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CHIRAL RECOGNITION OF THE ANGIOTENSIN II (AT_1) RECEPTOR BY A HIGHLY POTENT PHENOXYPROLINE OCTANOAMIDE

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Abstract: The synthesis and *in vitro* biological evaluation of a novel series of diastereomeric phenoxyproline octanoamides (**3a-h**) as angiotensin II (AT_1) receptor antagonists are reported.

The recent introduction of the nonpeptide angiotensin II (Ang II) receptor antagonist losartan has prompted the synthesis and evaluation of numerous structural analogs.[1,2] Most of the reported compounds are based on the biphenyltetrazole substructure of losartan or on the Takeda N-benzylimidazole series from which losartan was originally derived.[3] Recently, we described the synthesis and receptor affinity of a series of polysubstituted 4-aminoimidazole derivatives as novel structural antagonists of the AT_1 receptor.[4] These compounds (**1a-c**, Figure 1) possess only modest *in vitro* potency ($pA_2 = 7.0$) as measured by their ability to block Ang II induced contractile responses in rabbit aorta strips.[5] In order to take advantage of the structural novelty of the series, we began exhaustive structure activity relationship studies with the goal of significantly increasing the potency of these derivatives. Herein we report the discovery of a new class of highly potent and selective, chiral nonpeptide AT_1 antagonists, the effect of stereochemical modification on *in vitro* potency, and a structural comparison of these agents to losartan.

