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NEW POTENTIAL RENIN INHIBITORS WITH DIPEPTIDE REPLACEMENTS IN THE MOLECULE

IWONA WINIECKA[1], JADWIGA DUDKIEWICZ-WILCZYŃSKA[2], IZA ROMAN[2], and RYSZARD PARUSZEWSKI[1]*

[1]Department of Drug Chemistry, Medical University of Warsaw, Banacha 1, 02-097 Warszawa, Poland
[2]National Institute of Public Health, Chełmska 30/34, 00-725 Warszawa, Poland

Abstract: A series of eight non-peptidic potential renin inhibitors have been designed and synthesized. All of them contain dipeptide replacement: (3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) in their molecules. Four among them comprise two additional analogs of dipeptide: (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) and (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid (statine, Sta). All of the synthesized compounds contain also hydrophobic portions to receive a moderate lipophilicity of the molecules. Inhibitory activity of the compounds was measured *in vitro* by HPLC determination of Leu-Val-Tyr-Ser released from the N-acetyltetradecapeptide substrate by renin in the presence of the inhibitor. Asp-α(OEt)-(S,S)-ACHPA-εAhx-Iaa (**23**) shows inhibitory activity (7%) at the concentration of 1.0×10^{-2} M. The other synthesized compounds show no inhibitory activity up to this concentration.

Keywords: renin inhibitors, dipeptide replacement, HPLC, *in vitro* inhibitory activity determination

Hypertension is a major risk factor for cardiovascular and kidney diseases. The renin-angiotensin system plays a key role in the physiological regulation of blood pressure and intravascular volume through the action of octapeptide angiotensin II. The first step in this system is formation of decapeptide angiotensin I from substrate angiotensinogen, catalyzed by renin. In the next step, this decapeptide is decomposed to octapeptide angiotensin II under action of angiotensin converting enzyme (ACE). Excessive renin system activity may lead to hypertension. Drugs that inhibit the renin system activity, such as ACE inhibitors and angiotensin II receptor blockers, are highly effective in treatment of hypertension and related cardiovascular diseases. However, they stimulate compensatory renin release from the kidney, which results in an increase of level of angiotensin II. These drugs show also numerous side effects in the cured patients. Renin is a monospecific enzyme that displays remarkable specificity for its only known substrate angiotensinogen. Therefore, renin inhibitors can be a promising drugs for treatment of hypertension. Numerous trials to develop clinically effective direct renin inhibitors thwarted by the low potency, not stability or poor pharmacokinetic profiles of the synthesized compounds. There were peptide substrate analogues with amino acids substituted with non peptidic amino acids, peptide-like inhibitors with the transition state analogues and fully nonpeptidic inhibitors. The first in a new class of orally effective, nonpeptide direct renin inhibitors developed for the treatment of hypertension is aliskiren (2(S),4(S),5(S),7(S)-N-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)-phenyl]-octanamid hemifumarate) (1, 2)**.** It is an extremely potent competitive inhibitor with IC_{50} of 0.6×10^{-9} M and with high specificity for primate renin. Aliskiren shows favorable physicochemical properties, high aqueous solubility, low lipophilicity and is resistant to enzymatic degradation (3). Aliskiren is the first renin inhibitor registered at the US Food and Drug Administration. The structure of aliskiren is considerably different from the structure of 8–13 human angiotensinogen fragment. Nevertheles, it shows very high biological activity. Probably, it is a result of excellent accommodation of aliskiren to the active site of renin or exactly to a distinct subpocket specific to renin. Also pharmacokinetic and physicochemical properties, such as lipophilicity, solubility or resistance to degradation are beneficial for high activity of

