

NEW RENIN INHIBITORS – STABILITY AND ACTIVITY DETERMINATION.
PART IIDOROTA MARSZAŁEK^{1*}, ANNA GOLDNIK¹, ALEKSANDER P. MAZUREK², IWONA WINIECKA¹,
PAWEŁ JAWORSKI¹, SANDRA SZERSZAN¹, EWA KOZIKOWSKA¹ and TADEUSZ PAWLIK¹¹Department of Drug Chemistry, Medical University of Warsaw, 1 Banacha St., 02-097 Warszawa, Poland²National Medicines Institute, 30/34 Chełmska St., 00-725 Warszawa, Poland

Abstract: A series of new six pseudodipeptic potential renin inhibitors were synthesized. Enzymatic stability for all compounds (**1–6**) in homogenates (liver, kidney, lung) and body fluids (serum, gastric, intestinal juice) and α -chymotrypsin was determined. Compound **4** was stable, compound **5** was unstable and compounds **1**, **2**, **3**, **6** were partly unstable. Inhibitory activity of the compounds was measured *in vitro* by HPLC determination of lowering concentration of substrate (angiotensinogen) in the presence of renin and the potential renin inhibitor (compounds **1–6**). Compound **4**, **5**, **6** showed inhibitory activity (0.9×10^{-6} , 1.3×10^{-5} , 2.2×10^{-6} M, respectively). Other compounds showed no inhibitory activity up to 10^{-5} M.

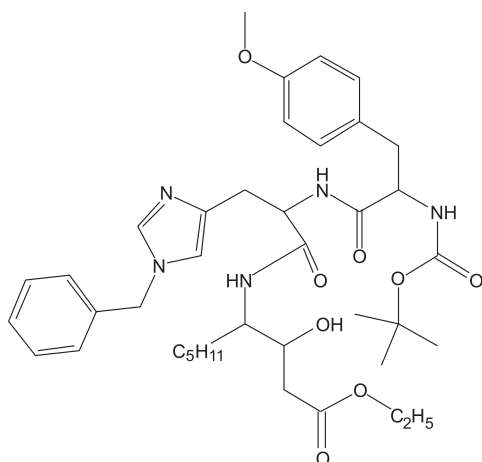
Keywords: HPLC, inhibition of renin activity, renin inhibitors

Hypertension is a major risk for cardiovascular and kidney diseases. Many of patients with hypertension, using antihypertensive drugs, do not have their blood pressure controlled to recommended target levels ($< 140/90$). Therefore, the renin-angiotensin system has been a highly attractive biological target for new drugs, which could regulate the blood pressure. Inhibition of angiotensin converting enzyme (ACE) has led to development of effective antihypertensive drugs. The other possibility to interrupt of renin-angiotensin system is to inhibit renin. Renin, an aspartyl protease, is involved in the first step of enzymatic cascade. Numerous laboratories have tried to develop clinically effective direct renin inhibitors of high potency and stability (**1–11**). The purpose of these trials was to search for new active inhibitors with simple structure, good bioavailability and easy synthesis. The first in a new class of oral, nonpeptide direct renin inhibitor for treatment of hypertension is aliskiren. The structure of aliskiren differs from the structure of 8–13 human angiotensinogen fragment. Searching for a new inhibitors, a series of compounds with dipeptide replacement [(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA)] in their molecules were synthesized (**12**). Some of them comprised two additional analogs of dipeptide: (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) and (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine). The inhibitory activity was low, so the authors conclude that renin inhibitors

ought to have one or two, but not three dipeptide analogs in the molecule. Bock et al. synthesized a series of statine-containing tetrapeptides, modified at the carboxy terminus with hydrophobic aromatic groups (**13**). The Phe amide group, known to be a site of hepatic metabolism, was replaced with nonpeptidic groups. It caused the reduction of peptide chain length but also lowering of activity. Searching for more active renin inhibitors the pentapeptide BOC-Phe-Phe-difluorostatone-Leu-Phe-NH₂ was synthesized (**14**). This compound contains a difluoromethylene ketone group. It showed an increase in inhibiting activity in animal models.

