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Design and optimization of renin inhibitors: Orally bioavailable alkyl amines

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ABSTRACT

Structure-based drug design led to the identification of a novel class of potent, low MW alkylamine renin inhibitors. Oral administration of lead compound **21l**, with MW of 508 and IC_{50} of 0.47 nM, caused a sustained reduction in mean arterial blood pressure in a double transgenic rat model of hypertension.

The renin angiotensin system (RAS, Fig. 1) plays an important role in the regulation of blood pressure and has been a prominent target for antihypertensive drugs.[1,2] Renin is a highly specific aspartic protease which catalyzes one step in the RAS, the cleavage of angiotensinogen which releases the decapeptide angiotensin I (Ang I). Renin has no other characterized activity and, while cathepsin D and tonin can also cleave angiotensinogen to Ang I, under normal conditions this activity is not pharmacologically relevant.[3]

Renin has long been recognized as a desirable target for antihypertensive therapy. Potent peptidomimetic inhibitors, for example, remikiren (RO 42-5892, **1**, Fig. 2) were discovered during the 1980s at many pharmaceutical companies.[4,5] None of these inhibitors, which typically had high MW and poor oral bioavailability, were developed as drugs. However, animal studies demonstrated that blood pressure lowering could be achieved with a renin inhibitor.[6]

More recent research has lead to the discovery of several classes of non-peptidic renin inhibitors.[7] Aliskiren (**2**) is the first marketed renin inhibitor.[8] Piperidine **3** and diaminopyrimidine **4** are representative of other chemotypes.[9,10] In this Letter we describe the structure based discovery and initial optimization of a novel class of potent, low MW, orally bioavailable renin inhibitors.

Starting with a published renin crystal structure (PDB code: 1rne), we applied Contour™, a computational structure-based design tool proprietary to Vitae Pharmaceuticals, to design novel ligands in the substrate binding site. Our goal was to identify molecules that contained an amine that would make the key interaction with one or both of the catalytic aspartic acids. This would provide a class of molecules that would have a water solubilizing charged atom, thus eliminating many of the issues associated with insoluble chemotypes. Strategically it was decided that optimization of the molecules would be limited to the interactions with the S_1, S_3 and S_3^{sp} pockets in order to minimize the molecular weights of the inhibitors while leaving S_2, S_4 and the prime side unoccupied. This approach was supported using Contour's scoring algorithm for predicting enzyme binding affinity. After a limited number of design and synthesis iterations, urea **5** (Fig. 3, IC_{50} = 663 nM), initially prepared as a mixture of isomers at the piperidine and benzyl ether centers, emerged as an attractive structure for further optimization. Our model of **5** bound to renin indicated that the preferred isomer would have the (R) stereochemistry at both the piperidine and the benzylic positions. It placed the terminal primary amine between the two catalytic aspartates, Asp32 and Asp215, the cyclohexylmethyl group in S_1, the benzyl group in S_3 and the methoxypropoxy side chain in S_3^{sp}. The importance of the methoxypropoxy group for potency was confirmed by the synthesis of **6** (IC_{50} = 44 μM).

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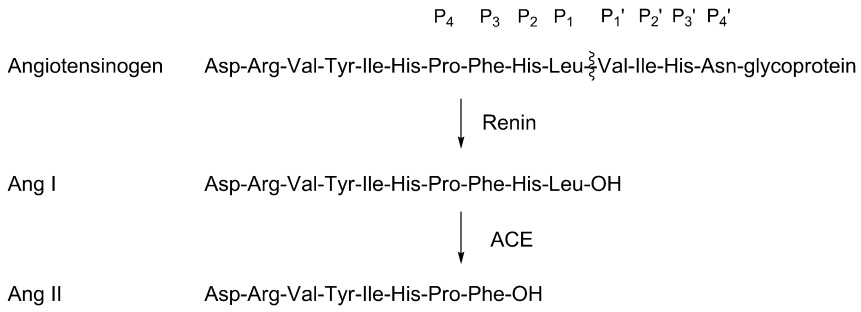


Figure 1. The renin angiotensin system.

