Structure−Activity Relationships for the Binding of Arylpiperazines and Arylbiguanides at 5-HT_3 Serotonin Receptors

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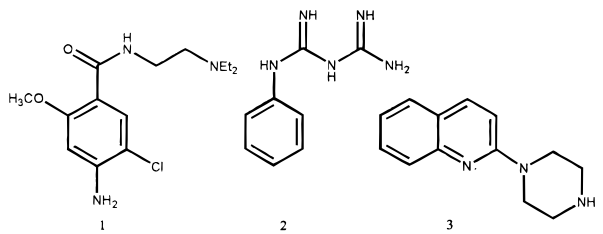
Arylpiperazines are nonselective agents that bind at 5-HT_3 serotonin receptors with moderate to high affinity, whereas 1-phenylbiguanide is a low-affinity but more selective 5-HT_3 agonist. In an attempt to enhance the affinity of the latter agent, and working with the assumption that similarities might exist between the binding of the two types of agents, we formulated structure−activity relationships for the binding of the arylpiperazines and then incorporated those substituents, leading to high affinity for the arylpiperazines, into 1-phenylbiguanide. A subsequent investigation examined the structure−activity relationships of the arylbiguanides and identified arylguanidines as a novel class of 5-HT_3 ligands. Although curious similarities exist between the structure−activity relationships of the arylpiperazines, arylbiguanides, and arylguanidines, it cannot be concluded that all three series of compounds are binding in the same manner. Furthermore, upon investigating pairs of compounds in the three series, the arylpiperazines behaved as 5-HT_3 antagonists (von Bezold−Jarisch assay) whereas the arylbiguanides and arylguanidines acted as 5-HT_3 agonists.

Selective ligands are typically required for characterizing the nature and function of neurotransmitter receptors, and it is fortunate that high-affinity 5-HT_3 serotonin receptor ligands have been identified/developed. This population of serotonin receptors has recently attracted widespread attention because of its possible involvement in chemotherapy-induced emesis, migraine, pain management, and certain mental disorders.[1−6] Most of the agents currently available, however, are 5-HT_3 antagonists. On the basis that metoclopramide (**1**) might produce some of its effects through a 5-HT_3 mechanism, Fozard[7] developed the first 5-HT_3-selective antagonist: MDL-72222. Since then, numerous other 5-HT_3 antagonists have been described; most of these antagonists are aryl- or heteroarylamide derivatives. The structure−activity relationships of 5-HT_3 antagonists have been extensively reviewed.[3−5]

Much less is known about 5-HT_3 agonists. Agents commonly employed as 5-HT_3 agonists include serotonin (5-HT) and 2-methyl 5-HT.[3] Serotonin binds with modest affinity at 5-HT_3 receptors (K_i = ca. 750 nM) and is a nonselective agent;[3,8] 2-methyl 5-HT, which binds with somewhat lower affinity (K_i = 1350 nM) than 5-HT, displays greater selectivity for 5-HT_3 receptors and retains agonist activity.[9] With the exception of 5-HT_3 receptors, most populations of 5-HT receptors are G-protein-coupled receptors;[3] 5-HT_3 receptors are ion channel receptors. Consequently, many of the agents that bind at 5-HT_3 receptors are fairly unique structurewise, and most agents that bind at other populations of 5-HT receptors display low affinity for 5-HT_3 receptors and vice versa.[1−5] Quaternized amines, for example, bind at 5-HT_3 receptors but not at other populations of 5-HT receptors.[4] Indeed, it has been demonstrated that the *N*,*N*,*N*-trimethyl quaternary amine derivative of 5-HT binds with 10 times the affinity of 5-HT, possesses a much more restrictive binding profile, and behaves as a 5-HT_3 agonist.[8,10]

Two other groups of agents that bind at 5-HT_3 receptors include the 1-arylpiperazines and the 1-arylbiguanides. However, whereas 5-HT_3 structure−activity relationships have been formulated for 5-HT_3 antagonists, e.g., ref 5, and for 5-HT-related derivatives, e.g., ref 10, relatively little is known about the arylpiperazines and arylbiguanides. In fact, 1-phenylbiguanide (**2**) was the only arylbiguanide available as a serotonergic agent for a number of years, and as a class, the arylpiperazines have never been specifically categorized as 5-HT_3 agonists or antagonists. One reason for the apparent lack of interest in the arylpiperazines as 5-HT_3 ligands is their high affinity for other populations of 5-HT receptors.[3,11] Quipazine (**3**), a nonselective serotonergic agent, was the first arylpiperazine demonstrated to bind at 5-HT_3 receptors.[12] Quipazine has been demonstrated to be an agonist in certain preparations[13] but an antagonist in others.[14−16]



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In the late 1980s we undertook a study aimed at identifying novel 5-HT_3 receptor agonists. We endeavored to formulate structure−activity relationships for the binding of arylpiperazines at 5-HT_3 receptors and reported the results of the first such study in 1989.[17] [^{3}H]Quipazine was briefly used as a radioligand to label 5-HT_3 receptors,[18] and it was the radioligand employed

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by us in our structure−activity study.[17] However, subsequent anecdotal reports suggested that [^{3}H]quipazine may not provide reliable results, and there now is evidence that this radioligand may be unstable under the conditions of the binding assay.[19] [^{3}H]Quipazine is no longer used to label 5-HT$_3$ receptors. Nevertheless, our earlier investigation identified two arylpiperazines, 1-(3-chlorophenyl)piperazine (mCPP, **4**) and 1-(2-naphthyl)piperazine (2-NP, **5**), as interesting agents for further study.[17] Although **4** and **5** bind with high affinity at 5-HT$_3$ receptors (5-HT$_3$ K_i = 20 and 30 nM, respectively), they are nonselective agents in that they bind at other populations of 5-HT receptors.[11] In 1987, Ireland and Tyers[15] described 1-phenylbiguanide (**2**) as a novel 5-HT$_3$ agonist. Although 1-phenylbiguanide displays relatively low affinity for 5-HT$_3$ receptors (K_i > 1000 nM), it represented the first 5-HT$_3$-selective agonist. Thus, 1-phenylbiguanide is a selective but low-affinity agonist, whereas certain arylpiperazines represent higher affinity but nonselective agents. Even though there is little evidence or *a priori* reason to suspect that arylpiperazines such as 1-phenylpiperazine, and arylbiguanides, such as 1-phenylbiguanide, should bind at 5-HT$_3$ receptors in a similar manner, this concept served as a starting point for our initial investigations, that is, if arylpiperazines and arylbiguanides utilize a common aromatic binding site on 5-HT$_3$ receptors, it might be possible to extrapolate the structure−activity relationships formulated for the arylpiperazines to the arylbiguanides so as to enhance their affinity for 5-HT$_3$ receptors. Early work in our laboratory, based on the results of our initial structure−activity study on arylpiperazines, led to two novel arylbiguanides: 1-(3-chlorophenyl)biguanide (**6**) and 1-(2-naphthyl)biguanide (**7**). Some of our preliminary results have been reported.[20]

Due to our use of [^{3}H]quipazine in the original arylpiperazine structure−activity study, we were no longer certain that the formulated structure−activity relationships were valid. We wished to repeat these studies using a newer radioligand to label 5-HT$_3$ receptors and to continue our investigations of the arylbiguanides. While our work was in progress, Kilpatrick et al.[12] reported that **6** is a high-affinity 5-HT$_3$ agonist. Morain et al.[21] have more recently described some of their structure−activity studies with aryl-modified arylbiguanides. However, apart from their study, a systematic and comprehensive structure−activity investigation of arylbiguanides has not yet been reported.

The purpose of the present report, then, is severalfold. First, we describe a structure−affinity study on arylpiperazines using [^{3}H]GR65630 as radioligand and, where possible, compare the present findings with the original[17] structure−affinity findings. Second, we describe the results of a structure−affinity study with the arylbiguanides where we investigate modifications of both the aryl and biguanide portions of the molecule. Finally, we examine the functional activity of certain selected compounds in order to determine if they are 5-HT$_3$ receptor agonists or antagonists. During the course of this investigation, arylguanidines were identified as a novel class of 5-HT$_3$ agonists.

