Published in final edited form as:
J Nat Prod. 2010 March 26; 73(3): 306–312. doi:10.1021/np9006124.

Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 3′-(Substituted Phenyl)epibatidine Analogues. Nicotinic Partial Agonists⊥

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Abstract

In 1992, John Daly et al. reported the isolation and structure determination of epibatidine. Epibatidine's unique structure and its potent nicotinic agonist activity have had a tremendous impact on nicotine receptor research. This research has led to a better understanding of the nicotinic acetylcholine receptor (nAChR) pharmacophore and to epibatidine analogues with potential as pharmacotherapies for treating various CNS disorders. In this study, we report the synthesis, receptor binding ([^{3}H]epibatidine and [^{125}I]iodoMLA), and in vivo pharmacological properties (mouse tail flick, hot plate, hypothermia, and spontaneous activity) of a series of 3′-(substituted phenyl) epibatidine analogues (**5a–m**). Results from these studies have added to the understanding of the nAChR pharmacophore and led to nicotinic partial agonists that may have potential for smoking cessation. All the analogues had affinities for the α4β2 nAChR similar to epibatidine (**1**). 3′-(3-Dimethylaminophenyl)epibatidine (**5m**) has a nicotinic partial agonist pharmacological profile similar to the smoking cessation drug varenicline. Other analogues are partial agonists with varying degrees of nicotinic functional agonist and antagonist activity. 3′-(3-Aminophenyl)epibatidine (**5j**) is a more potent functional agonist and antagonist in all tests than varenicline. 3′-(3-Fluorophenyl) epibatidine and 3′-(3-chlorophenyl)epibatidine (**5c** and **5e**) are more potent than varenicline when tested as agonists in four pharmacological tests and antagonists when evaluated against nicotine in the analgesia hot-plate test.

Since it is estimated that four million smoking-related deaths result annually from smoking-related diseases such as lung cancer, chronic obstructive pulmonary disease (COPD), and cardiovascular disease,[1] there is great interest in the development of pharmacotherapies for aiding people to stop smoking.2 In addition to nicotine (**2**) replacement therapy (NRT), the α4β2 nAChR partial agonist varenicline (**3**), and the antidepressant bupropion (**4**), which is also a noncompetitive nAChR antagonist, are the first-line treatment drugs for smoking cessation.[2–4]

Epibatidine (**1**), a structurally novel nicotinic acetylcholinergic compound, was isolated by Daly et al. from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*.[5] Its unique structure and potent nicotinic acetylcholine receptor (nAChR) activity has had a major impact

⊥Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland for his pioneering work on bioactive natural products.

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#Dr. Billy R. Martin died on June 8, 2008.

on nicotinic receptor research. A SciFinder® search on epibatidine reveals 1013 references from 1992 (original report on the isolation by Daly et al.) to September 2009. Even though epibatidine's acute toxicity limited its therapeutic potential,[6–9] it has served as a lead structure to develop pharmacotherapies for treating various CNS disorders including Alzheimer's and Parkinson's diseases, pain, schizophrenia, anxiety, depression, Tourette's syndrome, and smoking cessation.10

During the last several years, we have conducted structure-activity relationship (SAR) studies using epibatidine (**1**) as our lead structure to help characterize pharmacophores for the nAChR and to identify nAChR agonists, partial agonists, and antagonists as potential pharmacotherapies for treating smokers.[11–19] In a preliminary study, we reported that introduction of a phenyl group at the 3′-position on the 2-chloropyridine ring of epibatidine gave **5a**, which had high affinity for α4β2 nAChR but was 100–350-times less potent than epibatidine (**1**) in the mouse tail-flick, hot-plate, hypothermia, and spontaneous activity tests after acute administration.[11] The ability of **5a** to antagonize nicotine-induced antinociception was not tested in this study. In the present investigation, we report the nicotinic antagonist properties of **5a** and compare the nAChR binding and agonist/antagonist pharmacological properties of the 3′-(substituted phenyl)epibatidine analogues **5a–m** to those of the nAChR agonists, nicotine (**2**) and epibatidine (**1**), and the partial agonist varenicline (**3**). All analogues (**5a–m**) had high affinity for the α4β2 nAChR similar to epibatidine (**1**). Also like epibatidine, they also had weak affinity for the α7 nAChR. Compounds **5a–m** showed both agonist and antagonist activity in the mouse acute tail-flick, hot-plate, hypothermia, and spontaneous-activity functional tests and, thus, are partial nAChR agonists.

Results and Discussion

Chemistry

The synthesis of **5b**, **5d**, **5g**, **5i**, and **5k** is shown in Scheme 1. Palladium acetate-catalyzed coupling of *tert*-butoxycarbonyl-3′-bromoepibatidine (**6**)[14] with the appropriately substituted phenylboronic acid in dimethoxyethane (DME) in the presence of tris-(*o*-tolyl)phosphine and sodium carbonate gave the *tert*-butoxycarbonyl-protected 3′-(substituted phenyl)epibatidine analogues (**7**). Treatment of **7a–e** (X = F, Cl, NO_2, *t*BocNH, and CH_3O) with trifluoroacetic acid in methylene chloride removed the protecting *tert*-butoxycarbonyl group and afforded the desired 3′-(substituted phenyl)epibatidine analogues, **5b**, **5d**, **5g**, **5i**, and **5k**.

Scheme 2 outlines the synthesis of **5j** and **5m** starting with the previously reported 3′-(3-nitrophenyl)epibatidine (**5h**).[19] Reduction of **5h** with iron powder in ethanolic hydrogen chloride gave the desired **5j**. In order to prepare **5m**, **5h** was first converted to the *tert*-butoxycarbonyl-protected **8** using *tert*-butoxycarbonyl anhydride catalyzed by dimethylaminopyridine (DMAP) in methylene chloride containing a small amount of triethylamine. Reduction of **8** with nickel borohydride and hydrochloric acid in methanol provided the amino compound **9**, which was reductively methylated to the 7-*tert*-butoxycarbonyl-protected dimethylamino compound **10** using sodium cyanoborohydride and formaldehyde in acetonitrile. Treatment of **10** with trifluoroacetic acid in methylene chloride afforded **5m**. Target compounds **5c**, **5e**, and **5f** were all prepared from **5j** using various diazotization procedures (Scheme 3). Thus, diazotization of **5j** using sodium nitrite in 70% hydrogen fluoride-pyridine yielded **5c** and diazotization of **5j** using *n*-butyl nitrite with cuprous chloride or cuprous bromide in acetonitrile afforded **5e** and **5f**, respectively.

Biological Activity

The nAChR binding affinities and the functional nicotinic pharmacological properties of several 3′-(substituted phenyl)epibatidine analogues were determined. The K_i values for the

inhibition of [^{3}H]epibatidine and [^{125}I]iodoMLA binding at the α4β2 and α7 nAChRs, respectively, for compounds **5a–m** along with reference compounds (+)-and (−)-epibatidine [(+)-**1** and (−)-**1**], nicotine (**2**), and varenicline (**3**), are listed in Table 1. (+)-and (−)-Epibatidine with K_i values of 0.026 and 0.018 nM, respectively, have very similar affinities for the α4β2 nAChR.[15] Nicotine and varenicline have K_i values of 1.5 and 0.12 nM, respectively. Since the affinities of the epibatidine isomers are so similar, the (substituted phenyl)epibatidine analogues **5a–m** are only compared to the natural epibatidine isomer. The unsubstituted phenyl analogue **5a** has a K_i of 0.021 nM at the α4β2 nAChR, which is almost identical to that of epibatidine (K_i = 0.026 nM), and has 71- and 6-times higher affinity at the α4β2 nAChR than nicotine and varenicline, respectively. Substitution of the 3′-phenyl ring of **5a** with a 4- or 3-position electron-withdrawing or -releasing substituent had only small effects on binding affinity at the α4β2 nAChR. The K_i values varied from 0.008 and 0.009 nM for the 3-nitrophenyl (**5h**) and 3-dimethylamino (**5m**) analogues to 0.034 and 0.039 nM for the 4-aminophenyl (**5i**) and the 4-chlorophenyl (**5d**) analogues. In every case the 3′-(3-substituted phenyl) analogue had a slightly lower K_i value than the corresponding 3′-(4-substituted phenyl) analogue (compare **5c**, **5e**, **5h**, **5j**, and **5l** to the corresponding **5b**, **5d**, **5g**, **5i**, and **5k**). Similar to epibatidine, all analogues had relatively weak affinity for the α7 nAChR. The α7 nAChR K_i values varied from 30.5 nM for **5e** to 1100 nM for **5m**, compared to a K_i of 198 nM for epibatidine and 32.5 nM for varenicline. The 4-chlorophenyl analogue **5e** with a K_i of 30.5 nM had the highest affinity for the α7 nAChR but was still greater than 2000-fold selective for α4β2 nAChR relative to α7 nAChR.

