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Synthesis and pharmacological properties of novel hydrophilic 5-HT$_4$ receptor antagonists

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ABSTRACT

Serotonin (5-hydroxytryptamine, 5-HT) is an important signalling molecule in the human body. The 5-HT$_4$ serotonin receptor, coupled to the G protein G_s, plays important physiological and pathophysiological roles in the heart, urinary bladder, gastrointestinal tract and the adrenal gland. Both 5-HT$_4$ antagonists and agonists have been developed in the aim to treat diseases in these organs. 5-HT$_4$ agonists might have beneficial effects in the central nervous system (CNS) and therefore, 5-HT$_4$ antagonists might cause CNS side effects. In this study, we have developed new amphoteric 5-HT$_4$ antagonists. A series of cyclic indole amide derivatives possessing an oxazine ring and a piperidine alkane carboxylic acid side chain and the corresponding prodrug esters were synthesized and their binding to 5-HT$_4$ receptors and antagonist properties were evaluated. In addition, an indole ester without the oxazine ring and the corresponding indole amide derivatives were also tested. Octanol–water distribution (Log $D_{Oct7.4}$) was tested for some of the synthesized ligands. The main structure–affinity characteristics of the 5-HT$_4$ compounds tested were that the prodrug esters show higher affinity than their corresponding free acids, indole esters show higher affinity than the corresponding amides and ligands containing the oxazine ring in the indole skeleton show higher affinity than indole derivatives not containing the ring. One representative prodrug ester and its corresponding free acid were tested for binding on a panel of receptors and showed preserved selectivity for the 5-HT$_4$ receptor. These new molecules may be useful to target peripheral 5-HT$_4$ receptors.

1. Introduction

Serotonin (5-HT) is an important signalling molecule in the human body and has important effects both as a centrally acting neurotransmitter and a locally acting signalling molecule. The 14 different 5-HT receptors, divided into seven subfamilies (5-HT$_{1-7}$), are all G-protein-coupled receptors, except the 5-HT$_3$ receptor which is a ligand-gated ion channel. The 5-HT$_4$ receptor stimulates adenylyl cyclase (AC) through G_s.[1] There are at least 10 splice variants of the human 5-HT$_4$ receptor (5-HT$_{4(a-i)}$, $_{(hb)}$ and $_{(n)}$) with very similar pharmacological properties.[2–8]

In addition to the central nervous system (CNS), 5-HT$_4$ receptors are found in peripheral organs like the gastrointestinal tract, heart and urinary bladder, and may be implicated in diseases in these organs. 5-HT$_4$ agonists or partial agonists (e.g. cisapride, tegaserod) have shown effects in constipation-predominant irritable bowel syndrome (IBS) patients, whereas SB207266 (piboserod), an antagonist of 5-HT$_4$ receptors was effective in the preliminary clinical studies in patients with diarrhoea-predominant IBS.[9] In the heart atrium, 5-HT$_4$ antagonists may prevent atrial fibrillation as they block arrhythmic contractions induced by 5-HT.[10] In addition, a 5-HT$_4$-mediated inotropic response to serotonin was observed in the ventricle of porcine and failing human hearts[11] and both infarcted and failing rat hearts, but was absent in normal rat hearts.[12] In vivo cardiac function and *ex vivo* myocardial function improved in rats with heart failure treated for 6 weeks with the 5-HT$_4$ receptor antagonist SB207266 (piboserod),[13] in accordance with a proposed beneficial role of 5-HT$_4$ receptor antagonists in the treatment of heart failure.[14] This was supported by a recently concluded phase II clinical trial of 24-week treatment of heart failure patients with SB207266, which showed increased left ventricular ejection fraction in the patients treated with SB207266 compared to placebo.[15] The 5-HT$_4$ receptor mediates contractile effects in the urinary bladder. In clinical studies with the 5-HT$_4$ agonists metoclopramide and cisapride facilitation of urinary bladder emptying and occasional urinary incontinence was observed.[16] The

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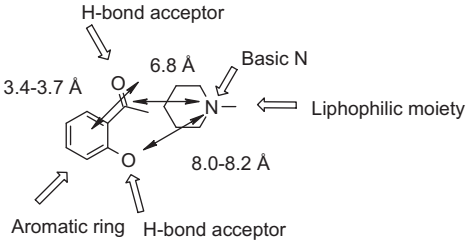


Figure 1. A proposed pharmacophore for the 5-HT$_4$ receptor (adapted from Clark).[20]

selective 5-HT$_4$ antagonist SB207266 antagonized the ability of 5-HT to enhance contraction in isolated human bladder strips.[17] Since 5-HT$_4$ receptors are involved in aldosterone secretion by the adrenal gland in man,[18] 5-HT$_4$ antagonists might be useful in treatment of hyperaldosteronism, which may cause harmful cardiovascular effects.

In the central nervous system (CNS), 5-HT$_4$ receptors are involved in the release of neurotransmitters such as acetylcholine (ACh), 5-HT and dopamine, and 5-HT$_4$ agonists may improve memory, cognitive and learning functions.[16] It is therefore possible that 5-HT$_4$ antagonists which access the CNS would have undesirable side effects.

Another problem in drug development is a possible QT prolongation due to human ether-a-go-go-related gene (hERG) potassium channel binding in the heart. Incorporation of a carboxylic acid group on benzamidine compounds with high hERG binding reduced this binding dramatically.[19] Since incorporating a carboxylic acid group in a 5-HT$_4$ antagonist molecule would thus potentially both reduce CNS penetration due to reduced lipophilicity and also minimise hERG binding, we decided to test whether such 5-HT$_4$ antagonists could be made without compromising 5-HT$_4$ receptor binding affinity, antagonist activity and selectivity.

Adhering to the structural requirements of 5-HT$_4$ antagonists (an aromatic ring, at least one hydrogen bond acceptor, a basic nitrogen and a hydrophobic group, see pharmacophore model in Fig. 1),[20] we synthesized 27 indole-derivatives and tested their binding affinity and antagonist activity at the 5-HT$_4$ receptor. Although the prodrug esters show better potencies than their corresponding free acids, the results demonstrate that this approach is feasible and may be useful in development of antagonists targeting 5-HT$_4$ receptors outside the CNS.

2. Chemistry

All of the new derivatives synthesized and tested had minor differences from potent and well known 5-HT$_4$ antagonists. Figure 2 shows known 5-HT$_4$ antagonists.

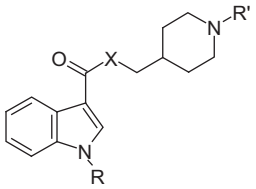
The new amphoteric compounds **4**, **8**, **26**–**32** and the prodrugs **7**, **9**–**25** are shown in Tables 1 and 2.

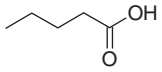
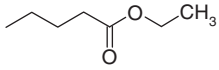
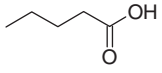
The target compound **4** was prepared according to Scheme 1. The piperidine intermediate **2** was prepared by N-alkylation of 4-piperidinemethanol with 2,2,2-trichloroethyl 4-bromobutyrate in acetone at reflux in the presence of potassium carbonate. The indole-3-carboxoyl chloride was prepared from the corresponding acid with oxalyl chloride in tetrahydrofuran according to Gaster et al.[21] The coupling of intermediate **2** with indole-3-carboxoyl chloride was achieved in a mixture of dichloromethane and tetrahydrofuran in the presence of triethylamine at room temperature. The trichloroethyl ester **3** was isolated after purification by column chromatography in 26% yield. Deprotection to the free acid **4** was achieved with zinc in a mixture of aqueous phosphate buffer and tetrahydrofuran in 84% yield.

Target compounds **7** and **8** were prepared according to Scheme 2. 1-Benzyl-4-aminomethylpiperidine[22] was coupled with indole-3-carboxoyl chloride in a mixture of dichloromethane and tetrahydrofuran in the presence of triethylamine at room temperature. The intermediate **5** was isolated after purification by column chromatography in 29% yield. The benzyl protected amine **5** was deprotected to intermediate **6** with hydrazine monohydrate in ethanol at reflux in 93% yield. The indole amide **7** was prepared by N-alkylation of **6** with ethyl 4-bromobutyrate in acetone at reflux in the presence of potassium carbonate in 75% yield. The free acid **8** was prepared by hydrolysis of the ethyl ester **7** with potassium hydroxide in a mixture of water and methanol at reflux in 61% yield.

Synthesis of target compounds **9** and **26** are outlined in Scheme 3. The prodrug esters **10**–**23** (Table 2) were all prepared by N-alkylation of 3,4-dihydro-*N*-[[4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide[23] (**6**) with different halo alkyl esters in acetone at reflux in the presence of potassium carbonate. The compounds were purified with column chromatography in 50–80% yield.

Table 1
Structures, pK_i-values from binding and pK_b-values from adenylyl cyclase assay of GR113808 and compounds **4**, **7** and **8**



Compound	X	R	R'	pK_i ± SEM[a]	pK_b ± SEM[b]
GR113808	O	Me		10.13 ± 0.07	9.24 ± 0.06
4	O	H		9.09 ± 0.16	8.16 ± 0.08
7	NH	H		6.97 ± 0.05	5.84 ± 0.21
8	NH	H		5.44 ± 0.01	4.62 ± 0.26

[a] n = 3, except for GR113808 where n = 5.
[b] n = 2, except for GR113808, where n = 12. Where n = 2, the values are given as pK_b ± half-range.

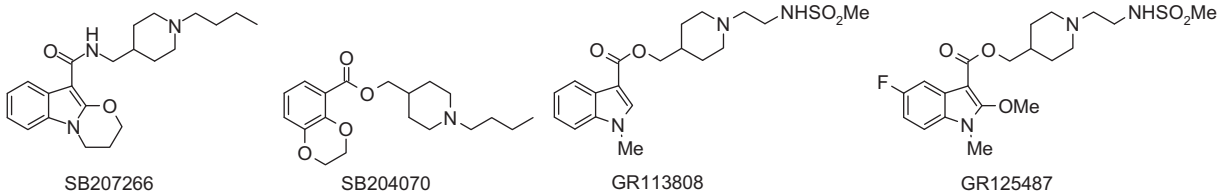
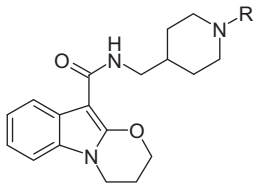


Figure 2. Known 5-HT$_4$ receptor antagonists.

Table 2
Structures, pK_i-values from competition of [^{3}H]GR113808 binding and pK_b-values from antagonism of 5-HT-stimulated adenylyl cyclase activity of SB207266 and compounds **9**–**32**



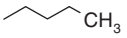
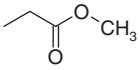
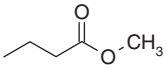
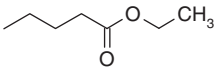
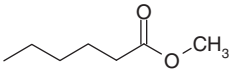
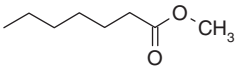
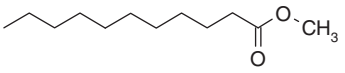
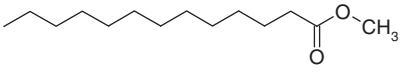
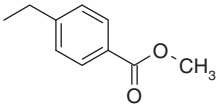
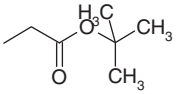
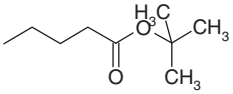
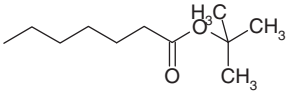
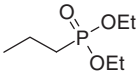
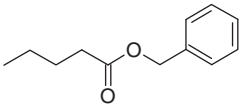
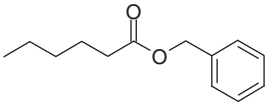
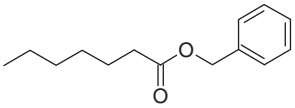
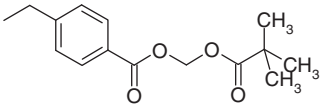
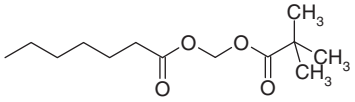
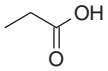
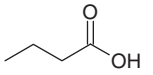
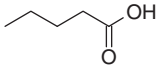
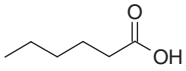
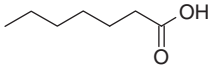
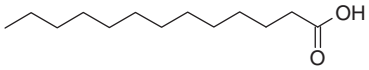
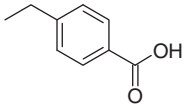
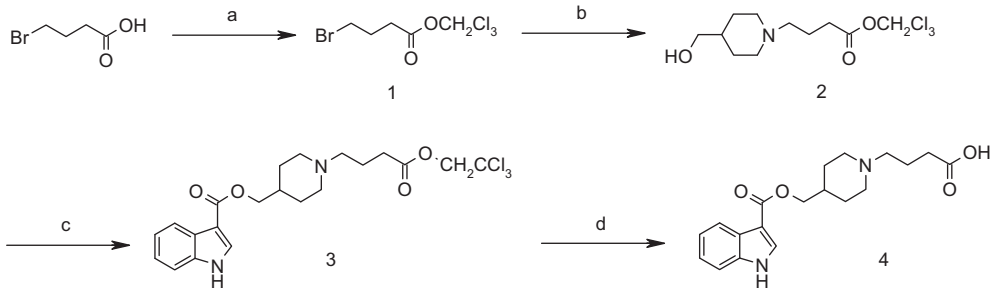
Compound	R	pK_i ± SEM[a]	pK_b ± SEM[b]
SB207266		10.28 ± 0.15	9.26 ± 0.08
9		–	–
10		–	–
11		9.99 ± 0.17	9.43 ± 0.18
12		–	–
13		9.97 ± 0.16	9.13 ± 0.12
14		9.71 ± 0.11	9.33 ± 0.06
15		9.47 ± 0.16	9.89 ± 0.12
16		–	–
17		9.12 ± 0.05	9.01 ± 0.20
18		9.63 ± 0.11	9.47 ± 0.29
19		10.27 ± 0.14	9.89 ± 0.16
20		8.90 ± 0.10	8.64 ± 0.18
21		10.37 ± 0.20	9.85 ± 0.15
22		11.84 ± 0.52	10.18 ± 0.20
23		9.85 ± 0.25	10.16 ± 0.22
24		9.47 ± 0.43	9.07 ± 0.18