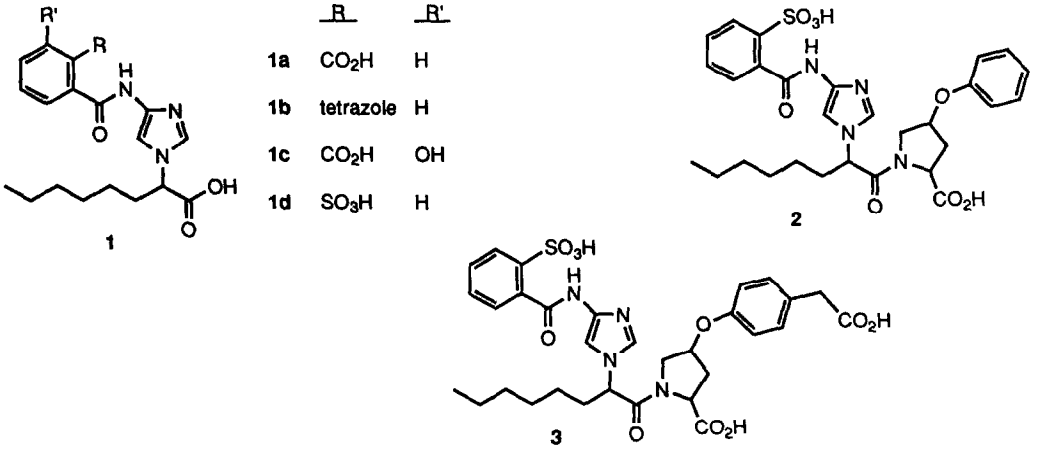


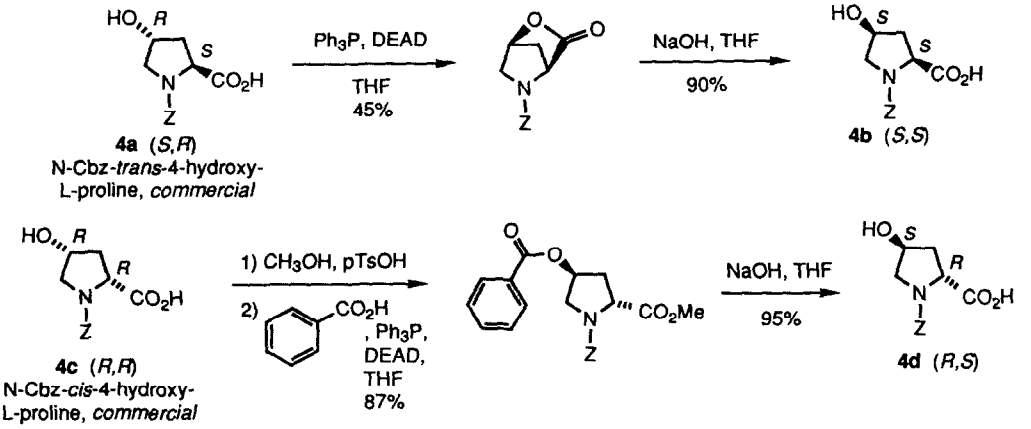
Figure 1

During the course of our work, we found that introduction of a 4-phenoxyproline residue via an amide linkage to **1** led to a compound (**2**) with 100-fold greater *in vitro* potency. Furthermore, SAR studies directed at the aryl ring yielded many potent antagonists representing a wide range of structural diversity.

One substitution of interest, which will be the focus of this communication, is the *para*-phenoxyacetic acid derivative **3**. An interesting structural consequence of introducing the phenoxyproline moiety is that these compounds possess three asymmetric centers. As a means of establishing the optimal stereochemistry at each center for interaction with the AT_1 receptor, we undertook the synthesis of all eight diastereomers of **3**.

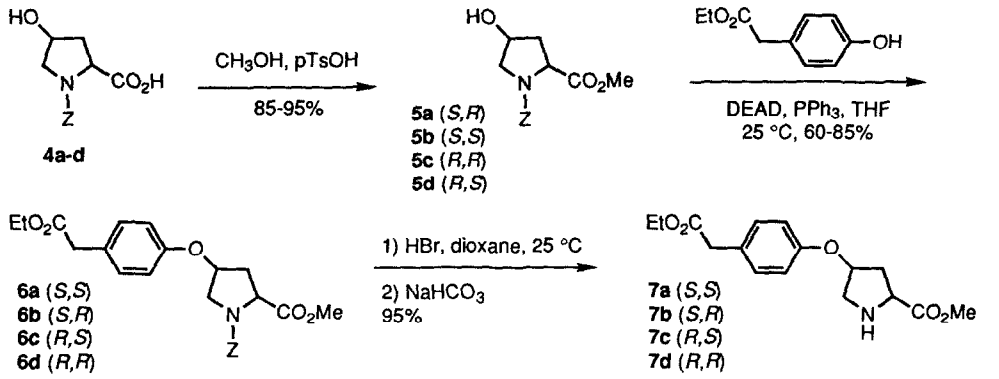
Chemistry Synthetic access to the eight diastereomers of **3** required the use of all four diastereomers of 4-hydroxyproline (**4a-d**, Scheme I) as starting materials. Whereas N-Cbz-*trans*-4-hydroxy-L-proline (**4a**) and N-Cbz-*cis*-4-hydroxy-D-proline (**4c**) were commercially available, **4b** and **4d** had to be prepared by stereochemical modification of **4a** and **4c**, respectively, as outlined in Scheme I.[6,7]

