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New renin inhibitors with hydrophilic C-terminus

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Four new peptide-based renin inhibitors, Boc-Phe(4-OMe)-MePhe-AHPPA-εAhx-EA (**11**), Boc-Phe(4-OMe)-MeLeu-AHPPA-εAhx-EA (**15**), Boc-Phe(4-OMe)-MePhe-Sta–εAhx-EA (**20**) and Boc-Phe(4-OMe)-MeLeu-Sta-εAhx-EA (**21**) have been synthesized in search of structures with improved biological properties. They were designed as compounds with moderate hydrophobicity (5.28, 4.79, 4.79 and 4.30), respectively. All synthesized inhibitors were resistant to chymotrypsin activity, all were poorly solubile in buffers pH 2.0 and pH 7.4. The inhibitory potency of renin activity *in vitro* of **11**, **15**, **20** and **21** expressed as IC_{50} was 7.0×10^{-4}, 7.5×10^{-5}, 6.0×10^{-4} and 2.5×10^{-4} M/l, respectively.

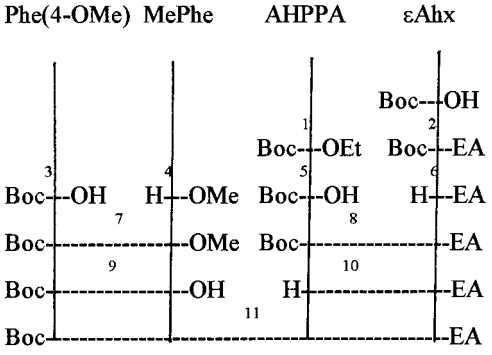
1. Introduction

Renin inhibitors may be useful drugs in the treatment of hypertension and heart failure especially because of their renal substrate specificity greater than that of angiotensin converting enzyme inhibitors. However, the therapeutic application is limited due to poor resorption from the gastrointestinal tract into the systemic circulation and rapid biliary excretion. Evidently the inhibitors should be widely resistant to proteolytic degradation. Protection of the inhibitors against proteolytic enzymes is possible by substitution of natural amino acids with unnatural ones. The activity depends on the complete structure of the inhibitor molecule. The molecular mechanisms of the biliary excretion are not well understood. Probably different endogenous carrier systems are involved in hepatocellular uptake and biliary excretion. Peptide-mimicking drugs differing in structure, size and physico-chemical properties are taken up and excreted into bile by different transport systems [1]. Oral absorption of peptide-based renim inhibitors depends upon their intestinal transport. It was shown that higher intensity correlates well with higher lipophilicity [2, 3]. However, limits of hydrophilicity are difficult to find.

Oral bioavailability can be limited by low solubility of the inhibitors. It is a serious problem, that compounds positively charged dissolve relatively well in acidic solution, but are precipitated in the small intestine at neutral pH. On the other hand, the inhibitors negatively charged show low intrinsic membrane permeability. Therefore structures of well absorbed renin inhibitors should probably be uncharged. Unfortunately, such molecules are rather poorly soluble. It is possible to overcome this problem using additives increasing the solubility, for instance cyclodextrins which improve the low solubility of some inhibitors [4]. Boyd et al. [5] synthesized a set of well absorbed nonpeptidic renin inhibitors which show bioavailability >50% in dog. Log P values of the best of them amount to 4–5. These results suggest to design renin inhibitor molecules of low molecular weight, uncharged and with Log P value within 4–5. These molecules must have structures securing renin affinity and resistance to proteolytic enzymes.

Scheme 1

Synthesis of Boc-Phe(4-OMe)-MePhe-AHPPA-εAhx-EA (**11**)



Boc = tert-Butyloxycarbonyl, MePhe = N-Methylphenylanine, AHPPA = phenylstatine = (3S, 4S)-4-amino-3-hydroxy-5-phenylpentanoic acid.

