Zatosetron, a Potent, Selective, and Long-Acting $5HT_3$ Receptor Antagonist: Synthesis and Structure-Activity Relationships

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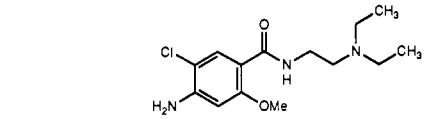
Antagonists of $5HT_3$ receptors are clinically effective in treating nausea and emesis associated with certain oncolytic drugs, including cisplatin. Moreover, these agents may be useful in pharmacological management of several central nervous system disorders, including anxiety, schizophrenia, dementia, and substance abuse. Our studies on aroyltropanamides led to the discovery that dihydrobenzofuranyl esters and amides are potent $5HT_3$ receptor antagonists. Simple benzoyl derivatives of tropine and 3α-aminotropane possessed weak $5HT_3$ receptor antagonist activity, as judged by blockade of bradycardia produced by iv injection of serotonin (5HT) to anesthetized rats. Within this series, use of benzofuran-7-carboxamide as the aroyl moiety led to a substantial increase of $5HT_3$ receptor affinity. The optimal $5HT_3$ receptor antagonist identified via extensive SAR studies was *endo*-5-chloro-2,3-dihydro-2,2-dimethyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-7-benzofurancarboxamide (*Z*)-2-butenedioate (zatosetron maleate). The 7-carbamyl regiochemistry, dimethyl substitution, chloro substituent, and endo stereochemistry were all crucial elements of the SAR. Zatosetron maleate was a potent antagonist of 5HT-induced bradycardia in rats (ED_{50} = 0.86 μg/kg iv). Low oral doses of zatosetron (30 μg/kg) produced long-lasting antagonism of $5HT_3$ receptors, as evidenced by blockade of 5HT-induced bradycardia for longer than 6 h in rats. Moreover, this compound did not produce hemodynamic effects after iv administration to rats, nor did it block carbamylcholine-induced bradycardia in doses that markedly blocked $5HT_3$ receptors. Thus, zatosetron is a potent, selective, orally effective $5HT_3$ receptor antagonist with a long duration of action in rats.

Introduction

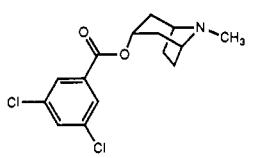
Biochemically distinct subtypes of serotonin receptors exist, and one of the major foci of serotonin (5-hydroxytryptamine, 5HT) research has been the discovery of selective ligands for these multiple 5HT receptor subtypes.[1,2] The existence of a 5HT receptor associated with peripheral enteric and afferent autonomic neurons has been known for over 3 decades, based upon the pioneering work of Gaddum and Picarelli.[3] However, our understanding of this 5HT receptor, now known as the $5HT_3$ receptor, has been dramatically augmented by the discovery of selective $5HT_3$ receptor antagonists.[4] Some of the more widely studied representatives of this class of 5HT receptor ligands include metoclopramide, MDL-72222, tropisetron (ICS 205-930), ondansetron (GR38032), and granisetron (Chart I; compounds 1-5, respectively). $5HT_3$ receptor antagonists are effective in ameliorating nausea and emesis induced by oncolytic drugs such as cisplatin, and ondansetron has been marketed in several countries for this indication.[5] Moreover, identification of $5HT_3$ receptors in the central nervous system (CNS)[6] has engendered considerable interest in the possible role of $5HT_3$ receptor antagonists in treatment of migraine, dementia, anxiety, schizophrenia, and substance abuse.[7-9]

For several years we have been studying a series of benzofuran-derived $5HT_3$ receptor antagonists. In this report, we will detail the structure-activity relationships (SAR), synthesis, and some aspects of the pharmacology of zatosetron (LY277359 maleate; *endo*-5-chloro-2,3-dihydro-2,2-dimethyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-7-benzofurancarboxamide (*Z*)-2-butenedioate; compound 10), a potent, selective, and long-acting compound from this series.[10,11]

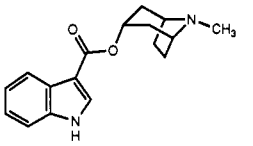
Chart I. Structures of Some $5HT_3$ Receptor Antagonists



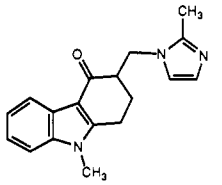
Metoclopramide
(1)



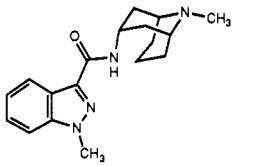
MDL-72222
(2)



Tropisetron
(3)



Ondansetron
(4)



Granisetron
(5)

Results and Discussion

Chemistry. The general synthetic procedures used in this study are best illustrated by the synthesis of the optimal compound zatosetron (Scheme I). Commercially available 5-chloro-2-hydroxybenzoic acid was converted into methyl ester 6 and then alkylated with 3-chloro-2-methylpropene to provide Claisen substrate 7. Compound

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(1) Bradley, P. B.; Engel, G.; Feniuk, W.; Fozard, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. *Neuropharmacology* **1986**, *25*, 563.
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(4) For a review see, Fozard, J. R. In *The Peripheral Actions of 5-Hydroxytryptamine*; Fozard, J. R., Ed.; Oxford University Press: Oxford, 1989; p 354.
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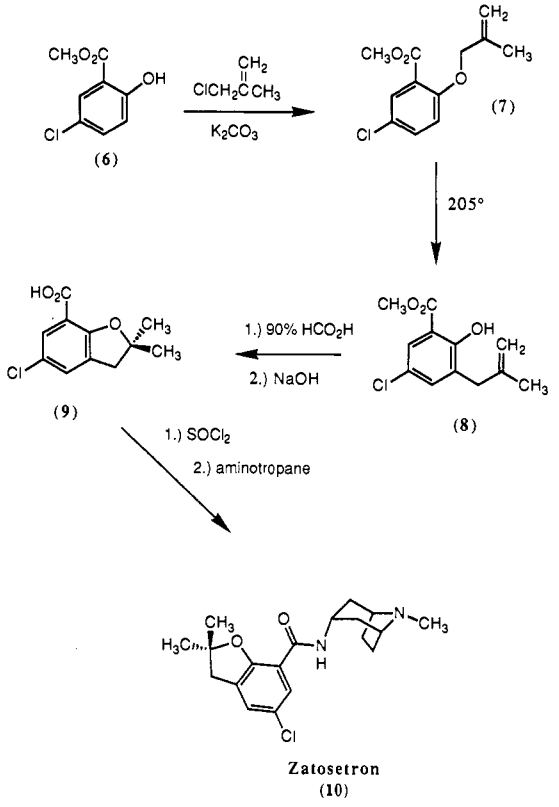
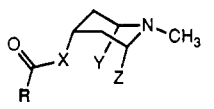
Scheme I. Synthesis of Zatosetron



7 was heated at reflux for 6 h in *N*-methylpyrrolidinone, resulting in a facile [3,3]-sigmatropic rearrangement to form phenol 8. Reaction of 8 with 90% formic acid, followed by sodium hydroxide saponification, generated 5-chloro-2,3-dihydro-2,2-dimethyl-7-benzofurancarboxylic acid (**9**), completing assembly of the aryl portion of zatosetron.[12] Reaction of the acid with thionyl chloride, followed by amide formation with 3α-aminotropane, completed the synthesis of zatosetron. The 3α-aminotropane was generated from tropinone and benzylamine following a published procedure,[13] and on the basis of NMR and TLC analyses, this material was contamined with some of the isomeric 3β-aminotropane. Thus, in the final product the desired endo isomer (**10**, zatosetron) was contaminated with approximately 15% of the exo amide. The exo isomer was easily distinguished from the endo isomer by ^{1}H NMR. For example, the *gem*-methyl substituents resonated at δ 1.32 and 1.38 for the exo and endo isomers, respectively. The proton α to the amide nitrogen in the endo isomer appeared as a well-defined multiplet at δ 4.40, whereas the corresponding proton in the exo isomer appeared as a less well-defined multiplet at δ 4.54. Moreover, the exo isomer

Table I. Tropane-Derived 5HT$_3$ Receptor Antagonists[a]