Table 2 (*continued*)

Compound	R	pK_i ± SEM[a]	pK_b ± SEM[b]
25		10.19 ± 0.25	9.83 ± 0.28
26		8.55 ± 0.04	7.91 ± 0.25
27		8.95 ± 0.16	8.08 ± 0.19
28		8.70 ± 0.08	7.96 ± 0.02
29		8.69 ± 0.13	8.10 ± 0.13
30		8.81 ± 0.15	7.94 ± 0.06
31		8.61 ± 0.02	8.97 ± 0.18
32		8.30 ± 0.15	7.25 ± 0.07

[a] $n = 3–6$.
[b] $n = 2–3$, except for SB207266 where $n = 13$. Where $n = 2$, the values are given as pK_b ± half-range.



Scheme 1. Reagents and conditions: (a) $HOCH_2CCl_3$, *p*TsOH, toluene, reflux; (b) 4-piperidinemethanol, K_2CO_3, acetone, reflux; (c) indole-3-carboxoyl chloride, NEt_3, tetrahydrofuran/dichloromethane, room temperature; (d) zinc dust, 1 M KH_2PO_3/tetrahydrofuran, room temperature.

The amphoteric drugs **26**–**32** (Table 2) were prepared by hydrolysis of the corresponding esters **10**–**23** with potassium hydroxide in a mixture of water and methanol in 60–70% yield.

The double esters **24** and **25** were prepared by alkylating the free acids **32** and **30**, respectively, with chloromethyl pivalate in dimethylformamide at reflux in the presence of caesium carbonate as outlined in Scheme 4. The compounds were purified with column chromatography in 59–66% yield.

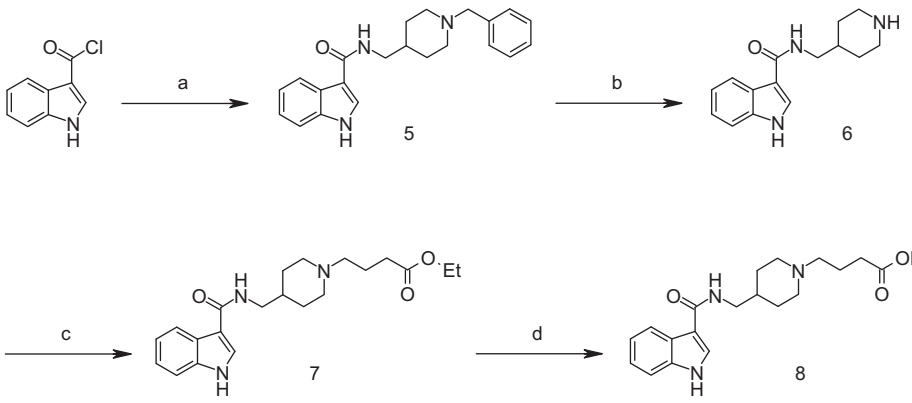
3. Pharmacology

Using membranes from HEK293 cells stably expressing the human 5-$HT_{4(b)}$ receptor,[24] competitive binding and concentration-dependent inhibition of 5-HT-stimulated adenylyl cyclase activity was studied for GR113808, SB207266 and compounds **4**, **7**, **8**, **11**, **13**–**15** and **17**–**32** to determine binding affinities and 5-HT_4 antagonist properties of the compounds. pK_i-values ($-$Log K_i) from competition of [^{3}H]GR113808 binding and pK_b-values ($-$Log K_b) from antagonism of 5-HT-stimulated adenylyl cyclase (AC) activity of GR113808, SB207266 and compounds **4** and **7**–**32** are summarized in Tables 1 and 2.

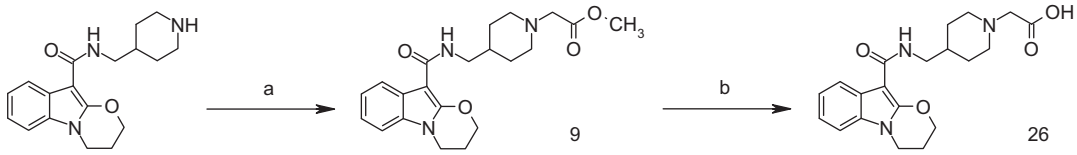
4. Results and discussion

The results from the binding experiments (Tables 1 and 2) demonstrate that many of the amphoteric compounds and their corresponding ester prodrugs possess moderate to high affinity for the 5-HT_4 receptor.

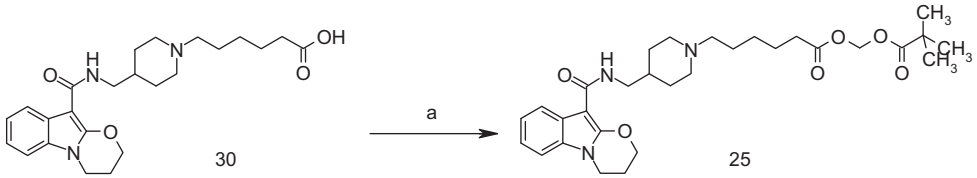
The amphoteric indole-3-carbonyl ester **4** has excellent receptor binding (pK_i 9.09 ± 0.16, pK_b 8.16 ± 0.08). The binding data for the corresponding amide **8** reveals much lower affinity measured in both binding and AC assays (pK_i 5.44 ± 0.01, pK_b 4.62 ± 0.26). Switching from an ester to an amide seems to have a detrimental effect on receptor binding (Fig. 3). This has also been shown for tropisetron, a ligand with affinity for both 5-HT_3 and 5-HT_4 receptors, where replacement of the ester with an amide linkage decreased the 5-HT_4 receptor affinity.[25] Other reports suggest that 5-HT_4 naphthalene derivatives with an ester linkage display higher



Scheme 2. Reagents and conditions: (a) 1-benzyl-4-aminomethylpiperidine, NEt_3, dichloromethane, room temperature; (b) $NH_2NH_2{\cdot}H_2O$, Pd–C 10%, EtOH, reflux; (c) ethyl 4-bromobutyrate, K_2CO_3, acetone, reflux; (d) KOH, H_2O/MeOH, reflux.



Scheme 3. Reagents and conditions: (a) methyl 2-bromoacetate, K_2CO_3, acetone, reflux; (b) KOH, H_2O/MeOH, reflux.



Scheme 4. Reagents and conditions: (a) chloromethyl pivalate, Cs_2CO_3, DMF, reflux.

affinities than their corresponding amides, and that this might be a result of conformational differences.[26] However, due to the possible lability of ester compounds in vivo, amide was preferred as linker between the aromatic indole and piperidine ring systems for compounds **9**–**32**.

The introduction of a [3,2-*a*] oxazino ring on indole amides **7** (pK_i 6.97 ± 0.05, pK_b 5.84 ± 0.21) and **8** (pK_i 5.44 ± 0.01, pK_b 4.62 ± 0.26) increases receptor binding. The corresponding [3,2-*a*] oxazino amides **11** (pK_i 9.99 ± 0.17, pK_b 9.43 ± 0.18) and **28** (pK_i 8.70 ± 0.08, pK_b 7.96 ± 0.02) show considerably higher binding affinity (Table 2, Fig. 4). These results might be explained by the pharmacophore model, where both the carbonyl group and the oxygen in the oxazino ring can act as hydrogen bond acceptors, but also because of a more favourable planar conformation for the oxazino derivatives.

The binding and AC data for the amphoteric compounds **26**–**31** reveal that they are promising candidates as antagonists for the 5-HT_4 receptor. Binding affinities determined from binding and AC assays range from pK_i 8.55 ± 0.04 to 8.95 ± 0.16 and pK_b 7.91 ± 0.25 to 8.97 ± 0.18, respectively. The pharmacophore model predicts a requirement of a hydrophobic group attached to the basic nitrogen in the piperidine ring. We therefore predicted increasing affinity to the receptor with increasing length and hydrophobicity of the alkyl chain attaching the hydrophilic group. However, the binding affinities do not seem to increase or decrease with increasing length of the side chain. The same lack of trend can be seen for the corresponding carboxylic esters **11** and **13**–**15**. Further molecular modelling studies are needed to investigate the role of the hydrophobic pocket in receptor–ligand interaction of the new ligands. Previous studies have shown the importance of the hydrophobic pocket in receptor binding.[27,28] Also the need for voluminous substituents to obtain selectivity for the 5-HT_4 receptor over 5-HT_3 has previously been reported.[29] Further synthetic and receptor binding studies are in progress to evaluate new amphoteric 5-HT_4 antagonists comprising different voluminous substituents with acidic moieties.

Amphoteric compounds in general have low bioavailability.[30] This reduced bioavailability is most likely caused by a lack of lipophilicity. The corresponding ester prodrugs to the amphoteric compounds were therefore prepared and since these compounds are expected to have some biological activity the receptor binding and AC activity were determined. The prodrug esters seem to display better potencies than their corresponding free acids. The pivolate ester **25** (pK_i 10.19 ± 0.25, pK_b 9.83 ± 0.28), the benzyl ester **23** (pK_i 9.85 ± 0.25, pK_b 10.16 ± 0.22), the *tert*-butyl ester **19** (pK_i 10.27 ± 0.14, pK_b 9.89 ± 0.16) and the methyl ester **13** (pK_i 9.97 ± 0.16, pK_b 9.13 ± 0.12) show better binding affinity than the corresponding free acid **30** (pK_i 8.81 ± 0.15, pK_b 7.94 ± 0.06). The same characteristic is seen for the ethyl ester **11** (pK_i 9.99 ± 0.17, pK_b 9.43 ± 0.18) and the methyl ester **15** (pK_i 9.47 ± 0.16, pK_b 9.89 ± 0.12) compared to their corresponding free acids **28** (pK_i 8.70 ± 0.08, pK_b 7.96 ± 0.02) and **31** (pK_i 8.61 ± 0.02, pK_b

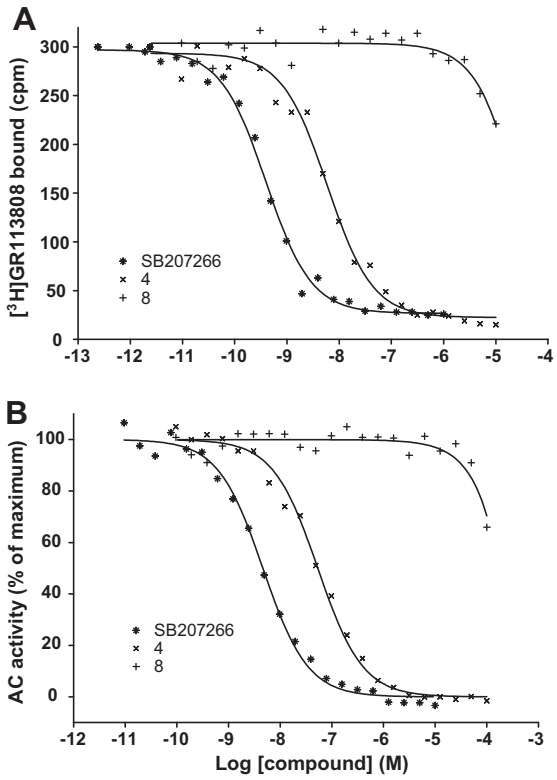


Figure 3. Competition binding curves (0.3 nM [^{3}H]GR113808 present) (panel A) and concentration–response curves of the inhibition of 5-HT (1 μM)-stimulated adenylyl cyclase (AC) activity (panel B) with an ester (ligand **4**) and the corresponding amide (ligand **8**). The data shown in panels A and B are from one representative experiment. The basal and the maximum 5-HT (1 μM)-stimulated AC activity in panel B was 25 and 148 pmol cAMP/mg protein/min, respectively.

