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NOVEL 2-(2'-FURO[3,2-*b*]PYRIDINYL) PYRROLIDINES: POTENT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR LIGANDS

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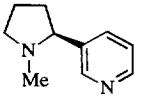
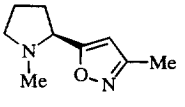
Abstract: A novel series of 2-(2'-furo[3,2-*b*]pyridinyl) pyrrolidines has been synthesized and evaluated as novel nicotinic acetylcholine receptor ligands. Changing the pyrrolidine stereochemistry and *N*-substitution pattern afforded analogs with K_i values ranging from 2.7 to 97 nM. Rubidium efflux studies revealed that these compounds had intrinsic activities ranging from 9–58% that of nicotine in the IMR-32 cell line and 6–81% in the K177 cell line. The N(*Me*)-2(*S*) analog **3a** demonstrated good selectivity in the K177 cell line ($\alpha_4\beta_2$ receptor) versus the IMR-32 cells ($\alpha_3\beta_x$ receptor) and TE 671 cells (α_1 neuromuscular receptor), and was a partial agonist with an EC_{50} value of 141 nM in dopamine release assay using rat striatal slices. © 1997 Elsevier Science Ltd.

The development of potent and selective neuronal nicotinic acetylcholine receptor (nAChR) modulators as potential therapeutic agents in the treatment of cognitive disorders continues to be an area of intense research.[1] Key criteria for effective and safe nicotinic agents include, among other things, high potency and selectivity for the appropriate nicotinic receptor subtype(s) modulating the desired biological activity. We previously reported on two classes of nAChR modulators; pyrrolidinyl isoxazoles (e.g., **I**, ABT-418)[2] and 3-pyridyl ethers (e.g., **II**, A-84543, Figure 1)[3] which have high affinity for nicotinic receptor subtypes and have been shown[2a,3b] to possess cognitive-enhancement activity in vivo.

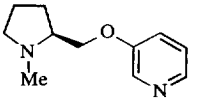
Figure 1. Structures of Several Classes of nAChR Ligands.



Nicotine



(I)
ABT-418



(II)
A-84543

The potent activity of these two series demonstrates that both the isoxazole ring and pyridine ether moiety can effectively substitute for the 3-pyridyl ring of nicotine, maintaining high nAChR affinity and good agonist activity. Structural comparison of these two classes raises questions regarding the similarities between the isoxazole ring and the pyridyl ether that allows both to be effective pharmacophores for the nicotinic receptor, as well as the dissimilarities in the SAR trends between the two classes (vide infra).[3a] We investigated

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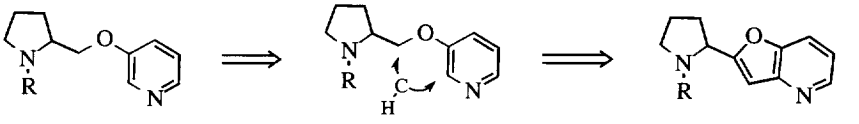
what effect conformational constraints on the 3-pyridyl moiety would have in terms of nAChR binding and functional activity. One approach to examining these constraints is to tether the carbon of the oxymethyl group to the pyridyl ring by inserting an additional sp-2 carbon, thus forming a furo[3,2-*b*]pyridine heterocycle (Figure 2). Herein, we report the synthesis and biological properties of a novel class of furo[3,2-*b*]pyridinyl pyrrolidines **III**.

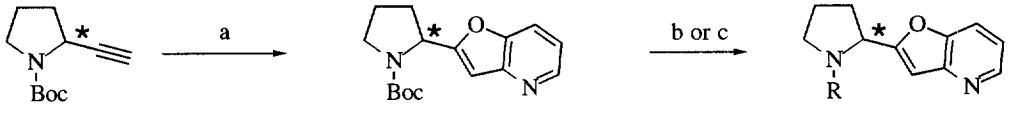
Figure 2. Furo[3,2-*b*] Pyridyl Pyrrolidine nAChR ligands.