Natural epibatidine has an ED_{50} of 0.0061 and 0.004 mg/kg in the tail-flick and hot-plate antinociception tests.[15] The unnatural epibatidine isomer has an ED50 of 0.0066 in the tail-flick test.[15] Epibatidine also has ED_{50} values of 0.004 and 0.001 mg/kg in the hypothermia and spontaneous-activity test. The ED_{50} values for nicotine (**2**) in the tail-flick, hot-plate, hypothermia, and spontaneous activity tests are 1.3, 0.65, 1.0, and 0.5 mg/kg, respectively. Varenicline (**3**) is inactive in the tail-flick and hot-plate tests but has ED_{50} values of 2.8 and 2.1 mg/kg in the hypothermia and spontaneous-activity tests. In addition, varenicline (**3**) antagonizes the nicotine-induced antinociception in the tail-flick and hot-plate tests with AD_{50} values of 0.2 and 470 μg/kg, respectively.[19]

With the exception of the 3-dimethylaminophenyl analogue **5m**, which had a profile like varenicline, all compounds showed weak agonists activity compared to epibatidine in the tail-flick, hot-plate, hypothermia, and spontaneous-activity tests. The 3-aminophenyl analogue **5j** with ED_{50} values of 0.34, 0.3, 0.31, and 0.03 mg/kg in the tail-flick, hot-plate, and hypothermia tests was the most potent analogue; however, this potency is 57-, 75-, 76- and 30-times weaker than epibatidine (**1**) in these four tests. The agonist potencies of these analogues were more similar to nicotine (**2**) than epibatidine. Similar to varenicline (**3**), **5m** did not have agonist activity in the tail-flick and hot-plate tests but had ED_{50} values of 3.3 and 2.0 mg/kg in the hypothermia and spontaneous-activity tests, respectively, compared to ED_{50} values of 2.8 and 2.1 mg/kg for varenicline (**3**) in these two tests. Most analogues did not exhibit pharmacological selectivity. For the most part they all produced similar potencies in all four tests. The most variation was in the spontaneous-activity test. For example, the 3-aminophenyl analogue **5j** was approximately an order of magnitude more potent in this test than in the other three tests.

The high binding affinity of compounds **5a–m** for α4β2 nAChRs combined with the relatively weak agonist potency suggested that these analogues might act as nAChR functional antagonists in vivo. Indeed, all analogues were potent antagonists with AD_{50} values of 0.14–20 μg/kg in the tail-flick test compared to 0.2 μg/kg for the partial agonist varenicline (**3**). In addition, the 3′-phenyl and 3′-(substituted phenyl) analogues **5a**, **5c**, **5e, 5h**, **5j**, and **5m**, respectively, were also potent antagonists in the hot-plate test with AD_{50} values of 26–560 μg/

kg compared to 470 μg/kg for varenicline (**3**). The three most potent analogues were the 3-aminophenyl (**5j**), 3-chlorophenyl (**5e**), and 3-fluorophenyl (**5c**), with AD_{50} values of 26, 80, and 86 μg/kg, respectively, which is 18-, 5.9-, and 5.5-times more potent as an antagonist than varenicline in the hot-plate test.

In summary, the addition of a 3′-phenyl or electron-withdrawing or -releasing 3′-(3- or 4-substituted phenyl) group to the highly potent nAChR agonist epibatidine (**1**) provided a series of analogues **5a–m**. Like epibatidine these compounds had high affinity for the α4β2 nAChR and weak affinity for the α7 nAChR. In contrast to the high potency of epibatidine (**1**), these analogues had agonist potency in the tail-flick, hot-plate, hypothermia, and spontaneous-activity tests in mice more like that of nicotine (**2**). On the other hand, like varenicline, these analogues were potent antagonists in the tail-flick test and to a lesser degree in the hot-plate test. Thus, similar to varenicline, these analogues are functional nAChR partial agonists in vivo. The 3-dimethylaminophenyl analogue **5m** has an agonist/antagonist profile most like varenicline. The 3-aminophenyl analogue **5j** is a more potent functional agonist and antagonist in all tests than varenicline. The 3-fluorophenyl and 3-chlorophenyl analogues **5c** and **5e**, respectively, are more potent than varenicline in all four agonist tests and the antagonist hot-plate test. Thus, compounds **5c**, **5e**, **5j**, and **5m** represent exciting lead structures for developing a new structural class of nicotinic partial agonists useful in the treatment of nicotine addiction (smokers) and possibly other CNS diseases and disorders.

Experimental Section

General Experimental Procedures

Melting points were determined on a Mel-temp (Laboratory Devices, Inc.) capillary tube apparatus. NMR spectra were recorded on a Bruker Avance 300 using tetramethylsilane as internal standard. Thin-layer chromatography was carried out on Whatman silica gel 60 plates. Visualization was accomplished under UV or in an iodine chamber. Microanalysis was carried out by Atlantic Microlab, Inc. Flash chromatography was carried out using silica gel 60 (230–400 mesh) using various solvent mixtures. CMA is 80% chloroform, 18% methanol, and 2% concentrated ammonium hydroxide.

The [^{3}H]epibatidine was purchased from Perkin Elmer Inc. (Boston, MA). The [^{125}]iodo-MLA was synthesized as previously reported.[20]

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(4-fluorophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (7a)

To a resealable reaction tube were added compound **6** (231 mg, 0.6 mmol), 4-fluorophenylboronic acid (168 mg, 1.2 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), tris(*o*-tolyl) phosphine (37 mg, 0.12 mmol), and Na_2CO_3 (159 mg, 1.5 mmol) in DME (2 mL) and H_2O (0.5 mL). The mixture was purged with argon, sealed, and heated in an 85 °C oil bath overnight. The reaction mixture was cooled, filtered through celite, and diluted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the resulting residue on a silica gel column using EtOAc-hexanes (1:1) yielded 217 mg (90%) of **7a** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.5–1.9 (5H, m), 2.02 (1H, m), 2.90 (1H, dd, J = 4.5, 8.7 Hz), 4.23 (1H, brs), 4.39 (1H, brs), 7.1–7.2 (2H, m), 7.4–7.5 (2H, m), 7.64 (1H, m), 8.27 (1H, m); ^{13}C NMR ($CDCl_3$) δ 28.6, 29.2, 30.1, 40.8, 45.2, 56.2, 62.2, 80.2, 115.6 (d, J_{CF} = 21.0 Hz), 131.5 (d, J_{CF} = 8.7 Hz), 135.9, 138.5, 140.9, 147.8, 155.2, 163.0 (d, J_{CF} = 247.7 Hz).

3′-(4-Fluorophenyl)epibatidine (5b) Hydrochloride

Compound **7a** (217 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (3 mL). TFA (3 mL) was added dropwise at 0 °C over 30 min. The mixture was stirred at room temperature for 4 h, poured

into a cold solution of conc. NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography of the resulting residue on a silica gel column using CH_2Cl_2-MeOH yielded 136 mg (83%) of **5b**. ^{1}H NMR ($CDCl_3$) δ 1.5–1.7 (5H, m), 1.94 (1H, dd, *J* = 9.0, 12.0 Hz), 2.82 (1H, dd, *J* = 5.1, 9.0 Hz), 3.62 (1H, brs), 3.81 (1H, brs), 7.1–7.2 (2H, m), 7.4–7.5 (2H, m), 7.76 (1H, d, *J* = 2.4 Hz), 8.29 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 30.2, 31.7, 40.6, 44.8, 56.8, 63.1, 115.6 (d, J_{CF} = 21.1 Hz), 131.6 (d, J_{CF} = 8.7 Hz), 135.7, 138.9, 141.8, 147.5, 148.0, 163.0 (d, J_{CF} = 247.8 Hz).

Compound **5b** (136 mg, 0.45 mmol) was dissolved in MeOH (4.6 mL) at room temperature. HCl (1 M in ether, 4.6 mL) was added. After stirring for 30 min, the solvent was removed, and the residue was recrystallized from a MeOH-ether mixture to give **5b**•HCl as a yellow solid; mp >200 °C (dec); *anal*. C 60.06%, H 5.16%, N 8.07%, calcd for $C_{17}H_{17}Cl_2FN_2$, C 60.19%, H 5.05%, N 8.26%.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(4-chlorophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (7b)

To a resealable reaction tube were added compound **6** (233 mg, 0.6 mmol), 4-chlorophenylboronic acid (188 mg, 1.2 mmol), Pd(OAc) (14 mg, 0.06 mmol), tris(*o*-tolyl) phosphine (37 mg, 0.12 mmol), and Na_2CO_3 (159 mg, 1.5 mmol) in DME (2 mL) and H_2O (0.5 mL). The mixture was purged with argon, sealed, and heated in an 85 °C oil bath overnight. The reaction mixture was cooled, filtered through celite, and diluted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the resulting residue on a silica gel column using EtOAc-hexanes (1:1) yielded 247 mg (98%) of **7b** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.5–1.9 (5H, m), 2.02 (1H, dd, *J* = 9.0, 12.3 Hz), 2.92 (1H, dd, *J* = 4.8, 9.0 Hz), 4.22 (1H, brs), 4.38 (1H, brs), 7.3–7.5 (4H, m), 7.63 (1H, d, *J* = 2.7 Hz), 8.28 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 28.6, 29.2, 30.0, 40.8, 45.2, 56.3, 62.2, 80.2, 128.9, 131.1, 131.5, 135.9, 138.4, 141.0, 147.9, 155.2.

