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# Binding of Nicotine and Homoazanicotine Analogues at Neuronal Nicotinic Acetylcholinergic (nACh) Receptors

G. Ferretti,[a,b] M. Dukat,[a] M. Giannella,[b] A. Piergentili,[b] M. Pigini,[b] W. Quaglia,[b] M. I. Damaj,[c] B. R. Martin[c] and R. A. Glennon[a,c,*]

[a]*Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298, USA*
[b]*Dipartimento di Scienze Chimiche, Universita di Camerino, Camerino 62032, Italy*
[c]*Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA*

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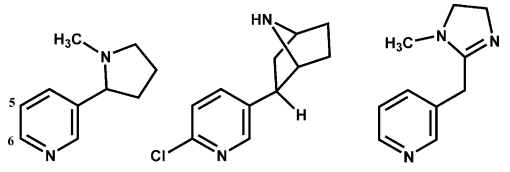
**Abstract**—A total of 20 substituted analogues of nicotine (**1a**) and homoazanicotine (**3a**) were examined in order to determine whether or not they might bind in a similar manner at α4β2 nicotinic acetylcholinergic (nACh) receptors. It was found that parallel structural changes in the two series resulted in parallel shifts in affinity. Evidence suggests that the two series are binding in a comparable fashion.

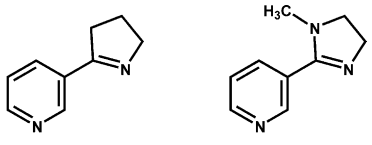

The past decade has witnessed increased interest in nicotinic cholinergic (nACh) receptors. This is based in part on the finding that neuronal nACh receptors can be structurally different from peripheral nACh receptors, and that nACh receptor agents might be of use in the treatment of pain, and certain neurological and mental disorders.[1–3] We have been examining the binding of nicotinic agents at α4β2 nACh receptors in order to identify those pharmacophoric features that contribute to binding. In the course of our studies we have examined nicotine (**1a**) analogues and the high-affinity nACh receptor ligand epibatidine (**2**).[4,5]



**1a** **2** **3a**

Recently, we found that insertion of a methylene bridge and introduction of an additional ring nitrogen atom results in a compound (homoazanicotine, **3a**; $K_i = 7.8$ nM) with high affinity for nACh receptors.[6] Compound **3a** retains some of the structural features of **2**, but not its overall geometry; this might explain its reduced affinity relative to **2** ($K_i = 0.05$ nM).[7] That is, the basic nitrogen atom of **3a** is removed from the pyridyl ring by two carbon atoms as it is in **2**, but **2** is a more conformationally constrained molecule.

Kim et al.[8] have concluded that there exists on nACh receptors a binding pocket of limited size associated with the pyrrolidine portion of nicotine analogues, and have shown that introduction of various pyrrolidine substituents can dramatically decrease affinity.



**4** **5**

Myosmine (**4**; $K_i = 3300 \pm 2000$ nM) also lacks significant affinity for α4β2 nACh receptors; however, introduction of an *N*-methyl substituent (i.e., azanicotine, **5**; $K_i = 206$ nM) results in enhanced affinity.[6] But **5** still binds with lower affinity than nicotine. Given that **3a** is somewhat sterically larger than nicotine, and that **5** binds with lower affinity than nicotine, compound **3a**

*Corresponding author. Tel.: +1-804-828-8487; fax: +1-804-828-7404; e-mail: glennon@hsc.vcu.edu

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might not have been expected to bind well. Yet, compound **3a** seems to represent the first member of a novel class of nicotinic ligands.[6,9]

Given the unlikelihood that **3a** should bind with high affinity, it was of interest to further pursue this series. In particular, we wished to determine whether or not **3a** binds at nACh receptors in a manner similar to that of **1a**. Parallel structural changes within two series of agents resulting in parallel shifts in affinity is often taken as evidence that two series of agents might be binding in a similar manner. Carroll et al.,[10] for example, have recently demonstrated for a series of six epibatidine (**2**) analogues, varying only with respect to the nature of substituents at the pyridine 6-position (i.e., the chloro position of **2**), that a significant correlation ($r = 0.99$) exists between their affinities and those reported for like-substituted nicotine analogues. They concluded that the epibatidine analogues and the corresponding nicotine analogues are binding in a similar fashion at α4β2 nACh receptors.

The affinities of a series of ten nicotine analogues were compared with those of the corresponding derivatives **3**. Introduction of halogen at the 6-position of nicotine results in retention of affinity, whereas a 6-methoxy group and benz-fusion at the *e*-face is somewhat less well tolerated.[4,11] Introduction of a 6-phenyl group substantially detracts from affinity as does a 2-methyl group; a *N*-methyl group appears optimal for affinity.[4] Analogues of **3a** were constructed that incorporated these same structural changes.

