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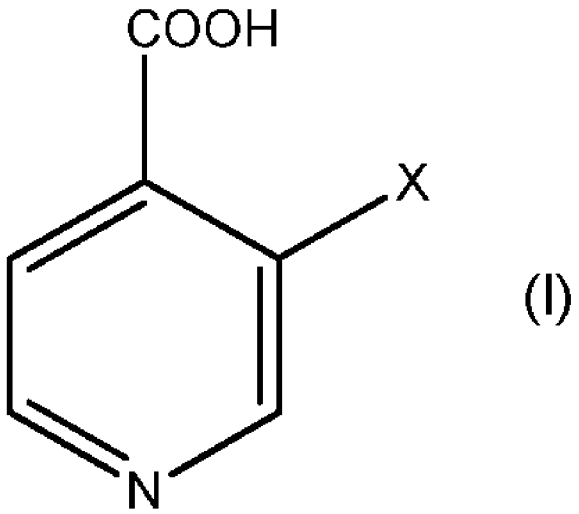
(54) Title: DEMETHYLASE ENZYMES INHIBITORS

(57) Abstract: A compound of formula (I) and its use as an inhibitor of one or more histone demethylase enzymes.



(I)

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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DEMETHYLASE ENZYMES INHIBITORS

Field of the Invention

The present invention relates to compounds, compositions, combinations and medicaments containing said compounds and processes for their preparation. The invention also relates to the use of said compounds, combinations, compositions and medicaments, for example as inhibitors of the activity of one or more JmjC domain-containing histone demethylase enzymes, to modify the epigenetic status of cells and/or the treatment of diseases and conditions mediated by said enzymes, in particular cancer, inflammation and autoimmune diseases.

Background of the Invention

The structure of chromatin is complex and dynamic and has a major effect on gene transcription. Chromatin is the complex combination of DNA and protein that makes up chromosomes. It is found inside the nuclei of eukaryotic cells and is divided between heterochromatin (condensed) and euchromatin (extended) forms. The major components of chromatin are DNA and proteins, including histones. The basic building blocks of chromatin are nucleosomes, each of which is composed of 146 base pairs of DNA wrapped around a histone octamer consisting of 2 copies of each H2A, H2B, H3 and H4. The functions of chromatin are to package DNA into a smaller volume to fit in the cell, to strengthen the DNA to allow mitosis and meiosis, and to serve as a mechanism to control expression and DNA replication. Chromatin contains genetic material serving as instructions to direct cell functions. Changes in chromatin structure are regulated by modifications on histone and DNA methylation. Epigenetic mechanisms do not change the DNA sequence but allow the genes to be expressed differently. Epigenetics modification include numerous mechanisms including DNA methylation and post-translational modification of N-terminal tails of histone proteins such as methylation, acetylation, phosphorylation and ubiquitination.

In recent years many of the target proteins involved in epigenetic control have been identified. These targets can be classified into families that read, write and erase covalent modification (e.g. methylation, acetylation) in histones.

Histone methylation is an abundant epigenetic modification of core histones found in eukaryotic organisms that has been linked to a number of cellular processes including DNA repair, cell cycle progression, cell differentiation and regulation of gene expression. This modification is catalysed by the specific histone methyltransferases (HMTs), lysine methyl transferase and arginine methyl transferase, which introduce methyl groups at lysine (K) or arginine (R) residues respectively. Thus far, histone methylation has been found to occur at six major sites, including histone H3 lysine 4 (H3K4), H3K9, H3K27, H3K36, H3K79 and H4K20. Unlike other modifications, the same lysine residue can be methylated to different degrees to include mono-, di- or trimethyl moieties, which may have different functional consequences. In general, lysine methylation at H3K4, H3K36 and H3K79 is associated with regions of transcriptionally active chromatin, whereas methylation at H3K9, H3K27

and H4K20 is associated with transcriptionally silenced regions (Martin C. and Zhang Y., Nature Rev. Mol. Cell Biol. 2005, 6, 838-849). H3K9 promoter methylation is considered a repressive mark for euchromatic genes (Nielsen et al., Nature 2001,412, 561-565; Shi et al., Nature 2003, 422, 735-738) and is also one of the landmark modifications associated with heterochromatin (Nakayama et al., Science 2001, 292, 110-113) but some studies have also identified association of H3K9 trimethylation (H3K9me3) with actively transcribed genes (Vakoc C. et al, Mol. Cell 2005, 19. 381-391).

Unlike other histone modifications such as acetylation, methylation used to be regarded as a permanent/irreversible modification. However, with the identification of histone demethylases, this process has been shown to be reversible and dynamically controlled. Histone methylation appears thus to be regulated by a complex network that involves a large number of site-specific methylases, demethylases and methyl recognition proteins, which play an important role in controlling the expression of genetic information through transcriptional changes and chromatin structure alterations. Since levels of lysine methylation are known to change during processes such as transcriptional regulation, it was proposed that specific enzymatic activity might remove the methyl groups (Bannister *et al*., 2002 Cell 109, 801-806). Recent work has confirmed the existence of enzymatic demethylation and two separate mechanisms of lysine demethylation have been demonstrated: amine oxidation by Lysine Specific Demethylase 1 (LSD1) and hydroxylation by JmjC-domain containing proteins, which indicate these proteins as being novel histone modifying enzymes that can remove methyl groups on lysines (Shi *et al*., 2004; Cell 119, 941-953; Tsukada *et al*., 2006, Nature 439, 811-816).

The Jumonji protein is the founding member of a group of proteins characterised by a novel structural motif, the JmjC domain. This is an extensive group of demethylase enzymes which can be defined into several families according to sequence similarity within the JmjC domain and the presence of other domains in the full length protein. The JmjC domain of several members of this family has been shown to possess lysine demethylation activity, which is dependent on iron (Fe (II)) and α-ketoglutarate as co-factors (Klose RJ *et al*, Nat Rev Genet. 2006 Sep; 7(9); 715-27). Unlike LSD1, which can only remove mono- and dimethyl lysine modifications, the JmjC-domain-containing histone demethylases (JHDMs) can remove all three histone lysine-methylation states.

JmjD3 (KDM6B) is one of the approximately 30 JmjC family members found in humans, and functions as a specific demethylase of lysine 27 of histone H3 (H3K27). JmjD3 can demethylate both the tri- and dimethylated H3K27-repressive histone marks, thereby facilitating gene transcription. This was first demonstrated in C. Elegans embryogenesis, where JmjD3 was shown to regulate gonadal development through modulation of HOX gene expression (Agger K *et al*, Nature 2007 Oct; 449(7163); 731-734). Further studies have placed JmjD3 at key cell fate decision checkpoints in T lymphocytes (Miller SA *et al*, Genes Dev. 2008 Oct; 22; 2280-2993) and macrophages (Ishii M *et al*, Blood 2009 Oct; 114(15); 3244-3254). In addition, JmjD3 has been demonstrated to regulate the differentiation state of the epidermis (Sen GL *et al*, Genes Dev. 2008 Jul; 22; 1865-1870) and to

activate the tumour suppressor, INK4A-Arf, in response stress induced signals (Agger K *et al*, Genes Dev. 2009 Apr; 23; 1171-1176). JmjD3 also appears to be involved in more acute, externally-driven, inflammatory processes. In macrophages, for example, JmjD3 is rapidly induced through an NF-kB-dependent mechanism in response to bacterial products and inflammatory stimuli (De Santa F *et al*, Cell 2007 Sept; 130; 1083-1094). Moreover, depletion experiments in these cells have demonstrated that JmjD3 participates directly in the inflammatory transcriptional response, although it remains unclear whether this is achieved through demethylation of H3K27me3 at target gene promoters (De Santa F *et al*, EMBO J. 2009 Sept; 28; 3341-3352).

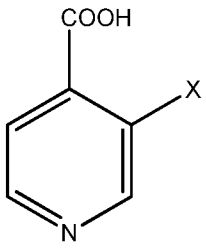
The JmjD2 family of histone demethylases consists of JmjD2A, JmjD2B, JmjD2C, JmjD2D, JmjD2E and JmjD2F, although the latter two genes appear to be pseudogenes (Katoh M (2004). Int J Oncol 24:1623-1628). JMJD2A (1064 aa), JMJD2B (1096 aa), and JMJD2C (1056 aa) proteins are closely related isoforms and consist of JmjN, JmjC, JD2H, and two TUDOR domains, while JMJD2D (523 aa), JMJD2E (506 aa) and JMJD2F (638 aa) consist of JmjN and JmjC domains. Among other substrates, the JmjD2 family members have been shown to catalyse demethylation of H3K9 and remove all three histone lysine-methylation states.

JmjC domain-containing proteins, have been implicated in tumorogenesis and thus have identified histone demethylases as targets of research for anti-cancer therapies (see for example WO2009/114011 and WO2010/043866).

The present inventors have discovered compounds which inhibit the activity at one or more JmjC-domain- containing histone demethylase enzymes.More particularly they inhibit the activity of one or more of the enzymes Jarid1c, JmjD2a, JmjD2c, JmjD2d, JmjD2e and JmjD3. Therefore these compounds may be useful in treating disorders associated with these enzymes including autoimmune diseases, cancer and inflammation.

Summary of the Invention

In one aspect of the present invention, there is provided a a compound of formula (I):



(I)

wherein

X is $-(R^1)_{0-1}-(R^2)_{0-1}-R^3$ or $-R^1-R^4$;

Each R^1 is independently NH, $N(CH_3)$, O;

R^2 is a linker group with a maximum length of 5 atoms between R^1 and R^3 and is selected from:

-CO-C_{1-6}alkyl-

-CO-

-CO-C_{1-6}alkyl-O-

-CO-C_{1-6}alkyl-S-

-CO-C_{1-6}alkyl-O-C_{1-6}alkyl-

-C_{1-6}alkyl-

-C_{1-6}alkyl-O

-C_{1-6}alkyl-SO_2-

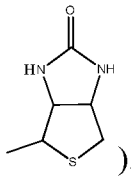
-C_{1-6}alkyl-NH-CO

-C_{1-3}alkyl-C_{3-6}cycloalkyl-C_{1-3}alkyl-O-

wherein each alkyl is straight chain or branched and may be optionally substituted by one or more substituents independently selected from phenyl or -OH;

R^3 is selected from:

a C_{6-12} mono or bicyclic aryl group, (each of which may be optionally substituted one or more times by substituents independently selected from halo, C_{1-6}alkyl, C_{1-6} haloalkyl, C_{1-6}alkoxy, $NHCOC_{1-3}$alkyl, -O-phenyl , -CH_2-phenyl, phenyl (optionally substituted by C_{1-3}alkyl), OH, NH_2, $CONH_2$, CN, -$NHCOC_{1-3}$alkylNH_2, –$NHCOC_{1-3}$alkyl, $NHCOOC_{1-3}$alkyl, -$NHSO_2C_{1-3}$alkyl, -SO_2C_{1-3}alkyl or

-$NHCOC_{1-3}$alkyl-$NHCOC_{1-4}$ alkyl 

).

a 5-12 membered mono or bicyclic heteroaryl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, -C_{1-6} alkyl, -oxo),

a 5 or 6 membered heterocyclic group containing one or more heteromoieties independently selected from N, S, SO, SO_2 or O and optionally fused to a phenyl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, C_{1-3}alkyl)

or a 3-7 membered cycloalkyl (including bridged cycloalkyl) and optionally fused to a phenyl group (and optionally substituted by one or more substituents independently selected from OH, phenyl, -CH_2 phenyl),

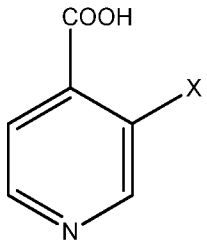
R^4 is selected from:

C_{1-6} straight chain or branched alkyl (optionally substituted by NH_2),

COC_{1-6} straight chain or branched alkyl;

or a pharmaceutically acceptable salt thereof for use in changing the epigenetic status of cells, treating cancer, inflammation or autoimmune diseases

In a further aspect there is a provided a compound of formula (1a)



(Ia)

wherein X is $-(R^1)_{0-1}-(R^2)_{0-1}-R^3$ or $-R^1-R^4$;

Each R^1 is independently NH, $N(CH_3)$, O;

R^2 is a linker group with a maximum length of 5 atoms between R^1 and R^3 and is selected from:

$-CO-C_{1-6}alkyl-$

-CO-

$-CO-C_{1-6}alkyl-O-$

$-CO-C_{1-6}alkyl-S-$

$-CO-C_{1-6}alkyl-O-C_{1-6}alkyl-$

$-C_{1-6}alkyl-$

$-C_{1-6}alkyl-O$

$-C_{1-6}alkyl-SO_2-$

$-C_{1-6}alkyl-NH-CO$

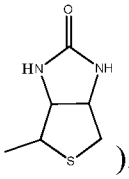
$-C_{1-3}alkyl-C_{3-6}cycloalkyl-C_{1-3}alkyl-O-$

wherein each alkyl is straight chain or branched and may be optionally substituted by one or more substituents independently selected from phenyl or -OH

R^3 is selected from:

a C_{6-12} mono or bicyclic aryl group, (each of which may be optionally substituted one or more times by substituents independently selected from halo, $C_{1-6}alkyl$, C_{1-6} haloalkyl, $C_{1-6}alkoxy$, $NHCOC_{1-3}alkyl$, -O-

phenyl , -CH_2-phenyl, phenyl (optionally substituted by C_{1-3}alkyl), OH, NH_2, $CONH_2$, CN, -$NHCOC_{1-3}$alkylNH_2, –$NHCOC_{1-3}$alkyl, $NHCOOC_{1-3}$alkyl, -$NHSO_2C_{1-3}$alkyl, -SO_2C_{1-3}alkyl or

-$NHCOC_{1-3}$alkyl-$NHCOC_{1-4}$ alkyl ).

a 5-12 membered mono or bicyclic heteroaryl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, oxo),

a 5 or 6 membered heterocyclic group containing one or more heteromoieties independently selected from N, S, SO, SO_2 or O and optionally fused to a phenyl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, C_{1-3}alkyl)

or a 5-7 membered cycloalkyl (including bridged cycloalkyl) and optionally fused to a phenyl group (and optionally substituted by one or more substituents independently selected from OH, phenyl, -CH_2 phenyl),

R^4 is selected from

C_{1-6} straight chain or branched alkyl (optionally substituted by NH_2),

COC_{1-6} straight chain or branched alkyl;

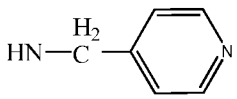
or a pharmaceutically acceptable salt thereof

with the proviso X is not

-NHCO-tert butyl

-NHCO-isobutyl

-OCH_2phenyl



-NHphenyl, or

-NHcyclohexyl

or a pharmaceutically acceptable salt thereof

In a further aspect of the present invention, there is provided a compound of formula (Ia), or a pharmaceutically acceptable salt thereof for use in therapy, in particular in changing the epigenetic status of cells, treating cancer, inflammation or autoimmune diseases.

In a further aspect of the present invention, there is provided a method of changing the epigenetic status of cells, treating cancer, inflammation or autoimmune diseases in a subject comprising administering a therapeutically effective amount of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof.

In a further aspect of the present invention, there is provided the use of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for use in changing the epigenetic status of cells, treating cancer, inflammation or autoimmune diseases.

In a further aspect there is provided a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one further therapeutic agent.

In a further aspect there is provided a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one further therapeutic agent for use in therapy, particularly for changing the epigenetic status of cells and treating cancer, inflammation or autoimmune diseases.

In a further aspect of the invention there is provided a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof for use in the treatment of cancer.

In a further aspect there is provided a method of treating cancer comprising administering to a human in need thereof a therapeutically effective amount of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof.

In a further aspect there is provided the use of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of cancer.

In a further aspect there is provided a combination comprising a compound of formula (I) or (Ia),or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent.

In a further aspect there is provided a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent, for use in therapy.

In a further aspect there is provided a combination comprising a compound of formula (I) or (Ia), or pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent, for use in treating cancer.

In a further aspect there is provided the use of a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent, in the manufacture of a medicament for the treatment of cancer.

In a further aspect there is provided a method of treating cancer, comprising administering to a human in need thereof a therapeutically effective amount of a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent.

In a further aspect there is provided a pharmaceutical composition comprising a combination comprising a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof and at least one further therapeutic agent, particularly at least one anti-neoplastic agent and one or more of pharmaceutically acceptable carriers, diluents and excipients.

In a further aspect there is provided a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof for use in the treatment of inflammation or autoimmune diseases.

In a further aspect there is provided a method of treating inflammation or autoimmune diseases comprising administering to human in need thereof, a therapeutically effect amount of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof.

In a further aspect there is provided the use of a compound of formula (I) or (Ia), or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of inflammation or autoimmune diseases.

Detailed Description of the Invention

Studies have identified JmjC domain-containing proteins as histone demethylases that mediate the reversal of methylation at histone H3K4, H3K9 and H3K36. Such inhibitors are therefore useful in changing the epigenetic status of cells resulting in inhibiting or activating chromatin remodelling by modifying histone methylation and thus in treating disorders associated with such modified histone methylation including cancer and other conditions associated with undesirable cell proliferation, autoimmune and inflammatory diseases or conditions and psychiatric disorders including depression.

As used herein, “a compound of formula (I)” or “a compound or formula (Ia)” includes all solvates, complexes, polymorphs, radiolabelled derivatives (including deuterated derivatives where one or more H are replaced by D), stereoisomers and optical isomers of the compounds of formula (I) or (Ia) and salts thereof. Compounds of formula (Ia) are all compounds of formula (I).

As used herein, the term "effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being

sought, for instance, by a researcher or clinician. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in improved treatment, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function.

The compounds of formula (I) may exist in solid or liquid form. In solid form, compound of the invention may exist in a continuum of solid states ranging from fully amorphous to fully crystalline. The term 'amorphous' refers to a state in which the material lacks long range order at the molecular level and, depending upon the temperature, may exhibit the physical properties of a solid or a liquid. Typically such materials do not give distinctive X-ray diffraction patterns and, while exhibiting the properties of a solid, are more formally described as a liquid. Upon heating, a change from solid to liquid properties occurs which is characterized by a change of state, typically second order ('glass transition'). The term 'crystalline' refers to a solid phase in which the material has a regular ordered internal structure at the molecular level and gives a distinctive X-ray diffraction pattern with defined peaks. Such materials when heated sufficiently will also exhibit the properties of a liquid, but the change from solid to liquid is characterized by a phase change, typically first order ('melting point').

The compound of formula (I) may exist in solvated and unsolvated forms. As used herein, the term "solvate" refers to a complex of variable stoichiometry formed by a solute (in this invention, a compound of formula (I) or a salt) and a solvent. Such solvents for the purpose of the invention may not interfere with the biological activity of the solute. The skilled artisan will appreciate that pharmaceutically acceptable solvates may be formed for crystalline compounds wherein solvent molecules are incorporated into the crystalline lattice during crystallization. The incorporated solvent molecules may be water molecules or non-aqueous such as ethanol, isopropanol, DMSO, acetic acid, ethanolamine, and ethyl acetate molecules. Crystalline lattice incorporated with water molecules are typically referred to as "hydrates". Hydrates include stoichiometric hydrates as well as compositions containing variable amounts of water. The present invention includes all such solvates.

The compounds of formula (I) may have the ability to crystallize in more than one form, a characteristic, which is known as polymorphism, and it is understood that such polymorphic forms ("polymorphs") are within the scope of the invention. Polymorphism generally can occur as a response to changes in temperature or pressure or both and can also result from variations in the crystallization process. Polymorphs can be distinguished by various physical characteristics known in the art such as x-ray diffraction patterns, solubility and melting point.

It is also noted that the compounds of formula (I) may form tautomers. It is understood that all tautomers and mixtures of tautomers of the compounds of the present invention are included within the scope of the compounds of the present invention.

As used herein, the term "histone demethylase inhibitor", or "inhibitor" refers to any compound or treatment capable of inhibiting or reducing the expression or activity of a histone demethylase. The inhibitor is preferably selective against one or more histone demethylase enzymes with no direct activity as any other histone modifying enzymes.

As used herein, the term "alkyl" and "alkylene" refers to a saturated hydrocarbon chain having the specified number of carbon atoms. C_{1-6}alkyl refers to an alkyl group having from 1 to 6 member atoms, for example 1 to 4 member atoms. Alkyl groups may be straight or branched. Representative branched alkyl groups have one, two, or three branches. Alkyl includes methyl, ethyl, propyl (n-propyl and isopropyl), butyl (n-butyl, isobutyl, and t-butyl), pentyl (n-pentyl, isopentyl, and neopentyl), and hexyl.

As used herein, the term "halo" refers to the halogen radical fluoro, chloro, bromo, or iodo.

As used herein, the term "haloalkyl" refers to a straight or branched chain alkyl group as defined above having the specified number of carbon atoms, carbon atoms respectively substituted with at least one halo group, halo being as defined herein. Examples of such branched or straight chained haloalkyl groups useful in the present invention include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl and n-butyl substituted independently with one or more halos, *e.g.,* fluoro, chloro, bromo and iodo.

As used herein, the term "heterocyclic group" refers to a non-aromatic ring having the specified number of member atoms being saturated or having one or more degrees of unsaturation and containing one or more heteroatoms selected from N or O.

As used herein, the term "aryl" refers to monocyclic carbocyclic groups and fused bicyclic carbocyclic groups having the specified number of carbon atoms and having at least one aromatic ring. Examples of aryl groups include phenyl and naphthyl.

As used herein, the term "heteroaryl" refers to an aromatic monocyclic ring, or to a fused bicyclic ring system wherein at least one ring is aromatic, having the specified number of ring atoms and containing at least one heteratom selected from N, O, and/or S. Examples of "heteroaryl" groups used herein include furanyl, thiophenyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, thiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, oxo-pyridyl, thiadiazolyl, isothiazolyl, pyridyl, pyridazyl, pyrazinyl, pyrimidyl, quinolinyl, isoquinolinyl, benzofuranyl, benzothiophenyl, indolyl, indazolyl.

As used herein the term alkoxy" refers to the group –OR where R is an alkyl group as defined above.

As used herein the term “cycloalkyl” refers to a non-aromatic cyclic hydrocarbon ring having the specified number of carbon atoms. Examples include but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl. Bridged cycloalkyl group are included.

As used herein, the term "member atoms" refers to the atom or atoms that form a chain or ring. Where more than one member atom is present in a chain and within a ring, each member atom is covalently bound to an adjacent member atom in the chain or ring. Atoms that make up a substituent group on a chain or ring are not member atoms in the chain or ring.

As used herein, the term "optionally substituted" indicates that a group may be unsubstituted or substituted with one or more substituents as defined herein.

As used herein, the term "substituted" in reference to a group indicates that a hydrogen atom attached to a member atom within a group is replaced. It should be understood that the term "substituted" includes the implicit provision that such substitution be in accordance with the permitted valence of the substituted atom and the substituent and that the substitution results in a stable compound (i.e. one that does not spontaneously undergo transformation such as by rearrangement, cyclization, or elimination). In certain embodiments, a single atom may be substituted with more than one substituent as long as such substitution is in accordance with the permitted valence of the atom. Suitable substituents are defined herein for each substituted or optionally substituted group.

As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

In one embodiment, X is $-R^1-R^4$.

In a further embodiment, when X is $-R^1-R^4$, R^1 is NH.

In one embodiment X is $-(R^1)_{0-1}-(R^2)_{0-1}-R^3$.

In one embodiment X is $-R^3$.

In one embodiment X is $-(R^1)-R^3$.

In one embodiment X is $-(R^1)-(R^2)-R^3$.

In one embodiment R^3 is napthyl, phenyl (optionally substituted as described above).

In one embodiment R^1 is NH

In one embodiment R^2 is selected from

-CO-

-$COCH_2$-
-$COCH_2CH_2$-
-$COCH_2CH_2CH_2$-
-$COCH(CH_3)CH_2CH_2$-
-$COCH(CH_3)$-
-CH_2-
-CH_2CH_2-
-CH_2CH_2 CH_2-
-$CH_2CH_2CH_2CH_2$-
-$COCH_2O$-
-$COCH_2CH_2O$-
-$COCH_2CH_2S$-
-$C(CH_3)_2$-
-$CH(CH_2CH_{2(}CH_3)$-
-$CH(CH_3)$-
-$CH_2CH(CH_3)$-
-$CH(CH_3)CH_2$-
-$CH_2CH_2CH_2CH$-(CH_3)-
-$CH_2CH(OH)CH_2O$-
-$CH_2CH_2CH_2$-O-
-$COCH_2OCH_2$
-CH_2CH_2NHCO-
-$CH_2CH_2CH_2SO_2$
-CH_2-cyclopropyl-CH_2O-
-CH_2-cyclohexyl-
-CH(phenyl)-
-CH_2 CH(phenyl)CH_2CH_2-

While embodiments for each variable have generally been listed above separately for each variable this invention includes those compounds in which several or each aspect in formula (I) is selected from each of the aspects listed above. Therefore, this invention is intended to include all combinations of aspects for each variable. These embodiments are also applicable to compounds of formula (Ia).

Specific examples of compounds formula (I) include the following:

3-{[(4-chlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid;
3-{[(4-methylphenyl)acetyl]amino}-4-pyridinecarboxylic acid;

3-[(3-phenylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-[(phenylcarbonyl)amino]-4-pyridinecarboxylic acid;
3-[(2,2-dimethylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-{[(phenyloxy)acetyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-methylphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(2-naphthalenylacetyl)amino]-4-pyridinecarboxylic acid;
3-{[4-(2-naphthalenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-bromophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(3,4-dichlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[(3,4-dichlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid;
3-({4-[3-(acetylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[4-(4-pyridinyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(2-methyl-4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(phenyloxy)propanoyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(phenylthio)propanoyl]amino}-4-pyridinecarboxylic acid;
3-({[3,4-bis(methyloxy)phenyl]acetyl}amino)-4-pyridinecarboxylic acid;
3-[(3,4-dihydro-2(1*H*)-isoquinolinylacetyl)amino]-4-pyridinecarboxylic acid;
3-[(1,3-dihydro-2*H*-isoindol-2-ylacetyl)amino]-4-pyridinecarboxylic acid;
3-[(2-phenylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[3-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-[(2-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(2-phenylpropyl)amino]-4-pyridinecarboxylic acid;
3-[(phenylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-2-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(4-phenylbutyl)oxy]-4-pyridinecarboxylic acid;
3-{[3-(2-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(trifluoromethyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(7-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(5-quinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(4-biphenylyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(3-aminophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-(3-phenylpropyl)-4-pyridinecarboxylic acid;, formate salt
3-({3-[(2-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(3-phenylpropyl)amino]-4-pyridinecarboxylic acid;
3-{[4-(3-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-[(phenylacetyl)amino]-4-pyridinecarboxylic acid;

3-(hexanoylamino)-4-pyridinecarboxylic acid;
3-({[(phenylmethyl)oxy]acetyl}amino)-4-pyridinecarboxylic acid;
3-[(2-methylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-[(3,3-dimethylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-[(5-phenylpentanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[4-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[4-(4-chlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylbutyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-4-phenylbutyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(4-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(2-aminoethyl)amino]-4-pyridinecarboxylic acid;
3-({2-[(phenylcarbonyl)amino]ethyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(2-naphthalenyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(4-bromophenyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-{[2-hydroxy-3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylpentyl)amino]-4-pyridinecarboxylic acid;
3-[({1-[(phenyloxy)methyl]cyclopropyl}methyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(phenylsulfonyl)propyl]amino}-4-pyridinecarboxylic acid;
3-{[(1-phenyl-3-pyrrolidinyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(diphenylmethyl)amino]-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-3-pyrrolidinyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[methyl(phenylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[2-(4-biphenylyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-[(2,4-diphenylbutyl)amino]-4-pyridinecarboxylic acid;
3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[1-(phenylmethyl)-1*H*-pyrazol-4-yl]amino}-4-pyridinecarboxylic acid;
3-({3-[(2-oxo-1,2,3,4-tetrahydro-6-quinolinyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[1-(phenylmethyl)-1*H*-1,2,4-triazol-3-yl]amino}-4-pyridinecarboxylic acid;, formate salt.
3-[(1*S*,4*R*)-bicyclo[2.2.1]hept-2-ylamino]-4-pyridinecarboxylic acid;
3-[(tetrahydro-2*H*-pyran-2-ylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(4-pyridinylmethyl)amino]-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-1*H*-pyrazol-4-yl]methyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-(2,3-dihydro-1*H*-inden-1-ylamino)-4-pyridinecarboxylic acid;
3-[(2-pyridinylmethyl)amino]-4-pyridinecarboxylic acid;
3-(3-biphenylylamino)-4-pyridinecarboxylic acid;

3-{[3-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-{[(3-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-({[2-(acetylamino)phenyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[(cyclohexylmethyl)amino]-4-pyridinecarboxylic acid;
3-({4-[4-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-[(4-{3-[(N-{[(1,1-dimethylethyl)oxy]carbonyl}-b-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[3-(b-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino}-4-pyridinecarboxylic acid;
3-[(3-biphenylylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)amino]-4-pyridinecarboxylic acid;
3-{[(1-phenylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-(2-pyridinylamino)-4-pyridinecarboxylic acid;
3-{[(2'-methyl-2-biphenylyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylcyclohexyl)amino]-4-pyridinecarboxylic acid;
3-(2-biphenylylamino)-4-pyridinecarboxylic acid;
3-{[4-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-[(2,2,6,6-tetramethyl-4-piperidinyl)amino]-4-pyridinecarboxylic acid;
3-(1,3-dihydro-2*H*-isoindol-2-yl)-4-pyridinecarboxylic acid;
3-(4-phenyl-1-piperazinyl)-4-pyridinecarboxylic acid;
3-(1,2,3,4-tetrahydro-1-naphthalenylamino)-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-3-piperidinyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[(4-biphenylylmethyl)amino]-4-pyridinecarboxylic acid;
3-(2,3-dihydro-1*H*-inden-2-ylamino)-4-pyridinecarboxylic acid;
3-[(1-cyclohexylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid;
3 3-[(3-{[3-(1-piperazinyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(6-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(3-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(methyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-fluorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(3-{[3-(phenyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-[(cyclopropylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(3-thienylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(3-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(1*H*-imidazol-4-ylmethyl)amino]-4-pyridinecarboxylic acid;

3-[(1-methylethyl)amino]-4-pyridinecarboxylic acid;
3-{[(1*R*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid;
3-[(1*H*-pyrazol-5-ylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(1-methylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(5-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(2-furanylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(1*S*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid;
3-(cyclobutylamino)-4-pyridinecarboxylic acid;
3-[(2-cyclopentyl-1-methylethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,4-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(2-thienylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,3-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(cyclopentylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,5-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(3-pentyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(4-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(2,6-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[3-({3-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(methylsulfonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-methylphenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(2-oxo-1(2*H*)-pyridinyl)propyl]amino}-4-pyridinecarboxylic acid;
3-[(4-cyclohexylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(2-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[4-(aminocarbonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-{[3-({4-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(8-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(1-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-aminopropyl)amino]-4-pyridinecarboxylic acid;
3-[(cyclobutylmethyl)amino]-4-pyridinecarboxylic acid;
3-(propylamino)-4-pyridinecarboxylic acid;
3-[methyl(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-({2-[2-(methyloxy)phenyl]ethyl}amino)-4-pyridinecarboxylic acid;
3-[(2-methylpropyl)amino]-4-pyridinecarboxylic acid;
3-(methylamino)-4-pyridinecarboxylic acid;
3-(butylamino)-4-pyridinecarboxylic acid;
3-[(2-cyclohexylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-1-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-(cyclopentylamino)-4-pyridinecarboxylic acid;

3-(cyclohexylamino)-4-pyridinecarboxylic acid;
3-[(2-cyclopentylethyl)amino]-4-pyridinecarboxylic acid;
3-[(2-cyclohexyl-1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-cyclohexyl-1-methylethyl)amino]-4-pyridinecarboxylic acid;
3-[(4-{3-[(N-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl]pentanoyl}-b-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid;
3-{[(1*R*,2*S*)-1-hydroxy-2,3-dihydro-1*H*-inden-2-yl]amino}-4-pyridinecarboxylic acid;
3-[(1-cyclohexylcyclopropyl)amino]-4-pyridinecarboxylic acid;
3-({2-[4-(2-thienyl)phenyl]ethyl}amino)-4-pyridinecarboxylic acid;
3-{[(2,4-difluorophenyl)carbonyl]amino}-4-pyridinecarboxylic acid;
Or a pharmaceutically acceptable salt thereof.

The compounds of formula (I) may be in the form of a pro-drug, for example an ester which upon administration to the patient is capable of providing a compound of formula (I). In one aspect the ester is a C_{1-6}alkyl ester.

The compounds of formula (I) may be in the form of a salt, e.g. a hydrochloride salt or formate salt.

Typically, the salts of the present invention are pharmaceutically acceptable salts. Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention. For a review on suitable salts see Berge et al, J. Pharm. Sci. 1977, 66, 1-19.

Suitable pharmaceutically acceptable salts can include acid addition salts.

A pharmaceutically acceptable acid addition salt can be formed by reaction of a compound of formula (I) with a suitable inorganic or organic acid (such as hydrobromic, hydrochloric, sulfuric, nitric, phosphoric, p-toluenesulfonic, benzenesulfonic, methanesulfonic, ethanesulfonic, naphthalenesulfonic such as 2-naphthalenesulfonic), optionally in a suitable solvent such as an organic solvent, to give the salt which is usually isolated for example by crystallisation and filtration. A pharmaceutically acceptable acid addition salt of a compound of formula (I) can comprise or be for example a hydrobromide, hydrochloride, sulfate, nitrate, phosphate, p-toluenesulfonate, benzenesulfonate, methanesulfonate, ethanesulfonate, naphthalenesulfonate (e.g. 2-naphthalenesulfonate) salt.

Other non-pharmaceutically acceptable salts, e.g. trifluoroacetates, may be used, for example in the isolation of compounds of formula (I), and are included within the scope of this invention.

The invention includes within its scope all possible stoichiometric and non-stoichiometric forms of the compounds of formula (I).

While it is possible that, for use in therapy, the compound of formula (I) or a pharmaceutically acceptable salt thereof may be administered as the raw chemical, it is possible to present the active ingredient as a pharmaceutical composition. Accordingly, the invention further provides pharmaceutical compositions comprising a compound of formula (I) or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable carriers, diluents, or excipients. The carrier(s), diluents(s) or excipient(s) must be acceptable in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. In accordance with another aspect of the invention there is also provided a process for the preparation of a pharmaceutical composition including a compound of formula (I), or pharmaceutically acceptable salts thereof, with one or more pharmaceutically acceptable carriers, diluents or excipients. The pharmaceutical composition can be for use in the treatment and/or prophylaxis of any of the conditions described herein.

Pharmaceutical compositions may be presented in unit dose forms containing a predetermined amount of active ingredient per unit dose. Preferred unit dosage compositions are those containing a daily dose or sub-dose, or an appropriate fraction thereof, of an active ingredient. Such unit doses may therefore be administered once or more than once a day. Such pharmaceutical compositions may be prepared by any of the methods well known in the pharmacy art.

Pharmaceutical compositions may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, inhaled, intranasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions 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 compositions 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.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Powders are prepared by reducing the compound to a suitable fine size and mixing with a similarly prepared pharmaceutical carrier such as an edible carbohydrate, as, for example, starch or mannitol. Flavouring, preservative, dispersing and colouring agent can also be present.

