Notes

Structure−Activity Studies on 2-Methyl-3-(2(*S*)-pyrrolidinylmethoxy)pyridine (ABT-089): An Orally Bioavailable 3-Pyridyl Ether Nicotinic Acetylcholine Receptor Ligand with Cognition-Enhancing Properties

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2-Methyl-3-(2(*S*)-pyrrolidinylmethoxy)pyridine, ABT-089 (**S-4**), a member of the 3-pyridyl ether class of nicotinic acetylcholine receptor (nAChR) ligands, shows positive effects in rodent and primate models of cognitive enhancement and a rodent model of anxiolytic activity and possesses a reduced propensity to activate peripheral ganglionic type receptors. The profiles of **S-4**, its *N*-methyl analogue, and the corresponding enantiomers across several measures of cholinergic channel function *in vitro* and *in vivo* are presented, together with *in vitro* metabolism and *in vivo* bioavailability data. On the basis of its biological activities and favorable oral bioavailability, **S-4** is an attractive candidate for further evaluation as a treatment for cognitive disorders.

Introduction

The potential ability for compounds acting at neuronal nicotinic acetylcholine receptors (nAChRs) to exert beneficial effects in central nervous system disorders has prompted a search for agents having improved safety and pharmacokinetic profiles relative to those of (*S*)-nicotine.[1−3] ABT-418 (**1**, Figure 1), an analogue of (*S*)-nicotine in which the pyridine ring is replaced by the 3-methyl-5-isoxazole moiety, has been shown to possess cognitive-enhancing and anxiolytic-like activities in animal models with an improved safety profile compared to that of nicotine.[4] Key objectives for ongoing efforts in this area have been to achieve further improvements in the margin of safety and to identify compounds with oral bioavailability.

Recently, we disclosed a novel series of 3-pyridyl ether compounds, including A-84543 (**2**) and A-85380 (**3**),[5] which possess subnanomolar affinity for nAChRs and stimulate ion flux at human nAChRs with efficacies similar to the efficacy of nicotine. A particularly attractive feature of this series was the opportunity for ready preparation of enantiomerically pure analogues from the commercially available chiral pool. During the course of structure−activity studies, numerous analogues of **2** and **3** were prepared and screened for binding affinity to central nAChRs, for activity in *in vitro* functional assays (ion flux), for effects on temperature and activity *in vivo*, and in rodent behavioral models of anxiolytic-like action and cognition-enhancing properties. On the basis of the hypothesis that ion flux in IMR-32 cells would serve as a model for activity at peripheral ganglionic receptors, and hence of potential cardiovascular and gastrointestinal liabilities,[6,7] it was of particular interest to identify compounds which elicited improvements in measures of cognitive enhancement and anxiolytic-like activity but showed reduced ability relative to **1**−**3** to stimulate ion flux in this cell line. Here we report that this strategy has led to the identification of **S-4** (ABT-089), a member of the 3-pyridyl ether series. The properties of **S-4** in *in vitro* binding and functional assays and in several behavioral tests are compared with those of **1**, **2**, **3**, and several close analogs of **S-4**. Further characterization in an aged primate model of cognitive enhancement demonstrates that **S-4** elicits favorable effects in this model. Moreover, **S-4** is shown to be orally bioavailable. Taken together, these data indicate that **S-4** is an orally bioavailable nAChR ligand with cognitive-enhancing properties and diminished potential for side effects mediated by peripheral ganglionic receptors.

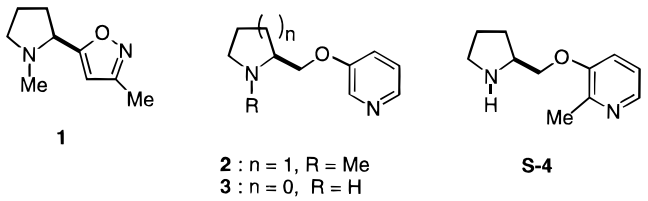

Figure 1. Structures of ABT-418 **1** and analogs **2**, **3**, and **S-4**.

