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Design and synthesis of potent macrocyclic renin inhibitors

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ABSTRACT

Two types of P1–P3-linked macrocyclic renin inhibitors containing the hydroxyethylene isostere (HE) scaffold just outside the macrocyclic ring have been synthesized. An aromatic or aliphatic substituent (P3sp) was introduced in the macrocyclic ring aiming at the S3 subpocket (S3sp) in order to optimize the potency. A 5–6-fold improvement in both the K_i and the human plasma renin activity (HPRA)IC_{50} was observed when moving from the starting linear peptidomimetic compound **1** to the most potent macrocycle **42** (K_i = 3.3 nM and HPRA IC_{50} = 7 nM). Truncation of the prime side of **42** led to 8–10-fold loss of inhibitory activity in macrocycle **43** (K_i = 34 nM and HPRA IC_{50} = 56 nM). All macrocycles were epimeric mixtures in regard to the P3sp substituent and X-ray crystallographic data of the representative renin macrocycle **43** complex showed that only the *S*-isomer buried the substituent into the S3sp. Inhibitory selectivity over cathepsin D (Cat-D) and BACE-1 was also investigated for all the macrocycles and showed that truncation of the prime side increased selectivity of inhibition in favor of renin.

The renin-angiotensin-aldosterone system (RAAS) plays a key role in the regulation of blood pressure and in the maintenance of sodium and volume homeostasis.[1] Inappropriate activation of the RAAS, as seen in disease states such as diabetes, is important in hypertension-induced cardiovascular disease and chronic kidney disease.[2,3] The aspartyl protease renin catalyzes the first and also rate-limiting cleavage of angiotensinogen in the RAAS and has long been recognized as a prominent target for antihypertensive therapy.[4] Since the 1980s a number of generations of linear peptidomimetic renin inhibitors have been developed by pharmaceutical companies,[5–7] but these compounds were hampered by high molecular weight and poor oral bioavailability. However, this development finally resulted in the linear nonpeptidomimetic aliskiren as an acceptable compromise between potency and drug-metabolism-pharmacokinetic (DMPK) properties.[8]

The linear peptidomimetic compound **1** (Fig. 1), which had earlier been synthesized and tested in our laboratory, showed potent renin inhibitory activity (K_i = 21 nM and HPRA IC_{50} = 38 nM), but characteristically suffered from high MW, low stability in human liver microsomes (HLM) (Clint ~260 μL/min/mg) and low permeability (Caco-2, p_{app} <0.4 × 10^{-6} cm/s). Furthermore, compound **1** had poor selectivity over Cat-D (K_i = 3.3 nM) and BACE-1 (IC_{50} = 30 nM). One possible way to favorably alter the DMPK properties of a flexible linear compound is to limit the number of

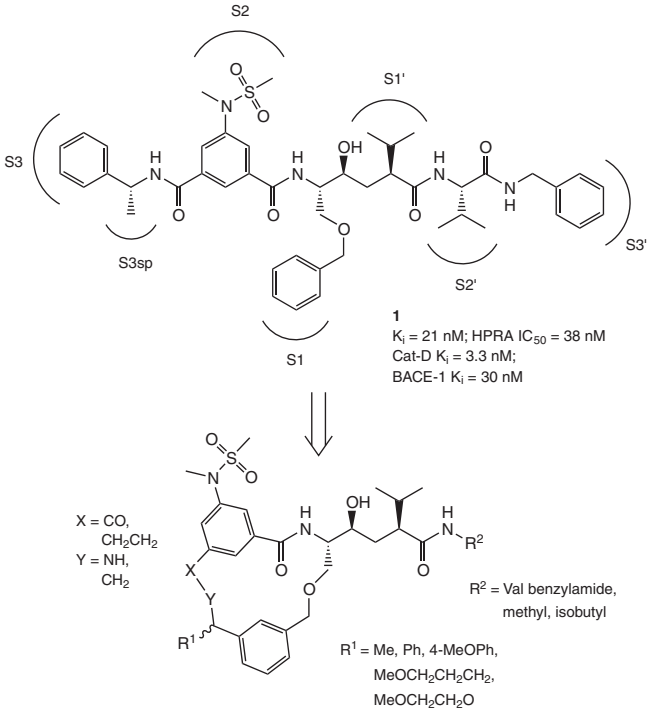

Figure 1. Development of lead compound **1** to the target macrocycles.

