Synthesis, Nicotinic Acetylcholine Receptor Binding, and Antinociceptive Properties of 2-*exo*-2-(2′-Substituted-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptanes. Novel Nicotinic Antagonist

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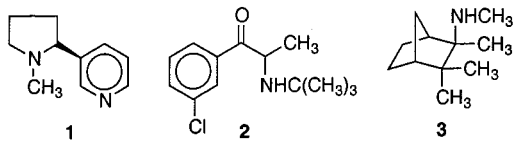
Received August 7, 2001

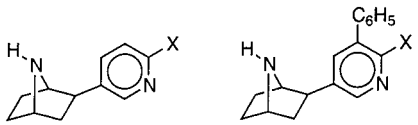
Abstract: A series of 2′-substituted-3′-phenyl epibatidine analogues were synthesized and evaluated for inhibition of binding at nicotine acetylcholine receptors and for antinociceptive properties in mice. The introduction of a bulky phenyl group at the 3′-position exerted a profound influence on both receptor binding and antinociceptive effects. Substitution of different groups at the 2′-position distinguished between agonist and antagonist properties. These results demonstrate that structural requirements for receptor activities and recognition are distinctively different.

Introduction. Cigarette smoking is responsible for over 430 000 deaths in the United States each year and is a major public health problem.[1,2] In addition to chronic obstructive pulmonary disease, smoking is known to cause cancer, heart disease, stroke, and pregnancy complications.[1,3] Even though most smokers want to quit, only about 3% can do so without the use of other intervention. It is now well established that the addiction experienced by smokers is due to the nicotine (**1**) present in the tobacco. Finding approaches for



controlling smoking addiction is presently a very active research area. At present, the major treatment to aid smokers from their addiction is replacement therapy using nicotine gum and patches and the antidepressant bupropion (**2**, Xyban). Alternative approaches under evaluation are the use of the noncompetitive nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine (**3**) as well as combined nicotine/mecamylamine treatment.[4]

Novel nAChR agonists and antagonists are needed to further characterize the various nAChR subtypes and as potential pharmacotherapies for treating smokers. The alkaloid epibatidine (**4a**, *exo*-2-(2′-chloro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane) was isolated from the skin of the Ecuadorian frog, *Epipedobates tricolor*, by Daly and co-workers[5] and was found to have very high affinity for $\alpha\beta$ nAChRs. Pharmacological studies show



4a, X = Cl
b, X = F
c, X = NH_2

5a, X = F
b, X = Cl
c, X = NH_2
d, X = $N(CH_3)_2$

that epibatidine acts as a potent nAChR agonist.[6,7] Although epibatidine is 200 times more potent than nicotine as an analgesic agent, it is also quite toxic, resulting in a limited therapeutic index. Nevertheless, it is an important lead structure for the development of potentially useful pharmacotherapy for treating nicotinic addiction. In this study we report the synthesis, nAChR binding affinity, and antinociception properties of 2-*exo*-2-(2′-substituted-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes (**5a**−**d**) and report that the 2′-fluoro analogue **5a** is a novel nicotine antagonist. To our knowledge, this is the first epibatidine analogue reported to show nAChR antagonist properties.

Chemistry. The compounds **5a**−**d** were synthesized as outlined in Scheme 1. Bromination of *tert*-butoxycarbonyl-2-*exo*-2-(2′-amino-5′-pyridinyl)-7-azabicyclo-[2.2.1]heptane (**6**)[8] using bromine in acetic acid provided the 2′-amino-3′-bromo intermediate **7**. Palladium acetate catalyzed reaction of **7** with phenylboronic acid in dimethoxyethane (DME) in the presence of tri-(2-tolyl)-phosphine and sodium carbonate gave the *tert*-butoxy protected 2′-amino-3′-phenyl analogue **8**. Diazotization of **8** using sodium nitrite in pyridine containing 70% hydrogen fluoride or concentrated hydrochloric acid in the presence of cuprous chloride yielded the 2′-fluoro-3′-phenyl and 2′-chloro-3′-phenyl analogues **5a** and **5b**, respectively. Resolution of **5a** using (+)- and (−)-di-*p*-toluoyl tartaric acid afforded (+)- and (−)-**5a**. Reductive methylation of **8** with formaldehyde using sodium cyanoborohydride yielded the *tert*-butoxy protected 2′-(dimethylamino)-3′-phenyl compound **9**. Treatment of **8** and **9** with trifluoroacetic acid yielded the desired analogues **5c**−**d**.