3′-(4-Chlorophenyl)epibatidine (5d) Hydrochloride

Compound **7b** (247 mg, 0.59 mmol) was dissolved in CH_2Cl_2 (3 mL). TFA (3 mL) was added dropwise at 0 °C over 30 min. The mixture was stirred at room temperature for 3 h, poured into a cold solution of NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography of the resulting residue on a silica gel column using CH_2Cl_2-MeOH yielded 147 mg (78%) of **5d** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.5–1.7 (5H, m), 1.94 (1H, dd, *J* = 9.0, 12.0 Hz), 2.82 (1H, dd, *J* = 5.1, 9.0 Hz), 3.62 (1H, brs), 3.80 (1H, brs), 7.3–7.5 (4H, m), 7.76 (1H, d, *J* = 2.4 Hz), 8.30 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 30.4, 31.7, 40.6, 44.8, 56.8, 63.1, 128.6, 128.8, 129.0, 131.1, 131.2, 131.5, 138.9, 141.8, 148.1.

Compound **5d** (147 mg, 0.46 mmol) was dissolved in MeOH (4.6 mL) at room temperature. HCl (1 M in ether, 4.6 mL) was added with a syringe pump over 50 min at room temperature. After stirring for 30 min, the solvent was removed. The residue was recrystallized from a MeOH-ether mixture to give **5d**•HCl as a yellow solid; mp >200 °C (dec.); *anal*. C 53.73%, H 4.90%, N 6.81%, calcd for $C_{17}H_{17}Cl_3N_2$•1.5 H_2O, C 53.35%, H 5.27%, N 7.32%.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(4-nitrophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (7c)

To a resealable reaction tube were added compound **6** (233 mg, 0.6 mmol), 4-nitrophenylboronic acid (200 mg, 1.2 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), tris(*o*-tolyl) phosphine (37 mg, 0.12 mmol), and Na_2CO_3 (159 mg, 1.5 mmol) in DME (2 mL) and H_2O (0.5 mL). The mixture was purged with argon, sealed, and heated in an 85 °C oil bath overnight. The reaction mixture was cooled and diluted with EtOAc. The organic phase was washed with

brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the resulting residue on a silica gel column using EtOAc-hexanes (1:1) yielded 239 mg (93%) of **7c** as a yellowish oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.5–1.9 (5H, m), 2.06 (1H, m), 2.97 (1H, dd, *J* = 4.5, 8.7 Hz), 4.24 (1H, brs), 4.40 (1H, brs), 7.6–7.7 (2H, m), 7.70 (1H, d, *J* = 2.4 Hz), 8.25–8.35 (2H, m), 8.35 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 28.6, 29.2, 30.0, 40.8, 45.1, 56.3, 62.2, 80.3, 123.8, 124.2, 130.8, 131.1, 138.3, 141.3, 144.4, 149.4, 155.2.

3′-(4-Nitrophenyl)epibatidine (5g) Hydrochloride

Compound **7c** (239 mg, 0.56 mmol) was dissolved in CH_2Cl_2 (3 mL). TFA (3 mL) was added dropwise at 0 °C over 30 min. The mixture was stirred at room temperature for 4 h, poured into a cold solution of NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography of the resulting residue on a silica gel column using CH_2Cl_2-MeOH yielded 126 mg (69%) of **5g** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.5–1.7 (5H, m), 1.97 (1H, dd, *J* = 9.0, 12.0 Hz), 2.72 (1H, brs), 2.85 (1H, dd, *J* = 5.1, 9.0 Hz), 3.66 (1H, brs), 3.84 (1H, m), 7.6–7.7 (2H, m), 7.86 (1H, d, *J* = 2.4 Hz), 7.25–7.35 (2H, m), 8.37 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 30.4, 31.7, 40.6, 44.7, 56.9, 63.2, 123.9, 130.9, 134.7, 138.8, 142.0, 144.6, 149.1.

Compound **5g** (126 mg, 0.38 mmol) was dissolved in MeOH (4 mL) at room temperature. HCl (1 M in ether, 4 mL) was added with a syringe pump over 50 min at room temperature. After stirring for 30 min, the solvent was removed. The residue was recrystallized from MeOH-ether to give **5g**•HCl as a yellow solid; mp 168–169 °C; *anal*. C 54.95%, H 4.78%, N 11.14%, calcd for $C_{17}H_{17}Cl_2N_3O_2$•0.25 H_2O, C 55.07%, H 4.76%, N 11.33%.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(4-*tert*-butoxylcarbonylaminophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (7d)

To a resealable reaction tube were added compound **6** (231 mg, 0.6 mmol), 4-(*N*-boc-amino) phenylboronic acid (284 mg, 1.2 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), tris(*o*-tolyl)phosphine (37 mg, 0.12 mmol), and Na_2CO_3 (159 mg, 1.5 mmol) in DME (2 mL) and H_2O (0.5 mL). The mixture was purged with argon, sealed, and heated in an 85 °C oil bath overnight. The reaction mixture was cooled, filtered through celite, and diluted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the resulting residue on a silica gel column using EtOAc-hexanes (1:1) yielded 285 mg (96%) of **7d**. ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.53 (9H, s), 1.5–1.9 (5H, m), 2.06 (1H, m), 2.90 (1H, dd, *J* = 4.8, 9.0 Hz), 4.22 (1H, brs), 4.38 (1H, brs), 6.78 (1H, brs), 7.3– 7.5 (4H, m), 7.62 (1H, d, *J* = 2.4 Hz), 8.24 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 28.6, 28.7, 29.1, 30.1, 40.7, 45.3, 56.3, 62.2, 80.2, 81.1, 118.4, 130.4, 132.4, 136.4, 138.5, 138.9, 140.8, 147.3, 147.8, 153.0, 155.3.

3′-(4-Aminophenyl)epibatidine (5i) Dihydrochloride

Compound **7d** (285 mg, 0.57 mmol) was dissolved in CH_2Cl_2 (3 mL). TFA (3 mL) was added at 0 °C over 30 min. The mixture was stirred at room temperature for 4 h, poured into a cold solution of conc. NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography of the resulting residue on a silica gel column using CH_2Cl_2-MeOH yielded 143 mg (84%) of **5i** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.5–1.7 (5H, m), 1.91 (1H, dd, *J* = 9.0, 12.0 Hz), 2.80 (1H, dd, *J* = 5.1, 9.0 Hz), 3.60 (1H, m), 3.77 (1H, m), 6.6–6.8 (2H, m), 7.2–7.3 (2H, m), 7.70 (1H, d, *J* = 2.4 Hz), 8.22 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 30.4, 31.6, 40.6, 45.0, 56.8, 63.1, 114.9, 127.8, 130.8, 136.7, 13838, 141.6, 146.9, 147.0, 147.5.

The free base **5i** (143 mg, 0.48 mmol) was dissolved in MeOH (5 mL) at room temperature. HCl (1 M in ether, 5 mL) was added with a syringe pump over 50 min at room temperature. After stirring for 30 min, the solvent was removed, and the residue was recrystallized from

MeOH-ether to give **5i**•HCl as a yellow solid; mp >265 °C (dec.); *anal*. C 50.89%, H 5.86%, N 10.14%, calcd for $C_{17}H_{20}Cl_3N_3$•1.5 H_2O, C 51.08%, H 5.80%, N 10.51%.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(4-methoxyphenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (7e)

To a resealable reaction tube were added compound **6** (231 mg, 0.6 mmol), 4-methoxyphenylboronic acid (182 mg, 1.2 mmol), $Pd(OAc)_2$ (14 mg, 0.06 mmol), tris(*o*-tolyl) phosphine (37 mg, 0.12 mmol), and Na_2CO_3 (159 mg, 1.5 mmol) in DME (2 mL) and H_2O (0.5 mL). The mixture was purged with argon, sealed, and heated in an 85 °C oil bath overnight. The reaction mixture was cooled, filtered through celite, and diluted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the resulting residue on a silica gel column using EtOAc-hexanes (1:1) yielded 223 mg (90%) of **7e** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.5–1.9 (5H, m), 2.01 (1H, dd, *J* = 9.0, 12.3 Hz), 2.91 (1H, dd, *J* = 4.8, 8.7 Hz), 3.84 (3H, s), 4.22 (1H, brs), 4.38 (1H, brs), 6.9–7.0 (2H, m), 7.3–7.4 (2H, m), 7.64 (1H, d, *J* = 2.4 Hz), 8.23 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 28.6, 29.2, 30.1, 40.7, 45.2, 55.7, 56.3, 62.2, 80.1, 114.1, 130.2, 130.9, 136.5, 138.6, 140.8, 147.2, 155.2, 160.0.

