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SYNTHESIS AND STRUCTURE–ACTIVITY RELATIONSHIPS OF PYRIDINE-MODIFIED ANALOGS OF 3-[2-((*S*)-PYRROLIDINYL)METHOXY]PYRIDINE, A-84543, A POTENT NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST

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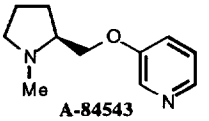
Abstract. Analogs of 3-[2-((*S*)-pyrrolidinyl)methoxy]pyridine (A-84543, **1**) with 2-, 4-, 5-, and 6-substituents on the pyridine ring were synthesized. These analogs exhibited K_i values ranging from 0.15 to > 9,000 nM when tested in vitro for neuronal nicotinic acetylcholine receptor binding activity. Assessment of functional activity at subtypes of neuronal nicotinic acetylcholine receptors indicates that pyridine substitution can have a profound effect on efficacy at these subtypes, and several subtype-selective agonists and antagonists have been identified.

Recent evidence indicating the therapeutic potential of nicotinic acetylcholine receptor modulators for the treatment of CNS disorders, as well as the diversity of neuronal nicotinic acetylcholine receptors (nAChRs), has suggested an opportunity to develop subtype-selective nAChR ligands for the treatment of specific CNS disorders with reduced side effect liabilities.[1–4] We have recently identified A-84543 (**1**), a member of a novel series of 3-pyridyl ether compounds, as a potent nicotinic acetylcholine receptor agonist.[5] Several compounds of this class were found to possess subnanomolar affinity for nAChRs and to activate specific subtype of neuronal nAChRs. More recently, a novel compound from the 3-pyridyl ether series, ABT-089, was found to show positive effects in rodent models of cognitive enhancement and anxiolytic activity and to possess a reduced propensity to activate peripheral ganglionic type receptors.[6] As part of our program to discover additional novel compounds in this series, structure-activity studies on A-84543 were undertaken. In this report, analogs substituted at the C2, C4, C5, or C6 position of the pyridine ring are described. These compounds have been screened for binding affinity to central nAChRs and assessed in in vitro functional (ion flux) assays. With regard to functional activity, the focus was on activity in two cell lines, K177 and IMR-32. The former cell line expresses the human brain nAChR subtype ($\alpha4\beta2$) having high affinity for (*S*)-nicotine, and the latter serves as a model for activity at human peripheral ganglionic receptors, which appear to at least partially mediate undesired cardiovascular and gastrointestinal effects of (*S*)-nicotine.[7,8]



A-84543

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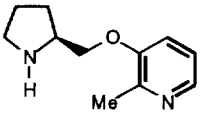

