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N_1H- and N_1-Substituted Phenylguanidines as α7 Nicotinic Acetylcholine (nACh) Receptor Antagonists: Structure−Activity Relationship Studies

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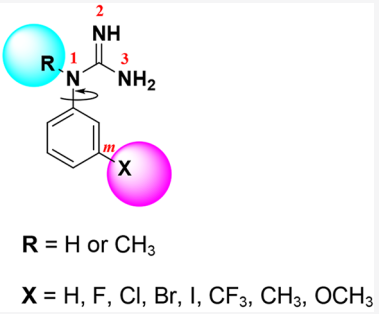
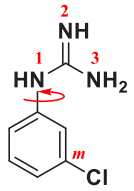
ABSTRACT: We previously reported that *N*-(3-chlorophenyl)guanidine (**1**) represents a novel α7 nicotinic ACh (nACh) receptor antagonist chemotype. In the present study, a small series of compounds was synthesized with the intent to investigate the structure−activity relationship (SAR). Preliminary data suggested that the N-methyl analog of **1**, **2**, was several times more potent. Therefore, the chloro group at the aryl 3-position of **1** and its N_1-methyl counterpart **2** were replaced with a number of substituents considering the electronic, lipophilic, and steric nature of the substituents. The potencies of the compounds to inhibit acetylcholine (ACh)-induced responses were obtained in *Xenopus laevis* oocytes expressing human α7 nicotinic ACh receptors (nAChRs) using a two-electrode voltage-clamp assay. We found that the nature of the 3-position substituents had relatively little (i.e., <10-fold) effect on potency, and the presence of an N_1-isopropyl substituent was tolerated. Here, we report the first SAR investigation of this novel α7 nAChR antagonist chemotype.



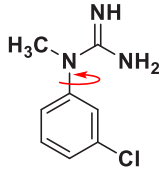
KEYWORDS: *α7 nAChR, SAR, electrophysiology, antagonist, phenylguanidines*

INTRODUCTION

Agents that antagonize the effect of acetylcholine (ACh) at nicotinic ACh receptors (nAChRs) are of interest for the treatment of neuropsychiatric,[1,2] neuroinflammatory,[3] and neurodegenerative disorders and as a novel therapeutic strategy for the treatment of cancer.[4,5] We have previously identified *m*-chlorophenylguanidine (*m*CPG, **1**) as a novel noncompetitive α7 nAChR antagonist chemotype.[6] That is, although **1** lacks affinity for α7 nAChR (i.e., K_i > 100 μM at [^{125}I]iodo-MLA-labeled receptors), it blocked the actions of ACh in electrophysiological and in vivo functional assays.[6]



*m*CPG (**1**)



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In an effort to better understand the action of **1** as a noncompetitive α7 nAChR antagonist, we initiated a structure−activity investigation. In a preliminary study, we found that the N_1-methyl analogue of **1** (i.e., **2**) was several times as potent as **1**.[7,8] Here, we examined a series of N_1−H 3-substituted phenylguanidines (Series I). Furthermore, knowing now that the N-methyl analogue **2** is active, (i) we prepared and examined a series of N_1-methyl analogs (Series II) and (ii) applied a Topliss approach[9] to examine several additional N_1-alkyl analogues.

CHEMISTRY

The phenylgunidine Series I analogues **1** and **3**−**9** were available from previous studies.[10,11] The *N*-methyl-*N*-phenylguanidine series analogues **10**−**16** were synthesized in a one-step reaction, as shown in Scheme 1, from their commercially available or previously reported N-methylanilines. Hydrochloride salts of the appropriate N-methylanilines (i.e., **23**−**29**; Scheme 1) were heated at reflux with cyanamide in absolute ethanol to yield the corresponding guanidines **10**−**16** as their hydrochloride salts. The hygroscopic hydrochloride salt of *N*-(3-methylphenyl)-*N*-methylguanidine (**15**) was converted to the nitrate salt with ammonium nitrate.

Commercially available anilines **32** and **34** were used in the preparation of **17** and **19**, respectively (Scheme 2). 3-Chloro-*N*-ethylaniline (**33**) was prepared via alkylation of **31** through a reductive amination step using acetaldehyde and pyridine-

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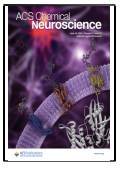
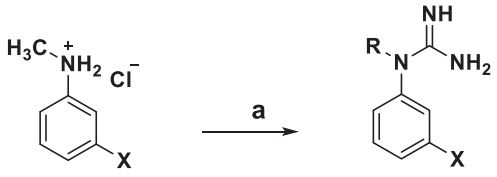


Scheme 1. Synthetic Route for N_1-Methylphenylguanidines[a] and Structures of Phenylguanidines 3−9 Series I



X :			R: H	X:	R: CH_3
	-H,	23	3	-H,	10
	-Br,	24	4	-Br,	11
	-I,	25	5	-I,	12
	-F,	26	6	-F,	13
	$-CF_3$	27	7	$-CF_3$,	14
	$-CH_3$,	28	8	$-CH_3$,	15
	$-OCH_3$,	29	9	$-OCH_3$,	16

[a]Reagents and conditions: (a) i. NH_2CN, EtOH, reflux; ii. (only for **15**) NH_4NO_3, H_2O.

borane as previously reported[12] and then utilized in the synthesis of **18**. For the preparation of **20** (Scheme 2), 3-chloroaniline (**31**) was treated with 2-bromopropane in the presence of an aqueous solution of NaOH, following the procedure of Koelsch and Britain[22] to yield the 3-chloro-*N*-isopropylaniline (**35**) followed by the condensation reaction with cyanamide. *N*-Cyclopentyl-*N*-phenylguanidine hydrochloride (**21**) and *N*-(3-chlorophenyl)-*N*-cyclopentylguanidine nitrate (**22**) were prepared in a reductive amination reaction (Scheme 2) of aniline (**30**) and 3-chloroaniline (**31**), respectively, and cyclopentanone in the presence of STAB (sodium triacetoxyborohydride). The hydrochloride salts of the alkylated anilines (i.e., **36** and **37**, respectively) were then heated at reflux with cyanamide to give **21** and **22**. The hygroscopic **22** hydrochloride salt was converted to the nitrate salt using ammonium nitrate.

