.1** and the tri-substituted thiophene **4.5**. The tri-substituted thiophene **4.5** showed inhibition of TNF-α gene expression at 1 μM concentration and higher (FIG 2). All of the tested analogues showed strong inhibition of LPS-induced IL-6 gene expression, including **4.1** and **4.5** as shown in FIG 3.

Effects on cellular functions relevant to cardiac remodelling

Measurement of neonatal rat cardiac myocyte hypertrophy

[00112] Neonatal rat cardiac myocyte (NCM) hypertrophy was determined by ^{3}H-leucine incorporation following a published protocol on the above synthesised compounds using either Angiotensin II (AngII, 100 nM) or TNF-α (10 ng/mL) as the stimulus. ^{3}H levels were counted in scintillation fluid on a beta counter to determine levels of ^{3}H-leucine incorporation. Both AngII and TNF-α are known activators of p38α MAPK and as expected AngII and TNF-α significantly stimulated NCM hypertrophy. All of the tested analogues dose-dependently suppressed both AngII- and TNF-α-induced NCM hypertrophy. FIGs 4 and 5 show the suppression of NCM hypertrophy by a number of compounds of the present invention, including compounds **4.1** and **4.5**.

Measurement of neonatal rat cardiac fibroblast collagen synthesis

[00113] The effect of the compounds on neonatal rat cardiac fibroblasts (NCF) collagen synthesis was determined by measuring ^{3}H-proline incorporation following a

published protocol using AngII (100 nM) as the stimulus. ^{3}H-proline incorporation was determined in the same way as the cardiac myocyte hypertrophy assay described above. AngII significantly stimulated NCF collagen synthesis. Compound **4.5** showed a dose-dependent inhibition of AngII-simulated NCF collagen synthesis (FIG 6). However, compound **4.1** did not suppress AngII-induced NCF collagen synthesis at 1 μM concentration.

Measurement of cell viability in neonatal rat cardiac fibroblasts

[00114] Measurement of NCF cell viability was carried out using a published method with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetraxolium bromide (MTT) assay. Both compounds **4.1** and **4.5** demonstrated good toxicity profiles having no affect on NCF cell viability at 10 and 25 μM concentration, respectively (FIG 9).

Effects on cellular functions related to renal fibrosis

Measurement of rat mesangial cell collagen synthesis

[00115] The effects of the compounds on rat mesangial cell (RMC) collagen synthesis were determined by measuring ^{3}H-proline incorporation following a published protocol using AngII (100 nM), uremic toxins: indoxyl sulphate (IS, 10μM), p-cresol sulphate (PCS, 100μM) and m-cresol sulphate (MCS, 100μM) as the stimulus. ^{3}H-proline incorporation was determined in the same way as the NCF collagen synthesis assay described above. AngII, IS, PCS and MCS significantly stimulated RMC collagen synthesis. Compound **4.5** showed a dose-dependent inhibition of AngII-, IS-, PCS- and MCS-simulated RMC collagen synthesis (FIG 8).

*In vitro metabolism of compounds **4.1** and **4.5***

[00116] The metabolic stability of compounds **4.1** and **4.5** was assayed in an in vitro assay which employed liver microsomes. Liver microsomes are valuable models for the determination of hepatic clearance because they contain a number of drug metabolising enzymes, such as the CYP450 enzymes. An *in vitro* metabolic stability study was conducted using human, rat and mouse liver microsomes as a prediction of the *in vivo* metabolic clearance. Table 2 shows the metabolic stability parameters for the two compounds. From the results, the rates of apparent compound degradation in human, rat and mouse liver microsomes were in agreement with the moderate to high microsome-predicted extraction ratios (E_H) for both compounds **4.1**

and **4.5**. Compound **4.5** had a better metabolic stability profile in all three species of liver microsomes. This compound had an acceptable intrinsic clearance and E_H values in human microsomes, though the half life is relatively short.

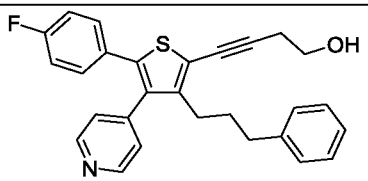
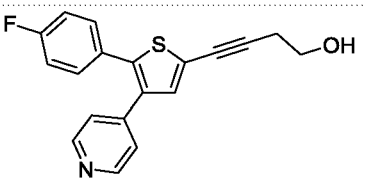
Compound	Species	Apparent Degradation half-life (min)	*In vitro* CL_{int} (µL/min/mg protein)	Microsome predicted E_H
 **4.1**	Human	8	219	0.92
	Rat	5*	319*	0.91*
	Mouse	5*	342*	0.94*
 **4.5**	Human	71	24	0.58
	Rat	16	110	0.78
	Mouse	9	184	0.89

Table 2: Metabolic stability parameters for compounds **4.1** and **4.5** based on nicotinamide adenine dinucleotide phosphate (NADPH)-dependent degradation profiles in human, rat and mouse liver microsomes. * Value is an approximation as concentrations were only above the analytical lower limit of quantification (LLQ) up to 5 min and degradation parameters were estimated using the initial two time points only (i.e. 2 and 5 min).

Acute Toxicity Study

[00117] An acute toxicity study of compound **4.5** was carried out. In this toxicity study, four male Sprague-Dawley (SD) rats received 150 mg/kg of compound **4.5** twice daily by oral gavage over a period of two weeks. The control group consisting of four male SD rats received 1% carboxymethylcellulose (CMC) in water. Blood samples were collected for analysis at 1 and 12 hours after the first dose and at the 7 and 14 day troughs. The rats were then sacrificed and organs collected for analysis. Individual body weights were measured at day -1, 2, 4, 7, 10 and 14. FIG 7 indicates the blood results from an acute toxicity study for **4.5**. FIG 10a shows the individual body weight gain of each rat over the course of two weeks. There was no difference in the average body weight compared to the vehicle group (FIG 10b). The weights of the kidneys, heart and liver were the same as the vehicle group but a loss in the mass of the spleen was observed (FIG 11).

[00118] The full blood, haemoglobin, haemocrit, red cell count, mean corpuscular haemoglobin, mean corpuscular volume, red cell distribution width, mean

corpuscular haemoglobin concentration and liver function were all assessed. No differences were observed between treated and vehicle groups. Given that liver toxicity has resulted in the failure of many p38α MAPK inhibitors, it was promising to see that there were no changes to liver function. The CDCO analysed blood samples using HPLC-MS to determine the concentrations of compound **4.5** in rat plasma at different time points (Table 3). An intake of 300mg/kg/day of compound **4.5** resulted in peak plasma levels two to nine times higher than the enzyme IC_{50} value. This suggests that there is a sufficient amount of compound in circulation. Overall, the results of the acute toxicity study indicate that compound **4.5** is well tolerated in rats.

Sample Time	Rat 1	Rat 2*	Rat 3	Rat 4
1 hour post first dose	1357	0.9	325	327
12 hours post first dose	8	7	147	5
7 day trough	5	424	58	6
14 day trough	7	105	32	14

Table 3: Concentrations (nM) of compound **4.5** in rat plasma. * The concentration profile for rat 2 appears atypical however data were confirmed by analysis of the second (duplicate) sample set from this animal. Data for rat 2 was excluded from the analysis.

Summary for Compound **4.1** and Related Compounds

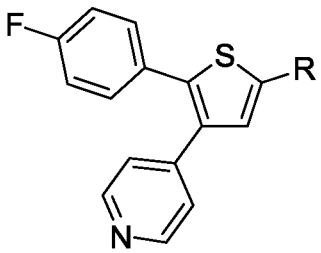
[00119] Most of the synthesised analogues tested above showed potent inhibitory activity against p38α MAPK and therefore, were expected to show anti-inflammatory properties. *In vitro*, the synthesised thiophene analogues were all shown to suppress IL-6 production from LPS-stimulated THP-1 cells in a dose dependent manner. Some inhibition of TNF-α production was also observed. Additional assays were conducted to determine whether the compounds could affect cardiac remodelling. Investigation into two major mechanisms of cardiac remodelling, cardiac myocyte hypertrophy and cardiac fibroblast collagen synthesis, have given positive results. Both AngII and TNF-α induced cardiac myocyte hypertrophy were inhibited by all synthesised analogues in a dose-dependent manner. At high concentrations, these compounds also suppress cardiac fibroblast collagen synthesis. Given that inflammatory cytokines are known to adversely affect cardiac remodelling, inhibition of the p38α MAPK pathway is a potential avenue for attenuating cardiac remodelling associated with cardiac disease. As an example, compound **4.5** dose-dependently inhibited Angiotensin II and uremic toxins (IS, PCS and MCS) stimulated rat mesangial cell collagen synthesis. Given that the renin angiotensin aldosterone system, inflammatory cytokines and uremic toxins are known

to adversely affect renal fibrosis, inhibition of the p38α MAPK pathway is a potential avenue for attenuating renal fibrosis associated with kidney disease.

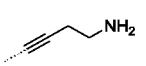
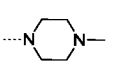
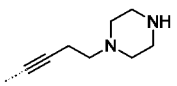
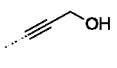
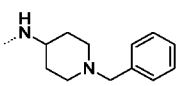
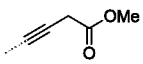
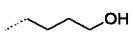
[00120] Furthermore, metabolic stability profiling of the two best compounds found that both compounds **4.1** and **4.5** had high degradation rates and moderate to high microsome-predicted extraction ratios. Compound **4.5** had a better metabolic stability profile than compound **4.1** in human, rat and mouse liver microsomes and subsequently was investigated in an acute toxicity study. This compound was well tolerated in the rat model. A dose of 300 mg/kg/day resulted in peak circulating concentration between 325 and 1357 nM which is well above the concentrations required to inhibit p38α MAPK, indicating that there was sufficient amount of compound in circulation.

Studies Towards Improved Thiophene p38α MAPK Inhibitors

[00121] Further experiments focused on potentially improving the physicochemical properties of the thiophene compounds by reducing molecular weight and lipophilicity. To guide this further work molecular modelling was carried out based around the thiphene core shown below:



[00122] A range of potential substituents were docked into the 1BL7 and 2EWA crystal structures to predict the binding conformation within the p38α MAPK active site. The results are shown in Table 4, below.

Rank	R*	Score	Rank	R*	Score	Rank	R*	Score
1		-8.83	17		-7.71	33		-7.26
2		-8.60	18		-7.67	34		-7.23
3		-8.31	19		-7.66	35		-7.22
4		-8.27	20		-7.66	36		-7.20

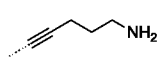
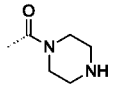
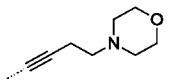
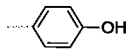
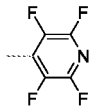
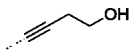
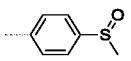
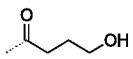
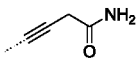
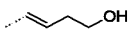
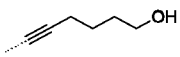
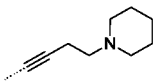
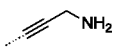
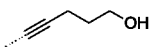
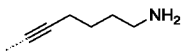
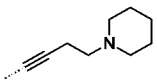
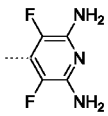
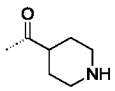
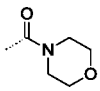
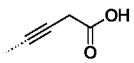
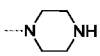
5		-8.21	21		-7.65	37		-7.20
6		-8.12	22		-7.61	38		-7.15
7		-7.96	23		-7.60	39		-7.10
8		-7.91	24		-7.54	40		-7.05
9		-7.91	25		-7.53	41		-7.05
10		-7.90	26		-7.48	42		-7.01
11		-7.85	27		-7.46	43		-6.94
12		-7.82	28		-7.46	44		-6.93
13		-7.80	29		-7.35	45		-6.86
14		-7.78	30		-7.35	46		-6.84
15		-7.73	31		-7.27	47		-6.81
16		-7.72	32		-7.26	48		-6.19

Table 4: Ensemble scores of designed compounds docked into the 1BL7-2EWA crystal structures. * Hashed line depicts the bond that is formed.

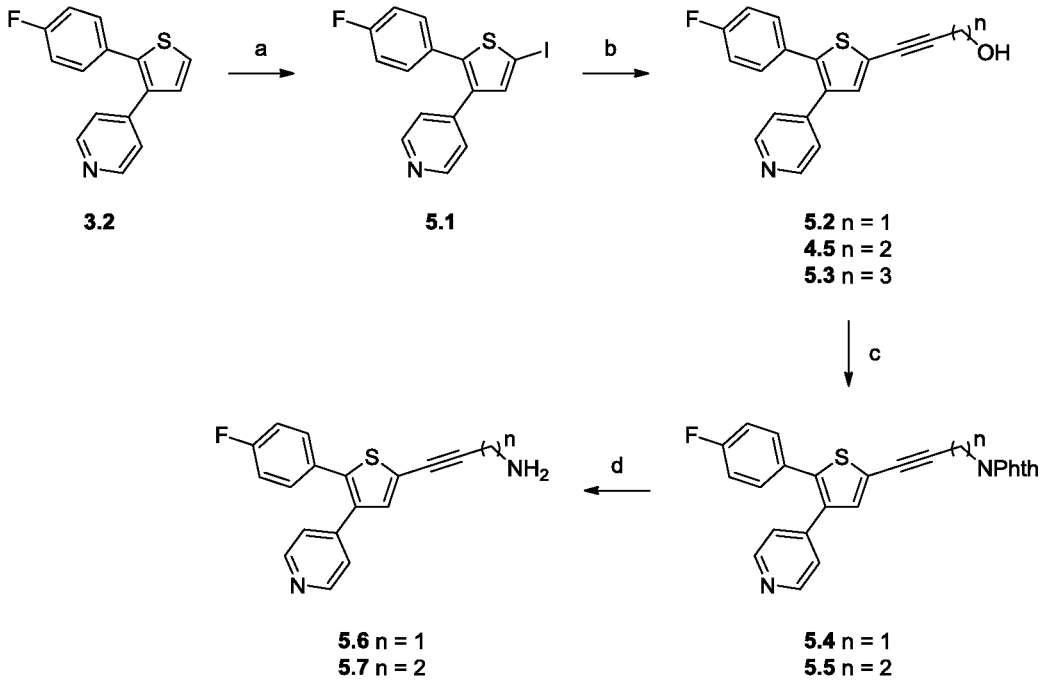
[00123] From the ensemble scores of the docked structures shown in Table 4, the best scoring compound contains a terminal amine as a replacement for the hydroxyl group. This analogue is able to form hydrogen bonding interactions with the surrounding Asp168 and Asn155 side chains. Generally the alkynyl amine analogues with differing chain lengths performed well in ensemble docking. Analogues in which the hydroxyl group was substituted with piperazine or morpholine moieties were also ranked highly. Using the rankings from the ensemble docking study the approach was undertaken to synthesise a range of compounds with differing chain lengths, terminal hydrogen bond donors, hydrogen bond acceptors in the α position and those with

increased flexibility to improve potency while also monitoring physicochemical properties such as lipophilicity.

Synthesis of tri-substituted thiophene analogues

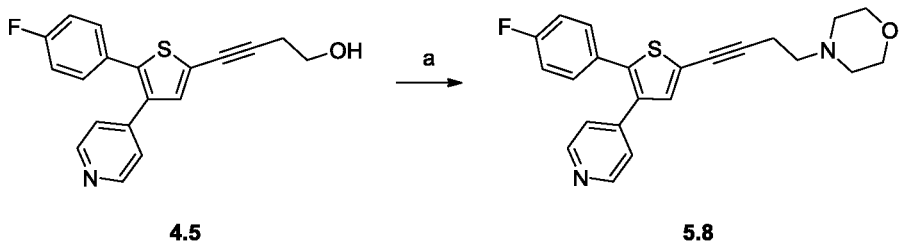
Modifications to chain length and replacement of the hydroxyl group

[00124] In order to investigate analogues with different chain lengths the synthesis of compound **4.5** was simplified (Scheme below). First, iodination of compound **3.2** at the α-position using silver nitrate and molecular iodine gave 2-iodothiophene **5.1** as a white powder in 74% yield. Sonogashira reaction of 2-iodothiophene **5.1** with the appropriate alkynyl alcohol gave the cross coupled product in high yields using copper iodide, triphenylphosphine, bis(triphenylphosphine)palladium(II) dichloride and triethylamine. The propargyl alcohol **5.2**, butynyl alcohol **4.5** and the pentynyl alcohol **5.3** were synthesised in 76%, 87% and 85% yield respectively. Results from the docking study found that the alkynyl amines were able to bind to the p38α MAPK structure in a low energy conformation. Therefore the synthesised alkynyl alcohol analogues were transformed into the corresponding alkynyl amines (as shown in the below scheme). To synthesise these analogues a Mitsunobu reaction was conducted in which *N,N*-diisopropylazodicarboxylate was added to a mixture of the alkynyl alcohol, phthalimide and triphenylphosphine. This reaction enabled substitution of the alcohol moiety for a phthalimide group producing the propargyl phthalimide **5.4** and butynyl phthalimide **5.5** in 51% and 93% yield, respectively. Cleavage of the phthalyl group with hydrazine monohydrate gave amines **5.6** and **5.7** in 78% and 75% yield respectively.



Synthesis of alkynyl alcohols **4.5, 5.2** and **5.3** and alkynyl amines **5.6** and **5.7**. Reagents and conditions: (a) $AgNO_3$, I_2, MeCN, rt, 16 h, 74%; (b) $PdCl_2(PPh_3)_2$, alkynyl alcohol, PPh_3, CuI, Et_3N, THF, reflux, 2 h, **5.2**: 76%, **4.5**: 87%, **5.3**: 85%; (c) Phthalimide, DIAD, PPh_3, THF, rt, 20 h, **5.4**: 51%, **5.5**: 93%; (d) $NH_2NH_2.H_2O$, MeOH/EtOH, **5.6**: 65%, **5.7**: 69%.

[00125] The alcohol group of compound **4.5** was also substituted with a morpholine (Scheme below). Compound **4.5** was treated with mesyl chloride then heated in morpholine at 100 °C to produce morpholine analogue **5.8** in 32% yield.

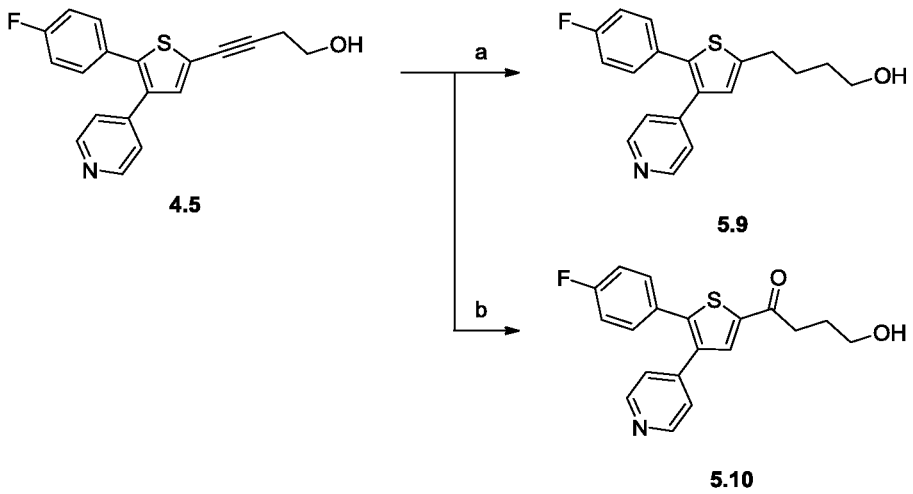


Synthesis of analogue **5.8**. Reagents and conditions: (a) MsCl, $CHCl_3$, 0 °C, 1 h, morpholine, 100 °C, 1 h, 32%.

Modifications to the alkyne functional group

[00126] To determine whether the alkyne functionality was needed for p38α MAPK binding, the butynyl substituent was reduced to increase flexibility. Reduction of the butynyl substituent was conducted using palladium on carbon under a hydrogen atmosphere to give butanol analogue **5.9** in 40% yield (Scheme below).

The alkyne of compound **4.5** was also hydrated to a ketone using sulfuric acid to give analogue **5.10** in good yield (90%).

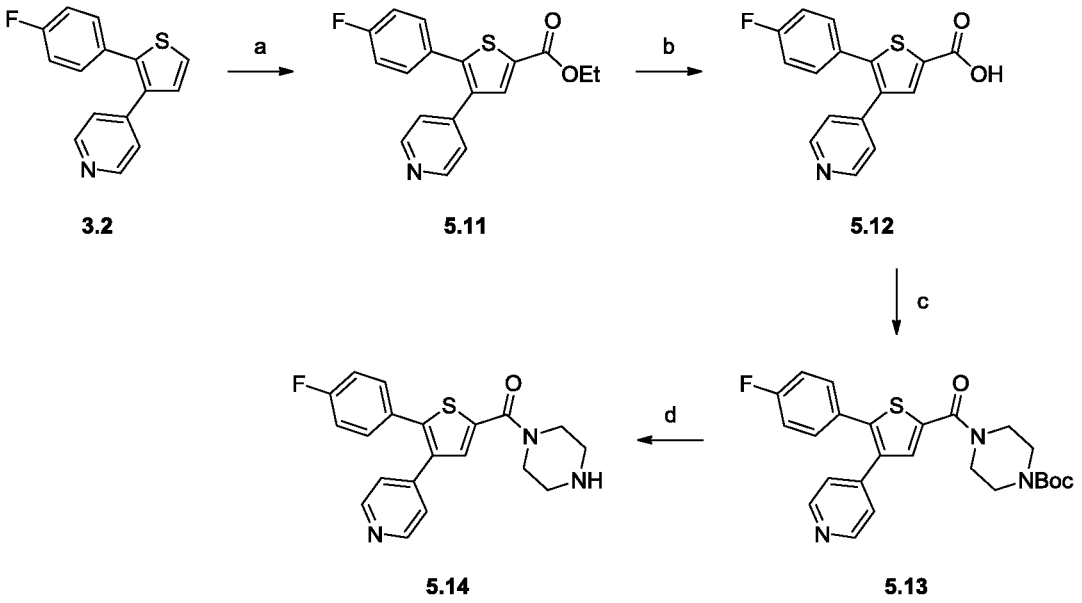


4.5 **5.9** **5.10**

Synthesis of analogues **5.9** and **5.10**. Reagents and conditions: (a) Pd/C, H_2, EtOH, rt, 3 d, 40%; (b) H_2SO_4, $(CH_3)_2CO$, 0°C - rt, 1.5 h, 71%.

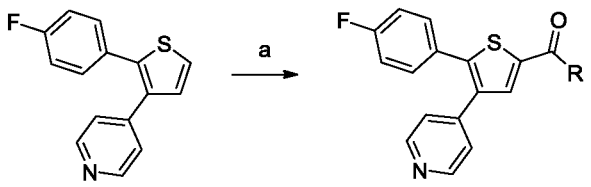
Carbonyl derivatives

[00127] The carboxypiperazine analogue **5.14** was ranked twenty-first in the docking study. To incorporate a carbonyl moiety in the α-position of the thiophene, compound **3.2** was reacted with *n*-butyllithium (Scheme below). The lithiated thiophene was reacted with ethyl chloroformate to form ethyl ester **5.11** in 55% yield. Hydrolysis of ethyl ester **5.11** with aqueous sodium hydroxide in ethanol gave acid **5.12** in 85% yield. Acid **5.12** was converted to the acid chloride using oxalyl chloride and a catalytic amount of *N,N*-dimethylformamide and then immediately reacted with 1-boc-piperazine to form the protected carboxypiperazine analogue **5.13** in 61% yield. Boc deprotection using trifluoroacetic acid in dichloromethane gave the desired carboxypiperazine **5.14** in 72% yield.



Synthesis of carboxypiperazine **5.14**. Reagents and conditions: (a) *n*BuLi, THF, -78 °C, 30 min, $ClCO_2Et$, rt, 3 h, 55%; (b) NaOH, EtOH, H_2O, 50 °C, 2 h, 85%; (c) $(COCl)_2$, DMF, DCM, rt, 2 h, 1-Boc-piperazine, DIPEA, DCM, rt, 4 h, 61%; (d) TFA, DCM, rt, 2 h, 72%.

[00128] Other carbonyl derivatives were synthesised using a Friedel-Crafts acylation reaction (Scheme below). The acid chloride was treated with aluminium chloride in dichloromethane, and then refluxed with compound **3.2**. Under these reaction conditions the acetyl chloride, benzoyl chloride and furoyl chloride all gave the desired analogues **5.15**, **5.16** and **5.17** in good yields.



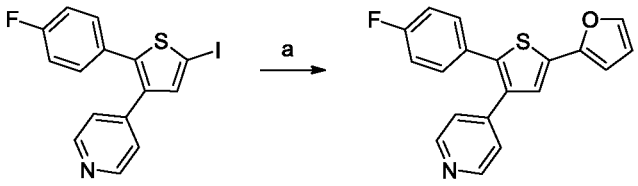
3.2

5.15 R = Me
5.16 R = Ph
5.17 R = 2-Furan

Synthesis of acyl analogues **5.15 - 5.17**. Reagents and conditions: (a) acid chloride, $AlCl_3$, DCM, reflux, 16 h, **5.15**: 67%. **5.16**: 86%, **5.17**: 85%.

Aromatic substituents

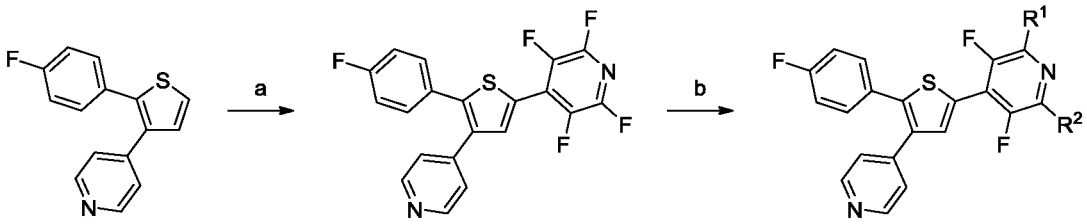
[00129] The furan analogue **5.18** was synthesised in high yield (91%) using a Suzuki coupling reaction described in the experimental section (Scheme below).



5.1 5.18

Synthesis of analogue **5.18**. Reagents and conditions: (a) furan-2-boronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, THF, μW, 100 °C, 90 min, 91%.

[00130] Compound **3.2** was reacted with *n*-butyllithium followed by pentafluoropyridine to give analogue **5.19** in good yield (65%). Monoamination occurred easily in aqueous ammonia using *N*-methyl-2-pyrrolidone as a co-solvent at 120 °C for 1 hour in a sealed tube. This gave analogue **5.20** quantitatively. Diamination of tetrafluoropyridine **5.19** progressed much slower and required harsh reaction conditions with only 70% conversion after heating the reaction at 150 °C over 3 days. The diamino analogue **5.21** was isolated in 36% yield.



3.2 **5.19** **5.20** R^1, R^2 = NH_2, F
5.21 R^1, R^2 = NH_2, NH_2

Synthesis of pyridine analogues **5.19 - 5.21**. Reagents and conditions: (a) *n*BuLi, THF, -78 °C, 30 min, pentafluoropyridine, rt, 2 h, 65%; (b) NH_3 (aq.), NMP, **5.20**: 120 °C, 1 hour, quant., **5.21**: 150 °C, 3 d, 50%

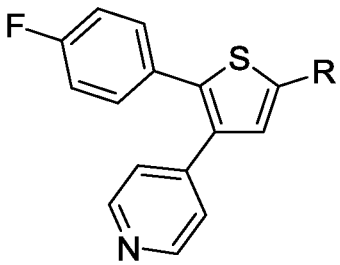
Biological Evaluation of Tri-substituted Thiophene Compounds

Binding affinity to p38α MAPK

[00131] The synthesised tri-substituted analogues were evaluated in the same fluorescence polarisation binding assay discussed above with binding affinities given in Table 5. The compounds showed stronger binding to the active form of the enzyme, consistent with the above description. Generally, the stronger the analogues bind to the inactive protein, the stronger they bind to active phosphorylated p38α MAPK. FIG 12 describes the SAR of the synthesised analogues. Small differences in affinity are observed when modifying the chain length of the alkynyl alcohol

substituent with K_i values of 0.63, 0.56 and 0.80 μM for the 3, 4 and 5 carbon spacer. When compared with disubstituted thiophene **3.2**, there are no significant improvements in binding affinity suggesting that the extra alkynyl alcohol functional group is not making key interactions within the binding pocket. However, a three-fold improvement in affinity is observed when converting the butynyl alcohol **4.5** to a butynyl amine **5.7** in which a K_i value of 0.19 μM was achieved. Loss of the terminal hydrogen bond donor reduces binding affinity which was exemplified by the morpholine analogue **5.8** (K_i 2.5 μM).

[00132] Reduction of the alkyne functional group to the fully saturated butyl group **5.9** improved binding affinity more than two-fold (K_i 0.25 μM). In addition, hydration of the alkyne to a ketone **5.10** maintained affinity with a K_i value of 0.67 μM. This suggests that the alkyne is not essential for p38α binding and increasing the flexibility improves affinity. Only aromatic substituents containing hydrogen bond donating functional groups were found to improve affinity. The tetrafluoropyridine analogue **5.19** had a K_i value of 0.80 μM which was further improved by the substitution of a fluoro group with an amine. This mono-aminopyridine analogue **5.20** had a K_i value of 0.20 μM. The diaminopyridine analogue **5.21** was the most potent analogue synthesised having a K_i value 0.16 μM.



Compound	R	$K_i \pm$ SEM (μM) to inactive p38α MAPK	$K_i \pm$ SEM (μM) to active p38α MAPK
RWJ67657	n.a.	0.21 ± 0.04	0.013 ± 0.006
3.2	H	5.0 ± 0.4	0.6 ± 0.1
5.1	I	1.9 ± 0.4	2.3
5.2	$C{\equiv}CCH_2OH$	2.1 ± 0.3	0.63 ± 0.04
4.5	$C{\equiv}C(CH_2)_2OH$	2.0 ± 0.2	0.56 ± 0.06
5.3	$C{\equiv}C(CH_2)_3OH$	1.7 ± 0.2	0.80 ± 0.04
5.6	$C{\equiv}CCH_2NH_2$	> 10.0	n.d.
5.7	$C{\equiv}C(CH_2)_2NH_2$	1.1 ± 0.2	0.19 ± 0.04
5.8	$C{\equiv}C(CH_2)_2$morpholine	2.2 ± 0.2	2.5
5.9	$(CH_2)_4OH$	0.98 ± 0.02	0.25 ± 0.04
5.10	$CO(CH_2)_3OH$	2.7 ± 0.3	0.67 ± 0.03
5.11	CO_2Et	6 ± 1	2.2
5.12	CO_2H	17 ± 2	1.4
5.14	carboxypiperazine	6.3 ± 0.4	0.9 ± 0.1
5.15	acetyl	6.4 ± 0.6	1.4
5.16	benzoyl	5 ± 2	2.8 ± 0.1
5.17	furoyl	5.9 ± 0.7	2.5
5.18	2-furan	> 10.0	1.7
5.19	2,3,5,6-tetrafluoropyridine	3.01 ± 0.03	0.8 ± 0.1
5.20	2-amino-3,5,6-trifluoropyridine	0.66 ± 0.06	0.21 ± 0.01
5.21	2,6-diamino-3,5-difluoropyridine	0.47 ± 0.01	0.16 ± 0.02

Table 5: Binding affinities to inactive and active p38α MAPK. n.d. not determined. For compounds with K_i < 10 μM to inactive p38α MAPK the binding assay was carried out in triplicate with n = 3 experiments. For compounds with K_i < 1 μM to active p38α MAPK the binding assay was conducted in duplicate with n = 2 - 3 experiments.

[00133] A number of compounds were selected for competition experiments to prove they were binding competitively with the SB203580-fluoroscein ligand and not to an allosteric site. The dose response curves are shown in FIG 14. Using differing concentrations of the inactive non-phosphorylated p38α MAPK (0.03 to 1000 nM) it was possible to determine the change in K_d value for the fluorescently labelled ligand. The affinity of SB203580-fluorescein to the inactive form of the p38α enzyme was observed to

decrease with increasing concentrations of the tested analogues, indicating that the synthesised analogues were competing for the same binding site as the fluoroprobe. All of the tested compounds showed competitive binding for the ATP pocket of p38α MAPK.

In vitro activation assay

[00134] To assess whether the compounds of the present invention could also inhibit the activation of p38α MAPK a number of select compounds were tested in an *in vitro* activation assay following a published method. As discussed above, inactive non-phosphorylated p38α MAPK was pre-incubated with the test compounds at 10 and 1 μM concentration for 30 minutes before the addition of ATP and MKK6. Western blot analysis shows whether the compounds were able to inhibit p38α activation. FIG 13 shows the western blots of compounds **4.5**, **5.9**, **5.10**, **5.16**, **5.20**, **5.21** and RWJ67657 (**1.49**). The assay was analysed by immunoblotting using antibodies against pan p38 MAPK and phosphorylated p38α MAPK. All of the tested compounds showed inhibition of p38α MAPK activation at 10 μM concentration. Compound **5.21** shows significant inhibition, being able to prevent MKK6 activation of p38α MAPK at 1 μM concentration. This coincides with the binding assay data which shows that compound **5.21** was the strongest binder of the analogue series having a K_i of 0.47 μM to inactive non-phosphorylated p38α MAPK.

[00135] The compounds of the first aspect, and particularly those having substitution at the 2-position (R_1 position) and hydrogen at the 3-position (R_2 position), have thereby been shown to be particularly efficacious in binding to the p38α MAPK enzyme and demonstrate good 'drug-like' characteristics in terms of their physicochemical characteristics which are further demonstrated in the experimental section.

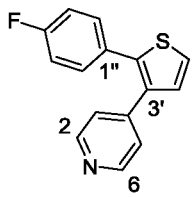
EXPERIMENTAL

General experimental

[00136] All chemical reagents were acquired from Sigma Aldrich, Fluka, Merck, Boron Molecular, and Matrix Scientific and were used without further purification. Flash chromatography was carried out using Scharlau silica gel 60, 0.06 - 0.20 mm (70 - 230 mesh ASTM). Melting points were determined using a Mettler Toledo MP50 melting point apparatus. NMR spectra were recorded on a 300 MHz Bruker Avance DPX 300 NMR spectrometer or a 400 MHz Bruker Avance Ultrashield Plus NMR spectrometer or a 600 MHz Varian Unity Inova NMR spectrometer. Chemical shifts (δ) were reported in parts per million (ppm) referenced to an internal standard of residual proteo-solvent (^{1}H NMR, ^{13}C NMR): $CDCl_3$ (7.26, 77.16), CD_3OD (3.31, 49.00) or d_6-DMSO (2.50, 39.52). Multiplicity is quoted as app. (apparent), br. (broad), s (singlet), d (doublet), t (triplet), q (quartet), p (pentet) and m (multiplet). Coupling constants (*J*) are given in Hertz (Hz). Where possible overlapped non-equivalent ^{13}C peaks were identified by ^{13}C-^{1}H HSQC and HMBC NMR and are indicated with (2C) after the identified overlapped signal. Low resolution mass spectrometry (LRMS) analyses were performed using a Micromass Platform II single quadrupole mass spectrometer equipped with an atmospheric pressure (ESI/APCI) ion source. Sample management was facilitated by an Agilent 1100 series high performance liquid chromatography (HPLC) system using MassLynx version 3.5 software. High resolution mass spectrometry (HRMS) analyses were carried out on a Waters Micromass LCT Premier XE Orthogonal Acceleration time-of-flight (TOF) mass spectrometer coupled to an Alliance 2795 Separation Module using MassLynx version 4.1 software. Liquid chromatography mass spectrometry (LCMS) was performed on an Agilent 1200 Series Separation Module fitted with a 6120 quadropole detector and a Phenomenex® Luna C8(2) 100 Å (50 × 4.6 mm, internal diameter) 5 µm column. Samples were run in a gradient of 5 - 100% buffer B in buffer A (buffer A: 0.1% aqueous formic acid; buffer B: 80% acetonitrile, 19.9% water, 0.1% formic acid) over 4 minutes, followed by isocratic 100% buffer B for 3 minutes then a gradient of 100 - 0% buffer B over 3 minutes at a flow rate of 0.5 mL/min. Agilent Chemstation software (version B.04.01) managed the running and processing of samples.

Analytical RP-HPLC was acquired on a Waters Millenium 2690 system fitted with a Phenomenex® Luna C8 100 Å (50 × 4.6 mm, internal diameter) 5 µm column with UV detection at 254 nm. Samples were run in a gradient of 20 - 100% buffer B in buffer A (buffer A: 0.1% aqueous trifluoroacetic acid; buffer B: 80% acetonitrile, 19.9% water, 0.1% trifluoroacetic acid) over 10 minutes, followed by isocratic 100% buffer B for 1 minute then a gradient of 100 - 20% buffer B over 1 minute followed by isocratic 20% buffer B for 10 minutes at a flow rate of 1.0 mL/minute. EmPowerPro managed the running and processing of samples. EmPowerPro managed the running and processing of samples. Microwave chemistry was performed using a Biotage Initiator Microwave Reactor according to manufacturer's instructions.

