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Laboratory note

Synthesis and renin inhibitory activity of novel angiotensinogen transition state analogues modified at the P_2-histidine position†

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Summary — With the aim of finding new renin inhibitors with improved bioavailability properties, two angiotensinogen transition state analogues **1a** and **1b**, containing a novel unnatural amino acid at the P_2 position, namely the (2*R*,3*S*)- and (2*S*,3*S*)-2-amino-3-(1,3-dithiolan-2-yl)-3-hydroxypropanoic acid (ADHPA), have been synthesized and tested for human renin inhibitory activity and for chemical and enzymatic stability. Only compound **1a** (the *S*-isomer) possessed a significant activity, which was lower than that of the corresponding histidyl derivative KRI-1314, and combined with a low stability to the gut enzyme chymotrypsin.

renin inhibitor / angiotensinogen transition state analogue / P_2-histidine position modification / 2-amino-3-(1,3-dithiolan-2-yl)-3-hydroxypropanoic acid

Introduction

The inhibition of renin, which catalyzes the first step in the formation of the vasoconstrictor peptide angiotensin II (AII) in the renin angiotensin system (RAS), was shown to be effective in lowering blood pressure [1, 2]. However, the therapeutic use of renin inhibitors is not yet allowed, mainly due to their insufficient oral absorption and rapid biliary excretion [3]. Only recently, some examples of inhibitors showing oral bioavailability in animal and human studies have appeared in the literature [4–6]. With the aim of overcoming these drawbacks, major efforts were focused on decreasing the peptidic character, the size and the lipophilicity of the inhibitors [7]. Recent studies indicate that the S_2 site in renin can accommodate a variety of side chains at the P_2 position affording a varied inhibitory activity [8–11].

In this paper we describe the synthesis and preliminary in vitro studies (human renin inhibitory activity) of two angiotensinogen transition state analogues **1a** and **1b** (fig 1) structurally related to the potent renin inhibitor KRI 1314 [12], in which the His at the P_2 position has been replaced with a novel unnatural heterocyclic amino acid, namely (2*R*,3*S*)- and (2*S*,3*S*)-2-amino-3-(1,3-dithiolan-2-yl)-3-hydroxypropanoic acid (ADHPA).

Chemistry

The target compounds **1a** and **1b** were synthesized by standard coupling procedures, starting from the appropriate amino-acid fragments **4**, **8**, **9** and **13**, whose syntheses are outlined in schemes 1–4.

The (2*R*,3*S*)- and (2*S*,3*S*)-2-amino-3-hydroxypropanoic acid methylesters **4** and **8** were prepared as pure diastereomers, starting from the known (2*R*,3*S*)-acid **2** [13], according to scheme 1, routes A and B, respectively.

The first attempt to obtain the (2*R*,3*S*)-methylester **4** from acid **2** by acid-catalyzed esterification in refluxing methanol failed. A partial decomposition of the oxazolidine ring occurred, affording a complex mixture of products, which were difficult to purify. The problem was overcome converting compound **2** into its *N*-Boc-methylester **3** (route A) by reaction with BOC-anhydride followed by treatment with diazomethane.

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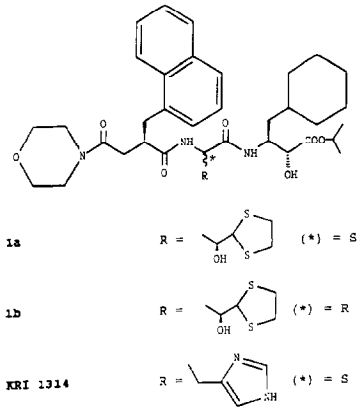


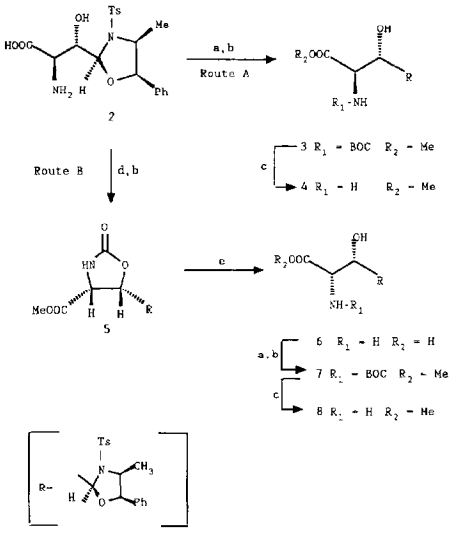
Fig 1. Structures of **1a**, **1b** and KRI 1314.