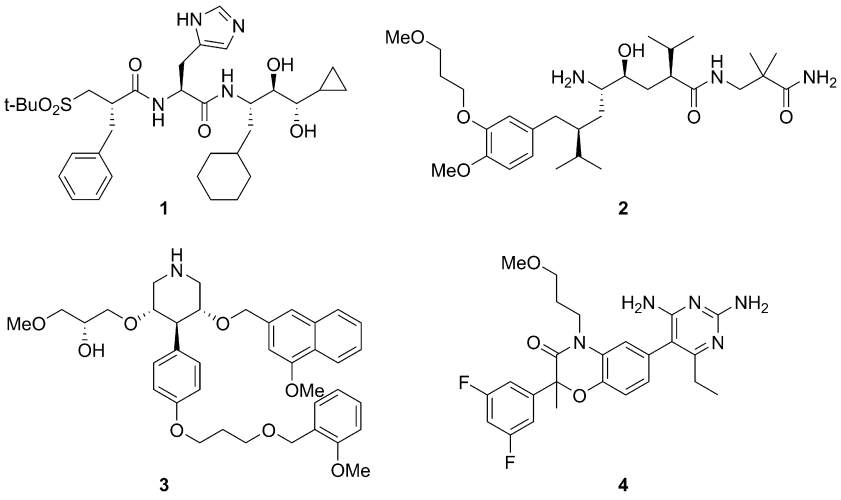


Figure 2. Renin inhibitors.

Our synthesis of the piperidine component of compounds of general structure **7** is shown in Scheme 1. (R)-Boc-nipecotic acid (**8**) was converted to its Weinreb amide and reacted with phenyl Grignard reagents or phenyllithiums to afford ketones **9**. Initially **9** (X = H) was reduced with $NaBH_4$ to give a ~1:1 mixture of alcohols **10** and **11**. In an effort to improve the ratio of **10**:**11**, several reducing agents were tested and (R)-(+)-2-methyl-CBS-oxazaborolidine with catecholborane[11] was identified as the most stereoselective affording a 13:1 mixture of **10** and **11** when X = H. The purity of **10** could be further improved by recrystallization. Alkylation of **10** was effected using large excesses of alkylating agent and NaH in refluxing THF. Finally, deprotection afforded piperidine **12**. The absolute configuration of the benzylic center was established by X-ray crystallography of *p*-bromobenzenesulfonyl derivative **13**.

The mono-protected diamines **17a** and **17b** were prepared from Boc protected aminoalcohol **14** via the mesylate **15** (Scheme 2). Treatment of **15** with NaN_3, followed by reduction, afforded primary amine **16a**, while treatment of **15** with methylamine directly afforded secondary amine **16b**. Amines **16a** and **16b** were protected as their Teoc derivatives and the Boc groups were removed using p-TsOH[12] to give **17a** and **17b**. Amines **17** were activated as their *p*-nitrophenylcarbamate derivatives and reacted with piperidines **12** to form the central urea linkage. Removal of the Teoc protecting group with Et_4NF unmasked the basic amine affording **7**.

Analogs **21**, with a tertiary alcohol at the benzylic position, were prepared as shown in Scheme 3. Ketone **9** was treated with 4-methoxybutylmagnesium chloride to afford **18** which was deprotected to afford piperidine **19**. The absolute configuration of the alcohol center in **19** was established by X-ray crystallography of *p*-bromobenzenesulfonyl derivative **20**. Reaction of **19** with the *p*-nitrophenylcarbamate derivative of **17** and deprotection using the same procedure described for **17**→**7** in Scheme 2 afforded **21**.

Immediately after the discovery of **5**, a number of analogs of general structure **7** were prepared as isomeric mixtures. The activity of these mixtures in the renin assay (data not shown) suggested that groups larger than Et at R^1 were poorly tolerated. At R^2 hydrogen and methyl gave better activity than larger alkyl groups. Small lipophilic aromatic substituents X at the *ortho* and *meta* positions gave promising increases in activity but at the *para* position only fluorine was tolerated. The aromatic substituent effects observed with the isomer mixtures, particularly the improvements in potency observed with small lipophilic substituents at the *meta* position, were consistent with our models and led us to favor *meta* substitution when synthesizing additional compounds (Tables 1 and 3).

