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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 2'-(R) AND (S) PYRROLIDINE-MODIFIED ANALOGS OF THE CHOLINERGIC CHANNEL ACTIVATOR, ABT-418

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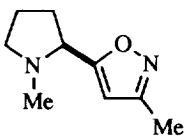
Abstract. 2'-(R) and (S) pyrrolidine modified analogs of the potent cholinergic channel activator (ChCA), 3-methyl-5-(2(S)-pyrrolidinyl)-isoxazole (ABT-418), **1**, were synthesized and tested *in vitro* for cholinergic channel receptor binding activity. The 4'-(S)-methyl analog of **1**, the most potent compound of the 4'-substituted analogs investigated, was 6-fold less potent than **1**.

Interest in neuronal nicotinic acetylcholine receptors (nAChRs) as potential molecular targets for the development of agents to treat CNS disorders has increased in recent years due to new findings related to the molecular biology of this receptor class[1] and the development of novel ligands.[2,3] We have recently reported on a series of isoxazole analogs, exemplified by ABT-418, that selectively interact with nAChRs.[2,4,5] ABT-418 differentially activates nAChR subtypes to elicit a variety of behavioral effects, including cognitive-enhancing and anxiolytic-like activity, in preclinical models.[2,6,7] Importantly, the compound has reduced side-effect liabilities compared to the classical nAChR agonist, (-)-nicotine.[6]

In the present report, 4'-substituted pyrrolidine analogs of ABT-418, and corresponding stereoisomers, were synthesized to further define the SAR of this novel cholinergic channel activator (ChCA) and to compare this series with the previously reported nicotine analogs.[8] In this regard, we have previously reported that addition of a methyl group to the C4' position of nicotine has a 3.7-fold change on its affinity for the $\alpha_4\beta_2$ subtype, one of the major nAChR subtypes.[8] Additionally, while it has been demonstrated that the (S)-enantiomer of ABT-418 is approximately 14-fold more potent than its corresponding (R)-enantiomer,[4] limited studies have been carried out with optical isomers of ABT-418 analogs. Thus, in the present study, the influence of stereochemistry and structural modification at the C4'position, and to a limited extent at the C5' position of the pyrrolidine ring of ABT-418 on the interactions with nAChRs were investigated. A variety of lipophilic and hydrophilic groups were placed at the C4' position to better understand the optimal substitution at this position for interaction with nAChRs.