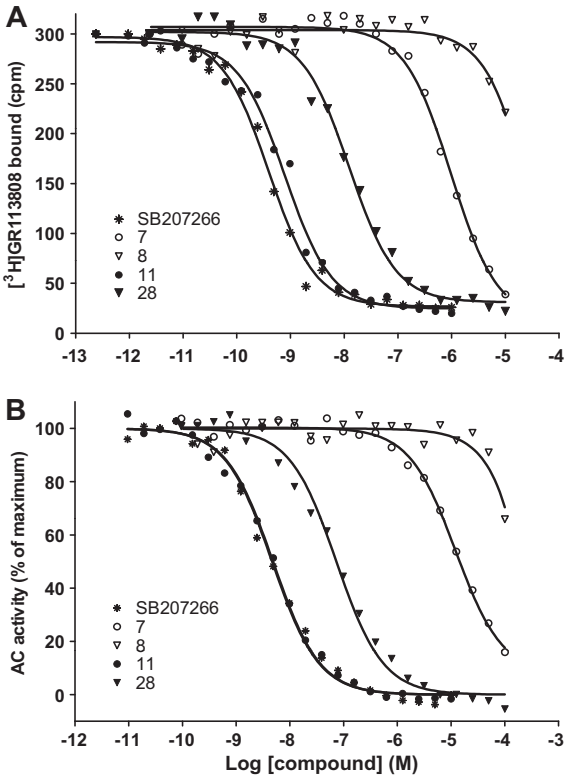


Figure 4. Competition binding curves (0.3 nM [^{3}H]GR113808 present) (panel A) and concentration–response curves of the inhibition of 5-HT (1 μM)-stimulated adenylyl cyclase (AC) activity (panel B) of ligands **7** and **8** not containing the oxazino ring in indole 2 position, and their respective corresponding drugs containing the oxazino ring, ligands **11** and **28**. Panel A: one representative experiment, panel B: data compiled from three representative experiments. The basal and the maximum 5-HT (1 μM)-stimulated AC activities in panel B was 21–27 and 127–149 pmol cAMP/mg protein/min, respectively.

8.97 ± 0.18), respectively. This seems to be a trend as all the esters show higher pK_i and pK_b values than their corresponding free acids (where this was tested) (Table 2, Fig. 5).

The amphoteric compounds **26**–**32** may be derivatized to prodrug esters to obtain sufficient bioavailability. These prodrug compounds could be either metabolized by first pass metabolism in the liver or be hydrolysed in plasma to free acid. Further investigations are necessary to address the degree of liver metabolism of the new ligands, but the plasma stability of the corresponding prodrugs of compound **28** were evaluated by incubating the ethyl ester **11**, benzyl ester **21** and *tert*-butyl ester **18** in bovine plasma. The concentration of the prodrugs and degradation products were followed by HPLC. The benzyl ester **21** and ethyl ester **11** were fully metabolized to the free acid **28** in bovine plasma after 10 h, and the half-life calculated to be approximately 20 and 30 min, respectively. The *tert*-butyl ester **18** was only metabolized to a minor extent, and the incubation time of 10 h was insufficient to obtain any half-life value. This is in agreement with earlier studies, where *tert*-butyl esters have been shown to be stable against hydrolysis in plasma.[31] However, further in vivo studies of **11**, **21** and **28** are necessary to obtain more precise bioavailability data.

Different methods have been used to predict the ability of a compound to reach the central nervous system. One of the most important factors used to explain the brain penetration is the partition coefficient (P) between octanol and water.[32] For ionizable compounds both the pK_a (acid dissociation constant) values and the partition coefficient is important, and the distribution coefficient between octanol and an aqueous buffer of pH 7.4 ($D_{Oct7.4}$) is therefore commonly used. According to literature, when Log $D_{Oct7.4}$ is smaller than zero, brain penetration is expected to be negligible, whereas compounds with good CNS penetration will have an optimal Log $D_{Oct7.4}$ value of approximately 2.[33] The logarithmic distribution coefficients for amphoteric compounds **26**–**31** were experimentally determined to range from −1.1 to −1.3, indicating low CNS penetration, whereas the distribution coefficient of the corresponding prodrug esters **13**, **24** and SB207266 were determined to 2.2–2.3 indicating potentially good CNS penetration (Table 3).

Cisapride, a partial 5-HT$_4$ agonist used in treatment of gastro-oesophageal reflux, has been withdrawn from the market or is now used with more restricted indications because of prolongation of QT intervals.[34] Due to structural similarities between 5-HT$_4$ agonists and antagonists it was decided to determine the effect of compounds **22** and **29** on hERG (human ether-a-go-go related gene) tail current. Compounds that inhibit hERG current have been shown to prolong the cardiac action potential and hence QT interval in man.[35] The benzyl ester prodrug **22** and the free carboxylic acid **29**, both tested at a concentration of 10 μM, reduced the hERG tail current to 6.6% ± 1.4% and 93.5% ± 2.5%, respectively. Assuming an inhibition curve of the hERG tail current with a Hill coefficient of one this would predict a $-\log IC_{50}$ of 6.15 ± 0.10 and 3.84 ± 0.18 for compounds **22** and **29**, respectively. Despite the small number of test compounds, incorporation of a free carboxylic acid group seems to be important in preventing hERG blockade. This supports data shown earlier by others.[19]

To reduce undesired side effects, it is important that the compounds are selective for the 5-HT$_4$ receptor. The free carboxylic acid compound **30** and the corresponding ester **13** were tested for binding to a panel of other receptors than the 5-HT$_4$ receptor;

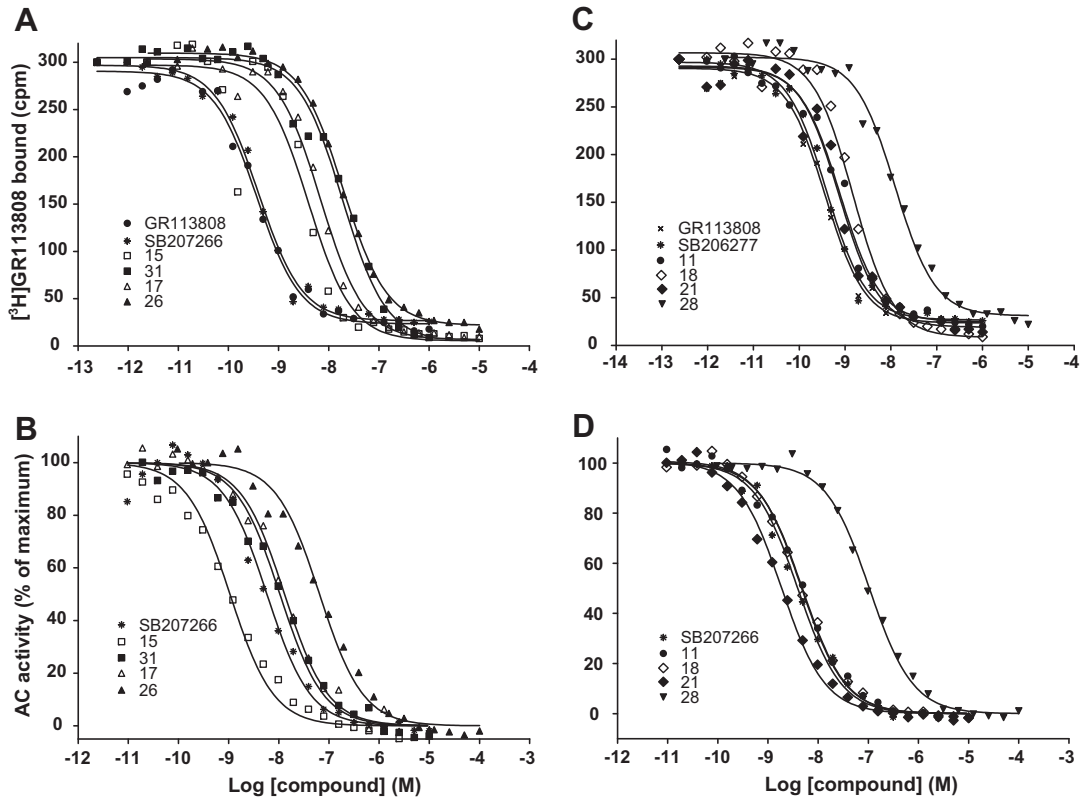


Figure 5. Competition binding curves (0.3 nM [^{3}H]GR113808 present) (panels A and C) and concentration–response curves of the inhibition of 5-HT (1 μM)-stimulated adenylyl cyclase activity (panels B and D) of the esters **15** and **17** and their respective corresponding free acids, ligands **31** and **26** (panels A and B) and of the free acid **28** and its corresponding esters **11**, **18** and **21** (panels C and D). Panels A and C: one representative experiment, panels B and D: data compiled from three and four representative experiments, respectively. The basal AC activities in panels B and D were 17–34 and 21–25 pmol cAMP/mg protein/min and the maximum 5-HT (1 μM)-stimulated AC activities were 90–158 and 125–154 pmol cAMP/mg protein/min, respectively.

the serotonin receptors 5-HT$_{1A,1B,1D,1E,2A,2B,2C,3,5A,6,7}$, the adrenergic receptors $\alpha_{1A,1B,1D}$, $\alpha_{2A,2B,2C}$, $\beta_{1,2,3}$, the dopamine receptors $D_{1,2,3,4,5}$, histamine receptors $H_{1,2,3,4}$, muscarinic acetylcholine receptors $M_{1,2,3,4,5}$, the opioid receptors DOR, MOR and KOR, Sigma 1 and 2, the benzodiazepine binding site, the GABA$_A$ receptor (muscimol site) and the monoamine transporters DAT (dopamine transporter) and SERT (serotonin transporter). The free carboxylic acid compound **30** did not show any significant binding (pK_i >5) to the selected receptors. The corresponding ester **13** showed binding to some of the selected receptors, but with low affinity (summarized in Table 4). Both compounds **13** and **30** show preserved selectivity for the 5-HT$_4$ receptor as the affinity for the 5-HT$_4$ receptor is at least 3 log units higher than for the other receptors tested, for example compound **13** shows a pK_i of 6.5 for the 5-HT$_{2B}$ receptor compared to 9.97 ± 0.16 for the 5-HT$_4$ receptor.

For all the drugs, except ligands **15**, **23** and **31**, the pK_i-values calculated from binding assays are higher than the pK_b-values calculated from adenylyl cyclase assays. These values are usually expected to be similar. Figure 6 shows the correlation between pK_i-values determined in binding assays and pK_b-values from adenylyl cyclase assays. By linear regression the slope was determined to be 0.99 ± 0.08 (r^2 = 0.86). As the slope is close to 1, the difference between pK_i and pK_b seems to be a methodological issue and not represent differences in the ability of the 5-HT$_4$ antagonists to bind to the receptor versus block an agonist activity. Recently, Mikami et al. demonstrated a lower binding affinity for 5-HT$_4$ antagonists when using [^{3}H]5-HT than when using [^{3}H]GR113808 in binding assays at the 5-HT$_{4(d)}$ receptor. The opposite was demonstrated with agonists, whereas partial agonists showed similar binding affinities with both radioligands.[36] If this is also true for the 5-HT$_{4(b)}$ receptor used in this study, this might explain why the 5-HT$_4$ antagonists in this study possess higher pK_i-values (competition with [^{3}H]GR113808) than pK_b-values (competition with 5-HT).

