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TETRAHYDROISOQUINOLINE AS A PHENYLALANINE REPLACEMENT IN RENIN INHIBITORS

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Abstract: A series of compounds incorporating the tetrahydroisoquinoline (THIQ) fragment was synthesized and evaluated for renin inhibition. Stereochemistry around the alpha carbon, as well as nitrogen substitution was investigated. Selected compounds were tested for in vivo activity in a conscious high renin cynomolgus monkey model.

The success of ACE inhibitors such as captopril and enalapril has demonstrated the importance of the renin-angiotensin system (RAS) in hypertension and led to a search for orally active renin inhibitors.[1,2] Inhibition of renin could be superior to that of ACE as an antihypertensive mechanism because renin is the first and rate limiting step in the RAS cascade. Additionally, inhibition of renin may yield a better side effect profile due to the fact that renin has only one known natural substrate, angiotensinogen.[3] ACE, on the other hand, is known to also cleave bradykinin and other peptides, in addition to angiotensin I.

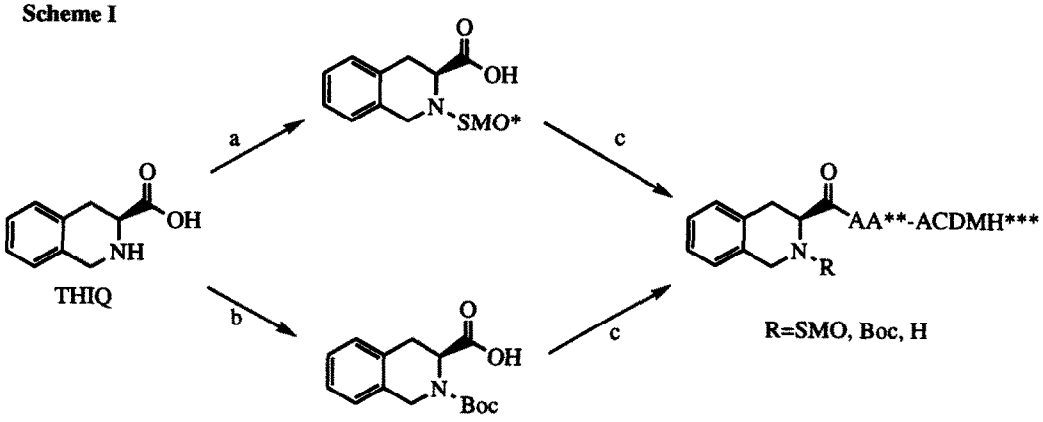
In our search for potent new renin inhibitors, our attention was drawn to the tetrahydroisoquinoline (THIQ) group. This moiety has been previously used in ACE inhibitors,[4,5] bradykinin antagonists,[6] and opioid antagonists[7] as a conformationally restricted phenylalanine (Phe) replacement. The P3 position of angiotensinogen is occupied by a Phe residue and it was hoped that the conformationally restricted surrogate THIQ group in a renin inhibitor might adopt a preferred binding mode at the S3 site of the renin enzyme, leading to improved potency when compared with the Phe P3 substituent.

Chemistry:

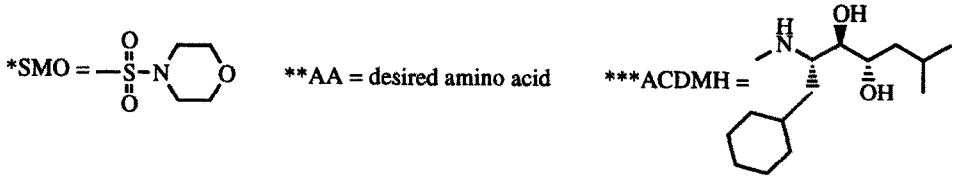
The compounds for this study were synthesized using standard peptide synthetic techniques employing DCC/HOBT coupling procedures as shown in Scheme I.[8] The reaction sequence entailed introduction of the nitrogen substituent group (Boc or sulfonylmorpholino) followed by DCC/HOBT coupling.

Scheme II shows the more complex synthesis of compounds 9 and 10, each containing an isosteric bond replacement. Following Boc protection of the THIQ amine, reaction with 1,1'-carbonyldiimidazole (CDI) and N,O-dimethylhydroxylamine·HCl afforded the N,O-dimethyl amide, 15, which underwent a Grignard reaction with 4-bromo-1-butene to form the alkene, 16. Oxidation to the carboxylic acid with $RuO_2/NaIO_4$ followed by DCC/HOBT coupling with ACDMH yielded 9. Reduction of the ketone with KBH_4 resulted in a mixture of diastereomeric alcohols, 10, which were not separable by chromatography on silica gel.

