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Expedited Articles

Identification and Initial Structure−Activity Relationships of (*R*)-5-(2-Azetidinylmethoxy)-2-chloropyridine (ABT-594), a Potent, Orally Active, Non-Opiate Analgesic Agent Acting via Neuronal Nicotinic Acetylcholine Receptors

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New members of a previously reported series of 3-pyridyl ether compounds are disclosed as novel, potent analgesic agents acting through neuronal nicotinic acetylcholine receptors. Both (*R*)-2-chloro-5-(2-azetidinylmethoxy)pyridine (ABT-594, **5**) and its *S*-enantiomer (**4**) show potent analgesic activity in the mouse hot-plate assay following either intraperitoneal (ip) or oral (po) administration, as well as activity in the mouse abdominal constriction (writhing) assay, a model of persistent pain. Compared to the *S*-enantiomer and to the prototypical potent nicotinic analgesic agent (±)-epibatidine, **5** shows diminished activity in models of peripheral side effects. Structure−activity studies of analogues related to **4** and **5** suggest that the N-unsubstituted azetidine moiety and the 2-chloro substituent on the pyridine ring are important contributors to potent analgesic activity.

Introduction

The alkaloid (−)-epibatidine (**1**), a natural product isolated from South American frogs, was discovered in the 1970s as a non-opioid analgesic agent with a potency 200-fold greater than that of morphine in mice[1] and was later shown to exert its antinociceptive actions via a neuronal nicotinic acetylcholine receptor (nAChR)-mediated mechanism.[2,3] Extensive pharmacological evaluations have demonstrated that **1** is a highly potent agonist at several nAChR subtypes, including $\alpha4\beta2$ and $\alpha7$, predominant subtypes in the central nervous system (CNS), as well as nAChRs in peripheral autonomic ganglia and skeletal muscle (reviewed in ref 4). In the spinal cord, **1** binds to specific nAChR sites and elicits agonist responses, including stimulation of excitatory neurotransmitter release.[5,6] However, compound **1** is toxic or even lethal at doses only slightly higher than its effective analgesic dose.[7−9] Another notable feature of **1** is the lack of stereoselectivity in its analgesic and other pharmacological actions, a property which contrasts with the nAChR-mediated actions of several other chiral nAChR ligands including (*S*)-nicotine and (+)-anatoxin.[10]

The identification of nAChR modulators having a wider separation between antinociceptive and toxic effects affords the possibility of developing novel analgesic agents lacking typical opioid liabilities such as tolerance, respiratory depression, and suppression of gastrointestinal motility. Analgesic properties have

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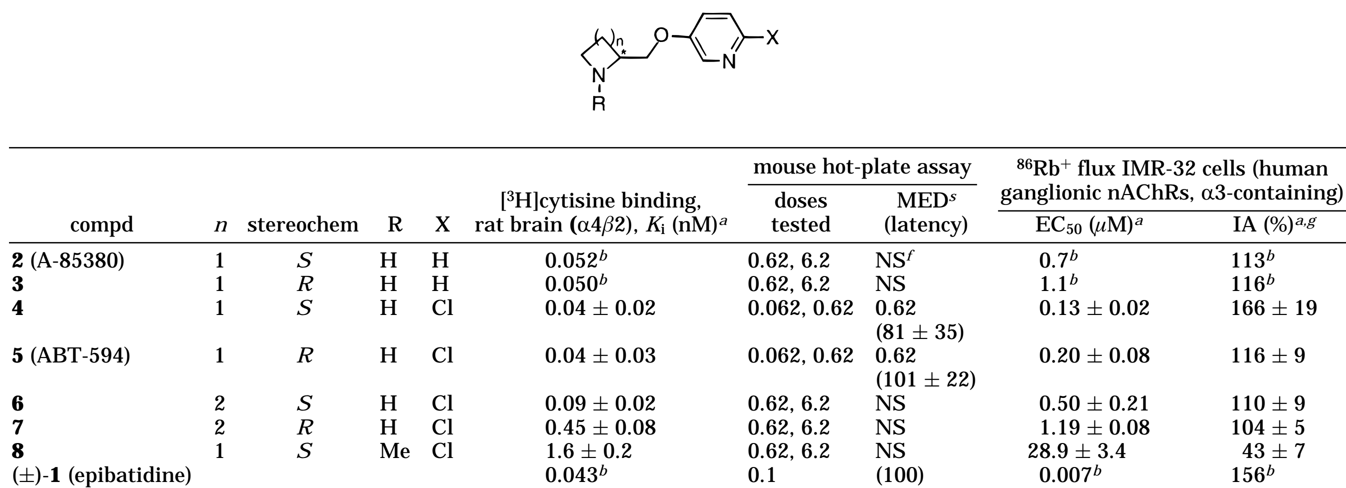
Table 1. Structures and Biological Data for 3-Pyridyl- and 2-Chloro-5-pyridyl Ethers

