

THIENO [2 , 3 -D] PYRIMIDINE DERIVATIVES AND THEIR USE TO TREAT ARRHYTHMIA**TECHNICAL FIELD**

The present invention relates to thienopyrimidine compounds which are potassium channel inhibitors. Pharmaceutical compositions comprising the compounds and their use in the treatment of arrhythmia are also provided.

BACKGROUND ART

Ion channels are proteins that span the lipid bilayer of the cell membrane and provide an aqueous pathway through which specific ions such as Na^+ , K^+ , Ca^{2+} and Cl^- can pass (Herbert, 1998). Potassium channels represent the largest and most diverse sub-group of ion channels and they play a central role in regulating the membrane potential and controlling cellular excitability (Armstrong & Hille, 1998). Potassium channels have been categorized into gene families based on their amino acid sequence and their biophysical properties (for nomenclature see Gutman *et al.*, 2003).

Compounds which modulate potassium channels have multiple therapeutic applications in several disease areas including cardiovascular, neuronal, auditory, renal, metabolic and cell proliferation (Shieh *et al.*, 2000; Ford *et al.*, 2002). More specifically potassium channels such as Kv4.3, Kir2.1, hERG, KCNQ1/minK, and Kv1.5 are involved in the repolarisation phase of the action potential in cardiac myocytes. These potassium channels subtypes have been associated with cardiovascular diseases and disorders including long QT syndrome, hypertrophy, ventricular fibrillation, and atrial fibrillation, all of which can cause cardiac failure and fatality (Marban, 2002).

The human delayed rectifier voltage gated potassium channel subunit, Kv1.5, is exclusively expressed in atrial myocytes and is believed to offer therapeutic opportunities for the management of atrial fibrillation for several different reasons (see review of Brendel and Peukert, 2002): (i) There is evidence that Kv1.5 underlies the cardiac ultrarapid delayed rectifier ($\text{K}_{\text{V(ur)}}$) physiological current in humans due to similar biophysical and pharmacological properties (Wang *et al.*, 1993; and Fedida *et al.*, 1993). This has been supported with antisense oligonucleotides to Kv1.5 which have been shown to reduce $\text{K}_{\text{V(ur)}}$ amplitude in human atrial myocytes (Feng *et al.*, 1997). (ii) electrophysiological recordings have demonstrated that $\text{K}_{\text{V(ur)}}$ is selectively expressed in atrial myocytes, and therefore avoids inducing potentially fatal ventricular arrhythmia

through interfering with ventricular repolarisation (Amos *et al.*, 1996; Li *et al.*, 1996; and Nattel, 2002). (iii) Inhibiting $K_{V(ur)}$ in atrial fibrillation-type human atrial myocytes prolonged the action potential duration compared to normal healthy human atrial myocytes (Courtemanche *et al.*, 1999). (iv) Prolonging the action potential duration by
5 selectively inhibiting $K_{v1.5}$ could present safer pharmacological interventions for protecting against atrial re-entrant arrhythmias such as atrial fibrillation and atrial flutter compared to traditional class III antiarrhythmics, by prolonging the atrial refractory period while leaving ventricular refractoriness unaltered (Nattel *et al.*, 1999, Knobloch *et al.*, 2002; and Wirth *et al.*, 2003). Class III antiarrhythmics have been widely reported as a
10 preferred method for treating cardiac arrhythmias (Colatsky *et al.*, 1990).

Traditional and novel class III antiarrhythmic potassium channel blockers have been reported to have a mechanism of action by directly modulating $K_{v1.5}$ or $K_{V(ur)}$. The known class III antiarrhythmics ambasilide (Feng *et al.*, 1997), quinidine (Wang *et al.*,
15 1995), clofilium (Malayev *et al.*, 1995) and bertosamil (Godreau *et al.*, 2002) have all been reported as potassium channel blockers of $K_{V(ur)}$ in human atrial myocytes. The novel benzopyran derivative, NIP-142, blocks $K_{v1.5}$ channels, prolongs the atrial refractory period and terminates atrial fibrillation and flutter in *in vivo* canine models (Matsuda *et al.*, 2001), and S9947 inhibited $K_{v1.5}$ stably expressed in both *Xenopus*
20 oocytes and Chinese hamster ovary (CHO) cells and $K_{V(ur)}$ in native rat and human cardiac myocytes (Bachmann *et al.*, 2001). Elsewhere, other novel potassium channel modulators which target $K_{v1.5}$ or $K_{V(ur)}$ have been described for the treatment of cardiac arrhythmias, these include biphenyls (Peukert *et al.* 2003), thiophene carboxylic acid amides (WO0248131), bisaryl derivatives (WO0244137, WO0246162), carbonamide
25 derivatives (WO0100573, WO0125189) anthranillic acid amides (WO2002100825, WO02088073, WO02087568), dihydropyrimidines (WO0140231), cycloalkyl derivatives (WO03063797), indane derivatives (WO0146155 WO9804521), tetralin benzocycloheptane derivatives (WO9937607), thiazolidone and metathiazanone derivatives (WO9962891), benzamide derivatives (WO0025774), isoquinoline
30 derivatives (WO0224655), pyridazinones derivatives (WO9818475 WO9818476), chroman derivatives (WO9804542), benzopyran derivatives (WO0121610, WO03000675, WO0121609, WO0125224, WO02064581), benzoxazine derivatives (WO0012492), and the novel compound A1998 purified from Ocean material (Xu & Xu,

2000). General voltage gated potassium channel inhibitors have been reported which could also modulate Kv1.5 (US05753676, US05821251, EP0743936B).

Thienopyrimidines have been reported to be useful as anti-inflammatory, anti-fungal,
5 anti-osteoporosis and anti-microbial agents, and as cardiovascular agents (acting through modulation of the phosphodiesterase group of enzymes or through modulation of the sodium/proton exchange system) amongst others.

Thieno[2,3-*d*]-pyrimidines substituted in the 4-position with an optionally substituted
10 benzylamine or phenethylamine moiety and in the 5-position with a methyl group may serve as anti-inflammatory or anti-osteoporosis agents (Katada *et al.*, 1999). Such compounds were shown to modulate the activity of several cell types including leukocytes, which originate from hematopoietic precursor cells in the bone marrow. Increased activity in leukocytes can lead to various inflammatory diseases; therefore
15 compounds cytotoxic to leukocytes could function as anti-inflammatory drugs. Such compounds are thought to suppress cellular activity by binding to integrins on the surface of leukocytes and preventing downstream cellular signalling events. Thieno[2,3-*d*]pyrimidines substituted in the 4-position with heteroarylthiols, aryl thiols, arylmethyl thiols, heteroarylamines, benzylamine, hydroxyl and chloro groups may also be useful
20 anti-inflammatory agents (Stewart *et al.*, 2001). This series of compounds were shown to inhibit induced expression of cell adhesion molecules on the luminal surface of vascular endothelial thus preventing the adhesion of leukocytes at the site of inflammation.

