NOVEL SPIROHETEROCYCLIC COMPOUNDS AS MGLU5 ANTAGONISTS

FIELD OF THE INVENTION

[001] This invention relates to novel spiroheterocyclic compounds having selective affinity for the mGlu5 subtype of metabotropic receptors, to pharmaceutical compositions comprising such compounds, and to methods of treatment therewith.

BACKGROUND OF THE INVENTION

[002] Lower urinary tract disorders encompass an assortment of syndromes that affect normal micturition. Lower urinary tract disorders may develop through a combination of pathological and/or age-related changes of the urogenital system, or other etiology, *e.g.*, neurological disorders. Individuals suffering from lower urinary tract disorders suffer from impaired quality of life, including embarrassment, poor self-perception, and a general reduction in emotional well-being, social function, and general health. Lower urinary tract disorders, moreover, may be associated with other physical ailments, including cellulitis, pressure ulcers, urinary tract infections, falls with fractures, sleep deprivation, social withdrawal, depression, and sexual dysfunction. Older individuals suffering from lower urinary tract disorders may require more care from health care providers, both family and profession, which may be a factor in decisions to place them in institutions.

[003] According to the U.S. National Institutes of Health (NIH), up to 35 million Americans are estimated to suffer from lower urinary tract disorders. Lower urinary tract disorders are more common among women than men (2:1) until age 80, after which men and women are equally affected. The prevalence of lower urinary tract disorders increases with age. By the age 65, lower urinary tract disorders affect 15% to 30% of all individuals and approximately 50% of individuals in long-term care.

[004] Agents with various modes of action have been used to treat lower urinary tract disorders. These include agents that act directly on the lower urinary tract, *e.g.*, antimuscarinics and alpha-1 antagonists, and agents that act through the central nervous system, *e.g.*, serotonin and/or noradrenaline reuptake inhibitors. According to the NIH, however, while some progress has been made in the diagnosis, management, and treatment of lower urinary tract disorders, these disorders frequently remain intractable. Thus there is a continued need for improved agents, formulations and therapies to treat lower urinary tract disorders.

[005] Glutamic acid, an excitatory amino acid, is present at synapses throughout the central nervous system and is known to act on at least two types of receptors: ionotropic and metabotropic glutamate receptors. The principle function of ionotropic glutamate receptors is that their activation forms ligand-gated ion channels and, thereby, directly mediates electrical signaling of nerve cells, producing rapid and relatively large conductance changes in the post-synaptic membranes. Metabotropic glutamate receptors (mGluRs) regulate electrical signaling indirectly, by influencing intracellular metabolic processes via G-proteins. Changes in the post-synaptic cell that are mediated through mGluRs are consequently relatively slow over time and are not linked to rapid and large changes in neuronal membrane conductance.

[006] Three subtypes of ionotropic glutamate receptors have been described, *i.e.*, the NMDA, AMPA and kainate subtypes.

[007] Eight subtypes of metabotropic glutamate receptors have been cloned. The subtypes are classified into three groups on the basis of sequence similarities, and pharmacological and biochemical properties (Spooren et al., *Trends Pharmacol. Sci.* 22: 331-337, 2001): Group I mGlu receptors (mGlu1 and mGlu5), Group II mGlu receptors (mGlu2 and mGlu3) and Group III mGlu receptors (mGlu4, mGlu6, mGlu7 and mGlu8).

[008] Group I receptor mGlu5 (either human or rat) is known to comprise at least two subtypes, "a" and "b". Subtype "b" is longer than subtype "a", because of an alternative splicing of a 32-amino-acid stretch in the C-terminal (intracellular) domain, 50 residues downstream of the beginning of the domain. The human mGlu5b is 1212 amino acids long, while the "a" form lacks the amino acids from 877 to 908 (n. 828 being the first of the intracellular domain). The rat mGlu5b is 1203 amino acids long, while the "a" form lacks the amino acids from 876 to 907 (n. 827 being the first of the intracellular domain). (Hermans and Challis, *Biochem. J.* 359: 465-484, 2001).

[009] The mGlu receptors, belonging to family 3 of GPCRs, are characterized by two distinct topological domains: a large extracellular N-terminal domain containing a Venus fly-trap module responsible for agonist binding and the 7-TM domain plus intracellular C-terminal domain that is involved in receptor activation and G-protein coupling.

[010] The 7-TMD of mGlu Group I receptors has been shown to form a binding pocket for positive and negative allosteric modulators; the negative ones have been identified thanks to high throughput screening technologies and act as non-competitive antagonists, having no effect on agonist binding. The most interesting property of these molecules, in addition to their high potency, is their remarkable subtype selectivity.

[011] The 7-TM binding region is located in a pocket-lined by TM-III, TM-V, TM-VI and TM-VII; this site corresponds to the retinal binding pocket in rhodopsin.

[012] Allosteric modulators of mGlu5 represent an exciting advance in demonstrating the potentiality for developing novel research tools and therapeutic agents that regulate activity of specific mGluR subtypes.

[013] The compounds of the instant invention include those reported herein as mGlu5 antagonists, which in many cases are actually negative allosteric modulators acting at the 7-TM binding region.

[014] WO 00/63166 discloses tricyclic carbamic acid derivatives useful for the treatment of different diseases, including urinary incontinence. The derivatives are disclosed to be agonists or antagonists of Group I mGlu receptors with specificity for the mGlu1 receptor.

[015] WO 01/32632 discloses pyrimidine derivatives useful for the treatment of different diseases, including urinary incontinence. The derivatives are disclosed as selective antagonists of the mGlu1 receptor with at least 10-fold selectivity for the mGlu1 receptor over the mGlu 5 receptor.

[016] WO 01/27070 discloses new bisarylacetamides useful for the treatment of urinary incontinence, among other conditions. The molecules are disclosed to be agonists or antagonists selective for the mGlu1 receptor.

[017] US 6,369,222 discloses heterocycloazepinyl pyrimidine derivatives useful for the treatment of urinary incontinence, among other conditions. The derivatives are disclosed to be antagonists of the mGlu1 receptor.

[018] The aforementioned applications and patent, therefore, disclose mGlu1 receptor antagonists as useful for treating urinary incontinence. None of the references, however, provide experimental support for treatment of urinary incontinence, either in human patients or in an animal model for lower urinary tract disease.

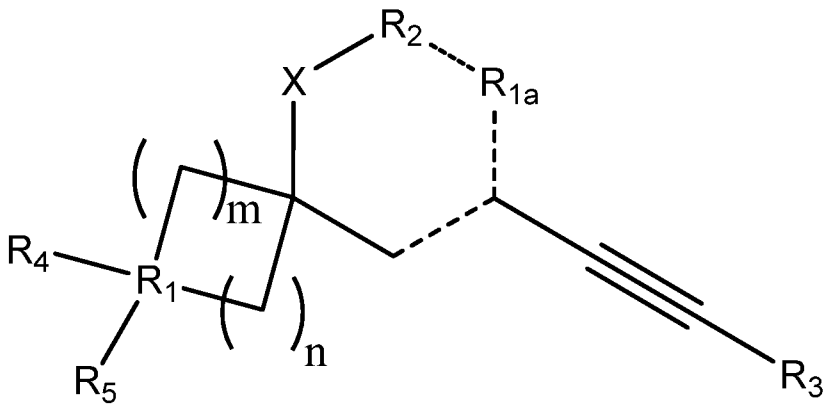
[019] We have tested the activity of selective mGlu1 and selective mGlu5 antagonists, in a rat model useful to detect activity on the lower urinary tract. Surprisingly, good activity was found for antagonists selective for the mGlu5 receptor, whereas two commercially available antagonists selective for mGlu1 receptor failed to exhibit an effect. An antagonist selective for Group II mGluR receptors also failed to exhibit an effect in the rat model. Given these results, selective mGlu5 antagonists can be an effective means to treat lower urinary tract disorders.

[020] There is a need in the art to develop novel compounds and compositions for the treatment of lower urinary tract disorders and for the alleviation of the symptoms associated

with such disorders. The present inventors have addressed this need through the development of novel heterocyclic compounds that are selective mGlu5 modulators, including selective mGlu5 antagonista. The compounds of the invention provide potent inhibition of the micturition reflex through a novel mechanism of action.

DESCRIPTION OF THE INVENTION

[021] The invention provides a compound having the general formula I



I

wherein:

X represents an oxygen or sulphur atom;

R_1 represents a carbon, nitrogen, oxygen or sulphur atom;

R_{1a} represents a CH, CH_2, N or NH moiety;

R_2 represents a valence bond or a CH or CH_2 group;

R_3 represents an optionally substituted C_1-C_6 alkyl group, an optionally substituted mono-, bi- or tricyclic C_1-C_{13} heterocyclic group containing 1 to 5 heteroatoms selected from N, O and S, an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group, an optionally substituted C_3-C_6 cycloalkyl group, or an optionally substituted C_3-C_6 cycloalkenyl group;

when R_1 represents a carbon atom:

R_4 represents a hydrogen atom, a halogen atom, an optionally substituted mono- or bicyclic C_1-C_9 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group;

R_5 represents a hydrogen or halogen atom, a cyano group, or an optionally substituted hydroxy, mercapto, amino, carbamoyl, C_1-C_6 alkyl or C_1-C_6 alkoxy group, or a group of

the formula –C(=O)–R_6 wherein R_6 represents an optionally substituted C_1-C_6 alkyl or C_1-C_6 alkoxy group, an optionally substituted mono- or bicyclic C_1-C_9 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group; or

R_4 and R_5 together represent an oxo group or an optionally substituted methylene or hydroxyimino group; or

R_4 and R_5 together with R_1 represent an optionally substituted unsaturated 4 to 6 membered ring containing from 0 to 3 heteroatoms selected from N, O and S;

when R_1 represents an oxygen atom:

R_4 and R_5 are absent;

when R_1 represents a nitrogen atom:

one of R_4 and R_5 is absent and the other is as defined above;

when R_1 represents a sulphur atom:

each of R_4 and R_5 independently is absent or represents an oxo group;

m is 1, 2 or 3;

n is 1 or 2;

when n is 2 or m is 2 or 3, the ring containing R_1 may be fused with an optionally substituted benzene ring; and

each ---- represents a single or double bond provided that one double bond extends from the carbon atom to which R_3-C≡C- is bonded and that no ring carbon atom bears two double bonds;

and solvates, hydrates, enantiomers, diastereomers, N-oxides and pharmaceutically acceptable salts of such compounds.

[022] The optional substituents are independently selected from halogen atoms and C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, mercapto, nitro, cyano, oxo, halo(C_1-C_6)alkyl, halo(C_1-C_6)alkoxy, C_1-C_6 alkylthio, C_1-C_6 alkylsulphonyl, C_1-C_6 alkylcarbonyl, sulphamoyl, C_1-C_6 alkylsulphamoyl, di(C_1-C_6)alkylsulphamoyl, (C_1-C_6)alkoxycarbonyl and (C_1-C_6)alkylcarbonyl(C_1-C_6)alkyl groups, and from groups of the formulae -NR*R*, -C(=O)NR*R*, -A, -O-A, -C(=O)A, -(CH_2)q-A, -NR**-A, -C(=O)NR**-A, -NR**C(=O)A and -O-C(=O)A wherein each R* independently represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, phenyl or benzyl group, R** represents a hydrogen atom or a C_1-C_6 alkyl group, qis an integer from 1 to 6 and A represents a phenyl group or a C_1-C_8 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S; each group A

being optionally substituted with from 1 to 3 groups independently selected from halo, hydroxy, cyano, nitro and C_1-C_6 alkyl.

[023] The most preferred compounds according to the invention are those in which X represents an oxygen atom, R_{1a} represents a nitrogen atom, R_2 represents a bond, and a double bond extends from R_{1a} to the carbon atom to which R_3-C≡C- is bonded. However, the compounds according to the invention in which X represents an oxygen atom, R_{1a} represents a CH group with a double bond extending from R_{1a} to the carbon atom to which R_3-C≡C- is bonded or a CH_2 group with a single bond extending from R_{1a} to the carbon atom to which R_3-C≡C- is bonded, and R_2 represents a CH_2 group are also preferred.

[024] R_3 preferably represents an alkyl group, an optionally substituted C_3-C_5 heteromonocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, an optionally substituted phenyl group, or a group of the formula –C(=O)–R_3 wherein R_3 is as defined in this sentence. More preferably, R_3 represents a methyl, isopropyl, butyl, phenyl, 3-(t.butyl)-phenyl, 2-chlorophenyl, 3-chlorophenyl, 3-chloro-5-fluorophenyl, 2-cyanophenyl, 3-cyanophenyl, 3-ethoxyphenyl, 3-ethylphenyl, 2-fluorophenyl, 3-fluorophenyl, 4-fluorophenyl, 3-hydroxyphenyl, 3-isopropylphenyl, 3-methoxyphenyl, 2-methylphenyl, 3-methylphenyl, 3-(3-methyl-[1,2,4]-oxadiazol-5-yl)-phenyl, 3-nitrophenyl, 3-trifluoromethoxyphenyl, 3-trifluoromethylphenyl, 2-pyridyl, 4-chloro-2-pyridyl, 5-chloro-2-pyridyl, 5-fluoro-2-pyridyl, 6-fluoro-2-pyridyl, 4-methyl-2-pyridyl, 6-methoxy-3-pyridyl, 6-methyl-2-pyridyl, 4-trifluoromethyl-2-pyridyl, 3-pyridyl, 6-fluoro-3-pyridyl, 2-furyl, 2-thienyl, 3-thienyl, 2-pyrazinyl, 2-methyl-1,3-thiazol-4-yl or 2-furoyl group.

[025] When R_1 represents a carbon atom, R_4 preferably represents a hydrogen atom, a halogen atom, an optionally substituted monocyclic C_3-C_5 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted phenyl group; and R_5 preferably represents a hydrogen or halogen atom, a cyano group, or an optionally substituted hydroxy, mercapto, amino, carbamoyl, C_1-C_6 alkyl or C_1-C_6 alkoxy group, or a group of the formula -C(=O)–R_6 wherein R_6 represents an optionally substituted C_1-C_6 alkyl group, an optionally substituted monocyclic C_3-C_5 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted phenyl group.

[026] When R_1 represents a carbon atom, R_4 more preferably represents a hydrogen or fluorine atom or a 2-pyridyl or 2-pyridylmethyl group, and R_5 more preferably represents a hydrogen or fluorine atom or a cyano, hydroxy or ethoxycarbonyl group.

[027] Alternatively, when R_1 represents a carbon atom, R_4 and R_5 together preferably represent an oxo, methylene, difluoromethylene, 2-pyridylmethylene, trimethylene,

tetramethylene, ethylenedioxy, propylenedioxy, oxyethyl, oxypropyl, methyleneoxymethylene, hydroxyimino, methoxyimino, 1-carboxy-3-hydroxy-propoxyimino or 2-oxo-tetrahydrofuran-3-yloxyimino group.

[028] Alternatively, when R_1 represents a carbon atom, R_4 and R_5 together preferably represent a group of the formula $-(Y_p)-$ wherein p is an integer of from 3 to 5 and each Y independently represents an oxygen atom or an optionally substituted methylene group, provided that no two adjacent Y moieties represent oxygen atoms. For example, $-(Y_p)-$ may be $-O-(CH_2)_2-O-$, $-O-(CH_2)_3-O-$, $-O-(CH_2)_2-$, $-O-(CH_2)_3-$, $-CH_2-O-CH_2-$, $-(CH_2)_3-$, $-(CH_2)_4-$, $-(CH_2)_5-$ or $-CH_2CH_2CF_2CH_2CH_2-$.

[029] When R_1 represents a nitrogen atom, R_4 preferably represents an optionally substituted C_3-C_5 heteromonocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, with R_5 being absent. Alternatively, R_5 preferably represents an alkoxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heterocyclylcarbonyl, carbamoyl, diakylcarbamoyl, N-alkoxy-N-alkylcarbamoyl or optionally substituted heterocyclylcarbamoyl group, with R_4 being absent.

[030] When R_1 represents a nitrogen atom, R_4 more preferably represents a 3-cyano-2-pyrazinyl, 3-nitro-2-pyridyl or 3-nitro-6-methyl-2-pyridyl group, with R_5 being absent. Alternatively, R_5 more preferably represents an ethoxycarbonyl, 3-chlorobenzoyl, 2-furoyl, 2-oxo-1-imidazolidinylcarbonyl, 3-methylsulphonyl-2-oxo-1-imidazolidinylcarbonyl, 4-methyl-1-piperazinylcarbonyl, piperidinocarbonyl, 1-pyrrolidinylcarbonyl, carbamoyl, dimethylcarbamoyl, diethylcarbamoyl, N-methoxy-N-methyl-carbamoyl, N-ethyl-N-isopropyl-carbamoyl or 4-pyridylcarbamoyl group, with R_4 being absent.

Definitions

[031] Except where stated otherwise, the following definitions apply throughout the present specification and claims. These definitions apply regardless of the whether a term is used by itself or in combination with other terms. For example, the definition of "alkyl" applies not only to alkyl as such but also to the alkyl portions of alkoxy, alkylamino, alkylthio, alkylcarbonyl etc. Furthermore, all ranges described for chemical group, for example, the ranges "from 1 to 20 carbon atoms" and "C_1-C_6 alkyl" include all combinations and subcombinations of ranges and specific numbers of carbon atoms therein.

[032] "Alkyl" means a straight chain or branched chain aliphatic hydrocarbon group having from 1 to 20 carbon atoms in the chain. Preferred alkyl groups have from 1 to 12 carbon atoms in the chain. More preferred alkyl groups have from 1 to 6 carbon atoms in the

chain. "Lower alkyl" means an alkyl group having about 1 to about 6 carbon atoms in the chain which may be straight or branched. Examples of suitable alkyl groups include methyl, ethyl, n-propyl, isopropyl, sec-butyl, n-butyl, and t-butyl.

[033] "Alkenyl" means a straight chain or branched chain aliphatic hydrocarbon group having at least one carbon-carbon double bond and having from 2 to 15 carbon atoms in the chain. Preferred alkenyl groups have from 2 to 12 carbon atoms in the chain. More preferred alkenyl groups have from 2 to 6 carbon atoms in the chain. "Lower alkenyl" means an alkenyl group having 2 to about 6 carbon atoms in the chain, which may be straight or branched. Examples of suitable alkenyl groups include ethenyl, propenyl, isopropenyl, n-butenyl, 1-hexenyl and 3-methylbut-2-enyl.

[034] "Alkynyl" means a straight chain or branched chain aliphatic hydrocarbon group having at least one carbon-carbon triple bond and having from 2 to 15 carbon atoms in the chain. Preferred alkynyl groups have from 2 to 12 carbon atoms in the chain. More preferred alkynyl groups have from 2 to 6 carbon atoms in the chain. "Lower alkynyl" means an alkynyl group having 2 to about 6 carbon atoms in the chain, which may be straight or branched. Examples of suitable alkynyl groups include ethynyl, propynyl and 2-butynyl.

[035] "Mono-, bi- or tricyclic aryl" means an aromatic monocyclic, bicyclic or tricyclic ring system comprising 6 to 14 carbon atoms. Bi- and tricyclic aryl groups are fused at 2 or 4 points or joined at one point via a bond or a heteroatom linker (O,S, NH, or N(C_1-C_6 alkyl) (e.g., biphenyl, 1-phenylnapthyl). The aryl group can be optionally substituted on the ring with one or more substituents, preferably 1 to 6 substituents, which may be the same or different. Examples of suitable aryl groups include phenyl and naphthyl.

[036] "Mono-, bi-, or tricyclic heterocyclic" means an aromatic or non-aromatic saturated mono bi or tricyclic ring system having from 2 to 14 ring carbon atoms, and containing from 1 to 5 ring atoms chosen from N, O and S, alone or in combination. Bi- and tricyclic heterocyclic groups are fused at 2 or 4 points or joined at one point via a bond or a heteroatom linker (O,S, NH, or N(C_1-C_6 alkyl). The "mono bi or tricyclic heterocyclic" can be optionally substituted on the ring by replacing an available hydrogen on the ring by one or more substituents which may be the same or different, There are no adjacent oxygen and or sulphur atoms present in the ring system. The nitrogen or sulphur atom of the heterocyclic can be optionally oxidized to the corresponding N-oxide, S-oxide or S-dioxide. Examples of suitable heterocyclics include furanyl, imidazolyl, isoxazolyl, oxadiazolyl, oxazolyl, pyrrolyl, pyridyl, pyrimidyl, pyridazinyl, thiazolyl, triazolyl, tetrazolyl, thienyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl,

benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, isoindolyl, acridinyl and benzoisoxazolyl, aziridinyl, piperidinyl, pyrrolidinyl, piperazinyl, tetrahydropyranyl, tetrahydrofuranyl, tetrahydrothiophenyl, morpholinyl and thiomorpholinyl.

[037] Heterocyclics with aromatic characteristics may be referred to as heteroaryls or heteroaromatics. Examples of suitable heteroaromatics include furanyl, imidazolyl, isoxazolyl, oxadiazolyl, oxazolyl, pyrrolyl, pyridyl, pyrimidyl, pyridazinyl, thiazolyl, triazolyl, tetrazolyl, thienyl, carbazolyl, benzimidazolyl, benzothienyl, benzofuranyl, indolyl, quinolinyl, benzotriazolyl, benzothiazolyl, benzooxazolyl, benzimidazolyl, isoquinolinyl, isoindolyl, acridinyl, benzoisoxazolyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, 3-phenylpyridine, 3-cyclohexylpyridine, 3-(pyridin-3-yl) morpholine, 3-phenylisoxazole and 2-(piperidin-1-yl)pyrimidine.

[038] "Mono or bicyclic cycloalkyl" means a monocyclic or bicyclic carbon ring system having from 3 to 14 carbon atoms, preferably from 3 to 6 carbon atoms. The cycloalkyl can be optionally substituted on the ring by replacing an available hydrogen on the ring by one or more substituents which may be the same or different. Examples of suitable monocyclic cycloalkyls include cyclopropyl, cyclopentyl, cyclohexyl and cycloheptyl. Examples of suitable multicyclic cycloalkyls include 1-decalinyl, norbornyl and adamantyl.

[039] "Cycloalkenyl" has a menaing corresponding to that of cycloalkyl, but with one or two double bonds within the ring (e.g., cyclohexenyl, cyclohexadiene).

[040] "Halogen" or "Hal" means fluorine, chlorine, bromine or iodine. Preferred are fluorine, chlorine or bromine, and more preferred are fluorine and chlorine.

[041] The term "acyl", whether used alone, or within a term such as "acylamino", denotes a radical provided by the residue after removal of hydroxyl from an organic acid. The term "acylamino" refers an amino radical substituted with an acyl group. An example of an "acylamino" radical is $CH_3C(=O)$-NH- where the amine may be further substituted with alkyl, aryl or aralkyl.

Stereochemistry of Compounds of the Invention

[042] Compounds of formula I may have one or more chiral centers and, depending on the nature of individual substituents, they can also have geometrical isomers. Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers". Stereoisomers that are not mirror images of one another are termed "diastereomers" and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has a chiral center, a pair of enantiomers is possible. An enantiomer can be characterized by the

absolute configuration of its asymmetric center and is described by the R--and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (-)-isomer respectively). A chiral compound can exist as either an individual enantiomer or as a mixture of enantiomers. A mixture containing equal proportions of the enantiomers is called a "racemic mixture". A mixture containing unequal portions of the enantiomers is described as having an "enantiomeric excess" (*ee*) of either the R or S compound. The excess of one enantiomer in a mixture is often described with a % enantiomeric excess (% *ee*) value determined by the formula:

$$\%\ ee = (R) - (S) / (R) + (S)$$

[043] The ratio of enantiomers can also be defined by "optical purity" wherein the degree at which the mixture of enantiomers rotates plane polarized light is compared to the individual optically pure R and S compounds. Optical purity can be determined using the following formula:

$$\text{Optical purity} = \text{enant.}_{major} / (\text{enant.}_{major} + \text{enant.}_{minor})$$

[044] The invention encompasses all individual isomers of compounds of formula I. The description or naming of a particular compound in the specification and claims is intended to include both individual enantiomers and mixtures, racemic or otherwise, thereof. Methods for the determination of stereochemistry and the resolution of stereoisomers are well-known in the art.

[045] For many applications, it is preferred to carry out stereoselective syntheses and/or to subject the reaction product to appropriate purification steps so as to produce substantially optically pure materials. Suitable stereoselective synthetic procedures for producing optically pure materials are well known in the art, as are procedures for purifying racemic mixtures into optically pure fractions. Those of skill in the art will further recognize that invention compounds may exist in polymorphic forms wherein a compound is capable of crystallizing in different forms. Suitable methods for identifying and separating polymorphisms are known in the art.

[046] Diastereisomers differ in both physical properties and chemical reactivity. A mixture of diastereomers can be separated into enantiomeric pairs based on solubility, fractional crystallization or chromatographic properties, e.g., thin layer chromatography, column chromatography or HPLC.

[047] Purification of complex mixtures of diastereomers into enantiomers typically requires two steps. In a first step, the mixture of diastereomers is resolved into enantiomeric

pairs, as described above. In a second step, enantiomeric pairs are further purified into compositions enriched for one or the other enantiomer or, more preferably resolved into compositions comprising pure enantiomers. Resolution of enantiomers typically requires reaction or molecular interaction with a chiral agent, e.g., solvent or column matrix. Resolution may be achieved, for example, by converting the mixture of enantiomers, e.g., a racemic mixture, into a mixture of diastereomers by reaction with a pure enantiomer of a second agent, i.e., a resolving agent. The two resulting diasteromeric products can then be separated. The separated diastereomers are then reconverted to the pure enantiomers by reversing the initial chemical transformation.

[048] Resolution of enantiomers can also be accomplished by differences in their non-covalent binding to a chiral substance, e.g., by chromatography on homochiral adsorbants. The noncovalent binding between enantiomers and the chromatographic adsorbant establishes diastereomeric complexes, leading to differential partitioning in the mobile and bound states in the chromatographic system. The two enantiomers therefore move through the chromatographic system, e.g, column, at different rates, allowing for their separation.

[049] Chiral resolving columns are well known in the art and are commercially available (e.g., from MetaChem Technologies Inc., a division of ANSYS Technologies, Inc., Lake Forest, CA). Enantiomers can be analyzed and purified using, for example, chiral stationary phases (CSPs) for HPLC. Chiral HPLC columns typically contain one form of an enantiomeric compound immobilized to the surface of a silica packing material.

[050] D-phenylglycine and L-leucine are examples of Type I CSPs and use combinations of π- π interactions, hydrogen bonds, dipole-dipole interactions, and steric interactions to achieve chiral recognition. To be resolved on a Type I column, analyte enantiomers must contain functionality complementary to that of the CSP so that the analyte undergoes essential interactions with the CSP. The sample should preferably contain one of the following functional groups: π -acid or π -base, hydrogen bond donor and/or acceptor, or an amide dipole. Derivatization is sometimes used to add the interactive sites to those compounds lacking them. The most common derivatives involve the formation of amides from amines and carboxylic acids.

[051] The MetaChiral ODM™ is an example of a type II CSP. The primary mechanisms for the formation of solute-CSP complexes is through attractive interactions, but inclusion complexes also play an important role. Hydrogen bonding, π - π interactions, and dipole stacking are important for chiral resolution on the MetaChiral™ ODM. Derivatization maybe necessary when the solute molecule does not contain the groups required for solute-

column interactions. Derivatization, usually to benzylamides, may be required for some strongly polar molecules like amines and carboxylic acids, which would otherwise interact strongly with the stationary phase through non-specific-stereo interactions.

[052] Compounds of formula I can be separated into diastereomeric pairs by, for example, separation by column chromatography or TLC on silica gel. These diastereomeric pairs are referred to herein as diastereomer with upper TLC Rf; and diastereomer with lower TLC Rf. The diastereomers can further be enriched for a particular enantiomer or resolved into a single enantiomer using methods well known in the art, such as those described herein.

[053] The relative configuration of the diastereomeric pairs can be deduced by the application of theoretical models or rules (e.g. Cram's rule, the Felkin-Ahn model) or using more reliable three-dimensional models generated by computational chemistry programs . In many instances, these methods are able to predict which diasteromer is the energetically favoured product of a chemical transformation. As an alternative, the relative configuration of the diastereomeric pairs can be indirectly determined by discovering the absolute configurations of a single enantiomer in one (or both) of the diastereomeric pair(s).

[054] The absolute configuration of the stereocenters can be determined by very well known method to those skilled in the art (e.g. X-Ray diffraction, circular dichroism). Determination of the absolute configuration can be useful also to confirm the predictability of theoretical models and can be helpful to extend the use of these models to similar molecules prepared by reactions with analogous mechanisms (e.g. ketone reductions and reductive amination of ketones by hydrides).

[055] The invention also encompasses stereoisomers of the Z-E type, and mixtures thereof due to R_2-R_3 substituents to the double bond not directly linked to the ring. Additional Z-E stereoisomers are encountered when m is not 1 and m and n are different. The Cahn-Ingold-Prelog priority rules are applied to determine whether the stereoisomers due to the respective position in the plane of the double bond of the doubly bonded substituents are Z or E. The stereoisomer is designated as *Z* (*zusammen* = together) if the 2 groups of highest priority lie on the same side of a reference plane passing through the C=C bond. The other stereoisomer is designated as *E* (*entgegen* = opposite).

[056] Mixture of stereoisomers of E-Z type can be separated (and/or characterized) in their components using classical method of purification that are based on the different chemico-physical properties of these compounds. Included in these method are fractional crystallization, chromatography carried out by low, medium or high pressure techniques, fractional distillation and any other method very well known to those skilled in the art.

Derivatives of Compounds of the Invention

[057] The invention further encompasses salts, solvates, hydrates, N-oxides, prodrugs and active metabolites of the compounds of formula I.

[058] The term "salts" can include acid addition salts or addition salts of free bases. For the purposes of isolation or purification it is possible to use pharmaceutically unacceptable salts. However, only the pharmaceutically acceptable, non-toxic salts are used therapeutically and they are therefore preferred. Examples of acids which may be employed to form pharmaceutically acceptable acid addition salts include salts derived from nontoxic inorganic acids such as nitric, phosphoric, sulphuric, or hydrobromic, hydroiodic, hydrofluoric, phosphorous, as well as salts derived from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyl alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulphonic acids, and acetic, maleic, succinic, or citric acids. Examples of such salts include napadisylate, besylate, sulphate, pyrosulphate, bisulphate, sulphite, bisulphite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, trifluoroacetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulphonate, toluenesulphonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulphonate, and the like. Also contemplated are salts of amino acids such as arginate and the like and gluconate, galacturonate (see, for example, Berge, *et al.* "Pharmaceutical Salts," *J. Pharma. Sci.* 1977;66:1).

[059] The phrase "pharmaceutically acceptable", as used in connection with compositions of the invention, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (*e.g.*, human). Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopeias for use in mammals, and more particularly in humans.

[060] Typically, a pharmaceutically acceptable salt of a compound of formula I may be readily prepared by using a desired acid or base as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. For example, an aqueous solution of an acid such as hydrochloric acid may be added to an aqueous suspension of a compound of formula I and the resulting mixture evaporated to

dryness (lyophilized) to obtain the acid addition salt as a solid. Alternatively, a compound of formula I may be dissolved in a suitable solvent, for example an alcohol such as isopropanol, and the acid may be added in the same solvent or another suitable solvent. The resulting acid addition salt may then be precipitated directly, or by addition of a less polar solvent such as diisopropyl ether or hexane, and isolated by filtration.

[061] The acid addition salts of the compounds of formula I may be prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the invention.

[062] Also included are both total and partial salts, that is to say salts with 1, 2 or 3, preferably 2, equivalents of base per mole of acid of formula I or salts with 1, 2 or 3 equivalents, preferably 1 equivalent, of acid per mole of base of formula I.

[063] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine.

[064] The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid.

[065] Compounds of the invention may have both a basic and an acidic center may and therefore be in the form of zwitterions or internal salts.

[066] Typically, a pharmaceutically acceptable salt of a compound of formula I may be readily prepared by using a desired acid or base as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent. For example, an aqueous solution of an acid such as hydrochloric acid may be added to an aqueous suspension of a compound of formula I and the resulting mixture evaporated to dryness (lyophilized) to obtain the acid addition salt as a solid. Alternatively, a compound of formula I may be dissolved in a suitable solvent, for example an alcohol such as isopropanol, and the acid may be added in the same solvent or another suitable solvent. The resulting acid

addition salt may then be precipitated directly, or by addition of a less polar solvent such as diisopropyl ether or hexane, and isolated by filtration.

