*Eur J Med Chem* (1996) 31, 669–673



# Synthesis of 1-(4-acyloxybenzoyloxyacetyl)-4-alkylpiperazines and 1-(4-acyloxybenzoyl)-4-alkylpiperazines as inhibitors of chymotrypsin

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(Received 4 September 1995; accepted 5 March 1996)

**Summary** — Sixteen new esters and amides of 4-acyloxybenzoic acids were prepared and screened as chymotrypsin inhibitors. Inhibiting activities on chymotrypsin differed markedly (>100–0.008 μM). Compounds which contained the 4-benzoyloxyacetyl moiety showed considerably higher activity then 4-acyloxybenzamides. Compounds **1c** and **11f** are also weak trypsin inhibitors.

**chymotrypsin / chymotrypsin inhibitor / 4-acyloxybenzoylester / 4-acyloxybenzamide**

## Introduction

4-Acyloxybenzamides and 4-acyloxybenzoyloxyacetamides have been described as chymotrypsin inhibitors and are considered to be potential drugs against pancreatitis [1]. Compounds of similar structure have been described for other indications [2]. There is little information about structure–activity relationships of these compounds, however. We prepared a set of 16 compounds **1a–g** and **2a–i** and measured their inhibiting activities on chymotrypsin and trypsin.

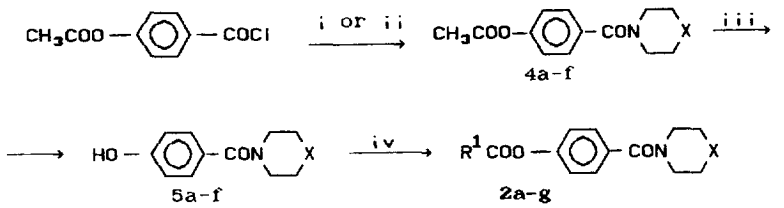
## Chemistry

*N*-Alkylpiperazines **3a–e** were prepared via reaction of piperazine monohydrohalogenide with a halogen derivative [3]. These compounds were reacted with 4-acetoxybenzoyl chloride to give 1-(4-acetoxybenzoyl)-4-alkylpiperazines **4a–e**; preparation of 4-acetoxybenzoyl-4-phenylpiperidine **4f** was carried out in the presence of triethylamine. After the acetoxy group was removed by potassium carbonate solution [4], the 1-(4-hydroxybenzoyl)-4-piperazines **5a–e** and 1-(4-hydroxybenzoyl)-4-phenylpyridine were obtained. These compounds were esterified with the corresponding acids via dicyclohexylcarbodiimide (DCC) coupling [5]. These oily substances were crystallized as fumarates to give final compounds **1a–f** (except **1g**) (scheme 1).

Chloroacetamides **6a,b** were prepared respectively from *N*-benzylpiperazine **3d** or *N*-isopropylpiperazine **3e** and chloroacetylchloride. 4-Hydroxybenzoic and 4-hydroxyphenyl acetic acid were selectively alkylated on the carboxyl with the corresponding halide derivatives in the presence of triethylamine to yield the esters **7a,c** respectively. These compounds were esterified by the corresponding acid via DCC coupling to give the final compounds **2a–h** which were recrystallized as fumarates. Compound **2i** was prepared from the potassium salt of **7a** and chloromethyl naphthalene (scheme 2). Physical data for all the compounds synthesized are presented in table IV, and the $^1$H NMR spectra of all the screened compounds are described in table V [6].

## Results and discussion

Inhibiting activities of compounds **1a–g** and **2a–i** are presented in table I. We first investigated the effect of the substituent on position 4 of the piperazine ring. No remarkable effect of this substitution was discovered; inhibiting activities of **1a–d** and **1f** vary from 43.5 to 12.22 μM. Substitution of the hydrogen of the indole ring in position 5 by a methoxy group increases the activity ca 25-fold (compare **1a** and **1e**). Substitution of basic nitrogen to a nonbasic moiety with similar shape leads to the inactive compound **1g**. We investigated the influence of an acyl group on the activity of **2a–i**. The most active inhibitor is **2a**, with a naphthylacetyl moiety. Compounds **2b,c,e** and **2h** do not show any remarkable differences in activity, but the substitution of an ester function with an ether linkage leads to an inactive compound (compare **2a** and **2i**). A