Biological Results. K_i values for the inhibition of [^{3}H]epibatidine ([^{3}H]**4a**) binding at the $\alpha\beta$ nAChR in male rat cerebral cortex for nicotine, (−)- and (+)-epibatidine [(−)- and (+)-**4a**, respectively], the 2-substituted analogues **4b**−**c**, and the 2′-substituted-3′-phenyl epibatidine analogues **5a**−**d** were determined using previously reported procedures and are listed in Table 1.[8] The 2′-chloro-3′-phenyl analogue **5b** with a K_i value of 0.021 nM is essentially identical to that of (+)- and (−)-**4a.** The K_i value of 0.24 nM for the 2′-fluoro-3′-phenyl (**5a**) analogue is 9-fold lower than that for (+)-**4a** or the 2′-fluoro epibatidine analogue **4b**. The K_i values of both (+)- and (−)-**5a** were essentially identical to that of racemic **5a**. The 2′-amino-3′-phenyl analogue

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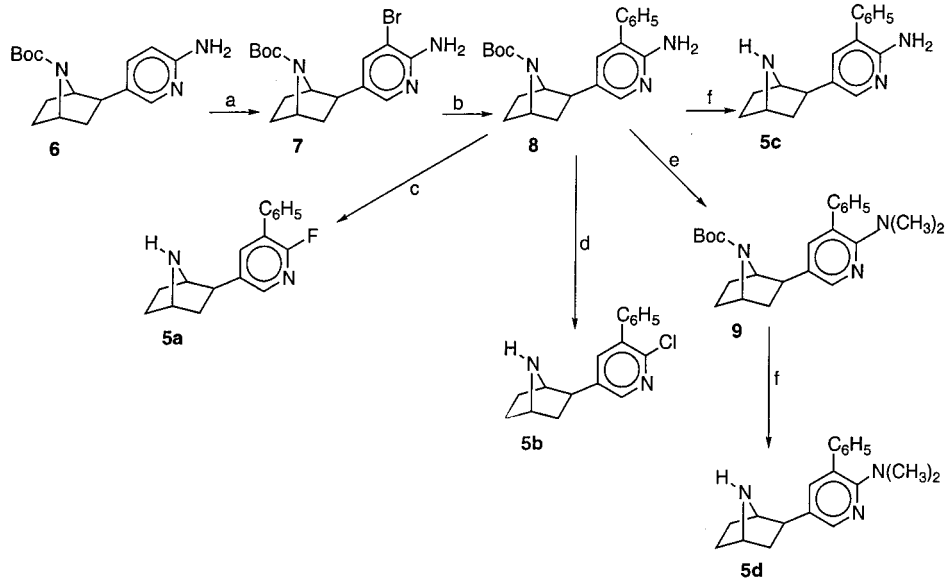
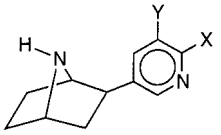
10.1021/jm015561v CCC: $20.00
Published on Web 10/25/2001

Scheme 1[a]



[a] Reagents: (a) Br_2, HOAc; (b) $C_6H_5B(OH)_2$, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, DME, Na_2CO_3; (c) $NaNO_2$, pyridine·HF; (d) $NaNO_2$, HCl, CuCl; (e) $NaBH_3CN$, H_2CO, CH_3OH; (f) CF_3CO_2H.

