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Letter

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Structure-Based Design of Substituted Piperidines as a New Class of Highly Efficacious Oral Direct Renin Inhibitors

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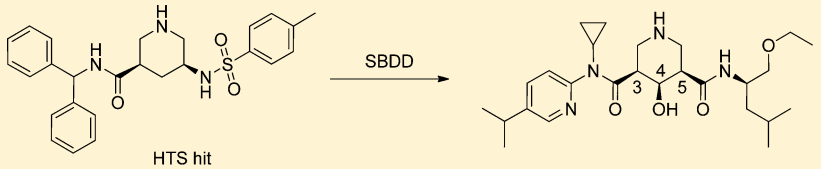
Supporting Information



SBDD = Structure-based Drug Design

ABSTRACT: A *cis*-configured 3,5-disubstituted piperidine direct renin inhibitor, (*syn*,*rac*)-**1**, was discovered as a high-throughput screening hit from a target-family tailored library. Optimization of both the prime and the nonprime site residues flanking the central piperidine transition-state surrogate resulted in analogues with improved potency and pharmacokinetic (PK) properties, culminating in the identification of the 4-hydroxy-3,5-substituted piperidine **31**. This compound showed high *in vitro* potency toward human renin with excellent off-target selectivity, 60% oral bioavailability in rat, and dose-dependent blood pressure lowering effects in the double-transgenic rat model.

KEYWORDS: *Renin inhibitor, structure-based design, aspartic protease, 3,5-piperidines*

Hypertension is a major risk factor for cardiovascular diseases (CVDs), such as congestive heart failure, stroke, and myocardial infarction, which are the leading causes of death in industrialized countries. However, despite several classes of therapeutic drugs available, less than 30% of hypertensive patients achieve currently recommended blood pressure goals.[1,2]

The renin-angiotensin-aldosterone system (RAAS) has long been established as being the key cascade in the regulation of blood pressure and homeostasis of body fluid volume. Renin, an aspartic protease, cleaves angiotensinogen to release inactive peptide angiotensin I (Ang-I). Cleavage of Ang-I by angiotensin converting enzyme (ACE) then generates angiotensin II (Ang-II), which induces the principal physiological effects of the RAAS, *i.e.* an increase of blood pressure by vasoconstriction, sodium retention and altering vascular resistance.[3] Renin controls the first and rate-limiting step of the RAAS and has high specificity for its substrate angiotensinogen. Blockade of renin has been considered to be a highly attractive paradigm to treat hypertension and to protect from end-organ damage.[4]

Since the early 1980s, substantial efforts had centered on peptidic or peptidomimetic scaffolds. However, these direct renin inhibitors (DRIs) suffered from lack of oral bioavailability and poor antihypertensive efficacy in clinical trials. To improve the unfavorable pharmacokinetic (PK) properties, a combination of crystallographic structure analyses and computational molecular modeling had been employed by Novartis to design nonpeptidic DRIs. This approach has led to the identification of aliskiren (Tekturna, Rasilez), a highly potent, selective, and long lasting antihypertensive DRI, which to date is the only marketed DRI for the treatment of hypertension.[5,6] Subsequently, a number of DRIs based on diverse transition-state surrogate structural motifs and scaffolds with different renin active-site binding topology have been reported.[7−9]

As part of our continued research efforts to identify novel lead scaffolds, we have successfully applied complementary approaches including enzymatic high-throughput screens (HTSs), a combined NMR/X-ray fragment based screen,[10] and *in silico* three-dimensional pharmacophore searches.[11] Compound **1**, a racemic *cis*-configured 3,5-disubstituted

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piperidine, originated as a weakly active HTS hit from a piperidine-based combinatorial library that had been specifically designed for aspartic proteases at Novartis (Figure 1).[12] We