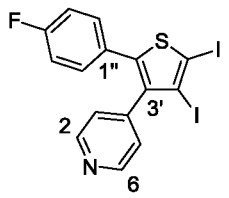
4-(2-(4-Fluorophenyl)thiophen-3-yl)pyridine (3.2)



[00137] To a solution of 2,3-dibromothiophene (2.38 mL, 20.7 mmol) in dimethylformamide (160 mL) was added 4-fluorophenylboronic acid (2.89 g, 20.7 mmol), sodium carbonate monohydrate (12.3 g, 99.2 mmol) and water (40 mL). The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium(II) chloride (0.725 g, 1.03 mmol) was added and the reaction mixture was heated at 70 °C for 3 hours. Pyridine-4-boronic acid (3.81 g, 31.0 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.725 g, 1.03 mmol) were added and the reaction mixture was heated at 120 °C for 16 hours. The reaction mixture was cooled to room temperature and passed through a plug of silica and washed with ethyl acetate (160 mL). The organic layer was washed with water (5 × 100 mL), then brine (50 mL), dried over magnesium sulfate and filtered. The organic layer was evaporated and the dimethylformamide removed by azeotrope with toluene to afford a yellow oil. The product was purified by column chromatography using a gradient elution (0

- 50% ethyl acetate/petroleum spirits) to afford a pale yellow solid. Recrystallisation in diethyl ether gave thiophene **3.2** (3.81 g, 71%) as a white powder. **3.2**: $C_{15}H_{10}FNS$ (M_r = 255.31); mp 95.8 - 97.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.51 (br. app. d, J = 5.6 Hz, 2H), 7.37 (d, J = 5.2 Hz, 1H), 7.28 - 7.23 (m, 2H), 7.18 (d, J = 5.3 Hz, 1H), 7.16 - 7.15 (m, 2H), 7.04 - 6.98 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.3 (d, $^1J_{CF}$ = 248.5 Hz), 149.8, 143.6, 139.7, 135.1, 130.9 (d, $^3J_{CF}$ = 8.1 Hz), 129.33 (d, $^4J_{CF}$ = 3.4 Hz), 129.27, 125.0, 123.4, 115.6 (d, $^2J_{CF}$ = 21.7 Hz); ESI-HRMS-TOF calcd for $C_{15}H_{11}FNS^+$ $(M+H)^+$ 256.0591, found 256.0589; ESI-LCMS R_t = 4.9 min, 256.1 $(M+H)^+$; RP-HPLC R_t = 6.5 min, 99%.

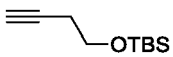
4-(2-(4-Fluorophenyl)-4,5-diiodothiophen-3-yl)pyridine (3.3)



[00138] To a solution of compound **3.2** (0.861 g, 3.37 mmol) in acetic acid (18 mL) was added mercuric acetate (3.23 g, 10.1 mmol). The solution was heated at 70 °C for 16 hours. Concurrently, iodine (5.14 g, 20.2 mmol) and potassium iodide (3.36 g, 20.2 mmol) were dissolved in water (38 mL) over 16 hours in a separate round bottom flask. After 16 hours, the acetic acid mixture was concentrated *in vacuo* and poured into ice/water (100 mL). The resulting white precipitate was filtered and washed with water then diethyl ether to afford the mercuric acetate intermediate as a white powder. The intermediate was added to the potassium triiodide solution. Tetrahydrofuran (1 mL) was added to break the surface tension and the mixture was stirred at room temperature for 16 hours. Saturated sodium thiosulfate (100 mL) was added and the resulting yellow solid was filtered and washed with water (50 mL). The solid was dissolved in tetrahydrofuran/ethyl acetate (100 mL) and washed further with saturated sodium thiosulfate (3 × 50 mL). The organic extract was dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford thiophene **3.3**.

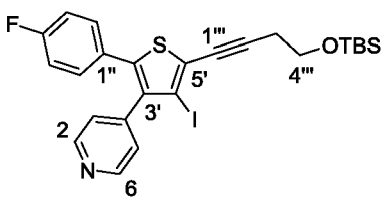
Recrystallisation from tetrahydrofuran/ethanol (1:1) afforded compound **3.3** (1.39 g, 81%) as yellow crystals. **3.3**: $C_{15}H_8FI_2NS$ (M_r = 507.10); mp 212.8 °C (decomposition); ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm) 8.59 - 8.58 (m, 2H), 7.20 - 7.12 (m, 6H); ^{13}C NMR (101 MHz, *d*$_6$-DMSO) δ (ppm) 162.0 (d, $^1J_{CF}$ = 247.0 Hz), 149.7, 145.1, 145.0, 140.1, 130.9 (d, $^3J_{CF}$ = 8.5 Hz), 128.4 (d, $^4J_{CF}$ = 3.2 Hz), 125.5, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 101.8, 87.6; ESI-HRMS-TOF calcd for $C_{15}H_9FI_2NS^+$ $(M+H)^+$ 507.8524, found 507.8521; ESI-LCMS R_t = 6.4 min, 507.9 $(M+H)^+$; RP-HPLC R_t = 8.4 min, > 99%.

(But-3-yn-1-yloxy)(tert-butyl)dimethylsilane (3.5)



[00139] Compound **3.5** was synthesised using a similar procedure by Nadeau *et al*. To a solution of 3-butyn-1-ol (3.00 g, 42.8 mmol) in dichloromethane (60 mL) was added imidazole (7.28 g, 107 mmol) and cooled to 5 °C. *tert*-Butyldimethylsilyl chloride (6.45 g, 42.8 mmol) was added and the reaction mixture was stirred at 25 °C for 16 hours. Dichloromethane (100 mL) was added and the mixture was washed with water (2 × 50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated *in vacuo* to afford compound **3.5** (7.73 g, 98%) as a colourless oil. **3.5**: $C_{10}H_{20}OSi$ (M_r = 184.35); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.74 (t, *J* = 7.1 Hz, 2H), 2.40 (td, *J* = 7.1, 2.7 Hz, 2H), 1.95 (t, *J* = 2.7 Hz, 1H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 81.6, 69.4, 61.9, 26.0, 23.0, 18.4, -5.2. Does not ionise in ESI-MS. Nb. ^{1}H NMR was consistent with literature data.

4-(5-(4-((*tert*-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-2-(4-fluorophenyl)-4-iodothiophen-3-yl)pyridine (3.6)



[00140] **Negishi coupling:** To a solution of alkyne **3.5** (2.06 mL, 9.97 mmol) in tetrahydrofuran (15 mL) was added dropwise *n*-butyllithium (1.2 M, 8.3 mL, 10 mmol) at 0 °C. The reaction mixture was stirred for 15 minutes. Zinc chloride (1.63 g, 12.0 mmol) was added and the reaction mixture was stirred at 0 °C for 15 minutes, then allowed to warm to room temperature for 15 minutes, at which time the zinc had dissolved. Concurrently, compound **3.3** (1.23 g, 2.43 mmol) was dissolved in tetrahydrofuran (18 mL) and nitrogen was bubbled through the solution for 30 minutes. The metallated alkyne solution was added dropwise to the thiophene solution followed by addition of tetrakis(triphenylphosphine)palladium(0) (0.283 g, 0.245 mmol). The mixture was stirred at 25 °C for 70 hours. Saturated ammonium chloride (7.5 mL) was added and the mixture was stirred for 15 minutes. Ethyl acetate (150 mL) was added and the mixture was washed with saturated sodium carbonate (3 × 40 mL) and brine (3 × 40 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography using a gradient elution (0 - 50% ethyl acetate/petroleum spirits) to afford thiophene **3.6** as a pale yellow solid (1.27 g, 94%).

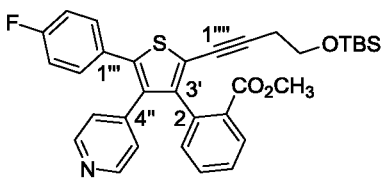
[00141] **Sonogashira coupling:** To a solution of compound **3.3** (1.30 g, 2.56 mmol) in tetrahydrofuran (13 mL) was added alkyne **3.5** (800 µL, 3.88 mmol), triphenylphosphine (0.010 g, 0.038 mmol), copper(I) iodide (0.027 g, 0.142 mmol), and bis(triphenyl-phosphine)palladium(II) dichloride (0.093 g, 0.132 mmol). The reaction mixture was bubbled with nitrogen for 15 minutes and heated at 120 °C for 2 hours. The reaction mixture was diluted with ethyl acetate (100 mL) and washed with water (3 × 50 mL) and brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting oil was purified by column chromatography using a gradient elution (0 - 50% ethyl acetate/petroleum spirits) to afford compound **3.6** as a white solid. Trituration with petroleum spirits gave compound **3.6** (1.19 g, 82%) as a white powder. **3.6**: $C_{25}H_{27}FINOSSi$ (M_r = 563.54); mp 96.6 - 97.7 °C; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.60 (br. app. d, J = 4.8 Hz, 2H, H2, H6), 7.13 - 7.11 (m, 2H), 7.10 - 7.05 (m, 2H), 6.94 - 6.89 (m, 2H), 3.87 (t, J = 6.9 Hz, 2H), 2.73

(t, J = 6.9 Hz, 2H), 0.92 (s, 9H,, 0.11 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.8 Hz), 150.1, 144.9, 140.1, 138.3, 130.9 (d, $^3J_{CF}$ = 8.3 Hz), 128.6 (d, $^4J_{CF}$ = 3.4 Hz), 125.7, 115.9 (d, $^2J_{CF}$ = 21.9 Hz), 96.9, 91.8, 75.7, 61.6, 26.1, 24.5, 18.5, -5.1; ESI-HRMS-TOF calcd for $C_{25}H_{28}FINOSSi^+$ $(M+H)^+$ 564.0684, found 564.0701; ESI-LCMS R_t = 7.8 min, 564.1 $(M+H)^+$; RP-HPLC R_t = 10.9 min, 97%.

General method for Suzuki coupling

[00142] To a solution of thiophene **3.6** (100 mg, 1.0 eq.) in tetrahydrofuran (3 mL) was added the boronic acid/pinacol ester (3.0 eq.) and sodium carbonate (1 M, 1 mL) in a 2 - 5 mL microwave vial. The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium (II) dichloride (0.10 eq.) was added, the vial was capped and the mixture was heated at 100 °C for 90 minutes in the microwave. The mixture was extracted with diethyl ether and concentrated *in vacuo*. The product was purified by column chromatography.

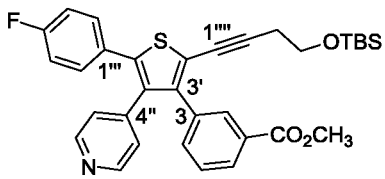
Methyl 2-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)benzoate (3.7)



[00143] Compound **3.7** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (67 mg, 0.12 mmol) and 2-(methoxycarbonyl)phenylboronic acid (65 mg, 0.36 mmol). The reaction time and temperature was changed to heating in the microwave at 100 °C for 1 hour, followed by 110 °C for a further hour. Purification using gradient column chromatography (0 - 50% ethyl acetate/petroleum spirits) gave compound **3.7** (14 mg, 21%) as a yellow oil. **3.7**: $C_{33}H_{34}FNO_3SSi$ (M_r = 571.78); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.31 (br. app. d, J = 4.7 Hz, 2H), 7.84 - 7.82 (m, 1H),

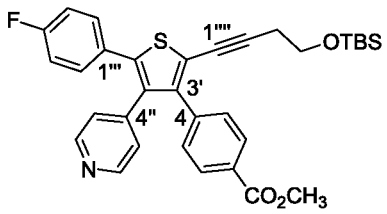
7.39 (app. td, J = 7.5, 1.6 Hz, 1H), 7.34 (app. td, J = 7.6, 1.5 Hz, 1H), 7.19 - 7.14 (m, 2H), 7.10 - 7.08 (m, 1H), 6.97 - 6.91 (m, 2H), 6.83 - 6.81 (m, 2H), 3.69 (s, 3H), 3.62 (app. td, J = 7.3, 3.9 Hz, 2H), 2.49 (t, J = 7.2 Hz, 2H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 167.4, 162.6 (d, $^1J_{CF}$ = 248.9 Hz), 149.6, 145.5, 143.9, 139.1, 136.3, 134.9, 132.0, 131.6, 131.4, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 130.2, 129.3 (d, $^4J_{CF}$ = 3.4 Hz), 127.9, 125.5, 120.1, 115.8 (d, $^2J_{CF}$ = 21.7 Hz), 94.7, 73.8, 61.6, 52.3, 26.0, 24.2, 18.4, -5.2; ESI-HRMS-TOF calcd for $C_{33}H_{35}FNO_3SSi^+$ $(M+H)^+$ 572.2086, found 572.2111; ESI-LCMS R_t = 7.4 min, 572.2 $(M+H)^+$; RP-HPLC R_t = 11.1 min, 95%.

Methyl 3-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)benzoate (3.8)



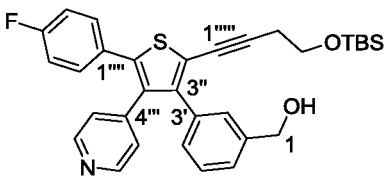
[00144] Compound **3.8** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (250 mg, 0.444 mmol) and 3-(methoxycarbonyl)phenylboronic acid (240 mg, 1.33 mmol). Purification using gradient column chromatography (20 - 50% ethyl acetate/petroleum spirits) gave compound **3.8** (226 mg, 89%) as a yellow solid. **3.8**: $C_{33}H_{34}FNO_3SSi$ (M_r = 571.78); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38 - 8.36 (m, 2H), 7.96 - 7.95 (m, 1H), 7.94 - 7.91 (m, 1H), 7.29 (app. td, J = 7.7, 0.5 Hz, 1H), 7.23 - 7.21 (m, 1H), 7.15 - 7.10 (m, 2H), 6.98 - 6.92 (m, 2H), 6.83 - 6.82 (m, 2H), 3.87 (s, 3H), 3.70 (t, J = 7.1 Hz, 2H), 2.56 (t, J = 7.1 Hz, 2H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 166.7, 162.7 (d, $^1J_{CF}$ = 249.3 Hz), 149.7, 144.1, 143.8, 139.9, 135.1, 134.7, 134.3, 131.4, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 130.1, 129.0 (d, $^4J_{CF}$ = 3.4 Hz), 128.7, 128.1, 125.7, 121.1, 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 94.9, 74.0, 61.6, 52.2, 25.9, 24.3, 18.3, -5.2; ESI-HRMS-TOF calcd for $C_{33}H_{35}FNO_3SSi^+$ $(M+H)^+$ 572.2086, found 572.2103; ESI-LCMS R_t = 7.6 min, 572.3 $(M+H)^+$; RP-HPLC R_t = 10.9 min, 95%.

Methyl 4-(2-(4-((*tert*-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)benzoate (3.9)



[00145] Compound **3.9** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (295 mg, 0.523 mmol) and 4-(methoxycarbonyl)phenylboronic acid (290 mg, 1.61 mmol). Purification using gradient column chromatography (0 - 50% ethyl acetate/petroleum spirits) gave compound **3.9** (299 mg, quant.) as a yellow solid. **3.9**: $C_{33}H_{34}FNO_3SSi$ (M_r = 571.78); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38 (br. app. d, J = 4.8 Hz, 2H), 7.94 - 7.91 (m, 2H), 7.23 - 7.20 (m, 2H), 7.15 - 7.10 (m, 2H), 7.00 - 6.93 (m, 2H), 6.85 - 6.83 (m, 2H), 3.91 (s, 3H), 3.71 (t, J = 6.9 Hz, 2H), 2.57 (t, J = 6.9 Hz, 2H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 166.9, 162.8 (d, $^1J_{CF}$ = 249.4 Hz), 149.8, 144.1, 143.7, 140.2, 139.6, 134.7, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 130.1, 129.4, 129.2, 129.0 (d, $^4J_{CF}$ = 3.5 Hz), 125.7, 121.4, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 95.2, 73.9, 61.6, 52.2, 26.0, 24.4, 18.4, -5.2; ESI-LRMS 572.5 $(M+H)^+$; RP-HPLC R_t = 10.1 min, 95%.

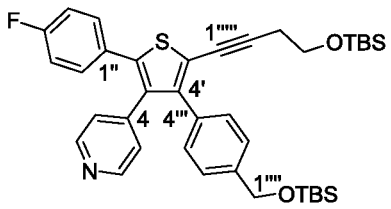
(3-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)methanol (3.10)



[00146] Compound **3.10** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (200 mg, 0.355 mmol) and 3-(hydroxymethyl)phenylboronic acid (162 mg, 1.06 mmol). Purification using gradient column chromatography (10 - 50% ethyl acetate/petroleum spirits) gave compound **3.10** (160 mg, 83%) as a yellow solid. **3.10**: $C_{32}H_{34}FNO_2SSi$

(M_r = 543.77); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.36 - 8.35 (m, 2H), 7.26 - 7.19 (m, 3H), 7.15 - 7.10 (m, 2H), 7.02 (app. dt, *J* = 7.0, 1.8 Hz, 1H), 6.98 - 6.92 (m, 2H), 6.84 - 6.82 (m, 2H), 4.58 (s, 2H), 3.73 (t, *J* = 6.9 Hz, 2H), 2.57 (t, *J* = 6.9 Hz, 2H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 248.6 Hz), 149.4, 145.1, 144.3, 141.3, 139.8, 134.9, 134.7, 131.3 (d, $^3J_{CF}$ = 7.8 Hz), 129.1, 128.7, 128.1, 126.1, 125.8, 120.6, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 94.4, 74.3, 64.8, 61.6, 26.0, 24.3, 18.4, -5.2; ESI-HRMS-TOF calcd for $C_{32}H_{35}FNO_2SSi^+$ $(M+H)^+$ 544.2136, found 544.2153; ESI-LCMS R_t = 6.9 min, 544.2 $(M+H)^+$; RP-HPLC R_t = 10.6 min, 98%.

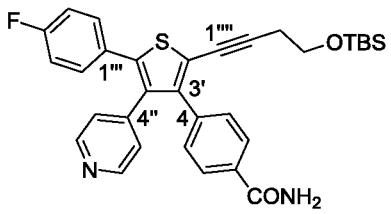
4-(5-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-4-(4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)-2-(4-fluorophenyl)thiophen-3-yl)pyridine (3.11)



[00147] Compound **3.11** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (110 mg, 0.195 mmol) and (4-(((*tert*-butyldimethylsilyl)oxy)methyl) phenyl)boronic acid (160 mg, 0.601 mmol). Purification using gradient column chromatography (0 - 40% ethyl acetate/petroleum spirits) gave thiophene **3.11** (113 mg, 88%) as a yellow solid. **3.11**: $C_{38}H_{48}FNO_2SSi_2$ (M_r = 658.04); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.36 - 8.35 (m, 2H), 7.19 (br. app. d, *J* = 8.5 Hz, 2H), 7.15 - 7.08 (m, 4H), 6.97 - 6.91 (m, 2H), 6.82 - 6.81 (m, 2H), 4.72 (s, 2H), 3.72 (t, *J* = 7.0 Hz, 2H), 2.57 (t, *J* = 7.0 Hz, 2H), 0.94 (s, 9H), 0.90 (s, 9H), 0.09 (s, 6H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.7 (d, $^1J_{CF}$ = 249.0 Hz), 149.8, 145.2, 144.1, 140.8, 139.7, 135.0, 133.4, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 130.0, 129.3 (d, $^4J_{CF}$ = 3.4 Hz), 125.8, 125.7, 120.5, 115.8 (d, $^2J_{CF}$ = 21.7 Hz), 94.3, 74.4, 64.9, 61.7, 26.1, 26.0, 24.4, 18.6, 18.5, -5.08, -5.13; ESI-LRMS 658.5 $(M+H)^+$; ESI-LCMS R_t = 10.2 min, 658.3 $(M+H)^+$; RP-HPLC R_t = 14.1 min, 99%.

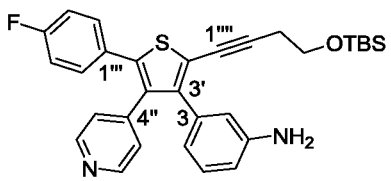
4-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-

4-yl)thiophen-3-yl)benzamide (3.12)



[00148] Compound **3.12** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (87 mg, 0.15 mmol) and 4-aminocarbonylphenylboronic acid (76 mg, 0.46 mmol). Purification using gradient column chromatography (50 - 100% ethyl acetate/petroleum spirits) gave thiophene **3.12** (69 mg, 80%) as a yellow solid. **3.12**: $C_{32}H_{33}FN_2O_2SSi$ (M_r = 556.77); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.39 - 8.37 (br. app. d, *J* = 4.2 Hz, 2H), 7.69 (app. d, *J* = 7.7 Hz, 2H), 7.23 (app. d, *J* = 7.7 Hz, 2H), 7.14 - 7.10 (m, 2H), 6.98 - 6.93 (m, 2H), 6.81 (br. app. d, *J* = 4.4 Hz, 2H), 6.04 (br. s, 1H), 5.70 (br. s, 1H), 3.72 (t, *J* = 6.7 Hz, 2H), 2.57 (t, *J* = 6.7 Hz, 2H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 169.1, 162.7 (d, $^1J_{CF}$ = 249.5 Hz), 149.9, 143.9, 143.7, 140.2, 138.7, 134.7, 132.3, 131.3 (d, $^3J_{CF}$ = 8.1 Hz), 130.4, 128.9 (d, $^4J_{CF}$ = 3.2 Hz), 127.2, 125.7, 121.4, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 95.2, 73.9, 61.6, 26.0, 24.4, 18.5, -5.1; ESI-LRMS 557.1 $(M+H)^+$; ESI-LCMS R_t = 9.2 min, 557.2 $(M+H)^+$; RP-HPLC R_t = 10.8 min, 83%.

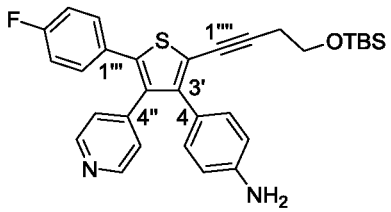
3-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)aniline (3.13)



[00149] Compound **3.13** was synthesised using the general method for Suzuki from thiophene **3.6** (598 mg, 1.06 mmol) and 3-aminophenylboronic acid (430 mg, 3.14 mmol). Purification using column chromatography in ethyl acetate gave compound **3.13** (403 mg, 72%) as a yellow solid. **3.13**: $C_{31}H_{33}FN_2OSSi$ (M_r = 528.76); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.37 - 8.36

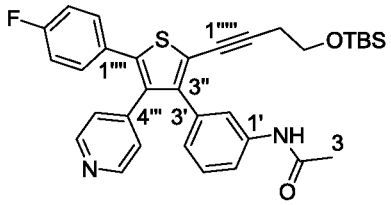
(m, 2H), 7.14 - 7.09 (m, 2H), 7.00 (app. t, J = 7.8 Hz, 1H), 6.97 - 6.91 (m, 2H), 6.85 - 6.83 (m, 2H), 6.56 (ddd, J = 8.0, 2.4, 1.0 Hz, 1H), 6.50 - 6.49 (m, 1H), 6.48 - 6.45 (m, 1H), 3.74 (t, J = 7.0 Hz, 2H), 3.56 (br. s, 2H), 2.58 (t, J = 7.0 Hz, 2H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 248.9 Hz), 149.6, 146.1, 145.5, 144.0, 139.6, 135.8, 134.9, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 129.3 (d, $^4J_{CF}$ = 3.4 Hz), 128.9, 125.7, 120.6, 120.3, 116.8, 115.8 (d, $^2J_{CF}$ = 21.7 Hz), 114.4, 94.3, 74.4, 61.7, 26.0, 24.4, 18.4, -5.1; ESI-LRMS 529.3 $(M+H)^+$; ESI-LCMS R_t = 9.1 min, 529.3 $(M+H)^+$.

4-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)aniline (3.14)



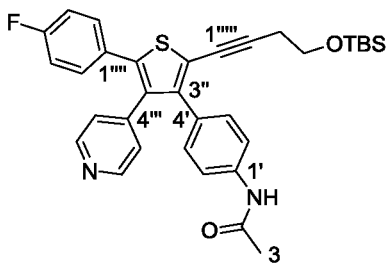
[00150] Compound **3.14** was synthesised using the general method for Suzuki from thiophene **3.6** (335 mg, 0.594 mmol) and 4-aminophenylboronic acid pinacol ester (392 mg, 1.79 mmol). Purification using gradient column chromatography (50 - 100% ethyl acetate/petroleum spirits) gave thiophene **3.14** (124 mg, 39%) as a beige solid. **3.14**: $C_{31}H_{33}FN_2OSSi$ (M_r = 528.76); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.37 - 8.36 (m, 2H), 7.13 - 7.08 (m, 2H), 6.95 - 6.89 (m, 4H), 6.85 - 6.83 (m, 2H), 6.54 - 6.51 (m, 2H), 3.74 (t, J = 7.0 Hz, 2H), 3.70 (br. s, 2H), 2.58 (t, J = 7.0 Hz, 2H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 248.7 Hz), 149.6, 145.8, 145.5, 144.4, 139.5, 134.9, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 131.1, 129.4 (d, $^4J_{CF}$ = 3.4 Hz), 125.9, 124.8, 119.4, 115.8 (d, $^2J_{CF}$ = 21.7 Hz), 114.6, 93.9, 74.7, 61.8, 26.0, 24.4, 18.4, -5.1; ESI-LRMS 529.4 $(M+H)^+$; ESI-LCMS R_t = 6.4 min, 529.2 $(M+H)^+$.

N-(3-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)acetamide (3.15)



[00151] To a solution of thiophene **3.13** (82 mg, 0.16 mmol) in pyridine (2 mL) was added acetic anhydride (0.41 mL, 4.3 mmol). The reaction mixture was stirred at room temperature for 3 hours. Water was added and the precipitate was filtered and washed with water. The crude product was dried under vacuum to give compound **3.15** (73 mg, 82%) as a pale yellow powder. **3.15**: $C_{33}H_{35}FN_2O_2SSi$ (M_r = 570.80); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.38 - 8.36 (m, 2H), 7.51 - 7.49 (m, 1H), 7.26 - 7.05 (m, 5H), 6.98 - 6.90 (m, 2H), 6.87 - 6.81 (m, 3H), 3.73 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 6.6 Hz, 2H), 2.13 (s, 3H), 0.89 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 168.5, 162.6 (d, $^1J_{CF}$ = 249.6 Hz), 149.5, 144.7, 144.0, 139.8, 138.0, 135.5, 134.6, 131.3 (d, $^3J_{CF}$ = 7.9 Hz), 129.1, 128.6, 125.9, 125.8, 121.2, 120.8, 119.0, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 94.6, 74.2, 61.6, 26.0, 24.6, 24.3, 18.4, -5.1; ESI-LRMS 572.0 $(M+H)^+$; ESI-LCMS R_t = 7.0 min, 571.3 $(M+H)^+$; RP-HPLC R_t = 9.5 min, 93%.

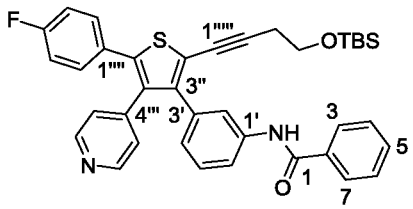
N-(4-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)acetamide (3.16)



[00152] Compound **3.16** was synthesised using the general method for Suzuki from thiophene **3.6** (100 mg, 0.177 mmol) and 4-acetamidophenylboronic acid pinacol ester (140 mg, 0.536 mmol). Purification using column chromatography in 5% methanol/dichloromethane gave

compound **3.16** (90 mg, 89%) as a yellow solid. **3.16**: $C_{33}H_{35}FN_2O_2SSi$ (M_r = 570.79); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.35 (app. d, *J* = 5.7 Hz, 2H), 8.06 (br. s, 1H), 7.40 (app. d, *J* = 8.5 Hz, 2H), 7.14 - 7.09 (m, 2H), 7.06 (app. d, *J* = 8.5 Hz, 2H), 6.96 - 6.91 (m, 2H), 6.83 - 6.82 (m, 2H), 3.72 (t, *J* = 6.9 Hz, 2H), 2.57 (t, *J* = 6.9 Hz, 2H), 2.15 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 168.6, 162.6 (d, $^1J_{CF}$ = 249.1 Hz), 149.6, 144.6, 144.5, 139.8, 137.7, 134.7, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 130.7, 130.4, 129.2 (d, $^4J_{CF}$ = 3.4 Hz), 125.9, 120.4, 119.0, 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 94.6, 74.2, 61.7, 26.0, 24.7, 24.4, 18.4, -5.1; ESI-LRMS 571.5 $(M+H)^+$; ESI-LCMS R_t = 7.1 min, 571.3 $(M+H)^+$; RP-HPLC 9.4 min, 95%.

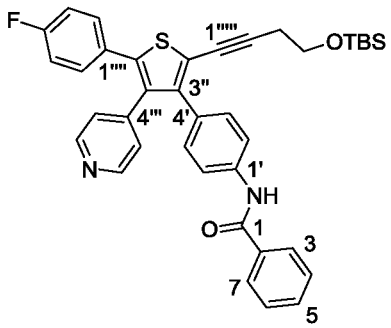
N-(3-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)benzamide (3.17)



[00153] To a solution of thiophene **3.13** (100 mg, 0.189 mmol) in ethyl acetate (3 mL) was added triethylamine (28 µL, 0.20 mmol). Benzoyl chloride (23 µL, 0.20 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was stirred at room temperature for 4 hours. Ethyl acetate (20 mL) was added and the organic extract was washed with water (3 × 10 mL), brine (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Recrystallisation from methanol gave compound **3.17** (105 mg, 88%) as pale pink crystals. **3.17**: $C_{38}H_{37}FN_2O_2SSi$ (M_r = 632.87); mp 195.3 - 196.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.39 - 8.37 (m, 2H), 7.84 - 7.81 (m, 2H), 7.67 (br. s, 1H), 7.61 - 7.44 (m, 5H), 7.26 - 7.22 (m, 1H), 7.16 - 7.11 (m, 2H), 7.00 - 6.93 (m, 2H), 6.89 - 6.86 (m, 3H), 3.73 (t, *J* = 7.0 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 165.8, 162.6 (d, $^1J_{CF}$ = 249.0 Hz), 149.7, 144.7, 143.9, 139.7, 138.0, 135.8, 135.1, 134.8, 131.9, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 129.2 (d, $^4J_{CF}$ = 3.4 Hz), 128.8, 128.7,

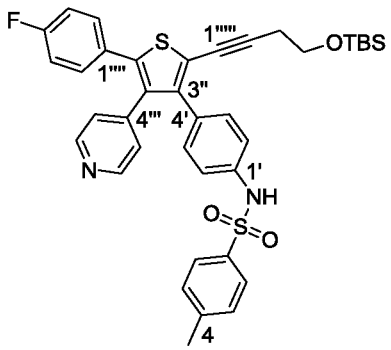
127.2, 126.3, 125.7, 121.7, 120.9, 119.4, 115.8 (d, $^{2}J_{CF}$ = 21.8 Hz), 94.7, 74.2, 61.7, 26.0, 24.4, 18.4, -5.2; ESI-HRMS-TOF calcd for $C_{38}H_{38}FN_2O_2SSi^+$ $(M+H)^+$ 633.2402, found 633.2404; ESI-LCMS R_t = 7.2 min, 633.3 $(M+H)^+$; RP-HPLC R_t = 10.8 min, 98%.

N-(4-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)benzamide (3.18)



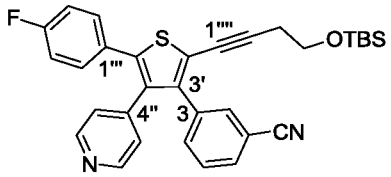
[00154] To a solution of thiophene **3.14** (150 mg, 0.284 mmol) in ethyl acetate (6 mL) was added triethylamine (42 µL, 0.30 mmol). Benzoyl chloride (35 µL, 0.30 mmol) was added to the reaction mixture at 0 °C. The reaction mixture was stirred at room temperature for 4 hours. Ethyl acetate (30 mL) was added and the organic extract was washed with water (3 × 15 mL), brine (15 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. Recrystallisation from methanol/water (9:1) gave compound **3.18** (162 mg, 90%) as yellow crystals. **3.18**: $C_{38}H_{37}FN_2O_2SSi$ (M_r = 632.87); mp 168.9 - 169.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.40 - 8.38 (m, 2H), 7.87 - 7.84 (m, 2H), 7.76 (br. s, 1H), 7.59 - 7.48 (m, 5H), 7.17 - 7.10 (m, 4H), 6.98 - 6.92 (m, 2H), 6.86 - 6.85 (m, 2H), 3.74 (t, *J* = 7.0 Hz, 2H), 2.59 (t, *J* = 7.0 Hz, 2H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 166.0, 162.6 (d, $^{1}J_{CF}$ = 249.1 Hz), 149.7, 144.6, 144.1, 139.8, 137.6, 135.1, 134.8, 131.9, 131.3 (d, $^{3}J_{CF}$ = 8.2 Hz), 130.8, 129.2 (d, $^{4}J_{CF}$ = 3.3 Hz), 128.8, 127.2, 125.8, 120.5, 119.5, 115.8 (d, $^{2}J_{CF}$ = 21.8 Hz), 94.6, 74.3, 61.7, 26.0, 24.4, 18.4, -5.1; ESI-HRMS-TOF calcd for $C_{38}H_{38}FN_2O_2SSi^+$ $(M+H)^+$ 633.2402, found 633.2409; ESI-LCMS R_t = 7.2 min, 633.3 $(M+H)^+$; RP-HPLC R_t = 12.1 min, 97%.

N-(4-(2-(4-((tert-butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)-4-methylbenzenesulfonamide (3.19)



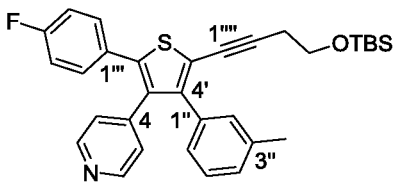
[00155] To a solution of thiophene **3.14** (100 mg, 0.189 mmol) in dichloromethane (5 mL) was added pyridine (137 µL, 1.70 mmol) and *p*-toluenesulfonyl chloride (59 mg, 0.31 mmol). The reaction mixture was stirred at room temperature for 20 hours. Aqueous hydrochloric acid (2.7 M, 20 mL) was added. The compound was extracted with dichloromethane (3 × 20 mL). The organic fractions were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by gradient column chromatography (0 - 50% ethyl acetate/petroleum spirits) gave thiophene **3.19** (119 mg, 92%) as a beige solid. **3.19**: $C_{38}H_{39}FN_2O_3S_2Si$ (M_r = 682.94); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.33 (app. d, *J* = 4.0 Hz, 2H), 7.64 (app. d, *J* = 7.5 Hz, 2H), 7.59 (br. s, 1H), 7.24 (app. d, *J* = 7.8 Hz, 2H), 7.12 - 7.08 (m, 2H), 7.00 - 6.92 (m, 6H), 6.76 (app. d, *J* = 4.4 Hz, 2H), 3.72 (t, *J* = 6.7 Hz, 2H), 2.56 (t, *J* = 6.6 Hz, 2H), 2.41 (s, 3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 162.7 (d, $^1J_{CF}$ = 249.2 Hz), 149.5, 144.2, 144.1, 144.0, 140.0, 136.4, 136.2, 134.6, 131.5, 131.3 (d, $^3J_{CF}$ = 8.1 Hz), 131.1, 129.7, 129.0 (d, $^4J_{CF}$ = 2.8 Hz), 127.4, 125.8, 120.7, 120.6, 115.9 (d, $^2J_{CF}$ = 21.7 Hz), 94.6, 74.1, 61.6, 26.0, 24.4, 21.7, 18.4, -5.1; ESI-LCMS R_t = 7.3 min, 683.3 $(M+H)^+$; RP-HPLC R_t = 9.9 min, 95%.

[00156] *3-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)benzonitrile (3.20)*



[00157] Compound **3.20** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (154 mg, 0.273 mmol) and 3-cyanophenylboronic acid (119 mg, 0.810 mmol). Purification using gradient column chromatography (10 - 50% ethyl acetate/petroleum spirits) gave thiophene **3.20** (130 mg, 88%) as a yellow solid. **3.20**: $C_{32}H_{31}FN_2OSSi$ (M_r = 538.76); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.42 - 8.40 (m, 2H), 7.60 (br. s, 1H), 7.58 - 7.52 (m, 1H), 7.32 (app. t, *J* = 7.7 Hz, 1H), 7.26 - 7.21 (m, 1H), 7.15 - 7.10 (m, 2H), 6.99 - 6.94 (m, 2H), 6.82 - 6.80 (m, 2H), 3.73 (t, *J* = 6.7 Hz, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.6 Hz), 150.0, 143.3, 142.5, 140.4, 136.2, 134.4, 133.6, 131.3 (d, $^3J_{CF}$ = 8.4 Hz), 131.2, 129.0, 128.7 (d, $^4J_{CF}$ = 3.1 Hz), 125.6, 121.8, 118.6, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 112.4, 95.7, 73.6, 61.5, 26.0, 24.3, 18.4, -5.2; ESI-LRMS 538.9 $(M+H)^+$; ESI-LCMS R_t = 7.7 min, 539.2 $(M+H)^+$; RP-HPLC R_t = 9.9 min, 95%.

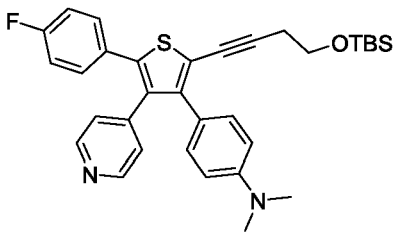
4-(5-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-2-(4-fluorophenyl)-4-(m-tolyl)thiophen-3-yl)pyridine (3.21)



[00158] Compound **3.21** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (158 mg, 0.280 mmol) and 3-tolylboronic acid (114 mg, 0.838 mmol). Purification using gradient column chromatography (0 - 40% ethyl acetate/petroleum spirits) gave compound **3.21** (144 mg, 97%) as a yellow solid. **3.21**: $C_{32}H_{34}FNOSSi$ (M_r = 527.77); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.38 - 8.36 (m, 2H), 7.17 - 7.02 (m, 4H), 6.99 - 6.87 (m, 4H), 6.84 - 6.82 (m, 2H), 3.73 (t, *J* = 7.1 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 2.24 (s,

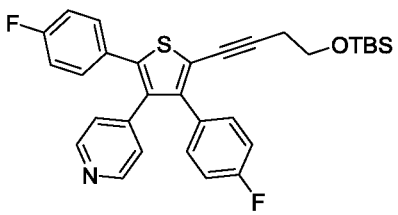
3H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (76 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 248.9 Hz), 149.7, 145.4, 144.1, 139.6, 137.4, 134.9, 134.6, 131.3 (d, $^3J_{CF}$ = 8.1 Hz), 130.8, 129.3 (d, $^4J_{CF}$ = 3.1 Hz), 128.3, 127.9, 127.1, 125.7, 120.3, 115.8 (d, $^2J_{CF}$ = 21.7 Hz), 94.1, 74.4, 61.7, 26.0, 24.4, 21.4, 18.4, -5.2; ESI-LCMS R_t = 7.6 min, 528.3 $(M+H)^+$; RP-HPLC R_t = 10.4 min, 90%.

