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***N'*-3-(Trifluoromethyl)phenyl Derivatives of *N*-Aryl-*N'*-methylguanidines as Prospective PET Radioligands for the Open Channel of the *N*-Methyl-D-aspartate (NMDA) Receptor: Synthesis and Structure–Affinity Relationships**

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Abstract

N-Methyl-D-aspartate (NMDA) receptor dysfunction has been linked to several neuropsychiatric disorders, including Alzheimer's disease, epilepsy, drug addiction, and schizophrenia. A radioligand that could be used with PET to image and quantify human brain NMDA receptors in the activated "open channel" state would be useful for research on such disorders and for the development of novel therapies. To date, no radioligands have shown well-validated efficacy for imaging NMDA receptors in human subjects. In order to discover improved radioligands for PET imaging, we explored structure–affinity relationships in *N'*-3-(trifluoromethyl)phenyl derivatives of *N*-aryl-*N'*-methylguanidines, seeking high affinity and moderate lipophilicity, plus necessary amenability for labeling with a positron-emitter, either carbon-11 or fluorine-18. Among a diverse set of 80 prepared *N'*-3-(trifluoromethyl)phenyl derivatives, four of these compounds (**13**, **19**, **20**, and **36**) displayed desirable low nanomolar affinity for inhibition of [³H](+)-MK801 at the PCP binding site and are of interest for candidate PET radioligand development.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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Detailed synthetic procedures and characterization for all synthesized compounds not described in the main text (PDF)

SMILES data (CSV)

INTRODUCTION

The *N*-methyl-D-aspartate (NMDA) receptor is a voltage and ligand-gated ion channel allowing for nonselective cation flux when activated.¹ The channel is regulated by at least six discrete binding sites for endogenous ligands that include glutamate, glycine, Mg²⁺, polyamine, Zn²⁺, and an allosteric modulator.^{1–3} NMDA receptors are heteromers derived from three main types of subunits, named GluN1, GluN2, and GluN3, where GluN1 is obligatory. Usually, a single NMDA receptor is composed of four subunits, often two GluN1 subunits plus two GluN2 subunits. There are eight variants of the GluN1 subunit produced by alternative gene splicing. Four distinct isoforms of the GluN2 subunit are expressed in vertebrates and are referred to as GluN2A through to GluN2D. There are currently two recognized subtypes of the GluN3 subunit, GluN3A and GluN3B. NMDA receptors play a pivotal role in synaptic long-term potentiation, which has significant effects on learning and memory as well as neuroplasticity, neurodevelopment, and neuroprotection.^{1–5} NMDA receptor dysfunction, via excessive activation followed by neuronal death, is indicated in neuropsychiatric disorders, such as stroke, neuropathic pain, epilepsy, drug addiction, Alzheimer's disease, and schizophrenia,^{1–5} making NMDA receptors a valuable therapeutic target.^{1–7}

The activation of the NMDA channel requires simultaneous binding of glutamate and glycine, whereas the subsequent depolarization of the postsynaptic terminal releases Mg²⁺, thus opening the channel and allowing access to a binding site within the channel, termed the phencyclidine (PCP) binding site.^{1,2} Because the PCP binding site is only exposed when the NMDA receptor is activated, this site is of special interest. Thus, brain-penetrant ligands capable of high-affinity binding to the PCP site would allow modulation of the activated channel. Moreover, the labeling of such radioligands with a positron-emitter might provide radioligands for quantifying NMDA receptors in their active states in both healthy and diseased brains with PET.

A plethora of radioligands from various structural classes has been developed as candidates for the imaging of the open channel of the NMDA receptor in vivo.^{8,9} Notably, these radioligands include [¹¹C]MK-801 ([¹¹C]**1**),¹⁰ [¹⁸F]MEM ([¹⁸F]**2**),¹¹ [¹²³I]CNS-1261 ([¹²³I]**3**),^{12,13} [¹¹C]CNS-5161 ([¹¹C]**4**),¹⁴ [¹⁸F]GE-179 ([¹⁸F]**5**),^{15,16} and recently [¹⁸F]PK-209 ([¹⁸F]**6**)^{17,18} (Chart 1). All current radioligands appear to suffer from at best low specific binding, whereas many others suffer from low brain uptake, high nonspecific binding, or confounding metabolism.^{8,9} Among these radioligands, members of the *N,N'*-diarylguanidine class have shown the highest affinity,^{12–20} and members of the *N*-(1-naphthyl)-*N'*-aryl-*N'*-methylguanidine subclass the most potential for molecular imaging.^{12,13,19,20} This is illustrated with the radioligand [¹²³I]**3**. SPECT studies with [¹²³I]**3**^{21–23} have displayed perhaps the best evidence of an NMDA-specific signal in vivo, albeit a small signal. [*N-Methyl*-¹¹C]**3** is, however, ineffective as a radioligand for imaging with PET, probably due to rapid deiodination in vivo to troublesome brain-penetrant radiometabolites.²⁴

Recently, we have shown that the 3-iodo substituent in **3** may be replaced with a trifluoromethyl substituent (**7**, Table 1) with an almost 2-fold improvement in binding

affinity at the PCP binding site.²⁰ Higher affinity PET radioligands with moderate lipophilicity are sought to improve the prospects of attaining a higher PCP-site specific signal in vivo. In this study, we sought to discover higher affinity ligands that would be amenable to labeling with carbon-11 ($t_{1/2}$ = 20.4 min) or fluorine-18 ($t_{1/2}$ = 109.8 min) for PET imaging, based on *N'*-3-trifluoromethyl members of the *N*-aryl-*N'*-methylguanidine class of ligands, and especially a subclass with naphthalen-1-yl as the *N*-aryl group. Here, we report the syntheses and structure–affinity relationships for a large set of such compounds. Several high affinity ligands were discovered and four with low nanomolar affinity. Among these ligands, 1-(6-fluoro-3-(trifluoromethyl)phenyl)-1-methyl-3-(naphthalen-1-yl)guanidine (**13**) was identified as particularly promising for PET radioligand development because of its exceptionally high affinity for the PCP binding site, moderate lipophilicity, and feasibility for labeling with either carbon-11 or fluorine-18.

