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RATIONAL DESIGN, SYNTHESIS, AND X-RAY STRUCTURE OF RENIN INHIBITORS WITH EXTENDED P1 SIDECHAINS

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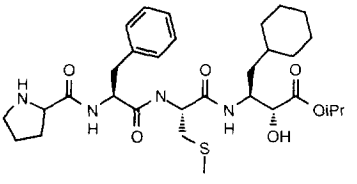
Abstract: Structural information from a complex of a tetrapeptide renin inhibitor, CP-85,339, with human renin, led to the design of inhibitors with extended P1 groups that span the S1-S3 active site pocket. A m-biphenyl sidechain at P1 and glycine at P3 led to a 160X increase in potency over the analogous phenyl compound. A crystal structure of this compound in complex with recombinant human renin (rHR) was solved.

Modulation of the renin-angiotensin system has proven to be an effective means of treating hypertension as evidenced by the commercial success of ACE inhibitors and recent clinical results with AII antagonists.[1,2] Interruption of the first and rate limiting step in this cascade, the conversion of angiotensinogen to angiotensin I (AI) by renin, has also been explored by numerous investigators.[3,4] However, renin inhibitors have not been successfully developed as orally active, cost effective treatment for cardiovascular disorders. Typical renin inhibitors have been transition-state inhibitors based on the sequence of angiotensinogen, and have therefore been peptidic molecules with molecular weight of >600. Many of these inhibitors suffer from poor bioavailability, short *in vivo* half lives and are expensive to prepare in significant quantities. Because of these factors, we were interested in utilizing recently obtained structural information to design "less peptidic" renin inhibitors with lower molecular weight, and superior pharmacokinetic properties. We used information derived from the X-ray structure of renin in complex with a conventionally designed inhibitor, CP-85,339, to design non-peptide inhibitors.[7] This work demsontrates the power of combining traditional synthetic chemistry with protein structure information.

Design: Plummer and coworkers have reported on the design of sidechains that could span the S1 and S3 pockets of human renin by substitution at the 4 position of the P1 cyclohexyl ring.[5] Their design was based on the hypothesis that the S1 and S3 pockets of renin form an extended lipophilic cavity filled by the P1 cyclohexylmethyl *and* P3 benzyl sidechains of peptidic inhibitors.[6] The X-ray structure of CP-85,339 in complex with rHR confirms their prediction of a contiguous S1-S3 pocket; the P1 and P3 sidechains make Van der Waals contact with each other, as well with the active site.[7] However, our analysis of the CP-85,339-renin complex structure indicated that both protons at the 4-position of the P1 cyclohexyl ring are in contact with the active site such that appendage of a substituent would force either the cyclohexyl ring or the enzyme to alter its position (Figure 1, top). The 3-axial position of the P1 cyclohexyl provided a more attractive vector to the S3 pocket. However, synthetic access to analogs of this type in the correct diastereomeric form was considered problematic. Based on analysis of the CP-85,339-renin X-ray structure we predicted that a phenyl sidechain at P1 would provide a better vector to the S3 pocket, but would not fill the S1 pocket as completely as the cyclohexyl sidechain. By modeling a P1 phenyl analog in the renin active site, we proposed extended P1 sidechains to fill the S1-S3 pocket. We suggested P1 phenyl groups substituted at the meta or para positions as targets for synthesis. From a conformational analysis of a m-biphenyl P1 sidechain in the enzyme active site we

