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5-HT₄ Receptor Ligands: Applications and New Prospects.

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Introduction

The physiological effect of serotonin (5-HT) is mediated in the central and periphery systems by seven subtypes of receptors. All but one are members of the G protein coupled receptor (GPCRs) family (Table 1). Thirteen genes coding for the 5-HT GPCRs have been characterised and only three 5-HT receptors (5-HT₄R, 5-HT₆R and 5-HT₇R) are coupled to G_s proteins and stimulate adenylyl cyclase activity giving rise to an increase of the intracellular cAMP.¹ Considerable interest has been devoted to the characterisation of 5-HT₄ receptors since their discovery^{2,3} in mouse colliculi neuronal cells and in the guinea-pig ileum, respectively. Their presence in the gut allowed to propose a mechanism responsible for the pharmacological activity of several gastrokinetic benzamide drugs, such as metoclopramide, zacopride or renzapride (Scheme 1). Several splice variants of the 5-HT₄ receptors have been cloned⁴ and they differ in the length of their C-terminal ends. When studied in heterologous expression systems, these receptors present some functional and pharmacological differences, in particular, the length of the C-terminal sequence of the receptor was shown implicated in its constitutive activity.⁵ 5-HT₄ receptors are localised in the central nervous system,⁶ heart,⁷ intestine,⁸ adrenal cortex⁹ or the bladder¹⁰ and several important physiological processes such as the release of acetylcholine in the hippocampus,¹¹ the increase of the Ca²⁺ and pacemaker currents in the atrium,¹² the initiation of the intestinal peristaltic reflex⁸ or the increase of the release of corticosterol in the adrenal gland,⁹ are mediated through the activation of these receptors. Consequently, 5-HT₄ receptors have been implicated in a variety pathological disorders and constitute a valuable target for the design of new drugs. To date, several advances have been made to develop new molecules, in particular to cure the irritable bowel syndrome (IBS)¹³ which is characterised by an altered bowel function with an alternation of constipation and diarrhoea due to the dysfunction of the intestinal serotonergic system. But

several other promising routes are currently under study, such as the prevention of atrial fibrillation¹⁴ with 5-HT₄ receptor antagonists, the improvement of the cognitive functions¹⁵ by the enhancement of the cholinergic transmission in the hippocampus with the 5-HT₄ receptor agonists or the treatment of the voiding disorders associated with detrusor hypocontractility.¹⁰ The first generation of the 5-HT₃ receptor antagonists benzamides which turned out to be ligands for both 5-HT₃ and 5-HT₄ receptors (Scheme 1) played a major role in the discovery of the potent and selective 5-HT₄ receptor ligands available to date. Previous recent reviews^{16,17} have covered the different aspects of the SAR (structure-activity relationships) of this field. The present paper describes the recent advances made in the medicinal chemistry and in the mechanisms proposed to explain the physiological role of 5-HT₄ receptors in the different tissues where they are present and the putative clinical applications of the agonists and antagonists.

Chemistry

The 5-HT₄ receptor ligands are related to the following chemical groups: benzimidazolones, benzamides, benzoic esters, aryl ketones, indole carboxylates or carboxamides and serotonin analogues.

Benzimidalone derivatives.

Initially, benzimidazole-1-carboxylic acid ester and amides **1** (scheme 2, X = O or NH) with the basic framework *endo* tropane or quinuclidine were described as potent 5-HT₃ receptor antagonists.¹⁸ In contrast with the reference compounds in the field such as MDL 72222¹⁹ and ICS 205-930²⁰ the amidic derivatives were more potent than the corresponding esters. On the other hand, like benzamides such as zacopride²¹ or renzapride²² the hydrogen bond between the NH amidic and the carbonyl group of benzimidazolone ring stabilised the coplanar

conformation. In contrast with ondansetron, these compounds were capable of enhancing the electric-field stimulated contraction of the intestine and possessed gastrokinetic properties.²³ It was demonstrated²⁴ that these properties were related to their ability to stimulate the 5-HT₄ receptors. Then, in the primary culture of mouse *Colliculi neurons*, the compounds BIMU 1 and BIMU 8 were found to be potent agonists ($EC_{50} = 360$ nM and 72 nM, respectively) to stimulate the production of cAMP mediated by this receptor in a range of concentrations similar to that of 5-HT ($EC_{50} = 360$ nM). The potency and the pharmacological profile of benzimidazolones were dependent upon the N-substitution of the benzimidazole ring as the NH compound, DAU 6215, was inactive in this bioassay and a competitive antagonist ($K_i = 220$ nM) of the cAMP production by BIMU 8 and 5-HT.²⁵ These pharmacological properties were confirmed in the gastrointestinal (GI) system and, in particular, in the relaxation of the carbachol contracted rat oesophagus,²⁶ an efficient model for the characterisation of the 5-HT₄ ligands. BIMU 1 and BIMU 8 were equipotent to 5-HT ($pEC_{50} = 8$ and 7.9 respectively) but with a weaker intrinsic activity. DAU 6215 inhibited the effect of 5-HT and BIMU 8 ($pA_2 = 6.9-7.2$) in this model confirming the identity between the central and peripheral receptors.²⁷ Although a combination of 5-HT₃ receptor antagonist and 5-HT₄ receptor agonist properties could have some advantage in clinical use, the search for selective compounds was mandatory to characterise correctly the 5-HT₄ receptors. In this respect, it was only recently²⁸ that selective compounds were designed in this benzimidazolone family. The tropane moiety was substituted by the piperazine ring linked to the benzimidazole group with the flexible ethyl chain (compounds **2** and **3**, scheme 2). This structural modification brought about a decrease of affinity for 5-HT₃ receptors while the affinity for 5-HT₄ receptors was maintained. As in the parent series, the pharmacological profile depended upon the nature of the substitution on the nitrogen atom of the benzimidazole ring: the compound **2** ($R = cC_3H_5$, $R' = i-C_3H_7$) was a selective ligand with 5-HT₄ receptor antagonist activity ($K_i = 6.7$ nM, $pK_i = 7.78$) and the

compound **3** ($R = i\text{-C}_3\text{H}_7$, $R' = \text{Me}$) was a selective high partial agonist with moderate affinity (intrinsic activity = 0.94, $K_i = 91.1 \text{ nM}$). More recently,²⁹ derivatives of 3-quinolocarboxamide **4**, a bioisosteric group of benzimidazolone, were synthesised as structural analogues of BIMU 8. Several potent and selective 5-HT₄ receptor agonists were obtained by introducing various substituents on the nitrogen atom of the tropane ring. This work demonstrated the ability of the binding site of the 5-HT₄ receptor to accept voluminous groups around the ionic anchorage site. The alkyl chains with different groups such as piperidine, OH, phenoxy or CN fitted correctly to give rise to agonists more potent than cisapride. TS-951K (**4**, $R = \text{OH}$, $n = 3$) was selected as a promising agent to alleviate symptoms of the gastrointestinal dysfunctions.³⁰

Benzamide derivatives

The parent compound of this class of compound was metoclopramide³¹ (Scheme 3) and the amides of 4-amino-5-chloro-2-methoxybenzoic acid were the key compounds in the discovery of 5-HT₄ receptors ligands. Metoclopramide was characterised as a gastric prokinetic agent possessing dopamine receptor antagonist properties. Clebopride,³² a potent gastrokinetic compound in the guinea-pig ileum bioassay, was designed by the modification of the amino chain of metoclopramide which was introduced into the piperidine framework. However, the presence of the N-benzyl substitution strengthened the affinity for the D₂ dopamine receptors and provided a highly potent D₂ receptor antagonist. Nevertheless, by introducing other substitutions such as the aryloxypropyl chain, new compounds were discovered which turned out to be potent gastrokinetic compounds devoid of antidopaminergic properties. Cisapride³³ was the first molecule of this family largely used in human clinic to treat gastrointestinal disorders.

The structural modifications of the 2-alkoxy chain of metoclopramide were also claimed to give compounds with potent gastrokinetic properties and weak affinity for the D₂ receptors in guinea-pig and rat. In particular, (S)-ML-1035, a sulfoxide derivative was described as equipotent to metoclopramide in the enhancement of guinea-pig ileum contraction and rat gastric emptying.^{34,35}

But more promising results were obtained with the derivatives of metoclopramide, such as BRL 20627,³⁶ BRL 24682,³⁷ renzapride,²² zacopride^{21,38} where the amino flexible chain was introduced into a rigid framework such as the quinolizidine, tropane or quinuclidine moieties. These compounds were potent 5-HT₃ receptor antagonists of the 5-HT-evoked Bezold-Jarisch reflex and more potent stimulants of the gastrointestinal motility than metoclopramide. On the other hand and, in contrast to metoclopramide, they were inactive on the dopaminergic receptors. Since 5-HT₃ receptors are present in the gastrointestinal system, blockade of these receptors might account for the gastrokinetic properties of these compounds. However, this putative mechanism was discarded³⁹ since several potent 5-HT₃ antagonists structurally different from benzamides had no effect on gastrointestinal motility. It was demonstrated³ that renzapride as well as 5-HT induced relaxation of the rat oesophagus and facilitated the peristaltic reflex in guinea-pig ileum. Simultaneously,² these compounds were shown to be full agonists of the newly characterised 5-HT₄ receptor in mouse embryo colliculi neurons which was positively coupled to adenylyl cyclase. In this bioassay, cisapride, renzapride, and zacopride behaved as agonists with pEC₅₀ values equal or inferior to that of 5-HT (7.14, 6.90 and 5.95, respectively) but with a superior efficacy (%: 142%, 133 % and 144%, respectively) while they were partial agonists in a different assay using 5-HT₄ receptor mediated cAMP response in guinea-pig hippocampal membranes.⁴⁰

The structural requirements implicated in the recognition of the benzamides by the D₂ and 5-HT₄ receptors were studied by comparing the pharmacological properties of BRL 20627 and

the phenyl-substituted indolizidine analogues **5** and **6** (Scheme 4) mimicking the restricted conformers of clebopride.⁴¹ Only the β -phenyl stereoisomer **5** retained the gastric prokinetic activity while the central dopamine antagonist activity was present in the α -phenyl stereoisomer **6**. Preliminary structural parameters implicated for the recognition by the 5-HT₄ and 5-HT₃ receptors were determined by King²² with the synthesis of benzamide analogues of the high energy conformations of the *cis* junction of the quinolizidine ring of BRL 20627 (Scheme 4). The compounds possessed an azabicyclic system in such a way that the amino group of the second ring was “tied back”. This induced the drop of the steric hindrance around the basic nitrogen atom.¹⁷ The most representative of this class of compounds was renzapride which can be viewed as an analogue of zacopride and which was characterised as a potent stimulant of the electrically evoked guinea pig ileum contraction and of the rat intragastric pressure. However, almost all of these compounds were also potent 5-HT₃ receptor antagonists in the 5-HT-evoked Bezold-Jarisch reflex, indicating that 5-HT₃ and 5-HT₄ receptors possessed common steric requirements around the anchorage point of the ammonium ion.

The first breakthrough to obtain more selective benzamides for 5-HT₄ versus 5-HT₃ receptors was reported by Flynn⁴² who used the pyrrolizidine ring as the basic constrained framework and synthesised SC-53116. This compound was characterised as a potent and efficient 5-HT₄ agonist in the rat tunica muscularis mucosae. With an EC₅₀ of 23.7 nM when tested in this preparation, SC-53116 was more potent than cisapride, renzapride, (S) and (R) zacopride and BIMU 8 (EC₅₀ = 55, 44, 173, 505 and 40.3 nM, respectively). Similarly to cisapride, SC-53116 had a moderate affinity for 5-HT₃ receptors (K_i = 152 nM) but was more selective due to the lack of affinity for the dopamine and adrenergic receptors. An enantioselectivity was observed as the *exo* (1-S, 8-S) enantiomer was 10-fold more potent than the corresponding antipode. The SAR developed in this series highlighted the close structural relationships

between the 5-HT₃ and 5-HT₄ ligands, as a modest structural variation led to large effect on the activity. Hence, shortening the amino chain gave rise to a potent 5-HT₃ receptor antagonist (SC-52246) with very weak affinity for the 5-HT₄ receptor. The comparison of SC-53116 with renzapride and zacopride indicated that 5-HT₃ receptor binding site preferred more compact azabicycles as the basic moiety. This point was clearly demonstrated with the series of azanoradamentane derivatives⁴³ which were 5-HT₄ receptor agonists but with a potent affinity for the 5-HT₃ receptor (SC-52491). The structural modification of the benzamide ring of SC-53116 led to a dramatic change of the pharmacological profile since the imidazopyridine carboxamide SC-53606 (Scheme 5) was a relatively selective potent antagonist of 5-HT₄ receptors.⁴⁴ Benzamides,⁴⁵ such as SK-951,⁴⁶ with an achiral pyrrolizidine were more recently described. The essential structural modification with regard to SC-53116 resided in the substitution of the classical 4-amino-5-chloro-2-methoxybenzoic acid by the 4-amino-2,3-dihydro-2-methylbenzo[b]-furan-7-carboxylic. SK-951 was claimed as a potent 5-HT₄ agonist (EC₅₀ = 14 nM) with a selectivity *versus* 5-HT₃ receptor (K_i = 420 nM) in *in vitro* and *in vivo* models.^{46,47}

Structural constraints were also introduced into the basic ethyl side-chain of metoclopramide in which the vicinal carbon atom of the basic nitrogen atom of the ethyl chain was introduced into a ring. Compound **7**⁴⁸ (Scheme 5) was described as a 5-HT₃ receptor antagonist (K_i = 9 nM) devoid of any affinity for the 5-HT₄ receptor while compound YM-47813⁴⁹ was shown to enhance the gastric motility and gastric emptying in dog when administered orally. On the other hand, the inclusion of the the cyclopropane ring⁵⁰ in the ethyl chain gave rise to compounds with the *trans* or *cis* stereochemistry which turned out to be inactive on the 5-HT₄ receptors.

Pertinent informations on the role of the steric requirements and of the orientation of the nitrogen atom lone pair of the basic framework was obtained from the macrocyclic benzamide

8⁵¹ which was compared to the flexible analogue piperidine derivative. In contrast with the derivatives in which the nitrogen substituent was not tied-back, **8** was a more potent 5-HT₄ receptor agonist (K_i (rat brain) = 29.7 nM, EC₅₀ (electrically-stimulated myenteric plexus) = 69 nM) than 5-HT₃ receptor antagonist (K_i = 53.2 nM).