The structures of targets **11**−**22** were confirmed by IR, ^{1}H NMR, and elemental analysis for C, H, N.

RESULTS

The nAChR antagonist actions of all compounds are shown in Figures 1, 2, and 4 and are reported in the form of half-maximal inhibitory concentrations (i.e., IC_{50} values, Tables 1 and 2).

For Series I and II, inhibitory potencies varied over a very narrow (<10-fold) range (Table 1). In both series, the aryl-unsubstituted compounds were slightly less potent (i.e., **3** and **10**), and the 3-iodo compounds (i.e., **5** and **12**) were somewhat more potent than chloro compounds **1** and **2**, respectively. An apparent discrepancy between the two series was the difference in potency between 3-CH_3 analogues **8** and **15**; whereas **8** is the least potent member in Series I, **15** is equipotent with **2**. As a consequence, data for these compounds were replicated and confirmed.

Parallel substituent modifications at the aryl 3-position of the two series were examined to determine if the two series might bind in a similar fashion. That is, if the two series are binding in a common manner, a relationship might exist between their potencies, and it would be expected that their potencies would covary.[13] Linear regressions and statistical analyses implemented here were conducted using GraphPad Prism software (version 5.04),[14] SPSS (Statistical Package for the Social Sciences; version 22.0), and JMP (John's Macintosh Program; version 11.2). Correlations expressing a p value of less than 0.05 were considered significant. A poor correlation (r = 0.474; n = 8) was found between pIC_{50} values of the Series I versus pIC_{50} values of the N_1-methyl Series II compounds (Figure 3).

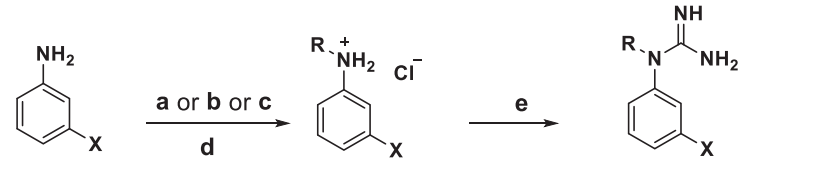
Follow-up statistical analysis was conducted to detect any potential outliers among the inhibitory potency values using Cook's D and Z score methods, specifically for the 3-CH_3 compounds (see Supporting Information). All IC_{50} values of the tested compounds, including the 3-CH_3 analogues, appeared to be within the acceptable range, and no single value exceeded the cutoffs.

If a methyl substituent is shown to improve the action of a compound in a given assay, certain other specific alkyl analogues should be examined as proscribed by the Topliss Operational Scheme.[9] These analogues include ethyl, isopropyl, and cyclopentyl.[9] Accordingly, these three N_1-alkyl analogues of **1** and its des-chloro counterpart **3** were explored. Figure 4 and Table 2 show data for these compounds; data for **3**, **1**, **10**, and **2**, from Table 1, are reproduced here for comparison.

The isopropyl analogues **19** and **20** were about twice as potent as **3** and **1**. Cyclopentyl analogues **21** and **22** were much less potent.

For the analysis of binding characteristics of selected compounds (i.e., **2** and **12**) to the native α7 nAChR in the

Scheme 2. Synthesis of Compounds 17−22[a]



X:	-H;	30					
	-Cl;	31					
R: C_2H_5;	X:	-H;	32	R: C_2H_5;	X:	-H;	17
		-Cl;	33			-Cl;	18
iC_3H_7;	X:	-H;	34	iC_3H_7;	X:	-H;	19
		-Cl;	35			-Cl;	20
cycloC_5H_9;	X:	-H;	36	cycloC_5H_9;	X:	-H;	21
		-Cl;	37			-Cl;	22

[a]Reagents and conditions: (a) acetaldehyde, pyridine-borane, MeOH, reflux; (b) 2-bromopropane, NaOH (15% aqueous), $ZnCl_2$ (saturated aqueous), 150 °C; (c) cyclopentanone, STAB, acetic acid, DCM, room temperature; (d) HCl/Et_2O; (e) i. NH_2CN, EtOH, reflux; ii. (only for compounds **19** and **22**) NH_4NO_3, H_2O.

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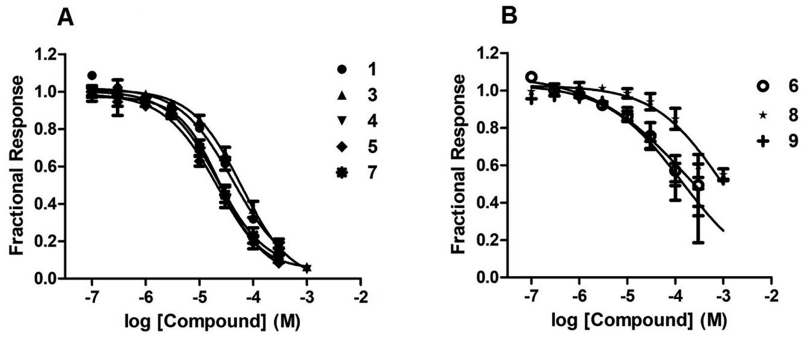

Figure 1. *Xenopus* oocytes expressing α7 nAChRs were coapplied with increasing concentrations of compounds (**1**, **3**, **4**, **5**, **6**, **7**, **8**, and **9**) with a fixed concentration of acetylcholine (100 μM). Data represent an $n \geq 4$ using oocytes from at least 2 different frogs. The increasing concentrations of compounds inhibited ACh induced responses as shown in dose response curves (1A and 1B) generated by using an inbuilt algorithm in GraphPad Prism. Dose response curves in 1A were unconstrained; however, due to insufficient data at the higher concentration of compounds **6**, **8**, and **9**, the dose response curves in 1B were constrained to bottom = 0.

