Novel and Potent 6-Chloro-3-pyridinyl Ligands for the α4β2 Neuronal Nicotinic Acetylcholine Receptor[‡]

Bachir Latli,[†] Kevin D'Amour, and John E. Casida*

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy, and Management, 114 Wellman Hall, University of California, Berkeley, California 94720-3112

1-[(6-Chloro-3-pyridinyl)methyl]-2-imidazolidine (**1**), the *N*-desnitro metabolite of the major insecticide imidacloprid, is known to have similar potency to that of (−)-nicotine as an inhibitor of [³H](−)-nicotine binding at the rat recombinant α4β2 neuronal nicotinic acetylcholine receptor (nAChR); IC$_{50}$ values in the present study are 3.8 nM for (−)-nicotine, 6.0 nM for **1**, and 155 nM for imidacloprid. Synthesis of new analogues of **1**, modified only in the heterocyclic moiety (five-, six-, or seven-membered rings with NH, S, O, and CH$_2$ substituents), gave compounds varying from 4-fold higher potency (2-iminothiazole analogue **10**) to >6000-fold less active than (−)-nicotine. Other potent *N*-[(6-chloro-3-pyridinyl)methyl] compounds are those in which the heterocyclic imine is replaced with pyrrolidine (**19**) (IC$_{50}$ 9 nM) or trimethylammonium (**22**) (IC$_{50}$ 18 nM). A novel conversion of (−)-nicotine to its 6-chloro analogue increased the potency 2-fold. These 6-chloro-3-pyridinyl compounds are of interest as novel nAChR probes and potential metabolites of candidate insecticides.

Introduction

The 6-chloro-3-pyridinyl moiety confers high potency to several types of compounds (Chart 1) acting at the nicotinic acetylcholine receptor (nAChR) as recognized independently in studies on natural product[1,2] and synthetic analgesics[3−5] and on structural optimization of nicotinoid insecticides.[6] More specifically, for the analgesics, (+)-epibatidine from the skin of an Ecuadorian frog, *Epipedobates tricolor*, is >1000 times more potent than morphine at blocking pain in mice,[1,2] and ABT-594 does not have the addictive effects of nicotine and has antinociceptive properties equal in efficacy to those of morphine.[4,5] Relative to the insecticidal activity, imidacloprid is one of the most important new synthetic insecticides of the past 3 decades and acts selectively at the insect versus the mammalian nAChR.[6]

Imidacloprid is converted in mammals to several metabolites including a small percent of the desnitro derivative **1** (Chart 1).[7] To our surprise, this metabolite is selective for the mammalian versus the insect nAChR and is similar in potency to that of (−)-nicotine at the mouse brain binding site for [³H](−)-nicotine.[8] In addition, the agonist potency of **1** is 4-fold greater than that of (−)-nicotine for inducing specific rubidium-86 ion efflux from intact human neuroblastoma SH-SY5Y cells.[9] These findings prompted us to synthesize a series of analogues of **1**. The 6-chloropyridinylmethyl moiety was retained and modifications were made in the imidazolidine portion. Analogues were also considered, including 6-chloronicotine as a derivative of (−)-nicotine. These compounds were assayed with rat recombinant α4β2 neuronal nAChR, the predominant high-affinity nicotine binding site in the brain, which was expressed

Chart 1. Structures of (−)-Nicotine and Four 6-Chloro-3-pyridinyl Ligands for nAChR
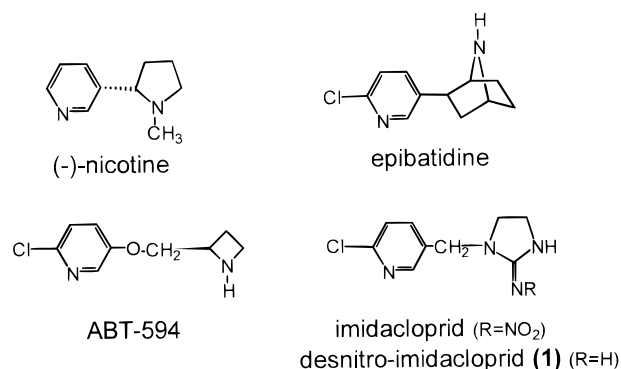
in baculovirus-infected Sf9 cells.[10] The study revealed compounds as potent as or more potent than **1** or (−)-nicotine in affinity binding assays. These analogues are of interest as novel and simple probes for the mammalian nAChR and as potential metabolites of candidate insecticides.

Results and Discussion

Modifications of Heterocyclic Imine Moiety of 6-Chloro-3-pyridinylmethyl Ligands 1−10 (Scheme 1). Most of the derivatives were synthesized straightforwardly from 2-chloro-5-(chloromethyl)pyridine (CCMP)[11,12] as the key compound. To prepare the guanidine-type analogues **1−3**, CCMP was first coupled to alkyldiamines. Monoalkylation is best achieved when one of the amino groups is protected by a readily removable substituent, such as *tert*-butylcarbonyl,[13] or when excess alkyldiamines are used. Hence, with excess 1,2-ethylenediamine, only 1-[(6-chloro-3-pyridinyl)methyl]-1,2-diaminoethane was isolated, but with 1 equiv of 1,3-diaminopropane the major product was 1,3-bis[(6-chloro-3-pyridinyl)methyl]-1,3-diaminopropane. The yield of 1-[(6-chloro-3-pyridinyl)methyl]-1,4-diaminobutane