25 Thieno[2,3-*d*]pyrimidines with a substituted hydrazine in the 4-position and a phenyl group in the 5 position (Hozien *et al.*, 1996), tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidines (Ismail *et al.*, 1995), thieno[2,3-*d*]pyrimidines which have a hydrogen, chloro, hydrazine, heterocyclyl, amino, methyl, ethyl or phenyl group in the 2-position, an alkylamino, alkylaryl amino, amino, dialkylamino or hydrazino substituent in the 4-
30 position, a hydrogen or methyl group in the 5-position, a hydrogen, methyl acetamide or phenyl group in the 6-position or a tetramethylene in the 5,6-position (GB7549025), and the lead series of 5-phenyl- and 5,6-tetramethylenethieno[2,3-*d*]pyrimidines with methyl or phenyl in the 2-position and alkylamino or arylamino in the 4- position (Konno *et al.*, 1989) have all been shown to have anti-microbial activity. Tetrahydrobenzothieno[2,3-

d]pyrimidine with the 2-oxo-3-pyrrolidinylmethylene-hydrazino moiety in the 4-position showed some herbicidal activity against velvet leaf (Ram *et al.*, 1981). It has also been reported that 4-chlorotetrahydrobenzothieno[2,3-*d*]pyrimidine is herbicidal, tetrahydrobenzothieno-[2,3-*d*]pyrimidines with a thiol, hydrazine, 2-fluoroanilino, 3-fluoroanilino or 4-diethylanilino substituent in the 4-position are bactericidal against *Streptococcus faecales* and tetrahydrobenzothieno[2,3-*d*]pyrimidines with a 2,4-dichlorobenzylamino or 2-fluoroanilino substituent in the 4-position are fungicidal against *Pythium* (Ram, 1979). Thieno[2,3-*d*]pyrimidines with a hydrogen, hydroxyl, thiol, halogen or cyano group in the 2-position, alkylamino, arylalkylamino or hydroxyalkyl amino groups in the 4-position, a hydrogen, alkyl or halogen in the 5- and/or 6- position or alkylene in the 5,6-position have been reported as tick-control agents (AU 521790).

Elsewhere, tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidines exhibited anti-tumour activity (Shehata *et al.*, 1996) and analgesic activity half that of aspirin (Moneer *et al.*, 1994), a series of thieno[2,3-*d*]pyrimidines with 4-alkylamino or arylamino, 5-H or 5-methyl, 6-methyl or 5,6-tetramethylene were shown to have potential as anticytokinins (Jordis *et al.*, 1986), a series of 5,6-dimethyl-thieno[2,3-*d*]pyrimidines and 5,6-tetramethylenethieno[2,3-*d*]pyrimidines, both substituted in the 2-position with arylamines or heterocyclic amines and in the 4-position with arylamines displayed blood platelet aggregation inhibiting properties (DD 226893), pyrano- and thiopyrano[3,4-*b*]thieno[5,4-*d*]pyrimidines with the 4-position substituted with amino, butylamine, aniline, cyclohexylamine, benzylamine, phenethylamine and 2-hydroxyethylamine have been reported to exhibit anticonvulsive activity (Noravyan *et al.*, 1977), and 4-[(Benzo-2,1,3-thiadiazolyl-4)amino]-5,6,7,8-tetrahydrobenzothieno-(2,3-*d*)-pyrimidine has been reported to possess anthelmintic activity in larval alveolar echinococcosis (RU 2116309).

Thieno[2,3-*d*]pyrimidines with a substituted amino group at the 4-position, hydrogen, alkyl or halo substitution at the 5 and 6-positions and an alkyl chain at the 2-position are claimed to be inhibitors of phosphodiesterase V and useful in the treatment of cardiovascular diseases and for disturbances in potency (DE10104802).

Elsewhere, 5-alkyl thieno[2,3-*d*]pyrimidines with a piperazinyl substituent at the 4-position were found to be inhibitors of the sodium/proton exchanger and useful in the treatment of various cardiovascular disorders, including angina pectoris and arrhythmia (WO 01/27107).

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4-[(phenyl)amino]-thieno[2,3-*d*]pyrimidines bearing a 5-thiophenyl substituent and a 2-methyl substituent were found to have molluscicidal activity (Hosni et al, Acta Poloniae Pharmaceutica, 1999, 56(1), 49-56).

10 Recently thienopyrimidines have also been reported as potent VEGFR inhibitors (Munchhof, 2004).

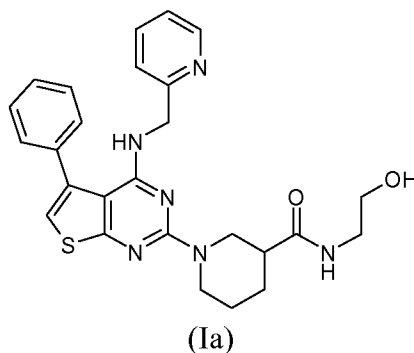
Several publications disclose compounds which are indicated as acting on potassium channels. Thus, US6531495 discloses 2'-aminomethylbiphenyl-2-carboxamides,
15 WO2002/100825 discloses anthranillic acid amides as antiarrhythmics and WO2002/036556 discloses acylaminoalkylbenzenesulfonamides as cardiovascular agents.

Thienopyrimidine compounds that are useful as potassium channel inhibitors,
20 particularly for inhibiting potassium channels Kv1.5 or Kv_(ur), are reported in WO 2004/111057.

DISCLOSURE OF THE INVENTION

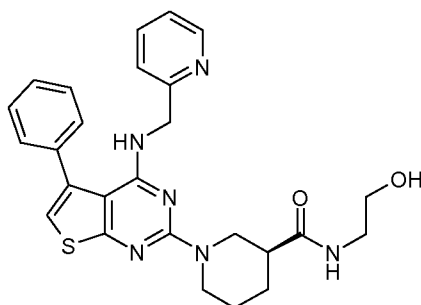
A first aspect of the invention provides a compound of formula (Ia)

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or a pharmaceutically acceptable ester or salt thereof.

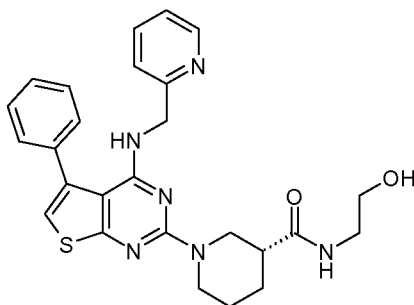
In one embodiment, the compound is of formula (Ib)



(Ib).

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In another embodiment, the compound is of formula (Ic)



(Ic).

- 10 In another embodiment, the compound of formula (Ia) comprises a mixture of the compounds of formulae (Ib) and (Ic). In a further embodiment, the compound of formula (Ia) comprises a racemic mixture of the compounds of formulae (Ib) and (Ic). In an alternative further embodiment, the compound of formula (Ia) comprises an enantiomeric excess of the compound of formula (Ib) or an enantiomeric excess of the
- 15 compound of formula (Ic).

A second aspect of the invention provides a pharmaceutical composition comprising at least one of the above compounds and, optionally, one or more pharmaceutically acceptable excipients.

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The compounds and compositions of the invention are potassium channel inhibitors that are particularly useful for inhibiting potassium channels Kv1.5 or Kv_(ur) for the treatment of cardiac arrhythmia in the atria such as atrial fibrillation. This invention is not limited

to treating cardiac arrhythmias, the compounds also being useful to treat diseases which require potassium channel inhibition (*e.g.* Shieh *et al.*, 2000; Ford *et al.*, 2002).

A third aspect of the invention therefore provides a method of potassium channel inhibition, comprising administering to a subject an effective amount of at least one compound or composition of the invention. This aspect of the invention further provides a compound or composition of the invention for use in potassium channel inhibition. In addition, this aspect of the invention further provides the use of a compound of the invention for the manufacture of a medicament for use in potassium channel inhibition.

As used herein, a “method of potassium channel inhibition” and “use in potassium channel inhibition” include methods and uses for treating or preventing a disorder which responds to the inhibition of potassium channel function. The disorder may be arrhythmia.

The compounds of the invention have advantageous properties over those of the prior art, in particular in terms of potency and/or selectivity.

DETAILED DESCRIPTION OF THE INVENTION

Racemic mixture

A “racemic mixture” contains approximately equal amounts of the compounds of formula (Ib) and formula (Ic). In other words, a compound or composition comprising a “racemic mixture” of the compounds of formula (Ib) and formula (Ic) contains an approximately 1:1, or 50:50, mixture of the compounds.