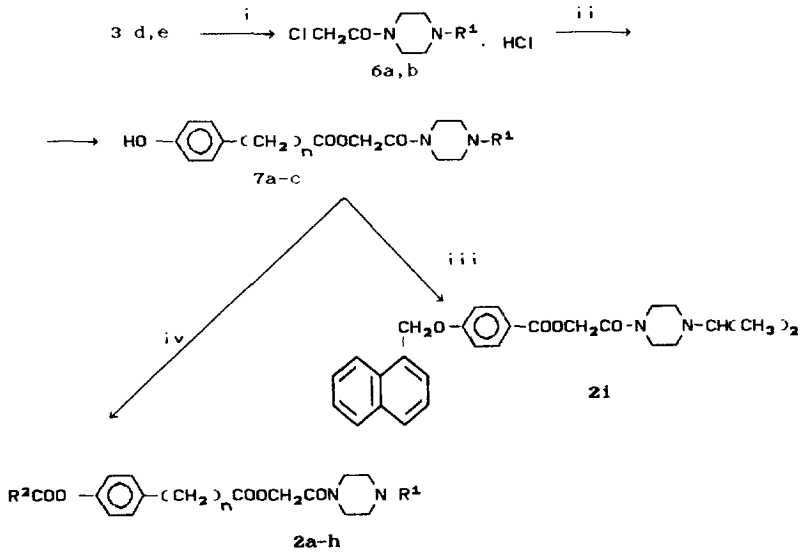


| *1* | *R* | *X* |
|---|---|---|
| **a** | 3-Indolylacetyl- | $NCH_2CH_2CH_3$ |
| **b** | 3-Indolylacetyl- | $NCH_2CH_2NEt_2$ |
| **c** | 3-Indolylacetyl- | $NCH_2CONEt_2$ |
| **d** | 3-Indolylacetyl- | $NCH_2C_6H_5$ |
| **e** | 5-Methoxy-3-indolylacetyl- | $NCH_2C_6H_5$ |
| **f** | 3-Indolylacetyl- | $NCH(CH_3)_2$ |
| **g** | 3-Indolylacetyl- | $CHC_6H_5$ |

| *3* | *X* |
|---|---|
| **a** | $NCH_2CH_2CH_3$ |
| **b** | $NCH_2CH_2NEt_2$ |
| **c** | $NCH_2CONEt_2$ |
| **d** | $NCH_2C_6H_5$ |
| **e** | $NCH(CH_3)_2$ |

| *4,5* | *X* |
|---|---|
| **a** | $NCH_2CH_2CH_3$ |
| **b** | $NCH_2CH_2NEt_2$ |
| **c** | $NCH_2CONEt_2$ |
| **d** | $NCH_2C_6H_5$ |
| **e** | $NCH(CH_3)_2$ |
| **f** | $CHC_6H_5$ |

**Scheme 1.** i: **3a–e**; ii: 4-phenylpiperidine/$Et_3N$; iii: $K_2CO_3$; iv: $R^1COOH$/DCC.



| *2* | *R¹* | *R²* | *n* |
|---|---|---|---|
| **a** | $CH(CH_3)_2$ | 1-Naphthylacetyl | 0 |
| **b** | $CH(CH_3)_2$ | 3-Indolylacetyl | 0 |
| **c** | $CH(CH_3)_2$ | 3-Indolylacetyl | 1 |
| **d** | $CH_2C_6H_5$ | 3-Indolylacetyl | 0 |
| **e** | $CH(CH_3)_2$ | 5-Methoxy-3-indolylacetyl | 0 |
| **f** | $CH(CH_3)_2$ | 3,4,5-Trimethoxycinnamoyl | 0 |
| **g** | $CH(CH_3)_2$ | Cinnamoyl | 0 |
| **h** | $CH(CH_3)_2$ | 5-Methoxy-2-methyl-3-indolylacetyl | 0 |

| *6* | *R¹* |
|---|---|
| **a** | $CH(CH_3)_2$ |
| **b** | $CH_2C_6H_5$ |

| *7* | *R¹* | *n* |
|---|---|---|
| **a** | $CH(CH_3)_2$ | 0 |
| **b** | $CH(CH_3)_2$ | 1 |
| **c** | $CH_2C_6H_5$ | 0 |

**Scheme 2.** i: $ClCH_2COCl$; ii: 4-hydroxybenzoic or 4-hydroxyphenylacetic acid/$Et_3N$; iii: 1-chlormethylnaphthalene/KO-*t*-Bu; iv: $R^2COOH$/DCC.