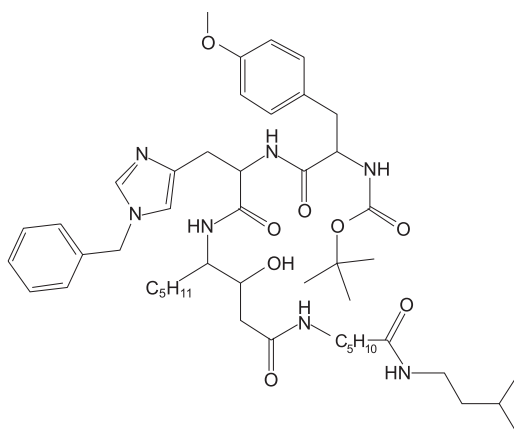
In our previous paper, we presented a series of new six pseudodipeptic potential renin inhibitors. Enzymatic stability for all compounds (**1–6**) in homogenates (liver, kidney, lung) and body fluids (serum, gastric, intestinal juice) and α -chymotrypsin was determined. Inhibitory activity of the compounds was measured *in vitro* by HPLC determination of lowering concentration of substrate (angiotensinogen) in the presence of renin and the potential renin inhibitor (compounds **1–6**). Compound **2** and **4** showed inhibitory activity 1.4×10^{-6} and 0.9×10^{-6} M, respectively, and compound **6** – 1.5×10^{-7} M. Other compounds showed no inhibitory activity up to 10^{-5} M. To continue search for the effective renin inhibitors, 6 new modified compounds of potentially inhibiting activity were synthesized. The chemical structures of them are presented in Figure 1.

* Corresponding author: e-mail: dorota.marszalek@wum.edu.pl; phone: 48 22 5720630, fax 48 22 5720697



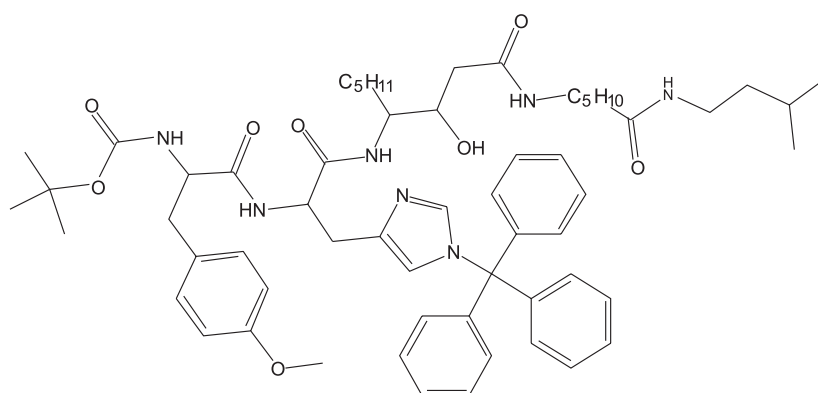
Boc-Phe(4-OMe)-His(N^{imm}BZL)-AHNA-OEt

Compound 1 – [N-(t-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{imm}benzylhistidyl-(3S,4S)-4-amino-3-hydroxynonanoyl-Ĺ-amino-hexanoic acid ethyl ester



