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The P_1 *N*-isopropyl motif bearing hydroxyethylene dipeptide isostere analogues of aliskiren are in vitro potent inhibitors of the human aspartyl protease renin

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ABSTRACT

Novel nonpeptide small molecule renin inhibitors bearing an *N*-isopropyl P_1 motif were designed based on initial lead structures **1** and aliskiren (**2**). (P_3–P_1)-Benzamide derivatives such as **9a** and **34**, as well as the corresponding P_1 basic tertiary amine derivatives **10** and **35** were found to display low nanomolar inhibition against human renin in vitro.

Hypertension is a major risk factor of high prevalence worldwide for cardiovascular diseases.[1] The pivotal functions of the renin–angiotensin–aldosterone system (RAAS) in cardiovascular (patho)physiology including the regulation of blood pressure, as well as electrolyte and body fluid homeostasis have been well established.[2] The RAAS has emerged as an important target for antihypertensive drug therapy, with angiotensin (Ang)-converting enzyme inhibitors (ACEi) and AT_1-receptor blockers (ARBs) having proven effectiveness in preventing cardiovascular and renal events.[3] Direct blockade of highly substrate-specific renin activity attenuates the formation of Ang I and Ang II, the principal mediator of the RAAS, even at elevated circulating plasma renin levels due to negative feedback mediated release from the kidney.[4,5] The attractiveness of the aspartyl protease renin as an advantageous target[6,7] with the potential to provide more effective suppression of the RAAS has stimulated vast efforts to develop small molecule, orally efficacious renin inhibitors.[8]

We have discovered recently several series of potent, highly soluble, and orally active non-peptide chemotype renin inhibitors, such as **1** and **2** (Fig. 1).[9] These transition-state mimetic (TSM) inhibitors emerged from an unprecedented topological structure-based design approach incorporating a direct spacer link of the P_1 and P_3 moieties and concomitant removal of the P_2–P_4 backbone of classical peptide-like renin inhibitors.[10–13] Aliskiren (**2**) is a highly potent selective inhibitor of human renin demonstrating sustained blood pressure lowering efficacy in vivo upon once-daily oral dosing.[12] It is the first direct renin inhibitor to be marketed as a new therapy for hypertension,[13] a hallmark of more than three decades of intense research efforts worldwide.

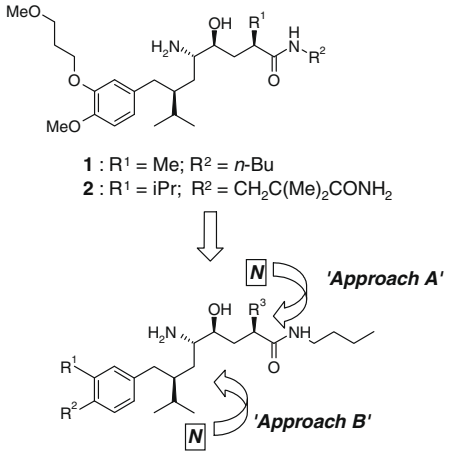

Figure 1. Renin inhibitors **1** and **2**, and 'simplification' strategies A and B by *N* versus chiral *CH** replacements at the P_1 and/or P_1' positions.

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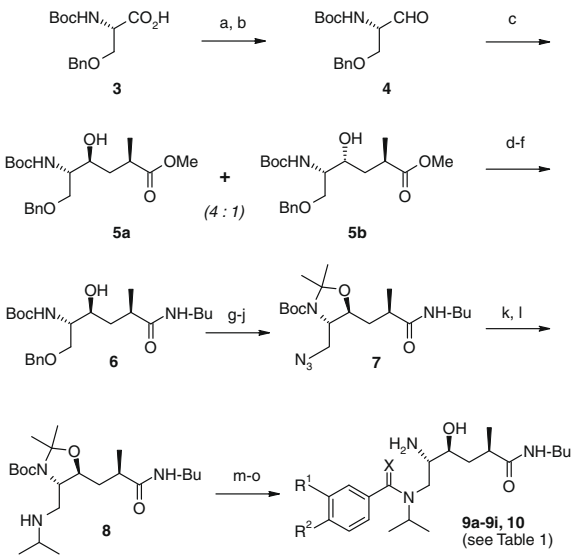
Our strategy during initial lead optimization of **1** and related analogues was to reduce the chemical complexity of these novel chemotype renin inhibitors by 'simplifying' structural elements of the key TSM pharmacophore. Computational modeling based on the X-ray structure of inhibitor **1** bound to the active site of recombinant human renin (rh-renin)[9,10] suggested replacement of the chirality center either at P_1' ('reversed carboxamides', approach A; Fig. 1),[14] or at P_1 and introduction of a functional spacer group to tether the substituted phenyl moiety (approach B) to be viable concepts.