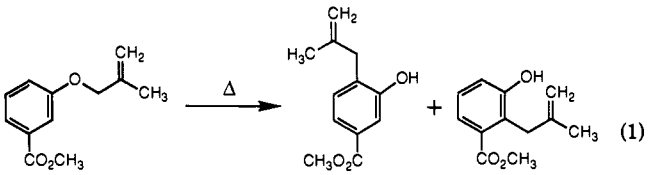


no.	R	X	Y	Z	% inhibn of 5HT-induced bradycardia (μg/kg iv)[b] 1000	100
14	Ph	O	(CH_2	CH_2)	88.1 ± 2.2	
15	Ph	O	H	H	0.0 ± 0	
16	Ph	CH_2	(CH_2	CH_2)		31.3 ± 18.4
17	4-(CF_3O)C_6H_4	O	(CH_2	CH_2)	4.7 ± 4.7	

[a] The stereochemistry was endo. [b] Compounds were administered via the femoral vein to urethane-anesthetized rats. 5HT (0.03 mg/kg iv) was then given; in control animals, this resulted in a 71.9% decrease in heart rate. Each compound was tested in groups of three or four animals and data represent means ± SEM for peak inhibition. For further details, consult the Experimental Section.

was slightly more mobile on silica gel TLC than zatosetron. Fortunately, the maleate salt of the exo isomer was very soluble in ethyl acetate, and this impurity was easily removed by formation of the maleate salt and recrystallization from ethanol/ethyl acetate. An authentic sample of 3β-aminotropane was prepared as previously described.[13,14] Amide formation between this substance and the acid chloride derived from **9** formed **50** (Table IV), the homogeneous exo isomer of zatosetron.

The 4- and 6-substituted benzamides (compounds **45** and **47**, Table IV) were prepared from 3-hydroxybenzoic acid, methyl ester. Alkylation with 3-chloro-2-methylpropene, followed by heat-induced sigmatropic rearrangement, yielded a mixture of regioisomers: Distillation



(1)

resulted in a 4/1 mixture of regioisomers **12** and **13**, which were then readily separated by HPLC; identities of these regioisomers were confirmed by ^{1}H NMR. The two regioisomers were then used to synthesize **45** and **47**, employing reaction sequences analogous to the one used in the synthesis of zatosetron. The 5-regioisomer **46** (Table IV) was easily prepared from 4-hydroxybenzoic acid. Syntheses of all remaining compounds in this study followed the general procedures used in the synthesis of zatosetron.

Structure–Activity Relationships. The activity of each compound was studied after intravenous administration to urethane-anesthetized rats.[11] The anesthetized rats were initially given a 30 μg/kg iv dose of 5HT, which produced a transient bradycardia. This 5HT-induced activation of the von Bezold–Jarisch reflex is mediated via 5HT$_3$ receptors in the right ventricle.[15] When heart rate returned to steady state (≤5 min), either test compound or saline was administered, and 15 minutes later rats were given another 30 μg/kg iv dose of 5HT. Peak 5HT-induced bradycardia was determined as a percent reduction in heart rate. In saline-treated animals, 5HT produced 72.6

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(10) A portion of these results have been presented in preliminary form: Lacefield, W. B.; Simon, R. L.; Pfeifer, W.; Robertson, D. W.; Bloomquist, W.; Cohen, M. L. 199th National Meeting of the American Chemical Society, Boston, MA, April 22–27, 1990; MEDI 102.
(11) For information on the pharmacology of zatosetron, see: Cohen, M. L.; Bloomquist, W.; Gidda, J. S.; Lacefield, W. B. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 350.
(12) For a general synthesis of 2,3-dihydro-7-benzofurancarboxylic acids, see: Anderson, W.; Christensen, H.; Gronvald, F. C.; Lundt, B. F. GB 1,314,325, May 9, 1969.
(13) Archer, S.; Lewis, T. R.; Unser, M. J. *J. Am. Chem. Soc.* **1957**, *79*, 4194.
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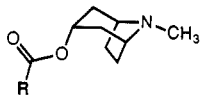
(15) Fozard, J. R.; Host, M. *Br. J. Pharmacol.* **1982**, *77*, 520P.

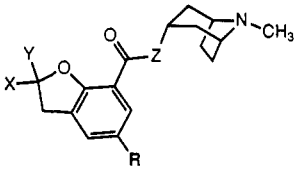
Table II. Aryl Alterations in Tropane-Derived $5HT_3$ Receptor Antagonists[a]



no.	R	% inhibn of 5HT-induced bradycardia (μg/kg iv)[b]				
		1000	100	30	10	3
14	Ph	88.1 ± 2.2				
18	biphenyl-2-yl	47.3 ± 20.9				
19	2-phenoxyphenyl	54.5 ± 18.4				
20	dibenzofuran-1-yl	66.3 ± 2.8	12.4 ± 10.1		0.0 ± 0	
21	dibenzofuran-3-yl	1.5 ± 1.5				
22	fluoren-1-yl	51.0 ± 13.4				
23	3-methylbenzofuran-7-yl				7.7 ± 1.2	
24	2-methylbenzofuran-7-yl				7.3 ± 14.5	
25	2,3-dihydro-2-methylbenzofuran-7-yl				30.6 ± 12.1	
26	2,3-dihydro-*trans*-2,3-dimethylbenzofuran-7-yl			78.7 ± 1.8	29.9 ± 10.0	1.8[c]
27	2,3-dihydro-*cis*-2,3-dimethylbenzofuran-7-yl			47.3 ± 8.9	32.7 ± 13.9	35.6[c]

[a] The stereochemistry was endo. When chiral centers were present in the aroyl moiety, the compounds were tested as racemates. [b] Compounds were administered via the femoral vein to urethane-anesthetized rats. 5HT (0.03 mg/kg iv) was then given; in control animals, this resulted in a 71.9% decrease in heart rate. Unless indicated otherwise, each compound was tested in groups of three to five animals and data represent means ± SEM for peak inhibition. For further details, consult the Experimental Section. [c] Data were derived from one animal.

Table III. Optimization of Benzofuran-Derived $5HT_3$ Receptor Antagonists[a]



no.	R	X	Y	Z	% inhibn of 5HT-induced bradycardia (μg/kg iv)[b]				
					100	10	3	1	0.3
28	H	H	H	O	51.2 ± 2.9	27.0 ± 11.8			
25	H	CH_3	H	O		30.6 ± 12.1			
29	H	CH_3	CH_3	O		38.0 ± 9.8	20.8 ± 8.7		
30	CH_3	H	H	O	67.2 ± 23.1	28.8 ± 14.6			
31	CH_3	CH_3	H	O	78.2 ± 15.3	66.7 ± 6.1	14.4 ± 9.1	3.3 ± 3.3	
32	CH_3O	CH_3	H	O		4.1 ± 5.2			
33	F	CH_3	H	O		32.2 ± 18.1			
34	Cl	CH_3	H	O		69.0 ± 7.4	50.8 ± 10.9	5.6 ± 1.9	
35	Cl	CH_3	CH_3	O		82.7 ± 6.0	19.3 ± 2.2	11.5 ± 11.5	
36	H	CH_3	H	NH	87.6[c]	22.3 ± 9.8			
37	H	CH_3	CH_3	NH		47.5 ± 7.3	13.7 ± 8.2		
38	Cl	CH_3	H	NH		80[c]	79.8 ± 3.1	58.1 ± 11.9	11.0 ± 7.9
zatosetron	Cl	CH_3	CH_3	NH			84.4 ± 3.5	66.2 ± 10.6	10.8 ± 5.4
39	F	CH_3	CH_3	NH		71.7 ± 13.9			
40	Br	CH_3	CH_3	NH		68.7 ± 19.4	15.9 ± 5.7		
41	CH_3	CH_3	CH_3	NH		87.1 ± 2.9	11.2 ± 8.4		
42	CH_3O	CH_3	H	NH		28.9 ± 11.9			
43	CH_3O	CH_3	CH_3	NH		2.9 ± 2.9			
44	OH	CH_3	CH_3	NH		54.7 ± 13.1			
tropisetron					70.6 ± 8.8	77.0 ± 6.1	54.2 ± 9.5	20.1 ± 12.1	
2					71.4 ± 11.9	9.7 ± 9.7			

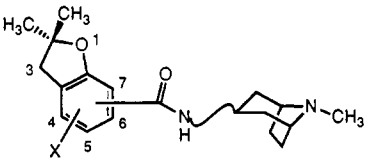
[a] The stereochemistry was endo. When chiral centers were present in the aroyl moiety, the compounds were tested as racemates. [b] Compounds were administered via the femoral vein to urethane-anesthetized rats. 5HT (0.03 mg/kg iv) was then given; in control animals, this resulted in a 71.9% decrease in heart rate. Unless indicated otherwise, each compound was tested in groups of 3–16 animals and data represent means ± SEM for peak inhibition. For further details, consult the Experimental Section. [c] Data were derived from one animal.

