Development of High-Affinity 5-HT$_3$ Receptor Antagonists. Structure-Affinity Relationships of Novel 1,7-Annelated Indole Derivatives. 1

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On the basis of the structures of ondansetron and GR 65,630, its ring-opened C-linked methylimidazole analogue, novel 1,7-annelated indole derivatives were synthesized as potential 5-HT$_3$ antagonists. Receptor binding studies show that all compounds display a high affinity for the 5-HT$_3$ receptors. In both series annelation results in compounds being 7 and 4 times more potent than the references ondansetron and GR 65,630, respectively. Similar to ondansetron, the 1,7-annelated indoles show little stereoselectivity. The (-)-isomers are only slightly more potent than the (+)-isomers. The receptor binding profile of *l*-10-[(2-methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one hydrochloride (**24b**) (INN cilansetron) shows that the compound displays, besides a high affinity for 5-HT$_3$ receptors (K_i = 0.19 nM), a weak affinity for σ-receptors (K_i = 340 nM), muscarine M$_1$ receptors (K_i = 910 nM), and 5-HT$_4$ receptors (K_i = 960 nM) and no affinity ($K_i \geq 5000$ nM) for all the other receptor types tested (n = 37). The new compounds fit the proposed necessary chemical template for binding: a heteroaromatic ring system, a coplanar carbonyl group, and a nitrogen center at well-defined distances. The enhanced potency of the annelated 1,7-indole derivatives indicates that the extra ring provides a favorable hydrophobic area for interaction with the 5-HT$_3$ receptor site. In vivo cilansetron is more potent and induces less central side effects than ondansetron. At present cilansetron is in clinical trials.

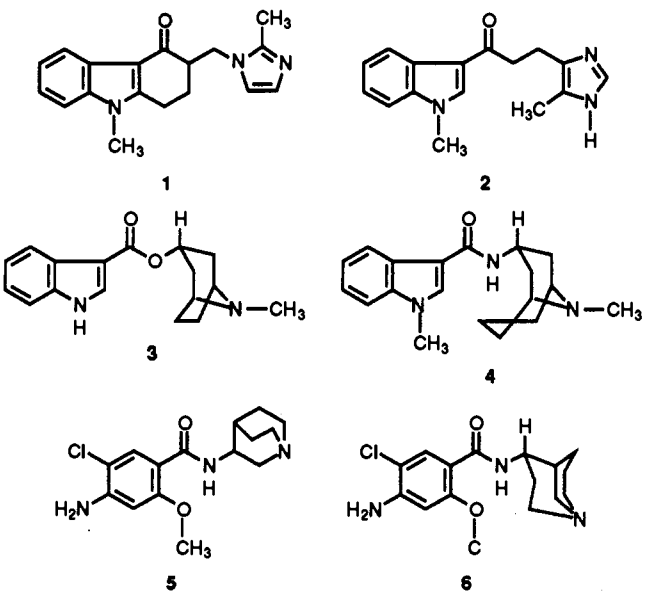
Introduction

Serotonin (5-hydroxytryptamine, 5-HT), involved in numerous physiological (e.g. feeding, hemodynamics, sleep) and pathophysiological (e.g. depression, anxiety, migraine) processes, interacts with various distinct membrane receptors.

At present four main subclasses, termed 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$, are well characterized. Two novel subclasses, designated 5-HT$_5$ and 5-HT$_6$, were recently identified by cloning. The 5-HT$_1$ receptors are further subdivided in 5-HT$_{1A-F}$, and the 5-HT$_2$ receptors in 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ (new name for the previously known 5-HT$_{1C}$ receptor).[1a-e] The 5-HT$_{1P}$ receptors demonstrated in the periphery[1f] still remain in the "orphan status". Most 5-HT receptors (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D\alpha}$, 5-HT$_{1D\beta}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_2$, 5-HT$_3$, 5-HT$_5$, and 5-HT$_6$ subtype) have now been cloned.[1a]

The majority of the 5-HT receptors belong to a G-protein linked receptor superfamily. Functionally 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_4$, and 5-HT$_6$ receptors regulate cAMP formation, whereas 5-HT$_{1C}$ (5-HT$_{2C}$) and 5-HT$_2$ receptors stimulate the hydrolysis of phosphoinositides. 5-HT$_3$ receptors are not linked to G-proteins but belong to a ligand-gated ion channel superfamily.[1a-e]

Receptor-binding studies have demonstrated that many compounds display a high affinity for 5-HT receptors, but only a few are selective (selectivity ratios ≥100) for one of the 5-HT receptor subtypes. The majority of compounds, including serotonin, display a high to moderate affinity for more than one 5-HT receptor subtype and are often not selective with respect to other (neuro-)transmitter receptors.[2] For the 5-HT$_3$ receptors, however, many potent and selective 5-HT$_3$ antagonists, such as ondansetron (1), GR 65,630 (2), tropisetron (3), granisetron (4), zacopride (5), and renzapride (6), have been reported.[3,4]



The rank order of potency is zacopride (K_i = 0.08 nM) > GR 65,630 (K_i = 0.28 nM) > tropisetron (K_i = 0.64 nM) > granisetron (K_i = 0.78 nM) = renzapride (K_i = 0.79 nM) > ondansetron (K_i = 1.6 nM) (Tulp, unpublished data). Selective 5-HT$_3$ agonists are still lacking.

The best-known 5-HT$_3$ antagonist is ondansetron, which is on the market as an antiemetic to prevent the cytotoxic drug-induced vomiting and in clinical trials to evaluate its potential use in anxiety, schizophrenia, drug abuse, and age-associated memory impairment.