We report herein our results on establishing a new series of non-peptide renin inhibitors based on the *N*-isopropyl benzamide P_1–P_3 motif and the corresponding *N*-isopropyl-*N*-benzylamine analogues (approach B). A divergent chemistry strategy provided ready access to a variety of analogues, starting from advanced versatilely protected amine TSM intermediates. This tactic afforded the opportunity for broad SAR investigation addressing structural modifications of the P_3–P_1 pharmacophore as well as the P_3^{sp} side chain.

The stereoselective synthesis of the key intermediate (2*R*,4*S*,5*S*)-**8** bearing a P_1' Me at the hydroxyethylene TSM portion is depicted in Scheme 1. Starting from commercial *O*Bn-protected *N*-BOC-serine **3**, aldehyde **4** was obtained in >85% yield over 2 steps with >95% ee (by chiral HPLC of the corresponding alcohol, obtained via $LiBH_4$/THF reduction, on Chiralcel OJ with *n*-hexane-isopropanol 96:4). Adaptation of the chiral homo-enolate addition to amino acid derived aldehydes, as reported by DeCamp et al.,[15] provided the hydroxyethylene moiety in an efficient and reliable manner on a multi-gram scale. Thus, metallation of 3-iodo-2(*R*)-methyl-propionic acid methyl ester with Zn(Cu)/Cl_3Ti(OiPr) and subsequent reaction (4 equiv of the homo-enolate species in excess) with **4** furnished the γ-hydroxy esters 4*S*-**5a**/4*R*-**5b** (4:1 ratio) in 50–60% overall yield.[16] Further transformation to the respective lactones under acidic conditions was followed by direct aminolysis with *n*-butylamine at 80 °C without detectable epimerization at C2. The desired 4(*S*)-**6** (mp 105 °C)[17] was isolated in high diastereomeric purity (>17:1 by RP-HPLC) after single crystallization from *n*-hexane–$CHCl_3$ in 40% yield from aldehyde **4**.



Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF, 70 °C, 95%; (b) DIBAL, toluene, −78 °C, >90%; (c) 3-iodo-2(*R*)-methyl-propionic acid methyl ester, Zn(Cu), $TiCl_4$–Ti(OiPr)$_4$, *N*,*N*-dimethyl acetamide, toluene, −20 °C; (d) AcOH, toluene, reflux. (e) *n*-$BuNH_2$, 80 °C; (f) recrystallisation from *n*-hexane–$CHCl_3$, 40% (4 steps from **3**); (g) 2-methoxypropene, cat. TsOH, CH_2Cl_2, rt, 97%. (h) H_2, cat. Pd/C 10%, MeOH; (i) MsCl, Et_3N, CH_2Cl_2 0 °C; (j) NaN_3, DMPU, 40 °C, 95% (3 steps); (k) H_2, cat. Pd/C 10%, MeOH; (l) acetone, $NaBH_3CN$, rt, >90% (2 steps); (m) 4-methoxy-3-(3-methoxy-propoxy)-benzaldehyde, Ti(OiPr)$_4$, $NaBH_3CN$, MeOH, 40%; or amide coupling to 3,4-substituted benzoic acids using HBTU, Et_3N, MeCN; (n) cat. TsOH, MeOH, rt; (o) 4 N HCl, dioxane, −20 °C; followed by lyophilisation.