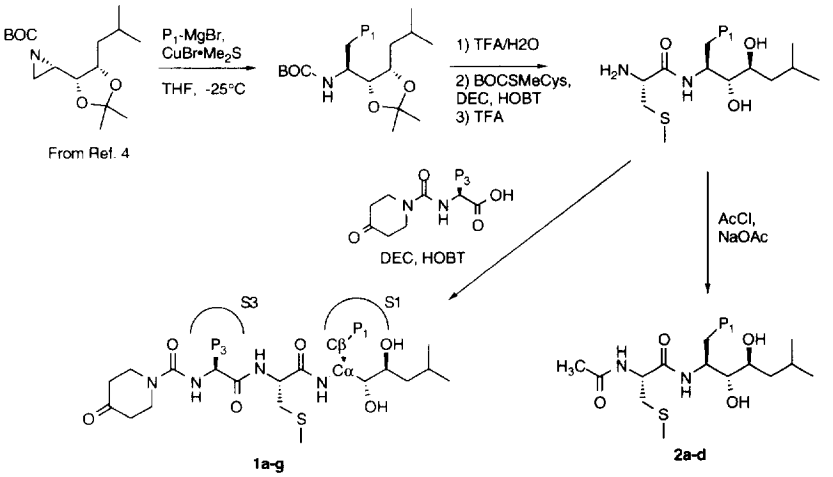


CP-85339

predicted that the sidechain would fit into the S1-S3 pocket in two conformations that differ by rotation about the Cα-Cβ bond. We also observed that the P4 proline residue is exposed to solvent and thus should not contribute significantly to the binding free energy of the ligand. Thus, we hypothesized that an inhibitor with an extended P1 sidechain could maintain sufficient *in vitro* potency even with the P4 and P3 groups removed.

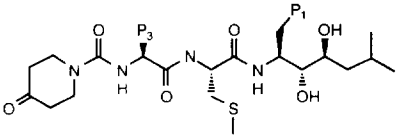
Synthesis/testing: To test these proposals, we prepared analogs with a variety of P1 sidechains. Initially, the peptidic backbone was not altered, and the P3 phenylalanine sidechain was truncated to glycine. Inhibitors with a variety of P1 sidechains were prepared as shown in Scheme I, using the aziridine based diol synthesis described by Chan and Hsiao.[8] Standard deprotection/coupling sequences were followed to incorporate these diols into potential inhibitors.

SCHEME I



Inhibitor **1a** is a subnanomolar inhibitor of rHR (Table I).[9] As anticipated, removal of the P3 benzyl sidechain (compound **1b**), which binds in the lipophilic S3 pocket, led to an 4000X loss in potency. Previous results with tri- and tetrapeptide inhibitors had demonstrated that P1 phenyl analogs are 10-50X less potent than corresponding P1 cyclohexyl analogs.[6,10,11] In this series, the P1 phenyl analog **1c** was approximately 13X less active than the cyclohexyl analog, supporting our prediction that the phenyl group does not completely fill the S1 pocket.

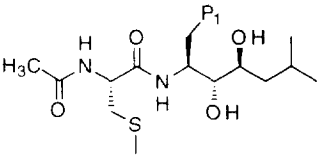
TABLE I Extended P1 Sidechains



Entry	P3 group	P1 group	IC_{50}, Recombinant Human Renin
1a	Ph-CH2	cyclohexyl	0.3 (±0.03) nM
1b	H	cyclohexyl	1200 (±200) nM
1c	H	phenyl	16000 (±3000) nM
1d	H	m-biphenyl	110 (±10) nM
1e	H	p-biphenyl	130 (±20) nM
1f	H	p-diphenylether	66 (±5.1) nM

When the P1 phenyl group was replaced with extended sidechains designed to span the S1-S3 pocket an increase in binding affinity of >100X was observed over the unsubstituted phenyl analog (see **1d** and **1e**). The diphenyl ether analog, **1f**, is approximately 2X more potent than **1e**, and 240X more potent than the unsubstituted phenyl, **1c**. This increased affinity for human renin was attributed to the ability of the second phenyl ring occupy the S3 pocket.