RESULTS AND DISCUSSION

Ligand Design

Our initial lead for this SAR study was **7** (Table 1),²⁰ a member of the *N*-(1-naphthyl)-*N'*-aryl-*N'*-methylguanidine class of compounds, and a 3-trifluoromethyl analogue of the 3-iodo compound **3**. We had found that replacement of the *N'*-3-iodo substituent of **3** with an *N'*-3-trifluoromethyl substituent gave nearly a 2-fold increase in binding affinity at the PCP binding site.²⁰ Retention of this 3-trifluoromethyl substituent in further analogues was seen as attractive for two main reasons. First, an aryl trifluoromethyl group is generally considered metabolically stable and would also evade the deiodination encountered in vivo with [¹²³I]**3**. Second, ligands bearing aryl trifluoromethyl groups are now plausible targets for labeling with fluorine-18 by recently developed no-carrier-added methods.^{25–29} Moreover, we aimed to retain a *N'*-methyl group, as this provides a site that is readily labeled with carbon-11.^{14,17,24}

Chemistry

The two primary components in the synthesis of *N,N',N'*-trisubstituted guanidines are a cyanamide and an amine hydrochloride. Two pathways for the synthesis of the cyanamides were used (Scheme 1). One pathway (A) is a single-step cyanation of the requisite arylamine with cyanogen bromide³⁰ and the other (B) a three-step process through a thiourea intermediate.^{31,32} The single-step pathway has several drawbacks, including moderate to low yields, tolerance of only a narrow range of substrates, and the need to handle noxious cyanogen bromide. The second pathway came to be preferred. This pathway tolerated a wider range of substrates and was overall much higher yielding. Also, the use of cyanogen bromide was avoided, and intermediates readily crashed out of solution, thus avoiding solvent-demanding flash chromatography. Once the desired *N*-arylcyanamides had been synthesized, they were either used in situ or converted into their corresponding *N*-methyl-*N*-arylcyanamides via alkylation with methyl iodide.³⁰

The (trifluoromethyl)arylamine hydrochlorides, required for coupling to the cyanamides, were either used directly or converted into their corresponding *N*-methylamine hydrochlorides (Scheme 2). Several methods were explored to prepare the *N*-methylamine

hydrochlorides. Direct alkylations of the amines with methyl iodide were initially attempted but resulted in large amounts of dimethylated byproducts, even when setting the stoichiometry of the reaction to use 0.90 equiv of methyl iodide. In order to prevent dimethylation, the use of a protecting group (e.g., *N*-boc or *N*-acetyl) was explored, but then the protection and deprotection steps became onerous. Finally, a one-pot reaction using trimethylorthoformate and catalytic amounts of sulfuric acid³³ was explored and successfully afforded all of the *N*-methylamine hydrochlorides with only limited amounts (<10%) of accompanying dimethylated byproduct.

N,N',N'-Trisubstituted guanidines were generally synthesized in one of two ways (Scheme 3), either by (A) heating an amine hydrochloride with the requisite *N*-methyl-*N*-arylcyanamide³⁰ or (B) by heating an *N*-arylcyanamide with the requisite *N*-methylamine hydrochloride.³³ In general, the latter method gave higher yields. For example, **7** was obtained by treating 1-naphthylamine hydrochloride with *N*-methyl-*N*-(3-(trifluoromethyl)phenyl)cyanamide at 130 °C in toluene in 16% yield but in over 2-fold higher yield (35%) by treating *N*-(naphthalen-1-yl)cyanamide with *N*-methyl-3-(trifluoromethyl)aniline hydrochloride under the same conditions.²⁰

Binding Studies

In vitro binding assays for the PCP site of the NMDA receptor were performed with rat brain membrane suspensions with [³H]*N*-(1-[thienyl]cyclohexyl)piperidine ([³H]TCP) as radioligand.²⁰ Initially, the percent inhibition of [³H]TCP binding was measured with test ligand at 1 μ M concentration. Usually, if the inhibition exceeded ~ 89%, a K_i value was determined, also with [³H]TCP as radioligand. Selected compounds showing high affinity (low K_i) versus [³H]TCP as the radioligand also had their K_i values measured with [³H]**1** as the radioligand.

Structure–Affinity Relationships

Initially, we explored whether prevention of the rotation of the *N'*-(3-(trifluoromethyl)phenyl) group would promote higher PCP binding site affinity by preparing the cyclized compounds **8** and **9** (Table 1). Both compounds showed lower binding affinity than **7**. The 4-trifluoromethyl compound **8** retained some binding affinity, but the 6-trifluoromethyl compound **9** lost all affinity. Therefore, a freely rotating *N'*-(3-(trifluoromethyl)-phenyl) group was deemed necessary and was retained in all newly prepared ligands.

Previous work on developing ligands from *N,N'*-biarylguanidines for the PCP binding site has shown that multiple substituents on the *N'*-aryl group may enhance binding affinity.²⁰ Therefore, we next explored whether further substitution of the *N'*-(3-(trifluoromethyl)phenyl) group would be beneficial. Initially, we investigated the addition of a fluoro substituent to minimize steric changes (Table 2). All of the prepared fluoro derivatives (**10–13**) displayed high (>60%) inhibition of [³H]TCP binding at micromolar concentration. The 6-fluoro compound **13** was found to have a higher binding affinity (K_i 13 nM vs [³H]TCP) than the previously reported²⁰ nonfluoro analogue **7** (18.3 nM) and over a

4-fold higher affinity than the next highest affinity isomer (i.e., 57.2 nM for the 5-fluoro compound, **12**). The affinity of **13** represents an almost 3-fold enhancement over that of **3**.

Analogues of the fluoro compounds were also prepared with heavier halogen substituents. All chloro (**14–17**) and bromo (**18–20**) analogues displayed moderate to high affinity (>50% inhibition) with the exception of the 4-chloro (**15**) and 4-bromo compounds (**18**; Table 2). K_i values (vs [^3H]TCP) were found to be 45.8 and 42.9 nM for the 5-bromo (**19**) and 6-bromo (**20**) compounds, respectively.

