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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.

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,** Fig. 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 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

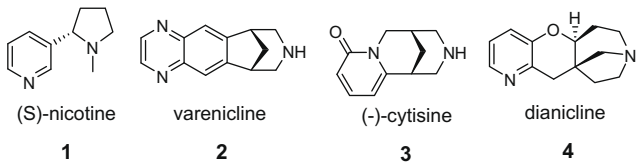

Figure 1. Nicotine and nicotinic ligands for smoking cessation.

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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 α6α4β2β3 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 Letter 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** (Fig. 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.

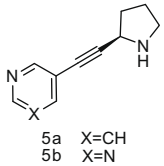


Figure 2. Alkynylpyrrolidines.

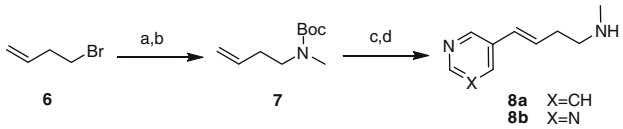
The metanicotines **8a** and **8b** were prepared by Heck coupling of alkene **7** with 3-bromopyridine or 5-bromopyrimidine according to a previously reported method (Scheme 1).[21]

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.

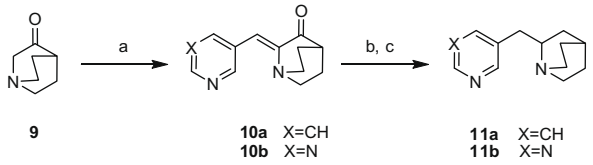
The readily available pyroglutamic acid **12**[22] was converted to alkene **14** by reduction, protecting group interconversion, Swern oxidation of the resulting alcohol **13** followed by olefination (Scheme 3). Treatment of **14** with 3-bromopyridine or 5-bromopyrimidine under Heck conditions gave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

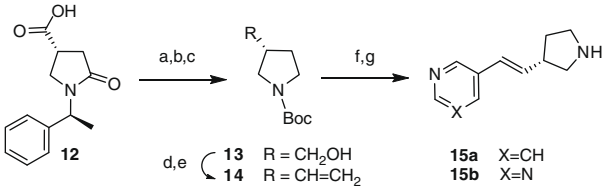
Compounds **23a** and **23b** were prepared according to similar procedures to those previously reported (Scheme 5).[24]



Scheme 1. Reagents and conditions: (a) $MeNH_2$, DMF, K_2CO_3; (b) $(BOC)_2O$, THF; (c) 3-bromopyridine or 5-bromopyrimidine, $Pd(OAc)_2$, P(*o*-tol)$_3$, Et_3N, CH_3CN; (d) TFA.



Scheme 2. Reagents and conditions: (a) 3-pyridine- or 5-pyrimidine-carboxaldehyde, KOH, MeOH; (b) Pd/C, H_2, MeOH; (c) N_2H_4, KOH, ethylene glycol.

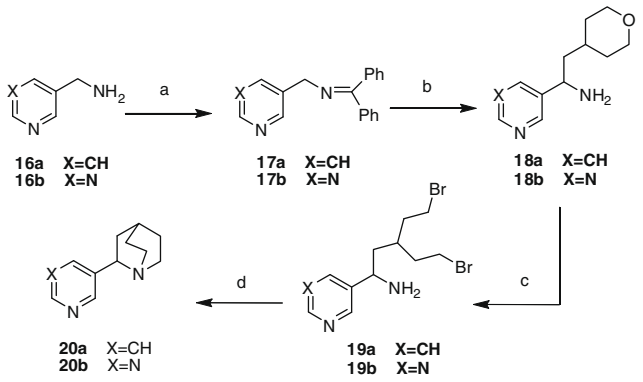


Scheme 3. Reagents and conditions: (a) $LiAlH_4$, THF; (b) Pd/C, H_2; (c) $(BOC)_2O$; (d) Swern Oxidation; (e) Ph_3PCH_3Br, nBuLi; (f) 3-bromopyridine or 5-bromopyrimidine, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, NMP; (g) TFA.

