Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

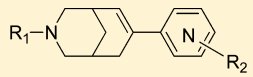
Structure−Activity Studies of 7-Heteroaryl-3-azabicyclo[3.3.1]non-6-enes: A Novel Class of Highly Potent Nicotinic Receptor Ligands

Scott R. Breining,*[,†,‡] Matt Melvin,[†,‡] Balwinder S. Bhatti,[†,‡] Gary D. Byrd,[†,§] Melanie N. Kiser,[†,∥] Christopher D. Hepler,[†,∥] Dawn N. Hooker,[†,∥] Jenny Zhang,[†,∥] Leslie A. Reynolds,[†,∥] Lisa R. Benson,[†,⊥] Nikolai B. Fedorov,[†,⊥] Serguei S. Sidach,[†,⊥] J. Pike Mitchener,[†,#] Linda M. Lucero,[¶] Ronald J. Lukas,[¶] Paul Whiteaker,[¶] and Daniel Yohannes*[,†,‡]

[†]Targacept, Inc. 200 East First Street, Suite 300, Winston−Salem, North Carolina 27101, United States

[‡]Department of Medicinal Chemistry, [§]Department of Analytical Research and Development, [∥]Department of Neurochemistry, [⊥]Department of Preclinical Research/Electrophysiology, [#]Department of Chemical Process and Development; [¶]Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix Arizona 85013, United States

ABSTRACT: The potential for nicotinic ligands with affinity for the $\alpha4\beta2$ or $\alpha7$ subtypes to treat such diverse diseases as nicotine addiction, neuropathic pain, and neurodegenerative and cognitive disorders has been exhibited clinically for several compounds while preclinical activity in relevant in vivo models has been demonstrated for many more. For several therapeutic programs, we sought nicotinic ligands with various combinations of affinity and function across both subtypes, with an emphasis on dual $\alpha4\beta2$−$\alpha7$ ligands, to explore the possibility of synergistic effects. We report here the structure−activity relationships (SAR) for a novel series of 7-heteroaryl-3-azabicyclo[3.3.1]non-6-enes and characterize many of the analogues for activity at multiple nicotinic subtypes.



R1=H, Me

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels distributed throughout the central and peripheral nervous systems. They are assembled from various combinations of subunits ($\alpha2$-$\alpha10$ and $\beta2$-$\beta4$) as homo- or heteromeric pentamers.[1] The $\alpha4\beta2$ nicotinic receptor subtype is the most widely distributed in brain, accounting for more than 90% of nicotine high affinity binding sites.[2] Regions of distribution include the cortex, substantia nigra, hippocampus, and ventral tegmental area.[3] The presence of $\alpha4\beta2$ receptors in these brain regions suggests therapeutic potential in treating disorders such as Parkinson's disease,[4] Alzheimer's disease[5] various pain states,[6] and nicotine addiction.[7] Nicotine (Figure 1, **1**), the classical nicotinic ligand,

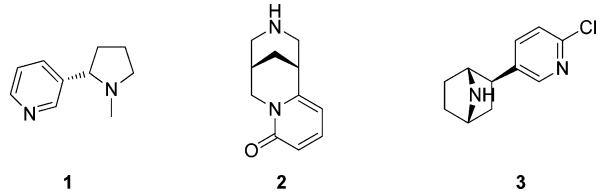


Figure 1. Naturally occurring nicotinic ligands.

is moderately selective for binding at the $\alpha4\beta2$ subtype ($\alpha4\beta2$ K_i = 3.5 nM, $\alpha7$ K_i = 244 nM, $\alpha3\beta4$ K_i = 167 nM).[8] Cytisine (**2**), a natural product present in *Laburnum* species and sold in European countries as a smoking cessation aid,[9] is also considered selective for the $\alpha4\beta2$ subtype ($\alpha4\beta2$ K_i = 1 nM, $\alpha7$ K_i = 8000 nM).[10] Epibatidine (**3**), the well-studied, extremely potent analgesic first isolated from the skin of Ecuadorian poison frogs,[11] is "pan-nicotinic", as it possesses extremely high affinity across all major nicotinic subtypes.[12] A number of $\alpha4\beta2$ receptor ligands have been evaluated clinically for treatment of a variety of conditions including Alzheimer's disease, pain, and nicotine addiction. Such compounds include dianicline (**4**),[13] tebanicline (**5**),[14] ABT-894 (**6**),[15] and AZD3480 (**7**)[16] (Figure 2), which represent a range of

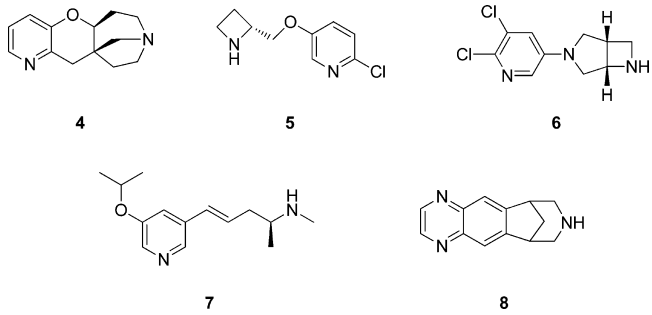


Figure 2. Clinical stage $\alpha4\beta2$ nicotinic ligands.

functional activity at $\alpha4\beta2$ from partial to full agonism. Additional robust validation comes from varenicline (**8**), the recently marketed nicotinic therapeutic, which is sold as a smoking cessation aid.[17]

The homomeric $\alpha7$ receptor, composed of five $\alpha7$ subunits, has more limited distribution within the brain but appears to play a prominent role in cognition, inflammatory processes,

Received: August 1, 2012
Published: October 1, 2012

schizophrenia, and pain pathways.[18,19] Results of several positive preclinical and clinical studies have been reported for $\alpha7$ agonists in these therapeutic areas.[20] $\alpha7$ receptors have also been identified as potentially influencing certain aspects of nicotine withdrawal.[21] Interestingly, the smoking cessation agent varenicline is a moderate affinity full agonist at $\alpha7$, albeit with rather low potency (K_i ~300 nM, 93% E_{max}, ~18 μM EC_{50}).[22] What if any role this activity plays in the in vivo profile of this compound is currently uncertain.

Activation of the ganglionic ($\alpha3\beta4$-containing) receptor subtype is considered responsible for GI and cardiovascular effects associated with exposure to nonselective nicotinics such as epibatidine.[23] The clinical failure of tebanicline has been attributed to poor tolerability as a result of inadequate $\alpha4\beta2$/$\alpha3\beta4$ selectivity,[24] while antagonism of $\alpha3\beta4$ receptors is thought to be responsible for the constipation experienced with higher doses of mecamylamine.[25] Therefore, interaction with the $\alpha3\beta4$ receptor appears to have undesirable consequences and seems best avoided.

Early efforts in our laboratories identified 7-pyridin-3-yl-3-azabicyclo[3.3.1]non-6-ene (Figure 3, **11a**) as a hybrid of 3-(3-pyridyl)-3,7-diazabicyclo[3.3.1]nonane (pyridyl bispidine, **9**)[26] and the metanicotine, TC-2403 (**10**).[27] Compound **9** is a high affinity pan-nicotinic compound,[28] while **10** is highly selective for $\alpha4\beta2$.[29] It was hoped that **11a** would possess a profile somewhere between the two extremes, retaining affinity at $\alpha4\beta2$ and $\alpha7$ but with reduced $\alpha3\beta4$ affinity; in fact, it was found to be a new pan-nicotinic compound, possessing high affinity for $\alpha4\beta2$, $\alpha7$ and $\alpha3\beta4$ subtypes.[30] Our strategy was to take advantage of the multiple sites for substitution on the pyridine ring to modulate interaction with the various nicotinic receptor subtypes, although the high residue homology between subtypes make this a challenging endeavor. Selecting a very high affinity pan-nicotinic scaffold for such modifications would potentially allow retention of high affinity at one or more receptor subtypes while diminishing binding at others, affording selectivity essentially by attrition. We report here the results of in vitro structure−activity relationship (SAR) studies performed on this novel scaffold in our efforts to identify subtype selective analogues.

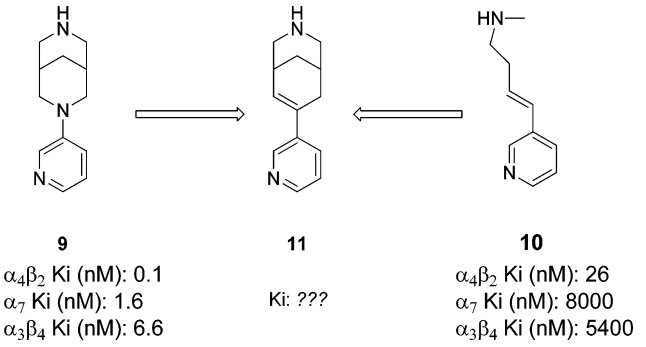


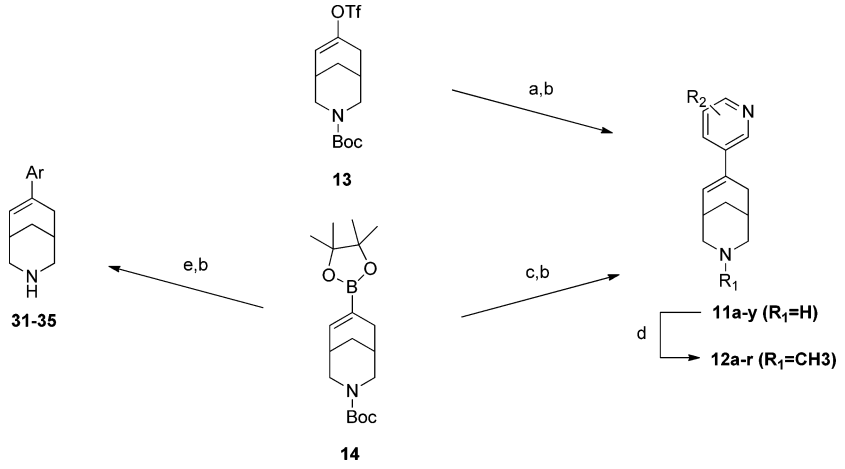
Figure 3. Conception of 7-pyridin-3-yl-3-azabicyclo[3.3.1]non-6-ene scaffold (**11a**).

CHEMISTRY

General Method for Suzuki Couplings. Scheme 1 illustrates the approach used for the preparation of the 7-heteroaryl-3-azabicyclo[3.3.1]non-6-enes **11a−y**, **12a−r**, and **31−35**. A number of the analogues were prepared by the Suzuki coupling[31] of vinyl triflate **13** with commercially available boronic acids. Because of the small number of commercially available heterocyclic boronic acids relative to bromoarenes, the vinyl boronate **14** was also utilized in Suzuki couplings to allow a wider variety of substitution on the heteroaromatic ring. This also allowed utilization of an array of noncommercial bromo-substituted heteroaryls. Many of the **11** analogues were further derivatized to the tertiary amines **12a−r** by *N*-methylation utilizing Eschweiler−Clark conditions.

Preparation of Scaffolds. Vinyl triflate **13** and vinyl boronate **14** were prepared according to Scheme 2 (independently and concurrent with our work, Lilly scientists reported a similar synthesis of **13**).[32] Rhodium-catalyzed hydrogenation of phenol diester **15** provided the corresponding saturated cyclohexanol diester which was oxidized to ketone **16** under Swern conditions. The ketone was protected as the dioxolane, and the esters subsequently reduced selectively to the dialdehyde **17** using diisobutylaluminum hydride under carefully controlled conditions. Bis-reductive amination of dialdehyde **17** with benzylamine and sodium triacetoxyborohy-

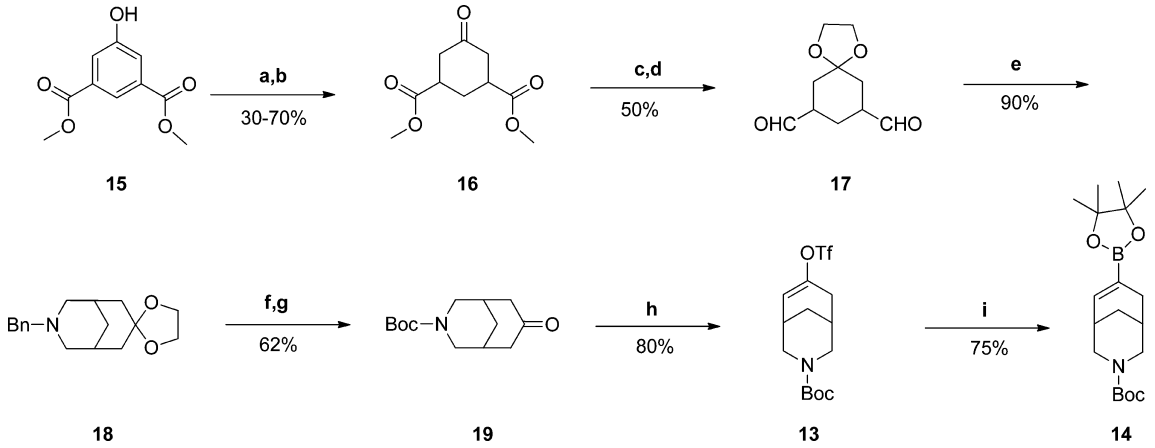
Scheme 1. General Method for Suzuki Couplings[a]



[a]Reagents and conditions: (a) pyridineboronic acid, $Pd(dppf)_2Cl_2$, K_2CO_3, DME, H_2O; (b) TFA; (c) bromopyridine, $Pd(dppf)_2Cl_2$, K_2CO_3, DME, H_2O; (d) CH_2O, HCO_2H; (e) bromoheteroarene, $Pd(dppf)_2Cl_2$, K_2CO_3, DME, H_2O.

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929−9945

Scheme 2. Preparation of Scaffolds 13 and 14[a]

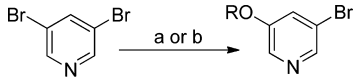


[a]Reagents and conditions: (a) Rh/Al_2O_3, H_2; (b) $(COCl)_2$, DMSO, Et_3N; (c) $HO(CH_2)_2OH$, $PhCH_3$, pTSA; (d) DiBAL-H; (e) $BnNH_2$, $Na(OAc)_3BH$, CH_2Cl_2; (f) Pd/C, NH_4CO_2H, EtOH, then $(BOC)_2O$; (g) acetone, pTSA; (h) KHMDS, Tf_2NPh, THF; (i) bispinacolborane, $Pd(OAc)_2$, dppf, KOAc, dioxane.

dride afforded amine **18**. Hydrogenolysis and treatment with di-*t*-butyldicarbonate was followed by ketal removal under mild conditions (acetone, catalytic PTSA) to give the desired BOC-protected ketone **19**. Vinyl triflate **13** was obtained by deprotonation with potassium hexamethyldisilazide and quenching of the enolate with *N*-phenyl-bis-trifluoromethanesulfonimide. Palladium catalyzed borylation of **13** with bispinacolatodiboron[33] gave vinylboronate **14**.

Preparation of Bromopyridines. A series of noncommercial 3-bromo-5-substituted-pyridines were prepared from 3,5-dibromopyridine **20** according to Scheme 3. Displacement of a single bromide, achieved with an alkoxide or phenoxide in DMF, gave ethers **21**–**30**.

Scheme 3. Preparation of 3-Alkoxy-5-bromopyridines and 3-Phenoxy-5-bromopyridines[a]



[a]Reagents and conditions: (a) ROH or ArOH, NaH, DMF; (b) ROH, K metal.

RESULTS AND DISCUSSION

The racemic parent compound, 7-pyridin-3-yl-3-azabicyclo[3.3.1]non-6-ene **11a**, displayed nanomolar or subnanomolar affinity across $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ subtypes (K_i = 0.17, 4.0, and 11.9 nM, respectively, Table 1). To develop an understanding of SAR around these three subtypes with the goal of producing a range of affinity profiles, a large number of analogues (>100) were prepared. Previous SAR reports on nicotinic ligands have indicated that substituents at the 5′- and 6′-positions of pyridine are generally well tolerated and play a significant role in modulating affinity, activity, and selectivity.[34] Therefore, much of our effort was devoted to exploration of these positions.

Because enantiomers of some nicotinic ligands have different affinity profiles (i.e., (*R*)- and (*S*)-nicotine), enantiomers of **11a** were separated and evaluated. Both enantiomers ((+)-**11a** and (−)-**11a**) resembled each other and racemic **11a** in affinity profile, although a minor (~5-fold) differential in $\alpha3\beta4$ affinity between enantiomers was noted. The $\alpha4\beta2$-to-$\alpha3\beta4$ selectivity ratio was 20-fold for (−)-**11a**, whereas for (+)-**11a**, the separation was 230-fold. Despite the >200× separation for (+)-**11a**, the 23 nM affinity at $\alpha3\beta4$ was still a matter of concern.

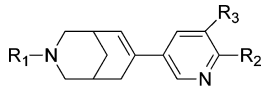
Several 5′-alkoxy substituted analogues were prepared and evaluated (compounds **11b**–**f**). Consistent with expectations, ethers at the 5′ position were well tolerated at $\alpha4\beta2$, exhibiting an affinity comparable to the unsubstituted parent. While 5-methoxy **11b** retained $\alpha7$ affinity, isopropoxy derivative **11c** displayed a 25× drop in $\alpha7$ affinity relative to **11a**. This would appear to be a steric phenomenon, although both the phenoxy (**11d**) and benzyloxy (**11e**) substituents exhibited a somewhat smaller loss in $\alpha7$ affinity (5× and 10×, respectively). A chloro group in position 6′ in combination with the 5-methoxy group (**11f**) was also well tolerated at $\alpha4\beta2$ but caused a 25× loss in $\alpha7$ affinity. Significantly, none of these substituents reduced $\alpha3\beta4$ affinity; in fact, **11d**, bearing a phenoxy group, possessed higher $\alpha3\beta4$ affinity than the parent.