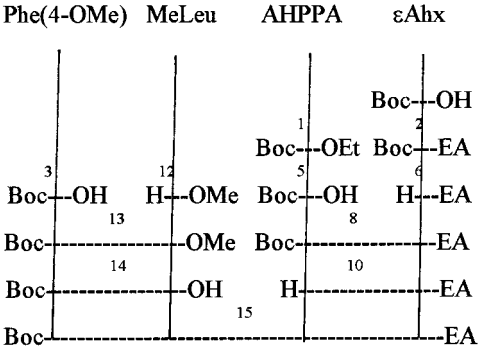
2. Investigations and results

2.1. Chemistry

The inhibitors **11**, **15**, **20**, **21** as well as their intermediates were synthesized as shown in Schemata 1–4. Amino acid esters **4**, **12** or amide **6** were coupled with N-Boc-amino acids **3**, **5**, **17** and esters or amides of N-Boc-substituted peptides were obtained (**7**, **8**, **13**, **18**). N-Boc-peptides with free carboxyl function (**9**, **14**) and esters or amides of peptides with liberated amino function (**10**, **19**) were obtained after deprotection. These intermediates were coupled (**9** + **10**, **14** + **10**, **9** + **19** and **14** + **19**) to receive the final N-Boc-peptide-amides **11**, **15**, **20** and **21**. General methods are given in the experimental part. Physical and analytical data of the synthesized compounds are given in Tables 1 and 2. The properties of derivatives obtained by removal of substituents blocking functional groups of the parent compounds are not given.

Scheme 2

Synthesis of Boc-Phe(4-OMe)-MeLeu-AHPPA-εAhx-EA (**15**)



εAhx = 6-aminohexanoic acid, EA = Ethanolamine.

Table 1: Physical and analytical data of synthesized compounds

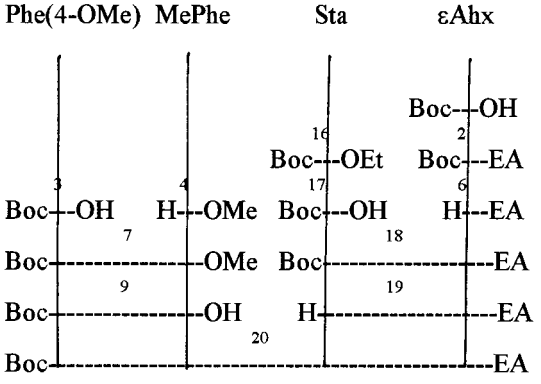
Compd.	Formula (mol. wt.)	SiO_2 column chrom. system for purif.	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ (c, MeOH)	TLC solv system (R_f)	HPLC (% of purity)
1 [6]	$C_{18}H_{26}O_5N$ (336.4)	D	32	85–88	−33.3 (1.2)	D (0.38)	nd
2	$C_{13}H_{26}O_4N_2$ (274.4)	B	33	oil	–	B (0.78)	nd
3 [7]	$C_{10}H_{13}O_3N$ (295.3)	B	90	60–65	+21.1 (1.2)	A (0.58)	nd
7 [8]	$C_{26}H_{34}O_6N_2$ (470.6)	B	44	124–126	−100.0 (1.0)	A (0.91)	nd
8	$C_{24}H_{39}O_6N_3$ (465.6)	B	39	107–109	−27.3 (1.1)	B (0.21)	nd
11	$C_{44}H_{61}O_9N_5$ (804.0)	B	32	75–80	−27.0 (1.0)	C (0.44)	95
13 [8]	$C_{23}H_{36}O_6N_2$ (436.5)	D	21	109–112	−42.6 (1.1)	E (0.55)	nd
15	$C_{41}H_{63}O_9N_5$ (770.0)	C	29	70–73	−27.4 (1.0)	C (0.53)	98
16 [9]	$C_{15}H_{29}O_5N$ (303.4)	D	35	oil	−36.4 (1.0)	D (0.10)	nd
18	$C_{21}H_{41}O_6N_3$ (431.6)	B	34	110–112	−38.3 (1.2)	B (0.08)	nd
20	$C_{41}H_{63}O_9N_3$ (770.0)	B	41	48–51	−25.2 (1.2)	C (0.40)	98
21	$C_{38}H_{65}O_9N_5$ (735.9)	C	35	53–55	−32.0 (1.5)	C (0.42)	96

The elemental analysis results were within ± 0.4% of theoretical values.

