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LETTER

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Discovery of VTP-27999, an Alkyl Amine Renin Inhibitor with Potential for Clinical Utility

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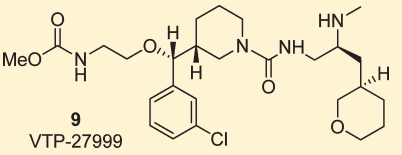
Supporting Information

ABSTRACT: Structure guided optimization of a series of nonpeptidic alkyl amine renin inhibitors allowed the rational incorporation of additional polar functionality. Replacement of the cyclohexylmethyl group occupying the S1 pocket with a (*R*)-(tetrahydropyran-3-yl)methyl group and utilization of a different attachment point led to the identification of clinical candidate **9**. This compound demonstrated excellent selectivity over related and unrelated off-targets, >15% oral bioavailability in three species, oral efficacy in a double transgenic rat model of hypertension, and good exposure in humans.



9
VTP-27999

KEYWORDS: Renin, aspartyl protease, hypertension, structure-based drug design

The renin angiotensin aldosterone system (RAAS) plays a major role in the control of vascular function and blood pressure.[1] Chronic stimulation of the RAAS leads to tissue inflammation and fibrosis with end organ dysfunction, particularly of the kidney.[2,3] Renin is a highly selective aspartyl protease secreted from the kidneys, which cleaves a decapeptide, angiotensin (Ang) I, from the N-terminus of angiotensinogen. Ang I is further processed by angiotensin converting enzyme (ACE) to give an octapeptide, Ang II, which binds to the angiotensin receptor type 1 (AT_1), leading to vasoconstriction and, with chronic stimulation, inflammation and fibrosis. Recently, local RAASs have been shown to function in various tissues, including kidney and heart.[4] Two major classes of antihypertensives, ACE inhibitors and AT_1 receptor blockers (ARBs), function by blocking the RAAS.[5] The high selectivity of renin and its activity at the top of the RAAS cascade suggest that a direct renin inhibitor (DRI) may offer a superior therapeutic profile to the ACE inhibitors and ARBs.[6,7] RAAS blockers are also used clinically for the treatment of chronic kidney disease (CKD).[8] Preliminary studies indicate that DRIs may provide better end organ protection in the treatment of CKD.[9,10]

Renin has proved to be a historically challenging target for medicinal chemists. During the 1980s, intensive efforts that focused on peptidomimetics led to the discovery of many potent compounds;[11,12] however, because of poor oral bioavailability, none of them was successfully developed as a drug.[13] Beginning in the mid-1990s, several classes of nonpeptidic renin inhibitors were reported.[13−17] One line of investigation culminated in the discovery of aliskiren (**1**, Figure 1), the first approved DRI, which was brought to market in 2007.[18]

We recently described the structure based discovery of a new class of nonpeptidic renin inhibitors represented by alkyl amine **2**.[19,20] Compound **2** had excellent potency against renin (Table 1). Its oral bioavailability in rat and monkey was just below 10%, and its clearance was medium in rat but low in cynomolgus monkey (Table 2). However, **2** had an IC_{50} of 4.6 μM against CYP3A4, which was considered undesirable. Reducing inhibition of CYP3A4, while retaining or improving oral bioavailability and excellent potency against plasma renin activity (PRA), became the major goal for the further optimization of **2**.

RESULTS AND DISCUSSION

Our strategy to accomplish this goal was to introduce additional polar functionality into **2** at positions which would allow favorable interactions with renin. We anticipated that the added polar functionality would cause unfavorable interactions with the largely hydrophobic binding pocket of CYP3A4.[21] We used the 1.9 Å resolution X-ray structure of **2** bound to human renin (PDB code: 3KM4), combined with our proprietary structure based drug design tool, Contour, to grow and score analogues of **2** within the renin binding site.