However the lack of bulk around the basic nitrogen was not an essential structural requirement to obtain 5-HT₄ receptor agonists. As already reported, and in contrast to the 5-HT₃ receptor, the 5-HT₄ receptor binding site can fit the linear flexible substituent on the basic nitrogen atom. Using this property, several potent and selective agonists of the 5-HT₄ receptors were designed in the benzamide series. Thus, mosapride⁵² (Scheme 6), a benzylmorpholine derivative, was shown to possess potent gastrokinetic properties mediated by the 5-HT₄ receptors with an affinity value of 84 nM measured in the myenteric plexus of the guinea pig ileum.⁵³ Preliminary SAR⁵⁴ studies on this compound showed that the 5-HT₄ receptor agonist properties were always present with the phenylbutyl chain confirming the existence of the large hydrophobic pocket around the cationic anchorage point in the binding site of the 5-HT₄ receptor. This point had been already emphasised with cisapride but it might be implicated in the lack of selectivity of this compound which interacted with the 5-HT₁, 5-HT₂ and adrenergic receptors. The role of this secondary binding site in the functionality of the 5-HT₄ receptor and the selectivity *versus* the other receptors were clearly demonstrated with new benzamides such as prucalopride⁵⁵ prepared from the metoclopramide like benzofuran acid and the N-methoxypropyl piperidine. This compound was particularly selective with pK_i values of 8.6 and 8.1 for the human 5-HT_{4(a)} and 5-HT_{4(b)} receptors, respectively. In different *in vitro* preparations of 5-HT₄ receptors, prucalopride was a potent agonist with pEC₅₀ values around 7.50. Similarly, Y-34959,⁵⁶ a benzamide of the aminomethylpiperidine framework, was characterised by the presence of the (1-methylindol-3-ylcarbonylamino)pentyl chain on the basic nitrogen atom. It was a potent and selective

ligand for the 5-HT₄ receptor (K_i (nM) D₂, 5-HT_{1A}, 5-HT₂, 5-HT₄ receptors = >1000, 110, >1000 and 0.3, respectively).

Benzoates

The interest of the ester derivatives for the characterisation of the 5-HT₄ receptors was shown with ICS 205-930, a potent 5-HT₃ antagonist which was the first surmountable antagonist of 5-HT₄ receptors² in the stimulation by 5-HT of cAMP formation in the primary culture of the mouse Colliculi neurons with a moderate activity ($pK_i = 6.2$). The first potent antagonist was described by Buchheit⁵⁷ who prepared the ester analogue of metoclopramide SDZ 205-557. In contrast with the parent compound the affinity for the 5-HT₄ receptor was dramatically increased and the compound antagonised the effect of 5-HT in the isolated guinea pig ileum longitudinal muscle preparation, with a pA_2 value of 7.4 while it was 5.6 in the contraction induced by the 5-HT₃ agonist 2-methyl serotonin.⁵⁸ However, the good selectivity of this compound was not confirmed in binding studies using mouse neuroblastoma NG 108-15 cells, which express the 5-HT₃ but not the 5-HT₄ receptor, since the affinity was similar in these cells to that observed for the 5-HT₄ receptors in the striatum.⁵⁹ With the goal to obtain more potent and selective compounds several SAR studies on the esters derived of benzamides were designed.^{60,61,62} They confirmed the preliminary results on the increase in affinity for the 5-HT₄ receptor with regard to the corresponding benzamide. In particular, ML 10302⁶³ (scheme 7) was characterised as a partial agonist equipotent to serotonin in the isolated guinea pig ileum longitudinal muscle preparation ($EC_{50} = 4$ nM (80%)) and in the carbachol-contracted rat oesophageal muscularis mucosae ($IC_{50} = 2.4$ nM (80%)). This compound was found to be selective on a large set of receptors with a weak affinity for the 5-HT₃ receptors ($K_i = 782$ nM). The introduction on the piperidine ring of various substituents modified the efficacy of the compounds and a complete drop in efficacy was observed with

the 3,5 dimethyl derivative. The *cis* compound (ML 10375) was characterised as a potent antagonist of 5-HT in the rat oesophageal muscularis mucosae preparation.⁶² The examination of the X-ray crystal structures of ML 10302 and ML 10375 showed a similar folded conformation of the ethyl chain giving the orientation of the hydrogen atom of the quaternary nitrogen atom similar to that of the azabicyclic benzamides such as zacopride or renzapride. The data on the X-ray crystal structures was confirmed by the structural analysis and showed the limited number of permissible conformations. The calculation of the minimum energy conformer indicated the putative equilibrium between the extended and the different folded conformations. An attractive hypothesis is that activation of the receptor is triggered by the propensity of a molecule such as ML 10302 to adopt the folded conformation in the binding site, mimicking the conformational restricted molecules such as zacopride or renzapride.⁶² On the other hand, ML 10375 might bind the receptor site in the extended conformation and be unable to give the active folded form because of the steric interactions of both methyl groups with the binding site. A superior homologue of ML 10302 was characterised as a potent antagonist of the 5-HT₄ receptor (RS 23597)⁶⁴ confirming the implication of the steric properties in the pharmacological profile of the molecules.

The possibility for various larger groups on the basic nitrogen atom to bind with the secondary binding site of the receptor was explored with the synthesis of the derivatives of ML 10302 where the piperidine ring was substituted by the different aryl and heteroarylpiperazines.⁶⁵ In contrast with ML 10302, these compounds were antagonists of the 5-HT-stimulated cAMP synthesis induced by activation of cloned human 5-HT₄ receptors isoforms and **9** was shown to antagonise the stimulatory effect of 5-HT on the L-type calcium current (I_{Ca}) in human cardiac myocytes ($K_D = 0.7$ nM).

The presence of the ester function is an important drawback for the administration *in vivo* of the compounds due to their putative short half-lives. A series of carbamates⁶⁶ derived from the

previous esters were prepared (Scheme 7). Conversely to the esters where the presence of the 4-amino-5-chloro substituents was mandatory to obtain active compounds, the *o*-alkoxyphenyl carbamates **10** were the most potent and characterised as 5-HT₄ receptor antagonist in the guinea-pig ileum. Similarly, aryl carbamates or ureas **11** in which the *o*-methoxy group was substituted by a heterocycle such as 1,2,4-oxadiazole were claimed as potent 5-HT₄ receptor antagonists.⁶⁷

Another attempt to overcome the problems related to the bioavailability of the esters was presented recently with the preparation of the 1,2,4-oxadiazol derivatives⁶⁸ as the biososteric moiety of the carbonyl function. Compound **12** (YM-53389) was a highly selective 5-HT₄ agonist equipotent to cisapride. It was shown to possess a significant activity that shortens the total gut transit time but without effect on the upper gastrointestinal propulsion.⁶⁹

An important series⁷⁰ of benzoate derivatives (Scheme 8) was obtained by the introduction of the *ortho* oxygen atom of benzoic acid within a five or six-membered ring **13** (X-Y = O-CH₂ or O-(CH₂)₂-) and the coupling with (1-butyl-4-piperidinyl)methylamine. Benzodioxan derivative SB 204070 was the most potent and highly selective antagonist of the 5-HT₄ receptors in the guinea-pig distal colon longitudinal muscle myenteric plexus preparation (LMMP).⁷¹ In this assay, this compound was characterised as a non surmountable antagonist with a pK_a value of 10.4, although its effect could be reversed upon washout of the drug. The antagonist effect of derivative SB 204070 was confirmed in the *in vivo* model of the conscious dog Heidenhain pouch where the drug inhibited the 5-HT-evoked response, in a dose-dependent manner.⁷² As previously reported for the other series, the corresponding amidic derivative of SB 204070, SB 205800, was less potent, although still active in the nanomolar range of concentrations (pK_a = 9). The high affinity and the selectivity of SB 204070 led to the development of the iodo derivative SB 207710 as a radioligand which binds the 5-HT₄ receptors with a high affinity (pK_i = 9.2).⁷³

Aromatic ketone derivatives

The design of aromatic ketones structurally related to the benzoates opened another way to overcome the problem of the metabolic hydrolysis of the esters and of their weak bioavailability. From the 5-HT₄ receptor antagonist RS-23597,⁶⁴ the corresponding ketone RS 17017⁷⁴ (scheme 9) was shown to be a partial agonist about one order of magnitude less potent than 5-HT in the relaxation of carbachol contracted rat oesophageal muscularis mucosae (pEC₅₀ = 7.4). However, more potent compounds⁷⁵ were obtained with the basic framework of GR 113808 substituted by *n*Bu (RS 67333) or (CH₂)₂NHSO₂Me (RS 67506). These compounds are highly selective for the 5-HT₄ receptor, although they also bind σ_1 and σ_2 receptors, and they behave as potent partial agonists in the model of relaxation of rat oesophagus (pEC₅₀ = 8.4 and 8.6 respectively). The 5-HT₄ receptor agonist activity was demonstrated *in vivo* in the piglet model of tachycardia.⁷⁶ In contrast to the 5-HT₃ receptor antagonists and 5-HT₄ receptor agonists renzapride and zacopride, RS 67506 enhanced the lower intestinal propulsion in mice, suggesting that blocking of 5-HT₃ receptors might not be suitable for the treatment of propulsion impairment in the colon.⁷⁷ Modification of the *o*-methoxy group of the 5-HT₄ receptor agonists RS 17017 and RS 67506, in particular by the substitution with the 3,5-dimethoxybenzyl group, led to a complete loss of agonist efficacy and to the highly potent 5-HT₄ receptor antagonists RS 67532 and RS 39604, respectively. These compounds inhibited the 5-HT mediated relaxation of carbachol contracted rat oesophageal muscularis (pK_i = 8.5 and pK_i = 9.2).⁷⁵ Moreover, the combination of the favourable effects of the benzodioxane moiety in the benzoic esters and of the previous results obtained with the ketones led to new antagonist derivatives, such as RS 100302 and RS 100235.⁷⁸

Indole carboxylic acid ester and amide derivatives

As reported previously, this class of compounds was derived from ICS 205-930 or tropisetron, a potent 5-HT₃ antagonist, which binds to 5-HT₄ receptors with a medium affinity (1 μM) and has an antagonist profile.² The substitution of the tropane moiety (Scheme 10) by the more flexible ethylamido chain and the more bulky piperidine ring gave rise to SB 203186,⁷⁹ a potent 5-HT₄ receptor antagonist in the piglet right atrium. However, an important advance was made with the synthesis of GR 113808⁸⁰ where the 4-piperidinylmethyl chain was introduced for the first time in the field of 5-HT₄ receptor ligands. This compound had a subnanomolar affinity and was very selective *versus* other receptors. GR 113808 constituted a very useful pharmacological tool for the characterisation of 5-HT₄ receptors, particularly with the tritiated molecule which was used for the localisation of 5-HT₄ receptors in various tissues and for receptor binding studies.⁸¹ A more elaborated compound GR 124487 was claimed as a more potent antagonist than GR 113808. Recently,⁸² naphthalene bioisosteric analogues were prepared but they were clearly less potent. In search for new compounds which would be effective orally so that they could be used as therapeutic drugs, SmithKlineBeecham synthesised a series of indole derivatives using the favourable results obtained with SB 204070. The idea was to mimic the benzodioxane ring on the indole by the introduction of an oxygen atom in the position 2 of the ring. The oxazolo, oxazino and oxazepino indoles hence obtained were potent antagonists of the 5-HT₄ receptors in the guinea-pig distal colon (LMMP preparation) and the derivative with the six-membered ring SB 207058 was the most potent (pIC₅₀ = 10.6).⁸³ As previously observed the conversion to the analogous amide was detrimental for the activity, although the effect on these cyclic compounds was less marked and SB 207266 (piboserod) retained a good level of activity (pIC₅₀ = 9.2). In contrast to SB

204070, the 5-HT response recovered its control level after washout of this drug, indicating a reversible antagonism. SB 207266 was highly potent in the model of the dog Heidenhain pouch when it was administered orally with a lasting time superior to 2 h indicating good orally bioavailability.⁸⁴

The influence of the constraints on the basic 4-piperidinylmethyl moiety chain was investigated with the synthesis of indolic esters of quinolizidine derivatives⁸⁵ which can mimic this basic group in the folded conformation. The best of these compounds were those obtained with a one methylene linker in the *para* position of the basic nitrogen atom between the acid and the azabicyclic group in the equatorial stereochemistry. The favourable influence of the oxazino[3,2-a]indole group was also confirmed with the synthesis of **14** which was highly potent in the guinea-pig distal colon LMMP preparation ($pIC_{50} = 9.5$) and inhibited the binding of [¹²⁵I] SB 207710 to piglet hippocampal membranes.

Several works about the structural analogues of the indole ring were undertaken to design compounds with a potential good bioavailability (Scheme 11) and consequently the search for the amidic derivatives was privileged. Imidazopyridine carboxamides or carboxylic esters **15**, with the basic framework derived from piperidine or quinolizidine rings were claimed as 5-HT₄ receptors antagonists.⁸⁶ An extensive SAR study on the indazole derivatives⁸⁷ was realised from a preliminary approach where it was demonstrated that indazole-3-carboxamide of *endo*-3-tropanamine **16** (Scheme 10) possessed a greater 5-HT₄ receptor affinity than tropisetron. As it had been reported¹⁸ in the benzimidazolone series, the N-isopropyl substitution gave the best compounds and the corresponding amides of the monocyclic piperidine linked directly on the ring or through a two methylene chain were potent 5-HT₄ receptor antagonists. The potency was largely increased by the introduction on the basic nitrogen atom of substituent with hydrogen donor group associated with a hydrophobic moiety, capable to bind with the accessory binding site already characterised in the 5-HT₄

receptor. One such compound, LY 353433, was a selective, potent and orally active 5-HT₄ receptor antagonist. A similar SAR study was performed on a series of benzimidazole-4-carboxamides by Lopez-Rodriguez⁸⁸ who found, in contrast with most previous studies that amides were more potent 5-HT₄ receptor antagonists than the corresponding esters, particularly the amides **17** of 6-chlorobenzimidazole-4-carboxylic acid.

