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Optimization of orally bioavailable alkyl amine renin inhibitors

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ABSTRACT

Structure-guided drug design led to new alkylamine renin inhibitors with improved in vitro and in vivo potency. Lead compound **21a**, has an IC_{50} of 0.83 nM for the inhibition of human renin in plasma (PRA). Oral administration of **21a** at 10 mg/kg resulted in >20 h reduction of blood pressure in a double transgenic rat model of hypertension.

The renin angiotensin aldosterone system (RAAS) plays an important role in regulating blood pressure.[1] The highly selective aspartyl protease, renin, is an important component of the RAAS and has long been recognized as a desirable target for antihypertensive drugs.[2–8] In 2007, the first direct renin inhibitor, aliskiren (**1**, Fig. 1), was approved for sale in the US.[9,10]

Recently, we reported the structure-based design of a new class of potent, low MW renin inhibitors including **2** and **3**.[11] Benzyl ether **2** had modest potency, especially in the presence of human plasma, but good oral bioavailability in rat, while benzyl alcohol **3** had excellent intrinsic potency but lower oral bioavailability in rat. Compound **3** was shown to cause a dose dependent reduction of mean blood pressure in double transgenic rats expressing both human renin and human angiotensinogen (dTGR). In the further optimization of **3**, our key objective was to improve activity in the presence of plasma (PRA), while maintaining or improving oral bioavailability.

Literature reports have indicated that decreasing log *P* often reduces plasma protein binding.[12–14] Our working hypothesis was that analogs of **3** incorporating additional polar groups would have reduced plasma protein binding and thus improved PRA. The S_3^{sp} pocket is a narrow, mostly hydrophobic channel, with some hydrogen bonding opportunities. In the X-ray structure of **3** bound to renin (PDB code: 3gw5), the ether oxygen of the 4-methoxybutyl group accepts a hydrogen bond from the backbone NH of Tyr14. Further inspection of the residues that form the S_3^{sp} pocket suggested that, in addition to this interaction, a suitably positioned donor in the ligand could interact with the carbonyl of Gly218. Workers at Roche had demonstrated that the terminus of the chain occupying S_3^{sp} pocket could be substituted with functional groups bearing hydrogen bond donors and acceptors, for example, OH, CH_3CONH with retention of activity.[15] Modeling of various chains R from the benzylic carbon of scaffold **4** into this region indicated that the 6–7 heavy atom chain seen in **2** and **3** would be optimal and that both the Tyr14 backbone NH and the Gly218 carbonyl could be engaged. Based on these studies, compounds of general structure **4**, wherein R was 4-hydroxybutyl (**5**, Scheme 1) and 3-(acylamino)propyl (**6**, Scheme 3) were selected for synthesis.

4-Hydroxybutyl compound **5** was prepared as shown in Scheme 1. Compound **7**, an intermediate in the previously reported synthesis of **3**,[11] was O-demethylated with $AlCl_3$ and *n*-Bu_4NI with concomitant loss of the Teoc group. The Teoc group was reintroduced to facilitate purification and subsequently cleaved with Et_4NF to afford **5**.

The synthesis of piperidines **13**, which comprise the left hand half of the target compounds **6**, is shown in Scheme 2. The cyclic disilyl protected amine **8** was converted to the corresponding Grignard reagent **9**. (*R*)-Boc-nipecotic acid **10** was converted to