Capsules are made by preparing a powder mixture, as described above, and filling formed gelatin sheaths. Glidants and lubricants such as colloidal silica, talc, magnesium stearate, calcium stearate or solid polyethylene glycol can be added to the powder mixture before the filling operation. A disintegrating or solubilizing agent such as agar-agar, calcium carbonate or sodium carbonate can also be added to improve the availability of the medicament when the capsule is ingested.

Moreover, when desired or necessary, suitable binders, glidants, lubricants, sweetening agents, flavours, disintegrating agents and colouring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum and the like. Tablets are formulated, for example, by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant and pressing into tablets. A powder mixture is prepared by mixing the compound, suitably comminuted, with a diluent or base as described above, and optionally, with a binder such as carboxymethylcellulose, an aliginate, gelatin, or polyvinyl pyrrolidone, a solution retardant such as paraffin, a resorption accelerator such as a quaternary salt and/or an absorption agent such as bentonite, kaolin or dicalcium phosphate. The powder mixture can be granulated by wetting with a binder such as syrup, starch paste, acadia mucilage or solutions of cellulosic or polymeric materials and forcing through a screen. As an alternative to granulating, the powder mixture can be run through the tablet machine and the result is imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc or mineral oil. The lubricated mixture is then compressed into tablets. The compounds of the present invention can also be combined with a free flowing inert carrier and compressed into tablets directly without going through the granulating or slugging steps. A clear or opaque protective coating consisting of a sealing coat of shellac, a coating of sugar or polymeric material and a polish coating of wax can be provided. Dyestuffs can be added to these coatings to distinguish different unit dosages.

Oral fluids such as solution, syrups and elixirs can be prepared in dosage unit form so that a given quantity contains a predetermined amount of the compound. Syrups can be prepared by dissolving the compound in a suitably flavoured aqueous solution, while elixirs are prepared through the use of a non-toxic alcoholic vehicle. Suspensions can be formulated by dispersing the compound in a non-toxic vehicle. Solubilizers and emulsifiers such as ethoxylated isostearyl alcohols and polyoxy ethylene sorbitol ethers, preservatives, flavor additive such as peppermint oil or natural sweeteners or saccharin or other artificial sweeteners, and the like can also be added.

Where appropriate, dosage unit compositions for oral administration can be microencapsulated. The composition can also be prepared to prolong or sustain the release as for example by coating or embedding particulate material in polymers, wax or the like.

The compounds of formula (I) or a pharmaceutically acceptable salt thereof may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Pharmaceutical compositions 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.

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

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

Dosage forms for nasal or inhaled administration may conveniently be formulated as aerosols, solutions, suspensions drops, gels or dry powders.

For compositions suitable and/or adapted for inhaled administration, it is preferred that the agent is in a particle-size-reduced form, and more preferably the size-reduced form is obtained or obtainable by micronisation. The preferable particle size of the size-reduced (e.g. micronised) compound or salt or solvate is defined by a D50 value of about 0.5 to about 10 microns (for example as measured using laser diffraction). Compositions adapted for administration by inhalation include the particle dusts or

mists. Suitable compositions wherein the carrier is a liquid for administration as a nasal spray or drops include aqueous or oil solutions/suspensions of the active ingredient which may be generated by means of various types of metered dose pressurised aerosols, nebulizers or insufflators.

Aerosol formulations, *e.g.* for inhaled administration, can comprise a solution or fine suspension of the agent in a pharmaceutically acceptable aqueous or non-aqueous solvent. Aerosol formulations can be presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomising device or inhaler. Alternatively the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve (metered dose inhaler) which is intended for disposal once the contents of the container have been exhausted.

Where the dosage form comprises an aerosol dispenser, it preferably contains a suitable propellant under pressure such as compressed air, carbon dioxide or an organic propellant such as a hydrofluorocarbon (HFC). Suitable HFC propellants include 1,1,1,2,3,3,3-heptafluoropropane and 1,1,1,2-tetrafluoroethane. The aerosol dosage forms can also take the form of a pump-atomiser. The pressurised aerosol may contain a solution or a suspension of the active compound. This may require the incorporation of additional excipients e.g. co-solvents and/or surfactants to improve the dispersion characteristics and homogeneity of suspension formulations. Solution formulations may also require the addition of co-solvents such as ethanol. Other excipient modifiers may also be incorporated to improve, for example, the stability and/or taste and/or fine particle mass characteristics (amount and/or profile) of the formulation.

For pharmaceutical compositions suitable and/or adapted for inhaled administration, the pharmaceutical composition may be a dry powder inhalable composition. Such a composition can comprise a powder base such as lactose, glucose, trehalose, mannitol or starch, the agent, (preferably in particle-size-reduced form, e.g. in micronised form), and optionally a performance modifier such as L-leucine or another amino acid, cellobiose octaacetate and/or metals salts of stearic acid such as magnesium or calcium stearate.

Aerosol formulations are preferably arranged so that each metered dose or “puff” of aerosol contains a particular amount of a compound of the invention. Administration may be once daily or several times daily, for example 2, 3 4 or 8 times, giving for example 1, 2 or 3 doses each time. The overall daily dose and the metered dose delivered by capsules and cartridges in an inhaler or insufflator will generally be double those with aerosol formulations.

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

Pharmaceutical compositions adapted for parental administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions 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.

It should be understood that in addition to the ingredients particularly mentioned above, the compositions 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.

Antisense or RNA interference molecules may be administered to the mammal in need thereof. Alternatively, constructs including the same may be administered. Such molecules and constructs can be used to interfere with the expression of the protein of interest, e.g., histone demethylase and as such, modify histone demethylation. Typically delivery is by means known in the art.

Antisense or RNA interference molecules can be delivered *in vitro* to cells or *in vivo*, e.g., to tumors of a mammal. Nodes of delivery can be used without limitations, including: intravenous, intramuscular, intraperitoncal, intra-arterial, local delivery during surgery, endoscopic, subcutaneous, and per os. Vectors can be selected for desirable properties for any particular application. Vectors can be viral or plasmid. Adenoviral vectors are useful in this regard. Tissue-specific, cell-type specific, or otherwise regulatable promoters can be used to control the transcription of the inhibitory polynucleotide molecules. Non-viral carriers such as liposomes or nanospheres can also be used.

A therapeutically effective amount of the compound of formula (I) or a pharmaceutically acceptable salt thereof will depend upon a number of factors including, for example, the age and weight of the subject, 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. In particular, the subject to be treated is a mammal, particularly a human.

The compound of formula (I) or a pharmaceutically acceptable salt thereof may be administered in a daily dose. 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.

Suitably, the amount of the compound of compound of formula (I) or a pharmaceutically acceptable salt thereof administered according to the present invention will be an amount selected from 0.01mg to 1000 mg per day (calculated as the free or unsalted compound).

The compounds of the formula (I) or a pharmaceutically acceptable salt thereof may be used in combination with or include one or more other therapeutic agents and may be administered either sequentially or simultaneously by any convenient route in separate or combined pharmaceutical compositions.

The compounds of formula (I) or a pharmaceutically acceptable salt thereof and further therapeutic agent(s) may be employed in combination by administration simultaneously in a unitary pharmaceutical composition including both compounds. Alternatively, the combination may be administered separately in separate pharmaceutical compositions, each including one of the compounds in a sequential manner wherein, for example, the compound of formula or a pharmaceutically acceptable salt thereof is administered first and the other second and *visa versa*. Such sequential administration may be close in time (e.g. simultaneously) or remote in time. Furthermore, it does not matter if the compounds are administered in the same dosage form, e.g. one compound may be administered topically and the other compound may be administered orally. Suitably, both compounds are administered orally.

The combinations may be presented as a combination kit. By the term “combination kit” “or kit of parts” as used herein is meant the pharmaceutical composition or compositions that are used to administer the combination according to the invention. When the agents of the combination are administered simultaneously, the combination kit can contain the agents in a single pharmaceutical composition, such as a tablet, or in separate pharmaceutical compositions. When the agents are not administered simultaneously, the combination kit will contain each agent in separate pharmaceutical compositions either in a single package or in separate pharmaceutical compositions in separate packages.

The combination kit can also be provided by instruction, such as dosage and administration instructions. Such dosage and administration instructions can be of the kind that are provided to a doctor, for example by a drug product label, or they can be of the kind that are provided by a doctor, such as instructions to a patient.

During a treatment regime, it will be appreciated that administration of each agent of the combination may be repeated one or more times.

When the combination is administered separately in a sequential manner wherein one is administered first and the other second or vice versa, such sequential administration may be close in time or remote in time. For example, administration of the other agent several minutes to several dozen minutes after the administration of the first agent, and administration of the other agent several hours to several days after the administration of the first agent are included, wherein the lapse of time is not

limited, For example, one agent may be administered once a day, and the other agent may be administered 2 or 3 times a day, or one agent may be administered once a week, and the other agent may be administered once a day and the like.

It will be clear to a person skilled in the art that, where appropriate, the other therapeutic ingredients(s) may be used in the form of salts, for example as alkali metal or amine salts or as acid addition salts, or prodrugs, or as esters, for example lower alkyl esters, or as solvates, for example hydrates, to optimise the activity and/or stability and/or physical characteristics, such as solubility, of the therapeutic ingredient. It will be clear also that, where appropriate, the therapeutic ingredients may be used in optically pure form.

When combined in the same composition it will be appreciated that the two compounds must be stable and compatible with each other and the other components of the composition and may be formulated for administration. When formulated separately they may be provided in any convenient composition, conveniently, in such a manner as known for such compounds in the art.

When the compound of compound of formula (I) or a pharmaceutically acceptable salt thereof is used in combination with further therapeutic agent or agents active against the same disease, condition or disorder the dose of each agent may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

In one embodiment in the methods and uses of the present invention the mammal is a human.

Provided herein are methods of changing the epigenetic status of cells and the treatment or prevention of cancer, inflammation and autoimmune diseases and conditions which may be improved by modulating the methylation status of histones, particularly H3K27 and thereby, e.g., modulate the level of expression of methylation repressed target genes. A method may comprise administering to a subject, e.g. a subject in need thereof, a therapeutically effective amount of an agent described herein.

Thus in one aspect there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for treating cancer, autoimmune and inflammatory diseases or conditions.

In one aspect the disease or condition is cancer.

Examples of cancer diseases and conditions in which compounds of formula (I), or a pharmaceutically acceptable salt or solvates thereof may have potentially beneficial antitumour effects include, but are not limited to, cancers of the lung, bone, pancreas, skin, head, neck, uterus, ovaries, stomach, colon,

breast, esophagus, small intestine, bowel, endocrine system, thyroid glad, parathyroid gland, adrenal gland, urethra, prostate, penis, testes, ureter, bladder, kidney or liver; rectal cancer; cancer of the anal region; carcinomas of the fallopian tubes, endometrium, cervix, vagina, vulva, renal pelvis, renal cell; sarcoma of soft tissue; myxoma; rhabdomyoma; fibroma; lipoma; teratoma; cholangiocarcinoma; hepatoblastoma; angiosarcoma; hemagioma; hepatoma; fibrosarcoma; chondrosarcoma; myeloma; chronic or acute leukemia; lymphocytic lymphomas; primary CNS lymphoma; neoplasms of the CNS; spinal axis tumours; squamous cell carcinomas; synovial sarcoma; malignant pleural mesotheliomas; brain stem glioma; pituitary adenoma; bronchial adenoma; chondromatous hanlartoma; inesothelioma; Hodgkin's Disease or a combination of one or more of the foregoing cancers.

The compounds of the compound of formula (I) or a pharmaceutically acceptable salt thereof may also be useful in the treatment of one or more diseases afflicting mammals which are characterized by cellular proliferation in the area of disorders associated with neo-vascularization and/or vascular permeability including blood vessel proliferative disorders including arthritis (rheumatoid arthritis) and restenosis; fibrotic disorders including hepatic cirrhosis and atherosclerosis; mesangial cell proliferative disorders include glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, proliferative retinopathies, organ transplant rejection and glomerulopathies; and metabolic disorders include psoriasis, diabetes mellitus, chronic wound healing, inflammation and neurodegenerative diseases.

In the embodiment, the compound of compound of formula (I) or a pharmaceutically acceptable salt thereof may be employed with other therapeutic methods of cancer treatment. In particular, in anti-neoplastic therapy, combination therapy with other chemotherapeutic, hormonal, antibody agents as well as surgical and/or radiation treatments other than those mentioned above are envisaged.

As indicated, therapeutically effective amounts of the compound of compound of formula (I) or a pharmaceutically acceptable salt thereof are discussed above. The therapeutically effective amount of the further therapeutic agents of the present invention will depend upon a number of factors including, for example, the age and weight of the mammal, the precise condition requiring treatment, the severity of the condition, the nature of the formulation, and the route of administration. Ultimately, the therapeutically effective amount will be at the discretion of the attendant physician or veterinarian. The relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

In one embodiment, the further anti-cancer therapy is surgical and/or radiotherapy.

In one embodiment, the further anti-cancer therapy is at least one additional anti-neoplastic agent.

Any anti-neoplastic agent that has activity versus a susceptible tumor being treated may be utilized in the combination. Typical anti-neoplastic agents useful include, but are not limited to, anti-microtubule agents such as diterpenoids and vinca alkaloids; platinum coordination complexes; alkylating agents such as nitrogen mustards, oxazaphosphorines, alkylsulfonates, nitrosoureas, and triazenes; antibiotic agents such as anthracyclins, actinomycins and bleomycins; topoisomerase II inhibitors such as epipodophyllotoxins; antimetabolites such as purine and pyrimidine analogues and anti-folate compounds; topoisomerase I inhibitors such as camptothecins; hormones and hormonal analogues; signal transduction pathway inhibitors; non-receptor tyrosine angiogenesis inhibitors; immunotherapeutic agents; proapoptotic agents; and cell cycle signaling inhibitors.

Anti-microtubule or anti-mitotic agents:

Anti-microtubule or anti-mitotic agents are phase specific agents active against the microtubules of tumor cells during M or the mitosis phase of the cell cycle. Examples of anti-microtubule agents include, but are not limited to, diterpenoids and vinca alkaloids.

Diterpenoids, which are derived from natural sources, are phase specific anti -cancer agents that operate at the G_2/M phases of the cell cycle. It is believed that the diterpenoids stabilize the β-tubulin subunit of the microtubules, by binding with this protein. Disassembly of the protein appears then to be inhibited with mitosis being arrested and cell death following. Examples of diterpenoids include, but are not limited to, paclitaxel and its analog docetaxel.

Paclitaxel, 5β,20-epoxy-1,2α,4,7β,10β,13α-hexa-hydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl-3-phenylisoserine; is a natural diterpene product isolated from the Pacific yew tree *Taxus brevifolia* and is commercially available as an injectable solution TAXOL®. It is a member of the taxane family of terpenes. Paclitaxel has been approved for clinical use in the treatment of refractory ovarian cancer in the United States (Markman et al., Yale Journal of Biology and Medicine, 64:583, 1991; McGuire et al., Ann. Intem, Med., 111:273,1989) and for the treatment of breast cancer (Holmes et al., J. Nat. Cancer Inst., 83:1797,1991.) It is a potential candidate for treatment of neoplasms in the skin (Einzig et. al., Proc. Am. Soc. Clin. Oncol., 20:46) and head and neck carcinomas (Forastire et. al., Sem. Oncol., 20:56, 1990). The compound also shows potential for the treatment of polycystic kidney disease (Woo et. al., Nature, 368:750. 1994), lung cancer and malaria. Treatment of patients with paclitaxel results in bone marrow suppression (multiple cell lineages, Ignoff, R.J. et. al, Cancer Chemotherapy Pocket Guide, 1998) related to the duration of dosing above a threshold concentration (50nM) (Kearns, C.M. et. al., Seminars in Oncology, 3(6) p.16-23, 1995).

Docetaxel, (2R,3S)- N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate; is commercially available as an injectable solution as TAXOTERE®. Docetaxel is indicated for the treatment of breast

cancer. Docetaxel is a semisynthetic derivative of paclitaxel *q.v.*, prepared using a natural precursor, 10-deacetyl-baccatin III, extracted from the needle of the European Yew tree.

Vinca alkaloids are phase specific anti-neoplastic agents derived from the periwinkle plant. Vinca alkaloids act at the M phase (mitosis) of the cell cycle by binding specifically to tubulin. Consequently, the bound tubulin molecule is unable to polymerize into microtubules. Mitosis is believed to be arrested in metaphase with cell death following. Examples of vinca alkaloids include, but are not limited to, vinblastine, vincristine, and vinorelbine.

Vinblastine, vincaleukoblastine sulfate, is commercially available as VELBAN® as an injectable solution. Although, it has possible indication as a second line therapy of various solid tumors, it is primarily indicated in the treatment of testicular cancer and various lymphomas including Hodgkin's Disease; and lymphocytic and histiocytic lymphomas. Myelosuppression is the dose limiting side effect of vinblastine.

Vincristine, vincaleukoblastine, 22-oxo-, sulfate, is commercially available as ONCOVIN® as an injectable solution. Vincristine is indicated for the treatment of acute leukemias and has also found use in treatment regimens for Hodgkin's and non-Hodgkin's malignant lymphomas. Alopecia and neurologic effects are the most common side effect of vincristine and to a lesser extent myelosupression and gastrointestinal mucositis effects occur.

Vinorelbine, 3',4'-didehydro -4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)], commercially available as an injectable solution of vinorelbine tartrate (NAVELBINE®), is a semisynthetic vinca alkaloid. Vinorelbine is indicated as a single agent or in combination with other chemotherapeutic agents, such as cisplatin, in the treatment of various solid tumors, particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. Myelosuppression is the most common dose limiting side effect of vinorelbine.

Platinum coordination complexes:

Platinum coordination complexes are non-phase specific anti-cancer agents, which are interactive with DNA. The platinum complexes enter tumor cells, undergo, aquation and form intra- and interstrand crosslinks with DNA causing adverse biological effects to the tumor. Examples of platinum coordination complexes include, but are not limited to, oxaliplatin, cisplatin and carboplatin.

Cisplatin, cis-diamminedichloroplatinum, is commercially available as PLATINOL® as an injectable solution. Cisplatin is primarily indicated in the treatment of metastatic testicular and ovarian cancer and advanced bladder cancer.

Carboplatin, platinum, diammine [1,1-cyclobutane-dicarboxylate(2-)-O,O'], is commercially available as PARAPLATIN® as an injectable solution. Carboplatin is primarily indicated in the first and second line treatment of advanced ovarian carcinoma.

Alkylating agents:

Alkylating agents are non-phase anti-cancer specific agents and strong electrophiles. Typically, alkylating agents form covalent linkages, by alkylation, to DNA through nucleophilic moieties of the DNA molecule such as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. Such alkylation disrupts nucleic acid function leading to cell death. Examples of alkylating agents include, but are not limited to, nitrogen mustards such as cyclophosphamide, melphalan, and chlorambucil; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine; and triazenes such as dacarbazine.

Cyclophosphamide, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, is commercially available as an injectable solution or tablets as CYTOXAN®. Cyclophosphamide is indicated as a single agent or in combination with other chemotherapeutic agents, in the treatment of malignant lymphomas, multiple myeloma, and leukemias.

Melphalan, 4-[bis(2-chloroethyl)amino]-L-phenylalanine, is commercially available as an injectable solution or tablets as ALKERAN®. Melphalan is indicated for the palliative treatment of multiple myeloma and non-resectable epithelial carcinoma of the ovary. Bone marrow suppression is the most common dose limiting side effect of melphalan.

Chlorambucil, 4-[bis(2-chloroethyl)amino]benzenebutanoic acid, is commercially available as LEUKERAN® tablets. Chlorambucil is indicated for the palliative treatment of chronic lymphatic leukemia, and malignant lymphomas such as lymphosarcoma, giant follicular lymphoma, and Hodgkin's disease.

Busulfan, 1,4-butanediol dimethanesulfonate, is commercially available as MYLERAN® TABLETS. Busulfan is indicated for the palliative treatment of chronic myelogenous leukemia.

Carmustine, 1,3-[bis(2-chloroethyl)-1-nitrosourea, is commercially available as single vials of lyophilized material as BiCNU®. Carmustine is indicated for the palliative treatment as a single agent or in combination with other agents for brain tumors, multiple myeloma, Hodgkin's disease, and non-Hodgkin's lymphomas.

Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, is commercially available as single vials of material as DTIC-Dome®. Dacarbazine is indicated for the treatment of metastatic

malignant melanoma and in combination with other agents for the second line treatment of Hodgkin's Disease.

Antibiotic anti-neoplastics :

Antibiotic anti-neoplastics are non-phase specific agents, which bind or intercalate with DNA. Typically, such action results in stable DNA complexes or strand breakage, which disrupts ordinary function of the nucleic acids leading to cell death. Examples of antibiotic anti-neoplastic agents include, but are not limited to, actinomycins such as dactinomycin, anthrocyclins such as daunorubicin and doxorubicin; and bleomycins.

Dactinomycin, also know as Actinomycin D, is commercially available in injectable form as COSMEGEN®. Dactinomycin is indicated for the treatment of Wilm's tumor and rhabdomyosarcoma.

Daunorubicin, (8S-cis-)-8-acetyl-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as a liposomal injectable form as DAUNOXOME® or as an injectable as CERUBIDINE®. Daunorubicin is indicated for remission induction in the treatment of acute nonlymphocytic leukemia and advanced HIV associated Kaposi's sarcoma.

Doxorubicin, (8S, 10S)-10-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-8-glycoloyl, 7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12 naphthacenedione hydrochloride, is commercially available as an injectable form as RUBEX® or ADRIAMYCIN RDF®. Doxorubicin is primarily indicated for the treatment of acute lymphoblastic leukemia and acute myeloblastic leukemia, but is also a useful component in the treatment of some solid tumors and lymphomas.

Bleomycin, a mixture of cytotoxic glycopeptide antibiotics isolated from a strain of *Streptomyces verticillus*, is commercially available as BLENOXANE®. Bleomycin is indicated as a palliative treatment, as a single agent or in combination with other agents, of squamous cell carcinoma, lymphomas, and testicular carcinomas.

Topoisomerase II inhibitors:

Topoisomerase II inhibitors include, but are not limited to, epipodophyllotoxins.

Epipodophyllotoxins are phase specific anti-neoplastic agents derived from the mandrake plant. Epipodophyllotoxins typically affect cells in the S and G_2 phases of the cell cycle by forming a ternary complex with topoisomerase II and DNA causing DNA strand breaks. The strand breaks accumulate and cell death follows. Examples of epipodophyllotoxins include, but are not limited to, etoposide and teniposide.

Etoposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-ethylidene-β-D-glucopyranoside], is commercially available as an injectable solution or capsules as VePESID® and is commonly known as VP-16. Etoposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of testicular and non-small cell lung cancers.

Teniposide, 4'-demethyl-epipodophyllotoxin 9[4,6-0-(R)-thenylidene-β-D-glucopyranoside], is commercially available as an injectable solution as VUMON® and is commonly known as VM-26. Teniposide is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia in children.

Antimetabolite neoplastic agents:

Antimetabolite neoplastic agents are phase specific anti-neoplastic agents that act at S phase (DNA synthesis) of the cell cycle by inhibiting DNA synthesis or by inhibiting purine or pyrimidine base synthesis and thereby limiting DNA synthesis. Consequently, S phase does not proceed and cell death follows. Examples of antimetabolite anti-neoplastic agents include, but are not limited to, fluorouracil, methotrexate, cytarabine, mecaptopurine, thioguanine, and gemcitabine.

5-fluorouracil, 5-fluoro-2,4- (1H,3H) pyrimidinedione, is commercially available as fluorouracil. Administration of 5-fluorouracil leads to inhibition of thymidylate synthesis and is also incorporated into both RNA and DNA. The result typically is cell death. 5-fluorouracil is indicated as a single agent or in combination with other chemotherapy agents in the treatment of carcinomas of the breast, colon, rectum, stomach and pancreas. Other fluoropyrimidine analogs include 5-fluoro deoxyuridine (floxuridine) and 5-fluorodeoxyuridine monophosphate.

Cytarabine, 4-amino-1-β-D-arabinofuranosyl-2 (1H)-pyrimidinone, is commercially available as CYTOSAR-U® and is commonly known as Ara-C. It is believed that cytarabine exhibits cell phase specificity at S-phase by inhibiting DNA chain elongation by terminal incorporation of cytarabine into the growing DNA chain. Cytarabine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other cytidine analogs include 5-azacytidine and 2',2'-difluorodeoxycytidine (gemcitabine).

Mercaptopurine, 1,7-dihydro-6H-purine-6-thione monohydrate, is commercially available as PURINETHOL®. Mercaptopurine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet unspecified mechanism. Mercaptopurine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. A useful mercaptopurine analog is azathioprine.

Thioguanine, 2-amino-1,7-dihydro-6H-purine-6-thione, is commercially available as TABLOID®. Thioguanine exhibits cell phase specificity at S-phase by inhibiting DNA synthesis by an as of yet

unspecified mechanism. Thioguanine is indicated as a single agent or in combination with other chemotherapy agents in the treatment of acute leukemia. Other purine analogs include pentostatin, erythrohydroxynonyladenine, fludarabine phosphate, and cladribine.

Gemcitabine, 2'-deoxy-2', 2'-difluorocytidine monohydrochloride (β-isomer), is commercially available as GEMZAR®. Gemcitabine exhibits cell phase specificity at S-phase and by blocking progression of cells through the G1/S boundary. Gemcitabine is indicated in combination with cisplatin in the treatment of locally advanced non-small cell lung cancer and alone in the treatment of locally advanced pancreatic cancer.

Methotrexate, N-[4[[(2,4-diamino-6-pteridinyl) methyl]methylamino] benzoyl]-L-glutamic acid, is commercially available as methotrexate sodium. Methotrexate exhibits cell phase effects specifically at S-phase by inhibiting DNA synthesis, repair and/or replication through the inhibition of dyhydrofolic acid reductase which is required for synthesis of purine nucleotides and thymidylate. Methotrexate is indicated as a single agent or in combination with other chemotherapy agents in the treatment of choriocarcinoma, meningeal leukemia, non-Hodgkin's lymphoma, and carcinomas of the breast, head, neck, ovary and bladder.

Topoisomerase I inhibitors:

Camptothecins, including, camptothecin and camptothecin derivatives are available or under development as Topoisomerase I inhibitors. Camptothecins cytotoxic activity is believed to be related to its Topoisomerase I inhibitory activity. Examples of camptothecins include, but are not limited to irinotecan, topotecan, and the various optical forms of 7-(4-methylpiperazino-methylene)-10,11-ethylenedioxy-20-camptothecin described below.

Irinotecan HCl, (4S)-4,11-diethyl-4-hydroxy-9-[(4-piperidinopiperidino) carbonyloxy]-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione hydrochloride, is commercially available as the injectable solution CAMPTOSAR®. Irinotecan is a derivative of camptothecin which binds, along with its active metabolite SN-38, to the topoisomerase I – DNA complex. It is believed that cytotoxicity occurs as a result of irreparable double strand breaks caused by interaction of the topoisomerase I : DNA : irintecan or SN-38 ternary complex with replication enzymes. Irinotecan is indicated for treatment of metastatic cancer of the colon or rectum.

Topotecan HCl, (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4',6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione monohydrochloride, is commercially available as the injectable solution HYCAMTIN®. Topotecan is a derivative of camptothecin which binds to the topoisomerase I – DNA complex and prevents religation of singles strand breaks caused by Topoisomerase I in response to torsional strain of the DNA molecule. Topotecan is indicated for second line treatment of metastatic carcinoma of the ovary and small cell lung cancer.

Hormones and hormonal analogues:

Hormones and hormonal analogues are useful compounds for treating cancers in which there is a relationship between the hormone(s) and growth and/or lack of growth of the cancer. Examples of hormones and hormonal analogues useful in cancer treatment include, but are not limited to, adrenocorticosteroids such as prednisone and prednisolone which are useful in the treatment of malignant lymphoma and acute leukemia in children ; aminoglutethimide and other aromatase inhibitors such as anastrozole, letrazole, vorazole, and exemestane useful in the treatment of adrenocortical carcinoma and hormone dependent breast carcinoma containing estrogen receptors; progestrins such as megestrol acetate useful in the treatment of hormone dependent breast cancer and endometrial carcinoma; estrogens, androgens, and anti-androgens such as flutamide, nilutamide, bicalutamide, cyproterone acetate and 5α-reductases such as finasteride and dutasteride, useful in the treatment of prostatic carcinoma and benign prostatic hypertrophy; anti-estrogens such as tamoxifen, toremifene, raloxifene, droloxifene, iodoxyfene, as well as selective estrogen receptor modulators (SERMS) such those described in U.S. Patent Nos. 5,681,835, 5,877,219, and 6,207,716, useful in the treatment of hormone dependent breast carcinoma and other susceptible cancers; and gonadotropin-releasing hormone (GnRH) and analogues thereof which stimulate the release of leutinizing hormone (LH) and/or follicle stimulating hormone (FSH) for the treatment prostatic carcinoma, for instance, LHRH agonists and antagagonists such as goserelin acetate and luprolide.

Signal transduction pathway inhibitors:

Signal transduction pathway inhibitors are those inhibitors, which block or inhibit a chemical process which evokes an intracellular change. As used herein this change is cell proliferation or differentiation. Signal tranduction inhibitors useful in the present invention include inhibitors of receptor tyrosine kinases, non-receptor tyrosine kinases, SH2/SH3domain blockers, serine/threonine kinases, phosphotidyl inositol-3 kinases, myo-inositol signaling, and Ras oncogenes.

Several protein tyrosine kinases catalyse the phosphorylation of specific tyrosyl residues in various proteins involved in the regulation of cell growth. Such protein tyrosine kinases can be broadly classified as receptor or non-receptor kinases.

Receptor tyrosine kinases are transmembrane proteins having an extracellular ligand binding domain, a transmembrane domain, and a tyrosine kinase domain. Receptor tyrosine kinases are involved in the regulation of cell growth and are generally termed growth factor receptors. Inappropriate or uncontrolled activation of many of these kinases, i.e. aberrant kinase growth factor receptor activity, for example by over-expression or mutation, has been shown to result in uncontrolled cell growth. Accordingly, the aberrant activity of such kinases has been linked to malignant tissue growth. Consequently, inhibitors of such kinases could provide cancer treatment methods. Growth factor receptors include, for example, epidermal growth factor receptor (EGFr), platelet derived growth factor

receptor (PDGFr), erbB2, erbB4, ret, vascular endothelial growth factor receptor (VEGFr), tyrosine kinase with immunoglobulin-like and epidermal growth factor homology domains (TIE-2), insulin growth factor –I (IGFI) receptor, macrophage colony stimulating factor (cfms), BTK, ckit, cmet, fibroblast growth factor (FGF) receptors, Trk receptors (TrkA, TrkB, and TrkC), ephrin (eph) receptors, and the RET protooncogene. Several inhibitors of growth receptors are under development and include ligand antagonists, antibodies, tyrosine kinase inhibitors and anti-sense oligonucleotides. Growth factor receptors and agents that inhibit growth factor receptor function are described, for instance, in Kath, John C., Exp. Opin. Ther. Patents (2000) 10(6):803-818; Shawver et al DDT Vol 2, No. 2 February 1997; and Lofts, F. J. et al, "Growth factor receptors as targets", New Molecular Targets for Cancer Chemotherapy, ed. Workman, Paul and Kerr, David, CRC press 1994, London.

Tyrosine kinases, which are not growth factor receptor kinases are termed non-receptor tyrosine kinases. Non-receptor tyrosine kinases useful in the present invention, which are targets or potential targets of anti-cancer drugs, include cSrc, Lck, Fyn, Yes, Jak, cAbl, FAK (Focal adhesion kinase), Brutons tyrosine kinase, and Bcr-Abl. Such non-receptor kinases and agents which inhibit non-receptor tyrosine kinase function are described in Sinh, S. and Corey, S.J., (1999) Journal of Hematotherapy and Stem Cell Research 8 (5): 465 – 80; and Bolen, J.B., Brugge, J.S., (1997) Annual review of Immunology. 15: 371-404.

SH2/SH3 domain blockers are agents that disrupt SH2 or SH3 domain binding in a variety of enzymes or adaptor proteins including, PI3-K p85 subunit, Src family kinases, adaptor molecules (Shc, Crk, Nck, Grb2) and Ras-GAP. SH2/SH3 domains as targets for anti-cancer drugs are discussed in Smithgall, T.E. (1995), Journal of Pharmacological and Toxicological Methods. 34(3) 125-32.

Inhibitors of Serine/Threonine Kinases including MAP kinase cascade blockers which include blockers of Raf kinases (rafk), Mitogen or Extracellular Regulated Kinase (MEKs), and Extracellular Regulated Kinases (ERKs); and Protein kinase C family member blockers including blockers of PKCs (alpha, beta, gamma, epsilon, mu, lambda, iota, zeta). IkB kinase family (IKKa, IKKb), PKB family kinases, akt kinase family members, and TGF beta receptor kinases. Such Serine/Threonine kinases and inhibitors thereof are described in Yamamoto, T., Taya, S., Kaibuchi, K., (1999), Journal of Biochemistry. 126 (5) 799-803; Brodt, P, Samani, A., and Navab, R. (2000), Biochemical Pharmacology, 60. 1101-1107; Massague, J., Weis-Garcia, F. (1996) Cancer Surveys. 27:41-64; Philip, P.A., and Harris, A.L. (1995), Cancer Treatment and Research. 78: 3-27, Lackey, K. et al Bioorganic and Medicinal Chemistry Letters, (10), 2000, 223-226; U.S. Patent No. 6,268,391; and Martinez-Iacaci, L., et al, Int. J. Cancer (2000), 88(1), 44-52.

Inhibitors of Phosphotidyl inositol-3 Kinase family members including blockers of PI3-kinase, ATM, DNA-PK, and Ku are also useful in the present invention. Such kinases are discussed in Abraham, R.T. (1996), Current Opinion in Immunology. 8 (3) 412-8; Canman, C.E., Lim, D.S. (1998), Oncogene

17 (25) 3301-3308; Jackson, S.P. (1997), International Journal of Biochemistry and Cell Biology. 29 (7):935-8; and Zhong, H. et al, Cancer res, (2000) 60(6), 1541-1545.

Also useful in the present invention are Myo-inositol signaling inhibitors such as phospholipase C blockers and Myoinositol analogues. Such signal inhibitors are described in Powis, G., and Kozikowski A., (1994) New Molecular Targets for Cancer Chemotherapy ed., Paul Workman and David Kerr, CRC press 1994, London.

Another group of signal transduction pathway inhibitors are inhibitors of Ras Oncogene. Such inhibitors include inhibitors of farnesyltransferase, geranyl-geranyl transferase, and CAAX proteases as well as anti-sense oligonucleotides, ribozymes and immunotherapy. Such inhibitors have been shown to block ras activation in cells containing wild type mutant ras , thereby acting as antiproliferation agents. Ras oncogene inhibition is discussed in Scharovsky, O.G., Rozados, V.R., Gervasoni, S.I. Matar, P. (2000), Journal of Biomedical Science. 7(4) 292-8; Ashby, M.N. (1998), Current Opinion in Lipidology. 9 (2) 99 – 102; and BioChim. Biophys. Acta, (19899) 1423(3):19-30.

As mentioned above, antibody antagonists to receptor kinase ligand binding may also serve as signal transduction inhibitors. This group of signal transduction pathway inhibitors includes the use of humanized antibodies to the extracellular ligand binding domain of receptor tyrosine kinases. For example Imclone C225 EGFR specific antibody (see Green, M.C. et al, Monoclonal Antibody Therapy for Solid Tumors, Cancer Treat. Rev., (2000), 26(4), 269-286); Herceptin ® erbB2 antibody (see Tyrosine Kinase Signalling in Breast cancer:erbB Family Receptor Tyrosine Kinases, Breast cancer Res., 2000, 2(3), 176-183); and 2CB VEGFR2 specific antibody (see Brekken, R.A. et al, Selective Inhibition of VEGFR2 Activity by a monoclonal Anti-VEGF antibody blocks tumor growth in mice, Cancer Res. (2000) 60, 5117-5124).