Scheme 1. Synthesis of 6-Chloro-3-pyridinylmethyl Ligands with Modifications in the Heterocyclic Imine Moiety (**1**−**10**) or Related Compounds (**11**−**14**)
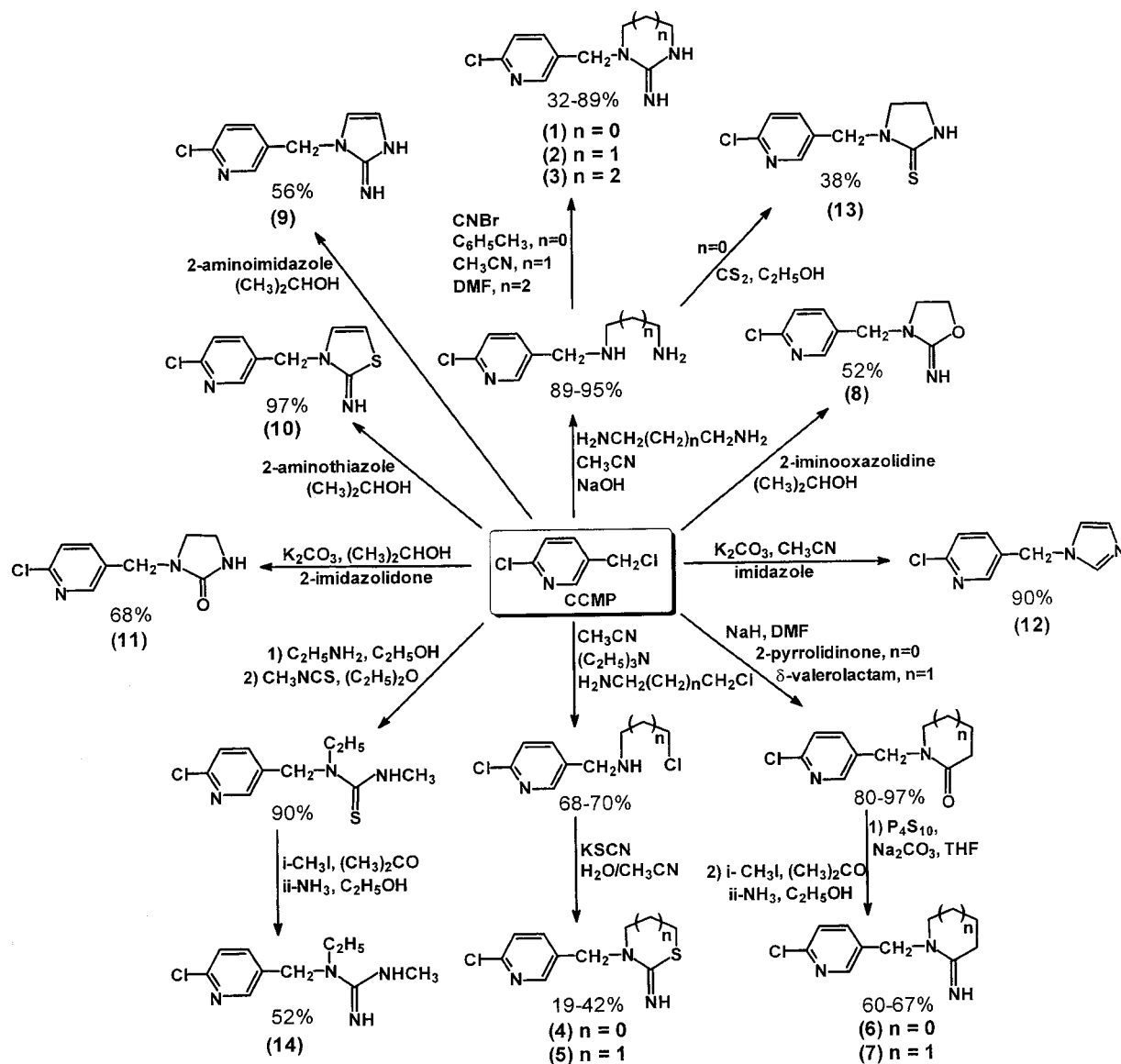
was greatly improved when using 2 equiv of 1,4-diaminobutane. The products from monoalkylation of 1,2-diaminoethane, 1,3-diaminopropane, and 1,4-diaminobutane were then reacted in anhydrous conditions with cyanogen bromide[14] using toluene, acetonitrile, and dimethylformamide (DMF), respectively, as the solvent.

Preparation of the iminothiazolidine and iminotetrahydrothiazine derivatives **4** and **5** was accomplished by coupling CCMP to the corresponding chloroalkyl-amine and then refluxing with potassium thiocyanate in water:acetonitrile (1:1). The iminopyrrolidine (**6**) and iminopiperidine (**7**) derivatives were obtained by first coupling CCMP to 2-pyrrolidinone and 2-piperidone using NaH in DMF. Second, the amide moieties were transformed to thioamides using P_4S_{10} and Na_2CO_3 in dry tetrahydrofuran (THF).[15] Compounds **6** and **7** were then produced by treatment with methyl iodide in acetone, and the resulting iodide salts were reacted with anhydrous ammonia in absolute ethanol.[15,16]

The imino-1,3-oxazolidine (**8**) was synthesized by refluxing CCMP with 2-imino-1,3-oxazolidine in 2-propanol.[17] Similarly, the 2-iminoimidazole (**9**) and 2-imi-

nothiazole (**10**) derivatives were produced by refluxing CCMP with either 2-aminoimidazole or 2-aminothiazole in 2-propanol in a type of coupling first reported by Raeymaekers et al.[18]

Structural Variations of 6-Chloro-3-pyridinylmethyl Ligands 11−14 (Scheme 1) and Related Compounds 15−17. The imidazolidone (**11**) and imidazole (**12**) derivatives were obtained as above from 2-imidazolidone or imidazole with CCMP. In synthesis of the 2-imidazolinethione analogue **13**, 1-[(2-chloro-5-pyridinyl)methyl]ethylenediamine was refluxed in ethanol with excess carbon disulfide.[19] The acyclic compound **14** was obtained by reacting CCMP with 70% ethylamine in water,[20] followed by treatment with methyl isothiocyanate,[21] and then reaction with methyl iodide in acetone followed by anhydrous ammonia in ethanol as before. The deschloro analogue (**15**) of **1** was produced as above in the guanidine-type analogues from 3-(chloromethyl)pyridine. Compounds **16** and **17** were synthesized according to the literature.[17]

Modifications of Heterocyclic Amine and Trialkylammonium Moieties of 6-Chloro-3-pyridinyl-

