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Synthesis and Pharmacological Characterization of a New Benzoxazole Derivative as a Potent 5-HT_3 Receptor Agonist

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Abstract—*N*-(2-Benzoxazol-2-yl-ethyl)-guanidine hydrochloride (**10**) was synthesized and pharmacologically tested. This compound showed high affinity for the 5-HT_3 receptor ($K_i = 0.77$ nM) and potently triggered the von Bezold–Jarisch reflex (BJR) in rats with an $ED_{50} = 0.52$ μg/kg iv and intrinsic activity next to 1 (i.a. = 0.94). This stimulant effect was abolished by pretreatment with the 5-HT_3 receptor antagonist granisetron and was subject to a rapid and pronounced tachyphylaxis, due to desensitization of the peripheric cardiac 5-HT_3 receptor. Consequently, **10** acts as an in vivo 5-HT_3 antagonist inhibiting the BJR responses evoked by submaximal doses of 5-HT with an $ID_{50} = 5.8$ μg/kg iv.

Introduction

In recent years much research effort has been made in the field of serotonin (5-HT) receptors. Until now seven families of 5-HT receptor have been recognised[1] and only the 5-HT_3 receptor belongs to the ligand-gate cation channel family.[2,3] The high affinity, selectivity and low incidence of side-effects of the 5-HT_3 receptor ligands have caused a huge interest in this receptor due to their potential therapeutic applications in a number of areas: emesis, anxiety, psychotic disorders, drug abuse, depression, migraine, pain, irritable bowel syndrome.[4–7]

Numerous selective antagonists among 5-HT_3 receptor ligands are currently available and they are of high therapeutic interest in the prevention and treatment of emesis associated with anticancer chemotherapy[8,9] and the prophylaxis of postoperative nausea and vomiting.[10] However, relatively little attention has been paid to the 5-HT_3 receptor agonists although several potent and selective agonists have also been identified such as 2-methyl-5-HT (**1**), *meta*-chlorophenylbiguanide (*m*-CPBG, **2**),[11] 2,3,5-trichloro-phenylbiguanide (2,3,5-trichloro-PBG, **3**),[11] YM 31636 (**4**),[12] SR 57227 (**5**),[13] S 21007 (**6**),[14] 5-chloro-7-methyl-2-(4-methyl-1-homopiperazinyl)benzoxazole (**7**),[15] 9-methyl-4-(4-methyl-piperazin-1-yl)pyrrolo[1,2-a]-quinoxaline (**8**),[16] 3,4,5-trichloro-phenylguanidine (3,4,5-trichloro-PG, **9**)[17] (Chart 1).

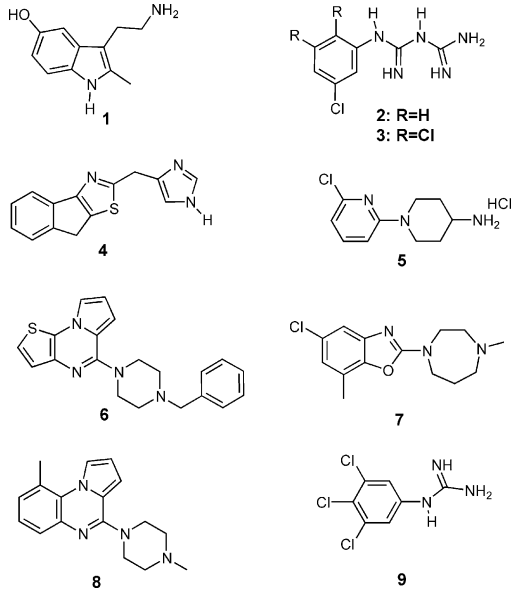

Chart 1.

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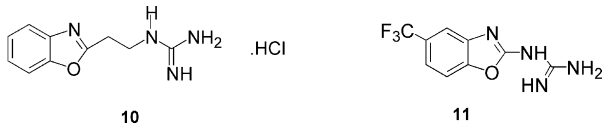


Chart 2.