Ondansetron is a racemate. The stereochemistry of the asymmetric carbon atom is not crucial for interaction with

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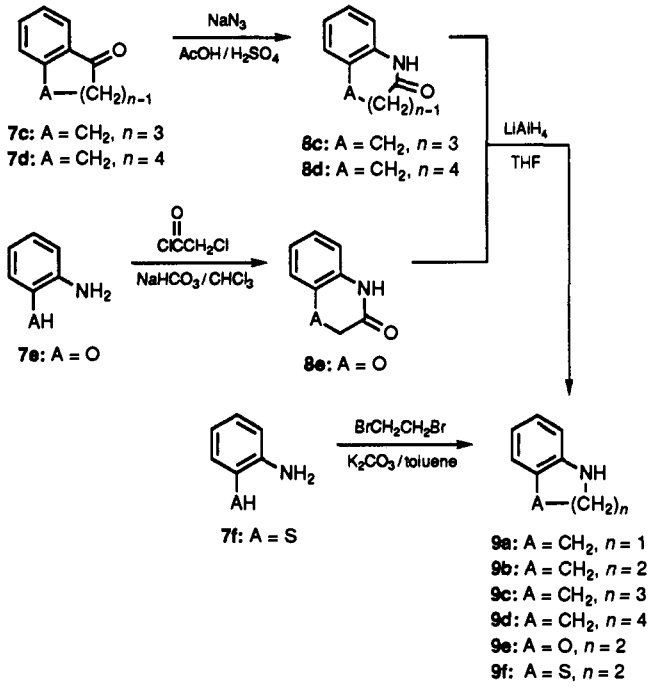
Scheme I



5-HT_3 receptors. Both isomers and the racemate display about the same high affinity for this receptor type.[5] Ondansetron, in contrast to the benzamide-derived 5-HT_3 antagonists zacopride and renzapride, does not interact with 5-HT_4 receptors.[6]

On the basis of the structures of ondansetron and its symmetrical ring-opened C-linked imidazole analogue GR 65,630 (**2**), we developed a series of 1,7-annelated indole derivatives as very potent 5-HT_3 antagonists.[7,8]

In this paper we present the chemistry, the affinity for the 5-HT_3 receptors (obtained by radioligand binding techniques), and the molecular modeling studies of this new class of 5-HT_3 receptor antagonists.

The structure–affinity relationships (SAR) will be discussed.

Chemistry

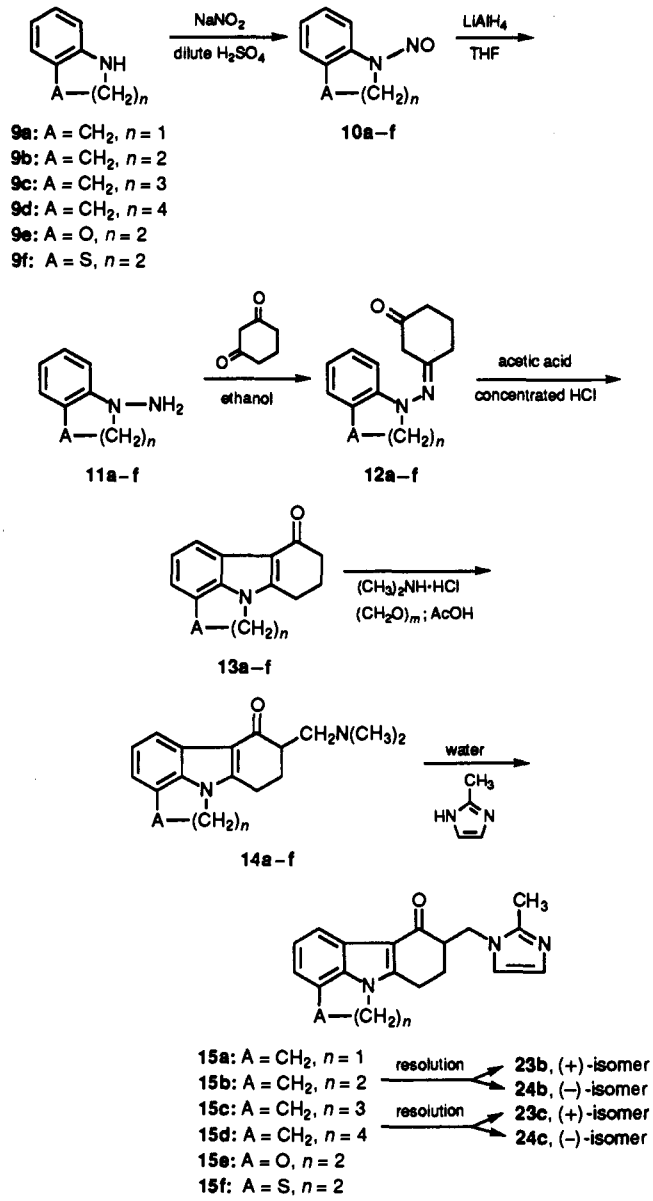
The starting materials (**9a–f**)[9] were prepared by known methods as shown in Scheme I. Scheme II shows the preparation of 1,7-annelated indole derivatives of ondansetron. Nitrosation of the starting materials (**9a–f**) followed by a reduction with $LiAlH_4$ afforded the hydrazine compounds (**11a–f**) in high yield. The indole derivatives (**13a–f**) were prepared by means of the Fischer indole synthesis with 1,3-cyclohexanedione.

The intermediate hydrazones (**12a–f**) were isolated and then converted into **13a–f**. A Mannich reaction followed by an exchange of the dimethylamino group (compounds **14a–f**) for 2-methylimidazole yielded the annelated ondansetron derivatives (**15a–f**).

