PYRAZOLES FOR THE TREATMENT OF GERD AND IBS

Field of the invention

The present invention relates to novel compounds having a positive allosteric $GABA_B$ receptor (GBR) modulator effect, methods for the preparation of said compounds and their use for the inhibition of transient lower esophageal sphincter relaxations, for the treatment of gastroesophageal reflux disease, as well as for the treatment of functional gastrointestinal disorders and irritable bowel syndrome (IBS).

Background of the invention

The lower esophageal sphincter (LES) is prone to relaxing intermittently. As a consequence, fluid from the stomach can pass into the esophagus since the mechanical barrier is temporarily lost at such times, an event hereinafter referred to as "reflux".

Gastroesophageal reflux disease (GERD) is the most prevalent upper gastrointestinal tract disease. Current pharmacotherapy aims at reducing gastric acid secretion, or at neutralizing acid in the esophagus. The major mechanism behind reflux has been considered to depend on a hypotonic lower esophageal sphincter. However, recent research (e.g. *Holloway & Dent (1990) Gastroenterol. Clin. N. Amer. 19, pp. 517-535)* has shown that most reflux episodes occur during transient lower esophageal sphincter relaxations (TLESR), i.e. relaxations not triggered by swallows. It has also been shown that gastric acid secretion usually is normal in patients with GERD.

Consequently, there is a need for a therapy that reduces the incidence of TLESR and thereby prevents reflux.

$GABA_B$-receptor agonists have been shown to inhibit TLESR, which is disclosed in WO 98/11885 A1.

$GABA_B$ receptor agonists

GABA (4-aminobutanoic acid) is an endogenous neurotransmitter in the central and peripheral nervous systems. Receptors for GABA have traditionally been divided into $GABA_A$ and $GABA_B$ receptor subtypes. $GABA_B$ receptors belong to the superfamily of G-protein coupled receptors (GPCRs).

The most studied $GABA_B$ receptor agonist baclofen (4-amino-3-(*p*-chlorophenyl)butanoic acid; disclosed in CH 449046) is useful as an antispastic agent. EP 356128 A2 describes the use of the $GABA_B$ receptor agonist (3-aminopropyl)methylphosphinic acid for use in therapy, in particular in the treatment of central nervous system disorders.

EP 463969 A1 and FR 2722192 A1 disclose 4-aminobutanoic acid derivatives having different heterocyclic substituents at the 3-carbon of the butyl chain. EP 181833 A1 discloses substituted 3-aminopropylphosphinic acids having high affinities towards $GABA_B$ receptor sites. EP 399949 A1 discloses derivatives of (3-aminopropyl)methylphosphinic acid, which are described as potent $GABA_B$ receptor agonists. Still other (3-aminopropyl)methylphosphinic acids and (3-aminopropyl)phosphinic acids have been disclosed in WO 01/41743 A1 and WO 01/42252 A1, respectively. Structure-activity relationships of several phosphinic acid analogues with respect to their affinities to the $GABA_B$ receptor are discussed in *J. Med. Chem. (1995), 38, 3297-3312*. Sulphinic acid analogues and their $GABA_B$ receptor activities are described in *Bioorg. & Med. Chem. Lett. (1998), 8, 3059-3064*. For a more general review on $GABA_B$ ligands, see *Curr. Med. Chem.-Central Nervous System Agents (2001), 1, 27-42*.

Positive allosteric modulation of $GABA_B$ receptors

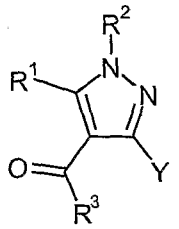
2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethylpropyl)phenol (CGP7930) and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2,2-dimethylpropanal (disclosed in US 5,304,685) have been described to exert positive allosteric modulation of native and recombinant $GABA_B$ receptor activity (*Society for Neuroscience, 30th Annual Meeting, New Orleans , La., Nov. 4-9, 2000: Positive Allosteric Modulation of Native and Recombinant $GABA_B$ Receptor Activity, S. Urwyler et al.; Molecular Pharmacol. (2001), 60, 963-971).*

N,N-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine has been described to exert positive allosteric modulation of the $GABA_B$ receptor (The Journal of Pharmacology and Experimental Therapeutics, 307 (2003), 322-330).

For a recent review on allosteric modulation of GPCRs, see: *Expert Opin. Ther. Patents (2001), 11, 1889-1904.*

Outline of the invention

The present invention relates to a compound of the general formula (I)



(I)

wherein

R^1 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, SO_3R^7, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or **R^1** represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups, wherein any aryl or heteroaryl group used in defining R^1 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

R^2 represents hydrogen, C_1–C_6 alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy; optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy,

halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^2 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

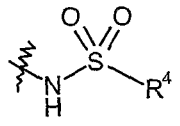
R^3 represents C_1-C_{10} alkoxy, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

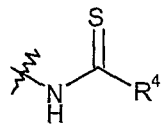
R^3 represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^3 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

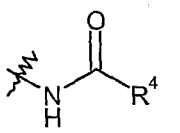
R^3 represents amino, optionally mono- or disubstituted with C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl or C_3-C_{10} cycloalkyl;

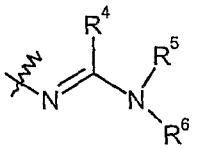
Y represents



;


;



; or


;

R^4 represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; C_1-C_{10} alkoxy; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, keto, carboxylic acid,

$CONR^8R^9$, NR^8COR^9, CO_2R^{10}, COR^{10}, nitrile, $SO_2NR^8R^9$, SO_2R^{11}, $NR^8SO_2R^9$, $NR^8C{=}ONR^9$ or one or two aryl or heteroaryl groups; or

R^4 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, $SO_2NR^8R^9$, $NR^8SO_2R^9$, SO_3R^7, nitrile or one or two aryl or heteroaryl groups, wherein said aryl or heteroaryl group used in defining R^4 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

R^5 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^5 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

R^6 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^6 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

or **R^5** and **R^6** together form a ring consisting of from 3 to 7 atoms selected from C, N and O, wherein said ring is optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s),

hydroxy, mercapto, nitro, keto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

$\mathbf{R^7}$ each and independently represents C_1-C_{10} alkyl;

$\mathbf{R^8}$ each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^9}$ each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^{10}}$ each and independently represents C_1-C_{10} alkyl, optionally substituted by aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^{11}}$ represents C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

wherein each of alkyl, alkenyl, alkynyl and cycloalkyl may independently have one or more carbon atom(s) substituted for O, N or S; wherein none of the O, N or S is in a position adjacent to any other O, N or S;

wherein each of alkyl, alkenyl, alkynyl, alkoxy and cycloalkyl may independently have one or more carbon atom(s) substituted by fluoro;

as well as pharmaceutically and pharmacologically acceptable salts thereof, and enantiomers of the compound of formula (I) and salts thereof;

with the exception of:

Pyrazole-4-carboxylic acid, 3-benzamido-1,5-diphenyl-, ethyl ester and 2-propenamide, N-(4-acetyl-5-methyl-1H-pyrazole-3-yl)-3-phenyl-.

In one embodiment of the present invention, $\mathbf{R^1}$ represents C_1–C_4 alkyl, optionally substituted by one aryl or two heteroaryl groups.