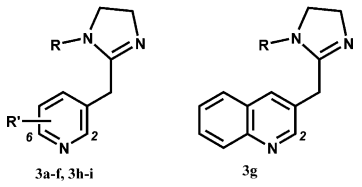
Compounds **3** were all prepared in a similar manner generally following that described for compound **3a**.[6,9] That is, the appropriate substituted-pyridine bearing a reactive functionality at the 3-position (e.g., a 6-substituted pyridyl-3-acetate methyl ester) was condensed with the necessary ethylenediamine derivative in the presence of trimethylaluminum to afford the compounds shown in Table 1. nACh receptor binding data were obtained, at least in triplicate, as previously described using rat brain (minus cerebellum) homogenates with [$^3$H](−)nicotine as radioligand.[6] Data are shown in Table 2.

As was the case with nicotine (**1a**), introduction of halogen at the 6-position of **3a** (i.e., **3b**, **3c**) was tolerated. The 6-methoxy and fused analogues (**3d** and **3g**, respectively) were somewhat less well tolerated, and the 6-phenyl (i.e., **3e**) and 2-methyl (i.e., **3f**) derivatives displayed low affinity. Also, as with nicotine, an *N*-methyl group appears optimal; demethylation (i.e., **3h**) or replacement of the *N*-methyl group with an ethyl (i.e., **3i**) or benzyl (i.e., **3j**) substituent, resulted in reduced affinity.

A plot of p$K_i$ values for the nicotine analogues versus the homoazanicotine analogues (Fig. 1) reveals the existence of a significant correlation ($r = 0.962$).

In order to obtain further support that homoazanicotine (**3a**) might bind in a manner similar to that of (−)nicotine, the two structures were modeled and

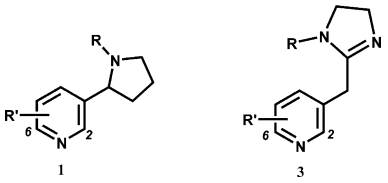
**Table 1.** Physicochemical properties for compounds **3**




| | R | R′ | Mp °C[a] | Emp. formula[b] |
|---|---|---|---|---|
| **3b** | $-CH_3$ | 6-Br | 160–161 | $C_{10}H_{12}BrN_3 \cdot C_2H_2O_4$ |
| **3c** | $-CH_3$ | 6-Cl | 124–125 | $C_{10}H_{12}ClN_3 \cdot 1.25C_2H_2O_4$ |
| **3d** | $-CH_3$ | 6-$OCH_3$ | 153–154 | $C_{11}H_{15}N_3O \cdot 2C_2H_2O_4$ |
| **3e** | $-CH_3$ | 6-Ph | 155–157 | $C_{16}H_{17}N_3 \cdot 2.5C_2H_2O_4$ |
| **3f** | $-CH_3$ | 2-$CH_3$ | 182–184 | $C_{11}H_{15}N_3 \cdot C_2H_2O_4$ |
| **3g** | $-CH_3$ | — | 134–136 | $C_{14}H_{15}N_3 \cdot 2.25C_2H_2O_4$ |
| **3h** | –H | –H | 180–182 | $C_9H_{11}N_3 \cdot 2C_2H_2O_4$ |
| **3i** | $-C_2H_5$ | –H | 168–169 | |
| **3j** | $-CH_2$-Ph | –H | 113–114 | |

[a]Compounds **3b**–**3d** were recrystallized from *i*PrOH, **3e**–**3g** from MeOH/EtOAc, and **3h** from absolute EtOH. Compounds **3i** and **3j** reported.[9]
[b]All compounds were isolated as oxalate salts and analyzed correctly for C, H, and N. Compound **3d** crystallized with 0.25 mol of iPrOH.

**Table 2.** Binding data for **1** and **3** derivatives at α4β2 nACh receptors




| | R | R′ | $K_i$, nM (±SEM)[a] | | | |
|---|---|---|---|---|---|---|
| | | | **1** | | **3** | |
| **a** | $-CH_3$ | –H | 1.3 | (0.3) | 7.8 | — |
| **b** | $-CH_3$ | 6-Br | 0.5 | — | 7.3 | (0.9) |
| **c** | $-CH_3$ | 6-Cl | 0.6 | — | 3.3 | (0.8) |
| **d** | $-CH_3$ | 6-$OCH_3$ | 22 | — | 46 | (3) |
| **e** | $-CH_3$ | 6-Ph | 9440 | — | 3260 | (12) |
| **f** | $-CH_3$ | 2-$CH_3$ | 2125 | (200) | 5420 | (1600) |
| **g** | $-CH_3$ | Fused[b] | 11 | — | 38 | (3) |
| **h** | –H | –H | 30 | (6) | 325 | (30) |
| **i** | $-C_2H_5$ | –H | 17 | — | 150 | (12) |
| **j** | $-CH_2$–Ph | –H | 360 | — | 850 | (90) |

[a]Where SEM is not shown, binding data were previously reported.[11,12]
[b]Compounds **1g** and **3g** represent 3-substituted quinoline analogues.