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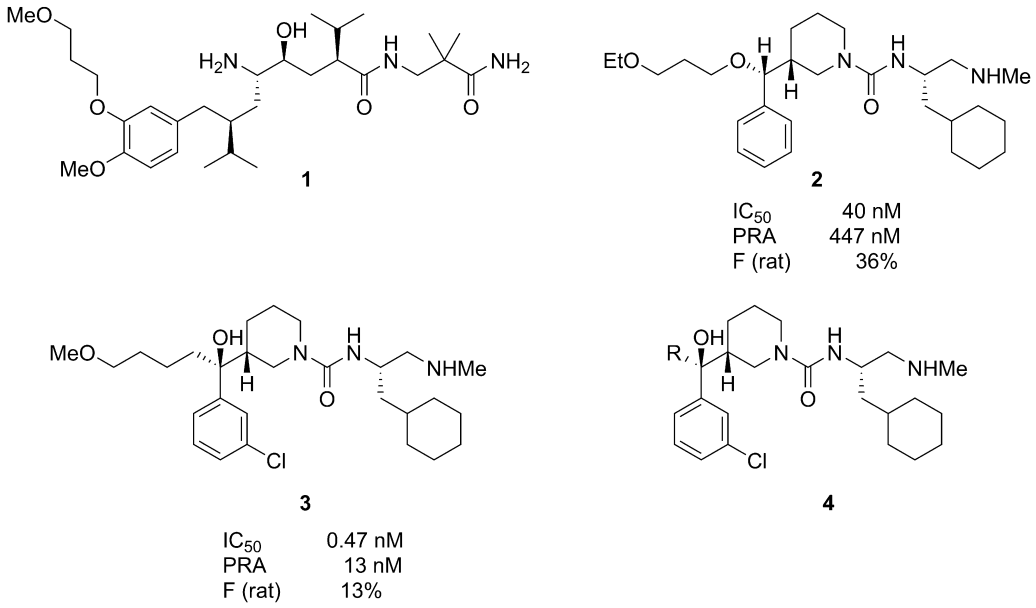
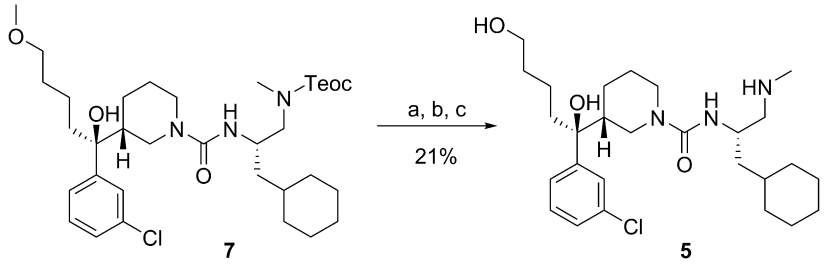
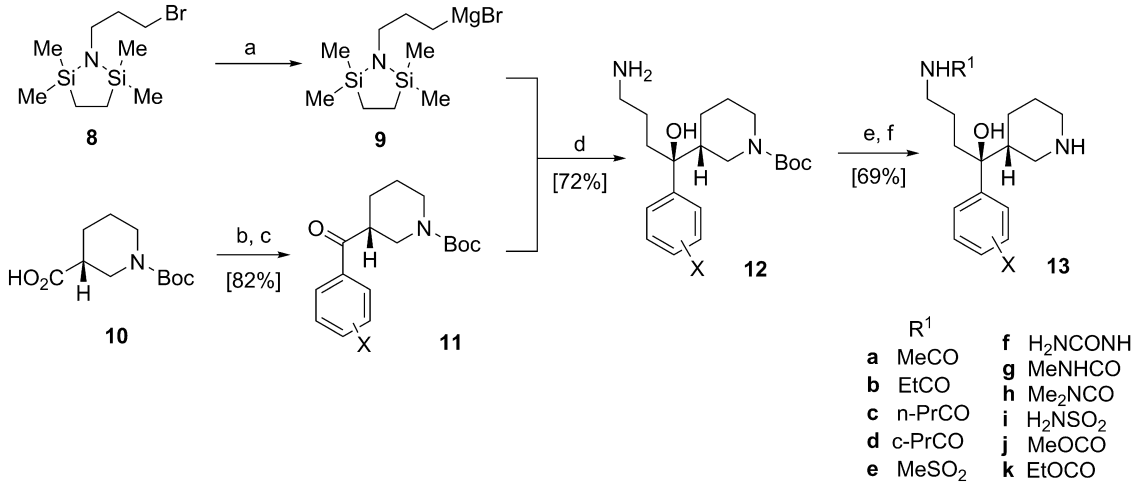


Figure 1. Nonpeptidic renin inhibitors.