**Table I.** Inhibiting activities of compounds **1a–g** and **2a–i** on chymotrypsin.

| *Compound* | *$IC_{50}$ (μM)* |
|---|---|
| **1a** | 20.210 |
| **1b** | 21.700 |
| **1c**[a] | 12.500 |
| **1d** | 43.500 |
| **1e** | 1.710 |
| **1f** | 12.220 |
| **1g** | >100 |
| **2a** | 0.008 |
| **2b** | 0.240 |
| **2c** | 1.800 |
| **2d** | 10.400 |
| **2e** | 0.610 |
| **2f**[a] | >100 |
| **2g** | >100 |
| **2h** | 26.173 |
| **2i** | >100 |

[a]Inhibiting activity on trypsin: **1c**: 10.46 μM; **2f**: 74.120 μM; inhibiting activities of all the other compounds on trypsin are over 100 μM.

**Table II.** Characteristic data for compounds **3a–e**.

| *Compound* | *Formula molecular weight* | *Yield (%)* | *Bp (°C) Pa* |
|---|---|---|---|
| **3a** | $C_7H_{16}N_2$<br>128.1 | 53 | 179–181<br>101 000 |
| **3b** | $C_{10}H_{23}N_3$<br>184.9 | 73 | 87–88<br>14 |
| **3c** | $C_{10}H_{21}N_3O$<br>199.1 | 64 | 103–104<br>14 |
| **3d** | $C_{11}H_{16}N_2$<br>175.4 | 78 | 89–90<br>16 |
| **3e** | $C_7H_{16}N_2$<br>128.1 | 57 | 177–178<br>101 000 |

**Table III.** Characteristic data for compounds **4a–e**, **5a–e**, **6a**, **6b** and **7a–c**.

| *Compound* *v* | *Formula molecular weight* | *Mp (°C) Recrystallization solvent* | *Yield (%)* |
|---|---|---|---|
| **4a** | $C_{16}H_{23}N_2ClO_3$<br>325.6 | 230–232<br>EtOH/EtOAc | 97 |
| **4b** | $C_{19}H_{30}N_3ClO_3$<br>382.5 | 151–153<br>EtOH/EtOAc | 97 |
| **4c** | $C_{19}H_{28}N_3OClO_4$<br>396.6 | 194–195<br>EtOH/EtOAc | 98 |
| **4d** | $C_{20}H_{23}N_2ClO_3$<br>373.5 | 291–221<br>EtOH/EtOAc | 96 |
| **4e** | $C_{16}H_{23}N_2ClO_3$<br>325.6 | 222–223<br>EtOH/EtOAc | 97 |
| **4f** | $C_{20}H_{21}NO_3$<br>323.1 | 147–148<br>EtOH/hexane | 81 |
| **5a** | $C_{14}H_{20}N_2O_2$<br>248.0 | 131–132<br>EtOH/hexane | 56 |
| **5b** | $C_{17}H_{27}N_3O_2$<br>305.0 | 86–88<br>TBME/$Me_2CO$ | 58 |
| **5c** | $C_{17}H_{25}N_3O_3$<br>319.1 | 186–187<br>$Me_2CO$/*i*PrOH | 66 |
| **5d** | $C_{18}H_{20}N_2O_2$<br>296.0 | 156–157<br>*i*PrOH/$H_2O$ | 67 |
| **5e** | $C_{14}H_{20}N_2O_2$<br>248.0 | 144–144.5<br>EtOH/hexane | 66 |
| **5f** | $C_{18}H_{19}NO_2$<br>281.0 | 167–168<br>*n*BuOAc/Hexane | 67 |
| **6a** | $C_9H_{18}N_2Cl_2O$<br>239.0 | 156–157<br>EtOAc/EtOH | 94 |
| **6b** | $C_{13}H_{18}N_2Cl_2O$<br>287.2 | 166–167<br>EtOAc/EtOH | 96 |
| **7a** | $C_{16}H_{22}N_2O_4$<br>306.1 | 166–168<br>*i*PrOH/$iPr_2O$ | 47 |
| **7b** | $C_{17}H_{24}N_2O_4$<br>320.0 | 93–94<br>TBME/$Me_2CO$ | 45 |
| **7c** | $C_{20}H_{22}N_2O_4$<br>354.1 | 205–206<br>*i*PrOH/$iPr_2O$ | 48 |