(III)
Furo[3,2-*b*]pyridine
Pyrrolidine

Methods: The synthesis of the furo[3,2-*b*]pyridinyl pyrrolidine analogs is outlined in Scheme 1. The synthesis of the enantiomerically pure starting Boc-protected pyrrolidinyl alkyne was previously reported.[1d] The key transformation of the alkyne to the furopyridine ring was accomplished via a palladium-mediated ring construction using the commercially available 3-hydroxy-2-iodo pyridine following the procedures of Kundu et al.[4] Subsequent treatment of the Boc-protected pyrrolidine **1** with either trifluoroacetic acid in methylene chloride or refluxing formic acid/formaldehyde (Eschweiler–Clarke conditions) afforded the desired *N*-H compounds **2** and the *N*-Me compounds **3**, respectively, in good yield.[5] These analogs were tested for nAChR binding using [^{3}H]-(-) -cytisine following the procedure of Pabreza[6] using a whole rat brain preparation. Release of ^{3}H-dopamine from rat striatal slices was measured as previously described.[2b] Compounds were evaluated for functional activity using an isotopic $^{86}Rb^+$ efflux assay in IMR-32, K177,[7] and TE 671[8] cells lines as described previously.

Scheme 1. Synthesis of Furo[3,2-*b*]pyridinyl Pyrrolidine Analogs.



1a * = (S)
1b * = (R)

2a R = H; * = (S)
2b R = H; * = (R)
3a R = Me; * = (S)
3b R = Me; * = (R)

Legend: (a) 2-Iodo-3-hydroxy pyridine/$(C_6H_5)_2PdCl_2$-CuI (b) TFA/CH_2Cl_2 (c) HCOOH/aq formalin, reflux

Table 1. Neuronal nAChR Binding and ^{86}Rb-Flux Studies of 2-(2'-furo[3,2-*b*]- pyridinyl) Pyrrolidine Analogs on IMR-32 and K177 Transfected Cells[a]

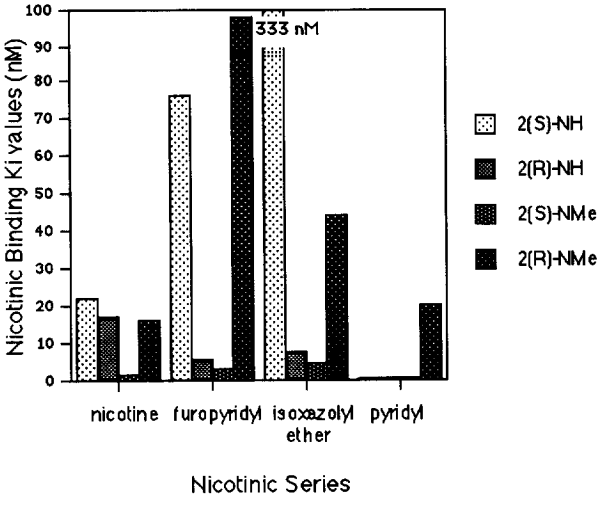
Compound	[^{3}H]-Cytisine Binding Ki (nM)[b]	^{86}Rb-Flux Human $\alpha_3\beta_x$ (IMR-32 cells)		^{86}Rb-Flux Human $\alpha_4\beta_2$ (K177 cells)	
		EC_{50} (μM)[c]	**% Intrinsic Activity[d]**	**EC_{50} (μM)[c]**	**% Intrinsic Activity[d]**
ABT-418	4.2 ± 0.6[f]	64 ± 12[f]	85 ± 4[f]	10.6 ± 1.1[g]	93 ± 8[g]
(-)-Nicotine[e]	1 ± 0.1	21 ± 3	(100)	5 ± 1	(100)
A-84543	0.15 ± 0.01	24 ± 3.7	77.2 ± 4.3	0.75 ± 0.2	100 ± 6
2a	75.8 ± 2.6	>1000	9.2 ± 4.5	>1000	7.4 ± 3.9
2b	5.11 ± 1.0	41.5 ± 13.5	57.8 ± 4.9	11 ± 2.2	30 ± 1.2
3a	2.73 ± 0.45	>1000	10.6 ± 4.5	3.2 ± 2.5	81 ± 15
3b[h]	97.9 ± 8.9	>1000	15.2 ± 4.9	>1000	5.7 ± 4.6