Serotonin analogues

Serotonin is a potent agonist of the 5-HT₄ receptors in the different biological preparation where this receptor is present. It could constitute a suitable structural model to design new agonists for this receptor. The activity obtained with the carbazimidamide **18**, a structural analogue of 5-HT where the guanidine group provided a stabilised basic function⁸⁹ confirmed the interest in this approach. **18** was 2.5-fold more potent than 5-HT with an efficacy of 150%, exhibiting a pD₂ value of 8.8 in the electrically stimulated longitudinal muscle preparation of the guinea-pig ileum. However, the presence of the guanidine function could be an important drawback for the bioavailability of the compound. A SAR study⁹⁰ of the indole carbazimidamide family showed the possibility to introduce lipophilic groups capable to interact with the secondary accessory hydrophobic site of the 5-HT₄ receptors. As already reported, this region of the receptor can accept a large set of substituents which clearly influence the pharmacological activity. Among a number of potent compounds, **19** was discovered as the most potent 5-HT₄ receptor full agonist described so far (pD₂ = 9.3). However, SDZ HTF 919 or tegaserod,⁹¹ a moderate partial agonist (pD₂ = 6.9) was selected for the clinical investigations in the disorders of the intestinal transit. The presence of the methoxy group in this compound mimics the structural analogy with 5-methoxytryptamine, a selective 5-HT₄ receptor agonist. The indole carbazimidamide derivatives shared no

recognition parameters with ligands of 5-HT₃ receptors and they were perfectly selective *versus* this receptor. However the structural analogy of these compounds with serotonin might explain why **19** was only 15-fold less potent on 5-HT_{1D} receptor than on the 5-HT₄ receptor. It is worth noting that, from the SAR study reported, the activity of these compounds resides only in the indole derivatives, since the carbazimidamide derivatives with the isosteric indole groups are either inactive or only weakly active. The introduction of a small substituent, such as Me or Et, on the aromatic ring of **19** brought about an interesting drop of the intrinsic activity⁹² since the compounds became 5-HT₄ receptor antagonists. **20** was a competitive and selective antagonist in the assay of the inhibition of 5-HT-induced contractions of guinea-pig ileum (pA₂ = 8.4). This last point highlighted the influence of the subtle structural modification of the ligands on their efficacy. Indeed, a small steric hindrance or constraint can hamper the rearrangement of the receptor during the activation step of the receptor producing a loss of efficacy and an antagonist profile for the molecules. This point has been reported recently with some agonists of 5-HT_{1A} and 5-HT₄ receptors^{62,93} which were transformed into the antagonists of the corresponding receptors by the introduction of a methyl group on the agonist structures.

Pharmacophore of the 5-HT₄ ligands and the binding receptor site.

The preliminary data on the structural parameters implicated in the recognition of the 5-HT₄ receptor binding site were influenced by the results obtained in the field of the 5-HT₃ receptors because a number of 5-HT₃ antagonists were also 5-HT₄ receptor agonists or antagonists. Therefore, the presence of an aromatic system, a hydrogen acceptor group such as the carbonyl function and a basic group were the essential characteristics of the 5-HT₄ receptor pharmacophore. It was structurally closely related to the model generated by Hibert⁹⁴ for the 5-HT₃ receptor antagonists. However several differences were emphasised during the

search for more selective ligands and the main ones concerned the structure of the basic chain. 5-HT₃ receptor site prefers the reduced steric hindrance around the basic nitrogen atom as in zacopride or renzapride while the 5-HT₄ receptor site can fit very voluminous groups on the basic nitrogen atom. The best illustration of this point is provided by compounds such as cisapride, prucalopride or LY 353433. The linker between the acceptor hydrogen group and the basic nitrogen atom possesses a variable length: six bonds in GR 113808, SB 207266 or RS 67506, five bonds in RS 67532 and four bonds in ML 10375 and SDZ 205-557.

In contrast with other members of the 5-HT receptor family, few molecular modelling studies have been published on 5-HT₄ receptors. In 1997, Lopez-Rodriguez⁹⁵ calculated the geometrical characteristics of a 3-D model of pharmacophore by the active analogue approach of two sets of 5-HT₃ and 5-HT₄ receptor ligands. It was characterised by the following parameters: a carbonyl function coplanar with the aromatic ring located to 3.6 Å from the centroid of the aromatic ring, a basic nitrogen atom separated by 8.0 Å from the centroid and by 5.4 Å from the oxygen atom of the carbonyl function, a hydrophobic accessory pocket capable to bind the voluminous substituent of the nitrogen atom. This study gave quantitative expression of what can be derived from a careful examination of the molecules and provided no information on the exact nature of the nitrogen atom substituent.

Few data were also available from SAR studies concerning the variation of the pharmacological profile of the molecules. For instance, the “tied back” structure of the basic framework of the 5-HT₄ receptor agonists of the first generation, such as zacopride or renzapride, which was related to the agonist profile. However, this structural property was not totally relevant of the pharmacological profile as several agonists such as cisapride or prucalopride possess a large substituent on the basic nitrogen atom.

An interesting structural analysis reported by Buchheit⁸⁹ compared the 3D structure of serotonin and zacopride and proposed a pharmacophore model where the basic nitrogen can

be located either 5.7 or 7.4 Å from the centre of the aromatic ring and 0.8 or 0.1 Å above the plane defined by this system. The carbamidazine **19** which embedded the guanidine function was capable to occupy simultaneously the two positions and to bind the putative hydrogen acceptor group and the charge acceptor located in the receptor site.

Recently,⁹⁶ a more direct approach of the nature of the interactions in the binding site and the ligands were reported with a site-directed mutagenesis and molecular modelling study. The new human 5-HT_{4(a)} isoform was expressed in COS-7 cells and several highly conserved amino acids residues in the G protein-receptor family were mutated. Docking experiments taking into account the results of the measurements of the affinity and functional activity of serotonin and GR 113808 were realised. Essential results (Fig 1) indicated that, for the binding of serotonin, Y302 in the seventh helix played a major role for the stabilisation of the consensus ionic interaction with D100 in the third helix and S197 on the fifth helix was the anchorage point with the hydroxyl function. For GR 113808, it was proposed that S197 binds the carbonyl group of the function ester. It is worth noting that the substitution D100N had a relative weak effect on GR 113808 binding which indicates a rather small contribution of the ionic interaction since it can be substituted by an hydrogen bond. Docking experiments showed that the indole ring was embedded in a pocket defined by the residues W146, F275, F276 and N279 as any mutations of these amino acids decreased dramatically the affinity of the ligand for its site and modified the functional activity of serotonin. These last characteristics were common with others serotonergic receptors, in particular the 5-HT₂ receptor.⁹⁷

Receptors, localisation, structure and functions

The 5-HT₄ receptor is a member of the seven trans-membrane-spanning G protein-coupled family of receptors (GPCR) and constitute an important sub-type of the class of the serotonin

receptors. Initially, the 5-HT₄ receptor was characterised in the neuronal cell culture⁹⁸ of mouse colliculi and it was shown to be positively coupled to adenylyl cyclase. The effect of serotonin was mimicked by the 5-HT₄ receptor agonists BIMU 1 and BIMU 8 and was blocked by the 5-HT₄ receptor antagonists DAU 6215.^{24,25} This positive effect of 5-HT₄ receptors on adenylyl cyclase is shared with the recently discovered 5-HT₆ and 5-HT₇ receptors.^{99,100} 5-HT₄ receptors are distributed in the guinea-pig and rat central nervous system (CNS) in two anatomically and functionally structures: the extrapyramidal motor system (striatum, globus pallidus and substantia nigra) and the mesolimbic system (nucleus accumbens and olfactory tubercle).^{6,101} They were characterised by using the binding bioassay with [³H] GR 113808 as radioligand.⁸¹ In human, their presence was shown in basal ganglia and in the caudate-putamen nuclei where the density is the highest ($B_{\max} = 225$ fmol/mg prot). Several areas with a substantial density of receptors were found: lenticular nucleus, substantia nigra, hippocampus and frontal cortex.^{102,103} Recently,¹⁰⁴ the mapping of the 5-HT₄ receptor in the post-mortem human brain was determined by using the localisation of the 5-HT₄ receptor mRNA and 5-HT receptor protein. The highest levels of 5-HT₄ receptors were found in caudate nucleus, putamen, nucleus accumbens and in the hippocampal formation. 5-HT₄ receptors are also present in the periphery,^{105,106,107} particularly in the overall gastrointestinal tract where they are implicated in the contraction or the relaxation of the smooth muscle. Saturable binding of [³H] GR 113808 was determined in the longitudinal muscle and myenteric plexus of guinea-pig, with a larger number of sites in the upper part of the intestine: duodenum > jejunum > ileum >> colon > rectum. In all cases, the number of binding sites in the intestine is inferior to that in brain. The presence of 5-HT₄ receptors in the intestine allowed to understand the mechanism of several benzamide gastrokinetic drugs, such as cisapride and metoclopramide.^{108,109} 5-HT₄ receptors are also present in the pig and human heart⁷ but they are exclusively located in the atrium where they

are responsible for the tachycardia and the positive inotropic effect observed after injection of 5-HT. Kaumann¹⁴ has suggested that 5-HT liberated from platelets could induce atrial arrhythmia by activation of these receptors. In the vascular system, 5-HT₄ receptors were characterised on the pulmonary vein and the mesenteric lymphatic system where they cause relaxation but too few data have been reported up to date to give a clear characterisation of this receptor in these tissues.¹¹⁰

It has been demonstrated that 5-HT₄ receptors are present in the human detrusor muscle and mediate the facilitation *via* a cholinergic mechanism of the contraction of the human bladder.¹⁰ 5-HT₄ receptors could be implicated in the urinary incontinence as this syndrome has been found to occur in patients treated with cisapride.

5-HT mediates the release of corticosterone and aldosterone *via* 5-HT₄ receptors on the human adrenal cortex.⁹ Zacopride caused the release of cortisol in the human adrenocortical slices¹¹¹ which was blocked only by high doses of tropisetron. To date, it is the only endocrine pathway which is regulated by this receptor sub-type.

In almost all tissues where the 5-HT₄ receptors are present, 5-HT or the corresponding agonists are capable of increasing cAMP synthesis.¹¹² This was the case in the hippocampus, the atrium, the oesophagus, the gut or the adrenal cortex. One of the characteristics of the 5-HT₄ receptors is their ability to desensitise after their stimulation. It was shown^{113,114} that 5-HT elicited the depolarisation of the pyramidal cells of CA1 region of hippocampus and a concentration-dependent reduction in the amplitude of the after-hyperpolarization (AHP) was emphasised. This effect was competitively inhibited by selective 5-HT₄ receptor antagonists such as GR 113808 and SDZ 205-507.¹¹⁵ Similarly in colliculi neurons¹¹⁶ the exposure of the receptors to 5-methoxytryptamine or 5-HT₄ receptor agonists was followed by a rapid and long inactivation which was related to the potency and the efficacy of the agonists. The desensitization was not dependent on the cAMP formed but on the mean occupancy time of

the receptor by the agonist and it was due to phosphorylation of the occupied receptor by β ARK or another specific receptor-dependent protein kinase.

A number of physiological processes can be triggered by an increase in intracellular cAMP. For instance, in mouse colliculi neurons,¹¹⁷ the activation of the 5-HT₄ receptors is followed by the inhibition of the voltage-activated K⁺ channel current caused by a phosphorylation reaction through cAMP-dependent protein kinase (PKA) (Fig. 2). Consequently, the action potentials are significantly prolonged, resulting in a loss of after-hyperpolarization (AHP). Prolongation of the cell membrane depolarisation would result in the activation of the voltage-dependent Ca²⁺ channels leading to an increase of Ca²⁺ influx which contributes to neurotransmitter release. This phenomenon might explain the positive effect of a 5-HT₄ receptor activation on the release of acetylcholine (ACh), in the CNS. In the atria, the increased production of cAMP induces activation via PKA of the L-type Ca²⁺ channel current (I_{Ca}).¹² In the intestine, the direct effect of an increase in cAMP concentration, via activation of 5-HT₄ receptors, is the relaxation of the smooth muscle. However, 5-HT₄ receptor activation can also induce contraction of the smooth muscle through the indirect release of ACh which results from activation of 5-HT₄ receptors located on the sensory neurones of the myenteric plexus.¹⁰⁵

The cloning and the structural characterisation of the rat 5-HT₄ receptors were described by Gerald^{118,119} who first isolated cDNA and expressed it in COS-7 cells. Two isoforms were first characterised. They were identical between the residues 1 and 359 and differed only in the C-terminal region. They were generated by the alternative splicing of a unique gene and named 5-HT_{4L} and 5-HT_{4S} for the long and short isoforms, respectively. More recently, a third shorter isoform was characterised¹²⁰ and the official nomenclature for these receptors was r5-HT_{4(b)}, r5-HT_{4(a)}, and r5-HT_{4(e)}. A similar diversity of isoforms was found for the mouse and four isoforms were described: 5-HT_{4(a)}, 5-HT_{4(b)}, 5-HT_{4(e)}, and 5-HT_{4(f)}.^{120,121} In 1997,

