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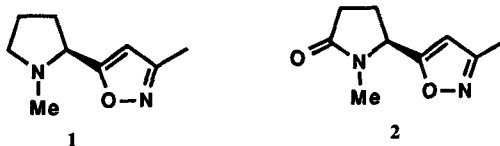
SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF PYRROLIDINE-MODIFIED ANALOGS OF THE POTENT CHOLINERGIC CHANNEL ACTIVATOR, ABT 418

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Abstract. 3' and 5'-substituted pyrrolidine analogs of the potent cholinergic channel activator (ChCA), 3-methyl-5-(2(S)-pyrrolidinyl)-isoxazole (ABT 418), **1**, were synthesized and tested *in vitro* for cholinergic channel receptor binding activity. Since the primary metabolism of **1** is oxidation at the C5' position, the effect of methyl substitution on the *in vitro* rate of metabolism was investigated.

Accumulating preclinical and clinical data suggests that compounds which activate neuronal nicotinic acetylcholine receptors (nAChRs) may have therapeutic utility in the treatment of a number of neurological disorders including Alzheimer's disease (AD)¹. We have recently reported the synthesis and pharmacological properties of ABT 418, a novel isotere of (-)-nicotine, which is currently in clinical development for the treatment of Alzheimer's disease^{2,3}. ABT 418 is a potent, stereoselective, cholinergic channel activator (ChCA) with cognitive enhancing and anxiolytic-like activity in a variety of preclinical models^{4,5,6}. Importantly, ABT 418 has a substantially reduced side-effect profile compared to (-)-nicotine⁵. ABT 418 is characterized by a rapid elimination half-life and poor oral bioavailability in rodent, dog and primate due to rapid metabolism⁷. Two primary routes of metabolism of ABT 418 have been identified.⁸ N-oxidation via microsomal NADPH-dependent flavin containing monooxygenase (FMO) to the (1R, 2S)-N-oxide and C-oxidation by cytochrome P-450 and aldehyde oxidase to a lactam (**2**) corresponding to the C5' position of the pyrrolidine ring. In the present study, 3' and 5'-substituted pyrrolidine analogs of ABT 418 were synthesized to further define the SAR of this novel ChCA. Additionally, in light of the rapid metabolism of ABT 418 we investigated the possibility that substituents at the C5' position of the pyrrolidine would protect the compound from enzymatic oxidation due to steric interactions.

