Short communication

Benzimidazole-2-carboxylic acid amides and esters: a new structural class of 5-HT₃ ligands

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Abstract – A series of novel benzimidazole-2-carboxylic acid amides and esters with a quinuclidine or a tropane moiety were synthesized and evaluated for in vitro affinity for the 5-HT₃, 5-HT₄ and D₂ receptors. Compounds **15a**, **13j** and **13h** exhibited affinity for the 5-HT₃ receptor (K_i = 20.2, 18.4 and 12.7 nM, respectively) and no significant affinity for both 5-HT₄ and D₂ receptors. The amide-ester replacement did not induce significant changes in the affinity profile. The enantioselectivity for the 5-HT₃ receptor was reversed with regard to the zacopride pattern and the (*R*)-enantiomer **13c** showed higher affinity (K_i = 56.4 nM) than the (*S*)-enantiomer **13d** (K_i = 242.3 nM). An increment of the steric hindrance around the nitrogen atom at the 1-position of the benzimidazole ring led to an improvement in the affinity. The 5-HT₃ receptor antagonist activity of compounds with higher affinity was performed by evaluating the inhibition of the 5-HT induced von Bezold-Jarisch reflex. They displayed moderate 5-HT₃ antagonist activity (ED₅₀ = 10.6–29.1 µg/kg i.v.). © Elsevier, Paris

benzimidazole-2-carboxamide / benzimidazole-2-carboxylate / tropane / quinuclidine / 5-HT₃ ligand

1. Introduction

In recent years the 5-HT₃ receptor subtype has attracted much attention because of the effectiveness of 5-HT₃ receptor antagonists in the treatment of chemotherapy-induced emesis [1, 2]. In addition, they are expected to be useful for the treatment of anxiety, schizophrenia, migraine and irritable bowel syndrome [3–5]. Some of these compounds, which proved to behave as 5-HT₃ receptor antagonists, also show affinity at the 5-HT₄ serotonin receptor subtype [6, 7]. Several 5-HT₃ antagonist benzamides have also dopamine receptor antagonist properties, which induce unfavorable effects in man such as central nervous system depression and extrapyramidal syndrome [8].

Several studies have suggested that there are three structural requirements for 5-HT₃ receptor antagonism: an aromatic ring, a linking acyl function and a basic nitrogen group [9–11]. Many of the reported 5-HT₃ receptor antagonists include an azabicycloalkane moiety,

indicating a particularly good fit between the receptor and these heterocycles, i.e. tropisetron 1, granisetron 2 and zacopride 3 (*figure 1*).

In order to find new 5-HT₃ receptor antagonists with selectivity over 5-HT₄ and D₂ receptors, we prepared a series of novel benzimidazole-2-carboxylic acid derivatives including a quinuclidine or a tropane moiety.

2. Chemistry

Benzimidazole-2-carboxylic acid **6** was synthesized as described in *figure 2*. 2-Hydroxymethylbenzimidazole **5** was prepared from 1,2-phenylenediamine **4** and glycolic acid, according to the Phillips procedure [12]. Oxidation of **5** with potassium permanganate afforded acid **6** [13]. 3-Aminotropane derivatives **9** and **10** were prepared using previously reported methods (*figure 3*) [14, 15]. The reaction of tropinone with hydroxylamine gave the oxime **8** which was reduced with lithium aluminum hydride to give the *endo* isomer **9**; sodium reduction of **8** afforded the *exo* isomer **10**. Other amines and alcohols used were commercially available. Amides **11** were

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Figure 1. 5-HT₃ receptor antagonists.

obtained from the acid **6** following a standard procedure, by coupling with the appropriate amine using carbonyldiimidazole (CDI) (*figure 4*). Amides **13** were synthesized by reaction of the corresponding amines with trimethylaluminium and subsequent treatment of the resulting dimethylaluminium amides with a carboxylic ester **12** [16]. Acid **6** was condensed with an alcohol using CDI and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give ester derivatives **14**; treatment of **14** with sodium hydride followed by reaction with an alkyl halide led to esters **15**.