**Table IV.** Characteristic data for compounds **1a–g** and **2a–h**.

| *Compound* | *Formula molecular weight* | *Mp (°C) Recrystallization solvent* | *Yield (%)* |
|---|---|---|---|
| **1a** | $C_{28}H_{31}N_3O_7$ 522.1 | 172–173 MeOH/$Et_2O$ | 57 |
| **1b** | $C_{31}H_{38}N_4O_7$ 578.0 | 155–156 MeOH/THF | 61 |
| **1c** | $C_{31}H_{36}N_3O_8$ 592.1 | 87–88 THF | 56 |
| **1d** | $C_{32}H_{31}N_3O_7$ 569.01 | 179–180 THF/MeOH | 59 |
| **1e** | $C_{33}H_{33}N_3O_8$ 599.0 | 175–176 $Et_2O$/MeOH | 61 |
| **1f** | $C_{28}H_{31}N_3O_7$ 522.1 | 194–195 THF/MeOH | 57 |
| **1g**[a] | $C_{28}H_{26}N_2O_3$ 437.9 | 230–234 THF/MeOH | 56 |
| **2a** | $C_{32}H_{34}N_2O_9$ 590.1 | 230–231 THF/MeOH | 39 |
| **2b** | $C_{30}H_{33}N_3O_9$ 579.1 | 211–212 THF/MeOH | 46 |
| **2c** | $C_{31}H_{35}N_3O_9$ 593.07 | 192–194 THF/MeOH | 46 |
| **2d** | $C_{34}H_{39}N_3O_9$ 627.01 | 205–208 EtOH/MeCN | 40 |
| **2e** | $C_{31}H_{35}N_3O_{10}$ 609.14 | 111–112 THF/MeOH | 50 |
| **2f** | $C_{32}H_{38}N_2O_{12}$ 642.22 | 155–157 THF | 51 |
| **2g** | $C_{29}H_{32}N_2O_9$ 552.06 | 230 EtOAc/EtOH | 55 |
| **2h** | $C_{32}H_{37}N_3O_{10}$ | 140–142 THF/MeOH | |

[a]Any fumarate.

remarkable difference is observed between the cinnamoylderivative **2g** ($IC_{50}$ on chymotrypsin is 27 μM) and the 3,4,5-trimethoxy cinnamoyl derivative **2f**, whose inhibiting activity on chymotrypsin is negligible; however, **2f** exhibits a weak inhibiting activity on trypsin. Compounds of group **2** containing the oxyacetyl moiety are better inhibitors of chymotrypsin than compounds of group **1**. Weak antitrypsin activities were observed only for **1c** and **2f**. Trypsin-inhibiting activities of all other compounds were less than 100 μM (tables I–IV).

## Experimental protocols

*Chemistry*

Melting points were measured on a Boetius apparatus and are uncorrected. Elemental analyses were carried out on a Carlo Erba 1106, a Specord UV-vis was used for measuring absorbance changes, and NMR spectra were recorded on a Varian 200.

All chemicals were supplied from Merck or Lachema, and enzymes and their substrates from Sigma. Solvents were dried by standard methods. The structure of all compounds was confirmed also by $^1H$ and $^{13}C$ NMR spectra [6] (table V).