Anti-angiogenic agents:

(i) Anti-angiogenic agents including non-receptorMEKngiogenesis inhibitors may alo be useful. Anti-angiogenic agents such as those which inhibit the effects of vascular edothelial growth factor, (for example the anti-vascular endothelial cell growth factor antibody bevacizumab [Avastin™], and compounds that work by other mechanisms (for example linomide, inhibitors of integrin αvβ3 function, endostatin and angiostatin);

Immunotherapeutic agents:

Agents used in immunotherapeutic regimens may also be useful in combination with the compounds of formula (I). Immunotherapy approaches, including for example ex-vivo and in-vivo approaches to increase the immunogenecity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte-macrophage colony stimulating factor, approaches to decrease T-cell anergy, approaches using transfected immune cells such as cytokine-transfected

dendritic cells, approaches using cytokine-transfected tumour cell lines and approaches using anti-idiotypic antibodies

Proapoptotoc agents:

Agents used in proapoptotic regimens (e.g., bcl-2 antisense oligonucleotides) may also be used in the combination of the present invention.

Cell cycle signalling inhibitors

Cell cycle signalling inhibitors inhibit molecules involved in the control of the cell cycle. A family of protein kinases called cyclin dependent kinases (CDKs) and their interaction with a family of proteins termed cyclins controls progression through the eukaryotic cell cycle. The coordinate activation and inactivation of different cyclin/CDK complexes is necessary for normal progression through the cell cycle. Several inhibitors of cell cycle signalling are under development. For instance, examples of cyclin dependent kinases, including CDK2, CDK4, and CDK6 and inhibitors for the same are described in, for instance, Rosania et al, Exp. Opin. Ther. Patents (2000) 10(2):215-230.

In one embodiment, the combination of the present invention comprises a compound of formula I or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine MEKngiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, and cell cycle signaling inhibitors.

In one embodiment, the combination of the present invention comprises a compound of formula I or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent which is an anti-microtubule agent selected from diterpenoids and vinca alkaloids.

In a further embodiment, the at least one anti-neoplastic agent agent is a diterpenoid.

In a further embodiment, the at least one anti-neoplastic agent is a vinca alkaloid.

In one embodiment, the combination of the present invention comprises a compound of formula I or a salt or solvate thereof and at least one anti-neoplastic agent, which is a platinum coordination complex.

In a further embodiment, the at least one anti-neoplastic agent is paclitaxel, carboplatin, or vinorelbine.

In a further embodiment, the at least one anti-neoplastic agent is carboplatin.

In a further embodiment, the at least one anti-neoplastic agent is vinorelbine.

In a further embodiment, the at least one anti-neoplastic agent is paclitaxel.

In one embodiment, the combination of the present invention comprises a compound of formula I or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent which is a signal transduction pathway inhibitor.

In a further embodiment the signal transduction pathway inhibitor is an inhibitor of a growth factor receptor kinase VEGFR2, TIE2, PDGFR, BTK, erbB2, EGFr, IGFR-1, TrkA, TrkB, TrkC, or c-fms.

In a further embodiment the signal transduction pathway inhibitor is an inhibitor of a serine/threonine kinase rafk, akt, or PKC-zeta.

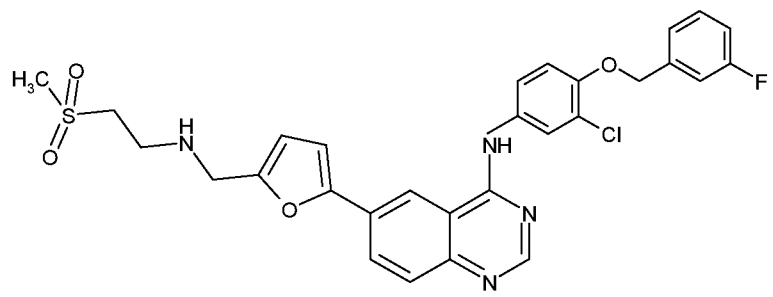
In a further embodiment the signal transduction pathway inhibitor is an inhibitor of a non- receptor tyrosine kinase selected from the src family of kinases.

In a further embodiment the signal transduction pathway inhibitor is an inhibitor of c-src.

In a further embodiment the signal transduction pathway inhibitor is an inhibitor of Ras oncogene selected from inhibitors of farnesyl transferase and geranylgeranyl transferase.

In a further embodiment the signal transduction pathway inhibitor is an inhibitor of a serine/threonine kinase selected from the group consisting of PI3K.

In a further embodiment the signal transduction pathway inhibitor is a dual EGFr/erbB2 inhibitor, for example N-{3-Chloro-4-[(3-fluorobenzyl) oxy]phenyl}-6-[5-({[2-(methanesulphonyl) ethyl]amino}methyl)-2-furyl]-4-quinazolinamine (structure below):



In one embodiment, the combination of the present invention comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof and at least one anti-neoplastic agent which is a cell cycle signaling inhibitor.

In further embodiment, cell cycle signaling inhibitor is an inhibitor of CDK2, CDK4 or CDK6.

In a further aspect, the disease or condition is autoimmune.

Autoimmune diseases associated with type 1 interferon include, but are not limited to Systemic lupus erythematosus, Psoriasis, insulin-dependent diabetes mellitus (IDDM), dermatomyositis and Sjogren's syndrome (SS).

In one aspect the disease or condition is inflammation.

Inflammation represents a group of vascular, cellular and neurological responses to trauma. Inflammation can be characterised as the movement of inflammatory cells such as monocytes, neutrophils and granulocytes into the tissues. This is usually associated with reduced endothelial barrier function and oedema into the tissues. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical event propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells which are present at the site of inflammation and is characterised by simultaneous destruction and healing of the tissue from the inflammatory process.

When occurring as part of an immune response to infection or as an acute response to trauma, inflammation can be beneficial and is normally self-limiting. However, inflammation can be detrimental under various conditions. This includes the production of excessive inflammation in response to infectious agents, which can lead to significant organ damage and death (for example, in the setting of sepsis). Moreover, chronic inflammation is generally deleterious and is at the root of numerous chronic diseases, causing severe and irreversible damage to tissues. In such settings, the immune response is often directed against self-tissues (autoimmunity), although chronic responses to foreign entities can also lead to bystander damage to self tissues.

The aim of anti-inflammatory therapy is therefore to reduce this inflammation, to inhibit autoimmunity when present and to allow for the physiological process or healing and tissue repair to progress.

The compound of formula (I) may be used to treat inflammation of any tissue and organs of the body, including musculoskeletal inflammation, vascular inflammation, neural inflammation, digestive system inflammation, ocular inflammation, inflammation of the reproductive system, and other inflammation, as exemplified below.

Musculoskeletal inflammation refers to any inflammatory condition of the musculoskeletal system, particularly those conditions affecting skeletal joints, including joints of the hand, wrist, elbow, shoulder, jaw, spine, neck, hip, knew, ankle, and foot, and conditions affecting tissues connecting muscles to bones such as tendons. Examples of musculoskeletal inflammation which may be treated with compounds of formula (I) include arthritis (including, for example, osteoarthritis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, acute and chronic infectious arthritis, arthritis associated with gout and pseudogout, and juvenile idiopathic arthritis), tendonitis, synovitis, tenosynovitis, bursitis, fibrositis (fibromyalgia), epicondylitis, myositis, and osteitis (including, for example, Paget's disease, osteitis pubis, and osteitis fibrosa cystic).

Ocular inflammation refers to inflammation of any structure of the eye, including the eye lids. Examples of ocular inflammation which may be treated with the compounds of formula (I) include blepharitis, blepharochalasis, conjunctivitis, dacryoadenitis, keratitis, keratoconjunctivitis sicca (dry eye), scleritis, trichiasis, and uveitis.

Examples of inflammation of the nervous system which may be treated with the compounds of formula (I) include encephalitis, Guillain-Barre syndrome, meningitis, neuromyotonia, narcolepsy, multiple sclerosis, myelitis and schizophrenia.

Examples of inflammation of the vasculature or lymphatic system which may be treated with the compounds of formula (I) include arthrosclerosis, arthritis, phlebitis, vasculitis, and lymphangitis.

Examples of inflammatory conditions of the digestive system which may be treated with the compounds of formula (I) include cholangitis, cholecystitis, enteritis, enterocolitis, gastritis, gastroenteritis, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), ileitis, and proctitis.

Examples of inflammatory conditions of the reproductive system which may be treated with the compounds of formula (I) include cervicitis, chorioamnionitis, endometritis, epididymitis, omphalitis, oophoritis, orchitis, salpingitis, tubo-ovarian abscess, urethritis, vaginitis, vulvitis, and vulvodynia.

The compound of formula (I) may be used to treat autoimmune conditions having an inflammatory component. Such conditions include acute disseminated alopecia universalise, Behcet's disease, Chagas' disease, chronic fatigue syndrome, dysautonomia, encephalomyelitis, ankylosing spondylitis,

aplastic anemia, hidradenitis suppurativa, autoimmune hepatitis, autoimmune oophoritis, celiac disease, Crohn's disease, diabetes mellitus type 1, giant cell arteritis, goodpasture's syndrome, Grave's disease, Guillain-Barre syndrome, Hashimoto's disease, Henoch-Schönlein purpura, Kawasaki's disease, lupus erythematosus, microscopic colitis, microscopic polyarteritis, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, opsocionus myoclonus syndrome, optic neuritis, ord's thyroiditis, pemphigus, polyarteritis nodosa, polymyalgia, rheumatoid arthritis, Reiter's syndrome, Sjogren's syndrome, temporal arteritis, Wegener's granulomatosis, warm autoimmune haemolytic anemia, interstitial cystitis, lyme disease, morphea, psoriasis, sarcoidosis, scleroderma, ulcerative colitis, and vitiligo.

The compound of formula (I) may be used to treat T-cell mediated hypersensitivity diseases having an inflammatory component. Such conditions include contact hypersensitivity, contact dermatitis (including that due to poison ivy), uticaria, skin allergies, respiratory allergies (hayfever, allergic rhinitis) and gluten-sensitive enteropathy (Celliac disease).

Other inflammatory conditions which may be treated with the agents include, for example, appendicitis, dermatitis, dermatomyositis, endocarditis, fibrositis, gingivitis, glossitis, hepatitis, hidradenitis suppurativa, iritis, laryngitis, mastitis, myocarditis, nephritis, otitis, pancreatitis, parotitis, percarditis, peritonoitis, pharyngitis, pleuritis, pneumonitis, prostatistis, pyelonephritis, and stomatisi, transplant rejection (involving organs such as kidney, liver, heart, lung, pancreas (e.g., islet cells), bone marrow, cornea, small bowel, skin allografts, skin homografts, and heart valve xengrafts, sewrum sickness, and graft vs host disease), acute pancreatitis, chronic pancreatitis, acute respiratory distress syndrome, Sexary's syndrome, congenital adrenal hyperplasis, nonsuppurative thyroiditis, hypercalcemia associated with cancer, pemphigus, bullous dermatitis herpetiformis, severe erythema multiforme, exfoliative dermatitis, seborrheic dermatitis, seasonal or perennial allergic rhinitis, bronchial asthma, contact dermatitis, astopic dermatitis, drug hypersensistivity reactions, allergic conjunctivitis, keratitis, herpes zoster ophthalmicus, iritis and oiridocyclitis, chorioretinitis, optic neuritis, symptomatic sarcoidosis, fulminating or disseminated pulmonary tuberculosis chemotherapy, idiopathic thrombocytopenic purpura in adults, secondary thrombocytopenia in adults, acquired (autroimmine) haemolytic anemia, leukaemia and lymphomas in adults, acute leukaemia of childhood, regional enteritis, autoimmune vasculitis, multiple sclerosis, chronic obstructive pulmonary disease, solid organ transplant rejection, sepsis. Preferred treatments include treatment of transplant rejection, rheumatoid arthritis, psoriatic arthritis, multiple sclerosis, Type 1 diabetes, asthma, inflammatory bowel disease, systemic lupus erythematosis, psoriasis, chronic obstructive pulmonary disease, and inflammation accompanying infectious conditions (e.g., sepsis).

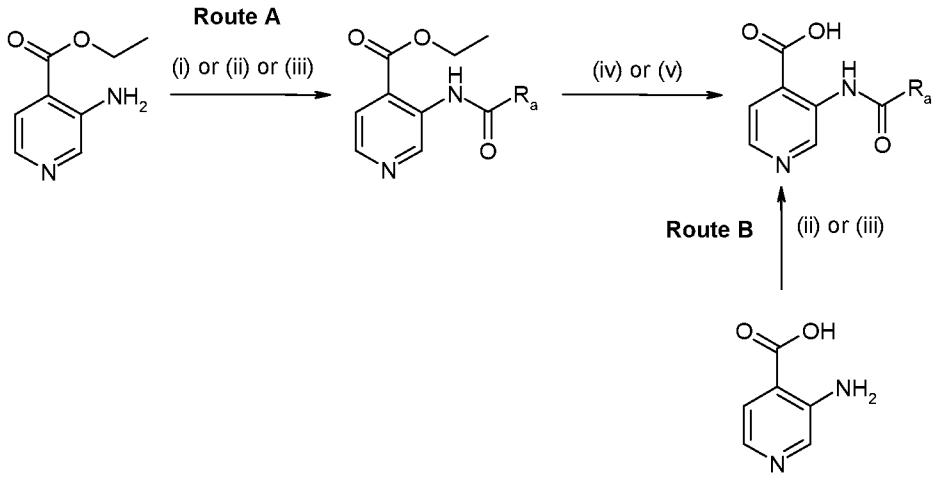
The compound of formula (I) and pharmaceutical compositions containing it may be used in combination with or include one or more other therapeutic agents, for example selected from NSAIDS,

corticosteroids, COX-2 inhibitors, cytokine inhibitors, anti-TNF agents, inhibitors oncostatin M, anti-malarials, immunsuppressive and cytostatics.

The methods of treatment and uses of the invention can be used in mammals, particularly in humans.

Compounds of formula (I) may be prepared by methods known in the art of organic synthesis as set forth in the schemes below and/or the specific Examples described below. In all of the methods, it is well understood that protecting groups for sensitive or reactive groups may be employed where necessary in accordance with general principles of chemistry. Protecting groups are manipulated according to standard methods of organic synthesis (T. W. Green and P. G. M. Wuts (1999) Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons). These groups are removed at a convenient stage of the compound synthesis using methods that are readily apparent to those skilled in the art. The selection of processes as well as the reaction conditions and order of their execution shall be consistent with the preparation of compounds of Formula (I).

Scheme 1 – Synthesis of 3-alkanoylamino-4-pyridinecarboxylic acids



(i) R_aCO_2H, 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane 2,4,6-trioxide, DIPEA, DCM

(ii) R_aCOCl, DMF with or without DIPEA and pyridine, or RaCOCl, aq. K_2CO_3 (sat.), water, DCM,

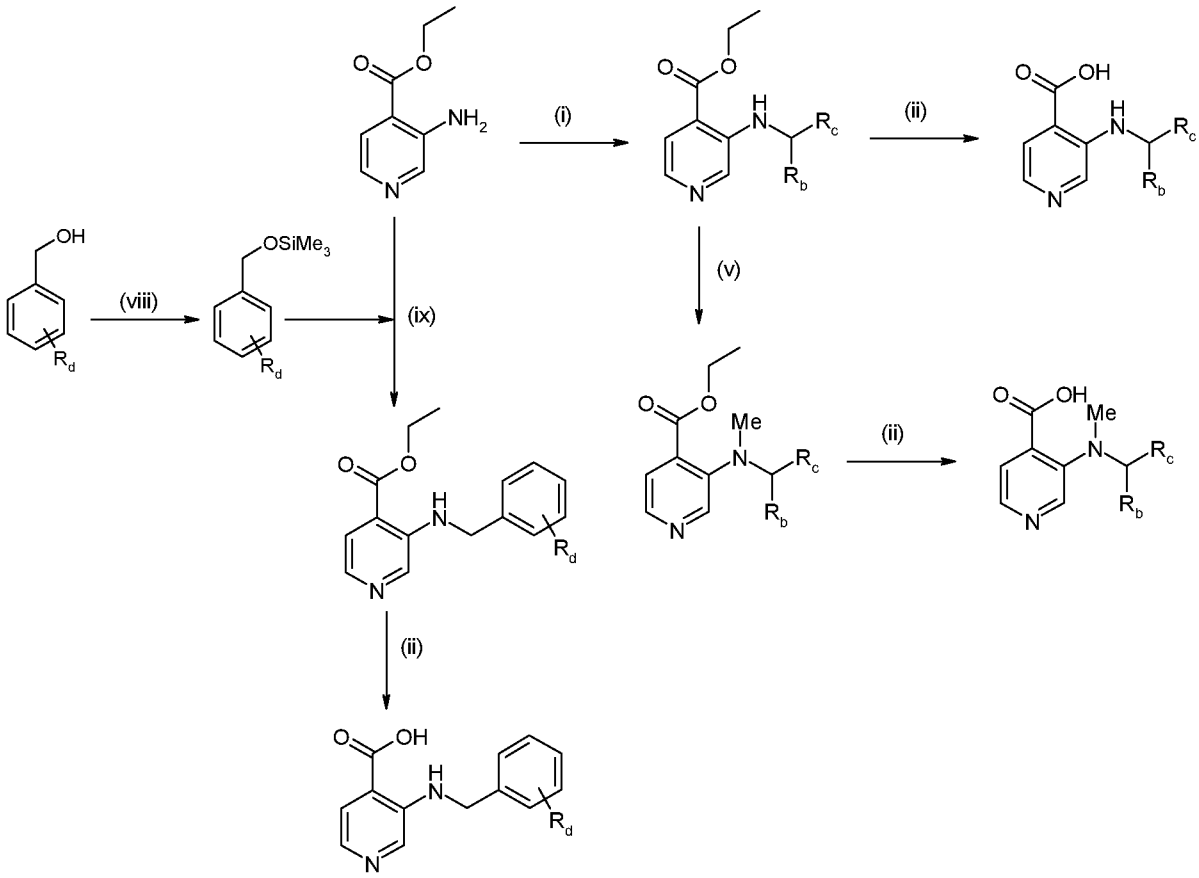
(iii) R_aCO_2H, Oxalyl chloride, DCM, DMF

(iv) LiOH monohydrate, THF and H_2O with or without MeOH

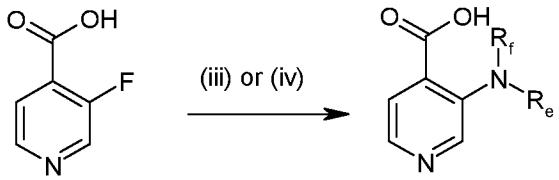
(v) 1M BBr_3 in DCM

Scheme 2 – Synthesis of 3-alkylamino-4-pyridinecarboxylic acids

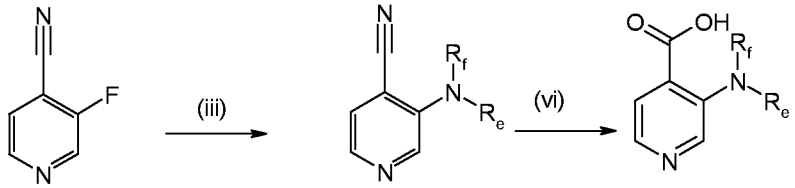
Route A



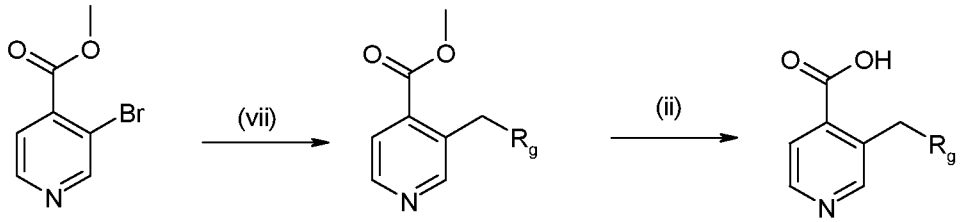
Route B



Route C



Route D



(i) R_bCOR_c, $NaBH(OAc)_3$, TFA, isopropylacetate

(ii) LiOH monohydrate, 1:1 THF/H_2O or LiOH monohydrate, 1:1:1 THF/H_2O/MeOH

(iii) R_eR_fNH, Heat or R_eR_fNH, DMSO, Heat, or R_eR_fNH, DIPEA, DMSO, Heat

(iv) R_eR_fNH, LiHMDS, -78°C to rt, THF

(v) 60% NaH, MeI, DMF

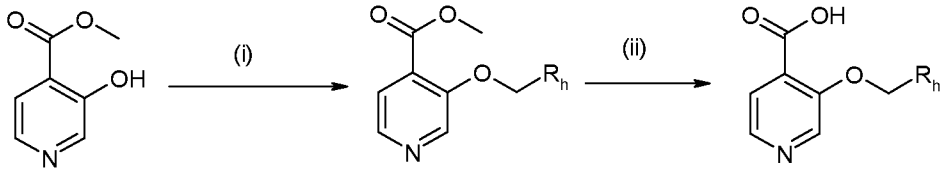
(vi) NaOH, water, EtOH, Heat

(vii) R_gCH_2 Zinc Bromide in THF,$Pd(OAc)_2$, S-Phos, heat

(viii) TMSI, $CHCl_3$

(ix) K_2CO_3, THF, 60°C, 4h

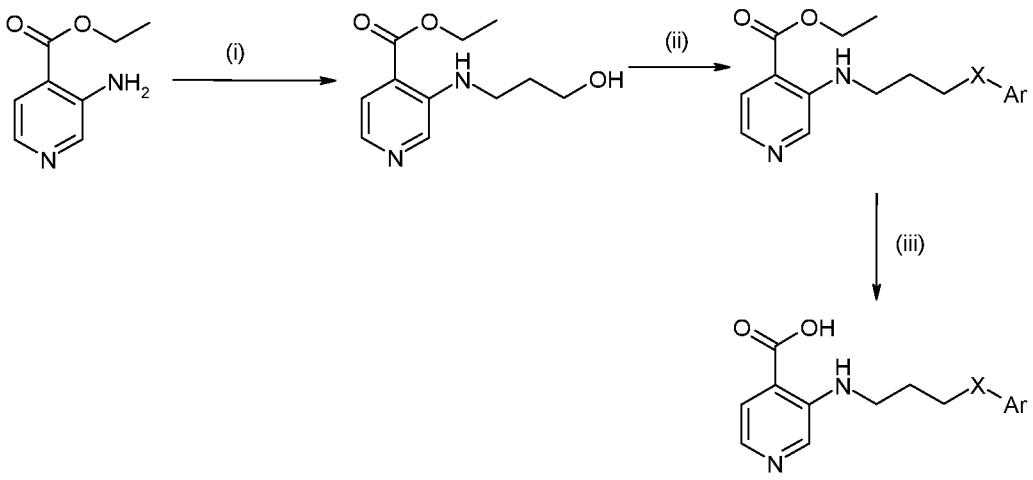
Scheme 3 – Synthesis of 3-alkyloxy-4-pyridinecarboxylic acids

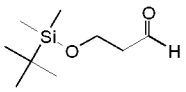


(i) R_hCH_2Br, Cs_2CO_3, DMF

(ii) LiOH monohydrate, 1:1 THF/H_2O or LiOH, 1:1:1 THF/H_2O/MeOH

Scheme 4 – Synthesis of 3-{[3-(aryloxy- and arylamino-)propyl]amino}-4-pyridinecarboxylic acids

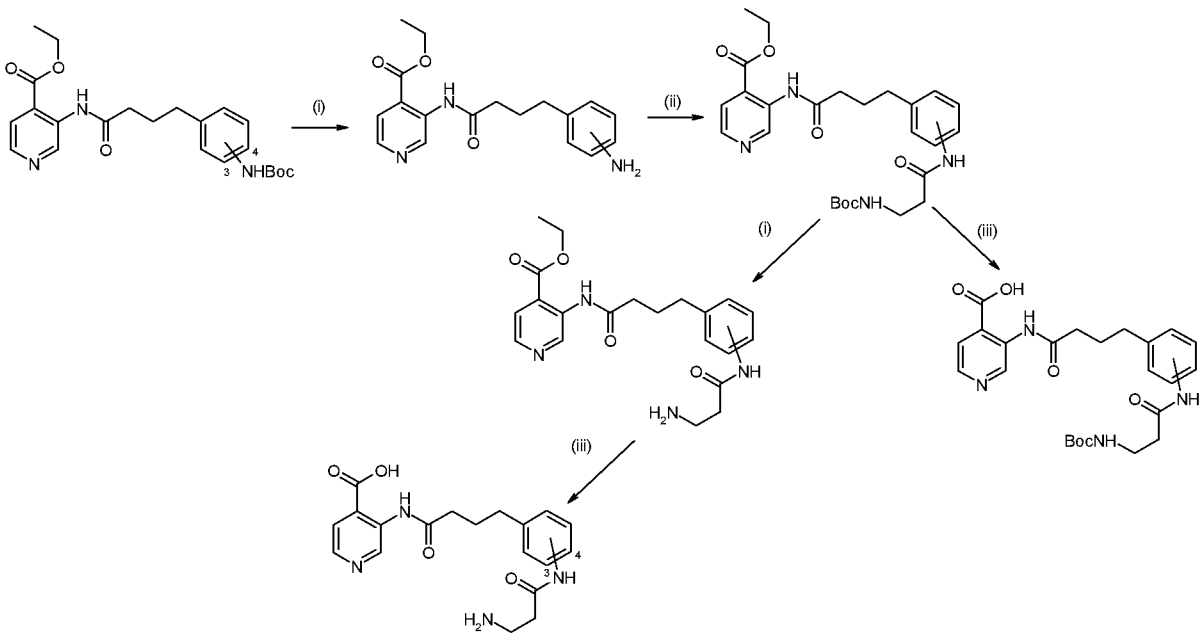


(i)  , $NaBH(OAc)_3$, TFA, isopropylacetate

(ii) ArXH, *bis*(1,1-dimethylethyl) -1,2-diazenedicarboxylate, PPh_3, THF, where X= O or N

(iii) LiOH monohydrate, 1:1 THF/H_2O or LiOH monohydrate, 1:1:1 THF/H_2O/MeOH

Scheme 5 – Synthesis of 3-({4-[3-(β–alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid and 3-({4-[4-(β–alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid and related compounds

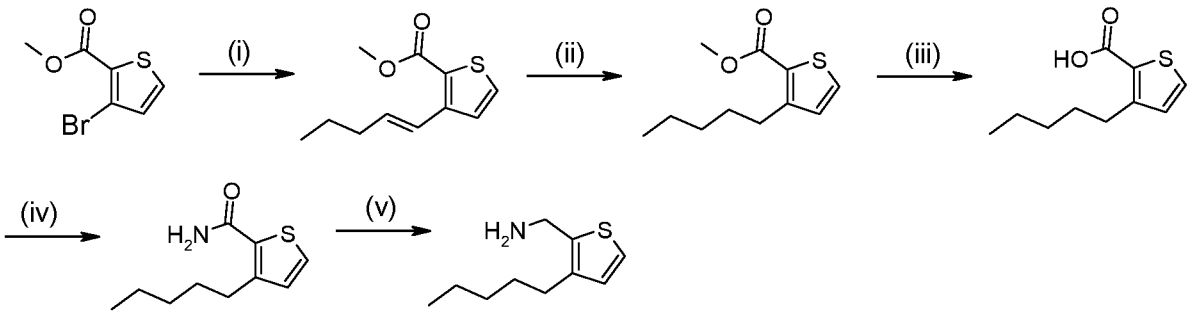


(i) TFA, DCM

(ii) *N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β–alanine, T3P, DIPEA, DCM

(iii) LiOH monohydrate, 1:1 THF/H_2O or LiOH monohydrate, 1:1:1 THF/H_2O/MeOH

Scheme 6 - Synthesis of [(3-pentyl-2-thienyl)methyl]amine



(i) (1*E*)-1-penten-1-ylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, water/dioxane, heat

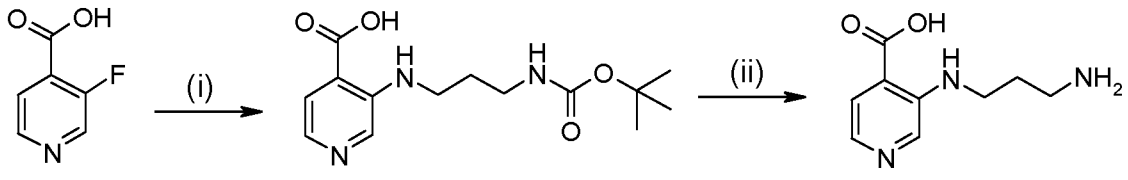
(ii) H_2, 10% Pd/C, EtOH

(iii) LiOH, water/dioxane, heat, overnight

(iv) Oxalyl chloride, heat, 18 hr then NH_4OH, 3 hr

(v) $LiAlH_4$ in THF

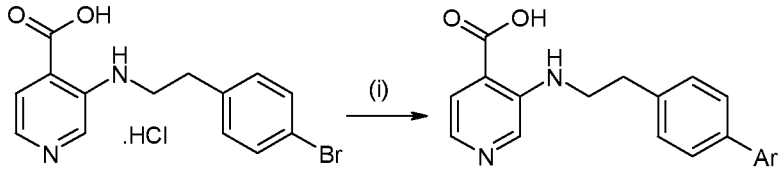
Scheme 7 – Synthesis of 3-[(3-aminopropyl)amino]-4-pyridinecarboxylic acid



(i) 1,1-dimethylethyl (3-aminopropyl)carbamate, 1,4-dioxane, Heat

(ii) TFA, DCM

Scheme 8 - 3-({2-[4-(aryl)phenyl]ethyl}amino)-4-pyridinecarboxylic acid



(i) Aryl boronic acid, sodium bicarbonate, 1,1'-Bis(diphenylphosphino)ferrocene]dichloro-palladium(II).DCM (1:1), 1,4-Dioxane/water heat

Examples

General

All temperatures are in °C.

DIPEA refers to *N,N* diisopropylethylamine

DCM refers to dichloromethane

DMSO refers to dimethylsulfoxide

DMF refers to *N,N*-dimethylformamide

DMA refers to *N,N*-dimethyl acetamide

DMAP refers to *N,N*-dimethyl-4-pyridinamine

Ether refers to diethyl ether

EtOAc refers to ethyl acetate

EtOH refers to ethanol

HPLC refers to high performance liquid chromatography

h and hr refer to time in hours

IPA refers to isopropylalcohol

LiHMDS refers to lithium hexamethyldisilazide

LiOH refers to lithium hydroxide

MDAP refers to mass-directed autopreparative HPLC

MeOH refers to methanol

min and mins refer to time in minutes

NMP refers to 1-methyl-2-pyrrolidinone

r.t. and rt refer to room temperature

Rt refers to retention time

s refers to time in seconds

SCX and SCX SPE refers to solid phase extraction (SPE) using aromatic benzene sulfonic acid SPE columns

S-Phos refers to 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl

TBTU refers to O-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate

THF refers to tetrahydrofuran

TFA refers to trifluoroacetic acid

T3P refers to 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane 2,4,6-trioxide

^{1}H NMR spectra were recorded using a Bruker NMR 400 MHz, referenced to tetramethylsilane.

LC/MS (Method A) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 0.1% v/v solution of Formic Acid in Water (Solvent A) and 0.1% v/v solution of Formic Acid in Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 3 – 100% B, 1.5-1.9min 100% B, 1.9 – 2.1min 3% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

LC/MS (Method B) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 10 mM Ammonium Bicarbonate in water adjusted to pH 10 with Ammonia solution (Solvent A) and Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 1 – 97% B, 1.5-1.9min 97% B, 1.9 – 2.1min 100% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

LC/MS (Method C) was conducted on an Acquity UPLC BEH C18 column (50mm x 2.1mm i.d. 1.7µm packing diameter) at 40 degrees centigrade, eluting with 0.1% v/v solution of Trifluoroacetic Acid in Water (Solvent A) and 0.1% v/v solution of Trifluoroacetic Acid in Acetonitrile (Solvent B) using the following elution gradient 0-1.5min 3 – 100% B, 1.5-1.9min 100% B, 1.9 – 2.0min 3% B at a flow rate of 1ml/min. The UV detection was a summed signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

LC/MS (Method D) was conducted on an Ace C18, 50 X 4.6 mm, 3.0 micron column, eluting with 0.1% v/v solution of Formic acid in Water (Solvent A) and 0.1% v/v solution of Formic acid in Acetonitrile (Solvent B) ,using the following elution gradient 0.01-4.00min 10 – 90% B, 4.00 - 5.00 min 90 - 100% B, 5.00-6.00 min 100% B, 6.00 – 6.01 min 100 – 10% B, 6.01 – 7.20 min 10% B. Column flow was 1 ml / min. The mass spectra were recorded on a SHIMADZU LCMS 2010 EV Spectrometer using Positive/negative electro spray.

MDAP (Method E). The HPLC analysis was conducted on an XBridge C18 column (100mm x 30mm i.d. 5µm packing diameter) at ambient temperature, eluting with 10mM Ammonium Bicarbonate in

water adjusted to pH 10 with Ammonia solution (Solvent A) and Acetonitrile (Solvent B) using an elution gradient of between 0 and 100% Solvent B over 15 or 25 minutes.

The UV detection was an averaged signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

MDAP (Method F). The HPLC analysis was conducted on a Sunfire C18 column (150mm x 30mm i.d. 5µm packing diameter) at ambient temperature, eluting with 0.1% v/v solution of Trifluoroacetic Acid in Water (Solvent A) and 0.1% v/v solution of Trifluoroacetic Acid in Acetonitrile (Solvent B) using an elution gradient of between 0 and 100% Solvent B over 15 or 25 minutes.

The UV detection was an averaged signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

MDAP (Method G). The HPLC analysis was conducted on a Sunfire C18 column (150mm x 30mm i.d. 5µm packing diameter) at ambient temperature, eluting with 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) using an elution gradient of between 0 and 100% Solvent B over 15 or 25 minutes.

The UV detection was an averaged signal from wavelength of 210nm to 350nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

Preparative HPLC (Method H) was carried out on Xbridge Shield 19x100mm, 5um column with averaged UV detection between 210 and 400 nm at ambient temperature. Column flow was 20 ml /min and mobile phase used was 10mM Ammonium Bicarbonate in water, adjusted to pH 10 with ammonia solution (A) and Acetonitrile (B), using an elution gradient of 0 - 0.5 min 10% B, 0.5 - 15 min 50% B, 15 – 15.2 min 99% B, 15.2 – 18.9 min 10% B, 19-20 min 10% B, The mass spectra were recorded using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer.

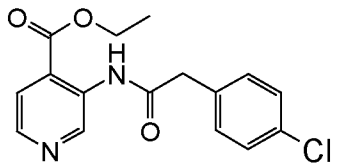
Preparative HPLC (Method I) was carried out on Waters 2487 using Phenomenex Luna C18, 100 X 21.2 mm, 5 micron column or an ACE C18 250 X 21.2 mm, 5 micron column with UV detection. Column flow was 21.0 mL /min. and mobile phase used were 0.1% Formic acid in water HPLC grade (A) and 0.1% Formic acid in Acetonitrile HPLC grade (B), or 0.1% TFA in water HPLC grade (A) and 0.1% TFA in Acetonitrile HPLC grade (B) using an appropriate elution gradient of Solvent A to B over a 10 to 20 min timeframe. Sample preparation was done in Water & Acetonitrile equal ratio.

