Design and Synthesis of Isoxazole Containing Bioisosteres of Epibatidine as Potent Nicotinic Acetylcholine Receptor Agonists

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An efficient synthesis of isoxazole containing isosteres of epibatidine is described. The synthesis proceeded from *N-tert*-butoxycarbonyl (Boc)-*exo*-2-(methoxycarbonyl)-7-azabicyclo[2.2.1]heptane (9). Compound 9 was reacted with the dilithium salt of an appropriately substituted oxime in tetrahydrofuran (THF). Cyclodehydration of the resultant β-keto oxime and deprotection of the *N*-Boc group in 5 N aqueous HCl afforded the isoxazole containing isosteres of epibatidine (6—8). The binding affinities of these compounds were determined at the nicotinic acetylcholine receptor for the displacement of [^{3}H]cytisine. The unsubstituted isoxazole containing isostere (6) showed the lower binding potency compared to the 3′-methylisoxazole isostere (7). Substitution with a phenyl group at the 3′-position of the isoxazole significantly reduced the binding potency. The *in vivo* toxicological studies of these analogs were also performed. The LD_{50} of the analogs ranged in the order: Me>H>Ph.

Key words epibatidine; isoxazole isosteres; binding affinity; toxicity

The alkaloid epibatidine (*exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane) (**1**; Fig. 1) was first isolated from the skin of the Ecuadorian poison frog, *Epipedobates tricolor*, by Daly and co-workers in minute quantities (0.5 mg from 750 frogs).[1] Preliminary biological assays showed that epibatidine was a very potent analgesic (200-500-fold more potent than morphine) with a non-opioid mechanism of action.[2] Apart from its analgesia effects, epibatidine induced several other effects consistent with potent actions at neuronal nicotinic receptors. It lowered body temperature in mice and decreased their locomotor activity.[2c,d] As with its analgesia effects, in all of the studies of epibatidine's behavioral and physiological effects so far reported, the (+) and (−)-enantiomers were virtually equipotent,[2c] suggesting that epibatidine's chiral center was not critical for its productive interaction with the neuronal nicotinic receptor.

Although epibatidine is the most potent neuronal nicotinic acetylcholine receptor (nAChR) ligand yet reported (K_i=0.04 nM at [^{3}H](−)-cytisine sites),[2a,b] it also displays high affinity for the neuromuscular junction nAChRs (K_i=2.7 nM)[3] and is highly toxic.[2d,4] Nevertheless, the discovery of epibatidine has rekindled interest in designing ligands, which would selectively interact with the neuronal nicotinic receptors and have no or minimal side-effects as compared to epibatidine. Since nicotinic receptors are involved in a diverse group of central and peripheral nervous system disorders, such selective nAChR agonists portend improved therapies for cognitive and attention disorders, Alzheimer's disease, Parkinson disease, anxiety, depression, smoking cessation, neuroprotection, schizophrenia, and analgesia.[5,6]

Bioisosterism is an important concept and serves as a valuable aid in structure–activity relationship studies and new drug design.[7] Based on molecular modeling and conformational studies we found that the pyridinyl pharmacophore of epibatidine could be replaced with an isoxazole pharmacophore. Computational properties of such compounds suggested them to be potent nicotinic agonists.[8] Besides, it was anticipated that such compounds might possess reduced toxicity compared to epibatidine. The choice of an isoxazole pharmacophore was further substantiated by the fact that ABT-418 (**2**; Fig. 1), which is currently under clinical development as a cognition enhancer for Alzheimer's disease, is an isoxazole isostere of nicotine (**3**; Fig. 1).[9] In this communication we wish to report an efficient synthesis and biological evaluation of some isoxazole isosteres of epibatidine (**6**—**8**; Fig. 1).

