Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 2-*exo*-2-(2′,3′-Disubstituted 5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes: Epibatidine Analogues

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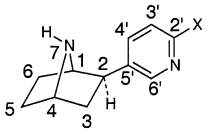
Received May 24, 2002

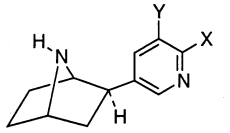
A number of 2′,3′-disubstituted epibatidine analogues were synthesized and evaluated in vitro for potency at nicotinic acetylcholine receptors (nAChRs) and in vivo for antinociception activity in the tail-flick and hot-plate models of acute pain and for their ability to affect core body temperature. Compounds that possessed electron-withdrawing groups (F, Cl, Br, and I) in both the 2′- and the 3′-positions showed affinities at the nAChR similar to epibatidine. However, in vivo efficacy did not correlate with affinity. 2-*exo*-(3′-Amino-2′-chloro-5′-pyridinyl)-7-azabicyclo-[2.2.1]heptane (**2i**), an epibatidine analogue possessing an electron-releasing amino group in the 3′-position, produced the highest affinity. Compound **2i** was also the most selective epibatidine analogue with a K_i of 0.001 nM at $\alpha\beta$ nAChRs, which is 26 times greater than that of epibatidine, and a $\alpha\beta/\alpha_7$ K_i ratio of 14 000, twice that of epibatidine. In vivo testing revealed that this compound potently inhibited nicotine-induced antinociception with AD_{50} values below 1 μg/kg. Surprisingly, this same compound was also an agonist at higher doses (ED_{50} ∼20 μg/kg). Thus, the addition of the 3′-amino group to epibatidine confers potent antagonist activity to the compound with little effect on agonist activity. 2,3-Disubstituted epibatidine analogues possessing a 2′-amino group combined with a 3′-bromo or 3′-iodo group showed in vitro and in vivo nAChR properties similar to nicotine.

Introduction

Epibatidine (**1a**, *exo*-2-(2′-chloro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane), a compound isolated from the skin of the Ecuadorian poisonous frog, *Epipedobates tricolor*, has high affinity for the $\alpha\beta$ nicotine acetylcholine receptor (nAChR).[1,2] The fact that nAChR plays a role in various neuropathological and physiological states, including Parkinson's disease, Alzheimer's disease, pain, tobacco dependency, schizophrenia, anxiety, and depression, has resulted in a renewed interest in characterizing the pharmacophore for the $\alpha\beta$ nAChR.[1,3−6] Recently, we reported the synthesis, nAChR binding properties, and antinociceptive effects of epibatidine (**1a**) and the *exo*-2-(2′-substituted-5′-pyridinyl)-7-azabicyclo-[2.2.1]heptanes (**1b**−**i**).[7] In this study, we report the synthesis, nAChR binding properties, and antinociception effects of the *exo*-2-(2′,3′-disubstituted-5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes (**2a**−**n**) and compare the results obtained with the 2′-monosubstituted compounds **1a**−**i**.



1a, X = Cl
b, X = F
c, X = Br
d, X = I
e, X = NH_2
f, X = OH
g, X = $N(CH_3)_2$
h, X = CF_3SO_3
i, X = H



2a, X = Y = F
b, X = F, Y = Cl
c, X = F, Y = Br
d, X = F, Y = I
e, X = Y = Cl
f, X = Cl, Y = F
g, X = Cl, Y = Br
h, X = Cl, Y = I
i, X = Cl, Y = NH_2
j, X = Y = Br
k, X = I, Y = Br
l, X = OH, Y = Br
m, X = NH_2, Y = Br
n, X = NH_2, Y = I

Chemistry

Scheme 1 outlines the synthesis of the intermediates **4**−**6**, which were used to prepare target compounds **2a**−**n**. The reductive palladium acetate-catalyzed addition of 3-amino-2-fluoro-5-iodopyridine (**11a**), 3-amino-2-chloro-5-iodopyridine (**11b**), and 2-amino-3,5-diiodopyridine[8] to 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]hept-2-ene (**3**) in dimethylformamide (DMF) containing tetrabutylammonium chloride and potassium formate at 100 °C provided the intermediates **4**−**6**. The 2-*exo* stereochemistry of **4**−**6** was established by comparison of the ^{1}H NMR to the spectrum of 7-*tert*-butoxycarbonyl-*exo*-2-(2′-chloro-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane.[9]

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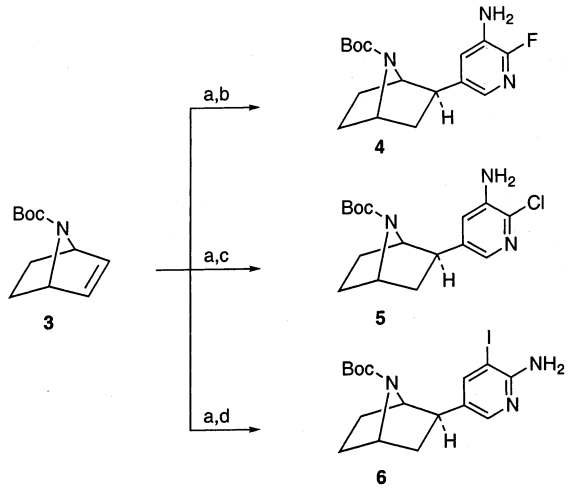
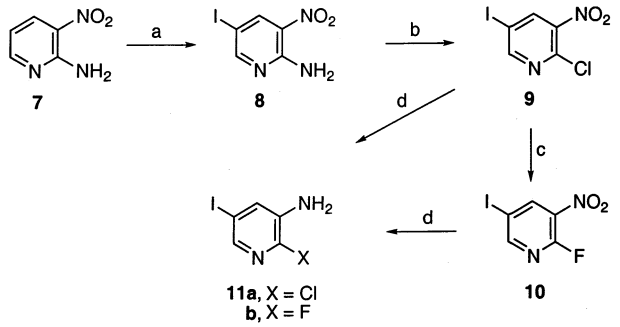
10.1021/jm0202268 CCC: $22.00
Published on Web 09/18/2002

Scheme 1[a]



[a] Reagents: (a) $Pd(OAc)_2$, $(nC_4H_9)_4N^+Cl^-$, KO_2CH, DMF, 100 °C, 12 h. (b) Compound **11b**. (c) Compound **11a**. (d) 2-Amino-3,5-diiodopyridine.