3′-(4-Methoxylphenyl)epibatidine (5k) Dihydrochloride

Compound **7e** (223 mg, 0.54 mmol) was dissolved in CH_2Cl_2 (3 mL). TFA (3 mL) was added dropwise at 0 °C over 30 min. The mixture was stirred at room temperature for 4 h, poured into a cold solution of conc. NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated to dryness. Flash chromatography of the resulting residue on a silica gel column using CH_2Cl_2-MeOH yielded 128 mg (76%) of **5k** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.5–1.7 (5H, m), 1.92 (1H, dd, *J* = 9.0, 12.3 Hz), 2.80 (1H, dd, *J* = 4.8, 8.7 Hz), 3.60 (1H, brs), 3.78 (1H, t, *J* = 3.6 Hz), 3.84 (3H, s), 6.9–7.0 (2H, m), 7.3–7.4 (2H, m), 7.74 (1H, d, *J* = 2.4 Hz), 8.26 (1H, d, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$) δ 30.1, 31.4, 40.3, 44.6, 55.3, 56.4, 62.8, 113.7, 130.1, 130.6, 136.0, 138.6, 141.4, 147.0, 147.2, 159.530.2, 31.7, 40.6, 44.8, 56.8, 63.1, 115.6 (d, *J* = 0.855 Hz), 131.6 (d, *J* = 0.33 Hz), 135.7, 138.9, 141.8, 147.5, 148.0, 161.4, 164.6.

The free base **5k** (128 mg, 0.41 mmol) was dissolved in MeOH (4 mL) at room temperature. HCl (1 M in ether, 4 mL) was added with a syringe pump over 20 min at room temperature. After stirring for 30 min, the solvent was removed, and the residue was recrystallized from a MeOH-ether mixture to give **5k**•2HCl as a yellow solid; mp >200 °C (dec.); *anal*. C 53.62%, H 5.63%, N 6.90%, calcd for $C_{18}H_{21}Cl_3N_2O$•H_2O, C 53.28%, H 5.71%, N 6.90%.

3′-(3-Aminophenyl)epibatidine (5j) Dihydrochloride

Compound **5h** (80 mg, 0.241 mmol), ethanol (2 mL), water (0.06 mL), and concentrated HCI (0.01 mL) were stirred at room temperature for 10 min. Iron powder (149.2 mg, 2.66 mmol) was added in small portions. The mixture was heated at 100 °C for 30 min, poured into a cold solution of sodium carbonate (aq. solution), and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with CMA-EtOAc (1:3) to yield 70 mg (95%) of **5j** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.48–1.70 (5H, m), 1.87–1.99 (1H, m), 2.79–2.84 (1H, dd), 3.61 (1H, brs), 3.78 (1H, brs), 6.70–6.74 (2H, m), 6.81 (1H, d, *J* = 6.0 Hz), 7.20 (1H, d, *J* = 6.0 Hz), 7.72 (s, 11:1), 8.27 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 30.3, 31.6, 40.6, 44.9, 56.8, 63.1, 115.2, 116.3, 119.9, 129.5, 137.0, 138.9, 139.2, 141.5, 146.7, 147.4, 147.7.

Compound **5j** (70 mg, 0.231 mmol) was dissolved in 4 mL of methanol, and 1 M HCI in ether (2 mL) was added. After stirring for 30 min, the solvent was removed. The residue was recrystallized from MeOH-ether (1:3) to yield **5j**•HCl as a yellow solid; mp 195 °C (dec.);

anal. C 51.08%, H 5.80%, N 10.51%, ca1cd for $C_{17}H_{20}Cl_3N_3$•1.5 H_20, C 51.14%, H 5.91%, N 9.61%.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(3-nitrophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (8)

A solution of compound **5h** (0.31 g, 0.94 mmol), *tert*-butoxycarbonyl anhydride (400 mg, 1.83 mmol), DMAP (10 mg), triethylamine (0.1 mL), and methylene chloride (5 mL) was stirred for 1 h, poured into 100 mL of 1 M K_2CO_3, and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column eluting with hexane-EtOAc (3:1) to yield 0.25 g (60%) of **8** as an oil. ^{1}H NMR ($CDCl_3$) δ 1.39 (9H, s), 1.50–1.70 (5H, m), 1.96–2.09 (1H, m), 2.93–2.97 (1H, m,), 4.23 (1H, brs), 4.39 (1H, brs), 7.64–7.70 (2H, m), 7.79–7.80 (1H, m), 8.31–8.35 (1H, m); ^{13}C NMR ($CDCl_3$) δ 27.8, 28.6 (CH_3-3), 31.3, 40.8, 45.2, 56.4, 62.3, 80.4, 123.6, 124.7, 129.8, 134.5, 135.9, 138.5, 139.6, 141.4, 147.5, 148.8, 149.1, 155.4.

7-*tert*-Butoxycarbonyl-2-*exo*-[3-(3-aminophenyl)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (9)

To a mixture of compound **8** (80 mg, 0.81 mmol), Ni_2B [56.1 mg, prepared from $Ni(OAc)_2$] in MeOH (3.2 mL) was added 1 M HCl (0.8 mL). The reaction mixture was heated at 60 °C for 30 min, poured into 100 mL of a solution of conc. NH_4OH-H_2O (1:1), and extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography eluting with hexane-EtOAc (3:1) to yield 70 mg (93%) of **9** as a yellow oil. ^{1}H NMR ($CDC1_3$) δ 1.40 (9H, s), 1.51–1.85 (5H, m), 1.97–2.04 (1H, m), 2.87–2.92 (1H, m), 3.77 (2H, brs), 4.21 (1H, brs), 4.38 (1H, brs), 6.70–6.74 (2H, m), 6.78–6.81 (1H, m), 7.20 (1H, t, *J* = 6.0 Hz), 7.62 (1H, s), 8.25 (1H, s); ^{13}C NMR ($CDC1_3$) δ 28.7 (CH_3-3), 29.2, 30.2, 40.7, 45.3, 56.3, 62.3, 80.3, 115.3, 116.3, 120.0, 129.6, 137.1, 138.6, 139.1, 140.7, 146.7, 147.5, 147.8.

7-*tert*-Butoxycarbonyl-2-*exo*-[3′-(3-dimethylamino)-5-pyridinyl]-7-azabicyclo[2.2.1]heptane (10)

A mixture of **9** (180 mg, 0.433 mmol), acetonitrile (12 mL), 37% aq. formaldehyde (1.54 mL), and $NaCNBH_3$ (488 mg, 7.76 mmol) was stirred for 3 h at room temperature. Glacial acetic acid (0.642 mL) was added and stirring continued overnight. The reaction mixture was poured into 100 mL of a solution of conc. NH_4OH-H_2O (1:1), extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on a silica gel column eluting with hexanes-EtOAc (3:l) to yield 179 mg (93%) of **10** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.39 (9H, s), 1.54–1.89 (5H, m), 1.97–2.01 (1H, m), 2.88–2.93 (1H, m), 2.98 (6H, s), 4.22 (1H, brs), 4.36 (1H, brs), 6.73–6.79 (3H, m), 7.29 (1H, d, *J* = 6.0 Hz), 7.65 (1H, s), 8.26 (1H, s).

3′-(3-Dimethylaminophenyl)epibatidine (5m) Dihydrochloride

Compound **10** (179 mg, 0.403 mmol) in methylene chloride (3 mL) was stirred at 0 °C for 15 min, then trifluroacetic acid (3 mL) was added. After stirring for 30 min, the reaction mixture was poured into 100 mL of a solution of conc. NH_4OH-H_2O (1:1), extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on a silica gel column eluting with CMA-EtOAc (1:5) to yield 132 mg (95%) of **5m**. ^{1}H NMR ($CDCl_3$) δ 1.49–1.69 (5H, m), 1.89–1.96 (1H, m), 2.79–2.84 (1H, m), 2.98 (6H, s), 3.61 (1H, brs), 3.77 (1H, brs), 6.75–6.78 (3H, m), 7.3 (1H, d, *J* = 6.0 Hz), 7.75 (1H, s), 8.29 (1H, s); ^{13}C NMR ($CDCl_3$) δ 30.5, 31.7, 40.7, 41.0 ($2CH_3$), 45.1, 56.8, 63.1, 112.6, 113.9, 117.9, 129.4, 137.6, 139.0, 141.6, 147.5, 147.6, 150.7. (One tertiary aromatic C was not observed.)

Compound **5m** (132 mg, 0.383 mmol) was dissolved in 4 mL of methanol and 1 M HCl in ether (4 mL) was added dropwise. After concentration the residue was recrystallized from a

MeOH-ether mixture (1:3) to give **5m**•2HCl; mp 69–72 °C; *anal*. C 54.23%, H 6.49%, N 9.51%, calcd for $C_{19}H_{24}Cl_3N_3$•1.25 H_2O, C 53.91%, H 6.31%, N 9.93%.