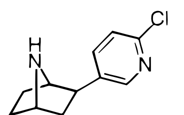


compd	*n*	stereochem	R	X	[^{3}H]cytisine binding, rat brain ($\alpha4\beta2$), K_i (nM)[a]	mouse hot-plate assay: doses tested	mouse hot-plate assay: MED[s] (latency)	^{86}Rb$^+$ flux IMR-32 cells (human ganglionic nAChRs, α3-containing): EC$_{50}$ (μM)[a]	^{86}Rb$^+$ flux IMR-32 cells (human ganglionic nAChRs, α3-containing): IA (%)[a,g]
2 (A-85380)	1	*S*	H	H	0.052[b]	0.62, 6.2	NS[f]	0.7[b]	113[b]
3	1	*R*	H	H	0.050[b]	0.62, 6.2	NS	1.1[b]	116[b]
4	1	*S*	H	Cl	0.04 ± 0.02	0.062, 0.62	0.62 (81 ± 35)	0.13 ± 0.02	166 ± 19
5 (ABT-594)	1	*R*	H	Cl	0.04 ± 0.03	0.062, 0.62	0.62 (101 ± 22)	0.20 ± 0.08	116 ± 9
6	2	*S*	H	Cl	0.09 ± 0.02	0.62, 6.2	NS	0.50 ± 0.21	110 ± 9
7	2	*R*	H	Cl	0.45 ± 0.08	0.62, 6.2	NS	1.19 ± 0.08	104 ± 5
8	1	*S*	Me	Cl	1.6 ± 0.2	0.62, 6.2	NS	28.9 ± 3.4	43 ± 7
(±)-**1** (epibatidine)					0.043[b]	0.1	(100)	0.007[b]	156[b]

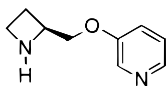
[a] Data are means ± SEM, $n \geq 3$. [b] Ref 14. [c] Highest dose reported is the highest dose tested that did not elicit overt signs of toxicity. [d] MED, minimum effective dose. [e] Expressed as percent of the latency observed for a 0.1 μmol/kg dose of (±)-**1** administered to a separate group of animals in the same experiment. This dose of (±)-**1** elicits a near-maximal effect for this compound in the mouse hot-plate assay. [f] NS = no significant effect observed at the doses tested. [g] IA, intrinsic activity, the maximum response observed as percent of response to 100 μM (*S*)-nicotine.

been reported for (*S*)-nicotine, but with much lower potency and shorter duration of action than **1**, and significant effects are not uniformly observed.[3,9,11] Other nAChR modulators, including (−)-cytisine (Cyt), *N*-methylcarbamoylcholine (MCC), (−)-lobeline, and *N,N*-dimethyl-*N'*-phenylpiperazinium, have been examined for antinociceptive effects, but these compounds also have side-effect liabilities, and under some conditions, Cyt, lobeline, and MCC have elicited hyperalgesic responses.[9,12,13]

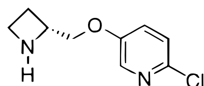
Previous reports from these laboratories have described a novel series of pyridyl ether compounds, including A-85380 (**2**), which possesses affinity for $\alpha4\beta2$ receptors comparable to that of (±)-**1**.[14,15] In this article, the evaluation of **2** as a potential analgesic agent is reported, together with further efforts to identify compounds in this series with potent analgesic activity and a satisfactory safety profile. This work has led to the identification of ABT-594 (**5**), a novel nAChR ligand which is shown to exert potent analgesic action in models of nociceptive and persistent pain and to possess substantially reduced activity at peripheral nAChRs compared to (±)-**1**.



