New Renin Inhibitors Containing Pseudodipeptidic Units in P₃–P₂ and P₁–P₁' Positions

Ryszard PARUSZEWSKI,^{*,a} Paweł JAWORSKI,^a Magdalena BODNAR,^a
Jadwiga DUDKIEWICZ-WILCZYŃSKA,^b and Iza ROMAN^b

^a Department of Drug Chemistry, Medical University; 02–097 Warszawa, 1 Banacha Str; Poland; and ^b National Institute of Public Health; 00–725 Warszawa, 30/34 Chelmska Str; Poland. Received October 21, 2004; accepted April 12, 2005

A series of four non-peptidic renin inhibitors have been designed and synthesized. All of them contain in their molecule (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), a hydrophobic portion at the C-terminus and a second dipeptide-like transition state analog or unnatural dipeptidic fragment at the N-terminus. Inhibitory activity of the compounds was measured *in vitro* by high performance liquid chromatography (HPLC). Their IC₅₀ (mM) values were: <10^{−3} (12), 1.0×10^{−6} (19), 4.0×10^{−4} (23) and 1.0×10^{−6} (29), respectively. All the compounds are stable against chymotrypsin.

Key words renin inhibitor; pseudodipeptidic unit; HPLC *in vitro* activity determination

Renin inhibitors may be useful drugs in the treatment of hypertension and heart failure, especially because of their high specificity for angiotensinogen. In the past a large number of peptidic renin inhibitors were designed and synthesized. Unfortunately, a majority of them showed poor oral absorption and rapid biliary uptake. The above limitations may result from the substrate-like structure of the synthesized inhibitors. Therefore, other structures of renin inhibitors were also sought.

Investigation of the crystal structure of human renin revealed that it is composed of two β -sheet domains and the cleft between them. This cleft extends over seven or eight residues of the substrate (S₄–S₃').^{1–3} Studies of the conformational structure of the cleft showed that the S₃–S₁ subsites forming a hydrophobic pocket constitute a large hydrophobic cavity.^{4,5} Other subsites (S₁'–S₃') are contained in the cleft, but their pockets are not well defined.

On the basis of these findings, inhibitors of a new type were designed and synthesized. The peptide fragment P₃–P₁ was replaced by a moderately hydrophobic moiety enclosing an aliphatic or alicyclic fragment showing affinity to the S₁ and aromatic fragment with affinity to the S₃ subsites.³ It was recently determined that respective substituents of the aromatic portion of the molecule are able strongly to enhance the binding affinity to renin and the selectivity of the inhibitor. This moiety was directly linked to the C-terminus of the transition state analog containing a hydroxyl moiety. The analog with a cyclohexyl substituent, used in numerous inhibitors, seemed especially worthy of note. This type of inhibitor did not contain residues at positions P₂' and P₃', but rather an amide aliphatic chain. The inhibitors obtained showed high *in vitro* affinity for renin and good bioavailability, and they were effective in lowering blood pressure in primates.

Aliskiren seems a very promising of new type compound.⁶ It is a highly potent and selective non-peptidic renin inhibitor *in vitro* and *in vivo*. Indeed, it contains only one peptide bond and is deprived of the P₄–P₁ fragment characteristic of the peptide inhibitors. Inhibiting of renin activity is due to hydrogen binding of the hydroxyl groups of the transition state analog to the catalytically active Asp 32 and Asp 215 residues of renin.³ Also, derivatives of 3,4-substituted

piperidine, which bind to a new enzyme active site conformation, are very potent renin inhibitors of a new type characterized by a complete non-peptidic structure.⁷ This conformation suggests that aspartic proteinase active sites have latent conformational flexibility.⁸ The most promising are two inhibitors with good pharmacodynamic and pharmacokinetic properties.⁹ However, there must be more possibilities for producing effective drugs free from side effects.