3′-(3-Fluorophenyl)epibatidine (5c) Hydrochloride

To a solution of **5j** (150 mg, 0.5 mmol) in 70% HF-pyridine (2.7 mL) at 0 °C was added $NaNO_2$ (266 mg, 3.9 mmol). The reaction mixture was stirred for 30 min at 0 °C and then heated to 100 °C for an additional hour. The reaction mixture was poured into 100 mL of a solution of NH_4OH-H_2O (1:1), extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel column chromatography eluting with CMA-EtOAc (1:3) to yield 40 mg (26%) of **5c** as an oil. ^{1}H NMR ($CDCl_3$) δ 1.53–1.67 (5H, m), 1.90–2.02 (1H, m), 2.79–2.83 (1H, m), 3.61 (1H, brs), 3.80 (1H, brs), 7.08–7.24 (1H, m), 7.38–7.42 (1H, m), 7.78 (1H, s), 8.31 (1H, s, pyridinyl); ^{13}C NMR ($CDCl_3$) δ 30.6, 31.9, 40.8, 44.9, 56.8, 63.2, 115.4 (d, J_{CF} = 20.9 Hz), 116.9 (d, J_{CF} = 22.3 Hz), 125.6 (d, J_{CF} = 2.9 Hz), 130.2 (d, J_{CF} = 8.2 Hz), 135.5, 138.9, 140.2 (d, J_{CF} = 7.7 Hz), 142.1, 147.3, 148.3, 162.8 (d, J_{CF} = 245 Hz).

Compound **5c** (40 mg, 0.131 mmol) was dissolved in 4 mL of methanol, and 1 M HCl in ether (4 mL) was added dropwise. The residue obtained on concentration was recrystallized from methanol and ether to give **5c**•HCl; mp 101–105 °C; *anal*. C 57.53%, H 5.43%, N 7.66%, calcd for $C_{17}H_{17}Cl_2FN_2$•0.75 H_2O, C 57.88%, H 5.29%, N 7.94%.

3′-(3-Chlorophenyl)epibatidine (5e) Hydrochloride

To a mixture of anhydrous CuCl (32.3 mg, 0.24 mmol), butyl nitrite (0.04 mL, 0.3 mmol), and anhydrous acetonitrile (10 mL) warmed to 65 °C was added **5j** (60.4 mg, 0.2 mmol) in 2 mL of anhydrous acetonitrile over 5 min. After 20 min, the cooled reaction mixture was poured into 10 mL of 20% HCl aq., stirred 10 min, and poured into 100 mL of a solution of conc. NH_4OH-H_2O (1:1), extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on a silica gel column eluting with CMA-EtOAc (1:5) to yield 50 mg (78%) of **5e**. ^{1}H NMR ($CDCl_3$) δ 1.50–1.72 (5H, m), 1.90–1.97 (1H, m), 2.79–2.83 (1H, m), 3.61 (−lH, brs), 3.79 (1H, brs), 7.34–7.43 (4H, m), 7.77 (1H, s), 8.30 (1H, s); ^{13}C NMR ($CDCl_3$) δ 30.6, 31.8, 40.8, 44.9, 56.8, 63.2, 128.1, 128.6, 129.8, 129.9, 134.5, 135.4, 138.9, 139.9, 142.1, 147.3, 148.4.

Compound **5e** (50 mg, 0.383 mmol) was dissolved in 4 mL of methanol, and 1 M HCI in ether (2 mL) was added. The residue obtained on concentration was recrystallized from CH_3OH-ether to give **5e**•HCl; mp 159 °C (dec.); *anal*. C 54.78%, H 5.08%, N 7.18%, calcd for $C_{17}H_{17}Cl_3N_2$•H_2O C 54.64%, H 5.12%, N 7.50%.

3′-(3-Bromophenyl)epibatidine (5f) Hydrochloride

To a mixture of anhydrous CuBr (53.6 mg, 0.24 mmol), butyl nitrite (0.04 mL, 0.3 mmol), and anhydrous acetonitrile (6 mL), warmed to 65 °C, was added **5j** (60.4 mg, 0.2 mmol) in 4 mL of anhydrous acetonitrile. After 20 min, the reaction mixture was poured into 10 mL of 20% HCl aq., stirred 10 min, poured into 100 mL of a solution of conc. NH_4OH-H_2O (1:1), extracted with CH_2Cl_2, dried (Na_2SO_4), and concentrated. The residue obtained was purified by flash chromatography on a silica gel column eluting with CMA-EtOAc (1:5) to yield 30 mg (41%) of **5f**. ^{1}H NMR ($CDC1_3$) δ 1.50–1.73 (5H, m), 1.90–1.99 (1H, m), 2.79–2.83 (1H, m), 3.61 (1H, brs), 3.80 (1H, brs), 7.31–7.41 (4H, m), 7.77 (1H, s), 8.31 (1H, s); ^{13}C NMR ($CDCl_3$) δ 30.6, 31.9, 40.8, 44.9, 56.8, 63.2, 122.6, 128.5, 130.2, 131.6, 132.6, 134.5, 135.3, 138.9, 140.2, 142.1, 148.4.

Compound **5f** (60 mg, 0.164 mmol) was dissolved in 4 mL of methanol, and 1 M HCl in ether (3 mL) was added. The residue obtained on concentration was recrystallized from CH_3OH-

ether to give **5f**•HCl; mp 117–120 °C (dec.); *anal*. C 49.57%, H 4.66%, N, 6.55%, calcd for $C_{17}H_{17}BrCl_2N_2$•0.75 H_2O, C 49.36%, H 4.51%, N 6.77%.

[^{3}H]Epibatidine Binding Assay

Adult male rat cerebral cortices (Pelfreeze Biological, Rogers, AK) were homogenized in 39 volumes of ice-cold 50 mM Tris buffer (pH 7.4 at 4 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ and sedimented at 37,000 g for 10 min at 4 °C. The supernatant was discarded, the pellet resuspended in the original volume of buffer, and the wash procedure repeated twice more. After the last centrifugation, the pellet was resuspended in 1/10 its original homogenization volume and stored at −80 °C until needed. In a final volume of 0.5 mL, each assay tube contained 3 mg wet weight male rat cerebral cortex homogenate (added last), 0.5 nM [^{3}H]epibatidine (NEN Life Science Products, Wilmington, DE) and one of 10–12 different concentrations of test compound dissolved in buffer (pH 7.4 at room temperature) containing 10% DMSO resulting in a final DMSO concentration of 1%. Total and nonspecific bindings were determined in the presence of vehicle and 300 μM (−)-nicotine, respectively. After a 4-h incubation period at room temperature, the samples were vacuum-filtered over GF/B filter papers presoaked in 0.03% polyethylenimine using a Brandel 48-well harvester and washed with 6 mL of ice-cold buffer. The amount of radioactivity trapped on the filter was determined by standard liquid scintillation techniques in a TriCarb 2200 scintillation counter (Packard Instruments, Meriden, CT) at approximately 50% efficiency. The binding data were fit using the nonlinear regression analysis routines in Prism (Graphpad, San Diego, CA). The K_i values for the test compounds were calculated from their respective IC_{50} values using the Cheng-Prusoff equation.

[^{125}I]Iodo-MLA Binding Assay

Adult male rat cerebral cortices (Pel-Freez Biologicals, Rogers, AK) were homogenized (polytron) in 39 volumes of ice-cold 50 mM Tris buffer (assay buffer; pH 7.4 at 4 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. The homogenate was centrifuged at 35,000 g for 10 min at 4 °C and the supernatant discarded. The pellet was resuspended in the original volume of buffer and the wash procedure repeated twice more. After the last centrifugation step, the pellet was resuspended in one-tenth the original homogenization volume and stored at −80 °C until needed. Triplicate samples were run in 1.4-mL polypropylene tubes (Matrix Technologies Corporation, Hudson, NH). Briefly, in a final volume of 0.5 mL, each assay sample contained 3 mg wet weight rat cerebral cortex (added last), 40–50 pM [^{125}I]MLA and 50 nM final concentration of test compound dissolved in buffer containing 10% DMSO, giving a final DMSO concentration of 1%. Total and nonspecific binding were determined in the presence of vehicle and 300 μM (−)-nicotine, respectively. After a 2-h incubation period on ice, the samples were vacuum-filtered using a Multimate 96-well harvester (Packard Instruments, Meriden, CT) onto GF/B filters presoaked for at least 30 min in assay buffer containing 0.15% bovine serum albumin. Each well was then washed with approximately 3.0 mL of ice-cold buffer. The filter plates were dried, and 30 μL of Microscint20 (Packard) was added to each well. The amount of radioligand remaining on each filter was determined using a TopCount 12-detector (Packard) microplate scintillation counter at approximately 70% efficiency.