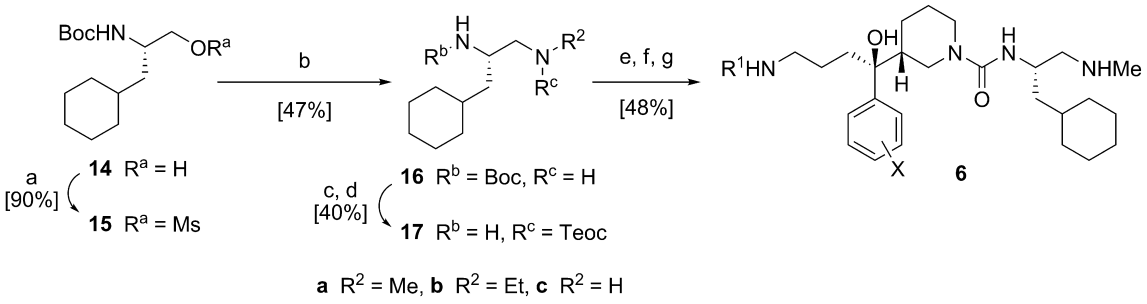


Scheme 1. Reagents and conditions: (a) $AlCl_3$, *n*-Bu_4NI, MeCN, 0 °C, 4 h; (b) Teoc-OSu, K_2CO_3, H_2O, CH_2Cl_2, rt, 2.5 h; (c) Et_4NF, MeCN, 80 °C, 2 h.



Scheme 2. Yields are shown for **13j** and are representative. Reagents and conditions: (a) Mg, THF, reflux, 2.5 h; (b) MeNHOMe·HCl, EDC; *i*-Pr_2NEt, CH_2Cl_2, rt, 16 h; (c) X-PhLi, THF, −70 °C to rt, 2 h; (d) THF, −70 °C to −8 °C, 16 h; (e) (**a**) Ac_2O, DMAP, Et_3N, CH_2Cl_2; (**b**) $(EtCO)_2O$, DMAP, Et_3N, CH_2Cl_2; (**c**) $(PrCO)_2CO$, DMAP, Et_3N, CH_2Cl_2; (**d**) *c*-$PrCO_2H$, EDC, CH_2Cl_2; (**e**) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (**f**) $PhOC(=O)NH_2$, dioxane, Δ; (**g**) (i) *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, THF; (ii) 33% $MeNH_2$ in EtOH; (**h**) (i) *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, THF; (ii) Me_2NH; (**i**) $H_2NSO_2NH_2$, dioxane, 110 °C; (**j**) MeOCOCl, DMAP, Et_3N, CH_2Cl_2; (**k**) EtOCOCl, *i*-Pr_2NEt, CH_2Cl_2; (f) 1:1 2 M aq HCl/MeCN, 16 h, rt.

its Weinreb amide and treated with various substituted phenyllithiums to afford ketones **11**. Addition of Grignard reagent **9** to ketone **11** afforded, after aqueous work up during which the silyl protecting group was lost, aminoalcohol **12** as the major product. The stereochemistry of the newly formed carbinol center in **12** was initially assigned based on the results obtained in analogous reactions reported previously.[11] This assignment was subsequently confirmed by an X-ray structure of derived compound **6a** (vide infra, Fig. 2). The primary amine at the terminus of the side chain of **12** was reacted with various electrophiles and the



Scheme 3. Yields are shown for **6j** and are representative. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2, −20 °C; (b) (**a**) 30% $MeNH_2$ in EtOH, 55 °C, 16 h; (**b**) $EtNH_2$, EtOH, Δ; (**c**) NaN_3, DMF, 80 °C, 16 h followed by H_2, Pd/C, MeOH; (c) Teoc-OSu, K_2CO_3, H_2O, CH_2Cl_2; (d) TsOH, EtOH, Et_2O, 60 °C; (e) *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2, 0 °C; (f) **13**, *i*-Pr_2NEt, 1:1 MeCN/CH_2Cl_2; (g) $Et_4N^+F^-$, MeCN, microwave, 100 °C, 10 min.