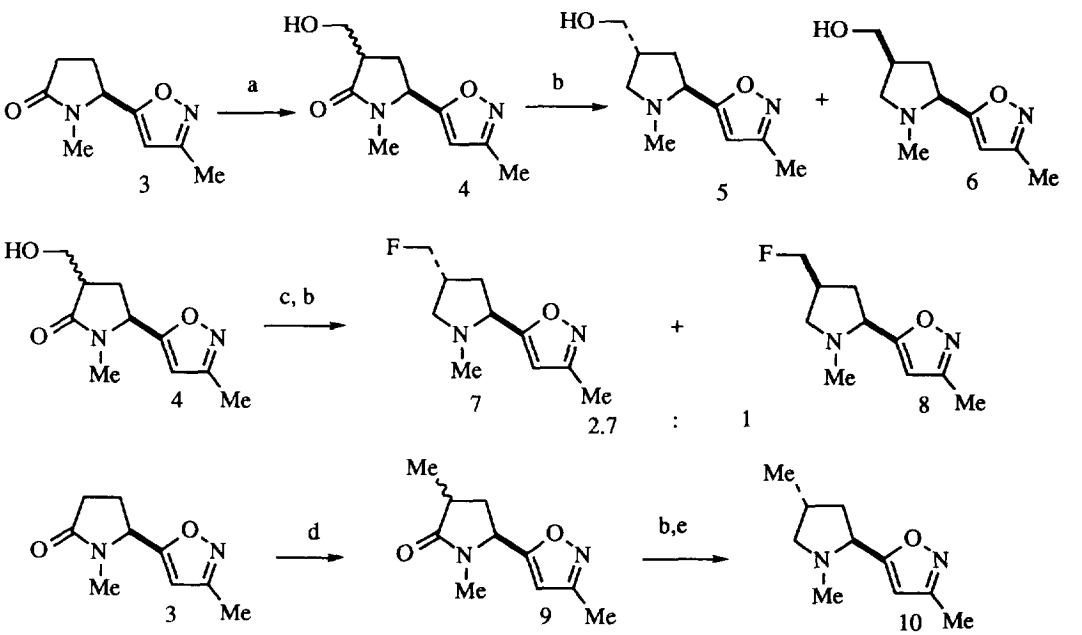


ABT-418

Chemistry: The 4'-substituted ABT-418 analogs in the S series were prepared from (S)-pyroglutamic acid. Thus, treatment of (S)-lactam **3**, which was derived from (S)-pyroglutamic acid according to the published

procedure,[9] with LDA at -78°C followed by addition of formaldehyde afforded the 3'-hydroxymethyl isoxazole **4**, as a mixture of two diastereoisomers. Reduction of the lactam with borane followed by cleavage of the borane complex with cesium fluoride provided the two hydroxymethyl isomers **5** and **6** in a 2.5:1 ratio which were readily separated by silica gel column chromatography ($CHCl_3$:CH_3OH = 40:1 then $CHCl_3$:CH_3OH = 20:1). The stereochemistry of diastereoisomers **5** and **6** was determined by NOE.[10] Treatment of compound **4** with DAST gave the corresponding fluoromethyl compound as a mixture of two isomers which was used directly for next reaction. Reduction of the lactam with borane followed by cleavage of the borane complex with cesium fluoride provided the two fluoromethylisoxazole isomers **7** and **8** in a 2.7:1 ratio which were readily separated via silica gel column chromatography ($CHCl_3$:CH_3OH = 20:1). The stereochemistry of compounds **7** and **8** was determined by NOE studies.[11] The trans-4'-methyl substituted analog was prepared by treatment of the enolate anion, which was generated by reaction of (S)-lactam **3** with LDA, with the methyl iodide followed by reduction with borane as described above. Although a very small amount of diastereoisomer of compound **9** could be detected, we failed to isolate the minor component of compound **10** after reduction.

Scheme 1[a]

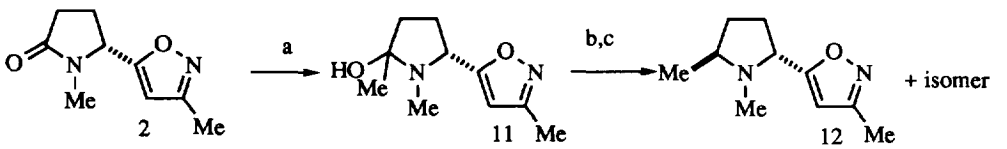


[a] Reagent: (a) LDA, H_2CO, -78 °C; (b) BH_3, THF; then CsF, EtOH; (c) DAST, CH_2Cl_2; (d) LDA, MeI, THF, -78 °C; (e) separation of the major isomer.

Preparation of (S)-5'-substituted analogs of ABT-418 was described previously.[5,12] Using the same methodology, the (R)-lactam **2** was converted to the 5'-methyl ABT-418 as a 8:1 mixture of two diastereoisomers, which were easily separated by silica gel column chromatography ($CHCl_3$:CH_3OH = 40:1 then

$CHCl_3$:CH_3OH = 20:1). The stereochemistry of compound (R)-**12** was determined by comparing its NMR spectrum with that of its enantiomer.[5]

Scheme 2[a]



[a] Reagent: (a) MeLi, THF, -78 °C; (b) $NaCNBH_3$, H^+; (c) separation of the major isomer.