LC/MS (Method J) was conducted on an Ace C18, 50 X 4.6 mm, 3.0 micron column, eluting with 0.1% v/v solution of Formic acid in Water (Solvent A) and 0.1% v/v solution of Formic acid in Acetonitrile (Solvent B) ,using the following elution gradient 0.01-4.0min 0.0 – 50% B, 4.0 - 5.00 min 50 - 100% B, 5.00-6.00 min 100% B, 6.00 – 6.01 min 100 – 0.0% B, 6.01 – 7.00 min 0.0% B. Column flow was 1 ml / min. The mass spectra were recorded on a SHIMADZU LCMS 2010 EV Spectrometer using Positive/negative electro spray.

LC/MS (Method K) was conducted on an Ace C18, 50 X 4.6 mm, 3.0 micron column, eluting with 0.05% w/v solution of Ammonium acetate in Water (Solvent A) and 0.05% w/v solution of Ammonium acetate in methanol (Solvent B) ,using the following elution gradient 0.01-4.00min 10 – 90% B, 4.00 - 5.00 min 90 - 100% B, 5.00-6.00 min 100% B, 6.00 – 6.01 min 100 – 10% B, 6.01 – 7.00 min 10% B. Column flow was 1 ml / min. The mass spectra were recorded on a SHIMADZU LCMS 2010 EV Spectrometer using Positive/negative electro spray.

LC/MS (Method L) LCMS was conducted on X-Bridge,C18, 150 X 4.6 mm, 5.0 micron column, eluting with 0.1% v/v solution of Formic acid in Water (Solvent A) and 0.1% v/v solution of Formic acid in Acetonitrile (Solvent B) ,using the following elution gradient 0.01-5.00min 10 – 90% B, 5.00 - 6.00 min 90 - 100% B, 6.00-10.00 min 100% B, 10.00 – 11.00 min 100 – 10% B, 11.01 – 12.00 min 10% B. Column flow was 1 ml / min. The mass spectra were recorded on a SHIMADZU LCMS 2010 EV Spectrometer using Positive/negative electro spray.

Intermediate 1: ethyl 3-{[(4-chlorophenyl)acetyl]amino}-4-pyridinecarboxylate

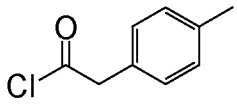
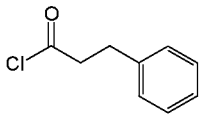
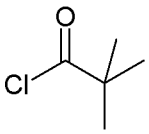
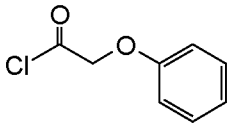
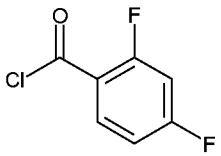


To a solution of ethyl 3-amino-4-pyridinecarboxylate (250 mg, 1.50 mmol) in DMF (2 ml) was added (4-chlorophenyl)acetyl chloride (0.242 ml, 1.66 mmol). The reaction mixture was stirred under nitrogen for 18 h then quenched with saturated sodium bicarbonate solution (20 ml) and extracted twice with ethyl acetate (2 x 50 ml). The combined organic layers were filtered through a hydrophobic frit then evaporated to dryness to give the crude product. The crude product was purified with column chromatography (eluted with 0-50% EtOAc in cyclohexane) to give the title compound as a viscous orange gum (188 mg, 39%).

LCMS (Method A): Rt = 1.08 mins, MH+ = 319.1

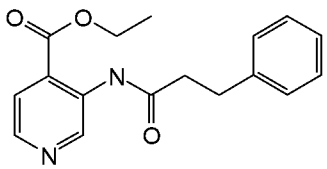
The Intermediates given in the following table were prepared in a manner similar to that described for

Intermediate 1. Some Intermediates required purification by MDAP (Method E, F or G) in addition to or instead of the aforementioned column chromatography. The preparation of intermediate 79 employed DIPEA (2 eq) and DMAP (0.068 eq) as bases.

Intermediate	Acid Chloride	Yield /%	LCMS
2 ethyl 3-{[(4-methylphenyl)acetyl]amino}-4-pyridinecarboxylate		62	Rt 1.06 mins, MH+ = 299.1 (Method A)
3 ethyl 3-[(3-phenylpropanoyl)amino]-4-pyridinecarboxylate		59	Rt 1.04 mins, MH+ = 299.2 (Method A)
4 ethyl 3-[(2,2-dimethylpropanoyl)amino]-4-pyridinecarboxylate		42	Rt 0.99 mins, MH+ = 251.1 (Method A)
5 *methyl 3-{[(phenyloxy)acetyl]amino}-4-pyridinecarboxylate		17	Rt 1.02 mins, MH+ 287.1 (Method A)
79 ethyl 3-{[(2,4-difluorophenyl)carbonyl]amino}-4-pyridinecarboxylate		16	Rt 1.09 mins, MH+ 307.0 (Method B)

* Methanol used in the transfer of material from one container to the next caused transesterification to the corresponding methyl ester.

Intermediate 6: ethyl 3-[(3-phenylpropanoyl)amino]-4-pyridinecarboxylate

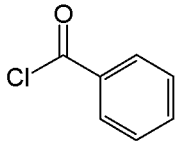


To a stirred mixture of ethyl 3-amino-4-pyridinecarboxylate (100 mg, 0.60 mmol) in DCM (3 ml) and saturated aq. potassium carbonate (3 ml) at room temperature was added 3-phenylpropanoyl chloride (0.116 ml, 0.78 mmol). The resultant biphasic solution was stirred rapidly for 16 h then partitioned between DCM (10 ml) and water (20 ml). The separated aqueous phase was extracted with DCM (2 x 20 ml) then the combined organic phase was passed through a hydrophobic frit then concentrated *in vacuo* to give a yellow oil. The sample was purified by column chromatography (eluted with 0-30% EtOAc in cyclohexane) to give the crude product as a colourless oil (144 mg). The crude product was

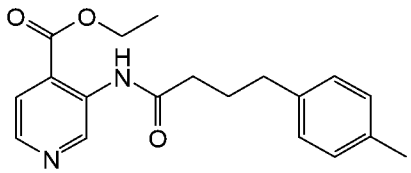
dissolved in 1:1 MeOH/DMSO (2 ml) then purified in two portions by MDAP using a formic modifier (Method G). The solvent was evaporated under vacuum to give a colouress oil which was dissolved in ethanol (10 ml) then loaded onto a 2 g aminopropyl column that had been preconditioned with two column volumes of ethanol. The aminopropyl column was washed with two column volumes of ethanol then combined fractions concentrated *in vacuo* to give the title compound as a colourless oil (105 mg, 59%).

LCMS (Method A): Rt = 1.04 mins, MH+ = 299.2

The Intermediate given in the following table was prepared in a manner similar to that described for Intermediate 6

Intermediate	Acid Chloride	Yield /%	LCMS
7 ethyl 3-[(phenylcarbonyl)amino]-4-pyridinecarboxylate		44	Rt 1.01 mins, MH+ = 271.2 (Method A)

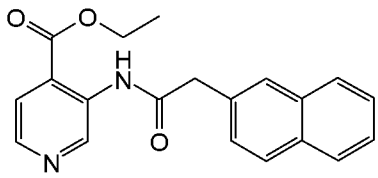
Intermediate 8: ethyl 3-{[4-(4-methylphenyl)butanoyl]amino}-4-pyridinecarboxylate



DMF (0.05 ml) was added dropwise over 10 s to a stirred solution of 3-(*p*-tolyl)butyric acid (322 mg, 1.81 mmol) and oxalyl chloride (0.19 ml, 2.26 mmol) in DCM (5 ml) at room temperature under nitrogen. The reaction mixture was stirred at room temperature 2 h then concentrated *in vacuo* to give a brown solid. The solid was dissolved in DMF (2 ml) then added dropwise over 30 s to a stirred suspension of ethyl 3-amino-4-pyridinecarboxylate (250 mg, 1.50 mmol) in DMF (3 ml) at 0 ºC under nitrogen. The reaction mixture was stirred at 0 ºC for 5 min then allowed to warm to room temperature over 1 h. Saturated sodium bicarbonate solution (10 ml) was added to the mixture portionwise over 2 min then the resultant mixture was partitioned with ethyl acetate (20 ml). The organic phase was isolated then the aqueous phase extracted with ethyl acetate (2 x 20 ml). The combined organic phase was passed through a hydrophobic frit then concentrated *in vacuo* to give the crude product. The crude product was purified with column chromatography (eluted with 0-40% EtOAc in cyclohexane) to give the title compound as a pale orange oil (310 mg, 63%).

LCMS (Method A): Rt = 1.21 mins, MH+ = 327.2

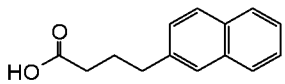
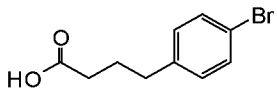
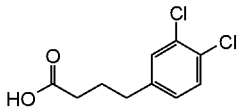
Intermediate 9: ethyl 3-[(2-naphthalenylacetyl)amino]-4-pyridinecarboxylate

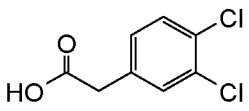
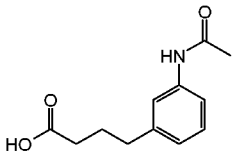
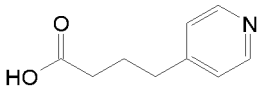
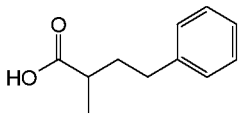
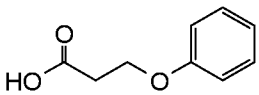
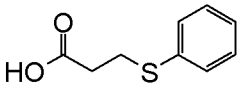
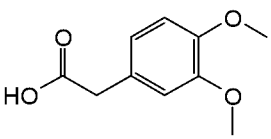
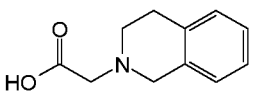
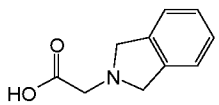
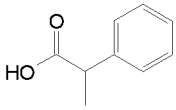


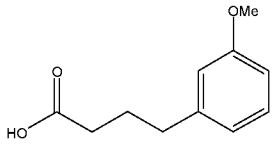
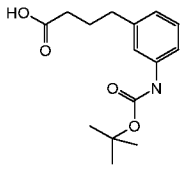
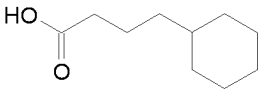
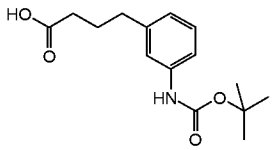
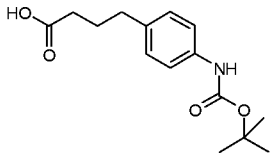
To a stirred solution of ethyl 3-amino-4-pyridinecarboxylate (250 mg, 1.50 mmol) and 2-naphthalenylacetic acid (280 mg, 1.50 mmol) and DIPEA (0.867 ml, 4.96 mmol) in DCM (10 ml) at room temperature under nitrogen was added 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane 2,4,6-trioxide (1340 mg, 50% by weight in ethyl acetate, 2.11 mmol) dropwise over 1 min. The mixture was allowed to stir for 76 h then saturated aq. sodium hydrogen carbonate solution (10 ml) was added, followed by chloroform (20 ml). The organic layer was isolated then the aqueous layer extracted twice with chloroform (2 x 20 ml). The combined organic phase was passed through a hydrophobic frit then concentrated *in vacuo* to give the crude product as a brown oil. The crude product was purified with column chromatography (eluted with 0-60% EtOAc in cyclohexane) to give the title compound as a pale yellow solid (391 mg, 78%).

LCMS (Method A): Rt = 1.13 mins, MH+ = 335.1

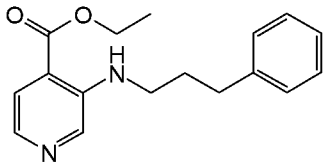
The Intermediates given in the following table were prepared in a manner similar to that described for Intermediate 9. Some Intermediates required purification by MDAP (Method E, F or G) in addition to or instead of the aforementioned column chromatography. After MDAP purification, an aminopropyl column was used in some cases to isolate the free base.

Intermediate	Acid	Yield /%	LCMS
10 3-{[4-(2-naphthalenyl)butanoyl]amino}-4-pyridinecarboxylic acid		13	Rt 1.25 mins, MH+ = 363.2 (Method A)
11 ethyl 3-{[4-(4-bromophenyl)butanoyl]amino}-4-pyridinecarboxylate		53	Rt 1.27 mins, MH+ = 391.1/393.1 (Method B)
12 ethyl 3-{[4-(3,4-dichlorophenyl)butanoyl]amino}-4-pyridinecarboxylate		48	Rt 1.28 mins, MH+ = 381.1/383.0 (Method A)

Intermediate	Acid	Yield /%	LCMS
13 ethyl 3-{[(3,4-dichlorophenyl)acetyl]amino}-4-pyridinecarboxylate		58	Rt 1.21 mins, MH+ = 353.0/355.0 (Method B)
14 ethyl 3-({4-[3-(acetylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylate		53	Rt 0.86 mins, MH+ = 370.2 (Method A)
15 ethyl 3-{[4-(4-pyridinyl)butanoyl]amino}-4-pyridinecarboxylate		58	Rt 0.53 mins, MH+ 314.1 (Method A)
16 ethyl 3-[(2-methyl-4-phenylbutanoyl)amino]-4-pyridinecarboxylate		35	Rt 1.23 mins, MH+ 327.2 (Method A)
17 ethyl 3-{[3-(phenyloxy)propanoyl]amino}-4-pyridinecarboxylate		24	Rt 1.03 mins, MH+ 315.1 (Method A)
18 ethyl 3-{[3-(phenylthio)propanoyl]amino}-4-pyridinecarboxylate		40	Rt 1.09 mins, MH+ 331.1 (Method A)
19 ethyl 3-({[3,4-bis(methyloxy)phenyl]acetyl}amino)-4-pyridinecarboxylate		61	Rt 0.89 mins, MH+ 345.2 (Method A)
20 ethyl 3-[(3,4-dihydro-2(1*H*)-isoquinolinylacetyl)amino]-4-pyridinecarboxylate		46	Rt 0.75 mins, MH+ 340.1 (Method A)
21 ethyl 3-[(1,3-dihydro-2*H*-isoindol-2-ylacetyl)amino]-4-pyridinecarboxylate		84	Rt 0.63 mins, MH+ 326.2 (Method A)
22 ethyl 3-[(2-phenylpropanoyl)amino]-4-pyridinecarboxylate		54	Rt 1.07 mins, MH+ 299.1 (Method A)

Intermediate	Acid	Yield /%	LCMS
23 ethyl 3-({4-[3-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylate		31	Rt 1.14 mins, MH+ 343.2 (Method B)
24 ethyl 3-({4-[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)phenyl]butanoyl}amino)-4-pyridinecarboxylate		67	Rt 1.20 mins, MH+ 428.3 (Method A)
42 ethyl 3-[(4-cyclohexylbutanoyl)amino]-4-pyridinecarboxylate		71	Rt 1.42 mins, MH+ 319.2 (Method B)
80 ethyl 3-({4-[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)phenyl]butanoyl}amino)-4-pyridinecarboxylate		71	Rt 1.18 mins, MH+ 428.1 (Method A)
81 ethyl 3-({4-[4-({[(1,1-dimethylethyl)oxy]carbonyl}amino)phenyl]butanoyl}amino)-4-pyridinecarboxylate		64	Rt 1.17 mins, MH+ 428 (Method A)

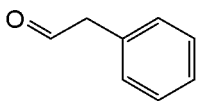
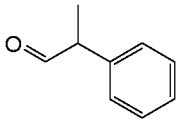
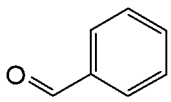
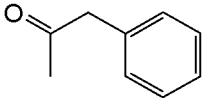
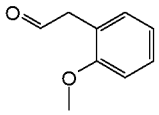
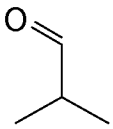
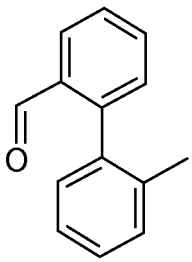
Intermediate 25: ethyl 3-[(3-phenylpropyl)amino]-4-pyridinecarboxylate

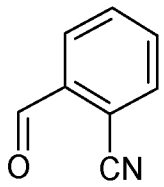
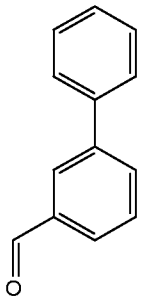


To a stirred solution of ethyl 3-amino-4-pyridinecarboxylate (250 mg, 1.50 mmol) and 3-phenylpropanal (0.218 ml, 1.66 mmol) and trifluoroacetic acid (0.695 ml, 9.03 mmol) in isopropyl acetate (3 ml) was added sodium triacetoxyborohydride (383 mg, 1.81 mmol) portionwise over 30 s then the mixture was stirred at room temperature under nitrogen for 30 min. The reaction mixture was quenched with saturated sodium hydrogen carbonate (20 ml) then partitioned with ethyl acetate (20 ml). The organic layer was isolated then the aqueous phase extracted twice with EtOAc (2 x 20 ml) then the combined organic phase passed through a hydrophobic frit then evaporated under reduced pressure to give the crude product as an orange oil. The crude product was purified with column chromatography (eluted with 0-20% EtOAc in cyclohexane) to give the title compound as a yellow oil (278 mg, 65%).

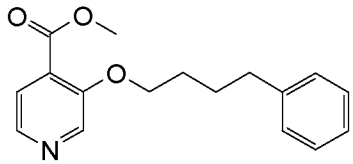
LCMS (Method A): Rt = 1.03 mins, MH+ = 285.1

The Intermediates given in the following table were prepared in a manner similar to that described for Intermediate 25. Some Intermediates required purification by MDAP (Method E, F or G) or preparative HPLC (Method I) in addition to or instead of the aforementioned column chromatography. After purification, an aminopropyl column was used in some cases to isolate the free base.

Intermediate	Aldehyde	Yield /%	LCMS
26 ethyl 3-[(2-phenylethyl)amino]-4-pyridinecarboxylate		58	Rt 0.94 mins, MH+ = 271.1 (Method A)
27 ethyl 3-[(2-phenylpropyl)amino]-4-pyridinecarboxylate		26	Rt 0.98 mins, MH+ = 285.1 (Method A)
28 ethyl 3-[(phenylmethyl)amino]-4-pyridinecarboxylate		78	Rt 0.92 mins, MH+ = 257.1 (Method A)
29 ethyl 3-[(1-methyl-2-phenylethyl)amino]-4-pyridinecarboxylate		9	Rt 1.02 mins, MH+ = 285.1 (Method A)
44 ethyl 3-({2-[2-(methyloxy)phenyl]ethyl}amino)-4-pyridinecarboxylate		25	Rt 1.00 mins, MH+ = 301.1 (Method A)
45 ethyl 3-[(2-methylpropyl)amino]-4-pyridinecarboxylate		31	Rt 0.85 mins, MH+ = 223.1 (Method A)
46 ethyl 3-{[(2'-methyl-2-biphenylyl)methyl]amino}-4-pyridinecarboxylate		32	Rt 7.20 mins, MH+ = 347 (Method D)

Intermediate	Aldehyde	Yield /%	LCMS
47 ethyl 3-{[(2-cyanophenyl)methyl]amino}-4-pyridinecarboxylate		30	Rt 5.17 mins, MH+ = 282 (Method D)
80 ethyl 3-[(3-biphenylylmethyl)amino]-4-pyridinecarboxylate		62	Rt 4.16 mins, MH+ = 333 (Method D)

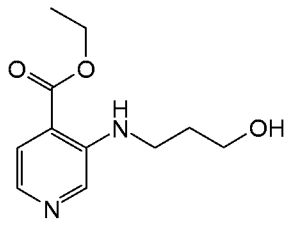
Intermediate 30: methyl 3-[(4-phenylbutyl)oxy]-4-pyridinecarboxylate



To a solution of methyl 3-hydroxy-4-pyridinecarboxylate (100 mg, 0.65 mmol) in DMF (2 ml) was added cesium carbonate (426 mg, 1.31 mmol) followed by (4-bromobutyl)benzene (139 mg, 0.65 mmol). The mixture was allowed to stir at room temperature for 16 h then concentrated *in vacuo*. The crude product was dissolved in DMSO (1 ml) and purified by MDAP using a formic modifier (Method G). Fractions containing desired product were loaded directly onto a 5g SCX-II SPE column that had been preconditioned with isopropylalcohol. The column was eluted with isopropylalcohol (3 x 50 ml) then with a 10% ammonia in water / 90% isopropylalcohol solution. Desired product eluted in ammonia based fractions which were combined then concentrated under reduced pressure to give the title compound as a pale brown gum (123mg, 66%).

LCMS (Method B): Rt = 1.19 mins, MH+ = 286.1

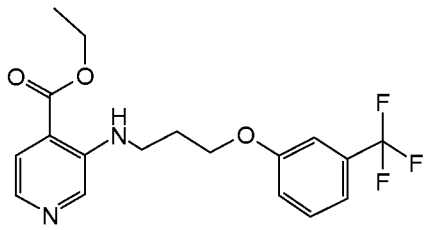
Intermediate 31: ethyl 3-[(3-hydroxypropyl)amino]-4-pyridinecarboxylate



To a stirred solution of 3-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}propanal (1.54g, 8.16 mmol), ethyl 3-amino-4-pyridinecarboxylate (0.68g, 4.08 mmol), TFA (1.89ml, 24.48 mmol) in isopropyl acetate (10ml) under nitrogen and at room temperature was added sodium triacetoxyborohydride (1.73g, 8.16 mmol) in a portionwise manner. The mixture was stirred for 16h, then quenched with saturated sodium hydrogen carbonate solution (50ml) and diluted with EtOAc (50ml). The organic phase was isolated then the aqueous phase reextracted with EtOAc (2 x 50ml) and the combined organic phase passed through a hydrophobic frit then concentrated under reduced pressure to give an orange oil. The crude product was purified with column chromatography (eluted with 0-8% MeOH in DCM) to give the title compound as a yellow solid (338mg, 37%).

LCMS (Method A): Rt = 0.48 mins, MH+ = 225.0

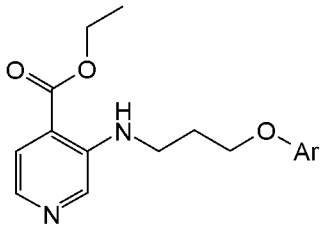
Intermediate 32: ethyl 3-[(3-{[3-(trifluoromethyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylate

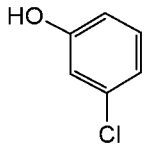
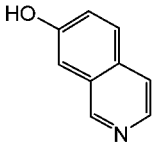
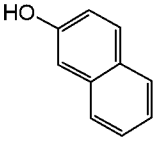
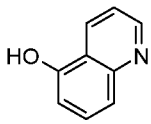
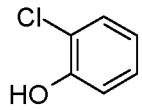
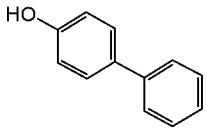
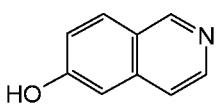


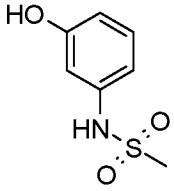
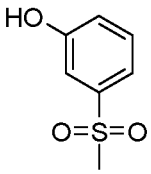
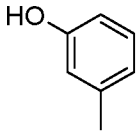
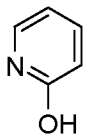
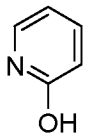
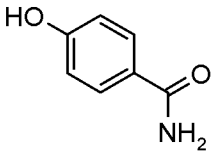
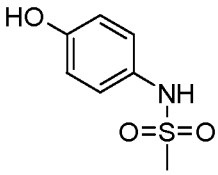
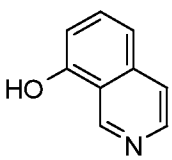
To a solution of ethyl 3-[(3-hydroxypropyl)amino]-4-pyridinecarboxylate (80mg, 0.36 mmol) in THF (2 ml) was added 3-(trifluoromethyl)phenol (0.043ml, 0.35 mmol) followed by triphenylphosphine (187mg, 0.71 mmol). The reaction mixture was flushed with nitrogen then cooled to around 0°C using an ice/water bath then a solution of bis(1,1-dimethylethyl) -1,2-diazenedicarboxylate (170mg, 0.45 mmol) in THF (1ml) was added dropwise. The reaction mixture was allowed to stir at room temperature overnight then TFA (0.200ml) added. The mixture was stirred at room temperature for ten minutes then concentrated under reduced pressure. The resulting residue was dissolved in MeOH then loaded onto a MeOH-preconditioned 10g SCX SPE column which was then eluted with MeOH then 2M ammonia in MeOH. Crude product eluted in ammonia based fractions which were combined then concentrated under reduced pressure. The crude product was purified with column chromatography (eluted with 25-75% EtOAc in cyclohexane) to give the title compound as a pale yellow oil (54 mg, 41%).

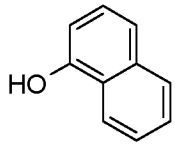
LCMS (Method A): Rt = 1.15 mins, MH+ = 369.0

The Intermediates given in the following table were prepared in a manner similar to that described for Intermediate 32. Some Intermediates required purification by MDAP (Method E, F or G) in addition to or instead of the aforementioned column chromatography. SCX SPE was excluded in some cases.

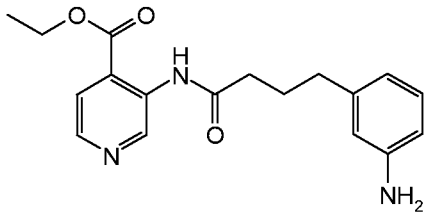


Intermediate	Phenol, ArOH	Yield /%	LCMS
33 ethyl 3-({3-[(3-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylate		41	Rt 1.11 mins, MH+ = 335.0/336.9 (Method A)
34 ethyl 3-{[3-(7-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylate		51	Rt 0.62 mins, MH+ = 352.1 (Method A)
35 ethyl 3-{[3-(2-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylate		93	Rt 1.15 mins, MH+ = 351.1 (Method A)
36 ethyl 3-{[3-(5-quinolinyloxy)propyl]amino}-4-pyridinecarboxylate		36	Rt 1.11 mins, MH+ = 352.2 (Method B)
37 ethyl 3-({3-[(2-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylate		54	Rt 1.30 mins, MH+ = 335.2/337.1 (Method B)
38 ethyl 3-{[3-(4-biphenylyloxy)propyl]amino}-4-pyridinecarboxylate		17	Rt 1.23 mins, MH+ = 377.1 (Method A)
48 ethyl 3-{[3-(6-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylate		Quantitative	Rt 0.59 mins, MH+ = 352.1 (Method A)

Intermediate	Phenol, ArOH	Yield /%	LCMS
49 ethyl 3-{[3-({3-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylate		22	Rt 0.94 mins, MH+ = 394.1 (Method B)
50 ethyl 3-[(3-{[3-(methylsulfonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylate		47	Rt 1.05 mins, MH+ = 379.1 (Method B)
51 ethyl 3-({3-[(3-methylphenyl)oxy]propyl}amino)-4-pyridinecarboxylate		35	Rt 1.32 mins, MH+ = 315.2 (Method B)
52 ethyl 3-{[3-(2-oxo-1(2*H*)-pyridinyl)propyl]amino}-4-pyridinecarboxylate		17	Rt 0.77 mins, MH+ = 302.1 (Method B)
53 3-{[3-(2-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid		48	Rt 1.12 mins, MH+ = 302.1 (Method B)
54 ethyl 3-[(3-{[4-(aminocarbonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylate		20	Rt 0.90 mins, MH+ = 344.1 (Method B)
55 ethyl 3-{[3-({4-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylate		35	Rt 0.91 mins, MH+ = 394.2 (Method B)
56 ethyl 3-{[3-(8-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylate		38	Rt 1.11 mins, MH+ = 352.2 (Method B)

Intermediate	Phenol, ArOH	Yield /%	LCMS
57 ethyl 3-{[3-(1-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylate		11	Rt 1.39 mins, MH+ = 351.2 (Method B)

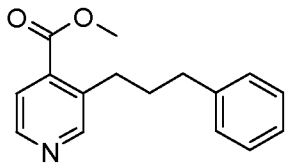
Intermediate 39: ethyl 3-{[4-(3-aminophenyl)butanoyl]amino}-4-pyridinecarboxylate



To a solution of ethyl 3-({4-[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)phenyl]butanoyl}amino)-4-pyridinecarboxylate (423mg, 0.99 mmol) in DCM (20ml) at room temperature was added TFA (0.762ml, 9.89 mmol) dropwise. The reaction mixture was stirred at room temperature for four hours, then saturated aqueous sodium hydrogen carbonate added (50ml) over 2 mins and the resultant biphasic mixture stirred rapidly for 10 mins. The organic phase was isolated then the aqueous phase reextracted using DCM (2 x 50ml) then the combined organic phases passed through a hydrophobic frit then concentrated under reduced pressure to give a yellow oil. The crude product was purified with column chromatography (eluted with 0-60% EtOAc in cyclohexane) to give the title compound as a yellow oil (255 mg, 79%).

LCMS (Method A): Rt = 0.70 mins, MH+ = 328.1

Intermediate 40: methyl 3-(3-phenylpropyl)-4-pyridinecarboxylate



To a mixture of methyl 3-bromo-4-pyridinecarboxylate (70mg, 0.32 mmol), palladium (II) acetate (5.5mg, 0.024 mmol) and S-Phos (13 mg, 0.032 mmol) in a microwave vial was added 0.5M bromo(3-phenylpropyl)zinc in THF (10.5ml, 5.25 mmol). The vial was sealed then the mixture heated at 80°C for 20 mins, then at 60°C for a further 10 mins and then at 80°C for a further 10 mins. Further 0.5M bromo(3-phenylpropyl)zinc in THF (10.5ml, 5.25 mmol) was added to the reaction mixture which was then heated at 80°C for a further 10 mins.

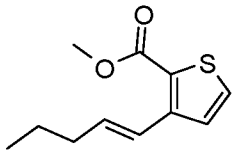
Separately, to a mixture of methyl 3-bromo-4-pyridinecarboxylate (758mg, 3.51 mmol), palladium (II) acetate (59mg, 0.26 mmol) and S-Phos (144mg, 0.35 mmol) in a microwave vial was added 0.5M

bromo(3-phenylpropyl)zinc in THF (10.5ml, 5.25 mmol). The vial was sealed then the mixture heated at 80°C for 20 mins.

The two reaction mixtures were combined then filtered using a phase separator, then partitioned between EtOAc (50ml) and ammonium chloride (25ml). The organic layer was isolated, washed with brine then dried over magnesium sulfate then filtered and concentrated under reduced pressure to give the crude product as an oil. The crude product was purified with column chromatography (eluted with 0-20% EtOAc in cyclohexane) then MDAP (Method E) to give the title compound as a yellow oil (118mg, 13%).

LCMS (Method B): Rt = 1.16mins, MH+ = 256.1

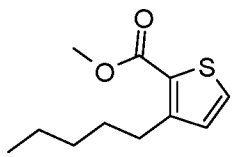
Intermediate 58: methyl 3-[(1*E*)-1-penten-1-yl]-2-thiophenecarboxylate



To a solution of tetrakis(triphenylphosphine) palladium (0) (1.307 g, 1.131 mmol) and methyl 3-bromothiophene-2-carboxylate (5 g, 22.6 mmol) in water (0.750 ml) and 1,4-dioxane (1.5ml) was added cesium carbonate (14.74 g, 45.2 mmol) and (1*E*)-1-penten-1-ylboronic acid (3.09 g, 27.1 mmol). The reaction mixture was heated at 110ºC for 12 hr then concentrated under reduced pressure and partitioned between EtOAc (15 ml) and water (15 ml). The aqueous layer was extracted three times with EtOAc (15 ml) then combined organic layers dried over sodium sulphate then concentrated under reduced pressure and dried under high vacuum to give crude product as a yellow oil. The crude material was purified by silica column chromatography, eluting with cyclohexane/DCM (0 to 100%) to give the title compound as a colourless liquid, 2.52g (53%).

LCMS (Method A): Rt = 1.29mins, MH+ = 211.1

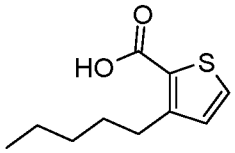
Intermediate 59: methyl 3-pentyl-2-thiophenecarboxylate



Methyl 3-[(1*E*)-1-penten-1-yl]-2-thiophenecarboxylate (2.52 g, 11.98 mmol) was diluted with EtOH (30 ml) then palladium on carbon (10% by weight, wet, 0.255 g) was added. The reaction mixture was flushed with hydrogen then left to stir under an atmosphere of hydrogen for 3 days then filtered on celite. The celite was washed four times with ethanol then the filtrate was evaporated under reduced pressure to give the title compound as a colourless liquid, 2.41 g (95%).

LCMS (Method A): Rt = 1.35mins, MH+ = 213.1

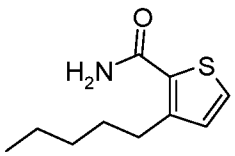
Intermediate 60: 3-pentyl-2-thiophenecarboxylic acid



To a solution of methyl 3-pentyl-2-thiophenecarboxylate (2.04 g, 9.61 mmol) in water (5 ml) and THF (5 ml) was added lithium hydroxide (1.878 g, 77 mmol) and the mixture stirred at 50ºC overnight. Further water (5 ml) was added to the reaction mixture which was stirred for a further 15 hr at 75ºC then allowed to cool to room temperature. The mixture was neutralized by the addition of 2M HCl then concentrated under reduced pressure to give a white solid, (7.26 g). The solid was dissolved in water (15 ml) then acidified to pH 2 and partitioned with EtOAc (20 ml). The organic layer was isolated then the aqueous layer reextracted with ethyl acetate (4 x 20 ml). Combined organic phases were dried over sodium sulphate then concentrated under reduced pressure to give the title compound as a yellow solid, 1.76 g (92%).

LCMS (Method B): Rt = 0.73 mins, MH- = 197.0

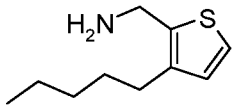
Intermediate 61: 3-pentyl-2-thiophenecarboxamide



To a solution of 3-pentyl-2-thiophenecarboxylic acid (320 mg, 1.614 mmol) in toluene (6 ml) at 0ºC and under nitrogen was added ethanedioyl dichloride (0.546 mL, 6.46 mmol) and the reaction mixture heated at 55ºC for 18 hr then concentrated under reduced pressure. The mixture was diluted with chloroform then concentrated under reduced pressure three times to give crude 3-pentyl-2-thiophenecarbonyl chloride as a brown oil (320 mg, 1.477 mmol). To a portion of the crude material (216 mg, 0.997) was added ammonium hydroxide (0.5 M solution in 1,4-dioxane, 20 ml, 10.0 mmol) and the resulting reaction mixture stirred for 3 hr at room temperature under nitrogen. The mixture was then filtered through celite and the filtrate washed twice with brine then dried over sodium sulphate and concentrated under reduced pressure. The resultant solid was redissolved in EtOAc then filtered under vaccum and the filtrate concentrated under reduced pressure to give the title compound as a white solid 192 mg (98%).