Some overall trends are apparent with respect to the effects of adding halo substituents to the N' -(3-trifluoromethyl)phenyl group of **7** on PCP binding site affinity. Thus, compounds with a halo substituent at position 5 or 6 (as in **12**, **13**, **16**, **17**, **19**, and **20**) generally show higher affinity than compounds with a halo substituent at position 2 or 4 (as in **10**, **11**, **14**, **15**, and **18**). For a halo substituent at position 6, affinity increases in the order $\text{Cl} < \text{Br} < \text{F}$, whereas at position 5 a bromo substituent gives highest affinity. The strong influence of a 6-fluoro substituent was further evident in the 4-bromo-6-fluoro-substituted compound **21**. This compound has much higher affinity than **18**, which only differs by lacking the 6-fluoro substituent.

We also explored the effects of adding a nonhalo substituent to the N' -(3-(trifluoromethyl)phenyl) group (Table 2). Among the prepared compounds, only the 4-, 5-, and 6-methyl-substituted compounds showed high binding affinities (>89% inhibition). The 5-methyl compound (**24**) has about twice the affinity (K_i 53 nM vs [^3H]TCP) of the 6-methyl compound (**25**) and about 5-fold higher affinity than the 4-methyl compound (**23**) but still 4-fold lower affinity than the 6-fluoro compound, **13**. A 2-, 4-, 5-, or 6-methoxy substituent, as in **26–29**, decreased binding affinity dramatically, as did a methylthio or ethoxy substituent at position 4 or 6 (**30–33**), or a nitrile substituent at position 4 (**34**). These substituents may influence the planarity of the anilino group. The compound with another trifluoromethyl substituent at position 5 (**35**) showed no affinity.

With N' -(3-(trifluoromethyl)-6-fluoro)phenyl identified as the preferred N' -aryl group, we began to explore structural changes to the other aryl group in **13** and its fluoro isomers. Replacement of the N -(1-naphthyl) group with an N -(2-(chloro-5-methylthiophenyl) group has been shown to be acceptable for high affinity, as in [^{18}F]**6** (Chart 1).^{17,18} Therefore, N -(2-(chloro-5-methylthiophenyl) analogues of **36–40** were prepared (Table 3). High affinity was found for the nonfluoro substituted compound (**36**). Contrary to expectation, the 6-fluoro compound **40** showed lower binding affinity than **36**. The binding affinity of **36** (14.2 nM) was found to be similar to that of **13** (13.0 nM). These results show that changes to the N -aryl side of the ligand may influence binding interactions on the opposite N' -aryl side and vice versa, thus impairing structural optimization efforts.

We next explored whether other alkylthio substituents might be preferred to the methylthio substituent in **36** (Table 4), especially as previous studies have suggested that bulkier thioether substituents might improve PCP binding site affinity.¹⁵ Among a prepared short series of linear alkylthio homologues, in which the S -alkyl group was increased up to butyl (**41–43**), binding affinity appeared lower than that for **36**. Branching of the alkyl chain had

an adverse effect on binding affinity, as seen by comparing the *S*-isopropyl compound (**44**) with the *S*-propyl compound (**42**), and the *S*-sec-butyl compound (**45**) with the *S*-butyl compound (**43**). Introduction of fluorine into the *S*-methyl substituent, as in **46**, markedly reduced affinity. By contrast, introduction of fluorine into the *S*-ethyl group, as in **47**, was almost without effect. Finally, it was found that an *S*-benzyl group, as in **48**, was well tolerated but offered no improvement over **36**.

Because of the benefit seen from replacing the methylthio substituent on the *N'*-aryl group of **5** with a trifluoromethyl substituent (**36**), we explored a similar replacement in the *N*-(2-(chloro-5-methylthiophenyl) group of **36**, as in **49**. However, this change resulted in some loss of affinity (compare **49** in Table 5 with **36** in Table 4). In attempts to improve upon the affinity of **49**, halo, methyl, or trifluoromethyl substitution was explored in the *N'*-(3-(trifluoromethyl)phenyl) group, as in compounds **50–59**. As for the *N*-(1-naphthyl) series, compounds with a 6-fluoro (**53**), 5-bromo (**54**), or 6-bromo (**55**) substituent displayed higher affinity than the nonhalo substituted compound (**49**, Table 5). However, unlike the *N*-(1-naphthyl) series, a 4-, 5-, or 6-methyl substituent was detrimental (**56–58**), as was a 5-trifluoromethyl substituent (**59**). Overall, with respect to binding affinity at the PCP binding site, substitutions in the *N'*-(3-(trifluoromethyl)-phenyl) group were best tolerated in the *N*-(1-naphthyl) series, followed by the *N*-(2-(chloro-5-(trifluoromethyl)phenyl) series and the *N*-(2-(chloro-5-methylthiophenyl) series.

The 2-chloro substituent in the *N*-(2-(chloro-5-methylthiophenyl) group of **49** was replaced with H, F, Br, Me, CF₃, or MeO in attempts to increase affinity for the PCP binding site (Table 6). Most of these compounds displayed moderately high affinity (>50% inhibition of [³H]TCP binding). The methyl (**82**), trifluoromethyl (**83**), and methoxy (**84**) analogues showed improved binding affinity over **49**. For the 2-fluoro or 2-bromo compounds, binding affinity was greatly enhanced by a 5-methyl substituent on the opposite ring (c.f., **67** vs **61**, and **78** vs **72**). For the 2-bromo compound, affinity was also enhanced by a 2-, 4-, 5-, or 6-fluoro substituent on the opposite ring (**73–76**).

Finally, replacement of the *N*-(1-naphthyl) group with another bi- or tricyclic group (1-(4-bromonaphthalen-1-yl), 1-(2,3,4*a*,8*a*-tetrahydrobenzo[*b*][1,4]dioxin-5-yl), 1-(4*a*,5,6,7,8,8*a*-hexahydronaphthalen-1-yl), or 1-(anthracen-1-yl) was explored (**85–88**) (Table 7). Each change abolished affinity. Therefore, the *N*-(1-naphthyl) group was found to be the optimal *N*-aryl substituent among those studied.

Some compounds with *K_i* values measured against [³H]TCP that were at least comparable with that of **3** (31.9 nM) also had *K_i* values measured against [³H]**1** as radioligand in order to allow direct comparison with the literature¹⁹ *K_i* value for **3** (4.65 nM vs [³H]**1** (Table 8). Four of these compounds (**13**, **19**, **20**, and **36**) were found to have low nanomolar *K_i* values that were very comparable with those of the two previously most advanced radioligands for imaging NMDA receptors in vivo, namely, [¹²³I]**3** for SPECT and [¹⁸F]**7** for PET. Ligand **13** showed the highest affinity (*K_i* = 1.29 nM), a value somewhat higher than that of ligand **7** (2.68 nM)²⁰ (Table 8).