*Preparation of N-alkylpiperazines **3a–e** [4]*

To a solution of dry piperazine (29.2 g, 0.2 M) in 96% ethanol (70 mL) was added conc HCl or HBr (0.1 mol) according to the halide required. The mixture was stirred under reflux and the halogen derivative added slowly (over ca 20 min). The bisdihydrohalogenated piperazine precipitated during reflux (1–5 h). The reaction mixture was left standing overnight at room temperature, and was then filtered and washed with ethanol (20 mL). The filtrate was evaporated in vacuo.The residue was dissolved in a saturated water solution of $K_2CO_3$ (50 mL) and extracted several (five to ten) times with dichloromethane. Compounds **3b** and **3e** were extracted continuously for 24 h. The dichloromethane solution was dried ($Na_2SO_4$) and evaporated in vacuo (table II). The samples for elemental analysis (ca 5 mL) were redistilled through a Vigreux column (0.3 × 5 cm) until analysis produced satisfactory results.

*General procedure for **4a–e***

A stirred solution of 4-acetoxybenzoyl chloride (3.95 g, 0.02 mol) in dry ether (50 mL) was cooled under a chlorcalcium tube to −15 °C. A solution of alkylpiperazine **3a–e** (0.02 mol) in dry ether (50 mL) was then added dropwise and the temperature maintained between −10 and −15 °C. The mixture was stirred for 10 min, then the precipitate was filtered, washed with ether and dried in vacuo. The analytical sample was recrystallized from ethanol/ethyl acetate, and the crude product was used in the next reaction step (see table III).

*Preparation of **4f***

4-Acetoxybenzoyl chloride (3.95 g, 0.02 mol) was dissolved in dry ether (50 mL) and treated as above. A solution of phenylpiperazine (3.1 g, 0.02 mol) and triethylamine (2.7 mL, 0.02 mol) in dry ether (50 mL) was added dropwise. Triethylammonium chloride was filtered, washed with ether and the filtrate evaporated in vacuo. The crude product was recrystallized (see table III).