Intermediate **6** was then subjected to *N*,*O*-acetal formation (97%), followed by hydrogenolysis of the benzylether, mesylate formation, and finally displacement with NaN_3 in DMPU to afford azide **7** in 83% (3 steps). Azide reduction and reductive amination with acetone/$NaBH_3CN$ yielded the secondary amine **8** in good overall yield. Inhibitors **9a**–**i** bearing a *tertiary N*-isopropyl-benzamide group at the P_1 position, as listed in Table 1, were prepared via the activated esters from the corresponding benzoic acids[18] using HBTU.[19] Removal of the *N*,*O*-acetal protecting group with cat. TsOH in MeOH proceeded uneventfully and in good isolated yields (>80%). *N*-BOC deprotection with 4 N HCl–dioxane was conducted at −20 °C in order to avoid formation of the dihydro-1,3-imidazole **37** as a major side product (Fig. 2, vide infra) affording the inhibitors as their mono-HCl salts after lyophilisation. Inhibitor **10** (di-HCl salt) bearing an *N*-isopropyl substituted tertiary amine was obtained by reductive alkylation of **8** with 4-methoxy-3-(3-methoxy-propoxy)-benzaldehyde[20] in the presence of a Lewis acid ($NaBH_3CN$, Ti(OiPr)$_4$; 40% yield), followed by two-step *N*,*O*-deprotection.

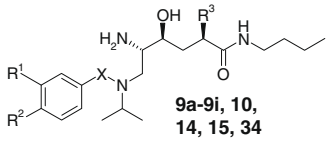
The sulfonamide analogue **14** was prepared according to Scheme 2. In brief, chlorosulfonylation of anisaldehyde **11** rendered **12** in high yield, which then was coupled to secondary amine **8** in the presence of DIEA as base. Baeyer–Villiger oxidation with *m*-chloro-perbenzoic acid and subsequent hydrolysis afforded intermediate **13,** which was O-alkylated to introduce the P_3^{sp} methoxypropoxy side chain. Final stepwise *N*,*O*-deprotection afforded **14** in good overall yield. **15** was obtained in a similar fashion via coupling of *p*-OMe-phenyl-sulfonylchloride to **8** (82% yield).

The synthesis of 3,4-disubstituted benzoic acids **18**, **20**, and **23–25** with a modified P_3 moiety was achieved according to standard protocols from readily available starting materials (Scheme 3).[18]

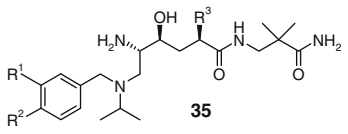
The synthesis of the *O*-silyl protected TSM intermediate (2*S*,4*S*,5*S*)-**33** with isopropyl at the P_1' position is described in Scheme 4. Thus, Evans alkylation of the lithium enolate derived from (*S*)-**27** with (*E*)-4-bromo-but-2-enyloxymethyl)-benzene[21] gave **28** in 77% yield with >95% de. Bromolactonization of **28** provided *trans*-**29** in high yield as the only isolated isomer. Displacement of the bromine in **29** under non-optimized reaction conditions afforded azide **30** (>97% ee)[22] in 30% isolated yield together with the elimination side product **31** (60%). The relative stereochemistry of lactones **29** and **30** was determined by ^1H and ^{13}C NMR as reported previously.[23] Azide **30** was then converted to the *N*-BOC protected lactone **32** by selective hydrogenation in the presence of $(BOC)_2O$ followed by *O*-debenzylation, mesylation, and final azide substitution. Inhibitor **34** was prepared from **33** by the reaction sequence analogous to the route described for **9a** in Scheme 1. Inhibitor **35** bearing a tertiary amine at P_1 was obtained as described in Scheme 4.