LCMS (Method B): Rt = 1.01 mins, MH+ = 198.2

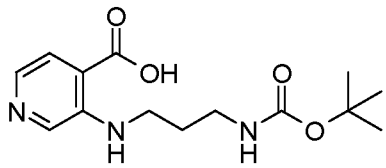
Intermediate 62: [(3-pentyl-2-thienyl)methyl]amine



To a solution of 3-pentyl-2-thiophenecarboxamide (192 mg, 0.973 mmol) in THF (3 mL) under nitrogen was added lithium aluminium hydride (1M in THF, 3 ml, 3.00 mmol). The reaction mixture was stirred for 18 hr at room temperature. The reaction was quenched by the addition of water (0.114 mL), then a solution of NaOH (15 %, 0.114 ml) and finally water (0.342 ml). The resultant mixture was filtered under reduced pressure. EtOAc (15 ml) was added to the filtrate which was then was washed with water (3 x 10 ml) and the organic layer dried over sodium sulphate then concentrated under reduced pressure to give the crude title compound as a yellow oil, 78 mg (44%).

1H NMR (400 MHz, DMSO-δ6) 7.21ppm (1H, d), 6.83ppm (1H, d), 3.81ppm (2H, s), 2.47ppm (2H, t, obscured by DMSO peak), 1.64-1.06 (6H, m, obscured by impurity peaks),0.86ppm (3H, t)

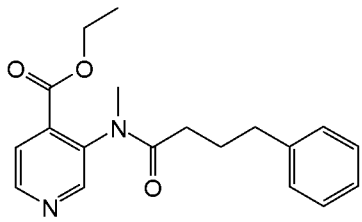
Intermediate 63: 3-{[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)propyl]amino}-4-pyridinecarboxylic acid



To a solution of 3-fluoro-4-pyridinecarboxylic acid (400mg, 2.83 mmol) in 1,4-Dioxane (3ml) was added 1,1-dimethylethyl (3-aminopropyl)carbamate (494 mg, 2.83 mmol) and the mixture heated to 120ºC using a microwave for 36 h. The mixture was allowed to cool to room temperature then concentrated by evaporation. The residue was purified by silica gel chromatography, eluting with 0-10% methanol in dichloromethane to give the title compound as a yellow oily solid, 227 mg (27% yield).

LCMS (Method A): Rt = 0.54 mins, MH+ = 296.1

Intermediate 64: ethyl 3-[methyl(4-phenylbutanoyl)amino]-4-pyridinecarboxylate

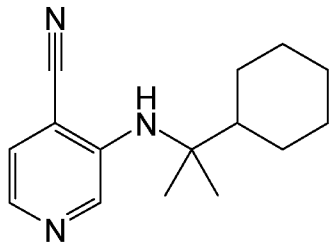


Sodium hydride (29 mg, 60% dispersion in mineral oil by weight, 0.72 mmol) was added in a single portion to a stirred solution of ethyl 3-[(4-phenylbutanoyl)amino]-4-pyridinecarboxylate (150 mg, 0.480 mmol) in DMF (5 ml) at 0 ºC and under nitrogen. The reaction mixture was stirred at 0 ºC for 15 min

then iodomethane (0.060 ml, 0.960 mmol) was added dropwise over 10 s and the resultant solution was stirred at 0 ºC for 30 min before being removed from the cooling bath and allowed to stir at rt for 30 min. Ammonium chloride (10 ml, saturated) and EtOAc (10 ml) were added and the the separated aqueous phase reextracted with EtOAc (2 x 20 mL). The combined organic phase was passed through a hydrophobic frit and then evaporated under reduced pressure to give an orange oil. The sample was purified by silica column chromatography, eluting with 0-50% EtOAc / cyclohexane to give the title compound as a colourless oil, 90 mg (57%).

LCMS (Method A): Rt = 1.01 mins, MH+ = 327.2

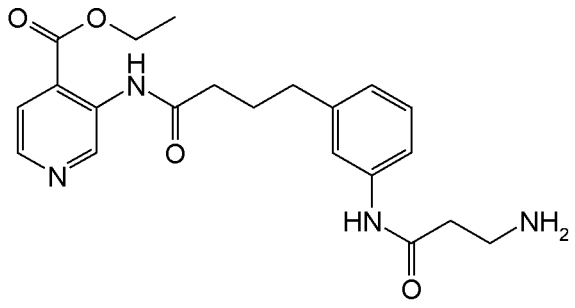
Intermediate 65: 3-[(1-cyclohexyl-1-methylethyl)amino]-4-pyridinecarbonitrile



To a mixture of 3-fluoro-4-pyridinecarbonitrile (100 mg, 0.819 mmol) in NMP (0.25 ml) under nitrogen was added (1-cyclohexyl-1-methylethyl)amine hydrochloride (218 mg, 1.229 mmol) and DIPEA (0.429 mL, 2.457 mmol), and the mixture heated to 100ºC using a microwave for 1 hr then at 150ºC for 2 hr, a further 4 hr and finally an additional 3 hr. The mixture was diluted with DMSO/acetonitrile then purified by MDAP (high pH, Method E) to give the title compound as a tan solid, 34 mg (16%).

LCMS (Method A): Rt = 1.23 mins, MH+ = 244.3

Intermediate 66: ethyl 3-({4-[3-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylate, formate salt.

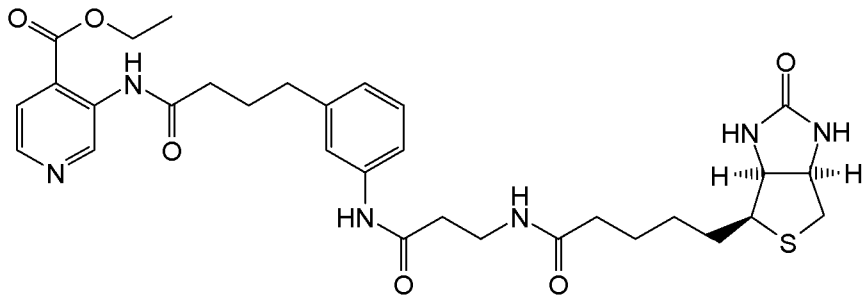


TFA (0.110 ml, 1.424 mmol) was added dropwise over 30 s to a stirred solution of ethyl 3-[(4-{3-[(*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylate (71 mg, 0.142 mmol) in DCM (10 ml) at rt and the mixture stirred at rt for 16 h, Sodium hydrogen

carbonate (saturated, aq, 10 ml) was added portionwise over 5 min and the resultant biphasic solution was stirred rapidly for 5 min. The separated aqueous phase was extracted with EtOAc (2 x 20 ml), then the combined organic phase passed through a hydrophobic frit and concentrated under reduced pressure to give a colourless oil. The oil was dissolved in 1:1 MeOH:DMSO (3 ml) then purified by MDAP (Method G) to give the title compound as a colourless oil, 24 mg (38%).

LCMS (Method A): Rt = 0.70 mins, MH+ = 399.1

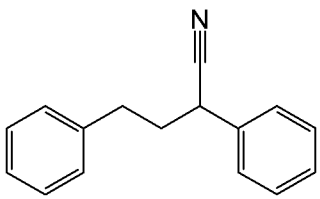
Intermediate 67: ethyl 3-[(4-{3-[(*N*-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}-β-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylate



TBTU (232 mg, 0.723 mmol) was added in a single portion to a stirred suspension of 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid (177 mg, 0.723 mmol) and DIPEA (0.574 ml, 3.29 mmol) in DMF (6 ml) at rt under nitrogen. The reaction mixture was stirred at room temperature for 15 min then a solution of ethyl 3-({4-[3-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylate (262 mg, 0.658 mmol) in DMF (4 ml) was added dropwise over 2 min. The resultant solution was stirred at rt for 1h and then saturated ammonium chloride (aq, 30 ml) and EtOAc (30 ml) were added. The separated aqueous phase was extracted with EtOAc (5 x 20 ml) and then DCM (3 x 30 ml) and the combined organic phase passed through a hydrophobic frit and evaporated under reduced pressure to give a yellow oil. The material was purified by silica column chromatography using a gradient of 0-15% EtOH / DCM to give the title compound as a white solid, 338 mg (82%).

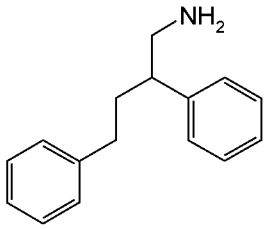
LCMS (Method A): Rt = 0.81 mins, MH+ = 625.4

Intermediate 68a: 2,4-diphenylbutanenitrile



2,4-diphenylbutanenitrile was prepared according to the method described in the Journal of Organic Chemistry, 2000 , Vol. 65 (17), page 5371 – 5381.

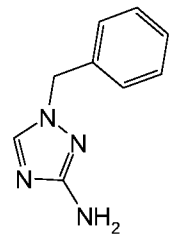
Intermediate 68b (2,4-diphenylbutyl)amine



2,4-diphenylbutanenitrile (300 mg, 1.36 mmol) was dissolved in EtOH (5 ml) and 37% HCl (5 ml, 165 mmol) and the resulting mixture hydrogenated overnight at atmospheric pressure using dichloropalladium (30 mg, 0.169 mmol) . The catalyst was removed by filtration through celite then volatiles removed under reduced pressure to give a yellow solid. The solid was dissolved in MeOH then loaded onto an SCX-cartridge which was eluted with MeOH then 2M ammonia in MeOH, Basic fractions were combined then concentrated under reduced pressure to give the title compound as a clear translucent oil, 174mg (57%).

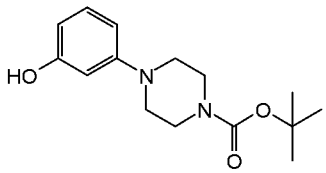
LCMS (Method B): Rt = 1.12 mins, MH+ = 226.1

Intermediate 69: 1-(phenylmethyl)-1*H*-1,2,4-triazol-3-amine



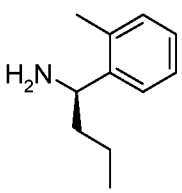
Available from Fluorochem Ltd.

Intermediate 70: 1,1-dimethylethyl 4-(3-hydroxyphenyl)-1-piperazinecarboxylate



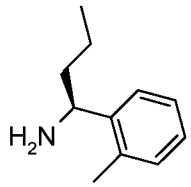
Available from Chem Impex International.

Intermediate 71: [(1*R*)-1-(2-methylphenyl)butyl]amine



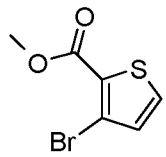
Available from APAC Pharmaceutical LLC.

Intermediate 72: [(1S)-1-(2-methylphenyl)butyl]amine



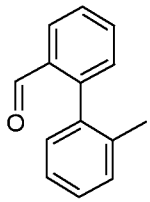
Available from APAC Pharmaceutical LLC.

Intermediate 73: methyl 3-bromo-2-thiophenecarboxylate



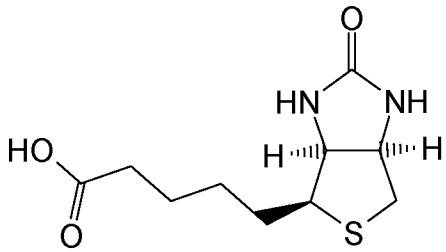
Available from Apollo Scientific Ltd.

Intermediate 74: 2'-methyl-2-biphenylcarbaldehyde



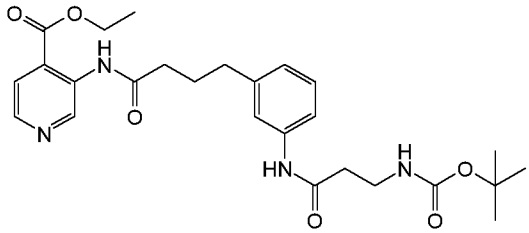
Available from Oakwood.

Intermediate 75: 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid



Available from Sigma Aldrich.

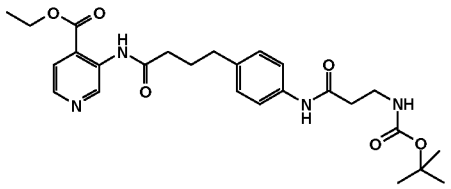
Intermediate 76: ethyl 3-[(4-{3-[(*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β–alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylate



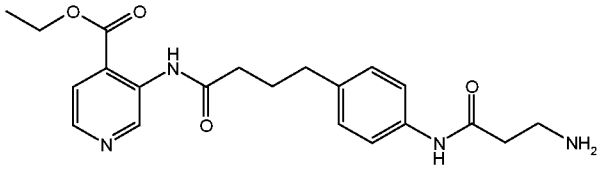
T3P (272 mg, 50% by weight in EtOAc, 0.428 mmol) was added dropwise over 30 s to a stirred solution of ethyl 3-{[4-(3-aminophenyl)butanoyl]amino}-4-pyridinecarboxylate (100 mg, 0.305 mmol), *N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β-alanine (58 mg, 0.307 mmol) and DIPEA (0.176 ml, 1.008 mmol) in DCM (10 ml) at rt under N_2. Following stirring at rt for 1 h, sat. aq. $NaHCO_3$ (20 mL) was added. The separated aqueous phase was extracted with CH_2Cl_2 (2 x 20 mL), the combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give a pale yellow oil. The sample was loaded in dichloromethane and purified by Biotage SP4 SNAP 25g silica using a gradient of 0-100% EtOAc / cyclohexane. The appropriate fractions were combined and evaporated under vacuum to give the title compound as a colourless oil (71mg, 47%).

LCMS (Method A):Rt = 1.04 min, MH+ = 499.2

The Intermediate given in the following table was prepared in a manner similar to that described for Intermediate 76.

Intermediate	Structure	Yield /%	LCMS
77 ethyl 3-[(4-{4-[(*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylate		93	Rt 1.03 mins, MH+ = 499.2 (Method A)

Intemediate 78: ethyl 3-({4-[4-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylate

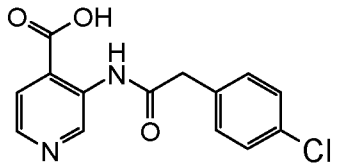


TFA (1.947 ml, 25.3 mmol) was added in a single portion to a stirred solution of ethyl 3-[(4-{4-[(*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylate (1.26 g, 2.53 mmol) in DCM (60 mL) at rt. Following stirring at rt for 30 min, sat. aq. $NaHCO_3$ (50 ml) and EtOAc (50 ml) were added. The separated aqueous phase was extracted with EtOAc (2 x 50 ml), the

combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give the desired compound as a pale yellow oil (710 mg, 71%).

LCMS (Method A): Rt = 0.67 min, MH+=399.1

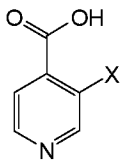
Example 1: 3-{[(4-chlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid hydrochloride

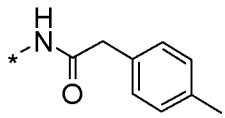
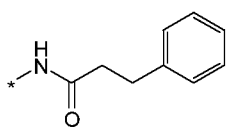


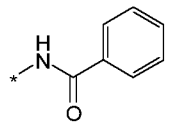
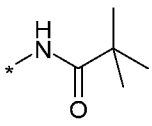
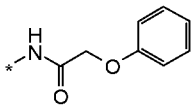
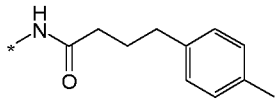
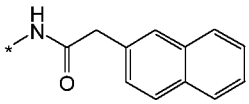
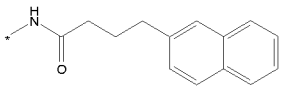
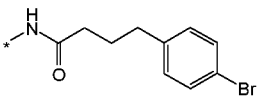
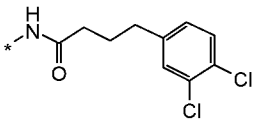
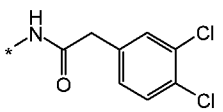
To a solution of ethyl 3-{[(4-chlorophenyl)acetyl]amino}-4-pyridinecarboxylate (184 mg, 0.58 mmol) in THF (10 ml) and water (3.33ml) was added lithium hydroxide monohydrate (48mg, 1.15 mmol). The reaction mixture was stirred overnight, then acidified with 2.0N aq. HCl and stirred for five minutes. The resulting orange suspension was filtered then washed with water (50 ml), then ethanol (30 ml) then ether (50 ml) and dried *in vacuo* to give the title compound as a white solid (221 mg, quantitative yield).

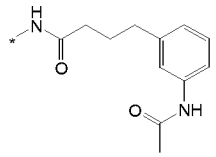
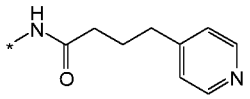
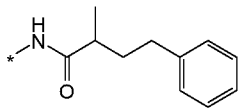
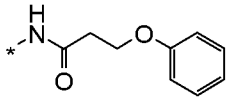
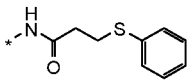
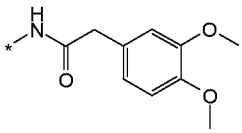
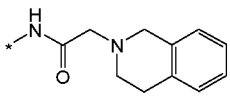
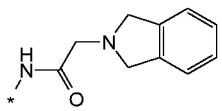
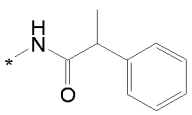
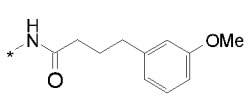
1H NMR (400 MHz, DMSO-δ6) 10.67ppm (1H, br s), 9.45ppm (1H, s), 8.43ppm (1H, d), 7.75ppm (1H, d), 7.47–7.35ppm (4H, m), 3.64–3.58ppm (2H, m).
LCMS (Method A): Rt = 0.65 mins, MH+ = 291.1

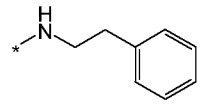
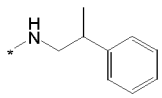
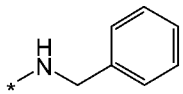
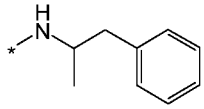
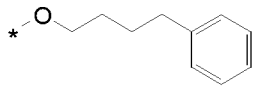
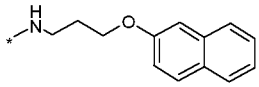
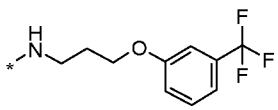
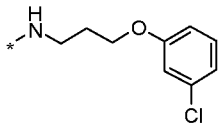
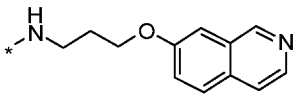
The Examples given in the following table were prepared in a manner similar to that described for Example 1. Some examples required purification by MDAP (Method E, F or G) in addition to or instead of the aforementioned column chromatography. After MDAP purification, an SCX column was used in some cases to isolate the free base.

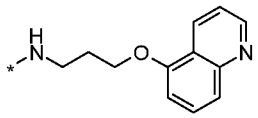
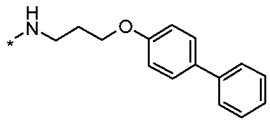
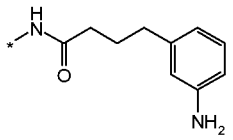
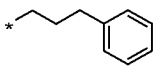
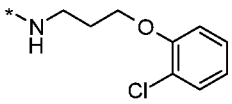
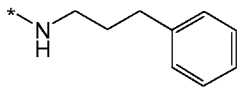
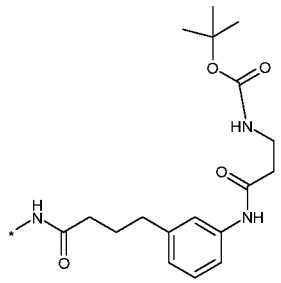
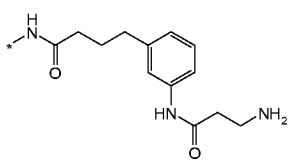
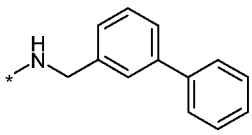


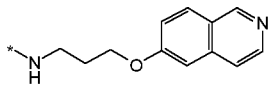
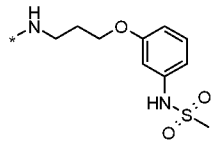
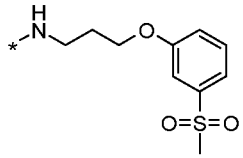
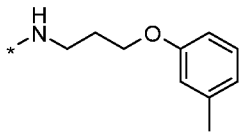
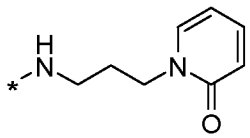
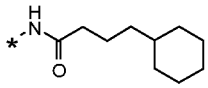
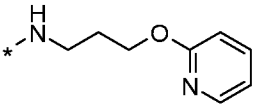
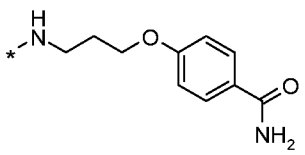
Example	X	Yield /%	LCMS
2 3-{[(4-methylphenyl)acetyl]amino}-4-pyridinecarboxylic acid		48	Rt 0.62 mins, MH+ = 271.1 (Method A)
3 3-[(3-phenylpropanoyl)amino]-4-pyridinecarboxylic acid		60	Rt 0.60 mins, MH+ = 271.1 (Method A)

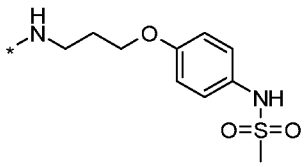
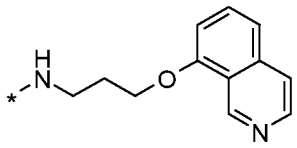
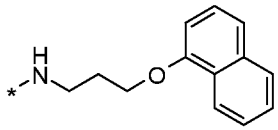
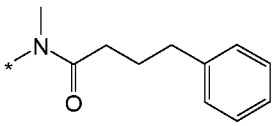
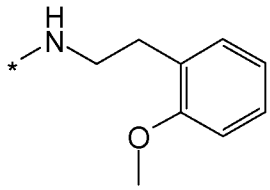
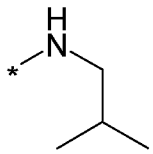
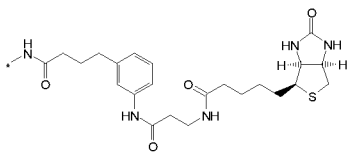
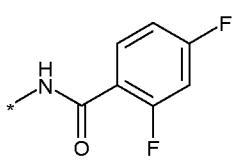
Example	X	Yield /%	LCMS
4 3-[(phenylcarbonyl)amino]-4-pyridinecarboxylic acid		58	Rt 0.51 mins, MH+ = 243.0 (Method A)
5 3-[(2,2-dimethylpropanoyl)amino]-4-pyridinecarboxylic acid		57	Rt 0.46 mins, MH+ = 223.1 (Method A)
6 3-{[(phenyloxy)acetyl]amino}-4-pyridinecarboxylic acid		56	Rt 0.57 mins, MH+ = 273.1 (Method A)
7 3-{[4-(4-methylphenyl)butanoyl]amino}-4-pyridinecarboxylic acid		55	Rt 0.74 mins, MH+ = 299.0 (Method A)
8 3-[(2-naphthalenylacetyl)amino]-4-pyridinecarboxylic acid		47	Rt 0.71 mins, MH+ = 307.1 (Method A)
9 3-{[4-(2-naphthalenyl)butanoyl]amino}-4-pyridinecarboxylic acid. 0.5 ammonium salt		57	Rt 0.77 mins, MH+ = 335.1 (Method B)
10 3-{[4-(4-bromophenyl)butanoyl]amino}-4-pyridinecarboxylic acid		25	Rt 0.76 mins, MH+ = 363.0/365.0 (Method B)
11 3-{[4-(3,4-dichlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid hydrochloride		94	Rt 0.85 mins, MH+ 353.0/355.1 (Method A)
12 3-{[(3,4-dichlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid hydrochloride		67	Rt 0.69 mins, MH+ 325.0/327.0 (Method B)

Example	X	Yield /%	LCMS
13 3-({4-[3-(acetylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid		43	Rt 0.53 mins, MH+ 342.1 (Method A)
14 3-{[4-(4-pyridinyl)butanoyl]amino}-4-pyridinecarboxylic acid		95	Rt 0.28 mins, MH+ 286.1 (Method A)
15 3-[(2-methyl-4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid		89	Rt 0.67 mins, MH+ 299.3 (Method B)
16 3-{[3-(phenyloxy)propanoyl]amino}-4-pyridinecarboxylic acid		54	Rt 0.58 mins, MH+ 287.1 (Method B)
17 3-{[3-(phenylthio)propanoyl]amino}-4-pyridinecarboxylic acid hydrochloride		35	Rt 0.63 mins, MH+ 302.9 (Method A)
18 3-({[3,4-bis(methyloxy)phenyl]acetyl}amino)-4-pyridinecarboxylic acid		28	Rt 0.51 mins, MH+ 317.1 (Method A)
19 3-[(3,4-dihydro-2(1*H*)-isoquinolinylacetyl)amino]-4-pyridinecarboxylic acid		99	Rt 0.45 mins, MH+ 312.1 (Method A)
20 3-[(1,3-dihydro-2*H*-isoindol-2-ylacetyl)amino]-4-pyridinecarboxylic acid		92	Rt 0.35 mins, MH+ 298.1 (Method A)
21 3-[(2-phenylpropanoyl)amino]-4-pyridinecarboxylic acid		85	Rt 0.60 mins, MH+ 271.1 (Method A)
22 3-({4-[3-(methyloxy)phenyl]butanoyl}amino)-4-		11	Rt 0.63 mins, MH+ 315.0 (Method B)

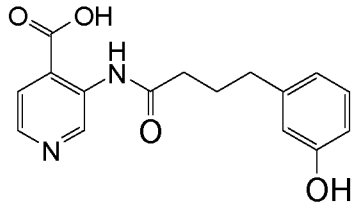
Example	X	Yield /%	LCMS
pyridinecarboxylic acid			
23 3-[(2-phenylethyl)amino]-4-pyridinecarboxylic acid		87	Rt 0.56 mins, MH+ = 243.0 (Method A)
24 3-[(2-phenylpropyl)amino]-4-pyridinecarboxylic acid		92	Rt 0.62 mins, MH+ = 257.1 (Method A)
25 3-[(phenylmethyl)amino]-4-pyridinecarboxylic acid		19	Rt 0.51 mins, MH+ = 229.1 (Method A)
26 3-[(1-methyl-2-phenylethyl)amino]-4-pyridinecarboxylic acid		99	Rt 0.60 mins, MH+ = 257.1 (Method A)
27 3-[(4-phenylbutyl)oxy]-4-pyridinecarboxylic acid		12	Rt 0.73min, MH+ 272.1 (Method A)
28 3-{[3-(2-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		51	Rt 0.80min, MH+ 323.1 (Method A)
29 3-[(3-{[3-(trifluoromethyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid hydrochloride		63	Rt 0.80min, MH+ 341.0 (Method A)
30 3-({3-[(3-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid hydrochloride		62	Rt 0.75min, MH+ 306.9/308.9 (Method A)
31 3-{[3-(7-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		95	Rt 0.39min, MH+ 324.0 (Method A)

Example	X	Yield /%	LCMS
32 3-{[3-(5-quinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		36	Rt 0.61min, MH+ 324.1 (Method B)
33 3-{[3-(4-biphenylyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		91	Rt 0.88min, MH+ 349.1 (Method A)
34 3-{[4-(3-aminophenyl)butanoyl]amino}-4-pyridinecarboxylic acid		31	Rt 0.38min, MH+ 300.0 (Method B)
35 3-(3-phenylpropyl)-4-pyridinecarboxylic acid, formate salt		66	Rt 0.57min, MH+ 242.1 (Method B)
36 3-({3-[(2-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid		68	Rt 0.75 min, MH+ 307.1/309.0 (Method B)
37 3-[(3-phenylpropyl)amino]-4-pyridinecarboxylic acid		50	Rt 0.66 min, MH+ 257.1 (Method A)
87 3-[(4-{3-[(*N*-{[(1,1-dimethylethyl)oxy]carbonyl}-β–alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid		Quantitative	Rt 0.73 min, MH+ 471.3 (Method A)
88 3-({4-[3-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid		100	Rt 0.43 min, MH+ 371.0 (Method A)
90 sodium 3-[(3-biphenylylmethyl)amino]-4-pyridinecarboxylate		7	Rt 2.79 mins, MH+ = 305 (Method D)

Example	X	Yield /%	LCMS
111 3-{[3-(6-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid		8	Rt 0.37 min, MH+ 324.0 (Method A)
137 3-{[3-({3-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		73	Rt 0.50 min, MH+ 366.0 (Method B)
138 3-[(3-{[3-(methylsulfonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid		69	Rt 0.58 min, MH+ 351.0 (Method B)
139 3-({3-[(3-methylphenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid hydrochloride		22	Rt 0.73 min, MH+ 287.1 (Method B)
140 3-{[3-(2-oxo-1(2*H*)-pyridinyl)propyl]amino}-4-pyridinecarboxylic acid. 0.33 Ammonium Salt		30	Rt 0.42min, MH+ 274.2 (Method B)
141 3-[(4-cyclohexylbutanoyl)amino]-4-pyridinecarboxylic acid hydrochloride		57	Rt 0.87min, MH+ 291.2 (Method A)
142 3-{[3-(2-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid		Quantitative	Rt 0.56min, MH+ 274.1 (Method B)
143 3-[(3-{[4-(aminocarbonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid hydrochloride		57	Rt 0.48min, MH+ 316.1 (Method B)

Example	X	Yield /%	LCMS
144 3-{[3-({4-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid		42	Rt 0.47min, MH+ 366.1 (Method B)
145 3-{[3-(8-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		57	Rt 0.62min, MH+ 324.1 (Method B)
146 3-{[3-(1-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid hydrochloride		12	Rt 0.80 mins, MH+ = 323.1 (Method B)
150 3-[methyl(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid		57	Rt 0.73min, MH+ 299.1 (Method A)
151 3-({2-[2-(methyloxy)phenyl]ethyl}amino)-4-pyridinecarboxylic acid		38	Rt 0.65min, MH+ 273.1 (Method A)
152 3-[(2-methylpropyl)amino]-4-pyridinecarboxylic acid		81	Rt 0.50min, MH+ 195.1 (Method A)
163 3-[(4-{3-[(*N*-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}-β-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid		94	Rt 0.57min, MH+ 597.3 (Method A)
167 3-{[(2,4-difluorophenyl)carbonyl]amino}-4-pyridinecarboxylic acid		36	Rt 0.54 min, MH+ 279.0 (Method A)

Example 38: 3-{[4-(3-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid

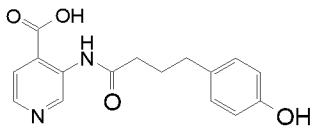


To a solution of ethyl 3-({4-[3-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylate (100 mg, 0.292 mmol) in DCM was added dropwise a 1M solution of boron tribromide (0.876 mL, 0.876 mmol) in DCM at 0 °C under N_2. The reaction mixture was allowed to warm to room temperature over 17hr. The reaction mixture was partitioned with water (5 mL), the organic layer was isolated using a hydrophobic frit, and the aqueous layer was re-extracted twice with DCM. The combined organic layers were concentrated *in vacuo* to give the crude product which was then purified by MDAP using an ammonium carbonate modifier (Method E). Fractions containing desired product were combined and then concentrated *in vacuo* to give the title compound as an off white solid (8 mg, 9%).

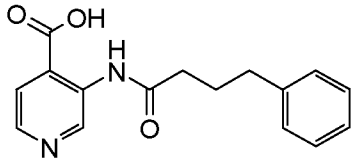
1H NMR (400 MHz, DMSO-δ6,) 11.10ppm (1H, br s), 9.40ppm (1H, s), 9.27ppm (1H, br s), 8.32ppm (1H, d), 7.74ppm (1H, d), 7.06ppm (1H, m), 6.62ppm, (3H, m), 2.54ppm (2H, t), 2.38ppm (2H, t), 1.88ppm (2H, m).

LCMS (Method B): Rt = 0.54 min, MH^+ = 301.1

The Example given in the following table was prepared in a manner similar to that described for Example 38.

Example	Structure	Yield /%	LCMS
39 3-{[4-(4-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid		4	Rt = 0.52 mins, MH+ = 301.0 (Method A)

Example 40: 3-[(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid

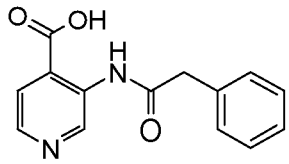


To a solution of 3-amino-4-pyridinecarboxylic acid (500 mg, 3.62 mmol) in DMF was added DIPEA (1.3 ml, 7.24 mmol) followed by pyridine (0.293 ml, 3.62 mmol) then 4-phenylbutanoyl chloride (0.596

ml, 3.62 mmol). The reaction mixture was stirred at room temperature for 3.5 days. The resulting solid was isolated by filtration then washed with ethyl acetate followed by methanol then dried *in vacuo* to give the title compound as an off-white solid (555mg, 54%).

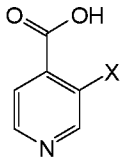
1H NMR (400 MHz, DMSO-δ6) 10.65ppm (1H, br s), 9.36 (1H, s), 8.41ppm (1H, d), 7.74ppm (1H, d), 7.34-7.14ppm (5H, m), 2.64ppm (2H, t), 2.41ppm (2H, t), 1.98-1.86ppm (2H, m).
LCMS (Method C): Rt = 0.67 min, MH^+ 285.1

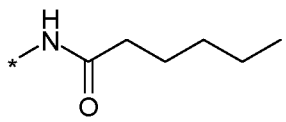
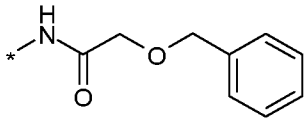
Example 41: 3-[(phenylacetyl)amino]-4-pyridinecarboxylic acid

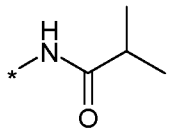
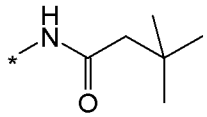


Phenyl acetyl chloride (0.240 mL, 1.81 mmol) was added dropwise to a stirred suspension of 3-amino-4-pyridinecarboxylic acid (250 mg, 1.81 mmol) in DMF (5 mL) at 0 ºC under N_2. Following stirring at 0 ºC for 5 min, the flask was removed from the cooling bath and allowed to stir at rt for 1 h. Water (20 mL) was added and the resultant suspension was stirred for 15 min. The suspension was filtered, then washed with H_2O (100 mL), followed by MeOH (100 mL) and finally Et_2O (100 mL). The solid was then dried under vacuum to give the title compound as a white solid (323 mg, 70%).
1H NMR (400 MHz, DMSO-δ6) 10.69ppm (1H, br s), 9.48ppm (1H, s), 8.42ppm (1H, d). 7.74ppm (1H, d), 7.36 (4H, m), 7.29 (1H, m 1H), 3.78 (2H, s).
LCMS (Method A): Rt = 0.51 min, MH^+ 257.1

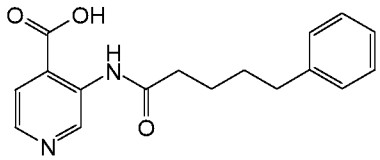
The Examples given in the following table were prepared in a manner similar to that described for Example 41.