Scheme 2[a]

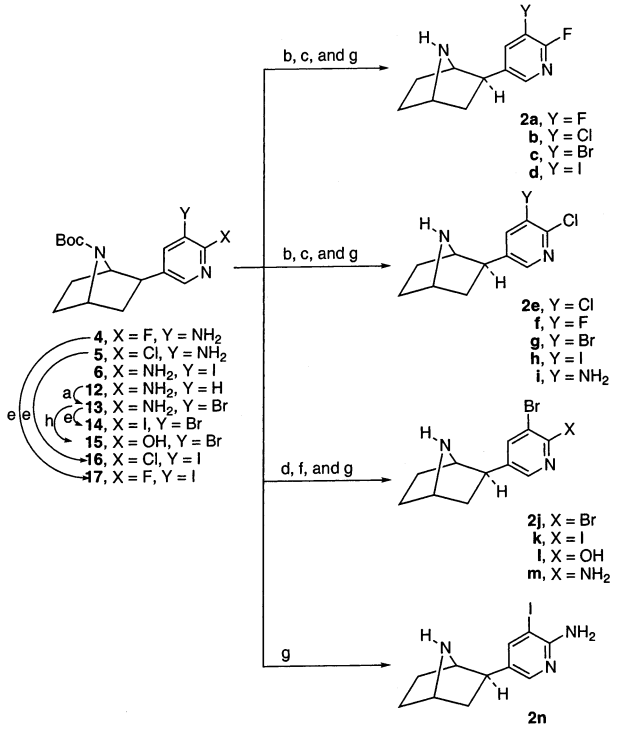


[a] Reagents: (a) I_2, H_5IO_6, aqueous HOAc, H_2SO_4. (b) HCl (concentrated aqueous), $NaNO_2$, CuCl. (c) KF, DMF, heat. (d) Fe, HCl (concentrated), C_2H_5OH.

The pyridines **10** and **11a**,**b** were prepared as shown in Scheme 2. Iodination of 2-amino-3-nitropyridine (**7**) using iodine in an acetic acid−sulfuric acid mixture containing hydroiodic acid yielded 2-amino-5-iodo-3-nitropyridine (**8**). Diazotization of **8** with sodium nitrite in concentrated hydrochloric acid containing cuprous chloride afforded 2-chloro-5-iodo-3-nitropyridine (**9**). Treatment of **9** with potassium fluoride in DMF provided 2-fluoro-5-iodo-3-nitropyridine (**10**). Reduction of **9** and **10** with iron in a concentrated hydrochloric acid−ethanol mixture provided the desired **11a**,**b**, respectively.

Scheme 3 outlines the routes used to prepare *tert*-butoxycarbonyl intermediates **13**−**17** and the conversion of **4**−**6** and **12**−**17** to target compounds **2a**−**n**. Bromination of *tert*-butoxycarbonyl-*exo*-2-(2′-amino-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**12**)[10] using bromine in acetic acid provided intermediate **13**. Diazotization of **4**, **5**, and **13** with isoamyl nitrite containing hydroiodic acid in methylene iodide gave **17**, **16**, and **14**, respectively. Diazotization of **13** with *tert*-butyl nitrite in DMF yielded intermediate **15**. Diazotization of **4** and **5** with sodium nitrite in pyridine containing 70% hydrogen fluoride−pyridine affected conversion of the 3-amino group to a fluoro group and deprotection of the N-*tert*-butoxycarbonyl group to give **2a**,**f**, respectively. Diazotization of **4**, **5**, and **13** with sodium nitrite in hydrochloric acid in the presence of cuprous chloride gave **2b**,**e**,**g**, respectively. Diazotization of **13** using sodium nitrite in hydrobromic acid in the presence of copper(I) bromide yielded **2j**. Treatment of **17**, **16**, **5**, **14**, **15**, **13**, and **6** with trifluoroacetic acid provided the **2d**,**h**,**i**,**k**−**n**, respectively.

Scheme 3[a]



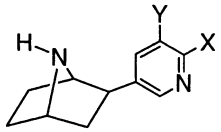
[a] Reagents: (a) Br_2, HOAc. (b) HF-pyridine, $NaNO_2$. (c) HCl, CuCl, $NaNO_2$. (d) HBr, $NaNO_2$, CuBr. (e) Isoamylnitrite, HI, CH_2I_2. (f) HCl, dioxane. (g) CF_3CO_2H. (h) *tert*-Butyl nitrite, DMF.

Biology

The K_i values for the inhibition of [^{3}H]epibatidine ([^{3}H]-**1**) binding at the $\alpha\beta$ nAChR in male rat cerebral cortex for the 2,3-disubstituted epibatidine analogues **2a**−**n** as well as for nicotine, (+)-epibatidine ((+)-**1a**), and reference compounds **1b**−**f** are listed in Table 1. The binding assays were conducted, and the K_i values were calculated as previously described.[7] The compounds were also evaluated at 50 nM for inhibition of [^{125}I]iodo-MLA binding at the α_7 nAChR using conditions previously reported except that the assays were run in a volume of 0.25 mL, and the final assay concentration of [^{125}I]iodo-MLA was 90 pM.[11] The K_i value was determined for any compound that inhibited [^{125}I]iodo-MLA binding by at least 50% using procedures similar to those described for [^{3}H]-**1** binding[7] and an [^{125}I]iodo-MLA K_d of 1.98 nM.