These early promising results prompted us to develop the stereoselective synthesis of piperidines **12** shown in Scheme 1 and prepare target compounds **7** as single isomers. Table 1 shows assay

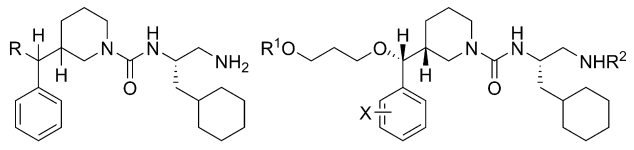
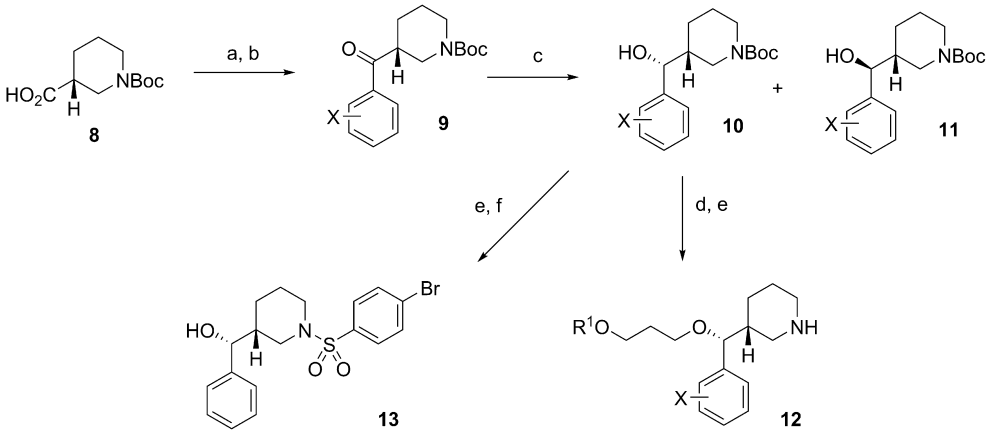
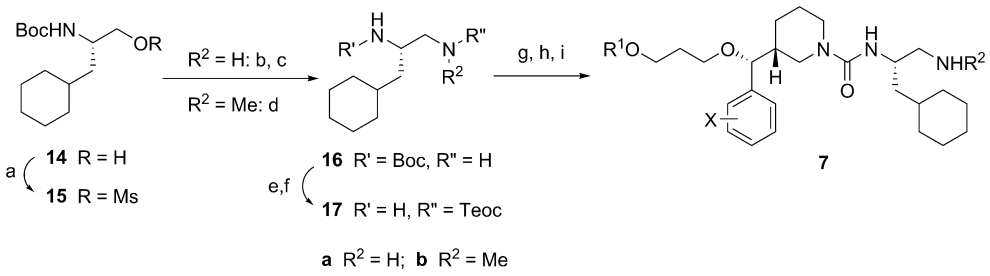


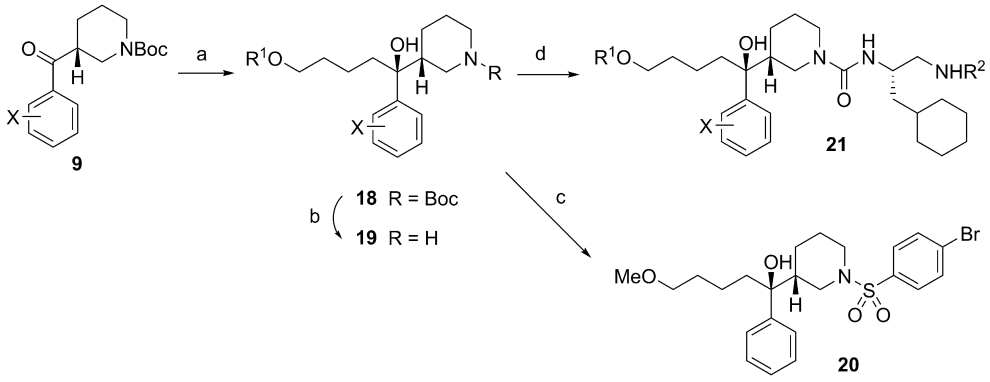
Figure 3. Prototype alkyl amine renin inhibitors.



