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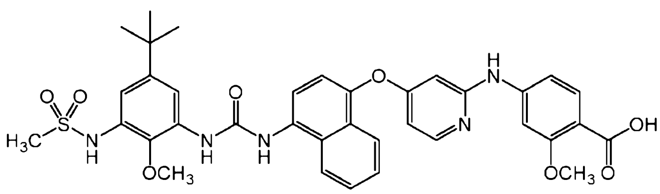
(54) **Title**: 4-(4-(4-PHENYLUREIDO-NAPHTHALEN-1-YL)OXY-PYRIDIN-2-YL)AMINO-BENZOIC ACID DERIVATIVE AS P38 KINASE INHIBITOR



I

(57) **Abstract**: There is provided a compound of formula I, which compound has antiinflammatory activity (e.g. through inhibition of one or more of members of: the family of p38 mitogen-activated protein kinase enzymes; Syk kinase; and members of the Src family of tyrosine kinases) and has use in therapy, including in pharmaceutical combinations, especially in the treatment of inflammatory diseases, including inflammatory diseases of the lung, eye and intestines.

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KINASE INHIBITOR

This invention relates, *inter alia*, to a compound which is an antiinflammatory agent (e.g. through inhibition of one or more of members of: the family of p38 mitogen-activated protein kinase enzymes (referred to herein as p38 MAP kinase inhibitors), for example the alpha kinase sub-type thereof; Syk kinase; and the Src family of tyrosine kinases). The invention also relates to the use of this compound in therapy, including in mono- and combination therapies, especially in the treatment of inflammatory diseases, including inflammatory diseases of the lung (such as asthma and chronic obstructive pulmonary disease (COPD)), eye (such as uveitis or keratoconjunctivitis sicca (dry eye disease, also known as xerophthalmia)) and gastrointestinal tract (such as Crohn's disease and ulcerative colitis).

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

Four p38 MAPK isoforms (alpha, beta, gamma and delta respectively) have been identified, each displaying different patterns of tissue expression. The p38 MAPK alpha and beta isoforms are found ubiquitously throughout the body, are present in many different cell types and are inhibited by a number of previously described small molecular weight compounds. Early classes of inhibitors were highly toxic due to the broad tissue distribution of these isoforms which resulted in off-target effects of the compounds. Some of the more recently identified inhibitors show improved selectivity for p38 MAPK alpha and beta isoforms and have wider safety margins.

p38 MAP kinase is believed to play a pivotal role in many of the signalling pathways that are involved in initiating and maintaining chronic, persistent inflammation in human disease, for example, in severe asthma, COPD and inflammatory bowel disease (IBD). There is now an abundant literature which demonstrates that p38 MAP kinase is activated by a range of pro-inflammatory cytokines and that its activation results in the recruitment and release of further pro-inflammatory cytokines. Indeed, data from some clinical studies demonstrate beneficial changes in disease activity in patients during treatment with p38 MAP kinase inhibitors. For instance, Smith describes the inhibitory effect of p38 MAP kinase inhibitors on TNFα (but not IL-8) release from human PBMCs (Smith, S. J., *Br. J. Pharmacol.,* 2006, **149**:393-404).

The use of inhibitors of p38 MAP kinase in the treatment of COPD and IBD has also been proposed. Small molecule inhibitors targeted to p38 MAPKα/β have proved to be effective in reducing various parameters of inflammation in:

- cells and tissues obtained from patients with COPD, who are generally corticosteroid insensitive (Smith, S. J., *Br. J. Pharmacol.,* 2006, **149**:393-404);
- biopsies from IBD patients (Docena, G. *et al.*, *J. Trans. Immunol.*, 2010, **162**:108-115); and
- *in vivo* animal models (Underwood, D. C. *et al.*, *Am. J. Physiol.,* 2000, **279**:L895-902; Nath, P. *et al., Eur. J. Pharmacol.,* 2006, **544**:160-167).

Irusen and colleagues also suggested the possibility of involvement of p38 MAPKα/β on corticosteroid insensitivity *via* the reduction of binding affinity of the glucocorticoid receptor (GR) in nuclei (Irusen, E. *et al.*, *J. Allergy Clin. Immunol.,* 2002, **109**:649-657). Clinical investigations in inflammatory diseases with a range of p38 MAP kinase inhibitors, including **AMG548**, **BIRB 796**, **VX702**, **SCIO469** and **SCIO323**, have been described (Lee, M. R. and Dominguez, C., *Current Med. Chem.,* 2005, **12**:2979-2994.). However, the major obstacle hindering the utility of p38 MAP kinase inhibitors in the treatment of human chronic inflammatory diseases has been the toxicity observed in patients. This has been sufficiently severe to result in the withdrawal from clinical development of many of the compounds progressed, including all those specifically mentioned above.

COPD is a condition in which the underlying inflammation is reported to be substantially resistant to the anti-inflammatory effects of inhaled corticosteroids. Consequently, a superior strategy for treating COPD would be to develop an intervention which has both inherent anti-inflammatory effects and the ability to increase the sensitivity of the lung tissues of COPD patients to inhaled corticosteroids. The recent publication of Mercado *et al.* (2007; *American Thoracic Society Abstract A56)* demonstrates that silencing p38 MAPK γ has the potential to restore sensitivity to corticosteroids. Thus, there may be a dual benefit for patients in the use of a p38 MAP kinase inhibitor for the treatment of COPD.

Many patients diagnosed with asthma or with COPD continue to suffer from uncontrolled symptoms and from exacerbations of their medical condition that can result in hospitalisation. This occurs despite the use of the most advanced, currently available treatment regimens, comprising of combination products of an inhaled corticosteroid and a long acting β-agonist. Data accumulated over the last decade indicates that a failure to manage effectively the underlying inflammatory component of the disease in the lung is the most likely reason that exacerbations occur. Given the established efficacy of corticosteroids as anti-inflammatory agents and, in particular, of inhaled corticosteroids in the treatment of asthma, these findings have provoked intense investigation. Resulting studies have identified that some environmental insults invoke corticosteroid-insensitive inflammatory changes in patients' lungs. An example is the response arising from virally-mediated upper respiratory tract infections (URTI), which have particular significance in increasing morbidity associated with asthma and COPD.

It has been disclosed previously that compounds that inhibit the activity of both the c-Src and Syk kinases are effective agents against rhinovirus replication (Charron, C.E. *et al.*, **WO 2011/158042**) and that compounds that inhibit p59-HCK are effective against influenza virus replication (Charron, C.E. *et al.*, **WO 2011/070369**). Taken together with inhibition of p38 MAPK, these are particularly attractive properties for compounds to possess that are intended to treat patients with chronic respiratory diseases.

Certain p38 MAPK inhibitors have also been described as inhibitors of replication of respiratory syncytial virus (Cass L. *et al.*, **WO 2011/158039**).

The precise etiology of IBD is uncertain, but is believed to be governed by genetic and environmental factors that interact to promote an excessive and poorly controlled mucosal inflammatory response directed against components of the luminal microflora. This response is mediated through infiltration of inflammatory neutrophils, dendritic cells and T-cells from the periphery. p38 has become an obvious target for investigation in IBD models as a consequence of its ubiquitous expression in inflammatory cells. Studies investigating the efficacy of p38 inhibitors in animal models of IBD and human biopsies from IBD patients indicated that p38 could be a target for the treatment of IBD (Hove, T. ten *et al., Gut, 2002,* **50**:507-512, Docena, G. *et al., J. Trans. Immunol,. 2010*, **162**:108-115). However, these findings are not completely consistent with other groups reporting no effect with p38 inhibitors (Malamut G. *et al., Dig. Dis. Sci, 2006,* **51**:1443-1453). A clinical study in Crohn's patients using the p38 alpha inhibitor **BIRB796** demonstrated potential clinical benefit with an improvement in C-reactive protein levels. However this improvement was transient, returning to baseline by week 8 (Schreiber, S. *et al., Clin. Gastro. Hepatology*, 2006, **4**:325-334). A small clinical study investigating the efficacy of CNI-1493, a p38 and Jnk inhibitor, in patients with severe Crohn's disease showed significant improvement in clinical score over 8 weeks (Hommes, D. *et al. Gastroenterology.* 2002 **122**:7-14).

T cells are known to play a key role in mediating inflammation of the gastrointestinal tract. Pioneering work by Powrie and colleagues demonstrated that transfer of naive CD4+ cells into severely compromised immunodeficient (SCID) animals results in the development of colitis which is dependent on the presence of commensal bacteria (Powrie F. *et al. Int Immunol.* 1993 **5**:1461-71). Furthermore, investigation of mucosal membranes from IBD patients showed an upregulation of CD4+ cells which were either Th1 (IFNg/IL-2) or Th2 (IL5/ TGFb) biased depending on whether the patient had Crohn's disease or ulcerative colitis (Fuss IJ. *et al. J Immunol.* 1996 **157**:1261-70.). Similarly, T cells are known to play a key role in inflammatory disorders of the eye with several studies reporting increased levels of T cell associated cytokines (IL-17 and IL-23) in sera of Beçhets patients (Chi W. *et al. Invest Ophthalmol Vis Sci.* 2008 **49**:3058-64). In support of these observations, Direskeneli and colleagues demonstrated that Beçhets patients have increased Th17 cells and decreased Treg cells in their peripheral blood (Direskeneli H. *et al.* J Allergy Clin Immunol. 2011 **128**:665-6).

One approach to inhibit T cell activation is to target kinases which are involved in activation of the T cell receptor signalling complex. Syk and Src family kinases are known to play a key role in this pathway, where Src family kinases, Fyn and Lck, are the first signalling molecules to be activated downstream of the T cell receptor (Barber EK. *et al. PNAS* 1989, **86**:3277-81). They initiate the tyrosine phosphorylation of the T cell receptor leading to the recruitment of the Syk family kinase, ZAP-70. Animal studies have shown that ZAP-70 knockout results in a SCID phenotype (Chan AC. *et al. Science.* 1994, **10**;264(5165):1599-601).

A clinical trial in rheumatoid arthritis patients with the Syk inhibitor Fostamatinib demonstrated the potential of Syk as an anti-inflammatory target with patients showing improved clinical outcome and reduced serum levels of IL-6 and MMP-3 (Weinblatt ME. *et al. Arthritis Rheum.* 2008 **58**:3309-18). Syk kinase is widely expressed in cells of the hematopoietic system, most notably in B cells and mature T cells. Through interaction with immunoreceptor tyrosine-based activation motifs (ITAM), it plays an important role in regulating T cell and B cell expansion as well as mediating immune-receptor signalling in inflammatory cells. Syk activation leads to IL-6 and MMP release – inflammatory mediators commonly found upregulated in inflammatory disorders including IBD and rheumatoid arthritis (Wang YD. et al *World J Gastroenterol* 2007; **13**: 5926-5932, Litinsky I *et al. Cytokine.* 2006 Jan **33**:106-10).

In addition to playing key roles in cell signalling events which control the activity of pro-inflammatory pathways, kinase enzymes are now also recognised to regulate the activity of a range of cellular functions, including the maintenance of DNA integrity (Shilo, Y. *Nature Reviews Cancer,* 2003, **3**: 155-168) and co-ordination of the complex processes of cell division. Indeed, certain kinase inhibitors (the so-called “Olaharski kinases”) have been found to alter the frequency of micronucleus formation *in vitro* (Olaharski, A. J. *et al., PLoS Comput. Biol.,* 2009, **5**(7), e1000446; doi: 10.1371/journal.pcbi.1000446). Micronucleus formation is implicated in, or associated with, disruption of mitotic processes and is therefore undesirable. Inhibition of glycogen synthase kinase 3α (GSK3α) was found to be a particularly significant factor that increases the likelihood of a kinase inhibitor promoting micronucleus formation. Also, inhibition of the kinase GSK3β with RNAi has been reported to promote micronucleus formation (Tighe, A. *et al., BMC Cell Biology,* 2007, **8:**34).

Whilst it may be possible to attenuate the adverse effects of inhibition of Olaharski kinases such as GSK3α by optimisation of the dose and/or by changing the route of administration of a molecule, it would be advantageous to identify further therapeutically useful molecules with low or negligible inhibition of Olaharski kinases, such as GSK 3α and/or have low or negligible disruption of mitotic processes (e.g. as measured in a mitosis assay).

Various compounds, including urea derivatives, are disclosed as inhibiting one or more kinases. Examples of such compounds may be found in WO 99/23091, WO 00/041698, WO 00/043384, WO 00/055139, WO 01/36403, WO 01/4115, WO 02/083628, WO 02/083642, WO 02/092576, WO 02/096876, WO 2003/005999, WO 2003/068223, WO 2003/068228, WO 2003/072569, WO 2004/014870, WO 2004/113352, WO 2005/005396, WO 2005/018624, WO 2005/023761, WO 2005/044825, WO 2006/015775, WO 2006/043090, WO 2007/004749, WO 2007/053394, WO 2013/050756, WO 2013/050757, WO 2014/027209, WO 2014/033446, WO 2014/033447, WO 2014/033448, WO 2014/033449, WO 2014/076484, WO 2014/140582 WO 2014/162121, WO 2014/162122, WO 2014/162126 and WO 2015/092423. Further examples may be found in articles published in:

- *Curr. Opin. Drug Devel.* (**2004**, *7(5)*, 600-616);
- *J. Med. Chem.* (**2007**, *50*, 4016-4026; **2009**, *52*, 3881-3891; and **2010**, *53*, 5639-5655);
- *Bioorg. Med. Chem. Lett.* (**2007**, *17*, 354–357; **2008**, *18*, 3251-3255; **2009**, *19*, 2386-2391; and **2010**, *20*, 4819-4824);
- *Curr. Top. Med. Chem.* (**2008**, *8*, 1452-1467);
- *Bioorg. Med. Chem.* (**2010**, *18*, 5738-5748);
- *Eur. J. Pharmacol.* (**2010**, *632*, 93-102);
- *J. Chem. Inf. Model.* (**2011**, *51*, 115-129); and

- *Br. J. Pharmacol.* (**2015**, *172,* 3805–3816).

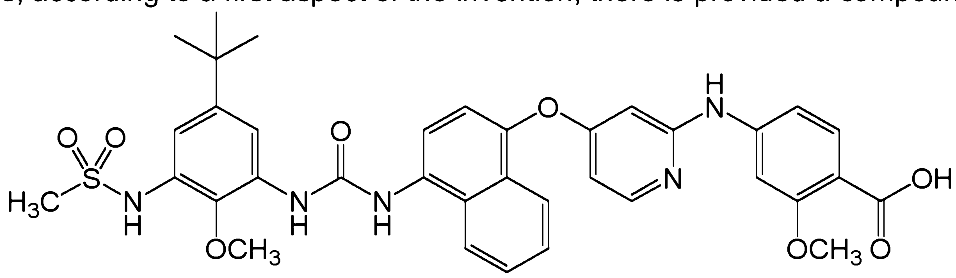
Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Nevertheless, there remains a need to identify and develop new kinase inhibitors, specifically alternative p38 MAP kinase inhibitors that are suitable for the treatment of inflammation. There is particularly a need for such inhibitors that have improved therapeutic potential over currently available treatments or, in particular, that exhibit a superior therapeutic index (e.g. inhibitors that are at least equally efficacious and, in one or more respects, are less toxic at the relevant therapeutic dose than previous agents).

We have now discovered, surprisingly, a benzoic acid aniline-possessing diarylurea inhibits one or more of p38 MAP kinase, Syk and Src family kinases and therefore possess good anti-inflammatory properties.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

Thus, according to a first aspect of the invention, there is provided a compound of formula I,



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or a pharmaceutically acceptable salt, solvate or isotopic derivative thereof,

which compound may be referred to hereinafter as "*the compound of the invention*".

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

Examples of pharmaceutically acceptable salts include acid addition salts derived from mineral acids and organic acids, and salts derived from metals.

For the avoidance of doubt, the compound of formula I may contain the stated atoms in any of their natural or non-natural isotopic forms. In this respect, embodiments of the invention that may be mentioned include those in which:

(a) the compound of formula I is not isotopically enriched or labelled with respect to any atoms of the compound; and

(b) the compound of formula I is isotopically enriched or labelled with respect to one or more atoms of the compound.

References herein to an "isotopic derivative" relate to the second of these two embodiments. In particular embodiments of the invention, the compound of formula I is isotopically enriched or labelled (with respect to one or more atoms of the compound) with one or more stable isotopes. Thus, compounds of the invention that may be mentioned include, for example, compounds of formula I that are isotopically enriched or labelled with one or more atoms such as deuterium or the like.

The compound of formula I may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

Unless otherwise specified, alkyl groups and alkoxy groups as defined herein may be straight-chain or, when there is a sufficient number (i.e. a minimum of three) of carbon atoms, be branched. Particular alkyl groups that may be mentioned include, for example, methyl, ethyl, n-propyl, iso-propyl, butyl, n-butyl and tert-butyl. Particular alkoxy groups that may be mentioned include, for example, methoxy, ethoxy, propoxy, and butoxy.

Unless otherwise specified, alkylene groups as defined herein may be straight-chain or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be branched. In particular embodiments of the invention, alkylene refers to straight-chain alkylene.

Unless otherwise stated, the point of attachment of aryl groups may be *via* any atom of the ring system. However, when aryl groups are bicyclic or tricyclic, they are linked to the rest of the molecule *via* an aromatic ring. C_{6-14} aryl groups include phenyl, naphthyl and the like. Embodiments of the invention that may be mentioned include those in which aryl is phenyl.

Unless otherwise specified, the term "*halo*" includes references to fluoro, chloro, bromo or iodo, in particular to fluoro, chloro or bromo, especially fluoro or chloro.

Unless the context clearly requires otherwise, throughout the description and the claims, the words “comprise”, “comprising”, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

The compound of formula I has the name 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid but may also be known by the chemical name 4-[[4-[[4-[[5-tert-butyl-3-

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(methanesulfonamido)-2-methoxyphenyl]-carbamoylamino]-1-naphthyl]oxy]-2-pyridyl]amino]-2-methoxy-benzoic acid.

Examples of salts of the compound of formula I include all pharmaceutically acceptable salts, such as, without limitation, acid addition salts of strong mineral acids such as HCl, H_2SO_4 and HBr salts (e.g. HCl or HBr salts) and addition salts of strong organic acids such as methanesulfonic acid.

Particular salts of the compound of formula I that may be mentioned include hydrochloric acid salts and sodium, ammonium, calcium, magnesium, N-methylglucamine ((2*R*,3*R*,4*R*,5*S*)-6-(methylamino)-hexane-1,2,3,4,5-pentol) or benethamine (N-benzyl-2-phenethylamine) salts (e.g. sodium or ammonium salts).

Thus, embodiments of the invention that may be mentioned include the hydrochloride, sodium, calcium, magnesium or ammonium salts of 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid.

More particular salts of the compound of formula I that may be mentioned include the hydrochloride, sodium and ammonium salts.

References herein to a compound of the invention (a compound of formula I) are intended to include references to the compound and to all pharmaceutically acceptable salts, solvates and/or tautomers of said compound, unless the context specifically indicates otherwise. In this respect, solvates that may be mentioned include hydrates.