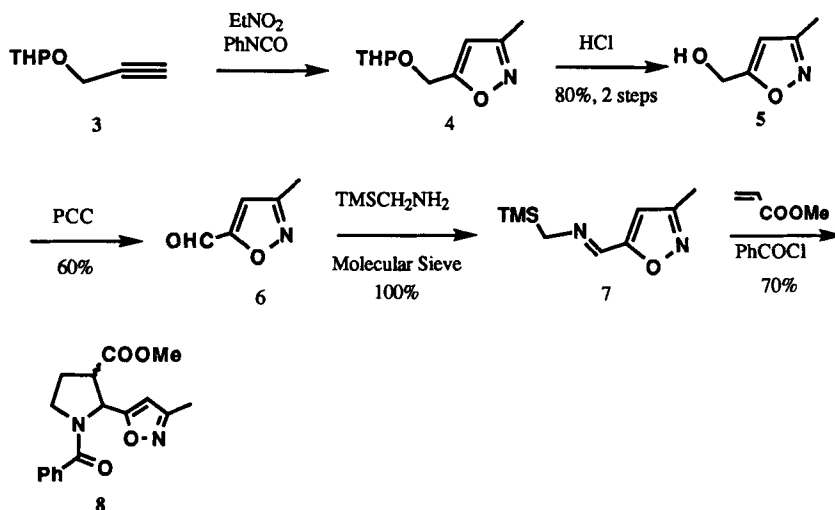


ABT-418

Chemistry: The key intermediate for the preparation of 3'-substituted ABT 418 was synthesized as outlined in Scheme 1. Protected propargyl alcohol **3** was treated with phenyl isocyanate and ethyl nitrite to give the crude 3-methyl isoxazole **4**. Cleavage of THP group with hydrogen chloride provided the alcohol **5** in 80% yield for the two steps reaction. Oxidation of **5** with pyridinium chlorochromate furnished the aldehyde **6**, which was converted to N-(silylmethyl)imine **7** upon treatment with N-(trimethylsilylmethyl)amine. The imine was then allowed to react with methyl acrylate. Unfortunately, attempts to synthesize the pyrrolidine via cycloaddition by

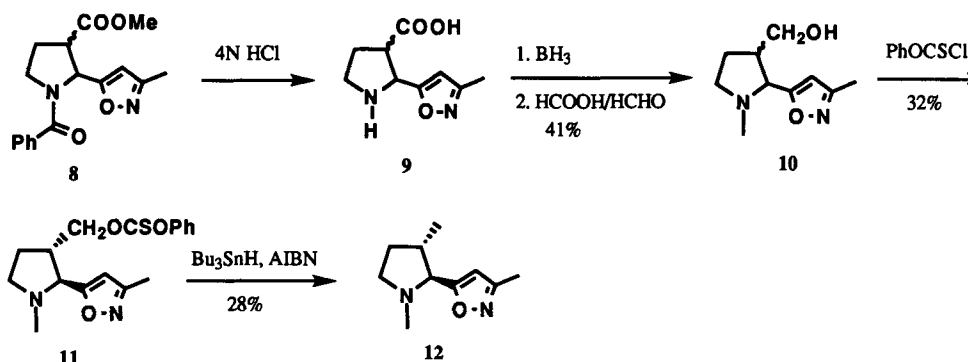
treatment with alkyl halides,⁹ CBZ-Cl,¹⁰ BOC₂O and methyl chloroformate failed to provide the desired product. After several trials, it was found that imine underwent 1,3-dipolar cycloaddition to methyl acrylate in the presence of benzoyl chloride to give pyrrolidine isoxazole **8** as a 2:1 mixture of *cis* and *trans* isomers which could not be separated on silica gel column. The mixture was therefore employed for further chemical transformations without additional purification.

Scheme 1



With racemic compound **8** well in hand, we then turned our attention to the preparation of 3'-substituted analogs depicted in Scheme 2.

Scheme 2

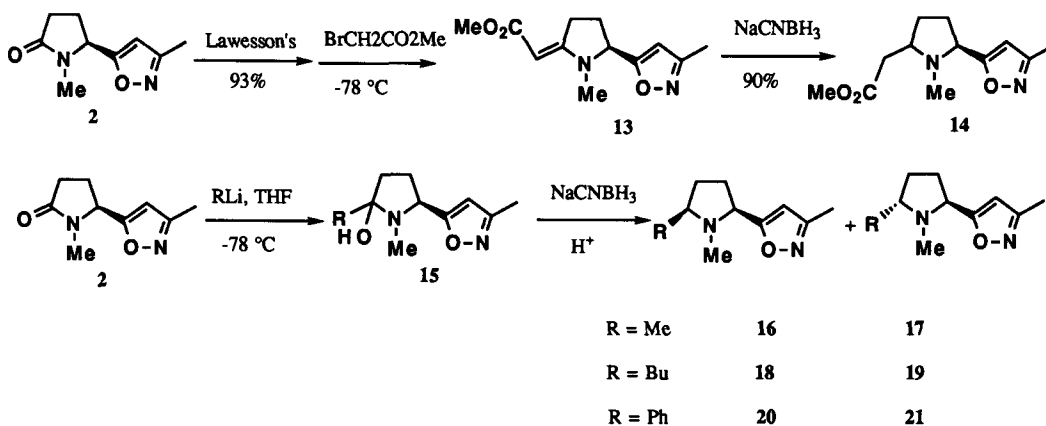


The protecting benzoyl group was removed by treating with hydrochloric acid. Compound **9** was then reduced with borane to give amino alcohol which was *N*-methylated with formic acid and formaldehyde to provide the alcohol **10**. The alcohol **10** was reacted with phenoxythiocarbonyl chloride to give compound **11**.

which could be separated on silica gel column chromatography. The trans thiocarbonate **11** was treated with tris(trimethylsilyl)silane¹¹ in the presence of AIBN to give the methyl analog **12**.

