Preparation and *In Vitro* Pharmacology of 5-HT4 Receptor Ligands. Partial Agonism and Antagonism of Metoclopramide Analogous Benzoic Esters☆

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Summary

Alicyclic ester analogues of the gastroprokinetic benzamide metoclopramide (**1**) and its ester congener SDZ 205557 (**2**), a 5-HT4 receptor antagonist, were prepared by *O*-alkylation of 4-amino-5-chloro-2-methoxybenzoate with *N*-(2-chloroethyl) substituted alicyclic amines. The bromo and iodo analogue of compound **13b** (2-(1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoate) were obtained by halogenation of dechloro-**13b** with *N*-halogenated succinimides. The series was evaluated in functional *in vitro* assays with regard to affinity for serotoninergic 5-HT4, 5-HT3 and muscarinic M3 receptors. The affinities for 5-HT3 and M3 receptors were below 6.0 (pK_B or pA_2). On 5-HT4 receptors in guinea-pig ileal longitudinal muscle and rat oesophagus, the majority of compounds revealed partial 5-HT4 receptor agonism susceptible to blockade by SDZ 205557, a reference 5-HT4 receptor antagonist (pK_B = 7.25 – 7.73 (guinea-pig ileum) and 7.09 – 7.43 (rat oesophagus)). The relative agonist potency was in the range of 5 – 303 % (5-HT: 100 %). Compound **13b** and its bromo analogue **17** were the most potent esters of the series. The enantiomers of **13g** ((*R*)- and (*S*)-2-(2-methyl-1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoate) interacted stereoselectively with 5-HT4 receptors and displayed enantiomeric potency ratios (*R*)/(*S*) of 4.3 – 8.7. There was an excellent correlation between (a) antagonist affinity on guinea-pig ileum and rat oesophagus, (b) relative agonist potency on guinea-pig ileum and rat oesophagus, and (c) between antagonist affinity and relative agonist potency within each assay ($r^2 > 0.91$). The new compounds may serve as academic tools in evaluating the functional role of 5-HT4 receptors. The selective partial 5-HT4 receptor agonists presented in this paper may be useful to restore physiological motility and secretion in the gut with reduced or absent propensity to elicit tachycardia and desensitization of the intestinal target receptor.

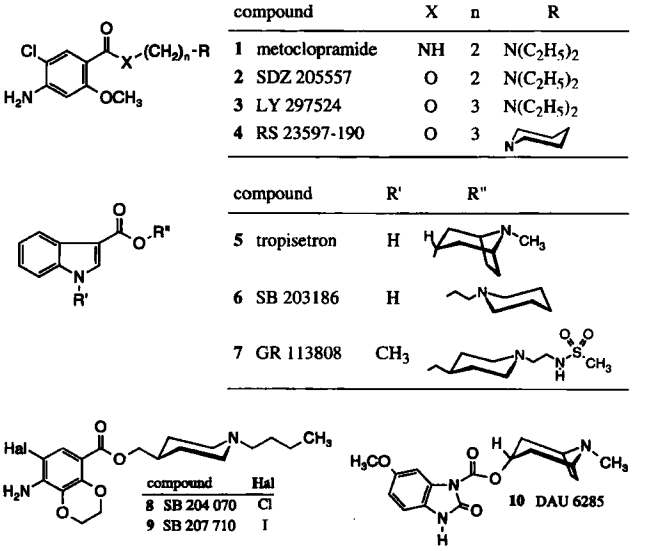
Introduction

The term '5-HT4 receptor' appeared for the first time in 1988 when the group of *Bockaert* presented evidence for the existence of another orphan receptor for serotonin (5-hydroxytryptamine, 5-HT) in fetal mouse colliculi neurons[1]. Since then, numerous 5-HT receptor mediated effects have been reported which display a pharmacological profile compatible with a 5-HT4 receptor related mechanism (for review see refs.[2-4]). Against this background, the recently revised nomenclature for 5-HT receptors[5] has officially introduced a fourth family of 5-HT receptors designated '5-HT4'.

Neuronally located 5-HT4 receptors have been discovered in the ileum of guinea-pig[6-9] and man[10] where they induce the release of acetylcholine[11,12] followed by a contraction of smooth muscle *via* muscarinic M3 receptors. 5-HT4 receptors are also found in the guinea-pig colon [13,14], rat oesophagus [15,16], frog adrenals[17], urinary bladder of man and rhesus monkey[18], and in the heart of pig[19–21] and man[22–25] where they evoke tachycardia[19–21], positive inotropy[22,23] and arrhythmias[25]. Activation of 5-HT4 receptors results in the formation of cyclic AMP *via* G proteins[1,23,26-28]. The cloning of a 5-HT4 receptor encoding cDNA has recently been claimed[29]. With the advent of potent radioligands, *viz.* [^{3}H]-GR 113808[30] and [^{125}I]-SB 207710[31], the existence of 5-HT4 receptors in the CNS of several species has been established[26,30,32-34] although their physiological role appears enigmatic at present.

Besides 5-HT and 5-methoxytryptamine (5-MeOT) which are full 5-HT4 receptor agonists, gastroprokinetic benzamides related to metoclopramide (**1**, Chart 1) have been reported as partial or full agonists for this receptor family, *e.g.* cisapride, renzapride and zacopride.[6,8,9,14,15,26] Historically tropisetron (**5**)[1], which is a potent 5-HT3 receptor blocker, and the more selective ester analogue of **1**, SDZ 205557 (**2**)[35,36] , were introduced as the first competitive 5-HT4 receptor antagonists. Recently developed ester-type 5-HT4 receptor antagonists possess improved selectivity, *e.g.* DAU 6285 (**10**)[37], LY 297524 (**3**)[38], RS 23597-190 (**4**)[39] and SB 203186 (**6**)[40], and nanomolar or subnanomolar affinity, such as GR 113808 (**7**)[41], SB 204070 (**8**)[42] and

Chart 1. Structures of metoclopramide (**1**), a partial 5-HT4 receptor agonist, and of 5-HT4 receptor antagonists **2** – **10**.



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SB 207710 (**9**)[43]. These antagonists represent powerful experimental tools and may find therapeutic application for the treatment of arrhythmias[4], bladder dysfunctions and CNS disorders[3].

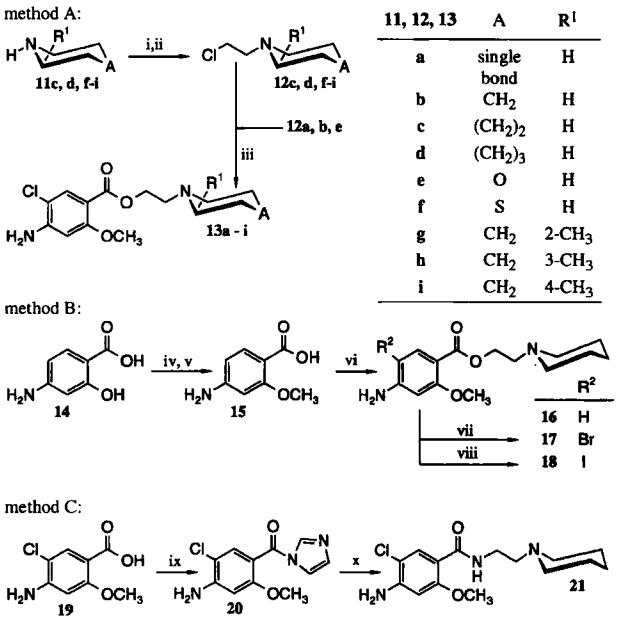
While newer antagonist developments display indole (**6**, **7**), benzimidazolone (**10**), benzodioxane (**8**, **9**) or imidazopyridine carboxylic acid functions[3] instead of the 4-amino-5-chloro-2-methoxybenzoic acid moiety of **1** and **2**, only few reports have been published concerning closer analogues of **2**. The higher homologue of **2**, ester **3**, and its piperidine analogue **4** antagonize 5-HT_4 receptor mediated effects with pK_B values of 7.7 – 7.8[3,38,39]. The present study is focused on the synthesis, pharmacological *in vitro* evaluation and structure-activity relationships of ester-type analogues of **1** and **2**, characterized by a *two-carbon* chain connecting ester and tertiary amine function. Alicyclic amine moieties have been chosen instead of the diethylamine structure of **1** and **2** (a) to try to enhance the 5-HT_4 receptor affinity, and (b) to introduce chirality and decide whether it will be worth-while studying the biological profile of stereoisomers in this series. A preliminary account on the antagonist properties of the title compounds has been presented in 1993 during the annual meeting of the German Pharmaceutical Society[44]. Independently, *Langlois et al.* have reported on the 5-HT_4 receptor partial agonism of compound **13b** (Chart 2)[45].