The mild acid-catalyzed removal of the BOC-protecting group gave pure methylester hydrochloride **4** in good yield.

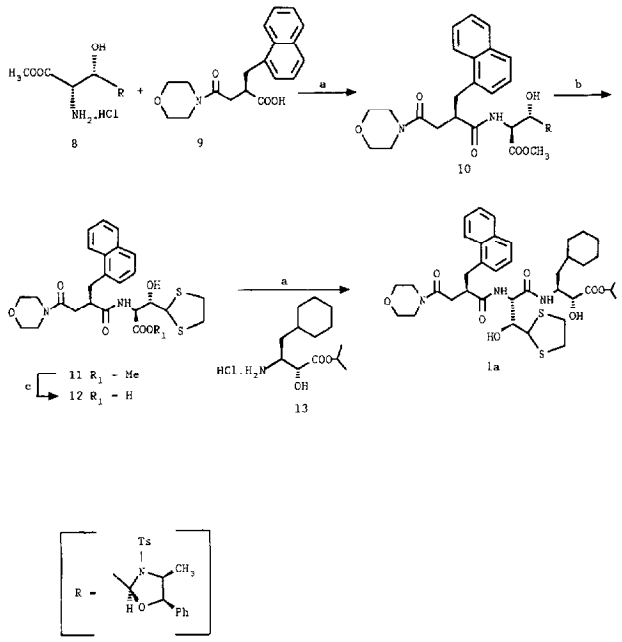
The synthesis of the diastereomeric (2*S*,3*S*)-methylester **8** is depicted in scheme 1, route B. Amino-acid derivative **2** was converted into the *cis*-oxazolidinone **5** according to a known procedure [13], which was isomerized to *trans*-oxazolidinone and saponified to yield **6**. The latter was finally converted into methylester **8** (via the *N*-BOC derivative **7**), as previously reported for **4**.

Inhibitor **1a** (*S* isomer) was prepared according to scheme 2. The amino derivative **8** was coupled with (*R*)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)-propanoic acid **9** [14] using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) to give intermediate **10**, which was converted into dithiolane **11** in the presence of 1,2-ethanedithiol and borontrifluoride etherate (BF_3•Et_2O). Finally, coupling of acid **12**, obtained from **11** by alkaline hydrolysis, with the known cyclohexylnorstatine isopropylester **13** [15] gave the desired pseudopeptide **1a**.

The same straightforward procedure could not be used for the preparation of the inhibitor **1b** (*R* isomer) in quantities needed for biological testing (scheme 3). In fact, after the first coupling step of amino acid **4** with **9** to give intermediate **14**, epimerization at the α-carbon atom intervened when methylester **14** was hydrolyzed to acid **15** with aqueous alkali. This finding was demonstrated by the formation of two products, respectively, **16a** and **16b**, from the coupling of the crude acid **15** with **13**. After chromatographic separation, **16a** and **16b** were reacted with ethanedithiol and BF_3•Et_2O. We recovered the desired compound **1b** (from **16b**) in poor yield, whereas the dithiolane derivative obtained from **16a** was identified as **1a**, previously obtained by the different route out-

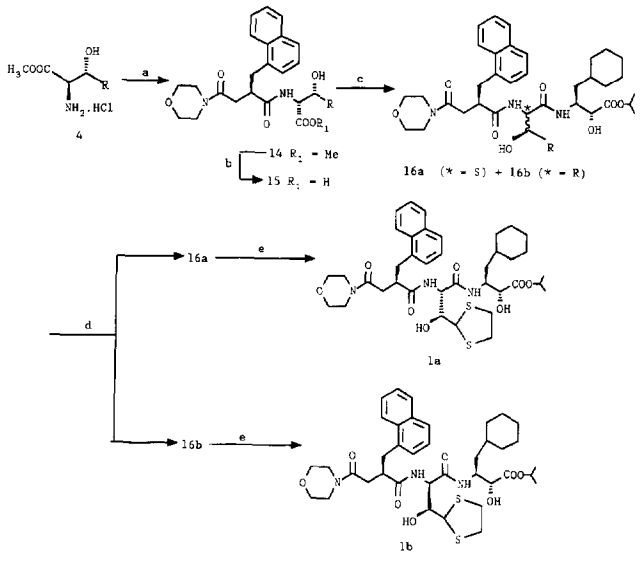


Scheme 1. (a) $(BOC)_2O$, Et_3N, MeOH, 50 °C; (b) CH_2N_2, MeOH, Et_2O; (c) aq HCl, dioxane, rt; (d) $COCl_2$, aq KOH, toluene, 0 °C; (e) aq KOH, MeOH, reflux.