Synthesis The key step in the synthesis of isoxazole isosteres (**6**—**8**; Fig. 1) of epibatidine was the condensation of *N*-protected-7-azabicyclo[2.2.1]heptan-2β-carboxylic acid methyl ester with the dilithium salt of an appropriately substituted oxime, followed by cyclodehydration and deprotec-

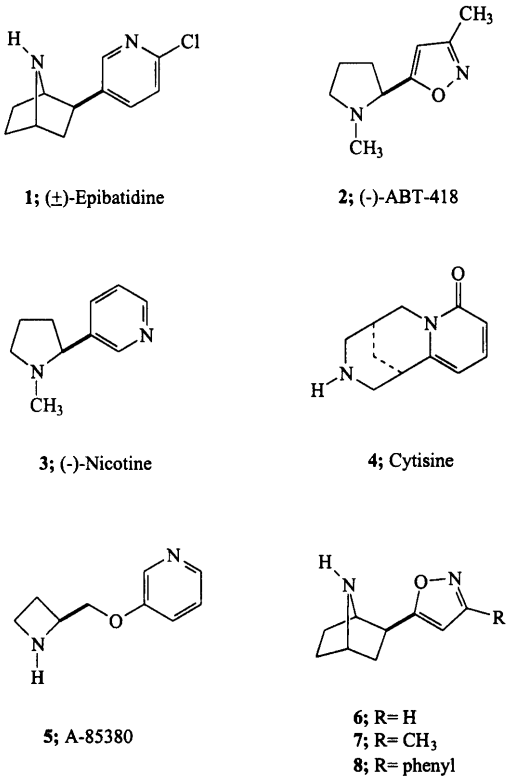

Fig. 1. Structures of Potent Nicotinic Acetylcholine Receptor Agonists

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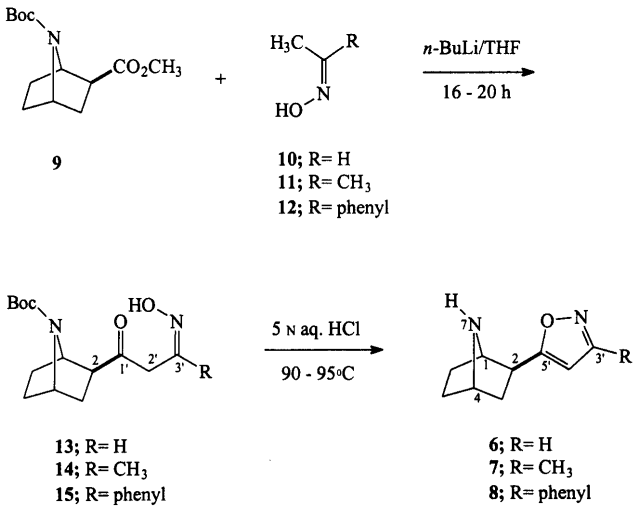

Chart 1

tion. Although a variety of synthetic approaches have been developed to construct the novel 7-azabicyclic system,[10] the *N*-protected-2β-carbomethoxy-7-azabicyclo[2.2.1]heptanes are not readily available. Treatment of the *exo* ester (**9**)[11] with the dilithium salt of acetaldehyde oxime (**10**), acetone oxime (**11**) or acetophenone oxime (**12**), which was generated by lithiation of the corresponding oxime with *n*-BuLi at 0 °C under dry nitrogen, afforded the β-keto oximes (**13**—**15**) as shown in Chart 1.[12] It should be noted here that the β-keto oxime **15** was generated from the reaction of acetophenone oxime (**12**) with **9** at room temperature. The reaction of acetone oxime (**11**) with **9**, however, did not go to completion at ambient temperature even after stirring for 72 h.[12b] The β-keto oximes **13** and **14** could only be generated by first refluxing the reaction mixtures for 4 and 1 h, respectively, and then continuing the reaction at ambient temperature for 16—20 h. The next step was cyclodehydration of the β-keto oximes. Previously described *in situ* reaction conditions for cyclodehydration of the β-keto oxime with tetrahydrofuran (THF)/H_2SO_4[12a] or conc. HCl[13] either did not yield any cyclized product or gave a complex mixture of products. Therefore, the β-ketoximes (**13**—**15**) were separated before subjecting to cyclodehydration. Cyclodehydration of the β-keto oximes was accomplished by heating in 5 N aqueous HCl at 90—95 °C for 2.5—3 h. The protective *N*-tert-butoxycarbonyl (Boc) group was also removed under the same reaction conditions. The isoxazole isosteres were then purified by column chromatography and converted into the bitartaric acid salts.