Results and Discussion

Chemistry. Alicyclic analogues of **2** were prepared by *O*-alkylation[46] of the benzoic acid **19** with alicyclic *N*-(2-chloroethyl) substituted amines **12a-g** which were either commercially available or prepared from the corresponding amino alcohols or secondary amines **11c,d,f-i**, respectively (Chart 2, method A). An alternative synthesis for **13b** has been reported recently.[45] Resolution of 2-methylpiperidine with (*R*)-(+)- and (*S*)-(–)-mandelic acid[47] afforded (*R*)-(–)-**11g** and (*S*)-(+)-**11g** which served as starting material for the preparation of (*R*)-(–)-**13g** and (*S*)-(+)-**13g**, respectively (method A). The absolute configuration of the enantiomers of **13g** is deduced from the known absolute configuration of (*R*)-**11g** and (*S*)-**11g** which correspond to (*S*)-(–)- and (*R*)-(+)-pipecolinic acid[48,49]. Although the enantiomeric purity of (*R*)- and (*S*)-**13g** has not been determined to date, the biological properties of the antipodes (Tables 2 and 4) indicate a sufficient enantiomeric excess. The 5-bromo and 5-iodo analogues of **13b** (**17** and **18**) were accessible by treatment of 5-dechloro-**13b** (**16**) with the respective *N*-halogenated succinimide as reported previously for the synthesis of 5-iodozacopride[50]. The amide-type hybrid of **1** and **13b** (**21**) was obtained from the reaction of the imidazolide of **19** (**20**) with *N*-(2-aminoethyl)piperidine (method C). Preparative and physicochemical properties of the title compounds are given in Table 1.

Pharmacology. The majority of compounds was evaluated in two functional assays for the interaction with 5-HT_4 receptors. Stimulated by recent reports concerning **13b** and zacopride related esters,[45] an investigation into the partial agonist properties of the title compounds on guinea-pig ileum and rat oesophagus was undertaken (Tables 2 and 3). Except **2** and **13d**, all compounds mimic 5-HT in contracting guinea-

Chart 2. Synthesis of metoclopramide related esters **13a – i** (method A), of the bromo and iodo analogue of **13b** (method B), and of the amide congener of **13b** (method C).[a]



[a]Experimental conditions, reagents and solvents: (i) $Br(CH_2)_2OH$, KOH in MeCN; (ii) 4-DMAP, $SOCl_2$ in $CHCl_3$; (iii) **19**, 1.25 equiv. Na in absol. *i*PrOH; (iv) $(Me_2O)_2SO_2$, K_2CO_3 in Me_2CO; (v) aqueous KOH; (vi) 1.25 equiv. Na in absol. *i*PrOH, 1 equiv. *N*-(2-chloroethyl)piperidine; (vii) *N*-bromosuccinimide, TFA in $CHCl_3$; (viii) *N*-iodosuccinimide, TFA in $CHCl_3$; (ix) CDI in absol. THF, N_2 atmosphere; (x) *N*-(2-aminoethyl)piperidine in absol. THF.

Table 1. Preparative and physicochemical properties of 5-HT_4 receptor ligands **13a-i**, **16-18**, **21** and of the imidazolide **20**.

compd	method[a]	yield[b] [%]	mp[c] [°C]	formula (C, H, N)[d]	MS (EI[e] or ^+FAB[f])
13a	A	17	102	$C_{14}H_{19}ClN_2O_3$	*299 (34), 98*
13b	A	54	137	$C_{15}H_{21}ClN_2O_3$	*313 (52), 112*
13c	A	31	132	$C_{16}H_{23}ClN_2O_3$	326 (7), 228
13d	A	16	118	$C_{17}H_{25}ClN_2O_3$	*341 (7), 140*
13e	A	50	143	$C_{14}H_{19}ClN_2O_4$	*315 (12), 184*
13f	A	42	152	$C_{14}H_{19}ClN_2O_3S$	*331 (12), 184*
(±)-**13g**	A	12	102	$C_{16}H_{23}ClN_2O_3$	*327 (19), 112*
(*R*)-(–)**13g**	A	21	90	$C_{16}H_{23}ClN_2O_3$	*327 (28), 126*
(*S*)-(+)**13g**	A	15	93	$C_{16}H_{23}ClN_2O_3$	*327 (20), 112*
(±)-**13h**	A	21	124	$C_{16}H_{23}ClN_2O_3$	*327 (25), 126*
13i	A	19	127	$C_{16}H_{23}ClN_2O_3$	326 (0.7), 112
16	B	14	64	$C_{15}H_{22}N_2O_3$	*279 (7), 150*
17	B	2	135	$C_{15}H_{21}BrN_2O_3$	*357 (26), 112*
18	B	31	64	$C_{15}H_{21}IN_2O_3$	*405 (16), 112*
20	C	75 – 97	124	$C_{11}H_{10}ClN_3O_2$	251 (4), 184
21	C	32	172	$C_{15}H_{22}ClN_3O_2$	*312 (52), 98*

[a] See experimental section. [b] Method A: calculated for the last synthetic step. [c] Crystallization solvent: ethyl acetate/petrolether (50 – 70 °C), except for **13a,b,e,f,h,i** (solvent: Et_2O). [d] All compounds gave sufficient elemental analyses for C, H, N, except **13f** (H, N; C: calcd, 50.8; found, 51.3). [e] EI: *m/z* (%) for [$M^{+\bullet}$] followed by *m/z* for 100 % peak. [f] ^+FAB: *m/z* (%) for [M+H^+] followed by *m/z* for 100 % peak (data given in italics).

Table 2. Partial 5-HT4 receptor agonism of the amides **1**, **21** and of related esters **2**, **13**, **16** – **18** on the *guinea-pig ileal longitudinal muscle* and the rat oesophagal *tunica muscularis mucosae* preparation.

	guinea-pig ileum				rat oesophagus			
		relative potency [%][a]		rel. E_{max}[b]		relative potency [%][a]		E_{max}
compound	*N*	mean	95 % c. l.[c]	[%]	*N*	mean	95 % c. l.[c]	[%]
5-HT		100		100		100		100
1	4	0.5	0.4–0.7	47 ± 7	6	0.8	0.4–1.7	72 ± 3
2	8			15 ± 4[d]	2			0
13a	6	20	9 – 46	20 ± 2				
13b	18	303[e]	254 – 362	44 ± 3	6	129	94 – 178	70 ± 4
13c	6	197[e]	119 – 327	42 ± 4	6	83	71 – 99	56 ± 7
13d	8	n. d.		<5	3			0
13e	6	6	4 – 10	41 ± 3				
13f	6	43	18 – 104	57 ± 6	4	19	12 – 30	70 ± 3
(±)-**13g**	4	117	46 – 297	42 ± 3				
(*R*)-(–)-**13g**	11	128[e]	105 – 156	53 ± 4	6	95	60 – 150	87 ± 1
(*S*)-(+)-**13g**	12	30	21 – 42	41 ± 2	6	12	10 – 14	84 ± 1
(±)-**13h**	6	113	90 – 143	42 ± 5	5	57	42 – 78	56 ± 6
13i	6	60	49 – 75	23 ± 5				
16	8	10	8 – 12	47 ± 7	5	7	5 – 10	45 ± 3
17	17	184[e]	156 – 216	39 ± 4	6	154[e]	107 – 221	50 ± 3
18	6	n. d.		17 ± 2	4	n. d.		<5
21	6	5	3 – 8	44 ± 6	5	6	4 – 9	81 ± 7

[a] Ratio [EC_{50}(5-HT)/EC_{50}(compound)]·100 %. [b] Calculated by normalizing the mean E_{max} of 5-HT treated control preparations to 100 %. [c] 95 % confidence limits. [d] The effect was observable at 1 – 100 µM **2** and must be attributed to a 5-HT4 receptor-independent mechanism since it was not sensitive to 5-HT4 receptor antagonists. [e] Significantly more potent than 5-HT ($P < 0.05$). n. d. not determined.