Enantiomeric excess

A compound or composition comprising an “enantiomeric excess” of the compound of formula (Ib) or of the compound of formula (Ic) comprises more of that enantiomer than the other (also known as a scalemic mixture).

The enantiomeric excess is the excess of one compound over the other, expressed as a percentage of the whole. For instance, a 98:2 mixture of the compound of formula (Ib) to the compound of formula (Ic) has a 96 % enantiomeric excess of the compound of formula (Ib). Thus, the compounds and compositions of the invention may comprise an enantiomeric excess of the compound of formula (Ib) of at least 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, or 99 %.

25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 % or up to 100 % (*i.e.* enantiomerically pure, up to the detection limit of purity). Alternatively, the compounds and compositions of the invention may comprise an enantiomeric excess of the compound of formula (Ic) of at least 5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %, 40 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 % or up to 100 %.

R and S nomenclature

As used herein, the term “R” or “S” isomer refers to the two possible enantiomers according to the Cahn-Ingold-Prelog system adopted by the International Union of Pure and Applied Chemistry (IUPAC). Thus, the compound of formula (Ib) is the “S-isomer” and the compound of formula (Ic) is the “R-isomer”.

Pharmaceutically acceptable ester or salt thereof

The term “pharmaceutically acceptable ester” includes compounds of the invention in which the hydrogen atom of the alcohol group may be replaced to form an ester (*e.g.* the hydrogen atom may be replaced by $-\text{C}(\text{O})\text{C}_{1-6}\text{alkyl}$).

The term “pharmaceutically acceptable salt” includes a salt prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic or organic acids and bases.

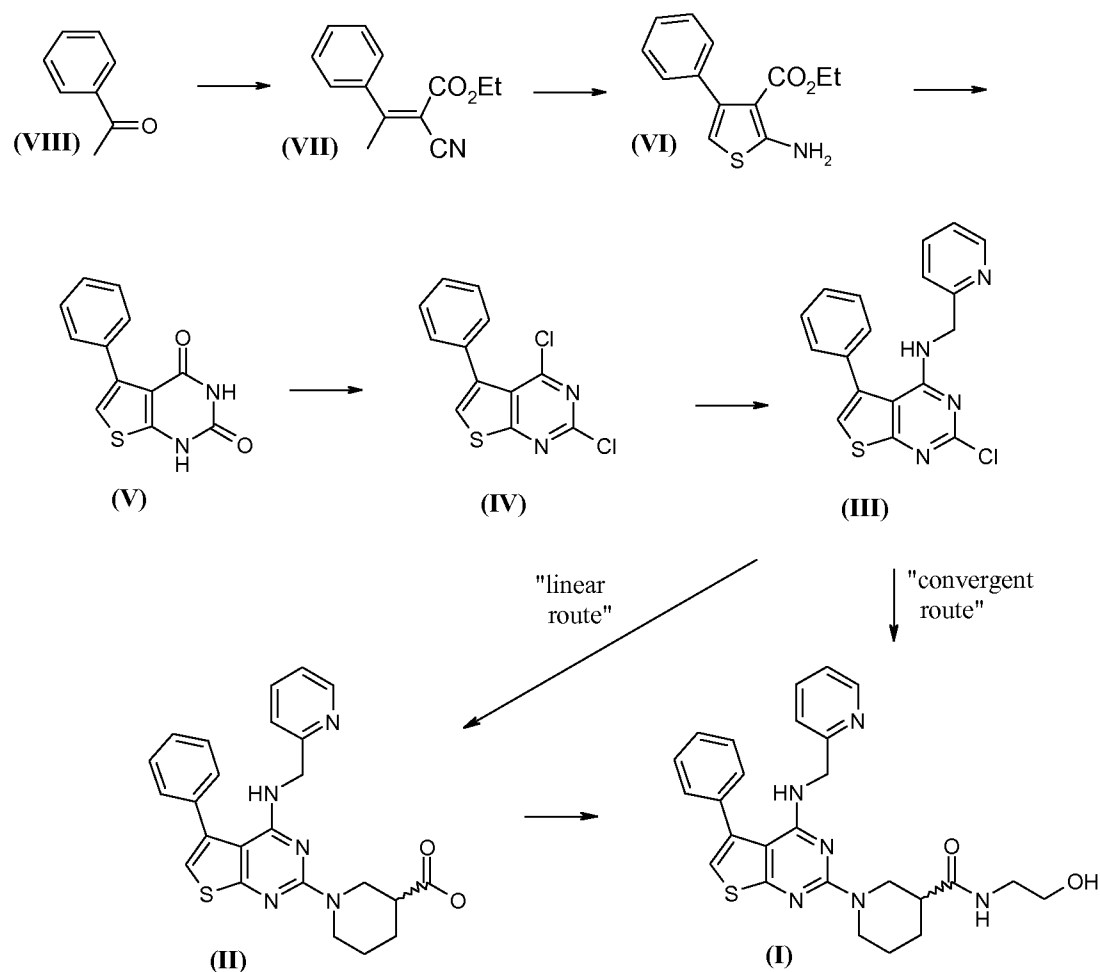
Pharmaceutically acceptable acid addition salts of the compounds of the invention include, but are not limited to, those of inorganic acids such as hydrohalic acids (*e.g.* hydrochloric, hydrobromic and hydroiodic acid), sulfuric acid, nitric acid, and phosphoric acids. In addition, pharmaceutically acceptable acid addition salts of the compounds of the invention include, but are not limited to, those of organic acids such as aliphatic, aromatic, carboxylic and sulfonic classes of organic acids, examples of which include: aliphatic monocarboxylic acids such as formic acid, acetic acid, propionic acid or butyric acid; aliphatic hydroxy acids such as lactic acid, citric acid, tartaric acid or malic acid; dicarboxylic acids such as maleic acid or succinic acid; aromatic carboxylic acids such as benzoic acid, p-chlorobenzoic acid, phenylacetic acid, diphenylacetic acid or triphenylacetic acid; aromatic hydroxyl acids such as o-hydroxybenzoic acid, p-

hydroxybenzoic acid, 1-hydroxynaphthalene-2-carboxylic acid or 3-hydroxynaphthalene-2-carboxylic acid; and sulfonic acids such as methanesulfonic acid, ethanesulfonic acid or benzenesulfonic acid. Other pharmaceutically acceptable acid addition salts of the compounds of the invention include, but are not limited to, those of
5 glycolic acid, glucuronic acid, furoic acid, glutamic acid, anthranilic acid, salicylic acid, mandelic acid, embonic (pamoic) acid, pantothenic acid, stearic acid, sulfanilic acid, algenic acid, and galacturonic acid.

Pharmaceutically acceptable basic salts of the compounds of the invention include, but
10 are not limited to, metal salts such as alkali metal or alkaline earth metal salts (*e.g.* sodium, potassium, magnesium or calcium salts) and zinc or aluminium salts. In addition, pharmaceutically acceptable basic salts of the compounds of the invention include, but are not limited to, salts formed with ammonia or pharmaceutically acceptable organic amines or heterocyclic bases such as ethanolamines (*e.g.*
15 diethanolamine), benzylamines, N-methyl-glucamine, amino acids (*e.g.* lysine) or pyridine.