Compounds **2a**−**n** were evaluated in two acute pain models, the tail-flick and the hot-plate tests, and the results for compounds **2a**−**n** along with results for epibatidine and nicotine are listed in the Table 1.[12] In the tail-flick method of D'Amour and Smith,[13] a control response (2−4 s) was determined for each mouse before treatment, and a test latency was determined after drug administration. The method used for the hot-plate test is a modification of those described by Eddy and Leimbach[14] and Atwell and Jacobson.[15] Mice were

Table 1. Radioligand Binding Data for 2′,3′-Disubstituted Epibatidine Analogues



compd[a]	X	Y	$\alpha\beta$ [^{3}H]-**1**[b] (K_i, nM) (hill slope)	α_7 [^{125}I]iodo-MLA[c] (K_i, nM) (hill slope)	ED_{50} mg/kg tail-flick[d]	ED_{50} mg/kg hot-plate[d]	ED_{50} mg/kg hypothermia[d]	AD_{50} tail-flick[d]	AD_{50} hot-plate[d]
2a	F	F	0.055 ± 0.001 (1.0 ± 0.01)		NT[e]	NT[e]	NT[e]		
2b	F	Cl	0.020 ± 0.0003 (0.8 ± 0.004)		NT[e]	NT[e]	NT[e]		
2c	F	Br	0.071 ± 0.005 (0.98 ± 0.01)		0.7 (0.5−1.0)	1.2 (0.7−2.1)	0.01 (0.005−0.1)	0% at 0.05	0% at 0.05
2d	F	I	0.051 ± 0.001 (0.78 ± 0.05)		0.7 (0.5−1.2)	0.9 (0.5−1.7)	0.3 (0.1−0.6)	0% at 0.05	0% at 0.05
2e	Cl	Cl	0.014 ± 0.002 (1.12 ± 0.03)	1.12 ± 0.10 (1.63 ± 0.41)	0.05 (0.03−0.07)	0.03 (0.01−0.04)	0.02 (0.01−0.07)		
2f	Cl	F	0.025 ± 0.002 (0.83 ± 0.11)	3.43 ± 0.43 (2.10 ± 0.15)	0.008 (0.005−0.01)	0.009 (0.005−0.01)	0.006 (0.002−0.009)		
2g	Cl	Br	0.013 ± 0.001 (0.95 ± 0.09)		0.03 (0.02−0.05)	0.02 (0.01−0.03)	0.04 (0.02−0.07)		
2h	Cl	I	0.008 ± 0.0006 (1.03 ± 0.06)		0.24 (0.15−0.4)	0.14 (0.07−0.2)	0.027 (0.01−0.08)	4% at 0.01	0% at 0.01
2i	Cl	NH_2	0.001 ± 0.0004 (0.74 ± 0.04)	13.9 ± 1.1 (1.42 ± 0.66)	0.02 (0.012−0.035)	0.02 (0.001−0.032)	0.017 (0.010−0.026)	0.00003 (0.000006−0.0001)	0.0006 (0.00006−0.0026)
2j	Br	Br	0.015 ± 0.0005 (0.90 ± 0.02)		NT[e]	NT[e]	NT[e]		
2k	I	Br	0.027 ± 0.002 (0.96 ± 0.05)		0.27 (0.19−0.6)	0.24 (0.1−0.5)	0.04 (0.01−0.07)	4% at 0.05	0% at 0.05
2l	OH	Br	14.4 ± 0.7 (1.10 ± 0.04)		1% at 10	9% at 10	0% at 10		
2m	NH_2	Br	0.29 ± 0.01 (1.0 ± 0.03)		0.07 (0.04−0.12)	0.05 (0.03−0.08)	0.075 (0.04−0.1)		
2n	NH_2	I	0.52 ± 0.02 (0.78 ± 0.08)	34.1 ± 2.0 (1.52 ± 0.03)	0.65 (0.4−1.0)	0.5 (0.3−0.9)	0.3 (0.1−0.6)		
1b	F	H	0.027[f]						
1c	Br	H	0.023[f]						
1d	I	H	0.070[f]						
1e	NH_2	H	1.3[f]						
1f	OH	H	107[f]						
			1.50 ± 0.30 (0.90 ± 0.4)	675[g]	1.5	0.50	1.2		
(+)-EB[h]	Cl	H	0.026 ± 0.002 (0.98 ± 0.05)	198[g,i]	0.006[j]	0.004[j]	0.004[j]		

[a] All of the compounds were tested as their hydrochloride salts. [b] Epibatidine K_d value of 0.020 nM. [c] K_i values determined for only those compounds showing greater than 50% inhibition in 50 nM screening assay. [^{125}I]MLA K_d = 1.98 nM. [d] Results were presented as ED_{50} (± confidence limits) in mg/kg or as a percent effect at the individual dose. [e] NT = not tested. [f] Data taken from ref 7. [g] Taken from ref 12. [h] (+)-EB is natural epibatidine hydrochloride. [i] K_i value is for (±)-EB. [j] Data taken from ref 18.

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 8−12 s. The reaction time was scored when the animal jumped or licked its paws. The mice were tested 5 min after sc injections of nicotinic ligands for the dose−response evaluation. Antinociceptive response was calculated as percentage of maximum possible effect (% MPE, where %MPE = [(test − control)/(maximum latency − control) × 100]). Eight mice per dose were injected sc and tested at various times thereafter to establish a time course when needed. The effects of the compounds on body temperature were also measured (Table 1). ED_{50} values with 95% confidence limits for behavioral data were calculated by unweighted least squares linear regression as described by Tallarida and Murray.[16] For the antagonist experiments, mice were pretreated sc with either saline or epibatidine analogues 10 min before nicotine. Nicotine was administered at a dose of 2.5 mg/kg, sc, and mice were tested 5 min later. AD_{50} values with 95% confidence limits were determined.

