Title: QUINOLINE DERIVATIVE AS TYROSINE KINASE INHIBITOR

Abstract: The present invention provides N,N(2-methyl-5-hydroxyphenyl)-(6-methanesulfonyl-quinolin-4-yl)-amine which exhibits protein tyrosine kinase inhibition, in particular p56lek activity, method(s) for its preparation, pharmaceutical compositions containing it and its use in medicine.
QUINOLINE DERIVATIVE AS TYROSINE KINASE INHIBITOR

The present invention relates to N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonyl-quinolin-4-yl)-amine which exhibits protein tyrosine kinase inhibition, in particular p56lck activity, method(s) for its preparation, pharmaceutical compositions containing it and its use in medicine.

Protein tyrosine kinases catalyse the phosphorylation of specific tyrosyl residues in various proteins involved in the regulation of cell growth and differentiation (A.F. Wilks, Progress in Growth Factor Research, 1990, 2, 97-111; S.A. Courtneidge, Dev. Supple, 1993, 57-64; J.A. Cooper, Semin. Cell Biol., 1994, 5(6), 377-387; R.F. Paulson, Semin. Immunol., 1995, 7(4), 267-277; A.C. Chan, Curr. Opin. Immunol., 1996, 8(3), 394-401). Protein tyrosine kinases can be broadly classified as receptor (e.g. EGF, c-erbB-2, c-met, tie-2, PDGFr, FGFr) or non-receptor (e.g. c-src, p56lck, ZAP70) kinases. Inappropriate or uncontrolled activation of many of these kinases, i.e. aberrant protein tyrosine kinase activity, for example by over-expression or mutation, has been shown to result in uncontrolled cell growth.


P56lck is indicated in disease conditions in which T cells are hyperactive e.g. transplant rejection, rheumatoid arthritis, SLE, graft vs. host disease, T-cell mediated hypersensitivity, multiple sclerosis, IBD, psoriasis, Hashimoto's thyroiditis, Guillain-Barre syndrome, cancer, dermatitis, allergic disorders, asthma, ischemic or reperfusion injury, allergic rhinitis, burns, myocardial
infarction, stroke, osteoarthritis, ulcerative colitis, Sjogren's syndrome, autoimmune hyperthyroidism, Grave's disease, Addison's disease, alopecia, anemia, autoimmune hypopituitarism, glomerulonephritis, urticaria, scleracierma, vasculitis, insulin-dependent diabetes and ARDS. The process of angiogenesis has been associated with a number of disease states (e.g. tumourogenesis, psoriasis, rheumatoid arthritis) and this has been shown to be controlled through the action of a number of receptor tyrosine kinases (L.K. Shawver, DDT, 1997, 2(2), 50-63).

The present invention provides N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine or a salt, solvate or physiologically functional derivative thereof.

Suitable pharmaceutically acceptable salts of N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine include acid addition salts formed with pharmaceutically acceptable organic or inorganic acids, for example those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids, and organic acids, such as tartaric, acetic, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic and methanesulfonic and arylsulfonic, for example p-toluenesulfonic, acids.

As used herein, the term "solvate" refers to a complex of variable stoichiometry formed by a solute (in this invention, N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine or a salt thereof or a physiologically functional derivative thereof) and a solvent. Solvents suitable for the purpose of the invention generally do not interfere with the biological activity of the solute. Examples of suitable solvents include water, methanol, ethanol and acetic acid. Preferably the solvent used is a pharmaceutically acceptable solvent. Examples of suitable pharmaceutically acceptable solvents include water, ethanol and acetic acid. Most preferably the solvent used is water.

N,N-(2-Methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine may exist as a physiologically functional derivative. The term "physiologically functional derivative" as used herein refers to an active metabolite of or any
pharmaceutically acceptable derivative of N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine, for example, an ester, which upon administration to a mammal, such as a human, is capable of providing (directly or indirectly) N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine. Such derivatives are clear to those skilled in the art, without undue experimentation, and with reference to the teaching of Burger's Medicinal Chemistry And Drug Discovery, 5th Edition, Vol 1: Principles And Practice, which is incorporated herein by reference.

