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3,5-Bicyclic aryl piperidines: A novel class of α4β2 neuronal nicotinic receptor partial agonists for smoking cessation

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Abstract—3,5-Bicyclic aryl piperidines are a new class of high-affinity α4β2 nicotinic receptor agents. We have sought nicotinic receptor partial agonists of the α4β2 nicotinic acetylcholine receptor for smoking cessation, and a number of compounds fulfill potency, selectivity, and efficacy requirements in vitro. In vivo, selected agents demonstrate potent partial agonist efficacy on the mesolimbic dopamine system, a key measure of therapeutic potential for smoking cessation.

More than half of the 1.25B current smokers worldwide will die from tobacco-related illness.[1] Despite this startling fact and the undisputed connection between tobacco use and disease, global smoking rates continue to rise. Usually beginning as an innocent act, smoking becomes habitual owing to the powerful dependence-producing effects of nicotine.[2] Unfortunately, the vast majority of smokers find tobacco dependence difficult to overcome, with >95% of unaided quit attempts ending in failure.

Smoking initiates and maintains a cycle of neurochemical events via nicotine's action as an agonist[3] of neuronal nicotinic acetylcholine receptors (nAChRs).[4] A number of studies point to the α4β2 subtype of the nAChRs as important to the dependence-producing effects of nicotine.[5] These reports have been recently bolstered by findings that certain α4 subtype mutants render transgenic mice hypersensitive to nicotine[6] and that β2 expression in the ventral tegmental area of the mesolimbic dopamine system of β2-deletion mutants restores wild type function in response to nicotine.[7]

Nicotine exposure from tobacco triggers the release of dopamine in the mesolimbic dopamine system.[8] As nicotine's concentration declines, the elevated dopamine levels subside, signaling the urge to smoke.[9] Lower dopaminergic tone from abstinence induces craving and withdrawal syndrome.[10,11] Hypothetically nicotine mediates reward and satisfaction through dopamine release, thereby providing relief from withdrawal and craving.[12] Peak and trough levels, or the overall dopaminergic tone, therefore, are managed through smoking. These fundamental effects of nicotine at nAChRs govern many subsequent responses, including receptor desensitization and upregulation and the dependence liability that results.

Although therapeutic advances have improved long-term quit rates compared to placebo in clinical studies, these rates remain disappointingly low.[13] Nicotine replacement therapy provides controlled-release nicotine and acts to suppress craving and withdrawal from smoking cessation, but it does not prevent activation of nAChRs by nicotine from smoking.[14] Bupropion has been shown to dampen the urge to smoke, but it permits a response to smoking.[15,16] Studies with nicotine vaccines that sequester nicotine to prevent nicotinic activation show promise, but these vaccines theoretically fail to address craving and withdrawal.[17]

We reasoned that a dual agent that relieved the craving and withdrawal syndrome while simultaneously attenuating the nicotine-induced effects of smoking could deliver

Keywords: α4β2 nicotinic receptor; nAChR; Partial agonist; Smoking cessation; Alkaloid; Varenicline.
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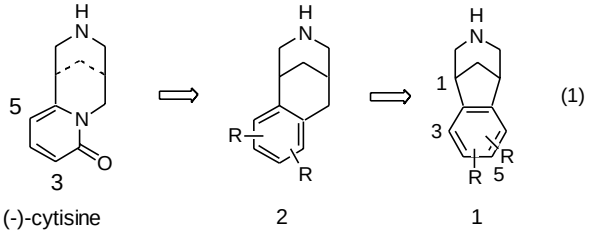
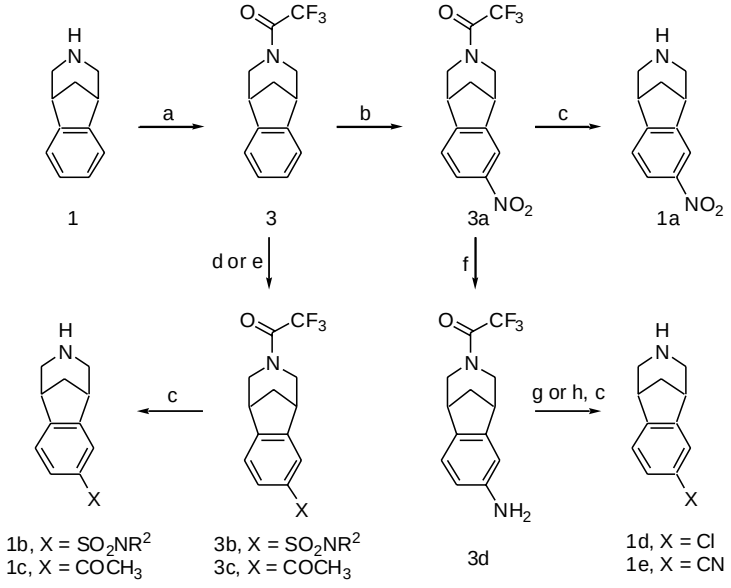
superior efficacy.[18] In theory, a partial agonist would act as a dual agent, diminishing the consequences of both nicotine exposure and its absence. By directly inhibiting nicotine induced dopaminergic activation through competitive α4β2 nAChR blockade, a partial agonist interrupts nicotine's agonist effects.[19] More important, by slightly elevating dopaminergic tone, a partial agonist would ease the craving and withdrawal syndrome that often precipitates relapse. Our efforts to discover partial agonists of the α4β2 nAChR, the high affinity nicotinic subtype in the brain,[3] have been recently reported.[20,21] Herein, we describe the strategy employed in the discovery of a series of promising compounds from which varenicline, a partial agonist of the α4β2 nAChR, has emerged as a clinical candidate for smoking cessation.[21]