[067] Those skilled in the art of organic chemistry will appreciate that many organic compounds can form complexes with solvents in which they are reacted or from which they are precipitated or crystallized. These complexes are known as “solvates”. For example, a complex with water is known as a “hydrate”. Solvates of the compound of the invention are within the scope of the invention. The salts of the compound of formula **I** may form solvates (*e.g.*, hydrates) and the invention also includes all such solvates. The meaning of the word "solvates" is well known to those skilled in the art as a compound formed by interaction of a solvent and a solute (i.e., solvation). Techniques for the preparation of solvates are well established in the art (see, for example, Brittain. *Polymorphism in Pharmaceutical solids.* Marcel Decker, New York, 1999.).

[068] The invention also encompasses N-oxides of the compounds of formulas I**.** The term "N-oxide" means that for heterocycles containing an otherwise unsubstituted sp^2 N atom, the N atom may bear a covalently bound O atom, i.e., -N→O. Examples of such N-oxide substituted heterocycles include pyridyl N-oxides, pyrimidyl N-oxides, pyrazinyl N-oxides and pyrazolyl N-oxides.

[069] The invention also encompasses prodrugs of the compounds of formula I**,** i.e., compounds which release an active parent drug according to formula I *in vivo* when administered to a mammalian subject. A prodrug is a pharmacologically active or more typically an inactive compound that is converted into a pharmacologically active agent by a metabolic transformation. Prodrugs of a compound of formula I are prepared by modifying functional groups present in the compound of formula I in such a way that the modifications may be cleaved *in vivo* to release the parent compound. *In vivo*, a prodrug readily undergoes chemical changes under physiological conditions (*e.g.*, are acted on by naturally occurring enzyme(s)) resulting in liberation of the pharmacologically active agent. Prodrugs include compounds of formula I wherein a hydroxy, amino, or carboxy group of a formula I compound is bonded to any group that may be cleaved *in vivo* to regenerate the free hydroxyl, amino or carboxy group, respectively. Examples of prodrugs include esters (*e.g.*, acetate, formate, and benzoate derivatives) of compounds of formula I or any other derivative which upon being brought to the physiological pH or through enzyme action is converted to the active parent drug. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described in the art (see, for example, Bundgaard. Design of Prodrugs. Elsevier, 1985).

[070] Prodrugs may be administered in the same manner as the active ingredient to which they convert or they may be delivered in a reservoir form, *e.g.*, a transdermal patch or other reservoir which is adapted to permit (by provision of an enzyme or other appropriate reagent) conversion of a prodrug to the active ingredient slowly over time, and delivery of the active ingredient to the patient.

[071] The invention also encompasses metabolites. A "metabolite" of a compound disclosed herein is a derivative of a compound which is formed when the compound is metabolised. The term "active metabolite" refers to a biologically active derivative of a compound which is formed when the compound is metabolised. The term "metabolised" refers to the sum of the processes by which a particular substance is changed in the living body. In brief, all compounds present in the body are manipulated by enzymes within the body in order to derive energy and/or to remove them from the body. Specific enzymes produce specific structural alterations to the compound. For example, cytochrome P450 catalyses a variety of oxidative and reductive reactions while uridine diphosphate glucuronyltransferases catalyse the transfer of an activated glucuronic-acid molecule to aromatic alcohols, aliphatic alcohols, carboxylic acids, amines and free sulphydryl groups. Further information on metabolism may be obtained from *The Pharmacological Basis of Therapeutics* , 9th Edition, McGraw-Hill (1996), pages 11-17.

[072] Metabolites of the compounds disclosed herein can be identified either by administration of compounds to a host and analysis of tissue samples from the host, or by incubation of compounds with hepatic cells *in vitro* and analysis of the resulting compounds. Both methods are well known in the art.

Use of the Compounds of the Invention to Treat Lower-Urinary Tract Disorders

[073] The nomenclature of lower urinary tract symptoms and pathologies used herein is set forth in Abrams et al., *Neurol. and Urodyn.* 21:167-178 (2002) and Andersson et al., *Pharmacol. Rev.* 56:581-631 (2004).

[074] Voiding dysfunctions can be roughly classified as disturbances of storage or emptying. Storage symptoms are experienced during the storage phase of the bladder, and include increased daytime frequency, nocturia (the waking at night one or more times to void), urgency (a sudden, compelling desire to pass urine that is difficult to defer), and urinary incontinence (the any involuntary leakage of urine). Urinary incontinence may be further characterized according to symptoms. Stress urinary incontinence is the involuntary leakage on effort or exertion, or on sneezing or coughing. Urge urinary incontinence is the involuntary

leakage of urine accompanied by or immediately preceded by urgency. Mixed urinary incontinence is the involuntary leakage of urine associated with urgency and also with exertion, effort, sneezing or coughing. Overflow incontinence is the involuntary leakage of urine occurring after the bladder capacity has been exceeded, *e.g.*, from a failure to empty. Enuresis also refers to any involuntary loss of urine. Nocturnal enuresis is the loss of urine occurring during sleep.

[075] Voiding symptoms include slow stream, splitting or spraying of the urine stream, intermittent stream (intermittency, *i.e.*, the stopping and restarting of urine flow during micturition, hesitancy (difficulty in initiating micturition resulting in a delay in the onset of voiding after the individual is ready to pass urine), straining and terminal dribble (a prolonged final part of micturition, when the flow has slowed to a trickle/dribble).

[076] Lower urinary tract disorders may further be categorized by a constellation of symptoms (*i.e.*, a syndrome) or by etiology. Individuals suffering from overactive bladder (OAB) syndrome, *e.g.*, typically suffer from symptoms of urgency, urge incontinence, increased daytime frequency or nocturia. OAB occurs as a result of detrusor muscle overactivity referred to as detrusor muscle instability. Detrusor muscle instability can arise from non-neurological abnormalities, such as bladder stones, muscle disease, urinary tract infection or drug side effects or can be idiopathic.

[077] Neurogenic overactive bladder (or neurogenic bladder) is a type of overactive bladder which occurs as a result of detrusor muscle overactivity referred to as detrusor hyperreflexia, secondary to known neurological disorders. Patients with neurological disorders, such as stroke, Parkinson's disease, diabetes, multiple sclerosis, peripheral neuropathy, or spinal cord lesions often suffer from neurogenic overactive bladder.

[078] Cystitis (including interstitial cystitis) is a lower urinary tract disorder of unknown etiology that predominantly affects young and middle-aged females, although men and children can also be affected. Symptoms of interstitial cystitis can include voiding symptoms, increased daytime frequency, urgency, nocturia or suprapubic or pelvic pain related to and relieved by voiding. Many interstitial cystitis patients also experience headaches as well as gastrointestinal and skin problems. In some cases, interstitial cystitis can also be associated with ulcers or scars of the bladder.

[079] Prostatitis and prostadynia are other lower urinary tract disorders that have been suggested to affect approximately 2-9% of the adult male population. Prostatitis is an inflammation of the prostate, and includes bacterial prostatitis (acute and chronic) and non-bacterial prostatitis. Acute and chronic bacterial prostatitis are characterized by inflammation

of the prostate and bacterial infection of the prostate gland, usually associated with symptoms of pain, increased daytime frequency and/or urgency. Chronic bacterial prostatitis is distinguished from acute bacterial prostatitis based on the recurrent nature of the disorder. Chronic non-bacterial prostatitis is characterized by inflammation of the prostate which is of unknown etiology accompanied by the presence of an excessive amount of inflammatory cells in prostatic secretions not currently associated with bacterial infection of the prostate gland, and usually associated with symptoms of pain, increased daytime frequency and/or urgency. Prostadynia is a disorder which mimics the symptoms of prostatitis absent inflammation of the prostate, bacterial infection of the prostate and elevated levels inflammatory cells in prostatic secretions. Prostadynia can be associated with symptoms of pain, increased daytime frequency and/or urgency.

[080] Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of the prostate that is very common in men over 40 years of age. BPH is thought to be due to excessive cellular growth of both glandular and stromal elements of the prostate. Symptoms of BPH can include increased frequency, urgency, urge incontinence, nocturia, and voiding symptoms, including slow stream, splitting or spraying of the urine stream, intermittency, hesitancy, straining and terminal dribble.

[081] Compounds according to the invention may be useful for the treatment of the aforesaid diseases or disorders of the lower urinary tract, especially neuromuscular dysfunctions of the lower urinary tract including urinary urgency, overactive bladder, increased urinary frequency, decreased urinary compliance (decreased bladder storage capacity), cystitis, interstitial cystitis, incontinence, urine leakage, enuresis, dysuria, urinary hesitancy and difficulty in emptying the bladder. By treatment, we mean treatment of any one or more of the above decsribed conditions, symptoms and/or syndromes.

[082] In the treatment of neuromuscular dysfunctions of the lower urinary tract, the compounds of the invention may be administered alone or in combination with another drug. Such other drug may be an antimuscarinic drug such as oxybuynin, tolterodine, darifenacin, solifenacin, trospium, imidafenacin, fesoterodine or temiverine; an α_1-adrenergic antagonist such as prazosin, doxazosin, terazosin, alfuzosin, silodosin or tamsulosin; a serotonin and/or noradrenalin reuptake inhibitor such as duloxetine, milnacipran, amoxapine, venlafaxine, des-venlafaxine, sibutramine, tesofensine or des-methylsibutramine; or a selective or non-selective COX inhibitor such as ibuprofen, naproxen, benoxaprofen, flurbiprofen, fenoprfen, ketoprofen, indoprofen, pirprofen, carprofen, tioxaprofe, suprofen, tiaprofenic acid, fluprofen, indomethacin, sulindac, tolmetin, zomepirac, diclofenac, fenclofenac, ibufenac, acetyl salicylic

acid, piroxicam, tenoxicam, nabumetone, ketorolac, azapropazone, mefenamic acid, tolfenamic acid, diflunisal, acemetacin, fentiazac, clidanac, meclofenamic acid, flufenamic acid, niflumic acid, flufenisal, sudoxicam, etodolac, salicylic acid, benorylate, isoxicam, 2-fluoro-α-methyl-[1,1'-biphenyl]-4-acetic acid 4-(nitrooxy)butyl ester, meloxicam, parecoxib or nimesulide.

Other Uses of the Compounds of the Invention

[083] Compounds according to the invention may also be useful for the treatment of migraine; for the treatment of gastroesophageal reflux disease (GERD); for the treatment of anxiety disorder; for the treatment of abuse, substance dependence and substance withdrawal disorder; for the treatment of neuropathic pain disorder; and for the treatment of fragile X syndrome disorders.

Pharmaceutical compositions comprising a compound of formula I

[084] While it is possible that a compound I may be administered as the bulk substance, it is preferable to present the active ingredient in a pharmaceutical formulation, *e.g.*, wherein the agent is in admixture with a pharmaceutically acceptable carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

[085] Accordingly, the invention further provides a pharmaceutical composition comprising a compound of formula I or a solvate, hydrate, enantiomer, diastereomer, N-oxide or pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier. The term "carrier" refers to a diluent, excipient, and/or vehicle with which an active compound is administered.

[086] A compound of formula I may be used in combination with other therapies and/or active agents. Accordingly, the invention provides, in a further aspect, a pharmaceutical composition comprising a compound of formula I or a solvate, hydrate, enantiomer, diastereomer, N-oxide or pharmaceutically acceptable salt thereof, a second active agent, and a pharmaceutically acceptable carrier.

[087] The pharmaceutical compositions may comprise as, in addition to, the carrier any suitable binder, lubricant, suspending agent, coating agent and/or solubilizing agent.

[088] Preservatives, stabilizers, dyes and flavouring agents also may be provided in the pharmaceutical composition. Antioxidants and suspending agents may be also used.

[089] The compounds of the invention may be milled using known milling procedures such as wet milling to obtain a particle size appropriate for tablet formation and for other

formulation types. Finely divided (nanoparticulate) preparations of the compounds of the invention may be prepared by processes known in the art, for example see WO 02/00196.

Routes of Administration and Unit Dosage Forms

[090] The routes for administration include oral (*e.g.*, as a tablet, capsule, or as an ingestible solution), topical, mucosal (*e.g.*, as a nasal spray or aerosol for inhalation), nasal, parenteral (*e.g.*, by an injectable form), gastrointestinal, intraspinal, intraperitoneal, intramuscular, intravenous, intrauterine, intraocular, intradermal, intracranial, intratracheal, intravaginal, intracerebroventricular, intracerebral, subcutaneous, ophthalmic (including intravitreal or intracameral), transdermal, rectal, buccal, epidural and sublingual. The compositions of the invention may be especially formulated for any of those administration routes. In preferred embodiments, the pharmaceutical compositions of the invention are formulated in a form that is suitable for oral delivery.

[091] There may be different composition/formulation requirements depending on the different delivery systems. It is to be understood that not all of the compounds need to be administered by the same route. Likewise, if the composition comprises more than one active component, then those components may be administered by different routes. By way of example, the pharmaceutical composition of the invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestible solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by multiple routes.

[092] Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile. For example, the compound of Formula I may be coated with an enteric coating layer. The enteric coating layer material may be dispersed or dissolved in either water or in a suitable organic solvent. As enteric coating layer polymers, one or more, separately or in combination, of the following can be used; *e.g.*, solutions or dispersions of methacrylic acid copolymers, cellulose acetate phthalate, cellulose acetate butyrate, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, cellulose acetate trimellitate, carboxymethylethylcellulose, shellac or other suitable enteric coating layer polymer(s). For environmental reasons, an aqueous

coating process may be preferred. In such aqueous processes methacrylic acid copolymers are most preferred.

[093] When appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges, which can be formulated in a conventional manner.

[094] When the composition of the invention is to be administered parenterally, such administration includes one or more of: intravenously, intraarterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intramuscularly or subcutaneously administering the agent; and/or by using infusion techniques.

[095] Pharmaceutical compositions of the invention can be administered parenterally, *e.g.*, by infusion or injection. Pharmaceutical compositions suitable for injection or infusion may be in the form of a sterile aqueous solution, a dispersion or a sterile powder that contains the active ingredient, adjusted, if necessary, for preparation of such a sterile solution or dispersion suitable for infusion or injection. This preparation may optionally be encapsulated into liposomes. In all cases, the final preparation must be sterile, liquid, and stable under production and storage conditions. To improve storage stability, such preparations may also contain a preservative to prevent the growth of microorganisms. Prevention of the action of micro-organisms can be achieved by the addition of various antibacterial and antifungal agents, *e.g.*, paraben, chlorobutanol, or acsorbic acid. In many cases isotonic substances are recommended, *e.g.*, sugars, buffers and sodium chloride to assure osmotic pressure similar to those of body fluids, particularly blood. Prolonged absorption of such injectable mixtures can be achieved by introduction of absorption-delaying agents, such as aluminium monostearate or gelatin.

[096] Dispersions can be prepared in a liquid carrier or intermediate, such as glycerin, liquid polyethylene glycols, triacetin oils, and mixtures thereof. The liquid carrier or intermediate can be a solvent or liquid dispersive medium that contains, for example, water, ethanol, a polyol (*e.g.*, glycerol, propylene glycol or the like), vegetable oils, non-toxic glycerine esters and suitable mixtures thereof. Suitable flowability may be maintained, by

generation of liposomes, administration of a suitable particle size in the case of dispersions, or by the addition of surfactants.

[097] For parenteral administration, the compound is best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

[098] Sterile injectable solutions can be prepared by mixing a compound of formula I with an appropriate solvent and one or more of the aforementioned carriers, followed by sterile filtering. In the case of sterile powders suitable for use in the preparation of sterile injectable solutions, preferable preparation methods include drying in vacuum and lyophilization, which provide powdery mixtures of the aldosterone receptor antagonists and desired excipients for subsequent preparation of sterile solutions.

[099] The compounds according to the invention may be formulated for use in human or veterinary medicine by injection (*e.g.*, by intravenous bolus injection or infusion or via intramuscular, subcutaneous or intrathecal routes) and may be presented in unit dose form, in ampoules, or other unit-dose containers, or in multi-dose containers, if necessary with an added preservative. The compositions for injection may be in the form of suspensions, solutions, or emulsions, in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, solubilizing and/or dispersing agents. Alternatively the active ingredient may be in sterile powder form for reconstitution with a suitable vehicle, *e.g.*, sterile, pyrogen-free water, before use.

[0100] The compounds of the invention can be administered (*e.g.*, orally or topically) in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, sustained-, pulsed-or controlled-release applications.

[0101] The compounds of the invention may also be presented for human or veterinary use in a form suitable for oral or buccal administration, for example in the form of solutions, gels, syrups, mouth washes or suspensions, or a dry powder for constitution with water or other suitable vehicle before use, optionally with flavouring and colouring agents. Solid compositions such as tablets, capsules, lozenges, pastilles, pills, boluses, powder, pastes, granules, bullets or premix preparations may also be used. Solid and liquid compositions for oral use may be prepared according to methods well-known in the art. Such compositions may

also contain one or more pharmaceutically acceptable carriers and excipients which may be in solid or liquid form.

[0102] The tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycolate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia.

[0103] Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

[0104] The compositions may be administered orally, in the form of rapid or controlled release tablets, microparticles, mini tablets, capsules, sachets, and oral solutions or suspensions, or powders for the preparation thereof. In addition to the new solid-state forms of pantoprazole of the invention as the active substance, oral preparations may optionally include various standard pharmaceutical carriers and excipients, such as binders, fillers, buffers, lubricants, glidants, dyes, disintegrants, odourants, sweeteners, surfactants, mold release agents, antiadhesive agents and coatings. Some excipients may have multiple roles in the compositions, *e.g.*, act as both binders and disintegrants.

[0105] Examples of pharmaceutically acceptable disintegrants for oral compositions include starch, pre-gelatinized starch, sodium starch glycolate, sodium carboxymethylcellulose, croscarmellose sodium, microcrystalline cellulose, alginates, resins, surfactants, effervescent compositions, aqueous aluminum silicates and cross-linked polyvinylpyrrolidone.

[0106] Examples of pharmaceutically acceptable binders for oral compositions include acacia; cellulose derivatives, such as methylcellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose or hydroxyethylcellulose; gelatin, glucose, dextrose, xylitol, polymethacrylates, polyvinylpyrrolidone, sorbitol, starch, pre-gelatinized starch, tragacanth, xanthane resin, alginates, magnesium–aluminum silicate, polyethylene glycol or bentonite.

[0107] Examples of pharmaceutically acceptable fillers for oral compositions include lactose, anhydrolactose, lactose monohydrate, sucrose, dextrose, mannitol, sorbitol, starch, cellulose (particularly microcrystalline cellulose), dihydro- or anhydro-calcium phosphate, calcium carbonate and calcium sulphate.

[0108] Examples of pharmaceutically acceptable lubricants useful in the compositions of the invention include magnesium stearate, talc, polyethylene glycol, polymers of ethylene oxide, sodium lauryl sulphate, magnesium lauryl sulphate, sodium oleate, sodium stearyl fumarate, and colloidal silicon dioxide.

[0109] Examples of suitable pharmaceutically acceptable odourants for the oral compositions include synthetic aromas and natural aromatic oils such as extracts of oils, flowers, fruits (*e.g.*, banana, apple, sour cherry, peach) and combinations thereof, and similar aromas. Their use depends on many factors, the most important being the organoleptic acceptability for the population that will be taking the pharmaceutical compositions.

[0110] Examples of suitable pharmaceutically acceptable dyes for the oral compositions include synthetic and natural dyes such as titanium dioxide, beta-carotene and extracts of grapefruit peel.

[0111] Examples of useful pharmaceutically acceptable coatings for the oral compositions, typically used to facilitate swallowing, modify the release properties, improve the appearance, and/or mask the taste of the compositions include hydroxypropylmethylcellulose, hydroxypropylcellulose and acrylate-methacrylate copolymers.

[0112] Examples of pharmaceutically acceptable sweeteners for the oral compositions include aspartame, saccharin, saccharin sodium, sodium cyclamate, xylitol, mannitol, sorbitol, lactose and sucrose.

[0113] Examples of pharmaceutically acceptable buffers include citric acid, sodium citrate, sodium bicarbonate, dibasic sodium phosphate, magnesium oxide, calcium carbonate and magnesium hydroxide.

[0114] Examples of pharmaceutically acceptable surfactants include sodium lauryl sulphate and polysorbates.

[0115] Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the agent may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

[0116] The compounds of the invention may also, for example, be formulated as suppositories *e.g.*, containing conventional suppository bases for use in human or veterinary medicine or as pessaries *e.g.*, containing conventional pessary bases.

[0117] The compounds according to the invention may be formulated for topical administration, for use in human and veterinary medicine, in the form of ointments, creams, gels, hydrogels, lotions, solutions, shampoos, powders (including spray or dusting powders), pessaries, tampons, sprays, dips, aerosols, drops (*e.g.*, eye ear or nose drops) or pour-ons.

[0118] For application topically to the skin, the agent of the invention can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, and water. Such compositions may also contain other pharmaceutically acceptable excipients, such as polymers, oils, liquid carriers, surfactants, buffers, preservatives, stabilizers, antioxidants, moisturizers, emollients, colourants, and odourants.

[0119] Examples of pharmaceutically acceptable polymers suitable for such topical compositions include acrylic polymers; cellulose derivatives, such as carboxymethylcellulose sodium, methylcellulose or hydroxypropylcellulose; natural polymers, such as alginates, tragacanth, pectin, xanthan and cytosan.

[0120] Examples of suitable pharmaceutically acceptable oils which are so useful include mineral oils, silicone oils, fatty acids, alcohols, and glycols.

[0121] Examples of suitable pharmaceutically acceptable liquid carriers include water, alcohols or glycols such as ethanol, isopropanol, propylene glycol, hexylene glycol, glycerol and polyethylene glycol, or mixtures thereof in which the pseudopolymorph is dissolved or dispersed, optionally with the addition of non-toxic anionic, cationic or non-ionic surfactants, and inorganic or organic buffers.

[0122] Examples of pharmaceutically acceptable preservatives include sodium benzoate, ascorbic acid, esters of p-hydroxybenzoic acid and various antibacterial and antifungal agents such as solvents, for example ethanol, propylene glycol, benzyl alcohol, chlorobutanol, quaternary ammonium salts, and parabens (such as methyl paraben, ethyl paraben and propyl paraben).

[0123] Examples of pharmaceutically acceptable stabilizers and antioxidants include ethylenediaminetetriacetic acid (EDTA), thiourea, tocopherol and butyl hydroxyanisole.

[0124] Examples of pharmaceutically acceptable moisturizers include glycerine, sorbitol, urea and polyethylene glycol.

[0125] Examples of pharmaceutically acceptable emollients include mineral oils, isopropyl myristate, and isopropyl palmitate.

[0126] The compounds may also be dermally or transdermally administered, for example, by use of a skin patch.

[0127] For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride.

[0128] As indicated, the compounds of the invention can be administered intranasally or by inhalation and is conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container, pump, spray or nebulizer with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134AT) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray or nebulizer may contain a solution or suspension of the active compound, *e.g.*, using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.*, sorbitan trioleate.

[0129] Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound and a suitable powder base such as lactose or starch.

[0130] For topical administration by inhalation the compounds according to the invention may be delivered for use in human or veterinary medicine via a nebulizer.

[0131] The pharmaceutical compositions of the invention may contain from 0.01 to 99% weight per volume of the active material. For topical administration, for example, the composition will generally contain from 0.01-10%, more preferably 0.01-1% of the active material.

[0132] The active agents can 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.

[0133] The pharmaceutical composition or unit dosage form of the invention may be administered according to a dosage and administration regimen defined by routine testing in the light of the guidelines given above in order to obtain optimal activity while minimizing

toxicity or side effects for a particular patient. However, such fine tuning of the therapeutic regimen is routine in the light of the guidelines given herein.

[0134] The dosage of the active agents of the invention may vary according to a variety of factors such as underlying disease conditions, the individual's condition, weight, gender and age, and the mode of administration. An effective amount for treating a disorder can easily be determined by empirical methods known to those of ordinary skill in the art, for example by establishing a matrix of dosages and frequencies of administration and comparing a group of experimental units or subjects at each point in the matrix. The exact amount to be administered to a patient will vary depending on the state and severity of the disorder and the physical condition of the patient. A measurable amelioration of any symptom or parameter can be determined by a person skilled in the art or reported by the patient to the physician. It will be understood that any clinically or statistically significant attenuation or amelioration of any symptom or parameter of urinary tract disorders is within the scope of the invention. Clinically significant attenuation or amelioration means perceptible to the patient and/or to the physician.

[0135] For example, a single patient may suffer from several symptoms of dysuria simultaneously, such as, for example, urgency and excessive frequency of urination or both, and these may be reduced using the methods of the invention. In the case of incontinence, any reduction in the frequency or volume of unwanted passage of urine is considered a beneficial effect of the present method of treatment.

[0136] The amount of the agent to be administered can range between about 0.01 and about 25 mg/kg/day, preferably between about 0.1 and about 10 mg/kg/day and most preferably between 0.2 and about 5 mg/kg/day. It will be understood that the pharmaceutical formulations of the invention need not necessarily contain the entire amount of the agent that is effective in treating the disorder, as such effective amounts can be reached by administration of a plurality of doses of such pharmaceutical formulations.

[0137] In a preferred embodiment of the invention, the compounds I are formulated in capsules or tablets, preferably containing 10 to 200 mg of the compounds of the invention, and are preferably administered to a patient at a total daily dose of 10 to 300 mg, preferably 20 to 150 mg and most preferably about 50 mg, for relief of urinary incontinence and other dysfunctions.

[0138] A pharmaceutical composition for parenteral administration contains from about 0.01% to about 100% by weight of the active agents of the invention, based upon 100% weight of total pharmaceutical composition.

[0139] Generally, transdermal dosage forms contain from about 0.01% to about 100% by weight of the active agents versus 100% total weight of the dosage form.

[0140] The pharmaceutical composition or unit dosage form may be administered in a single daily dose, or the total daily dosage may be administered in divided doses. In addition, co-administration or sequential administration of another compound for the treatment of the disorder may be desirable. To this purpose, the combined active principles are formulated into a simple dosage unit.

[0141] For combination treatment where the compounds are in separate dosage formulations, the compounds can be administered concurrently, or each can be administered at staggered intervals. For example, the compound of the invention may be administered in the morning and the antimuscarinic compound may be administered in the evening, or vice versa. Additional compounds may be administered at specific intervals too. The order of administration will depend upon a variety of factors including age, weight, gender and medical condition of the patient; the severity and aetiology of the disorders to be treated, the route of administration, the renal and hepatic function of the patient, the treatment history of the patient, and the responsiveness of the patient. Determination of the order of administration may be fine-tuned and such fine-tuning is routine in the light of the guidelines given herein.

Synthesis of the Compounds of the Invention

[0142] Compounds of formula I, and enantiomers, diastereomers, N-oxides, and pharmaceutically acceptable salts thereof, may be prepared by the general methods outlined hereinafter, said methods constituting a further aspect of the invention.

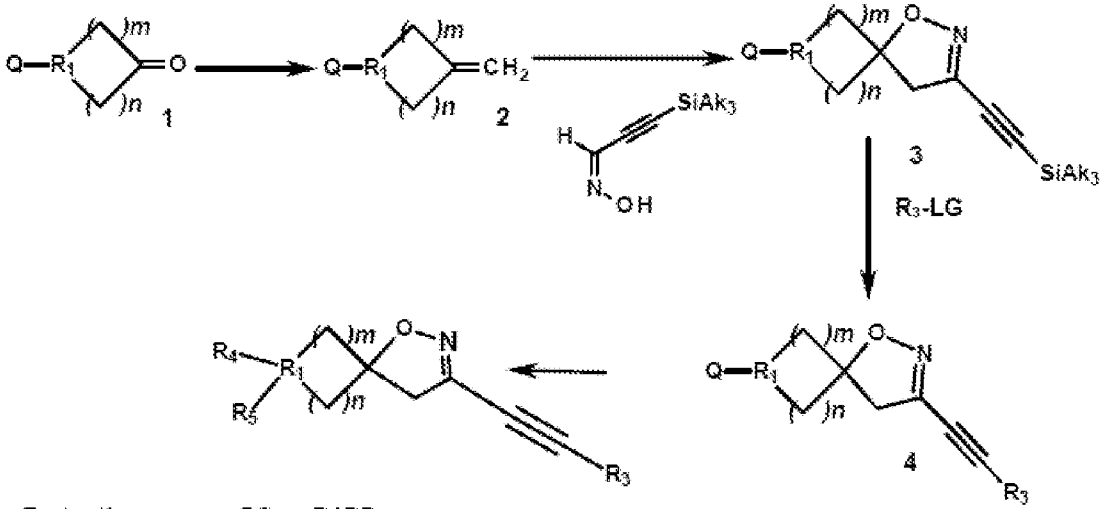
[0143] It will be appreciated by those skilled in the art that it may be desirable to use protected derivatives of intermediates used in the preparation of the compounds I. Protection and deprotection of functional groups may be performed by methods known in the art (see, for example, Green and Wuts *Protective Groups in Organic Synthesis.* John Wiley and Sons, New York, 1999). Hydroxy or amino groups may be protected with any hydroxy or amino protecting group. The amino protecting groups may be removed by conventional techniques. For example, acyl groups, such as alkanoyl, alkoxycarbonyl and aroyl groups, may be removed by solvolysis, *e.g.*, by hydrolysis under acidic or basic conditions. Arylmethoxycarbonyl groups (*e.g.*, benzyloxycarbonyl) may be cleaved by hydrogenolysis in the presence of a catalyst such as palladium-on-charcoal.

[0144] The synthesis of the target compounds is completed by removing any protecting groups which may be present in the penultimate intermediates using standard techniques, well-

known to those skilled in the art. The deprotected final products are then purified, as necessary, using standard techniques such as silica gel chromatography, HPLC on silica gel and the like, and/or by recrystallization.

[0145] The compounds of the invention are generally prepared according to the following scheme:

Scheme 1



Q = Protecting group - PG or R4R5

LG = I, Br, Cl, OMs, OTf, OTs

[0146] In Scheme 1, "Ak" represents a lower alkyl group, and the remaining variables are as defined for the general formula I or inside the scheme itself.

[0147] Cyclic ketones **1** are commercially available or easily prepared by standard methods known to those skilled in the art, e.g from piperidone (R_1 = N) with the carbonyl group that may or may not be previously protected (e.g. as a ketal, for example as 1,3-dioxolane) by simple nucleophilic substitution of activated haloaryls, haloalkyls or haloheteroaryls, that can be carried out in an appropriate solvent (such as n-butanol, DMF, N-methylpyrrolidone or N,N-dimethylacetamide) at a temperature between room temperature and the reflux of the selected solvent in the presence of a base, such as triethylamine or 4,4-dimethylaminopyridine. The reaction can be conducted also by microwave irradiation in a microwave apparatus to shorten the reaction time. An alternative general method for the preparation of piperidones from not activated haloaryls or haloheteroaryls (or triflates) is represented by the Buchwald–Hartwig amination using a palladium catalyst or by other metal catalyzed aminations. Very well known reductive amination procedures can be used where $R_4(R_5)_1$ is alkyl. Cyclic ketones **1** are reacted with the ylides, for example, obtained by the

addition of a base (e.g. LiHMDS or potassium tert-butoxide in an aprotic solvent like THF or diethyl ether) to, e.g., methyltriphenylphosphonium bromide, to afford exomethylene derivatives **2**. Methylene derivatives **2** are, in turn, cyclized using 1,3-dipolar cycloaddition methodology to the alkynyl derivatives **3** (Hao-Wei Shih et. al., Tetrahedron letters 49 (2008) 1008-1011, WO 20006/122770). To this end, trialkylsilylpropioladehydes oximes are converted *in situ* (or by isolating the intermediate) into the corresponding chloro or bromooximes (e.g. by the use of N-bromo succinimide or N-chlorosuccinimide or sodium hypochlorite or hypobromite or t.butylhypochlorite in an appropriate solvent such as dichloromethane or DMF with or without the addition of water), which are isolated or directly transformed inside the reaction vessel into the corresponding nitriloxides, by a mild base such as Na_2CO_3, triethylamine or ethlymagnesium bromide. Nitriloxides react quite easily with compounds **2** in a common solvent like dichloromethane or DMF usually at -78°C to 0° up to r.t. or heating till to the reflux of the chosen solvent, yielding compounds **3**.