± 1.9 and 71.9 ± 1.8% transient bradycardic responses for the first and second 30 μg/kg doses, respectively. Percent inhibition was calculated as the percent difference between the first and second 5HT-induced bradycardia and each animal served as its own control. Animals in which the first 5HT challenge produced less than a 50% reduction in heart rate were discarded to ensure reproducibility of results. Compounds were initially screened at 1000 μg/kg iv, and test doses became progressively lower as more potent $5HT_3$ receptor antagonists were identified.

Compounds of interest were subsequently tested orally. Rats were dosed orally with test compound or vehicle (distilled water) prior to anesthesia. All animals were dosed, after urethane-induced anesthesia, with 30 μg/kg iv of 5HT, and vehicle-treated animals served as the controls. In vehicle-treated animals this dose of 5HT produced a transient bradycardia of 71.3 ± 3.0%, consistent with control values in the iv studies. The SAR data are compiled in Tables I–VIII, and in some of the studies the prototypic compounds **2**, tropisetron, and ondansetron were included for comparison.

Because of the documented $5HT_3$ receptor blocking actions of certain tropanyl esters of structurally distinct aromatic carboxylic acids (e.g. **2** and tropisetron), we

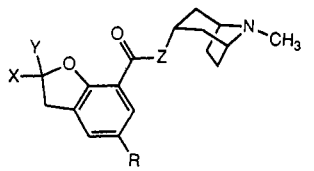
Table IV. Effects of Regio- and Stereochemistry in Benzofuran-Derived 5HT$_3$ Receptor Antagonists



no.	position: amide	position: X	stereochem	% inhibn of 5HT-induced bradycardia (μg/kg iv)[a]: 10	3	1	0.3
45	4	H	endo	2.1 ± 2.1			
46	5	H	endo	16.7 ± 7.8			
47	6	H	endo	0.0 ± 0			
37	7	H	endo	47.5 ± 7.3	13.7 ± 8.2		
48	7	4-Cl	endo	1.9 ± 1.9			
zatosetron	7	5-Cl	endo		84.4 ± 3.5	66.2 ± 10.6	10.8 ± 5.4
49	7	6-Cl	endo	0.8 ± 0.8			
50	7	5-Cl	exo	67.1 ± 1.4	25.9 ± 14.4		

[a] Compounds were administered via the femoral vein to urethane-anesthetized rats. 5HT (0.03 mg/kg iv) was then given; in control animals, this resulted in a 71.9% decrease in heart rate. Each compound was tested in groups of three to eight animals and data represent means ± SEM for peak inhibition. For further details, consult the Experimental Section.

Table V. Effects of 5HT$_3$ Receptor Antagonists after Oral Administration to Rats[a]



no.	R	X	Y	Z	% inhibn of 5HT-induced bradycardia (μg/kg po)[b]: 1000	300	100	30	10	3
tropisetron				O	85.0 ± 8.4	77.4 ± 8.2	39.8 ± 10.6		0.1 ± 0.1	
51[c]				O	84.2 ± 5.5	85.1 ± 7.7	23.9 ± 11.7			
52[d]				NH				84.4 ± 6.0	35.8 ± 19.7	
31	CH_3	CH_3	H	O	11.6 ± 6.2					
34	Cl	CH_3	H	O	94.7 ± 2.0	66.2 ± 17.6	33.1 ± 18.4			
38	Cl	CH_3	H	NH	94.6 ± 2.4	89.7 ± 7.0	74.6 ± 7.8	21.9 ± 11.5		
35	Cl	CH_3	CH_3	O				86.2 ± 4.5	73.9 ± 6.1	0.9 ± 0.7
zatosetron	Cl	CH_3	CH_3	NH				77.6 ± 6.3	57.1 ± 17.2	19.1 ± 14.7
ondansetron					73.4 ± 7.9	55.1 ± 12.5	18.2 ± 12.7	0.3 ± 0.3		

[a] The stereochemistry was endo. When chiral centers were present in the aroyl moiety, the compounds were tested as racemates. [b] Compounds were given by oral gavage to rats. Rats were then anesthetized and surgically prepared for the study. One hour after administration of the 5HT$_3$ receptor antagonist, 0.03 mg/kg of 5HT was given iv; in control animals, this resulted in a 71.3% decrease in heart rate. Each compound was tested in groups of 3–11 animals and data represent means ± SEM for peak inhibition. [c] 1*H*-Indazole-3-carboxylic acid, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester. This compound was synthesized as described in ref 20. [d] *N*-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-1*H*-indazole-3-carboxamide. This compound was synthesized as described in ref 20.

sought to understand the structural features in the aromatic portion of these molecules that governed 5HT$_3$ receptor interactions. 5HT$_3$ receptor affinity proved to be extremely sensitive to changes in this portion of the molecule.

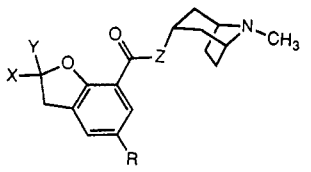
Initial compounds in the series (Table I) were studied at relatively high doses (1000 and 100 μg/kg iv). For example, the parent benzoyl derivative, **14**, inhibited 5HT-induced bradycardia by 88% after injection of 1000 μg/kg iv. Removal of the ethano bridge of the tropane ring resulted in piperidine derivative **15**, and this compound was devoid of 5HT$_3$ receptor antagonist activity following administration of 1000 μg/kg iv. These data suggested the importance of the conformationally restrained bicyclic amine for maximal 5HT$_3$ receptor affinity in this series, and this structural feature was retained in the remaining compounds. Other investigators have independently described the importance of the ethano bridge in tropane-derived 5HT$_3$ receptor antagonists.[16,17] Potency was retained when the ester oxygen was replaced with a methylene group (compound **16**). Finally, compound **17** first suggested the dramatic substituent effects which were often seen in this series of compounds; placement of a 4-(trifluoromethoxy) substituent on the phenyl ring of **14** dramatically attenuated activity of the compound after administration of 1000 μg/kg iv.

Substitution on the phenyl ring of **14** was investigated further (Table II). Addition of large, lipophilic phenyl (**18**) or phenoxy (**19**) substituents in the ortho position provided compounds which retained 5HT$_3$ receptor blocking properties at a dose of 1000 μg/kg iv. Moreover, these substituents could be used to form tricyclic ring systems (**20–22**), again without major perturbations in potency. In the two dibenzofuran-based compounds, the 1-yl regioisomer **20** was substantially more effective than the 3-yl isomer **21** (66 and 1.5% inhibition of 5HT-induced bradycardia, respectively). The greater potency of **20** relative

(16) For information on the SAR of **2**, see: Fozard, J. R.; in *The Peripheral Actions of 5-Hydroxytryptamine*; Fozard, J. R., Ed.; Oxford University Press: Oxford, 1989; p 364.

(17) For information on the SAR of tropisetron, see: Giger, R. K. A.; Donatsch, P.; Engel, G.; Richardson, B.; Stadler, P. A. *Proceedings of the VIII International Symposium on Medicinal Chemistry*; Uppsala, 1984; Vol. 2, p 133.