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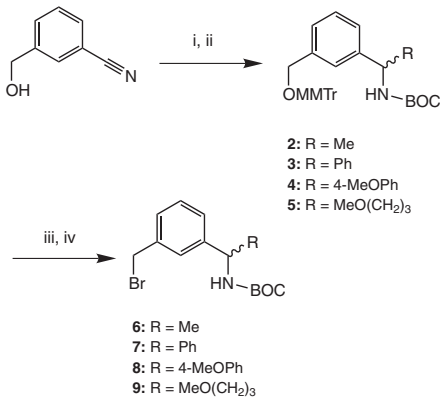
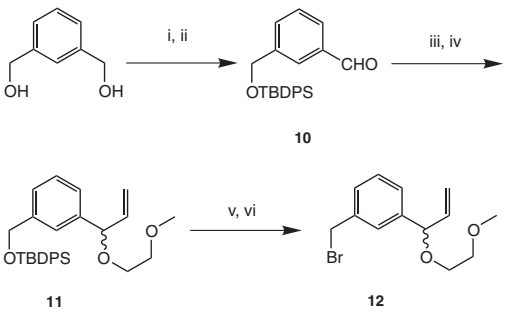
rotational bonds by rigidifying the compound.[9] This may be achieved by macrocyclization[10,11] and a number of research groups have employed various chemical strategies for macrocyclizations of linear peptidomimetic structures by bridging different P-moieties. Some common methods that have been employed are peptide coupling,[12,13] metathesis,[14–19] macrolactonization,[20] palladium coupling,[20] reductive amination[21] and [3 + 2] cycloaddition (click chemistry).[22,23]

In our present work with renin inhibitors, we decided to connect the P1 and P3 moieties in **1** (Fig. 1), either by peptide couplings or metathesis, with the intention of further improving the renin inhibitory activity and establishing selectivity over Cat-D and BACE-1 and also to alter the DMPK properties in a favorable way. We also wanted to reduce the molecular weight by truncating the prime side of the molecules. Computational modeling indicated that 15–16-membered macrocycles would have a suitable fit in the active site of renin as for **1** and that substituents at the newly formed benzylic position in the macrocycle would favorably aim into the renin S3sp. This positioning of a suitable substituent into the S3sp was earlier shown to be an important feature for excellent inhibitory activity of aliskiren against renin.[8]

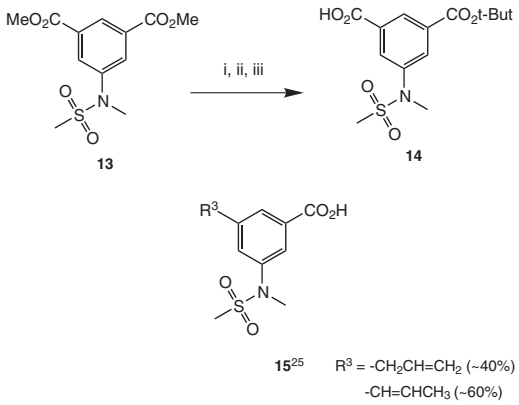
Schemes 1–3 display the synthesis of the key building blocks for the construction of the macrocycles, while Scheme 4 shows the synthesis of the final target macrocycles. For the functionalization at the 3-position of the P1 benzylic moiety, to be used for the amido linked macrocycles (**41**–**46**), 3-cyanobenzylalcohol was conveniently converted in four-steps to the O, N-protected intermediates **2** (R = Me), **3** (R = Ph) and **4** (R = 4-MeOPh), by the addition of aryl or alkyl Grignard reagents to the cyano group followed by reduction with $LiAlH_4$ and N-protection with Boc_2O (Scheme 1). These intermediates were then each treated with acid (80% acetic acid) and the generated benzylic alcohol was then converted to the corresponding bromide (Ph_3P, CBr_4) to give 3-functionalized benzylbromides **6** (R = Me), **7** (R = Ph) and **8** (R = 4-MeOPh). Similarly, 3-cyanobenzylalcohol was conveniently converted in four-steps to the O, N-protected intermediate **5** by addition of the O,C-bis-Mg reagent, followed by reduction and N-protection and methylation of the generated 3-hydroxy group by MeI and Ag_2O (Scheme 1). Acidic deprotection and bromination in the usual way gave the benzyl bromide **9**.