The compound of the invention (compound of formula I) is a p38 MAP kinase inhibitor (especially of the alpha subtype), Syk kinase and Src family kinases, e.g., Src and Lck, and is therefore useful in medicine, in particular for the treatment of inflammatory diseases. Further aspects of the invention that may be mentioned therefore include the following.

(a) A pharmaceutical formulation comprising a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

(b) A combination product comprising
   (A) a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, and
   (B) another therapeutic agent,

   wherein each of components (A) and (B) is formulated in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier.

   In this aspect of the invention, the combination product may be either a single (combination) pharmaceutical formulation or a kit-of-parts.

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Thus, this aspect of the invention encompasses a pharmaceutical formulation including a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, and another therapeutic agent, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier (which formulation is hereinafter referred to as a "combined preparation").

It also encompasses a kit of parts comprising components:

(i) a pharmaceutical formulation including a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier; and

(ii) a pharmaceutical formulation including another therapeutic agent, in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier,

which components (i) and (ii) are each provided in a form that is suitable for administration in conjunction with the other.

Component (i) of the kit of parts is thus component (A) above in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier. Similarly, component (ii) is component (B) above in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

(c) A process for preparing the pharmaceutical formulation of aspect (a) above, said process comprising the step of admixing the compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, with a pharmaceutically acceptable adjuvant, diluent or carrier.

Embodiments of this aspect of the invention that may be mentioned include those in which the pharmaceutically acceptable adjuvant, diluent or carrier is a topically acceptable adjuvant, diluent or carrier (and/or wherein the process is for preparing a topical pharmaceutical formulation, i.e. a pharmaceutical formulation that is adapted for topical administration).

(d) A compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, for use in medicine (or for use as a medicament or as a pharmaceutical).

(e) A compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention, for use in the treatment or prevention of an inflammatory disease.

(f) The use of

a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention,

for the preparation of a medicament for the treatment or prevention of an inflammatory disease modulated by one or more of p38 mitogen-activated protein kinase, Syk and Src family kinases.

(g) A method of treating or preventing an inflammatory disease modulated by one or more of p38 mitogen-activated protein kinase, Syk and Src family kinases, said method comprising administering to a subject an effective amount of

a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention.

(h) A method of sensitizing a subject to the anti-inflammatory effects of a corticosteroid, said method comprising administering to the subject an effective amount of

a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention.

Embodiments of this aspect of the invention that may be mentioned include those in which the subject is one who has become refractory to the anti-inflammatory effects of a corticosteroid.

References herein to “preventing an inflammatory disease” include references to preventing (or reducing the likelihood of) the recurrence of an inflammatory disease in a subject who has previously suffered from such a disease (e.g. a subject who has previously received treatment for that disease, for example treatment according to the method described in (g) above).

Thus, still further aspects of the invention that may be mentioned include the following.

(i) A compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention, for use in reducing the likelihood of the recurrence of an inflammatory disease in a subject who has previously received treatment for that disease (e.g. treatment with a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention).

(j) The use of

a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention,

for the preparation of a medicament for reducing the likelihood of the recurrence of an inflammatory disease in a subject who has previously received treatment for that disease (e.g. treatment with a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention).

(k) A method of reducing the likelihood of the recurrence of an inflammatory disease in a subject who has previously received treatment for that disease (e.g. treatment with a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention), said method comprising administering to said subject an effective amount of

a compound of formula I, as hereinbefore defined, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation or combination product as defined in connection with aspect (a) or (b) of the invention.

Formulations

In relation to aspects (a) and (b) above, diluents and carriers that may be mentioned include those suitable for parenteral, oral, topical, mucosal and rectal administration.

The pharmaceutical formulations and combination products of aspects (a) and (b) above may be prepared e.g. for parenteral, subcutaneous, intramuscular, intravenous, intra-articular, intravitreous, periocular, retrobulbar, subconjunctival, sub-Tenon, topical ocular or peri-articular administration, particularly in the form of liquid solutions, emulsions or suspensions; for oral administration, particularly in the form of tablets or capsules, and especially involving technologies aimed at furnishing colon-targeted drug release (Patel, M. M. *Expert Opin. Drug Deliv.* **2011**, *8* (10), 1247–1258); for topical e.g. pulmonary or intranasal administration, particularly in the form of powders, nasal drops or aerosols and transdermal administration; for topical ocular administration, particularly in the form of solutions, emulsions, suspensions, ointments, implants/inserts, gels, jellies or liposomal microparticle formulations (Ghate, D.; Edelhauser, H. F. *Expert Opin. Drug Deliv.* **2006**, *3* (2), 275–287); for ocular administration, particularly in the form of biodegradable and non-biodegradable implants, liposomes and nanoparticles (Thrimawithana, T. R. et al. *Drug Discov. Today* **2011**, *16* (5/6), 270–277); for mucosal administration e.g. to buccal, sublingual or vaginal mucosa, and for rectal administration e.g. in the form of a suppository or enema.

The pharmaceutical formulations and combination products of aspects (a) and (b) above may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA., (1985). Formulations for parenteral administration may contain as excipients sterile water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for nasal administration may be solid and may contain excipients, for example, lactose or dextran, or may be aqueous or oily solutions for use in the form of nasal drops or metered sprays. For buccal administration, typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinised starch, and the like.

Pharmaceutical formulations and combination products suitable for oral administration may comprise one or more physiologically compatible carriers and/or excipients and may be in solid or liquid form. Tablets and capsules may be prepared with binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or poly-vinylpyrrolidone; fillers, such as lactose, sucrose, corn starch, calcium phosphate, sorbitol, or glycine; lubricants, such as magnesium stearate, talc, polyethylene glycol, or silica; and surfactants, such as sodium lauryl sulfate. Liquid compositions may contain conventional additives such as suspending agents, for example sorbitol syrup, methyl cellulose, sugar syrup, gelatin, carboxymethyl-cellulose, or edible fats; emulsifying agents such as lecithin, or acacia; vegetable oils such as almond oil, coconut oil, cod liver oil, or peanut oil; preservatives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Liquid compositions may be encapsulated in, for example, gelatin to provide a unit dosage form.

Solid oral dosage forms include tablets, two-piece hard shell capsules and soft elastic gelatin (SEG) capsules. Such two-piece hard shell capsules may be made from, for example, gelatin or hydroxylpropyl methylcellulose (HPMC).

A dry shell formulation typically comprises of about 40% to 60% w/w concentration of gelatin, about a 20% to 30% concentration of plasticizer (such as glycerin, sorbitol or propylene glycol) and about a 30% to 40% concentration of water. Other materials such as preservatives, dyes, opacifiers and flavours also may be present. The liquid fill material comprises a solid drug that has been dissolved, solubilized or dispersed (with suspending agents such as beeswax, hydrogenated castor oil or polyethylene glycol 4000) or a liquid drug in vehicles or combinations of vehicles such as mineral oil, vegetable oils, triglycerides, glycols, polyols and surface-active agents.

The compound of the invention may be administered topically (e.g. to the lung, eye or intestines). Thus, embodiments of aspects (a) and (b) above that may be mentioned include pharmaceutical formulations and combination products that are adapted for topical administration. Such formulations include those in which the excipients (including any adjuvant, diluent and/or carrier) are topically acceptable.

Topical administration to the lung may be achieved by use of an aerosol formulation. Aerosol formulations typically comprise the active ingredient suspended or dissolved in a suitable aerosol propellant, such as a chlorofluorocarbon (CFC) or a hydrofluorocarbon (HFC). Suitable CFC propellants include trichloromonofluoromethane (propellant 11), dichlorotetrafluoroethane (propellant 114), and dichlorodifluoromethane (propellant 12). Suitable HFC propellants include tetrafluoroethane (HFC-134a) and heptafluoropropane (HFC-227). The propellant typically comprises 40% to 99.5% e.g. 40% to 90% by weight of the total inhalation composition. The formulation may comprise excipients including co-solvents (e.g. ethanol) and surfactants (e.g. lecithin, sorbitan trioleate and the like). Other possible excipients include polyethylene glycol, polyvinylpyrrolidone, glycerine and the like. Aerosol formulations are packaged in canisters and a suitable dose is delivered by means of a metering valve (e.g. as supplied by Bespak, Valois or 3M or alternatively by Aptar, Coster or Vari).

Topical administration to the lung may also be achieved by use of a non-pressurised formulation such as an aqueous solution or suspension. This may be administered by means of a nebuliser e.g. one that can be hand-held and portable or for home or hospital use (i.e. non-portable). The formulation may comprise excipients such as water, buffers, tonicity adjusting agents, pH adjusting agents, surfactants and co-solvents. Suspension liquid and aerosol formulations (whether pressurised or unpressurised) will typically contain the compound of the invention in finely divided form, for example with a D_{50} of 0.5-10 μm e.g. around 1-5 μm. Particle size distributions may be represented using D_{10}, D_{50} and D_{90} values. The D_{50} median value of particle size distributions is defined as the particle size in microns that divides the distribution in half. The measurement derived from laser diffraction is more accurately described as a volume distribution, and consequently the D_{50} value obtained using this procedure is more meaningfully referred to as a Dv_{50} value (median for a volume distribution). As used herein Dv values refer to particle size distributions measured using laser diffraction. Similarly, D_{10} and D_{90} values, used in the context of laser diffraction, are taken to mean Dv_{10} and Dv_{90} values and refer to the particle size whereby 10% of the distribution lies below the D_{10} value, and 90% of the distribution lies below the D_{90} value, respectively.

Topical administration to the lung may also be achieved by use of a dry-powder formulation. A dry powder formulation will contain the compound of the disclosure in finely divided form, typically with a mass mean aerodynamic diameter (MMAD) of 1-10 μm or a D_{50} of 0.5-10 μm e.g. around 1-5 μm. Powders of the compound of the invention in finely divided form may be prepared by a micronization process or similar size reduction process. Micronization may be performed using a jet mill such as those manufactured by Hosokawa Alpine. The resultant particle size distribution may be measured using laser diffraction (e.g. with a Malvern Mastersizer 2000S instrument). The formulation will typically contain a topically acceptable diluent such as lactose, glucose or mannitol (preferably lactose), usually of large particle size e.g. an MMAD of 50 μm or more, e.g. 100 μm or more or a D_{50} of 40-150 μm. As used herein, the term “lactose” refers to a lactose-containing component, including α-lactose monohydrate, β-lactose monohydrate, α-lactose anhydrous, β-lactose anhydrous and amorphous lactose. Lactose components may be processed by micronization, sieving, milling, compression, agglomeration or spray drying. Commercially available forms of lactose in various forms are also encompassed, for example Lactohale® (inhalation grade lactose; DFE Pharma), InhaLac®70 (sieved lactose for dry powder inhaler; Meggle), Pharmatose® (DFE Pharma) and Respitose® (sieved inhalation grade lactose; DFE Pharma) products. In one embodiment, the lactose component is selected from the group consisting of α-lactose monohydrate, α-lactose anhydrous and amorphous lactose. Preferably, the lactose is α-lactose monohydrate.

Dry powder formulations may also contain other excipients such as sodium stearate, calcium stearate or magnesium stearate.

A dry powder formulation is typically delivered using a dry powder inhaler (DPI) device. Examples of dry powder delivery systems include SPINHALER, DISKHALER, TURBOHALER, DISKUS and CLICKHALER. Further examples of dry powder delivery systems include ECLIPSE, NEXT, ROTAHALER, HANDIHALER, AEROLISER, CYCLOHALER, BREEZHALER/NEOHALER, MONODOSE, FLOWCAPS, TWINCAPS, X-CAPS, TURBOSPIN, ELPENHALER, MIATHALER, TWISTHALER, NOVOLIZER, PRESSAIR, ELLIPTA, ORIEL dry powder inhaler, MICRODOSE, PULVINAL, EASYHALER, ULTRAHALER, TAIFUN, PULMOJET, OMNIHALER, GYROHALER, TAPER, CONIX, XCELOVAIR and PROHALER.

In one embodiment the compound of the present invention is provided in a micronized dry powder formulation, for example further comprising lactose of a suitable grade optionally together with magnesium stearate, filled into a single dose device such as AEROLISER or filled into a multi dose device such as DISKUS.

The compound of the present invention may also be administered rectally, for example in the form of suppositories or enemas, which include aqueous or oily solutions as well as suspensions and emulsions. Such compositions are prepared following standard procedures, well known by those skilled in the art. For example, suppositories can be prepared by mixing the active ingredient with a conventional suppository base such as cocoa butter or other glycerides, e.g. Suppocire. In this case, the drug is mixed with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Generally, for compositions intended to be administered topically to the eye in the form of eye drops or eye ointments, the total amount of the inhibitor will be about 0.0001 to less than 4.0% (w/w).

Preferably, for topical ocular administration, the compositions administered according to the present invention will be formulated as solutions, suspensions, emulsions and other dosage forms. Aqueous solutions are generally preferred, based on ease of formulation, as well as a patient's ability to administer such compositions easily by means of instilling one to two drops of the solutions in the affected eyes. However, the compositions may also be suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions. Suspensions may be preferred for compounds that are sparingly soluble in water.

The compositions administered according to the present invention may also include various other ingredients, including, but not limited to, tonicity agents, buffers, surfactants, stabilizing polymer, preservatives, co-solvents and viscosity building agents. Preferred pharmaceutical compositions of the present invention include the inhibitor with a tonicity agent and a buffer. The pharmaceutical compositions of the present invention may further optionally include a surfactant and/or a palliative agent and/or a stabilizing polymer.

Various tonicity agents may be employed to adjust the tonicity of the composition, preferably to that of natural tears for ophthalmic compositions. For example, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, simple sugars, such as dextrose, fructose, galactose, and/or simply polyols, such as the sugar alcohols mannitol, sorbitol, xylitol, lactitol, isomaltitol, maltitol, and hydrogenated starch hydrolysates may be added to the composition to approximate physiological tonicity. Such an amount of tonicity agent will vary, depending on the particular agent to be added. In general, however, the compositions will have a tonicity agent in an amount sufficient to cause the final composition to have an ophthalmically acceptable osmolality (generally about 150-450 mOsm, preferably 250-350 mOsm and most preferably at approximately 290 mOsm). In general, the tonicity agents of the invention will be present in the range of 2 to 5% w/w (e.g. 2 to 4% w/w). Preferred tonicity agents of the invention include the simple sugars or the sugar alcohols, such as D-mannitol.

An appropriate buffer system (e.g. sodium phosphate, sodium acetate, sodium citrate, sodium borate or boric acid) may be added to the compositions to prevent pH drift under storage conditions. The particular concentration will vary, depending on the agent employed. Preferably however, the buffer will be chosen to maintain a target pH within the range of pH 5 to 8, and more preferably to a target pH of pH 5 to 7, or a target pH of 6.5 to 7.6.

Surfactants may optionally be employed to deliver higher concentrations of inhibitor. The surfactants function to solubilise the inhibitor and stabilise colloid dispersion, such as micellar solution, microemulsion, emulsion and suspension. Examples of surfactants which may optionally be used include polysorbate, poloxamer, polyoxyl 40 stearate, polyoxyl castor oil, tyloxapol, triton, and sorbitan monolaurate. Preferred surfactants to be employed in the invention have a hydrophile/lipophile/balance "HLB" in the range of 12.4 to 13.2 and are acceptable for ophthalmic use, such as TritonX114 and tyloxapol.

For example, a formulation of 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)-phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, for topical ocular administration may comprise:

(a) water;
(b) a surfactant (e.g. polyoxyl 40 stearate);
(c) a tonicity agent (e.g. mannitol); and

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(d) an appropriate buffer system (e.g. a phosphate buffer containing a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate) chosen to maintain a target pH within the range from 6.5 to 8.

In such topical ocular formulations, one or more (e.g. all) of the following may apply:

(i) 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)-phenyl)ureido)-naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid is present at a concentration in the range from 0.001 to 20 mg/mL (e.g. from 0.01 to 10 mg/mL, 0.1 to 2 mg/mL or, particularly, 1 mg/mL);

(ii) the surfactant (e.g. polyoxyl 40 stearate) is present at from 1 to 10% w/w (e.g. from 2 to 5% w/w, such as from 2.5 to 4% w/w or, particularly, 3% w/w);

(iii) the tonicity agent (e.g. mannitol) is present at from 1 to 15% w/w (e.g. from 2 to 10% w/w, such as from 3 to 6% w/w or, particularly, 4.5% w/w);

(iv) the buffer system used as a component of the formulation is an aqueous phosphate buffer (e.g. a 10 mM aqueous phosphate buffer) chosen to maintain a target pH within the range from 6.5 to 8.0 (e.g. within the range from 7.0 to 7.8 or, particularly, from 7.2 to 7.6).

Additional agents that may be added to the ophthalmic compositions of the present invention are demulcents which function as a stabilising polymer. The stabilizing polymer should be an ionic/charged example with precedence for topical ocular use, more specifically, a polymer that carries negative charge on its surface that can exhibit a zeta-potential of (–)10–50 mV for physical stability and capable of making a dispersion in water (i.e. water soluble). A preferred stabilising polymer of the invention would be polyelectrolyte, or polyelectrolytes if more than one, from the family of cross-linked polyacrylates, such as carbomers, polycarbophil and Pemulen(R), specifically Carbomer 974p (polyacrylic acid), at 0.1–0.5% w/w.

Other compounds may also be added to the ophthalmic compositions of the present invention to increase the viscosity of the carrier. Examples of viscosity enhancing agents include, but are not limited to: polysaccharides, such as hyaluronic acid and its salts, chondroitin sulfate and its salts, dextrans, various polymers of the cellulose family, vinyl polymers and acrylic acid polymers.

Topical ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, chlorobutanol, benzododecinium bromide, methyl paraben, propyl paraben, phenylethyl alcohol, edentate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% w/v. Unit dose compositions of the present invention will be sterile, but typically unpreserved. Such compositions, therefore, generally will not contain preservatives.

The medical practitioner, or other skilled person, will be able to determine a suitable dosage for the compound of the invention, and hence the amount of the compound of the invention that should be included in any particular pharmaceutical formulation (whether in unit dosage form or otherwise).

Embodiments of the invention that may be mentioned in connection with the combination products described at (b) above include those in which the other therapeutic agent is one or more therapeutic agents that are known by those skilled in the art to be suitable for treating inflammatory diseases (e.g. the specific diseases mentioned below).

For example, for the treatment of respiratory disorders (such as COPD or asthma), the other therapeutic agent is one or more agents selected from the list comprising:

- steroids (e.g. budesonide, beclomethasone dipropionate, fluticasone propionate, mometasone furoate, fluticasone furoate; a further example is ciclesonide);
- beta agonists, particularly beta2 agonists (e.g. terbutaline, salbutamol, salmeterol, formoterol; further examples are vilanterol, olodaterol, reproterol and fenoterol); and
- xanthines (e.g. theophylline).

For example, for the treatment of respiratory disorders (such as COPD or asthma), the other therapeutic agent is one or more agents selected from the list comprising:

- muscarinic antagonists (e.g. tiotropium, umeclidinium, glycopyrronium, aclidinium and daratropium, any of these for example as the bromide salt); and
- phosphodiesterase inhibitors.