Blondel¹²² followed by Clayesen¹²³ and Van den Wyngaert,¹²⁴ described the first human 5-HT₄ receptor isoform. Blondel¹²² isolated the receptor from human atrium and named h5-HT_{4(a)} as it was considered to be homologous to the short isoform described in rat. This receptor was transiently expressed in COS-7 and the response to 5-HT and some 5-HT₄ receptor agonists was identical to the pattern of stimulation of I_{Ca} obtained in response to these compounds in human atrial myocytes.¹²² Since then, several other splice variants were cloned h5-HT_{4(b)},⁴ two different h5-HT_{4(c)} isoforms,^{4,126} h5-HT_{4(d)},^{4,126} h5-HT_{4(e)},¹²⁷ (which will be called h5-HT_{4(e/g)} in the following, since this isoform was later renamed h5-HT_{4(g)}^{125,128} and replaced by another h5-HT_{4(e)} isoform¹²⁵ found more homologous to the mouse 5-HT_{4(e)} receptor¹²⁰), h5-HT_{4(f)},¹²⁵ and h5-HT_{4(n)}.¹²⁹ Whatever the animal species (mouse, rat or human) the different splice variants have an identical sequence up to Leu 358 whereas the length and the composition of the rest of C-terminus tail is specific for each variant (Fig. 3). For instance, in human, the C-terminal end is very short for the 5-HT_{4(d)}⁴ and 5-HT_{4(n)}¹²⁹ isoforms (respectively, only 2 and 1 amino acids after Leu 358) 5-HT_{4(d)} isoform (only 2 after Leu 358) and is 31 amino acids long for the 5-HT_{4(b)} variant.⁴ One of the characterised isoform 5-HT_{4(c)} might be the target of different protein kinases as several phosphorylation sites are present on its C-terminus tail.⁴ The tissue distribution revealed some degree of specificity. For instance, 5-HT_{4(a)}, 5-HT_{4(b)}, 5-HT_{4(c)} and 5-HT_{4(e/g)} receptors are all expressed in atrium, brain and intestine while the 5-HT_{4(a)} and 5-HT_{4(b)} receptor subtypes are the only receptors present in the bladder and kidney, respectively.⁴ On the other hand, the 5-HT_{4(d)} isoform was only characterised in the intestine. The different isoforms expressed in COS-7 cells displayed the classical profile of the 5-HT₄ receptors previously observed in the native tissues and showed an identical ability to stimulate adenylyl cyclase activity. No major difference could be found between the different isoforms as far as the *K_i* values of the agonists or the antagonist were only considered. On the other hand, some significant differences in the

functionality were observed with the agonists ML 10302 and renzapride. ML 10302 described as a potent agonist in GI tractus in rat was a weak partial agonist of each human isoform whereas renzapride was a full agonist on the 5-HT_{4(b)} and 5-HT_{4(d)} isoforms and a partial agonist on the 5-HT_{4(a)} and 5-HT_{4(e/g)} receptors.¹²⁷ These functional differences may explain the tissue-dependent specificities observed with benzamides which act as full or super agonists² in the mouse colliculi neurons and only partial agonists¹² in other systems such as human myocytes. An attractive hypothesis is that a given receptor subtype might convey a specific functional response in a particular tissue. However more information is needed to give clear conclusions on the exact repartition of the isoforms in the different tissues and the influence of the expression system on the efficacy of the ligands. Recently¹²⁸ using a real time quantitative RT-PCR method, the distribution of the different variants mRNA was investigated in the CNS, heart and gut in human. The data obtained suggested the predominant presence of h5-HT_{4(b)} receptor over the h5-HT_{4(a)} receptor in the different tissues studied. Interestingly, the existence of a new variant (h) was recently reported,¹²⁵ which possesses a 14 amino acid sequence inserted in the second extracellular loop and combined with the C-terminal sequence of the (b) variant, which led to its appellation 5-HT_{4(hb)}. This receptor has a pharmacological profile similar to those previously described in the competition binding assays for the other isoforms, but differs in its response to the reference antagonist GR 113808 which displays a partial agonistic activity on this new isoform.

The influence of the length of the C-terminal part in the activation of the receptor was shown by Claeysen⁵ who studied the constitutive activity of the different mouse receptor isoforms. This activity was inversely correlated to the length of the terminal sequences. Thus it was increased up to the level of the activity of the m5-HT_{4(f)} isoform by the progressive deletion of the C-terminal part of the m5-HT_{4(a)} receptor up to residue 359. Moreover, a dramatic increase of the activity was obtained with an additional 13 residues deletion. Therefore, it was

proposed that the common part of m5-HT₄ receptors up to residue 346 confers to the receptor a constitutive activity (state R*) and that the subsequent residues on the terminal end modulate this constitutive activity, modifying the equilibrium between the inactive state R and the active state R*. The propensity of the receptor to possess a high constitutive activity was characterized by an increase of the thermal denaturation rate.⁵ The studies made on several C-terminal mutants modifying the R*/R ratio demonstrated that the receptor in its active state R* denatures much faster than the protein in its resting state R and that the denaturation rate depended upon the J value of the equilibrium constant between the R and R* states. These studies on the constitutive activity of the different isoforms of 5-HT₄ receptors are important for a better understanding of the regulation mechanism of the signal transduction in the cell. As for other GPCRs,¹³⁰ constitutive activity of 5-HT₄ receptors may be implicated in a number of pharmacological and pathological processes and may point to the potential role for an inverse agonist, such as GR 125487 and ML 10375¹³¹ which reduce the constitutive activity of these receptors.

Recently, it was also shown that 5-HT₄ receptor agonists such as BIMU 8 was capable of stimulating the incorporation of palmitic acid in the 5-HT_{4(a)} receptor expressed in Sf 9 cells.¹³² This data emphasised the role of the membrane in the mechanism regulating the molecular events of the receptor and the signalling processes.

5-HT₄ receptors and the gastrointestinal system

5-Hydroxytryptamine has variable effects on the gastrointestinal tract: it induces both the contraction and relaxation of smooth muscle and stimulates the intramural nerve plexus. In

mammalian small intestine, 5-HT is stored in the enteric neurones and in the mucosal enterochromaffin cells and it is secreted into the lumen and the submucosal tissue. Once liberated, 5-HT can act as a local hormone or a neurotransmitter through the different receptor subtypes present in the gastrointestinal tract: 5-HT₁, 5-HT₂, 5-HT₃, 5-HT₄ and 5-HT₇.^{133,134,135,136,137}

5-HT_{1A} receptors have been demonstrated to be responsible for the hyperpolarizing response of the enteric neurons associated with the presynaptic inhibition of the release of ACh at the nicotinic receptors. It was also clearly demonstrated that 5-HT_{1D} receptor agonists, such as 5-CT or sumatriptan, are capable to stimulate the peristaltic reflex in the isolated guinea-pig ileum.¹³³ 5-HT₂ receptors, previously known as D receptors, are localised on smooth muscle and their activation mediates contraction of the guinea-pig longitudinal muscle. 5-HT₃ receptors¹³⁸ similarly to the 5-HT₄ receptors, are present at the presynaptic level on the enteric neurones and regulate positively the release of ACh. More recently, 5-HT₇ receptors have been characterized in the large intestine.¹³⁷

Activation of 5-HT₄ receptors in the GI system can cause various effects depending on the region studied and the animal species. 5-HT₄ receptor stimulation leads to the contraction of the guinea-pig ileum and colon⁸ and increases the reflex sensitivity of the isolated and intact guinea-pig ileum.¹³⁹ 5-HT₄ receptors were also implicated in the gastric emptying in rats¹⁴⁰ and in the 5-HTP (5-hydroxytryptophane)-induced defecation and diarrhoea in mice.¹⁴¹ Conversely, stimulation of 5-HT₄ receptors may lead to relaxation of rat oesophagus and terminal ileum³ and of human intestinal smooth muscle¹⁴² and to a decrease in the spontaneous activity of human colon.¹⁴³ These apparently opposite effects can be explained by the different localisation of the 5-HT₄ receptors. They are present on the interneurons of the myenteric plexus and mediate indirectly the release of ACh.¹⁴⁴ The relaxation is induced

directly by stimulation of the 5-HT₄ receptors present on the smooth muscle, the intensity of the effect depending upon the region studied.¹⁴⁵

Initially, the role of the 5-HT₄ receptors in the gut was demonstrated by Clarke³ who showed an activation of the peristaltic reflex of guinea-pig ileum by 5-HT₄ receptor agonists. 5-HT had a biphasic effect depending on the concentration, the first phase (10⁻⁹-10⁻⁷ M) involving activation of 5-HT₄ receptors while the second phase (> 1 μM) involved activation of 5-HT₃ receptors. The initial phase was inhibited by atropine and was insensitive to the blockade by ICS 205-930 (<1μM), granisetron and ondansetron excluding the contribution of 5-HT₃ receptors. Moreover, agonist-induced desensitisation protocols allowed a clear separation of the two response: thus, long exposure of guinea-pig ileum to 5-methoxytryptamine, a 5-HT₄ agonist, inhibited completely the first phase of the response to 5-HT but had no effect on the second phase; likewise exposure of the preparation to the 5-HT₃ receptor agonist, 2-methylserotonin, inhibited only the second phase of the response to 5-HT, but left unchanged the first phase.¹⁴⁶ The benzamides of first generation and the benzimidazolones BIMU 1 and BIMU 8, which possess both 5-HT₃ receptor antagonistic and 5-HT₄ receptor agonist properties, were found to mimic the effect of 5-HT on the first phase and to evoke the peristaltic reflex.³ These experiments were the first demonstration of the mechanism of action of benzamides which possess gastrokinetic properties and are used as drugs to increase the gastric emptying and the intestinal transit.¹⁰⁸

The presence of 5-HT₃ receptors in the guinea-pig ileum could be a drawback in the study of non-selective molecules. The characterisation of 5-HT₄ receptors in the rat oesophagus and, particularly, in the tunica muscularis mucosae constituted a clear improvement to select 5-HT₄ receptor agonists.³ In contrast to guinea-pig ileum, the 5-HT₃ agonist 2-methyl-5-HT possessed a very weak activity in this preparation. The assay evaluated the relaxation potency of the carbachol-contracted muscle preparation in the presence of cumulative doses of 5-HT

(ED₅₀ = 15.6 nM). Benzamides, such as renzapride and cisapride, were agonists equipotent to serotonin, and ICS 205-930 caused a rightward shift of the agonist effect curves. As demonstrated in the CNS, the 5-HT₄ receptors present in oesophagus mediated an increase of cAMP synthesis.¹⁴⁷ 5-HT and 5-HT₄ agonists caused a concentration-dependent increase of intracellular cAMP in the rank order of their efficacy indicating that the 5-HT₄ receptors were most likely localized post-synaptically on the smooth muscle cells. As reported in colliculi neurons, a rapid desensitisation of these non neuronal 5-HT₄ receptors was observed independently of the level of cAMP production, indicating a homologous rather than heterologous desensitisation process. This assay provided clear evidence for a marked heterogeneity among animal species of the response to 5-HT. Indeed, different responses to serotonin were found when tested on carbachol-contracted oesophagus of rat, guinea-pig, rabbit and dog: no effect was observed in dog, contractions were observed in guinea-pig and rabbit, and a relaxation was found in rat only.^{148,149,150}

5-HT₄ receptors are also localised in the guinea-pig stomach where 5-HT enhances the electrically induced contraction in the circular muscle strips from the fundus and corpus.^{151,152} However, the effect of 5-HT is rather complex due to the presence of different 5-HT receptors in this preparation: stimulation of 5-HT_{1A} and 5-HT₂ receptors by specific agonist (8-OH-DPAT and α -methyl-5-HT, respectively) inhibit contractions while 5-HT₄ and 5-HT₃ receptors agonists (renzapride, metoclopramide and 2-methyl-5-HT) increase the electrically induced contraction. Nevertheless, under physiological conditions, it is likely that the response to 5-HT is only mediated by 5-HT₄ receptors, unless large quantities of 5-HT are present. In rat, gastric 5-HT₄ receptors were implicated in the stimulation of the gastric emptying, a test largely used to select gastrokinetic drugs. However, several other data demonstrated an inhibitor role of 5-HT₃ receptors in rat stomach because several selective 5-

HT₃ receptor antagonists possess an ability to stimulate the gastric emptying in this animal species.¹⁵³

5-HT₄ receptors were identified in the distal colon longitudinal muscle myenteric plexus (LMMP) of guinea-pig, another very useful preparation for the characterisation of the ligands.¹⁵⁴ LMMP is a very sensitive model in which the pEC₅₀ value of serotonin is particularly high (9.2) compared to other tissues. As in the ileum, the effect of 5-HT on LMMP is blocked by atropine. This is consistent with the putative localisation of the 5-HT₄ receptors on the cholinergic neurons. Renzapride, cisapride and zacopride when they were tested in guinea-pig ileum have an intrinsic activity inferior to 1 while all benzamides tested in the distal colon behave as full agonists; in particular, (S)-zacopride was a full agonist distinct of the (R) enantiomer. The high intrinsic activity in this model may be attributed to the high density of the 5-HT receptors or to a more efficient coupling mechanism. However, the situation is complicated by the observation that mosapride, a benzamide 5-HT₄ receptor agonist, was shown to be active only in the upper gastrointestinal system and not in the colon.¹⁵⁵ On the other hand, not only 5-HT₄ receptors but also 5-HT₃ receptors contribute to the propulsion of the colon as evidenced in the guinea-pig where 5-HT₄ and 5-HT₃ receptor antagonists decreased with an additive effect the velocity of the propulsion while the agonists HTF 919 and prucalopride increased the propulsion.¹⁵⁶

In the recent years, the interest in drugs effective in relieving the different types of constipation, a major disorder of the gastrointestinal system in human, was considerably increased. In particular, the role of 5-HT₄ receptors in the regulation of the human intestine motility was investigated. While the 5-HT₄ receptors had been well characterised in guinea-pig large intestine,¹⁵⁷ the knowledge of their exact physiological role in human gut was limited. Their implication¹⁵⁸ was shown in the 5-HT-induced relaxation of the circular muscle of human intestine. These receptors are localised in the myenteric plexus and the muscle¹⁵⁹

and subsequently, the positive effect of 5-HT on cAMP synthesis was demonstrated.¹⁶⁰ Although 5-HT₇ receptors have been implicated in the relaxant effect of 5-HT,¹³⁷ Prins^{161,162} showed that the selective 5-HT₄ receptor agonists prucalopride and RO76186 elicited a clear dose-dependent relaxation of the KCl-precontracted circular muscle and that the potent selective 5-HT₄ receptor antagonists GR 113808, GR124487 and RS 39604 gave concentration-dependent rightward shift of the 5-HT dose-effect curve. A similar relaxant effect was observed in the canine isolated rectum circular smooth muscle, an assay which can constitute a predictive 5-HT₄ receptor model for the human colon.¹⁶³

The role of 5-HT₄ receptors on the induction of the contractility was clarified by the recent data which proposed more information about the mechanism regulating the initiation of the peristaltic reflex in the intestine. It was supposed that serotonin was released from the enterochromaffin cells of the epithelium of the mucosa in response to chemical or mechanical stimuli induced by the progression of the faecal bolus.¹⁶⁴ 5-HT acts on CGRP (calcitonin gene-related peptide)-containing sensory nerve terminal (Fig. 4) and liberates CGRP which brings about the release of excitatory (ACh, substance P, neurokinin A) and inhibitory (vasoactive intestinal peptide (VIP), pituitary adenylyl cyclase-activating peptide (PACAP) and NO) neurotransmitters.¹⁶⁵ In contrast with previously published data, it was proposed that 5-HT₄ receptors are located on the sensory CGRP neurones and not on the intermediate cholinergic neurones. Consequently the activation of the 5-HT₄ receptor located on the primary afferent neurones would stimulate an excitatory neurone producing contraction on the oral side and an inhibitory neurone on the caudad side. This results in the peristaltic reflex with contraction above and relaxation below the site of the stimulus.