Structure–activity relationship studies of the 5-HT_3 receptor agonists have generated several pharmacophoric hypotheses[17–21] to explain their interactions with this receptor. From these hypotheses three structural requirements for the 5-HT_3 agonism could be pointed out: a quaternary ammonium ion, a hydrogen bond acceptor and an aromatic site.

Some 5-HT_3 receptor agonists are able to cross the blood–brain barrier. This makes them ideal pharmacological tools to explore functional roles of central and peripheric 5-HT_3 receptors. Nowadays, the main interest on the 5-HT_3 receptor agonism is focused on diverse areas of psychoactivity, particularly depression, and gastrointestinal disorders.

As a part of a research project on 5-HT_3/5-HT_4 receptor ligands we have synthesized the benzoxazoleguanidine **10** (Chart 2) which has been characterized as a potent 5-HT_3 receptor agonist.

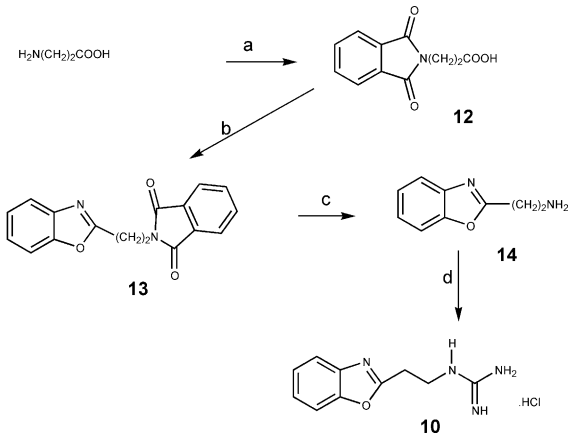
Compound **10** displays two moieties of interest, a guanidine group found in other ligands,[17,22,23] and a benzoxazole fragment related to a selective 5-HT_3 receptor partial agonistic activity.[15,18,24] or a weak gut related antagonism.[25] Morain et al.[11] have reported a guanidine derivative **11** (Chart 2), closely related to **10**, in which the guanidine group is directly linked to a substituted benzoxazole system, and surprisingly it lacks the affinity for the 5-HT_3 receptor. This fact suggests that the distance between the benzoxazole system and the guanidine fragment or the influence of the trifluoromethyl group placed on the aromatic ring or both play a fundamental role to achieve the fitting at the receptor. However, similar modifications (chain shorthening[22] or introduction of a trifluoromethyl group[17]) in arylguanidines did not produce significant reduction of affinity. Additional studies to know the structural requirement for the 5-HT_3 agonistic activity are required.

Results and Discussion

Synthesis

The preparation of the benzoxazolguanidine **10** was accomplished by the synthetic pathway illustrated in Scheme 1 using standard reactions.

The starting material was β-alanine which was converted into the corresponding phthalimide derivative **12** with phthalic anhydride in refluxing toluene.[26] The benzoxazole ring was subsequently obtained by cyclocondensation of the phthalimide acid derivative **12** and 2-aminophenol with polyphosphoric acid at 180 °C. Finally, deprotection of the amine in the phthalimido-



Scheme 1. Synthesis of compound **10**. Reagents: (a) Phthalic anhydride; (b) 2-aminophenol, polyphosphoric acid, 180 °C; (c) N_2H_4; (d) 1*H*-pyrazolecarboxamidine hydrochloride.

benzoxazole derivative **13** with hydrazine hydrate afforded the free amine **13** which was readily converted into the benzoxazolguanidine **10** by reaction with 1*H*-pyrazole-1-carboxamidine hydrochloride[27] and diisopropylethylamine in DMF.

