Title: WATHRANILIC ACID DERIVATIVES AND THEIR USE IN TREATMENT OF DISEASES OF LIPID METABOLISM, IN PARTICULAR DYSLIPIDAEMIA

Abstract: Therapeutically active anthranilic acid derivatives of Formula (I) wherein R1, R2, W, Y and Z are as defined in the specification, processes for the preparation of said derivatives, pharmaceutical formulations containing the active compounds and the use of the compounds in therapy, particularly in the treatment of diseases in which under-activation of the HMT74A receptor contributes to the disease or in which activation of the receptor will be beneficial, are disclosed.
ANTHRANILIC ACID DERIVATIVES AND THEIR USE IN TREATMENT OF DISEASES OF LIPID METABOLISM, IN PARTICULAR DYSLIPIDAEINA

The present invention relates to therapeutically active compounds which are anthranilic acid derivatives, processes for the manufacture of said derivatives, pharmaceutical formulations containing the active compounds and the use of the compounds in therapy, particularly in the treatment of diseases in which under-activation of the HM74A receptor contributes to the disease or in which activation of the receptor will be beneficial.

Dyslipidaemia is a general term used to describe individuals with aberrant lipoprotein profiles. Clinically, the main classes of compounds used for the treatment of patients with dyslipidaemia, and therefore at risk of cardiovascular disease are the statins, fibrates, bile-acid binding resins and nicotinic acid. Nicotinic acid (Niacin, a B vitamin) has been used clinically for over 40 years in patients with various forms of dyslipidaemia. The primary mode of action of nicotinic acid is via inhibition of hormone-sensitive triglyceride lipase (HSL), which results in a lowering of plasma non-esterified fatty acids (NEFA) which in turn alters hepatic fat metabolism to reduce the output of LDL and VLDL (low and very low density lipoprotein). Reduced VLDL levels are thought to lower cholesterol ester transfer protein (CETP) activity to result in increased HDL (high density lipoprotein) levels which may be the cause of the observed cardiovascular benefits. Thus, nicotinic acid produces a very desirable alteration in lipoprotein profiles; reducing levels of VLDL and LDL whilst increasing HDL. Nicotinic acid has also been demonstrated to have disease modifying benefits, reducing the progression and increasing the regression of atherosclerotic lesions and reducing the number of cardiovascular events in several trials.

The observed inhibition of HSL by nicotinic acid treatment is mediated by a decrease in cellular cyclic adenosine monophosphate (cAMP) caused by the G-protein-mediated inhibition of adenylyl cyclase. Recently, the G-protein coupled receptors HM74 and HM74A have been identified as receptors for nicotinic acid (PCT patent application WO02/84298; Wise et. al. J Biol Chem. 2003 278 (11) 9869-9874). The DNA sequence of human HM74A may be found in Genbank; accession number AY148884. Two other papers support this discovery, (Tunaru et. al. Nature Medicine 2003 (3) 352-255 and Soga et. al. Biochem Biophys Res Commun. 2003 303 (1) 364-369), however the nomenclature differs slightly. In the Tunaru paper what they term human HM74 is in fact HM74A and in the Soga paper HM74b is identical to HM74A. Cells transfected to express HM74A and/or HM74 gain the ability to elicit G, G-protein mediated responses following exposure to nicotinic acid. In mice lacking the homologue of HM74A (m-PUMA-G) nicotinic acid fails to reduce plasma NEFA levels.
We now present a group of anthranilic acid derivatives which are selective agonists of the nicotinic acid receptor HM74A and are thus of benefit in the treatment, prophylaxis and suppression of diseases in which under-activation of this receptor either contributes to the disease or in which activation of the receptor will be beneficial.

Summary of the Invention

The present invention provides therapeutically active anthranilic acid derivatives, in particular carbamate and urea derivatives, and the use of these derivatives in therapy, particularly in the treatment of diseases in which under-activation of the HM74A receptor contributes to the disease or in which activation of the receptor will be beneficial, in particular diseases of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. The compounds may also be of use in the treatment of inflammatory diseases or conditions, as set out further below.

Intermediates, formulations, methods and processes described herein form further aspects of the invention.

Detailed Description of the Invention

According to one aspect of this invention, we provide a compound of Formula (I)

\[
R^1 CO_2H \quad N \quad W \quad [Y]_m \quad Z \quad R^2
\]

or a salt, solvate or physiologically functional derivative thereof, wherein:

\(R^1\) represents hydrogen, halogen or \(C_1\text{-}C_6\text{alkyl}\);

\(R^2\) represents a 5, 6, 9 or 10-member saturated, partially saturated or unsaturated ring system optionally including from 1 to 3 heteroatoms independently selected from S, O and N;
W represents a linker selected from: \(-NR^3R^1-, -NR^2(CH_2)_n-, -NR^3SO_2-, -O-(CH_2)_n-\) and
\[\text{N} \quad \text{X} \quad (CH_2)_p \quad \text{N}\]

Y represents a 5 or 6-member aryl or heteroaryl ring;

Z represents \(-(CH_2)_n-, -(CH_2)_nO-, -O-(CH_2)_n-, -(CH_2)_nO-CH_2-\) or a bond;

X represents CH or N

n represents an integer selected from 1, 2, and 3;

m represents an integer selected from 0 and 1;

p represents an integer selected from 0, 1 and 2; and

R^3 represents hydrogen or C_1-C_4 alkyl.

The compounds are of use in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial, in particular diseases of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity.

In certain embodiments, R^1 represents hydrogen, fluorine or methyl (e.g. hydrogen).

In certain particular embodiments, W represents \(-O-(CH_2)_n-\) or
\[\text{N} \quad \text{X} \quad (CH_2)_p \quad \text{N}\]
In particular embodiments of the invention, n represents 1.

$R^3$ represents hydrogen or methyl in certain embodiments of the invention.

$R^2$ may represent an aryl, heteroaryl, biaryl, hetero-biaryl, fused aryl-cycloalkyl, fused heteroaryl-cycloalkyl, fused aryl-heterocycle or fused heteroaryl-heterocyclic ring system, as herein defined. In certain embodiments in which $R^2$ includes heteroatoms, 1 to 3 heteroatoms are present. The $R^2$ ring system may be joined to the Z linker unit via either a ring carbon atom or via a heteroatom, where present.

In certain compounds of the present invention in which the $R^2$ unit is a 10-member ring system, this is may be naphthyl or may have either 1 or 2 heteroatoms. Where 2 heteroatoms are present, particular embodiments will have both in the same ring of the fused system. In particular embodiments, the heteroatoms in a 10-member ring system are nitrogen atoms. In certain embodiments, a 10-member $R^2$ group is selected from the group consisting of:

![Chemical structures](image)

Where the $R^2$ unit is a 10-member ring system, this may be unsubstituted. In certain embodiments in which $R^2$ is a substituted 10-member ring system, the substituents are selected from $C_1$-$C_2$alkyl, (e.g. methyl), -C(O)Me, -O and $C_1$-$C_3$alkoxy (e.g. methoxy).

Where the $R^2$ unit is a 9-member ring system, this may be fused aryl-cycloalkyl, for example:

![Chemical structure](image)
optionally including up to 3 heteroatoms selected from S, O or N, or may be a 9-member fused aryl or heteroaryl system, optionally including up to 3 heteroatoms selected from S, O or N. In particular embodiments in which the R² unit is a 9-member ring system containing heteroatoms, these may be situated in the 5-member ring of the fused system. In particular embodiments where more than one heteroatom is present, they are both the same such as, for example, a benzimidazole derivative, although heterogeneous heteroaryl systems are also included. In certain embodiments, a 9-member R² group is selected from the group consisting of:

\[
\text{\begin{align*}
\text{NR}^4 & ; \\
\text{NR}^4 & ; \\
\text{NR}^4 & ; \\
\text{NR}^4 & ; \\
\text{NR}^4 & ; \\
\text{NR}^4 & ; \\
\end{align*}}
\]

wherein R⁴ represents hydrogen, methyl, CO₂H or CO₂Me.

Where the R² unit is a 9-member ring system, including those depicted above, this may be unsubstituted. In certain embodiments in which R² is a substituted 9-member ring system, the one or more substituents are selected from C₁-C₃alkyl (e.g. methyl); -C(O)Me; =O; C₁-C₃alkoxy (e.g. methoxy); CO₂H; and CO₂Me.

Where R² represents a 5 or 6 member heteroaryl ring, R² may be selected from thiophenyl, pyridinyl, pyrimidinyl, pyridazinyl, pyrazinyl, pyrazolyl, imidazolyl, oxazolyl and isoxazolyl. In certain other embodiments R² represents 5 or 6 member aryl for example phenyl. Where the R² unit represents a 5 or 6 member aryl or heteroaryl ring this may be unsubstituted. In certain embodiments in which the R² ring is substituted the one or more substituents are selected from halogen (e.g. fluorine), C₁-C₃alkyl (e.g. methyl), C₁-C₃alkoxy (e.g. methoxy), perfluoroC₁-C₃alkyl (e.g. trifluoromethyl), -NH-SO₂R³, CO₂H; and CO₂Me.

In certain embodiments, in which R² represents singly substituted phenyl, the substituent is at the meta or para position, for example para. In certain embodiments
in which $R^2$ represents doubly substituted phenyl, the substituents are at the para and meta, or at both meta positions.