(-)-epibatidine (**1**)



A-85380 (**2**)

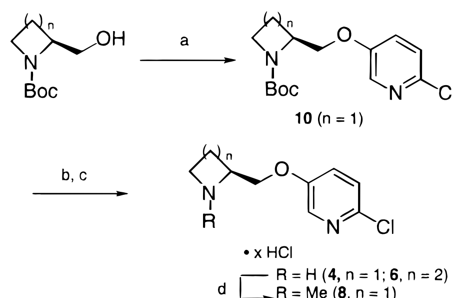


ABT-594 (**5**)

Chemistry

The structures of new compounds **4**−**8** are shown in Table 1. As illustrated in Scheme 1 for the *S*-stereochemical series, **4**−**8** were prepared using procedures analogous to those described previously.[14] Thus, Mitsunobu coupling of the known[16] 2-chloro-5-hydroxypyridine to the appropriate stereoisomer of *N*-Boc-2-azetidinemethanol[17] or *N*-Boc-2-pyrrolidinemethanol constituted the key ether-forming step for each compound. Boc deprotection was followed by conversion to a mono- or dihydrochloride salt. Reductive N-methylation of compound **4** was accomplished with formaldehyde in the presence of $NaBH_3CN$ to afford **8**. The preparation of 2-chloro-5-hydroxypyridine (**9**) from 5-amino-2-chloropyridine according to the literature method proceeded in low yield (<20%) in each of several trials.

Scheme 1[a]



[a] (a) PPh_3, DEAD, 2-chloro-5-hydroxypyridine (**9**), THF; (b) TFA, CH_2Cl_2 or 4 N HCl/dioxane; (c) recrystallization of hydrochloride or chromatography of free base and treatment with HCl/Et_2O; (d) aq HCHO, $NaBH_3CN$, HOAc.

Results and Discussion

Binding to the high-affinity nicotine binding site in rodent brain (principally the $\alpha4\beta2$ nAChR subtype) is commonly reported as an initial measure of the interaction of potential nAChR modulators with CNS nicotinic receptors. Accordingly, the potency of new compounds to displace [^{3}H]cytisine[18] from rat brain membranes is included for all new compounds (Table 1). However, since the nAChR subtype(s) mediating analgesia has not been identified, the mouse hot-plate assay was used as the primary screen to identify analgesic activity. In addition, to preliminarily assess the poten-

Table 2. Expanded in Vitro and in Vivo Profiles for Compounds **4** and **7** Compared with (±)-**1**

assay	**4**	**5**	(±)-**1**	**2**	**3**
		Binding K_i, nM[a]			
human α4β2 ([³H]cytisine, K177 cells)	0.034 ± 0.005	0.055 ± 0.005	0.05[i]	0.04[m]	
human α7 ([¹²⁵I]-α-Bgt, K28 cells)	2670 ± 1090	2060 ± 950	21[i]	148[m]	1275[m]
torpedo α1β1γδ ([¹²⁵I]-α-Bgt, electroplax)	3400 ± 1600	10000 ± 500	2.7[i]	314[m]	384[m]
		Functional Activity EC_{50}, μM (IA, %)[a]			
human α4β2 ($^{86}Rb^+$ flux, K177 cells)[b]	0.06 ± 0.02 (161 ± 4)	0.27 ± 0.13 (127 ± 13)	0.017 (156)[j]	0.7 (163)[j]	0.4 (159)[j]
human α7 (ion currents, oocytes)[c]	21 ± 7 (85 ± 6)	56 ± 20 (83 ± 7)	1.3 (59)[k]	8.9 (∼100)[m]	36 (∼100)[m]
human α1β1γδ ($^{86}Rb^+$ flux, TE671 cells)[d]	12.1 ± 0.3 (98 ± 9)	36 ± 12 (120 ± 20)	0.2 (∼140)[i]		
		In Vivo Effects[e]			
oral hot-plate (mouse) MED, po (μmol/kg)	6.2[f]	6.2[f]	0.5		
abdominal constriction (mouse) MED, ip (mmol/kg)	0.19[g]	0.19[g]	0.05		
increase in diastolic blood pressure (dog) (mm Hg), 10 nmol/kg, iv	67.3 ± 3.2[h]	23.2 ± 4.6[h]	110.7 ± 10.4[l]		
increase in heart rate (dog) (beats/min), 10 nmol/kg, iv	26.0 ± 7.8[h]	7.8 ± 2.9[h]	91.6 ± 41[l]		