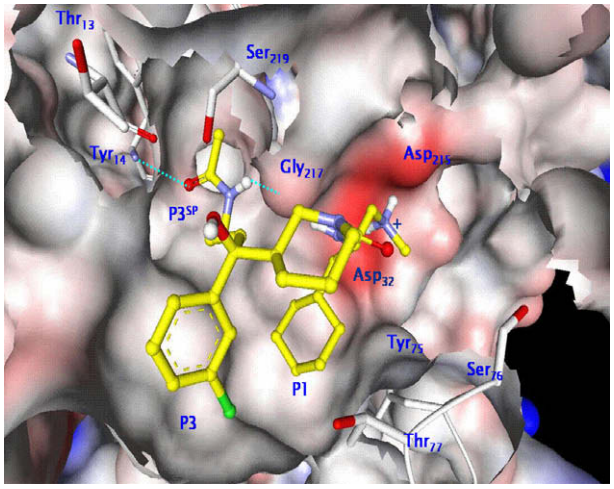


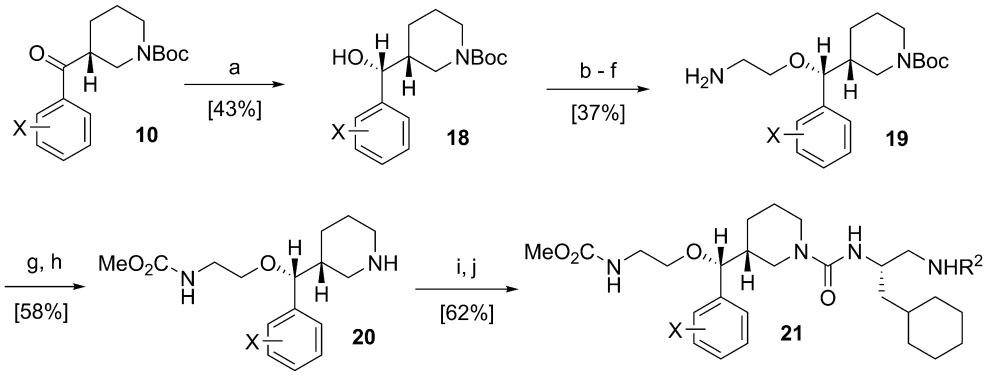
Figure 2. X-ray structure of **6a** bound to renin.

Boc group was removed under mild aqueous conditions to afford piperidines **13**.

The synthesis of the right hand diamine portion and the final assembly of target compounds **6** is depicted in Scheme 3. Commercially available Boc protected aminoalcohol **14** was converted to its mesylate **15** and the mesylate was displaced with methylamine or ethylamine to give mono-Boc protected diamines **16a** and **16b**, respectively. Alternatively, treatment of mesylate **15** with NaN_3 afforded an azide which was subjected to catalytic hydrogenation to give **16c**. Amine **16** was protected as its Teoc derivative and the Boc group was selectively removed with *p*-TsOH to afford **17**.[16] Primary amine **17** was activated as its *p*-nitrophenyl carbamate, reacted with piperidines **13** and deprotected with Et_4NF to give the desired target compounds **6**. Alternatively, carbonyl diimidazole was used to activate amine **17** for urea formation in some cases.

The synthesis of ureas **21**, in which the chain occupying S_3^{sp} is connected to the benzylic carbon by an ether linkage, is shown in Scheme 4. Ketone **11** was reduced stereoselectively with (*R*)-CBS borane to give a preponderance of the (*R*) alcohol **18**.[11,17] The side chain was elaborated as follows: alcohol **18** was alkylated with ethyl bromoacetate and the resulting ester was reduced to the corresponding primary alcohol with $NaBH_4$. The alcohol was converted to its mesylate which was displaced with sodium azide. Catalytic hydrogenation of the azide afforded primary amine **19**, which was acylated with methyl chloroformate and deprotected to afford piperidine **20**. The urea linkage of **21** was constructed as described for **6**, using the *p*-nitrophenyl carbamate derivative of **17a** followed by removal of the Teoc group with Et_4NF.