Compounds **3** and **5**, both of which vary in the group filling the S1 pocket,[22] emerged from our design process as attractive candidates to meet this goal. Despite the added polar groups, models of **3** and **5** bound to renin retained the key interactions and overall binding mode observed in the X-ray structure of **2** (PDB code: 3KM4). The tetrahydropyran ring of the (*R*)-(tetrahydropyran-3-yl)methyl compound **3** was predicted to retain the same pucker observed for the cyclohexane ring in the complex of **2** with renin and thus position the ring ether oxygen to accept a hydrogen bond from the side chain hydroxyl of Thr77 in the flap region of the protein. By contrast, the

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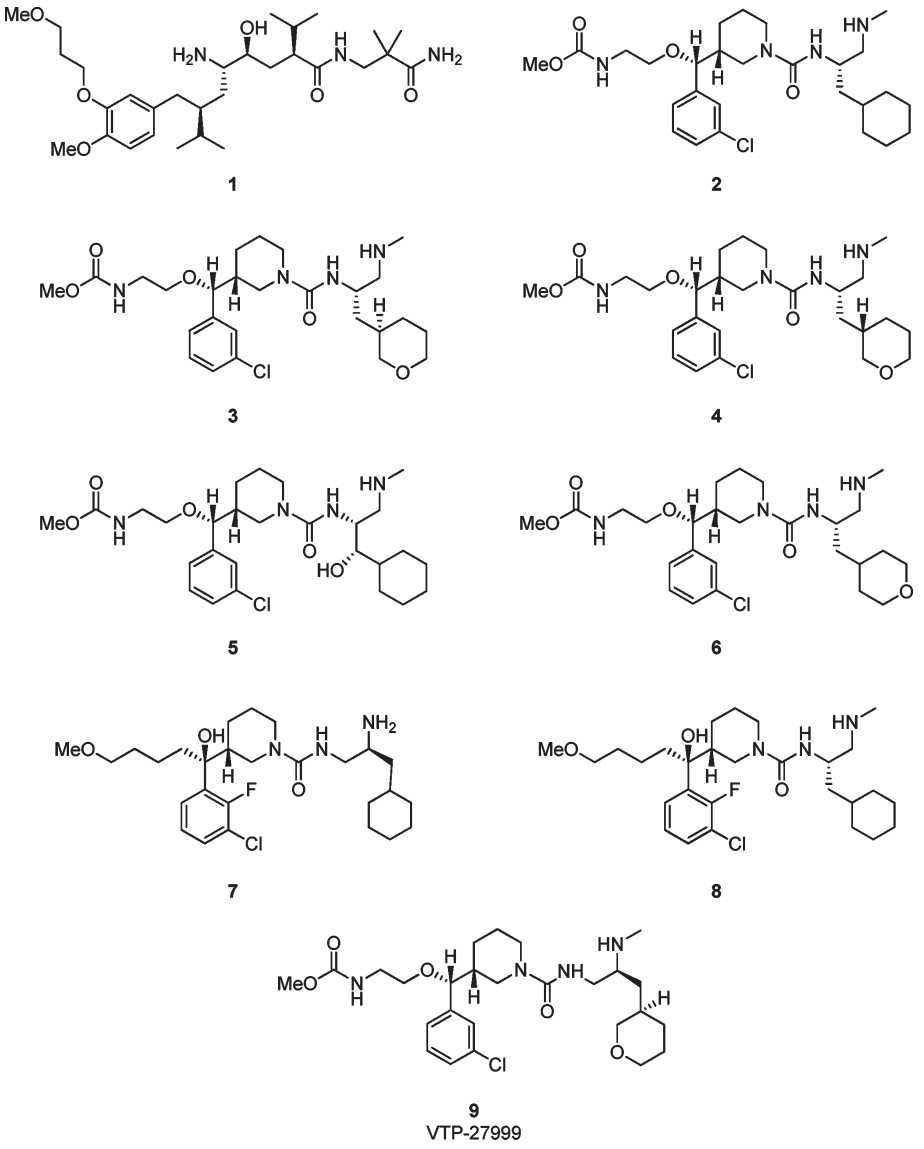

Figure 1. Aliskiren and alkyl amine renin inhibitors.

Table 1. Enzyme Inhibition[a,b]

compd no.	IC_{50}[c] (nM)	low renin IC_{50}[d] (nM)	PRA[e] (nM)	CYP3A4 IC_{50}[f] (nM)
1	0.40	0.53	0.65	
2	0.48		0.82	4600
3	0.56	0.42	0.48	28000
4	5.5	2.7	4.5	
5	0.33	0.15	1.06	7100
6	6.2	1.7	13.1	>30000
7	2.2	0.27	69	2910
8	0.73		9.4	
9	0.47	0.30	1.1	>30000

[a] Assay protocols are provided in the Supporting Information. [b] Assay results are the average of at least two replicates. [c] Inhibition of 0.3 nM of purified recombinant human renin in buffer was measured. [d] Inhibition of 36 pM of purified recombinant human renin in buffer was measured. [e] IC_{50} in the presence of human plasma. [f] Inhibition of CYP3A4 in human liver microsomes was measured.

corresponding ether oxygen in epimeric (*S*)-(tetrahydropyran-3-yl)-methyl compound **4** was unable to form this interaction and was predicted to be less potent. The hydroxy group of α-hydroxy-(cyclohexyl)methyl compound **5** was expected to donate a hydrogen bond to Asp32, one of the catalytic aspartates.