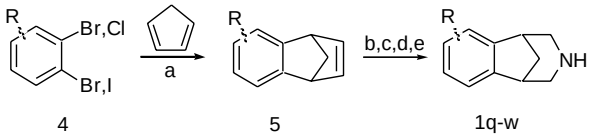


We began by considering ligands of nAChRs, many of which originate from natural sources.[22] Their role in plants is to protect against mammalian and insect predators by disturbing normal neuronal and peripheral AChR-mediated responses. We recently described the racemic total syntheses of the plant-derived, naturally occurring lupin alkaloid (−)-cytisine[23] and have extensively studied its functionalized derivatives.[24] In subsequent investigations we probed substituted phenyl ring analogs (e.g., **2**).[20] Although these compounds proved selective for the target α4β2 nAChR in affinity measures, analogs of **2** exhibited both decreased affinity relative to cytisine and poor in vivo partial agonist profiles. Herein we detail the synthesis, in vitro structure–activity relationships, and in vivo activity of novel α4β2 nicotinic receptor partial agonists based on benzazapine **1**. These efforts culminated in the identification of a clinical candidate for smoking cessation, varenicline.[21] Although differing structurally from **2** by only a single skeletal carbon atom, benzazapine derivatives (**1**) display potent affinity in vitro and nicotinic receptor partial agonist activity in vivo.

Before our studies, benzazapine **1** (R = H) had appeared only once in the medicinal literature in a 1979 study of morphine pharmacological probes.[25] While described as an inactive antinociceptive agent, benzazapine showed additional unexplained in vivo activity that we believed could be traced to underlying nicotinic pharmacology. Recognition of the clear structural similarity of **1** and **2** prompted our preparation of benzazapine **1**; we found that it displayed comparable affinity to **2** at the α4β2 nAChR (K_i 20 vs. 34 nM). Further studies of **1** revealed that the *N*-trifluoroacetamide protecting group was uniquely suited to electrophilic substitution chemistry (e.g., **3**), greatly facilitating analog synthesis (Scheme 1). With other *N*-alkyl and *N*-acyl-protecting groups, reaction progress was inhibited, even under forcing conditions. We presume that nitrogen-protecting groups that generate cationic intermediates upon activation by reagents inhibit aryl ring electrophilic chemistry. In contrast, *N*-trifluoroacetamide-protected derivative **3** converts to **3a** in high isolated yields as shown in Scheme 1. In addition, the symmetry of benzazapine **1** (and **3**) drove the relatively rapid exploration of analogs compared with **2**, which required regiochemically controlled total synthesis.[20] Given the structure–activity relationships of derivatives of **2** described in the previous report[20] and the observation that virtually all



Scheme 1. Reagents and conditions: (a) TFAA, py, CH_2Cl_2 (100%); (b) 1.3 equiv HNO_3, 2.6 equiv CF_3SO_2OH, −78 °C, CH_2Cl_2 (95%); (c) [1] Na_2CO_3, aq. MeOH (95%); [2] HCl, MeOH or EtOAc; (d) [1] SO_2ClOH, CH_2Cl_2, [2] HNR_2; (e) AcCl, $AlCl_3$; (f) H_2, $Pd(OH)_2$, EtOAc (94%); (g) HCl, $NaNO_2$, CuCl; (h) [1] HCl, $NaNO_2$, KI, [2] CuCN, NMP, 180 °C.