Table 3. Antagonism of the effects evoked by **1**, **13**, **16** - **18** and **21** (Table 2) by the 5-HT4 receptor blocker SDZ 205557 (**2**).

	guinea-pig ileum				rat oesophagus			
compound	*N*	pK_B for **2**	$-\log_{10} c$[a]	rel. E_{max}[b] (*N*)[c] [%]	*N*	pK_B for **2**	$-\log_{10} c$[a]	E_{max}[d] [%]
1	5	7.55 ± 0.09	7.3	35 ± 5				
13a	4	7.25 ± 0.12	7.0	17 ± 3				
13b	17	7.51 ± 0.05[e]	7.4	31 ± 9 (5)	4	7.29 ± 0.09	6.7	71 ± 3
			6.9	36 ± 7 (4)				
			6.4	29 ± 5 (4)				
			6.0	23 ± 1 (4)				
13c	4	7.38 ± 0.07	7.0	32 ± 2	4	7.14 ± 0.08	6.7	63 ± 8
13e	4	7.73 ± 0.09	7.0	20 ± 4				
13f	4	7.61 ± 0.05	7.0	51 ± 4	2	7.43; 7.21	6.7	76; 71
(*R*)-(–)-**13g**	6	7.57 ± 0.11	6.9	38 ± 1	4	7.38 ± 0.03	6.7	98 ± 3
(*S*)-(+)-**13g**	6	7.40 ± 0.08	6.9	32 ± 5	4	7.26 ± 0.10	6.7	91 ± 6
(±)–**13h**	4	7.30 ± 0.09	7.0	27 ± 6	1	7.15	6.7	80
13i	4	n. d.	7.0	<5				
16	4	7.54 ± 0.04	7.0	39 ± 10	3	7.12 ± 0.06	6.7	55 ± 1
17	19	7.69 ± 0.04[f]	7.4	29 ± 4 (7)	4	7.24 ± 0.01	6.7	65 ± 10
			7.0	27 ± 5 (4)				
			6.4	29 ± 5 (6)				
			6.2	28; 19				
18	2	n. d.	7.0	<5				
21	4	7.33 ± 0.16	7.0	51 ± 2	3	7.09 ± 0.06	6.7	75 ± 2

[a] Logarithmic concentration of antagonist **2**. [b] For 'relative E_{max}', see Table 2, footnote b. [c] (*N*) is the number of preparations treated with the indicated concentration when a set of different antagonist concentrations was used. [d] E_{max} was corrected for time dependent changes as monitored by untreated controls. [e] pA_2 value, slope $m = 1.00 \pm 0.10$. [f] pA_2 value, slope $m = 0.83 \pm 0.07$ (Significantly different from unity, $0.03 < P < 0.05$). n. d. not determined.

Table 4. 5-HT$_4$ receptor antagonism of reference compounds **1**, **2**, **4**, **5** and of title compounds **13**, **16** – **18** and **21**.[a]

	guinea-pig ileum					rat oesophagus				
compound	N	pK_B or pA_2 (slope m)	$-\log_{10} c$[d]	rel. E_{max}[b] [%]	(N)[e]	N	pK_B or pA_2 (slope m)	$-\log_{10} c$[d]	E_{max}[c] [%]	(N)[e]
1	6	5.65 ± 0.08	5.0	27; 23	(2)					
			5.3	74 ± 6	(4)					
2	13	7.49 ± 0.07	7.0	71 ± 10	(5)	15	7.34 ± 0.05	7.0	91 ± 3	(5)
		(m = 0.94 ± 0.08)	6.0	71 ± 5	(4)		(m = 0.95 ± 0.06)	6.0	98 ± 2	(5)
			5.0	39 ± 4	(4)			5.0	90 ± 4	(5)
4	6	7.39 ± 0.09	6.5	72 ± 5		2	7.67; 7.63	7.0; 6.0	108; 81	
5	14	6.52 ± 0.07	6.3	81 ± 9	(4)	19	6.60 ± 0.03	6.60	93 ± 4	(6)
		(m = 0.97 ± 0.27)	6.0	86 ± 9	(4)		(m = 0.98 ± 0.07)	6.27	97 ± 3	(4)
			5.7	86 ± 6	(6)			5.93	91 ± 9	(5)
								5.60	90 ± 8	(4)
13a	6	7.49 ± 0.05	6.0	73 ± 8		12	7.49 ± 0.07	7.0	80 ± 11	(4)
							(m = 0.95 ± 0.09)	6.0	85 ± 3	(4)
								5.0	80 ± 7	(4)
13b	19	8.50 ± 0.04	8.0	74 ± 7	(7)	21	8.41 ± 0.07[f]	8.5	92 ± 8	(5)
		(m = 1.06 ± 0.04)	7.0	53 ± 7	(6)		(m = 0.97 ± 0.06)	7.5	79 ± 4	(5)
			6.0	29 ± 6	(6)			6.5	80 ± 5	(6)
								5.5	89 ± 6	(5)
13c	6	8.58 ± 0.03	7.0	50 ± 8		8	8.24 ± 0.08	7.5	92 ± 4	(3)
							(m = 1.10 ± 0.08)	6.5	90; 76	
								5.5	83 ± 5	(3)
13d	14	8.47 ± 0.09	8.0	83 ± 7	(4)	6	7.87 ± 0.04[g]	7.0	74 ± 4	
		(m = 0.97 ± 0.15)	7.0	91 ± 10	(6)					
			6.5	59 ± 9	(4)					
13e	4	6.87 ± 0.19	6.0	70 ± 7		4	6.46 ± 0.16	5.5	90 ± 6	
13f	6	8.13 ± 0.08	7.0	58 ± 8		10	7.54 ± 0.09	7.5	90 ± 8	(3)
							(m = 0.97 ± 0.11)	6.5	76 ± 5	(3)
								5.5	63 ± 3	(4)
(±)-**13g**	6	8.07 ± 0.05	7.0	60 ± 7		6	8.20 ± 0.08	7.5	150; 80	
							(m = 1.02 ±0.09)	6.5	69; 65	
								5.5	67; 51	
(*R*)-(–)-**13g**	6	8.61 ± 0.08	7.0	29 ± 5						
(*S*)-(+)-**13g**	5	7.67 ± 0.06	7.0	63 ± 14						
(±)-**13h**	6	8.46 ± 0.06	7.0	67 ± 7		9	8.29 ± 0.12	7.5	102 ± 15	(3)
							(m = 1.15 ± 0.13)	6.5	97 ± 11	(3)
								5.5	107 ± 9	(3)
13i	6	8.38 ± 0.03	7.0	53 ± 5		8	8.01 ± 0.09	7.5	81 ± 6	(3)
							(m = 1.11 ± 0.09)	6.5	93; 85	
								5.5	86 ± 4	(3)
16	4	7.25 ± 0.04	6.0	71 ± 7						
17	8	8.74 ± 0.08	7.8	54; 46						
			7.5	121; 80						
			7.0	71 ± 10	(4)					
18	15	8.22 ± 0.07	7.8	78 ± 4	(4)	5	7.44 ± 0.05[g]	6.5	92 ± 4	
			7.5	127 ± 17	(4)					
			7.0	90 ± 8	(7)					
21	4	7.06 ± 0.10	6.0	61 ± 7						

[a] Agonist 5-HT. [b] For 'rel. E_{max}', see Table 2, footnote b. [c] See Table 3, footnote d. [d] Logarithmic concentration of antagonist. [e] See Table 3, footnote c. [f] pA_2 = 8.45 ± 0.06 *vs.* 5-methoxytryptamine; m = 1.13 ± 0.07 (N = 21); E_{max} = 87 ± 3 (0.03 μM), 85 ± 6 (0.3 μM), 90 ± 6 (3 μM) (N = 7 each). [g] *Incubation of antagonist: 120 min.*

Table 5. *5-HT3 receptor and muscarinic M3 receptor antagonism of reference compounds* **1, 2, 4, 5** *and of title compounds* **13, 16 - 18, 21.**