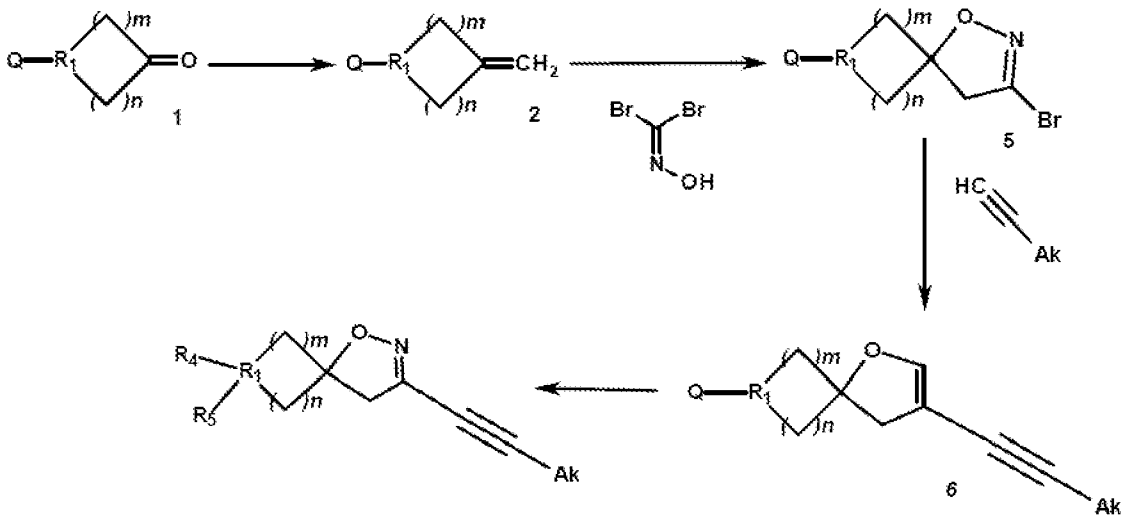
[0148] The silyl protecting group of **3** is then removed by treatment with tetrabutylammonium fluoride in THF at a temperature in the range from ambient temperature to reflux or by hydrolysis with a base (K_2CO_3 or KOH in MeOH) or other suitable method chosen from those reported in Greene-Wuts (Greene's Protective Groups in Organic Synthesis, 3rd Edition, Peter G. M. Wuts, Theodoura W. Greene 1999, Wiley Interscience page 654-659) and well known by those skilled in the art. The resultant acetylenic compounds are then transformed into compounds **4** by reacting them with R_3-LG following the well known Sonogashira procedure (Science of Synthesis, H. Heaney and S. Christie, October 2003, Vol. 3, Page 402 and following). This procedure uses cuprous iodide and a palladium complex chosen from $(Ph_3P)_2PdCl_2$, $(Ph_3P)_2Pd(OAc)_2$, $(Ph_3P)_4Pd$ (which can also be generated in situ *e.g.* from triphenylphosphine and $Pd(OAc)_2$) and all the other palladium complexes cited in the literature and used for this kind of reaction, in the presence of a base such as TEA, DEA, DIPEA, TMA, butylamine, piperidine. Solvents are chosen among THF, DME, DMF, DMA, EtOAc, DMSO, toluene and others suitable for the purpose of the reaction; or the same base in excess can be used as the reaction solvent. If one carries out the reaction in DMF or DME, the isolation of compounds **4** can be avoided by adding the tetrabutylammonium fluoride or tetrabutylammonium chloride directly to the reaction medium containing **3**, before the coupling (Sorensen, U.S., Pombo-Villar, E. Tetrahedron 2005, 61, 2697-2703). The R_3 substituents are introduced using aryl or heteroaryl halides (preferred in decreasing order iodide, bromide, chloride), aryl or heteroaryl triflates, alkyl halogenides or acyl chlorides, aroyl chlorides, heteroaroyl chlorides. Triflates are synthesized using very well known method to those skilled

in the art, *e.g.* from phenols or hydroxyaryls (heteroaryls) using trifluoromethanesulphonic anhydride in a chlorinated solvent or using N-phenyltriflimide in toluene or a chlorinated solvent in the presence or not of a base (*e.g.* TEA). Both processes can be accelerated with the aid of microwaves performing the reaction in a microwave oven. Other suitable leaving groups LG for R_3-LG are nonaflates, tosylates and potassium trifluoborates. Where R_3 is Ak an alternative procedure to Sonogashira coupling is the reaction of compounds **4** with lithium base (e.g. butyl lithium or LHDMS) in an appropriate solvent e.g. to produce the lithium alkinyl derivative, which on turn is reacted with a suitable electrophile like e.g. butyl iodide or the like in the same solvent or in alternative aprotic solvent like toluene at a temperature ranging from -20°C to the reflux of the chosen solvent.

[0149] If compounds **4** are obtained in which Q is a protecting group, they need further deprotection and N-arylation/alkylation or other kinds of derivatization steps to afford Compound **I.**

[0150] Compounds **4** can be obtained by directly reacting compounds **2** with appropriately derivatized propioladehyde oximes in which the R_3 group replaces the trialkylsilyl group.

Scheme 2



Q = Protecting group - PG or R1R4R5

LG = I, Br, Cl, OMs, OTf, OTs

[0151] In Scheme 2, "Ak" represents a lower alkyl group, and the remaining variables are as defined for the general formula I or inside the scheme itself.

Scheme 2 represents an alternative procedure to obtain compounds I wherein R_3 is Ak. Reacting dibromoformaldoxime as described above for the preparation of compounds **3,** one can synthesize compounds **5**. The bromooxazoline **5** can further be alkynylated by the use alkynes, an appropriate base such as sodium carbonate with the aid of Cu / CuI co-catalysis as described in detail within the experimental part. If Q is a protecting group in the resulting compounds **6,** they need further deprotection and N-arylation/alkylation or other kinds of derivatization steps to afford Compounds **I.**

[0152] Compounds **6** can be obtained by directly reacting compounds **2** with the appropriate alkylpropioladehyde oximes.

[0153] Other spiroheterocycle intermediates different from spirooxazolines can be synthesized following methods reported in the literature and cited in the experimental part as references.

[0154] The syntheses of other compounds not currently described in the general description above are well documented inside the experimental part of this invention which follows.

[0155] The free bases of formula **I**, their diastereomers or enantiomers can be converted to the corresponding pharmaceutically acceptable salts under standard conditions well known in the art. For example, the free base is dissolved in a suitable organic solvent, such as methanol, treated with, for example one equivalent of maleic or oxalic acid, one or two equivalents of hydrochloric acid or methanesulphonic acid, and then concentrated under vacuum to provide the corresponding pharmaceutically acceptable salt. The residue can then be purified by recrystallization from a suitable organic solvent or organic solvent mixture, such as methanol/diethyl ether.

[0156] The N-oxides of compounds of formula I can be synthesized by simple oxidation procedures well known to those skilled in the art.

EXAMPLES

[0157] The following examples illustrate the synthesis of the compounds of Formula **I** as described generally above. These examples are illustrative only and are not intended to limit the scope of the invention. The reagents and starting materials are readily available to one of ordinary skill in the art.

Example 1

3-Phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

3-Phenylprop-2-ynal oxime (Compound 1a)

A mixture of phenylpropargylaldehyde (859 mg, 6.6 mmol), hydroxylamine hydrochloride (4.59 g, 66 mmol), EtOH (48 ml) and water (12 ml) was stirred at r.t. for 24h. The reaction mixture was diluted with H_2O, extracted with Et_2O - EtOAc, washed with brine and evaporated to dryness *in vacuo* affording 0.96 g of the title compound (syn:anti 1:1) as a pasty brownish solid.

MS: $[M+H]^+ = 146.11$

3-Phenylethynyl-8-(t.butoxycarbonyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 1b)

To a solution of Compound 1a (0.35 g, 2.41 mmol) in 3 ml of DMF stirred at r.t. was added N-chlorosuccinimide (0.386 g, 2.89 mmol) and the solution became orange coloured. After 2 h stirring, the solution became yellow and, after additional 2 h stirring, it was cooled to 0°C and a solution of 1-(t.butoxycarbonyl)-4-methylene-piperidine (143 mg, 0.73 mmol) in 0.5 ml of DMF was added, followed by a solution of TEA in 0.5 ml of DMF. The cooling bath was removed and the reaction mixture was stirred at r.t. for 2 days. Afterwards, the reaction mixture was diluted with cold water, extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, evaporated to dryness *in vacuo*. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 7 : 3) affording 210 mg of the title product. Yield: 85.5%.

MS: $[M+H]^+ = 341.16$

3-Phenylethynyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 1c)

To a solution of Compound 1b (200 mg, 0.59 mmol) in CH_2Cl_2 (12 ml) stirred at 0°C was added trifluoroacetic acid (452 µl, 5.87 mmol) and the reaction mixture was stirred at 25 °C for 24 h, until the complete conversion of the reactant was observed by LC-MS. Water was added followed by 3N aq. NaOH to give a alkaline pH. Separation of the organic layer and extraction of the aqueous layer with CH_2Cl_2, washing with brine and drying over Na_2SO_4 the combined organic layers, afforded the title compound as beige solid, used in the next step without further purification.

MS: $[M+H]^+ = 241.29$

3-Phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

A solution of Compound 1c (50 mg, 0.21 mmol), 2-chloro-6-methyl-3-nitropyridine (39.5 mg, 0.23 mmol) and triethylamine (31.9 µL, 0.23 mmol) in N,N-dimethylacetamide (1.14 ml) was stirred at r.t. for 24 h and poured into water. The aqueous layer was extracted with EtOAc. The combined extracts were washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The crude product was purified by automated flash chromatography (SP1®TM – Biotage) eluting with a Petroleum Ether - Me_2CO 95 : 5 mixture. A further purification with CH_2Cl_2 as eluent afforded 47.7 mg of the title compound (67.7 %) as yellow solid.

MS: $[M+H]^+ = 377.22$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.50 (s, 3H), 3.00 (s, 2H), 3.50-3.71 (m, 4H), 6.63 (d, J=8Hz, 1H), 7.34-7.46 (m, 3H), 7.51-7.57 (m, 2H), 8.11 (d, 8Hz, 1H).

Example 2

3-Phenylethynyl-8-(3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

2-(4-Methylene-1-piperidyl)-3-nitropyridine (Compound 2a)

n-Butyllithium in hexane (2.5 M, 4.93 ml, 12.3 mmol) was dropped over 10 min. into a suspension of 98% methyltriphenylphosphonium bromide (4.65 g, 12.8 mmmol) in 40ml of THF stirred at -70°C. After 0.5h, a solution of 1-(3-nitro-2-pyridyl)-4-oxo-piperidine (2 g, 8.5 mmol) in 16 ml of THF was added and the reaction mixture was allowed to warm up to r.t.. After overnight resting, the mixture was quenched with an aqueous saturated solution of NH_4Cl, extracted with EtOAc, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The crude product was purified by automated flash chromatography (SP1®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 1:0 to 9:1 yielding 1.23 g of the title compound as yellow solid.

MS: $[M+H]^+ = 220.18$

3-Phenylethynyl-8-(3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared as described for Compound 1b but using Compound 2a instead of 1-(t.butoxycarbonyl)-4-methylene-piperidine. After the usual work-up, the mixture was purified by automated flash chromatography (SP1®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 95 : 5 to 7 : 3 yielding 25 mg of the title compound. Yield:

MS: $[M+H]^+ = 363.54$

^{1}H-NMR: ($CDCl_3$, δ): 1.90-2.11 (m, 4H), 3.00 (s, 2H), 3.51-3.70 (m, 4H), 6.76-6.83 (m, 1H), 7.34-7.46 (m, 3H), 7.47-7.51 (m, 2H), 8.14-8.18 (m, 1H), 8.36-8.38 (m, 1H).

Example 3

3-[(6-Methyl-2-pyridyl)-ethynyl]-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

6-Methyl-2-(4-methylene-1-piperidyl)-3-nitropyridine (Compound 3a)

The title product was synthesized following the procedure reported for Compound 2a but replacing 1-(3-nitro-2-pyridyl)-4-oxo-piperidine with 1-(6-methyl-3-nitro-2-pyridyl)-4-oxopiperidine. Yield: 62%.

MS: $[M+H]^+ = 233.12$

N-Hydroxy-3-trimethylsilyl-prop-2-ynimidoyl chloride (Compound 3b)

To a solution of 68 g (11.9 mmol) of 3-trimethylsilylprop-2-ynal oxime [Carreira, Erick M. et al., Organic Letters, Volume 7, Issue 10, 2005, Pages 2011-2014] in 11.9 ml of DMF stirred at r.t. was added N-chlorosuccinimide (1.99 g , 14.8 mmol). After 4 h stirring, the solution was poured into water and extracted with Et_2O. After the usual work-up, the residue (2.09 g) was used as it was for the next step.

3-(Trimethylsilyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 3c)

A solution of TEA (0.554 ml, 3.85 mmol) in 9.4 ml of dichloromethane was dropped into a solution of Compound 3b (1.67 g, 2.57 mmol) and Compound 3a (600 mg, 2.57 mmol) in 42 ml of dichloromethane stirred at 0°C. Afterwards, the reaction mixture was stirred at r.t. for 24 h; then it was diluted with cold water. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient CH_2Cl_2 - Petroleum Ether - EtOAc from 5 : 5 to 10 : 0) affording 641 mg of the title product . Yield: 67%.

MS: $[M+H]^+ = 273.43$

3-[(6-Methyl-2-pyridyl)-ethynyl]-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

A suspension of Compound 3c (58 mg, 0.16 mmol), sodium acetate trihydrate (42.5 mg, 0.31 mmol), 2-bromo-6-methylpyridine (26.8 mg, 0.16 mmol), tetrabutylammonium fluoride (40.8 mg, 0.156 mmol) and *tetrakis*(triphenylphosphine)palladium(0) (15.8 mg, 0.014 mmol) in 2.05 ml of DMF flushed with nitrogen was heated in a microwave oven (Biotage) at 120°C for 10 min. Dilution with EtOAc, washing with H_2O and drying over Na_2SO_4 followed by evaporation and purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 9 : 1 to 6 : 4) afforded 17.4 mg (28.5%) of the title compound as yellow oil.

MS: $[M+H]^+ = 392.33$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.07 (m, 4H), 2.48 (s, 3H), 2.66 (s, 3H), 3.04 (s, 2H), 3.50-3.68 (m, 4H), 6.62 (d, J=8Hz, 1H), 7.22-7.26 (m, 1H), 7.42-7.46 (m, 1H), 7.65-7.61 (m,1H), 8.10 (d, J=8Hz, 1H).

Example 4

3-(2-Thienylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 2-bromothiophene in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 9 : 1 to 6 : 4). Yield: 26.7%.

MS: $[M+H]^+ = 383.52$

Example 5

3-(2-Pyridylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 2-iodopyridine in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 9 : 1 to 4 : 6). Yield: 49.6%.

MS: $[M+H]^+ = 378.46$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.09 (m, 4H), 2.49 (s, 3H), 3.03 (s, 2H), 3.51-3.67 (m, 4H), 6.63 (d, J=8Hz,1H), 7.36-7.41 (m, 1H), 7.59-7.63 (m, 1H), 7.76-7.82 (m, 1H), 8.11 (d, J=8Hz, 1H), 8.66-8.69 (m, 1H).

Example 6

3-(2-Fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 2-fluoroiodobenzene in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 95 : 5 to 7 : 3). Yield: 42.6%.

MS: $[M+H]^+ = 395.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.50 (s, 3H), 3.02 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d, J=8Hz, 1H), 7.09-7.21 (m, 2H), 7.36-7.45 (m, 1H), 7.49-7.57 (m,1H), 8.11 (d, J=8Hz, 1H).

Example 7

3-(3-Fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-fluoroiodobenzene in place of 2-bromo-6-methylpyridine. Yield: 42.4%

MS: $[M+H]^+ = 395.41$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.50 (s, 3H), 2.99 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d J=8Hz, 1H), 7.09-7.17 (m, 1H), 7.20-7.27 (m, 1H), 7.30-7.39 (m, 2H), 8.11 (d, 8Hz, 1H).

Example 8

3-(4-Fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 4-fluoroiodobenzene in place of 2-bromo-6-methylpyridine. Yield: 37.7%

MS: $[M+H]^+ = 395.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.10 (m, 4H), 2.50 (s, 3H), 2.99 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d J=8Hz, 1H), 7.04-7.13 (m, 2H), 7.50-7.57 (m, 2H), 8.11 (d, J=8Hz, 1H).

Example 9

3-(2-Furylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 2-bromofuran in place of 2-bromo-6-methylpyridine. Purification of the

residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 95 : 5 to 6 : 4). Yield: 12.6%.

MS: $[M+H]^+ = 367.33$

Example 10

3-[(2-Methyl-1,3-thiazol-4-yl)-ethynyl]-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 4-bromo-2-methylthiazole in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 9 : 1 to 5 : 5). Yield: 17.6%.

MS: $[M+H]^+ = 398.35$

Example 11

3-(Prop-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

3-Ethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 11a)

To a solution of Compound 3c (360 mg, 0.,96 mmol) in 9 ml of THF was added tetrabutylammonium fluoride hydrate (269 mg, 0.963 mmol) and the reaction mixture was stirred at r.t. for 4h. After overnight resting, quenching with water, extraction with EtOAc and evaporation to dryness, the crude was purified by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 95 : 5 to 7 : 3), yielding compound 11a (0.231 g, 80 %).

MS: $[M+H]^+ = 301.36$

3-(Prop-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

Into a solution of Compound 11a (25 mg, 0.83 mmol) in THF (1.26 ml) kept under stirring at -78°C, butyllithium in hexane (2.5 M, 0.04 ml, 0.1 mmol) is dropped. After 30 min., methyl iodide (6.22 µL, 0.1 mmol) was addded. The cold bath was removed and the orange solution was allowed to reach r.t. After overnight resting, it was quenched with a saturated aqueous NH_4Cl solution, extracted with EtOAc, washed with H_2O, dried on Na_2SO_4 and evaporated to dryness *in vacuo*. The crude was purified by automated flash liquid chromatography

(Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 95 : 5 to 6 : 4) giving 6 mg of the title compound. Yield: 22.9 %.

MS: $[M+H]^+ = 315.32$

Example 12

3-(3-Chlorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-chloroiodobenzene in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 9 : 1 to 7 : 3). Yield: 55%.

MS: $[M+H]^+ = 411.16$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.50 (s, 3H), 2.99 (s, 2H), 3.50-3.70 (m, 4H), 6.64 (d, J=8Hz, 1H), 7.29-7.35 (m, 1H), 7.39-7.45 (m, 2H), 7.53 (m, 1H), 8.11 (d, 8Hz, 1H).

Example 13

3-(3-Methylphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-iodotoluene in place of 2-bromo-6-methylpyridine. Yellow solid. Yield: 59.6%

MS: $[M+H]^+ = 391.42$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.91-2.11 (m, 4H), 2.37 (s, 3H), 2.51(s, 3H), 2.99 (s, 2H), 3.52-3.70 (m, 4H), 6.64 (d, J=8Hz, 1H), 7.20-7.27 (m, 2H), 7.29-7.38 (m, 2H), 8.12 (d, J=8Hz, 1H).

Example 14

3-(3-Methoxyphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-methoxyiodobenzene in place of 2-bromo-6-methylpyridine. Yellow solid. Yield: 41%

MS: $[M+H]^+ = 407.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.09 (m, 4H), 2.49 (s, 3H), 3.00 (s, 2H), 3.52-3.70 (m, 4H), 3.84 (s, 3H), 6.63 (d, J=8Hz, 1H), 6.95-7.00 (m, 1H), 7.06-7.08 (m, 1H), 7.12-7.16 (m, 1H), 7.26-7.32(m, 1H), 8.11 (d, J=8Hz, 1H).

Example 15

3-(3-Cyanophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-iodobenzonitrile in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon®TM – Biotage) (Petroleum Ether – EtOAc gradient from 95 : 5 to 5 : 5). Yield: 66.5%.

MS: $[M+H]^+ = 402.23$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.09 (m, 4H), 2.49 (s, 3H), 3.00(s, 2H), 3.50-3.70 (m, 4H), 6.64 (d, J=8Hz, 1H), 7.49-7.55 (m, 1H), 7.67-7.71 (m, 1H), 7.74-7.78 (m, 1H), 7.81-7.83 (s, 1H), 8.11 (d, J=8Hz, 1H).

Example 16

3-(3-Thienylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-bromothiophene in place of 2-bromo-6-methylpyridine. Purification of the residue by automated flash liquid chromatography (Horizon™ – Biotage) (Petroleum Ether – EtOAc gradient from 7 : 3 to 3 : 7). Yellow solid. Yield: 66.5%.

MS: $[M+H]^+ = 383.22$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.01 (m, 4H), 2.49 (s, 3H), 2.98 (s, 2H), 3.50-3.70 (m, 4H), 6.62 (d, J=8Hz, 1H), 7.19-7.22 (m, 1H), 7.32-7.35 (m, 1H), 7.62-7.63 (m, 1H), 8.11 (d, J=8Hz, 1H).

Example 17

3-(3-Pyridylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 3-iodopyridine in place of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 7 : 3 to 3 : 7) affording the title product as yellow solid. Yield: 66.7%.

MS: $[M+H]^+ = 378.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.09 (m, 4H), 2.49 (s, 3H), 3.01(s, 2H), 3.50-3.70 (m, 4H), 6.63 (d, J=8Hz, 1H), 7.33-7.38 (m, 1H), 7.83-7.87 (m, 1H), 8.11 (d, J=8Hz, 1H), 8.63-8.65 (m, 1H), 8.78-7.80 (s, 1H).

Example 18

3-{3-(3-Methyl-[1,2,4]oxadiazol-5-yl)-phenylethynyl}-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 5-(3-bromo-phenyl)-3-methyl-[1,2,4]oxadiazole in place of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 6 : 4) affording the title product as yellow solid . Yield: 33.8%.

MS: $[M+H]^+ = 459.11$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.92-2.11 (m, 4H), 2.50-2.52 (m, 6H), 3.01(s, 2H), 3.52-3.70 (m, 4H), 6.64 (d, J=8Hz, 1H), 7.54-7.59 (m, 1H), 7.72-7.76 (m, 1H), 8.10-8.17 (m, 2H), 8.29-8.31 (m, 1H).

Example 19

3-(3-Methylbut-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diaza-spiro[4.5]dec-2-ene

3-Bromo-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 19a)

A suspension of 6-methyl-2-(4-methylene-1-piperidyl)-3-nitropyridine (Compound 3a, 350 mg, 1.5 mmol), sodium carbonate (1.26 g, 15 mmol) and dibromoformaldoxime (608 mg, 3 mmol) in 25 ml of EtOAc was stirred at r.t. for 2 days. Afterwards, the reaction mixture was washed with water, dried over sodium sulphate and evaporated to dryness *in vacuo*. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 9 : 1 to 6 : 4) giving 377 mg of the title product. Yield: 70.8%.

MS: $[M+H]^+ = 356.41$

3-(3-Methylbut-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diaza-spiro[4.5]dec-2-ene

A mixture of Compound 19a (50 mg, 0.141 mmol), 3-methyl-1-butyne (48 mg, 0.705 mmol), sodium carbonate (74.6 mg, 0.704 mmol) and Cu (44.8 mg, 0.705 mmol) was heated in a sealed vessel at 120°C for 32h, adding every 8h additional equal amounts of amounts of 3-methyl-1-butyne, sodium carbonate and Cu. Afterwards, the mixture was cooled, added with CH_2Cl_2, filtered on celite and evaporated to dryness *in vacuo*. The residue was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from

95 : 5 to 75 : 25 then isocratic Petroleum Ether - EtOAc from 9 : 1 giving 16 mg of the title product. Yield: 33.1%.

MS: $[M+H]^+ = 343.20$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.26 (d, 6H, J=8Hz), 1.86-2.05 (m, 4H), 2.49 (s, 3H), 2.73-2.81 (m, 1H), 2.86 (s, 2H), 3.48-3.65 (m, 4H), 6.62 (d, J=8Hz, 1H), 8.10 (d, J=8Hz, 1H).

Example 20

3-(Hex-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was synthesized following the method described for the compound of Example 19, but replacing 3-methyl-1-butyne with hex-1-yne. The residue was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 85 : 15) giving 7.8 mg of the title product. Yield: 27.9 %.

MS: $[M+H]^+ = 357.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 0.95-0.99 (m, 3H), 1.26-1.65 (m, 4H), 1.86-2.03 (m, 4H), 2.38-2.45 (m, 2H), 2.48 (s, 3H), 2.86 (s, 2H), 3.48-3.65 (m, 4H), 6.61 (d, J=8Hz, 1H), 8.10 (d, 8Hz, 1H).

Example 21

3-(2-Cyanophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 2-bromobenzonitrile in place of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 9 : 1 to 4 : 6) affording the title product as yellow oil . Yield: 74.8 %.

MS: $[M+H]^+ = 402.08$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.49 (s, 3H), 3.06 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d, J=8Hz, 1H), 7.48-7.54 (m, 1H), 7.60-7.74 (m, 3H), 8.11 (d, J=8Hz, 1H).

Example 22

3-(3-Chlorophenyl-ethynyl)-8-(3-cyano-2-pyrazinyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

3-Trimethylsilylethynyl-8-(t.butoxycarbonyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 22a)

The title compound was synthesized following the method reported for Compound 3c but using 1-(t.butoxycarbonyl)-4-methylenepiperidine instead of Compound 3a. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 6 : 4) affording the title product as white solid. Yield: 43.3%.

MS: $[M+H]^+ = 337.13$

3-(3-Chlorophenyl-ethynyl)-8-(t.butoxycarbonyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 22b)

The title compound was synthesized following the method reported for the compound of Example 3, substituting 2-bromo-6-methylpyridine with 1-chloro-3-iodobenzene and Compound 3c with Compound 22a. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 6 : 4) affording the title product as white solid . Yield: 76.9%.

MS: $[M+H]^+ = 375.14$

3-(3-Chlorophenyl-ethynyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene (Compound 22c)

The title compound was synthesized following the method reported for Compound 1c, substituting Compound 1b with Compound 22c. The crude residue was used without further purification in the next reaction. Yield: 90.4 %.

MS: $[M+H]^+ = 275.12$

3-(3-Chlorophenyl-ethynyl)-8-(3-cyano-2-pyrazinyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was prepared following the method described for the compound of Example 1, replacing Compound 1c with Compound 22d and 2-chloro-6-methyl-3-nitropyridine with 2-chloro-3-cyanopyrazine. Brownish dense oil. Yield: 83.3%.

MS: $[M+H]^+ = 378.14$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.00 (m, 2H), 2.10-2.15 (m, 2H), 2.99 (s, 2H), 3.65-3.78 (m, 2H), 4.20-4.27 (m, 2H), 7.29-7.35 (m, 1H), 7.39-7.45 (m, 2H), 7.54 (s, 1H), 8.05 (s, 1H), 8.28 (s, 1H).

Example 23

3-(2-Methylphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure reported for the compound of Example 3, replacing 2-bromo-6-methylpyridine with 2-bromotoluene. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 8 : 2) affording the title product as yellow solid . Yield: 29.6%.

MS: $[M+H]^+ = 391.09$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.46-2.50 (2s, 6H), 3.01 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d J=8Hz, 1H), 7.18-7.34 (m, 3H), 7.48-7.52 (m, 1H), 8.11 (d, J=8Hz, 1H).

Example 24

3-(2-Chlorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title product was prepared following the procedure reported above for the compound of Example 3, but using 1-chloro-2-iodobenzene in place of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 7 : 3) affording the title product as yellow oil . Yield: 57.2%.

MS: $[M+H]^+ = 410.97$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.90-2.11 (m, 4H), 2.49 (s, 3H), 3.03 (s, 2H), 3.50-3.70 (m, 4H), 6.63 (d J=8Hz, 1H), 7.26-7.38 (m, 2H), 7.44-7.48 (m, 1H), 7.56-7.60 (m,1H), 8.11 (d, J=8Hz, 1H).

Example 25

3-(2-Pyrazinyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing 2-bromo-6-methylpyridine with 2-iodopyrazine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 8 : 2 to 3 : 7) affording the title product as yellow solid . Yield: 28.1 %.

MS: $[M+H]^+ = 379. 22$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.93-2.11 (m, 4H), 2.51 (s, 3H), 3.03 (s, 2H), 3.51-3.71 (m, 4H), 6.64 (d J=8Hz, 1H), 8.12 (d, J=8Hz, 1H), 8.60 (dd, J=16Hz, 4Hz, 2H), 8.80 (s, 1H).

Example 26

3-Phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-8-azaspiro[4.5]dec-3-ene

3-Hydroxy-3-phenylethynyl-8-ethoxycarbonyl-1-oxa-8-azaspiro[4.5]decane (Compound 26a)

To a solution of 82 mg (0.362 mmol) of ethyl 3-oxo-1-oxa-8-azaspiro[4.5]decane-8-carboxylate [Edwin S. C. Wu et al., *J. Med. Chem.*, **1995**, *38* (9), pp 1558–1570] in THF (4 ml) was added dropwise a 1M solution of phenylethynylmagnesium bromide in THF (0.724 ml, 0.724 mmol), stirring at r.t. under anhydrous nitrogen atmosphere. The reaction mixture was stirred at r.t. for 4 h, then at 60°C for 6 h, then it was quenched with a saturated aqueous solution of ammonium chloride, extracted with EtOAc. The combined organic layers were washed with brine, dried over sodium sulphate and evaporated to dryness *in vacuo*. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 75 : 25 to 4 : 6) affording the title product as yellow solid . Yield: 87.2 %.
MS: $[M+H]^+ = 330.33$

3-Phenylethynyl-8-ethoxycarbonyl-1-oxa-8-azaspiro[4.5]dec-3-ene (Compound 26b)

To a solution of Compound 26a (93 mg, 0.282 mmol) stirred in pyridine (4 ml) at 0°C was added thionyl chloride (0.103 ml, 1.141 mmol) and the reaction mixture was stirred at 0° C to r.t. for 2 h. Afterwards, it was poured into water, acidified with 1M HCl and extracted with chloroform. The organic layers were combined, washed with brine, dried over sodium sulphate and evaporated to dryness to afford a crude, which was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 92 : 8 to 75 : 25) to give 18 mg of the title compound.
MS: $[M+H]^+ = 312.34$

3-Phenylethynyl-1-oxa-8-azaspiro[4.5]dec-3-ene (Compound 26c)

A mixture of Compound 26b (25 mg, 0.803 mmol), KOH (80 mg, 1.43 mmol), water (2 ml) and MeOH (4 ml) was stirred at reflux for 2 h. fterwards it was extracted with EtOAc. The organic layer was washed with brine, dried over sodium sulphate and evaporated to dryness *in vacuo.* The crude residue (19.2 mg) was used without any kind of purification in the next step.
MS: $[M+H]^+ = 240.10$

3-Phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-8-azaspiro[4.5]dec-3-ene

The title compound was prepared following the method described for the compound of Example 1, replacing Compound 1c with Compound 26c. The crude was purified by automated

flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 98 : 2 to 9 : 1) to give 8 mg of the title compound. Yield: 51%

MS: $[M+H]^+ = 376.14$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.75-1.87 (m, 2 H), 1.87-1.98 (m, 2 H), 2.48 (s, 3 H), 3.49 (ddd, *J*=13.39, 10.70, 3.06 Hz, 2 H), 3.68 (dt, *J*=13.45, 4.16 Hz, 2 H), 4.76 (d, *J*=2.20 Hz, 2 H), 6.13 (t, *J*=2.20 Hz, 1 H), 6.58 (d, *J*=8.31 Hz, 1 H), 7.32-7.40 (m, 3 H), 7.43-7.50 (m, 2 H), 8.08 (d, *J*=8.07 Hz, 1 H).

Example 27

3-(3-Chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

1-(t.butoxycarbonyl)-3-methylene-pyrrolidine (Compound 27a)

The title compound was synthesised following the method described above for Compound 2a, but using 1-(t.butoxycarbonyl)-3-pyrrolidinone instead of 1-(3-nitro-2-pyridyl)-4-oxopiperidine. After the usual work-up, the crude product was purified by automated flash chromatography (Horizon®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 95 : 5 to 7 : 3, yielding the title compound as brownish oil. Yield: 75.8 %.