The racemic compound **15b** was resolved with di-*p*-toluyl-L-tartaric acid and di-*p*-toluyl-D-tartaric acid, respectively, to give the pure enantiomers **23b** and **24b**. In an identical manner, compound **15c** was resolved into **23c** and **24c**. The optical purity was checked by ^1H NMR with (*R*)-(−)-TAE (9-anthryl-2,2,2-trifluoroethanol) as the complexing reagent. The absolute configuration of these compounds (**23**, **24**) has not yet been determined.

The 1,7-annelated indole derivatives of GR 65,630 were prepared according to Scheme III. Reaction of the starting

Scheme II

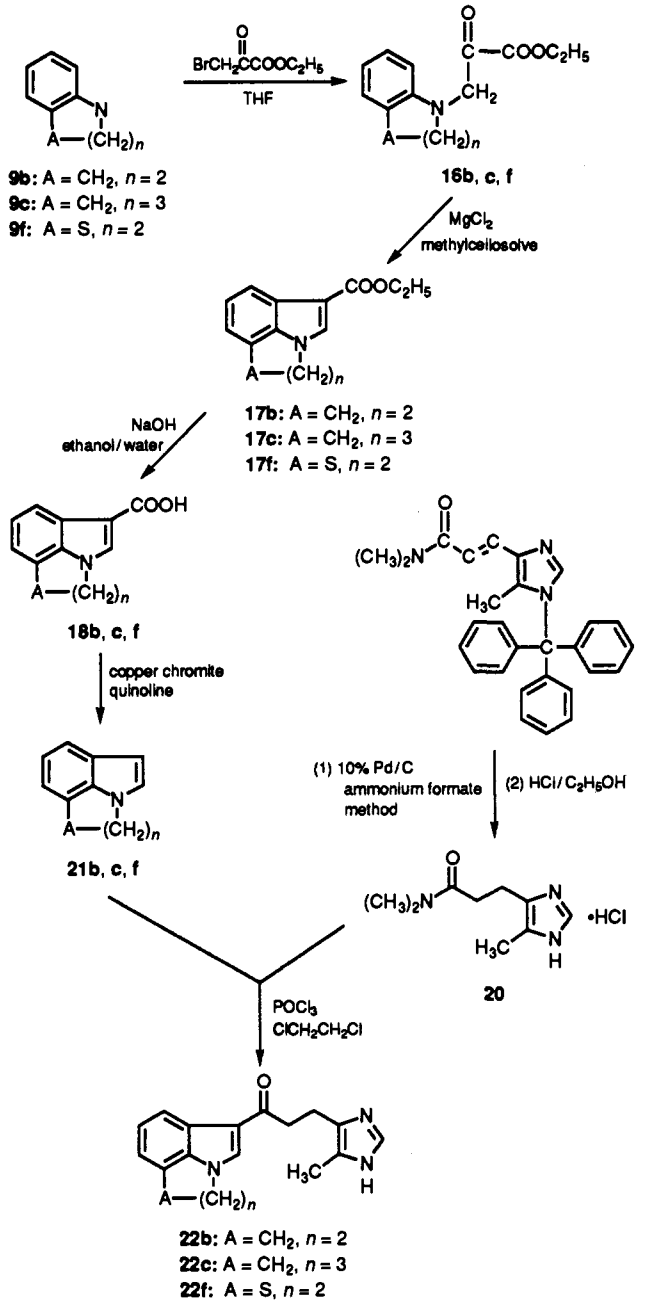


materials (**9b,c,f**) with ethylbromopyruvate followed by ring closure with $MgCl_2$ gave the indole derivatives **17b,c,f**, respectively. Hydrolysis of the ethoxycarbonyl group and subsequent decarboxylation with copper chromite yielded the compounds **21b,c,f**. These compounds were coupled with **20** to obtain the 1,7-annelated derivatives of GR-65,630 (**22b,c,f**).

Pharmacology

Structure–Affinity Relationships. The ability of the 1,7-annelated indole analogues of ondansetron to displace specific binding of [^3H]GR 65,630 from rat brain cortical membranes, determined according to the method of Kilpatrick et al.,[5] is presented in Table I. All compounds display a high affinity for 5-HT_3 receptors. In this series the 5- and 6-membered ring analogues **15a** and **15b** are about 7 times more potent and the azepine **15c** 2 times more potent than ondansetron (**1**). The affinity of the azocine **15d** is comparable to ondansetron. Heteroatoms can be introduced in the 1,7-annelated ring of **15b** with only a modest reduction in potency. The morpholino **15e** and thiomorpholino **15** analogues are 5 and 3 times less potent than the parent compound **15b**.

Scheme III



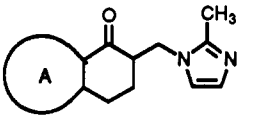
All the ondansetron-derived 1,7-annelated indole derivatives are racemates. Compounds **15b** and **15c** were selected for resolution into the enantiomers. Similar to ondansetron the binding of the 1,7-annelated indole derivatives to 5-HT$_3$ receptors shows little stereoselectivity. The (−)-isomers are 7 times (**24b** vs **23b**) and 1.5 times (**24c** vs **23c**) more potent than the (+)-isomers, making compound **24b** (INN cilansetron) the most potent 5-HT$_3$ compound of this series.

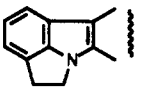
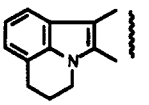
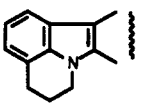
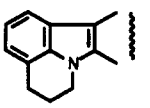
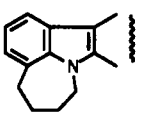
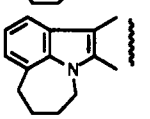
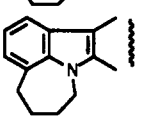
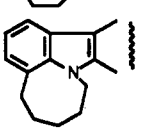
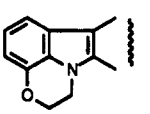
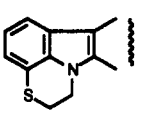
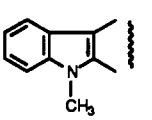
The affinity for the 5-HT$_3$ receptors of the 1,7-annelated indole analogues of GR 65,630 (**2**) is presented in Table II. The highest affinity is obtained in the 6-membered annealated indole analogue **22b**, which is 3.7 times as potent as the reference compound GR 65,630. In this series the thiomorpholine analogue **22** is somewhat less active than **22b**.