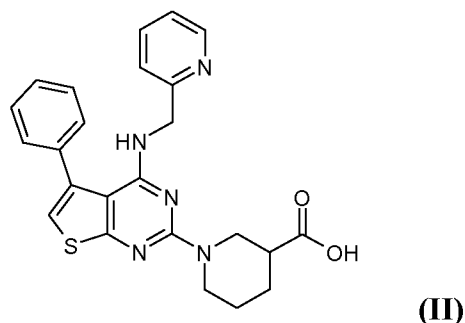
Synthesis

Compounds of formula (I) may be prepared as the racemate, as a scalemic mixture, or as a chirally pure enantiomer using routes described in the scheme 1 below:



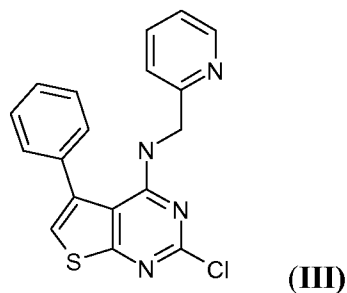
This includes the preparation of compounds of formula (I) using the “linear route” analogous to the synthetic route disclosed in WO2004/111057 from compounds of formula (II) and aminoethanol. Typically, this reaction is carried out using a coupling reagent such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) or 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) utilising standard methods familiar to those skilled in the art such as reaction in solvent such as tetrahydrofuran, acetonitrile or dimethylformamide at a range of temperatures from ambient to reflux temperature. Alternatively, compounds of formula (I) may be prepared from compounds of formula (III) by displacement of the 2-chloro substituent with a compound of formula (IX) in the presence of a base such as N,N-diisopropylethylamine

and a solvent such as N-methyl pyrrolidinone with conventional heating or microwave irradiation.



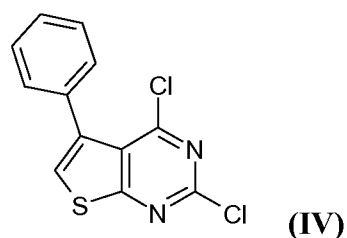
- 5 Compounds of formula (II) may be prepared from a compound of formula (III) by displacement of the 2-chloro substituent with commercially available nipecotic acid, in the presence of a base such as N,N-diisopropylethylamine and a solvent such as N-methyl pyrrolidinone with conventional heating or microwave irradiation.

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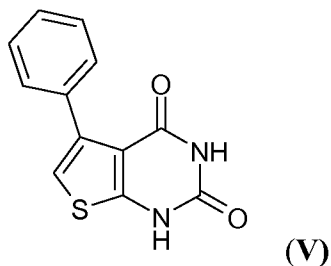


- Compounds of formula (III) are readily synthesised from compounds of formula (IV) by
 15 a nucleophilic substitution reaction with 2-aminomethylpyridine, optionally in the presence of a solvent and a base, and optionally at elevated temperature or with microwave irradiation. Preferably the solvent (if present) is an alcohol, preferably ethanol and the base is a hindered nitrogen base such as triethylamine. The reaction is carried out at ambient temperatures.

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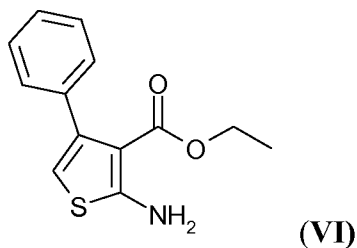


A compound of formula (IV) may be synthesised by reaction of a compound of formula
5 (V) with a chlorinating reagent such as phenylphosphonic dichloride or phosphorous
oxychloride.



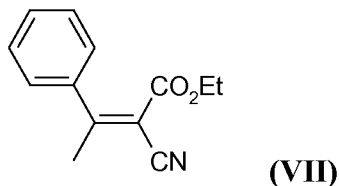
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Compounds of formula (V) may be synthesised by the reaction of a compound of
formula (VI) with an alkali metal cyanate, preferably potassium cyanate.



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A compound of formula (VI) can be prepared by the “Gewald reaction” in which a
compound of formula (VII) is reacted under basic conditions and in a suitable solvent
20 such as ethanol, with powdered sulphur. Preferably the base is diisopropylethylamine
(Hünig’s base) and the solvent may be an alcohol, preferably ethanol, and the reaction is
carried out between 25 and 65°C.

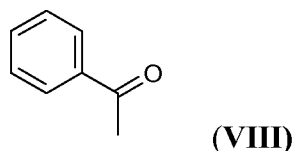


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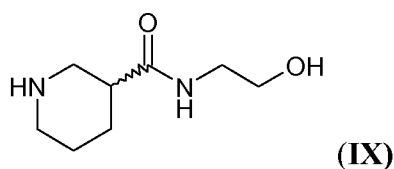
Compounds of formula (VII) can be prepared by the Knoevenagel condensation reaction
by heating compound of formula (VIII) with ethylcyanoacetate (NCCH₂CO₂Et) in the

presence of an acid and ammonium acetate in a suitable solvent such as toluene, optionally with azeotropic water removal. Preferably the acid is acetic acid. This gives the alkylidene cyano ester as a pair of (E and Z) geometric isomers.

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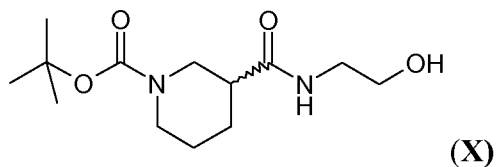
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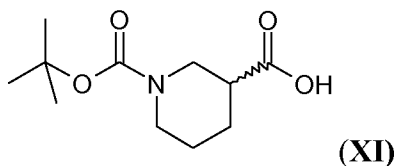
Compound of formula (IX) may be prepared from compound of formula (X) by hydrolysis of the *t*-butyl carbamate (BOC) protecting group with a strong acid in a solvent such as dichloromethane. Typically the acid is trifluoroacetic acid.

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25 Compound of formula (X) may be prepared from a compound of formula (XI) and aminoethanol. Typically, this reaction is carried out using a coupling reagent such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) or 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) utilising standard methods familiar to those skilled in the art such as reaction in solvent such as tetrahydrofuran,
30 acetonitrile or dimethylformamide at a range of temperatures from ambient to reflux temperature.

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5 *Pharmaceutical compositions*

As discussed herein, the compounds of the invention are useful in the treatment of various conditions. Thus, the second aspect of the invention provides a pharmaceutical composition or formulation comprising at least one compound of the invention and optionally one or more pharmaceutically acceptable excipients.

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Typical pharmaceutically acceptable excipients include:

- diluents, *e.g.* lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine;
- lubricants, *e.g.* silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol;
- binders, *e.g.* magnesium aluminum silicate, starch paste, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone;
- disintegrants, *e.g.* starches, agar, alginic acid or its sodium salt, or effervescent mixtures; and/or
- absorbants, colorants, and/or sweeteners.

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The compositions of the invention may be presented in unit dose forms containing a predetermined amount of each active ingredient per dose. Such a unit may be adapted to provide 5-100mg/day of the compound, preferably either 5-15mg/day, 10-30mg/day, 25-50mg/day 40-80mg/day or 60-100mg/day. For compounds of the invention, doses in the range 100-1000mg/day are provided, preferably either 100-400mg/day, 300-600mg/day or 500-1000mg/day. Such doses can be provided in a single dose or as a number of discrete doses. The ultimate dose will depend on the condition being treated, the route of administration and the age, weight and condition of the patient and will be at the doctor's discretion.

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The compositions of the invention 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
5 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
10 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
15 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
20 ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

For applications to the eye or other external tissues, for example the mouth and skin, the formulations are preferably applied as a topical ointment or cream. When formulated in
25 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 administration to the eye include eye
30 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
5 suppositories or 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
10 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
15 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.

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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
25 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
30 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 also include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration
5 may include flavouring agents.

MODES FOR CARRYING OUT THE INVENTION

The following protocols describe preparation of:-

- 10 1. racemate made using “convergent route” (Examples 1 to 6)
2. enantiomers made using “convergent route” (Examples 1 to 5 and 7 to 12)
3. enantiomers made using “linear route” (Examples 1 to 5 and 13 to 16)

Synthesis and determination of enantiomers

- 15 The desired enantiomerically pure compound was obtained by the careful selection of reagents and the use of appropriate experimental conditions and sequence in particular with regard to steps forming the chiral center and subsequent reaction. It was determined during the course of synthesis that the “linear route” was less prone to racemisation as the final amide forming bond could be carried out at lower temperature as opposed to the
20 “convergent route” which provided better yields but with detectable racemisation.