MS: $[M+H]^+ = 184.53$

3-Trimethylsilylethynyl-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 27b)

The title compound was synthesised following the method described above for Compound 3c, but using Compound 27a instead of Compound 3a. After the usual work-up, the crude product was purified by automated flash chromatography (Horizon®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 95 : 5 to 75 : 25 yielding the title compound. Yield: 82.9 %.

MS: $[M+H]^+ = 323.45$

3-(3-Chlorophenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 27c)

The title compound was prepared following the procedure reported for the compound of Example 3, but using 1-chloro-3-iodobenzene instead of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM –

Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 6 : 4) affording the title product as yellow oil. Yield: 14 %.

MS: $[M+H]^+ = 361.13$

3-(3-Chlorophenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 27d)

The title compound was synthesized following the method reported for Compound 1c, but substituting Compound 27c for Compound 1b. The crude residue was used without further purification in the next reaction. Yield: …..

MS: $[M+H]^+ = 261.11$

3-(3-Chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was prepared following the method described for the compound of Example 1, substituting Compound 27d for Compound 1c. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - Acetone from 95 : 5 to 9 : 1) to give the title compound as a dense yellow oil. Yield: 68.5%.

^{1}H-NMR: *($CDCl_3$, δ)*:_2.13-2.25 (m, 1 H), 2.46 (dd, *J*=12.84, 6.48 Hz, 1 H), 2.52 (s, 3 H), 3.17-3.34 (m, 2 H), 3.42 (d, *J*=12.47 Hz, 1 H), 3.69 (t, *J*=9.78 Hz, 1 H,) 3.82 (d, *J*=12.47 Hz, 1 H), 3.98 (td, *J*=10.94, 6.97 Hz, 1 H), 6.61 (d, *J*=8.07 Hz, 1 H), 7.30-7.36 (m, 1 H,) 7.37-7.45 (m, 2 H), 7.53 (s, 1 H), 8.07 (d, *J*=8.31 Hz, 1 H).

Example 28

3-(3-Chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

1-(t.butoxycarbonyl)-3-methylene-azetidine (Compund 28a)

To a suspension of methyltriphenylphosponium bromide (1.56 g, 4.37 mmol) in 30 ml of Et_2O stirred at 0°C was added potassium *tert*-butylate (0.459 g, 4.09 mmol). After 0.5h the cold bath was removed and the mixture was stirred for 1h at r.t.. Afterrwards, it was cooled off in water-ice bath, and 1-(t.butoxycarbonyl)-3-azetidinone (500 mg, 2.92 mmol) was added. The cold bath was removed. After overnight resting, the reaction mixture was quenched with a saturated solution of NH_4Cl in water, extracted with Et_2O, dried on Na_2SO_4, evaporated to dryness *in vacuo*. dissolvent. The residual crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - Acetone from 10 : 0 to 7 : 3) to give the title compound (426 mg) as a dense colourless oil. Yield: 86.2%.

MS: $[M+H]^+ = 170.45$

3-Trimethylsilylethynyl-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 28b)

The title compound was synthesised following the method described above for Compound 3c, but using Compound 28a instead of Compound 3a. After the usual work-up, the crude product was purified by automated flash chromatography (Horizon®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 95 : 5 to 6 : 4, yielding the title compound.

Yield: 62%.

MS: $[M+H]^+ = 309.16$

3-(3-Chlorophenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 28c)

The title compound was prepared following the procedure reported for the compound of Example 3, but using 1-chloro-3-iodobenzene instead of 2-bromo-6-methylpyridine. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 8 : 2 to 65 : 35) affording the title product as yellow oil . Yield: 24.3 %.

MS: $[M+H]^+ = 347.09$

3-(3-Chlorophenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 28d)

The title compound was synthesized following the method reported for Compound 1c, but substituting Compound 1b with Compound 28c. The crude residue was used without further purification in the next reaction step.

MS: $[M+H]^+ = 247.13$

3-(3-Chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 28d. The crude was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether – Dichloromethane 1 : 9) to give the title compound as a dense yellow oil. Yield: 68.5%

MS: $[M+H]^+ = 383.08$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.51 (s, 3 H), 3.49 (s, 2 H), 4.38 (d, *J*=10.76 Hz, 2 H), 4.49 (d, *J*=11.00 Hz, 2 H), 6.65 (d, *J*=8.31 Hz, 1 H), 7.33 (t, *J*=8.10 Hz, 1 H), 7.42 (t, *J*=8.07 Hz, 2 H),7.54 (s, 1 H), 8.17 (d, *J*=8.31 Hz, 1 H).

Example 29 and 30

4-Phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-4-ene and 4-Phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-3-ene

4-Methylene-9-(t.butoxycarbonyl)-1-oxa-9-azaspiro[5.5]undecane (Compound 29a)

To a solution of 1-Boc-piperidone (2.39 g, 12 mmol) and ethoxytrimethylsilane (3.75 ml, 24 mmol) in 80 ml of CCl_4 under stirring at 0°C was added trimethylsilyl trifluoromethylsulphonate (217 µl, 1.2 mmol) and trimethyl-(2-methylene-4-trimethylsilyloxybutyl)-silane (E. I. Marko et al., Journal of Organic Chemistry, 1992, 57, 2211-2213) dissolved in in 23 ml of CCl_4. After stirring at 0-5°C for 6h and overnight resting at 0°C, the reaction mixture was washed with water, dried on Na_2SO_4, filtered and the solvent was evaporated to dryness *in vacuo*. The residual 6.5 g of colourless oil was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether – EtOAc gradient from 99:1 to 85:15) to give 3.2 g of the title compound as colourless oil. Yield: 100%.

MS: $[M+H]^+ = 268.62$

4-Oxo-9-(t.butoxycarbonyl)-1-oxa-9-azaspiro[5.5]undecane (Compound 29b)

To a solution of Compound 29a (1.6 g, 5.99 mmol) in a mixture of dioxane – water (32.9 ml and 11 ml respectively) under stirring at r.t.was added a 4% sol. of osmium tetroxide in water (0.762 µl, 0.125 mmol). After 2h, was added portionwise milled sodium metaperiodate (2.56 g, 12 mmol) affording a suspension that tended to become clear gray from brownish. After 4h H_2O was added, extracting with EtOAc, washing with H_2O, drying over Na_2SO_4, evaporating the solvent to dryness. The residual tawny semi-solid was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether – EtOAc 7 : 3) to give 0.96 g of the title compound as a grey solid. Yield: 59.5 %

MS: $[M+H]^+ = 270.33$

4-Hydroxy-4-Phenylethynyl-9-(t.butoxycarbonyl)-1-oxa-9-azaspiro[5.5]undecane (Compound 29c)

The title compound was prepared following the method reported above for Compound 26a, substituting ethyl 3-oxo-1-oxa-8-azaspiro[4.5]decane-8-carboxylate with Compound 29b. After the usual work-up the oily residue was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether – EtOAc gradient 9: 1 to 6:4) to give 0.96 g of the title compound as a grey solid. Yield: 57.3 %

MS: $[M+H]^+ = 372.14$

4-Phenylethynyl-1-oxa-9-azaspiro[5.5]undec-3-ene and
4-Phenylethynyl-1-oxa-9-azaspiro[5.5]undec-4-ene (Compounds 29d)

The title compounds was prepared following the method reported here for Compound 26c, substituting Compound 26b with Compound 29c. After the usual work-up, the residual oil was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether – EtOAc gradient 9: 1 to 8:2) to give 0.49 g (33.6%) of the title compounds as a brownish oil, used in the next step without separation.

MS: $[M+H]^+ = 254.17$

4-Phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-4-ene and
4-Phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-3-ene

The title compounds were prepared following the method described for the compound of Example 1, replacing Compound 1c with Compounds 29d. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – CH_2Cl_2 from 4 : 6 to 3 : 7) to give 5 mg of the compound of Example 29 and 1 mg of the compound of Example 30.

Example 29

MS: $[M+H]^+ = 390.29$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.73-1.90 (m, 4 H), 2.32-2.39 (m, 2 H), 2.47 (s, 3 H), 3.38-3.50 (m, 2 H), 3.60-3.72 (m, 2 H), 3.87 (t, *J*=5.38 Hz, 2 H), 6.07 (s, 1 H), 6.57 (d, *J*=8.31 Hz, 1 H), 7.30-7.37 (m, 3 H), 7.42-7.50 (m, 2 H), 8.08 (d, *J*=8.07 Hz, 1 H).

Example 30

MS: $[M+H]^+ = 390.16$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.69-1.79 (m, 2 H), 1.95 (d, *J*=13.20 Hz, 2 H), 2.26 (d, *J*=1.96 Hz, 2 H), 2.48 (s, 3 H), 3.36-3.50 (m, 2 H), 3.58 - 3.70 (m, 2 H), 4.30 (d, *J*=2.69 Hz, 2 H), 6.20 (br. s., 1 H), 6.58 (d, *J*=8.31 Hz, 1 H), 7.31-7.41 (m, 3 H), 7.45 (dd, *J*=6.60, 2.93 Hz, 2 H), 8.08 (d, *J*=8.31 Hz, 1 H).

Example 31

3-(3-Chlorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

8-Methylene-1,4-dioxaspiro[4.5]decane (Compound 31a)

The title compound was prepared following the method reported above for Compound 2a, but using lithium-*bis*-trimethylsilylamide instead of butyl lithium and carrying out the reaction at -20°C. As starting material, 1,4-dioxaspiro[4.5]decan-8-one replaced 1-(3-nitro-2-pyridyl)-4-oxopiperidine. After the usual work-up the residue was purified by automated flash chromatography (Horizon®TM – Biotage; Petroleum Ether – EtOAc 95 : 5) to give the title compound as fluid clourless oil. Yield: 99.3 %.

MS: $[M+H]^+ = 155.13$

3-Trimethylsilylethynyl-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene (Compound 31b)

The title compound was synthesized using the method reported here for Compound 3c, but using Compound 31a in place of Compound 3a. After the usual work-up the residue was purified by automated flash chromatography (Horizon®TM – Biotage; gradient Petroleum Ether – EtOAc from 98 : 2 to 9 : 1) to give the title compound.

3-(3-Chlorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method reported above for the compound of Example 3, but replacing 2-bromo-6-methylpyridine with 1-chloro-3-iodobenzene. After the usual work-up, the residue was purified by automated flash chromatography (Horizon®TM – Biotage; Petroleum Ether – EtOAc 85 : 15) to give the title compound as a grey solid.

Yield: 80.1%.

Alternative procedure:

1-Chloro-3-(3,3-diethoxyprop-1-ynyl)-benzene (compound 31c)

A mixture of 1-chloro-3-iodobenzene (4 g, 16.8 mmol), propargylaldehyde diethyl acetal (2.66 ml, 18.5 mmol), *bis*(trifenilphosphine)palladium(II)dichloride (295 mg, 0.42 mmol), cuprous iodide (160 mg, 0.84 mmol) and triethylamine (60 ml) was stirred at r.t. for 3h. After 4h, the reaction mixture was quenched with H_2O and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4,and evaporated to dryness *in vacuo*. The residue was purified by automated flash chromatography (Horizon®TM – Biotage; Petroleum Ether – EtOAc 97 : 3) to give 4 g of the title compound as a fluid yellowish oil. Yield: 100%.

MS: $[M+H]^+ = 239.32$

3-(3-chlorophenyl)-prop-2-ynal (Compound 31d)

38.8 ml of water and 7.7 ml of trifluoroacetic acid were added to a solution of Compound 31c (4g, 16.7 mmol) in CH_2Cl_2. After 4 h under stirring, further 4 eq. of trifluoroacetic acid was added. After 24h, the conversion was completed, the 2 layers were separated, the organic layer was washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*.to afford the title compound as yellow-brownish oil, used in the next step without further purification.

MS: $[M+H]^+ = 165.35$

3-(3-Chlorophenyl)-prop-2-ynal oxime(Compound 31e)

The title compound was prepared following the method above described for Compound 1a, using Compound 31d instead of phenylpropargylaldehyde. The pale brown residue was used in the next step without further purification. Yield: 96.4%.

MS: $[M+H]^+ = 180.16$

3-(3-Chlorophenyl)-N-hydroxy-prop-2-ynimidoyl chloride (Compound 31f)

The title compound was prepared following the method herein described for Compound 3b, using Compound 31e instead of 3-trimethylsilylprop-2-ynal oxime. The pale brown residue was used in the next step without further purification. Yield: 96.4%.

3-(3-Chlorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 31a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (Horizon®TM – Biotage; gradient Petroleum Ether – Acetone from 95 : 5 to 9 : 1) to give the title compound as a grey solid. Yield: 64.8 %

MS: $[M+H]^+ = 332.14$

^{1}H-NMR: *($CDCl_3$, δ)*:_1.63-1.74 (m, 2 H), 1.88 (dd, *J*=13.94, 2.45 Hz, 2 H),1.96-2.10 (m, 4 H), 2.92 (s, 2 H), 3.92-4.05 (m, 4 H), 7.32 (d, *J*=7.58 Hz, 1 H), 7.35-7.44 (m, 2 H), 7.52 (s, 1 H).

Example 32

3-(3-Chlorophenyl-ethynyl)-8-oxo-1-oxa-2-azaspiro[4.5]dec-2-ene

A solution of the compound of Example 31 (0.76 g, 2.3 mmol) in 5 ml of trifluoroacetic acid was diluted with water (0.21 ml) and stirred at 0-4°C for 4 h, alkalinized with 3N NaOH, extracted with EtOAc, washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo* to afford the title compound as yellow-brownish oil. The residue was purified by automated flash chromatography (Horizon®TM – Biotage; Petroleum Ether – EtOAc 8 : 2) to give the title compound as a grey solid. Yield: 53.8 %

MS: $[M+H]^+ = 288.14$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.99-2.10 (m, 2 H), 2.28-2.44 (m, 4 H), 2.78-2.90 (m, 2 H), 3.03 (s, 2 H), 7.33 (t, *J*=7.60 Hz, 1 H), 7.38-7.46 (m, 2 H), 7.53 (s, 1 H).

Example 33

3-(3-Chlorophenyl-ethynyl)-8-methylene-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized using the same methodology described for Compound 31a, but starting from the compound of Example 32 instead of 1,4-dioxaspiro[4.5]decan-8-one. After the usual work-up, the residue was purified by automated flash chromatography (Horizon®TM – Biotage;gradient Petroleum Ether – Acetone EtOAc from 95 : 5 to 9 : 1) to give the title compound as a grey solid. Yield: 67.3 %

MS: $[M+H]^+ = 286.14$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.64-1.78 (m, 2 H) 1.93-2.06 (m, 2 H), 2.16-2.25 (m, 2 H), 2.50 (ddd, *J*=13.75, 9.60, 4.52 Hz, 2 H), 2.93 (s, 2 H), 4.74 (s, 2 H), 7.29-7.34 (m, 1 H), 7.35-7.43 (m, 2 H), 7.47-7.57 (m, 1 H).

Example 34

3-(3-Chlorophenyl-ethynyl)-8-methoxyimino-1-oxa-2-azaspiro[4.5]dec-2-ene

A mixture of the compound of Example 31 (80 mg, 0.28 mmol), O-methyl hydroxylamine hydrochloride (30.2 g, 0.361 mmol), MeOH (12 ml) and triethylamine (58 µl, 0.42 mmol) was

stirred at r.t. for 12h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with brine, dried over sodium sulphate and evaporated to dryness *in vacuo.* The residue was purified by automated flash chromatography (Horizon®TM – Biotage; gradient Petroleum Ether – EtOAC from 9 : 1 to 8 : 2) to give 56 mg of the title compound as yellow oil.

Yield: 63.6%

MS: $[M+H]^+ = 317.18$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.70-1.89 (m, 2 H), 2.04-2.20 (m, 2 H), 2.34 (dt, *J*=14.37, 4.68 Hz, 1 H), 2.48 (ddd, *J*=14.92, 11.25, 5.38 Hz, 1 H), 2.61 (ddd, *J*=14.49, 11.31, 5.01 Hz, 1H), 2.88-2.94 (m, 1 H), 2.95 (s, 2 H), 3.86 (s, 3 H), 7.29-7.35 (m, 1 H), 7.36-7.44 (m, 2 H), 7.51- 7.54 (m, 1 H).

Example 35

3-[(6-Methyl-2-pyridyl)-ethynyl]-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

3-[(6-methyl-2-pyridyl)-ethynyl]-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 35a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 3c with Compound 27b. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 75 : 25 to 40 : 60) affording the title product as colourless oil .

Yield: 66.2 %.

MS: $[M+H]^+ = 342.51$

3-[(6-methyl-2-pyridyl)-ethynyl]-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 35b)

The title compound was synthesized following the method reported for the Compound 1c, but substituting Compound 35a for Compound 1b and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next step.

Yield: 94.5 %.

MS: $[M+H]^+ = 242.33$

3-[(6-methyl-2-pyridyl)-ethynyl]-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was prepared following the method described for the compound of Example 1, replacing Compound 1c with Compound 35b. The crude was purified by automated RP chromatography (SP1®TM – Biotage; gradient MeCN – H_2O from 4 : 6 to 7 : 3) to give the title compound as yellow solid. Yield: 41.5 %.

MS: $[M+H]^+$ = 378.62

^{1}H-NMR: *($CDCl_3$, δ)*: 2.17 (d, 1H), 2.43 (dd, 1 H), 2.49 (s, 3 H), 2.63 (s, 3 H), 3.21-3.35 (m, 2 H), 3.42 (d, 1H), 3.63 (t, 1 H), 3.79 (d, 1H), 3.97 (m, 1H), 6.59 (d, 1H), 7.22 (d, 1H), 7.41 (d, 1H), 7.65 (t, 1H), 8.05 (d, 1H).

Example 36

3-Phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

3-Phenylethynyl-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 36a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 3c with Compound 27b and 2-bromo-6-methylpyridine with iodobenzene. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 60 : 40) affording the title product as colourless oil . Yield: 40.9%.

MS: $[M+H]^+$ = 327.35

3-Phenylethynyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 36b)

The title compound was synthesized following the method reported for the Compound 1c, substituting Compound 36a for Compound 1b and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next reaction step. Yield: 91.3 %.

MS: $[M+H]^+$ = 227.33

3-Phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 36b. The crude was purified by

automated chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to70 : 30) to give the title compound as yellow solid. Yield: 26.3%.

MS: $[M+H]^+ = 363.44$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.12-2.23 (m, 1 H), 2.39-2.53 (m, 1 H), 2.49 (s, 3 H), 3.18-3.32 (m, 2 H), 3.40 (d, 1H), 3.65 (dd, 1 H), 3.78 (d, 1 H), 3.96 (m, 1 H), 6.59 (d, 1 H), 7.34-7.46 (m, 3 H), 7.54 (dd, 2 H), 8.05 (d, 1 H).

Example 37

3-(3-Fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

3-(3-Fluorophenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 37a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 27b with Compound 3c and 2-bromo-6-methylpyridine with 1-fluoro-3-iodobenzene. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 60 : 40) affording the title product as colourless oil. Yield: 46.9%.

MS: $[M+H]^+ = 345.40$

3-(3-Fluorophenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 37b)

The title compound was synthesized following the method reported for the Compound 1c, but substituting Compound 1b with Compound 37a and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next reaction step. Yield: 91.8 %.

MS: $[M+H]^+ = 245.36$

3-(3-Fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 37b. The crude was purified by automated RP chromatography (SP1®TM – Biotage; gradient MeCN – ammonium carbonate buffer from 4: 6 to 7 : 3) to give the title compound as yellow solid. Yield: 74.9 %.

MS: $[M+H]^+ = 381.54$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.13-2.25 (m, 1 H), 2.45 (dd, 1 H), 2.52 (s, 3 H), 3.18-3.33 (m, 2 H), 3.41 (d, 1 H), 3.64-3.73 (m, 1 H), 3.81 (d, 1H), 3.98 (t, 1H), 6.61 (d, 1H), 7.10-7.17 (m, 1H) 7.21-7.27 (m, 1 H), 7.30-7.40 (m, 2 H), 8.07 (d, 1H).

Example 38

3-Phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

3-Phenylethynyl-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 38a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 3c with Compound 28b and 2-bromo-6-methylpyridine with 1-iodobenzene. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 8 : 2 to 65 : 35) affording the title product as colourless oil . Yield: 28.7%.

MS: $[M+H]^+ = 313.36$

3-Phenylethynyl-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 38b)

The title compound was synthesized following the method reported for Compound 1c, but substituting Compound 1b with Compound 38a and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next reaction step. Yield: 100 %.

MS: $[M+H]^+ = 213.25$

3-Phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

The title compound was prepared following the method described for the compound of Example 1, replacing Compound 1c with Compound 37b. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 1 : 0 to 7 : 3) to give the title compound as yellow oil. Yield: 20.9 %.

MS: $[M+H]^+ = 349.36$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.50 (s, 3 H), 3.49 (s, 2 H), 4.38 (d, *J*=10.76 Hz, 2 H), 4.49 (d, *J*=11.00 Hz, 2 H), 6.64 (d, *J*=8.31 Hz, 1 H), 7.34 - 7.48 (m, 3 H), 7.55 (d, *J*=6.60 Hz, 2 H), 8.17 (d, *J*=8.31 Hz, 1 H).

Example 39

3-(3-Hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

3-(3-Hydroxyphenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 39a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 27b with Compound 3c and 2-bromo-6-methylpyridine with 3-iodophenol. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 95 : 5 to 60 : 40) affording the title product as colourless oil . Yield: 31.4%.

MS: $[M+H]^+ = 343.51$

3-(3-Hydroxyphenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene (Compound 39b)

The title compound was synthesized following the method reported for the Compound 1c, but substituting Compound 1b with Compound 39a and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next reaction step. Yield: 84.8 %.

MS: $[M+H]^+ = 243.28$

3-(3-Hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 39b. The crude was purified by automated RP chromatography (SP1®TM – Biotage; gradient MeCN – ammonium carbonate buffer from 4 : 6 to 7 : 3) to give the title compound as yellow solid. Yield: 42.6 %.

MS: $[M+H]^+ = 379.40$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.18 (dd, 1 H), 2.45 (dd, 1H), 2.51 (s, 3H), 3.17-3.33 (m, 2 H), 3.40 (d, 1H), 3.67 (t, 1H), 3.80 (d, 1H), 3.98 (t, 1H), 6.60 (d, 1H), 6.87-6.95 (m, 1H), 6.97-7.03 (m, 1 H), 7.12 (d, 1H), 7.21-7.31 (m, 1 H), 8.06 (d, 1H).

Example 40

3-(3-Fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

3-(3-Fluorophenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 40a)

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 3c with Compound 28b and 2-bromo-6-methylpyridine with 1-fluoro-3-iodobenzene. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 1 : 0 to 60 : 40) affording the title product as colourless oil . Yield: 15.5%.

MS: $[M+H]^+ = 331.35$

3-(3-Fluorophenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 40b)

The title compound was synthesized following the method reported for Compound 1c, but substituting Compound 1b with Compound 40a and running the reaction in chloroform. The crude brownish oily residue was used without further purification in the next reaction step. Yield: 33.4%.

MS: $[M+H]^+ = 231.24$

3-(3-Fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 40b. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 9 : 1 to 5 : 5) to give the title compound as yellow oil. Yield: 36.9 %.

MS: $[M+H]^+ = 367.35$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.54 (s, 3 H) 3.50 (s, 2 H) 4.40 - 4.57 (m, 5 H) 6.66 (d, *J*=8.31 Hz, 1 H) 7.11 - 7.18 (m, 1 H) 7.25 (d, *J*=9.54 Hz, 1 H) 7.31 - 7.42 (m, 2 H) 8.19 (d, *J*=8.31 Hz, 1 H).

Examples 41 to 43

3-(3-Chlorophenyl-ethynyl)-8-(2-pyridylmethylene)-1-oxa-2-azaspiro[4.5]dec-2-ene and 3-(3-Chlorophenyl-ethynyl)-8-hydroxy-8-(2-pyridylmethyl)-1-oxa-2-azaspiro[4.5]dec-2-ene (Isomers 1 and 2)

To a suspension of triphenyl(2-pyridylmethyl)phosphonium chloride (81.1 mg, 0.19 mmol) stirred at - 20°C under anhydrous nitrogen atmosphere, in 2.5 ml was added dropwise lithium-bis-trimethylsilylamide (1M sol. in THF, 0.415 ml, 0.415 mmol). After 30 min, a solution of the compound of Example 32 (50 mg, 0.173 mmol) in 0.92 ml of anhydrous THF was added and the reaction mixture was stirred at -20°C for 1 hour and at r.t.. Then the reaction was heated at 65°C for 2h, cooled to r.t and quenched with NH_4Cl aqueous saturated solution, extracted with EtOAc, dried over sodium sulphate and evaporated to dryness. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 7 : 3 to 3 : 7) to give 24.5 mg of the compound of Example 41 (24.5 %) plus 6.1 mg of the compound of Example 42 and 3.3 mg of the compound of Example 43.

Example 41

MS: $[M+H]^+ = 363.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.72-1.91 (m, 2 H), 1.98-2.08 (m, 1 H),. 2.08-2.18 (m, 1 H), 2.31-2.43 (m, 1 H), 2.71 (t, *J*=9.78 Hz, 1 H), 2.81-3.06 (m, 4 H), 6.42 (br. s., 1 H), 7.14 (br. s., 1 H), 7.21 (d, *J*=7.58 Hz, 1 H), 7.32 (d, *J*=7.58 Hz, 1 H), 7.35-7.45 (m, 2 H), 7.53 (br. s., 1 H), 7.68 (br.s., 1 H), 8.61 (br. s., 1 H).

Example 42

MS: $[M+H]^+ = 381.18$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.56 (d, *J*=12.72 Hz, 2 H), 1.76-1.90 (m, 4 H), 1.95-2.11 (m, 2 H), 2.90 (s, 2 H), 2.96 (s, 2 H), 7.16 (d, *J*=7.82 Hz, 1 H), 7.18-7.25 (m, 1 H), 7.31 (d, *J*=7.58 Hz, 1 H), 7.35-7.44 (m, 2 H), 7.51 (s, 1 H), 7.62-7.74 (m, 1 H), 8.52 (d, *J*=4.65 Hz, 1 H)

Example 43

MS: $[M+H]^+ = 381.18$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.37-1.49 (m, 2 H), 1.62-1.72 (m, 2 H), 1.82 (d, *J*=13.45 Hz, 2 H), 2.23 (td, *J*=12.35, 3.67 Hz, 2 H), 2.93 (s, 2 H), 2.96 (s, 2 H), 7.16 (d, *J*=7.82 Hz, 1 H), 7.20-7.26 (m, 1 H), 7.32 (d, *J*=7.82 Hz, 1 H), 7.35-7.43 (m, 2 H), 7.51 (s, 1 H), 7.69 (t, *J*=7.58 Hz, 1 H)., 8.54 (d, *J*=4.65 Hz, 1 H).

Example 44

3-Phenylethynyl-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was synthesized using the same method described for Compound 1b but using Compound 31a instead of 1-(t.butoxycarbonyl)-4-methylene-piperidine. After the ususal work-up, the title product was purified by automated RP chromatography (Isolera®TM – Biotage; gradient buffer NH_4HCO_3 - MeCN from 40-60 a 20-80. Yield: 44.9 %.

MS: $[M+H]^+ = 298.2$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.64-1.73 (m, 2 H), 1.82-1.90 (m, 2 H), 1.96-2.10 (m, 4 H), 2.94 (s, 2 H), 3.92-4.04 (m, 4 H), 7.33-7.43 (m, 3 H), 7.53 (d, *J*=7.8, 2H).

Example 45

3-Phenylethynyl-8-oxo-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized using the method described for the compound of Example 32, but starting from the compound of Example 44 instead of the compound of Example 31. The crude was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether - EtOAc 8 : 2) to give the title product as white solid. Yield: 80%.

MS: $[M+H]^+ = 254.2$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.00-2.10 (m, 2 H), 2.29-2.44 (m, 4 H), 2.78-2.90 (m, 2 H), 3.05 (s, 2 H), 7.35-7.47 (m, 3 H), 7.55 (d, *J*=6.36 Hz, 2 H).

Example 46

3-Phenylethynyl-8-hydroxyimino-1-oxa-2-azaspiro[4.5]dec-2-ene

The title product was prepared according to the method described above for the preparation of Compound 1a, but using the compound of Example 45 instead of phenylpropargylaldehyde. The crude was purified by automated flash chromatography (SP1®TM – Biotage; Petroleum Ether - EtOAc 6 : 4) to give the title product. Yield: 80%.

MS: $[M+H]^+ = 269.2$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.71-1.89 (m, 2 H), 2.05-2.22 (m, 2 H), 2.31-2.41 (m, 1 H), 2.44-2.58 (m, 1 H), 2.58-2.69 (m, 1 H), 2.97 (s, 2 H), 2.97-3.05 (m,1 H), 7.34-7.46 (m, 3 H), 7.54 (d, *J*=7.09 Hz, 2 H).

Example 47

3-Phenylethynyl-8-(2-oxo-3-tetrahydrofuranyloxy-imino)-1-oxa-2-azaspiro[4.5]dec-2-ene

To a solution of the compound of Example 46 (50 mg, 0.186 mmol) in THF (5 ml) was added sodium hydride (60% oil dispersion, 7.81 mg, 0.195 mmol) and the mixture was stirred at r.t. for 1h. Afterwards, 2-bromo-*gamma*-butyrolactone (17.2 µl, 0.186 mmol) was added and the reaction mixture was stirred overnight quenched with water, and extracted with EtOAc. The combined organic layers were washed with brine and then evaporated to dryness *in vacuo* to afford a crude, which was purified by automated RP chromatography (Isolera®TM – Biotage; gradient buffer NH_4HCO_3 - MeCN from 40-60 a 30-70. Yield: 32 %

MS: $[M+H]^+ = 353.2$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.73-1.88 (m, 2 H), 2.05-2.22 (m, 2 H), 2.30-2.41 (m, 1 H), 2.41-2.70 (m, 4 H), 2.95-3.05 (m, 3 H), 4.27-4.37 (m,1 H), 4.43-4.53 (m, 1 H), 4.80-4.94 (m, 1 H), 7.34-7.45 (m, 3 H), 7.50-7.60 (m, 2 H).

Example 48

3-[(6-Methyl-2-pyridyl)-ethynyl]-8-cyano-8-(2-pyridyl)-1-oxa-2-azaspiro[4.5]dec-2-ene

4-Methylene-1-cyano-1-(2-pyridyl)-cyclohexane (Compound 48a)

The title compound was prepared following the method described for the compound of Example 2a, but replacing 1-(3-nitro-2-pyridyl)-4-oxo-piperidine with 4-oxo-1-cyano-1-(2-pyridyl)-cyclohexane. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 95 : 5 to 85 : 15) to give the title compound as yellow oil. Yield: 92.8 %.

MS: $[M+H]^+ = 199.25$

3-Trimethylsilylethynyl-8-cyano-8-(2-pyridyl)-1-oxa-2-azaspiro[4.5]dec-2-ene (Compound 48b)

The title compound was prepared following the method described for the compound of Example 1b, but using Compound 48a in place of 1-(t.butoxycarbonyl)-4-methylene-piperidine and replacing Compound 1a with Compound 3b. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 95 : 5 to 85 : 15) to give the title compound as yellow oil. Yield: 43.7 %.

MS: $[M+H]^+$ = 338.34

3-[(6-Methyl-2-pyridyl)-ethynyl]-8-cyano-8-(2-pyridyl)-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was prepared following the method described for the compound of Example 3, replacing Compound 3c with Compound 48b. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 65 : 35 to 4 : 6) to give the title compound as yellow oil. Yield: 43.4 %.

MS: $[M+H]^+$ = 357.17

^{1}H-NMR: *(CDCl3, δ)*: 2.06-2.25 (m, 6 H), 2.47-2.58 (m, 2 H), 2.61 (s, 3 H), 3.03 (s, 2 H), 7.20 (d, *J*=7.82 Hz, 1 H), 7.25-7.32 (m, 1 H), 7.40 (d, *J*=7.58 Hz, 1 H), 7.57 (d, *J*=8.07 Hz, 1 H), 7.62 (t, *J*=7.70 Hz, 1 H), 7.72-7.81 (m, 1 H), 8.66 (d, *J*=4.65 Hz, 1 H).

Example 49

3-[(5-Fluoro-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c and 2-bromo-5-fluoropyridine in place of 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 65 : 35 to 4 : 6) to give the title compound as yellow solid. Yield: 30.2 %.