3. Pharmacology

Compounds were evaluated for their in vitro 5-HT₃, 5-HT₄ and D₂ receptor affinities by radioligand binding assays [17–19]. For each compound, the ability to displace the specific ligand ([³H]-LY278584, [³H]-GR113808, and [³H]-Raclopride, respectively) from receptors was determined. The antagonist activity at the 5-HT₃ receptor was performed by evaluating the inhibition of the Bezold-Jarisch reflex evoked by 5-HT in urethane-anaesthetized rats (*table I*).



Figure 2. Synthesis of benzimidazole-2-carboxylic acid 6. Reagents: a) HO_2CCH_2OH , 4 N HCl, 40 min, reflux; b) KMnO₄, 10% NaOH, 3 h, room temperature.



Figure 3. Preparation of 3-aminotropane derivatives 9 and 10. Reagents: a) NH_2OH , pyridine, EtOH, 2 h, reflux; b) LiAlH₄, 10% H_2SO_4 , 2 h, reflux; c) Na, EtOH, 2 h, reflux.



Figure 4. Preparation of compounds **11**, **12**, **13**, **14** and **15**. Reagents: a) CDI, R_1NH_2 , DMF, 20 h, room temperature; b) HNa, R_2 Hal, DMF, 3 h, room temperature; c) Me₃Al, R_1NH_2 , PhCH₃, 3 h, reflux; d) CDI, DMF, DBU, R_1OH , 24 h, 60 °C; e) HNa, R_2 Hal, DMF, 3 h, room temperature.

4. Results and discussion

Most of the synthesized compounds exhibited moderate-to-high affinity for the 5-HT₃ receptor and no significant affinity for either the 5-HT₄ or the D₂ receptors.

The amide-ester replacement did not induce significant changes on the affinity. Compounds **11a** ($K_i = 172.3 \text{ nM}$) and **13j** ($K_i = 18.4 \text{ nM}$) showed a similar affinity value to **14a** ($K_i = 171.7 \text{ nM}$) and **15a** ($K_i = 20.2 \text{ nM}$), respectively. Only amide **11b** ($K_i = 143.9 \text{ nM}$) displayed ca. 2-fold higher affinity than its related ester **14b** ($K_i = 260.1 \text{ nM}$). A similar equipotency of amides and esters has been reported for several benzoic acid derivatives [20, 21].

With regard to the tropane moiety, the *endo*-derivative **11b** showed up to 1 000-fold more potent affinity for the 5-HT₃ receptor than the analogue *exo*-derivative **11c** (K_i > 1 000 nM). The inactivity of the axially substituted tropane derivatives is well known and has been reported [22]. On the other hand, the influence of the chirality of the quinuclidine moiety was examined. Whereas the (*R*)-enantiomer **13c** had higher affinity (K_i = 56.4 nM) than the racemate **13a** (K_i = 81.6 nM), the (*S*)-enantiomer **13d** showed weaker affinity (K_i = 242.3 nM). Consequently, the enantioselectivity for the 5-HT₃ receptor was reversed with regard to that of zacopride and related 3-quinuclidinyl amides in which the (*S*)-

enantiomers have higher affinity for the 5-HT₃ receptor than the (*R*)-enantiomers [23–25]. A similar inversion of enantioselectivity has been reported for naphtalimides, benzoylureas, and indolyl-2-acetamides [26–28]. The increase in the potency of the (*R*)-enantiomer could indicate the existence of a different and relevant bond between the compound and the receptor site.

With respect to the introduction of an alkyl substituent at the 1-position of the benzimidazole ring, an increment of the steric hindrance around the nitrogen atom led to an improvement in the affinity for the 5-HT₃ receptor; e. g. compare **11b** (K_i = 143.9 nM) and **13j** (K_i = 18.4 nM); **11a** (K_i = 172.3 nM) and **13i** (K_i = 24.4 nM); **14b** (K_i = 260.1 nM) and **15a** (K_i = 20.2 nM). However, replacement of the hydrogen in **11b** by a methyl group led to **13b** (K_i = 158.5 nM), with no change in the affinity. Besides, the affinity of the N-benzyl derivative **13j** (K_i = 18.4 nM) was somewhat lower to that of the isopropyl analogue **13h** (K_i = 12.7 nM). Moreover, compounds **13a** (K_i = 81.6 nM), **13e** (K_i = 65.2 nM) and **13g** (K_i = 70.4 nM) did not show any remarkable difference in the affinity.

