Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 1 pp. 59–69, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

NEW RENIN INHIBITORS CONTAINING PHENYLALANYLHISTIDYL-γ-AMINO ACID DERIVATIVES IN P_3 - P_1' POSITION

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Abstract: Five potential inhibitors of renin have been designed and obtained. In the molecule position P_3 – P_1', crucial for indicating inhibitory activity, all contain phenylalanylhistidylaminoalcanoyl group, ready for interaction with the hydrophobic pocket S_3 – S_1 of renin molecule. The aminoalcanoyl fragment consists of pseudodipeptidic units derivative of γ-amino acids: of 4-amino-3-hydroxybutanoic acid (AHBA) [**26**], 4-amino-5-(4-ethoxyphenyl)-3-hydroxypentanoic acid (AEPHPA) [**13**], 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) (1) and 4-amino-3-hydroxynonanoic acid (AHNA) [**21**]. At the P_3 – P_2 position of obtained compounds an unnatural fragment, derivative of Phe-His dipeptide, was placed ande isoamyl amid of 6-aminohexanoic acid was attached at the end of the molecule (εAhx-Iaa). The preliminary *in vitro* tests indicated that all compounds were inactive. However, they provided valuable information on P_3-P_2 fragment possible structure modification able to produce a resonable renin activity inhibition. All synthesized inhibitors were chymotrypsin-resistant.

Keywords: renin inhibitors, hydrophobic pocket, position P_3 - P_1', inhibitory activity, pseudodipeptide unit, γ-amino acid.

The rennin-angiotensin system (RAS) is an enzymatic hormonal mechanism regulating blood pressure and water-electrolyte management of the organism. Excessive activity of RAS constitutes the pathogenesis of cardiovascular diseases, such as arterial hypertension, congestive heart failure or ischemic heart disease. The crucial link of this system is renin, an enzyme belonging to the class of acidic aspartyl proteases. The key role of renin in the body comprises the conversion of the specific substrate – angiotensinogen – into the decapeptide angiotensin I, by breaking the bond between two amino acids Leu^{10} – Val^{11}. Angiotensin I is then transformed into octapeptide angiotensin II under the influence of angiotensin-converting enzyme (ACE). Angiotensin II exerts negative pressor activity on the cardiovascular system by specific receptors AT_1. Recent years have seen a dynamic development of drugs inhibiting this system, such as convertase inhibitors and antagonists of AT_1 receptor. The introduction of the first renin inhibitor aliskiren (2) into treatment in 2007 opened new perspectives for the treatment of cardiovascular system diseases. The results of contemporary tests conducted in various research facilities on the dependence of action on the structure of renin inhibitors indicate that apart from hydrogen bonds, the hydrophobic ligand – enzyme interaction has the essential impact on the activity (1).

To examine the influence of the interaction of the inhibitor molecule part at its P_3 – P_1' position with hydrophobic sites of renin active centre on the biological activity of the enzyme, we have designed and synthesized five new compounds (see Table 1):

Boc – Phe (4-OMe) – His (N^{im}Bzl) – ACHPA – εAhx – Iaa [**12**]
Boc – Phe (4-OMe) – His (N^{im}Trt) – AEPHPA – εAhx – Iaa [**20**]
Boc – Phe (4-OMe) – His (N^{im}Trt) – AHNA – εAhx – Iaa [**25**]
Boc – Phe (4-OMe) – His (N^{im}Bzl) – AHBA – εAhx – Iaa [**32**]
Boc – Phe (4-OMe) – His (N^{im}Bzl) – AHNA – εAhx – Iaa [**37**]

The compounds were designed on the basis of the amino acid sequence of angiotensinogen fragment 8 – 13,

8 9 10 11 12 13
Phe – His – Leu – Val – Ile – His –
P_3 P_2 P_1 P_1' P_2' P_3'

that in physiological conditions combines with renin as shown in Figure 11.

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Table 1. Physicochemical and analytical properties of the synthesized compounds.

Compd. No.	Structure	Formula m.w.	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ (c, MeOH)	TLC, R_f*	HPLC (% purity)	Log P
4	Boc-His(N^{im}Bzl)-ACHPA-OEt	$C_{31}H_{46}O_7N_4$ 586.71	67.00	144-145	-34.8 (1.0)	0.41 (A)	-	-
8	Boc-Phe(4-OMe)-His-(N^{im}Bzl)-ACHPA-OEt	$C_{39}H_{57}O_8N_5$ 747.44	48.00	Semi-solid	-36.0 (1.2)	0.38 (A)	-	-
12	Boc-Phe(4-OMe)-His(N^{im}Bzl)-ACHPA-Ahx-Iaa	$C_{50}H_{75}O_8N_7$ 902.20	26.00	Semi-solid	-25.0 (1.0)	0.35 (A)	99.57	6.14
14	Boc-Phe(4-OMe)-His(N^{im}Trt)-OMe	$C_{41}H_{44}O_6N_4$ 688.84	80.00	73-76	+18.2 (1.1)	0.64 (A) 0.21 (E)	-	-
15	Boc-AEPHPA-OEt	$C_{20}H_{31}O_6N$ 381.48	78.00	83-85	-22.0 (1.0)	0.16 (B)	-	-
18	Boc-Phe(4-OMe)-His(N^{im}Trt)-AEPHPA-OEt	$C_{55}H_{63}O_8N_5$ 933.65	39.00	94-96	-20.0 (1.0)	0.62 (A)	-	-
20	Boc-Phe(4-OMe)-His(N^{im}Trt)-AEPHPA-Ahx-Iaa	$C_{64}H_{81}O_9N_7$ 1091.41	28.00	Semi-solid	-15.0 (1.1)	0.71 (A)	99.78	9.50
21	Boc-AHNA-OEt	$C_{16}H_{32}O_5N$ 317.41	85.00	49-50	-4.7 (1.4)	0.38 (B)	-	-
23	Boc-Phe(4-OMe)-His(N^{im}-Trt)-AHNA-OEt	$C_{51}H_{63}O_8N_5$ 873.60	47.00	Oil	+28.0 (1.2)	0.64 (A)	-	-
25	Boc-Phe(4-OMe)-His(N^{im}Trt)-AHNA-Ahx-Iaa	$C_{60}H_{81}O_8N_7$ 1028.36	23.00	Oil	+23.0 (1.0)	0.68 (A)	99.04	9.28
26	Boc-AHBA-OEt	$C_{11}H_{21}O_5N$ 247.30	60.00	Semi-solid	-14.7 (1.0)	0.10 (B)	-	-
28	Boc-His(N^{im}Bzl)-AHBA-OEt	$C_{22}H_{34}O_6N_4$ 450.56	8.00	117-123	-21.5 (1.1)	0.63 (D)	-	-
30	Boc-Phe(4-OMe)-His(N^{im}Bz)l-AHBA-OEt	$C_{32}H_{45}O_8N_5$ 627.77	34.00	Semi-solid	-29.4 (1.0)	0.67 (C)	-	-