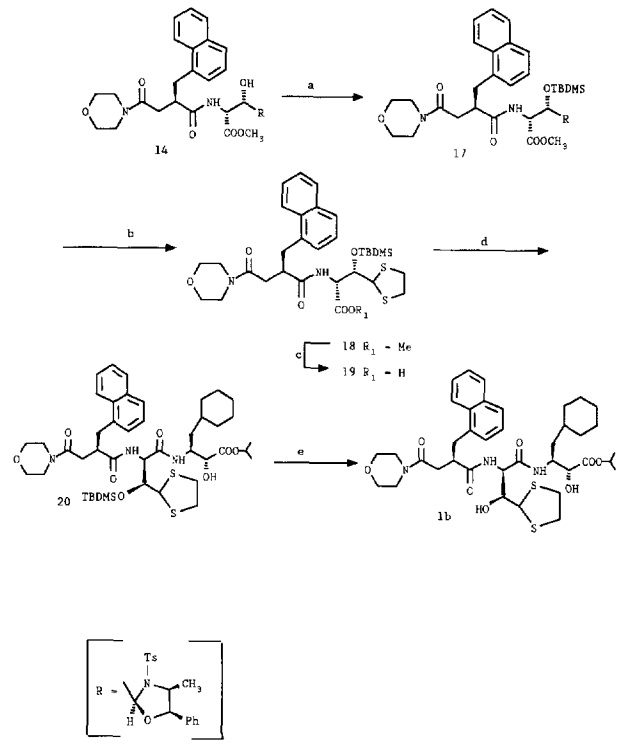


Scheme 2. (a) Et_3N, DCC, HOBT, CH_2Cl_2, 0 °C; (b) $HSCH_2CH_2SH$, BF_3•Et_2O, CH_2Cl_2, rt; (c) aq NaOH, MeOH, rt.

lined in scheme 2. We hypothesize that the base-catalyzed racemization could be explained by the formation of a cyclic intermediate involving the free hydroxyl group, unlike the usual formation of an



Scheme 3. (a) **9**, Et_3N, DCC, HOBT, CH_2Cl_2, 0 °C; (b) aq NaOH, MeOH, rt; (c) **13**, Et_3N, DCC, HOBT, CH_2Cl_2, 0 °C; (d) chromatographic separation; (e) $HSCH_2CH_2SH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt.



Scheme 4. (a) *t*-$BuMe_2SiOTf$, 2,6-lutidine, CH_2Cl_2, 0 °C; (b) $HSCH_2CH_2SH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt; (c) aq NaOH, MeOH, rt; (d) **13**, Et_3N, DCC, HOBT, CH_2Cl_2, 0 °C; (e) TBAF, THF, rt.

azalactone intermediate, generally invoked for peptide racemization [16]. In order to prevent epimerization the hydroxyl group of **14** was protected as *t*-butyldimethylsilylether to give intermediate **17**, which was in turn converted into the dithiolane **18** in the usual way (scheme 4). In the subsequent alkaline hydrolysis of **18** to acid **19** racemization did not take place. Finally, coupling of **19** with **13** gave intermediate **20**, from which pseudopeptide **1b** was obtained in good yield as a unique diastereomer, by removal of the silyl protective group.

Results and discussion

The renin inhibitory potency of the dithiolane derivatives **1a,b** and the reference compound KRI 1314 were determined in human plasma.

The data reported in table I and figure 2 indicate that significant inhibition of plasma renin activity (PRA) was exhibited only by compound **1a**, whereas compound **1b** was found practically ineffective in inhibiting PRA (less than 10% inhibition at 1 μM).

The different biological behaviour displayed by the two isomers **1a** (*S* configuration) and **1b** (*R* configuration) confirms the stereochemical requirements of the P_2 position in the renin inhibitors. The *S* configuration, also present in the reference compound KRI 1314, seems to be a fundamental condition for the activity in this and other series of related pseudopeptides [17].