We evaluated the 5-HT₃ receptor-antagonist activity of the compounds with higher affinity (13h, 13i, 13j and 15a) in the inhibition of the 5-HT induced von Bezold-Jarisch reflex assays in anaesthetized rats. They exhibited



R_2				A			В
Compound ^a	Х	\mathbb{R}^1	\mathbb{R}^2	5-HT ₃ affinity ^b	5-HT ₄ affinity	^b D ₂ affinity ^b	B-J reflex inhibition ^c
11a	NH	А	Н	172.3 ± 10.2	> 1000	> 1000	n.t. ^g
11b	NH	endo-B	Н	143.9 ± 15.6	> 1000	> 1000	n.t. ^g
11c	NH	exo-B	Н	> 1000	> 1000	> 1000	n.t. ^g
13ars	NH	А	Me	81.6 ± 10.9	> 1000	> 1000	n.t. ^g
13b	NH	endo-B	Me	158.5 ± 17.2	> 1000	> 1000	n.t. ^g
13c ^d	NH	A (R)	Me	56.4 ± 3.1	> 1000	> 1000	n.t. ^g
13d ^e	NH	A (S)	Me	242.3 ± 23.6	> 1000	> 1000	n.t. ^g
13e	NH	А	Et	65.2 ± 4.5	> 1000	> 1000	n.t. ^g
13f	NH	endo-B	Et	39.6 ± 5.7	> 1000	> 1000	n.t.
13g ^f	NH	А	i-Pr	70.4 ± 16.5	> 1000	> 1000	n.t. ^g
13h ^f	NH	endo-B	i-Pr	12.7 ± 1.9	> 1000	> 1000	12.8 ± 1.5
13i	NH	А	$PhCH_2$	24.4 ± 1.5	> 1000	> 1000	19.1 ± 3.6
13j	NH	endo-B	$PhCH_2$	18.4 ± 1.8	> 1000	> 1000	14.3 ± 2.8
14a	0	А	Н	171.7 ± 31.1	> 1000	> 1000	n.t. ^g
14b	0	endo-B	Н	260.1 ± 48.9	> 1000	> 1000	n.t. ^g
15a ^f	0	endo-B	$PhCH_2$	20.2 ± 4.2	> 1000	> 1000	10.6 ± 1.8
granisetron				1.8 ± 0.1	> 1000	> 1000	0.7 ± 0.2
tropisetron				1.5 ± 0.1	125.1 ± 15.8	> 1000	1.7 ± 0.3
cisapride				511.8 ± 36.4	101.2 ± 15.6	54.8 ± 4.8	n.t. ^g

^aC, H, N analyses were within $\pm 0.4\%$ of the theoretical values; ^bK_i (nM); ^cED₅₀ (μ g/kg i.v.); ^d[α]_D²⁰ + 27° (c = 0.19, EtOH); ^e[α]_D²⁰ -27° (c = 0.19, EtOH); ^ffumarate; ^gnot tested.

moderate 5-HT₃ antagonist activity, with ED_{50} values of 10.6–19.1 mg/kg i.v. (granisetron: 0.7 mg/kg i.v.).

We conclude that these compounds represent a new structural class of $5-HT_3$ ligands with selectivity over $5-HT_4$ and D_2 receptors and moderate $5-HT_3$ antagonist activity when intravenously administered. Studies are in progress in order to obtain more potent $5-HT_3$ receptor antagonists in this series.

5. Experimental protocols

5.1. Chemistry

Flash column chromatography was performed on silica gel, particle size 60 Å, mesh = 23–400 (Merck). Melting points were determined in open capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. Elemental analyses are within \pm 0.4% of the theoretical values. IR spectra were taken on a Perkin-Elmer 1310 instrument on KBr plates. ¹H- and ¹³C-NMR were recorded on a Bruker AC-200; chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Spectral data are consistent with assigned structures. Specific optical rotation at the sodium D line were measured in a 1 dm sample tube on a Perkin-Elmer 341 polarimeter.

The following compounds were prepared as described in the literature: benzimidazole-2-carboxylic acid **6** [13], *endo*-3-aminotropane **9** [15], and *exo*-3-aminotropane **10** [14].