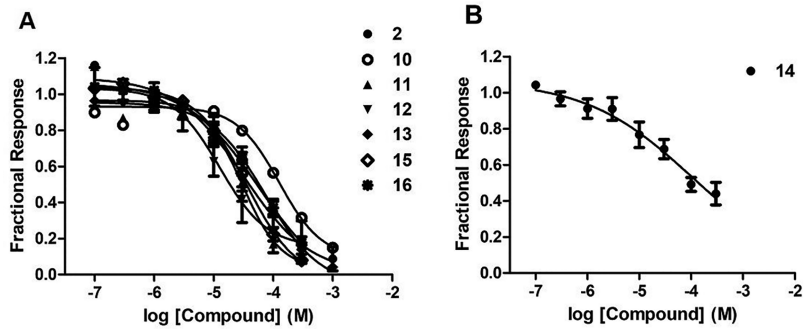

Figure 2. *Xenopus* oocytes expressing α7 nAChRs were coapplied with increasing concentrations of compounds (**2**, **10**, **11**, **12**, **13**, **14**, **15**, and **16**) with a fixed concentration of acetylcholine (100 μM). Data represent an $n \geq 4$ using oocytes from at least 2 different frogs. The increasing concentrations of compounds inhibited ACh induced responses as shown in dose response curves (2A and 2B) generated by using an inbuilt algorithm in GraphPad Prism. Dose response curves in 2A were unconstrained; however, due to insufficient data at the higher concentration of compound **14**, the dose response curve in 2B is constrained to bottom = 0.

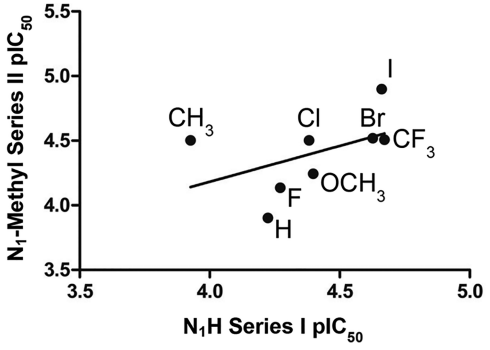

Figure 3. Plot of pIC_{50} values of the Series I versus the N_1-methyl analogue Series II (r = 0.474, p = 0.235, n = 8).

brain, autoradiography was conducted. Images of sections incubated with the same radiotracer, but with increasing concentrations of compounds **2** and **12**, showed that both compounds inhibited binding of the selective radiotracer to the native receptor site (Figure 5A). While compound **12** could displace virtually all binding at 100 μM concentrations, compound **2** only reduced the binding significantly at the same concentration. Quantitative analysis of binding of compound **2** (open bars) and **12** (squared) compared to total binding at 100% and unspecific binding at 0% (1 mM nicotine) showed that both compounds could displace the radiotracer, but compound **12** was more effective (Figure 5B).

When assayed against seven different nAChR populations (i.e., $\alpha2\beta2$, $\alpha2\beta4$, $\alpha3\beta2$, $\alpha4\beta2$, $\alpha3\beta4$, $\alpha4\beta2$, $\alpha4\beta4$) and 5-HT_3 receptors in radioligand binding studies, both parent **2** and the most potent inhibitor identified in these studies, i.e., **12**, lacked binding affinity at all eight (i.e., K_i > 10,000 nM). Furthermore, unlike **1** which binds at 5-HT_3 receptors with high affinity (K_i = 34 nM),[10] compound **12** lacked affinity for this receptor population.

DISCUSSION

Preliminary data suggested that **2** was several times more potent than **1** as a noncompetitive nAChR antagonist.[7,8] Accordingly, we examined a series of 3-substituted **1**-related compounds in comparison with their corresponding N_1-methyl counterparts (i.e., Series I and II) to better understand their structure−activity relationship (SAR). We also employed a two-electrode voltage-clamp assay system.

Considering the functional data from **1** and its *N*-methyl analogue **2**,[4] a small series of compounds was synthesized. The chloro group at the aryl 3-position of *N*-(3-chlorophenyl)-guanidine (**1**) and its N_1-methyl counterpart **2** were replaced with a number of substituents considering the electronic, lipophilic, and steric nature of the substituents.[15] The overall intent was to determine the effects of these substituents on the α7 nAChR action of **1** and **2** and, if possible, to conduct quantitative structure−activity relationship (QSAR) studies. The introduction of other halogen atoms (i.e., -F, -Br, and -I)

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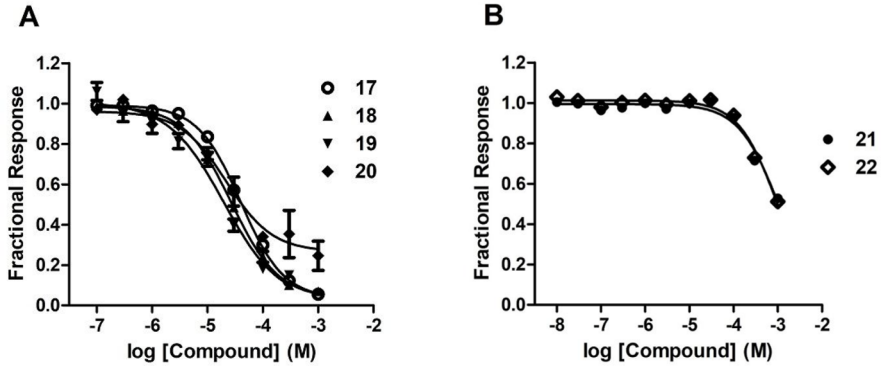
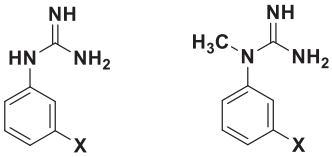


Figure 4. *Xenopus* oocytes expressing α7 nAChRs were coapplied with increasing concentrations of compounds (**17**, **18**, **19**, **20**, **21**, and **22**) with a fixed concentration of acetylcholine (EC_{50}). The increasing concentrations of compounds inhibited ACh induced responses as shown in dose response curves (4A and 4B) generated by using an inbuilt algorithm in GraphPad Prism. Dose response curves in 4A were unconstrained; however, due to insufficient data at the higher concentration of compounds **21** and **22**, the dose response curves in 4B were constrained to bottom = 0.