Electron withdrawing groups at the 5′-position were next evaluated (Table 2). 5-Fluoro substitution (**11g**) did not alter the affinity profile relative to **14a**. Thus, neither the hydrogen bonding potential nor the electron withdrawing character of the 5-fluoro group affect binding at the three subtypes evaluated. As with enantiomers of **11a**, no significant differential was seen between enantiomers (+)-**11g** and (−)-**11g**. Exchange of chlorine for fluorine also had little effect on binding profile (**11h**). Cyano and trifluoromethyl derivatives (**11i** and **11j**) retained high $\alpha4\beta2$ affinity but did experience a loss in $\alpha7$ affinity (K_i = 160 and 641 nM, respectively). Again, no modulation of $\alpha3\beta4$ affinity was noted, and enantiomers (+)-**11i** and (−)-**11i** were again essentially identical in profile. Finally, **11k**, bearing a 5-phenyl substituent, retained high binding affinity at $\alpha4\beta2$ and $\alpha3\beta4$ but demonstrated a complete loss $\alpha7$ affinity.

Several substituents at the 6′-position were also evaluated (Table 3). The fluoro and chloro congeners **11l** and **11m** maintained high affinity across all subtypes. Introduction of a 6-methoxy group (**11n**) severely diminished $\alpha7$ affinity and decreased $\alpha3\beta4$ affinity by nearly 100-fold relative to **11a**. This compound exhibited the highest $\alpha4\beta2$ selectivity relative to $\alpha7$

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929–9945

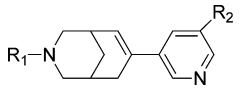
Table 1. Affinity Profiles for Unsubstituted Pyridines and 5′-Ethers[a]



compd	R1	R2	R3	$\alpha4\beta2$ K_i, nM[b]	$\alpha7$ K_i, nM[c]	$\alpha3\beta4$ K_i, nM[d]
11a	H	H	H	0.2 (0.02)	4.0 (1.4)	12 (4)
(+)-**11a**	H	H	H	0.1 (0.01)	1.3 (0.7)	23 (8)
(−)-**11a**	H	H	H	0.2 (0.01)	1.2 (0.1)	4.0 (0.3)
(+)-**12a**	CH_3	H	H	9.4 (2.1)	70 (5)	1796 (512)
(−)-**12a**	CH_3	H	H	2.1 (0.5)	74 (32)	534 (73)
11b	H	H	MeO	0.3 (0.1)	4.6 (1.2)	14.3 (7.6)
12b	CH_3	H	MeO	0.6 (0.1)	605 (283)	596 (203)
11c	H	H	iPrO	0.26 (0.04)	105 (54)	4.8 (0.1)
12c	CH_3	H	iPrO	2.2 (0.7)	>10000[e]	1726 (456)
11d	H	H	PhO	0.7 (0.2)	22 (17)	1.9 (0.2)
12d	CH_3	H	PhO	1.3 (0.5)	17 (5)	8.2 (1.4)
11e	H	H	BnO	0.7 (0.1)	43.9 (15.4)	44 (40)
12e	CH_3	H	BnO	1.7 (0.8)	4545 (1425)	711 (48)
11f	H	Cl	MeO	0.4 (0.1)	111.4 (1.6)	7.0 (5.5)
12f	CH3	Cl	MeO	4.4 (1.6)	7565 (3943)	1431 (462)

[a]Unless otherwise noted, represents a minimum of three assays. Values are reported (± SEM), using [^{3}H]-epibatidine as radioligand. [b]Human subunits expressed in SH-EP1 cells, [^{3}H]-nicotine as radioligand. [c]Human subunits expressed in HEK/RIC3 cells. [d]Human subunits expressed in SH-SY5Y cells. [e]Single HTS assay.

Table 2. Affinity Profiles for 5′-Pyridyl Electron Withdrawing Groups[a]



compd	R1	R2	$\alpha4\beta2$ K_i, nM[b]	$\alpha7$ K_i, nM[c]	$\alpha3\beta4$ K_i, nM[d]
11g	H	F	0.3 (0.1)	3.6 (0.5)	39 (34)
(+)-**11g**	H	F	0.2 (0.1)	3.3 (0.9)	8.3 (2.4)
(−)-**11g**	H	F	0.2 (0.03)	1.6 (0.2)	5.4 (0.9)
12g	CH_3	F	13 (5)	1007 (476)	1639 (196)
11h	H	Cl	0.4 (0.1)	15 (5)	40 (25)
12h	CH_3	Cl	9.6 (3.3)	8006 (358)	1831 (647)
11i	H	CN	0.6 (0.2)	160 (57)	47 (22)
(+)-**11i**	H	CN	0.3 (0.1)	73 (29)	15 (3)
(−)-**11i**	H	CN	0.3 (0.1)	79 (35)	17 (3)
12i	CH_3	CN	24 (5)	>10000[e]	4502 (1547)
11j	H	CF3	0.7 (0.3)	641 (208)	79 (27)
11k	H	Ph	0.8 (0.2)	3220 (155)	17 (2)

[a]Unless otherwise noted, represents a minimum of three assays. Values are reported (± SEM), using [^{3}H]-epibatidine as radioligand. [b]Human subunits expressed in SH-EP1 cells, [^{3}H]-nicotine as radioligand. [c]Human subunits expressed in HEK/RIC3 cells. [d]Human subunits expressed in SH-SY5Y cells. [e]Single HTS assay.

and $\alpha3\beta4$ of all compounds evaluated. **11o** indicates that a methyl group in this position is fairly well tolerated, although a 20× reduction in $\alpha7$ affinity resulted. Introduction of the sterically demanding 6-phenyl substituent (**11p**) reduced affinity to >100 nM at all three subtypes.

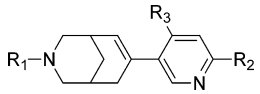
Finally, substituents at the 4′-position were evaluated. Introduction of 4-chloro (**11q**) or 4-cyano (**11r**) groups induced a modest loss in $\alpha4\beta2$ affinity (∼15-fold loss relative to **11a**). **11q** maintained high $\alpha7$ affinity (33 nM), while **11r**'s cyano group caused a considerable loss in $\alpha7$ affinity (330 nM). Both compounds also showed somewhat reduced $\alpha3\beta4$ affinity relative to **11a** (∼10-fold), but the compounds remained relatively nonselective.

Nicotine and the *des*-methyl analogue, nornicotine, possess very different affinity profiles, with nicotine possessing much higher (approximately an order of magnitude) $\alpha4\beta2$ and $\alpha7$ affinity.[35] Given this potential to modulate affinity by *N*-methylation,[34] many of the compounds in Tables 1−3 were also evaluated as their *N*-methyl versions (compounds **12a−r**, Tables 1−3). The *N*-methyl enantiomers (+)-**12a** and (−)-**12a** were much less potent than the parent *NH* compounds across all three subtypes, with little differentiation between the two enantiomers. The remaining compounds **12b−r** all demonstrated a loss of $\alpha4\beta2$ affinity relative to the secondary amine parent. Losses ranged from ∼2-fold to as great as 2000-fold (i.e., **12n** vs **11n**). $\alpha7$ affinities were similarly decreased for nearly all compounds. One notable exception was the 5-phenoxy analogue **12d**, which showed no loss of $\alpha7$ affinity. This is in stark contrast to methoxy, isopropoxy, and benzyloxypyridines (**12b,c,e**), which all showed greater than 100-fold losses. Affinity at $\alpha3\beta4$ decreased significantly (between 4 and 200-fold) for the tertiary amines across the entire series. Unfortunately, this reduction in $\alpha3\beta4$ affinity came at the expense of a similar loss in $\alpha4\beta2$ and/or $\alpha7$ affinity, such that affinity-based selectivity was not greatly altered relative to the parent secondary amines. In contrast to the secondary amines, many of the tertiary *N*-methyls do not possess a level of $\alpha3\beta4$ binding which is biologically relevant (>1 μM) but do maintain high $\alpha4\beta2$ affinity (<50 nM), indicating potential promise for enhanced tolerability. From the set of compounds presented in Tables 1−3, (+)-**12a** and (−)-**12a** were unique in balancing high $\alpha4\beta2$ affinity, moderate $\alpha7$ affinity, and low $\alpha3\beta4$ affinity, a profile which has been difficult to obtain for many nicotinic scaffolds.

In an effort to further enhance subtype selectivity, several heterocycles were explored as replacements for the 3-pyridyl attachment (Figure 4, Table 4). 4-Pyridyl (**31**) and quinoxalinyl (**35**) analogues exhibited a large loss in $\alpha4\beta2$ affinity, while pyrimidine (**32**), pyrazine (**33**), and chloropyridazine (**34**) replacements maintained high $\alpha4\beta2$ affinity (Table 4). Again, the $\alpha7$ receptor subtype was more sensitive to these

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929−9945

Table 3. Affinity Profiles for Substitution at Pyridine Positions 4′ and 6′.[a]



compd	R1	R2	R3	$\alpha4\beta2$ K_i, nM[b]	$\alpha7$ K_i, nM[c]	$\alpha3\beta4$ K_i, nM[d]
11l	H	F	H	0.4 (0.2)	20 (9)	28 (12)
12l	CH_3	F	H	18 (2)	1305 (240)	1305 (240)
11m	H	Cl	H	0.4 (0.2)	39 (16)	6.2 (4.0)
12m	CH_3	Cl	H	22 (4)	6530 (990)	986 (221)
11n	H	MeO	H	0.4 (0.1)	1849 (979)	328 (143)
12n	CH_3	MeO	H	797 (112)	>10000[e]	>10000[e]
11o	H	CH_3	H	0.3 (0.1)	80 (14)	25 (22)
11p	H	Ph	H	105 (4)	590 (219)	507 (195)
11q	H	H	Cl	3.0 (0.3)	33 (8)	128 (72)
12q	CH_3	H	Cl	1158 (434)	1421 (359)	7234 (3624)
11r	H	H	CN	2.8 (0.5)	330 (106)	103 (55)
12r	CH_3	H	CN	3418 (2281)	4847 (655)	ND

[a]Unless otherwise noted, represents a minimum of three assays. Values are reported (± SEM), using [^{3}H]-epibatidine as radioligand. [b]Human subunits expressed in SH-EP1 cells, [^{3}H]-nicotine as radioligand. [c]Human subunits expressed in HEK/RIC3 cells. [d]Human subunits expressed in SH-SY5Y cells. [e]Single HTS assay.

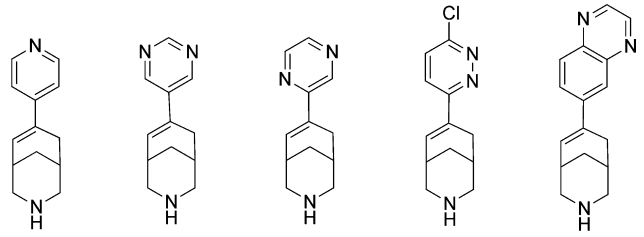


Figure 4. Alternate heterocycles.

Table 4. Affinity Profiles for Alternate Heterocycles[a]

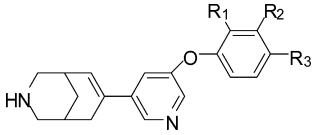
compd	$\alpha4\beta2$ K_i, nM[b]	$\alpha7$ K_i, nM[c]	$\alpha3\beta4$ K_i, nM[d]
31	56 (10)	>10000[e]	459 (17)
32	0.2 (0.03)	19 (4)	58 (51)
33	1.1 (0.3)	281 (160)	828 (471)
34	0.4 (0.1)	77 (10)	73 (44)
35	85 (16)	>10000[e]	5736 (850)

[a]Unless otherwise noted, represents a minimum of three assays. Values are reported (± SEM), using [^{3}H]-epibatidine as radioligand. [b]Human subunits expressed in SH-EP1 cells, [^{3}H]-nicotine as radioligand. [c]Human subunits expressed in HEK/RIC3 cells. [d]Human subunits expressed in SH-SY5Y cells. [e]Single HTS assay.

modifications, resulting in substantial affinity losses, although pyrimidine was reasonably well tolerated. No enhancement in selectivity over $\alpha3\beta4$ was achieved with pyridine replacements **31**, **32**, **34**, or **35**, so these modifications were not pursued further. Pyrazine **33** did exhibit good $\alpha4\beta2$ vs $\alpha3\beta4$ selectivity, but $\alpha3\beta4$ did not separate sufficiently from $\alpha7$ to provide a useful dual affinity ligand.

After establishing the basic series SAR and noting the general lack of $\alpha3\beta4$ selectivity, we looked for further ways to enhance selectivity while retaining high $\alpha7$ affinity. A potential handle for optimization was recognized in the 5′-phenoxypyridyl substituent. Because the phenoxy group was well tolerated and substitutions on the phenyl ring could be readily explored, a large number of analogues were prepared; a representative set are presented in Table 5. Introduction of a methoxy, fluoro, or

Table 5. Affinity Profile for Substituted Phenoxy Analogues[a]



compd	R1	R2	R3	$\alpha4\beta2$ K_i, nM[b]	$\alpha7$ K_i, nM[c]	$\alpha3\beta4$ K_i, nM[d]
11d	H	H	H	0.7 (0.2)	22 (17)	1.9 (0.2)
11s	MeO	H	H	1.0 (0.3)	30 (7)	56 (41)
11t	H	MeO	H	0.9 (0.1)	13 (4)	15 (9)
11u	H	H	MeO	1.4 (0.4)	23 (2)	27 (17)
11v	Cl	H	H	0.9 (0.3)	39 (4)	26 (18)
11w	H	Cl	H	1.0 (0.5)	13 (3)	22 (14)
11x	H	H	Cl	1.9 (0.6)	23 (8)	40 (25)
11y	H	H	F	0.6 (0.1)	7.2 (1.1)	31 (25)

[a]Unless otherwise noted, represents a minimum of three assays. Values are reported (± SEM), using [^{3}H]-epibatidine as radioligand. [b]human subunits expressed in SH-EP1 cells, [^{3}H]-nicotine as radioligand. [c]human subunits expressed in HEK/RIC3 cells. [d]human subunits expressed in SH-SY5Y cells.

chloro group at the 2-, 3-, or 4-positions had no significant effect on binding profile. This is suggestive of a deep pocket in the receptor which accommodates the phenyl group but does not provide opportunities for polar interactions with residues which would influence binding. The lack of modulation of affinity profile also indicates that this feature is conserved across subtypes, although $\alpha3\beta4$ seemed slightly less tolerant of substituents on the phenoxy ring. It was also noted that introduction of the 5′-phenoxy moiety to this scaffold led to undesirable hERG activity; therefore, this modality was not explored further.

After evaluating compounds for binding affinity SAR, select compounds were characterized with respect to *functional* SAR. We hoped to identify compounds with $\alpha4\beta2/\alpha3\beta4$ functional selectivity >100-fold based on EC_{50} values. The $\alpha4\beta2$ receptor has been shown to possess two distinct stoichiometries: $(\alpha4)3(\beta2)2$ (low sensitivity, LS) and $(\alpha4)2(\beta2)3$ (high sensitivity, HS).[36] The LS and HS monikers reflect sensitivity to activation by acetylcholine (EC_{50} ~100 and ~1 μM,

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929–9945

Table 6. Calcium Flux Function at $\alpha4\beta2$ (HS and LS) and $\alpha3\beta4$ Subtypes[a]

compd	$\alpha4\beta2$ HS max %[b]	$\alpha4\beta2$ HS EC_{50}, nM	$\alpha4\beta2$ LS max %[b]	$\alpha4\beta2$ LS EC_{50}, nM	$\alpha3\beta4$ max %[c]	$\alpha3\beta4$ EC_{50}, nM
(+)-**11a**	111 (5)	2.3 (0.1)	131 (16)	35 (12)	139 (18)	73 (23)
(−)-**11a**	114 (4)	2.3 (1.1)	129 (12)	12 (4)	142 (6)	37 (9)
(+)-**12a**	53 (4)	6661 (1370)	17 (6)	>10000[d]	25 (2)	>10000[d]
(−)-**12a**	19 (9)	>10000[d]	11 (4)	7664 (2854)	31 (6)	7531 (2474)
11b	72 (24)	49 (19)	100 (5)	53 (18)	145 (9)	105 (17)
12b	19 (6)	2660 (49)	15 (1)	5133 (2136)	27 (4)	3641 (369)
11c	75 (12)	164 (62)	54 (14)	562 (165)	160 (39)	69 (15)
12c	1.0 (0.5)	>10000[d]	3.3 (0.9)	>10000[d]	15 (4)	>10000[d]
11d	52 (15)	62 (30)	30 (11)	654 (564)	101 (2)	23 (6)
12d	42 (13)	183 (88)	9.5 (3.8)	>10000[d]	103 (4)	100 (26)
(+)-**11g**	100 (9)	29 (10)	119 (10)	50 (16)	140 (11)	155 (33)
(−)-**11g**	112 (12)	34 (14)	120 (12)	36 (16)	153 (2)	111 (20)
(+)-**11i**	95 (5)	256 (124)	88 (8)	4189 (2297)	136 (13)	355 (127)
(−)-**11i**	92 (4.7)	107 (53)	89 (9)	343 (251)	144 (9)	447 (54)
11k	33 (14)	85 (40)	5.3 (0.9)	>10000[d]	61 (5)	181 (40)
11l	104 (6)	85 (21)	136 (18)	85 (21)	140 (7)	99 (52)
12l	22 (5)	>10000[d]	9.4 (5.3)	>10000[d]	25 (3)	>10000[d]
11m	98 (7)	54 (18)	152 (36)	35 (5)	141(9)	16 (4)
11n	91 (10)	3462 (1708)	20 (6)	>10000[d]	61 (8)	1890 (230)
11o	107 (16)	75 (1)	112 (14)	79 (8)	149 (10)	93 (48)
11p	19 (8)	>10000[d]	18 (6)	9660 (7259)	17(3)	10861 (1683)
11q	46 (6)	2004 (1304)	12.8 (0.5)	>10000[d]	105 (2)	377 (185)
11r	55 (1)	3250 (2213)	27 (19)	>10000[d]	130 (10)	166 (33)
32	116 (8)	240 (205)	145 (40)	162 (83)	147 (12)	198 (78)
34	118 (13)	80 (9)	160 (47)	595 (507)	135 (4)	149 (37)