Table VI. Inhibition of 5HT-Induced Bradycardia by $5HT_3$ Receptor Antagonists[a,b]



no.	R	X	Y	Z	ED_{50} values (μg/kg) iv	ED_{50} values (μg/kg) po	dose ratio: po/iv
tropisetron				O	3.1	127	42
51[c]				O	2.6	159	62
52[d]				NH	2.2	13	6.4
31	CH_3	CH_3	H	O	12.6	5168	410
34	Cl	CH_3	H	O	4.2	179	43
38	Cl	CH_3	H	NH	1.0	63	65
35	Cl	CH_3	CH_3	O	4.6	8.8	1.9
zatosetron	Cl	CH_3	CH_3	NH	0.9	9.2	11
ondansetron					3.3	316	96

[a] The stereochemistry was endo. When chiral centers were present in the aroyl moiety, the compounds were tested as racemates. [b] The iv and po ED_{50} values were calculated from the group-derived data presented in Tables III and V, respectively. [c] 1*H*-Indazole-3-carboxylic acid, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester. This compound was synthesized as described in ref 20. [d] *N*-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide. This compound was synthesized as described in ref 20.

to **21** suggested that the ortho orientation of the carboxylate moiety and ether oxygen was important. This was reminiscent of the SAR of the *ortho*-methoxybenzamides such as metoclopramide and related dopamine receptor antagonists.[18]

Because of this regiochemical result, the ortho orientation between the carboxylate moiety and ether oxygen was maintained, and the SAR of the dibenzofuran was examined further. Removal of the distal benzo moiety and saturation of the furan ring improved potency; for example, compound **25**, the 2,3-dihydro-2-methylbenzofuran, displayed substantial $5HT_3$ receptor antagonist potency; it inhibited 5HT-induced bradycardia by 31% after administration of 10 μg/kg iv (Table II). Comparison of **25** with its unsaturated congener, **24**, revealed the importance of saturation at positions 2 and 3; doses of 10 μg/kg iv of **24** and **25** inhibited 5HT-induced bradycardia by 7 and 31%, respectively. Because of the improved antagonist potency of **25**, we used the 2,3-dihydrobenzofuran as the aromatic framework for the remainder of the SAR study (Tables III and IV). The parent, unsubstituted dihydrobenzofuran (**28**), inhibited 5HT-induced bradycardia by 51 and 27% at doses of 100 and 10 μg/kg iv, respectively. Three key variables in the SAR were now examined: effects of mono or *gem*-methyl groups at position 2, effects of electronically distinct substituents at position 5, and the issue of ester vs amide tethers linking the benzofuran and tropane pharmacophores. With no substituent at position 5, effects of the methyl groups were not significant; for example, compounds **25**, **28**, and **29** (10 μg/kg iv) produced 31, 27, and 38% inhibition of 5HT-induced bradycardia, respectively. In the amide series, the mono (**36**) and dimethyl (**37**) compounds (10 μg/kg iv) caused 22 and 48% inhibition of 5HT-induced bradycardia, respectively (Table III).

Several small substituents were incorporated into position 5 of the dihydrobenzofuran ring to preclude the possibility of metabolic hydroxylation at this site (Table III). A 5-methyl substituent was tolerated (e.g. **30**, **31**), but 5-methoxy tended to be deleterious (e.g., **32**, **42**, **43**). Halogens, and in particular chlorine, at position 5 had beneficial effects on intrinsic activity. For example, in the ester species, the 5-chloro substituent increased the percent inhibition of 5HT-induced bradycardia from 38 to 83% for compounds **29** and **35**, respectively (10 μg/kg, iv), and in the amide series it increased the response from 14 to 84% for compound **37** and zatosetron, respectively (3 μg/kg, iv). In the series of esters bearing a monomethyl substituent at position 2, the efficacy at 10 μg/kg iv followed the order Cl > CH_3 > F = H > CH_3O (compounds **34**, **31**, **33**, **25**, and **32**, respectively). In the series of amides bearing *gem*-methyl substituents at position 2, the efficacy at 10 μg/kg iv followed the order Cl > CH_3 > F = Br > H = OH > CH_3O (compounds zatosetron, **41**, **39**, **40**, **37**, **44**, and **43**, respectively). These data demonstrate that substituent effects have substantial and predictable influences on potency within benzofuran-derived $5HT_3$ receptor antagonists. Moreover, these substituent-effect data concur with results presented by other groups on structurally related $5HT_3$ receptor antagonists,[16] and resemble some of the substituent effects seen with benzamide D_2 receptor antagonists.[18]

Amide tethers linking the aromatic and bicyclic amine pharmacophores were preferred in this series. For example, the ester and amide tethers could be directly compared using compounds **35** and zatosetron. The amide zatosetron, at both 1 and 3 μg/kg iv, was more effective than the corresponding ester as a $5HT_3$ receptor antagonist (Table III). Additional examples where the amide appeared to be more effective than its ester analogue include (ester and amide) **29** and **37**, **34** and **38**, and **32** and **42**. Whether this reflects differences between amides and esters in absolute affinity at the $5HT_3$ receptor or pharmacokinetic differences could not be determined from these data. However, in a series of indazole-derived $5HT_3$ receptor antagonists, the ester and amide congeners had comparable affinity for central $5HT_3$ receptors in radioligand binding assays.[19,20] The decreased potency of esters versus amides in these dihydrobenzofuran $5HT_3$ receptor antagonists may reflect the greater susceptibility of the esters to plasma hydrolytic enzymes during the present series of in vivo comparisons. However, it is of interest that Nelson and Thomas have noted a close linear correlation between activity of a series of $5HT_3$ receptor antagonists in blocking 5HT-mediated von Bezold–Jarisch reflex in vivo and their absolute affinity for rat brain $5HT_3$ receptors in vitro.[21] Therefore, more data would be required to determine the precise reason for the superiority of the amides in these dihydrobenzofuran $5HT_3$ receptor antagonists.

After discovering the beneficial effects of *gem*-methyl substituents at position 2 and a chloro substituent at position 5, we reexamined several stereo- and regiochemical issues (Table IV). The position of the carboxamide moiety on the dihydrodimethylbenzofuran was systematically varied, and by comparing the activities of these regioisomers after iv administration to rats (10 μg/kg), the 7-dihydrobenzofuran was clearly preferred. In the 7-dihydrobenzofuran series, the 4- and 6-chloro regioisomers of zatosetron were essentially devoid of $5HT_3$ receptor antagonist activity at the 10 μg/kg dose, which underscored

(18) For a seminal review of the medicinal chemistry of metoclopramide and related D_2 receptor antagonists, see: De Paulis, T. *Proceedings of the VIIIth International Symposium on Medicinal Chemistry*; Uppsala, 1984; Vol. 1, p 405.

(19) Wong, D. T.; Robertson, D. W.; Reid, L. R. *Eur. J. Pharmacol.* **1989**, *166*, 107.

(20) Robertson, D. W.; Bloomquist, W.; Cohen, M.; Reid, L. R.; Schenck, K.; Wong, D. T. *J. Med. Chem.* **1990**, *33*, 3176.

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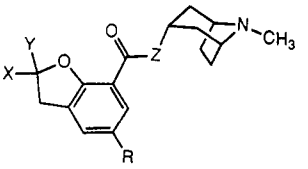
Table VII. Duration of Action of 5HT$_3$ Receptor Antagonists after Oral Administration to Rats[a,b]



no.	R	X	Y	Z	dose (mg/kg po)	% inhibn of 5HT-induced bradycardia vs time (h) 0.5	1	3	6	16
tropisetron				O	0.3	86.6 ± 5.8	77.4 ± 8.2	65.2 ± 9.1	17.4 ± 16.6	
52[c]				NH	0.03	78.4 ± 9.5	84.4 ± 6.0	7.4 ± 7.4	0.0 ± 0[d]	
31	CH_3	CH_3	H	O	10.0		74.8 ± 9.4	0.0 ± 0		
34	Cl	CH_3	H	O	0.3	92.6 ± 2.4	66.2 ± 17.6	60.5 ± 10.5	0.0 ± 0	
38	Cl	CH_3	H	NH	0.1	87.0 ± 5.9	74.6 ± 7.8	75.2 ± 7.6	71.4 ± 9.8	0 ± 0
35	Cl	CH_3	CH_3	O	0.03		86.2 ± 4.5	58.0 ± 19.6	20.6 ± 14.2	
zatosetron	Cl	CH_3	CH_3	NH	0.03	86.4 ± 2.7	77.6 ± 6.3	61.1 ± 16.9	81.4 ± 7.5	0 ± 0
ondansetron					1.0	74.3 ± 14.5	73.4 ± 7.9	6.5 ± 3.4		

[a] The stereochemistry was endo. When chiral centers were present in the aroyl moiety, the compounds were tested as racemates. [b] Compounds were administered by oral gavage to rats. Animals were then anesthetized and surgically prepared for the study. At the indicated times, 0.03 mg/kg iv of 5HT was given; in control animals, this resulted in a 71.3% decrease in heart rate. Unless indicated otherwise, each compound was tested in groups of 3–11 animals and the data represent means ± SEM for peak inhibition. For further details, consult the Experimental Section. [c] *N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide. This compound was synthesized as described in ref 20. [d] Data were derived from two animals.