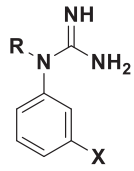
Table 1. Inhibitory Potencies (IC_{50} Values) of the 3-Substituted Series I and the N_1-Methyl Series II Phenylguanidines for α7 nAChRs[a]



IC_{50} [μM]	95% CI [μM]	Series I	X	Series II	IC_{50} [μM]	95% CI [μM]
42	26–65	**1**	Cl	**2**	32	16–63
60	41–90	**3**	H	**10**	130	99–159
24	20–28	**4**	Br	**11**	30	18–51
21	14–34	**5**	I	**12**	13	6–26
170	57–520	**6**	F	**13**	73	36–151
21	14–32	7	CF_3	**14**	102	34–310
900	570–1400	**8**	CH_3	**15**	35	22–56
130	54–320	**9**	OCH_3	**16**	58	14–230

[a]The data were obtained by using a nonlinear curve fitting algorithm from the dose response curves shown in Figures 1 and 2. Data represent an $n \geq 4$ using oocytes from at least 2 different frogs.

Table 2. Inhibitory Potency (IC_{50} Values) of the N_1-Alkylphenylguanidines for α7 nAChRs[a]



R	X	compound	IC_{50} [μM]	95% CI [μM]
H	H	**3**	60	41–90
H	Cl	**1**	42	26–65
CH_3	H	**10**	130	99–159
CH_3	Cl	**2**	32	16–63
C_2H_5	H	**17**	40	35–46
C_2H_5	Cl	**18**	27	23–31
$CH(CH_3)_2$	H	**19**	20	11–37
$CH(CH_3)_2$	Cl	**20**	22	10–47
cyclopentyl	H	**21**	1000	870–1300
cyclopentyl	Cl	**22**	960	850–1100

[a]The data were obtained by using nonlinear curve fitting algorithm from the dose response curves shown in Figure 4.

at the 3-position will allow for testing mainly the effect of substituent size variation over a relatively fixed range of electron-withdrawing effects. A recent study on a number of α7 nAChR modulators described the diverse impact of different halogen atoms at the same ring position on the compounds' pharmacological properties.[16] That is, alteration of the halogen atom influenced not only potency but also the functional action of the compound.

Our previous findings[7,8] for investigating the inhibitory effect of phenylguanidines at rat α7 nAChRs were obtained using the whole-cell configuration of the patch-clamp technique on cells expressed in stably transfected human embryonic kidney (HEK) 293 cells. Functional data in the current project were obtained by evaluating the potencies of the compounds in inhibiting ACh-induced responses in *Xenopus laevis* oocytes expressing human α7 nAChRs using an automated two-electrode voltage-clamp assay incorporating rapid perfusion (20 mL/min) and a vertical flow recording chamber. This system has been demonstrated to provide a more rapid solution exchange and enhanced rise times in response to an agonist.[17] Despite the rapid exchange time, however, recorded potencies were expected to be lower compared to those (e.g., for **1**) from the previously used HEK 293 cells, because oocyte surface components, such as the large number of invaginations in the membrane and the surrounding vitelline membrane and follicle cells, decrease the accessibility of the compounds.[18] Nevertheless, the current assay should allow a rapid determination of SAR.

In Series I, the potencies of the analogues as hα7 nAChR antagonists only varied over a 6-fold range, and the most potent compounds (i.e., **4**, **5**, **7**) were only twice as potent as **1**. The 3-position substituents were varied in a manner (i.e., with varying physicochemical properties) such that a QSAR study might be conducted. However, the narrow range of potencies precluded a QSAR study.

Series I substituents were also examined in the N_1-methyl series with the expectation that Series II might be somewhat more potent. This turned out not to be the case. For Series II, potencies varied only over a 10-fold range. Both Series I and II have a single bond between the phenyl ring and the anilinic N atom; thus, they can easily rotate. We have previously shown that **1** can exist in four low-energy rotamers. Furthermore, if

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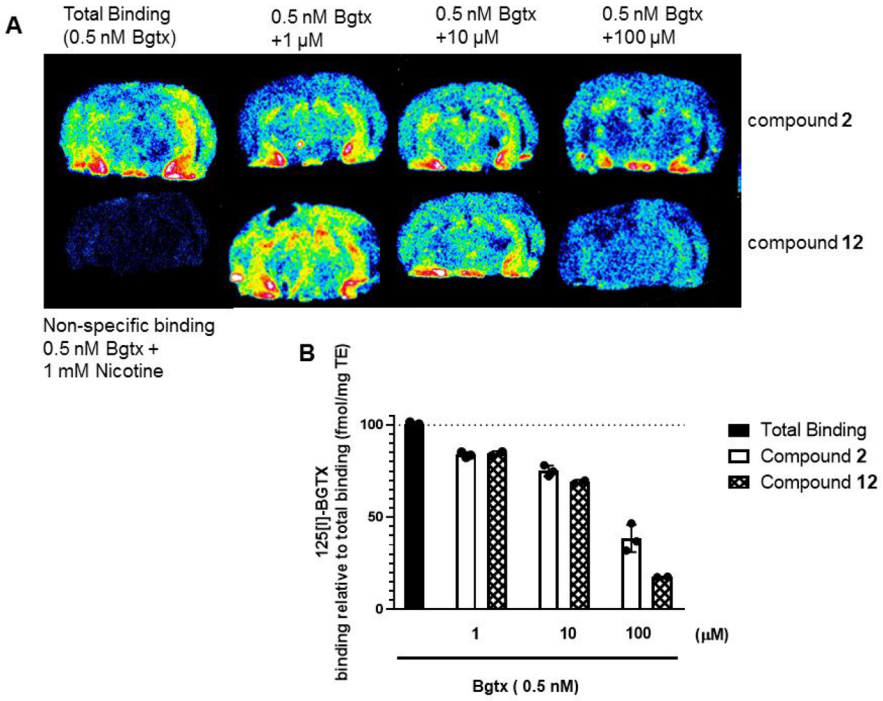