* Corresponding author: e-mail: ryszard.paruszewski@neostrada.pl

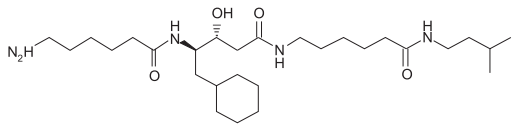
aliskiren. It seems, that the mentioned conditions could be accomplished by different structures. Our intention was to search for new active inhibitors with simple structure, good bioavailability and easy synthesis. It was intended to reach this purpose by coupling non-peptidic fragments with good affinity to active site of renin. Previously, a moderately active *in vitro* renin peptidic inhibitors were obtained. Between them were compounds with dipeptide replacements at P_1-P_1' as well at P_2'-P_3' positions and with activity *in vitro* in the range of 10^{-5} – 10^{-6} M (4). Other previously obtained inhibitors with two dipeptide replacements showed activity *in vitro* also at the similar concentration (5). All the obtained compounds have also different extensions at the N-, C- or both termini of the fragment binding to the active site of renin (positions P_3-P_2- or -P_2'-P_3'). The extensions are mainly hydrophobic linear substituents used to correct the affinity and activity. The purpose also was to study relations between the structure of the amino acids as well as the incorporated substituents and biological activity of the inhibitors. Because introduction of the second dipeptide analog into the inhibitor molecule succeeded in an increase of the inhibitory activity, now it was decided to synthesize four potential inhibitors (**18, 36, 43, 50**) with three such units. The hydrophobic active site of renin extending over seven residues (S_4-S_3') can accommodate 7 amino acid units of the substrate (6, 7). Therefore, we supposed that the molecule built from three analogs of dipeptide probably can also be accommodated to the active site. All three dipeptide analogs have hydroxyl groups necessary to bind to the catalytically active Asp 32 and Asp 215 residues of renin and all three are of hydrophobic character. It is difficult to say which one could be bound. However, taking under consideration a size of hydrophobic cleft, it may be supposed that it is the central dipeptide analog, it means ACHPA. Also it was decided to lengthen the molecule at the N-, C- or both termini of this fragment by incorporation of different substituents – ε-aminohexanoic acid (εAhx), isoamylamide group (Iaa), isovaleryl group (Iva) or some another extensions. The purpose was to correct the receptor affinity and may be activity, as well to study the relation between the length of the molecule and its activity. Four successive potential inhibitors (**9, 23, 25, 32**) were synthesized to examine an influence of lengthening of dipeptide analog ACHPA molecule at the both termini upon the biological activity. These inhibitors can bind by its hydroxyl group of ACHPA to both Asp residues of renin. Their hydrophobic substituents can comprise, as was supposed, in the hydrophobic cleft of the active site. The result of incorporation of such extensions can be interesting, because some of them have no side chain at all, but are very flexible.

EXPERIMENTAL

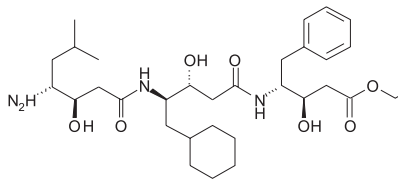
Chemistry

The structures of the inhibitors are shown in Fig. 1. The inhibitors (**9**, **18**, **23**, **25**, **32**, **36**, **43**, **50**) as well as their intermediates were synthesized as shown in Schemes 1–8 in Fig. 2. The methods are given in the section entitled – syntheses. Physicochemical and analytical data of the inhibitors, as well as newly synthesized intermediates (**8**, **17**, **20–22**, **24**, **26**, **31**, **35**, **42**, **46–49**) are presented in Tables 1 and 2. Properties of some derivatives (**15**, **28**, **29**, **34**, **40**), which are not a final products, are not given. Data of the other synthesized compounds are consistent with those described elsewhere: **6**, **13**, **14** (5); **1** (8); **2**, **4**, **12**, **16** (9, 10); **3**, **5**, **33** (11); **7** (12); **10**, **30** (13); **11** (14); **27** (15); **37–39**, **41** (16); **44**, **45** (17).

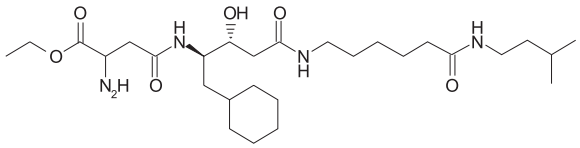
The amino acid, some derivatives (Boc-α(OH)-OBzl, **19)** and reagents were purchased from Aldrich. Renin from porcine kidney and *N*-acetyl-renin substrate tetradecapeptide were obtained from Sigma. Solvents were of analytical purity. Tetrahydrofuran (THF) was distilled from Na/benzophenone under N_2. Dichloromethane and dimethylformamide (DMF) were dried over 4 Å molecular sieves. The peptides were synthesized by the *N,N*-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt) method of fragment condensation in solution. All synthesized compounds were separated and purified by column chromatography (CC) on silica gel (Merck, grade 230 to 400 mesh). TLC was carried out on 0.25 mm thickness silica gel plates (Merck, silica gel 60 F_{254}. The solvent systems used in TLC and CC were $CHCl_3$/MeOH in various ratios. The spots were visualized with 0.3% ninhydrin in EtOH/AcOH (97 : 3, v/v). Elemental analyses were performed on a Perkin-Elmer Microanalyser. Melting points were determined in a Böetius apparatus. ^{1}H NMR spectra were recorded on a Bruker DM 400 MHz Avance 400 WB spectrometer. Chemical shifts were measured as δ units (ppm) relative to tetramethylsilane (TMS). Optical rotations were measured at the Na-D line with a Polamat (Carl Zeiss, Jena) polarimeter in a 5 cm polarimeter cell. HPLC analyses of synthesized inhibitors were performed on a Shimadzu apparatus equipped with a pump LC-10AT, detector UV SPD-6A and recorder Chromax 2001. The