	5-HT3 receptor antagonism[a] (guinea-pig ileum)				M3 receptor antagonism[b] (guinea-pig ileum)			
compound	*N*	pK_B	E_{max} [%]	$-\log_{10} c$[c]	*N*	parameter	mean	(slope *m*)
1	2	5.18; 5.18	95; 90	(4.3)	3	pK_B	3.87 ± 0.10	
2	4	5.68 ± 0.03	87 ± 2	(5.0)	8	pK_B	4.51 ± 0.05	
4[d]	8	<5.00	107 ± 3	(5.0)				
5	25[e]	7.84 ± 0.03	[f]	(8 – 6)	6	pK_B	5.12 ± 0.11	
13a	4	5.41 ± 0.11	93 ± 2	(5.0)	4	pK_B	4.18 ± 0.08	
13b	4	5.90 ± 0.07	86 ± 1	(5.0)	6	pK_B	4.31 ± 0.06	
13c	4	5.75 ± 0.06	84 ± 3	(5.0)	6	pK_B	4.67 ± 0.06	
13d	4	5.62 ± 0.09	94 ± 4	(5.5)	12	pA_2	5.11 ± 0.05[g]	(*m* = 0.89 ± 0.11)
13e	4	4.64 ± 0.09	90 ± 1	(4.5)	3	pK_B	<4.0	
13f	4	5.33 ± 0.06	86 ± 3	(5.0)	4	pD'_2	3.97 ± 0.14	
(*R*)-(–)-**13g**	8	5.84 ± 0.06	92 ± 2	(5.5)	14	pA_2	5.02 ± 0.06	(*m* = 1.00 ± 0.14)
(*S*)-(+)-**13g**	7	5.97 ± 0.03	94 ± 3	(5.5)	14	pA_2	5.19 ± 0.06	(*m* = 0.87 ± 0.14)
(±)-**13h**	4	5.44 ± 0.10	78 ± 3	(5.0)	12	pA_2	5.31 ± 0.08	(*m* = 0.82 ± 0.08)[h]
13i	4	5.64 ± 0.16	87 ± 2	(5.0)	12	pA_2	5.06 ± 0.06	(*m* = 0.78 ± 0.13)
16	4	4.46 ± 0.10	98 ± 2	(4.8)	8	pK_B	4.64 ± 0.04	
17	4	5.85 ± 0.17	101 ± 4	(5.5)	3	pK_B	4.78 ± 0.07	
18	4	5.59 ± 0.06	93 ± 4	(5.5)	3	pK_B	4.93 ± 0.02	
21	6	5.12 ± 0.05	99 ± 3	(5.0)	4	pK_B	4.11 ± 0.13	

[a] Agonist 5-HT. [b] Agonist carbachol. [c] Logarithmic concentration of antagonist. [d] Data taken from Eglen et al.[39] Radioligand binding studies: pK_I = 5.7 (5-HT3 receptors on rat cerebral cortex) and pK_I <4.5 (M3 receptors on rat submaxilliary gland).[39] [e] pA_2 value; slope *m* = 1.00 ± 0.04. [f] 102 ± 2 (0.01 μM), 99 ± 3 (0.03 μM), 88 ± 4 (0.1 μM), 98 ± 5 (0.3 μM), 90 ± 10 (1 μM) (*N* = 5 each). [g] pD'_2 = 3.45 ± 0.25 (*N* = 3, 100 μM). [h] Significantly different from unity ($P < 0.05$).

pig ileal longitudinal muscle, and in relaxing rat oesophagal *tunica muscularis mucosae* preparations precontracted by carbachol. With regard to the maximum effect, all compounds must be classified as partial agonists (Table 2). The agonist potency covers a range of approximately two orders of magnitude (relative potency 5 – 303 %, 5-HT: 100 %). The prominent results reported for **13b**[45] are confirmed by this study (pEC_{50} = 8.40 on the electrically field stimulated guinea-pig ileum[45] *vs.* 8.40 on the quiescent ileum (this study, Figure 1C); relative potency on the rat oesophagus 154 %[45] *vs.* 129 % (this study)). The 5-HT4 receptor mediated nature of the effects was verified by competition experiments using SDZ 205557 (**2**)[35,36], a competitive 5-HT4 receptor antagonist (0.04 – 10 μM) (Figure 1C). pK_B values from single point experiments or pA_2 values are in accordance with literature data[35,36] (Table 3: 7.25 – 7.73 on the guinea-pig ileum, and 7.09 – 7.43 on the rat oesophagus, respectively). Therefore, it is beyond doubt that the serotonin-like effects of the partial agonists studied can be attributed to the stimulation of 5-HT4 receptors in both tissues.

Affinity estimates were also made using the new ligands as antagonists of 5-HT evoked effects (Table 4)[44]. Typical sets of the respective concentration-effect curves for 5-HT in the absence and presence of antagonists are presented in Figure 1A,B (guinea-pig ileum) and Figure 2A,B (rat oesophagus) for **2** and **13b**, respectively. The maximum effects elicited by 5-HT in the guinea-pig ileum assay are usually depressed in a concentration dependent manner in the presence of antagonists (*e.g.* by **13b**, Figure 1B). On the contrary, the maximal oesophagal relaxation is less affected by the presence of antagonist (Table 4, Figure 2A,B).

There is a good correlation between the results obtained on the guinea-pig ileum and the rat oesophagus assay (Figure 3A,B). On an average, both antagonist affinity and partial agonist potency on the guinea-pig 5-HT4 receptor amount to approximately 1.7-fold of the values observed for the rat 5-HT4 receptor. The same trend is displayed by the range of pK_B or pA_2 values measured for SDZ 205557 in both species (Table 3). The most prominent discrimination resides in the more lipophilic compounds of the series, *viz.* the iodinated **18** (ΔpK_B = 0.78), **13d** (ΔpK_B = 0.60), and the thiomorpholine derivative **13f** (ΔpK_B = 0.59), respectively. These factors may be indicative of slightly different 5-HT4 receptor proteins in guinea-pig and rat. However, more species-selective antago-

nist tools and comparable results from additional tissues are required to support this hypothesis. As expected from receptor theory[51], the pEC_{50} values calculated for partial agonists are in good agreement with pK_B or pA_2 values measured for these ligands as antagonists of 5-HT evoked effects. The linear relationship between antagonist affinity and partial agonist potency is displayed in Figure 3C,D for both assays.

5-HT_4 receptors have been reported to be subject to a rapidly developing tachyphylaxis after prolonged contact with full agonists, such as 5-HT or 5-methoxytryptamine, or with prokinetic benzamide partial agonists (for review see[3]). Surprisingly the ester-type partial agonists of this study do *not* induce desensitization under the experimental conditions involved. Otherwise, after an incubation time of 30 min (guinea-pig ileum) or 60 min (rat oesophagus), the response to 5-HT would have been completely abolished. The antagonist concentration dependent dextral shift of the 5-HT curves is compatible with a simple competitive interaction although the maximum depression for 5-HT in the guinea-pig model may indicate a 'desensitization' with respect to the amplitude of the contractile effect. Other possible explanations for this phenomenon of depression in the guinea-pig ileum assay may be the very short contact time (15 – 30 s) after injection of the agonist or partial agonist dose into the organ bath, or the establishment of a 'hemiequilibrium state' frequently observed for the interaction of partial agonists with usually surmountable, competitive antagonists.[52]

With regard to receptor selectivity all compounds were checked for interaction with 5-HT_3 receptors and muscarinic M_3 receptors on the guinea-pig ileum. The pK_B values for 5-HT_3 receptor antagonism are below 6.0 (Table 5) although this result should be interpreted with caution since 5-HT_3 receptor antagonists usually display lower affinity in the guinea-pig compared with other species.[3,36] However, the functional affinity of **13b** (pK_B = 5.90) matches the pK_i of 6.11 reported for the displacement of [^{3}H]-granisetron from rat posterior cortex membranes.[45] The potential interaction of 5-HT_4 receptor ligands with muscarinic M_3 receptors can obscure the interpretation of the results on the guinea-pig ileum (5-HT_3/5-HT_4) and rat oesophagus assay. These ligands, at concentrations that block ileal M_3 receptors on the smooth muscle cell, mask their interference with neuronal 5-HT_3/5-HT_4 receptors and therefore render a precise analysis of data impossible. Similar problems occur on the oesophagus model where the cumulative addition of M_3 receptor blockers would directly attenuate the carbachol induced tone and thus simulate 5-HT_4 receptor agonist properties. Fortunately M_3 receptor affinities in the series were smaller than 5.5 (Table 5) and allowed to study most of the compounds in concentrations up to 10 μM.

