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Conformationally-Restricted Analogues and Partition Coefficients of the 5-HT_3 Serotonin Receptor Ligands *meta*-Chlorophenylbiguanide (*m*CPBG) and *meta*-Chlorophenylguanidine (*m*CPG)

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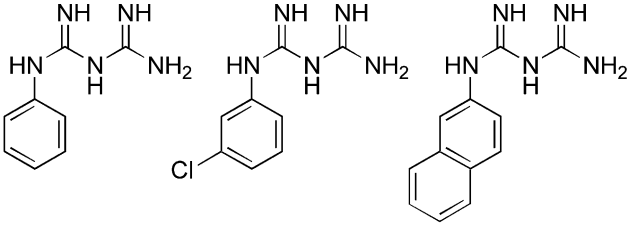
Abstract—The present investigation examined two features of arylbiguanide and arylguanidine 5-HT_3 ligands: conformation and partition coefficients. Several conformationally-constrained analogues of *m*CPBG (**2**) and *m*CPG (**11**; $K_i = 32$ nM) were prepared and of these only 2-amino-5-chloro-3,4-dihydroquinazoline (**14**; $K_i = 34$ nM) retained high affinity. The partition coefficient of compound **11** ($LogP_{app} = -0.64$) was less than that of its corresponding arylbiguanide **2** ($LogP_{app} = -0.38$). The quinazoline structure may represent a pharmacologically-active conformation of these agents, and the arylbiguanides were found more lipid soluble than their arylguanidine counterparts at physiological pH.

Serotonin (5-HT) receptors are divided into seven major families (5-HT_1–5-HT_7) and all are G-protein coupled except for 5-HT_3 receptors; the latter belong to the ion channel superfamily of receptors.[1–3] 5-HT_3 receptor antagonists have found therapeutic application in the treatment of emesis,[2,3] but much less is known about the potential therapeutic utility of 5-HT_3 ligands with agonist activity. One of the first 5-HT_3 ligands identified was phenylbiguanide (**1**). Although it was widely used as an agonist in preclinical studies for several years, its affinity for 5-HT_3 receptors was only in the low micromolar range.[2] *meta*-Chlorophenylbiguanide (*m*CPBG; **2**)[2,4] represented a significant advance in that it is an agonist that binds at 5-HT_3 receptors with higher affinity ($K_i < 50$ nM). It has also been demonstrated that the chlorophenyl substituent of **2** can be replaced by a 2-naphthyl group (i.e., **3**) with retention of affinity.[5,6] Compared in the same investigation, the K_i values determined for **1**–**3** were 1200, 18, and 14 nM, respectively.[7]



1 **2** **3**

The biguanide moiety common to **1**–**3** is conformationally flexible. In order to identify possible biologically-preferred conformations that might account for the binding of such compounds at 5-HT_3 receptors, we synthesized and examined several conformationally restricted analogues (see Fig. 1). Another issue addressed was the lipid solubility of the arylbiguanides. Evidence

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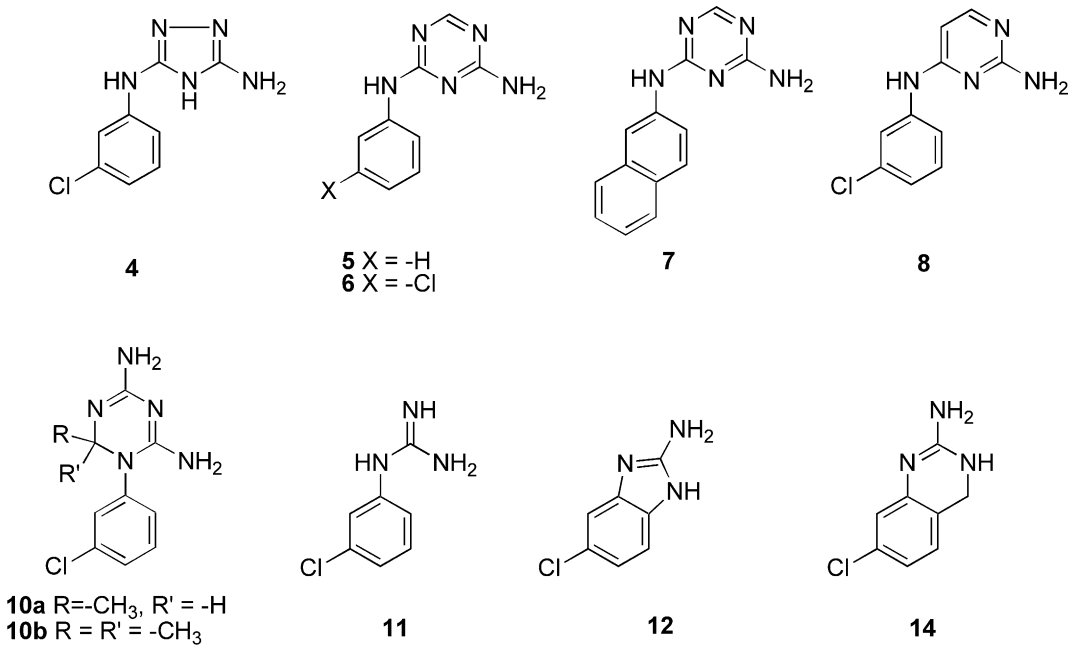