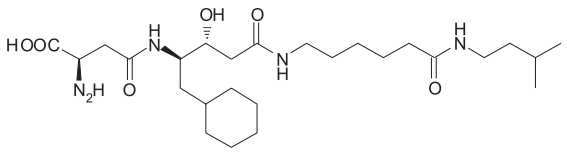
9. ε-Aminoheksanoyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid isoamyl amide. (H-εAhx-(S,S)-ACHPA-εAhx-Iaa)



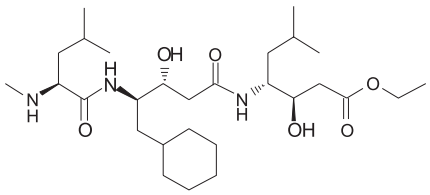
18. (3S,4S)-4-Amino-3-hydroxy-6-methylheptanoyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-(3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid ethyl ester (H-(S,S)-Sta-(S,S)-ACHPA-(S,S)-AHPPA-OEt)



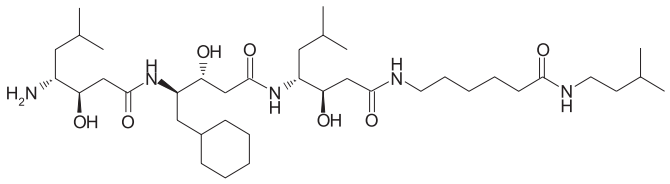
23. α-Ethoxyaspartyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-ε-amino-hexanoic acid isoamylamide (H-Asp-α-(OEt)-(S,S)-ACHPA-εAhx-Iaa)



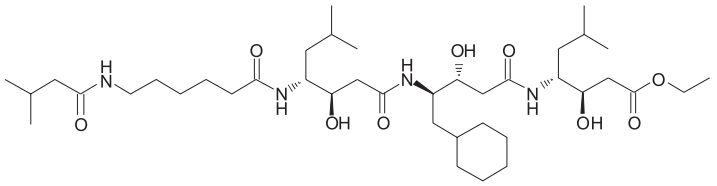
25. α-Hydroxyaspartyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-ε-aminohexanoic acid isoamylamide (H-Asp-α-(OH)-(S,S)-ACHPA-εAhx-Iaa)



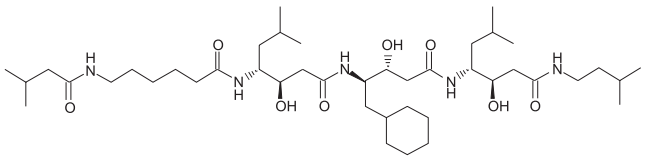
32. N-Methylleucyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid ethyl ester (H-MeLeu-(S,S)-ACHPA-(S,S)-Sta-OEt)



36. (3S,4S)-4-amino-3-hydroxy-6-methylheptanoyl-(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoyl-ε-aminohexanoic acid isoamylamide (H-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-ε-Ahx-Iaa)



43. N-isovaleryl-ε-aminohexanoyl-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoyl-(3S,4S)-amino-5-cyclohexyl-3-hydroxypentanoyl-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid ethyl ester (Iva-εAhx-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-OEt)



50. N-isovaleryl-ε-aminohexanoyl-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoyl-(3S,4S)-amino-5-cyclohexyl-3-hydroxypentanoyl-(3S,4S)-4-amino-3-hydroxy-6- methylheptanoic acid isoamylamide (Iva-εAhx-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-Iaa)

Figure 1. Structures of the synthesized inhibitors

peaks were recorded at 210 nm and the solvent system was 1% CH_3COOH/MeOH (10 : 90, v/v). HPLC determination of tetrapeptide released from the substrate *N*-acetyl-tetradecapeptide was performed using the same apparatus equipped with two LC-10 AT pumps, a detector UV-VIS SPD-10 A and a controller/recorder SCL-10 A. The peaks were recorded at 210 nm. A reversed phase HPLC method with linear gradient, from 16% to 100% phase B in 14 minute with a flow rate of 1.5 mL/min was used. The mobile phase A consisted of 0.01 M ammonium acetate, the mobile phase B consisted of 0.15% (v/v) acetic acid/acetonitrile : water (60:40).

Syntheses

Introduction of the *N-tert*-butyloxycarbonyl (Boc) group

This group was introduced by generally used method with Boc-azide.

Removal of the *N-tert*-Boc group

Boc-amino acid or Boc-peptide (1 mmol) in a solution of 4 M HCl in dioxane (3 – 5 mL) was stirred at room temperature for 30 min. The solution was concentrated *in vacuo*, then the residue was evaporated twice with ethyl ether and dried *in vacuo*.