In certain embodiments, $R^2$ is selected from the group consisting of:
In certain embodiments in which Y is aryl, for example C6 aryl (e.g. phenyl), Y is linked through the 1 and 4, the 1 and 3 or the 1 and 2 positions. In certain embodiments in which Y is heteroaryl, for example a 5 member heteroaryl ring (e.g. 1, 2, 4 oxadiazolyl, 1,2,4-thiadiazolyl or 1, 3 thiazolyl), Y may be linked through the 3 and 5 or the 2 and 5 positions. Y may be unsubstituted or may carry one or more substitutions selected from C1-3alkyl.

It is to be understood that the present invention includes any combination of particular embodiments and covers all combinations of particular substituents described hereinabove.

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

As used herein, the terms "halogen" or "halo" refer to fluorine, chlorine, bromine and iodine.

As used herein, the term "alkyl" (when used as a group or as part of a group) refers to a straight or branched hydrocarbon chain containing the specified number of carbon atoms. For example, C1-C3alkyl means a straight or branched hydrocarbon chain containing at least 1 and at most 3 carbon atoms. Examples of alkyl as used herein include, but are not limited to; methyl (Me), ethyl (Et), n-propyl, i-propyl.

As used herein, the term "alkoxy" (when used as a group or as part of a group) refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Examples of alkoxy as used herein include, but are not limited to; methoxy, ethoxy, n-propoxy, i-propoxy and the like.

As used herein, the term "biaryl" (when used as a group or as part of a group) refers to a group containing two aromatic rings which have two atoms in common. Examples of fused biaryl as used herein include, but are not limited to naphthyl and indyl. Said biaryl groups may be optionally substituted – where not otherwise specified, the substitutions may be one or more groups selected from C1-C3alkyl, C1-C3alkoxy, –C(O)Me, CO2H, CO2Me and =O.

As used herein, the term "hetero-biaryl" (when used as a group or as part of a group) refers to a biaryl group which contains one or more nitrogen, sulphur, or oxygen heteroatoms. Examples of hetero-biaryl as used herein include, but are not limited to;
quinoline, isoquinoline, quinoxaline, benzimidazole, indolizine, indole and benzothiophene groups. Said hetero-biaryl groups may be optionally substituted — where not otherwise specified, the substitutions may be one or more groups selected from \( \text{C}_{1-3}\text{alkyl}, \text{C}_{1-3}\text{alkoxy}, -\text{C(O)Me}, \text{CO}_2\text{H}, \text{CO}_2\text{Me} \) and \( =\text{O} \).

As used herein, the term "fused aryl-cycloalkyl" (when used as a group or as part of a group) refers to a group containing one aromatic ring and one alicyclic ring which have two atoms in common. Examples of fused aryl-cycloalkyl as used herein include, but are not limited to;

![Diagram of a fused aryl-cycloalkyl group]

Said fused aryl-cycloalkyl groups may be optionally substituted — where not otherwise specified, the substitutions may be one or more groups selected from \( \text{C}_{1-3}\text{alkyl}, \text{C}_{1-3}\text{alkoxy}, -\text{C(O)Me}, \text{CO}_2\text{H}, \text{CO}_2\text{Me} \) and \( =\text{O} \).

As used herein, the term "fused heteroaryl-cycloalkyl" (when used as a group or as part of a group) refers to a fused aryl-cycloalkyl group, the aryl ring of which contains one or more nitrogen, sulphur, or oxygen heteroatoms. Said fused heteroaryl-cycloalkyl groups may be optionally substituted — where not otherwise specified, the substitutions may be one or more groups selected from \( \text{C}_{1-3}\text{alkyl}, \text{C}_{1-3}\text{alkoxy}, -\text{C(O)Me}, \text{CO}_2\text{H}, \text{CO}_2\text{Me} \) and \( =\text{O} \).

As used herein, the term "fused aryl-heterocycle" (when used as a group or as part of a group) refers to a fused aryl-cycloalkyl group, the alicyclic ring of which contains one or more nitrogen, sulphur, or oxygen heteroatoms. Examples of fused aryl-heterocycle as used herein include, but are not limited to; benzodioxolane, indoline. Said fused aryl-heterocycle groups may be optionally substituted — where not otherwise specified, the substitutions may be one or more groups selected from \( \text{C}_{1-3}\text{alkyl}, \text{C}_{1-3}\text{alkoxy}, -\text{C(O)Me}, \text{CO}_2\text{H}, \text{CO}_2\text{Me} \) and \( =\text{O} \).

As used herein, the term "fused heteroaryl-heterocyclic" (when used as a group or as part of a group) refers to a fused aryl-cycloalkyl group, which contains one or more nitrogen, sulphur, or oxygen heteroatoms either present as an atom shared between the two rings, or one or more heteroatoms being present in each ring. Said fused heteroaryl-heterocyclic groups may be optionally substituted — where not otherwise specified, the substitutions may be one or more groups selected from \( \text{C}_{1-3}\text{alkyl}, \text{C}_{1-3}\text{alkoxy}, -\text{C(O)Me}, \text{CO}_2\text{H}, \text{CO}_2\text{Me} \) and \( =\text{O} \).
As used herein, the term "physiologically functional derivative" refers to any pharmaceutically acceptable derivative of a compound of the present invention, for example an ester or an amide thereof, and includes any pharmaceutically acceptable salt, ester, or salt of such ester of a compound of formula (I) which, upon administration to a mammal, such as a human, is capable of providing (directly or indirectly) a compound of formula (I) or an active metabolite or residue thereof. It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide physiologically functional derivatives thereof at any of the functional groups in the compounds, and that the compounds of formula (I) may be so modified at more than one position.

As used herein, the term "pharmaceutically acceptable" used in relation to an ingredient (active ingredient or excipient) which may be included in a pharmaceutical formulation for administration to a patient, refers to that ingredient being acceptable in the sense of being compatible with any other ingredients present in the pharmaceutical formulation and not being deleterious to the recipient thereof.

As used herein, the term "solvent" refers to a complex of variable stoichiometry formed by a solute (in this invention, a compound of formula (I), a salt thereof or a physiologically functional derivative thereof) and a solvent. Such solvents for the purposes of the present invention may not interfere with the biological activity of the solute. Examples of suitable solvents include water, methanol, ethanol and acetic acid. Preferably the solvent used is a pharmaceutically acceptable solvent. Examples of suitable pharmaceutically acceptable solvents include water, ethanol and acetic acid. Most preferably the solvent used is water, in which case the solvate may be referred to as a hydrate of the solute in question.

It will be appreciated that, for pharmaceutical use, the "salt or solvate" referred to above will be a pharmaceutically acceptable salt or solvate. However, other salts or solvates may find use, for example, in the preparation of a compound of formula (I) or in the preparation of a pharmaceutically acceptable salt or solvate thereof.

Pharmaceutically acceptable salts include those described by Berge, Bighley and Monkhouse, J. Pharm. Sci., 1977, 66, 1-19. Suitable pharmaceutically acceptable salts include acid addition salts formed from the addition of inorganic acids or organic acids, preferably inorganic acids. Examples of suitable acid addition salts include hydrochlorides, hydrobromides, sulphates and acetates. Further representative examples of pharmaceutically acceptable salts include those formed from maleic, fumaric, benzoic, ascorbic, pamoic, succinic, bismethylenesaliclyc, methanesulfonic, ethanedisulfonic, propionic, tartaric, salicylic, citric, gluconic, aspartic, stearic, palmitic, itaconic, glycolic, p-amino benzoic, glutamic, benzenesulfonic, cyclohexylsulfamic, phosphoric and nitric acids. Suitable pharmaceutically acceptable salts also include
alkali metal salts formed from the addition of alkali metal bases such as alkali metal hydroxides. An example of a suitable alkali metal salt is a sodium salt.

Compounds of the present invention are of potential therapeutic benefit in the treatment and amelioration of the symptoms of many diseases of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia. As such, the compounds may also find favour as therapeutics for coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity. The use of a compound of Formula (I) in the treatment of one or more of these diseases is a further aspect of the present invention.

Furthermore, it is also believed that the HM74 and HM74A receptors are involved in 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 with regards to disease typically is referred to as chronic inflammation and can last up to a lifetime. Such chronic inflammation may manifest itself through disease symptoms. The aim of anti-inflammatory therapy is therefore to reduce this chronic inflammation and allow for the physiological process of healing and tissue repair to progress.

Thus, a further aspect of the present invention resides in the use of a compound of Formula (I) or a salt, solvate or physiologically functional derivative thereof as defined above in the treatment of inflammatory diseases or conditions of the joint, particularly arthritis (e.g. rheumatoid arthritis, osteoarthritis, prosthetic joint failure), or the gastrointestinal tract (e.g. ulcerative colitis, Crohn's disease, and other inflammatory bowel and gastrointestinal diseases, gastritis and mucosal inflammation resulting from infection, the enteropathy provoked by non-steroidal anti-inflammatory drugs), of the lung (e.g. adult respiratory distress syndrome, asthma, cystic fibrosis, or chronic obstructive pulmonary disease), of the heart (e.g. myocarditis), of nervous tissue (e.g. multiple sclerosis), of the pancreas, (e.g. inflammation associated with diabetes mellitus and complications thereof), of the kidney (e.g. glomerulonephritis), of the skin (e.g. dermatitis, psoriasis, eczema, urticaria, burn injury), of the eye (e.g. glaucoma) as well as of transplanted organs (e.g. rejection) and multi-organ diseases (e.g. systemic lupus erythematos, sepsis) and inflammatory sequelae of viral or bacterial infections and inflammatory conditions associated with atherosclerosis and following
hypoxic or ischaemic insults (with or without reperfusion), for example in the brain or in ischaemic heart disease.