Chemistry

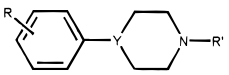
Many of the compounds necessary for this investigation were on hand as a result of previous studies, were commercially available, or were prepared according to literature procedures (see the Experimental Section for details). Pyridine derivative **11** was synthesized by catalytic hydrogenation of the product obtained from the Grignard reaction of 2-methoxybromobenzene and *N*-benzyl-4-piperidone. The quaternary compounds **14** and **16** were synthesized by exhaustive N-methylation of the corresponding phenylpiperazines with iodomethane. The piperazine derivatives **17**,[22] **18**, and **19**[22,23] were prepared using the procedure described by Brewster et al.[24] where bis(2-chloroethyl)amine hydrochloride was used to alkylate the appropriate aniline. 2-[1-(4-Methylpiperazinyl)]pyridines **24**−**26** were obtained by alkylation of *N*-methylpiperazine with the corresponding halopyridine.[25] The *N*,*N*-dimethyl analog of arylbiguanide **34** was prepared by the general and well-known reaction of an aromatic amine mineral acid salt and appropriately substituted dicyandiamide. The reverse amide **36** and amide **37** were prepared by reaction of the appropriate amine with acid halides which were then converted by Gabriel synthesis[26] to the phthalimides and hydrolyzed with hydrazine hydrate to the corresponding amines. Although the free base and nitrate of compound **44** have been reported,[27] we synthesized its oxalate salt in a manner similar to that of **39**, that is, the appropriate halide was converted to its nitrile and then reduced with $LiAlH_4$ to the corresponding amine. The *N*-methyl (**55**) and *N*,*N*-dimethyl (**56**) derivatives of compound **54** are mentioned in the literature; however, we were unable to find any physical characterization of these compounds. The secondary amine **55** was prepared from **54** via a carbamate intermediate which was subsequently reduced to the desired target with $LiAlH_4$. The tertiary amine **56** was prepared from **54** by reductive methylation using an Eschweiler−Clarke procedure. Guanidine **60** was prepared by the general method described by Short and Darby.[28]

Results

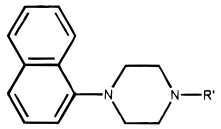
Arylpiperazines. Radioligand binding data for the arylpiperazine derivatives are presented in Table 1. Of the compounds examined, 10 are common to this and the original[17] study. This allowed some comparisons to be made between the two studies. Of the 10 compounds, binding data for five (**3**−**5**, **20**, **21**) differed between the two investigations by only 2- or 3-fold. However, the affinity of the parent phenylpiperazine **8** is approximately 7-fold lower than reported earlier, and the affinities of **9**, **10**, **12**, and **13** are >23-, 8-, 5-, and 18-fold lower. While there generally seems to be a good qualitative agreement between the two studies, the original study overestimated the affinity of certain arylpiperazines for 5-HT$_3$ receptors. The only case where this may have been of consequence is with **8** because, being the parent member of the series, its affinity was compared to that of other arylpiperazines in making structure−activity comparisons. Thus, although some of the quantitative statements made in the original study may now need to be revised, many of the original conclusions still seem to apply.

1-Phenylpiperazine (**8**) binds with low affinity (K_i = 3000 nM; Table 1). Replacement of the less basic nitrogen atom of **8** with a carbon atom results in reduced affinity (**9**, K_i > 10 000 nM). Methoxy substitution at the phenyl 2- or 4-position (**10**, **12**) has little effect on

Table 1. Binding of Aryl-Modified Piperazines at 5-HT$_3$ Receptors



compd	R	Y	R′	K_i, nM (SEM)
8	H	N	H	3000 (50)
9	H	CH	H	>10000
10	2-OMe	N	H	2500 (530)
11	2-OMe	C-OH	H	>10000
12	4-OMe	N	H	1640 (480)
13	4-Cl	N	H	2160 (170)
4	3-Cl	N	H	62 (5)
14	3-Cl	N	Me_2^+	1450 (380)
15	3-CF_3	N	H	1390 (425)
16	3-CF_3	N	Me_2^+	>10000
17	2,4-$(OMe)_2$	N	H	>10000
18	2-Me, 4-OMe	N	H	660 (10)
19	2-OMe, 5-Cl	N	H	40 (4)



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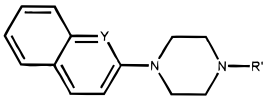

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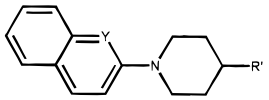
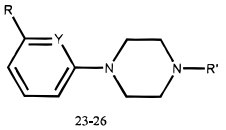


22

20			H	175 (15)
5		CH	H	32 (8)
3		N	H	2 (1)
21		N	Me	3 (1)
22		N	H	>10000



23-26

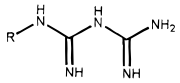
23	Cl	N	H	15 (5)
24	Cl	N	Me	38 (1)
25	Me	N	Me	560 (90)
26	I	N	Me	48 (3)

affinity, whereas 2,4-dimethoxy substitution (i.e., **17**) is detrimental to binding. Replacement of the less basic nitrogen atom of **10** with a C-OH group (i.e., **11**) also reduces affinity. Introduction of a 4-chloro group (i.e., **13**) seems inconsequential relative to **8**, whereas introduction of the 3-chloro (i.e., **4**, K_i = 62 nM) enhances affinity by 50-fold. The 3-trifluoromethyl derivative **15** binds with 2 times the affinity of its parent **8** but with a 22-fold lower affinity than the corresponding 3-chloro derivative **4**. The influence of the *m*-chloro substituent is also seen when the 2-methoxy-5-chloro compound **19** (K_i = 40 nM) is compared with the monosubstituted 2-methoxy compound **10** (K_i = 2500 nM). As mentioned in the introduction, the quaternary amine analog of 5-HT binds with 10-fold higher affinity than 5-HT itself. Consequently, we prepared the *N*,*N*-dimethyl quaternary amine analogs of **4** and **15**; however, affinity was decreased in both cases (i.e., **14** and **16**, K_i = 1450 and > 10 000 nM, respectively).

Introduction of the 2-methyl-4-methoxy substituents seems to enhance affinity (**18**, K_i = 660 nM) relative to the 4-methoxy derivative **12** (K_i = 1640 nM) suggesting that substitution may be tolerated at the 2-position. Consequently we examined 1-(1-naphthyl)piperazine (**20**, K_i = 175 nM) and found it to bind with 17-fold higher affinity than the parent phenylpiperazine **8**. 1-(2-Naphthyl)piperazine (2-NP, **5**, K_i = 32 nM) binds with even higher affinity and with an affinity approximately 100 times greater than that of **8**. The lack of affinity of **22** suggests that the N4 nitrogen atom is required for binding. Introduction of a nitrogen atom into the ring as with quipazine (**3**) and its *N*-methyl derivative **21** (K_i = 2 and 3 nM, respectively) produces the highest affinity members of the series. Finally, the quinoline-type nitrogen atom of **3** was incorporated into **4** (K_i = 62 nM) to afford the 6-chloro-2-pyridyl derivative **23**; compound **23** (K_i = 15 nM) was found to bind with 4 times the affinity of **4** and 200 times the affinity of **8**. The related *N*-methyl derivative **24** (K_i = 38 nM) binds with similar affinity as does the 6-iodo counterpart **26** (K_i = 48 nM). In contrast, the 6-methyl derivative **25** (K_i = 560 nM) binds with reduced affinity.