***In Vitro* Pharmacology**: In brain, two major classes of nAChR exist. They are divided into those labeled by [^{3}H](-)-nicotine or [^{3}H]cytisine, and those labeled with high affinity by [^{125}I]α-bungarotoxin (α–BgT).[13] The distribution of high affinity nicotine binding sites in rat brain coincides with the distribution the α4β2 subunit combination in rat brain[13,14] and is consistent with the finding that greater than 90% of the high affinity nAChR binding sites in rat can be precipitated by antibodies raised against the α4 and β2 subunits.[15] A good correlation has also been reported between the distribution of α7 mRNA and that of the high affinity binding sites for α–BgT in rodent brain.[13,16]

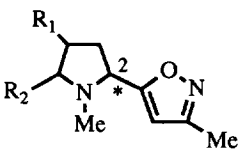
Our previous studies have shown that ABT-418 (**1**) potently interacts (K_i = 3 nM) with the putative α4β2 subtype of the nAChR but has lower affinity (K_i > 10,000 nM) for the putative α7 nAChR subtype.[2] Thus, in the present study, the interaction of all analogs with the putative α4β2 subtype was investigated. The ability of the most potent analogs to displace [^{125}I]α-BgT binding was also examined.

The effect of substitution at the C4' position on binding affinity was examined. Substituents were varied with respect to size, electronic character and hydrophobic properties in order to determine the overall effect on ligand binding affinity. As shown in Table 1, replacement of C4' hydrogen of **1** with a *trans*-methyl group [i.e. (S)-**10**] resulted in a 6-fold drop in binding affinity towards the [^{3}H]-cytisine binding site. This is similar to our findings with the 4'-methylnicotine analog which exhibts a 4-fold reduction in binding potency compared with that of (-)-nicotine.[8] Changing the 4'-methyl substituent to a fluoromethyl group [(S)-**7**] increased the K_i value to 155 nM (51-fold decrease in affinity compared to (S)-**1**). A 10-fold decrease was observed when nicotine was substituted with fluoromethyl at C4' position. While replacement of the fluoro group of (S)-**7** with a polar hydroxy functionality [i.e.(S)- **5**] resulted in approximate 2200-fold decrease in binding potency, a 136-fold decrease was observed with the corresponding nicotinic analog. The deleterious effect of these substituents might be due to steric occlusion in this region of the receptor. It may be that the steric volume of a fluoromethyl group represents the upper limit which may be accommodated by the space in the receptor ligand binding domain since a large decrease in binding was observed in going from fluoromethyl to hydroxymethyl group. Thus, steric factors appear to be important for optimal binding potency for the substituents at the C4' position of the pyrrolidine ring of (S)-ABT-418. These results also indicate that (S)-isoxazole analogs are more sensitive to the substituents at C4' position than the corresponding (S)-nicotine analogs. In the (R)-series, replacement of the C4'-*trans*-methyl

group [i.e. (R)-**10**] with a hydroxymethyl group [i.e.(R)- **5**] resulted in a 12-fold drop in binding affinity towards the [^{3}H]-cytisine binding site. This result is similar to our findings with the S enantiomers.

To examine whether pyrrolidine modified ABT-418 analogs have the same stereochemical bias to nicotinic receptors at C2' position as we observed with ABT-418 (**1**) and its corresponding enantiomer,[4] the enantiomers of compounds **5**, **6**, **8**, **10** and **12** also were evaluated for the affinity at receptor binding sites. Table 1 reveals that (S)-4'-fluoromethyl (**8**), (S)-4'-methyl (**10**) and (S)-5'-methyl (**12**) analogs were more potent than the corresponding (R)-enantiomers. Thus, this result is consistent with the finding we obtained with ABT-418 (**1**) and its enantiomer. However, the difference in the potency diminished when a larger functional group was placed at C4' position of pyrrolidine ring. As demonstrated from the table, (S)-4'-*trans*-hydroxymethyl (**5**) and (S)-4'-*cis*-hydroxymethyl (**6**) analogs possess the similar binding affinities to those of their corresponding R enantiomers.