Scheme 1. Synthesis of piperidine ether intermediates. Reagents and conditions: (a) MeNHOMe·HCl, CDI, Et_3N, THF; (b) X-Ph-MgX or X-Ph-Li, THF, −70 °C to rt; (c) (*R*)-CBS borane, catecholborane, PhMe, −14 °C, 16 h; (d) $R^1O(CH_2)_3X$ (5–10 equiv), NaH (5–10 equiv), THF, reflux; (e) 2 M aq HCl, MeCN, rt; (f) *p*-$BrC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt.



Scheme 2. Synthesis of ether analogs. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, −20 °C; (b) NaN_3, DMF, 80 °C, 16 h; (c) H_2, Pd/C, MeOH; (d) 30% $MeNH_2$ in EtOH, 55 °C, 16 h; (e) TeocOSu, K_2CO_3, H_2O, CH_2Cl_2; (f) TsOH, EtOH, Et_2O, 60 °C; (g) *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C; (h) **12**, *i*-Pr_2NEt, 1:1 MeCN/CH_2Cl_2; (i) $Et_4N^+F^-$, MeCN, μwave, 100 °C, 10 min.



Scheme 3. Synthesis of piperidine alcohol analogs. Reagents and conditions: (a) $R^1O(CH_2)_4MgCl$, THF, −70 °C; (b) 2 M aq HCl, MeCN; (c) *p*-$BrC_6H_4SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C to rt; (d) **17**, Scheme 1 steps g–i.

results for eleven benzyl ethers of general structure **7**. Compounds with ethyl at R^1 proved more potent than those with methyl (**7b** vs **7a**; **7j** vs **7e**; **7k** vs **7g**) or hydrogen (**7i** vs **7d**). Introduction of a chloro substituent at the 3-position of the phenyl ring improved potency over hydrogen (**7c** vs **7b**) and fluorine (**7h** vs **7j**), while introduction of a chlorine at the ortho position reduced potency (**7k** vs **7i**). Methyl and hydrogen at R^2 were essentially equiactive (**7h** vs **7b**; **7i** vs **7c**). Four analogs were assayed against renin in the presence of human plasma (PRA) resulting in 7–34 fold loss in potency. Encouragingly, **7h** was 35% orally bioavailable in the rat (Table 2), and had a half life of 5.9 h.

Our model of **7** bound to renin revealed that the benzyl ether oxygen, introduced for reasons of synthetic ease, had a repulsive interaction with the backbone carbonyl of Gly217. Our design indicated that the OH of synthetically accessible tertiary alcohols of general structure **21** (Scheme 3) would donate a hydrogen bond to the γ oxygen of Ser219, while removing the repulsive interaction of the ether oxygen with Gly217.

The first compound tested was **21a** (Table 3), the direct analog of **7b**, which had IC_{50} = 38 nM. In the tertiary alcohol series R^1 = Me and H were more potent than R^1 = Et (**21b**, **21c** vs **21a**; **21e** vs **21d**) and a further increase in potency was obtained with R^2 = Me (**21d** vs **21b**). Introduction of chlorine at the *ortho* position of the benzene ring maintained potency (**21f**) while fluorine gave a slight increase in potency (**21g**). A broader range of substituents was explored at the *meta* position (**21h**–**p**) from which the chloro and

Table 1
SAR of ether series

Compd	R^1	X	R^2	IC_{50}[a,b] (nM)	PRA[b,c] (nM)
7a	Me	H	H	308	
7b	Et	H	H	55	
7c	Et	3-Cl	H	5.5	190
7d	H	3-Cl	Me	764	
7e	Me	3-F	Me	58	
7f	Me	2-F-3-Cl	Me	39	369
7g	Me	2,3-DiCl	Me	609	
7h	Et	H	Me	40	447
7i	Et	3-Cl	Me	4	
7j	Et	3-F	Me	11	84
7k	Et	2,3-DiCl	Me	125	

[a] See Ref. 13 for assay protocol.
[b] Average of at least two replicates.
[c] See Ref. 14 for assay protocol.