5.1.1. General procedure for compounds 11

To a solution of benzimidazole-2-carboxylic acid **6** (10 mmol) in DMF (40 mL), 1,1'-carbonyldiimidazole (11 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, and then an appropriate amine (11 mmol) was added. Stirring was continued overnight and then the solvent evaporated. The residue was poured into water (30 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried (Na₂SO₄) and the solvent was evaporated under reduced pressure to yield the crude product **11** which was further purified by silica gel column chromatography (MeOH:NH₄OH, 98:2).

5.1.1.1. N-(1-azabicyclo[2.2.2]octan-3-yl)-1H-benzimidazole-2-carboxamide **11a**

Yield: 67%; m.p.: 145–147 °C. ¹H NMR (CDCl₃) 1.54 (m, 1H, CH₂), 1.75 (m, 2H, CH₂), 1.89 (m, 1H, CH₂), 2.10 (m, 1H, CH₂), 2.72–3.02 (m, 5H, CH₂), 3.48 (m, 1H, CH), 4.21 (m, 1H, CH), 7.32–7.36 (m, 2H, ar), 7.51 (m, 1H, ar), 7.77 (m, 1H, ar), 7.89 (br d, 1H, CONH), 12.25 (br s, 1H, NH). ¹³C NMR (CDCl₃) 19.97, 25.66, 25.88, 46.45, 47.14, 47.26, 55.05, 112.37, 120.19, 123.34, 124.75, 134.48, 142.65, 145.20, 158.47.

5.1.1.2. endo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3yl)-1H-benzimidazole-2-carboxamide **11b**

Yield: 73%; m.p.: 183–185 °C. ¹H NMR (CDCl₃) 1.76–2.43 (m, 8H, CH₂), 2.38 (s, 3H, CH₃), 3.25 (m, 2H, CH), 4.39 (m, 1H, CH), 7.28–7.34 (m, 2H, ar), 7.54 (m, 1H, ar), 7.81 (m, 1H, ar), 8.15 (br d, 1H, CONH), 12.30 (br s, 1H, NH). ¹³C NMR (CDCl₃) 25.70, 36.17, 40.16, 41.83, 59.83, 112.28, 120.31, 123.07, 124.56, 134. 35, 142.76, 145.22, 158.82.

5.1.1.3. exo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3-yl)-1H-benzimidazole-2-carboxamide **11c**

Yield: 71%; m.p.: 250–252 °C. ¹H NMR (DMSO d_6) 1.42–2.14 (m, 8H, CH₂), 2.20 (s, 3H, CH₃), 3.06 (m, 2H, CH), 4.13 (m, 1H, CH), 7.24 (m, 2H, ar), 7.58 (m, 2H, ar), 8.70 (br d, 1H, CONH), 12.25 (br s, 1H, NH). ¹³C NMR (DMSO- d_6) 26.36, 35.46, 38.38, 39.91, 59.69, 112.94, 120.49, 123.37, 124.92, 134.84, 142.82, 145.06, 158.61.

5.1.2. General procedure for compounds 13

To an ice-cooled solution of benzimidazole-2carboxylic acid **6** (30 mmol) in 60 mL of DMF, HNa (60% suspension, 66 mmol) was added, and the mixture was stirred for 30 min. The appropriate alkyl halide (66 mmol) was added dropwise and the resulting suspension was stirred for 3 h at room temperature. Aqueous sodium hydroxide (10%, 20 mL) was slowly added and the mixture extracted with dichloromethane (3×20 mL). The combined organic layers were dried (Na₂SO₄) and concentrated, and compound **12** was isolated by silica gel chromatography (Cl₂CH₂:MeOH, 9:1).

To a solution of an appropriate amine (11 mmol) in 30 mL of toluene, a solution of trimethylaluminium in toluene (2 M, 11 mmol) was added, and the resulting mixture was stirred for 1 h at room temperature. Compound **12** (9 mmol) was added, and the mixture was heated to reflux for 3 h and then cooled. Water (10 mL) was added dropwise, and the resulting precipitate was filtered off. Evaporation of the filtrate under reduced pressure and further purification by silica gel chromatography (MeOH:NH₄OH, 98:2) afforded **13**.