The present findings essentially confirm (at least qualitatively) and extend the results of our earlier structure−activity study.[17] Most importantly, there appears to be something unique about the 3-position of the phenylpiperazines because the presence of a chloro group at this position enhances affinity by about 50-fold. This effect cannot be mimicked by incorporation of a trifluoromethyl group. However, fusion of the *c*-face of **8** with a benzene ring, to give 2-NP (**5**), results in a high-affinity compound. Affinity is further enhanced by introduction of a ring nitrogen (i.e., **3**, K_i = 2 nM). A ring nitrogen atom also enhances the affinity of **4** (i.e., **23**) but to a somewhat lesser extent. Perhaps the most unexpected finding is that quaternization of the terminal amine of the arylpiperazines (i.e., **14**, **16**) results in reduced affinity; this is unlike what was seen with 5-HT itself.[8]

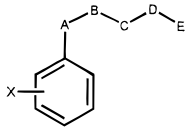
Table 2. Binding of Arylbiguanides at 5-HT$_3$ Receptors



compd	R	K_i, nM (SEM)
2	phenyl	1200 (40)
27	2-Cl-phenyl	62 (4)
6	3-Cl-phenyl	17 (4)
28	4-Cl-phenyl	200 (20)
29	3-NO_2-phenyl	220 (40)
30	3-Me-phenyl	780 (50)
31	4-Me-phenyl	>10000
32	3-Cl-4-Me-phenyl	225 (25)
33	2-OMe-5-Cl-phenyl	126 (33)
7	2-naphthyl	12 (2)

Arylbiguanides. 1. Aryl Substitution. 1-Phenylbiguanide (PBG; **2**) and several of its aryl-substituted derivatives were examined (Table 2). PBG (**2**) binds with low affinity (K_i = 1200 nM). Incorporation of a chloro group at the 2-, 3-, or 4-position results in enhanced affinity (**27**, **6**, **28**, K_i = 62, 17, and 200 nM, respectively). The effect of the 3-chloro group could not be mimicked by replacement with a (nearly equilipophilic) methyl group (i.e., **30**, K_i = 780 nM) or the electron-withdrawing nitro group (i.e., **29**, K_i = 220 nM). The 4-methyl derivative **31** lacks affinity; however, introduction of a chloro group to give the 3-chloro-4-methyl derivative **32** (K_i = 225 nM) again improves binding. The 2-methoxy-5-chloro derivative **33** (K_i = 126 nM) binds with 10 times the affinity of **2**. The

Table 3. Binding of Biguanide Derivatives at 5-HT_3 Receptors



compd	A	B	C	D	E	X	K_i, nM (SEM)
6	NH	C(=NH)	NH	C(=NH)	NH_2	3-Cl	17 (4)
34	NH	C(=NH)	NH	C(=NH)	NMe_2	3-Cl	>10000
35	NH	C(=O)	NH	C(=NH)	NH_2	3-Cl	1250 (22)
36	NH	C(=O)	CH_2	CH_2	NH_2	3-Cl	>10000
37	C(=O)	NH	CH_2	CH_2	NH_2	3-Cl	>10000
38	NH	CH_2	CH_2	CH_2	NH_2	3-Cl	>10000
39	CH_2	CH_2	CH_2	CH_2	NH_2	3-Cl	>10000
40	CH_2	CH_2	NH	C(=NH)	NH_2	3-Cl	40 (2)
41	CH_2	CH_2	NH	C(=NH)	NH_2	2-Cl	150 (30)
42	CH_2	CH_2	NH	C(=NH)	NH_2	4-Cl	243 (27)
43	CH_2	NH	C(=NH)	NH_2		3-Cl	1200 (12)
44	CH_2	CH_2	CH_2	NH_2		3-Cl	>10000
45	NH	C(=NH)	NH_2			3-Cl	35 (5)

2-naphthyl derivative **7** (K_i = 12 nM) binds with 100-fold greater affinity than **2**.

There are some similarities and differences between the binding of the arylpiperazines and the arylbiguanides. In both series, introduction of a 3-chloro group results in enhanced affinity (50- and 70-fold) as compared to the parent aryl-unsubstituted compound. In both series, the 2-naphthyl derivatives bind with 100-fold greater affinity than their respective parent. In both series, the electron-withdrawing chloro group cannot be replaced with another electron-withdrawing group with retention of affinity. However, 2-methoxy-5-chloro substitution results in a 75-fold increase in affinity in the arylpiperazine series, whereas it produces only a 10-fold increase in affinity in the arylbiguanide series. At this time, it cannot be conclusively stated that the aromatic portions of the two series of agents are binding in a similar manner (or at the same site); however, there are certain curious trends to suggest that this may be possible.

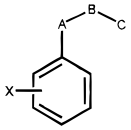
Morain et al.[21] recently published the results of their structure−activity studies on arylbiguanides. Although they examined a total of 24 aryl-modified biguanides, there are only three substituted phenylbiguanide derivatives common to the two investigations. For these three compounds, K_i values are nearly identical between the two studies: **27** (K_i = 69 nM[21] as compared to 62 nM in Table 2), **6** (13 nM[21] as compared to 17 nM), and **28** (170 nM[21] as compared to 200 nM). Morain et al.[21] found that the 3-chloro group could not be replaced by a trifluoromethyl group with retention of high affinity (i.e., this replacement resulted in a 54-fold decrease in affinity); they further identified the 2,3,5-trichloro derivative of **2** (K_i = 0.44 nM) as the highest affinity member of the series. Krijzer and Tulp[29] had earlier reported that the 3,4-dichloro derivative of phenylbiguanide is a high-affinity (K_i = 40 nM) 5-HT_3 agonist; Morain et al.[21] reported a K_i of 20 nM for this compound. All of these findings attest to the importance of a *m*-chloro group.

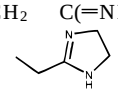
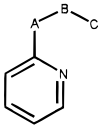
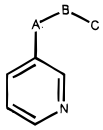
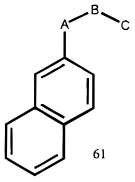
2. Biguanide Modification. With the 3-chloro group present, the biguanide portion of **6** (K_i = 17 nM) was modified (Table 3). Introduction of a terminal *N*,*N*-dimethyl group (i.e., **34**) abolishes affinity. Replacement of the N2 nitrogen atom with oxygen (**35**, K_i = 1250 nM) decreases affinity by 73-fold. Further modification of the biguanide chain as in **36**−**39** also abolishes affinity. Interestingly, the *N*-(2-phenylethyl)guanidine derivative **40** binds with high affinity (K_i = 40 nM) suggesting that the intact biguanide moiety is not required for binding. The affinity of the 3-chloro derivative **40** was compared with those of its 2- and 4-chloro positional isomers **41** and **42**, respectively. The 2-chloro derivative **41** and the 4-chloro derivative **42** bind with approximately 4- and 6-fold lower affinity, respectively, than **40**. Shortening of the phenylethyl portion of **40** to benzyl (**43**, K_i = 1200 nM) reduces affinity by 30-fold, and further modification, as with **44**, abolishes affinity. In an investigation of the binding requirements of 5-HT_3 antagonists, Rizzi et al.[30] found that the deschloro derivative of **43** (i.e., *N*-benzylguanidine; K_i > 10 000 nM) does not bind at 5-HT_3 receptors. The higher affinity of **43** suggests once again that the *m*-chloro group contributes to binding. However, removing the ethyl chain of **40** to give the (3-chlorophenyl)guanidine derivative **45** (K_i = 35 nM) results in a high-affinity compound. Because **45** is essentially a biguanide lacking the terminal amidine portion of the molecule, its structure−activity relationships were examined in further detail.

Arylguanidines. Compound **45** appears to be the first member of a novel class of serotonergic ligands; we investigated the effect of both aryl substitution and modification of the guanidine moiety (Table 4). Removal of the 2-chloro group to give the parent 1-phenylguanidine (**46**, K_i = 2340 nM) reduces affinity by nearly 70-fold. Of the three different chloro-substituted compounds (**45**, **47**, **48**), 3-chloro substitution seems to be optimal. As seen with the other series of compounds, the 3-chloro substituent cannot be replaced by trifluoromethyl with retention of affinity; the trifluoromethyl derivative **49** (K_i = 5770 nM) binds with 165-fold lower affinity than **45**. Likewise, replacement of this chloro substituent with methyl or methoxy groups (**50**, **51**) results in low-affinity compounds.