Esterification and hydrolysis

Boc-amino acids were esterified with CH_3I or C_2H_5I as described earlier (16). Boc-ACHPA-OEt, Boc-AHPPA-OEt and Boc-Sta-OEt were formed from mono-ethyl malonate used to prepare these compounds (13). Alkaline hydrolysis of ester group was carried out as described earlier (16).

Coupling reaction with DCC/HOBt

The coupling was performed in a commonly used manner by fragment condensation as shown in schemes 1–8.

Alkylamide formation

Boc-amino acid (1 mmol) in solution of dry THF (4–5 mL) was stirred and N-methylmorpholine (1 mmol) was added. The reaction mixture was cooled to –15 – –20°C with stirring under nitrogen. Isobutyl chloroformate (1 mmol) in THF (2 mL) was added dropwise to this solution. Then, alkylamine (1 mmol) in THF (2 mL) was added dropwise with the temperature of the reaction mixture maintained at –15 – –20°C. The solution was stirred at this temperature for 30 min, then warmed to room temperature and stirred for 1 h. Then, THF was removed under reduced pressure, the residue was dissolved in a mixture of 20 mL of ethyl acetate and 20 mL of water and the water layer was extracted twice with 20 ml of ethyl acetate. The combined extracts were washed with 10% citric acid solution, then with water, then with 10% sodium hydrocarbonate solution and with saturated sodium chloride solution, dried, filtered and concentrated. The obtained solid residue was crystallized from n-hexane to give the pure product.

N-acylation reaction *via* symetrical anhydrides

A solution of amino acid ester hydrochloride (1 mmol) in DMF (5 mL) was cooled to 0°C and neutralized with triethylamine (1 mmol). Then the symmetrical anhydride (2 mmol) was added and stirring was continued for 4 h at 4°C and for 15 h at room temperature. The solvent was removed under reduced pressure to yield a solid residue. It was dissolved in ethyl ether. After several hours in refrigerator the precipitate was filtered, washed with small portions of cold ether and dried.

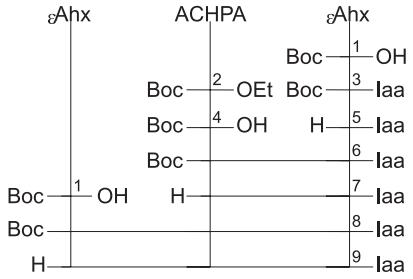
Biochemical assay

Determination of inhibition of renin activity

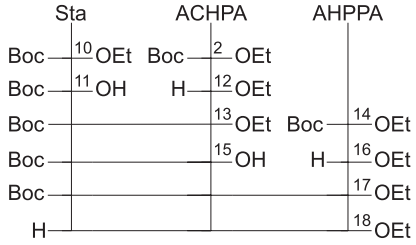
Renin inhibiting activity of the synthesized potential inhibitors was determined *in vitro* according to the method of Galen et al. (18) with modification consisting of a change of spectrofluorimetric to chromatographic method (HPLC) (19) for determination of Leu-Val-Tyr-Ser released from *N*-acetyl-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser effected by the action of renin in the presence of the inhibitor tested. The course of determination is described in the previous paper (17). The activity was tested at the concentration of the compound = 10^{-2} M and is reported as a percent of the inhibiting action.

RESULTS AND DISCUSSION

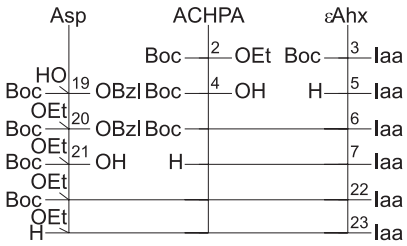
Physicochemical data of the synthesized compounds are given in Tables 1 and 2. Determination of inhibition of renin activity have been performed in preliminary test *in vitro*. The results of activity determination showed that only H-Asp-α-(OEt)-(S,S)-ACHPA-εAhx-Iaa (**22**) has inhibitory activity – 7% in concentration 10^{-2} M. Other synthesized compound are inactive up to this concentration. A lack of activity showed especially by them with three dipeptide replacements (**18, 36, 43 50**) is an unpleasant surprise, although the similar previously synthesized inhibitors showed activity in the range of 10^{-5} – 10^{-6} M. However, the solubility of the examined compounds is poor, although they were



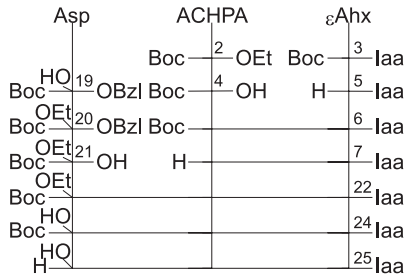
Synthesis of H-εAhx-[(S,S)-ACHPA]-εAhx-Iaa **[9]**



