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β-Lactam derivatives as enzyme inhibitors: Derivatives of (*E*)-2-[(*RS*)-1-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionic acid

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The 1,4-diaryl disubstituted azetidin-2-one (β-lactam) **1** is transformed into the 3-methylidene derivative (*E*)-2-[(*RS*)-1-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionic acid (**3**), and then, using the DCC/NHS method reacted with amino acid esters and dipeptide esters forming 3-(peptidyl)-β-lactams **5** and **7**. Structures and properties are evaluated mainly by spectroscopic methods and discussed. As molecular modeling experiments might suggest a potential activity as inhibitors of PPE(HLE), a number of selected compounds has been tested in an enzyme assay. But none of them showed any remarkable inhibitory activity. Evaluation of the data was done with the new program EnKinPlot.

1. Introduction

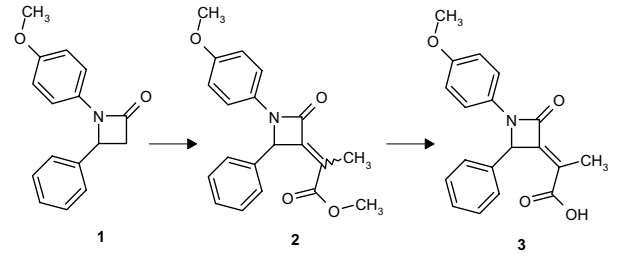
The serine protease elastase (HLE) and some cysteine proteases have been found involved in the pathogenesis of a large number of diseases [1]. Following the concept of development of protease inhibitors bearing a β-lactam ring as the chemically reactive group allowing a nucleophilic attack at the active site of the enzyme, and a peptide moiety responsible for the recognition by the enzyme [2], we now report about syntheses and properties of some peptidyl derivatives of (*E*)-2-[(*RS*)-1-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionic acid. Amino acid residues used are Val, Ile, Pro, and Ala.

2. Investigations, results and discussion

2.1. Chemistry

The β-lactam **1** was prepared from 4-methoxybenzalaniline and ethyl bromoacetate according to *Gilman* and *Speeter* [3], but in THF and not in toluene. It was silylated with CTMS and LDA giving a *cis*/*trans* mixture [4] of 1-(4-methoxyphenyl)-4-phenyl-3-(trimethylsilyl)-azetidin-2-one, from which by olefination with methyl 2-oxopropionate the 3-methylidene derivative **2** was obtained according to Peterson [5] as an *E*/*Z* mixture. Separation of the isomers by CC [5] yielded a ratio $E:Z = 1:1$, while by fractional crystallization a ratio of 3:1 was obtained. Furthermore, by NOE experiments we could demonstrate that the *Z*-isomer has a m.p. 142–145 °C, while the *E*-isomer melts at 165–168 °C. As demonstrated by Schirmeister [6] the hydrolysis **2**→**3** (Scheme 1) can be performed with porcine liver esterase, but as the reaction of the *E*-isomer was very slow we decided to hydrolyze **2***E* with NaOH in acetone, and **3** was isolated with 88% yield. The structure of **3** was established by the IR band at 1732 cm^{-1} indicating the β-lactam ring structure, by the signal at $\delta = 5.87$ ppm of the proton at C-4 in the ^{1}H NMR spectrum (d_6-DMSO), and by the mass spectrum (EI) giving $m/z = 323$.

Scheme 1



The dipeptide esters **6** were prepared by standard procedures in dichloromethane using DCC and triethylamine [7]. The reaction of **3** with amino acid esters or dipeptide esters **6** either can be done in a one-pot-procedure with DCC and N-hydroxysuccinimide, or in a two-step-procedure after preparation and isolation of the active ester **4**. In all reactions with amino acid esters the one-pot-procedure gave better yields of the products **5** (Scheme 2, Table 1).

All compounds **5** were isolated as mixtures of diastereomers as could be seen from their ^{1}H NMR spectra. The signals of the protons of the aromatic methoxy group were found at $\delta = 3.73$ ppm, those of the methyl group at the double bond around $\delta = 2.3$ ppm. As for the signal of 4-H around $\delta = 5.7$ ppm for the signal of the methyl protons usually two signals were found indicating the diastereomeric mixture. The α-protons of the amino acid part gave in all cases a singulet around $\delta = 4.4$ ppm, establishing that no racemization had occurred during the reaction. The β-lactam ring was characterized by a strong band around $\nu = 1735$ cm^{-1} in the IR spectra of all compounds **5**. The ratio of diastereomers was determined by HPLC methods using either a RP18 column or a (S,S)-Whelk-01 column.

Scheme 2

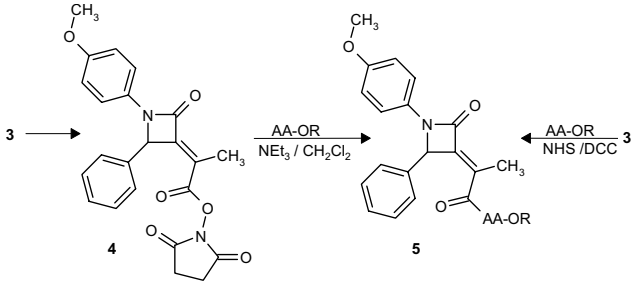


Table 1: β-Lactam amino acid esters 5

Compd.	AA-OR	Yield (%)	M.p. (°C)	Ratio of diastereomers
5a	Ala-OCH_2-C_6H_5	32	92–97	1 : 1[a]
5b	Ile-OCH_3	58	110–113	1 : 0.8[b]
5c	Phe-OCH_2-C_6H_5	43	110–115	1 : 0.5[b]
5d	Val-OCH_2-C_6H_5	45	134–138	1 : 1.2[b]
5e	Val-OCH_3	34	137–142	1 : 1[b]

[a] from ^{1}H NMR spectra, [b] from HPLC

The reaction of the β-lactam **3** with dipeptide esters was done in a similar way as that with amino acid esters. First, the Boc-protected dipeptide esters **6** were deprotected with trifluoroacetic acid, and then the dipeptide ester triflates were added to a mixture of **3** with DCC and NHS.

By this procedure the β-lactam dipeptide esters **7** were obtained in yields around 35% (Scheme 3, Table 2). All compounds were isolated after CC as light yellow solids. From HPLC experiments it became evident that all compounds were obtained as mixtures of diastereomers. We did not separate the diastereomers. All compounds were characterized by IR and NMR spectra.