Example	X	Yield /%	LCMS
42 3-(hexanoylamino)-4-pyridinecarboxylic acid		61	Rt = 0.60 mins, MH+ = 237.1 (Method A)
43 3-({[(phenylmethyl)oxy]acetyl}ami		57	Rt = 0.58 mins, MH+ = 287.1 (Method A)

Example	X	Yield /%	LCMS
no)-4-pyridinecarboxylic acid			
44 3-[(2-methylpropanoyl)amino]-4-pyridinecarboxylic acid		65	Rt = 0.37 mins, MH+ = 209.1 (Method A)
45 3-[(3,3-dimethylbutanoyl)amino]-4-pyridinecarboxylic acid		24	Rt = 0.54 mins, MH+ = 237.1 (Method A)

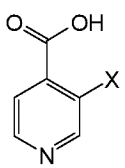
Example 46: 3-[(5-phenylpentanoyl)amino]-4-pyridinecarboxylic acid



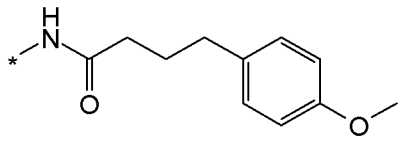
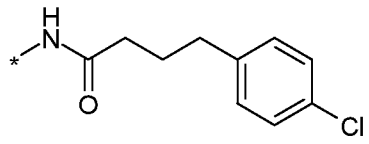
DMF (0.05ml) was added dropwise over 10 s to a solution of 5-phenylpentanoic acid (387 mg, 2.17 mmol) and oxalyl chloride (0.238 ml, 2.71 mmol) in DCM (5 ml) under nitrogen. The reaction mixture was stirred at room temperature for 2 h then concentrated *in vacuo* to give a yellow oil. The oil was dissolved in DMF (1 ml) then added dropwise to a stirred suspension of 3-amino-4-pyridinecarboxylic acid (250 mg, 1.81 mmol) in DMF (4 mL) at 0 ºC under nitrogen. After 5 min stirring the reaction mixture was allowed to warm to room temperature over 1 h then filtered through a plug of cotton wool. Water (10 ml) was added to the filtrate and the resultant suspension stirred for 15 min then filtered, washed with water (100 ml), then methanol (100 ml), then ether (100 ml). The solid was dried *in vacuo* to give the title compound as a white solid (370mg, 69%).

1H NMR (400 MHz, DMSO-δ6) 10.59ppm (1H, br s), 9.40ppm (1H, s), 8.41ppm (1H, d), 7.75ppm (1H, d), 7.32-7.13ppm (5H, m), 2.61ppm (2H, t), 2.44ppm (2H, t), 1.72-1.50ppm (4H, m).
LCMS (Method A): Rt = 0.75 mins, MH+ = 299.1

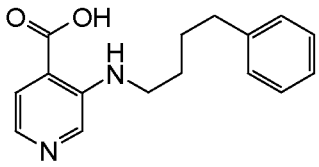
The Examples given in the following table were prepared in a manner similar to that described for Example 46. Some examples required recrystallisation from hot DMF.



Example	X	Yield /%	LCMS

Example	X	Yield /%	LCMS
47 3-({4-[4-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid		49	Rt 0.66 mins, MH+ = 315.2 (Method A)
48 3-{[4-(4-chlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid		32	Rt 0.77 mins, MH+ = 319.0/320.9 (Method A)

Example 49: 3-[(4-phenylbutyl)amino]-4-pyridinecarboxylic acid

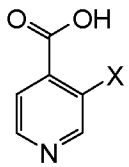


To 3-fluoro-4-pyridinecarboxylic acid (200 mg, 1.42 mmol) was added (4-phenylbutyl)amine (0.5 ml, 3.16 mmol) then the reaction mixture was heated in a Biotage Initiator microwave at 130 °C for 1 hour. The reaction mixture was diluted with methanol (1.5 ml) then purified in two portions by MDAP using a formic modifier (Method G). Fractions containing desired product were combined then concentrated *in vacuo* and the resulting material dissolved in isopropyl alcohol then loaded onto an SCX-II SPE column that had been preconditioned with isopropyl alcohol. The SPE column was eluted with three column volumes of isopropyl alcohol followed by three column volumes of 10% ammonia in isopropyl alcohol. Desired product eluted in the first two ammonia washes which were combined then concentrated *in vacuo* to give the title compound as a pale yellow solid (99 mg, 26%).

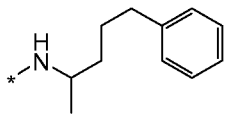
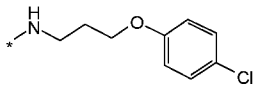
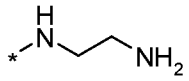
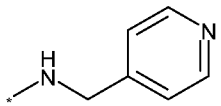
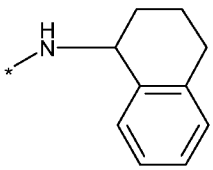
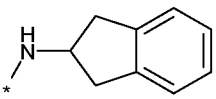
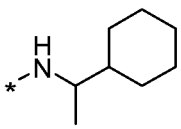
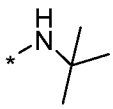
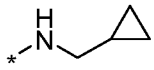
1H NMR (400 MHz, DMSO-δ6) 8.24ppm (1H, s), 7.81ppm (1H, d), 7.54ppm (1H, d), 7.32-7.13ppm (5H, m), 3.17ppm (2H, m, obscured by water peak), 2.64ppm (2H, t), 1.75-1.55ppm (4H, m).

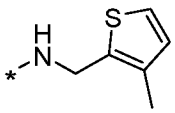
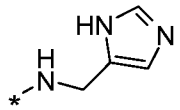
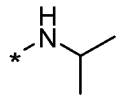
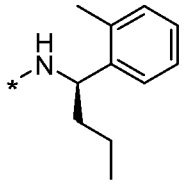
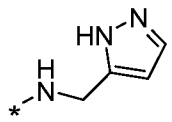
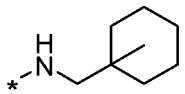
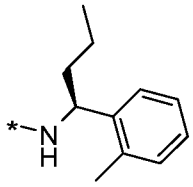
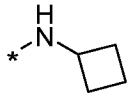
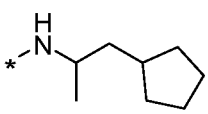
LCMS (Method A): Rt = 0.74 mins, MH+ = 271.1

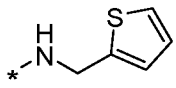
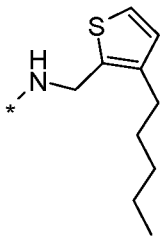
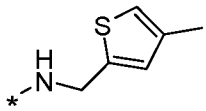
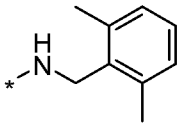
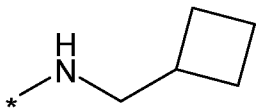
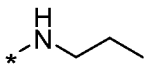
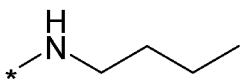
The Examples given in the following table were prepared in a manner similar to that described for Example 49. In the preparation of certain Examples, DIPEA was employed as a base:

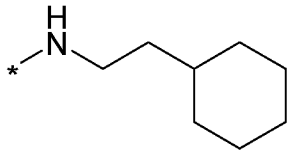
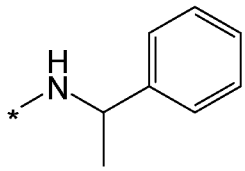
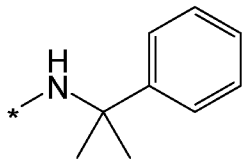
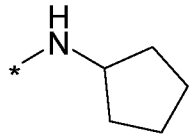
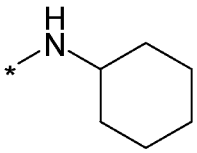
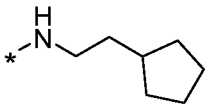


Example	X	Yield /%	LCMS

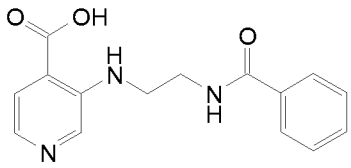
Example	X	Yield /%	LCMS
50 3-[(1-methyl-4-phenylbutyl)amino]-4-pyridinecarboxylic acid		7	Rt 0.78 mins, MH+ = 285.1 (Method A)
51 3-({3-[(4-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid		2	Rt 0.75 mins, MH+ = 307.0/309.0 (Method A)
52 3-[(2-aminoethyl)amino]-4-pyridinecarboxylic acid		41	Rt 0.17 mins, MH+ = 182.0 (Method C)
76 3-[(4-pyridinylmethyl)amino]-4-pyridinecarboxylic acid		6	Rt 1.07 mins, MH+ = 230 (Method D)
104 3-(1,2,3,4-tetrahydro-1-naphthalenylamino)-4-pyridinecarboxylic acid		16	Rt 2.38 mins, MH+ = 269 (Method D)
107 3-(2,3-dihydro-1*H*-inden-2-ylamino)-4-pyridinecarboxylic acid		3	Rt 2.23 mins, MH+ = 255 (Method D)
108 3-[(1-cyclohexylethyl)amino]-4-pyridinecarboxylic acid		33	Rt 0.70 mins, MH+ = 249.2 (Method A)
109 3-[(1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid		11	Rt 0.46 mins, MH+ = 195.1 (Method B)
116 3-[(cyclopropylmethyl)amino]-4-pyridinecarboxylic acid		23	Rt 0.40 mins, MH+ = 193.1 (Method A)

Example	X	Yield /%	LCMS
118 3-{[(3-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid		21	Rt 0.60 mins, MH+ = 249.1 (Method B)
119 3-[(1*H*-imidazol-4-ylmethyl)amino]-4-pyridinecarboxylic acid		11	Rt 0.33 mins, MH+ = 219.2 (Method B)
120 3-[(1-methylethyl)amino]-4-pyridinecarboxylic acid		8	Rt 0.37 mins, MH+ = 181.1 (Method A)
121 3-{[(1*R*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid		19	Rt 0.78 mins, MH+ = 285.1 (Method B)
122 3-[(1*H*-pyrazol-5-ylmethyl)amino]-4-pyridinecarboxylic acid		9	Rt 0.35 mins, MH+ = 219.2 (Method B)
123 3-{[(1-methylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid		10	Rt 0.73 mins, MH+ = 249.2 (Method B)
126 3-{[(1*S*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid		6	Rt 0.78 mins, MH+ = 285.1 (Method B)
127 3-(cyclobutylamino)-4-pyridinecarboxylic acid		20	Rt 0.48 mins, MH+ = 193.1 (Method B)
128 3-[(2-cyclopentyl-1-methylethyl)amino]-4-		7	Rt 0.75 mins, MH+ = 249.1 (Method B)

Example	X	Yield /%	LCMS
pyridinecarboxylic acid			
130 3-[(2-thienylmethyl)amino]-4-pyridinecarboxylic acid		42	Rt 0.53 mins, MH+ = 235.1 (Method B)
134 3-{[(3-pentyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid		10	Rt 0.85 mins, MH+ = 305.2 (Method B)
135 3-{[(4-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid		20	Rt 0.61 mins, MH+ = 249.1 (Method B)
136 3-{[(2,6-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid		50	Rt 0.72 mins, MH+ = 257.1 (Method B)
148 3-[(cyclobutylmethyl)amino]-4-pyridinecarboxylic acid		3	Rt 0.50 mins, MH+ = 207 (Method A)
149 3-(propylamino)-4-pyridinecarboxylic acid		36	Rt 0.38 mins, MH+ = 181.1 (Method A)
154 3-(butylamino)-4-pyridinecarboxylic acid		3	Rt 0.47 mins, MH+ = 195.2 (Method A)

Example	X	Yield /%	LCMS
155 3-[(2-cyclohexylethyl)amino]-4-pyridinecarboxylic acid		18	Rt 0.75 mins, MH+ = 249.2 (Method A)
156 3-[(1-phenylethyl)amino]-4-pyridinecarboxylic acid		54	Rt 0.56 mins, MH+ = 243.1 (Method A)
157 3-[(1-methyl-1-phenylethyl)amino]-4-pyridinecarboxylic acid		4	Rt 0.59 mins, MH+ = 257.2 (Method A)
158 3-(cyclopentylamino)-4-pyridinecarboxylic acid		19	Rt 0.47 mins, MH+ = 207.1 (Method A)
159 3-(cyclohexylamino)-4-pyridinecarboxylic acid		72	Rt 0.55 mins, MH+ = 221.2 (Method A)
160 3-[(2-cyclopentylethyl)amino]-4-pyridinecarboxylic acid		25	Rt 0.68 mins, MH+ = 235.2 (Method A)

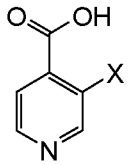
Example 53: 3-({2-[(phenylcarbonyl)amino]ethyl}amino)-4-pyridinecarboxylic acid hydrochloride

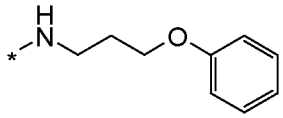
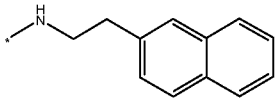
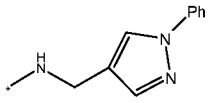


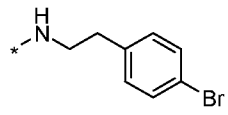
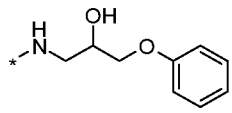
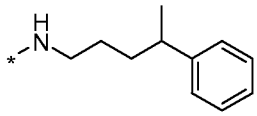
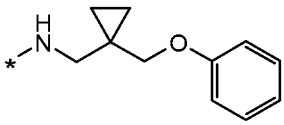
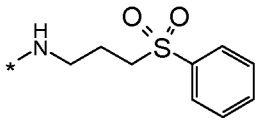
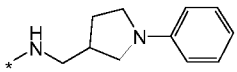
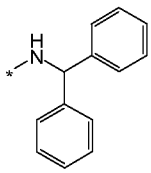
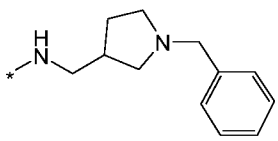
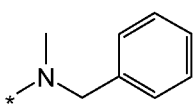
3-fluoro-4-pyridinecarboxylic acid (100mg, 0.71 mmol), *N*-(2-aminoethyl)benzamide hydrochloride (142mg, 0.71 mmol), DIPEA (0.186ml, 1.06 mmol) in DMSO (0.5ml) were heated at 160°C for 2.5 hours using a Biotage Initator microwave. The mixture was diluted to 3 ml then purified in three portions by MDAP using a formic modifier (Method G). Fractions containing desired product were combined then concentrated under vacuum and the resulting residue dissolved in DMSO (1ml) then loaded onto a 2 g aminopropyl column which was then washed with three column volumes of isopropyl alcohol followed by ten column volumes of 10% aqueous 2N HCl in isopropyl alcohol. Product eluted in the HCl fractions which were combined then concentrated under reduced pressure to give the title compound as a yellow solid (34mg, 15%).

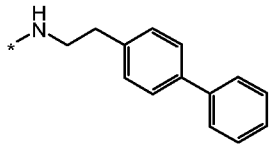
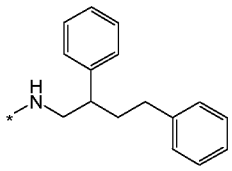
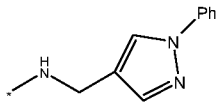
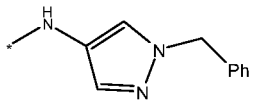
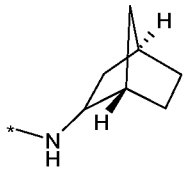
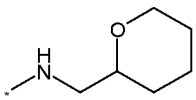
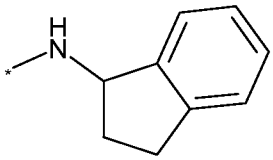
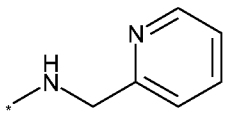
1H NMR (400 MHz, DMSO-δ6) 8.78ppm (1H, t), 8.59ppm (1H, s), 8.00-7.93ppm (2H, m), 7.87-7.80ppm (2H, m), 7.56-7.42ppm (3H, m), 3.57ppm (2H, t), 3.51ppm (2H, t).
LCMS (Method A): Rt = 0.45 mins, MH+ = 286.1

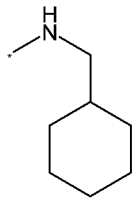
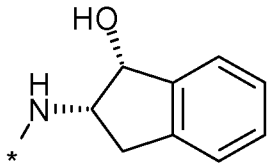
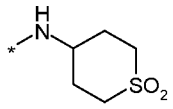
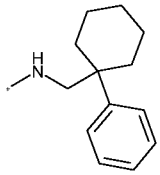
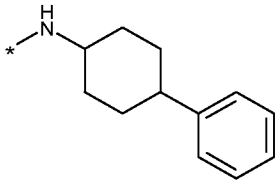
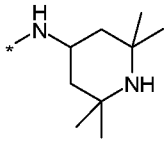
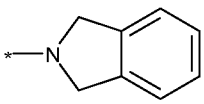
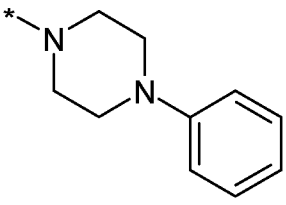
The Examples given in the following table were prepared in a manner similar to that described for Example 53. In some cases DIPEA was omitted. In some cases DMSO was replaced with IPA, 2-methyl-2-propanol or 1,4-dioxane. In some cases the aminopropyl SPE workup step was used before MDAP, or the aminopropyl SPE workup step was excluded altogether:.

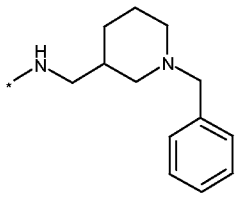
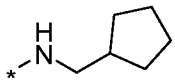


Example	X	Yield /%	LCMS
54 3-{[3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid		10	Rt 0.63 mins, MH+ = 273.1 (Method A)
55 3-{[2-(2-naphthalenyl)ethyl]amino}-4-pyridinecarboxylic acid, ammonium salt		8	Rt 0.73 mins, MH+ = 293.1 (Method B)
56 3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid		22	Rt 0.61 mins, MH+ = 295.1 (Method B)

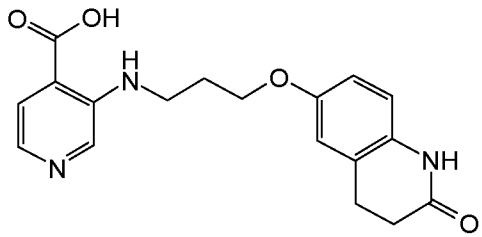
Example	X	Yield /%	LCMS
57 3-{[2-(4-bromophenyl)ethyl]amino}-4-pyridinecarboxylic acid		1	Rt 0.74 mins, MH+ = 321.0/323.0 (Method B)
58 3-{[2-hydroxy-3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid		16	Rt 0.55 mins, MH+ = 289.1 (Method B)
59 3-[(4-phenylpentyl)amino]-4-pyridinecarboxylic acid		5	Rt 0.75 mins, MH+ = 285.1 (Method B)
60 3-[({1-[(phenyloxy)methyl]cyclopropyl}methyl)amino]-4-pyridinecarboxylic acid		11	Rt 0.68 mins, MH+ = 299.2 (Method B)
61 3-{[3-(phenylsulfonyl)propyl]amino}-4-pyridinecarboxylic acid		12	Rt 0.51 mins, MH+ = 321.1 (Method B)
62 3-{[(1-phenyl-3-pyrrolidinyl)methyl]amino}-4-pyridinecarboxylic acid		3	Rt 0.71 mins, MH+ = 298.1 (Method B)
63 3-[(diphenylmethyl)amino]-4-pyridinecarboxylic acid		2	Rt 0.76 mins, MH+ = 305.0 (Method A)
64 3-({[1-(phenylmethyl)-3-pyrrolidinyl]methyl}amino)-4-pyridinecarboxylic acid		7	Rt 0.62 mins, MH+ = 312.1 (Method B)
65 3-[methyl(phenylmethyl)amino]-4-pyridinecarboxylic acid		20	Rt 0.49 mins, MH+ = 243.1 (Method B)

Example	X	Yield /%	LCMS
66 3-{[2-(4-biphenylyl)ethyl]amino}-4-pyridinecarboxylic acid		5	Rt 0.80 mins, MH+ = 319.1 (Method B)
67 3-[(2,4-diphenylbutyl)amino]-4-pyridinecarboxylic acid		11	Rt 0.84 mins, MH+ = 347.1 (Method B)
68 3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid		22	Rt 0.61 mins, MH+ = 295.1 (Method B)
69 3-{[1-(phenylmethyl)-1*H*-pyrazol-4-yl]amino}-4-pyridinecarboxylic acid		2	Rt 0.56 mins, MH+ = 295.1 (Method B)
73 3-[(1S,4R)-bicyclo[2.2.1]hept-2-ylamino]-4-pyridinecarboxylic acid		20	Rt 2.10 mins, MH+ = 233 (Method D)
74 3-[(tetrahydro-2*H*-pyran-2-ylmethyl)amino]-4-pyridinecarboxylic acid		4	Rt 1.60 mins, MH+ = 237 (Method D)
79 3-(2,3-dihydro-1*H*-inden-1-ylamino)-4-pyridinecarboxylic acid		4	Rt 2.24 mins, MH+ = 255 (Method D)
80 3-[(2-pyridinylmethyl)amino]-4-pyridinecarboxylic acid		22	Rt 1.86 mins, MH+ = 230 (Method J)

Example	X	Yield /%	LCMS
85 3-[(cyclohexylmethyl)amino]-4-pyridinecarboxylic acid		16	Rt 2.34 mins, MH+ = 235 (Method D)
89 3-{[(1S,2R)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino}-4-pyridinecarboxylic acid		7	Rt 1.77 mins, MH+ = 271 (Method D)
91 3-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)amino]-4-pyridinecarboxylic acid		15	Rt 1.09 mins, MH+ = 271 (Method D)
92 3-{[(1-phenylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid		7	Rt 2.83 mins, MH+ = 311 (Method D)
97 3-[(4-phenylcyclohexyl)amino]-4-pyridinecarboxylic acid		2	Rt 2.82 mins, MH+ = 297 (Method D)
101 3-[(2,2,6,6-tetramethyl-4-piperidinyl)amino]-4-pyridinecarboxylic acid		8	Rt 1.93 mins, MH+ = 278 (Method K)
102 3-(1,3-dihydro-2*H*-isoindol-2-yl)-4-pyridinecarboxylic acid		3	Rt 3.05 mins, MH+ = 241 (Method L)
103 3-(4-phenyl-1-piperazinyl)-4-pyridinecarboxylic acid		2	Rt 1.68 mins, MH+ = 284 (Method D)

Example	X	Yield /%	LCMS
105 3-({[1-(phenylmethyl)-3-piperidinyl]methyl}amino)-4-pyridinecarboxylic acid		13	Rt 1.37 mins, MH+ = 326 (Method D)
132 3-[(cyclopentylmethyl)amino]-4-pyridinecarboxylic acid		9	Rt 0.58 mins, MH+ = 221.1 (Method A)

Example 70: 3-({3-[(2-oxo-1,2,3,4-tetrahydro-6-quinolinyl)oxy]propyl}amino)-4-pyridinecarboxylic acid hydrochloride

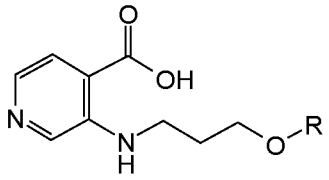


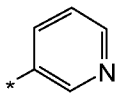
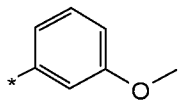
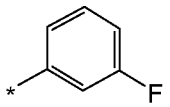
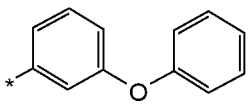
To a solution of ethyl 3-[(3-hydroxypropyl)amino]-4-pyridinecarboxylate (50mg, 0.22 mmol) in THF (2ml) was added 6-hydroxy-3,4-dihydro-2(1*H*)-quinolinone (36mg, 0.22 mmol) followed by triphenylphosphine (59mg, 0.22 mmol) then a solution of bis(1,1-dimethylethyl) -1,2-diazenedicarboxylate (103mg, 0.45 mmol) in THF (1ml). The reaction mixture was stirred under nitrogen for 2.5 hours then further triphenylphosphine (59mg, 0.22 mmol) was added to the reaction mixture which was allowed to stir for 22 hours. Lithium hydroxide monohydrate (19mg, 0.45 mmol) then water (1ml) was added directly to the reaction mixture which was stirred at room temperature for 5.5 hours. The reaction mixture was concentrated under a stream of nitrogen then MeOH added, followed by seven drops of water then one drop of HCl. The resulting suspension was filtered under reduced pressure, then further aqueous 2M HCl (2ml) was added to the filtrate and the resulting solid filtered under vacuum using a hydrophobic frit then washed with aqueous 2M HCl (2ml), followed by diethyl ether (2ml), to give the title compound as a yellow solid (11mg, 13%).

1H NMR (400 MHz, DMSO-δ6) 9.87ppm (1H, s), 8.40ppm (1H, s), 8.02-7.94ppm (2H, m), 7.70-7.51 (2H, m), 6.86-6.73ppm (2H, m), 4.04ppm (2H, t), 3.51ppm (2H, t), 2.83ppm (2H, t), 2.40ppm (2H, t), 2.20-2.00ppm (2H, m).
LCMS (Method A): Rt = 0.46 mins, MH+ = 342.1

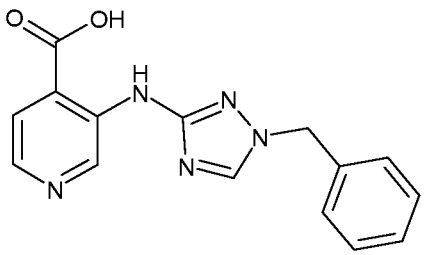
The Examples given in the following table were prepared in a manner similar to that described for

Example 70. In some cases the product was obtained after a further purification using MDAP (Method E, F or G):



Example	R	Yield /%	LCMS
112 3-{[3-(3-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid		14	Rt 0.50mins, MH+ = 274.0 (Method B)
113 3-[(3-{[3-(methyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid hydrochloride		19	Rt 0.65 mins, MH+ = 303.0 (Method A)
114 3-({3-[(3-fluorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid		11	Rt 0.70 mins, MH+ = 291.1 (Method B)
115 3-[(3-{[3-(phenyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid		2	Rt 0.89 mins, MH+ = 365.0 (Method A)

Example 72: 3-{[1-(phenylmethyl)-1*H*-1,2,4-triazol-3-yl]amino}-4-pyridinecarboxylic acid, formate salt.

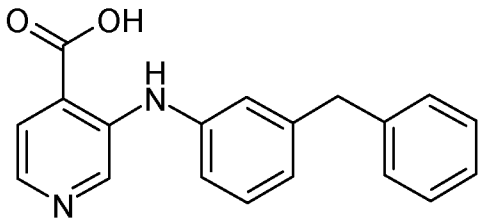


A mixture of 3-fluoro-4-pyridinecarboxylic acid (96 mg, 0.68 mmol) and 1-(phenylmethyl)-1*H*-1,2,4-triazol-3-amine (119 mg, 0.68 mmol) under nitrogen was dissolved in THF (3 ml) then cooled to -78 °C. LiHMDS (2.391 mL, 2.39 mmol) was then added then the resulting mixture allowed to reach rt and

left standing overnight. The mixture was cooled to -78 °C then further 3-fluoro-4-pyridinecarboxylic acid (48.2 mg, 0.342 mmol) was added followed by more LiHMDS (1 ml, 1.00 mmol). The resulting mixture was allowed to reach rt and left standing overnight. The reaction was quenched by slow addition to a stirred solution of 4.0 M HCl in dioxan (2 ml, 8.00 mmol) and the resulting mixture stirred for 5 min. Volatiles were removed under reduce pressure to afford a bright yellow powder which was purified using silica column chromatography, eluting with a gradient of 20% of 2M ammonia in MeOH/ DCM then MDAP (Method E) and finally MDAP (Method G) to give the title compound 11mg (5%).

LCMS (Method B): Rt = 0.58 mins, MH+ = 296.1

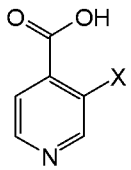
Example 78: Sodium 3-{[3-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylate

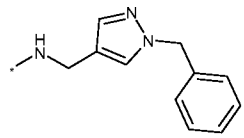


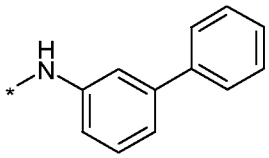
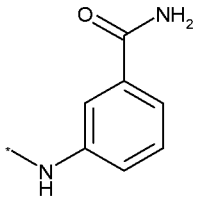
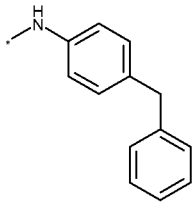
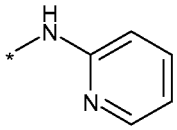
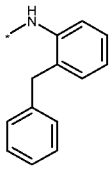
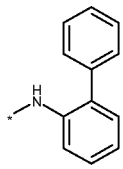
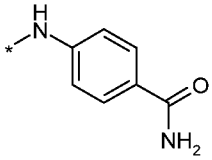
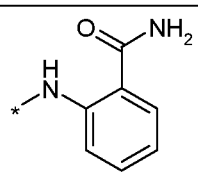
3-Fluoro-4-pyridinecarboxylic acid (40 mg, 0.28 mmol and 3-(phenylmethyl)aniline (52 mg, 0.28 mmol) and THF (0.75ml) were stirred under nitrogen at -78°C. A solution of LiHMDS (1M in THF, 1 ml, 0.99 mmol)was added to the reaction mixture which was stirred for 15 min at 78°C and then left to stir for 18 hr at 24°C. A solution of NaOH (2 ml, aqueous) was added and the mixture stirred at 24°C for 30 min then concentrated. The mixture was triturated with ether-dichloromethane (9:1, 20 ml) then concentrated to give the title compound, 65mg (76%).

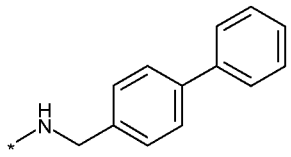
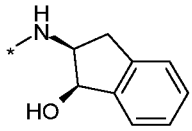
LCMS (Method D): 2.86 mins, MH+ = 305

The Examples given in the following table were prepared in a manner similar to that described for Example 78 with additional MDAP (Method E, F or G) or preparative HPLC (Method I) purification steps. To prepare the sodium salts indicated in the table, 2M NaOH was added to the free acid, the reaction stirred for 3 hr then the solid triturated with ether to give the title compound.

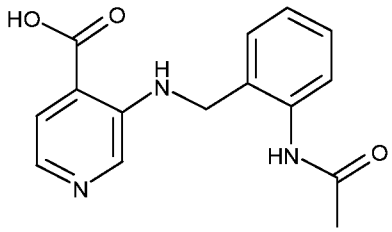


Example	X	Yield /%	LCMS
77 3-({[1-(phenylmethyl)-1*H*-pyrazol-4-yl]methyl}amino)-4-pyridinecarboxylic		6	Rt 2.01 mins, MH+ = 309 (Method D)

Example	X	Yield /%	LCMS
acid			
81 3-(3-biphenylylamino)-4-pyridinecarboxylic acid		2	Rt 2.69 mins, MH+ = 291 (Method D)
82 3-{[3-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid		15	Rt 2.01 mins, MH+ = 258 (Method D)
93 sodium 3-{[4-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylate		94	Rt 4.79 mins, MH+ = 305 (Method D)
94 sodium 3-(2-pyridinylamino)-4-pyridinecarboxylate		86	Rt 2.77 mins, MH+ = 216 (Method J)
96 sodium 3-{[2-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylate		94	Rt 2.63 mins, MH+ = 305 (Method K)
98 sodium 3-(2-biphenylylamino)-4-pyridinecarboxylate		71	Rt 4.32 mins, MH+ = 291 (Method K)
99 sodium 3-{[4-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylate		75	Rt 2.02 mins, MH- = 258 (Method J)
100 3-{[2-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid		56	Rt 1.31 mins, MH+ = 258 (Method D)

Example	X	Yield /%	LCMS
106 3-[(4-biphenylylmethyl)amino]-4-pyridinecarboxylic acid		6	Rt 4.80 mins, MH- = 305 (Method D)
164 3-{[(1*R*,2*S*)-1-hydroxy-2,3-dihydro-1*H*-inden-2-yl]amino}-4-pyridinecarboxylic acid		6	Rt 1.85 mins, MH- = 271 (Method D)

Example 84: 3-({[2-(acetylamino)phenyl]methyl}amino)-4-pyridinecarboxylic acid

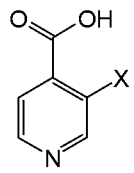


To a solution of *N*-[2-(hydroxymethyl)phenyl]acetamide (90 mg, 0.545 mmol) in chloroform (2.0 ml) at 0 to 10°C was added iodo(trimethyl)silane (90 ul, 0.654 mmol) dropwise. The reaction mixture was stirred at 25°C for 2hr then concentrated to give crude *N*-(4-{[(trimethylsilyl)oxy]methyl}-3-pyridinyl)acetamide.

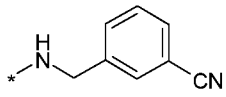
A solution of crude *N*-(4-{[(trimethylsilyl)oxy]methyl}-3-pyridinyl)acetamide (180 mg, 0.759 mmol), potassium carbonate (166mg, 1.204mmol) and ethyl 3-amino-4-pyridinecarboxylate (100 mg, 0.602 mmol) in THF (1.0ml) was stirred at 60°C for 4 hr. The reaction mixture was allowed to cool to 25°C then diluted with water (1 ml) then extracted with EtOAc (2 x 10 ml).. The aqueous layer was purified by preparative HPLC (Method I) to give the title compound, 9.0 mg (8%) .

LCMS (Method D): Rt = 1.29 min, MH+ 286

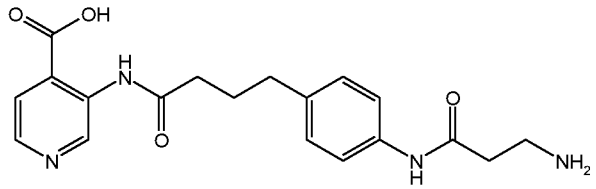
The Example given in the following table was prepared in a manner similar to that described for Example 84:



Example	X	Yield /%	LCMS

83 3-{[(3-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid		3	Rt 1.60 mins, MH+ = 254 (Method D)

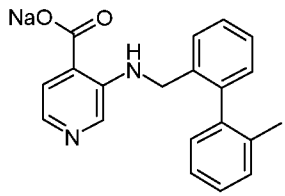
Example 86: 3-({4-[4-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid



Lithium hydroxide hydrate (147 mg, 3.51 mmol) was added in a single portion ot a stirred solution of ethyl 3-({4-[4-(β–alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylate (700 mg, 1.757 mmol) in THF (50 ml) and water (16 ml) at rt. Following stirring at rt for 30 min, 2 M aq. HCl (20 mL) was added. The resultant solution was evaporated under reduced pressure to give a yellow solid. The solid was dissolved in MeOH (50 mL) and loaded onto an SCX column that had been prewashed with MeOH (2 CV). The column was eluted with MeOH (3 CV), followed by 2 M NH_3 in MeOH (2 CV). The methanolic ammonia fractions were combined and evaporated under reduced pressure to give the title compound as a white solid (621 mg, 95%).

LCMS (Method A): Rt = 0.40 min, MH+=371.0,

Example 95: sodium 3-{[(2'-methyl-2-biphenylyl)methyl]amino}-4-pyridinecarboxylate

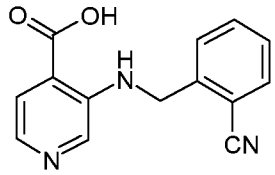


To a solution of ethyl 3-{[(2'-methyl-2-biphenylyl)methyl]amino}-4-pyridinecarboxylate (30 mg, 0.087 mmol) in THF (2.0 ml) at 0°C was added NaOH (2M, 0.1 ml, 0.173 mmol) at 0°C. The reaction mixture was stirred at 25°C for 24 hr then concentrated by evaporation and the resulting solid washed with diethyl ether to give the title compound as a white solid, 107 mg (quantitative yield).

