

A Combination of Thermodynamic and Structural Information Guides Optimization of Substituted Diaminopyrimidine Renin Inhibitors

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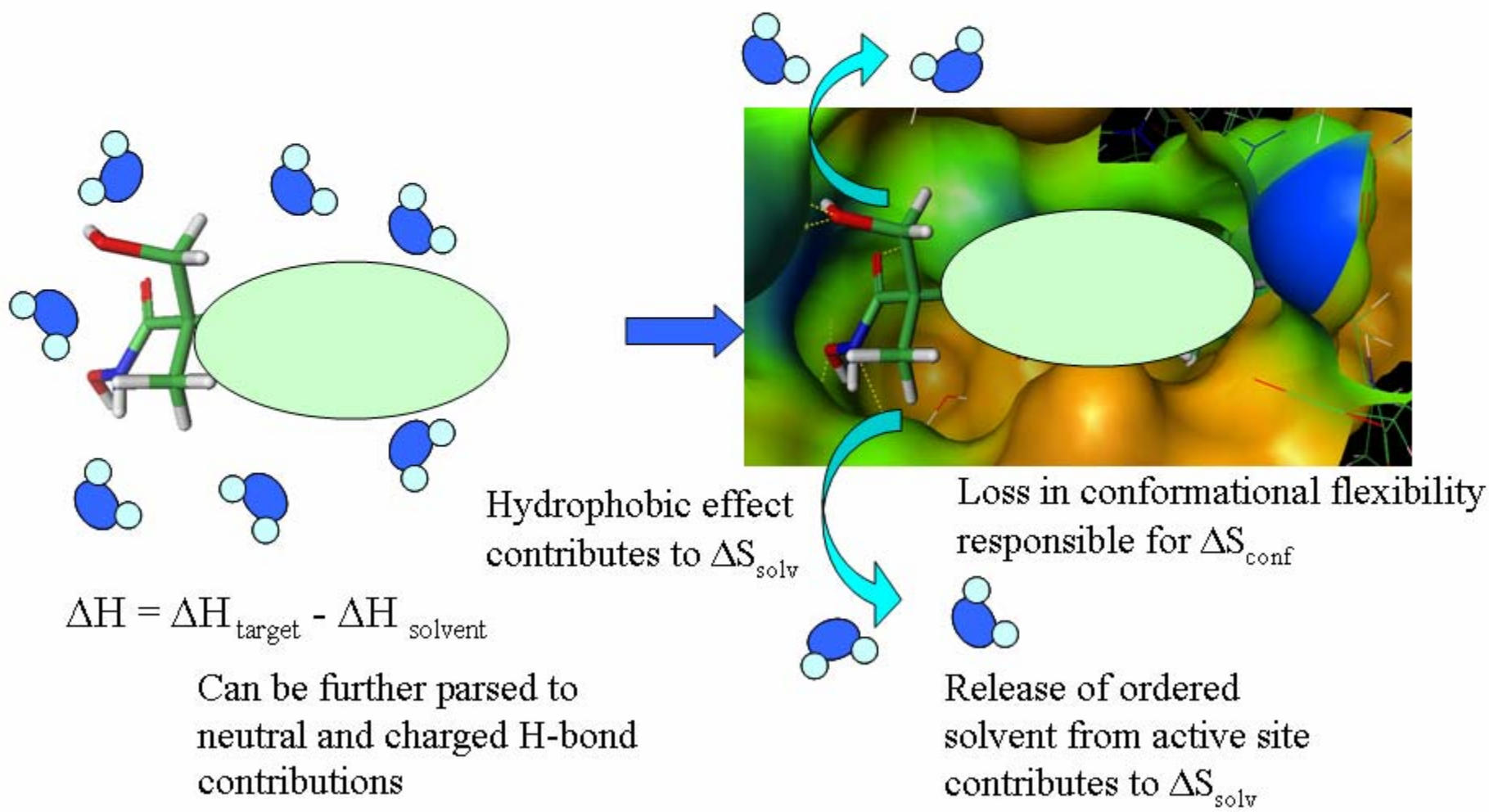


Overview

- Brief review of structure and thermodynamic considerations in enzyme / receptor inhibitor design
- Hypertension and renin pharmacology
- Structure and thermodynamic guided design of small molecule renin inhibitors

Thermodynamics of Ligand Binding

$$\Delta G = -RT \ln K_a = \Delta H - T\Delta S = \Delta H - T\Delta S_{\text{solv}} - T\Delta S_{\text{conf}}$$



Dissecting the Driving Forces for Binding

- **Binding enthalpy, ΔH** , is primarily comprised of the strength of ligand interactions with protein (van der Waals, H-bonds, electrostatic) relative to those existing with solvent

- π/π and ionic interactions contribute significant binding enthalpy
- H-bonds formed need to be well positioned to balance enthalpy loss due to desolvation of a polar group

Net ΔH for H-bond formation:

- -1 kcal/M for neutral (amide / amide) H-bond of ideal geometry¹

- -2 kcal/M for ionic (ie. carboxylate / amide) H-bond of ideal geometry¹

- Distance important for van der Waals attraction: too close, large repulsion; too far, affinity is weak
- Removal of a H₂O molecule from an active site can result in a loss of binding enthalpy but forming H₂O mediated H-bonds from a ligand to H₂O molecules deep in an active site can add significant enthalpy

¹Dudley Williams, et.al. (2004) J.Mol.Biol. 340, 373-383

Representative Desolvation Enthalpies of Polar Functional Groups²

	ΔH^* kcal/M
NH ₂	7.9
NH	9.4
OH	8.7
CO	5.5
COOH	8.4
CH ₃	0.57

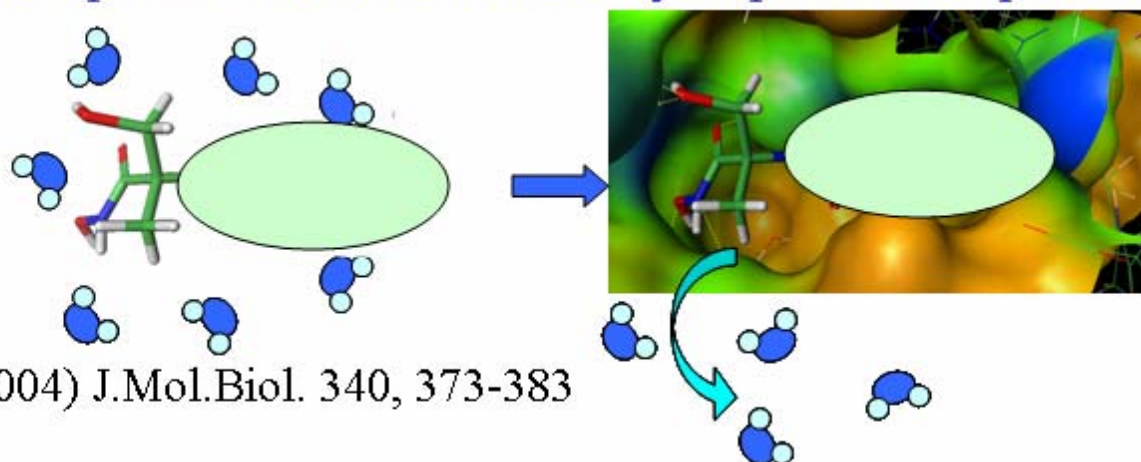
When burying polar functional groups in a hydrophobic active site, significant desolvation enthalpies must be offset by forming appropriately positioned H-bonds with the target, **otherwise the net result is a loss of binding enthalpy**

²Cabani et al (1981) J. Sol. Chem. 10, 563

* Converted to kcal/M, 4.18 kcal / kJ

Dissecting the Driving Forces for Binding

- **Binding entropy, ΔS** , is mainly comprised of changes in solvation entropy and changes in conformational entropy
 - Removal of ordered solvent molecules from the active site upon binding results in a gain in entropy (can be as large as 2 kcal/M), partially offset by the loss in conformational entropy of the drug and protein side-chains
 - 0.3 - 1 kcal/M penalty for restriction of each rotatable bond¹
 - Burial of each \AA^2 of nonpolar surface area contributes ~ -0.04 kcal/M to binding free energy¹ (ie, $-\text{CH}_3$ at 53\AA^2 can provide -2.2 kcal/M)
 - The overall entropy gain is usually larger for hydrophobic ligands
 - Tends to push lead optimization toward more hydrophobic compounds