Our intention is search for new active inhibitors with simple structure, good bioavailability, inexpensive and easy synthesis. We intend to reach this purpose by coupling non-peptidic fragments with good affinity to some regions of the substrate. We previously obtained several *in vitro* active renin peptidic inhibitors.^{10–12} Between them are compounds with pseudodipeptidic units at P₁–P₁' as well at P₂'–P₃' positions and activity *in vitro* in the limits of 10^{−5} M/L.¹² Other previously obtained inhibitors with ϵ Ahx ethylamide¹¹ or ϵ Ahx-isoamylamide¹³ also at the P₂'–P₃' positions showed inhibitory activity at a concentration of 10^{−5} M/L. On the other hand, substitution of only an isoamylamide group at this position produces an inactive compound.¹⁴ Substitution of the ϵ Ahx-amide fragment appeared equally effective as a pseudodipeptidic unit, but was obtained more easily and much more cheaply. But the presence of a pseudodipeptidic unit, ideally with an alicyclic ring, is necessary at the P₁–P₁' position.

All things considered, we designed a series of inhibitors enclosing three different pseudodipeptidic units at the P₃–P₂ positions. We wanted to find the most effective one at this position. The units are in the successive compounds: (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA), (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine, Sta) and unnatural dipeptide Phe(4-OMe)-MeLeu. All of them contain at the P₁–P₁' positions ACHPA, whose hydroxyl group is presumed to be bound to two Asp residues. Chains of all four inhibitors are lengthened at the C-terminus by linkage of ϵ -aminohexanoic acid (ϵ Ahx) isoamylamide (Iaa).

Chemistry The inhibitors (12, 19, 23, 29) as well as their intermediates were synthesized as shown in Schemata 1–4 (Fig. 2). Their structures are shown in Fig. 1. General

* To whom correspondence should be addressed. e-mail: rpbw@farm.amwaw.edu.pl

Fig. 1. Structures of the Synthesized Renin Inhibitors

methods are given in the experimental section. Physicochemical and analytical data of the newly synthesized compounds (7, 11, 12, 15, 18, 19, 22, 23, 26, 28, 29) are presented in Tables 2 and 3. Properties of derivatives (5, 6, 9, 10, 13, 16, 17, 20, 21, 25, 27) obtained by removal of the substituent blocking functional groups of the parent compounds (N-*tert*-Boc or ester) are not given. Data of the other synthesized compounds are consistent with those described elsewhere (3, 8,¹⁰ 4,¹⁵ 14,¹¹ 24¹⁶).

Biochemical Assays. Determination of Inhibitor Stability Stability was determined as described earlier¹² against chymotrypsin in a solution of ammonium carbonate (pH 6.9) after incubation for 4 h at 37 °C. Analysis of incubates was carried out by HPLC. The results of the experiments are

Table 1. Stability and Activity of the Synthesized Compounds

Compound	Stability $t_{1/2}$ min (chymotrypsin)	IC ₅₀ (M/l)
12	Stable ^{a)}	1.0×10^{-6}
19	Stable ^{a)}	$< 10^{-3}$
23	Stable ^{a)}	4.0×10^{-4}
29	Stable ^{a)}	1.0×10^{-6}
Boc-Phe-His-OMe	<10	
Boc-Val-Tyr-OMe	<10	

a) Stable means that no detectable degradation was found at 4 h.

Scheme 1

Synthesis of HCl . Phe(4-OMe)-MeLeu-(SS)-ACHPA-εAhx-Iaa

Scheme 3

Synthesis of HCl . (SS)-ACHPA-(SS)-ACHPA-εAhx-Iaa

Scheme 2

Synthesis of HCl . (SS)-AHPPA-(SS)-ACHPA-εAhx-Iaa

Scheme 4

Synthesis of HCl . (SS)-Sta-(SS)-ACHPA-εAhx-Iaa

Fig. 2. Schemata of the Synthesized Renin Inhibitors

given in Table 1.

Determination of Inhibition of Renin Activity Renin-inhibiting activity of the compounds was determined *in vitro* according to the method of Galen *et al.*¹⁷⁾ with modification consisting of a change from a spectrofluorimetric to chromatographic (HPLC)¹⁸⁾ method of determination of the 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.¹⁹⁾ The activity is designed in terms of IC₅₀, which is the molecular concentration of the tested inhibitor causing 50% inhibition of the control renin activity. The results are given in Table 1.