Structure–activity relationship (SAR) data based on IC_{50}s for inhibition of rh-renin, as well as of endogenous renin in human plasma, is summarized for a set of representative inhibitors in Table 1. We were gratified to find the in vitro potency of the P_1 *N*-isopropyl benzamide **9a** to be comparable to that of the corresponding P_1 carba-analogue **1** in the purified renin assay, albeit enzyme affinity of **9a** dropped fivefold in the plasma assay as compared to **1**. Extending previous SAR[10,24] by modification of the P_3^{sp} side chain revealed that the phenol ether oxygen of **9a** is not important for binding interactions to purified renin (**9b** to **9d**). The ether link in **9a** was replaced by CH_2 to afford an in vitro highly potent inhibitor of rh-renin, **9b**, which however was 15-fold less active in the presence of plasma.[25] A similar potency loss in the plasma assay using angiotensinogen as the endogenous substrate has been observed in several other non-peptide

Table 1
In vitro enzyme inhibition data for human renin



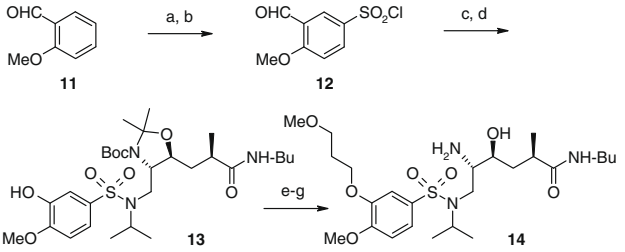
9a-9i, 10, 14, 15, 34



35

No.	X	R^1	R^2	R^3	IC_{50} (nM), purified[a]	IC_{50} (nM), plasma[b]
1					1	1
2					0.6	0.6
9a	CO	$O(CH_2)_3OMe$	OMe	Me	2	5
9b	CO	$(CH_2)_4OMe$	OMe	Me	1	15
9c[d]	CO	$CH{=}CH(CH_2)_2OMe$	OMe	Me	1	4
9d	CO	CH_2NHCO_2Me	OMe	Me	3	2
9e	CO	$(CH_2)_2NHCO_2Me$	OMe	Me	100	nd[c]
9f	CO	$O(CH_2)_3OMe$	Me	Me	8	13
9g	CO	$O(CH_2)_3OMe$	Et	Me	2	7
9h	CO	$O(CH_2)_3OMe$	Br	Me	8	21
9i	CO	$O(CH_2)_3OMe$	CCH	Me	6	14
34	CO	$O(CH_2)_3OMe$	Et	*i*Pr	1	10
14	SO_2	$O(CH_2)_3OMe$	OMe	Me	300	nd[c]
15	SO_2	H	OMe	Me	>100,000	nd[c]
10	CH_2	$O(CH_2)_3OMe$	OMe	Me	13	14
35		$O(CH_2)_3OMe$	OMe	iPr	7	7

[a] Purified rh-renin assay at pH 7.4.[11]
[b] Inhibition of endogenous renin in plasma from human.[11]
[c] Not determined.
[d] Stereoisomer ratio *Z*:*E* = 10:1. All compounds showed similar IC_{50}s against marmoset plasma renin, with high selectivity to bovine cathepsin D and porcine pepsin (IC_{50} >100 μM). **9a**, **9g**, **9h**, **10** had IC_{50} >20 μM for rat renin (for **35**, IC_{50} = 1.4 μM). Calculated log *P* for **9a**: 2.74; **9b**: 3.60; **9g**: 4.08; **34**: 5.00; **35**: 2.94.



Scheme 2. Reagents and conditions: (a) $HC(OEt)_3$, NH_4Cl, EtOH, reflux, 90%. (b) $ClSO_3H$, 3 h at −5 °C, then 2 h at rt, 80%; (c) **8**, DIEA, CH_2Cl_2, 0 °C, 16 h, 72%; (d) MCPBA, $CHCl_3$, 48 h, rt; then Et_3N, MeOH, rt, 2 d, 55%. (e) $MeO(CH_2)_3I$, K_2CO_3, MeCN, reflux, 18 h, 82%. (f) CSA, MeOH, rt, 89%; (g) 4 N HCl–dioxane, 0 °C, 2 h, 82%.