Scheme 2. Reagents and conditions: (a) Mg, THF or *n*-BuLi, hydrocarbon; (b) OsO_4, NMO, aq acetone 89%; (c) $NaIO_4$ aq DCE; (d) $BnNH_2$, $NaBH(OAc)_3$ DCE 82–86%; (e) H_2 or NH_4HCO_2, $Pd(OH)_2$, HCl, MeOH 88–95%.

high-affinity nicotinic agonists possess electron-deficient heteroaromatic π-systems,[22] we prepared **3a** via nitration of **3**. Deprotection afforded **1a**, which proved to be significantly more potent than nitro analogs of **2** (vide infra).[20] The partial agonist activity of **1a** in vivo stimulated further exploration of benzazapine derivatives as reported herein.[26]

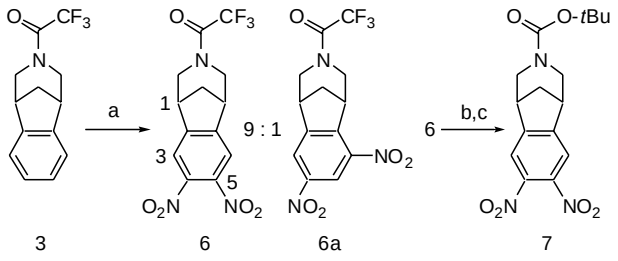
Additional mono-substituted analogs were prepared by standard transformations. Sulfonylation[27] and Friedel–Crafts acylation[28] provided direct access to carbon and sulfur substitution (Scheme 1). Aniline **3d** was converted to halide[29] and nitrile derivatives using Sandmeyer chemistry.[30] Dichlorination occurs selectively to produce a single *ortho*-dichloro analog.[31] Access to other substituents was accomplished via appropriately substituted benzyne Diels–Alder chemistry (Scheme 2)[32] followed by piperidine synthesis,[33] allowing ready access to substitution at one or more aromatic positions.

When **3** is exposed to excess nitronium triflate (>2 equiv), a highly reactive nitrating agent, the unexpectedly efficient conversion to *ortho*-dinitroderivative **6** occurs with ~9:1 selectivity (Scheme 3). We attribute this dramatic result to the steric and electronic influences of the benzazapine ring system, which decreases the reactivity of the 3-position relative to the distal 5-position of mono-nitrated intermediate **3a**. This fortuitous finding facilitated the exploration of fused heterocyclic derivatives including quinoxalines, benzimidazoles, and benzoxazoles. Additional fused heterocycles, including benzothiaxoles, quinolines, isoquinolines, and benzisoxazoles, were accessed using contemporary methodology.

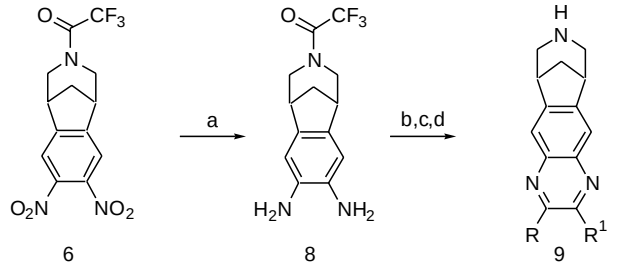
Quinoxaline derivatives were prepared from dianiline **8** via condensation with 1,2-dione derivatives (Scheme 4).

Benzimidazole synthesis involved two routes (Scheme 5). In the first route, *N*-*t*-Boc dinitro derivative **7** was converted to **12** on exposure to amines or anilines in warm THF by displacement of a single nitro group.[34] Reduction of **12** and ring closure[35] afforded either the unsubstituted (R^1 hydrogen) or the alkyl-substituted derivatives after acidic *N*-*t*-Boc deprotection. This route allowed for the R^2 substituents to be aryl groups or hindered alkyl groups. The second route involved benzimidazole formation (**10** → **11**) followed by alkylation on the benzimidazole nitrogen to introduce R^2 substituents.[36]

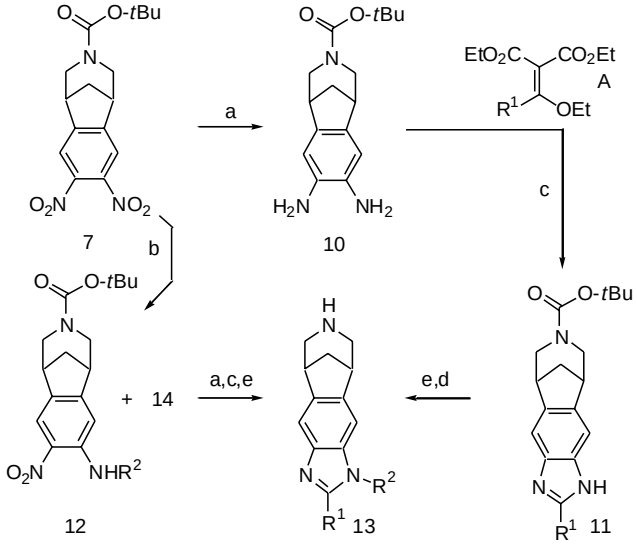
Benzoxazole syntheses involved displacement of a single nitro group by KOAc in warm DMSO. After nitro group reduction, the *o*-aminophenol was acylated and



Scheme 3. Reagents and conditions: (a) 2.3 equiv HNO_3, 4.6 equiv CF_3SO_2OH, −78 to 20 °C, CH_2Cl_2 (77%); (b) Na_2CO_3, aq MeOH (95%); (c) *t*-Boc_2O, Na_2CO_3, H_2O, CH_2Cl_2 (95%).