Table 3
SAR of tertiary alcohol series

Compd	R^1	X	R^2	IC_{50}[a,b] (nM)	PRA[b,c] (nM)
21a	Et	H	H	38	490
21b	Me	H	H	21	165
21c	H	3-Cl	Me	21	315
21d	Me	H	Me	7	64
21e	Et	H	Me	16	162
21f	Me	2-Cl	Me	7	151
21g	Me	2-F	Me	4	61
21h	Me	3-Me	Me	2.5	67
21i	Me	3-Et	Me	9	285
21j	Me	3-MeO	Me	13	88
21k	Me	3-F	Me	3	47
21l	Me	3-Cl	Me	0.47	13
21m	Me	3-Br	Me	0.13	12
21n	Me	3-CN	Me	37	86
21o	Me	3-CF_3	Me	7	389
21p	Me	3-CF_3O	Me	69	
21q	Me	3-Cl	Et	6	154
21r	Me	3-Cl	CH_2CF_3	46	1400
21s	Me	2,3-DiF	Me	0.14	20
21t	Me	2-F-3-Cl	Me	0.10	27
21u	Me	2-F-5-Me	Me	3.5	67
21v	Me	3-F-5-Cl	Me	0.9	28
2				0.5	0.6

[a] See Ref. 13 for assay protocol.
[b] Average of at least two replicates.
[c] See Ref. 14 for assay protocol.

bromo analogs (**21l** and **21m**) stand out as the most promising, particularly because they retain good activity when assayed in the presence of human plasma. Introduction of an ethyl group at R^2 (**21q** vs **21l**) somewhat reduced potency while the larger, electron withdrawing 2,2,2-trifluoroethyl (**21r**) had an even more substantial deleterious effect. Selected examples of disubstituted analogs are shown. The 2,3-difluoro (**21s**), 2-fluoro-3-chloro(**21t**) and 2-fluoro-5-chloro (**21v**) analogs all had subnanomolar intrinsic potency but were less potent in the presence of human plasma than **21l** and **21m**. The potencies of **21l**, **21m**, **21s** and **21t** are equal or superior to aliskiren (**2**)[8] against renin in buffer but weaker in the presence of plasma.

Rat PK parameters were determined for six analogs in the tertiary alcohols series (Table 2). Oral bioavailability ranged from 5.5% to 48.3%, AUC values were large and $t_{1/2}$, where they could be calculated, were in excess of 5 h. However, the compounds were all rapidly cleared. Based on its good potency in the presence of plasma, superior AUC, relatively low clearance and acceptable oral bioavailability, **21l** was selected for advanced evaluation. Notably, **21l** was 10×more orally bioavailable in rat than aliskiren (**2**).[15]

The solubility of the fumarate salt of **21l** in water was shown to be >1 mg/mL. The compound showed >1000× selectivity over the aspartic proteases pepsin, β-secretase, cathepsin D and cathepsin E. The IC_{50} of **21l** against the HERG channel in a patch clamp assay was >30 μM. PK parameters of **21l** were determined in dog and cynomolgus monkey (Table 4). As in rat, the compound was absorbed rapidly and reached peak plasma concentrations in approximately 1–3 h. The oral bioavailability in monkey was modest (17%) and similar to that observed in rat. However, in dog it was significantly higher (38%). The lower oral bioavailability in rat was due, at least in part, to a combination of high clearance and incomplete absorption from the gastrointestinal tract. In both dog and monkey, the apparent terminal half lives ($t_{1/2}$) following oral dosing of **21l** were both ~16 h.