[a] Data are means ± SEM, $n \geq 3$. IA, intrinsic activity, the maximum response observed as percent of response to standard agonist, as indicated. [b] Maximum response relative to 100 μM (*S*)-nicotine. [c] Maximum response relative to 10 mM acetylcholine; the maximum response of (*S*)-nicotine in this assay is 74% that of acetylcholine. [d] Maximum response is relative to 1 mM (*S*)-nicotine. [e] MED, minimum effective dose. [f] Dose range tested: 0.19−6.2 μmol/kg; at 6.2 μmol/kg, po, **4** and **5** were ca. 65% as efficacious as at 0.62 μmol/kg, ip. [g] Dose range tested: 0.062−0.62 μmol/kg. [h] Mean values ± SEM for $n = 6$. [i] Data from ref 4. [j] Data from ref 14. [k] Data from ref 31. [l] Mean values ± SEM for $n = 3$. [m] Data from ref 15.

tial for effects on the cardiovascular[19] and gastrointestinal[20] systems, compounds also were evaluated for functional activation of human sympathetic ganglionic-type nAChRs in IMR-32 cells.[21]

Although compound **2** possesses affinity for central nAChRs comparable to that of epibatidine,[14,15] this analogue does not elicit a significant antinociceptive effect in the mouse hot-plate assay at doses up to 6.2 μmol/kg, a dose 62-fold higher than that at which epibatidine shows a robust effect (Table 1). Compound **3**, the enantiomer of **2**, similarly has high affinity for central α4β2 nAChRs and also does not show a significant analgesic effect at doses up to 6.2 μmol/kg.

In contrast to the results with **2** and **3**, the corresponding 6-chloro analogues **4** and **5** demonstrated analgesic effects at 0.62 μmol/kg comparable to that of epibatidine at 0.1 μmol/kg. On the other hand, the effect of the chloro substituent of **4** or **5** on affinity for the [³H]-cytisine binding site is small, in analogy to results reported for (+)-**1**.[3] Also, like epibatidine,[3] little enantioselectivity is observed for these compounds with respect to analgesic activity. Compounds **4** and **5** are more potent and (in the case of **4**) more efficacious than the corresponding deschloro analogues to activate cation flux in IMR-32 cells. It is noteworthy that some enantioselectivity is observed for **4** vs **5** in IMR-32 cells, suggesting the potential for a difference in propensity for these compounds to elicit peripheral side effects (vide infra). In comparison with (±)-**1**, compound **5** is approximately 6-fold less potent in the hot-plate assay but nearly 30-fold less potent and also less efficacious at ganglionic-like nAChRs in IMR-32 cells. Both the analgesic effect and the activity in IMR-32 cells elicited by **4** and **5** were blocked by the noncompetitive nAChR antagonist mecamylamine; the analgesic effects were not blocked by the opiate antagonist naloxone (data not shown).

Table 1 shows the effects of azacycle ring size and azetidine N-methylation on activity in the above tests. Pyrrolidine analogues **6** and **7** failed to show significant activity in the hot-plate assay at the doses where **4** and **5** showed robust effects and also were inactive at 10-fold higher doses. In the in vitro tests, some decrease in potency resulted from the increased ring size, but this decrease was in no case greater than 12-fold. Interestingly, unlike the corresponding azetidines, pyrrolidines **6** and **7** exhibit modest stereoselectivity with respect to binding affinity. N-Methylation of **4** (compound **8**) results in a substantial attenuation of [³H]cytisine binding affinity and ganglionic-like activity, as well as loss of analgesic activity. Thus, within the 2-chloro-5-pyridyl ether series, potent analgesic efficacy resides nonstereoselectively in N-unsubstituted azetidines **4** and **5** and not in the corresponding pyrrolidines.

Additional in vitro and in vivo data for compounds **4** and **5** compared with (±)-**1**, **2**, and **3** are presented in Table 2. The affinities of these compounds for [³H]-cytisine binding sites in cells expressing human α4β2 receptors are similar to those found in rat brain (cf. Table 1), further supporting correlations established previously for binding affinities in these two systems.[22] In cells expressing human α7 nAChRs, compounds **4** and **5** exhibit, respectively, 127- and 98-fold lower affinities than (±)-**1**. Comparison with the values for **2** and **3** shows that the chloro substituent has an appreciable detrimental effect on affinity for the α7 nAChR only in the *S*-stereochemical series. In *Torpedo californica* membranes, which express nAChRs similar to those found in mammalian skeletal muscle, (±)-**1** dis-

places [^{125}I]-α-Bgt with nanomolar potency, whereas **4** and **5** are approximately 1250- and 3700-fold weaker, respectively, and deschloro analogues **2** and **3** have intermediate affinities.