5'-Substituted analogs of ABT 418 were prepared in accordance with Scheme 3. The vinylogous methyl ester **13** was synthesized from thiolactam which derived from lactam by treatment with Lawesson's reagent. Reduction of carbon-carbon double bond was achieved by reaction with sodium cyanoborohydride to provide **14** as a 3/1 mixture of epimers at C5'. Using previously reported methodology,¹² the lactam **2** was reacted with an alkyl lithium or phenyllithium in THF at -78°C to give the amino alcohol intermediate **15**, which could be isolated in purified form via silica gel column chromatography. However, as a general practice crude compound **15** was immediately treated with sodium cyanoborohydride in the presence of hydrochloric acid to provide the 5'-substituted ABT 418 as a mixture of two diastereoisomers, which were easily separated by silica gel column chromatography.

Scheme 3

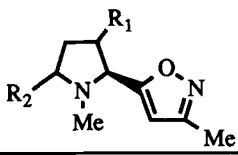


In Vitro Pharmacology: Previous studies have shown that ABT 418 (**1**) potently interacts ($K_i = 3 \text{ nM}$) with the $\alpha 4\beta 2$ subtype of nAChRs but has very low affinity ($K_i > 10,000 \text{ nM}$) for the $\alpha 7$ nAChR subtype⁴. In the present study a series of analogs containing substitutions at the 3'- and 5'-position of **1** were evaluated for their nAChR binding properties. Substituents were varied with respect to size, electronic character and hydrophobic properties in order to determine the overall effect on ligand binding affinity. The binding affinity of each compound was determined by measuring the displacement of [^3H]($-$)-cytisine and [^{125}I]alpha-bungarotoxin (α -BgT) from whole rat brain membranes as described.¹³ [^3H]($-$)-cytisine has been shown to bind with high affinity to the $\alpha 4\beta 2$ subtype of nAChRs while α -BgT displays a marked selectivity towards the $\alpha 7$ subtype¹.

As shown in Table 1, replacement of C5' hydrogen of **1** with an alkyl or phenyl group resulted in a significant decrease in affinity towards the [^3H]($-$)-cytisine binding site. Thus, the cis-methyl analog **16** showed a 23-fold decrease in affinity, whereas the trans-isomer showed a ca. 1700-fold decrease. Phenyl and butyl substituents (compound **18-21**) were not well tolerated in either orientation, although the trend toward lower affinity for analogs in which the 5'-substituent is cis to the isoxazole ring was still maintained. We assumed that

a combination of steric and conformational effects may account for the observed results. Since the 5'-position is directly adjacent to the pyrrolidine nitrogen atom, which is believed to constitute a critical binding point,^{14,15} close contact with the receptor protein in this region is quite plausible.

Table 1 Binding data for pyrrolidine modified ABT-418 analogs



Compound	R ₁	R ₂	[³ H]Cytisine Binding K _i (nM) ^a
1	H	H	3.0 ± 0.4
10	(β)-CH ₂ OH	H	> 10,000
12	(β)-Me	H	> 10,000
11	(β)-CH ₂ OCSOPh	H	840 ± 99
14	H	(α,β)-CH ₂ CO ₂ Me	3,500 ± 1000
16	H	(β)-Me	5,000 ± 840
17	H	(α)-Me	71 ± 13
18	H	(β)-Bu	3,666 ± 230
19	H	(α)-Bu	1020 ± 253
20	H	(β)-Ph	>10,000
21	H	(α)-Ph	8,000 ± 900

^a The ability of compounds to displace [³H](-)-cytisine binding to whole rat brain membranes was performed as described¹³. Values are the means ± S. E. M; n=3-4. In all cases, Hill co-efficient number were close to unity indicative of an interaction with a single class of binding sites.