In conclusion, a new series of amphoteric 3,4-dihydro-*N*-[[4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide derivatives **26**–**32** have been prepared and show promising binding affinities and potencies as novel 5-HT$_4$ antagonists. The logarithmic octanol/buffer distribution coefficients of the amphoteric compounds may predict reduced CNS distribution. Additionally the hydrophilic characteristics of the free carboxylic acids might also reduce binding to the hERG potassium channel. In summary the hydrophilic properties may predict reduced probability of CNS side effects as well as reduced probability of hERG channel blockade. To obtain acceptable bioavailability it may be necessary to give the amphoteric derivatives as a prodrug ester **9**–**25**. The results from the binding studies of the corresponding esters showed higher receptor binding than the corresponding free acid. Further studies with amphoteric 5-HT$_4$ antagonists to optimise the pharmacological binding profile and to better clarify bioavailability are in progress.

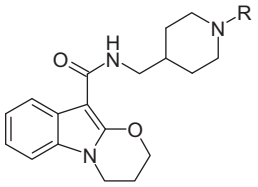
5. Experimental section

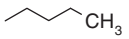
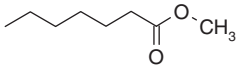
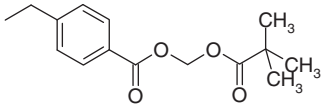
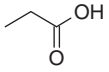
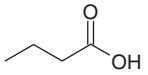
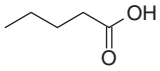
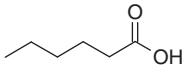
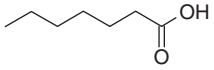
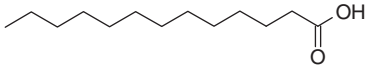
5.1. Pharmacology

5.1.1. Membrane preparation

The development of human embryonic kidney (HEK293) cell lines stably expressing human 5-HT$_{4(b)}$ receptors was described and published previously.[24] The stably transfected HEK293 cells were cultured in 150 mm cell culture dishes. The cells were

Table 3
Log $D_{Oct7.4}$ values of 5-HT_4 antagonists



Compound	R	Log $D_{Oct7.4}$
SB207266		2.2
13		2.2
24		2.3
26		−1.1
27		−1.3
28		−1.2
29		−1.3
30		−1.3
31		[a]

[a] Micelle formation. Log $D_{Oct7.4}$ value not determined.

washed twice with 10 ml ice cold Hanks Balanced Salt Solution (HBSS) and scraped with a rubber scraper in 10 ml HBSS. The cells were collected by centrifugation at 800*g* for 5 min at 4 °C. After centrifugation the cells were resuspended in 1 ml ice cold STE (27% sucrose (w/w), 50 mM Tris–HCl, pH 7.5 at 20 °C, 5 mM EDTA) and homogenised with Ultra-Turrax T8 (IKA Labortechnik) 30 s chilled in ice-water. The homogenate was centrifuged for 20 min at 27,000*g* at 4 °C. The supernatant was discarded and the pellet resuspended in 1 ml ice cold TE (50 mM Tris–HCl pH 7.5 at 20 °C, 1 mM EDTA) using a cold Dounce glass-glass homogeniser, 10 strokes with a tight-fitting pestle, aliquoted and snap-frozen in liquid N_2 and stored at −70 °C until use for assays. The membranes were re-homogenised in a cold Dounce glass-glass homogeniser before each assay. In radioligand binding assays and adenylyl cyclase assays, cell lines expressing the 5-$HT_{4(b)}$ receptor at high and low levels, respectively, were used.[24]

5.1.2. Radioligand binding assay for the 5-HT_4 receptor

The radioligand binding assays were performed in 96-well, round bottom microtiter plates with the total reaction volumes of 50–200 μl, containing the 5-HT_4 specific ligand [^{3}H]GR113808 (0.3 nM) in the presence of 21 different concentrations of cold ligand in the range of 1–100 μM to 9.5×10^{-13}–9.5×10^{-11} M, 50 mM Tris–HCl (pH 7.5 at 20 °C), 1 mM EDTA, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM ascorbic acid, 0.1% BSA, 100 μM GTP and membranes. The plates were incubated at room temperature for 1 h and the membranes harvested onto Multiscreen® FC filter (Millipore) presoaked in 0.3% polyethyleneimine, with a Packard Cell Harvester (Packard Instrument Co.). The membranes were washed 6–8 times with cold (4 °C) washing buffer (50 mM Tris–HCl, pH 7.0 at 20 °C, 2 mM $MgCl_2$). Filters were dried and 20 μl MicroScint scintillation fluid (Packard Instrument Co.) were added to each well. The filters were counted in a Top-Count Liquid scintillation counter (Packard). In radioligand binding assays, a cell line expressing the 5-$HT_{4(b)}$ receptors at high levels (5000 fmol/mg protein) was used. In all experiments, GR113808 or SB207266 were used as controls.

5.1.3. Adenylyl cyclase assay

The adenylyl cyclase activity was measured by determining the conversion of [α-^{32}P]-ATP to [^{32}P]cyclic AMP in membranes from a cell line expressing the h5-$HT_{4(b)}$ receptor. Adenylyl cyclase activity was measured in 10 μl of crude membrane preparations in a final volume of 50 μl in the presence of 0.1 mM [α-^{32}P]-ATP, 1 mM cAMP, 1 mM EDTA, 1 mM [^{3}H]cAMP (ca 10,000 cpm/assay), 4 mM $MgCl_2$, 1 mM IBMX (3-isobutyl-1-methylxanthine), 20 μM GTP and an ATP-regenerating system consisting of 20 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase and 40 U/ml myokinase. Ligands were used in 21 different concentration in the range of 1–100 μM to 9.5×10^{-13}–9.5×10^{-11} M inhibiting the adenylyl cyclase activity stimulated by 1 μM 5-HT. Incubations were at 32 °C for 20 min. The reaction products were separated by sequential chromatography on Dowex 50 cation exchanger and on neutral aluminium oxide (Alumina) columns and cAMP formed quantified as originally described by Salomon et al.[37] with minor modifications.[38] In adenylyl cyclase assay a cell line expressing the 5-$HT_{4(b)}$ receptor at low levels (400 fmol/mg protein) were used. In all experiments, GR113808 or SB207266 were used as controls.

5.1.4. Analysis of binding and adenylyl cyclase data

Binding and adenylyl cyclase data were analysed by non-linear regression using Microsoft® Excel with the Solver add-in, using the below equations.

5.1.4.1. Competitive binding assay. The data were fit to the equation:

$$K_i = (IC_{50})/(1 + (L/K_d))$$

K_i is the affinity constant of the unlabelled competitor for the receptor. IC_{50} is the concentration of unlabelled ligand that inhibits the binding of the radioactive ligand by 50%. L is the concentration of the radiolabelled ligand used. K_d is the affinity constant for the radiolabelled ligand.

5.1.4.2. Activation of adenylyl cyclase. The data were fit to the equation:

$$Y = a + (b - a)x/(c + x)$$

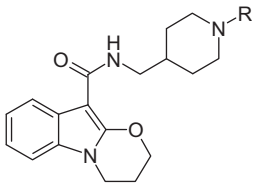
where a is basal adenylyl cyclase activity, b is maximal adenylyl cyclase activity stimulated by the agonist, c is EC_{50} (agonist concentration at 50% of maximal response) and x is the concentration of agonist.

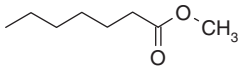
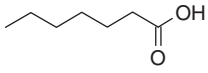
5.1.4.3. Inhibition of 5-HT-evoked adenylyl cyclase stimulation. The data were fit to the equation:

$$Y = a + (b - a)/(1 + x/c)$$

where a is basal adenylyl cyclase activity in the presence of saturating concentrations of antagonist, b is maximal adenylyl cyclase activity stimulated by the agonist in the absence of antagonist, c is the IC_{50} of the antagonist (antagonist concentration when it inhibits 50% of the maximal response of the agonist) and x is the concentration of antagonist.

Table 4
pK_i determined for ligands **13** and **30**



Receptor	pK_i ligand **13** R = 	pK_i ligand **30** R = 	pK_i (reference substance)
Serotonin receptors			
5-HT_{1A}	<5	<5	
5-HT_{1B}	<5	<5	
5-HT_{1D}	5.5	<5	8.5 (ergotamine)
5-HT_{1E}	<5	<5	
5-HT_{2A}	5.2	<5	7.8 (chlorpromazine)
5-HT_{2B}	6.5	<5	8.7 (methysergide)
5-HT_{2C}	<5	<5	
5-HT_3	5.6	<5	8.7 (zacopride)
5-HT_{5A}	<5	<5	
5-HT_6	<5	<5	
5-HT_7	<5	<5	
Adrenergic receptors			
α_{1A}	<5	<5	
α_{1B}	<5	<5	
α_{1D}	<5	<5	
α_{2A}	<5	<5	
α_{2B}	5.9	<5	8.1 (yohimbine)
α_{2C}	<5	<5	
β_1	<5	<5	
β_2	<5	<5	
β_3	<5	<5	
Dopamine receptors			
D_1	<5	<5	
D_2	<5	<5	
D_3	<5	<5	
D_4	<5	<5	
D_5	<5	<5	
Histamine receptors			
H_1	5.9	<5	8.5 (chlorpheniramine)
H_2	5.5	<5	6.5 (cimetidine)
H_3	<5	<5	
H_4	<5	<5	
Muscarinic acetylcholine receptors			
M_1	<5	<5	
M_2	5.6	<5	8.6 (atropine)
M_3	<5	<5	
M_4	<5	<5	
M_5	<5	<5	
Opioid receptors			
DOR	<5	<5	
KOR	<5	<5	
MOR	<5	<5	
Sigma1	6.4	<5	8.7 (haloperidol)
Sigma2	<5	<5	
BDZ site	<5	<5	
$GABA_A$ receptor (muscimol site)	<5	<5	
Monoamine transporters			
DAT	<5	<5	
SERT	<5	<5	

The K_d values of the ligands were calculated using the equation:

$$K_d = a(1/(1 + x/b))$$

Where a is the IC_{50} value for the antagonist, b is the EC_{50} value of the agonist and x is the concentration of agonist.[39]

5.1.5. Protein measurement

The protein concentrations in the membrane preparations were measured with the Micro BCA Protein Assay Reagent Kit (Uptima) using bovine serum albumin (BSA) as standard.

5.1.6. Selectivity testing on other receptors than the 5-HT_4 receptor

Receptor binding profiles and pK_i determinations were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-C (NIMH PDSP). The NIMH PDSP is directed by Bryan L. Roth MD, Ph.D. at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscol at NIMH, Bethesda, MD, USA. For experimental details please refer to the PDSP web site http://pdsp.med.unc.edu/ and click on 'Binding Assay'.

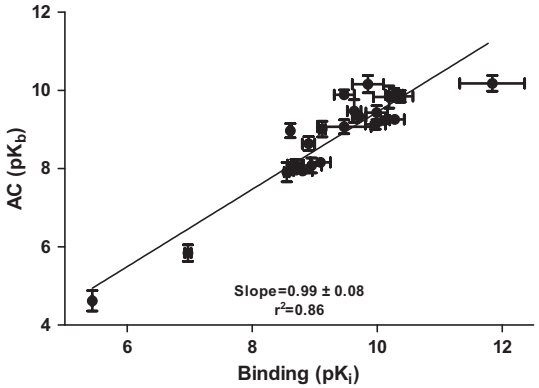


Figure 6. Correlation between the estimated affinities from competition binding (pK_i) and the inhibition of 5-HT-stimulated adenylyl cyclase (AC) activity (pK_b). Linear regression analysis produced a slope of 0.99 ± 0.08 (r^2 = 0.86).

5.1.7. Radiochemicals

[^{3}H]GR113808 (81–84 Ci/mmol), [α-^{32}P]ATP (400–3000 Ci/mmol) and [2,8-^{3}H]cAMP (42 Ci/mmol) were from GE Healthcare (Little Chalfont, Buckinghamshire, UK).

5.1.8. Drugs

5-Hydroxytryptamine hydrochloride (5-HT, serotonin) was from Sigma–Aldrich (St. Louis, MO, USA). GR113808 (1-methyl-1*H*-indole-3-carboxylic acid, [1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl ester) maleate was from Tocris (Avonmouth, UK). The other compounds tested were synthesized by Drug Discovery Laboratory AS (DDL) (Oslo, Norway).

5.1.9. Effect on hERG tail current

The experimental work was carried out at Huntingdon Life Sciences (Cambridgeshire, England). The test compounds were tested in an in vitro system of HEK293 cells stably transfected with hERG ion channel cDNA. Using the patch-clamp technique, peak hERG tail current amplitude was measured prior to and following exposure to a nominal concentration of 10 μM of the compounds, vehicle (0.1% DMSO) and 50 nM terfenadine as control.