4-(2-(4-(Tert-butyldimethylsilyloxy)but-1-ynyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)-N,N-dimethylaniline (3.22)



[00159] Compound **3.22** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (158 mg, 0.280 mmol) and 4-(dimethylamino)phenylboronic acid (139 mg, 0.841 mmol). Purification using gradient column chromatography (20 - 50% EtOAc/hexane) gave compound **3.22** (130 mg, 83%) as a yellow solid. **3.22**: $C_{33}H_{37}FN_2OSSi$ (M_r = 556.81); ^{1}H NMR (300 MHz, *d*$_6$-DMSO) δ (ppm) 8.40 - 8.38 (m, 2H), 7.22 - 7.12 (m, 4H), 6.95 - 6.93 (m, 4H), 6.57 (d, *J* = 8.9 Hz, 2H), 3.70 (t, *J* = 6.4 Hz, 2H), 2.87 (s, 6H), 2.58 (t, *J* = 6.4 Hz, 2H), 0.87 (s, 9H), 0.05 (s, 6H); ESI-LRMS 557.7 $(M+H)^+$.

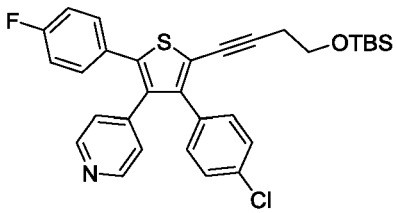
4-(5-(4-(tert-Butyldimethylsilyloxy)but-1-ynyl)-2,4-bis(4-fluorophenyl)thiophen-3-yl)pyridine (3.23)



[00160] Compound **3.23** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (151 mg, 0.268 mmol) and 4-fluorophenylboronic acid (112 mg, 0.804 mmol). Purification using gradient

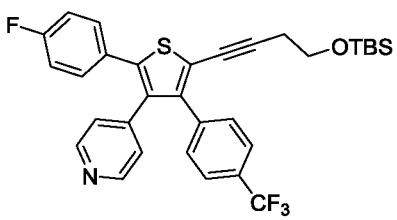
column chromatography (20 - 50% EtOAc/hexane) gave compound **3.23** (123 mg, 86%) as a yellow solid. **3.23**: $C_{31}H_{31}F_2NOSSi$ (M_r = 531.73); ^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm) 8.40 - 8.38 (m, 2H), 7.25 - 7.07 (m, 8H), 6.96 - 6.94 (m, 2H), 3.67 (t, *J* = 6.3 Hz, 2H), 2.58 (t, *J* = 6.3 Hz, 2H), 0.85 (s, 9H), 0.02 (s, 6H); ESI-LRMS 532.1 $(M+H)^+$.

4-(5-(4-(tert-Butyldimethylsilyloxy)but-1-ynyl)-4-(4-chlorophenyl)-2-(4-fluorophenyl)thiophen-3-yl)pyridine (3.24)



[00161] Compound **3.24** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (135 mg, 0.240 mmol) and 4-chlorophenylboronic acid (112 mg, 0.719 mmol). Purification using gradient column chromatography (20 - 50% EtOAc/hexane) gave compound **3.24** (80 mg, 61%) as a yellow solid. **3.24**: $C_{31}H_{31}ClFNOSSi$ (M_r = 548.19); ^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm) 8.41 - 8.39 (m, 2H), 7.34 (d, *J* = 8.5 Hz, 2H), 7.25 - 7.14 (m, 6H), 6.97 - 6.95 (m, 2H), 3.67 (t, *J* = 6.2 Hz, 2H), 2.59 (t, *J* = 6.2 Hz, 2H), 0.84 (s, 9H), 0.02 (s, 6H); ESI-LRMS 548.0 $(M+H)^+$.

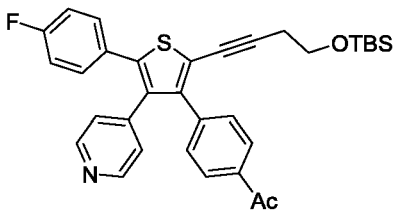
4-(5-(4-(tert-Butyldimethylsilyloxy)but-1-ynyl)-2-(4-fluorophenyl)-4-(4-(trifluoromethyl)phenyl)thiophen-3-yl)pyridine (3.25)



[00162] Compound **3.25** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (125 mg, 0.222 mmol) and 4-(trifluoromethyl)phenylboronic acid (126 mg, 0.665 mmol). Purification using gradient column chromatography (20 - 50% EtOAc/hexane) gave compound

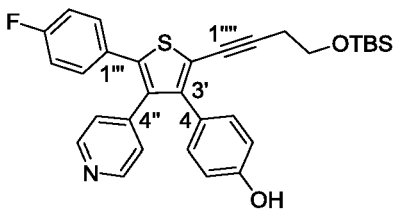
3.25 (90 mg, 70%) as a yellow solid. **3.25**: $C_{32}H_{31}F_4NOSSi$ (M_r = 581.74); ^1H NMR (300 MHz, d_6-DMSO) δ (ppm) 8.41 - 8.39 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.26 - 7.15 (m, 4H), 6.99 - 6.97 (m, 2H), 3.65 (t, *J* = 6.2 Hz, 2H), 2.59 (t, *J* = 6.2 Hz, 2H), 0.82 (s, 9H), -0.01 (s, 6H); ESI-LRMS 582.0 $(M+H)^+$.

1-(4-(2-(4-(Tert-butyldimethylsilyloxy)but-1-ynyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)ethanone (3.26)



[00163] Compound **3.26** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (142 mg, 0.252 mmol) and 4-acetylphenylboronic acid (124 mg, 0.756 mmol). Purification using gradient column chromatography (20 - 50% EtOAc/hexane) gave compound **3.26** (63 mg, 45%) as a yellow solid. **3.26**: $C_{33}H_{34}FNO_2SSi$ (M_r = 555.78); ^1H NMR (300 MHz, d_6-DMSO) δ (ppm) 8.39 (br. app. d, *J* = 4.6 Hz, 2H), 7.85 (app. d, *J* = 7.8 Hz, 2H), 7.29 - 7.15 (m, 6H), 6.98 - 6.96 (m, 2H), 3.66 (t, *J* = 6.1 Hz, 2H), 2.58 (t, *J* = 6.3 Hz, 2H), 2.54 (s, 3H), 0.83 (s, 9H), 0.01 (s, 6H); ESI-LRMS 556.3 $(M+H)^+$.

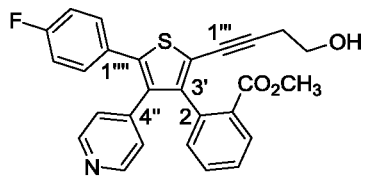
4-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)phenol (3.27)



[00164] Compound **3.27** was synthesised using the general method for Suzuki coupling from thiophene **3.6** (101 mg, 0.179 mmol) and 4-

hydroxyphenylboronic acid (76 mg, 0.55 mmol). Purification using gradient column chromatography (20 - 50% ethyl acetate/petroleum spirits) gave thiophene **3.27** (67 mg, 71%) as a white solid. **3.27**: $C_{31}H_{32}FNO_2SSi$ (M_r = 529.75); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.90 (br. s, 1H), 8.36 - 8.34 (m, 2H), 7.16 - 7.10 (m, 2H), 6.99 - 6.89 (m, 6H), 6.65 - 6.61 (m, 2H), 3.75 (t, *J* = 7.0 Hz, 2H), 2.60 (t, *J* = 7.0 Hz, 2H), 0.90 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (76 MHz, d_6-DMSO) δ (ppm) 161.9 (d, $^1J_{CF}$ = 246.1 Hz), 156.9, 149.5, 145.2, 143.3, 138.5, 135.4, 131.3 (d, $^3J_{CF}$ = 8.3 Hz), 130.8, 128.9 (d, $^4J_{CF}$ = 2.7 Hz), 125.5, 124.7, 118.3, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 114.8, 94.9, 74.0, 61.1, 25.8, 23.6, 18.0, -5.3; ESI-LRMS 530.0 $(M+H)^+$; ESI-LCMS R_t = 7.1 min, 530.3 $(M+H)^+$; RP-HPLC R_t = 9.5 min, 95%.

Methyl 2-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzoate (3.28)



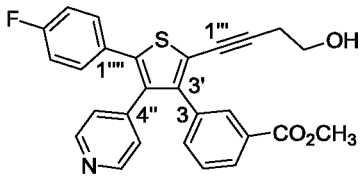
[00165] Compound **3.28** was synthesised using the general method for Suzuki from thiophene **3.55** (50 mg, 0.11 mmol) and 2-(methoxycarbonyl)phenylboronic acid (65 mg, 0.36 mmol). After heating in the microwave at 100 °C for 90 minutes, the reaction was not complete. Therefore the reaction was heated in the microwave at 120 °C for a further hour. Purification using gradient column chromatography (0 - 6% methanol/chloroform) followed by preparative HPLC gave compound **3.28** (13 mg, 26%) as a yellow oil. **3.28**: $C_{27}H_{20}FNO_3S$ (M_r = 457.52); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.24 (br. app. d, *J* = 4.7 Hz, 2H), 7.84 - 7.81 (m, 1H), 7.47 (app. td, *J* = 7.5, 1.5 Hz, 1H), 7.41 (app. td, *J* = 7.6, 1.4 Hz, 1H), 7.27 - 7.22 (m, 2H), 7.17 - 7.14 (m, 1H), 7.08 - 7.02 (m, 2H), 6.97 - 6.95 (m, 2H), 3.70 (s, 3H), 3.55 (td, *J* = 6.9, 1.6 Hz, 2H), 2.49 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR

(101 MHz, CD_3OD) δ 169.1, 164.1 (d, $^1J_{CF}$ = 247.8 Hz), 149.7, 146.7, 146.2, 140.6, 137.2, 136.1, 133.2, 132.9, 132.8, 132.6 (d, $^3J_{CF}$ = 8.4 Hz), 131.1, 130.5 (d, $^4J_{CF}$ = 3.5 Hz), 129.2, 127.3, 121.4, 116.7 (d, $^2J_{CF}$ = 22.1 Hz), 95.7, 74.3, 61.3, 52.8, 24.4; ESI-HRMS-TOF calcd for $C_{27}H_{21}FNO_3S^+$ $(M+H)^+$ 458.1221, found 458.1222; ESI-LCMS R_t = 5.3 min, 458.2 $(M+H)^+$; RP-HPLC R_t = 7.0 min, 97%.

General procedure for TBS deprotection

[00166] To the TBS protected starting material (100 mg, 1.0 eq.) in methanol (10 mL) was added ammonium fluoride (3.0 - 4.0 eq.). The reaction mixture was refluxed for 16 hours. The crude product was concentrated, extracted into ethyl acetate and washed with water. The organic fraction was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography.

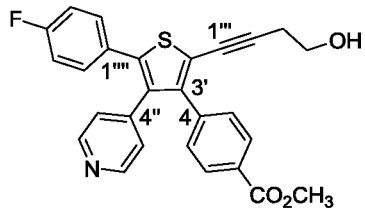
Methyl 3-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen -3-yl)benzoate (3.29)



[00167] Compound **3.29** was synthesised from compound **3.8** (462 mg, 0.808 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 10% methanol/chloroform afforded thiophene **3.29** (361 mg, 98%) as a white powder. **3.29**: $C_{27}H_{20}FNO_3S$ (M_r = 457.52); mp 148.6 - 150.1 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.39 - 8.37 (m, 2H), 7.86 - 7.82 (m, 2H), 7.41 (app. t, J = 7.7 Hz, 1H), 7.32 (app. dt, J = 7.7, 1.6 Hz, 1H), 7.26 - 7.21 (m, 2H), 7.19 - 7.14 (m, 2H), 6.97 - 6.96 (m, 2H), 4.87 (t, J = 5.6 Hz, 1H), 3.81 (s, 3H), 3.47 (td, J = 6.9, 5.7 Hz, 2H), 2.52 - 2.48 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 165.8, 162.0 (d, $^1J_{CF}$ = 247.0 Hz), 149.6,

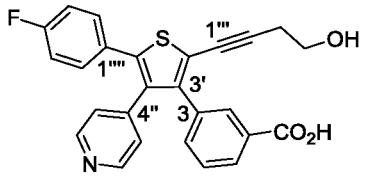
143.8, 142.9, 139.0, 135.1, 134.6, 134.3, 131.3 (d, $^{3}J_{CF}$ = 8.5 Hz), 130.5, 129.4, 128.6, 128.6 (d, $^{4}J_{CF}$ = 5.4 Hz), 128.3, 125.5, 119.9, 115.9 (d, $^{2}J_{CF}$ = 21.9 Hz), 96.2, 73.3, 59.4, 52.2, 23.6; ESI-HRMS-TOF calcd for $C_{27}H_{21}FNO_3S^+$ $(M+H)^+$ 458.1221, found 458.1236; ESI-LCMS R_t = 5.4 min, 458.2 $(M+H)^+$; RP-HPLC R_t = 7.3 min, > 99%.

Methyl 4-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen -3-yl)benzoate (3.30)



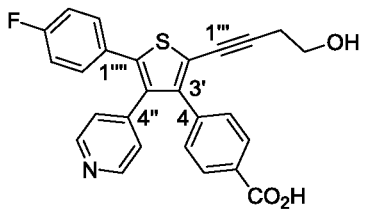
[00168] Compound **3.30** was synthesised from compound **3.9** (593 mg, 1.04 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 10% methanol/chloroform afforded compound **3.30** (478 mg, 99%) as a white powder. **3.30**: $C_{27}H_{20}FNO_3S$ (M_r = 457.52); mp 218.3 - 219.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38 - 8.37 (m, 2H), 7.95 - 7.92 (m, 2H), 7.24 - 7.21 (m, 2H), 7.15 - 7.10 (m, 2H), 6.99 - 6.93 (m, 2H), 6.81 - 6.80 (m, 2H), 3.90 (s, 3H), 3.71 (app. q, *J* = 6.2 Hz, 2H), 2.64 (t, *J* = 6.3 Hz, 2H), 1.64 (t, *J* = 6.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 166.9, 162.8 (d, $^{1}J_{CF}$ = 249.3 Hz), 149.8, 144.5, 143.6, 140.4, 139.5, 134.7, 131.3 (d, $^{3}J_{CF}$ = 8.2 Hz), 130.1, 129.4, 129.3, 128.8 (d, $^{4}J_{CF}$ = 3.6 Hz), 125.6, 121.0, 116.0 (d, $^{2}J_{CF}$ = 21.8 Hz), 94.4, 74.7, 60.9, 52.3, 24.3; ESI-HRMS-TOF calcd for $C_{27}H_{21}FNO_3S^+$ $(M+H)^+$ 458.1221, found 458.1212; ESI-LCMS R_t = 5.4 min, 458.1 $(M+H)^+$; RP-HPLC R_t = 7.4 min, 99%.

3-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzoic acid (3.31)



[00169] The starting thiophene **3.29** (200 mg, 0.47 mmol) was dissolved in ethanol (10 mL). Water (10 mL) was added followed by sodium hydroxide (70 mg, 1.75 mmol). The reaction mixture was heated at 50 °C for 2 hours. The reaction mixture was concentrated *in vacuo* to remove the ethanol. The mixture was acidified with hydrochloric acid (1 M) to pH 2. The resulting precipitate was filtered and dried under vacuum to afford compound **3.31** (155 mg, 80%) as a white powder. **3.31**: $C_{26}H_{18}FNO_3S$ (M_r = 443.49); mp 257.4 - 259.2 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.43 - 8.42 (m, 2H), 7.84 (app. dt, J = 7.7, 1.4 Hz, 1H), 7.78 - 7.76 (m, 1H), 7.40 (app. t, J = 7.7 Hz, 1H), 7.34 (app. dt, J = 7.8, 1.4 Hz, 1H), 7.26 - 7.23 (m, 2H), 7.20 - 7.16 (m, 2H), 7.04 - 7.03 (m, 2H), 3.47 (t, J = 6.9 Hz, 2H), 2.52 - 2.49 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 166.8, 162.1 (d, $^1J_{CF}$ = 246.8 Hz), 148.9, 144.0, 143.7, 139.1, 135.0, 134.3, 133.9, 131.4 (d, $^3J_{CF}$ = 8.5 Hz), 130.6, 130.6, 128.6 (d, $^4J_{CF}$ = 3.3 Hz), 128.5, 128.4, 125.8, 119.9, 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 96.1, 73.3, 59.4, 23.6; ESI-HRMS-TOF calcd for $C_{26}H_{19}FNO_3S^+$ $(M+H)^+$ 444.1064, found 444.1075; ESI-LCMS R_t = 5.1 min, 444.1 $(M+H)^+$; RP-HPLC R_t = 6.7 min, > 99%.

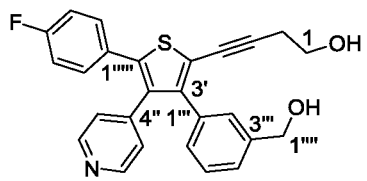
4-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzoic acid (3.32)



[00170] The starting thiophene **3.30** (100 mg, 0.219 mmol) was dissolved in ethanol (5 mL). Water (5 mL) was added followed by sodium hydroxide (35 mg, 0.87 mmol). The reaction mixture was heated at 50 °C for 2 hours. The

reaction mixture was concentrated *in vacuo* to remove the ethanol. The mixture was acidified with hydrochloric acid (1 M) to pH 2. The resulting precipitate was filtered and dried under vacuum to afford compound **3.32** (89 mg, 92%) as a white powder. **3.32**: $C_{26}H_{18}FNO_3S$ (M_r = 443.49); ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.71 (br. app. d, J = 5.3 Hz, 2H), 7.87 (app. d, J = 8.2 Hz, 2H), 7.48 (br. app. d, J = 5.9 Hz, 2H), 7.30 - 7.26 (m, 4H), 7.23 - 7.18 (m, 2H), 3.50 (t, J = 6.7 Hz, 2H), 2.53 (t, J = 6.7 Hz, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 166.9, 162.4 (d, $^1J_{CF}$ = 247.7 Hz), 150.3, 143.6, 143.2, 141.1, 137.8, 133.2, 131.8 (d, $^3J_{CF}$ = 8.7 Hz), 130.11, 130.07, 129.2, 128.1, 127.8 (d, $^4J_{CF}$ = 3.0 Hz), 120.8, 116.2 (d, $^2J_{CF}$ = 21.9 Hz), 97.1, 72.8, 59.3, 23.6; ESI-HRMS-TOF calcd for $C_{26}H_{19}FNO_3S^+$ $(M+H)^+$ 444.1064, found 444.1071; ESI-LCMS R_t = 5.1 min, 444.1 $(M+H)^+$; RP-HPLC R_t = 6.7 min, 98%.

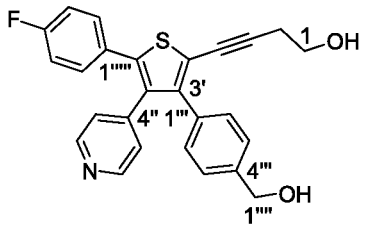
4-(5-(4-Fluorophenyl)-3-(3-(hydroxymethyl)phenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.33)



Compound **3.33** was synthesised from compound **3.10** (119 mg, 0.219 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 5% methanol/chloroform afforded compound **3.33** (78 mg, 83%) as a white powder. **3.33**: $C_{26}H_{20}FNO_2S$ (M_r = 429.51); mp 189.0 - 190.6 °C; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.29 - 8.28 (m, 2H), 7.27 - 7.20 (m, 5H), 7.07 - 7.01 (m, 2H), 6.99 - 6.96 (m, 3H), 4.53 (s, 2H), 3.63 (t, J = 6.7 Hz, 2H), 2.57 (t, J = 6.7 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD) δ (ppm) 164.1 (d, $^1J_{CF}$ = 247.9 Hz), 149.8, 146.7, 146.5, 142.7, 141.1, 136.2, 136.1, 132.7 (d, $^3J_{CF}$ = 8.4 Hz), 130.5 (d, $^4J_{CF}$ = 3.5 Hz), 130.0, 129.8, 129.0, 127.6, 127.2, 121.8, 116.7 (d, $^2J_{CF}$ = 22.1 Hz), 95.5, 74.9, 64.9, 61.4, 24.5; ESI-HRMS-TOF calcd for $C_{26}H_{21}FNO_2S^+$ $(M+H)^+$ 430.1272, found 430.1259; ESI-

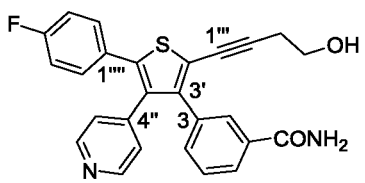
LCMS R_t = 5.0 min, 430.2 $(M+H)^+$; RP-HPLC R_t = 6.7 min, > 99%.

4-(5-(4-Fluorophenyl)-3-(4-(hydroxymethyl)phenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.34)



[00171] Compound **3.34** was synthesised from compound **3.11** (98 mg, 0.15 mmol) following the general procedure for TBS deprotection. Recrystallisation from methanol afforded thiophene **3.34** (37 mg, 58%) as white crystals. **3.34**: $C_{26}H_{20}FNO_2S$ (M_r = 429.51); mp 235 - 236.7 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.39 - 8.38 (m, 2H), 7.24 - 7.14 (m, 6H), 7.09 (app. d, J = 8.2 Hz, 2H), 6.96 - 6.95 (m, 2H), 5.18 (br. s, 1H), 4.88 (br. s, 1H), 4.46 (s, 2H), 3.50 (t, J = 6.8 Hz, 2H), 2.53 - 2.49 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 162.0 (d, $^1J_{CF}$ = 246.6 Hz), 149.3, 144.8, 143.5, 141.9, 138.8, 135.3, 132.4, 131.3 (d, $^3J_{CF}$ = 8.5 Hz), 129.4, 128.8 (d, $^4J_{CF}$ = 3.0 Hz), 126.0, 125.6, 119.3, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 95.5, 73.6, 62.6, 59.5, 23.6; ESI-HRMS-TOF calcd for $C_{26}H_{21}FNO_2S^+$ $(M+H)^+$ 430.1272, found 430.1285; ESI-LCMS R_t = 5.0 min, 430.2 $(M+H)^+$; RP-HPLC R_t = 6.2 min, > 99%.

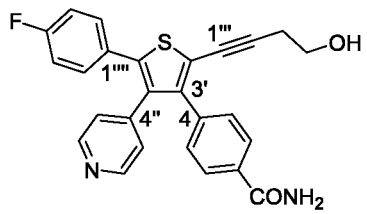
3-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzamide (3.35)



[00172] To a suspension of acid **3.31** (80 mg, 0.18 mmol) in dimethylformamide (2 mL) was added (benzotriazol-1-

yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (119 mg, 0.269 mmol), *N,N*-diisopropylethylamine (46 µL, 0.27 mmol) and ammonium carbonate (90 mg, 0.93 mmol). The reaction mixture was stirred at room temperature for 16 hours. The solvent was evaporated *in vacuo*. Purification by column chromatography in 5% methanol/chloroform followed by recrystallisation from methanol afforded compound **3.35** (44 mg, 55%) as white crystals. **3.35**: $C_{26}H_{19}FN_2O_2S$ (M_r = 442.51); mp 239.4 - 240.8 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.38 - 8.37 (m, 2H), 7.88 (br. s, 1H), 7.78 - 7.75 (m, 2H), 7.35 - 7.31 (m, 2H), 7.26 - 7.15 (m, 5H), 6.95 - 6.94 (m, 2H), 4.88 (br. s, 1H), 3.47 (t, *J* = 6.8 Hz, 2H), 2.51 - 2.48 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 167.4, 162.0 (d, $^1J_{CF}$ = 247.2 Hz), 149.6, 144.6, 142.9, 138.8, 135.3, 134.2, 134.0, 132.3, 131.3 (d, $^3J_{CF}$ = 8.5 Hz), 129.1, 128.7 (d, $^4J_{CF}$ = 3.3 Hz), 127.9, 126.7, 125.5, 119.7, 115.9 (d, $^2J_{CF}$ = 21.9 Hz), 95.9, 73.4, 59.4, 23.6; ESI-HRMS-TOF calcd for $C_{26}H_{20}FN_2O_2S^+$ $(M+H)^+$ 443.1224, found 443.1244; ESI-LCMS R_t = 4.9 min, 443.2 $(M+H)^+$; RP-HPLC R_t = 6.4 min, 99%.

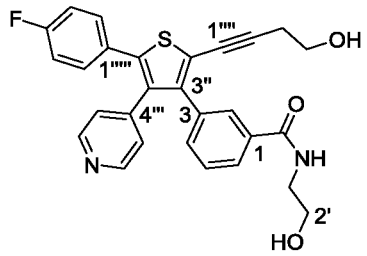
4-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzamide (3.36)



[00173] Compound **3.36** was synthesised from compound **3.12** (49 mg, 0.09 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 5% methanol/chloroform gave thiophene **3.36** (27 mg, 70%) as a white powder. **3.36**: $C_{26}H_{19}FN_2O_2S$ (M_r = 442.51); mp 251.3 - 254.2 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.40 - 8.39 (m, 2H), 7.95 (br. s, 1H), 7.77 - 7.74 (m, 2H), 7.37 (br. s, 1H), 7.25 - 7.15 (m, 6H), 6.97 - 6.95 (m, 2H), 4.89 (t, *J* = 5.6 Hz, 1H), 3.49 (td, *J* = 6.9, 5.6 Hz, 2H), 2.54 - 2.49 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 167.5, 162.0 (d, $^1J_{CF}$ = 246.6 Hz),

149.6, 144.2, 143.0, 138.9, 137.0, 135.2, 133.2, 131.4 (d, $^{3}J_{CF}$ = 8.4 Hz), 129.6, 128.6 (d, $^{4}J_{CF}$ = 3.2 Hz), 127.2, 125.5, 119.9, 115.9 (d, $^{2}J_{CF}$ = 21.9 Hz), 96.1, 73.4, 59.4, 23.6; ESI-HRMS-TOF calcd for $C_{26}H_{20}FN_2O_2S^+$ $(M+H)^+$ 443.1224, found 443.1242; ESI-LCMS R_t = 4.9 min, 443.2 $(M+H)^+$; RP-HPLC R_t = 6.4 min, 97%.

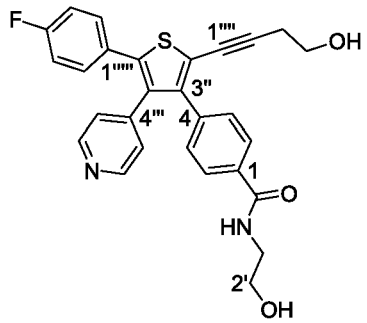
3-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)-N-(2-hydroxyethyl)benzamide (3.37)



[00174] To a suspension of compound **3.31** (80 mg, 0.18 mmol) in acetonitrile (2 mL) was added (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (120 mg, 0.271 mmol), *N,N*-diisopropylethylamine (47 µL, 0.27 mmol) and ethanolamine (50 µL, 0.90 mmol). The reaction mixture was stirred at room temperature for 16 hours. Ethyl acetate (10 mL) was added and the mixture was washed with water (3 × 10 mL) and the aqueous fraction further extracted with ethyl acetate (3 × 10 mL). The combined organic fractions were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by gradient column chromatography (0 - 10% methanol/chloroform) afforded compound **3.37** (50 mg, 57%) as a white powder. **3.37**: $C_{28}H_{23}FN_2O_3S$ (M_r = 486.56); mp 140.0 - 142.5 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.38 - 8.34 (m, 3H), 7.80 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.33 (app. t, J = 7.7 Hz, 1H), 7.25 - 7.14 (m, 5H), 6.96 - 6.94 (m, 2H), 4.88 (t, J = 5.6 Hz, 1H), 4.71 (t, J = 5.6 Hz, 1H), 3.51 - 3.44 (m, 4H), 3.34 - 3.28 (m, 2H), 2.51 - 2.48 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 165.8, 162.0 (d, $^{1}J_{CF}$ = 246.7 Hz), 149.6, 144.5, 142.9, 138.8, 135.3, 134.3, 134.2, 132.1, 131.3 (d, $^{3}J_{CF}$ = 8.5 Hz), 128.8, 128.7 (d, $^{4}J_{CF}$

= 3.1 Hz), 127.9, 126.4, 125.5, 119.8, 115.9 (d, $^{2}J_{CF}$ = 21.8 Hz), 96.0, 73.4, 59.7, 59.4, 42.2, 23.6; ESI-HRMS-TOF calcd for $C_{28}H_{24}FN_2O_3S^+$ $(M+H)^+$ 487.1486, found 487.1508; ESI-LCMS R_t = 4.9 min, 487.2 $(M+H)^+$; RP-HPLC R_t = 6.2 min, > 99%.

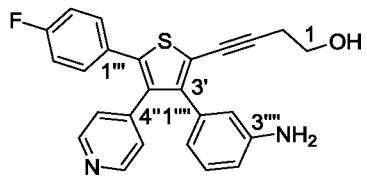
4-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)-N-(2-hydroxyethyl)benzamide (3.38)



[00175] To a suspension of compound **3.32** (60 mg, 0.14 mmol) in acetonitrole (1.5 mL) was added (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (90 mg, 0.20 mmol), *N,N*-diisopropylethylamine (35 µL, 0.20 mmol) and ethanolamine (38 µL, 0.68 mmol). The reaction mixture was stirred at room temperature for 16 hours. Ethyl acetate (10 mL) was added and the mixture was washed with water (3 × 10 mL) and the aqueous fraction further extracted with ethyl acetate (3 × 10 mL). The combined organic fractions was dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by gradient column chromatography (0 - 10% methanol/chloroform) afforded compound **3.38** (30 mg, 45%) as a white powder. **3.38**: $C_{28}H_{23}FN_2O_3S$ (M_r = 486.56); mp 218.7 - 220.9 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.43 - 8.39 (m, 3H), 7.74 (app. d, *J* = 8.5 Hz, 2H), 7.25 - 7.15 (m, 6H), 6.96 - 6.95 (m, 2H), 4.88 (t, *J* = 5.6 Hz, 1H), 4.70 (t, *J* = 5.7 Hz, 1H), 3.52 - 3.46 (m, 4H), 3.35 - 3.28 (m, 2H), 2.53 - 2.49 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 165.8, 162.0 (d, $^{1}J_{CF}$ = 247.0 Hz), 149.6, 144.1, 142.9, 138.9, 136.8, 135.2, 133.5, 131.3 (d, $^{3}J_{CF}$ = 8.5 Hz), 129.5, 128.6 (d, $^{4}J_{CF}$ = 3.2 Hz), 126.9, 125.5, 119.8, 115.9 (d, $^{2}J_{CF}$ =

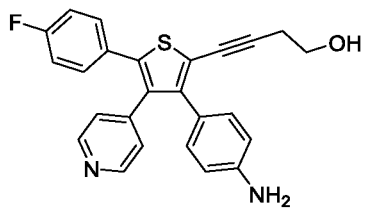
21.8 Hz), 96.1, 73.3, 59.7, 59.4, 42.2, 23.6; ESI-HRMS-TOF calcd for $C_{28}H_{24}FN_2O_3S^+$ $(M+H)^+$ 487.1486, found 487.1483; ESI-LCMS R_t = 4.9 min, 487.2 $(M+H)^+$; RP-HPLC R_t = 6.1 min, 97%.

4-(3-(3-Aminophenyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.39)



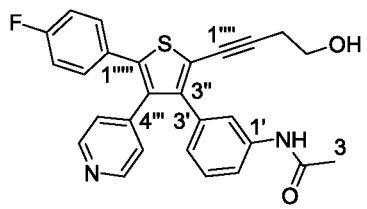
[00176] Compound **3.39** was synthesised from compound **3.13** (100 mg, 0.189 mmol) following the general procedure for TBS deprotection. Purification using gradient column chromatography (0 - 5% methanol/ethyl acetate) gave compound **3.39** (48 mg, 62%) as a yellow powder. **3.39**: $C_{25}H_{19}FN_2OS$ (M_r = 414.50); mp 142.5 - 143.8 °C; ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.29 - 8.27 (m, 2H), 7.22 - 7.16 (m, 2H), 7.05 - 6.93 (m, 5H), 6.64 - 6.61 (m, 2H), 6.37 (d, J = 7.5 Hz, 2H), 3.64 (t, J = 6.8 Hz, 2H), 2.58 (t, J = 6.8 Hz, 2H); ^{13}C NMR (76 MHz, CD_3OD) δ (ppm) 164.1 (d, $^1J_{CF}$ = 247.8 Hz), 149.7, 148.5, 147.3, 146.6, 140.9, 136.9, 136.2, 132.6 (d, $^3J_{CF}$ = 8.3 Hz), 130.6 (d, $^4J_{CF}$ = 3.1 Hz), 129.6, 127.5, 121.5, 121.0, 118.2, 116.7 (d, $^2J_{CF}$ = 22.1 Hz), 115.8, 95.2, 75.0, 61.4, 24.6; ESI-HRMS-TOF calcd for $C_{25}H_{20}FN_2OS^+$ $(M+H)^+$ 415.1275, found 415.1293; ESI-LCMS R_t = 4.9 min, 415.2 $(M+H)^+$; RP-HPLC R_t = 5.5 min, 96%.

4-(3-(4-Aminophenyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.40)



[00177] Compound **3.40** was synthesised from compound **3.14** (83 mg, 0.16 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.40** (56 mg, 86%) as yellow powder. **3.40**: $C_{25}H_{19}FN_2OS$ (M_r = 414.49); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.30 - 8.28 (m, 2H), 7.23 - 7.16 (m, 2H), 7.06 - 6.96 (m, 4H), 6.90 - 6.86 (m, 2H), 6.61 - 6.56 (m, 2H), 3.65 (t, *J* = 6.9 Hz, 2H), 2.58 (t, *J* = 6.9 Hz, 2H); ^{13}C NMR (76 MHz, *d*$_6$-DMSO) δ (ppm) 161.9 (d, $^1J_{CF}$ = 246.3 Hz), 149.4, 148.1, 145.8, 143.6, 138.2, 135.3, 131.3 (d, $^3J_{CF}$ = 8.4 Hz), 130.4, 129.1 (d, $^4J_{CF}$ = 2.6 Hz), 125.6, 121.2, 117.5, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 113.1, 94.7, 74.2, 59.6, 23.7; ESI-HRMS-TOF calcd for $C_{25}H_{20}FN_2OS^+$ $(M+H)^+$ 415.1275, found 415.1273; RP-HPLC R_t = 5.3 min, 90%.

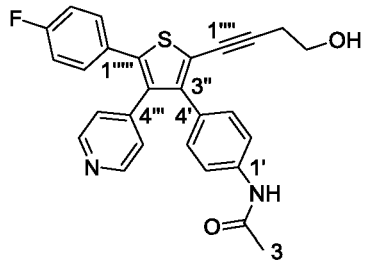
N-(3-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)acetamide (3.41)



[00178] Compound **3.41** was synthesised from compound **3.15** (65 mg, 0.11 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (0 - 10% methanol/chloroform) afforded compound **3.41** (47 mg, 90%) as white powder. **3.41**: $C_{27}H_{21}FN_2O_2S$ (M_r = 456.54); mp 139.4 - 143.2 °C; ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm) 9.89 (s, 1H), 8.38 - 8.36 (m, 2H), 7.53 (s, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.24 - 7.13 (m, 5H), 6.93 - 6.91 (m, 2H), 6.69 (d, *J* = 7.7 Hz, 1H), 4.87 (t, *J* = 5.6 Hz, 1H), 3.48 (app. q, *J* = 6.5 Hz, 2H), 2.51 (t, *J* = 6.9 Hz, 2H), 2.00 (s, 3H); ^{13}C NMR (101 MHz, *d*$_6$-DMSO) δ (ppm) 168.3, 162.0 (d, $^1J_{CF}$ = 246.8 Hz), 149.5, 145.0, 143.0, 139.1, 138.7, 135.3, 134.6, 131.4 (d, $^3J_{CF}$ = 8.4 Hz), 128.8 (d, $^4J_{CF}$ = 3.2 Hz), 128.2, 125.4, 124.3, 120.2, 119.4, 118.1, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 95.7, 73.5, 59.5, 24.0, 23.6; ESI-HRMS-TOF calcd for $C_{27}H_{22}FN_2O_2S^+$

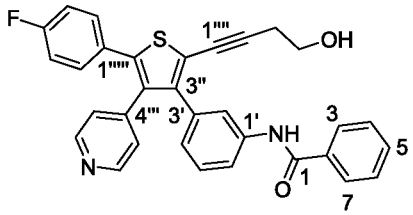
$(M+H)^+$ 457.1381, found 457.1394; ESI-LCMS R_t = 5.0 min, 457.2 $(M+H)^+$; RP-HPLC R_t = 6.5 min, > 99%.

