Published in final edited form as:

Bioorg Med Chem Lett. 2009 August 01; 19(15): 4359–4363. doi:10.1016/j.bmcl.2009.05.085.

Evaluation of structurally diverse neuronal nicotinic receptor ligands for selectivity at the $\alpha 6^*$ subtype

Scott R. Breining^a, Merouane Bencherif^b, Sharon R. Grady^c, Paul Whiteaker^{c,†}, Michael J. Marks^c, Charles R. Wageman^c, Henry A. Lester^d, and Daniel Yoharmes^a

^aDepartment of Medicinal Chemistry, Targacept, Inc., 200 East First St., Suite 300, Winston-Salem, NC 27101

^bPreclinical Research, Targacept, Inc., 200 East First St, Suite 300, Winston-Salem, NC 27101

^cInstitute for Behavioral Genetics, University of Colorado, Boulder, Colorado 80309-0447

^dDepartment of Biology, California Institute of Technology, 1200 E. California Blvd. Pasadena, CA 91125

Abstract

Direct comparison of pyridine versus pyrimidine substituents on a small but diverse set of ligands indicates that the pyrimidine substitution has the potential to enhance affinity and/or functional activity at $\alpha 6$ subunit-containing neuronal nicotinic receptors (NNRs) and decrease activation of ganglionic nicotinic receptors, depending on the scaffold. The ramifications of this structure-activity relationship are discussed in the context of the design of small molecules targeting smoking cessation.

Graphical Abstract

Direct comparison of pyridine versus pyrimidine substituents on a small but diverse set of ligands indicates that the pyrimidine substitution has the potential to enhance affinity and/or functional activity at $\alpha 6^*$ neuronal nicotinic receptors (NNRs) and decrease activation of ganglionic nicotinic receptors, depending on the scaffold. The ramifications of this structure-activity relationship are discussed in the context of the design of small molecules targeting smoking cessation.

Correspondence to: Scott R. Breining; Daniel Yoharmes.

[†]Current address: Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ 85013, USA

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Supplementary Data

Spectral data of the synthesized compounds (¹H NMR, ¹³C NMR, and LCMS) as well as general experimental information and details for the $\alpha 7$ binding assay. Supplementary data associated with this article can be found, in the online version, at doi:

Smoking is a leading cause of premature mortality in developed countries.¹ Smoking is also a difficult addiction to overcome, with an unaided relapse rate of approximately 80% within the first month of abstinence.² Nicotine (**1**, Figure 1) is widely recognized as the agent responsible for mediating smoking addiction. Currently, several FDA-approved pharmacological options exist for treatment of nicotine addiction. These include nicotine replacement therapy bupropion, and the recently approved drug Chantix® (varenicline, **2**). While not approved for use in the United States, cytisine (**3**), a natural product, has been used for many years as a smoking cessation aid in Eastern European countries.³ Dianicline (**4**) was a compound under advanced clinical investigation by Sanofi-aventis for smoking cessation,⁴ but was discontinued from clinical development in 2008.

Activation of mesolimbic dopaminergic neurons leads to dopamine release, initiating a physiological response that contributes to the reinforcing effects of nicotine.⁵ While nicotine can interact with several neuronal nicotinic receptor (NNR) subtypes in the mesolimbic and nigrostriatal pathway, including $\alpha 4^*$, $\alpha 6^*$ (the asterisk denotes the presence of additional subunits and/or stoichiometries) and $\alpha 7$ receptors, convincing evidence shows that $\alpha 4$ and/or $\beta 2$ subunits are crucial in the reinforcing effects of nicotine.⁶ Cytisine (**3**),³ varenicline (**2**)⁷ and dianicline (**4**)⁴ all produce varying degrees of nicotinic acetylcholine receptor activation, particularly at the $\alpha 4\beta 2^*$ subtype. Varenicline (**2**) apparently acts *via* simultaneous activation and antagonism of the $\alpha 4\beta 2^*$ receptor.⁵ Elucidation of the exact mechanism is complicated by the fact that in addition to $\alpha 4\beta 2^*$ activity, varenicline also interacts with $\alpha 7$ and $\alpha 6\beta 2^*$ receptors.⁸ Compounding this complexity is the presence of an $\alpha 4\text{-}\beta 2$ interface within a subset of the $\alpha 6^*$ receptors (i.e., the $\alpha 6\alpha 4\beta 3$ but not the $\alpha 6\beta 2\beta 3$).