MS: $[M+H]^+$ = 317.13

^{1}H-NMR: *($CDCl_3$, δ)*: 1.58-1.78 (m, 2 H), 1.88 (dd, *J*=13.82, 2.32 Hz, 2 H), 1.95-2.09 (m, 4 H), 2.95 (s, 2 H), 3.92-4.04 (m, 4 H), 7.45 (td, *J*=8.19, 2.93 Hz, 1 H), 7.58 (dd, *J*=8.68, 4.52 Hz, 1 H), 8.51 (d, *J*=2.93 Hz, 1 H).

Example 50

3-[(6-Methyl-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – EtOAc from 65 : 35 to 4 : 6) to give the title compound as yellow solid. Yield: 47.1 %.

MS: $[M+H]^+$ = 313.08

^{1}H-NMR: *($CDCl_3$, δ)*: 1.63-1.72 (m, 2 H), 1.80-1.91 (m, 2 H), 1.95-2.09 (m, 4 H), 2.61 (s, 3 H), 2.95 (s, 2 H), 3.92-4.04 (m, 4 H), 7.19 (d, *J*=7.82 Hz, 1 H), 7.39 (d, *J*=7.58 Hz, 1 H), 7.62 (t, *J*=7.70 Hz, 1 H).

Example 51

3-[(6-Fluoro-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c and 2-bromo-6-fluoropyridine in place of 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 65 : 35 to 4 : 6) to give the title compound as yellow solid. Yield: 51.1 %.

MS: $[M+H]^+$ = 317.00

^{1}H-NMR: *($CDCl_3$, δ)*: 1.62-1.75 (m, 2 H), 1.88 (dd, *J*=13.69, 2.45 Hz, 2 H), 1.95-2.13 (m, 4 H), 2.94 (s, 2 H), 3.88-4.06 (m, 4 H), 6.99 (dd, *J*=8.31, 2.69 Hz, 1 H), 7.45 (dd, *J*=7.46, 2.08 Hz, 1 H), 7.82 (q, *J*=7.91 Hz, 1 H).

Example 52

3-[(6-Fluoro-3-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c and 5-bromo-2-fluoropyridine in place of 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 8 : 2 to 5 : 5) to give the title compound as yellow solid. Yield: 37.2%.

MS: $[M+H]^+$ = 317.00

^{1}H-NMR: *($CDCl_3$, δ)*: 1.64-1.77 (m, 2 H), 1.89 (dd, *J*=13.94, 2.45 Hz, 2 H), 1.96-2.12 (m, 4 H), 2.94 (s, 2 H), 3.87-4.08 (m, 4 H), 6.98 (dd, *J*=8.44, 3.06 Hz, 1 H), 7.83-8.00 (m, 1 H), 8.41 (d, *J*=1.96 Hz, 1 H).

Example 53

3-(3-Nitrophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c and 3-bromo-nitrobenzene in place of 2-bromo-6-methylpyridine. The crude was purified by automated flash

chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 8 : 2 to 5 : 5) to give the title compound as yellow solid. Yield: %.

MS: $[M+H]^+$ = 343.08

^{1}H-NMR: *($CDCl_3$, δ)*: 1.67-1.76 (m, 2 H), 1.82-1.93 (m, 2 H), 1.96-2.11 (m, 4 H), 2.95 (s, 2 H), 3.93-4.05 (m, 4 H), 7.58 (t, *J*=8.07 Hz, 1 H), 7.83 (d, *J*=7.58 Hz, 1 H), 8.26 (dd, *J*=8.31, 1.22 Hz, 1 H), 8.38 (s, 1 H).

Example 54

3-[(2-Methyl-1,3-thiazol-4-yl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

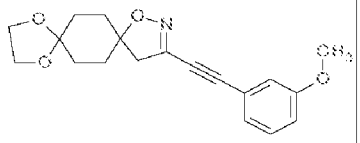
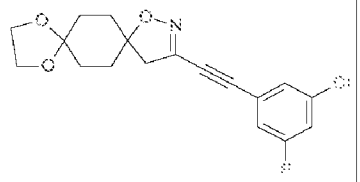
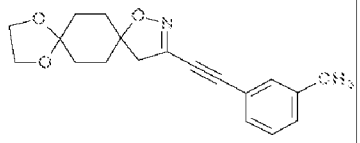
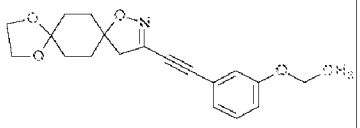
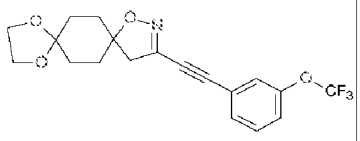
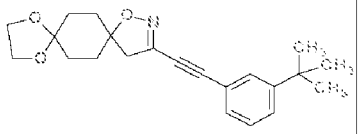
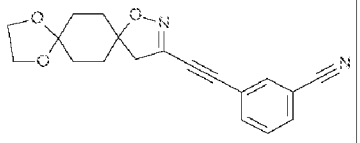
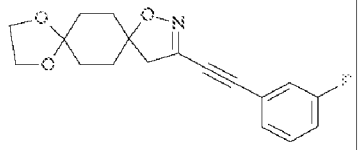
The title compound was prepared following the method described for the compound of Example 3, but using Compound 31b in place of Compound 3c and 4-bromo-2-methyl-1,3-thiazole for 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 6 : 4 to 5 : 5) to give the title compound as yellow solid. Yield: 60.6%.

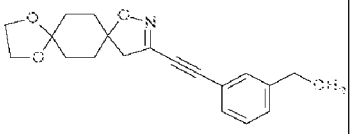
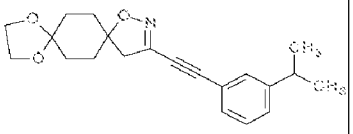
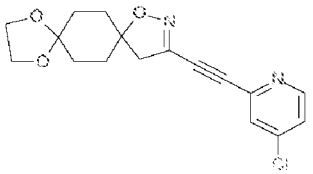
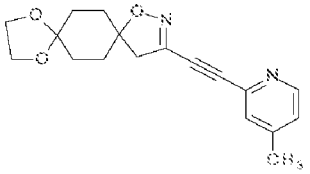
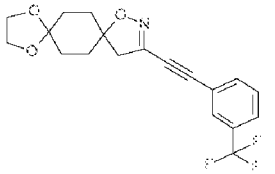
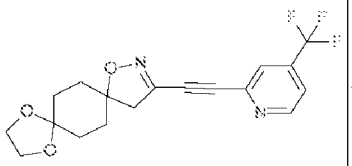
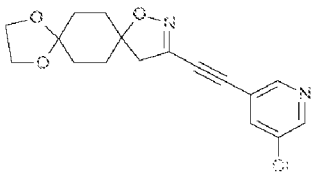
MS: $[M+H]^+$ = 319.23

^{1}H-NMR: *($CDCl_3$, δ)*: 1.63-1.77 (m, 2 H), 1.86 (dd, *J*=13.57, 2.32 Hz, 2 H), 1.92-2.11 (m, 4 H), 2.75 (s, 3 H), 2.92 (s, 2 H), 3.91-4.06 (m, 4 H), 7.48 (s, 1 H).

Examples 55 to 69

Following the method described for the compound of Example 3, but using Compound 31b insteaed of Compound 3c and using the appropriate haloderivative, the Compounds of Examples 55 to 69 were synthesised. Purification was effected by automated flash chromatography (SP1®TM – Biotage). The following Table 1 describes the structure and the analytical characterization of the Compounds of Examples 55 to 69.

Example	Structure	Reagent	MS: $[M+H]^+$	1H-NMR CDCl3(δ),
55		3-iodomethoxybenzene	328.15	1.64 - 1.73 (m, 2 H), 1.88 (d, J=13.20 Hz, 2 H), 1.96 - 2.10 (m, 4 H), 2.94 (s, 2 H), 3.83 (s, 3 H), 3.94 - 4.04 (m, 4 H), 6.93 - 6.99 (m, 1 H), 7.05 (s, 1 H), 7.13 (d, 7.58 Hz, 1 H), 7.25 - 7.32 (m, 1 H)
56		3-chloro-5-fluorobromobenzene	349.89	1.64 - 1.74 (m, 2 H), 1.82 - 1.92 (m, 2 H), 1.95 - 2.09 (m, 4 H), 2.92 (s, 2 H), 3.94 - 4.04 (m, 4 H), 7.10 - 7.18 (m, 2 H), 7.32 (s, 1 H)
57		3-methyliodobenzene	312.2	1.64 - 1.73 (m, 2 H), 1.81 - 1.91 (m, 2 H), 1.96 - 2.11 (m, 4 H), 2.37 (s, 3 H), 2.93 (s, 2 H), 3.93 - 4.04 (m, 4 H), 7.19 - 7.30 (m, 2 H), 7.31 - 7.38 (m, 2 H)
58		3-ethoxybromobenzene	342.14	1.44 (t, J=6.97 Hz, 3 H), 1.64 - 1.74 (m, 2 H), 1.80 -1.92 (m, 2 H), 1.95 - 2.10 (m, 4 H), 2.93 (s, 2 H), 3.93 - 4.02 (m, 4 H), 4.05 (q, J=7.09 Hz, 2 H), 6.91 - 6.98 (m, 1 H), 7.04 (s, 1 H), 7.11 (d, J=7.58 Hz, 1 H), 7.26 (t, J=8.30 Hz, 1 H)
59		3-trifluoromethoxyiodobenzene	382.01	1.64 - 1.74 (m, 2 H), 1.82 - 1.92 (m, 2 H), 1.96 - 2.11(m, 4 H), 2.94 (s, 2 H), 3.93 - 4.05 (m, 4 H), 7.26 (d, J=8.07 Hz, 2 H), 7.36 - 7.49 (m, 3 H)
60		3-*tert*-butyliodobenzene	354.1	1.34 (s, 9 H), 1.63 - 1.74 (m, 2 H), 1.81 - 1.92 (m, 2 H), 1.97 - 2.11 (m, 4 H), 2.94 (s, 2 H), 3.93 - 4.04 (m, 4 H), 7.26 - 7.38 (m, 2 H), 7.44 (d, J=7.58 Hz, 1 H), 7.56 (s, 1 H)
61		3-iodobenzonitrile	323.22	1.64 - 1.74 (m, 2 H), 1.82 - 1.93 (m, 2 H), 1.96 - 2.11(m, 4 H), 2.93 (s, 2 H), 3.92 - 4.05 (m, 4 H), 7.51 (t, J=7.95 Hz, 1 H), 7.68 (d, J=7.82 Hz, 1 H), 7.74 (d, J=7.82 Hz, 1 H), 7.80 (s, 1 H)
62		3-fluoroiodobenzene	316.19	1.63 - 1.74 (m, 2 H), 1.81 - 1.92 (m, 2 H), 1.95 - 2.11 (m, 4 H), 2.93 (s, 2 H), 3.93 - 4.05 (m, 4 H), 7.08 - 7.16 (m, 1 H), 7.22 (d, J=9.54 Hz, 1 H), 7.29 - 7.39 (m, 2 H)

Example	Structure	Reagent	MS: $[M+H]^+$	1H-NMR CDCl3(δ),
63		1-bromo-3-ethylbenzene	326.26	1.26 (t, J=7.58 Hz, 3 H), 1.64 - 1.74 (m, 2 H), 1.81 -1.91 (m, 2 H), 1.96 - 2.10 (m, 4 H), 2.66 (q, J=7.58 Hz, 2 H), 2.93 (s, 2 H), 3.94 - 4.04 (m, 4 H), 7.21 - 7.31 (m, 2 H), 7.35 (d, J=7.34 Hz, 1 H), 7.38 (s, 1 H)
64		1-bromo-3-isopropylbenzene	340.18	1.27 (d, J=6.85 Hz, 6 H), 1.64 - 1.73 (m, 2 H), 1.88 (dd, J=13.94, 2.69 Hz, 2 H), 1.96 - 2.11 (m, 4 H), 2.91 (spt, J=6.80 Hz, 1 H), 2.94 (s, 2 H), 3.94 - 4.04 (m, 4H), 7.24 - 7.29 (m, 1 H), 7.29 - 7.33 (m, 1 H), 7.33 - 7.37 (m, 1 H), 7.41 (s, 1 H)
65		2-bromo-4-chloropyridine	333.15	1.62 - 1.74 (m, 2 H), 1.80 - 1.93 (m, 2 H), 1.94 - 2.10 (m, 4 H), 2.95 (s, 2 H), 3.91 - 4.05 (m, 4 H), 7.35 (d, J=5.13 Hz, 1 H), 7.58 (s, 1 H), 8.54 (d, J=5.14 Hz, 1H)
66		2-bromo-4-methylpyridine	313.14	1.60 - 1.73 (m, 2 H), 1.79 - 1.92 (m, 2 H), 1.95 - 2.09 (m, 4 H), 2.39 (s, 3 H), 2.95 (s, 2 H), 3.99 (dd, J=7.70, 4.03 Hz, 4 H), 7.10 - 7.18 (m, 1 H), 7.40 (s, 1 H), 8.50 (d, J=4.89 Hz, 1 H)
67		3-trifluoromethylbromobenzene	366.13	1.64 - 1.75 (m, 2 H), 1.82 - 1.93 (m, 2 H), 1.96 - 2.11 (m, 4 H), 2.94 (s, 2 H), 3.94 - 4.05 (m, 4 H), 7.52 (t, J=7.82 Hz, 1 H), 7.66 (d, J=7.82 Hz, 1 H), 7.70 (d, J=7.58 Hz, 1 H), 7.80 (s, 1 H)
68		2-bromo-4-trifluoromethylpyridine	324.09	2.32 - 2.42 (m, 2 H), 2.42 - 2.56 (m, 2 H), 2.93 - 3.09 (m, 2 H), 3.03 (s, 2 H), 3.44 - 3.57 (m, 2 H), 7.31 - 7.36 (m, 1 H), 7.42 (dt, J=6.85, 1.59 Hz, 2 H), 7.53 (t, J=1.59 Hz, 1 H)
69		3-iodo-4-chloropyridine	333.15	1.63 - 1.74 (m, 2 H), 1.89 (d, J=12.47 Hz, 2 H), 1.96 - 2.12 (m, 4 H), 2.94 (s, 2 H), 3.93 - 4.05 (m, 4 H), 7.82 (s, 1 H), 8.58 (s, 1 H), 8.63 (s, 1 H)

Example 70

3-(2-Furoyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

3-Ethynyl-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene (Compound 70a)

The title product was prepared by the same method illustrated for Compound 11a, but starting from Compound 31b instead of Compound 3c. It was used in the next step without further purification.

MS: $[M+H]^+$ = 222.37

3-(2-Furoyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

A mixture of Compound 70a (60 mg, 0.27 mmol), 2-furoyl chloride (40.2 µl, 0.41 mmol), triethylamine (75.5 µl, 0.54 mmol), *bis*(triphenylphosphine)palladium(II)dichloride (11.4 mg, 0.016 mmol) and CuI (11.4 mg, 0.16 mmol) in 4 ml of anhydrous THF was stirred under nitrogen for 3 h. Afterwards, the reaction mixture was poured into water, extracted with EtOAc. The organic solvent was washed with brine, dried over sodium sulphate and evaporated to dryness *in vacuo*. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – EtOAc from 7 : 3 to 5 : 5) to give 85.5 mg (23.4 %) of the title compound.

MS: $[M+H]^+$ = 316.64

^{1}H-NMR: *($CDCl_3$, δ)*: 1.67-1.75 (m, 2 H), 1.82-1.93 (m, 2 H), 1.93-2.11 (m, 4 H), 2.95 (s, 2 H), 3.92-4.05 (m, 4 H), 6.64 (d, *J*=2.93 Hz, 1 H), 7.47 (d, *J*=3.42 Hz, 1 H), 7.73 (s, 1H).

Example 71

3-Phenylethynyl-8-(1-carboxy-3-hydroxy-propoxyimino)-1-oxa-2-azaspiro[4.5]dec-2-ene

To a solution of the Compound of Example 47 (14 mg, 0.4 mmol) in 1.75 ml of dioxane was added 1M sodium hydroxide (40 µl, 0.4 mmol) and the reaction mixture was stirred at 40°C for 2h. After evaporation to dryness, the crude was purified by automated RP flash chromatography (SP1®TM – Biotage; gradient 20 mM aqueous ammonium carbonate – MeCN from 1:0 to 6:4) to give 10 mg of the title compound as a white solid.

MS: $[M+H]^+$ = 371.06

^{1}H-NMR: *($DMSO\text{-}d_6$, δ)*: 1.69-1.97 (m, 6 H), 2.17-2.28 (m, 1 H), 2.28-2.39 (m, 1 H), 2.45-2.58 (m, 1 H), 2.58-2.72 (m, 1 H), 3.11 (d, *J*=5.14 Hz, 2 H), 3.43-3.57 (m, 2 H) 4.38 (t, *J*=5.26 Hz, 1 H), 7.41-7.54 (m, 3 H), 7.54-7.61 (m, 2 H).

Example 72

3-(3-Chlorophenyl-ethynyl)-1,8-dioxa-2-azaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure described for Compound 1b, but using Compound 31e instead of Compound 1a and 4-methylene-tetrahydropyran (Tetrahedron, 46(7), 2411-2424, 1990) instead of 1-(t.butoxycarbonyl)-4-methylene-piperidine. After the usual work-up, the residue was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – EtOAc from 10 : 1 to 8 : 2) to give the title compound as a yellowish solid. Yield: 23.7 %.

MS: $[M+H]^+ = 276.7$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.79-1.89 (m, 2 H), 1.89-1.99 (m, 2 H), 2.95 (s, 2 H), 3.69 - 3.80 (m, 2 H), 3.92 (ddd, 2 H), 7.29-7.36 (m, 1 H), 7.41 (t, 2H), 7.52 (s, 1 H).

Example 73

3-[(6-Methyl-2-pyridyl)-ethynyl]-1,8-dioxa-2-azaspiro[4.5]dec-2-ene

3-(3-Trimethylsylylbut-1-ynyl)-1,8-dioxa-2-azaspiro[4.5]dec-2-ene (Compound 73a)

The title compound was synthesized using the method described above for Compound 3c, but using 4-methylenetetrahydropyran instead of Compound 3a. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 75 : 25) to give the title compound as brownish oil. Yield: 30.2%.

MS: $[M+H]^+ = 238.21$.

^{1}H-NMR: *($CDCl_3$, δ)*: 0.24-0.27 (m, 9 H), 1.79 (ddd, J=13.45, 9.05, 4.16 Hz, 2 H), 1.90 (dt, J=13.40, 4.40 Hz, 2 H), 2.86 (s, 2 H), 3.71 (dt, J=11.74, 4.65 Hz, 2 H), 3.89 (ddd, J=11.80, 8.86, 3.30 Hz, 2 H).

3-[(6-Methyl-2-pyridyl-)ethynyl]-1,8-dioxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized using the method described above for the Compound of Example 3, but replacing Compound 3c with Compound 73a. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 8 : 2 to 4 : 6) to give the title compound as yellow solid. Yield: 41.7 %.

MS: $[M+H]^+ = 257.19$.

^{1}H-NMR: *($CDCl_3$, δ)*: 1.78-1.89 (m, 2 H), 1.89-2.00 (m, 2 H), 2.61 (s, 3 H), 2.97 (s, 2 H), 3.68-3.80 (m, 2 H), 3.85-3.98 (m, 2 H), 7.20 (d, J=7.82 Hz, 1 H), 7.39 (d, J=7.58 Hz, 1 H), 7.62 (t, J=7.70 Hz, 1 H).

Example 74

3-[(4-Chloro-2-pyridyl)-ethynyl]-1,8-dioxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized using the method described above for the Compound of Example 3, but replacing Compound 3c with Compound 73a and using 2-bromo-4-chloropyridine in place of 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 8 : 2 to 35 : 65) to give the title compound.Yield: 67 %.

MS: $[M+H]^+ = 277.12$.

^{1}H-NMR: *($CDCl_3$, δ)*: 1.82-1.90 (m, 2 H), 1.90-1.99 (m, 2 H), 2.97 (s, 2 H), 3.75 (dt, J=11.68, 4.68 Hz, 2 H), 3.91 (ddd, J=11.86, 8.93, 3.18 Hz, 2 H), 7.35 (dd, J=5.38, 1.96 Hz, 1 H), 7.58 (d, J=1.71 Hz, 1 H), 8.55 (d, J=5.38 Hz, 1 H).

Example 75

3-(3-Chlorophenyl-ethynyl)-1-oxa-8-thia-2-azaspiro[4.5]dec-2-ene 8,8-dioxide

4-Methylene-1,1-dioxo-thiane (Compound 75a)

The title product was synthesized following the procedure reported for Compound 2a, but using 1,1,4-trioxo-thiane in place of 1-(3-nitro-2-pyridyl)-4-oxo-piperidine. After the usual work-up, the residue was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 85: 15 to 6 : 4) to give the title compound.. Yield: 79%

MS: $[M+H]^+ = 147.21$.

^{1}H-NMR: ($CDCl_3$, δ): 2.58 (t, J=6.30 Hz, 4 H), 3.11 (t, J=6.30, 4 H), 4.92 (s, 2 H).

3-Trimethylsilylethynyl-1-oxa-8-thia-2-azaspiro[4.5]dec-2-ene 8,8-dioxide(Compound 75b)

The title compound was synthesized using the method described above for Compound 3c, but replacing Compound 3a with Compound 75a. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 7 : 3) to give the title compound as a white solid brownish oil. Yield: 40.1 %.

MS: $[M+H]^+ = 286.25$.

^{1}H-NMR: *($CDCl_3$, δ)*: 0.26 (s, 9 H), 2.25-2.37 (m, 2 H), 2.43 (td, J=14.00, 2.90 Hz, 2 H), 2.93 (s, 2 H), 2.95-3.04 (m, 2 H), 3.47 (td, J=13.57, 3.91 Hz, 2 H).

3-(3-Chlorophenyl-ethynyl)-1-oxa-8-thia-2-azaspiro[4.5]dec-2-ene 8,8-dioxide

The title compound was synthesized according to the method described above for the Compound of Example 3, but replacing Compound 3c with Compound 75b and using 1-chloro-3-iodobenzene instead of 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 85 : 15 to 65 : 35) to give the title compound.Yield: 54 %.

MS: $[M+H]^+$ = 323.87.

^{1}H-NMR: *($CDCl_3$, δ)*: 2.32-2.42 (m, 2 H), 2.42 - 2.56 (m, 2 H), 2.93-3.09 (m, 2 H), 3.03 (s, 2 H), 3.44-3.57 (m, 2 H), 7.31-7.36 (m, 1 H), 7.42 (dt, J=6.85, 1.59 Hz, 2 H), 7.53 (t, J=1.59 Hz, 1 H).

Example 76

3-(3-Chlorophenyl-ethynyl)-1,7-dioxa-2-azaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and 3-methylene-tetrahydopyran (Kirmse, W.; Rode, K. Chemische Berichte,120, 1987, 847 – 848) instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (Horizon®TM – Biotage;gradient Petroleum Ether – EtOAc from 1 : 0 to 8 : 2) to give the title compound as a grey solid. Yield: 4%

MS: $[M+H]^+$ = 276.25

^{1}H-NMR: *($CDCl_3$, δ)*: 1.62-1.74 (m, 1 H), 1.86-2.09 (m, 3 H), 2.90 (d,1 H), 3.11 (d, 1 H), 3.52-3.67(m, 3 H), 3.73-3.82 (m, 1 H), 7.29-7.35 (m, 1 H),,7.37-7.44 (m, 2 H), 7.52 (t, 1 H).

Example 77

3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene – Isomer A

8-Methylene-1-oxaspiro[4.5]decane (Compound 77a)

The title compound was prepared following the procedure reported for Compound 2a, but using 1-oxaspiro[4.5]decan-8-one for 1-(3-nitro-2-pyridyl)-4-oxo-piperidine. After the usual work-

up, the residue was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – EtOAc from 100: 5 to 75 : 25) to give the title compound.
Yield: 29%.
^{1}H-NMR: (*CDCl3,* δ): 1.50-1.60 (m, 2 H); 1.67-1.75 (m, 4 H); 1.88-2.00 (m, 2 H); 2.08-2.18 (m, 2 H); 2.31-2.43 (m, 2 H); 3.86 (t, *J*=6.7 Hz, 2 H); 4.65 (s, 2 H).

3-Trimethysilylethynyl-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene
(Compound77b - isomers A and B)
The title compounds were synthesized using the method described above for Compound 3c, but replacing Compound 3a with Compound 77a. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 75 : 25) to give the title compound as yellowish oils. Yield: 17.6 % of the first eluted Isomer A and 11.6% of the Isomer B.
MS: $[M+H]^+$ = 292.48
^{1}H-NMR: Isomer A, (*$CDCl_3$,* δ): ppm 0.25 (s, 9 H); 1.58-1.70 (m, 4 H); 1.70-1.77 (m, 2 H); 1.79-1.91 (m, 4 H); 1.90-2.00 (m, 2 H); 2.80 (s, 2 H); 3.78 - 3.93 (m, 2 H).
^{1}H-NMR: Isomer B, (*$CDCl_3$,* δ): ppm 0.25 (s, 9 H); 1.46 (ddd, *J*=13.6, 9.8, 4.0 Hz, 2 H); 1.58-1.67 (m, 2 H); 1.67-1.75 (m, 2 H); 1.82-2.00 (m, 4 H); 2.07 (ddd, *J*=13.7, 10.0, 4.2 Hz, 2 H); 2.83 (s, 2 H); 3.77-3.93 (m, 2 H).

3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene – Isomer A
The title compound was synthesized using the method described above for the Compound of Example 3, but replacing Compound 3c with Compound 77b – Isomer A and substituting 1-chloro-3-iodobenzene for 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 95 : 5 to 8 : 2) to give the title compound as ivory solid.Yield: 69 %.
MS: $[M+H]^+$ = 330.03
^{1}H-NMR: *(CDCl3,* δ): 1.49 (ddd, *J*=13.51, 9.72, 3.91 Hz, 2 H); 1.62-1.70 (m, 2 H); 1.70-1.77 (m, 2 H); 1.87-2.00 (m, 4 H); 2.12 (ddd, *J*=13.33, 9.66, 4.16 Hz, 2 H); 2.92 (s, 2 H); 3.86 (t, *J*=6.72 Hz, 2 H); 7.29-7.34 (m, 1 H); 7.36-7.43 (m, 2 H); 7.51 (s, 1 H).

Example 78
3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene – Isomer B

The title compound was synthesized using the method described above for the Compound of Example 3, but replacing Compound 3c with Compound 77b – Isomer B and substituting 1-chloro-3-iodobenzene for 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 95 : 5 to 8 : 2) to give the title compound as pale yellow solid.Yield: 33.5 %.

MS: $[M+H]^+ = 330.18$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.61-1.68 (m, 2 H); 1.71-1.78 (m, 2 H); 1.80-2.01 (m, 8 H); 2.89 (s, 2 H); 3.85 (t, *J*=6.75 Hz, 2 H); 7.27-7.33 (m, 1 H); 7.35-7.43 (m, 2 H); 7.52 (t, *J*=1.66 Hz, 1 H).

Example 79

3-(3-Methylphenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was synthesized using the method described above for the Compound of Example 3, but substituting Compound 77b – Isomer B for Compound 3c and 3-iodotoluene for 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum ether – EtOAc from 95 : 5 to 8 : 2) to give the title compound as ivory solid.Yield: 67.6%.

MS: $[M+H]^+ = 310.24$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.49 (ddd, *J*=13.39, 9.84, 3.91 Hz, 2 H); 1.67 (dd, *J*=12.47, 4.65 Hz, 2 H); 1.70-1.77 (m, 2 H); 1.85-2.00 (m, 4 H); 2.12 (ddd, *J*=13.14, 9.60, 3.91 Hz, 2 H); 2.37 (s, 3 H); 2.92 (s, 2 H); 3.86 (t, *J*=6.72 Hz, 2 H); 7.17-7.30 (m, 2 H); 7.34 (d, *J*=11.98 Hz, 2 H).

Example 80

3-(3-Hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

3-Ethynyl-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 80a)

A solution of Compound 28b (557 mg, 1.81 mmol) and tetrabutylammonium fluoride hydrate (759 mg, 2.72 mmol) in 10 ml of THF was stirred at r.t. for 4h. After the usual work-up with EtOAc – water the crude residue was used in the next step without further purification.

MS: $[M+H]^+ = 238.4$

3-(3-Hydroxyphenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 80b)

The title compound was synthesized according to the method described above for the Compound of Example 3, but substituting Compound 80a for Compound 3c and 3-iodophenol for 2-bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 8 : 2 to 65 : 35) to give the title compound as yellowish glassy solid.Yield: 28.8%.

MS: $[M+H]^+ = 329.4$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.48 (s, 9 H), 3.40 (s, 2 H), 4.07 (d, *J*=10.3 Hz, 2 H), 4.31 (d, *J*=10.0 Hz, 2 H), 4.99 (s, 1 H), 6.91 (ddd, 1 H), 6.98 - 7.02 (m, 1 H), 7.12 (d, *J*=7.8 Hz, 1 H), 7.28 (t, *J*=8.1 Hz, 1 H).

3-(3-Hydroxyphenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 80c)

The title compound was synthesized following the method reported for the Compound 1c, but replacing Compound 1b with Compound 80b. The crude residue was used without further purification in the next reaction. Yield: 90.4 %.

MS: $[M+H]^+ = 229.3$

3-(3-Hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 80c. After the ususal work-up, the title product was purified by automated RP chromatography (SP1®TM – Biotage; gradient buffer NH_4HCO_3 - MeCN from 20-80 a 40-60. Yield: 26 %.

MS: $[M+H]^+ = 365.05$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.51 (s, 3 H), 3.49 (s, 2 H), 4.38 (d, 2 H), 4.49 (d, 2 H), 4.83 (br. s., 1 H), 6.65 (d, 1 H), 6.92 (d, 1 H), 7.01 (s, 1 H), 7.14 (d, 1 H), 7.23-7.32 (m, 5 H), 8.18 (d, 1H).

Example 81

3-(3-Cyanophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

3-(3-Cyanophenyl-ethynyl)-7-(t.butoxycarbonyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 81a)

The title compound was synthesized using the method described above for the Compound of Example 3, but substituting Compound 80a for Compound 3c and 3-iodobenzonitrile for 2-

bromo-6-methylpyridine. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum ether – EtOAc from 9 : 1 to 6 : 4) to give the title compound as yellowish glassy solid.Yield: 84.5 %.

MS: $[M+H]^+ = 338.4$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.47 (s, 9 H), 3.39 (s, 2 H), 4.06 (dd, *J*=9.8, 1.2 Hz, 2 H), 4.32 (dd, *J*=9.8, 1.2 Hz, 2 H), 7.48-7.55 (m, 1 H), 7.69 (ddd, *J*=7.9, 1.4 Hz, 1 H), 7.74 (ddd, *J*=7.9, 1.4 Hz, 1 H), 7.78 - 7.83 (m, 1 H).

3-(3-Cyanophenyl-ethynyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene (Compound 81b)

The title compound was synthesized following the method reported for Compound 1c, but replacing Compound 1b with Compound 81a. The crude residue was used without further purification in the next reaction. Yield: 90.4%.

MS: $[M+H]^+ = 238.3$

3-(3-Cyanophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene

The title compound was prepared following the method described for the compound of Example 1, but replacing Compound 1c with Compound 81b. After the usual work-up, the title product was purified by automated RP chromatography (SP1®TM – Biotage; gradient buffer NH_4HCO_3 - MeCN from 20-80 a 40-60. Yield: 72.5%. Yellowish solid.

MS: $[M+H]^+ = 374.11$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.52 (s, 3 H), 3.50 (s, 2 H), 4.38 - 4.44 (m, 1 H), 4.49-4.54 (m, 1 H), 6.66 (d, *J*=8.31 Hz, 1 H), 7.53 (dd,*J*=7.90 Hz, 1 H), 7.71 (ddd, *J*=7.80, 1.50 Hz, 1 H), 7.76 (ddd, *J*=7.80, 1.30 Hz, 1 H), 7.83 (dd, *J*=1.30 Hz, 1 H), 8.18 (d, *J*=8.31 Hz, 1 H).