Scheme 1. Reagents and conditions: (i) MMTrCl, pyridine, rt, 98%; (ii) *Method A* (for **2**–**4**): RMgBr, ether, reflux, then $LiAlH_4$, THF, reflux, then Boc_2O, EtOAc, rt, 77–91% in three-steps; *Method B* (for **5**): $ClMg(CH_2)_3OMgBr$, THF, reflux, then $LiAlH_4$, THF, reflux, then Boc_2O, EtOAc, rt, 60% in three-steps; MeI, Ag_2O, reflux, 52%; (iii) 80%AcOH, rt, 92–97%; (iv) Ph_3P, CBr_4, DCM, rt, 58–89%.



Scheme 2. Reagents and conditions: (i) TBDPSiCl, DIPEA, DCM, rt, 90%; (ii) Dess-Martin periodane, DCM, rt, 79%; (iii) CH_2=CHMgBr, THF, rt, 74%; (iv) NaH, $MeOCH_2CH_2Br$, TBAI, THF, rt, 73%; (v) TBAF, THF, rt, 86%; (vi) Ph_3P, CBr_4, DCM, rt, 76%.

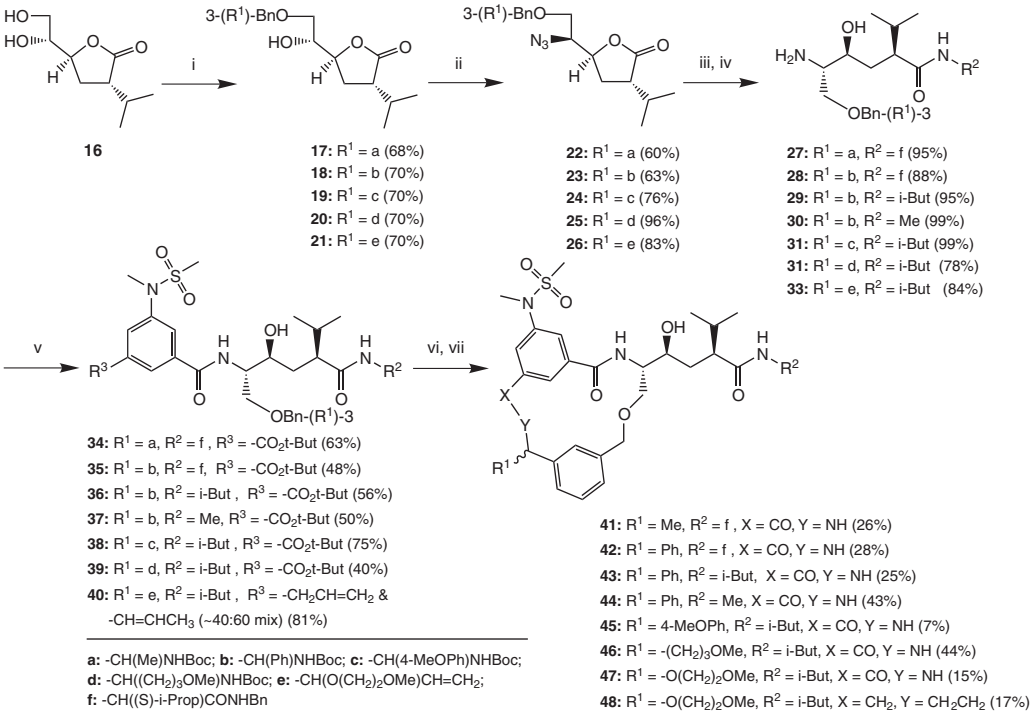


Scheme 3. Reagents and conditions: (i) NaOH, H_2O, MeOH, THF, rt, 80%; (ii) *t*-butOH, EDAC, DMAP, DMF, rt, 46%; (iii) NaOH, H_2O, MeOH, THF, rt, 85%.

For the 3-functionalisation of the P1 benzylic moiety, to be used for macrocyclization through metathesis (macrocycles **47, 48**), 1,3-bis-hydroxymethylbenzene was monosilylated and then oxidized to the aldehyde **10**, which was reacted with vinyl magnesium bromide, followed by alkylation of the resulting secondary alcohol with 2-methoxyethyl bromide to give intermediate **11**. This compound was deprotected by fluoride ion and the resulting benzylic alcohol was brominated in the usual way to give the 3-functionalized benzyl bromide **12** (Scheme 2).

The benzoic acid moiety harboring the P2 sulfonamide group needed to be modified for engagement in macrocyclization (Scheme 3). The methylsulfonamido dimethylester **13**, which was prepared according to published procedure,[24] was converted in three-steps (33%) to the mono *t*-butylester mono carboxylic acid derivative **14**, which was used for macrocyclizations through peptide couplings giving the amido linked macrocycles **41**–**46**. The benzoic acid derivative **15**[25] (Scheme 3) provided the olefinic handle in the intermediate **40** necessary for the metathesis reaction leading to the macrocyclic compounds **47** and **48**.