Recent data show that the $\alpha 6\beta 2^*$ NNRs contribute to the effect of nicotine on dopamine release in the nucleus accumbens.⁹ These observations have led to questions regarding the role of $\alpha 6\beta 2$ functional activity in mediating nicotine addiction.¹⁰ While previous work using subunit-null mutant mice has separately implicated the $\beta 2$ and $\alpha 4$ subunits in the heteropentameric receptors involved in addiction, this paper reports on the relative contribution of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ receptors in modulating mesolimbic and nigrostriatal dopamine release within a set of diverse compounds. Such data could guide discovery of a next-generation smoking cessation candidate with an optimum profile, perhaps overcoming the presently available therapies' shortcomings, which include poor tolerability, complex titration schedule, and potential safety issues.¹¹ While the pharmacological requirements for

binding to the $\alpha 4\beta 2^*$ NNRs are reasonably well established, less is known about the structural requirements for ligand binding to and activation of $\alpha 6\beta 2^*$ receptors.¹² This dearth of understanding about the structure-activity relationship (SAR) for $\alpha 6\beta 2^*$ ligands is additionally complicated by uncertainties about the precise subunit composition of $\alpha 6\beta 2^*$ receptors in rodents and primates.¹³ The continued need for $\alpha 6^*$ -selective ligands with a range of functional activity for study in models of nicotine addiction as well as other disease states has motivated the initial $\alpha 6\beta 2^*$ SAR report detailed herein.

During the initial screening of a diverse set of sixteen compounds selected from Targacept's compound library, several hits with nanomolar to micromolar affinity at the $\alpha 6\beta 2^*$ subtype were identified and subsequently profiled for functional activity. Among the compounds profiled were alkynylpyrrolidines **5a** and **5b** (Figure 2).¹⁴ In measurements of dopamine (DA) release, while the pyridine analog **5a** possessed a modest level (42%) of efficacy with respect to $\alpha 6^*$ -mediated DA release, the pyrimidine analog **5b** demonstrated a relative >3 fold enhancement (Table 1).¹⁵

This initial observation led to the hypothesis that a pyrimidine substituent could confer $\alpha 6\beta 2^*$ functional selectivity onto other scaffolds. Therefore, an additional set of pyridine and pyrimidine pairs were identified, synthesized²⁰ and evaluated to complete a pyridine-pyrimidine matrix on a small, structurally diverse set of scaffolds to evaluate this hypothesis.

The metanicotines **8a** and **8b** were prepared by Heck coupling of alkene **7** with 3-bromopyridine or 5-bromopyrimidine according to a previously reported method (Scheme 1).²¹

The preparation of quinuclidines **11a** and **11b** is illustrated in Scheme 2. Quinuclidinone **9** was condensed under basic conditions with 3-pyridinecarboxaldehyde or 5-pyrimidinecarboxaldehyde to give vinylquinuclidinones **10a** or **10b**. Hydrogenation under standard conditions afforded the saturated ketone intermediates, which were subjected to Wolff-Kishner conditions to give products **11a** and **11b**, respectively.

The readily available pyroglutamic acid **12**²² was converted to alkene **14** by reduction, protecting group interconversion, Swern oxidation of the resulting alcohol **13** followed by olefination (Scheme 3). Treatment of **14** with 3-bromopyridine or 5-bromopyrimidine under Heck conditions gave substituted vinylpyrrolidines **15a** and **15b**, respectively.

Preparation of both chiral and racemic forms of compound **20a** (Scheme 4) has been previously reported.²³ Application of this same methodology likewise afforded the desired pyrimidine analog **20b**.

Compounds **23a** and **23b** were prepared according to similar procedures to those previously reported (Scheme 5).²⁴

The collection of pyridine/pyrimidine compound pairs was first evaluated for binding affinity across several nicotinic receptor subtypes (Table 2). All of the compounds possessed high affinity at $\alpha 4\beta 2^*$. A slight drop in affinity for the pyrimidine analogs relative to the corresponding pyridines at the $\alpha 4\beta 2^*$ subtype was noted for all except **23b**. A much wider

range of affinities was observed for the $\alpha 6\beta 2^*$ subtype, from nanomolar to micromolar binding; again, the pyrimidine analogs showed a trend toward slightly reduced affinity with the exception of **23b**. In general, the compounds displayed selectivity for the $\alpha 4\beta 2^*$ subtype relative to $\alpha 6\beta 2^*$ and $\alpha 7$. Two noteworthy compounds are quinuclidine **20a**, which possesses high affinity across all three subtypes, and **20b**, wherein high affinity is retained for $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ while $\alpha 7$ affinity is diminished.