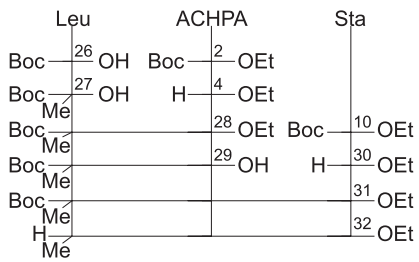
Synthesis of H-[(S,S)-Sta]-[(S,S)-ACHPA]-[(S,S)-AHPPA]-OEt **[18]**



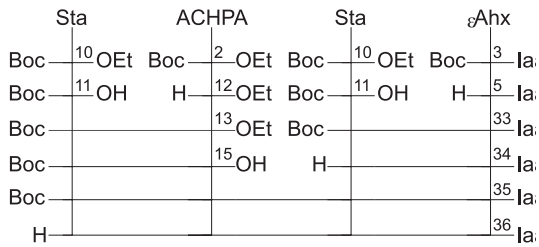
Synthesis of H-Asp(OEt)[(S,S)-ACHPA]-εAhx-Iaa **[23]**



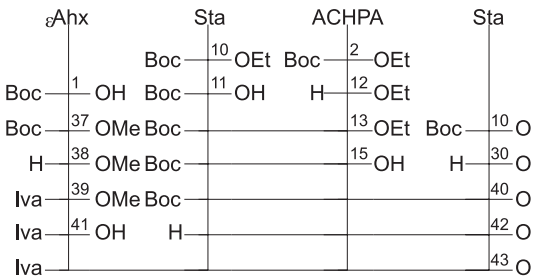
Synthesis of H-Asp(OH)[(S,S)-ACHPA]-εAhx-Iaa **[25]**



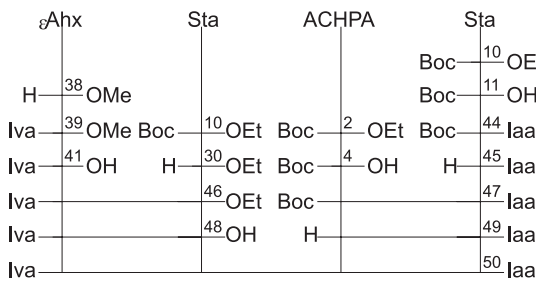
Synthesis of H-MeLeu-[(S,S)-ACHPA]-[(S,S)-Sta]-OEt **[32]**



Synthesis of H-[(S,S)-Sta]-[(S,S)-ACHPA]-[(S,S)-Sta]- εAhx-Iaa **[36]**



Synthesis of Iva-εAhx-[(S,S)-Sta]-[(S,S)-ACHPA]-[(S,S)-Sta]-OEt **[43]**



Synthesis of Iva-εAhx-[(S,S)-Sta]-[(S,S)-ACHPA]-[(S,S)-Sta-Iaa] **[50]**

Fig. 2. Schemes of the inhibitors synthesis

Table 1. Physicochemical and analytical data of some of the synthesized compounds.