Figure 5. (A) Representative images of rat brain sections exposed to [^{125}I]-α-bungarotoxin (Bgtx) alone or together with 1 mM nicotine showing total and unspecific binding to α7 nAChR binding sites. As previously demonstrated, specific binding is observed in the cerebral cortex, the hippocampus, and the hypothalamic mammillary nuclei. Images of sections incubated with the same radiotracer, but increasing concentrations of compounds **2** and **12**, showed that both compounds inhibited binding of the selective radiotracer to the native receptor site at high concentrations. The autoradiograms revealed that, while compound **12** displaced binding in all areas at 100 μM concentration, significant binding remained in high-binding areas such as the ventral hippocampus and the mammillary nuclei when displaced with compound **2** at the same concentration. (B) Quantitative analysis of binding in the rat brain section(s) of compound **2** (open bars) and **12** (squared) compared to total binding (black bar) at 100% and unspecific binding at 0% (1 mM nicotine) in three brain sections. Dots represent the individual results of three separate experiments. The graph showed that both compounds could displace the radiotracer; however, where compound **12** nearly eliminated binding, specific binding remained after displacement with compound **2**.

two series of compounds bind to the receptor in the same manner, parallel structural changes in both series should result in parallel shifts in affinity/activity, and the correlation would be of 1 or close to 1. Figure 3 shows that there is no correlation between activities in these two series (statistically insignificant correlation). Combined, the rotameric existence of the compounds and the poor correlation of their activities support our speculations that compounds might interact with the receptors in a slightly different manner. In addition, follow-up studies with **2** and **12** (Figure 5) showed that their actions cannot be solely attributed to competitive antagonism, because concentrations exceeding their EC_{50} values (Table 1) were required to substantially displace the radioligand.

Following the Topliss approach, several N_1-alkylphenylguanidines were examined, and N_1-isopropyl analogues **19** and **20** were more potent than their N_1H parents **3** and **1**, respectively. Compounds with a larger N_1-cyclopentyl substituent (i.e., **21**, **22**) displayed considerably reduced potency indicating limited tolerance for N_1 bulk.

CONCLUSIONS

Compound **1** is a novel α7 nAChR antagonist chemotype, and various other phenylguanidines are shown here to retain this action. Overall, however, it would seem that (i) the nature of the 3-position substituent has relatively little (i.e., <10-fold) effect on potency, and (ii) the presence of an N_1-isopropyl substituent doubles the potency of **1**. The most potent compound identified, **12**, was only 3-fold more potent than **1**. However, unlike **1**, it showed little affinity (K_i > 10 000 nM) for other nACh or 5-HT_3 receptors.

Because rotameric binding might obfuscate the results of this study, future studies should focus on 4-substituted phenylguanidines. In addition, particular attention should be paid to the possibility that N_1H and N_1-alkyl derivatives might interact with the receptors in a slightly different manner.

EXPERIMENTAL SECTION

Synthesis. Melting points (mps) were taken in glass capillary tubes using a Thomas-Hoover melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Bruker ARX 400 MHz spectrometer at which peak positions are given in parts per million (δ) downfield from the internal standard tetramethylsilane (TMS), followed by the splitting pattern (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, m = multiplet), coupling constant (Hz), and integration. Infrared spectra were obtained on a Thermo Nicolet iS10 FT-IR. Purity of compounds was determined by elemental analysis performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements, and the obtained values are within 0.4% of theoretical values. Reactions were monitored by thin-layer chromatography (TLC) on silica gel GHLF plates (250 μm, 2.5 cm × 10 cm; Analtech Inc. Newark, DE), and Flash chromatography was performed on a CombiFlash Companion/TS (Teledyne Isco Inc. Lincoln, NE) using packed silica gel (Silica Gel 230−400 mesh) columns (RediSep Rf Normal-phase Silica Flash Column, Teledyne Isco Inc., Lincoln, NE). Electrospray ionization-mass spectroscopy (ESI-MS) profiles were recorded using a Waters Acquity TQD (tandem quadrupole) spectrometer in positive ion mode.

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The nitrate salts of *m*-chlorophenylguanidine (**1**) and **7**, the hydrochloride salts of **2**, phenylguanidine (PG; **3**), **4**−**6**, **8**, **10**, and the hemisulfate salt **9** were available from previous studies[10,11] and used as such. *N*-Ethyl-*N*-phenylguanidine hydrochloride (**17**) was prepared according to a literature procedure.[19]

*N-(3-Bromophenyl)-N-methylguanidine Hydrochloride (**11**).* Cyanamide (154 mg, 3.66 mmol) was added to a solution of 3-bromo-*N*-methylaniline hydrochloride (**24**) (407 mg, 1.83 mmol) in absolute EtOH (5 mL). The reaction mixture was allowed to stir and heated at reflux for 24 h; then, the solvent was removed under reduced pressure. The resultant oily residue was crystallized with H_2O to give 178 mg (37%) of the desired product as white crystals: mp 278−280 °C; IR (diamond, cm^{-1}): 3118 (NH), 3276 (NH_2); ^1H NMR (DMSO-d_6): δ 3.26 (s, 3H, CH_3), 7.4 (qd, J = 8.06, 1.00 Hz, 1H, ArH), 7.47 (t, J = 7.96 Hz, 1H, ArH), 7.64 (td, J = 7.92, 1.32 Hz, 1H, ArH), 7.68 (t, J = 1.86 Hz, 1H, ArH). Anal. Calcd ($C_8H_{10}BrN_3$·HCl) C, 36.32; H, 4.19; N, 15.88. Found: C, 36.23; H, 4.28; N, 15.77.