In vitro functional assays for the human α4β2, human α7, and putative human α1β1γδ nAChR subtypes indicate that (±)-**1** and **2**−**5** generally act as agonists at these subtypes, with efficacies in some cases exceeding that of (*S*)-nicotine. It is evident from comparing the activities of **2** and **3**, which were inactive in the hot-plate assay, with those of compound **5**, in particular, that potent functional activity at α4β2 nAChRs does not provide a clear prediction of analgesic activity. Functional activity at the α7 nAChR also does not appear to be predictive, consistent with the failure of the α7 antagonist methyllycaconitine to block the analgesic actions of epibatidine.[9] Thus, based on the data presented in Tables 1 and 2, it is not apparent that activity in any of the in vitro assays representing major neuronal nAChRs (sympathetic ganglionic-like, α4β2, or α7) correlates with analgesic activity. The most obvious rationale is that the analgesic activity is mediated by a nAChR subtype different from those represented by the in vitro assays. However, differences in susceptibility of the compounds to metabolic processes cannot be ruled out, and it should be appreciated that the functional assays are based on human, not rodent, nAChR subtypes. In TE671 cells, which express human muscle-type nAChRs,[23] compounds **4** and **5** are 60- and 180-fold less potent, respectively, than (±)-**1**, suggestive of a reduced potential for **4** or **5** to elicit side effects related to actions at skeletal muscle nAChRs. In general, the data in Table 2 indicate that relative functional potencies are not well-correlated with relative binding affinities in systems representing the same nAChR subtype. The disparities in these measures may reflect interactions with different states of the respective nAChRs[24] or alternatively could be a consequence of species differences (α1β1γδ) or differences in the expression system (α7).

Further evaluation of analgesic effects in vivo reveals that compounds **4** and **5** are effective in the hot-plate assay also following oral administration, whereby the minimally effective dose (MED) for each compound is 10-fold higher than that found with ip administration (Table 2). Compounds **4** and **5** also show significant effects in the mouse abdominal constriction model of persistent pain at 0.19 μmol/kg, ip, a dose approximately 4-fold higher than the minimally effective dose for (±)-**1**. The effects of compounds **4** and **5** on cardiovascular parameters in anesthetized dogs also are shown in Table 2, and whereas both compounds have improved profiles compared to (±)-**1**, the observation of differential responses to **4** vs **5** is noteworthy. Thus, administration of the *R*-enantiomer **5** results in both a significantly lower change in diastolic blood pressure as well as a smaller change in heart rate compared to the effects of the *S*-enantiomer **4**. A current hypothesis is that cardiovascular effects are related to the potency and efficacy of compounds to activate human sympathetic ganglionic-like nAChRs in IMR-32 cells (Table 1).

Conclusion

In summary, compounds **4** and **5** have been identified as members of the pyridyl ether series of nAChR modulators having potent, orally effective analgesic activity in mice. On the basis of blockade by mecamylamine, the analgesic properties are mediated by neuronal nAChRs. The chloro substituent and the azetidine ring moieties of **4** and **5** are important structural elements for potent analgesic activity in this series. Compounds **4** and **5** are less potent than (±)-**1** to activate peripheral skeletal muscle-type nAChRs and nAChRs in sympathetic ganglia, with compound **5** showing 180- and 28-fold lower potencies, respectively, than (±)-**1** in these measures. Whereas little stereoselectivity is exhibited with respect to the analgesic activities of **4** and **5**, compound **5** has less intrinsic activity than **4** at nAChRs found in autonomic ganglia and furthermore elicits less pronounced cardiovascular effects in anesthetized dogs. While such stereoselectivity would not have been anticipated based on the general precedents set by **1**[4] and **2**,[15] the observation of enhanced separation of analgesic effects from activation of peripheral ganglionic nAChRs and elicitation of cardiovascular effects appears to represent an important advance, both in the recognition that such separation is feasible as well as in the potential usefulness of **5** as a therapeutic agent. Thus **5** (ABT-594) represents an attractive candidate for further evaluation[25−28] as an analgesic agent with a novel mechanism of action for the management of pain states.

Experimental Section

General. Proton magnetic resonance spectra were obtained on a Nicolet QE-300 (300 MHz) or a General Electric GN-300 (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) downfield relative to Me_4Si as an internal standard. Mass spectra were obtained with a Hewlett-Packard HP5965 spectrometer; CI/NH_3 indicates chemical ionization mode in the presence of ammonia. Combustion analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Melting points were determined on a Buchi melting point apparatus with a silicone oil bath and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. Chromatographies were carried out in flash mode using silica gel 60 (230−400 mesh) from E. Merck.