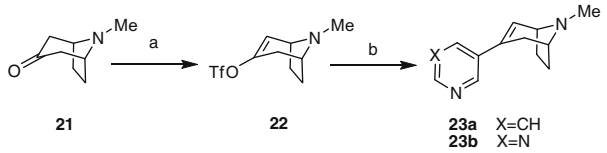
Table 1
In-vitro profile of compounds **5a** and **5b** at NNR subtypes

#	α4β2* K_i[a] (nM)	α7 K_i[b] (nM)	α6β2* K_i[c] (nM)	α4β2* DA E_{max}[d] (%)	α4β2* DA EC_{50}[d] (μM)	α6β2* DA E_{max}[e] (%)	α6β2* DA EC_{50}[e] (μM)
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 MII in mouse olfactory tubercles, striatum and superior colliculus.[18]
[d] Measured in striatal synaptosomes as α-conotoxin MII-resistant DA release.[19]
[e] Measured in striatal synaptosomes as α-conotoxin MII-sensitive DA release.[20]



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, $Pd(Ph_3P)_4$, LiCl, DME, Na_2CO_3.

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 $\alpha4\beta2^*$. A slight drop in affinity for the pyrimidine analogs relative to the corresponding pyridines at the $\alpha4\beta2^*$ subtype was noted for all except **23b**. A much wider range of affinities was observed for the $\alpha6\beta2^*$ 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 $\alpha4\beta2^*$ subtype relative to $\alpha6\beta2^*$ and $\alpha7$. Two noteworthy compounds are quinuclidine **20a,** which possesses high affinity across all three subtypes, and **20b**, wherein high affinity is retained for $\alpha4\beta2^*$ and $\alpha6\beta2^*$ while $\alpha7$ 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 $\alpha4\beta2^*$ receptor subtype (122%, 8.3 μM EC_{50}), it has no functional activity at $\alpha6\beta2^*$. In contrast, pyrimidine **8b** is a partial agonist (73%, 5.9 μM) at $\alpha4\beta2^*$-mediated dopamine release as well as via $\alpha6\beta2^*$ (80% E_{max}), albeit with low potency (37 μM EC_{50}). In the case of quinuclidines **11a/b**, both are potent, full antagonists of both $\alpha4\beta2^*$ and $\alpha6\beta2^*$-mediated dopamine release. For vinylpyrrolidine pair **15a/b**, both analogs exhibited similar levels of efficacy and potency for both $\alpha4\beta2^*$ and $\alpha6\beta2^*$-mediated dopamine release. We note that the relatively low $\alpha6\beta2^*$ affinity of **15b** (325 nM) still translates to good potency (530 nM EC_{50}). Two possible explanations exist for this. First, $\alpha6\beta2^*$ may be analogous to $\alpha4\beta2^*$ wherein the K_i value reflects binding to desensitized state(s) and the EC_{50} value indicates binding to the functional state of the receptor. Perhaps for $\alpha6\beta2^*$ these two states are more similar than for $\alpha4\beta2^*$. Another possibility is that EC_{50} values in the complex $\alpha6\beta2^*$ forms (e.g., $\alpha6\alpha4\beta3\beta2$ responsible for mediating dopamine release in the functional assay may reflect cooperativity of both α subunits and may therefore differ significantly from values expected for an $\alpha6\beta2^*$-containing receptor.

Quinuclidines **20a** and **20b** are intriguing compounds in that they possess relatively low efficacy but high potency at $\alpha4\beta2^*$-mediated dopamine release, while they are very potent, full agonists at $\alpha6\beta2^*$-mediated dopamine release. We believe that this is the first report of full agonists with functional selectivity (both efficacy and potency) for the $\alpha6\beta2^*$ subtype. Finally, tropinone derivatives **23a** and **23b** are both moderately potent partial agonists at $\alpha4\beta2^*$-mediated dopamine release. Both compounds also possess appreciable efficacy (50 and 77%) and robust potency (200 and 100 nM, respectively) at $\alpha6\beta2^*$-mediated dopamine release.