The release of CGRP was found to be inhibited by 5-HT₄ antagonists in human intestine and rat colon,¹⁶⁶ although a similar inhibitory effect was obtained in guinea-pig colon with either 5-HT₃ or 5-HT₄ antagonists.

The additional relaxation induced by activation of the 5-HT₄ receptors present on the smooth muscle of the intestine or the colon in the different species studied can induce some misleading conclusions on the pharmacological profile of the 5-HT₄ receptors agonists or antagonists. Indeed, overall effects of 5-HT is a balance between the excitatory and the inhibitory effects. Moreover, 5-HT₄ receptors were found to play a minor role in the normal gut function because administration of potent 5-HT₄ receptor antagonist, such as SB-207266, had no effect on the level of defecation in mice.¹⁶⁷ However, when the intestinal function was disturbed by abnormal level of 5-HT produced by stress or administration of 5-HTP, SB-207266 was capable to inhibit the intestinal disfunction.¹⁶⁷

Up to date, gastrokinetic drugs with 5-HT₄ serotonergic properties are used in human clinic for the treatment of gastro-oesophageal reflux disease and different gastric stasis. Cisapride, which is particularly efficient in those different disorders, was also demonstrated to be capable to stimulate the peristalsis at the colonic level and to induce defecation in constipated patients.^{168,169} Recently, the interest for drugs acting on the gut motility and particularly on the disorders of the small bowel and the colon has largely increased. The irritable bowel syndrome (IBS) is a disorder which has been clinically well characterised and which represents a prevalence of 10% in the Western countries and 40% of the consultations in gastroenterologists.^{170,171} It is a complex dysfunction of the intestine with three components: the hypersensitivity of the gut, the altered motility and the psychosocial disorder.¹⁷² In general the patients may present either diarrhoea or constipation or an alternance of both. Consequently, 5-HT₄ receptor agonists or partial agonists seem to be suitable for the treatment of IBS in which the intestinal propulsion impairment dominates while 5-HT₄ receptor antagonists can be effective in the patients with important diarrhoea. Several data¹⁷³ have been recently reported on the clinical activity of 5-HT₄ ligands in patients with IBS. SDZ HTF 919 or tegaserod, a partial 5-HT₄ receptor agonist, showed in healthy subject a clear effect on

the total colonic transit time (5 mg twice a day)¹⁷⁴ and a significant improvement in phase III clinical trials in patient with constipation-predominant IBS.^{92,175} Prucalopride was shown to be effective in healthy subjects to accelerate the colonic transit without modification of the gastric emptying and the small bowel transit.¹⁷⁶ However in patients with severe constipation, a dose-dependent effect of acceleration of the overall transit time was observed¹⁷⁷ and several double-blind clinical studies demonstrated the significant efficacy of the compound in the mean weekly stool frequency.¹⁷⁸ Diarrhoea may be the common side-effect observed with the 5-HT₄ receptor agonists which precludes their use in patients with diarrhoea-predominant IBS. The visceral hypersensitivity observed with these patient may be mediated partially through activation of 5-HT₄ receptors and it can be treated with 5-HT₄ receptor antagonists. SB-207266 or piboserod was effective in the preliminary clinical studies by increasing the oro-caecal transit time toward normal values with the improvement of the symptoms.^{179,180} A recent study in healthy volunteers demonstrated the relatively weak effect of the 5-HT₄ antagonism on the colonic transit in normal physiological conditions. However, a marked effect was observed on the colonic transit in the presence of the 5-HT₄ agonist cisapride, indicating the interest of this compound in the pathophysiological conditions.¹⁸¹

The role of 5-HT is not limited to the motor function of the intestine. Indeed 5-HT was shown to be a potent intestinal stimulant secretagogue for intestinal chloride ion and is implicated in normal physiological conditions for the secretion of water in the digestion process. 5-HT is also a mediator of diarrhoea in the carcinoid syndrome where the large release of 5-HT was emphasised. The luminal membrane of the epithelial cells of the small intestine contains an ion channel: a cAMP-dependent Cl⁻ channel known as cystic fibrosis transmembrane regulator (CFTR). The increase of the intracellular concentrations cAMP results in the secretion of the Cl⁻ ions in the lumen and subsequently of Na⁺ ions which lead to water secretion. Abnormal activation of the cAMP-dependent Cl⁻ channel can be triggered by

cholera toxin which maintains the channel in the opened state causing a massive secretion of water that is manifest as severe diarrhea.¹⁸² It was found that 5-HT stimulates the short-circuit current (SCC), a method for the evaluation of the index of the mucosal electrolyte secretion.^{183,184} However, the results depended on the animal species. In the guinea-pig, the 5-HT effect was both tetrodotoxin-sensitive and -insensitive and was mediated by 5-HT₄ receptors since the effect of 5-HT was mimicked by 5-methoxytryptamine and SC 53116 and inhibited by GR 113808 or high concentration of ICS 205-930. This indicates both a neuronal and nonneuronal localisation of 5-HT₄ receptors mediating the secretion. Similar experiments performed in rat^{185,186} and in human.^{187,188} led to the conclusion that 5-HT₄ receptors were not located on neurones. Recently, it was demonstrated that activation of the human jejunal mucosa preparation by stroking induced a 5-HT release and Cl⁻ secretion. The increase of the 5-HT concentration by the release of 5-HT was associated with a positive change of the short-circuit which was blocked by inhibitors of the chloride secretion. The change of the SCC was inhibited by micromolar concentrations of tropisetron and SDZ 205-557 but not by the antagonists of the 5-HT_{1P}, 5-HT_{2A} or 5-HT₃ receptors.¹⁸⁹ The most likely mechanism to explain these results is that the mechanical deformation induces a 5-HT release from the enterochromaffin cells which activates the 5-HT₄ receptors present on the enteric sensory neurones. Consequently, a number of evidences indicate that 5-HT is implicated in the gastrointestinal disorders consequent to a release of water in the intestine, like in diarrhoea. The animal models, such as 5-HTP-induced diarrhoea in mice, demonstrated clearly the role of 5-HT₄ receptors.¹⁴¹ Hence, 5-HT₄ receptor antagonists can be valuable drugs to treat these disorders by acting at the same time on the control of the intestinal motricity and the excessive release of water.

Finally, 5-HT present in enterochromaffin cells can be released during treatment with cytotoxic drugs and this results in episodes of emesis which is an important problem in the

treatment of patients with cancer. The role of the 5-HT₃ receptors in the initiation of emesis and its inhibition by 5-HT₃ receptor antagonists have been largely documented.¹⁹⁰ However, emesis is only reduced by about 60% with selective 5-HT₃ antagonists used during the chemotherapy treatment, which led to the proposal that 5-HT₄ receptors could be also implicated in this phenomenon. Thus, molecules combining 5-HT₃ and 5-HT₄ receptor antagonist properties could be more beneficial for this type of treatment¹⁹¹

5-HT₄ receptors and the cardiovascular system

There is a large species dependent heterogeneity in the number and subtypes of 5-HT receptors expressed in the heart. Activation of the 5-HT₃ receptors was shown to produce bradycardia through the activation of the Bezold-Jarisch reflex¹⁹² while the activation of 5-HT₁ and 5-HT₂ receptors in cat, rabbit, rat and dog¹⁹³ produce tachycardia. Selective activation of 5-HT₄ receptors also produces tachycardia but only in human and pig atrium.^{7,194,195} In human and pig atrial strips, 5-HT induces a rapid increase in contractile force mimicking the positive inotropic effect of (-) isoprenaline, a β -adrenergic receptor agonist. The pEC₅₀ value for 5-HT is 6.6 and its effect is not antagonised by none of the 5-HT₁, 5-HT₂, 5-HT₃ and β -adrenergic receptor antagonists, except by ICS 205-930. However the maximum inotropic effect of 5-HT is smaller than that of (-)isoprenaline suggesting a weaker coupling efficiency between receptor and effector or a lower density of receptors.¹⁹⁶ Binding studies have shown that the density of 5-HT₄ receptors in human atrium was 10 and 5 times lower than the density of β_1 and β_2 receptors, respectively.¹⁹⁷

The stimulation of 5-HT₄ receptors in piglet isolated right atrium constitutes useful method to evaluate the potency and the pharmacological profile of 5-HT₄ receptor ligands. For instance, renzapride and cisapride behave as partial agonists in this model, while (-) zacopride is a full

agonist.¹⁹⁸ Similarly, pharmacological differences were observed with benzimidazolones: BIMU 1 is full agonist and BIMU 8 only partial agonist. The selective 5-HT₄ antagonists SB 203186, DAU 6215, SDZ 205-557 caused a parallel rightward shift of the 5-HT concentration-effect curves and the slopes of the Schild plots were not significantly different from 1, indicating a competitive antagonism.¹⁹⁹

An important aspect of cardiac response to 5-HT is the absence of effect of 5-HT or 5-HT₄ receptor agonists in the ventricular tissue of pig and human^{200,201,202} although mRNA²⁰³ coding for 5-HT_{4(a)} and 5-HT_{4(b)} receptors has recently been found in human ventricle. Thus, the lack of effect of 5-HT₄ agonists in human ventricle is attributed either to the absence of 5-HT₄ receptors in this tissue, or to the lack of functional coupling, making it therefore unlikely that 5-HT₄ receptor agonists will ever be used in the therapy of heart failure.

A mechanism was proposed to explain the effect of 5-HT *via* 5-HT₄ receptors on human cardiac cells.^{12,204} Exposure of human isolated atrial myocytes to 5-HT induced an increase of both whole-cell^{4,12} and single-channel Ca²⁺ current (I_{Ca}) through voltage-gated L-type Ca²⁺ channels.²⁰⁴ This effect is due to 5-HT₄-receptor mediated activation of adenylyl cyclase, cAMP synthesis and phosphorylation of the Ca²⁺ channels by cAMP-dependent protein kinase A (PKA) increasing their opening probability. The mechanism proposed is similar to that described for the β-adrenoreceptor, suggesting that 5-HT and (-) isoprenaline share a common signalling pathway. In addition to activating the L-type Ca²⁺ current I_{Ca}⁺, there is also evidence that 5-HT stimulates the so-called pacemaker current I_f in atrial myocytes isolated from human patients.^{205,206} I_f is an inward current activated by hyperpolarisation and carried via channels with mixed permeability to K⁺ and Na⁺. In control human myocytes, this current activates at potentials too negative to play a significant role in pacing. However, when 5-HT is applied, cAMP generation via activation of 5-HT₄ receptors leads to a progressive shift to more depolarised potentials of the activation curve of I_f. This allows a significant contribution

of the I_f current to the diastolic depolarization, leading to a positive chronotropic effect. It was demonstrated that the effect of 5-HT on the I_f current was completely inhibited by the 5-HT₄ receptor antagonist DAU 6285 and GR 124487.

In 1994, it was proposed¹⁴ an hypothetical role for 5-HT in the triggering of atrial arrhythmia through the activation of 5-HT₄ receptors. Using a model of arrhythmic contractions in human isolated atrial strips, 5-HT was found to induce arrhythmic contractions with a higher incidence in tissues obtained from patients treated with β -blockers.²⁰⁷ Arrhythmias were totally blocked by SB 203186, a potent and selective 5-HT₄ receptor antagonist, and the blockade was surmounted by increasing 5-HT concentration. It was supposed that the treatment by β -blockers induced a sensitisation of 5-HT₄ receptors as well as any other step involved in the cAMP cascade. Subsequent experiments testing the effect of 5-HT on isolated right atrial strips from patients chronically treated or not treated with β -blockers demonstrated significantly lower EC₅₀ value for 5-HT in patients treated (pEC₅₀ = 7.9) than in control patients (pEC₅₀ = 7.3).²⁰⁸ Through activation of Ca²⁺ channels, 5-HT stimulation of the 5-HT₄ receptor might lead to intracellular Ca²⁺ overload, a condition generally associated with the generation of arrhythmia. These experiments suggested that, under pathological conditions such as the enlarged atria of the elderly, endocardial lesions or carcinoid tumours, 5-HT released from platelets may cause atrial arrhythmias.

The 5-HT-evoked positive chronotropic effect was characterised *in vivo* in piglets and minipigs.²⁰⁹ The effect was clearly antagonised by SB 203186 when it was administered iv or intraduodenum, indicating a good bioavailability of the 5-HT₄ antagonist despite the presence of the ester function. However, it was not possible to induce *in vivo* arrhythmias in piglets and minipigs by bolus injection of 5-HT. This lack of effect was most likely due to the very low density of 5-HT₄ receptors in pig as compared to that found in human.