2.2. Physico-chemical and biochemical assays

The activities of the obtained renin inhibitors were assayed *in vitro* by a spectrofluorimetric determination of Leu-Val-Tyr-Ser under action of renin in the presence of the tested inhibitor according to Glen et al. [10]. Their stability was determined in pH 6.0 ammonium carbonate solution after incubation for 4 h at 37 °C. Analysis of incubates was carried out by HPLC. Solubilities of the synthesized renin inhibitors were measured in buffers of pH 7.4 and 2.0. Hydrophobicities of the inhibitors expressed as Log P value were determined as partition coefficient among 1-octanol and pH 7.4 buffer and alsoc calculated by a computer method. Because of difficulties of inhibitor solubility the calculated values are considered as more accurate. All results are given in Table 3.

Scheme 3
Synthesis of Boc-Phe(4-OMe)-MePhe-Sta-εAhx-EA **(20)**



Sta = statine = (3S,4S)-4-amino-3-hydroxy-6-methyl-heptanoic acid.

3. Discussion

As mentioned above, an effective renin inhibitor must be resistant to enzymatic degradation, strongly active, well absorbed after oral administration and at least slow excreted with bile. We have designed and synthesized four inhibitors, proteolytic resistance of which is the result of the use of unnatural amino acids. Substitution of Phe(4-OMe) at the P_3 position and MePhe or MeLeu at the P_2 position stabilizes the P_2–P_3 peptide bond against chymotrypsin action. To use of N-methylated amino acids at the P_2 position is possible because the amide hydrogen of the P_2 amino acid is not included in a hydrogen bond [11]. Sta or AHPPA which are pseudodipeptidic units in the