Molecular Modeling The molecular modeling studies utilized Spartan 5.0 (Wavefunction, Inc.) running under IRIX 6.4 on an SGI Octane Workstation. The built-in Sybyl molecular force field was used to minimize the structures. Conformational search was performed using the systematic searching method. The energies of the conformations as well as the N–N distances were plotted against the conformer structures. Following minimum energy conformational analysis, the root mean square (RMS) deviation was determined by superimposing the lowest energy conformers of epibatidine (**1**), and **6**. This step was performed using the Alchemy 2000 (Tripos) software running under Windows NT 4.0 on a Pentium 200 mHz Pro PC. For superimposition three atom pairs were picked: three atoms of compound **6** (bridgehead nitrogen atom, C-2 carbon atom of the 7-azabicyclic ring attached to the isoxazole ring and the nitrogen atom of the isoxazole ring) and three atoms of epibatidine (bridgehead nitrogen atom, C-2 carbon atom of the 7-azabicyclic ring attached to the pyridine ring and the nitrogen atom of the pyridine ring).

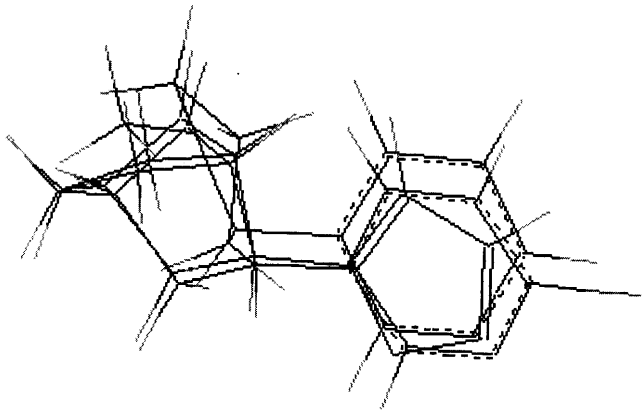

Fig. 2. Superimposition of Epibatidine (**1**), Nicotine (**3**), and Isoxazole Isostere (**6**)

Table 1. Relative Potency of Isoxazole Isosteres of Epibatidine for Displacement of [^{3}H]Cytisine from Rat Cortical Membranes and LD_{50} in CD-1 Mice

Compound	IC_{50} (nM)[a]	LD_{50} (mg/kg)[b]
Epibatidine	0.331±0.1	0.196
Cytisine	1.11±0.1	–
Nicotine	9.22±0.2	27
6	3.17±0.09	2.23
7	1.33±0.08	1.82
8	147±1.0	>130[c]

a) IC_{50} values are mean of ±SD of two to four replicate determinations. *b*) LD_{50} values are the 50% of mice (n=10 per dose) exhibiting seizures and death. *c*) No mice died or exhibited seizures and convulsions at this dose.

Nicotinic Receptor Binding The *in vitro* nicotinic receptor binding affinities of the synthesized compounds were determined for the displacement of [^{3}H]cytisine in rat cerebral cortical tissue. As shown in Table 1, epibatidine was the most potent of all the compounds. Substitution of the pyridine pharmacophore with an unsubstituted isoxazole ring led to a 9.6-fold decrease in the binding potency at the nicotinic receptor. Compound **7** was the most potent of all the isoxazole isosteres at the nicotinic receptor and was comparable to cytisine. However, both compounds **6** and **7** were more potent than nicotine, 2.9 and 6.9-fold, respectively. The binding potencies of compounds **6**—**8** were 4 to 445-fold less than epibatidine.

Lethality The LD_{50} of the isoxazole isosteres was determined in CD-1 mice (n=10) and compared with epibatidine and nicotine. As shown in Table 1, compounds **6** and **7** were 11.3 and 9.2-fold less toxic than epibatidine, respectively. Compound **8** did not cause any death at the dose of 130 mg/kg body weight; it only produced sedation without affecting any vital signs.

Discussion

High-resolution 3-D structures of the nicotinic receptors located on cell membranes are not yet known. However, both