5.2. Log $D_{Oct7.4}$ measurements

The 5-HT$_4$ prodrugs and corresponding amphoteric drugs (5 mg of each compound), were added to a mixture of aqueous 1 M Tris buffer with pH 7.4 (1.0 ml) and *n*-octanol (1.0 ml) at ambient temperature. The mixture was vigorously shaken, allowed to reach equilibrium for 3 days at room temperature before 0.1 ml of the aqueous and *n*-octanol solution was removed. The samples were diluted and the ligand concentration in the two solutions determined by HPLC.

5.3. Metabolic stability in bovine plasma

The tested compounds were added to bovine plasma (Sigma–Aldrich) and incubated at 37 °C. The initial concentration was 40 μM. At different time intervals, 0.25 ml of plasma was removed and 10 μl H_3PO_4 added to remove any drug–protein complex. CH_3CN (0.25 ml) was added to the plasma samples and the mixtures centrifuged at 12,000 rpm (mini Spin plus, Eppendorf AG, Hamburg, Germany) for 3 min. The supernatant was removed and the concentration of the compound that remained was determined by HPLC.

5.4. Chemistry

^{1}H NMR spectra were recorded on a Bruker Spectrospin Avance 200 or 300 MHz spectrometer and the corresponding ^{13}C NMR spectra at 50 and 75 MHz, respectively. Chemical shifts are reported in parts per million relative to internal tetramethylsilane. Coupling constants (*J*) are reported in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Electron spray mass spectra were recorded at Vitas AS, Oslo, Norway. GC analyses were carried out on a Shimadzu GC-14A gas chromatograph equipped with a flame-ionization detector and a 30 m × 0.25 μm i.d. glass capillary column coated with poly(dimethylsiloxane), at a flow rate of nitrogen of 40 ml/min. The column temperature was ranged from 80 °C to 200 °C at 8 °C/min. The temperatures of the injector and detector were 250 °C. Analytical thin layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254) with detection with UV-light or iodine. For flash chromatography, Fluka silica gel type 60 (size 200–400 mesh) was used. All solvents and reagents were of analytical or reagent grade and were obtained from commercial sources.

The following known intermediates and reference compounds were synthesized according to literature procedures: 1-benzyl-4-aminomethylpiperidine,[22] methyl 2-(3-chloropropoxy)-indole-3-carboxylate, methyl 3,4-dihydro-2*H*-[1,3]oxazino[3,2-*a*]-indole-10-carboxylate, 3,4-dihydro-*N*-[[1-(phenylmethyl)-4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide, 3,4-dihydro-*N*-[[4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]-indole-10-carboxamide.[23]

Spectral data of all described compounds were consistent with the proposed structures.

5.4.1. 2,2,2-Trichloroethyl 4-bromobutyrate (1)

2,2,2-Trichloroethanol (14.94 g, 0.10 mol) was added to a stirred solution of 4-bromobutyric acid (3.34 g, 20.0 mmol) and *p*-toluenesulfonic acid monohydrate (7.60 g, 40.0 mmol) in toluene (50 ml). The reaction mixture was heated to reflux with a Dean–Stark trap attached for 6 h. Water was removed continuously. The reaction mixture was cooled to room temperature and concentrated in vacuo. The mixture was added CH_2Cl_2 (75 ml) and washed with H_2O (3 × 25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo to leave an oil. The residue was distilled to leave the title compound as a colourless oil (4.77 g, 79.9%) (bp 100 °C at 0.5 mmHg). ^{1}H NMR ($CDCl_3$): δ 4.74 (s, 2H), 3.48 (t, 2H), 2.65 (t, 2H), 2.21–2.13 (m, 2H).

5.4.2. 2,2,2-Trichloroethyl 4-[1-hydroxymethylperidinyl]butyrate (2)

Trichloroethyl ester **1** (4.47 g, 15.0 mmol) was added to a stirred suspension of 4-piperidinemethanol (1.72 g, 15.0 mmol) and K_2CO_3 (4.14 g, 30.0 mmol) in acetone (100 ml). The reaction mixture was heated at reflux for 3 h, cooled to room temperature and filtered. The filtrate was concentrated in vacuo, the residue added CH_2Cl_2 (75 ml) and washed with brine (25 ml) and H_2O (2 × 25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo to leave the title compound as a viscous oil (4.70 g, 94.1%). ^{1}H NMR ($CDCl_3$): δ 4.74 (s, 2H), 3.50 (d, 2H), 2.92 (d, 2H), 2.52–2.35 (m, 4H), 1.97–1.70 (m, 7H), 1.52–1.45 (m, 1H), 1.32–1.23 (m, 2H).

5.4.3. 2,2,2-Trichloroethyl 4-[1*H*-indole-3-carboxylate]-1-methylpiperidinylbutanoate (3)

Oxalyl chloride (1.84 ml, 20.7 mmol) and DMF (one drop) was added to a stirred suspension of indole-3-carboxylic acid (2.90 g, 18.0 mmol) in CH_2Cl_2 (75 ml) and stirred at room temperature

for 2 h, then concentrated in vacuo to leave the acid chloride as a yellow solid. This was dissolved in a mixture of CH_2Cl_2 (30 ml) and THF (10 ml) and added dropwise (30 min) to a stirred solution of trichloroethyl ester **2** (4.98 g, 15.0 mmol) and NEt_3 (1.82 g, 18.0 mmol) in CH_2Cl_2 (30 ml). The reaction mixture was stirred at room temperature overnight, treated with brine (25 ml) and 10% aqueous $NaHCO_3$ solution (25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was separated with flash chromatography (EtOAc) to leave the title compound as a pale yellow solid (1.83 g, 25.6%). Conversion to the hydrochloride salt was effected using ethereal HCl. ^{1}H NMR ($CDCl_3$): δ 9.02 (br s, 1H), 8.22–8.18 (m, 1H), 7.92 (d, 1H), 7.48–7.41 (m, 1H), 7.35–7.28 (m, 2H), 4.77 (s, 2H), 4.24 (d, 2H), 3.03 (d, 2H), 2.59–2.44 (q, 5H), 2.13–1.85 (m, 7H), 1.60–1.43 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 171.7, 165.5, 136.2, 131.5, 125.7, 122.8, 121.7, 121.0, 111.7, 108.0, 94.8, 73.7, 67.9, 57.5, 53.1, 35.4, 31.7, 28.8, 21.8. MS (ES): 477.1 $[M+H]^+$.

5.4.4. 4-[1*H*-Indole-3-carboxylate]-1-methylpiperidinylbutanoic acid (4)

Zn-powder (0.66 g, 10.0 mmol) was added to a stirred solution of trichloroethyl ester **3** (0.48 g, 1.0 mmol) in THF (25 ml) and aqueous 1 M KH_2PO_4 (5 ml). The resulting mixture was stirred at room temperature for 24 h, and then filtered through a pad of kieselguhr and the filtrate evaporated in vacuo. The residue was separated with flash chromatography (EtOAc/MeOH 2:1) to leave the title compound as a white solid (0.29 g, 84.2%). Conversion to the hydrochloride salt was effected using ethereal HCl. ^{1}H NMR (DMSO-d_6): δ 11.98 (s, 1H), 8.08–7.97 (m, 2H), 7.47 (d, 1H), 7.20–7.17 (m, 2H), 4.11 (d, 2H), 2.96 (d, 2H), 2.50–2.37 (m, 4H), 2.05 (t, 2H), 1.77–1.66 (m, 6H), 1.42–1.35 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 171.7, 165.5, 136.2, 131.5, 125.7, 122.8, 121.7, 121.0, 111.7, 108.0, 94.8, 73.7, 67.9, 57.5, 53.1, 35.4, 31.7, 28.8, 21.8. HRSM (TOF MS ES+) for $C_{19}H_{24}N_2O_4$ $[M+H]^+$: calcd: 345.1809; found: 345.1822.

5.4.5. *N*-[1-Benzyl-4-piperidinyl]methyl indole-3-carboxamide (5)

Indole-3-carboxoyl chloride (5.56 g, 30.95 mmol) dissolved in a mixture of CH_2Cl_2 (50 ml) and THF (20 ml) was added dropwise to a solution of 1-benzyl-4-aminomethylpiperidine (6.33 g, 30.95 mmol) and NEt_3 (3.13 g, 30.95 mol) in CH_2Cl_2 (50 ml). The reaction mixture was stirred at room temperature overnight, and then treated with brine (25 ml) and 10% aqueous $NaHCO_3$ solution (25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was separated with flash chromatography (EtOAc) to leave the title compound as a pale yellow solid (3.17 g, 29.6%). ^{1}H NMR ($CDCl_3$): δ 9.96 (s, 1H), 7.97–7.95 (m, 1H), 7.67 (d, J = 2.8 Hz, 1H), 7.44–7.22 (m, 8H), 6.24 (t, J = 5.9 Hz, 1H), 3.51 (s, 2H), 3.40 (t, J = 5.9 Hz, 2H), 2.92 (br d, 2H), 1.98 (t, J = 10.9 Hz, 2H), 1.78–1.67 (m, 3H), 1.44–1.30 (m, 2H).

5.4.6. *N*-[4-Piperidinyl]methyl indole-3-carboxamide (6)

Hydrazine monohydrate (0.36 ml) followed by 10% palladium on activated charcoal (M-type, 0.40 g) was added to a stirred solution of *N*-[1-(benzyl)-4-piperidinyl]methyl]indole-3-carboxamide (1.74 g, 5.0 mmol) in EtOH (20 ml) and heated to reflux for 2 h. The reaction mixture was cooled to room temperature and filtered through a pad of kieselguhr. The filtrate was evaporated in vacuo to leave the title compound as a white solid (1.18 g, 92.7%). ^{1}H NMR (DMSO-d_6): δ 11.56 (br s, 1H), 8.15–8.12 (m, 1H), 8.03 (s, 1H), 7.85 (t, J = 5.7 Hz, 1H), 7.43–7.40 (m, 1H), 7.15–7.08 (m, 2H), 3.12 (t, J = 6.0 Hz, 2H), 2.94–2.90 (m, 2H), 2.55–2.49 (m, 1H), 2.4–2.36 (m, 2H), 1.64–1.60 (m, 3H), 1.06–1.01 (m, 2H).

5.4.7. Ethyl 4-[(1*H*-indole-3-carbonyl)amino]-1-metylpiperidinylbutanoate (7)

Ethyl 4-bromobutyrate (0.81 g, 4.1 mmol) was added to a stirred suspension of *N*-[4-piperidinyl]methyl]indole-3-carboxamide (1.07 g, 4.1 mmol) and K_2CO_3 (2.29 g, 16.5 mmol) in acetone (50 ml) and heated to reflux for 24 h. The mixture was cooled to room temperature and filtered. The filtrate was evaporated in vacuo and the residue added CH_2Cl_2 (75 ml) and washed with H_2O (3×25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was separated with flash chromatography (CH_2Cl_2/MeOH (9: 1) to leave the title compound as a yellow solid (1.15 g, 75.2%). ^{1}H NMR ($CDCl_3$): δ 9.70 (br s, 1H), 8.00–7.95 (m, 1H), 7.78 (d, J = 2.8 Hz, 1H), 7.49–7.44 (m, 1H), 7.30–7.25 (m, 2H), 6.33 (t, J = 5.8 Hz, 1H), 4.14 (q, 2H), 3.40 (t, 2H), 2.97 (d, 2H), 2.44–2.31 (m, 4H), 1.99–1.76 (m, 7H), 1.43–1.24 (m, 5H), ^{13}C NMR (75 MHz, $CDCl_3$):δ 173.4, 165.7, 136.4, 128.1, 124.7, 122.7, 121.4, 119.8, 112.1, 60.3, 57.8, 53.2, 44.8, 36.1, 32.2, 29.7, 21.9, 14.2. HRSM (TOF MS ES+) for $C_{19}H_{24}N_3O_4$ $[M+H]^+$: calcd: 372.2282; found: 372.2294.