The IR spectra showed the carbonyl band of the β-lactam ring between $\nu = 1734$ and $1744\ cm^{-1}$, and those of the peptide bonds between $\nu = 1634$ and $1693\ cm^{-1}$. The ^1H NMR spectra clearly indicated the mixture of diastereomers by double signals of the protons of the methyl group at the double bond around $\delta = 2.25–2.35$ ppm, of the aromatic methoxy group protons between $\delta = 3.72$ and 3.74 ppm, and of proton 4-H between $\delta = 5.4$ and 5.8 ppm. On the other hand, the signals of the amino acid residue protons demonstrated that no racemization had occurred during the synthesis. The ratio of diastereomers was obtained from HPLC experiments.

2.2. Biological evaluation

The enzyme inhibitor complex of PPE, PBD Code: 1NES, was used as the basis of our calculations. To minimize the calculation time all solvent molecules were omitted, the structure of our compounds were optimized by MM+, and all calculations were done in vacuo. Finally, in the first series of calculations only binding to the catalytic triade His-57, Asp-102 and Ser-195 was calculated. These calculations showed that also Thr-41 and Gly-193 should be involved in the binding of the inhibitors. Under these conditions, the complexes between PPE and the compounds **7a**, **7c**, **7e**, **7f** and **7g** were calculated again, but the interactions found were very low. Nevertheless all derivatives **5** and **7** were tested with the standard procedure [1]. None of the compounds showed any remarkable inhibitor activity.

Scheme 3

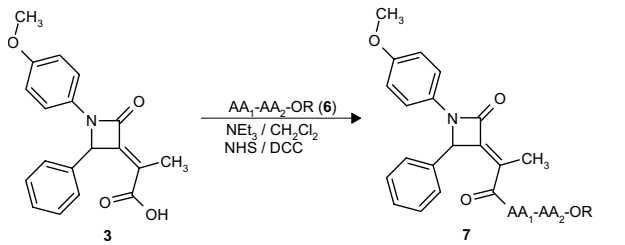


Table 2: β-Lactam dipeptide esters 7

Compd.	AA_1-AA_2-OR	Yield (%)	M.p. (°C)	Ratio of diastereomers[a]
7a	Phe-Val-OC_2H_5	33	150–152	1 : 1.25
7b	Phe-Val-OCH_3	31	115–120	1 : 1.25
7c	Pro-Val-OCH_2-CO-C_6H_5	31	160	1 : 1
7d	Val-Ile-OCH_3	36	155–158	1 : 0.8
7e	Val-Phe-OC_2H_5	35	185–188	1 : 1
7f	Val-Pro-OCH_2-C_6H_5	33	150–155	1 : 1.7
7g	Val-Val-OCH_3	37	160–166	1 : 0.9
7h	Val-Val-OCH_2-CO-C_2H_5	28	168–174	1 : 1

[a] from HPLC

3. Experimental

3.1. Chemistry

3.1.1. Apparatus and reagents

Mp: PHMK 80/2747 (Küstner, Dresden) apparatus, not corrected. IR Spectra: Perkin-Elmer FTIR 1600; in KBr (cm^{-1}), if not noted otherwise. NMR Spectra: Bruker DPX 200 (200 MHz), *ARX 300* (300 MHz) for ^1H; Bruker DPX 200 (50 MHz) for ^{13}C; δ (ppm) rel. to TMS as internal standard, J in Hz; ^1H-values and ^{13}C-values from spectra in $CDCl_3$, if not noted otherwise. Mass Spectra: Intectra AMD 402/3. Optical rotation: Polatronic D. Elementary analyses: Perkin-Elmer Analyzer 2400 CHN, Pharmazeutisches Institut der Universität Greifswald. TLC on Merck DC-Alufolien, Silica Gel 60 F_{254}, Nr. 5554. CC with Silica Gel 60 Merck Nr. 7734 or 9385. HPLC with LaChrom apparatus series 7000 Merck Hitachi, LiChrospher 250-4, RP-18,5 µm, and LiChroCART 250-4, (S,S)-Whelk-O1, 5 µm.

PPE (≈ 200 U/mg) was purchased from Serva, Suc-$(Ala)_3$-pNA from Fluka.

All the results of elemental analyses were in an acceptable range.

Tetrahydrofuran (THF) was stored with $CaCl_2$, then refluxed with Na and benzophenone, and distilled prior to use. Other solvents were dried/purified according to literature procedures. LDA (lithium diisopropylamide) was freshly prepared by mixing of equivalent amounts of freshly distilled diisopropylamine and n-butyllithium (15% in hexane) at −78 °C.

Abbreviations: CC = Column chromatography; CTMS = Chlorotrimethylsilane; DCC = Dicyclohexyl Carbodiimide; DPPA = Diphenylphosphoroazidate; EtOAc = Ethyl acetate; NHS = N-Hydroxysuccinimide; ar = aromatic.

3.1.2. (E/Z)-Methyl 2-[(RS)-1-(4-methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionate (**2**)

Synthesis as described earlier [5], but extraction with EtOAc instead of $CHCl_3$, and separation of isomers by fractional crystallization from methanol.

(*E*)-**2**: Yield: 700 mg (20%). Yellow platelets. M.p. 165–168 °C. – IR: $\nu = 3063, 2942, 2849$ (CH), 1725, 1709 (CO). – ^1H NMR: $\delta = 2.29$, 3.57 (2 s, each 3 H, CH_3), 3.73 (s, 3 H, OCH_3), 5.60 (s, 1 H, 4-H), 6.75–7.41 (m, 9 H, ar H). – ^{13}C NMR: $\delta = 14.4$ (CH_3), 51.7 (CH_3), 55.4 (C-4), 65.0 (OCH_3), 114.4, 118.6, 126.3, 127.7, 128.7, 130.7, 160.4 (ar C), 136.4, 148.9 (C=C), 156.4, 165.8 (CO). – MS (EI, 70 eV): m/z = 337. – HREIMS: Calcd. 337.13141; found 337.12578.

(*Z*)-**2**: Yield: 300 mg (9%). Yellow needles. M.p. 142–145 °C. – IR: $\nu = 3082, 3059, 2952, 2833$ (CH), 1740 (CO). – ^1H NMR: $\delta = 1.73$ (s, 3 H, CH_3), 3.75 (s, 3 H, OCH_3), 3.89 (s, 3 H, CH_3), 5.37 (s, 1 H, 4-H), 6.76–7.44 (m, 9 H, ar H). – ^{13}C NMR: $\delta = 14.9$ (CH_3), 52.3 (CH_3), 55.4 (C-4), 62.6 (OCH_3), 114.4, 118.4, 127.5, 128.6, 129.2, 133.3, 160.4 (ar C), 135.7, 148.3 (C=C), 156.4, 166.3 (CO).