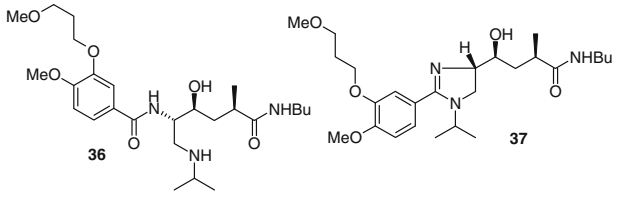
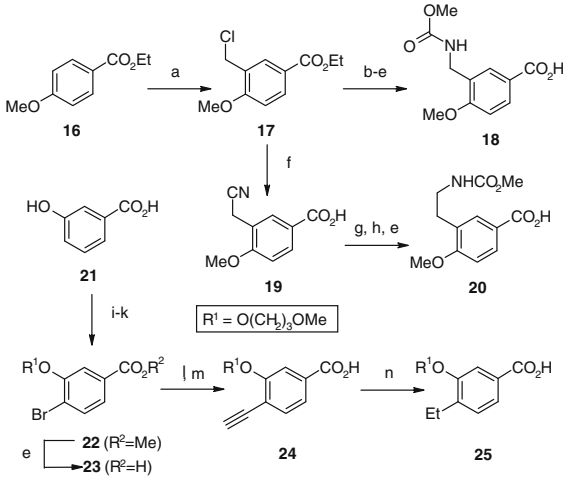


Figure 2. Decomposition products of inhibitor **9a**.

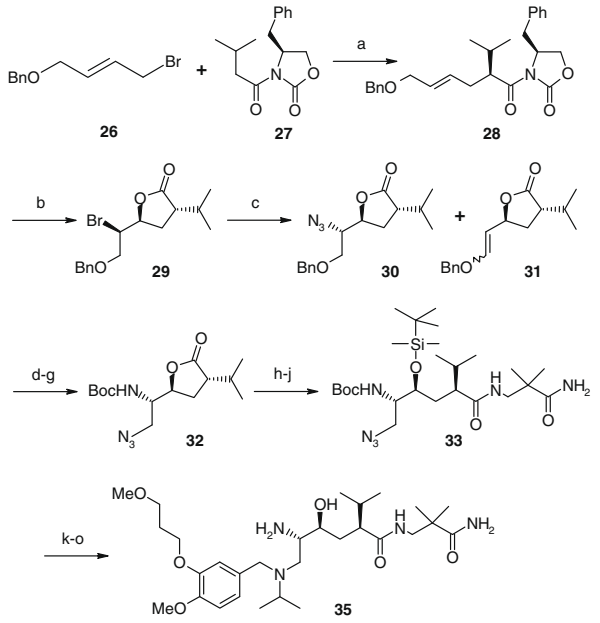


Scheme 3. Reagents and conditions: (a) $(CHO)_n$, HCl, $CHCl_3$, rt, 86%; (b) NaN_3, KI, DMPU, 100 °C, 81%; (c) Ph_3P, THF; then H_2O, 91%; (d) MeOCOCl, DIEA, dioxane, 60%; (e) 1 N NaOH, MeOH; (f) NaCN, KI, MeCN, 100 °C, 76%; (g) H_2, RaNi, 79%; (h) MeOCOCl, CH_2Cl_2, 0 °C to rt, 4 h, 87%; (i) Br_2, AcOH, rt, 12 h, 15%; (j) H_2SO_4, MeOH, reflux, 18 h, 95%; (k) $MeO(CH_2)_3I$, K_2CO_3, reflux, 16 h, 98%; (l) trimethylsilylacetylene, cat. $Cl_2Pd(PPh_3)_2$, CuI, Et_3N, rt, 15 h, 100%; (m) 1 N KOH, MeOH, 97%; (n) H_2, cat. Pd/C 10%, EtOH, 77%.

compound series upon minor structural modifications leading to a subtle increase in overall hydrophobicity of the inhibitors.[10,11,26]

A methyl carbamate group tethered to the phenyl by a methylene spacer provided highly active **9d** with comparable enzyme affinities in both in vitro assays. Both the NH and the MeO oxygen of the carbamate were found in reasonable H-bonding distance to the Ser219 side chain OH according to modeling. In contrast, the homologous carbamate **9e** with a two-carbon tether was only weakly active against rh-renin.