Structure-activity considerations. It is obvious both from the literature[35,45] and from this study that the 5-HT_4 receptor affinity of metoclopramide and analogues is enhanced by introduction of an ester instead of an amide function by approximately 1.5 – 2 orders of magnitude (**1** → **2**; **21** → **13b**). This increase is accompanied by a complete loss of intrinsic activity for diethylamine derivatives (**1** → **2**) but not for piperidines (**21** → **13b**). In the ester series characterized by a *three*-carbon chain connecting the ester and tertiary amine function, diethylamine and piperidine derivative are

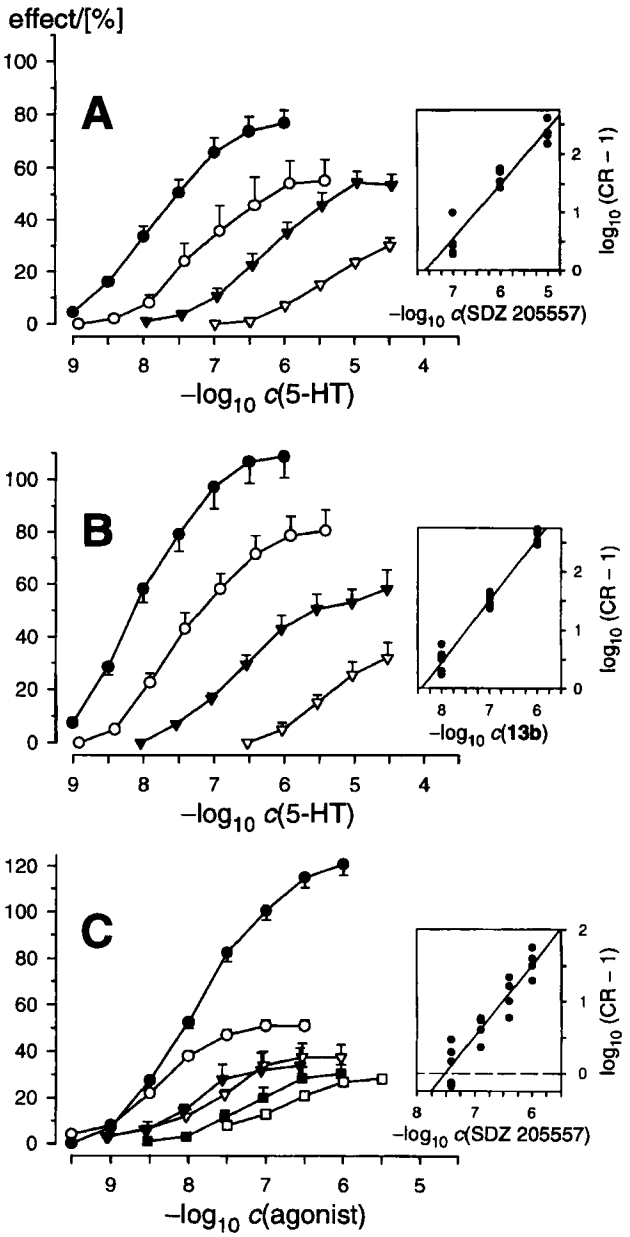


Figure 1. Contraction of quiescent guinea-pig ileal longitudinal muscle with adhering myenteric plexus in the absence and presence of 5-HT_4 receptor antagonists. Results are expressed as percent of the response observed in the second priming experiment with 5-HT (0.3 μM) for each strip. Antagonists were incubated for 30 min. *Schild* plot slopes were not significantly different from 1.00. pA_2 values were calculated after imposing the unity constraint (Tables 3 and 4). (**A**) Contraction evoked by 5-HT in the absence (●, N = 13) and presence of SDZ 205557 (μM): 0.1 (○, N = 5), 1 (▼, N = 4), and 10 (▽, N = 4). *Inset*: *Schild* plot for SDZ 205557 (N = 13). (**B**) Contraction evoked by 5-HT in the absence (●, N = 17) and presence of **13b** (μM): 0.01 (○, N = 7), 0.1 (▼, N = 6), and 1 (▽, N = 6). *Inset*: *Schild* plot for **13b** (N = 19). (**C**) Contraction evoked by 5-HT (●, N = 18) and **13b** (○, N = 18) in the absence of antagonist. Mean E_{max} and pEC_{50} were 121 ± 5 % and 7.92 (●), and 51 ± 3 % and 8.40 (○), respectively. Curves for **13b** were shifted to the right by increasing concentrations of SDZ 205557 (μM): 0.04 (▼, N = 5), 0.12 (▽, N = 4), 0.4 (■, N = 4), and 1 (□, N = 4). *Inset*: *Schild* plot for the competition of SDZ 205557 with **13b** (N = 17).

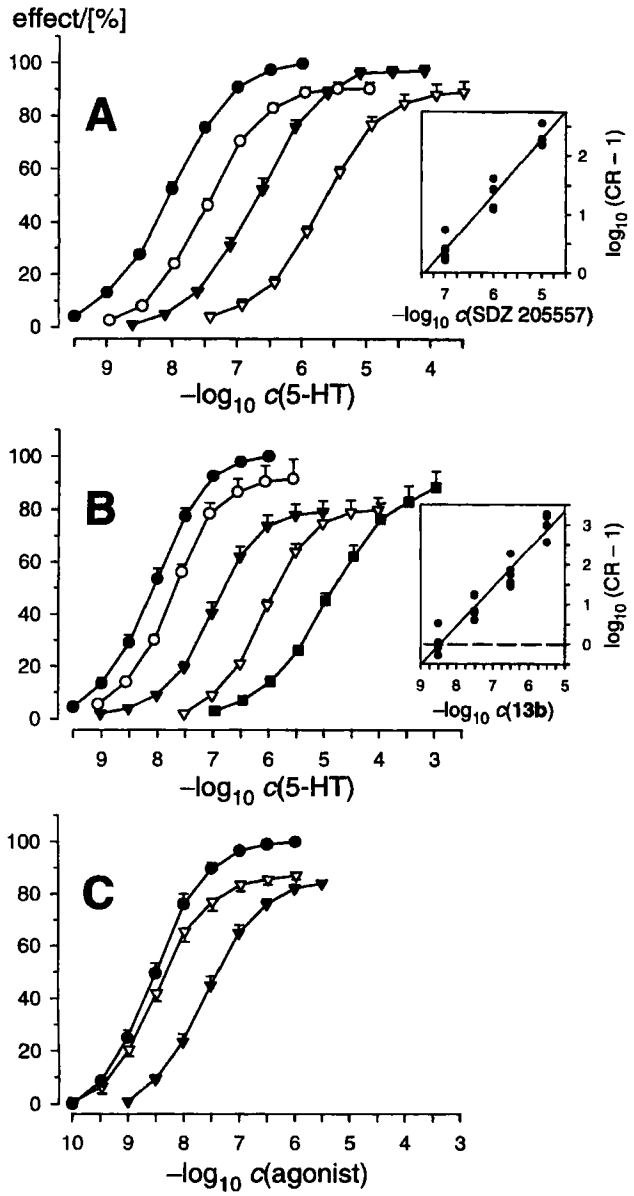


Figure 2. Relaxation of longitudinal strips of rat oesophagal *tunica muscularis mucosae* to increasing concentrations of 5-HT$_4$ receptor agonists in the absence and presence of antagonists. Antagonists were incubated for 60 min. *Schild* plot slopes were not significantly different from 1.00. pA_2 values were calculated after imposing the unity constraint (Table 4). (**A**) Relaxation evoked by 5-HT in the absence (●, N = 14) and presence of SDZ 205557 (μM): 0.1 (○, N = 5), 1 (▼, N = 5), and 10 (▽, N = 5). *Inset: Schild* plot for SDZ 205557 (N = 15). (**B**) Relaxation evoked by 5-HT in the absence (●, N = 12) and presence of **13b** (μM): 0.003 (○, N = 5), 0.03 (▼, N = 5), 0.3 (▽, N = 6), and 3 (■, N = 5). *Inset: Schild* plot for **13b** (N = 21). (**C**) Relaxation evoked by 5-HT (●, N = 12, pooled first curves), (*R*)-**13g** (▽, N = 6, second curves), and (*S*)-**13g** (▼, N = 6, second curves). The enantiomeric potency ratio of (*R*)- and (*S*)-**13g** was 8.0 (95 % confidence limits: 5.3 – 12.2).