Compound lipophilicity is an important parameter influencing PET radioligand performance, especially with regard to the ability of a compound to enter the brain adequately without excessive nonspecific binding.^{34–36} Generally, moderate lipophilicity, represented by a log *D* value in the 2–3 range, is considered desirable. The computed log *D* values for ligands **13**, **19**, **20**, and **36**, like those of **3**, **5**, and **7**, fall into this range (Table 8). Ligands **54**, **55**, **67**, **75**, **78**, and **80** showed not only much lower affinity versus [³H]**1** as radioligand but also appreciably higher computed log *D* values, which render these ligands unattractive for PET radioligand development.

CONCLUSIONS

This study identified 12 *N*-(aryl)-*N'*-(3-(trifluoromethyl))-*N'*-methylguanidines that at micromolar concentration displayed high inhibition (>90%) of [³H]TCP binding to the PCP binding site of the NMDA receptor, demonstrating that this is a potent subclass of receptor ligands. Compounds with an *N*-naphthyl group showed the greatest tolerance for structural modification in the opposed *N'*-(3-(trifluoromethyl)phenyl) group. Four ligands (**13**, **19**, **20**, and **36**) were found to have nanomolar affinity for NMDA receptors, moderate lipophilicity, as well as amenability to labeling with carbon-11 or fluorine-18 for PET imaging. These properties are similar or superior to those of [¹²³I]**3** and [¹⁸F]**5**, the only other radioligands so far considered promising for imaging the open channel of the NMDA receptor in human subjects.^{13,16} Ligand **13** has an especially favorable combination of properties for PET radioligand development.

EXPERIMENTAL SECTION

Materials and Methods

Reagents and solvents were purchased commercially and used without further purification. All reactions were performed under an inert atmosphere in oven-dried glassware and monitored by TLC on silica layers (0.2 mm; Polygram Sil G/UV254; Grace; Deerfield, IL) visualized under UV light (254 nm). Column chromatography was performed on silica gel (SiliFlash F60, 230–400 mesh; Silicycle; Quebec City, CA). Melting points were taken in open-ended capillaries with an SMP10 melting point apparatus (Stuart; Staffordshire, UK). ¹H-(400 MHz) and ¹³C-(100 MHz) NMR spectra were recorded in the solvent later indicated on an Avance 400 instrument (Bruker; Billerica, MA). Chemical shifts are given in parts per million (ppm) (δ) relative to the signal for TMS. All new compounds were analyzed by HRMS at the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois (Urbana–Champaign, IL) using a Micromass Q-ToF Ultima instrument for ESI (Waters Corp; Columbia, MD). Compound purity was determined with HPLC on a LC-20AD instrument (Shimadzu; Columbia, MD) equipped with a diode array detector (254 nm), and a Gemini C18 column (5 μ m; 110 Å; 4.6 $\times$ 250 mm; Phenomenex; Torrance, CA) eluted with MeOH/10 mM aq ammonium carbonate (4:1 v/v) at 1 mL/min. The purity of all final compounds was 95%. Chromatographic mobile phase compositions are given by volume. cLog *D* values (for pH 7.4) were calculated using ACD/Percepta Platform prediction software (ACD/Laboratories).

Preparation of Requisite Amines

2-Chloro-5-(methylthio)-aniline (89)—Et₃N (3.8 g, 38 mmol) and diphenylphosphoryl azide (7.4 g, 26.8 mmol) were added dropwise to a suspension of 2-chloro-5-(methylthio)benzoic acid (5.0 g, 24.7 mmol) in *t*-butanol (20 mL) at rt. The reaction mixture was heated to reflux for 6 h, then cooled to rt, and concentrated under reduced pressure. The residue was diluted with THF (12.5 mL) and aq HCl (10% w/v; 12.5 mL) and heated to reflux for a second time. After 16 h, the reaction mixture was cooled to rt and concentrated under reduced pressure. The crude residue was cooled to 0 °C and basified to pH ~ 12 with aq NaOH (20% w/v). The mixture was extracted with EtOAc (15 mL × 4), and the combined organic extracts were washed with water (20 mL) and dried (MgSO₄). The MgSO₄ was filtered off, and the filtrate was then concentrated under reduced pressure. The crude product was purified on a silica gel column eluted with Et₂O/hexane (4:1) to afford **89** (3.2 g, 74%) as a white solid. All characterization data agreed with literature data.³⁷

3-Amino-4-chlorobenzenethiol (90)—4-Chloro-3-nitrobenzenesulfonyl chloride (1.7 g, 6.6 mmol) was added portion-wise to a cold (0 °C) solution of tin(II) chloride dihydrate (13.5 g, 59.4 mmol) in aq HCl (37% w/v; 10 mL). The reaction mixture was then warmed to rt over 15 min and then refluxed for 1 h. The reaction mixture was cooled to rt, diluted with water (100 mL), and carefully neutralized by the slow addition of sodium bicarbonate. The aq layer was then extracted with chloroform (50 mL × 4), and the combined organic layers were dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/dichloromethane (7:3) to afford **90** (0.4 g, 40%) as a white solid. All characterization data agreed with literature data.¹⁵

5,5'-Disulfanediyldis(2-chloroaniline) (91)—4-Chloro-3-nitrobenzenesulfonyl chloride (1.7 g, 6.6 mmol) was added portion-wise to a cold (0 °C) solution of tin(II) chloride dihydrate (13.5 g, 59.4 mmol) in aq HCl (37% w/v; 10 mL). The reaction mixture was then warmed to rt over 15 min, heated at 125 °C for 3 h, and finally cooled to rt. The precipitate was filtered off and dissolved in water (85 mL). Iodine solution (50 mg/mL) was added portion-wise until complete conversion into disulfide was observed by TLC. The solution was filtered, and water was added to the precipitate. The resulting mixture was then stirred and neutralized with aq NaOH (10% w/v). The mixture was extracted with Et₂O (50 mL × 4), dried (MgSO₄), and filtered. The crude product was recrystallized from Et₂O/hexane to afford **91** (0.9 g, 71%) as a light purple solid. All characterization data agreed with literature data.³⁸