Replacement of the six heavy atom 4-methoxybutyl chain with a five heavy atom 4-hydroxybutyl chain led to a 45 × loss in activity (Table 1, **3** vs **5**), presumably because the chain is shorter than optimal and no acceptor is suitably positioned to interact with the hydrogen of the OH group. Incorporation of an amide functionality into a six or seven heavy atom chain (**6a**, **6b**) restored excellent potency. A 1.8-Å resolution X-ray structure of **6a** bound to renin validated the model we had developed (Fig. 2, PDB code: 3km4). The amide NH of **6a** donates a hydrogen bond to the carbonyl oxygen of Gly218, while the amide carbonyl accepts a hydrogen bond from the backbone NH of Tyr14. Other key interactions between **6a** and renin were similar to those seen in the previously reported X-ray structure of **3** bound to renin (PDB code: 3gw5): the tertiary alcohol donates a hydrogen bond to Ser219, the urea carbonyl



Scheme 4. Yields are shown for **21a** and are representative. Reagents and conditions: (a) (*R*)-CBS borane, catecholborane, PhMe, −14 °C, 16 h; (b) $BrCH_2CO_2Et$, NaH, THF, 0 °C to rt, 1 h; (c) $NaBH_4$, MeOH, rt, 3 h; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C, 3 h; (e) NaN_3, DMF, 80 °C, 16 h; (f) H_2 (1 atm), 10% Pd/C, EtOAc; (g) MeOCOCl, Et_3N, CH_2Cl_2; (h) 1:1 2 M aq HCl/MeCN; (i) **17**, *p*-$NO_2C_6H_4OCOCl$, *i*-Pr_2NEt, CH_2Cl_2; (j) $Et_4N^+F^-$, MeCN, microwave, 100 °C, 10 min.

Table 1
SAR of tertiary alcohols **6**

Compd no.	R^1	X	IC_{50}[a,b] (nM)	PRA[b,c] (nM)
3[d]	–		0.47	13
5[e]	–		21	315
6a	MeC(=O)–	Cl	1.3	16
6b	EtC(=O)–	Cl	1.5	21
6c	PrC(=O)–	Cl	57	
6d	*c*-PrC(=O)–	Cl	17	79
6e	$MeSO_2$–	Cl	177	
6f	$H_2NC(=O)$–	Cl	31	200
6g	MeNHC(=O)–	Cl	46	141
6h	$Me_2NC(=O)$–	Cl	2260	
6i	H_2NSO_2–	Cl	46	297
6j	MeOC(=O)–	Cl	0.18	3.7
6k	EtOC(=O)–	Cl	14	170

[a] See Ref. 18 for assay protocol.
[b] Average of at least two replicates.
[c] See Ref. 19 for assay protocol.
[d] See Figure 1 for structure of **3**.
[e] See Scheme 1 for structure of **5**.

forms a water mediated hydrogen bond with the backbone NHs of Ser77 and Thr78, the urea NH donates a hydrogen bond to the backbone carbonyl of Gly217 and the terminal $-N^+H_2Me$ group is positioned between the catalytic carboxylates of Asp32 and Asp215.[11] The steric limitations on substituents filling the S_3^{sp} pocket were further demonstrated by the diminished potency of the eight heavy atom butanoamide chain in **6c** and the branched cyclopropanecarboxamide **6d**. Furthermore, methylation of the amide NH of **6a** caused a >3000× loss in potency (data not shown). Replacement of the carbonyl in **6a** with a sulfonyl group to give **6e** led to >100× loss in activity. Ureas **6f** and **6g**, and sulfamide **6i** demonstrated that additional hydrogen bond donors are not well accommodated, while the dimethylurea **6h** again showed the lack of tolerance for branching. Finally, methyl and ethyl carbamates **6j** and **6k** had superior intrinsic potency to the isosteric amides **6b** and **6c**. In addition, the PRA of methyl carbamate **6j** was threefold better than that of lead compound **3**. Interestingly, the SAR reported by Märki et al. differed somewhat from that of our compounds. In our series, the analogs with chains terminating with H_2NCONH–, H_2NSO_2NH– and CH_3SO_2NH– groups (**6e**, **6f**, and **6i**) suffered >20× losses in potency compared to acetamide **6a**, whereas the corresponding literature analogs differed in potency only by 2×.[15]