In particular, the compounds of Formula (I) are useful in the treatment and prevention of inflammation, and cardiovascular diseases or conditions including atherosclerosis, arteriosclerosis, hypertriglycerideremia, and mixed dyslipidaemia.

Thus, there is also provided the use of a compound of Formula (I) or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof, in the manufacture of a medicament for the treatment of disorders of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglycerideraemia. The compounds are also provided for use in the treatment of coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity.

Nicotinic acid has a significant side effect profile, possibly because it is dosed at high level (gram quantities daily). The most common side effect is an intense cutaneous flushing. The compounds of the present invention preferably exhibit reduced side effects compared to nicotinic acid. HM74A has been identified as a high affinity receptor for nicotinic acid whilst HM74 is a lower affinity receptor. The compounds of the present invention are selective for HM74A by which is meant that they show greater affinity for HM74A than for HM74.

The potential for compounds of formula (I) to activate HM74A may be demonstrated, for example, using the following enzyme and in vitro whole cell assays:

**In-vitro testing**

For transient transfections, HEK293T cells (HEK293 cells stably expressing the SV40 large T-antigen) are maintained in DMEM containing 10% foetal calf serum and 2mM glutamine. Cells are seeded in 90mm culture dishes and grown to 60-80% confluence (18-24h) prior to transfection. Human HM74A (GenBank™ accession number AY148884) is subcloned in to a mammalian expression vector (pcDNA3; Invitrogen) and transfected using Lipofectamine reagent. For transfection, 9µg of DNA is mixed with 30µl Lipofectamine in 0.6ml of Opti-MEM (Life Technologies Inc.) and incubated at room temperature for 30min prior to the addition of 1.6ml of Opti-MEM. Cells are exposed to the Lipofectamine/DNA mixture for 5h and 6ml of 20% (v/v) foetal calf serum in DMEM is then added. Cells are harvested 48h after transfection. Pertussis
toxin treatment is carried out by supplementation into media at 50ng/ml for 16h. All transient transfection studies involve co-transfection of receptor together with the G\textsubscript{V}G protein, G\textsubscript{ot}\textalpha. 

For generation of stable cell lines the above method is used to transfect CHO-K1 cells seeded in six well dishes grown to 30% confluence. These cells are maintained in DMEM F-12 HAM media containing 10% foetal calf serum and 2mM glutamine. 48h post-transfection the media is supplemented with 400\mu g/ml Geneticin (G418, Gibco) for selection of antibiotic resistant cells. Clonal CHO-K1 cell lines stably expressing HM74A are confirmed by [\textsuperscript{35}S]-GTP\gamma S binding measurements, following the addition of nicotinic acid.

**P2 membrane preparation** - Plasma membrane-containing P2 particulate fractions are prepared from cell pastes frozen at –80°C after harvest. All procedures are carried out at 4°C. Cell pellets are resuspended in 1 ml of 10mM Tris-HCl and 0.1mM EDTA, pH 7.5 (buffer A) and by homogenisation for 20s with a Ultra Turrax followed by passage (5 times) through a 25-gauge needle. Cell lysates are centrifuged at 1,000g for 10 min in a microcentrifuge to pellet the nuclei and unbroken cells and P2 particulate fractions are recovered by microcentrifugation at 16,000g for 30min. P2 particulate fractions are resuspended in buffer A and stored at –80°C until required.

**[\textsuperscript{35}S]-GTP\gamma S binding** - Assays are performed at room temperature either in 96-well format as described previously (Wieland, T. and Jakobs, K.H. (1994) Methods Enzymol. 237, 3-13) or in an adapted protocol carried out in 384-well format.

96-well format: Briefly, membranes (10 \mu g per point) are diluted to 0.083 mg/ml in assay buffer (20 mM HEPES, 100 mM NaCl, 10 mM MgCl\textsubscript{2}, pH7.4) supplemented with saponin (10 mg/l) and pre-incubated with 10 \mu M GDP. Various concentrations of nicotinic acid or related molecules are added, followed by [\textsuperscript{35}S]-GTP\gamma S (1170 Ci/mmol, Amersham) at 0.3 nM (total vol. of 100 \mu l) and binding is allowed to proceed at room temperature for 30 min. Non-specific binding is determined by the inclusion of 0.6 mM GTP. Wheatgerm agglutinin SPA beads (Amersham) (0.5 mg) in 25\mu l assay buffer are added and the whole is incubated at room temperature for 30 min with agitation. Plates are centrifuged at 1500 g for 5 min and bound [\textsuperscript{35}S]-GTP\gamma S is determined by scintillation counting on a Wallac 1450 microbeta Trilux scintillation counter.

384-well format: Briefly, the dilution of standard or test compounds are prepared and added to a 384-well plate in a volume of 10\mu l. Membranes (HM74A or HM74) are diluted in assay buffer (20mM HEPES, 100mM NaCl, 10mM MgCl\textsubscript{2}, pH7.4) supplemented with saponin (60\mu g/ml), Leadseeker WGA beads (Amersham; 250\mu g/well) and 10\mu M GDP, so that the 20\mu l volume added to each well contains 5\mu g of membranes. [\textsuperscript{35}S]-GTP\gamma S (1170 Ci/mmol, Amersham) is diluted (1:1500) in assay
buffer and 20μl added to each well. Following the addition of the radioligand, the plates are sealed, pulse spun and incubated for 4 hours at room temperature. At the end of the incubation period the plates are read on a Leadseeker machine (VIEWLUX PLUS; Perkin-Elmer) to determine the levels of specific binding.

In-vivo testing

HM74A agonists are tested in male Sprague-Dawley rats (200-250 grammes) which have been fasted for at least 12 hours prior to the study. The compounds are dosed intravenously (5ml/kg) or by oral gavage (10ml/kg). Blood samples (0.3ml tail vein bleed) are taken pre-dose and at three times post-dose (times ranging from 15 minutes to 8 hours post-dose). Each blood sample is transferred to a heparin tube (Becton Dickinson Microtainer, PST LH) and centrifuged (10,000 g for 5 minutes) to produce a plasma sample. The plasma samples are assayed for levels of non-esterified fatty acids (NEFA) using a commercially available kit (Randox). Inhibition of plasma NEFA levels, relative to pre-dose levels, is used as a surrogate for HM74A agonist activity.

In order to determine whether compounds of the invention exhibit theflushing response associated with nicotinic acid, they are dosed to anaesthetised guinea-pigs. Nicotinic acid is used as positive control. Male Dunkin Hartley guinea pigs (300-800g) are fasted for 12 hours prior to being anaesthetised with a mixture of Ketamine hydrochloride (Vetalar, 40mg/kg i.m.), Xylazine (Rompun, 8mg/kg i.m.) and sodium pentobarbitone (Sagatal, 30mg/kg i.p.). Following anaesthesia a tracheostomy is performed and the animals are mechanically ventilated with room air (10-12mL/kg, 60 breaths/min). A jugular vein, and a carotid artery, are cannulated for intravenous administration of test compound and collection of blood respectively. An infra-red temperature probe (Extech Instruments) is placed 3-5mm from the tip of the left ear. Temperature measurements are recorded every minute from 5 minutes prior to test compound or nicotinic acid and up to 40 minutes post-administration of test compound or nicotinic acid. Data is automatically collected on a Psion computer before being transferred for data analysis within an Excel spreadsheet. Prior to, and at frequent time points after compound administration, blood samples (0.3ml) are taken via the carotid arterial cannula and transferred to Microtainer (BD) tubes containing lithium heparin. The samples are mixed thoroughly on a blood roller and then stored on ice prior to centrifugation at 1200g for 5 minutes.

Compounds according to Formula (I) have been synthesised (see synthetic examples below).

As indicated above, compounds of Formula (I) are useful in human or veterinary medicine, in particular as activators of HM74A, in the management of dyslipidaemia and hyperlipoproteinaemia.
Thus, there is provided as a further aspect of the present invention a compound of formula (I) or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof, for use in human or veterinary medicine, particularly in the treatment of disorders of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, hypertriglyceridaemia, coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity.

It will be appreciated that references herein to treatment extend to prophylaxis, prevention of recurrence and suppression of symptoms as well as the treatment of established conditions.

According to another aspect of the invention, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof, in the manufacture of a medicament for the treatment of disorders of lipid metabolism including dislipidaemia or hyperlipoproteinaemia. In particular, the use is provided of a compound of Formula (I) in the manufacture of a medicament for the treatment of diabetic dyslipidaemia, mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, as well as the cardiovascular indications associated with type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity.

It is to be understood that this aspect of the present invention includes, with respect to the use of compounds of Formula (I) in the manufacture of a medicament, any combination of particular embodiments and covers all combinations of particular substituents of compounds of Formula (I) described hereinabove.