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the Beers and Reich[14] and the Sheridan[15] models have proposed two essential elements of the nicotinic pharmacophore: a hydrogen bond acceptor (*i.e.*, pyridine lone pair of nicotine) and a charged species, *e.g.*, a protonated or quaternary nitrogen. These models suggested an internitrogen distance of around 4.85 Å in nicotine. The subsequent discovery of epibatidine as an exceptionally high-affinity ligand for nicotinic receptor has suggested that the optimal internitrogen distance for a high-affinity ligand may be closer to 5.54 Å,[16] Recently, compound A-85380 (**5**; Fig. 1) with higher internitrogen distance (6.29 Å) was found to possess subnanomolar affinity for the nicotinic receptors.[17] Given the importance of the distance between both binding sites, we designed the isoxazole isosteres of epibatidine. The molecular mechanics based conformational studies of these compounds (**6**—**8**) revealed an internitrogen distance of 5.14 Å, well within the range proposed by the above models.[8] Furthermore, superimposition of **6** on epibatidine after minimum-energy conformational analysis indicated a good fit. The RMS deviation was 0.14 (Fig. 2). We also measured the distances between bridgehead nitrogen and isoxazole oxygen in **6**—**8**, because the oxygen in the isoxazole ring could also participate as H-bond acceptor. This distance is, however, shorter (4.28 Å) than proposed by the above models; therefore, participation of the isoxazole oxygen in H-bonding appears unlikely.

The binding potencies of compounds **6**—**8** were determined by displacement of [^{3}H]cytisine from rat cortical membranes. The choice of the ligand [^{3}H]cytisine was based on its higher affinity for neuronal nicotinic receptors, relatively low nonspecific binding, slow rate of dissociation from the receptor.[18] Substitution of the pyridine ring with an unsubstituted isoxazole ring in epibatidine (*i.e.*, **6**) caused a 9.6-fold reduction in binding potency compared to epibatidine. The 3′-methylisoxazole containing isostere **7** was 2.38-fold more potent than **6**. Compound **7** was equipotent to cytisine and only 4-fold less potent than epibatidine. Both compounds **6** and **7** were, however, more potent than nicotine, 2.9 and 6.9-fold, respectively. Recently, binding affinity of **7** has been reported to be 10-fold less than epibatidine (0.6 *vs.* 0.058 nM, respectively) for the displacement of [^{3}H]nicotine.[13] This discrepancy in binding potency, however, could arise due to the different radioligands used in the two studies. Introduction of a phenyl group at the 3′-position of **6** (*i.e.*, **8**) significantly reduced the binding affinity, 445-fold less than epibatidine. This result was unexpected because an introduction of a methyl group at 3′-position in **6** had increased the binding potency by 2.38-fold. It could be speculated that while a lipophilic functionality may be beneficial for better binding of the isoxazole analogs of epibatidine; a bulky lipophilic group such as a phenyl ring may not be tolerated at the nicotinic receptor. Further evidence will be required to define the size and lipophilicity of the functional groups, which would enhance the binding potency of the isoxazole analogs of epibatidine at the nicotinic receptor. Nevertheless, the present investigation points to the fact that a lipophilic group, which is smaller than a phenyl ring might be appropriate for good binding of the isoxazole analogs of epibatidine at the nicotinic receptor.

Toxicity is one of the major concerns in developing nicotinic agonists. Therefore, we determined the LD_{50} of the isoxazole analogs of epibatidine in CD-1 mice. As shown in Table 1, the replacement of the pyridine ring in epibatidine with an isoxazole ring (**6**—**8**) caused significant reduction in toxicity. The LD_{50} of compounds **6** and **7** were 11.3 and 9.2-fold less compared to epibatidine, respectively. The most important observation was that the introduction of a methyl group at the 3′-position of **6** (*i.e.*, compound **7**) while it increased the binding potency by 2.38-fold, it only increased toxicity slightly. Compound **8** did not cause death at the dose level of 130 mg/kg body weight. Higher doses were not studied and thought to be of little significance. Furthermore, it is important to note that while compounds **6** and **7** were less toxic than epibatidine, they still exhibited convulsions, seizures, and bronchial spasm similar to epibatidine. However, analog **8** was qualitatively different and exhibited no convulsions, seizures, and bronchial spasm. The animals appeared to be sedated and showed no signs of distress. Therefore, modifications at the 3′-position of the isoxazole analog **6** with lipophilic groups less bulky than a phenyl group might lead to high-affinity ligands with reduced toxicity for the nicotinic receptor. On the other hand appropriate substitutions in the phenyl ring of compound **8** might lead to ligands for the nicotinic receptor with qualitatively different properties.

It is important to note that the methylisoxazole analog of nicotine, ABT-418 (**2**; Fig. 1) was 3-fold less potent than nicotine for the displacement of [^{3}H]cytisine in rat brain, but its LD_{50} was only 2-fold less than nicotine.[9] This indicates that the 7-azabicyclic[2.2.1]heptane ring system of the isoxazole isosteres may be the primary determinant of the binding potency at the nicotinic receptor, whereas an isoxazole pharmacophore might be responsible for reduction in binding potency as well as toxicity. Furthermore, an important finding is that a methylisoxazole in epibatidine produced much reduced lethality compared to a methylisoxazole in nicotine.