The synthesis of the analogues in Figure 1 was accomplished by formation of the central urea linkage from the appropriate left-hand piperidine and a suitably protected right-hand diamine. The final steps in the synthesis of **3** from piperidine **10**[19] and protected diamine **11** are shown in Scheme 1. Complete synthetic schemes and procedures are provided in the Supporting Information.

Compounds **3** and **5** both retained potency against renin in the absence or presence of plasma comparable to that of **2** (Table 1). Compound **3** was a substantially weaker inhibitor of CYP3A4 than **2**, while **5** did not differ significantly from **2**. As predicted, **4** was less potent than **3**. Analogue **6** was also prepared because the (tetrahydropyran-4-yl)methyl group has the advantage of not introducing an additional chiral center. Interestingly, although **6** was less potent against the enzyme in buffer than **2** or **3**, it experiences only a 2× loss in potency when assayed in the presence of human plasma and its CYP3A4 IC_{50} value was >30 μM. Pharmacokinetic parameters of **3** were determined in rat and cynomolgus monkey (Table 2). The

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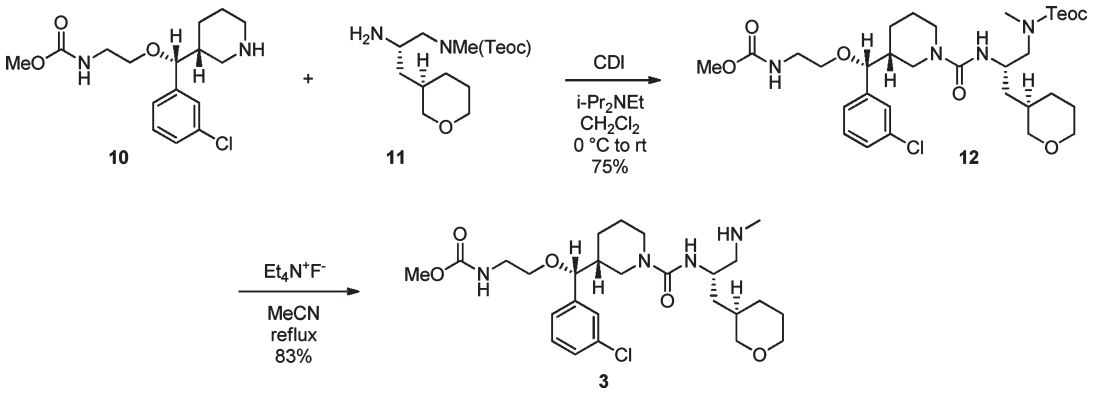
Table 2. PK Parameters[a]

compd no.	species	IV dose (mg/kg)	oral dose (mg/kg)	oral $AUC_{(0-t)}$ (ng·h/mL)	IV $t_{1/2}$ (h)	IV CL (mL/min·kg)	V_{ss} (L/kg)	F (%)
1	rat[b]	2.0	10.0	21	39.6	55	62	1.2
2	rat	1.6	8.0	301	16.2	36	19	9.4
3	rat	1.5	8.2	167	18.3	48	39	7.5
7	rat	0.8	4.0	269	51.0	47	101	29.5
8	rat	1.6	8.2	226	15.9	30	16	5.5
9	rat	2.0	10.0	477	7.3	128	38	37
1	monkey[b]	1.0	2.0	154	14.7	2.5	1.5	1.4
2	monkey	1.0	1.0	130	23.3	11.7	9.8	9.9
3	monkey	1.0	2.0	172	14.0	9.5	5.0	5.3
9	monkey	1.0	2.0	404	12.3	29.6	4.7	18
1	dog	1.0	1.0	149	16.3	11.0	4.7	11
9	dog	0.5	1.0	484	9.9	12.9	4.0	41

[a] Compounds were administered as fumarate salts. [b] PK parameters for **1** in rat, marmoset, and dog have been reported previously. Concentrations of **1** were determined by a bioassay. See ref 18.