Chemistry. New compounds **S-4**, its *N*-methyl analogue [2-methyl-3-[[((*S*)-1-methyl-2-pyrrolidyl)methyl]-oxy]pyridine, **S-5**], and the corresponding enantiomers [2-methyl-3-[((*R*)-2-pyrrolidylmethyl)oxy]pyridine, **R-4**] and [2-methyl-3-[[((*R*)-1-methyl-2-pyrrolidyl)methyl]-

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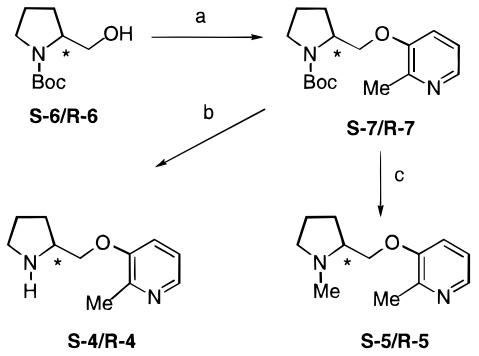
Scheme 1[a]



[a] (a) 2-Methyl-3-pyridinol, diethyl azodicarboxylate, triphenylphosphine, THF; (b) TFA, CH_2Cl_2; (c) $(HCHO)_n$, HCO_2H, 70 °C.

Table 1. Radioligand Binding and Functional Properties of Cholinergic Channel Ligands[a]

	K_i (nM)		$^{86}Rb^+$ flux, human α3β4x (IMR-32 cells)	
compound	[^{3}H]cytisine binding	[^{125}I]-α-Bgt binding (K28 cells)	EC_{50} (μM)	% max[b]
S-4 (ABT-089)	16.7 ± 3	>10000	150 ± 20	8 ± 4
S-5	27.7 ± 2	>10000	25 ± 6	29 ± 11
R-4	39 ± 4	>10000	50 ± 11	11 ± 5
R-5	3000 ± 360	>10000	NT	NT
2 (A-84543)	0.28 ± 0.05	1530 ± 320	63 ± 9	100 ± 10
1 (ABT-418)	4.2 ± 0.6	4050 ± 795	64 ± 12	85 ± 4
(*S*)-nicotine[c]	1 ± 0.1	1610 ± 220	21 ± 3	(100)

[a] Values represent mean ± SEM; $n = 3-5$. [b] % max represents the maximal efficacy of the compounds relative to 100 μM nicotine. NT = not tested. [c] Data from refs 5 and 17.

oxy]pyridine, **R-5**] were synthesized by employing the Mitsunobu reaction as a key step as described previously (Scheme 1).[5] Following Mitsunobu couplings, *N*-Boc-protected derivatives (**S-7** or **R-7**) were deprotected by trifluoroacetic acid to give *N*-H products (**S-4** or **R-4**). *N*-Methylpyrrolidines were prepared by reductive methylation of the *N*-Boc-protected secondary amines with formaldehyde and formic acid. Final compounds were isolated and characterized as dihydrochlorides.

Results and Discussion

***In Vitro* Pharmacology.** Binding affinities to [^{3}H]-(−)-cytisine sites and to α7 homomeric receptors are shown in Table 1 for **S-4**, **R-4**, **S-5**, **R-5**, and reference compounds **1** and **2**, together with functional data measured at peripheral ganglionic-type receptors in IMR-32 cells. Compounds **S-4**, **R-4**, and **S-5** are all in a similar potency range for binding to [^{3}H]-(−)-cytisine sites, whereas **R-5** possesses much lower affinity. This pattern of affinities with respect to stereochemistry and *N*-methylation is similar to that observed for the corresponding 2-desmethyl series,[5] although the compounds of the present series are consistently 100−300-fold weaker than those lacking the 2-methyl substituent. None of analogues **S-4**, **R-4**, **S-5**, and **R-5** showed detectable affinity for human α7 receptors at up to 10 μM.

With respect to functional activity, whereas **1** and **2** elicited maximal responses at the ganglionic subtype comparable to that of (*S*)-nicotine, **S-4**, **R-4**, and **S-5** showed comparatively low efficacy at this subtype. It is apparent by comparison of the functional data with