For the “linear route”, pure enantiomers of the nipecotic acid were obtained by classical resolution of cheap commercially available racemic nipecotic acid using 1-(S)-camphor sulfonic acid as the resolving agent, determining ee analysis after forming a BOC
25 derivative of a sample.

The enantiomeric purity was determined by Chiral HPLC.

Analytical methods

- 30 Proton magnetic resonance (^1H NMR) spectra were recorded on a Varian 400MHz Mercury Plus spectrometer. All spectra were determined in dmso-d₆ unless otherwise stated and chemical shifts are reported in (sigma) units downfield from the internal standard tetramethylsilane (TMS) and interproton coupling constants are reported in

Hertz (Hz), splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; dd, doublet of doublet; dt, doublet of triplet; bs, broad singlet; dq, doublet of quartet.

IR spectra were determined on a Perkin Elmer Spectrum One instrument.

- 5 Mass spectra were determined on an Agilent 6310 Ion trap instrument.

HPLC analysis (method (a)) was carried out on a Waters 2695 system using ZORBAX SB C-18 (4.6 x 50mm) column;

Mobile phase: A: 0.05%TFA (AQ,) B: 0.05% TFA (MeCN); T%B: 0/20, 5/90, 8/90, 8.1/20; flow rate 1.0mL/min;

- 10 Chiral column: Chiralpak IC (4.6X250mm)5u, mobile phase: A: Hexane, B: EtOH (70:30); flow rate 0.8ml/min run over 40 minutes.

Melting points were determined on an EX-Melt instrument (Model: MPA120).

- Alternatively, HPLC analysis (method (b)) was carried out with: Waters 616 fluid
15 handling system, Waters 996 photodiode array detector.

Chiral column: Daicel Chiralpak AD-H (Chiral technologies) reporting chiral purity at 244nm; mobile phase 80%Hexanes:20%EtOH; flow rate 0.8ml/min; temp 40 °C.

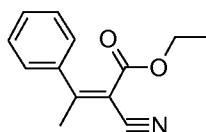
Mass spectra were determined on an Agilent 1100 series instrument (Model: G1946C).

- 20 Using the information outlined herein the following compounds can be synthesised which are given by way of example only. The pharmacological profile of compounds of the present invention can readily be assessed by those skilled in the art using routine experimentation, such as procedures and techniques illustrated herein and described in detail in Ford *et al.*, 2002.

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Example 1

(Z)-2-Cyano-3-phenyl-but-2-enoic acid ethyl ester (VII)



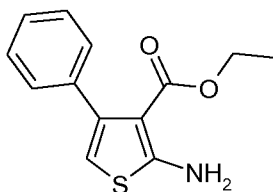
30

A stirred mixture of acetophenone (VIII) (180g, 1.5mol), ethyl cyanoacetate (170g, 1.3mol), ammonium acetate (23.1g), acetic acid (72g) and toluene (300ml) was heated under reflux for 18 hours while water was removed from the reaction by azeotropic distillation. The mixture was allowed to cool to ambient temperature, toluene (100ml) was added, then the mixture was washed with water (3 x 100ml). The combined aqueous washings were shaken with toluene (50ml), then the combined toluene solutions were dried over magnesium sulphate, filtered and the solvent was removed *in vacuo*. The residual oil was distilled under reduced pressure to give 2-cyano-3-phenyl-but-2-enoic acid ethyl ester as an oil (309g) which was used without further purification.

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Example 2

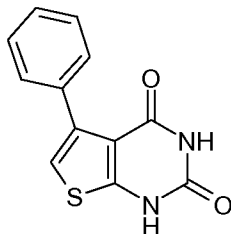
2-Amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (VI)



15

2-Cyano-3-phenyl-but-2-enoic acid ethyl ester (513.25g, 2.3mol) was added at ambient temperature to a vigorously-stirred suspension of powdered sulfur (76g, 2.3mol) in ethanol (500ml). Diethylamine (200ml) was added in portions over 20 minutes, during which time the temperature of the reaction rose to 62°C. The mixture was allowed to cool to 36°C, then it was heated to 50°C and stirring at that temperature was continued for 1hr. After this time, stirring was discontinued, the hot solution was removed by decantation from unreacted sulfur, then it was allowed to cool to ambient temperature. The resulting solid was collected by filtration, washed with a little cold ethanol and dried *in vacuo* to give 2-amino-4-phenylthiophene-3-carboxylic acid ethyl ester as an orange solid (195g) which was used without further purification.

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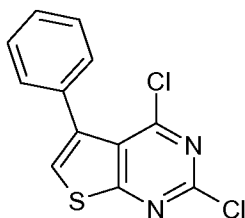
Example 3**5-Phenyl-1H-thieno[2,3-d]pyrimidine-2,4-dione (V)**

5

2-Amino-4-phenyl-thiophene-3-carboxylic acid ethyl ester (2.0g, 8.1mmol), and Potassium Cyanate (Aldrich, 2.0g, 24.3mmol) were added to glacial acetic acid (VWR, 20ml) and stirred at ambient temperature for 18h. The reaction was diluted with water (50ml) and the resultant precipitate filtered, washed with water and dried to a damp cake. The solid was suspended in water (100ml) and made alkaline to pH 12-14 by the addition of concentrated sodium hydroxide. The resultant suspension was heated at 100°C for 2h with stirring, then cooled to ambient temperature and acidified by the addition of glacial acetic acid. The resulting solid was collected by filtration, washed with water and dried in vacuo at 40°C to give 5-Phenyl-1H-thieno[2,3-d]pyrimidine-2,4-dione as a white solid. Yield = (1.1g, 56%).

Example 4**2,4-Dichloro-5-phenyl-thieno[2,3-d]pyrimidine (IV)**

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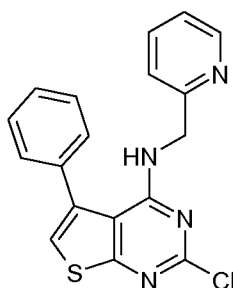


A stirred mixture of 5-Phenyl-1H-thieno[2,3-d]pyrimidine-2,4-dione (1.07g, 4.39mmol) and phenyl phosphonic dichloride (Aldrich, 10ml, excess) was heated at 150°C for 7h then allowed to stand at ambient temperature for 18hrs. The resulting dark solution was

poured into ice-water and extracted with DCM (3 x 150ml). The combined extracts were washed with saturated sodium hydrogen carbonate solution (150ml) and dried (MgSO₄). The solvent was removed in vacuo and the oily residue triturated with 40-60°C petroleum ether to give 2,4-Dichloro-5-phenyl-thieno[2,3-d]pyrimidine as a pale yellow solid. Yield = (0.82g, 66%).

Example 5

(2-Chloro-5-phenyl-thieno[2,3-d]pyrimidin-4-yl)-pyridin-2-ylmethyl-amine (III)



10

A mixture of 2,4-Dichloro-5-phenyl-thieno[2,3-d]pyrimidine (1.77g, 6.3mmol), 2-aminomethylpyridine (Aldrich, 782μl, 7.6mmol), and triethylamine (VWR, 1.06ml, 7.63 mmol) were refluxed in ethanol (30ml) for 3 hrs. On cooling, the reaction was poured into water (300ml) and stirred for 1 hr. The resulting precipitate was filtered, washed with water (2 x 30ml) and dried under vacuum at 40°C to give (2-Chloro-5-phenyl-thieno[2,3-d]pyrimidin-4-yl)-pyridin-2-ylmethyl-amine as a pale-yellow solid. Yield = (1.55g, 70%).