Table 1. cont.

Compd. No.	Structure	Formula m.w.	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ (c, MeOH)	TLC, R_f^*	HPLC (% purity)	Log P
32	Boc-Phe(4-OMe)-His(N^{im}Bzl)-AHBA-Ahx-Iaa	$C_{43}H_{63}O_8N_7$ 806.04	21.00	Semi-solid	-22.0 (1.2)	0.75 (A)	95.96	7.21
33	Boc-His(N^{im}Bzl)-AHNA-OEt	$C_{29}H_{44}O_6N_4$ 662.82	61.00	97 – 98	-16.0 (1.0)	0.42 (E)	-	-
35	Boc-Phe(4-OMe)-His(N^{im}Bzl)-AHNA-OEt	$C_{36}H_{53}O_8N_5$ 707.36	32.00	Oil	-31.3 (1.0)	0.57 (C)	-	-
37	Boc-Phe(4-OMe)-His(N^{im}Bzl)-AHNA-Ahx-Iaa	$C_{48}H_{71}O_8N_7$ 874.12	19.00	Semi-solid	-26.4 (1.2)	0.71 (D)	96.97	5.92

The elemental analysis results were within ±0.4% of theoretical values. Hydrophobicity of the compounds expressed as log P value was calculated by a computational method (24). Mobile phase systems (v/v) were: CH_3Cl-MeOH 95 : 5 (A), hexane-AcOEt 80 : 20 (B), CH_3Cl-MeOH 50 : 50 (C), CH_3Cl-MeOH 90 : 10 (D), CH_3Cl-MeOH 98 : 2 (E)

In the molecule position P_3 – P_1', crucial for the inhibitory activity, all planned compounds contain non-peptide derivatives of phenylalanylhistidyl-γ-amino acids. In order to increase stability, Leu-Val dipeptide in position P_1 - P_1' sensitive to proteolytic activity of renin, was replaced with hydrophobic pseudodipeptide derivatives of unnatural γ-amino acids AHBA, AHNA, AEPHPA, ACHPA. Previously obtained renin inhibitors containing 4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) exhibited medium or high inhibitory activity (1, 3). Inhibitors containing 4-amino-3-hydroxybutanoic acid (AHBA), 4-amino-3-hydroxynonanoic acid (AHNA), 4-amino-5-(4-ethoxyphenyl)-3-hydroxypentanoic acid (AEPHPA) have not been obtained and tested so far. We have assumed that the structural differences between hydrophobic groups in the applied pseudopeptides would allow us to determine the most optimal interaction of these molecule's fragments with the hydrophobic pocket S_1. In position P_1 the compounds [**25**, **37**] contain unbranched aliphatic side chain (fragment C_5 - C_9 in AHNA molecule). Probably, the long flexible chain, having adopted sufficiently beneficial conformation, will adjust well to the hydrophobic pocket, similarly to cyclohexyl substituent in position 5 of ACHPA molecule, which is confirmed by high inhibitory activity of obtained inhibitors containing this pseudopeptide (4, 5). This beneficial biological effect arises from the capacity of C - C bonds to change position around carbon atom C_5 and the cyclohexane ring capacity to adopt the "chair" conformation, which results in the most beneficial adjustment of the hydrophobic pocket to hydrocarbon radical. It seems interesting to compare the biological activity of the inhibitor with the cycloaliphatic ring [**12**] with compound [**20**] containing an aromatic 4-ethoxyphenyl substituent in position 5 of AEPHPA molecule. Theoretically, lower affinity of the flat, rigid aromatic ring to the hydrophobic pocket, as compared to the cycloaliphatic ring, may be balanced by a small aliphatic ethoxy substituent present in position 4 of the phenyl ring, due to the possible presence of specific subpockets in site S_1, similarly to the case of site S_3. The presence of a moderately polar ethoxy group of the side chain, in fragments P_1 of the inhibitor molecules, due to its probable participation in the hydrogen bonding in sub pockets, may impact not only the activity, but also pharmacokinetic properties such as solubility at physiological pH and bioavailability. Designing compound [**32**] (of minimum hydrophobicity) without a side chain and with a 4-carbon frame of 4-amino-3-hydroxybutanoic acid is to answer the question of whether and in