apparently equipotent and devoid of agonist properties (**3**[38], **4**[39]). Their lower homologues, **2** and **13b**, differ significantly with respect to affinity and partial agonism (Tables 2 and 4). For **13b**, 5-HT$_4$ receptor affinity estimates up to pK_i = 8.97 (radioligand binding experiments) have been reported.[45] The rank order of affinity for homologous alicyclic

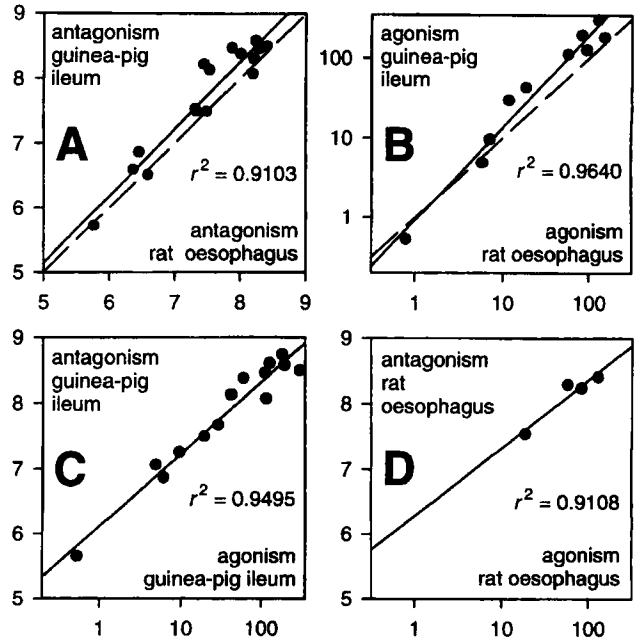


Figure 3. Linear relationship between (**A**) antagonist affinity (pK_B or pA_2) for the guinea-pig ileum and the rat oesophagus 5-HT$_4$ receptor (N = 16), (**B**) agonist potency (relative to 5-HT = 100) in the guinea-pig ileum and the rat oesophagus assay (N = 10), (**C**) antagonist affinity (pK_B or pA_2) and relative agonist potency in the guinea-pig ileum assay (N = 14), (**D**) antagonist affinity (pK_B or pA_2) and relative agonist potency in the rat oesophagus assay (N = 4). The dashed line represents the line of identity (**A**, **B**). On an average, pharmacological parameters on the guinea-pig ileum were significantly greater than on the rat oesophagus 5-HT$_4$ receptor by 1.72-fold (**A**, antagonist affinity, 95 % confidence limits: 1.25 – 2.37) and 1.67-fold (**B**, relative agonist potency, 95 % confidence limits: 1.23 – 2.28), respectively. For panel **A**, data points of four unpublished compounds related to **2** have been included in the calculation. The other data points (**A** – **D**) correspond to the values presented in Tables 2 and 4.

derivatives of **2** is **2** = **13a** < **13b** ≈ **13c** ≈ **13d** on the guinea-pig 5-HT$_4$ receptor. The eight-membered ring of **13d** apparently abolishes the intrinsic 5-HT$_4$ receptor activity which is observed for **13a-c**. The insertion of a heteroatom in the piperidine moiety of **13b** attenuates the affinity by *ca.* two orders of magnitude (**13b** > **13f** > **13e**) but leaves the intrinsic activity (E_{max}) unchanged. Substitution of the piperidine ring with methyl groups ((±)-**13g**, (±)-**13h**, and **13i**, respectively) hardly affects the 5-HT$_4$ receptor affinity (ΔpK 0.04 – 0.43 (guinea-pig ileum) and 0.12 – 0.40 (rat oesophagus)). In the ileum assay the relative maximum effect is reduced for the 4-methyl derivative **13i** compared with (±)-**13g** and (±)-**13h**. The activity profile of **13b** is significantly altered when the 5-chloro substituent is replaced by hydrogen (**16**), bromine (**17**), and iodine (**18**). The impact on the maximum effect depends on the test system used (guinea-pig ileum: E_{max} for **13b** ≈ **16** ≈ **17** > **18** > 0 %; rat oesophagus: E_{max} for **13b** > **16** ≈ **17** > **18** = 0 %) while the order of affinity is **13b** ≈ **17** > **18** > **16** for both systems.

The enantiomers of **13g** stereoselectively interfere with 5-HT$_4$ receptors (Tables 2 and 4) and display enantiomeric potency ratios of 4.3 – 8.7. (*R*)-(–)-**13g** is the eutomer. Related enantiomeric ester analogues of zacopride possess nearly indistinguishable agonist properties on the electrically stimu-

lated guinea-pig ileum but display moderate stereodiscrimination in a 5-HT_4 receptor binding assay (ratio (*R*)/(*S*) = 5.4)[45]. A major drawback of these zacopride derivatives is their nanomolar affinity for 5-HT_3 receptors.[45] Thus it will be a promising task to prepare enantiomeric derivatives of the ester series presented in this paper.

Acknowledgement

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Experimental

Chemistry. General Procedures. Melting points were determined on a digital melting point apparatus Electrothermal IA 9200 and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 300 (300 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from internal TMS. Signals of protons exchangeable with D_2O are denoted by an asterisk (*). IR spectra (KBr) were measured on a Perkin-Elmer 1420 spectrophometer. $^+$FAB MS spectra were obtained on a Finnigan MAT CH5DF (Xe, DMSO/glycerol), EI MS spectra on a Kratos MS 25 Rf (250 °C, 70 eV) and a Varian MAT CH7 (170 °C, 70 eV), respectively. Optical rotations were measured on a Perkin-Elmer 241 MC. Elemental analyses (C, H, N) for novel compounds were within ±0.4 % of the theoretical values unless otherwise indicated. Chromatographic purifications on a preparative scale were performed with a Chromatotron 7924 (Harrison Research), using glass rotors with 4 mm layers of silica gel PF_{254} containing gypsum (Merck). No attempts were made to optimize yields. Secondary amines **11c,d,f-i**, *N*-(2-chloroethyl)amines **12b,e**, and the amino alcohol corresponding to **12a** were obtained from commercial sources as well as the 4-aminobenzoic acids **14** and **19**. Typical examples for syntheses according to methods A – C (Chart 2) are given below.

Method A. 2-(2-Methyl-1-piperidinyl)ethyl 4-amino-5-chloro-2-methoxybenzoate (13g). Bromoethanol (7.50 g, 60 mmol) was dropped into a mixture of (±)-**11g** (4.96 g, 50 mmol) and KOH (2.81 g, 50 mmol) in MeCN (30 mL). The suspension was stirred overnight at room temp., partitioned between H_2O and $CHCl_3$ and extracted three times with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, evaporated and the crude amino alcohol was redissolved in $CHCl_3$. After addition of 4-DMAP (10 mg, 0.8 mmol), $SOCl_2$ (8.33 g, 70 mmol) was dropped into the mixture at 5 °C and the solution was stirred for 2 h at room temp.. Aqueous KOH (*w* = 0.05, 50 mL) was added and the crude (±)-*N*-(2-chloroethyl)-2-methylpiperidine ((±)-**12g**) was isolated as described in the first step for the corresponding amino alcohol.

4-Amino-5-chloro-2-methoxybenzoic acid (**19**) (4.03 g, 20 mmol) was added to a solution of sodium (0.58 g, 25 mmol) in 50 mL absol. *i*PrOH and stirred for 15 min. Crude (±)-**12g**, redissolved in 30 mL absol. *i*PrOH, was added and the solution was stirred for 2 h at 70 °C. After evaporation to dryness the residue was dissolved in H_2O and extracted three times with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and concentrated, and (±)-**13g** was isolated by chromatography (Chromatotron, eluent: CH_2Cl_2/MeOH 97/3 (*V/V*), NH_3 atmosphere) and subsequent crystallization from ethyl acetate/petrolether (50 – 70 °C) (see Table 1); ^{1}H NMR ($CDCl_3$): δ = 7.83 (s, 1H, Ph-6-H), 6.29 (s, 1H, Ph-3-H), 4.45 (s*, 2H, NH_2), 4.35 (t, *J* = 6.4 Hz, 2H, OCH_2), 3.85 (s, 3H, OCH_3), 3.00 (m, 2H, CH_2N), 2.83 (m, 1H, Pip-6-H_{eq}), 2.38 (m, 2H, Pip-2-H_{ax}, Pip-6-H_{ax}), 1.59–1.67 (m, 4H, Pip-3-H_{eq}, Pip-4-H_{eq}, Pip-5-H_{eq}, Pip-5-H_{ax}), 1.29 (m, 2H Pip-4-H_{ax}, Pip-3-H_{ax}), 1.12 (d, *J* = 6.2 Hz, 3H, CH_3).