$C_{20}H_{19}NO_4$ (337.4)

3.1.3. (E)-2-[(RS)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionic acid (**3**)

700 mg (2.1 mmol) of (*E*)-**2** was dissolved in 20 ml of acetone, 25 ml of 0.1 N NaOH was added, and the mixture was stirred at 40 °C for 30–60 min. The mixture was extracted with EtOAc, the organic layer was separated, the aqueous layer was acidified with HCl and extracted again. The combined organic layers were dried (Na_2SO_4), and the solvent was evaporated in vacuo. Yield: 600 mg (88%). Yellow solid. M.p. 210–213 °C (MeOH). – IR: $\nu = 3072, 3032, 2997, 2956, 2904, 2831$ (CH), 1732, 1699 (CO). – ^1H NMR ([d_6]DMSO): $\delta = 2.15$ (s, 3 H, CH_3), 3.77 (s, 3 H, OCH_3), 5.87 (s, 1 H, 4-H), 6.75–7.44 (m, 9 H, ar H). – ^{13}C NMR ([d_6]DMSO): $\delta = 14.07$ (CH_3), 55.14 (C-4), 63.66 (OCH_3), 114.40, 118.39, 126.81, 127.82, 128.30, 130.05, 159.99 (ar C), 136.60, 147.74 (C=C), 155.90, 166.16 (CO). – HPLC: 8.14 min (RP-18, acetonitril/1% HOAc 1 : 1). – MS (EI, 70 eV): m/z = 323. – HREIMS: Calcd. 323.11575; found 323.11049.

$C_{19}H_{17}NO_4$ (323.4)

3.1.4. (E)-1-{2-[(RS)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyloxy}-pyrrolidin-2,5-dione (**4**)

1 mmol of **3** and 2 mmol of NHS were dissolved in 50 ml of CH_2Cl_2 and cooled to 0 °C, 1.1 mmol of DCC in 10 ml of CH_2Cl_2 was added, the mixture was stirred for 2 h at 0 °C, than 24 h at room temp. The precipitate was separated, the filtrate concentrated and purified by CC (EtOAc/cyclohexane 1 : 1, $R_f = 0.41$). Yield: 350 mg (83%). Yellow solid. M.p. 204-207 °C (MeOH). – IR: $\nu = 3454, 3326, 2928, 2850$ (CH), 1767, 1737 (CO). – ^1H NMR: $\delta = 2.43$ (s, 3 H, CH_3), 2.80 (s, 4 H, CH_2), 3.74 (s, 3 H, OCH_3), 5.66 (s, 1 H, 4-H), 6.75–7.44 (m, 9 H, ar H). – ^{13}C NMR: $\delta = 14.11$ (CH_3), 25.54 (CH_2), 55.41 (C-4), 65.24 (OCH_3), 114.40, 118.39, 126.81, 127.82, 128.30, 130.05, 159.99 (ar C), 136.60, 147.74 (C=C), 155.90, 166.16 (CO). – HPLC: 3.73 min (RP-18, acetonitril/water 6 : 2). – MS (EI, 70 eV): m/z = 420. – HREIMS: Calcd. 420.13214; found 420.13491.

$C_{23}H_{20}N_2O_6$ (420.4)

3.1.5. Synthesis of β-lactam amino acid esters **5**

1 eq of **3** and 2 eq of NHS were suspended in 50 ml of CH_2Cl_2 and cooled to −10 °C. With stirring, 1.1 eq of DCC was added, and after 30 min, 1.2 eq of the amino acid ester salt and 1.2 eq. of TEA were added. Stirring was continued for 6-8 h at −10 °C and then for 3-5 d at room temp. Then, the mixture was filtered, the solvent was concentrated, the residue was dissolved in EtOAc, filtered, and washed with dil. HCl (2 × 25 ml), a satd. solution of Na_2CO_3 (25 ml), and a satd. solution of NaCl (25 ml). The organic layer was dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by CC (EtOAc/CH_2Cl_2 1 : 1).

3.1.5.1. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-alanine benzyl ester (**5a**)

From 420 mg (1.3 mmol) of **3**, and 550 mg (1.6 mmol) of L-Ala-OBn-p-tosylate. Yield: 200 mg (32%). Light yellow solid. M.p. 92–97 °C. – $R_f = 0.68$. – IR: ν = 3335 (NH), 3064, 3030, 2931, 2836 (CH), 1736 (CO). – ^{1}H NMR: δ = 1.14, 1.17 [2s, 3 H, CH_3(Ala)], 2.33, 2.35 (2s, 3 H, CH_3), 3.73 (s, 3 H, OCH_3), 4.44 [m, 1 H, α-H(Ala)], 5.14 [m, 2 H, CH_2(benzyl)], 5.58, 5.62 (2s, 1 H, 4-H), 5.98, 6.04 (2d, 1 H, NH), 6.75–7.45 (m, 9 H, ar H). – ^{13}C NMR: δ = 14.68 (CH_3), 17.74, 18.10 [CH_3(Ala)], 48.16 [α-C(Ala)], 55.38 (C-4), 64.18 (OCH_3), 67.30 [CH_2(benzyl)], 114.39, 118.51, 127.80, 128.12, 128.28, 128.76, 128.93, 130.83, 160.81 (ar C), 136.28, 144.39 (C=C), 156.36, 164.80, 172.39 (CO). – HPLC: 5.62 min (RP-18, acetonitril/water 6 : 2). – MS (EI, 70 eV): m/z = 484. – HREIMS: Calcd. 484.19983; found 484.19667.
$C_{29}H_{28}N_2O_5$ (484.6)

3.1.5.2. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-isoleucine methyl ester (**5b**)

From 310 mg (0.95 mmol) of **3**, and 210 mg (1.1 mmol) of L-Ile-OMe-HCl. Yield: 250 mg (58%). Light yellow solid. M.p. 110–113 °C. – $R_f = 0.64$. – IR: ν = 3426 (NH), 3033, 2962, 2876 (CH), 1735 (CO). – ^{1}H NMR: δ = 0.74, 0.79 [m, 6 H, CH_3(Ile)], 1.18 [m, 2 H, CH_2(Ile)], 1.53, 1.67 [m, 1 H, β-H(Ile)], 2.28, 2.30 (2s, 3 H, CH_3), 3.59 (s, 3 H, CH_3) 3.66 (s, 3 H, OCH_3), 4.37 [m, 1 H, α-H(Ile)], 5.61, 5.63 (2s, 1 H, 4-H), 5.93, 5.98 (2d, 1 H, NH), 6.68-7.47 (m, 9 H, ar H). – ^{13}C NMR: δ = 11.42 [CH_3(Ile)], 14.73, 14.89 (CH_3), 15.21 [CH_3(Ile)], 25.16 [CH_2(Ile)], 37.68, 37.89 [β-C(Ile)], 52.10 [α-C(Ile)], 55.39 (C-4), 56.34, 56.54 (CH_3), 64.14, 64.27 (OCH_3), 114.37, 118.54, 127.85, 128.15, 128.59, 128.80, 130.83, 130.95, 160.83 (ar C), 136.09, 145.04, 145.75 (C=C), 156.33, 164.61, 164.83, 172.10 (CO). – HPLC: 12.11, 14.63 min [(S,S)-Whelk-01, hexane/isopropanol 4 : 6]. – MS (EI, 70 eV): m/z = 450. – HREIMS: Calcd. 484.19983; found 484.19667.
$C_{26}H_{30}N_2O_5$ (450.5)