In conclusion, some isoxazole analogs of epibatidine were synthesized. A methyl group at the 3′-position (**7**) of compound **6** increased the binding potency by 2.38-fold, but did not increase the LD_{50} significantly. A phenyl group at the 3′-position (**8**) significantly reduced the binding potency at the nicotinic receptor. The toxic effects of **8** were qualitatively different than epibatidine, nicotine, **6** and **7**. The present data, though limited in scope, are significant and demonstrate that the potency of epibatidine at the nicotinic acetylcholine receptor can be separated from its toxic effects.

Experimental

All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, unless otherwise noted. Acetophenone oxime was purchased from Lancaster, Windham, NH. [^{3}H]cytisine HCl (39.7 Ci mM^{-1}) was purchased from Dupont NEN products, Inc., Boston, MA. THF was dried by distillation over CaH_2. The HPLC grade solvents were obtained from Fisher Scientific, St. Louis, MO. Petroleum ether refers to pentanes with a boiling point range of 30—60 °C. Chromatography refers to flash chromatography on silica gel (Silica gel 60 Å, 230—400 mesh). Thin layer chromatography (TLC) utilized silica gel plates with fluorescent indicator (Eastman Kodak Co., Rochester, NY).

The melting points were determined on a Thomas–Hoover capillary melting point apparatus and are not corrected. Elemental analysis was performed by Midwest Micro Lab Ltd., Indianapolis, IN. The ^{1}H-NMR spectra were recorded on a Varian 300 MHz broad band mercury spectrometer. Electron impact (EI)-MS was performed on a Hewlett-Packard GC-MS, Model 5895 (Palo Alto, CA).

***exo*-7-(*tert*-Butoxycarbonyl)-2-(methoxycarbonyl)-7-azabicyclo[2.2.1]-**

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heptane (9) This compound was synthesized in three steps starting from *N*-Boc-pyrrole and 3-bromopropiolic acid methyl ester.[11] ^{1}H-NMR ($CDCl_3$): δ 4.51 (m, 1H, C(1)-H), 4.30 (m, 1H, C(4)-H), 3.70 (s, 3H, OCH_3), 2.55 (dd, J=9.0, 5.1 Hz, 1H, C(2)-H), 2.28 (m, 1H, C(3)-H), 1.80 (m, 2H, C(5,6)-H), 1.61 (dd, J=12.5, 8.7 Hz, 1H, C(3)-H), 1.43 (s, 9H, $C(CH_3)_3$), 1.42 (m, overlapping 2H, C(5,6)-H) ppm.

***exo*-7-(*tert*-Butoxycarbonyl)-7-azabicyclo[2.2.1]heptan-1,3-propanedione-3-oxime (13)** To the solution of acetaldehyde oxime (**10**; 70.8 mg, 1.2 mM) in dry THF (2.0 ml) was added *n*-BuLi (1.5 ml, 2.4 mM; 1.6 M solution in hexanes) dropwise at 0 to −5 °C under dry N_2. After stirring the mixture at 0 °C for 2 h, a solution of the *exo* ester **13** (102 mg, 0.4 mM) in THF (2.0 ml) was added slowly while maintaining the temperature at 0 to 5 °C. The reaction mixture was allowed to warm to room temperature and then heated to a gentle reflux (60—65 °C). After refluxing for 4 h, the mixture was cooled to room temperature and continued to stir at room temperature for 16 h. Monitoring of the reaction by TLC (20% EtOAc/petroleum ether) showed no starting material. The stirring was stopped and 2.0 ml of 10% aqueous HCl was added carefully after cooling the reaction mixture in an ice bath. The contents were transferred to a separatory funnel and the two layers were separated. The aqueous layer was washed with ether (5 ml), then basified with $NaHCO_3/Na_2CO_3$ (solid) to pH *ca.* 9—10 and extracted with CH_2Cl_2 (4×10 ml). The combined organic layers were dried ($MgSO_4$) and concentrated to afford crude product (150 mg). Purification over silica gel (2.0 g) (EtOAc/petroleum ether 1 : 8 to 1 : 1) afforded 110 mg of an oil (97.5%). EI-MS (12 eV DIP): *m/z* 282 (M^+, $C_{14}H_{22}N_2O_4$). ^{1}H-NMR ($CDCl_3$): δ 7.20 (d, J=1.8 Hz, 1H, C(3′)-H), 4.49 (m, 1H, C(1)-H), 4.27 (m, 1H, C(4)-H), 2.81 (d, J=1.5 Hz, 2H, C(2′)-H_2), 2.15 (dd, J=8.7, 6.0 Hz, 1H, C(2)-H), 1.81 (m, 1H, C(3)-H), 1.68 (m, 2H, C(5,6)-H), 1.45 (m, 1H, C(3)-H), 1.44 (s, 9H, $C(CH_3)_3$), 1.28 (m, 2H, C(5,6)-H) ppm. *Anal.* Calcd for $C_{14}H_{22}N_2O_4$; C, 59.59; H, 7.79; N, 9.92. Found: C, 59.89; H, 7.61; N, 9.69.