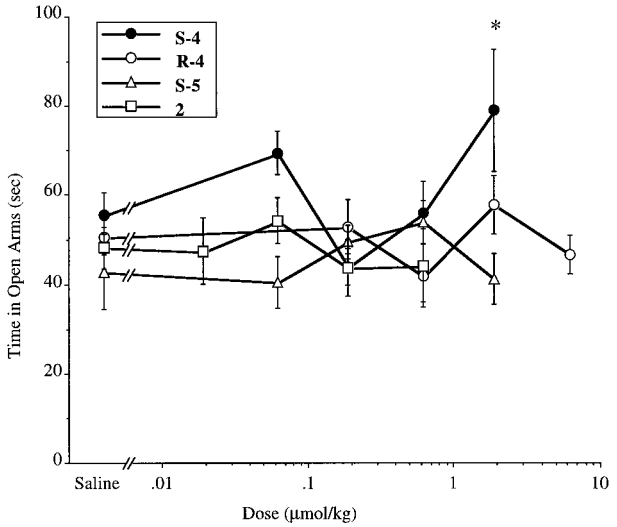


Figure 2. The effects of **2**, **S-4**, **S-5**, and **R-4** on elevated plus-maze exploration in mice. Shown are the mean times spent in the open arms in seconds (±SEM). Significant improvement in retention test performance was observed for **S-4** [$F(5,69) = 3.21$; $p < 0.05$]. *Significantly different from appropriate saline group, $p < 0.05$.

those from the previous report[5] that the 2-methyl substituent on the pyridine ring of **S-4**, **R-4**, and **S-5** is principally responsible for the reduced activity at ganglionic-type receptors. Thus, compound **S-5** shows only 29% of the response in IMR-32 cells compared to **2**. Similarly, the 2-desmethyl analogues of **S-4** and **R-4** possessed efficacy comparable to that of (*S*)-nicotine in IMR-32 assay,[5] whereas **S-4** and **R-4** stimulated only 8% and 11% of the nicotine response, respectively. A separate report describes more detailed *in vitro* characterization of **S-4** and **R-4**, including evidence that several effects of **S-4** (dopamine release, ion flux in TE671 cells and neuroprotective properties) are mediated by cholinergic channels.[8] Interestingly, **S-4** is not a potent activator of ion flux mediated by human α4β2 receptors.[8]

***In Vivo* Pharmacology.** Compounds **2**, **S-4**, **R-4**, and **S-5** were evaluated in the elevated plus maze as a screen for anxiolytic-like activity (Figure 2). Compound **S-4** showed a significant effect in this assay at 1.9 μmol/kg, whereas **2**, **R-4**, and **S-5** did not show a significant effect at the doses tested. By comparison, **1** and (*S*)-nicotine showed activity in this assay at 0.19 and 0.62 μmol/kg, respectively.[9] Compounds **2**, **S-4**, **R-4**, and **S-5** also were evaluated for their effects on locomotor activity in mice (Figure 3). None of **S-4**, **R-4**, or **S-5** showed significant reduction in locomotor activity at doses up to 190 μmol/kg. In contrast, compound **2** caused significantly reduced locomotor activity at 6.2 and 19 μmol/kg, doses similar to those at which (*S*)-nicotine had a significant effect.[9]

Compound **S-4** was further characterized in the inhibitory avoidance assay as a preliminary screen for possible cognitive enhancing activity, as well as for hypothermic effects and ability to elicit seizures. Data comparing **S-4** in these measures with **1** and (*S*)-nicotine are presented in Table 2. Compound **S-4** elicited a significant effect in the inhibitory avoidance assay at 0.62 μmol/kg, whereas (*S*)-nicotine and **1** produced similar effects at the same and at a 10-fold lower dose, respectively. Compound **S-4** did not elicit a hypothermic

Table 2. *In Vivo* Pharmacological Properties of **S-4** Compared to Those of **1** and (*S*)-Nicotine

	ED_{min}, μmol/kg, ip[a]		
assay	**1**	**S-4**	(*S*)-nicotine
mouse inhibitory avoidance	0.062[b]	0.62	0.62[b]
locomotor activity	NS[c] (0.0062−6.2)	NS[c] (1.9−19)	0.62[b]
hypothermia	19[b]	>62	6.2[b]
seizure	62[d] [51−75]	774[d] [670−894]	41[d] [34−49]

[a] ED_{min} is defined as the minimum dose of the drug that elicited a statistically significant response. [b] Data from ref 9. [c] NS = no significant effect in indicated dose range. [d] (ED_{50}, μmol/kg [95% CI].