Figure 1. Arylbiguanides and arylguanidines analogues examined in this study.

suggests that **1** displays poor brain penetration; hence, we determined the partition coefficients of several derivatives for comparison with their arylguanidine counterparts.

Results and Discussion

Conformationally-constrained analogues

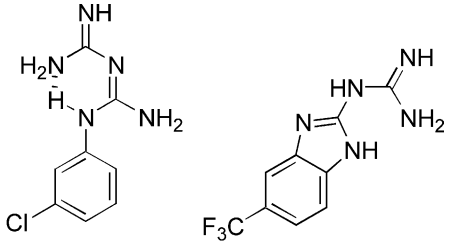
Radioligand binding data[9] are summarized in Table 1. The first compound examined was the triazole **4**[8] in which the biguanide moiety was constrained in a five-membered ring system. Compound **4** (K_i > 10,000 nM) was found to lack affinity for 5-HT_3 receptors relative to its ring-open counterpart **2** (K_i = 18 nM). Compounds **1**–**3** were also conformationally constrained in a six-membered ring (i.e., triazines **5**–**7**).[10] None of these analogues displayed measurable affinity (K_i > 10,000 nM in each case) for 5-HT_3 receptors. It is true that in addition to fixing the conformation of the compounds the basicity of **4**–**7** has likely been altered relative to that of their parents; however, at this time it is not known what role the basicity of the various nitrogen atoms plays in the binding of arylbiguanides at 5-HT_3 receptors. For example, compound **8** (K_i = 1,460 nM), a pyrimidine analogue of **6**, lacks one of the ring nitrogen atoms yet binds with an affinity similar to that of unsubstituted derivative **1**.

Table 1. Radioligand binding data for compounds examined

Compd	5-HT_3 affinity K_i, nM (±SEM)[a]
4	> 10,000
5	> 10,000
6	> 10,000
7	> 10,000
8	1460 (290)
10a	> 10,000
10b	> 10,000
11	32 (6)
12	725 (75)
14	34 (8)

[a]SEM not provided where K_i values are > 10,000 nM.

It has been suggested that arylbiguanides might form an intramolecular hydrogen bond to afford structures such as **9**.[11] Several dihydrotriazines were prepared (i.e., **10**)[12] to mimic the general structure of **9**. Both **10a** and **10b** (K_i > 10,000 nM) lacked 5-HT_3 receptor affinity.



9 **13**

We have previously found that the biguanide portion of the arylbiguanides can be abbreviated to a guanidine moiety with retention of 5-HT_3 receptor affinity.[5,7] For example, **11** (*meta*-chlorophenylguanidine, *m*CPG; K_i = 32 nM; Table 1) is the guanidine analogue of **2**. It was thought that this smaller structure might aid in minimizing the conformational possibilities of the flexible chain. The first conformationally restricted analogue of **11** to be considered was benzimidazole **12**. Morain et al.[13] have previously shown that benzimidazole **13** binds at 5-HT_3 receptors only in the low micromolar range. However, because replacement of the chloro group of **2** with a $-CF_3$ group dramatically reduces its affinity, it is not known if the low affinity of **13** is attributable to its restricted conformation or to the

presence of the trifluoromethyl substituent. Hence, **12**[14] was prepared and examined to address this issue. Benzimidazole **12** ($K_i = 725$ nM) displayed 20-fold lower affinity for 5-HT_3 receptors than **11**; yet, its affinity was higher than that of any of the conformationally-constrained analogues examined up to this point.