Additionally, the present invention provides the use of a compound of formula (I) or a physiologically acceptable salt or solvate thereof, in the manufacture of a medicament for the treatment of inflammatory diseases or conditions of the joint, particularly arthritis (e.g. rheumatoid arthritis, osteoarthritis, prosthetic joint failure), or of the gastrointestinal tract (e.g. ulcerative colitis, Crohn's disease, and other inflammatory bowel and gastrointestinal diseases, gastritis and mucosal inflammation resulting from infection, the enteropathy provoked by non-steroidal anti-inflammatory drugs), of the lung (e.g. adult respiratory distress syndrome, asthma, cystic fibrosis, or chronic obstructive pulmonary disease), of the heart (e.g. myocarditis), of nervous tissue (e.g.
multiple sclerosis), of the pancreas, (e.g. inflammation associated with diabetes mellitus and complications thereof, of the kidney (e.g. glomerulonephritis), of the skin (e.g. dermatitis, psoriasis, eczema, urticaria, burn injury), of the eye (e.g. glaucoma) as well as of transplanted organs (e.g. rejection) and multi-organ diseases (e.g. systemic lupus erythematosus, sepsis) and inflammatory sequelae of viral or bacterial infections and inflammatory conditions associated with atherosclerosis and following hypoxic or ischaemic insults (with or without reperfusion), for example in the brain or in ischaemic heart disease.

In a further or alternative aspect there is provided a method for the treatment of a human or animal subject with a condition where under-activation of the HM74A receptor contributes to the condition or where activation of the receptor will be beneficial, which method comprises administering to said human or animal subject an effective amount of a compound of Formula (I) or a physiologically acceptable salt or solvate thereof.

Again, is to be understood that this aspect of the present invention includes, with respect to the use of compounds of Formula (I) in a method of treatment, any combination of particular embodiments and covers all combinations of particular substituents of compounds of Formula (I) described hereinabove.

More particularly, the present invention provides a method for the treatment of disorders of lipid metabolism including dislipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa, obesity, which method comprises administering to said human or animal subject an effective amount of a compound of Formula (I) or a physiologically acceptable salt or solvate thereof. As such, these compounds may also find favour in methods for the treatment of coronary artery disease, thrombosis, angina, chronic renal failure, peripheral vascular disease and stroke, which methods comprise administering to said human or animal subject an effective amount of a compound of Formula (I).

The amount of a HM74A modulator which is required to achieve the desired biological effect will, of course, depend on a number of factors, for example, the mode of administration and the precise clinical condition of the recipient. In general, the daily dose will be in the range of 0.1mg - 1g/kg, typically 0.1 - 100mg/kg. An intravenous dose may, for example, be in the range of 0.01mg to 0.1g/kg, typically 0.01mg to 10mg/kg, which may conveniently be administered as an infusion of from 0.1μg to 1mg, per minute. Infusion fluids suitable for this purpose may contain, for example, from 0.01μg to 0.1mg, per millilitre. Unit doses may contain, for example, from 0.01μg
to 1g of a HM74A modulator. Thus ampoules for injection may contain, for example, from 0.01μg to 0.1g and orally administrable unit dose formulations, such as tablets or capsules, may contain, for example, from 0.1mg to 1g. No toxicological effects are indicated/expected when a compound of the invention is administered in the above mentioned dosage range.

A compound of the present invention may be employed as the compound *per se* in the treatment of a disease where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial, but is preferably presented with an acceptable carrier in the form of a pharmaceutical formulation. The carrier must, of course, be acceptable in the sense of being compatible with the other ingredients of the formulation and must not be deleterious to the recipient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the HM74A modulator as a unit-dose formulation, for example, a tablet, which may contain from 0.05% to 95% by weight of the HM74A modulator.

The formulations include those suitable for oral, rectal, topical, buccal (e.g. sublingual) and parenteral (e.g. subcutaneous, intramuscular, intradermal or intravenous) administration.

There is also provided according to the invention a process for preparation of such a pharmaceutical composition which comprises mixing the ingredients.

Formulations suitable for oral administration may be presented in discrete units, such as capsules, cachets, lozenges or tablets, each containing a predetermined amount of a HM74A modulator; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. In general, the formulations are prepared by uniformly and intimately admixing the active HM74A modulator with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the product. For example, a tablet may be prepared by compressing or moulding a powder or granules of the HM74A modulator optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or granules optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). Moulded tablets may be made by moulding, in a suitable machine, the powdered compound moistened with an inert liquid diluent.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize- starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for
example, potato starch, croscarmellose sodium or sodium starch glycolate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan monooleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or preservatives, for example, methyl or propyl p-hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising a HM74A modulator in a flavoured base, usually sucrose and acacia or tragacanth, and pastilles comprising the HM74A modulator in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations of the present invention suitable for parenteral administration conveniently comprise sterile aqueous preparations of an HM74A modulator, preferably isotonic with the blood of the intended recipient. These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection. Such preparations may conveniently be prepared by admixing the HM74A modulator with water and rendering the resulting solution sterile and isotonic with the blood. Injectable compositions according to the invention will generally contain from 0.1 to 5% w/w of the HM74A modulator.

Thus, formulations of the present invention suitable for parenteral administration comprising a compound according to the invention may be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or toxicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.
Formulations suitable for rectal administration are preferably presented as unit-dose suppositories. These may be prepared by admixing a HM74A modulator with one or more conventional solid carriers, for example, cocoa butter or glycerides and then shaping the resulting mixture.

Formulations suitable for topical application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which may be used include vaseline, lanolin, polyethylene glycols, alcohols, and combinations of two or more thereof. The HM74A modulator is generally present at a concentration of from 0.1 to 15% w/w of the composition, for example, from 0.5 to 2%.

By topical administration as used herein, we include administration by insufflation and inhalation. Examples of various types of preparation for topical administration include ointments, creams, lotions, powders, pessaries, sprays, aerosols, capsules or cartridges for use in an inhaler or insufflator or drops (e.g. eye or nose drops).

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents and/or solvents. Such bases may thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil or a solvent such as a polyethylene glycol. Thickening agents which may be used include soft paraffin, aluminium stearate, cetostearyl alcohol, polyethylene glycols, microcrystalline wax and beeswax.

Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents or thickening agents.

Powders for external application may be formed with the aid of any suitable powder base, for example, talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents.

Spray compositions may be formulated, for example, as aqueous solutions or suspensions or as aerosols delivered from pressurised packs, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, 1,1,1,2,3,3,3-heptafluoropropane, 1,1,1,2- tetrafluorethane, carbon dioxide or other suitable gas.

Capsules and cartridges for use in an inhaler or insufflator, of for example gelatin, may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.
The pharmaceutical compositions according to the invention may also be used in combination with other therapeutic agents, for example in combination with other classes of dyslipidaemic drugs (e.g. statins, fibrates, bile-acid binding resins or nicotinic acid).

The compounds of the instant invention may be used in combination with one or more other therapeutic agents for example in combination with other classes of dyslipidaemic drugs e.g. 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) or fibrates or bile acid binding resins or nicotinic acid. The invention thus provides, in a further aspect, the use of such a combination in the treatment of diseases where under-activation of the HM74A receptor contributes to the disease or where activation of the receptor will be beneficial and the use of a compound of formula (I) or a pharmaceutically acceptable salt, solvate or physiologically functional derivative thereof in the manufacture of a medicament for the combination therapy of disorders of lipid metabolism including distlipidaemia or hyperlipoproteinaemia such as diabetic dyslipidaemia and mixed dyslipidaemia, heart failure, hypercholesterolaemia, cardiovascular disease including atherosclerosis, arteriosclerosis, and hypertriglyceridaemia, type II diabetes mellitus, type I diabetes, insulin resistance, hyperlipidaemia, anorexia nervosa or obesity.

When the compounds of the present invention are used in combination with other therapeutic agents, the compounds may be administered either sequentially or simultaneously by any convenient route.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above optimally together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

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

When in combination with a second therapeutic agent active against the same disease, the dose of each component may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.
The invention thus provides, in a further aspect, a combination comprising a compound of formula (I) or a physiologically acceptable salt or solvate thereof together with another therapeutically active agent.

The combination referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier thereof represent a further aspect of the invention.

The compounds of formula (I) and salts and solvates thereof may be prepared by the methodology described hereinafter, constituting a further aspect of this invention.

ABBREVIATIONS

THF  Tetrahydrofuran
TFA  Trifluoroacetic Acid
DMSO  Dimethylsulphoxide
HBTU  O-Benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
CDI  Carbonyl diimidazole
PyHOTs  Pyridinium tosylate

Method A

A process for preparing carbamate compounds of the present invention is set out in scheme (a):

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{O} \\
\text{R} & \quad \text{OH} & \quad \text{R}
\end{align*}
\]

wherein R represents \((Y)_n-Z-R^2\) as defined above.

Accordingly, a process according to the invention for preparing a compound of formula (I) comprises:

(i) reaction of acid-protected isocyanate with alcohol followed by base hydrolysis of the methyl ester;
(ii) where desired or necessary converting a resultant free acid or base compound of formula (I) into a physiologically acceptable salt form or vice versa or converting one salt form into another physiologically acceptable salt form.

Method B

A process for preparing urea derivatives of the present invention in which W represents piperazinyl is set out in scheme (b):

\[
\begin{align*}
\text{Scheme (b)}
\end{align*}
\]

wherein Ar represents R² as defined above.