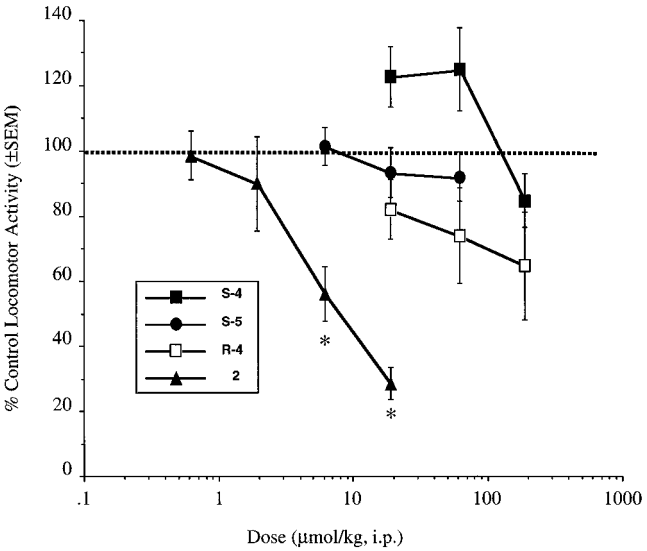


Figure 3. The effects of **2**, **S-4**, **S-5**, and **R-4** on locomotor activity measured for 15 min in an open field beginning approximately 2 min after injection. Shown are mean percent of saline control activity counts (infrared beam breaks) ± SEM. Significant effects were noted for **2** [$F(5,42) = 6.65$; $p < 0.0001$], but not for **S-4**, **S-5**, and **R-4**. *Significantly different from appropriate saline group, $p < 0.05$.

effect at doses up to 62 μmol/kg, compared to significant effects elicited by **1** and (*S*)-nicotine at 19 and 6.2 μmol/kg, respectively. Moreover, compound **S-4** was >10-fold less potent to elicit seizure activity than either **1** or (*S*)-nicotine.

The effect of **S-4** in the delayed matching-to-sample task is shown in Figure 4. The data are normalized to the actual percent correct for each delay interval. In aged monkeys, **S-4** given orally 90 min prior to behavioral evaluation (average "best dose" = 38 ± 14 nmol/kg) improves impaired performance of the task,with a robust enhancement seen primarily at the medium delay interval. Broader behavioral assessment of **S-4** in rats and monkeys is the subject of a separate report.[10]

Metabolism and Pharmacokinetics

The half-lives of compounds **1**, **S-4**, **R-4**, **S-5**, and **R-5** in the presence of dog liver slices *in vitro* and in dogs *in vivo* are shown in Table 3. The *N*-desmethyl compounds are more stable than the corresponding *N*-methyl compounds, whereas the effect of stereochemistry is minimal under the conditions examined. Compound **1** is substantially less stable than **S-5** or **R-5**. Since it has been established that the primary sites of metabolism of **1** occur on the pyrrolidine ring,[11,12] it appears that the pyridyloxymethyl appendage confers

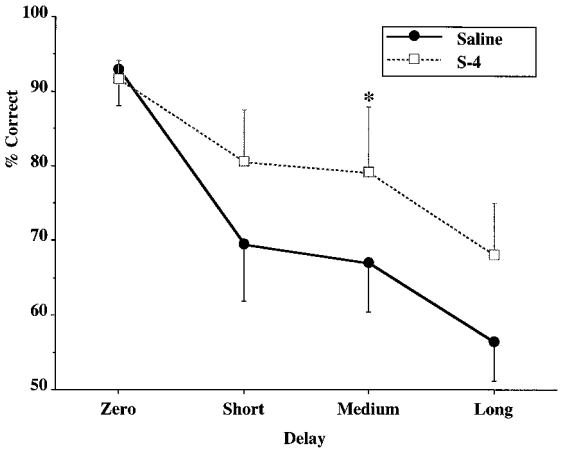


Figure 4. The effects of **S-4** on delayed matching-to-sample (DMTS) in monkeys. Shown are the mean correct in percentage (± SEM). Significant improvement in DMTS test performance was observed for **S-4** [$F(1,2) = 35.00$; $p = 0.027$] at medium delay. *Significantly different from appropriate saline group, $p = 0.027$.

Table 3. Half-Lives of Compounds **S-4**, **1**, and **S-5**−**R-5** *in Vitro* and *in Vivo* and Their Oral Bioavailabilities

compound	$t_{1/2}$ (h) in dog liver slices (% remaining after 24 h)[b]	$t_{1/2}$ (h) following iv administration to dogs	oral bioavailability in dog (%)[a,c]
S-4	>24 (77)	1.6	61.5 ± 27.5[d]
S-5	9 (32)	0.31	5.8 ± 3.6
R-4	>24 (72)	1.4	66.0 ± 14.3
R-5	8.5 (32)	0.61	4.9 ± 0.4
1	3 (2.9)	0.21	1.2 ± 1.2

[a] Data provided as the mean ± SEM following a single 500 nmol/kg oral or intravenous dose in dog. [b] Half-life from *in vitro* dog liver slice experiments (% remaining after 24 h incubation). [c] Values are mean of three dogs, unless otherwise indicated. [d] Values are mean of six dogs.

a stabilizing influence on the pyrrolidine ring relative to the effect of the isoxazole substituent.