6-(Trifluoromethyl)indoline (92)—Sodium cyanoborohydride (679 mg, 10.8 mmol) was added portion-wise to a stirred solution of 6-(trifluoromethyl)indole (1.0 g, 5.4 mmol) in glacial acetic acid (10 mL) at rt. After 3 h, the reaction mixture was diluted with water (20 mL), cooled to 0 °C, and basified with aq NaOH (40% w/v). The reaction mixture was then extracted with dichloromethane (30 mL × 3). The combined organic extracts were dried (MgSO₄), filtered, and concentrated under reduced pressure to afford **92** (1.1 g, 89%) as a brown solid. All characterization data agreed with literature data.³⁹

General Method for the Preparation of *N*-Methylanilines

General Method A: Illustrated by the Synthesis of *N*-Methyl-3-

(trifluoromethyl)aniline (93).²⁰—Three drops of sulfuric acid were added to a solution of 3-(trifluoromethyl)aniline (5.0 g, 31 mmol) and trimethyl orthoformate (4.9 g, 46.5 mmol). This reaction mixture was slowly heated to 120 °C to allow MeOH to be distilled off. After 2 h of being stirred at 120 °C, the reaction mixture was heated to 170 °C. After 30 min, the reaction mixture was cooled to 100 °C, and aq HCl (10% w/v; 25 mL) was added. The reaction mixture was then refluxed for 3 h, cooled in an ice-bath to 0 °C, and then basified with aq 20% NaOH (w/v). The reaction mixture was extracted with EtOAc (30 mL × 3), dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel column with gradient elution (hexane/EtOAc; 1:0 to 1:10), to afford **93** (1.7 g, 25%) as a colorless oil: ¹H NMR (CD₃OD) δ 7.95 (br s, 1H), 7.91–7.82 (m, 3H), 3.17 (s, 3H); ¹³C NMR (CD₃OD) δ 139.4, 133.6 (q, *J*_{C-F} = 33 Hz), 132.8, 127.6 (q, *J*_{C-F} = 4 Hz), 127.4, 124.7 (q, *J*_{C-F} = 271 Hz), 120.6 (q, *J*_{C-F} = 4 Hz), 37.8; HRMS *m/z* 176.0683 [(M + H)⁺; calcd for C₈H₈F₃N, 176.0687].

Compounds **S2–S25** were also prepared by this method, as described in Supporting Information.

Preparation of Thioethers

General Method B: Illustrated by the Synthesis of 2-Chloro-5-(ethylthio)aniline

Hydrochloride (94)—Potassium carbonate (207 mg, 1.50 mmol) and ethyl iodide (195 mg, 1.30 mmol) were added sequentially to a cold (0 °C) solution of **90** (200 mg, 1.30 mmol) in DMF (5 mL). The reaction mixture was warmed to rt, stirred overnight, diluted with water (15 mL), and extracted with EtOAc (20 mL × 3). The combined organic extracts were washed with brine (20 mL × 2), dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude residue was diluted with Et₂O (5 mL). Ethereal HCl (1 M) was then added dropwise until no further precipitation occurred. The precipitate was then filtered off to afford **94** (268 mg, 96%) as a white solid: mp 171–172 °C; ¹H NMR (CD₃OD) δ 7.51(d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.34 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.05 (q, *J* = 7.2 Hz, 2H), 1.35 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (CD₃OD) δ 140.4, 131.9, 131.8, 129.5, 124.9, 123.8, 27.8, 14.4; HRMS *m/z* 188.0299 [(M + H)⁺; calcd for C₈H₁₀ClNS, 188.0301].

Compounds **S26–S31** were also prepared by this method, as described in Supporting Information.

Preparation of Requisite Cyanamides

N-(Naphthalen-1-yl)-cyanamide (95)—This was prepared from cyanogen bromide and 1-naphthylamine as previously described.²⁰

N-(3-(Trifluoromethyl)phenyl)cyanamide (96)—A solution of cyanogen bromide in acetonitrile (5 M; 3.9 mL, 19.4 mmol), diluted further in Et₂O (10 mL), was added dropwise to a cold (0 °C) solution of (3-trifluoromethyl)aniline (5.0 g, 31 mmol) in Et₂O (20 mL), refluxed for 24 h, and cooled to rt. The resultant precipitate was filtered off and washed with copious EtOAc. The washes were then combined with the filtrate and washed with cold aq

HCl (1 M; 25 mL $\times$ 2), water (25 mL), and brine (25 mL). The organic layer was dried (MgSO_4), filtered, and concentrated under reduced pressure. The resultant crude solid was recrystallized from ethanol and water to afford **96** (1.5 g, 42%) as a white solid. All characterization data agreed with literature data.⁴⁰

N-Methyl-N-(3-(trifluoromethyl)phenyl)cyanamide (97)—A suspension of **96** (1.5 g, 8.1 mmol) and sodium hydride (0.39 g, 16.2 mmol) in THF (40 mL) was refluxed for 2 h. The reaction mixture was then cooled to 0 °C, and iodomethane (2.9 g, 20.3 mmol) was added dropwise. The reaction mixture was then warmed to rt, stirred for an additional 16 h, diluted with MeOH (20 mL) and water (40 mL), and extracted with chloroform (20 mL $\times$ 3). The combined organic phases were washed with water (10 mL) and brine (10 mL), dried (MgSO_4), filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/EtOAc (3:1) to afford **97** (1.1 g, 68%) as a white solid. All characterization data agreed with literature data.⁴⁰

General Method C: Illustrated by the Three-Step Synthesis of N-(2-Bromo-5-(trifluoromethyl)phenyl)cyanamide (100)