Accordingly, a process according to the invention for preparing a compound of formula (I) comprises:

a) Reaction of protected piperazinyl amine group with an acid-protected isocyanate to form the urea

b) Removal of the amine protecting group using hydrogenation

c) Reductive amination of the amine and then base hydrolysis of the methyl ester.

d) where desired or necessary converting a resultant free acid or base compound of formula (I) into a physiologically acceptable salt form or vice versa or converting one salt form into another physiologically acceptable salt form.

Method C
A process for preparing urea derivatives of the present invention in which W represents piperazinyl or piperidinyl is set out in scheme (c):

```
\[ \text{Scheme (c)} \]
```

wherein Ar represents R² as defined above.

Accordingly, a process according to the invention for preparing a compound of formula (I) comprises:

(i) Reaction of an amine with an acid-protected isocyanate to form the urea
(ii) Hydrolysis of the methyl ester using base
(iii) where desired or necessary converting a resultant free acid or base compound of formula (I) into a physiologically acceptable salt form or vice versa or converting one salt form into another physiologically acceptable salt form.

Method C

A process for preparing urea derivatives of the present invention in which W represents piperazinyl, m = 0 and Z represents a bond is set out in scheme (d):
Accordingly, a process according to the invention for preparing a compound of formula (I) comprises:

a) Nucleophilic displacement of the chloride using an amine
b) Base hydrolysis of the methyl ester
c) where desired or necessary converting a resultant free acid or base compound of formula (I) into a physiologically acceptable salt form or vice versa or converting one salt form into another physiologically acceptable salt form.

The following non-limiting examples illustrate the present invention:

Synthetic Examples

A. Example compounds synthesised using Method A

Example 1: 2-{{[(2,3-dihydro-1,4-benzodioxin-6-ylmethyl)oxy]carbonyl}amino} benzoic acid

2,3-Dihydro-1,4-benzodioxin-6-ylmethanol (0.025g, 0.15mmol) was treated with a solution of methyl 2-isocyanatobenzoate (0.04g, 0.24mmol) and triethylamine
(0.035ml, 0.25mmol) in THF (1ml). After stirring at ambient temperature for 18 hours
the mixture was heated at 60°C for 3 hours then cooled. The mixture was
subsequently treated with a solution of lithium hydroxide (0.011g, 0.46mmol) in a
mixture of methanol (0.65ml) and water (0.65ml) then heated at 45°C for 3 hours.
The mixture was cooled, filtered and the filtrate acidified to about pH 4 using 2M
aqueous hydrochloric acid. The precipitated material was filtered off and from this the
product was obtained using reverse-phase HPLC. This gave the title compound as a
white solid (0.0047g, 10%). NMR; δH (400MHz, d6-DMSO) 4.23(s, 4H), 5.04(s, 2H),
6.84-6.94(m, 3H), 7.11(t, 1H, J=7.3Hz), 7.60(dt, 1H, J=1.5, 7.0Hz), 7.97(dd, 1H, J=1.5,
8.0Hz), 8.28(d, 1H, J=8.3Hz), 10.77(s, 1H), 13.71(br s, 1H); m/z 347[MNH₄⁺].

The following examples 2-8 were also prepared using the above procedure, method A:

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Example No.</th>
<th>Compound: R =</th>
<th>yield</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td><img src="image" alt="Br" /></td>
<td>4.1mg (6.9%)</td>
<td>397, 399 [MNH₄⁺]</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="N" /></td>
<td>3.4mg (7.0%)</td>
<td>323 [MH⁺]</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="O" /></td>
<td>0.62mg (1.2%)</td>
<td>m/z 340 [MH⁺]</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="H" /></td>
<td>18.4mg (39%)</td>
<td>314 [MH⁺] &amp; 331 [MNH₄⁺]</td>
</tr>
<tr>
<td>Example No:</td>
<td>Compound: R =</td>
<td>yield</td>
<td>m/z</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>-------------</td>
<td>------------</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Structure" /></td>
<td>6.3mg (11.3%)</td>
<td>369 [MH⁺];</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Structure" /></td>
<td>12.3mg (21.5%)</td>
<td>381 [MNH₄⁺]</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Structure" /></td>
<td>0.63mg (1.3%)</td>
<td>m/z 329 [MH⁺];</td>
</tr>
</tbody>
</table>

Analytical data:

**Example 2:** 2-[[3-bromo-4-(methyloxy)phenyl]methyl]oxy)carbonyl]amino]benzoic acid

NMR δ_H (400MHz, d⁶-DMSO) 3.84(s, 3H), 5.10(s, 2H), 7.10(t, 1H, J=7.6Hz), 7.13(d, 1H, J=8.6Hz), 7.44(dd, 1H, J=2.0, 8.6 Hz), 7.60(dt, 1H, J=1.5, 8.6Hz), 7.67(d, 1H, J=2.0Hz), 7.96(dd, 1H, J=1.5, 8.1Hz), 8.27(d, 1H, J=8.6Hz), 10.82(s, 1H), 13.72(br s, 1H); HPLC rt 3.77

**Example 3:** 2-[[3-quinolinylmethyl]oxy]carbonyl]amino]benzoic acid

NMR δ_H (400MHz, d⁶-DMSO) 5.41(s, 2H), 7.11(t, 1H, J=7.8Hz), 7.57-7.65(m, 2H), 7.78(dt, 1H, J=1.5, 7.6Hz), (dd, 1H, J=1.5, 7.8Hz), 8.02-8.05(m, 2H), 8.27(d, 1H, J=8.3Hz), 8.43(d, 1H, J=1.5Hz), 8.98(d, 1H, J=2.0Hz), both exchangeable protons not observed to δ_H 13; HPLC rt 3.44 mins.

**Example 4:** 2-[[3-phenyl-1,2,4-oxadiazol-5-yl]methyl]oxy]carbonyl]amino]benzoic acid

HPLC rt 3.74 mins.

**Example 5:** 2-[[4-(1-methylethyl)phenyl]methyl]oxy]carbonyl]amino]benzoic acid
NMR $\delta_H$ (600MHz, $d^6$-DMSO) 1.20(d, 6H, $J=6.8$ Hz), 2.85-2.97(m, 1H), 5.14(s, 2H), 7.11(t, 1H, $J=7.6$ Hz), 7.27(d, 2H, $J=7.6$ Hz), 7.35(d, 2H, $J=7.9$ Hz), 7.60(t, 1H, $J=7.9$ Hz), 7.98(d, 1H, $J=7.9$ Hz), 8.28(d, 1H, $J=8.3$ Hz), 10.81(br s, 1H), one exchangeable proton not observed to $\delta_H$ 13.

**Example 6:** 2-[[[[4-methyl-2-phenyl-1,3-thiazol-5-yl)methyl]oxy]carbonyl]amino]benzoic acid

NMR $\delta_H$ (600MHz, $d^6$-DMSO) 2.48(s, 3H), 5.39(s, 2H), 7.09(t, 1H, $J=7.6$ Hz), 7.48-7.49(m, 2H), 7.57(t, 1H, $J=7.9$ Hz), 7.76(d, 1H, $J=3.8$ Hz), 7.91-7.92(m, 1H), 7.97(d, 1H, $J=7.6$ Hz), 8.25(d, 2H, $J=8.3$ Hz), both exchangeable protons not observed to $\delta_H$ 13.

**Example 7:** 2-[[[[3-chloro-4-[(1-methylethyl)oxy]phenyl]methyl]oxy]carbonyl]amino) benzoic acid

NMR $\delta_H$ (600MHz, $d^6$-DMSO) 1.29(d, 6H, $J=5.7$ Hz), 4.67(m, 1H), 5.10(s, 2H), 7.11(s, 1H), 7.18(s, 1H), 7.35(t, 1H, $J=8.3$ Hz), 7.50(d, 1H, $J=7.9$ Hz), 7.60(t, 1H, $J=7.9$ Hz), 7.97(d, 1H, $J=7.9$ Hz), 8.27(d, 1H, $J=7.9$ Hz), both exchangeable protons not observed to $\delta_H$ 13.

**Example 8:** 2-[[[1,3-benzothiazol-2-ylmethyl]oxy]carbonyl]amino)benzoic acid

HPLC rt 3.72 mins.