The centroid-to-amine distance of the flexible **11** is calculated to range from 3.7 to 4.9 Å.[7] That for **12** (centroid to NH_2) is calculated to be 4.5 Å. Because the centroid-to-amine distance of **14** is calculated to be 4.9 Å, it was thought that **14** might better mimic the more extended form of **11**. Compound **14** ($K_i = 34$ nM), the ring-expanded analogue of **12**, was examined and found to bind with enhanced affinity, and with an affinity nearly identical to that of **11** itself. Of the various conformationaly-constrained analogues examined, only quinazoline **14** was found to bind with low nanomolar affinity.

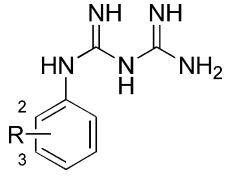
Partition coefficients

Intuitively, it might have been thought that the arylguanidines, lacking the additional amidine moiety of the arylbiguanides, would be more lipid soluble than their corresponding arylbiguanides. This was not found to be the case. Three pairs of arylbiguanides and arylguanidines were examined (Table 2) and in each case the arylbiguanides were found to be more lipid soluble than their corresponding arylguanidines at physiological pH. In both cases, the unsubstituted compounds (**1** and **16**) were the most soluble in aqueous media; the monochloro derivatives **2** and **11** were more lipid soluble, and the trichloro derivatives **15** and **17** were the most lipid soluble.

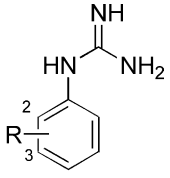
There is relatively limited literature information on the lipid solubility of arylbiguanides and their ability to penetrate the blood–brain barrier. In an ex vivo study, Bachy et al.[15] found that **1** and **2** displayed poor brain penetration in mice as determined by measuring their ability to displace [^{3}H]granisetron 30 min following systemic administration. A number of years ago we unsuccessfully attempted to train rats to discriminate **1** from vehicle in a two-lever operant procedure (unpublished findings); the inability of **1** to establish stimulus control of behavior might now be explained by its low lipophilicity and inability to penetrate the blood–brain barrier. However, **2** has been used as a training drug in drug discrimination studies using rats and its stimulus effects appeared to be centrally mediated.[16] Likewise, **2** substituted for arylguanidine **11** in rats trained to discriminate **11** from vehicle; here too, it was shown that the stimulus effect is likely centrally mediated.[6] In contrast, it might be noted that the 4-chloro analogue of **2** (i.e., 3,4-dichlorophenylbiguanide), which binds with higher affinity than **2** and which is presumably more lipophilic than **2**, failed to serve as a discriminative stimulus.[17] Thus, while certain of these agents might penetrate the blood–brain barrier only with difficulty, there is also evidence indicating that some have central actions.

Compounds with LogP values in the 1.5–2.5 range will, in general, readily enter the brain; in fact, some compounds with significantly lower LogP values can penetrate the blood–brain barrier and display central actions.[19] An optimum value for LogP is most crucial for agents with low receptor affinity.[19] Nevertheless, the LogP values obtained for **2** and **11** are certainly not inconsistent with the concept that they might penetrate the blood–brain barrier only with difficulty.

Table 2. $LogP_{app}$ of several arylbiguanides and arylguanidines[18]



1, **2**, **15**



11, **16**, **17**

Compd	R	$LogP_{app}$ (SEM)
1	H	−1.17 (0.04)
2	3-Cl	−0.38 (0.02)
15	3,4,5-Cl_3	1.47 (0.01)
16	H	−1.32 (0.18)
11	3-Cl	−0.64 (0.08)
17	3,4,5-Cl_3	1.16 (0.02)

Summary

The present investigation provides evidence that quinazoline analogue **14**, the first member of a novel series of 5-HT_3 ligands, likely represents a biologically active conformation of the arylguanidines. Also, the arylbiguanides were found to possess greater lipid solubility than their corresponding arylguanidines; as expected, introduction of multiple chloro groups resulted in the most lipophilic derivatives. Because both the arylbiguanides and aryl guanidines displayed low lipid solubility, future studies will focus on the synthesis of quinazoline analogues of these agents and, in particular, on multi chloro-substituted analogues. This study, then, demonstrates how the future construction of novel, brain-penetrant 5-HT_3 ligands with enhanced lipid solubility might be achieved. Such studies are now underway in our laboratories.