¹Dudley Williams, et.al. (2004) J.Mol.Biol. 340, 373-383

Change in Binding Affinity Can Also be Factored by Free Energy Contributions

$$\Delta G = \Delta G_{t+r} + n\Delta G_r + A\Delta G_h + \sum \Delta G_p + \sum \Delta G_{ionic}^1$$

- ΔG_{t+r} – loss of rotational and translational entropy of the ligand when it binds to receptor. When comparing two ligands is usually regarded as common to both associations and term is removed
 - Therefore, usually compare 2 ligands with one change to determine $\Delta\Delta G$
- $n\Delta G_r$ - loss due to restriction of rotatable bonds (n, # rotors * 0.3-1 kcal/M)
- $A\Delta G_h$ - gain due to burial of hydrophobic surface area (A, area in \AA^2 * -0.04 kcal/M)
- $\sum \Delta G_p$ - gain due to hydrogen bonds
- $\sum \Delta G_{ionic}$ - gain due to ionic interactions

¹Dudley Williams, et.al. (2004) J.Mol.Biol. 340, 373-383

Additional Terms and Simplification of Free Energy Equation

- ΔG_{conf} – loss due to conformational strain, can be removed when bound conformation is close in energy to energy minimized conformation of free ligand³
- ΔG_{vdW} – loss due to van der Waals repulsions³
- Multivariate equation, but can often be simplified to:

$$\Delta G = n\Delta G_r + A\Delta G_h + \sum \Delta G_p + \sum \Delta G_{\text{ionic}}$$

Loss from restriction of rotatable bonds

Contribution from hydrophobic burial (includes expulsion of ordered water)

Contribution from H-bonds formed

Contribution from ionic interactions

³Stephen Holroyd, ... Dudley Williams (1993) Tetrahedron 49(41), 9171-9182

What Can be Gained from Measuring Binding Thermodynamics?

- Aid in template selection (enthalpic vs entropic)
- Enthalpic differences in binding have been used to differentiate agonists and antagonists⁴
- When comparing binding of inhibitors with designed structural changes, thermodynamics can
 - Be used to estimate binding strength of specific binding interactions
 - Provide a quick guide to whether an H-bond was formed with the protein
 - Identify thermodynamic hot-spots in the active site (areas that provide good binding enthalpy or entropy)
 - When co-structures are available data provide even more detailed information on contributions of specific interactions to binding strength
- Aid in design of optimal interactions

⁴Searle, Williams (1992) Bioorganic & Med Chem Letters 2(9), 993-996

Use of Thermodynamics in Lead Evaluation

- Traditional lead optimization can lead to drug binding that is entropically driven
 - For example, designing ligands with restricted conformations pre-fit to the active site pocket and adding hydrophobicity to make interactions with solvent less favorable
 - Entropically driven binding can be detrimental since protein mutations can result in large decreases in activity - small changes in the active site are not well accommodated by a rigid inhibitor

Enthalpically Driven Binding is Advantageous

- Lead compounds that exhibit favorable binding enthalpy provide several advantages.
 - **More flexible ligands can better accommodate changes (mutations) in the active site (important for antibacterials and antivirals).**
 - Favorable ΔH indicates good electrostatic interactions of compound with target.
 - Binding affinity of an enthalpic lead can be enhanced by introducing conformational restraints, hydrophobicity, or additional enthalpic interactions.
- Literature suggests it is more difficult to increase ligand affinity when binding interactions are entropically driven. Such ligands are highly hydrophobic and rigid⁵

⁵Velazquez-Campoy, et.al. (2001) Therm. Acta. 380, 217-227

Isothermal Titration Calorimetry (ITC) Assay for Lead Optimization

- Capacity with an auto-ITC:
 - ~ 15-20 compounds / day
- Current assay format:
 - 96 deep well assay plates
- Compound and Macromolecule Requirements:
 - 2.0 ml of macromolecule between 5 and 20 μM
 - 0.7 ml of compound at 100 - 200 μM

Hypertension Statistics

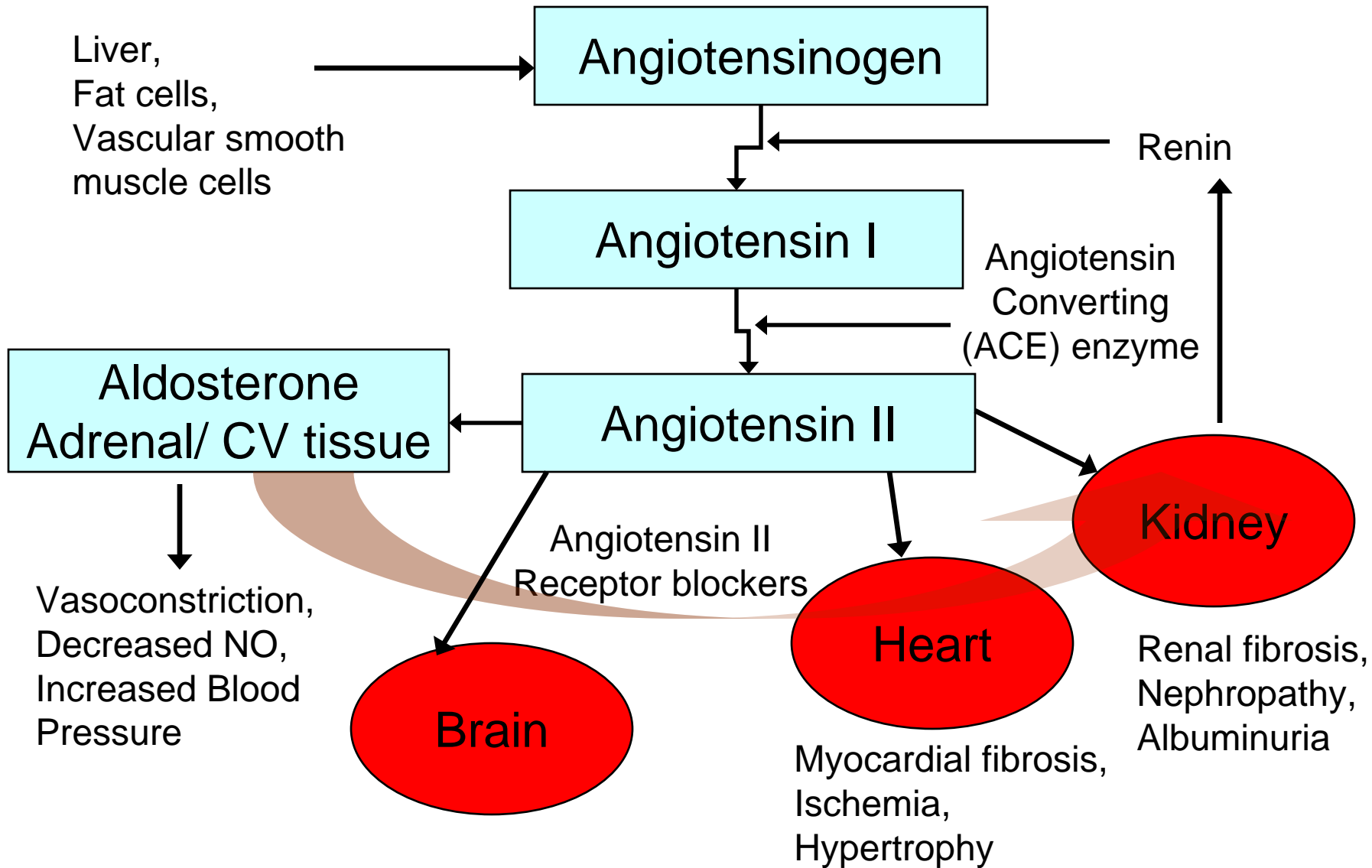
- ~ 65 million people have high blood pressure
 - Defined as systolic ≥ 140 mm or diastolic ≥ 90 mm
- Of these, only 63% knew they were hypertensive
- Estimated that 46 million people did not have their blood pressure under control