Scheme 2. Synthesis of 6-Chloro-3-pyridinylmethyl Ligands with Modifications in the Heterocyclic Imine and Trialkylammonium Moieties (**18−23**)
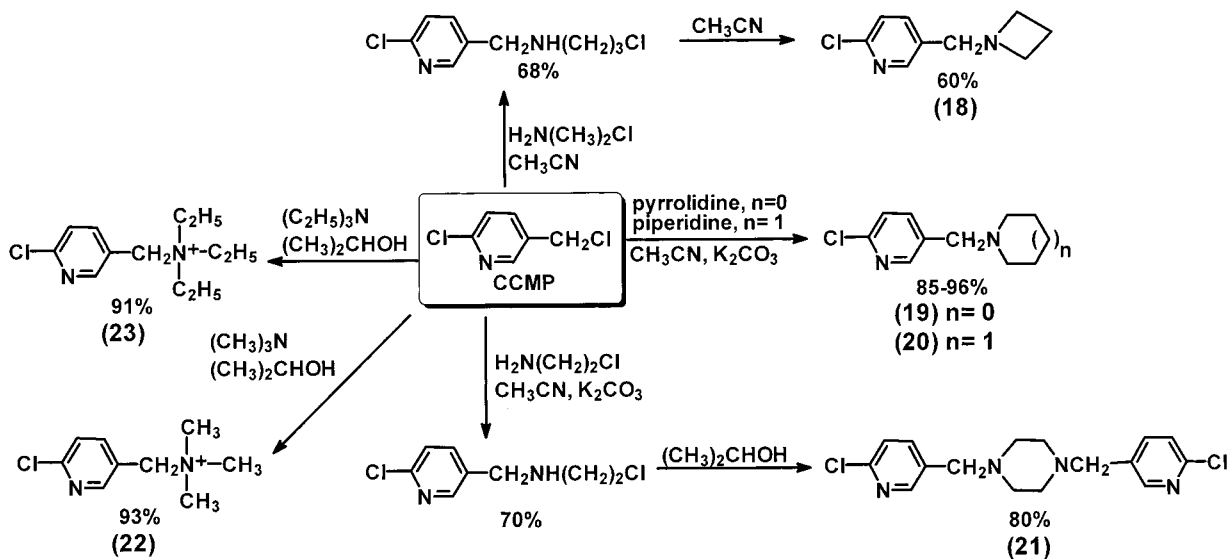
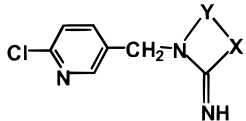**Scheme 3.** Synthesis of 6- and 2-Chloronicotine (**24** and **25**)
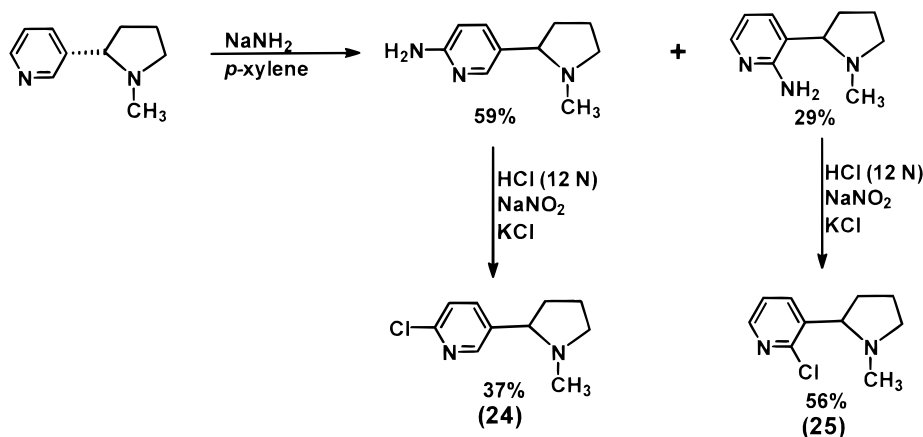
methyl Ligands **18−23** (Scheme 2). The azetidine derivative **18** was prepared by coupling CCMP to 3-chloropropylamine in the usual manner, followed by cyclization in refluxing acetonitrile. The pyrrolidine and piperidine derivatives **19** and **20** were obtained by refluxing pyrrolidine and piperidine with CCMP in acetonitrile in the presence of K_2CO_3. The 1,4-disubstituted-piperazine **21** was produced on refluxing 1-[(6-chloro-3-pyridinyl)methyl]-2-chloroethylamine in 2-propanol. For ammonium derivatives **22** and **23**, CCMP was refluxed with either trimethylamine or triethylamine in 2-propanol.

6- and 2-Chloronicotine (24 and 25) (Scheme 3). Racemic 6-chloronicotine has been prepared although the experimental details were not published.[22] Here, 6- and 2-chloronicotine were prepared from (−)-nicotine in two steps. First, (−)-nicotine was converted to 6- and 2-aminonicotine as reported[23] with product separation by silica gel flash chromatography. The amino compounds were then converted to the chlorides by diazotization (12 N HCl, NaNO₂) in the presence of excess KCl.
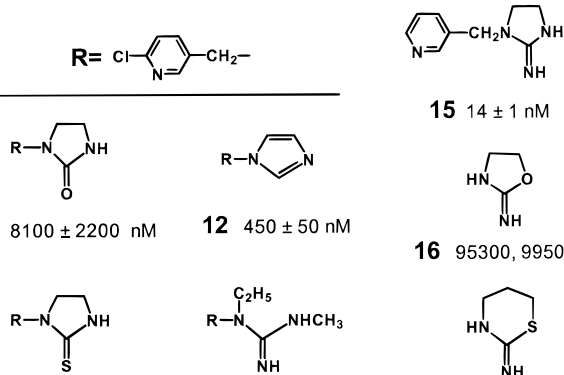
Structure−Activity Relationships. General. This research was based on imidacloprid, its desnitro metabolite **1**, and (−)-nicotine which gave concentrations for 50% inhibition (IC$_{50}$ values) of 155, 6.0, and 3.8 nM,

respectively, in competition assays with [³H](−)-nicotine and rat recombinant α4β2 neuronal nAChR. It examined a series of **1** analogues with emphasis on the 6-chloro-pyridinyl moiety even though other bioisosteric replacements for the pyridine ring also conferred high potency nAChR ligands.[3,8,24] Structure−activity studies in the nicotine series have previously assumed that binding to the nAChR was associated with a pyrrolidine nitrogen atom (a cationic center), a pyridine nitrogen atom (an electronegative atom), planarity of the pyridine ring, and the distance between the two nitrogen atoms.[25−27]

Modifications of Heterocyclic Imine Moiety of 6-Chloro-3-pyridinylmethyl Ligands 1−10 (Table 1). Analogues of **1** modified only in the heterocyclic moiety (five-, six-, and seven-membered rings with NH, S, O, and CH₂ substituents) varied from 4-fold more potent to 16-fold less potent than (−)-nicotine. Where compared, the five-membered ring conferred higher potency than the corresponding six-membered ring, and in the single example the seven-membered ring compound **3** was even more potent. While the five-membered ring resembles the pyrrolidine moiety in nicotine, the seven-membered ring may also adopt a conformation that fits this portion of the binding site. An X substituent of S or CH₂ was generally preferred over NH or O.

Table 1. Effect of Modifications of Heterocyclic Imine Moiety on the Potency of 6-Chloro-3-pyridinylmethyl Ligands **1**−**10** as Inhibitors of [^{3}H](−)-Nicotine Binding to $\alpha4\beta2$ nAChR

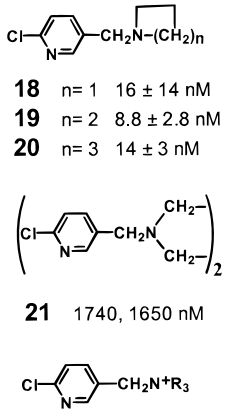| no.	X	Y	IC$_{50}$ (nM $\pm$ SD, $n = 4$)
1	NH	(CH$_2$)$_2$	6.0 $\pm$ 0.5
2	NH	(CH$_2$)$_3$	58, 63[a]
3	NH	(CH$_2$)$_4$	2.8 $\pm$ 1.0
4	S	(CH$_2$)$_2$	2.3 $\pm$ 0.3
5	S	(CH$_2$)$_3$	14 $\pm$ 4
6	CH$_2$	(CH$_2$)$_2$	3.5 $\pm$ 0.7
7	CH$_2$	(CH$_2$)$_3$	11 $\pm$ 5
8	O	(CH$_2$)$_2$	7.2 $\pm$ 2.0
9	NH	CH=CH	23 $\pm$ 9
10	S	CH=CH	0.91 $\pm$ 0.40

[a] Individual values for two determinations.