Acetamide **6a** and methyl carbamate **6j** were selected for rat PK (Table 2) but, disappointingly, both had negligible oral bioavailability. Noting that benzyl ether **2** had better oral bioavailability than tertiary alcohol **3**, we prepared compounds of general structure **21** (Scheme 4), in which the chain occupying S_3^{sp} is attached through an ether oxygen and the methyl carbamate feature at the terminus of the chain is maintained.

Methyl carbamate **21a** (Table 3) had superior PRA to the corresponding tertiary alcohol analog **6j**. The *m*-chloro substituent could be replaced with F, Me or H with retention of subnanomolar intrinsic potency (**21b**–**21d**, respectively) but with inferior PRA. The effect of changing R^2 from methyl to hydrogen (**21e**, **21f**) and to ethyl (**21g**, **21h**) was investigated in both the *m*-chloro and *m*-fluoro phenyl analogs. The *m*-chloro primary amine analog **21e** had the best PRA of these four analogs but was inferior to methylamine **21a**. Compound **21a** had comparable enzyme potency and PRA to aliskiren (**1**)

Following up on the favorable properties of **21a**, a number of analogs with disubstituted phenyl rings occupying S_3 were prepared. Earlier SAR and modeling had indicated that, in addition to the favorable effect of chlorine at the 3-position, small lipophilic substituents such as F and Cl could be accommodated at the 2-, 5-, and 6-positions. The 3,5-difluorophenyl and 3-chloro-5-fluorophenyl analogs **21n** and **21o** both had excellent intrinsic potency although their PRA values were slightly poorer than **21a**.

Rat PK studies were performed on four benzyl ether compounds of general structures **21** (Table 2). Gratifyingly, reintroduction of the ether linkage into the chain occupying S_3^{sp} restored oral bioavailability (**21a** vs **6j**). The IV clearance of **21a** was comparable to that of lead compound **3**. *m*-Fluoro analog **21b** had greater oral bioavailability and AUC than **21a** despite higher IV clearance, while primary amine analog **21e** had comparable oral bioavailability to **21a**, lower AUC and higher IV clearance. 3-Chloro-5-fluoro analog **21o** had comparable oral bioavailability and IV clearance to **21a** but a considerably lower AUC value. Oral bioavailabilities for compounds **21** in rat uniformly exceeded that for aliskiren (**1**).

Table 3
SAR of benzyl ethers **21**

Compd no.	X	R^2	IC_{50}[a,b] (nM)	PRA[b,c] (nM)
21a	3-Cl	Me	0.48	0.82
21b	3-F	Me	0.59	1.8
21c	3-Me	Me	0.47	4.0
21d	H	Me	1.17	2.9
21e	3-Cl	H	0.48	2.6
21f	3-F	H	0.58	3.3
21g	3-Cl	Et	0.87	7.7
21h	3-F	Et	2.10	18.0
21i	2,3-diF	Me	1.7	6.2
21j	2,5-diF	Me	0.62	2.7
21k	2-F-3-Cl	Me	0.61	3.4
21l	2-F-5-Cl	Me	0.62	2.1
21m	3,4-diF	Me	2.5	16.6
21n	3,5-diF	Me	0.23	1.2
21o	3-F-5-Cl	Me	0.52	1.5
1			0.53	0.65

[a] See Ref. 18 for assay protocol.
[b] Average of at least two replicates.
[c] See Ref. 19 for assay protocol.

Table 2
Rat PK parameters[a]

Compd	Oral C_{max} (ng/mL)	Oral t_{max} (h)	Oral $AUC_{(0-t)}$ (ng h/mL)	Oral $t_{1/2}$ (h)	IV CL (mL/min kg)	Vss (L/kg)	*F* (%)
2	91	3.0	832	5.9	59	22	35.8
3	73	3.3	472	nd	33	nd	12.9
6a	nd[b]	nd	nd	nd	23	117	nd
6j	5	0.9	10	1.8	34	29	0.3
21a	94	3.0	301	9.7	36	19	9.4
21b	123	2.0	380	12.7	58	61	22.1
21e	63	4.0	201	7.8	53	51	7.2
21o	54	4.0	166	7.2	41	38	13.1
1	4	0.1	21	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.