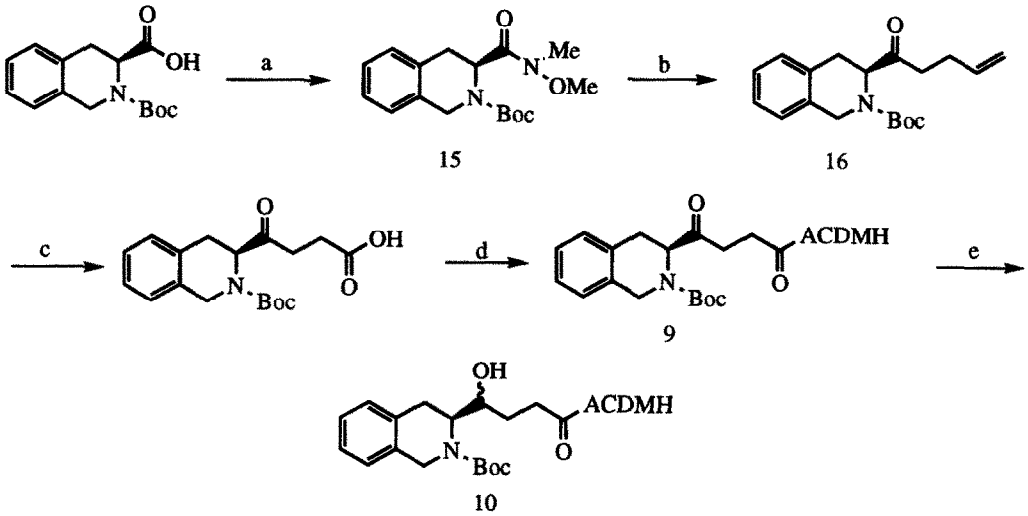
Scheme I



a $O(CH_2CH_2)_2N-SO_2Cl/Me_4N^+OH^-$ b $Boc_2O/NaOH$ c AA-ACDMH/DCC/HOBT for R=H, second step HCl



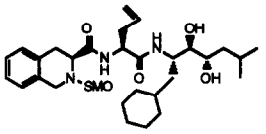
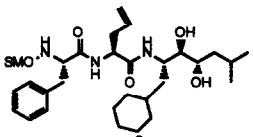
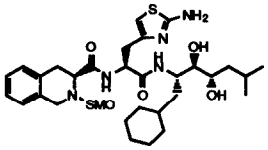
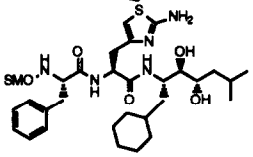
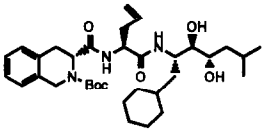
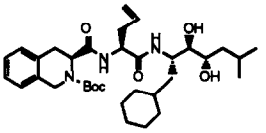
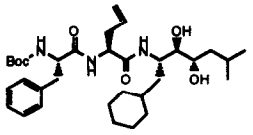
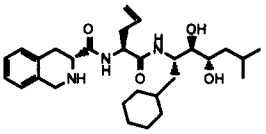
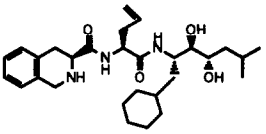
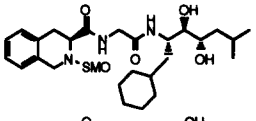
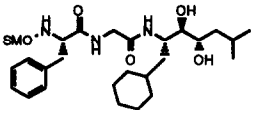
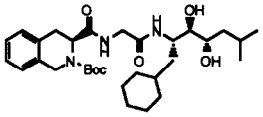
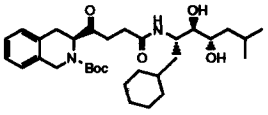
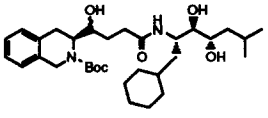
Scheme II



a MeNHOMe•HCl/CDI/N-Me piperidine b $CH_2=CHCH_2CH_2Br/Mg$ c $RuO_2/NaIO_4$

d ACDMH/DCC/HOBT e $KBH_4/EtOH/H_2O$

Table I

	Structure	Renin Activity IC_{50} (nM)		Structure	Renin Activity IC_{50} (nM)
1		2.89	11		0.16
2		2.71	12		0.36
3		325			
4		146	13		0.42
5		373			
6		11.5			
7		101	14		3.6
8		11.9% at 10^{-6}M			
9		9.8% at 10^{-6}M			
10		0.0% at 10^{-6}M			