2-Chloro-5-hydroxypyridine (9). 2-Chloro-5-aminopyridine (156 mmol; Aldrich) was treated with $NaNO_2$ under acidic conditions according to the procedure of Effenberger et al.[16] The crude product was purified by chromatography (Et_2O/hexane, 4:1) to afford the title compound as a pale-yellow solid in 8% yield: ^{1}H NMR (MeOH-d_4) δ 7.22 (m, 2H), 7.85 (m, 1H); MS (CI/NH_3) *m/z* 130/132 (M + H)$^+$, 147/149 (M + NH_4)$^+$.

***(S)*-2-Chloro-5-[[1-(*tert*-butyloxycarbonyl)-2-azetidinyl]-methoxy]pyridine (10).** To a stirred solution of triphenylphosphine (8.57 g, 32.7 mmol) in THF (110 mL) at 0 °C was added diethyl azodicarboxylate (5.1 mL, 32.7 mmol). After the mixture stirred for 15 min, a solution of (*S*)-1-(*tert*-butyloxycarbonyl)-2-azetidinemethanol[14] (6.1 g, 5.1 mmol) in THF (33 mL) was added followed by addition of **9** (3.0 g, 23.16 mmol). The mixture was allowed to warm to room temperature and stir for 4 h. The solvent was evaporated, and the residue was chromatographed (EtOAc/hexane, 1:6 to 1:4) to afford a viscous pink oil (7.4 g, 107%): ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 9H), 2.15 (m, 1H), 2.32 (m, 1H), 3.77 (t, J = 7.7 Hz, 2H), 4.20 (dd, J = 3.3, 11.0 Hz, 1H), 4.36 (dd, J = 4.4, 11.0 Hz, 1H), 4.44 (m, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.48 (dd, J = 3.3, 8.8 Hz, 1H), 8.14 (d, J = 3.3 Hz, 1H); MS (CI/NH_3) *m/z* 299/301 (M + H)$^+$, 316/318 (M + NH_4)$^+$.

***(S)*-2-Chloro-5-(2-azetidinylmethoxy)pyridine (4), Dihydrochloride.** Compound **10** (1.02 g, 3.43 mmol) was treated with 4.5 N HCl/dioxane (10 mL) at room temperature for 0.5 h. The solvent was removed, and the residue was recrystallized from MeOH/Et_2O to yield colorless needles (340

mg, 36%): mp 113−115 °C; ^{1}H NMR (D_2O) δ 2.68 (q, J = 8.5 Hz, 2H), 4.02−4.19 (m, 2H), 4.42 (d, J = 4.4 Hz, 2H), 4.93 (m, 1H), 7.47 (d, J = 8.9 Hz, 1H), 7.57 (dd, J = 8.9, 3.0 Hz, 1H), 8.15 (d, J = 3.0 Hz, 1H); MS (CI/NH_3) m/z 199/201 (M + H)$^+$. Anal. ($C_9H_{11}ClN_2O$·2HCl) C, H, N.

4, Hydrochloride. Crude azetidine dihydrochloride, obtained as described above, was converted to the free base by extraction from aqueous K_2CO_3 and then chromatographed (MeOH/$CHCl_3$, 1:9, then MeOH/$CHCl_3$/concentrated NH_4OH, 1:9:0.05). The monohydrochloride was prepared by treatment of the purified free base in Et_2O with ca. 1 equiv of HCl in Et_2O and recrystallization from MeOH/Et_2O: ^{1}H NMR (D_2O) δ 2.65−2.74 (m, 2H), 4.03−4.30 (2, 2H), 4.43 (d, J = 4.4 Hz, 2H), 4.95 (m, 1H), 7.48 (d, J = 8.9 Hz, 1H), 7.56 (dd, J = 3.0, 8.9 Hz, 1H), 8.18 (d, J = 3.0 Hz, 1H); MS (CI/NH_3) m/z 199 (M + H)$^+$. Anal. ($C_9H_{11}ClN_2O$·HCl) C, H, N.