In functional measurements of dopamine release, the results for the metanicotine pair **8a/b** are quite striking. While pyridine **8a** is a full agonist at DA release mediated via the $\alpha 4\beta 2^*$ receptor subtype (122%, 8.3 μ M EC₅₀), it has no functional activity at $\alpha 6\beta 2^*$. In contrast, pyrimidine **8b** is a partial agonist (73%, 5.9 μ M) at $\alpha 4\beta 2^*$ -mediated dopamine release as well as *via* $\alpha 6\beta 2^*$ (80% EMax), albeit with low potency (37 μ M EC₅₀). In the case of quinuclidines **11a/b**, both are potent, full antagonists of both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ -mediated dopamine release. For vinylpyrrolidine pair **15a/b**, both analogs exhibited similar levels of efficacy and potency for both $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ -mediated dopamine release. We note that the relatively low $\alpha 6\beta 2^*$ affinity of **15b** (325 nM) still translates to good potency (530 nM EC₅₀). Two possible explanations exist for this. First, $\alpha 6\beta 2^*$ may be analogous to $\alpha 4\beta 2^*$ wherein the K_i value reflects binding to desensitized state(s) and the EC₅₀ value indicates binding to the functional state of the receptor. Perhaps for $\alpha 6\beta 2^*$ these two states are more similar than for $\alpha 4\beta 2^*$. Another possibility is that EC₅₀ values in the complex $\alpha 6\beta 2^*$ forms (eg $\alpha 6\alpha 4\beta 3\beta 2$) responsible for mediating dopamine release in the functional assay may reflect cooperativity of both subunits and may therefore differ significantly from values expected for an $\alpha 6\beta 2^*$ -containing receptor.

Quinuclidines **20a** and **20b** are intriguing compounds in that they possess relatively low efficacy but high potency at $\alpha 4\beta 2^*$ -mediated dopamine release, while they are very potent full agonists at $\alpha 6\beta 2^*$ -mediated dopamine release. We believe that this is the first report of full agonists with functional selectivity (both efficacy and potency) for the $\alpha 6\beta 2^*$ subtype. Finally, tropinone derivatives **23a** and **23b** are both moderately potent partial agonists at $\alpha 4\beta 2^*$ -mediated dopamine release. Both compounds also possess appreciable efficacy (50 and 77%) and robust potency (200 and 100 nM, respectively) at $\alpha 6\beta 2^*$ -mediated dopamine release.

A secondary, albeit extremely important goal in optimizing the pharmacological profile for smoking cessation was to improve functional selectivity for $\alpha 6\beta 2^*$ *vs.* ganglionic receptor activation. Activation of the ganglionic $\alpha 3\beta 4^*$ subtype may cause some of the side effects of nicotine and nicotinic ligands.²⁵ Enhanced selectivity for central *vs.* peripheral sites, particularly with respect to the cardiovascular system, is therefore anticipated to improve tolerability *in vivo*. The compounds of this study were therefore evaluated for functional activity at the $\alpha 3\beta 4^*$ subtype, and the functional potencies (EC₅₀s) compared with those for $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ activation to generate selectivity ratios (Table 3). Most compounds exhibited fairly high efficacy at $\alpha 3\beta 4^*$, with little difference between the pyridine and pyrimidine analogs or across the various chemotypes. The exceptions were the metanicotines **8a/b** and the related vinylpyrrolidines **15a/b**. Our current hypothesis is that the greater degree of flexibility of these scaffolds is less well tolerated in the $\alpha 3\beta 4^*$ binding domain. Significant differences in potency occurred both between scaffolds and for

pyridines versus pyrimidines. Notably, for $\alpha 4\beta 2^*$ moving from pyridine to pyrimidine generally increased EC_{50} 's (*decreased* potency). Fairly wide variances in functional selectivity across the compound set were noted (0.4 to 127 $\times$ for $\alpha 3\beta 4^*$ vs. $\alpha 4\beta 2^*$ and 0.26 to 410 $\times$ for $\alpha 3\beta 4^*$ vs. $\alpha 6\beta 2^*$). It may be asked whether the two scaffolds produce different cation- π interactions within the conserved aromatic box of the various subtypes investigated here.²⁶ Exchanging pyrimidine for pyridine enhanced functional selectivity for $\alpha 4\beta 2^*$ -mediated dopamine release relative to ganglionic activation in half the cases; with respect to $\alpha 6\beta 2^*$ -mediated dopamine release relative to ganglionic activation, the selectivity improvements were more modest (2–4 $\times$) but also more consistent.