Further, for the treatment of gastrointestinal disorders (such as Crohn's disease or ulcerative colitis), the other therapeutic agent may be, for example, one or more agents selected from the list comprising:

- 5-aminosalicylic acid, or a prodrug thereof (such as sulfasalazine, olsalazine or balsalazide);
- corticosteroids (e.g. prednisolone, methylprednisolone, or budesonide);
- immunosuppressants (e.g. cyclosporin, tacrolimus, methotrexate, azathioprine or 6-mercaptopurine);
- anti-TNFα antibodies (e.g. infliximab, adalimumab, certolizumab pegol or golimumab);
- anti-IL12/IL23 antibodies (e.g. ustekinumab) or small molecule IL12/IL23 inhibitors (e.g. apilimod);
- anti-α4β7 antibodies (e.g. vedolizumab);
- toll-like receptor (TLR) blockers (e.g. BL-7040; Avecia (Cambridge, UK));
- MAdCAM-1 blockers (e.g. PF-00547659);
- antibodies against the cell adhesion molecule α4-integrin (e.g. natalizumab);
- antibodies against the IL2 receptor α subunit (e.g. daclizumab or basiliximab);
- anti-Smad7 antibodies (e.g. mongersen (GED0301; all-P-ambo-2'-deoxy-P-thioguanylyl-(3'→5')-P-thiothymidylyl-(3'→5')-2'-deoxy-5-methyl-P-thiocytidylyl-(3'→5')-2'-deoxy-P-thioguanylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-P-thiothymidylyl-(3'→5')-P-thiothymidylyl-(3'→5')-2'-deoxy-Pthiocytidylyl-(3'→5')-P-thiothymidylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-

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2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-5-methyl-P-thiocytidylyl-(3'→5')-2'-deoxy-Pthioguanylyl-(3'→5')-2'-deoxy-P-thiocytidylyl-(3'→5')-2'-deoxy-Pthioadenylyl-(3'→5')-2'-deoxy-P-thioguanylyl-(3'→5')-2'-deoxycytidine));

- sphingosine 1-phosphate receptor 1 (S1P1) modulators (e.g. ozanimod ((S)-5-(3-(1-((2-hydroxyethyl)amino)-2,3-dihydro-1H-inden-4-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile), amiselimod (MT1303; 2-amino-2-{2-[4-(heptyloxy)-3-(trifluoromethyl)phenyl]ethyl}propane-1,3-diol) or APD334 (2-[7-[4-cyclopentyl-3-(trifluoromethyl)benzyloxy]-1,2,3,4-tetrahydrocyclopenta[b]indol-3(R)-yl]acetic acid));
- JAK inhibitors (e.g. tofacitinib, baricitinib (1-(ethylsulfonyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-azetidineacetonitrile), filgotinib (N-[5-[4-[(1,1-dioxo-1,4-thiazinan-4-yl)methyl]phenyl]-[1,2,4]triazolo[1,5-a]pyridin-2-yl]cyclopropanecarboxamide), peficitinib (4-(((1R,2r,3S,5s,7s)-5-hydroxyadamantan-2-yl)amino)-1H-pyrrolo[2,3-b]pyridine-5-carboxamide) or R348 (see, for example, US 2014/0206708));
- STAT3 inhibitors (e.g. TAK-114; (3E)-1-methyl-3-(2-oxo-1H-indol-3-ylidene)indol-2-one);
- receptor-interacting protein-1 (RIP1) kinase inhibitors (e.g. GSK2982772);
- Syk inhibitors and prodrugs thereof (e.g. fostamatinib and R-406);
- Phosphodiesterase-4 inhibitors (e.g. tetomilast);
- HMPL-004;
- probiotics;
- microbiome modulators (e.g. SGM1019);
- Dersalazine;
- semapimod/CPSI-2364; and
- protein kinase C inhibitors (e.g. AEB-071)

(e.g. for the treatment of gastrointestinal disorders (such as Crohn's disease or ulcerative colitis), the other therapeutic agent may be, for example, one or more agents selected from the list comprising:

- 5-aminosalicylic acid, or a prodrug thereof (such as sulfasalazine, olsalazine or balsalazide);
- corticosteroids (e.g. prednisolone, methylprednisolone, or budesonide);
- immunosuppressants (e.g. cyclosporin, tacrolimus, methotrexate, azathioprine or 6-mercaptopurine);
- anti-TNFα antibodies (e.g. infliximab, adalimumab, certolizumab pegol or golimumab);
- anti-IL12/IL23 antibodies (e.g. ustekinumab) or small molecule IL12/IL23 inhibitors (e.g. apilimod);
- anti-α4β7 antibodies (e.g. vedolizumab);
- MAdCAM-1 blockers (e.g. PF-00547659);
- antibodies against the cell adhesion molecule α4-integrin (e.g. natalizumab);
- antibodies against the IL2 receptor α subunit (e.g. daclizumab or basiliximab);
- JAK3 inhibitors (e.g. tofacitinib or R348);
- Syk inhibitors and prodrugs thereof (e.g. fostamatinib and R-406);
- Phosphodiesterase-4 inhibitors (e.g. tetomilast);
- HMPL-004;

- probiotics;
- Dersalazine;
- semapimod/CPSI-2364; and
- protein kinase C inhibitors (e.g. AEB-071)).

For the treatment of eye disorders (such as uveitis and keratoconjunctivitis sicca (dry eye)), the other therapeutic agent may be, for example, one or more agents selected from the list comprising:
- corticosteroids (e.g. dexamethasone, prednisolone, triamcinolone acetonide, difluprednate or fluocinolone acetonide);
- glucocorticoid agonists (e.g. mapracorat);
- immunosuppressants (e.g. cyclosporin, voclosporin, azathioprine, methotrexate, mycophenolate mofetil or tacrolimus);
- anti-TNFα antibodies (e.g. infliximab, adalimumab, certolizumab pegol, ESBA-105 or golimumab);
- anti-IL-17A antibodies (e.g. secukinumab);
- mTOR inhibitors (e.g. sirolimus);
- VGX-1027;
- adenosine A3 receptor agonists (e.g. CF-101);
- lifitegrast;
- IL1 blockers (e.g. EBI-005; Hou *et al*. *PNAS* 2013, **110**(10), 3913-3918);
- RGN-259 (Thymosin β4);
- SI-614;
- OTX-101;
- JNK inhibitors (e.g. XG-104);
- MAP kinase signalling inhibitors (e.g. DA-6034; {[2-(3,4-dimethoxyphenyl)-5-methoxy-4-oxochromen-7-yl]oxy}acetic acid);
- mucin stimulators (e.g. rebamipide; 2-[(4-chlorobenzoyl)amino]-3-(2-oxo-1H-quinolin-4-yl)propanoic acid);
- MIM-D3 (Tavilermide; see, for example, US 2013/0345395);
- JAK inhibitors (e.g. tofacitinib, baricitinib (1-(ethylsulfonyl)-3-[4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl]-3-azetidineacetonitrile), filgotinib (N-[5-[4-[(1,1-dioxo-1,4-thiazinan-4-yl)methyl]phenyl]-[1,2,4]triazolo[1,5-a]pyridin-2-yl]cyclopropanecarboxamide), peficitinib (4-(((1R,2r,3S,5s,7s)-5-hydroxyadamantan-2-yl)amino)-1H-pyrrolo[2,3-b]pyridine-5-carboxamide) or R348 (see, for example, US 2014/0206708)); and
- protein kinase C inhibitors (e.g. AEB-071).

(e.g. for the treatment of eye disorders (such as uveitis and keratoconjunctivitis sicca (dry eye)), the other therapeutic agent may be, for example, one or more agents selected from the list comprising:
- corticosteroids (e.g. dexamethasone, prednisolone, triamcinolone acetonide, difluprednate or fluocinolone acetonide);
- glucocorticoid agonists (e.g. mapracorat);-

- immunosuppressants (e.g. cyclosporin, voclosporin, azathioprine, methotrexate, mycophenolate mofetil or tacrolimus);
- anti-TNFα antibodies (e.g. infliximab, adalimumab, certolizumab pegol, ESBA-105 or golimumab);
- anti-IL-17A antibodies (e.g. secukinumab);
- mTOR inhibitors (e.g. sirolimus);
- VGX-1027;
- adenosine A3 receptor agonists (e.g. CF-101);
- lifitegrast;
- JAK3 inhibitors (e.g. tofacitinib or R348); and
- protein kinase C inhibitors (e.g. AEB-071)).

In particular embodiments, for the treatment of eye disorders (such as uveitis and keratoconjunctivitis sicca (dry eye)), the other therapeutic agent may be, for example, one or more agents selected from the list comprising:

- corticosteroids (e.g. dexamethasone, prednisolone, triamcinolone acetonide, difluprednate or fluocinolone acetonide);
- immunosuppressants (e.g. cyclosporin, voclosporin, azathioprine, methotrexate, mycophenolate mofetil or tacrolimus);
- anti-TNFα antibodies (e.g. infliximab, adalimumab, certolizumab pegol, ESBA-105 or golimumab);
- anti-IL-17A antibodies (e.g. secukinumab);
- mTOR inhibitors (e.g. sirolimus);
- VGX-1027;
- JAK inhibitors (e.g. tofacitinib, baricitinib, filgotinib, peficitinib or R348) (e.g. JAK3 inhibitors such as tofacitinib or R348); and
- protein kinase C inhibitors (e.g. AEB-071).

<u>Medical Uses</u>

The compound of the invention may be used as monotherapies for inflammatory diseases, or in combination therapies for such diseases.

Thus, embodiments of aspects (e) to (g) above that may be mentioned include those in which the compound of formula I (or pharmaceutically acceptable salt, solvate or isotopic derivative thereof) is the sole pharmacologically active ingredient utilised in the treatment.

However, in other embodiments of aspects (e) to (g) above, the compound of formula I (or pharmaceutically acceptable salt, solvate or isotopic derivative thereof) is administered to a subject who is also administered one or more other therapeutic agents (e.g. wherein the one or more other therapeutic agents are as defined above in connection with combination products).

When used herein, the term "*inflammatory disease*" specifically includes references to any one or more of the following:

(i) lung diseases or disorders having an inflammatory component, such as cystic fibrosis, pulmonary hypertension, lung sarcoidosis, idiopathic pulmonary fibrosis or, particularly, COPD (including chronic bronchitis and emphysema), asthma or paediatric asthma;

(ii) skin diseases or disorders having an inflammatory component, such as atopic dermatitis, allergic dermatitis, contact dermatitis or psoriasis;

(iii) nasal diseases or disorders having an inflammatory component, such as allergic rhinitis, rhinitis or sinusitis;

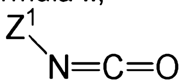
(iv) eye diseases or disorders having an inflammatory component, such as conjunctivitis, allergic conjunctivitis, glaucoma, diabetic retinopathy, macular oedema (including diabetic macular oedema), central retinal vein occlusion (CRVO), dry and/or wet age related macular degeneration (AMD), post-operative cataract inflammation, or, particularly, keratoconjunctivitis sicca (dry eye, also known as xerophthalmia), uveitis (including posterior, anterior and pan uveitis), corneal graft and limbal cell transplant rejection; and

(v) gastrointestinal diseases or disorders having an inflammatory component, such as gluten sensitive enteropathy (coeliac disease), eosinophilic esophagitis, intestinal graft versus host disease or, particularly, Crohn's disease or ulcerative colitis.

References herein to diseases having an inflammatory component include references to diseases that involve inflammation, whether or not there are other (non-inflammatory) symptoms or consequences of the disease.

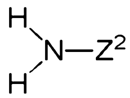
According to a further aspect of the invention there is provided a process for the preparation of the compound of formula I which process comprises:

(a) reaction of a compound of formula II,



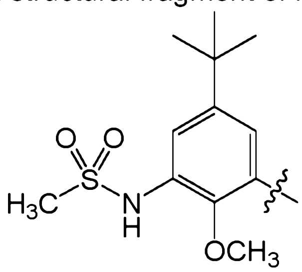
II

with a compound of formula III,



III

wherein one of Z^1 and Z^2 is a structural fragment of formula IV



IV

and the other of Z^1 and Z^2 is a structural fragment of formula V

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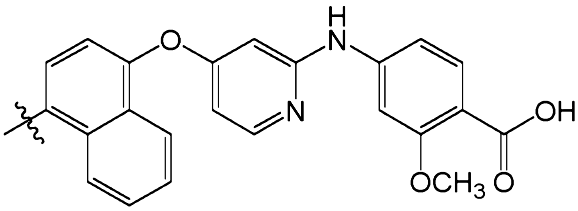
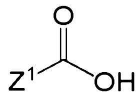


V

for example under conditions known to those skilled in the art, such as at a temperature from ambient (e.g. 15 to 30°C) to about 110°C in the presence of a suitable organic solvent (e.g. a polar aprotic solvent such as DMF, THF, 1,4-dioxane, or mixtures thereof);

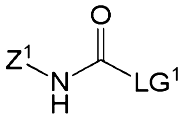
(b) reaction of a compound of formula IIa,



IIa

wherein Z^1 is as defined above, with a suitable azide-forming agent (i.e. a suitable source of a leaving group and activated azide ion, such as diphenyl phosphorazidate; see, for example, *Tetrahedron* **1974**, *30*, 2151–2157) under conditions known to those skilled in the art, such as at sub-ambient to ambient temperature (e.g. from an initial temperature of about -5 to 5°C to ambient temperature post-reaction) in the presence of an amine base (e.g. triethylamine or a sterically hindered base such as *N*,*N*-diisopropylethylamine) and a suitable organic solvent (e.g. a polar aprotic solvent such as DMF, THF, 1,4-dioxane, or mixtures thereof), which reaction is followed, without isolation, by thermal rearrangement (e.g. under heating) of the intermediate acyl azide (of formula Z^1-C(O)-N_3) e.g. at ambient temperature (such as from 15 to 30°C) to provide, *in situ*, a compound of formula II, which compound is then reacted with a compound of formula III, as defined above, to provide the compound of formula I;

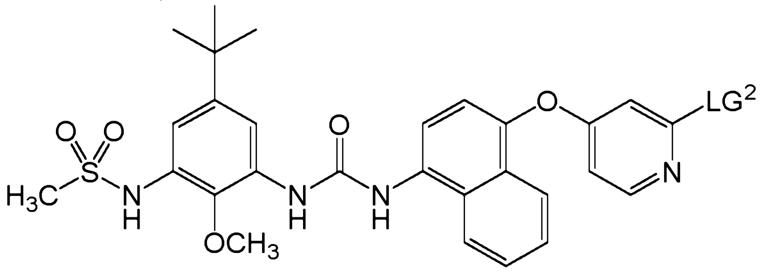
(c) reaction of a compound of formula IIb,



IIb

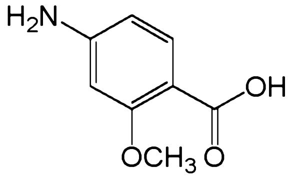
wherein LG^1 represents a suitable leaving group (e.g. imidazolyl, chloro, or aryloxy, such as phenoxy) and Z^1 is as defined above, with a compound of formula III, as defined above, for example under conditions known to those skilled in the art, such as at ambient temperature (e.g. from ambient to 80°C), optionally in the presence of an amine base (e.g. triethylamine or a sterically hindered base like *N*,*N*-diisopropylethylamine) and a suitable organic solvent (e.g. an aprotic solvent, such as dichloromethane or an ester such as isopropyl acetate);

(d) reaction of a compound of formula VI,



VI

wherein LG^2 represents a suitable leaving group (e.g. a halo group such as chloro or bromo), with a compound of formula VII,



VII

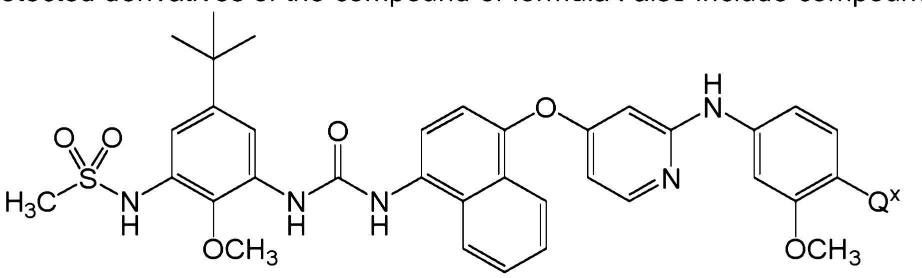
for example under conditions known to those skilled in the art (e.g. as described in *J. Am. Chem. Soc.* **2011**, *133*, 15686–15696), such as at elevated temperature (e.g. from 50 to 110°C) in the presence of a suitable organic solvent (e.g. a polar aprotic solvent such as DMF, THF, 1,4-dioxane, or mixtures thereof) and, optionally, an acidic catalyst (e.g. a sulfonic acid such as *para*-toluenesulfonic acid);

(e) deprotection of a protected derivative of the compound of formula I, under conditions known to those skilled in the art, wherein the protected derivative bears a protecting group on an O- or N-atom of the compound of formula I (and, for the avoidance of doubt, a protected derivative of one compound of formula I may or may not represent another compound of formula I).

Examples of protected derivatives of the compound of formula I include those where:

- an O-atom is protected with a benzyl group, which benzyl group may be removed by hydrogenation, for example in the presence of a palladium catalyst (such as Pd/C);
- an O-atom of a carboxylic acid is protected with an alkyl group (such as methyl, ethyl or *tert*-butyl), which alkyl group may be removed by either basic hydrolysis (e.g. for methyl or ethyl groups, by a hydrolysis reaction using an alkali metal hydroxide such as sodium hydroxide) or acid hydrolysis (e.g. for a *tert*-butyl group, by a hydrolysis reaction using an acid such as trifluoroacetic acid).

Protected derivatives of the compound of formula I also include compounds of formula VIIa,

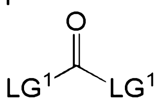


VIIa

wherein Q^x represents -C(O)OR$^{4'}$ and R$^{4'}$ represents a C_{1-4} alkyl group (e.g. a C_4 alkyl group or a C_{1-3} alkyl group, such as methyl).

Compounds of formula II may be prepared according to or by analogy with methods known to those skilled in the art, for example by reaction of a compound of formula IIa, as defined above, with an azide-forming agent, followed by rearrangement of the intermediate acyl azide (as described at (b) above; see, for example, *Tetrahedron* **1974**, *30*, 2151–2157).