Table VIII. Comparative in Vitro Affinities of Selected 5HT$_3$ Receptor Antagonists for 5HT$_3$ Receptors in the Guinea Pig Ileum[a]

compound	$-\log K_B$ ± SEM	compound	$-\log K_B$ ± SEM
tropisetron	8.06 ± 0.05	35	7.92 ± 0.10
31	7.32 ± 0.09	38	8.27 ± 0.12
34	7.88 ± 0.21	ondansetron	7.04 ± 0.04
zatosetron	8.78 ± 0.10	51	7.71 ± 0.14

[a] Values are means ± SEM for K_B = [B]/(dose ratio − 1) where [B] = concentration of antagonist and dose ratio = ED_{50} of the agonist after the antagonist, divided by the ED_{50} of the agonist before the antagonist. The number of tissues examined for each antagonist varied between four and seven.

the importance of the 5-chloro regiochemistry. The stereochemistry of the azabicyclo[3.2.1]octane ring was examined next. Compound **50**, the exo isomer of zatosetron, appeared to be considerably less potent than its endo counterpart. Zatosetron and **50**, at 3 μg/kg iv, caused 84 and 26% inhibition of 5HT-induced bradycardia, respectively. Fozard, as well as other investigators, have reached similar conclusions about the importance of the endo stereochemistry in tropane-derived 5HT$_3$ receptor antagonists.[16,17]

Selection of Zatosetron. These SAR studies indicated several benzofuran-derived compounds were potent 5HT$_3$ receptor antagonists after intravenous administration; however, additional in vivo comparisons were required to select the best candidate for development. The issue of oral activity was explored because of our interest in treating several chronic CNS disorders with 5HT$_3$ receptor antagonists. In the first set of experiments, **31**, a relatively early compound in the SAR, was compared to reference compounds **2** and tropisetron, after iv and po dosing. All three compounds inhibited 5HT-induced bradycardia in urethane-anesthetized rats. After iv administration, compound **31** was approximately 4-fold less potent than tropisetron, but both compounds were considerably more potent than **2** as 5HT$_3$ receptor antagonists (Figure 1). The two most potent compounds, tropisetron and **31**, were then compared 1 h after oral administration, and **31** was approximately 40-fold less potent than tropisetron (Figure 2). These data demonstrated that among ester 5HT$_3$ receptor antagonists, po/iv ratios can vary tremendously: the po/iv dose ratio for tropisetron was approximately 42, while the analogous value for **31** was considerably higher.

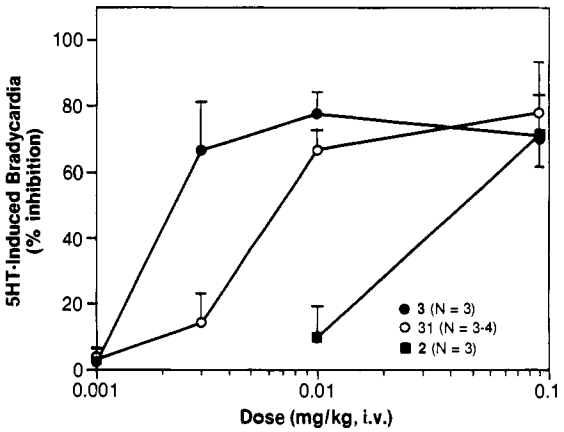


Figure 1. Inhibition of serotonin-induced bradycardia following iv administration of 5HT$_3$ receptor antagonists to urethane-anesthetized rats. Points are mean values and vertical bars represent standard errors of the mean. Serotonin (30 μg/kg iv) was injected following administration of the indicated doses of the 5HT$_3$ receptor antagonists.

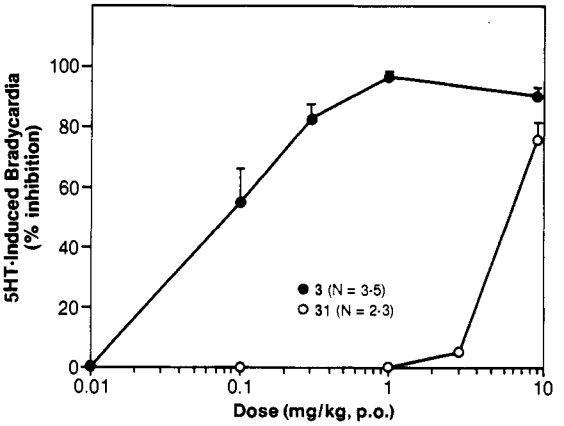


Figure 2. Inhibition of serotonin-induced bradycardia following oral administration of 5HT$_3$ receptor antagonists to rats. Points are mean values and vertical bars represent standard errors of the mean. Serotonin (30 μg/kg iv) was injected one hour following administration of the indicated doses of the 5HT$_3$ receptor antagonists.

Because of these findings, oral activities of several esters and their corresponding amide congeners were examined;

all compounds were evaluated 1 h after dosing, and the data are presented in Table V. It was evident from these studies that both esters and amides can produce potent, orally effective $5HT_3$ receptor antagonists (e.g. **35** and zatosetron). To enable more quantitative comparisons among these compounds, the po data (Table V) as well as analogous iv data (Table III) were transformed into ED_{50} values, and po/iv dose ratios were calculated; these values are presented in Table VI. By comparing ED_{50} values for tropisetron and **51**, it is apparent that indazoles are bioisosteric with indoles in $5HT_3$ receptor antagonists, a fact that has been previously reported.[22,23] Replacement of the ester of **51** with an amide to form **52** only modestly improved intrinsic potency (iv ED_{50} values were 2.57 and 2.17 μg/kg, respectively), but the oral ED_{50} value of the ester was 1 order of magnitude higher than that of the amide (po ED_{50} values were 159.8 and 13.8 μg/kg, respectively), resulting in a substantially improved po/iv dose ratio for the amide. However, the po/iv dose ratio for the amide zatosetron was 10.7, whereas the value for the corresponding ester **35** was 1.9, a surprisingly low value. Oral potency of these two agents was comparable, indicating that the relatively low po/iv dose ratio of the ester resulted from the lower iv $5HT_3$ receptor potency of **35** relative to zatosetron.

Duration of action in rats for these select compounds was studied next, and the data are presented in Table VII. The compounds were given orally at doses that produced similar antagonism of $5HT_3$ receptors (70–80% inhibition of 5HT-induced bradycardia), and block of 5HT-induced bradycardia was measured vs time. The two benzofuran amides, **38** and zatosetron, had the longest apparent pharmacological half-lives, and robust blockade of cardiac $5HT_3$ receptors was still demonstrable 6 h after oral administration. In contrast, $5HT_3$ receptor blockade by ondansetron and tropisetron had dissipated 3 and 6 h postadministration, respectively. This was also the case for the ester congeners of zatosetron.

From these studies, zatosetron was the optimal compound of the series as measured by potency, po/iv dose ratio, and duration of action. However, all these studies involved block of cardiac $5HT_3$ receptors, and we explored further the actions of zatosetron and a select group of congeners against $5HT_3$ receptor-mediated responses in a different tissue. Several publications have suggested that there may be differences among $5HT_3$ receptors located in different tissues.[24,25] This hypothesis was based upon the different apparent receptor affinities of $5HT_3$ receptor antagonists in the guinea pig ileum, the rabbit heart, and the rabbit vagus nerve.[24,25] However, others have suggested that these pharmacological differences do not reflect actual differences among $5HT_3$ receptors in these tissues, but relate to the complex effects of 5HT in the guinea pig ileum.[26-28] To examine this question further, some of these

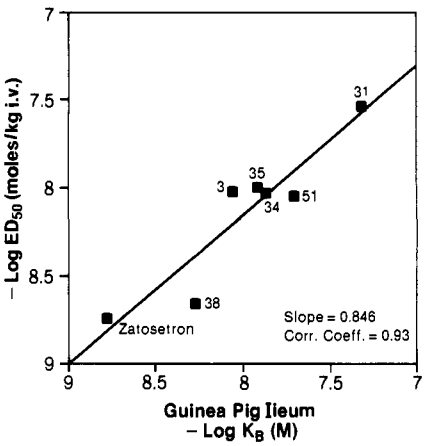


Figure 3. Correlation of the abilities of structurally diverse compounds to block $5HT_3$ receptors in rat heart and guinea pig ileum. The $-\log K_B$ values for inhibition of 2-methylserotonin-induced contractions of guinea pig ileum and $-\log ED_{50}$ values for inhibition of 5HT-induced bradycardia in rat heart were obtained as described in the Experimental Section. Each point represents data derived from at least three tissues or animals. The correlation coefficient for the least squares linear regression line was 0.93.

benzofuran derivatives were evaluated as $5HT_3$ receptor antagonists in the guinea pig ileum. In this tissue, 2-methylserotonin caused a contraction that was mediated by activation of $5HT_3$ receptors,[29] and apparent antagonist dissociation constants ($-\log K_B$) were generated for block of this response (Table VIII). All compounds were potent $5HT_3$ receptor antagonists in this tissue, and zatosetron proved to be the most potent ($-\log K_B = 8.78$). Zatosetron was almost 2 orders of magnitude more potent than ondansetron as an antagonist of ileal $5HT_3$ receptors. Importantly, the rank order of potency of these compounds was similar against 5HT-mediated responses at rat cardiac $5HT_3$ receptors and at guinea pig ileal $5HT_3$ receptors (compare Tables VI and VIII).