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

Compd. no.	Solvent	Chemical shifts δ, ppm
4	$CDCl_3$	0.72-1.84 (m, 13H, C_6H_{11}, $2HC_5$); 1.24 (t, 3H, CH_3); 1.42 (s, 9H, C_4H_9); 2.28-2.56 (m, 2H, $2HC_2$); 2.92-3.12 (m, 2H, 2HCβ); 4.05-4.20 (m, 2H, OCH_2); 4.26-4.36 (m, 1H, $1HC_3$); 5.03 (d, *J* = 2Hz, 2H, CH_{2Bzl}); 6.26 (d, *J* = 4 Hz, 1H, NH); 6.59 (d, *J* = 10Hz, 1H, NH); 6.75 (s, 1H, HC^{im}); 7.26 (s, 5H C_6H_5); 7.5 (s, 1H, HC^{im}).
8	$CDCl_3$	0.72-1.86 (m, 15H, $C_6H_{11,}$ $2HC_{2,}$ $2HC_5$); 1.25 (t, 3H, CH_3); 1.36 (s, 9H, C_4H_9); 2.73-3.24 (m, 4H, 2HCβHis, HCαPhe, HC_4); 3.78 (s, 3H, OCH_3); 4.14 (q, 2H, OCH_2); 4.20-4.73 (m, 4H, $2HC_β$Phe, HCαHis, HC_3); 5.01 (s, 2H, CH_2Bzl); 6.64-6.91 (m, 3H, CH^{im}, 2×NH); 6.92-7.20 (m, 4H, C_6H_4); 7.27 (s, 5H, C_6H_5); 7.33 (s, 1H, CH^{im}); 8.25 (d, *J* = 12Hz, 1H, NH).
12	$CDCl_3$	0.72-1.85 (m, 28H, $C_6H_{11,}$ $2HC_5$, 2×CH_3Iaa, $2HC_β$Iaa, HC γ Iaa, 3×CH_2Ahx), 1.36 (s, 9H, C_4H_9); 1.86-2.00 (d, *J* = 12Hz, 2H, $2HC_2$); 3.47 (s, br, 2H, $2HC_β$ His); 4.08 (s, 3H, OCH_3); 4.24-4.51 (m, 10H, CH_2Phe, HC α His, $HC_{3,}$ 2HCαIaa, 2×CH_2Ahx); 4.76 (s, 2H, CH_2 Bzl); 6.64 (d, *J* = 8Hz, 4H, C_6H_4); 6.82 (d, *J* = 8Hz, 1H, NH); 7.78-7.94 (m, 4H, 2×HC^{im}, 2×NH); 8.08 (d, *J* = 8Hz, 1H, NH).
14	$CDCl_3$	1.34 (s, 9H, C_4H_9); 2.86-3.12 (m, 4H, 2×CH_2); 3.59 (s, 3H, OCH_3 ester); 3.72 (s, 3H, OCH_3 ether); 4.11 (q, 1H, HC_2); 4.39 (d, *J* = 5Hz, 1H, NH); 4.77 (q, 1H, CH), 5.23 (d, *J* = 6Hz, 1H, NH); 6.53 (s, 1H, CH^{im}); 7.02-7.38 (m, 20H, C_6H_4, 3×C_6H_5, CH^{im}).
15	$CDCl_3$	1.24 (t, 3H, CH_3 ester); 1.39 (t, 3H, CH_3 ether); 1.41 (s, 9H, C_4H_9); 2.32-2.35 (m, 1H, CH); 2.53-2.62 (m, 1H, CH); 2.84 (d, *J* = 9Hz, 2H, $2HC_5$); 3.62-3.96 (m, 2H, CH_2); 4.00 (q, 2H, OCH_2 ester); 4.13 (q, 2H, OCH_2 ester); 4.92 (d, *J* = 10Hz, 1H, NH); 6.81; 7.14 (dd, *J* = 8Hz, 8 = Hz, 4H, C_6H_4).
18	$CDCl_3$	1.99 (t, 3H, CH_3); 1.33 (s, 9H, C_4H_9); 1.35 (t, 3H, CH_3); 2.39-2.57 (m, 2H, CH_2); 2.75-3.02 (m, 5H, 2×$CH_{2,}$ CH); 3.41 (d, *J* = 3,41Hz, 1H, CH); 3.76 (s, 3H, OCH_3); 3.94-4.12 (m, 6H, 2×OCH_2, CH_2); 4.30 (q, 1H, CH); 4.66-4.78 (m, 1H, CH); 5.41 (s, br, 1H, NH); 6.78-7.45 (m, 21H, 3×C_6H_5, C_6H_4, 2×CH^{im}); 7.72 (s br, 1H, NH); 7.87 (s br, 1H, NH).
20	$CDCl_3$	0.87 (d, *J* = 6Hz, 6H, 2×CH_3Iaa); 1.12-1.46 (m, 8H, $2HC_α$Iaa, HCγIaa, HC_3, 2×CH_2Ahx); 1.24 (s, 9H, C_4H_9); 1.47-1.83 (m, 12H, $2HC_2$, $2HC_5$, $2HC_β$Iaa, 3×CH_2Ahx); 2.16 (t, 3H, CH_3); 2.62-3.00 (m, 2H, 2×HC); 3.79 (s, 3H, OCH_3); 3.89 (s, 2H, CH_2); 3.99 (q, 2H, OCH_2); 4.18 (q, 1H,HC_4); 4.51 (d, *J* = 5Hz, 2H, CH_2); 5.08 (s, 1H, NH); 5.93(s, 1H, NH); 6.65 (s, 1H, NH); 6.75-6.94 (m, 5H, C_6H_4, NH); 6.95-7.50 (m, 21H, 3×C_6H_5, C_6H_4, 2×CH^{im}); 9.39 (s, br, 1H, NH).
21	$CDCl_3$	0.88 (t, 3H, CH_3); 1.24-1.40 (m, 7H, CH_3 ester, 2×CH_2); 1.44 (s, 9H, C_4H_9); 2.43-2.60 (m, 2H, CH_2); 3.50 (s, 1H, NH); 4.06 (s, 1H, CH); 4.16 (q, 2H, OCH_2); 4.77 (d, *J* = 9.6Hz, 1H, NH).
23	$CDCl_3$	0.87 (t, 3H, CH_3 ester); 1.10-1.38 (m, 7H, CH_3, 2×CH_2) 1.41 (s, 9H, C_4H_9); 2.96-3.01 (m, 2H, CH_2His); 3.58-3.65 (m, 2H, CH_2 Phe(4-OMe)); 3.77 (s, 3H, OCH_3); 4.13 (q, 2H, OCH_2); 4.80-4.87 (m, 1H, CH); 6.03 (d, *J* = 9.6Hz, 1H, NH); 6.19(d, *J* = 9.6Hz, 1H, NH); 6.81 (d, *J* = 8.4Hz, 1H, NH); 7.10-7.50 (m, 21H, $C_6H_{4,}$ 3×$C_6H_{5,}$ 2×CH^{im}).
25	$CDCl_3$	0.82-1.56 (m, 26H, C_4H_9, $2HC_5$, 2×CH_3 Iaa, $2HC_β$ Iaa, HCγIaa, 3×CH_2Ahx); 1.92 (d, *J* = 10.2Hz, 2H CH_2); 3.4-3.6 (m, 2H, CH_2His); 3.8 (q, 2H, 2×CH); 4.02-4.40 (m, 5H, CH_2Phe(4-OMe), OCH_3); 6.20-6.22 (dd, *J* = 9.6Hz, *J* = 9.6Hz, 1H, NH); 6.66 (d, *J* = 8.4Hz, 2H, $2HC^{im}$); 7.24-8.20 (m, 21H, $C_6H_{4,}$ 3×C_6H_5, 2×HC^{im}); 7.10-7.16 (m, 1H, NH).
26 28	$CDCl_3$ $CDCl_3$	1.20-1.31 (m, 3H, CH_3 ester); 1.45 (s, 9H, C_4H_9); 2.41-2.58 (m, 2H, CH_2); 3.03-3.39 (m, 2H, CH_2); 4.06-4.28 (m, 3H, OCH_2, CH); 5.04 (s, br,1H, NH). 1.25 (s, 3H, CH_3 ester); 1.41 (s, 9H, C_4H_9); 1.90-1.94 (m, 2H, CH_2); 3.11 (d, 2H, CH_2); 4.07-4.20 (m, 3H, HCβ, OCH_2); 4.45 (s,br, 1H, CHα His); 5.09 (s, 2H, CH_2Bzl); 5.85 (s, br, 1H, NH); 5.88 (s, br, 1H, NH); 6.74 (s, 1H, HC^{im}); 7.15-7.37 (m, 5H, C_6H_5), 7.74 (s, 1H, HC^{im}).
30	$CDCl_3$	1.25-1.42 (m, 12H, C_4H_9, CH_3 ester); 1.9 (d, *J* = 9Hz, 2H, $2HC_2$); 3.05 (d, *J* = 5.4Hz, 2H, $2HC_β$His); 3.4-3.8 (m, 9H, CH_2Phe, CH_2Bzl, HC_aHis, HC_3, OCH_3); 4.95-4.38 (m, 2H, OCH_2); 4.55 (d, *J* = 7.2Hz, 1H, HC); 4.95 (d, *J* = 7.2Hz, 1H, HC); 6.78-7.13 (m, 7H, C_6H_5, $2HC^{im}$).