Scheme I



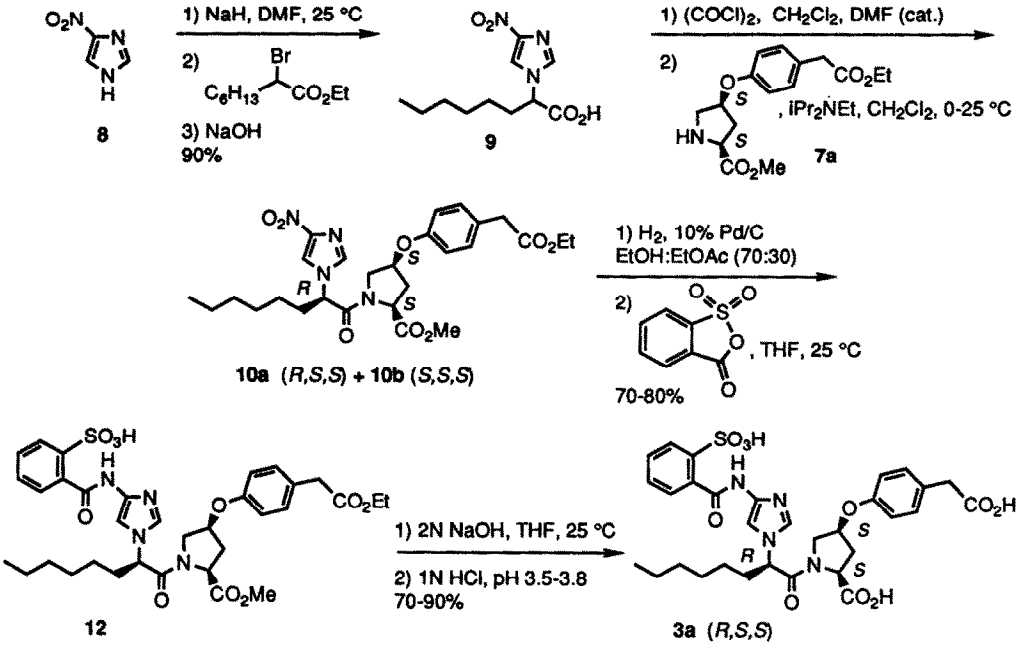
Preparation of the 4-phenoxyproline derivatives **7a-d** is described in Scheme II. Thus, esterification of **4a-d** (CH_3OH, pTsOH) yielded methyl ester derivatives **5a-d** in 85-95% yield. These compounds were then reacted with ethyl-4-hydroxyphenylacetate under Mitsunobu conditions (DEAD, Ph_3P, THF) to provide the four diastereomeric 4-phenoxyproline derivatives **6a-d**.[7,8] Cleavage of the Cbz protecting groups under acidic conditions (HBr, dioxane, 25 °C, then $NaHCO_3$) gave amino esters **7a-d** in good yield.

Scheme II



The transformation of **7a-d** to **3** is exemplified for **7a** in Scheme III. 4-Nitroimidazole **8** was alkylated with ethyl-2-bromooctanoate (NaH, DMF, 25 °C) to give exclusive N-(1) alkylation. The crude product was hydrolyzed under alkaline conditions to give, upon work-up, 2-(4-nitroimidazole)-octanoic acid **9** in 90% yield. This material was converted to the acid chloride and reacted directly with proline ester **7a** at 0°C in the presence of Hünig's base. Work-up provided a 1:1 mixture of diastereomeric nitroimidazole derivatives **10a** and **10b** which were separated by chromatography. Each isomer was isolated in approximately 33% overall yield. The absolute stereochemistry of **10a** was assigned (*R,S,S*) based on X-ray analysis of a closely related compound in the series (differing only at the *para* position of the proline 4-aryloxy group, -H vs. $-CH_2CO_2Et$) and comparison of ^1H NMR spectral data and HPLC profile of the two compounds. It follows, therefore, that the absolute stereochemistry of **10b** is (*S,S,S*). The final steps in the sequence for the preparation of **3** are demonstrated for **10a**. Catalytic reduction of **10a** provided an intermediate 4-aminoimidazole derivative that was treated with sulfobenzoic anhydride to give sulfonic acid **12** in ~75% yield. Lastly, the carboxylic acid esters of **12** were hydrolyzed under alkaline conditions to provide **3a**, isolated as a white solid following adjustment of the solution pH.

Scheme III



Summarized in Figure 2 is the stereochemical origin of all eight diastereomers (**3a-h**). Absolute stereochemical assignments for **3c-h** are based on the following arguments. Isomers **3g** and **3h** are enantiomers of **3b** and **3a**, respectively, and, as expected, the enantiomeric pairs provide identical ^1H NMR spectra as well as HPLC profiles. Thus, these isomers are assigned as **3g** (*R,R,R*) and **3h** (*S,R,R*). The assignment of the remaining isomers is based on an independent synthesis of **3c** and **3e** using the (*R*) enantiomer of octanoic acid **9**, prepared by chemical resolution. Comparison of these samples to those prepared employing racemic **9** establish the stereochemistry of **3c** and **3e** as (*R,S,R*) and (*R,R,S*), respectively. Finally, enantiomeric relationships confirm the assignment of **3d** as (*S,S,R*) and **3f** as (*S,R,S*).

