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The Binding of Arylguanidines at 5-HT_3 Serotonin Receptors: A Structure–Affinity Investigation

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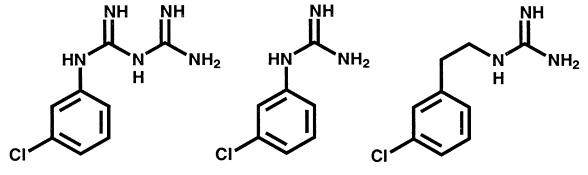
Abstract—The 5-HT_3 receptor binding affinities of nine pairs of aryl-substituted arylguanidines and arylbiguanides were examined and the results suggest the likelihood that both classes of agents utilize common receptor binding features. The effects of structural modification were also examined using CoMFA. 1-(3,4,5-Trichlorophenyl)guanidine (5-HT_3 $K_i = 0.7$ nM) was identified as a very high-affinity arylguanidine. The structures of the high-affinity arylguanidines are inconsistent with current 5-HT_3 pharmacophore models.

Of the seven major families of serotonin (5-HT) receptors, the 5-HT_3 receptors are unique in that they are ion channel receptors rather than G-protein coupled receptors.[1,2] 5-HT_3 receptors are thought to play a role in emesis, and evidence suggests that they could be involved in migraine, memory, and anxiety.[1–3] Numerous 5-HT_3 antagonists have been identified.[2,3] However, the armamentarium of 5-HT_3 agonists is relatively limited and what is available is either of low affinity, of limited selectivity, and/or has difficulty penetrating the blood–brain barrier[1,2,4] (but see also refs 5 and 6). The first selective 5-HT_3 agonist was phenylbiguanide (**1a**; 5-HT_3 K_i ca. 1000 nM). Shortly after its introduction, 1-(3-chlorophenylbiguanide) (*m*CPBG, **1d**) and 2-naphthylbiguanide (**2**) were introduced as 5-HT_3 ligands with higher affinity than **1a**.[7–9] *m*CPBG (**1d**) is now considered a prototypical 5-HT_3 agonist.



We have previously found that the entire biguanide moiety is unnecessary for 5-HT_3 receptor binding.[8] For example, **3d** (*MD*-354, $K_i = 35$ nM) and 1-(2-naphthyl)-guanidine (**4**, $K_i = 25$ nM) bind at 5-HT_3 receptors with high affinity, and behave as 5-HT_3 agonists.[8,10] Removal of the 3-chloro group reduced affinity (i.e., phenylguanidine; **3a**, $K_i = 2{,}340$) by >60-fold, and moving the chloro group to the 2- or 4-position decreased affinity by several-fold.[8] Similar results were obtained with the corresponding phenylbiguanides suggesting that the two series might interact at 5-HT_3 receptors in such a fashion that they likely share common aryl binding sites. Another finding is that a guanidine moiety, common to both series, is seemingly important for binding. In contrast, guanidine **5** ($K_i = 40$ nM)[8] binds with an affinity comparable to that of **3d** and questions whether the guanidine portion of **3d** mimics the *proximal* guanidine portion of **1d** or that of **5** when it interacts with 5-HT_3 receptors. In other words, do the aryl portions of **1**-series and **3**-series compounds bind in a common manner at the receptors, or is the guanidine portion of **3d** binding such that it mimics the terminal (i.e., *distal*) guanidine moiety of **1d**? With the latter possibility, there is more than one way in which this can occur, but in no case can it occur in a manner where the aryl portions of the molecules are superimposed. The primary purpose of the present investigation, then, was to examine several additional analogues of **1** and **3**, and to determine the influence of various aryl substituents on 5-HT_3 receptor affinity. If the aryl

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rings of **1**-series and **3**-series compounds utilize a common binding site, parallel substituent changes would be expected to result in parallel shifts in 5-HT_3 receptor affinity. Additional studies were conducted to determine the role of aryl substituents, the importance of the guanidine moiety, and the necessity of an NH function for 5-HT_3 receptor binding, and CoMFA was conducted to better appreciate how structural features might contribute to the binding of these compounds.