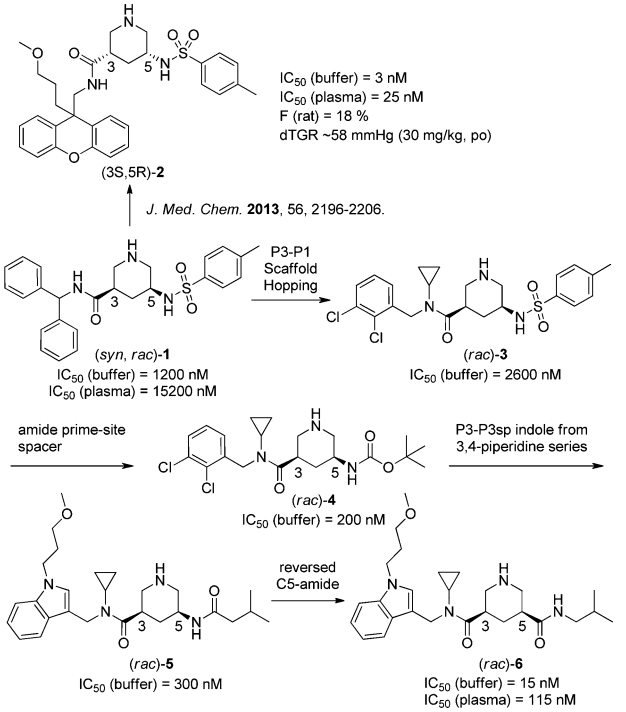


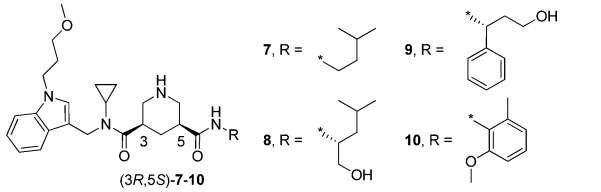
Figure 1. Discovery of (*rac*)-**6** from the HTS hit (*syn*,*rac*)-**1**.

previously reported that X-ray crystallography employing (*rac*)-**1** and recombinant human renin (rh-renin) resulted in a complex structure with only the (3*S*,5*R*)-enantiomer of **1** bound in the active site and with the flap β-hairpin loop in a closed conformation (see Supporting Information, Figure S1). Subsequently, merging this piperidine scaffold with a second fragment-based screening hit led to (3*S*,5*R*)-**2** with enhanced *in vitro* potency (IC_{50} = 3 nM).[10] We report herein the results of an alternative approach to optimize screening hit **1**, which led to the discovery of several potent and orally bioavailable DRIs.

Initially, the highly lipophilic diphenylmethylamine P3−P1 pharmacophore of **1** was replaced by the *N*-cyclopropyl *N*-dichlorobenzyl amide motif, previously reported for 3,4-piperidine-based DRIs to fill the large contiguous S3−S1 binding pocket,[13] to provide (*rac*)-**3** (Figure 1). Replacement of the C5-sulfonamide spacer by a carbamate linker identified the 10-fold more potent (*rac*)-**4**. The X-ray cocrystal structure of (*rac*)-**4** with rh-renin showed only the (3*R*,5*S*)-enantiomer bound, opposite to the observation made for (*rac*)-**1** (see Supporting Information, Figure S1). With the aim of improving potency by filling the nonsubstrate S3 subpocket (S3sp), extending perpendicular from the S3 pocket, we next replaced the dichlorophenyl moiety by an indole scaffold substituted at the ring nitrogen by a methoxypropyl side chain[14] to afford (*rac*)-**5**. Further optimization of (*rac*)-**5** by introduction of the reversed amide spacer at the 5-position of the central piperidine provided (*rac*)-**6**, exhibiting a marked increase in potency. Computational modeling suggested that the higher inhibitory affinity is due to the reversed amide carbonyl group forming an H-bond to the backbone nitrogen of Ser_{76} of the flap β-hairpin.[15]

We next investigated the optimization of the C5-amide moiety with various α-branched amines occupying the S1′-S2′ pockets (Table 1). Incorporation of an H-bond donor or