N-(4-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)acetamide (3.42)



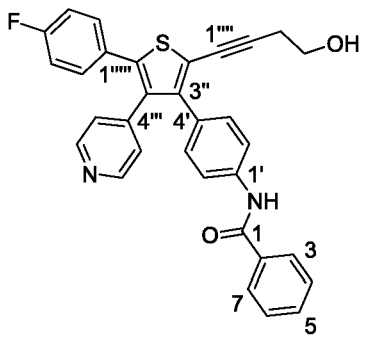
[00179] Compound **3.42** was synthesised from compound **3.16** (84 mg, 0.15 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (0 - 10% methanol/chloroform) afforded thiophene **3.42** (52 mg, 77%) as a white powder. **3.42**: $C_{27}H_{21}FN_2O_2S$ (M_r = 456.53); mp 251.4 - 253.3 °C; ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm) 9.96 (s, 1H), 8.39 - 8.38 (m, 2H), 7.46 (app. d, *J* = 8.7 Hz, 2H), 7.23 - 7.14 (m, 4H), 7.04 (app. d, *J* = 8.7 Hz, 2H), 6.94 - 6.92 (m, 2H), 4.88 (t, *J* = 5.6 Hz, 1H), 3.50 (app. q, J = 6.3 Hz, 2H), 2.53 - 2.49 (m, 2H), 2.02 (s, 3H); ^{13}C NMR (101 MHz, *d*$_6$-DMSO) δ (ppm) 168.4, 162.0 (d, $^1J_{CF}$ = 246.7 Hz), 149.5, 144.8, 143.2, 138.7, 138.6, 135.3, 131.3 (d, $^3J_{CF}$ = 8.4 Hz), 130.1, 128.8 (d, $^4J_{CF}$ = 3.2 Hz), 128.7, 125.5, 118.9, 118.2, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 95.5, 73.7, 59.5, 24.0, 23.6; ESI-HRMS-TOF calcd for $C_{27}H_{22}FN_2O_2S^+$ $(M+H)^+$ 457.1381, found 457.1390; ESI-LCMS R_t = 5.1 min, 457.2 $(M+H)^+$; RP-HPLC R_t = 6.6 min, > 99%.

N-(3-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)benzamide (3.43)



[00180] Compound **3.43** was synthesised from compound **3.17** (100 mg, 0.158 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (0 - 5% methanol/chloroform) afforded thiophene **3.43** (78 mg, 95%) as a white powder. **3.43**: $C_{32}H_{23}FN_2O_2S$ (M_r = 518.61); mp 204.4 - 206.3 °C; ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm) 10.23 (s, 1H), 8.40 - 8.38 (m, 2H), 7.92 - 7.90 (m, 2H), 7.82 (br. s, 1H), 7.67 (br. d, *J* = 8.1 Hz, 1H), 7.61 - 7.51 (m, 3H), 7.26 - 7.15 (m, 5H), 6.97 - 6.96 (m, 2H), 6.75 (d, *J* = 7.7 Hz, 1H), 4.86 (t, *J* = 5.6 Hz, 1H), 3.50 (app. q, *J* = 6.4 Hz, 2H), 2.53 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, *d*$_6$-DMSO) δ (ppm) 165.6, 162.0 (d, $^1J_{CF}$ = 246.5 Hz), 149.5, 145.0, 143.0, 139.0, 138.7, 135.3, 135.0, 134.6, 131.6, 131.4 (d, $^3J_{CF}$ = 8.4 Hz), 128.8 (d, $^4J_{CF}$ = 3.2 Hz), 128.4, 128.1, 127.7, 125.4, 124.9, 121.5, 119.5, 119.4, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 95.8, 73.5, 59.5, 23.7; ESI-HRMS-TOF calcd for $C_{32}H_{24}FN_2O_2S^+$ $(M+H)^+$ 519.1537, found 519.1525; ESI-LCMS R_t = 5.4 min, 519.2 $(M+H)^+$; RP-HPLC R_t = 8.1 min, 99%.

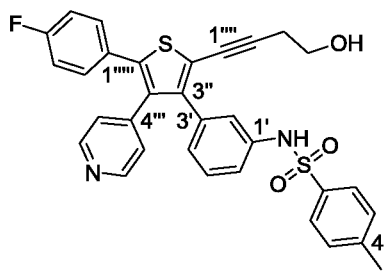
N-(4-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)benzamide (3.44)



[00181] Compound **3.44** was synthesised from compound **3.18** (150 mg, 0.237 mmol) following the general procedure for TBS deprotection. Purification

by gradient column chromatography (0 - 5% methanol/chloroform) afforded compound **3.44** (110 mg, 89%) as white powder. **3.44**: $C_{32}H_{23}FN_2O_2S$ (M_r = 518.61); mp 248.6 - 250.5 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 10.28 (s, 1H), 8.42 - 8.40 (m, 2H), 7.93 - 7.91 (m, 2H), 7.68 (app. d, J = 8.6 Hz, 2H), 7.61 - 7.50 (m, 3H), 7.27 - 7.15 (m, 4H), 7.12 (app. d, J = 8.6 Hz, 2H), 6.98 - 6.96 (m, 2H), 4.90 (t, J = 5.5 Hz, 1H), 3.52 (app. q, J = 6.4 Hz, 2H), 2.54 (t, J = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 165.7, 162.0 (d, $^1J_{CF}$ = 246.8 Hz), 149.6, 144.7, 143.2, 138.6, 138.5, 135.3, 134.9, 131.6, 131.3 (d, $^3J_{CF}$, J = 8.5 Hz), 130.0, 129.4, 128.8 (d, $^4J_{CF}$ = 3.3 Hz), 128.4, 127.6, 125.6, 119.6, 119.1, 115.8 (d, $^2J_{CF}$ = 21.9 Hz), 95.6, 73.7, 59.5, 23.7; ESI-HRMS-TOF calcd for $C_{32}H_{24}FN_2O_2S^+$ $(M+H)^+$ 519.1537, found 519.1559; ESI-LCMS R_t = 5.4 min, 519.2 $(M+H)^+$; RP-HPLC R_t = 7.1 min, 98%.

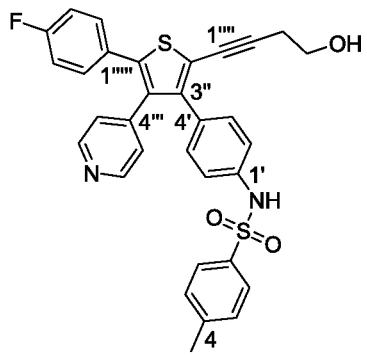
N-(3-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)-4-methylbenzenesulfonamide (3.45)



[00182] To a solution of thiophene **3.13** (98 mg, 0.19 mmol) in dichloromethane (5 mL) was added pyridine (91 µL, 1.1 mmol) and *p*-toluenesulfonyl chloride (45 mg, 0.23 mmol). The reaction mixture was stirred at room temperature for 24 hours. Aqueous hydrochloric acid (2.7 M, 20 mL) was added. The compound was extracted with dichloromethane (3 × 20 mL). The organic fractions were combined and dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by gradient column chromatography (0 - 50% ethyl acetate/petroleum spirits) gave compound **3.45** (62 mg, 59%) as a beige powder. **3.45**: $C_{32}H_{25}FN_2O_3S_2$ (M_r = 568.68); mp 212.7 - 214.6 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 10.18 (s, 1H), 8.34 - 8.33 (m, 2H), 7.51

(app. d, J = 8.3 Hz, 2H), 7.34 (app. d, J = 8.0 Hz, 2H), 7.23 - 7.14 (m, 4H), 7.08 (app. t, J = 7.9 Hz, 1H), 7.00 (br. app. t, J = 1.8 Hz, 1H), 6.95 (ddd, J = 8.1, 2.1, 0.9 Hz, 1H), 6.83 - 6.82 (m, 2H), 6.71 (app. dt, J = 7.7, 1.2 Hz, 1H), 4.88 (br. s, 1H), 3.47 (t, J = 6.9 Hz, 2H), 2.50 - 2.46 (m, 2H), 2.36 (s, 3H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 162.0 (d, $^{1}J_{CF}$ = 246.6 Hz), 149.3, 144.4, 143.2, 142.9, 138.8, 137.6, 136.6, 135.2, 135.1, 131.3 (d, $^{3}J_{CF}$ = 8.5 Hz), 129.6, 128.7, 128.7 (d, $^{4}J_{CF}$ = 3.4 Hz), 126.6, 125.4, 125.3, 121.4, 119.6, 119.0, 115.8 (d, $^{2}J_{CF}$ = 21.8 Hz), 95.8, 73.2, 59.5, 23.6, 21.0; ESI-HRMS-TOF calcd for $C_{32}H_{26}FN_2O_3S_2^+$ $(M+H)^+$ 569.1363, found 569.1370; ESI-LCMS R_t = 5.6 min, 569.2 $(M+H)^+$; RP-HPLC R_t = 7.6 min, > 99%.

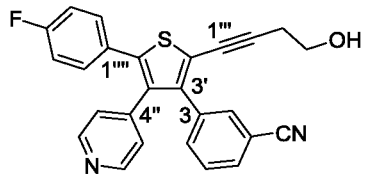
N-(4-(5-(4-fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)-4-methylbenzenesulfonamide (3.46)



[00183] Compound **3.46** was synthesised from compound **3.19** (119 mg, 0.174 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 100% ethyl acetate/petroleum spirits) followed by recrystallisation from methanol afforded compound **3.46** (68 mg, 69%) as white crystals. **3.46**: $C_{32}H_{25}FN_2O_3S_2$ (M_r = 568.68); mp 244.3 - 246.4 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 10.24 (s, 1H), 8.34 (br. app. d, J = 3.9 Hz, 2H), 7.59 (app. d, J = 8.3 Hz, 2H), 7.33 (app. d, J = 8.1 Hz, 2H), 7.21 - 7.13 (m, 4H), 6.99 - 6.94 (m, 4H), 6.87 (br. app. d, J = 5.6 Hz, 2H), 4.90 (br. s, 1H), 3.45 (t, J = 6.9 Hz, 2H), 2.51 - 2.46 (m, 2H), 2.36 (s, 3H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 162.0 (d, $^{1}J_{CF}$ = 246.7 Hz), 149.4, 144.5, 143.3,

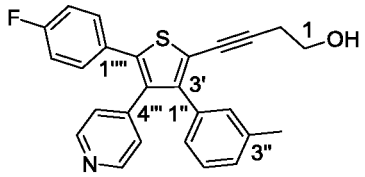
143.0, 138.6, 137.2, 136.5, 135.2, 131.3 (d, $^{3}J_{CF}$ = 8.4 Hz), 130.5, 129.7, 129.6, 128.7 (d, $^{4}J_{CF}$ = 3.2 Hz), 126.7, 125.4, 119.2, 119.1, 115.8 (d, $^{2}J_{CF}$ = 21.8 Hz), 95.6, 73.5, 59.4, 23.6, 21.0; ESI-HRMS-TOF calcd for $C_{32}H_{26}FN_2O_3S_2^+$ $(M+H)^+$ 569.1363, found 569.1382; ESI-LCMS R_t = 5.7 min, 569.2 $(M+H)^+$; RP-HPLC R_t = 7.3 min, 95%.

3-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)benzonitrile (3.47)



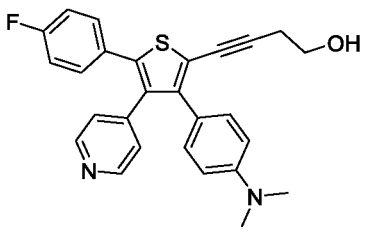
[00184] Compound **3.47** was synthesised from compound **3.20** (53 mg, 0.10 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 3% methanol/dichloromethane afforded compound **3.47** (40 mg, 96%) as a yellow solid. **3.47**: $C_{26}H_{17}FN_2OS$ (M_r = 424.49); mp 184.0 - 185.9 °C; ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.34 (app. d, *J* = 3.5 Hz, 2H), 7.65 - 7.61 (m, 2H), 7.46 - 7.39 (m, 2H), 7.25 - 7.21 (m, 2H), 7.07 - 7.01 (m, 4H), 3.65 (t, *J* = 6.5 Hz, 2H), 2.59 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (76 MHz, CD_3OD) δ (ppm) 164.2 (d, $^{1}J_{CF}$ = 248.2 Hz), 150.1, 145.9, 144.1, 141.7, 137.5, 135.9, 135.8, 134.7, 132.7 (d, $^{3}J_{CF}$ = 8.4 Hz), 132.3, 130.3, 130.1 (d, $^{4}J_{CF}$ = 2.5 Hz), 127.5, 122.9, 119.3, 116.8 (d, $^{2}J_{CF}$ = 22.2 Hz), 113.3, 96.6, 74.2, 61.3, 24.5; ESI-HRMS-TOF calcd for $C_{26}H_{18}FN_2OS^+$ $(M+H)^+$ 425.1118, found 425.1118; ESI-LCMS R_t = 5.5 min, 425.2 $(M+H)^+$; RP-HPLC R_t = 7.2 min, 99%.

4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)-3-(m-tolyl)thiophen-2-yl)but-3-yn-1-ol (3.48)



[00185] Compound **3.48** was synthesised from compound **3.21** (50 mg, 0.10 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 3% methanol/dichloromethane afforded thiophene **3.48** (37 mg, 94%) as a yellow powder. **3.48**: $C_{26}H_{20}FNOS$ (M_r = 413.51); mp 177.1 - 179.0 °C; ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.30 - 8.28 (m, 2H), 7.24 - 7.19 (m, 2H), 7.14 - 7.00 (m, 5H), 6.98 - 6.96 (m, 2H), 6.89 (d, *J* = 7.4 Hz, 1H), 3.63 (t, *J* = 6.8 Hz, 2H), 2.57 (t, *J* = 6.8 Hz, 2H), 2.24 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ (ppm) 164.1 (d, $^1J_{CF}$ = 247.7 Hz), 149.8, 146.9, 146.6, 141.1, 138.8, 136.2, 136.0, 132.6 (d, $^3J_{CF}$ = 8.4 Hz), 131.8, 130.5 (d, $^4J_{CF}$ = 3.4 Hz), 129.3, 128.9, 128.2, 127.6, 121.6, 116.7 (d, $^2J_{CF}$ = 22.1 Hz), 95.2, 75.0, 61.4, 24.5, 21.3; ESI-HRMS-TOF calcd for $C_{26}H_{21}FNOS^+$ $(M+H)^+$ 414.1322, found 414.1317; ESI-LCMS R_t = 5.5 min, 414.2 $(M+H)^+$; RP-HPLC R_t = 7.0 min, 98%.

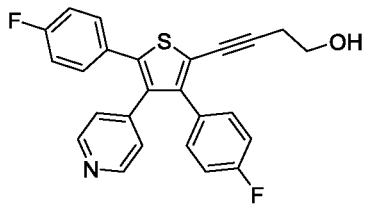
4-(3-(4-(Dimethylamino)phenyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.49)



[00186] Compound **3.49** was synthesised from compound **3.22** (63 mg, 0.11 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.49** (31 mg, 62%) as yellow powder. **3.49**: $C_{27}H_{23}FN_2OS$ (M_r = 442.55); ^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm) 8.40 - 8.38 (m, 2H), 7.22 - 7.12 (m, 4H), 6.96 - 6.93 (m, 4H), 6.57 (d, *J* = 8.9 Hz, 2H), 4.88 (t, *J* = 5.5 Hz, 1H),

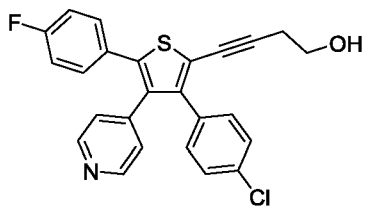
3.52 (app. q, J = 6.4 Hz, 2H), 2.87 (s, 6H), 2.55 - 2.49 (m, 2H); ESI-HRMS-TOF calcd for $C_{27}H_{24}FN_2OS^+$ $(M+H)^+$ 443.1588, found 443.1566.

4-(3,5-bis(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.50)



[00187] Compound **3.50** was synthesised from compound **3.23** (110 mg, 0.207 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.50** (81 mg, 94%) as yellow powder. **3.50**: $C_{25}H_{17}F_2NOS$ (M_r = 417.47); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.33 - 8.31 (m, 2H), 7.25 - 7.15 (m, 4H), 7.08 - 6.97 (m, 6H), 3.66 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ (ppm) 164.1 (d, $^1J_{CF}$ = 247.8 Hz), 163.6 (d, $^1J_{CF}$ = 246.3 Hz), 149.9, 146.4, 145.6, 141.2, 136.1, 133.1 (d, $^3J_{CF}$ = 8.2 Hz), 132.6 (d, $^3J_{CF}$ = 8.5 Hz), 132.3 (d, $^4J_{CF}$ = 3.3 Hz), 130.4 (d, $^4J_{CF}$ = 3.4 Hz), 127.6, 122.0, 116.7 (d, $^2J_{CF}$ = 22.0 Hz), 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 95.7, 74.7, 61.3, 24.5; ESI-HRMS-TOF calcd for $C_{25}H_{18}F_2NOS^+$ $(M+H)^+$ 418.1072, found 418.1063; RP-HPLC R_t = 6.9 min, 95%.

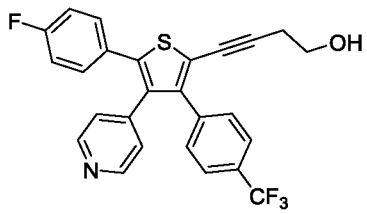
4-(3-(4-Chlorophenyl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.51)



[00188] Compound **3.51** was synthesised from compound **3.24** (81 mg, 0.15 mmol) following the general procedure for TBS deprotection. Purification

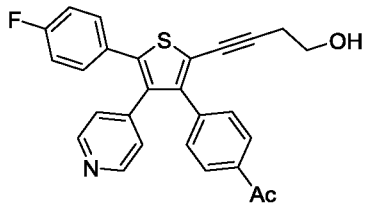
by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.51** (60 mg, 94%) as yellow powder. **3.51**: $C_{25}H_{17}ClFNOS$ (M_r = 433.93); ^{1}H NMR (400 MHz, DMSO) δ 8.42 - 8.40 (m, 2H), 7.37 - 7.33 (m, 2H), 7.24 - 7.13 (m, 6H), 6.96 (dd, *J* = 4.4, 1.6 Hz, 2H), 4.89 (br. s, 1H), 3.50 (dd, *J* = 10.2, 6.4 Hz, 2H), 2.54 - 2.49 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 162.0 (d, $^1J_{CF}$ = 246.8 Hz), 149.6, 143.6, 142.9, 138.9, 135.1, 133.0, 132.4, 131.5, 131.3 (d, $^3J_{CF}$ = 8.5 Hz), 128.6 (d, $^4J_{CF}$ = 3.1 Hz), 128.1, 125.5, 119.7, 115.9 (d, $^2J_{CF}$ = 21.9 Hz), 96.2, 73.2, 59.4, 23.6; ESI-HRMS-TOF calcd for $C_{25}H_{18}ClFNOS^+$ $(M+H)^+$ 434.0776, found 434.0784; RP-HPLC R_t = 7.2 min, 95%.

4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)-3-(4-(trifluoromethyl)phenyl)thiophen-2-yl)but-3-yn-1-ol (3.52)



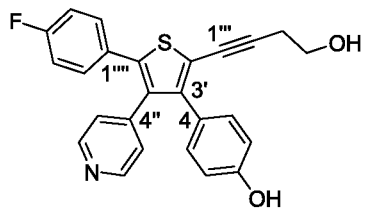
[00189] Compound **3.52** was synthesised from compound **3.25** (91 mg, 0.16 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.52** (58 mg, 79%) as yellow powder. **3.52**: $C_{26}H_{17}F_4NOS$ (M_r = 467.48); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.33 (br. app. d, *J* = 5.1 Hz, 2H), 7.58 (app. d, *J* = 8.3 Hz, 2H), 7.37 (app. d, *J* = 8.1 Hz, 2H), 7.25 - 7.21 (m, 2H), 7.08 - 6.99 (m, 4H), 3.64 (t, *J* = 6.7 Hz, 2H), 2.59 (t, *J* = 6.7 Hz, 2H); ^{13}C NMR (76 MHz, MeOH) δ 164.2 (d, $^1J_{CF}$ = 248.0 Hz), 150.1, 146.1, 144.9, 141.6, 140.1, 136.0, 132.7 (d, $^3J_{CF}$ = 8.5 Hz), 131.8, 130.6 (d, $^2J_{CF}$ = 32.3 Hz), 130.1 (d, $^4J_{CF}$ = 3.5 Hz), 127.5, 126.0 (q, $^3J_{CF}$ = 3.8 Hz), 125.6 (d, $^1J_{CF}$ = 271.2 Hz), 122.8, 116.8 (d, $^2J_{CF}$ = 22.1 Hz), 96.3, 74.3, 61.3, 24.5; ESI-HRMS-TOF calcd for $C_{26}H_{18}F_4NOS^+$ $(M+H)^+$ 468.1040, found 468.1051; RP-HPLC R_t = 7.4 min, > 99%.

1-(4-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenyl)ethanone (3.53)



[00190] Compound **3.53** was synthesised from compound **3.26** (60 mg, 0.11 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (20 - 50% EtOAc/hexane) afforded compound **3.53** (31 mg, 65%) as yellow powder. **3.53**: $C_{27}H_{20}FNO_2S$ (M_r = 441.52); ^{1}H NMR (300 MHz, CD_3OD) δ (ppm) 8.31 (br. app. d, J = 5.3 Hz, 2H), 7.92 - 7.88 (m, 2H), 7.34 - 7.30 (m, 2H), 7.26 - 7.20 (m, 2H), 7.09 - 6.99 (m, 4H), 3.64 (t, J = 6.7 Hz, 2H), 2.60 - 2.56 (m, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ (ppm) 199.9, 164.2 (d, $^1J_{CF}$ = 248.0 Hz), 150.0, 146.1, 145.3, 141.7, 141.1, 137.3, 135.9, 132.7 (d, $^3J_{CF}$ = 8.3 Hz), 131.5, 130.2 (d, $^4J_{CF}$ = 3.3 Hz), 129.2, 127.5, 122.6, 116.8 (d, $^2J_{CF}$ = 22.1 Hz), 96.3, 74.5, 61.3, 26.7, 24.5; ESI-HRMS-TOF calcd for $C_{27}H_{21}FNO_2S^+$ $(M+H)^+$ 442.1272, found 442.1288; RP-HPLC R_t = 6.6 min, 95%.

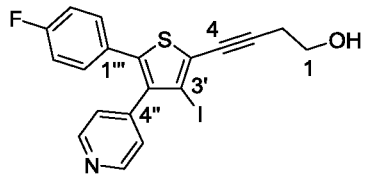
4-(5-(4-Fluorophenyl)-2-(4-hydroxybut-1-yn-1-yl)-4-(pyridin-4-yl)thiophen-3-yl)phenol (3.54)



[00191] Compound **3.54** was synthesised from compound **3.27** (53 mg, 0.10 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 3% methanol/dichloromethane afforded compound **3.54** (40 mg, 96%) as a white powder. **3.54**: $C_{25}H_{28}FNO_2S$ (M_r = 415.48); mp 248.1 - 251.0 °C; ^{1}H NMR (300 MHz, d_6-DMSO) δ (ppm) 9.53 (s,

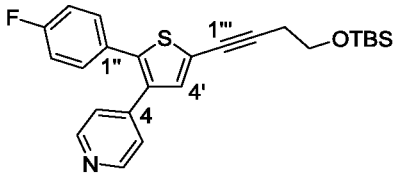
1H), 8.37 (app. d, *J* = 4.3 Hz, 2H), 7.21 - 7.11 (m, 4H), 6.98 - 6.87 (m, 4H), 6.62 (app. d, *J* = 8.5 Hz, 2H), 4.87 (t, *J* = 5.5 Hz, 1H), 3.48 (app. q, *J* = 6.7 Hz, 2H), 2.52 - 2.48 (m, 2H); ^{13}C NMR (76 MHz, CD_3OD) δ (ppm) 162.0 (d, $^{1}J_{CF}$ = 246.8 Hz), 156.9, 149.5, 145.2, 143.4, 138.5, 135.4, 131.4 (d, $^{3}J_{CF}$ = 8.4 Hz), 130.9, 129.0 (d, $^{4}J_{CF}$ = 2.4 Hz), 125.6, 124.8, 118.4, 115.9 (d, $^{2}J_{CF}$ = 21.8 Hz), 114.9, 95.2, 73.9, 59.6, 23.7; ESI-HRMS-TOF calcd for $C_{25}H_{19}FNO_2S^+$ $(M+H)^+$ 416.1115, found 416.1120; ESI-LCMS R_t = 5.1 min, 416.2 $(M+H)^+$; RP-HPLC R_t = 6.6 min, 99%.

4-(5-(4-Fluorophenyl)-3-iodo-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (3.55)



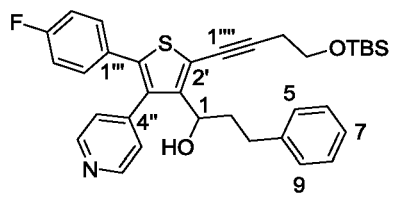
[00192] Compound **3.55** was synthesised from compound **3.6** (155 mg, 0.275 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (0 - 10% methanol/chloroform) followed by recrystallisation from methanol afforded compound **3.55** (117 mg, 95%) as a yellow crystals. **3.55**: $C_{19}H_{13}FINOS$ (M_r = 449.28); mp 187.2 - 189.3 °C; ^{1}H NMR (400 MHz, *d*$_6$-DMSO) δ (ppm) 8.61 - 8.59 (m, 2H), 7.22 - 7.12 (m, 6H), 4.98 (t, *J* = 5.6 Hz, 1H), 3.63 (td, *J* = 6.8, 5.7 Hz, 2H), 2.67 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, *d*$_6$-DMSO) δ (ppm) 162.1 (d, $^{1}J_{CF}$ = 247.2 Hz), 149.8, 144.3, 139.2, 138.9, 131.0 (d, $^{3}J_{CF}$ = 8.6 Hz), 128.2 (d, $^{4}J_{CF}$ = 3.3 Hz), 125.5, 124.2, 115.9 (d, $^{2}J_{CF}$ = 21.9 Hz), 97.6, 94.8, 75.1, 59.5, 23.8; ESI-HRMS-TOF calcd for $C_{19}H_{14}FINOS^+$ $(M+H)^+$ 449.9819, found 449.9833; ESI-LCMS R_t = 5.4 min, 450.0 $(M+H)^+$; RP-HPLC R_t = 6.5 min, 99%.

4-(5-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-2-(4-fluorophenyl)thiophen-3-yl)pyridine (4.3)



[00193] To a solution of thiophene **3.6** (50 mg, 0.089 mmol) in anhydrous tetrahydrofuran (1 mL) was added isopropylmagnesium chloride lithium chloride complex solution (0.83 M in tetrahydrofuran, 0.12 mL, 0.10 mmol) at -78 °C. The reaction mixture was stirred for 1 hour at -78 °C. Methanol (1 mL) was added and the mixture was diluted with diethyl ether (10 mL), washed with water (3 × 10 mL) and aqueous layer further extracted with diethyl ether (3 × 10 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (0 - 50% ethyl acetate/petroleum spirits) to give compound **4.3** as a yellow oil (30 mg, 77%). **4.3**: $C_{25}H_{28}FNOSSi$ (M_r = 437.65); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.51 (app. d, J = 5.8 Hz, 2H), 7.25 - 7.19 (m, 2H), 7.17 (s, 1H), 7.12 - 7.10 (m, 2H), 7.03 - 6.97 (m, 2H), 3.83 (t, J = 6.9 Hz, 2H), 2.68 (t, J = 6.9 Hz, 2H), 0.93 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 162.9 (d, $^1J_{CF}$ = 247.9 Hz), 150.2, 143.5, 140.1, 135.0, 133.3, 131.3 (d, $^3J_{CF}$ = 8.2 Hz), 129.1 (d, $^4J_{CF}$ = 3.4 Hz), 123.8, 123.7, 116.1 (d, $^2J_{CF}$ = 21.7 Hz), 93.4, 74.1, 61.6, 26.1, 24.3, 18.5, -5.1; ESI-HRMS-TOF calcd for $C_{25}H_{29}FNOSSi^+$ $(M+H)^+$ 438.1718, found 438.1724.

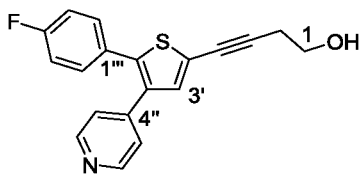
1-(2-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)-3-phenylpropan-1-ol (4.4)



[00194] To a solution of thiophene **3.6** (0.22 g, 0.39 mmol) in tetrahydrofuran (0.4 mL) was added isopropylmagnesium chloride lithium

chloride complex solution (0.83 M in tetrahydrofuran, 0.53 mL) at -78 °C. The reaction mixture was stirred at -78 °C for 30 minutes. Hydrocinnamaldehyde (55 µL, 0.42 mmol) was added and the reaction mixture was stirred at 0 °C for 1 hour. Saturated ammonium chloride was added and the mixture was extracted with diethyl ether, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting oil was purified by flash chromatography using gradient elution (0 - 70% ethyl acetate/hexane) to afford compound **4.4** as a yellow oil (0.14 g, 64%). **4.4**: $C_{34}H_{38}FNO_2SSi$ (M_r = 571.83); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.46 - 8.45 (m, 2H), 7.24 - 7.14 (m, 3H), 7.06 - 7.01 (m, 4H), 6.99 - 6.97 (m, 2H), 6.93 - 6.82 (m, 2H), 4.54 (td, *J* = 8.8, 5.2 Hz, 1H), 3.83 (t, *J* = 6.6 Hz, 2H), 2.70 (t, *J* = 6.6 Hz, 2H), 2.69 - 2.52 (m, 2H), 2.29 - 2.18 (m, 1H), 2.04 - 1.95 (m, 1H), 0.93 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.5 (d, $^1J_{CF}$ = 247.7 Hz), 149.8, 147.6, 144.1, 141.4, 140.0, 135.0, 131.0 (d, $^3J_{CF}$ = 8.1 Hz), 128.9 (d, $^4J_{CF}$ = 3.3 Hz), 128.4 (s, 2C), 126.0, 125.7, 119.5, 115.7 (d, $^2J_{CF}$ = 21.6 Hz), 97.9, 73.6, 68.8, 61.6, 38.6, 32.2, 26.0, 24.5, 18.4, -5.1; ESI-HRMS-TOF calcd for $C_{34}H_{39}FNO_2SSi^+$ $(M+H)^+$ 572.2449, found 572.2462; ESI-LCMS R_t = 7.2 min, 572.3 $(M+H)^+$; RP-HPLC R_t = 11.9 min, 96%.

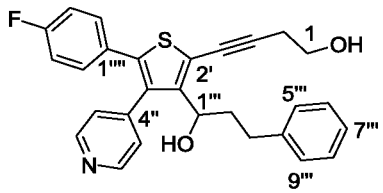
4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (4.5)



[00195] T To a solution of aryl iodide **5.1** (2.57 g, 6.74 mmol) in tetrahydrofuran (26 mL) was added 3-butyn-1-ol (0.77 mL, 10 mmol), triphenylphosphine (18 mg, 0.07 mmol), copper(I) iodide (64 mg, 0.34 mmol) and triethylamine (9.4 mL, 67 mmol). The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium(II) dichloride (237 mg, 0.338 mmol) was added and the reaction mixture was refluxed for 2

hours. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (3 × 10 mL) and brine (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting crude product was purified by gradient column chromatography (50 - 100% ethyl acetate/petroleum spirits) to afford compound **4.5** (1.90 g, 87%) as a yellow solid. Recrystallisation from methanol gave white crystals. **4.5**: $C_{19}H_{14}FNOS$ (M_r = 323.39); mp 153.2 - 155.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.52 - 8.50 (m, 2H), 7.25 - 7.20 (m, 3H), 7.12 - 7.10 (m, 2H), 7.03 - 6.97 (m, 2H), 3.84 (app. q, *J* = 5.5 Hz, 2H), 2.75 (t, *J* = 6.3 Hz, 2H), 1.82 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.4 Hz), 149.9, 143.6, 140.3, 134.8, 133.4, 131.1 (d, $^3J_{CF}$ = 8.2 Hz), 128.8 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 123.4, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 93.1, 74.4, 60.8, 24.3; ESI-HRMS-TOF calcd for $C_{19}H_{15}FNOS^+$ $(M+H)^+$ 324.0853, found 324.0862; ESI-LCMS R_t = 4.9 min, 324.2 $(M+H)^+$; RP-HPLC R_t = 6.3 min, 96%.

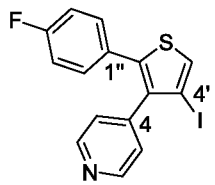
4-(5-(4-Fluorophenyl)-3-(1-hydroxy-3-phenylpropyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (4.6)



[00196] Compound **4.6** was synthesised from compound **4.4** (44 mg, 0.08 mmol) following the general procedure for TBS deprotection. Purification by gradient column chromatography (0 - 5% methanol/dichloromethane) afforded **4.6** as a yellow oil (35 mg, 99%). **4.6**: $C_{28}H_{24}FNO_2S$ (M_r = 457.56); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.46 - 8.45 (m, 2H), 7.26 - 7.15 (m, 3H), 7.08 - 7.01 (m, 4H), 6.96 - 6.94 (m, 2H), 6.92 - 6.86 (m, 2H), 4.53 (dd, *J* = 9.1, 4.6 Hz, 1H), 3.85 (t, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 5.6 Hz, 2H, 2.73 - 2.67 (m, 1H), 2.62 - 2.55 (m, 1H), 2.40 - 2.30 (m, 1H), 2.04 - 1.95 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 249.2 Hz), 150.0, 148.1, 143.9, 141.3, 139.8, 134.9, 131.0 (d, $^3J_{CF}$ = 8.2 Hz), 128.9 (d, $^4J_{CF}$ = 3.4 Hz), 128.533, 128.526,

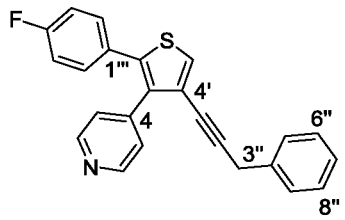
126.1, 125.6, 119.3, 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 97.6, 75.0, 68.8, 61.1, 38.5, 32.2, 24.5; ESI-HRMS-TOF calcd for $C_{28}H_{25}FNO_2S^+$ $(M+H)^+$ 458.1585, found 458.1599; ESI-LCMS R_t = 5.5 min, 458.2 $(M+H)^+$; RP-HPLC R_t = 7.2 min, > 99%.

4-(2-(4-Fluorophenyl)-4-iodothiophen-3-yl)pyridine (4.16)



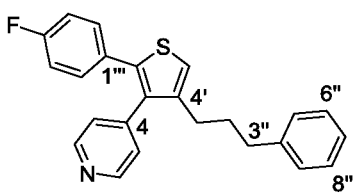
[00197] To a solution of thiophene **3.3** (0.20 g, 0.39 mmol) in anhydrous tetrahydrofuran (5 mL) was added isopropylmagnesium chloride lithium chloride complex solution (0.95 M in tetrahydrofuran, 0.45 mL, 0.43 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 1 hour. Methanol (5 mL) was added and the mixture was concentrated *in vacuo*. The crude product was diluted with diethyl ether (10 mL), washed with water (10 mL) and extracted with diethyl ether (3 × 10 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Purification by flash column chromatography using gradient elution (0 - 50% ethyl acetate/petroleum spirits) gave compound **4.16** (0.14 g, 95%) as a yellow powder. **4.16**: $C_{15}H_9FINS$ (M_r = 381.21); mp 129.3 - 131.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.60 - 8.58 (app. d, J = 4.5 Hz, 2H), 7.53 (s, 1H), 7.13 - 7.07 (m, 4H), 6.94 - 6.89 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.7 (d, $^1J_{CF}$ = 249.3 Hz), 149.9, 144.6, 140.3, 138.2, 130.9 (d, $^3J_{CF}$ = 8.3 Hz), 128.8 (d, $^4J_{CF}$ = 3.4 Hz), 128.6, 125.8, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 83.2; ESI-HRMS-TOF calcd for $C_{15}H_{10}FINS^+$ $(M+H)^+$ 381.9557, found 381.9559; ESI-LCMS R_t = 5.9 min, 382.0 $(M+H)^+$; RP-HPLC R_t = 7.2 min, 95%.

4-(2-(4-Fluorophenyl)-4-(3-phenylprop-1-yn-1-yl)thiophen-3-yl)pyridine (4.19)



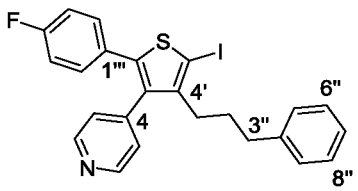
[00198] Compound **4.16** (0.55 g, 1.4 mmol) was dissolved in tetrahydrofuran (5.5 mL). Copper(I) iodide (15 mg, 0.079 mmol), triphenylphosphine (8 mg, 0.001 mmol), 3-phenyl-1-propyne (0.27 mL, 2.2 mmol) and triethylamine (5.5 mL) were added. The reaction mixture was bubbled with nitrogen for 30 minutes. Bis(triphenylphosphine)palladium(II) dichloride (60 mg, 0.085 mmol) was added and the reaction mixture was heated at reflux for 4 hours. Water (20 mL) was added and the mixture was extracted with ethyl acetate (3 × 20 mL). The organic layers were concentrated *in vacuo* and the product was purified by flash column chromatography using gradient elution (0 - 40% ethyl acetate/petroleum spirits) to give compound **4.19** as a yellow oil (0.15 g, 28%). **4.19**: $C_{24}H_{16}FNS$ (M_r = 369.46); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.49 - 8.48 (m, 2H), 7.49 (s, 1H), 7.29 - 7.13 (m, 9H), 6.99 - 6.93 (m, 2H), 3.71 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.7 (d, $^1J_{CF}$ = 248.9 Hz), 149.6, 143.4, 140.1, 136.4, 136.3, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 129.3 (d, $^4J_{CF}$ = 3.4 Hz), 128.7, 128.1, 128.0, 126.9, 125.3, 124.0, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 89.8, 77.4, 25.9; ESI-HRMS-TOF calcd for $C_{24}H_{17}FNS^+$ $(M+H)^+$ 370.1060, found 370.1068; ESI-LCMS R_t = 5.9 min, 370.2 $(M+H)^+$; RP-HPLC R_t = 8.4 min, 95%.