In another embodiment of the present invention, $\mathbf{R^1}$ represents aryl, optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, SO_3R^7, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

According to another embodiment of the present invention, $\mathbf{R^1}$ represents unsubstituted phenyl.

In a further embodiment of the present invention, $\mathbf{R^2}$ represents C_1-C_4 alkyl.

In yet a further embodiment of the present invention, $\mathbf{R^1}$ and $\mathbf{R^2}$ form a ring consisting of 5 or 6 atoms selected from C, O and N.

In one embodiment of the present invention, $\mathbf{R^3}$ represents C_1-C_4 alkoxy, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

According to yet another embodiment of the present invention, $\mathbf{R^3}$ represents C_1–C_{10} alkyl, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

In another embodiment of the present invention, $\mathbf{R^4}$ represents C_1–C_7 alkyl, C_2–C_7 alkenyl, C_2–C_7 alkynyl or C_3–C_7 cycloalkyl, optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic

acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile, amide, sulphonamide, urea or one or two aryl or heteroaryl groups, wherein said aryl or heteroaryl group used in defining R^4 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups.

In yet another embodiment of the present invention, $\mathbf{R^4}$ represents C_1–C_4 alkyl, optionally substituted by one or two aryl or heteroaryl groups.

In a further embodiment of the present invention, $\mathbf{R^4}$ represents C_1–C_4 alkyl, substituted by one or two aryl or heteroaryl groups.

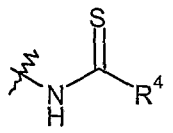
In yet a further embodiment of the present invention, $\mathbf{R^4}$ represents aryl or heteroaryl, optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

According to one embodiment of the present invention, $\mathbf{R^5}$ represents C_{1-4} alkyl. According to yet another embodiment of the present invention, $\mathbf{R^5}$ represents methyl.

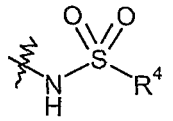
In another embodiment of the present invention, $\mathbf{R^6}$ represents C_{1-4} alkyl. In yet another embodiment of the present invention, $\mathbf{R^6}$ represents methyl.

According to another embodiment of the present invention, $\mathbf{R^5}$ and $\mathbf{R^6}$ form a ring consisting of 5 or 6 atoms selected from C, O and N.

According to yet another embodiment of the present invention, **Y** represents



According to a further embodiment of the present invention, **Y** represents



The present invention also relates to a compound according to claim 1, selected from ethyl 3-[(2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl)amino]-1-ethyl-1*H*-pyrazole-4-carboxylate and ethyl 1-ethyl-3-[(2-phenylbutanoyl)amino]-1*H*-pyrazole-4-carboxylate.

The compounds of formula (I) above are useful as positive allosteric $GABA_B$ receptor modulators as well as agonists.

The molecular weight of compounds of formula (I) above is generally within the range of from 300 g/mol to 700 g/mol.

It is to be understood that the present invention also relates to any and all tautomeric forms of the compounds of formula (I).

The general terms used in the definition of formula (I) have the following meanings:

C_1-C_{10} alkyl is a straight or branched alkyl group, having from 1 to 10 carbon atoms, for example methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, secondary butyl, tertiary butyl, pentyl, isopentyl, hexyl or heptyl. The alkyl groups may contain one or more heteroatoms selected from O, N and S, i.e. one or more of the carbon atoms may be substituted for such a heteroatom. Examples of such groups are methyl-ethylether, methyl-ethylamine and methyl-thiomethyl. The alkyl group may form part of a ring. One or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom.

C_1-C_4 alkyl is a straight or branched alkyl group, having from 1 to 4 carbon atoms, for example methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, secondary butyl or tertiary butyl. The alkyl groups may contain one or more heteroatoms selected from O, N and S, i.e. one or more of the carbon atoms may be substituted for such a heteroatom. Examples

of such groups are methyl-ethylether, methyl-ethylamine and methyl-thiomethyl. The alkyl group may form part of a ring. One or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom.

C_2-C_{10} alkenyl is a straight or branched alkenyl group, having 2 to 10 carbon atoms, for example vinyl, isopropenyl and 1-butenyl. The alkenyl groups may contain one or more heteroatoms selected from O, N and S, i.e. one or more of the carbon atoms may be substituted for such a heteroatom. One or more of the hydrogen atoms of the alkenyl group may be substituted for a fluorine atom.

C_2-C_{10} alkynyl is a straight or branched alkynyl group, having 2 to 10 carbon atoms, for example ethynyl, 2-propynyl and but-2-ynyl. The alkynyl groups may contain one or more heteroatoms selected from O, N and S, i.e. one or more of the carbon atoms may be substituted for such a heteroatom. One or more of the hydrogen atoms of the alkynyl group may be substituted for a fluorine atom.

C_3-C_{10} cycloalkyl is a cyclic alkyl, having 3 to 10 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl. The cycloalkyl may also be unsaturated. The cycloalkyl groups may have one or more heteroatoms selected from O, N and S, i.e. one or more of the carbon atoms may be substituted for such a heteroatom. One or more of the hydrogen atoms of the cycloalkyl group may be substituted for a fluorine atom.

C_1-C_{10} alkoxy is an alkoxy group having 1 to 10 carbon atoms, for example methoxy, ethoxy, n-propoxy, n-butoxy, isopropoxy, isobutoxy, secondary butoxy, tertiary butoxy, pentoxy, hexoxy or a heptoxy group. The alkoxy may be cyclic, partially unsaturated or unsaturated, such as in propenoxy or cyclopentoxy. The alkoxy may be aromatic, such as in benzyloxy or phenoxy.

C_1-C_{10} thioalkoxy is a thioalkoxy group having 1 to 10 carbon atoms, for example thiomethoxy, thioethoxy, n-thiopropoxy, n-thiobutoxy, thioisopropoxy, thioisobutoxy, secondary thiobutoxy, tertiary thiobutoxy, thiopentoxy, thiohexoxy or thioheptoxy group.

The thioalkoxy may be unsaturated, such as in thiopropenoxy or aromatic, such as in thiobenzyloxy or thiophenoxy.

The term aryl is herein defined as an aromatic ring having from 6 to 14 carbon atoms including both single rings and polycyclic compounds, such as phenyl, benzyl or naphtyl. Polycyclic rings are saturated, partially unsaturated or saturated.

The term heteroaryl is herein defined as an aromatic ring having 3 to 14 carbon atoms, including both single rings and polycyclic compounds in which one or several of the ring atoms is either oxygen, nitrogen or sulphur, such as furanyl, thiophenyl or imidazopyridine. Polycyclic rings are saturated, partially unsaturated or saturated.

Halogen(s) as used herein is selected from chlorine, fluorine, bromine or iodine.

The term keto is defined herein as a divalent oxygen atom double bonded to a carbon atom. Carbon atoms are present adjacent to the carbon atom to which the divalent oxygen is bonded.

When the compounds of formula (I) have at least one asymmetric carbon atom, they can exist in several stereochemical forms. The present invention includes the mixture of isomers as well as the individual stereoisomers. The present invention further includes geometrical isomers, rotational isomers, enantiomers, racemates and diastereomers.

Where applicable, the compounds of formula (I) may be used in neutral form, e.g. as a carboxylic acid, or in the form of a salt, preferably a pharmaceutically acceptable salt such as the sodium, potassium, ammonium, calcium or magnesium salt of the compound at issue.