In conclusion, we provide novel SAR data on affinity and function for a diverse group of nicotinic ligands in $\alpha 6\beta 2^*$ containing NNR subtypes. Direct comparison of pyridine versus pyrimidine substituents on this set of scaffolds indicates that this substitution has the potential to enhance $\alpha 6\beta 2^*$ affinity and/or functional activity and to decrease ganglionic activation, depending on the scaffold. Additionally, we have identified two scaffolds with functional selectivity for $\alpha 6\beta 2^*$ (exemplified by compounds **20a/b** and **23a/b**). Both may serve as tools to explore the role of $\alpha 6\beta 2^*$ receptors in various disease states and as leads for further optimization of $\alpha 6\beta 2^*$ activity. The present scaffolds should be investigated with a larger and more diverse set of molecules to test the SAR conclusions around $\alpha 6\beta 2^*$ affinity and function, and identify additional selective compounds. An $\alpha 6\beta 2^*$ selective ligand may provide a valuable tool in a repertoire of therapies needed for drug addiction and movement disorders such as Parkinson's and Huntington's diseases. An appropriately labeled $\alpha 6\beta 2^*$ selective molecule may also become a useful PET ligand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NCDDG grant DA019375 from the National Institutes of Health to HAL, MJM and MB.

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Figure 1.
Nicotine and nicotinic ligands for smoking cessation.

Figure 2.
Alkynylpyrrolidines.

Scheme 1.

Reagents and conditions: (a) MeNH₂, DMF, K₂CO₃; (b) (Boc)₂O, THF; (c) 3-bromopyridine or 5-bromopyrimidine, Pd(OAc)₂, P(o-tol)₃, Et₃N, CH₃CN; (d) TFA.

Scheme 2.

Reagents and conditions: (a) 3-pyridine- or 5-pyrimidine-carboxaldehyde, KOH, MeOH; (b) Pd/C, H₂, MeOH; (c) N₂H₄, KOH, ethylene glycol.

Scheme 3.

Reagents and conditions: (a) LiAlH₄, THF; (b) Pd/C, H₂; (c) (Boc)₂O; (d) Swern Oxidation; (e) Ph₃PCH₃Br, nBuLi; (f) 3-bromopyridine or 5-bromopyrimidine, Pd(OAc)₂, P(o-tolyl)₃, NMP; (g) TFA.

Scheme 4.

Reagents and conditions: (a) benzophenone imine; (b) LDA, 4-bromomethyltetrahydropyran; (c) HBr; (d) K₂CO₃, EtOH.

Scheme 5.

Reagents and conditions: (a) LDA, Trf_2NPh ; (b) pyridine-3-boronic acid or pyrimidine-5-boronic acid, $\text{Pd}(\text{Ph}_3\text{P})_4$, Li Cl, DME, Na_2CO_3 .

Table 1.*In-vitro* profile of Compounds **5a** and **5b** at NNR subtypes.

#	$\alpha 4\beta 2^*$ Ki (nM) ^a	$\alpha 7$ Ki (nM) ^b	$\alpha 6\beta 2^*$ Ki (nM) ^c	$\alpha 4\beta 2^*$ DA EMax % ^d	$\alpha 4\beta 2^*$ DA EC ₅₀ (uM) ^d	$\alpha 6\beta 2^*$ DA EMax % ^e	$\alpha 6\beta 2^*$ DA EC ₅₀ (uM) ^e
5a	20 ± 3	733 ± 299	242 ± 43	120 ± 18	13 ± 9	42 ± 8	0.7 ± 0.3
5b	30 ± 8	5770 ± 1170	340 ± 144	64 ± 7	3.2 ± 1.5	134 ± 29	1.2 ± 1.4

^a Measured by displacement of epibatidine in mouse cortex.¹⁶^b Measured using [¹²⁵I]-bungarotoxin in mouse hippocampal membranes.¹⁷^c Measured using [¹²⁵I]- α -Conotoxin Mil in mouse olfactory tubercles, striatum and superior colliculus.¹⁸^d Measured in striatal synaptosomes as α -conotoxin Mil-resistant DA release.¹⁹^e Measured in striatal synaptosomes as α -conotoxin Mil-sensitive DA release.²⁰

Table 2.*In-vitro* profile of pyridine (a) / pyrimidine (b) pairs at NNR subtypes.