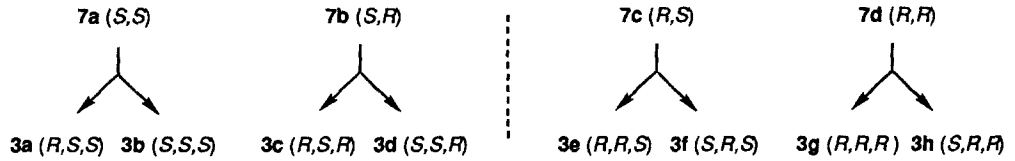


Figure 2

Biological Evaluation Compound mediated antagonism of Ang II *in vitro* was determined in isolated rabbit thoracic aorta as described in detail previously.[9] Antagonist potency was determined by calculation of K_B using competitive (**3c-3h**) or noncompetitive (**3a** and **3b**) theory.[10] There were marked differences among diastereomers in their ability to antagonize Ang II *in vitro* (Table 1). The (*R,S,S*) diastereomer **3a** was significantly more potent ($pK_B = 9.9$) than the (*S,S,S*) diastereomer **3b** ($pK_B = 8.3$), and both were significantly more potent than any of the other six diastereomers all of which had pK_Bs less than 7. There was more than a three order of magnitude difference in the potency between **3a** and its enantiomer **3h** (*S,R,R*). The (*R,S,S*) diastereomer **3a** was a highly selective nonsurmountable antagonist of Ang II because it lacked activity *in vitro* against the effects of KCl, norepinephrine, or serotonin. For comparison, the competitive antagonist losartan yielded a pK_B of 8.2 (n = 30) under the same conditions.

Table 1. Dissociation Constants of Diastereomers†

Compound	$K_B \pm$ S.E. (nM)	(n)
3a (*R,S,S*)	**0.12** ± **0.03**[a]	(12)
3b (*S,S,S*)	**4.5** ± **0.09**[b]	(10)
3c (*R,S,R*)	**503** ± **104**[c]	(8)
3d (*S,S,R*)	**555** ± **147**[c]	(8)
3e (*R,R,S*)	**156** ± **37**[d]	(8)
3f (*S,R,S*)	**761** ± **224**[c]	(8)
3g (*R,R,R*)	**191** ± **26**[d,c]	(8)
3h (*S,R,R*)	**489** ± **210**[d,c]	(7)

† K_B values with the same superscript letter are not significantly ($p > 0.05$) different from each other. Number of individual tissues are in parentheses.

Thus, both asymmetric centers on the proline ring are required to possess the (*S*) configuration; either alone is insufficient for maximum activity. The stereocenter bearing the octyl side chain is less sensitive to stereochemical change; however, activity shows a definite preference for the (*R*) configuration. The marked selectivity exhibited by **3a** vs. **3h** suggests a highly stereospecific interaction between the ligand and its receptor.

Structural Considerations Several subsites in the AT_1 receptor are important for recognition and binding of ligands.[9,11] These subsites include a lipophilic pocket that accommodates an alkyl chain and a basic residue that interacts electrostatically with an acidic group. By spatial analogy, molecular modeling studies of Ang II have shown that these subsites can accommodate the side chain of Ile_5 and the C-terminal carboxyl group of Phe_8, respectively.[12] Losartan and most other AT_1 receptor antagonists reported to date fill these subsites. Interestingly, compound **3a** not only is able to fill these sites, but also reaches an additional, previously unknown, subsite of the AT_1 receptor. Figure 3, which was produced with the SYBYL molecular modeling program,[13,14] illustrates the overlap of the structures of **3a** and losartan. As described above, the phenoxyproline side chain of **3a** contributes significantly to the molecule's affinity for

the AT_1 receptor and, as can be appreciated from Figure 3, stereospecifically accesses a site of the receptor that losartan cannot.