The compounds of formula (I) are useful as positive allosteric GBR ($GABA_B$ receptor) modulators. A positive allosteric modulator of the $GABA_B$ receptor is defined as a compound which makes the $GABA_B$ receptor more sensitive to GABA and $GABA_B$ receptor agonists by binding to the $GABA_B$ receptor protein at a site different from that

used by the endogenous ligand. The positive allosteric GBR modulator acts synergistically with an agonist and increases potency and/or intrinsic efficacy of the $GABA_B$ receptor agonist. It has also been shown that positive allosteric modulators acting at the $GABA_B$ receptor can produce an agonistic effect. Therefore, compounds of formula (I) can be effective as full or partial agonists.

A further aspect of the invention is a compound of the formula (I) for use in therapy.

As a consequence of the $GABA_B$ receptor becoming more sensitive to $GABA_B$ receptor agonists upon the administration of a positive allosteric modulator, an increased inhibition of transient lower esophageal sphincter relaxations (TLESR) for a $GABA_B$ agonist is observed. Consequently, the present invention is directed to the use of a positive allosteric $GABA_B$ receptor modulator according to formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the preparation of a medicament for the inhibition of transient lower esophageal sphincter relaxations (TLESRs).

A further aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the prevention of reflux.

Still a further aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of gastroesophageal reflux disease (GERD).

Effective management of regurgitation in infants would be an important way of preventing, as well as curing lung disease due to aspiration of regurgitated gastric contents, and for managing failure to thrive, *inter alia* due to excessive loss of ingested nutrient. Thus, a further aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of lung disease.

Another aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the management of failure to thrive.

Another aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention of asthma, such as reflux-related asthma.

A further aspect of the invention is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention of laryngitis or chronic laryngitis.

A further aspect of the present invention is a method for the inhibition of transient lower esophageal sphincter relaxations (TLESRs), whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to subject in need of such inhibition.

Another aspect of the invention is a method for the prevention of reflux, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such prevention.

Still a further aspect of the invention is a method for the treatment of gastroesophageal reflux disease (GERD), whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

Another aspect of the present invention is a method for the treatment or prevention of regurgitation, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

Yet another aspect of the invention is a method for the treatment or prevention of regurgitation in infants, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

Still a further aspect of the invention is a method for the treatment, prevention or inhibition of lung disease, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment. The lung disease to be treated may *inter alia* be due to aspiration of regurgitated gastric contents.

Still a further aspect of the invention is a method for the management of failure to thrive, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

A further aspect of the invention is a method for the treatment or prevention of asthma, such as reflux-related asthma, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

A further aspect of the invention is a method for the treatment or prevention of laryngitis or chronic laryngitis, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

A further embodiment is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of a functional gastrointestinal disorder (FGD). Another aspect of the invention is a method for the treatment of a functional gastrointestinal disorder, whereby an effective amount of a

compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject suffering from said condition.

A further embodiment is the use of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of functional dyspepsia. Another aspect of the invention is a method for the treatment of functional dyspepsia, whereby an effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject suffering from said condition.

Functional dyspepsia refers to pain or discomfort centered in the upper abdomen. Discomfort may be characterized by or combined with upper abdominal fullness, early satiety, bloating or nausea. Etiologically, patients with functional dyspepsia can be divided into two groups:

1- Those with an identifiable pathophysiological or microbiologic abnormality of uncertain clinical relevance (e.g. *Helicobacter pylori* gastritis, histological duodenitis, gallstones, visceral hypersensitivity, gastroduodenal dysmotility)
2- Patients with no identifiable explanation for the symptoms.

Functional dyspepsia can be diagnosed according to the following:
At least 12 weeks, which need not be consecutive within the preceding 12 months of

1- Persistent or recurrent dyspepsia (pain or discomfort centered in the upper abdomen) and
2- No evidence of organic disease (including at upper endoscopy) that is likely to explain the symptoms and
3- No evidence that dyspepsia is exclusively relieved by defecation or associated with the onset of a change in stool frequency or form.

Functional dyspepsia can be divided into subsets based on distinctive symptom patterns, such as ulcer-like dyspepsia, dysmotility-like dyspepsia and unspecified (non-specific) dyspepsia.

Currently existing therapy of functional dyspepsia is largely empirical and directed towards relief of prominent symptoms. The most commonly used therapies still include antidepressants.

A further aspect of the invention is the use of a compound according to formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention of irritable bowel syndrome (IBS), such as constipation predominant IBS, diarrhea predominant IBS or alternating bowel movement predominant IBS.

A further aspect of the invention is a method for the treatment or prevention of irritable bowel syndrome (IBS), whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

IBS is herein defined as a chronic functional disorder with specific symptoms that include continuous or recurrent abdominal pain and discomfort accompanied by altered bowel function, often with abdominal bloating and abdominal distension. It is generally divided into 3 subgroups according to the predominant bowel pattern:

1- diarrhea predominant
2- constipation predominant
3- alternating bowel movements.

Abdominal pain or discomfort is the hallmark of IBS and is present in the three subgroups. IBS symptoms have been categorized according to the Rome criteria and subsequently modified to the Rome II criteria. This conformity in describing the symptoms of IBS has helped to achieve consensus in designing and evaluating IBS clinical studies.
The Rome II diagnostic criteria are:

1- Presence of abdominal pain or discomfort for at least 12 weeks (not necessarily consecutively) out of the preceding year
2- Two or more of the following symptoms:
a) Relief with defecation

b) Onset associated with change in stool frequency

c) Onset associated with change in stool consistency

A further aspect of the invention is the use of a compound according to formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention CNS disorders, such as anxiety.

A further aspect of the invention is a method for the treatment or prevention of CNS disorders, such as anxiety, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

A further aspect of the invention is the use of a compound according to formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention of depression.

A further aspect of the invention is a method for the treatment or prevention of depression, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

A further aspect of the invention is the use of a compound according to formula (I), optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment or prevention of dependency, such as alcohol or nicotine dependency.

A further aspect of the invention is a method for the treatment or prevention of dependency, such as aclohol dependency, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

For the purpose of this invention, the term "agonist " should be understood as including full agonists as well as partial agonists, whereby a "partial agonist" should be understood as a compound capable of partially, but not fully, activating $GABA_B$ receptors.

The wording "TLESR", transient lower esophageal sphincter relaxations, is herein defined in accordance with *Mittal, R.K., Holloway, R.H., Penagini, R., Blackshaw, L.A., Dent, J., 1995; Transient lower esophageal sphincter relaxation. Gastroenterology 109, pp. 601-610.*

The wording "reflux" is defined as fluid from the stomach being able to pass into the esophagus, since the mechanical barrier is temporarily lost at such times.