Table 2. Effect of Structural Variations on the Potency of 6-Chloro-3-pyridinylmethyl Ligands **11**−**14** and Related Compounds **15**−**17** as Inhibitors of [^{3}H](−)-Nicotine Binding to $\alpha4\beta2$ nAChR[a]

[a] IC$_{50}$ $\pm$ SD, $n = 4$, or individual values for two determinations.

When a double bond was introduced into the five-membered ring, once again S conferred higher potency than CH$_2$, and in fact, the 2-iminothiazole analogue **10** was the most potent compound examined (IC$_{50}$ 0.91 nM). The double bond in **9** lowered the potency of **1** in $\alpha4\beta2$ nAChR assays but with imidacloprid increased the insecticidal activity to selected pests.[28]

Structural Variations of 6-Chloro-3-pyridinylmethyl Ligands 11−14 and Related Compounds 15−17 (Table 2). When the imino nitrogen of **1** was replaced with oxygen or sulfur as in **11** and **13**, respectively, the inhibitory potency dropped >1000-fold, and when deleted like in **12** the potency loss was 75-fold. This may be due to the imino group but not the oxygen or sulfur serving as a hydrogen bond donor. Opening the ring as in **14**, a potential metabolite of yet another insecticide (nitenpyram),[29] gave a moderate potency. The chlorine atom on the pyridine ring enhanced the potency but not to a large extent, e.g., compounds **1** and **15**, which was not surprising since the chlorine sometimes can be replaced by other halogen atoms or even a methyl group without reducing the activity significantly as an insecticide[29,30] or nAChR ligand.[8,22,31] Compounds **16** and **17** were >10000-fold less active at the nAChR than their 6-chloro-3-pyridi-

Table 3. Effect of Modifications of Heterocyclic Amine and Trialkylammonium Moieties on the Potency of 6-Chloro-3-pyridinylmethyl Ligands **18**−**23** as Inhibitors of [^{3}H](−)-Nicotine Binding to $\alpha4\beta2$ nAChR[a]

[a] IC$_{50}$ $\pm$ SD, $n = 4$, or individual values for two determinations.

nylmethyl derivatives **8** and **5**, respectively, yet **16** and **17** were moderately active as inhibitors of the human nitric oxide synthase isoforms.[17]

Modifications of Heterocyclic Amine and Trialkylammonium Moieties of 6-Chloro-3-pyridinylmethyl Ligands 18−23 (Table 3). Early investigations on *N*-substituted (3-pyridyl)alkylamines established the structural requirements for nicotine-like activity: i.e., the distance between the nitrogen atoms has to be close to that of nicotine, the amino group has to be tertiary, and the molecule should not be too lipophilic,[25,32] features also important for imidacloprid analogues.[6,33,34] Similar requirements were established for the *N,N*-dialkyl substituents of 3-pyridylmethylamine in the nAChR of insects and *Torpedo*.[35,36] In the present study with substituted 6-chloro-3-pyridinylmethylamine, the pyrrolidine analogue **19** was more potent than the piperidine **20** (also noted previously in the 3-pyridinylmethylamine series)[22] and the azetidine **18** was of similar activity. The 1,4-disubstituted piperazine **21** was of low activity, suggesting that the nAChR did not tolerate sterical encumbrance even if the addition was a mirror image of disubstituted 6-chloro-3-pyridinylmethylamine. There are many nAChR modulators that have the trialkylammonium moiety, prompting the synthesis of the chloropyridinylmethyl trimethylammonium compound **22** and its triethylammonium analogue **23**. The trimethylammonium derivative **22** was much more potent than the triethylammonium compound **23** (this study) which may be too lipophilic in contrast to the *N,N*-dialkyl-3-pyridinylmethylamines, where the order for activity was methyl, ethyl ≫ diethyl > dimethyl and the 6-chloro substituent on the pyridinyl moiety lowered the potency of the methyl, ethyl compound.[22] Importantly, the trimethylammonium compound was one-fifth as potent as (−)-nicotine.

6- and 2-Chloronicotine (24 and 25) (Table 4). Substitution on the pyridine ring of nicotine was an attractive way for introducing photolabile groups in the preparation of photoaffinity probes, radioisotopes of hydrogen and iodine, and other groups.[37−41] The substituents were introduced primarily at the 5-position,

Table 4. Effect of the Chloro Substituent on the Potency of
(−)-Nicotine Analogues **24** and **25** as Inhibitors of
[³H](−)-Nicotine Binding to α4β2 nAChR[a]

	unsubst.	3.8 ± 0.8 nM
24	6-Cl	1.9 ± 0.4 nM
25	2-Cl	3200 ± 1400 nM

[a] IC_{50}, nM. For comparison, the IC_{50} for (±)-epibatidine Is 0.44
nM (IC_{50} ± SD, $n = 4$).

while the 6-position received less attention than in the
epibatidine series in which chlorine was replaced with
iodine-125, fluorine-18, and a methyl group.[1] Thus,
when a halogen (fluorine, chlorine, and bromine) was
introduced at the 6-position, the halogenated racemic
nicotine analogues were more potent than nicotine in
receptor affinity and other functional parameters.[22] In
our study we prepared 6-chloronicotine (**24**) and 2-chlo-
ronicotine (**25**). Compound **24** was 2-fold more potent
than (−)-nicotine in α4β2 nAChR assays and was also
more toxic to mice by the intraperitoneal route (data
not shown). In contrast, 2-chloropyridinyl compound **25**
had an IC_{50} of 3.2 μM, consistent with the earlier
observation[42] that on adding a methyl group in position
2 or 4, the activity of these two compounds was
significantly lower than that of nicotine. In conclusion,
this study reports the synthesis of new ligands with high
potency at the α4β2 neuronal nAChR.