Results and Discussion

Parallel structural modification

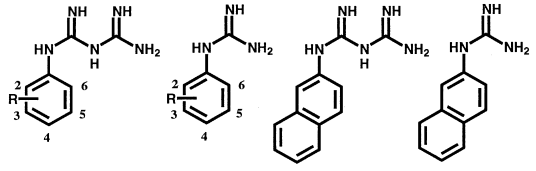
5-HT_3 receptor binding data were obtained for the arylbiguanide series (**1**-series) and arylguanidine series (**3**-series) compounds (**1a**–**h** and **3a**–**h**; Table 1). Parallel structural changes resulted in parallel shifts in affinity. Figure 1 shows a plot of the 5-HT_3 pK_i values for nine arylbiguanides versus those of their corresponding arylguanidines, and there is a high correlation ($r = 0.932$) between the affinities of the nine pairs of compounds.

Role of aryl substituents

Replacement of the 3-Cl group of **1d** with 3-CF_3 results in a 54-fold decrease in affinity.[11] In the arylguanidine series, this same replacement resulted in a 75-fold decrease (i.e., **6**; Table 1). This may be taken as yet another example of parallelism between the affinities of the arylbiguanides and the arylguanidines. This finding for the arylbiguanides, and now for the arylguanidines, has raised the question of the *role* of aryl substituents on binding. Replacement of the 3-Cl group of **3d** with a more effective electron withdrawing group (as in the 3-CF_3-containing **6**; $K_i = 2440$ nM) resulted in decreased affinity and in a compound with an affinity similar to the unsubstituted parent **3a**. Replacement of the chloro group with a lipophilic methyl group (i.e., 1-(3-methylphenyl)guanidine; $K_i = 6{,}520$ nM)[8] also results in decreased affinity. It appears that 3-position substituents are probably not contributing to affinity simply by virtue of their electronic or lipophilic character. Curiously, however, introduction of a 3-CF_3 function enhanced the affinity of the 4-chloro compound **3e** by nearly an order of magnitude (from 325 nM for **3e** to 36 nM for **8**; Table 1).

Introduction of an electron withdrawing -Cl or -CF_3 group at the aryl 4-position increased the affinity of the resulting compound by, typically, about 10-fold (e.g., **3a**→**3e**, **3d**→**3f**, **3g**→**3h**). In contrast, arylguanidines bearing electron donating substituents at the 4-position (i.e., **9** and **10**) bind with an affinity similar to that of the unsubstituted parent **3a**, but the methylenedioxy derivative **11** binds with nearly 10 times the affinity of **3a**. Although there is some evidence that the electronic character of the ring might influence binding, at this time it is not clear exactly what these effects are. There is also an indication that the lipophilicity of the 4-position substituent plays a role in binding, but additional compounds will need to be investigated.

Table 1. 5-HT_3 receptor binding affinities of arylbiguanide and arylguanidine analogues



1 3, 6–11 2 4

Compd	R	Arylbiguanides[a]		Arylguanidines[a]	
		K_i (nM)	(SEM)	K_i (nM)	(SEM)
1a/3a	–H	1200	—	2340	—
1b/3b	4-Me	890	(80)	442	(40)
1c/3c	2-Cl	62	—	190	—
1d/3d	3-Cl	18	(2)	32	(6)
1e/3e	4-Cl	210	(20)	326	(50)
1f/3f	3,4-di-Cl	12[b]	(1)	3.1	(0.2)
1g/3g	3,5-di-Cl	1.8	(0.4)	5.0	(1.4)
1h/3h	3,4,5-tri-Cl	2.7	(0.1)	0.7	(0.1)
2/4		14	(2)	18	(3)
6	3-CF_3	—		2440	(950)
7	4-CF_3	—		230	(35)
8	3-CF_3; 4-Cl	—		36	(7)
9	4-OMe	—		990	(65)
10	3,4-di-OMe	—		2710	(650)
11	3-OCH_2O-4	—		274	(28)

[a]Where SEM are not provided, K_i values are from a prior study[8] and are included here only for comparison. We previously reported for **6** $K_i = 5700$ nM; compound **6** was re-synthesized and re-evaluated in the present study.