The wording "GERD", gastroesophageal reflux disease, is defined in accordance with *van Heerwarden, M.A., Smout A.J.P.M., 2000; Diagnosis of reflux disease. Baillière's Clin. Gastroenterol. 14, pp. 759-774.*

Functional gastrointestinal disorders, such as functional dyspepsia, can be defined in accordance with *Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Mueller-Lissner SA. C. Functional Bowel Disorders and Functional Abdominal Pain. In: Drossman DA, Talley NJ, Thompson WG, Whitehead WE, Coraziarri E, eds. Rome II: Functional Gastrointestinal Disorders: Diagnosis, Pathophysiology and Treatment. 2 ed. McLean, VA: Degnon Associates, Inc.; 2000:351-432 and Drossman DA, Corazziari E, Talley NJ, Thompson WG and Whitehead WE. Rome II: A multinational consensus document on Functional Gastrointestinal Disorders. Gut 45(Suppl.2), II1-II81.9-1-1999.*

Irritable bowel syndrome (IBS) can be defined in accordance with *Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Mueller-Lissner SA. C. Functional Bowel Disorders and Functional Abdominal Pain. In: Drossman DA, Talley NJ, Thompson WG, Whitehead WE, Coraziarri E, eds. Rome II: Functional Gastrointestinal Disorders: Diagnosis, Pathophysiology and Treatment. 2 ed. McLean, VA: Degnon Associates, Inc.; 2000:351-432 and Drossman DA, Corazziari E, Talley NJ, Thompson WG and Whitehead*

WE. Rome II: A multinational consensus document on Functional Gastrointestinal Disorders. Gut 45(Suppl.2), II1-II81.9-1-1999.

A "combination" according to the invention may be present as a "fix combination" or as a "kit of parts combination".

A "fix combination" is defined as a combination wherein (i) a compound of formula (I); and (ii) a $GABA_B$ receptor agonist are present in one unit. One example of a "fix combination" is a pharmaceutical composition wherein (i) a compound of formula (I) and (ii) a $GABA_B$ receptor agonist are present in admixture. Another example of a "fix combination" is a pharmaceutical composition wherein (i) a compound of formula (I) and (ii) a $GABA_B$ receptor agonist; are present in one unit without being in admixture.

A "kit of parts combination" is defined as a combination wherein (i) a compound of formula (I) and (ii) a $GABA_B$ receptor agonist are present in more than one unit. One example of a "kit of parts combination" is a combination wherein (i) a compound of formula (I) and (ii) a $GABA_B$ receptor agonist are present separately. The components of the "kit of parts combination" may be administered simultaneously, sequentially or separately, i.e. separately or together.

The term "positive allosteric modulator" is defined as a compound which makes a receptor more sensitive to receptor agonists by binding to the receptor protein at a site different from that used by the endogenous ligand.

The term "therapy" and the term "treatment" also include "prophylaxis" and/or prevention unless stated otherwise. The terms "therapeutic" and "therapeutically" should be construed accordingly.

Pharmaceutical formulations

The compound of formula (I) can be formulated alone or in combination with a $GABA_B$ receptor agonist.

For clinical use, the compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is in accordance with the present invention suitably formulated into pharmaceutical formulations for oral administration. Also rectal, parenteral or any other route of administration may be contemplated to the skilled man in the art of formulations. Thus, the compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, is formulated with a pharmaceutically and pharmacologically acceptable carrier or adjuvant. The carrier may be in the form of a solid, semi-solid or liquid diluent.

In the preparation of oral pharmaceutical formulations in accordance with the invention, the compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, to be formulated is mixed with solid, powdered ingredients such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture is then processed into granules or compressed into tablets.

Soft gelatine capsules may be prepared with capsules containing a mixture of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, with vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance(s) mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, in a mixture with a vegetable oil, paraffin oil, or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

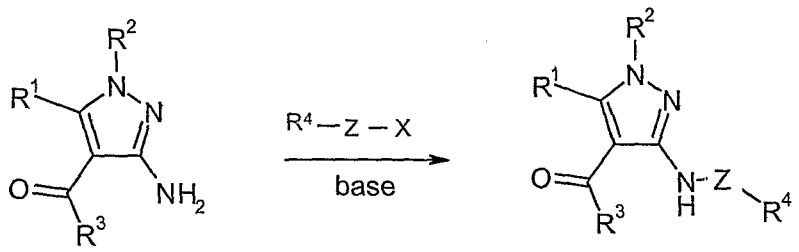
Liquid preparations for oral administration may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions, containing a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, and the remainder of the formulation consisting of sugar or sugar alcohols, and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethyl cellulose or other thickening agents. Liquid preparations for oral administration may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients and/or buffering ingredients and are dispensed into unit doses in the form of ampoules or vials. Solutions for parenteral administration may also be prepared as a dry preparation to be reconstituted with a suitable solvent extemporaneously before use.

In one aspect of the present invention, a compound of formula (I), optionally in combination with a $GABA_B$ receptor agonist, may be administered once or twice daily, depending on the severity of the patient's condition. A typical daily dose of the compounds of formula (I) is from 0.1 to 100 mg per kg body weight of the subject to be treated, but this will depend on various factors such as the route of administration, the age and weight of the patient as well as of the severity of the patient's condition.

Methods of preparation

The compounds according to formula (I) of the present invention, when Y= -NH-Z-R^4 and wherein R^1, R^2, R^3 and R^4 are defined as above, Z is $-SO_2-$, -C(S)- or –C(O)-, may be prepared by the following general methods:

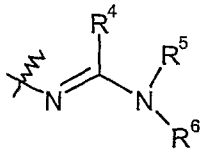


(II) X = reactive functionality (e.g. Cl) (Ia)

Scheme 1

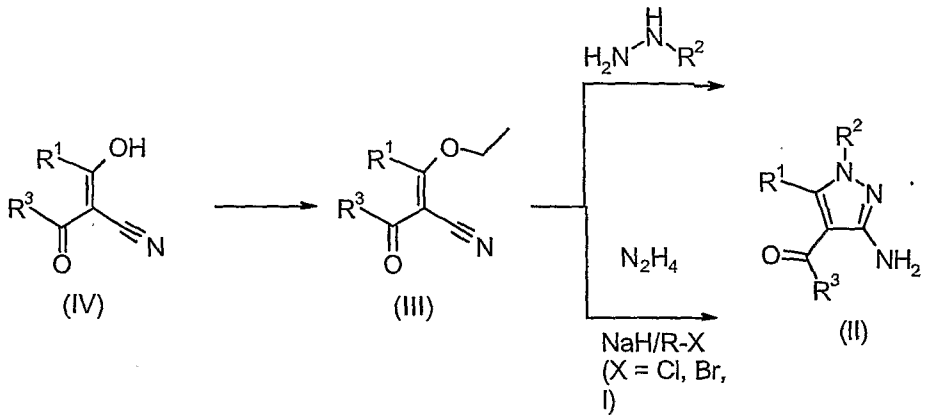
wherein aminopyrazoles (II) efficiently are converted into (Ia), using electrophiles such as acyl chlorides, sulfonylchlorides, carbamoylchlorides, isocyanates or isothiocyanates (typically 1.0 - 2.0 equivalents) in organic solvents such as THF or the like. The reaction is performed either in the presence of bases such as triethylamine and temperatures of 25 – 50°C or in the presence of polymer-supported diisopropylethylamine (PS-DIPEA; 1.5-3 equivalents) at ambient temperature to 50°C with agitation over 4-18 hours. Filtration of the reaction mixture over the nucleophilic anion exchange resin Isolute-NH2, elution with THF and evaporation *in vacuo* yields the desired products as oils or amorphous solids.