5.1.2.1. N-(1-azabicyclo[2.2.2]octan-3-yl)-1-methyl-1Hbenzimidazole-2-carboxamide **13a**

Yield: 64%; m.p.: 143–145 °C. ¹H NMR (CDCl₃) 1.58 (m, 1H, CH₂), 1.72 (m, 2H, CH₂), 1.83 (m, 1H, CH₂), 2.05 (m, 1H, CH₂), 2.53–2.99 (m, 5H, CH₂), 3.43 (m, 1H, CH), 4.15 (m, 1H, CH), 4.22 (s, 3H, CH₃), 7.31–7.42 (m, 3H, ar), 7.45–7.79 (m, 1H, ar), 7.89 (br d, 1H, CONH). ¹³C NMR (CDCl₃) 20.18, 25.82, 25.84, 31.96, 46.64, 46.84, 47.49, 55.79, 110.37, 120.46, 123.43, 124.51, 136.95, 140.87, 143.25, 159.69.

5.1.2.2. endo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3yl)-1-methyl-1H-benzimidazole-2-carboxamide **13b**

Yield: 74%; m.p.: 143–145 °C. ¹H NMR (DMSO- d_6) 1.68–2.09 (m, 8H, CH₂), 2.15 (s, 3H, CH₃), 3.03 (m, 2H, CH), 4.05 (m, 1H, CH), 4.12 (m, 3H, CH₃), 7.28–7.38 (m, 2H, ar), 7.64–7.74 (m, 2H, ar), 8.32 (br d, 1H, CONH). ¹³C NMR (DMSO- d_6) 25.51, 31.69, 35.30, 39.07, 40.74, 59.22, 111.22, 120.04, 123.03, 124.06, 136.68, 140.48, 143.88, 158.46.

5.1.2.3 N-(1-azabicyclo[2.2.2]octan-3-yl)-1-ethyl-1Hbenzimidazole-2-carboxamide **13e**

Yield: 68%; m.p.: 120–122°C. ¹H NMR (CDCl₃) 1.48 (t, 3H, CH₃, J = 7.2 Hz), 1.50 (m, 1H, CH₂), 1.72 (m, 2H, CH₂), 1.85 (m, 1H, CH₂), 2.05 (m, 1H, CH₂), 2.55–2.99 (m, 5H, CH₂), 3.42 (m, 1H, CH), 4.14 (m, 1H, CH), 4.78 (q, 2H, CH₂, J = 7.2 Hz), 7.27–7.48 (m, 3H, ar), 7.77 (m, 1H, ar), 7.81 (br d, 1H, CONH). ¹³C NMR (CDCl₃) 15.51, 20.20, 25.84, 25.85, 40.40, 46.67, 46.88, 47.41, 55.72, 110.45, 120.56, 123.36, 124.46, 135.96, 141.05, 142.78, 159.47.

5.1.2.4. endo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3yl)-1-ethyl-1H-benzimidazole-2-carboxamide **13f**

Yield: 57%; m.p.: 197–199 °C. ¹H NMR (DMSO- d_6) 1.43 (t, 3H, CH₃, J = 7.2 Hz), 2.02–2.45 (m, 8H, CH₂), 2.15 (s, 3H, CH₃), 3.63 (m, 2H, CH), 4.05 (m, 1H, CH), 4.81 (q, 2H, CH₂, J = 7.2 Hz), 7.28–7.48 (m, 2H, ar), 7.75–7.84 (m, 2H, ar), 8.82 (br d, 1H, CONH). ¹³C NMR DMSO- d_6) 15.53, 23.68, 32.33, 37.56, 39.07, 40.74, 60.22, 111.19, 120.23, 123.04, 124.21, 135.51, 140.74, 143.78, 159.18.

5.1.2.5. N-(1-azabicyclo[2.2.2]octan-3-yl)-1-isopropyl-1H-benzimidazole-2-carboxamide (fumarate) **13g**

Yield: 51%; m.p.: 122 °C (dec). ¹H NMR (DMSO- d_6) 1.54 (d, 6H, CH₃, J = 6.9 Hz), 1.53 (m, 1H, CH₂), 1.73 (m, 2H, CH₂), 1.87 (m, 1H, CH₂), 2.06 (m, 1H, CH₂), 2.79–3.22 (m, 5H, CH₂), 3.42 (m, 1H, CH), 4.21 (m, 1H, CH), 5.64 (m, 1H, CH), 7.21–7.38 (m, 2H, ar), 7.68–7.87 (m, 2H, ar), 9.21 (br d, 1H, CONH). ¹³C NMR (DMSO- d_6) 18.36, 21.01, 23.27, 25.26, 40.76, 45.16, 45.52, 48.15, 51.03, 110.45, 120.56, 123.36, 124.46, 135.96, 141.05, 142.78, 159.47.