Scheme 4
Synthesis of Boc-Phe(4-OMe)-MeLeu-Sta-εAhx-EA **(21)**

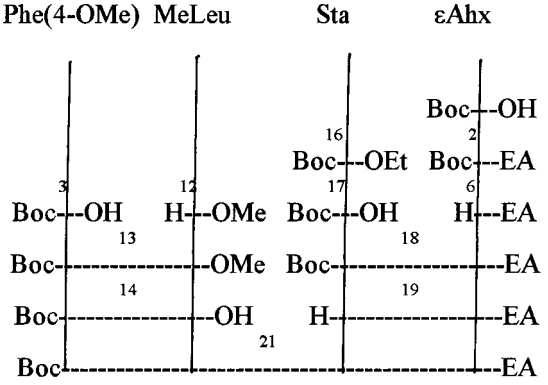


Table 2: ^{1}H NMR spectra of the synthesized compounds

Compd.	Chemical shifts δ value (ppm), $CDCl_3$
1	1.18–1.32 (m, 12 H, C_4H_9, CH_3 ester), 2.55 (d, J = 6 Hz, 2 H, 2 HC_5 AHPPA), 2.93 (d, J = 4 Hz, 2 H, 2 HC_2 AHPPA), 3.72–4.02 (m, 2 H, HC_3 AHPPA, HC_4 AHPPA), 4.18 (q, J = 7 Hz, 2 H, OCH_2), 4.54 (d, J = 8 Hz, 1 H, NH), 7.22 (s, 5 H, C_6H_5).
2	1.25–1.78 (m, 15 H, C_4H_9, 3 · CH_2Ahx), 2.22 (t, J = 7 Hz, 2 H, CH_2Ahx), 3.08 (1, J = 7 Hz, 2 H, CH_2Ahx), 3.43 (q, J = 4 Hz, 2 H, CH_2EA), 3.68 (q, J = 4 Hz, 2 H, CH_2EA), 4.78 (s br, 1 H, NH), 6.70 (s br, 1 H, NH).
3	1.48 (s, 9 H, C_4H_9), 2.96 (d, J = 6 Hz, 2 H, 2 HC_3 Phe(4-OMe)), 3.72 (s, 3 H, OCH_3), 4.36 (s br, 1 H, HC_2 Phe(4-OMe)), 4,85 (d, J = 7 Hz, 1 H, NH), 6.55, 7.96 (dd, J = 7 Hz, 7 Hz, 4 H total C_6H_5).
7	1.40 (s. 9 H, C_4H_9), 2.75 (s, 3 H, N-CH_3), 2.78–2.92 (m, 2 H, 2 HC_3 Phe(4-OMe)), 3.20–3.33 (d, J = 3 Hz, 2 H, 2 HC_3 MePhe), 3.70 (s, 3 H, OCH_3 ester), 3.77 (s, 3 H, OCH_3 eter), 4.60–4.83 (m, 1 H, HC_2 Phe(4-OMe)), 5.05–5.25 (m, 2 H, HC_2 MePhe, NH). 6.85, 7.10 (dd, J = 7 Hz, 7 Hz, 4 H total, C_6H_4), 7.20 (s, 5 H, C_6H_5).
8	1.20–1.67 (m, 15 H, C_4H_9, 3 · CH_2Ahx), 2.16–2.23 (m, 4 H, CH_2Ahx, 2 HC_5AHPPA) 2.89 (d, J = 7 Hz, 2 H, 2 HC_2AHPPA), 3.20 (s br, 2 H, CH_2Ahx), 3.39 (q, J = 4 Hz, 2 H, CH_2EA), 3.68 (m, 3 H, Ch_2EA, HC_4 AHPPA), 3.94 (d, J = 4 Hz, 1 H, HC_3 AHPPA), 5.08 (d, J = 6 Hz, 1 H, NH), 6.31 (s br, 1 H, NH), 6.51 (s br, 1 H, NH), 7.25 (s, 5 H, C_6H-5).
11	1.25–1.74 (m, 15 H, C_4H_9, 3 · CH_2Ahx), 1.90–1.98 (m, 2 H, 2 HC_5 AHPPA), 2.-19–2.30 (m, 4 H, 2 HC_3 Phe(4-OMe), CH_2Ahx), 2.40–2.55 (m, 1 H, HC_4 AHPPA), 2.75–2.97 (m, 5 H, CH_2Ahx, N–CH_3), 3.10–3.30 (m, 4 H, 2 HC_2 AHPPA, 2 HC_3 MePhe), 3.40 (q, J = 2.5 Hz, 2 H, CH_2EA), 3.68 (q, J = 2.5 Hz, 2 H, CH_2EA), 3.76 (d, J = 1.5 Hz, 3 H, OCH_3), 3.90–4.25 (m, 2 H, HC_3 AHPPA, OH), 4.56 (t, J = 4 Hz, 1 H, HC_2 MePhe), 4.80 (q, J = 5 Hz, 1 H, HC_2 Phe(4-OMe)), 5.00 (t, J = 5 Hz, 1 H, NH), 5.11–5.32 (m, 1 H NH), 6.16–6.45 (m, 2 H, 2 · NH), 6.74–6.91 (m, 4 H, C_6H_4), 7.23 (s br, 10 H, 2 · C_6H_5).
13	0.75–1.00 (m, 6 H, 2 · CH_3 MeLeu), 1.42 (d, J = 2 Hz, 9 H, C_4H_3), 1.50–1.80 (m, 3 H, 2 $HC_3C_4H_9$ MeLeu, HC_4 MeLeu), 2.77, 2.83 (d, J = 6 Hz, 3 H total, N–CH_3), 2.84–3.05 (m, 2 H, 2 HC_3 Phe(4-OMe)), 3.68 (s, 3 H, OCH_3 ester), 3.77 (s, 3 H, OCH_3 eter), 4.83 (t, J = 7 Hz, 1 H, HC_2 MeLeu), 5.02–5.43 (m, 2 H, NH, HC_2 Phe(4-OMe)), 6.78, 7.11 (dd, J = 7 Hz, 7 Hz, 4 H total, C_6H_4).