[a]Represents a minimum of three assays unless otherwise indicated. Values are reported (± SEM) relative to nicotine. [b]Human subunits expressed in SH-EP1 cells. [c]Human subunits expressed in SH-SY5Y cells. [d]Could not be reliably determined.

respectively). In vitro, the expression of HS stoichiometry can be greatly enhanced by reduced temperature conditions or chronic nicotine exposure.[37] The presence and distribution of the forms across brain regions is not known, but it is presumed that the stoichiometric ratio has in vivo consequences.[38] A number of nicotinic ligands, including many of the compounds illustrated in Figures 1 and 2, have previously been profiled for activity at $\alpha4\beta2$ HS and LS and display a differential in functional selectivity.[39] Thus, data is provided for representative ligands of the 7-heteroaryl-3-azabicyclo[3.3.1]non-6-enes series in Table 6. Data were obtained in calcium flux FLIPR assays for both HS and LS forms of $\alpha4\beta2$ and $\alpha3\beta4$ (using human subunits expressed in SH-EP1 cells and SH-SY5Y cells, respectively; max % values are reported relative to activation induced by 10 μM nicotine). The enantiomers (+)-**11a** and (−)-**11a** both demonstrated full, potent agonism at HS and LS $\alpha4\beta2$. Potency was a bit higher for both compounds at HS (EC_{50} ~2 nM for each) than at LS (EC_{50} of 34 nM for (+)-**11a**, 12 nM for (−)-**11a**). Unfortunately, there was rather poor functional separation for $\alpha4\beta2$ vs $\alpha3\beta4$, even using the more potent HS EC_{50} values (32-fold for (+)-**11a**, 16-fold for (−)-**11a**). Additionally, this does not take into account the likelihood that $\alpha3\beta4$ receptors also exhibit multiple stoichiometries which may exhibit similar differentials.[40] Introduction of a 5-methoxy (**11b**) group reduced the maximal response to 75% and somewhat reduced potency at $\alpha4\beta2$ HS but did not change the response at $\alpha3\beta4$ or $\alpha4\beta2$ LS. Replacement of the methoxy with the bulkier isopropoxy **11c** did not change the $\alpha4\beta2$ HS profile significantly relative to **11b** but produced a further loss of efficacy and potency at LS; no effect on $\alpha3\beta4$ function was observed. Introduction of the 5-phenoxy group (**11d**) provided a further drop in efficacy at HS and LS (max % = 52 and 30, respectively) and some loss of potency. This compound was also a full agonist at $\alpha3\beta4$, with very high potency (EC_{50} of 23 nM). The electron-withdrawing fluoro substituent in enantiomers (+)-**11g** and (−)-**11g** did not significantly alter the functional profile relative to the unsubstituted parent, although there was a slight reduction in $\alpha4\beta2$ HS EC_{50} (~15-fold). As with **11a**, there was no differential in activity between enantiomers. Replacement of fluoro with cyano caused a further modest loss in potency at HS for each enantiomer ((+)-**11i** and (−)-**11i**). At LS, there was a difference in potency between the two enantiomers, but both enantiomers were equipotent, full agonists at $\alpha3\beta4$. The bulky phenyl substituent in **11k** induced a loss of both potency and efficacy at $\alpha4\beta2$ and $\alpha3\beta4$. The loss of activity at LS was most profound, with no significant agonist activity remaining (5% activation).

Within 6-pyridyl substituents, the 6-fluoro- and 6-chloropyridines (**11l,m**) were potent, full agonists at $\alpha4\beta2$ and $\alpha3\beta4$. The 6-methoxy congener **11n** retained full agonism at HS, albeit with a large loss in potency (3462 nM), while at LS, **11n** exhibited very weak partial agonism (max % = 20, EC_{50} >10 μM) and $\alpha3\beta4$ potency also dropped sharply (1890 nM EC_{50}). The 6-methyl pyridine **11o** exhibited full, potent agonism at both $\alpha4\beta2$ stoichiometries as well as at $\alpha3\beta4$, while introduction of the bulky 6-phenyl substituent (**11p**) resulted in low efficacy across the board. As pyrimidine and chloropyridazine replacements for pyridine were well tolerated with respect to affinity, these compounds were also evaluated for their functional activity. Although both **32** and **34** were somewhat less potent than **11a**, they retained full activation of $\alpha4\beta2$ HS/LS and $\alpha3\beta4$.

Table 7. $\alpha4\beta2$ and $\alpha7$ Function in Electrophysiology for Select Compounds[a,b]

compd	$\alpha4\beta2$ max %[c]	$\alpha4\beta2$ EC_{50}, μM	$\alpha7$ max %[d]	$\alpha7$ EC_{50}, μM
(+)-**11a**	32 (17−34)	0.05 (0.05−1.6)	28 (22−40)	0.02 (0.016−0.039)
(−)-**11a**	40 (37−48)	0.1 (0.084−0.22)	20 (14−26)	0.03 (0.019−0.062)
(+)-**12a**	18 (15−20)	3.1 (1.4−6.8)	87 (60−114)	6 (0.9−9.9)
(−)-**12a**	19 (15−22)	3.5 (0.99−13)	100 (99−103)	3.8 (1.4−2.4)
11c	11 (10−12)	(1.7−1.4)	92 (85−100)	2.8 (2.2−3.3)
12c	10 (7−12)	3.5 (0.6−20)	60 (25−109)	21.3 (6−80)
11d	9 (3−15)	0.59 (0.14−2.5)	31 (24−37)	0.35 (0.2−0.5)

[a]Minimum of three cells recorded. Range of values given in parentheses. [b]max % is % of maximal response relative to 1 mM ACh. [c]Human subunits expressed in SH-EP1 cells. [d]Rat subunits expressed in GH4C1 cells.

Table 8. DA Release and $\alpha6/3\beta2\beta3$* Affinity for Select Compounds[a]

compd	DA max %[b]	DA EC_{50}, nM	$\alpha6/3\beta2\beta3$* K_i, nM	$\alpha6/3\beta2\beta3$* max %[b]	$\alpha6/3\beta2\beta3$* EC_{50}, (nM)
(+)-**11a**	49 (5)	1.4 (0.4)	1.0 (0.1)	82 (27)	15 (0.4)
(−)-**11a**	73 (3)	0.95 (0.25)	0.95 (0.04)	110 (29)	14 (4)
(+)-**12a**	13 (3)	855 (467)	320 (24)	9 (5)	>10000
(−)-**12a**	27 (10)	>10000[c]	300 (42)	7 (2)	>10000
11c	86 (16)	1090 (403)	2.6 (0.4)	3 (2)	>10000
12c	63 (20)	879 (358)	690 (95)	0.2 (0.2)	>10000
11d	57 (11)	718 (545)	4.8 (1.7)	0	>10000

[a]Reported as (± SEM). [b]relative to 10 μM nicotine. [c]Could not be reliably determined.

A number of *N*-methyl versions were also evaluated for functional response. (+)-**12a** demonstrated a sharp drop in efficacy and potency at HS (53%, 6.6 μM), LS (17%, >10 μM), and $\alpha3\beta4$ (25%, >10 μM) relative to (+)-**11a**. While the functional selectivity for $\alpha4\beta2$ vs $\alpha3\beta4$ was actually *reduced* relative to that for (+)-**11a**, the low efficacy and potency at $\alpha3\beta4$, coupled with the lack of binding affinity (1796 nM K_i), make this measure of selectivity less relevant. A similar trend was observed for antipode (−)-**12a**, although a further reduction in HS activity was observed (19%, >10 μM EC_{50}). In all the remaining cases, *N*-methylation consistently reduced efficacy and potency across subtypes. As with binding affinity, there seems to be a complex interplay of the *N*-methyl group and the pyridine substituent in modulating agonist activity, suggesting alternate binding modes depending on pyridine substitution pattern.

In comparing K_i values with EC_{50}s, it was noted that the EC_{50}/K_i ratio for $\alpha4\beta2$ HS ranged from about 14 to more than 20000 (average ∼2300) and for LS from 40 to >20000 (average ∼11000). In contrast, the EC_{50}/K_i ratio for $\alpha3\beta4$ ranged from 1 to 30 (average ∼10). Within this series, binding and potency for activation are more closely related for the $\alpha3\beta4$ subtype such that separation based on affinity does not translate to appropriate functional selectivity.[41]

To more fully characterize the functional profiles of compounds of interest, $\alpha7$ activation was determined by electrophysiology (rat subunits expressed in GH4C1 cell line); activation of $\alpha4\beta2$ was also performed by electrophysiology (human subunits expressed in SH-EP1 cell line) to complement calcium flux data. Enantiomers (+)-**11a** and (−)-**11a** possessed only minor differences in efficacy and potency at $\alpha4\beta2$ (Table 7). The potency and efficacy were far less than that measured by calcium flux methodology. This discrepancy between function measured by ion flux and in electrophysiology is a typical observation. An important caveat is that values in calcium flux assays are reported relative to nicotine, while they are reported relative to acetylcholine for electrophysiology. With respect to $\alpha7$, while very potent (20−30 nM EC_{50}), neither compound exhibited much $\alpha7$ efficacy (maximal response <30%). Consistent with the $\alpha4\beta2$ results in the calcium flux assay, *N*-methylation of (+)- and (−)-**11a** caused a further drop in efficacy (32−18% and 40−19%, respectively) and large loss in potency (62-fold and 35-fold, respectively) for (+)-**12a** and (−)-**12a**. Surprisingly, an enhancement of $\alpha7$ efficacy was observed for (+)-**12a** and (−)-**12a**, although with much weaker potency (6 and 3.8 μM, respectively). **11c**, **12c**, and **11d** together indicate that both methylation and introduction of pyridine substituents decrease potency and efficacy at $\alpha4\beta2$ function, while these same modifications more subtly modulate $\alpha7$ activity.

Nicotine modulation of dopamine release within the mesolimbic system is recognized as a key factor in initiating and maintaining nicotine addiction.[42] There is also evidence that decreased dopamine levels in the striatum may be associated with pain states.[43] Both $\alpha4\beta2$ and $\alpha6$-containing receptors have been identified as playing an important role in modulating dopamine release from dopaminergic neurons,[44−46] although dopamine release from striatal tissue is driven to a large extent by $\alpha4\beta2$ receptors (∼60% of nicotine's response).[47] $\alpha6$-containing receptors are heteromeric, possessing complex subunit combinations such as $\alpha6\beta2\beta3$* and $\alpha4\alpha6\beta2\beta3$*; the localization and pharmacology of these $\alpha6$-containing receptors have also implicated them as targets for the treatment of Parkinson's disease as well as nicotine addiction.[48] Select compounds from this series were therefore assayed for their potency and efficacy in inducing dopamine release in vitro from striatal synaptosomes as well as for binding affinity and function in an $\alpha6/\alpha3\beta2\beta3$ chimera (Table 8).[49] The dopamine release efficacy proved moderate for (+)-**11a** and (−)-**11a** (∼49% and ∼73%, respectively), but at a very potent ∼1 nM EC_{50}. These values are in alignment with the observed $\alpha4\beta2$ full agonism in the calcium flux assay. Both enantiomers exhibited ∼1 nM affinity and potent, full agonism in the $\alpha6/3\beta2\beta3$ chimera.

Dopamine release E_{max} was reduced for both **12a** enantiomers relative to the corresponding **11a** enantiomers,

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929−9945

and both **12a** enantiomers also exhibited greatly reduced $\alpha6/3\beta2\beta3$ affinity and functional response. **11c** exhibited a greater dopamine release E_{max} than either enantiomer of **11a** but with 1000-fold lower potency, while high $\alpha6/3\beta2\beta3$ affinity was maintained (2.6 nM). This high affinity did not translate into meaningful $\alpha6/3\beta2\beta3$ agonism The tertiary amine version of **11c** (compound **12c**) exhibited similar efficacy and potency to **11c**, but affinity did drop off greatly at $\alpha6/3\beta2\beta3$. Overall, the results presented in Tables 7 and 8 indicate that for these compounds, there are no consistent trends between: (1) $\alpha7$ affinity and efficacy/potency, (2) dopamine release and $\alpha4\beta2$ or $\alpha6/\alpha3\beta2\beta3$ affinity, (3) $\alpha6/\alpha3\beta2\beta3$ affinity and efficacy, and (4) agonism and DA release efficacy/potency. There does appear to be a relationship between dopamine release and $\alpha4\beta2$ efficacy which suggests that, at least in this set of compounds, DA release is being driven through the $\alpha4\beta2$ manifold. This is further supported by an apparent general correspondence between $\alpha4\beta2$ EC_{50} and DA release EC_{50}. The relevance of the affinity and function observed in this $\alpha6/\alpha3\beta2\beta3$ chimera to native $\alpha6$* receptors is admittedly unknown. Support exists for the relevancy,[50] however, $\alpha6$ subtypes which also contain an $\alpha4$ subunit have also been shown to play an important role in eliciting dopamine release.[51] On the basis of this small set, it also appears that affinity and efficacy at $\alpha6/\alpha3\beta2\beta3$ are particularly sensitive to structural modifications. One final observation worth mentioning is that for the two enantiomers of **11a**, the ratio of EC_{50} to K_i was ~15-fold. It would be interesting to further explore this relationship as mentioned previously for $\alpha4\beta2$ and $\alpha3\beta4$. Unfortunately, the remaining compounds do not have meaningful EC_{50} values, precluding further comparison.

CONCLUSIONS

The affinity data presented here for 7-heteroaryl-3-azabicyclo[3.3.1]non-6-enes indicate that, consistent with related series, $\alpha4\beta2$ and $\alpha3\beta4$ receptors are fairly tolerant of pyridine substituents and replacement of pyridine with other related heterocycles,[34] while $\alpha7$ is more sensitive to structural variations. This makes it difficult to achieve affinity-based separation from $\alpha3\beta4$, particularly if retention of $\alpha7$ affinity is desired. With respect to functional activity, there is a lack of direct correlation between affinity and functional efficacy and potency, but there are some consistent trends. Typically, functional potency (EC_{50}) is observed at ~10−1000× the K_i for $\alpha4\beta2$, while less difference exists between $\alpha3\beta4$ EC_{50} and K_i (~1−30×). Comparisons between $\alpha7$ and $\alpha4\beta2$ or $\alpha3\beta4$ cannot be reasonably made because there are differences in assay format or subunit species used. In terms of functional SAR, variation of pyridine ring substituents does little to modulate efficacy and potency, with substituents generally decreasing EC_{50}s at $\alpha4\beta2$ HS and LS relative to the unsubstituted pyridine. The LS form seems to be a bit more sensitive than the HS with respect to pyridine substitution, such that many of the compounds presented here possess a modest preference for activation of the HS stoichiometry. *N*-Methylation in this series tends to confer a greater degree of separation between $\alpha4\beta2$ and $\alpha3\beta4$ activation, but it has proven difficult to achieve a balance between high affinity and functional activity at both $\alpha4\beta2$ and $\alpha7$ while maintaining reasonable $\alpha3\beta4$ separation. In conclusion, we have identified a novel series with a range of affinity and functional activity at $\alpha4\beta2$, $\alpha7$, $\alpha6/\alpha3\beta2\beta3$, and $\alpha3\beta4$ receptor subtypes, although a clear, interpretable SAR did not emerge. From the extensive evaluation presented here, the compounds most closely aligned with our original objectives (high affinity at $\alpha4\beta2$ and $\alpha7$, partial agonist profile, minimal interaction at $\alpha3\beta4$) are the enantiomers of **12a**. How the affinity and functional profiles of compounds from this series translate into potency, efficacy, and tolerability in in vivo models of CNS disorders will be the subject of a future paper.