Table 1. Optimization of the C5-Amide Moiety Filling the S1′-S2′ Pockets



cmpd[a]	renin IC_{50} (nM) (buffer/plasma)	*in vitro* CL_{int} (μL/min/mg, human/rat)	rat F (%) (*i.v.*/*p.o.* 2/6 mg/kg)
7	3/40	194/141	29
8	3/8	157/111	0.4
9	0.4/1	173/109	<0.1
10	0.4/2	679/533	nd

[a]All compounds were used as their free base.

acceptor into the prime-site amide moiety was anticipated to target an H-bond with Gly_{34} as well as to reduce lipophilicity, thereby increasing plasma renin potency. Indeed, compounds **8** and **9** displayed IC_{50} values of 8 and 1 nM, respectively, in the presence of plasma. However, these compounds suffered from low oral bioavailability in rat (<1%) with medium clearance in rat liver microsomes (RLM). An *o*,*o*-disubstituted aniline moiety was also found to fill the prime-site efficiently, however, compound **10** showed high *in vitro* clearance. In addition, we noticed that the potential for metabolism of the 3-amino-methylindole moiety could lead to generation of a reactive electrophilic intermediate.[16] We therefore returned our focus to alternative P3−P1 moieties.

At this stage, the development of a versatile synthetic route was required to facilitate optimization of both the prime- and the nonprime binding motifs (Scheme 1). We established a stereoselective synthesis of both (*S*,*R*)- and (*R*,*S*)-configured *cis*-piperidine enantiomers **12** and **13** based on an asymmetric desymmetrization approach starting from the *meso*-cyclic anhydride **11**.[17] Cinchona base-catalyzed methanolysis of **11**, followed by recrystallization with (*R*)- and (*S*)-1-phenylethyl-amine provided the enantiomerically pure 3,5-piperidine carboxylic acids (3*S*,5*R*)-**12** and (3*R*,5*S*)-**13** with >99% ee, respectively.[18] Subsequent amide coupling of (3*S*,5*R*)-**12** with sterically hindered *N*-cyclopropyl anilines required extensive optimization. A mixed anhydride coupling method using TcBocCl (2,2,2-trichloro-1,1-dimethylethyl chloroformate) in the presence of magnesium bromide proved optimal in providing amides **14**. Alkaline hydrolysis followed by coupling to the prime-site moiety afforded intermediates **16**. *N*-Boc deprotection using HCl gave the target products **17**. Similarly, the reversed order of coupling steps starting from (3*R*,5*S*)-**13** could be employed.

Next, we revisited the *N*-cyclopropyl *N*-dichlorobenzyl amide P3−P1 binding motif. Modeling and SAR studies led to replacement of the 3-aminomethylindole with 4-isopropyl-3-(3-methoxypropoxy)anilide. Compound **21** afforded IC_{50}s of 0.3 and 2.0 nM against rh-human renin in buffer and in the presence of plasma, respectively (Table 2). The potency of **21** is believed to be a result of the fluorine atom forming an H-bond with the hydroxyl group of Thr_{77}. Unfortunately, inhibitor