Example 82

3'-(3-Chlorophenyl-ethynyl)-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole]

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and 3-methylene-indan-1-ol (Angew Chem, Int Ed, 48(33), 2009, 6148-6151) instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (Horizon®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 1 : 1) to give the title compound as a brownish oil. Yield: 74%

The title compound was characterized as a 83:17 diastereoisomeric mixture by ^{1}H-NMR spectrum.

MS: $[M+H]^+ = 324.01$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.25 (dd, *J*=13.94, 4.89 Hz, 1 H), 2.57 (dd, *J*=14.30, 3.79 Hz, 0.21 H), 2.74 (dd, *J*=14.31, 6.48 Hz, 0.21 H), 3.02 (dd, *J*=13.94, 6.60 Hz, 1 H), 3.39 (dd, 0.42) 3.50 (dd, 2 H) 5.12-5.25 (m, 0.21 H), 5.50 (t, *J*=5.62 Hz, 1 H), 7.30-7.38 (m, 1 H), 7.38 - 7.52 (m, 6 H), 7.56 (s, 1 H).

Example 83

3'-(3-Chlorophenyl-ethynyl)-2,3-dihydro-3-oxo-4'H-spiro[indene-1,5'-isoxazole]

A solution of oxalyl chloride (64.6 µl, 0.74 mmol) in 5 ml of anhydrous CH_2Cl_2 was cooled to -60°C; DMSO (105 µl, 1.48 mmol) was dropped so as to keep the reaction at the same temperature. After 15 min. the compound of Example 82 (174 mg, 0.49 mmol) dissolved in 1 ml of anhydrous CH_2Cl_2 was added and after 30 minutes TEA (0.413 ml, 2.96 ml) was added dropwise. Turning off the cooling and heating up to r.t., the reaction mixture was stirred for 3 hours, then it was quenched with H_2O, extracted with CH_2Cl_2 and dried on Na_2SO_4._The residue from evaporation was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 1 : 1) to give the title compound as a brownish oil. Yield: 50 %

MS: $[M+H]^+ = 322.2$

^{1}H-NMR: *($CDCl_3$, δ)*: 3.01 (d, *J*=18.83 Hz, 1 H), 3.30 (d, *J*=18.83 Hz, 1 H),l 3.48 (d, *J*=17.36 Hz, 1 H), 3.63 (d, *J*=17.36 Hz, 1 H), 7.31-7.38 (m, 1 H), 7.40-7.49 (m, 2 H), 7.55-7.57 (m, 1H), 7.60 (td, *J*=7.40, 1.10 Hz, 1 H), 7.69-7.73 (m, 1 H), 7.75-7.83 (m, 2 H).

Example 84

3'-(3-Chlorophenyl-ethynyl)-2,3-dihydro-3-methoxyimino-4'H-spiro[indene-1,5'-isoxazole]

To a solution of the Compound of Example 83 (0.14 g, 0.44 mmol) in 1.5 ml of pyridine was added methoxylamine (0.04 g, 0.48 mmol). After 4 hours stirring at r.t., the reaction was checked by TLC and considered to completion. It was diluted with water, acidified by 1N HCl and extracted with EtOAc. After drying on Na_2SO_4 and evaporation to dryness, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 1 : 1) to give the title compound as a colourless oil. Yield: 75.4%.

MS: $[M+H]^+ = 351.44$

^{1}H-NMR: *($CDCl_3$, δ)*: 3.19 (d, 1 H), 3.34-3.41 (m, 1 H), 3.43 (d, 1 H), 3.51-3.57 (m, 1 H), 4.02 (s, 3 H), 7.30-7.37 (m, 1 H), 7.39-7.51 (m, 5 H), 7.56 (t, 1H), 7.71-7.77 (m, 1 H)

Example 85-86

3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene (Isomer 1) and 3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene (Isomer 2)

7-Methylene-1-oxaspiro[3.5]nonane (Compound 85a)

N-(p.toluenesulphonyl)-dimethylsulphoximine (1.49 g, 6.01 mmol) and anhydrous DMSO (5 ml) were added to NaH 60% oil dispersion (0.22 g, 5.46 mmol) kept under stirring under anhydrous inert gas atmosphere in a flamed 3 necks flask. The mixture became greenish, and then changed to yellow. After stirring at 35°C for 10h, a solution of 4-methylenecyclohexanone (0.2 g, 1.82 mmol) in 5 ml of DMSO was added and the reaction mixture was stirred at 50°C for 20h. Afterwards, the reaction mixture was diluted with a saturated solution of NaCl and extracted with Et_2O (3 times). After drying on Na_2SO_4 and evaporation to dryness, the residue was purified by automated flash chromatography (Isolera®TM – Biotage; isocratic Petroleum Ether – EtOAc 9 : 1) to give 0.252 g of the title compound as a colourless oil. Yield: 27.8 %.

MS: $[M+H]^+ = 138.21$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.80 - 1.99 (m, 4 H) 2.05 - 2.15 (m, 2 H) 2.27 - 2.37 (m, 2 H) 2.41 (t, *J*=7.7 Hz, 2 H) 4.55 (t, *J*=7.8 Hz, 2 H) 4.66 (s, 2 H).

3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene (Isomer 1) and 3-(3-Chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene (Isomer 2)

The title compounds were prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 85a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 6 : 4) to give a first group of fractions affording the less polar diastereoisomer (Example 86; 43.9 %) followed by a third group of fractions containing the more polar diastereoisomer (Example 85; 25.4%).

Example 85

MS: $[M+H]^+ = 315.80$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.63 (ddd, *J*=13.4, 9.4, 4.2 Hz, 2 H) 1.78 - 1.90 (m, 2 H) 2.00 - 2.10 (m, 2 H) 2.16 (ddd, *J*=13.2, 9.3, 4.2 Hz, 2 H) 2.42 (t, *J*=7.8 Hz, 2 H) 2.89 (s, 2 H) 4.55 (t, *J*=7.8 Hz, 2 H) 7.31 (dd, *J*=7.9 Hz, 1 H) 7.36 - 7.43 (m, 2 H) 7.51 (t, *J*=1.8 Hz, 1 H)

Example 86

MS: $[M+H]^+ = 315.80$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.79 - 1.88 (m, 4 H) 1.97 - 2.06 (m, 4 H) 2.43 (t, *J*=7.8 Hz, 2 H) 2.89 (s, 2 H) 4.54 (t, *J*=7.8 Hz, 2 H) 7.27 -.34 (m, 1 H) 7.36 - 7.43 (m, 2 H) 7.49 - 7.53 (m, 1 H)

Example 87

3-(3-Chlorophenyl-ethynyl)-1,9,13-trioxa-2-azadispiro[4.2.5.2]pentadec-2-ene

A solution of the compound of Example 32 (0.41 g, 1.42 mmol), 1,3-propandiol (0.15g, 1.95 mmol) and *p*-toluensulphonic acid.H_2O (7.5 mg, 0.04 mmol) in 30 ml of benzene was stirred at reflux for 10.5 h, removing water with a Dean-Stark Apparatus. Dilution with water, washing with aq. 5% $NaHCO_3$ and drying the organic layer over Na_2SO_4 afforded the title compound as dense yellow oil. This was further purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 7 : 3) giving a white solid.

Yield: 75.3%.

MS: $[M+H]^+ = 346.27$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.77 (d, *J*=5.4 Hz, 4 H); 2.00 (d, *J*=7.6 Hz, 6 H); 2.91 (s, 2 H); 3.92 (s, 2 H); 3.96 (s, 2 H); 7.31 (s, 1 H); 7.36 -7.44 (m, 2 H); 7.52 (s, 1 H).

Example 88

3'-[(6-Methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole]

3'-Trimethylsilylethynyl-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole]

(Compound 88a)

The title compound was prepared following the procedure described for Compound 3c, using 3-methyleneindan-1-ol (Angew. Chem., Int. Ed., 48(33), 2009, 6148-6151) instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 1 : 1) to give the title compound as a brownish oil. Yield: 79.9%

MS: $[M+H]^+ = 286.38$

3'-[(6-Methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole]

The title compound was synthesized using the method described above for the compound of Example 3, but substituting Compound 88a for Compound 3c and replacing 2-bromo-6-methylpyridine with 3-iodophenol. The crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 4 : 6) to give the title compound as a brownish oil.Yield: 67.6%.

MS: $[M+H]^+ = 305.31$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.64 (s, 3H), 3.02 (d, 1H) 3.35 (d, 1H), 3.55 (d, 1H), 3.65 (d, 1H), 5,41-5.45(m, 1H), 7.23 (d, 1H), 7.44 (d, 1H), 7.55-7.62 (m, 1H), 7.63-7.72 (m, 2H), 7.76 (d, 1H), 7.80 (d, 1H).

Example 89

3-(3-Chlorophenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene

8-Methylenespiro[4.5]decane (Compound 89a)

The title compound was prepared following the method reported above for Compound 31a. As starting material spiro[4.5]decan-8-one replaced 1,4-dioxaspiro[4.5]decan-8-one. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; CH_2Cl_2) to give the title compound as a colourless oil. Yield: 98.5%.

^{1}H-NMR: *($CDCl_3$, δ)*: 1.37 - 1.49 (m, 8 H); 1.63 (dt, *J*=7.2, 3.6 Hz, 4 H); 2.16 (t, *J*=6.4 Hz, 4 H); 4.61 (s, 2 H).

3-(3-Chlorophenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 89a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (ISOLERA®TM – Biotage; gradient Petroleum Ether – EtOAc from 97 : 3 to 9 : 1) to give the title compound as a white-yellowish solid. Yield: 58.5%.

MS: $[M+H]^+ = 328.29$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.31 - 1.41 (m, 2 H); 1.46 (dt, *J*=19.7, 6.9 Hz, 4 H); 1.59 - 1.76 (m, 8 H); 1.84 - 1.94 (m, 2 H); 2.89 (s, 2 H); 7.26 - 7.34 (m, 1 H); 7.35 - 7.45 (m, 2 H); 7.51 (s, 1 H).

Example 90

3-(3-Chlorophenyl-ethynyl)-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

1,1-Difluoro-4-methylenecyclohexane (Compound 90a)

A methyl triphenyl phosphonium bromide/sodium amide mixture (MTP instant ylide, 0.49 g, 1.24 mmol) was suspended in anhydrous Et_2O (3 ml) stirring for 1h at r.t.. Afterwards, the reaction mixture was cooled at 0°C and a solution of 4,4-difluorocyclohexanone (0.145 g, 1.08 mmol) in 3 ml of Et_2O was dropped into. Stirring at r.t. was maintained for 16h, then the reaction mixture was filtered and the filtrate was used in the next step without further purification.

3-(3-Chlorophenyl-ethynyl)-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3a and Compound 90a instead of Compound 3b. After the usual work-up, the residue was purified by automated flash chromatography (ISOLERA®TM – Biotage; gradient Petroleum Ether – EtOAc from 99 : 1 to 95 : 5) to give the title compound as a yellowish solid. Yield: 41.9%.

MS: $[M+H]^+ = 310.35$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.82 - 1.92 (m, 2 H), 1.97 - 2.14 (m, 4 H), 2.14 - 2.34 (m, 2 H), 2.95 (s, 2 H), 7.33 (d, *J*=7.6 Hz, 1 H), 7.41 (t, *J*=7.1 Hz, 2H), 7.52 (s, 1 H).

Example 91

3-(3-Methylphenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene

1-(3,3-Diethoxyprop-1-yn-1-yl)-3-methylbenzene(Compound 91a)

The title compound was synthesized following the procedure reported for Compound 31c, but using 3-iodotoluene instead of 3-chloroiodobenzene. After the usual work-up, the residue was purified by automated flash chromatography (ISOLERA®TM – Biotage; gradient Petroleum Ether – EtOAc from 100 : 3 to 100 : 7) to give the tile compound as a yellowish oil.

Yield: 94%.

^{1}H-NMR: *($CDCl_3$, δ)*: 1.30 (t, *J*=6.8 Hz, 6 H); 2.34 (s, 3 H); 3.69 (quin, *J*=7.8 Hz, 2 H); 3.85 (quin, *J*=7.7 Hz, 2 H); 5.51 (s, 1 H); 7.16 (d, *J*=7.6 Hz, 1 H); 7.22 (t, *J*=7.6 Hz, 1 H); 7.27 - 7.36 (m, 2 H).

3-(3-methylphenyl)prop-2-ynal (Compound 91b)

The title compound was synthesized following the procedure reported for Compound 31d, but starting from Compound 91a instead of Compound 31c. The crude title compound was used in the next step without further purification.

MS: $[M+H]^+ = 145.12$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.39 (s, 3 H); 7.30 - 7.35 (m, 2 H); 7.40 - 7.49 (m, 2 H); 9.44 (s, 1 H).

N-hydroxy-3-(3-methylphenyl)prop-2-yn-1-imine (Compound 91c)

The title compound was synthesized following the procedure reported for Compound 31e but starting from Compound 91b instead of Compound 31d. The crude title compound (brownish oil) was used in the next step without further purification.

MS: $[M+H]^+ = 160.19$

^{1}H-NMR: *($CDCl_3$, δ)*: **mix E/Z 1/2**: 2.37 (s, 1.5 H); 2.37 (s, 3 H); 7.04 (s, 1 H); 7.18 - 7.31 (m, 3 H); 7.31 - 7.42 (m, 3 H); 7.62 (s, 0.5 H).

N-hydroxy-3-(m-tolyl)-prop-2-ynimidoyl chloride(Compound 91d)

The title compound was prepared following the method herein described for Compound 3b, using Compound 91c instead of 3-trimethylsilylprop-2-ynal oxime. The residue was used in the next step without further purification. Yield: 96.4%.

3-(3-Methylphenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 91d instead of Compound 3b and Compound 89a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (ISOLERA®TM – Biotage; gradient Petroleum Ether – EtOAc from 97 : 3 to 9 : 1) to give the title compound as a white-yellowish solid. Yield: 58.5%.

MS: $[M+H]^+ = 308.28$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.31 - 1.52 (m, 6 H); 1.53 - 1.76 (m, 9 H); 1.87 (dd, *J*=12.0, 3.2 Hz, 2 H); 2.37 (s, 3 H); 2.90 (s, 2 H); 7.17 -7.23 (m, 1 H); 7.26 (dd, *J*=7.6 Hz, 1 H); 7.31 - 7.38 (m, 2 H).

Example 92

3-[(6-Methoxy-3-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene

The title product was obtained following the procedure described for the compound of Example 3, but starting from Compound 70a instead of Compound 3c, and substituting 5-iodo-2-methoxypyridine for 2-bromo-6-methylpyridine. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 4 : 6) to give the title compound as a brownish oil solid. Yield: 45%.

MS: $[M+H]^+ = 329.23$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.63-1.74 (m, 2 H), 1.77-1.92 (m, 2 H), 1.98-2.11 (m, 4 H), 2.93 (s, 2H), 3.92-4.05 (m, 4 H), 4.00 (s, 3H), 6.77 (d, 1 H), 7.70 (d, 1 H), 8.36 (s, 1 H).

Example 93

3'-[(6-Methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-oxo-4'H-spiro[indene-1,5'-isoxazole]

The title product was obtained by Swern's oxidation as described in Example 83, starting from the compound of Example 88 intead of the compound of Example 82. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 1 : 1) to give the title compound as a brownish oil solid. Yield: 50.3 %.

MS: $[M+H]^+ = 303.21$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.63 (s, 3H), 2.99 (d, 1H) 3.29 (d, 1H), 3.51 (d, 1H), 3.65 (d, 1H), 7.23 (d, 1H), 7.44 (d, 1H), 7.55-7.62 (m, 1H), 7.63-7.72 (m, 2H), 7.76 (d, 1H), 7.80 (d, 1H).

Example 94

3'-[(6-Methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-methoxyimino-4'H-spiro[indene-1,5'-isoxazole]

The title product was obtained the method described for the compound of Example 84, but starting from the Compound of Example 93 instead of the Compound of Example 83. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 1 : 1) to give the title compound. Yield: 82%.

MS: $[M+H]^+ = 332.15$

^{1}H-NMR: *($CDCl_3$, δ)*: 2.59 (s, 3H), 3.15 (d, 1H), 3.34-3.45 (m, 2H), 3.49-3.58 (m, 1H), 4.00 (s, 3H), 7.19 (d, 1H), 7.37-7.49 (m, 4H), 7.61 (t, 1H), 7.69-7.74 (m, 1 H).

Example 95

3-(3-Chlorophenyl-ethynyl)-7-dimethylcarbamoyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene

To a solution of Compound 27d (0.12 g, 0.46 mmol) in 10 ml of dichloromethane was added TEA (0.02 ml, 1.38 mmol) followed by N,N-dimethylchloroformamide (0.051 ml, 0.59 mmol). The reaction mixture was stirred at r.t. for 4 h. Afterwards, it was evaporated to dryness and purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 3 : 7 to 0:1) to give the title compound as a pale yellow solid. Yield: 68.8%.

MS: $[M+H]^+ = 332.3$

^{1}H-NMR: *(*DMSO-*d6*, *δ*):ppm 1.95 - 2.18 (m, 2 H) 2.75 (s, 6 H) 3.22 - 3.41 (m, 3 H) 3.41 - 3.46 (m, 1 H) 3.51 (td, *J*=10.2, 7.0 Hz, 1 H) 3.58 (d, *J*=11.7 Hz, 1 H) 7.46 - 7.53 (m, 1 H) 7.54 - 7.61 (m, 2 H) 7.68 (t, *J*=1.7 Hz, 1 H).

Example 96

3-(3-Chlorophenyl-ethynyl)-7-carbamoyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene

To a solution of Compound 27d (0.125 g, 0.48 mmol) in 10 ml of methanol was added trimethylsylylisocyanate (0.097 ml, 0.72 mmol). The reaction mixture was stirred at r.t. for 20 h. Afterwards, it was evaporated to dryness and purified by automated RP chromatography (SP01®TM – Biotage; MeCN – ammonium carbonate buffer 38:62) to give the title compound as white solid. Yield: 55%.

MS: $[M+H]^+ = 304.3$

^{1}H-NMR: *(CDCl$_3$, δ)*: ppm 2.13 (dt, *J*=13.1, 9.2 Hz, 1 H) 2.33 - 2.45 (m, 1 H) 3.12 - 3.20 (m, 1 H) 3.20 - 3.28 (m, 1 H) 3.55 (d, *J*=11.5 Hz, 1 H) 3.58 - 3.68 (m, 2 H) 3.84 (d, *J*=11.5 Hz, 1 H) 4.44 (br. s., 2 H) 7.32 (t, *J*=7.8 Hz, 1 H) 7.38 - 7.45 (m, 2 H) 7.53 (t, *J*=1.6 Hz, 1 H)

Example 97

3-(3-Chlorophenyl-ethynyl)-1-oxa-2-aza-dispiro[4.1.3.1]undec-2-ene

6-methylene-spiro[3.3]heptane (Compound 97a)

The title compound was prepared following the methodology described for Compound 28a replacing 1-(t.butoxycarbonyl)-3-azetidinone with spiro[3.3]heptan-6-one. The reaction crude was used in the next step without further purification.

MS: $[M+H]^+ = 108.18$

3-(3-Chlorophenyl-ethynyl)-1-oxa-2-aza-dispiro[4.1.3.1]undec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31e instead of Compound 3b and Compound 97a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; Petroleum Ether – EtOAc 97 : 3; then DCM 100%) to give the title compound as a white-yellowish solid. Yield: 20.7%.

MS: $[M+H]^+ = 285.77$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.82 - 1.94 (m, 2 H), 2.02 - 2.11 (m, 4 H), 2.23 - 2.32 (m, 2 H), 2.49 - 2.57 (m, 2 H), 3.15 (s, 2 H), 7.31 (t, *J*=7.8 Hz, 1 H), 7.34 - 7.43 (m, 2 H), 7.50 - 7.54 (m, 1 H).

Example 98

3-(3-Methylphenyl-ethynyl)-1,9,13-trioxa-2-azadispiro[4.2.5.2]pentadec-2-ene

9-Methylene-1,5-dioxaspiro[5.5]undecane (Compound 98a)

The title compound was prepared following the methodology described for Compound 28a, but replacing 1-(t.butoxycarbonyl)-3-azetidinone with 1,5-dioxaspiro[5.5]undecan-9-one. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; Et_2O) to give the title compound as a pale yellow oil. Yield: 96.3%.

^{1}H-NMR: *($CDCl_3$, δ)*: 1.76 (quin, *J*=5.4 Hz, 2 H); 1.89 (t, *J*=6.4 Hz, 4 H); 2.23 (t, *J*=6.2 Hz, 4 H); 3.95 (t, *J*=5.5 Hz, 4 H); 4.67 (s, 2 H).

3-(3-Methylphenyl-ethynyl)-1,9,13-trioxa-2-azadispiro[4.2.5.2]pentadec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 91d instead of Compound 3b and Compound 97a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 7 : 3) to give the title compound as a pasty solid. Yield: 20.7%.

MS: $[M+H]^+ = 325.97$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.71 - 1.82 (m, 4 H); 1.89 - 2.08 (m, 6 H); 2.37 (s, 3 H); 2.91 (s, 2 H); 3.94 (dt, *J*=18.5, 5.5 Hz, 4 H); 7.17 -7.30 (m, 2 H); 7.30 - 7.39 (m, 2 H).

Example 99

3-(3-Chlorophenyl-ethynyl)-1-oxa-2-azaspiro[4.3]oct-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and methylenecyclobutane instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 9 : 1) to give the title compound as a pale yellow solid. Yield: 80.4 %.

MS: $[M+H]^+ = 245.71$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.58 - 1.71 (m, 1 H), 1.89 (dtt, *J*=11.3, 9.9, 9.9, 3.5, 3.5 Hz, 1 H), 2.18 - 2.28 (m, 2 H), 2.52 - 2.64 (m, 2 H), 3.22 (s, 2 H), 7.32 (t, *J*=7.6 Hz, 1 H), 7.36 - 7.44 (m, 2 H), 7.52 (t, *J*=1.6 Hz, 1 H).

Example 100

3-(3-Chlorophenyl-ethynyl)-1,10-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene

Diethyl 4-methylenecyclohexane-1,1-dicarboxylate (Compound100a)

The title compound was prepared following the method described for Compound 31a, but using diethyl 4-oxocyclohexane-1,1-dicarboxylate in place of 1,4-dioxaspiro[4.5]decan-8-one. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 8 : 2) to give the title compound as a colourless oil. Yield: 88.1%.

MS: $[M+H]^+ = 240.30$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.28 (t, *J*=7.2 Hz, 6 H) 2.05 - 2.15 (m, 4 H) 2.19 - 2.27 (m, 4 H) 4.22 (q, *J*=7.1 Hz, 4 H) 4.68 (s, 2 H).

1,1-bis(Hydroxymethyl)-4-methylene-cyclohexane (Compound 100b)

To a suspension of $LiAlH_4$ (0.166 g, 4.4 mmol) in anhydrous Et_2O (10 ml) stirred a 0°C was added a solution of Compound 100a (100 mg, 2.08 mmol) in Et_2O (10 ml). The reaction mixture was stirred at r.t. for 2 h. Additional 0.5 equivalents of $LiAlH_4$ were added and the reaction was stirred at r.t. for 1 h. Afterwards, it was quenched, by adding THF/H_2O (2 ml : 1.3 ml), then 2N HCl (30 ml). Extraction with EtOAc, drying over Na_2SO_4 and evaporation to dryness afforded a crude which was purified by automated flash chromatography (SP01®TM – Biotage; gradient Dichloromethane – Methanol gradient from 1 : 0 to 95 : 5) to give the title compound as a white solid. Yield: 92.1 %.

MS: $[M+H]^+ = 156.22$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.51 (t, *J*=6.6 Hz, 4 H) 2.02 (br. s, 2 H) 2.19 (t, *J*=6.1 Hz, 4 H) 3.70 (s, 4 H) 4.67 (s, 2 H).

[1-(Hydroxymethyl)-4-methylidenecyclohexyl]methyl 4-methylbenzenesulphonate (Compound 100c)

To a suspension of Compound 100b (0.25 g, 1.6 mmol) and pyridine (0.40 ml) in dichloromethane was added at 0°C a solution of *p*-toluenesulphonyl chloride (0.336 g, 1.76 mmol) in 1 ml of dichloromethane. The reaction mixture was stirred at 0°C for 30 min., then at r.t. for 3.5 h.. Dilution with dichloromethane, washing with 1M aqueous $NaHSO_4$ and brine, and drying with Na_2SO_4, afforded, after evaporation, a crude. This was purified by automated flash chromatography (SP01®TM – Biotage; gradient Dichloromethane – Methanol from 1 : 0 to 95 : 5) to give the title compound.

MS: $[M+H]^+ = 310.41$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.39 - 1.53 (m, 4 H) 1.98 - 2.24 (m, 4 H) 2.48 (s, 3 H) 3.57 (s, 2 H) 4.01 (s, 2 H) 4.65 (s, 2 H) 7.38 (m, *J*=8.1 Hz, 2 H) 7.83 (d, *J*=8.3 Hz, 2 H).

7-Methylidene-2-oxaspiro[3.5]nonane (Compound 100d)

To a solution of compound 100c (0.320 g, 1.03 mmol) in THF (2 ml) stirred at 0°C was added sodium hydride (0.082 g, 2.06 mmol). After 30 min. stirring, the mixture was heated at 60°C for 5 h. After dilution with water, extraction with Et_2O and evaporation to dryness, the crude was used in the next step without further purification. Yield: 77.3 %.

3-(3-Chlorophenyl-ethynyl)-1,10-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 100d instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 8 : 2 to 1 : 1) to give the title compound as a pale-yellow solid. Yield: 67.6%.

MS: $[M+H]^+ = 315.80$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.52 - 1.69 (m, 2 H) 1.82 - 1.95 (m, 4 H) 2.01 - 2.14 (m, 2 H), 2.87 (s, 2 H) 4.45 (d, *J*=3.4 Hz, 4 H) 7.29 - 7.35 (m, 1 H), 7.36 - 7.44 (m, 2 H), 7.51 (t, *J*=1.6 Hz, 1 H).

Example 101

3-[(4-Chloro-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

3-[(4-Chloro-2-pyridyl)-ethynyl]-8-oxo-1-oxa-2-azaspiro[4.5]dec-2-ene (Compound 101a)

The title compound was synthesized using the method described for the compound of Example 32, but starting from the compound of Example 65 instead of the compound of Example 31. The crude was used in the next step without further purification.

MS: $[M+H]^+ = 289.7$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 1.97 - 2.10 (m, 2 H) 2.24 - 2.45 (m, 4 H) 2.74 - 2.90 (m, 2 H) 3.05 (s, 2 H) 7.36 (dd, *J*=5.4, 2.0 Hz, 1 H) 7.59 (d, *J*=1.5 Hz, 1 H) 8.55 (d, *J*=5.4 Hz, 1 H).

3-[(4-Chloro-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

Into a solution of Compound 101a (0.085 g, 0.29 mmol) in 5 ml of dichloromethane stirred at r.t. under a nitrogen stream was dropped diethylaminosulphurtrifluoride (0.163 ml, 1.23 mmol). Stirring was continued overnight. Afterwards, a saturated aqueous solution of $NaHCO_3$ (5 ml) was added, extracting with DCM (3 x 5 ml), drying the organic layer with Na_2SO_4 and evaporating to dryness. The residue was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 6 : 4) to give the title compound as a yellow oil containing a main impurity. This oil was purified again using RP chromatography (Isolera®TM – Biotage; aq. NH_4HCO_3/AcCN 55:45) affording the title compound a yellowish-white solid. Yield: 14.2%.

MS: $[M+H]^+ = 311.4$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 1.82 - 1.96 (m, 2 H) 2.02 - 2.14 (m, 4 H) 2.14 - 2.35 (m, 2 H) 2.98(s, 2 H) 7.37 (dd, *J*=5.4, 2.0 Hz, 1 H) 7.59 (d, *J*=1.5 Hz, 1 H) 8.55 (d, *J*=5.4 Hz, 1 H).

Example 102

3-[(4-Chloro-2-pyridyl)-ethynyl]-8-ethoxycarbonyl-1-oxa-2-azaspiro[4.5]dec-2-ene

Ethyl 4-methylidenecyclohexanecarboxylate (Compound 102 a)

This intermediate was prepared following the procedure described Compound 31a. As starting material ethyl 4-oxocyclohexane-1-carboxylate replaced 1,4-dioxaspiro[4.5]decan-8-one. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM –

Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 4 : 6) to give the title compound as a colourless oil. Yield: 68.7 %.

MS: $[M+H]^+ = 169.50$

3-trimethylsilylethynyl-8-ethoxycarbonyl-1-oxa-2-azaspiro[4.5]dec-2-ene (Compound 102b)

The title compound was synthesized using the method described above for Compound 3c, but replacing Compound 3a with Compound 102a. The crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 98 : 2 to 40 : 60) to give the title compound as colourless oil. Yield: 49.3%.

MS: $[M+H]^+ = 308.55$

3-[(4-Chloro-2-pyridyl)-ethynyl]-8-ethoxycarbonyl-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized following the procedure described for the compound of Example 3, but substituting Compound 102b for Compound 3c. The residue was purified by automated RP chromatography (Isolera®TM – Biotage; aq. NH_4HCO_3/AcCN 55:45) affording the title compound. Yield: 29%.

MS: $[M+H]^+ = 327.16$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.29 (t, 3H), 1.60-1.72 (m, 2H) 1.74-1.84 (m, 2H), 1.84-1.92 (m, 2H), 2.09 (dt, 2H), 2.42-2.51 (m, 1H), 2.62 (s, 3H), 2.96 (s, 2H), 4.17 (q, 2H), 7.20 (d, 1H), 7.39 (d, 1H), 7.63 (t, 1H).

Example 103

3'-(3-Chlorophenyl-ethynyl)-1H,4'H-spiro[isochroman-4,5'-isoxazole]

4-methyleneisochroman (Compound 103a)

This intermediate was prepared following the procedure described Compound 31a. As starting material isochroman-4-one replaced 1,4-dioxaspiro[4.5]decan-8-one. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 0 : 1) to give the title compound as a colourless oil. Yield: 69.8%.

MS: $[M+H]^+ = 146.19$

^{1}H-NMR: *($CDCl_3$, δ)*: 4.48 (s, 2 H) 4.84 (s, 2 H) 5.04 (s, 1 H) 5.63 (s, 1 H) 7.02 - 7.09 (m, 1 H) 7.22 - 7.28 (m, 2 H) 7.67 - 7.75 (m, 1 H)

3'-(3-Chlorophenyl-ethynyl)-1H,4'H-spiro[isochroman-4,5'-isoxazole]

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 103a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; Petroleum Ether – EtOAc from 97 : 3) followed by automated RP chromatography (SP01®TM – Biotage; gradient aq. NH_4HCO_3/AcCN from 40 : 60 to 20 : 80) affording the title compound. Yield: 15.8%.

MS: $[M+H]^+ = 323.78$

^{1}H-NMR: *(DMSO-d_6, δ)*: 3.36 - 3.43 (m, 1 H) 3.46 - 3.54 (m, 1 H) 3.86 (d, *J*=12.0 Hz, 1 H) 3.96 (d, *J*=12.0 Hz, 1 H) 4.69 - 4.77 (m, 1 H) 4.77 - 4.85 (m, 1 H) 7.11 - 7.18 (m, 1 H) 7.31 - 7.38 (m, 2 H) 7.43 - 7.54 (m, 2 H) 7.56 - 7.62 (m, 2 H) 7.71 (t, *J*=1.7 Hz, 1 H).

Example 104

3-(3-Chlorophenyl-ethynyl)-7-(4-methyl-1-piperazinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

To a solution of Compound 27d (150 mg, 0.58 mmol) in dichloromethane (10 ml) was added TEA (0.248 ml, 1.73 mmol) followed by 4-methyl-1-piperazinecarbonyl chloride (122 mg, 0.748 mmol). The reaction mixture was kept under stirring for 20 h. After evaporation, the residue was purified by automated isocratic RP chromatography (SP01®TM – Biotage; NH_4HCO_3/AcCN 55: 45) affording 91 mg of the title compound as a gummy oil. Yield: 49%.