For compounds according to formula (I) of the present invention when Y =



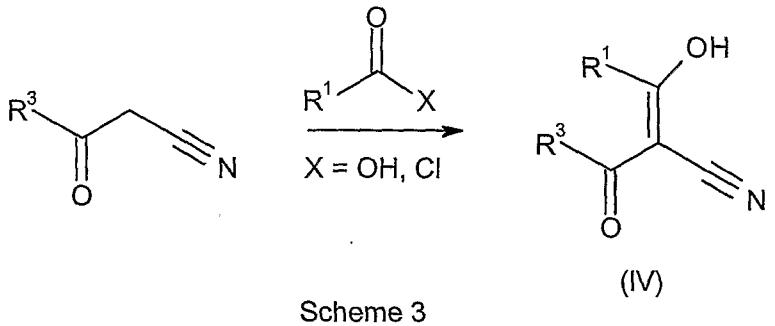
, the preparation is done according to methods familiar to the man skilled in the art.

The aminopyrazoles (II) are prepared from intermediates (III) by heating the reagent with either substituted hydrazines or hydrazine itself followed by alkylation of the thus generated unsubstituted pyrazoles with alkyl halides in the presence of a base such as sodium hydride. In these reactions, both regioisomeres are being produced which can be separated by chromatography (as indicated in Scheme 2; Literature: Australian J. Chem. 1985, 38, 221 – 230; Helvetica Chimica Acta 1959, 42, 349 - 359). The intermediates (III) can be prepared by reaction of (IV) with ethanol under standard Mitsunobu reaction conditions (Literature: D.L. Huges in Organic Reactions, Vol 42, p. 335 – 656, 1992.).



Scheme 2

The intermediates (IV) are accessible through known literature procedures. For example, ketonitriles can be converted to the intermediates (IV) via reaction with diethyl phosphorocyanidate and carboxylic acids as described by Shiori (J. Org. Chem. 1978, 43, 3631 - 3632), or via reaction with acid chlorides as described by Rappoport (J. Org. Chem. 1982, 47, 1397 – 1408) (see Scheme 3).

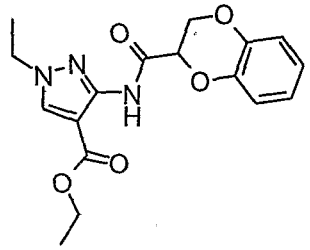


Scheme 3

EXAMPLES

Example 1:

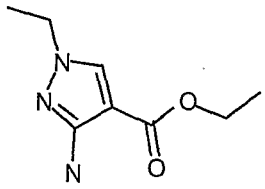
Synthesis of ethyl 3-[(2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl)amino]-1-ethyl-1*H*-pyrazole-4-carboxylate



The 1*H*-pyrazole-4-carboxylate (0.03 mmol) was dissolved in 2 mL dry THF. Then, PS-DIEA (25 mg, 0.09 mmol) followed by the acid chloride (0.07 mmol) were added. The reaction mixture was shaken at room temperature for 48 hours. The reaction mixture was filtered through an isolute-NH2 ion exchange column and the solvent was evaporated. The crude material was purified by flash chromatography using 30% EtOAc:heptane as an eluent to provide the desired product. Yield: 37.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H), 7.79 (s, 1H), 7.18-7.08 (m), 7.00-6.86 (m), 4.86 (d, 1H), 4.66 (d, 1H), 4.40-4.15 (m), 1.53 (t, 3H), 1.38 (t, 3H).
MS *m/z* 346.12 $(M+H)^+$.

Example 2:

Synthesis of ethyl 3-amino-1-ethyl-1*H*-pyrazole-4-carboxylate (used as intermediate)



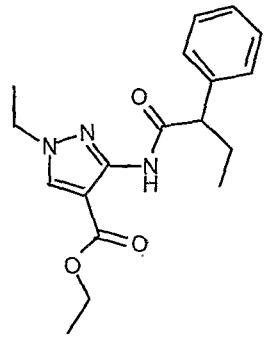
To a solution of ethyl 3-amino-1*H*-pyrazole-4-carboxylate (0.96 mmol; commercially available from ACROS) in MeCN was added sodium hydride (60%, 46 mg) at room temperature. After 30 minutes of stirring, a solution of ethyl iodide (0.96 mmol) in MeCN was added dropwise. The reaction mixture was stirred at room temperature for 48 hours. Then, the reaction was stopped by the addition was water, followed by dichloromethane.

The phases were separated using a phase separator. The organic phase was collected, dried over $MgSO_4$, the solvent was evaporated and the crude mixture was purified by flash chromatography using 30% EtOAc:heptane as an eluent to give a mixture of regioisomers that were directly used in next synthesis steps without any further separation. Yield: 68.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (s, 1H), 5.50 (s, 1H), 4.28-4.17 (m), 4.10-3.91 (m), 3.37-3.18 (m), 1.92 (s, 1H), 1.48-1.18 (m). MS *m/z* 184.15 $(M+H)^+$.

The following example was synthesized according to the examples described above.

Example 3:

ethyl 1-ethyl-3-[(2-phenylbutanoyl)amino]-1*H*-pyrazole-4-carboxylate



Yield: 33.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.43-7.20 (m), 4.28-4,07 (m), 3.44 (s, 1H), 2.36-2.16 (m), 1.98-1.75 (m), 1.47 (t, 3H), 1.29 (t, 3H) 0.93 (t, 3H).
MS *m/z* 330.27 $(M+H)^+$.

Analysis

LC-MS analysis was performed using a Micromass 8 probe MUX-LTC ESP+ system, purity being determined by single wavelength (254nm) UV detection. Chromatography was performed over an XterraTM MS C8 3.5um, 4.6 x30 mm column, 8 in parallel. The flow of 15ml/min was split over the 8 columns to give a flow rate of 1.9ml/min. The 10-minute chromatography gradient was as follows:

Mobile Phase A: 95% ACN + 5% 0,010 M NH_4OAc

Mobile Phase B: 5% ACN + 95% 0,010 M NH_4OAc

10 min	0,0 min	0% A

8,0 min 100% A
9,0 min 100% A
9,1 min 0% A

NMR analysis was performed at 400MHz.

Biological evaluation

Effects of the positive allosteric $GABA_B$ receptor modulator in a functional *in vitro* assay.

The effect of GABA and baclofen on intracellular calcium release in CHO cells expressing the $GABA_{B(1A,2)}$ receptor heterodimer was studied in the presence or absence of the positive allosteric modulator. The positive allosteric modulator according to the invention increased both the potency and the efficacy of GABA.

The potency of the compounds i.e. the ability of the compounds to reduce the EC_{50} of GABA was revealed by the concentration required to reduce GABA´s EC_{50} by 50 %. These potencies were similar to the potency reported for CGP7930 (can be purchased from Tocris, Northpoint, Fourth Way, Avonmouth, Bristol, BS11 8TA, UK) by Urwyler *et al.* CGP7930 increases the potency of GABA from EC_{50} of about 170-180 nM to EC_{50} of about 35-50 nM.

EXPERIMENTAL PROCEDURES

Materials

Nut mix F-12 (Ham) cell culture media, OPTI-MEM I reduced serum medium, Fetal bovine serum (FBS), penicillin/streptomycin solution (PEST), geneticin, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (buffer),1 M solution), Hank´s Balanced Salt Solution (HBSS) and zeocin were from Life technologies (Paisley, Scotland); Polyethyleneimine, probenicid, baclofen and γ-aminobutyric acid (GABA) were from Sigma (St Louis, USA); Fluo-3 AM was from Molecular Probes (Oregon, USA). 4-Amino-n-[2,3-^{3}H]butyric acid ([^{3}H]GABA) was from Amersham Pharmacia Biotech (Uppsala, Sweden).