5.1.2.6. endo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3yl)-1-isopropyl-1H-benzimidazole-2-carboxamide (fumarate) **13h**

Yield: 53%; m.p.: 183–185 °C. ¹H NMR (DMSO- d_6) 1.57 (d, 6H, CH₃, J = 6.92 Hz), 2.04–2.40 (m, 8H, CH₂), 2.15 (s, 3H, CH₃), 3.61 (m, 2H, CH), 4.01 (m, 1H, CH), 5.70 (m, 1H, CH), 7.24–7.35 (m, 2H, ar), 7.74–7.86 (m, 2H, ar), 8.77 (br d, 1H, CONH). ¹³C NMR (DMSO- d_6) 21.01, 23.84, 32.44, 37.68, 40.75,48.16, 60.19, 113.39, 120.53, 122.62, 123.75, 134.03, 141.45, 144.44, 159.90.

5.1.2.7. N-(1-azabicyclo[2.2.2]octan-3-yl)-1-phenylmethyl-1H-benzimidazole-2-carboxamide **13i**

Yield: 64%; m.p.: 179–181°C. ¹H NMR (DMSO- d_6) 1.24 (m, 1H, CH₂), 1.59 (m, 2H, CH₂), 1.75 (m, 1H, CH₂), 1.86 (m, 1H, CH₂), 2.60–3.12 (m, 5H, CH₂), 3.40 (m, 1H, CH), 4.07 (m, 1H, CH), 5.96 (s, 2H, CH₂), 7.15–7.38 (m, 7H, ar), 7.63 (m, 1H, ar), 7.76 (m, 1H, ar), 8.87 (br d, 1H, CONH). ¹³C NMR (DMSO- d_6) 19.88, 25.62, 25.86, 46.37, 46.62, 46.95, 47.46, 53.06, 111.68, 120.17, 123.13, 124.30, 126.98, 127.43, 128.53, 135.76, 137.31, 140.80, 143.93, 159.60.

5.1.2.8. endo-N-(8-methyl-8-azabicyclo[3.2.1]octan-3yl)-1-phenylmethyl-1H-benzimidazole-2-carboxamide **13j**

Yield: 70%; m.p.: 125–127°C. ¹H NMR (DMSO- d_6) 1.68–2.09 (m, 8H, CH₂), 2.14 (s, 3H, CH₃), 3.02 (m, 2H, CH), 4.03 (m, 1H, CH), 5.95 (s, 2H, CH₂), 7.17–7.36 (m, 7H, ar), 7.60 (m, 1H, ar), 7.77 (m, 1H, ar), 8.41 (br d, 1H, CONH). ¹³C NMR (DMSO- d_6) 25.48, 35.21, 39.91, 41.16, 47.53, 59.20, 111.72, 120.27, 123.24, 124.36, 126.95, 127.42, 128.52, 135.94, 137.23, 140.67, 143.63, 158.48.

5.1.3. General procedure for compounds 14

To a solution of benzimidazole-2-carboxylic acid **6** (10 mmol) in DMF (40 mL), 1,1'-carbonyldiimidazole (11 mmol) was added. After stirring the reaction mixture for 3 h at room temperature, the corresponding alcohol (11 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (11 mmol) were added, and the mixture was heated at 60 °C for 18 h. Solvent was removed under reduced pressure and the residue was extracted with Cl_2CH_2 (3 × 20 mL). The combined organic layers were dried (Na₂SO₄) and evaporated to give a residue which was purified by silica gel chromatography (Cl_2CH_2 : triethylamine, 95:5) to yield **14**.

5.1.3.1. 1-azabicyclo[2.2.2]octan-3-yl benzimidazole-2carboxylate **14a**

Yield: 42%; m.p.: 128 °C (dec). ¹H NMR (DMSO- d_6) 1.40 (m, 1H, CH₂), 1.63 (m, 2H, CH₂), 2.88 (m, 1H, CH₂), 2.17 (m, 1H, CH₂), 2.61–2.87 (m, 5H, CH₂), 3.27 (m, 1H, CH), 5.12 (m, 1H, CH), 7.23–7.40 (m, 2H, ar), 7.60–7.79 (m, 2H, ar). ¹³C NMR (DMSO- d_6) 19.12, 24.31, 25.21, 46.18, 46.93, 54.57, 73.68, 112.72, 114.65, 121.60, 124.51, 139.81, 142.06, 149.96, 159.92.