*N-(3-Iodophenyl)-N-methylguanidine Hydrochloride (**12**).* This was prepared in the same manner as that of compound **11** from 3-iodo-*N*-methylaniline hydrochloride (**25**)[20] except that the product crystallized without the addition of H_2O. The residue was recrystallized from absolute EtOH to give 25% of the desired product as brown crystals: mp 285−287 °C; IR (diamond, cm^{-1}): 3121 (NH), 3280 (NH_2); ^1H NMR (DMSO-d_6): δ 3.25 (s, 3H, CH_3), 7.30 (t, J = 8.16 Hz, 1H, ArH), 7.41 (td, J = 8.00, 1.50 Hz, 1H, ArH), 7.79 (m, 1H, ArH), 7.80 (m, 1H, ArH). Anal. Calcd ($C_8H_{10}IN_3$·HCl) C, 30.84; H, 3.56; N, 13.49. Found: C, 31.07; H, 3.71; N, 13.28.

*N-(3-Fluorophenyl)-N-methylguanidine Hydrochloride (**13**).* This was prepared in the same manner as that of compound **11** from 3-fluoro-*N*-methylaniline hydrochloride (**26**). The residue was recrystallized from *i*-PrOH to give 22% of the desired product as white crystals: mp 197−199 °C; IR (diamond, cm^{-1}): 3123 (NH), 3288 (NH_2); ^1H NMR (DMSO-d_6): δ 3.27 (s, 3H, CH_3), 7.24 (dd, J = 7.36, 0.96 Hz, 1H, ArH), 7.29 (td, J = 8.60, 2.40 Hz, 1H, ArH), 7.35 (d, J = 9.88 Hz, 1H, ArH), 7.56 (q, J = 8.06 Hz, 1H, ArH). Anal. Calcd ($C_8H_{10}FN_3$·HCl) C, 47.18; H, 5.44; N, 20.63. Found: C, 47.08; H, 5.57; N, 20.84.

*N-(3-Trifluoromethyl)-N-methylguanidine Hydrochloride (**14**).* This was prepared in the same manner as that of compound **11** from *N*-methyl-3-trifluoromethylaniline hydrochloride (**27**).[21] The residue was recrystallized from 1-butanol to give 21% of the target as white crystals: mp 247−249 °C; IR (diamond, cm^{-1}): 3120 (NH), 3282 (NH_2); ^1H NMR (DMSO-d_6): δ 3.29 (s, 3H, CH_3), 7.72 (m, 2H, ArH), 7.79 (d, J = 7.68 Hz, 1H, ArH), 7.82 (s, 1H, ArH). Anal. Calcd ($C_9H_{10}F_3N_3$·HCl) C, 42.62; H, 4.37; N, 16.57. Found: C, 42.66; H, 4.30; N, 16.52.

*N-(3-Methylphenyl)-N-methylguanidine Nitrate (**15**).* This was prepared in the same manner as that of compound **11** from 3-methyl-*N*-methylaniline hydrochloride (**28**). The resultant oily residue which failed to crystallize was dissolved in H_2O followed by addition of NH_4NO_3. The solvent was removed under reduced pressure, and the resultant semisolid was recrystallized from H_2O and then from a mixture of *i*-PrOH/Et_2O to give a 6% yield of the target as white crystals: mp 139−141 °C; IR (diamond, cm^{-1}): 3172 (NH), 3345 (NH_2); ^1H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 3.25 (s, 3H, CH_3), 7.16 (d, J = 7.88 Hz, 1H, ArH), 7.19 (s, 1H, ArH), 7.26 (d, J = 7.60 Hz, 1H, ArH), 7.40 (t, J = 7.72 Hz, 1H, ArH). Anal. Calcd ($C_9H_{13}N_3$·HNO_3) C, 47.78; H, 6.24; N, 24.77. Found: C, 47.69; H, 6.29; N, 24.86.

*N-(3-Methoxyphenyl)-N-methylguanidine Hydrochloride (**16**).* This was prepared in the same manner as that of compound **11** from 3-methoxy-*N*-methylaniline hydrochloride (**29**). The resulting residue was recrystallized from a mixture of absolute EtOH/Et_2O to give 47% of the desired product as off-white crystals: mp 219−221 °C; IR (diamond, cm^{-1}): 3122 (NH), 3292 (NH_2); ^1H NMR (DMSO-d_6): δ 3.27 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 6.93 (dq, J = 7.80, 0.72 Hz, 1H, ArH), 6.98 (t, J = 2.14 Hz, 1H, ArH), 7.01 (dt, J = 8.28, 1.84 Hz, 1H, ArH), 7.42 (t, J = 8.06 Hz, 1H, ArH). Anal. Calcd ($C_9H_{13}N_3O$·HCl) C, 50.12; H, 6.54; N, 19.48. Found: C, 50.12; H, 6.60; N, 19.45.