***exo*-7-(*tert*-Butoxycarbonyl)-7-azabicyclo[2.2.1]heptan-1,3-butanedione-3-oxime (14)** To the solution of acetone oxime (**11**; 58.4 mg, 0.8 mM) in dry THF (2.0 ml) was added *n*-BuLi (1.0 ml, 1.6 mM; 1.6 M solution in hexanes) dropwise at 0 to −5 °C under dry N_2. After stirring the mixture at 0 °C for 45 min, a solution of the *exo* ester **9** (102 mg, 0.4 mM) in THF (2.0 ml) was added slowly while maintaining 0 to 5 °C temperature. The reaction mixture was allowed to warm to room temperature and then heated to a gentle reflux (60—65 °C). After refluxing for 1 h, the reaction was allowed to occur at room temperature for another 16 h. The stirring was stopped and 2.0 ml of 10% aqueous HCl was added carefully after cooling the reaction mixture in an ice bath. The contents were transferred to a separatory funnel and the two layers were separated. The aqueous layer was washed with ether (2×5 ml), then basified with $NaHCO_3/Na_2CO_3$ (solid) to pH *ca.* 9—10 and extracted with CH_2Cl_2 (4×10 ml). The combined organic layers were dried ($MgSO_4$) and concentrated to afford crude product (140 mg). Purification over silica gel (2.0 g) (EtOAc/petroleum ether 1 : 8 to 1 : 1) afforded 80 mg of an oil (67.7%). EI-MS (12 eV DIP): *m/z* 296 (M^+, $C_{15}H_{24}N_2O_4$). ^{1}H-NMR ($CDCl_3$): δ 4.41 (m, 1H, C(1)-H), 4.25 (m, 1H, C(4)-H), 2.69 (dd, J=9.9, 6.6 Hz, 1H, C(2)-H), 2.05 (m, 1H, C(3)-H), 2.00 (s, 2H, C(2′)-H_2), 1.99 (s, 3H, C(4′)-H_3), 1.79 (m, 2H, C(5,6)-H), 1.68 (m, 1H, C(3)-H), 1.44 (s, 9H, $C(CH_3)_3$), 1.43 (m, 2H, C(5,6)-H) ppm. *Anal.* Calcd for $C_{15}H_{24}N_2O_4$; C, 60.83; H, 8.10; N, 9.45. Found: C, 60.62; H, 8.01; N, 9.68.