Truncated Inhibitors



2a P_1 = cyclohexyl IC_{50} = 5100 (±800) nM
2b P_1 = m-biphenyl IC_{50} = 1600 (±200) nM
2c P_1 = p-biphenyl IC_{50} = 1100 (±160) nM

We next investigated truncation of the P3-P4 region of these inhibitors. Excision of the N-terminal ketopiperidine glycine fragment of **1b**, and capping of the P2 S-methyl cysteine with an acetyl group (**2a**) led to an ~5X loss in affinity for human renin vs. **1b**. This confirms the small contribution of the polar, but solvent exposed, P4 sidechain to the binding free energy of the inhibitor. The biphenyl analogs **2b** and **2c** were slightly (~3-5X) more potent than **2a**, which has the more potent cyclohexyl P1 sidechain.

Crystallography: Compound **1d** was co-crystallized with rHR and the structure solved to a resolution of 2.8Å.[12,13,14] This crystal contains two renin molecules per asymmetric unit, and thus affords two independent views of the same inhibitor bound to the enzyme.

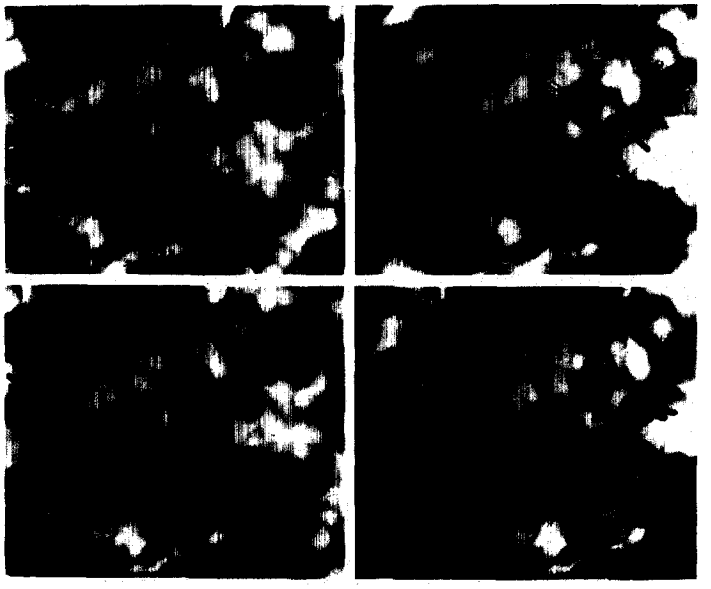


Figure1) top: CP-85,339 complexed to rH renin.[7] A molecular surface of the active site is shown in white. Hydrogen atoms have been added to show that the hydrogens at the 4 position of the cyclohexyl ring are close to the surface of the enzyme (see text). bottom: The X-ray structures of **1d** (red and yellow) overlaid with the CP-85,339 structure (light blue).

Figure 1 (bottom) shows these two independent structures of **1d** overlaid with the single published structure of CP-85,339. The backbone of **1d** overlaps well with the backbone of the CP-85,339-rHR complex described previously. The observation of two conformations of the P1 sidechain in the **1d**/rHR complex confirms our prediction from the conformational analysis. The biphenyl sidechain of **1d** spans but does not completely fill the S1-S3 pockets, as predicted. This suggests that further refinement of the extended P1 sidechain of these truncated inhibitors could result in improved potency. The X-ray structure of **1d** supports inclusion of additional substituents on the terminal phenyl of the sidechain to fill the S1-S3 pocket more completely.

Summary: X-ray structural information of a tripeptide renin inhibitor enabled the structure-based design of inhibitors with extended P1 sidechains that are up to 240X more potent than the corresponding P1 phenyl analog. The 2.8Å X-ray structure of the m-biphenyl inhibitor **1d**, confirmed that the biaryl sidechain occupies the S1-S3 pocket and verified the existence of two binding conformations. Removal of the solvent exposed P3-P4 region of "tripeptide" renin inhibitors did not significantly reduce potency. Extended P1 sidechains provided truncated analogs that were only slightly more potent than the standard cycohexyl analog. The X-ray structure of **1d** in complex with rHR suggests that additional increases in potency could be achieved by optimization of substituents on each of the biaryl rings to more completely fill the S1-S3 cavity.