Results and Discussion

A number of epibatidine analogues have been prepared, and their nAChR binding properties have been determined using various radioligands.[5] We recently reported that replacement of the 2′-chloro group of epibatidine with a fluoro, bromo, or iodo group provided analogues with affinity for the $\alpha\beta$ nAChR essentially identical to that of epibatidine; see compounds **1b**−**d** in the table. In addition, we reported that replacement of the 2′-chloro group with an amino group resulted in an analogue with nearly the same affinity as nicotine whereas replacement with a hydroxyl group afforded an analogue with very low affinity for the $\alpha\beta$ nAChR. In this study, we report the effects on binding affinity and antinociception activity of adding a number of 3′-substitutes to epibatidine (**1a**) and its analogues **1b**−**f**.

The addition of the electron-withdrawing 3′-fluoro (**2f**), 3′-chloro (**2e**), 3′-bromo (**2g**), and 3′-iodo (**2h**) groups as well as the electron-releasing 3′-amino (**2i**) to **1a** all leads to highly potent epibatidine analogues. The 2′-chloro, 3′-amino analogue **2i** has a K_i value of 0.001 nM (26-fold greater than that of epibatidine) and thus is the highest affinity analogue tested. Similarly, the addition of a 3′-fluoro (**2a**), 3′-chloro (**2b**), 3′-bromo (**2c**), or 3′-iodo (**2d**) to the 2′-fluoro analogues of epibatidine (**1b**) all leads to analogues with affinity for $\alpha\beta$ nAChR similar to epibatidine (**1a**) and the 2-fluoro analogue (**1b**). The addition of a 3′-bromo group to the 2′-bromo (**1c**), the 2′-iodo (**1d**), and the 2′-hydroxy (**1f**) resulted in analogues **2j**−**l** that possess slightly higher binding affinity relative to the parent compounds. The addition of a 3′-bromo or 3′-iodo group to **1e** to give **2m**,**n** results in a 3−5-fold greater affinity for the $\alpha\beta$ nAChR than **1e**.

The compounds **2e**,**f**,**i**,**n** inhibited [^{125}I]iodo-MLA binding by at least 50% in our initial screen. The compounds with the highest affinity for the α7 nAChR were **2e** (K_i = 1.12 nM) and **2f** (K_i = 3.43 nM), both with 2′,3′ electron-withdrawing groups. Compound **2i**, substituted with a 2′-chloro and a 3′-amino and the most potent $\alpha\beta$ nAChR ligand, had a K_i value of 13.9 nM. Compound **2n** had the lowest affinity of these four compounds with a K_i of 34 nM. All four compounds had Hill slopes between 1.0 and 2.1. Compound **2i**, with a $\alpha\beta/\alpha_7$ K_i ratio of ∼14 000 was 2 orders of magnitude more selective for the $\alpha\beta$ nAChR than the other three compounds. This level of selectivity is similar to but greater than that seen for epibatidine, which in our assays is 7600 times more selective for the $\alpha\beta$ nAChR. Thus, **2i** is not only the most potent compound at the $\alpha\beta$ nAChR that we have synthesized but it is also the most selective.

All of the compounds tested in the tail-flick, hot-plate, and hypothermia tests were nAChR agonists with the exception of **2l**, which was inactive in all tests. Most of the analogues did not exhibit pharmacological selectivity. They all produced similar potency in both antinociceptive assays. The 2′-chloro, 3′-fluoro analogue **2f** with ED_{50} values of 0.008, 0.009, and 0.006 mg/kg in the tail-flick, hot-plate, and hypothermia tests, respectively, as compared to 0.006, 0.004, and 0.004 mg/kg for epibatidine, was the most potent analogue. However, the 2′,3′-dichloro (**2e**) and 2′-chloro-3′-bromo (**2g**) analogues were 3−8-fold less potent than epibatidine in all three tests. The 2′-fluoro, 3′-iodo (**2d**), 2′-chloro, 3′-iodo (**2h**), 2′-iodo, 3′-bromo (**2k**), and 2′-amino, 3′-iodo (**2n**) analogues were 35−225-fold less potent in the three tests. The 2′-fluoro, 3′-bromo analogue (**2c**) and 2′-iodo, 3′-bromo analogues (**2k**) were 45−300-fold times less potent than epibatidine in the tail-flick and hot-plate test but were only 3−10 times less potent in the hypothermia test. It is difficult to predict the structural features responsible for this selectivity given that it occurs in only two compounds. These two 3′-bromo analogues have fluoro and iodo substituents at C-2′, which suggest that substituent size and electronegativity are not the critical factors. With the exception of **2c** in the hot-plate test and **2l** in all three tests, all of the analogues were more potent than nicotine. The effects of these epibatidine analogues were all blocked by mecamylamine, a nicotinic antagonist (data not shown).