[b]Known to bind at 5-HT_3 receptors ($K_i = 20$–40 nM); other high-affinity analogues also have been reported.[11,12]

Necessity of an NH function

In an earlier study, we found that replacement of the aniline NH of **3d** by –CH_2– (i.e., **12**), or replacement of the imino NH by a carbonyl oxygen atom (C=NH→C=O; i.e., **13**), resulted in decreased affinity.[8] Likewise, replacement of the aniline NH by –CH_2– reduced the affinity of **1d**.[8,9] Compound **14** ($K_i > 10{,}000$ nM), a cyclic analogue of *m*CPBG (**1d**), also lacks affinity for 5-HT_3 receptors.[9] The results indicate that an

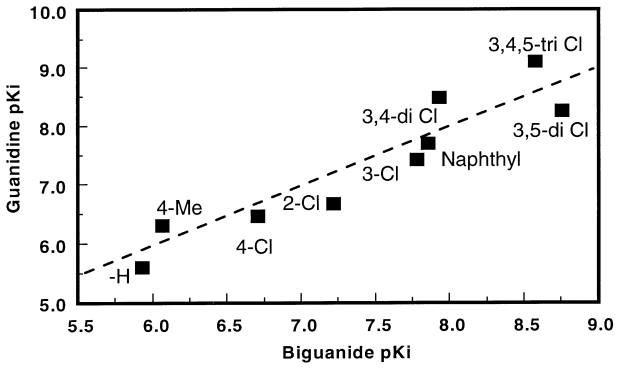
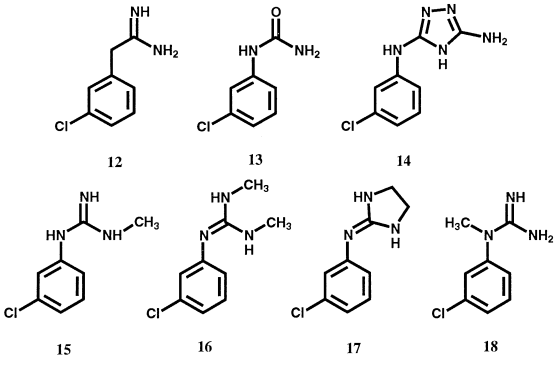


Figure 1. Relationship between the 5-HT_3 receptor affinities (pK_i values) of arylbiguanides **1a**–**h** and **2**, and their corresponding arylguanidines **3a**–**h** and **4**, respectively ($r = 0.932$; $n = 9$).

anilino NH function contributes to binding. Either reduced basicity, as with **13**, or the loss of hydrogen bonding capability could explain these affinity-reducing effects. Compound **14** possesses an anilino nitrogen atom, but conformational restriction into an unfavored binding conformation could account for its low affinity. We prepared several additional guanidine-related analogues to further explore the necessity of an NH function for 5-HT_3 receptor binding. Compound **15** ($K_i = 450 \pm 40$ nM) binds with 14-fold reduced affinity relative to **3d**; however, compounds **16** and **17** ($K_i > 10{,}000$ nM) lacked affinity. The guanidines might require an NH group for binding, but it is uncertain which NH group is important because **18** ($K_i = 6200 \pm 90$ nM) also binds with very low affinity. However, the issue is further complicated by possible tautomerism of the guanidine or alkylguanidine moiety (and is an even greater issue with arylbiguanides).[13] It would seem that either an NH group is required for binding (possibly via formation of a hydrogen bond with the receptor), that the steric bulk introduced by the alkyl substituents of **16**–**18** is not tolerated by the receptor, or perhaps both. In any event, any alteration of the guanidine function (as shown here or as reported earlier)[8] results in dramatically decreased affinity.