**Example 9:** 2-[[[4-biphenyl)methyl]oxy]carbonyl]amino)benzoic acid

![Chemical Structure](image)

a) methyl 2-[[[4-biphenyl)methyl]oxy]carbonyl]amino)benzoate

To a solution of 1,1'-biphenyl-4-ylmethanol (96.8mg, 0.52mmole, 1.05equiv) in tetrahydrofuran (5ml) was added methyl 2-isocyanatobenzoate (88mg, 0.5mmole,
1equiv) the mixture was stirred overnight at room temperature under an atmosphere of nitrogen. The mixture was then heated at gentle reflux over the weekend. On cooling 2N ammonia in methanol (2ml) was added and the reaction mixture stirred at room temperature for 15 mins. Evaporation of the solvent gave the title compound as a pale yellow solid 180mg (100%); m/z 362.1 [MH⁺], 379.2 [MNH₄⁺].

b) 2-(((4-biphenyl)methyl)oxy)carbonyl]amino)benzoic acid

To a solution of methyl 2-(((4-biphenyl)methyl)oxy)carbonyl]amino)benzoate (180mg, 0.5mmol, 1equiv) in dioxan (5ml) was added a 2M solution of sodium hydroxide (0.275ml, 1.1equiv). The solution was heated at 75οC for 3 hr when a further equivalent of a 2M sodium hydroxide solution (0.25ml) was added, after another 3 hr heating the reaction mixture was cooled, filtered and acidified with HCl. The reaction mixture was diluted with ethyl acetate washed with brine, dried and evaporated to dryness. The resulting yellow solid was chromatographed on a 10g aminopropyl SPE and eluted with methanol to methanol + 10% acetic acid, to afford the title compound as a pale yellow solid (122mg, 71%). □□ (400MHz, CDCl3) 5.28 (2H, s), 7.07 (1H, t, J=7 Hz), 7.36 (1H, apt, J=7.5Hz), 7.45 (2H, t, J=8 Hz), 7.52 (2H, app d, J=8 Hz), 7.61 (5H, t, J=8 Hz), 8.1 (1H, dd, J=1 and 8 Hz), 8.51 (1H, d, J=8.5 Hz), 10.41 (1H, s) one exchangeable proton not observed to □□ 13; m/z 365.2 [MNH₄⁺], 346.2 [M-H]-

Example compounds synthesised using Method B

Example 10: 2-(((4-(1-benzothien-2-ylmethyl)-1-piperazinyl]carbonyl]amino)benzoic acid

![Chemical structure]

a) Methyl 2-(((4-(phenylmethyl)-1-piperazinyl]carbonyl]amino)benzoate

To a solution of methyl 2-isocyanatobenzoate (4.0g, 22.6mmol, 1equiv) in MeCN (90ml) was added 1-(phenylmethyl)piperazine (4.2g, 23.9mmol, 1.06equiv) and the reaction mixture heated at 45οC for 3h. The mixture was cooled to room temperature then concentrated under reduced pressure to give a white solid which was which was
purified by Biotage™ chromatography (eluting with cyclohexane/ethyl acetate 3:2) to
give the title compound (7.7g, 96%) as a white solid; LC/MS: m/z 354.4 [MH⁺].

b) Methyl 2-[(1-piperazinylcarbonyl)amino]benzoate

A mixture of methyl 2-((4-(phenylmethyl)-1-piperazinyl[carbonyl]amino)benzoate (4.1g,
11.6mmol, 1equiv), 10% palladium hydroxide on carbon (0.4g, 10% bw) and ethanol
(100ml) was stirred under an atmosphere of hydrogen for 16h. The reaction mixture
was filtered through Celite™ and the filtrate was concentrated under reduced pressure
to give the title compound (3.0g, 100%) as white solid; LC/MS: m/z 264.3 [MH⁺]


5
c) 2-((4-(1-benzothien-2-ylmethyl)-1-piperazinyl[carbonyl]amino)benzoic acid

Methyl 2-[(1-piperazinylcarbonyl)amino]benzoate (0.040g, 0.15mmol, 1equiv) and 1-
benzothioephene-2-carbaldehyde (0.037g, 0.23mmol, 1.2equiv) were dissolved in THF
(3 ml) in an Alltech™ tube and MP-triacetoxyborohydride resin added (0.184g, 0.4
mmol, 1.9equiv), followed by acetic acid (0.036g, 0.6mmol, 4equiv) and the mixture
was shaken for 16 hr. PS-tosylhydrazine resin was added (0.164g, 0.46mmol,
3.1equiv) followed by MP-isocyanate resin (0.184g, 0.38mmol, 2.5equiv) and the
mixture shaken for 19 hr. The reaction mixture was filtered, the resin washed with
DCM (6 x 8ml) and the combined organic fractions concentrated under reduced
pressure with heating at 30°C.

The crude material was treated with methanol (1ml), THF (1ml), water (1ml) and LiOH
(0.019g, 0.45mmol, 3equiv) and heated to 50°C for 4 hr. After cooling, solvent was
removed under reduced pressure and the crude material de-salted using SPE (C18,
eluting with acetonitrile) and evaporated under a stream of nitrogen to give the title
compound (0.011g, 14%) as a cream solid; δH (400MHz, d8-MeOD): 8.22 (1H, dd, J=8
and 1 Hz), 7.99 (1H, dd, J=8 and 1.5Hz), 7.80 (1H, d, J=7 Hz), 7.71 (1H, dd, J=7 and
1.5Hz), 7.32-7.22 (4H, m), 6.91 (1H, m), 3.86 (2H, s), 3.62 – 3.60 (4H, m), 2.61–2.58
(4H, m), both exchangeable protons not observed to δH 13; LC/MS: m/z 396.2 [MH⁺].

Similarly the following compounds examples 11-17 were prepared using Method B,
with an additional purification step using SCX-SPE as appropriate (eluting with
ammonia:methanol, 1:9):
<table>
<thead>
<tr>
<th>Example No.</th>
<th>Compound: Ar</th>
<th>yield</th>
<th>m/z [MH⁺]</th>
</tr>
</thead>
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<tr>
<td>11</td>
<td><img src="image1.png" alt="Image" /></td>
<td>26.0mg (30%)</td>
<td>397.3</td>
</tr>
<tr>
<td>12</td>
<td><img src="image2.png" alt="Image" /></td>
<td>54.4mg (62%)</td>
<td>408.3</td>
</tr>
<tr>
<td>13</td>
<td><img src="image3.png" alt="Image" /></td>
<td>88.1mg (92%)</td>
<td>446.3</td>
</tr>
<tr>
<td>14</td>
<td><img src="image4.png" alt="Image" /></td>
<td>59.8mg (64%)</td>
<td>433.2</td>
</tr>
<tr>
<td>15</td>
<td><img src="image5.png" alt="Image" /></td>
<td>64.1mg (76%)</td>
<td>354.3</td>
</tr>
<tr>
<td>16</td>
<td><img src="image6.png" alt="Image" /></td>
<td>18.2mg (24%)</td>
<td>346.2</td>
</tr>
<tr>
<td>17</td>
<td><img src="image7.png" alt="Image" /></td>
<td>39.3mg (47%)</td>
<td>391.3</td>
</tr>
</tbody>
</table>

**Example 11**: 2-(((4-(1,3-benzothiazol-2-ylmethyl)-1-piperazinyl)carbonyl)amino)benzoic acid

NMR δH (400MHz, d6-DMSO) 14.26 (1H, s), 8.25 (1H, d, J=8 Hz), 8.07 (1H, dd, J=8 and 1Hz), 7.96-7.88 (2H, m), 7.53-7.38 (2H, m), 7.15 (1H, td, J=7.5 and 1.5Hz), 6.76 (1H, td, J=7.5 and 1Hz), 4.01 (2H, s), 3.54 – 3.50 (4H, m), 2.6 –2.58 (4H, m), one exchangeable proton not seen to δH 14.5.
Example 12: 2-[[4-[(4-fluoro-1-naphthalenyl)methyl]-1-piperazinyl]carbonyl]amino]benzoic acid

NMR $\delta_{H}$ (400MHz, d$^4$-MeOD) 8.35 (1H, d, J=8 Hz), 8.22 (1H, d, J=8 Hz), 8.08 (1H, d, J=8 Hz), 7.99 (1H, d, J=7.5 Hz), 7.62–7.56 (2H, m), 7.42 (1H, t, J=6.5 Hz), 7.29 (1H, t, J=8 Hz), 7.14–7.09 (1H, m), 6.91 (1H, t, J=8 Hz), 3.93 (2H, s), 3.56–3.54 (4H, m), 2.57–2.55 (4H, m), both exchangeable protons not seen to $\delta_{H}$ 13.


NMR $\delta_{H}$ (400MHz, d$^4$-MeOD) 8.22 (1H, d, J=8 Hz), 8.00 (1H, dd, J=8 Hz and 1.5Hz), 7.47 (2H, d, J=7.5 Hz), 7.38–7.21 (5H, m), 7.04(2H, d, J=8 Hz), 6.95–6.89 (2H, m), 5.11 (2H, s), 3.65(2H, s), 3.57–3.55 (4H, m), 2.56–2.53 (4H, m), both exchangeable protons not observed to $\delta_{H}$ 13.

Example 14: 2-[[4-(2-[(methylsulfonyl)amino]phenyl)methyl]-1-piperazinyl]carbonyl]amino)benzoic acid

NMR $\delta_{H}$ (400MHz, d$^4$-MeOD) 8.22 (1H, d, J=8 Hz), 8.00 (1H, dd, J=8 Hz and 1Hz), 7.48 (1H, d, J=8 Hz), 7.33–7.28 (2H, m), 7.23 (1H, d, J=8 Hz), 7.09 (1H, td, J=8 and 1Hz), 6.92 (1H, td, J=8 and 1Hz), 3.73 (2H, s), 3.63–3.61 (4H, m), 3.07 (3H, s), 2.56–2.54 (4H, m), three exchangeable protons not observed to $\delta_{H}$ 13.

Example 15: 2-[[4-[(4-methylphenyl)methyl]-1-piperazinyl]carbonyl]amino]benzoic acid

NMR $\delta_{H}$ (400MHz, d$^4$-MeOD): 8.21 (1H, d, J=8 Hz), 7.99 (1H, d, J=8 Hz), 7.29 (1H, td, J=8 and 1Hz), 7.22 (2H, d, J=8 Hz), 7.14 (2H, d, J=8 Hz), 6.91 (1H, td, J=7 and 1Hz), 3.58–3.56 (4H, m), 3.51 (2H, s), 2.50–2.48 (4H, m), 2.31 (3H, s), both exchangeable protons not observed to $\delta_{H}$ 13.