3.5.1.3. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-phenylalanine benzyl ester (**5c**)

From 480 mg (1.5 mmol) of **3**, and 770 mg (1.8 mmol) of L-Phe-OBn-p-tosylate. Yield: 360 mg (43%). Light yellow solid. M.p. 110–115 °C. $R_f = 0.71$. – IR: ν = 3408 (NH), 3299, 3061, 3028, 2950, 2836 (CH), 1729, 1702 (CO). – ^{1}H NMR: δ = 2.23 (s, 3 H, CH_3), 2.87 [dd, 2 H, β-H(Phe)], 3.04 [dd, 2 H, β-H(Phe)], 3.73 (s, 3 H, OCH_3), 4.71 [m, 1 H, α-H(Phe)], 5.10 [s, 2 H, CH_2(benzyl)], 5.62 (s, 1 H, 4-H), 5.88 (m, 1 H, N–H), 6.75–7.42 (m, 19 H, ar H). – ^{13}C NMR: δ = 14.43 (CH_3), 36.88, 37.33 [CH_2(Phe)], 53.24, 53.41 [α-C(Phe)], 55.39 (C-4), 64.24 (OCH_3), 67.37 [CH_2(benzyl)], 114.39, 118.53, 127.18, 128.01, 128.66, 128.80, 129.15, 130.88, 135.08, 135.55, 160.77 (ar C), 136.39, 144.49 (C=C), 156.36, 164.64, 170.87 (CO). – HPLC: 20.50, 21.51 min (RP-18, acetonitril/KH_2PO_4-buffer 1 : 1). – MS (EI, 70 eV): m/z = 560. – HREIMS: Calcd. 560.23114; found 560.22541.
$C_{35}H_{32}N_2O_5$ (560.7)

3.5.1.4. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valine benzyl ester (**5d**)

From 450 mg (1.3 mmol) of **3**, and 590 mg (1.5 mmol) of L-Val-OBn-p-tosylate. Yield: 300 mg (45%). Light yellow solid. M.p. 134–138 °C. $R_f = 0.73$. – IR: ν = 3402, 3318 (NH), 3034, 2962, 2835 (CH), 1741 (CO). – ^{1}H NMR: δ = 0.55, 0.64, 0.65, 0.81 [2dd, 6 H, CH_3(Val)], 1.98 [m, 1 H, β-H(Val)], 2.34, 2.37 (2s, 3 H, CH_3), 3.73 (s, 3 H, OCH_3), 4.46 [m, 1 H, α-H(Val)], 5.10 [s, 2 H, CH_2(benzyl)], 5.63, 5.68 (2s, 1 H, 4-H), 5.98, 6.07 (2d, 1 H, NH), 6.75-7.45 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.71 (CH_3), 17.58, 17.83, 18.45, 18.81 [CH_3(Val)], 31.20, 31.41 [β-C(Val)], 55.40 (C-4), 56.92, 57.24 [α-C(Val)], 64.24 (OCH_3), 67.18 [CH_2(benzyl)], 114.38, 118.55, 127.83, 128.14, 128.58, 128.84, 130.97, 135.16, 160.81 (ar C), 136.13, 145.03, 145.89 (C=C), 156.35, 164.71, 164.98, 171.40 (CO). – HPLC: 16.59, 17.35 min (RP-18, acetonitril/KH_2PO_4-buffer 1 : 1). – MS (EI, 70 eV): m/z = 512. – HREIMS: Calcd. 512.23114; found 512.23127.
$C_{35}H_{32}N_2O_5$ (512.6)

3.1.5.5. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valine methyl ester (**5e**)

From 330 mg (1.0 mmol) of **3**, and 200 mg (1.2 mmol) of L-Val-OMe-HCl. Yield: 150 mg (34%). Light yellow solid. M.p. 137–142 °C. $R_f = 0.56$. – IR: ν = 3356 (NH), 3070, 3032, 2962, 2872 (CH), 1734 (CO). – ^{1}H NMR: δ = 0.58, 0.68, 0.73, 0.84 [2dd, 6 H, CH_3(Val)], 1.99 [m, 1 H, β-H(Val)], 2.36, 2.38 (2s, 3 H, CH_3), 3.67 (s, 3 H, CH_3), 3.73 (s, 3 H, OCH_3), 4.42 [m, 1 H, α-H(Val)], 5.71 (s, 1 H, 4-H), 5.98, 6.03 (2d, 1 H, N-H), 6.75–7.54 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.71 (CH_3), 17.73, 17.98, 18.47, 18.79 [CH_3(Val)], 31.24, 31.40 [β-C(Val)], 52.15, 52.25 [CH_3(Val)], 55.39 (C-4), 56.93, 57.19 [α-C(Val)], 64.34 (OCH_3), 114.39, 118.56, 127.88, 128.13, 128.59, 128.80, 130.85, 160.85 (ar C), 136.11, 136.21, 145.35, 145.94 (C=C), 156.36, 164.70, 164.90, 172.11 (CO). – HPLC: 10.05, 12.51 min [(S,S)-Whelk-01, hexane/propanol 4 : 6]. – MS (EI, 70 eV): m/z = 436. HREIMS: Calcd. 436.19983; found 436.19514.
$C_{25}H_{28}N_2O_5$ (436.5)

3.1.6. Synthesis of dipeptide esters **6**

1 eq N-Boc amino acid, 1.2 eq amino acid ester salt, and 1.2 eq TEA were dissolved in 20 ml of CH_2Cl_2 and cooled to −10 °C. 1.1 eq DCC in 10 ml of CH_2Cl_2 was added, and the mixture was stirred 2 h at −10 °C, and then 12 h at room temp. Then, the precipitate was separated, and the mixture was washed with 1 M HCl (50 ml), a satd. solution of Na_2CO_3 (50 ml) and a satd. solution of NaCl (50 ml). The organic layer was dried (Na_2SO_4), evaporated in vacuo, and the residue was purified by CC (EtOAc/cyclohexane 1 : 1).