***exo*-7-(*tert*-Butoxycarbonyl)-7-azabicyclo[2.2.1]heptan-1,3-(3-phenyl)-propanedione-3-oxime (15)** To the solution of acetophenone oxime (**12**; 108 mg, 0.8 mM) in dry THF (2.0 ml) was added *n*-BuLi (1.0 ml, 1.6 mM; 1.6 M solution in hexanes) dropwise at 0 to −5 °C under dry N_2. After stirring the mixture at 0 °C for 1.5 h, a solution of the *exo* ester **9** (102 mg, 0.4 mM) in THF (2.0 ml) was added slowly while maintaining 0 to 5 °C temperature. The reaction mixture was allowed to warm to room temperature and continued to stir for 20 h. The stirring was stopped and 2.0 ml of 10% aqueous HCl was added carefully after cooling the reaction mixture in an ice bath. The contents were transferred to a separatory funnel and the two layers were separated. The aqueous layer was washed with ether (2×5 ml), then basified with $NaHCO_3/Na_2CO_3$ (solid) to pH *ca.* 9—10 and extracted with CH_2Cl_2 (4×10 ml). The combined organic layers were dried ($MgSO_4$) and concentrated to afford crude product (230 mg). Purification over silica gel (2.0 g) (EtOAc/petroleum ether 1 : 8 to 1 : 1) afforded 120 mg of an oil (83.9%). EI-MS (12 eV DIP): *m/z* 358 (M^+, $C_{20}H_{26}N_2O_4$). ^{1}H-NMR ($CDCl_3$): δ 7.64 (m, 2H, aromatic), 7.38 (m, 3H, aromatic), 4.56 (m, 1H, C(1)-H), 4.28 (m, 1H, C(4)-H), 3.19 (s, 2H, C(2′)-H2), 2.60 (dd, J=8.7, 5.7 Hz, 1H, C(2)-H), 1.82 (m, 1H, C(3)-H), 1.75 (m, 2H, C(5,6)-H), 1.50 (m, 1H, C(3)-H), 1.47 (s, 9H, $C(CH_3)_3$), 1.44 (m, 2H, C(5,6)-H) ppm. *Anal.* Calcd for $C_{20}H_{26}N_2O_4$; C, 67.05; H, 7.25; N, 7.81. Found: C, 67.34; H, 7.19; N, 7.49.

***exo*-2-(5-Isoxazolyl)-7-azabicyclo[2.2.1]heptane (6)** The β-keto oxime **13** (80 mg, 0.28 mM) was dissolved in 3.0 ml of 5 N aqueous HCl and heated at 90—95 °C. After heating for 2.5 h, the reaction mixture was cooled in an ice bath. It was then neutralized with $Na_2CO_3/NaHCO_3$ to pH *ca.* 8—9 and extracted with EtOAc (5×10 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum to afford 40 mg of crude product. Purification over silica gel column gave 30 mg (65.3%) oil. EI-MS (12 eV DIP): *m/z* 163.9 (M^+, $C_9H_{12}N_2O$). ^{1}H-NMR ($CDCl_3$): δ 8.14 (d, J=1.5 Hz, 1H, C(3′)-H), 6.05 (d, J=1.5 Hz, C(4′)-H), 3.93 (m, 1H, C(1)-H), 3.88 (m, 1H, C(4)-H), 3.16 (dd, J=9.0, 5.7 Hz, 1H, C(2)-H), 2.04 (m, 1H, C(3)-H), 1.83 (m, 2H, C(5,6)-H), 1.51 (m, 1H, C(3)-H), 1.36, 1.34 (m, overlapping 2H, C(5,6-H) ppm. *Anal.* Calcd for $C_9H_{12}N_2O$; C, 65.87; H, 7.31; N, 17.06. Found: C, 65.47; H, 7.34; N, 16.79.

***exo*-2-(3-Methyl-5-isoxazolyl)-7-azabicyclo[2.2.1]heptane (7)** The β-keto oxime **14** (80 mg, 0.27 mM) was dissolved in 2 ml of 5 N aqueous HCl and heated at 90—95 °C. After heating for 3 h, the reaction mixture was cooled in an ice bath. It was then neutralized with $Na_2CO_3/NaHCO_3$ to pH *ca.* 8—9 and extracted with EtOAc (5×10 ml). The combined extracts were dried ($MgSO_4$) and concentrated *in vacuo* to afford 35 mg of crude product. Purification over silica gel column gave 30 mg (62.5%) oil. EI-MS (12 eV DIP): *m/z* 178 (M^+, $C_{10}H_{14}N_2O$). ^{1}H-NMR ($CDCl_3$): δ 5.81 (s, 1H, C(4′)-H), 3.78 (m, 1H, C(1)-H), 3.73 (m, 1H, C(4)-H), 3.01 (dd, J=9.0, 4.8 Hz, 1H, C(2)-H), 2.25 (s, 3H, C(3′)-H3), 1.92 (dd, J=12.6, 9.0 Hz, 1H, C(3)-H), 1.77 (m, 2H, C(5,6)-H), 1.69 (m, 1H, C(3)-H), 1.47, 1.42 (m, overlapping 2H, C(5,6-H) ppm. *Anal.* Calcd for $C_{10}H_{14}N_2O$; C, 67.43; H, 7.86; N, 15.72. Found: C, 67.67, H, 7.66; N, 15.43.