Compd. No.	Structure	Formula m.w.	Yield [%]	M.p. [°C]	$[\alpha]^{20}_D$ (c, MeOH)	TLC, R_f (m. ph.)*	HPLC % purity	Log P
8	Boc-εAhx-(S,S)-ACHPA-εAhx-Iaa	$C_{28}H_{54}O_4N_4$ 510.74	17	oil	–28.4	0.85 (B)	–	–
9	HCl × H-εAhx-(S,S)-ACHPA-εAhx-Iaa	$C_{23}H_{46}O_2N_4Cl$ 448.14	99	semi-solid	–24.1 (F)	0.56	91	2.25
17	Boc-(S,S)-Sta-(S,S)-ACHPA-(S,S)-AHPPA-OEt	$C_{37}H_{61}O_9N_3$ 691.90	20	oil	–16.9	0.20 (A)	–	–
18	HCl × H-(S,S)-Sta-(S,S)-ACHPA-(S,S)-AHPPA-OEt	$C_{32}H_{53}O_7N_3Cl$ 591.90	95	78–82	–20.0	0.64 (F)	88	2.58
20	Boc-Asp-α(OEt)-OBzl	$C_{18}H_{25}O_6N$ 351.37	89	oil	–11.0	0.60 (C)	–	–
21	Boc-Asp-α(OEt)-OH	$C_{11}H_{19}O_6N$ 261.28	46	98–103	–16.0	0.86 (C)	–	–
22	Boc-Asp-α(OEt)-(S,S)-ACHPA-εAhx-Iaa	$C_{38}H_{58}O_8N_4$ 638.85	35	semi-solid	–17.8	0.15 (B)	–	–
23	HCl × H-Asp-ε(OEt)-(S,S)-ACHPA-εAhx-Iaa	$C_{28}H_{50}O_6N_4Cl$ 573.40	99	148–150	–11.1	0.46 (F)	87	1.32
24	Boc-Asp-α(OH)-(S,S)-ACHPA-εAhx-Iaa	$C_{31}H_{56}O_8N_4$ 612.81	96	69–77	–20.0	0.14 (B)	–	–
25	HCl × H-Asp-α(OH)-(S,S)-ACHPA-εAhx-Iaa	$C_{26}H_{48}O_6N_4Cl$ 547.36	98	88	–11.7	0.29 (F)	94	0.94
26	Boc-MeLeu-(S,S)-ACHPA-OEt	$C_{25}H_{46}O_6N_2$ 470.65	38	oil	–25.0	0.87 (B)	–	–
31	Boc-MeLeu-(S,S)-ACHPA-(S,S)-Sta-OEt	$C_{33}H_{59}O_8N$ 625.85	31	oil	–30.8	0.42 (B)	–	–
32	HCl × H-MeLeu-(S,S)-ACHPA-(S,S)-Sta-OEt	$C_{28}H_{51}O_6N_3Cl$ 525.73	99	82–86	–10.0	0.58 (F)	97	2.81
35	Boc-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-εAhx-Iaa	$C_{48}H_{81}O_9N_5$ 812.13	73	oil	–20.7	0.11 (B)	–	–
36	HCl × H-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-εAhx-Iaa	$C_{38}H_{73}O_7N_5Cll$ 716.06	90	74–78	–16.3	0.63 (F)	99	2.46
42	HCl × H-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-OEt	$C_{29}H_{55}O_7N_3Cl$ 595.22	92	semi-solid	–18.0	0.30 (F)	–	–
43	Iva-εAhx-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-OEt	$C_{39}H_{74}O_9N_3$ 743.04	45	56–62	–19.3	0.93 (E)	94	3.42
46	Iva-εAhx-(S,S)-Sta-OEt	$C_{12}H_{40}O_5N_2$ 400.55	39	oil	–46.7	0.45 (C)	–	–
47	Boc-(S,S)-ACHPA-(S,S)-Sta-Iaa	$C_{29}H_{55}O_6N_3$ 541.77	56	173–176	–25.3	0.69 (C)	–	–
49	HCl × H-(S,S)-ACHPA-(S,S)-Sta-Iaa	$C_{24}H_{47}O_4N_3Cl$ 479.17	87	semi-solid	–23.0	0.68 (F)	–	–
50	Iva-εAhx-(S,S)-Sta-(S,S)-ACHPA-(S,S)-Sta-Iaa	$C_{43}H_{81}O_8N_5$ 808.32	24	oil	–39.8	0.55 (D)	99	6.03

Elemental analyses were within the limit of theoretical values. Hydrophobicity of the compounds expressed as log P value was calculated by a computer method. *Mobile phase systems (v/v) were: $CHCl_3$ – MeOH: (A) 97:3, (B) 95:5, (C) 90:10, (D) 50:50; hexane – AcOEt: (E) 70:30; butanol-pyridine-water: (F) 65:35:65 (organic phase).

Table 2. ^{1}H NMR spectra of the synthesized compounds.