12 13 14 15 16 17 18

CoMFA study

A CoMFA analysis was undertaken with the arylguanidines and arylbiguanides to provide some additional insight regarding the influence of substituent groups on 5-HT_3 binding (Fig. 2). A total of 33 compounds were included in the study (see Fig. 3 for a description of compounds included) and K_i values spanned nearly a 10,000-fold range. Because of the relationship shown in Figure 1, it was felt justifiable to include arylbiguanides and arylguanidines in the same analysis. The results support the idea that binding is sensitive to the electronic and lipophilic character of substituents at the aryl 3-position. Lipophilic substituents at the 4-position also seem to contribute to binding; Figure 2 shows a limited hydrophobic area associated with this position. Substituents at the 5-position might also make a contribution to the interaction. Nevertheless, the possibility of rotameric binding cannot be excluded—either between series or even within a series. For example, because both the 3- and 5-positions are *meta* to the 'anilino' nitrogen atom, it is difficult to know, given this limited data set, if all *meta*-substituted compounds are oriented at the receptor in a similar manner. In any event, the model provides a starting point for further exploration of such compounds.

Pharmacophore models

Yamada et al.[14] have recently described a pharmacophore model that applies to 5-HT_3 agonists; although the model was not based on arylbiguanides, they have commented that their model accounts for the binding of *m*CPBG (**1d**). Daveu et al.[15] have described a somewhat related model to account for the binding of a series of 5-HT_3 partial agonists. In both instances, the aromatic centroid-to-amine distance (i.e., 7.5 and 6.5 Å, respectively) exceeds that calculated for the arylguanidines (e.g., **3d**: 3.7–4.9 Å). Although the first model was for-

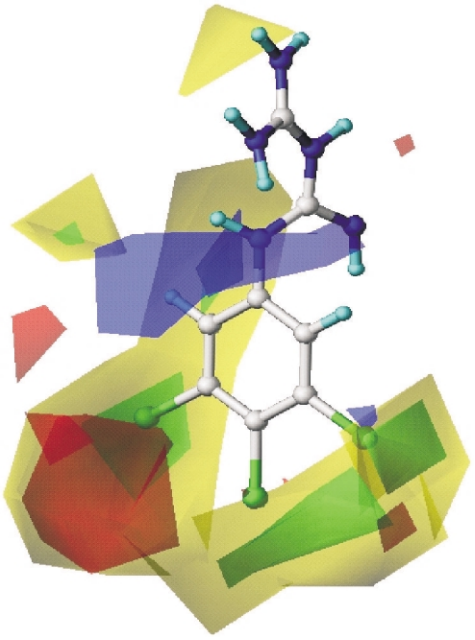
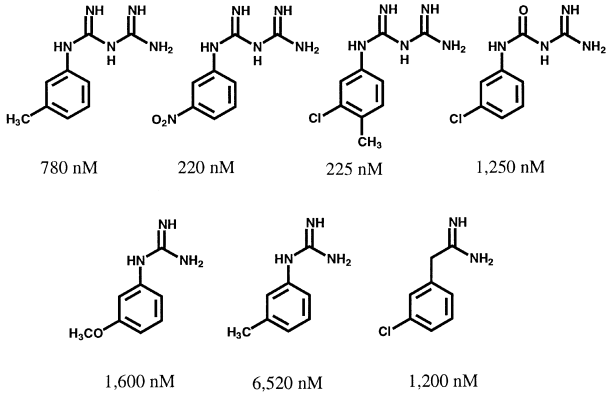


Figure 2. Results of a CoMFA study with the arylguanidines and arylbiguanides ($n = 33$, $q^2 = 0.584$, $r^2 = 0.851$; optimal number of components = 5; 79.5% steric and 20.5% electronic). The structure of compound **1h** is shown embedded within the model, and is drawn such that the aryl 3-position is oriented to the left. Green areas represent regions where bulky substituents are predicted to improve affinity, whereas the yellow regions are those where bulky substituents are disfavored. Red shaded areas are those where more negatively charged substituents are predicted to improve affinity, and blue areas are those where more positively charged substituents are favored.