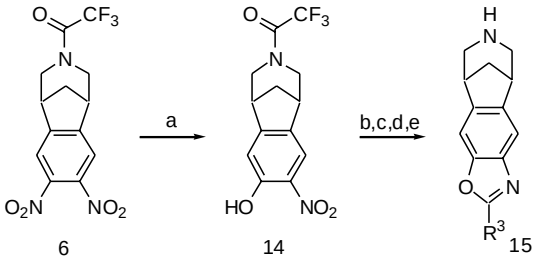
Scheme 4. Reagents and conditions: (a) H_2, $Pd(OH)_2$; (b) glyoxal, H_2O, 80 °C or butane 2,3-dione, 40–60%; (c) Na_2CO_3, aq MeOH (95%); (d) HCl, EtOAc.



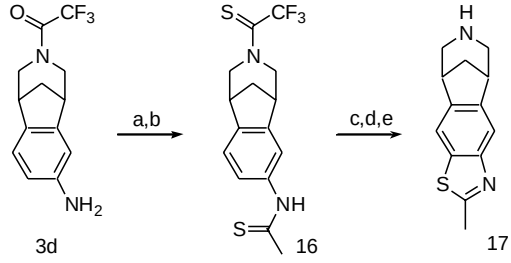
Scheme 5. Reagents and conditions: (a) H_2, $Pd(OH)_2$; (b) R^2NH_2, THF, Δ; (c) **A**, 10/1 EtOH/AcOH, Δ; (d) R^2I or R^2Br, NaOH, DMSO, Δ; (e) HCl, EtOAc.

then cyclized thermally under acidic conditions (Scheme 6).[37]

Benzothiazole **17** was prepared via oxidative ring closure[38] of dithioamide **16**,[39] itself readily formed by treating *N*-trifluoroacetamide-protected aniline acetamide, derived from **3d**, with Lawesson's reagent. The trifluorothioacetamide was removed in an oxidative cyclization, and final salt formation completed the synthesis (Scheme 7).



Scheme 6. Reagents and conditions: (a) 2.3 equiv KOAc, 100 °C, DMSO (97%); (b) H_2, $Pd(OH)_2$, EtOAc; (c) [1] R^3COCl, TEA, [2] PPTs, xyl, Δ; or $R^3C(OEt)_3$, PPTs, xyl, Δ; (d) Na_2CO_3, aq MeOH (95%); (e) HCl, EtOAc.



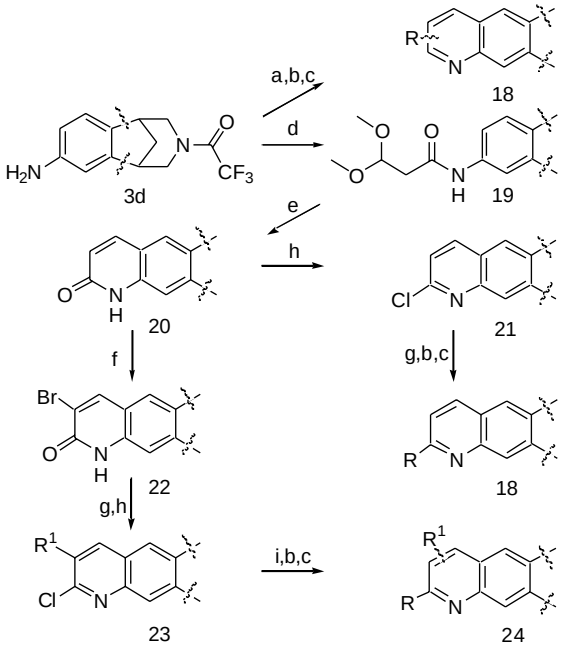
Scheme 7. Reagents and conditions: (a) AcCl, Et_3N, CH_2Cl_2(87%); (b) Lawesson's reagent; (44%); (c) K_3FeCN_6; (d) *t*-Boc_2O, Na_2CO_3, H_2O, CH_2Cl_2; (e) HCl, EtOAc; (c,d,e 14%).

Quinoline syntheses were accomplished as shown in Scheme 8. Aniline **3d** was converted via reaction with acrolein and its derivatives to give 2-, 3- or 4-substituted alkyl analogs **18** (**24**) following known procedures.[40] Alternatively, a variation of established methods[41] gave intermediate **19**, which was readily converted to 2-quinolone **20** by mild acid-catalyzed ring closure. This intermediate served to introduce 2-aryl substituents via Suzuki reaction with chloro-derivative **21**. 3-Aryl analogs were conveniently prepared via bromination of **20**, affording **22**, which was then converted using Suzuki coupling, chlorination (**23**), and hydrogenation to give 3-aryl derivatives **24**.