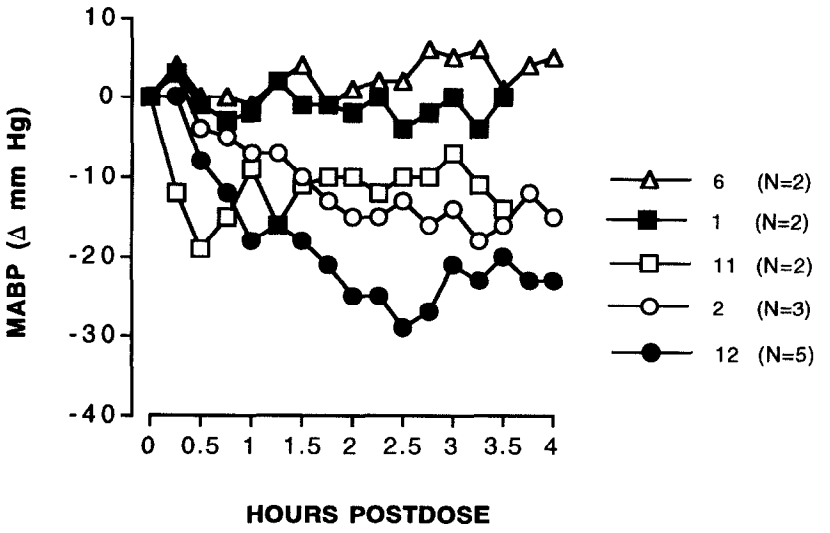
Pharmacology:

The results of in vitro renin inhibition (monkey plasma) for the compounds are shown in Table I.[9] Selected P3 Phe containing compounds are listed for comparison. As the data in Table I illustrate, it was found that THIQ substitution at P3 resulted in compounds with binding affinities ranging from micromolar to low nanomolar. We had speculated that restricting the allowed conformations of the aromatic side chain might improve affinity, however, comparison of the THIQ compound 1 with its phenylalanine analog 11 showed the latter to be the more active. This was also found to be the case with the other analog pairs of 2 vs 12, 4 vs 13, and 7 vs 14. In each case the phenylalanine analog was at least seven times more potent at inhibiting renin.

The importance of stereochemistry at the alpha carbon of THIQ was investigated through the compound pairs of 3 vs 4 and 5 vs 6. The modeling[10] of the R and S isomers in the renin model[11] prior to synthesis predicted that the R-THIQ analog would be more active than the S derivative based on the steric fit of the derivatives. The in vitro experimental results for both pairs of Boc protected and free amine diastereomers, however, showed that the opposite was true, with the S isomer exhibiting greater potency than the R.

Comparison of the in vitro renin inhibition data for compounds 1-8 with regard to the nitrogen substituent of THIQ leads to the conclusion that SMO substitution afforded the best affinity while the Boc containing compounds produced only micromolar activity. The Boc-(R) isomer, 3, shows a slightly better activity versus the free amine, 5, but this was not the case with the S isomers (4 and 6). Compound 6 is the only example we are aware of, where a free nitrogen in the P3 position of a renin inhibitor does not decrease in vitro potency. In both cases it was found that the free amines were more soluble in an aqueous medium than the Boc substituted analogs.[12] Isosteric P2-P3 bond containing analogs 9 and 10 did not show an improvement in activity over the parent analog, 8. The decreased activity could be due to the isosteric nature of the P2-P3 bond or possibly due to the lack of a P2 substituent.

Figure I



The in vivo oral activity of compounds 1, 2, 6, 11, and 12[13] are shown in Figure I. The data were collected using a conscious, high renin, normotensive, male cynomolgus monkey model.[14] It was hoped the increased aqueous solubility of 6 might improve oral activity, however, the compound did not show any decrease in blood pressure. Addition of a SMO group to the nitrogen also did not improve oral potency (1). The P3 Phe analog, 11, showed modest oral activity, so in this case the THIQ was detrimental to oral efficacy despite reasonable receptor binding affinity. Likewise, when (S)-2-amino-4-thiazolylalanine was substituted at P2 with THIQ at P3, a decrease in efficacy compared to the P3 Phe analog was seen (2 vs 12). Compound 2, the most potent in vitro renin inhibitor, showed the best efficacy of the compounds tested in this series.

Conclusions:

We have shown that substitution of the conformationally restricted THIQ in the P3 position of renin inhibitors can yield compounds that possess good in vitro potency (1,2). In this series, stereochemistry parallels the natural amino acid preference, contrary to the original modeling predictions. Substitution on the nitrogen was not necessary to maintain in vitro potency and the unsubstituted derivatives possessed greater aqueous solubility. Oral efficacy of the compounds was decreased as compared to the unrestricted Phe analogs. Compound 2 was the most potent THIQ containing analog in vivo.

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