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A Comparison of the Binding of Three Series of Nicotinic Ligands

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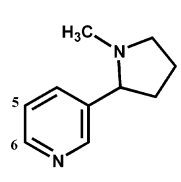
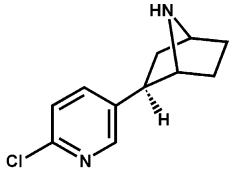
Abstract—A total of 24 aryl-substituted analogues of nicotine (**1a**) and two related series of nicotinic ligands, aminomethylpyridines **3** and ether analogues **8**, were examined to determine if they bind at α4β2 nACh receptors in a common manner. A modest correlation ($r = 0.785$) was found between the affinities of the nicotine analogues and derivatives of **3**, but little correlation ($r = 0.348$) was found with analogues **8**. However, a modest correlation ($r = 0.742$) exists between the binding of analogues **3** and **8**. It seems that **1**-series and **8**-series compounds bind differently but that the **3**-series compounds share some intermediate binding similarity with both.

There has been great interest in the past decade in the development and refinement of α4β2 nicotinic cholinergic (nACh) receptor pharmacophores. This interest derives from the possible therapeutic application of nicotinic ligands as antinociceptive agents, anorectic agents and for their potential use in the treatment of mental disorders (e.g., as antipsychotic agents and cognition enhancers).[1]



1a



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The first explicit pharmacophore for nicotinic agents was that proposed by Beers and Reich,[2] who implicated (i) a coulombic interaction involving a charged amine moiety (e.g., the pyrrolidine amine of nicotine, **1a**) and (ii) a hydrogen bond acceptor (e.g., the pyridine nitrogen atom of nicotine)—specifically, a point on a vector directed from the hydrogen bond acceptor toward a hydrogen bond donor 3 Å distant on the receptor was defined as being 5.9 Å from the cationic head. Sheridan and co-workers refined this model and defined distances between, in the case of nicotine (**1a**), the pyrrolidine nitrogen atom, the pyridine nitrogen atom, and a dummy atom that comprised the near-centroid of the pyridine ring; an internitrogen (N–N) distance of 4.8 Å was found to be optimal.[3] With the discovery of epibatidine (**2**) and newer nicotinic agents, it was proposed that the nicotinic receptors could accommodate larger N–N distances, and that a distance of 5.1–5.5 Å might be optimal (reviewed in refs 4 and 5). Due to the conformational flexibility of epibatidine and other nicotinic agents, later models suggested that N–N distances of either 4.6 or 6.3 Å could explain binding.[4] Koren et al.[6] favored the shorter distance on the basis that although certain nicotinic agents could achieve either the 'long distance' or the 'short distance', the highest affinity agents could only adopt conformations with the shorter distance. Subsequently, Tønder et al.[7] identified a series of high affinity nicotinic agents that possessed N–N distances > 4.8 Å. To account for this, they suggested that the distance between specific ligand/receptor features, rather than the N–N distance, is essential for binding. To some extent, this recalls the earlier concept of Beers and Reich, but Tønder et al.[7] provided a more detailed analysis of these interactions. In the latter model, ligands are assumed to bind to the receptor at points *a* and *b*, where *a* is a site 2.9 Å from the cationic head and *b* is a site 2.9 Å from the hydrogen bond acceptor; an *a*–*b* distance of 7.0–8.0 Å is thought to be optimal for high-affinity binding. There is a certain appeal to this model in that it accounts for the binding of agents with short and long N–N distances

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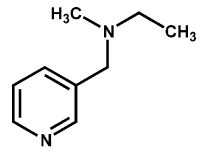
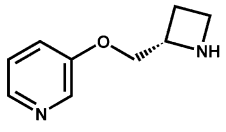
because a key feature is the direction of vectors associated with the two nitrogen atoms and not the N–N distance itself that is important. A newer model utilizes similar basic concepts to define a four-element model—two elements of which are receptor-associated features.[8]