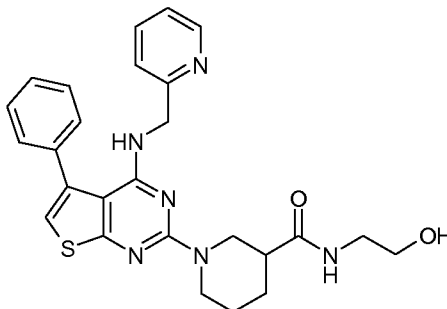
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Racemate – convergent route

Example 6

(Racemic) 1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide (Ia)

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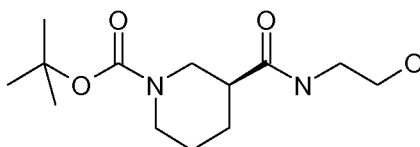


(2-Chloro-5-phenyl-thieno[2,3-d]pyrimidin-4-yl)-pyridin-2-ylmethyl-amine (44mg, 0.124mmol), Piperidine-3-carboxylic acid(2-hydroxyethyl)amide (Fluorochem, 32mg, 0.188mmol, 1.5eq) and N,N-diisopropylethylamine (Aldrich, 0.188mmol) were dissolved in N-Methyl Pyrrolidinone (1.5ml) in a Biotage microwave tube and heated to 200°C and maintained at this temperature for 30 min. On cooling, the solvents were removed in vacuo. The residue was triturated with DCM (2x10ml) and the extracts combined, concentrated and purified by prep TLC (eluent 10% MeOH/DCM) to give the product as a yellow oil, which slowly solidified on standing to a waxy solid. The waxy solid may be converted to a free-flowing powder by stirring in diethyl ether for 1-2h (0.5g in 10ml). Yield=18.3mg (30%)

20 *Enantiomers – convergent route*

Example 7

(S)-3-(2-Hydroxy-ethylcarbamoyl)-piperidine-1-carboxylic acid tert-butyl ester



25

(S)-Piperidine-1,3-dicarboxylic acid 1-tert-butyl ester (500mg, 2.2mmol), HATU (833 mg, 2.2mmol) and Di-isopropylethylamine (761 μ L, 4.4mmol) were stirred in dry DCM (10ml) in an ice bath for 5 min, then at room temperature for 5 min. Ethanolamine (198 μ L, 3.28mmol) was added and the reaction stirred at room temperature for 3 hrs. The
5 reaction was diluted with DCM (40ml), washed with water (50ml), the DCM layer separated and dried (MgSO_4) and concentrated. The residue was columned on silica (20g isolate). Eluting: MeOH/DCM 0-5% 5CV, MeOH/DCM 5%-5% 10CV, MeOH/DCM 5-10% 5CV. TLC visualized with KMnO_4 . This yielded the product as a clear oil (327mg).

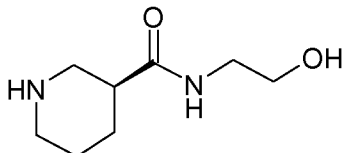
10 Similarly was prepared:

Example 8

(R)-3-(2-Hydroxy-ethylcarbamoyl)-piperidine-1-carboxylic acid tert-butyl ester

Example 9

15 **(S)-Piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide**



The product of the above reaction was stirred in 1:1 TFA/DCM for 2hrs then
20 concentrated *in vacuo* to an oil. This was dissolved in MeOH (5ml) and loaded onto a 5g SCX cartridge. The cartridge was washed with MeOH (10ml), then the product eluted with 2M NH_3 /MeOH (10ml). The fraction was concentrated to give a white solid. Yield = 260mg.

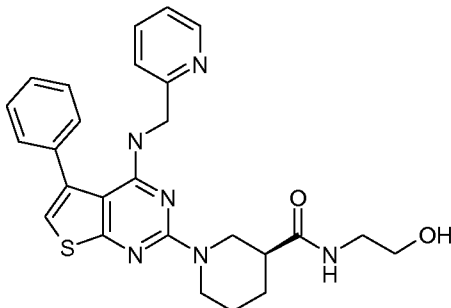
25 Similarly was prepared:

Example 10

(R)-Piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide

Example 11

(S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide (Ib)



- 5 (S)-Piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide was reacted with (2-Chloro-5-phenyl-thieno[2,3-d]pyrimidin-4-yl)-pyridin-2-ylmethyl-amine as in Example 6 above to give (S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide as a yellow foam (188mg).

- 10 Similarly was prepared:

Example 12

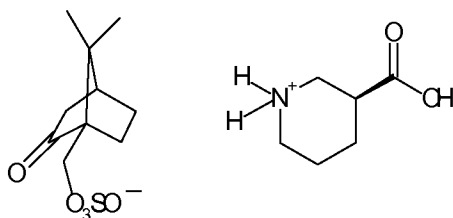
(R)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide (Ic)

- 15 *Enantiomers – linear route via*

Example 13

(S)-Nipecotic acid-(S)-Camphorsulfonate salt

Chiral resolution of (S) Nipecotic acid from commercial racemic mixture

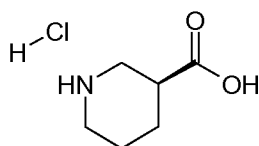


To a solution of (S)-camphorsulfonic acid (18kg, 77mol) in acetone (127kg) at 55–58 °C, a solution of (R,S)-nipecotic acid (10kg, 77mol) in water (20kg) was quickly charged. The mixture was maintained at 55–58 °C until all solids were dissolved. The

solution was slowly cooled to 20–25 °C to precipitate the salt, then stirred overnight, and isolated. To further increase the diastereomeric purity, the resulting salt was re-crystallized from acetone (16kg) and water (4kg) at 55–58 °C. Again, the hot solution was cooled to 20–25 °C, stirred overnight, and isolated to give the purified (S)-nipecotic acid-(S)-camphorsulfonate salt (14kg).

Example 14

(S)-Piperidine-3-carboxylic acid hydrochloride



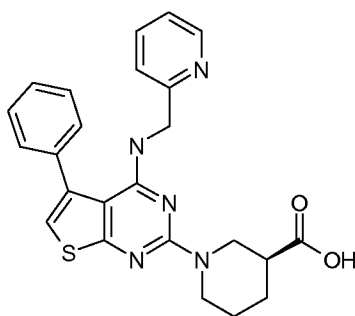
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(S)-Piperidine-1,3-dicarboxylic acid 1-tert-butyl ester (20kg, 87.2mol) was slurried in acetic acid (189kg) and cooled to 15 °C. An excess of hydrogen chloride gas (9.6kg) was charged and stirred for ~4 hours to complete deprotection. The slurry was isolated and filter-cake rinsed with acetic acid (2 x 31.5kg). The filter cake was then vacuum dried to obtain product (14.4kg).

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Example 15

(S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid



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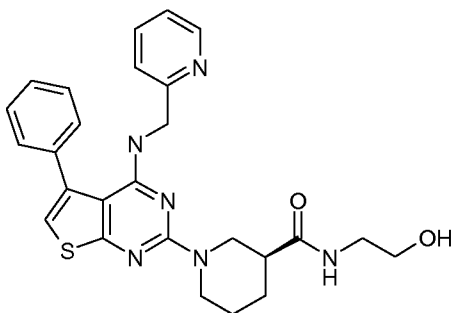
(2-Chloro-5-phenyl-thieno[2,3-d]pyrimidin-4-yl)-pyridin-2-ylmethyl-amine (5.9kg, 16.7mol) and (S)-nipecotic acid hydrochloride (4.15kg, 25.1mol) were dissolved in butyronitrile (13.9kg). An excess of diisopropylethylamine (8.6kg, 66.9mol) was added and the mixture heated to 110 °C for 24 to 48 hours to complete reaction. With coupling complete (<2% nipecotic acid remaining), the reaction was cooled to room temperature

25

and water (29kg) was charged. The mixture pH was adjusted to ~10 with 25% aqueous sodium hydroxide (4.5L) and the layers separated. The product aqueous layer was extracted twice with ethyl acetate (15.9L) then methylene chloride (23.5kg) was added to the aqueous layer and the pH adjusted to ~2.5 with concentrated hydrochloric acid (6.3kg). The layers were separated and the aqueous layer re-extracted with methylene chloride (2 x 15.7kg). The methylene chloride layers were combined and washed with water (18kg) then dried over sodium sulfate (5.9kg) and product solution was held for processing in next step (Example 16).