The animal model used to evaluate the antihypertensive efficacy of **21l** was a double transgenic rat (dTGR).[16,17] These rats contain copies of both the human angiotensinogen and human renin genes. They produce high levels of Ang II which leads to a steady increase in blood pressure, and also generates significant end organ damage, over the life of the rats.

Compound **21l** was tested in a telemetered dTGR cohort ($n = 4$) in a repeat dose study. On the first day of the study, animals were treated with vehicle and BP was monitored over the initial 24 h. On subsequent days, animals were given **21l** by oral gavage (60 mg/kg). Data in Figure 4 show the BP changes for the first three days of the study. Vehicle produced no effect and the BP reflected the diurnal changes over the first 24 h. Dosing with **21l** at days 2 (24–48 h) and 3 (48–72 h) produced significant BP lowering. The pharmacodynamic effect was reproducible and the BP returned to baseline within 24 h post dose.

Finally, an X-ray structure of **21l** bound to renin was determined at 2 Å resolution (Fig. 5, PDB code: 3gw5). The X-ray structure was in very close agreement with the model of **21l** in the protein generated using Contour™.

In conclusion, we have described the structure-based discovery of a novel series of potent, selective, non-peptidic, low MW renin

Table 2
Rat pharmacokinetic parameters[a,b]

Compd	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0\text{-}t)}$ (ng h/mL)	$AUC_{(inf)}$ (ng h/mL)	$T_{1/2}$ (h)	IV CL (mL/min kg)	V_{ss} (L/kg)	F (%)
7h	91	3.0	832	882	5.9	59	22	35.8
21b	28	2.3	221	192	5.1	46	56	7.5
21d	101	2.0	547	689	nd	66	nd	33.6
21e	135	3.0	745	833	8.7	79	55	48.3
21k	83	2.7	433	510	9.6	59	nd	21.1
21l	73	3.3	472	530	nd[b]	33	nd	12.9
21t	28	4.3	226	254	8.0	30	16	5.5
2	4	0.1	21	31	13.5	55	62	1.2

[a] Compounds were administered as fumarate salts. PO dose 10 mg/kg. IV dose 2 mg/kg.
[b] nd = not determined.

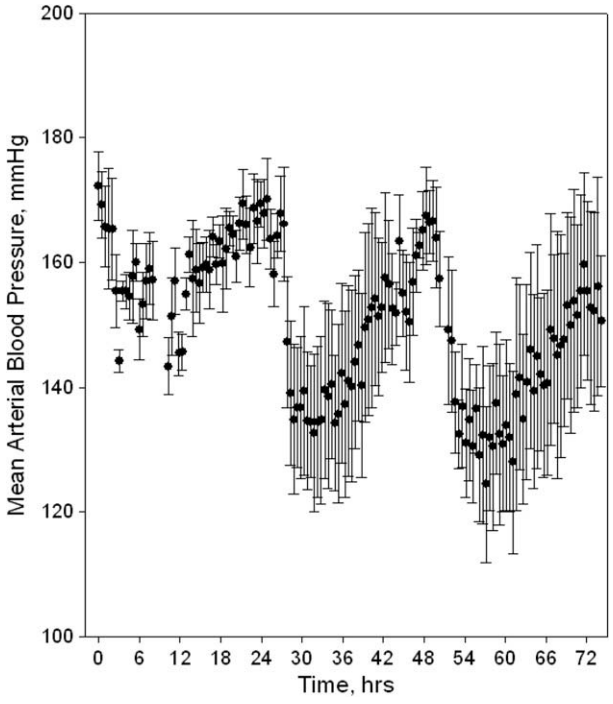


Figure 4. Effect of **21l** on blood pressure in dTGR. Vehicle dosed at $t = 0$ h. **21l** (60 mg/kg PO) dosed at $t = 24$ h and $t = 48$ h.