Example 16: 2-[[4-(2-thienylmethyl)-1-piperazinyl]carbonyl]amino)benzoic acid

NMR $\delta_{H}$ (400MHz, d$^4$-MeOD) 8.21 (1H, dd, J=1 and 8.5 Hz), 7.99 (1H, dd, J=8 and 1.5Hz), 7.33–7.27 (2H, m), 6.98–6.89 (3H, m), 3.78 (2H, s), 3.60 – 3.57 (4H, m), 2.55–2.53 (4H, m), both exchangeable protons not observed to $\delta_{H}$ 13.
Example 17: 2-([4-(3-quinolinylmethyl)-1-piperazinyl]carbonyl)amino)benzoic acid

NMR $\delta_H$ (400MHz, d$_4$-MeOD) 8.89 (1H, d, J=2 Hz), 8.31 (1H, s), 8.22 (1H, d, J=8 Hz), 8.04-7.94 (3H, m), 7.76 (1H, td, J=7 and 1.5Hz), 7.62 (1H, td, J=7 and 1Hz), 7.29 (1H, td, J=7 and 2Hz), 6.91(1H, td, J=7 and 1Hz), 3.80 (2H, s), 3.62–3.60 (4H, m), 2.60–2.58 (4H, m), both exchangeable protons not observed to $\delta_H$ 13.

Example compounds synthesised using Method C

Example 18: 2-([4-(1,2,4-benzotriazin-3-yl)-1-piperazinyl]carbonyl)amino)benzoic acid

Methyl 2-isocyanatobenzoate (0.035g, 0.20mmol, 1equiv) and 3-(1-piperazinyl)-1,2,4-benzotriazaine (0.065g, 0.26mmol, 1.2equiv) were stirred in anhydrous MeCN for 4 hr at 45°C under an atmosphere of nitrogen. The solvent was removed under reduced pressure using a vacuum centrifuge.

The reaction mixture was treated with methanol (1ml), THF (1ml), water (1ml) and LiOH (0.017g, 0.40mmol, 2equiv) and heated to 50°C for 4 hr. After cooling, solvent was removed under reduced pressure and the crude solid purified by recrystallisation from boiling DMSO/methanol (1:1) to yield the title compound as a yellow solid (0.041g, 54%); $\delta_H$ (600MHz, DMSO): 11.19 (1H, br s), 8.42 (1H, d, J=8 Hz), 8.27 (1H, d, J=8 Hz), 7.97 (1H, d, J=8 Hz), 7.87 (1H, t, J=7.5 Hz), 7.66 (1H, d, J=8 Hz), 7.54 (2H, m), 7.03 (1H, t, J=7.5 Hz), 4.12–4.10 (4H, br s), 3.72–3.71 (4H, m), one exchangeable proton not observed to $\delta_H$ 13; LC/MS : m/z 379.2 [MH$^+$].

Similarly the following compounds examples 19-26 were prepared using Method C, and isolated as sodium salts after purification using C18-SPE. The crude product was dissolved in a mixture of 2N NaOH with MeCN/H$_2$O or DMSO/MeOH then loaded onto the cartridge and eluted with 0.1% NH$_3$ in water and MeCN (0.5% NH$_3$).
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<th>m/z</th>
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<td>19</td>
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<td></td>
<td>13.8mg (14%)</td>
<td>377.2 [MH⁺]</td>
</tr>
<tr>
<td>20</td>
<td>N</td>
<td></td>
<td>44.5mg (43%)</td>
<td>382.2 [MH⁺]</td>
</tr>
<tr>
<td>21</td>
<td>N</td>
<td></td>
<td>72.4mg (71%)</td>
<td>376.2 [MH⁺]</td>
</tr>
<tr>
<td>22</td>
<td>N</td>
<td></td>
<td>10.8mg (10%)</td>
<td>394.2 [MH⁺]</td>
</tr>
<tr>
<td>23</td>
<td>N</td>
<td></td>
<td>41.3mg (40%)</td>
<td>383.2 [MH⁺]</td>
</tr>
<tr>
<td>24</td>
<td>N</td>
<td></td>
<td>85.0mg (77%)</td>
<td>410.1 [MH⁺]</td>
</tr>
<tr>
<td>25</td>
<td>N</td>
<td></td>
<td>71.5mg (68%)</td>
<td>390.3 [MH⁺]</td>
</tr>
<tr>
<td>26</td>
<td>C</td>
<td></td>
<td>62.9mg (67%)</td>
<td>339.2 [MH⁺]</td>
</tr>
</tbody>
</table>
Example 19: Sodium 2-[[4-(1-isooquinoliny1)-1-piperaziny1]carbonyl]amino]benzoate

NMR $\delta_{H}$ (600MHz, d$^6$-DMSO) 8.30–8.34 (1H, m), 8.17 (1H, d, J=8.3Hz), 8.13 (1H, d, J=5.6 Hz), 7.90–7.94 (2H, m), 7.73 (1H, t, J=7.6Hz), 7.63 (1H, t, J=7.6Hz), 7.42 (1H, d, J=5.7Hz), 7.35 (1H, d, J=5.3Hz), 6.79–6.84 (1H, m), 3.74–3.76 (4H, m), 2.39–2.41 (4H, m), one exchangeable proton not observed.

Example 20: Sodium 2-[[4-(1-benzothien-3-yl)-1-piperaziny1]carbonyl]amino]benzoate

NMR $\delta_{H}$ (600MHz, d$^6$-DMSO) 3.08 (4H, s), 3.71 (4H, s), 6.84 (1H, t, J=7.6Hz), 6.99 (1H, s), 7.26 (1H, t, J=7.2Hz), 7.33–7.42 (2H, m), 7.83 (1H, d, J=7.6Hz), 7.92–7.95 (2H, m), 8.33 (1H, d, J=8.3Hz), 13.55 (1H, br s).

Example 21: Sodium 2-[[4-(1-naphthalenyl)-1-piperaziny1]carbonyl]amino]benzoate

NMR $\delta_{H}$ (600MHz, d$^6$-DMSO) 3.05-3.08 (4H, m), 3.71 (4H, s), 6.82 (1H, t, J=7.6Hz), 7.16 (1H, d, J=7.2Hz), 7.22 (1H, t, J=6.8Hz), 7.44 (1H, t, J=7.93Hz), 7.50-7.56 (2H, m), 7.62 (1H, d, J=8.3Hz), 7.91 (1H, d, J=7.9Hz), 7.94 (1H, d, J=7.9Hz), 8.20 (1H, d, J=7.9Hz), 8.31 (1H, d, J=7.9Hz), 13.97 (1H, br s)

Example 22: Sodium 2-[[4-(trifluoromethyl)phenyl]-1-piperaziny1]carbonyl]amino] benzoate

NMR $\delta_{H}$ (600MHz, d$^6$-DMSO) 3.14-3.18 (4H, m), 3.61-3.64 (4H, m), 6.82 (1H, t, J=6.8Hz), 7.00 (1H, d, J=9.1Hz), 7.11 (1H, d, J=8.7Hz), 7.22-7.26 (2H, m), 7.52 (1H, d, J=8.7Hz), 7.92 (1H, d, J=7.6Hz), 8.30 (1H, d, J=8.3Hz), 13.85 (1H, br s)

Example 23: Sodium 2-[[4-(1,3-benzothiazol-2-yl)-1-piperaziny1]carbonyl]amino] benzoate

NMR $\delta_{H}$ (600MHz, d$^6$-DMSO) 3.16-3.18 (4H, m), 3.64-3.66 (4H, m), 6.80 (1H, t, J=7.6Hz), 7.09 (1H, t, J=7.6Hz), 7.18 (1H, t, J=8.3Hz), 7.29 (1H, t, J=7.6Hz), 7.49 (1H, d, J=7.9Hz), 7.78 (1H, d, J=7.6Hz), 7.92 (1H, d, J=7.6Hz), 8.27 (1H, d, J=8.3Hz), 14.35 (1H, br s)
Example 24: Sodium 2-({4-(3-phenyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl}[carbonyl]amino)benzoate

NMR $\delta_{H}$ (600MHz, $d^5$-DMSO), 3.65-3.69 (8H, m), 6.80 (1H, t, J=8.1Hz), 7.19 (1H, dt, J=6.8Hz and 1.8Hz), 7.47-7.49 (3H, m), 7.93 (1H, dd, J=7.8Hz and 1.8Hz), 8.11-8.14 (2H, m), 8.28 (1H, d, J=7.6Hz), 14.40 (1H, br s)

Example 25: Sodium 2-({4-(1-naphthalenylmethyl)-1-piperazinyl}[carbonyl]amino)benzoate

NMR $\delta_{H}$ (600MHz, $d^5$-DMSO) 8.30 (1H, d, J=8 Hz), 8.26 (1H, d, J=8 Hz), 7.92 (2H, t, J=9 Hz), 7.85 (1H, d, J=7.5 Hz), 7.57-7.45 (4H, m), 7.17 (1H, t, J=7.5 Hz), 6.78 (1H, t, J=7.5 Hz), 3.92 (2H, s), 3.46-3.44 (4H, br m), 2.48-2.46 (4H, br m), one exchangeable proton not observed to $\delta_{H}$ 13.