***exo*-2-(3-Phenyl-5-isoxazolyl)-7-azabicyclo[2.2.1]heptane (8)** The β-keto oxime **15** (110 mg, 0.30 mM) was dissolved in 2.0 ml of 5 N aqueous HCl and heated at 90—95 °C. After stirring at the same temperature for 3 h, the reaction mixture was cooled in an ice bath. It was then neutralized with $Na_2CO_3/NaHCO_3$ to pH *ca.* 8—9 and extracted with EtOAc (5×10 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum to afford 35 mg of crude product. Purification over silica gel column afforded 50 mg (67.8%) oil. EI-MS (12 eV DIP): *m/z* 240 (M^+, $C_{15}H_{16}N_2O$). ^{1}H-NMR ($CDCl_3$): δ 7.78 (m, 2H, aromatic), 7.42 (m, 3H, aromatic), 6.30 (s, 1H, C(4′)-H), 3.82, 3.80 (m, overlapping 2H, C(1,4)-H), 3.10 (dd, J=8.7, 5.1 Hz, 1H, C(2)-H), 1.92 (dd, J=12.2, 8.7 Hz, 1H, C(3)-H), 1.88, 1.82 (m, overlapping 2H, C(5,6)-H), 1.70 (m, 1H, C(3)-H), 1.48, 1.45 (m, overlapping 2H, C(5,6-H) ppm. *Anal.* Calcd for $C_{15}H_{16}N_2O$; C, 75.01; H, 6.66; N, 11.66. Found: C, 75.21; H, 6.57; N, 11.46.

Nicotinic Receptor Binding Assay. Displacement of [^{3}H]Cytisine Binding [^{3}H]Cytisine binding was performed according to the method of Pabreza *et al.*[18] Briefly, cortical membranes were isolated from rat brains (Sprague-Dawley) and homogenized in 40 volumes (ml/g wet weight of tissue) of ice-cold 50 mM Tris–HCl buffer, pH 7.0, containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2.5 mM $CaCl_2$ with a Polytron cell homogenizer, (setting 6, 10). The homogenate was centrifuged twice at 40000×***g*** for 10 min at 4 °C. The pellet was resuspended in fresh ice-cold buffer at each centrifugation. The final pellet was suspended in buffer (175 mg of original wet tissue/ml). Aliquots (50 μl) of freshly prepared homogenate containing 600—900 μg of protein were added to test tubes containing the same buffer or buffer plus 10 μl of competitor and 2.0 nM of [^{3}H]cytisine (20 μl) and incubated in triplicates for 75 min at 2 °C. Final volume of the reaction mixture was 250 μl. Nicotine bitartarate (10 μM) was used to define nonspecific binding. The potency of test compounds for nicotinic binding was determined from the ability of a wide range of concentrations (10^{-12} to 10^{-6} M) of the unlabeled test compound to displace [^{3}H]cytisine binding. The binding reaction was terminated by rapid vacuum filtration through Whatman GF/B filter paper presoaked in 0.05% PEI (polyethylenimine) solution and mounted on a Brandel cell harvester. The filters were washed three times with ice-cold buffer and then counted in a scintillation counter after overnight incubation. Data was analyzed with EBDA and LIGAND software from Biosoft. Mean±SD IC_{50} values were determined from two to four separate experimental determinations for each compound.

Lethality Male CD-1 mice 6—7 weeks of age were purchased from Charles River Laboratories (Wilmington, MA). Mice were group housed (n=5 per cage) on hardwood litter (Sanichips) in standard plexiglas mouse cages in an AAALAC-accredited Animal Care Facility. Animals were maintained under a 12 h light/dark cycle and given access to *ad libitum* to a standard rodent pellet chow (Lab Blox, Oklahoma City, OK) and water. At least one week of recovery from shipping trauma was allowed before mice were used in experiments. Each animal was used only once. Nicotine-di-*d*-tartarate, cytisine, (±)-epibatidine (RBI, Natick, MA) and the test compounds were dissolved in 0.9% NaCl (Abbott Laboratories, North Chicago, IL). Freshly prepared solutions were administered intraperitoneally (i.p.) at a

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constant volume to weight ratio (0.1 ml per 10 g body weight). The toxic effects and other behavioral changes were scored over a 30 min period post dosing.

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