Next, various small hydrophobic residues such as alkyl and halogen were investigated as replacements of the *para*-OMe group which interacts with the S_3 site of renin (Table 1, **9f–i**). The P_3 ethyl derivative **9g** was equipotent to **9a** in both assays, whereas a three- to four -fold increase in IC_{50}s was observed for **9f**, **9h**, and **9i**. Inhibitor **34** with a P_1' isopropyl substituted hydroxyethylene isostere showed similar in vitro activity as the P_1' Me analogue **9g**. The relative increase in IC_{50} of **9g** in presence of plasma was surprisingly



Scheme 4. Reagents and conditions: (a) $LiN(TMS)_2$, THF, −78 °C, 77%; (b) NBS, 1:1 DME–H_2O, 0 °C to rt, 15 h, 74%; (c) NaN_3, DMPU, 40 °C, 18 h; **30** (30%) and **31** (60%); (d) H_2, cat. Pd/C 10%, BOC_2O, EtOAc, 82%; (e) H_2, cat. Pd/C 10%. (f) MsCl, Et_3N, CH_2Cl_2; (g) NaN_3, DMPU, 60 °C, 20 h, 95% (3 steps from **30**); (h) 1 M LiOH, 2:1 DME–H_2O, rt, 3 h; (i) TBDMSiCl, imidazole, DMF, >80% (2 steps); (j) HBTU, Et_3N, $H_2NCH_2C(CH_3)_2CONH_2$, DMF, 89%. (k) H_2, cat. Pd/C 10%, EtOAc; (l) acetone, $NaBH_3CN$, 4 Å MS, MeOH, 74%. (2 steps); (m) 4-methoxy-3-(3-methoxy-propoxy)-benzylbromide[18], K_2CO_3, DMF, 50%; (n) Bu_4NF, THF, rt, 63%; (o) 4 N HCl–dioxane, rt, 90%.

more pronounced, in contrast to the SAR observed for the inhibitor **1** series.[10]

The sulfonamide P_3–P_1 spacer was explored with the aim to identify potent inhibitors with improved physicochemical properties including crystallinity and chemical stability in plasma (vide infra). Compound **14** with an IC_{50} of 0.3 μM towards rh-renin was found to be greater than 100 times less potent compared to the carboxamide analogue **9a** (Table 1). Compound **15** lacking the P_3^{sp} side chain did not show significant enzyme affinity up to 100 μM. These results confirm previous findings,[24,27] that binding interactions to the contiguous S_3–S_1 hydrophobic pocket of renin alone are not sufficient for strong enzyme affinity, and further suggest the methoxypropoxy group of inhibitor **14** to interact with the non-substrate P_3^{sp} binding site. Computational docking of **14** into the renin active site predicted a similar binding pose in an energy-minimized conformation as was observed by X-ray for inhibitor **1** complexed to rh-renin.[9,10] The modeling pose suggested a H-bond of a sulfonyl oxygen to the flap Thr_{77} NH, but also an unfavorable interaction of the sulfonyl with Gly_{217}. This could explain at least in part the marked drop in potency of **14** versus **9a**.

Interestingly, the 3,4-dialkoxybenzyl substituted tertiary amine **10** showed high potency with similar affinities to recombinant and plasma renin (Table 1).[28] Compared to benzamide **9a**, compound **10** was only threefold less active in vitro in the physiologically more relevant plasma renin assay (IC_{50}s of 14 vs 5 nM, respectively). The increased potency of **9a** over **10** was considered to be due to a H-bond interaction of the spacer carbonyl to Thr_{77} of the flap domain in its closed conformation. Other contributing factors could be a potential intramolecular H-bond favoring a solution conformation of **9a** similar to its enzyme-bound conformation, or different desolvatation energies for **9a** and **10** required for binding into the solvent-shielded hydrophobic P_3–P_1 site of the catalytic cleft. To the best of our knowledge, **10** is the first reported low nanomolar inhibitor of renin, which incorporates a basic amine at the P_1 position. This encouraging result prompted us, subsequent to the discovery of the highly potent development candidate **2** bearing an optimized P_2' carboxamide moiety[11], to explore its corresponding basic P_1 analogue **35**.[28] We envisaged that the P_1' isopropyl group of the dipeptide TSM, replacing the smaller Me group in **10**, would improve the in vitro enzyme inhibition, and in particular oral in vivo potency, as demonstrated previously for the lead series.[11] Indeed inhibitor **35** showed strong inhibition in both human renin in vitro assays with IC_{50}s of 7 nM. However, **35** was only equipotent to the P_1' Me analogue towards plasma renin, and more than 10-fold less active as compared to **2** (Table 1). The significant reduction in the potency of **35** limited its potential attractiveness for more extensive investigations.