5.1.3.2. endo-8-methyl-8-azabicyclo[3.2.1]octan-3-yl benzimidazole-2-carboxylate **14b**

Yield: 49%; 106 °C (dec). ¹H NMR (DMSO- d_6) 1.92–2.32 (m, 8H, CH₂), 2.30 (s, 3H, CH₃), 3.21 (m, 2H, CH), 5.29 (m, 1H, CH), 7.27–7.40 (m, 2H, ar), 7.72 (m, 2H, ar), 12.21 (br s, 1H, NH). ¹³C NMR (DMSO- d_6) 25.92, 36.63, 40.52, 59.82, 70.58, 115.70, 116.67, 121.85, 123.91, 138.59, 142.16, 151.01, 159.80.

5.1.4. endo-8-methyl-8-azabicyclo[3.2.1]octan-3-yl-1phenylmethyl-1H-benzimidazole-2-carboxylate (fumarate) **15a**

To an ice-cooled solution of **14b** (4.56 mmol, 1.3 g) in DMF (40 mL), HNa (60% suspension, 6 mmol, 0.25 g) was slowly added, and the mixture was stirred for 30 min. Benzyl bromide (6 mmol, 0.7 mL) was added dropwise, and the solution was stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuo and the residue was treated with H₂O (30 mL) and extracted with CHCl₃/ⁱPrOH (4/1). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give a residue which was purified by silica gel chromatography (Cl₂CH₂:triethylamine, 1:1) to provide **15a** (65%). ¹H NMR (DMSO-*d*₆) 1.91–2.42 (m, 8H, CH₂), 2.43 (s, 3H, CH₃), 3.71 (m, 2H, CH), 5.20 (m, 1H, CH), 5.89 (m, 2H, CH₂), 7.16–7.42 (m, 7H, ar), 7.67 (m, 1H, ar), 7.85 (m, 1H, ar). ¹³C NMR (DMSO- d_6) 24.35, 33.52, 37.70, 48.20, 59.96, 67.23, 111.86, 121.21, 123.62, 125.46, 126.68, 127.51, 134.86, 136.01, 137.06, 140.86, 141.30, 158.85.

5.2. Pharmacology

5.2.1. 5-HT₃ receptor binding assay [17]

Adult male Wistar rats weighing 220–280 g, were used. Animals were killed by decapitation and the whole brain, with the exception of the brainstem and cerebellum, was quickly removed and the various areas dissected, weighed and immediately frozen at -70 °C. Enthorinal cortex used for the binding experiments was homogenized with an Ultra-Turrax (setting 5 for 20 s) in 10 volumes of ice-cold 0.32 M sucrose buffer, centrifuged at 1 000 g for 10 min (4 °C) and the supernatant was

recentrifuged at 17 000 g for 20 min (4 °C). The resulting pellet was resuspended in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4), incubated at 37 °C for 10 min and then centrifuged three times more at $48\ 000\ g$ for 10 min (4 °C). The final pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer containing 5 mM CaCl₂ and 0.1% ascorbate, and was stored at -70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer with 10 µM pargyline (final dilution 1:40, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [³H]-LY278584 (Amersham, 60–85 Ci/mmol) in buffer (final concentration 2 nM). Non-specific binding was determined using 10 µM cold 5-HT. Binding experiments were initiated by addition of 0.8 mL of membrane suspension (500-600 µg of protein). After incubation for 30 min at 25 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% polyethylenimine in 50 mM Tris-HCl buffer), using a Brandel Cell Harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris-HCl (pH 7.4) buffer. Filters were placed in polyethylene scintillation vials (with 5 mL of scintillation cocktail), equilibrated, and filter-retained radioactivity measured in a liquid scintillation counter (Kontron Beta V). IC550 and Ki values were calculated using the computer program EBDA (McPherson).