Results and Discussion

So far, activity of the synthesized inhibitors have been tested *in vitro*, which it is not a expensive procedure. Our modification¹⁸⁾ of the method of Galen *et al.*¹⁷⁾ allows for

Table 2. Physicochemical and Analytical Data of the Synthesized Compounds

No	Compound	Formula MW	Yield (%)	mp (°C)	$[\alpha]_D^{20}$ (c, MeOH)	TLC, <i>R_f</i> Solv. syst.	H HPLC % purity	Log P
7.	Boc-Phe(4-OMe)-MeLeu(SS)-ACHPA-OEt	C ₃₆ H ₅₈ O ₈ N ₃ 660.84	74	45—46	−35.4 (1.0)	0.42 A	n.d.	—
11.	Boc-Phe(4-OMe)-MeLeu(SS)-ACHPA-εAhx-Iaa	C ₄₄ H ₇₅ O ₈ N ₅ 802.08	71	About 58	−25.7 (1.4)	0.32 B	n.d.	—
12.	HCl. Phe(4-OMe)-MeLeu(SS)-ACHPA-εAhx-Iaa	C ₃₉ H ₆₈ O ₆ N ₅ Cl 738.42	85	61—64	−20.0 (1.2)	0.35 B	100	5.55
15.	Boc-(SS)-ACHPA-εAhx-Iaa	C ₂₇ H ₅₁ O ₅ N ₃ 497.70	73	Semi solid	−35.0 (1.2)	0.38 B	n.d.	—
18.	Boc-(SS)-AHPPA(SS)-ACHPA-εAhx-Iaa	C ₃₈ H ₆₄ O ₇ N ₄ 688.92	79	60—62	−36.8 (1.3)	0.22 B	n.d.	—
19.	HCl. (SS)-AHPPA(SS)-ACHPA-εAhx-Iaa	C ₃₃ H ₅₇ O ₅ N ₄ Cl 625.27	78	86—90	−26.0 (1.0)	0.50 B	100	4.39
22.	Boc-(SS)-ACHPA(SS)-ACHPA-εAhx-Iaa	C ₃₈ H ₇₀ O ₇ N ₄ 625.27	70	55—58	−40.0 (1.6)	0.24 B	n.d.	—
23.	HCl. (SS)-ACHPA(SS)-ACHPA-εAhx-Iaa	C ₃₃ H ₆₃ O ₅ N ₄ Cl 631.31	67	89—94	−23.6 (1.1)	0.48 B	100	4.33
26.	Boc-(SS)-Sta(SS)-ACHPA-OEt	C ₂₆ H ₄₈ O ₇ N ₂ 500.66	54	Semi solid	−75.0 (1.2)	0.46 A	n.d.	—
28.	Boc-(SS)-Sta(SS)-ACHPA-εAhx-Iaa	C ₃₅ H ₆₆ O ₇ N ₄ 654.91	69	53—56	−38.0 (1.0)	0.25 B	n.d.	—
29.	HCl. (SS)-Sta(SS)-ACHPA-εAhx-Iaa	C ₃₀ H ₅₉ O ₅ N ₄ Cl 591.25	72	71—74	−28.0 (1.2)	0.48 B	100	3.65

(A)=CHCl₃:MeOH 98:2, (B)=CHCl₃:MeOH 80:20. The elemental analyses were within ±0.4% of the theoretic value.