Table 4
Monkey PK of **21a**, **3**, and **1**[a]

Compd	Oral C_{max} (ng/mL)	Oral t_{max} (h)	Oral $AUC_{(0-t)}$ (ng h/mL)	Oral $t_{1/2}$ (h)	IVCL (mL/min kg)	Vss (L/kg)	F (%)
21a[b,c]	33.3	1.0	129.7	29.2	11.7	9.8	9.9
3[b,d]	116	2.7	615[e]	15	5.2	6.1	16
1[b,d]	29.5	1.3	154	9.8	2.5	1.5	1.4

[a] Compounds were administered as fumarate salts.
[b] IV dose = 1 mg/kg.
[c] PO dose 1 mg/kg.
[d] PO dose 2 mg/kg. IV dose 1 mg/kg.
[e] t = 32 h.

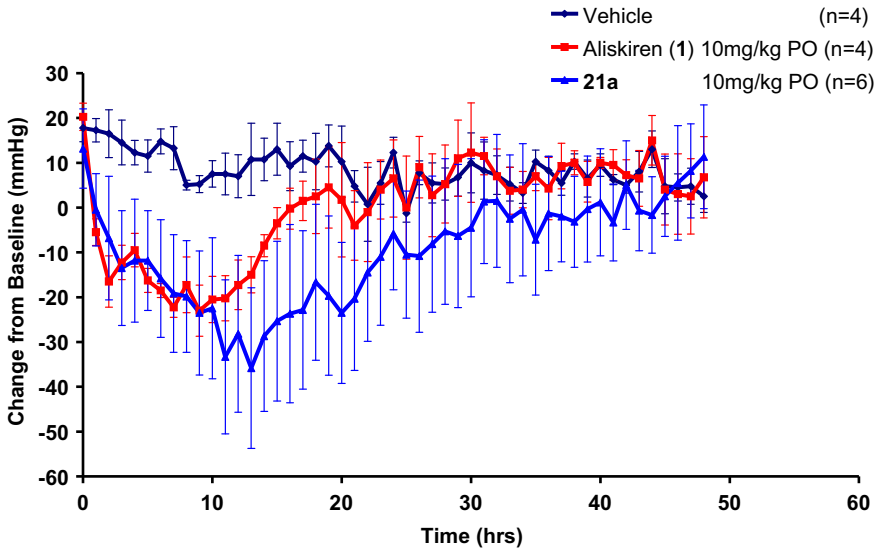


Figure 3. Effect of **21a** and aliskiren (**1**) on mean arterial pressure in double transgenic rats.

Based on its good PRA and rat PK parameters, compound **21a** was selected for advanced in vitro and in vivo evaluation. The compound showed >1000× selectivity over the aspartic proteases β-secretase, cathepsin D and cathepsin E. Its half-life in human liver microsomes was >30 min. The cynomolgus monkey PK parameters of **21a**, **3**, and aliskiren (**1**) are compared in Table 4. While the oral bioavailability of **21a** is lower than that of **3**, it is substantially better than that of aliskiren (**1**). Notably, **21a** has a half-life of 29 h in cynomolgus monkey.

Compound **21a** was studied in an animal model of hypertension. In the dTGR,[20] a 10 mg/kg oral dose gave a statistically significant reduction in mean blood pressure compared to vehicle lasting for >20 h (Fig. 3). The magnitude and duration of blood pressure lowering were greater than that observed with the same dose of aliskiren (**1**) and achieved a statistically significant difference over the 24 h interval.

In conclusion, we have described the structure-based discovery of renin inhibitors with improved plasma renin activity. Oral administration of **21a** elicited sustained reductions in mean blood pressure in an animal model of hypertension.

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18. 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).

19. 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/h) ~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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