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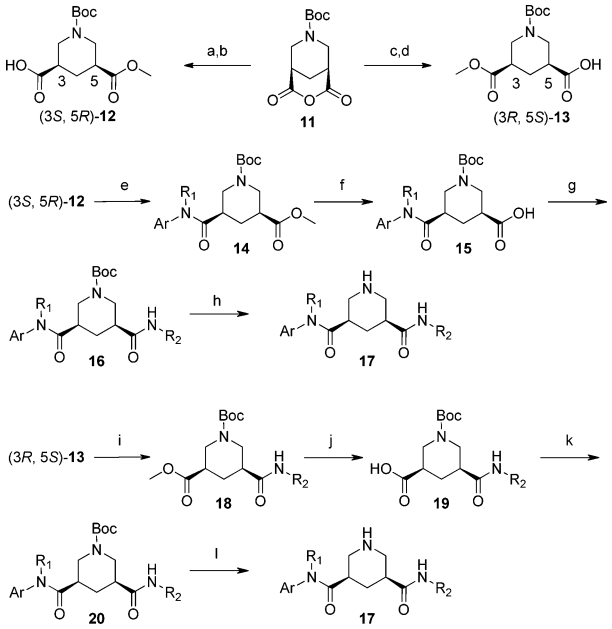
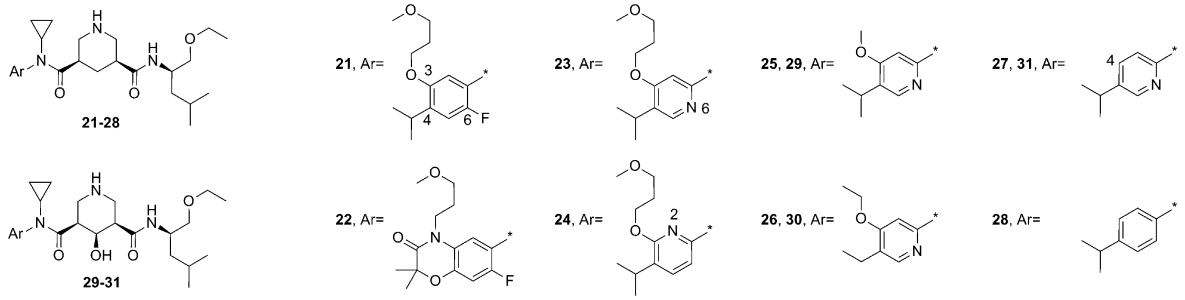
Scheme 1. Stereoselective Synthesis of 3,5-Piperidine Analogues[a]



[a]Reagents and conditions: (a) MeOH, $(DHQ)_2AQN$, 95%, 63% ee; (b) recrystallization with (*R*)-1-phenylethylamine, >99% ee; (c) MeOH, $(DHQD)_2AQN$, 91%, 75% ee; (d) recrystallization with (*S*)-1-phenylethylamine, >99% ee; (e) TcBocCl, Et_3N, CH_3CN then ArR_1-NH, $MgBr_2$; (f) *aq*. LiOH, THF; (g) R_2-NH_2, EDCI·HCl, HOAt, DMF; (h) HCl in dioxane; (i) R_2-NH_2, EDCI·HCl, HOAt, DMF; (j) *aq*. LiOH, THF; (k) ArR_1-NH, BopCl, Et_3N, CH_2CH_2; (l) HCl in dioxane.

21 suffered from high clearance in RLM as well as hERG binding, presumably due to the more lipophilic character of the molecule. We therefore explored the less hydrophobic 1,4-benzoxazinone motif as a P3 pharmacophore.[19] Inhibitor **22** (Table 2) showed not only high *in vitro* potency but also high clearance in RLM, translating into high *in vivo* clearance in rat. Replacement of the 6-fluoroaniline with 6-aminopyridine offered an alternative H-bond acceptor for Thr_{77}, and also reduced the lipophilicity. As a result, the 6-aminopyridine P3 pharmacophore in **23** retained high potency while improving *in vitro* and *in vivo* clearance in rats. The regioisomeric 2-aminopyridine analogue **24** was found to be less potent and metabolically less stable than **23**. We suspected that the S3sp-filling alkoxy side chain of **23** potentially constitutes a metabolically weak spot, and furthermore that its high number of rotatable bonds may limit cell permeability. Truncation of the S3sp side chain provided **25** and **26**, which were found to retain high *in vitro* potency despite the suboptimal occupancy of S3sp. Most remarkably, the P3-aminopyridine analogue **27** bearing no side chain at the C4 position still exhibited high *in vitro* potency, while the corresponding P3-aniline analogue **28** was 4 and >1000-fold less active in the buffer and plasma renin assays, respectively. These results suggested that the H-bond interaction of the P3-pyridyl nitrogen atom with the OH of Thr_{77} fully compensates for the lack of binding interactions to the S3sp cavity. In addition, the more hydrophilic P3-aminopyridine moiety led to a reduction in the plasma IC_{50} shift. Compound **27** also showed improved *in vitro* metabolic stability and oral bioavailability in the rat. However, **27** (logD (pH 6.8) = 4.9) showed modest affinity to the hERG channel as determined by a dofetilide binding assay,[20] as compared to the less hydrophobic compounds **25** (logD (pH 6.8) = 2.2) and **26** (logD (pH 6.8) = 2.1). Therefore, introduction of a polar