In many of the pharmacophore models developed to explain the binding of nicotinic agents at nACh receptors, the assumption has been made that the pyridine rings (or equivalent structures) of various agents are superimposable. Pyridine-ring superimposition, although important, may be less stringent in the 'vector' models, but even these models require some degree of ring overlap.[8] There is support for this concept. For example, replacement of the pyridine nitrogen atom of nicotinic agents with an sp^2-hybrized carbon atom results in a dramatic decrease in affinity.[4] Relocation of the pyridine nitrogen atom of various agents (including epibatidine)[9] to another position can have a similar effect.[4,5] This argues that the nitrogen atom is a contributor to binding and that its position in the ring is of consequence to affinity. There is a commonly held tenet that parallel structural changes within two series of agents can result in parallel shifts in affinity if the two series are binding in a similar fashion. Can it be safely assumed that this is the case for nicotinic analogues? Carroll et al.[10] have recently reported for a series of six epibatidine analogues, varying only with respect to the nature of the substituent at the pyridine 6-position, that a significant correlation ($r = 0.99$) exists between their affinities and those reported for like-substituted nicotine analogues. They concluded that epibatidine analogues and nicotine analogues are binding in a similar fashion.



3a



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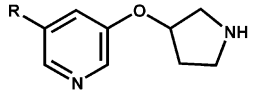

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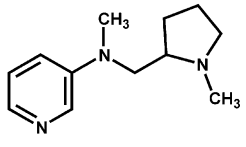

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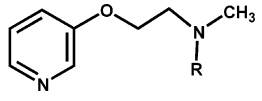
One early explanation for the enhanced affinity of epibatidine (**2**) over nicotine was that the chloro group of the latter makes a significant contribution to binding. To test this hypothesis, '6-chloronicotine' [2-chloro-5-(*N*-methylpyrrolidin-2-yl)pyridine, **1g**] was prepared and evaluated.[11] It was concluded that the high affinity of epibatidine was not solely attributable to the presence of the chloro group,[11] and it has since been shown that *des*-chloroepibatidine binds with similar affinity as epibatidine.[10] Nevertheless, the added chloro substituent of 6-chloronicotine was tolerated by nACh receptors and its presence at least doubled the affinity of nicotine.[11] Since then, it has become relatively common practice to introduce a chloro group α to the pyridine nitrogen atom of various nACh ligands. Interestingly, introduction of this function has been shown to have varying effects. For example, addition of a 6-chloro group halved the affinity of **3a**, a ring-opened analogue of nicotine, from 28 to 41 nM, whereas it had no effect on the affinity of A-85380 (**4**; $K_i = 0.05$ nM) but decreased the affinity of its *N*-methyl analogue ($K_i = 0.45$ nM) by 3-fold (reviewed in ref 5). In a series of compounds **5** where the 5-position substituent was varied, introduction of a 6-chloro group enhanced affinity from about 2- to 25-fold,[12] whereas a 6-chloro group decreased the affinity of **6** by nearly 100-fold (from 8.9 to 870 nM).[13]

Some of these affinity shifts are relatively small. Furthermore, these shifts reflect alteration of a single substituent (i.e., H→Cl). We have previously reported that compound **7**, the structural parent of numerous chain-extended nicotine analogues (including **4** and **5**), binds at nACh receptors;[14] compound **8a** is simply a homologue of **7**. If compounds such as nicotine (**1a**), **3a** and **8a** bind at nACh receptors in a common manner, parallel substituent changes should result in parallel shifts in affinity. Thus, we examined a total of 24 members of the three series where the 5- and 6-position substituents were varied to determine what effect these structural alterations would have on affinity, and whether parallel structural changes would result in parallel affinity shifts. We had previously reported[15] the effect of 6-position alteration on the affinity of nicotine and this served as a starting point for the present investigation.



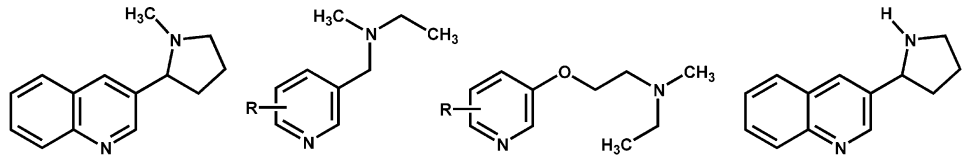
7, R = -CH_3
8a, R = -CH_2CH_3

One of the simplest structural relatives of nicotine is its ring-opened counterpart **3** (Table 1). Comparing the affinities of the nine derivatives of **1** with those of **3** (Table 2), there is a modest correlation ($r = 0.785$) between affinities within the two series. In contrast, there is little correlation between the affinities of the nicotine analogues **1** and those of the pyridyl ethers **8** ($r = 0.348$; $n = 6$); it seems unlikely, then, that these two series are binding with overlapping pyridyl rings.