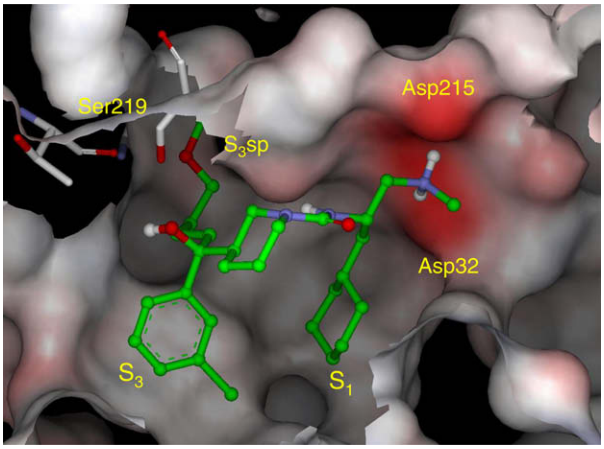


Figure 5. X-ray structure of **21l** bound to renin.

inhibitors. The ability to generate potent inhibitors by occupying only three specificity pockets in the enzyme active site using an amine interaction with the aspartates has validated our initial design hypothesis. Moreover, the prototype compound **21l** was shown to be orally bioavailable in three species and caused reproducible lowering of blood pressure in a transgenic animal model of hypertension.

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13. The activity of renin inhibitors in vitro was measured using the following test protocol: All reactions were carried out in a flat bottom white opaque microtiter plate. A 4 μL aliquot of 400 μM renin substrate (DABCYL-γ-Abu-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-EDANS) in 192 μL assay buffer (50 mM BES, 150 mM NaCl, 0.25 mg/mL bovine serum albumin, pH 7.0) was added to 4 μL of test compound in DMSO at various concentrations ranging from 10 μM to 1 nM final concentrations. Next, 100 μL of trypsin-activated recombinant human renin (final enzyme concentration of 0.2–2 nM) in assay buffer was added, and the solution was mixed by pipetting. The increase in fluorescence at 495 nm (excitation at 340 nm) was measured for 60–360 min at rt using a Perkin–Elmer Fusion microplate reader. The slope of a linear portion of the plot of fluorescence increase as a function of time was then determined, and the rate was used to calculate % inhibition in relation to uninhibited control. The % inhibition values were plotted as a function of inhibitor concentration, and the IC_{50} was determined from a fit of this data to a four parameter equation. The IC_{50} is defined as the concentration of a particular inhibitor that reduces the formation of product by 50% relative to a control sample containing no inhibitor. (Wang G. T. et al. *Anal. Biochem.* **1993**, *210*, 351; Nakamura, N. et al. *J. Biochem. (Tokyo)* **1991**, *109*, 741; Murakami, K. et al. *Anal Biochem.* **1981**, *110*, 232).
14. The activity of renin inhibitors in vitro in human plasma was measured by the decrease in plasma renin activity (PRA) levels observed in the presence of the compounds. Incubation mixtures contained in the final volume of 250 μL 95.5 mM *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, pH 7.0, 8 mM EDTA, 0.1 mM neomycin sulfate, 1 mg/ml sodium azide, 1 mM phenylmethanesulfonyl fluoride, 2% DMSO and 87.3% of pooled mixed-gender human plasma stabilized with EDTA. For plasma batches with low PRA (less than 1 ng/ml/hr) ~2 pM of recombinant human renin was added to achieve PRA of 3–4 ng/ml/h. The cleavage of endogenous angiotensinogen in plasma was carried out at 37 °C for 90 min and the product angiotensin I was measured by competitive radioimmunoassay using DiaSorin PRA kit. Uninhibited incubations containing 2% DMSO and fully inhibited controls with 2 μM of isovaleryl-Phe-Nle-Sta-Ala-Sta-OH were used to derive the % inhibition for each concentration of inhibitors. The % inhibition values were plotted as a function of inhibitor concentration, and the IC_{50} was determined from a fit of this data to a four parameter equation. The IC_{50} is defined as the concentration of a particular inhibitor that reduces the formation of product by 50% relative to a control sample containing no inhibitor.
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