Institut für Pharmazeutische Chemie[1], Philipps-University Marburg and Hans-Knöll-Institut für Naturstoff-Forschung e. V.[2], Jena, Germany

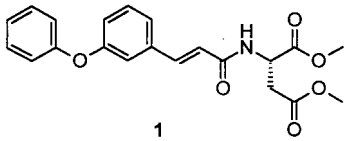
# Structure activity relationship of antiproliferative *N*-acyl-aspartic acid dimethyl ester. 2. Variation of the aspartyl moiety

P. WISSNER[1], H.-M. DAHSE[2] and M. SCHLITZER[1]

Structural variations of the L-aspartic acid substructure of (*S*)-N-3-phenoxycinnamoylaspartic acid dimethyl ester which shows a selective antiproliferative activity against THP-1 tumor cells, demonstrated that the L-aspartic acid moiety is absolutely mandatory for antiproliferative activity as well as for selectivity.

## 1. Introduction

We have recently described some *N*-acyl aspartic acid dimethyl esters which display interesting antiproliferative activity in selectively inhibiting the growth of THP-1 cells [1], a human acute monocytic leukaemic cell line. In this first part of our study we addressed the effect of variations of the *N*-acyl residue on antiproliferative efficiency and selectivity while leaving the aspartic acid dimethyl ester substructure unchanged. The 3-phenoxycinnamoyl derivative **1** turned out to be the most acitive compound of that series. In the second part of our study we used **1** as a lead structure and replaced the aspartic acid ester moiety by several amino acid esters.



**1**

## 2. Investigations, results and discussion

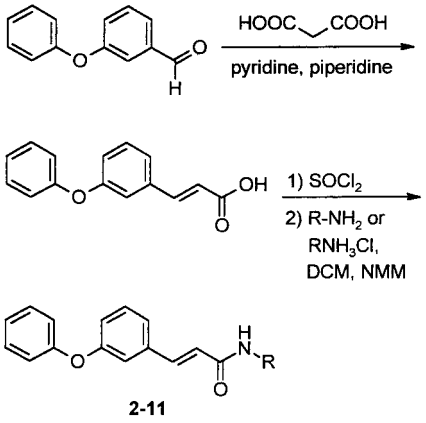
3-Phenoxycinnamic acid was prepared from commercially available 3-phenoxybenzaldehyde and malonic acid in a knoevenagel condensation [2]. After transformation into the corresponding acid chloride, this was reacted with different amino acid esters and amino acid ester hydrochlorides, respectively to yield the target compounds.

The target compounds were assayed against cell lines K-562 (human chronic myeloid leukaemic cell line), THP-1 (human acute monocytic leukaemic cell line), L-929 (mouse fibroblast cell line) and HeLa (human cervix carcinoma cell line) for their antiproliferative effects. The cells were incubated with ten concentrations of the test compounds ranging from 50 μg/ml to 0.1 μg/ml.

The results of the antiproliferation assays of the new cinnamic amides **2**–**11** derived from lead structure **1** are dispayed in the Table. As can clearly be deliniated, antiproliferative efficiency as well as selectivity is absolutely dependent on the presence of the L-aspartic acid moiety. Inversion of the stereochemistry as with the D-aspartic acid ester **2** resulted in a sharp drop in activity and in an almost complete loss of selectivity. Replacement of the L-aspartyl moiety by monobasic amino acid ester (compounds **3**, **4**, **5**, **8**) resulted in inactive (**3**, **8**) or weakly antiproliferative (**4**, **5**) compounds, the latter without any selectivity towards THP-1 cells. Removal of the methylene group from the aspartyl moiety of **1** resulted in the aminomalonic acid derivative **6**, which preserved some of the selectivity of **1** towards THP-1 cells but is more than tenfold less active. In contrast, introduction of an additional methylene group as in the glutamic acid derivative **7** led to a complete loss of selectivity which is combined with an overall weak antiproliferative activity. Some activity and selectivity towards L-929 and K-562 cells, respectively, is seen with the aromatic amino acid derivatives **9** and **10**, a result which may be worth persueing.

In summary, this series of modifications of our lead structure **1** has shown that the L-aspartic acid substructure is absolutely mandatory for the observed selective antiproliferative activity of **1** against THP-1 tumor cells.