Step 1. N-(2-Bromo-5-(trifluoromethyl)phenyl)carbamothioyl)benzamide (98): A solution of benzoyl isothiocyanate (3.4 g, 20.8 mmol) in acetone (42 mL) was added dropwise to a stirred solution of 2-bromo-5-(trifluoromethyl)aniline (5.0 g, 20.8 mmol) in acetone (100 mL) and stirred at rt for 21 h. Hexane (100 mL) was then added to the reaction mixture, and the resultant precipitate was filtered off and washed liberally with water and hexane. The crude solid was recrystallized from Et_2O and hexane to afford **98** (7.0 g, 83%) as a white solid: mp 155–156 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 12.74 (s, 1H), 11.98 (s, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.03–7.99 (m, 3H), 7.69 (tt, J = 7.6, 1.2 Hz, 1H), 7.62 (dd, J = 8.4, 1.6 Hz, 1H), 7.56 (t, J = 7.2 Hz, 2H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 180.7, 168.5, 138.0, 133.9, 133.3, 131.8, 128.8, 128.6 (q, $J_{\text{C-F}}$ = 35 Hz), 128.5, 125.3 (q, $J_{\text{C-F}}$ = 4 Hz), 124.8 (q, $J_{\text{C-F}}$ = 3 Hz), 124.2, 123.6 (q, $J_{\text{C-F}}$ = 270 Hz); HRMS m/z 402.9732 [(M + H)⁺; calcd for $\text{C}_{15}\text{H}_{10}\text{BrF}_3\text{N}_2\text{OS}$, 402.9728].

Step 2. 1-(2-Bromo-5-(trifluoromethyl)phenyl)thiourea (99): Compound **98** (7.0 g, 17.4 mmol) was added to a solution of aq NaOH (5%; 70 mL) at 90 °C, stirred at 90 °C for 20 min, and then filtered while still hot. The filtrate was then cooled to rt and acidified with aq HCl (10% w/v). Ammonium hydroxide was then added portion-wise until the pH reached 8. The resultant precipitate was filtered off and washed with water and hexane to afford **99** (4.8 g, 92%) as a white solid: mp 179–180 °C; ^1H NMR ($\text{DMSO}-d_6$) δ 9.43 (s, 1H), 8.14 (br s, 1H), 8.08 (d, J = 2.0 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.58 (br s, 1H), 7.49 (dd, J = 8.4, 2.0 Hz, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 182.1, 138.6, 133.7, 128.0 (q, $J_{\text{C-F}}$ = 32 Hz), 125.8 (q, $J_{\text{C-F}}$ = 4 Hz), 124.1, 123.6 (q, $J_{\text{C-F}}$ = 271 Hz), 123.5 (q, $J_{\text{C-F}}$ = 4 Hz); HRMS m/z 298.9455 [(M + H)⁺; calcd for $\text{C}_8\text{H}_6\text{BrF}_3\text{N}_2\text{S}$, 298.9465].

Step 3. N-(2-Bromo-5-(trifluoromethyl)phenyl)cyanamide (100): Iodine (1.7 g, 6.7 mmol) was added slowly over 15 min to a solution of **99** (2.0 g, 6.7 mmol) and Et_3N (1.4 g, 13.4 mmol) in EtOAc (16 mL). Once the addition was complete, the reaction mixture was stirred at rt for 10 min, and then the resultant precipitate was filtered off and washed with

EtOAc. The filtrate was then combined with the EtOAc wash, and the mixture was washed with water (20 mL) and brine (20 mL), dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude product was purified on a silica gel column eluted with hexane/EtOAc (9:1) to afford **100** (1.3 g, 74%) as a white solid: mp 133–135 °C; ¹H-NMR (DMSO-*d*₆) δ 10.19 (br s, H), 7.88 (d, *J* = 8.4 Hz, H), 7.36 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.33 (d, *J* = 1.2 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 138.0, 134.8, 129.6 (q, *J*_{C-F} = 33 Hz), 123.4 (q, *J*_{C-F} = 270 Hz), 120.8 (q, *J*_{C-F} = 4 Hz), 114.2, 112.8 (q, *J*_{C-F} = 4 Hz), 111.1; HRMS *m/z* 263.9507 [(M + H)⁺; calcd for C₈H₄BrF₃N₂, 263.9510].

Compounds **S32–S37** were also prepared by this method, as described in Supporting Information.

Preparation of Guanidines

General Method D: Illustrated by the Synthesis of 1-Methyl-3-(naphthalen-1-yl)-1-(3-(trifluoromethyl)phenyl)guanidine (7)—Compound **93** (0.35 g, 2 mmol) was dissolved in Et₂O (5 mL) and converted into its hydrochloride salt by the dropwise addition of ethereal HCl (1 M). The resultant precipitate was filtered off, dried for 10 min, and used in the subsequent reaction. A mixture of **93** hydrochloride (212 mg, 1 mmol), **95** (185 mg, 1.1 mmol), and toluene (2 mL) was heated to 130 °C, stirred for 21 h, cooled to rt, and diluted with MeOH (5 mL). The solution was concentrated onto silica gel and purified on a silica gel column with gradient elution (EtOAc to EtOAc/MeOH, 4:1). The fractions containing the desired guanidine were collected, concentrated under reduced pressure, and applied to a second silica gel column eluted with Et₃N/EtOAc/hexanes (5:20:75) to afford **7** (132 mg, 35%) as a white solid: mp 184–185 °C; ¹H NMR (CDCl₃) δ 8.08–8.05 (m, 1H), 7.76–7.73 (m, 1H), 7.53 (br s, 1H), 7.46–7.44 (d, *J* = 8.4 Hz, 1H), 7.41–7.38 (m, 5H), 7.38–7.31 (m, 1H), 6.91 (dd, *J* = 7.24, 1.04 Hz, 1H), 3.86 (br s, 2H), 3.44 (s, 3H); ¹³C NMR (CDCl₃) δ 150.2, 146.3, 145.7, 134.8, 132.0 (q, *J*_{C-F} = 34 Hz), 130.2, 129.7, 128.9, 128.0, 126.5, 126.1, 125.2, 124.1, 123.1 (q, *J*_{C-F} = 3 Hz), 122.4 (q, *J*_{C-F} = 3 Hz), 122.3, 117.5, 38.8; HRMS *m/z* 344.1371 [(M + H)⁺; calcd for C₁₉H₁₆F₃N₃, 344.1375].

Compounds **3**, **8**, **9**, **11–20**, **22–45**, and **48–88** were also prepared by method D, as described in Supporting Information.