Scheme 1. Preparation of 3[a]



[a] Teoc = 2-(trimethylsilyl)ethoxycarbonyl; CDI = carbonyl diimidazole.

oral bioavailability of **3** in both species was lower than that of **2** but superior to that of **1**.[18] Further synthesis was directed toward the identification of a compound that combined the potency and minimal CYP3A4 inhibition of **3** with improved oral bioavailability.

In the course of earlier modeling and SAR studies around **2**, it had been shown that the cyclohexylmethyl susbstituent that fills the S1 pocket could also be attached to the carbon atom α to the basic amine, albeit with a modest loss in potency. For example, primary amine **7** had an IC_{50} of 2.2 nM against human renin in buffer. Intriguingly, **7** had significantly greater oral bioavailability in rat than the related regioisomeric compound **8**[20], in which the cyclohexylmethyl moiety is attached to the carbon atom β to the basic amine. Modeling indicated that **9**, in which an (*R*)-(tetrahydropyran-3-yl)-methyl group is attached in the (*S*) configuration α to the basic amine, suitably positioned the pyran oxygen to accept a hydrogen bond from Thr 77. Compound **9** proved to have comparable potency to **1** and **3** against renin both in buffer and in the PRA assay (Table 1) and had an IC_{50} value >30 μM against CYP3A4. Encouragingly, the oral bioavailabilities of **9** in rat and monkey were 37% and 18%, respectively, surpassing both **1** and **3** (Table 2). Furthermore, the oral bioavailability of **9** in dog was greater than that of **1**. The free fraction of **9** in plasma was determined in four species, including human, and ranged from 22% in dog to 29% in monkey. These results are consistent with the observed 3× loss in potency when enzyme inhibition was measured in the absence and presence of plasma.

Compound **9** was selected for further in vitro and in vivo evaluation. At a 10 μM concentration, **9** caused <10% inhibition of three other human aspartyl proteases: β-secretase, cathepsin D, and cathepsin E. In addition, on the basis of its PRA potency, **9** demonstrated >1000× selectivity for renin, over a panel of >150 receptors, ion channels, and enzymes.[23,24]

Compound **9** was nonmutagenic in the Ames assay, when tested in either the presence or absence of S9-mix, and was not genotoxic in the mouse lymphoma L5178Y TK± test system.

The efficacy of **9** at 10 mg/kg po was compared to **1** at the same dose in double transgenic rats (dTGR)[25] engineered to express human renin and angiotensinogen (Figure 2). These animals have severe hypertension dependent on human renin. Compound **9** provided a greater reduction in mean arterial blood pressure (MAP) at t = 24 h and a longer duration of action.

In a single ascending dose study in normal volunteers, dose proportionate increases in plasma drug levels of **9** were observed with doses from 40 mg to 1000 mg.[26]

X-ray crystal structures were obtained of both **3** (PDB code: 3Q5H, Figure 3A) and **9** (PDB code: 3Q4B Figure 3B) bound to renin. The general binding modes and interactions observed in both

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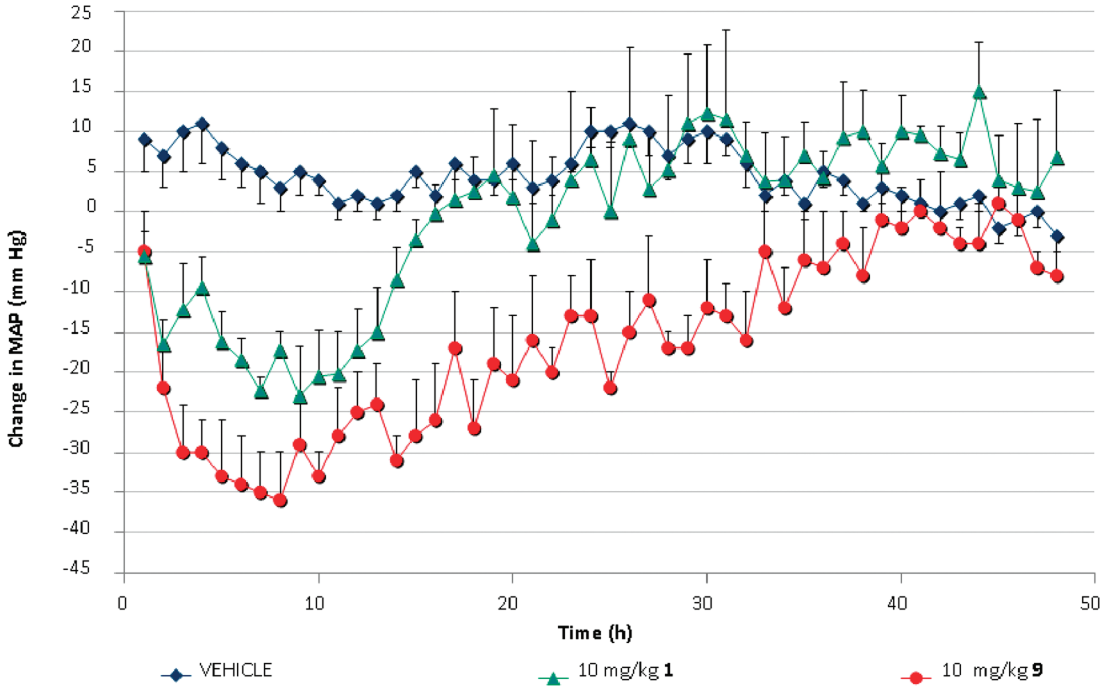