The present invention thus also provides N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine and pharmaceutically acceptable salts or solvates thereof, or physiologically functional derivatives thereof, for use in therapy.

A further aspect of the invention provides a method of treatment of a human or animal subject suffering from a disorder mediated by aberrant protein tyrosine kinase activity which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof.

A further aspect of the invention provides a method of treatment of a human or animal subject suffering from a disorder mediated by aberrant p56lck activity which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof.

A further aspect of the invention provides a method of treatment of a human or animal subject suffering from rheumatoid arthritis which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof.
A further aspect of the present invention provides the use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of a disorder mediated by aberrant protein tyrosine kinase activity.

A further aspect of the present invention provides the use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of a disorder mediated by aberrant p56lck activity.

A further aspect of the present invention provides the use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of rheumatoid arthritis.

The aberrant p56lck activity referred to herein is any p56lck activity that deviates from the normal p56lck activity expected in a particular mammalian subject. Aberrant p56lck activity may take the form of, for instance, an abnormal increase in activity, or an aberration in the timing and or control of p56lck activity. Such aberrant activity may result then, for example, from overexpression or mutation of the protein kinase or ligand leading to inappropriate or uncontrolled activation of the receptor. Furthermore, it is also understood that unwanted p56lck activity may reside in an abnormal source. That is, the level of p56lck activity does not have to be abnormal to be considered aberrant, rather the activity derives from an abnormal source.

Whilst it is possible for N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, its salts, solvates or physiologically functional derivatives of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine to be administered as the raw chemical, it is preferred to present it in the form of a pharmaceutical formulation.
According to a further feature of the present invention there is provided a pharmaceutical formulation comprising N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, together with one or more pharmaceutically acceptable carriers, diluents or excipients.

Pharmaceutical formulations may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose. Such a unit may contain for example 0.5mg to 1g, preferably 35mg to 700mg of a compound of the formula (I) depending on the condition being treated, the route of administration and the age, weight and condition of the patient.

Pharmaceutical formulations may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such formulations may be prepared by any method known in the art of pharmacy, for example by bringing into association the active ingredient with the carrier(s) or excipient(s).

Pharmaceutical formulations adapted for oral administration may be presented as discrete units such as capsules or tablets; powders or granules; solutions or suspensions in aqueous or non-aqueous liquids; edible foams or whips; or oil-in-water liquid emulsions or water-in-oil liquid emulsions.

Pharmaceutical formulations adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3(6), 318 (1986).

Pharmaceutical formulations adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.
For treatments of the eye or other external tissues, for example mouth and skin, the formulations are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical formulations adapted for topical administrations to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent.

Pharmaceutical formulations adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

Pharmaceutical formulations adapted for rectal administration may be presented as suppositories or as enemas.

Pharmaceutical formulations adapted for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical formulations adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

Pharmaceutical formulations adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-
oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Preferred unit dosage formulations are those containing a daily dose or sub-dose, as herein above recited, or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

The animal requiring treatment with N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, its salt, solvate or physiologically functional derivative is usually a mammal, such as a human being.

A therapeutically effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, its salt, solvate or physiologically functional derivative of the present invention will depend upon a number of factors including, for example, the age and weight of the animal, the precise condition requiring treatment and its severity, the nature of the formulation, and the route of administration, and will ultimately be at the discretion of the attendant physician or veterinarian. However, an effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine for the treatment of rheumatoid arthritis, will generally be in the range of 0.1 to 100 mg/kg body weight of recipient (mammal) per day and more usually in the range of 0.5 to 10 mg/kg body weight per day. Thus, for a 70kg adult mammal, the actual amount per day would usually be from 35 to 700 mg and this amount may
be given in a single dose per day or more usually in a number (such as two, three, four, five or six) of sub-doses per day such that the total daily dose is the same. An effective amount of a salt or solvate of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine may be determined as a proportion of the effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine per se.