Comparison of the in vitro potencies between **1a** and the reference KRI 1314 shows that substitution of His by ADHPA is unfavourable to inhibitory activity, in contrast to previous results obtained with other alkylthio groups [11]. Compound **1a** was further studied with regard to stability to artificial gastric and intestinal juice and to the gut enzyme chymotrypsin, the degradation of renin inhibitors being a possible limiting factor for their low oral activity [18, 19]. Even if it was stable to artificial juices, a partial degradation occurred in the presence of chymotrypsin (see *Experimental protocols*). These findings dissuaded us from undertaking further investigations on compound **1a**.

Table I. In vitro renin inhibitory activity.

Compound	IC_{50} *(μM)*
1a	0.75 ± 0.14
1b	ND[a]
KRI 1314	0.066 ± 0.011

[a]ND = not determined; less than 10% inhibition at 1 μM.

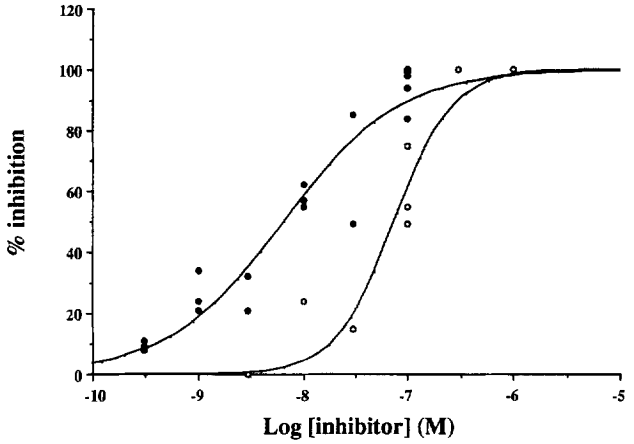


Fig 2. Inhibition of human plasma renin activity by compound **1a** (open symbols) and KRI 1314 (closed symbols). Single experimental points are reported.

Experimental protocols

Chemistry

Melting points were determined with a Büchi 510 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Brucker AC 200 instrument with the chemical shifts reported in δ downfield from tetramethylsilane with multiplicity, number of protons and coupling constants in hertz. Mass spectroscopic analyses (FAB) were performed on a VG 7070 EQHF instrument. Analyses, indicated by the symbols of the elements, were within ±0.4% of theoretical values. High pressure liquid chromatography (HPLC) analyses were performed on a Waters 600 E apparatus with a UV detector at 229 nm and a μBondapack C_{18} Waters column (3.9 × 300 mm). Solvent mixtures of CH_3CN and H_2O were used (system A: CH_3CN/H_2O 6:4; system B: CH_3CN/H_2O 1:1; system C: CH_3CN/H_2O 7:3) with a flow rate of 1.5 mL/min. Retention time (t_R) in minutes and HPLC assay are reported. TLC analyses were performed on silica-gel plates (Merck 60 F254). Flash column chromatography (FC) was carried out using silica gel (Merck 60, 230–400 mesh).

(2R,3S)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(2S,4S,-5R)-4-methyl-3-[(4-methylphenyl)sulfonyl]-5-phenyl oxazolidin-2-yl]-3-hydroxypropanoic acid methylester ***3***

To a stirred solution of (2*R*,3*S*)-2-amino-3-[(2*S*,4*S*,5*R*)-4-methyl-3-[(4-methylphenyl)sulfonyl]-5-phenyl-oxazolin-2-yl]-3-hydroxy propanoic acid **2** [13] (1.0 g, 2.4 mmol) in 10 mL MeOH, were added Et_3N (0.27 g, 2.6 mmol) and $(BOC)_2O$ (1.0 g, 4.8 mmol). The brown solution obtained was stirred for 1 h at 50 °C and then cooled to room temperature and concentrated under vacuum. The resulting oil was partitioned between AcOEt and cold 0.1 N HCl, the aqueous phase was separated and extracted twice with AcOEt. The combined organic phases were dried (Na_2SO_4) and evaporated in vacuo to afford crude *N*-BOC-amino-acid derivative. The residue was dissolved in 10 mL of MeOH cooled to 0 °C and treated with an excess of CH_2N_2 in Et_2O. After evaporation and purification by FC (toluene/*i*-Pr_2O 75:25) compound **3** was obtained as a white foam (0.72 g, 60%). Anal $C_{26}H_{34}N_2O_8S$ (C, H, N); ^{1}H NMR ($CDCl_3$): 7.83 (d, 2H, *J* = 8.4 Hz), 7.44 (d, 2H, *J* = 8.4 Hz), 7.35–7.20 (m, 5H), 5.51 (m, 1H), 5.21 (d, 1H, *J* = 6.2 Hz), 4.82 (m, 1H), 4.35–4.04 (m, 3H), 3.80 (s, 3H), 2.47 (s, 3H), 1.47 (5, 9H), 0.85 (t, 3H, *J* = 6.8 Hz).