We rendered these comparisons more quantitative by plotting the $-\log K_B$ data from the block of guinea pig ileal $5HT_3$ receptors vs $-\log ED_{50}$ data for the compounds as antagonists of rat cardiac $5HT_3$ receptors (Figure 3). The correlation coefficient was 0.93, demonstrating that an excellent correlation exists for these two pharmacological effects of tropane-derived $5HT_3$ receptor antagonists. The highly significant ($p < 0.01$) correlation was impressive since the data involved comparisons from two species, and the usual complications of comparing in vitro and in vivo responses. These data do not support the hypothesis of major differences between $5HT_3$ receptors located in rat heart and guinea pig ileum. However, it should be noted that the structurally distinct $5HT_3$ receptor antagonist, ondansetron, was less potent in the guinea pig ileum (see Tables VI and VIII) than would have been predicted on the basis of the correlative data involving tropane-derived $5HT_3$ receptor antagonists (zatosetron, its congeners, and **3**). Similar correlative studies using additional ondansetron congeners would be of obvious interest. Although these data did not reveal differences between $5HT_3$ receptors in rat heart and guinea pig ileum, they did demonstrate that zatosetron was the optimal benzofuran-de-

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(28) For a recent review which summarizes the information on possible subtypes of $5HT_3$ receptors, see: Kirkpatrick, G. J.; Bunce, K. T.; Tyers, M. B. *Med. Res. Rev.* **1990**, *4*, 441.

(29) Cohen, M. L.; Bloomquist, W.; Gidda, J. S.; Lacefield, W. B. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 197.

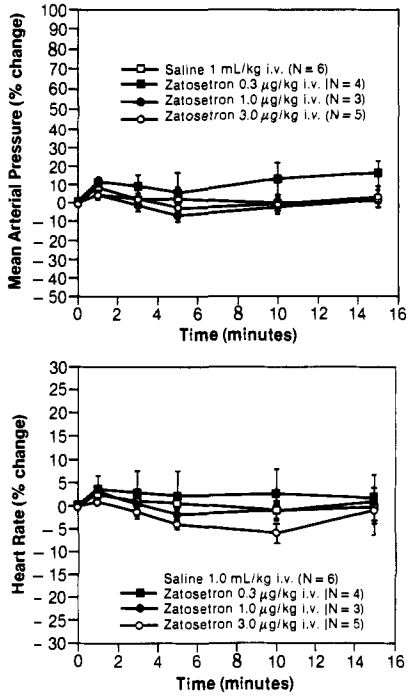

Figure 4. Effect of zatosetron (0.3, 1.0, and 3.0 μg/kg) on mean arterial blood pressure (MAP) and heart rate (HR) for 15 min after iv administration (upper and lower panels, respectively). Control values for MAP (mmHg) and HR (beats/min) were 93.6 ± 5.8 (n = 12) and 375.5 ± 10.0 (n = 12), respectively. Points are mean values and vertical bars represent the standard errors of the mean for the number of rats indicated in parentheses.

rived $5HT_3$ receptor antagonist using two species and two different tissues.

Pharmacological Selectivity of Zatosetron. For $5HT_3$ receptor antagonists to be useful in treating chronic CNS diseases, selectivity becomes of paramount importance in order to minimize side-effect liabilities of the drug candidate. Therefore, zatosetron was tested for anticholinergic activity in urethane-anesthetized rats and in isolated guinea pig ileum. Whereas zatosetron blocked 5HT-mediated contractions in the guinea pig ileum with a $-\log K_B$ of 8.78, it did not affect carbamylcholine-induced contractions at concentrations up to 10^{-6} M.[11] Thus, zatosetron displays at least 500-fold in vitro selectivity vis-à-vis these two receptors. In vivo, zatosetron (1000 μg/kg iv) also did not inhibit carbamylcholine (10 μg/kg iv) induced bradycardia in urethane-anesthetized rats, documenting that zatoseton has minimal, if any, anticholinergic liabilities.

The hemodynamic effects of zatosetron were studied in urethane-anesthetized rats (Figure 4). As previously noted, zatosetron blocked 5HT-induced bradycardia in this model with an ED_{50} of 0.86 μg/kg iv. However, at doses up to and including 3 μg/kg iv zatosetron produced no statistically significant alterations in heart rate or mean arterial blood pressure (lower and upper panels, respectively). Thus, although this $5HT_3$ receptor antagonist blocks the bradycardic effects of 5HT, it has minimal intrinsic hemodynamic effects.

With regard to gastrointestinal effects, zatosetron did not produce gastrointestinal prokinesis, whereas zacopride significantly increased gastric emptying in rats.[11] Thus, zatosetron would not be expected to produce gastrointestinal side effects at clinically relevant doses.

Conclusions and Summary. This paper documents the SAR that led to the discovery of zatosetron, a potent, selective, and long-acting $5HT_3$ receptor antagonist. Although numerous publications have appeared on the biochemistry and pharmacology of tropane-derived $5HT_3$ receptor antagonists (e.g. **2** and **3**), there are fewer publications on the SAR of these agents.[16,17,20,30-34] In this paper we have defined some of the key structural features which govern $5HT_3$ receptor antagonist activity within this chemical class. In dihydrobenzofuran-derived $5HT_3$ antagonists, *gem*-methyl substituents at position 2 and a 5-chloro substituent led to an optimally substituted dihydrobenzofuran moiety. Amide analogues were superior to ester derivatives, and strict stereo- and regiochemical requirements had to be met for optimal $5HT_3$ receptor blockade. Zatosetron blocked 5HT-induced bradycardia after iv administration, and the ED_{50} was 0.86 μg/kg iv, suggesting zatosetron is 3–4-fold more potent than tropisetron or ondansetron when administered intravenously.[29] Moreover, the compound was well-absorbed orally and had a long duration of action. Finally, we have demonstrated the pharmacological selectivity of zatosetron as measured by its lack of hemodynamic, anticholinergic, or gastrointestinal effects. The potency, selectivity, and long