Pharmacological Activity

Radioligand binding studies

Radioligand binding data for **10** and the reference drugs are shown in Table 1. Compound **10** binds to the 5-HT_3 receptor in rat entorhinal cortex labelled with [^{3}H]-LY278584 with high affinity ($K_i = 0.77$ nM). Its K_i value

Table 1. Affinity values of compound **10** and other 5-HT_3 receptor ligands

Drugs	K_i (nM)	Radioligand	Tissue
10	0.77±0.09[a]	[^{3}H]-LY278584	Rat entorhinal cortex
Granisetron[b]	1.76±0.15[a]	[^{3}H]-LY278584	Rat entorhinal cortex
Ondansetron[b]	2.00±0.30[a]	[^{3}H]-LY278584	Rat entorhinal cortex
Tropisetron[b]	1.55±0.10[a]	[^{3}H]-LY278584	Rat entorhinal cortex
Lerisetron[b]	0.62±0.08[a]	[^{3}H]-LY278584	Rat entorhinal cortex
(*S*)-Zacopride[b]	0.49±0.03[a]	[^{3}H]-LY278584	Rat entorhinal cortex
5-HT[c]	42±7[d]	[^{3}H]-BRL43694	N1E-115 cells
1[c]	350±30[d]	[^{3}H]-BRL43694	N1E-115 cells
2[c]	13±2[d]	[^{3}H]-BRL43694	N1E-115 cells
3[c]	0.44±0.09[d]	[^{3}H]-BRL43694	N1E-115 cells
4[c]	0.21±0.05[e]	[^{3}H]-Ramosetron	Cloned human
5[c]	115±31[f]	[^{3}H]-Zacopride	Rat cerebral cortex
6[c]	2.8±0.3[g]	[^{3}H]-Granisetron	N1E-115 cells
7[c]	1.1±0.1[h]	[^{3}H]-GR65630	Rat cerebral cortex
8[c]	0.79±0.1[i]	[^{3}H]-Zacopride	Rat cerebral cortex
9[c]	0.7±0.1[j]	[^{3}H]-GR65630	NG108-15 cells

[a]All values are the mean±SEM of data from 2–3 independent experiments performed in triplicate in our laboratory.
[b]5-HT_3 receptor antagonist.
[c]5-HT_3 receptor agonist. K_i values were previously reported and are included only for comparison.
[d]See ref 11.
[e]See ref 12.
[f]See ref 13.
[g]See ref 14.
[h]See ref 15.
[i]See ref 16.
[j]See ref 17.

is comparable to those of granisetron ($K_i = 1.76$ nM), ondansetron ($K_i = 2.00$ nM), tropisetron ($K_i = 1.55$ nM), lerisetron ($K_i = 0.62$ nM) and (*S*)-zacopride ($K_i = 0.49$ nM), all of them potent serotonin 5-HT_3 receptor antagonists. Also, the affinity shown by **10** was similar to the most potent 5-HT_3 agonists described up to date such as **4** ($K_i = 0.21$ nM),[12] **3** ($K_i = 0.44$ nM),[11] **8** ($K_i = 0.79$ nM)[16] and **9** ($K_i = 0.70$ nM).[17]

On the other hand, **10** did not show significant affinity for the serotonin 5-HT_4 and dopamine D_2 receptors at 1 μM concentration (data not shown).

In vivo experiments

The 5-HT_3 receptor agonistic effect of **10** was evaluated in vivo on the BJR in anaesthetized rats and compared to that of 5-HT. The obtained value of $ED_{50} = 18.6$ μg/kg iv for 5-HT was similar to that found in literature.[13,28] Like 5-HT, cumulative doses of **10** elicited the BJR, but a bell-shaped dose–response curve was obtained as shown in Figure 1. The maximum percentage of bradycardia induced by the highest dose of 5-HT was estimated as the 100% effect (about 75–80% of reduction in heart rate (HR) at 100–333 μg/kg iv). **10** At 1 μg/kg iv showed 65% of the previously defined maximum effect. Thus, a value of intrinsic activity (i.a.) relative to 5-HT of 0.65 was estimated, suggesting a partial agonistic behaviour at the 5-HT_3 receptor. But at higher doses each cumulative and succesive injection of **10** produced progressively smaller responses, so that at the dose of 33 μg/kg iv the BJR response was not significant with respect to the saline-induced effect. This finding also suggests that the induction of rapid tachyphylaxis may be due to receptor desensitization. In this case, its initial partial agonistic potential could have been understimated.

