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Original article

Synthesis and structure-activity relationship study of the new set of trypsin-like proteinase inhibitors

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Abstract – A new set of 25 trypsin-like proteinase inhibitors was prepared and the inhibiting activity on trypsin, thrombin, plasmin and urokinase was measured. The structure-activity relationship is discussed. High inhibiting activities were observed in 4-guanidinobenzoic acid esters only. The replacement of this moiety for N-formamidinyl-isonipecotic acid or an arginine moiety caused almost total loss of the activity. In the series of 4-guanidinobenzoic acid esters, any important influence of the ester-groups reactivity was observed. The trypsin-thrombin selectivity in the compounds with the guanidine-remote carboxylic function was also observed. © 1999 Éditions scientifiques et médicales Elsevier SAS

trypsine-like proteinases / inhibitors of proteinases / guanidine derivatives

1. Introduction

Trypsin-like proteinases are serine proteinases for which hyperactivity can cause a variety of damage to health [1]. Recently, the activity of the urokinase type plasminogen activator (uPA) was discovered as an important starting proteinase in the proteolytic cascade of tumour invasion and metastases [2]. Inhibitors of these enzymes are of interest as potential therapeutics in various diseases.

Recently, we have reported a set of 4-guanidinobenzoic acid esters with considerable inhibiting activity on trypsin [3]. Continuing in this research we have synthesised a further set of compounds to demonstrate the influence of either ester group reactivity or guanidine-bearing function moiety.

2. Chemistry

Synthesis of the compounds **2a–h** was performed via selective esterification of the hydroxyaromatic acid with the corresponding halogen derivative, and followed by esterification of the produced compounds **1a–h** with 4-guanidinobenzoic acid mesylate via the dicyclohexyl carbodiimide (DCC) method as described [3].

Reaction of the 4-aminobenzoic acid with chloroacetyl morpholine in the presence of triethylamine in acetonitrile gave a moderate yield of the corresponding ester **1l**. After diazotation and nucleophile substitution with potassium ethyl dithiocarbonate and amonolysis of the intermediate, the thiole **1m** was produced. This was esterified with 4-GBA/DCC to get **2l**. Compound **2i** was prepared by hydrogenolysis of benzylester **2c**, compounds **2j** and **2k** were prepared by acidic hydrolysis of the tert. butyl esters **2g** and **2h** (*figure 1*).

Compounds **4a–e** were prepared via activation of the free hydroxyaromatic acid with N-ethyl-N´-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDI) followed by aminolysis of the activated complex with the corresponding amine. The hydroxyaromatic amides **3a–e** were esterified by 4-guanidinobenzoic acid mesylate and DCC (*figure 2*).

Synthesis of compounds **7a** and **b** is mentioned in *figure 3*. N-chloroacetyl morpholine was reacted with potassium ethyl dithiocarbonate in ethanol. Following amonolysis with ammonia yielded N-mercaptoacetyl morpholine **5a**. BOC-glycine was activated with ethyl chloroformate and reacted with morpholine. After acidic