EXPERIMENTAL SECTION

Biology. *Binding Assays.* Binding affinity was obtained at human $\alpha4\beta2$, $\alpha7$, and $\alpha3\beta4$ receptor subtypes, heterologously expressed in transfected nAChR-null mammalian cell lines, for all compounds. The binding assays were performed as previously described.[52] The $\alpha4\beta2$ assay was conducted in membranes prepared from a SH-EP1 human cell line expressing human $\alpha4$ and $\beta2$ subunits[53] using [^{3}H]-nicotine (L-(−)-[*N*-methyl-^{3}H]-nicotine, 69.5 Ci/mmol, Perkin-Elmer Life Sciences, Waltham, MA). The cell line was obtained from Dr. Ron Lukas (Barrow Neurological Institute). The affinity at the $\alpha7$ subtype was measured in a membrane preparation from a HEK/RIC3 cell line[54] expressing human subunits (Jon Lindstrom, U. Pennsylvania) using [^{3}H]-epibatidine (52 Ci/mmol, Perkin-Elmer Life Sciences) as the reference ligand. Affinity at the ganglionic subtype was measured in SH-SY5Y cells expressing human $\alpha3$ and $\beta4$ subunits using [^{3}H]-epibatidine as the radioligand.[55] Selected compounds were assayed for affinity at the $\alpha6$* subtype using [^{3}H]-epibatidine as the radioligand. For this assessment, a novel cell line expressing $\alpha6/3\beta2\beta3$ subtype nAChRs in a human SH-EP1 cell line was constructed. $\alpha6/3$ denotes a chimeric subunit composed of the extracellular, ligand-binding domain of the $\alpha6$ subunit fused to the first transmembrane domain and following sequence of the $\alpha3$ nAChR subunit; this approach reproducibly increases expression compared to that seen for native $\alpha6$ subunits while retaining $\alpha6$-like pharmacology.[56] Wild-type SH-EP1 cells were transfected with nAChR subunit clones using the cationic polymer Effectene (Qiagen, Valencia, CA). nAChR subunit genes optimized for vertebrate expression were synthesized by GeneArt, Inc. (Burlingame, CA) and were delivered in the pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA; pcDNA3.1zeo for $\alpha6/\alpha3$ subunit, pcDNA3.1hygro for the $\beta2$ subunit, pcDNA3.1neo for the $\beta3$ subunit). Triple transfectants expressing $\alpha6/3$, $\beta2$, and $\beta3$ subunits were selected for, using selection with zeocin (0.25 mg/mL, Invitrogen), hygromycinB (0.4 mg/mL, 0.13 mg/mL biologically active hygromycin, Calbiochem, San Diego, CA), and with G418 sulfate to a final concentration of 0.6 mg/mL (A.G. Scientific, San Diego, CA). The polyclonal pool of survivors from this selection round was then used to pick monoclones. Clones were screened for radioligand binding using [^{3}H]epibatidine binding and for function using $^{86}Rb^+$ efflux assays, and a clone exhibiting consistently high nAChR expression was selected. These cells were maintained in medium supplemented with zeocin, hygromycin, and G418 (described below) to maintain positive selection of transfectants, as low passage number (1−40 from our frozen stocks) cultures to ensure stable expression of phenotype. Cells were typically passaged once weekly by splitting just-confluent cultures 1/20−1/40 to maintain cells in proliferative growth.

The SH-EP1 cell lines and SH-SY5Y cells were maintained in a proliferative growth phase in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, California) with 10% horse serum (Invitrogen), 5% fetal bovine serum (HyClone, Logan UT), 1 mM sodium pyruvate, and 4 mM L-glutamine. For maintenance of stable transfectants, the $\alpha4\beta2$ cell medium was supplemented with 0.25 mg/mL zeocin and 0.13 mg/mL hygromycin B. The $\alpha6/3\beta2\beta3$ cell line medium was supplemented with zeocin and hygromycin B at the same concentration, with the addition of G418 at 0.6 mg/mL. HEK-human $\alpha7$/RIC3 cells were maintained in proliferative growth phase in Dulbecco's Modified Eagle's Medium (Invitrogen) with 10% fetal bovine serum (HyClone, Logan, UT), 1 mM sodium pyruvate, 4 mM L-glutamine, 0.6 mg/mL Geneticin, and 0.5 mg/mL zeocin.

Cells were harvested in ice-cold PBS, pH 7.4, then homogenized with a Polytron (Kinematica GmbH, Switzerland). Homogenates were

centrifuged at 40000*g* for 20 min (4 °C). The pellet was resuspended in PBS and protein concentration determined using the Pierce BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL). Membranes were reconstituted from frozen stocks and incubated for 2 h on ice in 150 μL of assay buffer (PBS) in the presence of competitor compound (0.001 nM to 100 μM) and radioligand. Incubation was terminated by rapid filtration on a multimanifold tissue harvester (Brandel, Gaithersburg, MD) using GF/B filters presoaked in 0.33% polyethyleneimine (w/v) to reduce nonspecific binding. Filters were washed three times with ice-cold PBS, and the retained radioactivity was determined by liquid scintillation counting. Binding data were expressed as percent total control binding. Replicates for each point were averaged and plotted against the log of drug concentration. The IC_{50} (concentration of the compound that produces 50% inhibition of binding) was determined by least-squares nonlinear regression using GraphPad Prism software (GraphPAD, San Diego, CA). K_i was calculated using the Cheng−Prusoff equation.[57]

Functional Assays. Functional response was evaluated at the $\alpha4\beta2$, $\alpha6/3\beta2\beta3$, and ganglionic subtypes using a fluorescence calcium flux assay (FLIPR Tetra). The $\alpha4\beta2$ response was evaluated in SH-EP1 cells expressing human subunits To determine independently the response at high sensitivity ($(\alpha4)2(\beta2)3$ stoichiometry) and low sensitivity ($(\alpha4)3(\beta2)2$ stoichiometry) forms of the receptor, temperature-induced upregulation was utilized to increase expression of the high sensitivity form. Thus, growing the cells at the standard 37°C temperature leads to a predominance of the LS form, while a reduced temperature of 29°C leads to an excess of the HS form. For determination of functional activity at the $\alpha6/3\beta2\beta3$ subtype, the SH-EP1 human $\alpha6/3\beta2\beta3$ cell line was employed. For determination of functional activity at the ganglionic subtype, the SH-SY5Y cell line expressing human $\alpha3$ and $\beta4$ subunits was employed. Then 24−48 h prior to each experiment, cells were plated in 96-well black-walled, clear-bottom plates (Corning, Corning, NY) at 60−100000 cells/well. On the day of the experiment, growth medium was gently removed, 200 μL of 1× FLIPR Calcium 4 Assay reagent (Molecular Devices, Sunnyvale, CA) in assay buffer (20 mM HEPES, 7 mM TRIS base, 4 mM $CaCl_2$, 5 mM D-glucose, 0.8 mM $MgSO_4$, 5 mM KCl, 0.8 mM $MgCl_2$, 120 mM *N*-methyl D-glucamine, and 20 mM NaCl, pH 7.4, for SH-EP1-human $\alpha4\beta2$ cells or 10 mM HEPES, 2.5 mM $CaCl_2$, 5.6 mM D-glucose, 0.8 mM $MgSO_4$, 5.3 mM KCl, and 138 mM NaCl, pH 7.4, with TRIS-base for all other cell lines) was added to each well and plates were incubated at 37 °C for 1 h (29 °C for the 29 °C-treated SH-EP1-human $\alpha4\beta2$ cells). The plates were removed from the incubator and allowed to equilibrate to room temperature. Plates were transferred to a FLIPR Tetra fluorometric imaging plate reader (Molecular Devices) for addition of compound and monitoring of fluorescence (excitation 485 nm, emission 525 nm). The amount of calcium flux was compared to both a positive (nicotine) and negative control (buffer alone). The positive control was defined as 100% response, and the results of the test compounds were expressed as a percentage of the positive control.

Dopamine release studies were performed using striatal synaptosomes obtained from rat brain as previously described.[58] Striatal tissue from two rats (female, Sprague−Dawley, weighing 150−250 g) was pooled and homogenized in ice-cold 0.32 M sucrose (5 mL) containing 5 mM HEPES, pH 7.4, using a glass/glass homogenizer. The tissue was then centrifuged at 1000*g* for 10 min. The pellet was discarded, and the supernatant was centrifuged at 12500*g* for 20 min. The resulting pellet was resuspended in ice-cold perfusion buffer containing monoamine oxidase inhibitors (128 mM NaCl, 1.2 mM KH_2PO_4, 2.4 mM KCl, 3.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM HEPES, 1 mM ascorbic acid, 0.02 mM pargyline HCl, and 10 mM glucose, pH 7.4) and centrifuged for 15 min at 23000*g*. The final pellet was resuspended in perfusion buffer (2 mL) for immediate use. The synaptosomal suspension was incubated for 10 min in a 37 °C shaking incubator to restore metabolic activity. [^{3}H]Dopamine ([^{3}H]DA, specific activity = 28.0 Ci/mmol, NEN Research Products) was added at a final concentration of 0.1 μM, and the suspension was incubated at 37 °C for another 10 min. Aliquots of perfusion buffer (100 μL) and tissue (100 μL) were loaded into the suprafusion chambers of a Brandel Suprafusion system (series 2500, Gaithersburg, MD). Perfusion buffer (room temperature) was pumped into the chambers at a rate of approximately 0.6 mL/min for a wash period of 8 min. Competitor compound (10 pM to 100 nM) was applied in the perfusion stream for 8 min. Nicotine (10 μM) was then applied in the perfusion stream for 48 s. Fractions (12 s each) were continuously collected from each chamber throughout the experiment to capture basal release and agonist-induced peak release and to re-establish the baseline after the agonist application. The perfusate was collected directly into scintillation vials to which scintillation fluid was added. Released [^{3}H]DA was quantified by scintillation counting. For each chamber, the integrated area of the peak was normalized to its baseline. [^{3}H]DA release was expressed as a percentage of release obtained with control nicotine in the absence of competitor. Within each assay, each test compound concentration was replicated using two chambers; replicates were averaged. The compound concentration resulting in half maximal inhibition (IC_{50}) of specific ion flux was defined.

Electrophysiology Materials and Methods. *GH4C1 Rat α7 Cells.* GH4C1, a stable cell line expressing the rat *a*7-nAChR subunit with a single mutation at T6′S[59] was used to measure whole-cell currents.

Subclonal Human Epithelial-*hα4β2*Cells. Established techniques were used to introduce human $\alpha4$ (S452) and $\beta2$ subunits (kindly provided by Dr. Ortrud Steinlein, Institute of Human Genetics, University Hospital, Ludwig-Maximilians-Universitat, Munich, Germany) and subcloned into pcDNA3.1-zeocin and pcDNA3.1-hygromycin vectors, respectively, into native NNR-null SHEP1 cells to create the stably transfected, monoclonal subclonal human epithelial (SH-EP1)-*hα4β2* cell line heterologously expressing human $\alpha4\beta2$ receptors. Cell cultures were maintained at low passage numbers (1−26 from frozen stocks to ensure the stable expression of the phenotype) in complete medium augmented with 0.5 mg/mL zeocin and 0.4 mg/mL hygromycin (to provide a positive selection of transfectants) and passaged once weekly by splitting the just-confluent cultures 1:20 to maintain cells in proliferative growth. Reverse transcriptase−polymerase chain reaction, immunofluorescence, radioligand-binding assays, and isotopic ion flux assays were conducted recurrently to confirm the stable expression of $\alpha4\beta2$ NNRs as message, protein, ligand-binding sites, and functional receptors, respectively.

Cell Handling. After removal from the incubator, the medium was aspirated, and cells were trypsinized for 3 min, washed thoroughly twice with recording medium, and resuspended in 2 mL of external solution. Cells were gently triturated to detach them from the plate and transferred into 4 mL test tubes from which cells were placed in the Dynaflow chip mount on the stage of an inverted Zeiss microscope (Carl Zeiss Inc., Thornwood, NY). On average, 5 min was necessary before the whole-cell recording configuration was established. To avoid modification of the cell conditions, a single cell was recorded per single load. To evoke short responses, agonists were applied using a Dynaflow system (Cellectricon, Inc., Gaithersburg, MD), where each channel delivered pressure-driven solutions at either 50 or 150 psi.

Electrophysiology. Conventional whole-cell current recordings, together with a computer-controlled Dynaflow system (Cellectricon, Inc.) for fast application and removal of agonists, were used in these studies. In brief, the cells were placed in a silicon chip bath mount on an inverted microscope (Carl Zeiss Inc.). Cells chosen for analysis were continuously perfused with standard external solution (60 μL/min). Glass microelectrodes (3−5 MΩ resistance between the pipet and extracellular solutions) were used to form tight seals (>1 GΩ) on the cell surface until suction was applied to convert to conventional whole-cell recording. The cells were then voltage-clamped at holding potentials of −60 mV, and ion currents in response to application of ligands were measured. On average, the whole-cell recording stabilized within <5 min. Responses were evoked by placing the cell in front of the agonist-containing channel for 1 s (for $\alpha4\beta2$) and 0.5 s (for $\langle\alpha7$) and 30 s washout between applications. Whole-cell currents recorded with an Axon 700A amplifier were filtered at 1 kHz and sampled at 5 kHz by an ADC board 1440 (Molecular Devices) and stored on the hard disk of a PC computer. Whole-cell access resistance was less than

20 MΩ. Data acquisition of whole-cell currents was done using a Clampex 10 (Molecular Devices, Sunnyvale, CA), and the results were plotted using Prism 5.0 (GraphPad Software Inc., San Diego, CA). The experimental data are presented as the mean ± SEM, and comparisons of different conditions were analyzed for statistical significance using Student's *t* tests. All experiments were performed at room temperature (22 ± 1 °C). Concentration−response profiles were fit to the Hill equation and analyzed using Prism 5.0. No differences in the fraction of responsive cells could be detected among experimental conditions. More than 90% of the cells responded to acetylcholine (ACh), and every cell presenting a measurable current was taken into account. Neuronal $\alpha4\beta2$ receptor dose−response curves could be described by the sum of two empirical Hill equations comparable with methods described previously:[60]

$$y = I_{max}\frac{\alpha 1}{1+\left(\frac{EC_{50H}}{x}\right)^{nH1}} + \frac{1-a1}{1+\left(\frac{EC_{50L}}{x}\right)^{nH2}} \quad (1)$$

where I_{max} is the maximal current amplitude, and x is the agonist concentration. EC_{50H}, nH1, and a1 are the half-effective concentration, the Hill coefficient, and the percentage of receptors in the HS state. EC_{50L} and nH2 are the half-effective concentration and the Hill coefficient in the LS state. A single Hill equation,

$$y = I_{max}\frac{1}{\left(1+\frac{EC_{50}}{x}\right)^{nH}} \quad (2)$$

was used for comparison of the fit with eq 1. I_{max}, EC_{50}, and nH have the same meanings. Single Hill equation was used for all $\alpha7$ receptors and for $\alpha4\beta2$ receptors when appropriate. Data are expressed relative to maximal acetylcholine response.

Solutions and Drug Application. The standard external solution contained: 120 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 25 mM D-glucose, and 10 mM HEPES and was adjusted to pH 7.4 with Tris base. In the experiments, ACh was applied as an agonist without atropine because literature data indicate that 1 μM atropine sulfate did not affect ACh-induced currents and because atropine itself has been reported to block nicotinic receptors.[61] For all conventional whole-cell recordings, Tris electrodes were used and filled with solution containing: 110 mM Tris phosphate dibasic, 28 mM Tris base, 11 mM EGTA, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 4 mM Mg-ATP, pH 7.3. To initiate whole-cell current responses, nicotinic agonists were delivered by placing cells from the control solution to agonist-containing solution and back so that solution exchange occurred within 10−50 ms (based on 10−90% peak current rise times). Intervals between drug applications (0.5−1 min) were adjusted specifically to ensure the stability of receptor responsiveness (without functional rundown), and the selection of pipet solutions used in most of the studies described here was made with the same objective. The reagents used in the present studies, including ACh, were purchased from Sigma-Aldrich (St. Louis, MO). All standards were prepared daily from stock solutions.

Chemistry. *General.* Unless otherwise specified, all reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed on prepacked columns supplied by Analogix. Preparative HPLC separations were performed on a Gilson 215 liquid handler using a gradient of acetonitrile and water containing 0.1% TFA or a gradient of methanol/2-propanol in water containing 0.1% TFA. The purity of all final compounds was determined to be >95% by analytical LCMS on a Waters 2695 separation module running an acetonitrile−water gradient, paired with both a 2996 diode array detector and micromass ZQ mass spectrometer run in electrospray positive ionization mode. Proton NMR spectra were recorded on a Varian VNMR at 300 or 400 MHz in the solvent indicated.

Separation of Enantiomers. In cases where individual enantiomers were desired, racemic material was separated by chiral supercritical fluid chromatography (SFC) on a ChiralPak AD-H 50 mm × 250 mm column, eluting with 70% CO_2 and 30% of a mixture of 75% ethanol/25% methanol/0.5% diethylamine at a flow rate of 250 g/min. Chiral purity was determined by HPLC on a ChiralPak AD column (4.6 mm × 250 mm, 10 μm), eluting with IPA/EtOH (70:30 V/V) containing 0.1% diethylamine at a flow rate of 2 mL/min (monitored by UV at 250 nM). Enantiomers exhibited a chiral purity of >95%. Optical rotation was determined for each enantiomer. Absolute assignment of stereochemistry was made only for (+)-**11a**. This was determined by single crystal X-ray diffraction on the *p*-bromobenzamide derivative, which allowed assignment as (1*R*,5*S*) with a quality factor of 1 (R_1 = 4.49%).

General Method A: Suzuki Coupling with Vinyl Triflate ***13*** *Followed by Deprotection.* To *tert*-butyl 7-(trifluoromethylsulfonyloxy)-3-azabicyclo[3.3.1]non-7-ene-3-carboxylate **13**, the pyridylboronic acid (1.2 equiv), $Pd(OAc)_2$ (0.02 equiv), and 1,1′-bis-(diphenylphosphino)ferrocene (0.02 equiv) were added and the solids dissolved in 1,2-dimethoxyethane (~10 mL/mmol substrate). Potassium carbonate (3 equiv) was added, and the reaction was degassed (prime/purge with N_2). The solution was heated under reflux for 4 h, cooled, and poured in to H_2O. The mixture was extracted with Et_2O, and the combined organic layers dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified on an Analogix silica gel column, eluting with a gradient of hexane to 4:1 Hex/EtOAc. A solution of the resulting BOC-protected product was taken up in 1:1 dichloromethane/trifluoroacetic acid (~1 mL/mmol). The reaction mixture was stirred at 25 °C for 16 h and then concentrated to dryness and partitioned between saturated sodium bicarbonate and dichloromethane. The aqueous phase was extracted three times with dichloromethane, and the organic phases combined, dried over Na_2SO_4, filtered, and concentrated to dryness. Products were repurified by reverse phase HPLC as necessary to achieve >95% purity.