General Method E: Illustrated by the Two-Step Synthesis of 3-(2-Chloro-5-(fluoromethylthio)phenyl)-1-methyl-1-(3-(trifluoromethyl)phenyl)guanidine (46)

Step 1. 1,1'-(5,5'-Disulfanediy)bis(2-chloro-5,1-phenylene))bis(3-methyl-3-(3-(trifluoromethyl)phenyl)guanidine) (101): Compound **91** (407 mg, 1.28 mmol) was dissolved in Et₂O (5 mL) and converted into its hydrochloride salt by the dropwise addition of ethereal HCl (1 M). The resultant precipitate was filtered off, dried for 10 min, and used in the subsequent reaction. A mixture of **91** dihydrochloride (488 mg, 1.25 mmol), **97** (1.0 g, 5 mmol), and toluene (2 mL) was heated to 130 °C. The reaction mixture was stirred at 130 °C for 21 h, cooled to rt, and diluted with MeOH (5 mL). This solution was concentrated onto silica gel and purified on a silica gel column with gradient elution, EtOAc/MeOH (1:0, 4:1). The fractions containing the desired guanidine were collected, concentrated under reduced pressure, and applied to a second silica gel column eluted with

Et₃N/EtOAc/hexanes (5:20:75) to afford **101** (132 mg, 35%) as an off-white solid: mp 70–72 °C; ¹H NMR (CDCl₃) δ 7.58–7.50 (m, 8H), 7.32–7.30 (m, 2H), 7.09–7.06 (m, 4H), 3.93 (br s, 4H), 3.36 (s, 6H); ¹³C NMR (CDCl₃) δ 150.5, 147.3, 145.1, 136.0, 132.1 (q, *J*_{C–F} = 32 Hz), 130.6, 130.2, 130.0, 127.1, 124.0, 123.6 (q, *J*_{C–F} = 271 Hz), 123.4 (q, *J*_{C–F} = 3 Hz), 123.0 (q, *J*_{C–F} = 4 Hz), 122.9, 37.8; HRMS *m/z* 717.0859 [(M + H)⁺; calcd for C₃₀H₂₄Cl₂F₆N₆S₂, 717.0863].

Step 2. 3-(2-Chloro-5-(fluoromethylthio)phenyl)-1-methyl-1-(3-

(trifluoromethyl)phenyl)guanidine (46): Sodium borohydride (32 mg, 0.84 mmol) was added to a solution of **101** (150 mg, 0.21 mmol) and fluoroiodomethane (69 mg, 0.43 mmol) in EtOH (9 mL). The reaction mixture was heated at 60 °C for 17 h, then cooled to rt, and concentrated under reduced pressure. The crude product was purified on a silica gel column eluted with Et₃N/acetone/hexane (5:5:90) to afford **46** (49 mg, 60%) as a pale yellow oil: ¹H NMR (CDCl₃) δ 7.59 (s, 1H), 7.54–7.49 (m, 3H), 7.34 (d, *J* = 8.40 Hz, 1H), 7.15 (d, *J* = 2.40 Hz, 1H), 7.05 (dd, *J* = 8.40, 2.40 Hz, 1H), 5.77 (s, 1H), 5.64 (s, 1H), 4.05 (br s, 2H), 3.45 (s, 3H); ¹³C NMR (CDCl₃) δ 150.7, 146.9, 145.0, 133.5 (d, *J*_{C–F} = 3 Hz), 132.2 (q, *J*_{C–F} = 32 Hz), 130.6, 130.2, 130.0, 127.5, 126.4 (d, *J*_{C–F} = 2 Hz), 125.2 (d, *J*_{C–F} = 2 Hz), 123.6 (q, *J*_{C–F} = 271 Hz), 123.4 (q, *J*_{C–F} = 4 Hz), 123.0 (q, *J*_{C–F} = 4 Hz), 88.3 (d, *J*_{C–F} = 216 Hz), 39.0; HRMS *m/z* 392.0599 [(M + H)⁺; calcd for C₁₆H₁₄ClF₄N₃S, 392.0611].

Compound **47** was also prepared by method E, step 2, as described in Supporting Information.

Binding Assays

In vitro binding assays for the PCP site of the NMDA receptor were performed with rat brain membrane suspensions by Caliper Life Sciences (Hanover, MD) with [³H]TCP as radioligand or in-house with [³H]**1** as reference radioligand. For in-house assays, **1** was dissolved in Tris buffer (5 mM; pH 7.4). Stock solutions of test ligands were freshly prepared by first dissolving the ligand in EtOH. [³H]**1** solution (specific activity, 22.5 Ci/mmol; PerkinElmer; 100 μL), L-glutamate solution (50 μL), and glycine solution (50 μL) were added to each assay tube to provide final assay tube concentrations of 0.50 nM, 100 μM, and 30 μM, respectively. Each stock solution of test ligand was serially diluted in Tris buffer (5 mM; pH 7.4), and an aliquot (100 μL) was added to each assay tube to provide the following final assay tube concentrations: 0.001, 0.01, 0.03, 0.10, 0.30, 1.0, 3.0, 10, 30, 100, 1000, and 10000 nM. After the addition of the rat brain homogenate preparation (700 μL; ~150 μg/mL tissue final concentration), the mixtures were incubated at 23 °C for 2 h. Nonspecific binding was determined in the presence of **1** (30 μM; 100 μL). One or two competitive binding assays were performed in triplicate. Each test ligand was assayed once along with control samples to determine the *K*_D of **1** with GraphPad Prism using “One-Site Homologous Fitting”. The percent inhibitions for each of the test ligands and in some cases the *K*_i values were determined with GraphPad Prism version 5 for Windows (GraphPad Software; San Diego, CA, USA) with “One Site-Fit log IC₅₀” and “One Site-Fit Ki” curve-fitting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PCP	phencyclidine
SAR	structure–affinity relationship
TCP	<i>N</i> -(1-[thienyl]cyclohexyl)piperidine

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Scheme 1. Synthesis of Arylcyanamides and *N*Methylcyanamides^a

^aReagents and conditions: (i) cyanogen bromide, Et₂O, reflux; (ii) sodium hydride, methyl iodide, THF, reflux; (iii) benzoyl isothiocyanate, acetone, rt; (iv) 5% aq NaOH, 90 °C; (v) I₂, Et₃N, EtOAc, rt.