[a]All final compounds were fully characterized by ^{1}H NMR and MS, with elemental analyses within ±0.4% of theoretical values, unless otherwise noted. [b]The compounds were tested for nAChR binding in a whole rat brain preparation using [^{3}H]-cytisine following a modification of the procedure of Pabreza et al.[6] Values represent mean ±SEM. [c]Values represent mean ± SEM. The isotopic rubidium efflux assay employed to assess functional activity of nAChRs expressed in IMR-32 and K177 transfected cells was performed as described.[7] [d]Percent intrinsic activity relative to 100 μM (*S*)-nicotine. [e]Values from ref 3a. [f]Values from ref 3b. [g]Values from ref 2e. [h]Calcd. for $C_{12}H_{14}N_2O$•2HCl•0.5H_2O: C, 50.72; H, 6.03; N, 9.86. Found: C, 50.33; H, 6.21; N, 8.87. HRMS calcd. 203.1184; observed 203.1180.

Results and Discussion. As shown in Table 1, the furopyridine analogs had good binding affinity for the nAChR labeled by cytisine ($\alpha_4\beta_2$), having K_i values ranging from 97.9 nM to 2.7 nM. The effect of *N*-substitution on the binding affinity is strongly dependent on the pyrrolidine stereochemistry, with the binding affinity increasing upon *N*-methylation in the (*S*) series (**2a** vs. **3a**) but decreasing in the (*R*) series (**2b** vs. **3b**). These trends are similar to the SAR found with the isoxazole series as well as with isomers of nicotine itself[2a] and is dissimilar to the pyridyl ether class (see Chart) in which the 2(*S*)-NH and 2(*S*)-NMe and 2(*R*)-NH compounds are nearly equipotent.[3a,9] Interestingly, compound **3a**, which has the same *N*-substituent and stereochemistry as **ABT-418** and (-)-nicotine, demonstrated the highest affinity (K_i = 2.73 nM) of the four isomers. In terms of overall receptor affinity these furopyridines are more similar to isoxazole pyrrolidine and nicotine analogs (i.e., low nanomolar range) and not as potent as the pyridyl ethers, which are often in the low picomolar range. We found that replacement of the furopyridine nitrogen atom with an sp2 carbon to give the corresponding

benzofuran analogs afforded compounds with >100 nM affinity for the $\alpha_4\beta_2$ nAChR (data not shown), highlighting the importance of the furopyridine nitrogen for good nAChR receptor binding affinity. All four compounds had receptor binding values of >10,000 nM against the α-bungarotoxin sensitive α_7 subtype (data not shown).

Chart. Effects of Pyrrolidine Stereochemistry and *N*-Substitution on Neuronal nAChR Binding of Four Classes of Nicotinic Analogs.