#	$\alpha 4\beta 2^*$ Ki (nM) ^a	$\alpha 7$ Ki ¹⁵ (nM) ^b	$\alpha 6\beta 2^*$ Ki (nM) ^c	$\alpha 4\beta 2^*$ DA EMa x% ^d	$\alpha 4\beta 2^*$ DA EC ₅₀ (uM) ^d	$\alpha 6\beta 2^*$ DA EMax % ^g	$\alpha 6\beta 2^*$ DA EC ₅₀ (uM) ^g
8a	25 ± 7	>10k	1550 ± 214	122 ± 26	8.3 ± 4.3	0	NA
8b	69 ± 19	>10k	1060 ± 370	73 ± 3	5.9 ± 1.0	80 ± 18	37 ± 3
11a	16 ± 2	449 ± 161	85 ± 24	98 ± 11 ^e	0.026 ± 0.007 ^f	96 ± 19 ^e	0.85 ± 0.76 ^f
11b	59 ± 21	2590 ± 430	115 ± 42	92 ± 5 ^e	0.32 ± 0.06 ^f	91 ± 8 ^e	2.5 ± 1.1 ^f
15a	11 ± 4	3160 ± 940	184 ± 57	109 ± 11	4.4 ± 1.9	66 ± 20	0.8 ± 1.0
15b	24 ± 3	>10k	325 ± 70	133 ± 18	19 ± 1	48 ± 13	0.53 ± 0.45
20a	0.5 ± 0.1	69 ± 10	1.1 ± 0.3	29 ± 2	0.034 ± 0.007	109 ± 14	0.007 ± 0.001
20b	1.8 ± 0.8	1100 ± 220	8 ± 4	43 ± 7	0.45 ± 0.50	104 ± 10	0.09 ± 0.05
23a	9.8 ± 3.0	2110 ± 400	55 ± 4	75 ± 14	3.4 ± 3.0	50 ± 7	0.2 ± 0.2
23b	1.2 ± 0.5	9100 ± 3170	17 ± 3	45 ± 4	1.8 ± 0.7	77 ± 9	0.10 ± 0.08

^a Measured by displacement of epibatidine in mouse cortex.¹⁷^b Measured using [¹²⁵I]-bungarotoxin in mouse hippocampal membranes.¹⁸^c Measured using [¹²⁵I]- α -Conotoxin Mil in mouse olfactory tubercles, striatum and superior colliculus.¹⁹^d Measured in striatal synaptosomes as conotoxin Mil-resistant DA release.²⁰^e Antagonist IMax %.^f Ki for inhibition^g Measured in striatal synaptosomes as α -conotoxin MII sensitive DA release.²⁰

Table 3.

Functional selectivity for pyridine-pyrimidine pairs.

#	$\alpha 3\beta 4^*$ Emax %	$\alpha 3\beta 4^*$ EC ₅₀ uM ^a	Functional Selectivity $\alpha 3\beta 4^* / \alpha 4\beta 2^*$ EC ₅₀ ratio	Functional Selectivity $\alpha 3\beta 4^* / \alpha 6\beta 2^*$ EC ₅₀ ratio
5a	81 ± 7	5.6 ± 1.3	0.4	8
5b	74 ± 3	34 ± 3	10.6	28
8a	45 ± 9	218 ± 81	26	NA
8b	4 ± 2	9.5 ± 2.3	1.6	0.26
11a	99 ± 2	3.3 ± 0.1	127 ^b	3.9 ^b
11b	106 ± 6	28 ± 4	87.5 ^b	11.2 ^b
15a	58 ± 4	16 ± 3	3.6	20
15b	97 ± 13	161 ± 48	8.5	304
20a	106 ± 13	0.4 ± 0.2	11.8	57
20b	95 ± 1	2.4 ± 0.05	5.3	26.7
23a	71 ± 9	21 ± 7	6.2	105
23b	35 ± 5	41 ± 12	22.7	410

^a Measured by ACh release in interpeduncular nucleus tissue.²⁷^b The ratios for **11a**, **11b** reflect EC₅₀/Ki.