(*R*)-1-(*tert*-Butyloxycarbonyl)-2-azetidinemethanol (11). A solution of (*R*)-1-(benzyloxycarbonyl)azetidine-2-carboxylic acid[14] (8.8 g, 37.5 mmol) in MeOH was stirred in the presence of 10% Pd/C under a H_2 balloon for 3 days. The catalyst was removed by filtration and washed with MeOH and H_2O. The combined filtrates were concentrated, and the residue was dissolved in 1:1 H_2O/dioxane (180 mL). The mixture was treated with NEt*i*-Pr_2 (7.8 mL, 45.1 mmol) and di-*tert*-butyl dicarbonate (9.8 g, 45.1 mmol) and stirred at room temperature. Following consumption of the starting material (TLC; $CHCl_3$/MeOH/concentrated NH_4OH, 10:4:1), the mixture was partitioned between EtOAc and 15% NaOH; then the separated aqueous phase was acidified with 2 N HCl and extracted with CH_2Cl_2 (2×). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated. The residue was dissolved in THF (75 mL) under N_2, and the solution was stirred during dropwise addition of 1.0 M BH_3 in THF (56 mL, 56 mmol). After stirring for an additional 0.5 h, the mixture was treated by careful addition of saturated aqueous $NaHCO_3$ and then partitioned between H_2O and CH_2Cl_2. The separated aqueous phase was extracted with additional CH_2Cl_2; then the combined organic phases were dried ($MgSO_4$) and concentrated. The residue was chromatographed (EtOAc/hexane, 1:2) to afford a colorless oil (3.71 g, 53%): ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 9H), 1.93 (m, 1H), 2.17 (m, 1H), 3.68−3.92 (m, 4H), 4.41−4.50 (m, 1H); MS (CI/NH_3) 188 (M + H)$^+$; $[\alpha]^{23}_D$ +21° (c 0.90, $CHCl_3$).

General Procedure for Compounds 5−7. Compound **11**, (*S*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinemethanol,[14] and (*R*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinemethanol[14] were converted respectively to compounds **5−7** in analogous fashion to the preparation of **4**.

(*R*)-2-Chloro-5-(2-azetidinylmethoxy)pyridine (5), hydrochloride: mp 116−117 °C; $[\alpha]^{23}_D$ +8.6° (c 0.52, MeOH); ^{1}H NMR (D_2O) δ 2.65−2.76 (m, 2H), 4.03−4.21 (m, 2H), 4.42 (d, J = 4.1 Hz, 2H), 4.96 (m, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.56 (dd, J = 3.0, 8.8 Hz, 1H), 8.15 (d, J = 3.0 Hz, 1H); MS (CI/NH_3) m/z 199/201 (M + H)$^+$. Anal. ($C_9H_{11}ClN_2O$·HCl) C, H, N.

(*S*)-2-Chloro-5-(2-pyrrolidinylmethoxy)pyridine (6), dihydrochloride: mp 136−138 °C; $[\alpha]^{23}_D$ +12.7° (c 0.60, MeOH); ^{1}H NMR (D_2O) δ 1.90−2.35 (m, 4H), 3.27−3.46 (m, 2H), 4.11 (m, 1H), 4.24 (dd, J = 5.8, 11.0 Hz, 1H), 4.46 (dd, J = 3.0, 11.0 Hz, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.52 (dd, J = 8.8, 3.0 Hz, 1H), 8.13 (d, J = 3.0 Hz, 1H); MS (CI/NH_3) m/z 213/215 (M + H)$^+$, 230/232 (M + NH_4)$^+$. Anal. ($C_{10}H_{13}ClN_2O$·2HCl) C, H, N.

(*R*)-2-Chloro-5-(2-pyrrolidinylmethoxy)pyridine (7), hydrochloride: mp 157−159 °C; ^{1}H NMR (D_2O) δ 1.88−2.34 (m, 4H), 3.41 (t, J = 7.2 Hz, 2H), 4.11 (m, 1H), 4.45 (dd, J = 7.7, 10.7 Hz, 1H), 7.43−7.53 (m, 2H), 8.10 (d, J = 3.0 Hz, 1H); MS (CI/NH_3) m/z 213/215 (M + H)$^+$, 230/232 (M + NH_4)$^+$. Anal. ($C_{10}H_{13}ClN_2O$·1.3HCl) C, H, N.