(2S,3S)-2-[[(1,1-Dimethylethoxy)carbonyl]amino]-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)sulfonyl]-5-phenyloxazolidin-2-yl]-3-hydroxypropanoic acid methylester 7

Compound **7** was obtained analogously to **3** and purified by FC (toluene/*i*-Pr_2O 7:3). Yield 50%. Anal $C_{26}H_{34}N_2O_8S$ (C, H, N); ^{1}H NMR ($CDCl_3$) δ: 7.83 (d, 2H, *J* = 8.4 Hz), 7.45–7.02 (m, 7H), 5.42 (d, 1H, *J* = 8.0 Hz), 4.98 (d, 1H, *J* = 7.4 Hz), 4.76 (d, 1H, *J* = 10.1 Hz), 4.32–4.05 (m, 3H), 3.82 (s, 3H), 2.48 (s, 3H), 1.47 (s, 9H), 0.88 (d, 3H, *J* = 6.8 Hz).

(2R,3S)-2-Amino-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)-sulfonyl)]-5-phenyloxazolidin-2-yl]3-hydroxypropanoic acid methylester hydrochloride ***4***

To a solution of **3** (2.3 g, 4.3 mmol) in 30 mL dioxane was added 10 mL of 4 N HCl and the mixture was stirred for 3 h at room temperature. The solvent was evaporated and the yellow oil obtained was dissolved in MeOH and dried under vacuum until an ivory solid was formed. Then Et_2O was added and the slurry obtained was stirred for 1 h. After filtration and drying, pure hydrochloride **4** was obtained as a white powder (1.9 g, 95%). Anal $C_{21}H_{26}N_2O_6S$·HCl (C, H, N); mp 150–155 °C (dec.); ^{1}H NMR ($CDCl_3$ + D_2O) δ: 7.85 (d, 2H, *J* = 8.3 Hz), 7.45–7.02 (m, 7H), 5.48 (d, 1H, *J* = 7.6 Hz), 4.72 (s, 1H), 4.52 (d, 1H, *J* = 7.6 Hz), 4.25 (d, 1H, *J* = 5.2 Hz), 4.01 (m, 1H), 3.82 (s, 3H), 2.34 (s, 3H), 0.80 (d, 3H, *J* = 6.8 Hz).

(2S,3S)-2-Amino-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)-sulfonyl]-5-phenyloxazolidin-2-yl]-3-hydroxypropanoic acid methylester hydrochloride ***8***

Compound **8** was obtained analogously to **4**. Yield 97%. Anal $C_{21}H_{26}N_2O_6S$·HCl (C, H, N); mp 123–126 °C (dec.); ^{1}H NMR ($CDCl_3$ + D_2O) δ: 7.95 (d, 2H, *J* = 8.4 Hz), 7.35 (d, 2H, *J* = 8.4 Hz), 7.32–7.04 (m, 5H), 5.62 (d, 1H, *J* = 6.8 Hz), 4.51 (s broad, 1H), 4.33 (d, 1H, *J* = 6.2 Hz), 4.15 (d, 1H, *J* = 5.4 Hz), 3.97 (m, 1H), 3.84 (s, 3H), 2.37 (s, 3H), 0.85 (d, 3H, *J* = 7.0 Hz).