Conversion of the guanidine moiety of **45** to a urea (i.e., **52**) abolishes affinity; most other modifications of the guanidine moiety also abolish affinity (i.e., **53**−**56**). The amidine **57** (K_i = 1200 nM), in which one of the nitrogen atoms of **45** has been replaced by a methylene group, binds with 35-fold reduced affinity, whereas cyclization to the imidazoline **58** abolishes affinity. The 2-pyridyl analog **59** is inactive, but interestingly the

Table 4. Binding of Arylguanidines at 5-HT$_3$ Receptors



compd	A	B	C	X	K_i, nM (SEM)
46	NH	C(=NH)	NH_2	H	2340 (150)
47	NH	C(=NH)	NH_2	2-Cl	190 (90)
45	NH	C(=NH)	NH_2	3-Cl	35 (5)
48	NH	C(=NH)	NH_2	4-Cl	320 (150)
49	NH	C(=NH)	NH_2	3-CF_3	5700 (2300)
50	NH	C(=NH)	NH_2	3-Me	6520 (1035)
51	NH	C(=NH)	NH_2	3-OMe	1600 (300)
52	NH	C(=O)	NH_2	3-Cl	>10000
53	N=	CH	NH_2	3-Cl	>10000
54	CH_2	CH_2	NH_2	3-Cl	>10000
55	CH_2	CH_2	NH-Me	3-Cl	>10000
56	CH_2	CH_2	$N(Me)_2$	3-Cl	>10000
57	CH_2	C(=NH)	NH_2	3-Cl	1200 (96)
58				3-Cl	>10000
	 59		 60		 61
59	NH	C(=NH)	NH_2		>10000
60	NH	C(=NH)	NH_2		2910 (740)
61	NH	C(=NH)	NH_2		25 (2)

3-pyridyl analog displays about the same affinity (**60**, K_i = 2910 nM) as the unsubstituted parent **46**. The 2-naphthyl derivative **61** (K_i = 25 nM) binds with nearly 100-fold higher affinity than **46**.

This class of compounds is rather interesting for several reasons. As with the arylpiperazines and the arylbiguanides, introduction of a 3-chloro group results in enhanced affinity and replacement of phenyl with 2-naphthyl increases affinity by 100-fold. Also, as with the other two series, the 3-chloro substituent cannot be replaced with a trifluoromethyl group without a significant decrease in affinity. Differences also exist. For example, whereas introduction of a 4-chloro group enhances affinity by about 7-fold in the latter two series, it has essentially no effect in the arylpiperazine series; also, replacement of the phenyl group by 2-pyridyl enhances the affinity of the arylpiperazines but reduces the affinity of the arylguanidines.

Functional Studies. Compounds **2**, **4**−**7**, **45**, and **61** were examined in the von Bezold−Jarisch assay.[31] After several preliminary trials, compound **6** was subsequently used as control in all other experiments; **6** consistently produced the bradycardic response at doses of 0.9−20 μg/kg. Results for the other compounds examined are reported in Table 5 relative to **6**. 1-Phenylbiguanide (**2**) and the naphthyl biguanide **7** were about one-half as potent as **6;** guanidine **45** was somewhat more potent than, and **61** was essentially equipotent with, **6**. The agonist effects of these compounds could be antagonized by the 5-HT$_3$ antagonist tropisetron (data not shown). The two piperazine derivatives **4** and **5** were ineffective as agonists at doses of up to 200 μg/kg. In fact, at doses of 20−120 μg/kg, both agents were able to attenuate the effects of **6** when given in combination (data not shown). It would seem then that the two piperazines **4** and **5** lack agonist activity and possess some antagonist character. In contrast, both arylbiguanides and both arylguanidines act as 5-HT$_3$ agonists.

Table 5. 5-HT$_3$ Receptor Stimulant Potencies in Rat (von Bezold−Jarisch Reflex)

		relative potency[a]	
compd	*n*	potency	range
2	5	0.45	0.3−0.6
4	3	<0.02	
5	3	<0.02	
6	21	1.0	
7	4	0.5	0.3−0.7
45	7	0.74	0.5−1.3
61	5	1.0	0.5−1.5

[a] Stimulant potency relative to compound **6** = 1. Compound **6** produced relfex bradycardia in all preparations at concentrations of 0.9−20 μg/kg and was used as a reference standard in all experiments. Tropisetron at doses of 2−10 μg/kg markedly reduced or fully antagonized the stimulant effects of **2**, **6**, **7**, **45**, and **61**. Compounds **4** and **5** produced no detectable effect.

Table 6. 5-HT$_3$ Receptor Stimulant Potencies in the Rabbit Bladder Preparation

compd	*n*	minimal dose[a]
2	2	1−10
5	1	30
6	6	6.6
7	4	1−10
45	4	30−150
61	2	2.5−15

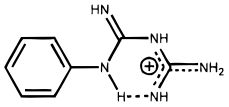
[a] Minimal effective dose in μg/kg. The minimal effective dose for **6** was determined to be 6.6 ± 1.8 μg/kg. Potencies of other compounds are reported as dose ranges. Compound **4** was not examined. The stimulant effect of all compounds was antagonized by tropisetron at doses of 5−10 μg/kg; antagonism of **61** was not examined.

In the rabbit bladder assay,[32] compound **6** produced a stimulant effect with a minimal effective dose of 6.6 (±1.8) μg/kg (Table 6). Compounds **2** and **7** produced similar effects at the 1−10 μg/kg dose range. Compound **45** was somewhat less potent (minimal effective dose range = 30−150 μg/kg), whereas **61** (2.5−15 μg/kg) seems nearly equipotent with **6**. The stimulant effects of each of these compounds was antagonized by tropisetron. At 30 μg/kg, the piperazine derivative **5** displayed agonist activity; although its potency is low, its effect was antagonized by tropisetron. Compound **4** was not examined. These results show that the two arylbiguanides and the two arylguanidines act as agonists in this preparation. In conclusion, both assays reveal that the two arylbiguanides and the two arylguanidines are 5-HT$_3$ agonists.

Discussion

Previously, it was not known with certainty whether or not arylpiperazines represented 5-HT$_3$ agonists or antagonists. Even in the absence of knowledge concerning the functional activity of these agents, it was not unreasonable to suspect that there may be some structural similarity between the arylpiperazines and arylbiguanides with respect to the manner in which they interact with 5-HT$_3$ receptors, that is, it is possible that structural relationships might exist between the two structure types even if one class represents agonists and the other antagonists. We expected that structure−activity information derived from the high-affinity but nonselective arylpiperazines might be applied to en-

hancing the affinity of the more 5-HT_3-selective, yet low-affinity, 1-phenylbiguanide (**2**). We posed the question: Is there any structural similarity between arylpiperazines and arylbiguanides? One can envision that arylbiguanides, particularly in a protonated form, might exist as hydrogen-bonded structures (e.g., **62**). The delocalized cationic center of **62** might mimic the 4-position nitrogen atom of an arylpiperazine at the receptor, and molecular modeling studies have shown that the two structures can be superimposed.[20] Interestingly, 25 years ago, Shapiro and co-workers[33] proposed such a cyclic conformation to account for some of the ultraviolet absorption spectra of various arylbiguanides. Although this cyclic structure represents only one of numerous solution conformations, and is not supported by the solid state structures of such compounds as determined by X-ray crystallography,[34−36] it does offer a reasonable explanation for why structural similarities might exist.



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The results of our original structure−activity studies on arylpiperazines were essentially confirmed and extended in the present investigation. Taken together, they suggest that 1-phenylpiperazine (**8**, $K_i = 3000$ nM) binds with modest affinity and that introduction of a 3-chloro group, or replacement of the phenyl ring with a 2-naphthyl group, enhances affinity by 50−100-fold (i.e., **4** $K_i = 62$ nM, **5** $K_i = 32$ nM, respectively). Replacement of phenyl by a 2-quinolinyl group, to afford quipazine, results in the highest affinity member of the series (**3**, $K_i = 2$ nM). The present results also support the original suggestion that the presence of the 4-position piperidine nitrogen atom is critical for 5-HT_3 binding (i.e., **22**, $K_i > 10\,000$).