(*S*)-2-Chloro-5-[(1-methyl-2-azetidinyl)methoxy]pyridine (8), Hydrochloride. The free base of compound **4** (628 mg, 2.67 mmol) in a mixture of H_2O (4 mL) and HOAc (1 mL) was treated with 37% HCHO (2 mL) and $NaCNBH_3$ (500 mg, 8 mmol). The stirred solution was treated with additional HOAc as necessary to maintain the acidity at approximately pH 5. When the reaction was complete (TLC), 2 N HCl (2 mL) was added, and the mixture was extracted with EtOAc. The aqueous phase was made basic with K_2CO_3 and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated, and the residue was chromatographed (MeOH/$CHCl_3$, 2:98 to 5:95) to afford the free base (210 mg, 37%). The base (203 mg) in Et_2O (12 mL) was treated with ca. 1 equiv of HCl in Et_2O to give the monohydrochloride (222 mg, 93%): mp 108−110 °C; ^{1}H NMR (D_2O) δ 2.55−2.73 (m, 2H), 2.97 (s, 3H), 4.00 (m, 1H), 4.23 (m, 1H), 4.39−4.52 (m, 2H), 4.78 (m, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.56 (dd, J = 3.3, 8.8 Hz, 1H), 8.16 (d, J = 3.3 Hz, 1H); MS (CI/NH_3) m/z 213/215 (M + H)$^+$, 230/232 (M + NH_4)$^+$. Anal. ($C_{10}H_{13}ClN_2O$·1.2HCl) C, H, N.

In Vitro Assays. The following assays were performed according to previously described procedures: displacement of [^{3}H]cytisine from rat whole brain[29] or K177 cells expressing human $\alpha 4\beta 2$ nAChRs;[22] displacement of [^{125}I]-α-bungarotoxin (α-Bgt) from K28 cells expressing human $\alpha 7$ homomers;[29] displacement of [^{125}I]-α-Bgt from *Torpedo* electroplax;[30] $^{86}Rb^+$ efflux from the human neuroblastoma cell line IMR-32;[14] $^{86}Rb^+$ efflux from the human medulloblastoma cell line TE671;[30] $^{86}Rb^+$ efflux from K177 cells expressing recombinant $\alpha 4\beta 2$ receptors;[14] channel currents at $\alpha 7$ homomers expressed in oocytes.[31] Intrinsic activities in ion flux assays are expressed as percent of the maximal response of (*S*)-nicotine at a concentration of 100 μM (K177 and IMR-32 cells) or 1 mM (TE671 cells). Intrinsic activities for $\alpha 7$ channel currents are expressed as percent of the maximal response of acetylcholine at a concentration of 10 mM.

Mouse Hot-Plate Assay. An automated hot-plate analgesia monitor (model AHP16AN, Omnitech Electronics, Inc., Columbus, OH) was used, with the hot-plate temperature maintained at 55 °C. Separate groups of eight mice were utilized for each dose group. Drugs were dissolved in 0.9% sterile saline and administered ip or po in a volume of 10 mL/kg 30 min prior to testing. Doses were selected in log unit increments up to 62 μmol/kg, with the highest dose reported being that which did not elicit overt signs of toxicity, e.g., seizures or death. An increase in the latency until the tenth jump relative to the saline control group was considered an antinociceptive (i.e., analgesic) effect. A cutoff time of 180 s was utilized. (±)-Epibatidine (0.1 μmol/kg, ip) was administered to a separate group of animals as a positive control in all experiments.

Mouse Abdominal Constriction Assay. Each mouse was given an ip injection of the chemical irritant phenyl-*p*-quinone (68 μmol/kg dissolved in 5% EtOH, 10 mL/kg). Beginning 5 min after this injection, animals were observed for the presence of characteristic stretching or writhing responses for a 10-min period. Mice displaying one or more of these responses were designated as responders. Test compounds were dissolved in 0.9% sterile saline and injected ip (10 mL/kg) 30 min before the injection of phenyl-*p*-quinone. A reduction in the number of animals responding to phenyl-*p*-quinone relative to the number responding in the saline control group was considered an antinociceptive effect. Separate groups of 10 mice were used for each dose group. (±)-Epibatidine (0.05 μmol/kg, ip) was used as a positive control in all experiments.

Measurement of Cardiovascular Effects in Dogs. Male beagle dogs were anesthetized with pentobarbital (35 mg/kg, iv) followed by constant iv infusion of pentobarbital (5 mg/kg/h). The animals were ventilated with room air by means of a mechanical respiration pump. Blood pressure was measured using a dual tip micromanometer catheter (Millar, model SPC-770, 7F) inserted into the heart left ventricle via the carotid artery. Compounds were injected into the right femoral vein via catheter. Hemodynamic variables were computed using XYZ Real Time Spreadsheet software on a signal processing workstation (Modular Instruments, Inc.). Sixty minutes were allowed following surgery to achieve a steady-state baseline for the measured variables. Test compounds were administered by iv bolus (10 nmol/kg) and compared for their relative

ability to elicit changes in blood pressure and heart rate over a 5-min data collection period.

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