Table 2. SAR Data for Inhibitors Modified at P3−P1, including *in Vivo* Rat Pharmacokinetics (PK) (*i.v.* 2 mg/kg, *p.o.* 6 mg/kg)



cmpd	renin IC_{50} (nM) (buffer/plasma)[a]	*in vitro* CL_{int} (μL/min/mg, human/rat)[a]	*i.v.* PK parameters[b] CL (L/h/kg)	Vd_{ss} (L/kg)	$T_{1/2}$ (h)	*p.o.* PK parameters[b] oral C_{max}/dose (nM/mg/kg)	oral AUC_{inf}/dose (nM*h/mg/kg)	*F* (%)	$hERG_{bind.}$ IC_{50} (μM)[a]
21	0.3/2.0	115/214	nd	nd	nd	nd	nd	nd	5.5
22	0.3/3.0	61/198	7.3	12	1.9	12	49	20	>30
23	0.4/1.0	53/96	4.8	11	4.3	18	93	19	>30
24	0.8/6.0	289/252	nd	nd	nd	nd	nd	nd	3.3
25	0.2/0.4	15/101	2.4	4.3	7.0	68	324	39	>30
26	0.2/0.6	46/62	1.4	4. 6	7.4	155	690	44	>30
27	0.9/1.1	<3.4/13	3.6	7.2	6.5	63	296	45	7
28	4.0/5340	nd/nd	nd	nd	nd	nd	nd	nd	nd
29	0.8/2.0	22/142	2.4	6.1	7.7	74	511	61	>30
30	0.6/2.5	22/54	0.74	2.6	5.9	252	1630	60	>30
31	0.2/0.6	<3.4/52	2.4	4	6.8	136	552	61	>30

[a]Determined for the HCl salt of each compound. [b]The following salts were used for *in vivo* PK studies in rat: **22**, HCl; **23**, HCl; **25**, tartrate; **26**, tartrate; **27**, fumarate; **30**, fumarate; **31**, succinate; inhibitor **29** was administered as its free base.

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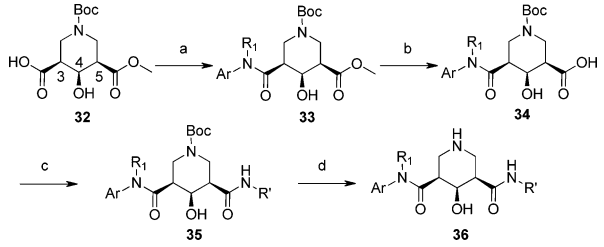
hydroxyl group to the 4-position of the central piperidine ring was investigated. Inhibitors **29**, **30** and **31** (logD (pH 6.8) = 0.93) all retained the *in vitro* inhibitory potency against human renin as well as a favorable hERG profile. These 4-OH analogues also showed better oral bioavailability compared to their corresponding nonhydroxylated analogues (**25**, **26** and **27**, respectively, Table 2).

The synthesis of inhibitors bearing the core (3*S*,4*S*,5*R*)-4-hydroxy-3,5-piperidine scaffold started from (3*S*,4*S*,5*R*)-**32**, which was obtained by the published procedure,[21] followed by recrystallization with (*R*)-1-phenylethylamine to increase the enantiomeric excess to >99%. Amide coupling of **32** with the respective P3−P1 anilines was carried out using acid chloride or TcBocCl mediated activation of the carboxylic acid function. Alkaline ester hydrolysis of the resulting intermediate **33**, followed by the second amide coupling step to attach the prime-site moiety and deprotection afforded inhibitors **36** (Scheme 2).