Heart Disease and Stroke Statistics – 2006 Update, American Heart Association

Hypertension Treatment Options

- Lifestyle modification – lowering salt intake, reducing weight, and increasing exercise
- Pharmaceutical intervention
 - Diuretics, α -blockers, α/β -blockers, β -blockers, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and as of March 2007 - renin inhibition

Renin Angiotensin System Overview

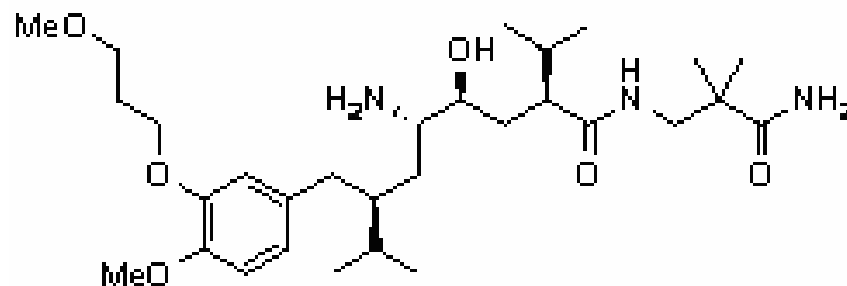
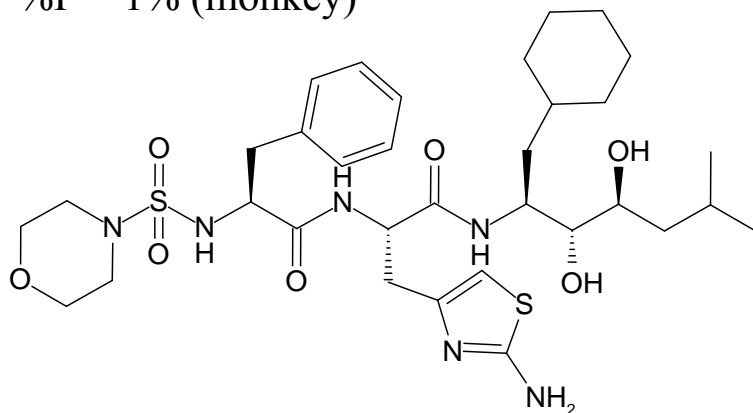


Historical Renin Inhibitors

CI-992

IC₅₀=0.38nM

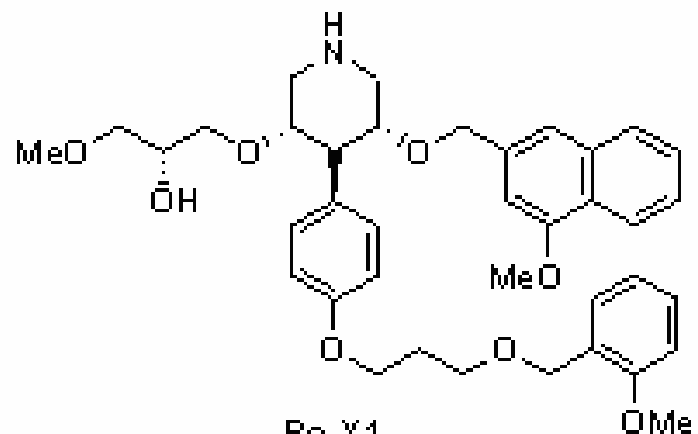
%F = 1% (monkey)



Aliskiren (SPP-100)

IC₅₀ = 0.6 nM

%F = 2% (Rat), 33% (Dog), 3% (Human)

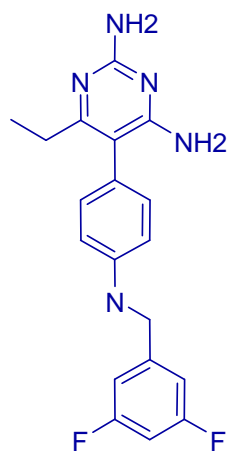


Ro-X1

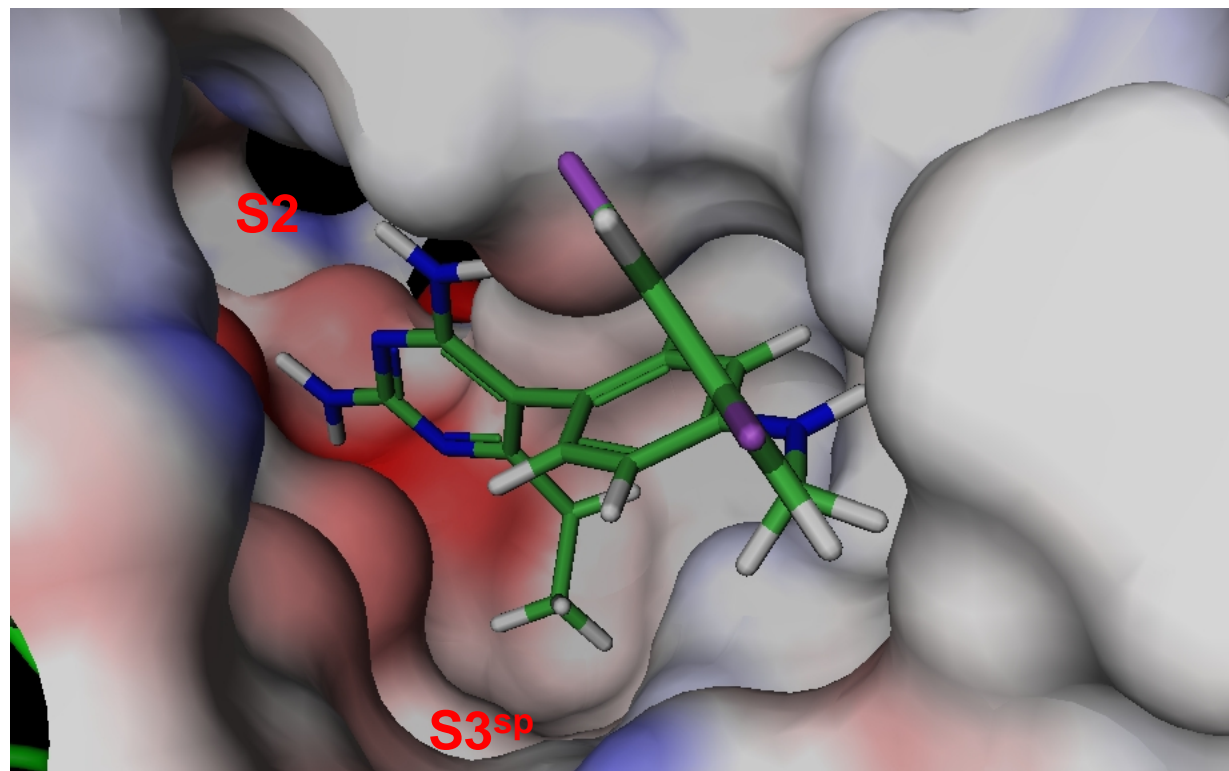
IC₅₀ = 0.07 nM

%F = 6% (dog)