A secondary, albeit extremely important goal in optimizing the pharmacological profile for smoking cessation was to improve functional selectivity for $\alpha6\beta2^*$ versus ganglionic receptor activation. Activation of the ganglionic $\alpha3\beta4^*$ subtype may cause some of the side effects of nicotine and nicotinic ligands.[25] Enhanced selectivity for central versus 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 $\alpha3\beta4^*$ subtype, and the functional potencies (EC_{50}s) compared with those for $\alpha4\beta2^*$ and $\alpha6\beta2^*$ activation to generate selectivity ratios (Table 3). Most compounds exhibited fairly high efficacy at $\alpha3\beta4^*$, with little difference between the pyridine and pyrimidine analogs or across the various chemotypes. The exceptions were

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

#	$\alpha4\beta2^*$ K_i[a] (nM)	$\alpha7$ K_i[b15] (nM)	$\alpha6\beta2^*$ K_i[c] (nM)	$\alpha4\beta2^*$ DA E_{max}[d] (%)	$\alpha4\beta2^*$ DA EC_{50}[d] (μM)	$\alpha6\beta2^*$ DA E_{max}[g] (%)	$\alpha6\beta2^*$ DA EC_{50}[g] (μM)
8a	25 ± 7	>10 k	1550 ± 214	122 ± 26	8.3 ± 4.3	0	NA
8b	69 ± 19	>10 k	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	>10 k	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 MII in mouse olfactory tubercles, striatum and superior colliculus.[19]
[d] Measured in striatal synaptosomes as conotoxin MII-resistant DA release.[20]
[e] Antagonist I_{max}%.
[f] K_i for inhibition.
[g] Measured in striatal synaptosomes as α-conotoxin MII-sensitive DA release.[20]

Table 3
Functional selectivity for pyridine–pyrimidine pairs

#	$\alpha3\beta4^*$ E_{max}[a] (%)	$\alpha3\beta4^*$ EC_{50}[a] (μM)	Functional selectivity $\alpha3\beta4^*/\alpha4\beta2^*$ EC_{50} ratio	Functional selectivity $\alpha3\beta4^*/\alpha6\beta2^*$ 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}/K_i.

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 $\alpha3\beta4^*$ binding domain. Significant differences in potency occurred both between scaffolds and for pyridines versus pyrimidines. Notably, for $\alpha3\beta4^*$, moving from pyridine to pyrimidine generally increased EC_{50} (decreased potency). Fairly wide variances in functional selectivity across the compound set were noted (0.4 to 127 fold for $\alpha3\beta4^*$ verses $\alpha4\beta2^*$ and 0.26 to 410 fold for $\alpha3\beta4^*$ vs $\alpha6\beta2^*$). 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 $\alpha4\beta2^*$-mediated dopamine release relative to ganglionic activation in half the cases; with respect to $\alpha6\beta2^*$-mediated dopamine release relative to ganglionic activation, the selectivity improvements were more modest (2–4 fold) but also more consistent.

In conclusion, we provide novel SAR data on affinity and function for a diverse group of nicotinic ligands in $\alpha6\beta2^*$ containing NNR subtypes. Direct comparison of pyridine versus pyrimidine substituents on this set of scaffolds indicates that this substitution has the potential to enhance $\alpha6\beta2^*$ affinity and/or functional activity and to decrease ganglionic activation, depending on the scaffold. Additionally, we have identified two scaffolds with functional selectivity for $\alpha6\beta2^*$ (exemplified by compounds **20a/b** and **23a/b**). Both may serve as tools to explore the role of $\alpha6\beta2^*$ receptors in various disease states and as leads for further optimization of $\alpha6\beta2^*$ activity. The present scaffolds should be investigated with a larger and more diverse set of molecules to test the SAR conclusions around $\alpha6\beta2^*$ affinity and function, and to identify additional selective compounds. An $\alpha6\beta2^*$ 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 $\alpha6\beta2^*$ selective molecule may also become a useful PET ligand.

Acknowledgments

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Supplementary data

Supplementary data (spectral data of the synthesized compounds (^{1}H NMR, ^{13}C NMR, and LC–MS) as well as general experimental information and details for the α7 binding assay) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.085.

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