The correlation between binding affinity and pharmacological potency for the compounds **2a**−**n** is not as positive as normally seen with a series of high affinity analogues. As mentioned above, the affinities of the dihalide analogues differed no more than 9-fold; yet, their pharmacological potencies varied as much as 100-fold. Even the lowest affinity C-2′ amino derivatives (analogues **2m**,**n**) demonstrated a greater difference in pharmacological potency than expected based upon binding affinity. This divergence between binding affinity and potency for the highest affinity ligands occured for both tail-flick and hot-plate responses because these effects were most likely mediated by different nAChR subtypes.[17] There are several possible explanations for the apparent lack of correlation between agonist affinity and in vivo efficacy. One is that there is a physiological "ceiling" for the in vivo endpoints that prevents the full effect of the compounds from being measured. Thus, as affinity increases, the ED_{50} values tend to cluster around the highest affinity compounds. Another possible explanation is that each compound may have similar affinity for the different nAChR subtypes mediating these physiological effects or these analogues may bind to a receptor population different from epibatidine. In the binding assays, we are limited to the subtype binding profile of [^{3}H]-**1**, and our tissue homogenate contains predominantly the $\alpha_4\beta_2$ nAChR subtype. Thus, these new compounds may have a nAChR subtype binding profile different from epibatidine. Alternatively, the compounds may have differential binding affinity to the various conformations of nicotinic receptors (desensitized or inactive state, for example). Of course, there can be numerous factors that must be taken into account when comparing in vivo and in vitro data. However, the close structural similarity of these analogues suggests that pharmacodynamic factors, rather than pharmacokinetics, are the important determinants.

We recently reported that 2-*exo*-2-(2′-fluoro-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**18**), derived by the addition of a bulky 3′-phenyl group to **1b**, was devoid of agonist activities but was a potent nicotinic antagonist in both the tail-flick and the hot-plate test.[18] Because compounds **2c**,**d**,**h**,**i**,**k** all showed agonist activity below that expected from the K_i values for inhibition of [^{3}H]-**1** binding, they were evaluated for nAChR antagonist activity in the tail-flick and hot-plate test at low doses. Compounds **2c**,**d**,**k**, at a dose of 0.05 mg/kg, did not block the activity of 2.5 mg/kg of nicotine in either test. Similarly, compound **2h** lacked antagonist activity at a dose of 0.01 mg/kg in both tests. In sharp contrast, the 3′-amino-2′-chloro compound **2i** (RTI-7527-33) was a potent nAChR antagonist in both tests with AD_{50} values of 30 and 600 ng/kg in the tail-flick and hot-plate tests, respectively. As compared to other nicotinic antagonists, the potency of **2i** in blocking nicotine's effect in the tail-flick test is 1500 times higher than the noncompetitive antagonist mecamylamine and 16 600 times more potent than the competitive antagonist dihydro-β-erythroidine.[19] Thus, to our knowledge, **2i** is the most potent nicotinic antagonist ever reported. Moreover, **2i** has very unique nicotinic pharmacological properties in that it also produced agonist effects when examined at much higher doses (Table 1). Indeed, it is

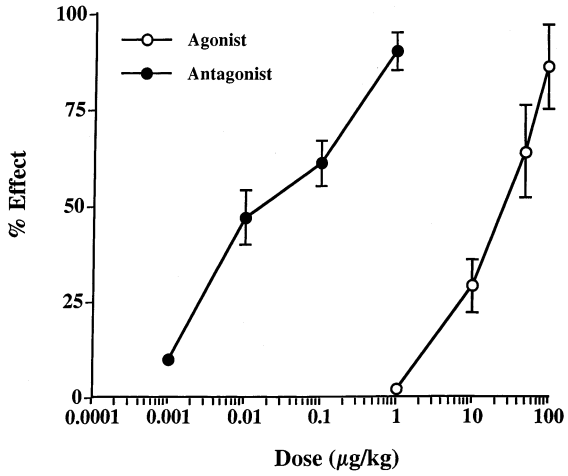
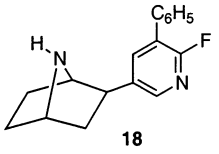


Figure 1. Comparison of agonist and antagonist potencies of **2i** in the tail-flick tests after systematic (sc) administration in mice. The antagonistic effect of **2i** was assessed by blocking the effects of 2.5 mg/kg nicotine.

only three times less potent than epibatidine as an agonist in both the tail-flick and the hot-plate tests. In addition, the separation between agonist/antagonist properties of **2i** is greater than 650 and 35 times for the tail-flick (Figure 1) and the hot-plate tests, respectively. Thus, it is a potent nAChR ligand with mixed potent agonist/antagonist activity with an affinity for the $\alpha\beta$ nAChR subtype 26 times greater than that of epibatidine. These results would be more easily explained if **2i** was a partial and not full agonist in the antinociception assays. One possible explanation is that **2i** is binding to two different nAChR subtypes that are both involved in mediating antinociception in the tail-flick and hot-plate models of pain. As such, **2i** could act as a higher affinity antagonist at one subtype and a relatively lower affinity agonist at the other with the agonist overriding the antagonist pathway.



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In summary, we have synthesized a number of epibatidine analogues and evaluated them for their in vitro and in vivo nAChR properties. Analogues that possessed electron-withdrawing groups in both the 2′- and the 3′-position showed high affinity for the $\alpha\beta$ nAChR receptor in vitro and showed agonist activity in vivo. However, the agonist activity did not correlate well with their binding affinities. The most interesting compound studied was the 2′-chloro-3′-amino analogue (2-*exo*-(2′-chloro-3′-amino-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane, (**2i**)), which had both antagonist and agonist properties. Compound **2i** appears to be the most potent nAChR antagonist reported to date.

Experimental Section

Melting points were determined on a Mel-temp (Laboratory Devices, Inc.) capillary tube apparatus. NMR spectra were recorded on a Bruker Avance 300 or AMX 500 Spectrometer 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.