4-(2-(4-Fluorophenyl)-4-(3-phenylpropyl)thiophen-3-yl)pyridine (4.18)



[00199] To a dry three-neck round bottom flask was added a solution of thiophene **4.19** (105 mg, 0.284 mmol) in ethanol (20 mL). The round bottom flask was evacuated and flushed with nitrogen. Palladium on carbon (10% w/w, approx. 10 mg) was added and the round bottom flask was evacuated and flushed with nitrogen three times, then evacuated and flushed with hydrogen three times. The reaction mixture was stirred at room temperature for 3 days under hydrogen. The product was filtered through celite and the solvent was evaporated *in vacuo* to afford thiophene **4.18** (106 mg, quant.) as a yellow oil. **4.18**: $C_{24}H_{20}FNS$ (M_r = 373.49); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 - 8.53 (m, 2H), 7.27 - 7.23 (m, 2H), 7.19 - 7.16 (m, 1H), 7.13 - 7.05 (m, 7H), 6.93 - 6.88 (m, 2H), 2.57 (t, J = 7.6 Hz, 2H), 2.50 (t, J = 7.8 Hz, 2H), 1.79 (app. p, J = 7.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.2 (d, $^1J_{CF}$ = 248.1 Hz), 149.9, 145.1, 142.2, 141.8, 140.3, 135.8, 130.9 (d, $^3J_{CF}$ = 8.1 Hz), 129.9 (d, $^4J_{CF}$ = 3.4 Hz), 128.39, 128.35, 125.9, 125.3, 120.4, 115.6 (d, $^2J_{CF}$ = 21.6 Hz), 35.4, 31.4, 29.1; ESI-HRMS-TOF calcd for $C_{24}H_{21}FNS^+$ $(M+H)^+$ 374.1373, found 374.1380; ESI-LCMS R_t = 6.1 min, 374.2 $(M+H)^+$; RP-HPLC R_t = 8.7 min, 97%.

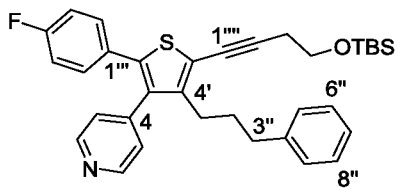
4-(2-(4-Fluorophenyl)-5-iodo-4-(3-phenylpropyl)thiophen-3-yl)pyridine (4.20)



[00200] To a solution of compound **4.18** (81 mg, 0.22 mmol) in acetonitrile (3 mL) was added iodine (65 mg, 0.26 mmol). The reaction mixture was stirred until the iodine had dissolved. Silver nitrate (48 mg, 0.28 mmol) was added to the solution resulting in formation of a yellow precipitate. The reaction mixture was stirred at room temperature for 1 hour and the precipitate filtered. The resulting filtrate was evaporated *in vacuo* and the crude product was diluted with ethyl acetate (20 mL), washed with saturated sodium thiosulfate (20 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo* to give

compound **4.20** (108 mg, quant.). Recrystallisation in methanol afforded the desired compound **4.20** as yellow crystals. **4.20**: $C_{24}H_{19}FINS$ (M_r = 499.39); mp 140.1 - 141.9; ^{1}H NMR (400 MHz, d_6-DMSO + NaOH) δ (ppm) 8.51 - 8.50 (m, 2H), 7.21 - 7.08 (m, 9H), 6.99 - 6.97 (m, 2H), 2.46 - 2.37 (m, 4H), 1.56 - 1.49 (m, 2H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 161.8 (d, $^1J_{CF}$ = 246.1 Hz), 149.9, 145.7, 143.7 (2C), 141.1, 136.1, 130.8 (d, $^3J_{CF}$ = 8.4 Hz), 129.1 (d, $^4J_{CF}$ = 3.2 Hz), 128.3, 128.1, 125.8, 125.1, 115.8 (d, $^2J_{CF}$ = 21.8 Hz), 77.6, 34.8, 30.4, 30.3; ESI-HRMS-TOF calcd for $C_{24}H_{20}FINS^+$ $(M+H)^+$ 500.0340, found 500.0360; ESI-LCMS R_t = 6.8 min, 500.1 $(M+H)^+$; RP-HPLC R_t = 10.1 min, 99%. Nb. NaOH added to d_6-DMSO sample to remove broadening of the pyridyl proton signals.

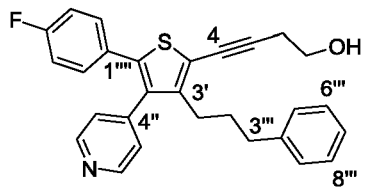
4-(5-(4-((tert-Butyldimethylsilyl)oxy)but-1-yn-1-yl)-2-(4-fluorophenyl)-4-(3-phenylpropyl)thiophen-3-yl)pyridine (4.2)



[00201] Compound **4.20** (80 mg, 0.16 mmol) was dissolved in tetrahydrofuran (1 mL). Copper(I) iodide (3 mg, 0.02 mmol), triphenylphosphine (1 mg, 0.004 mmol), alkyne **3.5** (19 µL, 0.24 mmol) and triethylamine (1 mL) were added. The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium(II) dichloride (8 mg, 0.01 mmol) was added and the reaction mixture was heated at reflux for 2 hours. Water (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 10 mL). The organic layers were concentrated *in vacuo* and the product was purified by column chromatography using gradient elution (0 - 40% ethyl acetate/petroleum spirits) to give compound **4.2** as a yellow solid (52 mg, 58%). **4.2**: $C_{34}H_{38}FNOSSi$ (M_r = 555.83); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.52 (br. s, 2H), 7.24 - 7.20 (m, 2H), 7.17 - 7.13 (m, 1H), 7.08 - 7.00 (m, 6H), 6.92 - 6.86 (m, 2H), 3.82 (t, J = 7.0 Hz, 2H), 2.68 (t, J = 7.0 Hz, 2H), 2.60 - 2.56 (m, 2H),

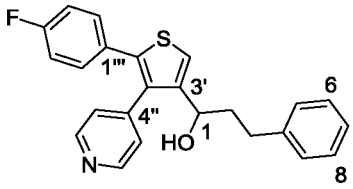
2.51 (t, J = 7.4 Hz, 2H), 1.71 - 1.62 (m, 2H), 0.93 (s, 9H), 0.11 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.4 (d, $^1J_{CF}$ = 248.6 Hz), 150.1, 146.0, 144.8, 141.8, 139.2, 135.6, 130.9 (d, $^3J_{CF}$ = 8.1 Hz), 129.4 (d, $^4J_{CF}$ = 3.5 Hz), 128.4 (2C), 125.9, 125.2, 119.5, 115.7 (d, $^2J_{CF}$ = 21.7 Hz), 95.2, 74.0, 61.9, 35.6, 31.2, 28.2, 26.0, 24.4, 18.5, -5.1; ESI-HRMS-TOF calcd for $C_{34}H_{39}FNOSSi^+$ $(M+H)^+$ 556.2500, found 556.2508; ESI-LCMS R_t = 8.5 min, 556.3 $(M+H)^+$; RP-HPLC R_t = 12.8 min, 99%.

4-(5-(4-Fluorophenyl)-3-(3-phenylpropyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-ol (4.1)



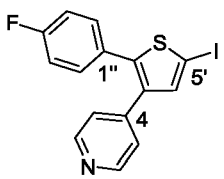
[00202] Compound **4.1** was synthesised from compound **4.2** (46 mg, 0.083 mmol) following the general procedure for TBS deprotection. Purification by column chromatography in 50% ethyl acetate/petroleum spirits afforded **4.1** (35 mg, 96%) as a yellow solid. **4.1**: $C_{28}H_{24}FNOS$ (M_r = 441.56); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 (br. s, 2H), 7.25 - 7.21 (m, 2H), 7.18 - 7.14 (m, 1H), 7.08 - 7.00 (m, 6H), 6.92 - 6.86 (m, 2H), 3.80 (br. s, 2H), 2.74 (t, J = 6.3 Hz, 2H), 2.60 - 2.56 (m, 2H), 2.52 (t, J = 7.4 Hz, 1H), 1.85 (br. s, 1H), 1.72 - 1.63 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.5 (d, $^1J_{CF}$ = 248.9 Hz), 150.0, 146.3, 144.8, 141.7, 139.5, 135.5, 130.9 (d, $^3J_{CF}$ = 8.2 Hz), 129.2 (d, $^4J_{CF}$ = 3.4 Hz), 128.4 (2C), 125.9, 125.3, 119.1, 115.7 (d, $^2J_{CF}$ = 21.7 Hz), 94.5, 74.6, 61.1, 35.6, 31.2, 28.2, 24.4; ESI-HRMS-TOF calcd for $C_{28}H_{25}FNOS^+$ $(M+H)^+$ 442.1635, found 442.1625; ESI-LCMS R_t = 5.8 min, 442.2 $(M+H)^+$; RP-HPLC R_t = 8.5 min, 99%.

1-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-3-yl)-3-phenylpropan-1-ol (4.17)



[00203] To a solution of thiophene **4.16** (97 mg, 0.25 mmol) in anhydrous tetrahydrofuran (1 mL) was added isopropylmagnesium chloride lithium chloride complex solution (1.2 M in THF, 0.24 mL, 0.29 mmol) at -78 °C. The reaction mixture was stirred at -78 °C for 30 minutes. Hydrocinnamaldehyde (36 µL, 0.28 mmol) was added and the mixture was stirred at 0 °C for 1 hour. Saturated ammonium chloride (5 mL) was added and the mixture was extracted with ethyl acetate (3 × 5 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (20 - 60% ethyl acetate/petroleum spirits) to give compound **4.17** (62 mg, 63%) as a yellow oil. **4.17**: $C_{24}H_{20}FNOS$ (M_r = 389.49); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 - 8.47 (m, 2H), 7.41 (d, J = 0.6 Hz, 1H), 7.25 - 7.13 (m, 3H), 7.12 - 7.06 (m, 2H), 7.06 - 7.00 (m, 4H), 6.94 - 6.87 (m, 2H), 4.56 (app. dd, J = 8.4, 4.4 Hz, 1H), 2.78 - 2.54 (m, 2H), 2.06 - 1.85 (m, 2H), 1.66 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.4 (d, $^1J_{CF}$ = 248.5 Hz), 149.7, 145.8, 144.7, 141.4, 141.1, 134.5, 131.0 (d, $^3J_{CF}$ = 8.1 Hz), 129.6 (d, $^4J_{CF}$ = 3.5 Hz), 128.5, 128.4, 126.0, 125.5, 121.1, 115.7 (d, $^2J_{CF}$ = 21.7 Hz), 67.7, 39.5, 32.1; ESI-HRMS-TOF calcd for $C_{24}H_{21}FNOS^+$ $(M+H)^+$ 390.1322, found 390.1342; ESI-LRMS 390.4 $(M+H)^+$; ESI-LCMS R_t = 5.3 min, 390.2 $(M+H)^+$; RP-HPLC R_t = 7.9 min, 93%.

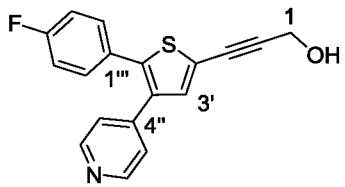
4-(2-(4-Fluorophenyl)-5-iodothiophen-3-yl)pyridine (5.1)



[00204] To a solution of compound **3.2** (2.88 g, 11.3 mmol) in acetonitrile (87 mL) was added iodine (3.15 g, 12.4 mmol). Silver nitrate (2.30 g, 13.6 mmol) was added to the suspension resulting in formation of a yellow

precipitate. The reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was filtered and the resulting filtrate was evaporated *in vacuo*. Chloroform (100 mL) was added and the mixture was washed with aqueous sodium thiosulfate (100 mL). The organic layer was evaporated *in vacuo* and the crude compound was purified by column chromatography in diethyl ether to afford compound **5.1**. Recrystallisation in methanol afforded the desired compound **5.1** (3.20 g, 74%) as white crystals. **5.1**: $C_{15}H_9FINS$ (M_r = 381.21); mp 147.6 - 149.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.52 - 8.50 (m, 2H), 7.32 (s, 1H), 7.22 - 7.17 (m, 2H), 7.11 - 7.09 (m, 2H), 7.03 - 6.98 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 163.0 (d, $^1J_{CF}$ = 249.4 Hz), 150.2, 145.8, 142.6, 139.0, 137.2, 131.1 (d, $^3J_{CF}$ = 8.2 Hz), 128.6 (d, $^4J_{CF}$ = 3.5 Hz), 123.5, 116.1 (d, $^2J_{CF}$ = 21.8 Hz), 73.2; ESI-HRMS-TOF calcd for $C_{15}H_{10}FINS^+$ $(M+H)^+$ 381.9557, found 381.9568; ESI-LCMS R_t = 5.6 min, 381.9 $(M+H)^+$; RP-HPLC R_t = 6.8 min, 99%.

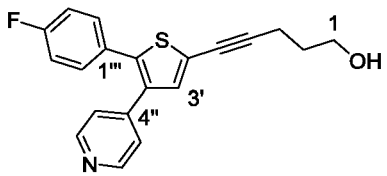
3-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)prop-2-yn-1-ol (5.2)



[00205] To a solution of aryl iodide **5.1** (500 mg, 1.31 mmol) in tetrahydrofuran (5 mL) was added propargyl alcohol (116 µL, 1.97 mmol), triphenylphosphine (3 mg, 0.01 mmol), copper(I) iodide (13 mg, 0.068 mmol) and triethylamine (5.0 mL, 36 mmol). The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium(II) dichloride (46 mg, 0.066 mmol) was added and the reaction mixture was refluxed for 2 hours. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (3 × 10 mL), brine (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting crude product was purified by column chromatography in 50% ethyl acetate/petroleum spirits to afford compound **5.2** (308 mg, 76%) as a yellow foam. **5.2**: $C_{18}H_{12}FNOS$ (M_r = 309.36); ^{1}H NMR

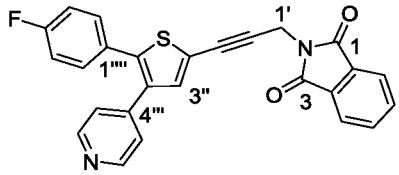
(400 MHz, $CDCl_3$) δ (ppm) 8.53 - 8.51 (m, 2H), 7.24 (s, 1H), 7.24 - 7.20 (m, 2H), 7.12 - 7.11 (m, 2H), 7.03 - 6.98 (m, 2H), 4.54 (s, 2H), 2.17 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.9 (d, $^1J_{CF}$ = 249.7 Hz), 149.9, 143.6, 141.3, 134.9, 134.1, 131.2 (d, $^3J_{CF}$ = 8.3 Hz), 128.7 (d, $^4J_{CF}$ = 3.5 Hz), 123.7, 122.6, 116.1 (d, $^2J_{CF}$ = 21.8 Hz), 93.7, 77.6, 51.3; ESI-HRMS-TOF calcd for $C_{18}H_{13}FNOS^+$ $(M+H)^+$ 310.0696, found 310.0710; ESI-LCMS R_t = 4.9 min, 310.0 $(M+H)^+$; RP-HPLC R_t = 5.5 min, 99%.

5-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)pent-4-yn-1-ol (5.3)



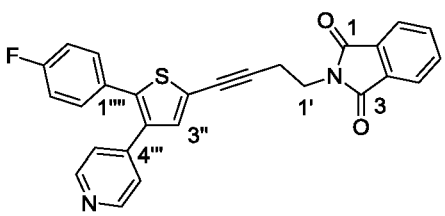
[00206] To a solution of aryl iodide **5.1** (200 mg, 0.525 mmol) in tetrahydrofuran (2 mL) was added 4-pentyn-1-ol (73 µL, 0.79 mmol), triphenylphosphine (1.4 mg, 5.0 µmol), copper(I) iodide (5.0 mg, 0.026 mmol) and triethylamine (2.0 mL, 14 mmol). The reaction mixture was bubbled with nitrogen for 15 minutes. Bis(triphenylphosphine)palladium(II) dichloride (18 mg, 0.026 mmol) was added and the reaction mixture was refluxed for 2 hours. The reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (3 × 10 mL), brine (10 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting crude product was purified by column chromatography in chloroform to afford compound **5.3** (151 mg, 85%) as a yellow foam. **5.3**: $C_{20}H_{16}FNOS$ (M_r = 337.41); ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.50 (app. d, *J* = 5.6 Hz, 2H), 7.24 - 7.18 (m, 2H), 7.18 (s, 1H), 7.12 - 7.10 (m, 2H), 7.03 - 6.97 (m, 2H), 3.82 (br. t, *J* = 5.9 Hz, 2H), 2.60 (t, *J* = 7.0 Hz, 2H), 1.92 - 1.85 (m, 2H), 1.55 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.3 Hz), 150.0, 143.6, 140.0, 134.9, 133.2, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 128.9 (d, $^4J_{CF}$ = 3.5 Hz), 123.8, 123.7, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 95.6, 73.4, 61.4, 31.4, 16.4; ESI-HRMS-TOF $(M+H)^+$ calcd for $C_{20}H_{17}FNOS^+$ $(M+H)^+$ 338.1009, found 338.1026; ESI-LCMS R_t = 5.1 min, 338.0 $(M+H)^+$; RP-HPLC R_t = 6.2 min, > 99%.

2-(3-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)prop-2-yn-1-yl)isoindoline-1,3-dione (5.4)



[00207] Compound **5.2** (188 mg, 0.608 mmol) was dissolved in tetrahydrofuran (6.1 mL). The solution was cooled to 0 °C. Phthalimide (179 mg, 1.22 mmol) and triphenylphosphine (239 mg, 0.911 mmol) were added. Diisopropyl azodicarboxylate (179 µL, 0.909 mmol) was added over 15 minutes. The reaction mixture was stirred at room temperature for 20 hours. Water (100 µL) was added and the solvent evaporated *in vacuo*. The crude compound was purified by column chromatography in ethyl acetate to afford compound **5.4** (136 mg, 51%) as a yellow foam. **5.4**: $C_{26}H_{15}FN_2O_2S$ (M_r = 438.48); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.50 - 8.49 (m, 2H), 7.91 (app. dd, J = 5.5, 3.0 Hz, 2H), 7.76 (app. dd, J = 5.5, 3.1 Hz, 2H), 7.26 (s, 1H), 7.22 - 7.17 (m, 2H), 7.09 - 7.07 (m, 2H), 7.02 - 6.96 (m, 2H), 4.72 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 167.1, 162.8 (d, $^1J_{CF}$ = 249.5 Hz), 150.1, 143.1, 141.3, 135.0, 134.8, 134.3, 132.1, 131.2 (d, $^3J_{CF}$ = 8.3 Hz), 128.7 (d, $^4J_{CF}$ = 3.5 Hz), 123.7, 123.5, 121.8, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 88.3, 75.7, 28.0; ESI-HRMS-TOF calcd for $C_{26}H_{16}FN_2O_2S^+$ $(M+H)^+$ 439.0911, found 439.0910; ESI-LCMS R_t = 5.7 min, 439.1 $(M+H)^+$; RP-HPLC R_t = 7.3 min, 95%.

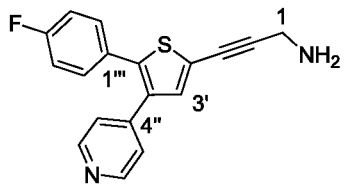
2-(4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-yl)isoindoline-1,3-dione (5.5)



[00208] Compound **4.5** (200 mg, 0.618 mmol) was dissolved in tetrahydrofuran (6.2 mL). The solution was cooled to 0 °C. Phthalimide

(182 mg, 1.24 mmol) and triphenylphosphine (243 g, 0.926 mmol) were added. Diisopropyl azodicarboxylate (183 µL, 0.929 mmol) was added over 15 minutes. The reaction mixture was stirred at room temperature for 20 hours. Water (100 µL) was added and the solvent evaporated *in vacuo*. The crude compound was purified by column chromatography in ethyl acetate to afford compound **5.5** (261 mg, 93%) as a yellow foam. **5.5**: $C_{27}H_{17}FN_2O_2S$ (M_r = 452.50); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.51 - 8.49 (m, 2H), 7.88 (app. dd, J = 5.5, 3.0 Hz, 2H), 7.73 (app. dd, J = 5.5, 3.1 Hz, 2H), 7.22 - 7.17 (m, 2H), 7.13 (s, 1H), 7.10 - 7.08 (m, 2H), 7.02 - 6.96 (m, 2H), 3.98 (t, J = 7.1 Hz, 2H), 2.88 (t, J = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 168.0, 162.7 (d, $^1J_{CF}$ = 249.3 Hz), 149.7, 143.5, 140.4, 134.8, 134.1, 133.5, 132.0, 131.1 (d, $^3J_{CF}$ = 8.2 Hz), 128.8 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 123.4, 123.0, 115.9 (d, $^2J_{CF}$ = 21.8 Hz), 91.7, 74.8, 36.5, 19.7; ESI-HRMS-TOF calcd for $C_{27}H_{18}FN_2O_2S^+$ $(M+H)^+$ 453.1068, found 453.1066; ESI-LCMS R_t = 5.7 min, 453.1 $(M+H)^+$; RP-HPLC R_t = 7.3 min, 95%.

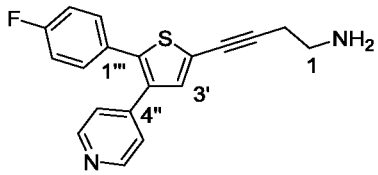
3-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)prop-2-yn-1-amine (5.6)



[00209] Compound **5.4** (136 mg, 0.310 mmol) was dissolved in methanol (6.1 mL). Hydrazine monohydrate (151 µL, 3.10 mmol) was added and the reaction mixture was stirred at room temperature for 20 hours. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography in ethyl acetate/methanol/triethylamine (15:4:1) to afford compound **5.6** (62 mg, 65%) as a brown foam. **5.6**: $C_{18}H_{13}FN_2S$ (M_r = 308.37); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.52 - 8.51 (m, 2H), 7.25 - 7.20 (m, 3H), 7.12 - 7.11 (m, 2H), 7.03 - 6.97 (m, 2H), 3.70 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.9 (d, $^1J_{CF}$ = 249.4 Hz), 150.2, 143.4, 140.7, 135.1, 133.7, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 128.9 (d, $^4J_{CF}$ = 3.5 Hz), 123.7, 123.0, 116.1 (d, $^2J_{CF}$ = 21.8 Hz), 95.8, 75.1, 32.5; ESI-HRMS-TOF calcd for $C_{18}H_{14}FN_2S^+$ $(M+H)^+$

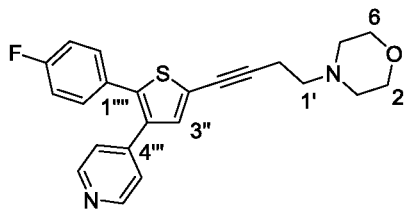
309.0856, found 309.0855; ESI-LCMS R_t = 4.1 min, 309.1 $(M+H)^+$; RP-HPLC R_t = 4.7 min, 82%.

4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-amine (5.7)



[00210] Compound **5.5** (149 mg, 0.329 mmol) was dissolved in methanol (6.6 mL). Hydrazine monohydrate (160 mL, 3.29 mmol) was added and the reaction mixture was stirred at room temperature for 20 hours. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography in ethyl acetate/methanol/triethylamine (15:4:1) to afford compound **5.7** (70 mg, 69%) as a yellow foam. **5.7**: $C_{19}H_{15}FN_2S$ (M_r = 322.40); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.51 (app. d, *J* = 5.6 Hz, 2H), 7.24 - 7.20 (m, 3H), 7.12 - 7.10 (m, 2H), 7.02 - 6.97 (m, 2H), 2.95 (br. s, 2H), 2.61 (t, *J* = 6.4 Hz, 2H) 1.59 (br. s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.2 Hz), 150.1, 143.4, 140.1, 135.0, 133.5, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 128.9 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 123.4, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 93.8, 74.4, 41.0, 24.8; ESI-HRMS-TOF calcd for $C_{19}H_{16}FN_2S^+$ $(M+H)^+$ 323.1013, found 323.1013; ESI-LCMS R_t = 4.2 min, 323.1 $(M+H)^+$; RP-HPLC R_t = 4.1 min, 98%.

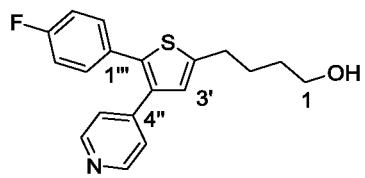
4-(4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)but-3-yn-1-yl)morpholine (5.8)



[00211] To a solution of thiophene **4.5** (195 mg, 0.603 mmol) in chloroform (5 mL) was added triethylamine (112 µL, 0.804 mmol). The reaction mixture was cooled to 0 °C and methanesulfonyl chloride (57 µL, 0.74 mmol) was added. The reaction mixture was stirred for 1 hour. Saturated sodium hydrogen

carbonate (20 mL) was added and the mixture was extracted with chloroform (3 × 20 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. Morpholine (2.0 mL, 23 mmol) was added and the reaction mixture was stirred at 100 °C for 1 hour. The mixture was diluted with diethyl ether (20 mL) and filtered. The filtrate was extracted with hydrochloric acid (3 M, 20 mL). The aqueous layer was made basic with sodium hydroxide and extracted with ethyl acetate (3 × 20 mL). The organic layers were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The product was purified by column chromatography using 5% methanol/chloroform to give thiophene **5.8** as a yellow oil (75 mg, 32%). **5.8**: $C_{23}H_{21}FN_2OS$ (M_r = 392.49); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.50 (app. d, J = 5.8 Hz, 2H), 7.24 - 7.17 (m, 2H), 7.17 (s, 1H), 7.11 - 7.10 (m, 2H), 7.02 - 6.96 (m, 2H), 3.74 - 3.72 (m, 4H), 2.71 - 2.62 (m, 4H), 2.54 - 2.52 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.2 Hz), 150.1, 143.4, 140.1, 135.0, 133.3, 131.2 (d, $^3J_{CF}$ = 8.2 Hz), 128.9 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 123.6, 116.0 (d, $^2J_{CF}$ = 21.8 Hz), 94.1, 73.9, 67.0, 57.3, 53.5, 18.1; ESI-HRMS-TOF calcd for $C_{23}H_{22}FN_2OS^+$ $(M+H)^+$ 393.1431, found 393.1430; ESI-LCMS R_t = 4.3 min, 393.1 $(M+H)^+$; RP-HPLC R_t = 5.0 min, 99%.

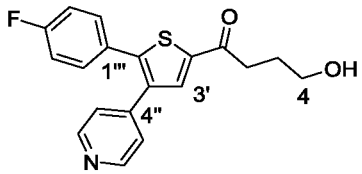
4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)butan-1-ol (5.9)



[00212] To a dry three-neck round bottom flask was added a solution of thiophene **4.5** (200 mg, 0.618 mmol) in ethanol (20 mL). The round bottom flask was evacuated and flushed with nitrogen. Palladium on carbon (10% w/w, approx. 10 mg) was added and the round bottom flask was evacuated and flushed with nitrogen three times, then evacuated and flushed with hydrogen three times. The reaction mixture was stirred at room temperature for 3 days. The product was filtered through celite and the solvent was evaporated *in vacuo*. The resulting crude product was purified by column chromatography

10% methanol/chloroform to afford thiophene **5.9** as a white solid (82 mg, 40%). **5.9**: $C_{19}H_{18}FNOS$ (M_r = 327.42); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.47 (app. d, *J* = 5.9 Hz, 2H), 7.24 - 7.19 (m, 2H), 7.14 - 7.12 (m, 2H), 7.01 - 6.95 (m, 2H), 6.87 (br. s, 1H), 3.71 (t, *J* = 6.4 Hz, 2H), 2.88 (t, *J* = 7.4 Hz, 2H), 1.86 - 1.67 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.6 (d, $^1J_{CF}$ = 248.2 Hz), 150.0, 145.2, 144.3, 137.6, 134.9, 131.1 (d, $^3J_{CF}$ = 8.1 Hz), 129.9 (d, $^4J_{CF}$ = 3.5 Hz), 126.8, 123.7, 115.9 (d, $^2J_{CF}$ = 21.7 Hz), 62.6, 32.2, 29.9, 27.9; ESI-HRMS-TOF calcd for $C_{19}H_{19}FNOS^+$ $(M+H)^+$ 328.1166, found 328.1165; ESI-LCMS Rt = 4.8 min, 328.0 $(M+H)^+$; RP-HPLC R_t = 5.8 min, 98%.

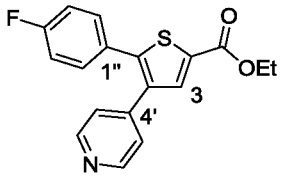
1-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)-4-hydroxybutan-1-one (5.10)



[00213] A solution of compound **4.5** (100 mg, 0.309 mmol) was dissolved in acetone (10 mL) and added dropwise to sulfuric acid (10 M, 0.77 mL) at 0 °C in an ice bath. The reaction mixture was allowed to warm to room temperature and stirred for 1.5 hours. The reaction mixture was concentrated *in vacuo* and then diluted with ethyl acetate (20 mL). The mixture was washed with water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography 10% methanol/chloroform to afford thiophene **5.10** as a pale yellow solid (75 mg, 71%). **5.10**: $C_{19}H_{16}FNO_2S$ (M_r = 341.40); mp 126.2 - 128.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.56 - 8.54 (m, 2H), 7.78 (s, 1H), 7.29 - 7.24 (m, 2H), 7.15 - 7.14 (m, 2H), 7.06 - 7.01 (m, 2H), 3.77 (br. t, *J* = 5.7 Hz, 2H), 3.10 (t, *J* = 7.0 Hz, 2H), 2.08 - 2.02 (m, 2H), 1.84 (br. s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 193.2, 163.1 (d, $^1J_{CF}$ = 250.5 Hz), 150.0, 147.7, 143.1, 142.7, 136.1, 133.7, 131.1 (d, $^3J_{CF}$ = 8.4 Hz), 128.5 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 116.2 (d, $^2J_{CF}$ = 21.9 Hz), 61.6, 35.6, 27.3; ESI-HRMS-TOF calcd for $C_{19}H_{17}FNO_2S^+$ $(M+H)^+$ 342.0959, found 342.0960; ESI-LCMS Rt = 4.5 min, 342.2 $(M+H)^+$; RP-HPLC R_t

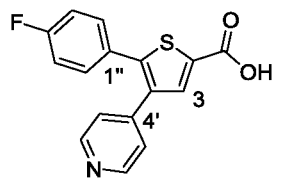
= 5.0 min, 95%.

Ethyl 5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophene-2-carboxylate (5.11)



[00214] A solution of *n*-butyllithium in hexanes (1.1 M, 4.0 mL, 4.4 mmol) was added to a solution of thiophene **3.2** (1.0 g, 3.9 mmol) in tetrahydrofuran (50 mL) at -78 °C. The reaction mixture was stirred for 30 minutes. Ethyl chloroformate (0.42 mL, 4.4 mmol) was added and the reaction mixture was stirred for 3 hours. Water (50 mL) was added and the tetrahydrofuran was evaporated *in vacuo.* The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic extracts were washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography in 50% ethyl acetate/petroleum spirits to afford compound **5.11** (0.71 g, 55%) as a yellow oil. **5.11**: $C_{18}H_{14}FNO_2S$ (M_r = 327.37); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 - 8.52 (m, 2H), 7.84 (s, 1H), 7.29 - 7.24 (m, 2H), 7.15 - 7.14 (m, 2H), 7.06 - 7.00 (m, 2H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.40 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 163.1 (d, $^1J_{CF}$ = 250.1 Hz), 161.8, 150.2, 146.2, 143.0, 135.9, 135.0, 132.9, 131.2 (d, $^3J_{CF}$ = 8.3 Hz), 128.7 (d, $^4J_{CF}$ = 3.5 Hz), 123.5, 116.2 (d, $^2J_{CF}$ = 21.9 Hz), 61.6, 14.4; ESI-HRMS-TOF calcd for $C_{18}H_{15}FNO_2S^+$ $(M+H)^+$ 328.0802, found 328.0800; ESI-LCMS R_t = 5.4 min, 328.0 $(M+H)^+$; RP-HPLC R_t = 6.3 min, 99%.

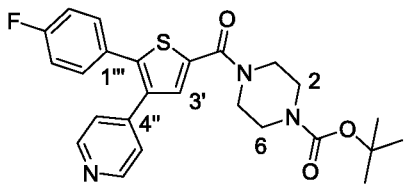
5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophene-2-carboxylic acid (5.12)



[00215] A mixture of ester **5.11** (600 mg, 1.83 mmol) and sodium hydroxide (290 mg, 7.25 mmol) in ethanol/water (1:1, 60 mL) was heated at

50 °C for 2 hours. The reaction mixture was concentrated *in vacuo* to remove the ethanol. The mixture was acidified with 1 M hydrochloric acid. The resulting precipitate was filtered and dried under vacuum to afford **5.12** as a white powder (468 mg, 85%). **5.12**: $C_{16}H_{10}FNO_2S$ (M_r = 299.32); mp 294.0 - 296.2 °C; ^{1}H NMR (400 MHz, d_6-DMSO) δ (ppm) 8.52 - 8.51 (m, 2H), 7.89 (s, 1H), 7.40 - 7.35 (m, 2H), 7.29 - 7.23 (m, 4H); ^{13}C NMR (101 MHz, d_6-DMSO) δ (ppm) 162.5, 162.5 (d, $^1J_{CF}$ = 247.3 Hz), 150.0, 145.1, 142.2, 136.0, 134.8, 133.8, 131.4 (d, $^3J_{CF}$ = 8.6 Hz), 128.7 (d, $^4J_{CF}$ = 3.2 Hz), 123.5, 116.2 (d, $^2J_{CF}$ = 21.9 Hz); ESI-HRM-TOF calcd for $C_{16}H_{11}FNO_2S^+$ $(M+H)^+$ 300.0489, found 300.0498; ESI-LCMS Rt = 4.7 min, 300.0 $(M+H)^+$; RP-HPLC R_t = 4.9 min, 99%.

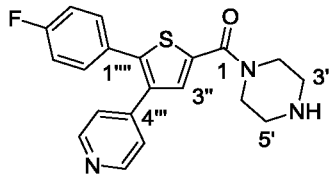
tert-Butyl 4-(5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophene-2-carbonyl)piperazine-1-carboxylate (5.13)



[00216] Dimethylformamide (20 µL) was added to a compound **5.12** (170 mg, 0.570 mmol) in dichloromethane (10 mL). Oxalyl chloride (195 µL, 2.27 mmol) was added dropwise under nitrogen. The reaction mixture was stirred at room temperature for 2 hours forming an orange solution. The solvent was evaporated *in vacuo* to give the acid chloride intermediate. Dichloromethane (5 mL) was added to the acid chloride intermediate followed by *N,N*-diisopropylethylamine (129 µL, 0.739 mmol) and 1-Boc-piperazine (116 mg, 0.623 mmol). The reaction mixture was stirred at room temperature for 4 hours and then poured into water (15 mL) and acidified to pH 1 with 1 M hydrochloric acid. The mixture was extracted with dichloromethane (3 × 20 mL) and the combined organic layers were concentrated *in vacuo*. The crude product was purified by flash column chromatography with gradient elution (0 - 10% methanol/chloroform) to afford compound **5.13** as a white solid (160 mg, 61%). **5.13**: $C_{25}H_{26}FN_3O_3S$ (M_r = 467.56); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm)

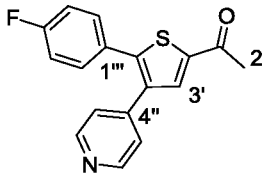
8.54 - 8.53 (m, 2H), 7.36 (s, 1H), 7.28 - 7.23 (m, 2H), 7.14 - 7.12 (m, 2H), 7.06 - 7.00 (m, 2H), 3.80 - 3.77 (m, 4H), 3.55 - 3.52 (m, 4H), 1.48 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 163.1, 163.0 (d, $^1J_{CF}$ = 249.9 Hz), 154.6, 150.2, 143.2, 143.1, 135.9, 135.1, 131.2 (d, $^3J_{CF}$ = 8.3 Hz), 131.0, 128.5 (d, $^4J_{CF}$ = 3.4 Hz), 123.6, 116.2 (d, $^2J_{CF}$ = 21.9 Hz), 80.6, 43.6, 43.4, 28.4; ESI-LCMS R_t = 5.3 min, 468.1 $(M+H)^+$; RP-HPLC R_t = 6.6 min, 98%. Nb. ^{13}C NMR signals at 43.6 and 43.4 ppm were identified from the HSQC experiment.

(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)(piperazin-1-yl)methanone (5.14)



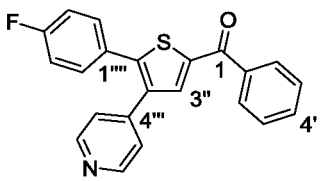
[00217] Compound **5.13** (93 mg, 0.20 mmol) was dissolved in dichloromethane (5 mL). Trifluoroacetic acid (1 mL) was added and the reaction mixture was stirred at room temperature for 2 hours. The mixture was poured into aqueous sodium hydroxide (1 M, 30 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography with gradient elution (10 - 20% methanol/chloroform) to afford compound **5.14** (53 mg, 72%) as a yellow oil. **5.14**: $C_{20}H_{18}FN_3OS$ (M_r = 367.44); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 - 8.52 (m, 2H), 7.36 (s, 1H), 7.28 - 7.22 (m, 2H), 7.14 - 7.12 (m, 2H), 7.06 - 7.00 (m, 2H), 3.97 - 3.95 (m, 4H), 3.14 - 3.10 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 163.1 (d, $^1J_{CF}$ = 250.0 Hz), 163.1, 150.2, 143.4, 143.2, 135.6, 135.1, 131.3 (d, $^3J_{CF}$ = 8.3 Hz), 131.2, 128.5 (d, $^4J_{CF}$ = 3.5 Hz), 123.6, 116.3 (d, $^2J_{CF}$ = 21.9 Hz), 45.3 (2C); ESI-HRMS-TOF calcd for $C_{20}H_{19}FN_3OS^+$ $(M+H)^+$ 368.1227, found 368.1228; ESI-LCMS R_t = 3.8 min, 368.1 $(M+H)^+$; RP-HPLC 5.7 min, 97%.