ABT-089

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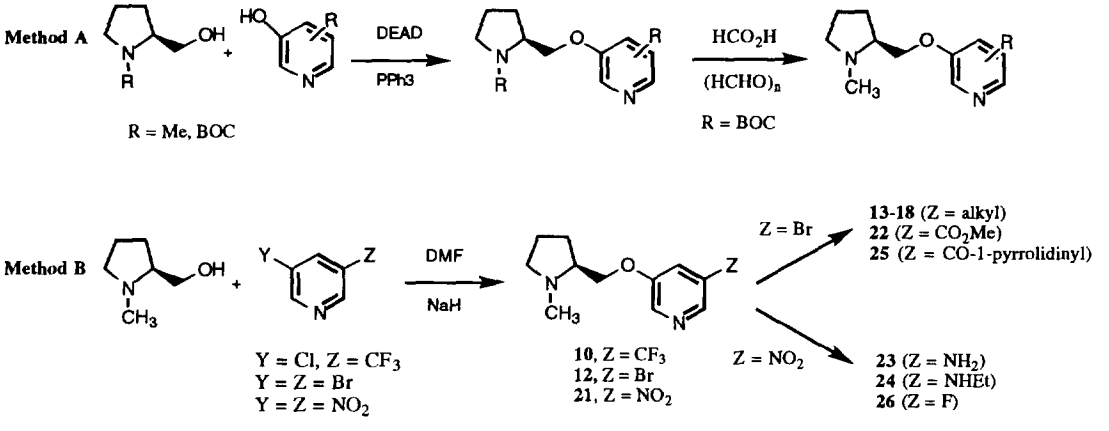
Methods: Pyridine substituted analogs of **1**, tabulated in Table 1, were synthesized by one of two general methods as outlined in Scheme 1. The 2-, 4-, 6-, and some 5-substituted analogs were synthesized (Method A) from the appropriately substituted 3-hydroxypyridine and an appropriate (*S*)-prolinol derivative using Mitsunobu chemistry as described previously.[5] The 2-substituted-3-hydroxypyridines were commercially available, whereas the 4-substituted 3-hydroxypyridines were prepared by metallation of the 3-*N,N*-diethylcarbamate of 3-hydroxypyridine with *sec*-BuLi [9] and addition of suitable electrophiles followed by carbamate hydrolysis. 2-Fluoro-3-hydroxypyridine was prepared from 2-amino-5-hydroxypridine[10] by diazotization with sodium nitrite and hydrogen fluoride, whereas the corresponding 2-chloro- and 2-bromo-3-hydroxypyridines were synthesized by a known procedure[11] from 5-amino-2-chloropyridine and 2-bromo-5-nitropyridine, respectively. 3-Hydroxypyridines bearing the 5-Cl, and 6-Me substituents are commercially available. The 6-hydroxymethyl substituent of **31** was incorporated via oxidation of a 6-methyl substituent via the corresponding N-oxide.[12] 5-CF_3- (**10**), 5-Br- (**12**) and 5-NO_2-pyridine (**21**) analogs were prepared (Method B) by treatment respectively of known 3-chloro-5-trifluoromethylpyridine, 3,5-dibromopyridine or 3,5-dinitropyridine[13] with the sodium salt of 1-methyl-2(*S*)-pyrrolidinylmethanol. Additional 5-substituted analogs were prepared using **12** or **21** as key intermediates. Thus, 5-alkyl analogs (**13-18**) were prepared from **12** employing a nickel-catalyzed Grignard coupling reaction.[14] Carbonylation of **12** with CO in the presence of palladium(0) catalyst furnished the 5-carbomethoxypyridine analog **22**, and treatment of compound **22** with pyrrolidine gave the corresponding amide **25**. Reaction of **12** with tri-*n*-butyl-(1-ethoxyvinyl)tin under palladium(0) catalysis followed by acid hydrolysis afforded acetyl derivative **19**. Hydrogenation of compound **21** provided the corresponding 5-aminopyridine **23**, which was converted to *N*-ethylamino analog **24** by acetylation followed by reduction with borane. Diazotization of compound **23** with boron trifluoride and *t*-butyl nitrite provided the diazonium salt, which was subsequently decomposed to furnish the 5-fluoropyridine **26**.



Scheme 1

Results and Discussion: A major subtype of nAChR in the brain is labeled with high affinity by [^{3}H](-)-nicotine and [^{3}H](-) cytisine and is composed of α4 and β2 subunits.[15] The affinities of new analogs for this subtype, as reflected by displacement of [^{3}H](-)-cytisine from rat brain membranes, are shown in Table 1. In general, compounds having substituents at the C2 position had substantially reduced receptor binding affinity

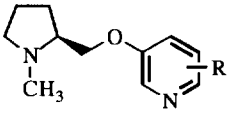
compared to **1**. The 2-methylpyridyl analog **2** possessed 152-fold weaker affinity than **1**, and the 2-Br (**3**), 2-thiomethyl (**4**), 2-NO_2 (**5**), and 2-I (**6**) analogs were weaker still, with each having affinity ≥ 3000-fold lower than **1**. Although the 4-methyl (**8**) and 4-bromo (**9**) analogs also were less potent than compound **1**, these analogs still had affinities in the low- to mid-nanomolar range. In contrast, the bulky 4-diethylcarbamyl moiety (compound **7**) was not well tolerated.