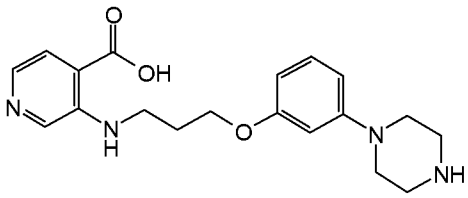
LCMS (Method D): Rt = 2.82 mins, MH+ 319

The Example given in the following table was prepared in a manner similar to that described for Example 95 with an additional preparative HPLC purification step (Method I):

Example	Structure	Yield /%	LCMS

75 3-{[(2-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid		11	Rt 1.78mins, MH+ = 254 (Method D)

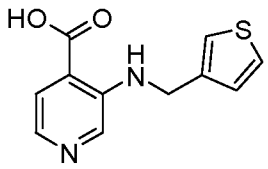
Example 110: 3 3-[(3-{[3-(1-piperazinyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid



A solution of triphenylphosphine (94 mg, 0.357 mmol) in THF (3 mL) under nitrogen was cooled using an ice-water bath before bis(1,1-dimethylethyl) -1,2-diazenedicarboxylate (82 mg, 0.357 mmol) was added. The yellow reaction mixture was stirred for 5 min before ethyl 3-[(3-hydroxypropyl)amino]-4-pyridinecarboxylate (80 mg, 0.357 mmol) was added the reaction mixture was stirred. Further 1,1-dimethylethyl 4-(3-hydroxyphenyl)-1-piperazinecarboxylate (99 mg, 0.357 mmol) was added and the reaction mixture stirred for 5 min then allowed to warm to room temperature over 18 h. The reaction mixture was cooled using an ice-water bath then triphenylphosphine (94 mg, 0.357 mmol) was added. After 5 min of stirring, bis(1,1-dimethylethyl) -1,2-diazenedicarboxylate (82 mg, 0.357 mmol) was added and the reaction mixture stirred for a further 10 min then 1,1-dimethylethyl 4-(3-hydroxyphenyl)-1-piperazinecarboxylate (99 mg, 0.357 mmol) was added. The reaction mixture was stirred for 5min then allowed to warm to room temperature for 30 hr then refluxed for 24 hr. The reaction mixture was concentrated under reduced pressure then purified using silica column chromatography. The crude intermediate 1,1-dimethylethyl 4-(3-{[3-({4-[(ethyloxy)carbonyl]-3-pyridinyl}amino)propyl]oxy}phenyl)-1-piperazinecarboxylate was eluted with a 0-50% ethyl acetate/cyclohexane solvent system.
The crude intermediate was dissolved in a mixture of THF (1.0ml) and water (1.0ml) then lithium hydroxide monohydrate (29.9 mg, 0.713 mmol) was added and the reaction mixture stirred at room temperature for 19 hours. The reaction mixture was concentrated under a stream of nitrogen then HCl (aq., 2N, 2ml) and the mixture stirred for 3 hr then concentrated under a stream of nitrogen. The crude material was purified by MDAP using a high pH modifier (Method E) to give the title compound (42.5 mg, 33 %) as an orange solid.

LCMS (Method A): Rt = 0.42 mins, MH+ = 357.1

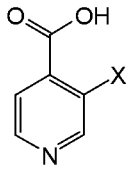
Example 117: 3-[(3-thienylmethyl)amino]-4-pyridinecarboxylic acid hydrochloride

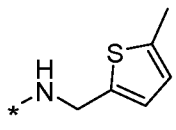
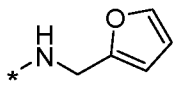
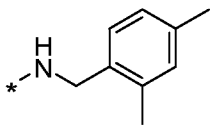
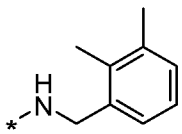
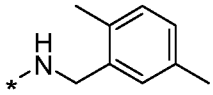


To a mixture of 3-fluoro-4-pyridinecarboxylic acid (200 mg, 1.417 mmol) and *N*-ethyl-*N*-(1-methylethyl)-2-propanamine (619 µl, 3.54 mmol) was added (3-thienylmethyl)amine (198 mg, 1.701 mmol). The reaction mixture was heated using a microwave 160ºC for 4hr then diluted with DMSO (4 ml) and purified by MDAP (High pH modifier, Method E) to give the free base compound as a white solid (83 mg). This was dissolved in 1,4-Dioxane (3 ml) then 2N HCl in ether (178 µl, 0.356 mmol) was added and the resulting solution concentrated under reduced pressure to give the title compound as a yellow solid (92 mg, 24%)

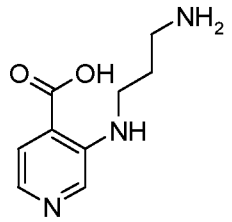
LCMS (Method B): Rt = 0.55 mins, MH+ = 235.1

The Examples given in the following table were prepared in a manner similar to that described for Example 117.



Example	X	Yield /%	LCMS
124 3-{[(5-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid hydrochloride		20	Rt 0.62 mins, MH+ = 249.0 (Method B)
125 3-[(2-furanylmethyl)amino]-4-pyridinecarboxylic acid hydrochloride		10	Rt 0.49 mins, MH+ = 219.1 (Method B)
129 3-{[(2,4-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid hydrochloride		29	Rt 0.72 mins, MH+ = 257.0 (Method B)
131 3-{[(2,3-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid hydrochloride		36	Rt 0.71 mins, MH+ = 257.0 (Method B)
133 3-{[(2,5-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid hydrochloride		49	Rt 0.71 mins, MH+ = 257.1 (Method B)

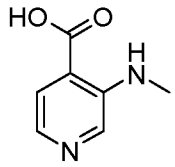
Example 147: 3-[(3-aminopropyl)amino]-4-pyridinecarboxylic acid



To a solution of 3-{[3-({[(1,1-dimethylethyl)oxy]carbonyl}amino)propyl]amino}-4-pyridinecarboxylic acid (45 mg, 0.152 mmol) in DCM (3ml) was added TFA (0.1 ml, 1.298 mmol) and the mixture stirred at room temperature for 16h. The reaction mixture was concentrated under reduced pressure and the residue dissolved in MeOH (5 ml) and loaded onto an SCX SPE cartridge, washed with MeOH then 2N ammonia solution in MeOH. Fractions containing desired product were combined then concentrated under reduced pressure to give the title compound as a pale yellow solid, 21 mg (71 % yield).

LCMS (Method C): Rt = 0.18 mins, MH+ = 196.1

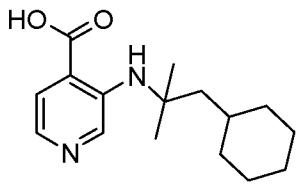
Example 153: 3-(methylamino)-4-pyridinecarboxylic acid



Methylamine (0.321 mL,3.90 mmol, 40% solution in water) was added to a 3-fluoro-4-pyridinecarboxylic acid (250 mg, 1.772 mmol) and 1,4-Dioxane (0.3 ml) and the mixture heated using a microwave to 125 ºC for 1.5 hr then 2 hr and finally an additional 6 hr. The mixture was allowed to cool then concentrated by evaporation. Water (10 ml) was added then the mixture acidifed to pH 3 using 37% HCl. The resulting bright yellow precipitate was filtered then washed with water (10 ml) to give the title compound as a yellow solid, 114 mg (42%).

1H NMR (400 MHz, DMSO-δ6) 8.20ppm (1H, s), 7.84ppm (1H, d), 7.55ppm (1H, d), 2.93ppm (3H, s).

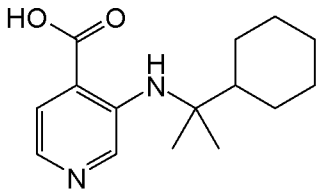
Example 161: 3-[(2-cyclohexyl-1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid hydrochloride



To 3-fluoro-4-pyridinecarbonitrile (99 mg, 0.811 mmol) and *N*-ethyl-*N*-(1-methylethyl)-2-propanamine (0.850 mL, 4.86 mmol) were added (2-cyclohexyl-1,1-dimethylethyl)amine hydrochloride (622 mg, 3.24 mmol) and NMP (0.4 ml) under nitrogen. The reaction mixture was heated using a microwave for 4 hr at 160ºC. To the stirred reaction mixture was added EtOH (2 ml,), water (2 ml) and NaOH (454 mg, 11.35 mmol). The vessel was heated using a microwave at 160ºC for a further 4 hr then water (10ml) and EtOH (10ml) were added. The reaction mixture was neutralized by HCl then concentrated under reduced. The resultant solid was dissolved in DMSO (5ml) then filtered. The filtrate was purified by MDAP (High pH modifier, Method E) then resulting material dissolved in 1,4-dioxane (2 ml) and HCl (1M in ether, 0.185ml) added. The mixture was concentrated under a stream of nitrogen then material dried under high vacuum to give the title compound, 47mg (19%).

LCMS (Method B): Rt = 0.83 mins, MH+ = 277.2

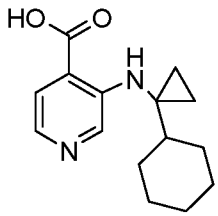
Example 162: 3-[(1-cyclohexyl-1-methylethyl)amino]-4-pyridinecarboxylic acid



To 3-[(1-cyclohexyl-1-methylethyl)amino]-4-pyridinecarbonitrile (32 mg, 0.131 mmol) in EtOH (2 ml) under nitrogen was added NaOH (12.7 mg, 0.318 mmol). The mixture wsa heated to 100ºC using a microwave for 1 hour. The mixture was allowed to stand at room temperature for 4 days then additional NaOH (21.0 mg, 0.526 mmol) was added and mixture heated using a microwave to 140ºC for 2 hr then at 140ºC for a further 4 hr. Additional NaOH (50 mg, 1.250 mmol) was added and mixture heated using a microwave at 140ºC for 4 hr then at 140ºC for an additional 4 hr. The reaction mixture was diluted with DMSO/acetonitrile then purified using MDAP (high pH modifier, Method E) to give the title compound as a cream solid, 11mg (29%).

LCMS (Method A): Rt = 0.74 mins, MH+ = 263.2

Example 165: 3-[(1-cyclohexylcyclopropyl)amino]-4-pyridinecarboxylic acid

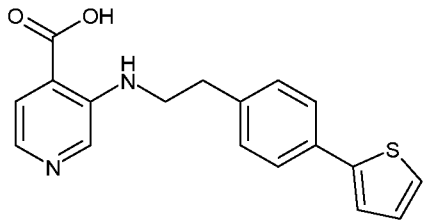


To 3-fluoro-4-pyridinecarbonitrile (85 mg, 0.696 mmol) in *N*-ethyl-*N*-(1-methylethyl)-2-propanamine (0.730 mL, 4.18 mmol) was added (1-cyclohexylcyclopropyl)amine hydrochloride (489 mg, 2.78

mmol) and NMP (0.6 ml). The reaction mixture was stirred under nitrogen and heated using a microwave for 4 hr at 160ºC. EtOH (2 ml), water (0.5 ml) and NaOH (390 mg, 9.75 mmol) were added then the reaction mixture heated using a microwave for 4 hr at 160ºC. The reaction mixture was neutralized using 2N HCl (aq), then concentrated under reduced pressure. The resultant solid was dried under high vacuum to give a grey solid which was dissolved in water then purified in two batches using a C18 column, eluting with acetonitrile in water (10 to 100%). Combined crude material was purified by MDAP (high pH modifier, Method E) to give the title compound as a white solid, 10.2 mg (6%).

LCMS (Method A): Rt = 0.71 mins, MH+ = 261.1

Example 166: 3-({2-[4-(2-thienyl)phenyl]ethyl}amino)-4-pyridinecarboxylic acid



To a microwave vial with a stirrer was added 2-thienylboronic acid (358 mg, 2.80 mmol), 3-{[2-(4-bromophenyl)ethyl]amino}-4-pyridinecarboxylic acid hydrochloride (500 mg, 1.398 mmol), sodium bicarbonate (352 mg, 4.19 mmol), 1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II).DCM (1:1) (114 mg, 0.140 mmol), 1,4-Dioxane (2 ml) and water (0.5 ml). The reaction vial was sealed and heated in a microwave to 110 °C for 1 hr then allowed to cool to room temperature. The reaction mixture was concentrated under reduced pressure then IPA (2 ml) was added, followed by 2N HCl dropwise to neutralise the mixture. The reaction mixture was then diluted using IPA (30 ml), passed through an SCX SPE column, eluting with IPA then 10% ammonia/IPA solution. Fractions containing product were combined then concentrated under reduced pressure to give the crude product, 345mg, as a brown solid. The brown solid was purified by preparative HPLC (Method H) to give the title compound, 9 mg (2%) as a brown solid.

LCMS (Method B): Rt = 0.81 mins, MH+ = 325.1

JMJD2x RapidFire™ High Throughput Mass Spectrometry Demethylase Assay Protocol

The JMJD2 RapidFire™ mass spectrometric assays monitor demethylation of a histone H3 peptide containing tri-methylated K9, by recombinant Jumonji D2 family demethylase enzymes.

Protein Preparation (JMJD2a, JMJD2c, JMJD2d and JMJD2e)

JMJD2a Protein Preparation

JMJD2a DNA encoding the catalytic domain (residues–359) was subcloned from pNIC-JMJD2a (1-359), obtained from the SGC into an in house vector for baculovirus expression (pFB-Flag) using NdeI and BamHI restriction sites, resulting in a protein containing an N-terminal Flag-6His tag followed by a TEV-protease cleavage site. pFB-Flag-6H-Tev JMJD2a (1-359) was transposed into the baculovirus genome using the BAC-to-BAC technology (Invitrogen). Bacmid DNA was transfected into *Spodoptera frugiperda* (Sf9) cells using Cellfectin II (Invitrogen), and expression was performed at a 1L scale in Excel 420 media (SAFC Biosciences). The culture, at a cell concentration of $3.8xe^6$ cells/ml, was infected with P1 recombinant Baculovirus at a nominal multiplicity of infection of 3 and incubated for 48 hours. The cells were removed from the media by centrifugation at 2500g for 20 minutes, and the cell pellet was frozen for subsequent purification.

The pellet from the Baculovirus culture was resuspended in buffer A (50mM Tris-HCl pH7.8, 300mM NaCl, 10% glycerol, 50mM Imidazole, 10uM Ammonium Iron II Sulphate,Benzonase (100ul/1L) and 1ul/ml Protease Inhibitor Cocktail Set III (Calbiochem 539134)). Cells were lysed by Dounce Homogenisation, on ice, and centrifuged at 25,000 x g for 90 minutes at 4°C. The 25,000 x g supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02). The column was washed with ten column volumes of buffer A containing 50mM imidazole. Bound protein was eluted from the column using buffer A containing 300mM imidazole over 10 column volumes. Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

Eluted JMJD2a protein from the HisTRAP column was loaded onto a HiLoad 26/20 Superdex 200 prep grade size exclusion column (GE Healthcare 17-1069-01), equilibrated with buffer B (50mM Tris-HCl pH7.8, 150mM NaCl, 0.5mM DTT, 10% glycerol, 10uM Ammonium Iron II Sulphate and 1ul/ml Protease Inhibitor Cocktail Set III (Calbiochem 539134)). Fractions containing JMJD2a were pooled and concentrated (Ultrafree-15 30kDa).

Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry. *(Observed MW +43 Da, JMJD2a +1 acetylation)*

<u>JMJD2c Protein Preparation</u>

JMJD2c DNA encoding the catalytic domain (residues 1–366) was amplified by PCR to introduce an N-terminal Flag-6H-Tev tag and cloned into pFB1 vector (Invitrogen). pFB1-Flag-6H-Tev-JMJD2c 1-366 was transposed into the baculovirus genome using the BAC-to-BAC technology (Invitrogen). Bacmid DNA was transfected into *Spodoptera frugiperda* (Sf9) cells using Cellfectin II (Invitrogen), and expression was performed at a 20L scale in Excel 420 media (SAFC Biosciences). The culture, at a cell concentration of $5.8xe^6$ cells/ml, was infected with P1 recombinant Baculovirus at a nominal multiplicity of infection of 3 and incubated for 48 hours. The cells were removed from the media by centrifugation at 2500g for 20 minutes, and the cell pellet was frozen for subsequent purification.

The pellet from the Baculovirus culture was resuspended in buffer A (50mM Tris, 300mM NaCl , 5% Glycerol, 1ul/ml Protease Inhibitor Cocktail Set III (Calbiochem), pH8.0). Cells were lysed by Dounce Homogenisation, on ice, and centrifuged at 100,000 x g for 90 minutes at 4°C. The 100,000 x g supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02). The column was washed with ten column volumes of buffer A, followed by ten column volumes of buffer A containing 30mM Imidazole. Bound protein was eluted from the column using buffer A containing 180mM Imidazole. Eluted JMJD2c protein from the HisTrap column was loaded onto a Superdex 75 column prep grade size exclusion column (GE Healthcare), equilibrated with buffer A (50mM Tris-HCl, 150mM NaCl, 5% Glycerol, pH8.0). Fractions containing JMJD2c were pooled and concentrated (YM10 MWCO Amicon filter).

Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

JMJD2d Protein Preparation

JMJD2d DNA encoding the catalytic domain (residues 1–366) was amplified by PCR to introduce an N-terminal Flag-6H-Tev tag and cloned into the pFB-CT/TOPO vector (Invitrogen. pFB1-Flag-6H-Tev-JMJD2d 1-366 was transposed into the baculovirus genome using the BAC-to-BAC technology (Invitrogen). Bacmid DNA was transfected into *Spodoptera frugiperda* (Sf9) cells using Cellfectin II (Invitrogen), and expression was performed at a 20L scale in Excel 420 media (SAFC Biosciences). The culture, at a cell concentration of $3.3xe^6$ cells/ml, was infected with P1 recombinant Baculovirus at a nominal multiplicity of infection of 3 and incubated for 44 hours. The cells were removed from the media by centrifugation at 2500g for 20 minutes, and the cell pellet was frozen for subsequent purification.

The pellet from the Baculovirus culture was resuspended in buffer A (PBS pH8.1, 10mM β-mercaptoethanol, 0.1mM benzamidine, 0.1mM PMSF and 10uM $Fe(NH_4)_2(SO_4)_2$).

Cells were lysed by Dounce Homogenisation, on ice, followed by sonication on ice, for 4min at 40% amplitude ¾" probe, 9.9 sec on/off. The homogenate was centrifuged at 100,000 x g for 90 minutes at 4°C. The 100,000 x g supernatant was applied to Ni NTA resin (Qiagen) by end over end mixing overnight at 4°C. The resin was allowed to settle under gravity then packed in a XK16 column. The column was washed with ten column volumes of buffer A, followed by ten column volumes of buffer A containing 20mM Imidazole. Bound protein was eluted from the column using buffer A containing 300mM Imidazole.

Eluted JMJD2d protein from the NiNTA column was loaded onto a Superdex pg200 column prep grade size exclusion column (GE Healthcare), equilibrated with buffer B (20mM BisTrisPropane pH 7.8, 100mM NaCl, 10mM β-mercaptoethanol, 10uM $Fe(NH_4)_2(SO_4)_2$, 5% glycerol). Fractions containing JMJD2d were pooled and concentrated (Ultrafree-15 30kDa).

Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

JMJD2e Protein Preparation

An expression vector containing the JMJD2e DNA catalytic domain (residues 1–336) was obtained from the SGC (see details in PDB entry 2W2I). Expression was performed in Rosetta2(DE3)pLysS using modified Terrific Broth containing 50 µg/ml Kanamycin, and 34 µg/ml chloramphenicol. Media was inoculated with 5 ml overnight culture and grown at 37°C until it reached OD_{600}= 0.8. The protein expression was induced with 0.2 mM IPTG and grown for a further 20 hours at 18°C.

The pellet from the *E. coli* culture was resuspended in 6ml/g of lysis buffer (50mM Hepes, 500mM NaCl , 50mM Imidazole , 0.5mM TCEP , 5% Glycerol ,1ug/ml BLAP (bezamidine, leupeptin, aprotinin, pepstatin), 1mg/ml lysozyme, pH7.5). The sample was then lysed by sonication (10secs on / 10secs off) for 10mins. The resulting lysate was centrifuged at 100,000xg for 90mins. The lysate supermatant was harvested and then passed through a 10ml NiNTA HiTrap column (GE Healthcare) at 0.5ml/min . The column was washed back to baseline with buffer A (50mM Hepes, 500mM NaCl , 0.5mM TCEP, 5% Glycerol, 1ug/ml BLAP , pH8.0) and then eluted using buffer B (50mM Hepes, 500mM NaCl , 100mM EDTA, 0.5mM TCEP , 5% Glycerol , 1ug/ml BLAP , pH8.0). Fractions containing JMJD2e were was incubated with 1mg of TEV protease (GE Healthcare) overnight by dialysing against 10mM Hepes , 500mM NaCl , 0.5mM TCEP, 5% Glycerol, pH7.5 . This material was then analysed by SDS PAGE to see if full cleavage had taken place and then the pool was recontacted with NiNTA resin (as above) to remove the cleaved His tag and uncleaved JMJD2e. The non absorbed fraction was harvested and concentrated (YM10 MWCO Amicon filter). JMJD2e protein from the TEV cleavage was loaded onto a Superdex p75 column prep grade size exclusion column (GE Healthcare), equilibrated with buffer C (10mM Hepes, 500mM NaCl, 5% Glycerol, 10uM $Fe(NH_4)_2(SO_4)_2$, pH7.5). Fractions containing JMJD2e were pooled and concentrated (YM10 MWCO Amicon filter).
Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

Peptide Synthesis

Peptide sequence: ARTAQTAR**K**STGGIA (the bold underlined lysine corresponds to K9 in human histone 3 and is tri-methylated): ARTAQTAR-K(TriMe)-STGGIA

The peptide was supplied by Cambridge Research Biochemicals. In detail, the protected peptide was assembled on a solid-phase synthesiser using preloaded Wang resin and utilising standard Fmoc synthesis protocols. The crude peptide was obtained after cleavage from the resin with a mixture of trifluoroacetic acid (TFA), triisopropylsilane and water (95:2.5:2.5) for 3 hours at room temperature and was then purified using a C18 reverse-phase column utilising a 0.1%TFA-buffered water/acetonitrile gradient. The resulting fractions were analysed and fractions which were >95% pure by analytical HPLC and giving the correct molecular weight (mw) (by MALDiTOF mass

spectroscopy) were pooled and freeze dried. The final material was analysed by HPLC and MALDI-TOF mass spectroscopy.

The trimethyl lysine was incorporated into the sequence during assembly as Fmoc-Lys(TriMe)-OH. This was made by reaction of a suitably protected alpha-nitrogen lysine with an excess of methyl chloride or bromide to form the quaternary salt.
This peptide is suitable for use as a substrate for all JmjD2 family H3K9 demthylases.

Compound plate preparation:

For all JMJD2 RapidFire™ assays, compounds were diluted to 10mM in DMSO, and a 1:3, 11 point serial dilution performed across a 384 well hibase plate (Greiner Bio-one, Stonehouse, UK). 100nl of this dilution series was then transferred into a 384 well V-base plate (Greiner Bio-one) using the Echo™ acoustic dispenser (Labcyte Inc, Sunnyvale, CA, USA) to create the assay plate. 100nl DMSO was added to column 6 and 18.

Assay method:

For all JMJD2 RapidFire™ assays; 60µl of 0.5% trifluoroacetic acid (TFA) solution (Applied Biosystems, Warrington, UK) was added to column 18 of assay plates to generate a 100% inhibition control.

High enzyme concentration format: 5µl of an enzyme solution containing either 0.5 µM JMJD2a, 0.175µM JMJD2c, 0.375 µM JMJD2d or 0.075µM JMJD2e enzyme (all prepared in-house) and 0.25mg/ml BSA (Sigma-Aldrich, Dorset, UK), diluted in a buffer of 50mM HEPES pH 7.0 and varying concentrations of NaCl (both Sigma-Aldrich) was added to each well of the plate using a Multidrop Combi® dispenser (Thermo Fisher Scientific, Waltham, MA, USA). NaCl concentration was assay dependent (no NaCl was added in the case of JMJD2c, 100mM for JMJD2a and JMJD2d and 50mM for JMJD2e).

Low enzyme concentration format: 5µl of an enzyme solution containing either 0.05 µM JMJD2a, 0.025µM JMJD2c, 0.05 µM JMJD2d or 0.01µM JMJD2e enzyme (all prepared in-house) and 0.25mg/ml BSA (Sigma-Aldrich, Dorset, UK), diluted in a buffer of 50mM MES (2-(N-Morpholino)-ethanesulfonic acid 4-Morpholineethanesulfonic acid monohydrate) pH 7.0 and varying concentrations of NaCl (both Sigma-Aldrich) was added to each well of the plate using a Multidrop Combi® dispenser (Thermo Fisher Scientific, Waltham, MA, USA). NaCl concentration was assay dependent (no NaCl was added in the case of JMJD2c, 100mM for JMJD2a and JMJD2d and 50mM for JMJD2e).

Plates were incubated for 10 minutes at room temperature, after which time 5µl of a substrate solution containing 100µM ascorbic acid (Sigma-Aldrich), 100µM ammonium iron (II) sulphate (Fisher

Scientific Waltham, MA, USA), 20μM alphaketoglutarate (Sigma-Aldrich) and H3K9Me3 peptide at 2 x K_m concentration (prepared in-house), all diluted in assay buffer as above, was added to each well of the plate using a Multidrop Combi® dispenser, to initiate the reaction. Concentrations quoted are 2 x final assay concentrations. K_m concentrations for the H3K9Me$_3$ peptide were determined with the following results:

Assay	$K_{m\ (Obs)}$
JMJD2a	50 μM
JMJD2c	10 μM
JMJD2d	25 μM
JMJD2e	20 μM

Table 1: K_m determinations for H3K9Me$_3$ peptide in the various JMJD2 RapidFire™ demethylase assays.

Plates were centrifuged at 1000 rpm for 1 minute and incubated at room temperature for the duration of the linear phase of the reaction (usually 10 minutes). After this time, the reaction was quenched by the addition of 30μl of a 0.5% TFA solution to all wells except column 18 (to which this was previously added) using a Multidrop Combi ®dispenser. Plates were centrifuged at 1000 rpm for 5 minutes before analysis.

Plates were analysed by RapidFire™ high throughput solid phase extraction system (Biocius Life Sciences, MA, USA) coupled to a QqQ mass spectrometer (AB Sciex, Cheshire, UK) to generate relative peak areas of H3K9Me$_3$ substrate and H3K9Me$_2$ product. Peaks were integrated using the RapidFire™ integrator software before percentage conversion of H3K9Me$_3$ substrate to H3K9Me$_2$ product was calculated as shown below:

$$\%\ Conversion = (H3K9Me_2\ peak\ area)/(H3K9Me_3\ peak\ area + H3K9Me_2\ peak\ area))^{x\ 100}$$

Data were further analysed within Activitybase software (IDBS) using a four parameter curve fit of the following form:

$$y = \frac{a-d}{1+\left(\frac{x}{c}\right)^b} + d$$

where *a* is the minimum, *b* is the Hill slope, *c* is the XC_{50} and *d* is the maximum. Data are presented as the mean pXC_{50} (negative $\log_{10}$ of the molar XC_{50}).

JMJD3 MALDI-TOF Demethylase Assay Protocol

The JMJD3 MALDI-TOF mass spectrometric assay monitors demethylation of a histone H3 peptide containing tri-methylated K27, by recombinant Jumonji D3 demethylase enzyme.

JMJD3 Protein Preparation

JMJD3 DNA encoding the catalytic domain (residues 1141–1682), and lacking residues 1637-1675 (a splice variant deletion; GenBank BC009994) was cloned into the pFB-HTb vector (Invitrogen) using BamHI and XhoI restriction sites, which encodes for a protein with an N-terminal 6His tag followed by a TEV-protease cleavage site. pFB1-HTb-JMJD3-6H 1141-1682 (del 1637-1675) was transposed into the baculovirus genome using the BAC-to-BAC technology (Invitrogen). Bacmid DNA was transfected into *Spodoptera frugiperda* (Sf9) cells using Cellfectin II (Invitrogen), and expression was performed at a 1L scale in Excel 420 media (SAFC Biosciences). The culture, at a cell concentration of 3.8xe^6 cells/ml, was infected with P1 recombinant Baculovirus at a nominal multiplicity of infection of 3 and incubated for 48 hours. The cells were removed from the media by centrifugation at 2500g for 20 minutes, and the cell pellet was frozen for subsequent purification.

The pellet from the Baculovirus culture was resuspended in buffer A (20mM Tris pH 8.5, 300mM NaCl, 10% glycerol, 1ul/ml Protease Inhibitor Cocktail Set III (Calbiochem 539134) and 10uM $Fe(NH4)_2(SO_4)_2$).

Cells were lysed by Dounce Homogenisation, on ice, and centrifuged at 100,000 x g for 90 minutes at 4°C. The 100,000 x g supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02). The column was washed with ten column volumes of buffer A, followed by ten column volumes of buffer A containing 20mM Imidazole. Bound protein was eluted from the column using a linear gradient of 20-250mM Imidazole over twenty column volumes. The JMJD3 protein was eluted between 100mM and 200mM Imidazole.

Eluted JMJD3 protein from the HisTRAP column was concentrated fourfold (Amicon Ultrafree-15 30kDa, Millipore UFC903024) and loaded onto a HiLoad 26/20 Superdex 200 prep grade size exclusion column (GE Healthcare 17-1069-01), equilibrated with buffer B (20mM Tris pH 8.0, 150mM NaCl, 5% glycerol, 0.5mM TCEP, 2mM α-Ketoglutaric acid sodium salt (Sigma K1875) and 10uM $Fe(NH_4)_2(SO_4)_2$).

Fractions containing JMJD3 were pooled and concentrated (Ultrafree-15 30kDa).

Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

Peptide Synthesis

Peptide sequence : ATKAAR**K**SAPATGGVKKPHRYRPG (the bold underlined lysine corresponds to K27 in human histone 3 and is tri-methylated): ATKAAR-K(TriMe)-SAPATGGVKKPHRYRPG

The peptide was supplied by Cambridge Research Biochemicals. In detail, the protected peptide was assembled on a solid-phase synthesiser using preloaded Wang resin and utilising standard Fmoc

synthesis protocols. The crude peptide was obtained after cleavage from the resin with a mixture of trifluoroacetic acid (TFA), triisopropylsilane and water (95:2.5:2.5) for 3 hours at room temperature and was then purified using a C18 reverse-phase column utilising a 0.1%TFA-buffered water/acetonitrile gradient. The resulting fractions were analysed and fractions which were >95% pure by analytical HPLC and giving the correct molecular weight (mw) (by MALDiTOF mass spectroscopy) were pooled and freeze dried. The final material was analysed by HPLC and MALDiTOF mass spectroscopy.

The trimethyl lysine was incorporated into the sequence during assembly as Fmoc-Lys(TriMe)-OH. This was made by reacting a suitably protected alpha-nitrogen lysine with an excess of methyl chloride or bromide to form the quaternary salt.

Compound plate preparation:

Compounds were diluted to 10mM in DMSO, and a 1:3, 11 point serial dilution performed across a 384 well hibase plate (Greiner Bio-one, Stonehouse, UK). 100nl of this dilution series was then transferred into a 384 well V-base plate (Greiner Bio-one) using the Echo™ acoustic dispenser (Labcyte Inc, Sunnyvale, CA, USA) to create the assay plate. 100nl DMSO was added to column 6 to generate a high control.

Assay method:

10ul of a 1% trifluoroacetic acid (TFA)solution was added to column 18 of compound plates to generate a 100% inhibition control, before 5ul of an enzyme solution containing 1.2uM 6H-Tev-Flag-JMJD3 and 200uM CHAPS (Sigma-Aldrich, St. Louis, MO) diluted in a buffer of 20mM Tris HCL and 150mM NaCl (both Sigma-Aldrich) was added to each well of the plate using a Multidrop Combi® dispenser (Thermo Fisher Scientific, Waltham, MA, USA). This gives a final assay concentration of 600nM JMJD3 enzyme and 100uM CHAPS.

Plates were incubated for 15 minutes at room temperature, after which time 5ul of a substrate solution containing 4mM ascorbic acid (Sigma -Aldrich), 100uM ammonium iron (II) sulphate (Fisher Scientific Waltham, MA, USA), 70uM alphaketoglutarate (Sigma-Aldrich) and 30uM $H3K27Me_3$ peptide, all diluted in a buffer of 50mM Hepes, was added to each well of the plate using a Multidrop Combi® dispenser, to initiate the reaction. Final assay concentrations are 2mM ascorbic acid, 50uM ammonium iron (II) sulphate, 35uM alphaketoglutarate and 15uM $H3K27Me_3$ peptide.

After precisely 8 minutes, the reaction was quenched by the addition of 10ul of a 1% TFA solution to all wells except column 18 (to which this was previously added) using a Multidrop Combi ®dispenser.

Samples were diluted 1:100 in 5% acetonitrile using the Biomek® FX (Beckman Coulter, Brea, CA, USA) and 1ul of this dilution transferred on to a 384 well PAC target (Bruker Daltonics, Bremen, Germany) again using the Biomek® FX. The target was washed by immersing in a solution of 10mM ammonium phosphate (Sigma-Aldrich) and 0.1% TFA for 10 seconds before being dried at room

temperature and analysed by MALDI-TOF (Ultraflex III, Bruker Daltonics) to generate relative peak areas of $H3K27Me_3$ substrate and $H3K27Me_2$ product.

JMJD3 RapidFire™ High Throughput Mass Spectrometry Demethylase Assay Protocol

MATERIALS AND METHODS

Materials

Ascorbic acid, α-ketoglutarate, dimethyl sulfoxide (DMSO), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Gillingham, Dorset, UK). Trifluoroacetic acid (TFA) was from Applied Biosystems (Warrington, UK). Acetonitrile and ammonium iron (II) sulphate were purchased from Fisher Scientific (Loughborough, UK). H_3K_{27}-Me_3 peptide was synthesized and HPLC-purified to >95% purity by Cambridge Research Biochemicals (Cambridge, UK).

Expression and purification of JMJD3

JMJD3 DNA encoding the catalytic domain (residues 1142–1682), and lacking residues 1638-1675 (a splice variant deletion; GenBank BC009994) was cloned into the pFB-HTb vector (Invitrogen) using BamHI and XhoI restriction sites, which encodes for a protein with an N-terminal 6His tag followed by a TEV-protease cleavage site. pFB1-HTb-JMJD3-6H 1142-1682 (del 1638-1675) was transposed into the baculovirus genome using the BAC-to-BAC technology (Invitrogen). Bacmid DNA was transfected into *Spodoptera frugiperda* (Sf9) cells using Cellfectin II (Invitrogen), and expression was performed at a 1L scale in Excel 420 media (SAFC Biosciences). The culture, at a cell concentration of $3.8xe^6$ cells/ml, was infected with P1 recombinant Baculovirus at a nominal multiplicity of infection of 3 and incubated for 48 hours. The cells were removed from the media by centrifugation at 2500g for 20 minutes, and the cell pellet was frozen for subsequent purification.