Tail-flick Test

Antinociception was assessed by the tail-flick method of D'Amour and Smith.[21] A control response (2–4 sec) was determined for each mouse before treatment, and a test latency was determined after drug administration. In order to minimize tissue damage, a maximum latency of 10 sec was imposed. Antinociceptive response was calculated as percent maximum possible effect (% MPE), where %MPE = [(test-control)/(10-control)] × 100. Groups of eight to twelve

animals were used for each dose and for each treatment. The mice were tested 5 min after s.c. injections of epibatidine analogues for the dose-response evaluation. Eight to twelve mice were treated per dose and a minimum of four doses were performed for dose-response curve determination.

Hot-plate Test

Mice were placed into a 10 cm wide glass cylinder on a hot plate (Thermojust Apparatus) maintained at 55.0 °C. Two control latencies at least 10 min apart were determined for each mouse. The normal latency (reaction time) was 8 to 12 sec. Antinociceptive response was calculated as percent maximum possible effect (% MPE), where %MPE = [(test-control)/40-control) × 100]. The reaction time was scored when the animal jumped or licked its paws. Eight mice per dose were injected s.c. with epibatidine analogues and tested 5 min thereafter in order to establish a dose-response curve.

Locomotor Activity

Mice were placed into individual Omnitech photocell activity cages (28 × 16.5 cm) 5 min after s.c. administration of either 0.9% saline or epibatidine analogues. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 10 min. Data were expressed as number of photocell interruptions.

Body Temperature

Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and 30 min at different times after the s.c. injection of either saline or epibatidine analogues. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21–24 °C from day to day.

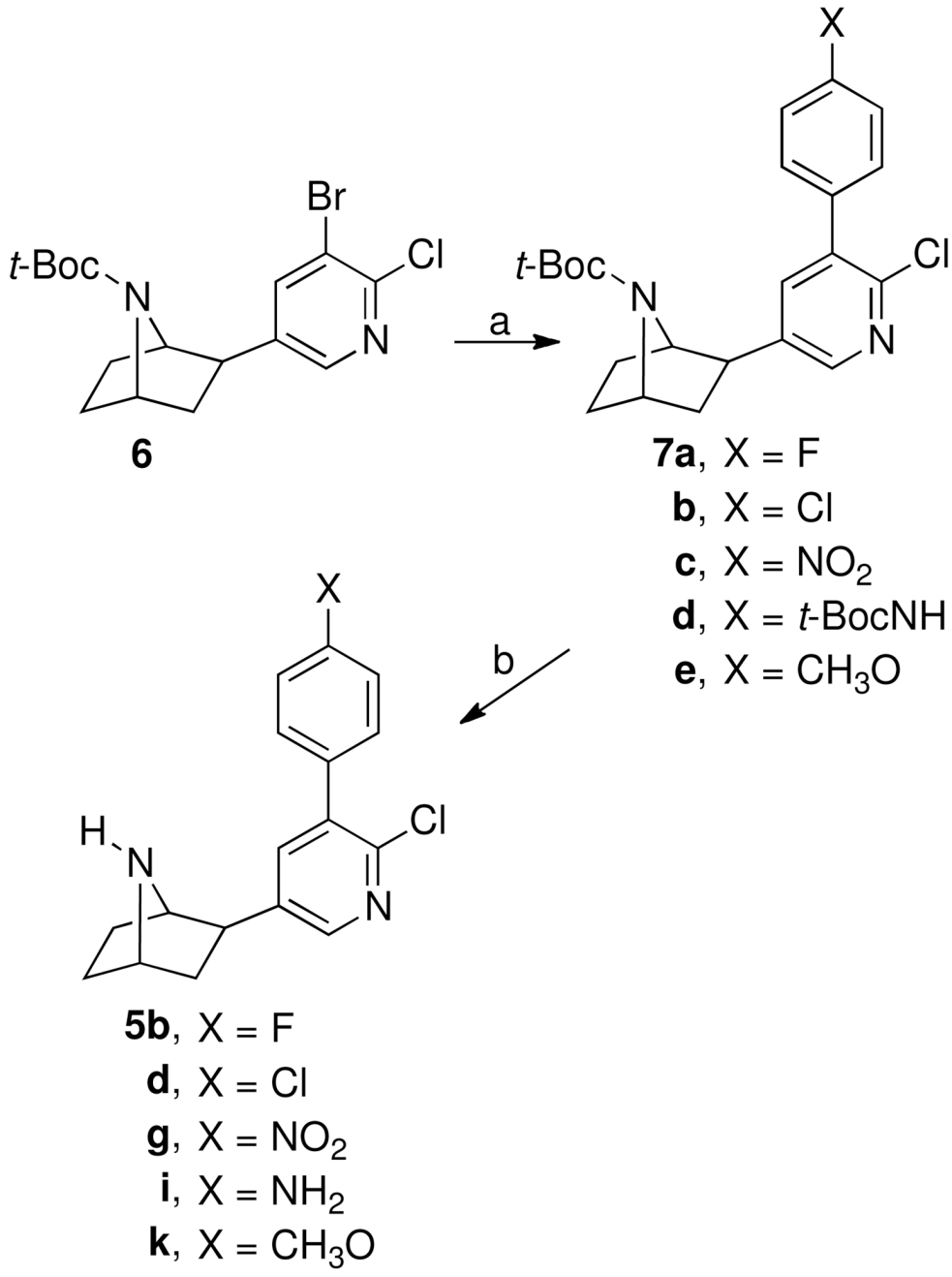
Acknowledgments

This research was supported by the National Institute on Drug Abuse Grant DA12001.

References and Notes

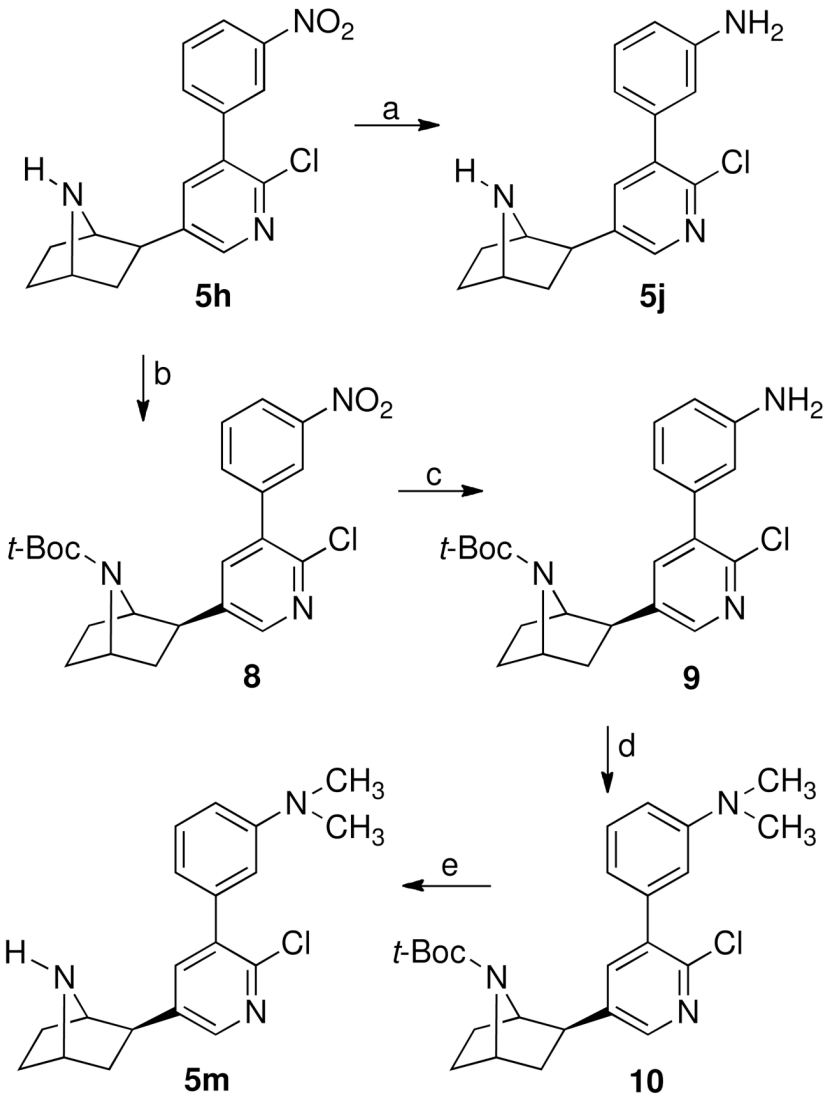
1. Ezzati M, Lopez AD. Lancet 2003;362:847–852. [PubMed: 13678970]
2. Henningfield JE, Fant RV, Buchhalter AR, Stitzer ML. CA Cancer J. Clin 2005;55:281–299. [PubMed: 16166074]
3. Keating GM, Siddiqui MAA. CNS Drugs 2006;20:945–960. [PubMed: 17044731]
4. Niaura R, Jones C, Kirkpatrick P. Nat. Rev. Drug Discov 2006;5:537–538. [PubMed: 16883648]
5. Spande TF, Garraffo HM, Edwards MW, Yeh HJC, Pannell L, Daly JW. J. Am. Chem. Soc 1992;114:3475–3478.
6. Molina PE, Ding YS, Carroll FI, Liang F, Volkow ND, Pappas N, Kuhar M, Abumrad N, Gatley SJ, Fowler JS. Nucl. Med. Biol 1997;24:743–747. [PubMed: 9428600]
7. Rao TS, Correa LD, Reid RT, Lloyd GK. Neuropharmacology 1996;35:393–405. [PubMed: 8793901]
8. Bonhaus DW, Bley KR, Broka CA, Fontana DJ, Leung E, Lewis R, Shieh A, Wong EH. J. Pharmacol. Exp. Ther 1995;272:1199–1203. [PubMed: 7891333]
9. Sullivan JP, Decker MW, Brioni JD, Donnelly-Roberts D, Anderson DJ, Bannon AW, Kang CH, Adams P, Piattoni-Kaplan M, Buckley MJ, Gopalakrishnan M, Williams M, Arneric SP. J. Pharmacol. Exp. Ther 1994;271:624–631. [PubMed: 7965777]
10. Lloyd GK, Williams M. J. Pharmacol. Exp. Ther 2000;292:461–467. [PubMed: 10640281]
11. Carroll FI. Bioorg. Med. Chem. Lett 2004;14:1889–1896. [PubMed: 15050621]
12. Carroll FI, Brieaddy LE, Navarro HA, Damaj MI, Martin BR. J. Med. Chem 2005;48:7491–7495. [PubMed: 16279810]