7-*tert*-Butoxycarbonyl-2-*exo*-(3′-amino-2′-fluoro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (4). To a resealable reaction vessel containing degassed DMF (5 mL) were added 0.500 g (2.56 mmol) of **3**, 788 mg (3.36 mmol) of **11b**, $Pd(OAc)_2$ (50 mg, 0.22 mmol), tetrabutylammonium chloride (116 mg, 0.417 mmol), and potassium formate (288 mg, 3.42 mmol). The reaction tube was sealed under nitrogen, placed into a 105 °C oil bath, and stirred for 2 h. The reaction was then diluted with ethyl acetate and filtered through a Celite pad, and the organics were washed with 1:1 NH_4OH:H_2O (150 mL). The combined organic extracts were dried with sodium sulfate and concentrated, and the residue was purified by flash chromatography on silica gel using a 2:1 hexane:ethyl acetate as the eluent to yield 366 mg (46%) of **4** as a colorless solid; mp 80−82 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.44 (s, 9H), 1.44−1.60 (m, 2H), 1.70−1.88 (m, 3 H), 1. 95 (dd, J = 9.0, 12.3 Hz, 1H), 2.78 (dd, J = 5.2, 8.9 Hz, 1H), 3.86 (br s, 2H), 4.13 (br s, 1H), 4.34 (br s, 1H), 7.11 (d, 1H, J_{HF} = 10.5 Hz, pyridyl CH), 7.37 (s, 1H, pyridyl CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.19 (3C), 28.63, 29.59, 40.3, 44.67, 55.9, 61.97, 79.63, 122.78 (J_{CF} = 5.2 Hz), 129.34 (J_{CF} = 28.6 Hz), 133.23 (J_{CF} =13.1 Hz), 139.73 (J_{CF} = 4.1 Hz), 149.72, 154.32 (J_{CF} = 118 Hz). Anal. ($C_{16}H_{22}FN_3O_2$) C, H, N.

7-*tert*-Butoxycarbonyl-2-*exo*-(3′-amino-2′-chloro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (5). To a resealable reaction vessel containing degassed DMF (35 mL) were added 2.50 g (0.013 mol) of **3**, 4.90 g (0.019 mol) of **11a**, tetrabutylammonium chloride (0.67 g, 0.0024 mol), potassium formate (2.10 g, 0.025 mol), and $Pd(OAc)_2$ (0.13 g, 0.00058 mol). The reaction vessel was sealed under nitrogen, placed into a 95 °C oil bath, and stirred for 94 h. The reaction mixture was cooled, added to 1:1 NH_4OH (200 mL), and extracted with CH_2Cl_2 to afford 6.0 g of a red oil. This oil was purified by column chromatography on silica gel using 65% hexane/EtOAc as eluent to yield 2.4 g (45%) of **5** as a tan solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.43 (s, 9H), 1.44−1.61 (m, 2H), 1.70−1.85 (m, 3H), 1.95 (dd, J = 9.0, 12.3 Hz, 1H), 2.77 (dd, J = 5.1, 9.0 Hz, 1H), 4.15 (br s, 3H), 4.34 (br s, 1H), 7.05 (s, 1H, pyridyl CH), 7.65 (s, 1H, pyridyl CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.15 (3C), 28.59, 29.58, 40.6, 44.63, 55.97, 61.80, 79.62, 120.71, 134.77, 137.38, 139.30, 141.33, 155.18.

7-*tert*-Butoxycarbonyl-2-*exo*-(2′-amino-3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (6). To a resealable reaction vessel containing DMF (16 mL) were added 796 mg (4.08 mmol) of **3**, 2.91 g (8.41 mmol) of 2-amino-3,5-diiodopyridine,[8] $Pd(OAc)_2$ (55 mg, 0.24 mmol), tetrabutylammonium chloride (284 mg, 1.02 mmol), and potassium formate (690 mg, 8.2 mmol). The reaction tube was sealed under nitrogen, placed into an 85 °C oil bath, and stirred for 16 h. The reaction was then diluted with ethyl acetate and filtered through a Celite pad, and the organics were extracted with 1:1 NH_4OH:H_2O (150 mL). The combined organic extracts were dried with sodium sulfate and concentrated, and then, the residue was purified by flash chromatography on silica gel using 1:9 triethylamine:diethyl ether as eluent to yield 0.108 g (6%) of **6** as a colorless solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.48 (s, 9H), 1.50−1.60 (m, 2H), 1.70−1.90 (m, 4H), 2.62 (dd, J = 5.3, 8.6 Hz, 1H), 4.34 (br s, 1H), 4.38 (br s, 1H), 4.71 (br s, 2H), 7.69 (s, 1H, pyridyl CH), 8.09 (s, 1H, pyridyl CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.3 (3C), 29.8, 31.3, 37.2, 43.6, 55.4, 59.4, 80.2, 125.8, 142.3, 151.2, 154.0, 155.1.

2-Amino-5-iodo-3-nitropyridine (8). A mixture of 30.4 g (0.219 mole) of **7**, acetic acid (140 mL), water (30 mL), sulfuric acid (4.2 mL), and H_5IO_6 (10.5 g, 0.046 mol) was allowed to stir at 90 °C for 15 min. Iodine crystals (22.8 g, 0.090 mol) were added in portions. After it was stirred for 1 h, the reaction mixture was poured into saturated sodium thiosulfate solution and extracted with ethyl acetate. The organic layer was separated, dried with sodium sulfate, and then evaporated to give 57.5 g (99%) of **8** as an orange solid; mp 213-215 °C. ^{1}H NMR (DMSO): δ (ppm) 8.03 (br s, 2H), 8.53 (d, J = 2.0 Hz,

1H), 8.58 (d, J = 2.0 Hz, 1H). ^{13}C NMR (DMSO): δ (ppm) 74.18, 127.92, 141.31, 152.50, 160.88. Anal. ($C_5H_4IN_3O_2$) C, H, N.