Table 3. ¹H-NMR Spectra of the Synthesized Compounds

Compound	Chemical shifts δ (ppm)
7	(CDCl ₃): 0.81—0.89 (m, 6H, 2×CH ₃), 1.16 (t, <i>J</i> =9.4 Hz, 3H, −O−CH ₂ −CH ₃), 1.24—1.68 (m, 12H, 6×CH ₂), 1.40 (s, 9H, C ₄ H ₉), 2.76 (s, 3H, N−CH ₃), 3.78 (d, <i>J</i> =5.7 Hz, 3H, −O−CH ₃), 4.16 (q, <i>J</i> =6.5 Hz, 2H, −O−CH ₂ −CH ₃), 5.97 (d, <i>J</i> =9.3 Hz, 1H, NH), 6.85, 7.11 (dd, <i>J</i> =9 Hz, 9 Hz, 4H, C ₆ H ₄), 7.43 (d, <i>J</i> =9.3 Hz, 1H, NH).
11	(CDCl ₃): 0.80—0.92 (m, 12H, 4×CH ₃), 1.16—1.80 (m, 21H, 10×CH ₂ , CH(CH ₃) ₂), 1.40 (s, 9H, C ₄ H ₉), 2.14 (t, <i>J</i> =6.7 Hz, 2H, CH ₂), 2.84 (s, 3H, N−CH ₃), 3.23—3.28 (m, 4H, 2×CH ₂), 3.78 (d, <i>J</i> =3.9 Hz, 3H, −O−CH ₃), 6.00 (d, <i>J</i> =9.3 Hz, 1H, NH), 6.50 (brs, 1H, NH), 6.70 (brs, 1H, NH), 6.86, 7.13 (dd, <i>J</i> =9.4 Hz, 9.4 Hz, 4H, C ₆ H ₄), 7.49 (d, <i>J</i> =9.3 Hz, 1H, NH).
12	(CD ₃ OD): 0.90—1.00 (m, 12H, 4×CH ₃), 1.20—1.90 (m, 21H, 10×CH ₂ , CH(CH ₃) ₂), 2.22 (t, <i>J</i> =7.1 Hz, 2H, CH ₂), 3.00 (s, 3H, N−CH ₃), 3.18—3.22 (m, 4H, 2×CH ₂), 3.79 (s, 3H, −O−CH ₃), 6.92, 7.25 (dd, <i>J</i> =9.5 Hz, 9.5 Hz, 4H, C ₆ H ₄).
15	(CDCl ₃): 0.91 (d, <i>J</i> =5.8 Hz, 6H, 2×CH ₃), 1.13—1.62 (m, 21H, 10×CH ₂ , CH(CH ₃) ₂), 1.44 (s, 9H, C ₄ H ₉), 2.20 (t, <i>J</i> =7.0 Hz, 2H, CH ₂), 3.25—3.28 (m, 4H, 2×CH ₂), 4.82 (d, <i>J</i> =8.5 Hz, 1H, NH), 5.67 (brs, 1H, NH), 6.52 (brs, 1H, NH).
18	(CDCl ₃): 0.91 (d, <i>J</i> =7.3 Hz, 6H, 2×CH ₃), 1.16—1.58 (m, 21H, 10×CH ₂ , CH(CH ₃) ₂), 1.39 (s, 9H, C ₄ H ₉), 2.15 (t, <i>J</i> =7.1 Hz, 2H, CH ₂), 3.22—3.26 (m, 4H, 2×CH ₂), 5.02 (d, <i>J</i> =10.1 Hz, 1H, NH), 5.53 (brs, 1H, NH), 6.08 (d, <i>J</i> =10.0 Hz, 1H, NH), 6.46 (brs, 1H, NH), 7.23 (s, 5H, C ₆ H ₅).
19	(CD ₃ OD): 0.90 (d, <i>J</i> =6.0 Hz, 6H, 2×CH ₃), 1.21—1.61 (m, 21H, 10×CH ₂ , CH(CH ₃) ₂), 2.16 (t, <i>J</i> =7.3 Hz, 2H, CH ₂), 3.16—3.20 (m, 4H, 2×CH ₂), 7.26 (brs, 5H, C ₆ H ₅).
22	(CDCl ₃): 0.91 (d, <i>J</i> =7.0 Hz, 6H, 2×CH ₃), 1.11—1.58 (m, 33H, 16×CH ₂ , CH(CH ₃) ₂), 1.41 (s, 9H, C ₄ H ₉), 2.15 (t, <i>J</i> =6.5 Hz, 2H, CH ₂), 3.21—3.29 (m, 4H, 2×CH ₂), 4.81 (d, <i>J</i> =10.0 Hz, 1H, NH), 5.61 (brs, 1H, NH), 6.22 (d, <i>J</i> =8.5 Hz, 1H, NH), 6.71 (brs, 1H, NH).
23	(CD ₃ OD): 0.90 (brs, 6H, 2×CH ₃), 1.15—1.63 (m, 33H, 16×CH ₂ , CH(CH ₃) ₂), 2.17 (t, <i>J</i> =6.5 Hz, 2H, CH ₂), 3.16—3.30 (m, 4H, 2×CH ₂).
26	(CDCl ₃): 0.92 (d, <i>J</i> =5.4 Hz, 6H, 2×CH ₃), 1.26 (t, <i>J</i> =7.2 Hz, 3H, −O−CH ₂ −CH ₃), 1.17—1.80 (m, 16H, 7×CH ₂ , 2×CH(CH ₃) ₂), 1.44 (s, 9H, C ₄ H ₉), 2.15 (t, <i>J</i> =6.9 Hz, 2H, CH ₂), 3.52—3.30 (m, 4H, 2×CH ₂), 4.15 (q, <i>J</i> =6.8 Hz, 2H, −O−CH ₂ −CH ₃), 4.85 (d, <i>J</i> =9.7 Hz, 1H, NH), 6.40 (d, <i>J</i> =9.7 Hz, 1H, NH).
28	(CDCl ₃): 0.91 (brs, 12H, 4×CH ₃), 1.15—1.55 (m, 24H, 11×CH ₂ , 2×CH(CH ₃) ₂), 1.43 (s, 9H, C ₄ H ₉), 2.14 (t, <i>J</i> =6.7, 2H, CH ₂), 3.22—3.28 (m, 4H, 2×CH ₂), 4.88 (d, <i>J</i> =8.6 Hz, 1H, NH), 5.73 (brs, 1H, NH), 6.36 (d, <i>J</i> =8.6 Hz, 1H, NH), 6.89 (brs, 1H, NH).
29	(CD ₃ OD): 0.90 (d, <i>J</i> =5.8 Hz, 12H, 4×CH ₃), 1.20—1.80 (m, 24H, 11×CH ₂ , 2×CH(CH ₃) ₂), 2.22 (t, <i>J</i> =6.5 Hz, 2H, CH ₂), 3.23—3.26 (m, 4H, 2×CH ₂).