Experimental Section

Chemistry. General. Silica gel TLC for analysis was
performed with precoated plastic sheets with fluorescent
indicator; all R_f values reported are for development with
10% methanol/chloroform. Preparative TLC utilized precoated
silica gel GF plates. NMR spectra were recorded for $CDCl_3$ or
CD_3OD solutions with the Bruker AM-300 spectrometer.
Chemical shifts (δ in ppm) are reported for ¹H at 300 MHz
and for ¹³C at 75 MHz relative to internal tetramethylsilane
and $CDCl_3$, respectively. Mass spectra were acquired by GC/
MS with a Hewlett-Packard 5971A or 5985B instrument in
the electron impact (EI) mode (70 eV, 200 °C). Fast atom
bombardment (FAB)-MS (both low and high resolution, LR and
HR, respectively) was conducted with the Fisons ZAB2-EQ
spectrometer. Melting points recorded on a Fisher-Johns appa-
ratus are uncorrected. Reagents were from Aldrich Chemical
Co. (Milwaukee, WI) except EDTA and (−)-nicotine (tartrate
salt) from Sigma Chemical Co. (St. Louis, MO) and 2-chloro-
ethylamine HCl from Lancaster (Windham, NH). Solvents
were reagent or HPLC grade. [*N-methyl*-³H](−)-Nicotine ([³H]-
(−)-nicotine) at 78 Ci/mmol was purchased from DuPont NEN
Research Products (Boston, MA). Each compound was >98%
pure based on TLC and ¹H and ¹³C NMR integrations.

**1-[(6-Chloro-3-pyridinyl)methyl]-2-iminoimidazoli-
dine (1).** Prepared as before.[14]

**1-[(6-Chloro-3-pyridinyl)methyl]-1,3-diaminopro-
pane.** CCMP (1.62 g, 10 mmol) in acetonitrile (15 mL) was
added dropwise to a solution of 1,3-diaminopropane (0.835 mL,
10 mmol) in acetonitrile (20 mL) over a period of 2 h at 25 °C.
The resulting mixture was stirred overnight. A 30% aqueous
solution of NaOH (2.0 mL) was added, and the mixture was
concentrated in vacuo. The residue was extracted with chlo-
roform, dried (MgSO₄), filtered, and concentrated under re-
duced pressure to give 1.87 g of a viscous oil. Purification by
preparative TLC with 10% methanol/chloroform gave 0.86 g
of 1,3-bis[(6-chloro-3-pyridinyl)methyl]-1,3-diaminopropane (R_f
= 0.40) and 0.35 g of the desired monoalkylated product (R_f =
0.16).

**1-[(6-Chloro-3-pyridinyl)methyl]-2-iminotetrahydro-
pyrimidine (2).** The above monoalkylated diamine (144 mg,
0.72 mmol) was dissolved in acetonitrile (5 mL) and added
dropwise to a solution of cyanogen bromide (80 mg, 0.75 mmol)
in acetonitrile (3 mL) at 25 °C. The mixture was stirred
overnight. The resulting precipitate was filtered off and
washed with acetonitrile (3 × 10 mL). The solid was dried
under reduced pressure to give 70 mg (32% yield) of the
product, mp = 220 °C. FAB-LR: MH⁺ (225, 45%), MH⁺ +
2(15%), 200(100%), 202(33%). FAB-HR: $C_{10}H_{13}ClN_4H^+$, calcd
225.0907, found 225.0909.

1-[(6-Chloro-3-pyridinyl)methyl]-1,4-diaminobutane.
CCMP (324 mg, 2.0 mmol) was added in acetonitrile (10 mL)
over a period of 2 h to a solution of 1,4-diaminobutane (352
mg, 4.0 mmol) in acetonitrile (10 mL) containing triethylamine
(0.5 mL), and the resulting mixture was stirred overnight at
25 °C. After the usual workup, the desired product was isolated
by preparative TLC with 10% methanol/chloroform to give 380
mg in 89% yield as a yellowish oil, R_f = 0.13, and 80 mg of
1,4-bis[(6-chloro-3-pyridinyl)methyl]-1,4-diaminobutane as a
pale resin, R_f = 0.40.

**1-[(6-Chloro-3-pyridinyl)methyl]-2-iminotetrahydrodi-
azepine (3).** The above monoalkylated diamine (178 mg, 0.83
mmol) in DMF (5 mL) was added at 25 °C to cyanogen bromide
(90 mg, 0.85 mmol) in DMF (2 mL), and the resulting solution
was stirred overnight. The product was isolated by concentra-
tion in vacuo and then preparative TLC as above to give 46
mg in 53% yield based on the reacting diamine, mp = 125 °C.
FAB-LR: MH⁺ (239, 100%), MH⁺ + 2(32%), 154(40%), 156-
(11%). FAB-HR: $C_{11}H_{15}ClN_4H^+$, calcd 239.1063, found 239.1059.

**1-[(6-Chloro-3-pyridinyl)methyl]-2-chloroethyl-
amine**. A solution of CCMP (1.62 g, 10 mmol), 2-chloroethy-
lamine HCl (1.3 g, 11.2 mmol), and triethylamine (1.53 mL,
11 mmol) in acetonitrile (20 mL) was stirred at 25 °C for 40 h.
The desired product was isolated as before by preparative TLC
using 10% methanol/chloroform (R_f = 0.60) to give 1.0 g in
70% yield based on reacted CCMP.

**1-[(6-Chloro-3-pyridinyl)methyl]-2-iminothiazoli-
dine (4).** A solution of the above amine (205 mg, 1.0 mmol)
and potassium thiocyanate (100 mg, 1.0 mmol) in acetonitrile
(5 mL) and water (5 mL) was refluxed for 3 h. The product
was isolated by preparative TLC with 10% methanol/chloro-
form to give 62 mg of 1,4-bis[(6-chloro-3-pyridinyl)methyl]-
piperazine (R_f = 0.66) and 50 mg of the desired compound in
19% yield, mp = 190 °C, R_f = 0.16. FAB-LR: MH⁺ (228, 100%),
MH⁺ + 2(34%). FAB-HR: $C_9H_{10}ClN_3SH^+$, calcd 228.0362,
found 228.0365.

**1-[(6-Chloro-3-pyridinyl)methyl]-3-chloropropyl-
amine**. A solution of CCMP (1.62 g, 10 mmol), 3-chloropro-
pylamine (1.7 g, 13 mmol), and triethylamine (1.8 mL, 13
mmol) in acetonitrile (20 mL) was stirred at 55 °C overnight
before it was concentrated under reduced pressure and worked
up as usual. Purification by preparative TLC with 10%
methanol/chloroform gave 1.5 g of a yellowish oil in 68% yield,
R_f = 0.50.

**1-[(6-Chloro-3-pyridinyl)methyl]-2-iminotetrahydrothi-
azine (5).** A solution of the above compound (219 mg, 1.0
mmol) and potassium thiocyanate (100 mg, 1.02 mmol) in
acetonitrile (5 mL) and water (5 mL) was refluxed overnight.
The solution was then treated as above to give 101 mg of a
solid product in 42% yield, mp = 215 °C. FAB-LR: MH⁺ (242,
100%), MH⁺ + 2(34%). FAB-HR: $C_{10}H_{12}ClN_3SH^+$, calcd
242.0518, found 242.0518.