3.1.6.1. Boc-L-phenylalanyl-L-valine ethyl ester (**6a**)

From 3.04 g (11.5 mmol) Boc-L-Phe, and 2.50 g (13.8 mmol) L-Val-OEt-HCl. Yield: 2.3 g (51%). $[a]_D^{25} = -18°$ (c = 2, MeOH). $R_f = 0.78$. M.p. 118–120 °C [8]. HPLC: 26.62 min (RP-18, acetonitril/water 1 : 1).
$C_{21}H_{32}N_2O_5$ (392.5)

3.1.6.2. Boc-L-phenylalanyl-L-valine methyl ester (**6b**)

From 3.65 g (10.2 mmol) Boc-L-Phe, and 2.05 g (12.2 mmol) L-Val-OMe-HCl. Yield: 2.4 g (62%). $[a]_D^{25} = -15.5°$ (c = 2, MeOH). $R_f = 0.70$. M.p. 114–117 °C [9]. HPLC: 18.65 min (RP-18, acetonitril/water 1 : 1).
$C_{20}H_{30}N_2O_5$ (378.5)

3.1.6.3. Boc-L-prolyl-L-phenylalanine phenacyl ester (**6c**)

From 0.90 g (4.2 mmol) Boc-L-Pro, and 2.00 g (5.0 mmol) L-Phe-O-phenacyl ester triflate. Yield: 0.65 g (32%). = −57.5° (c = 2, MeOH). $R_f = 0.36$. M.p. 85–90 °C. – IR: ν = 3392 (NH), 2977, 2895 (CH), 1764, 1699, 1670 (CO). – ^{1}H NMR: δ = 1.41 [s, 9 H, $C(CH_3)_3$], 1.72–2.17 [m, 4 H, β-H, γ-H(Pro)], 3.13, 3.44, 5.04 (ABX, $J_{AX} = 7.9$ Hz, $J_{BX} = 5.3$ Hz, $J_{AB} = 14.1$ Hz, 3 H, 2 β-H(Phe), α-H(Phe)], 3.25 [m, 2 H, δ-H(Pro)], 4.22 [m, 1 H, α-H(Pro)], 5.34, 5.51 (dd, J = 16.4 Hz, 2 H, CH_2), 6.47 (bs, 1 H, N-H), 7.42–7.93 (m, 10 H, ar H). – HPLC: 4.77 min (RP-18, acetonitril/water 8 : 2).
$C_{27}H_{32}N_2O_6$ (480.6)

3.1.6.4. Boc-L-prolyl-L-valine phenacyl ester (**6d**)

From 1.00 g (4.6 mmol) Boc-L-Pro, and 2.00 g (5.7 mmol) L-Val-O-phenacyl ester triflate. Yield: 1.00 g (52%). $[a]_D^{25} = -95°$ (c = 1.31, MeOH). $R_f = 0.34$. M.p. 96 °C. – IR: ν = 3332 (NH), 3077, 2968, 2933 (CH), 1748, 1685, 1648 (CO). – ^{1}H NMR ([d_6]DMSO): δ = 0.99, 1.02 [d, J = 6.6 Hz, 6 H, CH_3(Val)], 1.33 [s, 9 H, $C(CH_3)_3$], 1.78 [m, 4 H, β-H(Val), 2 γ-H(Pro), β-H(Pro)], 2.20 [m, 1 H, β-H(Pro)], 3.24 [m, 2 H, δ-H(Pro)], 4.29, 4.37 [m, 2 H, α-H(Val), α-H(Pro)], 5.48, 5.60 (dd, J = 16.8 Hz, 2 H, CH_2), 7.52–8.00 (m, 5 H, ar H), 8.16 [m, 1 H, N-H(Val)]. – HPLC: 20.72 (acetonitril/water 1 : 1).
$C_{23}H_{32}N_2O_6$ (432.5)

3.1.6.5. Boc-L-valyl-L-isoleucine methyl ester (**6e**)

From 2.17 g (10.0 mmol) Boc-L-Val, and 2.18 g (12.0 mmol) L-Ile-OMe-HCl. Yield: 2.60 g (75%). $[a]_D^{25} = -28.5°$ (c = 2, MeOH). $R_f = 0.56$. M.p. 120–122 °C [10]. HPLC: 6.43 min (RP-18, acetonitril/water 7 : 3).
$C_{17}H_{32}N_2O_5$ (344.5)

3.1.6.6. Boc-L-valyl-L-phenylalanine ethyl ester (**6f**)

From 1.97 g (9.1 mmol) Boc-L-Val, and 2.50 g (10.9 mmol) L-Phe-OEt-HCl. Yield: 1.80 g (51%). $[a]_D^{25} = +31°$ (c = 1, CH_2Cl_2). $R_f = 0.82$. M.p. 110–113 °C [8]. HPLC: 23.52 min (RP-18, acetonitril/water 1 : 1).
$C_{21}H_{32}N_2O_5$ (392.5)

3.1.6.7. Boc-L-valyl-L-valine methyl ester (**6g**)

From 2.20 g (10.1 mmol) Boc-L-Val, and 2.04 g (12.1 mmol) L-Val-OMe-HCl. Yield: 2.18 g (66%). $[a]_D^{25} = -34°$ (c = 2, MeOH). $R_f = 0.69$. M.p. 157–160 °C [11]. HPLC: 11.47 min (RP-18, acetonitril/water 1 : 1).
$C_{16}H_{30}N_2O_5$ (330.4)

3.1.6.8. Boc-L-valyl-L-valine phenacyl ester (**6h**)

From 0.93 g (4.3 mmol) Boc-L-Val, and 1.80 g (5.0 mmol) L-Val-O-phenacyl ester triflate. Yield: 0.98 g (52%). $[a]_D^{25} = -58.5°$ (c = 2, MeOH). $R_f = 0.69$. M.p. 143–145 °C. – IR: ν = 3336 (NH), 1747, 1700, 1691, 1655 (CO). – ^{1}H NMR: δ = 0.96 [t, 6 H, CH_3(Val)], 1.05 [d, 6 H, CH_3(Val)], 1.44 [s, 9 H, $C(CH_3)_3$], 2.15 [q, 1 H, β-H(Val)], 2.40 [m, 1 H, β-H(Val)], 3.91, 3.95 [dd, 1 H, α-H(Val)], 4.73, [dd, 1 H, α-H(Val)], 5.06 [d, 1 H, N-H(Val)], 5.29, 5.52 (dd, J = 16.4 Hz, 2 H, CH_2), 6.40 [d, 1 H, N-H(Val)], 7.45–7.92 (m, 5 H, ar H). – HPLC: 29.05 min (RP-18, acetonitril/water 1 : 1).
$C_{23}H_{34}N_2O_6$ (434.5)

3.1.6.9. Boc-L-valyl-L-proline benzyl ester (**6i**)

Synthesis from 2.0 g (9.2 mmol) of Boc-L-Val and 2.45 g (10.1 mmol) L-Pro-OBn-HCl, using DDPA, see ref. [12]. Yield: 1.50 g (40%). M.p. 74–76 °C (ref. 79 °C). $[a]_D^{25} = -94°$ (c = 1, MeOH). $R_f = 0.25$ (EtOAc/cyclohexane 1 : 1). HPLC: 29.89 min (RP-18, acetonitril/water 1 : 1).
$C_{22}H_{32}N_2O_5$ (404.5)