Generation of cell lines expressing the $GABA_B$ receptor

$GABA_BR1a$ and $GABA_BR2$ were cloned from human brain cDNA and subcloned into pCI-Neo (Promega) and pALTER-1 (Promega), respectively. A $GABA_BR1a$-$G_{\alpha qi5}$ fusion protein expression vector was constructed using the pCI-Neo-$GABA_BR1a$ cDNA plasmid and pLEC1-$G_{\alpha qi5}$ (Molecular Devices, CA). In order to make the $G_{\alpha qi5}$ pertussis toxin insensitive, Cys356 was mutated to Gly using standard PCR methodology with the primers 5'-GGATCCATGGCATGCTGCCTGAGCGA-3´ (forward) and 5'-GCGGCCG CTCAGAAGAGGCCGCCGTCCTT-3' (reverse). The $G_{\alpha qi5mut}$ cDNA was ligated into the BamHI and NotI sites of pcDNA3.0 (Invitrogen). The $GABA_B$ R1a coding sequence was amplified by PCR from pCI-Neo-$GABA_BR1a$ using the primers, 5'-GGATCCCCGGGGAGCCGGGCCC-3' (forward) and 5´-GGATCCCTTATAAAGCAAATGCACTCGA-3' (reverse) and subcloned into the BamHI site of pcDNA3.0-$G_{\alpha qi5mut}$.

In order to optimise the Kozak consensus sequence of $GABA_BR2$, *in situ* mutagenesis was performed using the Altered Sites Mutagenesis kit according to manufacturer's instruction (Promega) with the following primer, 5'-GAATTCGCACCATGGCTTCCC-3'. The optimised $GABA_BR2$ was then restricted from pALTER-1 with Xho I + Kpn I and subcloned into the mammalian expression vector pcDNA3.1(-)/Zeo (Invitrogen) to produce the final construct, pcDNA3.1(-)/Zeo-$GABA_BR2$.

For generation of stable cell lines, CHO-K1 cells were grown in Nut mix F-12 (Ham) media supplemented with 10% FBS, 100 U/ml Penicillin and 100 µg/ml Streptomycin at 37° C in a humidified CO_2-incubator. The cells were detached with 1 mM EDTA in PBS and 1 million cells were seeded in 100 mm petri dishes. After 24 hours the culture media was replaced with OptiMEM and incubated for 1 hour in a CO_2-incubator.
For generation of a cell line expressing the $GABA_BR1a/GABA_BR2$ heterodimer, $GABA_BR1a$ plasmid DNA (4 µg) $GABA_BR2$ plasmid DNA (4 µg) and lipofectamine (24 µl) were mixed in 5 ml OptiMEM and incubated for 45 minutes at room temperature. The cells were exposed to the transfection medium for 5 hours, which then was replaced with culture medium. The cells were cultured for an additional 10 days before selection agents

(300 µg/ml hygromycin and 400 µg/ml geneticin) were added. Twenty-four days after transfection, single cell sorting into 96-well plates by flow cytometry was performed using a FACS Vantage SE (Becton Dickinson, Palo Alto, CA). After expansion, the $GABA_B$ receptor functional response was tested using the FLIPR assay described below. The clone with the highest functional response was collected, expanded and then subcloned by single cell sorting. The clonal cell line with the highest peak response in the FLIPR was used in the present study.

For generation of a stable cell line expressing $GABA_BR1a$-$G_{\alpha qi5}$ fusion protein and $GABA_BR2$, $GABA_BR1a$-$G_{\alpha qi5mut}$ plasmid DNA (8 µg) $GABA_BR2$ plasmid DNA (8 µg) and lipofectamine (24 µl) were mixed in 5 ml OptiMEM and incubated for 45 minutes at room temperature. The cells were exposed to the transfection medium for 5 hours, which then was replaced with culture medium. After forty-eight hours, the cells were detached and seeded in 6 well plates (2000 cells/well) and grown in culture medium supplemented with geneticin (400 µg/ml) and zeocin (250 µg/ml). After 4 days, cells from single colonies were collected and transferred to a 24-well plate. After 10 days, the cell clones were seeded in T-25 flasks and grown for another 16 days before they were tested for $GABA_B$ receptor mediated functional response. The clones that showed the highest peak response were collected and subcloned by seeding the cells in 6-well plates (1000 cells/well) and repeating the steps described above. The clonal cell line that gave the highest peak response in the FLIPR was used in the present study.

Measurement of $GABA_B$ receptor dependent release of intracellular calcium in the FLIPR

Measurement of $GABA_B$ receptor dependent release of intracellular calcium in the fluorescence imaging plate reader (FLIPR) was performed as described by Coward et al. *Anal. Biochem. (1999) 270, 242-248*, with some modifications. Transfected CHO cells were cultivated in Nut Mix F-12 (HAM) with Glutamax-I and supplemented with 10%, 100 U/ml penicillin and 100 µg/ml streptomycin, 250 µg/ml zeocin and 400 µg/ml geneticin. Twenty-four hours prior to the experiment the cells (35,000 cells/well) were seeded in black-walled 96-well poly-D-lysine coated plates (Becton Dickinson, Bedford, UK) in culture medium without selection agents. The cell culture medium was aspirated

and 100 µl of Fluo-3 loading solution (4 µM Fluo-3, 2.5 mM probenecid and 20 mM Hepes in Nut Mix F-12 (Ham)) was added. After incubation for 1 hour at 37°C in a 5 % CO_2 incubator, the dye-solution was aspirated and the cells were washed 2 times with 150 µl of wash solution (2.5 mM probenecid and 20 mM Hepes in HBSS) followed by addition of 150 µl of wash solution. The cells were then assayed in a fluorescence imaging plate reader (Molecular Devices Corp., CA, USA). Test compounds were diluted to 50 µM concentrations in HBSS containing 20 mM Hepes and 5% DMSO and added in a volume of 50 µl. The fluorescence was sampled every second for 60 s (10 s before and 50 s after the addition of test compound) before GABA (50 µl 7.6 nM-150 µM) was added and sampling continued every sixth second for additional 120 seconds.

GTPγS

[^{35}S]-GTPγS binding assays were performed at 30°C for 45min in membrane buffer (100mM NaCl, 5mM, 1mM EDTA, 50mM HEPES, pH 7.4) containing 0.025µg/µl of membrane protein (prepared from the cell lines described above) with 0.01% bovine serum albumin (fatty acid free), 10µM GDP, 100µM DTT and 0.53nM [^{35}S]-GTPγS (Amersham-Pharmacia Biotech) in a final volume of 200µl. Non-specific binding was determined in the presence of 20µM GTPγS. The reaction was started by the addition of GABA at concentration between 1mM and 0.1nM in the presence or absence of the required concentration of PAM. The reaction was terminated by addition of ice-cold wash buffer (50mM Tris-HCl, 5mM $MgCl_2$, 50mM NaCl, pH 7.4) followed by rapid filtration under vacuum through Printed Filtermat A glass fiber filters (Wallac) (0.05% PEI treated) using a Micro 96 Harvester (Skatron Instruments). The filters were dried for 30 min at 50°C, then a paraffin scintillant pad was melted onto the filters and the bound radioactivity was determined using a 1450 Microbeta Trilux (Wallac) scintillation counter.