Crystal Structure of HTS Hit Bound to Renin

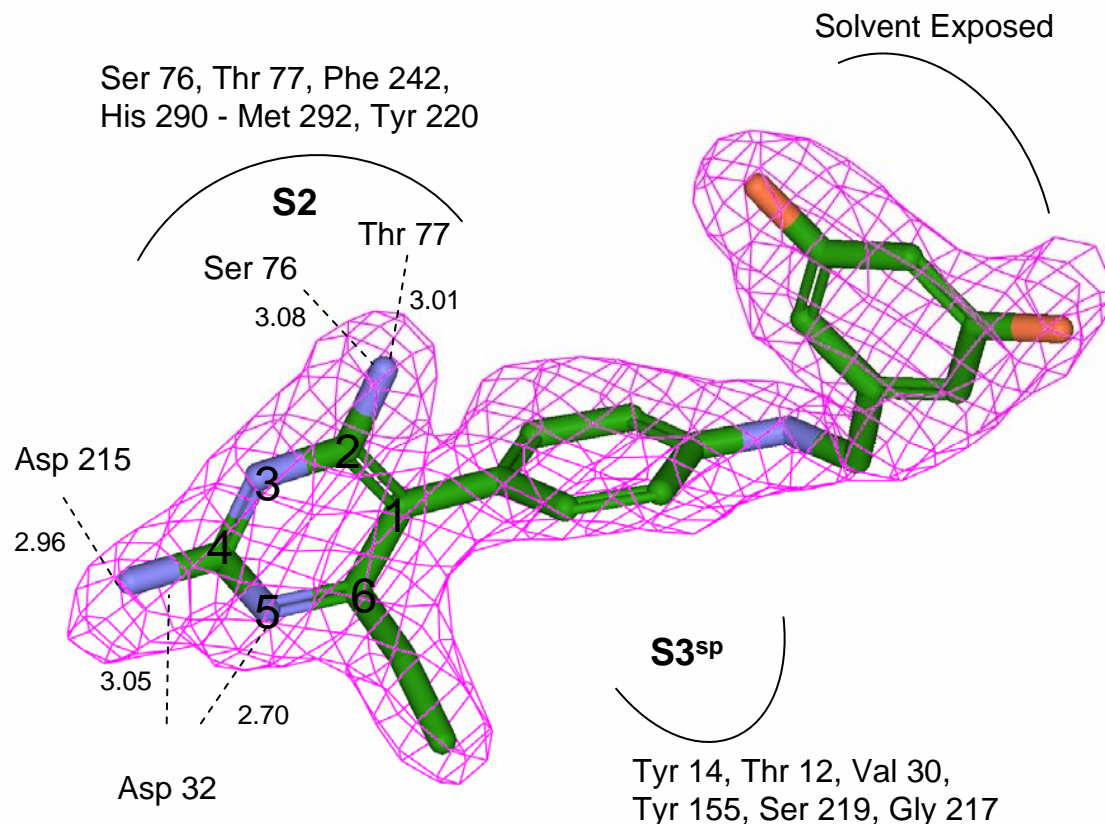


HTS lead



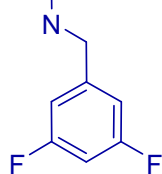
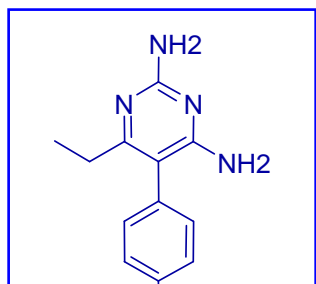
Co-structure of HTS hit bound to renin showing the Connolly surface of the binding site color coded according to electrostatic potential. Red indicates negatively charged and blue positively charged surface area. A large hydrophobic pocket S2 and the mouth of the smaller hydrophobic S3 subpocket are identified.

Electron Density Map of HTS Hit Bound to Renin

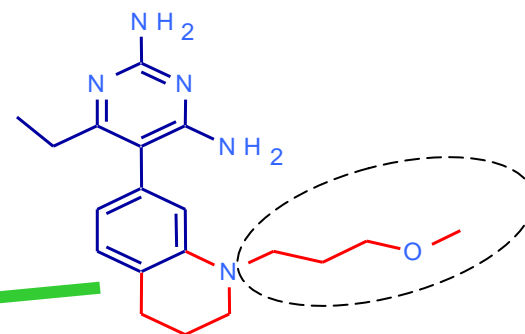
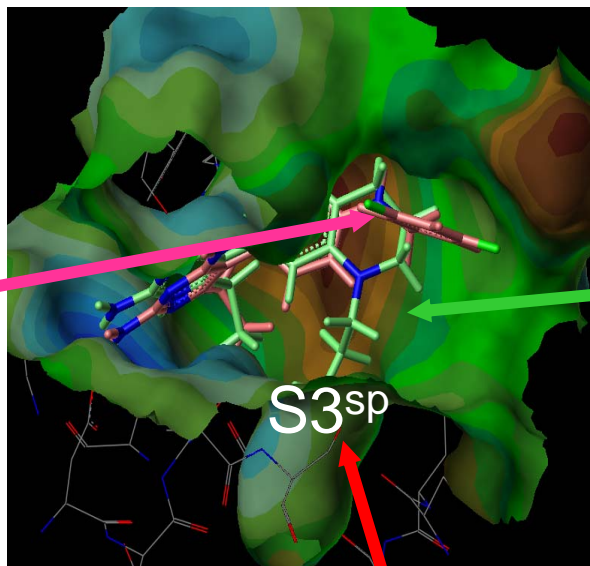


View of the OMIT (Fo-Fc) electron density map of HTS hit (rotated from previous slide) in the renin complex, atom colors: carbon - green, nitrogen - blue, oxygen - red and fluorine – orange. Ring numbering of the diaminopyrimidine ring is provided. Also shown are residues involved in the S2 and S3^{sp} pockets and residues involved in H-bond formation with the HTS hit.

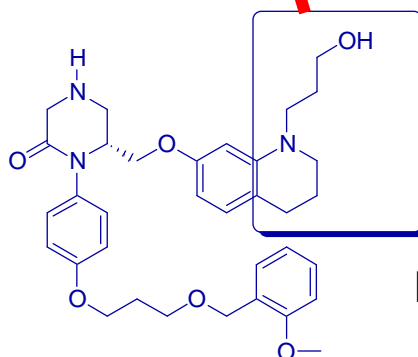
1st Steps to Improve Potency - Combining Selected Features of Two Renin Inhibitors to Fill S3



HTS hit
 IC_{50} : 6600 nM
X-ray Structure



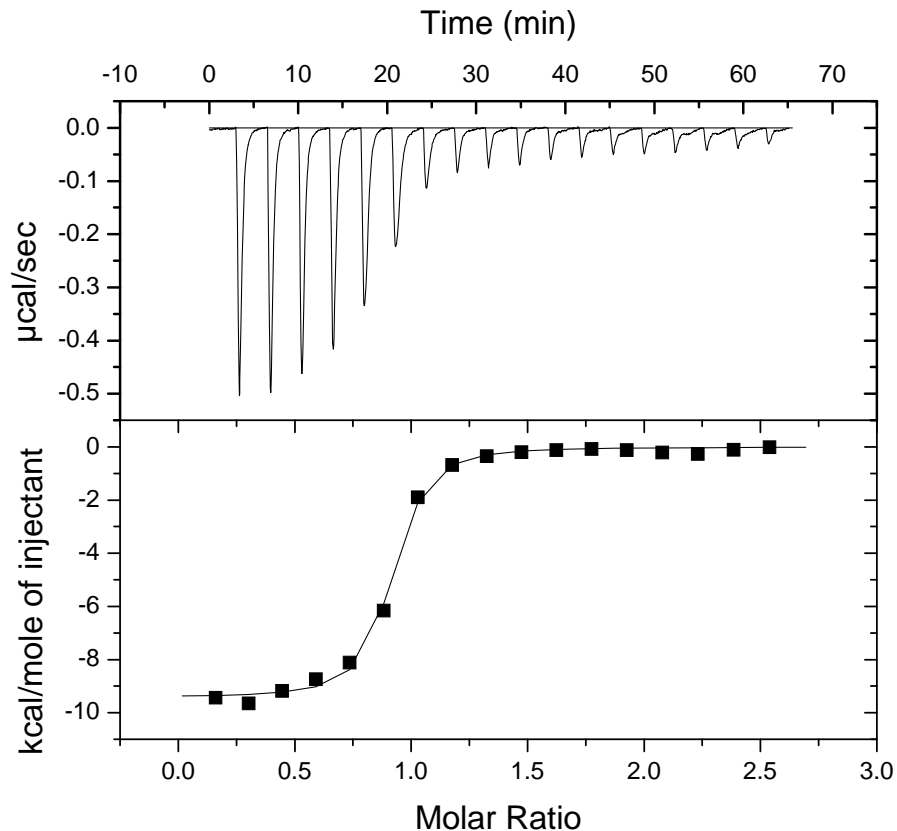
Modelling suggests
ether would extend into
the S3 subpocket