We had established a primate in vivo pharmacology model in telemetered sodium-depleted marmosets (*Callthrix jacchus*) for non-invasive measurement of blood pressure (BP), heart rate, and motor activity in freely moving animals to study oral efficacy of renin inhibitors and other RAS blocking agents.[29] Inhibitor **9a**, administered orally in saline solution at 3 mg/kg to normotensive marmosets pre-treated with furosemide ($N = 5$; 6 mg/kg/d for 1 week), caused a significant change in mean arterial pressure (MAP), compared to vehicle-treated animals, with a rapid onset of action and paralleled by partial inhibition of plasma renin activity (PRA). The maximal depressor effect ΔMAP of −18 mmHg observed 90 min post-dose was similar to ΔMAP_{max} of inhibitor **1** at the 3 mg/kg po dose in this model. However, and in marked contrast to the sustained BP lowering potency of **1** over up to 8 h,[10] **9a** had only short-lasting effects, with complete recovery to baseline after 4 h post-dose. Total peak plasma exposure was found to be low (0.07 μmol/L at 1.5 h post-dose). The inferior potency of **9a** in vivo suggested a poor pharmacokinetics profile due to low oral absorption and/or rapid clearance in marmosets.

The chemical stability of inhibitors **9a** and **9g** in aqueous phosphate or Tris buffer solutions at pH 6.0, 7.4, and 8.0 was investigated. Both compounds showed significant decomposition up to 30% and 12%, respectively, after 24 h at 37 °C. A trans-acylation reaction to form the secondary amide **36** (Fig. 2) was found to be the major decomposition pathway under weakly acidic conditions, whereas in neutral or slightly basic solutions the formation of the dihydro-1,3-imidazole derivative **37**[30] was observed in addition. In DMSO at 100 °C, **9a** was converted exclusively into **36**[31] within 1 h. **36** (rh-renin $IC_{50} = 0.67$ μM) was stable in aqueous buffers over a broad pH range. Additional studies are required to demonstrate whether the limited stability of **9a** in physiological buffer may at least partially contribute to the short duration of action in vivo in marmosets.

In summary, the synthesis, the chemical stability and in vitro SAR for inhibition of human renin has been explored for a novel series of 'symmetrized' hydroxyethylene dipeptide mimetics. From this work, the first low nanomolar renin inhibitors **10** and **35** with a basic tertiary amine at the P_1 position are described. The (P_3–P_1)-benzamide analogue **9a** showed potent but transient blood pressure lowering effects in Na-depleted marmosets. In subsequent studies as part of our continued renin inhibitor program, the *N*-isopropyl benzamide (P_3–P_1)-pharmacophore, as in **9a**, has been combined with a pyrrolidine-based center scaffold as TSM surrogate to afford highly potent candidate inhibitors with promising in vivo activity profiles.[8,18] A full account of this more recent work will be disclosed in due course.