The enantiomers of **13g** were prepared analogously from (*R*)- and (*S*)-**11g**. Crystallization of (±)-**11g** (5.00 g, 50 mmol) with (*R*)-(+)-mandelic acid (7.61 g, 50 mmol) from EtOH/Et_2O (1.6 + 3.2 mL per g of salt) was repeated three times and gave dextrorotatory (*R*)-**11g** (*R*)-mandelate, yield 3.15 g (50 %); mp 119 °C (115 – 116 °C[47]); $[\alpha]_D^{20}$ = +73.5° (*c* = 1.0, H_2O) (+70.8°[47]). The same procedure with (*S*)-(−)-mandelic acid afforded levorotatory (*S*)-**11g** (*S*)-mandelate, yield 3.92 g (62 %), mp 118 °C (116 – 118°C[47]); $[\alpha]_D^{20}$ = −71.7° (*c* = 1.0, H_2O) (−72.0°[47]). The diastereomeric salts were dissolved in aqueous KOH (*w* = 0.05) and the enantiomeric secondary amines were exctracted with Et_2O. After drying (Na_2SO_4) and removal of the solvent, (*R*)-(−)-**11g** and (*S*)-(+)-**11g** were used for the preparation of (*R*)-(−)-**13g** and (*S*)-(+)-**13g**, as described above for the racemate (Table 1): $[\alpha]_D^{20}$ = −26.6° (*c* = 0.75, $CHCl_3$) for (*R*)-(−)-**13g**, $[\alpha]_D^{20}$ = +24.4° (*c* = 1.03, $CHCl_3$) for (*S*)-(+)-**13g**.

Method B. 2-(1-Piperidinyl)ethyl 4-amino-2-methoxybenzoate (16) and 5-bromo derivative 17. Dimethylsulfate (20 mL, 210 mmol) was dropped into a suspension of 4-aminosalicylic acid (**14**) (15.32 g, 100 mmol) and K_2CO_3 (29.02 g, 210 mmol) in Me_2CO and the mixture was stirred for 2 h at 50 °C[53]. After addition of concentrated acetic acid (1 mL) the solvent was evaporated and the residue partitioned between ethyl acetate and aqueous $NaHCO_3$ solution (*w* = 0.05). The organic layers were dried (Na_2SO_4) and concentrated. The crude methyl 4-amino-2-methoxybenzoate was hydrolyzed with aqueous KOH (*w* = 0.05) for 30 min at 60 °C. The pH was adjusted to 4 – 5 (0.1 N-HCl) and the crude 4-amino-2-methoxybenzoic acid (**15**)[53] was extracted with CH_2Cl_2. After drying (Na_2SO_4) the solvent was removed and **15** was crystallized from MeOH, overall yield 5.02 g (30 %); mp 148 °C (149 – 150 °C[53]). ^{1}H NMR ($CDCl_3$): δ = 10.56 (s*, 1H, COOH), 7.94 (d, *J* = 8,5 Hz, 1H, Ph-6-H), 6.36 (d, *J* = 8.5 Hz, 1H, Ph-5-H), 6.24 (s, 1H, Ph-3-H), 4.31 (s*, 2H, NH_2), 4.00 (s, 3H, OCH_3).

O-Alkylation of **15** with **12b** and subsequent isolation of **16** was carried out as described for method A (see Table 1); ^{1}H NMR ($CDCl_3$): δ =7.73 (d, *J* = 8.4 Hz, 1H, Ph-6-H), 6.21 (m, 2H, Ph-3-H, Ph-5-H), 4.38 (t, *J* = 6.2 Hz, 2H, OCH_2), 4.07 (s*, 2H, NH_2), 3.85 (s, 3H, OCH_3), 2.73 (t, *J* = 6.2 Hz, 2H, CH_2N), 2.51 (t, *J* = 4.9 Hz, 4H, 2Pip-2-CH_2), 1.60 (quint, *J* = 5.6 Hz, 4H, 2 × Pip-3-CH_2), 1.44 (m, *J* = 5.3 Hz, 2H, Pip-4-CH_2).

For the preparation of the 5-bromo analogue **17**, compound **16** (0.53 g, 2 mmol) was dissolved in $CHCl_3$ (10 mL). *N*-Bromsuccinimide (0.36 g, 2 mmol) and TFA (2.28 g, 20 mmol) were added and the mixture was stirred overnight at ambient temp.. After addition of aqueous KOH (*w* = 0.05) the product was extracted with $CHCl_3$, and the organic solution was dried (Na_2SO_4) and concentrated. Chromatographic purification (Chromatotron, eluent CH_2Cl_2/MeOH 95/5 (*V/V*), NH_3 atmosphere) and crystallization from ethyl acetate/petrolether (50 – 70 °C) afforded **17** (see Table 1); ^{1}H NMR ($CDCl_3$): δ = 7.97 (s, 1H, Ph-6-H), 6.28 (s, 1H, Ph-3-H), 4.49 (s*, 2H, NH_2), 4.38 (t, *J* = 6.1 Hz, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 2.73 (t, *J* = 6.2 Hz, 2H, CH_2N), 2.52 (m, 4H, 2 × Pip-2-CH_2), 1.60 (m, 4H, 2 × Pip-3-CH_2), 1.45 (m, 2H, Pip-4-CH_2).

Method C. *N*-[2-(1-Piperidinyl)ethyl]-4-amino-5-chloro-2-methoxybenzamide (21). Educt **19** (4.03 g, 20 mmol) and CDI (4.05 g, 25 mmol) [54] in absol. THF (50 mL) were stirred for 40 min under N_2 atmosphere. The reaction mixture was partitioned between H_2O and $CHCl_3$ and extracted three times with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and the imidazolide **20** crystallized from the concentrated solution (see Table 1); ^{1}H NMR ($CDCl_3$): δ = 7.91 (s, 1H, Ph-6-H), 7.46 (2 s, 2H, Imi-2-H, Imi-5-H), 7.07 (s, 1H, Imi-4-H), 6.34 (s, 1H, Ph-3-H), 4.62 (s*, 2H, NH_2), 3.74 (s, 3H, OCH_3).

Compound **20** (0.50 g, 2 mmol) and *N*-(2-aminoethyl)piperidine (0.26 g, 2 mmol) were allowed to react in stirred absol. THF overnight at room temp.. Aqueous KOH (*w* = 0.05) was added and the benzamide **21** was extracted three times with $CHCl_3$. After drying (Na_2SO_4) and evaporation, compound **21** was purified as described for method A and crystallized from ethyl acetate/petrolether (50 – 70 °C) (see Table 1); ^{1}H NMR ($CDCl_3$): δ = 8.23 (s, 1H, NH), 8.11 (s, 1H, Ph-6-H), 6.29 (s, 1H, Ph-3-H), 4.39 (s*, 2H, NH_2), 3.91 (s*, 3H, OCH_3), 3.51 (q, *J* = 5.6 Hz, 2H, $HNCH_2$), 2.50 (t, *J* = 5.9 Hz, 2H, NCH_2), 2.42 (m, 4H, 2Pip-2-CH_2), 1.59 (m, 4H, 2 × Pip-3-CH_2), 1.47 (m, 2H, Pip-4-CH_2).

Pharmacology. Drugs. The following compounds were purchased: 5-methoxytryptamine (Aldrich, Steinheim, Germany); metoclopramide hydrochloride (Caelo, Hilden, Germany); choline chloride, 5-hydroxytryptamine creatinine sulphate (Janssen, Beerse, Belgium); atropine sulphate, cocaine hydrochloride (Merck, Darmstadt, Germany); carbachol, corticosterone, pargyline hydrochloride (Sigma, Deisenhofen, Germany). Gratuitous samples of drugs were kindly donated by Glaxo, Greenford, UK (ondansetron hydrochloride) and Sandoz, Basle, Switzerland (tropisetron hydrochloride, methysergide hydrogenmaleate). SDZ 205557 and RS 23597-190 were synthesized in house according to method C and A, respectively (Chart 2).