Modeling. The minimum-energy conformations of all the compounds illustrated in Tables I and II were calculated with the "maximin2" routine in the SYBYL 5.4 software using the Tripos force field.[10,11] In Figure 1 the (*R*)-enantiomers of the 1,7-annelated indole analogues in their minimum-energy conformation were superimposed in the minimum-energy conformation of (*R*)-ondansetron. There is only a slight conformational change between the newly synthesized compounds and (*R*)-ondansetron. An increase in hydrophobicity, especially at position 7, increases affinity, whereas ring systems with parts far out of the aromatic plane (7- and 8-membered rings) decrease activity.

The fit of GR 65,630 and the 1,7-annelated indole analogues of GR 65,630 in their minimum-energy conformation is illustrated in Figure 2. The conformational resemblance is obvious. The SAR of the 1,7-annelated

Table I. Displacement of [^{3}H]GR 65,630[5] Binding to 5-HT$_3$ Receptors in Rat Brain Cortical Membranes by 1,7-Annelated Indole Analogues of Ondansetron



no.	A	K_i ± SEM, nM
15a (±)		0.23 ± 0.04
15b (±)		0.25 ± 0.08
23b (+)		1.40 ± 0.40
24b (−)		0.19 ± 0.01
15c (±)		0.75 ± 0.05
23c (+)		0.93 ± 0.25
24c (−)		0.64 ± 0.17
15d (±)		1.10 ± 0.40
15e (±)		1.30 ± 0.30
15f (±)		0.77 ± 0.17
1 (±)		1.60 ± 0.20

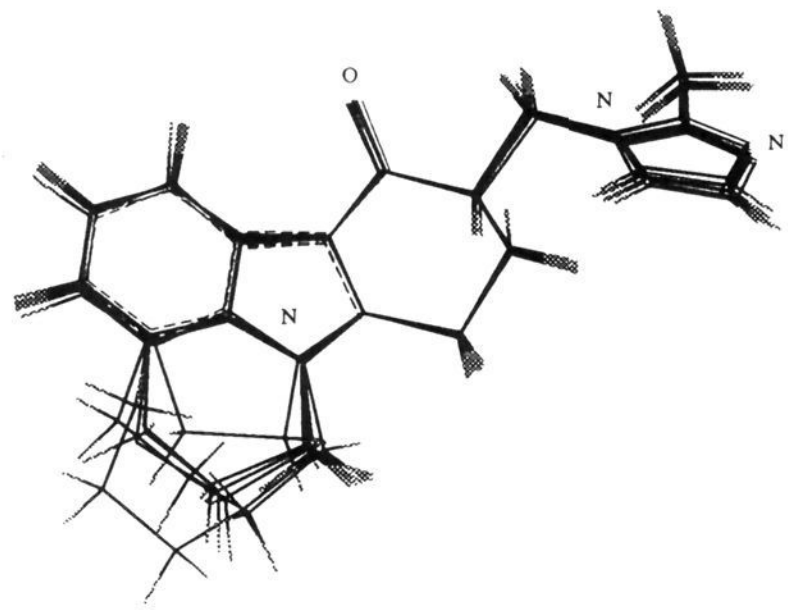


Figure 1. Fit of the (*R*)-enantiomers of 1,7-annelated indole analogues of ondansetron of Table I and ondansetron in their minimum energy conformations.

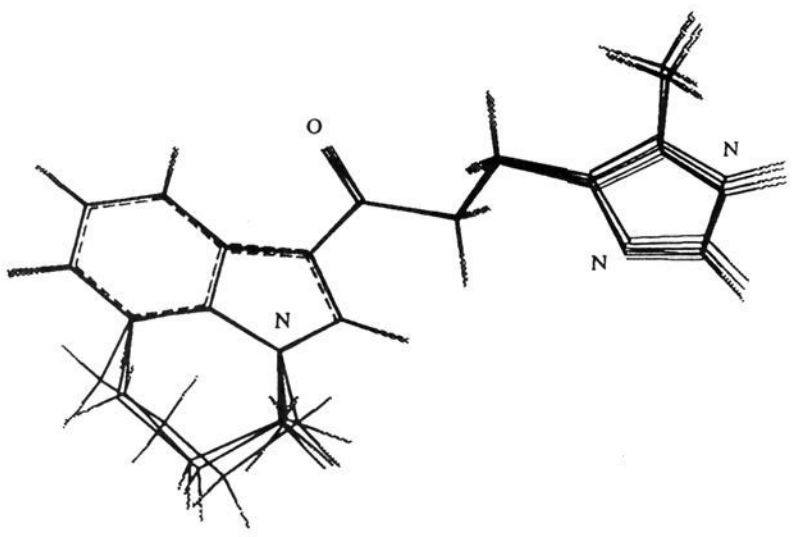
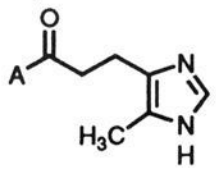
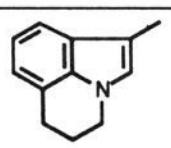
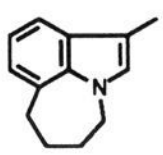
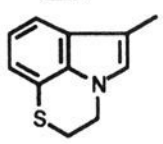
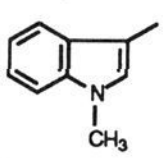


Figure 2. Fit of the (*R*)-enantiomers of 1,7-annelated indole analogues of GR 65,630 of Table II and GR 65,630 in their minimum energy conformations.