Curiously, there exists a slight correlation ($r = 0.742$; $n = 6$) between the affinities of **3a**–**f** and their pyridyl ether counterparts **8a**–**f**. How can this be explained? One explanation is that the correlation is fortuitous.

Another possible explanation is that the series-**3** compounds bind somewhat similarly to series **1**, and that series **8** binds somewhat similarly to series **3**. That is, the modes of binding of the **1**-series and **8**-series compounds are different, but the **3**-series compounds bind in a manner intermediate between the two. However, there are clear differences between the affinities of **3** and **8** upon alteration of the terminal amine substituents. For example, we have already found that shortening the

Table 1. Physicochemical properties of compounds investigated in the present study[a]

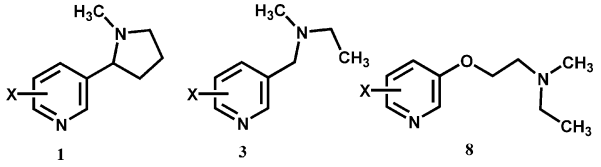


1f 3 8 9

	R	Yield (%)	Mp (°C)	Recrystallization solvent	Empirical formula[b]
(−)**1f**	—	11	228–230	EtOH–Et_2O	$C_{14}H_{16}N_2$·2HCl
(+)**1f**	—	26	228–230	EtOH–Et_2O	$C_{14}H_{16}N_2$·2HCl
3b	5-Br	95	157–158	Et_2O	$C_9H_{13}BrN_2$·1.25$C_2H_2O_4$
3c	5-OCH_3	33	128–129	EtOH–Et_2O	$C_{10}H_{16}N_2O$·2$C_2H_2O_4$[d]
3d	6-Br	96	153–155	Et_2O	$C_9H_{13}BrN_2$·1.5HCl
3f	Fused[c]	84	179–180	EtOH–Et_2O	$C_{13}H_{16}N_2$·2HCl
8b	5-Br	83	100–101 (dec)	Acetone	$C_{10}H_{15}BrN_2O$·1.5$C_2H_2O_4$
8c	5-OCH_3	38	108–109	Acetone	$C_{11}H_{18}N_2O_2$·1.25$C_2H_2O_4$[e]
8d	6-Br	79	134–135	Acetone	$C_{10}H_{15}BrN_2O$·$C_2H_2O_4$
8e	6-OCH_3	83	121–122	Acetone	$C_{11}H_{18}N_2O_2$·$C_2H_2O_4$
8f	Fused[c]	94	178–179	EtOH–Et_2O	$C_{14}H_{18}N_2O$·2HCl[f]
9	—	26	184–186	EtOH–Et_2O	$C_{13}H_{14}N_2$·2HCl

[a]Methods of synthesis discussed in Methods section.
[b]All compounds analyzed within 0.4% of theory for C, H, N.
[c]A 5,6-fused isoquinoline analogue.
[d]Crystallized with 0.5 mol H_2O.
[e]Crystallized with 0.25 mol H_2O.
[f]Crystallized with 1.0 mol H_2O.

Table 2. Binding data for nACh receptor ligands



1 3 8

	X	K_i, nM (±SEM)[a]					
		1		**3**		**8**	
a	–H	1.3	(0.2)	28		22	
b	5-Br	6.9		850	(140)	447	(32)
c	5-OCH_3	14		115	(20)	16	(1)
d	6-Br	0.45		62	(6)	290	(40)
e	6-OCH_3	22		640		4070	(1900)
f	Fused[c]	11.2	(2.1)	1110	(220)	2470	(300)
g	–Cl	0.63		41		—[b]	
h	–F	1.03		93		—	
i	6-CH_3	1.8		66		—	

[a]Where SEM is not shown, binding data have been previously reported;[15] synthesis of compounds **1b** and **1c** has been reported.[16]
[b]Not determined.
[c]A 5,6-fused isoquinoline analogue.