To minimize the desensitizer effect and to check the real agonistic potential of **10** a non-cumulative dose–response curve was obtained administering only one dose to each rat (Fig. 2). In this assay a full agonistic character with an $ED_{50} = 0.52$ μg/kg iv and an i.a. = 0.94 not significantly different from the unity, was observed. The calculated ED_{50} values indicate that **10** is about 35 times more potent than 5-HT to induce the BJR in anaesthetized rats.

The potency of **10** to induce the BJR in urethane-anaesthetised rats results similar or slightly superior to **2** (ED_{50} from 0.7 to 1 μg/kg iv)[28] and clearly superior to **1** (ED_{50} from 5 to 27 μg/kg iv).[13] Agonists **5** and **6** with ED_{50} values of 8 μg/kg iv and higher than 120 μg/kg iv respectively[13,14] are less potent than **10**.

Recently, Whalen et al.[29] have reported functional evidence for tachyphylaxis in BJR responses induced in conscious rats by five consecutive iv injections of 100 μg/kg of two structurally different 5-HT_3 receptor agonists **1** and **2**. To determine whether the agonistic effect of **10** is subjected to tachyphylaxis, a similar experiment using anaesthetized rats was carried out.

Five cumulative successive submaximal doses of **10** (1 μg/kg iv) and 5-HT (30 μg/kg iv), graphically calculated, were administered and the induced changes in BJR responses registered and plotted (Fig. 3). The reductions in HR caused by the first injection of the compounds were similar, with percentages of 67% for **10** and 72% for 5-HT. Contrary to 5-HT, each consecutive administration of **10** produced progressively smaller responses and finally the fifth injection was not able to induce a significant response. This fact suggests that this difference could be associated with a different interaction of both agonists with the 5-HT_3 binding sites, as already described for **6**, a 5-HT_3 agonist structurally unrelated to **10** but with high affinity for the 5-HT_3 receptor.[14]

Structurally related to **10** is the derivate **7**, that has been reported[15] as a 5-HT_3 receptor partial agonist in the gut. This compound does not induce the BJR in the anaesthetised rat at the dose that blocks the BJR induced by 5-HT but acts as a 5-HT_3 receptor antagonist.

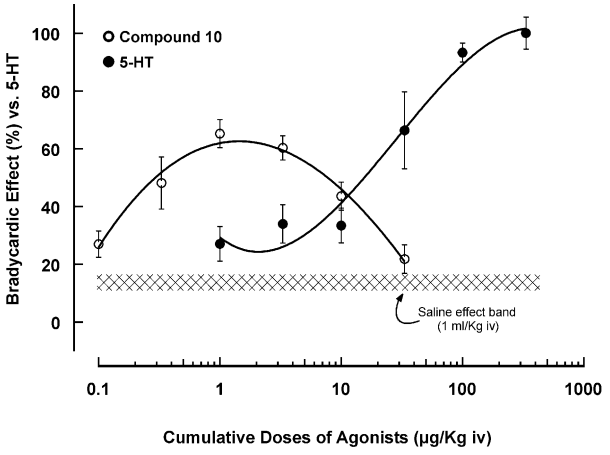


Figure 1. Cumulative dose-dependent bradycardic effect (BJR) elicited by 5-HT (1 to 333 μg/kg iv) and compound **10** (0.1 to 33 μg/kg iv) in anaesthetized rats. The effect is expressed as a percentage of the maximum obtained by 5-HT. Each point is the mean ± SEM ($n = 8$ rats/drug). The saline effect band is the effect induced by bolus injection of sterile saline.