**Scheme 1**




**2-11**

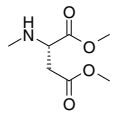
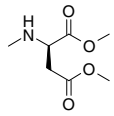
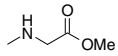
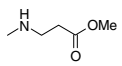
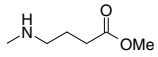
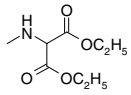
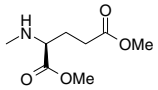
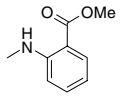
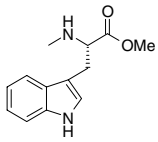
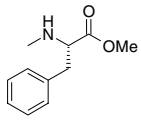
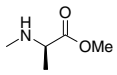
## 3. Experimental

Melting points were determined on an electric variable heater (Mel-Temp II) and are not corrected. $^1$H- and $^{13}$C NMR spectra were recorded on a Jeol JMN-GX-400 and a Jeol JMN-LA-500 spectrometer, using tetramethylsilane as internal standard. MS were obtained with a Vacuum Generators VG 7070 H using a Vector 1 data aquisition system from Teknivent or an AutoSpec mass spectrometer from Micromass. IR spectra were recorded on a Nicolet 510P FT-IR-spectrometer. Microanalyses were obtained from a CH analyzer according to Dr. Salzer from Labormatic and from a Hewlett Packard CHN-analyser type 185. All compounds gave satisfactory elemental analysis (±0.4% of the theoretical values). CC was carried out using silica gel 60 (62–200 μm) from Merck. 3-Phenoxycinnamic acid was prepared as described [2] and transformed into its acid chloride using standard thionyl chloride methodology.

### *3.1. Preparation of the compounds* **2**–**11**

The appropriate amino acid ester hydrochloride was dissolved in a mixture of dry $CH_2Cl_2$ (50–100 ml) and *N*-methylmorpholine (NMM) (0.25 ml per mmol) and cooled to 0 °C. After addition of 1 equivalent of 3-phenoxycinnamic acid chloride, the solution was stirred overnight. The mixture was washed successively with 2N citric acid, sat. $NaHCO_3$ solution and brine and dried over $MgSO_4$. The solvent was removed in vacuo.

**Table: Antiproliferative activity ($GI_{50}$ (μM)) of compounds 1–11**

| Compd. | –NH–R | L-929 | K-562 | HeLa | THP-1 |
|---|---|---|---|---|---|
| **1** |  | 93.9 | 99.6 | 130.4 | 1.3 |
| **2** |  | 76.7 | 101.2 | >130 | 68.8 |
| **3** |  | >160 | >160 | >160 | >160 |
| **4** |  | 57.5 | 45.5 | 52.6 | 50.4 |
| **5** |  | 63.9 | 39.2 | 57.7 | 57.5 |
| **6** |  | 61.9 | 67.7 | >126 | 17.8 |
| **7** |  | 45.5 | 84.5 | 105.4 | 54.1 |
| **8** |  | >134 | >134 | >134 | 99.6 |
| **9** |  | 7.9 | 34.0 | 77.0 | 40.2 |
| **10** |  | 18.7 | 14.9 | 84.7 | 55.0 |
| **11** |  | 29.8 | 15.7 | 26.1 | 17.2 |

Arithmetic means of three measurements. Values are estimated to be reliable within ±1%

*3.1.1. (R)-N-3-Phenoxycinnamoylaspartic acid dimethyl ester* (**2**)

Prepared from (R)-aspartic acid dimethyl ester hydrochloride (593 mg, 3 mmol) and 3-phenoxycinnamic acid chloride (776 mg, 3 mmol). Compound **2** was a beige solid with a m.p. of 60 °C. Yield: 897 mg (78%). IR (KBr): ν = 3320, 3030, 2965, 1765, 1730, 1655, 1620, 1580, 1520, 1490, 1440 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) δ = 2.93 (dd, J = 18, 4 Hz, 1 H, $CH_2$), 3.10 (dd, J = 18, 4 Hz, 1 H, $CH_2$), 3.68 (s, 3 H, $OCH_3$), 3.76 (s, 3 H, $OCH_3$), 4.96–5.00 (m, 1 H, CH), 6.39 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.72 (d, 1 H, J = 8 Hz, NH), 6.98–7.03 (m, 3 H, H aromat.), 7.11–7.15 (m, 2 H, H aromat.), 7.19–7.22 (m, 1 H, H aromat.), 7.28–7.37 (m, 1 H, H aromat.), 7.57 (d, 1 H, J = 15.5 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 36.1 ($CH_2$), 48.6 (CH), 52.0, 52.8 (2 × $OCH_3$), 117.3, 119.1, 120.1, 120.6, 122.9, 123.7, 129.9, 130.1, 136.4, 141.3 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.2 (CONH), 171.1, 171.6 (2 × $\underline{C}OOCH_3$). MS: m/z (%) = 383 (13, $M^+$), 224 (18), 223 (100).
$C_{21}H_{21}NO_6$ (383.4)