N-ethyl group of **8a** to a methyl group (i.e., **7**; $K_i = 21$ nM) has no effect on affinity, whereas the same alteration of **3a** reduces affinity by 20-fold;[5] likewise, removal of the *N*-alkyl groups of **8a** to afford the primary amine has little effect on affinity ($K_i = 35$ nM), whereas the corresponding change in the **3** series results in a >350-fold decrease in affinity.[14] Hence, the pyridyl rings of the **3**-series and **8**-series nicotinic ligands may be binding in a somewhat similar manner but the terminal amine portions might be interacting in a different manner to accommodate the aryl moiety.

Although the focus of the study was on the commonality of binding modes between series, the possibility exists that members *within* a series do not bind in a common manner. Compound **1f** was examined in greater detail because it bears 'substituents' both at the 5- and 6-positions. *N*-Demethylation of nicotine decreases affinity by approximately 15-fold.[4,5] Furthermore, the *S*(−)enantiomer of nicotine binds with about 20- to 50-fold higher affinity than *R*(+)nicotine.[4,5] If **1f** binds in a similar fashion as nicotine, similar effects should be observed. We found that *N*-desmethyl **1f** (**9**; $K_i = 93 \pm 2$ nM) binds with nearly 10-fold lower affinity than **1f**, and that (−)**1f** ($K_i = 2.9 \pm 0.3$ nM) binds with about 90-fold higher affinity than (+)**1f** ($K_i = 265 \pm 20$ nM). These results are not inconsistent with a common mode of binding for **1f** and nicotine.

Additional comparisons will be required to sort through the issues raised. For example, the ether oxygen atom of the more flexible **8**-series compounds contributes to binding (i.e., its replacement by a methylene group dramatically decreases affinity[5]) and aryl substituents could influence its electronic character. Nevertheless, on the basis of the comparisons with the **1**- and **8**-series compounds, it can be concluded that the two series are probably not binding in the same manner because parallel structural changes in the aromatic rings did not result in parallel shifts in receptor affinity. Prior evidence suggests that the presence and location of the pyridine ring N is important for binding in each of the three series.[5] Thus, the compounds might utilize a common hydrogen bonding site on the receptor, but the direction of the vector towards the site need not involve atom-by-atom ring superimpositions. Alternatively, the **1**-series and **8**-series compounds might be binding in an

entirely different manner. These factors need to be taken into account in future molecular modeling studies and proposals of possible pharmacophore models.

Methods

The benz-fused analogue of nicotine **1** was prepared according to a literature procedure by Ide and coworkers[17] except that *N*-vinylpyrrolidin-2-one and NaH were used in the first step of the preparation of the intermediate benz-fused myosmine. Reduction of the intermediate with $NaBH_4$ gave **9**. Methylation of **9** via an Eschweiler–Clarke reaction resulted in **1f**. Adapting the method of Gerlach[18] the optical isomers (−)**1f** ($[\alpha]_D^{28} = -131.5°$; *c* 0.92, CH_3OH) and (+)**1f** ($[\alpha]_D^{28} = +126.5°$; *c* 0.64, CH_3OH) were obtained by optical resolution of racemic **1f** using the (+)di-*O*,*O*′-*p*-toluyl-L-tartaric acid and (−)di-*O*,*O*′-*p*-toluyl-D-tartaric acid, respectively.

Analogues **8b**, **8d**, and **8f** were synthesized from the substituted pyridin-3-ols[19,20] or quinolin-3-ols.[21] Although reported, 5-bromopyridin-3-ol was prepared using a new approach: 3,5-dibromopyridine was treated with the sodium salt of 4-methoxybenzyl alcohol in DMF followed by deprotection in TFA to give the desired intermediate. 6-Bromopyridine-3-ol was synthesized using the Naumann and Langhals[21] method for the preparation of quinoline-3-ol. The desired ethers were obtained via Williamson synthesis using dibromoethane[22] and aminated with *N*-methylethylamine to give the final products.

Derivative **3b** was prepared from 5-bromonicotinic acid by a previously reported method.[23] In the case of **3d** and **3f**, 2-bromo-5-methylpyridine and 3-methylquinoline, respectively, were brominated with NBS followed by *N*-alkylation. Sodium methoxide was used to synthesize **3c**, **8c**, and **8e** from the corresponding bromo analogues **3b**, **8b**, and **8d**, respectively.

The binding assay was performed as previously reported using rat brain homogenates with [^{3}H]nicotine as radioligand.[16] Correlations were obtained using Lotus Freelance Graphics (Cambridge, MA, USA).

Acknowledgement

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