N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine and its salts, solvates and physiologically functional derivatives may be employed alone or in combination with other therapeutic agents for the treatment of the above-mentioned conditions. Combination therapies according to the present invention thus comprise the administration of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, and at least one other pharmaceutically active agent. N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine and the other pharmaceutically active agent(s) may be administered together or separately and, when administered separately this may occur simultaneously or sequentially in any order. The amounts of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine and the other pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine may be prepared by the general methods outlined hereinafter.

N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonfyl-quinolin-4-yl)-amine may be prepared by reacting a compound of formula (II)
with 3-hydroxy-6-methyl aniline. The halogen may be fluorine, chlorine, bromine or iodine. Preferably, the halogen is chlorine.

Compounds of formula (II) may be prepared by halogenating the compound of formula (III)

Convenient halogenating agents include inorganic acid halides, for example, phosphorus (III) chloride, phosphorus (V) oxychloride and phosphorus (V) chloride. Preferably, thionyl chloride is used.

The compound of formula (III) may be prepared by the decarboxylation of a compound of formula (IV)

The decarboxylation may be affected by heating the compound of formula (IV) in the presence of an inert solvent or diluent such as an ether, for example diphenyl ether, at an elevated temperature.

The compound of formula (IV) may be prepared by the reacting a compound of formula (V)
with a base at reflux, and then acidifying the resulting mixture. The base is preferably sodium hydroxide solution.

The compound of formula (V) may be prepared by the cyclisation of a compound of formula (VI)

The cyclisation may be affected by heating the compound of formula (VI) in the presence of an inert solvent or diluent such as an ether, for example diphenyl ether, at an elevated temperature.

The compound of formula (VI) may be prepared by reacting a compound of formula (VII)

with diethylethoxymethylenealmonate at elevated temperature.

The compound of formula (VII) may be prepared by reducing a compound of formula (VIII)
with a suitable reducing agent, such as a sulfide, or zinc, tin or iron in the presence of acid, or hydrogen in the presence of a suitable catalyst, for example palladium, 10% on activated carbon. A preferred reducing agent is iron powder and acetic acid.

The compound of formula (VIII) may be prepared by reacting a compound of formula (IX)

![Chemical Structure](image)

with sodium sulfinate in the presence of an inert solvent such as dimethylacetamide. The halogen may be fluorine, chlorine, bromine or iodine. Preferably, the halogen is fluorine.

Certain embodiments of the present invention will now be illustrated by way of example only. The physical data given for N,N-(2-methyl-5-hydroxyphenyl)-(6-methanesulfonyl-quinolin-4-yl)-amine is consistent with the assigned structure of this compound.

$^1$H NMR spectra were obtained at 500MHz on a Bruker AMX500 spectrophotometer, on a Bruker spectrophotometer at 300MHz, on a Bruker AC250 or Bruker AM250 spectrophotometer at 250MHz and on a Varian Unity Plus NMR spectrophotometer at 300 or 400MHz. J values are given in Hz. Mass spectra were obtained on one of the following machines: VG Micromass Platform (electrospray positive or negative), HP5989A Engine (thermospray positive) or Finnigan-MAT LCQ (ion trap) mass spectrometer. Analytical thin layer chromatography (tlc) was used to verify the purity of some intermediates which could not be isolated or which were too unstable for full characterisation, and to follow the progress of reactions. Unless otherwise stated, this was done using silica gel (Merck Silica Gel 60 F254). Unless otherwise stated, column chromatography for the purification of some compounds used Merck Silica gel (Art. 1.09385, 230-400 mesh), and the stated solvent system.
All temperatures are in °C.
DMSO refers to dimethylsulfoxide
THF refers to tetrahydrofuran
HPLC refers to high performance liquid chromatography

Intermediate 1

4-Methanesulfonyl nitrobenzene

4-Fluoronitrobenzene (46.2g) in dimethylacetamide (450mL) was treated with sodium sulfinate (41.83g) and stirred at room temperature overnight. After 5 hours at 50°C, water (1800mL) was added and the precipitated solid collected by filtration at the pump to give 54.5g of 4-methanesulfonyl nitrobenzene as a white solid; m/z 202 (M+1)⁺.