Compounds of formula IIb may be prepared reaction of a compound of formula VIII,



VIII

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wherein LG^1 is as hereinbefore defined, with a compound of formula IX,

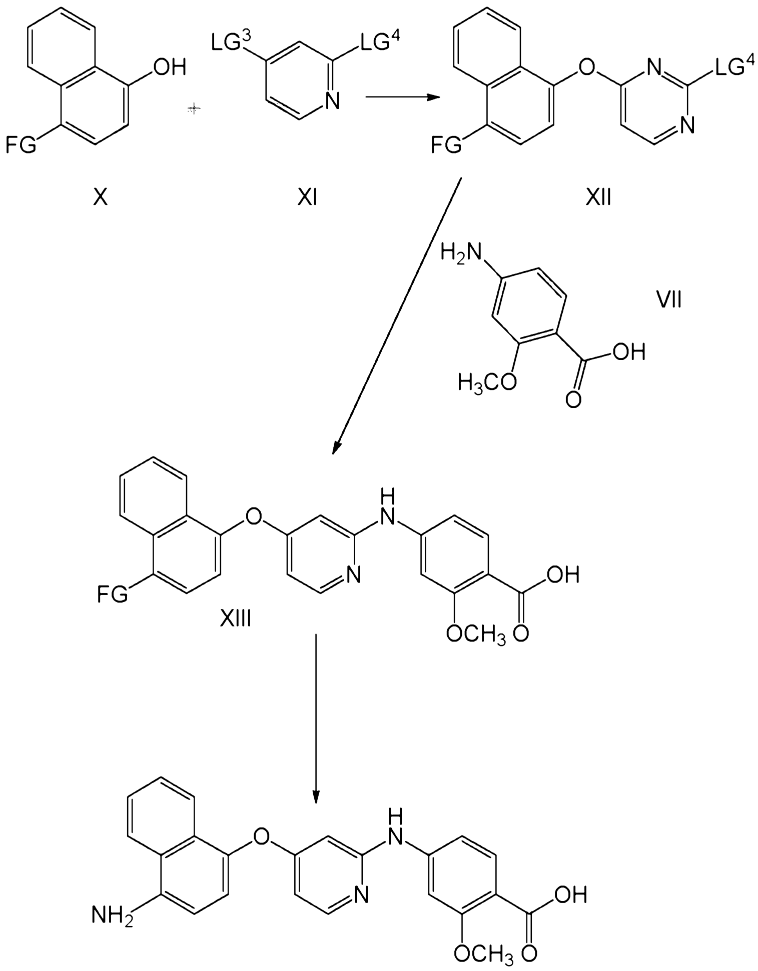
$$Z^1{-}NH_2 \quad \text{IX}$$

wherein Z^1 is as hereinbefore defined, for example under conditions known to those skilled in the art.

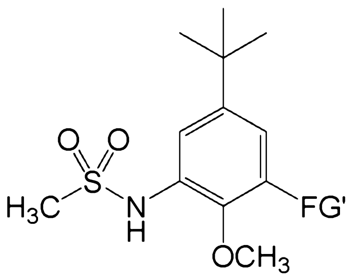
Amines of formula IX may be prepared from carboxylic acids of formula IIa through the route described in (b) above, where the intermediate isocyanate II is hydrolysed with water to give a carbamic acid that loses carbon dioxide to furnish IX. By the same token, the intermediate isocyanate II can be reacted with an alcohol, such as *t*-butanol, to generate a protected version of IX.

The compound of formula III in which Z^2 represents a structural fragment of formula V, or the compound of formula IX in which Z^1 represents a structural fragment of formula V, may be synthesised employing the route outlined in Scheme 1 (see, for example: WO 2003/072569; and WO 2008/046216), wherein LG^3 and LG^4 represent leaving groups, e.g. halogen or methanesulfonyl, and FG represents a real or latent NH_2 group, i.e., a group that is readily transformed into an NH_2 group, such as nitro or a protected variant NH–PG^2, where PG^2 is a typical protecting group (see, for example: Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley, 4th revised edition, 2006; ISBN-10: 0471697540), e.g. a carbamate ester or carboxamide. The sequence starts with the base-mediated S_NAr displacement of LG^3 in XI by the aroxides formed when X is treated with base to generate ethers XII. The remaining halogen or methanesulfonyl substituent (LG^4) of the ether XII is then displaced (i) by an amine of formula VII in a second S_NAr reaction or (ii) via a Buchwald coupling (see, for example, WO 2009/017838) with an amine of formula VII to furnish the desired compound (when FG is NH_2), or XIII (when FG is nitro or NH–PG^2). When FG is nitro in XIII, the NH_2 group may be revealed by a reduction reaction, typically done through hydrogenation employing a suitable catalyst, e.g. palladium on carbon, or employing dissolving metal conditions, such as with iron in glacial acetic acid. Alternatively, when FG is a protecting group, the NH_2 group may be revealed by a deprotection reaction. Although only depicted as taking place in the final step of the sequence, it should be noted that the unmasking of the latent NH_2 group represented by FG can take place at any stage in the synthetic route shown in Scheme 1.

Scheme 1



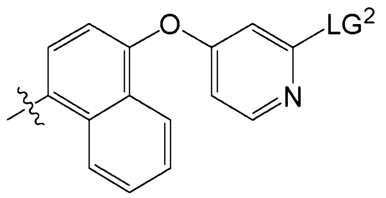
In a similar manner, amines of formula IX in which Z^1 represents a structural fragment of formula IV may be synthesised by conversion of a latent to a real NH_2 group in a compound of formula XIIIa,



XIIIa

wherein FG' is as defined for FG above, except that it does not represent NH_2.

Compounds of formula VI may be synthesised by analogy with the compound of formula I (see, for example, alternative processes (a) to (c) above). For example, compounds of formula VI can be prepared by reaction of a compound of formula IIx with a compound of formula IIIx, wherein the compounds of formulae IIx and IIIx take the same definitions as the compounds of formulae II and III, with the exception that one of Z^1 and Z^2 represents a structural fragment of formula IV, as hereinbefore defined, and the other of Z^1 and Z^2 represents a structural fragment of formula Va,



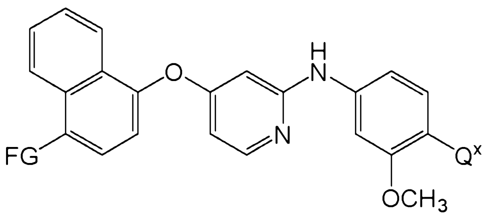
Va

Compounds of formula VIIa may be prepared by analogy with the procedures described herein for preparation of the compound of formula I (see, for example, processes (a) to (d) and Scheme 1 above).

For example, the $-CO_2H$ group may be replaced by Q^x in:

- the structural fragment of formula V (to give a structural fragment of formula Vp, and corresponding compounds of formulae IIp, IIap, IIbp and IIIp, in which Z^1 and Z^2 are replaced by Z^{1p} and Z^{2p}, respectively, wherein one of Z^{1p} and Z^{2p} is a structural fragment of formula IV, as defined above, and the other of Z^{1p} and Z^{2p} is a structural fragment of formula Vp); or
- the compound of formula VII (to give a compound of formula VIIp).

Alternatively, compounds of formula VIIa may be prepared by converting, in a compound of formula XIIIb



XIIIb

,

the group FG to NH_2, wherein FG and Q^x are as hereinbefore defined (e.g. by converting FG to NH_2 as described above in connection with Scheme 1), followed by reaction with, for example, a compound of formula IIb where Z^1 represents a structural fragment of formula IV.

It will be understood by persons skilled in the art that compounds represented by formulae II, IIx and IIb are generally reactive intermediates. These intermediates may be formed *in situ* and reacted directly, without isolation, with compounds of formula III to provide the compound of formula I. Furthermore, it will be understood by those skilled in the art that the use of appropriate protective groups may be required during the processes described above for any of the groups Z^1 and Z^2 which possess chemically-sensitive functional groups, for example, a hydroxyl group or an amino function.

Many of the compounds illustrated in the Schemes are either commercially available, or can be obtained using the cited procedures, or can be readily prepared by conventional methods by those skilled in the art. See for example Regan, J. et al.; *J. Med. Chem.* **2003**, *46*, 4676–4686, WO 2000/043384, WO 2007/053346, WO 2007/087448, WO 2007/089512, WO 2009/117080 and WO 2014/027209.

Novel intermediates as described herein form an aspect of the invention. In this respect, further aspects of the invention relate to:

(i) a compound of formula II, IIa or IIb as hereinbefore defined, wherein Z^1 represents a structural fragment of formula V, or a salt or protected derivative thereof;

(ii) a compound of formula III as hereinbefore defined, wherein Z^2 represents a structural fragment of formula V, or a salt or protected derivative thereof;

(iii) a compound of formula VIIa as hereinbefore defined, or a salt or protected derivative thereof; and

(v) a compound of formula XIII or XIIIb as hereinbefore defined, or a salt or protected derivative thereof.

Protected derivatives of the compounds of formulae III, VII, XIII and XIIIb include those in which the essential NH_2 group (or NH_2 group represented by FG) is protected. In this respect, such protected derivatives include amides or, particularly, carbamates of those compounds. For example, those protected derivatives include compounds in which a H-atom of the NH_2 group is replaced by:

R'-C(O)-, wherein R' is H, C_{1-8} alkyl, phenyl or benzyl, which latter two groups are optionally substituted by one or more groups selected from halo, hydroxy, methyl and methoxy; or

R''-O-C(O)-, wherein R'' is *tert*-butyl, phenyl, benzyl or fluorenyl, which latter three groups are optionally substituted by one or more groups selected from halo, hydroxy, methyl and methoxy.

Protected derivatives of the compounds of formulae II, IIa, IIb, III, VII and XIII in which R^4 represents $-CO_2H$ additionally (or alternatively) include those in which the carboxyl moiety is protected. In this respect, such protected derivatives also include esters (e.g. C_{1-8} alkyl esters, such as ethyl or, particularly, methyl esters) of such compounds.

Those skilled in the art will appreciate that compounds of formula III in which Z^2 represents a structural fragment of formula V may be protected at the essential NH_2 group and/or, when R^4 represents $-CO_2H$, at the carboxyl moiety. In this respect, for example, particular protected derivatives of compounds of formula III in which Z^2 represents a structural fragment of formula V that may be mentioned include:

methyl 4-((4-((4-aminonaphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate; and

methyl 4-((4-((4-((tert-butoxycarbonyl)amino)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate.

Both methyl 4-((4-((4-aminonaphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate and methyl 4-((4-((4-((tert-butoxycarbonyl)amino)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate are also compounds of formula XIIIb (in which Q^x represents $-CO_2CH_3$ and, respectively, FG represents NH_2 or NH-PG^2, in which PG^2 represents *tert*-butyloxycarbonyl).

Alternative embodiments of the invention relate to a compound that is either:

(i) a protected derivative of compound of formula III in which Z^2 represents a structural fragment of formula V; or

(ii) a compound of formula XIIIb, or a protected derivative thereof,

provided that said compound is not:
methyl 4-((4-((4-aminonaphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate; or
methyl 4-((4-((4-((tert-butoxycarbonyl)amino)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate.

Still further embodiments of the invention relate to a compound of formula VIIa as hereinbefore defined, provided that said compound either:

(a) is; or

(b) is not

methyl 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate.

The aspects of the invention described herein (e.g. the above-mentioned compounds, combinations, methods and uses) may have the advantage that, in the treatment of the conditions described herein, they may be more convenient for the physician and/or patient than, be more efficacious than, be less toxic than, have better selectivity over, have a broader range of activity than, be more potent than, produce fewer side effects than, have a better pharmacokinetic and/or pharmacodynamic profile than, have more suitable solid state morphology than, have better long term stability than, or may have other useful pharmacological properties over, similar compounds, combinations, methods (treatments) or uses known in the prior art for use in the treatment of those conditions or otherwise.

The compound of the invention may additionally (or alternatively):

- exhibit a long duration of action and/or persistence of action (e.g. in comparison to other previously disclosed p38 MAP kinase inhibitors such as, for example, **BIRB796**);
- exhibit potent inhibition of Syk (e.g. they may have an IC_{50} against Syk of 500 nM or less, such as 350 nM or less);
- not strongly inhibit GSK 3α (e.g. they may have an IC_{50} against GSK 3α of 1,000 nM or greater; such as 1,500, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000 or 10,000 nM or greater);
- target a smaller portion of the kinome, i.e., with improved selectivity, as illustrated by lowered KinomeScan Selectivity Scores;
- maintain a relatively high local drug concentration between doses (e.g. a high local concentration relative to other previously disclosed p38 MAP kinase inhibitors such as, for example, **BIRB796**);
- exhibit properties that are particularly suited to topical/local administration (e.g. following topical/local administration, the generation of high target tissue concentrations but low plasma concentrations of the compound of formula I and/or rapid clearance of the compound of formula I from plasma, for example as a result of high renal or hepatic extraction);
- exhibit little or no β-catenin induction and/or inhibition of mitosis in cells;
- not produce increases in binucleated cells containing micronuclei in the human lymphocyte *in vitro* micronucleus test;

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- exhibit little or no time-dependent inhibition of members of the cytochrome P450 superfamily;
- show improved chemical stability in the presence of water (e.g. stability to hydrolysis in aqueous mixtures at elevated temperatures) compared to previously disclosed p38 MAP kinase inhibitors such as, for example, **BIRB796**;
- following administration to a patient, give rise to metabolites associated with little or no safety (e.g. toxicity) concerns;
- display reduced ocular irritancy or toxicity in both preclinical species and humans following topical administration;
- exhibit good aqueous solubility and/or cellular permeability;
- be more readily formulated in aqueous solution in the pH range 7–8 with lower quantities of solubilising excipients;
- have a high degree of crystallinity; and/or
- exhibit little or no hygroscopicity in the solid state.

Experimental Methods

General Procedures

All starting materials and solvents were obtained either from commercial sources or prepared according to the literature citation. Unless otherwise stated all reactions were stirred. Organic solutions were routinely dried over anhydrous magnesium sulfate. Hydrogenations were performed on a Thales H-cube flow reactor under the conditions stated or under a balloon of hydrogen. Microwave reactions were performed in a CEM Discover and Smithcreator microwave reactor, heating to a constant temperature using variable power microwave irradiation.

Normal phase column chromatography was routinely carried out on an automated flash chromatography system such as CombiFlash Companion or CombiFlash RF system using pre-packed silica (230-400 mesh, 40-63 µm) cartridges. SCX was purchased from Supelco and treated with 1M hydrochloric acid prior to use. Unless stated otherwise the reaction mixture to be purified was first diluted with MeOH and made acidic with a few drops of AcOH. This solution was loaded directly onto the SCX and washed with MeOH. The desired material was then eluted by washing with 1% NH_3 in MeOH.

Analytical Methods

Analytical HPLC was carried out using a Waters Xselect CSH C18, 2.5 µm, 4.6x30 mm column eluting with a gradient of 0.1% Formic Acid in MeCN in 0.1% aqueous Formic Acid or a Waters Xbridge BEH C18, 2.5 µm, 4.6x30 mm column eluting with a gradient of MeCN in aqueous 10 mM Ammonium Bicarbonate. UV spectra of the eluted peaks were measured using either a diode array or variable wavelength detector on an Agilent 1100 system.

Analytical LCMS was carried out using a Waters Xselect CSH C18, 2.5 µm, 4.6x30 mm column eluting with a gradient of 0.1% Formic Acid in MeCN in 0.1% aqueous Formic Acid or a Waters Xbridge BEH C18, 2.5 µm, 4.6x30 mm column eluting with a gradient of MeCN in aqueous 10 mM Ammonium Bicarbonate. UV and mass spectra of the eluted peaks were measured using a variable wavelength detector on either an Agilent 1200 or an Agilent Infinity 1260 LCMS with 6120 single quadrupole mass spectrometer with positive and negative ion electrospray.

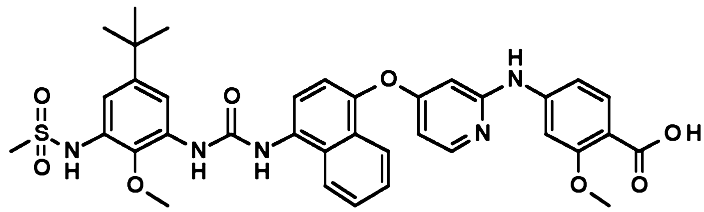
Preparative HPLC was carried out using a Waters Xselect CSH C18, 5 µm, 19x50 mm column using either a gradient of either 0.1% Formic Acid in MeCN in 0.1% aqueous Formic Acid or a gradient of MeCN in aqueous 10 mM Ammonium Bicarbonate or employing a Waters Xbridge BEH C18, 5 µm, 19x50 mm column using a gradient of MeCN in aqueous 10 mM Ammonium Bicarbonate. Fractions were collected following detection by UV at a single wavelength measured by a variable wavelength detector on a Gilson 215 preparative HPLC or Varian PrepStar preparative HPLC or by mass and UV at a single wavelength measured by a ZQ single quadrupole mass spectrometer, with positive and negative ion electrospray, and a dual wavelength detector on a Waters FractionLynx LCMS.

^{1}H NMR Spectroscopy: ^{1}H NMR spectra were acquired on a Bruker Avance III spectrometer at 400 MHz. Either the central peaks of chloroform-*d*, dimethylsulfoxide-*d*$_6$ or an internal standard of tetramethylsilane were used as references.

Preparation of the Compound of the Invention

Example 1

4-((4-((4-(3-(5-(tert-Butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid



(i) tert-Butyl (4-((2-chloropyridin-4-yl)oxy)naphthalen-1-yl)carbamate

Method 1

A mixture of 4-((2-chloropyridin-4-yl)oxy)naphthalen-1-amine (see, for example, Ito, K. *et al.,* **WO 2010/112936**, 07 Oct 2010; 1000 mg, 3.69 mmol) and di-tert-butyl dicarbonate (750 mg, 3.44 mmol) in t-BuOH (10 mL) was stirred at reflux for 18 h. The mixture was diluted with water (15 mL) and the solid collected by filtration. The solid was triturated in diethyl ether to yield the sub-title compound (1002 mg) as a pale grey solid.

^{1}H NMR (DMSO-d6) 400 MHz, δ: 9.37 (s, 1H), 8.28 (d, 1H), 8.16 (d, 1H), 8.82 (dd, 1H), 7.66 (d, 1H), 7.66-7.54 (m, 2H), 7.40 (d, 1H), 7.03 (d, 1H), 6.91 (dd, 1H), 1.52 (s, 9H).