5-HT4 Receptor affinity (rat oesophagal *tunica muscularis mucosae*). Male Sprague-Dawley rats (280 – 350 g) were killed by cervical dislocation. The entire oesophagus was excised and the outer striated muscle layer of the *tunica propria* was removed by microdissection, leaving behind the inner smooth muscle tube of the *tunica muscularis mucosae*.[55] The muscle tube was divided into a proximal (cervical), middle (thoracic), and distal (abdominal) segment. From each segment 2 – 3 strips were cut longitudinally and set up under a resting force of 1.5 mN in 20 mL organ baths filled with Tyrode solution (37 °C, gassed with 95 % O_2 – 5 % CO_2) for continuous recording of isometric changes in tension, as previously described.[15] The Tyrode solution contained [mM]: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.42, $NaHCO_3$ 11.9 and *D*-glucose 5.0. During a stabilization period of 30 min the strips were exposed to 100 mM pargyline. Thereafter, the force remained unchanged and the preparations were allowed to equilibrate for further 30 min. During the stabilization and the equilibration period the strips were washed with 200 mL Tyrode solution every 15 min. All experiments were conducted in the presence of 30 μM cocaine, 30 μM corticosterone and 1 μM methysergide, respectively. The strips were contracted with a submaximal concentration of carbachol (3 μM). After a stable response to carbachol had been attained (15 – 35 min), a cumulative concentration-effect curve to 5-hydroxytryptamine (5-HT, 0.3 – 1000 nM) or 5-methoxytryptamine (5-MeOT, 1 – 3000 nM), respectively, was performed. The maximum relaxation of the carbachol-induced tone by addition of agonist in each strip during the first curve was arbitrarily set as 100 %.

For the determination of antagonist affinity, up to three concentration-effect curves were performed at intervals of 90 min on each strip, the first in the absence, and the second and third in the presence of increasing concentrations of antagonist. Antagonists were incubated for 60 min except for **13d** and **18** (120 min). One strip of each portion of the oesophagus served as a control preparation to monitor changes in sensitivity. In time control experiments for 5-HT the pEC_{50} was calculated as 8.02 ± 0.07 for the first curve, 8.00 ± 0.06 (maximum effect 100 ± 2 %) for the second and 7.82 ± 0.07 (maximum effect 81 ± 3.5 %, $P < 0.05$) for the third curve ($N = 15$). In time control experiments for 5-MeOT the pEC_{50} was calculated as 7.67 ± 0.05 for the first curve and 7.50 ± 0.05 (maximum effect 92.5 ± 2 %, $P < 0.05$) for the second curve ($N = 14$). Dextral shift and depression observed in the presence of antagonist in the third curve to 5-HT or the second curve to 5-MeOT, respectively, were corrected individually using the desensitization measured for the respective control strip.

For the determination of partial agonist potency, three concentration-effect curves were performed, the first using 5-HT, the second and third using the potential partial agonist. The third curve was recorded after 60 min incubation of the 5-HT4 receptor antagonist SDZ 205557 (**2**; 0.2 μM). One preparation of each oesophagal portion remained untreated to monitor time-dependent changes of sensitivity.

5-HT4 Receptor affinity (guinea-pig ileum, longitudinal muscle). Dunkin-Hartley guinea-pigs of either sex (350 – 650 g) were stunned and exsanguinated. The small intestine was rapidly dissected. Strips of longitudinal muscle with adhering myenteric plexus, 1.5 – 2 cm in length and proximal to the ileocaecal junction, were suspended isometrically under a force of 7.5 mN in 20 mL organ baths filled with Tyrode solution (see above, 37 °C, aerated with 95 % O_2 – 5 % CO_2). In all experiments, 1 μM choline, 10 μM ondansetron to block 5-HT_3 receptors, and 1 μM methysergide to block 5-HT_1 and 5-HT_2 receptors[8] were present in the bath solution. When high 5-HT concentrations (10 and 30 μM) had to be applied in the presence of 5-HT4 antagonists, the concentration of ondansetron was doubled to suppress the break-through of a 5-HT_3 mediated response. After a stabilization period of 30 min the strips were challenged twice with 0.3 μM 5-HT followed by an intensive 8 min wash-out (320 mL) during a period of 45 min in order to establish tissue viability. During the following 30 min the strips were incubated with antagonist or vehicle. At least two strips of the same animal served as control preparations.

Non-cumulative concentration-effect curves for 5-HT[56] or for potential agonists were constructed by adding increasing concentrations of agonist (5-HT: 0.001 – 1 μM). Each concentration was left in contact with the tissue for approx. 15 – 30 s followed by a wash-out with 200 mL Tyrode solution in order to minimize desensitization. The next higher concentration of agonist was applied 12 – 15 min later. Each strip was used to establish only one concentration-effect curve. Antagonists were added after each wash-out. Effects were expressed relative to the percentage of the maximum response to 5-HT (0.3 μM) in the second priming experiment. The dextral shift induced by antagonists was estimated by comparison of individual pEC_{50} values of antagonist treated preparations with mean pEC_{50} values of control strips of the same animal and portion of the intestine. Agonist potency was estimated by comparison of individual pEC_{50} values of strips receiving test agonist with mean pEC_{50} values of strips contracted by 5-HT. The mean pEC_{50} value of control organs treated with 5-HT amounted to 7.97 ± 0.03 (maximum effect 116 ± 2 %, $N = 90$).

5-HT3 Receptor affinity (guinea-pig ileum, longitudinal muscle). Strips of longitudinal muscle with adhering myenteric plexus, 1.5 – 2 cm in length and proximal to the ileocaecal junction, were mounted as described for the 5-HT4 experiments on guinea-pig ileum (see above) in 20 mL organ baths filled with Tyrode solution (37 °C, aerated with 95 % O_2 – 5 % CO_2) containing 1 μM choline. In all experiments, 10 μM 5-methoxytryptamine (5-MeOT) was present for the desensitization of 5-HT4 receptors[7] and 1 μM methysergide to block 5-HT_1 and 5-HT_2 receptors.[8] After a stabilization period of 30 min the strips were challenged twice with 3 μM 5-HT followed by an intensive 10 min wash-out (400 mL) during a period of 50 min in order to establish tissue viability.

Non-cumulative concentration-effect curves for 5-HT[56] were constructed by adding increasing concentrations of 5-HT (1 – 30 μM). Each concentration was left in contact with the tissue for 15 – 30 s followed by wash-out with 200 mL Tyrode solution. The next higher concentration of 5-HT was applied 15 min later. Each strip was used to establish two concentration-effect curves, the first for 5-HT in the absence and the second for 5-HT in the presence of antagonist 60 min after the first curve. Antagonists were added 30 min before the second concentration-effect curve and were retained in the bath fluid during construction of the second curve. Effects were expressed as percentage of the maximum response to 5-HT in the first curve. In time control experiments in the presence of 5-MeOT, the pEC_{50} for 5-HT was calculated as 5.57 ± 0.02 for the first curve and 5.50 ± 0.03 for the second curve (maximum effect 97 ± 1 %, $N = 24$).

M_3 Receptor affinity (guinea-pig ileum, quiescent whole segments). Whole segments of guinea-pig ileum, 2 – 3 cm in length, were suspended isotonically (preload 5 mN) in 20 mL organ baths filled with Tyrode solution (see above, 37 °C, aerated with 95 % O_2 – 5 % CO_2). After a stabilization period of 20 min the preparations were challenged three times with 1 μM carbachol during a period of 45 min in order to establish a constant tissue response. Up to four cumulative concentration-effect curves for carbachol (first curve 0.003 – 10 μM) were constructed in the absence and presence of increasing concentrations of antagonist. Incubation time was 5 – 10 min for antagonist concentrations above 1 μM. In time control experiments in the absence of antagonist, four successive curves for carbachol on the same preparation were superimposable ($N = 6$). For atropine, a pA_2 value of 9.11 ± 0.03 (slope constrained to unity, $N = 30$) was determined.

Pharmacological parameters. Results are given as arithmetic mean ± s.e.m. unless otherwise indicated. Relative maximum effects (E_{max}), pK_B and pD'_2 values were calculated according to *van Rossum*.[57] pA_2 ± s.e. value and slope parameter (m ± s.e.) were obtained from the *Schild* plot regression[58,59] for at least three different concentrations of antagonist. For the calculation of pA_2 values, the *Schild* plot slope was forced to unity unless it was significantly different from unity.[59] *Students t*-test served to discover significant differences of pharmacological parameters ($P < 0.05$).

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