Intermediate 2

4-Methanesulfonylanilineline

4-Methanesulfonyl nitrobenzene (30g) as a suspension in acetic acid (300mL) was treated with iron powder (33.36g) portionwise at 0°C. The ice bath was removed and after 1 hour, the acetic acid was removed in vacuo. The residue was suspended in water (300mL) and solid sodium hydroxide was added until the pH>10. The aqueous phase was extracted with ethyl acetate, combined organic phases were washed with brine, dried (MgSO₄), filtered and concentrated in vacuo to give 4-methanesulfonylaniliniline (22.3g); m/z 172 (M+1)⁺.

Intermediate 3

Diethyl-N-(4-methanesulfonylphenyl)-aminomethylene malonate

4-Methanesulfonylaniliniline (40.3g) was treated with diethylethoxymethylene malonate (300mL) at 130°C for 2 hours. The mixture was cooled, diluted with cyclohexane and the precipitated solid filtered and dried
to give diethyl-N-(4-methanesulfonylphenyl)-aminomethylenemalonate (69g) as a colourless solid; m/z 342 (M+1⁺).

**Intermediate 4**

3-Carboethoxy-6-methanesulfonyl quinolinyl-4-one

Diethyl-N-(4-methanesulfonylphenyl)-aminomethylenemalonate (36.4g) was added portionwise to refluxing diphenyl ether over 5 minutes. After 15 minutes at 230°C, the mixture was cooled, diluted with cyclohexane and filtered to give 3-carboethoxy-6-methanesulfonyl quinolinyl-4-one (32.41g) as a colourless solid; m/z 296 (M+1⁺).

**Intermediate 5**

(6-Methanesulfonyl quinolinyl-4-one-3-yl)carboxylic acid

3-Carboethoxy-6-methanesulfonyl quinolinyl-4-one (48.15) in ethanol (300mL) was treated with sodium hydroxide solution (19.59g in 300mL) and heated at reflux for two hours. After cooling, the mixture was acidified with 2N HCl and the (6-methanesulfonyl quinolinyl-4-one-3-yl)carboxylic acid (43g) was collected by filtration; m/z 288 (M+1⁺).

**Intermediate 6**

6-Methanesulfonlyl quinolin-4-one

(6-Methanesulfonyl quinolinyl-4-one-3-yl)carboxylic acid (10g) was added to diphenyl ether (200mL) at reflux. After one hour, the solution was cooled and cyclohexane (1000mL) was added. The precipitated solid 6-methanesulfonlyl quinolin-4-one (6.32g) was filtered and dried at the pump; m/z 223 (M⁺).

**Intermediate 7**

4-Chloro-6-methanesulfonylquinoline
6-Methanesulfonyl quinolin-4-one (6.32g) was suspended in thionyl chloride (60mL) and 3 drops of DMF added. The mixture was heated at reflux for 3 hours. The mixture was allowed to cool and the thionyl chloride was removed in vacuo. Subsequent addition and removal in vacuo of toluene was followed by trituration from 2N sodium hydroxide to give 4-chloro-6-methanesulfonylquinoline (6.36g) as a pale brown solid; δH [DMSO] 9.03(1H, d), 8.73(1H, d), 8.33(2H, m), 7.97(1H, d), 3.38(3H,s); m/z 242 (M+1)^+.

Example 1

N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine

4-Chloro-6-methanesulfonylquinoline (2.057g) and 3-hydroxy-6-methyl aniline (1.134g) in acetonitrile (15mL) were heated at reflux under N₂. After 20 hours, the acetonitrile was removed in vacuo, the residues taken up in methanol and absorbed onto silica. Purification by chromatography on silica eluting with dichloromethane/ethanol/ammonia (150:8:1) gave the title compound as an off-white solid (1.778g); δH [D₆] DMSO 9.52(1H,s), 9.33(1H,s), 9.19(1H,d), 8.57(1H,dd), 8.18(1H,dd), 8.11(1H,d), 7.26(1H,d), 6.77(2H,m), 6.29(1H,dd), 3.40(3H,s), 2.11(3H,s); m/z 329 (M+1)^+.