Table 1. Radioligand Binding Data for 2-*exo*-2-(2′-Substituted-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes (**5a**−**d**)



compd	X	Y	[^{3}H]epibatidine (K_i, nM)	Hill slope
(−)-**1**			1.50 ± 0.30	0.90 ± 0.4
(+)-**4a**[a]	Cl	H	0.026 ± 0.002	0.98 ± 0.05
(−)-**4a**[a]	Cl	H	0.018 ± 0.001	1.02 ± 0.07
4b[a]	F	H	0.027 ± 0.001	0.90 ± 0.02
4c[a]	NH_2	H	1.3 ± 0.1	1.0 ± 0.01
(±)-**5a**	F	C_6H_5	0.24 ± 0.02	0.98 ± 0.05
(+)-**5a**	F	C_6H_5	0.24 ± 0.02	1.13 ± 0.13
(−)-**5a**	F	C_6H_5	0.26 ± 0.05	0.78 ± 0.05
5b	Cl	C_6H_5	0.021 ± 0.005	0.81 ± 0.02
5c	NH_2	C_6H_5	0.33 ± 0.05	0.93 ± 0.03
5d	$(CH_3)_2N$	C_6H_5	57.5 ± 7.5	0.84 ± 0.10

[a] Taken from Carroll et al. *J. Med. Chem.* **2001**, *44*, 2229−2237.

5c with a K_i value of 0.33 nM is 13-fold lower than that for **4a** but is four times more potent than the 2′-amino epibatidine analogue **4c** which has a K_i value of 1.3 nM. Dimethylation of **5c** to give the 2′-*N*,*N*-dimethyl analogues (**5d**) resulted in a 150-fold loss of affinity relative to the 2′-amino epibatidine analogue **4c**.

Compounds **5a**−**d** were tested for antinociception using the tail-flick and hot-plate tests in male ICR mice using previously reported procedures.[9] The effects of the compounds on body temperature and locomotor activity were also measured (Table 2). In contrast to nicotine and epibatidine, the 2′-fluoro-3′-phenyl analogue (**5a**), its optical isomers, and the 2′-amino-3′-phenyl analogue (**5c**) were not active in the tail-flick and hot-plate tests. Furthermore, the 2′-chloro-3′-phenyl analogue **(5b)** was 117 less potent than (−)-epibatidine [(−)-**4a**]. Compound **5b** was also inactive after intrathecal injection, which suggests that the lack of activity is not due to pharmacokinetic properties. Similar differences in potency were found on the body temperature and locomotor activity decrease (Table 2). The pharmacological action of these analogues on body temperature and locomotor activity were blocked by pretreatment with the nicotinic antagonist mecamylamine (1 mg/kg, sc). The 2′-fluoro-3′-phenyl analogue (±)-**5a** and its optical analogues were evaluated as nicotinic antagonists in the tail-flick and hot-plate tests. Compounds (±)-**5a**, (+)-**5a**, and (−)-**5a** blocked the antinociceptive effect of nicotine in the tail-flick test after sc administration with AD_{50} values of 0.5, 1.0, and 0.08 mg/kg, respectively. In the hot-plate test, (±)-**5a**, (+)-**5a**, and (−)-**5a** blocked the antinociceptive effect of nicotine with AD_{50} values of 1.2, 2.4, and 0.7 mg/kg, respectively. In contrast, the 2′-amino-3′-phenyl analogue (**5c**) failed to block nicotine's effects in both analgesic tests.

Discussion. Synthetic methods have been developed for the preparation of *tert*-butoxycarbonyl-2-*exo*-2-(2′-amino-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane (**8**). This key intermediate was used to prepare the 3′-phenyl epibatidine analogue **5b** as well as several other 3′-phenyl substituted epibatidine analogues where the 2′-chloro group in **5b** is replaced by a fluoro (**5a**), amino (**5c**), or dimethylamino (**5d**) group. Radioligand binding studies show that compound **5b** where a phenyl group has been added to the 3′-postion of epibatidine has a K_i value which is essentially the same as that of epibatidine. Replacement of the 2′-chloro with the smaller, highly electronegative fluoro group to give **5a** or with the electron releasing amino group to give **5c** resulted in some loss in binding affinity; however, all three analogues **5a**−**c** exhibited greater affinity than nicotine. Surprisingly, unlike epibatidine, which is a potent analgesic, these 3′-phenyl substituted analogues have dramatically reduced analgesic activity or no analgesic activity. In addition, each differed regarding their pharmacological profiles. For example, a striking feature of these high affinity analogues is their lack of lethality at the doses tested. Whereas epibatidine is lethal in the low μg/kg dose range, these analogues were nonlethal up to 15 mg/kg.