The synthesis of macrocycles **41**–**48** is depicted in Scheme 4. Starting by regioselective monobenzylations of the chirally pure dihydroxylactone **16**[26] by the 3-functionalized benzyl bromides **6**–**9** and **12** gave the benzylethers **17**–**21** in 59–70% yields. The secondary hydroxyl group was converted into the azido group with inverted configuration either by triflation followed by reaction with NaN_3 (*Method A*, worked for **17**–**19** but sluggishly for **20** and **21**) or by a Mitsunobu type reaction (*Method B*, for **20**, **21**) with yields of all azides **22**–**26** in the range of 60–96%. The lactone function of these compounds was opened either by a reaction with (2S)-2-amino-*N*-benzyl-3-methyl-butanamide, with isobutyl amine or methyl amine to give the open chained azido alcohols (57–95% yields), which were reduced to the corresponding amino alcohols, either by hydrogenation with Lindlar catalyst to give **27** (95%), **28** (88%), **29** (95%), **30** (99%), **31** (99%) and **32** (78%), or by triphenylphosphine to give **33** (84%). These crude amino alcohols



Scheme 4. Reagents and conditions: (i) **6**–**9**,**12**, Bu_2SnO, toluene, reflux; (ii) *Method A* (for **17**–**19**): Tf_2O, pyridine, DCM, 0 °C, then NaN_3, DMF, 70 °C; *Method B* (for **20**,**21**): Ph_3P, DIAD, DPPA, THF, 0 °C–rt; (iii) *Method A* (for $NH_2CH((S)$-isopr)CONHBn): 2-hydroxypyridine, DIPEA, DMF, 70 °C, (57–63%); *Method B* (for isobutylamine): neat, 70 °C, (57–95%); *Method C* (for methylamine): 40% aq$MeNH_2$, 70 °C, (84%); (iv) *Method A* (for azides derived from **22**–**25**): $Pd/CaCO_3$, H_2 (1 atm), EtOH, rt; *Method B* (for azide derived from **26**): Ph_3P, MeOH, H_2O, rt; (v) **14** or **15**, DCC, pentafluorphenol, rt; (vi) *Method A* (for **34**–**37**, **39**): TFA, DCM, rt, (crude); *Method B* (for **38**): 100% HCO_2H, dioxane, rt, (crude); (vii) *Method A* (for **34**–**39**): HATU, DIPEA, DMF, rt, 0.001 M substr. concn; *Method B* (for **40**): H–G-II catalyst, DCE, reflux, then H_2 (1 atm), Pd/C, EtOH, rt.