*3.1.2. N-3-Phenoxycinnamoylglycine methyl ester* (**3**)

Prepared from glycine methyl ester hydrochloride (377 mg, 3 mmol) and 3-phenoxycinnamic acid chloride (776 mg, 3 mmol). Compound **3** was a white solid with a m.p. of 106 °C. Yield: 810 mg (86%). IR (KBr): ν = 3255, 3075, 2955, 1735, 1675, 1655, 1610, 1575, 1490 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ = 3.77, (s, 3 H, $OCH_3$), 4.17 (d, 1 H, J = 5.4 Hz, $CH_2$), 6.30 (s, br., 1 H, NH), 6.41 (d, 1 H, J = 16 Hz, vinyl-α-H), 6.99–7.03 (m, 3 H, H aromat.), 7.12–7.15 (m, 2 H, H aromat.), 7.22 (m, 1 H, H aromat.), 7.31 (m, 1 H, H aromat.), 7.33–7.38 (m, 2 H, H aromat.), 7.59 (d, 1 H, J = 16 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 41.5 ($CH_2$), 52.5 ($OCH_3$), 117.3, 119.1, 120.1, 120.5, 123.0, 123.7, 129.9, 130.1, 136.4, 141.2 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.7 (CONH), 170.5 ($\underline{C}OOCH_3$). MS: m/z (%) = 311 (72, $M^+$), 312 (15), 310 (20), 224 (21), 223 (100).
$C_{18}H_{17}NO_4$ (311.4)

*3.1.3. N-3-Phenoxycinnamoyl-β-alanine methyl ester* (**4**)

Prepared from β-alanine methyl ester hydrochloride (279 mg, 2 mmol) and 3-phenoxycinnamic acid chloride (517 mg, 2 mmol). Compound **4** was a white solid with a m.p. of 89 °C. Yield: 553 mg (85%). IR (KBr): ν = 3270, 3055, 2950, 1730, 1660, 1620, 1580, 1545, 1485 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ = 2.61, (t, J = 6 Hz, 2 H, $CH_2$), 3.64, (q, J = 6 Hz, 2 H, $CH_2$), 3.71, (s, 3 H, $OCH_3$), 6.26 (s, br., 1 H, NH), 6.31 (d, 1 H, J = 16 Hz, vinyl-α-H), 6.98–7.03 (m, 3 H, H aromat.), 7.12–7.16 (m, 2 H, H aromat.), 7.22 (m, 1 H, H aromat.), 7.12–7.16 (m, 1 H, H aromat.) 7.34–7.38 (m, 2 H, H aromat.), 7.56 (d, 1 H, J = 16 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 33.7 ($CH_2$), 34.9 ($CH_2$), 51.8 ($OCH_3$), 117.2, 119.1, 119.9, 121.2, 122.9, 123.6, 129.8, 130.1, 136.6, 140.5 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.5 (CONH), 173.2 ($\underline{C}OOCH_3$). MS: m/z (%) = 325 (88, $M^+$), 294 (10), 238 (10), 224 (38), 223 (100), 102 (18).
$C_{19}H_{19}NO_4$ (325.4)

*3.1.4. N-3-Phenoxycinnamoyl-γ-aminobutyric acid methyl ester* (**5**)

Prepared from γ-aminobutyric acid methyl ester hydrochloride (614 mg, 4 mmol) and 3-phenoxycinnamic acid chloride (1.035 g, 4 mmol). Compound **5** was a light powder with a m.p. of 70 °C. Yield: 1.12 g (82%). IR (KBr): ν = 3255, 3060, 2950, 2875, 1735, 1655, 1615, 1550, 1490 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ = 1.90 (m, 2 H, $CH_2$), 2.41 (t, J = 7 Hz, 2 H, $CH_2$), 3.42 (m, 2 H, $CH_2$), 3.67 (s, 3 H, $OCH_3$), 6.03 (s,br., 1 H, NH), 6.33 (d, 1 H, J = 16 Hz, vinyl-α-H), 6.96–7.05 (m, 3 H, H aromat.), 7.11–7.17 (m, 2 H, H aromat.), 7.21 (m, 1 H, H aromat.), 7.31 (d, 1 H, J = 8 Hz, H aromat.) 7.33–7.38 (m, 2 H, H aromat.), 7.55 (d, 1 H, J = 16 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 24.6 ($CH_2$), 31.5 ($CH_2$), 39.2 ($CH_2$), 51.8 ($OCH_3$), 117.3, 119.1, 119.9, 121.3, 122.9, 123.6, 129.9, 130.1, 136.6, 140.3 (C aromat.,vinyl.), 156.7, 157.7 (aryl-CO), 165.8 (CONH), 174.0 ($\underline{C}OOCH_3$). MS: m/z (%) = 339 (44, $M^+$), 238 (12), 224 (50), 223 (100), 116 (17).
$C_{20}H_{21}NO_4$ (339.42).