Armed with this information, we prepared and examined the 3-chloro derivative of 1-phenylbiguanide (**2**, $K_i = 1200$ nM) and found it to bind with markedly enhanced affinity (**6**, $K_i = 17$ nM). Kilpatrick et al.[12] have independently reported that **6** binds with high affinity at 5-HT_3 receptors. 1-(2-Naphthyl)biguanide (**7**, $K_i = 12$ nM) also binds with high affinity. In both series, the effect of the chloro group could not be mimicked by another electron-withdrawing group. Morain et al.[21] observed a similar phenomenon upon replacement of the chloro group with a trifluoromethyl group in the biguanide series and have suggested that the inductive and mesomeric properties of the chloro group may play a major role in the acidic character of the molecules and that this might be important for 5-HT_3 binding. They also suggested that delocalization of electrons between the biguanide moiety and the aromatic ring contributes to binding due to a decrease in affinity upon insertion of a methylene group between the two components. However, as shown in Table 3, compound **40** ($K_i = 40$ nM), which lacks an NH substituent attached directly to the 3-chlorophenyl ring, still binds with high affinity. Although aryl substitution may influence the acidity of an attached nitrogen, the presence of such a nitrogen attached directly to the aromatic ring does not appear to be critical for binding.

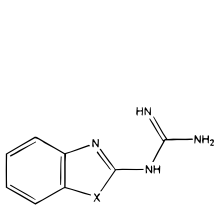
Structure−activity studies with arylbiguanides identified several arylguanidines as high-affinity 5-HT_3 ligands. As with the arylpiperazines and arylbiguanides, the 3-chloro derivative **45** and the naphthyl derivative **61** are among the higher affinity members of the series. From this perspective, these are two features that are shared by all three series of compounds. All three series may bind in a common manner, but this is difficult to envision. Obviously, additional work needs to be done. However, in comparing the high-affinity arylbiguanides and arylguanidines, the only nonaromatic feature shared by the two series is that substituent "C" (see Tables 3 and 4) contains NH (e.g., **6**, **40**, **45**). But, this alone is insufficient for binding (e.g., see **54**, $K_i > 10\,000$ nM). The guanidino moiety (i.e., as found in **6**, **40**, and **45**) is evidently important for binding. However, if the guanidino portion of all three compounds is overlayed, the aromatic portions cannot be aligned. In contrast, if the aromatic portions are aligned (and there is reason to believe this is possible in some cases on the basis of parallel substituent effects), the "C-substituent" NH is common and may constitute a center of diffuse charge with which to interact at a common feature of the receptor.

When our work began, it was known that 1-phenylbiguanide represents a 5-HT_3 agonist; however, the functional activity of arylpiperazines as a class was unclear. More recently, it has been shown that quipazine acts as a 5-HT_3 antagonist in certain instances and as an agonist in others (see the introduction). Specifically, quipazine behaves as a 5-HT_3 agonist as reflected by its ability to increase [^{14}C]guanidinium uptake in NG 108-15 cells;[13] NG 108-15 cells also served as the source of 5-HT_3 receptors for the present investigation. The possibility exists, then, that certain arylpiperazines might constitute 5-HT_3 agonists. Nevertheless, for the purpose of the present investigation, we wished to specifically determine if the *m*-chlorophenyl and 2-naphthyl analogs in the piperazine, biguanide, and guanidine series act as 5-HT_3 agonists or antagonists, that is, we wished to compare the actions of arylpiperazines **4** and **5** with those of arylbiguanides **6** and **7** and arylguanidines **45** and **61**. The commonly used 1-phenylbiguanide (**2**) was included for purpose of comparison. The von Bezold−Jarisch reflex is an *in vivo* assay that consists of a reflex and transient bradycardia evoked by 5-HT that activates vagal afferent nerve terminals within the heart; as an assay, it represents the first and still the most convenient *in vivo* assay for 5-HT_3 receptor function.[31] The effect can be induced by 5-HT and 5-HT_3 agonists such as 2-methyl 5-HT and 1-phenylbiguanide (**2**), and the effect evoked by these agents is antagonized by 5-HT_3 antagonists.[31] The actions of 5-HT on isolated ganglia are complex and may involve, at least in part, 5-HT_3 receptors.[31] In an older study, it was shown that 1-phenylbiguanide stimulates pelvic ganglia producing bladder stimulation in animals.[32] This effect, which is also evoked by 5-HT, can be antagonized by the 5-HT_3 antagonist tropisetron (unpublished data).

Functional studies with 1-phenylbiguanide (**2**), the 3-chloro and naphthyl derivatives of phenylpiperazine (i.e., **4** and **5**), phenylbiguanide (i.e., **6** and **7**), and phenylguanidine (i.e., **45** and **61**) indicate that both arylpiperazines behave as antagonists whereas the

arylbiguanides and arylguanidines act as 5-HT$_3$ agonists in the von Bezold−Jarisch assay. The stimulant effects of the agonists were effectively antagonized by the 5-HT$_3$ antagonist tropisetron. In the rabbit bladder assay, again the arylbiguanides and the arylguanidines served as agonists, and their effects could be antagonized by tropisetron.

Initially, it may seem surprising that the arylguanidines bind at 5-HT$_3$ receptors and, further, that they are 5-HT$_3$ agonists, in light of a report by Morain et al.[21] that guanidines lack affinity for 5-HT$_3$ receptors (K_i = 36 000−>100 000 nM); however, the compounds examined by Morain and co-workers were heterocyclic (i.e., benzimidazole, benzothiazole, benzoxazole; e.g., **63** where X = NH, S, and O) guanidines and not simple arylguanidines of the type examined here. Rizzi et al.[30] have found that even the structurally simpler *N*-benzylguanidine lacks affinity for 5-HT$_3$ receptors. But the same group developed guanidine **64** as a 5-HT$_3$ antagonist and demonstrated that it binds with high affinity (K_i = 3.3 nM).[38] Interestingly, they also found in the von Bezold−Jarisch assay that **64** is a mixed agonist/antagonist.



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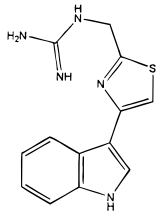

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These results suggest that with the appropriate substituents guanidines can bind at 5-HT$_3$ receptors and that they may contribute to agonist action. Furthermore, a search of the literature reveals that guanidine (and biguanide) derivatives were reported over 40 years ago as possessing serotonergic agonist activity, but this work seems to have laid dormant. For example, certain biguanides produce a fall in blood pressure and heart rate through what was speculated to be a serotonergic mechanism (see Fastier[37] for a review). Gyermek[32] demonstrated that **61** (and **8**) mimics 5-HT in stimulating the inferior mesenteric ganglion of the cat and the pelvic ganglia of the dog and that these effects are inhibited by 5-HT antagonists. Perhaps the significance of these studies went unrecognized because they were reported prior to the discovery of 5-HT$_3$ receptors. With the realization that arylguanidines bind at 5-HT$_3$ receptors, that they act as agonists in the von Bezold−Jarisch assay, and that their effects can be antagonized by the 5-HT$_3$ antagonist tropisetron, it seems that we have rediscovered a "new" class of 5-HT$_3$ agonists. Indeed, it may be the presence of the guanidine moiety that imparts mixed agonist/antagonist activity to **64**, a compound originally designed as a 5-HT$_3$ antagonist.