3.1.7. Synthesis of β-lactam dipeptide esters **7**

a) At room temp., 2 ml of trifluoroacetic acid was added to 10 mmol of **6**, the mixture was stirred for 1 h, and then evaporated in vacuo. The residue was used without further purification.
b) 1 eq of **3** and 2 eq of NHS were suspended in 50 ml of CH_2Cl_2, and cooled to −10 °C. With stirring, 1.1 eq of DCC was added, and after 30 min, 1.2 eq of the dipeptide ester triflate (from a) was added. Stirring was continued for 6–8 h at −10 °C, and then for 3–5 d at room temp. After filtration, the solution was concentrated in vacuo, the residue was dissolved in EtOAc, filtered, and washed with dil. HCl (2 × 25 ml), a satd. solution of Na_2CO_3 (25 ml), and a satd. solution of NaCl (25 ml). The organic layer was dried (Na_2SO_4), and evaporated in vacuo. The residue was purified by CC, EtOAc/CH_2Cl_2 1: 4, if not noted otherwise.

3.1.7.1. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-phenylalanyl-L-valine ethyl ester (**7a**)

From 330 mg (1.0 mmol) of **3** and **6a**. Yield: 200 mg (33%). Light yellow solid. M.p. 150–152 °C. $R_f = 0.65$. – IR: ν = 3312 (NH), 3061, 3030, 2963, 2934, 2873 (CH), 1734, 1634 (CO). – ^{1}H NMR: δ = 0.73, 0.77, 0.84, 0.87 [2dd, 6 H, CH_3(Val)], 1.27 (t, 3 H, CH_3), 2.04 [m, 1 H, β-H(Val)], 2.26 (s, 3 H, CH_3), 2.83 [m, 2 H, β-H(Phe)], 3.72 (s, 3 H, OCH_3), 4.18 (m, 2 H, CH_2), 4.46 [m, 2 H, α-H(Val), α-H(Phe)], 5.63, 5.71 (2s, 1 H, 4-H), 6.29 (m, 2 H, 2 NH), 6.75–7.46 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.23, 14.55 (CH_3), 17.77, 18.84 [CH_3(Val)], 31.07 [β-C(Val)], 37.72, 37.96 [CH_2(Phe)], 53.99 [α-C(Phe)], 54.89, 55.37 (C-4), 57.44 [α-C(Val)], 64.04 (OCH_3), 64.32 (CH_2), 114.36, 118.53, 127.13, 127.82, 128.09, 128.51, 128.60, 128.68, 128.92, 129.21, 129.32, 130.88, 160.70 (ar C), 135.75, 136.28, 144.95, 145.85 (C=C), 156.38, 164.78, 165.01, 170.09, 170.35, 171.11, 171.27 (CO). – HPLC: 44.91, 48.15 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 597. – HREIMS: Calcd. 597.28387; found 597.28438.
$C_{35}H_{39}N_3O_6$ (597.7)

3.1.7.2. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-phenylalanyl-L-valine methyl ester (**7b**)

From 330 mg (1.0 mmol) of **3** and **6b**. Yield: 180 mg (31%). Light yellow solid. M.p. 115–120 °C (MeOH). – $R_f = 0.47$ (EtOAc/cyclohexane 1 : 1). – IR: ν = 3319 (NH), 3060, 3030, 2958, 2873 (CH), 1734, 1662 (CO). – ^{1}H NMR: δ = 0.71, 0.75, 0.81, 0.85 [2dd, 6 H, CH_3(Val)], 2.07 [m, 1 H, β-H(Val)], 2.25, 2.26 (2s, 3 H, CH_3), 2.83 [m, 2 H, β-H(Phe)], 3.72 (m, 6 H, 2 OCH_3), 4.37 [m, 1 H, α-H(Val)], 4.53 [m, 1 H, α-H(Phe)], 5.63, 5.69 (m, 1 H, 4-H), 6.11, 6.34 (2m, 2 H, 2 NH), 6.75–7.47 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.57 (CH_3), 17.77, 18.84 [CH_3(Val)], 31.03 [β-C(Val)], 37.96 [CH_2(Phe)], 52.19 (OCH_3), 54.12 [α-C(Phe)], 54.88, 55.37 (C-4), 57.47 [α-C(Val)], 64.04, 64.35 (OCH_3), 114.38, 118.53, 127.09, 127.84, 128.13, 128.62, 128.74, 128.96, 129.32, 130.90, 160.72 (ar C), 135.83, 136.29, 145.01, 145.85 (C=C), 156.34, 164.81, 165.02, 170.09, 170.31, 171.65 (CO). – HPLC: 30.43, 32.35 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 583. – HREIMS: Calcd. 583.26825; found 583.26171.
$C_{34}H_{37}N_3O_6$ (583.7)

3.1.7.3. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-prolyl-L-valine phenacyl ester (**7c**)

From 490 mg (1.5 mmol) of **3** and **6d**. Yield: 300 mg (31%). Light yellow solid. M.p. 160 °C. $R_f = 0.33$. – IR: ν = 3031 (CH), 1740, 1699, 1679 (CO). – HPLC: 37.21, 39.72 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): *m/z* = 637. – HREIMS: Calcd. 637.27881; found 637.28400.