Table 2. cont.

Compd. no.	Solvent	Chemical shifts δ, ppm
32	$CDCl_3$	0.91-1.92 (m, 26H, C_4H_9, $2HC_5$, $2\times CH_3$Iaa, $2HC_\beta$Iaa, HC_γIaa, $3\times CH_2$Ahx); 1.93-2.15 (m, 4H, $2HC_2$ Ahx, $2HC_5$ (Phe-4OMe); 3.01 (s, br, 2H, $2HC_\beta$His); 3.41-3.59 (m, 1H, HC); 3.67-3.83 (m, 5H, OCH_3, CH_2Phe); 4.12-4.19 (m, 4H, CH_2Bzl, 2HCPhe); 4.5 (d, *J* = 6.3 Hz, 1H, HC), 4.9 (d, *J* = 7.2Hz, 1H, HC); 5.12-5.38 (m, 7H, C_6H_5, $2HC^{im}$).
33	$CDCl_3$	0.85 (t, 3H, CH_3), 1.18-1.38 (m, 16H, C_4H_9, CH_3ester, $2\times CH_2$); 2.32-2.52 (m, 2H, $2HC_2$); 2.88-3.22 (m, 2H, $2HC_\beta$); 4.5 (s, 1H, HC_3); 5.11 (d, *J* = 5.2Hz, 2H, CH_2Bzl); 5.4-5.73 (m, 4H, HC_αHis, CH_3); 5.92 (s, 1H, NH); 6.16 (s, 1H, NH); 6.83 (d, *J* = 8.7Hz, 1H, CH^{im}); 7.03-7.90 (m, 8H, C_6H_5, CH, 2CH); 7.68 (d, *J* = 10.2Hz, 1H, HC^{im}).
35	$CDCl_3$	0.85 (t, 3H, CH_3 ester); 1.2-1.34 (m, 16H, C_4H_9, $2HC_2$ $2HC_5$, CH_3); 1.5-1.6 (m, 2H, $2HC_2$); 2.26-2.44 (m, 2H, $2HC_\beta$); 2.78-3.18 (m, 5H, HC_αHis, HC_3, CH_2Bzl, HC_αPhe); 3.74 (s, 3H, OCH_3); 4.12 (q, 2H, OCH_3); 4.30 (d, *J* = 6Hz, 1H, NH); 4.76 (q, 1H, HC_3); 5.06 (d, *J* = 12Hz, 2H CH_2Bzl); 5.36 (d, *J* = 6.4Hz, 1H, NH); 5.6-5.9 (s, br, 4H HC_αHis, CH_3); 7.06 (d, *J* = 8.6Hz, 1H, HC^{im}); 7.14-7.40 (m, 12H, C_6H_4, C_6H_5, CH, 2HC); 7.75 (d, *J* = 4Hz, 1H, HC^{im},); 8.04 (d, *J* = 6.6Hz, 1H, NH).
37	$CDCl_3$	0.76-0.96 (m, 6H, $2\times CH_3$Iaa); 1.02-1.76 (m, 26H, $2HC_\alpha$Iaa, HC_γIaa, $2HC_3$, C_4H_9, $2HC_2$, $2HC_5$, $2HC_\beta$Iaa, $3\times CH_2$Ahx); 2.43 (s, 2H, $2HC_\beta$); 2.9-3.1 (m, 2H, CH_2His); 3.3-3.5 (s, br, 1H, CH); 3.72 (s, 3H, OCH_3); 3.6-3.75 (m, 5H, CH_2Phe, CHPhe, CH_2Bzl); 3.9 (d, *J* = 9Hz, 1H, CH); 4.48 (s, 1H, NH); 4.95 (s, 1H, NH); 6.72 (d, *J* = 7.8Hz, 1H, HC^{im}); 7.2-7.32 (m, 11H, C_6H_4, C_6H_5, 2HC); 7.33 (d, *J* = 4.8 Hz, 1H, HC^{im}).