**Table V.** $^1$H NMR spectra for compounds **1a–e** and **2a–i** (in methanol),

| *Compound* | *δ (ppm)* |
|---|---|
| **1a** | 1.71t (*J* = 5.72 Hz, 3H), 2.55m (2H), 3.55bs (4H), 4.12q (*J* = 4.36 Hz, 2H), 4.22bs (4H), 4.72s (2H), 7.6s (1H), 7.91d (*J* = 7.72 Hz, 2H), 8.00–8.43m (9H), 8.55d (*J* = 7.73 Hz, 2H), 10.59bs (1H) |
| **1b** | 1.1t (*J* = 5.72 Hz, 6H), 2.41–2.65m (8H), 2.9d (*J* = 6.42 Hz, 2H), 3.1bs (4H), 3.22q (*J* = 5.73 Hz, 4H), 4.05s (2H), 6.78d (*J* = 7.65 Hz, 2H), 6.95–7.45m (9H), 7.61d (*J* = 7.65 Hz, 2H), 8.00bs (1H), 11.00bs (1H) |
| **1c** | 1.00t (*J* = 4.59 Hz, 3H), 1.1t (*J* = 4.58 Hz, 3H), 3.05–3.55m (10H), 4.00s (2H), 7.00d (*J* = 7.48 Hz, 2H), 7.11–7.45m (9H), 7.61d (*J* = 7.47 Hz, 2H), 11.00bs (1H) |
| **1d** | 3.01bs (4H), 3.55s (2H), 3.65bs (4H), 4.04s (2H), 6.95d (*J* = 7.36 Hz, 2H), 7.05–7.45m (1H), 7.60d (*J* = 7.37 Hz, 2H), 11.05bs (1H) |
| **1e** | 3.11bs (4H), 3.72s (2H), 3.81bs (4H), 3.93s (3H), 4.05s (2H), 7.01d (*J* = 7.22 Hz, 2H), 7.1–7.45m (13H), 7.70d (*J* = 7.23 Hz, 2H), 11.00bs (1H) |
| **1f** | 1.11d (*J* = 8.72 Hz, 6H), 3.36bs (4H), 3.78bs (4H), 3.92m (1H), 4.00s (2H), 7.00d (*J* = 7.48 Hz, 2H), 7.01–7.44m (9H), 7.55d (*J* = 7.49 Hz, 2H), 11.05bs (1H) |
| **1g** | 1.5–2.0m (5H), 2.79–2.85m (4H), 4.05s (2H), 6.40s (2H), 6.85d (*J* = 5.85 Hz, 2H), 7.10–7.44m (12H), 11.01bs (1H) |
| **2a** | 1.00d (*J* = 4.72 Hz, 6H), 2.55bs (4H), 2.77m (1H), 3.33bs (4H), 4.5s (2H), 5.04s (2H), 6.61s (2H), 7.30d (*J* = 7.89 Hz, 2H), 7.50–7.81m (4H), 7.92–8.22m (5H) |
| **2b** | 1.00d (*J* = 4.70 Hz, 6H), 2.55bs (4H), 2.77m (1H), 3.33bs (4H), 4.01s (2H), 5.00s (2H), 6.60s (2H), 7.01–7.45m (5H), 7.55d (2H), 8.01d (2H) |
| **2c** | 0.98d (*J* = 4.46 Hz, 6H), 2.5bs (4H), 2.78m (1H), 3.32s (4H), 3.42bs (4H), 3.72s (2H), 5.06s (2H), 7.01–7.45m (5H), 7.65–7.81m (2H), 8.01d (*J* = 7.28 Hz, 2H), 11.05s (1H) |
| **2d** | 2.60bs (4H), 3.11s (2H), 3.38bs (4H, 4.5s (2H), 5.04s (2H), 6.66s (2H), 7.00–7.38m (10H), 7.45d (*J* = 7.65 Hz, 2H), 8.05d (*J* = 7.68 Hz, 2H), 11.00bs (1H), |
| **2e** | 1.00d (*J* = 4.65 Hz, 6H), 2.55bs (4H), 2.89m (1H), 3.05s (13H), 3.38bs (4H), 3.89s (2H), 5.01bs (2H), 6.59s (2H), 6.82–7.11m (6H), 8.00d (*J* = 7.56 Hz, 2H), 10.30bs (1H), |
| **2f** | 1.00d (*J* = 4.68 Hz, 6H), 2.68bs (4H), 2.85m (1H), 3.05s (2H), 3.55bs (4H), 3.76s (3H), 4.03s (6H), 4.80s (2H), 6.58s (2H), 7.10d (*J* = 8.34 Hz, 2H), 7.15s (2H), 7.35m, 7.58d (*J* = 7.36 Hz, 2H), 11.00bs (1H) |
| **2g** | 0.78d (*J* = 4.72 Hz, 6H), 2.36bs (4H), 2.50m (1H), 3.32bs (4H), 4.45s (2H), 6.31s (2H), 6.60d (*J* = 9.78 Hz, 2H), 7.05–7.20m (5H), 7.42–7.65m (4H), 7.86d (*J* = 7.25 Hz, 2H), 11.01bs (1H) |
| **2h** | 1.05d (*J* = 4.78 Hz, 6H), 1.92s (3H), 2.55bs (4H), 2.78m (1H), 3.05s (3H), 3.45bs (4H), 3.78s (2H), 5.03bs (2H), 6.61s (2H), 6.70dd (1H), 6.85d (*J* = 6.56 Hz, 1H), 7.01d (*J* = 2.56 Hz, 1H), 7.30d (*J* = 7.87 Hz, 2H), 8.00d (*J* = 7.86 Hz, 2H), 10.55bs (1H) |
| **2i** | 1.01d (*J* = 4.78 Hz, 6H), 2.87bs (4H), 2.5m (1H), 3.38bs (4H), 4.05s (2H), 5.06s (2H), 6.71s (2H), 7.25d (*J* = 7.87 Hz, 2H), 7.50–7.85m (7H), 8.05d (*J* = 7.88 Hz, 2H), 11.00bs (1H) |

*General procedure for preparation of **5a–f***

A solution of one of the compounds **4a–f** (0.01 mol) in methanol (30 mL) was added to a saturated solution of potassium carbonate (30 mL). The mixture was stirred for 1 h, then partially evaporated in vacuo and water added (100 mL). The mixture was extracted with ethyl acetate (five times). The extract was dried over sodium sulfate and evaporated in vacuo. The corresponding product was recrystallized (table III).