Scheme 2. Synthesis of *N*-Methyl-(trifluoromethyl)arylamine Hydrochlorides^a

^aReagents and conditions: (i) (a) (1) trimethylorthoformate, H₂SO₄, 120 °C; (2) 170 °C; (3) 10% aq HCl, reflux; (b) Et₂O/HCl.

Scheme 3. Synthesis of *N,N',N'*-Trisubstituted Guanidines^a

^aReagents and conditions: (i) toluene, 130 °C.

Chart 1.
Some Prominently Studied Radioligands for the NMDA Receptor

Table 1Inhibition of [^3H]TCP Binding at the PCP Binding Site by **7** and Structurally Rigid Analogues

ligand	R ¹	R ²	inhibition (%) ^a
7 ^b			107
8	CF ₃	H	61.5
9	H	CF ₃	-4.5

^aAt 1.0 μM (mean from two experiments).^bFrom ref 20.

Table 2

Inhibition of [^3H]TCP Binding at the PCP Binding Site by **3** and Substituted *N*-(1-Naphthyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines and Selected K_i Values

ligand	R	inhibition (%) ^a	[^3H]TCP K_i (nM) ^{b, c}
3 ^d		106	31.9
7 ^d	2-H	107	18.3
10	2-F	74.2	
11	4-F	62.1	
12	5-F	82.7	57.2
13	6-F	91.0	13.0
14	2-Cl	54.1	
15	4-Cl	0.1	
16	5-Cl	87.9	
17	6-Cl	61.2	
18	4-Br	21.6	
19	5-Br	98.2	45.8
20	6-Br	99.8	42.9
21	4-Br, 6-F	75.4	
22	2-Me	41.2	
23	4-Me	89.4	256
24	5-Me	104	53
25	6-Me	93.8	104
26	2-OMe	41.0	
27	4-OMe	3.5	
28	5-OMe	-1.0	
29	6-OMe	1.9	
30	4-SMe	31.4	
31	6-SMe	7.9	
32	4-OEt	35.5	
33	6-OEt	5.5	
34	4-CN	0.2	
35	5-CF ₃	-0.2	

^a At 1.0 μM (the mean from two experiments).

^b Binding affinities measured for compounds with >89% inhibition and **12**.

^cThe mean from two experiments.

^dData from ref 20.

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Table 3

Inhibition of [³H]TCP Binding at the PCP Site Binding Site by Substituted *N*-(2-(Chloro-5-methylthiophenyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines and *K_i* Value for 36

ligand	R	inhibition (%) ^a	[³ H]TCP <i>K_i</i> (nM) ^{b, c}
36	2-H	96.1	14.2
37	2-F	51.4	
38	4-F	21.1	
39	5-F	43.2	
40	6-F	73.6	

^a At 1.0 μ M (mean from two experiments).

^b Binding affinity for the compound with >90% inhibition.

^c The mean from two experiments.

Table 4

Inhibition of [^3H]TCP Binding at the PCP Binding Site by Substituted *N*-(2-(Chloro-5-alkylthiophenyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines

ligand	R	inhibition (%) ^a
36 ^b	Me	96.1
41	Et	86.4
42	Pr	62.1
43	Bu	82.7
44	iPr	22.6
45	^s Bu	48.8
46	CH ₂ F	57.5
47	CH ₂ CH ₂ F	82.1
48	Bn	88.6

^a At 1.0 μM (mean from two experiments).

^b From Table 3; shown for ease of comparison.

Table 5

Inhibition of [^3H]TCP Binding at the PCP Binding Site by Substituted *N*-(2-(Chloro-5-(trifluoromethyl)phenyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines

ligand	R	inhibition (%) ^a
49		71.2
50	2-F	-8.7
51	4-F	21.1
52	5-F	43.2
53	6-F	84.8
54	5-Br	99.0
55	6-Br	93.0
56	4-Me	42.3
57	5-Me	66.4
58	6-Me	38.0
59	5-CF ₃	46.2

^a At 1.0 μM (the mean from two experiments).

Table 6

Inhibition of [^3H]TCP Binding at the PCP Binding Site by Substituted *N*-(5-(Trifluoromethyl)phenyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines

ligand	X	R	inhibition (%) ^a
60	H		53.9
61	F		59.4
62	F	2-F	36.2
63	F	4-F	24.9
64	F	5-F	20.9
65	F	6-F	44.5
66	F	4-Me	39.1
67	F	5-Me	95.3
68	F	6-Me	33.4
69	F	5-Br	27.7
70	F	6-Br	30.2
71	F	5-CF ₃	34.0
72	Br		62.0
73	Br	2-F	71.0
74	Br	4-F	89.1
75	Br	5-F	98.0
76	Br	6-F	72.4
77	Br	4-Me	31.3
78	Br	5-Me	99.0
79	Br	6-Me	85.5
80	Br	5-Br	99.0
81	Br	6-Br	93.4
82	Me		85.8
83	CF ₃		89.0
84	OMe		79.6

^a At 1.0 μM (mean from two experiments).

Table 7

Inhibition of [^3H]TCP Binding at the PCP Binding Site by *N*-(Polycyclyl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines

Ligand	Ar	Inhibition(%) ^a
85		-2.6
86		3.8
87		8.1
88		-7.3

^a At 1.0 μM (the mean from two experiments).

Table 8

Affinities and Computed Lipophilicities of Selected *N*-(Aryl)-*N'*-(3-(trifluoromethyl)phenyl)-*N'*-methylguanidines at the PCP Binding Site Measured with [³H]1

ligand	[³ H]1 <i>K_i</i> ^a (nM)	clogD ^a
3	1.65 ± 0.16 ^b	2.72
5	0.91 ± 0.05 ^b	2.58
7	2.68 ± 0.26 ^b	2.23
13	1.29 ± 0.03 ^b	2.27
19	3.42 ± 0.82 ^c	2.73
20	4.7 ± 0.4 ^c	2.86
36	3.25 ± 0.63 ^c	2.79
54	62 ± 11 ^b	4.07
55	233 ± 43 ^b	4.30
67	243 ± 82 ^b	3.06
75	49 ± 16 ^b	3.49
78	29 ± 4 ^b	3.54
80	43 ± 23 ^b	4.26

^aComputed with ACD Laboratories software.

^bMean ± SD from 3 experiments.

^cMean ± SD from 6 experiments.