what way the hydrophobic activity of only a fragment C_1-C_4 of molecule with probable domination of the hydrogen bond OH bounding the designed rennin inhibitors to S_1 area of rennin fragment. In all obtained compounds, there is an unnatural fragment Phe (4-OMe)- His (N^{im}Bzl) [**12**, **32**, **37**] and Phe (4-OMe)- His (N^{im}Trt) [**20**, **25**] at position P_3 - P_2. In the digestive tract the natural dipeptide is subject to proteolysis catalyzed by chymotrypsin. To achieve stability of renin inhibitors in the digestive tract the site P_3 - P_2 should be modified in such a way so as to prevent or significantly hamper formation of the hydrogen and hydrophobic bonds of this fragment with the active centre of chymotrypsin. The substitution of the phenyl ring in position 4 with methoxy group (Phe (4-OMe)) decreases the affinity to chymotrypsin without hampering its capacity to bind with the active centre of renin (6). Position P_3 absolutely requires the presence of the aromatic ring, which is evidenced by previous synthesis of active inhibitors containing in this position 4-metoxyphenyl ring (7), as well as obtaining inactive inhibitors devoid of this ring (8). This arises from the fact that the crucial element of the active site S_3 is the subpocket S_{3sp} (Figure 11), which is specific to the phenyl ring (9, 10).

It is now a well-known fact that the structural modification of position P_2 plays a significant role for the activity and selectivity of analogs. A large, bifurcated hydrophobic pocket S_2 of renin may adjust both hydrophobic and polar pharmacophores, thus enabling significant modification of the enzyme – ligand interaction. The fact that the conformation of the side chain in P_2 is not strictly dependent on the location of the bond shows that exact prediction of the enzyme – ligand interaction is relatively difficult (1, 11-13). Our team previously obtained active inhibitors of renin containing aliphatic amino acids with a small hydrophobic side chain instead of histidine (His) in position P_2 (7, 14, 15). This fact suggests that apart from the formation of a hydrogen bonding with the nitrogen atom of the imidazole ring, there is a possibility of simultaneous presence of significant hydrophobic reaction with a hydrophobic subpocket in site S_2. To verify these hypotheses, in designed inhibitors, we introduced into their position P_2 a histidine molecule substituted at the nitrogen atom in the imidazole ring with large substituents with hydrophobic properties. It seems interesting to compare the location of the benzyl substituent (Bzl) with one rigid aromatic ring [**12**, **32**, **37**] in the subpocket S_2, with the analogue containing a bifurcated 3-ring triphenylmethyl substituent (Trt) characterized by increased hydrophobicity [**20**, **25**].

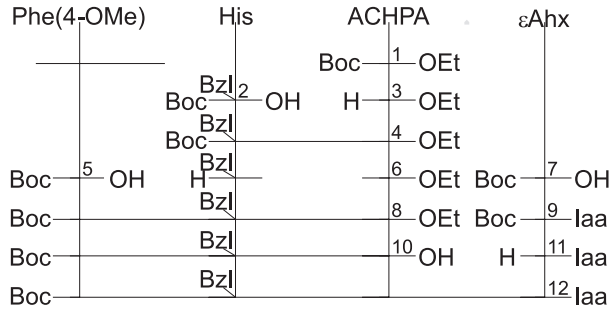

Figure 1. Synthesis of Boc–Phe (4-OMe)–His (N^{im}Bzl)–ACHPA–εAhx–Iaa [**12**]

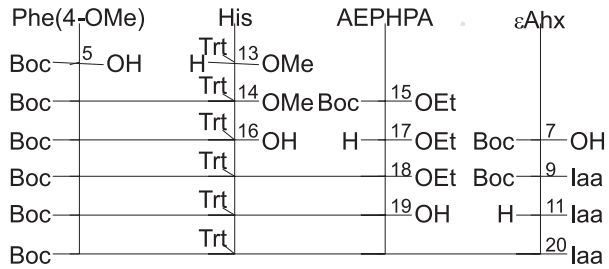

Figure 2. Synthesis of Boc–Phe (4-OMe)–His (N^{im}Trt)–AEPHPA–εAhx–Iaa [**20**]