Binds S3 subpocket

IC_{50} : 29 nM

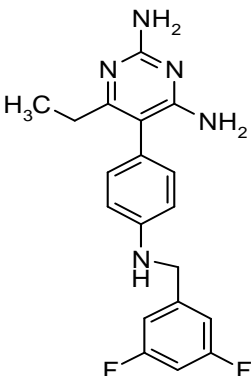
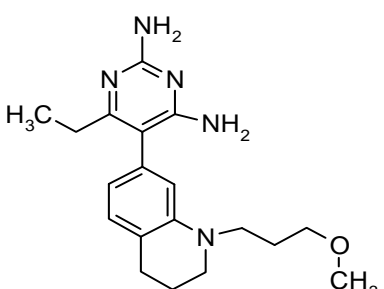
ITC Data for Injections of Renin into Diaminopyrimidine Inhibitor



“Reverse” titrations performed since compound solubilities $< 50\mu\text{M}$

Isothermal titration calorimetry results for 8 μL injections of 204 μM renin into 10 μM compound in 20 mM Tris, pH 7.0, 100 mM NaCl at 28°C. Top panel shows the change in enthalpy per injection of renin into compound. Bottom panel shows the integrated enthalpies and the results from non-linear regression fitting. Thermodynamic parameters from non-linear regression analysis: $N = 0.86$, $K_a = 2.10 \times 10^7 \text{ M}^{-1}$, $\Delta H = -9.44 \text{ kcal/M}$.

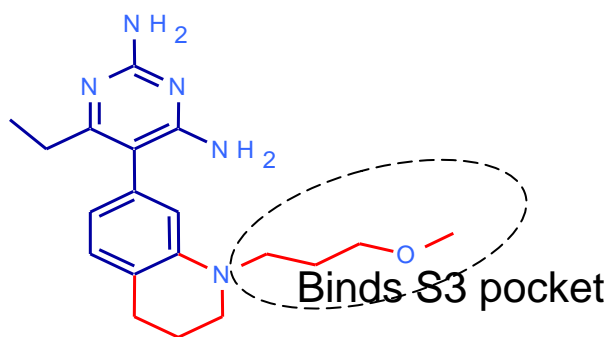
Binding Thermodynamics of Renin Inhibitors Obtained by Isothermal Titration Calorimetry

Structure	T (C)	K _a (M ⁻¹)	K _d (nM)	ΔG (kcal/M)	ΔH (kcal/M)	T ΔS (kcal/M)	IC ₅₀ (nM) ^a
	28	2.80E+05	3571	-7.50	-9.50	-2.00	6560
	28	1.87E+06	535	-8.63	-14.50	-5.87	691

Calorimetry indicated ~7x improved affinity and 5 kcal/M increased enthalpy but 4 kcal/M decreased entropy (restricting ether appendage)

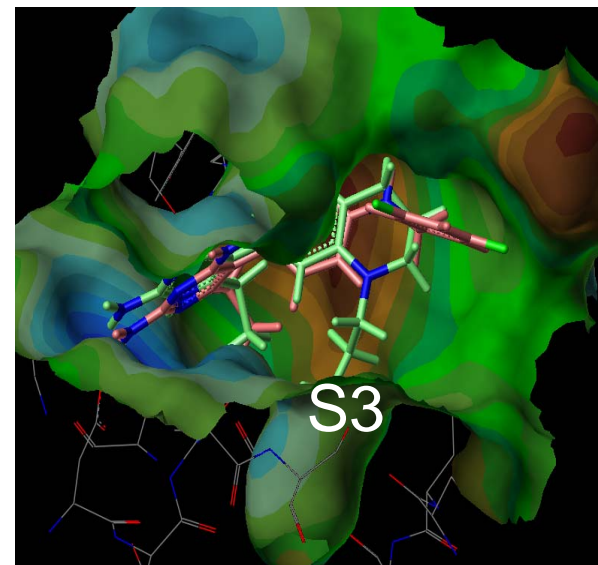
^aIC₅₀'s determined in duplicate using a GFP assay, Holsworth, et al. Bioorg. Med. Chem. 13 (2005) 2657-2664

After synthesizing compound with an ether appendage, crystal structure confirmed modeled binding mode

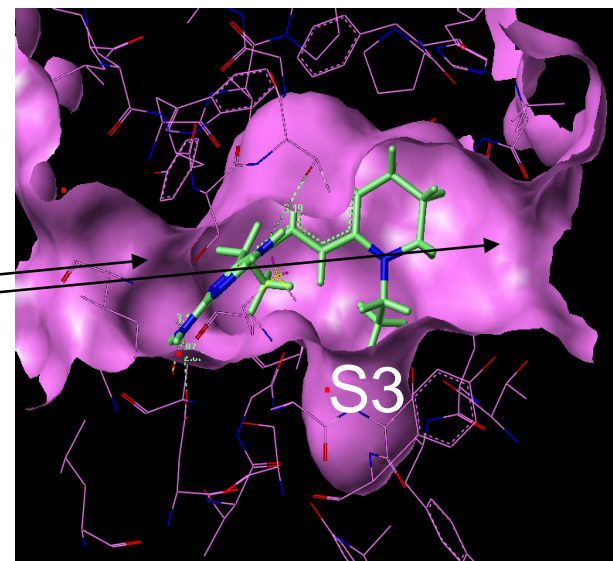


IC50: 690 nM

Potential to improve affinity by extending into S2 and into solvent channel and rigidifying ether appendage