were then coupled with the benzoic acid derivative **14** (for **27**–**32**) or with benzoic acid derivative **15** (for **33**), through a first in situ activation of the carboxylic acid function with DCC and pentafluorphenol followed by mixing the resulting pentafluorphenylester with the respective amino compounds (**27**–**33**), to give the respective benzamido derivatives **34** (63%), **35** (48%), **36** (56%), **37** (50%), **38** (75%), **39** (40%) and **40** (81%). The Boc and the *t*-butyl groups were then removed, either with trifluoracetic acid (TFA) (**34**–**37**, **39**) or with 100% formic acid (HCO_2H) (**38**) and the crude amino acids obtained after evaporations were each dissolved in DMF to high dilution (0.001 M) and treated with the coupling agent HATU to give after HPLC purification macrocyclic compounds **41** (26%), **42** (28%), **43** (25%), **44** (43%), **45** (7%) and **46** (44%). Intermediate **40** was actually a mixture of two different propene isomers (allyl and 1-propenyl) and was subjected to metathesis reaction with Hoveyda–Grubbs second generation catalyst (H–G-II catalyst) under high dilution conditions to give a corresponding mixture of the unsaturated macrocyclic intermediates. This mixture was subjected to hydrogenation on palladium charcoal to give the final macrocycles **47** (15%, 15-membered) and **48** (17%, 16-membered), after HPLC separation.

All synthesized inhibitors were screened against renin, Cat-D and BACE-1 to determine the respective K_i or IC_{50} values (Table 1, Fig. 3). The macrocycles were all 15-membered, except for **48**, which was 16-membered. The macrocycles were epimeric mixtures at the position of the P3sp substituent in the ring suggesting that the renin potencies reported herein could be improved with diastereomerically pure compounds having the correct stereochemistry for fit into the S3sp. Attempts were made to chromatographically separate the diastereomers, both among the intermediates **34**–**40** and the final compounds **41**–**48**, but these attempts were unsuccessful. However, X-ray crystallography data from the renin macrocycle **43** complex with 2.0 Å resolution showed that only the isomer of **43** with *S* stereochemistry at the benzylic position turned the phenyl group into the S3sp (PDB-code: 3OWN, Fig. 2). We consider this orientation of the P3sp group to be a contributing factor for the renin potency seen in most of these macrocycles. This is balanced against binding interactions

Table 1
Renin, cathepsin D and BACE-1 inhibition data. (Fig. 3)

Compd	X	Y	R^1	R^2	Renin K_i[a] (nM)	Cathepsin D K_i (nM)	BACE-1 IC_{50} (nM)
1					21	3.3	30
41	C=O	NH	Me	ValBnamide	94	13	50
42	C=O	NH	Ph	ValBnamide	3.3 (7.0)	0.75	240
44	C=O	NH	Ph	Methyl	340	1700	>10000
43	C=O	NH	Ph	Isobutyl	34 (56)	200	3700
45	C=O	NH	4-MeOPh	Isobutyl	130	760	3100
46	C=O	NH	$MeO(CH_2)_3$	Isobutyl	450	460	1900
48	(CH_2CH_2)	CH_2	$MeO(CH_2)_2O$	Isobutyl	940	>5000	>10000
47	CH_2	CH_2	$MeO(CH_2)_2O$	Isobutyl	>5000	>5000	>10000

[a] In brackets: HPRA IC_{50} (nM).