General Method B: Suzuki Couplings with Vinyl Boronate ***14*** *Followed by Deprotection.* To 0.1 mL of 0.5 M $Pd(OAc)_2$ in DME in a screw cap vial was added 0.1 mL of a solution of 0.5 M DPPF in DME, 0.5 mL of a 1 M solution of *tert*-butyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-azabicyclo[3.3.1]non-7-ene-3-carboxylate **14** in DME, 0.625 mL of 2N potassium carbonate in water, and the appropriate bromopyridine (0.6 mL of a 1 M solution in DME). The vial was capped, and the mixture was shaken with heating at 106 °C for ~15 h. Samples were cooled to ambient temperature and diluted with 1.5 mL of water and 1.5 mL of ethyl acetate. Following extraction, the layers were separated by centrifugation and the organic layer concentrated to dryness. The residue was taken up in 1 mL of dichloromethane and treated with 0.5 mL of TFA. After 1 h, the mixture was extracted with 2 × 1 mL of water, and the aqueous layers combined and concentrated to dryness. The residue was purified by reverse phase HPLC to give the products as trifluoroacetate salts unless otherwise noted.

General Method C: N-Methylation of Free Base. To the appropriate free base was added formic acid (88%, 2 mL/mmol) and an aqueous solution of formaldehyde (37%, 2 mL/mmol) and the mixture heated under reflux with stirring until completion of reaction. Aliquots were examined by LC/MS for complete conversion to *N*-methylated product. The reaction mixture was cooled and concentrated to give a dark-brown oily residue and off-white solid residue of paraformaldehyde. This was triturated with hot methanol and filtered, and the filtrate was concentrated. The resulting residue was partitioned between dichloromethane and 10% aqueous sodium hydroxide and the aqueous layer extracted again with dichloromethane. The extracts were combined, washed with brine, dried over sodium sulfate, filtered, and concentrated to give products which were typically further purified by reverse phase HPLC to give final compounds as TFA salts unless otherwise indicated.

*7-(Pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**11a**).* Prepared according to general method A. Compound **14a** was obtained as an off-white solid (16.4 g; 97%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, J = 2 Hz, 1H), 8.54 (m, 2H), 7.90 (dd, J = 5.8 Hz, 8.2 Hz, 1H), 6.49 (d, J = 6.3 Hz, 1H), 3.27 (d, J = 13 Hz, 1H), 3.19−3.11 (m, 3H), 2.9−2.81 (m, 2H), 2.48−2.43 (m, 2H), 1.94 (d, J = 13 Hz, 1H), 1.72 (d, J = 13.3 Hz, 1H). HRMS (ES) m/z Calcd for $C_{13}H_{16}N_2$ + H: 201.1392. Found: 201.1394.

*(1R,5S)-7-(Pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((+)-**11a**).* Prepared according to general method A. ^{1}H NMR (400 MHz, CD_3OD) δ 9.01 (br s, 1H), 8.78 (s, 1H), 8.76 (s, 1H), 8.10 (br s, 1H), 6.65 (d, J = 4.2 Hz, 1H), 3.43 (d, J = 12.9 Hz, 1H), 3.35−3.25 (m, 3H), 3.01 (dd, J = 6.3 Hz, 18 Hz, 1H), 2.93 (s, 1H), 2.69 (d, J = 18 Hz, 1H), 2.58 (s, 1H), 2.05 (d, J = 13 Hz, 1H), 1.94 (d, J = 13.3 Hz, 1H). ^{13}C NMR (400 MHz, D_2O) δ142.8, 139.5, 139.3, 137.8, 135.2, 129.9, 126.9, 49.9, 45.7, 31.0, 27.3, 26.1, 24.8. HRMS (ES) m/z Calcd for $C_{13}H_{16}N_2$ + H: 201.1392. Found: 201.1389. $[\alpha]_D^{20}$ = +36.70° (c = 1.0, MeOH).

*(1S,5R)-7-(Pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((−)-**11a**).* Prepared according to general method A. ^{1}H NMR (400 MHz, CD_3OD) δ 9.01 (br s, 1H), 8.78 (s, 1H), 8.76 (s, 1H), 8.10 (br s, 1H), 6.65 (d, J = 4.2 Hz, 1H), 3.43 (d, J = 12.9 Hz, 1H), 3.35−3.25 (m, 3H), 3.01 (dd, J = 6.3 Hz, 18 Hz, 1H), 2.93 (s, 1H), 2.69 (d, J = 18 Hz, 1H), 2.58 (s, 1H), 2.05 (d, J = 13 Hz, 1H), 1.94 (d, J = 13.3 Hz, 1H). HRMS (ES) m/z Calcd for $C_{13}H_{16}N_2$ + H: 201.1392. Found: 201.1394. $[\alpha]_D^{20}$ = −35.83° (c = 1.0, MeOH).

*7-(5-Methoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt (**11b**).* Prepared according to general method A. ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (d, J = 1.5 Hz, 1H), 8.27 (d, J = 2.8 Hz, 1H), 8.05 (t, J = 2 Hz, 1H), 6.50 (d, J = 6.3 Hz, 1H), 3.91 (s, 3H), 3.30 (d, J = 13 Hz, 1H), 3.25−3.15 (m, 3H), 2.95−2.8 (m, 2H), 2.5−2.45 (m, 2H), 1.85 (q, J = 13 Hz, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{18}N_2O$ + H: 231.1497. Found: 231.1507.

*7-(5-Isopropoxy-pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt (**11c**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.33 (s, 1H), 8.22 (s, 1H), 7.98 (t, J = 2.3 Hz, 1H), 6.47 (d, J = 6.3 Hz, 1H), 4.78−4.68 (m, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H), 1.27 (d, J = 5.8 Hz, 6H). HRMS (ES) m/z Calcd for $C_{16}H_{22}N_2O$ + H: 259.1810. Found: 259.1815.

*7-(5-Phenoxy-pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt (**11d**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (d, J = 1.6 Hz, 1H), 8.28 (d, J = 2.7 Hz, 1H), 8.04 (t, J = 2 Hz, 1H), 7.43 (t, J = 8 Hz, 2H), 7.27 (t, J = 8 Hz, 1H), 7.14 (d, J = 1.8 Hz, 2H), 6.42 (d, J = 6.7 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{19}H_{20}N_2O$ + H: 293.1654. Found: 293.1665.

*7-(5-(Benzyloxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11e**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 8.24 (s, 1H), 7.58 (s, 1H), 7.47−7.31 (m, 5H), 6.37 (d, J = 5.3 Hz, 1H), 5.22 (s, 2H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{20}H_{22}N_2O$ + H: 307.1810. Found: 307.1811.

*7-(6-Chloro-5-methoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11f**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 1.7 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 6.39 (d, J = 6.7 Hz, 1H), 3.97 (s, 3H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{14}H_{17}ClN_2O$ + H: 265.1108. Found: 265.1103.

*7-(5-Fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11g**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.62 (d, J = 2 Hz, 1H), 8.58 (m, 1H), 8.38 (m, 1H), 6.52 (d, J = 6.7 Hz, 1H), 3.26−3.08 (m, 4H), 2.82 (dd, J = 6.2 Hz, 19.2 Hz, 2H), 2.43 (s, 1.5H), 2.38 (s, 0.5 H), 1.83−1.72 (m, 2H). MS (ES) m/z 219 (M + H)$^+$. HRMS (ES) m/z Calcd for $C_{13}H_{15}FN_2$ + H: 219.1298. Found: 219.1295.

*(+)-7-(5-Fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((+)-**11g**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.91 (br s, 1H), 8.47 (d, J = 9 Hz, 1H), 6.66 (d, J = 6.2 Hz, 1H), 3.42 (d, J = 13 Hz, 1H), 3.35 (m, 3H), 2.99 (dd, J = 6.2 Hz, 18.3 Hz, 1H), 2.92 (br s, 1H), 2.65 (d, J = 19 Hz, 1H), 2.57 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.93 (d, J = 12.9 Hz, 1H). $[\alpha]_D^{20}$ = +35.42° (c = 1.0, MeOH). HRMS (ES) m/z Calcd For $C_{13}H_{15}FN_2$ + H: 219.1298. Found: 219.1291.

*(−)-7-(5-Fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((−)-**11g**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.91 (br s, 1H), 8.47 (d, J = 9 Hz, 1H), 6.66 (d, J = 6.2 Hz, 1H), 3.42 (d, J = 13 Hz, 1H), 3.35 (m, 3H), 2.99 (dd, J = 6.2 Hz, 18.3 Hz, 1H), 2.92 (br s, 1H), 2.65 (d, J = 19 Hz, 1H), 2.57 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.93 (d, J = 12.9 Hz, 1H). $[\alpha]_D^{20}$ = −31.85° (c = 1.0, MeOH). HRMS (ES) m/z Calcd for $C_{13}H_{15}FN_2$ + H: 219.1298. Found: 219.1292.

*7-(5-Chloropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11h**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.48 (d, J = 2.4 Hz, 1H), 8.65 (d, J = 2.4 Hz, 1H), 7.99 (t, J = 2 Hz, 1H), 6.42 (d, J = 6.3 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{13}H_{15}ClN_2$ + H: 235.1002. Found: 235.1004.

*7-(5-Cyanopyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**11i**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.95 (d, J = 2 Hz, 1H), 8.82 (d, J = 2 Hz, 1H), 8.29 (t, J = 2 Hz, 1 H), 6.47 (d, J = 6 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{14}H_{15}N_3$ + H: 226.1344. Found: 226.1343.

*(+)-7-(5-Cyanopyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((+)-**11i**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (br s, 2H), 8.62 (s, 1H), 6.58 (d, J = 6 Hz, 1H), 3.40 (d, J = 12 Hz, 1H), 3.31 (m, 3H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.91 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.56 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.93 (d, J = 12.9 Hz, 1H). $[\alpha]_D^{20}$ = +31.69° (c = 1.0, MeOH). HRMS (ES) m/z Calcd for $C_{14}H_{15}N_3$ + H: 226.1344. Found: 226.1338.

*(−)-7-(5-Cyanopyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt ((−)-**11i**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 9.17 (br s, 2H), 8.62 (s, 1H), 6.58 (d, J = 6 Hz, 1H), 3.40 (d, J = 12 Hz, 1H), 3.31 (m, 3H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.91 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.56 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.95 (d, J = 12.9 Hz, 1H). $[\alpha]_D^{20}$ = −28.44° (c = 1.0, MeOH). HRMS (ES) m/z Calcd for $C_{14}H_{15}N_3$ + H: 226.1344. Found: 226.1337.

*7-(5-(Trifluoromethyl)pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**11j**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.91 (s, 1H), 8.80 (s, 1H), 8.21 (s, 1H), 6.47 (d, J = 6.3 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.02 (d, J = 12.5 Hz, 1H), 1.95 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{14}H_{15}F_3N_2$ + H: 269.1266. Found: 269.1266.

*7-(5-Phenylpyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, HCl Salt (**11k**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.81 (s, 1H), 8.70 (d, 1.5 Hz, 2H), 7.69−7.62 (m, 2H), 7.54−7.46 (m, 3 H), 6.56 (d, J = 6.2 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{19}H_{20}N_2$ + H: 277.1705. Found: 277.1710.

*7-(6-Fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**11l**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (s. 1H), 8.08 (t, J = 7.4 Hz, 1H), 7.07 (dd, J = 8.2, 2.4 Hz, 1H), 6.32 (d, J = 6.1 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{13}H_{15}FN_2$ + H: 219.1298. Found: 219.1295.

*7-(6-Chloropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11m**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.48 (d, J = 2 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 6.38 (d, J = 5.8 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{13}H_{15}ClN_2$ + H: 235.1002. Found: 235.0996.

dx.doi.org/10.1021/jm3011299 | J. Med. Chem. 2012, 55, 9929−9945

7-(6-Methoxypyridin-3-yl)-3-aza-bicyclo[3.3.1]non-6-ene (**11n**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 7.84 (dd, J = 8.6, 2.7 Hz, 1H), 6.8 (d, J = 8.6 Hz, 1H), 6.23 (d, J = 6.7 Hz, 1H), 3.91 (s, 3H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 1.92 (dd, J = 12.3, 31.8 Hz, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{18}N_2O$ + H: 231.1497. Found: 231.1491.

7-(6-Methylpyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11o**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.69 (d, J = 1.6 Hz, 1H), 8.39 (dd, J = 2.3, 8.2 Hz, 1H), 7.74 (d, J = 8.6 Hz, 1H), 6.52 (d, J = 5.9 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.68 (s, 3H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{14}H_{18}N_2$ + H: 215.1548. Found: 215.1544.

7-(6-Phenylpyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, Free Base (**11p**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, J = 1.8 Hz, 1H), 8.51 (s, 1H), 8.0 (dd, J = 2.3, 8.7 Hz, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.85 (d, J = 8.2 Hz, 1H), 7.51−7.42 (m, 3H), 6.43 (d, J = 6.3 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 1.99 (q, J = 12.9 Hz, 2H). HRMS (ES) m/z Calcd for $C_{19}H_{20}N_2$ + H: 277.1705. Found: 277.1703.

3-(3-Azabicyclo[3.3.1]non-6-en-7-yl)-isonicotinonitrile (**11r**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.92 (s, 1H), 8.69 (d, J = 4.6 Hz, 1H), 7.76 (d, J = 5.1 Hz, 1H), 6.29 (d, J = 6.6 Hz, 1H), 3.36 (dd, J = 12.5, 46 Hz, 2H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (s, 1H), 2.65−2.7−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd For $C_{14}H_{15}N_3$ + H: 226.1344. Found: 226.1334.

7-(5-(2-Methoxyphenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11s**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 7.38 (s, 1H), 7.27−7.02 (m, 4H), 6.31 (d, J = 6.6 Hz, 1H), 3.77 (s, 3H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd For $C_{20}H_{22}N_2O_2$ + H: 323.1760. Found: 323.1762.

7-(5-(3-Methoxyphenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11t**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 7.56 (s, 1H), 7.32 (t, J = 8.2 Hz, 1H), 6.77 (d, J = 6.6 Hz, 1H), 6.62−6.59 (m, 2H), 6.36 (d, J = 6.3 Hz, 1H), 3.79 (s, 3H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{20}H_{22}N_2O_2$ + H: 323.1760. Found: 323.1755.

7-(5-(4-Methoxyphenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11u**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H), 8.15 (s, 1H), 7.45 (s, 1H), 7.01 (dd, J = 9, 23 Hz, 4H), 6.32 (d, J = 5.5 Hz, 1H), 3.80 (s, 3H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{20}H_{22}N_2O_2$ + H: 323.1760. Found: 323.1755.

7-(5-(2-Chlorophenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11v**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H), 8.14 (s, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.46 (s, 1H), 7.38 (m, 1H), 7.26 (m, 1H), 7.18 (d, J = 8.2 Hz, 1H), 6.35 (d, J = 6.3 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{19}H_{19}ClN_2O$ + H: 327.1264. Found: 327.1263.

7-(5-(3-Chlorophenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11w**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 1H), 8.24 (d, J = 3 Hz, 1H), 7.58 (s, 1H), 7.38 (s, 1H), 7.19 (d, J = 7.8 Hz, 1H), 7.06 (t, J = 2 Hz, 1H), 6.98 (d, J = 8.3 Hz, 1H), 6.36 (d, J = 5.5 Hz, 1H), 3.40−3.28 (m, 4H), 2.92−2.87 (m, 1H), 2.82 (s, 1H), 2.58−2.49 (m, 2H), 1.96 (q, J = 12 Hz, 2H). HRMS (ES) m/z Calcd for $C_{19}H_{19}ClN_2O$ + H: 327.1264. Found: 327.1259.

7-(5-(4-Chlorophenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11x**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H), 8.22 (s, 1H), 7.54 (s, 1H), 7.39 (d, J = 9 Hz, 2H), 7.05 (d, J = 9 Hz, 2H), 6.35 (d, J = 6.6 Hz, 1H), 3.40−3.26 (m, 3H), 2.95−2.75 (m, 2H), 2.6−2.45 (m, 2H), 2.25−2.15 (m, 1H), 1.95−1.90 (m, 2H). HRMS (ES) m/z Calcd for $C_{19}H_{19}ClN_2O$ + H: 327.1264. Found: 327.1257.

7-(5-(4-Fluorophenoxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**11y**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.44 (d, J = 2.7 Hz, 1H), 8.18 (d, J = 2.7 Hz, 1H), 7.49 (d, J = 2.3 Hz, 1H), 7.17−7.07 (m, 4H), 6.33 (d, J = 6.3 Hz, 1H), 3.40−3.26 (m, 4H), 2.95−2.76 (m, 21H), 2.56−2.45 (m, 2H), 1.95−1.84 (m, 2H). HRMS (ES) m/z Calcd for $C_{19}H_{19}FN_2O$ + H: 311.1560. Found: 311.1548.