2-Chloro-5-iodo-3-nitropyridine (9). A mixture of 2.51 g (9.48 mmol) of **8** and concentrated HCl (20 mL) was stirred at room temperature for 10 min. Sodium nitrite (13 g, 188 mmol) was then slowly added followed by CuCl (1.0 g, 10 mmol) with stirring continued overnight. The mixture was poured into 1:1 NH_4OH:H_2O, extracted with ethyl acetate, dried over sodium sulfate, and concentrated. The crude residue was purified by flash chromatography on silica gel using 9:1 hexane:ethyl acetate as eluent to yield 984 mg (36%) of **9** as a colorless solid; mp 77−79 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.50 (d, J = 2.0 Hz, 1H), 8.82 (d, J = 2.0 Hz, 1H). ^{13}C NMR (DMSO): δ (ppm) 89.6, 141.7, 142.9, 144.9, 158.33. Anal. ($C_5H_2ClIN_2O_2$) C, H, N.

3-Amino-2-fluoro-5-iodopyridine (11b). A mixture of 230 mg (0.809 mmol) of **9** in ethanol (1 mL), water (6 drops), and concentrated HCl (0.020 mL) was stirred at room temperature for 10 min in a round bottom flask. Iron powder (500 mg, 8.95 mmol) was then added in small portions, and the reaction was placed into a 100 °C oil bath for 20 min. The iron was removed by filtration and washed with ethanol, and then, the combined ethanol layers were concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel using 9:1 hexane:ethyl acetate as eluent to give 190 mg (92%) of **11b** as a colorless solid; mp 129 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 4.15 (br s, 2H), 7.34 (d, J = 2.0 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm) 91.41, 129.67, 136.31, 140.77, 143.90. Anal. ($C_5H_4ClIN_2$) C, H, N.

2-Fluoro-5-iodo-3-nitropyridine (10). A mixture of 3.0 g (0.015 mol) of **9**, KF (9.2 g, 0.158 mol), and DMF (15 mL) was stirred at 45 °C for 18 h. The mixture was poured into saturated brine, stirred for 10 min, and extracted with EtOAc. The organic layer was separated, dried with Na_2SO_4, and concentrated to yield 2.5 g (89%) of **10** as a tan solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 8.70 (s, 1H), 8.77 (d, J = 7.7 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm) 86.66 (J_{CF} = 21.5 Hz), 144.24, 152.96, 156.97, 158.27 (J_{CF} = 58 Hz). Anal. ($C_5H_2FIN_2O_2$) C, H, N.

3-Amino-2-chloro-5-iodopyridine (11a). A mixture of 1.74 g (6.49 mmol) of **10** in ethanol (13 mL), water (2 mL), and concentrated HCl (0.20 mL) was added to a 25 mL round bottom flask and allowed to stir. Iron (3.6 g, 64.4 mmol) was added in portions to the reaction mixture followed by heating at 80 °C for 30 min. The iron was then removed and washed with ethanol over a fritted filter while the ethanol washings were concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel using 1:2 hexane:ethyl acetate as eluent to give 821 mg (53%) of **11a** as a colorless solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 3.94 (br s, 2H), 7.36 (dd, J_{HF} = 2.0. 9.8 Hz, 1H), 7.70 (t, J = 1.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm) 87.84, 131.43, 140.21 (J_{HF} = 13.3 Hz), 150.34, 154.05.

7-*tert*-Butoxycarbonyl-2-*exo*-(2′-amino-3′-bromo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (13). To a stirred solution of 968 mg (3.30 mmol) of **12**[10] in methylene chloride (8 mL) and acetic acid (7 mL) under nitrogen at 0 °C was added bromine (0.260 mL, 5.05 mmol) followed by triethylamine (0.260 mL). After the reaction was stirred for 16 h, the mixture was poured into a 1:1 NH_4OH:H_2O (100 mL) solution and extracted 3× with chloroform. The combined organic extracts were dried with magnesium sulfate and concentrated, and then, the residue was purified by flash chromatography using 4:1 ether triethylamine to give 1.04 g (85%) of **13** as a colorless solid; mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.44 (s, 9H), 1.40−1.55 (m, 2H), 1.70−1.84 (m, 3H), 1.90 (dd, J = 9.0, 12.3 Hz, 1H), 2.70 (dd, J = 4.8, 8.8 Hz, 1H), 4.08 (br s, 1H), 4.33 (br s, 1H), 7.62 (s, 1H, pyridyl CH), 7.83 (s, 1H, pyridyl CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.3 (3C), 28.7, 29.7, 40.3, 44.6, 55.7, 62.0, 79.7, 104.6, 132.9, 138.8, 145.5,154.0, 154.9. Anal. ($C_{16}H_{22}BrN_3O_2$) C, H, N.

7-*tert*-Butoxycarbonyl-2-*exo*-(3′-bromo-2′-iodo-5′-pyridinyl)-7-azabicyclo[2−2.1]heptane (14). To a solution of 209 mg (0.565 mmol) of **13** in isoamyl nitrite (1.2 mL) and diiodomethane (4 mL) was added hydroiodic acid (0.020 mL). The reaction was allowed to stir overnight. The mixture was then poured into a solution of 1:1 NH_4OH:H_2O (20 mL) and extracted with chloroform. The combined organic layers were dried with sodium sulfate and concentrated, and the residue was purified via flash chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (45:9:1) as eluent to give 85 mg (31%) of **14** as a colorless solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.45 (s, 9H), 1.50−1.65 (m, 2H), 1.70−1.90 (m, 3H), 1.99 (dd, J = 9.0, 12.4 Hz, 1H), 2.81 (dd, J = 4.9, 8.9 Hz, 1H), 4.17 (br s, 1H), 4.39 (br s, 1H), 7.79 (s, 1H, pyridyl CH), 8.18 (s, 1H, pyridyl CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.2 (3C), 28.4, 29.5, 40.2, 57.0, 61.6, 80.1, 120.8, 129.6, 138.2, 142.2, 147.5, 154.9.