Table IX. Physical Data for Compounds of Tables I–IV

no.	recryst solvent	mp, °C	formula[a,b]
14	$EtOH/Et_2O$	264–269	$C_{15}H_{19}NO_2 \cdot HCl$
15	$EtOH/Et_2O$	213–215	$C_{13}H_{17}NO_2 \cdot HCl$
16	$EtOH/Et_2O$	102–104	$C_{16}H_{21}NO \cdot C_4H_4O_4$
17	$EtOH/Et_2O$	276–277	$C_{16}H_{16}F_3NO_3 \cdot HCl$
18	$EtOH/Et_2O$	212–214	$C_{21}H_{23}NO_2 \cdot HCl$
19	$EtOH/Et_2O$	119.5–121	$C_{21}H_{23}NO_3 \cdot C_4H_4O_4$
20	$EtOH/H_2O/Et_2O$	301–302	$C_{21}H_{21}NO_3 \cdot HCl$
21	$EtOH/Et_2O$	298–300	$C_{21}H_{21}NO_3 \cdot HCl$
22	$EtOH/Et_2O$	194–195	$C_{22}H_{23}NO_2 \cdot C_4H_4O_4$
23	$EtOH/Et_2O$	145–147	$C_{18}H_{21}NO_3 \cdot C_4H_4O_4$
24	$EtOH/H_2O$	204–206	$C_{18}H_{21}NO_3 \cdot C_4H_4O_4$
25	$EtOH/EtOAc/Et_2O$	178–179.5	$C_{18}H_{23}NO_3 \cdot C_4H_4O_4$
26	$EtOH/H_2O$	166–167.5	$C_{19}H_{25}NO_3 \cdot C_4H_4O_4$
27	$EtOH/H_2O$	178–180	$C_{19}H_{25}NO_3 \cdot C_4H_4O_4$
28	$EtOH/Et_2O$	182–184	$C_{17}H_{21}NO_3 \cdot C_4H_4O_4$
29	$EtOH/Et_2O$	174–176	$C_{19}H_{25}NO_3 \cdot C_4H_4O_4$
30	$EtOH/Et_2O$	178.5–179	$C_{18}H_{23}NO_3 \cdot C_4H_4O_4$
31	$EtOH/Et_2O$	161–164	$C_{19}H_{25}NO_3 \cdot C_4H_4O_4$
32	$EtOH/Et_2O$	162–164	$C_{19}H_{25}NO_4 \cdot C_4H_4O_4$
33	$EtOH/Et_2O$	154–156	$C_{18}H_{22}FNO_3 \cdot C_4H_4O_4$
34	$EtOH/Et_2O$	170–172	$C_{18}H_{22}ClNO_3 \cdot C_4H_4O_4$
35	$EtOH/Et_2O/EtOAc$	175–177	$C_{19}H_{24}ClNO_3 \cdot C_4H_4O_4$
36	$EtOH/Et_2O$	144–146	$C_{18}H_{23}N_2O_2 \cdot C_4H_4O_4$
37	$EtOH/Et_2O$	163–164	$C_{19}H_{26}N_2O_2 \cdot C_4H_4O_4$
38	$EtOH/Et_2O$	196–198	$C_{18}H_{22}ClN_2O_2 \cdot C_4H_4O_4$
zatosetron	$EtOAc/EtOH$	184–186	$C_{19}H_{25}ClN_2O_2 \cdot C_4H_4O_4$
39	$EtOH/EtOAc$	178–180	$C_{19}H_{25}FN_2O_2 \cdot C_4H_4O_4$
40	$EtOAc$	185–187	$C_{19}H_{25}BrN_2O \cdot C_4H_4O_4$
41	$EtOH/EtOAc/Et_2O$	173–174	$C_{20}H_{28}N_2O_2 \cdot C_4H_4O_4$
42	$EtOH/Et_2O$	172–174	$C_{19}H_{26}N_2O_3 \cdot C_4H_4O_4$
43	$EtOH/EtOAc/Et_2O$	189–190	$C_{20}H_{28}N_2O_3 \cdot C_4H_4O_4$
44	$EtOH/Et_2O$	228–231	$C_{19}H_{26}N_2O_3 \cdot C_4H_4O_4$
45	$EtOH/EtOAc$	182–184	$C_{19}H_{26}N_2O_2 \cdot C_4H_4O_4$
46	$EtOH/Et_2O$	193–195	$C_{19}H_{26}N_2O_2 \cdot C_4H_4O_4$
47	$EtOH/EtOAc$	174–175	$C_{19}H_{26}N_2O_2 \cdot C_4H_4O_4$
48	$EtOH/Et_2O$	198–200	$C_{19}H_{25}ClN_2O_2 \cdot C_4H_4O_4$
49	$EtOH/Et_2O$	198.5–200	$C_{19}H_{25}ClN_2O_2 \cdot C_4H_4O_4$
50	$EtOH/Et_2O$	129–130.5	$C_{19}H_{26}ClN_2O_2 \cdot {}^1/_2H_2O \cdot C_4H_4O_4$

[a] C, H, N analyses were within ±0.4% of theoretical values. [b] The *Z* isomer of $C_4H_4O_4$ (maleic acid) was used.

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duration of action of zatosetron should enable this compound to be used to further define effects mediated via $5HT_3$ receptors in animals and man.

Experimental Section

Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are not corrected. Microanalytical data were provided by the Physical Chemistry Department of Lilly Research Laboratories; only symbols of elements analyzed are given, and they were within 0.4% of theoretical values unless indicated otherwise. Physical data for all final compounds are compiled in Table IX.

Except where noted, a standard procedure was used for product isolation. This involved quenching by addition to water, filtration, or exhaustive extraction with a solvent (washing of extract with aqueous solutions, on occasion), drying over an anhydrous salt, and evaporation of solvent under reduced pressure. Particular solvents, aqueous washes (if needed), and drying agents are mentioned in parentheses after the phrase "product isolation". Compounds **2**, tropisetron, and ondansetron were prepared according to published procedures.[35-37]

Synthesis of Zatosetron. The synthetic methods used in this study are best illustrated by the synthesis of zatosetron.

5-Chlorosalicylic Acid, Methyl Ester (6). Gaseous hydrogen chloride was passed through a solution of 5-chlorosalicylic acid (400 g, 2.33 mol) in 2 L of methanol until the solution was saturated, and then the reaction mixture was refluxed overnight. Solvent was removed under reduced pressure, and product isolation (ethyl acetate, 10% sodium bicarbonate, water, Na_2SO_4), followed by distillation, provided 369 g (85%) of **6** as a clear liquid with bp 110–111 °C (5 mmHg). Anal. ($C_8H_7ClO_3$) C, H.

5-Chloro-2-[(2-methylprop-1-en-3-yl)oxy]benzoic Acid, Methyl Ester (7). 3-Chloro-2-methylpropene (182 g, 2.0 mol) was added rapidly to a mixture of 5-chlorosalicylic acid, methyl ester (369 g, 1.98 mol), and potassium carbonate (276 g, 2.0 mol) in 1.5 L of acetone, and the reaction mixture was refluxed overnight. Product isolation (water, ethyl acetate, 10% sodium bicarbonate, water, Na_2SO_4) and distillation provided 137.7 (29%) of **7** with bp 111–112 °C (0.2 mmHg). Anal. ($C_{12}H_{13}ClO_3$) C, H.

5-Chloro-3-(2-methylprop-1-en-3-yl)salicylic Acid, Methyl Ester (8). A solution of **7** (137.7 g) in 200 mL of *N*-methylpyrrolidinone was heated at reflux for 7 h. Distillation provided 112.5 g (81.7%) of **8** as a clear liquid with bp 128–134 °C (2.0 mmHg). Anal. ($C_{12}H_{13}ClO_3$) C, H.

5-Chloro-2,3-dihydro-2,2-dimethylbenzofuran-7-carboxylic Acid (9). A solution of **8** (52.5 g) in 150 mL of 90% formic acid was refluxed for 3 h. Solvent was then removed under reduced pressure to afford cyclized product as an oil.

A mixture of this oil, sodium hydroxide (40 g), and 250 mL of water was refluxed for 3 h. Additional water was added and the reaction mixture was extracted with ethyl acetate (discarded). The aqueous layer was then acidified with concentrated hydrochloric acid. Product isolation (ethyl acetate, water) and recrystallization from ethyl acetate/hexane provided 39.4 g (79%) of **9** as a white solid with mp 159–161 °C. Anal. ($C_{11}H_{11}ClO_3$) C, H.

***endo*-5-Chloro-2,3-dihydro-2,2-dimethyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-7-benzofurancarboxamide (Zatosetron, LY277359).** A solution of **9** (24.9 g, 0.11 mol) in thionyl chloride (357 g, 3.0 mol) was refluxed for 3 h. Thionyl chloride was removed under reduced pressure. Toluene was added and removed under reduced pressure; this process was repeated three times.

A solution of 3α-aminotropane[13] (28.0 g, 0.2 mol) in 100 mL of toluene was added dropwise to a solution of the acid chloride in 1 L of toluene, and the reaction mixture was heated under reflux overnight. The reaction was cooled, water was added, and 10% sodium hydroxide was used to basify the mixture. The mixture was extracted with ether/ethyl acetate and the organic layer was then washed twice with 6 N hydrochloric acid. The combined acid washes were then rendered basic with 10% sodium hydroxide. Product isolation (ether/ethyl acetate, water, Na_2SO_4), formation of the maleic acid salt, and recrystallization from ethanol/ether provided 30.1 g (59%) of zatosetron maleate as white crystals with mp 184–186 °C. Anal. ($C_{19}H_{25}ClN_2O_2 \cdot C_4H_4O_4$) C, H, N.

3-[(2-Methylprop-1-en-3-yl)oxy]benzoic Acid, Methyl Ester (11). A mixture of 3-hydroxybenzoic acid, methyl ester (216.6 g, 1.43 mol), 3-chloro-2-methylpropene (135 g, 1.5 mol), and potassium carbonate (207 g, 1.5 mol) in 1 L of acetone was heated under reflux overnight. The reaction mixture was cooled to room temperature and then poured onto ice. Product isolation (ethyl acetate, water, 10% sodium bicarbonate, water, sodium sulfate) and distillation provided 182.1 g (63%) of product as a colorless oil with bp 92–95 °C (0.1 mmHg). Anal. ($C_{12}H_{14}O_3$) C, H.