The C5 position appears to tolerate a broad range of substituents. Thus, 5-methyl analog **13** had three-fold lower affinity compared to **1**, and no further reduction in binding potency was observed upon substitution with larger alkyl groups, including isobutyl and n-hexyl (cf. **14, 15, 16, 17, 18**). Halo substitution (cf. **11, 12, 26**) resulted in two- to five-fold reductions in binding affinity. In general, introduction of electron withdrawing groups (CF_3, NO_2, $COCH_3$, CO_2Me, CO-pyrrolidine) caused reductions in binding potencies compared with **1**, but many of these analogs still possessed affinities in the low nanomolar range (cf. **10, 19, 21, 22, 25**). Regarding electron-donating groups, the binding potency of the NH_2 analog **23** was similar to the methyl analog (**13**), but NHEt analog **24** was weaker than its isosteric *n*-Pr compound **16**.

The effect of substituents on the binding affinities at C6 position of pyridine ring also was examined. Substitution with 6-methyl (**28**) decreased the affinity to 1.5 nM (ten-fold less potent than **1**), while 6-bromo substitution (**30**) resulted in a 13-fold decrease in binding potency. Introduction of a chloro group at the C6 position (**29**) resulted in a four-fold decrease in binding potency compared with **1**. Interestingly, replacement of the methyl group of **28** with a hydroxymethyl functionality caused a 60-fold decrease in binding potency (**28** vs **31**).

We conclude that the position of substitution of a given functional group has a substantial effect on nAChR binding affinity. Thus, the 2-methyl analog **2** is significantly less potent in binding than its 5- or 6-substituted counterpart (**13** or **28**), while the 4-methyl analog **8** exhibits intermediate activity. The same trend is observed with bromo analogs (compounds **3**, **9**, **12**, and **30**).

Table 1. Binding Data for Pyridine Substituted Analogs



Compound	R	Method	[^{3}H]-Cytisine Binding K_i (nM)[a]
1	H	A	0.15 ± 0.05
2	2-Me	A	22.9 ± 3.4
3	2-Br	A	451 ± 55
4	2-SMe	A	559 ± 84
5	2-NO_2	A	9000 ± 577
6	2-I	A	606 ± 21
7	4-$CONEt_2$	A[b]	2833 ± 655
8	4-Me	A	4.12 ± 0.30
9	4-Br	A	28 ± 3.8
10	5-CF_3	B	1.83 ± 0.28

11	5-Cl	A	0.60 ± 0.04
12	5-Br	B	0.27 ± 0.02
13	5-Methyl	B	0.48 ± 0.02
14	5-Ethyl	B	0.32 ± 0.06
15	5-Butyl	B	0.16 ± 0.04
16	5-Propyl	B	0.12 ± 0.03
17	5-*i*-Butyl	B	0.21 ± 0.02
18	5-n-Hexyl	B	0.32 ± 0.11
19	5-CH_3CO	From **12**[c]	1.19 ± 0.05
20	5-Br-1-oxide	From **12**[d]	34.8 ± 5.2
21	5-NO_2	B	4.65 ± 0.46
22	5-CO_2Me	From **12**[e]	1.45 ± 0.13
23	5-NH2	From **21**[f]	0.63 ± 0.06
24	5-NHEt	From **23**[g]	3.70 ± 1.53
25	5-CO-pyrrolidine	From **22**[h]	19.9 ± 1.8
26	5-F	From **23**[i]	0.69 ± 0.06
27	6-F	A	2.56 ± 0.42
28	6-Me	A	1.26 ± 0.11
29	6-Cl	A	0.64 ± 0.09
30	6-Br	A	1.91 ± 0.37
31	6-CH2OH	Via 6-Me-*N*-oxide j	97.35 ± 15.53

a. The ability of compounds to displace [^{3}H](-)-cytisine binding to whole rat brain membranes was performed as described. Values are the means ± S. E. M; n=3-4. In all cases, the Hill co-efficient was close to unity indicative of an interaction with a single class of binding sites. b. *sec*-BuLi, $ClCONEt_2$; c. $CH_2CH(OEt)SnBu_3$, $Pd(PPh_3)_4$; d. *m*-CPBA; e. CO, MeOH, $Pd(PPh_3)_2Cl_2$; f. H_2, Pd/C; g. 1. AcCl, NEt_3 2. BH_3/THF; h. pyrrolidine, reflux; i. BF_3, *t*-BuONO; j. 1. *m*-CPBA; 2. Ac_2O; 3. K_2CO_3/MeOH