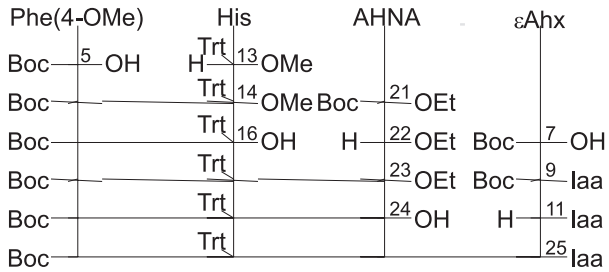

Figure 3. Synthesis of Boc–Phe (4-OMe)–His (N^{im}Trt)–AHNA–εAhx–Iaa [**25**]

Moreover, the presence of spatially large side chains in P_2 may protect the peptide bond in position P_3 - P_2 against the activity of chymotrypsin. In all five renin inhibitors obtained by us, the tert-butoxycarbonyl (t-Boc) substituent was placed at the N-end, and isoamylamide of 6-aminohexanoic acid (εAhx-Iaa) was introduced into position P_2' - P_3'. It is assumed that hydrophobic grouping with branched alkyl structure (t-Boc) may improve the absorption from the digestive tract due to the increase in lipophilicity (16). Branched alkylamide of the unnatural amino acid of linear and flexible structure (εAhx-Iaa)

placed in position P_2' - P_3' protects the C-end of the molecule from enzymatic degradation and may have inhibitory effect due to good affinity to the hydrophobic site S_2' - S_3' of the active centre of renin, which is confirmed by the numerous active and stable inhibitors containing εAhx-Iaa at C-end that we have obtained in recent years (3, 17-19).

EXPERIMENTAL

Chemistry

The structures of inhibitors considered in the present work are shown in Figures 6 to 10. The inhibitors [**12**, **20**, **25**, **32**, **37**] as well as their intermediates were synthesized according to the schemes presented in Figures 1 to 5. The applied methods are

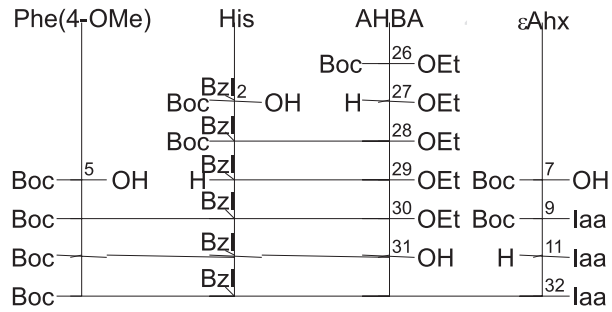

Figure 4. Synthesis of Boc–Phe (4-OMe)–His (N^{im}Bzl)–AHBA–εAhx–Iaa [**32**]

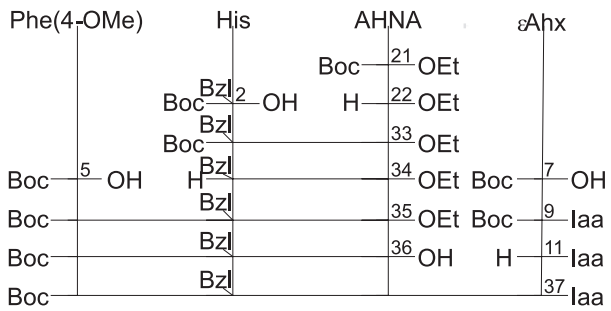

Figure 5. Synthesis of Boc–Phe (4-OMe)–His (N^{im}Bzl)–AHNA–εAhx–Iaa [**37**]

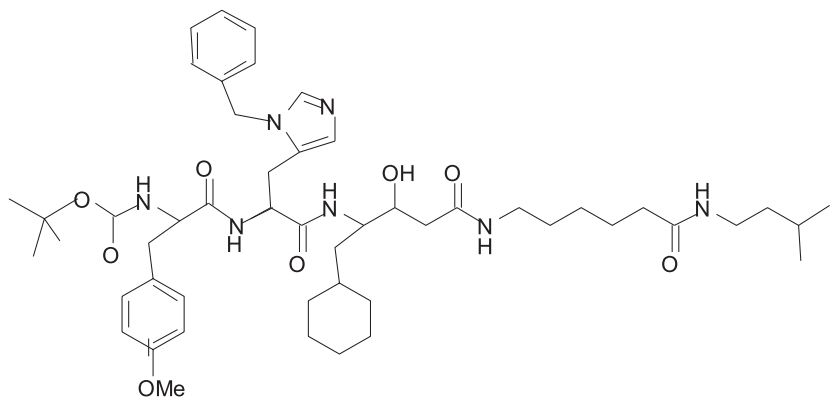

Figure 6. [N-(tert-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{im}benzylhistidyl-(3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoyl-ε-aminohexanoic acid isoamylamide Boc–Phe (4-OMe)–His (N^{im}Bzl)–ACHPA–εAhx–Iaa [**12**]