10 Example 16

(S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide (Ib)



15 (S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid solution (7.4kg, 16.63mol) (from Example 15) was cooled to 0 °C and diisopropylamine (4.51kg, 35mol) and ethanolamine (2.03kg, 33.3mol) were added. Maintaining the reaction temperature below 10 °C, bezotriazolyl tetramethyluronium-BF₄ (TBTU) (5.9kg, 18.3mol) was charged in portions then stirred
20 at ~5 °C until the coupling was complete. The reaction solution was then filtered to remove TBTU salts and washed with water (22.2 L), followed by two washes with citric acid/sodium hydroxide buffer aqueous solution (pH ~5) (2.88kg, 15mol), and finally with a brine solution (4L). Subsequently, the mixture was charged with butyronitrile (17.4L) and partially stripped to precipitate the diastereomeric product. The slurry was
25 filtered to remove diastereoisomer and the filtrates stripped further to ~1/2 volume. To the mix heptanes (30.4L) was charged to precipitate product and the slurry cooled to room temperature. The slurry was filtered, rinsed with heptanes (10.1L), and vacuum dried to obtain product (4.1kg).

(R)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide (Ic) may be prepared according to a route analogous to Examples 13 to 16.

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Example 17

Analytical data for the compounds represented by the above examples are shown in the table below.

Ex	NMR spectrum ¹ H (400MHz; dms0-d6)	HPLC (RT) mins	Mass Spec (M ⁺)	Chiral HPLC (method (a))	MP (°C)	FT-IR λ _{max} (cm ⁻¹)
2	0.91 (3H, t), 3.96 (2H,q), 6.15 (1H, s)7.3 (5H, m)	4.8	248 (99.5%)			
3	6.67 (1H, s), 7.3 (3H, m), 7.47 (2H, m)	(uplc)	245 (98.9%)			
4	7.51 (5H, m), 7.99 (1H, s)	(uplc)	282 (92%)			
5	4.64 (2H, s), 7.07 (1H, m), 7.23 (1H, m), 7.4 (1H, d), 7.55 (6H, m), 7.75 (1H, dt), 8.21 (1H, m)	(uplc 1.75)	353 (97.6%)			
6	1.3 (1H, m), 1.6 (2H, m), 1.8 (1H, m), 2.3 (1H, m), 2.8 (2H, m), 3.1 (2H, m), 3.4 (2H, m), 4.6 (5H, m), 6.3 (1H,m), 6.95 (1H,s), 7.2-7.3 (2H,	2.67 (uplc 0.91)	489 (99.8%)		190- 194	3338, 3298, 3098, 3009, 2931, 2847, 1642, 1556, 1517, 1504, 1484, 1438, 1386, 1321,

	m), 7.5 (5H, m), 7.7 (1H, m), 7.9 (1H, m), 8.3 (1H, m)					1300, 1255, 1220, 1203, 1139, 1062
11 (S)	1.3 (1H, m), 1.6 (2H, m), 1.8 (1H, m), 2.3 (1H, m), 2.8 (2H, m), 3.2 (2H, m), 3.4 (2H, m), 4.6 (5H, m), 6.3 (1H, t), 6.95 (1H,s), 7.2 (1H, dd), 7.3 (1H, d), 7.5 (5H, m), 7.7 (1H, dd), 7.9 (1H, m), 8.3 (1H, m)	-	489.3	98.3% RT=18.4 min	61- 65	3426, 3357, 1649
12 (R)	1.3 (1H, m), 1.6 (2H, m), 1.8 (1H, m), 2.3 (1H, m), 2.8 (2H, m), 3.2 (2H, m), 3.4 (2H, m), 4.6 (5H, m), 6.3 (1H, t), 6.95 (1H,s), 7.2 (1H, dd), 7.3 (1H, d), 7.5 (5H, m), 7.7 (1H, dd), 7.9 (1H, m), 8.3 (1H, m)	-	489.3	97.6% RT= 14.87 MIN	71- 76	3425, 3352, 1649

Example 18**Kv1.5 Electrophysiology Method**

The ability of the compounds of the invention to inhibit the Kv1.5 potassium channel
5 was measured in an electrophysiology experiment, using recombinant cells expressing the channel of interest in a whole cell patch clamp experiment.

The external bathing solution contained (in mM): 150 NaCl, 10 KCl, 3 MgCl₂, 1 CaCl₂, 10 HEPES, pH 7.4. Patch pipettes were filled with an electrode solution of composition
10 (in mM): 160 KCl, 0.5 MgCl₂, 10 HEPES, 1 EGTA, pH 7.2 with KOH.

Compounds were dissolved in DMSO (100 %) and freshly made up in the external bath at the desired concentration (final DMSO concentration = 0.1%). All experiments were conducted at room temperature.

5

For whole-cell patch-clamp studies cells (CHO stably transfected with hKv1.5) were seeded onto glass coverslips before recordings were made. Cells were seeded in sterile 30 mm Petri dishes at a density to enable isolated cells to be selected for patch clamp experiments. The dishes were stored in a humidified, gassed (5 % CO₂) incubator at 10 37 °C until use.

Whole-cell patch-clamp recordings of membrane currents were made following gigaohm seal formation between the patch electrode and the cell using HEKA EPC-9/10 amplifiers controlled by Pulse Software (Ver8.5x/8.6x, HEKA, Germany). Coverslips 15 seeded with cells were placed in a recording chamber mounted on the stage of an inverted microscope. During the experiment the cell of interest was continuously superfused with bath solution delivered via a cannula placed in close proximity to the cell to enable control of the extracellular solution environment. Only those cells with a current >500pA were used for experiments. During experiments total series resistance 20 did not exceed 10 MΩ and was compensated by a minimum of 70 %. Leak subtraction was performed online using a P/n protocol in Pulse.

Electrophysiology voltage-step protocols and analysis of data was performed as follows. Data was sampled at 5kHz, and filtered with a -3 dB bandwidth of 2.5kHz. Cells were 25 held at a voltage of -80mV. Currents were evoked by a depolarising voltage step to 0mV (900ms) before repolarisation first to -40mV (100ms) before returning to -80mV. The command waveform was repeatedly applied every 5s throughout the experiment. Mean currents during 75-95% of the depolarising step to 0mV were analysed using Pulsefit software (v8.x, HEKA, Germany). The voltage protocol was applied to achieve a stable 30 current baseline in bath before the test substance was superfused via the cannula; fluid exchange took approximately 15 s. The test substance was allowed to equilibrate during which time voltage protocol was repeatedly applied and recorded. Percentage inhibition

of the current in the presence of test substance was calculated relative to the control pre-drug value.