LCMS m/z 371 $(M+H)^+$ (ES^+); 369 $(M-H)^-$ (ES^-)

Method 2

2-Chloro-4-fluoropyridine (33 mL, 365 mmol) was added to a mixture of tert-butyl (4-hydroxynaphthalen-1-yl)carbamate (85 g, 328 mmol) and Cs_2CO_3 (139 g, 426 mmol) in DMSO (600 mL) and stirred at rt for 24h. Water (1L) was added, the mixture stirred for 1h, then the precipitate filtered off. The reaction was repeated on a further 85 g scale of naphthol. The combined precipitates were washed with water (2 L), ether (4 x 400 mL) and dried under vacuum at 70°C for 72 h to afford the sub-title compound (201.6 g) as a light grey solid.
^{1}H NMR (400 MHz, DMSO-d6) δ 9.38 (s, 1H), 8.28 (d, 1H), 8.16 (d, 1H), 7.82 (d, 1H), 7.67-7.56 (m, 3H), 7.40 (d, 1H), 7.03 (d, 1H), 6.92 (dd, 1H), 1.52 (s, 9H).
LCMS m/z 371 (M+H)$^+$ (ES$^+$); 369 (M-H)$^-$ (ES$^-$)

<u>(ii) Methyl 4-((4-((4-((tert-butoxycarbonyl)amino)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate</u>

<u>Method 1</u>

A suspension of the product from step (i) above (2.0 g, 5.39 mmol), methyl 4-amino-2-methoxybenzoate (1.0 g, 5.52 mmol), BINAP (300 mg, 0.482 mmol) and cesium carbonate (3.5 g, 10.74 mmol) in 1,4-dioxane (30 mL) was degassed with nitrogen for 10 min. Pd_2dba_3 (200 mg, 0.218 mmol) was added and the mixture was heated to 90°C overnight. The mixture was diluted with diethyl ether (60 mL) and filtered. The filtrate was then washed with water (2 x 100 mL), and saturated brine (50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield the crude product as a red foam. The crude product was purified by chromatography on the Companion (80 g column, 20-50% EtOAc in hexane) to afford the sub-title compound (2.34 g) as a yellow foam.
^{1}H NMR (400 MHz, DMSO-d6) δ: 9.38 (s, 1H), 9.36 (s, 1H), 8.18 (d, 1H), 8.14 (d, 1H), 7.83 (d, 1H), 7.54-7.66 (m, 5H), 7.37 (d, 1H), 7.22 (dd, 1H), 6.69 (dd, 1H), 6.15 (d, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 1.53 (s, 9H).
LCMS m/z 516 (M+H)$^+$ (ES$^+$)

<u>Method 2</u>

A mixture of methyl 4-amino-2-methoxybenzoate (10.8 g, 59.6 mmol), the product from step (i) above (20.09 g, 54.2 mmol) and potassium carbonate (15 g, 109 mmol) in DMF (300 mL) was degassed with N_2 for 10 min. BrettPhos G3 precatalyst (1 g, 1.103 mmol) was added and the mixture heated at 85°C for 3 h. The mixture was cooled then partitioned between DCM (500 mL) and water (800 mL). The organic layer was washed with water (500 mL), dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was triturated with ether, filtered and dried to afford the sub-title compound (21.7 g) as a grey solid.
^{1}H NMR (400 MHz, DMSO-d6) δ 9.38 (s, 1H), 9.36 (s, 1H), 8.18 (d, 1H), 8.14 (d, 1H), 7.83 (d, 1H), 7.54-7.66 (m, 5H), 7.38 (d, 1H), 7.22 (dd, 1H), 6.69 (dd, 1H), 6.14 (d, 1H), 3.74 (s, 3H), 3.71 (s, 3H), 1.53 (s, 9H).
LCMS m/z 516 (M+H)$^+$ (ES$^+$)

<u>(iii) Methyl 4-((4-((4-aminonaphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate</u>

TFA (7 mL, 91 mmol) was added to a solution of the product from step (ii) above (2.34 g, 4.08 mmol) in DCM (50 mL) and the reaction stirred for 2 h. The solvents were evaporated and the residue partitioned between sat $NaHCO_3$ soln. (100 mL) and DCM (60 mL). The organics were separated, dried ($MgSO_4$), filtered and the solvent evaporated to afford the sub-title compound (1.5 g) as a pale brown foam.

LCMS m/z 416 $(M+H)^+$ (ES^+)

(iv) Phenyl (5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)carbamate

Phenyl chloroformate (0.750 mL, 5.98 mmol) was added to a stirred solution of N-(3-amino-5-(tert-butyl)-2-methoxyphenyl)methanesulfonamide (see, for example, Cirillo, P. F. *et al*., **WO 2002/083628**, 24 October 2002; 1.5 g, 5.51 mmol) and $NaHCO_3$ (1.0 g, 11.90 mmol) in THF (15 mL) and DCM (15 mL) and the mixture was stirred for 2 h. The mixture was washed with water (20 mL) and the organic layer separated, dried ($MgSO_4$), filtered and evaporated to a brown foam which was stirred in cyclohexane (20 mL) to afford the sub-title compound (2.05 g) as a colourless solid.

LCMS m/z 393 $(M+H)^+$ (ES^+); 391 $(M-H)^-$ (ES^-)

(v) Methyl 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)-naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate

Method 1

Triethylamine (20 µL, 0.143 mmol) was added to a solution of the product from step (iv) above (300 mg, 0.764 mmol) and the product from step (ii) above (300 mg, 0.722 mmol) in iPrOAc (15 mL) at 65°C (block temperature) and the mixture stirred overnight. The reaction was cooled to rt and concentrated in vacuo affording a pale brown foam. The foam was slurried in Et_2O (10 mL) for 2 h and the resulting solid collected by filtration, washing with further portions of Et_2O, affording the sub-title compound (433 mg) as a pale pink solid.

LCMS m/z 358 $(M+2H)^{2+}$ (ES^+)

Method 2

Triethylamine (600 µL, 4.30 mmol) was added to a solution of the product from step (iv) above (9.0 g, 22.93 mmol) and the product from step (iii) above (9.0 g, 21.66 mmol) in iPrOAc (300 mL) at 65°C (block temperature) and the mixture stirred for 24h. The reaction was cooled to room temperature and concentrated in vacuo affording a brown foam. The crude product was purified by chromatography on the Companion (330g column, 1-5% MeOH in DCM) to afford the sub-title compound (13.2 g) as a pale pink solid.

^{1}H NMR (400 MHz, DMSO-d6) δ: 9.40 (s, 1H), 9.35 (s, 1H), 916 (s, 1H), 8.93 (s, 1H), 8.31 (d, 1H), 8.17-8.20 (m, 2H), 8.13 (d, 1H), 7.87 (d, 1H), 7.69-7.73 (m, 1H), 7.60-7.63 (m, 2H), 7.53 (d, 1H), 7.41 (d, 1H), 7.24 (dd, 1H), 7.03 (d, 1H), 6.69 (dd, 1H), 6.17 (d, 1H), 3.81 (s, 3H), 3.74 (s, 3H), 3.71 (s, 3H), 3.10 (s, 3H), 1.27 (s, 9H).

LCMS m/z 714 $(M+H)^+$ (ES^+)

(vi) 4-((4-((4-(3-(5-(tert-Butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid

Method 1

An aqueous solution of lithium hydroxide monohydrate (25 mg, 0.596 mmol) in water (3 mL) was added to a solution of the product from step (v) above (433 mg, 0.540 mmol) in THF (2mL) and methanol (1 mL) and the mixture was stirred at rt overnight. Lithium hydroxide monohydrate (25 mg, 0.596 mmol) was added and stirring was continued for a further 3 days. The mixture was concentrated under reduced pressure to remove THF and methanol then diluted with water (25 mL). A solution of citric acid monohydrate (250 mg, 1.190 mmol) in water (5 mL) was added and the resulting precipitate was collected by filtration to yield the title compound (360 mg) as a pale pink solid.

^{1}H NMR (400 MHz, DMSO-d6) δ 11.96 (br s, 1H), 9.39 (s, 1H), 9.31 (s, 1H), 9.14 (s, 1H), 8.91 (s, 1H), 8.30 (d, 1H), 8.19 (d, 1H), 8.18 (d, 1H), 8.12 (d, 1H), 7.87 (d, 1H), 7.71 (ddd, 1H), 7.63 (d, 1H), 7.61 (ddd, 1H), 7.51 (d, 1H), 7.40 (d, 1H), 7.22 (dd, 1H), 7.02 (d, 1H), 6.68 (dd, 1H), 6.16 (d, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 3.10 (s, 3H), 1.27 (s, 9H). 90% purity

LCMS m/z 700 $(M+H)^{+}$ (ES^{+})

<u>Method 2</u>

To a stirred solution of the product from step (v) above (33.4 g, 45.9 mmol) in THF (300 mL) was added NaOH (6M aq.) (85.0 mL, 510 mmol). MeOH (60 mL) was added and stirring continued for 28h. The reaction was concentrated *in vacuo* affording a yellow solid. The material was suspended in water (200 mL) and acidified with 6M HCl (100 mL) causing a white solid to precipitate. The solid was collected by filtration, washing with water. The resulting solid was dried on the frit for 1 h then further dried at 40°C under vacuum affording the title compound as the hydrochloride salt as a white solid.

^{1}H NMR (of hydrochloride salt; 400 MHz, DMSO-d6) δ: 9.80 (s, 1H), 9.59 (s, 1H), 9.15 (s, 1H), 9.02 (s, 1H), 8.37 (d, 1H), 8.13-8.18 (m, 3H), 7.86 (d, 1H), 7.70-7.74 (m, 1H), 7.62-7.66 (m, 2H), 7.44 (d, 1H), 7.35 (s, 1H), 7.10 (d, 1H), 7.03 (d, 1H), 6.78 (d, 1H), 6.26 (d, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 3.10 (s, 3H), 1.27 (s, 9H).

LCMS m/z 700 $(M+H)^{+}$ (ES^{+})

The hydrochloride salt was loaded in 2.0 g batches, dissolved in THF, onto a pre-conditioned cartridge of SCX resin (20 g of resin, conditioned in MeCN). The resin was washed with MeCN then the product released in 1% NH_3 in MeOH. The NH_3 fractions were combined and concentrated *in vacuo*, affording the title compound (30 g) as the free acid as a pale pink solid.

^{1}H NMR (of free acid; 400 MHz, DMSO-d6) δ: 9.56 (s, 1H), 9.28 (s, 1H), 9.00 (s, 1H), 8.34 (d, 1H), 8.16-8.17 (m, 2H), 8.11 (d, 1H), 7.86 (d, 1H), 7.69-7.71 (m, 1H), 7.57-7.63 (m, 2H), 7.48 (d, 1H), 7.40 (d, 1H), 7.21 (dd, 1H), 7.03 (d, 1H), 6.66 (dd, 1H), 6.16 (d, 1H), 3.81 (s, 3H), 3.73 (s, 3H), 3.09 (s, 3H), 1.27 (s, 9H).

LCMS m/z 700 $(M+H)^{+}$ (ES^{+})

The free acid (1.0 g, 1.386 mmol) was suspended in an aqueous solution of NaOH (0.057 g, 1.414 mmol) in water (25 mL). MeOH (5 mL) was added and the mixture stirred until homogeneity was achieved. The resulting solution was diluted with MeOH (20 mL) and concentrated *in vacuo*, affording a pale grey solid. The material was suspended in MeCN (5 mL), to which water (0.5 mL) was added and the suspension stirred over the weekend. The suspension was filtered and the resulting solid washed with MeCN (2 x 3 mL) and dried under vacuum at 50°C, affording the title compound as the sodium salt (940 mg) as a white solid.
^{1}H NMR (of sodium salt; 400 MHz, DMSO-d6) δ: 9.68 (s, 1H), 9.07 (s, 1H), 9.02 (s, 1H), 8.35 (d, 1H), 8.08-8.13 (m, 2H), 8.02 (d, 1H), 7.85 (d, 1H), 7.64-7.68 (m, 1H), 7.57-7.61 (m, 1H), 7.37-7.43 (m, 2H), 7.30 (s, 1H), 7.11 (dd, 1H), 7.03 (d, 1H), 6.61 (dd, 1H), 6.12 (d, 1H), 3.80 (s, 3H), 3.65 (s, 3H), 2.96 (s, 3H), 1.25 (s, 9H).
LCMS m/z 700 $(M+H)^+$ (ES^+)

Biological Testing: Experimental Methods

Enzyme Binding Assays (Kinomescan)

Kinase enzyme binding activities of the compound disclosed herein may be determined using a proprietary assay which measures active site-directed competition binding to an immobilized ligand (Fabian, M.A. *et al*., *Nature Biotechnol*., 2005, **23**:329-336). These assays may be conducted by DiscoverX (formerly Ambit; San Diego, CA). The percentage inhibition produced by incubation with a test compound may be calculated relative to the non-inhibited control.

Enzyme Inhibition Assays

The enzyme inhibitory activities of the compound disclosed herein are determined by FRET using synthetic peptides labelled with both donor and acceptor fluorophores (Z-LYTE, Invitrogen Ltd., Paisley, UK).

p38 MAPKα Enzyme Inhibition
The following two assay variants can be used for determination of p38 MAPKα inhibition.

Method 1
The inhibitory activities of test compounds against the p38 MAPKα isoform (MAPK14: Invitrogen) are evaluated indirectly by determining the level of activation / phosphorylation of the down-stream molecule, MAPKAP-K2. The p38 MAPKα protein (80 ng/mL, 2.5 µL) is mixed with the test compound (2.5 µL of either 4 µg/mL, 0.4 µg/mL, 0.04 µg/mL or 0.004 µg/mL) for 2 hr at RT. The mix solution (2.5 µL) of the p38α inactive target MAPKAP-K2 (Invitrogen, 600 ng/mL) and FRET peptide (8 µM; a phosphorylation target for MAPKAP-K2) is then added, then the kinase reaction is initiated by adding ATP (40 µM, 2.5µL). The mixture is incubated for 1 hr at RT. Development reagent (protease, 5 µL) is added for 1 hr prior to detection in a fluorescence microplate reader (Varioskan® Flash, ThermoFisher Scientific).

Method 2

This method follows the same steps as Method 1 above, but utilises a higher concentration of the p38 MAPKα protein (2.5 µL of 200 ng/mL protein instead of 2.5 µL of 80 ng/mL protein) for mixing with the test compound (tested at either 1 µg/mL, 0.1 µg/mL, 0.01 µg/mL or 0.001 µg/mL).

p38 MAPKγ Enzyme Inhibition

The inhibitory activities of the compound of the invention against p38MAPKγ (MAPK12: Invitrogen), are evaluated in a similar fashion to that described hereinabove. The enzyme (800 ng/mL, 2.5 µL) is incubated with the test compound (2.5 µL of either 4 µg/mL, 0.4 µg/mL, 0.04 µg/mL, or 0.004 µg/mL) for 2 hr at RT. The FRET peptides (8 µM, 2.5 µL), and appropriate ATP solution (2.5 µL, 400 µM) are then added to the enzymes / compound mixtures and the whole is incubated for 1 hr. Development reagent (protease, 5 µL) is added for 1 hr prior to detection in a fluorescence microplate reader (Varioskan® Flash, Thermo Scientific).

c-Src and Syk Enzyme Inhibition

The inhibitory activities of the compound of the invention against c-Src and Syk enzymes (Invitrogen), are evaluated in a similar fashion to that described hereinabove. The relevant enzyme (3000 ng/mL or 2000 ng/mL respectively, 2.5 µL) is incubated with the test compound (either 1 µg/mL, 0.1 µg/mL, 0.01 µg/mL, or 0.001 µg/mL, 2.5 µL each) for 2 hr at RT. The FRET peptides (8 µM, 2.5 µL), and appropriate ATP solutions (2.5 µL, 800 µM for c-Src, and 60 µM ATP for Syk) are then added to the enzymes / compound mixtures and the mixture incubated for 1 hr. Development reagent (protease, 5 µL) is added for 1 hr prior to detection in a fluorescence microplate reader (Varioskan® Flash, ThermoFisher Scientific).

GSK 3α Enzyme Inhibition

The following two assay variants can be used for determination of GSK 3α inhibition.

<u>Method 1</u>

The inhibitory activities of the compound of the invention against the GSK 3α enzyme isoform (Invitrogen), are evaluated by determining the level of activation / phosphorylation of the target peptide. The GSK3-α protein (500 ng/mL, 2.5 µL) is mixed with the test compound (2.5 µL at either 4 µg/mL, 0.4 µg/mL, 0.04 µg/mL, or 0.004 µg/mL) for 2 hr at RT. The FRET peptide (8 µM, 2.5 µL), which is a phosphorylation target for GSK3α, and ATP (40 µM, 2.5 µL) are then added to the enzyme / compound mixture and the resulting mixture incubated for 1 hr. Development reagent (protease, 5 µL) is added for 1 hr prior to detection in a fluorescence microplate reader (Varioskan® Flash, ThermoFisher Scientific).

In all cases, the site-specific protease cleaves non-phosphorylated peptide only and eliminates the FRET signal. Phosphorylation levels of each reaction are calculated using the ratio of coumarin emission (donor) over fluorescein emission (acceptor), for which high ratios indicate high phosphorylation and low ratios indicate low phosphorylation levels. The percentage inhibition of each reaction is calculated relative to non-inhibited control and the 50% inhibitory concentration (IC_{50} value) is then calculated from the concentration-response curve.

Method 2

This method follows the same steps as Method 1 above, but utilises a shorter period of mixing of the test compound (105 minutes instead of 2 hours) with the GSK3-α protein. In addition, the concentrations of test compound employed are either 10 µg/mL, 1 µg/mL, 0.1 µg/mL, or 0.01 µg/mL

Cellular Assays

The compound of the invention was studied using one or more of the following assays.

(a) *Inhibition of p38 MAPKα and Lck in Jurkat cells*

Jurkat T cells were cultured in starve medium (RPMI 1640 + 5% FBS) for 24 h prior to the experiment. Cells were harvested and resuspended at $10x10^6$ *cells*/mL in starve medium and then plated into round-bottomed 96 well plates at $1x10^6$ *cells*/well. Serial dilutions of test compound were added (1% final DMSO concentration) for 2 h prior to stimulation. Following pre-incubation with compound, cells were stimulated with H_2O_2 (0.05% final) for 5 min. The reaction was stopped by centrifugation at 2000 rpm (3 min, 4 °C), then the supernatant was removed and 100 µL of cold fix/perm solution (BD Fix/Perm kit #554714) added. Plates were incubated for 20 min at 4 °C before centrifugation and washing with supplied 1x wash medium (BD Fix/Perm kit #554714). Cells were stained for either phospho-p38α (T180/182), supplied by Cell Signalling Technology (9211s), or phospho-Lck (Y394), supplied by R&D (MAB7500). Antibodies were diluted to 5 µg/mL (R&D) or 1:200 (Cell Signalling Technology) in wash medium, before being incubated 1 h at 4 °C in the dark. Following 3 repeat washes with ice cold wash buffer, secondary antibody (anti-rabbit-FITC #F1362 or anti-mouse-FITC #F2883, both from Sigma) was added at a dilution of 1:1000 and incubated for 1 h at 4 °C in the dark. Cells were washed 3x times in cold wash buffer then, following a final wash in cold PBS, were resuspended in 150 µL cold PBS. Cells were analysed by flow cytometry using BD Accuri C6.

(aa) *LPS-induced TNFα / IL-8 release in d-U937 cells*

U937 cells, a human monocytic cell line, are differentiated to macrophage-type cells by incubation with phorbol myristate acetate (PMA; 100 ng/mL) for 48 to 72 hr. Cells are pre-incubated with final concentrations of test compound for 2 hr and are then stimulated with 0.1 µg/mL of LPS (from *E. Coli*: 0111:B4, Sigma) for 4 hr. The supernatant is collected for determination of TNFα and IL-8 concentrations by sandwich ELISA (Duo-set, R&D systems). The inhibition of TNFα production is calculated as a percentage of that achieved by 10 µg/mL of **BIRB796** at each concentration of test compound by comparison against vehicle control. The relative 50% effective concentration (REC_{50}) is determined from the resultant concentration-response curve. The inhibition of IL-8 production is calculated at each concentration of test compound by comparison with vehicle control. The 50% inhibitory concentration (IC_{50}) is determined from the resultant concentration-response curve.