Compound	Solvent	Chemical shifts δ, ppm)
8	$CDCl_3$	0.85 (d, *J* = 6.4 Hz, 6H, 2×CH_3), 0.90–1.38 (m, 10H, 5×CH_2), 1.40 (s, 9H, C_4H_9), 2.16 (t, *J* = 8.0 Hz, 4H, 2×CH_2), 3.06–3.18 (m, 2H, CH_2), 3.21–3.30 (m, 4H, 2×CH_2), 4.85 (s br, 1H, NH), 5.20 (s br, 1H, NH), 6.37 (s br, 1H, NH), 6.50 (d, *J* = 5.8 Hz, 1H, NH).
9	CD_3OD	0.92 (d, *J* = 6.4 Hz, 6H, 2×CH_3), 0.96–1.52 (m, 10H, 5×CH_2), 2.15–2.26 (m, 4H, 2×CH_2), 2.90–2.98 (m, 2H, CH_2), 3.15–3.25 (m, 4H, 2×CH_2), 3.60 (s br, 1H, NH), 3.98 (s br, 1H, NH).
17	$CDCl_3$	0.92, 0.94 (dd, *J* = 3.2 Hz, 3.2 Hz, 6H, 2×CH_3), 1.10–1.18 (m, 19H, CH_3, 8×CH_2), 1.45 (s, 9H, C_4H_9), 2.33–2.55 (m, 6H, 3×CH_2), 3.60–3.80 (m, 3H, 3×CH), 3.95–4.07 (m, 3H, 3×CH), 4.12 (q, *J* = 6.4 Hz, 2H, -OCH_2), 4.78 (d, *J* = 9Hz, 1H, NH), 6.12 (d, *J* = 9Hz, 1H, NH), 6.64 (d, *J* = 9 Hz, 1H, NH), 7.21–7.28 (m, 5H, C_6H_5).
18	$CDCl_3$	0.99 (d, *J* = 6.7 Hz, 6H, 2×CH_3), 1.10–2.95 (m, 25H, 11×CH_2, CH_3), 3.60–3.75 (m, 3H, 3×CH), 4.08 (q, *J* = 6.0 Hz, 2H, -OCH_2), 7.13–7.20 (m, 2H, 2×NH), 7.35–7.45 (s br, 1H, NH), 7.25 (s, 5H, C_6H_5).
20	$CDCl_3$	1.25 (t, *J* = 7.0 Hz, 3H, CH_3), 1.44 (s br, 9H, C_4H_9), 2.29, 3.10 (dd, *J* = 10.2 Hz, 10.2 Hz, 2H, CH_2), 4.10 (q, *J* = 3.2 Hz, 2H, -OCH_2), 4.60–4.70 (m, 1H, CH), 5.14 (d, *J* = 2.9 Hz, 2H, CH_2), 5.55 (d, *J* = 6.0 Hz, 1H, NH), 7.34–7.38 (m, 5H, C_6H_5).
21	$CDCl_3$	1.27 (t, *J* = 7.2 Hz, 3H, CH_3), 1.45 (s, 9H, C_4H_9), 2.86, 3.08 (dd, *J* = 9 Hz, 9Hz, 2H, CH_2), 4.42 (q, *J* = 3.2 Hz, -OCH_2), 4.56 (s br, 1H, CH), 5.50 (d, *J* = 3.2 Hz, 1H, NH).
22	$CDCl_3$	0.91 (d, *J* = 4.7 Hz, 6H, 2×CH_3), 1.13–1.77 (m, 20H, 9×CH_2, 2×CH), 1.25–1.29 (m, 3H, CH_3), 1.44 (s, 9H, C_4H_9), 2.18 (t, *J* = 6.7 Hz, 2H, CH_2), 3.25 (s br, 4H, 2×CH_2), 3.94–4.08 (m, 2H, 2×CH), 4.20 (q, *J* = 6.7 Hz, 2H, -OCH_2), 5.74 (m, 2H, 2×NH), 6.11 (s br, 1H, NH), 6.58 (s br, 1H, NH).
23	CD_3OD	0.94 (d, *J* = 3.6 Hz, 6H, 2×CH_3), 1.18–1.82 (m, 20H, 9×CH_2, 2×CH), 1.32 (t, *J* = 4.4 Hz, 3H, CH_3), 2.18 (t, *J* = 5.5 Hz, 2H, CH_2), 2.97 (s br, 2H, CH_2), 3.98 (s br, 2H, 2×CH), 4.30 (q, *J* = 6.5 Hz, 2H, -OCH_2).
24	$CDCl_3$	0.91 (d, *J* = 5.9 Hz, 6H, 2x×CH_3), 1.17–1.74 (m, 20H, 9×CH_2, 2×CH), 1.44 (s, 9H, C_4H_9), 2.25 (s br, 2H, CH_2), 3.18 (s br, 1H, NH), 3.25, (s br, 2H, CH_2), 4.00 (s br, 2H, 2×NH), 4.25 (s br, 1H, NH).
25	CD_3OD	0.92 (d, *J* = 5.9 Hz, 6H, 2×CH_3), 1.16–1.75 (m, 20H, 9×CH_2, 2×CH), 2.18 (t, J=3.4 Hz, 2H, CH_2), 2.95 (t, J=5.3 Hz, 2H, CH_2).
28	$CDCl_3$	0.90, 0,93 (dd, *J* = 5.3 Hz, 5.3 Hz, 6H, 2×CH_3), 1.15–1.80 (m, 12H, 6×CH_2), 2.43 (d, *J* = 3.9 Hz, 2H, CH_2), 1.23–1.31 (m, 3H, CH_3), 1,46 (s, 9H, C_4H_9), 2.73 (s br, 3H, N-CH_3), 4.14 (q, *J* = 7.0 Hz, -OCH_2), 4.65 (s br, 1H, NH).
31	$CDCl_3$	0.90–1.00 (m, 12 H, 4×CH_3), 1.10–1.80 (m, 14H, 6×CH_2, 2×CH), 1.26 (t, *J* = 4.8 Hz, 3H, CH_3), 1.48 (s, 9H, C_4H_9), 2.28–2.34 (m, 2H, CH_2), 2.42–2.53 (m, 2H, CH_2), 2.75 (d, *J* = 5.6 Hz, 3H, N-CH_3), 4.14 (q, *J* = 6 Hz, 2H, -OCH_2), 4.66 (s br, 1H, NH), 6.53 (s br, 1H, NH).
32	$CDCl_3$	0.90–1.00 (m, 12H, 4×CH_3), 1.10–1.90 (m, 14H, 6×CH_2, 2×CH), 1.26 (t, *J* = 4.0, 3H, CH_3), 2.78 (s br, 3H, N-CH_3), 4.14 (q, *J* = 6.0 Hz, 2H, -OCH_2), 7.26 (s br, 1H, NH), 8.01 (s br, 1H, NH).
35	$CDCl_3$	0.85–0.88 (m, 18H, 6×CH_3), 1.00–1.75 (m, 37H, 12×CH_2, 4×CH, C_4H_9), 2.19 (s br, 2H, CH_2), 5.20 (s br, 1H, NH), 6.60–6.72 (m, 3H, 3×NH).
36	$CDCl_3$	0.90–1.10 (m, 18H, 6×CH_3), 1.13–1.90 (m, 28H, 12×CH_2, 4×CH), 3.90–4.40 (m, 4H, 4×NH).
42	*CD_3OD*	0.90–1.05 (m, 12H, 4×CH_3), 1.10–1.95 (m, 19H, 8×CH_2, 3×CH).
43	$CDCl_3$	0.82–0.85 (m, 18H, 6×CH_3), 1.16–1.19 (m, 3H, CH_3), 1.20–2.50 (m, 21H, 9×CH_2, 3×CH), 4.06 (q, *J* = 6.4 Hz, 2H, -OCH_2), 6.25–6.52 (m, 2H, 2×NH), 6.75 (s br, 1H, NH), 7.03 (s br, 1H, NH).
46	$CDCl_3$	0.85–0.92 (m, 12H, 4×CH_3), 1.21 (t, *J* = 6.0 Hz, 3H, CH_3), 2.18 (t, *J* = 7.6 Hz, 2H, CH_2), 4.10 (q, *J* = 9.0 Hz, 2H, -OCH_2), 6.17 (s br, 1H, NH), 6.24 (s br, 1H, NH).
47	$CDCl_3$	0.89–0.95 (m, 12H, 4×CH_3), 1.00–1.80 (m, 19H, 8×CH_2, 3×CH), 1.46 (s, 9H, C_4H_9), 3.20 (s br, 1H, NH), 3.45 (s br, 1H, NH), 3.66 (d, *J* = 8.9 Hz, 1H, NH).
49	CD_3OD	0.90–0.95 (m, 12H, 4×CH_3), 1.00–1.85 (m, 19H, 8×CH_2, 3×CH).
50	CD_3OD	0.85–1.05 (m, 24H, 8×CH_3), 1.10–2.05 (m, 31H, 12×CH_2, 5×CH).