These furopyridine compounds were evaluated for functional activity by measuring $^{86}Rb^+$ efflux in several cell lines. These studies were carried out using IMR-32 cells and K177 transfected cells, which are reported to possess $\alpha_3\beta_x$[10] and $\alpha_4\beta_2$[7] nicotinic receptor subtypes, respectively. The $\alpha_4\beta_2$ nicotinic receptor subtype accounts for over 90% of the high affinity binding sites for nicotine in rat brain,[11] whereas the neuroblastoma-derived IMR-32 cells may serve as a model for nAChR subtypes mediating some of the undesirable cardiovascular and gastrointestinal side-effects found with (*S*)-nicotine. All four compounds had low intrinsic activity in the IMR-32 cells, ranging from basal levels (0–25%) up to 58% that of nicotine. Compound **2b** showed moderate potency in this cell line, having an EC_{50} of 41 μM, whereas the other three compounds had EC_{50} values of >1000 μM. A wider range of functional activity was observed in the K177 cells, where values ranged for basal levels (e.g., **2a**, **3b**), low levels (**2b**), to nearly full agonist activity (**3a**). Compound **3a** was unique in that it demonstrated good functional selectivity for the two cell lines, having near full agonist activity (81% intrinsic activity/EC_{50} = 3.2 μM) in the K177 cells and very low functional activity in the IMR-32 cells (EC_{50} > 1000 μM). These results are in contrast to the $^{86}Rb^+$-flux studies with the 3-(2-

pyrrolidinylmethoxy)pyridine analogs in which the 2(*R*)-NMe isomer had <20% maximal intrinsic activity and the other three isomers had full intrinsic activity in both cell lines, and no cell-type functional selectivity was observed.[3a] Likewise, neither **ABT-418** nor (-)-nicotine exhibited any functional selectivity in these two cell lines. The good binding potency and unique functional selectivity of **3a** prompted us to investigate this compound further.

The nAChR-mediated release of dopamine from rat striatal slices has been suggested to involve activation of the α_3 nicotinic subunit,[12] and it is believed that the addiction liabilities and locomotor effects of (-)-nicotine may be mediated by dopamine release.[13] Evaluation of compound **3a** in this assay revealed that it was a partial agonist (43% intrinsic activity), having an EC_{50} of 141 nM as compared to 380 nM and 40 nM for **ABT-418** and (-)-nicotine, respectively. Compound **3a** was also evaluated for functional activity ($^{86}Rb^+$ efflux) in TE 671 cells, which contain an α_1 neuromuscular nAChR subtype.[14] As shown in Table 2, **3a** showed very weak activity in this cell line, having an EC_{50} value of greater than 100 μM and only basal levels of functional activity.

Table 2. Dopamine Release and ^{86}Rb-Flux (TE 671 Cells) Studies of nAChR Analogs.

	[^{3}H]-Dopamine Release (Rat Striatal Slices)		^{86}Rb-Flux Human α_1 (TE 671 Cells)[a]	
Compound	**EC_{50} (nM)[b]**	**% Intrinsic Activity**	**EC_{50} (μM)[b]**	**% Intrinsic Activity**
ABT-418	380[c]	100[c]	411 ± 116[d]	38[d]
(-)-Nicotine	40[c]	(100)	180 ± 30[d]	(100)
A-84543	107 ± 42	74	34 ± 9.5	40
3a	141 ± 43	43	>100 μM (n = 3)	18.9

[a]Compounds were assayed as described in ref 8. Percent intrinsic activity relative to (*S*)-nicotine at 1 mM. [b]Values represent mean ±SEM. [c]Values from ref 2b; Percent intrinsic activity relative to (*S*)-nicotine at 100 nM. [d]Values from ref 2e

In summary, a novel series of 2-(2'-furo[3,2-*b*]- pyridinyl) pyrrolidines has been synthesized and shown to be potent nAChR ligands. Although these analogs were originally designed based on structural elements of the pyridyl ether class of nicotinic ligand, the potency and SAR trends for nAChR binding of these analogs appear qualitatively more like the isoxazole/nicotinic classes than the pyridyl ethers. However, in terms of functional activity this class distinguishes itself from previous classes with its own unique profile. Of particular interest, compound **3a** has been shown to have high binding affinity for the cytisine ($\alpha_4\beta_2$) binding site and functional selectivity for the $\alpha_4\beta_2$ receptor-containing K177 cell line versus the $\alpha_3\beta_x$-bearing IMR-32 cell line or the α_1-bearing TE 671 cells. Further research exploring the SAR and pharmacology of these furopyridine analogs, as well as other conformationally constrained analogs, is currently underway.

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