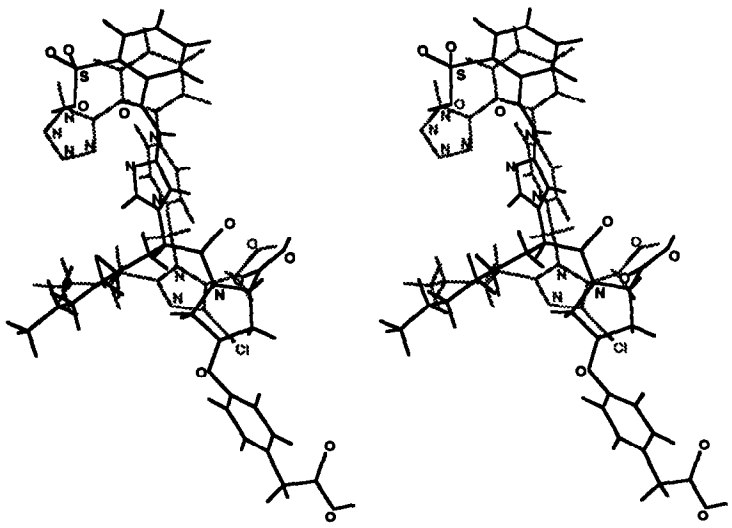


Figure 3
Stereo view of overlap of **3a** (black) and losartan (gray) obtained by flexibly fitting the ortho acidic groups (sulfonic acid and tetrazole), the alkyl chains (hexyl and butyl), and the hydrogen bonding groups (proline carboxyl and hydroxymethyl). The shape and dimensions of the two compounds are somewhat similar except for the 4-phenoxyproline side chain.

In summary, we have identified a novel series of chiral nonpeptide AT_1 antagonists that interact with the receptor in a highly stereospecific manner. Furthermore, these ligands define a new subsite of the AT_1 receptor not accessed by losartan. A more extensive discussion of the chemistry, SAR, and *in vivo* pharmacology of these agents will be the subject of future reports from our laboratories.

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10. (a) According to Waud (ref. 10b, eq. 25), a reasonable function to model an empirical dose-response curve is the 3 parameter logistic: **response = max/[1 + (ED50 • (1/a))^S]** (Eq. 1), where max = the maximum possible response, a = the agonist concentration, and s = steepness of the sigmoidal curve. If a second dose response curve is generated after adding a competitive antagonist, then Waud (ref. 10b, eq. 14) indicates the following equation relates equally effective agonist concentrations: **1/a = (1/A) • (1 + (B/K_B))** (Eq. 2), where B = antagonist concentration, K_B = dissociation constant of the antagonist, and A = agonist concentration equally effective in the presence of antagonist. Equation 2 may be substituted into equation 1 giving the following dose response equation in the presence of a competitive antagonist: **response = max/[1 + (ED50 • (1/A) • (1 + (B/K_B))^S]** (Eq. 3). If a second dose response curve is generated after adding a noncompetitive antagonist, Kenakin (ref. 10c, eq. 45) suggests the following modification to equation 2: **1/a = [(1/A) • (1 + (B/K_B)) + int]** (Eq. 4), where int = intercept term for the linear equation. Equation 4 may be substituted into equation 1 giving the following dose-response equation in the presence of a noncompetitive antagonist: **response = max/[1 + (ED50 • ((1/A) • (1 + (B/K_B))+ int))^S]** (Eq. 5). For competitive antagonists, equations 1 and 3 were simultaneously fit to pairs of dose-response curves without and with antagonist, respectively. For noncompetitive antagonists (compounds **3a** and **3b**), equations 1 and 5 were fit simultaneously. The curve fitting and estimation of K_B were done by the nonlinear least squares methodology available in the software package JMP (ref. 10d). The estimated K_B values (after logarithmic transformation) were compared among diastereomers using analysis of variance with the Tukey-Kramer (ref. 10d) method for all pairwise comparisons. (b) Waud, D. R. In *Advances in General and Cellular Pharmacology*; Narahashi, L. T.; Bianchi, C. P., Eds.; Plenum, New York: 1976; Vol. 1, Chapter 4, pp. 145-178. (c) Kenakin, T. P. *Pharmacol. Rev.* **1984**, *36*, 165-222. (d) JMP User's Guide: Version 2 of JMP; SAS Institute, Inc.: Cary, NC, 1989; Chapter 18, pp. 427-450.
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