Boc-Phe(4-OMe)-His(N^{imm}BZL)-AHNA-εAhx-Iaa

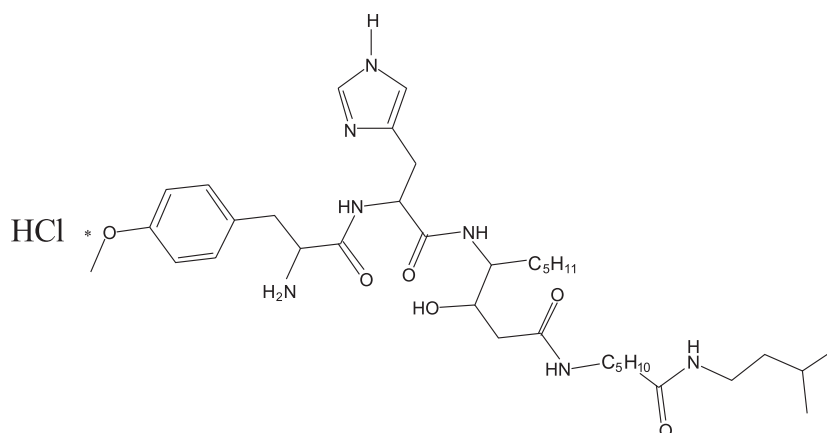
Compound 2 – [N-(t-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{imm}benzylhistidyl-(3S,4S)-4-amino-3-hydroxynonanoyl-ε-amino-hexanoic acid isoamylamide



Boc-Phe(4-OMe)-His(N^{imm}trityl)-AHNA-εAhx-Iaa

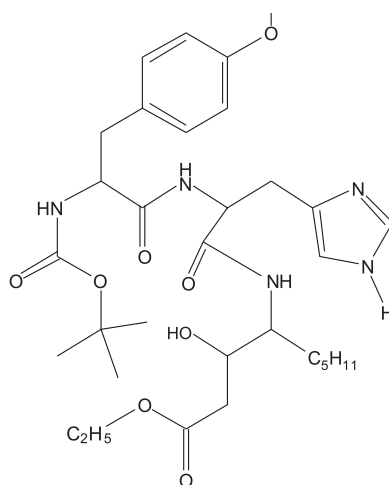
Compound 3 – [N-(t-butoxycarbonyl)-4-methoxyphenylalanyl]-N^{imm}tritylhistidyl-(3S, 4S)-4-amino-3-hydroxynonanoyl-ε-amino-hexanoic acid isoamylamide

Figure 1. Chemical structure of 6 new renin inhibitors



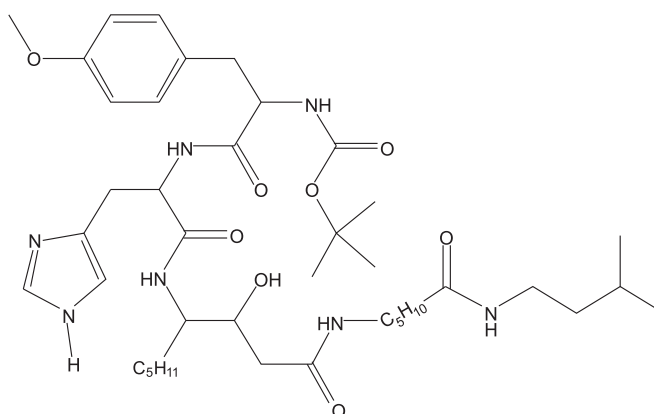
Phe(4-OMe)-His-AHNA-εAhx-Iaa × HCl

Compound 4 – 4-methoxyphenylalanyl-histidyl-(3S,4S)-4-amino-3-hydroxynonanoyl-ε-aminohexanoic acid isoamylamide hydrochloride



Boc-Phe(4-OMe)-His-AHNA-OEt

Compound 5 – [N-(t-butoxycarbonyl)-4-methoxyphenylalanyl]-(3S,4S)-4-amino-3-hydroxynanoylethyl ester



Boc-Phe(4-OMe)-His-AHNA-εAhx-Iaa

Compound 6 – [N-(t-butoxycarbonyl)-4-methoxyphenylalanyl]-(3S,4S)-4-amino-3-hydroxynonanoyl-ε-aminohexanoic acid isoamylamide