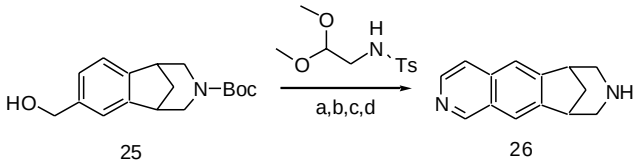
Isoquinoline preparation followed a related electrophilic cyclization strategy.[42] Benzyl alcohol **25** was accessed from the trifluoromethyl derivative conversion to the acid through treatment with warm sulfuric acid[43] and reduction with borane. Mitsunobu coupling with *N*-(2,2-dimethoxy-ethyl)-4-methyl-benzenesulfonamide gave an intermediate that readily cyclized in warm sulfuric acid to afford, after deprotection, isoquinoline **26** (Scheme 9).

Finally, benzisoxazole derivative **29** was accessed from **3c** via Baeyer–Villiger oxidation[44] and subsequent Fries rearrangement[45] to give **27**. Oxime formation and acylation gave **28**, which was converted by the action of NaH in DMF to the fused ring system (Scheme 10).[46] Deprotection and salt formation complete the synthesis.

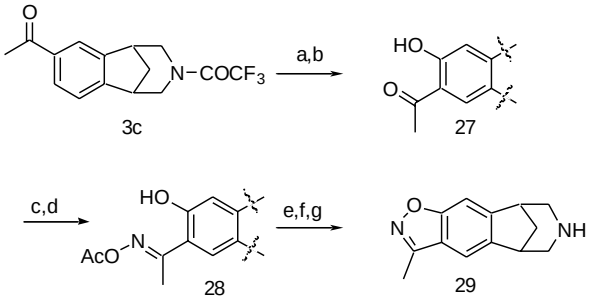
In vitro K_i values for 3,5-bicyclic aryl piperidine analogs at the α4β2 nAChR subtype were determined using radi-



Scheme 8. Reagents and conditions: (a) acrylates, $FeCl_3$, $ZnCl_2$, EtOH, 65 °C, (15–52%); (b) Na_2CO_3, aq MeOH; (c) HCl, EtOAc; (d) $(MeO)_2CHCH_2CO_2COCF_3$, aq $NaHCO_3$, toluene, (100%); (e) TFA, 20 °C (83%); (f) Br_2 AcOH; (g) 1.5 equiv $ArB(OH)_2$, 5 mol%, $Pd(PPh_3)_4$, 5 equiv Na_2CO_3, 95%EtOH/H_2O, 6–18 h, 90 °C; (h) $POCl_3$ (93%); (i) H_2, $Pd(OH)_2$, MeOH (100%).



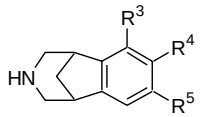
Scheme 9. Reagents and conditions: (a) PPh_3, $((CH_2)_5NCON)_2$, toluene (55%); (b) H_2SO_4, 80 °C, (18%); (c) *t*-Boc_2O, Na_2CO_3, H_2O, CH_2Cl_2; (d) HCl, EtOAc; (b,c,d 14%).



Scheme 10. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2,40 °C, (69%); (b) $AlCl_3$ 170 °C, 2 h (24%). (c) H_2NOH HCl, NaOAc, MeOH, 65 °C (93%); (d) Ac_2O, Et_3N, CH_2Cl_2(87%); (e) NaH, DMF; (f) Na_2CO_3, aq MeOH; (g) HCl, EtOAc (13%).

oligand displacement experiments (Tables 1 and 2).[47,48] A range of K_i values from 0.10 to 1700 nM at the α4β2 nAChR were observed for mono- and di-substituted derivatives (Table 1). Mono-substitution at the R^4-position and R^4–R^5-di-substitution confer greater

Table 1. In vitro affinity and agonist/antagonist activity of derivatives of **1** at the α4β2 nAChR subtype



Compound	R^3	R^4	R^5	Affinity α4β2 (K_i (nm))[a]	Functional activity (*h*α4β2 in oocytes)	
					Efficacy (%10 μM nicotine)[b]	Inhibition of nicotine (%)[c]
1	H	H	H	20[d]	0	35
1a	H	NO_2	H	0.75[d]	64	36
1b	H	Cl	H	0.20/1.6[d]	2	68
1c	H	Cl	Cl	0.10	4	81
1d	H	NH_2	H	100	0	25
1e	H	NHAc	H	75	—	—
1f	H	$SO_2N(CH_2)_2$	H	2.0	51	4
1g	H	$SO_2N(CH_2)_4$	H	3.1	32	24
1h	H	$COCH_3$	H	0.17	86	11
1i	H	CN	H	0.5	82	115[e]
1j	H	OH	H	2.8	27	32
1k	H	F	H	1.1	16	50
1l	H	F	F	0.28/0.28[d]	10	78
1m	H	CH_3	H	1.6	14	19
1n	H	CF_3	H	0.33	22	50
1o	H	2-pyr	H	68[d]	—	—
1p	H	3-pyr	H	120[d]	—	—
1q	CF_3	H	H	39	2	31
1r	F	H	H	200	—	—
1s	OH	H	H	1700	—	—
1t	Ph	H	H	1600/>500[d]	—	—