15	0.77–0.89 (m, 6 H, 2 · CH_3 MeLeu), 1.25–1.70 (m, 18 H, C_4H_9, 3 · CH_2Ahx, 2 HC_3 MeLeu, HC_4 MeLeu), 2.07–2.32 (m, 4 H, 2 HC_5 AHPPA, CH_2Ahx), 2.49–2.51 (m, 1 H, HC_4 AHPPA), 2.69–2.94 (m, 7 H, N–CH_3, CH_2Ahx, 2 HC_3 Phe(4-OMe)), 3.15–3.70 (m, 6 H, 2 · CH_2EA, 2 HC_2 AHPPA), 3.77 (t, J = 1 Hz, 3 H, OCH_3), 3.92–4.20 (s br, 2 H, HC_3 AHPPA, OH), 4.67 (t, J = 1.5 Hz, 1 H, HC_2 MeLeu), 5.02–5.30 (m, 2 H, HC_2 Phe(4-OMe), NH), 6.56 (s br, 1 H, NH), 6.75–7.26 (m, 9 H, C_6H_4, C_6H_5), 7.42 (t, J = 4 Hz, 1 H, NH).
16	0.90 (d, J = 7 Hz, 6 H, 2 · CH_3 Sta), 1.22 (t, J = 7 Hz, 3 H, CH_3 ester), 1.53 (s, 9 H, C_4H_9), 1.62–2.04 (m, 3 H, 2 HC_5 Sta, HC_6 Sta), 2.52–2.68 (d, J = 6.5 Hz, 2 H, 2 HC_2 Sta), 3.54–3.72 (q, J = 7 Hz, 1 H, HC_4 Sta), 4.05–4.42 (m, 3 H, HC_3 Sta, OCH_2), 4.82 (d, J = 10 Hz, 1 H, NH).
18	0.91 (d, J = 2.5 Hz. 6 H, 2 · CH_3 Sta), 1.25–1.70 (m, 18 H, C_4H_9, 3 · CH_2Ahx, HC_6 Sta, 2 HC_5 Sta), 2.22 (t, J = 4 Hz, 2 H, CH_2Ahx), 2.34 (d, J = 2 Hz, 2 H, 2 HC_2 Sta), 3.25 (d, J = 2 Hz, 2 H, CH_2Ahx), 3.41 (q, J = 2.5 Hz, 2 H, CH_2EA), 3.57 (d br, J = 2 Hz, 1 H, HC_4 Sta), 3.80 (m, 2 H, CH_2EA), 3.94 (t, J = 3 Hz, 1 H, HC_3 Sta), 4.87 (d, J = 5 Hz, 1 H,. NH), 6.40 (s br, 1 H, NH), 6.75 (s br, 1 H, NH)
20	0.85–0.89 (m, 6 H, 2 · CH_3 Sta), 1.16–1.72 (m, 18 H, C_4H_9, 3 · CH_2 Sta, HC_6 Sta, HC_5 Sta), 1.95–2.40 (m, 6 H, CH_2Ahx, 2 HC_2 Sta, 2 HC_3 Phe(4-OMe)), 2.48–2.93 (m, 5 H, N–CH_3, 2 HC_3 MePhe), 3.05–3.50 (m, 5 H, CH_2Ahx, HC_4 Sta, CH_2EA), 3.60–3.77 (m, 5 H, OCH_3, CH_2EA), 3.83 (t, J = 7 Hz, 1 H, HC_3Sta), 4.33–4.75 (m, 2 H, NH, HC_2 MePhe), 4.91–5.34 (m, 2 H, HC_2 Phe(4-OMe), NH), 6.15–6.46 (m, 2 H, 2 · NH). 6.72–7.28 (m, 9 H, C_6H_4, C_6H_5).
21	0.84–0.96 (m, 6 H, 2 · CH_3Sta), 1.21–1.70 (m. 21 H, 2 HC_5 Sta, HC_6 Sta, C_4H_9, 3 · CH_2Ahx, 2 HC_3 MeLeu, HC_4 MeLeu), 2.21 (q, J = 1.5 Hz, 2 H, CH_2Ahx), 2.27–2.30 (m, 2 H, 2 HC_2Sta), 2.76–2.82 (m, 2 H, 2 HC_3 Phe(4-OMe)), 2.84 (s, 3 H, N–CH_3), 3.18–3.34 (m, 3 H, HC_4 Sta, CH_2Ahx), 3.42 (q, J = 1 Hz, 2 H, CH_2EA), 3.71 (q, J = 1 Hz, 2 H, CH_2EA), 3.78 (d, J = 1 Hz, 3 H, OCH_3), 3.84–4.00 (m, 1 H, HC_3 Sta), 4.58–4.79 (m, 1 H, HC_2 MeLeu), 5.07–5.11 (m, 1 H, NH), 5.26–5.31 (q, J = 1.5 Hz, 1 H, HC_2 Phe(4-OMe)), 6.32–6.47 (m, 2 H, 2 · NH), 6.64–6.75 (m, 1 H, NH), 6.82–7.15 (m, 4 H, C_6H_4).