Figure 1. cont

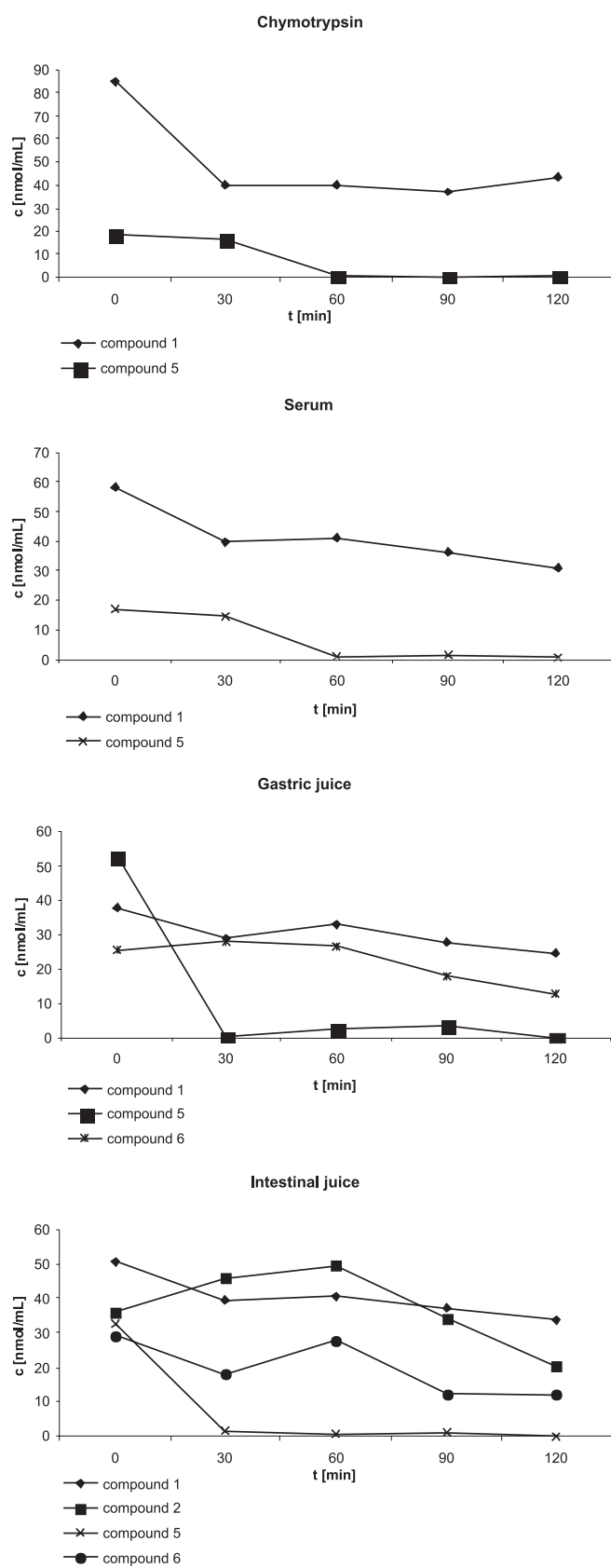


Figure 2. Stability of compound 1-6 in body fluids and organs (the plots concentration vs. time)