very precise determination. *In vivo* assay of the inhibitory activity of the most promising compounds will be performed at the end of the entire research project. Four obtained inhibitors are stable against chymotrypsin. Their molecular weights are not too high (550–590 g/mol as free base, except for **12** which is about 700), and lipophilicity is moderate (Log P values in the range of 3.65–5.55). The inhibitors containing two pseudodipeptidic units have one peptide bond between them and another one linking the unnatural ϵ Ahx. An additional peptide bond couples the hydrophobic isoamylamide (Iaa) substituent with the basic structure of the compounds. These mentioned features seems to favor the activity of the designed inhibitors. The rather high activity of compound **29** (with ϵ Ahx-Iaa fragment at the C-terminus) suggests that this linear hydrophobic portion of the inhibitor prefers (SS)-ACHPA but not (SS)-Sta at the active site of renin inhibitor. Therefore, ϵ Ahx-Iaa fragment appears to be very important for the action. The potent activity of compound **29** and rather poor activity of **19** and **23** shows that the aliphatic chain of Sta at the N-terminal fragment of compound **29** assures the desired effect, in contrast with the alicyclic ACHPA (compound **23**), and especially the aromatic AHPPA (compound **19**) rings. This probably results from a higher affinity of the aliphatic portion to the S_2 subpocket, in spite of a strong preference of aromatic residues to the S_3 subpocket. This hypothesis seems to be confirmed by the presence of the 7(*S*)-isopropyl group in the structure of Aliskiren, whose position suggests affinity to the S_2 subpocket.⁶ Also, the micromolar activity of compound **12** with MeLeu in the P_2 position supports our reasoning. It is a matter of debate whether the pseudodipeptide Sta could be substituted with a simpler (and cheaper) aliphatic fragment to produce potent nonpeptidic inhibitors with improved bioavailability. One conclusion from our investigation is that it is possible to obtain with good yield a moderately active renin inhibitor of a simple structure. The search for such compound, but with higher potency, is under way.