The presence of 5-HT₄ receptors in human atrium and their potential role in the generation of arrhythmia or tachycardia is a serious concern when using 5-HT₄ receptor agonists as gastrokinetic agents. For instance, metoclopramide, renzapride or cisapride which are used clinically, all behave as partial agonists of the human atrial 5-HT₄ receptors.¹⁹⁹ However, the undesirable cardiac side-effects of the 5-HT₄ receptor agonists are not necessarily related to their stimulant effect on cardiac 5-HT₄ receptors. For instance, cisapride, a non selective 5-HT₄ receptor agonist, produces arrhythmogenic effects when administered in human which are not related to activation of cardiac 5-HT₄ receptors. Indeed, cisapride behaves as a blocker of the delayed rectifying K⁺ current (I_{KR}), an effect which leads to a lengthening of the action potential,²¹⁰ a prolongation of the electrocardiographic QT and polymorphous ventricular tachycardia such as torsades de pointes.²¹¹ However, this deleterious effect of cisapride on QT interval is not encountered by the structurally related drug mosapride,²¹² or by other 5-HT₄ receptor agonists such as ML 10302,²¹³ TS-951²¹⁴ or TKS159 (4-amino-5-chloro-2-methoxy-N-[(2S,4S)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide).²¹⁵

The recent characterisation of several human 5-HT₄ receptor isoforms with some degree of specificity in their tissue distribution may confer a different potency and/or efficacy for a given 5-HT₄ receptor ligand when tested in different tissues.^{4,122} For instance, ML 10302 behaves as a potent stimulant of intestine *in vitro* and *in vivo* and as a partial and weak stimulant of I_{Ca} in isolated human atrial myocytes (1 μM ML 10302 and 1 μM 5-HT increased I_{Ca} by, respectively, 17.4 % and 157% with regard to the basal level).¹²² Similarly, cisapride and renzapride behave partial agonists on the 5-HT_{4(e/g)} isoform while they behave as full agonists on the 5-HT_{4(c)} and 5-HT_{4(d)} isoforms.^{126,127} However, in the case of ML 10302, a similar pharmacological profile was obtained on 5-HT_{4(a)}, 5-HT_{4(b)} and 5-HT_{4(e/g)} variants.^{122,127} which are all expressed in heart,¹²⁸ indicating that another mechanism must account for the tissue-specificity of the drug. A difference in the level of expression of a given

receptor in different tissues might provide a possible explanation for the change in the profile of the molecules.¹²³

As shown above, 5-HT₄ receptors are connected to the electrical activity of human atrium, via activation of two inward currents, I_{Ca} and I_f, making these receptors potential candidates for the generation of atrial arrhythmia. However, down regulation of 5-HT₄ receptors was observed in atrial tissues of patients with atrial fibrillation (AF) which may provide a feedback mechanism for the stabilisation of the arrhythmia.²¹⁶ This is accompanied by a general reduction of the transcription of L-type Ca²⁺ channel subunits. The overall reduction of 5-HT₄ receptor and Ca²⁺ channel transcription may contribute to the shortening of the action potential, providing a general protective effect against excessive intracellular Ca²⁺ accumulation and for stabilisation of AF.²¹⁶

Altogether, these data highlighted the interest of the 5-HT₄ receptor antagonists as potential antiarrhythmic drugs in human for the prevention or treatment of human atrial fibrillation. A recent study by Rahme²¹⁷ confirmed this hypothesis. RS-100302, a new 5-HT₄ receptor antagonist, inhibited the atrial flutter and fibrillation induced by rapid right pacing in the juvenile pigs and the effect was reversed by cisapride, a partial 5-HT₄ receptor agonist. It is worth noting the absence of ventricular electrophysiological effects confirming that the only target of the 5-HT₄ receptor ligands in the heart was located on atria and consequently minimum ventricular side-effects can be expected with such a drug. Consequently the 5-HT₄ receptor antagonists have the potential to become a valuable alternative to the β-adrenergic and Ca²⁺ channel blockers for the treatment of AF, without depressive effect on the ventricle.²¹⁸

5-HT₄ receptors and the central nervous system

As reported previously, 5-HT₄ receptors are expressed in the region of the limbic system and the nigro-striatal pathways. They were shown to be implicated in the long-term blockade of K⁺ channels¹¹⁷ resulting in the opening of Ca²⁺ channels and release of neurotransmitters such as ACh,^{219,220} serotonin²²¹ and dopamine.²²² Consequently, 5-HT₄ receptors are potentially involved in a number of physiological processes of the central nervous system, such as learning, memory, depression or anxiety and agonists or antagonist of these receptors can be valuable pharmacological tools to explain the mechanisms of these processes.

The increase of cAMP mediated by the stimulation of the 5-HT₄ receptors in the hippocampus is supposed to induce long-term potentiation of the neurons which plays an essential role in the cellular mechanism of learning and memory. Several behavioural studies have demonstrated that the 5-HT₄ receptor agonists can be considered as cognitive and learning function enhancers. For instance, metoclopramide, cisapride and SR-17 (endo 8-methyl-8-azabicyclo[3.2.1]octan-3-yl benzofuran-3-carboxylate), a bioisosteric analogue of ICS 205-930, were shown to be active in the mouse passive avoidance test in the range of the doses of piracetam.²²³ However, due to the lack of specificity of the compounds used, it was impossible to conclude that 5-HT₄ receptors were solely involved in the effect of these drugs. More convincing evidence in favour of the role of the 5-HT₄ receptors was given by the effect of BIMU-1 which was active on the social olfactory recognition test and on the olfactory associative task, both effects being inhibited by the 5-HT₄ receptor antagonist GR 124487 and not by the 5-HT₃ receptor antagonist ondansetron.^{117,224,225} The selective hydrophobic 5-HT₄ receptor agonist RS 67333 but not the hydrophilic 5-HT₄ receptor agonist RS 67506 was shown to reverse in the rat the atropine induced-deficit performance in the Morris water maze, an effect which was inhibited by GR 113-808.²²⁶ More recently,²²⁷ the role of 5-HT₄ receptors in the neuronal mechanism of memory enhancement and the cognitive process was clearly demonstrated with SC 53116 which increased the spike amplitude in the hippocampal CA1

neurones, the tetanus-induced long term potentiation and the concentration dependent release of ACh. The effects of SC 53116 was blocked by the 5-HT₄ receptor antagonists GR 113808. On the other hand, application of GR 113808 alone had no effect on the release of ACh indicating that the 5-HT₄ receptors are not constitutively coupled to the cholinergic neuronal system. This latter result indicates that 5-HT₄ receptor agonists may prevent the memory deficit but exert little influence on the memory process itself. The beneficial effect of 5-HT₄ receptor agonists on the memory deficit was confirmed *in vivo* by a behavioural study in rat where SC 53116 improved the scopolamine-induced deficit in the passive avoidance test.²²⁷ However, these results are in contrast with those reported on the activity of 5-HT₄ receptor ligands RS 17017, RS 67333 and RS 67532 in the olfactory associative discrimination task in rats.²²⁸ In this study, the antagonist RS 67532 induced a marked impairment in the learning performance observed with regard to the control while the deficit induced by the antagonist was reversed by the administration of the agonists RS 17017 and RS 67333. Moreover, the hydrophobic compound RS 67333 administered alone gave a significant improvement in the learning of the task and the memory performance with regard to the control. These results were confirmed with RS 67333 in the Morris water maze where improvement of the learning rate was demonstrated.²²⁹

The above mentioned memory-enhancing effects of 5-HT₄ receptors agonists may be relevant for the treatment of memory dysfunction in patients suffering from Alzheimer's disease. Indeed, a binding study reported a decrease of the density of 5-HT₄ receptors in the hippocampus²³⁰ of patients with Alzheimer's disease. Recent data²³¹ indicated another potential promising way to alleviate symptoms of the Alzheimer's disease with 5-HT₄ receptor agonists. In CHO cells expressing the neuronal h5-HT_{4(e/g)} receptor, 5-HT was found to stimulate the release of the non-amyloidogenic soluble amyloid precursor protein (sAPP α)

which exerts neuroprotective and enhancing memory effects. This process was blocked by GR 113808 and SB 204070.

Recently, indeloxazine, an inhibitor of 5-HT and noradrenaline reuptake, was shown to increase the release of ACh in the rat frontal cortex. The effect was mediated through 5-HT₄ receptors since it was blocked by the selective antagonists GR 113808 and RS 23597 and not by 5-HT_{1A/1B/2A/2C/3} antagonists. Thus, 5-HT₄ receptors may also play a role in the effects of antidepressants, by increasing ACh release upon an increase of the level of 5-HT in the frontal cortex.²³²

Since modulation of the concentration of 5-HT in the CNS is a common mechanism involved in the action of anxiolytic and antidepressant agents, the role of 5-HT₄ was examined. In 1996, Barnes²²¹ showed that a systemic administration of the 5-HT₄ receptor agonist renzapride induced a clear and dose-dependent increase of the extracellular levels of 5-HT (200%) in the rat hippocampus. This effect was antagonised by the 5-HT₄ receptor antagonist GR 125487D which, when used alone, reduced the concentration of 5-HT to 80% below basal level. Although the previous study might suggest the involvement of 5-HT₄ receptors in the effects of antidepressants, a more recent study suggests the opposite. In this study,²³³ the potent and selective 5-HT₄ receptor antagonist SB 204070 was inactive on the effect of fluoxetine, a selective uptake inhibitor, on the decrease of the immobility time in the forced swim test. On the other hand, SB 204070 and GR 113808 were shown to possess marked anxiolytic properties in two animal models: the rat social interaction test²³⁴ and the elevated plus-maze.²³⁵ However the effect was species dependent since in the BKW mice the 5-HT₄ receptor antagonists SDZ 205-557, GR 113808 and SB 204070 were unable to modify the behaviour of the mice in the light/dark test box and caused an inhibition of the disinhibitory behaviour of diazepam.²³⁶

Using the microdialysis technique,²³⁷ it was shown that 5-methoxytryptamine, a 5-HT₄ receptor agonist, increased the release of dopamine and that this effect could be blocked by a high concentration of tropisetron. This result was confirmed with renzapride and (S)-zacopride by *in vitro* studies in rat striatal slices and *in vivo* studies using microdialysis.^{222,238} The localisation of the 5-HT₄ receptors in the extrapyramidal forebrain areas allowed to propose the hypothesis that the increase in dopamine release could play a role in the modulation of the reward and motor behaviour processes. Preliminary data indicated that the antagonist GR 113808 reduced the ethanol intake in alcohol-preferring rats suggesting a role of 5-HT₄ receptors in the regulation of the alcohol intake control.²³⁹ On the other hand, no antagonist effect of SB-204070 was found in pharmacological test implicating the basal forebrain, such as the stimulation of the motor activity by amphetamine or nicotine or the cocaine intracranial self-stimulation reward threshold. As previously reported, such a lack of activity of a 5-HT₄ receptor antagonists may reflect the absence of 5-HT tone in the area considered.²⁴⁰ Nevertheless, the partial 5-HT₄ receptor agonist RS 67333 and the antagonist SDZ 205-557, when injected in the accumbens nucleus in rat were found to decrease significantly the hyperactivity induced by cocaine injection.²⁴¹

In summary, the data available in the literature so far, indicate that 5-HT₄ receptor ligands may play in the future an important role in the treatment of several pathological disorders of the CNS. In particular, 5-HT₄ receptor agonists seem to be promising molecules to overcome deficits of the memory and of the learning tasks. Although no clinical data is available to date, the observed decrease in the density of 5-HT₄ receptors in patients with Alzheimer disease²³⁰ indicates a potential therapeutic use for these new molecules. On the other hand, further work is needed to confirm the promising results obtained with different 5-HT₄ receptors ligands in different animal models on anxiety, mood or drug dependence.

5-HT₄ receptors and urinary tract

Normal bladder functions requires the simultaneous occurrence of detrusor relaxation and sphincter contraction during the filling phase and the converse during micturition. The descending bulbospinal pathway is an inhibitory circuit driven by the release of 5-HT which inhibits the bladder contraction through the activation of 5-HT_{1A} receptors. Indeed, it was clearly demonstrated that the application of 5-HT_{1A} antagonists in rat releases the inhibition of contraction and increases the capacity of the bladder through a control exerted at the central level.²⁴²

However, 5-HT receptors are also present on the isolated bladder and may contribute to the peripheral effects of 5-HT. As already observed in several other tissues, the types of 5-HT receptors present in bladder vary depending on the animal species: in the guinea-pig, potentiation of the electrically evoked bladder is mediated by 5-HT_{2A}, 5-HT₃ and 5-HT₄ receptors,²⁴³ in the rabbit by 5-HT₃ receptors²⁴⁴ and in human by 5-HT₄ receptors.²⁴⁵ Stimulation of 5-HT₄ receptors leads to activation of cholinergic neurons, release of ACh, and ACh-mediated contraction of the bladder. Several cases have been reported in clinical studies showing a facilitation of bladder emptying and, occasionally, urinary incontinence in patients treated with metoclopramide²⁴⁶ or cisapride.²⁴⁷ However, the nature of the pharmacological response of the activation of 5-HT₄ receptors depend also on the animal species, since 5-HT was found to inhibit the electrically-evoked contraction of the bladder strip in *Rhesus* and *Cynomolgus* monkeys. This effect was mimicked by BIMU 8 and renzapride and it was competitively inhibited by the reference 5-HT₄ antagonists, such as GR 113808, DAU 6285 and RS 23597-190.²⁴⁸

A complete pharmacological characterisation²⁴⁹ of the role of 5-HT₄ receptors in the human isolated detrusor muscle was realised by evaluating the activities of reference agonists, such as 5-HT, 5-MeOT, BIMU 8, zacopride and cisapride, in the isolated detrusor muscle. All

these compounds induced bladder contraction with a rank order of potency comparable to that observed in other tissues ($pEC_{50} = 8.02, 6.02, 6.86, 5.67$ and 6.61 , respectively). In addition, 5-HT and 5-MeOT behaved as full agonists while the other compounds had intermediate intrinsic activity, unexpectedly RS 23597 which was described as a 5-HT₄ antagonist behaved as a partial agonist. The 5-HT effect was competitively antagonised by GR 125487 with a pA_2 value of 9.75. Recently, the detrusor muscle of pig was shown an efficient model to study potential drugs to be used in human clinic: 5-HT increases the electrical field stimulated cholinergic response of the pig bladder strips and the effect is inhibited by RS-100235 and GR-113808.²⁵⁰

In conclusion, the pharmacological and clinical data reported with 5-HT₄ receptor agonists provide a rationale for the development of new therapeutic drugs for the management of the voiding disorders associated with the detrusor hypocontractility in human.¹⁰