	Compound	Kv1.5 IC ₅₀ (nM)
Racemate	1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide	9
(S) enantiomer (Ib)	(S)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide	27
(R) enantiomer (Ic)	(R)-1-{5-Phenyl-4-[(pyridin-2-ylmethyl)-amino]-thieno[2,3-d]pyrimidin-2-yl}-piperidine-3-carboxylic acid (2-hydroxy-ethyl)-amide	5

5

Example 19

Selectivity screening

A compound of the invention and a comparative compound were screened in the following assays:

- 10 1. Nav1.5; screened on the Sophion QPatch using CHO cells expressing hNav1.5 currents, stably transfected with heterologous hNav1.5 cDNA.
2. Kv4.3; screened by manual whole cell patch clamp using CHO cells expressing hKv4.3 currents, stably transfected with heterologous Kv4.3 cDNA.
3. hERG; screened by manual whole cell patch clamp using HEK293 cells expressing
15 hERG currents, stably transfected with heterologous hERG cDNA.
4. Kir3.1/3.4; screened by manual whole cell patch clamp using HEK293 cells expressing rKir3.1/3.4 currents, stably transfected with heterologous rKir3.1 and rKir3.4 cDNA.
5. KCNQ1; screened by manual whole cell patch clamp using CHO cells expressing
20 hKCNQ1/hmink currents, stably transfected with heterologous hKCNQ1/hmink cDNA.

6. Kir2.1; screened by manual whole cell patch clamp using HEK293 cells expressing hKir2.1 currents, stably transfected with heterologous hKir2.1 cDNA.
7. Cav1.2; screened using GH3 cells or HEK293 cells expressing hCav1.2 currents, stably transfected with heterologous hCav1.2 cDNA.
- 5 The selectivity ratios for Kv1.5 as compared to the above ion channels are shown below:

Ion Channel	Compound of the invention	Comparative compound
Nav1.5	>350x	~120x
Kv4.3	~500x	17x
hERG	~275x	54x
Kir3.1/3.4	~265x	~42x
KCNQ1	~1200x	~300x
Kir2.1	>400x	>1245x
Cav1.2	>1200x	>1245x

Example 20

Inhibition of the I_{Kur} current in dissociated human atrial myocytes

Isolation of human atrial myocytes

- 10 Specimens of human atrial appendage (either right or left) were obtained from patients undergoing a range of cardiac surgical procedures. Tissue was obtained from consenting patients from Papworth Hospital NHS Trust, Cambs. UK. following approval from the Local Research Ethical Approval Committee. The mechano-enzymatic isolation of myocytes was performed using a modified protocol as described by Wang *et al.* (1993)
- 15 and Dobrev *et al.* (2005). Isolated myocytes were suspended in a modified 'Kraftebrühe' (KB) solution until use.

Recording system

- Myocytes were placed into a small-volume recording chamber with a glass-coverslip base, mounted on the stage of an inverted microscope. During the experiment, the cell of
- 20 interest was constantly superfused with bath solution delivered via a cannula placed in close proximity to the cell to enable control of the extracellular solution environment.

Whole-cell patch-clamp recordings of membrane currents were made using a HEKA EPC-9/10 amplifier following Gigaohm seal formation between the patch electrode and the myocyte. Glass patch-pipettes were pulled from borosilicate glass. Only rod-shaped, striated myocytes were selected for use. Capacitance and series resistance were compensated using Pulse software. Voltage-clamp commands were generated using Pulse software and data were recorded onto the hard disk of a PC. Leak subtraction was not performed and cells with significant leak were rejected. Experiments were performed at room temperature. To minimise contamination from other ionic currents, experimental solutions contained 10 mM tetraethylammonium chloride (I_K), 100 nM atropine ($I_{K,ACh}$), 200 μ M $CdCl_2$ ($I_{Ca,L}$; and $I_{Cl,Ca}$), 0.5 mM $BaCl_2$ (I_{K1} and $I_{K,ACh}$). Blockers were used at a concentration that would not be expected to affect I_{Kur} . The sodium current (I_{Na}) was suppressed by using a choline chloride based bath. Depolarising voltage-step were applied every 10s to elicit an outward potassium current composed of a transient and sustained component. The sustained current sensitive to 300 μ M 4-AP was defined as the ultra-rapid delayed rectifier current, I_{Kur} .

Ionic current	Compound of the invention	Comparative compound
hI_{Kur}	11 nM	154 nM

Abbreviations

HGNC	HUGO Gene Nomenclature Committee
$KV_{(ur)}$	Cardiac Ultrarapid Delayed Rectifier
CHO	Chinese Hamster Ovary Cells
IP_3	Inositol Triphosphate
CRAC	Ca^{2+} -Release-Activated- Ca^{2+} Current
DMEM	Dulbecco's Modified Eagle media
DMSO	Dimethyl sulphoxide
FCS	Fetal Calf Serum
EBSS	Earls Balanced Salt Solution
WCPC	Whole-Cell Patch-Clamp
HEK293	Human Embryonic Kidney 293 Cells

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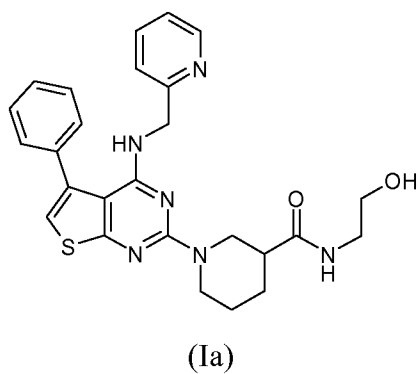
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CLAIMS:

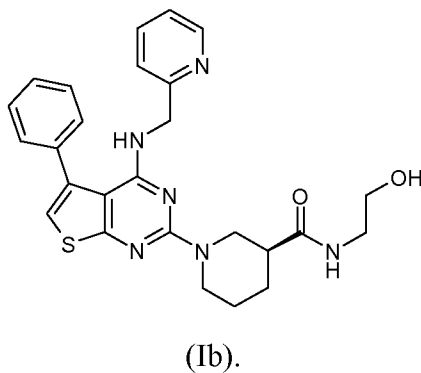
1. A compound of formula (Ia)

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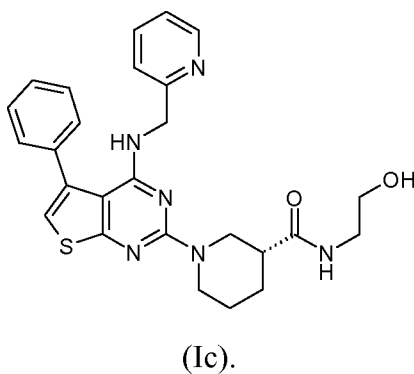
or a pharmaceutically acceptable ester or salt thereof.

- 10 2. The compound of claim 1, wherein the compound is of formula (Ib)



3. The compound of claim 1, wherein the compound is of formula (Ic)

15



4. The compound of claim 1, wherein the compound of formula (Ia) comprises a mixture of the compounds of formulae (Ib) and (Ic).
5. The compound of claim 4, wherein the compound of formula (Ia) comprises a racemic mixture of the compounds of formulae (Ib) and (Ic).
6. The compound of claim 4, wherein the compound of formula (Ia) comprises an enantiomeric excess of the compound of formula (Ib).
- 10 7. The compound of claim 4, wherein the compound of formula (Ia) comprises an enantiomeric excess of the compound of formula (Ic).
8. A pharmaceutical composition comprising at least one compound as claimed in any one of claims 1 to 7 and, optionally, one or more pharmaceutically acceptable excipients.
- 15 9. A compound or composition as claimed in any one of claims 1 to 8 for use in therapy.
- 20 10. A method of potassium channel inhibition, comprising administering to a subject an effective amount of at least one compound or composition as claimed in any one of claims 1 to 8.
11. The method of claim 10, wherein the method is for treating or preventing arrhythmia.
- 25 12. A compound or composition as claimed in any one of claims 1 to 8 for use in potassium channel inhibition.
- 30 13. The compound or composition as claimed in claim 12, wherein the compound or composition is for use in the treatment or prevention of arrhythmia.

14. The use of a compound as claimed in any one of claims 1 to 7 for the manufacture of a medicament for use in potassium channel inhibition.
15. The use of claim 14 wherein the medicament is for use in the treatment or
5 prevention of arrhythmia.