*3.1.5. N-3-Phenoxycinnamoylaminomalonic acid diethyl ester* (**6**)

Prepared from aminomalonic acid diethyl ester hydrochloride (847 mg, 4 mmol) and 3-phenoxycinnamic acid chloride (1.035 g, 4 mmol). Compound **6** was a white powder with a m.p. of 119 °C. Yield: 1.391 g (85%). IR (KBr): ν = 3290, 2990, 1740, 1655, 1615, 1590, 1580, 1530, 1490 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ = 1.30 (m, 6 H, 2 × $CH_3$), 4.30 (m, 4 H, 2 × $CH_2$), 5.28 (d, 1 H, J = 7 Hz, CH), 6.45 (d, 1 H, J = 16 Hz, vinyl-α-H), 6.68 (d, 1 H, J = 7 Hz, NH), 7.00–7.04 (m, 3 H, H aromat.), 7.12–7.17 (m, 2 H, H aromat.), 7.23 (m, 1 H, H aromat.), 7.31–7.39 (m, 3 H, H aromat.), 7.60 (d, 1 H, J = 16 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 14.0 (2 × $CH_3$), 56.6 (CH), 62.7 (2 × $CH_2$), 117.4, 119.1, 119.2, 119.9, 120.2, 123.0, 123.7, 129.9, 130.2, 136.3, 141.9 (C aromat., vinyl.), 156.6, 157.9 (aryl-CO), 165.1 (CONH), 166.3 (2 × $\underline{C}OOC_2H_5$). MS: m/z (%) = 397 (54, $M^+$), 224 (33), 223 (100), 197 (33).
$C_{22}H_{23}NO_6$ (397.5)

*3.1.6. (S)-N-3-Phenoxycinnamoylglutamic acid dimethyl ester* (**7**)

Prepared from glutamic acid dimethyl ester hydrochloride (423 mg, 2 mmol) and 3-phenoxycinnamic acid chloride (517 mg, 2 mmol). Compound **7** was a yellow resin. Yield: 676 mg (85%). IR (film): ν = 3295, 3060, 2955, 1940, 1740, 1630, 1440 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ = 2.01–2.11 (m, 1 H, $CH_2$), 2.23–2.31 (m, 1 H, $CH_2$), 2.36–2.51 (m, 2 H, $CH_2$), 3.67 (s, 3 H, $OCH_3$), 3.78 (s, 3 H, $OCH_3$), 4.74–4.80 (m, 1 H, CH), 6.38 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.48 (d, 1 H, J = 7 Hz, NH), 6.99–7.03 (m, 3 H, H aromat.), 7.11–7.16 (m, 2 H, H aromat.), 7.22 (m, 1 H, H aromat.), 7.31–7.39 (m, 3 H, H aromat.), 7.57 (d, 1 H, J = 15.5 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) δ = 27.4 ($CH_2$), 30.1 ($CH_2$), 51.8, 51.9 (2 × $OCH_3$), 52.6 (CH), 117.4, 119.1, 120.1, 120.6, 123.0, 123.7, 129.9, 130.1, 136.4, 141.2 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.4 (CONH), 172.4, 173.4 (2 × $\underline{C}OOCH_3$). MS: m/z (%) = 397 (17, $M^+$), 224 (18), 223 (100), 174 (34).
$C_{22}H_{23}NO_6$ (397.5).