7-*tert*-Butoxycarbonyl-2-*exo*-(3′-bromo-2′-hydroxy-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (15). To a warm solution (40 °C) of 1.12 g (0.0030 mol) **13** in DMF (5 mL) was added 90% *tert*-butyl nitrite (0.55 mL, 0.0042 mol). The reaction mixture was warmed to 65 °C, stirred for 40 min, and then cooled to room temperature and added to 1 M $KHSO_4$ (200 mL). The reaction mixture was extracted with ethyl acetate, separated, dried ($NaSO_4$), and concentrated to afford 1.09 g (98%) of **15** as an amorphous solid.

7-*tert*-Butoxycarbonyl-2-*exo*-(2′-chloro-3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (16). A solution of 130 mg (0.401 mmol) of **5** in methylene iodide (2.0 mL) and isoamyl nitrite (1.0 mL) was allowed to stir at room temperature for 30 min. HI (0.012 mL) was then added. After 3 h, the reaction was decanted into 1:1 NH_4OH:H_2O and then extracted with chloroform three times. The combined organic extracts were dried with sodium sulfate and concentrated, and the residue was purified by flash chromatography on silica gel using 9:1 hexane:ethyl acetate as eluent to give 74 mg (42%) of **16** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.46 (s, 9H), 1.52−1.62 (m, 2H), 1.70−1.92 (m, 3H), 1.99 (dd, J = 1.7, 10.8 Hz, 1H), 2.82 (dd, J = 4.8, 8.8 Hz, 1H), 4.17 (br s, 1H), 4.39 (br s, 1H), 8.12 (s, 1H, pyridyl CH), 8.23 (s, 1H, pyridyl CH). ^{13}C NMR ($CDC1_3$): δ (ppm) 28.29 (3C), 28.72, 29.66, 40.28, 44.44, 55.8, 61.69, 80.05, 94.73, 141.45, 147.21, 147.73, 152.16, 154.84.

7-*tert*-Butoxycarbonyl-2-*exo*-(2′-fluoro-3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (17). A solution of 105 mg (0.540 mmol) of **4** in methylene iodide (2.0 mL) and isoamyl nitrite (1.0 mL) was allowed to stir at room temperature for 30 min. HI (0.012 mL) was then added. After 24 h, the reaction was decanted into 1:1 NH_4OH:H_2O and then extracted 3× with chloroform. The combined organic extracts were dried with sodium sulfate and concentrated, and then, the residue was purified by flash chromatography on silica gel using 9:1 hexane:ethyl acetate as eluent to give 49 mg (22%) of **17** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.46 (s, 9H), 1.35−1.90 (m, 5H), 2.00 (dd, J = 9.0, 12.4 Hz, 1H), 2.85 (dd, J = 4.8, 8.9 Hz, 1H), 4.16 (br s, 1H), 4.39 (br s, 1H), 8.01 (s, 1H, pyridyl CH), 8.14 (dd, J_{HF} = 2.0, 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.27 (3C), 28.70, 29.60, 40.39, 44.37, 55.82, 61.80, 80.02, 140.82 (J_{CF} = 5.0 Hz), 145.62(J_{CF} =13 Hz), 148.48,154.89, 158.91, 162.62.

2-*exo*-(2′,3′-Disubstituted 5′-pyridinyl)-7-azabicyclo-[2.2.1]heptanes (2a−n). Compounds **2a**−**n** were synthesized using procedures similar to those reported for 2-*exo*-(2′-sub-

Table 2. Molecular Formula for Compounds **2a**−**n**

compd[a]	molecular formula[b]	compd[a]	molecular formula[b]
2a	$C_{11}H_{13}ClF_2N_2$	**2h**	$C_{11}H_{13}Cl_2IN_2$
2b	$C_{11}H_{13}Cl_2FN_2 \cdot 0.5H_2O$	**2i**	$C_{11}H_{15}Cl_2N_3 \cdot H_2O$
2c	$C_{11}H_{13}BrClFN_2$	**2j**	$C_{11}H_{13}Br_2ClN_2$
2d	$C_{11}H_{13}ClFIN_2$	**2k**	$C_{11}H_{13}BrClIN_2$
2e	$C_{11}H_{13}Cl_3N_2$	**2l**	$C_{11}H_{14}BrClN_2O$
2f	$C_{11}H_{13}Cl_2FN_2$	**2m**	$C_{11}H_{16}BrCl_2N_3 \cdot 1.25H_2O$
2g	$C_{11}H_{13}BrCl_2N_2$	**2n**	$C_{11}H_{14}IN_3 \cdot 0.25H_2O$

[a] All of the compounds except **2n**, which was a free base, were characterized as their hydrochloride salts. Detailed experimental procedures for the synthesis of each compound along with mp and ^{1}H NMR data are provided in Supporting Information. [b] Analytical results were within 0.4% of theoretical value.

stituted 5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes.[7] The molecular formulas for the salts characterized are listed in Table 2.

[^{3}H]-1 Binding Assay. The [^{3}H]-**1** binding assays were conducted as previously reported.[7]

[^{125}I]Iodo-MLA Binding Assay.[11] These assays were conducted as previously described with the following modifications: the assay volume was 0.25 mL, the assays were run in Corning 3371 round bottom polypropylene plates (Corning, Inc., Corning, NY), the final radioligand concentration was 90 pM, the assay buffer contained 0.15% bovine serum albumin, and the K_i values were calculated from competition binding curve IC_{50} values using the Cheng−Prusoff equation[20] and an [^{125}I]iodo-MLA K_d of 1.98 nM.

Acknowledgment. This research was supported by the Nationa1 Institute on Drug Abuse Grant DA12001.

Supporting Information Available: Detailed experimental procedures for the synthesis of **2a**−**n**. This material is available free of charge via the Internet at http://pub.acs.org.

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JM0202268