(b) *LPS-induced TNFα / IL-8 release in PBMC cells*

Peripheral blood mononuclear cells (PBMCs) from healthy subjects are separated from whole blood using a density gradient (Lymphoprep, Axis-Shield Healthcare). The PBMCs are seeded in 96 well plates and treated with compounds at the desired concentration for 2 hours before addition of 1 ng/mL LPS (Escherichia Coli 0111:B4 from Sigma Aldrich) for 24 hours under normal tissue culture conditions (37°C, 5%CO_2). The supernatant is harvested for determination of IL-8 and TNFα concentrations by sandwich ELISA (Duo-set, R&D systems) and read on the fluorescence microplate reader (Varioskan® Flash, ThermoFisher Scientific). The concentration at 50% inhibition (IC_{50}) of IL-8 and TNFα production is calculated from the dose response curve.

(c) *IL-2 and IFN gamma release in CD3/CD28 stimulated PBMC cells*

PBMCs from healthy subjects are separated from whole blood using a density gradient (Lymphoprep, Axis-Shield Healthcare). Cells are added to a 96 well plate pre-coated with a mixture of CD3/CD28 monoclonal antibodies (0.3 μg/mL eBioscience and 3 μg/mL BD Pharmingen respectively). Compound at the desired concentration is then added to the wells and the plate left for 3 days under normal tissue culture conditions. Supernatants are harvested and IL-2 and IFN gamma release determined by Sandwich ELISA (Duo-set, R&D System). The IC_{50} is determined from the dose response curve.

(d) *IL-1β-induced IL-8 release in HT29 cells*

HT29 cells, a human colon adenocarcinoma cell line, are plated in a 96 well plate (24 hr) and pre-treated with compounds at the desired concentration for 2 hours before addition of 5 ng/mL of IL-1β (Abcam) for 24 hours. Supernatants are harvested for IL-8 quantification by Sandwich ELISA (Duo-set, R&D System). The IC_{50} is determined from the dose response curve.

(e) *LPS-induced IL-8 and TNFα release in primary macrophages*

PBMCs from healthy subjects are separated from whole blood using a density gradient (Lymphoprep, Axis-Shield Healthcare). Cells are incubated for 2hrs and non-adherent cells removed by washing. To differentiate the cells to macrophages, they are incubated with 5 ng/mL of GM-CSF (Peprotech) for 7 days under normal tissue culture conditions. Compounds are then added to the cells at the desired concentration for a 2 hour pre-treatment before stimulation with 10 ng/mL LPS for 24 hours. Supernatants are harvested and IL-8 and TNFα release determined by Sandwich ELISA (Duo-set, R&D System). The IC_{50} is determined from the dose response curve.

(f) *Poly I:C-induced ICAM-1 expression in BEAS2B cells*

Poly I:C is used in these studies as a simple, RNA virus mimic. Poly I:C-Oligofectamine mixture (1 μg/mL Poly I:C, ± 2% Oligofectamine, 25 μL; Invivogen Ltd., San Diego, CA, and Invitrogen, Carlsbad, CA, respectively) is transfected into BEAS2B cells (human bronchial epithelial cells, ATCC). Cells are pre-incubated with final concentrations of test compounds for 2 hr and the level of ICAM1 expression on the cell surface is determined by cell-based ELISA. At a time point 18 hr after poly I:C transfection, cells are fixed with 4% formaldehyde in PBS and then endogenous peroxidase is quenched by the addition of washing buffer (100 μL, 0.05% Tween in PBS: PBS-Tween) containing 0.1% sodium azide and 1% hydrogen peroxide. Cells are washed with wash-buffer (3 x 200 μL) and after blocking the wells with 5%

milk in PBS-Tween (100 µL) for 1 hr, the cells are incubated with anti-human ICAM-1 antibody (50 µL; Cell Signalling Technology, Danvers, MA) in 1% BSA PBS overnight at 4°C.

The cells are washed with PBS-Tween (3 x 200 µL) and incubated with the secondary antibody (100 µL; HRP-conjugated anti-rabbit IgG, Dako Ltd., Glostrup, Denmark). The cells are then incubated with substrate (50 µL) for 2-20min, followed by the addition of stop solution (50 µL, 1N H_2SO_4). The ICAM-1 signal is detected by reading the absorbance at 450 nm against a reference wavelength of 655 nm using a spectrophotometer. The cells are then washed with PBS-Tween (3 x 200 µL) and total cell numbers in each well are determined by reading absorbance at 595 nm after Crystal Violet staining (50 µL of a 2% solution in PBS) and elution by 1% SDS solution (100 µL) in distilled water. The measured OD 450-655 readings are corrected for cell number by dividing with the OD595 reading in each well. The inhibition of ICAM-1 expression is calculated at each concentration of test compound by comparison with vehicle control. The 50% inhibitory concentration (IC_{50}) is determined from the resultant concentration-response curve.

(g) *Cell mitosis assay*

Peripheral blood mononucleocytes (PBMCs) from healthy subjects are separated from whole blood (Quintiles, London, UK) using a density gradient (Histopaque®-1077, Sigma-Aldrich, Poole, UK). The PBMCs (3 million cells per sample) are subsequently treated with 2% PHA (phytohaemagglutinin, Sigma-Aldrich, Poole, UK) for 48 hr, followed by a 20 hr exposure to varying concentrations of test compounds. At 2 hr before collection, PBMCs are treated with demecolcine (0.1 µg/mL; Invitrogen, Paisley, UK) to arrest cells in metaphase. To observe mitotic cells, PBMCs are permeabilised and fixed by adding Intraprep (50 µL; Beckman Coulter, France), and stained with anti-phospho-histone 3 (0.26 ng/L; #9701; Cell Signalling, Danvers, MA) and propidium iodide (1 mg/mL; Sigma-Aldrich, Poole, UK) as previously described (Muehlbauer P.A. and Schuler M.J., *Mutation Research*, 2003, **537**:117-130). Fluorescence is observed using an ATTUNE flow cytometer (Invitrogen, Paisley, UK), gating for lymphocytes. The percentage inhibition of mitosis is calculated for each treatment relative to vehicle (0.5% DMSO) treatment.

(h) *Rhinovirus-induced IL-8 release and ICAM-1 expression*

Human rhinovirus RV16 is obtained from the American Type Culture Collection (Manassas, VA). Viral stocks are generated by infecting HeLa cells with HRV until 80% of the cells are cytopathic.

BEAS2B cells are infected with HRV at an MOI of 5 and incubated for 2 hr at 33°C with gentle shaking to promote absorption. The cells are then washed with PBS, fresh media added and the cells are incubated for a further 72 hr. The supernatant is collected for assay of IL-8 concentrations using a Duoset ELISA development kit (R&D systems, Minneapolis, MN).

The level of ICAM-1 expressing cell surface is determined by cell-based ELISA. At 72 hr after infection, cells are fixed with 4% formaldehyde in PBS. After quenching endogenous peroxidase by adding 0.1% sodium azide and 1% hydrogen peroxide, wells are washed with wash-buffer (0.05% Tween in PBS: PBS-Tween). After blocking well with 5% milk in PBS-Tween for 1 hr, the cells are incubated with anti-human ICAM-1 antibody in 5% BSA PBS-Tween (1:500) overnight. Wells are washed with PBS-Tween and incubated with the secondary antibody (HRP-conjugated anti-rabbit IgG, Dako Ltd.). The ICAM-1 signal is detected by adding substrate and reading at 450 nm with a reference wavelength of 655 nm using a spectrophotometer. The wells are then washed with PBS-Tween and total cell numbers in each well are determined by reading absorbance at 595 nm after Crystal Violet staining and elution with 1% SDS solution. The measured $OD_{450\text{-}655}$ readings are corrected for cell number by dividing with the OD_{595} reading in each well. Compounds are added 2 hr before HRV infection and 2 hr after infection when non-infected HRV is washed out.

(i) *Assessment of HRV16 induced Cytopathic Effect (CPE) in MRC5 cells*
MRC5 cells are infected with HRV16 at an MOI of 1 in DMEM containing 5% FCS and 1.5 mM $MgCl_2$, followed by incubation for 1 hr at 33ºC to promote adsorption. The supernatants are aspirated, and then fresh media added followed by incubation for 4 days. Where appropriate, cells are pre-incubated with compound or DMSO for 2 hr, and the compounds and DMSO added again after washout of the virus.

Supernatants are aspirated and incubated with methylene blue solution (100 µL, 2% formaldehyde, 10% methanol and 0.175% Methylene Blue) for 2 hr at RT. After washing, 1% SDS in distilled water (100 µL) is added to each well, and the plates are shaken lightly for 1-2 hr prior to reading the absorbance at 660 nm. The percentage inhibition for each well is calculated. The IC_{50} value is calculated from the concentration-response curve generated by the serial dilutions of the test compounds.

(j) *In vitro RSV virus load in primary bronchial epithelial cells*
Normal human bronchial epithelial cells (NHBEC) grown in 96 well plates are infected with RSV A2 (Strain A2, HPA, Salisbury, UK) at a MOI of 0.001 in the LHC8 Media:RPMI-1640 (50:50) containing 15 mM magnesium chloride and incubated for 1 hr at 37°C for adsorption. The cells are washed with PBS (3 x 200 µL), then fresh media (200 µL) is added and incubation continued for 4 days. Where appropriate, cells are pre-incubated with the compound or DMSO for 2 hr, and then added again after washout of the virus.

The cells are fixed with 4% formaldehyde in PBS solution (50 µL) for 20 min, washed with WB (3 x 200 µL) (washing buffer, PBS including 0.5% BSA and 0.05% Tween-20) and incubated with blocking solution (5% condensed milk in PBS) for 1 hr. Cells are then washed with WB (3 x 200 µL) and incubated for 1 hr at RT with anti-RSV (2F7) F-fusion protein antibody (40 µL; mouse monoclonal, lot 798760, Cat. No.ab43812, Abcam) in 5% BSA in PBS-tween. After washing, cells are incubated with an HRP-conjugated secondary antibody solution (50 µL) in 5% BSA in PBS-Tween (lot 00053170, Cat.No. P0447, Dako) and then TMB substrate added (50 µL; substrate reagent pack, lot 269472, Cat. No. DY999, R&D Systems, Inc.). This reaction is stopped by the addition of 2N H_2SO_4 (50 µL) and the resultant signal is determined colourimetrically (OD: 450 nm with a reference wavelength of 655 nm) in a microplate reader (Varioskan® Flash, ThermoFisher Scientific).

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Cells are then washed and a 2.5% crystal violet solution (50 µL; lot 8656, Cat. No. PL7000, Pro-Lab Diagnostics) is applied for 30 min. After washing with WB, 1% SDS in distilled water (100 µL) is added to each well, and plates are shaken lightly on the shaker for 1 hr prior to reading the absorbance at 595 nm. The measured $OD_{450-655}$ readings are corrected to the cell number by dividing the $OD_{450-655}$ by the OD_{595} readings. The percentage inhibition for each well is calculated and the IC_{50} value is calculated from the concentration-response curve generated from the serial dilutions of compound.

(k) _Cell viability assay: MTT assay_

Differentiated U937 cells are pre-incubated with each test compound (final concentration 1 µg/mL or 10 µg/mL in 200 µL media indicated below) under two protocols: the first for 4 hr in 5% FCS RPMI1640 media and the second in 10% FCS RPMI1640 media for 24 h. The supernatant is replaced with new media (200 µL) and MTT stock solution (10 µL, 5 mg/mL) is added to each well. After incubation for 1 hr the media are removed, DMSO (200 µL) is added to each well and the plates are shaken lightly for 1 hr prior to reading the absorbance at 550 nm. The percentage loss of cell viability is calculated for each well relative to vehicle (0.5% DMSO) treatment. Consequently an apparent increase in cell viability for drug treatment relative to vehicle is tabulated as a negative percentage.

(l) _Human biopsy assay_

Intestinal mucosa biopsies are obtained from the inflamed regions of the colons of IBD patients. The biopsy material is cut into small pieces (2–3 mm) and placed on steel grids in an organ culture chamber at 37°C in a 5% CO_2/95% O_2 atmosphere in serum-free media. DMSO control or test compounds at the desired concentration are added to the tissue and incubated for 24 hr in the organ culture chamber. The supernatant is harvested for determination of IL-6, IL-8, IL-1β and TNFα levels by R&D ELISA. Percentage inhibition of cytokine release by the test compounds is calculated relative to the cytokine release determined for the DMSO control (100%).

(m) _Accumulation of β catenin in d-U937 cells_

U937 cells, a human monocytic cell line, are differentiated into macrophage-type cells by incubation with PMA (100 ng/mL) for between 48 to 72 hr. The cells are then incubated with either final concentrations of test compound or vehicle for 18 hr. The induction of β-catenin by the test compounds is stopped by replacing the media with 4% formaldehyde solution. Endogenous peroxide activity is neutralised by incubating with quenching buffer (100 µL, 0.1% sodium azide, 1% H_2O_2 in PBS with 0.05% Tween-20) for 20 min. The cells are washed with washing buffer (200 µL; PBS containing 0.05% Tween-20) and incubated with blocking solution (200 µL; 5% milk in PBS) for 1 hr, re-washed with washing buffer (200 µL) and then incubated overnight with anti-β-catenin antibody solution (50 µL) in 1% BSA/PBS (BD, Oxford, UK).

After washing with washing buffer (3 x 200 µL; PBS containing 0.05% Tween-20), cells are incubated with a HRP-conjugated secondary antibody solution (100 µL) in 1% BSA/PBS (Dako, Cambridge, UK) and the resultant signal is determined colourimetrically (OD: 450 nm

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with a reference wavelength of 655 nm) using TMB substrate (50 µL; R&D Systems, Abingdon, UK). This reaction is stopped by addition of 1N H_2SO_4 solution (50 µL). Cells are then washed with washing buffer and 2% crystal violet solution (50 µL) is applied for 30 min. After washing with washing buffer (3 x 200 µL), 1% SDS (100 µL) is added to each well and the plates are shaken lightly for 1 hr prior to measuring the absorbance at 595 nm (Varioskan® Flash, Thermo-Fisher Scientific).

The measured $OD_{450\text{-}655}$ readings are corrected for cell number by dividing the $OD_{450\text{-}655}$ by the OD_{595} readings. The percentage induction for each well is calculated relative to vehicle, and the ratio of induction normalised in comparison with the induction produced by a standard control comprising the **Reference compound** *N*-(4-(4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy)pyridin-2-yl)-2-methoxyacetamide (1 µg/mL), which is defined as unity.

(n) *T cell proliferation*

PBMCs from healthy subjects are separated from whole blood using a density gradient (Lymphoprep, Axis-Shield Healthcare). The lymphocyte fraction is first enriched for CD4+ T cells by negative magnetic cell sorting as per the manufacturer's instructions (Miltenyi Biotec 130-091-155). Naïve CD4+ T cells are then separated using positive magnetic selection of CD45RA+ cells using microbeads as per the manufacturer's instructions (130-045-901). Cells are plated at $2x10^5$ cells per well in 100 µL RPMI/10%FBS on 96 well flat bottomed plate (Corning Costar). 25 µL of test compound are diluted to the appropriate concentration (8x final concentration) in normal medium and added to duplicate wells on the plate to achieve a dose response range of 0.03 ng/mL – 250 ng/mL. DMSO is added as a negative control. Plates are allowed to pre-incubate for 2 hours before stimulation with 1 µg/mL anti-CD3 (OKT3; eBioscience). After 72 h, the medium in each well is replaced with 150 µL of fresh medium containing 10 µM BrdU (Roche). After 16 h, the supernatant is removed, the plate is dried and the cells fixed by adding 100 µL of fix/denature solution to each well for 20 min as per the manufacturer's instructions (Roche). Plates are washed once with PBS before addition of the anti-BrdU detection antibody and incubated for 90mins at room temperature. Plates are then washed gently 3x with the wash buffer supplied and developed by addition of 100 µL of substrate solution. The reaction is stopped by addition of 50 µL of 1 M H_2SO_4 and read for absorbance at 450 nm on a plate reader (Varioskan® Flash, ThermoFisher Scientific). The IC_{50} is determined from the dose response curve.

(o) *IL-2 and IFNγ release in CD3/CD28 stimulated LPMC cells from IBD patients*

Lamina propria mononuclear cells (LPMCs) are isolated and purified from inflamed IBD mucosa of surgical specimens or from normal mucosa of surgical specimens as follows:

The mucosa is removed from the deeper layers of the surgical specimens with a scalpel and cut in fragments of size 3-4mm. The epithelium is removed by washing the tissue fragments three times with 1 mM EDTA (Sigma-Aldrich, Poole, UK) in HBSS (Sigma-Aldrich) with agitation using a magnetic stirrer, discarding the supernatant after each wash. The sample is subsequently treated with type 1A collagenase (1 mg/mL; Sigma-Aldrich) for 1 h with stirring at 37°C. The resulting cell suspension is then filtered using a 100 µm cell strainer, washed twice, resuspended in RPMI-1640 medium (Sigma-Aldrich) containing 10% fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin, and used for cell culture.

Freshly isolated LPMCs (2x10^5 cells/well) are stimulated with 1 μg/mL α-CD3/α-CD28 for 48 h in the presence of either DMSO control or appropriate concentrations of compound. After 48 h, the supernatant is removed and assayed for the presence of TNFα and IFNγ by R&D ELISA. Percentage inhibition of cytokine release by the test compounds is calculated relative to the cytokine release determined for the DMSO control (100%).

(p) *Inhibition of cytokine release from myofibroblasts isolated from IBD patients*

Myofibroblasts from inflamed IBD mucosa are isolated as follows:

The mucosa is dissected and discarded and 1 mm-sized mucosal samples are cultured at 37°C in a humidified CO_2 incubator in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 20% FBS, 1% non-essential amino acids (Invitrogen, Paisley, UK), 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL gentamycin, and 1 μg/mL amphotericin (Sigma-Aldrich). Established colonies of myofibroblasts are seeded into 25-cm^2 culture flasks and cultured in DMEM supplemented with 20% FBS and antibiotics to at least passage 4 to provide a sufficient quantity for use in stimulation experiments.

Subconfluent monolayers of myofibroblasts, seeded in 12-well plates at 3x10^5 cells per well, are starved in serum-free medium for 24 h at 37°C, 5%CO_2, before being cultured for 24 h in the presence of either DMSO control or appropriate concentrations of compound. After 24 h, the supernatant is removed and assayed for the presence of IL-8 and IL-6 by R&D ELISA. Percentage inhibition of cytokine release by the test compounds is calculated relative to the cytokine release determined for the DMSO control (100%).

(q) *Human neutrophil degranulation*

Neutrophils are isolated from human peripheral blood as follows:

Blood is collected by venepuncture and anti-coagulated by addition of 1:1 EDTA:sterile phosphate buffered saline (PBS, no Ca+/Mg+). Dextran (3% w/v) is added (1 part dextran solution to 4 parts blood) and the blood allowed to stand for approximately 20 minutes at rt. The supernatant is carefully layered on a density gradient (Lymphoprep, Axis-Shield Healthcare) and centrifuged (15 mins, 2000rpm, no brake). The supernatant is aspirated off and the cell pellet is re-suspended in sterile saline (0.2%) for no longer than 60 seconds (to lyse contaminating red blood cells). 10 times volume of PBS is then added and the cells centrifuged (5 mins, 1200 rpm). Cells are re-suspended in HBSS+ (Hanks buffered salt solution (without phenol red) containing cytochalasin B (5 μg/mL) and 1 mM $CaCl_2$) to achieve 5 x 10^6 cells/mL.