*N-(3-Chlorophenyl)-N-ethylguanidine Hydrochloride (**18**).* Cyanamide (0.13 g, 3.12 mmol) was added to a solution of 3-chloro-*N*-ethylaniline hydrochloride (**33**)[12] (0.3 g, 1.56 mmol) in absolute EtOH (10 mL). The stirred reaction mixture was heated at reflux for 24 h. Upon cooling, the reaction mixture was evaporated under reduced pressure to obtain a residue that was recrystallized from a mixture of absolute EtOH/Et_2O to give 0.048 g (13%) of the desired product as white crystals: mp 186−189 °C; IR (diamond, cm^{-1}): 3077, 3187 (NH), 3274 (NH_2); ^1H NMR (DMSO-d_6): δ 1.07 (t, J = 7.16 Hz, 3H, CH_3) 3.66 (q, J = 7.12, 7.16 Hz, 2H, CH_2), 7.34 (m, 1H, ArH), 7.54 (m, 3H, ArH). Anal. Calcd ($C_9H_{12}ClN_3$·HCl) C, 46.17; H, 5.60; N, 17.95. Found: C, 45.93; H, 5.64; N, 17.72.

*N-Isopropyl-N-phenylguanidine Nitrate (**19**).* An aqueous solution of cyanamide (50%; 1.5 mL) was added to a solution of *N*-isopropylaniline hydrochloride (**34**) (100 mg, 0.58 mmol) in absolute EtOH (9 mL). The stirred reaction mixture was heated at reflux for 25 h and then cooled to 0 °C (freezer) for 20 h. Solvent was removed under reduced pressure, and the resulting oily residue was dissolved in H_2O (1 mL) followed by addition of NH_4NO_3 (108 mg, 1.34 mmol). The solution was concentrated under reduced pressure, and the residue was dissolved in H_2O (5 mL) and washed with Et_2O (3 × 20 mL), followed by evaporation of H_2O under reduced pressure. The resulting solid was recrystallized from H_2O and then from absolute EtOH to give 30 mg (21%) of the desired product as white crystals: mp 164−165 °C; IR (diamond, cm^{-1}): 2159, 3172 (NH), 3319 (NH_2); ^1H NMR (DMSO-d_6): δ 1.05 (d, J = 6.52 Hz, 6H, CH_3), 4.4 (m, 1H, CH), 7.3 (m, 2H, ArH), 7.6 (m, 3H, ArH). Anal. Calcd ($C_{10}H_{15}N_3$·HNO_3) C, 49.99; H, 6.71; N, 23.32. Found: C, 49.85; H, 6.64; N, 23.27.

*N-(3-Chlorophenyl)-N-isopropylguanidine Hydrochloride (**20**).* Cyanamide (1.02 g, 24.26 mmol) was added to a solution of 3-chloro-*N*-isopropylaniline (**35**)[22] (2.50 g, 12.13 mmol) in absolute EtOH (30 mL). The reaction mixture was allowed to stir at reflux for 24 h. The solution was concentrated under reduced pressure, and anhydrous Et_2O (25 mL) was added. The white precipitate was collected by filtration and recrystallized from *i*-PrOH to give 0.51 g (17%) of the desired product as white crystals: mp 272−274 °C; IR (diamond, cm^{-1}): 3108 (NH), 3269 (NH_2); ^1H NMR (DMSO-d_6): δ 1.04 (d, J = 6.56 Hz, 6H, CH_3), 4.36 (m, 1H, CH), 7.28 (td, J = 7.72, 1.62 Hz, 1H, ArH), 7.45 (t, J = 1.84 Hz, 1H, ArH), 7.58 (m, 2H, ArH). Anal. Calcd ($C_{10}H_{14}ClN_3$·HCl) C, 48.40; H, 6.09; N, 16.93. Found: C, 48.68; H, 6.11; N, 17.06.

*N-Cyclopentyl-N-phenylguanidine Hydrochloride (**21**).* Cyanamide (0.64 g, 15.17 mmol) was added to a solution of *N*-cyclopentylaniline hydrochloride (**36**)[23] (1.5 g, 7.58 mmol) in absolute EtOH (15 mL). The stirred reaction mixture was heated at reflux for 24 h. After it cooled, the solvent was evaporated under reduced pressure to obtain a residue that was washed with cyclohexane and recrystallized from THF to give 0.15 g (9%) of the desired product as white crystals: mp 209−211 °C; IR (diamond, cm^{-1}): 2956, 3117 (NH), 3276 (NH_2); ^1H NMR (DMSO-d_6): δ 1.20 (m, 2H, CH_2), 1.43 (m, 4H, CH_2), 1.92 (m, 2H, CH_2), 4.31 (m, 1H, CH), 7.28 (m, 2H, ArH), 7.53 (m, 3H, ArH). Anal. Calcd ($C_9H_{12}ClN_3$·HCl·0.1 C_6H_{12}) C, 65.94; H, 9.06; N, 13.44. Found: C, 65.98; H, 9.13; N, 13.15.

*N-(3-Chlorophenyl)-N-cyclopentylguanidine Nitrate (**22**).* Cyanamide (0.72 g, 17.23 mmol) was added to a solution of 3-chloro-*N*-cyclopentylaniline hydrochloride (**37**) (2.0 g, 8.61 mmol) in absolute EtOH (15 mL). The stirred reaction mixture was heated at reflux for 24 h. The solvent was removed under reduced pressure, and the residue was dissolved in H_2O (8 mL). The solution was washed with Et_2O (3 × 20 mL), followed by evaporation of H_2O under reduced pressure. The resultant oily residue was dissolved in H_2O (8 mL), followed by addition of NH_4NO_3 (0.12 g, 1.44 mmol). The solvent was evaporated under reduced pressure, and the resultant semisolid was recrystallized from EtOH to give 0.34 g (15%) of the desired product as white crystals: mp 225−227 °C; IR (diamond, cm^{-1}): 2969, 3175 (NH), 3346 (NH_2); ^1H NMR (DMSO-d_6): δ 1.19 (m,

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2H, CH_2), 1.45 (m, 4H, CH_2), 1.94 (m, 2H, CH_2), 4.27 (m, 1H, CH), 7.29 (dd, J = 1.64, 7.52 Hz, 1H, ArH), 7.48 (s, 1H, ArH), 7.55 (m, 2H, ArH). Anal. Calcd ($C_{12}H_{16}ClN_3 \cdot HNO_3$) C, 47.93; H, 5.70; N, 18.63. Found: C, 48.06; H, 5.59; N, 18.61.