Table 2. Pharmacological Properties of 2-*exo*-2′-Substituted-3′-phenyl-5′-pyridinyl)-7-azabicyclo[2.2.1]heptanes (**5a**−**d**)[a]

	ED_{50}				AD_{50}	
compd	tail-flick	hot-plate	hypothermia	hypomotility	tail-flick	hot-plate
(−)-**4**	0.006 (0.001−0.01)	0.004 (0.001−0.008)	0.004 (0.002−0.008)	0.001 (0.0005−0.005)		
(−)-**1**	1.3 (0.5−1.8)	0.65 (0.25−0.85)	1 (0.6−2.1)	0.5 (0.15−0.78)		
(±)-**5a**	3% @ 15	4% @ 15	−0.5 °C @ 10	4.7 (3.5−8.5)	0.5 (0.25−1.5)	1.2 (0.9−2.1)
(+)-**5a**	7% @ 15	8% @ 15	−0.4 °C @ 10	NT[b]	1.0 (0.5−1.5)	2.4 (1.9−3.8)
(−)-**5a**	5% @ 15	10% @ 15	−0.8 °C @ 10	NT[b]	0.08 (0.03−0.2)	0.7 (0.1−2.5)
5b	0.7 (0.5−1.0)	1.0 (0.5−2.0)	0.40 (0.1−0.9)	0.35 (0.2−0.85)		
5c	2% @ 10	15% @ 10	−0.5 °C @ 10	NT[b]	IA[c]	IA[c]

[a] Results are presented as ED_{50} or AD_{50} values (± confidence limits) in mg/kg or as percent effect at the individual mg/kg dose. [b] Not tested. [c] Inactive.

Since (±)-**5a** and its optical isomers (+)- and (−)-**5a** show high affinity for the nAChR and are devoid of agonist effects, they are ideal candidates for antagonists. Indeed, **5a** proved to be effective in antagonizing the antinociceptive effects of nicotine with a potency similar to that of mecamylamine.[10] In contrast to the agonist activity of epibatidine, the antagonist effect of **5a** was enantioselective with (−)-**5a** being 13 times more potent than (+)-**5a**.

These finding are intriguing in light of the fact that supraspinal nicotine analgesia (as measured in the hot-plate test) was eliminated in $\alpha_4\beta_2$ receptor knock-out mice, whereas spinal nicotine analgesia was attenuated.[11] Retention of analgesic activity in these mutant mice suggested the possible role of multiple AChR's in nicotine spinal analgesia, a notion consistent with the different enantioselectivities of **5a** in the tail-flick and hot-plate procedures.

The degree to which the 3′-phenyl substituent influences the interaction with the nicotine receptors is best illustrated through a comparison of analogues with and without this moiety. For example, the 2′-chloro-3′-phenyl analogue **5b** exhibited receptor affinity potency similar to epibatidine (**4a**) which lacks the 5′-phenyl group[8] but with reduced activity in the in vivo assays. However, 2-*exo*-2-(2′-fluoro-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane (**4b**) lacking the 3′-phenyl substituent has receptor affinity and analgesic potency equivalent to that of epibatidine,[8] whereas addition of the 3′-phenyl to give **5a** reduced receptor affinity an order of magnitude and changed the pharmacological profile from an agonist to an antagonist. Interestingly, the receptor affinity and potency (inactive in both series) of the 2-*exo*-2-(2′-amino-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**4c**) was not altered by the addition of the 3′-phenyl to give **5c**.

Conclusion. It is clear that introduction of a bulky phenyl group at the 3′-position exerted a profound influence on both receptor binding (recognition) and receptor activation. The mere presence of this group further defines the volume map of the pharmacophore and identifies an important lead structure for future structure−activity relationship strategies. Moreover, it provides key insights into structural requirements for activation of the receptor. Substitutions of different halides at the 2′-position distinguished between agonist and antagonist properties, while a 2′-amino exhibited neither property. These findings demonstrate that structural requirements for receptor activation and recognition are distinctively different. Further studies addressing the question of nAChR subtypes responsible for various in vivo effects are currently underway.

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

Supporting Information Available: Data for compounds **5a**−**d** include (1) HPLC trace, (2) ^{1}H NMR spectra, and (3) electrospray mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM015561V