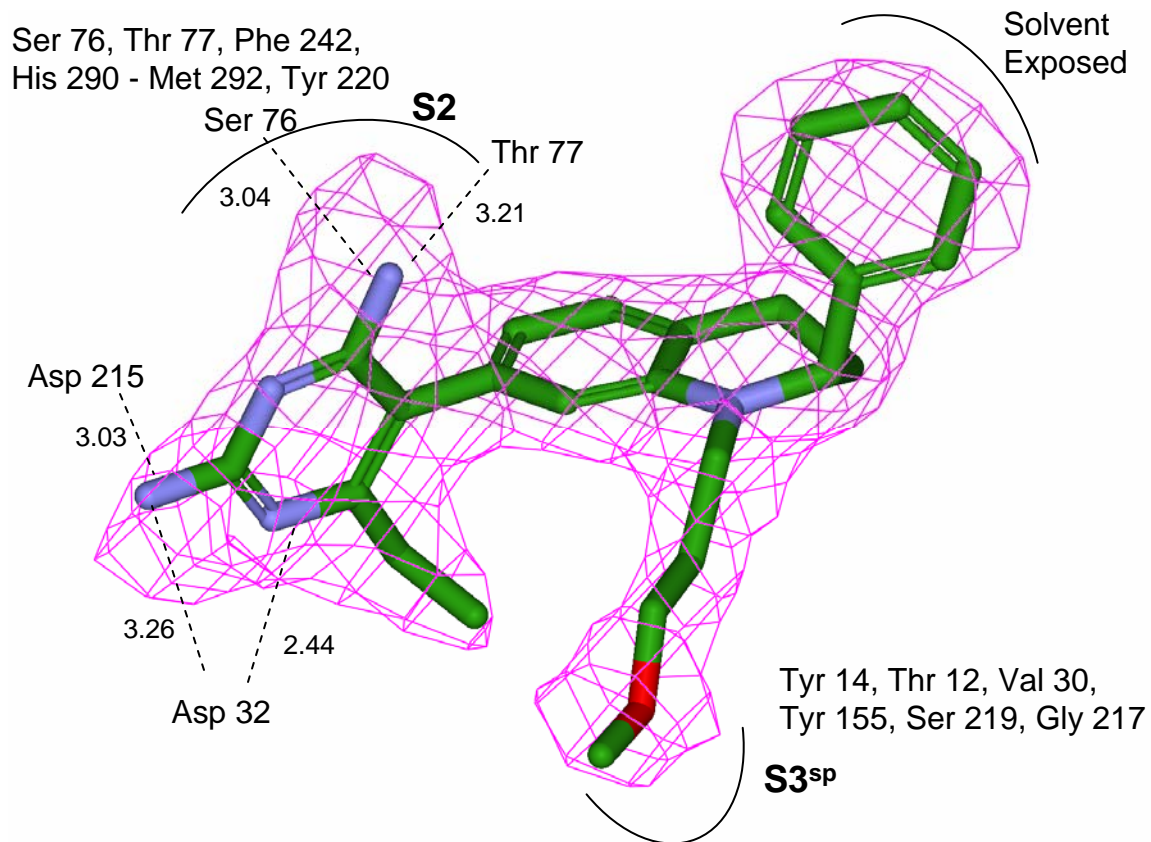


Overlay of X-ray Structure for HTS hit with model for cmpd with S3 extension

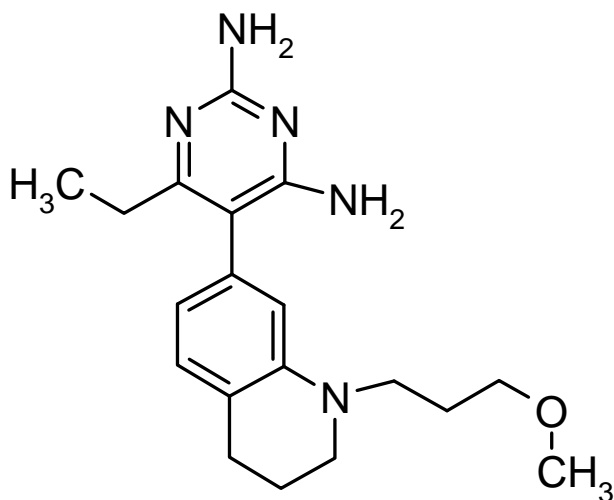


Crystal structure of cmpd with S3 extension

Electron Density Map of Diaminopyrimidine with S3 Extension Bound to Renin



Good Physical Properties for Non-Peptidic Renin Inhibitor



IC₅₀ = 690 nM

MW = 341

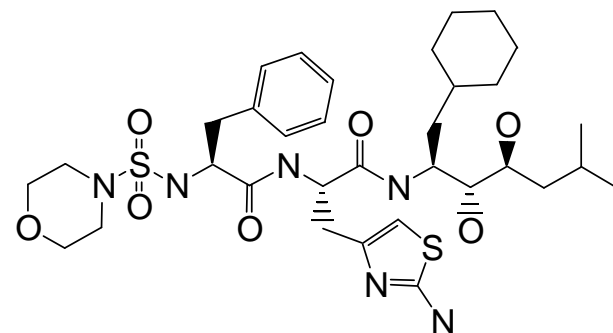
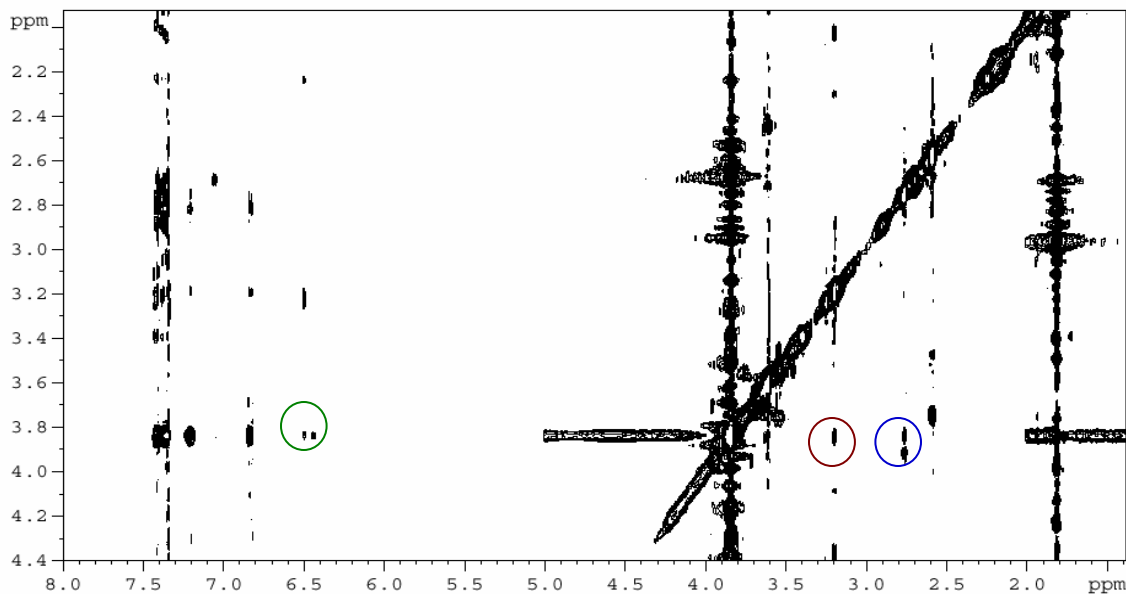
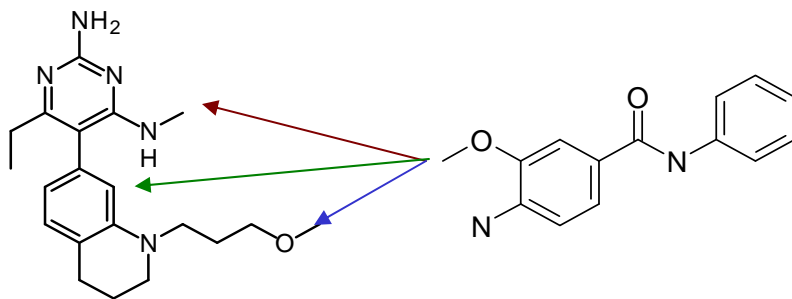
clogP = 3.35

Solubility = 59.3 ug/mL

CYP3A4 IC₅₀ = 2.8 uM

NMR Auxillary Screen Identified Compound Extending into S2 Pocket

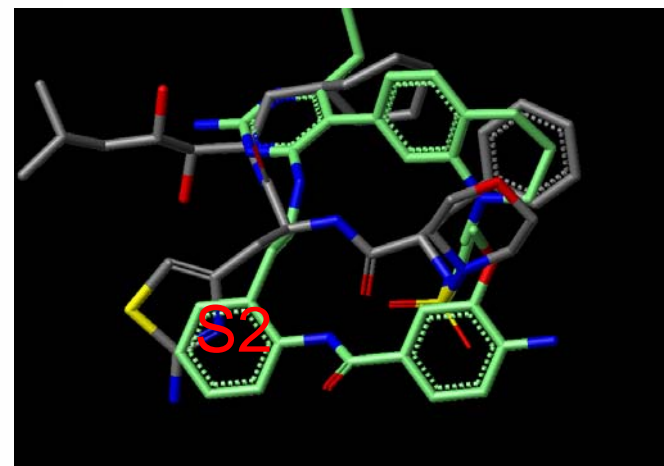
Inter-Ligand NOE's



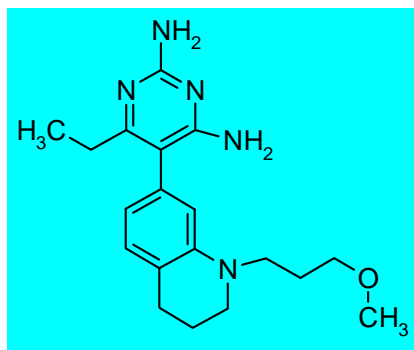
CI-992

Auxiliary hit does not bind the
renin/CI-992 complex

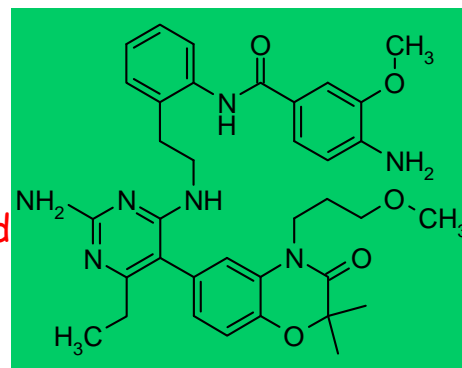
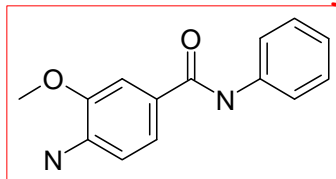
modeling ↓