780 nM 220 nM 225 nM 1,250 nM

1,600 nM 6,520 nM 1,200 nM

Figure 3. Additional compounds included in the CoMFA study. The CoMFA study included all 26 compounds described in the present manuscript (compounds in Table 1 and **15** and **18**), and the seven compounds shown here for which data were previously reported.[8]

mulated to describe the actions of 5-HT_3 agonists at gut receptors, and the latter model was based on a series of heterocyclic amines, neither model seems to account for the binding of the types of compounds described herein. That is, neither model accommodates the shorter centroid-to-amine distance of the arylguanidines.

In summary, the present investigation identified similarities between the binding of arylbiguanides and arylguanidines at 5-HT_3 receptors, and examined the effect on binding of several *N*-methylated arylguanidines. The conclusion reached is that certain arylbiguanides and arylguanidines likely bind in a similar manner (i.e., with common 5-HT_3 receptor aryl binding features), and that the arylguanidines fit neither of the currently proposed 5-HT_3 receptor agonist pharmacophore models. In the course of this study several novel high-affinity 5-HT_3 receptor ligands, particularly compound **3h** ($K_i = 0.7$ nM), were identified.

Experimental

Synthesis

The following compounds were re-synthesized in the manner previously reported: **1b–e** HCl, **3b** HCl, **3c–e** nitrate, **2** HCl and **4**.[8] Compounds **1f–h** HCl,[16] **3f** HCl, **3g** nitrate, **9** nitrate, **10** HCl, and **11** HCl,[17] **3b** HCl,[18] and **6** nitrate[19] were prepared according to literature procedures. Compounds **3h** HCl hemihydrate (mp 246–247 °C; absolute EtOH) and **7** HCl (mp 163–164 °C; absolute EtOH/anhyd Et_2O) were prepared using standard techniques,[19] as was **8** HCl (mp 204–207 °C; absolute EtOH);[19] all three analyzed within 0.4% of theory for C, H, and N. Compounds **15** (mp 75–79 °C; absolute EtOH/anhyd Et_2O), **16** (mp 103–105 °C; anhyd MeOH/anhyd Et_2O), and **17** (mp 149–151 °C; absolute EtOH) were obtained as their oxalate salts, and compound **18** HCl (mp 261–262 °C; anhyd MeOH/anhyd Et_2O) was prepared by the general procedure of King and Tonkin[17] beginning with *N*-methyl-3-chloroaniline; all analyzed within 0.4% of theory for C, H, and N.

Radioligand binding assay

The assay was performed in triplicate as previously reported[8] using NG108-15 cells (obtained from Dr. Marshall Nirenberg; NIH) which express the 5-HT_3 receptor.[20] [^{3}H]GR65630 (84.2 Ci/mmol; New England Nuclear, Boston, MA) (1 nM) was used as radioligand and 1 μM tropisetron (ICS 205–930) was used to define nonspecific binding. The IC_{50} values obtained 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.

Molecular modeling

The structure of phenylbiguanide hydrochloride (**1a**) was constructed from its X-ray coordinates[13a] using the CRYSTAL interface of the SYBYL molecular modeling program [SYBYL Molecular Modeling Package, Version 6.6 (1999); Tripos Inc., St. Louis, MO, USA]. The structure was then manually abbreviated to the free base. After molecular mechanics minimization (MINIMIZE), the charges were calculated by the Gasteiger–Huckel algorithm. The structures of the other arylbiguanides and arylguanidines were then manually constructed by the addition or subtraction of groups as needed, subjected to molecular mechanics minimization (MINIMIZE), and charges were calculated using the Gasteiger–Huckel algorithm. The series of compounds were overlayed into a database using a least squares method based on three common points: (1) the aryl 3-position, (2) the aryl 5-position, and (3) the carbon atom (cationic) attached to the 'anilino' nitrogen (N_{pl3}) atom. The CoMFA column was constructed using the default parameters in SYBYL.