P_1–P_1^l position in place of -Leu-Val-stabilize this fragment against renin hydrolytic activity. They ensure simultaneously a high affinity of the substrate to the enzyme. In this way proteolytical stability of the designed inhibitors was obtained, but their inhibitory activity against renin activity is rather moderate. Because of their poor solubility the *in vitro* determined activity could be too low. The core of the synthesized inhibitors is the structure: Phe(4-OMe)-MePhe-Sta–εAhx. Use of this fragment with ester substituent at the C-terminus previously led to inhibitors with nanomolar activity [12]. Now we use this fragment as a basic structure. Selection of substituents blocking the N- and C-termini of this structure resulted from our intention to obtain hydrophobicities in the limits of Log P 4–5. The reason was, that some nonpeptidic inhibitors showing a bioavailability above 50% in dogs [5] have Log P value in these limits. This seemed to confirm the correctness of our intention. We chose a hydrophobic substituent at the N-terminus – a Boc-group- and a hydrophilic one at the C-terminus – an OH-group. Application of both resulted in receiving the demanded whole molecule hydrophobi-

Table 3: Physico-chemical and biochemical properties of synthesized renin inhibitors

Compd.	Solubility (mg/ml)		Stability (chymotrypsin)	IC_{50} (M/l)	Log P*	Log P**
	pH 2.0	pH 7.4				
11	<1	<1	Stable	7.0×10^{-4}	>1	5.28
15	<1	<1	Stable	7.5×10^{-5}	>1	4.79
20	<1	<1	Stable	6.0×10^{-4}	>1	4.79
21	<1	<1	Stable	2.5×10^{-4}	>1	4.30

Log P* = log of the value of the partition coefficient $\frac{\text{peak area in 1-octanol}}{\text{peak area in buffer pH 7.4}}$

Log P** = value calculated by computer method

city. Poor solubility of the designed structures resulting from substitution with the Boc-group could decrease its inhibitory activity, but the elimination of the positive charge of the N-terminus in the N-Boc-substituted inhibitors is able to decrease the biliary excretion. We recognized the verification of this possibility as promising. The mean inhibitory activity of the obtained compounds with Log P values near 4 or 5 as well as the previously synthesized [13] with Log P values about 2 and 7 shows that hydrophobicity is not a decisive aspect for potency. This failed our expectations, that it should be possible to find a direct correlation between hydrophobicity and inhibitory potency of renin activity. Also the removal of polarity did not increase the potency of the molecules. Comparing the activities of the renin inhibitors of similar structures synthesized now and previously we consider that potency is determined by their solubility (in addition to correct structure). A good absorption after oral administration and not too rapid biliary excretion of the renin inhibitors depends undoubtedly on their hydrophobicity, polarity and solubility. Therefore we intend to look for improved biological properties by correlation of those three parameters.