MS: $[M+H]^+ = 357.44$

^{1}H-NMR: *(*DMSO-*d*6, δ):ppm 1.98 - 2.15 (m, 2 H) 2.18 (s, 3 H) 2.22 - 2.37 (m, 4 H) 3.08 - 3.17 (m, 2 H) 3.17 - 3.23 (m, 2 H) 3.23 - 3.41 (m, 3 H) 3.45 (d, *J*=11.7 Hz, 1 H) 3.53 (td, *J*=10.2, 7.0 Hz, 1 H) 3.60 (d, *J*=12.0 Hz, 1 H) 7.46 - 7.53 (m, 1 H) 7.54 - 7.61 (m, 2 H) 7.66 - 7.71 (m, 1 H).

Example 105

3-(3-Chlorophenyl-ethynyl)-7-(2-oxo-1-imidazolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with 2-oxo-1-imidazolidinecarbonyl chloride. After evaporation, the crude was purified by automated flash chromatography

(SP01®TM – Biotage; gradient EtOAc-MeOH from 95 : 5 to 8:2) to afford a yellowish solid. Yield: 51.3%.

MS: $[M+H]^+ = 373.3$

^{1}H-NMR: *(*DMSO-d_6, δ*):* ppm 2.04 - 2.23 (m, 2 H) 3.21 - 3.42 (m, 4 H) 3.49 - 3.79 (m, 6 H) 7.22 (s, 1 H) 7.46 - 7.53 (m, 1 H) 7.54 - 7.61 (m, 2 H) 7.66 - 7.71 (m, 1 H)

Example 106

3-(3-Chlorophenyl-ethynyl)-7-(1-pyrrolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with 1-pyrrolidinylcarbonyl chloride (97%). After the usual work-up, the crude was purified twice by automated flash chromatography (SP01®TM – Biotage; $CHCl_3$-MeOH/NH_3 100:0.5 then 100:0.3,) to afford an ivory solid. Yield: 72.8%.

MS: $[M+H]^+ = 358.35$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.77 - 1.96 (m, 4 H); 2.01 (dt, *J*=13.0, 9.2 Hz, 1 H); 2.32 (ddd, *J*=12.9, 6.7, 3.2 Hz, 1 H); 3.13 (d, *J*=17.1 Hz,1 H); 3.23 (d, *J*=17.4 Hz, 1 H); 3.34 - 3.49 (m, 4 H); 3.58 - 3.65 (m, 1 H); 3.71 (s, 2 H); 3.66 - 3.76 (m, 1 H); 7.32 (t, *J*=8.1 Hz, 1 H); 7.37 - 7.45 (m, 2 H); 7.53 (t, *J*=1.7 Hz, 1 H).

Example 107

3-(3-Chlorophenyl-ethynyl)-7-piperidinocarbonyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with piperidinocarbonyl chloride. After the usual work-up, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient petroleum ether – EtOAc 7 : 3 to 3 : 7) to afford a yellowish oil. Yield: 37.4%.

MS: $[M+H]^+ = 372.3$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 1.50 - 1.69 (m, 6 H) 1.99 (dt, *J*=13.0, 9.0 Hz, 1 H) 2.24 - 2.34 (m,1 H) 3.07 - 3.17 (m, 1 H) 3.17 - 3.33 (m, 5 H) 3.56 (ddd, *J*=10.6, 8.2, 2.9 Hz, 1 H) 3.60 - 3.65 (m, 1 H) 3.65 - 3.72 (m, 1 H) 3.72 - 3.77 (m, 1 H) 7.32 (t, *J*=7.8 Hz, 1 H) 7.38 - 7.44 (m, 2 H) 7.53 (t, *J*=1.7 Hz,1 H)

Example 108

3-(3-Chlorophenyl-ethynyl)-7-(3-methylsulphonyl-2-oxo-1-imidazolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with 3-methanesulphonyl-2-oxo-1-imidazolidinylcarbonyl chloride. After evaporation, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient petroleum ether – EtOAc 1 : 1 to 1 : 9) to afford a grey solid. Yield: 67.3%.

MS: $[M+H]^+ = 451.3$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 2.08 - 2.23 (m, 1 H) 2.40 (dd, *J*=13.0, 6.8 Hz, 1 H) 3.12 - 3.27 (m,2 H) 3.34 (s, 3 H) 3.62 - 4.10 (m, 8 H) 7.33 (t, *J*=8.3 Hz, 1 H) 7.42 (t, *J*=6.6 Hz, 2 H) 7.53 (s, 1 H).

Example 109

3-(3-Chlorophenyl-ethynyl)-7-(N-ethyl-N-isopropyl-carbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

A solution of Compound 27d (100 mg, 0.38 mmol) and DIPEA (0.074 ml, 0.07 mmol)) in 4 ml of CH_2Cl_2 was dropped over a period of 45 min at r.t. into a triphosgene (39.9 mg, 0.13 mmol) solution in CH_2Cl_2 (1 ml). The reaction was stirred for 10 min.then it was dropped into a solution of ethylisopropylamine (0.070 ml, 0.576 mmol) and DIPEA (0.074 ml, 0.07 mmol) in CH_2Cl_2 (1 ml). After overnight resting, the reaction was concentrated under reduced pressure and the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient petroleum ether – EtOAc 8 : 21 to 4 : 6) obtaining 25 mg as a yellow thick oil. Yield: 17.4%

MS: $[M+H]^+ = 373.88$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.15 (t, *J*=7.1 Hz, 3 H) 1.19 (d, *J*=6.6 Hz, 3 H) 1.24 (d, *J*=6.6 Hz, 3 H) 2.02 (dt, *J*=13.1, 9.1 Hz, 1 H) 2.26 - 2.36 (m, 1 H) 3.06 (dq, *J*=14.2, 7.1 Hz, 1 H) 3.11 - 3.34 (m, 3 H) 3.54 - 3.62 (m, 1 H) 3.62 - 3.78 (m, 3 H) 3.96 (quin, *J*=6.7 Hz, 1 H) 7.29 - 7.36 (m, 1 H) 7.41 (t, *J*=7.6 Hz, 2 H) 7.50 - 7.55 (m, 1 H).

Example 110

3-(3-Chlorophenyl-ethynyl)-7-(N-methoxy-N-methyl-carbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with N-methoxy-N-

methylcarbamoyl chloride. After evaporation, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient petroleum ether – EtOAc 8 : 2 to 4 : 6) to afford a pale yellow solid. Yield: 54.1%.

MS: $[M+H]^+ = 348.3$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 1.98 - 2.09 (m, 1 H) 2.34 (dddd, *J*=12.9, 6.6, 3.2, 1.5 Hz, 1 H) 3.06 (s, 3 H) 3.15 (d, *J*=17.4 Hz, 1 H) 3.23 (d, *J*=16.9 Hz, 1 H) 3.63 (s, 3 H) 3.67 (d, *J*=12.2 Hz, 1 H) 3.69 - 3.82 (m, 2 H) 3.85 (dd, *J*=12.2, 1.5 Hz, 1 H) 7.30 - 7.35 (m, 1 H) 7.38 - 7.45 (m, 2 H) 7.53 (t, *J*=1.7 Hz, 1 H).

Example 111

3-(3-Chlorophenyl-ethynyl)-7-(4-pyridylcarbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 109, but substituting 4-aminopyridine for ethylisopropylamine. After the usual work-up and evaporation, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient EtOAc - MeOH/NH3 from 98:2 to 85:15) to afford a pale yellow solid. Yield: 16.4%.

MS: $[M+H]^+ = 381.3$

^{1}H-NMR: *($CDCl_3$, δ)*: ppm 2.20 (dt, *J*=13.1, 9.4 Hz, 1 H) 2.43 - 2.54 (m, 1 H) 3.16 - 3.25 (m, 1 H) 3.25 - 3.34 (m, 1 H) 3.70 (d, *J*=11.7 Hz, 1 H)3.77 - 3.86 (m, 2 H) 4.02 (d, *J*=10.5 Hz, 1 H) 7.30 - 7.37 (m, 1 H) 7.39 - 7.46 (m, 2 H) 7.50 - 7.57 (m, 1 H) 7.66 (d, *J*=5.6 Hz, 2 H) 8.40 (d, *J*=5.9 Hz, 2 H).

Example 112

3-(3-Chlorophenyl-ethynyl)-7-ethoxycarbonyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene

The title compound was synthesized using the method described for the compound of Example 104, but replacing 4-methyl-1-piperazinecarbonyl chloride with ethyl chloroformate. After evaporation, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient petroleum ether – EtOAc 9 : 1 to 1 : 1) to afford a pale yellow oil. Yield: 67.3 %.

MS: $[M+H]^+ = 332.79$

^{1}H-NMR: *($DMSO\text{-}d_6$, δ)*: 1.19 (t, *J*=6.7 Hz, 3 H) 2.15 (br. s., 2 H) 3.17 - 3.28 (m, 1 H) 3.32 - 3.44 (m, 2 H) 3.44 - 3.63 (m, 3 H) 4.05 (q, *J*=6.6 Hz, 2 H) 7.46 - 7.53 (m, 1 H) 7.54 - 7.60 (m, 2 H) 7.68 (s, 1 H).

Example 113

3-[(6-Methyl-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

3-Trimethylsilylethynyl-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene (Compound 113a)

The title compound was synthesised following the method described above for Compound 3c, but using Compound 90a instead of Compound 3a. After the usual work-up, the crude product was purified by automated flash chromatography (Horizon®TM – Biotage) eluting with a Petroleum Ether – EtOAc gradient from 99 : 1 to 95 : 5. Yield: 34.1%.

3-[(6-Methyl-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure reported for the compound of Example 3, but replacing Compound 3c with Compound 113a. After the usual work-up, the crude was purified by automated flash chromatography (SP1®TM – Biotage; gradient Petroleum Ether - EtOAc from 9 : 1 to 7 : 3) affording the title product as yellow solid.

Yield: 36.5%.

MS: $[M+H]^+ = 291.39$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.78 - 1.94 (m, 2 H), 2.01 - 2.12 (m, 4 H), 2.12 - 2.35 (m, 2 H), 2.62 (s, 3 H), 2.98 (s, 2 H), 7.21 (d, *J*=7.8 Hz,1 H), 7.40 (d, *J*=7.6 Hz, 1 H), 7.64 (t, *J*=7.8 Hz, 1 H).

Example 114

3-(3-Chlorophenyl-ethynyl)-1,7-dioxa-2-azaspiro[4.3]oct-2-ene

3-Methyleneoxetan (Compound 114a)

The title compound was prepared following the methodology described for Compound 28a, but replacing 1-(t.butoxycarbonyl)-3-azetidinone with 3-oxetanone. The reaction crude was used in the next step without further purification.

3-(3-Chlorophenyl-ethynyl)-1,7-dioxa-2-azaspiro[4.3]oct-2-ene

The title compound was prepared following the procedure described for Compound 3c, but using Compound 31f instead of Compound 3b and Compound 114a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from from 90:10 to 75:25), followed

by automated RP chromatography (SP01®TM – Biotage; eluent aq. $(NH_4)_2CO_3$ 1.6 g/l – AcCN 1:1, affording the title compound as a yellow solid. Yield: 3.7% from 3-oxetanone.

MS: $[M+H]^+$ = 248.38

^{1}H-NMR: *(CDCl3, δ)*: 3.49 (s, 2 H), 4.73 (d, *J*=7.8 Hz, 2 H), 5.05 (d, *J*=8.1 Hz, 2 H), 7.32 (t, *J*=7.8 Hz, 1 H), 7.38 - 7.44 (m, 2 H),7.50 - 7.55 (m, 1 H).

Example 115

3-(3-Chlorophenyl-ethynyl)-1-oxa-3-azaspiro[4.5]dec-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and 1-methylenecyclohexane instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 9 : 1). Another purification was required to obtain pure title compound, carried out with a gradient Petroleum Ether – Et_2O from 1 : 0 to 9 : 1. Yield: 90.5%.

MS: $[M+H]^+$ = 273.76

^{1}H-NMR: *(DMSO-d6, δ)*: 1.33 - 1.54 (m, 4 H) 1.54 - 1.73 (m, 6 H) 2.98 (s, 2 H) 7.48 (t, *J*=7.8 Hz, 1 H) 7.51 - 7.59 (m, 2 H) 7.66 (t, *J*=1.6 Hz, 1 H).

Example 116

3-(3-Chlorophenyl-ethynyl)-8-difluoromethylene-1-oxa-2-azaspiro[4.5]dec-2-ene

A solution of tris-dimethylaminophosphine in 2.6 ml of anhydrous THF was added under a nitrogen atmosphere to a solution of dibromodifluoromethane in 3.9 ml of anhydrous THF at 0°C over 15 min. The reaction mixture was stirred at r.t. for 30 min. and then a solution of the Compound of Example 32 (0.275 g, 0.956 mmol) was dropped into it. After overnight resting, the solution was evaporated off and the residue was taken up with EtOAc. Washing with water, drying the organic layer with Na_2SO_4 and evaporating to dryness *in vacuo* afforded a crude which was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 8 : 2) giving 52 mg of the title compound.

Yield: 13.7%.

MS: $[M+H]^+$ = 322.20

^{1}H-NMR: *(DMSO-d6, δ)*: 1.65 - 1.86 (m, 4 H), 2.11 - 2.30 (m, 4 H), 3.05 (s, 2 H), 7.49 (t, *J*=7.8 Hz, 1 H), 7.52 - 7.60 (m, 2 H), 7.65 - 7.69 (m, 1H).

Example 117

3-(3-Chlorophenyl-ethynyl)-8-carbamoyl-1-oxa-2-azaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 96, but replacing Compound 27d with Compound 22c. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 1 : 0 to 93 : 7) giving the title compound as a white solid. Yield: 76.9 %.

MS: $[M+H]^+ = 318.25$

^{1}H-NMR: (*DMSO-d6, δ*): 1.64 - 1.72 (m, 4 H) 3.06 (s, 2 H) 3.31 - 3.46 (m, 4 H) 5.98 (s, 2 H) 7.49 (t, *J*=8.1 Hz, 1 H) 7.53 - 7.59 (m, 2 H) 7.65-7.74 (m, 1 H).

Example 118

3-(3-Chlorophenyl-ethynyl)-8-(2-furoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 96, but replacing Compound 27d with Compound 22c and using and 2-furoyl chloride instead of methylisocyanate. After the usual work-up the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 95 : 5 to 8 : 2) giving 105 mg of the title compound. Yield: 60.2%.

MS: $[M+H]^+ = 369.30$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.84 (t, *J*=5.6 Hz, 4 H), 3.12 (s, 2 H), 3.66 (br. s., 2 H), 3.75 - 3.90 (m, 2 H), 6.63 (dd, *J*=3.4, 1.7 Hz, 1 H), 7.00 (d, *J*=3.4 Hz, 1 H), 7.49 (t, *J*=8.3 Hz, 1 H), 7.53 - 7.62 (m, 2 H), 7.68 (t, *J*=1.6 Hz, 1 H), 7.84 (d, *J*=1.0 Hz, 1 H).

Example 119

3-(3-Chlorophenyl-ethynyl)-8-(N-methoxy-N-methyl-carbamoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized using the method described for the compound of Example 110, but substituting Compound 22c for Compound 27d. After evaporation, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient petroleum ether – EtOAc 8 : 2 to 5 : 5) to afford a pale yellow oil. Yield: 74%.

MS: $[M+H]^+ = 362.30$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.76 (t, *J*=5.6 Hz, 4 H), 2.84 (s, 3 H), 3.08 (s, 2 H), 3.36 - 3.49 (m, 4 H), 3.54 (s, 3 H), 7.49 (t, *J*=7.8 Hz, 1 H), 7.53 - 7.59 (m, 2 H), 7.67 (t, *J*=1.7 Hz, 1 H).

Example 120

3-(3-Chlorophenyl-ethynyl)-8-diethylcarbamoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 96 but replacing Compound 27d with Compound 22c and methylisocyanate with N,N-diethylchloroformamide. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 8 : 2 to 6 : 4) giving the title compound. Yield: 47.1%.

MS: $[M+H]^+ = 374.34$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.05 (t, *J*=7.0 Hz, 6 H), 1.75 (t, *J*=5.6 Hz, 4 H), 3.06 (s, 2 H), 3.08 - 3.17 (m, 6 H), 3.17 - 3.25 (m, 2 H), 7.49 (t, *J*=7.8 Hz, 1 H), 7.52 - 7.60 (m, 2 H), 7.65 - 7.69 (m, 1 H).

Example 121

3-(3-Chlorophenyl-ethynyl)-8-(3-chlorobenzoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 96 but replacing Compound 27d with Compound 22c and methylisocyanate with 3-chlorobenzoyl chloride. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 8 : 2 to 6 : 4) giving 52 mg of the title compound as a pale yellow oil. Yield: 67.5%.

MS: $[M+H]^+ = 413.25$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.82 (br. s., 4 H), 3.10 (br. s., 2 H), 3.33 - 3.66 (m, 3 H), 3.83 (br. s., 1 H), 7.36 - 7.41 (m, 1 H), 7.45 - 7.61 (m, 6 H), 7.67 (t, *J*=1.6 Hz, 1 H).

Example 122

3-(3-Chlorophenyl-ethynyl)-8-ethoxycarbonyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 112 but substituting Compound 22c for Compound 27d. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 8 : 2) giving the title compound as a white solid.

Yield: 62%.

MS: $[M+H]^+ = 347.27$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.19 (t, *J*=7.1 Hz, 3 H), 1.73 (t, *J*=5.7 Hz, 4 H), 3.07 (s, 2 H), 3.37 - 3.55 (m, 4 H), 4.05 (q, *J*=7.1 Hz, 2 H), 7.49 (t, *J*=8.6 Hz, 1 H), 7.53 - 7.61 (m, 2 H), 7.67 (t, *J*=1.7 Hz, 1 H).

Example 123

3-(3-Chlorophenyl-ethynyl)-8-(1-pyrrolidinylcarbonyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method herein described for the compound of Example 106 but substituting Compound 22d for Compound 27c. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 7 : 3 to 3 : 7) giving 52 mg of the title compound as a pale yellow solid. Yield: 72.6%.

MS: $[M+H]^+ = 372.42$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.67 - 1.83 (m, 8 H), 3.07 (s, 2 H), 3.15 - 3.29 (m, 8 H), 7.49 (t, *J*=7.8 Hz, 1 H), 7.52 - 7.61 (m, 2 H), 7.67 (t, *J*=1.7 Hz,1 H).

Example 124

3-(3-Chlorophenyl-ethynyl)-8-dimethylcarbamoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene

The title compound was synthesized following the method described for the compound of Example 95 but substituting Compound 22d for Compound 27c. After the usual work-up, the crude was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 3 : 7 to 0 : 1) giving the title compound as a pale yellow solid. Yield: 66.2%.

MS: $[M+H]^+ = 346.29$

^{1}H-NMR: *(DMSO-d6, δ)*: 1.75 (t, *J*=5.5 Hz, 4 H), 2.75 (s, 6 H), 3.07 (s, 2 H), 3.10 - 3.27 (m, 4 H), 7.49 (t, *J*=7.6 Hz, 1 H), 7.53 - 7.60 (m, 2 H), 7.67 (t, *J*=1.6 Hz, 1 H).

Example 125

3-(3-Chlorophenyl-ethynyl)-8-(t.butoxycarbonyl)-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene

1-(t.butoxycarbonyl)-3-methoxy-4-methylene-pyrrolidine (Compound 125a)

NaH was added to a solution of 1-(t.butoxycarbonyl)-3-hydroxy-4-methylene-pyrrolidine (0.257 g, 1.29 mmol), prepared as described by L Alcaraz et. al., Organic Letters, 2001, vol. 3, no. 25, p. 4051 – 4054) in 8 ml of THF, stirred at r.t. under a nitrogen atmosphere. After 0.5 h stirring at r.t., methyl iodide (0.057 g, 1.42 mmol) was added and the resulting mixture was stirred at r.t. for 2 h. After resting overnight, the reaction was quenched with water. The reaction mixture was extracted with EtOAc, and the extracts were washed with brine, dried over Na_2SO_4 and evaporated to dryness affording a crude, which was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 9 : 1 to 7 : 3) giving 165 mg of the title compound. Yield: 60 %.

MS: $[M+H]^+$ = 391.42

3-(3-Chlorophenyl-ethynyl)-8-(t.butoxycarbonyl)-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene

The title compound was prepared following the procedure described for Compound 3c, using Compound 31f instead of Compound 3b and Compound 125a instead of Compound 3a. After the usual work-up, the residue was purified by automated flash chromatography (Isolera®TM – Biotage; gradient Petroleum Ether – EtOAc from 8 : 2 to 1 : 1) to give the title compound as a pale-yellow solid. Yield: 67.6 %.

MS: $[M+H]^+$ = 391.42

^{1}H-NMR: *(DMSO-d_6, δ)*: 1.42 (s, 9 H), 3.22 - 3.28 (m, 1 H), 3.33 (s, 3 H), 3.35 - 3.57 (m, 5 H), 3.75 (br. s., 1 H), 7.50 (t, *J*=7.6 Hz, 1 H), 7.54 - 7.61 (m, 2 H), 7.70 (t, *J*=1.6 Hz, 1 H).

Example 126

3-(3-Chlorophenyl-ethynyl)-8-ethoxycarbonyl-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene

3-(3-Chlorophenyl-ethynyl)-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene (Compound 126a)

The title compound was synthesized following the method reported for Compound 1c, but replacing Compound 1b with the compound of Example 125 and using chloroform as the solvent. The crude residue was used without further purification in the next reaction.

MS: $[M+H]^+$ = 291.24

3-(3-Chlorophenyl-ethynyl)-8-ethoxycarbonyl-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene

The title compound was synthesized following the method described above for the compound of Example 104 but replacing Compound 27d with Compound 126a and 4-methyl-1-piperazinecarbonyl chloride with ethyl chloroformate. After the usual work-up, the crude was purified by automated flash chromatography (SP01®TM – Biotage; gradient Petroleum Ether – EtOAc from 8 : 2 to 1 : 1) giving the title compound. Yield: 52.6 %.

MS: $[M+H]^+ = 363.43$

^{1}H-NMR: *($CDCl_3$, δ)*: 1.29 (q, *J*=6.6 Hz, 3 H), 3.00 (dd, *J*=17.9, 12.5 Hz, 1 H), 3.42 (s, 3 H), 3.50 - 3.84 (m, 6 H), 4.12 - 4.23 (m, 2H), 7.33 (t, *J*=7.6 Hz, 1 H), 7.38 - 7.45 (m, 2 H), 7.54 (t, *J*=1.5 Hz, 1 H).

Example 127

Affinity of Selected Antagonists for mGlu5 Receptor Subtype

Radioligand Binding Assay at metabotropic glutamate receptor 5 in rat brain.

Methods

[0158] a) Membrane preparation: male Sprague Dawley rats (200-300g, Charles River, Italy) were killed by cervical dislocation and the forebrain (cortex, striatum and hippocampus) was homogenized (2x20 sec) in 50 vols of cold 50 mM Tris buffer pH 7.4, using a Politron homogenizer (Kinematica). Homogenates were centrifuged at 48000xg for 15 min, resuspended in 50 vols of the same buffer, incubated at 37°C for 15 min and centrifuged and resuspended two more times. The final pellets were frozen and stored at -80°C until use.

[0159] b) Binding assay: pellets from rat forebrain were resuspended in 100 vols of 20mM HEPES, 2 mM $MgCl_2$, 2mM $CaCl_2$, pH 7.4. The membranes were incubated in a final volume of 1 ml for 60 min at 25°C with 4 nM [^{3}H]MPEP in the absence or presence of competing drugs. Non-specific binding was determined in the presence of 10 µM MPEP (Spooren W. *et al.*, Trends Pharmacol Sci. 22, 331-337, 2001). The incubation was stopped by the addition of cold Tris buffer pH 7.4 and rapid filtration through 0.5% polyethyleneimine pretreated Filtermat 1204-401 (Wallac) filters. The filters were then washed with cold buffer and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

[0160] c) Data Analysis: the inhibition of specific binding of the radioligands by the tested compounds was analyzed to estimate the inhibitory concentration 50% (IC_{50}) value by using the non-linear curve-fitting software Prism 4.0 (Graphpad, San Diego, CA). The IC_{50}

value was converted to an affinity constant (Ki) by the equation of Cheng & Prusoff (Cheng, Y.C.& Prusoff, W.H. Biochem. Pharmacol. 22, 3099-3108, 1973).

Results

[0161] The affinity (Ki) of the compounds of the instant invention for mGlu5 receptor is between 0.1 and 1000 nM. For instance, Compound of Example 12 has a Ki of 3.88 nM and Compound of Example 110 has a Ki of 1.49 nM.

Example 128

Affinity of Selected Antagonists for mGlu1 Receptor Subtype

Radioligand Binding Assay at metabotropic glutamate receptor 1 in rat brain.

Methods

[0162] a) Membrane preparation: male Sprague Dawley rats (200-300g, Charles River, Italy) were killed by cervical dislocation and the cerebella were homogenized (2x20 sec) in 50 vols of cold 50 mM Tris buffer pH 7.4, using a Politron homogenizer (Kinematica). Homogenates were centrifuged at 48000xg for 15 min, resuspended in 50 vols of the same buffer, incubated at 37°C for 15 min and centrifuged and resuspended two more times. The final pellets were frozen and stored at -80°C until use.

[0163] b) Binding assay: pellets from rat cerebellum were resuspended in 50 mM Tris, 1.2 mM $MgCl_2$, 2mM $CaCl_2$, pH 7.4; membranes were incubated in a final volume of 1 ml for 30 min at 0°C with 1.5 nM [^{3}H] R214127 in absence or presence of competing drugs. Non-specific binding was determined in the presence of 1 μM R214127 (Lavreysen H et al Mol. Pharmacol. 63:1082-1093, 2003). The incubation was stopped by the addition of cold Tris buffer pH 7.4 and rapid filtration through 0.5% polyethyleneimine pretreated Filtermat 1204-401 (Wallac) filters. The filters were then washed with cold buffer and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

[0164] c) Data Analysis: the inhibition of specific binding of the radioligands by the tested compounds was analyzed to estimate the inhibitory concentration 50% (IC_{50}) value by using the non-linear curve-fitting software Prism 4.0 (Graphpad, San Diego, CA). The IC_{50} value was converted to an affinity constant (Ki) by the equation of Cheng & Prusoff (Cheng, Y.C.& Prusoff, W.H. Biochem. Pharmacol. 22, 3099-3108, 1973).

Results

[0165] The affinity of the compounds of the instant invention for mGlu1 receptor is at least 10 times lower than their affinity for mGlu5 receptor.

Example 129

Affinity of Selected Antagonists for Group II (mGlu2+ mGlu3) Receptor Subtypes

Radioligand Binding Assay at Group II metabotropic glutamate receptors in rat brain.

Methods

[0166] a) Membrane preparation: male Sprague Dawley rats (200-300g, Charles River, Italy) were killed by cervical dislocation and the forebrain (cortex, striatum and hippocampus) was homogenized (2x20 sec) in 50 vols of cold 50 mM Tris buffer pH 7.4, using a Politron homogenizer (Kinematica). Homogenates were centrifuged at 48000xg for 15 min, resuspended in 50 vols of the same buffer, incubated at 37°C for 15 min and centrifuged and resuspended two more times. The final pellets were frozen and stored at -80°C until use.

[0167] b) Binding assay: pellets of rat forebrain were washed three times with ice-cold assay buffer (10 mM potassium phosphate + 100 nM potassium bromide, ph 7,6). Final pellets were resuspended in 200 vols of the assay buffer and membranes incubated in a final volume of 1 ml for 30 min at 0°C with 1 nM [^{3}H]LY341495 in the absence or presence of competing drugs. Non-specific binding was determined in the presence of 1 mM l-glutamate (Wright R.A. et al. J. Pharmacol. Exp. Ther. 298:453-460, 2001; Mutel V et al. J.Neurochem. 75, 2590-2601, 2000). The incubation was stopped by the addition of cold Tris buffer pH 7.4 and rapid filtration through 0.5% polyethyleneimine pretreated Filtermat 1204-401 (Wallac) filters. The filters were then washed with cold buffer and the radioactivity retained on the filters was counted by liquid scintillation spectrometry.

[0168] c) Data Analysis: the inhibition of specific binding of the radioligands by the tested compounds was analyzed to estimate the inhibitory concentration 50% (IC_{50}) value by using the non-linear curve-fitting software Prism 4.0 (Graphpad, San Diego, CA). The IC_{50} value was converted to an affinity constant (Ki) by the equation of Cheng & Prusoff (Cheng, Y.C.& Prusoff, W.H. Biochem. Pharmacol. 22, 3099-3108, 1973).

Results

[0169] The compounds of the instant invention did not affect [^{3}H]LY341495 binding to Group II (mGlu2+ mGlu3) metabotropic glutamate receptors up to 1000 nM.

Example 130

Determination of Functional Activity at mGlu5 receptor as Accumulation of Inositol Phosphate

[0170] To determine the mode of action (agonist, antagonist or inverse agonist) of the test compounds at mGlu5 receptor, the concentration dependence of the stimulation of inositol phosphate production in response to the agonist (glutamate or quisqualic acid) was compared in the absence and presence of different concentrations of the test compounds themselves, measured in cells expressing mGlu5 receptor.

[0171] The cells were preincubated with the glutamate-degrading enzyme (1U/ml glutamate pyruvate transaminase) and 2 mM pyruvate to avoid the possible action of glutamate released from the cells. The stimulation was then conducted in a medium containing 10 mM LiCl, and different concentrations of the agonist (glutamate or quisqualic acid) or compounds to be tested for agonistic activity.

[0172] When antagonist activity was studied, test compounds were added to cell cultures 20 min prior to the addition of the agonist and further incubated in the presence of the agonist.

[0173] The incubation was stopped adding ice cold perchloric acid then samples were neutralized, centrifuged and the supernatant utilized for the determination of inositol phosphate (IP) accumulation using the The Biotrak D-*myo*-Inositol 1,4,5-trisphosphate assay system from Amersham Biosciences. D-*myo*-Inositol 1,4,5-trisphosphate (IP_3) may be measured in the range 0.19-25 pmol (0.08-10.5 ng) per tube. In the assay, unlabelled IP_3 competes with a fixed amount of [^{3}H]-labelled IP_3 for a limited number of bovine adrenal IP_3 binding proteins. The bound IP_3 is then separated from the free IP_3 by centrifugation, which brings the binding protein to the bottom of the tube. The free IP_3 in the supernatant can then be discarded by simple decantation, leaving the bound fraction adhering to the tube. Measurement of the radioactivity in the tube enables the amount of unlabelled IP_3 in the sample to be determined by interpolation from a standard curve.

[0174] EC_{50}/IC_{50} were determined by nonlinear regression analysis using the software Prism 4.0 (Graphpad, San Diego, CA).

Results

The compounds of the instant invention showed antagonistic activity.

Example 131

Effect on Cystometry in Conscious Rats

Methods:

[0175] Male Sprague-Dawley rats [Crl: CD® (SD) IGS BR] of 300-400g b.w. supplied by Charles River Italia were used. The animals were housed with free access to food and water and maintained on a forced 12-hour-light/12-hour-dark cycle at 22-24°C of temperature, except during the experiment. To quantify urodynamic parameters in conscious rats, cystometrographic studies were performed according to the procedure previously reported (Guarneri et al., Pharmacol. Res. 24: 175, 1991).

[0176] Briefly, the rats were anaesthetised by intraperitoneal administration of 3 ml/kg of Equithensin solution (pentobarbital 30 mg/kg and chloral hydrate 125 mg/kg) and placed in a supine position. An approximately 10mm long midline incision was made in the shaved and cleaned abdominal wall. The urinary bladder was gently freed from adhering tissues, emptied and then cannulated via an incision in the bladder body, using a polyethylene cannula (0.58mm internal diameter, 0.96mm external diameter) which was permanently sutured with silk thread. The cannula was exteriorised through a subcutaneous tunnel in the retroscapular area, where it was connected to a plastic adapter in order to avoid the risk of removal by the animal. For drug testing, the rats were utilised one day after implantation.