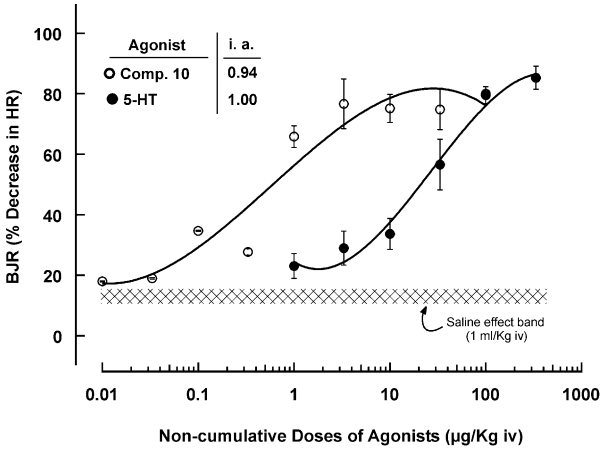


Figure 2. Non-cumulative dose-dependent BJR response elicited by 5-HT (1 to 333 μg/kg iv) and compound **10** (0.01 to 33 μg/kg iv) in anaesthetized rats. The effect is expressed as a percentage of decrease in HR. Each point is the mean ± SEM ($n = 5$ rats/dose).

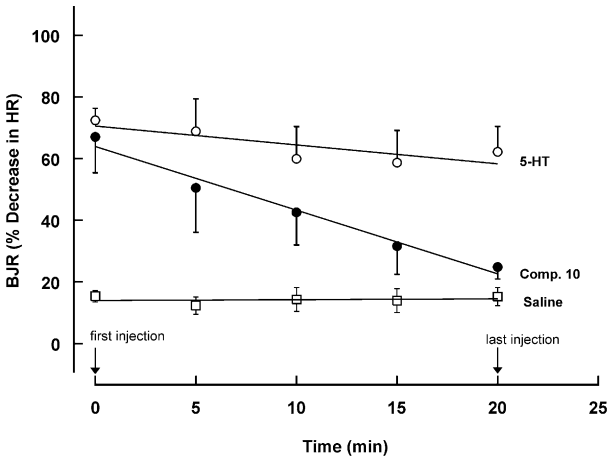


Figure 3. Time–effect regression lines obtained after five consecutive intravenous injections of submaximal doses of compound **10** (1 μg/kg), 5-HT (30 μg/kg) or sterile saline (1 mL/kg). The effect is expressed as a percentage of decrease in HR. Each point is the mean ± SEM ($n = 6$ rats/drug).

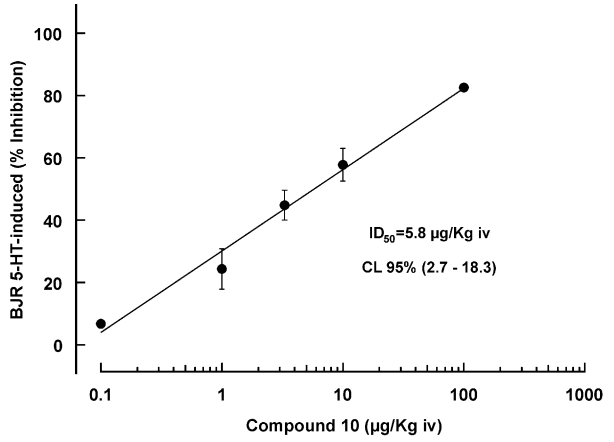


Figure 4. Log dose-inhibition regression line obtained with intravenous injections of compound **10**, 5 min before of a submaximal dose of 5-HT (30 μg/kg iv) (1 mL/kg). The effect is expressed as a percentage of inhibition. Each point is the mean ± SEM ($n = 5$ rats/dose).

It should be considered that the performance of 5-HT_3 receptor full agonist, partial agonist or antagonist is conditioned by the type of assay and the nature of the tissue used. The wide found response diversity could reflect the existence of subtypes of 5-HT_3 receptors,[16] a suggestion[30] that still requires additional studies.

To date, all 5-HT_3 full and partial agonists, characterized by their responses in the BJR in rats, have in common that their effects are abolished by a previous treatment with a 5-HT_3 antagonist. In our study, the effect (65–70% decrease in HR) registered by a submaximal dose (1 μg/kg iv) of **10** was completely antagonised (95% of inhibition) by 10 μg/kg iv of granisetron administered 5 min before. This finding corroborates that it acts stimulating the 5-HT_3 receptors (data not shown).