1-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)ethanone (5.15)



[00218] A mixture of acetyl chloride (84 µL, 1.2 mmol) and aluminium chloride (329 mg, 2.46 mmol) in dichloromethane (10 mL) was stirred at room temperature for 30 minutes. Thiophene **3.2** (201 mg, 0.787 mmol) in dichloromethane (5 mL) was added and the reaction mixture was refluxed for 16 hours. The reaction mixture was poured into ice water (100 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting product was purified by gradient column chromatography (50 - 70% diethyl ether/petroleum spirits) to afford compound **5.15** (155 mg, 66%). **5.15**: $C_{17}H_{12}FNOS$ (M_r = 297.35); mp 167.7 - 168.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.56 (app. d, J = 5.4 Hz, 2H), 7.72 (s, 1H), 7.30 - 7.25 (m, 2H), 7.16 - 7.14 (m, 2H), 7.06 - 7.00 (m, 2H), 2.60 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 190.4, 163.3 (d, $^1J_{CF}$ = 250.5 Hz), 150.4, 147.9, 143.14, 143.06, 136.4, 134.2, 131.2 (d, $^3J_{CF}$ = 8.4 Hz), 128.7 (d, $^4J_{CF}$ = 3.6 Hz), 123.6, 116.3 (d, $^2J_{CF}$ = 21.9 Hz), 26.8; ESI-HRMS-TOF $(M+H)^+$ calcd for $C_{17}H_{13}FNOS^+$ $(M+H)^+$ 298.0696, found 298.0699; ESI-LCMS R_t = 5.0 min, 298.0 $(M+H)^+$; RP-HPLC R_t = 5.4 min, > 99%.

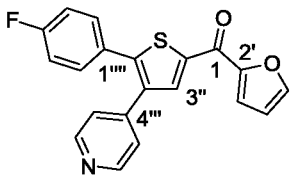
(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)(phenyl)methanone (5.16)



[00219] A mixture of benzoyl chloride (136 µL, 1.17 mmol) and aluminium chloride (320 mg, 2.40 mmol) in dichloromethane (10 mL) was stirred at room temperature for 30 minutes. Thiophene **3.2** (195 mg, 0.764 mmol) in dichloromethane (5 mL) was added and the reaction mixture was refluxed for

16 hours. The reaction mixture was poured into ice water (100 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting product was purified by column chromatgraphy (50% diethyl ether/petroleum spirits) to afford compound **5.16** (243 mg, 89%). **5.16**: $C_{22}H_{14}FNOS$ (M_r = 359.42); mp 151.4 - 153.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.55 (app. d, J = 5.2 Hz, 2H), 7.93 - 7.90 (m, 2H), 7.68 (s, 1H), 7.65 - 7.61 (m, 1H), 7.56 - 7.51 (m, 2H), 7.34 - 7.29 (m, 2H), 7.16 - 7.14 (m, 2H), 7.08 - 7.02 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 187.8, 163.4 (d, $^1J_{CF}$ = 250.6 Hz), 150.4, 148.3, 143.1, 142.3, 137.7, 136.4, 136.3, 132.7, 131.3 (d, $^3J_{CF}$ = 8.4 Hz), 129.3, 128.8, 128.7 (d, $^4J_{CF}$ = 3.5 Hz), 123.7, 116.4 (d, $^2J_{CF}$ = 21.9 Hz); ESI-HRMS-TOF calcd for $C_{22}H_{14}FNOS^+$ $(M+H)^+$ 360.0853, found 360.0860; ESI-LCMS R_t = 5.6 min, 360.0 $(M+H)^+$; RP-HPLC R_t = 6.8 min, 99%.

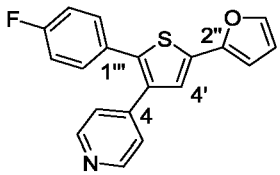
(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)(furan-2-yl)methanone (5.17)



[00220] A mixture of furoyl chloride (116 µL, 1.18 mmol) and aluminium chloride (313 mg, 2.35 mmol) in dichloromethane (10 mL) was stirred at room temperature for 30 minutes. Thiophene **3.2** (200 mg, 0.783 mmol) in dichloromethane (5 mL) was added and the reaction mixture was refluxed for 16 hours. The reaction mixture was poured into ice water (100 mL) and extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (50 mL), dried over magnesium sulfate, filtered and concentrated *in vacuo*. The resulting product was purified in 50% diethyl ether/petroleum spirits to afford compound **5.17** (233 mg, 85%). **5.17**: $C_{20}H_{12}FNO_2S$ (M_r = 349.38); mp 157.6 - 158.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.58 - 8.56 (m, 2H), 8.23 (s, 1H), 7.71 (dd, J = 1.7, 0.8 Hz, 1H), 7.47 (dd, J = 3.6, 0.8 Hz, 1H), 7.34 - 7.29 (m, 2H), 7.21 - 7.20 (m, 2H), 7.08 - 7.02 (m,

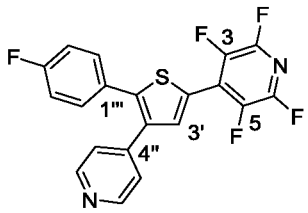
2H), 6.65 (dd, J = 3.6, 1.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 172.9, 163.3 (d, $^1J_{CF}$ = 250.5 Hz), 152.5, 150.3, 148.2, 146.8, 143.3, 140.8, 136.5, 135.7, 131.3 (d, $^3J_{CF}$ = 8.3 Hz), 128.7 (d, $^4J_{CF}$ = 3.5 Hz), 123.7, 119.3, 116.3 (d, $^2J_{CF}$ = 21.9 Hz), 112.9; ESI-HRMS-TOF $(M+H)^+$ calcd for $C_{20}H_{13}FNO_2S^+$ $(M+H)^+$ 350.0646, found 350.0660; ESI-LCMS R_t = 5.3 min, 350.0 $(M+H)^+$; RP-HPLC R_t = 6.2 min, 99%.

4-(2-(4-Fluorophenyl)-5-(furan-2-yl)thiophen-3-yl)pyridine (5.18)



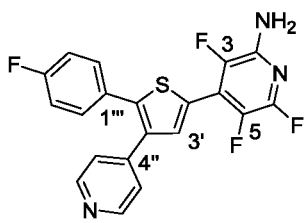
[00221] To a solution of thiophene **5.1** (200 mg, 0.525 mmol) in tetrahydrofuran (6 mL) was added furan-2-boronic acid (178 mg, 1.59 mmol) and sodium carbonate (1 M, 2 mL). The mixture was bubbled with nitrogen for 5 minutes. Bis(triphenylphosphine)palladium(II) dichloride (39 mg, 0.056 mmol) was added. The reaction mixture was heated in the microwave at 100 °C for 1.5 hours. Diethyl ether (20 mL) was added and the reaction mixture was washed with water (2 × 20 mL). The aqueous layer was further extracted with diethyl ether (2 × 20 mL) and the combined organic layers were evaporated *in vacuo*. The crude product was purified by gradient column chromatography (50 - 80% diethyl ether/petroleum spirits) to afford compound **5.18** (154 mg, 91%) as a yellow foam. **5.18**: $C_{19}H_{12}FNOS$ (M_r = 321.37); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.53 (br. s, 2H), 7.45 (dd, J = 1.8, 0.7 Hz, 1H), 7.31 (s, 1H), 7.31 - 7.23 (m, 2H), 7.20 - 7.18 (m, 2H), 7.04 - 6.99 (m, 2H), 6.57 (dd, J = 3.4, 0.6 Hz, 1H), 6.48 (dd, J = 3.4, 1.8 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 162.8 (d, $^1J_{CF}$ = 249.0 Hz), 150.1, 148.6, 143.9, 142.3, 138.3, 135.8, 133.2, 131.1 (d, $^3J_{CF}$ = 8.2 Hz), 129.3 (d, $^4J_{CF}$ = 3.5 Hz), 124.6, 123.7, 116.1 (d, $^2J_{CF}$ = 21.8 Hz), 112.0, 105.9; ESI-HRMS-TOF calcd for $C_{19}H_{13}FNOS^+$ $(M+H)^+$ 322.0696, found 322.0712; ESI-LCMS R_t = 5.5 min, 322.0 $(M+H)^+$; RP-HPLC R_t = 6.9 min, 95%.

2,3,5,6-Tetrafluoro-4-(5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)pyridine (5.19)



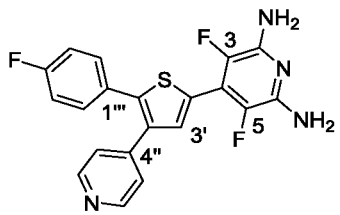
[00222] To a solution of thiophene **3.2** (350 g, 1.37 mmol) in tetrahydrofuran (13.7 mL) was added *n*-butyllithium in hexanes (1.06 M, 1.42 mL, 1.51 mmol) dropwise at -78 °C. The reaction mixture was stirred for 30 minutes and pentafluoropyridine (180 µL, 1.65 mmol) was added. The reaction mixture was stirred for a further 2 hours. The solvent was evaporated and the resulting product was taken up in water (40 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography in 50% ethyl acetate/petroleum spirits to afford compound **5.19** (365 mg, 65%) as a yellow solid. **5.19**: $C_{20}H_9F_5N_2S$ (M_r = 404.36); mp 137.5 - 139.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.61 - 8.59 (m, 2H), 7.94 (s, 1H), 7.35 - 7.30 (m, 4H), 7.12 - 7.06 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 163.4 (d, $^1J_{CF}$ = 250.7 Hz), 150.2, 145.8 - 143.0 (m, 2C), 143.0, 138.5 (app. dd, $^1J_{CF}$ = 261.1 Hz, $^2J_{CF}$ = 35.1 Hz), 136.2, 134.7 (t, $^4J_{CF}$ = 7.2 Hz), 131.3 (d, $^3J_{CF}$ = 8.4 Hz), 128.0 (d, $^4J_{CF}$ = 3.6 Hz), 126.0, 123.7, 116.4 (d, $^2J_{CF}$ = 21.9 Hz); ESI-HRMS-TOF calcd for $C_{20}H_{10}F_5N_2S^+$ $(M+H)^+$ 405.0479, found 405.0478; ESI-LCMS R_t = 6.3 min, 405.1 $(M+H)^+$; RP-HPLC R_t = 7.3 min, > 99%.

3,5,6-Trifluoro-4-(5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)pyridin-2-amine (5.20)



[00223] To a solution of compound **5.19** (100 mg, 0.247 mmol) in *N*-methyl-2-pyrrolidone (3 mL) was added aqueous ammonia (25%, 9 mL) in a microwave vial. The reaction mixture was sealed and heated on a hotplate at 120 °C for 1 hour. The resulting precipitate was filtered and dried to give compound **5.20** (99 mg, quant.) as a yellow solid. **5.20**: $C_{20}H_{11}F_4N_3S$ (M_r = 401.38); mp 209.7 - 211.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.57 - 8.55 (m, 2H), 7.84 (s, 1H), 7.36 - 7.28 (m, 4H), 7.11 - 7.04 (m, 2H), 4.61 (br. s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2 (d, $^1J_{CF}$ = 250.0 Hz), 150.0, 147.8 - 145.2 (m), 143.9 - 143.8 (m), 143.6, 143.9 - 141.2 (m) , 140.0 - 137.4 (m), 135.7, 133.6 (t, $^4J_{CF}$ = 6.8 Hz), 131.3 (d, $^3J_{CF}$ = 8.3 Hz), 128.4 (d, $^4J_{CF}$ = 3.5 Hz), 127.41 - 127.37 (m), 123.8, 121.9, 116.3 (d, $^2J_{CF}$ = 21.9 Hz); ESI-HRMS-TOF calcd for $C_{20}H_{12}F_4N_3S^+$ $(M+H)^+$ 402.0683, found 402.0682; ESI-LCMS R_t = 5.4 min, 402.1 $(M+H)^+$; RP-HPLC R_t = 6.8 min, 96%.

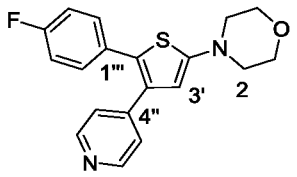
3,5-Difluoro-4-(5-(4-fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)pyridine-2,6-diamine (5.21)



[00224] To a solution of compound **5.19** (100 mg, 0.247 mmol) in *N*-methyl-2-pyrrolidone (3 mL) was added aqueous ammonia (25%, 9 mL) in a microwave vial. The reaction mixture was sealed and heated on a hotplate at 150 °C for 3 days. Ethyl acetate (20 mL) was added and the mixture was washed with water (3 × 20 mL). The aqueous fraction was extracted with ethyl acetate (20 mL). The combined organic layer was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography in 10% methanol/chloroform to afford compound **5.21** (35 mg, 36%). **5.21**: $C_{20}H_{13}F_3N_4S$ (M_r = 398.41); mp 228.2 - 230.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.55 - 8.54 (m, 2H), 7.75 (s, 1H), 7.33 - 7.28 (m, 2H), 7.21 - 7.19 (m, 2H), 7.07 - 7.01 (m, 2H), 4.31 (br. s, 4H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ (ppm) 163.0 (d, $^1J_{CF}$ = 249.4 Hz), 150.2, 143.6, 142.6 - 142.2 (m), 135.5, 135.3 (d, $^1J_{CF}$ = 245.2 Hz), 132.9 (t, $^4J_{CF}$ = 6.6 Hz), 131.3 (d, $^3J_{CF}$ = 8.3 Hz), 128.9 (d, $^4J_{CF}$ = 3.5 Hz), 128.6, 123.8, 118.6, 116.2 (d, $^2J_{CF}$ = 21.8 Hz); ESI-HRMS-TOF calcd for $C_{20}H_{14}F_3N_2S^+$ $(M+H)^+$ 399.0886, found 399.0883; ESI-LCMS R_t = 5.0 min, 399.1 $(M+H)^+$; RP-HPLC R_t = 5.8 min, 99%.

4-(5-(4-Fluorophenyl)-4-(pyridin-4-yl)thiophen-2-yl)morpholine (5.23)



[00225] A mixture of compound **5.1** (150 mg, 0.393 mmol), tris(dibenzylideneacetone)dipalladium(0) (7.2 mg, 7.9 µmol, 2 mol%), xantphos (4.6 mg, 7.9 µmol, 2 mol%), sodium *tert*-butoxide (53 mg, 0.55 mmol), toluene (1.2 mL) and morpholine (41 µL, 0.47 mmol) was stirred at 60 °C for 32 hours in a sealed tube. Ethyl acetate (10 mL) was added and the mixture was washed with water (3 × 10 mL). The organic layer was dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude product was purified by gradient column chromatography (50 - 100% ethyl acetate/petroleum spirits, then 1% methanol/ethyl acetate) to afford compound **5.23** (7.5 mg, 6%). **5.23**: $C_{19}H_{17}FN_2OS$ (M_r = 340.41); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.50 - 8.48 (m, 2H), 7.21 - 7.12 (m, 4H), 7.00 - 6.91 (m, 2H), 6.19 (s, 1H), 3.90 - 3.83 (m, 4H), 3.20 - 3.15 (m, 4H).

Biochemistry

Fluorescence polarisation (FP) assay

[00226] FP signals were measured with a PHERAstar microplate reader (BMG Labtech) using black, low-binding half-area 96 well plates (Corning). Each binding data point was carried out in duplicate or triplicate. Data were recorded in millipolarisation (mP) units and measured at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Tris(hydroxymethyl)aminomethane (Tris) assay buffer was used in all binding

experiments and consisted of 10 mM Tris, 50 nM potassium chloride, and 3.5 mM 3-((3-cholamidopropyl)dimethylammonium)-1-propanesulfonate (Chaps) at pH 8.0. The recombinant inactive and active His-tag p38α MAPK used in the ligand binding assays were prepared as described by Bukhtiyarova *et al.*

Determination of K_d for the fluorescently labelled ligand

[00227] The method used to determine the K_d for the fluorescently labelled ligand was similar to the method by Munoz *et al.* 5 nM of the fluoroprobe was incubated at room temperature for 1 hour with increasing concentrations of inactive non-phosphorylated p38α MAPK (0 - 1000 nM) in the presence of 10% dimethylsulfoxide. The K_d value to inactive p38α MAPK was calculated from 3 independent experiments and determined to be 13 ± 1 nM. To determine the affinity of the fluoroprobe to active phosphorylated p38α MAPK, 5 nM of fluoroprobe was incubated at room temperature for 2 hours with increasing concentrations of active phosphorylated p38α MAPK (0 - 500 nM) in the presence of 10% dimethylsulfoxide. The K_d value to active p38α MAPK was calculated from 3 independent experiments and determined to be 36 ± 2 nM.

Ligand binding experiments

[00228] Test compounds were prepared from dimethylsulfoxide stocks (10 mM). To determine the K_i values, the test compounds (final concentration ranging from 10 nM - 500 μM), 5 nM of fluoroprobe and 50 nM of inactive p38α MAPK were added to each well. Plates were incubated at room temperature for 1 hour and FP signals were recorded. For binding experiments to active p38α MAPK, the FP signals were recorded after 2 hours incubation at room temperature. Data is represented as mean ± SEM from two to three independent experiments. A list of K_i values for the synthesised analogues is given below in table 6.

Compound	K_i ± SEM (μM) to inactive p38α	K_i ± SEM (μM) to active p38α	IC_{50} (mean, μM)
RWJ67657	0.21 ± 0.04	0.013 ± 0.006	n.d.
3.2	5.0 ± 0.4	0.6 ± 0.1	n.d.

3.3	> 10	> 10	n.d.
3.28	> 10	8.7	n.d.
3.29	> 10	3.0	n.d.
3.30	> 10	> 10	n.d.
3.31	> 10	7.1	n.d.
3.32	> 10	> 10	n.d.
3.33	> 10	1.3 ± 0.2	n.d.
3.34	> 10	3.5	n.d.
3.35	> 10	2.2 ± 0.3	n.d.
3.36	> 10	2 ± 1	n.d.
3.37	1.9 ± 0.2	2 ± 1	n.d.
3.38	> 10	1.7 ± 0.2	n.d.
3.39	> 10	3.2	n.d.
3.40	> 10	> 10	n.d.
3.41	2.6 ± 0.6	2.0 ± 0.1	n.d.
3.42	> 10	> 10	n.d.
3.43	> 10	> 10	n.d.
3.44	> 10	> 10	n.d.
3.45	> 10	> 10	n.d.
3.46	> 10	> 10	n.d.
3.47	> 10	1.8 ± 0.4	n.d.
3.48	> 10	> 10	n.d.
3.49	> 10	> 10	n.d.
3.50	> 10	0.9 ± 0.4	n.d.
3.51	> 10	> 10	n.d.
3.52	> 10	> 10	n.d.
3.53	> 10	2.2 ± 0.3	n.d.
3.54	> 10	3.4	n.d.
3.55	> 10	> 10	n.d.
4.1	2.3 ± 0.2	0.6 ± 0.1	0.18
4.5	2.0 ± 0.2	0.56 ± 0.06	0.16
4.6	> 10	3.9	4.72
4.17	1.9 ± 0.3	0.99 ± 0.09	0.26
4.18	1.5 ± 0.5	0.72 ± 0.03	0.27
4.19	> 10	9.4	n.d.
5.1	1.9 ± 0.4	2.3	n.d.
5.2	2.1 ± 0.3	0.63 ± 0.04	n.d.
5.3	1.7 ± 0.2	0.80 ± 0.04	n.d.
5.6	> 10	n.d.	n.d.
5.7	1.1 ± 0.2	0.19 ± 0.04	n.d.
5.8	2.2 ± 0.2	2.5	n.d.
5.9	0.98 ± 0.02	0.25 ± 0.04	n.d.
5.10	2.7 ± 0.3	0.67 ± 0.03	n.d.
5.11	6 ± 1	2.2	n.d.
5.12	17 ± 2	1.4	n.d.
5.14	6.3 ± 0.4	0.9 ± 0.1	n.d.
5.15	6.4 ± 0.6	1.4	n.d.
5.16	5 ± 2	2.8 ± 0.1	n.d.
5.17	5.9 ± 0.7	2.5	n.d.
5.18	> 10	1.7	n.d.
5.19	3.01 ± 0.03	0.8 ± 0.1	n.d.
5.20	0.66 ± 0.06	0.21 ± 0.01	n.d.
5.21	0.47 ± 0.01	0.16 ± 0.02	n.d.

Table 6: Complete binding affinity and enzyme inhibition data of synthesised analogues

Competition experiments

[00229] Competition experiments were carried out to determine whether the synthesised analogues were competing with the fluoroprobe for the ATP binding pocket of p38α MAPK. In these experiments, the test compounds were analysed at two concentrations, with a constant concentration of fluoroprobe and increasing concentration of inactive p38α MAPK. Test compounds were prepared from dimethylsulfoxide stocks (10 mM). The test compound (final concentration differs between ligands) and SB203580-fluoroscein (5 nM) were added to each well. The competition binding assay was started by the addition of inactive p38α MAPK (final concentration 0.03 - 1000 nM), and the FP signals were measured after 1 hour of incubation at room temperature, where each binding data point was performed in duplicate. Graphs for the competition experiments are shown in FIG 14 indicating that all compounds bind competitively with SB203580-fluoroscein ligand to inactive p38α MAPK.

In vitro activation assay (phosphorylation of p38α MAPK)

[00230] Inactive p38 MAPK (500 ng) was pre-incubated with test inhibitors (1 and 10 μM) for 30 minutes in Tris buffer. The reaction was started by the addition of active MKK6 (5 ng) and ATP (100 μM). After 30 minutes incubation at room temperature, the reaction was stopped with ethylenediaminetetraacetic acid (EDTA) (10 mM). Phosphorylation of p38 MAPK was analysed by Western blotting.

Western blotting

[00231] Reaction samples were mixed 1:1 with Laemmli loading buffer, including β-mercaptoethanol (Biorad). Samples were heated at 95 °C for 5 minutes and 80 ng of protein was resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels (Biorad) at 200 V for 90 minutes. Proteins were transferred (100 V, 1 hour) to a polyvinylidene difluoride (PVDF) membrane (Merck). Membranes were incubated in blocking buffer (5% skim milk in Tris-buffered saline

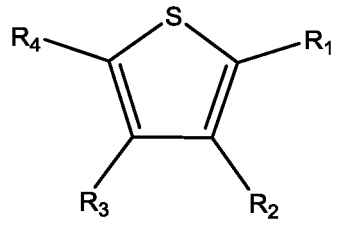
with Tween 20 (TBST), 1 hour, room temperature) and washed in TBST. Membranes were incubated with primary antibodies against total and phosphorylated p38 MAPK (1:1,000, Cell Signaling Technology) overnight at 4 °C. Membranes were washed 4 x 15 minutes in TBST and probed with a secondary anti-mouse antibody at 1:2,000 dilution for 1 hour at room temperature, followed by washing in TBST (4 x 15 minutes). Signal detection was done with Western Lightning plus-ECL enhanced chemiluminescent substrate (Perkin Elmer) with the ChemiDoc Imaging System (BioRad).

[00232] The above description of various embodiments of the present invention is provided for purposes of description to one of ordinary skill in the related art. It is not intended to be exhaustive or to limit the invention to a single disclosed embodiment. As mentioned above, numerous alternatives and variations to the present invention will be apparent to those skilled in the art of the above teaching. Accordingly, while some alternative embodiments have been discussed specifically, other embodiments will be apparent or relatively easily developed by those of ordinary skill in the art. Accordingly, this patent specification is intended to embrace all alternatives, modifications and variations of the present invention that have been discussed herein, and other embodiments that fall within the spirit and scope of the above described invention.

[00233] In the claims which follow and in the preceding description of the invention, except where the context clearly requires otherwise due to express language or necessary implication, the word “comprise”, or variations thereof including “comprises” or “comprising”, is used in an inclusive sense, that is, to specify the presence of the stated integers but without precluding the presence or addition of further integers in one or more embodiments of the invention.

ITEMISED LISTING OF EMBODIMENTS

1. A compound of formula (I), or a pharmaceutically acceptable salt thereof:



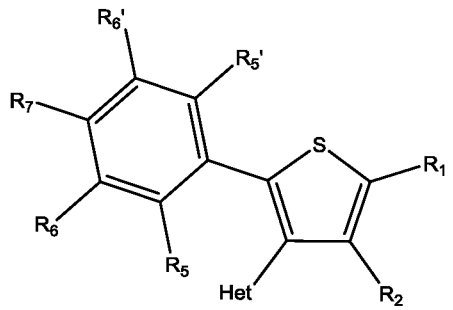
Formula (I)

wherein, R_1 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, alkanoyl, carboalkoxy, acyloxy, aryl, aroyl, heteroaryl, heteroaroyl, heterocyclyl, heterocycloyl, cycloalkyl, O-alkyl and O-aryl, O-heteroaryl, amino and amido, all of which groups may be substituted or unsubstituted;

R_2 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, alkylaryl, heterocyclyl and heteroaryl, all of which may be substituted or unsubstituted; and

R_3 and R_4 are independently selected from the group consisting of aryl, heteroaryl, heterocyclyl and cycloalkyl, all of which groups may be substituted or unsubstituted.

2. The compound of item 1 wherein the compound is a compound of formula (II), or a pharmaceutically acceptable salt thereof:



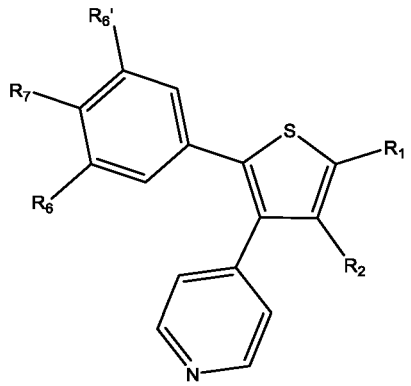
Formula (II)

wherein R_1 and R_2 are as described in item 1;

Het is selected from the group consisting of C_5-C_7 heteroaryl and C_5-C_7 heterocyclyl, each of which groups may be substituted or unsubstituted; and

R_5, R_5', R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo, haloalkyl, hydroxy and nitro.

3. The compound of item 1 or item 2 wherein the compound is a compound of formula (III), or a pharmaceutically acceptable salt thereof:



Formula (III)

wherein R_1, R_2, R_6, R_6' and R_7 are as described in item 1 or item 2 for formula (I) or formula (II).

4. The compound of any one of the preceding items wherein R_1 is selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_2-C_{12} alkanoyl, C_5-C_7 aryl, C_5-C_7 aroyl, C_5-C_7 heteroaryl, C_5-C_7 heteroaroyl, C_5-C_7 heterocyclyl, C_5-C_7 heterocycloyl and C_5-C_7 cycloalkyl, all of which groups may be substituted or unsubstituted.

5. The compound of any one of the preceding items wherein R_1 is selected from the group consisting of C_2-C_6 alkynyl, C_2-C_6 alkanoyl, C_5-C_6 aryl, C_5-C_6 aroyl, C_5-C_6 heteroaryl, C_5-C_6 heteroaroyl, C_5-C_6 heterocyclyl and C_5-C_6 heterocycloyl, all of which groups may be substituted or unsubstituted.

6. The compound of any one of the preceding items wherein R_2 is selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_5-C_7 aryl and alkyl-C_5-C_7 aryl, all of which may be substituted or unsubstituted.

7. The compound of any one of the preceding items wherein R_2 is hydrogen.

8. The compound of any one of the preceding items wherein R_3 is selected from the group consisting of C_5-C_7 heteroaryl and C_5-C_7 heterocyclyl, each of which groups may be substituted or unsubstituted.

9. The compound of any one of the preceding items wherein R_3 is selected from the group consisting of C_6 nitrogen heteroaryl and C_6 nitrogen heterocycyl, each of which groups may be substituted or unsubstituted.

10. The compound of any one of the preceding items wherein R_3 is selected from the group consisting of pyridyl, piperidyl, pyrazyl, pyrimidyl and pyridazyl, each of which groups may be substituted or unsubstituted.

11. The compound of any one of the preceding items wherein R_4 is substituted or unsubstituted C_5-C_7 aryl or C_5-C_7 heteroaryl.

12. The compound of any one of the preceding items wherein R_4 is substituted or unsubstituted phenyl.

13. The compound of any one of the preceding items wherein R_4 is phenyl substituted with a substituent selected from the group consisting of halo, haloalkyl, hydroxy and nitro.

14. The compound of any one of the preceding items wherein Het is selected from the group consisting of pyridyl, piperidyl, pyrazyl, pyrimidyl and pyridazyl, each of which groups may be substituted or unsubstituted.

15. The compound of any one of the preceding items wherein Het is selected from the group consisting of pyridyl, piperidyl and pyrimidyl, each of which groups may be substituted or unsubstituted.

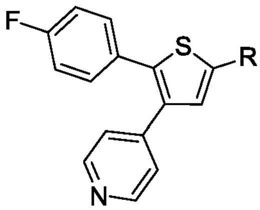
16. The compound of any one of the preceding items wherein R_5, R_5', R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo, haloalkyl, hydroxy and nitro

17. The compound of any one of the preceding items wherein R_5, R_5', R_6, R_6' and R_7 are independently selected from the group consisting of hydrogen, halo and haloalkyl.

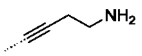
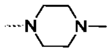
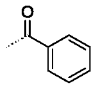
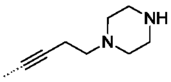
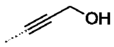
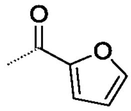
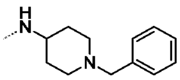
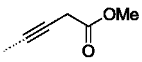
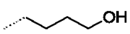
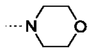
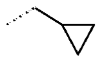
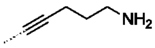
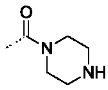
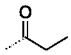
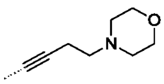
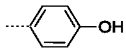
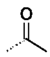
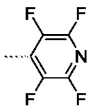
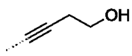
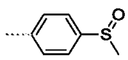
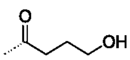
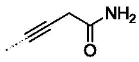
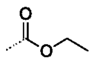
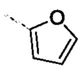
18. The compound of any one of the preceding items wherein R_5, R_5', R_6 and R_6' are hydrogen.

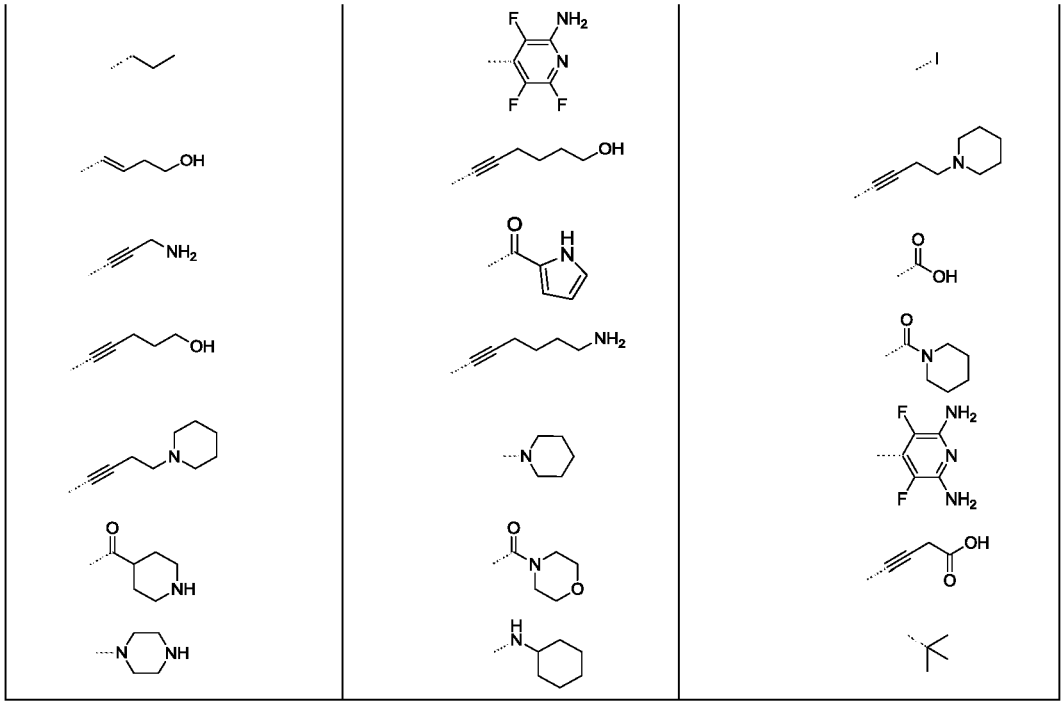
19. The compound of any one of the preceding items wherein R_7 is selected from the group consisting of halo and haloalkyl.

20. The compound of any one of the preceding items wherein the compound is a compound of the below formula, or a pharmaceutically acceptable salt thereof:



wherein R is selected from the groups shown in the below table

R	R	R
		
		
		
		
		
		
		
		
Me	H	



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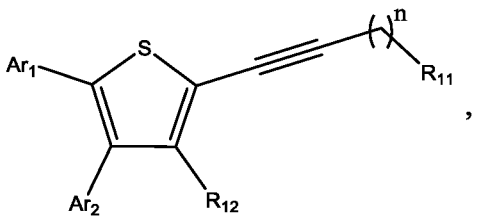
21. The compound of any one of the preceding items wherein the compound, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of:



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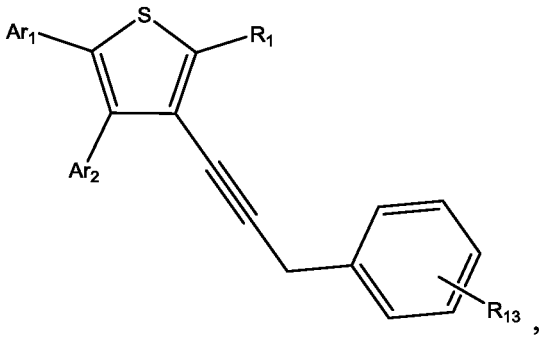

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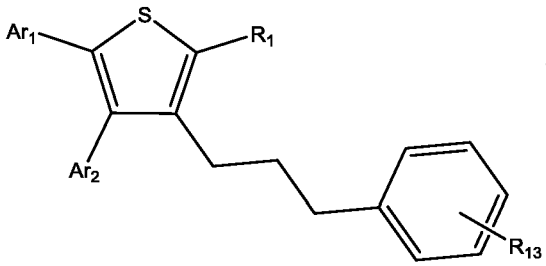

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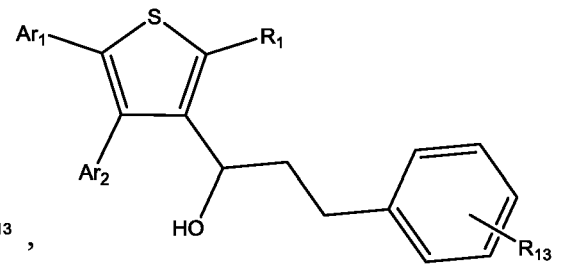

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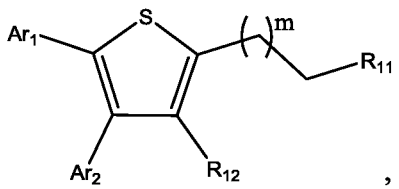

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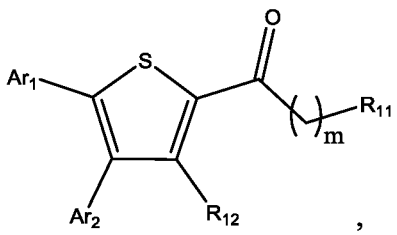

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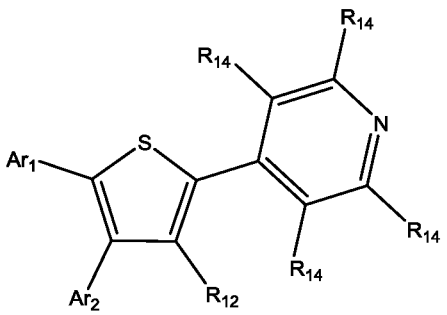

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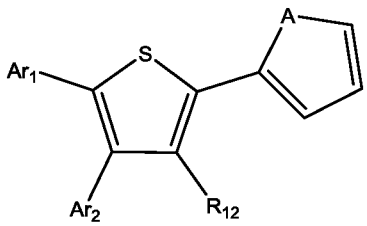


wherein, Ar_1 and Ar_2 are independently substituted or unsubstituted aryl or heteroaryl, A is selected from oxygen, sulphur or nitrogen, n is 1 or 2, m is 0 to 6, R_1 is as described in any one of the embodiments for formula (I) to (III),

R_{11} is selected from the group consisting of hydroxy, amino, C_1-C_6 alkyl, phenyl, furan, morpholine, piperazine and *N*-phthalimide;

R_{12} is selected from the group consisting of hydrogen, alkylphenyl and hydroxyalkyl phenyl wherein the phenyl ring may be substituted with R_{13};

R_{13}, when present, is selected from the group consisting of halo, amino, hydroxy, haloalkyl, C_1-C_6 alkyl and C_1-C_6 alkanoyl; and

each incidence of R_{14} is independently selected from the group consisting of hydrogen, fluoro, chloro, bromo, iodo, amino and aminoalkyl.

22. The compound of any one of the preceding items wherein Ar_2 is substituted or unsubstituted pyridyl.

23. The compound of any one of the preceding items wherein A is preferably oxygen.

24. The compound of any one of the preceding items wherein R_{12} is preferably hydrogen.

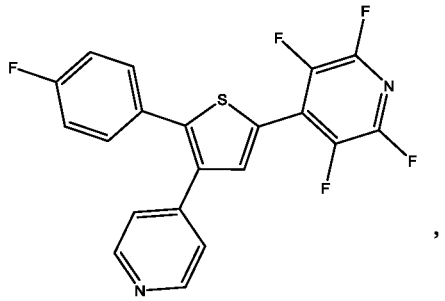
25. The compound of any one of the preceding items wherein it is preferred that R_{13} is not present, that is, only hydrogens are attached to the ring carbons.

26. The compound of any one of the preceding items wherein each incidence of R_{14} is independently selected from fluoro or amino.