5 x 10^4 cells are added to each well of a V-bottom 96 well plate and are incubated (30 mins, 37°C) with the appropriate concentration of test compound (0.3 – 1000 ng/mL) or vehicle (DMSO, 0.5% final conc). Degranulation is stimulated by addition of fMLP (final concentration 1 μM). After a further incubation (30 mins, 37 °C), the cells are removed by centrifugation (5 mins, 1500 rpm) and the supernatants transferred to a flat bottom 96 well plate. An equal

volume of tetramethylbenzidine (TMB) is added and, after 10 mins, the reaction terminated by addition of an equal volume of sulphuric acid (0.5 M) and absorbance read at 450 nm (background at 655nm subtracted). The 50% inhibitory concentration (IC_{50}) is determined from the resultant concentration-response curve.

(r) *Cell cytotoxicity assay*

1 × 10^5 Jurkat cells (immortalised human T lymphocytes) are added to the appropriate number of wells of a 96 well plate in 100 µL of media (RPMI supplemented with 10% foetal bovine serum). 1 µL of DMSO control (final concentration 1.0% v/v) or test compound (final concentration 20, 5 or 1 µg/mL) is added to the wells and incubated at 37°C, 5% CO_2. After 24 hours, the plate is centrifuged at 1200 rpm for 3 minutes and the supernatant discarded. Cells are then resuspended in 150 µL (final concentration 7.5 µg/mL) of propidium iodide (PI) in PBS and incubated at 37°C, 5% CO_2 for 15 minutes. After 15 minutes, cells are analysed by flow cytometry (BD accuri) using the FL3 window. The % viability is calculated as the % of cells that are PI negative in the test wells normalised to the DMSO control.

In Vivo Screening: Pharmacodynamics and Anti-inflammatory Activity

(i) *LPS-induced neutrophil accumulation in mice*

Non-fasted Balb/c mice are dosed by the intra tracheal route with either vehicle, or the test substance at the indicated times (within the range 2-8 hr) before stimulation of the inflammatory response by application of an LPS challenge. At T = 0, mice are placed into an exposure chamber and exposed to LPS (7.0 mL, 0.5 mg/mL solution in PBS) for 30 min. After a further 8 hr, the animals are anesthetized, their tracheas cannulated and BALF extracted by infusing and then withdrawing from their lungs 1.0 mL of PBS *via* the tracheal catheter. Total and differential white cell counts in the BALF samples are measured using a Neubauer haemocytometer. Cytospin smears of the BALF samples are prepared by centrifugation at 200 rpm for 5 min at RT and stained using a DiffQuik stain system (Dade Behring). Cells are counted using oil immersion microscopy. Data for neutrophil numbers in BAL are represented as mean ± S.E.M. (standard error of the mean). The percentage inhibition of neutrophil accumulation is calculated for each treatment relative to vehicle treatment.

(ii) *Cigarette smoke model*

A/J mice (males, 5 weeks old) are exposed to cigarette smoke (4% cigarette smoke, diluted with air) for 30 min/day for 11 days using a Tobacco Smoke Inhalation Experiment System for small animals (Model SIS-CS; Sibata Scientific Technology, Tokyo, Japan). Test substances are administered intra-nasally (35 µL of solution in 50% DMSO/PBS) once daily for 3 days after the final cigarette smoke exposure. At 12 hr after the last dosing, each of the animals is anesthetized, the trachea cannulated and bronchoalveolar lavage fluid (BALF) is collected. The numbers of alveolar macrophages and neutrophils are determined by FACS analysis (EPICS® ALTRA II, Beckman Coulter, Inc., Fullerton, CA, USA) using anti-mouse MOMA2 antibody (macrophage) or anti-mouse 7/4 antibody (neutrophil).

(iii) *DSS-induced colitis in mice*

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Non-fasted, 10-12 week old, male BDF1 mice are dosed by oral gavage twice daily with either vehicle, reference item (5-ASA) or test compound one day before (Day -1) stimulation of the inflammatory response by treatment with dextran sodium sulphate (DSS). On Day 0 of the study, DSS (5% w/v) is administered in the drinking water followed by BID dosing of the vehicle (5 mL/kg), reference (100 mg/kg) or test compound (5 mg/kg) for 7 days. The drinking water with DSS is replenished every 3 days. During the study, animals are weighed every day and stool observations are made and recorded as a score, based on stool consistency. At the time of sacrifice on Day +6, the large intestine is removed and the length and weight are recorded. Sections of the colon are taken for either MPO analysis, to determine neutrophil infiltration, or for histopathology scoring to determine disease severity.

(iv) _TNBS-induced colitis in mice_

Non-fasted, 10-12 week old, male BDF1 mice are dosed by oral gavage twice daily with either vehicle (5 mL/kg), reference item (Budesonide 2.5 mg/kg) or test compound (1 or 5 mg/kg) one day before (Day -1) stimulation of the inflammatory response by treatment with 2,4,6-trinitrobenzenesulphonic acid (TNBS) (15 mg/mL in 50% ethanol / 50% saline). On Day 0 of the study TNBS (200 µL) is administered intra-colonically *via* a plastic catheter with BID dosing of the vehicle, reference or test compound continuing for 2 or 4 days. During the study, animals are weighed every day and stool observations are made and recorded as a score, based on stool consistency. At the time of sacrifice on Day 2 (or Day 4), the large intestine is removed and the length and weight recorded. Sections of the colon are taken for histopathology scoring to determine disease severity.

(v) _Adoptive transfer in mice_

On Study day 0, female Balb/C mice are terminated and spleens obtained for CD45RB^{high} cell isolation (Using SCID IBD cell Separation protocol). Approximately 4X10^{5} cells/mL CD45RB^{high} cells are then injected intraperitoneally (100 µL/mouse) into female SCID animals. On study day 14, mice are weighed and randomized into treatment groups based on body weight. On Day 14, compounds are administered BID, via oral gavage, in a dose volume of 5 mL/kg. Treatment continues until study day 42, at which point the animals are necropsied 4 hours after the morning administration. The colon length and weight are recorded and used as a secondary endpoint in the study as a measurement of colon oedema. The colon is then divided into six cross-sections, four of which are used for histopathology scoring (primary endpoint) and two are homogenised for cytokine analysis. Data shown is the % inhibition of the induction window between naïve animals and vehicle animals, where higher inhibition implies closer to the non-diseased, naïve, phenotype.

(vi) _Endotoxin-induced uveitis in rats_

Male, Lewis rats (6-8 weeks old, Charles River UK Limited) are housed in cages of 3 at 19-21°C with a 12 h light/dark cycle (07:00/19:00) and fed a standard diet of rodent chow and water ad libitum. Non-fasted rats are weighed, individually identified on the tail with a permanent marker, and receive a single intravitreal administration into the right vitreous humor (5 µL dose volume) of 100 ng/animal of LPS (Escherichia coli 0111:B4 prepared in PBS, Sigma Aldrich, UK) using a 32-gauge needle. Untreated rats are injected with PBS. Test compound

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or vehicle (4% polyoxyl 40 stearate, 4% mannitol in PBS (pH 7.4)) are administered by the topical route onto the right eye (10 µL) of animals 1 hour prior to LPS, at the time of LPS administration, and 1, 2 and 4 hours post LPS administration. Before administration, the solution to be administered is sonicated to ensure a clear solution. 6 hours after LPS dosing, animals are euthanized by overdose with pentobarbitone (via cardiac puncture). Immediately after euthanasia, 10 µL of aqueous humor is collected from the right eye of the rats by puncture of the anterior chamber using a 32 gauge needle under a surgical microscope. The aqueous humor is diluted in 20 µL of PBS and total cell counts are measured immediately using a Countess automated cell counter (Invitrogen). Following collection of the aqueous humour, the right eye of each animal is enucleated and dissected into front (anterior) and back (posterior) sections around the lens. Each section is weighed and homogenised in 500 µL of sterile phosphate buffered saline followed by 20 minutes centrifugation at 12000 rpm at 4°C. The resulting supernatant is divided into 3 aliquots and stored at -80°C until subsequent cytokine analysis by R&D DuoSet ELISA.

Summary of In Vitro and In Vivo Screening Results

Test Compound Example No.	**Dissociation Constant (nM)**		
	Lck	**p38 MAPKα**	**Syk**
Example 1	4.2	2.8	7.1

Table 1: Dissociation constants for selected kinases determined by LeadHunter Discover Services (DiscoveRx Corporation, Fremont, CA), using the KINOMEscan™ technology.

Studies conducted by LeadHunter Discover Services (DiscoveRx Corporation, Fremont, CA) using the KINOMEscan™ technology determined that compound of Example 1 did not have any significant effect on the binding of the kinases B-Raf and B-Raf (V600E) to their standard ligands. Moreover, this compound showed improved selectivity compared to the **Reference Compound** *N*-(4-(4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy)pyridin-2-yl)-2-methoxyacetamide (WO 2010/112936), as evidenced by lower selectivity scores (Table 1a).

	KinomeScan Selectivity Scores / number of individual kinase hits					
	50 nM			500 nM		
Compound	S(35)	S(10)	S(1)	S(35)	S(10)	S(1)
Reference Compound	0.174 / 67	0.083 / 32	0.018 / 7	0.370 / 143	0.272 / 105	0.117 / 45
Ex. 1	0.186 / 75	0.072 / 29	0.005 / 2	0.347 / 140	0.251 / 101	0.089 / 36

Table 1a: KinomeScan Selectivity score data at 50 and 500 nM; S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested); S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested); S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested)

Test Compound Example No.	IC50 Values for Enzyme Inhibition (nM)			
	p38 MAPKα	c-Src	Syk	GSK3α
1	11	14	5	115

Table 1b. Results from *in vitro* p38 MAPKα (Method 2), c-Src, Syk and GSK3α (Method 2) inhibition assays

Test Compound Example No.	PBMCs		
	IL-8	IL-2	IFNγ
1	6.1	1125.5	9.5

Table 2. Inhibition of cytokine release in stimulated cells (assays (b), (c) and (d) above).

As illustrated in Table 3 below, the compound of the example of the present invention is markedly less active than the **Reference Compound** (*N*-(4-(4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy)pyridin-2-yl)-2-methoxyacetamide; WO 2010/112936) in assay (g) above, which measures impact on cell division (mitosis) in PBMCs. Similarly, the compound of the example of the present invention is substantially less cytotoxic than the **Reference Compound**, displaying enhanced viabilities in cell cytotoxicity assay (r) above (Table 3).

Test compound	% Inhibition of mitosis at 5 μg/mL	% Viability at 1 μg/mL	% Viability at 5 μg/mL	% Viability at 20 μg/mL
Reference compound	87.8[a]	23.5	18.9	17.3
1	2.4	94.6	96.1	95.8

Table 3: Effect of the compound of the invention on cell division in PBMCs (NT = not tested) and on Jurkat cell viability

[a] See, for example, the value reported in WO 2013/050757.

As illustrated in Table 4 below, the compound of Example 1 significantly and dose-dependently reduced cellular infiltration, as revealed by lowered cell counts, and cytokine IL-1β levels in both the anterior and posterior segments of the eyes of rats treated with intravitreal endotoxin LPS (see assay (vi) above).

Treatment	n	IL-1β (pg/mL) Anterior tissue	IL-1β (pg/mL) Posterior tissue	Cell counts ($x10^5$/mL)
Non-diseased	5	14.1 ± 6.3	30.8 ± 11.3	1.8 ± 0.2
Vehicle control	8	1636.6 ± 145.1	877.3 ± 115.6	69.9 ± 5.4
Example 1 (1 mg/mL)	8	367.3 ± 100.4	188.1 ± 54.7	21.9 ± 5.0
Example 1 (0.1 mg/mL)	8	791.2 ± 131.9	327.4 ± 61.4	30.4 ± 6.7
Example 1 (0.01 mg/mL)	8	980.0 ± 110.8	740.5 ± 56.2	43.5 ± 6.3
Example 1 (0.001 mg/mL)	8	1558.1 ± 145.7	867.9 ± 120.8	63.6 ± 7.0

Table 4: Dose-dependent effect of the compound of Example 1 on IL-1β levels and cell counts in the eyes of LPS-stimulated rats. Data are reported as means ± SEM.

Summary of Additional Studies

Determination of Solubilities in Fasted-State Simulated Colonic Fluid (FaSSCoF)

The solubility of the compound of the invention in FaSSCoF at pH 6.5 is determined using a modification of a previously-reported procedure (Vertzoni, M., et al. *Pharm. Res.* **2010**, *27*, 2187–2196). In place of the bile salt extract employed in the original procedure (which extract is no longer available), the modified procedure uses a mixture of sodium taurochlorate (0.15 g), glycocholic acid (0.15 g), ursodeoxycholic acid (0.05 g), cholic acid (0.05 g), and glycodeoxycholic acid (0.05 g). These five bile acids are ground together with a mortar and pestle to produce a fine white powder that is incorporated into the FaSSCoF, as outlined below.

FaSSCoF medium: Tris(hydroxymethyl)aminomethane (Tris; 0.275 g) and maleic acid (0.44 g) are dissolved in water (35 mL) to give a solution whose pH is adjusted to 6.5 by treatment with 0.5M NaOH (ca. 12 mL). The solution is then made up to 50 mL with water. A portion of this Tris/maleate buffer solution (ca. 25 mL) is added to a 0.5 L round-bottomed flask, before being treated with 0.00565 g of the bile acid mixture described above. Solutions of phosphatidylcholine (0.0111 g) in DCM (0.15 mL) and palmitic acid (0.0013 g) in DCM (0.15 mL) are added, then the organic solvent is evaporated off under reduced pressure at 40°C until a clear solution, with no perceptible DCM odour, is achieved. The volume of the evaporated solution is adjusted to 50 mL by addition of the remainder of Tris/maleate buffer, then BSA (0.115 g) is added, before being dissolved by gentle agitation.

Solubility Determination: Test compounds are suspended in the pH 6.5 FaSSCoF medium to give a maximum final concentration of 2–10 mg/mL. The suspensions are equilibrated at 25°C for 24 h, before being filtered through a glass fibre C filter. The filtrates are then diluted as appropriate for injection and quantification by HPLC with reference to a standard. Different volumes of the standard, diluted and undiluted sample solutions are injected and the solubilities are calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

FaSSCoF solubilities are shown in Table 5 below, which reveals that the compound of the Example exhibited solubilities in the FaSSCoF medium at pH 6.5 of in excess of 0.01 mg/mL. For the compound of the Example of the present invention, pH 6.5 FaSSCoF solubilities were superior to those of Reference Compound A, 3-((4-((4-(3-(3-(tert-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)pyrimidin-2-yl)amino)-5-ethynyl-N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzamide {Fyfe, M. C. T., WO 2014/140582}.

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Test Compound Example No.	pH 6.5 FaSSCoF Solubility (mg/mL)			
	Run 1	**Run 2**	**Run 3**	**Run 4**
Reference Compound A	<0.001	<0.001		
1	0.016	0.018	0.010	0.011
1 (sodium salt)	0.031	0.032	0.020	0.025
1 (hydrochloride salt)	0.016	0.015	–	–

Table 5: Solubilities measured for the compound of the Example of the present invention in FaSSCoF at pH 6.5.

Determination of Pharmacokinetic Parameters

Studies were conducted by Sai Life Sciences (Hinjewadi, Pune, India) to investigate the systemic pharmacokinetics and total colon tissue distribution of the compound of the invention. In particular, pharmacokinetic studies were carried out in male C57BL/6 mice following a single oral administration of the compound.

The data catalogued in Table 6 reveal that the compound of the invention achieves substantial colonic concentrations, while, in contrast, systemic plasma or blood exposures are very low or negligible.

Cpd. Ex. No.		**Mx**	**Time (h)**							
D	**M**		**0.5**	**1**	**2**	**4**	**6**	**8**	**12**	**24**
1	1	Pl	44.9	49.2	13.0	5.2	1.9	0.6	5.3	0.0
(Na salt)		TC	4.3	9.3	52.4	10,403	4,049	12,898	535	41.4

Table 6: Mean plasma or blood concentrations (ng/mL) or total colon levels (ng/g) obtained following oral administration of the compound of the invention to mice at 5 mg/kg. Vehicle = 0.1% Tween 80 in 0.5% methylcellulose solution prepared in water.

Key to Table 6

D = compound administered (dosed)
M = compound measured
Mx = matrix
Pl = plasma
Bd = blood
TC = total colon

hERG Inhibition Studies

The compound of the invention was tested for inhibition of the human ether a go-go (hERG) channel using IonWorks™ patch clamp electrophysiology at Essen Bioscience (Welwyn Garden City, England).

Example	IC_{50} (μM)	% Inhibition at Top Concentration	Top Concentration
1	>3.3	–4	3 μM

Table 7: hERG inhibition data for the compound of the invention

Acute Eye Irritation/Corrosion Study

The objective of the acute eye irritation/corrosion study was to assess the possible irritation or corrosion potential of the compound of Example 1 at two selected dose levels (0.1 and 1.0 mg/mL), in comparison to vehicle (4% w/v polyoxyethylene 40 stearate/4% w/v mannitol/phosphate buffer (pH 7.4) solution), after one treatment day (Phase 1) or three consecutive treatment days (Phase 2) with four daily administrations (4-hours apart) by the ocular route (bi-lateral instillations of 40 µL/eye/instillation) in the eyes of albino New Zealand White rabbits (13–15 weeks at initiation of dosing; 2 males and 2 females per dose group).

During the study, there were no unscheduled deaths, nor test item-related clinical signs. Furthermore, there were no effects on body weight, nor on food consumption.

In Phase 1, ocular reactions were limited mainly to conjunctival redness (grade 1 or 2) and occasionally to chemosis (grade 1) and discharge (grade 1), in all groups, after instillation of the vehicle or of the test item, Example 1, formulation at any dose levels. These scores were slight to moderate. There were no differences in the frequency, severity and incidence between the Example 1-treated animals and the vehicle controls. The conjunctival redness was the most frequent reaction, and was yet present (at grade 1) before the start of dosing. This local reaction is known to occur spontaneously in the albino rabbit during ocular studies and is related to the numerous ocular examinations undergone on the animals. Chemosis and discharge were sporadically observed in all groups after the first instillation and over the 3-day observation period thereafter. In addition, congestion of the iris was occasionally and unilaterally observed in the eyes of the two high dose group rabbits (1 mg/mL) and one vehicle group female. The Draize examination confirmed the integrity of the cornea after a single treatment day and the photomotor reflex was normal for all animals on all occasions. In summary, the local tolerability of the formulations was thus considered to be acceptable after a single dosing day. Similar local reactions were observed after instillation of the vehicle or formulations containing the compound of Example 1, indicative of a moderate vehicle-related effect on the ocular tolerance.