The tachyphylaxis induced by **10** suggests that this compound could also act as an in vivo antagonist. A pretreatment with **10** was able to inhibit in a dose-dependent manner the BJR responses evoked by submaximal doses (30 μg/kg iv) of 5-HT (Fig. 4) with an $ID_{50} = 5.8$ μg/kg iv. This value is in accordance with its affinity for the 5-HT_3 receptor and is lower than those of 5-HT_3 antagonists presented in Table 1.

Conclusions

The presented results indicate that **10** shows high affinity for the 5-HT_3 receptor in rat entorhinal cortex membranes and behaves as a full agonist about 35 times more potent than 5-HT in functional 5-HT_3 receptor-mediated studies (BJR in anaesthetized rats). The stimulant effect is abolished by granisetron and it is subject to a rapid tachyphylaxis, probably due to desensitization mechanisms that induce loss of responsiveness of cardiopulmonary vagal afferents. Compound **10** is able to inhibit the 5-HT induced BJR acting as a potent 5-HT_3 antagonist in vivo. Because of such properties it could be a useful pharmacological tool to explore functional roles and desensitization mechanisms of central and peripheral 5-HT_3 receptors. A receptor binding profile is underway to investigate its selectivity. Although **10** exhibits a high affinity for the 5-HT_3 receptor, several analogues are being prepared to elucidate the structural requirements for the 5-HT_3 receptor agonistic activity and to evaluate their potential activities in the neuropsychopharmacology and gastrointestinal areas.

Experimental

Chemistry

All starting materials were obtained from commercial sources. When necessary, solvents were purified and/or dried by standard procedures. Thin layer chromatography was performed on 0.2 mm Merck precoated plates of silica gel 60 F_{254} and spots were visualised with UV light or I_2. Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 230–400 (Merck). High performance liquid chromatograms were obtained in a Waters LCM-1 apparatus using a C-18 reverse phase Nucleosil 120 column (data not shown). Melting points were determined in open capillary tubes on a Büchi SMP-20 apparatus and were uncorrected. Elemental analyses (C, H and N) were within 0.4% of the theoretical values. Infrared (IR) spectra (in KBr) were taken on a Perkin–Elmer 1310 spectrophotometer. ^{1}H- and ^{13}C NMR were recorded on a Bruker AC-200. ^{1}H NMR chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane, which was used as an internal standard. ^{13}C NMR chemical shifts (δ) are reported in ppm relative to the solvent and converted to the TMS scale, using the value 76.91 for $CDCl_3$. The structures of all compounds were consistent with their analytical and spectroscopic data.

The following compounds were prepared as described in the literature: 3-(2-phthalimido)propionic acid **12**[26] and 1*H*-pyrazole-1-carboxamidine hydrochloride.[27]

Preparation of 2-[2-(2-phthalimido)ethyl]benzoxazole 13. A solution of 3-(2-phthalimido)propionic acid **12** (8.8 g, 40 mmol) and *o*-aminophenol (4.4 g, 10 mmol) in polyphosphoric acid (20 g) was heated at 180 °C for 2 h. Afterwards the reaction mixture was allowed to cool and poured into water (400 mL). This aqueous mixture was made basic until pH 12 with 50% sodium hydroxide solution. The obtained precipitate was filtered off, washed thoroughly with water and dried, yielding the desired product (8 g, 68.5% yield). Mp 171–4 °C. ^{1}H NMR ($CDCl_3$): (δ) 7.85 (2H, m); 7.75 (2H, m); 7.60 (1H, m); 7.42 (1H, m); 7.31 (2H, m); 4.22 (2H, t, $J=7.2$ Hz); 3.35 (2H, t, $J=7.2$ Hz). ^{13}C NMR ($CDCl_3$): (δ) 167.8, 163.3, 150.8, 141.0, 134.0, 131.8, 124.7, 124.1, 123.3, 119.6, 110.3, 35.0, 27.7. Anal. calcd for $C_{17}H_{12}N_2O_3$: C, 69.86; H, 4.14; N, 9.58. Found: C, H, N.