4. Experimental

4.1. Chemistry

The amino acids and reagents were purchased from Aldrich. Boc-MeLeu and Boc-MePhe were obtained according to Cheung et al. [14]. Phe(4-OMe) was synthesized using Behr's method [7], Sta and AHPPA using Maibaum's method [9]. Renin from porcine kidney, N-acetyl renin substrate tetradecapeptide Ac-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH and chymotrypsin type I S from bovine pancreas were obtained from Sigma. Solvents were of analytical purity. THF was distilled from Na/benzophenone under N_2, CH_2Cl_2 and DMF were dried over molecular sieves 4 Å. The peptides were synthesized by the DCCI/HOBt (N,N^1-dicyclohexylcarbodiimide/l-hydroxybenzotriazole) method in solution [15]. M.p.'s were determined with a Boetius apparatus. Optical rotations were measured at the Na-D line with a Polamat (Carl Zeiss, Jena) polarimeter in a 5 cm polarimeter cell. ^{1}H NMR spectra were recorded on UNITY plus Varian 200 or Varian 500 spectrometer. Chemical shifts were measured as δ units (ppm) relative to tetramethylsilane. Elemental analyses were performed on a Perkin-Elmer Microanalyser model 2400 CHNS. TLC was carried out on 0.25 mm thickness plates (Merck Kieselgel 60 F 254). The spots were visualized with 0.3% ninhydrin in EtOH/AcOH 97 : 3 or 7% phosphomolybdic acid in MeOH. Column chromatography (CC) was carried out under gravity on silica gel (Merck, grade 230 to 400 mesh). The solvent systems used in TLC and CC were: $CHCl_3$/MeOH 90 : 10 (A), 95 : 5 (B), CH_2Cl_2/MeOH 95 : 5 (C), hexane/EtOAc 50 : 50 (D), 80 : 20 (E). HPLC data were obtained on a Techma-Robot Typ 302 apparatus equipped with a silica gel ODS 10 μm (3.8 × 250 mm) column, UV detector Typ LCD 2040 (Laboratorni Pristroje, Praha) at 210 nm and computer registrator/recorder CHROMA (POLLAB Warszawa). The solvent system was Me/OH 70 : 30 used at flow rate of 1.9 ml/min.
Spectrofluorimetric determination of Leu-Val-Tyr-Ser released from substrate tetradecapeptide was performed on a Shimadzu apparatus with Fluram solution according to the method of Galen et al. [10]. The fluorescence was detected at 395–495 nm.

4.1.1. Introduction of the Boc-group

This group was introduced as usual [16].

4.1.2. Esterification reaction

Boc-amino acids were esterified with CH_3I as described earlies [13].

4.1.3. N-tert-Butyloxycarbonyl-ε-aminohexanoic acid ethanolamide (Boc-εAhx-EA)

Boc-εAhx (2 mmol, 0.262 g) was dissolved in dry THF (10 ml) and *N*-methylmorpholine (2 mmol, 0.202 g, 0.22 ml) was added. This solution was stirred under N_2 and chilled to −15 °C. Isobutyl chloroformate (2 mmol, 0.270 g, 0.26 ml) was added dropwise to keep the temperature bellow −15 °C. Ethanolamine (2 mmol, 0.122 g, 0.12 ml) was added in small portions and the reaction mixture was kept stirring at −15 to −20 °C for 30 min. and at RT for 1 h. The solution was concentrated in vacuo, the residue was dissolved in $CHCl_3$ (20 ml). This solution was washed with 5% HCl (20 ml), 5% $NaHCO_3$ solution (20 ml), saturated NaCl solution (20 ml), dried with anh. $MgSO_4$, filtered and evaporated in vacuo. The oily residue was dried under vacuo. Data of obtained product are given in Tables 1 and 2.

4.1.4. Coupling reaction with DCCI/HOBt

The amino acid or peptide ester hydrochloride (1 mmol) was dissolved in CH_2Cl_2 (5 ml) and neutralized at 0 °C with TEA (1 mmol). Boc-amino acid or Boc-peptide (1 mmol) and HOBt (1.5 mmol) were added followed by a solution of DCCI (1.1 mmol) in CH_2Cl_2 (5 ml). The reaction mixture was stirred at 0 °C for 2–4 h and left at RT overnight. DCU Dicyclohexylurea (DCU) was filtered off and the filtrate was evaporated in vacuo. The residue was dissolved in $CHCl_3$, washed successively with 5% HCl, 5% $NaHCO_3$, saturated NaCl solution, dried with anh. $MgSO_4$ and concentrated in vacuo. The peptide was purified by silica gel CC to yield the pure product.

4.1.5. Removal of the Boc-group

Boc-amino acid or peptide (1 mmol) in solution of 4 M HCl in dioxane (3–5 ml) was stirred at RT for 30 min. The solution was concentrated in vacuo, the residue was re-evaporated twice with ether and then dried in vacuo [17].

4.1.6. Alkaline hydrolysis of amino acid or peptide esters

Hydrolysis was carried out as described earlier [13].

4.2. Physico-chemical and biochemical assays

4.2.1. Inhibition of renin activity

The course of determination is described in detail in a previous paper [13]. The renin inhibitory activity is designed in terms of IC_{50} (which is the molecular concentration of the tested inhibitor causing 50% inhibition of control renin activity).