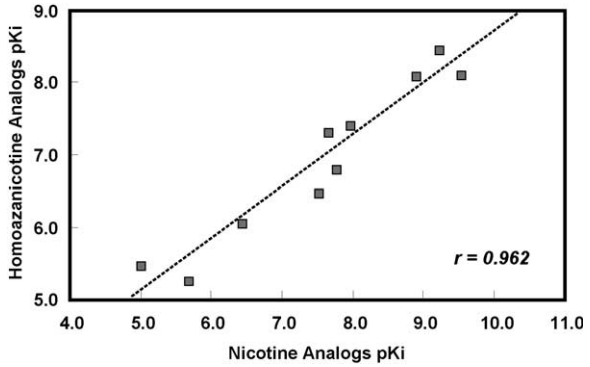



**Figure 1.** Relationship between the α4β2 nACh receptor affinities of derivatives of nicotine (**1a**) and the affinities of their corresponding homoazanicotine (**3a**) analogues ($r = 0.962$; $n = 10$; Freelance Graphics).

graphically superimposed.[13] The internitrogen (N–N) distance in nicotine has been argued to be of importance[14,15] Others have alternatively proposed, particularly to account for the binding of agents with long N–N distances, that the distance separating the termini of vectors drawn from these nitrogen atoms to receptor features can influence binding.[16-19] In any event, the calculated N–N distance in nicotine and many related analogues is 4.8(±0.3) Å.[5,15] In the present investigation, the N–N distance of a model of nicotine constructed from its X-ray crystal structure[20] following energy minimization was 4.8 Å. This distance in **3a**, for the lowest energy conformer of the rotamer with the *N*-methyl group on the same side of the molecule as (−)nicotine, was calculated to be 4.5 Å. Hence, the internitrogen distance of **3a** is somewhat shorter than that believed to be optimal for (−)nicotine. Flexible superimposition of the two molecules indicated a reasonable fit (rms = 0.154). However, the presence of a positively charged imidazoline moiety could result in dispersal of the charge over the amidinium portion of the molecule; thus, it is possible that the carbocationic nature of the imidazolinium species might be a better nicotine mimic for receptor interaction. The distance between the pyridine nitrogen atom and the $C_{2'}$ atom of the lowest energy conformer of the carbocation of protonated **3a** is calculated to be 4.6 Å; superimposition with (−)nicotine also provided a reasonable fit of selected atoms (rms = 0.069). The present results, both empirical and computational, are not inconsistent with the possibility that **3a** can mimic (−)nicotine upon binding at nACh receptors.

In summary, the α4β2 nACh receptor affinities of 10 pairs of substituted derivatives of nicotine (**1a**) and homoazanicotine (**3a**) were compared. Substituents were varied at the pyridine 2- and 6-positions, and at the pyrrolidine nitrogen atom. Affinities ($K_i$ values) spanned nearly a 20,000-fold range. In general, the homoazanicotine analogues **3** displayed about 10-fold lower affinity than the corresponding nicotine analogues. Nevertheless, the structure–affinity relationships between the two series seem to be similar and there was a significant correlation between the affinities of these two series of compounds. Computational studies with **3a** indicate that it possesses an N–N distance similar to that of nicotine. Evidence would suggest that the two series might be binding at nACh receptors in a comparable fashion.

## Acknowledgements

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13. X-Ray cooridinates[20] of nicotine hydrogen iodide were utilized in the construction of protonated nicotine. The CRYSTAL interface of the SYBYL molecular modeling program [SYBYL Molecular Modeling Package, Version 6.7 (2001); Tripos Inc., St. Louis, MO, USA] was applied, followed by molecular mechanics minimization (MINIMIZE) and calculation of charges by the Gasteiger–Huckel algorithm. The structure of homoazanicotine was built by modification of nicotine using standard bonds length and angles within BUILD/SKETCH molecule command followed by minimization and charge calculation in a manner similar to that of nicotine. Conformational search was performed using the SYSTEMATIC SEARCH command: the two rotatable bonds were rotated in 15°-increments including the starting conformation. The result was ANALYZED with the SYSTEMATIC SEARCH command. The minimized X-ray structure of nicotine was superimposed (FIT-ATOM root mean square) on the lowest energy conformer of protonated homoazanicotine (**3a**) corresponding to the spatial arrangement and orientation of nicotine. The stereochemistry about the $sp^3$ protonated nitrogen atoms of both molecules is consistent with that of the X-ray structure of (−)nicotine (i.e., *R*). Due to the likelihood of positively charged carbon occurring in protonated homoazanicotine, two modes of superimposition were investigated: $N_{pyr}$, Du-atom (i.e., aryl centroid), $N_{Me}$ for both molecules, and $N_{Me}$ of the pyrrolidine ring and C-$sp^2$ of the imidazoline ring for the alternate possibility. It might be noted that other low-energy conformers of **3a** were identified that give better rms values than those shown here; these other conformers also possess N–N distances closer to that found for (−)nicotine.
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