*3.1.7. (S-(3-Phenoxycinnamoyl)-2-aminobenzoic acid methyl ester* (**8**)

Prepared from anthranilic acid methyl ester (302 mg, 2 mmol) and 3-phenoxycinnamic acid chloride (517 mg, 2 mmol). Compound **8** was a beige solid with a m.p. of 121 °C. Yield: 553 mg (74%). IR (KBr): $\nu = 3265$, 3040, 2955, 1675, 1630, 1605, 1590, 1535, 1490, 1440 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta = 3.94$ (s, 3 H, $OCH_3$), 6.58 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.99–7.04 (m, 3 H, H aromat.), 7.07–7.16 (m, 2 H, H aromat.), 7.22–7.24 (m, 1 H, H aromat.), 7.31–7.38 (m, 4 H, H aromat.), 7.54–7.59 (m, 1 H, H aromat.), 7.70 (d, 1 H, J = 15.5 Hz, vinyl-β-H), 8.04–8.06 (m, 1 H, H aromat.), 8.85 (d, 1 H, J = 7.4 Hz), 11.36 (s br., 1 H, NH). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta = 52.4$ ($OCH_3$), 114.9, 118.1, 118.9, 120.2, 120.6, 122.6, 122.7, 123.0, 123.5, 129.8, 130.1, 130.8, 134.7, 136.5, 141.5, 141.7 (C aromat.,vinyl.), 156.9, 157.7 (aryl-CO), 164.2 (CONH), 168.9 (COOCH$_3$). MS: m/z (%) = 373 (59, $M^+$), 224 (16), 223 (100), 151 (46).
$C_{23}H_{19}NO_4$ (373.4)

*3.1.8. (S)-N-3-Phenoxycinnamoyltryptophane methyl ester* (**9**)

Prepared from tryptophane methyl ester hydrochloride (509 mg, 2 mmol) and 3-phenoxycinnamic acid chloride (517 mg, 2 mmol). Compound **9** was a light solid with a m.p. of 70 °C. Yield: 661 mg (75%). IR (KBr): $\nu = 3275$, 3055, 2950, 1735, 1660, 1620, 1575, 1515, 1490, 1455, 1440. $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta = 3.33–3.43$ (m, 2 H, $CH_2$), 3.70 (s, 3 H, $OCH_3$), 5.05–5.11 (m, 1 H, CH), 6.20 (d, 1 H, J = 7.8 Hz, NH), 6.28 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.96–7.03 (m, 4 H, H aromat.), 7.07–7.19 (m, 5 H, H aromat.), 7.28–7.37 (m, 4 H, H aromat.), 7.51–7.58 (m, 2 H, vinyl-β-H, H aromat.), 8.22 (s br., 1 H, indol-NH). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta = 27.7$ ($CH_2$), 52.4 ($OCH_3$), 53.2 (CH), 110.0, 111.3, 117.4, 118.6, 119.1, 119.7, 120.0, 120.9, 122.3, 122.8, 122.9, 123.6, 127.6, 129.9, 130.1, 136.1, 136.4, 141.0 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.2 (CONH), 172.3 (COOCH$_3$). MS: m/z (%) = 440 (3, $M^+$), 223 (16), 201 (65), 131 (10), 130 (100).
$C_{27}H_{24}N_2O_4$ (440.5)

*3.1.9. (S)-N-3-Phenoxycinnamoylphenylalanine methyl ester* (**10**)

Prepared from phenylalanine methyl ester hydrochloride (431 mg, 2 mmol) and 3-phenoxycinnamic acid chloride (517 mg, 2 mmol). Compound **10** was a yellow resin. Yield: 614 mg (76%). IR (film): $\nu = 3275$, 3065, 3030, 2950, 2855, 1755, 1670, 1570, 1515, 1490, 1440. $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta = 3.14–3.25$ (m, 2 H, $CH_2$), 3.75 (s, 3 H, $OCH_3$), 5.00–5.04 (m, 1 H, CH), 6.06 (d, 1 H, J = 7.3 Hz, NH), 6.33 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.99–7.04 (m, 3 H, H aromat.), 7.09–7.16 (m, 4 H, H aromat.), 7.20–7.38 (m, 7 H, H aromat.), 7.57 (d, 1 H, J = 15.5 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta = 37.9$ ($CH_2$), 52.4 ($OCH_3$), 53.3 (CH), 117.4, 119.1, 120.2, 120.6, 123.0, 123.7, 127.2, 128.6, 129.3, 129.9, 130.1, 135.1, 135.8, 136.4, 141.2 (C aromat., vinyl.), 156.7, 157.8 (aryl-CO), 165.0 (CONH), 172.0 (COOCH$_3$). MS: m/z (%) = 401 (13, $M^+$), 239 (49), 238 (34), 224 (16), 223 (100), 218 (13).
$C_{25}H_{23}NO_4$ (401.5).