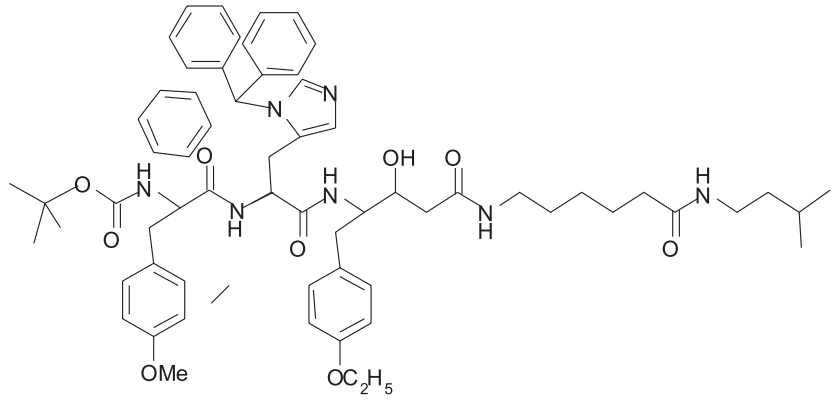

Figure 7. [N-(tert-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{im}tritylhistidyl-(3S, 4S)-4-amino-5-(4-ethoxyphenyl)-3-hydroxypentanoyl-ε-aminohexanoic acid isoamylamide Boc – Phe (4-OMe) – His (N^{im}Trt) – AEPHPA – εAhx – Iaa [**20**]

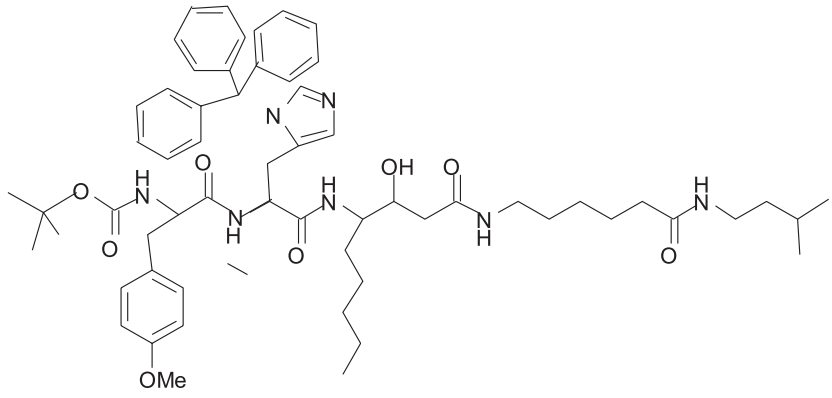

Figure 8. [N-(tert-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{im}tritylhistidyl-(3S, 4S)-4-amino-3-hydroxynonanoyl-ε-aminohexanoic acid isoamylamide Boc – Phe (4-OMe) – His (N^{im}Trt) – AHNA – εAhx – Iaa [**25**]

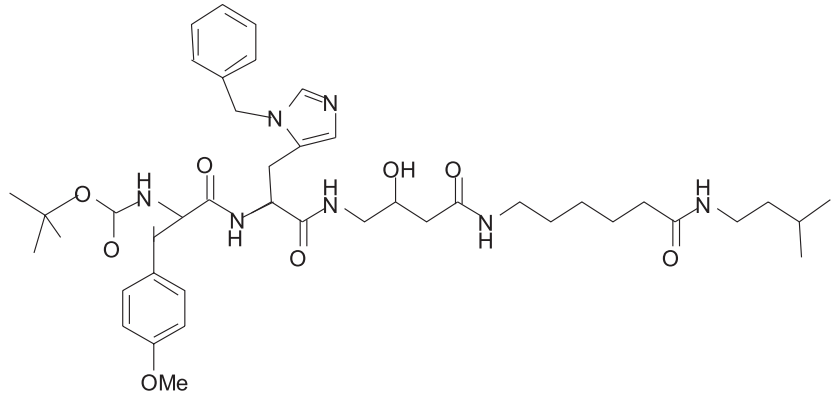

Figure 9. [N-(tert-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{im}benzylhistidyl-(3S, 4S)-4-amino-3-hydroxybutanoyl-ε-aminohexanoic acid isoamylamide Boc – Phe (4-OMe) – His (N^{im}Bzl) – AHBA – εAhx – Iaa [**32**]

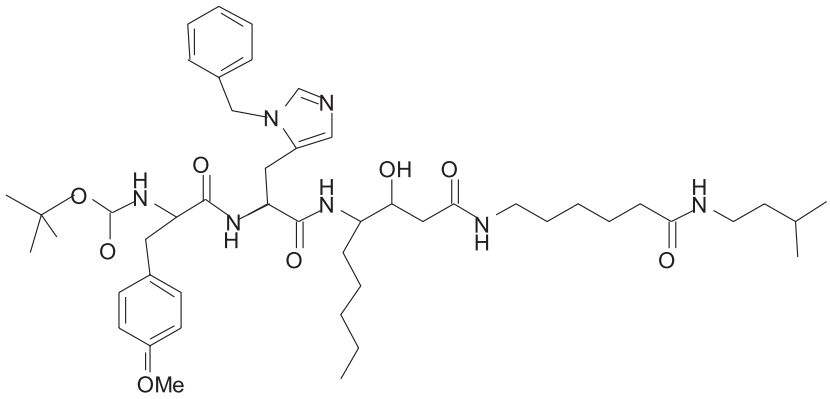

Figure 10. [N-(tert-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{im}benzylhistidyl-(3S, 4S)-4-amino-3-hydroxynonanoyl-ε-aminohexanoic acid isoamylamide Boc – Phe (4-OMe) – His (N^{im}Bzl) – AHNA – εAhx – Iaa [**37**]