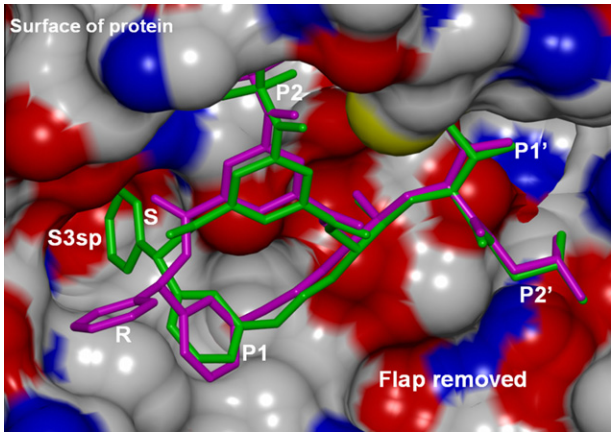


Figure 2. Superimposed X-ray structures of the **R** (magenta) and **S** (green) isomers of **43** bound to renin. 2.0 Å resolution. (PDB-code: 3OWN).

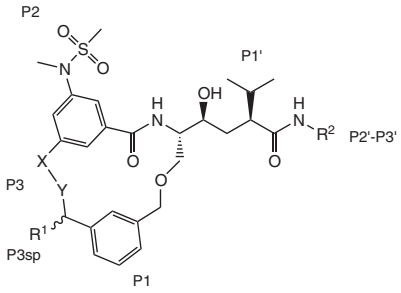


Figure 3.

in other parts of the ligand-active site complex, giving thus the net potency reflected in the measured K_i values. We find it plausible that this *S*-orientation seen for **43** is also important for activity for the rest of the macrocycles in this series.

Another explanation of the difference in activity between **41** and **42** (Table 1, Fig. 3) is that the possibility that the methyl group is not spacious enough for filling the S3sp, but the phenyl group is. Unfortunately, also the Cat-D activity followed the same trend, **42** having the highest potency towards this anti-target (K_i = 0.75 nM). Enzymes of this class are known to have flexibility in the S3 region and the sub pocket of Cat-D would be expected to expand in a similar manner to that seen in BACE-1 and renin when challenged with the larger P3sp groups, such as a phenyl. These enzymes have differences in subsites other than S3 and S3sp, which could influence the overall binding of our macrocycles and the selectivity ratios observed. Furthermore, since isomeric mixtures were tested herein, some of the activity may be attributed to the isomer which does not have the R^1 group in the S3sp.

In order to address the issues with selectivity and permeability some prime side truncated compounds were prepared. A fivefold selectivity over Cat-D was observed in **44** (renin K_i = 340 nM; Cat-D K_i = 1700 nM), where the prime side Val-benzyl amide has been replaced by a methyl group, but with a 100-fold penalty on renin potency compared to **42** (Table 1, Fig. 3). The corresponding penalty for Cat-D was over 2000-fold, indicating that ligand binding on the prime side is particularly crucial for Cat-D in this series of ligands. By replacing the NH–methyl group with the more lipophilic NH–isobutyl group, which may have the possibility to reach into the S2′ pocket, both the renin and Cat-D potencies were recovered with about 10-fold improvement compared to **44** (**43**, renin K_i = 34 nM; Cat-D K_i = 200 nM) and with marginally higher renin/Cat-D selectivity ratio.

During this investigation we observed that truncation of the prime side significantly weakened the BACE-1 activity, and in some cases abolished it (Table 1, Fig. 3). Interestingly, the renin/BACE-1 selectivity ratio decreases **43** → **45** → **46**, which seems to hint at BACE-1 being more prone to accept straight chain aliphatic linkers into its S3sp, as part of the explanation for improved binding to BACE-1, while the opposite holds true for renin.