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References and Notes:

(1) Ashton, W. T. *Exp. Opin. Invest. Drugs* **1994**, *3*, 1105.
(2) Brunner, H. R.; Delacrétaz, E.; Nussberger, J.; Burnier, M.; Waeber, B. *J. Hypertens.* **1994**, *12*, S29.
(3) Klienert, H. *Exp. Opin. Invest. Drugs* **1994**, *3*, 1087. Raddatz, P. *Exp. Opin. Ther.Pat.* **1994**, *4*, 1347. Boyd, S. A.; Fung, A. K. L.; Baker, W. R.; Mantei, R. A.; Stein, H. H.; Cohen, J.; Barlow, J. L.; Klinghofer, V.; Weeale, J. L.; Verburg, K. M.; Polakowski, J. S.; Adler, A. L.; Calzadilla, S. V.; Kovar, P.; Yao, Z.; Hutchins, C. W.; Denissen, J. F.; Grabowski, B. A.; Cepa, S.; Hoffman, D. J.; Garren, K. W.; Kleinert, H. D. *J. Med. Chem.* **1994**, *37*, 2991.
(4) Smith, S. A.; Al-Barazanji, K. A.; Buckingham, R. E.; Cassidy, F.; Coldwell, M. C.; Finney, F. J. L.; Hadley, M. S.; Ham, P.; Lawrence, S. A.; Nash, D. J.; Tingley, E. J. R.; Watson, J. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1291.
(5) Plummer, M.; Shahripour, A.; Kaltenbronn, J. S.; Lunney, E. A.; Steinbaugh, B. A.; Hamby, J. M.; Hamilton, H. W.; Sawyer, T. K.; Humblet, C.; Doherty, A. M.; Taylor, M. D.; Hingorani, G.; Batley, B. L.; Rapundalo, S. T. *J. Med. Chem.* **1995**, *38*, 2893.
(6) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 532.
(7) Dhanaraj, V.; Dealwis, C. G.; Frazao, C.; Badasso, M.; Sibanda, B. L.; Tickle, I. J.; Cooper, J. B.; Driessen, H. P. C.; Newman, M.; Aguilar, C.; Wood, S. P.; Blundell, T. J.; Hobart, P. M.; Geoghegan, K. F.; Ammirati, M. J.; Danley, D. E.; O'Connor, B. A.; Hoover, D. J. *Nature* **1992**, *357*, 466.
(8) Chan, M. F.; Hsiao, C.-N. *Tetrahedron Lett.* **1992**, *33*, 3567.
(9) Contillo, L. G.; Singelton, D. H.; Andrews, G. C.; Spencer, R. W.; Faraci, W. S.; Martin, W. H.; Stock, I. A. *Technique Prot. Chem.* **1994**, *5*, 493.
(10) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137.
(11) Martin, W. A.; Stock, I. A.; Lefker, B. L.; Hada, W. A.; Wright, A. S. Unpublished results.
(12) Coordinates are available from the authors upon request. Cubic crystals (space group P213, a=143.0Å) were grown using the protocol described previously.[7] Data to 2.8Å resolution were collected from a single crystal on a Rigaku rotating anode generator, using an RAXIS-II imaging plate detector. Difference maps calculated using rHR coordinates from ref. 7 showed unambiguous electron density into which the inhibitor could be modelled. Rigid-body and simulated annealing refinement were done using X-PLOR.[13] The final R-factor (R-factor = $100 \times \Sigma_{hkl} \| |F_{obs}| - |F_{calc}| \| / \Sigma_{hkl} |F_{obs}|$) is 19.7% for all data ($F > 2\sigma$) between 10-2.8Å. The model does not include any water molecules.
(13) Brünger, A. T. *X-PLOR, Version 3.1, A System for X-ray Crystallography and NMR*; Yale University Press: New Haven, 1992.
(14) Attempts to obtain suitable crystals of other inhibitors complexed with human renin were unsuccessful.

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