Calculations

GABA dose-response curves in the presence and absence of test compounds were constructed using the 4-parameter logistic equation, $y=y_{max} + ((y_{min}-y_{max})/1+(x/C)^D)$, where $C=EC_{50}$ and D=slope factor.

The potency of PAM in GTPγS assays was determined by plotting the log EC_{50} for GABA against the log concentration of the positive allosteric modulator in the presence of which the measurement was performed.

Generally, the potency of the compounds of formula (I) ranges from EC_{50}s between 20 μM and 0.001 μM.

Effect of compounds in IBS model (colorectal distension)

Colorectal Distension (CRD)

For CRD, a 3 cm polyethylene balloon with a connecting catheter (made in-house) is inserted in the distal colon, 2 cm from the base of the balloon to the anus, during light isoflurane anaesthesia (Forene®, Abbott Scandinavia AB, Sweden). The catheter is fixed to the base of the tail with tape. At the same time, an intravenous catheter (Neoflon®, Becton Dickinson AB, Sweden) is inserted in a tail vein for compounds administration. Thereafter, rats are placed in Bollman cages and allowed to recover from sedation for at least 15 min before starting the experiments.

During the CRD procedure, the balloons are connected to pressure transducers (P-602, CFM-k33, 100 mmHg; Bronkhorst Hi-Tec, Veenendal, The Netherlands). A customized barostat (AstraZeneca, Mölndal, Sweden) is used to control the air inflation and intraballoon pressure. A customized computer software (PharmLab on-line 4.0.1) running on a standard PC is used to control the barostat and to perform data collection and storage. The distension paradigm generated by the barostat are achieved by generating pulse patterns on an analog output channel. The CRD paradigms use consisted on repeated phasic distensions, 12 times at 80 mmHg, with a pulse duration of 30 s at 5 min intervals.

Responses to CRD are assessed by recording and quantitation of phasic changes in intraballoon pressure during the distending pulses. Pressure oscillations during the isobaric inflation of the intracolonic balloon reflect abdominal muscle contractions associated to the distension procedure and, therefore, are considered a valid assessment of the visceromotor response (VMR) associated to the presence of pain of visceral origin.

Data Collection and Analysis

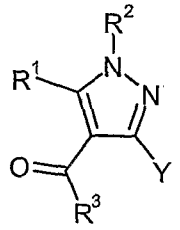
The balloon pressure signals are sampled at 50 Hz and afterwards subjected to digital filtering. A highpass filter at 1 Hz is used to separate the contraction-induced pressure changes from the slow varying pressure generated by the barostat. A resistance in the airflow between the pressure generator and the pressure transducer further enhance the pressure variations induced by abdominal contractions of the animal. In addition, a band-stop filtere at 49-51 Hz is used to remove line frequency interference. A customized computer software (PharmLab off-line 4.0.1) is used to quantify the phasic changes of the balloon pressure signals. The average rectified value (ARV) of the balloon pressure signals is calculated for the 30 s period before the pulse (baseline activity) and for the duration of the pulse (as a measure of the VMR to distension). When performing pulses analysis, the first and last second of each pulse are excluded since they reflect artefact signals produced by the barostat during inflation and deflation of the balloon and do not originate from the animal.

Results

The effect of the positive allosteric modulators is examined on the VMR to isobaric CRD in rats. A paradigm consisting of 12 distensions at 80 mmHg is used. The compounds are administered at a dose of 1 to 50 μmol/kg and VMR responses to CRD compared to the vehicle control.

Claims

1. A compound of the general formula (I)



(I)

wherein

R^1 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, SO_3R^7, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^1 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups, wherein any aryl or heteroaryl group used in defining R^1 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

R^2 represents hydrogen, C_1–C_6 alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy; optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^2 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

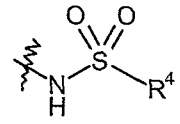
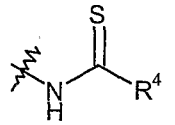
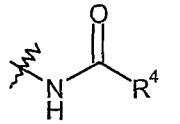
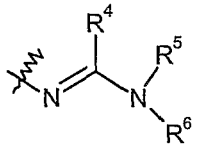
$\mathbf{R^3}$ represents C_1-C_{10} alkoxy, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

$\mathbf{R^3}$ represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

$\mathbf{R^3}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

$\mathbf{R^3}$ represents amino, optionally mono- or disubstituted with C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl or C_3-C_{10} cycloalkyl;

Y represents


;

;

; or

;

$\mathbf{R^4}$ represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; C_1-C_{10} alkoxy; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, keto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, COR^{10}, nitrile, $SO_2NR^8R^9$, SO_2R^{11}, $NR^8SO_2R^9$, $NR^8C{=}ONR^9$ or one or two aryl or heteroaryl groups; or

$\mathbf{R^4}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, $SO_2NR^8R^9$, $NR^8SO_2R^9$, SO_3R^7, nitrile or one or two aryl or heteroaryl groups, wherein said aryl or heteroaryl group used in defining R^4 may be further substituted by one or more of

halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

$\mathbf{R^5}$ represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or
$\mathbf{R^5}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

$\mathbf{R^6}$ represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or
$\mathbf{R^6}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

or $\mathbf{R^5}$ and $\mathbf{R^6}$ together form a ring consisting of from 3 to 7 atoms selected from C, N and O, wherein said ring is optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, keto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

$\mathbf{R^7}$ each and independently represents C_1-C_{10} alkyl;

$\mathbf{R^8}$ each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

R^9 each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

R^{10} each and independently represents C_1-C_{10} alkyl, optionally substituted by aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

R^{11} represents C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

wherein each of alkyl, alkenyl, alkynyl and cycloalkyl may independently have one or more carbon atom(s) substituted for O, N or S; wherein none of the O, N or S is in a position adjacent to any other O, N or S;

wherein each of alkyl, alkenyl, alkynyl, alkoxy and cycloalkyl may independently have one or more carbon atom(s) substituted by fluoro;

as well as pharmaceutically and pharmacologically acceptable salts thereof, and enantiomers of the compound of formula (I) and salts thereof;

with the exception of:
Pyrazole-4-carboxylic acid, 3-benzamido-1,5-diphenyl-, ethyl ester and 2-propenamide, N-(4-acetyl-5-methyl-1H-pyrazole-3-yl)-3-phenyl-.

2. A compound according to claim 1, wherein R^1 represents C_1–C_4 alkyl, optionally substituted by one aryl or two heteroaryl groups.

3. A compound according to claim 1, wherein R^1 represents aryl, optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10}

alkoxy, C_1-C_{10} thioalkoxy, SO_3R^7, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

4. A compound according to claim 3 wherein $\mathbf{R^1}$ represents unsubstituted phenyl.

5. A compound according to any one of claims 1-4, wherein $\mathbf{R^2}$ represents C_1-C_4 alkyl.

6. A compound according to claim 1, wherein $\mathbf{R^1}$ and $\mathbf{R^2}$ form a ring consisting of 5 or 6 atoms selected from C, O and N.

7. A compound according to any one of claims 1-6, wherein $\mathbf{R^3}$ represents C_1-C_4 alkoxy, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

8. A compound according to any one of claims 1-6, wherein $\mathbf{R^3}$ represents C_1–C_{10} alkyl, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

9. A compound according to any one of claims 1-8, wherein $\mathbf{R^4}$ represents C_1–C_7 alkyl, C_2–C_7 alkenyl, C_2–C_7 alkynyl or C_3–C_7 cycloalkyl, optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile, amide, sulphonamide, urea or one or two aryl or heteroaryl groups, wherein said aryl or heteroaryl group used in defining R^4 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups.