*3-Chloro-N-cyclopentylaniline Hydrochloride (**37**).* Cyclopentanone (2.8 mL, 31.35 mmol), acetic acid (0.6 mL, 15.68 mmol), and sodium triacetoxyborohydride (6.6 g, 31.35 mmol) were added to a solution of 3-chloroaniline (**31**) (1.6 mL, 15.68 mmol) in dichloromethane (15 mL), and the reaction mixture was allowed to stir at room temperature for 1.5 h. The mixture was washed with saturated aqueous sodium bicarbonate solution (3 × 25 mL), and the solvent was evaporated under reduced pressure and dried under vacuum for 7 h to yield 2.7 g of the free base of **37** as a yellow oil. The free base was dissolved in Et_2O, and a saturated solution of HCl in Et_2O (50 mL) was added. The precipitate was collected by filtration and recrystallized from EtOAc to give 3.0 g (98%) of the product as white crystals: mp 115−117 °C; IR (diamond, cm^{-1}): 1589, 2628 (NH); ^1H NMR (DMSO-d_6): δ 1.58 (m, 4H, CH_2), 1.72 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 3.78 (m, 1H, CH), 7.01 (m, 3H, ArH), 7.27 (m, 1H, ArH). The product was used without further characterization for synthesis of compound **22**.

Electrophysiology. Functional characterization of all the compounds were performed using two-electrode voltage-clamp assay using *Xenopus* oocytes that expressed α7 receptors as described previously.[24−27] Briefly, ovarian lobes were surgically harvested from *X. laevis* and washed twice in Ca^{2+}-free Barth's solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl2, and 5 mM HEPES, pH 7.4). These lobes were enzymatically defolliculated to release oocytes by shaking for 90 min in 1.5% collagenase (Sigma-Aldrich) dissolved in Ca^{2+}-free Barth's Buffer. Oocytes were washed, and debris were separated and stored at 19 °C until needed for microinjection. cRNA was synthesized in vitro using an appropriate cDNA and the mMESSAGE MACHINE mRNA synthesis kit (Ambion). Each oocyte was injected with 50 nL of mRNA (0.3 μg/μL concentration) and incubated at 19 °C for at least 24−36 h prior to electrophysiological recording. Oocytes were placed in the recording chamber perfused with ND-96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 2 mM phosphate, pH 7.4) at a flow rate of 20 mL/min. Two electrodes (1−2 MΩ) filled with 3 M KCl corresponding to the current and voltage electrodes were inserted, and the holding potential was clamped at −60 mV using a Warner instruments amplifier. Ligands were prepared in ND-96 buffer and then injected into the chamber. Current responses were recorded using an Axon Instruments A/D board and PClamp software. The oocyte recording system used automated sampling and injection to maintain consistent perfusion rates and times and a vertical recording chamber that provides enhanced stability and the ability to use higher flow rates. This system has been described and evaluated in a prior publication.[17] A dose response curve was determined using pooled peak amplitudes of individual responses and analyzed using nonlinear curve fitting with variable slope and constraints as mentioned in figures using GraphPad Prism software.

Compounds were initially applied alone, in the absence of ACh, to determine whether they were capable of activating the receptors (agonist action). None of the test compounds showed any ability to activate receptors when applied alone at concentrations up to 1 mM. Test compounds were then evaluated for their ability to inhibit ACh induced responses (antagonist action) by coapplication of ACh with increasing concentrations of above compounds, and IC_{50} values were determined. Competition experiments used a standard concentration of 100 μM ACh.

Autoradiography. Here, 20 μm of rat brain cryostat sections (Sprague−Dawley male) were incubated for 2 h in 0.5 nM [^{125}I]Tyr54-monoiodo-α-bungarotoxin (2200 Ci/mmol, PerkinElmer) in 50 mM Tris buffer, pH 7.3, to assess total binding as described earlier.[28] For determination of displacement, increasing concentrations of compounds **2** and **12** were added to the incubation. After incubation with the radiotracer with and without the selected compounds, the slides were briefly washed in the same buffer, followed by short washes in ice-cold binding buffer, and they were rinsed briefly in ice-cold distilled water. Finally, the slides were dried for 24 h and, together with [^{125}I] microscale standards, exposed to FUJI imaging phosphor plates overnight. The FUJI imaging plates were scanned by Fujifilm Image Reader (BAS-2500 V1.8), and autoradiograms were analyzed with ImageJ software (Version 2.0.0, NIH). Nonspecific binding was determined by adding 1 mM S(−)-nicotine (Sigma-Aldrich, Denmark) in the incubation solution.

Competition Binding Assay. Binding assays for **2** and **12** were performed by the Psychoactive Drug Screening Program (PDSP). For experimental details, see the PDSP website http://pdsp.med.unc.edu/ and click on "Binding Assay".

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschemneuro.1c00212.

Statistical analysis of potential outliers using Cook's D and Z score methods (PDF)

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Author Contributions

O.I.A. and S.K. are co-first authors. M.D. conceived the idea, supervised the work, and prepared the first draft of the manuscript. O.I.A. synthesized all N_1-phenylguanidine analogues under the supervision of M.D. S.K. performed electrophysiological studies on α7 nAChR receptors under the supervision of M.K.S. S.S.A. performed the autoradio-

graphic studies under the supervision of J.D.M. All authors contributed to the preparation of this manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

nAChR, nicotinic acetylcholine receptor; mCPG, *N*-(3-chlorophenylguanidine); SAR, structure−activity relationship; STAB, sodium triacetoxyborohydride; Bgtx, [^{125}I]-α-bungarotoxin

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