In summary, then, we have examined the structure−activity relationships for the binding of arylpiperazines at 5-HT$_3$ receptors and, where possible, compared the results with those from our original[17] investigation. It would seem that the original binding assay using [^{3}H]-quipazine as radioligand provided results that overestimated the affinities of certain compounds. Nevertheless, the qualitative, if not quantitative, conclusions are consistent between the two studies. The identification of *m*-chlorophenyl and 2-naphthyl substituents as being important for binding led to the synthesis of the corresponding arylbiguanide derivatives **6** and **7** (ref 20 and present study). Of the arylbiguanides investigated (Tables 2 and 3), none bind with higher affinity than **6** and **7**. A detailed structure−activity investigation identified arylguanidines as retaining 5-HT$_3$ affinity, and subsequently, the (*m*-chlorophenyl)guanidine **45** and the 2-naphthylguanidine **61** were found to be the highest affinity members of the series. Finally, all four of these compounds (**6**, **7**, **45**, **61**) were shown to act as 5-HT$_3$ agonists (it might be noted, however, that the selectivity of these compounds for 5-HT$_3$ versus other populations of 5-HT receptors has yet to be determined). Thus, these studies provide structure−activity data on several classes of 5-HT$_3$ ligands, indicate how arylguanidines may be structurally related to the arylbiguanides, and show that the arylguanidines **45** and **61** can act as 5-HT$_3$ agonists. These results should also prove useful in future molecular modeling studies aimed at understanding how 5-HT$_3$ ligands interact with 5-HT$_3$ receptors.

Experimental Section

Synthesis. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on either a JEOL FX90Q or QE300 spectrometer with tetramethylsilane as an internal standard. Assigned structures are consistent with spectral data. Elemental analysis was performed by Atlantic Microlab (Norcross, GA), and values are within 0.4% of theory.

Compounds **3** maleate,[39] **5** HCl,[39] **10** HCl,[40] **20** HCl,[39] **21** dimaleate,[41] and **22** HCl[39] were previously prepared in our laboratories. The arylpiperazine **23** HCl was prepared according to the method of Lumma and Saari.[42] The arylbiguanides **6** HCl,[43,44] **7** HCl,[46] **27** HCl,[44] **28** HCl,[44] **29** HCl,[44] **30** HCl,[43] **31** HCl,[43] **32** HCl,[45] and **33** HCl[45] were prepared according to literature procedures. The modified analogs phenylbiguanide **35** HCl,[45] **38** 2HCl,[47] **40** hemisulfate,[48] **41** hemisulfate,[48] **42** hemisulfate,[28] **43** HCl,[28] and **45** nitrate[49] are known, and methods of preparation were employed from cited references. The arylguanidines **46** nitrate,[50] **47** nitrate,[51] **48** nitrate,[52] **49** nitrate,[53] **50** hemisulfate,[53] **51**,[54] **52**,[55] **53** HCl,[56] **54** HCl,[57] **57** HCl,[58] **58** HCl,[59] **59** hemisulfate,[60] and **61**[61] were synthesized using the literature procedures. Compounds **2**, **4**, **8**, **9**, **12**, **13**, and **15** were commercially available (Aldrich Chemical Co.).

4-Hydroxy-4-(2-methoxyphenyl)piperidine Hydrochloride (11). A solution of 2-methoxybromobenzene (2.0 g, 10.7 mmol) in anhydrous Et_2O (10 mL) was added to a mixture of Mg (0.29 g, 11.8 mmol) and a catalytic amount of I_2 in anhydrous Et_2O (20 mL) under N_2 at room temperature. The stirred mixture was heated at reflux for 5 h. To this cloudy solution was added *N*-benzyl-4-piperidone (2.0 g, 10.7 mmol) in anhydrous Et_2O (15 mL) at 0 °C, and the mixture was heated at reflux (water bath) for 1.5 h. The crude product was isolated by acid−base extraction and purified by distillation (bp 87−95 °C/0.55 mmHg). This oily product was used in the next step without further characterization. A solution of this intermediate (0.5 g) in absolute EtOH (60 mL) was debenzylated by hydrogenation over 30% Pd/C (0.1 g) for 24 h, at 45 psi. The heterogeneous mixture was filtered through a Celite pad to removed the catalyst. The filtrate was evaporated under reduced pressure to afford a white solid. The crude free base was converted to the HCl salt and recrystallized from absolute EtOH to yield 0.25 g (70%) of **11**: mp 231−233 °C; ^{1}H-NMR (DMSO-d_6) δ 2.5−3.3 (m, 9H, CH_2, CH), 3.82 (s, 3H, CH_3), 6.85−7.6 (m, 4H, Ar-H), 9.05 (bs, 1H, NH). Anal. ($C_{12}H_{17}NO_2$·HCl) C,H,N.

1-(3-Chlorophenyl)-4,4-dimethylpiperazinium Iodide (14). Compound **14** was prepared as a white crystalline material in 31% yield by alkylation of 1-(3-chlorophenyl)-piperazine with MeI in a manner similar to that described for **16**: mp 199−201 °C (MeOH); ^{1}H-NMR (DMSO-d_6) δ 3.2 (s,

6H, CH_3) 3.5 (s, 8H, CH_2), 6.8−7.4 (m, 4H, Ar-H). Anal. ($C_{12}H_{18}N_2ClI$) C,H,N.

1-[3-(Trifluoromethyl)phenyl]-4,4-dimethylpiperazinium Iodide (16). A mixture of 1-(α,α,α-trifluoro-*m*-tolyl)-piperazine (1.15 g, 5 mmol), MeI (1.43 g, 10 mmol), and K_2CO_3 (0.35 g, 2.5 mmol) was heated at reflux for 3 h. The reaction mixture was poured onto 5 mL of H_2O and washed with 3 × 10-mL portions of Et_2O. The product that precipitated from the aqueous solution upon cooling was collected by filtration and recrystallized from MeOH to give 0.51 g (27%) of **16** as white crystals: mp 224−225 °C; ^{1}H-NMR (DMSO-d_6) δ 3.1 (s, 6H, CH_3), 3.6 (s, 8H, CH_2), 7.0−7.6 (m, 4H, Ar-H); ^{13}C-NMR (DMSO-d_6) δ 150.13 (ArC-N), 130.49 (ArC-CF_3), 119.59 (CF_3), 116.17 (ArC), 116.12 (ArC), 111.86 (ArC), 111.81 (ArC), 60.44 ($(CH_2)_2N^+$), 50.75 ($(CH_3)_2N^+$), 42.00 ($(CH_2)_2N$). Anal. ($C_{13}H_{18}N_2F_3I$) C,H,N.

1-(2-Methyl-4-methoxyphenyl)piperazine Hydrochloride (18). Anhydrous K_2CO_3 (4 g, 29 mmol) and bis(2-chloroethyl)amine hydrochloride (5.2 g, 29 mmol) were added to a stirred solution of freshly distilled 2-methyl-4-methoxyaniline (4 g, 29 mmol) in diglyme (25 mL) at room temperature. The reaction mixture was heated at reflux for 18 h, cooled to room temperature, and poured into H_2O (100 mL). The aqueous mixture was made basic (ca. pH 14) with a saturated KOH solution and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with H_2O (3 × 100 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure to give an oily residue. Vacuum distillation afforded 3.6 g (60%) of **18** as a free base, bp 105 °C/0.05 mmHg. Preparation of the hydrochloride salt gave **18** as white crystals: mp 255 °C dec; ^{1}H-NMR ($CDCl_3$) δ 2.15 (s, 1H, NH), 2.30 (s, 3H, Ar-CH_3), 2.55−3.30 (m, 8H, N-CH_2), 3.75 (s, 3H, OCH_3), 6.8 (d, 2H, J = 9.0 Hz, Ar-H), 7.10 (d, 1H, J = 9.0 Hz, Ar-H). Anal. ($C_{12}H_{18}N_2O$·HCl) C,H,N.