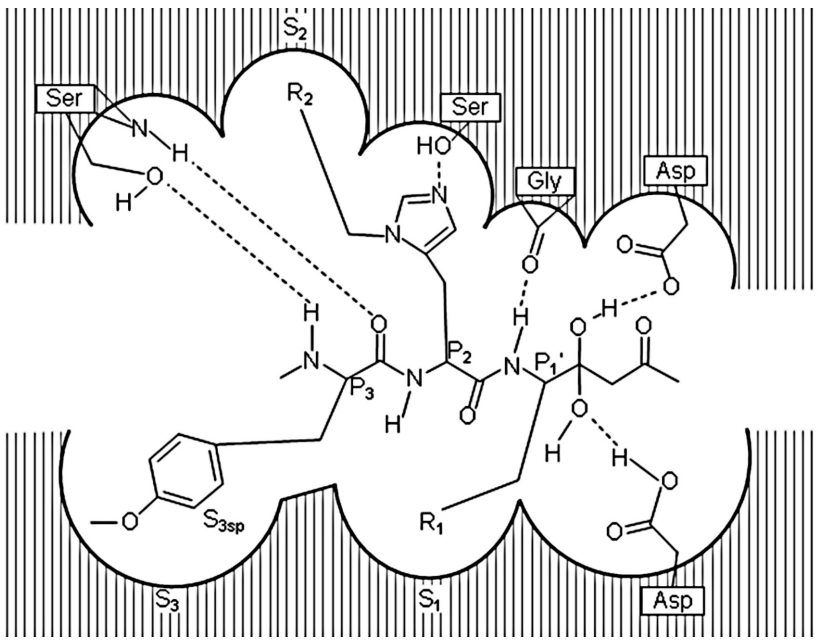

Figure 11. Schematic interaction mode of P_3-P_1 fragment with hydrophobic pocket of renin active center; R_1 = cyclohexyl, butyl or 4-ethoxyphenyl, R_2 = benzyl or trityl

specified below in the syntheses section. Physicochemical properties of the inhibitors, as well as their newly synthesized intermediates [**4**, **8**, **14**, **15**, **18**, **21**, **23**, **26**, **28**, **30**, **33**, **35**] are presented in Tables 1 and 2.

Reagents Boc - Phe (4-OMe) - OH, His (N^{im}Bzl) - OEt, His (N^{im}Trt) - OEt, porcine kidney renin and N-acetylrenin substrate tetradecapeptide were acquired from recognized vendor. ACHPA was synthesized according to the Maibaum protocol (20). 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 (21). Column chromatography (CC) on silica gel (Merck, grade 230 to 400 mesh) was used to separate and purify all synthesized com-

pounds. TLC was carried out on 0.25 mm thickness silica gel plates (Merck, silica gel 60 F254). 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). Perkin-Elmer Microanalyser was used to carry out elemental analyses. Böetius apparatus was used to determine melting points. Bruker DM 300 MHz Avance 300 WB spectrometer was applied to record ^{1}H NMR spectra. Chemical shifts were measured relative to tetramethylsilane (TMS) as δ units (ppm). Optical rotations were measured at the Na-D line with the use of AP-300 (Atago) polarimeter in a 5 cm polarimeter cell. HPLC analyses of purity and activity of synthesized inhibitors were performed on a Shimadzu apparatus equipped with a pump LC-10AT, detector UV SPD-10A and recorder Chromax 2001. The peaks were recorded at 213 nm. The separation was carried out in the reverse phase system (Ultrasphere C8, Wild Pore C8, Symmetry C18) with various mobile phases.

Syntheses

Introduction of the N-tert-Boc group

This group was introduced in a commonly used manner (22).

Removal of the N-tert-Boc group

Boc-amino acid or Boc-peptide (1 mmol) in a solution of 4 M HCl in dioxane was stirred at room temperature for 30 min. The solution was concentrated *in vacuo*, then the residue was evaporated twice with diethyl ether and dried *in vacuo* (23).

Esterification and hydrolysis

Boc-amino acids were esterified with CH_3I or C_2H_5I as described earlier (16). Boc – ACHPA – OEt, Boc – AHNA – OEt, Boc – AEPHPA – OEt and Boc – AHBA – OEt were formed from monoethyl malonate used to prepare these compounds (18). Alkaline hydrolysis of ester group was carried out as described earlier (20).

Coupling reaction with DCC/HOBt

The coupling was performed in a commonly used manner by fragment condensation as shown in schemes in Figures 1-5.

Biochemical assay

Determination of the inhibition of renin activity

Renin inhibiting activity of the synthesized potential inhibitors was determined *in vitro*. HPLC method was used to determine the concentration of renin substrate The activity of the compounds was tested in the following concentrations: 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9},10^{-10}, 10^{-11} M. All synthesized compounds showed no inhibiting activity, even in the highest tested concentration 10^{-5} M.

RESULTS AND DISCUSSION

Preliminary *in vitro* tests of the synthesized compounds showed a lack of renin-inhibiting effect of all five new potential inhibitors and their resistance to chymotrypsin. However, the lack of inhibitory activity of compounds: [**12**, **20**, **25**, **32**, **37**] containing histidine with hydrophobic benzyl and trityl (Trt) substituents in position P_2 is likely to result from the difficulty for big rigid and ring-type substituents to fit to subpocket S_2. Therefore, it should be assumed that straight or branched flexible alkyl chains capable of adopting a proper conformation in subpocket S_2 may be the most optimal substituent of imidazole nitrogen atom of histidine. Quite contrary, a big hydrophobic trityl or benzyl substituent constitutes an effective spatial obstacle which in combination with Phe (4-OMe) in position P_3 results in stabilization of P_3 - P_2. Low solubility of these compounds at certain concentration causing difficulties during *in vitro* activity tests may be yet another reason for absence of biological activity of designed and obtained inhibitors. This low solubility is caused by high lipophilicity of a molecule resulting from the presence of hydrophobic side chains and hydrophobic branched structures of N- and C-end, however, the objective of our tests was to verify the determinants of maximum interaction of the molecule with all hydrophobic sites of the active centre of renin and the influence of this interaction on inhibitory activity and stability of inhibitors.

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Received: 18. 03. 2013