Table II. Displacement of [^{3}H]GR 65,630[5] Binding to 5-HT$_3$ Receptors in Rat Brain Cortical Membranes by 1,7-Annelated Indole Analogues of GR 65,630



no.	A	K_i ± SEM, nM
22b		0.075 ± 0.008
22c		0.33 ± 0.13
22f		0.19 ± 0.05
2		0.28 ± 0.07

indole moiety is very similar to the SAR of the 1,7-annelated indole analogues of ondansetron.

Receptor Selectivity. Cilansetron (**24b**) and ondansetron were evaluated for affinity at 39 other (neuro-)transmitter receptors. The receptor binding profile of both drugs is presented in Table III. It is obvious that cilansetron and ondansetron are highly selective for 5-HT$_3$ receptors. Cilansetron displays a weak affinity for σ receptors (K_i = 340 nM), muscarine M$_1$ receptors (K_i = 910 nM), and 5-HT$_4$ receptors (K_i = 960 nM), which is in comparison to the high affinity for 5-HT$_3$ receptors (K_i = 0.19 nM) of no significance.

Ondansetron is somewhat less selective with respect to σ receptors.

Table III. Receptor Binding Profiles of Cilansetron and Ondansetron

receptor	cilansetron K_i (nM)[a]	ondansetron K_i (nM)	[^{3}H]ligand	ref
α_1-adrenergic	5600	3400	prazosine	2
α_2-adrenergic	>10000	>10000	clonidine	2
imidazoline-I$_2$	>10000	>10000	idazoxan	12a
$\beta_{1,2}$-adrenergic	>10000	>10000	DHA[b]	2
dopamine-D$_1$	>10000	>10000	dopamine	2
dopamine-D$_2$	>10000	>10000	spiperone	2
dopamine-D$_3$	>10000	>10000	spiperone	12b
5-HT$_{1A}$	>10000	>10000	8-OH-DPAT	2
5-HT$_{1B}$	>10000	3700	serotonin	2
5-HT$_{1C}$	4900	5000	serotonin	2
5-HT$_{1D}$	>10000	20000	serotonin	2
5-HT$_2$	7800	>10000	spiperone	2
5-HT$_3$	0.19	1.6	GR 65,630	5
5-HT$_4$	960	1200	GR 113,808	12c
5-HT$_{uptake}$	>10000	>10000	paroxetine	2
tryptamine	>10000	>10000	tryptamine	2
melatonin	>10000	>10000	2-iodo-melat	12d
histamine-H$_1$	6500	>10000	mepyramine	2
histamine-H$_3$	>10000	>10000	N^α-Me-histam	12e
muscarine-M$_1$	910	5100	pirenzepine	12f
muscarine-M$_2$	>10000	>10000	N-Me-SCOPOL	12f
muscarine-M$_3$	>10000	>10000	N-Me-SCOPOL	12f
μ-opiate	8500	2900	naloxone	2
κ-opiate	>10000	>10000	EKC	2
δ-opiate	>10000	>10000	dadle	2
benzodiazepine	>10000	>10000	diazepam	2
TRH	>10000	>10000	MeTRH	2
leukotriene-D$_4$	>10000	>10000	LTD$_4$	12f
interleukine-6	>10000	>10000	IL-6	12g
sigma (σ)	340	680	(+)-3-PPP	12h
CCK$_B$ (central)	>10000	>10000	CCK-8	2
CCK$_A$ (peripheral)	>10000	>10000	CCK-8	2
substance P	>10000	>10000	substance P	12f
GABA$_A$	>10000	>10000	DH-muscimol	2
glycine	>10000	>10000	strychnine	2
glycine$_{strych.ins.}$	>10000	>10000	5,7-diClkyn.acid	12i
NMDA	>10000	>10000	CGS 19755	12j
Ca^{2+}(DHP)$_{brain}$	>10000	>10000	nitrendipine	12f
Ca^{2+}(VER)$_{brain}$	5400	>10000	D-888	12f
Na^+(BTX)$_{brain}$	>10000	>10000	batrachotox	12k

[a] For the sake of clarity, SEM values omitted. [b] Dihydroalprenolol.

Functional Correlates for 5-HT$_3$ Receptors. All annelated 1,7-indole derivatives are antagonists of the von Bezold–Jarisch reflex[3] (unpublished data). Further pharmacological profiling of cilansetron (**24b**) shows that this compound is a potent, competitive 5-HT$_3$ antagonist in vitro as well as in vivo. Some data are presented in Table IV. In the rat isolated vagus nerve (RVN) and guinea pig isolated ileum (GpI), cilansetron is about 10 times as potent as ondansetron (for details of methods, see ref 3). In the von Bezold–Jarisch reflex test (BJR) in unrestrained conscious rats, cilansetron is orally active at a dose 6 times lower than that of ondansetron. In the primary observation test (POT) in mice (indicative for central side effects), the lowest effective dose (LED) affecting behavioral parameters is 10 times higher for cilansetron than for ondansetron.

Table IV. Activity of Cilansetron and Ondansetron in Functional Tests for 5-HT$_3$ Antagonism[a] and in the Primary Observation Test[13]

test	cilansetron	ondansetron
RVN[a]	pA_2[e] = 9.94	pA_2 = 8.99
GpI[b]	pA_2 = 7.80	pA_2 = 6.80
BJR[c]	ED_{10}[f] = 26 μg/kg po	ED_{10} = 165 μg/kg po
POT[d]	LED[g] = 30 mg/kg po	LED = 3 mg/kg po

[a] Rat isolated vagus nerve. [b] Guinea pig isolated ileum. [c] Von Bezold–Jarisch reflex in unrestrained concious rats. [d] Primary observation test in mice. [e] The pA_2 is the negative logarithm of the molar concentration of an antagonist which necessitates the doubling of the agonist dose to counteract the effect of that antagonist and restore the original response. [f] Dose of antagonist which inhibits the by the 2-Me-5-HT induced slowing of the heart rate by 10 beats/5 s. [g] Lowest effective dose affecting behavioral parameters in at least half of the animals.