4.2.2. Stability determination

Stability of the obtained renin inhibitors was assayed in pH 6.0 NH_4HCO_3 solution after incubation for 4 h at 37 °C. Analysis of incubates was carried out by HPLC. Peak areas of inhibitors in incubates were compared with peak areas of the standard solutions. The values were determined in 3 separate assays. The differences among the peak areas in limits ±5% were recognized as a determination error. The accurate course of determination is described in detail in ref. [13].

4.2.3. Solubility determination

Solubility of the synthesized renin inhibitors was determined in pH 7.4 phosphate buffer and in pH 2 acetate buffer. The sample of the compound was magnetically stirred for 3 h at 25 °C and filtered. Concentration of the inhibitor was measured by HPLC. The accurate course of determination is described in ref. [13].

4.2.4. Partition coefficient determination

Inhibitor (5 mg) was shaken with 1-octanol (1 ml) and pH 7.4 phosphate buffer (1 ml) for 1 h at 25 °C. The resulting solutions were centrifuged for 5 min. Portions of both phases (0.5 ml) were separated. Octanol was removed by blowing and the residue was dissolved in the HPLC mobile phase (0.5 ml). The inhibitor concentration was measured in both phases by HPLC (detection at 210 nm). Peak areas were compared with peak areas of standard solutions containing 1.25 mg of inhibitor dissolved in 1 ml of the HPLC mobile phase.

4.2.5. Log P value calculation by computer method

Computational calculations were performed on a Pentium-S, 100 Mhz PC. Structures of inhibitors were built within the HyperChem 4.5 program. The semiempirical method PM 3 was used for single point calculation. Geometry optimization was performed by Polak-Ribier method. Log P as a measure of hydrophobicity of optimized structure was calculated using QSAR Properties program, Chem Plus, extension for HyperChem (Hypercube, Inc.). Calculation of Log P in this program was carried out using atomic parameters derived by Ghose et al. [18, 19].

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Synthesis and biological screening of new 1,3-diphenylpyrazoles with different heterocyclic moieties at position-4

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1,3-Diphenyl-1*H*-pyrazole-4-carboxaldehyde (**1**) was reacted with barbituric acid, thiobarbituric acid, some activated nitriles and/or acetophenone to give the condensation products **2a**, **b**, **3a**–**c** and **4**, respectively. The reaction of **1** with hydrazine hydrate, semicarbazide or thiosemicarbazide afforded the corresponding azomethines **5a**–**c**. The compounds **3a**, **b**, **4** and **5a**, **c** were subjected for different sequence reactions to produce the title compounds. The antibacterial and antifungal activity of some selected derivatives were evaluated.

1. Introduction

The pyrazole nucleus in general and its chemistry [1–5] has found considerable attention during the decades due to outstanding biological activities as antianxiety [6, 7], antipyretic, analgesic and antiinflammatory drugs [8, 9] as well as its good antibacterial and antifungal properties [10–14]. All these findings prompted us to introduce several pharmacophores such as pyran, pyridine, pyrimidine or thiazole moieties into the pyrazole system hoping to get compounds with enhanced potency. Also, some of the synthesized compounds were screened *in vitro* for their antibacterial and antifungal activities.

2. Investigations, results and discussion

2.1. Chemistry

Our approach to the target heterocyclic compounds was achieved by the synthesis of 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehyde (**1**) from acetophenone phenylhydrazone and Vilsmeier reagent [15]. Compound **1** was reacted with some active methylene compounds namely; barbituric acid, thiobarbituric acid, malononitrile, ω-cyanoacetophenone or cyanothioacetamide to give the condensation products **2a**, **b** and **3a**–**c**, respectively. Also, the chalcone **4** was prepared by condensation of **1** with acetophenone as reported before [14]. On the other hand, the reaction of **1** with hydrazine hydrate, semicarbazide and thiosemicarbazide furnished the corresponding azomethines **5a**–**c** (Scheme 1).

Most of the latter compounds (**3a**, **b**, **4** and **5a**, **c**) were used as key intermediates in the synthesis of the desired pyrazoles via their interaction with different reagents.

Scheme 1