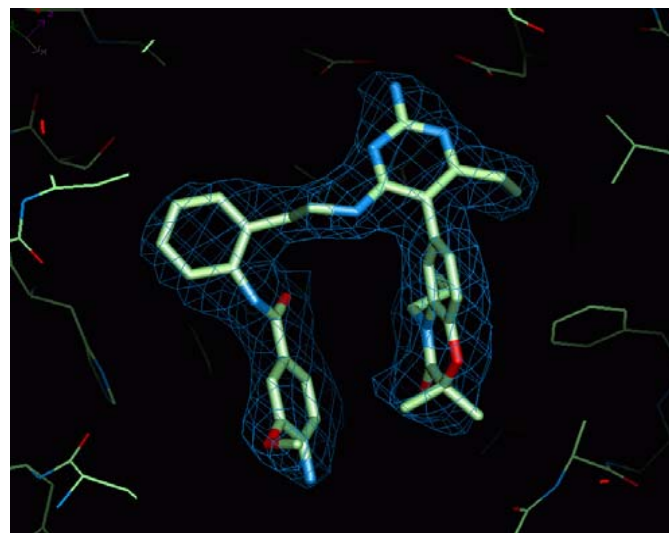
Sythetic Effort Completed to Add Substituted Aryl-Benamide to Core Diaminopyrimidine to Extend into the S2 Pocket



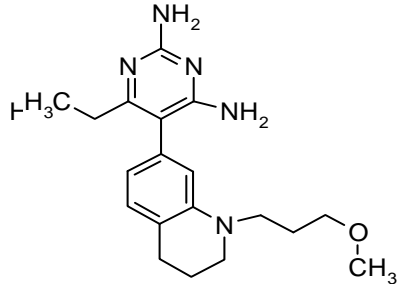
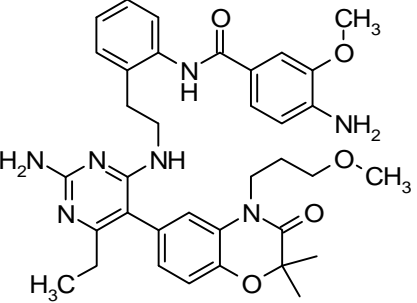
Synthetic chemistry effort combined scaffold with NMR screening hit



Renin / Inhibitor Crystal Structure



Thermodynamics Suggested Structural Alterations of S2 Substituent to Improve Renin Affinity

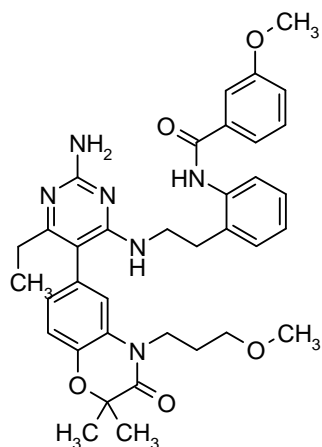
Structure	T (C)	K _a (M ⁻¹)	K _d (nM)	ΔG (kcal/M)	ΔH (kcal/M)	T ΔS (kcal/M)	IC ₅₀ (nM) ^a
	28	1.87E+06	535	-8.63	-14.50	-5.87	691
	28	3.68E+06	272	-9.04	-2.10	6.94	336

Calorimetry indicated ~2x improved affinity, significant loss in enthalpy but large favorable entropy (expulsion of ordered H₂O)

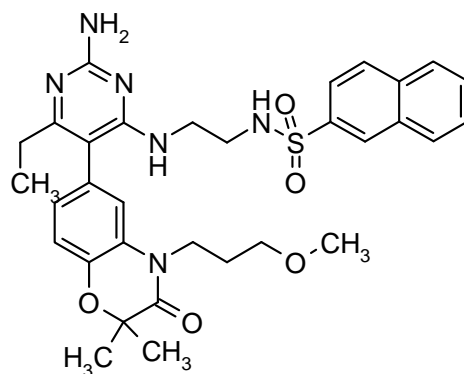
Calorimetry suggested main binding force was mainly hydrophobic effect and change in protein to flap-open conformation, no significant H-bonds were formed in the S2 pocket, therefore, need to correctly position polar functionality in S2

^aIC₅₀'s determined in duplicate using a GFP assay, Holsworth, et al. Bioorg. Med. Chem. 13 (2005) 2657-2664

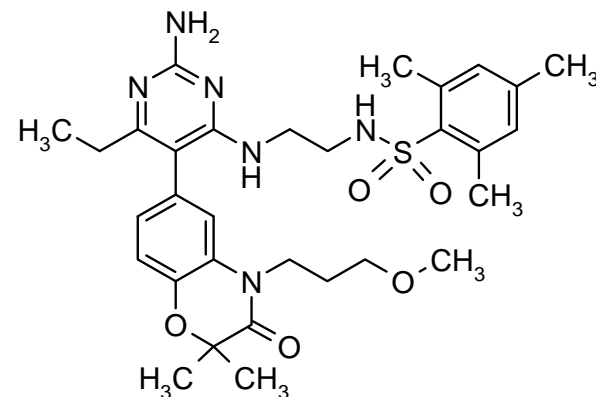
Synthetic Efforts to Position Polar S2 Substituents for H-Bonding Yielded Improved Affinities



$IC_{50} = 350 \text{ nM}$

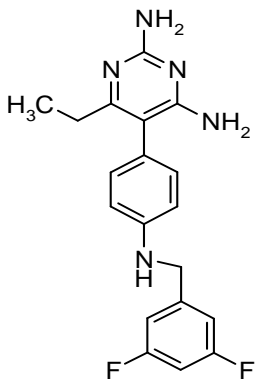
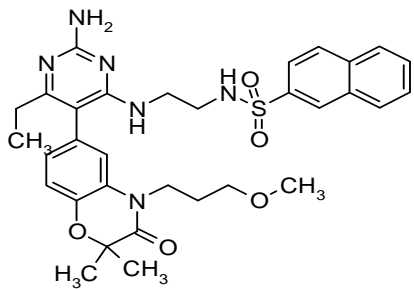


Improved S2 binding
 $IC_{50} = 23 \text{ nM}$



Further optimized S2 binding
 $IC_{50} = 1 \text{ nM}$

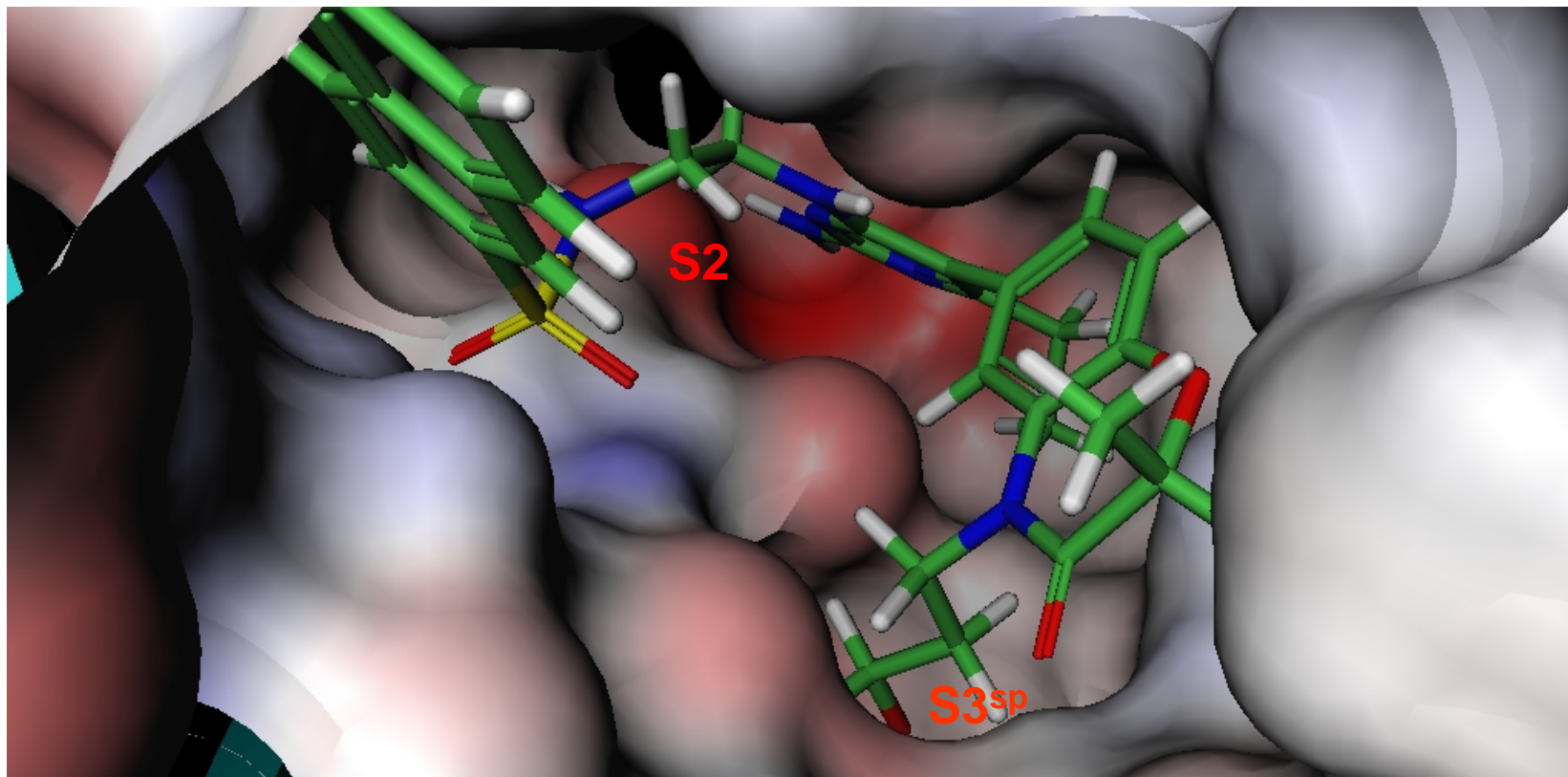
Aryl Sulfonamide Formed H-bonds with Ser219, Tyr220, and Thr77 in S2 Pocket Improving Affinity