3-Hydroxy-4-(2-methylprop-1-en-3-yl)benzoic Acid, Methyl Ester, and 3-Hydroxy-2-(2-methylprop-1-en-3-yl)benzoic Acid, Methyl Ester (12 and 13). A solution of 3-(2-methylprop-1-en-3-yloxy)benzoic acid, methyl ester (182.1 g), was heated at reflux for 6 h. Distillation provided 148 g (82%) of product as a mixture of the 2- and 4-regioisomers (bp 120–140 °C, 1.0 mmHg). A 31.5-g portion was purified by HPLC (toluene, silica gel) to provide 4.4 g of the less mobile 4-regioisomer and 19.2 g of the more mobile 2-regioisomer. Both regioisomers were clear viscous oils. Anal. ($C_{12}H_{14}O_3$) C, H.

Pharmacological Methods. Serotonin-Induced Activation of von Bezold–Jarisch Reflex in Urethane-Anesthetized Rats. Male Sprague–Dawley rats (200–300 g, Harlan Industries) were anesthetized with urethane (1.25 g/kg ip), a tracheotomy was performed, and an endotracheal tube was inserted (PE240). The carotid artery was cannulated and connected to a Gould Statham P23 1D pressure transducer via a 23-gauge needle. A femoral vein was exposed, cannulated with polyethylene tubing (PE50), and used for iv drug administration. Heart rate and blood pressure were monitored using the pressure tranducer signal and a cardiotachometer coupler (Beckman 9857B).

For iv evaluation of $5HT_3$ receptor antagonists, an initial response to 5HT or carbamylcholine was generated, and agonist-induced bradycardia was measured at peak effect. When agonist-induced bradycardia returned to steady state (within 5 min), either antagonist or saline was administered, and agonist-induced bradycardia was elicited again 5 or 15 min after antagonist or saline administration. For oral studies, fasted rats were dosed (5 mL/kg) by gavage with either antagonist or vehicle; 15–20 min before 5HT challenge, rats were anesthetized with urethane and surgically prepared as indicated above. For studies in-

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volving the duration of drug action, the 5HT$_3$ receptor antagonist or vehicle was administered orally to conscious rats. Rats were then anesthetized, surgically prepared, and challenged with 5HT at the time indicated after oral administration of the 5HT$_3$ receptor antagonist. Compounds were prepared daily in either distilled water or saline, with subsequent dilutions in physiological saline.

For inhibition of 5HT-induced changes in heart rate, statistical significance between mean values was determined with Student's t test for paired data. Statistical significance was assumed when $p < 0.05$.

Isolation of Ileal Smooth Muscle. Male guinea pigs (200–500 g) (Charles River Laboratories, Portage MI) were killed by cervical dislocation. Longitudinal sections of the guinea pig ileum (2–3 cm long) were used. To stabilize the baseline contraction, segments of guinea pig ileum were placed between two electrodes consisting of a stainless steel rod (bottom) and a circular platinum wire (top). Square-wave impulses (0.1 Hz) at 40 V and 0.7 ms in duration were provided by a Grass S44 stimulator (Grass Instruments, Quincy, MA).

Tissues were mounted in organ baths containing 10 mL of modified Krebs' solution of the following composition (in mM): 118.2 NaCl, 4.6 KCl, 1.6 $CaCl_2 \cdot 2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10.0 dextrose, 24.8 $NaHCO_3$. Tissue bath solutions were maintained at 37 °C and equilibrated with 95% O_2–5% CO_2. Each tissue was placed under optimum resting force and allowed to equilibrate for approximately 1 h before exposure to drugs. Isometric contractions were recorded as changes in grams of force on a Beckman dynograph (Beckman Instruments, Fullerton, CA) with Statham UC-3 transducers (Statham Medical Instruments, Los Angeles, CA).

Determination of Apparent Antagonist Dissociation Constants in Guinea Pig Ileum. Noncumulative (tissues were washed between agonist additions) contractile concentration–response curves for 2-methylserotonin, a selective 5HT$_3$ receptor agonist,[24] were obtained by stepwise increases in concentrations. EC_{50} values were taken as the concentration of 2-methylserotonin that produced half-maximal contraction. After control responses to 2-methylserotonin were obtained, ilea were incubated with appropriate concentrations of antagonist for 1 h. Noncumulative responses to 2-methylserotonin were then repeated in the presence of the antagonist that was added in the wash buffer between 2-methylserotonin additions. In each tissue, only one antagonist concentration was examined, and in each experiment one tissue was not treated with the antagonist and served as a control to correct for time-related changes in sensitivity.

Apparent antagonist dissociation constants (K_B) were determined for each concentration of antagonist according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1)$$

where [B] is concentration of antagonist and dose ratio is EC_{50} of the agonist in the presence of the antagonist divided by control EC_{50}. These results were then expressed as the negative logarithm of K_B (i.e., $-\log K_B$). These calculations were performed according to the procedures of Bemis.[38]

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Registry No. **6**, 4068-78-4; **7**, 136954-96-6; **8**, 136954-97-7; **9**, 123656-27-9; **10**, 123482-22-4; **10**·$C_4H_4O_4$, 123482-23-5; **11**, 136954-98-8; **12**, 136954-99-9; **13**, 136955-00-5; **14**, 19145-60-9; **14**·HCl, 55623-27-3; **15**, 16597-30-1; **15**·HCl, 40378-58-3; **16**, 97618-84-3; **16**·$C_4H_4O_4$, 136955-01-6; **17**, 136955-02-7; **17**·HCl, 136955-03-8; **18**, 136955-04-9; **18**·HCl, 136955-05-0; **19**, 136955-06-1; **19**·$C_4H_4O_4$, 136955-07-2; **20**, 136955-08-3; **20**·HCl, 136955-09-4; **21**, 136955-10-7; **21**·HCl, 136955-11-8; **22**, 136955-12-9; **22**·$C_4H_4O_4$, 136955-13-0; **23**, 136955-14-1; **23**·$C_4H_4O_4$, 136955-15-2; **24**, 136955-16-3; **24**·$C_4H_4O_4$, 136955-17-4; **25**, 136955-18-5; **25**·$C_4H_4O_4$, 136955-19-6; **26**, 136955-20-9; **26**·$C_4H_4O_4$, 137036-94-3; **27**, 137036-95-4; **27**·$C_4H_4O_4$, 137119-39-2; **28**, 136955-21-0; **28**·$C_4H_4O_4$, 136955-22-1; **29**, 123655-56-1; **29**·$C_4H_4O_4$, 123655-57-2; **30**, 136955-23-2; **30**·$C_4H_4O_4$, 136955-24-3; **31**, 136955-25-4; **31**·$C_4H_4O_4$, 136955-26-5; **32**, 136955-27-6; **32**·$C_4H_4O_4$, 136955-28-7; **33**, 136955-29-8; **33**·$C_4H_4O_4$, 136955-30-1; **34**, 136955-31-2; **34**·$C_4H_4O_4$, 136955-32-3; **35**, 123655-58-3; **35**·$C_4H_4O_4$, 123655-59-4; **36**, 136955-33-4; **36**·$C_4H_4O_4$, 136955-34-5; **37**, 123655-44-7; **37**·$C_4H_4O_4$, 123655-45-8; **38**, 136955-35-6; **38**·$C_4H_4O_4$, 136955-36-7; **39**, 123655-88-9; **39**·$C_4H_4O_4$, 123655-89-0; **40**, 123656-21-3; **40**·$C_4H_4O_4$, 123656-22-4; **41**, 123655-50-5; **41**·$C_4H_4O_4$, 123655-51-6; **42**, 136955-37-8; **42**·$C_4H_4O_4$, 136955-38-9; **43**, 123655-86-7; **43**·$C_4H_4O_4$, 123655-87-8; **44**, 123656-08-6; **44**·$C_4H_4O_4$, 123656-09-7; **45**, 123655-52-7; **45**·$C_4H_4O_4$, 123655-53-8; **46**, 123655-48-1; **46**·$C_4H_4O_4$, 123655-49-2; **47**, 123655-54-9; **47**·$C_4H_4O_4$, 123655-55-0; **48**, 123655-46-9; **48**·$C_4H_4O_4$, 123655-47-0; **49**, 123655-92-5; **49**·$C_4H_4O_4$, 123655-93-6; **50**, 123655-68-5; **50**·$C_4H_4O_4$, 123655-69-6; **51**, 107007-94-3; **52**, 109216-57-1; 5-chlorosalicylic acid, 321-14-2; 3-chloro-2-methylpropene, 563-47-3; 3α-aminotropane, 87571-88-8; 3-hydroxybenzoic acid, methyl ester, 19438-10-9.

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