(1R,5S)-3-Methyl-7-(pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, Monofumarate ((+)-**12a**). Prepared according to general method C. The product obtained from Suzuki coupling (0.95 g, 89%) was taken up in 8 mL of warm THF and added all at once to a solution of fumaric acid (5 mmol, 0.58 g) in 10 mL of hot THF. The product precipitated immediately as an off-white solid. After cooling for 1 h, the solvent was decanted and the residue taken up in a minimum amount of hot ethanol (∼15 mL) and allowed to cool slowly. The crystalline solid was filtered and washed with cold ethanol, then ether, and dried in vacuo to give 0.9 g of monofumarate. ^{1}H NMR (400 MHz, CD_3OD) δ 8.69 (br s, 1H), 8.46 (br s, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.43 (br s, 1H), 6.60 (s, 2H), 6.43 (d, J = 6.3 Hz, 1H), 3.55 (d, J = 12.5 Hz, 1H), 3.46 (d, J = 12 Hz, 1H), 3.22 (dd, J = 3 Hz, 12.5 Hz, 1H), 3.17 (dd, J = 3.6 Hz, 12.1 Hz, 1H), 2.94 (dd, J = 12.5 Hz, 19.5 Hz, 1H), 2.89 (br s, 1H), 2.81 (s, 3H), 2.67 (d, J = 19.4 Hz, 1H), 2.57 (br s, 1H), 1.97−1.89 (m, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{18}N_2$ + H: 215.1548. Found: 215.1544. $[\alpha]_D^{20}$ = +22.27° (c = 1.0, MeOH).

(1S,5R)-3-Methyl-7-(pyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene, Monofumarate ((−)-**12a**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.69 (br s, 1H), 8.46 (br s, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.43 (br s, 1H), 6.60 (s, 2H), 6.43 (d, J = 6.3 Hz, 1H), 3.55 (d, J = 12.5 Hz, 1H), 3.46 (d, J = 12 Hz, 1H), 3.22 (dd, J = 3 Hz, 12.5 Hz, 1H), 3.17 (dd, J = 3.6 Hz, 12.1 Hz, 1H), 2.94 (dd, J = 12.5 Hz, 19.5 Hz, 1H), 2.89 (br s, 1H), 2.81 (s, 3H), 2.67 (d, J = 19.4 Hz, 1H), 2.57 (br s, 1H), 1.97−1.89 (m, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{18}N_2$ + H: 215.1548. Found: 215.1540. $[\alpha]_D^{20}$ = −23.07° (c = 1.0, MeOH).

3-Methyl-7-(5-methoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12b**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.30 (s, 1H), 7.88 (s, 1H), 7.41 (s, 1H), 6.27 (d, J = 6.3 Hz, 1H), 3.91 (s, 3H), 3.54−3.43 (m, 2H), 3.23−3.18 (m, 2H), 2.93−2.88 (m, 2H), 2.83 (s, 3H), 2.64−2.56 (m, 2H), 1.98−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{15}H_{20}N_2O$ + H: 245.158. Found: 245.162.

3-Methyl-7-(5-isopropoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12c**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 8.15 (s, 1H), 7.51 (s, 1H), 6.41 (d, J = 6.6 Hz, 1H), 3.54−3.43 (m, 3H), 3.23−3.18 (m, 2H), 2.93−2.88 (m 2H), 2.82 (s, 3H), 2.64−2.56 (m, 2H), 1.98−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{17}H_{24}N_2O$ + H: 273.1967. Found: 273.1958.

3-Methyl-7-(5-phenoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12d**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.44 (s, 1H), 8.18 (d, J = 2.3 HZ, 1H), 7.56 (s, 1H), 7.41 (m, 2H), 7.19 (m, 1H), 7.07 (m, 2H), 6.36 (dd, J = 14.1 Hz, 5.5 Hz, 1H), 3.54−3.43 (m, 3H), 3.23−3.18 (m, 2H), 2.93−2.88 (m 2H), 2.82 (s, 3H), 2.64−2.56 (m, 2H), 1.98−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{20}H_{22}N_2O$ + H: 307.1810. Found: 307.1800.

3-Methyl-7-(5-(benzyloxy)pyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12e**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H), 8.31 (d, J = 1.9 Hz, 1H), 7.72 (s, 1H), 7.48−7.46 (m, 2H), 7.41−7.32 (m, 3H), 6.46 (d, J = 6.7 Hz, 1H), 5.25 (s, 2H), 3.55 (d, 11.9 Hz, 1H), 3.47 (d, J = 12 Hz, 1H), 3.34−3.20 (m, 2H), 2.89 (br s, 2H), 2.83 (s, 3H), 2.59 (br s, 2H), 1.98−1.89 (m, 2H). HRMS (ES) m/z Calcd for $C_{21}H_{24}N_2O$ + H: 321.1967. Found: 321.1962.

3-Methyl-7-(6-chloro-5-methoxypyridin-3-yl)-3-azabicyclo[3.3.1]-non-7-ene (**12f**). Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 1.7 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 6.41 (d, J = 6.6 Hz, 1H), 3.97 (s, 3H), 3.40−3.26 (m, 4H), 2.97

(dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.82 (s, 3H), 2.64 (d, J = 18.3 Hz, 1H), 2.54 (br s, 1H), 1.97−1.91 (m, 2H). HRMS (ES) m/z Calcd for $C_{15}H_{19}ClN_2O$ + H: 279.1264. Found: 279.1273.

*3-Methyl-7-(5-fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12g**).* Prepared according to general method C. Obtained 1.5 g, 79% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.54 (s, 1H), 8.38 (s, 1H), 7.74 (d, J = 10.4 Hz, 1H), 6.47 (d, J = 6.3 Hz, 1H), 3.52 (d, 13.3 Hz, 1H), 3.45 (d, J = 13.3 Hz, 1H), 3.22−3.19 (m, 2H), 2.9−2.83 (m, 3H), 2.82 (s, 3H), 2.59 (m, 2H), 1.93−1.91 (m, 2H). HRMS (ES) m/z Calcd For $C_{14}H_{17}FN_2$ + H: 233.1454. Found: 233.1447.

*3-Methyl-7-(5-chloropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12h**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.5 (d, J = 2.1 Hz, 1 H), 8.46 (d, J = 2 Hz, 1H), 7.97 (d, J = 1.9 Hz, 1H), 6.44 (d, J = 5.9 Hz, 1H), 3.52 (d, 11.8 Hz, 2H), 3.43 (d, J = 11 Hz, 2H), 3.22−3.18 (m, 2H), 2.95−2.89 (m, 2H), 2.82 (s, 3H), 2.64−2.58 (m, 2H), 1.96−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{17}ClN_2$ + H: 249.1159. Found: 249.1150.

*5-(3-Methyl-3-azabicyclo[3.3.1]non-7-en-7-yl)nicotinonitrile (**12i**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.92 (d, J = 2 Hz, 1 H), 8.81 (s, 1H), 8.26 (d, J = 2 Hz, 1H), 6.49 (d, J = 6.6 Hz, 1H), 3.52 (d, 11 Hz, 2H), 3.43 (d, J = 11 Hz, 2H), 3.21−3.17 (m, 2H), 2.95−2.89 (m, 2H), 2.82 (s, 3H), 2.64−2.58 (m, 2H), 1.96−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{15}H_{17}N_3$ + H: 240.1501. Found: 240.1496.

*3-Methyl-7-(6-fluoropyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**12l**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1 H), 8.05 (m, 1H), 7.06 (d, J = 5.9 Hz, 1H), 6.34 (d, J = 6.6 Hz, 1H), 3.52 (d, 11 Hz, 2H), 3.43 (d, J = 11 Hz, 2H), 3.21−3.17 (m, 2H), 2.95−2.89 (m, 2H), 2.82 (s, 3H), 2.64−2.58 (m, 2H), 1.96−1.88 (m, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{17}FN_2$ + H: 233.1454. Found: 233.1445.

*3-Methyl-7-(6-chloropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12m**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (d, J = 2.3 Hz, 1 H), 7.93 (d, J = 2.3 Hz, 1H), 7.43 (d, J = 8.2 Hz, 1H), 6.41 (d, J = 5.8 Hz, 1H), 3.52 (d, 12.5 Hz, 1H), 3.45 (d, J = 12.5 Hz, 1H), 3.22−3.17 (m, 2H), 2.90−2.85 (m, 3H), 2.81 (s, 3H), 2.63 (s, 1H), 2.58 (s, 1H), 1.92−1.87 (m, 2H). HRMS (ES) m/z Calcd for $C_{14}H_{17}ClN_2$ + H: 249.1159. Found: 249.1150.

*3-Methyl-7-(6-methoxypyridin-3-yl)-3-azabicyclo[3.3.1]non-6-ene (**12n**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (d, J = 2.3 Hz, 1 H), 7.83 (dd, J = 8.6 Hz, 2.3 Hz, 1H), 6.78 (d, J = 9 Hz, 1H), 6.25 (d, J = 5.5 Hz, 1H), 3.88 (s, 3H), 3.52 (d, 12.5 Hz, 1H), 3.45 (d, J = 12.5 Hz, 1H), 3.22−3.17 (m, 2H), 2.90−2.85 (m, 3H), 2.81 (s, 3H), 2.63 (s, 1H), 2.58 (s, 1H), 1.92−1.87 (m, 2H). HRMS (ES) m/z Calcd for $C_{15}H_{20}N_2O$ + H: 245.1654. Found: 245.1645.

*3-Methyl-7-(4-chloropyridin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**12q**).* Prepared according to general method C. HRMS (ES) m/z Calcd for $C_{14}H_{17}ClN_2$ + H: 249.1159. Found: 249.1154.

*3-(3-Methyl-3-azabicyclo[3.3.1]non-7-en-7-yl)isonicotinonitrile (**12r**).* Prepared according to general method C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (s, 1H), 8.67 (d, J = 5 Hz, 1H), 7.74 (d, J = 5 Hz, 1H), 6.31 (d, J = 6.3 Hz, 1H), 3.60 (d, J = 11.8 Hz, 1H), 3.49 (d, J = 11.3 Hz, 1H), 3.06−2.94 (m, 2H), 2.88 (s, 3H), 2.65−2.57 (m, 2H), 1.98−1.94 (m, 2H). HRMS (ES) m/z Calcd for $C_{15}H_{17}N_3$ + H: 240.1501. Found: 240.1505.

*tert-Butyl 7-(Trifluoromethylsulfonyloxy)-3-azabicyclo[3.3.1]non-7-ene-3-carboxylate (**13**).* A solution of lithium bis(trimethylsilyl)-amide (1.05 M in THF, 150 mmol, 142.3 mL) in THF (300 mL) was cooled to −78 °C, and a solution of *tert*-butyl 7-oxo-3-azabicyclo-[3.3.1]nonane-3-carboxylate **19** (30 g, 125.4 mmol) in THF (150 mL) was added dropwise, maintaining the internal temperature of the reaction below −60 °C. The reaction was stirred for 30 min at −78 °C, and then a solution of *N*-phenylbis(trifluoromethanesulphonimide) (138 mmol, 49.3 g) in THF (150 mL) was added dropwise over 1 h. The reaction mixture was allowed to stir for a further 30 min at −78 °C, followed by gradual warming to −10 °C. The mixture was stirred at this temperature for 3 h and quenched by pouring into 750 mL of brine. The organic phase was collected, and the aqueous phase was extracted with ether (3 × 75 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was purified in three portions on a 400 g Analogix column (Basic Alumina, Act. II−III), eluting with hexane. The appropriate fractions were concentrated to obtain the desired triflate (37.2 g; 80%) as a white, crystalline solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (dd, J = 7.7, 17.3 Hz, 1H), 4.27 (d, J = 13.3 Hz, 0.5H), 4.12 (d, J = 13.3 Hz, 0.5 H), 4.04 (d, J = 13.3 Hz, 0.5 H), 3.88 (d, J = 12.9 Hz, 0.5H), 2.94 (t, J = 15.3 Hz, 1H), 2.83 (t, J = 11.7 Hz, 1H), 2.69−2.55 (m, 2H), 2.33 (t, J = 18.7 Hz, 1H), 2.18 (br m, 1H), 1.81−1.69 (m, 2H), 1.45 (s, 4.5 H), 1.42 (s, 4.5 H). MS (ES) m/z 371 $(M + H)^+$.

*tert-Butyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-azabicyclo[3.3.1]non-7-ene-3-carboxylate (**14**).* To *tert*-butyl 7-(trifluoromethylsulfonyloxy)-3-azabicyclo[3.3.1]non-7-ene-3-carboxylate **13** (20 g, 53.9 mmol) in dioxane (320 mL) were added 1,1′-bis(diphenylphosphino)ferrocene (2.69 mmol, 1.49 g), (1,1′-bis-(diphenylphosphino)ferrocene)palladium(II) chloride (2.69 mmol, 2.20 g), and potassium acetate (161.6 mmol, 15.9 g). The mixture was degassed with nitrogen and heated at 80 °C for 16 H. The reaction was cooled, poured into brine (300 mL), and extracted with EtOAc (3 × 150 mL). The organic phase was dried over sodium sulfate, filtered, and concentrated to dryness. The crude reaction was chromatographed in three portions on a 200 g Analogix silica gel column, eluting with 100% hexane followed by a gradient to 85:15 hexane/diethyl ether. The appropriate fractions were concentrated to obtain **14** (14.2 g; 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, J = 5.5 Hz, 0.5 H), 6.52 (d, J = 5.5 Hz, 0.5 H), 4.23 (d, J = 12.5 Hz, 0.5H), 4.08 (d, J = 13.3 Hz, 0.5H), 4.02 (d, J = 12.5 Hz, 0.5H), 3.87 (d, J = 12.5 Hz, 0.5H), 2.96−2.75 (m, 2H), 2.41−2.16 (m, 3H), 1.93 (d, J = 10.6 Hz, 1H), 1.75−1.61 (m, 2H), 1.41 (s, 4.5 H), 1.39 (s, 4.5 H), 1.26 (s, 12H).

*Dimethyl 5-Oxocyclohexane-1,3-dicarboxylate (**16**).* Diester **15** (5.4 g, 25.7 mmol) was charged to a Parr flask with acetic acid (1 mL) and methanol (100 mL). The flask was purged with nitrogen, and rhodium on alumina (0.54 g) was added. The reaction mixture was purged with hydrogen and pressurized to 55 PSI. Shaking was continued for 16 h. An aliquot was examined by LC/MS and indicated mostly product, contaminated with a small amount of dehydroxylated side product. The mixture was filtered and the filtrate concentrated to dryness. The residue was partitioned between 1N NaOH (100 mL) and chloroform (200 mL). The chloroform layer was washed with water, dried over sodium sulfate, filtered, and concentrated. The product was obtained as an oil (4.3 g, 77%) and used directly. To a solution of DMSO (182 mL, 2.6 mol) in dry DCM (470 mL), oxalyl chloride (111 mL, 1.3 mol) in dry DCM (340 mL) was added at −60 °C. After 1 h, a solution of the above diester (91.6 g, 0.42 mol) in DCM (400 mL) was added slowly at a rate that maintained the reaction temperature. The reaction mixture was stirred for 2 h, and then triethylamine (475 mL, 3.4 mol) was added at a rate that kept the temperature below 0 °C. The mixture was stirred for 2 h before ice water was added to quench the reaction. The mixture was warmed to room temperature and the phases separated. The organic phase was washed with 2 M HCl (2 × 500 mL) and then brine (500 mL), dried with $MgSO_4$, filtered, and evaporated in vacuo. The residue was recrystallized from ethyl acetate (50 mL) and the solid triturated with Et_2O (150 mL) and dried to give **16** as a white solid (63.2 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (6H, s), 2.15−2.5 (6H, m), 1.30−1.60 (2H, m). MS (ES) m/z 215 $(M + H)^+$.

*1,4-Dioxaspiro[4,5]decane-7,9-dicarbaldehyde (**17**).* A mixture of compound **16** (40 g, 186 mmol), ethylene glycol (20 mL, 372 mmol), and catalytic *p*-toluenesulfonic acid (680 mg) was heated under reflux in toluene (600 mL) for 3 h with azeotropic removal of water. The cooled solution was washed with aqueous potassium carbonate and then brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The product was purified by chromatography on silica gel, eluting with 5:1 hexane/ethyl acetate to give a white solid (38 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45−1.70 (3H, m), 1.95−2.05 (2H, m), 2.20−2.35 (1H, m), 2.60−2.75 (2H, m), 3.70 (6H, s), 3.90−4.05 (4H, m). MS (ES) m/z 259 $(M + H)^+$.

This ketal intermediate (38.5 g, 0.149 mol) was dissolved in dry DCM (600 mL) and cooled to −78 °C under nitrogen atmosphere. A 1.0 M solution of DIBAL in hexane (300 mL, 0.3 mol) was added at

such a rate that the temperature did not exceed −65 °C. On complete addition, the reaction mixture was stirred at −78 °C for 1 h. The resulting suspension was filtered. The solvent was removed in vacuo, and the resulting residue was purified by chromatography on SiO_2, eluting with Et_2O to give **17** as a colorless oil (17.7 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.63 (2H, s), 4.02−3.90 (4H, m), 2.70−2.60 (2H, m), 2.35−2.28 (2H, m), 1.50−1.40 (2H, m), 1.30−1.20 (2H, m). MS (ES) m/z 199 $(M + H)^+$.

*3-Benzyl-3-azaspiro[bicyclo[3.3.1]nonane-7,2′-[1,3]dioxolane (**18**).* Compound **17** (32.4 g, 0.164 mol) was dissolved in DCM (800 mL) and benzylamine (35.0 g, 0.33 mol) was added at ambient temperature. After stirring for 30 min, sodium triacetoxyborohydride (173 g, 0.82 mol) was added. The reaction mixture was then stirred overnight. The reaction was basified to pH 9−10 with 2 M NaOH (720 mL, 1.44 mol). The mixture was shaken, and the phases were separated. The organic phase was washed with brine, dried with $NaSO_4$, filtered, and evaporated in vacuum to give the residue, which was purified by chromatography on SiO_2, eluting with 5:1 hexane/ethyl acetate to give **19** as a colorless oil (40 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.30 (m, 2H), 7.30−7.15 (m, 3H), 3.90−3.80 (m, 4H), 3.75−3.70 (s, 2H), 2.90−2.80 (d, 2H), 2.50−2.40 (d, 2H), 2.10−1.98 (m, 5H), 1.90−1.75 (m, 2H), 1.40−1.30 (d, 1H). MS (ES) m/z 274 $(M + H)^+$.