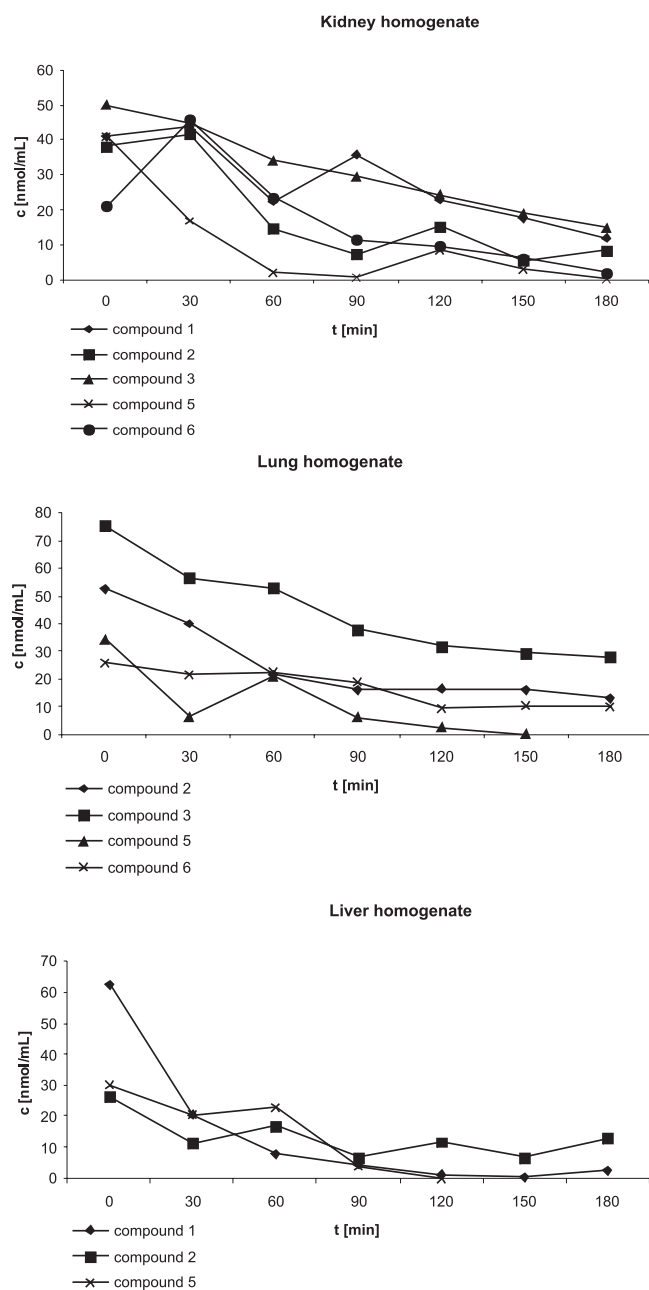


Figure 2. cont.

The purpose of this study was to determine the stability of the new synthesized compounds in homogenates of body organs and body fluids (*in vitro*) and to check the inhibiting activity of six potential renin inhibitors (*in vitro*).

EXPERIMENTAL

Materials and reagents

New renin inhibitors synthesized in the

Department of Drug Chemistry, Medical University of Warsaw are presented in Table 1. Chemical structures are shown in Figure 1.

Renin human and α -chymotrypsin from bovine pancreas were purchased from Sigma. Angiotensinogen was purchased from Bachem.

Apparatus and methods

The details are the same as in the preceding paper.

Table 1. Characterization of new synthesized renin inhibitors.

Compd. no.	Compound	Formula	M _r
1	Boc-Phe(4-OMe)-His(BZL)-AHNA-OEt	C ₃₈ H ₅₅ N ₃ O ₇	693.89
2	Boc-Phe(4-OMe)-His(BZL)-AHNA-Ahx-Iaa	C ₄₇ H ₇₁ N ₇ O ₈	862.12
3	Boc-Phe(4-OMe)-His(trit)-AHNA-Ahx-Iaa	C ₆₀ H ₈₁ N ₇ O ₈	1028.36
4	Phe-(4-OMe)-His-AHNA-Ahx-Iaa · HCl	C ₃₅ H ₅₈ N ₇ O ₆ Cl	708.36
5	Boc-Phe(4-OMe)-His-AHNA-OEt	C ₃₁ H ₄₉ O ₇ N ₅	603.761
6	Boc-Phe(4-OMe)-His-AHNA-Ahx-Iaa	C ₄₀ H ₆₅ O ₈ N ₇	772.001

Boc – *tert*-butoxycarbonyl, Iaa – isoamylamide, Ahx – 6-aminohexanoic acid, AHNA – 4-amino-3-hydroxynonanoic acid, Phe(4-OMe) – 4-methoxyphenylalanine

Table 2. Parameters of chromatographic determination and validation procedure of determined compounds.