10. A compound according to claim 9, wherein $\mathbf{R^4}$ represents C_1–C_4 alkyl, optionally substituted by one or two aryl or heteroaryl groups.

11. A compound according to claim 10, wherein $\mathbf{R^4}$ represents C_1–C_4 alkyl, substituted by one or two aryl or heteroaryl groups.

12. A compound according to any one of claims 1-8, wherein $\mathbf{R^4}$ represents aryl or heteroaryl, optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups.

13. A compound according to any one of claims 1-12, wherein $\mathbf{R^5}$ represents C_{1-4} alkyl.

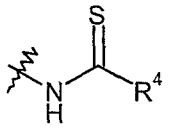
14. A compound according to claim 13, wherein $\mathbf{R^5}$ represents methyl.

15. A compound according to any one of claims 1-14, wherein $\mathbf{R^6}$ represents C_{1-4} alkyl.

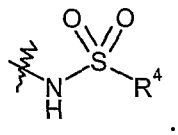
16. A compound according to claim 15, wherein $\mathbf{R^6}$ represents methyl.

17. A compound according to any one of claims 1-12, wherein $\mathbf{R^5}$ and $\mathbf{R^6}$ form a ring consisting of 5 or 6 atoms selected from C, O and N.

18. A compound according to any one of claims 1-12, wherein **Y** represents



19. A compound according to any one of claims 1-12, wherein **Y** represents

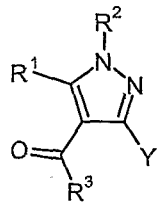


20. A compound according to claim 1, selected from

ethyl 3-[(2,3-dihydro-1,4-benzodioxin-2-ylcarbonyl)amino]-1-ethyl-1*H*-pyrazole-4-carboxylate and ethyl 1-ethyl-3-[(2-phenylbutanoyl)amino]-1*H*-pyrazole-4-carboxylate.

21. A pharmaceutical composition comprising a compound according to any one of claims 1-20 and a pharmaceutically acceptable carrier or diluent.

22. A compound of the general formula (I)



(I)

wherein

R^1 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, SO_3R^7, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^1 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups, wherein any aryl or heteroaryl group used in defining R^1 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

R^2 represents hydrogen, C_1–C_6 alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy; optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

$\mathbf{R^2}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

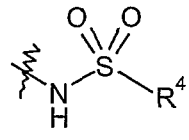
$\mathbf{R^3}$ represents C_1-C_{10} alkoxy, optionally substituted by one or more of C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

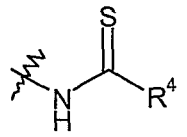
$\mathbf{R^3}$ represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, C_3–C_{10} cycloalkyl, keto, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

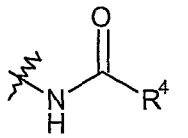
$\mathbf{R^3}$ represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

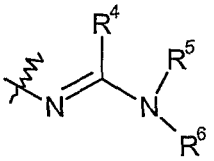
$\mathbf{R^3}$ represents amino, optionally mono- or disubstituted with C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl or C_3-C_{10} cycloalkyl;

Y represents



;


;


; or


;

$\mathbf{R^4}$ represents C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; C_1-C_{10} alkoxy; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, keto, carboxylic acid,

$CONR^8R^9$, NR^8COR^9, CO_2R^{10}, COR^{10}, nitrile, $SO_2NR^8R^9$, SO_2R^{11}, $NR^8SO_2R^9$, $NR^8C{=}ONR^9$ or one or two aryl or heteroaryl groups; or

R^4 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, $SO_2NR^8R^9$, $NR^8SO_2R^9$, SO_3R^7, nitrile or one or two aryl or heteroaryl groups, wherein said aryl or heteroaryl group used in defining R^4 may be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy, wherein said C_1-C_{10} alkyl may be further substituted by one or two aryl or heteroaryl groups;

R^5 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^5 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

R^6 represents hydrogen, C_1–C_{10} alkyl; C_2–C_{10} alkenyl; C_2–C_{10} alkynyl; or C_3–C_{10} cycloalkyl, each optionally substituted by one or more of C_1-C_{10} alkoxy, C_3–C_{10} cycloalkyl, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups; or

R^6 represents aryl or heteroaryl, each optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10} alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

or **R**^{5} and **R**^{6} together form a ring consisting of from 3 to 7 atoms selected from C, N and O, wherein said ring is optionally substituted by one or more of C_1-C_{10} alkyl, C_2–C_{10}

alkenyl, C_2–C_{10} alkynyl, C_3–C_{10} cycloalkyl, C_1-C_{10} alkoxy, C_1-C_{10} thioalkoxy, halogen(s), hydroxy, mercapto, nitro, keto, carboxylic acid, $CONR^8R^9$, NR^8COR^9, CO_2R^{10}, nitrile or one or two aryl or heteroaryl groups;

$\mathbf{R^7}$ each and independently represents C_1-C_{10} alkyl;

$\mathbf{R^8}$ each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^9}$ each and independently represents hydrogen, C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^{10}}$ each and independently represents C_1-C_{10} alkyl, optionally substituted by aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

$\mathbf{R^{11}}$ represents C_1-C_{10} alkyl, aryl or heteroaryl, wherein said aryl or heteroaryl may optionally be further substituted by one or more of halogen(s), C_1-C_{10} alkyl, C_1-C_{10} alkoxy or C_1-C_{10} thioalkoxy;

wherein each of alkyl, alkenyl, alkynyl and cycloalkyl may independently have one or more carbon atom(s) substituted for O, N or S; wherein none of the O, N or S is in a position adjacent to any other O, N or S;

wherein each of alkyl, alkenyl, alkynyl, alkoxy and cycloalkyl may independently have one or more carbon atom(s) substituted by fluoro;

as well as pharmaceutically and pharmacologically acceptable salts thereof, and enantiomers of the compound of formula (I) and salts thereof, for use in therapy.

23. Use of a compound as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of gastroesophageal reflux disease (GERD).

24. Use of a compound as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the prevention of reflux.

25. Use of a compound as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the inhibition of transient lower esophageal sphincter relaxations (TLESRs).

26. Use of a compound as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of a functional gastrointestinal disorder.

27. Use according to claim 26 wherein said functional gastrointestinal disorder is functional dyspepsia.

28. Use of a compound as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, for the manufacture of a medicament for the treatment of irritable bowel syndrome (IBS).

29. Use according to claim 28 wherein said IBS is constipation predominant IBS.

30. Use according to claim 28 wherein said IBS is diarrhea predominant IBS.

31. Use according to claim 28 wherein said IBS is alternating bowel movement predominant IBS.

32. A method for the treatment of gastroesophageal reflux disease (GERD), whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I) as

defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

33. A method for the treatment of a functional gastrointestinal disorder, whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I) as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.

34. A method for the treatment of irritable bowel syndrome (IBS), whereby a pharmaceutically and pharmacologically effective amount of a compound of formula (I) as defined in any one of claims 1-20 or claim 22, optionally in combination with a $GABA_B$ receptor agonist, is administered to a subject in need of such treatment.