5-HT₄ receptors and adrenal gland

The first evidence for a role of 5-HT₄ receptors in the adrenal gland came from the finding that 5-HT stimulated the production of corticosteroides such as corticosterone and aldosterone in the adrenal glands of frog. This effect of 5-HT was demonstrated to be mediated by 5-HT₄ receptors since it was mimicked by zacopride, cisapride, renzapride, BIMU 1 and BIMU 8.^{251,252} These drugs behaved as full or partial agonists in the model with the rank order of potency; (S) zacopride > BIMU 8 = (R,S) zacopride > BIMU 1 = (R) zacopride. Only high doses of ICS 205-930 were capable to block the production of aldosterone or corticosterone while the selective 5-HT₃ antagonist MDL 72222 was totally inactive to inhibit this effect. As already reported in other tissues, the effect of 5-HT in adrenal glands appears to be mediated by stimulation of cAMP synthesis followed by activation of PKA and stimulation of the Ca²⁺ channels.²⁵³ Similar effects were observed in human treated with metoclopramide^{254,255} which

induced a transient increase of the plasmatic aldosterone concentration. The implication of 5HT₄ receptors was demonstrated in the human adrenal cortex where 5-HT and zacopride, as previously observed in frog, mediated the increase of aldosterone secretion.²⁵⁶ The administration of zacopride²⁵⁷ in normal volunteers induced an increase of plasma aldosterone concentration without any modification in renin and ACTH levels. However, the increase of aldosterone level was only transient since the level of the steroid returned to basal during chronic administration of zacopride.^{258,259} This data can explain why there was no change in blood pressure in patients during chronic administration of cisapride.²⁶⁰ As reported in other tissues, the transient effect of 5-HT₄ receptor activation on aldosterone secretion likely reflects the desensitisation of the receptors. Hyperaldosteronism can exert deleterious effects on the cardiovascular system under pathological conditions. Since the inhibition of the renin-angiotensin system by inhibitors of the converting enzyme is not sufficient to restore a normal level of aldosterone, the combination of this therapy with a 5-HT₄ receptor antagonist could constitute a new approach to treat cardiovascular disorders, such as heart failure or hypertension.²⁶¹

Conclusions and perspectives

Since the pharmacological characterisation of the 5-HT₄ receptors in brain and intestine, considerable progress have been made in the design of several potent and selective compounds from a relatively limited number of chemical leads and in the understanding of the functions of these receptors. Interest of this class of receptors resides in the existence of several isoforms distributed in various central and peripheral tissues and in the putative role of these different variants in the regulation mechanism of the signal transduction and cell function. Preliminary data indicate the presence of 5-HT₄ receptors in major organs and

tissues although large qualitative and quantitative difference exist in their distribution. For instance, 5-HT_{4(a)} and 5-HT_{4(b)} receptors are the only isoforms present in the bladder and kidney, respectively, and 5-HT_{4(d)} receptor was only characterised in intestine. To date, no information is available on the specific role of the different isoforms and on the possible implication in pathological disorders of the variability in tissues distribution. Potent 5-HT₄ receptor agonists are now available. However, when tested for their binding capacity on expressed receptors, none of these agonists show any specificity for any given 5-HT₄ receptor isoform, which is understandable since the binding site is structurally identical in all isoforms. Surprisingly, when tested for their capacity to activate cAMP synthesis or to modulate cellular function, the efficacy of a given 5-HT₄ receptor agonist depends strongly on the variant considered. A partial explanation for this observation may reside in the allosteric influence of the C-terminal end on the rest of the receptor; Indeed truncation of the C-terminal end of a given 5-HT₄ receptor was shown recently to increase its constitutive activity. This is particularly relevant, since the main difference between the 5-HT₄ receptor variants reside in their C-terminal composition and length. Therefore, a specific pattern of expression of the different 5-HT₄ receptor isoforms in a given tissue may confer to the cell a unique mechanism to finely regulate signal transduction and function. This may also provide a clue for the development of “selective” ligands, as the selectivity for a particular receptor isoform may not be revealed in binding studies but rather in functional studies (e.g; as observed with renzapride). The elucidation of the structural factors concerned in this fascinating phenomenon is a challenge for the medicinal chemist. Also intriguing for the chemist is the close structural similarity between some agonists and antagonists which differ in their chemical structure by subtle steric factors. However, to date, it is not realistic to imagine the design of selective ligands for a given isoform.

It is worth also noting that the localisation and the functionality of the 5-HT₄ receptors are animal species-dependent and that the potential drugs have to be selected on the animal models relevant of the clinical activity.

Originally designed to cure gastrointestinal disorders, 5-HT₄ receptor agonists and antagonists available to date constitute worthwhile pharmacological tools to understand the physiological and pathophysiological role of these receptors in the GI. Several drugs are currently in development to treat constipation or the IBS syndrome, a complex disorder of the GI system where the inactivation and the activation of the peristaltic reflex was closely mixed. It will be of interest to observe the effects in human of the partial agonists, such tegaserod, which are currently in clinical investigations. But several other promising drugs are under study, in particular the use of antagonists, in the prevention or treatment of atrial arrhythmia, a major pathological disorder of people suffering of heart failure or other cardiovascular problems related to platelet aggregation. Development of a new class of antiarrhythmic drugs, potentially devoid of ventricular effects constitutes a promising progress in cardiovascular therapeutic. Finally, a number of other pharmacological studies allow to predict a good future for 5-HT₄ receptor ligands with potential therapeutic applications for treatments of the memory disorders, hypertension or the dysfunction of the urinary tract.

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Table 1. The different 5-HT receptor subtypes.

	5-HT₁	5-HT₂	5-HT₃	5-HT₄	5-HT₅	5-HT₆	5-HT₇
Subtypes	5-HT _{1A} , 5-HT _{1B} , 5-HT _{1D} , 5-HT _{1E} , 5-HT _{1F}	5-HT _{2A} , 5-HT _{2B} , 5-HT _{2C}			5-HT _{5A} , 5- HT _{5B}		
Signalling pathways	^a cAMP ↓	^b IP ₃ ↑	^c Ion channel ↑	^d cAMP ↑	^e cAMP	^d cAMP ↑	^d cAMP ↑

Table legend

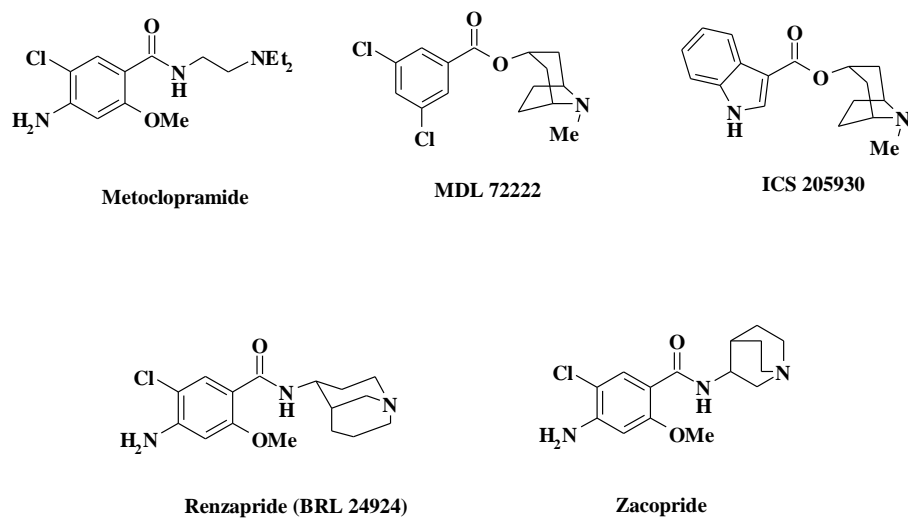
^a Decrease of cAMP synthesis. ^b Stimulation of the phospholipase C. ^c Ion channel. ^d Increase of cAMP synthesis. ^e A possible negative coupling with adenylyl cyclase.

Table 2. The 5-HT receptors in the gastrointestinal system

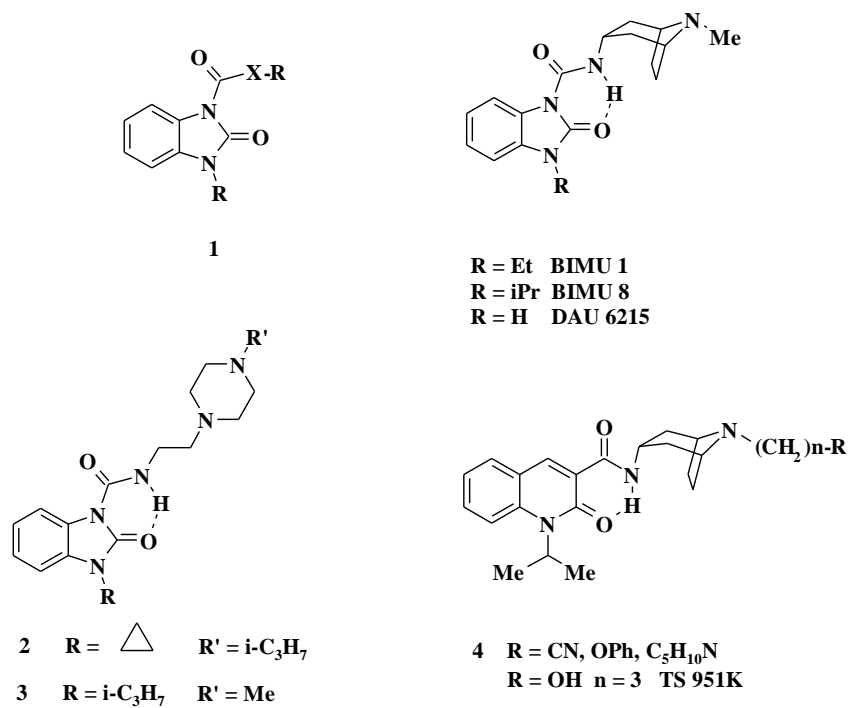
	5-HT _{1A}	5-HT _{1D}	5-HT ₂	5-HT ₃	5-HT ₄	5-HT ₇
Oesophagus					- Relaxation (rat) - Contraction (G-P)	
Stomach	- Relaxation (G-P)			- Antagonist increase the gastric emptying. - Increases the electrically induced contraction (G-P).	- Increases the electrically induced contraction (G-P).	
Small intestine	- Relaxation	- Activation of the peristaltic reflex (G-P)	Contraction of the smooth muscle (G-P)	- Contraction of the smooth muscle (G-P), increases the peristaltic reflex (G-P). ^o	- Activation of the peristaltic reflex (G-P) - Relaxation of the smooth muscle (rat).	
Colon	- Relaxation			- Increases the peristaltic reflex (G-P).	- Contraction of the distal colon (G-P). - relaxation of the circular smooth muscle.	- Relaxation of the colonic circular smooth muscle.

Table 3. Effects mediated by activation of 5-HT₄ receptors in the gastrointestinal system of different animal species

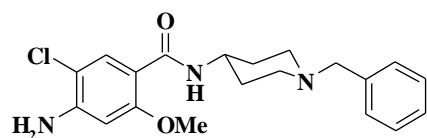
	Oesophagus	Stomach	Ileum	Distal colon
Guinea-pig	Contraction	- Potentiation of the electrically evoked contraction.	- Contraction of the isolated longitudinal and circular muscles. - Stimulation of the peristaltic reflex. - Facilitation of the circular muscle contraction.	- Density of the 5-HT ₄ receptors was superior to that on the the smooth muscle. - Contraction of the distal colon LMMP.
Rat	Relaxation	- Stimulation of the gastric emptying. - Contraction of the stomach fundus strips.	- Relaxation	- Not present
Dog	No effect	- Increases the antral motor activity and the gastric emptying.		- Increases the electrical field- evoked contraction of the longitudinal muscle in the proximal colon and the inhibition in the distal part. - Increases the relaxation of the rectum circular smooth muscle.
Human		- Contraction.	- Relaxation of the circular muscle.	- Relaxation of the circular muscle. - Increases the electrical field-evoked contraction of the longitudinal muscle.



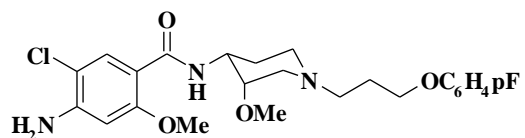
Scheme 1. The 5-HT₃ receptor antagonists of the first generation.



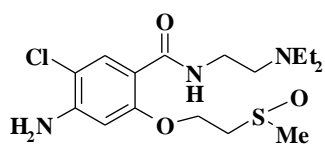
Scheme 2. 2-Benzimidazolone and quinoline carboxamide derivatives as 5-HT₄ agonists and antagonists



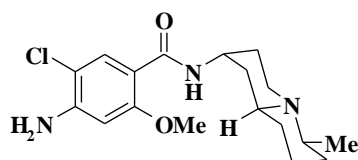
Clebopride



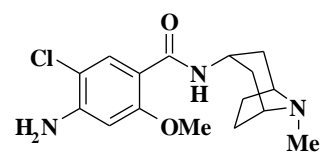
Cisapride



(S)-ML-1035

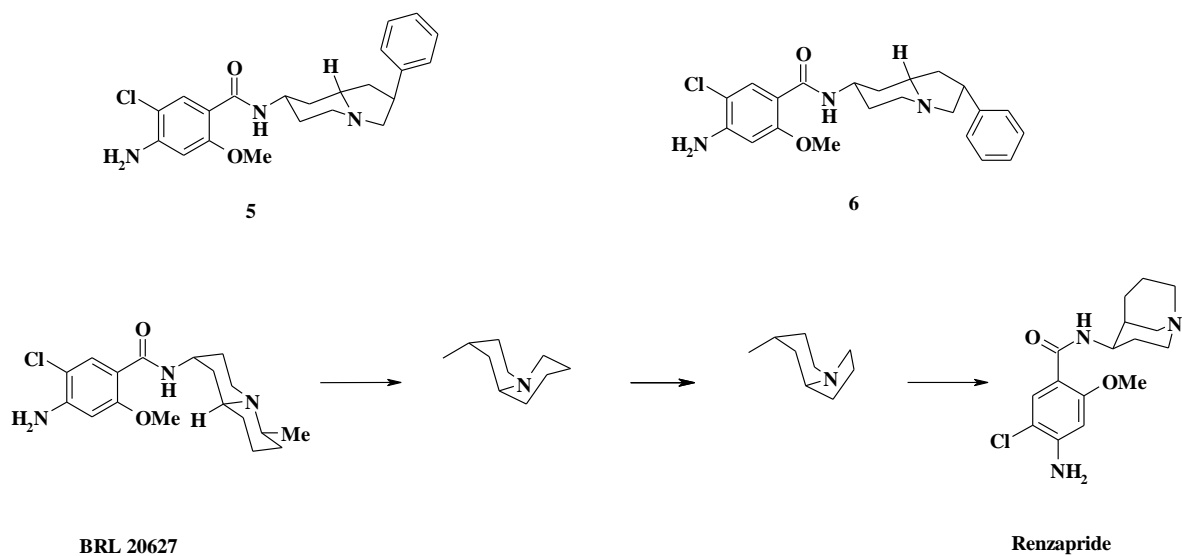


BRL 20627

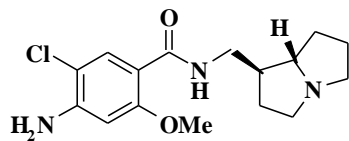


BRL 24682

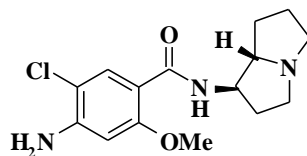
Scheme 3. Gastrokinetic benzamides of the first generation



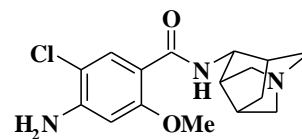
Scheme 4. The influence of the stereochemistry of the basic framework for the recognition by the 5-HT₄ and the D₂ receptors. The structural intermediates between BRL 20627 and renzapride.