*3.1.10. (R)-N-3-Phenoxycinnamoylalanine methyl ester* (**11**)

Prepared from (R)-alanine methyl ester hydrochloride (419 mg, 3 mmol) and 3-phenoxycinnamic acid chloride (776 mg, 3 mmol). Compound **11** was a light-yellow solid. Yield: 871 mg (80%). IR (KBr): $\nu = 3270$, 3065, 2980, 2930, 1730, 1660, 1625, 1580, 1550, 1490, $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta = 1.46$ (d, 3 H, J = 7.1 Hz, $CH_3$), 3.77 (s, 3 H, $OCH_3$), 4.67–4.78 (m, 1 H, CH), 6.30 (d, 1 H, J = 7.3 Hz, NH), 6.38 (d, 1 H, J = 15.5 Hz, vinyl-α-H), 6.98–7.03 (m, 3 H, H aromat.), 7.11–7.16 (m, 2 H, H aromat.), 7.19–7.22 (m, 1 H, H aromat.), 7.29–7.30 (m, 3 H, H aromat.), 7.57 (d, 1 H, J = 15.5 Hz, vinyl-β-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta = 18.6$ ($CH_3$), 48.2 ($OCH_3$), 52.6 (CH), 117.3, 119.2, 120.1, 120.8, 123.0, 123.7, 129.9, 130.1, 136.5, 141.0 (C aromat.,vinyl.), 156.7, 157.8 (aryl-CO), 165.0 (CONH), 173.6 (COOCH$_3$). MS: m/z (%) = 325 (30, $M^+$), 266 (13), 224 (23), 223 (100).
$C_{19}H_{19}NO_4$ (325.4)

### *3.2. Pharmacological screening*

*3.2.1. Cells and culture conditions*

Cells of established suspended cell lines K-562, THP-1, and L-929 were cultured in RPMI 1640 medium (GIBCO BRL 42402–016), supplemented with 100 U/ml penicillin G sodium salt/100 μg/ml streptomycin-sulfate (GIBCO BRL 15140-114) and 10% heat inactivated fetal bovine serum (GIBCO BRL 10500-064), and L-glutamine (GIBCO BRL 25030-024) at 37 °C in high density polyethylene flasks (NUNC 156340). HeLa cells were grown in RPMI 1640 culture medium (GIBCO BRL 21875-034) supplemented with 100 U/ml penicillin G sodium salt/100 μg/ml streptomycin-sulfate (GIBCO BRL 15140-114), 10% heat inactivated fetal bovine serum (GIBCO BRL 10500-064), and 10 ml/l non-essential amino acid (GIBCO BRL 11140-035) at 37 °C in high density polyethylene flasks (NUNC 156340)

*3.2.2. Test conditions*

For each experiment with K-562 and THP-1 cells, approximately 10,000 cells were seeded with 0.1 ml RPMI 1640 culture medium (GIBCO BRL 21875-34) but without HEPES, into 96-well microplates (NUNC 163320). The adherent cells of L-929 and HeLa were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA. L929 and HeLa cells were seeded with approximately 10,000 cells per 0.1 ml RPMI 1640 per well of the 96-well microplates. The plates were previously prepared with ten dilutions of the test compounds in 0.1 ml RPMI 1640. The cells were incubated for 72 h at 37 °C in a humidified atmosphere and 5% $CO_2$.

*3.2.3. Methods of evaluation*

Suspension cultures of K-562 and THP-1 cells in microplates were analysed by an electronic cell analyser system CASY1 (Schärfe, Reutlingen, Germany). The principle of measurement and evaluation of data were described [3]. The 0.2 ml content of each well in the microplate was diluted 1 : 50 with CASYTON (Schärfe, Reutlingen, Germany). Every count/ml was automatically calculated from the arithmetic mean of three successive counts of 0.4 ml each. From the dose response curves the $GI_{50}$ values (concentration which inhibited cell growth by 50%) were calculated with the software for data evaluation CASYSTAT (Schärfe). The monolayer of the adherent cell lines L-929 and HeLa werde fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gentle washing the stain was eluted by 0.2 ml of 0.33 N HCl in the wells. The optical densities were measured at 630 nm in a DYNATECH MR 7000 microplate reader. Comparison of different value were performed with Microsoft EXCEL.

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Priv.-Doz. Dr. M. Schlitzer
Institut für Pharmazeutische Chemie
Marbacher Weg 6
D-35032 Marburg
schlitze@mailer.uni-marburg.de