Table 1 Binding data for pyrrolidine modified ABT-418 analogs



Compound	Stereochemistry at C2	R_1	R_2	[^{3}H]Cytisine Binding K_i (nM)[a]
(S)-1	S	H	H	3.0 ± 0.4
(S)-10	S	*trans*-Me	H	19 ± 1.0
(S)-7	S	*trans*-CH_2F	H	155 ± 6
(S)-8	S	*cis*-CH_2F	H	1,683 ± 159
(S)-5	S	*trans*-CH_2OH	H	6646 ± 2714
(S)-6	S	*cis*-CH_2OH	H	6036 ± 600
(S)-12	S	H	*trans*-Me	71 ± 8
(R)-1	R	H	H	7.4 ± 0.7
(R)-10	R	*trans*-Me	H	946 ± 112
(R)-8	R	*cis*-CH_2F	H	16,946 ± 5150
(R)-5	R	*trans*-CH_2OH	H	10883 ± 2670
(R)-6	R	*cis*-CH_2OH	H	8136 ± 902
(R)-12	R	H	*trans*-Me	166 ± 43

[a] The ability of compounds to displace [^{3}H](-)-cytisine binding to whole rat brain membranes was performed as described.[17] Values are the means ± S. E. M; n=3-4. In all cases, the Hill co-efficient was not significantly different from unity indicative of an interaction with a single class of binding sites.

Previous studies[5] have shown that configuration at C5' position affects the binding potency. In the present study, the effect of configuration at C4' position of pyrrolidine ring on [^{3}H]-cytisine binding can also be

assessed (Table 1). In the case of (S)-hydroxymethyl analogs, the *cis* isomers [(S)-**5**] and *trans* isomers [(S)-**6**] displayed similar binding affinities. A small but significant difference (*trans* is 2-fold more potent) with respect to C4' stereochemistry was previously observed with the corresponding nicotine analogs. In contrast to hydroxymethyl analogs, somewhat greater differences were observed with fluoromethyl analogs [(S)-**7** is 10.8-fold more potent than (S)-**8**]. This latter result is in accord with our previous findings on the 5'-substituted analogs of ABT-418, where [^{3}H]-cytisine binding affinities for *trans* isomers were more potent than *cis* isomers.[5] With (R)-hydroxymethyl analogs, *cis* [(R)-**5**] and *trans* isomers [(R)-**6**)] displayed similar binding affinities.

Several representative compounds, namely, (S)-**10**, (R)-**10** and (S)-**12**, were also investigated for their ability to displace [^{125}I]α-BgT binding to rat brain membranes. All of the compounds were found to display very weak affinity toward this nAChR subtype. K_i values for the (S)-**10**, (R)-**10** and (S)-**12** analogs were 15690 ± 3100 nM, 11980 ± 1567 nM and 19675 ± 3482 nM (mean ± S.E.M.; n=3), respectively. While the binding affinity of these ligands for the putative α7 subtype is much lower than that found for the putative α4β2 subtype, it is important to note that nicotine, presumably the endogenous α7 ligand, also displaces [^{125}I[α-BgT binding to rat brain with relatively weak affinity (K_i = 5000 nM).

In conclusion, we have shown that the configuration at C4' and C2' position of pyrrolidine ring of ABT-418 has a profound effect on the binding potency when substituents are smaller than fluoromethyl group. The effect was diminished when substituents are larger than fluoromethyl. In addition, the steric volumes of substituents at C4' position play an important role in determining the binding affinities in both S and R series.

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10. The stereochemistry of alcohol **6** was determined by NOSEY and DQCOSY. H3a' (β configuration) was first identified by using DQCOSY and NOSEY. A NOE was observed between H3a' and H2'. Therefore, the stereochemistry of H3a' is β. We have also seen an NOE between H3a' and the methylene proton of CH_2OH. Thus, the stereochemistry of CH_2OH is assigned as β. Based on this analysis, the configuration of CH_2OH should be β.
11 The stereochemistry of fluoromethyl compound **7** was determined by NOSEY and DQCOSY. H2 (α configuration) was first identified by using DQCOSY. A NOE was observed between H3a' and H2'. Therefore, the stereochemistry of H3a' is α. We have also seen a NOE between H3a' and the methylene proton of CH_2F. Thus, the stereochemistry of CH_2OH is assigned as α. Basing on this analysis, the configuration of CH_2F should be α.
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