Scheme 2. Stereoselective Synthesis of 4-Hydroxy-3,5-piperidine Analogues[a]



[a]Reagents and conditions: (a) (i) (1-chloro-2-methylpropenyl)-dimethylamine, CH_2Cl_2 or TcBocCl, Et_3N, THF, (ii) P1−P3 amine, $MgBr_2$ (for TcBoc method); (b) *aq.* LiOH, THF; (c) R′-NH_2, EDCI·HCl, HOAt, DMF; (d) HCl in dioxane.

Inhibitor **31** was selected for further *in vitro* and *in vivo* evaluation based on its overall attractive profile. The compound was highly selective against a panel of recombinant human aspartic proteases comprising pepsins A and C, cathepsins D and E, as well as BACE 1 and BACE 2 (all IC_{50}s >30 μM). Furthermore, high selectivity over a panel of >60 receptors, ion channels, and enzymes including CYP3A4, 2C9, and 2D6 (all IC_{50} >30 μM) was achieved. Parent **31** as well as the corresponding aminopyridine P3−P1 fragment were non-mutagenic in the Ames test, both in the presence and absence of S9 mix. Compound **31** exhibited weak inhibitory activity against hERG, as well as the cardiac sodium (Nav1.5) and L-type calcium (Cav1.2) channels (IC_{50}s of >30 μM) as measured by *in vitro* patch clamp assays. The succinate salt is crystalline (mp 122 °C), chemically stable in bulk during 3 days at 80 °C, and highly soluble in water at the pH range between 1 and 9 (>10 mg/mL). Single oral dosing of **31** as its succinate salt to telemetered double-transgenic rats (dTGRs)[22] at 1, 3, 10, 30, and 100 mg/kg resulted in a dose-dependent and long-lasting reduction in mean arterial blood pressure (MAP) with peak changes in MAP of 33, 42, 52, 63, and 66 mmHg, respectively (Figure 2). Remarkably, a significant blood pressure lowering effect was still observed 42 h after the 100 mg/kg oral dose was administered.

The crystal structure of rh-renin in complex with **31** revealed the ligand to bind in an extended conformation, occupying the S3 to S2′ site of the enzyme active site (Figure 3). The basic nitrogen atom of the piperidine ring interacts with both catalytic aspartic acid residues, Asp_{32} and Asp_{215}, which

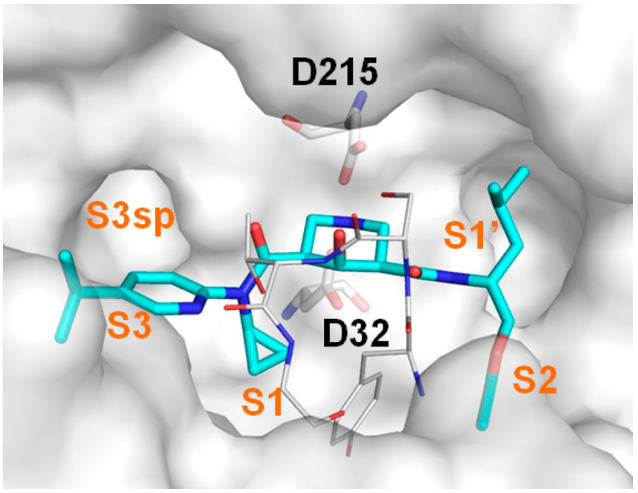


Figure 3. Crystal structure of **31** (shown as a stick model in cyan color; PDB code 4Q1N) bound to rh-renin. Shown is the solvent accessible surface of the active site of renin, the catalytic aspartate residues Asp_{32} and Asp_{215}, as well as the residues of the flap β-hairpin with carbon atoms colored in gray. The flap residues Tyr_{75}, Ser_{76}, Thr_{77}, and Gly_{78} have been omitted from the protein surface calculation. The oxygen and nitrogen atoms are colored in red and blue, respectively.