Figure 2. Mean arterial pressure (MAP) responses to **1** and **9** in dTGR.

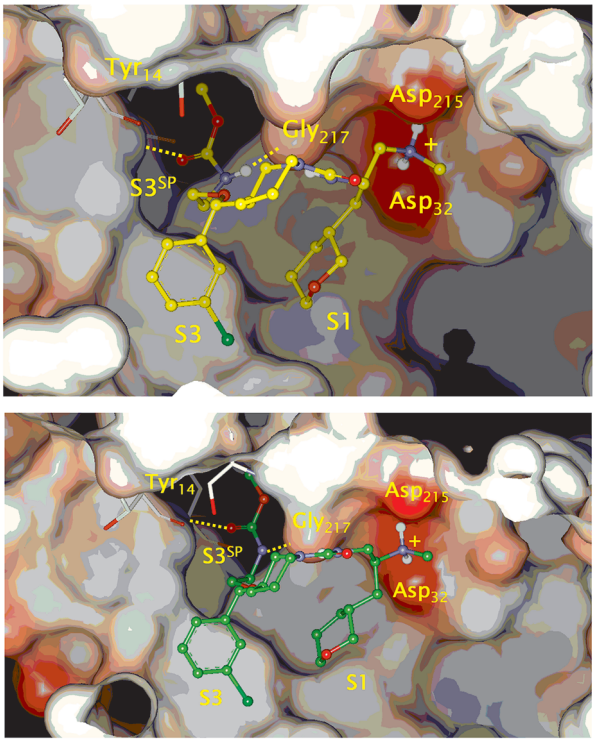

Figure 3. Crystal structures of **3** and **9** bound to renin: (A) Compound **3** bound to renin, PDB code: 3Q5H. (B) Compound **9** bound to renin, PDB code: 3Q4B. Selected hydrogen bonds are depicted as dashed yellow lines. The molecular surface corresponding to certain amino acid residues from the flap region of the binding site, including Thr77, was not rendered to provide an unobstructed view of the inhibitor and its interactions.

complexes were as expected on the basis of molecular modeling and the previously published X-ray crystal structures of members of the alkyl amine chemotype (PDB codes: 3KM4, 3GW5).[19,20] Thus, in both cases, the protonated secondary amine is positioned between the catalytic aspartates, Asp32 and Asp215. The urea and carbamate NH's both donate hydrogen bonds to the backbone carbonyl oxygen of Gly217. These favorable hydrogen bonds, and an intraligand hydrogen bond between the carbamate NH and the ether oxygen of the group occupying the $S3^{sp}$ subsite, serve to mitigate the repulsion between the carbonyl of Gly217 and the ether oxygen of the ligands. The tetrahydropyran oxygens of both **3** and **9** form hydrogen bonds to Thr77, validating our design.

CONCLUSION

Structure based optimization of a series of nonpeptidic alkyl amine renin inhibitors allowed the rational incorporation of additional polar functionality and a change in the attachment point of the group occupying the S1 pocket. The additional polar functionality maintained potency against renin, while improving the selectivity of the compounds, and led to the identification of clinical candidate **9**. This compound demonstrated >1000× selectivity over related and unrelated off-targets, >15% oral bioavailability in three species, and oral efficacy in a dTGR model of hypertension.

ASSOCIATED CONTENT

Supporting Information. Representative synthetic schemes and procedures; NMR, MS, and HPLC data on new compounds; assay protocols; and crystallography procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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