5.2.2. 5- HT_4 receptor binding assay [18]

Adult male Dunkin-Hartley guinea-pigs weighing 350-400 g were used. Animals were killed by decapitation and the whole brain, with the exception of the brainstem and cerebellum, was quickly removed and the various areas dissected, weighed and immediately frozen at -70 °C. Striatum used for the binding experiments was homogenized with an Ultra-Turrax (setting 5 for 20 s) in 15 volumes of ice-cold 50 mM Hepes buffer (pH 7.4) and centrifuged at 48 000 g for 20 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Hepes buffer (pH 7.4) and was stored at -70 °C until use. At the time of the experiment, the membranes were diluted in the same ice-cold buffer (final dilution 1:80, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [³H]GR-113808 (Amersham, 60-85 Ci/mmol) in buffer (final concentration 0.1 nM). Non-specific binding was determined using 10 µM cold 5-HT. Binding experiments were initiated by addition of 0.8 mL of 421

membrane suspension (800–900 µg of protein). After incubation for 30 min at 25 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass pretreated filters (1% polyethylenimine in 50 mM Hepes buffer), using a Brandel Cell Harvester. Filters were placed in polyethylene scintillation vials (with 5 mL of scintillation cocktail), equilibrated, and filter-retained radioactivity measured in a liquid scintillation counter (Kontron Beta V). IC₅₀ and K_i values were calculated using the computer program EBDA (McPherson).

5.2.3. D₂ receptor binding assay [19]

Adult male Wistar rats weighing 220-280 g were used. Animals were killed by decapitation and the whole brain, with the exception of the brainstem and cerebellum, was quickly removed and the various areas dissected, weighed and immediately frozen at -70 °C. Striatum used for the binding experiments was homogenized with an Ultra-Turrax (setting 5 for 20 s) in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7), centrifuged at 48 000 g for 10 min (4 °C). The resulting pellet was resuspended in 50 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7), incubated at 37 °C for 10 min and then centrifuged once more at 48 000 g for 10 min (4 °C). The resulting pellet was resuspended in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) and was stored at -70 °C until use. At the time of experiment, the membranes were diluted in the same ice-cold buffer with 10 µM pargyline (final dilution 1:150, wt/vol). Competition assays were performed in triplicate, in a final volume of 1 mL. To each assay tube were added the following: 0.1 mL of the displacer drug concentration (0.1 mL of vehicle if no competing drug was added) and 0.1 mL of [³H]Raclopride (NEN, 60-87 Ci/mmol) in buffer (final concentration 1 nM). Non-specific binding was determined using 1 µM cold (+)-butaclamol. Binding experiments were initiated by addition of 0.8 mL of membrane suspension (300-400 µg of protein). After incubation for 60 min at 25 °C the reaction was stopped for vacuum filtration through Whatman GF/B glass filters, using a Brandel Cell Harvester, followed by two washes with 5 mL of ice-cold 50 mM Tris-HCl (pH 7.7) buffer. Filters were placed in polyethylene scintillation vials (with 5 mL of scintillation cocktail), equilibrated, and filter-retained radioactivity measured in a liquid scintillation counter (Kontron Beta V). IC₅₀ and K_i values were calculated using the computer program EBDA (McPherson).

5.2.4. Inhibition of the von Bezold-Jarisch reflex

Adult male Wistar rats weighing 220–300 g and fasted for 18 h were used. Rats were anaesthetized with urethane (1.25 g/kg i.p.) and placed on a heating table (Hugo Sachs Elektronik, Freiburg, Germany) to maintain body temperature at 37 °C. The trachea and right jugular vein were cannulated to facilitate respiration and drug administration, respectively. The carotid artery was also cannulated and connected to a Goul Statham P23Db pressure transducer to record blood pressure. The heart rate was measured using the blood pressure signal and a cardiotachometer coupler and recorded on a Graphtec Linearcorder WR3101 (Hugo Sachs Elektronik, Freiburg, Germany). The B-J reflex was evoked by rapid bolus i.v. injection of 5-HT (30 µg/kg). When 5-HT-induced bradycardia (65–75% fall of heart rate from control heart rate) returned to pretreatment levels (within 5 min), either antagonist or saline were administered (1 mL/kg) and 5-HT-induced bradycardia was elicited again 5 min later. Drugs were prepared daily and were dissolved in distilled water or 0.1 M tartaric acid, with subsequent dilutions in physiological saline. ED₅₀ values were calculated from 3-5 doses (4-5 rats per dose) by a linear-regression analysis.

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