*tert-Butyl 7-Oxo-3-azabicyclo[3.3.1]nonane-3-carboxylate (**19**).* Compound **18** (7.2 g, 26 mmol) was taken up in ethanol (20 mL). $Pd(OH)_2$ (5.3 g) and ammonium formate were added and the mixture heated under reflux for 2 h. The mixture was concentrated to dryness by rotary evaporation and the residue taken up in dichloromethane (20 mL). Triethylamine (8.0 g, 80 mmol) and DMAP (0.3 g, 3 mmol) were added, and the mixture was cooled to 0 °C. A solution of $(Boc)_2O$ (11.5 g, 53 mmol) in dichloromethane (5 mL) was added dropwise. The mixture was warmed to room temperature and stirred overnight. The reaction mixture was concentrated by rotary evaporation and the residue purified by silica gel chromatography, eluting with 5:1 hexane/ethyl acetate to give the ketal as a white solid (6 g, 80%). The product was taken up in acetone (30 mL), and catalytic *p*-toluenesulfonic acid was added (0.03 g, 0.17 mmol). The mixture was stirred at ambient temperature overnight and then concentrated by rotary evaporation. The residue was dissolved in ethyl acetate and washed successively with saturated aq $NaHCO_3$ and brine. The organic extract was dried over sodium sulfate, filtered, and concentrated to give a pale-yellow oil that crystallized on standing (4.0 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ4.08−4.04 (d, 2H), 2.88 (s, 2H), 2.41 (s, 4H), 2.30 (s, 2H), 1.98−1.83 (q, 2H), 1.43 (s, 9H). MS (ES) m/z 240 $(M + H)^+$.

*3-Bromo-5-isopropoxypyridine (**21**).* Potassium metal (6.6 g, 169 mmol) was dissolved in dry 2-propanol (60 mL) under nitrogen. The resulting potassium isopropoxide was heated with 3,5-dibromopyridine **20** (20 g, 84 mmol) and catalytic copper powder (1 g, 5 wt %) at 140 °C in a sealed glass tube for 14 h. The reaction mixture was cooled to ambient temperature and extracted with diethyl ether (4 × 200 mL). The combined ether extracts were dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The resulting crude product was purified by chromatography over aluminum oxide, eluting with ethyl acetate/hexane (1:9, v/v). Selected fractions were combined and concentrated by rotary evaporation, producing a pale-yellow oil (13 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J = 2 Hz, 1H), 8.2 (d, J = 2 Hz, 1H), 7.34 (t, J = 2 Hz, 1H), 4.56 (m, 1H), 1.36 (d, J = 7.5 Hz, 6H). MS (ES) m/z 216, 218 $(M + H)^+$.

*3-Bromo-5-Benzyloxypyridine (**22**).*[62] Sodium hydride (1 mol, 40.9 g of 60% in oil) was added to 800 mL of DMF and cooled in an ice bath. Benzyl alcohol (105 mL, 1 mol) was added slowly and stirred for 1 h at room temperature after complete addition. Solid 3,5-dibromopyridine **21** (200 g, 846 mmol) was added to the mixture, which was stirred for 16 h. The mixture was quenched by the addition of 500 mL of saturated ammonium chloride solution, followed by 400 mL of water, and the mixture extracted with 5 × 300 mL of ether. The extracts were combined and washed with brine (6 × 300 mL of 50% saturated), dried with $MgSO_4$, filtered, and concentrated. The residue was recrystallized from ether to afford the product as a white solid (161 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (m, 2H), 7.43−7.33 (m, 6H), 5.09 (s, 2H). MS (ES) m/z 264, 266 $(M + H)^+$.

*5-Bromo-3-phenoxypyridine (**23**).* Sodium hydride (1.35 g of 80% in mineral oil, 45 mmol) was added to a stirred solution of phenol (4.3 g, 45 mmol) in DMF (30 mL) at 0 °C, under nitrogen. The mixture was stirred at room temperature for 3 h, treated with 3,5-dibromopyridine **21** (4.0 g, 16.9 mmol), and heated at 100 °C for 48 h. The reaction mixture was cooled to room temperature, poured into a mixture of water (100 mL) and 5 M sodium hydroxide (10 mL), and extracted with ether (3 × 60 mL). The combined ether extracts were dried (Na_2SO_4), filtered, and concentrated by rotary evaporation to a pale-yellow semisolid (4.9 g). This was chromatographed on a silica gel (200 g) column with hexane/ethyl acetate/chloroform (8:1:1, v/v) as eluant to give a colorless oil (2.9 g, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, J = 1.3 Hz, 1H), 8.33 (d, J = 1.3 Hz, 1H), 7.43−7.38 (m, 3H), 7.21 (t, J = 7.4 Hz, 1H), 7.05 (d, J = 7.4 Hz, 2H). MS (ES) m/z 250, 252 $(M + H)^+$.

*3-Bromo-5-(2-methoxyphenoxy)-pyridine (**24**).* Prepared according to the procedure for **23** but using 2-methoxyphenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.26 (s, 1H), 7.28−7.09 (m, 2H), 7.07−6.99 (m, 3H), 3.80 (s, 3H). MS (ES) m/z 280, 282 $(M + H)^+$.

*3-Bromo-5-(3-methoxyphenoxy)-pyridine (**25**).* Prepared according to the procedure for **23** but using 3-methoxyphenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 8.32 (s, 1H), 7.43 (s, 1H), 7.27−7.31 (m, 1H), 6.77−6.72 (m, 1H), 6.62−6.60 (m, 2H), 3.81 (s, 3H). MS (ES) m/z 280, 282 $(M + H)^+$.

*3-Bromo-5-(4-methoxy)phenoxypyridine (**26**).* Prepared according to the procedure for **23** but using 4-methoxyphenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.28 (s, 1H), 7.32 (m, 1H), 7.0 (d, J = 9.0 Hz, 2H), 6.92 (d, J = 9.4 Hz, 2H), 3.83 (s, 3H). MS (ES) m/z 280, 282 $(M + H)^+$.

*3-Bromo-5-(2-chloro)phenoxypyridine (**27**).* Prepared according to the procedure for **23** but using 2-chlorophenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.4 (d, J = 2.4 Hz, 1H), 8.3 (d, J = 2.4 Hz, 1H), 7.52 (dd, J = 8.2 Hz, 1.2 Hz, 1H), 7.29−7.19 (m, 3H), 7.1 (dd, J = 1.2, 8.2 Hz, 1H). MS (ES) m/z 284, 286, 288 $(M + H)^+$.

*3-Bromo-5-(3-chloro)phenoxypyridine (**28**).* Prepared according to the procedure for **23** but using 3-chlorophenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 2 Hz, 1H), 8.33 (d, J = 2.4 Hz, 1H), 7.45 (t, J = 2.4 Hz, 1H), 7.32 (t, J = 8 Hz, 1H), 7.19 (dd, J = 1.2, 7.2 Hz, 1H), 7.05 (t, 2 Hz, 1H), 6.93 (dd, J = 1.6, 7.4 Hz, 1H). MS (ES) m/z 284, 286, 288 $(M + H)^+$.

*3-Bromo-5-(4-chloro)phenoxypyridine (**29**).* Prepared according to the procedure for **23** but using 4-chlorophenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 2.3 Hz, 1H), 8.32 (d, J = 2.3 Hz, 1H), 7.41 (t, J = 2.3 Hz, 1H), 7.36 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 9 Hz, 2H). MS (ES) m/z 284, 286, 288 $(M + H)^+$.

*3-Bromo-5-(4-fluorophenoxy)pyridine (**30**).* Prepared according to the procedure for **23** but using 4-fluorophenol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.4 (d, J = 2 Hz, 1H), 8.3 (d, J = 2.4 Hz, 1H), 7.37 (t, J = 2.4 Hz, 1H), 7.14−7.05 (m, 2H), 7.0−7.05 (m, 2H). MS (ES) m/z 268, 270 $(M + H)^+$.

*7-(Pyridin-4-yl)-3-azabicyclo[3.3.1]non-7-ene (**31**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.67 (d, J = 6.6 Hz, 2H), 7.92 (d, J = 6.6 Hz, 2H), 6.86 (d, J = 5.8 Hz, 1H), 3.40−3.26 (m, 4H), 3.05−2.88 (m, 2H), 2.65 (d, J = 18.4 Hz, 1H), 2.55 (s, 1H), 1.95−1.84 (m, 2H). HRMS (ES) m/z Calcd for $C_{13}H_{16}N_2$ + H: 201.1392. Found: 201.1384.

*7-(Pyrimidin-5-yl)-3-azabicyclo[3.3.1]non-7-ene (**32**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 9.05 (s, 1H), 8.91 (s, 2H), 6.47 (d, J = 5.1 Hz, 1H), 3.40−3.26 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{12}H_{15}N_3$ + H: 202.1344. Found: 202.1343.

*7-(Pyrazin-2-yl)-3-azabicyclo[3.3.1]non-7-ene (**33**).* Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 9.03 (s, 1H), 8.85 (s, 2H), 6.42 (d, J = 6.5 Hz, 1H), 3.45−3.28 (m, 4H), 2.97 (dd, J = 6.6 Hz, 19 Hz, 1H), 2.88 (br s, 1H), 2.65−2.52 (m, 2H), 2.04 (d, J = 12.5 Hz, 1H), 1.97 (d, J = 12.9 Hz, 1H). HRMS (ES) m/z Calcd for $C_{12}H_{15}N_3$ + H: 202.1344. Found: 202.1339.

7-(6-Chloropyridazin-3-yl)-3-azabicyclo[3.3.1]non-7-ene (**34**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (d, J = 9.4 Hz, 1H), 7.76 (d, J = 9.4 Hz, 1H), 6.85 (d, J = 5.5 Hz, 1H), 3.40−3.26 (m, 4H), 3.05−2.88 (m, 2H), 2.65−2.55 (m, 2H), 2.04−1.95 (m, 2H). HRMS (ES) m/z Calcd for $C_{12}H_{14}ClN_3$ + H: 236.0955. Found: 236.0958.

6-(3-Azabicyclo[3.3.1]non-7-en-7-yl)quinoxaline (**35**). Prepared according to general method B. ^{1}H NMR (400 MHz, CD_3OD) δ 8.85 (dd, J = 12.9 Hz, 2 Hz, 2H), 8.14−8.03 (m, 3H), 6.62 (d, J = 7 Hz, 1H), 3.41−3.28 (m, 2H), 3.12 (d, J = 6.6 Hz, 1H), 3.07 (d, J = 6.3 Hz, 1H), 2.91 (br s, 1H), 2.77 (d, J = 18.3 Hz, 1H), 2.64 (s, 1H), 2.57 (br s, 1H), 2.02−1.99 (m, 2H). HRMS (ES) m/z Calcd For $C_{16}H_{17}N_3$ + H: 252.1501. Found: 252.1499.

AUTHOR INFORMATION

Corresponding Author

*For S.R.B.: Phone, 336-723-4019; E-mail, srbreining@gmail.com. For Targacept, contact D.Y.: Phone, (336) 480-2190; E-mail, daniel.yohannes@targacept.com.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

BOC, *tert*-butyloxycarbonyl; DCM, dichloromethane; DME, dimethoxyethane; DMF, dimethylformamide; DPPF, diphenylphosphinoferrocene; HTS, high throughput screening; PTSA, *p*-toluenesulfonic acid; SAR, structure−activity relationship

REFERENCES

(1) Anand, R.; Conroy, W. G.; Schoepper, R.; Whiting, P.; Lindstrom, J. Neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes have a pentameric quaternary structure. *J. Biol. Chem.* **1991**, *266*, 11192−11198.

(2) Jensen, A. A.; Frolund, B.; Liljefors, T.; Krogsgaard-Larsen, P. J. Neuronal Nicotinic Acetylcholine Receptors: Structural Revelations, Target Identifications, and Therapeutic Inspirations. *J. Med. Chem.* **2005**, *48*, 4705−4745.

(3) Nashmi, R.; Lester, H. A. CNS Localization of Neuronal Nicotinic Receptors. *J. Mol. Neurosci.* **2006**, *30*, 181−184.

(4) Quik, M.; Huang, L. Z.; Parameswaran, N.; Bordia, T.; Campos, C.; Perez, X. A. Multiple roles for nicotine in Parkinson's disease. *Biochem. Pharmacol.* **2009**, *78*, 677−685.

(5) (a) Decker, M. W.; Gopalakrishnan, M.; Meyer, M. D. The potential of neuronal nicotinic acetylcholine receptor agonists for treating CNS conditions. *Expert Opin. Drug Discovery* **2008**, *3*, 1027−1040. (b) Oddo, S.; LaFerla, F. M. The role of nicotinic acetylcholine receptors in Alzheimer's Disease. *J. Physiol.* **2006**, *99*, 172−179.

(6) Rowbotham, M. C.; Duan, W. R.; Thomas, J.; Nothaft, W.; Backonja, M.-M. A randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of ABT-594 in patients with diabetic peripheral neuropathic pain. *Pain* **2009**, *146*, 245−252.

(7) DeBiasi, M.; Salas, R. Influence of neuronal nicotinic receptors over nicotine addiction and withdrawal. *Exp. Biol. Med.* **2008**, *233*, 917−929.

(8) Grady, S. R.; Drenan, R. M.; Breining, S. R.; Yohannes, D.; Wageman, C. R.; Fedorov, N. B.; McKinney, S.; Whiteaker, P.; Bencherif, M.; Lester, H. A.; Marks, M. J. Structural Differences determine the relative selectivity of nicotinic compounds for native $\alpha4\beta2$, $\alpha6\beta2$, $\alpha3\beta4$ and $\alpha7$ nicotinic acetylcholine receptors. *Neuropharmacology* **2010**, *58*, 1054−66.

(9) (a) Etter, J.-F. Cytisine for smoking cessation: a literature review and a meta-analysis. *Arch. Intern. Med.* **2006**, *166*, 1553−1559. (b) Etter, J-F; Lukas, R. J.; Benowitz, N. L.; West, R.; Dresler, C. M. Cytisine for smoking cessation: a research agenda. *Drug Alcohol Depend.* **2008**, *92*, 3−8.

(10) Abin-Carriquiry, J. A.; Voutilainen, M. H.; Barik, J.; Cassels, B. K.; Iturriaga-Vasquez, P.; Bermudez, I.; Durand, C.; Dajas, F.; Wonnacott, S. C3-Halogenation of cytisine generates potent and efficacious nicotinic receptor agonists. *Eur. J. Pharmacol.* **2006**, *536*, 1−11.

(11) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. Epibatidine: a novel (chloropyridyl)-azabicycloheptane with potent analgesic activity from an Ecuadoran poison frog. *J. Am. Chem. Soc.* **1992**, *114*, 3475−3478.

(12) Daly, J. W. Nicotinic agonists, antagonists, and modulators from natural sources. *Cell. Mol. Neurobiol.* **2005**, *25*, 513−552.

(13) Cohen, C.; Bergis, O. E.; Galli, F.; Lochead, A. W.; Jegham, S.; Biton, B.; Leonardon, J.; Avenet, P.; Sgard, F.; Besnard, F.; Graham, D.; Coste, A.; Oblin, A.; Curet, O.; Voltz, C.; Gardes, A.; Caille, D.; Perrault, G.; George, P.; Soubrie, P.; Scatton, B. SSR591813, a novel selective and partial $\alpha4\beta2$ nicotinic receptor agonist with potential as an aid to smoking cessation. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 407−420.

(14) Sorbera, L. A.; Revel, L.; Leeson, P. A.; Castaner, J. ABT-594. *Drugs Future* **2001**, *26*, 927−934.

(15) Jain, K. Modulators of nicotinic acetylcholine receptors as analgesics. *Curr. Opin. Invest. Drugs* **2004**, *5*, 76−81.

(16) Owen, R. T.; Serradell, N.; Rosa, E. Ispronicline: nicotinic acetylcholine $\alpha4\beta2$ agonist treatment of cognition disorders. *Drugs Future* **2008**, *33*, 197−202.

(17) Rollema, H; Coe, J. W.; Chambers, L. K.; Hurst, R. S.; Stahl, S. M.; Williams, K. E. Rationale, pharmacology and clinical efficacy of partial agonists of $\alpha4\beta2$ nACh receptors for smoking cessation. *Trends Pharmacol. Sci.* **2007**, *28*, 316−325.

(18) (a) Ripoll, N.; Bronnec, M.; Bourin, M. Nicotinic receptors and schizophrenia. *Curr. Med. Res. Opin.* **2004**, *20*, 1057−1074. (b) Leonard, S. Consequences of low levels of nicotinic acetylcholine receptors in schizophrenia for drug development. *Drug Dev. Res.* **2003**, *60*, 127−136. (c) Martin, L. F.; Kem, W. R.; Freedman, R. $\alpha7$ nicotinic receptor agonists: potential new candidates for treatment of schizophrenia. *Psychopharmacology* **2004**, *174*, 54−64.

(19) Lippiello, P. M.; Bencherif, M.; Hauser, T. A.; Jordan, K. G.; Letchworth, S. R.; Mazurov, A. A. Nicotinic receptors as targets for therapeutic discovery. *Exp. Opin. Drug Discovery* **2007**, *2*, 1185−1203.