27. The compound of any one of the preceding items wherein Ar_1 is 4-fluorophenyl.

28. The compound of any one of the preceding items wherein Ar_2 is 4-pyridyl.

29. The compound of any one of the preceding items wherein the compound is selected from the group consisting of: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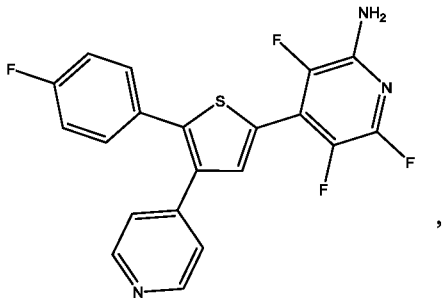
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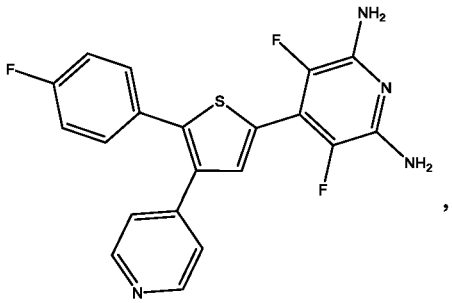
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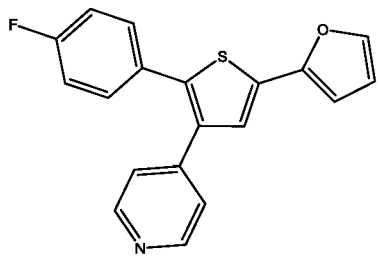
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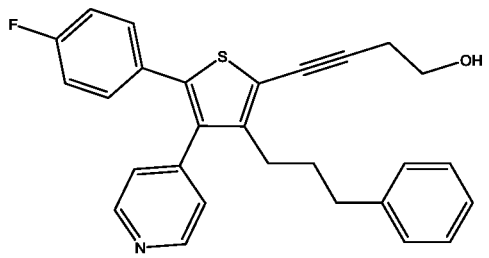
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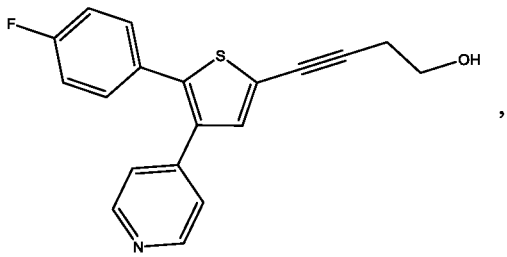
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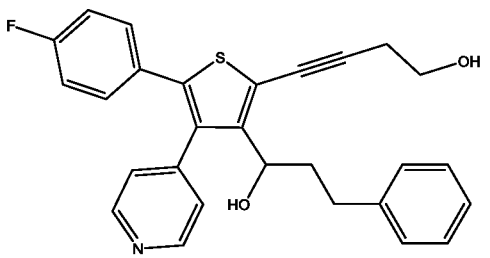
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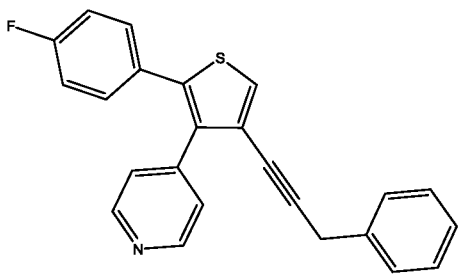
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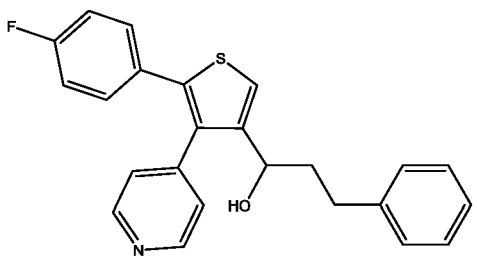
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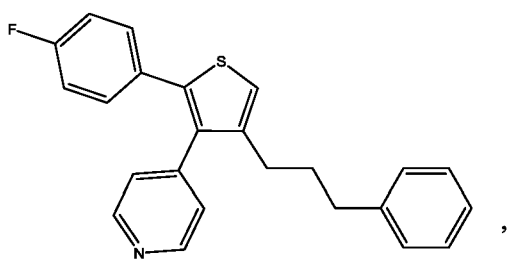
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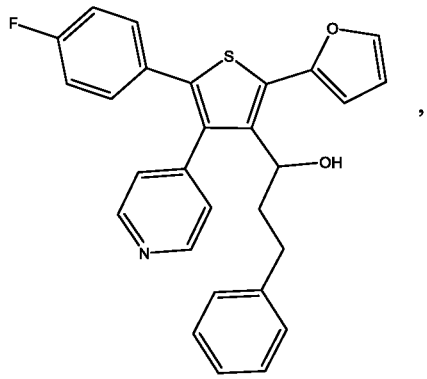
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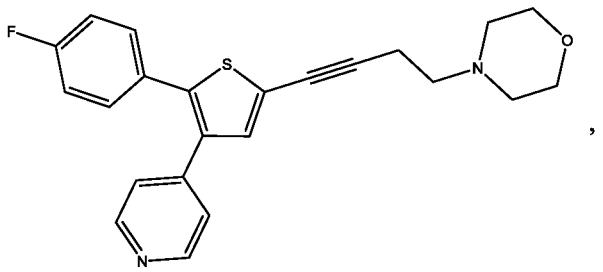
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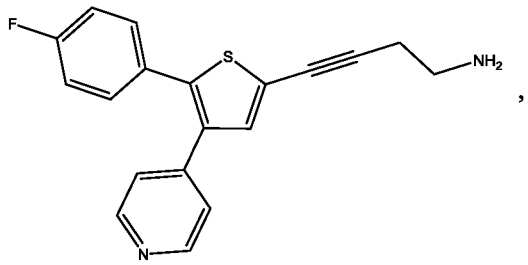
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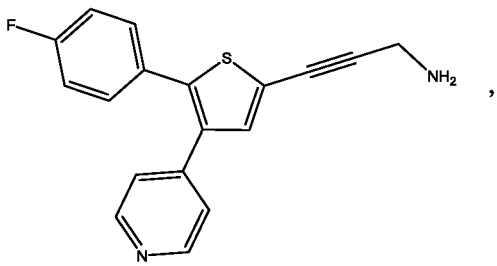
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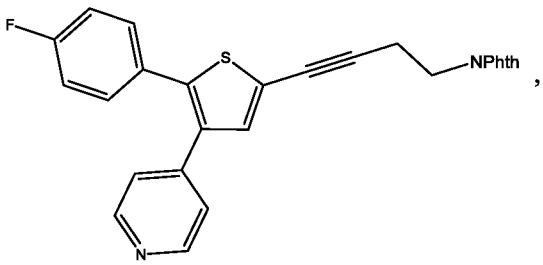
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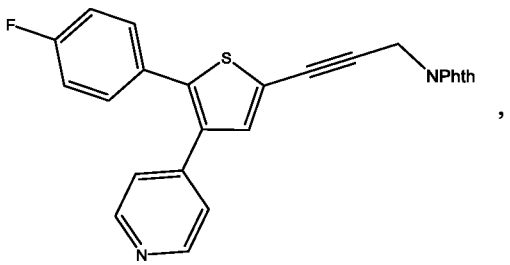
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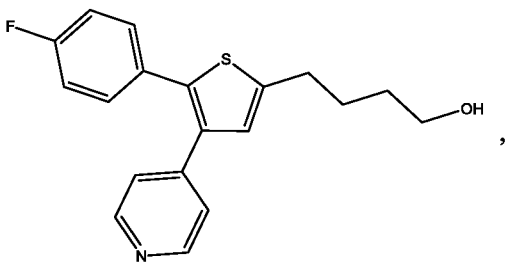
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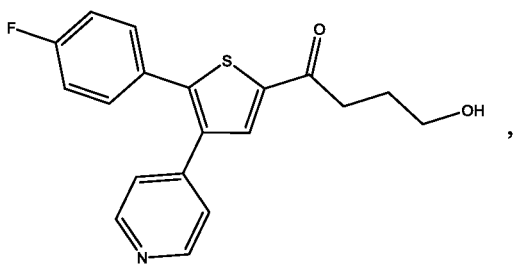
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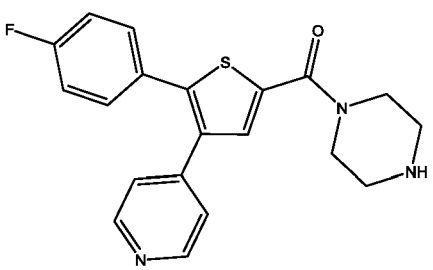
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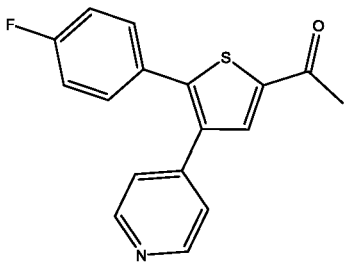
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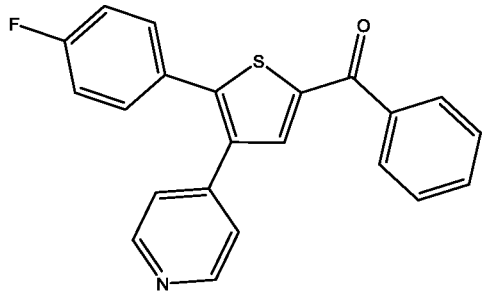

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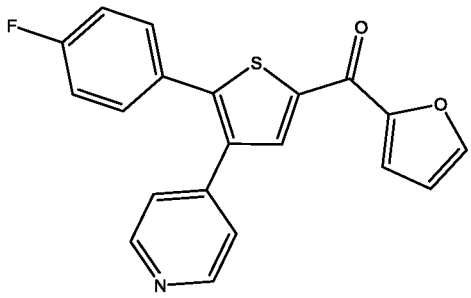


or a pharmaceutically acceptable salt thereof.

30. The compound of any one of the preceding items wherein the compound of the first aspect is a non-naturally occurring compound

31. A pharmaceutical composition comprising an effective amount of a compound of any one of items 1 to 30, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent and/or excipient.

32. The pharmaceutical composition of any one of items 1 to 30 wherein the pharmaceutical composition is for the treatment or prophylaxis of a disease, disorder or condition responsive to MAPK inhibition, preferably p38 MAPK inhibition, more preferably p38α MAPK inhibition.

33. A method of treating a patient suffering from a disease, disorder or condition responsive to MAPK inhibition including the step of administering an effective amount of a compound of any one of items 1 to 30, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of any one of items 31 or 32, to the patient.

34. A compound of any one of items 1 to 30, or a pharmaceutically effective salt thereof, or the pharmaceutical composition of any one of items 31 or 32 for use in the treatment of a disease, disorder or condition responsive to MAPK inhibition.

35. Use of a compound of any one of items 1 to 30, or a pharmaceutically effective salt thereof, in the manufacture of a medicament for the treatment of a disease, disorder or condition responsive to MAPK inhibition.

36. The method, compound or use of any one of items 33 to 35 wherein the disease, disorder or condition is responsive to p38 MAPK inhibition.

37. The method, compound or use of any one of items 33 to 36 wherein the disease, disorder or condition is responsive to p38α MAPK inhibition.

38. The method, compound or use of any one of items 33 to 37 wherein the method is a method of reducing inflammation, or use is a use in treating inflammation, in a patient by inhibiting MAPK, particularly by inhibiting p38 MAPK, more particularly by inhibiting p38α MAPK.

39. The method, compound or use of any one of items 33 to 38 wherein the disease, disorder or condition is selected from the group consisting of arthritis, inflammatory bowel disease, asthma, psoriasis, myocardial injury, stroke, cancer, Alzheimer's disease, HIV, COPD, multiple myeloma, myelodysplastic syndrome, acute respiratory distress syndrome, coronary heart disease, acute coronary syndrome, major depressive disorder, dental pain, artherosclerosis, neuropathic pain and inflammation associated with any one or more of the aforementioned diseases, disorders or conditions.

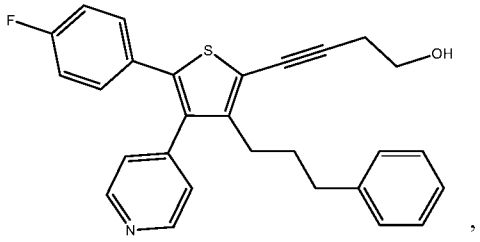
40. The method, compound or use of any one of items 33 to 39 wherein the patient is a domestic or livestock animal or a human.

41. A complex of a compound of any one of items 1 to 30, or a pharmaceutically effective salt thereof, with a p38 MAPK enzyme.

42. The complex of embodiment 40 wherein the p38 MAPK enzyme is a p38α MAPK enzyme.

CLAIMS

1. A compound selected from the group consisting of:



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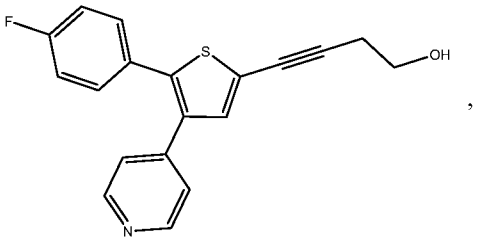

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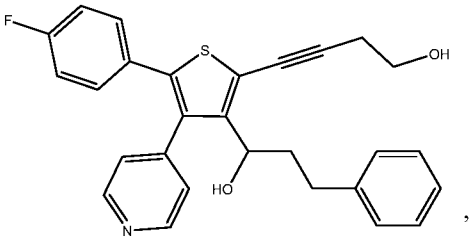

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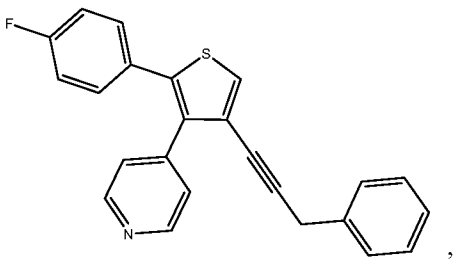

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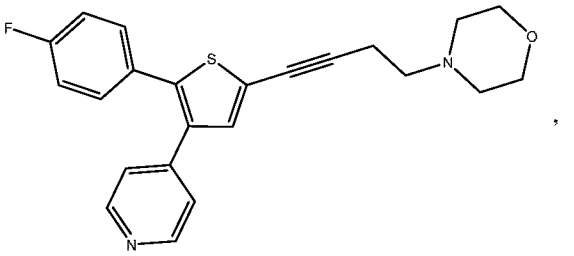

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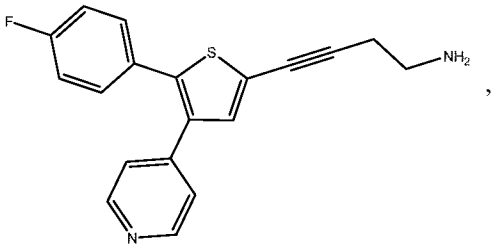

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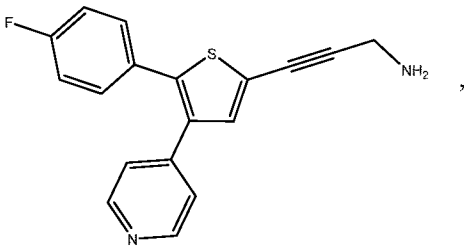

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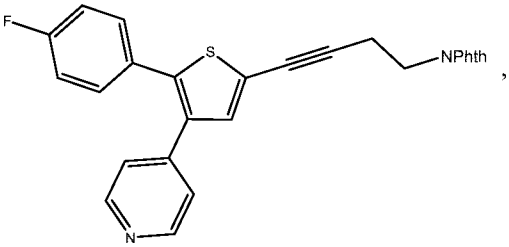

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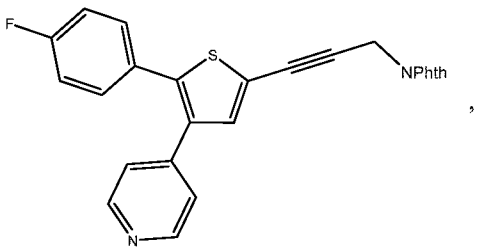

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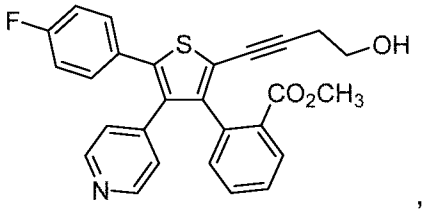
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OH
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N

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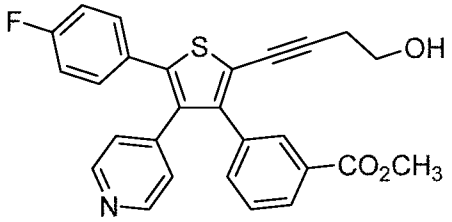
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CO_2CH_3
N

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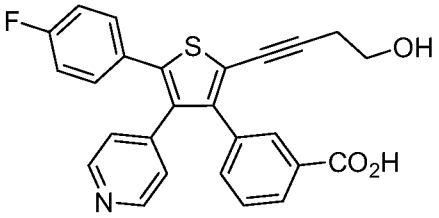
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OH
CO_2H
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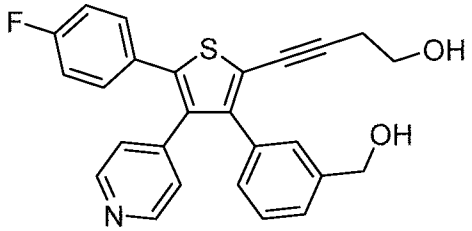
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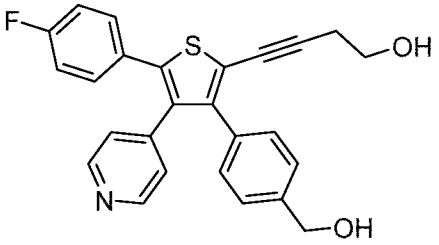
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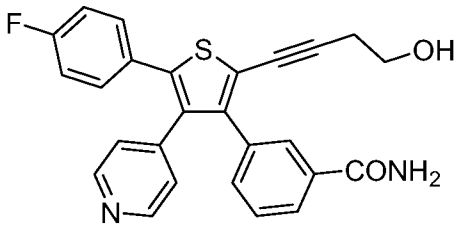
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N

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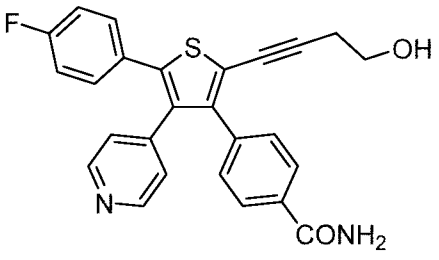
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$CONH_2$

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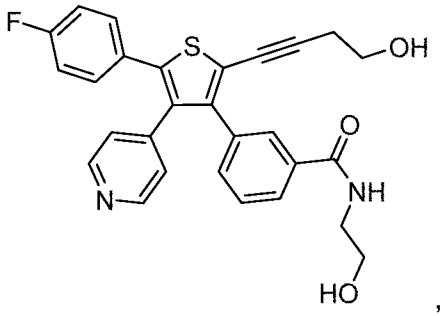
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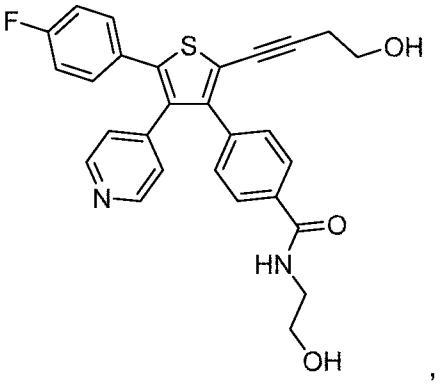
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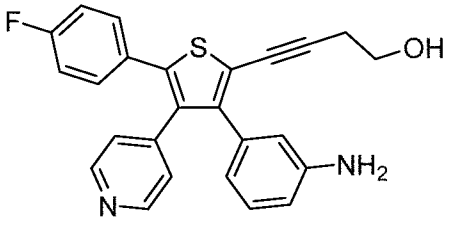

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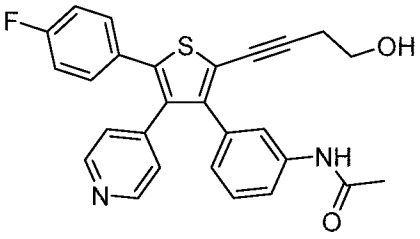

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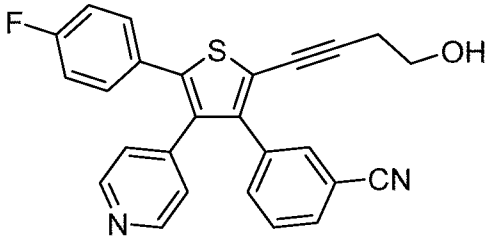

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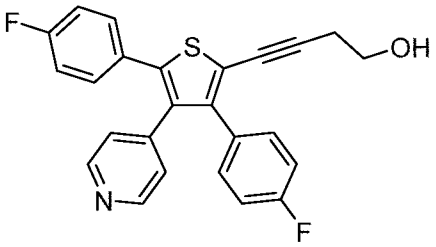

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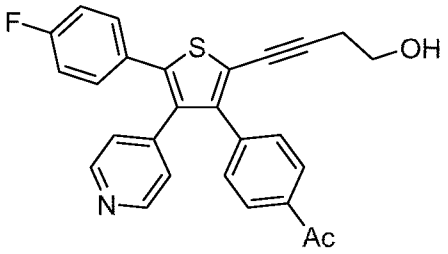

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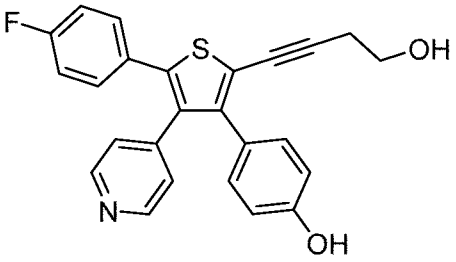

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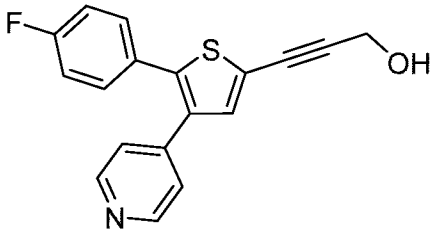

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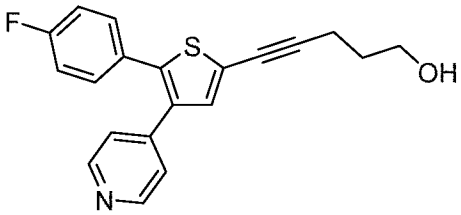

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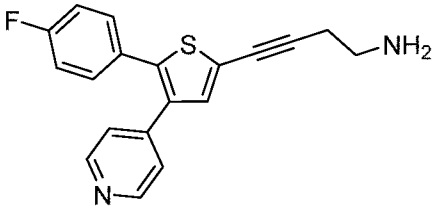
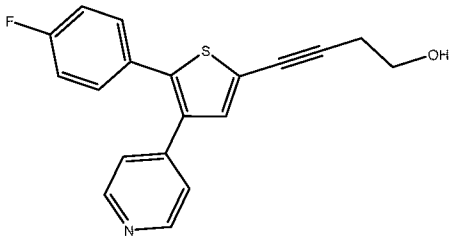


or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein the compound is selected from the group consisting of:



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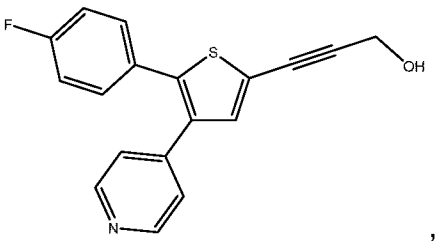

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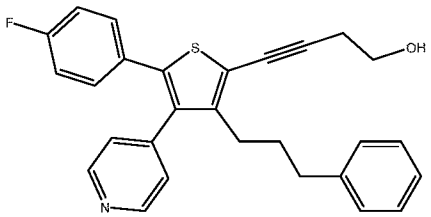

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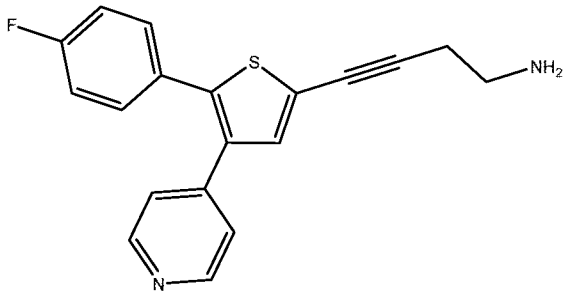

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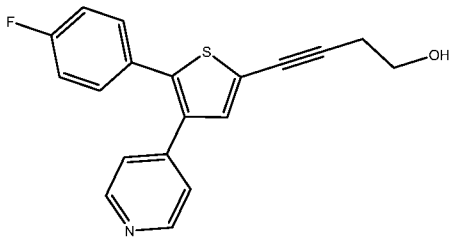
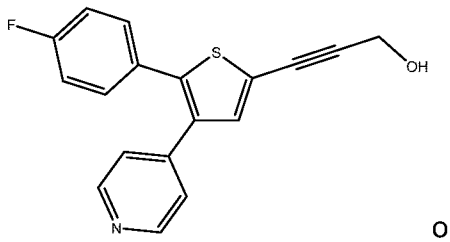
or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 or 2 wherein the compound is



or a pharmaceutically acceptable salt thereof.

4. The compound of claim 1 or 2 wherein the compound is



or a pharmaceutically acceptable salt thereof.

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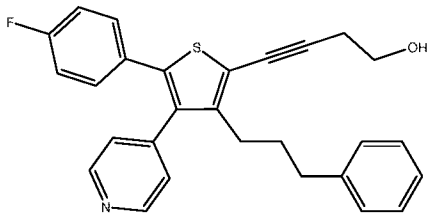
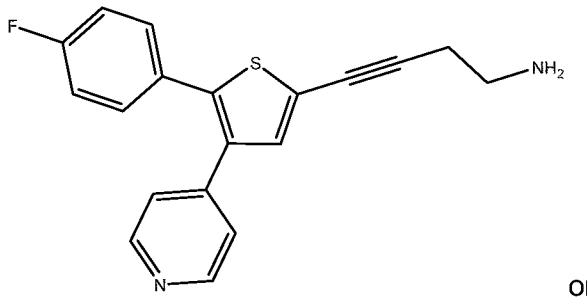
5. The compound of claim 1 or 2 wherein the compound is



or a pharmaceutically acceptable salt thereof.

6. The compound of claim 1 or 2 wherein the compound is



or a pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition comprising an effective amount of a compound according to any one of claim 1 to claim 6, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, diluent and/or excipient.

8. A method of treating a patient suffering from a disease, disorder or condition responsive to MAPK inhibition including the step of administering an effective amount of a compound according to any one of claim 1 to claim 6, or a pharmaceutically effective salt thereof, or the pharmaceutical composition according to claim 7, to the patient.

9. The method of claim 8 wherein the disease, disorder or condition is inflammation.

10. The method of claim 8 wherein the disease, disorder or condition is selected from the group consisting of arthritis, inflammatory bowel disease, asthma, psoriasis, myocardial injury, stroke, cancer, Alzheimer's disease, HIV, COPD, multiple myeloma, myelodysplastic syndrome, acute respiratory distress syndrome, coronary heart disease, acute coronary syndrome, major depressive

disorder, dental pain, artherosclerosis, neuropathic pain and inflammation associated with any one or more of these diseases, disorders or conditions.

11. The method of any one of claim 8 to claim 10 wherein the patient is a human.

12. Use of the compound of any one of claim 1 to claim 6 or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the the treatment of a disease, disorder or condition responsive to MAPK inhibition.

13. The use of claim 12 wherein the disease, disorder or condition is inflammation.

14. The use of claim 12 wherein the disease, disorder or condition is selected from the group consisting of arthritis, inflammatory bowel disease, asthma, psoriasis, myocardial injury, stroke, cancer, Alzheimer's disease, HIV, COPD, multiple myeloma, myelodysplastic syndrome, acute respiratory distress syndrome, coronary heart disease, acute coronary syndrome, major depressive disorder, dental pain, artherosclerosis, neuropathic pain and inflammation associated with any one or more of these diseases, disorders or conditions.

15. Use of the compound of any one of claim 1 to claim 6 or a pharmaceutically acceptable salt thereof, for the the treatment of a disease, disorder or condition responsive to MAPK inhibition.

16. The use of claim 15 wherein the disease, disorder or condition is inflammation.

17. The use of claim 15 wherein the disease, disorder or condition is selected from the group consisting of arthritis, inflammatory bowel disease, asthma, psoriasis, myocardial injury, stroke, cancer, Alzheimer's disease, HIV, COPD, multiple myeloma, myelodysplastic syndrome, acute respiratory distress syndrome, coronary heart disease, acute coronary syndrome, major depressive disorder, dental pain, artherosclerosis, neuropathic pain and inflammation associated with any one or more of these diseases, disorders or conditions.

18. A complex of a compound according to any one of claim 1 to claim 6, or a pharmaceutically acceptable salt thereof, with a p38 MAPK enzyme.

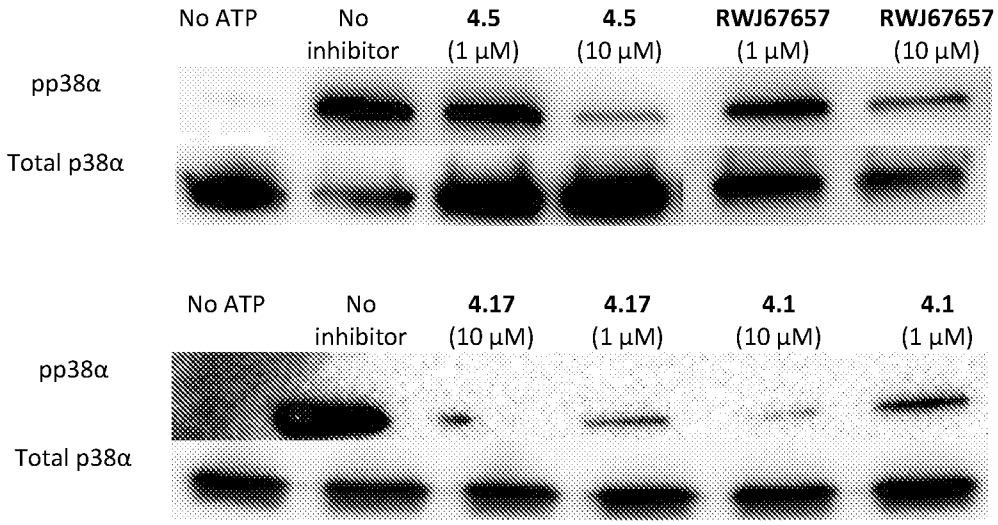

No ATP
No inhibitor
4.5 (1 µM)
4.5 (10 µM)
RWJ67657 (1 µM)
RWJ67657 (10 µM)
pp38α
Total p38α
No ATP
No inhibitor
4.17 (10 µM)
4.17 (1 µM)
4.1 (10 µM)
4.1 (1 µM)
pp38α
Total p38α

FIG 1

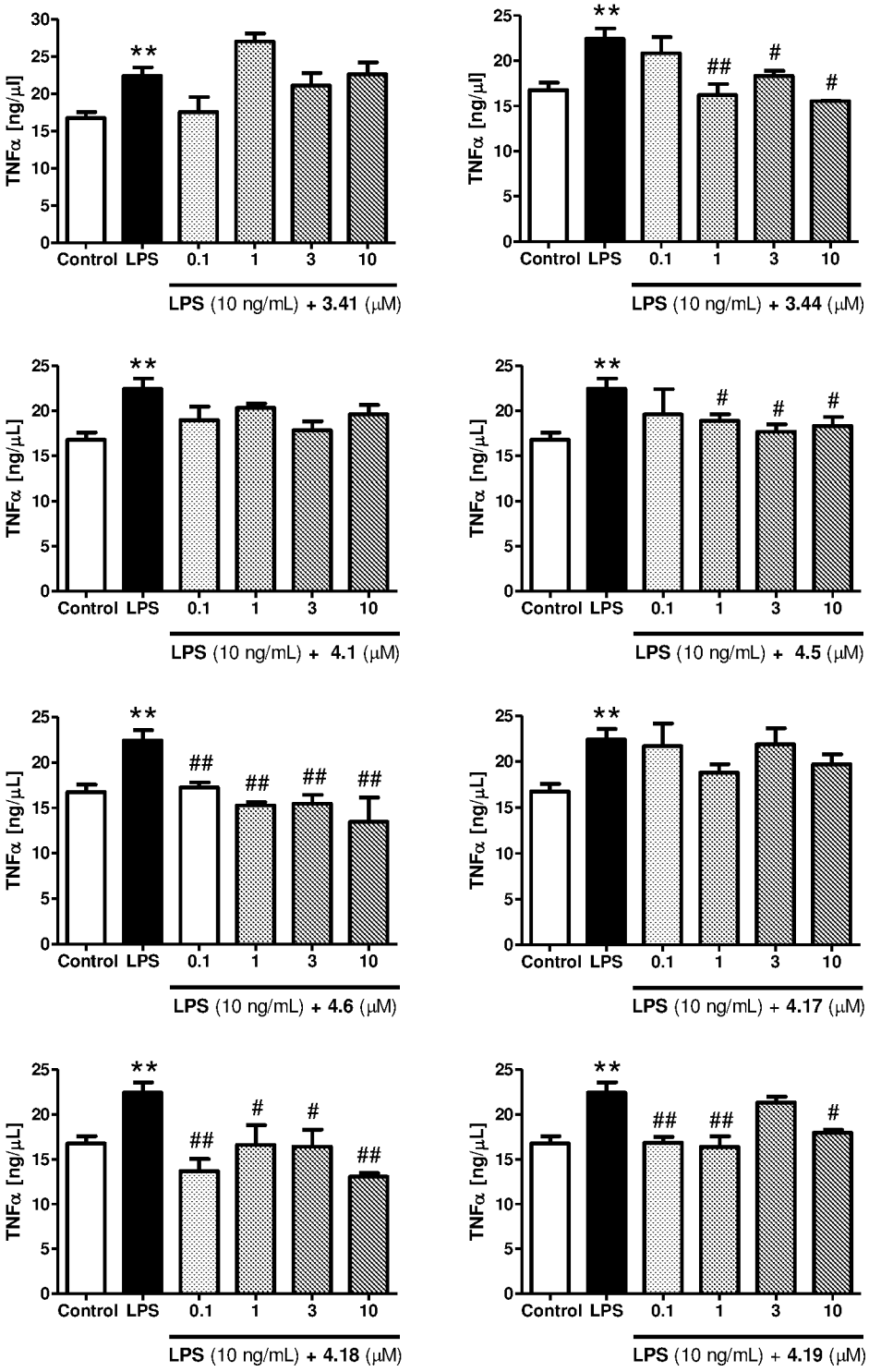

TNFα [ng/μl]
Control LPS 0.1 1 3 10
LPS (10 ng/mL) + 3.41 (μM)
LPS (10 ng/mL) + 3.44 (μM)
LPS (10 ng/mL) + 4.1 (μM)
LPS (10 ng/mL) + 4.5 (μM)
LPS (10 ng/mL) + 4.6 (μM)
LPS (10 ng/mL) + 4.17 (μM)
LPS (10 ng/mL) + 4.18 (μM)
LPS (10 ng/mL) + 4.19 (μM)

FIG 2

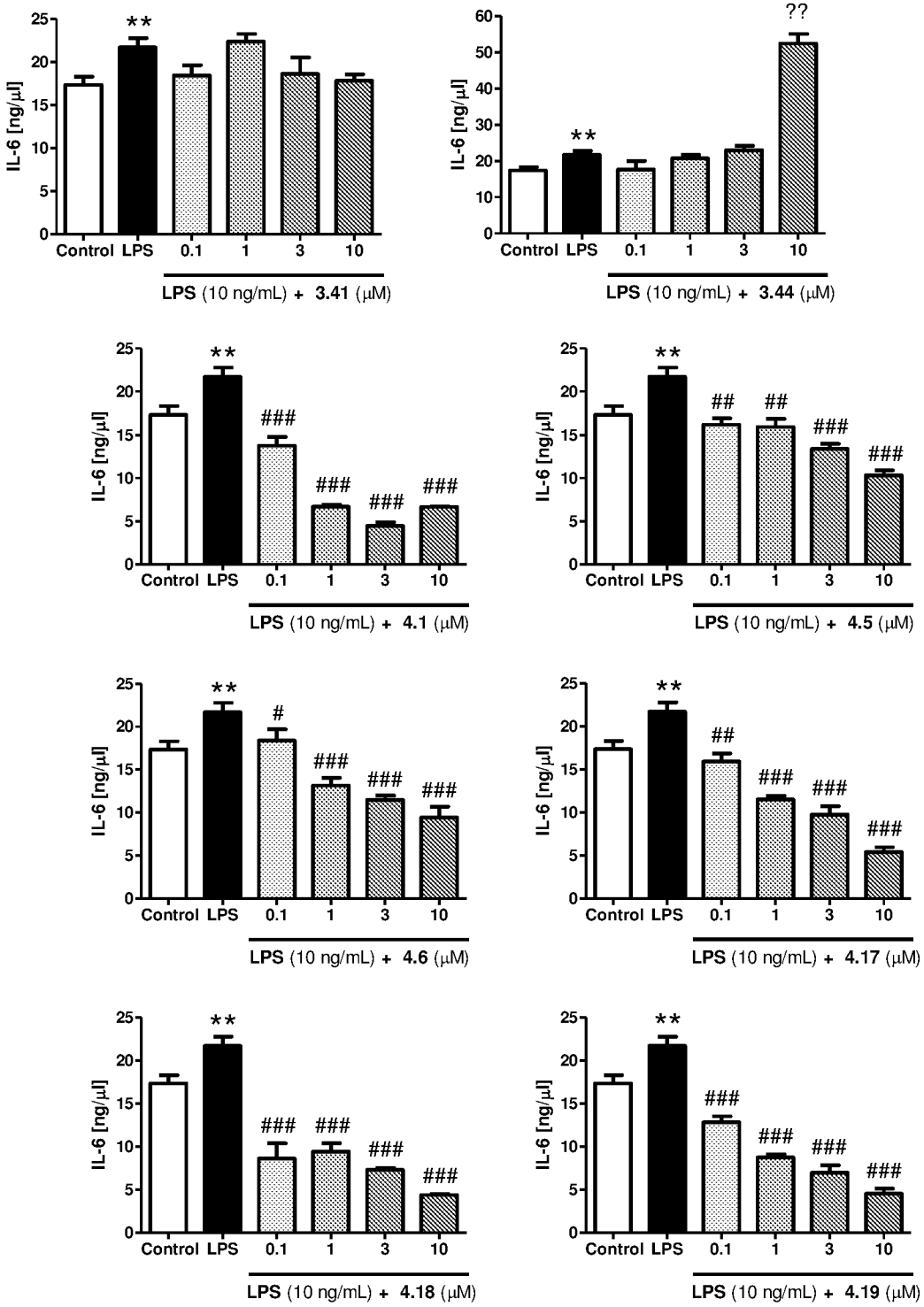

IL-6 [ng/µl]
Control
LPS
0.1
1
3
10
**
??
###
##
#
LPS (10 ng/mL) + 3.41 (µM)
LPS (10 ng/mL) + 3.44 (µM)
LPS (10 ng/mL) + 4.1 (µM)
LPS (10 ng/mL) + 4.5 (µM)
LPS (10 ng/mL) + 4.6 (µM)
LPS (10 ng/mL) + 4.17 (µM)
LPS (10 ng/mL) + 4.18 (µM)
LPS (10 ng/mL) + 4.19 (µM)