The ability of these analogs to functionally activate nAChRs was investigated using cell lines that express the human α4β2 subunit combination (K177 cells) or human ganglionic-like (α3-containing) nAChRs (IMR 32 cells). With the exception of compound **13** (5-Me), all of the analogs tested stimulated cation efflux with low efficacy at human ganglionic-like receptors. At the α4β2 subtype, most of the compounds tested showed reduced efficacy relative to **1**. An exception is 6-chloro analog **29**, which stimulated cation efflux with high potency and efficacy at human α4β2, and thus represents a novel nAChR ligand with selectivity over the ganglionic subtype. Regarding positional isomers, the position of substitution exerted some effect on the potency at the α4β2 subtype. As demonstrated in Table 2, 5-, and 6-bromopyridine analogs (**12** and **30**) were more potent and efficacious than the corresponding 2- and 4-bromo analogs (**3** and **9**). In contrast, only small differences were observed among the potencies of the methyl analogs, although the 2-methyl compound also showed markedly lower efficacy (cf. **2**, **8**, **13**, **28**).

In addition to **29**, several other compounds exhibited significant nAChR subtype selectivity at the functional level with respect to either efficacy or potency. 2-Methylpyridine analog **2** is four-fold more potent in stimulating the α4β2 subtype than the ganglionic-like subtype, and 4-methyl (**8**), 5-ethyl (**14**),and 6-bromo (**30**) analogs were both more potent and more efficacious at the α4β2 subtype. In contrast to the 5-methyl analog, the 5-*n*-propyl (**15**) and 5-*n*-butyl (**16**) compounds stimulated cation efflux with low potency and efficacy at both subtypes. Thus, increasing steric volume at C5 position had the effect of diminishing agonist properties in both cell lines.

Table 2. Functional Properties of Pyridine Substituted Analogs[a]

		^{86}Rb+ Flux Human α4β2 K177 Cells		^{86}Rb+ Flux Human α3βx IMR-32 Cells	
Compound	R	EC_{50} (μM)[a]	% Max[b]	EC_{50} (μM)	% Max
(S)-nicotine		5 ± 1	(100)	21 ± 3	(100)
1	H	0.75 ± 0.2	100 ± 6	19.4 ± 4.6	73.2 ± 4
2	2-Me	4.8 ± 0.2	26.7 ± 1.9	18.2 ± 7.1	27.7 ± 0.9
3	2-Br	> 1,000	12.3 ± 3.3	> 1,000	7.1 ± 0.5
8	4-Me	2.1 ± 0.5	73.9 ± 6.7	> 1,000	12.0 ± 4.6
9	4-Br	> 1,000	14.1 ± 2.1	> 1,000	17.0 ± 11.9
12	5-Br	5.95 ± 0.7	41.8 ± 1.0	N.T.[c]	N.T.
13	5-Me	2.3 ± 0.4	77.0 ± 2.0	2.9 ± 2.3	78.8 ± 21.3
14	5-Et	1.0 ± 0.3	33.7 ± 5.6	> 1,000	15.2 ± 1.1
15	5-Pr	> 1,000	14 ± 2.8	>1,000	8.7[d]
16	5-Bu	> 1,000	13 ± 4.6	> 1,000	2.5[d]
28	6-Me	2.2 ± 0.4	54.4 ± 9.2	13.4 ± 6.2	30.9 ± 3.8
29	6-Cl	1.4 ± 1.1	108.2 ± 11.6	27.1 ± 9	17.9 ± 1.9
30	6-Br	2.6 ± 0.8	42.5 ± 13.1	>1,000	10.6 ± 1.3

a Values represent mean ± S.E.M. n = 3–5. b. % Max represents the maximal efficacy of the compounds relative to 100 μM nicotine. c. NT: not tested. d. (n = 2) for these values

Since the *n*-propyl (**15**) and *n*-butyl (**16**) analogs displayed very low efficacy (< 20%) at both subtypes, they were tested for antagonist properties in both preparations. At the α4β2 subtype, **15** and **16** antagonized

Table 3. Antagonist Properties of Pyridine Substituted Analogs[a]

Compound	R	^{86}Rb+ Flux Human α4β2 K-177 Cells IC50 (μM)[b]	^{86}Rb+ Flux Human α3βx IMR-32 Cells IC50 (μM)[b]
15	5-Pr	0.64 ± 0.31	5.0 ± 0.6
16	5-Bu	2.4 ± 1.3	3.2 ± 0.1

a. Compounds were tested against 100 μM of (-)-nicotine. b. Values represent mean ± S.E.M. n = 3–5.