13. Carroll FI, Lee JR, Navarro HA, Brieaddy LE, Abraham P, Damaj MI, Martin BR. J. Med. Chem 2001;44:4039–4041. [PubMed: 11708907]
14. Carroll FI, Lee JR, Navarro HA, Ma W, Brieaddy LE, Abraham P, Damaj MI, Martin BR. J. Med. Chem 2002;45:4755–4761. [PubMed: 12361403]
15. Carroll FI, Liang F, Navarro HA, Brieaddy LE, Abraham P, Damaj MI, Martin BR. J. Med. Chem 2001;44:2229–2237. [PubMed: 11405659]
16. Carroll FI, Ma W, Yokota Y, Lee JR, Brieaddy LE, Navarro HA, Damaj MI, Martin BR. J. Med. Chem 2005;48:1221–1228. [PubMed: 15715488]
17. Carroll FI, Ware R, Brieaddy LE, Navarro HA, Damaj MI, Martin BR. J. Med. Chem 2004;47:4588–4594. [PubMed: 15317468]
18. Abdrakhmanova G, Damaj MI, Carroll FI, Martin BR. Mol. Pharmacol 2006;69:1945–1952. [PubMed: 16505153]
19. Carroll FI, Yokota Y, Ma W, Lee JR, Brieaddy LE, Burgess JP, Navarro HA, Damaj MI, Martin BR. Bioorg. Med. Chem 2008;16:746–754. [PubMed: 17964169]
20. Navarro HA, Xu H, Zhong D, Abraham P, Carroll FI. Synapse 2002;44:117–123. [PubMed: 11954042]
21. D'Amour FE, Smith DL. J. Pharmacol. Exp. Ther 1941;72:74–79.



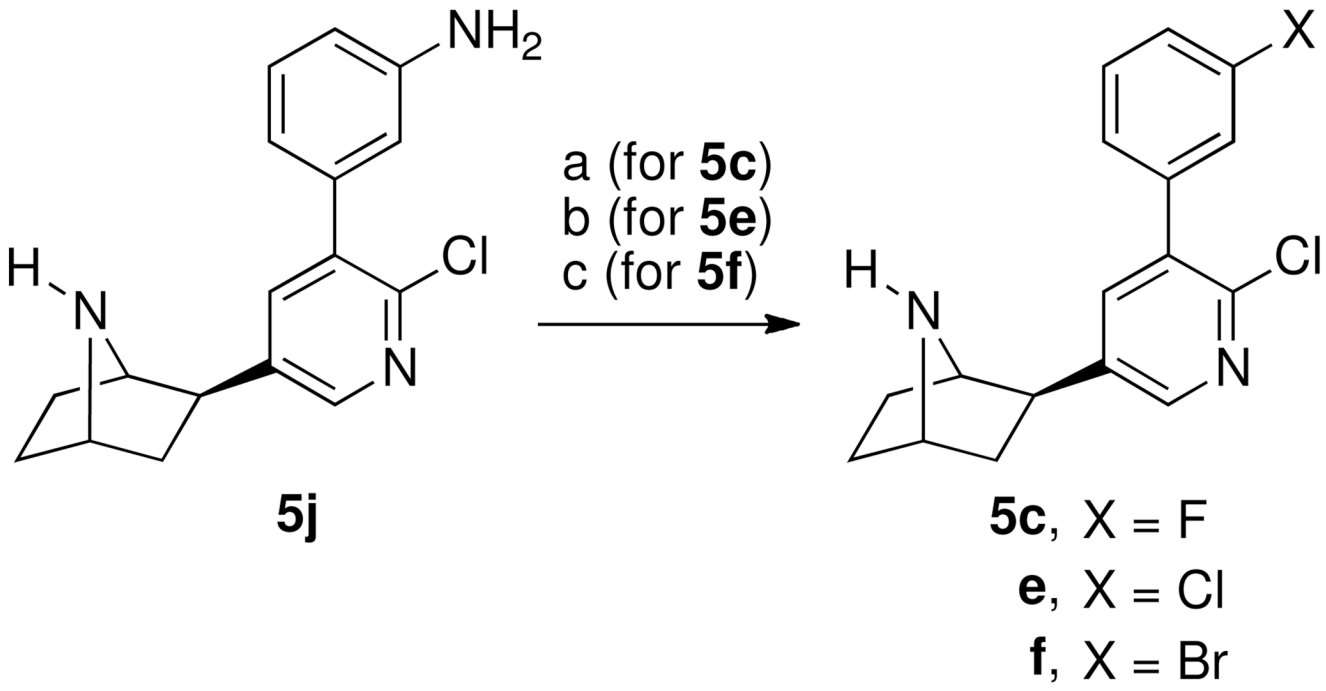
Scheme 1a.

[a] Reagents: (a) $Pd(OAc)_2$, $P(o\text{-}tolyl)_3$, Na_2CO_3, $(X)C_6H_4B(OH)_2$, DME; (b) CF_3CO_2H



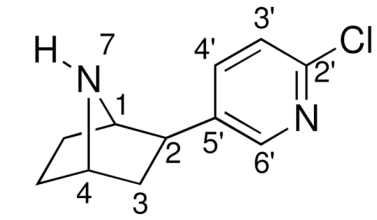
Scheme 2a.

[a] Reagents: (a) Fe, HCl, C_2H_5OH; (b) $(Boc)_2O$, DMAP, $(C_2H_5)_3N$, CH_2Cl_2; (c) Ni_2B, CH_3OH, HCl; (d) $NaCNBH_3$, H_2CO, CH_3CN; (e) CF_3CO_2H, CH_2Cl_2

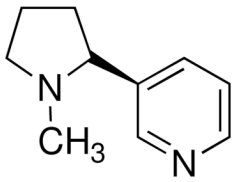


Scheme 3a.

[a] Reagents: (a) HF pyridine, $NaNO_2$; (b) nC_4H_9ONO, CuCl, CH_3CN, 65 °C; (c) nC_4H_9ONO, CuBr, CH_3,CN, 65 °C



(1*R*,2*R*,4*S*)-epibatidine (**1**)



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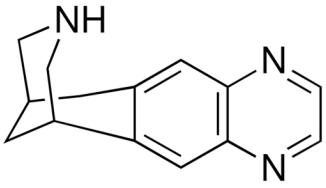

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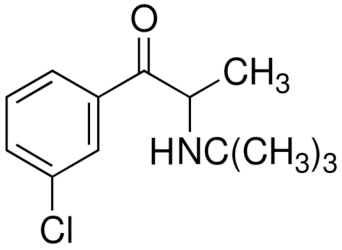

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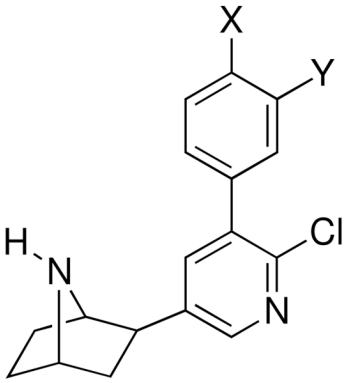


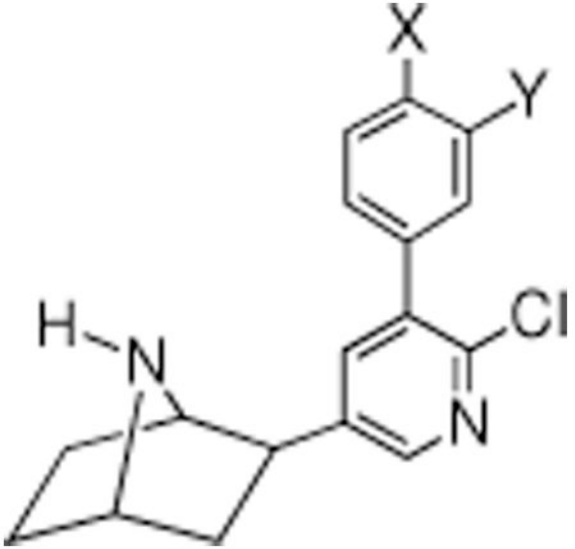
5a, X = Y = H
b, X = F, Y = H
c, X = H, Y = F
d, X = Cl, Y = H
e, X = H, Y = Cl
f, X = H, Y = Br
g, X = NO_2, Y = H
h, X = H, Y = NO_2
i, X = NH_2, Y = H
j, X = H, Y = NH_2
k, X = CH_3O, Y = H
l, X = H, Y = CH_3O
m, X = H, Y = $(CH_3)_2N$

Scheme 4.