Experimental

The amino acids and reagents were purchased from Aldrich. Boc-ACHPA-OEt, Boc-AHPPA-OEt and Boc-Sta-OEt were synthesized according to the protocol of Maibaum *et al.*,¹⁶ Phe(4-OMe) using the method of Behr's *et al.*²⁰ and Boc-MeLeu using that of Cheung *et al.*²¹ Renin from porcine kidney, *N*-acetyl renin substrate tetradecapeptide 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 . Dichloromethane and DMF were dried over 4 Å molecular sieves. The inhibitors 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, Kieselgel 60 F-254). The solvents 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). Elemental analyses were performed on a Perkin-Elmer Micro-analyser. Melting points were determined in a Bötius apparatus. 1H -NMR spectra were recorded on a Bruker DM 400 MHz spectrometer. Chemical shifts were measured as δ units (ppm) relative to tetramethylsilane. 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 the inhibitors were performed on an apparatus equipped with a pump (Techma-Robot, Warsaw), UV detector LCD 2040 (Laboratormi Pistroje, Praha) and computer registrar/recorder (Chroma Pollab, Warsaw). The peaks were recorded at 210 nm and the solvent system was 1% CH_3COOH /MeOH (10:90). The same method, apparatus and equipment were used for stability determination. HPLC determination of tetrapeptide released from the substrate *N*-

acetyl-tetradecapeptide was performed using the Shimadzu apparatus equipped with two LC-10AT vp pumps, an automatic sampler SIL-10 AD vp, detector UV-VIS SPD-10 A and controller/recorder SCL-10 A. The peaks were recorded at 210 nm, and the following gradient systems—A: 0.01 M ammonium acetate, B: acetonitrile + 1.5 ml acetic acid/1 l (0–12 min. 10–60% B) at 25 °C were used.

Introduction of the *N*-tert-Boc Group This group was introduced by the Schwyzter *et al.* method²² using 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 re-evaporated twice with ethyl ether and dried *in vacuo*.

Esterification Reaction Boc-amino acids were esterified with CH_3I as described earlier.¹³ Boc-ACHPPA-OEt, Boc-AHPPA-OEt and Boc-Sta-OEt were formed from mono-ethyl malonate used to prepare these compounds.¹⁶

Alkaline Hydrolysis of Ester Group Hydrolysis was carried out as described earlier.¹³

Coupling Reaction with DCCI/HOBt The coupling was performed by fragment condensation as shown in schemata 1–4, performed in a commonly used manner described earlier.^{11,12}

Acknowledgement This investigation was supported in part by the Warsaw Medical University (Grant FW-22/W/02-04).