The effect of substitution at the C3' position on binding affinity was also examined. Replacement of hydrogen at the C3' position of **1** with a methyl group **12** gave a big drop in the binding affinity. Replacement of the methyl group with a hydroxymethyl functionality (i.e. **10**) also resulted in a decrease in binding potency. Interestingly, changing the substituent to a bulky phenoxycarbonylhydroxymethyl group provided compound **11** which displayed weak affinity for the receptor (Table 1). Clearly, simple steric effect can't account for the low affinity of **10** and **12**.

In contrast to the effects of the analogs on the binding affinity of the α4β2 subtype labeled by (-)-cytisine, the weak affinity observed for **1** at the [¹²⁵I]α-BgT binding site was not altered by the substitutions described in this study (data not shown).

Since ABT 418 undergoes metabolic oxidation at the C5' position of the pyrrolidine⁶, the effect of methyl substitution at this position on the overall rate of NADPH-dependent metabolism was examined using rat hepatic S-9 fraction⁸. The *in vitro* half-life (Table 2) for the cis-methyl analog **16** was similar to that for ABT 418, whereas the *in vitro* half-life of the trans-methyl substituted compound **17** was significantly shorter than ABT 418. These findings indicate that C5' methyl substitution, while chemically obstructing the principle site of ABT

418 metabolism, i.e. C-oxidation to the lactam **2**, does not reduce the overall susceptibility of these compounds to biotransformations. In fact, the observation that these compounds have *in vitro* half lives similar to and even shorter than ABT 418 raises the question whether that C5' methyl substitution actually enhances the rate by which these compounds are metabolized by alternative routes (e.g., pyrrolidine N-oxidation). Moreover, the differences in metabolic rates between the *cis* and *trans*-methyl substituted compounds indicate that metabolism by these alternative routes of metabolism is influenced by the orientation of the methyl substitution.

Table 2. In Vitro Half-Life of ABT 418 and C5' Methyl-Substituted Pyrrolidine Analogs

<u>Compound</u>	<u>In Vitro Half-Life^a</u> <u>(mins)</u>
1	47.3 ± 1.5
16	48.3 ± 3.3
17	26.0 ± 1.7*

^aThe half-lives shown are normalized to 2 mg S-9 protein. Values represent the means ± S.E.M.; n=3. * Significantly different to **1**, *p* < 0.05.

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13. [³H](-)-Cytisine binding conditions were as follows: samples containing 100-200 µg of rat brain membranes, 1.25 nM [³H](-)-cytisine (30 Ci/mmol) and various concentrations of drug were incubated in a final volume of 500 µl for 75 mins. at 4°C in duplicate. Non-specific binding was determined in the presence of 10 µM (-)-nicotine. To assess the ability of compounds to interact with the [¹²⁵I]α-BgT binding site, rat brain membranes (200 µg) were incubated with 1.9 nM [¹²⁵I] α- Bgt (106 Ci/mmol) and the various concentrations of drug in triplicate for 3 hours at 37°C. Non-specific binding was determined in the presence of 1 µM unlabeled α-BgT. In competition experiments, the drug concentration producing 50% inhibition (IC₅₀) of radioligand binding and the Hill coefficient (n_H) were determined from plots of log (B₀ - B)/B versus log (concentration of drug), where B₀ and B are specific binding in the absence and presence of competitor, respectively using a four parameter logistics program in RS/1 (Bolt Beranek and Newman Inc. Cambridge, MA). Inhibition constant (K_i) values were determined using the Cheng-Prusoff equation ($K_i = IC_{50}/(1 + [L]/K_d)$) where [L] is the radioligand concentration and K_d is the affinity of the receptor for the radioligand.
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