*General procedure for **1a–g** and **2a–h***

To a stirred solution of the corresponding acid (0.01 mol) and the hydroxy derivative **5a–f** or **7a–c** in a mixture of dry acetonitrile (20 mL) and dry dichloromethane (20 mL) at 0 °C, protected by a calcium chloride tube, was added DCC (2.06 g, 0.01 mol) and 4-dimethylaminopyridine (50 mg). The mixture was stirred overnight at room temperature. The precipitated dicyclohexylurea was filtered and washed with dichlorome-

thane. The filtrate was evaporated in vacuo. The residual oil was dissolved in tetrahydrofuran (30 mL) and fumaric acid was added (1.16 g, 0.01 mol). The miture was refluxed for 5 min, and allowed to stand in the refrigerator overnight (–20 °C). The precipitated product was filtered, washed with tetrahydrofuran and recrystallized (see table IV).

*Preparation of chloracetamides **6a,b***
Compounds **6a,b** were prepared in the same manner as described for **4a–e**, using 0.05 mol of chloracetyl chloride and 0.05 mol of *N*-alkylpiperazine. An analytical sample was obtained upon recrystallization from ethyl acetate/ethanol (table III).

*Preparation of esters **7a–c***
4-Hydroxybenzoic or 4-hydroxyphenyl acetic acid (0.1 mol) was suspended in dry acetonitrile (150 mL), and triethylamine (16.7 mL, 0.15 mol) and respectively **6a** or **6b** (0.05 mol) were added. The mixture was refluxed for 5 h, evaporated to half of its original volume, poured into 15% aqueous $Na_2CO_3$ (150 mL) and extracted with ethylacetate (four times).The extract was dried ($Na_2SO_4$) and evaporated in vacuo. The crude product was recrystallized (see table III).

*Preparation of **2i***
To a solution of potassium *tert*-butoxide (1.29 g, 0.01 mol) in *tert*-butanol (50 mL) was added **7a** (0.01 mol) and 1-chloromethyl naphthalene (2 g, 0.11 mol). The mixture was stirred under reflux for 6 h, then left to stand overnight at room temperature. The solid was filtered, and the filtrate evaporated in vacuo. The oil was subjected to column chromatography of Kieselgel 40–60 μm (50 × 5 cms) and eluted with 300 mL ethyl acetate/ethanol, 10:1 v/v, then 450 mL ethyl acetate/ethanol/triethylamine, 10:1:1 v/v/v. The fraction containing the product was evaporated and the product was recrystallized as a fumarate (see the procedure for **1a–g** and **2a–h**). Mp 83–84 °C (MeOH/THF).

Element anal ($C_{31}H_{34}N_2O_8$) $M_r$ = 528.35: %C calc 66.19, found 66.12; %H calc 6.04, found 5.98; %N calc 4.98, found 4.95. $^1$H NMR (MeOH, δ [ppm]): 0.95d ($J$ = 5.78 Hz, 6H), 2.55 bs (4H), 3.51 bs (4H), 5.05 s (2H), 5.72 s (2H), 6.58 s (2H), 7.36 d ($J$ = 6.71 Hz, 2H), 7.51–7.73 m (5H), 7.91–8.11 m (4H).

*Inhibiting activities*

Inhibiting activities were screened according to [7]. Benzoyl tyrosin ethyl ester hydrochloride (BTEE) was used as substrate for chymotrypsin, and tosyl arginine methyl ester was used for trypsin. The reaction mixture consisted of 1.5 mL buffer (0.1 M Tris-HCl pH = 7.8 in 50% MeOH (v/v) without $Ca^{2+}$ or another activator), and 1.4 mL substrate solution 0.01 M BTEE in 50% MeOH (v/v). The reaction was started by addition of enzyme solution (1 mg of chymotrypsin/mL 0.001 N HCl). The increase in the absorbance at 256 nm was measured over 4 min. The $IC_{50}$ value was obtained by least-squares analysis.

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