The *in vivo* half-lives and the oral bioavailabilities of **S-4**, **R-4**, **S-5**, and **R-5** reflect their stabilities *in vitro*. Thus, the *N*-desmethyl compounds **S-4** and **R-4** have half-lives approximately 2.5−5-fold longer than those of the corresponding *N*-methyl analogs **S-5** and **R-5**, and oral bioavailabilities of **S-4** and **R-4** are 62% and 66%, respectively, compared to less than 10% for compounds **S-5** and **R-5**. As was found in *in vitro* studies, the effect of stereochemistry is minimal, although for the *N*-methyl analogues, **R-5** has a somewhat longer half-life than **S-5**. A possible interpretation of the data is that first pass metabolism could play a substantial role in reducing the *in vivo* half-lives and bioavailabilities of **1**, **S-5**, and **R-5**.

Summary

Compound **S-4** is a member of the 3-pyridyl ether class of nAChR ligands that exhibits positive effects in rodent and primate models of cognitive enhancement and in a rodent model of anxiolytic-like activity. In addition, **S-4** has a reduced propensity compared to (*S*)-nicotine to activate peripheral ganglionic-like nAChRs and to elicit seizures or effects on temperature and locomotor activity. On the basis of its biological activities and favorable oral bioavailability, **S-4** is an attractive candidate for further evaluation as an orally active agent for treatment of cognitive deficits.

Experimental Section

General Chemistry Methods. 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) relative to Me_4Si as an internal standard unless otherwise indicated. Mass spectra were obtained with a Hewlett-Packard HP5965 spectrometer. The above determinations were performed by the Analytical Research Department, Abbott Laboratories, and elemental analyses were performed by Robertson Microlit Laboratiories, Inc., Madison, NJ. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values.

Thin-layer chromatography (TLC) was carried out by using E. Merck precoated silica gel F-254 plates (thickness 0.25 mm). Flash chromatography was carried out using Merck silica gel 60, 200−400 mesh.

Melting points are uncorrected and were determined on a Buchi melting point apparatus. Optical rotation data were obtained on a Perkin-Elmer Model 241 polarimeter. All reactions were performed under anhydrous conditions unless otherwise noted.

The following abbreviations are used in the Experimental Section: THF for tetrahydrofuran, D_2O for deuterium oxide, $CDCl_3$ for deuteriochloroform, DMSO-d_6 for deuteriodimethyl sulfoxide, BOC for *tert*-butoxycarbonyl, TFA for trifluoroacetic acid, and DEAD for diethyl azodicarboxylate.

(*S*)-1-(*tert*-Butoxycarbonyl)-2-pyrrolidinemethanol and (*R*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinemethanol were prepared according to the literature procedure[13,14] starting from commercially available L-proline and D-proline, which were purchased from Aldrich.

2-Methyl-3-(2(*S*)-pyrrolidinylmethoxy)pyridine Dihydrochloride (S-4). To a solution of triphenylphosphine (3.83 g, 14.6 mmol) in 40 mL of anhydrous THF at 0 °C was added diethyl azodicarboxylate (2.30 mL, 14.6 mmol) dropwise. The mixture was stirred at 0 °C for 30 min and then brought to room temperature. (*S*)-1-BOC-2-pyrrolidinemethanol (1.96 g, 9.75 mmol) and 2-methyl-3-hydroxypyridine (1.60 g, 14.6 mmol) were added to the reaction vessel, and the mixture was stirred for 16 h. Solvent was removed *in vacuo*, and the residue was diluted with hexane and sonicated for 30 min. The resulting precipitate was filtered and washed with hexane. The hexane was removed *in vacuo*, and the residue was purified by silica gel flash chromatography (ethyl acetate) to give 1.42 g (50% yield) of the title compound as a pale yellow oil: TLC $R_f = 0.50$ (EtOAc); MS (DCI/NH_3) *m/e* 293 $(M + H)^+$.