5.4.8. General procedure for the alkali hydrolysis of ester to free acid

5.4.8.1. 4-[(1H-Indole-3-carbonyl)amino]-1-metylpiperidinylbutanoic acid (8). Ethyl 4-[(1*H*-indole-3-carbonyl)amino]-1-metylpiperidinylbutanoate (**7**) (0.45 g, 1.20 mmol) was added to a mixture of 2 M aqueous NaOH solution (1.2 ml) and MeOH (5 ml) and heated to reflux for 2 h. The reaction mixture was cooled to room temperature, concentrated in vacuo and dropwise added 10% aqueous HCl to pH 2. The precipitate was filtered off, washed with water and dried in vacuo to leave the title compound as a white crystalline solid (0.21 g, 61.1%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.63 (s, 1H), 8.13 (d, 1H), 8.05 (d, 1H), 7.97 (t, 1H), 7.41 (d, 1H), 7.15–7.05 (m, 2H), 3.14 (t, 2H), 3.02 (d, 2H), 2.50 (t, 2H), 2.26 (t, 2H), 2.17 (t, 2H), 1.75–1.53 (m, 5H), 1.31–1.21 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 174.4, 164.6, 136.0, 127.5, 126.1, 121.6, 120.9, 120.1, 111.7, 110.5, 57.0, 52.2, 43.6, 35.5, 33.4, 28.7, 20.9. HRSM (TOF MS ES+) for $C_{19}H_{25}N_3O_3$ $[M+H]^+$: calcd: 344.1969; found: 344.1989.

5.4.9. General procedure for the synthesis of 3,4-dihydro-*N*-[[4-piperidinyl]methyl]-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide derivatives 9–23

5.4.9.1. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylacetate (9). Methyl bromoacetate (0.50 g, 3.3 mmol) was added to a stirred suspension of *N*-(4-piperidylmethyl) 3,4-dihydro-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carboxamide (1.05 g, 3.0 mmol) and K_2CO_3 (1.65 g, 12.0 mmol) in acetone (30 ml) and heated to reflux for 24 h. The mixture was cooled to room temperature and filtered. The filtrate was evaporated in vacuo and the residue added CH_2Cl_2 (50 ml) and washed with H_2O (3×25 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was separated with flash chromatography (CH_2Cl_2/MeOH (9:1) to leave the title compound as a yellow oil (0.62 g, 54.2%). ^{1}H NMR ($CDCl_3$): δ 8.32 (d, J = 7.5 Hz, 1H), 7.28–7.06 (m, 3H), 6.54 (t, J = 5.9 Hz, 1H), 4.51 (t, J = 5.2 Hz, 2H), 4.06 (t, J = 6.2 Hz, 2H), 3.72 (s, 3H), 3.34 (t, J = 6.2 Hz, 2H), 3.23 (s, 2H), 2.98–2.94 (m, 2H), 2.37–2.30 (m, 2H), 2.22–2.15 (m, 2H), 1.79–1.63 (m, 3H), 1.51–1.39 (m, 2H). ^{13}C NMR $CDCl_3$): δ 170.8, 164.7, 149.1, 131.0, 125.5, 122.0, 120.9, 120.5, 107.4, 89.1, 66.7, 59.7, 53.3, 51.6, 44.2, 38.8, 35.7, 29.7, 21.1. HRSM (TOF MS ES+) for $C_{21}H_{27}N_3O_4$ $[M+H]^+$: calcd: 386.2074; found: 386.2086.

5.4.9.2. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylpropanoate (10). Yield: 0.72 g (60.3%). ^{1}H NMR ($CDCl_3$): δ 8.32 (d, J = 7.5 Hz, 1H),

7.28–7.06 (m, 3H), 6.54 (t, J = 5.9 Hz, 1H), 4.51 (t, J = 5.2 Hz, 2H), 4.06 (t, J = 6.2 Hz, 2H), 3.72 (s, 3H), 3.34 (t, J = 6.2 Hz, 2H), 2.98–2.94 (m, 2H), 2.72 (m, 2H), 2.54 (m, 2H), 2.37–2.30 (m, 2H), 2.22–2.15 (m, 2H), 1.79–1.63 (m, 3H), 1.51–1.39 (m, 2H). ^{13}C NMR $CDCl_3$): δ 173.2, 165.2, 149.6, 131.5, 126.0, 122.5, 121.4, 121.0, 107.8, 89.5, 67.2, 54.0, 53.6, 52.0, 44.6, 39.3, 36.4, 32.2, 30.1, 21.6. HRSM (TOF MS ES+) for $C_{22}H_{29}N_3O_4$ $[M+H]^+$: calcd: 400.2231; found: 400.2244.

5.4.9.3. Ethyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbutanoate (11). Yield: 0.84 g (65.7%). ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 7.5 Hz, 1H), 7.30–7.09 (m, 3H), 6.58 (t, J = 5.9 Hz, 1H), 4.55 (t, J = 5.2 Hz, 2H), 4.20–4.07 (m, 4H), 3.35 (t, J = 6.1 Hz, 2H), 3.07–3.01 (m, 2H), 2.47–2.32 (m, 6H), 2.09–1.50 (m, 10H), 1.27 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 173.2, 164.7, 149.2, 131.0, 125.5, 122.0, 120.9, 120.6, 107.4, 89.0, 66.8, 60.2, 57.6, 53.2, 44.1, 38.9, 35.9, 32.1, 29.4, 21.7, 21.1, 14.1. HRSM (TOF MS ES+) for $C_{24}H_{33}N_3O_4$ $[M+H]^+$: calcd: 428.2544; found: 428.2564. Conversion to the hydrochloride salt was effected with ethereal HCl. The precipitate was collected and recrystallized from acetone to leave the HCl salt as a white crystalline solid.

5.4.9.4. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylpentanoate (12). Yield: 0.79 g (62.3%). ^{1}H NMR ($CDCl_3$): δ 8.31 (d, J = 7.5 Hz, 1H), 7.28–7.08 (m, 3H), 6.57 (t, J = 5.9 Hz, 1H), 4.54 (t, J = 5.2 Hz, 2H), 4.09 (t, J = 6.1 Hz, 2H), 3.67 (s, 3H), 3.34 (t, J = 6.1 Hz, 2H), 3.08–3.04 (m, 2H), 2.49–2.44 (m, 2H), 2.39–2.32 (m, 4H), 2.15–2.08 (m, 2H), 1.84–1.49 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 173.8, 164.8, 149.2, 131.0, 125.5, 122.0, 120.8, 120.6, 107.4, 89.0, 66.8, 58.0, 53.2, 51.4, 44.0, 38.9, 35.6, 33.6, 29.1, 25.6, 22.7, 21.1. HRSM (TOF MS ES+) for $C_{24}H_{33}N_3O_4$ $[M+H]^+$: calcd: 428.2544; found: 428.2560.

5.4.9.5. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylhexanoate (13). Yield: 1.11 g (84.5%). ^{1}H NMR ($CDCl_3$): δ 8.27 (d, J = 7.5 Hz, 1H), 7.23–7.09 (m, 3H), 6.52 (t, J = 5.9 Hz, 1H), 4.49 (t, J = 5.1 Hz, 2H), 4.03 (t, J = 6.2 Hz, 2H), 3.62 (s, 3H), 3.30 (t, J = 6.2 Hz, 2H), 3.03–2.99 (m, 2H), 2.42–2.25 (m, 6H), 2.06–2.00 (m, 2H), 1.75–1.29 (m, 10H). ^{13}C NMR ($CDCl_3$): δ 174.1, 164.7, 149.1, 131.0, 125.5, 122.0, 121.0, 120.6, 107.4, 89.1, 66.8, 58.7, 53.5, 51.4, 44.3, 38.9, 36.2, 33.9, 29.8, 27.1, 26.4, 24.7, 21.2. HRSM (TOF MS ES+) for $C_{25}H_{35}N_3O_4$ $[M+H]^+$: calcd: 442.2700; found: 442.2699.

5.4.9.6. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinyldecanoate (14). Yield: 1.19 g (80.2%). ^{1}H NMR ($CDCl_3$): δ 8.27 (d, J = 7.5 Hz, 1H), 7.24–7.12 (m, 3H), 6.70 (t, J = 5.9 Hz, 1H), 4.60 (t, J = 5.1 Hz, 2H), 4.13 (t, J = 6.2 Hz, 2H), 3.67 (s, 3H), 3.58–3.51 (m, 2H), 3.35 (t, J = 6.2 Hz, 2H), 2.92–2.87 (m, 2H), 2.68–2.60 (m, 2H), 2.44–2.34 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.13–1.70 (m, 8H), 1.65–1.56 (m, 2H), 1.29 (br s, 10H). ^{13}C NMR ($CDCl_3$): δ 174.2, 165.1, 149.7, 131.1, 125.4, 122.2, 120.8, 120.6, 107.6, 77.2, 67.0, 52.7, 51.4, 43.9, 38.9, 33.9, 33.6, 29.0, 28.9, 28.8, 28.7, 26.7, 24.8, 23.4, 21.1. HRSM (TOF MS ES+) for $C_{29}H_{43}N_3O_4$ $[M+H]^+$: calcd: 498.3326; found: 498.3340. Conversion to the hydrochloride salt was effected using ethereal HCl.

5.4.9.7. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinyldodecanoate (15). Yield: 1.16 g (73.8%). ^{1}H NMR ($CDCl_3$): δ 8.25 (d, J = 7.5 Hz, 1H), 7.17–7.00 (m, 3H), 6.50 (t, J = 5.9 Hz, 1H), 4.45 (t, J = 5.1 Hz, 2H), 3.98 (t, J = 6.2 Hz, 2H), 3.61 (s, 3H), 3.28 (t, J = 6.2 Hz, 2H), 3.01–2.98 (m, 2H), 2.39–2.33 (m, 2H), 2.31–2.22 (m, 4H), 2.06–1.98 (m, 2H), 1.77–1.38 (m, 9H), 1.22 (br s, 14H). ^{13}C NMR ($CDCl_3$): δ 174.2, 164.7, 149.2, 131.0, 125.4, 121.9, 120.7, 120.5, 107.4, 88.9, 66.7, 58.7, 53.2, 51.3, 50.4, 44.0, 38.8, 35.8, 34.0, 29.4, 29.3, 29.2, 29.1, 29.0, 27.4, 26.2, 24.8, 21.0. HRSM (TOF MS ES+) for $C_{31}H_{47}N_3O_4$ $[M+H]^+$: calcd: 526.3639; found: 526.3639.

5.4.9.8. Methyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbenzoate (16). Yield: 1.04 g (75.1%). ^{1}H NMR ($CDCl_3$): δ 8.26 (d, J = 7.5 Hz, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.1 Hz, 2H), 7.16–7.03 (m, 3H), 6.49 (t, J = 5.9 Hz, 1H), 4.48 (t, J = 5.1 Hz, 2H), 4.03 (t, J = 6.2 Hz, 2H), 3.89 (s, 3H), 3.61 (s, 2H), 3.29 (t, J = 6.2 Hz, 2H), 2.94–2.90 (m, 2H), 2.31–2.28 (m, 2H), 2.14–2.10 (m, 2H), 1.76–1.72 (m, 3H), 1.25–1.19 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 166.8, 164.7, 149.2, 131.0, 129.5, 129.3, 125.5, 122.0, 120.8, 120.6, 107.5, 89.0, 66.8, 62.3, 53.2, 52.4, 52.0, 44.0, 38.8, 35.7, 29.4, 21.1. HRSM (TOF MS ES+) for $C_{27}H_{31}N_3O_4$ $[M+H]^+$: calcd: 462.2387; found: 462.2385.

5.4.9.9. t-Butyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylacetate (17). Yield: 0.89 g (69.6%). ^{1}H NMR ($CDCl_3$): δ 8.32 (dd, 1H), 7.21–7.05 (m, 3H), 6.53 (t, 1H), 4.50 (t, 2H), 4.04 (t, 2H), 3.33 (t, 2H), 3.11 (s, 2H), 2.96 (br d, 2H), 2.34–2.31 (m, 2H), 2.21–2.12 (m, 2H), 1.77–1.49 (m, 3H), 1.46–1.40 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 169.8, 164.7, 149.1, 131.0, 125.5, 121.9, 120.9, 120.5, 107.4, 89.1, 80.8, 66.7, 60.3, 53.2, 44.2, 38.8, 35.9, 29.9, 28.0, 21.1. HRSM (TOF MS ES+) for $C_{24}H_{33}N_3O_4$ $[M+H]^+$: calcd: 428.2544; found: 428.2556.