(4R,5S)-5-[(2S,4S,5R)-4-Methyl-3-[(4-methylphenyl)sulfonyl]-5-phenyloxazolidin-2-yl]-4-(methoxycarbonyl)oxazolidin-2(3H)-one ***5***

Compound **5** was obtained according to the procedure reported in the literature [13]. Yield 75%. Anal $C_{22}H_{24}N_2O_7S$ (C, H, N); ^{1}H NMR ($CDCl_3$) δ: 7.91–7.05 (m, 9H), 6.05 (bs, 1H), 5.62 (dd, 1H, *J* = 4.4 Hz, 2.1 Hz), 4.73 (d, 1H, *J* = 2.1 Hz), 4.70 (d, 1H, *J* = 4.4 Hz), 4.61 (d, 1H, *J* = 5.6 Hz), 4.92 (m, 1H), 3.72 (s, 3H), 2.42 (s, 3H), 0.71 (d, 3H, *J* = 7.2 Hz)

(2S,3S)-2-Amino-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)-sulfonyl]-5-phenyloxazolidin-2-yl]-3-hydroxypropanoic acid hydrochloride ***6***

Compound **6** was obtained according to the procedure reported in the literature [13]. Yield 80%. Anal $C_{20}H_{24}N_2O_6S$·HCl (C, H, N); ^{1}H NMR (DMSO-d_6): 0.75 (d, 3H, *J* = 6.6 Hz), 2.43 (s, 3H), 3.80 (bs, 1H), 4.00 (d, 1H, *J* = 5.4 Hz), 4.32 (m, 2H), 5.25 (d, 1H, *J* = 5.4 Hz), 7.20–7.47 (m, 5H), 7.52 (d, 2H, *J* = 7.8 Hz), 7.97 (d, 2H, *J* = 7.8 Hz).

*General procedure for the preparation of acids **12**, **15** and **19***
To a solution of the appropriate methyl ester (**11**, **14**, **18**) (1 mmol) in 10 mL MeOH, was added 1 N NaOH (5 mmol). The mixture was stirred at room temperature until TLC analyses indicated completion and successively evaporated under vacuum. The residue was dissolved in H_2O, acidified with 0.1 N HCl to pH 4 and extracted with AcOEt. The organic extracts were dried (Na_2SO_4) and evaporated under vacuum. After drying, the crude acids obtained (**12**, **15** and **19**) were utilized in the next coupling reaction without further purification.

*General procedure of coupling for compounds **1a**, **10**, **14** and **20***
To a stirred solution of the appropriate amino derivative hydrochloride (1 mmol) in 10 mL dry CH_2Cl_2 cooled to 0 °C and under N_2 atmosphere, were added sequentially Et_3N (1 mmol), a solution of the carboxylic acid derivative (obtained as mentioned above by hydrolysis of the corresponding methylester) (1 mmol) dissolved in 5 mL dry CH_2Cl_2, HOBT (1.2 mmol) and DCC (1 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 h and then the solid was filtered off and the filtrate evaporated under vacuum. After purification by flash chromatography, pure di- and tri-pseudopeptides were obtained.

*Methyl (2S,3S)-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)-sulfonyl]-5-phenyloxazolidin-2-yl]-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-hydroxypropanoate **10***
Purified by FC (CH_2Cl_2/MeOH 98:2). Yield 50%. Anal $C_{40}H_{45}N_3O_9S$ (C, H, N); ^1H NMR ($CDCl_3$): 0.85 (d, 3H, J = 6.8 Hz), 2.42 (s, 3H), 2.35–2.50 (m, 1H), 2.85 (dd, 1H, J = 9.6 Hz, J = 1.6 Hz), 3.15–4.30 (m, 14H), 3.72 (s, 3H), 5.15 (m, 2H), 6.78 (d, 1H, J = 8.8 Hz), 7.05–7.55 (m, 11H), 7.72–7.91 (m, 4H), 8.15 (d, 1H, J = 7.4 Hz). HPLC: system B, t_R 11.14, assay 95.3%.

*Isopropyl (2R,3S)-4-cyclohexyl-2-hydroxy-3-[N-[(2S,3S)-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-(1,3-dithiolan-2-yl)-3-hydroxypropanoyl]amino] butanoate **1a***
Purified by FC (CH_2Cl_2/MeOH 98:2). Yield 68%. Anal $C_{38}H_{53}N_3O_8S_2$ (C, H, N); mp: 112–117 °C (dec); MS (FAB): *m/e* 744 (MH^+); HPLC: system A, t_R 8.09, assay 99.5%.

*Methyl (2R,3S)-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)-sulfonyl]-5-phenyloxazolidin-2-yl]-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-hydroxypropanoate **14***
Purified by FC (hexane/AcOEt 30:70). Yield 58%. Anal $C_{40}H_{45}N_3O_9S$ (C, H, N); MS (FAB+): *m/e* 744 (MH^+); HPLC: system A, t_R 8.29, assay 95.1%.