(20) Mazurov, A.; Hauser, T.; Miller, C. H. Selective $\alpha7$ nicotinic acetylcholine receptor ligands. *Curr. Med. Chem.* **2006**, *13*, 1567−1584.

(21) Salas, R.; Main, A.; Gangitano, D. A.; DeBiasi, M. Decreased withdrawal symptoms but normal tolerance to nicotine in mice null for the $\alpha7$ nicotinic acetylcholine receptor subunit. *Neuropharmacology* **2007**, *53*, 863−869.

(22) Mihalak, K. B.; Carroll, F. I.; Luetje, C. W. Varenicline is a partial agonist at $\alpha4\beta2$ and a full agonist at $\alpha7$ neuronal nicotinic receptors. *Mol. Pharmacol.* **2006**, *70*, 801−805.

(23) Sullivan, J. P.; Bannon, A. W. Epibatidine: Pharmacological Properties of a Novel Nicotinic Acetylcholine Receptor Agonist and Analgesic Agent. *CNS Drug Rev.* **1996**, *2*, 21−39.

(24) Meyer, M. D. Neuronal nicotinic acetylcholine receptors as a target for the treatment of neuropathic pain. *Drug Dev. Res.* **2006**, *67*, 355−359.

(25) Young, J. M.; Shytle, R. D.; Sanberg, P. R.; George, T. P. Mecamylamine: new therapeutic uses and toxicity/risk profile. *Clin. Ther.* **2001**, *23*, 532−565.

(26) (a) Bunnelle, W. H.; Barlocco, D.; Daanen, J. F.; Dart, M. J.; Meyer, M. D.; Ryther, K. B.; Schrimpf, M. R.; Sippy, K. B.; Toupence, R. B. Preparation of diazabicyclic central nervous system (CNS) active agents for use in pharmaceutical compositions. US Pat. Appl. Publ. 2003225268, 2003. (b) Clark, T. J.; Dull, G. M.; Lynm, D.; Miao, L.; Miller, C. H.; Schmitt, J. D. Pharmaceutical Compositions and Methods for Use. US Patent 6,440,970, 2002.

(27) Bencherif, M.; Byrd, G.; Caldwell, W. S.; Hayes, J. R.; Lippiello, P. M. Pharmacological Characterization of RJR-2403: A Nicotinic

Agonist with Potential Therapeutic Benefit in the Treatment of Alzheimer's Disease. *CNS Drug Rev.* **1997**, *3*, 325−345.
(28) Unpublished data. Determined at Targacept by same methods employed for experimental compounds in this manuscript.
(29) Bencherif, M.; Lovette, M. E.; Fowler, K. W.; Arrington, S.; Reeves, L.; Caldwell, W. S.; Lippiello, P. M. RJR-2403: a nicotinic agonist with CNS selectivity I. In vitro characterization. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1413−1421.
(30) (a) Grinevich, V. P.; Haubner, A.; Fedorov, N.; Bencherif, M. Effects of TC-5653, a potent $\alpha4/\alpha6$ nicotinic receptor partial agonist, on striatal dopamine release. Society for Neuroscience 38th Annual Meeting, Washington, D.C., November 15−19, 2008, Poster 328.16/C46. (b) Breining, S. R.; Bhatti, B. S.; Hawkins, G. H.; Miao, L.; Mazurov, A.; Phillips, T. Y.; Miller, C. H. Pharmaceutical Compositions and Methods for Relieving Pain and Treating Central Nervous System Disorders. US Patent 7,402,592, 2008.
(31) Wustrow, D. J.; Wise, L. D. Coupling of Arylboronic acids with a Partially Reduced Pyridine Derivative. *Synthesis* **1991**, 993−995.
(32) Martin, F. M.; Flynn, C. J.; Richards, S. J. Azabicycloalkane derivatives useful as nicotinic acetylcholine receptor agonists. US Pat. Appl. Pub. US2008/0261999, 2008.
(33) Eastwood, P. R. A versatile synthesis of 4-aryl tetrahydropyridines via palladium mediated Suzuki cross coupling with cyclic vinyl boronates. *Tetrahedron Lett.* **2000**, *41*, 3705−3708.
(34) (a) Bunnelle, W. H.; Tietje, K. R.; Frost, J. M.; Peters, D.; Ji, J.; Li, T.; Scanio, M. J. C.; Shi, L.; Anderson, D. J.; Dyhring, T.; Gronlien, J. H.; Ween, H.; Thorin-Hagene, K.; Meyer, M. D. Octahydropyrrolo-[3,4-*c*]pyrrole: a diamine scaffold for construction of either $\alpha4\beta2$ or $\alpha7$-selective nicotinic acetylcholine receptor (nAChR) ligands—substitutions that switch subtype selectivity. *J. Med. Chem.* **2009**, *52*, 4126−4141. (b) Frost, J. M.; Bunnelle, W. H.; Tietje, K. R.; Anderson, D. J.; Reuter, L. E.; Curzon, P.; Surowy, C. S.; Ji, J.; Daanen, J. F.; Kohlhaas, K. L.; Buckley, M. J.; Henry, R. F.; Dyhring, T.; Ahring, P. K.; Meyer, M. D. Synthesis and structure−activity relationships of 3,8-diazabicyclo[4.2.0]octane ligands, potent nicotinic acetylcholine receptor agonists. *J. Med. Chem.* **2006**, *49*, 7843−7853.
(35) Values for (*S*)-nornicotine obtained from ref 12. $\alpha4\beta2$ K_i = 23 nM, $\alpha7$ $K_i \geq$ 10000 nM, $\alpha3\beta4$ K_i = 610 nM. Values are comparable with those obtained internally at Targacept.
(36) Nelson, M. E.; Kuryatov, A.; Choi, C. H.; Zhou, Y.; Lindstrom, J. Alternate stoichiometries of alpha4beta2 nicotinic acetylcholine receptors. *Mol. Pharmacol.* **2003**, *63*, 332−341.
(37) Moroni, M.; Zwart, R.; Sher, E.; Cassels, B. K.; Bermudez, I. alpha4beta2 nicotinic receptors with high and low acetylcholine sensitivity: pharmacology, stoichiometry, and sensitivity to long-term exposure to nicotine. *Mol. Pharmacol.* **2006**, *70*, 755−768.
(38) Gotti, C.; Moretti, M.; Meinerz, N. M; Clementi, F.; Gaimarri, A.; Collins, A. C.; Marks, M. J. Partial deletion of the nicotinic cholinergic receptor $\alpha4$ or $\beta2$ subunit genes changes the acetylcholine sensitivity of receptor-mediated ^{86}Rb efflux in cortex and thalamus and alters relative expression of $\alpha4$ and $\beta2$ subunits. *Mol. Pharmacol.* **2008**, *73*, 1796−1807.
(39) Anderson, D. J.; Malysz, J.; Gronlien, J. H.; El Kouhen, R.; Hakerud, M.; Wetterstrand, C.; Briggs, C. A.; Gopalakrishnan, M.. Stimulation of dopamine release by nicotinic acetylcholine receptor ligands in rat brain slices correlates with the profile of high, but not low, sensitivity $\alpha4\beta2$ subunit combination. *Biochem. Pharmacol.* **2009**, *78*, 844−851.
(40) (a) George, A. A.; Lucero, L. M.; Damaj, M. I.; Lukas, R. J.; Chen, X.; Whiteaker, P. Function of Human $\alpha3\beta4\alpha5$ Nicotinic Acetylcholine Receptors Is Reduced by the $\alpha5$(D398N) Variant. *J. Biol. Chem.* **2012**, *287*, 25151−25162. (b) Grishin, A. A.; Wang, C.-I. A.; Muttenthaler, M.; Alewood, P. F.; Lewis, R. J.; Adams, D. J. α-Conotoxin AuIB Isomers Exhibit Distinct Inhibitory Mechanisms and Differential Sensitivity to Stoichiometry of $\alpha3\beta4$ Nicotinic Acetylcholine Receptors. *J. Biol. Chem.* **2010**, *285*, 22254−22263. (c) Krashia, P.; Moroni, M.; Broadbent, S.; Hofmann, G.; Kracun, S.; Beato, M.; Groot-Kormelink, P. J.; Sivilotti, L. G. Human $\alpha3\beta4$ Neuronal Nicotinic Receptors Show Different Stoichiometry If They Are Expressed in *Xenopus* Oocytes or Mammalian HEK293 Cells. *PLoS One* **2010**, *5*, 13611.
(41) Others have reported discrepancies between affinity and potency. See, for example: Sippy, K. B.; Anderson, D. J.; Bunnelle, W. H.; Hutchins, C. W.; Schrimpf, M. R. Preparation and characterization of *N*-(3-pyridinyl) spirocyclic diamines as ligands for nicotinic acetylcholine receptors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1682−1685.
(42) Benowitz, N. L. Clinical Pharmacology of Nicotine: Implications for Understanding, Preventing, and Treating Tobacco Addiction. *Clin. Pharmacol. Ther.* **2008**, *83*, 531−541.
(43) Bunnelle, W. H.; Daanen, J. F.; Ryther, K. B.; Schrimpf, M. R.; Dart, M. J.; Gelain, A.; Meyer, M. D.; Frost, J. M.; Anderson, D. J.; Buckley, M.; Curzon, P.; Cao, Y.-J.; Puttfarcken, P.; Searle, X.; Ji, J.; Putman, C. B.; Surowy, C.; Toma, L.; Barlocco, D. Structure−Activity Studies and Analgesic Efficacy of *N*-(3-Pyridinyl)-Bridged Bicyclic Diamines, Exceptionally Potent Agonists at Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **2007**, *50*, 3627−3644.
(44) Exley, R.; Clements, M. A.; Hartung, H.; McIntosh, J. M.; Cragg, S. J. $\alpha6$-Containing Nicotinic Acetylcholine Receptors Dominate the Nicotine Control of Dopamine Neurotransmission in Nucleus Accumbens. *Neuropsychopharmacology* **2008**, *33*, 2158−2166.
(45) Kuryatov, A.; Olale, F.; Cooper, J.; Choi, C.; Lindstrom, J. Human $\alpha6$ AChR subtypes: subunit composition, assembly, and pharmacological responses. *Neuropharmacology* **2000**, *39*, 2570−2590.
(46) Drenan, R. M.; Grady, S. R.; Whiteaker, P.; McClure-Begley, T.; McKinney, S.; Miwa, J. M.; Bupp, S.; Heintz, N.; McIntosh, J. M.; Bencherif, M.; Marks, M. J.; Lester, H. A. In vivo activation of midbrain dopamine neurons via sensitized, high-affinity $\alpha6$* nicotinic acetylcholine receptors. *Neuron* **2008**, *60*, 123−126.
(47) Cao, Y.-J; Surowy, C. S.; Puttfarcken, P. S. Different nicotinic acetylcholine receptor subtypes mediating striatal and prefrontal cortical [3H]-dopamine release. *Neuropharmacology* **2005**, *48*, 72−79.
(48) (a) Yang, K.; Jin, G.; Wu, J. Mysterious $\alpha6$-containing nAChRs: function, pharmacology, and pathophysiology. *Acta Pharmacol. Sin.* **2009**, *30*, 740−751. (b) Wu, J.; Lukas, R. J. Naturally-expressed nicotinic acetylcholine receptor subtypes. *Biochem. Pharmacol.* **2011**, *82*, 800−807. (c) Tuesta, L.; Fowler, C. D.; Kenny, P. J. Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behavior. *Biochem. Pharmacol.* **2011**, *82*, 984−995. (d) Quik, M.; Perez, X. A.; Grady, S. R. Role of $\alpha6$ nicotinic receptors in CNS dopaminergic function: relevance to addiction and neurological disorders. *Biochem. Pharmacol.* **2011**, *82*, 873−882.
(49) (a) Letchworth, S. R.; Whiteaker, P. Progress and challenges in the study of $\alpha6$-containing nicotinic acetylcholine receptors. *Biochem. Pharmacol.* **2011**, *82*, 862−872. (b) Capelli, A. M.; Castelletti, L.; Chen, Y. H.; Van der Keyl, H.; Pucci, L.; Oliosi, B.; Salvagno, C.; Bertani, B.; Gotti, C.; Powell, A.; Mugnaini, M. Stable expression and functional characterization of a human nicotinic acetylcholine receptor with $\alpha6\beta2$ properties: discovery of selective antagonists. *Br. J. Pharmacol.* **2011**, *163*, 313−329.
(50) See, for example: (a) McIntosh, J. M.; Azam, L.; Staheli, S.; Dowell, C.; Lindstrom, J. M.; Kuryatov, A.; Garrett, J. E.; Marks, M. J.; Whiteaker, P. Analogs of alpha-conotoxin MII are selective for alpha6-containing nicotinic acetylcholine receptors. *Mol. Pharmacol.* **2004**, *65*, 944−952. (b) Dowell, C.; Olivera, B. M.; Garrett, J. E.; Staheli, S. T.; Watkins, M.; Kuryatov, A.; Yoshikami, D.; Lindstrom, J. M.; McIntosh, J. M. Alpha-conotoxin PIA is selective for alpha6 subunit containing nicotinic acetylcholine receptors. *J. Neurosci.* **2003**, *23*, 8445−8452.
(51) Drenan, R. M.; Grady, S. R.; Steele, A. D.; McKinney, S.; Patzlaff, N. E.; McIntosh, J. M.; Marks, M. J.; Miwa, J. M.; Lester, H. A. Cholinergic modulation of locomotion and striatal dopamine release is mediated by alpha6alpha4* nicotinic acetylcholine receptors. *J. Neurosci.* **2010**, *30*, 9877−9889.
(52) Hauser, T. A.; Hepler, C. D.; Kombo, D. C.; Grinevich, V. P.; Kiser, M. N.; Hooker, D. N.; Zhang, J.; Mountfort, D.; Selwood, A.; Akireddy, S. R.; Letchworth, S. R.; Yohannes, D. Comparison of acetylcholine receptor interactions of the marine toxins, 13-

desmethylspirolide C and gymnodimine. *Neuropharmacology* **2012**, *62*, 2238−49.
(53) Eaton, J. B.; Peng, J.; Schroeder, K. M.; George, A. A.; Fryer, J. D.; Krishnan, C.; Buhlman, L.; Kuo, Y.; Steinlein, O.; Lukas, R. J. Characterization of human $\alpha4\beta2$- nicotinic acetylcholine receptors stably and heterologously expressed in native nicotinic receptor-null SH-EP1 human epithelial cells. *Mol. Pharmacol.* **2003**, *64*, 1283−1294.
(54) Mukherjee, J.; Kuryatov, A.; Moss, S. J.; Lindstrom, J. M.; Anand, R. Mutations of cytosolic loop residues impair assembly and maturation of $\alpha7$ nicotinic acetylcholine receptors. *J. Neurochem.* **2009**, *110*, 1885−1894.
(55) Pakkanen, J. S.; Nousiainen, H.; Yli-Kauhaluoma, J.; Kylänlahti, I.; Möykkynen, T.; Korpi, E. R.; Peng, J.-H.; Lukas, R. J.; Ahtee, L.; Tuominen, R. K. Methadone increases intracellular calcium in SH-SY5Y and SH-EP1-h$\alpha7$ cells by activating neuronal nicotinic acetylcholine receptors. *J. Neurochem.* **2005**, *94*, 1329−1341.
(56) (a) Kuryatov, A.; Olale, F.; Cooper, J.; Choi, C.; Lindstrom, J. Human alpha 6 AChR subtypes: subunit composition, assembly, and pharmacological responses. *Neuropharmacology* **2000**, *39*, 2570−2590. (b) McIntosh, J. M.; Azam, L.; Staheli, S.; Dowell, C.; Lindstrom, J. M.; Kuryatov, A.; Garrett, J. E.; Marks, M. J.; Whiteaker, P. Analogs of alpha-conotoxin MII are selective for alpha 6-containing nicotinic acetylcholine receptors. *Mol. Pharmacol.* **2004**, *65*, 944−952.
(57) Cheng, Y.; Prusoff, W. H. Relation between the inhibition constant (K_i) and the concentration of inhibitor which causes fifty percent inhibition (IC_{50}) of an enzymic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(58) Bencherif, M.; Schmitt, J. D.; Bhatti, B. S.; Crooks, P.; Caldwell, W. S.; Lovette, M. E.; Fowler, K.; Reeves, L.; Lippiello, P. M. The heterocyclic substituted pyridine derivative (±)-2-(-3-pyridinyl)-1-azabicyclo[2.2.2]octane (RJR-2429): a selective ligand at nicotinic acetylcholine receptors. *J. Pharmacol. Exp.Ther.* **1998**, *284*, 886−894.
(59) Placzek, A. N.; Grassi, F.; Meyer, E. M.; Papke, R. L. An $\alpha7$ nicotinic acetylcholine receptor gain-of-function mutant that retains pharmacological fidelity. *Mol. Pharmacol.* **2005**, *68*, 1863−1876.
(60) Covernton, P. J.; Connolly, J. G. Multiple components in the agonist concentration−response relationships of neuronal nicotinic acetylcholine receptors. *J. Neurosci. Methods* **2000**, *96*, 63−70.
(61) Liu, Q; Yu, K. W.; Chang, Y. C.; Lukas, R. J.; Wu, J. Agonist-induced hump current production in heterologously-expressed human $\alpha4\beta2$-nicotinic acetylcholine receptors. *Acta Pharmacol. Sin* **2008**, *29*, 305−319.
(62) Wasicak, J. T.; Garvey, D. S.; Holladay, M. W.; Lin, N. H.; Ryther, K. B. 7a-Heterocycle substituted hexahydro-1*H*-pyrrolizine compounds useful in controlling chemical synaptic transmission. US Patent 5,733,912, 1998.