The second class of macrocycles represented by **47** and **48** (Table 1, Fig. 3) had only poor or no inhibitory activity against renin, Cat-D and BACE-1. The more flexible macrocycle **48** still allowed for sub micromolar potency in case of renin. As shown in **47** and **48**, increased lipophilicity in the macrocyclic ring, facing the S3 region, together with limited interactions on the prime side, was not well tolerated by any of these three aspartyl proteases.

In terms of DMPK a modest twofold improvement of the HLM stability was observed for **42** (Clint ~110 μL/min/mg) over **1** (Clint ~260 μL/min/mg) and the permeability was still low despite a decrease in rotational bonds (**42**, Caco-2, p_{app} <0.4 × 10^{-6} cm/s). The permeability value for the prime side truncated **43** remained basically the same as for **42** despite the decrease in molecular weight.

In order to gain some insights into the P-glycoprotein (Pgp)-efflux of these macrocycles, permeability assays with the MDCK wild type (wt) and human Pgp-enriched MDCK cells (MDR-1)[27] were performed on the representative **42** and **43** and the BA/AB ratios were assessed. Unfortunately, both MDCK cell types generated p_{app}-values in the lower range of 0.1 − 2.0 × 10^{-6} cm/s. This is in line with the results from the earlier Caco-2 assays and indicates that these macrocycles have too low intrinsic permeability to be able to generate reliable BA/AB ratios from both MDCK cell types (**42**, wt = 2.3, MDR-1 = 12.0; **43**, wt = 2.0, MDR-1 = 10.0). However, judging from these ratios, macrocycles **42** and **43** may be Pgp-substrates. For comparison, analogous MDCK assays were also run on aliskiren as a reference (BA/AB ratio: wt = 0.7; MDR-1 = 1.5). This compound gave also very low p_{app}-values (<1), thus again raising concern about the reliability of the BA/AB ratios. Nevertheless, the small difference we obtained between the wt and MDR-1 BA/AB ratios might indicate that aliskiren is not a Pgp-substrate, which would be in contrast to earlier work indicating that aliskiren is a Pgp-substrate, based on in vitro Pgp ATPase activity assays[28].

The described synthetic route provided two types of macrocyclic hydroxyethylene isosteres (HE) and the route gave possibility for smooth decoration of the macrocyclic ring with different substituents. In total 8 macrocyclic renin inhibitors were synthesized. The most potent inhibitor **42** exhibited a renin K_i value of 3.3 nM. There was no selectivity over Cat-D in this case, but a 75-fold selectivity over BACE-1 was recorded. A modest sixfold selectivity of inhibition of renin over Cat-D was achieved with the prime side truncated compound **43** followed by a fivefold selectivity for **44**. Based on results from crystallography, we judge that the *S*-configuration of the P3sp group in our macrocycles is important for inhibitory activity against renin, but is also important for Cat-D, thus creating low or modest selectivity ratios. This configuration of the P3sp group also plays a role in BACE-1, but is seemingly less significant when judging from the activity data.

The measurements of HLM values of the most renin potent examples, **42** and **43**, showed that macrocyclization gave metabolically more stable compounds compared to the starting linear compound **1**, but no improvement of permeability (Caco-2) was achieved. The assessment of the BA/AB ratios for representative **42** and **43** from MDCK wt/MDCK MDR-1 assays implies that these macrocycles may be Pgp-substrates while aliskiren under same conditions may not be. However, bearing in mind the low intrinsic permeability of **42**, **43** and aliskiren this conclusion is to be treated with caution.

All the results presented in this paper points out that further optimizations are needed for these types of macrocyclic compounds in order to further improve pharmacokinetic properties and in particular renin/Cat-D inhibitory selectivity ratios, while sustaining and or improving on the already good nanomolar renin potencies.

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