—, not determined.
[a] [^{3}H]Nicotine; *h*α4β2 nAChR in HEK293 cells.
[b] Percent response of 10 μM test compound relative to 10 μM (−)-nicotine (SEM ⩽ 10%).
[c] Percent response of 10 μM test compound against 10 μM nicotine (SEM ⩽ 10%).
[d] [^{3}H]Nicotine; rat cortex (N = 2–4).
[e] Potentiation of the nicotine response.

affinity and selectivity for the α4β2 nAChR subtype than those exhibited by the parent compound, **1a** (R^4 = H). Substitution at the R^3-position is poorly tolerated, as each group is >100× less potent than the corresponding R^4-derivative. Electron-withdrawing substitution favors potent affinity, whereas electron-donating groups such as hydroxyl-, amino-, and AcNH- impart weaker affinity. Dichloro- and difluoro-substituted derivatives at the R^4- and R^5-positions also exhibit potent affinity (**1c** and **1l**, respectively).

In vitro affinity values for fused heterocyclic derivatives appear in Table 2. The results for these electron-deficient heterocycles are consistent with those of the structurally related R^4- and R^5-di-substituted derivatives (Table 1), and electron-withdrawing substitution is again favored. Except in *N*-substituted benzimidazoles, aryl and electron-donating groups generally display lower affinity within any heterocyclic family. All heterocyclic families have examples with high affinity.

The in vitro functional agonist, partial agonist, and antagonist properties of compounds were evaluated in *Xenopus* oocytes expressing the *h*α4β2 nAChR and appear in Tables 1 and 2.[49] Owing to concerns regarding receptor desensitization that might result from repeated exposure, we examined a single test concentration of 10 μM, below the EC_{50} of nicotine (∼15 μM[21]), an agonist at the α4β2 nAChR.[3] This paradigm permits the identification of partial agonists that have less maximal efficacy than nicotine but exhibit greater functional potency. Thus, a full concentration response curve is not required for this two-step analog screening process. For example, at 10 μM, compounds that appeared to be high efficacy agonists or antagonists relative to 10 μM nicotine were eliminated from consideration as suitable partial agonists. Antagonist effects were evaluated by measuring a compound's ability to inhibit the current evoked by nicotine (10 μM). Values are expressed as the percentage reversal of nicotine's effect. Compounds exhibiting both partial agonist effects alone and antagonism of nicotine's effects at these concentrations were considered for in vivo evaluation.

3,5-Bicyclic aryl piperidine derivatives exhibit a range of functional activity in these measures, from antagonist (**1b**,**c**) to agonist (**1i**) (Table 1). 6,5-Fused heterocyclic analogs also exhibit a range of functional activity from agonist to antagonist at this concentration relative to 10 μM nicotine (**13**, **15**, **17**, and **29**). The functional properties appear to be remarkably sensitive to structural change as illustrated by benzimidazole and

benzisoxazole derivatives. For instance, all C-2 hydrogen-substituted analogs are partial agonists; however, C-2 methyl analogs are consistently agonists with greater efficacy than nicotine itself at 10 μM (as reflected by values greater than 100%: compare derivatives of **13** and **15**; Table 2, Fig. 1). Benzothiazole derivative **17** is a particularly efficacious agonist (405% rel. 10 μM nicotine). With further increases in C-2 substituent size, the affinity and efficacy of analogs decline—for instance, 2-ethyl-benzoxazole, isopropyl, and benzylbenzisoxazole derivatives exhibit partial agonist activity and reduced affinity (**13m**, **15b**–**e**).

6,6-Fused heterocycles also span a broad range of affinity and functional efficacy (Table 2). Analogs exhibiting partial agonist activity at 10 μM include quinoxalines, quinolines, and isoquinolines (e.g., **9a**, **9h**, **24i**, and **26**).

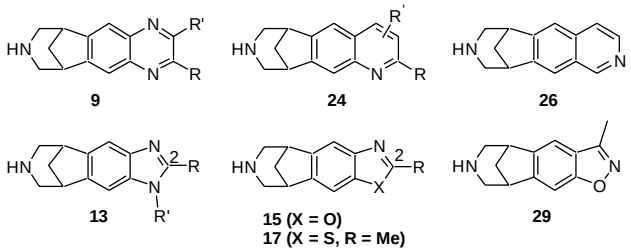


Figure 1. Fused heterocyclic analogs.