A comprehensive report on the pharmacology and safety is in preparation and will be published elsewhere.

Conclusions

Annelation of the 1,7-positions of the indole nucleus present in ondansetron and GR 65,630 results in an increase in affinity for the 5-HT$_3$ receptors, without influencing selectivity. These structurally new 5-HT$_3$ antagonists, with prototype cilansetron, belong to the most potent and selective 5-HT$_3$ antagonists known in literature.

Experimental Section

Chemistry. General Directions. Melting points were determined on a Mettler FP 62 melting point apparatus and are not corrected. Thin-layer analyses were done on E. Merck silica gel F-254 plates of 0.25-mm thickness. Micro analytical data were provided by TNO, Zeist; only symbols of elements analyzed are given, and they were within 0.4% of the theoretical values unless indicated otherwise.

Chromatography and flash chromatography were carried out on E. Merck silica gel 60, respectively 70–230 and 230–400 mesh.

1-Amino-1,2,3,4-tetrahydroquinoline (11b). A solution of **10b** (25 g, 154 mmol) (prepared from **9b**[9] and sodium nitrite in diluted sulfuric acid and dichloromethane) in THF (175 mL) was added over 30 min to a boiling mixture of $LiAlH_4$ (11.7 g, 309 mmol) and THF (385 mL). After boiling for a further hour, the reaction mixture was cooled, and then water (11.7 mL), 2 N sodium hydroxide (23.4 mL), and water (23.4 mL) were added (below 25 °C). Then the mixture was boiled for 15 min, filtered, and evaporated, giving nearly pure **11b** (21.8 g, 96%). According to this method, the following were prepared: **11a** (yield 98%; R_f 0.45, CH_2Cl_2/MeOH, 95/5), **11c** (yield 97%; R_f 0.2 ether/petroleum ether, 1/1), **11d** (yield 92%; R_f 0.4 CH_2Cl_2/MeOH, 95/5), **11e** (yield 96%; R_f 0.1 CH_2Cl_2), **11f** (yield 98%; R_f 0.1 CH_2Cl_2).

5,6,8,9,10,11-Hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one (13b). A mixture of 1-amino-1,2,3,4-tetrahydroquinoline (46.3 g, 313 mmol) and 1,3-cyclohexanedione (38 g, 329 mmol) in absolute ethanol (500 mL) was boiled for 1 h. The reaction mixture was then evaporated to dryness, and the residue was dissolved in methanol. Ethyl acetate was added, and the mixture was left to crystallize overnight at 0 °C. After vacuum filtration, 68.1 g (90%) of the hydrazone (**12b**), having a melting point of 153–156 °C, was obtained. The hydrazone **12b** (68.1 g) was mixed with acetic acid (600 mL) and concentrated hydrochloric acid (100 mL), refluxed for 1 h, and evaporated to dryness. The residue was taken up in dichloromethane and washed with water. The organic phase was concentrated, and the residue was chromatographed over 2500 g of silica gel with ethyl acetate as an eluting agent. The fractions with R_f 0.3 (thin layer) were collected. **13b** (37.7 g, 60%) was obtained with a melting point of 173–174 °C. According to this method, the following were prepared: **12a** (yield 89%; mp 185–187 °C; R_f 0.45 CH_2Cl_2/MeOH, 95/5), **12c** (yield 80%; mp 181–183.5 °C dec; R_f 0.5 CH_2Cl_2/MeOH, 90/10), **12d** (yield 60%; mp 210 °C dec; R_f 0.25 CH_2Cl_2/MeOH, 95/5), **12e** (yield 72%; mp 160–162 °C; R_f 0.18 CH_2Cl_2/MeOH, 95/5), **12f** (yield 85%; oil; R_f 0.23 CH_2Cl_2/MeOH, 95/5), **13a** (yield 89%; mp 208–210 °C; R_f 0.3 ethyl acetate), **13c** (yield 63%; R_f 0.4 CH_2Cl_2/MeOH, 97/3), **13d** (yield 49%; mp 153–155 °C; R_f 0.4 CH_2Cl_2/MeOH, 97/3), **13e** (yield 36%; mp 194–197 °C; R_f 0.25 ethyl acetate) **13f** (yield 37%; mp 201–203 °C; R_f 0.27 ethyl acetate).

***d,l*-10-[(Dimethylamino)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one Hydrochloride (14b).** A mixture of **13b** (6.75 g, 30 mmol), paraformaldehyde (1.8 g, 60 mmol), dimethylamine hydrochloride (5.4 g, 66 mmol), and acetic acid (90 mL) was stirred for 3 h at 100 °C. After evaporating to dryness the residue was treated with 2 N NaOH and dichloromethane. The organic solution was washed with water and evaporated. The residue was chromatographed over silica gel with methanol containing 3% triethylamine as an eluting agent. The fractions with R_f 0.35 (thin layer) were collected and evaporated. The residue was dissolved in a mixture of ethanol (25 mL) and concentrated hydrochloric acid (2 mL), giving **14b** as its hydrochloric salt (5.1 g, 53%) with a melting point of 208–209 °C.

According to this method, the following were prepared: **14a** (yield 45%; mp 222 °C dec), **14c** (yield 72%; mp 202–203 °C), **14d** (yield 95%; mp 209–210 °C), **14e** (yield 60%; mp 219–223 °C), **14f** (yield 34%; mp 205–207 °C).