Isomer I: ^{1}H NMR: δ = 1.00, 1.04 [dd, 6 H, CH_3(Val)], 1.36 [m, 1 H, β-H(Pro)], 1.65 [m, 3 H, β-H(Val), 2 γ-H(Pro)], 2.26 (s, 3 H, CH_3), 2.38 [m, 1 H, β-H(Pro)], 3.13 [m, 2 H, 2 δ-H(Pro)], 3.74 (s, 3 H, OCH_3), 4.31, 4.33 [dd, 1 H, α-H(Pro)], 4.57, 4.62 [dd, 1 H, α-H(Val)], 5.27, 5.53 [AB, J = 16.3 Hz, 2 H, CH_2(ester)], 5.45 (s, 1 H, 4-H), 6.27 (d, 1 H, NH), 6.75–7.93 (m, 14 H, ar H). – ^{13}C NMR: δ = 15.62 (CH_3), 17.26, 19.24 [CH_3(Val)], 24.20 [γ-C(Pro)], 26.28 [β-C(Pro)], 30.92 [β-C(Val)], 47.38 [δ-C(Pro)], 55.44 (C-4), 57.30 [α-C(Val)], 58.85 [α-C(Pro)], 62.16 (OCH_3), 66.37 (CH_2), 114.45, 118.33, 126.53, 127.77, 128.93, 130.88, 132.82, 133.99, 160.06 (ar C), 135.75 , 139.77 (C=C), 156.28, 168.47, 170.39, 171.19, 191.10 (CO).
Isomer II: ^{1}H NMR: δ = 0.86, 0.93 [dd, 6 H, CH_3(Val)], 1.32 [m, 1 H, β-H(Pro)], 1.60 [m, 3 H, β-H(Val), 2 γ-H(Pro)], 2.26 (s, 3 H, CH_3), 2.34 [m, 1H, β-H(Pro)], 3.10 [m, 2 H, δ-H(Pro)], 3.74 (s, 3 H, OCH_3), 4.39, 4.43 [dd, 1 H, α-H(Pro)], 4.66, 4.70 [dd, 1 H, α-H(Val)], 5.52 (s, 1 H, 4-H), 5.43, 5.58 [AB, J = 16.0 Hz, 2 H, CH_2(ester)], 6.22 (d, 1 H, N−H), 6.75–7.93 (m, 14 H, ar H). – ^{13}C NMR: δ = 16.15 (CH_3), 17.84, 19.95 [CH_3(Val)], 24.78 (γ-C(Pro)], 26.94 [β-C(Pro)], 30.62 [β-C(Val)], 47.72 [δ-C(Pro)], 55.42 (C-4), 56.92 [α-C(Val)], 58.26 [α-C(Pro)], 62.77 (OCH_3), 66.57 (CH_2), 114.43, 118.33, 126.43, 127.80, 128.94, 129.38, 130.87, 132.09, 134.14, 160.34 (ar C), 136.08 , 140.56 (C=C), 156.21, 168.11, 168.71, 169.84, 170.85, 171.39, 191.75 (CO).
$C_{37}H_{39}N_3O_7$ (637.7)

3.1.7.4. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valyl-L-isoleucine methyl ester (**7d**)

From 330 mg (1.0 mmol) of **3** and **6e**. Yield: 200 mg (36%). Light yellow solid. M.p. 155–158 °C. $R_f = 0.51$ (EtOAc/cyclohexane 1 : 1). – IR: ν = 3064, 3033 (CH), 1739, 1657 (CO). – ^{1}H NMR: δ = 0.66, 0.92 [m, 12 H, CH_3(Val), CH_3(Ile)], 1.18 [m, 2 H, CH_2(Ile)], 1.85 [m, 2 H, β-H(Val), β-H(Ile)], 2.34, 2.37 (2s, 3 H, CH_3), 3.73 (m, 6 H, 2 OCH_3), 4.11, 4.25 [m, 1 H, α-H(Val)], 4.54 [m, 1 H, α-H(Ile)], 5.69, 5.72 (m, 1 H, 4-H), 6.19, 6.36 (2m, 2 H, 2 N-H), 6.75–7.55 (m, 14 H, ar H). – ^{13}C NMR: δ = 11.59 [CH_3(Ile)], 14.65 (CH_3), 15.46 [CH_3(Ile)], 17.85, 18.64 [CH_3(Val)], 25.20 [CH_2(Ile)], 31.83 [β-C(Val)], 37.61 [β-C(Ile)], 52.15 (OCH_3), 55.36 (C-4), 57.87 [α-C(Ile)], 58.75 [α-C(Val)], 64.03, 65.64 (OCH_3), 114.36, 118.58, 127.85, 128.17, 128.48, 128.88, 129.89, 130.82, 130.94, 160.76, 160.86 (ar C), 136.18, 136.28, 145.10, 146.19 (C=C), 156.30, 164.94, 170.62, 170.79, 172.04, 172.22 (CO). – HPLC: 34.73, 36.13 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 549. – HREIMS: Calcd. 549.28387; found 549.27572.
$C_{31}H_{39}N_3O_6$ (549.7)

3.1.7.5. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valyl-L-phenylalanine ethyl ester (**7e**)

From 330 mg (1.0 mmol) of **3** and **6f**. Yield: 210 mg (35%). Light yellow solid. M.p. 185–188 °C (dec.). $R_f = 0.55$. – IR: ν = 3302, 3062, 3032, 2965, 2932 (CH), 1744, 1658 (CO). – ^{1}H NMR: δ = 0.61, 0.63, 0.68, 0.84 [m, 6 H, CH_3(Val)], 1.24 (m, 3 H, OCH_3), 1.81 [m, 1 H, β-H(Val)], 2.32, 2.36 (2s, 3 H, CH_3), 2.90, 3.04, 4.79 [ABX, J_{AB} = 13.8 Hz, 3 H, 2 β-H(Phe), α-H(Phe)], 3.73 (s, 3 H, OCH_3), 4.11 [m, 3 H, α-H(Val), CH_2], 5.56, 5.87 (2s, 1 H, 4-H), 6.11 [m, 2 H, 2 N-H), 6.76–7.54 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.35 (CH_3), 14.59 (OCH_3), 17.79, 18.18, 18.61, 19.04 [CH_3(Val)], 30.62, 31.45 [β-C(Val)], 37.88, 38.06 [CH_2(Phe)], 53.13 [α-C(Phe)], 55.39 (C-4), 57.93, 58.49 [α-C(Val)], 61.66 (CH_2), 64.25, 64.36 (OCH_3), 114.38, 118.53, 127.13, 127.31, 127.59, 127.84, 128.18, 128.53, 128.68, 128.95, 129.28, 130.78, 130.96, 160.82 (ar C), 135.61, 136.25, 145.34, 146.15 (C=C), 156.35, 164.61, 164.84, 170.07, 170.16, 171.11 (CO). – HPLC: 40.31, 42.85 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 597. – HREIMS: Calcd. 597.28387; found 597.28403.
$C_{35}H_{39}N_3O_6$ (597.7)

3.1.7.6. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valyl-L-proline benzyl ester (**7f**)