6-Chloro-2-[1-(4-methylpiperazinyl)]pyridine Maleate (24). A solution of 2,6-dichloropyridine (1.5 g, 10.1 mmol), *N*-methylpiperazine (1.0 g, 10 mmol), and NEt_3 (1.0 g, 10 mmol) in *n*-BuOH (100 mL) was heated at reflux for 20 h and allowed to cool to room temperature. The NEt_3·HCl was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The oily residue was dissolved in Et_2O (100 mL), and the solution was washed well with H_2O (3 × 50 mL) and dried ($MgSO_4$). The filtrate was treated with a methanolic solution of maleic acid; the precipitate was collected by filtration and recrystallized from absolute EtOH to give 2.0 g (62%) of the crystalline maleate salt: mp 144−146 °C. Anal. ($C_{10}H_{14}ClN_3$·$C_4H_4O_4$) C,H,N.

6-Methyl-2-[1-(4-methylpiperazinyl)]pyridine Maleate (25). Compound **25** was obtained by alkylation of *N*-methylpiperazine with 6-chloro-2-picoline in a manner similar to that described for **24**. The product was recrystallized from absolute EtOH to yield 0.12 g (49%) of **25**: mp 140−141 °C. Anal. ($C_{11}H_{17}N_3$·$C_4H_4O_4$) C,H,N.

6-Iodo-2-[1-(4-methylpiperazinyl)]pyridine Maleate (26). A mixture of 2,6-diiodopyridine (0.32 g, 1 mmol), *N*-methylpiperazine (0.1 g, 1 mmol), and NEt_3 (0.1 g, 1 mmol) in *n*-BuOH (20 mL) was heated under reflux for 16 h; the solution was allowed to cool to room temperature and filtered, and the filtrate was evaporated to dryness under reduced pressure. The solid residue was treated with dilute HCl (10%), and the solution was again filtered. The filtrate was made alkaline to pH 8−9 with NaOH and extracted with Et_2O (3 × 15 mL). The combined Et_2O extracts were dried ($MgSO_4$), filtered, and treated with a saturated solution of maleic acid in Et_2O. The precipitate was collected by filtration, dried, and recrystallized from absolute EtOH/anhydrous Et_2O to give 0.1 g (35%) of **26** as a crystalline solid: mp 138−139 °C. Anal. ($C_{10}H_{14}IN_3$·$C_4H_4O_4$) C,H,N.

***N,N*-Dimethyl-(3-chlorophenyl)biguanide Hydrochloride (34).** A mixture of 3-chloroaniline hydrochloride (1.97 g, 12 mmol) and dimethyl dicyandiamide (1.37 g, 12 mmol) in H_2O (10 mL) was heated at reflux for 2 h and filtered while hot. After cooling in an ice bath, the aqueous solution was allowed to stand at 5 °C overnight. The white precipitated product was collected by filtration. Recrystallization from absolute EtOH afforded 2.34 g (70%) of the title compound **34** as white crystals: mp 258−259 °C. Anal. ($C_{10}H_{14}N_5$·HCl) C,H,N.

***N*-(3-Aminopropionyl)-3-chloroaniline Oxalate (36).** The title compound **36** was prepared in a similar manner to that of **37** in 15% yield: mp 169−170 °C (EtOH:anhydrous Et_2O). Anal. ($C_9H_{11}N_2ClO$·$C_2H_2O_4$·0.5 H_2O) C,H,N.

***N*-(2-Aminoethyl)-3-chlorobenzamide Hydrochloride (37).** 3-Chlorobenzoyl chloride (1.75 g, 10 mmol) in THF (10 mL) was added in a dropwise manner to a solution of 2-bromoethylamine (2.04 g, 10 mmol) and NEt_3 (2.02 g, 20 mmol) in THF (20 mL). The mixture was allowed to stir at room temperature for 6 h; then the precipitate (NEt_3·HBr) was removed by filtration. The solvent was removed in vacuo, and the residue was dissolved in Et_2O (15 mL), washed with H_2O (3 × 15 mL), and dried ($MgSO_4$). After removal of the solvent, the residue was recrystallized from benzene:hexane (5:1) to afford 1.00 g (33%) of *N*-(2-bromoethyl)-3-chlorobenzamide (mp 82−84 °C) as an intermediate which was used in the next step without further characterization.

Potassium phthalimide (0.70 g, 3.8 mmol) was added to a solution of the above intermediate bromide (1.00 g, 3.8 mmol) in dry DMF (3 mL). The reaction was slightly exothermic, with the temperature rising to 80 °C. Stirring was allowed to continue for 12 h, and the temperature was slowly decreased to 25 °C. After the addition of $CHCl_3$ (20 mL), the mixture was poured into H_2O (100 mL). The aqueous phase was separated and extracted with $CHCl_3$ (2 × 10 mL). The combined extract was washed with 0.2 N NaOH (20 mL) and H_2O (20 mL) and dried (Na_2SO_4). The solvent was removed in vacuo, and the white crystalline residue was triturated with Et_2O (30 mL) to give 0.50 g (40%) of *N*-(phthalimidoethyl)-3-chlorobenzamide (mp 165−168 °C).

Hydrazine hydrate (0.08 g, 1.5 mmol) was added to a solution of the above phthalimide (0.50 g, 1.5 mmol) in 95% EtOH (10 mL). The reaction mixture was heated at reflux for 3 h and then cooled to 0 °C (ice bath). The cold solution was acidified to pH 1−2 with ice-cold concentrated HCl and filtered. The solid was washed with 95% EtOH (3 × 5 mL). Evaporation of the combined filtrate and washings gave 0.05 g (16%) of compound **37**: mp 205−207 °C, after recrystallization from absolute EtOH. Anal. ($C_9H_{11}N_2ClO$·HCl) C,H,N.

4-(3-Chlorophenyl)butylamine Fumarate (39). The method was adapted from **44**. 3-(3-Chlorophenyl)propyl bromide[62] was converted to its nitrile and then reduced with $LiAlH_4$ and treated with an ethereal solution of fumaric acid to afford 0.02 g (6%) of the title compound **39**: mp 169−170 °C. Anal. ($C_{10}H_{14}NCl$·$C_4H_4O_4$) C,H,N.

3-(3-Chlorophenyl)propylamine Oxalate (44). 3-(3-Chlorophenyl)ethanol (1.00 g, 6.4 mmol) in 48% HBr (10 mL) and H_2SO_4 (3 drops) was heated at reflux for 3 h. The reaction mixture was cooled to room temperature, poured into H_2O (60 mL), extracted with $CHCl_3$ (3 × 25 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to afford 1.25 g (89%) of 3-(3-chlorophenyl)ethyl bromide as an oil. IR spectral data indicated lack of an OH signal.

The above intermediate (1.25 g, 5.7 mmol) in MeOH (10 mL) was added at once to a solution of NaCN (0.56 g, 11.3 mmol) in H_2O (1 mL). The reaction mixture was heated at gentle reflux (water bath) for 30 min; then the solvent was removed in vacuo, and the residue was dissolved in Et_2O (5 mL). The precipitate was removed by filtration, and the filtrate was evaporated to dryness to give 0.55 g (58%) of 3-(3-chlorophenyl)ethyl nitrile as an oily intermediate. IR spectroscopy indicated the presence of a CN signal at 2242 cm^{-1}.

The above nitrile (0.17 g, 10 mmol) in dry THF (5 mL) was added in a dropwise manner to a stirred and cooled (ice bath) suspension of $LiAlH_4$ (0.05 g, 13 mmol) in dry THF (3 mL) under an N_2 atmosphere. After the addition was complete, the reaction mixture was heated at reflux for 2 h. Excess $LiAlH_4$ was decomposed by the addition of H_2O (0.2 mL), 15% NaOH (0.2 mL), and H_2O (0.6 mL), and the inorganic material was removed by filtration. The solution was dried ($MgSO_4$), and THF was removed under reduced pressure to afford an oily residue of the title compound. The crude product was purified by Kugelrohr distillation to afford 0.07 g (41%) of **44**

as a free base. The free base was converted to its oxalate salt and recrystallized from absolute EtOH: mp 148−152 °C. Anal. ($C_9H_{12}ClN\cdot C_2H_2O_4\cdot 0.5\ H_2O$) C,H,N.