FIG 3

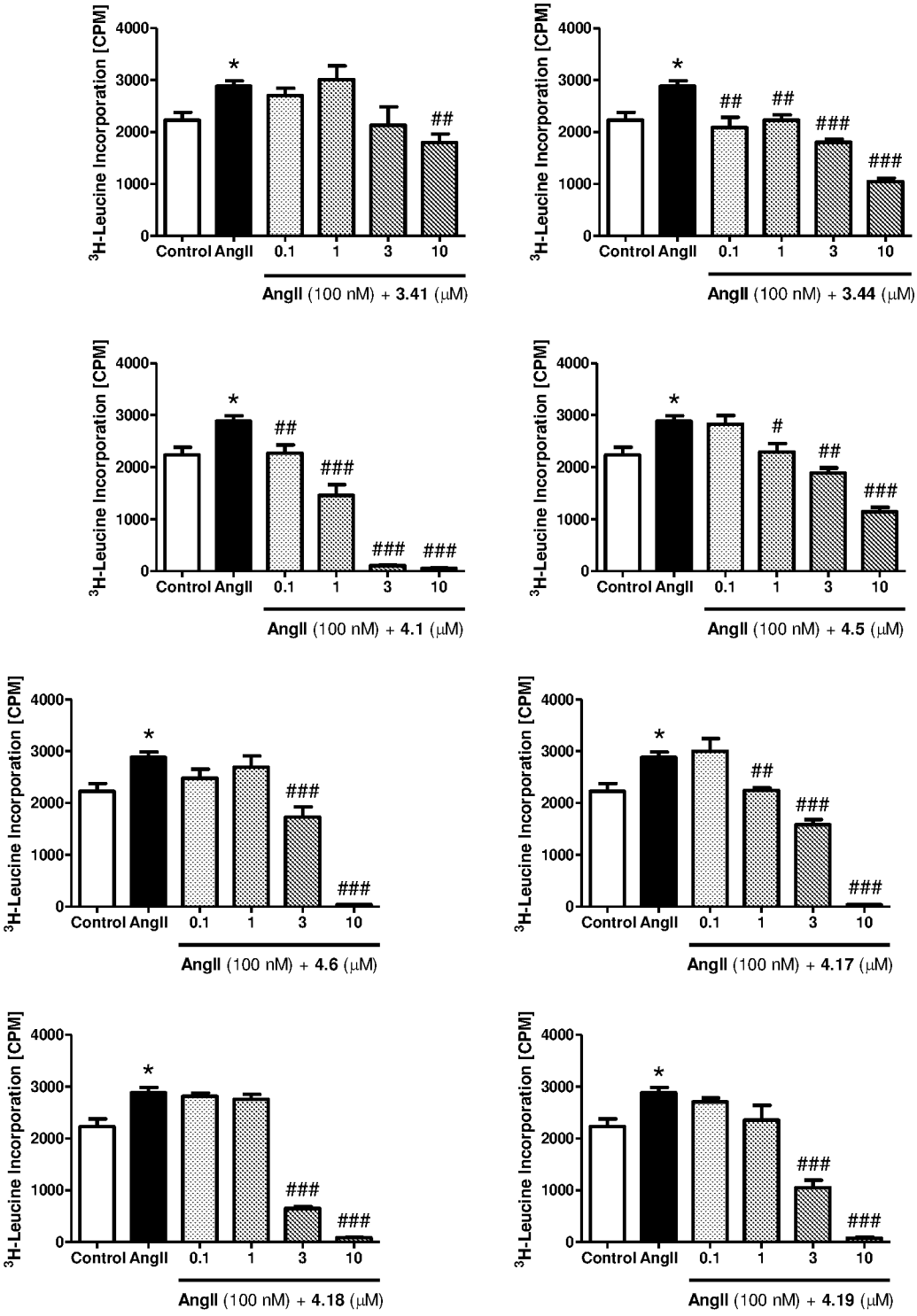

^{3}H-Leucine Incorporation [CPM]
4000
3000
2000
1000
0
Control AngII
0.1
1
3
10
AngII (100 nM) + 3.41 (μM)
AngII (100 nM) + 3.44 (μM)
AngII (100 nM) + 4.1 (μM)
AngII (100 nM) + 4.5 (μM)
AngII (100 nM) + 4.6 (μM)
AngII (100 nM) + 4.17 (μM)
AngII (100 nM) + 4.18 (μM)
AngII (100 nM) + 4.19 (μM)

FIG 4

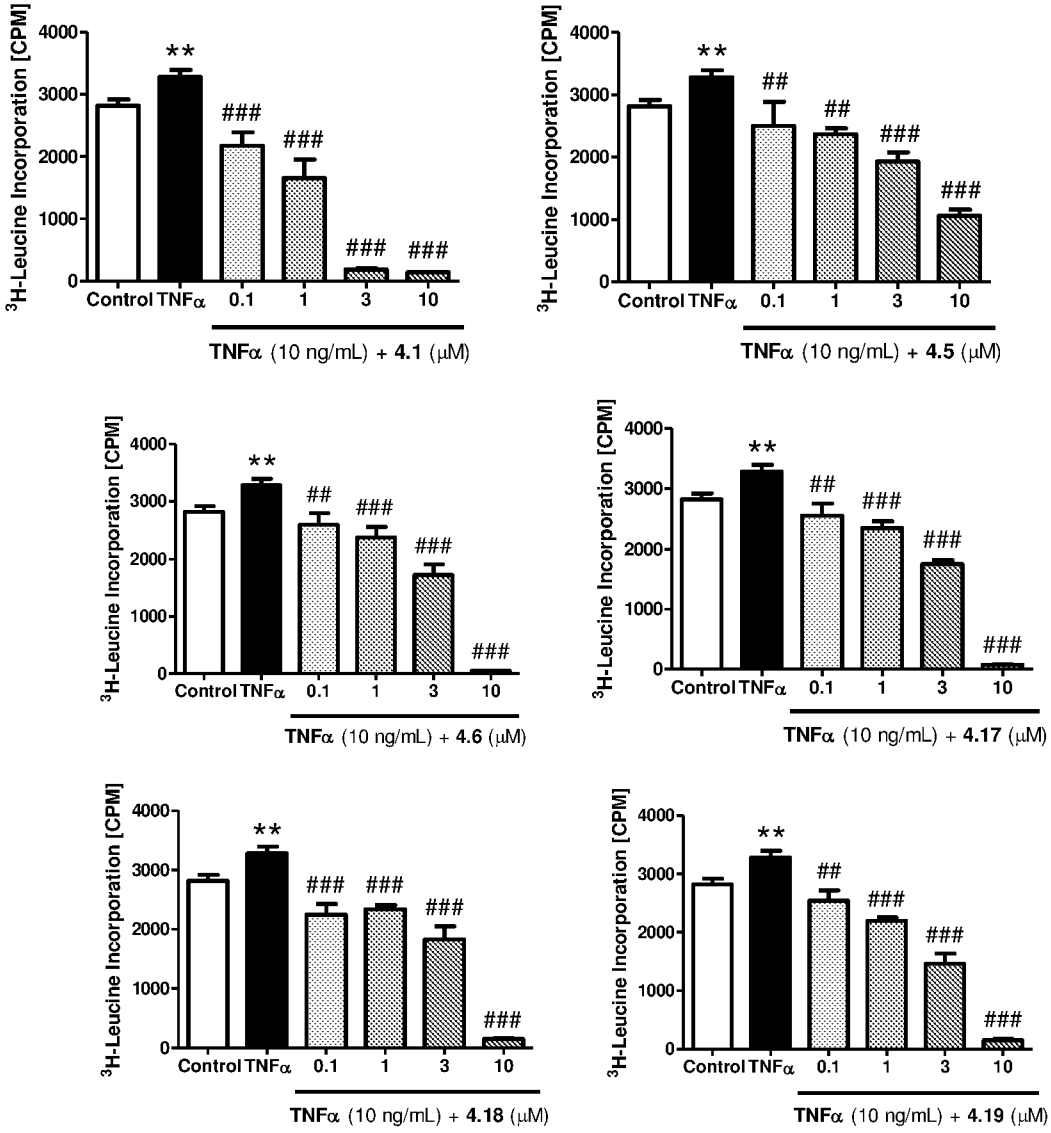

^{3}H-Leucine Incorporation [CPM]
4000
3000
2000
1000
0
**
###
Control TNFα 0.1 1 3 10
TNFα (10 ng/mL) + 4.1 (μM)
##
TNFα (10 ng/mL) + 4.5 (μM)
TNFα (10 ng/mL) + 4.6 (μM)
TNFα (10 ng/mL) + 4.17 (μM)
TNFα (10 ng/mL) + 4.18 (μM)
TNFα (10 ng/mL) + 4.19 (μM)

FIG 5

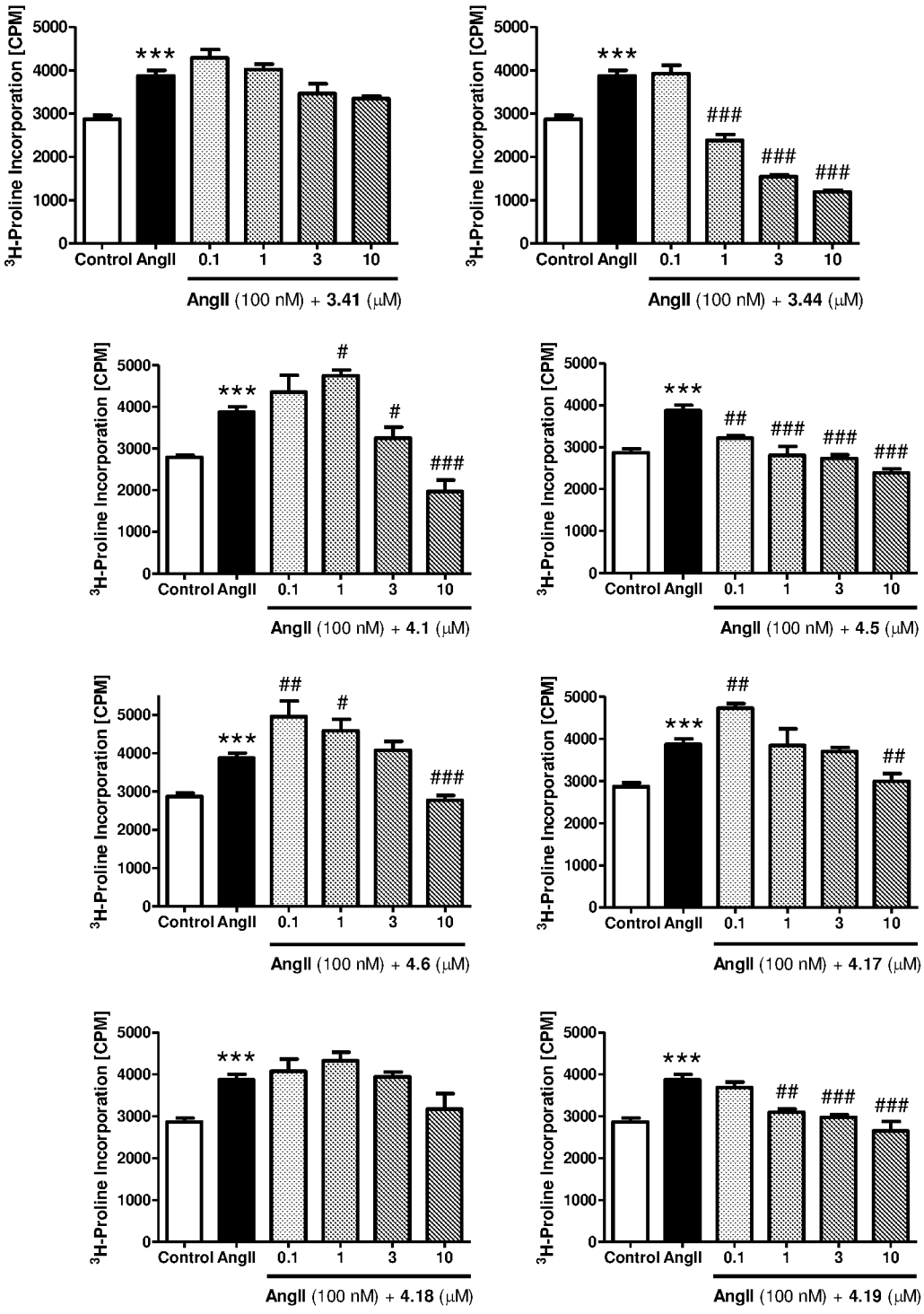

^{3}H-Proline Incorporation [CPM]
5000
4000
3000
2000
1000
0
***
###
#
##
Control AngII
0.1
1
3
10
AngII (100 nM) + 3.41 (μM)
AngII (100 nM) + 3.44 (μM)
AngII (100 nM) + 4.1 (μM)
AngII (100 nM) + 4.5 (μM)
AngII (100 nM) + 4.6 (μM)
AngII (100 nM) + 4.17 (μM)
AngII (100 nM) + 4.18 (μM)
AngII (100 nM) + 4.19 (μM)

FIG 6

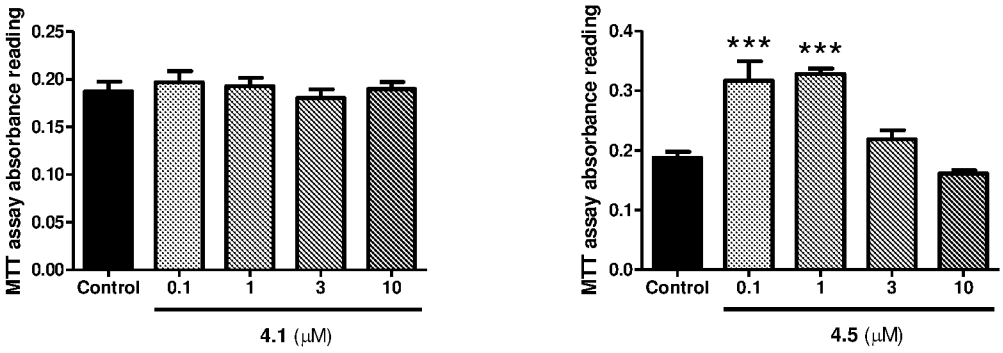

MTT assay absorbance reading
0.25
0.20
0.15
0.10
0.05
0.00
Control
0.1
1
3
10
4.1 (μM)
MTT assay absorbance reading
0.4
0.3
0.2
0.1
0.0
***
***
Control
0.1
1
3
10
4.5 (μM)

FIG 7

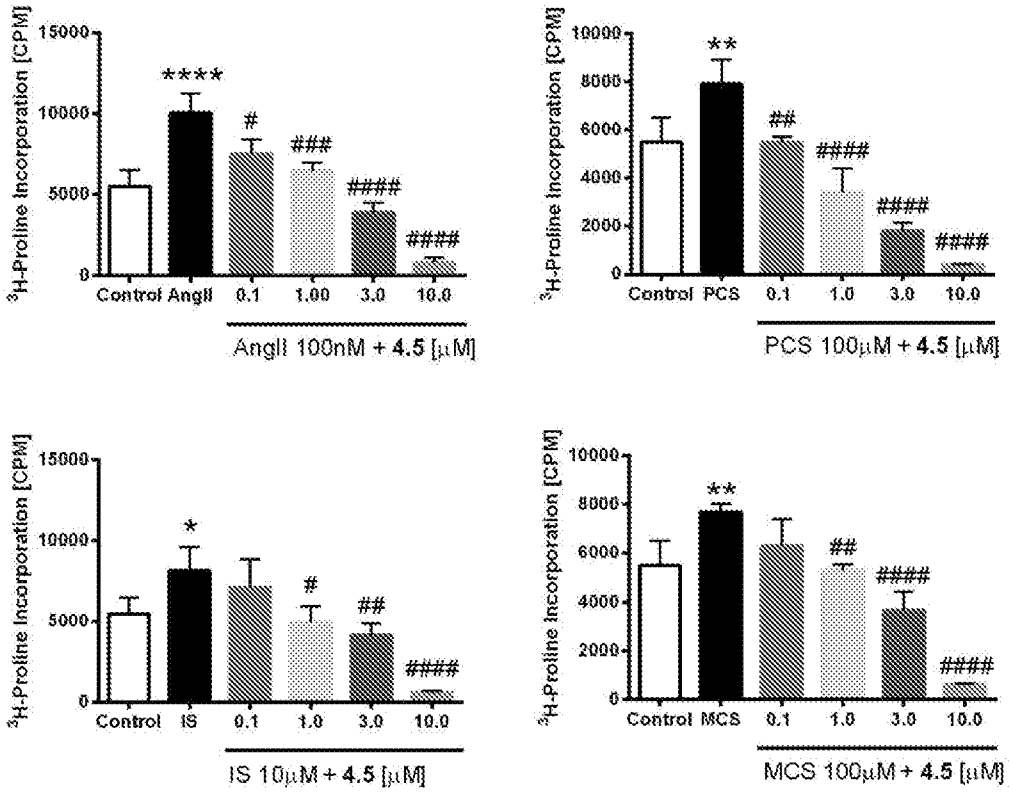

^{3}H-Proline Incorporation [CPM]
15000
10000
5000
0
****
#
###
####
####
Control AngII 0.1 1.00 3.0 10.0
AngII 100nM + 4.5 [μM]
^{3}H-Proline Incorporation [CPM]
10000
8000
6000
4000
2000
0
**
##
####
####
####
Control PCS 0.1 1.0 3.0 10.0
PCS 100μM + 4.5 [μM]
^{3}H-Proline Incorporation [CPM]
15000
10000
5000
0
*
#
##
####
Control IS 0.1 1.0 3.0 10.0
IS 10μM + 4.5 [μM]
^{3}H-Proline Incorporation [CPM]
10000
8000
6000
4000
2000
0
**
##
####
####
Control MCS 0.1 1.0 3.0 10.0
MCS 100μM + 4.5 [μM]

FIG 8

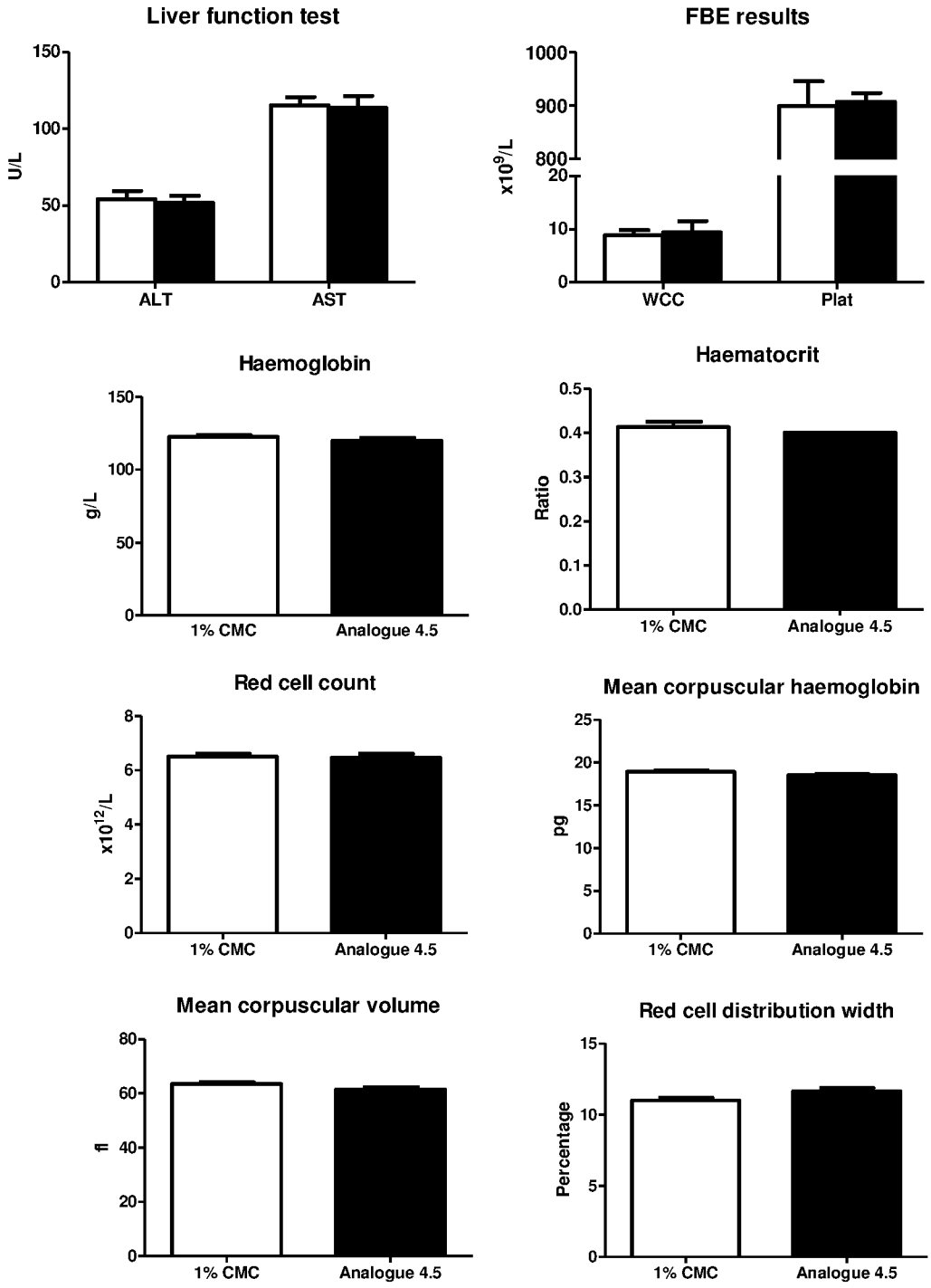
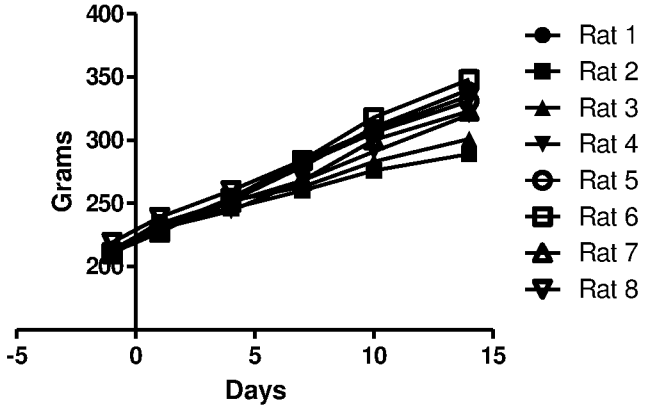
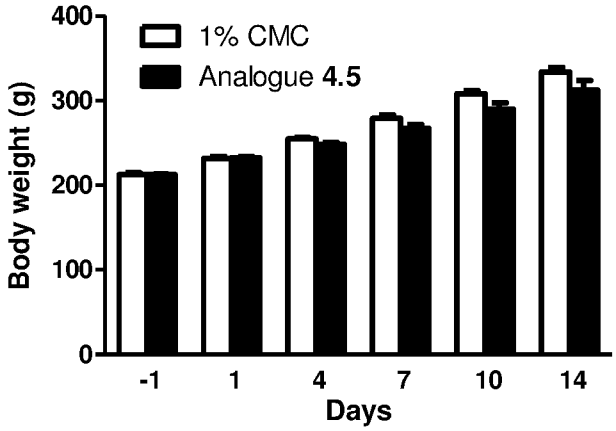

Liver function test
U/L
150
100
50
0
ALT
AST
FBE results
x10^9/L
1000
900
800
20
10
0
WCC
Plat
Haemoglobin
g/L
150
100
50
0
1% CMC
Analogue 4.5
Haematocrit
Ratio
0.5
0.4
0.3
0.2
0.1
0.0
1% CMC
Analogue 4.5
Red cell count
x10^12/L
8
6
4
2
0
1% CMC
Analogue 4.5
Mean corpuscular haemoglobin
pg
25
20
15
10
5
0
1% CMC
Analogue 4.5
Mean corpuscular volume
fl
80
60
40
20
0
1% CMC
Analogue 4.5
Red cell distribution width
Percentage
15
10
5
0
1% CMC
Analogue 4.5

FIG 9



(a)



(b)

FIG 10 (a) and (b)

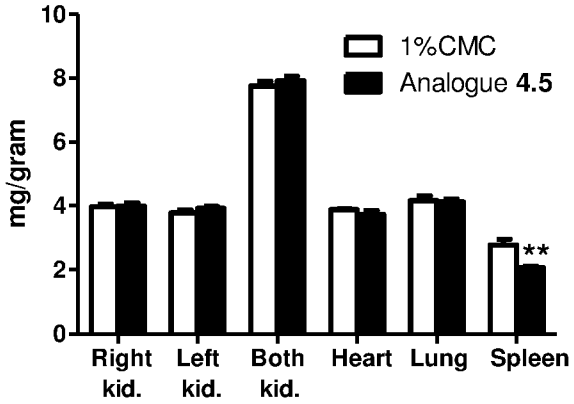


FIG 11

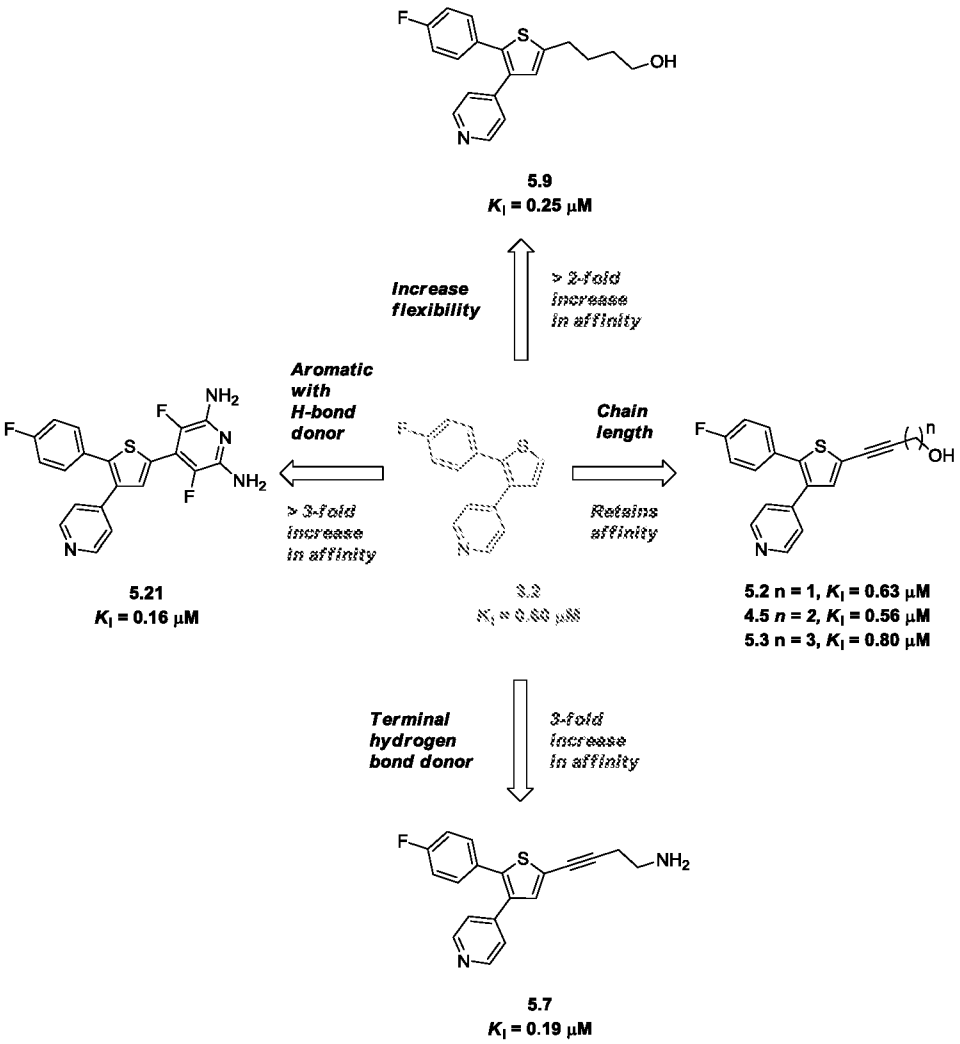

5.9
K_I = 0.25 μM
Increase flexibility
> 2-fold increase in affinity
Aromatic with H-bond donor
> 3-fold increase in affinity
Chain length
Retains affinity
5.21
K_I = 0.16 μM
5.2 n = 1, K_I = 0.63 μM
4.5 n = 2, K_I = 0.56 μM
5.3 n = 3, K_I = 0.80 μM
Terminal hydrogen bond donor
3-fold increase in affinity
5.7
K_I = 0.19 μM

FIG 12

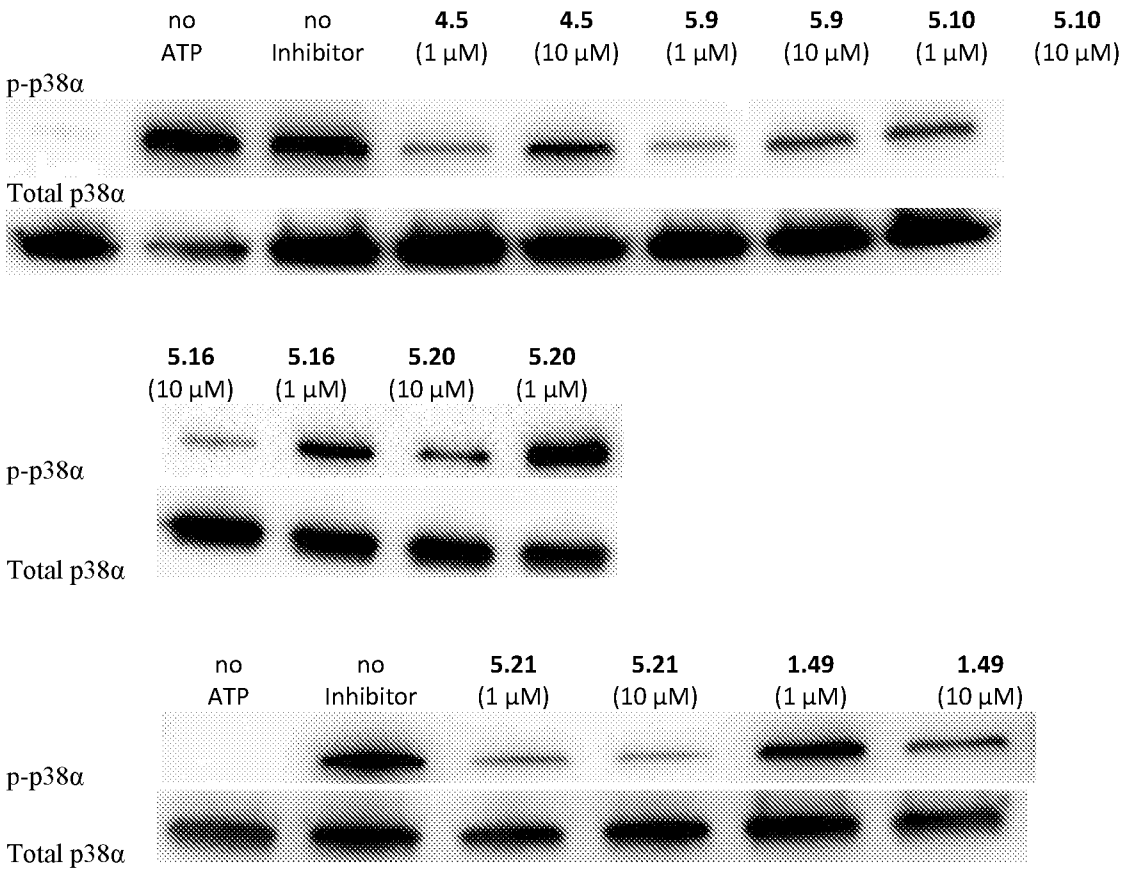

no ATP
no Inhibitor
4.5 (1 μM)
4.5 (10 μM)
5.9 (1 μM)
5.9 (10 μM)
5.10 (1 μM)
5.10 (10 μM)
p-p38α
Total p38α
5.16 (10 μM)
5.16 (1 μM)
5.20 (10 μM)
5.20 (1 μM)
p-p38α
Total p38α
no ATP
no Inhibitor
5.21 (1 μM)
5.21 (10 μM)
1.49 (1 μM)
1.49 (10 μM)
p-p38α
Total p38α

FIG 13

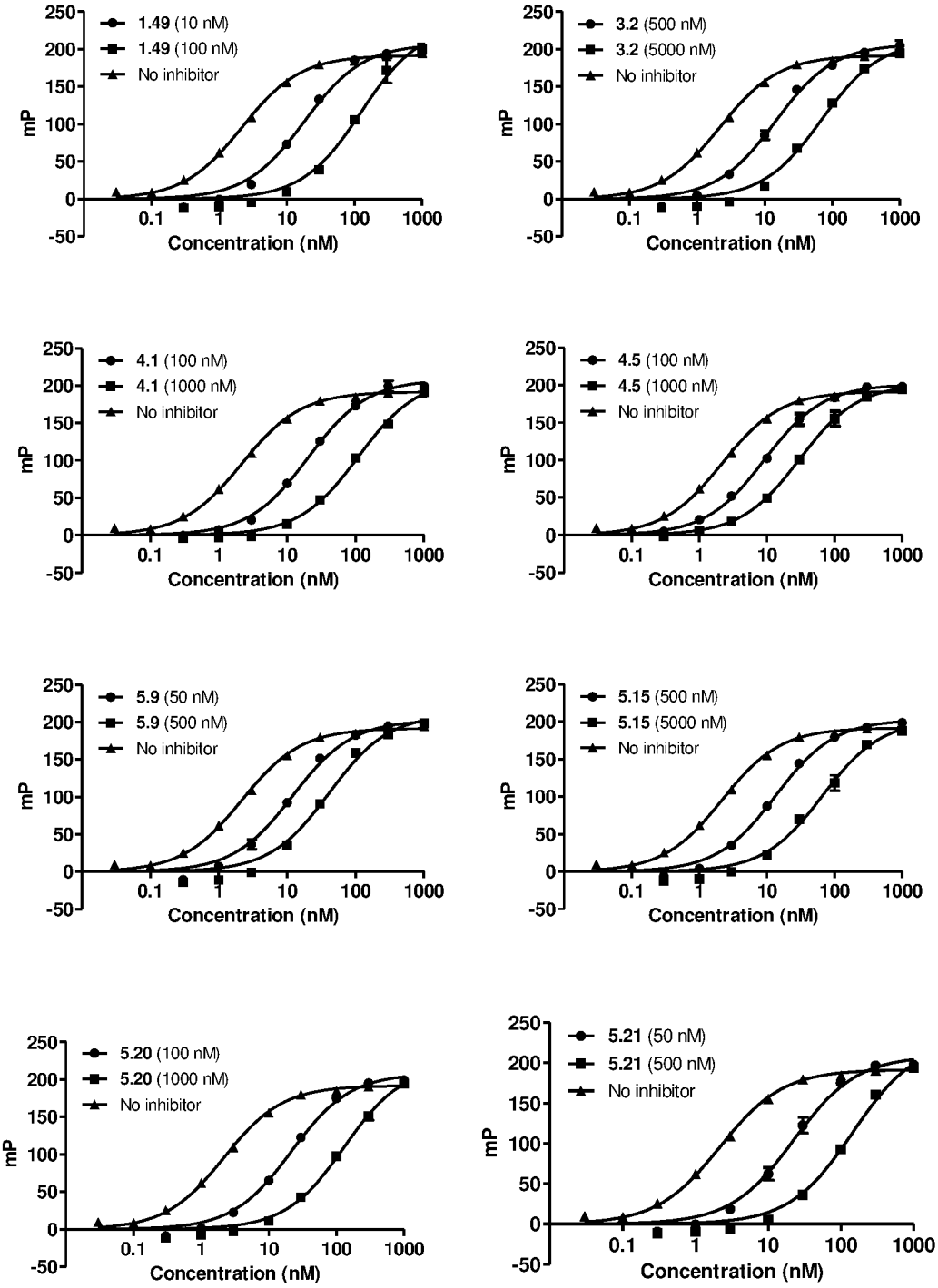

1.49 (10 nM)
1.49 (100 nM)
No inhibitor
3.2 (500 nM)
3.2 (5000 nM)
No inhibitor
4.1 (100 nM)
4.1 (1000 nM)
No inhibitor
4.5 (100 nM)
4.5 (1000 nM)
No inhibitor
5.9 (50 nM)
5.9 (500 nM)
No inhibitor
5.15 (500 nM)
5.15 (5000 nM)
No inhibitor
5.20 (100 nM)
5.20 (1000 nM)
No inhibitor
5.21 (50 nM)
5.21 (500 nM)
No inhibitor
mP
Concentration (nM)
250
200
150
100
50
0
-50
0.1
1
10
100
1000

FIG 14