References

- Sibanda B. L., Blundell T., Hobart P. M., Fogliano M., Bindra J. S., Dominy B. W., Chirgwin J. M., *FEBS*, **174**, 102–111 (1984).
- Boyd S. A., Fung A. K. L., Mantei R. A., Stein H. H., Cohen J., Barlow J. L., Klinghofer V., Weeale J. L., Verburg K. M., Polakowski J. S., Adler A. L., Calzadilla S., Kovar P., Yao Z., Hutchins C. W., Denissen J. F., Grabowski B. A., Cepa S., Hoffman D. J., Garren K. W., Kleinert H. D., *J. Med. Chem.*, **37**, 2991–3007 (1994).
- Rahuel J., Rasetti V., Maibaum J., Rüeger H., Göschke R., Cohen N.-C., Stutz S., Cumin F., Fuhrer W., Wood J. M., Grütter M. G., *Chem. Biol.*, **7**, 493–504 (2000).
- Leffer B. A., Hada W. A., Wright A. S., Martin W. H., Stock I. A., Schulte G. K., Pandit J., Danley D. E., Ammirati M. K., Sneddon S. F., *Bioorg. Med. Chem. Lett.*, **5**, 2623–2626 (1995).
- Rosetti V., Cohen N.-C., Rüeger H., Göschke R., Maibaum J., Cumin F., Fuhrer W., Wood J. M., *Bioorg. Med. Chem. Lett.*, **6**, 1589–1594 (1996).
- Wood J. M., Maibaum J., Rahuel J., Grütter M. G., Cohen N.-C., Rasetti V., Rüeger H., Göschke R., Stutz S., Fuhrer W., Schilling W., Rigollier P., Yamaguchi Y., Cumin F., Baum H.-P., Schnell C. R., Herold P., Mah R., Jensen C., O'Brien E., Stanton A., Bedigian M. P., *Biochem. Biophys. Res. Commun.*, **308**, 698–705 (2003).
- Vieira E., Binggeli A., Breu V., Bur D., Fischli W., Güller R., Hirth G., Märki H. P., Müller M., Oefner C., Scalone M., Stadler H., Wilhelm M., Wostl W., *Bioorg. Med. Chem. Lett.*, **9**, 1397–1402 (1999).
- Oefner C., Binggeli A., Breu V., Bur D., Clozel J.-P., D'Arcy A., Dorn A., Fischli W., Güller R., Hirth G., Märki H. P., Mathews S., Müller M., Ridley R. G., Stadler H., Vieira E., Wilhelm M., Winkler F. K., Wostl W., *Chem. Biol.*, **6**, 127–131 (1999).
- Märki H. P., Binggeli A., Bittner B., Bohnen-Lang V., Breu V., Bur D., Coassolo Ph., Clozel J. P., D'Arcy A., Doebelli H., Fischlo W., Funk Ch., Forichecker J., Giller T., Grüninger F., Guenzi A., Güller R., Hartung T., Hirth G., Jenny Ch., Kansy M., Klinkhammer U., Lave T., Lohri B., Luft F. C., Mervaala E. M., Müller M., Montavon F., Oefner Ch., Qiu C., Reichel A., Sanvald-Ducray P., Scalone M., Schleimer M., Schmidt R., Stadler H., Treiber A., Valdenaire O., Vieira E., Waldmeier P., Wiegand-Chou R., Wilhelm M., Wostl W., Zell M., Zell R., *Farmaco*, **56**, 21–27 (2001).
- Paruszcwski R., Jaworski P., Tautt J., Dudkiewicz J., *Boll. Chim. Farm.*, **5**, 301–308 (1994).
- Paruszcwski R., Jaworski P., Winiecka I., Tautt J., Dudkiewicz J., *Pharmazie*, **54**, 102–106 (1999).
- Paruszcwski R., Jaworski P., Winiecka I., Tautt J., Dudkiewicz J., *Chem. Pharm. Bull.*, **50**, 850–853 (2002).
- Paruszcwski R., Jaworski P., Tautt J., Dudkiewicz J., *Pharmazie*, **52**, 206–209 (1997).
- Paruszcwski R., Jaworski P., Tautt J., Dudkiewicz J., *Boll. Chim. Farm.*, **133**, 301–308 (1994).
- Boger J., Payne L. S., Perlow D. S., Lohr N. S., Poe M., Blaine E. H., Ulm E. H., Schorn T. W., LaMount B. I., Lien T.-K., Kawai M., Rich

- D. H., Veber D. F., *J. Med. Chem.*, **28**, 1779—1790 (1985).
- 16) Maibaum J., Rich D. H., *J. Org. Chem.*, **53**, 869—873 (1988).
- 17) Galen F. X., Devaux C., Grogg P., Menard J., Corvol P., *Biochim. Biophys. Acta*, **523**, 485—493 (1978).
- 18) Dudkiewicz-Wilczyńska J., Roman I., Paruszewski R., *Acta Pol. Pharm.*, **61**, 171—174 (2004).
- 19) Paruszewski R., Tautt J., Dudkiewicz J., *Pol. J. Pharmacol.*, **45**, 75—82 (1993).
- 20) Behr L. D., Clarke H. J., *J. Am. Chem. Soc.*, **54**, 1630—1637 (1932).
- 21) Cheung S. T., Benoiton N. L., *Can. J. Chem.*, **55**, 906—910 (1977).
- 22) Schwyzer R., Sieber P., Kappeler H., *Helv. Chim. Acta*, **42**, 2622—2634 (1959).