The pellet from the Baculovirus culture was resuspended in buffer A (20mM Tris pH 8.0, 300mM NaCl, 10% glycerol and 1ul/ml Protease Inhibitor Cocktail Set III (Calbiochem 539134)). Cells were lysed by Dounce Homogenisation, on ice, and centrifuged at 100,000 x g for 90 minutes at 4°C. The 100,000 x g supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02). The column was washed with ten column volumes of buffer A, followed by ten column volumes of buffer A containing 20mM Imidazole. Bound protein was eluted from the column using a linear gradient of 20-250mM Imidazole over twenty column volumes. The JMJD3 protein was eluted between 100mM and 200mM Imidazole.

Eluted JMJD3 protein from the HisTRAP column was concentrated threefold (Amicon Ultrafree-15 30kDa, Millipore UFC903024) and loaded onto a HiLoad 26/20 Superdex 200 prep grade size exclusion column (GE Healthcare 17-1071-01), equilibrated with buffer B (20mM Tris pH 8.0, 150mM NaCl and 5% glycerol).

Fractions containing JMJD3 were pooled and concentrated (Ultrafree-15 30kDa).
Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by mass spectrometry.

Preparation of compound screening plates

For dose-response curves, 3-fold serial dilutions were prepared from 10 mM compound solutions in DMSO across 384 well HiBase plates (Greiner Bio-one, Stonehouse, UK). A 100nl of dilution series were transferred into 384 well V base assay plates (Greiner Bio-one, Stonehouse, UK) giving a concentration range between 100 μM and 1.7 nM. Columns 6 and 18 of the assay plates were reserved for the high and low controls, respectively. The high controls had 100 nL of DMSO, but no compound, while the low controls also had 100 nL of DMSO but the enzyme was acid-inactivated by pretreatment with 0.5% (v/v) TFA. Compounds and DMSO were dispensed using the Echo™ acoustic dispenser (Labcyte Inc, Sunnyvale, CA, USA).

RapidFire® mass spectrometry assay.

In this assay, disappearance of the trimethylated peptide substrate and formation of the dimethylated product are both monitored by mass spectrometry. For all assays 60μl of 0.5% trifluoroacetic acid (TFA) solution (Applied Biosystems, Warrington, UK) was added to column 18 of assay plates to generate a 100% inhibition control.

Assays were performed by initially dispensing 5 μL of enzyme solution containing 0.15 μM JMJD3, 0.25 mg/mL BSA and 100μM CHAPS in 50 mM Hepes pH 7.0 into the 384-well plates containing 100 nL compound. Plates were allowed to incubate for 10 minutes at ambient temperature before reactions were initiated by the addition of 5 μL of substrate solution containing 50 μM ascorbate, 50 μM Fe2+, 10 μM α-ketoglutarate, and 30 μM peptide also in 50 mM Hepes pH 7.0. Plates were centrifuged at 1000 rpm for 1 minute and the reactions incubated at room temperature for 6 minutes, before being quenched by the addition of 60 μL of a 0.5% (w/v) TFA solution. Plates were centrifuged at 1000 rpm for 5 minutes before analysis. All solutions were dispensed using a Multidrop Combi® dispenser (Thermo Fisher Scientific, Waltham, MA, USA).

Assay plates were transferred onto a high-throughput RapidFire200 integrated autosampler/solid phase extraction (SPE) system (Agilent Technologies Inc., Wakefield, MA, USA) coupled to an API4000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada). Solvent A was water containing 0.01% (v/v) TFA and 0.09% (v/v) formic acid. Solvent B was acetonitrile/water (8:2, v/v) containing 0.01% (v/v) TFA and 0.09% (v/v) formic acid. Samples were aspirated under vacuum directly from 384-well assay plates for 500 ms. The sample was then loaded onto a C4 solid phase extraction cartridge to remove non-volatile buffer salts, using solvent A at a flow rate of 1.5 mL/min for 3 s. The retained analytes were eluted to the mass spectrometer by washing the cartridge with solvent B at 1.25 mL/min for 3 s. The cartridge was re-equilibrated with

solvent A for 500 ms at 1.5 mL/min. The entire sampling cycle was approximately 7 seconds per well, enabling analysis of a 384 well plate in approximately 45 minutes.

The mass spectrometer was operated in positive electrospray MRM mode. MRM transitions, collision energy, and declustering potential (Q1/Q3/CE/DP) for each species were as follows. K27Me3: 418.3/414.8/21/70; K27Me2: 519.7/515.0/21/70. A dwell time of 50 ms was used for all of the MRM transitions. The mass spectrometer was operated with a spray voltage of 3500 V and at a source temperature of 650 °C. The peaks detected by mass spectrometry were approximately 1.2 s wide at half-height and they were integrated and processed using the RapidFire® peak integration software.

Data analysis

Data were analyzed using the ActivityBase Suite (ID Business Solutions Ltd, Surrey, UK). The extent of enzymatic turnover of the tri-methylated substrate to di-methylated product was expressed as percent conversion as shown in Eq. (1):

$$\% \text{ Conversion} = 100 \times Me2/(Me2 + Me3) \quad (1)$$

where Me2 and Me3 represent the integrated peak areas of the di- and trimethylated peptides. Data were then fitted to Eq. (2) to determine Ic50 values

$$\% \text{ Inhibition} = (a - d)/[1 + ([I]/IC50)h] + d \quad (2)$$

where a is the uninhibited value, d is the fully inhibited value, [I] is the inhibitor concentration, IC50 is [I] that gives ½ × (a – d), and h is the Hill slope.

Determination of the effect of the compounds on Jarid1c

Principle of the assay

In the competitive binding assay test compounds are added directly into a cell lysate. Various concentrations of test compounds were added to cell lysate samples and allowed to bind to the proteins contained in the lysate sample. At the same time the affinity matrix was added to capture proteins not bound to the test compound. After the incubation time the beads with captured proteins were separated from the lysate. Bead-bound proteins were then eluted and the presence of Jarid1c was detected and quantified using specific antibodies and the Odyssey Infrared Detection system. Further experimental protocols can be found in WO2011/018241.

Protocols

<u>Preparation of the affinity matrix (immobilization of compound on beads)</u>

NHS-activated Sepharose 4 Fast Flow (Amersham Biosciences, 17-0906-01) was equilibrated with anhydrous DMSO (Fluka, 41648, $H_20 \leq 0.005\%$). 5 mL of settled beads were transferred into a 50 mL Falcon tube, then 250 µl of a 100 mM solution of the immobilization compound (**Example 86**)

dissolved in DMSO and 75 µL of triethylamine (Sigma, T-0886, 99% pure) were added. This leads to a final concentration of the immobilization compound of 5 µmol/mL beads. The beads were incubated at room temperature in darkness on an end-over-end shaker (Roto Shake Genie, Scientific Industries Inc.) for 16 – 20 hours. The coupling efficiency was determined by HPLC analysis of non-immobilized compound in the supernatant. Non-reacted NHS-groups on the beads were blocked by incubation with aminoethanol at room temperature on the end-over-end shaker over night. Beads were washed twice with 5 to 10 volumes of DMSO and stored in *iso*propanol at -20°C. These beads were used as the affinity matrix in the following experiments.

Equilibration of the affinity matrix

The affinity matrix was washed three times with 5 to 10 volumes of DP buffer (50 mM Tris-HCl pH 7.4, 5% Glycerol, 1.5 mM $MgCl_2$, 150 mM NaCl, 1 mM Na_3VO_4, 0.4% Igepal® CA-630, 10 µM $FeCl_2$, 200 µM sodium-L-ascorbate). Beads were collected by centrifugation (4 min at 300 x g in a Heraeus centrifuge) and finally resuspended in DP buffer to prepare a 10% beads slurry.

Preparation of test compounds

Stock solutions of test compounds were prepared in DMSO corresponding to a 50-fold higher concentration compared to the final desired test concentration (e.g. a 10 mM stock solution was prepared for a final test concentration of 200 µM). This dilution scheme resulted in a final DMSO concentration of 2%. As positive control 2.5 mM **Example 86** (final assay concentration: 50 µM) was applied, as negative control DMSO was added.

Cell culture

HUT-78 cells (ATCC TIB-161, Manassas, VA, USA) were grown in spinner flasks (Integra Bioscience 182101, Zizers, Switzerland) in IMDM medium (Invitrogen 21980.065, Carlsbad, CA, USA) supplemented with 20% fetal calf serum (Invitrogen 10270) up to a cell concentration of $1x10^6$ cells/ml.

Preparation of cell lysate (nuclear extract)

Cell pellets were resuspended in 4 volumes of hypotonic buffer (10 mM TRIS-Cl, pH 7.4, 1.5 mM $MgCl_2$ (Sigma M-1028), 10 mM KCl , 25 mM NaF (Sigma S7920), 1 mM Na_3VO_4 (Sigma S6508). The cells were allowed to swell for 3 minutes (swelling checked under microscope) and then centrifuged for 5 minutes at 2,350 rpm (Heraeus). The supernatant was discarded and the pellet was resuspended in 2 volumes of hypotonic buffer supplemented with protease inhibitors. Cells were homogenized by 10 to 15 strokes in a homogenizer (VWR SCERSP885300-0015, Radnor, PA, USA) and the homogenate was centrifuged for 10 minutes at 3,300 rpm. The supernatant was discarded and the nuclei were washed in 3 volumes of hypotonic buffer supplemented with protease inhibitors (1 tablet for 25 ml; Roche 13137200, Basel, Switzerland) and centrifuged for 15 minutes at 10.000 rpm

in a SLA-600TC rotor (Sorvall 74503). The pellet was resuspended in 1 volume of extraction buffer (50 mM TRIS-Cl, pH 7.4, 1.5 mM $MgCl_2$, 20 % glycerol (Merck Z835091), 420 mM NaCl (Sigma S5150), 25 mM NaF, 1 mM Na_3VO_4, 400 units/ml of DNAse I (Sigma D4527), and protease inhibitors (Roche Diagnostics, #11 873 580 001; 1 tablet for 25 ml)) and then homogenized first with 20 strokes with a homogenizer and then by 30 minutes gentle mixing at 4°C. The homogenate was then centrifuged for 30 minutes at 10,000 rpm in a SLA-600TC rotor. The supernatant was diluted in dilution buffer (1.8 ml buffer per 1 ml supernatant; 50 mM TRIS-Cl, pH 7.4, 1.5 mM $MgCl_2$, 25 mM NaF, 1 mM Na_3VO_4, 0.6 % Igepal® CA-630 (Sigma, I3021) and protease inhibitors (1 tablet for 25 ml)). After 10 minutes incubation on ice, the lysate was centrifuged for 1 hour at 33,500 rpm in a Ti50.2 rotor (Beckman Coulter LE90K, 392052, Brea, CA, USA) and the supernatant was frozen in liquid nitrogen and stored at -80°C.

Dilution of cell lysate

For a typical experiment one nuclear lysate aliquot containing 120 mg of protein was thawed in a 21 °C water bath and then kept at 4°C. The protein concentration of the nuclear lysate was typically 3.5 – 5.5 mg/ml. After thawing, sodium-L-ascorbate (Merck KGa, 1.03861) and $FeCl_2$ (Sigma, A4034) were added to the nuclear lysate at final concentration of 200 µM and 10 µM, respectively. The final buffer composition was 50 mM TRIS pH 7.4, 5% Glycerol, 150 mM NaCl, 25 mM NaF, 1.5 mM $MgCl_2$, 0.4% Igepal® CA-630, 200 µM sodium-L-ascorbate, 10 µM $FeCl_2$ and protease inhibitors (1 tablet for 25 ml lysate).

Incubation of cell lysate with test compound and affinity matrix

To a 384 well filter plate (Multiscreen HTS, HV Filter Plates, Millipore, MZHVN0W50) the following components were added per well: 2.5 µL affinity matrix (as 10% beads slurry), 1.5 µL of compound solution, and 50 µL of diluted cell lysate in a final assay volume of 75.5 µL. Plates were sealed and incubated for two hours at 4°C on an end-over-end shaker (Roto Shake Genie, Scientific Industries Inc.). Afterwards the wells of the plate were washed twice with 60 µL DP buffer per well. The filter plate was placed on top of a collection plate (Greiner bio-one, polypropylene microplate 384 well V-shape, 781 280) and the beads were eluted with 20 µL elution buffer (100 mM Tris, pH 7.4, 4% SDS, 0.01% Bromophenol blue, 20% glycerol, 50 mM DTT). The eluate was stored at -20 °C or directly used for spotting on a nitrocellulose membrane.

Detection and quantification of eluted Jarid1c

Jarid1c in the eluates was detected and quantified by spotting the eluate on nitrocellulose membranes and using a first antibody directed against the protein of interest (Jarid1c) and a fluorescently labeled secondary antibody (anti-rabbit IRDye™ antibody). The Odyssey Infrared Imaging system from LI-COR Biosciences (Lincoln, Nebraska, USA) was operated according to instructions provided by the

manufacturer (Schutz-Geschwendener et al., 2004. Quantitative, two-color Western blot detection with infrared fluorescence. Published May 2004 by LI-COR Biosciences, www.licor.com).

After spotting of the eluates the nitrocellulose membranes (BioTrace NT; PALL, BTNT30R) were first blocked by incubation with Odyssey blocking buffer (LICOR, 927-40000) for 1 hour at room temperature. Blocked membranes were then incubated for 16 hours at 4°C with the first antibody diluted in Odyssey blocking buffer supplemented with 0.4% Tween® 20. Afterwards the membranes were washed three times for 5 minutes with 15 mL PBS buffer containing 0.1% Tween® 20 (Sigma, T2700) at room temperature. Then the membranes were incubated for 60 minutes at room temperature with the detection antibody (IRDye™ labelled antibody from LI-COR) diluted in Odyssey blocking buffer (LICOR 927-40000) containing 0.2% Tween® 20. Afterwards the membranes were washed four times for 5 minutes each with PBS buffer containing 0.1% Tween® 20 at room temperature. Then the membrane was rinsed twice with PBS buffer to remove residual Tween® 20. The membranes were scanned with the Odyssey Infrared Imaging system. Fluorescence signals were recorded and analyzed according to the instructions of the manufacturer. Concentration response curves were computed with BioAssay and Tibco Spotfire software.

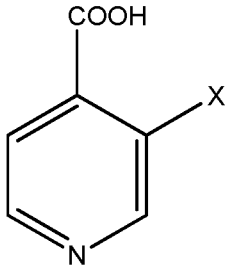
Sources and dilutions of antibodies:

Protein target	**Primary antibody (dilution)**	**Secondary antibody (dilution)**
Jarid1c	Rabbit anti-Jarid1c, Novus Biologicals; Littleton, CO, USA, catalogue number 22270002 (1:1000)	IRDeye800 goat anti-rabbit LI-COR, Lincoln, USA; catalogue number 926-32221 (1:5000)

The compounds of examples 1-166 have an average IC_{50} value in at least one of the JmjD2a, JmjD2c, JmjD2d, JmjD2e, JmjD3 or Jarid1c assays of < 100 µM.

Claims

1. A compound of formula (I)



Wherein

X is $-(R^1)_{0-1}-(R^2)_{0-1}-R^3$ or $-R^1-R^4$;

Each R^1 is independently NH, $N(CH_3)$, O;

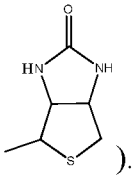
R^2 is a linker group with a maximum length of 5 atoms between R^1 and R^3 and is selected from:

$-CO-C_{1-6}alkyl-$
$-CO-$
$-CO-C_{1-6}alkyl-O-$
$-CO-C_{1-6}alkyl-S-$
$-CO-C_{1-6}alkyl-O-C_{1-6}alkyl-$
$-C_{1-6}alkyl-$
$-C_{1-6}alkyl-O-$
$-C_{1-6}alkyl-SO_2-$
$-C_{1-6}alkyl-NH-CO-$
$-C_{1-3}alkyl-C_{3-6}cycloalkyl-C_{1-3}alkyl-O-$

wherein each alkyl is straight chain or branched and may be optionally substituted by one or more substituents independently selected from phenyl or -OH;

R^3 is selected from:

a C_{6-12} mono or bicyclic aryl group, (each of which may be optionally substituted one or more times by substituents independently selected from halo, $C_{1-6}alkyl$, C_{1-6} haloalkyl, $C_{1-6}alkoxy$, $NHCOC_{1-3}alkyl$, -O-phenyl , $-CH_2$-phenyl, phenyl (optionally substituted by $C_{1-3}alkyl$), OH, NH_2, $CONH_2$, CN, $-NHCOC_{1-3}alkylNH_2$, $–NHCOC_{1-3}alkyl$, $NHCOOC_{1-3}alkyl$, $-NHSO_2C_{1-3}alkyl$, $-SO_2C_{1-3}alkyl$ or

-NHCOC$_{1-3}$alkyl-NHCOC$_{1-4}$ alkyl ).

a 5-12 membered mono or bicyclic heteroaryl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, -C_{1-6} alkyl, -oxo),

a 5 or 6 membered heterocyclic group containing one or more heteromoieties independently selected from N, S, SO, SO_2 or O and optionally fused to a phenyl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, C_{1-3}alkyl)

or a 3-7 membered cycloalkyl (including bridged cycloalkyl) and optionally fused to a phenyl group (and optionally substituted by one or more substituents independently selected from OH, phenyl, CH_2 phenyl),

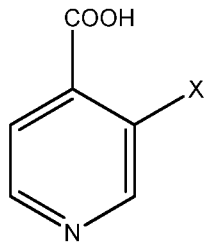
R^4 is selected from:

C_{1-6} straight chain or branched alkyl (optionally substituted by NH_2),

COC_{1-6} straight chain or branched alkyl;

or a pharmaceutically acceptable salt thereof for use in changing the epigenetic status of cells, treating cancer inflammation or autoimmune diseases

2 A compound of formula (Ia):



(Ia)

Wherein

X is -$(R^1)_{0-1}$-$(R^2)_{0-1}$-R^3 or -R^1-R^4;

Each R^1 is independently NH, N(CH_3), O;

In a further aspect of the present invention, there is provided a compound of formula (Ia), or a pharmaceutically acceptable salt thereof for use in therapy, in particular in changing the epigenetic status of cells, treating cancer, inflammation or autoimmune diseases.

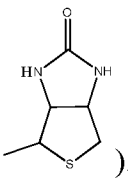
R^2 is a linker group with a maximum length of 5 atoms between R^1 and R^3 and is selected from:

-CO-C_{1-6}alkyl-
-CO-
-CO-C_{1-6}alkyl-O-
-CO-C_{1-6}alkyl-S-
-CO-C_{1-6}alkyl-O-C_{1-6}alkyl-
-C_{1-6}alkyl-
-C_{1-6}alkyl-O
-C_{1-6}alkyl-SO_2-
-C_{1-6}alkyl-NH-CO
-C_{1-3}alkyl-C_{3-6}cycloalkyl-C_{1-3}alkyl-O-

wherein each alkyl is straight chain or branched and may be optionally substituted by one or more substituents independently selected from phenyl or -OH

R^3 is selected from:

a C_{6-12} mono or bicyclic aryl group, (each of which may be optionally substituted one or more times by substituents independently selected from halo, C_{1-6}alkyl, C_{1-6} haloalkyl, C_{1-6}alkoxy, $NHCOC_{1-3}$alkyl, -O-phenyl , -CH_2-phenyl, phenyl (optionally substituted by C_{1-3}alkyl), OH, NH_2, $CONH_2$, CN, -$NHCOC_{1-3}$alkylNH_2, –$NHCOC_{1-3}$alkyl, $NHCOOC_{1-3}$alkyl, -$NHSO_2C_{1-3}$alkyl, -SO_2C_{1-3}alkyl or

-$NHCOC_{1-3}$alkyl-$NHCOC_{1-4}$ alkyl 

).

a 5-12 membered mono or bicyclic heteroaryl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, oxo),

a 5 or 6 membered heterocyclic group containing one or more heteromoieties independently selected from N, S, SO, SO_2 or O and optionally fused to a phenyl group (optionally substituted by one or more substituents independently selected from phenyl, CH_2phenyl, C_{1-3}alkyl)

or a 5-7 membered cycloalkyl (including bridged cycloalkyl) and optionally fused to a phenyl group (and optionally substituted by one or more substituents independently selected from OH, phenyl, CH_2 phenyl),

R^4 is selected from

C_{1-6} straight chain or branched alkyl (optionally substituted by NH_2),

COC_{1-6} straight chain or branched alkyl;

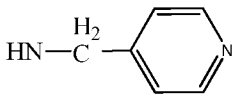
or a pharmaceutically acceptable salt thereof

with the proviso X is not

-NHCO-tert butyl

-NHCO-isobutyl

-OCH_2phenyl



-NHphenyl, or

-NHcyclohexyl

or a pharmaceutically acceptable salt thereof

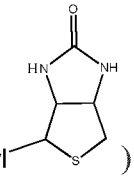
3. A compound or salt according to claim 2 wherein X is R^1-R^4

4. A compound or salt according to claim 3 wherein R^1 is NH

5. A compound or salt according to claim 2 wherein X is $(R^1)_{0-1}$-$(R^2)_{0-1}$-R^3

6. A compound or salt according to claim 5 wherein X is R^1-R^2-R^3

7. A compound or salt according to claim 6 wherein R^1 is NH

8. A compound or salt according to claims 5-6 wherein R^3 is napthyl or phenyl wherein the phenyl is optionally substituted one or more times with substituents independently selected from halo, -C_{1-6}alkyl, -C_{1-6}haloalkyl, -C_{1-6}alkoxy, -$NHCOC_{1-3}$alkyl, -O-phenyl,-CH_2-phenyl, -phenyl (optionally substituted by -C_{1-3}alkyl), -OH, -NH_2, -$CONH_2$, -CN, -$NHCOC_{1-3}$alkylNH_2 or –$NHCOC_{1-3}$alkyl$NHCOOC_{1-3}$alkyl, -$NHSO_2C_{1-3}$alkyl, -SO_2C_{1-3} alkyl or -C_{1-6} alkyl$NHCOC_{1-4}$alkyl )

9. A compound or salt according to claims 5-8 wherein R^2 is

-CO-
-$COCH_2$-
-$COCH_2CH_2$-
-$COCH_2CH_2$ CH_2-
-$COCH(CH_3)CH_2CH_2$-
-$COCH(CH_3)$-
- CH_2-
- CH_2CH_2-
- CH_2CH_2 CH_2-
- CH_2CH_2 CH_2-CH_2-
- $COCH_2O$-
-$COCH_2CH_2O$-
-$COCH_2CH_2S$-
-$C(CH_3)_2$-
-$CH(CH_2CH_2CH_3)$-
-$CH(CH_3)$-
-$CH_2CH(CH_3)$-
-$CH(CH_3)CH_2$-
-CH_2CH_2 $CH_2CH(CH_3)$-
-$CH_2CH(OH)CH_2O$-
-$CH_2CH_2CH_2O$-
-$COCH_2OCH_2$-
-CH_2CH_2NHCO-
-$CH_2CH_2CH_2SO_2$
-CH_2-cyclopropyl-CH_2O-
-CH_2-cyclohexyl-
-CH_2(phenyl)-
-CH_2CH(phenyl)CH_2 -CH_2-

10. A compound according to claims 2 selected from

3-{[(4-chlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid;

3-{[(4-methylphenyl)acetyl]amino}-4-pyridinecarboxylic acid;

3-[(3-phenylpropanoyl)amino]-4-pyridinecarboxylic acid;

3-[(phenylcarbonyl)amino]-4-pyridinecarboxylic acid;
3-[(2,2-dimethylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-{[(phenyloxy)acetyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-methylphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(2-naphthalenylacetyl)amino]-4-pyridinecarboxylic acid;
3-{[4-(2-naphthalenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-bromophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(3,4-dichlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[(3,4-dichlorophenyl)acetyl]amino}-4-pyridinecarboxylic acid;
3-({4-[3-(acetylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[4-(4-pyridinyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(2-methyl-4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(phenyloxy)propanoyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(phenylthio)propanoyl]amino}-4-pyridinecarboxylic acid;
3-({[3,4-bis(methyloxy)phenyl]acetyl}amino)-4-pyridinecarboxylic acid;
3-[(3,4-dihydro-2(1*H*)-isoquinolinylacetyl)amino]-4-pyridinecarboxylic acid;
3-[(1,3-dihydro-2*H*-isoindol-2-ylacetyl)amino]-4-pyridinecarboxylic acid;
3-[(2-phenylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[3-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-[(2-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(2-phenylpropyl)amino]-4-pyridinecarboxylic acid;
3-[(phenylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-2-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(4-phenylbutyl)oxy]-4-pyridinecarboxylic acid;
3-{[3-(2-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(trifluoromethyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(7-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(5-quinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(4-biphenylyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(3-aminophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-(3-phenylpropyl)-4-pyridinecarboxylic acid;, formate salt
3-({3-[(2-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(3-phenylpropyl)amino]-4-pyridinecarboxylic acid;
3-{[4-(3-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(4-hydroxyphenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-[(phenylacetyl)amino]-4-pyridinecarboxylic acid;
3-(hexanoylamino)-4-pyridinecarboxylic acid;

3-({[(phenylmethyl)oxy]acetyl}amino)-4-pyridinecarboxylic acid;
3-[(2-methylpropanoyl)amino]-4-pyridinecarboxylic acid;
3-[(3,3-dimethylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-[(5-phenylpentanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[4-(methyloxy)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[4-(4-chlorophenyl)butanoyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylbutyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-4-phenylbutyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(4-chlorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(2-aminoethyl)amino]-4-pyridinecarboxylic acid;
3-({2-[(phenylcarbonyl)amino]ethyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(2-naphthalenyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(4-bromophenyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-{[2-hydroxy-3-(phenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylpentyl)amino]-4-pyridinecarboxylic acid;
3-[({1-[(phenyloxy)methyl]cyclopropyl}methyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(phenylsulfonyl)propyl]amino}-4-pyridinecarboxylic acid;
3-{[(1-phenyl-3-pyrrolidinyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(diphenylmethyl)amino]-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-3-pyrrolidinyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[methyl(phenylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[2-(4-biphenylyl)ethyl]amino}-4-pyridinecarboxylic acid;
3-[(2,4-diphenylbutyl)amino]-4-pyridinecarboxylic acid;
3-{[(1-phenyl-1*H*-pyrazol-4-yl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[1-(phenylmethyl)-1*H*-pyrazol-4-yl]amino}-4-pyridinecarboxylic acid;
3-({3-[(2-oxo-1,2,3,4-tetrahydro-6-quinolinyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[1-(phenylmethyl)-1*H*-1,2,4-triazol-3-yl]amino}-4-pyridinecarboxylic acid;, formate salt.
3-[(1*S*,4*R*)-bicyclo[2.2.1]hept-2-ylamino]-4-pyridinecarboxylic acid;
3-[(tetrahydro-2*H*-pyran-2-ylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(4-pyridinylmethyl)amino]-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-1*H*-pyrazol-4-yl]methyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-(2,3-dihydro-1*H*-inden-1-ylamino)-4-pyridinecarboxylic acid;
3-[(2-pyridinylmethyl)amino]-4-pyridinecarboxylic acid;
3-(3-biphenylylamino)-4-pyridinecarboxylic acid;
3-{[3-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;

3-{[(3-cyanophenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-({[2-(acetylamino)phenyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[(cyclohexylmethyl)amino]-4-pyridinecarboxylic acid;
3-({4-[4-(β-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-[(4-{3-[(N-{[(1,1-dimethylethyl)oxy]carbonyl}-b-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid;
3-({4-[3-(b-alanylamino)phenyl]butanoyl}amino)-4-pyridinecarboxylic acid;
3-{[(1*S*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl]amino}-4-pyridinecarboxylic acid;
3-[(3-biphenylylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)amino]-4-pyridinecarboxylic acid;
3-{[(1-phenylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[4-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-(2-pyridinylamino)-4-pyridinecarboxylic acid;
3-{[(2'-methyl-2-biphenylyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(phenylmethyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-[(4-phenylcyclohexyl)amino]-4-pyridinecarboxylic acid;
3-(2-biphenylylamino)-4-pyridinecarboxylic acid;
3-{[4-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-{[2-(aminocarbonyl)phenyl]amino}-4-pyridinecarboxylic acid;
3-[(2,2,6,6-tetramethyl-4-piperidinyl)amino]-4-pyridinecarboxylic acid;
3-(1,3-dihydro-2*H*-isoindol-2-yl)-4-pyridinecarboxylic acid;
3-(4-phenyl-1-piperazinyl)-4-pyridinecarboxylic acid;
3-(1,2,3,4-tetrahydro-1-naphthalenylamino)-4-pyridinecarboxylic acid;
3-({[1-(phenylmethyl)-3-piperidinyl]methyl}amino)-4-pyridinecarboxylic acid;
3-[(4-biphenylylmethyl)amino]-4-pyridinecarboxylic acid;
3-(2,3-dihydro-1*H*-inden-2-ylamino)-4-pyridinecarboxylic acid;
3-[(1-cyclohexylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid;
3 3-[(3-{[3-(1-piperazinyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(6-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(3-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(methyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-fluorophenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-[(3-{[3-(phenyloxy)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-[(cyclopropylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(3-thienylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(3-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(1*H*-imidazol-4-ylmethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methylethyl)amino]-4-pyridinecarboxylic acid;

3-{[(1*R*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid;
3-[(1*H*-pyrazol-5-ylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(1-methylcyclohexyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(5-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(2-furanylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(1*S*)-1-(2-methylphenyl)butyl]amino}-4-pyridinecarboxylic acid;
3-(cyclobutylamino)-4-pyridinecarboxylic acid;
3-[(2-cyclopentyl-1-methylethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,4-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(2-thienylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,3-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-[(cyclopentylmethyl)amino]-4-pyridinecarboxylic acid;
3-{[(2,5-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(3-pentyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(4-methyl-2-thienyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[(2,6-dimethylphenyl)methyl]amino}-4-pyridinecarboxylic acid;
3-{[3-({3-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[3-(methylsulfonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-({3-[(3-methylphenyl)oxy]propyl}amino)-4-pyridinecarboxylic acid;
3-{[3-(2-oxo-1(2*H*)-pyridinyl)propyl]amino}-4-pyridinecarboxylic acid;
3-[(4-cyclohexylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-{[3-(2-pyridinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-{[4-(aminocarbonyl)phenyl]oxy}propyl)amino]-4-pyridinecarboxylic acid;
3-{[3-({4-[(methylsulfonyl)amino]phenyl}oxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(8-isoquinolinyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-{[3-(1-naphthalenyloxy)propyl]amino}-4-pyridinecarboxylic acid;
3-[(3-aminopropyl)amino]-4-pyridinecarboxylic acid;
3-[(cyclobutylmethyl)amino]-4-pyridinecarboxylic acid;
3-(propylamino)-4-pyridinecarboxylic acid;
3-[methyl(4-phenylbutanoyl)amino]-4-pyridinecarboxylic acid;
3-({2-[2-(methyloxy)phenyl]ethyl}amino)-4-pyridinecarboxylic acid;
3-[(2-methylpropyl)amino]-4-pyridinecarboxylic acid;
3-(methylamino)-4-pyridinecarboxylic acid;
3-(butylamino)-4-pyridinecarboxylic acid;
3-[(2-cyclohexylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-methyl-1-phenylethyl)amino]-4-pyridinecarboxylic acid;
3-(cyclopentylamino)-4-pyridinecarboxylic acid;
3-(cyclohexylamino)-4-pyridinecarboxylic acid;

3-[(2-cyclopentylethyl)amino]-4-pyridinecarboxylic acid;
3-[(2-cyclohexyl-1,1-dimethylethyl)amino]-4-pyridinecarboxylic acid;
3-[(1-cyclohexyl-1-methylethyl)amino]-4-pyridinecarboxylic acid;
3-[(4-{3-[(N-{5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl]pentanoyl}-b-alanyl)amino]phenyl}butanoyl)amino]-4-pyridinecarboxylic acid;
3-{[(1*R*,2*S*)-1-hydroxy-2,3-dihydro-1*H*-inden-2-yl]amino}-4-pyridinecarboxylic acid;
3-[(1-cyclohexylcyclopropyl)amino]-4-pyridinecarboxylic acid;
3-({2-[4-(2-thienyl)phenyl]ethyl}amino)-4-pyridinecarboxylic acid;
3-{[(2,4-difluorophenyl)carbonyl]amino}-4-pyridinecarboxylic acid;

Or a pharmaceutically acceptable salt thereof.

11. A compound or salt according to claims 2-10 for use in therapy

12. A compound or salt according to claims 2-10 for use or a pharmaceutically acceptable salt thereof for use in changing the epigenetic status of cells, treating cancer inflammation or autoimmune diseases

13. A pharmaceutical composition comprising a compound or salt according to claims 2-10 or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable diluent or carrier.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/055730

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D213/79 C07D401/04 C07D401/12 C07D405/12 C07D409/12
C07D497/04 A61K31/44 A61K31/4412 A61P29/00 A61P35/00
A61P37/00

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)
C07D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/043866 A2 (ISIS INNOVATION [GB]; SCHOFIELD CHRISTOPHER JOSEPH [GB]; MCDONOUGH MIC) 22 April 2010 (2010-04-22) see claim 1 formula (III)	1-13
A	ROSE N. R. ET AL: "Inhibitor scaffolds for 2-oxoglutarate-dependent histone lysine demethylase", JOURNAL OF MEDICINAL CHEMISTRY, vol. 51, 23 October 2008 (2008-10-23), pages 7053-7056, XP002679249, the whole document	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 2012	16/07/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bérillon, Laurent

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HAMADA S.: "Design, synthesys, enzyme-inhibitory activity, and effect on human cancer cells of a novel series of Jumonji domain-containing protein 2 histone demethylase inhibitors", JOURNAL OF MEDICINAL CHEMISTRY, vol. 53, 14 July 2010 (2010-07-14), pages 5629-5638, XP002679250, the whole document	1-13
X	STAUFFER K. J.: "9-Hydroxyazafluorenes and their use in thrombin inhibitors", JOURNAL OF MEDICINAL CHEMISTRY, vol. 48, 24 February 2005 (2005-02-24), pages 2282-2293, XP002679251, see preparation of 19a,b on page 2290, compound in step 2	2
X	PAVIA M. R.: "3-Phenoxypyridine 1-oxides as anticonvulsant agents", JOURNAL OF MEDICINAL CHEMISTRY, vol. 31, 1988, pages 841-847, XP002679252, see scheme VI, page 843, starting material	2
X	CALE A. D. ET AL.: "Benzo- and pyrido-1,4-oxazepin-5-ones and -thiones: synthesis and structure-activity relationships of a new series of H1 antihistamines", JOURNAL OF MEDICINAL CHEMISTRY, vol. 32, 1989, pages 2178-2199, XP002679253, see method D on page 2192, intermediate compound	2
X	VILLANI F. J. ET AL.: "Derivatives of 10,11-dihydro-5-H-dibenzo-[a,d]cyclohepten e and related compounds. III Azaketones (1,2)", JOURNAL OF HETEROCYCLIC CHEMISTRY, vol. 8, 1971, pages 73-78, XP002679254, see compound 9b on page 74	2
X	WO 2007/068637 A1 (NERVIANO MEDICAL SCIENCES SRL [IT]; BANDIERA TIZIANO [IT]; LOMBARDI BO) 21 June 2007 (2007-06-21) see page 103, step 2, title compound	2

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/055730

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010043866 A2	22-04-2010	NONE	
WO 2007068637 A1	21-06-2007	AU 2006326063 A1	21-06-2007
		CA 2631907 A1	21-06-2007
		EP 1963326 A1	03-09-2008
		JP 2009523707 A	25-06-2009
		US 2009163503 A1	25-06-2009
		US 2011288088 A1	24-11-2011
		WO 2007068637 A1	21-06-2007