Example 26: Sodium 2-({4-(phenylmethyl)-1-piperidinyl}[carbonyl]amino)benzoate

NMR $\delta_{H}$ (600MHz, $d^5$-DMSO) 11.30 (1H, br s), 8.37 (1H, d, J=8 Hz), 7.95 (1H, d, J=8 Hz), 7.48 - 7.45 (1H, m), 7.28 (2H, t, J=7 Hz), 7.20 - 7.17 (3H, m), 6.96 (1H, m), 4.04 (2H, d, J=13 Hz), 2.83 (2H, t, J=13 Hz), 2.55-2.51 (2H, m), 1.76 (1H, br s), 1.61 (2H, d, J=12 Hz), 1.17-1.10 (2H,m).

Example compound synthesised using Method D

Example 27: 2-({4-(2-quinoxaliny1)-1-piperazinyl}[carbonyl]amino)benzoic acid

\[
\begin{align*}
\text{HO} & \quad \text{HN} \\
\text{O} & \quad \text{N} \\
\text{N} & \quad \text{N}
\end{align*}
\]

a) Methyl 2-({4-(2-quinoxaliny1)-1-piperazinyl}[carbonyl]amino)benzoate

Methyl 2-((1-piperazinyl)[carbonyl]amino)benzoate (0.015g, 0.06mmol, 1equiv) and 2-chloroquinoxaline (0.010g, 0.06mmol, 1equiv) were heated together in NMP (0.35ml) at 125°C for 16 hr. The crude material was purified by SCX-SPE (eluting methanol/ammonia, 90/10), then further purified using mass directed preparative
h.p.l.c. to give the title compound as a yellow solid (0.009g, 38%); LC/MS: m/z 392.3 [MH⁺].

b) 2-((4-(2-quinoxaliny1)-1-piperazinyl)carbonyl)amino)benzoic acid Methyl 2-((4-(2-
quinoxaliny1)-1-piperazinyl)carbonyl)amino)benzoate (0.009g, 0.02mmol, 1equiv) was treated with methanol (1ml), THF (1ml), water (1ml) and LiOH (0.027g, 0.64mmol, 32equiv) and heated to 50°C for 5 hr. After cooling, solvent was removed under reduced pressure and the crude material de-salted using SPE (C18, eluting with methanol) and evaporated under a stream of nitrogen to give the title compound (0.004g, 53%) as a yellow solid: δ_H (400MHz, MeOD): 8.72 (1H, s), 8.26 (1H, d, J=8.5 Hz), 8.02 (1H, d), 7.85–7.82 (1H, m), 7.71–7.68 (1H, m), 7.63–7.60 (1H, m), 7.45–7.41 (1H, m), 7.32 (1H, t, J=8 Hz), 6.94 (1H, t, J=8 Hz), 3.95–3.92 (4H, m), 3.79–3.77 (4H, m), both exchangeable protons not observed to δ_H 13; LC/MS: m/z 378.2 [MH⁺].

All publications, including but not limited to patents and patent applications, cited in
this specification are herein incorporated by reference as if each individual publication
were specifically and individually indicated to be incorporated by reference herein as
though fully set forth.

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

1. A compound selected from: a compound of Formula (I)

\[
\text{CO}_2\text{H} \quad \text{N} \quad \text{W} \quad \text{Y} \quad \text{Z} \quad \text{R}^2
\]

and a salt, solvate or physiologically functional derivative thereof, wherein:

- \( \text{R}^1 \) represents hydrogen, halogen or \( \text{C}_1-\text{C}_3 \text{alkyl} \);

- \( \text{R}^2 \) represents a 5, 6, 9 or 10-member saturated, partially saturated or unsaturated ring system optionally including from 1 to 3 heteroatoms independently selected from S, O and N;

- \( \text{W} \) represents a linker selected from: \(-\text{NR}^3\text{R}^4-, -\text{NR}^3\text{(CH}_2\text{)}_n-, -\text{NR}^2\text{SO}_2-; -\text{O-}(\text{CH}_2\text{)}_n-\)

- \( \text{Y} \) represents a 5 or 6-member aryl or heteroaryl ring;

- \( \text{Z} \) represents \(-\text{(CH}_2\text{)}_n-, -(\text{CH}_2\text{)}_n\text{O-}, -(\text{CH}_2\text{)}_n\text{O-CH}_2-, -(\text{CH}_2\text{)}_n\text{O-CH}_2- \) or a bond;

- \( \text{X} \) represents CH or N

- \( n \) represents an integer selected from 1, 2, and 3;

- \( m \) represents an integer selected from 0 and 1;

- \( p \) represents an integer selected from 0, 1 and 2; and

- \( \text{R}^3 \) represents hydrogen or \( \text{C}_1-\text{C}_4 \text{alkyl} \).
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D319/18  C07D215/20  C07D271/10  C07D277/20  C07D277/68
A61K31/33     A61P9/10

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 practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category*</th>
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<td>DATABASE CHEMCATS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; XP002376946 retrieved from STN Order Number AL CE 0666 abstract</td>
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</table>

Further documents are listed in the continuation of Box C. 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 document 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

*"Y" document member of the same patent family

Date of the actual completion of the international search

12 April 2006

Date of mailing of the international search report

09/05/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5816 Patentliaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 940-9040, Tx. 31 651 epo nl
Fax: (+31-70) 940-3016

Authorized officer

Zellner, A
<table>
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<tr>
<td>X</td>
<td>&amp; &quot;RARA CHEMICALS CATALOGUE&quot; 27 September 2004 (2004-09-27), RARE-CHEMICALS GMBH, SCHULSTRASSE 6, 24214 GETTENDORF, GERMANY</td>
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<td>X</td>
<td>TAKANO, YASUO ET AL: &quot;Synthesis and AMPA receptor antagonistic activity of a novel class of quinoxalinecarboxylic acid with a substituted phenyl group at the C-7 position&quot; BIOORGANIC &amp; MEDICINAL CHEMISTRY LETTERS, 13(20), 3521-3525 CODEN: BMCEL8; ISSN: 0960-894X, 2003, XP002376938 page 3524; compound 15J</td>
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<td>WO 93/18006 A1 (NARHEX LIMITED, HONG KONG) 16 September 1993 (1993-09-16) page 33; example 4A</td>
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<tr>
<td>X</td>
<td>TAKANO, YASUO ET AL: &quot;Design, synthesis, and AMPA receptor antagonistic activity of a novel 6-nitro-3-oxoquinoxaline-2-carboxylic acid with a substituted phenyl group at the 7 position&quot; BIOORGANIC &amp; MEDICINAL CHEMISTRY, 13(20), 5841-5863 CODEN: BMECEP; ISSN: 0960-0896, 2005, XP002376939 page 5858; example 25n</td>
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<td>NEUMANN, U. ET AL: &quot;Inhibition of human chymase by 2-amino-3,1-benzoxazin-4-ones&quot; BIOORGANIC &amp; MEDICINAL CHEMISTRY, 9(4), 947-954 CODEN: BMECEP; ISSN: 0960-0896, 2001, XP002376940 page 952; compound 19</td>
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<td>X</td>
<td>STAIGER, ROGER P. ET AL: &quot;Isatoic anhydride. III. Reactions with primary and secondary amines&quot; JOURNAL OF ORGANIC CHEMISTRY, 18, 1427-39 CODEN: JOCEAH; ISSN: 0022-3263, 1953, XP002376941 Compound III, R=Benzyl (Tab I)</td>
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<td>WO 97/30019 A (SHAMAN PHARMACEUTICALS, INC) 21 August 1997 (1997-08-21) the whole document</td>
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<td>WO 2005/016867 A (SMITHKLINE BEECHAM CORPORATION; CAMPBELL, MATHEW; HATLEY, RICHARD, JON) 24 February 2005 (2005-02-24) the whole document</td>
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</table>
Continuation of Box II.2

Claims Nos.: –

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claim 1 may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, the search was performed taking into consideration the non-compliance in determining the extent of the search of claim 1.

The documents cited "X" in the International Search Report only represent an arbitrary selection of documents retrieved. The search can only be considered complete for compounds wherein W is selected from piperazin and piperidin.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.
# INTERNATIONAL SEARCH REPORT

**Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   
   see FURTHER INFORMATION sheet PCT/ISA/210

3. □ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. □ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- □ The additional search fees were accompanied by the applicant's protest.
- □ No protest accompanied the payment of additional search fees.
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<td>AT 309994 T</td>
<td>15-12-2005</td>
</tr>
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<td>BR 0207795 A</td>
<td>23-03-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2438737 A1</td>
<td>12-09-2002</td>
</tr>
<tr>
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<td></td>
<td>CN 1527824 A</td>
<td>08-09-2004</td>
</tr>
<tr>
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<td></td>
<td>DE 60207399 D1</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>WO 02070500 A1</td>
<td>12-09-2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2004529899 T</td>
<td>30-09-2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX PA03007865 A</td>
<td>04-12-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2130754 A1</td>
<td>12-09-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 7504654 T</td>
<td>25-05-1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RU 2126794 C1</td>
<td>27-02-1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5679688 A</td>
<td>21-10-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5942504 A</td>
<td>24-08-1999</td>
</tr>
<tr>
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