the stimulatory effect of 100 μM (*S*)-nicotine with IC_{50} values of 0.64 and 2.4 μM, respectively. At the ganglionic-like subtype, compounds **15** and **16** antagonized cation efflux mediated by 100 μM (*S*)-nicotine with IC_{50} values of 5 μM and 3.2 μM, respectively. Thus, compound **15** represents a novel structural class of nAChR antagonists with significant subtype selectivity at the functional level.

In summary, we have shown that varying the substituent pattern of the pyridyl moiety of A-84543 alters binding and particularly functional properties of compounds. Regarding monomethyl and monobromo positional isomers, the order of binding potency is 5-substituted>6-substituted>4-substituted>2-substituted. We have also demonstrated that preparation of agonists or antagonists with subtype selectivity could be achieved by varying the substituents on the pyridine ring. The 6-chloro analog **29** was identified as a selective α4β2 agonist, the 4-methyl analog **8** as a selective α4β2 partial agonist and 5-propyl analog **15** was identified as a moderately selective α4β2 antagonist.

References and Notes

1. Holladay, M. W.; Lebold, S. A.; Lin, N.-H. *Drug Devel. Res.* **1995**, *35*, 191-213.
2. Arneric, S. P.; Sullivan, J. P.; Williams, M. In *Psychopharmacology: Fourth Generation of Progress;* Bloom, E. B.; Kuper, D. J. Eds.; Raven: New York, 1995; pp 95–110.
3. Meyer, E. M.; de Fiebre, C. M.; Hunter, B. E.; Simpkins, C. E.; Frauworth, N.; de Fiebre, N. E. C. *Drug Devel. Res.* **1994**, *31*, 127-134.
4. Garvey, D. S.; Wasicak, J. T.; Decker, M. W.; Brioni, J. D.; Buckley, M. J.; Sullivan, J. P.; Carrera, G. M.; Holladay, M. W.; Arneric, S. P.; Williams, M. *J. Med. Chem.* **1994**, *37*, 1055-1059.
5. Abreo, M. A.; Lin, N.-H.; Garvey, D. S.; Gunn, D. E.; Hettinger, A.-M.; Wasicak, J. T.; Pavlik, P. A.; Martin, Y. C.; Donnelly-Roberts, D. L.; Anderson, D. J.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. *J. Med. Chem.* **1996**, *39*, 817-825.
6. Lin, N.-H.; Gunn, D. E.; Ryther, K. B.; Garvey, D. S.; Donnelly-Roberts, D. L.; Decker, M.; Brioni, J. D.; Buckley, M. J.; Rodrigues, D.; Marsh, K.; Anderson, D. J.; Buccafusco, J. J.; Prendergast, M. A.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. *J. Med. Chem.* **1997**, *40*, 385-390.
7. McBride, P. E. *Med. Clin. N. Am.* **1992**, *76*, 333-353.
8. Carlson, G. M.; Ruddon, R. W.; Hug, C. C., Jr.; Schmiege, S. K.; Bass, P. *J. Pharmacol. Exp. Ther.* **1970**, *173*, 377-383.
9. Snieckus, V. *Heterocycle* **1980**, *14*, 1649.
10. Moore, J. A.; Marasia, F. J. *J. Am. Chem. Soc.* **1959**, *81*, 6049-6055.
11. Effenberger, F.; Krebs, A.; Willrett, P. Z. *Chem. Ber.* **1992**, *125*, 1131-1140.
12. Vozza, J. F. *J. Org. Chem.* **1962**, *27*, 3856.
13. Wozniak, M.; Baranski, A.; Szpakiewicz, B. *Liebigs Ann. Chem.* **1993**, *1*, 7-10.
14. Tamao, K.; Kodama, S.; Nakajima, I.; Kumada, M. *Tetrahedron* **1982**, *22*, 3347-3353.
15. Flores, C. M.; Rogers, S. W.; Pabreza, L. A.; Wolfe, B. B.; Kellar, K. *J. Mol. Pharm.* **1991**, *41*, 31-37.