*Isopropyl (2R,3S)-4-cyclohexyl-2-hydroxy-3-[N-[(2R,3S)-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-(1,3-dithiolan-2-yl)-3-[(1,1-dimethylethyl)dimethylsilyloxy]propanoyl]amino]butanoate **20***
Purified by FC (hexane/AcOEt 65:35). Yield 54%. Anal $C_{44}H_{67}N_3O_8S_2Si$ (C, H, N); ^1H NMR ($CDCl_3$) (selected values) δ: 0.14 (s, 3H), 0.20 (s, 3H), 0.81 (s, 9H); 2.85–3.75 (m, 18H), 4.00–4.22 (m, 3H), 4.29 (d, 1H, J = 4.2 Hz), 4.40 (m, 2H), 4.85 (dd, 1H, J = 3.9 Hz; J = 2.7 Hz), 5.05 (m, 1H), 7.20–7.50 (m, 5H), 7.80–7.95 (m, 2H), 8.15 (m, 1H).

*Methyl (2S,3S)-3-(1,3-dithiolan-2-yl)-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-hydroxypropanoate **11***
To a stirred solution of **10** (0.20 g, 0.27 mmol) in 5 mL of dry CH_2Cl_2 under N_2 atmosphere was added 1,2-ethanedithiol (0.3 mL, 3.5 mmol) and BF_3•Et_2O (0.33 mL, 2.7 mmol). After 24 h the same quantity of the last two reagents were added and the reaction mixture was stirred for an additional 24 h. Then 5% $NaHCO_3$ aqueous solution was added, the organic phase was separated and the aqueous phase extracted with CH_2Cl_2.

The combined organic phases were washed with brine and evaporated under vacuum. The crude product was purified by FC (hexane/AcOEt 30:70) to yield **11** as a white foam (0.075 g, 54%). Anal $C_{26}H_{32}N_2O_6S_2$ (C, H, N); MS (FAB+) *m/e* 533 (MH^+); ^1H NMR ($CDCl_3$) (selected values) δ: 4.35 (d, 1H, J = 10.1 Hz, -SC*H*S); 4.95 (d, 1H, J = 8.9 Hz, -C*H*COOCH$_3$); 5.08 (dd, J = 10.1 Hz, 1.1 Hz, -C*H*OH); HPLC: system B, t_R 3.87, assay 96.2%.

*Methyl (2R,3S)-3-(1,3-dithiolan-2-yl)-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]propanoate **18***
Obtained analogously to **11** and purified by FC (hexane/AcOEt 60:40). Yield 48%. Anal $C_{32}H_{46}N_2O_6S_2Si$ (C, H, N); ^1H-NMR ($CDCl_3$) δ: 0.05 (s, 3H), 0.06 (s, 3H), 2.50–2.80 (m, 3H), 3.05–3.70 (m, 20H), 3.71 (s, 3H), 4.51 (d, 1H, J = 9.1 Hz), 5.03 (d, 1H, J = 8.3 Hz), 6.54 (d, 1H, J = 8.9 Hz), 7.28–7.91 (m, 6H), 8.23 (d, 1H, J = 8.5 Hz).

*Methyl (2R,3S)-3-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-3-[(2S,4S,5R)-4-methyl-3-[(4-methylphenyl)sulfonyl]-5-phenyloxazolidin-2-yl]-2-[N-[(2R)-3-morpholine-carbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]propanoate **17***
To a stirred solution of **14** (0.50 g, 0.67 mmol) in 20 mL of dry CH_2Cl_2 (20 mL) cooled to 0 °C under N_2 atmosphere was added 2,6-lutidine (0.6 mL, 5.3 mmol) and *t*-BuMe$_2$SiOTf (0.45 mL, 2.0 mmol). After 1 h at 0 °C the reaction mixture was quenched with MeOH and the solvent was evaporated. The residue was dissolved in AcOEt, washed first with 1 N HCl and then with brine and finally dried (Na_2SO_4). Evaporation of the solvent yielded the target compound **17** as a white foam (0.42 g, 78%). Anal $C_{46}H_{59}N_3O_9SSi$ (C, H, N); ^1H NMR ($CDCl_3$) δ: 0.10 (s, 3H); 0.15 (s, 3H); 0.81 (d, 3H, J = 7.1 Hz), 0.83 (s, 9H), 2.43 (s, 3H),; 2.50–2.89 (m, 5H), 3.35–3.73 (m, 12H), 3.76 (s, 3H), 3.88 (d, 1H, J = 5.2 Hz), 5.21–5.35 (m, 3H), 6.80 (d, 1H, J = 7.5 Hz), 7.10–7.85 (m, 10H), 8.22 (d, 1H, J = 7.5 Hz)