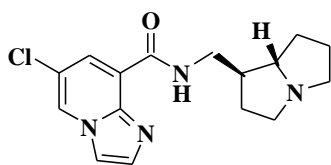
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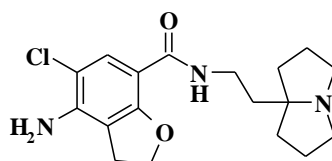
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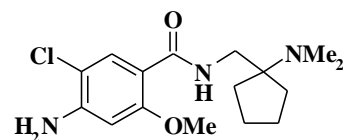
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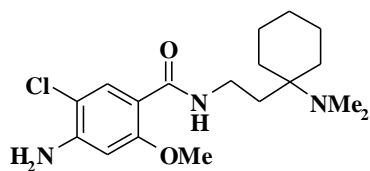
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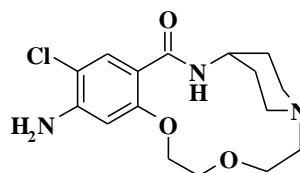
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7

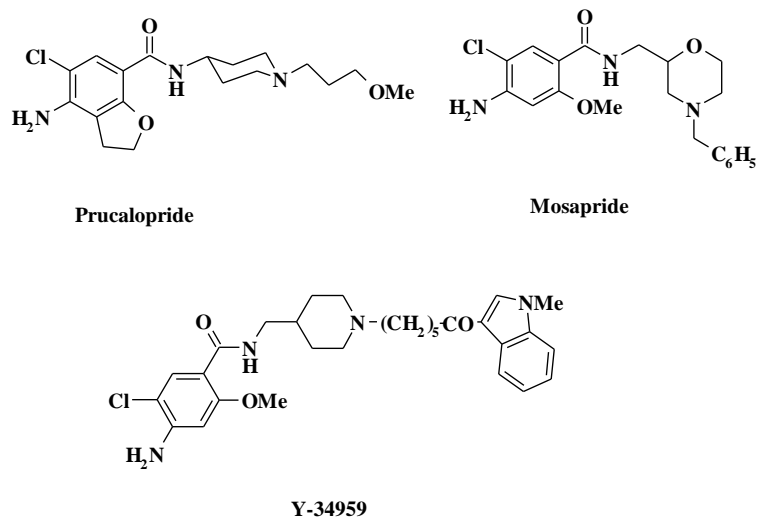


YM-47813

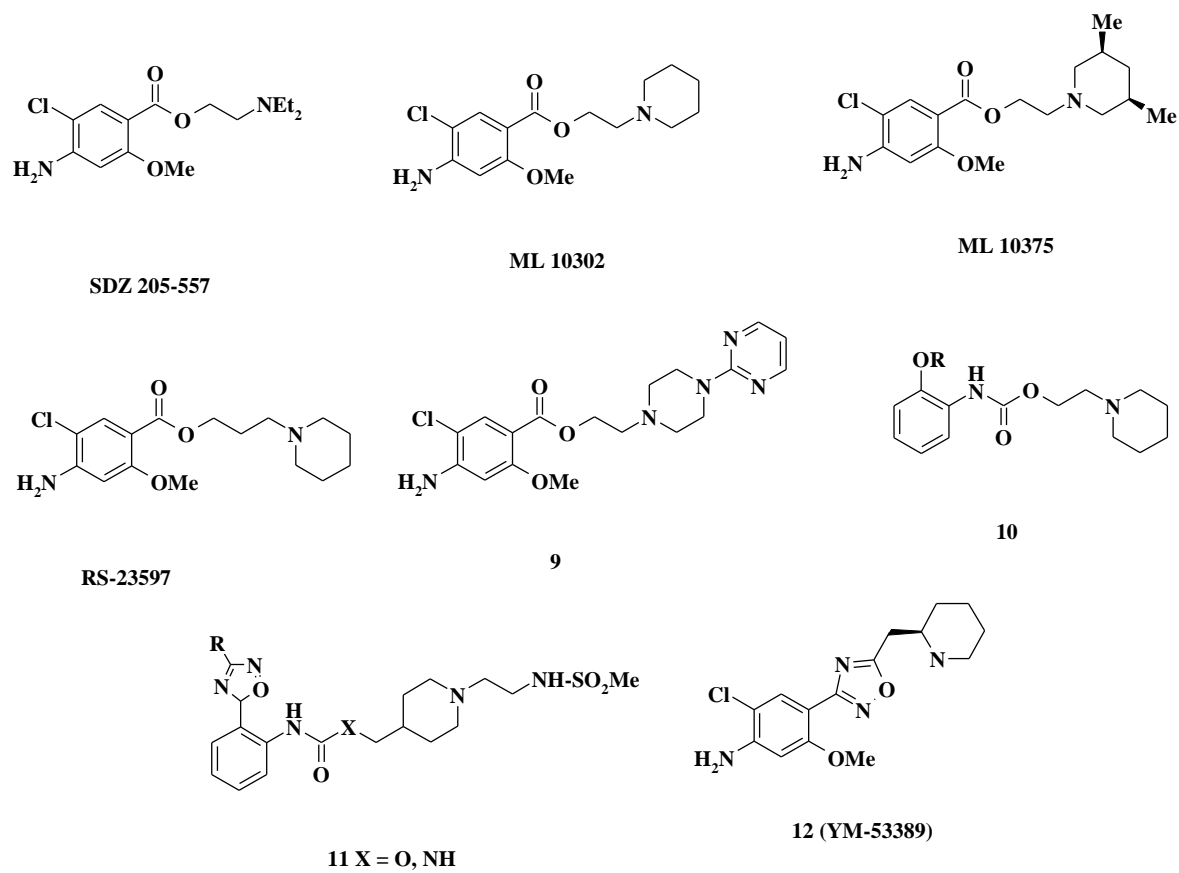


8

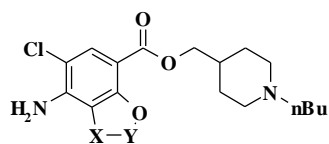
Scheme 5. Second generation of benzamide derivatives with structural modification of the basic chain.



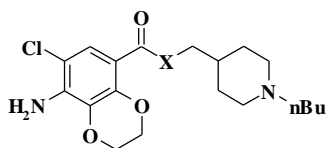
Scheme 6. Benzamides with a large substituent on the basic nitrogen atom as 5-HT₄ receptor agonists



Scheme 7. Benzoate and carbamate derivatives as selective 5-HT₄ receptor agonists and antagonists

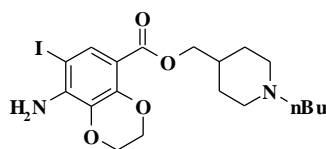


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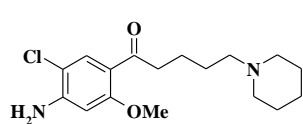
X = O SB 204070

X = NH SB 205800

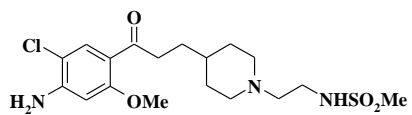


SB 207710

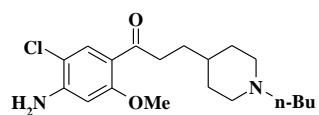
Scheme 8. Benzoate derivatives as selective and potent 5-HT₄ receptor antagonists.



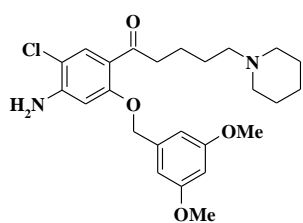
RS 17017



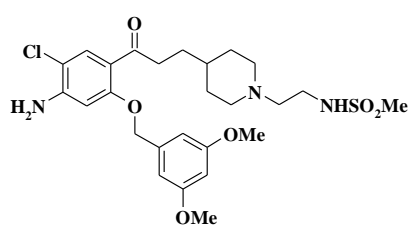
RS 67506



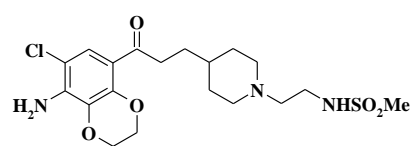
RS 67333



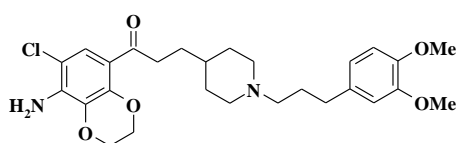
RS 67532



RS 39604

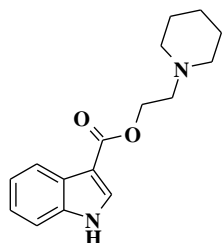


RS 100302

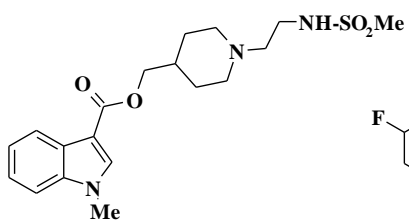


RS 100235

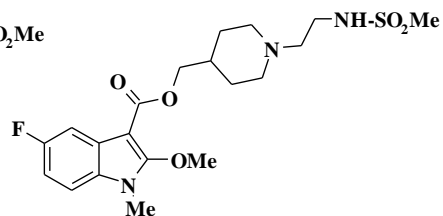
Scheme 9. Ketone derivatives as 5-HT₄ receptor agonists and antagonists



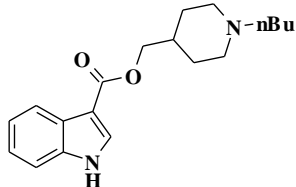
SB 203186



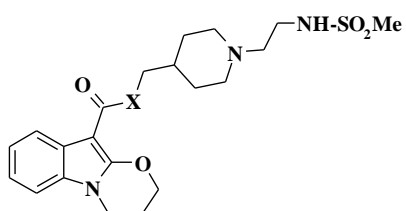
GR 113808



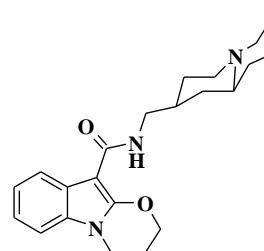
GR 124487



SB 204139

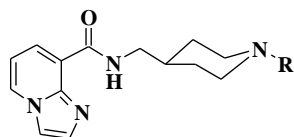


X = O SB 207058
X = NH SB 207266

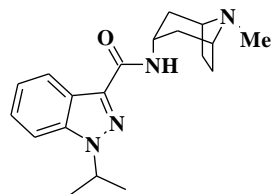


14

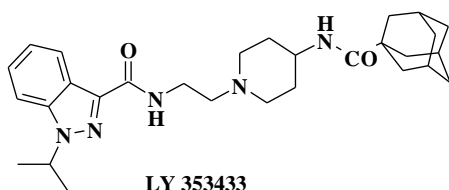
Scheme 10. Indole amides and esters as 5-HT₄ receptor antagonists



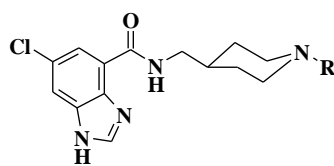
15



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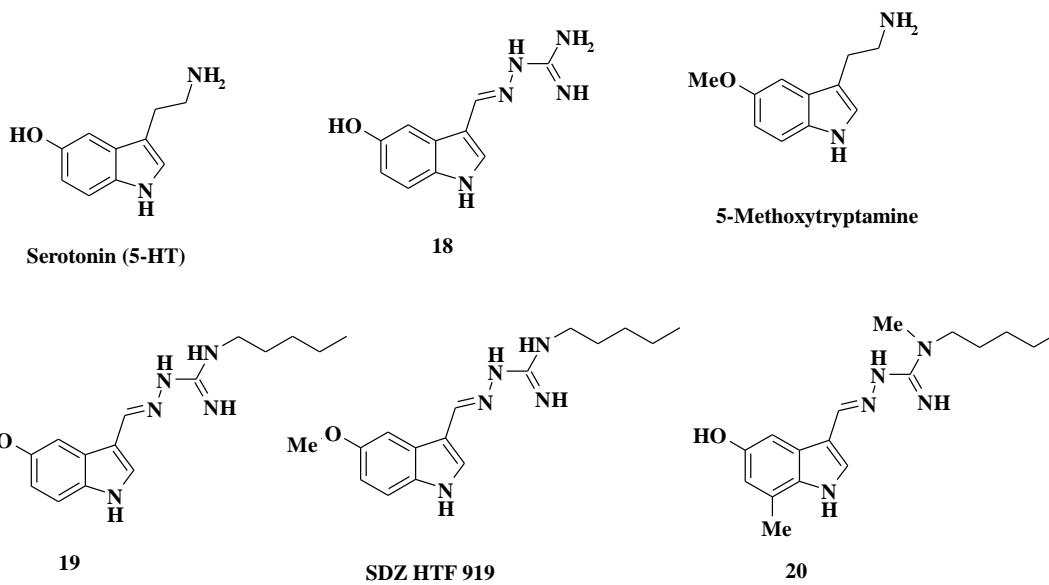
LY 353433



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R = nBu
 R = nPent
 R = (CH₂)₂NHSO₂Me
 R = CH₂CH(CH₃)₂

Scheme 11. Imidazopyridine, indazole and benzimidazole derivatives as 5-HT₄ receptor antagonists.



Scheme 12. Carbazimidamide derivatives as 5-HT₄ receptor agonists or antagonists.