Preparation of 2-benzoxazol-2-yl-ethylamine 14. To a suspension of 2-[2-(2-phthalimido)ethyl]benzoxazole **13** (6.5 g, 22.2 mmol) in ethanol (50 mL) hydrazine hydrate (1.2 mL, 24.7 mmol) was added. The mixture was refluxed for 2 h, allowed to cool, filtered and the obtained solid washed with ethanol. The filtrate was concentrated and purified by flash chromatography (CH_2Cl_2:MeOH 9:1) to give 2-(2-aminoethyl)benzoxazole **14** as an oil (2.3 g, 64% yield). ^{1}H NMR ($CDCl_3$): (δ) 7.58 (1H, m); 7.40 (1H, m); 7.20 (2H, m); 3.15 (2H, t, $J=7.0$ Hz); 2.95 (2H, t, $J=7.0$ Hz); 2.10 (2H, br s, D_2O exchange). ^{13}C NMR ($CDCl_3$): (δ) 165.4, 150.5, 141.1, 124.4, 124.0, 119.4, 110.1, 39.1, 32.5. Anal. calcd for $C_9H_{10}N_2O$: C, 66.65; H, 6.21; N, 17.27. Found: C, H, N.

Preparation of *N*-(2-benzoxazol-2-yl-ethyl)-guanidine hydrochloride 10. 1*H*-Pyrazole-1-carboxamidine hydrochloride (1 g, 6.8 mmol) was added to a solution of 2-benzoxazol-2-yl-ethylamine **14** (1.1 g, 6.7 mmol) and diisopropylethylamine (4.75 mL, 27 mmol) in DMF (10 mL). The reaction mixture was stirred at room temperature under N_2 for 24 h. Subsequently, diethylether (75 mL) was added, the upper layer was decanted and the remaining oil solidified by treatment with CH_2Cl_2. The solid was colected, washed with diethylether, CH_2Cl_2, and $CHCl_3$:^{i}PrOH (4:1) and dried to yield **10** (0.86 g, 52.6% yield). Mp 183–186 °C. ^{1}H NMR ($CDCl_3$): (δ) 7.80 (1H, m, D_2O exchange); 7.65 (2H, m); 7.45 (6H, m, 4H exchange with D_2O); 3.65 (2H, m); 3.20 (2H, t, $J=6.5$ Hz). ^{13}C NMR ($CDCl_3$): (δ) 164.2, 157.2, 150.2, 140.7, 124.9, 124.4, 119.3, 110.6, 37.7, 28.0. Anal. calcd for $C_{10}H_{12}N_4O$.HCl: C, 49.90; H, 5.44; N, 23.28. Found: C, H, N.

Pharmacological Methods

In vitro. Binding assays

Bindings to serotonin 5-HT_3 and 5-HT_4 receptors and dopamine D_2 receptors were determined by radioligand assays according to previously published procedures.[31–33] The affinity for the serotonin 5-HT_3 receptor was determined by displacement of [^{3}H]-LY278584 binding in membranes from rat entorhinal cortex, using 15 mg (original wet weight) of membranes and 2 nM [^{3}H]-LY278584. Nonspecific binding was defined in the presence of 10 μM 5-HT. The incubation time was 30 min at 25 °C. The affinity for the serotonin 5-HT_4 receptor was determined by displacement of [^{3}H]-GR113808 binding in membranes from guinea-pig striatum, using 10 mg (original wet weight) of membranes and 0.2 nM [^{3}H]-GR113808. Nonspecific binding was defined in the presence of 100 μM 5-HT. The incubation time was 30 min at 25 °C. The affinity for the dopamine D_2 receptor was determined by displacement of [^{3}H]-raclopride binding in membranes from rat striatum, using 5 mg (original wet weight) of membranes and 1 nM [^{3}H]-raclopride. Nonspecific binding was defined in the presence of 1 μM (+)-butaclamol. The incubation time was 60 min at 25 °C. After incubation, the reaction was stopped by vacuum filtration through Whatman GF/B presoaked filters (1% polyethylenimine) using a 24 Brandel Cell Harvester. The filters were counted by a Kontron Betamatic V liquid scintillation counter in 5 mL of aqueous counting scintillant (Ecoscint-H, National Diagnostic). Data from binding assays were plotted as log concentration vs percentage specific bound and analyzed by nonlinear regression techniques (GraphPad v1.00). The IC_{50} values were obtained from 2–3 separate competition experiments with samples in triplicate, using 10–12 different concentrations of drugs. For all of them, K_i values were calculated from the Cheng and Prusoff standard equation.[34]