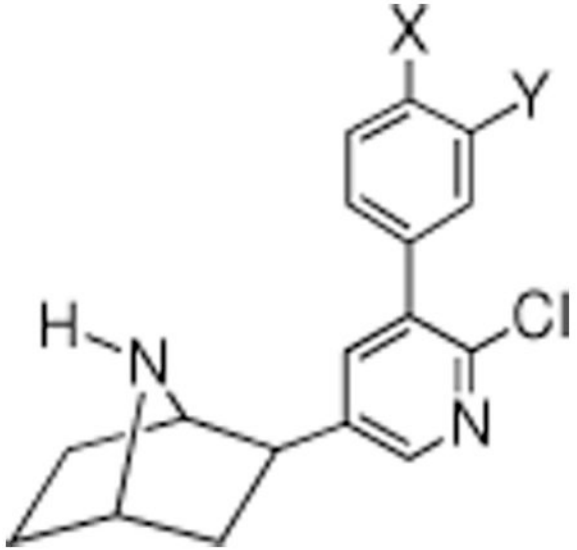
J Nat Prod. Author manuscript; available in PMC 2011 March 26.

Table 1

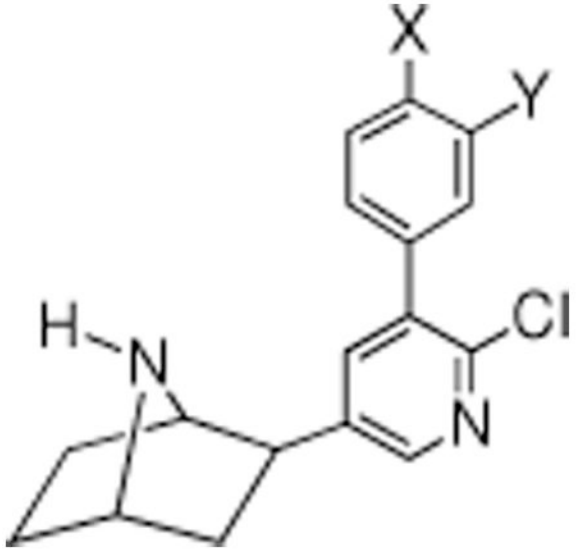
Comparison of Nicotine, Epibatidine, and Varenicline Radioligand Binding and Antinociception Data to 3′-(Substituted Phenyl)epibatidine Analogues (**5a–m**)



compound	X	Y	αβ [^{3}H]epibatidine (K_i, nM)	α_7 [^{125}I]iodoMLA (K_i, nM)[b]	ED_{50} mg/kg tail-flick[c]	ED_{50} mg/kg hot-plate[c]	ED_{50} mg/kg hypothermia[c]	ED_{50} mg/kg spontaneous activity[c]	AD_{50} (μg/kg)[c]	
									tail-flick	hot-plate
(+)-epibatidine [(+)-**1**][d]			0.026 ± 0.002[e]	198	0.0061[e]	0.004[e]	0.004[e]	0.001[e]		
(−)-epibatidine [(−)-**1**][d]			0.018 ± 0.001[e]		0.0066[e]					
nicotine (**2**)[e]			1.5 ± 0.3		1.3	0.65	1.0	0.5		
varenicline (**3**)[f]			0.12 ± 0.02	32.5 ± 1.3	11% @ 10	10% @ 10	2.8	2.1	0.2	470
5a	H	H	0.021 ± 0.005	260 ± 5.0	0.7[g] (0.5–1.0)	1.0[g] (0.5–2.0)	0.4[g] (0.1–0.9)	0.35[g] (0.2–0.85)	280 (80–900)	30% @ 10,000
5b	F	H	0.017 ± 0.003	309 ± 13	2.48 (1.7–3.5)	1.27 (0.8–1.9)	0.33 (0.22–0.67)	0.33 (0.15–0.7)	0.5 (0.06–29)	30% @ 100



compound	X	Y	αβ [^{3}H]epibatidine (K_i, nM)	α_7 [^{125}I]iodoMLA (K_i, nM)[b]	ED_{50} mg/kg tail-flick[c]	ED_{50} mg/kg hot-plate[c]	ED_{50} mg/kg hypothermia[c]	ED_{50} mg/kg spontaneous activity[c]	AD_{50} (μg/kg)[c]	
									tail-flick	hot-plate
5c	H	F	0.012 ± 0.001	250 ± 44	1.0 (0.7–1.5)	0.43 (0.2–0.94)	0.19 (0.1–0.7)	0.07 (0.05–0.10)	3.9 (5–30)	86 (20–300)
5d	Cl	H	0.039 ± 0.005	209 ± 50	2.6 (2.3–3.3)	2 (1.4–2.7)	0.89 (0.5–1.2)	0.53 (0.3–0.9)	1 (0.1–27)	10% @100
5e	H	Cl	0.013 ± 0.001	30.5 ± 84	3.4 (2.6–4.3)	4.2 (1.5–11.6)	0.38 (0.2–1)	0.06 (0.02–0.13)	2.2 (4–15)	80 (30–200)
5f	H	Br	0.016 ± 0.003	85 ± 10	0.54 (0.44–0.67)	2.2 (1.2–3.8)	0.67 (0.5–0.9)	0.11 (0.06–0.22)	10 (7–90)	220 (50–600)
5g	NO_2	H	0.015 ± 0.001	250 ± 25	0.46 (0.33–0.64)	0.38 (0.2–0.5)	0.23 (0.1–0.6)	0.24 (0.1–0.5)	7 (3–10)	17% @ 50
5h	H	NO_2	0.008 ± 0.0003	229 ± 43	4.6 (3.3–6.5)	3.2 (2.4–4.5)	1.4 (1–2.4)	0.9 (0.7–1.2)	0.25 (0.02–0.7)	180 (29–1078)
5i	NH_2	H	0.034 ± 0.001	234 ± 17	5 (3.4–7.2)	4.5 (3.6–5.7)	1.1 (0.8–1.9)	0.52 (0.11–2.3)	6 (5–7)	0% @100
5j	H	NH_2	0.017 ± 0.001	256 ± 46	0.34 (0.24–0.46)	0.3 (0.17–0.53)	0.31 (0.2–0.5)	0.03 (0.009–0.12)	0.14 (0.05–0.4)	26 (5–90)
5k	CH_3O	H	0.022 ± 0.008	175 ± 10	2.36 (2–3.7)	1.1 (0.5–2.7)	0.79 (0.55–1.1)	0.43 (0.1–1.3)	10 (1–9)	10% @ 200



compound	X	Y	αβ [^{3}H]epibatidine (K_i, nM)	α_7 [^{125}I]iodoMLA (K_i, nM)[b]	ED_{50} mg/kg tail-flick[c]	ED_{50} mg/kg hot-plate[c]	ED_{50} mg/kg hypothermia[c]	ED_{50} mg/kg spontaneous activity[c]	AD_{50} (μg/kg)[c]	
									tail-flick	hot-plate
5l	H	CH_3O	0.019 ± 0.005	558 ± 50	0.7 (0.3–1.2)	0.8 (0.3–1.7)	0.7 (0.5–1.1)	0.2 (0.18–0.23)	29 (10–60)	0% @100
5m	H	$(CH_3)_2N$	0.009 ± 0.001	1100 ± 157	0% @ 2	0% @ 2	3.3 (2.1–5.6)	2.0 (0.7–5.8)	20 (10–50)	560 (100–9200)

[a] All epibatidine analogues were tested as hydrochloride salts, and all were racemates.

[b] Data represent means ± SE from at least three independent experiments.

[c] Results are provided as ED_{50} or AD_{50} values (± confidence limits) or as a percent effect at the individual dose.

[d] Compound (+)-**1** is the natural epibatidine hydrochloride, and (−)-**1** is the enantiomeric epibatidine hydrochloride.

[e] Data taken from Ref. 15.

[f] Data taken from Ref. 19.

[g] Data taken from Ref. 13.