Biological Data

Insect cell pellets infected with the appropriate recombinant baculovirus directing the expression of the relevant kinases were homogenised in 40 mM HEPES (pH 7.4), 100 mM NaCl 2 mM EDTA, 10% glycerol, 0.1 mM vanadate and protease inhibitors. The 100 000 g supernatant was stored at -85°C. Stored lysates were thawed on ice, ATP and MgCl₂ (0.1 mM and 10 mM) were added. Following incubation on ice the kinase was diluted in 40 mM HEPES (pH 7.4). The kinase reaction mixture contained 40 mM HEPES (pH 7.4), 10 mM MgCl₂, 0.05 mM, ATP, and substrate peptide. The reaction was stopped by the addition of EDTA (25 mM). The amount of phosphopeptide was quantitated by homogeneous time resolved fluorescence as described in Kolb et al. (1998) Drug Discovery Today 3:333-342. An increase in the level of fluorescence indicates an increase in kinase activity.
To screen compounds for modulation of kinase activity the order of addition of assay reagents and timings were:
Appropriate dilution of compound to well, Enzyme to well, 15 min pre-incubation, Substrates (ATP, peptide) added, 30 min incubation, Reaction stopped with EDTA, HTRF reagents (APC, Eu labelled antibody (antiPY antibody)) added, Stand for 20 mins, read signal on plate reader.
Using this assay the IC50s against the following kinases were determined as follows:-
Mu Lck 10nM, Hu Src 18nM, Mu Lyn 16nM, Mu FynT 5nM

Cell data (IC50)
The Lck cell-based assay measures the production of IL-2 from mouse T-cells after stimulation with either ovalbumin (antigen) or Ionomycin and PMA (I/PMA). The latter, an indirect stimulus of IL-2 production gives a measure of specificity of assayed compounds.

Cells are prepared from DO.10 mice spleens and adjusted to a count of 1.5x10^6 cells/ml. Concentration-effect curves are constructed using doubling dilutions from top concentrations of 10μM compound for the antigen and 50μM compound for I/PMA. The cells are incubated with the assayed compounds and either antigen or I/PMA at 37°C (95% air, 5% CO₂) overnight in 96 well plates.

The supernatants are then removed from the cells and IL-2 content measured using a sandwich Elisa. Optical Density is then established at 450nm in a plate reader.

The human lymphoma J6 Jurkat cell line were cultured in RPMI1640 medium containing 10% FCS, L-Glutamine and antibiotics, and harvested as required. They were washed in RPMI1640 medium containing 30mM HEPES and suspended in this medium at 100x10^6/ml and kept until required at 37°C. 100μl aliquots were placed in minifuge tubes containing 1μl of either DMSO or drug at 100x final concentration (in DMSO) and incubated at 37°C for 10 minutes with occasional mixing. 10μl of anti-mouse IgG at 0.4mg/ml (in PBS) was added to each tube, mixed and incubated for 2 minutes before 10μl of PBS (for the basal controls) or anti-CD3 (at 0.4mg/ml in PBS) was added, mixed, and incubated for
1 minute before stopping the reactions by snap-freezing in liquid nitrogen. 300μl of Octyl-Glucopyranoside lysis buffer OGLB was added to each tube and kept on ice as the samples thawed, and then vortexed periodically over a period of ~30 minutes. The samples were then centrifuged at ~13000g for 5 minutes at 4°C and the lysate removed to fresh tubes. Protein determinations were then performed (Biorad Protein Determination Kit), equivalent 'protein' transferred to fresh tubes and made up to 300μl with OGLB. 10μl of anti-zeta and 40μl of 10% Protein A Sepharose (in PBS) were then added and the tubes tumbled overnight at 4°C.