To a solution of the compound from above (0.407 g, 1.39 mmol) in methylene chloride (2 mL) at 0 °C was added TFA (2 mL). The mixture was stirred at this temperature for 40 min, allowed to reach room temperature, and stirred for an additional 30 min. Saturated K_2CO_3 was added, and the product was extracted with CH_2Cl_2 (3×). The organic layer was dried over $MgSO_4$, and the crude product was purified by silica gel flash chromatography (100% $CHCl_3$ to 1:9 MeOH/$CHCl_3$ and finally 0.1:1:9 NH_4OH/MeOH/$CHCl_3$) to give 0.236 g (88%) of the title compound as a pale yellow oil: MS (DCI/NH_3) *m/e* 193 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (t, $J = 3$ Hz, 1H), 7.07 (m, 2H), 3.95−3.85 (m, 2H), 3.58 (m, 1H), 3.10-2.97 (m, 2H), 2.48 (s, 3H), 2.23 (br s, 1H), 1.99 (m, 1H), 1.90−1.78 (m, 2H), 1.67−1.58 (m, 1H).

The free base from above was dissolved in diethyl ether and brought to 0 °C with stirring. The solution was treated with diethyl ether saturated with hydrogen chloride gas. The solvent was removed *in vacuo*. The resulting salt was triturated with diethyl ether (2×) and dried under vacuum to give a beige powder: mp >250 °C (decomposition occurs at 250 °C and higher); $[\alpha]^{25}_D = +25.50°$ ($c = 1$, MeOH); MS (DCI/NH_3) *m/e* 193 $(M + H)^+$; ^{1}H NMR (D_2O, 300 MHz) δ 2.22−1.96 (m, 3H), 2.32 (m, 1H), 2.62 (s, 3H), 3.46−3.42 (m, 2H), 4.22 (m, 1H), 4.34 (dd, $J = 11$, 8 Hz, 1H), 4.58 (dd, $J = 11$, 3 Hz, 1H), 7.67 (dd, $J = 8.50$, 5.50 Hz, 1H), 7.86 (d, $J = 7.5$ Hz, 1H),8.17 (d, $J = 5.5$ Hz, 1H). Anal. ($C_{11}H_{16}N_2O$·2 HCl) C, H, N.

2-Methyl-3-(2(*R*)-pyrrolidinylmethoxy)pyridine Dihydrochloride (R-4). To a solution of triphenylphosphine (7.87 g, 30 mmol) in 120 mL of anhydrous THF at 0 °C was added DEAD (4.7 mL, 30 mmol) dropwise. The mixture was stirred at 0 °C for 30 min and then brought to room temperature. (*R*)-1-BOC-2-pyrrolidinemethanol (4.03 g, 20 mmol) and 2-methyl-3-hydroxypyridine (3.27 g, 30 mmol) were added to the reaction vessel, and the mixture was stirred for 16 h. Solvent was removed *in vacuo*, and the residue was diluted with hexane and sonicated for 30 min. The resulting precipitate was filtered and washed with hexane. The hexane was removed *in vacuo*. The residue was purified by silica gel flash chromatography (hexane/ethyl acetate, 9:1 to 7:3) to give 1.8 g (31% yield) of the title compound as a pale yellow oil: TLC $R_f = 0.50$ (EtOAc); MS (DCI/NH_3) *m/e* 293 $(M + H)^+$.

To a solution of the product from above (0.40 g, 1.35 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added TFA (3 mL). The reaction mixture was stirred at this temperature for 40 min. The temperature was raised to room temperature, and the reaction mixture was stirred for an additional 30 min. Once the starting material was consumed, saturated K_2CO_3 was added and the product was extracted from the aqueous phase with CH_2Cl_2 (3×). The organic layer was then dried over $MgSO_4$. The resulting crude material was purified by silica gel flash chromatography (100% $CHCl_3$ to MeOH/$CHCl_3$, 1:9) to give 0.196 g (55%) of product as a pale yellow oil: MS (DCI/NH_3) *m/e* 193 $(M + H)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.07 (t, $J = 3$ Hz, 1H), 7.07 (m, 2H), 3.95−3.85 (m, 2H), 3.58 (m, 1H), 3.10-2.97 (m, 2H), 2.48 (s, 3H), 2.23 (br s, 1H), 1.99 (m, 1H), 1.90−1.78 (m, 2H), 1.63 (m, 1H).