***N*-Methyl-2-(3-chlorophenyl)ethylamine Hydrochloride (55).** Methyl chloroformate (0.31 g, 3.3 mmol) in dry THF (5 mL) was added dropwise to a solution of 2-(3-chlorophenyl)-ethylamine (0.50 g, 3.2 mmol) and NEt_3 (0.66 g, 6.5 mmol) in dry THF (5 mL) at 0 °C under an N_2 atmosphere. The solution was allowed to stir at 0 °C for 30 min and at room temperature for 14 h. The $NEt_3\cdot HCl$ was removed by filtration, and the solvent was removed in vacuo. The residue was dissolved in H_2O (15 mL) and then extracted with Et_2O (3 × 10 mL). The combined ethereal portions were washed with 10% HCl (15 mL), brine (15 mL), and H_2O (20 mL), dried (K_2CO_3), and evaporated to afford 0.65 g (96%) of *N*-carbomethoxy-2-(3-chlorophenyl)ethylamine as an oil which was used in the next step without further characterization.

A solution of the above *N*-carbomethoxy intermediate (0.45 g, 2.1 mmol) in dry THF (4 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (0.11 g, 2.7 mmol) in THF (3 mL) at 0 °C under an N_2 atmosphere. After the addition was complete, the mixture was heated at reflux for 2 h. The excess $LiAlH_4$ was decomposed by the addition of H_2O (0.5 mL), 15% NaOH (0.5 mL), and H_2O (1.5 mL), and the inorganic material was removed by filtration. The solution was dried ($MgSO_4$), and the THF was removed under reduced pressure to afford 0.24 g (67%) of the title compound **55** as an oil. The free base was converted to its HCl salt: mp 134−135 °C (EtOH:Et_2O). Anal. ($C_9H_{12}ClN\cdot HCl$) C,H,N.

***N,N*-Dimethyl-2-(3-chlorophenyl)ethylamine Oxalate (56).** Formic acid (88%, 0.69 g, 15 mmol), followed by formaldehyde (36%, 0.39 g, 13 mmol), was slowly added to 2-(3-chlorophenyl)ethylamine (0.78 g, 5 mmol) at 0 °C. The resulting solution was stirred at 80 °C for 24 h, cooled to 0 °C, and acidified with 6 N HCl (2 mL). The mixture was extracted with Et_2O (3 × 10 mL), basified to pH 10 by the addition of 50% aqueous NaOH, and extracted with Et_2O (3 × 10 mL). The pooled ether extracts were washed with H_2O (5 mL) and dried ($MgSO_4$), and solvent was removed under reduced pressure. The resulting residue was dissolved in EtOH:Et_2O (1:1) (2 mL) and treated with an ethereal solution of oxalic acid to give 0.38 g (28%) of the title compound **56**: mp 144−146 °C. Anal. ($C_{10}H_{14}ClN\cdot C_2H_2O_4$) C,H,N.

***N*-(3-Pyridyl)guanidine Hydrochloride (60).** The title compound was prepared by the general method of Short and Darby.[28] A mixture of 3-aminopyridine (0.47 g, 3.5 mmol) and cyanamide (0.18 g, 4.3 mmol) in concentrated HCl (2 mL) was heated in an oil bath to 180 °C over a 1-h period and held at that temperature for 3 h. The residue was triturated with MeOH:Et_2O; then the solid was washed with acetone (to remove unreacted amine) and recrystallized from EtOH:Et_2O to give 0.40 g (38%) of the title compound **60**: mp 210−213 °C. Anal. ($C_6H_8N_4\cdot 2HCl$) C,H,N.

Pharmacology. 1. Radioligand Binding. NG 108-15 cells, which express the 5-HT$_3$ receptor,[63] were obtained from Dr. Marshall Nirenberg (National Institutes of Health). The cells were grown in Dulbecco's modified Eagle's medium with fetal bovine serum (10%), penicillin/streptomycin (200 units/mL), L-glutamine (2 nM/mL), hypoxanthine (25 μM), aminopterin (0.5 μM), and thymidine (4 μM), in cell culture flasks, to confluence. The cells were harvested in 50 mM Tris HCl buffer, 0.5 mM EDTA, and 10 nM $MgSO_4$ and centrifuged at 9000*g* for 25 min. The pellet was resuspended in buffer, incubated at 37 °C for 15 min, and centrifuged again at 9000*g* for 20 min. The pellets were stored at −40 °C until used.

Radioligand binding assays were performed in triplicate in a 2.0-mL volume containing 1 nM [^{3}H]GR65630 (New England Nuclear); 1 μM tropisetron (ICS 205-930) was used to define nonspecific binding, with varying concentrations of competing drug and membranes prepared from NG 108-15 cells (100 mg of protein/mL). A 64% specific binding signal was produced using 1 nM [^{3}H]GR65630 (84.2 Ci/mmol). Assay tubes were incubated for 30 min at room temperature; suspensions were filtered on Schleicher & Schuell 32 glass fiber filters (presoaked in 0.1% poly(ethylenimine)) and washed with 10 mL ice-cold buffer. The filters were counted by a Beckman 3801 liquid scintillation counter in 5 mL of aqueous counting scintillant (Ecoscint, National Diagnostic). Data from binding assays were plotted as log concentration vs percent inhibition and analyzed by nonlinear least squares techniques in which 100% maximal inhibition was assumed at high test compound concentrations. The IC_{50} values obtained from such data treatment were used to calculate apparent inhibition constants from the following equation: $K_i = IC_{50}/1 + ([c]/K_D)$, where $[c]$ is the concentration of radioligand employed in the binding assay and K_D is its receptor dissociation constant (K_D = 0.7 nM for [^{3}H]GR65630).

2. von Bezold−Jarisch Bradycardia Reflex in Rats. White albino Harlan rats (200−300 g) were anesthetized with ethylurethane (1.5 g/kg, ip). The trachea was cannulated, and when necessary, artificial ventilation was provided. A tail vein was cannulated with a no. 25 "butterfly ST" needle (Abbott Laboratories, N. Chicago, IL) for iv injection. Needle (no. 25) electrodes were inserted subcutaneously to record ECG (Module AEC 3101, Western Graphtec (WG) Co., Irvine, CA). Lead II ECG signals were coupled with a Cardiotachograph (KF 3101-02 module), and responses were recorded on a WG Linearcorder WR 3101 polygraph. A constant body temperature was maintained. Agents were dissolved in normal saline, and stock solutions were stored under refrigerated conditions; appropriate dilutions were made prior to use. Agents were injected in dose ranges of 1−200 μg/kg in a total volume of 0.3 mL and "washed in" with an additional 0.4 mL of normal saline. The reflex bradycardic responses were elicited at 15−20-min intervals. Minimal effective doses of the agents (producing 10−20% bradycardia) were determined by interpolation between two to three doses for each agent. To identify the bradycardic response with 5-HT$_3$ receptor stimulation, each stimulant agent was tested at least once at 2 and 20 min after the administration of the 5-HT$_3$ antagonist tropisetron (5−10 μg/kg, iv).

3. Rabbit Bladder Experiments. White male adult New Zealand rabbits (1.8−2.5 kg) were anesthetized with 1.2 g/kg of ethylurethane (ip) and, when necessary, supplemented with sodium pentobarbital (10−20 mg/kg) injected into a marginal ear vein. The trachea was cannulated for artificial ventilation. The bladder was exposed from a lower midline incision of the abdomen, and a no. 12 gauge plastic catheter was inserted into the apex of the bladder. The urethra was clamped off. Pressure changes of the bladder were monitored via a Gould P 2310 pressure transducer with a WG, AUT 3510 pressure module on a WG polygraph. Agents were injected with close intraarterial administration into the cannulated femoral artery in the 1−200 μg/kg dose range in each animal at 15−20-min intervals. Minimal effective doses producing twitchlike contractions were determined as described earlier in dog and cat preparations.[64] To identify the bladder contractions as being 5-HT$_3$ receptor mediated, tropisetron (5−10 μg/kg) was administered intraarterially 1 min prior to the administration of an effective dose of each stimulant agent to produce complete blockade.

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