1-[(6-Chloro-3-pyridinyl)methyl]-2-pyrrolidinone. NaH
(138 mg, 3.0 mmol, 50% oil dispersion) was washed three times
with hexane and then suspended in DMF (5 mL). To this was
added a solution of 2-pyrrolidinone (190 μL, 2.5 mmol) in DMF
(2 mL) slowly at 0 °C. The resulting mixture was further
stirred for 1 h before CCMP (324 mg, 2.0 mmol) in DMF (2
mL) was added dropwise at this temperature. The mixture was
then stirred overnight as the ice melted. The reaction mixture
was poured into water, extracted with chloroform, dried
(MgSO₄), filtered, and concentrated in vacuo. Purification by

preparative TLC with 10% methanol/chloroform gave the desired product in 97% yield, $R_f = 0.53$.

1-[(6-Chloro-3-pyridinyl)methyl]-2-pyrrolidinethione. To a suspension of P_4S_{10} (1.02 g, 2.3 mmol) in dry THF (10 mL) was added Na_2CO_3 (238 mg, 2.25 mmol) at 25 °C. The mixture turned to a yellow solution in a few minutes and was further stirred for 30 min. To this solution was added the above pyrrolidinone derivative (0.4 g, 1.9 mmol) in THF (5 mL), and the solution was stirred at 25 °C for 3 h. A solution of 10% Na_2HPO_4 (10 mL) was added, and the organic phase was extracted with ethyl acetate, dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was isolated by preparative TLC with 10% methanol/chloroform to give 280 mg in 68% yield, $R_f = 0.60$.

1-[(6-Chloro-3-pyridinyl)methyl]-2-iminopyrrolidine (6). To a solution of the above thione (226 mg, 1.0 mmol) in acetone (10 mL) was added methyl iodide (70 μL, 1.1 mmol) at 25 °C. The resulting solution was stirred for 48 h before it was concentrated under reduced pressure. The oil residue was then dissolved in absolute ethanol (10 mL), and anhydrous ammonia was bubbled in slowly for 20 min. The reaction flask was then sealed and stirred at 25 °C for 48 h. After concentration in vacuo the product was isolated by preparative TLC with 10% methanol/chloroform to give the product in 99% yield, mp = 165 °C, $R_f = 0.03$. FAB-LR: MH^+ (210, 100%), $MH^+ + 2$(33%). FAB-HR: $C_{10}H_{12}ClN_3H^+$, calcd 210.0798, found 210.0802.

1-[(6-Chloro-3-pyridinyl)methyl]-2-piperidone. This compound was prepared as above from δ-valerolactam (2-piperidone) (0.60 g, 6.0 mmol) and CCMP (0.81 g, 5.0 mmol) in 90% yield (0.90 g) as a yellowish oil, $R_f = 0.60$.

1-[(6-Chloro-3-pyridinyl)methyl]-2-thiopiperidone. This product was prepared from the above lactam (0.4 g, 1.78 mmol), P_4S_{10} (1.0 g, 2.25 mmol), and Na_2CO_3 (238 mg, 2.25 mmol) in dry THF (10 mL) to give 280 mg of a yellow oil in 65% yield, $R_f = 0.73$.

1-[(6-Chloro-3-pyridinyl)methyl]-2-iminopiperidine (7). The aforementioned procedure was used to prepare this compound from the thione (89 mg, 0.37 mmol) and methyl iodide (26 μL, 0.4 mmol) in acetone (5 mL). Then, anhydrous ammonia was added in ethanol (5 mL) to give 73 mg of a hygroscopic product in 93% yield, $R_f = 0.03$. FAB-LR: MH^+ (224, 100%), $MH^+ + 2$(33%). FAB-HR: $C_{11}H_{14}ClN_3H^+$, calcd 224.0954, found 224.0952.

1-[(6-Chloro-3-pyridinyl)methyl]-2-imino-1,3-oxazolidine (8). To a suspension of NaH (112 mg, 2.4 mmol, 50% oil dispersion) in DMF (5 mL) was added oxazolidine **17** (see below) (294 mg, 2.4 mmol) in DMF (2 mL) at 0 °C. The mixture was stirred for 1 h; then CCMP (190 mg, 1.73 mmol) was added in dry DMF (3 mL) dropwise. The resulting mixture was stirred overnight as the ice melted. Water (10 mL) was added, and the reaction was extracted with chloroform, dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was isolated by preparative TLC as above to give 130 mg of a hygroscopic material in 52% yield. FAB-LR: MH^+ (212, 100%), $MH^+ + 2$(33%). FAB-HR: $C_9H_{10}ClN_3OH^+$, calcd 212.0591, found 212.0597.

1-[(6-Chloro-3-pyridinyl)methyl]-2-iminoimidazole (9). 2-Aminoimidazole sulfate (1.0 g, 7.57 mmol) was dissolved in a saturated solution of K_2CO_3. The solution was then concentrated in vacuo to give a solid residue which was extracted with hot ethanol. The ethanolic solution was then concentrated to give 0.71 g of 2-aminoimidazole as a brownish oil (86% crude yield). The free base (100 mg, 1.2 mmol) and CCMP (162 mg, 1.0 mmol) were dissolved in 2-propanol (10 mL) and refluxed for 40 h. The mixture was then concentrated in vacuo, and the product was isolated by preparative TLC (2.0 mm plate) using 10% methanol/chloroform as eluent to give 116 mg of the desired product in 56% yield, mp = 188 °C, $R_f = 0.10$. MS-FAB-LR: MH^+ (209, 100%), $MH^+ + 2$(34%). FAB-HR: $C_9H_9ClN_4H^+$, calcd 209.0594, found 209.0593.

1-[(6-Chloro-3-pyridinyl)methyl]-2-iminothiazole (10). A solution of CCMP (230 mg, 1.42 mmol) and 2-aminothiazole (156 mg, 1.56 mmol) in 2-propanol (6 mL) was refluxed for 40

h. The mixture was then cooled to 25 °C and concentrated in vacuo. The solid residue was purified by preparative TLC (2.0-mm plate) with 10% methanol/chloroform to give 310 mg of a yellowish solid in 97% yield, mp = 205−206 °C, $R_f = 0.13$. MS-FAB-LR: MH^+ (226, 100%), $MH^+ + 2$(228, 34%). FAB-HR: $C_9H_8ClN_3SH^+$, calcd 226.0206, found 226.0210.

1-[(6-Chloro-3-pyridinyl)methyl]-2-imidazolidone (11). A mixture of CCMP (162 mg, 1.0 mmol), 2-imidazolidone (225 mg, 2.61 mmol), and K_2CO_3 (345 mg, 2.5 mmol) in 2-propanol (10 mL) was refluxed for 48 h. The solvent was then evaporated in vacuo, and the residue was dissolved in water, extracted with chloroform, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The product was isolated as usual to give 53 mg in 68% yield based on reacted CCMP, mp = 115 °C, $R_f = 0.50$. MS-EI-LR: M^+(211, 100%), $M^+ + 2$(32%). EI-HR: $C_9H_{10}ClN_3O$, calcd 211.0512, found 211.0512.

1-[(6-Chloro-3-pyridinyl)methyl]imidazole (12). A mixture of CCMP (1.62 g, 10 mmol), imidazole (0.715 g, 10.5 mmol), and K_2CO_3 (2.1 g, 15 mmol) in acetonitrile (20 mL) was refluxed overnight. Then, it was cooled to 25 °C and concentrated in vacuo. To the residue was added water (10 mL), and the mixture was extracted with chloroform, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Purification as usual gave 1.2 g of a viscous oil which solidified upon standing at 25 °C, mp = 95 °C, $R_f = 0.40$. EI-LR: M^+ (193, 58%), $M^+ + 2$(195, 20%), 126(100%), 128(32%). EI-HR: $C_9H_8ClN_3$, calcd 193.0407, found 193.0416; for ^{35}Cl, calcd 195.0377, found 195.0389 for ^{37}Cl. Anal. C, H, N.

1-[(6-Chloro-3-pyridinyl)methyl]-2-imidazolidinethione (13). 1-[(6-Chloro-3-pyridinyl)methyl]-1,2-diaminoethane (prepared as described in ref 14) (0.64 g, 3.45 mmol) and carbon disulfide (3.3 mL, 54 mmol) in ethanol (20 mL) were refluxed for 4 h. The solution was then concentrated in vacuo, and the product was isolated by preparative TLC as above to give 0.3 g in 38% yield as a cream-colored solid, mp = 188 °C, $R_f = 0.33$. EI-LR: M^+ (227, 100%), $M^+ + 2$(37%). EI-HR: $C_9H_{10}ClN_3S$, calcd 227.0284, found 227.0280. Anal. C, H, N.

1-[(6-Chloro-3-pyridinyl)methyl]-1-(ethylamino)-2-imino-3-methylamine (14). A solution of the thiourea derivative (100 mg, 0.41 mmol) in acetone (5 mL) was treated with methyl iodide (28 μL, 0.45 mmol) and stirred at 25 °C for 60 h. The solution was concentrated in vacuo and then dissolved in ethanol, and anhydrous ammonia was bubbled in for 20 min. The reaction flask was sealed, and the solution was stirred overnight. The product was isolated as before in 52% yield (48 mg), mp = 190 °C. FAB-LR: MH^+ (227, 100%), $MH^+ + 2$(229, 33%). FAB-HR: $C_{10}H_{15}ClN_4H^+$, calcd 227.1063, found 227.1056.

1-(3-Pyridinylmethyl)-1,2-diaminoethane. To a suspension of 3-(chloromethyl)pyridine HCl (2.3 g, 14 mmol) in acetonitrile (30 mL) was added an aqueous solution of NaOH (30%, 2 mL). The resulting brownish mixture was further stirred for 30 min, and then water (20 mL) was added and extracted with chloroform. The organic extract was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was dissolved in methanol (30 mL), and ethylenediamine (3.0 g, 50 mmol) was added dropwise in methanol (30 mL) at 25 °C. Stirring was continued overnight, then NaOH (30% solution, 2 mL) was added, and the mixture was concentrated in vacuo. Water was added to the residue which was then extracted with chloroform. The usual workup followed by silica gel purification gave 0.44 g of a pale-yellow oil in 21% yield.

1-(3-Pyridinylmethyl)-2-iminoimidazolidine (15). To a solution of the above diamine (0.44 g, 2.93 mmol) in dry toluene (10 mL) was added at 25 °C and with stirring a solution of cyanogen bromide (0.32 g, 3.0 mmol) in toluene (5 mL). The resulting mixture was stirred overnight and then concentrated in vacuo and the solid residue purified as usual to give 0.53 g in 67% yield of a slightly orange solid, mp = 182 °C. FAB-LR: MH^+ (177, 100%). FAB-HR: $C_9H_{12}N_4H^+$, calcd 177.1140, found 177.1144.

2-Imino-1,3-oxazolidine (16). A mixture of 2-chloroethylamine HCl (3.5 g, 30 mmol) and sodium cyanate (2.0 g, 30 mmol) in water (10 mL) was stirred at 90 °C for 3 h. The

solution was concentrated under reduced pressure at 25 °C and the semisolid residue extracted with hot ethanol. Anhydrous ether was added to the combined ethanolic solutions to give crystals (needles) in 76% yield (2.8 g), mp = 105 °C.

2-Iminotetrahydrothiazine (17). A solution of 3-bromopropylamine HBr (2.19 g, 10 mmol) and potassium thiocyanate (0.972 g, 10 mmol) in water (10 mL) was heated at 90 °C for 3 h. The product was obtained as before from ether/ethanol in 96% yield (1.9 g) as a white powder, mp = 131 °C.

1-[(6-Chloro-3-pyridinyl)methyl]azetidine (18). A solution of 1-[(6-chloro-3-pyridinyl)methyl]-3-chloropropylamine (219 mg, 1.0 mmol) in acetonitrile (5 mL) was refluxed overnight. The product was isolated in the usual manner as a pale-yellow oil in 60% yield (110 mg), R_f = 0.53. MS-FAB-LR: MH$^+$ (183, 100%), MH$^+$ + 2(34%). FAB-HR: $C_9H_{11}ClN_2H^+$, calcd 183.0689, found 183.0649.

1-[(6-Chloro-3-pyridinyl)methyl]pyrrolidine (19). CCMP (162 mg, 1.0 mmol), pyrrolidine (167 μL, 2.0 mmol), and K_2CO_3 (138 mg, 1.0 mmol) in acetonitrile (10 mL) were refluxed overnight. The mixture was cooled to 25 °C, and water was added. Extraction with chloroform, drying (MgSO$_4$), filtration, and concentration in vacuo gave a residue which was purified by preparative TLC using 10% methanol/chloroform to obtain a yellowish oil in 96% yield, R_f = 0.56. MS-FAB-LR: MH$^+$ (197, 100%), MH$^+$ + 2(27%). FAB-HR: $C_{10}H_{13}ClN_2H^+$, calcd 197.0846, found 197.0846. Anal. C, H, N.

1-[(6-Chloro-3-pyridinyl)methyl]piperidine (20). The above procedure was used to prepare this compound from CCMP (162 mg, 1.0 mmol), piperidine (200 μL, 2.0 mmol), and K_2CO_3 (138 mg, 1.0 mmol) in acetonitrile (10 mL) to give 180 mg after silica gel purification in 85% yield as a yellow oil which solidified upon standing at 25 °C, mp = 48 °C, R_f = 0.60. MS-FAB-LR: MH$^+$ (211, 100%), MH$^+$ + 2(30%), 154-(74%), 156(22%). FAB-HR: $C_{11}H_{15}ClN_2H^+$, calcd 211.1002, found 211.1005. Anal. C, H, N.

1,4-Bis[(6-chloro-3-pyridinyl)methyl]piperazine (21). 1-[(6-Chloro-3-pyridinyl)methyl]-2-chloroethylamine (205 mg, 1.0 mmol) in 2-propanol (10 mL) was refluxed overnight in the presence of 1.0 equiv of K_2CO_3. The product was isolated as before in 80% yield after silica gel chromatography as a white powder, mp = 125 °C, R_f = 0.66. MS-EI-LR: M$^+$ (336, 11%), 210(M$^+$ − C_5H_3NCl-CH$_2$, 45%), 212(15%), 126(210 − (NCH$_2$CH$_2$)$_2$, 100%), 128(32%). FAB-LR: MH$^+$ (337, 100%), MH$^+$ + 2(60%). EI-HR: $C_{16}H_{18}Cl_2N_4$, calcd 336.0908, found 336.0906. Anal. C, H, N.

1-[(6-Chloro-3-pyridinyl)methyl]-1,1,1-trimethylammonium Chloride (22). A solution of CCMP (180 mg, 1.11 mmol) and trimethylamine (excess, 70% solution in water) in 2-propanol (10 mL) was refluxed overnight and then concentrated. The product was isolated by silica gel chomatography as a hygroscopic colorless solid in 93% yield (230 mg). MS-FAB-LR: [M − Cl]$^+$ (185, 100%), [M − Cl]$^+$ + 2(32%). FAB-HR: [M − Cl]$^+$ $C_9H_{14}ClN_2^+$, calcd 185.0845, found 185.0846.

1-[(6-Chloro-3-pyridinyl)methyl]-1,1,1-triethylammonium Chloride (23). As before, a solution of CCMP (190 mg, 1.19 mmol) and triethylamine (182 μL, 1.3 mmol) in 2-propanol (10 mL) was refluxed overnight. Concentration under reduced pressure followed by preparative TLC with 10% chloroform/methanol gave 280 mg of a hygroscopic colorless product in 91% yield. MS-FAB-LR: [M − Cl]$^+$ (227, 100%), [M − Cl]$^+$ + 2(34%). FAB-HR: [M − Cl]$^+$ $C_{12}H_{20}ClN_2^+$, calcd 227.1315, found 227.1336.

6- and 2-Aminonicotine. To a solution of (−)-nicotine (98%) (5.0 g, 32 mmol) in *p*-xylene (10 mL) was added sodium amide (5 mL, 50% suspension in xylenes). The mixture was then heated at 130 °C for 3 h. The resulting brownish mixture was cooled to 25 °C, and concentrated HCl (12 N) was added carefully until pH 1. The organic phase was extracted with ether, and the aqueous phase was made again alkaline (pH 14) with 20% NaOH and then extracted with ether. The combined ether solutions were dried (MgSO$_4$), filtered, and concentrated in vacuo to give 5.0 g of a brownish slurry. Purification by flash chomatography using 0−10% methanol/chloroform as eluent gave 1.56 g of 2-aminonicotine (29% yield)

as a pale-yellow oil, R_f = 0.60. 6-Aminonicotine was obtained in 59% yield (3.20 g) as a yellowish oil, R_f = 0.36.

6-Chloronicotine (24). To 6-aminonicotine (0.44 g, 2.5 mmol) in concentrated HCl (12 N, 3 mL) stirred at 0 °C was added a solution of NaNO$_2$ (0.44 g, 6.4 mmol) in water (3 mL) over a 1 h period. Then KCl (0.37 g, 4.9 mmol) in water (2 mL) was added slowly. The resulting solution was made basic gradually by the addition of aqueous NaOH (6 N, 4.5 mL). The product was extracted with chloroform, dried (MgSO$_4$), filtered, and concentrated in vacuo. Purification by flash chromatography gave a pale-yellow oil in 37% yield (180 mg), R_f = 0.66. FAB-LR: MH$^+$ (197, 100%), MH$^+$ + 2(27%). FAB-HR: $C_{10}H_{13}ClN_2H^+$, calcd 197.0846, found 197.0846.

2-Chloronicotine (25). The above procedure on the same scale gave compound **25** in 56% yield (274 mg) as a pale-yellow oil, R_f = 0.66. FAB-LR: MH$^+$ (197, 100%), MH$^+$ + 2(26%). FAB-HR: $C_{10}H_{13}ClN_2H^+$, calcd 197.0845, found 197.0842. Anal. C, H, N.

Biology. Production of Recombinant α4β2 nAChR.[43] Recombinant baculovirus stocks containing inserts for the rat α4 and β2 nAChR subunits were independently amplified.[10] Production of the α4β2 nAChR was achieved by infection of Sf9 insect cells with each recombinant viral stock. Cells were harvested 48−72 h postinfection by centrifugation for 10 min at 100g, then washed in phosphate-buffered saline, and homogenized in a small volume of buffer (20 mM Tris-HCl, pH 7.4, 118 mM NaCl, 4.8 M KCl, 2.5 mM CaCl$_2$, 1.2 mM MgSO$_4$, and 1.0 mM EDTA) followed by centrifugation for 10 min at 300g. The final step was centrifugation of the supernatant at 37000g. The membrane pellet was resuspended in buffer and adjusted to 200 μg of total protein/mL. These nAChR preparations were used immediately for binding assays or stored as single-experiment aliquots at −80 °C.

Binding Assays.[43] The standard assay involved incubation of 1 nM [^{3}H](−)-nicotine and various concentrations of inhibitor with 20 μg of α4β2 nAChR preparation in 0.25 mL of buffer for 30 min at 22 °C. Reactions were terminated by addition of 3 mL of ice-cold 0.9% NaCl followed by rapid vacuum filtration though Filtermat B glass fiber filters and two 3-mL washes on the filter. Filters were presoaked at least 60 min in 0.1% w/v poly(ethylenimine) to reduce nonspecific binding. Specific binding was defined as total binding with radioligand alone minus nonspecific binding determined with 1 μM (−)-nicotine. IC$_{50}$ values were determined by iterative nonlinear least-squares regression using the SigmaPlot program (Jandel Scientific Software, San Rafael, CA). The results reported are mean values for four determinations with standard deviations or are two individual values when only two experiments were made.

Supporting Information Available: ^{1}H and ^{13}C NMR spectral data for each of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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