The free base from above was dissolved in diethyl ether and brought to 0 °C with stirring. The solution was treated with diethyl ether saturated with hydrogen chloride gas. The solvent was removed *in vacuo*. The resulting salt was triturated with diethyl ether (2×) and dried under vacuum to give a beige powder: mp > 250 °C (decomposition occurs at 250 °C and higher); $[\alpha]^{25}_D = -25.5°$ ($c = 1$, MeOH); MS (DCI/NH_3) *m/e* 193 $(M + H)^+$; ^{1}H NMR (D_2O, 300 MHz) δ 8.17 (d, $J = 5.5$ Hz, 1H), 7.86 (d, $J = 7.5$ Hz, 1H), 7.67 (dd, $J = 8.50$, 5.50 Hz, 1H), 4.58 (dd, $J = 11$, 3 Hz, 1H), 4.34 (dd, $J = 11$, 8 Hz, 1H), 4.22 (m, 1H), 3.46−3.42 (m, 2H), 2.62 (s, 3H), 2.32 (m, 1H), 2.22−196 (m, 3H). Anal. Calcd for ($C_{11}H_{16}N_2O$·2HCl) C, H, N.

2-Methyl-3-(1-methyl-2(*S*)-pyrrolidinylmethoxy)pyridine Dihydrochloride (S-5). To a sample of **S-4** from above (658 mg, 2.25 mmol) were added formic acid (1 mL) and formaldehyde (2 mL), and the reaction mixture was stirred at 80 °C for 3 h. The reaction was quenched with water and saturated K_2CO_3, and the mixture was extracted with methylene chloride. The extract was dried over $MgSO_4$ and reduced in volume, and the residue was purified by chromatography on silica gel (MeOH/ $CHCl_3$, 1:19 to 1:9). Removal of the solvent and conversion of the residue into the salt with HCl in ether gave the title compound: mp 202−205 °C; $[\alpha]^{25}_D = +6.38°$ ($c = 0.02$, MeOH); MS (DCI/NH_3) *m/e* 193 $(M + H)^+$; ^{1}H NMR (D_2O, 300 MHz) δ 2.08−2.27 (m, 3H), 2.45 (m, 1H), 2.55 (s, 3H), 3.09 (s, 3H), 3.31 (br s, 1H), 3.76 (br s, 1H), 4.04 (br s, 1H), 4.36 (dd, $J = 7$, 11 Hz, 1H), 4.58 (dd, $J = 3$, 11 Hz, 1H), 7.50 (dd, $J = 5$, 8.5 Hz, 1H), 7.65 (dd, $J = 1$, 8.5 Hz, 1H), 8.12 (d, $J = 1$, 5 Hz, 1H). Anal. ($C_{12}H_{18}N_2O$·2HCl) C, H, N.

2-Methyl-3-[[(1-methyl-2(*R*)-pyrrolidinyl)methyl]oxy]-pyridine Dihydrochloride (R-5). 2-Methyl-3-[[(1-BOC-2(*R*)-pyrrolidinyl)methyl]oxy]pyridine (1.4 g, 4.7 mmol) was dissolved in formic acid (5 mL) and formalin (10 mL), heated at reflux for 2 h, and cooled to room temperature. The reaction mixture was poured into water (60 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The pH was adjusted to 10 with K_2CO_3. The aqueous mixture was extracted with CH_2Cl_2 (3 × 10 mL), the combined extracts were dried over $MgSO_4$, and the solvent was removed. The residue was chromatographed on silica gel ($CHCl_3$/MeOH, 95:5), and the HCl salt was formed in Et_2O/EtOH to yield 0.78 g (60%) of a hygroscopic solid: mp 203−207 °C; $[\alpha]^{25}_D = -5.73°$ ($c = 1$, MeOH); MS (DCI/NH_3) *m/z* 207 $(M + H)^+$; NMR (D_2O, 300 MHz) δ 2.26 (m, 1H), 2.35 (m, 1H), 2.45 (m, 1H), 2.6 (s, 3H), 3.09 (s, 3H), 3.9 (m, 1H), 3.77 (m, 1H), 4.03 (m, 1H) 4.47 (m, 1H) 4.46 (m, 1H), 4.65 (m, 1H), 7.76 (dd, $J = 5.5$, 8.4 Hz, 1H), 7.97 (d, $J = 8.4$ Hz, 1H), 8.21 (dd, $J = 1.1$, 5.9 Hz, 1H). Anal. Calcd ($C_{12}H_{18}N_2O_4$·2HCl·0.4H_2O) C, H, N.