5.4.9.10. t-Butyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbutanoate (18). Yield: 0.96 g (70.8%). ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 7.5 Hz, 1H), 7.24–7.10 (m, 3H), 6.57 (t, J = 5.9 Hz, 1H), 4.55 (t, J = 5.1 Hz, 2H), 4.12 (t, J = 6.2 Hz, 2H), 3.35 (t, J = 6.2 Hz, 2H), 3.05–3.01 (m, 2H), 2.45–2.35 (m, 4H), 2.26 (t, J = 7.3 Hz, 2H), 2.12–2.04 (m, 2H), 1.87–1.79 (m, 5H), 1.48–1.37 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.5, 164.7, 149.2, 130.9, 125.4, 121.8, 120.7, 120.4, 88.8, 80.1, 66.7, 57.7, 53.2, 44.1, 38.7, 35.9, 33.2, 29.4, 27.9, 27.8, 21.8, 21.0. HRSM (TOF MS ES+) for $C_{26}H_{37}N_3O_4$ $[M+H]^+$: calcd: 456.2857; found: 456.2855.

5.4.9.11. t-Butyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylhexanoate (19). Yield: 0.99 g (68.3%). ^{1}H NMR ($CDCl_3$): δ 8.23 (dd, 1H), 7.19–7.03 (m, 3H), 6.57 (t, J = 6.2 Hz, 1H), 4.51 (t, J = 5.1 Hz, 2H), 4.05 (t, J = 6.2 Hz, 2H), 3.33–3.17 (m, 4H), 2.64–2.54 (m, 2H), 2.33–2.28 (m, 4H), 2.16 (t, J = 7.2 Hz, 2H), 1.83–1.51 (m, 9H), 1.39–1.28 (m, 11H). ^{13}C NMR ($CDCl_3$): δ 172.77, 164.92, 149.47, 131.08, 125.45, 122.06, 120.70, 107.56, 88.82, 80.08, 77.20, 66.93, 57.76, 52.71, 43.61, 38.91, 35.15, 34.69, 28.03, 27.81, 26.55, 24.70, 24.51, 21.13. HRSM (TOF MS ES+) for $C_{28}H_{41}N_3O_4$ $[M+H]^+$: calcd: 484.3170; found: 484.3163.

5.4.9.12. Diethyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylethylphosphonate (20). Yield: 1.00 g (70.4%). ^{1}H NMR ($CDCl_3$): δ 8.30 (d, J = 7.5 Hz, 1H), 7.23–7.10 (m, 3H), 6.59 (t, J = 5.9 Hz, 1H), 4.56 (t, J = 5.1 Hz, 2H), 4.17–4.06 (m, 6H), 3.35 (t, J = 5.9 Hz, 2H), 3.09 (br d, 2H), 2.86–2.78 (m, 2H), 2.39–2.33 (p, J = 5.5 Hz, 2H), 2.27–2.08 (m, 4H), 1.88–1.60 (m, 3H), 1.58–1.50 (m, 2H), 1.33 (t, J = 7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 163.5, 149.8, 131.5, 125.9, 122.5, 121.3, 121.1, 107.9, 89.4, 67.3, 62.4, 62.3, 53.2, 52.1, 44.3, 39.4, 35.9, 29.3, 21.6, 16.9, 16.8. HRSM (TOF MS ES+) for $C_{24}H_{36}N_3O_5P$ $[M+H]^+$: calcd: 478.2465; found: 478.2462.

5.4.9.13. Benzyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbutanoate (21). Yield: 1.06 g (72.8%). ^{1}H NMR ($CDCl_3$): δ 8.29 (dd, 1H), 7.34–7.29 (m, 5H), 7.18–7.06 (m, 3H), 6.53 (t, J = 5.9 Hz, 1H), 5.08 (s, 2H), 4.49 (t, J = 5.1 Hz, 2H), 4.04 (t, J = 6.2 Hz, 2H), 3.29 (t, J = 6.2 Hz, 2H), 2.96 (br d, 2H), 2.42–2.26 (m, 6H), 2.04–1.96 (m, 2H), 1.91–1.84 (m,

2H), 1.77–1.55 (m, 3H), 1.44–1.37 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 173.1, 164.7, 149.2, 135.8, 131.0, 128.5, 128.1, 125.5, 122.0, 120.8, 120.6, 107.4, 89.0, 66.8, 66.1, 57.6, 53.2, 44.1, 38.8, 35.9, 32.1, 29.4, 21.7, 21.1. HRSM (TOF MS ES+) for $C_{29}H_{35}N_3O_4$ $[M+H]^+$: calcd: 490.2700; found: 490.2694.

5.4.9.14. Benzyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylpentanoate (22). Yield: 1.05 g (69.9%). ^{1}H NMR ($CDCl_3$): δ 8.26 (d, J = 7.5 Hz, 1H), 7.33–7.28 (m, 5H), 7.20–7.05 (m, 3H), 6.55 (t, J = 5.9 Hz, 1H), 5.07 (s, 2H), 4.51 (t, J = 5.1 Hz, 2H), 4.06 (t, J = 6.2 Hz, 2H), 3.30 (t, J = 6.2 Hz, 2H), 3.10 (br d, 2H), 2.52–2.47 (m, 2H), 2.38–2.28 (m, 4H), 2.19 (t, 2H), 1.83–1.80 (m, 3H), 1.65–1.56 (m, 6H). HRSM (TOF MS ES+) for $C_{30}H_{37}N_3O_4$ $[M+H]^+$: calcd: 504.2857; found: 504.2848.

5.4.9.15. Benzyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylhexanoate (23). Yield: 1.09 g (70.5%). ^{1}H NMR ($CDCl_3$): δ 8.25 (d, J = 7.5 Hz, 1H), 7.33–7.27 (m, 5H), 7.19–7.06 (m, 3H), 6.56 (t, J = 5.9 Hz, 1H), 5.06 (s, 2H), 4.49 (t, J = 5.1 Hz, 2H), 4.03 (t, J = 6.2 Hz, 2H), 3.29 (t, J = 6.2 Hz, 2H), 3.12 (br d, 2H), 2.52–2.47 (m, 2H), 2.34–2.21 (m, 6H), 1.83–1.80 (m, 3H), 1.65–1.57 (m, 6H), 1.33–1.26 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 173.24, 164.88, 149.37, 135.88, 131.02, 128.47, 128.11, 125.43, 122.04, 120.72, 120.62, 107.52, 88.83, 66.85, 66.06, 57.93, 52.91, 43.79, 38.87, 35.04, 33.92, 28.31, 26.66, 25.08, 24.44, 21.09. HRSM (TOF MS ES+) for $C_{31}H_{39}N_3O_4$ $[M+H]^+$: calcd: 518.3013; found: 518.3010.

5.4.9.16. Pivaloylmethyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbenzoate (24). NaI (catalytic amount) was added to a stirred suspension of 4-[(3,4-dihydro-2*H*-[1,3]oxazino[3,2-*a*]indole-10-carbonyl)-amino]-1-methylpiperidinylbutanoic acid (**28**) (0.63 g, 1.30 mmol) and Cs_2CO_3 (1.69 g, 5.20 mmol) in DMF (5 ml) and stirred at room temperature for 0.5 h. Chloromethyl pivalate (0.23 g, 1.56 mmol) was added to the reaction mixture and heated to reflux for 12 h, then cooled to room temperature and filtered. The filtrate was concentrated in vacuo and the residue separated with flash chromatography (CH_2Cl_2/MeOH, 9:1) to leave the title compound as an oil. Yield: 0.43 g (59.3%). Conversion to the hydrochloride salt was effected using ethereal HCl. ^{1}H NMR ($CDCl_3$): δ 8.33 (d, J = 7.5 Hz, 1H), 8.02 (d, J = 7.7 Hz, 2H), 7.48 (br s, 2H), 7.25–7.10 (m, 3H), 6.56 (t, J = 5.9 Hz, 1H), 6.00 (s, 2H), 4.54 (t, J = 5.1 Hz, 2H), 4.12 (t, J = 6.2 Hz, 2H), 3.60 (br s, 2H), 3.35 (t, J = 6.2 Hz, 2H), 2.96–2.89 (m, 2H), 2.41–2.35 (m, 2H), 2.18–1.40 (m, 7H), 1.24 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 166.8, 164.7, 149.2, 131.0, 129.5, 129.3, 125.5, 122.0, 120.8, 120.6, 107.5, 89.0, 66.8, 62.3, 53.2, 52.4, 52.0, 44.0, 38.8, 35.7, 29.4, 21.1. HRSM (TOF MS ES+) for $C_{32}H_{39}N_3O_6$ $[M+H]^+$: calcd: 562.2912; found: 562.2902.

5.4.9.17. Pivaloylmethyl 4-[(3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylhexanoate (25) was prepared in a manner similar to compound 24. Yield: 0.47 g (66.3%). ^{1}H NMR ($CDCl_3$): δ 8.23 (d, J = 7.5 Hz, 1H), 7.17–7.01 (m, 3H), 6.53 (t, J = 5.9 Hz, 1H), 5.69 (s, 1H), 4.46 (t, J = 5.1 Hz, 2H), 3.98 (t, J = 6.2 Hz, 2H), 3.28 (t, J = 6.2 Hz, 2H), 3.09 (br d, 2H), 2.51–2.46 (m, 2H), 2.33–2.15 (m, 6H), 1.81–1.53 (m, 9H), 1.31–1.26 (m, 2H), 1.16 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 176.9, 171.8, 164.7, 149.2, 130.9, 125.3, 121.8, 120.5, 120.4, 107.4, 88.6, 79.1, 66.7, 57.7, 52.7, 43.6, 38.7, 38.5, 35.1, 33.5, 28.3, 26.7, 26.4, 25.0, 24.0, 20.9. HRSM (TOF MS ES+) for $C_{30}H_{43}N_3O_6$ $[M+H]^+$: calcd: 542.3225; found: 542.3227. Conversion to the hydrochloride salt was effected using ethereal HCl.

Compounds **26**–**32** were prepared in a manner similar to compound **8**.

5.4.9.18. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylacetic acid (26). Yield: (0.29 g, 63.4%). ^{1}H NMR (CD_3OD): δ 8.09–8.06 (m, 1H), 7.25–7.09 (m, 4H), 4.60 (t, J = 5.1 Hz, 2H), 4.15 (t, J = 6.1 Hz, 2H), 3.65–3.59 (m, 4H), 3.39–3.35 (m, 2H), 2.99 (t, 2H), 2.40–2.33 (m, 2H), 2.04–1.80 (m, 4H), 1.70–1.57 (m, 2H). ^{13}C NMR (CD_3OD): δ 169.7, 167.6, 152.0, 132.9, 126.6, 122.8, 121.8, 120.9, 109.2, 89.0, 68.5, 54.0, 44.0, 40.1, 35.5, 28.0, 22.2. HRSM (TOF MS ES+) for $C_{20}H_{25}N_3O_4$ $[M+H]^+$: calcd: 372.1918; found: 372.1920.

5.4.9.19. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylpropanoic acid (27). Yield: (0.62 g, 67.1%). ^{1}H NMR (DMSO-d_6): δ 10.62 (br s, 1H), 8.08–8.03 (m, 1H), 7.32–7.27 (m, 1H), 7.13–7.06 (m, 2H), 6.96 (t, J = 5.9 Hz, 1H), 4.55 (t, J = 5.1 Hz, 2H), 4.11 (t, J = 6.1 Hz, 2H), 3.34–3.31 (m, 2H), 3.19–3.10 (m, 4H), 2.85–2.76 (m, 4H), 2.27–2.24 (m, 2H), 1.79–1.75 (m, 3H), 1.59–1.51 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 173.2, 164.7, 149.2, 131.0, 125.5, 122.0, 120.9, 120.6, 107.4, 89.0, 66.8, 60.2, 57.6, 53.2, 50.6, 44.1, 38.9, 35.9, 32.1, 29.4, 21.7, 21.1, 14.1. HRSM (TOF MS ES+) for $C_{21}H_{27}N_3O_4$ $[M+H]^+$: calcd: 386.2074; found: 386.2078.

5.4.9.20. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbutanoic acid (28). Yield: (0.33 g, 72.9%). ^{1}H NMR (DMSO-d_6): δ 12.31 (br s, 1H), 10.25 (br s, 1H), 8.10–8.04 (m, 1H), 7.32–7.26 (m, 1H), 7.14–7.04 (m, 2H), 6.96 (t, J = 5.9 Hz, 1H), 4.59 (t, J = 5.1 Hz, 2H), 4.15 (t, J = 6.1 Hz, 2H), 3.43–3-01 (m, 8H), 2.38–2.28 (m, 4H), 1.97–1.81 (m, 5H), 1.68–1.55 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 174.3, 164.6, 150.6, 131.8, 126.1, 122.0, 120.7, 120.4, 109.3, 88.7, 67.9, 56.1, 52.4, 43.7, 34.9, 31.5, 27.7, 21.4, 19.7. HRSM (TOF MS ES+) for $C_{22}H_{29}N_3O_4$ $[M+H]^+$: calcd: 400.2231; found: 400.2225.