used as hydrochloride salts. This can exert influence on the results of HPLC activity determination. A substantial difference in the structure of the previously obtained moderately active inhibitors and these synthesized at present is a number of coupled dipeptide analogues. A comparison of the structures seems to suggest that may be the active site of renin is not large enough to hold the inhibitor fragment built from three dipeptide analogs. Other possibility is that the side chain of some dipeptide analogs does not fit to the specific subpocket in the active site of renin. It looks that incorporated extensions have no influence upon activity of these compounds. A lack of activity of compounds **9, 23, 25 and 32** containing one or two dipeptide analogs shows that the structure of the substituents in positions P_3 and P_2 is very important for activity. It is confirmed by a quite good activity of the previously synthesized inhibitors: Phe(4-OMe)-MeLeu-(S,S)-AHPPA-(S,S)-Sta-OEt, $IC_{50} = 1.0 \times 10^{-5}$ M, Iva-εAhx-Phe(4-OMe)-MeLeu-(S,S)-Sta-εAhx-Iaa, $IC_{50} = 5.0 \times 10^{-6}$ M and εAhx-Phe(4-OMe)-His-(S,S)-Sta-εAhx-Iaa, $IC_{50} = 7.5 \times 10^{-6}$ M. In any case, in the issue of this research and in comparison with previous results it is possible to conclude that biologically active renin inhibitors ought to have one or two, but not three dipeptide analogs in the molecule. Other conclusion is that at P_2 position ought to be some branched substituent resistant to enzymatic degradation, for instance N-methyl leucine (MeLeu), and at P_3 position another substituent also resistant to enzymatic degradation. The presence and structure of extensions at P_2' and P_3' seem to be not specially important for this group of inhibitors.

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