Biology. Interaction with high-affinity nicotine binding

sites, mainly composed of α4 and β2 subunits,[15] was measured by displacement of [^{3}H]-(−)-cytisine from a preparation of whole rat brain.[16] Interaction with the α7 subtype was assessed by displacement of [^{125}I]-α-bungarotoxin from K28 cells, which stably express human α7 homooligomers.[17] Interaction with ganglionic-type receptors, thought to contain α3 subunits in combination with β2 or β4 and possibly α5,[18] was measured using a $^{86}Rb^+$ flux functional assay in a human neuroblastoma-derived IMR-32 cell line, as described previously.[19]

All animal studies were conducted in accord with AALAC procedures as approved by the Institutional Animal Care and Use Committee at Abbott Laboratories. Body temperature and open field locomotor activity were measured in the same mice. Beginning 4 min after an ip injection of compounds, horizontal and vertical activity counts were recorded for 15 min in an open field (41 × 41 cm) using photobeam activity monitors (San Diego Instrument, San Diego, CA). Body temperature was measured immediately after the mice were removed from the open field (approximately 20 min after drug injection) using a rectal probe (YSI TeleThermometer, Yellow Spring Instrument Co., Yellow Spring, OH) inserted 3 cm.

Anxiolytic-like activity was evaluated in mice using the elevated plus-maze as previously described.[9,20] Briefly, the maze consists of two open arms, to which rodents are naturally averse, and two closed arms. The extent to which exploration of the open arms is increased as a result of drug administration (ip, 30 min prior to the test) is taken as a reflection of anxiolytic-like action of the drug. Inhibitory avoidance testing was conducted as described previously.[9] Briefly, mice were trained to avoid the dark side of a dark−light chamber by administration of a mild foot shock. The latency to enter the dark chamber during a subsequent test session was used as the index of memory of the training experience. Test compounds were administered (ip) 15 min before the beginning of the training session.

Delayed matching-to-sample (DMTS) was evaluated in monkeys as previously described.[21] Stimuli on the test panels were 2.54 cm diameter colored disks (red, yellow, or green). Each trial began with the illumination of the sample key by one of three colored stimuli. A key-press extinguished the sample light and initiated one of four preprogrammed delay intervals, during which no disks were illuminated. Following the delay interval, two choice lights located below the sample key were illuminated. One of the choice stimuli matched the hue of the sample light. Key-presses of choice stimuli that matched the hue of the sample stimulus were rewarded by a 30 mg banana-flavored pellet. Monkeys completed 96 trials on each day of testing. Four possible delay intervals between a monkey's response to the sample light and the presentation of the two choice lights were employed. Short, medium, and long delay intervals were individually adjusted for each monkey to produce stable performance approximating the following performance levels: short (75-84% correct), medium (65-74% correct), and long (55−64% correct). The duration of the short, medium, and long delay intervals in this experiment were 5, 10, and 15 s, respectively, for the first monkey; 5, 10, and 20 s for the second monkey; and 5, 15, and 30 s for the third monkey. Performance for 0 s delay trials averaged 85−100% correct. ABT-089 was dissolved in saline and placed on a sugar cube which was fed to each monkey 90 min prior to the start of DMTS testing. Monkeys were tested 5 days per week with drug being administered on a maximum of two of these days.

Metabolism and Pharmacokinetics. The stability of compounds in the presence of dog liver slices *in vitro* was carried out by employing a previously published procedure.[11] Compounds at a final concentration of 12 μg/mL were incubated with liver tissue at 37 °C for various times up to 24 h. At the required time, the medium was removed, transferred to separate tubes, and immediately frozen in a 2-propanol/dry ice bath. The samples were stored frozen until required for assay by HPLC.[22]

The pharmacokinetic behavior of each compound was evaluated in beagle dog, fasted overnight prior to dosing and throughout the duration of the study; animals were permitted free access to water. Groups of dogs ($n = 3-6$) received a 500 nmol/kg dose administered as either an oral dose (by gavage) or as a slow bolus intravenous dose in the saphenous vein. Blood samples were obtained from the jugular vein of each animal at selected time points for the 8 h postdosing interval. Plasma concentrations of parent drug were determined by reverse phase HPLC with either electrochemical detection (compounds **1**, **S-5**, and **R-5**) or fluorescence detection following derivatization with 7-fluoro-4-nitro-2-oxa-1,3-diazole[23] (compounds **S-4** and **R-4**).

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