*Isopropyl (2R,3S)-4-cyclohexyl-2-hydroxy-3-[N-[(2R,3S)-2-[N-[(2R)-3-morpholinecarbonyl-2-(1-naphthalenylmethyl)propanoyl]amino]-3-(1,3-dithiolan-2-yl)-3-hydroxypropanoyl]amino] butanoate **1b***
To a stirred solution of **20** (0.24 g, 0.28 mmol) in 8 mL dry THF, 0.6 mL of 1 M THF solution of TBAF was added dropwise. After stirring 4 h at room temperature the solvent was evaporated and the remaining oil was partitioned between H_2O and AcOEt. The organic phase was separated, washed with brine and dried (Na_2SO_4). After evaporation of the solvent under vacuum, the crude product was purified by FC (hexane/AcOEt 80:20) to yield **1b** as a white powder (0.13 g, 62%). mp 112–117 °C (dec.); Anal $C_{38}H_{53}N_3O_8S_2$ (C, H, N); MS (FAB): *m/e* 744 (MH^+); ^1H NMR ($CDCl_3$) δ: 0.75–1.95 (m, 19H), 2.56 (d, 1H, J = 14 Hz), 2.90–3.30 (m, 17H), (dd, 1H, J = 9.2 Hz, J = 4.8 Hz), 4.82 (m, 1H), 6.05 (d, 1H, J = 9 Hz), 7.09 (d, 1H,

J = 10 Hz), 7.25–7.63 (m, 4H), 7.77 (dd, 1H, J = 7.6 Hz), 7.88 (dd, 1H, J = 7.6), 8.02 (dd, 1H, J = 8 Hz); HPLC: system A, t_R 8.54, assay 99.3%.

*Preparation of compounds **1a** and **1b** according to scheme 3*
Compound **14** (0.51 g, 0.68 mmol) was saponified according to the general procedure of hydrolysis to give the crude acid derivative **15**, in turn coupled with the amino derivative **13** to yield the two distinct pseudotripeptitides **16a** and **16b**. After separation by FC (CH_2Cl_2/MeOH 98:2) and analytical characterization (**16a**: MS (FAB+): *m/e* 897 (MH^+), HPLC: system C, t_R 8.27; **16b**: MS (FAB+): *m/e* 897 (MH^+), HPLC: system C, t_R 7.31) they were separately reacted with ethanedithiol and BF_3·Et_2O according to the general procedure, to give after purification, respectively **1a** (0.05 g, 13%) (from **16a**) and **1b** (0.06 g, 10%) (from **16b**).

Pharmacology

In vitro inhibition of human plasma renin activity
Venous blood from healthy volunteers was collected on K_3EDTA in chilled vacutainer. Plasma obtained by centrifugation was used fresh or frozen (thawing at ≤ 4 °C, no refreezing). Inhibitors were added as aqueous solutions in the concentration range 1–1000 nM. Angiotensin I, formed after incubation at pH 6.0 (maleate buffer in the presence of the peptidase inhibitor PMSF) for 90 min at 37 °C, was measured by radioimmunoassay (Clinical Assays PRA RIA Kit, Incstar Corp, Stillwater, MN, USA). PRA inhibition was calculated from the ratio of angiotensin I formed in the presence and in the absence of inhibitor, after correction for the basal content of peptide. IC_{50} values were computed from the inhibition/log concentration curves with the MacALLFIT program (Consorzio M Negri Sud, Chieti, Italy).

Stability studies
Artificial gastric and intestinal juice. Chemical stability testing were performed by using the method reported in the literature [18].

Chymotrypsin. A solution of compound **1a** in MeOH (5 mg/mL) was prepared and an aliquot of 50 µL was added to 3 mL of a 0.03 M Na_2HPO_4/0.1 M NaCl solution at pH 6.8 containing 0.1 mg/mL of chymotrypsin (Sigma C-4129), and the mixture was incubated at 37 °C. After the incubation time (0, 30, 60, 120, 180 min) samples were removed, diluted with CH_3CN and analysed by HPLC using the experimental conditions (system A) reported in *Chemistry* above.

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