***d,l*-10-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one (15b).** **14b** (3.2 g, 10 mmol) was mixed with 2-methylimidazole (2.5 g, 30 mmol) and water (30 mL). The mixture was stirred for 20 h at 100 °C. After cooling at 0 °C the product was isolated by filtration, dried, and chromatographed over silica gel with dichloromethane containing 5% methanol as eluting agent. A 2.8-g amount (87%) of **15b** was obtained with a melting point of 183–184 °C. Anal. $C_{20}H_{21}N_3O_1$: C, H, N.

According to this method, the following were prepared: **15a** (yield 88%; mp 226–227 °C. Anal. $C_{19}H_{19}N_3O_1$: C, H, N), **15c** (yield 49%; mp 188.5–191 °C. Anal. $C_{21}H_{23}N_3O_1$: C, H, N), **15d** (hydrochloride; yield 37%; mp 231–231.5 °C. Anal. $C_{22}H_{26}Cl_1N_3O_1$: C, H, N), **15e** (yield 78%; mp 230–231 °C. Anal. $C_{19}H_{19}N_3O_2$: C, H, N), **15f** (yield 86%; mp 213–215 °C. Anal. $C_{19}H_{19}N_3O_1S_1$: C, H, N, S).

Resolution. *l*-10-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one d-Di-*p*-toluyl-D-tartaric Acid. A solution of *d*-di-*p*-toluyl-D-tartaric acid monohydrate (12.5 g) in warm methanol (125 mL) was added to a solution of **15b** (9.8 g) in warm methanol (210 mL). The mixture was stirred for 20 h at 20 °C and thereafter for 1 h at 0–5 °C. The crystallized product was isolated by filtration, washed with cold methanol and petroleum ether, and dried. Yield: 18.7 g. The obtained salt was dissolved in DMF (465 mL) while being heated. An amount of warm water (230 mL) was added slowly, and the mixture was cooled to room temperature while being stirred. After a night at room temperature, the crystallized product was isolated by filtration, washed with cold DMF/water (2:1), with absolute ethanol, and with ether, and dried. Yield: 14.6 g. This crystallization procedure was repeated twice using 25 mL of the (2:1) mixture of DMF and water per 1 g of the above salt (yield 7.9 g) having a melting point of 155–157 °C (dec), and $[\alpha]^5_D$ = +76° (c = 0.3, methanol).

***l*-10-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one Hydrochloride (24b).** A solution of hydrochloric acid (0.82 g) in absolute ethanol (15 mL) was added to a suspension of *l*-10-[(2-methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido-[3,2,1-*jk*]carbazol-11-one *d*-di-*p*-toluyl-D-tartaric acid (7.8 g) in absolute ethanol (75 mL). The solution so obtained was evaporated almost to dryness under reduced pressure and at a temperature below 45 °C. The residue was stirred in ethyl acetate. The crystals were isolated by filtration and washed with ethyl acetate. Then the crystals were stirred with 2-propanol, isolated by filtration, washed with 2-propanol and with petroleum ether, and dried. **24b**: Yield 3.6 g; mp 226–228 °C; $[\alpha]^5_D$ = −5.0° (c = 1.8, methanol); optical purity ≥98.5%. Anal. $C_{20}H_{22}Cl_1N_3O_1$: C, H, N.

According to this method, the following compounds were obtained.

d-10-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-5,6,8,9,10,11-hexahydro-4*H*-pyrido[3,2,1-*jk*]carbazol-11-one hydrochloride (23b): from 15.0 g of the racemate, using 19.0 g of *l*-di-*p*-toluyl-L-tartaric acid monohydrate; yield 4.15 g; mp 226–228 °C dec; $[\alpha]^5_D$ = +4.4° (c = 1.7, methanol); optical purity ≥97.5%. Anal. $C_{20}H_{22}Cl_1N_3O_1$: C, H, N.

l-11-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-4,5,6,7,9,10,11,-12-octahydroazepino[3,2,1-*jk*]carbazol-12-one hydrochloride (24c): from 7.2 g of the racemate, using 8.35 g of *d*-di-*p*-toluyl-D-tartaric acid monohydrate; yield 0.8 g, mp 213.5–214.5 °C dec; $[\alpha]^5_D$ = −3.3° (c = 1.79, methanol); optical purity 94%. Anal. $C_{21}H_{24}Cl_1N_3O_1$: C, H, N.

d-11-[(2-Methyl-1*H*-imidazol-1-yl)methyl]-4,5,6,7,9,10,11,-12-octahydroazepino[3,2,1-*jk*]carbazol-12-one hydrochloride (23c): from 13.1 g of the racemate, using 15.9 g of *l*-di-*p*-toluyl-L-tartaric acid monohydrate; yield 3.7 g; mp 216–217 °C dec; $[\alpha]^5_D$ = +3.1° (c = 1.95, methanol); optical purity 100%. Anal. $C_{21}H_{24}Cl_1N_3O_1$: C, H, N.

