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Evaluation of structurally diverse neuronal nicotinic receptor ligands for selectivity at the α6* subtype

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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 α6 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 α6* 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.

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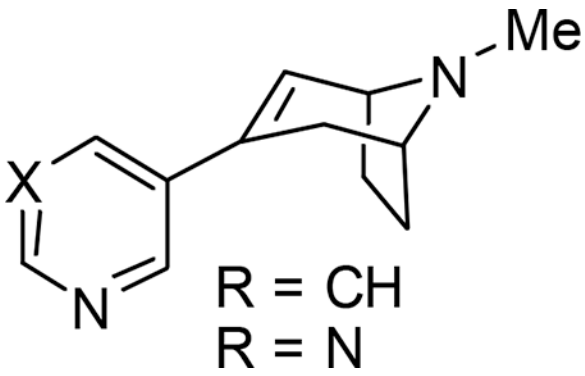
Supplementary Data

Spectral data of the synthesized compounds (1H NMR, 13C NMR, and LCMS) as well as general experimental information and details for the α7 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 α4*, α6* (the asterisk denotes the presence of additional subunits and/or stoichiometries) and α7 receptors, convincing evidence shows that α4 and/or β2 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 *α4β2** subtype. Varenicline (**2**) apparently acts *via* simultaneous activation and antagonism of the α4β2* receptor.[5] Elucidation of the exact mechanism is complicated by the fact that in addition to α4β2* activity, varenicline also interacts with α7 and α6β2* receptors.[8] Compounding this complexity is the presence of an α4-β2 interface within a subset of the α6* receptors (i.e., the α6α4β3 but not the α6β2β3).

Recent data show that the α6β2* 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 α6β2 functional activity in mediating nicotine addiction.[10] While previous work using subunit-null mutant mice has separately implicated the β2 and α4 subunits in the heteropentameric receptors involved in addiction, this paper reports on the relative contribution of α4β2* and α6β2* 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 α4β2* NNRs are reasonably well established, less is known about the structural requirements for ligand binding to and activation of α6β2* receptors.[12] This dearth of understanding about the structure-activity relationship (SAR) for α6β2* ligands is additionally complicated by uncertainties about the precise subunit composition of α6β2* receptors in rodents and primates.[13] The continued need for α6* -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 α6β2* 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 α6β2* 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 α6* - 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 α6β2* functional selectivity onto other scaffolds. Therefore, an additional set of pyridine and pyrimidine pairs were identified, synthesized[20] 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 intennediates, 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, Swem oxidation of the resulting alcohol **13** followed by olefmation (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 α4β2*. A slight drop in affinity for the pyrimidine analogs relative to the corresponding pyridines at the α4β2* subtype was noted for all except **23b**. A much wider

range of affinities was observed for the α6β2* 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 α4β2* subtype relative to α6β2* and α7. Two noteworthy compounds are quinuclidine **20a,** which possesses high affinity across all three subtypes, and **20b,** wherein high affinity is retained for α4β2* andα6β2* while α7 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 α4β2* receptor subtype (122%, 8.3 μM EC50), it has no functional activity at α6β2*. In contrast, pyrimidine **8b** is a partial agonist (73%, 5.9 μM) at α4β2*-mediated dopamine release as well as *via* α6β2* (80% EMax), albeit with low potency (37 μM EC_{50}). In the case of quinuclidines **11a/b,** both are potent, full antagonists of both α4β2* and α6β2* -mediated dopamine release. For vinylpyrrolidine pair **15a/b,** both analogs exhibited similar levels of efficacy and potency for both α4β2* and α6β2* -mediated dopamine release. We note that the relatively low α6β2* affinity of **15b** (325 nM) still translates to good potency (530 nM EC50). Two possible explanations exist for this. First, α6β2* may be analogous to α4β2* wherein the Ki value reflects binding to desensitized state(s) and the EC_{50} value indicates binding to the functional state of the receptor. Perhaps for α6β2* these two states are more similar than for α4β2* Another possibility is that EC50 values in the complex α6β2* forms (eg α6α4β3β2) responsible for mediating dopamine release in the functional assay may reflect cooperativity of both a subunits and may therefore differ significantly from values expected for an α6β2* -containing receptor.

Quinuclidines **20a** and **20b** are intriguing compounds in that they possess relatively low efficacy but high potency at α4β2*-mediated dopamine release, while they are very potent full agonists at α6β2* -mediated dopamine release. We believe that this is the first report of full agonists with functional selectivity (both efficacy and potency) for the α6β2* subtype. Finally, tropinone derivatives **23a** and **23b** are both moderately potent partial agonists at α4β2*-mediated dopamine release. Both compounds also possess appreciable efficacy (50 and 77%) and robust potency (200 and 100 nM, respectively) at α6β2* -mediated dopamine release.