Acknowledgments

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16. *Typical procedure for the preparation of* **5a**/**5b**: To a suspension of Zn(Cu) (43.3 g) in toluene (130 mL) was added a solution of 3-iodo-2(*R*)-methyl-propionic acid methyl ester (92.5 g, 0.41 mol) in *N*,*N*-dimethyl acetamide (69 mL) and toluene (560 mL) at rt over 10 min. The suspension was stirred at rt for 1 h and then refluxed for 5 h. To a solution of $Ti(OiPr)_4$ (30.1 mL) in toluene (105 mL) and CH_2Cl_2 (530 mL) was added $TiCl_4$ (33.3 mL) at <25 °C. The solution was stirred at rt for 15 min, followed by addition of the Zn-homoenolate solution while keeping the temperature below −20 °C. Then, a solution of **4** (26.4 g, 0.09 mol) in CH_2Cl_2 (130 mL) was added below −35 °C, and the mixture was stirred at −20 °C for 2 days. Standard workup afforded a 4:1 mixture of **5a**/**5b** (27.7 g, 77%) as colorless oil.
17. *Analytical data for 4*(*S*)-**6**: TLC R_f 0.33 (hexane/EtOAc 1:1); mp 105 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.65 (t, J = 8 Hz, 1H), 7.36–7.27 (m, 5H), 6.29 (d, J = 12 Hz, 1H), 4.46 (s, 2H), 3.58–3.48 (m, 3H), 3.05–3.00 (m, 2H), 2.47–2.44 (m, 1H), 1.66–1.60 (m, 1H), 1.39 (s, 9H), 1.36–1.20 (m, 6H), 0.99 (d, J = 8 Hz, 3H), 0.85 (t, J = 8 Hz, 3H). HRMS $[M+H]^+$ calcd 423.2854; found 423.2853.
18. For the preparation of other 3,4-disubstituted benzoic acid intermediates, see: Ehrhardt, C.; Irie, O.; Lorthiois, E. L. J.; Maibaum, J. K.; Ostermann, N.; Sellner, H. PCT Int. Appl. WO2006/100036(A1); *Chem. Abstr.* **2006**, *145*, 377192.
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21. Bromide **26** was prepared from (E)-but-2-ene-1,4-diol by mono-benzylation (NaH, BnBr, DMF, −20 °C, 35%) and bromination (NBS, Ph_3P, CH_2Cl_2, −78 °C, 91%).
22. Enantiomeric purity of (3*S*,5*S*,1′*R*)-**30** was determined by chiral HPLC using its (3*R*,5R,1′*S*)-enantiomer prepared from (*R*)-configured **27** according to Scheme 4.
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25. The P_3^{sp} methoxybutyl side chain substituted analogue of **2** was subsequently identified as preclinical candidate with high oral potency in Na-depleted marmosets.[11]
26. Plasma IC_{50} shifts in related series could be only in part rationalized by plasma protein binding (PPB).[11] Human %PPB for **9**, **34**, **35** were not determined.
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28. A recent patent application has disclosed related renin inhibitors including compounds **10** and **35**: Miyazaki, S.; Nakamura, Y.; Nagayama, T.; Tokui, T. PTC Int. Appl. WO2007/148775(A1); *Chem. Abstr.* **2007**, *148*, 100512.
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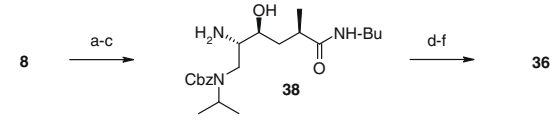
30. Derivative **37**, isolated as major side product from *N*-BOC-**9a** deprotection with 4 N HCl–dioxane at rt showed weak affinity to rh-renin (IC_{50} = 1 μM).
31. The trans-acyl decomposition product **36** was synthesized according to the following reaction scheme:



Reaction conditions: (a) Cbz–Cl, Et_3N, CH_2Cl_2, 0 °C to rt, 18 h, (72%); (b) cat. *p*-TsOH, MeOH, 58%; (c) TFA, CH_2Cl_2, 0 °C to rt, 3 h, 75%; (d) 4-methoxy-3-(3-methoxy-propoxy)-benzoic acid, HOBT, EDC, *N*-methylmorpholine, DMF, rt, 16 h, 78%; (e) H_2, cat. Pd/C, BOC_2O, MeOH, rt, 7 h, 89%. (f) 4 N HCl–dioxane, rt, 69%.