Preparation of 1-(2,3-Dihydropyrrolo[1,2,3-*de*][1,4]benzothiazin-6-yl)-3-(5-methylimidazol-4-yl)propan-1-one Hydrochloride (22f). 2,3-Dihydropyrrolo[1,2,3-*de*][1,4]benzothiazin-6-carboxylic Acid Ethyl Ester (17f). To a solution of **9f**[9] (20 g, 132 mmol) in THF (60 mL) was added in 30 min bromoethyl pyruvate (10 mL, 80–85%, 65 mmol) with stirring. After the mixture was stirred for 24 h at room temperature, the crystallized product was isolated by filtration and washed with THF. The filtrate was concentrated by evaporation under reduced pressure, giving **16f** (24.6 g, oil; R_f 0.25 dichloromethane), sufficient pure for the next reaction. Anhydrous magnesium chloride (6.2 g, 65 mmol) was mixed with methylcellosolve (100 mL), and the mixture was stirred for 15 min at 125 °C. Thereafter a solution of **16f** (24.6 g, max. 0.65 mmol) in methylcellosolve (30 mL) and THF (10 mL) was added at 125 °C in 60 min. The mixture was stirred for a further 5 h at reflux temperature and then concentrated by evaporation. The residue was shaken with a mixture of dichloromethane, ether, and 2 N hydrochloric acid. The organic solution was washed with a 5% sodium bicarbonate solution and evaporated under reduced pressure. The residue was chromatographed over silica gel (1500 mL) with dichloromethane as an eluting agent. The fractions with an R_f = 0.2 (thin layer, CH_2Cl_2) were collected and evaporated, giving **17f** (7.6 g, 47% from **9f**).

2,3-Dihydropyrrolo[1,2,3-*de*][1,4]benzothiazine-6-carboxylic Acid (18f). **17f** (5.1 g, 20.6 mmol) was mixed with ethanol (30 mL), water (20 mL), and sodium hydroxide (1.8 g, 45 mmol), and the mixture was refluxed for 2 h. Thereafter the mixture was cooled, diluted with water (250 mL), and shaken with dichloromethane. The aqueous solution was filtered and treated with concentrated hydrochloric acid (4 mL). The solid substance was isolated by filtration, washed with water, and dried. **18f**: yield 4.0 g (88%); mp 220–222 °C dec.

According to this method, the following were prepared: **18b** (yield 94%; mp 221–224 °C dec), **18c** (yield 73%; mp 232 °C dec).

2,3-Dihydropyrrolo[1,2,3-*de*][1,4]benzothiazine (21f). A mixture of **18f** (4.0 g, 18.3 mmol), copper chromite (1.5 g), and quinoline (20 mL) was stirred for 3 h at 185 °C. Thereafter the mixture was cooled, diluted with dichloromethane, and filtered over hyflo. The filtrate was washed two times with 2 N hydrochloric acid (100 mL) and with 2 N sodium hydroxide (25 mL) and then evaporated. The residue was chromatographed over silica gel with dichloromethane as an eluting agent. The fractions with R_f = 0.57 (dichloromethane) were collected. **21f**: yield 2.9 g (91%); mp 75–76 °C.

According to this method, the following were prepared: **21b** (yield 93%; mp 82–83 °C), **21c** (yield 89%; mp 36–37 °C).

1-(2,3-Dihydropyrrolo[1,2,3-*de*][1,4]benzothiazin-6-yl)-3-(5-methylimidazol-4-yl)propan-1-one Hydrochloric Acid (22f). A mixture of **19** (4.2 g, 10 mmol) (prepared according EP 242 973), 10% Pd on carbon (2 g), and ammonium formate (3.2 g) in methanol (100 mL) was refluxed for 2 h. Then the mixture was filtered over hyflo. The filtrate was diluted with absolute ethanol, containing HCl (70 mmol), and evaporated under reduced pressure. Then toluene was added, and the mixture was evaporated under reduced pressure, giving crude **20**.

To a suspension of the product **20** (max. 10 mmol) in 1,2-dichloroethane (50 mL) was added phosphorus oxychloride (2 mL, 21 mmol). After the mixture was stirred for 10 min at 20 °C, **21f** (1.75 g, 10 mmol) was added. The mixture was refluxed for 5 h, cooled, and diluted with water (70 mL) and dichloromethane (50 mL). The aqueous solution was rendered alkaline and shaken with dichloromethane. The organic solvent was evaporated, and the residue was chromatographed over silica gel with dichloromethane containing 10 volume percent ethanol and 1 volume percent ammonia (25% in water) as an eluting agent. The fractions with R_f = 0.25 were collected and evaporated under reduced pressure. The residue was dissolved in absolute ethanol (10 mL) and ethyl acetate (10 mL). Then a solution of HCl (0.35 g) in absolute ethanol (3 mL) was added. The solid substance was isolated by filtration, washed with ethyl acetate, and dried. **22f**: yield 1.1 g (32%); mp 223–224 °C. Anal. $C_{17}H_{18}Cl_1N_3O_1S_1$: C, H, N.

According to this method, the following were prepared: **22b** (yield 53%; mp 250–252 °C. Anal. $C_{19}H_{20}Cl_1N_3O_1$: C, H, N), **22c** (yielded 32%; mp 240–241 °C. Anal. $C_{19}H_{22}Cl_1N_3O_1$: C, H, N).

Receptor Binding Assays. All binding assays were performed according to well-documented methods summarized in Table III. Drug solutions [Tecan automatic dilution robot (Type 5032, Tecan AG, Switzerland)] were pipetted in all displacement experiments, while the [^{3}H]ligand solutions and tissue suspensions were pipetted automatically by a Filterprep 101 (Ismatec, Zürich, Switzerland), which further performed the assays up to and including the addition of Scintillation Emulsifier-299 (Packard) to the glassfiber filters (Whatman GF/B), collected in plastic minivials (Packard). Overnight equilibration was followed by counting of the content of the vials for tritium in a liquid scintillation counter (Packard B460). Concentrations of unlabeled drug causing 50% displacement of the specific binding of a [^{3}H]label (IC_{50} values) were obtained by computerized log-probit linear regression analysis of data obtained in experiments in which four to six different concentrations of the test compound were used. Inhibition constant (K_i) values were calculated using the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + S/K_d)$ in which S represents the concentration of the [^{3}H]label. Average K_i values were calculated from at least three values obtained from independent experiments; that is, experiments performed on different days with different membrane preparations. All incubations were performed in triplicate.

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