[0177] On the day of the experiment, the rats were placed in modified Bollman cages, *i.e.*, restraining cages that were large enough to permit the rats to adopt a normal crouched posture, but narrow enough to prevent turning around. After a stabilisation period of about 20 minutes, the free tip of the bladder cannula was connected through a T-shaped tube to a pressure transducer (Statham P23XL) and to a peristaltic pump (Gilson Minipuls 2) for continuous infusion of a warm (37°C) saline solution into the urinary bladder, at a constant rate of 0.1 ml/minute. The intraluminal-pressure signal during infusion of saline into the bladder (cystometrogram) was continuously recorded on a polygraph (Rectigraph-8K San-ei with BM614/2 amplifier from Biomedica Mangoni) or stored on PC by data acquisition system (PowerLab, Chart 4 software, AD Instruments). From the cystometrogram, bladder volume capacity (BVC) was evaluated. BVC (in ml) is defined as the volume of saline infused into the bladder necessary to induce detrusor contraction followed by micturition. Basal BVC value was evaluated as the mean of the values observed in the cystometrograms recorded in an initial period of 30-60 minutes. At this point in the assay, the infusion was interrupted and the test

compounds were administered orally by a stomach tube. The bladder infusion restarted and changes in BVC were evaluated from the mean values obtained in the cystometrograms observed during 1, 2, and 3 hours after treatment. The compounds were administered in a volume of 2 ml/kg. Groups of control animals received the same amount of vehicle corresponding to a solution 0.5% methocel in water.

[0178] Under the given test conditions, measurement of BVC is equivalent to measurement of interval time between micturitions.

Statistical Analysis

[0179] Each experimental group was composed of 4-11 animals. All data were expressed as mean ± standard error. The percent change of BVC *versus* the basal value, as well as Δ value (difference in ml) of BVC (BVC at time "x" minus basal value), were also evaluated for each rat/time. In the figures, data are reported as % change *versus* the basal value.

[0180] Statistical analysis on BVC values, as well as on Δ values, was performed by S.A.S./STAT software, version 6.12. The difference between vehicle and active treatment effect was evaluated on Δ values of BVC, whereas the difference between the values at different times *versus* the basal values was evaluated on original BVC data.

Results

[0181] The compound of the invention, administered at 0.1 to 10 mg/kg p.o. proved effective in increasing the bladder volume capacity.

[0182] The reference compound MTEP, orally administered at the dose of 1 mg/kg showed only a slight increase of bladder volume capacity, whereas the dose of 3 mg/kg induced a sustained increase of this parameter, which resulted statistically significant from the vehicle group after 3 hours from treatment.

[0183] The activity of compounds of the invention and reference standard was expressed as MED (*i.e.* Minimal Effective Dose that induces statistically significant increase of bladder volume capacity). MTEP showed a MED of 3. For some compounds of the invention MED was equal or better. For instance the Compound of Example 78 showed a MED of 3.

Example 132

Plasma Extravasation in the Dura Mater of Rats Induced by Electrical Stimulation of the Trigeminal Ganglion

[0184] Electrical stimulation of the trigeminal ganglion induces inflammation in the dura mater which causes plasma extravasation. This animal model is widely accepted for testing drugs useful in migraine.

[0185] Male Wistar rats weighing 175-190 g are anaesthetised with 50 mg/kg i.p. of pentobarbital and the jugular vein is cannulated for injection of drugs. The animals are placed in a stereotaxic frame. Symmetrical boreholes are drilled 3.0 mm laterally and 3.2 mm posteriorly from bregma and the electrodes are lowered 9.5 mm from dura mater. The test compound or control-vehicle solution are administered intravenously 10 min prior to electrical stimulation of the right trigeminal ganglion (5 min; 2.0 mA, 5 Hz, 5 ms duration and Evans blue (30 mg/kg i.v.), is given 5 min prior to electrical stimulation as a marker of plasma protein extravasation. 15 minutes after the end of the stimulation period the animals are perfused with 50 ml saline via the left cardiac ventricle to remove intravascular Evans blue. The dura mater is removed, blotted dry and weighed. Tissue Evans blue is extracted in 0.3 ml formamide at 50° C. for 24 h. Dye concentrations are measured with a spectrophotometer at 620 nm wavelength, interpolated on a standard curve and expressed as ng Evans blue content per mg tissue weight.

[0186] Extravasation is expressed as the quotient calculated by dividing the Evan's blue content of the stimulated side by the Evan's blue content of the unstimulated side.

Example 133

GERD model in dogs

[0187] Beagle dogs are equipped with a chronic esophagostomy to allow passage of a manometric catheter and a pH probe along the esophagus and the stomach.

Following recording of the basal pressure of the Lower Esophageal Sphincter and the stomach, compounds under evaluation and vehicle for control are administered by intravenous route.

[0188] Transient Lower Esophageal Sphincter Relaxations (TLESRs) and acid reflux are induced by infusion of an acidified meal followed by stomach distension using a peristaltic pump infusing air at 40 ml/min, in accordance to Stakeberg J. and Lehmann A., (Neurogastroenterol. Mot. (1999) 11: 125-132). Active compounds reduce dose-dependently the frequency of TLESRs and TLESRs associated with acid reflux. The activity is determined as % inhibition of both parameters as compared to vehicle control.

Example 134

Vogel Conflict Test in Rat

The method, which detects anxiolytic activity, follows that described by Vogel et al. as "Anxiolytics increase punished drinking" (Vogel J.R., Beer B., Clody D.E. A simple and reliable conflict procedure for testing anti-anxiety agents Psychopharmacologia, 21, 1-7, 1971).

[0189] Rats were deprived of water for approximately 48 hours and were then placed individually into a transparent Plexiglas enclosure (15 x 32 x 34 cm) with a floor consisting of stainless steel bars (0.4 cm) spaced 1 cm apart. The back wall of the enclosure was made of opaque Plexiglas thereby concealing the observer from the experimental animal. In the centre of the opposite wall, 5 cm above the floor, a metal water spout protruded into the cage and was connected to one pole of a shock generator. The other pole of the shock generator was connected to the metal grid floor.

[0190] The rat was left to explore until it founded the water spout. Then, every time it drank, it received a slight electric shock (1.7 mA, 1 s) 2 seconds after it started lapping. The number of punished drinks was counted during a 3 minute test.

The test was performed blind.

[0191] The test compounds were administered p.o. 60 minutes before the test, and compared with a vehicle control group.

Example 135

Operant alcohol self-administration training

[0192] The method was used to assess a substance abuse model and to detect the activity of the compounds of the invention in preventing this behavior.

[0193] Rats were trained to orally self-administer ethanol by using a modification of a training protocol described previously by Samson (1986). Briefly, rats were deprived of water for 12 h prior to training sessions for three consecutive days and were trained to respond for a 0.1-ml drop of 0.2% (w/v) saccharin solution on both levers under a fixed ratio 1 (FR1) schedule of reinforcement. After this initial training, water deprivation was terminated, and animals had free access to food and water in their home cages throughout the subsequent training and testing. Non-deprived rats were given two additional saccharin sessions to confirm that they had acquired responding for saccharin before ethanol self administration training started. Then, during the next three sessions, responses at the right lever resulted in the delivery of 0.1 ml of 5% (w/v) ethanol + 0.2% saccharin solution. Responses at the left lever were

recorded but had no programmed consequences. Thereafter, the concentration of ethanol was increased first to 8% and then to 10% w/v and the concentration of saccharin was decreased until saccharin was eliminated completely from the drinking solution.

[0194] The final schedule of reinforcement for the 10% w/v ethanol concentration was similar to the training schedule except that a stimulus light was added. Thus, during the 30-min sessions responses on the active lever resulted in the delivery of 0.1 ml of ethanol and, in addition, in the illumination of the stimulus light for 3 s. The left lever remained inactive. When rats had reached stable ethanol self-administration under these conditions, the effects of the tested compounds after i.p. administration on ethanol self administration were examined. The agonists were administered 30 min before start of the self-administration session.

Example 136

Neuropathic pain test (Bennett) in the rat

The method, which detects analgesic activity in rats with neuropathic pain, follows that described by Bennett and Xie (Bennett G.J., Xie Y.K., A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain, 33, 87 - 107, 1988).

[0195] Chronic constriction injury of the common sciatic nerve in rats is associated with hyperalgesia, allodynia and spontaneous pain, and constitutes therefore a model for peripheral neuropathic pain in humans. Antihyperalgesics reduce these chronic signs of pain hypersensitivity.

[0196] Rats (150 - 200 g) were anesthetized (sodium pentobarbital 40 mg/kg i.p.) and an incision at mid-thigh level was performed to expose the common left sciatic nerve. Four ligatures spaced 1 mm apart were loosely tied around the sciatic nerve. The wound was then sutured. The rats were allowed to recover. One week after the surgery, when the chronic pain state was fully installed, rats were submitted consecutively to tactile and thermal stimulation of both hindpaws.

[0197] For tactile stimulation, the animal was placed under an inverted acrylic plastic box (17 x 11 x 14 cm) on a grid floor. The tip of an electronic Von Frey probe was then applied with increasing force to the non-inflamed and inflamed hindpaws and the force inducing paw-withdrawal was automatically recorded. This procedure was carried out 3 times and the mean force per paw was calculated.

[0198] For thermal stimulation, the apparatus consists of individual acrylic plastic boxes (17 x 11 x 14 cm) placed upon an elevated glass floor. A rat was placed in the box and

left free to habituate for 10 minutes. A mobile infrared radiant source (96 ± 10 mW/cm²) was then focused first under the non-lesioned and then the lesioned hindpaw and the paw-withdrawal latency was automatically recorded. In order to prevent tissue damage the heat source was automatically turned off after 45 seconds.

[0199] Prior to receiving drug treatment all animals were submitted to tactile stimulation of the hindpaws and assigned to treatment groups matched on the basis of the pain response of the lesioned hindpaw.

[0200] 8 rats were studied per group. The test was performed blind.

[0201] Test compounds were administered p.o. 60 minutes before the test, and compared with a vehicle control group (0.5% carboxymethylcellulose (CMC) in distilled water).

Example 137

Limbic epileptogenesis in a mouse model

[0202] FMRP (fragile X mental retardation protein) plays a critical role in suppressing limbic epileptogenesis and predict that the enhanced susceptibility of patients with FXS to epilepsy is a direct consequence of the loss of an important homeostatic factor that mitigates vulnerability to excessive neuronal excitation.

[0203] Kindling experiments were conducted in FMR1 mutant mice (knockout mice with reduced expression of mGluR5) during the light phase of the cycle on 12-week-old adult mice.

[0204] A twisted bipolar electrode was implanted into the right amygdale (coordinates: 2.9 mm lateral and 1.2 mm posterior to bregma, 4.6 mm below dura) of animals under pentobarbital (60 mg/kg) anesthesia. Animals were then given 10 days to recover. The electrographic seizure threshold (EST) for each individual mouse was determined by applying 1-s train of 1-ms biphasic rectangular pulses at 60 Hz beginning at 50 lA. Additional stimulations increasing by 10 lA were administered at 2-min intervals until an electrographic seizure lasting at least 5 s was evoked. Stimulations at the EST intensity were subsequently applied once daily. EEGs and behavioral seizures were observed and recorded. The severity of the behavioral manifestations of seizures was classified according to the criteria of Racine (1972). Fully kindled is defined by the occurrence of 3 consecutive seizures of class 4 or greater. All surgery and kindling procedures were performed blind to genotype.

[0205] Unstimulated control animals of each genotype underwent surgical implantation of an electrode in the amygdala and were handled identically but were not stimulated. Electrode placement was confirmed by methyl green pyronine-Y staining. Data derived from animals with correct electrode placement were analyzed.

[0206] Tested drugs were administered by intraperitoneal injection 30 min before a class 5 seizure-inducing stimulation.

Example 138

6-hydroxydopamine–lesioned rats

[0207] This model is a reliable and robust model for reproducing Parkinson's lesions in the brain and for studying the protecting effects of drugs.

[0208] Female Sprague-Dawley rats (180 – 220 g) underwent stereotaxic surgery to produce lesions of the nigrostriatal system. Within each experimental group, half of the animals received an intrastriatal injection of 6-hydroxydopamine (6-OHDA) with 0.01% ascorbic acid in saline (lesioned animals) while the remaining animals received an intrastriatal injection of 0.01% ascorbic acid in saline (control animals). Surgical procedure for unilateral intrastriatal injection of 6-OHDA: rats were anaesthetized with sodium pentobarbitone (60 mg/ kg, i.p.) and placed in a Kopf stereotaxic apparatus, where the head was constrained to a tilted skull position (−3.0 mm). An incision was made on the midline of the scalp and a burr hole drilled through the skull at the appropriate coordinates. Through this, an intracerebral injection was delivered into the left striatum using a 30 gauge blunt-tipped cannula. Stereotaxic coordinates for injection were: 0.3 mm anterior and 3.0 mm lateral from Bregma, and 5.2 mm ventral from the cortical surface, according to the atlas of Paxinos & Watson Lesioned rats received 4 µl of 2.5 µg/ µl 6-OHDA/0.01% (w/ v) ascorbic acid, while control rats received 4 µl of saline/0.01% (w/ v) ascorbic acid. Injections were delivered at a rate of 0.6 µl/ min and the needle left in position for 10 min following injection before being withdrawn slowly. After sealing the skull, the incision was closed and the animals allowed to recover.

[0209] Tested compounds were administered for 14 days before the induced lesions. Protection of tested compound from loss of striatal dopaminergic nerve terminals was assessed by [^{3}H]-mazindol autoradiography.

[0210] Seven days after stereotaxic surgery, rats were lightly anaesthetized (CO_2/O_2: 80/20) and decapitated. Brains were rapidly removed and frozen over liquid nitrogen, then stored at −40°C prior to sectioning.

[0211] A Reichert Jung cryostat was used to cut consecutive coronal 14 µm sections of striatum at level +0.30 mm from Bregma according to the atlas of Paxinos & Watson. Sections were thaw mounted onto poly-L-lysine coated slides, then stored at −20°C until use.

[^{3}H]-mazindol autoradiography was used to visualize dopaminergic nerve terminals within sections of striatum taken from rat brains. All autoradiographic steps were carried out at 4°C to reduce non-specific binding.

[0212] Slide mounted sections of striatum were preincubated for 15 min in 50 mM Tris-HCl solution (pH 7.9) containing 120 mM NaCl and 5 mM KCl. Sections were then incubated for 60 min with 4 nM [^{3}H]-mazindol in 50 mM Tris-HCl solution (pH 7.9) containing 300 mM NaCl and 5 mM KCl. Desipramine (DMI; 300 nM) was included in all incubation solutions to prevent non-selective [^{3}H]-mazindol binding at noradrenergic uptake sites. Nomifensine (100 µM), a selective inhibitor of dopamine uptake sites, was used to determine non-specific binding. Sections were washed twice (2×3 min) in ice-cold incubation buffer to remove excess [^{3}H]-mazindol and dried under a stream of cold, dry air.

[0213] Once dry, radiolabelled sections were apposed to Hyperfilm-^{3}H and exposed for 21 days to allow an image of striatal dopaminergic nerve terminal density to develop on the film. Following the exposure period, films were developed for 5 min in Phenisol X-ray developer, rinsed briefly in a weak solution of stopbath and fixed in Hypam X-ray fixer for 10 min.

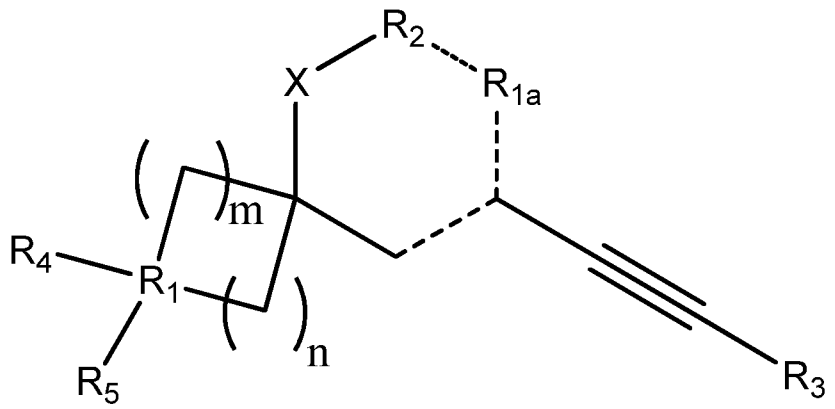
Computer-assisted densitometry was used to quantify the optical density of film images. The system was calibrated using [^{3}H]-standards, so that optical density measurements were made in nCi mm^{-2}. Specific binding was determined by subtracting the non-specific binding image from that of total binding, and was measured in the entire striatum.

Data analysis

The mean optical density and standard error of the mean were determined from independent measurements taken in at least three consecutive coronal sections of striatum for each animal.

CLAIMS

1. A compound having the general formula I



I

wherein:

X represents an oxygen or sulphur atom;

R_1 represents a carbon, nitrogen, oxygen or sulphur atom;

R_{1a} represents a CH, CH_2, N or NH moiety;

R_2 represents a valence bond or a CH or CH_2 group;

R_3 represents an optionally substituted C_1-C_6 alkyl group, an optionally substituted mono-, bi- or tricyclic C_1-C_{13} heterocyclic group containing 1 to 5 heteroatoms selected from N, O and S, an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group, an optionally substituted C_3-C_6 cycloalkyl group, or an optionally substituted C_3-C_6 cycloalkenyl group;

when R_1 represents a carbon atom:

R_4 represents a hydrogen atom, a halogen atom, an optionally substituted mono- or bicyclic C_1-C_9 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group;

R_5 represents a hydrogen or halogen atom, a cyano group, or an optionally substituted hydroxy, mercapto, amino, carbamoyl, C_1-C_6 alkyl or C_1-C_6 alkoxy group, or a group of the formula –C(=O)–R_6 wherein R_6 represents an optionally substituted C_1-C_6 alkyl or C_1-C_6 alkoxy group, an optionally substituted mono- or bicyclic C_1-C_9 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted mono-, bi- or tricyclic C_6-C_{14} aryl group; or

R_4 and R_5 together represent an oxo group or an optionally substituted methylene or hydroxyimino group; or

R_4 and R_5 together with R_1 represent an optionally substituted unsaturated 4 to 6 membered ring containing from 0 to 3 heteroatoms selected from N, O and S;

when R_1 represents an oxygen atom:

R_4 and R_5 are absent;

when R_1 represents a nitrogen atom:

one of R_4 and R_5 is absent and the other is as defined above;

when R_1 represents a sulphur atom:

each of R_4 and R_5 independently is absent or represents an oxo group;

m is 1, 2 or 3;

n is 1 or 2;

when n is 2 or m is 2 or 3, the ring containing R_1 may be fused with an optionally substituted benzene ring; and

each ---- represents a single or double bond provided that one double bond extends from the carbon atom to which R_3-C≡C- is bonded and that no ring carbon atom bears two double bonds;

the optional substituents being independently selected from halogen atoms and C_1-C_6 alkyl, C_1-C_6 alkoxy, hydroxy, mercapto, nitro, cyano, oxo, halo(C_1-C_6)alkyl, halo(C_1-C_6)alkoxy, C_1-C_6 alkylthio, C_1-C_6 alkylsulphonyl, C_1-C_6 alkylcarbonyl, sulphamoyl, C_1-C_6 alkylsulphamoyl, di(C_1-C_6)alkylsulphamoyl, (C_1-C_6)alkoxycarbonyl and (C_1-C_6)alkylcarbonyl(C_1-C_6)alkyl groups, and from groups of the formulae -NR*R*, -C(=O)-NR*R*, -A, -O-A, -C(=O)-A, -(CH_2)q-A, -NR**-A, -C(=O)NR**-A, -NR**C(=O)-A and -O-C(=O)-A wherein each R* independently represents a hydrogen atom or a C_1-C_6 alkyl, C_1-C_6 alkylcarbonyl, phenyl or benzyl group, R** represents a hydrogen atom or a C_1-C_6 alkyl group, q is an integer from 1 to 6 and A represents a phenyl group or a C_1-C_8 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S; each group A being optionally substituted with from 1 to 3 groups independently selected from halo, hydroxy, cyano, nitro and C_1-C_6 alkyl;

and solvates, hydrates, enantiomers, diastereomers, N-oxides and pharmaceutically acceptable salts of such compounds.

2. A compound according to claim 1 in which X represents an oxygen atom, R_{1a} represents a nitrogen atom, R_2 represents a bond, and a double bond extends from R_{1a} to the carbon atom to which R_3-C≡C- is bonded.

3. A compound according to claim 1 in which X represents an oxygen atom, R_{1a} represents a CH group with a double bond extending from R_{1a} to the carbon atom to which R_3-C≡C- is bonded or a CH_2 group with a single bond extending from R_{1a} to the carbon atom to which R_3-C≡C- is bonded, and R_2 represents a CH_2 group.

4. A compound according to any one of claims 1 to 3 in which R_3 represents an alkyl group, an optionally substituted C_3-C_5 heteromonocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted phenyl group; or R_3 represents a group of the formula –C(=O)–R_3 wherein R_3 is as defined in this claim.

5. A compound according to any one of claims 1 to 3 in which R_3 represents a methyl, isopropyl, butyl, phenyl, 3-(t.butyl)-phenyl, 2-chlorophenyl, 3-chlorophenyl, 3-chloro-5-fluorophenyl, 2-cyanophenyl, 3-cyanophenyl, 3-ethoxyphenyl, 3-ethylphenyl, 2-fluorophenyl, 3-fluorophenyl, 4-fluorophenyl, 3-hydroxyphenyl, 3-isopropylphenyl, 3-methoxyphenyl, 2-methylphenyl, 3-methylphenyl, 3-(3-methyl-[1,2,4]-oxadiazol-5-yl)-phenyl, 3-nitrophenyl, 3-trifluoromethoxyphenyl, 3-trifluoromethylphenyl, 2-pyridyl, 4-chloro-2-pyridyl, 5-chloro-2-pyridyl, 5-fluoro-2-pyridyl, 6-fluoro-2-pyridyl, 4-methyl-2-pyridyl, 6-methoxy-3-pyridyl, 6-methyl-2-pyridyl, 4-trifluoromethyl-2-pyridyl, 3-pyridyl, 6-fluoro-3-pyridyl, 2-furyl, 2-thienyl, 3-thienyl, 2-pyrazinyl, 2-methyl-1,3-thiazol-4-yl or 2-furoyl group.

6. A compound according to any one of claims 1 to 5 in which R_1 represents a carbon atom, R_4 represents a hydrogen atom, a halogen atom, an optionally substituted monocyclic C_3-C_5 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted phenyl group; and R_5 represents a hydrogen or halogen atom, a cyano group, or an optionally substituted hydroxy, mercapto, amino, carbamoyl, C_1-C_6 alkyl or C_1-C_6 alkoxy group, or a group of the formula –C(=O)–R_6 wherein R_6 represents an optionally substituted C_1-C_6 alkyl group, an optionally substituted monocyclic C_3-C_5 heterocyclic group containing from 1 to 3 heteroatoms selected from N, O and S, or an optionally substituted phenyl group.

7. A compound according to any one of claims 1 to 5 in which R_1 represents a carbon atom, R_4 represents a hydrogen or fluorine atom or a 2-pyridyl or 2-pyridylmethyl group, and R_5 represents a hydrogen or fluorine atom or a cyano, hydroxy or ethoxycarbonyl group.

8. A compound according to any one of claims 1 to 5 in which R_1 represents a carbon atom; and R_4 and R_5 together represent an oxo, methylene, difluoromethylene, 2-pyridylmethylene, trimethylene, tetramethylene, ethylenedioxy, propylenedioxy, oxyethyl, oxypropyl, methyleneoxymethylene, hydroxyimino, methoxyimino, 1-carboxy-3-hydroxy-propoxyimino or 2-oxo-tetrahydrofuran-3-yloxyimino group.

9. A compound according to any one of claims 1 to 5 in which R_1 represents a carbon atom, and R_4 and R_5 together represent a group of the formula $-(Y_p)-$ wherein p is an integer of from 3 to 5 and each Y independently represents an oxygen atom or an optionally substituted methylene group, provided that no two adjacent Y moieties represent oxygen atoms.

10. A compound according to claim 9 in which R_4 and R_5 together represent a group of the formula -O-CH_2CH_2-O-, -O-$CH_2CH_2CH_2$-O-, -O-CH_2CH_2-, -O-$CH_2CH_2CH_2$-, -CH_2-O-CH_2-, -$CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2$-, -$CH_2CH_2CH_2CH_2CH_2$- or -$CH_2CH_2CF_2CH_2CH_2$-,

11. A compound according to any one of claims 1 to 5 in which R_1 represents a nitrogen atom; R_4 represents an optionally substituted C_3-C_5 heteromonocyclic group containing from 1 to 3 heteroatoms selected from N, O and S; and R_5 is absent.

12. A compound according to any one of claims 1 to 5 in which R_1 represents a nitrogen atom; R_4 represents a 3-cyano-2-pyrazinyl, 3-nitro-2-pyridyl or 3-nitro-6-methyl-2-pyridyl group; and R_5 is absent.

13. A compound according to any one of claims 1 to 5 in which R_1 represents a nitrogen atom; R_4 is absent; and R_5 represents an alkoxycarbonyl, optionally substituted arylcarbonyl, optionally substituted heterocyclylcarbonyl, carbamoyl, diakylcarbamoyl, N-alkoxy-N-alkylcarbamoyl or optionally substituted heterocyclylcarbamoyl group.

14. A compound according to any one of claims 1 to 5 in which R_1 represents a nitrogen atom; R_4 is absent; and R_5 represents an ethoxycarbonyl, 3-chlorobenzoyl, 2-furoyl, 2-oxo-1-imidazolidinylcarbonyl, 3-methylsulphonyl-2-oxo-1-imidazolidinylcarbonyl, 4-methyl-1-piperazinylcarbonyl, piperidinocarbonyl, 1-pyrrolidinylcarbonyl, carbamoyl, dimethylcarbamoyl, diethylcarbamoyl, N-methoxy-N-methyl-carbamoyl, N-ethyl-N-isopropyl-carbamoyl or 4-pyridylcarbamoyl group.

15. Any one of the following compounds according to claim 1:

3-phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-phenylethynyl-8-(3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-[(6-methyl-2-pyridyl)-ethynyl]-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-thienylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-pyridylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(4-fluorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-furylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-[(2-methyl-1,3-thiazol-4-yl)-ethynyl]-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(prop-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-methylphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-methoxyphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-cyanophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-thienylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-Pyridylethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-{3-(3-methyl-[1,2,4]oxadiazol-5-yl)-phenylethynyl}-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-methylbut-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diaza-spiro[4.5]dec-2-ene,

3-(hex-1-ynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-cyanophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(3-cyano-2-pyrazinyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-methylphenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-chlorophenyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(2-Pyrazinyl-ethynyl)-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-phenylethynyl-8-(6-methyl-3-nitro-2-pyridyl)-1-oxa-8-azaspiro[4.5]dec-3-ene,

3-(3-chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene,

4-phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-4-ene,

4-phenylethynyl-9-(6-methyl-3-nitro-2-pyridyl)-1-oxa-9-azaspiro[5.5]undec-3-ene,

3-(3-chlorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-oxo-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-methylene-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-methoxyimino-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-[(6-methyl-2-pyridyl)-ethynyl]-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-phenylethynyl-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene,

3-(3-hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-fluorophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(2-pyridylmethylene)-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-hydroxy-8-(2-pyridylmethyl)-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-phenylethynyl-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-phenylethynyl-8-oxo-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-phenylethynyl-8-hydroxyimino-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-phenylethynyl-8-(2-oxo-3-tetrahydrofuranyloxy-imino)-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-[(6-methyl-2-pyridyl)-ethynyl]-8-cyano-8-(2-pyridyl)-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-[(5-fluoro-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-[(6-methyl-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-[(6-fluoro-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-[(6-fluoro-3-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-nitrophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-[(2-methyl-1,3-thiazol-4-yl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-methoxyphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-chloro-5-fluorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-methylphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-ethoxyphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,

3-(3-trifluoromethoxyphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-t.butylphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-cyanophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-fluorophenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-ethylphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-isopropylphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-[(4-chloro-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-[(4-methyl-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-trifluoromethylphenyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-[(4-trifluoromethyl-2-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-[(5-chloro-3-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(2-furoyl-ethynyl)-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-phenylethynyl-8-(1-carboxy-3-hydroxy-propoxyimino)-1-oxa-2-azaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-1,8-dioxa-2-azaspiro[4.5]dec-2-ene,
3-[(6-methyl-2-pyridyl)-ethynyl]-1,8-dioxa-2-azaspiro[4.5]dec-2-ene,
3-[(4-chloro-2-pyridyl)-ethynyl]-1,8-dioxa-2-azaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-1-oxa-8-thia-2-azaspiro[4.5]dec-2-ene 8,8-dioxide,
3-(3-chlorophenyl-ethynyl)-1,7-dioxa-2-azaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-methylphenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-hydroxyphenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene,
3-(3-cyanophenyl-ethynyl)-7-(6-methyl-3-nitro-2-pyridyl)-1-oxa-2,7-diazaspiro[4.3]oct-2-ene,
3'-(3-chlorophenyl-ethynyl)-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole],
3'-(3-chlorophenyl-ethynyl)-2,3-dihydro-3-oxo-4'H-spiro[indene-1,5'-isoxazole],
3'-(3-chlorophenyl-ethynyl)-2,3-dihydro-3-methoxyimino-4'H-spiro[indene-1,5'-isoxazole],
3-(3-chlorophenyl-ethynyl)-1,9-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene,
3-(3-chlorophenyl-ethynyl)-1,9,13-trioxa-2-azadispiro[4.2.5.2]pentadec-2-ene,
3'-[(6-methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-hydroxy-4'H-spiro[indene-1,5'-isoxazole],
3-(3-chlorophenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-(3-chlorophenyl-ethynyl)-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene,
3-(3-methylphenyl-ethynyl)-1-oxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3-[(6-methoxy-3-pyridyl)-ethynyl]-1,9,12-trioxa-2-azadispiro[4.2.4.2]tetradec-2-ene,
3'-[(6-methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-oxo-4'H-spiro[indene-1,5'-isoxazole],

3'-[(6-methyl-2-pyridyl)-ethynyl]-2,3-dihydro-3-methoxyimino-4'H-spiro[indene-1,5'-isoxazole],

3-(3-chlorophenyl-ethynyl)-7-dimethylcarbamoyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-carbamoyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-1-oxa-2-aza-dispiro[4.1.3.1]undec-2-ene,

3-(3-methylphenyl-ethynyl)-1,9,13-trioxa-2-azadispiro[4.2.5.2]pentadec-2-ene,

3-(3-chlorophenyl-ethynyl)-1-oxa-2-azaspiro[4.3]oct-2-ene,

3-(3-chlorophenyl-ethynyl)-1,10-dioxa-2-azadispiro[4.2.3.2]tridec-2-ene,

3-[(4-chloro-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-[(4-chloro-2-pyridyl)-ethynyl]-8-ethoxycarbonyl-1-oxa-2-azaspiro[4.5]dec-2-ene,

3'-(3-chlorophenyl-ethynyl)-1H,4'H-spiro[isochroman-4,5'-isoxazole],

3-(3-chlorophenyl-ethynyl)-7-(4-methyl-1-piperazinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(2-oxo-1-imidazolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(1-pyrrolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-piperidinocarbonyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(3-methylsulphonyl-2-oxo-1-imidazolidinylcarbonyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(N-ethyl-N-isopropyl-carbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(N-methoxy-N-methyl-carbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-(4-pyridylcarbamoyl)-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-(3-chlorophenyl-ethynyl)-7-ethoxycarbonyl-1-oxa-2,7-diazaspiro[4.4]non-2-ene,

3-[(6-methyl-2-pyridyl)-ethynyl]-8,8-difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-1,7-dioxa-2-azaspiro[4.3]oct-2-ene,

3-(3-chlorophenyl-ethynyl)-1-oxa-3-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-difluoromethylene-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-carbamoyl-1-oxa-2-azaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(2-furoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(N-methoxy-N-methyl-carbamoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-diethylcarbamoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,

3-(3-chlorophenyl-ethynyl)-8-(3-chlorobenzoyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-8-ethoxycarbonyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-8-(1-pyrrolidinylcarbonyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,
3-(3-chlorophenyl-ethynyl)-8-dimethylcarbamoyl-1-oxa-2,8-diazaspiro[4.5]dec-2-ene,
3-(3-Chlorophenyl-ethynyl)-8-(t.butoxycarbonyl)-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene or
3-(3-Chlorophenyl-ethynyl)-8-ethoxycarbonyl-6-methoxy-1-oxa-2,8-diazaspiro[4.4]non-2-ene,
or a pharmaceutically acceptable salt thereof.

16. A pharmaceutical composition comprising a compound according to any one of claims 1 to 14 or a solvate, hydrate, enantiomer, diastereomer, N-oxide or pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier.