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Original article

Synthesis, receptor binding and QSAR studies on 6-substituted nicotine derivatives as cholinergic ligands

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Abstract – Nicotine **1** binds at nicotinic acetylcholinergic receptors (nAChRs) but relatively little is known regarding its structure–affinity relationships at central receptors. The present study focuses on the pyridine 6-position of nicotine. Earlier studies from our laboratories suggested that the electronic (σ) and/or lipophilic (π) nature of the 6-position substituent might influence nAChR affinity. To examine this in greater detail, we prepared and evaluated a series of 6-substituted nicotine analogs. The various analogs were found to bind at nAChRs with affinities (K_i values) ranging from 0.45 to > 10000 nM. It was demonstrated, for fifteen of these analogs, that affinity could not be explained on the basis of either σ or π. However, a combination of π and Δ MOL VOL (representative of the volume of the 6-position substituent) accounted for affinity ($r = 0.970$, $n = 15$). The basicity of the pyridine nitrogen atom was also examined by determining the pK_a values of several representative analogs. Consistent with the above studies examining σ, as well as with previously published studies on peripheral nAChR binding, pK_a alone did not account for variation in affinity. It would appear that lipophilic substituents at the pyridine 6-position contribute to nAChR affinity of nicotine analogs, but that affinity is further modulated by the steric size of this substituent in that increased size results in decreased affinity. © Elsevier, Paris

nicotine / nicotinic cholinergic receptors / nAChR binding / structure–affinity relationships / QSAR

1. Introduction

Nicotinic acetylcholinergic receptors (nAChRs) have long held the interest of investigators attempting to define nicotinic versus muscarinic cholinergic function. Efforts have also focussed on gaining an appreciation for the abuse character of nicotine **1**, a typical nAChR ligand. More recently has come the realization that nAChRs may be involved in a variety of therapeutically targetable actions. For example, nicotinic cholinergic receptors may be involved in appetite, anxiety, nociception, schizophrenias and several other mental and neurological disorders [1–3]. Indeed, nicotine itself has been shown to produce a number of beneficial effects, but its therapeutic application may be limited by the abuse liability and toxicities associated with the agent [4, 5]. However, there is no a priori reason to believe that all therapeutic effects of nicotine are inextricably linked to its toxic effects. Furthermore, current thinking is that there exists various subpopulations of the pentameric nAChRs that differ in subunit identity and composition [6, 7]. Thus, we and others are attempting to exploit nAChR ligands for their therapeutic potential [7, 8].

Several years ago Daly and co-workers reported that epibatidine **2** represents a novel naturally occurring antinociceptive agent with morphine-like actions that apparently produces its effects via a non-opiate mechanism [9]. We were intrigued with the potential structural similarities between **1** and **2**, veiled though they may be, and compared the two structures using molecular modeling techniques. Indeed, structural similarities were identified, and it was shown that the two structures could be overlayed such that the chlorine-bearing carbon atom of epibatidine could be superimposed on the 6-position carbon atom of nicotine [10, 11]. This led to the synthesis of (±)-6-chloronicotine and to the subsequent demonstra-

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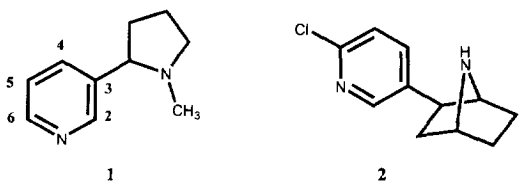


Figure 1. Structures of nicotine **1** and epibatidine **2**.

tion that this agent binds with several-fold higher affinity than (–)-nicotine (K_i = 0.63 nM and 2.4 nM, respectively) [10], and that it produces nicotine-like antinociceptive effects with fifteen times the potency of nicotine [12, 13]. (±)-6-Chloronicotine is also 20-fold more potent than (–)-nicotine when administered via the intrathecal route [13]. That these actions could be antagonized by the noncompetitive nicotinic antagonist mecamylamine [12] and the competitive nicotinic antagonist dihydro-β-erythroidine [13] supports the notion of nAChR involvement *(figure 1)*.

The above findings prompted the synthesis of several additional 6-substituted nicotine analogs including 6-bromonicotine (K_i = 0.45 nM), 6-fluoronicotine (K_i = 1.03 nM), 6-methylnicotine (K_i = 1.8 nM) and 6-methoxynicotine (K_i = 22 nM) [12]. With respect to their antinociceptive actions as measured in the tail-flick assay in mice, the bromo derivative was equipotent with the chloro analog, the fluoro and methyl derivatives were essentially equipotent with (–)-nicotine, and the methoxy derivative was much less active [12]. Initially, we thought that the 6-substituted nicotine analogs might bind with nAChRs in such a manner that the 6-position region was situated near a region of bulk tolerance and that observed differences in antinociceptive effects could be explained on the basis of differential rates of absorption and distribution of the various 6-substituted compounds. However, the reduced affinity of the 6-methoxy derivative suggested that the region of bulk tolerance was either limited in size or sensitive to the electronic nature of the substituents [12]. We performed a Hansch analysis and found that affinity (pK_i) was related both to the lipophilicity (i.e., π value) and electronic nature (i.e., σ value) of the 6-position substituent [14]. That is, as the lipophilicity or electron withdrawing nature of the 6-position substituent increased, affinity increased. However, the small number of compounds precluded the inclusion of both independent variables in the same relating equation. Furthermore, we found a significant internal correlation between the π and σ values of the substituents being examined [14]. That is, only one of the two parameters might actually be important for binding and the second might simply serve as a surrogate for the other. Thus, the purpose of the present investigation was to (a) prepare several additional examples of 6-substituted nicotine analogs so that a minimum of two independent variables could be employed in examining potential relating equations, (b) select substituents such that there was no internal correlation between the various π and σ values, and to (c) repeat the Hansch analysis to determine the influence of 6-position substituent lipophilicity and electronic character on nAChR affinity.

2. Chemistry

Compounds **3–6** and **9** were available from a previous study [12]. Thirteen additional analogs were prepared for examination in the present study. The free bases of the (–)-6-ethyl- (i.e., (–)-**7**) and (–)-6-*n*-propyl- (i.e., (–)-**8**) analogs were prepared by free radical alkylation of (S)-(–)-nicotine as reported by Seeman et al. [15]. Compound **16** (6-hydroxynicotine) was obtained by the use of drastic reaction conditions in an attempted synthesis of 6-methoxynicotine **9**. The key compound for the synthesis of all other analogs was 6-aminonicotine **11** *(figure 2)* obtained from S-(–)-nicotine in a Chichibabin [16] reaction. The acetamido, **18** and sulfonamido, **19**, analogs were prepared by amidation of **11** with acetic anhydride and methanesulfonyl chloride, respectively. Analog **14** was derived from amide **18** by reduction with $LiAlH_4$. Although the free base of 6-nitronicotine **12** has been reported [17] no detail of its synthesis was available. The nitro analog **12** was obtained by treating **11** with Caro's acid. The required 6-formylnicotine **13** was prepared from 6-bromonicotine **3** in a one-step reaction via directed lithiation. 6-Bromonicotine **3** was converted into nitrile **10** by heating at reflux with copper(I)-cyanide in the aprotic solvent DMF. Hydrolysis of **10** with sodium hydroxide, under different conditions, afforded either amide **17** and/or carboxylic acid **20**. It should be noted that although the elemental analysis for the oxalate salt of **20** was incorrect for H and N, its structure was supported by its ^{1}H-NMR spectrum and by conversion of **20** via an acid chloride to ester **15**.

3. Results

A total of nineteen compounds were now available for investigation; nAChR radioligand binding data were obtained for all new compounds and the results are shown in *table I*. A total of fifteen compounds were then examined in Hansch analysis fashion; these included racemic nicotine **1** and compounds **3–15** and **17**. Com-

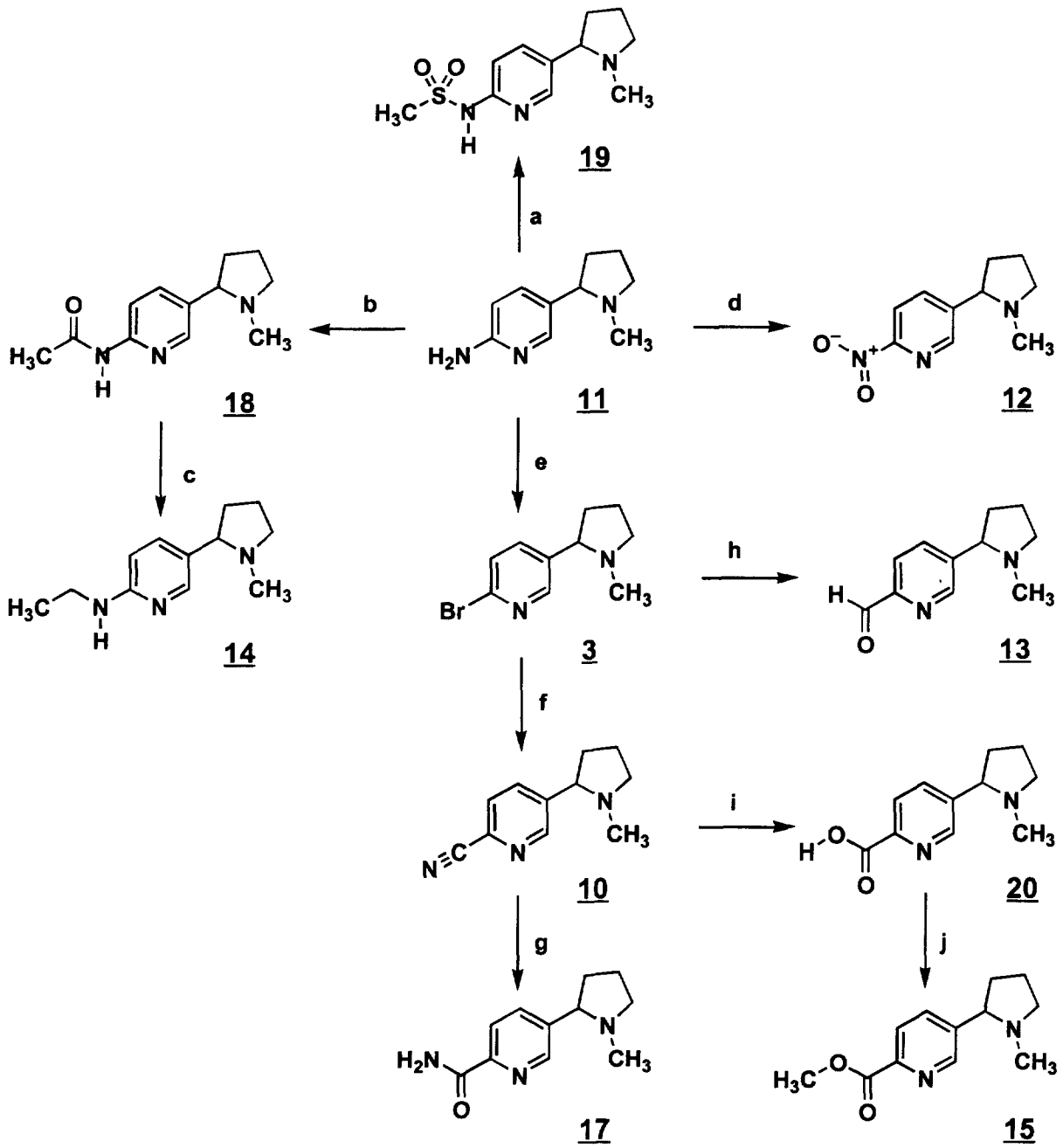
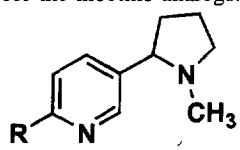


Figure 2. (a) $CH_3SO_2Cl/CHCl_3$; (b) $(CH_3CO)_2O/NEt_3$; (c) $LiAlH_4/Et_2O$; (d) H_2SO_4/Caro's acid; (e) $NaNO_2$/HBr; (f) CuCN/DMF; (g) NaOH/70% EtOH; (h) nBuLi/DMF; (i) NaOH/70% EtOH, Δ; (j) $SOCl_2$/MeOH, Δ.

pounds **18–20** could not be utilized due to their indeterminate affinities (i.e., $K_i > 10000$ nM). Compound **16** was also excluded from the analysis for reasons that will be discussed below. Attempted correlation of the affinities (pK_i values) of the fifteen compounds with the σ or π values of their 6-position substituents resulted in correlation coefficients (r values) of 0.092 and 0.592, respectively. The correlation coefficient was not improved when

Table I. Radioligand binding data (actual and predicted) for the nicotine analogs.



	R	Included [a]	K_i, nM	(SEM)	Δ Mol Vol [b]	Actual pK_i	Predicted pK_i [c]
1	–H	+	1.26	(± 0.2)	0.0	8.90	9.05
3	–Br	+	0.45	[d]	17.1	9.35	8.96
4	–Cl	+	0.63	[d]	11.7	9.20	9.13
5	–F	+	1.03	[d]	2.3	8.99	9.07
6	$-CH_3$	+	1.8	[d]	16.3	8.74	8.66
(–)-7	$-C_2H_5$	+	5.6	(± 0.3)	32.9	8.25	8.13
(–)-8	$-C_3H_7$	+	17.3	(± 3.8)	48.9	7.76	7.72
9	$-OCH_3$	+	22.0	[d]	23.8	7.67	7.48
10	–CN	+	29.0	(± 4.0)	12.7	7.54	7.55
11	$-NH_2$	+	81.0	(± 15.3)	13.6	7.09	6.70
12	$-NO_2$	+	142	(± 17)	17.8	6.85	7.56
13	–CHO	+	118	(± 18)	17.1	6.93	7.17
14	$-NHC_2H_5$	+	730	(± 74)	45.3	6.14	6.20
15	$-COOCH_3$	+	866	(± 97)	41.2	6.06	6.36
16	–OH		1072	(± 80)	7.4	5.97	7.77
17	$-CONH_2$	+	1344	(± 82)	26.9	5.87	5.53
18	$-NHCOCH_3$		> 10000		43.6	< 5.0	4.90
19	$-NHSO_2CH_3$		> 10000		51.4	< 5.0	4.11
20	–COOH		> 10000		19.7	< 5.0	2.58

[a] Included in the derivation of the relating equation; [b] difference in molecular volume, in Å^3, from nicotine (see text); [c] pK_i value calculated from the relating equation (1); [d] K_i value previously reported.

σ plus π was examined together (r = 0.593).The relationship between pK_i and substituent size (i.e., Δ MOL VOL) was examined due to the possibility that the overall size of the 6-position substituents might play a role in binding; however, the correlation coefficient was only r = 0.561. Thus, σ, π, σ + π and Δ MOL VOL accounted for 0%, 35%, 35% and 32%, respectively, of the variation in affinity. But a combination of π plus Δ MOL VOL resulted in a relating equation that accounted for 94% of the variation in affinity:

$$pK_i = 9.05\ (\pm 0.15) + 1.19\ (\pm 0.11)\ \pi - 0.067\ (\pm 0.006)\ \Delta\ \text{MOL VOL}, \quad (1)$$

r = 0.970; F = 95.3; SE = 0.31; n = 15,

that is, a combination of π and substituent size seemingly accounted for affinity as shown in *figure 3*.

With the exception of the 6-ethyl and 6-*n*-propyl derivatives (–)-**7** and (–)-**8**, respectively, all compounds were examined as their racemic mixtures. In order to determine if there might exist a significant affinity difference between the (–)-isomers and their racemates, the affinity of racemic **8** (K_i = 25 ± 6 nM) was measured and compared with that of (–)-**8** as control (replicate determination K_i = 22 ± 7 nM). Due to the similarity in K_i

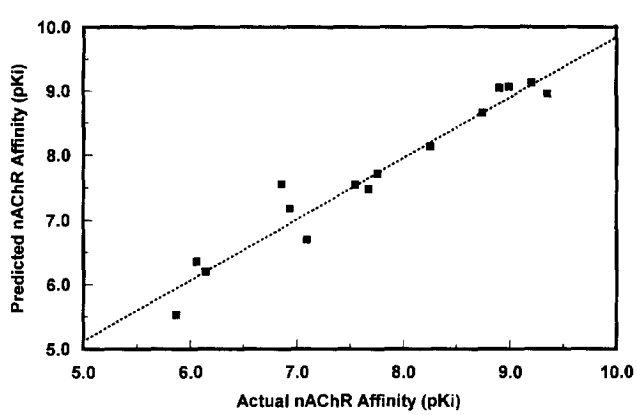


Figure 3. Relationship between actual and predicted (via equation (1)) nAChR affinities (pK_i values) for the fifteen 6-substituted nicotine analogs identified in *table I* (r = 0.970).

Table II. pK_a values for selected nicotine analogs.

	R [a]	pK_a	(± SEM)
1	–H	2.93	(0.03)
4	–Cl	–1.3	(0.1)
6	$-CH_3$	3.78	(0.09)
(–)-7	$-C_2H_5$	3.69	(0.03)
(–)-8	$-C_3H_7$	3.65	(0.06)
9	$-OCH_3$	1.34	(0.04)
18	$-NHCOCH_3$	2.2	(0.1)

[a] R = 6-position substituent; see *table I* for general structure.

values for (–)-**8** and (±)-**8**, inclusion of the two chiral agents in the present study probably did not obscure any correlation.

As a further attempt to determine whether electronic factors might influence binding, we determined the pK_a values of a representative number of compounds. The results are shown in *table II.*

4. Discussion

In the present investigation we determined the nAChR affinities of a series of 6-substituted nicotine derivatives. Three of these agents, amide **18,** sulfonamide **19** and acid **20**, failed to display significant affinity (i.e., K_i > 10000 nM). Another analog, 6-hydroxynicotine **16**, was found to bind with modest affinity (K_i = 1062 nM). α-Hydroxypyridines can co-exist in a keto form with the equilibrium lying in favor of the latter [18]; because it was unknown how much of the two tautomers of **16** was present under the conditions of the binding assay, it was felt that inclusion of the binding data for **16** might lead to spurious results in the Hansch analysis. Consequently, **16** was not considered in the analysis.

4.1. Hansch analysis

A total of fifteen derivatives were subsequently examined by Hansch analysis in an attempt to identify physicochemical properties that might influence nAChR binding. Of these fifteen analogs, K_i values spanned a 3000-fold range from 0.45 nM to 1344 nM, and there was no internal correlation between the σ and π values of the substituents ($r = 0.165$; $n = 15$). A preliminary analysis on the initially reported partial series of compounds [14] suggested that affinity might be related to either (or both) the σ or π values of the 6-position substituents. In the present investigation, with a series of fifteen analogs, neither the σ values nor the π values of the 6-position substituents accounted for more than 35% of the variability in the binding data. This was true even for a combination of σ and π values.

We had previously speculated that the size of the 6-position substituent might be involved in binding [12]. Thus, we sought a possible correlation between affinity and Δ MOL VOL. Although there was some indication that steric size might contribute to binding (i.e., r = 0.561), alone it explained only 32% of the data variation. However, a combination of π plus Δ MOL VOL resulted in a related equation (equation (1)) that accounted for 94% of the variation in binding data. It would appear then that lipophilicity is an important contributor to binding, but that increased bulk of a lipophilic 6-position substituent tends to decrease affinity. In other words, the size of the 6-position substituent seems to modulate the effect of a lipophilic requirement.

4.2. Dissociation constants

Rondahl and Ingman [19] have reported that there is no correlation between the pK_a values determined for nicotine derivatives (i.e., nicotine and 5-substituted nicotine derivatives and related analogs) and the biological activities of these agents. Nevertheless, they were primarily concerned with the nicotine-like effects of these compounds on isolated guinea pig vas deferens. In an attempt to empirically determine whether the basicity of the pyridine nitrogen atom of 6-substituted nicotine analogs plays a role in the binding of these compounds at central nAChRs, we measured the pK_a values of a representative number of compounds. The pK_a value determined for nicotine (pK_a = 2.93) was not different, depending upon the conditions employed, from those commonly reported (i.e., pK_a = 2.85 to 3.4) [19–24]. The results in *table II* show pK_a values for several additional compounds. It is concluded that there is no significant relationship (i.e., r = 0.117; n = 7) between the pK_a values and nAChR affinities of the agents examined.

4.3. Summary

We have shown that 6-substituted derivatives of nicotine bind at nAChRs with varying affinity. Additionally, affinity seems to be related to the lipophilicity of the 6-position substituent and is further influenced by the size of this substituent (as shown by equation (1)). Electronic factors appear to play a less significant role as indicated by the lack of a relationship between affinity and either the electron withdrawing/donating properties of the 6-position substituent or the pK_a of the pyridine nitrogen atom. It has been suggested that the pyridine nitrogen atom of nicotine and certain nicotine analogs forms a hydrogen bond with some binding feature on the

nAChR [8]. The observation that replacement of the pyridine nitrogen atom of nicotine with an sp^2-hybridized carbon atom results in a significantly decreased affinity [8] is consistent with this concept. However, the nature of the hydrogen bond interaction may be an all-or-none effect. That is, although the presence of a hydrogen-bonding pyridine nitrogen atom may be necessary for high-affinity binding, binding does not appear to be directly dependent upon the base strength of the pyridine nitrogen atom or the electronic character of the adjacent 6-position substituent. Alternatively, the size or steric bulk of this substituent may overshadow the role of basicity by interfering with an optimal drug–receptor interaction. This may be due to limited bulk tolerance in the receptor with the region associated with the 6-position of the nicotinic analogs, and/or certain of the 6-position substituents might sterically interfere with hydrogen bond formation.

5. Experimental protocols

5.1. Synthesis

Elemental analyses were performed by Atlantic Microlab (Norcross, GA) and all results (except for **20**) are within ±0.4% of theory. Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. Proton NMR (^{1}H-NMR) spectra were recorded on a GE QE-300 FT NMR; chemical shifts are reported as ppm, and trimethylsilane was used as an internal standard. Spectral data are consistent with assigned structures. Thin-layer chromatography was performed on precoated silica gel glass plates (60F254, Merck).

5.1.1. (–)-6-Ethyl-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride [(–)-7]

The free base of (–)-**7** was prepared according to the procedure of Seeman et al. [15] and converted to its hydrochloride salt. Recrystallization from a mixture of 2-PrOH/EtOAc/Et_2O (50:30:20) afforded 0.35 g (25%) of (–)-**7** as white crystals; m.p. 213–217 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.31–1.36 (t, 3H); 2.14–2.28 (m, 3H); 2.35–2.40 (m, 1H); 2.65 (d, 3H); 3.00–3.10 (q, 2H); 3.15–3.20 (m, 1H); 3.6–3.7 (m, 1H); 4.6–4.7 (m, 1H); 7.90–8.01 (d, 1H); 8.87–8.90 (d, 1H); 9.06 (s, 1H). $[\alpha]_D^{30}$ (free base) = –150.3° (c = 0.26, CH_2Cl_2) (Lit. [15] $[\alpha]_D^{20}$ = –160°). Anal. ($C_{12}H_{18}N_2$•2HCl) C, H, N.

5.1.2. (–)-6-n-Propyl-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride [(–)-8]

The free base of (–)-**8** was synthesized using the method of Seeman et al. [15] and converted to its hydrochloride salt. Recrystallization from a mixture of 2-PrOH/Et_2O gave (–)-**8** in 30% yield as ivory-colored crystals; m.p. 227–230 °C. ^{1}H-NMR (D_2O) δ: 1.19–1.24 (t, 3H); 2.04–2.11 (m, 2H); 2.60–2.67 (br m, 3H); 2.90–2.94 (m, 1H); 3.12 (br s, 3H); 3.30–3.36 (t, 2H); 3.63–3.67 (m, 1H); 4.18 (m, 1H); 4.92–4.97 (m, 1H); 8.29–8.32 (d, 1H); 8.90–8.93 (d, 1H); 9.19 (s, 1H). $[\alpha]_D^{37}$ (free base) = –148° (c = 0.25, CH_2Cl_2) (Lit. [15] $[\alpha]_D^{20}$ = –150°). Anal. ($C_{13}H_{20}N_2$•2HCl) C, H, N.

5.1.3. (±)-6-n-Propyl-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride [(±)-8]

A solution of myosmine [25] (2.25 g, 15 mmol) in 10% H_2SO_4 (20 mL, 36 mmol) and $AgNO_3$ (0.55 g, 3 mmol) was allowed to stir at 70 °C under an N_2 atmosphere. Butanoic acid (2.71 g, 31 mmol) and a solution of ammonium peroxydisulfate (7.10 g, 31 mmol) in H_2O (15 mL) were independently added (from two addition funnels) over a 10-min period. When the addition was complete, stirring and heating (70 °C) were continued for additional 1 h. The reaction mixture was cooled to room temperature, basified with cold NH_4OH (35 mL) and extracted with $CHCl_3$ (3 × 10 mL). The organic portions were combined, and the solvent was removed in vacuo. The crude compound (probably a mixture of 2-, 4- and 6-propyl isomers, as well as 2,6- and 4,6-dipropyl isomers) was purified by column chromatography (eluent: acetone/hexane, 1:20) to afford 0.50 g (17%) of 6-propylmyosmine as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.94–0.99 (t, 3H); 1.69–1.81 (m, 2H); 2.00–2.10 (m, 2H); 2.78–2.83 (t, 2H); 2.85–2.98 (m, 2H); 4.03–4.09 (m, 2H); 7.18–7.21 (d, 1H); 8.09–8.12 (dd, 1H); 8.88–8.89 (d, 1H).

Sodium borohydride (0.19 g, 0.5 mmol) was added portionwise over a 10-min period with vigorous stirring, to a cooled solution of the above intermediate 6-propylmyosmine (0.43 g, 2.3 mmol) in CH_3OH/CH_3COOH, 4:1 (5 mL) at –40 °C (dry ice/acetone bath). During the course of the addition, the temperature rose to –20 °C. After warming to room temperature, most of the solvent was removed under reduced pressure; H_2O (15 mL) was added and the solution was made basic (pH = 10) with 15% NaOH and extracted with CH_2Cl_2 (2 × 30 mL). The organic portions were combined, washed with brine (5 mL), dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was treated with 37% HCHO (5.5 mL) and 97% HCOOH (5.5 mL) and heated at reflux for 2 h, cooled to room temperature and extracted with Et_2O (3 × 30 mL). The aqueous portion was basified (pH = 10–11) with aqueous $NaHCO_3$ and solid K_2CO_3 and extracted with $CHCl_3$ (3 × 30 mL). The combined organic portion was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The crude product was

purified by column chromatography (eluent: acetone/hexane, 1:10) to give 0.28 g (60%) of the title compound as a yellow oil. The hydrochloride salt was prepared to afford (±)-**8** as a light brown powder; m.p. 215–217 °C (2-PrOH/anhydrous Et_2O). ^{1}H-NMR (D_2O) δ: 1.19–1.24 (t, 3H); 2.04–2.11 (s, 2H); 2.60–2.67 (m, 3H); 2.90–2.94 (m, 1H); 3.12 (s, 3H, N–CH_3); 3.30–3.36 (t, 2H); 3.63–3.67 (m, 1H); 4.18 (br s, 1H); 4.92–4.97 (m, 1H); 8.29–8.32 (d, 1H); 8.90–8.93 (d, 1H); 9.11 (s, 1H). Anal. ($C_{13}H_{20}N_2$•2HCl) C, H, N.

*5.1.4. 6-Cyano-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride **10***

Copper (I) cyanide (1.11 g, 12.39 mmol) was added to a stirred solution of 6-bromonicotine **3** [12] (2.00 g, 8.28 mmol) in DMF (40 mL) and the mixture was heated at reflux for 4 h. The reaction mixture was filtered hot and the residue was washed with hot CH_2Cl_2 (100 mL). The solvent was remove in vacuo. The residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH, 100:0.5) to give 0.55 g (35%) of a pale-yellow oil. The free base was converted to its hydrochloride salt; m.p. 166–168 °C after recrystallization from 2-PrOH/anhydrous Et_2O. ^{1}H-NMR ($CDCl_3$) δ: 1.78 (m, 1H); 2.29 (m, 1H); 2.56 (m, 2H); 2.74 (s, 3H); 3.09 (br m, 1H); 4.16 (t, 2H); 7.87 (d, 1H); 8.82 (d, 1H); 8.98 (s, 1H). Anal. ($C_{11}H_{13}N_3$•HCl) C, H, N.

*5.1.5. 6-Nitro-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride **12***

A solution of 6-aminonicotine **11** (0.71 g, 4 mmol) in anhydrous MeOH (5 mL) was treated with a methanolic solution of HCl and allowed to stir for 15 min at room temperature. The solvent was removed in vacuo and the solid residue was dissolved in concentrated H_2SO_4 (10 mL). This was added in a dropwise manner to a cooled solution of Caro's acid [17] (4.6 g, 40 mmol) at 5 °C over a period of 15 min. The resulting mixture was allowed to warm to room temperature and was kept for 3 days at that temperature. The reaction mixture was poured onto ice and made basic with 10% NaOH solution (pH 9–10); the mixture was extracted with CH_2Cl_2 (3 × 200 mL), the organic portion was washed with brine (20 mL) and dried (Na_2SO_4). The filtrate was evaporated in vacuo and the residue was purified by column chromatography (eluent: acetone/hexane, 1:1) to give 0.58 g (70%) of ivory-colored crystals of the title compound as its free base; m.p. 74–75 °C after crystallization from hexane (Lit. [17] m.p. 78.5–79 °C). The hydrochloride salt **12** was prepared as ivory-colored crystals; m.p. 146–148 °C (absolute EtOH/anhydrous Et_2O). ^{1}H-NMR (CD_3OD) δ: 2.35–2.47 (m, 3H); 2.66–2.71 (m, 1H); 2.86 (s, 3H); 3.37–3.43 (q, 1H); 4.70–4.74 (t, 1H); 4.90–4.99 (m, 1H); 8.42–8.44 (d, 1H); 8.55–8.57 (d, 1H); 8.80 (d, 1H). Anal. ($C_{10}H_{13}N_3O_2$•HCl•0.5H_2O) C, H, N.

*5.1.6. 6-Formyl-3-(1-methyl-2-pyrrolidinyl)pyridine maleate **13***

n-BuLi (2.8 mL, 7.04 mmol) was added to a stirred solution of 6-bromonicotine **3** [12] (0.85 g, 3.52 mmol) in dry THF(20 mL) under an N_2 atmosphere at –78 °C. The reaction mixture was allowed to stir for 1h at –78 °C, then DMF (0.28 g, 3.87 mmol) was added and stirring was continued for additional 0.5 h under the same conditions. The cold mixture was poured directly into a stirred 5% $NaHCO_3$ solution (25 mL) and the mixture was extracted with Et_2O (3 × 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford 0.65 g of crude product as a yellow oil. Purification by column chromatography (silica gel 62 mesh, eluent: $CHCl_3$/MeOH, 100:1) gave 0.22 g (33%) of the title compound as a free base. ^{1}H-NMR ($CDCl_3$) δ: 1.68–1.75 (m, 1H); 1.82–1.89 (m, 2H); 1.94–2.05 (m, 1H); 2.19 (s, 3H, NCH_3); 2.22–2.30 (m, 1H); 3.21–3.28 (m, 2H); 7.87–7.89 (dd, 1H); 7.93–7.95 (d, 1H); 8.71–8.72 (d, 1H); 10.08 (s, 1H, CHO). The maleate salt was prepared to afford **13** as a light-brown powder; m.p. 127–130 °C (MeOH/Et_2O). Anal. ($C_{11}H_{14}N_2O$•$C_4H_4O_4$) C, H, N.

*5.1.7. 6-Ethylamino-3-(1-methyl-pyrrolidinyl)pyridine oxalate **14***

A solution of **18** free base (1.4 g, 6.5 mmol) in anhydrous Et_2O (50 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (0.5 g, 13.6 mmol) in anhydrous Et_2O (100 mL) (at such rate that the Et_2O in the flask boiled gently), over a 1 h period, under an N_2 atmosphere. When the addition was complete, stirring and heating were continued for additional 1 h. Excess $LiAlH_4$ was decomposed at 0 °C by the successive dropwise addition of H_2O (0.5 mL), 15% NaOH (0.5 mL) and H_2O (1.5 mL). The insoluble salts were removed by filtration and washed with Et_2O (3 × 35 mL). The organic portions were combined, and the solvent was removed under reduced pressure. The crude compound was purified by acid (10% HCl) base (15% NaOH) extraction with Et_2O (3 × 100 mL); the organic portion was dried (K_2CO_3) and the solvent was removed in vacuo to give a yellow oily residue of the free base (0.9 g; 89%). The oxalate salt was prepared and recrystallized from 2-PrOH/anhydrous Et_2O to afford **14** as air-sensitive yellow crystals. ^{1}H-NMR (D_2O) δ: 1.15 (t, 3H); 1.55–2.09 (m, 5H); 2.19 (s, 3H); 2.80–2.95 (m, 1H); 3.10–3.30 (m, 2H); 3.60 (t, 1H); 6.20 (m, 1H, partially

exchanged); 7.10 (d, 1H); 7.50 (dd, 1H); 8.10 (d, 1H). Anal. ($C_{12}H_{19}N_3 \cdot 2C_2H_2O_4 \cdot H_2O$) C, H, N.

5.1.8. Methyl 3-(1-methyl-2-pyrrolidinyl)pyridine-6-carboxylate hydrochloride ***15***

Thionyl chloride (0.6 g, 5.08 mmol) was added to a stirred solution of acid **20** (0.5 g, 2.42 mmol) in dry CH_3OH (10 mL). The reaction mixture was heated at reflux for 2 h; the solvent was evaporated under reduced pressure and the residue was treated with 10% $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic portion was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by column chromatography (eluent: acetone/hexane, 2:1) to afford 0.38 g (70%) of a yellow oil of the title compound as a free base. The hydrochloride salt was prepared to give **15** as a light brown crystals; m.p. 157–160 °C (absolute EtOH/anhydrous Et_2O). ^{1}H-NMR (CD_3OD) δ: 2.36–2.51 (m, 3H); 2.63 (br s, 1H); 2.83 (s, 3H); 3.35–3.38 (br m like, 1H); 3.91–3.94 (m, 1H); 4.01 (s, 3H); 4.61 (br m like, 1H); 8.26–8.35 (dd, 2H); 8.86 (s, 1H). Anal. ($C_{12}H_{16}N_2O_2 \cdot 1HCl \cdot 0.25H_2O$) C, H, N.

5.1.9. 6-Hydroxy-3-(1-methyl-2-pyrrolidinyl)pyridine hydrochloride ***16***

A mixture of 6-methoxynor-nicotine [12] (0.22 g, 1.21 mmol), 37% formaldehyde (6 mL), and 95–97% formic acid (6 mL) were heated with stirring at 100–102 °C for 25 h. The residue obtained upon evaporation of the reaction mixture was basified by treatment with a saturated aqueous solution of K_2CO_3 to pH 10–11, then extracted with $CHCl_3$ (3 × 15 mL). The combined organic portion was washed with brine, dried (K_2CO_3), and the solvent was removed in vacuo. The oily residue was purified by column chromatography (eluent: $CHCl_3$/hexane, 8:1) to give 0.12 g (65%) of the free base of the title compound as a pale-yellow oil. The free base was converted to the hydrochloride salt; m.p. 198–205 °C (absolute EtOH/anhydrous Et_2O). IR (free base, neat): 1664 (pyridone C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.74 (br m, 2H); 1.92 (m, 1H); 2.11 (m, 1H); 2.16 (s, 1H); 2.26 (m, 1H); 2.84 (t, 1H); 3.18 (t, 1H); 6.62 (d, 1H); 7.31 (s, 1H); 7.57 (d, 1H). Anal. ($C_{10}H_{14}N_2O \cdot 2HCl$) C, H, N.

5.1.10. 3-(1-Methyl-2-pyrrolidinyl)pyridine-6-carboxamide oxalate ***17***

Sodium hydroxide (0.5 g) was added to a stirred solution of 6-cyanonicotine **10** (0.5 g, 2.6 mmol) in 70% EtOH (50 mL). The reaction mixture was heated at reflux for 2 h; the solvent was evaporated under reduced pressure and the residue was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure to afford the crude product. Recrystallization from anhydrous Et_2O/hexane gave 0.4 g (77%) of the title compound as its free base; m.p. 136–138 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.66–2.06 (m, 4H); 2.18 (s, 3H, N–CH_3); 2.22–2.38 (m, 1H); 3.15–3.28 (m, 2H); 6.17 (br s, 1H, CONH, D_2O exchangeable); 7.83–7.86 (d, 1H); 7.87 (s, 1H, CONH, D_2O exchangeable); 8.15–8.18 (d, 1H); 8.51 (s, 1H). The oxalate salt was prepared to afford **17** as a white powder; m.p. 135–137 °C (absolute EtOH/anhydrous Et_2O). Anal. ($C_{11}H_{15}N_3O \cdot C_2H_2O_4 \cdot 1H_2O$) C, H, N.

5.1.11. 3-(1-Methyl-2-pyrrolidinyl)pyridine-6-acetamide ***18***

NEt_3 (0.4 mL, 2.82 mmol) and acetic anhydride (0.23 g, 2.25 mmol) were added successively in a dropwise manner to a solution of 6-amino-nicotine [16] (0.2 g, 1.13 mmol) in dry THF (10 mL) at 0 °C under an N_2 atmosphere. The reaction mixture was allowed to stir for 10 min and was then heated at reflux for 1 h. After cooling to room temperature, the reaction mixture was quenched with saturated $NaHCO_3$ (pH 9–10) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic portions were dried ($MgSO_4$), and the solvent was removed under reduced pressure. The oily residue was purified by flash chromatography (eluent: $CHCl_3/CH_3OH$, 9.5:0.5) to afford 0.22 g (80%) of the desired amide as a colorless oil. An anhydrous Et_2O solution of the free base was treated with a saturated solution of HCl gas in Et_2O to give a white solid of hydrochloride salt. Recrystallization from 2-PrOH/anhydrous Et_2O (1:1) gave 0.2 g (70%) of **18**; m.p. 208–210 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (s, 3H, NCH_3); 2.12–2.31 (m, 3H); 2.32–2.48 (m, 1H); 2.58 (br s, 3H, $COCH_3$); 3.08–3.22 (m, 1H); 3.65–3.82 (m, 1H); 4.32–4.44 (m, 1H); 7.25 (br s, 1H, NHCO); 8.08 (d, 1H); 8.25 (d, 1H); 8.55 (br s, 1H). Anal. ($C_{12}H_{17}N_3O \cdot 2HCl$) C, H, N.

5.1.12. 6-Sulfonamido-3-(1-methyl-2-pyrrolidinyl) pyridine hydrochloride ***19***

A solution of methane-sulfonyl chloride (0.25 g, 2.2 mmol) in $CHCl_3$ (10 mL) was added in a dropwise manner to a cooled mixture of 6-aminonicotine [16] (0.30 g, 1.7 mmol) and NEt_3 (0.55 mL, 4.0 mmol) at 0 °C (ice-NaCl bath) under an N_2 atmosphere. The reaction was allowed to proceed at this temperature for an additional 30 min, and was then quenched with a saturated $NaHCO_3$ solution (50 mL) followed by extraction with $CHCl_3$ (3 × 50 mL). The organic portions were combined, washed with brine (2 × 50 mL), dried ($MgSO_4$), and the solvent was removed under reduced

pressure. The residue was purified by column chromatography (eluent: $CHCl_3$/MeOH, 9.5:0.5) to give the title compound as its free base. The hydrochloride salt was prepared and recrystallized from 2-PrOH/anhydrous Et_2O to afford 0.13 g (55%) of **19** as air-sensitive yellow crystals; ^{1}H-NMR (D_2O) δ: 1.95–2.09 (m, 5H); 2.18 (s, 3H); 3.1–3.3 (m, 1H); 3.58 (s, 3H); 7.8 (d, 1H); 8.1 (dd, 1H); 8.58 (s, 1H, amide-H). Anal. ($C_{11}H_{17}N_3SO_2$•2HCl) C, H, N.

*5.1.13. 3-(1-Methyl-2-pyrrolidinyl)pyridine-6-carboxylic acid oxalate **20***

Sodium hydroxide (0.6 g, 15 mmol) was added to a stirred solution of 6-cyanonicotine **10** (0.6 g, 3.2 mmol) in 70% EtOH (60 mL). The reaction mixture was heated at reflux for 3 h, and the solvent was evaporated under reduced pressure. The basic residue was neutralized with 37% HCl (1.48 mL) and the solvent was removed in vacuo. The residue was dissolved in dry MeOH and inorganic salts were removed by filtration; the solvent was evaporated under reduced pressure to give a crude product. Purification by column chromatography (silica gel 62 mesh, eluent: acetone/dry MeOH, 1:1) gave 0.5 g (75%) of the desired compound as its free base (m.p.: decomposition > 200 °C). A methanolic solution of the amine was treated with a methanolic solution of oxalic acid to afford the crude salt; recrystallization from 2-PrOH/anhydrous Et_2O gave the target compound as a yellow powder; m.p. 185–188 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.21 (br m, 3H); 2.49–2.52 (br m, 1H); 2.67 (s, 3H, N–CH_3); 3.23–3.28 (br m, 1H); 3.79–3.81 (br m, 1H); 4.57–4.60 (br m, 1H); 8.11–8.14 (d, 1H); 8.41–8.43 (d, 1H); 8.94 (s, 1H); 11.3–11.5 (br s, 1H, COOH). Anal. ($C_{11}H_{14}N_2O_2$•0.5$C_2H_2O_4$•1.25H_2O) [calculated/found: C, 52.62/52.46; H, 6.44/6.02; N, 10.23/10.73%].

5.2. Hansch analysis

Analysis was performed by the method of Hansch et al. [26, 27] using the linear regression and multiple linear regression tools of SigmaStat version 2.0 for Windows. For the substituent constants employed, there was no internal correlation between σ and π values (r = 0.165; n = 15). There was also no internal correlation between π and the Δ MOL VOL values employed (r = 0.401; n = 15).

5.3. pK_a determinations

The experimental procedure for the determination of pK_a values was a general spectrophotometric method [28] as applied to the measurement of pK_a values for 2-substituted pyridines [29, 30]. For each nicotine analog, the spectra (190 to 350 nm) of the unprotonated and protonated species were obtained in 0.01 M KOH and 1 M HCl, respectively, except for 6-methoxy- and 6-chloronicotine (3 M HCl was used in place of 1 M HCl for 6-methoxy nicotine; see below for 6-chloronicotine). The pH measurements were obtained on a Corning Ion Analyzer 255 using a Corning Semimicro glass electrode that was standardized immediately before use with Fisher standard buffers (pH 4.01 and 7.00). Absorption spectra and absorbencies at various wavelength were recorded on a Varian DMS 100 UV–Visible Spectrophotometer at 25 (± 2) °C. Absorbances were measured in a series of buffer solutions with pH values near the estimated pK_a of the analog. Buffers were made such that ionic strength was constant (I = 0.01) [31]. The pK_a value was calculated using the equation:

$$pK_a = pH + \log [(A - A_M) / (A_I - A)]$$

where A is the absorbance of the analog at a given pH, A_M is the absorbance of the unprotonated analog, and A_I is the absorbance of the protonated analog. For 6-chloronicotine, the spectra and absorbances were measured in solutions with high HCl concentrations that precluded accurate measurement of pH; hence, the Hammett acidity function was used as a measure of pH [32]. The spectra of the protonated species was determined in concentrated HCl and that of the unprotonated species in 0.01 M KOH and three measurements in 3.6 M, 4.1 M and 4.5 M HCl. All results represent, at a minimum, triplicate determinations.

5.4. Molecular modeling studies

Molecular models of the nicotine analogs were constructed, using Sybyl (Version 6.2, Tripos Inc., St. Louis, Missouri), from the template molecule of (–)-nicotine which was energy minimized using the Tripos force field (MAXIMIN2) to a gradient of 0.05 kcal/mol × A and with Gasteiger–Huckel charges. The volume of each energy-minimized analog was estimated using the Volume Contour function within Sybyl. The difference in the molecular volume attributed to each substituent, Δ MOL VOL, was calculated by subtracting the volume of (–)-nicotine (154.6 Å^3) from the volume of the nicotine analog. The conformation of the pyrrolidine ring was essentially the same for all of the analogs.

5.5. Radioligand receptor binding

The binding assay was conducted as previously described [12]. In brief, rat brain without cerebellum was homogenized in 10 volumes of ice-cold 0.05 M Na–K

phosphate buffer (pH 7.4) and centrifuged at 17000 *g* (4 °C) for 30 min. The pellet was resuspended in 20 volumes of ice-cold glass-distilled H_2O and allowed to incubate on ice for 60 min before centrifugation as described above. The resulting pellet was resuspended to a final tissue concentration of 40 mg/mL of buffer. [^{3}H]Nicotine was incubated with 0.5 mL of tissue homogenate in a final incubation volume of 1 mL for 2 h at 0 °C. The samples were rapidly filtered through Whatman GF/C filters. Specific binding was defined as the difference in the amount of binding in the presence and absence of 100 μM (–)-nicotine tartrate. Following three consecutive washes with ice-cold buffer the filters were allowed to air-dry and were placed in scintillation vials for quantitation of radioactivity. Following transformation of the data by the Scatchard method, the K_D and B_{max} values were determined using the program LIGAND [33]. Displacement of tritiated nicotine binding at 1 nM was determined in the presence of increasing concentrations of various ligands and converted to percent displacement of specific binding. The IC_{50} values were determined from a plot of log concentration versus percent displacement and converted to K_i values by the method of Cheng and Prusoff [34]. All K_i values represent a minimum of triplicate determinations.

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[14] The compounds included in the initial Hansch analysis were nicotine and its 6-fluoro, 6-chloro, 6-bromo, 6-methyl and 6-methoxy derivatives. 6-Aminonicotine, used as an intermediate in the synthesis of the 6-halogeno derivatives, was also included. Receptor affinity (pK_i) was found to be correlated both with π ($r = 0.875$; $n = 7$) and σ ($r = 0.952$; $n = 7$). An internal correlation ($r = 0.896$) was found for the σ and π values employed for the original series of compounds.

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