Compared to parent quinoxaline **9a** (varenicline), which displays high affinity, selectivity, and a desirable in vitro partial agonist profile, aryl-substituted examples are generally less potent. A similar trend to 6,5-fused heterocycles is observed within the quinoline derivatives: affin-

Table 2. In vitro affinity and agonist/antagonist activity of heterocycles at the α4β2 nAChR subtype

Compound	R	R′	Affinity	Functional activity (*h*α4β2 in oocytes)	
			α4β2 (K_i (nM))[a]	Efficacy (%10 μM nicotine)[b]	Inhibition of nicotine (%)[c]
9a	H	H	0.11/0.06[d]	68	32
b	Ph	H	390[d]	1.7	66
c	3-pyr	H	360[d]	0.2	11
d	4-PhCl	H	78[d]	—	—
e	4-PhOMe	H	26[d]	—	—
f	2,4-diClPh	H	88[d]	—	—
g	OH	H	<6.0[d]	—	—
h	Me	Me	0.55	18	22
24a	H	H	1.6[d]	—	—
b	OH	H	8.5[d]	—	—
c	Cl	H	0.27[d]	300	250[e]
d	Me	H	0.76[d]	160	140[e]
e	OMe	H	30[d]	104	167[e]
f	H	3-Me	0.65[d]	100	148[e]
g	H	3-Et	1.2[d]	11	82
h	H	3-Ph	132[d]	8.4	20
i	H	4-Me	1.8[d]	31	73
26	H	H	2.3[d]	11	48
13a	H	H	0.15	—	—
b	H	Me	0.75	—	—
c	H	Pr	0.36	96	39
d	H	Bu	0.20	64	36
e	H	*i*-Bu	0.30	84	36
f	H	Ph	0.14	9	85
g	Me	H	0.10	98	164[e]
h	Me	Me	0.31	180	139[e]
i	Me	Pr	0.19	200	143[e]
j	Me	*i*-Bu	0.28	190	140[e]
k	Me	Neo-pentyl	0.75	1	49
l	Me	Ph	<5.0	45	64
m	Ph	H	14[d]	32	29
15a	H	—	0.16	16	61
b	Me	—	0.15	180	190[e]
c	Et	—	12[d]	19	33
d	*i*-Pr	—	1.9/7.6[d]	18	21
e	Bn	—	34/100[d]	—	—
17	Me	—	0.12	405	206[e]
29	Me	—	0.13	130	21

—, not determined.

[a] [^{3}H]Nicotine; *h*α4β2 nAChR in HEK293 cells.

[b] Percent response of 10 μM test compound relative to 10 μM (−)-nicotine (SEM ⩽ 10%).

[c] Percent response of 10 μM test compound against 10 μM nicotine (SEM ⩽ 10%).

[d] [^{3}H]Nicotine; rat cortex (N = 2–4).

[e] Potentiation of the nicotine response.

ity decreases with bulkier substitution, whereas electron-withdrawing substituents confer increased affinity compared to electron-donating substituents (e.g., **24c**, **24b**).

The in vitro affinity of the bicyclic aryl piperidine analogs shown in Tables 1 and 2 is generally greater than that of the cytisine skeletal analogs (**2**).[20] Nitro-derivative **1a**, for instance, is significantly more potent and selective than the nitrated derivatives of **2** (α4β2 K_i: 0.75 vs. 5, 6, 13 nM).[20] In both series of compounds, small electron-deficient substitution confers high affinity. Significant effects on agonist activity at 10 μM are observed with structural change. One pronounced example within the 6,5-fused heterocyclic analogs is the impact of methyl substitution at the C-2 position of benzimidazoles, benzoxazoles, and benzothiazoles which suggests that consistent interactions at the ligand–receptor level are involved in this series of compounds.

Subtype selectivity measures appear in Table 3. As observed with analogs of **2**,[20] the derivatives of **1** in Tables 1 and 2 are selective for the α4β2 nAChR, exhibiting greater affinity than for other neuronal α3β4, α7, and peripheral α1βγδ nAChR subtypes. These compounds were further evaluated in vivo.

In vivo measures of a compound's agonist and antagonist activity were measured by their effects on the mesolimbic dopamine system and appear in Figure 2 for the compounds shown in Table 3. Dopamine turnover was determined as the change in postmortem concentrations of dopamine and its metabolites in the nucleus accumbens of male Sprague–Dawley rats (200–300 g).[50] The maximum dopamine turnover response for nicotine was found at a dose of 1 mg/kg s.c. and was used to characterize the response evoked by an agonist. A maximum well-tolerated dose of 5.6 mg/kg s.c. was determined for cytisine alone and revealed (partial) agonist activity of 40% of the nicotine response. Selected bicyclic aryl piperidines demonstrate similar partial agonist profiles (Fig. 2, filled bars). The data reveal the agonist