A secondary, albeit extremely important goal in optimizing the pharmacological profile for smoking cessation was to improve functional selectivity for α6β2* *vs,* ganglionic receptor activation. Activation of the ganglionic α3β4* 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 α3β4* subtype, and the functional potencies (ECT_{50}s) compared with those for α4β2* and α6β2* activation to generate selectivity ratios (Table 3). Most compounds exhibited fairly high efficacy at α3β4*, 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 α3β4* binding domain. Significant differences in potency occurred both between scaffolds and for

pyridines versus pyrimidines. Notably, for α4β2* 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× for α3β4* *vs.* α4β2* and 0.26 to 410x for α3β4* *vs.* α6β2*). 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 α4β2*-mediated dopamine release relative to ganglionic activation in half the cases; with respect to α6β2* -mediated dopamine release relative to ganglionic activation, the selectivity improvements were more modest (2–4x) but also more consistent.

In conclusion, we provide novel SAR data on affinity and function for a diverse group of nicotinic ligands in α6β2* containing NNR subtypes. Direct comparison of pyridine versus pyrimidine substituents on this set of scaffolds indicates that this substitution has the potential to enhance α6β2* affinity and/or functional activity and to decrease ganglionic activation, depending on the scaffold. Additionally, we have identified two scaffolds with functional selectivity for α6β2* (exemplified by compounds **20a/b** and **23a/b).** Both may serve as tools to explore the role of α6β2* receptors in various disease states and as leads for further optimization of α6β2* activity. The present scaffolds should be investigated with a larger and more diverse set of molecules to test the SAR conclusions around α6β2* affinity and function, and identify additional selective compounds. An α6β2* 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 α6β2* selective molecule may also become a useful PET ligand.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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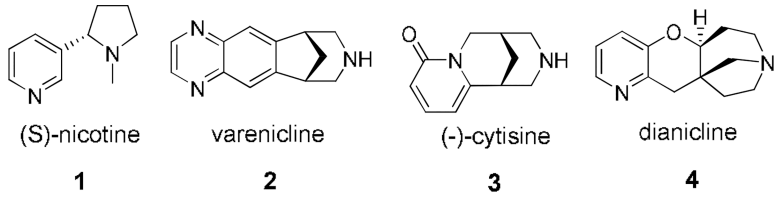


Figure 1.
Nicotine and nicotinic ligands for smoking cessation.

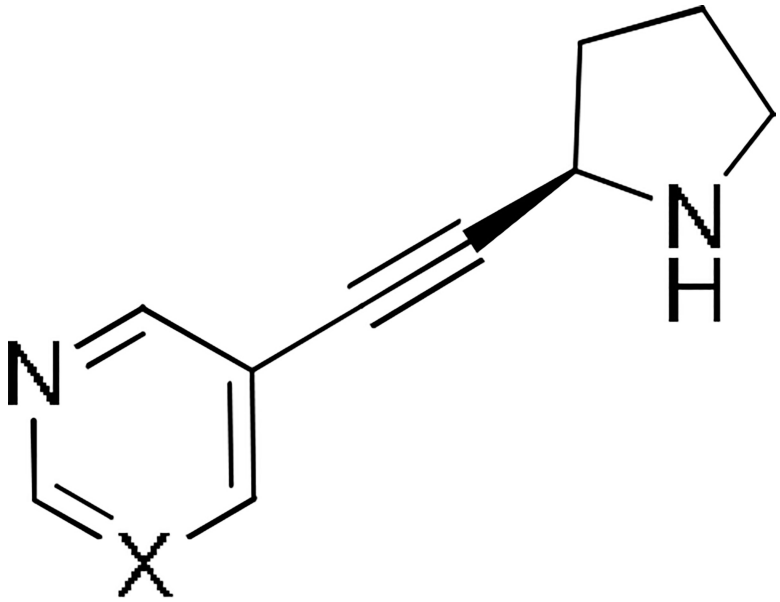
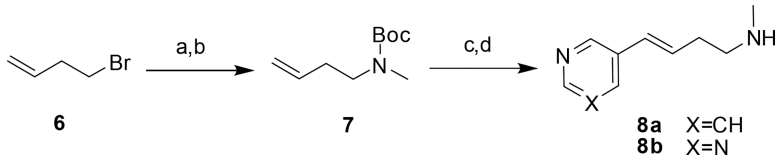
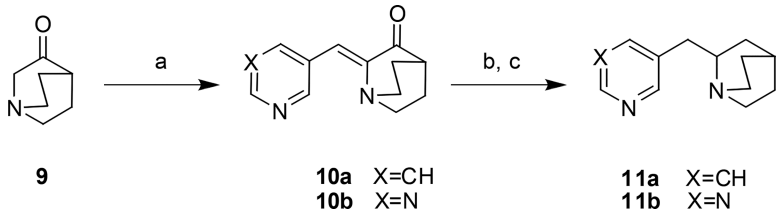


Figure 2.
Alkynylpyrrolidines.



Scheme 1.