5.4.9.21. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylpentanoic acid (29). Yield: 0.33 g (69.7%). ^{1}H NMR (DMSO-d_6): δ 8.04–8.01 (m, 1H), 7.27–7.25 (m, 1H), 7.09–7.01 (m, 2H), 6.92 (t, J = 5.9 Hz, 1H), 4.56 (t, J = 5.1 Hz, 2H), 4.10 (t, J = 6.1 Hz, 2H), 3.32–3.29 (m, 2H), 3.22–3.17 (m, 2H), 2.90–2.85 (m, 2H), 2.80–2.72 (m, 2H), 2.26–2.21 (m, 4H), 1.79–1.46 (m, 10H). ^{13}C NMR (DMSO-d_6): δ 174.2, 163.7, 149.8, 130.9, 125.2, 121.1, 119.9, 119.5, 108.4, 87.8, 67.1, 61.9, 51.0, 42.7, 34.1, 33.3, 26.5, 25.5, 22.9, 21.9. HRSM (TOF MS ES+) for $C_{23}H_{31}N_3O_4$ $[M+H]^+$: calcd: 414.2384; found: 414.2387.

5.4.9.22. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylhexanoic acid (30). Yield: 0.35 g (68.3%). ^{1}H NMR (DMSO-d_6): δ 8.03–8.01 (m, 1H), 7.26–7.24 (m, 1H), 7.08–7.01 (m, 2H), 6.91 (t, J = 5.9 Hz, 1H), 4.55 (t, J = 5.1 Hz, 2H), 4.11 (t, J = 6.1 Hz, 2H), 3.32–3.28 (m, 2H), 3.21–3.16 (m, 2H), 2.84–2.80 (m, 2H), 2,51–2.49 (m, 2H), 2.30–2.19 (m, 4H), 1.77–1.44 (m, 9H), 1.30–1.22 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 174.4, 163.7, 149.8, 130.9, 125.2, 121.1, 119.8, 119.5, 108.4, 87.8, 67.1, 55.6, 51.1, 48.5, 42.7, 33.5, 26.7, 25.7, 24.0, 23.2, 20.6. HRSM (TOF MS ES+) for $C_{24}H_{33}N_3O_4$ $[M+H]^+$: calcd: 428.2544; found: 428.25439.

5.4.9.23. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinyldodecanoic acid (31). Yield: 0.43 g (70.3%). ^{1}H NMR (DMSO-d_6): δ 8.05–8.01 (m, 1H), 7.27–7.23 (m, 1H), 7.09–7.01 (m, 2H), 6.88 (t, J = 5.9 Hz, 1H), 4.55 (t, J = 5.1 Hz, 2H), 4.11 (t, J = 6.1 Hz, 2H), 3.24–3.15 (m, 4H), 2.75–2.70 (m, 2H), 2.58–2.54 (m, 2H), 2.28–2.25 (m, 2H), 2.16 (t, 2H), 1.76–1.44 (m, 9H), 1.23 (br s, 14H). ^{13}C NMR ($CDCl_3$): δ 172.5, 171.5, 161.7, 147.7, 128.9, 123.2, 119.1, 117.8, 117.5, 106.4, 85.8, 65.1, 54.1, 49.5, 31.7, 26.8, 26.7, 26.6, 26.5, 26.4, 22.5. HRSM (TOF MS ES+) for $C_{30}H_{45}N_3O_4$ $[M+H]^+$: calcd: 512.3483; found: 512.3476.

5.4.9.24. 4-[(3,4-Dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carbonyl)amino]-1-methylpiperidinylbenzoic acid (32). Yield: 0.38 g (71.3%). ^{1}H NMR (DMSO-d_6): δ 8.03–8.00 (m, 1H), 7.88 (d, 2H), 7.40 (d, 2H), 7.26–7.23 (m, 1H), 7.07–7.00 (m, 2H), 6.79 (t, 1H), 4.54 (t, 2H), 4.10 (t, 2H), 3.50 (br s, 2H), 3.15 (d, 2H), 2.84 (d, 2H), 2.27–2.23 (m, 2H), 2.09–2.03 (m, 2H), 1.65–1.52 (m, 3H), 1.31–1.21 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.5, 164.4, 150.5, 131.8, 130.0, 129.6, 126.0, 121.9, 120.7, 120.4, 109.3, 88.8, 88.7, 68.0, 53.4, 36.6, 30.0, 21.4. HRSM (TOF MS ES+) for $C_{26}H_{29}N_3O_4$ $[M+H]^+$: calcd: 448.2231; found: 448.2234.

6. Conflicts of Interest

The compounds described in the present paper are described in WO2005061483 (Klaveness, J.; Levy, F. O.; Brudeli, B.). The potential use of 5-HT$_4$ antagonists for treatment of heart failure is described by a published patent application WO03097065 (Levy, F. O.). These patent families belong to the Norwegian biotech company Serodus AS where all the authors are shareholders and FOL is board member.

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References and notes

1. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A. *Pharmacol. Rev.* **1994**, *46*, 157.
2. Bender, E.; Pindon, A.; van Oers, I.; Zhang, Y. B.; Gommeren, W.; Verhasselt, P.; Jurzak, M.; Leysen, J.; Luyten, W. *J. Neurochem.* **2000**, *74*, 478.
3. Blondel, O.; Vandecasteele, G.; Gastineau, M.; Leclerc, S.; Dahmoune, Y.; Langlois, M.; Fischmeister, R. *FEBS Lett.* **1997**, *412*, 465.
4. Claeysen, S.; Faye, P.; Sebben, M.; Lemaire, S.; Bockaert, J.; Dumuis, A. *NeuroReport* **1997**, *8*, 3189.
5. Blondel, O.; Gastineau, M.; Dahmoune, Y.; Langlois, M.; Fischmeister, R. *J. Neurochem.* **1998**, *70*, 2252.
6. Mialet, J.; Berque-Bestel, I.; Sicsic, S.; Langlois, M.; Fischmeister, R.; Lezoualc'h, F. *Br. J. Pharmacol.* **2000**, *131*, 827.
7. Vilaro, M. T.; Domenech, T.; Palacios, J. M.; Mengod, G. *Neuropharmacology* **2002**, *42*, 60.
8. Brattelid, T.; Kvingedal, A. M.; Krobert, K. A.; Andressen, K. W.; Bach, T.; Hystad, M. E.; Kaumann, A. J.; Levy, F. O. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2004**, *369*, 616.
9. De Ponti, F.; Tonini, M. *Drugs* **2001**, *61*, 317.
10. Kaumann, A. J. *Trends Pharmacol. Sci.* **1994**, *15*, 451.
11. Brattelid, T.; Qvigstad, E.; Lynham, J. A.; Molenaar, P.; Aass, H.; Geiran, O.; Skomedal, T.; Osnes, J.-B.; Levy, F. O.; Kaumann, A. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2004**, *370*, 157.
12. Qvigstad, E.; Brattelid, T.; Sjaastad, I.; Andressen, K. W.; Krobert, K. A.; Birkeland, J. A.; Sejersted, O. M.; Kaumann, A. J.; Skomedal, T.; Osnes, J.-B.; Levy, F. O. *Cardiovasc. Res.* **2005**, *65*, 869.
13. Birkeland, J. A.; Sjaastad, I.; Brattelid, T.; Qvigstad, E.; Moberg, E. R.; Krobert, K. A.; Bjørnerheim, R.; Skomedal, T.; Sejersted, O. M.; Osnes, J. B.; Levy, F. O. *Br. J. Pharmacol.* **2007**, *150*, 143.
14. Levy, F. O.; Qvigstad, E.; Krobert, K. A.; Skomedal, T.; Osnes, J. B. *Neuropharmacology* **2008**, *55*, 1066.
15. Kjekshus, J.; Torp-Pedersen, C.; Gullestad, L.; Køber, L.; Edvardsen, T.; Olsen, I. C.; Sjaastad, I.; Qvigstad, E.; Skomedal, T.; Osnes, J.-B.; Levy, F. O. *Eur. J. Heart Fail.* **2009**.
16. Langlois, M.; Fischmeister, R. *J. Med. Chem.* **2003**, *46*, 319.
17. Darblade, B.; Behr-Roussel, D.; Gorny, D.; Lebret, T.; Benoit, G.; Hieble, J. P.; Brooks, D.; Alexandre, L.; Giuliano, F. *World J. Urol.* **2005**, *23*, 147.
18. Lefebvre, H.; Contesse, V.; Delarue, C.; Vaudry, H.; Kuhn, J. M. *Horm. Metab. Res.* **1998**, *30*, 398.
19. Zhu, B. Y.; Jia, Z. J.; Zhang, P.; Su, T.; Huang, W.; Goldman, E.; Tumas, D.; Kadambi, V.; Eddy, P.; Sinha, U.; Scarborough, R. M.; Song, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5507.
20. Clark, R. D. In *5-HT$_4$ Receptors in the Brain and Periphery*; Eglen, R. M., Ed.; Springer: Berlin, Heidelberg, New York, 1998; pp 1–48.
21. Gaster, L. M.; Joiner, G. F.; King, F. D.; Wyman, P. A.; Sutton, J. M.; Bingham, S.; Ellis, E. S.; Sanger, G. J.; Wardle, K. A. *J. Med. Chem.* **1995**, *38*, 4760.
22. Contreras, J. M.; Rival, Y. M.; Chayer, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Med. Chem.* **1999**, *42*, 730.
23. Fedouloff, M.; Hossner, F.; Voyle, M.; Ranson, J.; Powles, J.; Riley, G.; Sanger, G. *Bioorg. Med. Chem.* **2001**, *9*, 2119.
24. Bach, T.; Syversveen, T.; Kvingedal, A. M.; Krobert, K. A.; Brattelid, T.; Kaumann, A. J.; Levy, F. O. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2001**, *363*, 146.
25. Schaus, J. M.; Thompson, D. C.; Bloomquist, W. E.; Susemichel, A. D.; Calligaro, D. O.; Cohen, M. L. *J. Med. Chem.* **1998**, *41*, 1943.
26. Diouf, O.; Depreux, P.; Chavatte, P.; Poupaert, J. H. *Eur. J. Med. Chem.* **2000**, *35*, 699.
27. Lopez-Rodriguez, M. L.; Murcia, M.; Benhamu, B.; Viso, A.; Campillo, M.; Pardo, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2807.
28. Rivail, L.; Giner, M.; Gastineau, M.; Berthouze, M.; Soulier, J. L.; Fischmeister, R.; Lezoualc'h, F.; Maigret, B.; Sicsic, S.; Berque-Bestel, I. *Br. J. Pharmacol.* **2004**, *143*, 361.
29. Lopez-Rodriguez, M. L.; Morcillo, M. J.; Benhamu, B.; Rosado, M. L. *J. Comput. Aided Mol. Des.* **1997**, *11*, 589.
30. Sakaguchi, J.; Iwsaki, N.; Iwanaga, Y.; Saito, T.; Takahara, E.; Kato, H.; Hanaoka, M. *Chem. Pharm. Bull. (Tokyo)* **2001**, *49*, 424.
31. Kahns, A. H.; Buur, A.; Bundgaard, H. *Pharm. Res.* **1993**, *10*, 68.
32. Levin, V. A. *J. Med. Chem.* **1980**, *23*, 682.
33. ter Laak, A. M.; Tsai, R. S.; Donnq-Op den Kelder, G. M.; Carrupt, P.-A.; Testa, B.; Timmerman, H. *Eur. J. Pharm. Sci.* **1994**, *2*, 373.
34. Layton, D.; Key, C.; Shakir, S. A. *Pharmacoepidemiol. Drug Saf.* **2003**, *12*, 31.
35. Yao, X.; Anderson, D. L.; Ross, S. A.; Lang, D. G.; Desai, B. Z.; Cooper, D. C.; Wheelan, P.; McIntyre, M. S.; Bergquist, M. L.; MacKenzie, K. I.; Becherer, J. D.; Hashim, M. A. *Br. J. Pharmacol.* **2008**, *154*, 1446.
36. Mikami, T.; Sugimoto, H.; Naganeo, R.; Ohmi, T.; Saito, T.; Eda, H. *J. Pharmacol. Sci.* **2008**, *107*, 251.
37. Salomon, Y.; Londos, C.; Rodbell, M. *Anal. Biochem.* **1974**, *58*, 541.
38. Bockaert, J.; Hunzicker-Dunn, M.; Birnbaumer, L. *J. Biol. Chem.* **1976**, *251*, 2653.
39. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.