The immunoprecipitates (pellets) were then collected, washed 3x with OGLB and then solubilised by adding 20μl 2x sample loading buffer (containing 10mM sodium orthovanadate and DTT), vortexing and heating at 100°C for 10 minutes. Proteins were then separated on 4-20% gradient SDS-PAGE gels (Novex) and then transferred to PVDF membranes (Millipore), which were then blocked using 1% BSA (in PBS) for 30 minutes. 4G10 anti-phosphotyrosine antibody (1/2500 dilution) was added and incubated with constant mixing for ~1 hour, and then the membranes were washed extensively with PBS/0.1%Tween. HRP conjugated anti-mouse IgG (1/100000 dilution) was then added and the membranes incubated overnight at 4°C.

The membranes were then washed extensively again and then incubated with ECL+Plus reagent (Amersham) for 5 minutes before detection and quantification using the Storm system (Molecular Dynamics).

IC50 in this assay was found to be IL-2 240nM, P/I 2.58μM, zeta chain phosphorylation 330nM

**In vivo efficacy**

This model involves systemic reactivation of an acute inflammatory reaction in a joint injected previously with a sub-arthritisogenic dose of PG-PS (peptidoglycan-polysaccharide from Streptococcus pyogenes).

Male Lewis rats were injected intra-articularly with 5μg of PG-PS into the right ankle joint, causing a mild inflammatory reaction. Approximately two weeks later,
the rats were injected intravenously with 200μg of PG-PS. This treatment reactivates inflammation in the previously injected joint only. Ankle diameters were measured at the time of reactivation (Day 0), then daily on Days 1, 2 and 3. Results were expressed as change in ankle diameter (or ankle swelling) by subtracting the starting value measured on Day 0. Inhibition of the anti-inflammatory activity of compounds was tested for statistical significance using One Way Analysis of Variance followed by Dunnett’s test (treatment versus control). Body weights were recorded daily to monitor any potential toxicity caused by the treatment. Compounds were given on days 0, 1 and 2, with the first dose being administered 1 hour prior to reactivation and continuing once, twice or three times daily (at 24, 12 or 8 hour intervals respectively). The experiment was terminated on Day 3 with the final ankle diameters being measured, and, if required, tissue/blood samples also taken for subsequent histological/toxicological analysis, pharmacokinetic profiling or ex-vivo assay.

Using a range of doses of the compound, the EC50 in the PG-PS model (rat) was found to be 0.04mg/kg.

The application of which this description and claim(s) forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process or use claims and may include, by way of example and without limitation, one or more of the following claim(s):
Claims

1. N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a salt, solvate or physiologically functional derivative thereof.

2. A pharmaceutical formulation comprising N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, together with one or more pharmaceutically acceptable carriers, diluents or excipients.

3. N,N-(2-Methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, for use in therapy.

4. The use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of a disorder mediated by aberrant protein tyrosine kinase activity.

5. The use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of a disorder mediated by aberrant p56lck activity.

6. The use of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine, or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof, in the preparation of a medicament for the treatment of rheumatoid arthritis.

7. A method of treatment of a human or animal subject suffering from a disorder mediated by aberrant protein tyrosine kinase activity which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically
acceptable salt or solvate thereof, or a physiologically functional derivative thereof.

8. A method of treatment of a human or animal subject suffering from a disorder mediated by aberrant p56lck activity which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof.

9. A method of treatment of a human or animal subject suffering from rheumatoid arthritis which comprises administering to said subject an effective amount of N,N-(2-methyl-5-hydroxyphenyl)–(6-methanesulfonyl-quinolin-4-yl)-amine or a pharmaceutically acceptable salt or solvate thereof, or a physiologically functional derivative thereof.
## A. CLASSIFICATION OF SUBJECT MATTER

**IPC** 7 C07D215/44 A61K31/4706

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

**Minimum documentation searched** (classification system followed by classification symbols)

**IPC** 7 C07D A61K

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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**Date of the actual completion of the international search**

13 June 2002

**Date of mailing of the international search report**

21/06/2002

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentixam 2 NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 spo nl, Fax (+31-70) 340-3016

**Authorized officer**

Fanni, S
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