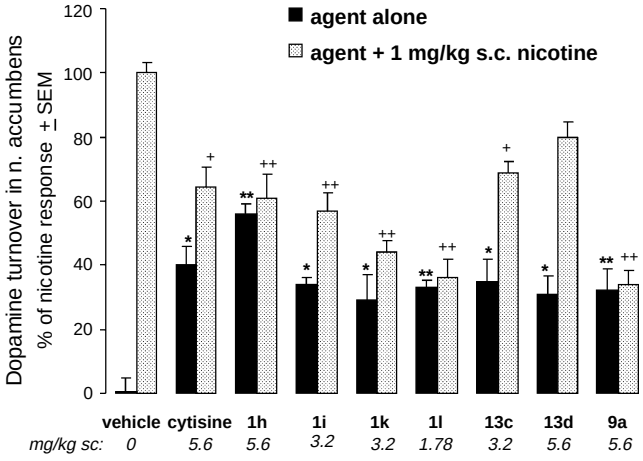


Figure 2. Effects of (−)-nicotine, (−)-cytisine, and selected agents on dopamine turnover in rat nucleus accumbens 1 h post-dose. All values are expressed as percentages of the effect of 1.0 mg/kg s.c. nicotine (100%) ± SEM (N = 5–10). Each compound was administered at the indicated dose (mg/kg s.c.) alone (filled bars) and with 1 mg/kg s.c. nicotine (shaded bars). *p < .05 agent alone vs vehicle; **p < .01 agent alone vs vehicle; ^{+}p < .05 and ^{++}p < .01: agent with nicotine vs nicotine alone (one-way ANOVA with post hoc Dunnett's test).

responses at maximum well-tolerated test doses; all are well below the effect of a maximum well-tolerated dose of nicotine.

Partial agonist activity was further demonstrated in vivo by evaluating the antagonist properties of (−)-cytisine and selected agents. Their ability to attenuate nicotine's effect on the mesolimbic dopamine system was determined in animals concurrently treated with 1 mg/kg s.c. nicotine. All compounds reduced the nicotine-induced increase in dopamine turnover in the nucleus accumbens (Fig. 2, shaded bars). The extent of reversal was incomplete for many analogs, especially **1i**, **13c**, and **13d**, suggesting incomplete receptor occupancy at these maximum well-tolerated doses. However, **1h**, **1l**, and **9a** (varenicline) fully blocked nicotine's effect: increases in dopamine turnover with these agents after 1 h were

Table 3. In vitro affinity at nAChR subtypes and agonist/antagonist activity at the α4β2 receptor

Compound	Affinity at nAChR subtypes (K_i (nM))				Functional activity (*h*α4β2 nAChR in oocytes)	
	α4β2[a]	α3β4[b]	α1βγδ[c]	α7[d]	Efficacy (%10 μM nicotine)[e]	Inhibition of nicotine (%)[f]
nic	1.6	530	6270	630	100	—
cyt	0.23	840	250	1420	56	30
1h	0.17	69	650	—	86	11
1i	0.50	122	520	—	82	115[g]
1k	1.1	1190	—	—	16	50
1l	0.28	1000	67	1200	10	78
13c	0.36	25	>910	—	96	39
13d	0.20	151	—	—	64	36
9a	0.11	240	3540	617	68	32

—, not determined.
[a] [^{3}H]Nicotine; *h*α4β2 nAChR in HEK293 cells (N = 2–4).
[b] [^{3}H]Epibatidine; IMR32 cells.
[c] [^{125}I]-α-Bungarotoxin; electroplax.
[d] [^{125}I]-α-Bungarotoxin; IMR32 cells.
[e] Percent response of 10 μM test compound relative to 10 μM (−)-nicotine (SEM ⩽ 10%).
[f] Percent response of 10 μM test compound against 10 μM nicotine (SEM ⩽ 10%).
[g] Potentiation of the nicotine response.

the same alone and in the presence of nicotine. These data demonstrate that all of these compounds are partial agonists and that more potent in vivo activity is observed with **1h**, **1l**, and **9a** than with (−)-cytisine.[51]

More effective therapeutic approaches for smoking cessation are needed than are currently available, as half of today's smokers will die of smoking-related illness. We have pursued selective nicotinic receptor partial agonists for this indication that are expected to provide sufficient dopaminergic tone to overcome the craving and withdrawal syndrome experienced upon quitting, while simultaneously attenuating the reinforcing actions of nicotine. The discovery that benzazapine **1** is a nicotinic agent has led to the study of a series of bicyclic aryl piperidines, or modified benzazapine derivatives, that exhibit significant affinity at the α4β2 nAChR. In vitro activity of the analogs is sensitive to structural modification, showing a striking dependence on both the steric and the electronic nature of appended residues. Furthermore, the series exhibits a range of partial agonist activity and potency in vivo. The methods reported here have identified agents with desirable in vivo partial agonist profiles, such as **1h**, **1l**, and **9a**, which are partial agonists alone and fully block nicotine's effect in vivo. From this series of compounds, varenicline (**9a**) has been progressed to human clinical trials as a smoking cessation treatment.[21]

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.035.

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