Structure	T (C)	K _a (M ⁻¹)	K _d (nM)	ΔG (kcal/M)	ΔH (kcal/M)	T ΔS (kcal/M)	IC50 (nM) ^a
	28	2.80E+05	3571	-7.50	-9.50	-2.00	6560
	28	1.27E+07	79	-9.78	-9.35	0.43	27

Overall, 45X increase in affinity, similar dH and improved binding entropy

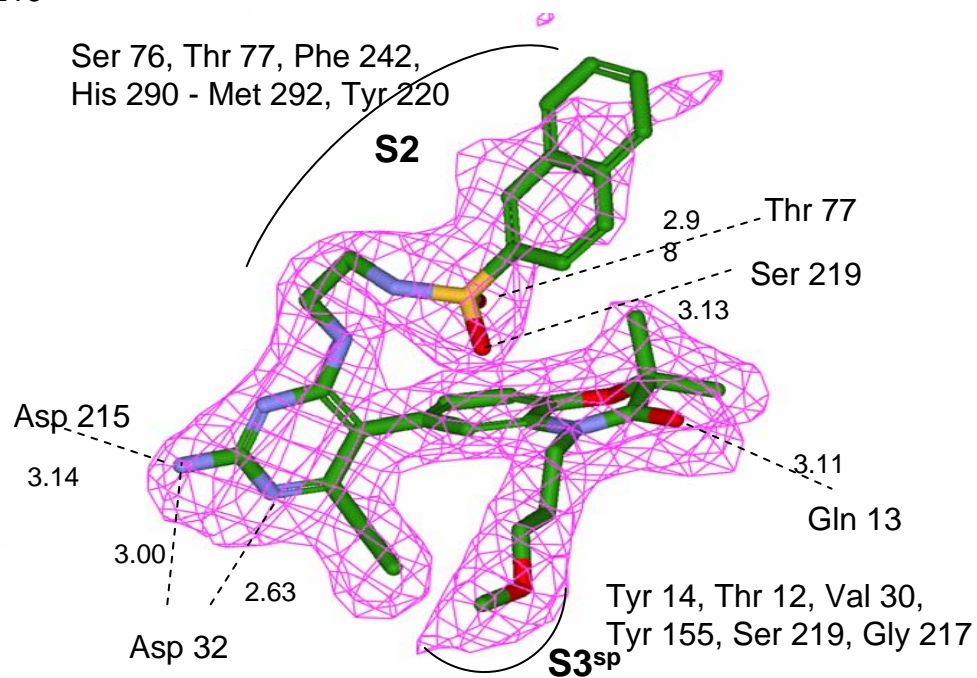
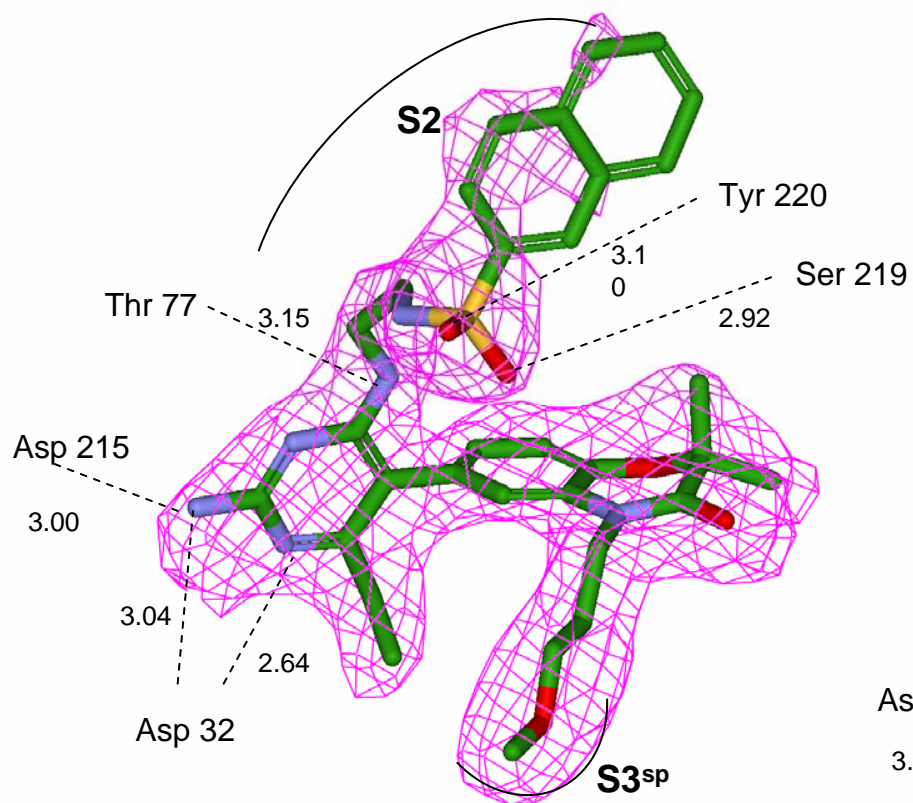
Improved enthalpy compared to aryl benzamide S2 extension that didn't form productive H-bonds and now gained binding entropy

Co-Structure of Aryl Sulfonamide Bound to Renin



Connolly surface of the binding site color coded according to electrostatic potential. Extensions of the aryl sulfonamide into the S2 pocket and the methoxypropyl ether into the S3 subpocket are shown

X-Ray Structure Indicated Two Different Orientations of Aryl Sulfonamide in the S2 Pocket



Summary

- Renin inhibitor affinities improved > 6000 fold from initial 6 uM lead
- Appendage for S2 pocket identified from NMR screen
- Combination of thermodynamic and structural data provided unique insights into inhibitor design not obtained by either technique alone
 - S3^{sp} extension provided improved affinity due mainly to increased binding enthalpy from van der Waals interactions but additional gains could be obtained by restricting rotation of the ether appendage
 - Insight into lack of improvement in affinity with initial aryl-benzamide extension into S2, mainly hydrophobic effect and lack of H-bonds
 - H-bonding of arylsulfonamide improved binding enthalpy of S2 extension and retained entropic advantage detected with S2 pocket derivatives
- Additional information provided in the following article:
 - Sarver, et.al. (2007) *Anal. Biochem* **360**, 30-40.

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