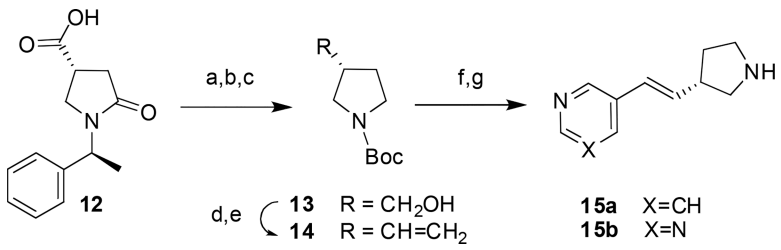
Reagents and conditions: (a) $MeNH_2$, DMF, K_2C0_3; (b) $(B0C)_2O$, THF; (c) 3-bromopyridine or 5-bromopyrimidine, $Pd(OAc)_2$, $P(o\text{-}tol)_3$. Et_3N, CH_3CN; (d) TFA.



9 **10a** X=CH **10b** X=N **11a** X=CH **11b** X=N

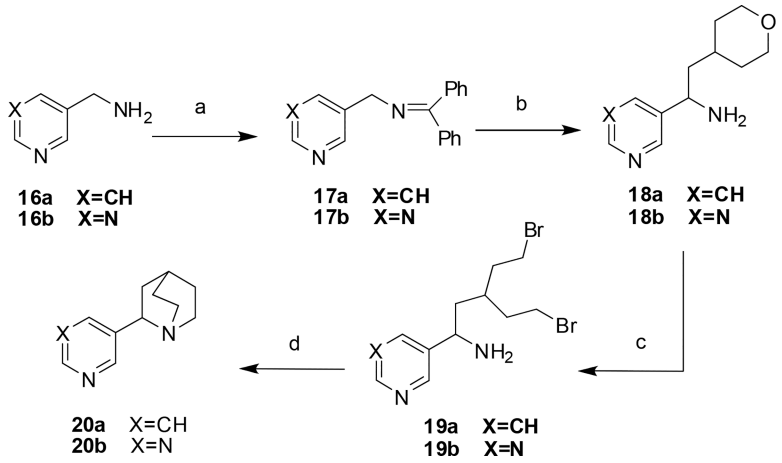
Scheme 2.

Reagents and conditions: (a) 3-pyridine- or 5-pyrimidine-carboxaldehyde, KOH, MeOH; (b) Pd/C, H_2, MeOH; (c) N_2H4, KOH, ethylene glycol.



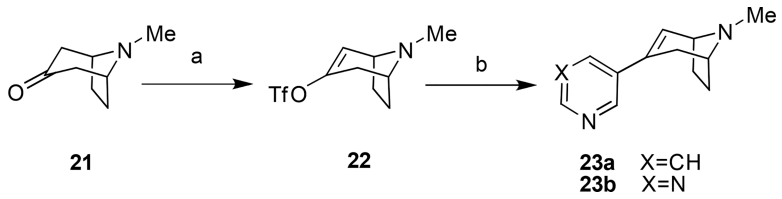
Scheme 3.

Reagents and conditions: (a) $LiAlH_4$, THF; (b) Pd/C, H_2; (c) $(B0C)_2O$; (d) Swem Oxidation; (e) Ph_3PCH_3Br, nBuLi; (f) 3-bromopyridine or 5-bromopyrimidine, $Pd(OAc)_2$, $P(o\text{-}tolyl)_3$, NMP; (g) TFA.



Scheme 4.

Reagents and conditions: (a) benzophenone imine; (b) LDA, 4-bromomethyltetrahydropyran; (c) HBr; (d) K_2CO_3, EtOH.



Scheme 5.

Reagents and conditions: (a) LDA, Tf_2NPh; (b) pyridine-3-boronic acid or pyrimidine-5-boronic acid, $Pd(Ph_3P)_4$, Li Cl, DME, Na_2CO_3.

Table 1.

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

#	α4β2* Ki (nM)[a]	α7 Ki (nM)[b]	α6β2* Ki (nM)[c]	α4β2* DA EMax %[d]	α4β2* DA EC_{50} (uM)[d]	α6β2* DA EMax %[e]	α6β2* 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 [^{125}I]-bungarotoxin in mouse hippocampal membranes.[17]

[c] Measured using [^{125}I]-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.

#	α4β2* Ki (nM)[a]	α7 Ki[15] (nM)[b]	α6β2* Ki (nM)[c]	α4β2* DA EMa x%[d]	α4β2* DA EC_{50} (uM)[d]	α6β2* DA EMax %[g]	α6β2* 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 [^{125}I]-bungarotoxin in mouse hippocampal membranes.[18]

[c] Measured using [^{125}I]-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.

#	α3β4* Emax %[a]	α3β4* EC_{50} uM[a]	Functional Selectivity α3ß4* / α4β2* EC_{50} ratio	Functional Selectivity α3β4* / α6β2* 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 EC_{50}/Ki.