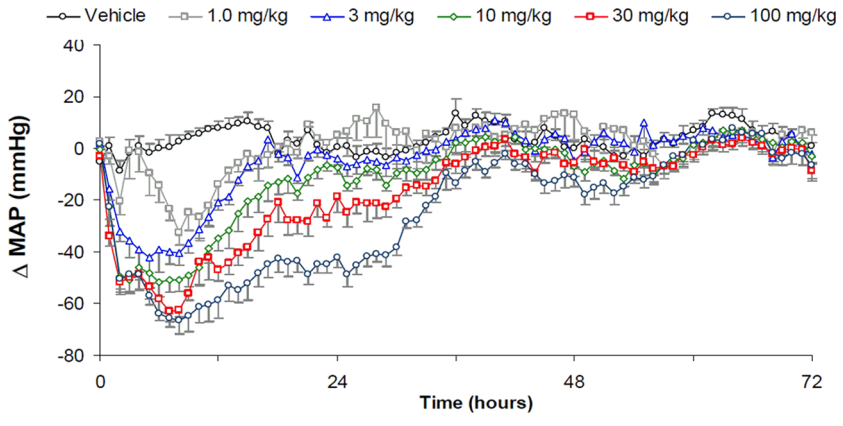


Figure 2. *In vivo* blood-pressure lowering oral efficacy of **31** (succinate salt) in telemetered dTGRs.

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functions as a transition-state surrogate. The 4-hydroxyl group is H-bonded to Ser_{76} of the flap being in a closed conformation.[23] The *N*-cyclopropyl group and the isopropyl-substituted pyridyl moiety are positioned in the large contiguous hydrophobic S1/S3 pocket, leaving the S3sp unoccupied. The pyridyl nitrogen is in close H-bond distance to the side chain hydroxyl of Thr_{77} located at the tip of the flap domain. The data support the notion that this interaction is indeed crucial for strong binding affinity, in particular for inhibitors lacking interactions with the S3sp site. The carbonyl oxygen of the prime-site amide linker forms an H-bond to the backbone nitrogen of the flap Ser_{76}, while the amide NH functions as an H-bond donor to the carbonyl oxygen of Gly_{34}. The *sec*-butyl makes hydrophobic interactions to S1′, and the terminal ethoxyethane group occupies the S2′ pocket.

In summary, novel highly potent *cis*-configured (3*R*,5*S*)-piperidine-based DRIs were derived from a weakly active HTS hit (compound **1**). Structure-based modification of the prime-site linker and optimization of the P3−P1 and P1′-P2′ portions guided by X-ray crystallography significantly improved the *in vitro* potency. The discovery of the *N*-alkyl substituted 6-pyridyl as a novel high affinity P3 scaffold even in the absence of a P3sp side chain constituted a breakthrough of our extensive optimization efforts. The additional introduction of a hydroxyl group on the piperidine core furnished several inhibitors with improved PK profiles in rats. Inhibitor **31** demonstrated high *in vitro* potency and off-target selectivity, an attractive physicochemical and *in vitro* ADME profile, as well as excellent oral bioavailability and long elimination half-life in rat. Inhibitor **31** showed high efficacy in the dTGR model with a dose-dependent sustained blood pressure reduction observed for more than 24 h after single oral dose administration.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details for compounds synthesized, experimental procedures for biological assays, *in vivo* pharmacokinetics, *in vivo* pharmacology, and X-ray crystallographic information for the rh-renin−inhibitor complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

RAAS, renin−angiotensin−aldosterone system; ACE, angiotensin converting enzyme; ADME, absorption, distribution, metabolism, and excretion; DRI, direct renin inhibitor; HTS, high-throughput screening; rh-renin, recombinant human renin; Ac_2O, acetic anhydride; Boc, *tert*-butoxycarbonyl; $(Boc)_2O$, di-*tert*-butyl dicarbonate; BopCl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; DMF, dimethylformamide; dTGR, double transgenic rat; EDCI·HCl, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride; Et_3N, triethylamine; EtOAc, ethyl acetate; EtOH, ethyl alcohol; hERG, the human *ether-à-go-go*-related gene; HOAT, 1-hydroxy-7-azabenzotriazol; mp, melting point; PK, pharmacokinetics; RLM, rat liver microsomes; TcBocCl, 2,2,2-trichloro-1,1-dimethylethyl chloroformate

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