Experimental

Melting points (uncorrected) were obtained with a Thomas Hoover apparatus. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Microanalyses were performed by Atlantic Microlab (GA, USA) for the indicated elements, and the results are within 0.4% of theory. Reactions and product mixtures were routinely monitored by thin-layer chromatography on silica gel precoated F_{254} Merck plates. Several of the compounds used in the present study were known and were prepared according to literature procedures; the literature citations are provided in the text.

2-Amino-4-(3-chlorophenyl)aminopyrimidine hydrochloride (8). A mixture of 2-amino-4-chloropyrimidine (2.0 g, 20 mmol), 3-chloroaniline (1.6 mL, 20 mmol) and concd HCl (0.1 mL) in H_2O (15 mL) was heated at reflux for 2 h. The clear dark solution was warmed with charcoal powder and filtered hot. The filtrate was allowed to cool to room temperature, and made strongly alkaline by the addition of 10 N NaOH. The precipitated product was collected and recrystallized from benzene to afford 3.2 g (94%) of product (mp 126–128 °C) which was converted to its HCl salt: mp 191–194 °C; IR (KBr, cm^{-1}): 3340, 1560. ^1H NMR (DMSO-d_6) δ 6.22 (d, 1H, Pyr–H), 6.90 (br s, 2H, D_2O-exchangeable), 7.11 (d, 1H, ArH), 7.40 (m, 1H, ArH), 7.80 (d, 1H, ArH), 7.90 (s, 1H, ArH), 8.05 (d, 1H, Pyr–H), 9.40 (s, 1H, NH, D_2O-exchangeable). Anal. ($C_{10}H_9ClN_4$) C, H, N.

2-Amino-7-chloro-3,4-dihydroquinazoline hydrochloride (14). Following the general procedure of Grosso et al.[20] a suspension of S-methylisothiouronium sulphate (3.6 g, 18 mmol) and Na_2CO_3 (2.14 g, 20 mmol) in dry dioxane was heated until solution was complete. Chloroisatoic anhydride (3.56 g, 13 mmol) was added to the reaction mixture; the white suspension, which became yellow, was heated at reflux for further 20 h. The reaction mixture was allowed to cool at room temperature, poured into H_2O (60 mL), and allowed to stir for 15 min. The product was recovered by suction filtration and the filter cake was dried under vacuum at 60 °C for 24 h. The product was recrystallized from dioxane to yield 1.50 g (40%) of the desired quinazolinone: mp > 300 °C.

The quinazolinone (0.75 g, 3.82 mmol) was added to 1 M BH_3–THF (12.25 mL) and the resulting solution was heated at reflux under N_2 for 2 h. The borate complex and excess reagent were hydrolyzed by the dropwise addition of 6 N HCl (3 mL), and the acidic solution was basified with 6 N NaOH (12 mL). The mixture was concentrated and the residue was extracted with hot chloroform (3 × 10 mL). The combined extracts were concentrated under vacuum to yield 0.15 g (21%) of a white solid: mp 230 °C. The solid in absolute EtOH (10 mL) was titrated to pH ≈3 with 37% aqueous HCl. Solvent was removed under vacuum and the residue was dissolved in absolute EtOH (10 mL) and evaporated to dryness under vacuum; the process was repeated three more times. The residue was recrystallized from absolute EtOH/anhydrous Et_2O to yield 0.07 g (38%) of **14**: mp 240–242 °C; IR (KBr, cm^{-1}): 3180, 3078, 1620. ^1H NMR (DMSO-d_6) δ 4.72 (s, 2H, CH_2Ar), 6.98–7.01 (d, 1H, ArH), 7.76 (s, 1H, ArH), 8.66 (bs, 2H, NH, D_2O-exchangeable), 11.12 (s, 1H, NH, D_2O-exchangeable). Anal. ($C_8H_8ClN_3$ HCl) C, H, N.

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$23 \pm 1.0\,^{\circ}C$. Concentrations of compound in the aqueous phase were measured via spectrophotometer and the results represent the means of duplicate determinations run in triplicate. See do Amaral et al.[21] and references therein for greater experimental detail. Compounds were prepared as previously described.[7]

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