In Phase 2, the main ocular reactions were limited to conjunctival redness (grade 1) in all groups after instillation of the vehicle or of the test item formulations at any dose levels. This score was slight and was noted without any meaningful difference in the incidence and frequency between groups throughout the 3-day treatment period. The severity was occasionally higher (grade 2) in the vehicle group than in the test item-treated groups. This conjunctival redness was persistent and was still observed before the first instillation on the following day. In addition, congestion of the iris was occasionally observed in the eyes of the two high dose group rabbits and one vehicle group female. No discharge was observed in any animals at all occasions. The Draize examination confirmed the integrity of the cornea during the 3-day treatment period. The photomotor reflex was normal for all animals on all occasions. In summary, the local tolerability of the formulations was thus considered to be acceptable without any aggravation for the 3 treatment days. Similar local reactions were observed after instillation of the vehicle alone or formulations containing the compound of Example 1, indicative of the moderate vehicle-related effect on the ocular tolerance.

Mutagenicity Assessment (Bacterial Reverse Mutation Screen)

Studies were conducted by Sequani (Ledbury, Herefordshire, UK) to assess the compound of Example 1 *in vitro* for its ability to induce mutations in four histidine dependent auxotrophic mutants of *Salmonella typhimurium*, strains TA1535, TA1537, TA98 and TA100 and one tryptophan dependent auxotrophic mutant of *Escherichia coli*, WP2 *uvrA*.

The mutation screen was conducted using the plate incorporation method and was performed in both the presence and absence of S-9 mix (a liver post-mitochondrial fraction derived from the livers of Aroclor 1254 treated rats). The bacteria were exposed to the compound of Example 1 dissolved in dimethylsulphoxide, which solvent was also used as the negative control. The positive Control chemicals were Sodium Azide (TA1535 and TA100), 9-Aminoacridine (TA1537), 2-Nitrofluorene (TA98) and 4-Nitroquinoline-N-Oxide (WP2 *uvrA*) in the absence of S-9 mix and 2-Aminoanthracene (all strains) in the presence of S-9 mix.

The doses of the compound of Example 1 used in the mutation test under plate incorporation conditions were 15, 50, 150, 500 or 1500 µg/plate in all strains in the presence and absence of S-9 mix.

The compound of Example 1 was analysed up to the limit of solubility of 1500 µg/plate in all strains in the presence and absence of S-9 mix, under plate incorporation conditions.

Precipitation was observed at 500 µg/plate in TA1537 and TA98 in the presence of S-9 mix, and at 1500 µg/plate in all strains in the presence and absence of S-9 mix. There was also a reduction in the mean colony count at 500 µg/plate and 1500 µg/plate in TA98 and at 1500 µg/plate in TA1535, in the presence of S-9 mix, indicating toxicity of the test item to the bacteria.

There were no dose-related or statistically significant increases in revertant numbers observed in any strain at any dose level of the compound of Example 1, in the presence or absence of S-9 mix, under plate incorporation conditions. This indicates the absence of any mutagenic effects for the compound of Example 1 under the conditions of the test.

Hydrolytic Stability Study

Chemical stability of the compound of the invention can be assessed in a mixture of DMSO and water (3:1) at a test compound concentration of 1 mg/mL.

General HPLC procedure

Agilent, Waters X-Select C18, 2.5 µm, 4.6x30 mm column, 4 min method, 5-95% MeCN/water (0.1% formic acid). Flow rate 2.5 mL/min. Column Oven Temperature 40°C. Detection 254 nm.

Sample preparation

A 1.0 mg sample of test compound is dissolved in 750 µL of DMSO. Water (250 µL) is added slowly, ensuring no precipitation occurred.

Recording stability

A 50 µL aliquot of the test solution is removed and analysed in duplicate by 5 µL HPLC injections. The peak area for the test compound is recorded following manual integration of the corresponding UV trace.

The test solution is heated to 60°C, with stirring, and 50 µL aliquots removed for HPLC analysis at 5 and 24 h timepoints. In all cases, 5 µL injections are used and the samples analysed in duplicate.

The peak areas for the test compounds are recorded at both subsequent timepoints and the % decomposition calculated from the % change in peak area over time.

Reference Compound B (3-ethynyl-5-((4-((4-(3-(3-isopropyl-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)pyrimidin-2-yl)amino)-*N*-(2-morpholinoethyl)benzamide; Cariou, C. A. M., et al, WO 2014/027209) is included in each stability study as a control to validate the study.

Stability of pharmaceutical formulations

20mL of 1mg/mL stock solutions were prepared, in duplicate, of the sodium (Na) and hydrochloride (HCl) salts of the compound of Example 1 as follows: The appropriate quantities of each salt were mixed with 10 mM pH 7.2 phosphate buffer containing 4.5% mannitol and 3% polyoxyl 40 stearate. The samples were sonicated to achieve clear solutions having the following properties: Osmolality (mOsm/kg): 310 (Na), 314 (HCl); pH: 7.00 (Na), 7.05 (HCl). 0.5mL of the stock solutions were diluted to 1 mL with 20% DMSO in water & injected for purity analysis by HPLC. The remaining stock solutions were then split into aliquots of 0.5 mL in HPLC vials, and stored at various conditions in duplicate. Samples were stored at 5 and 25°C, before being analysed by HPLC at 1, 2 and 4 weeks. Separate samples were stored at 40°C and analysed at 4 weeks. The analysis shown in the Table below reveals that the Compound of Example 1 is stable in solution at 5°C.

Test substance	Sample	Original Purity (%)	Temp (°C)	Purity (%) at week n		
				n = 1	n = 2	n = 4
Example 1, sodium salt	1	98.6	5	98.2	98.4	98.3
			25	98.4	98.1	97.6
			40	-	-	76.0
	2	98.5	5	98.4	98.5	98.4
			25	98.5	98.1	97.6
			40	-	-	73.7
Example 1, hydrochloride salt	1	98.1	5	98.2	98.1	97.9
			25	98.0	97.7	97.2
			40	-	-	79.2
	2	98.1	5	98.2	98.0	98.0
			25	98.0	97.6	97.4
			40	-	-	80.4

Abbreviations

AcOH	glacial acetic acid
aq	aqueous
5-ASA	5-aminosalicylic acid
ATP	adenosine-5'-triphosphate
BALF	bronchoalveolar lavage fluid
BID	bis in die (twice-daily)
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
br	broad
BrdU	5-bromo-2'-deoxyuridine
BSA	bovine serum albumin
CatCart®	catalytic cartridge
CDI	1,1-carbonyl-diimidazole
COPD	chronic obstructive pulmonary disease
d	doublet
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMEM	Dulbecco’s modified eagle medium
DMF	N,N-dimethylformamide

DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
d-U937 cells	PMA differentiated U-937 cells
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
(ES^-)	electrospray ionization, negative mode
(ES^+)	electrospray ionization, positive mode
Et	ethyl
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FACS	fluorescence-activated cell sorting
FBS	foetal bovine serum
FCS	foetal calf serum
fMLP	formyl-methionyl-leucyl-phenylalanine
FRET	fluorescence resonance energy transfer
GSK3α	glycogen synthase kinase 3α
HBEC	primary human bronchial epithelial cells
HBSS	Hank's balanced salt solution
HPLC	high performance liquid chromatography
HPMC	hydroxypropylmethylcellulose
h or hr	hour(s)
HATU	2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	hydroxybenzotriazole
HRP	horseradish peroxidise
HRV	human rhinovirus
ICAM-1	inter-cellular adhesion molecule 1
IFNγ	interferon-γ
IL	interleukin
iPrOAc	isopropyl acetate
JNK	c-Jun N-terminal kinase
LC	liquid chromatography
Lck	lymphocyte-specific protein tyrosine kinase
LPS	lipopolysaccharide
m	multiplet
$(M+H)^+$	protonated molecular ion
MAPK	mitogen-activated protein kinase
MAPKAP-K2	mitogen-activated protein kinase-activated protein kinase-2
mCPBA	*meta*-chloroperbenzoic acid

Me	methyl
MeCN	acetonitrile
MeOH	methanol
MHz	megahertz
min or mins	minute(s)
MMAD	mass median aerodynamic diameter
MOI	multiplicity of infection
MPO	myeloperoxidase
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MS	mass spectrometry
m/z	mass-to-charge ratio
NMP	N-methyl pyrrolodinone
NMR	nuclear magnetic resonance (spectroscopy)
OD	optical density
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
Ph	phenyl
PHA	phytohaemagglutinin
PMA	phorbol myristate acetate
pTSA	4-methylbenzenesulfonic acid (*para*-toluenesulfonic acid)
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	quartet
rt or RT	room temperature
RP HPLC	reverse phase high performance liquid chromatography
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute
RSV	respiratory syncytial virus
s	singlet
sat or satd	saturated
SCID	severe combined immunodeficiency
SCX	solid supported cation exchange (resin)
SDS	sodium dodecyl sulfate
S_NAr	nucleophilic aromatic substitution
Syk	Spleen tyrosine kinase
t	triplet
T3P	1-propanephosphonic acid cyclic anhydride
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
$TCID_{50}$	50% tissue culture infectious dose
TEA	triethylamine
THF	tetrahydrofuran

TFA	trifluoroacetic acid
TGFβ	transforming growth factor beta
TIPS	triisopropylsilyl
TMB	3,3',5,5'-tetramethylbenzidine
TMS-Cl	trimethylsilyl chloride
TNFα	tumor necrosis factor alpha

Prefixes *n*-, *s*-, *i*-, *t*- and *tert*- have their usual meanings: normal, secondary, *iso*, and tertiary.

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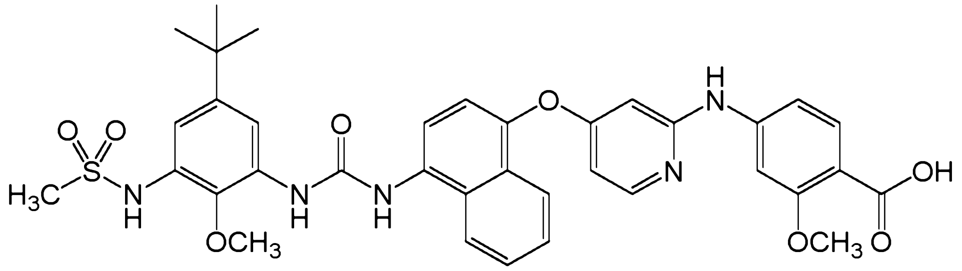
Claims

1. A compound of formula I,



I

or a pharmaceutically acceptable salt, solvate or isotopic derivative thereof.

2. A compound according to Claim 1 that is 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoic acid.

3. A pharmaceutical formulation comprising a compound as defined in Claim 1, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

4. A combination product comprising
   (A) a compound as defined in Claim 1, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, and
   (B) another therapeutic agent,

   wherein each of components (A) and (B) is formulated in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier.

5. A method of treating or preventing an inflammatory disease modulated by one or more of p38 mitogen-activated protein kinase, Syk and Src family kinases, said method comprising administering to a subject an effective amount of a compound as defined in Claim 1, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or a pharmaceutical formulation as defined in Claim 3, or a combination product as defined Claim 4.

6. A method according to Claim 5, wherein the inflammatory disease is selected from the list comprising cystic fibrosis, pulmonary hypertension, lung sarcoidosis, idiopathic pulmonary fibrosis, COPD (including chronic bronchitis and emphysema), asthma, paediatric asthma, atopic dermatitis, allergic dermatitis, contact dermatitis or psoriasis, allergic rhinitis, rhinitis, sinusitis, conjunctivitis, allergic conjunctivitis, keratoconjunctivitis sicca (dry eye or xerophthalmia), glaucoma, diabetic retinopathy, macular oedema (including diabetic macular oedema), central retinal vein occlusion (CRVO), dry and/or wet age related macular degeneration (AMD), post-operative cataract inflammation, uveitis (including posterior, anterior and pan uveitis), corneal graft and limbal cell transplant rejection, gluten sensitive enteropathy (coeliac disease),

eosinophilic esophagitis, intestinal graft versus host disease, Crohn's disease and ulcerative colitis.

7. A method according to Claim 5 or Claim 6, wherein the inflammatory disease is diabetic retinopathy, macular oedema, uveitis, keratoconjunctivitis sicca (dry eye or xerophthalmia), Crohn's disease or ulcerative colitis.

8. A method according to Claim 5 or Claim 6, wherein the inflammatory disease is keratoconjunctivitis sicca (dry eye or xerophthalmia).

9. A method according to Claim 5 or Claim 6, wherein the inflammatory disease is diabetic retinopathy or diabetic macular oedema.

10. The use of

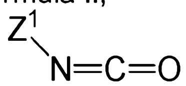
a compound as defined in Claim 1, or pharmaceutically acceptable salt, solvate or isotopic derivative thereof, or

a pharmaceutical formulation as defined in Claim 3 or a combination product as defined Claim 4,

for the preparation of a medicament for the treatment or prevention of an inflammatory disease modulated by one or more of p38 mitogen-activated protein kinase, Syk and Src family kinases, as defined in any one of Claims 5 to 9.

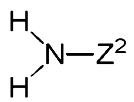
11. A process for the preparation of a compound of formula I, as defined in Claim 1, which process comprises:

(a) reaction of a compound of formula II,



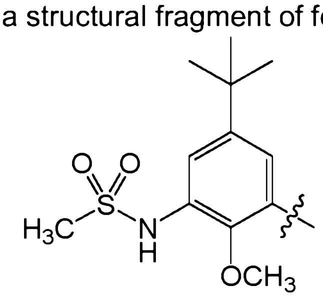
II

with a compound of formula III,



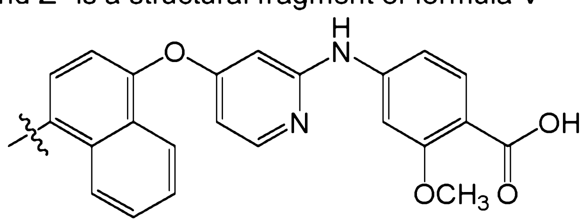
III

wherein one of Z^1 and Z^2 is a structural fragment of formula IV



IV

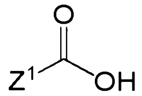
and the other of Z^1 and Z^2 is a structural fragment of formula V



V

;

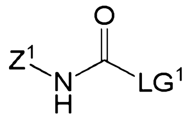
(b) reaction of a compound of formula IIa,


IIa

wherein Z^1 is as defined above, with a suitable azide-forming agent,

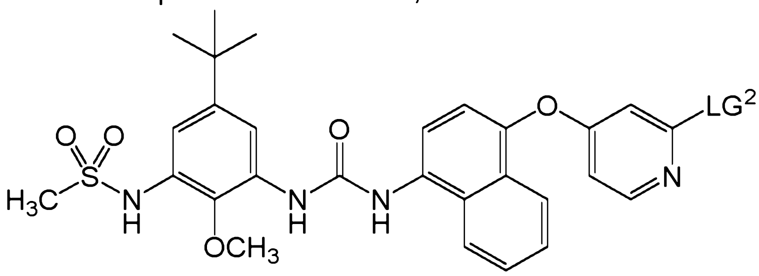
which reaction is followed, without isolation, by thermal rearrangement of the intermediate acyl azide (of formula Z^1-C(O)-N_3) to provide, *in situ*, a compound of formula II, which compound is then reacted with a compound of formula III as defined above;

(c) reaction of a compound of formula IIb,

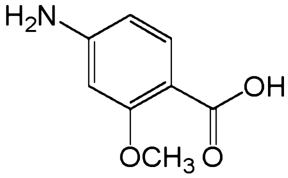

IIb

wherein LG^1 represents a leaving group and Z^1 is as defined above, with a compound of formula III, as defined above;

(d) reaction of a compound of formula VI,


VI

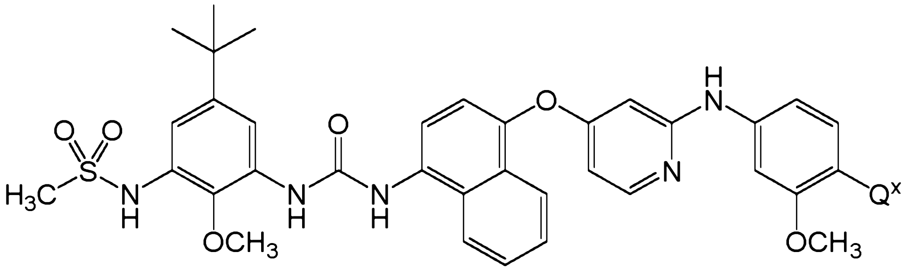
wherein LG^2 represents a leaving group, with a compound of formula VII,


VII

; or

(e) deprotection of a protected derivative of the compound of formula I, wherein the protected derivative bears a protecting group on an O- or N-atom of the compound of formula I.

12. A process according to Claim 11, wherein the protected derivative of the compound of formula I is a compound of formula VIIa,


VIIa

wherein Q^x represents -C(O)O-C_{1-4} alkyl.

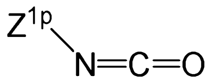
13. A compound of formula VIIa, as defined in Claim 12, or a salt thereof.

14. A compound of formula VIIa, or a salt thereof, as claimed in Claim 13, wherein said compound is methyl 4-((4-((4-(3-(5-(tert-butyl)-2-methoxy-3-

(methylsulfonamido)phenyl)ureido)naphthalen-1-yl)oxy)pyridin-2-yl)amino)-2-methoxybenzoate.

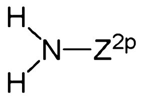
15. A process for the preparation of a compound of formula VIIa, as defined in Claim 12, which process comprises:

(a) reaction of a compound of formula IIp,



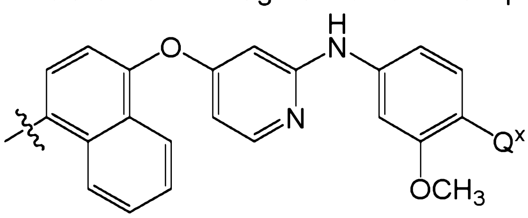
IIp

with a compound of formula IIIp,



IIIp

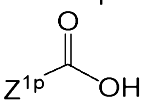
wherein one of Z^{1p} and Z^{2p} is a structural fragment of formula IV, as defined in Claim 11, and the other of Z^{1p} and Z^{2p} is a structural fragment of formula Vp



Vp

wherein Q^x is as defined in Claim 12;

(b) reaction of a compound of formula IIap

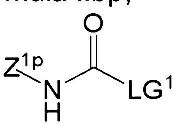


IIap

wherein Z^{1p} is as defined above, with a suitable azide-forming agent,

which reaction is followed, without isolation, by thermal rearrangement of the intermediate acyl azide (of formula Z^{1p}-C(O)-N_3) to provide, *in situ*, a compound of formula II, which compound is then reacted with a compound of formula IIIp as defined above;

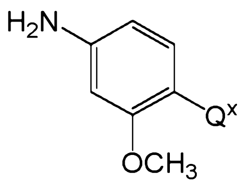
(c) reaction of a compound of formula IIbp,



IIbp

wherein LG^1 represents a leaving group and Z^{1p} is as defined above, with a compound of formula IIIp, as defined above; or

(d) reaction of a compound of formula VI, as defined in Claim 11, with a compound of formula VIIp,



VIIp

wherein Q^x is as defined in Claim 12.