Compd. no.	Column	Mobile phase (v/v/v)	r	Recovery (%)	CV (%)	Extraction
1	Beckman Ultrasphere Octyl (150 × 4.6 mm)	ACN–H ₂ O–H ₃ PO ₄ (42 : 58 : 0.1)	0.9988	103.14 ± 8.12	4.96 ± 2.37	alkaline 71%
2	Beckman Ultrasphere Octyl (150 × 4.6 mm)	MeOH–H ₂ O–H ₃ PO ₄ (65 : 35 : 0.1)	0.9926	100.36 ± 5.03	6.8 ± 1.64	alkaline 80%
3	Discovery Wide Pore C ₈ (150 × 4.6 mm)	MeOH–H ₂ O–H ₃ PO ₄ (40 : 60 : 0.1)	0.9999	100.13 ± 1.87	5.11 ± 1.65	acidic 67%
4	Beckman Ultrasphere Octyl (150 × 4.6 mm)	ACN–acetate buffer pH 4.0 (40 : 60)	0.9932	101.99 ± 9.05	5.82 ± 1.65	acidic 67%
5	Symmetry C ₁₈ (150 × 4.6 mm)	ACN–H ₂ O–H ₃ PO ₄ (80 : 20 : 0.1)	0.9908	100.84 ± 5.95	4.06 ± 2.95	acidic 64%
6	Beckman Ultrasphere Octyl (150 × 4.6 mm)	ACN–H ₂ O–H ₃ PO ₄ (80 : 20 : 0.1)	0.9940	98.17 ± 6.00	6.83 ± 2.83	alkaline 47%

Table 3. Stability of compounds 1–6.

Compd. no.	α-Chymotrypsin	Serum	Gastric juice	Intestinal juice	Kidney homogenate	Lung homogenate	Liver homogenate
1	unstable	first-order reaction t _{0.5} = 157 min	unstable	first-order reaction t _{0.5} = 239 min	unstable	stable	unstable
2	stable	stable	stable	unstable	unstable	first-order reaction t _{0.5} = 92 min	unstable
3	stable	stable	stable	stable	first-order reaction t _{0.5} = 103 min	first-order reaction t _{0.5} = 122 min	no results*
4	stable	stable	stable	stable	stable	stable	stable
5	unstable	first-order reaction t _{0.5} = 25.7 min	unstable	unstable	unstable	unstable	unstable
6	stable	stable	unstable	unstable	first-order reaction t _{0.5} = 50 min	first-order reaction t _{0.5} = 111.8 min	stable

* it was not possible to isolate compound 3 from biological matrix due to very strong hydrophobic bonds.

Table 4. Inhibiting activity of compounds 1–6.

Compound no.	Human renin IC ₅₀ (M)
1	inactive > 10 ⁻⁵
2	inactive > 10 ⁻⁵
3	inactive > 10 ⁻⁵
4	0.9 × 10 ⁻⁶
5	1.3 × 10 ⁻⁸
6	2.2 × 10 ⁻⁶

Determination of enzymatic stability of compounds 1–6 *in vitro*

The stability of compounds 1–6 in body fluids and organ homogenates was examined similarly as described in the preceding paper.

Determination of inhibition activity vs. human renin of compounds 1–6

Renin inhibiting activity of the synthesized potential inhibitors 1–6 was determined *in vitro* similarly as described in the preceding paper. The results are presented in Table 4.

RESULTS AND DISCUSSION

Determination of enzymatic stability and determination of inhibiting activity of compounds 1–6 against human renin have been performed in test *in vitro*. According to the results of that investigation it was shown that compound 1, 2 and 3 showed no inhibiting activity. The presence of large trityl substituent of His in the structure of compound 3 caused increased strength of hydrophobic bonds with biological matrix, which made extraction of that compound from liver homogenate impossible. A removal of Boc group of Phe and substituent of His in compound 4 caused that it was stable in all homogenates and body fluids. It is possible to conclude that active renin inhibitors should contain amino acid His without any substituents. Compound 5, which contained ester bond in C-terminus, was totally metabolically unstable but showed the highest activity IC₅₀ = 1.3 × 10⁻⁸ M. A removal of Boc group and conversion of compound 4 to hydrochloride increased twice the inhibiting activity. It could be caused by an increase of solubility. This compound was also more stable. The Ahx-Iaa substituent in C-terminus of compounds 2 and 3 protected C-terminus and increased the metabolic stability but reduced inhibiting activity.

The search for other compounds with expected higher potency will be continued.

Acknowledgments

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