In vivo. Induction or inhibition of the von Bezold–Jarisch reflex in rats

Adult male Wistar rats weighing 250–320 g fasted for 18 h were used. They were anaesthetized with urethane (1.50 g/kg ip) and placed on a heating table to maintain their body temperature at 37 °C. The trachea and right jugular vein were cannulated to facilitate the respiration and drug administration, respectively. The carotid artery was also cannulated and connected to a ISOTEC™ pressure transducer to record blood pressure (BP) (Hugo Sachs Elektronik, Freiburg, Germany). The HR was measured using the BP signal and a cardiotachometer coupler and recorded on a Graphtec Linearcorder WR3101 (Hugo Sachs Elektronik, Freiburg, Germany). Both parameters (BP and HR) were also monitorized and analyzed using a PowerLab/8SP unit (ADInstruments) connected to WR3101 recorder. The BJR was evoked by bolus intravenous injection of 5-HT or **10** dissolved in sterile saline.

In agonism studies, a cumulative dose–response curve to 5-HT and **10** was initially constructed on independent groups of rats. The BJR responses produced by each injection of the drugs were allowed to subside completely before another injection was given. Each injection or dose was given normaly 5–6 min apart. The intensity of the BJR was expressed in percentage of bradycardia considering the basal or initial HR value and the final one obtained after 5-HT or **10** iv administration. For each drug a dose–response curve from 6

cumulative doses (8 rats/drug) was plotted estimating as the 100% effect the maximum percentage of bradycardia induced by the highest dose of 5-HT. This value was about 80% of reduction in HR.

Subsequently, to minimize a probable desensitizer effect in the ability of test compound to evoke the BJR, another set of randomized experiments were evaluated in a non-cumulative way. Thus, after the initial period of stabilization of BP and HR parameters only one BJR response per rat was recorded for each bolus iv injection of drug. In this experiment the ED_{50} and i.a. values from 8 doses of **10** (5 rats/dose) were calculated and compared with 5-HT.

To determine whether the BJR responses elicited by 5-HT or **10** are subject to rapid tachyphylaxis, submaximal and equiactives doses for each agonist were selected. Five consecutive and cumulative bolus iv injections of saline (1 mL/kg), 5-HT (30 μg/kg) or **10** (1 μg/kg) were given every 5 min to rats (6 rats/agonist) distributed *at random*. For each agonist a time-percentage of bradycardia curve was constructed to analyze the variation of the BJR response.

To confirm that the agonist effect of **10** was 5-HT_3 receptor dependent, another series of rats were used in which the 5-HT_3 antagonist granisetron was administered intravenously at 10 μg/kg 5 min before a submaximal dose of **10** which, when given alone, reduced the HR about 65–70%.

Finally, to evaluate the 5-HT_3 antagonistic activity of **10** a first BJR by bolus iv injection of a submaximal dose of 5-HT (30 μg/kg) was evoked in each rat. When the 5-HT-induced bradycardia (65–70% fall in HR) returned to pretreatment levels (within 5 min) either **10** or saline were administered and a second BJR was elicited with the same 5-HT dose 5 min later. An ID_{50} value from 5 doses (5 rats/dose) was calculated from the lineal-regression of the log dose-inhibition line.

Acknowledgements

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