From 330 mg (1.0 mmol) of **3** and **6i**. Yield: 200 mg (33%). Light yellow solid. M.p. 150–155 °C. $R_f = 0.41$. – IR: ν = 3440, 3326 (NH), 3031, 2930, 2850 (CH), 1735, 1723 (CO). – ^{1}H NMR: δ = 0.58, 0.63, 0.71, 0.91 [m, 6 H, CH_3(Val)], 1.64 [m, 4 H, β-H(Val), 2 γ-H(Pro), β-H(Pro)], 1.98 [m, 1 H, β-H(Pro)], 2.33, 2.35 (2s, 3 H, CH_3), 3.56 [m, 2 H, δ-H(Pro)], 3.72 (s, 3 H, OCH_3), 4.50 [m, 2 H, α-H(Pro), α-H(Val)], 5.15 [dd, 2 H, CH_2), 5.69, 5.70 (2s, 1 H, 4-H), 6.24, 6.41 (2d, 1 H, N-H), 6.74–7.55 (m, 14 H, ar H). – ^{13}C NMR: δ = 14.53, 14.66 (CH_3), 17.92, 19.09 [CH_3(Val)], 24.99 [γ-C(Pro)], 29.06 [β-C(Pro)], 31.79 [β-C(Val)], 47.26 [δ-C(Pro)], 55.36 (C-4), 55.78 [α-C(Val)], 58.94 [α-C(Pro)], 64.33, 64.41 (OCH_3), 66.96 [CH_2(ester)], 114.38, 118.53, 127.13, 127.31, 127.59, 127.84, 128.18, 128.53, 128.68, 128.95, 129.28, 130.78, 130.96, 160.82 (ar C), 135.61, 136.25, 145.34, 146.15 (C=C), 156.35, 164.61, 164.84, 170.07, 170.16, 171.11 (CO). – HPLC: 48.83, 56.91 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 609. – HREIMS: Calcd. 609.28387; found 609.28411.
$C_{36}H_{39}N_3O_6$ (609.7)

3.1.7.7. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valyl-L-valine methyl ester (**7g**)

From 330 mg (1.0 mmol) of **3** and **6g**. Yield: 200 mg (37%). Light yellow solid. M.p. 160–166 °C. $R_f = 0.34$. – IR: $\nu = 3305$ (NH), 3034, 2962, 2873, 2837 (CH), 1738, 1660 (CO). – ^{1}H NMR ([d_6]DMSO): $\delta = 0.61$, 0.73, 0.83, 0.91 [m, 12 H, CH_3(Val)], 2.00 [m, 2 H, β-H(Val)], 2.19, 2.23 (2s, 3 H, CH_3), 3.59, 3.62 (2s, 3 H, OCH_3), 3.68 (s, 3 H, OCH_3), 4.16 [m, 2 H, α-H(Val)], 5.76, 5.85 (2s, 1 H, 4-H), 6.75–7.45 (m, 9 H, ar H), 7.82, 7.94, 8.16, 8.27 (4d, 2 H, 2 N-H). – ^{13}C NMR: $\delta = 14.65$ (CH_3), 17.84, 18.38, 18.68, 18.93, 19.04 [CH_3(Val)], 31.02, 31.69 [β-C(Val)], 52.23 (OCH_3), 55.37 (C-4), 57.22, 57.96, 58.82 [α-C(Val)], 64.12, 64.45 (OCH_3), 114.37, 118.56, 127.85, 128.17, 128.51, 129.90, 130.84, 130.98, 160.85 (ar C), 136.16, 136.30, 145.29, 146.16 (C=C), 156.36, 164.81, 170.68, 170.80, 172.16 (CO). – HPLC: 21.10, 22.02 min (RP-18, acetonitril/water 1 : 1). – MS (EI, 70 eV): m/z = 535. – HREIMS: Calcd. 535.26825; found 535.26670.
$C_{30}H_{37}N_3O_6$ (535.6)

3.1.7.8. *E*-1-{2-[(*RS*)-1-(4-Methoxyphenyl)-2-oxo-4-phenylazetidin-3-ylidene]propionyl}-L-valyl-L-valine phenacyl ester (**7h**)

From 330 mg (1.0 mmol) of **3** and **6h**. Yield: 180 mg (28%). Light yellow solid. M.p. 168–174 °C. $R_f = 0.37$. – IR: $\nu = 3311$ (NH), 3061, 2959, 2934, 2872 (CH), 1740, 1693, 1660 (CO). – ^{1}H NMR: $\delta = 0.65$, 0.68, 0.75, 0.89, 0.94, 0.96, 1.05 [8d, 12 H, CH_3(Val)], 1.89 [m, 2 H, β-H(Val)], 2.34, 2.37 (2s, 3 H, CH_3), 3.71, 3.73 (2s, 3 H, OCH_3), 4.13, 4.26 [2dd, 1 H, α-H(Val)], 4.66 [m, 1 H, α-H(Val)], 5.39 [AB, J = 16.4 Hz, 2 H, CH_2(ester)], 5.69, 5.71 (2s, 1 H, 4-H), 6.18, 6.33 (2t, 2 H, 2 NH), 6.73–7.91 (m, 14 H, ar H). – ^{13}C NMR: $\delta = 14.56$, 14.64 (CH_3), 17.41, 17.82, 18.48, 18.64, 18.84, 18.99, 19.11 [CH_3(Val)], 30.99, 31.48 [β-CH(Val)], 55.37, 56.10 (C-4), 57.08, 57.84, 58.50 [α-CH(Val)], 64.30 (OCH_3), 66.47 [CH_2(ester)], 114.34, 118.51, 127.70, 128.10, 128.36, 128.50, 128.69, 128.91, 129.73, 130.86, 133.93, 134.05, 160.79 (ar C), 136.19, 145.68 (C=C), 157.93, 163.68, 164.79, 165.40, 170.79, 170.90, 172.72 (CO). – HPLC: 5.34, 5.55 min (RP-18, acetonitril/water 8 : 2). – MS (EI, 70 eV): m/z = 639. – HREIMS: Calcd. 639.29443; found 639.29251.
$C_{37}H_{41}N_3O_7$ (639.7)

3.2. Enzyme test

The assays were performed at PPE concentrations of 7.5 μg/ml and substrate concentrations between 0.18–1.40 mM of Suc-Ala-Ala-Ala-pNA in 0.1 M tris-buffer, pH 8.0. Stock solutions of PPE were prepared by dissolving the enzyme in 1 mM acetic acid. The rate of enzymatic hydrolysis was monitored at 405 nm continuously for 10 min via the release of *p*-nitroaniline at 25 °C at 405 nm ($\varepsilon = 9960\ l\ mol^{-1}\ cm^{-1}$). The K_m-value was calculated to be 1.2 mM. Both substrate and inhibitor were dissolved in DMF, and all assays were performed with the same final concentration of DMF. Enzyme, inhibitor and substrate were mixed and the time-dependent increase in the UV-absorption was monitored over 10 min (final concentrations [I]: **5a**: 0.23 mM; **5b**: 0.23 mM; **5c**: 0.09 mM; **5d**: 0.21 mM; **5e**: 0.25 mM; **7a**: 0.17 mM; **7b**: 0.18 mM; **7c**: 0.16 mM; **7d**: 0.29 M; **7e**: 0.27 mM; **7f**: 0.27 mM; **7g**: 0.30 mM; **7h**: 0.26 mM). Without inhibitor no significant decrease in enzyme activity occurred during the time of the assay (steady-state conditions). For each inhibitor, two independent experiments were performed, each with five different inhibitor concentrations and ten substrate concentrations, respectively. The evaluation of data was done with the program EnKinPlot [13].

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