Acknowledgements

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References and Notes

1. Glennon, R. A.; Dukat, M.; Westkaemper, R. B. In *Psychopharmacology, a Generation of Progress*; Watson, S. J., Ed.; Lippincott-Raven: New York, 1998 (CD ROM Version).
2. Gozlan, H. In *Serotonin Receptors and Their Ligands*; van Wijngaarden, I. O. B., Soudin, W., Eds.; Elsevier: Amsterdam, 1997; pp 221–258.
3. King, F. D., Jones, B. J., Sanger, G. J., Eds.; *5-Hydroxytryptamine-3 Receptor Antagonists*; CRC: Boca Raton, FL, 1994.
4. (a) Dukat, M.; Miller, K.; Teitler, M.; Glennon, R. A. *Med. Chem. Res.* **1991**, *1*, 271. (b) Glennon, R. A.; Young, R.; Dukat, M. *Pharmacol. Biochem. Behav.* **1992**, *41*, 361. (c) Glennon, R. A.; Bondarev, M.; Roth, B. *Med. Chem. Res.* **1999**, *9*, 108.
5. MKC-733 *Drugs Future* **1999**, *24*, 996.
6. Ito, H.; Kiso, T.; Miyata, K.; Kamato, T.; Yuki, H.; Akuzawa, S.; Nagakura, Y.; Yamano, M.; Suzuki, M.; Naitoh, Y.; Sakai, H.; Iwaoka, K.; Yamaguchi, T. *Eur. J. Pharmacol.* **2000**, *409*, 195.
7. Kilpatrick, G. J.; Butler, A.; Burridge, J.; Oxford, W. *Eur. J. Pharmacol.* **1990**, *182*, 193.
8. Dukat, M.; Abdel-Rahman, A. A.; Ismaiel, A. M.; Ingher, S.; Teitler, M.; Gyermek, L.; Glennon, R. A. *J. Med. Chem.* **1996**, *39*, 4017.
9. Dukat, M.; Westkaemper, R. B.; Teitler, M.; Glennon, R. A. *Abstracts*, 2nd International Symposium on Serotonin, From Cell Biology to Pharmacology and Therapeutics, Houston, TX, September 15–18, 1992; p 42.
10. Dukat, M.; Young, R.; Darmani, N. N.; Ahmed, B.; Glennon, R. A. *Psychopharmacology (Berlin)* **2000**, *150*, 200.
11. Morain, P.; Abraham, C.; Portevin, B.; DeNanteuil, G. *Mol. Pharmacol.* **1994**, *46*, 732.
12. Krijzer, F. N. C.; Tulp, M. T. M. *Eur. J. Pharmacol.* **1990**, *183*, 1969.
13. (a) For example, see: Brown, C. J.; Sengier, L. *Acta Cryst.* **1984**, *C40*, 1294. (b) Barker, J.; Powell, H. R. *Acta Cryst.*

1995, *C51*, 1714. (c) Dalpiaz, A.; Ferretti, V.; Gilli, P.; Bertolasi, V. *Acta Cryst*. **1996**, *B52*, 509.
14. Yamada, M.; Sato, Y.; Kobayashi, K.; Konno, F.; Soneda, T.; Watanabe, T. *Chem. Pharm. Bull. (Tokyo)* **1998**, *46*, 445.
15. Daveu, C.; Bureau, R.; Baglin, I.; Prunier, H.; Lancelot, J.-C.; Rault, S. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 362.
16. Curd, F. H.; Rose, F. L. *J. Chem. Soc*. **1946**, 362.
17. King, H.; Tonkin, I. M. *J. Chem. Soc*. **1946**, 1063.
18. Braun, C. E. *J. Am. Chem. Soc*. **1933**, *55*, 1280.
19. Kreutzberger, A.; Tantawy, A. *Arch. Pharm*. **1979**, *312*, 426.
20. Hoyer, D.; Neijt, H. C. *Eur. J. Pharmacol*. **1987**, *143*, 291.