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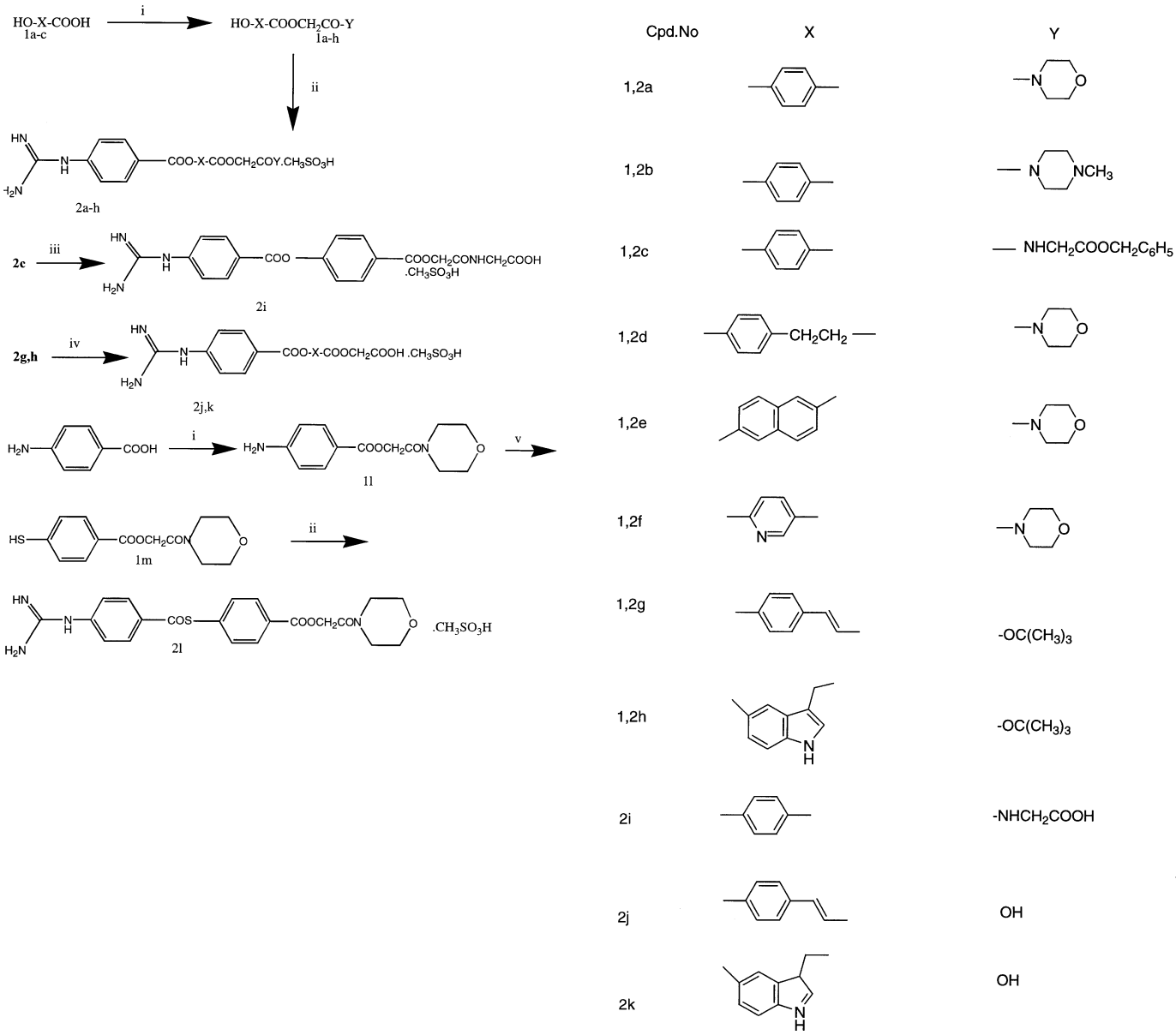
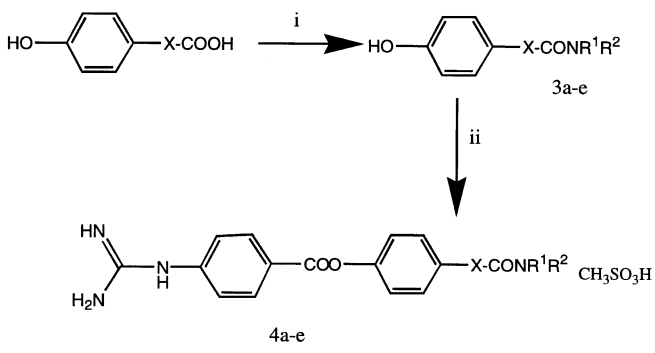


Figure 1. i: Halogen-CH_2COY/MeCN, TEA. ii: 4-Guanidinobenzoic acid mesylate/DCC/pyridide/RT. iii: H_2/Pd-C. iv: CF_3COOH/DCM/anisole. v: $NaNO_2$, HCl, 2.KSCSOEt, 3.NH_3.

hydrolysis of the BOC group, the free N-aminoacetyl morpholine was reacted with 4-acetoxybenzoyl chloride. Then, one-pot cleavage of the protective acetyl group via aqueous methanolic sodium carbonate gave **6a**, as well as **6b** which was prepared via the same reactions. Esterification by 4-guanidinobenzoic acid mesylate/DCC gave **7a** and **b** (*figure 3*).

The sodium salt of 4-mercaptoanisole was the starting material for synthesis of compounds **10** and **11**. It reacted with ethyl chloroacetate to give the corresponding ester which was deprotected with hydrobromic acid, both on phenolic and carboxylic hydroxyl, to give the acid **8**. The acid **8** was esterified via the usual method with chloroacetyl morpholine to give compound **9**. This was esteri-



Cpd.No.	R^1	R^2	X
3,4a		$-CH_2CH_2OCH_2CH_2-$	$-CH_2CH_2-$
3,4b		$-CH_2CH_2N(CH_3)CH_2CH_2-$	$-CH_2CH_2-$
3,4c	$-CH_2CON(CH_2CH_2)_2O$	-H	$-CH_2CH_2-$
3,4d		$-CH_2CH_2OCH_2CH_2-$	(E)-CH=CH-
3,4e	$-CH_2C_6H_5$	-H	(E)-CH=CH-

Figure 2. i: EDI/amine/TEA/MeCN. ii: 4-Guanidinobenzoic acid mesylate/DCC/pyridine.

fied to get compound **10**. After oxidation of 10 with hydrogen peroxide-acetic acid, compound **11** was prepared (*figure 4*).

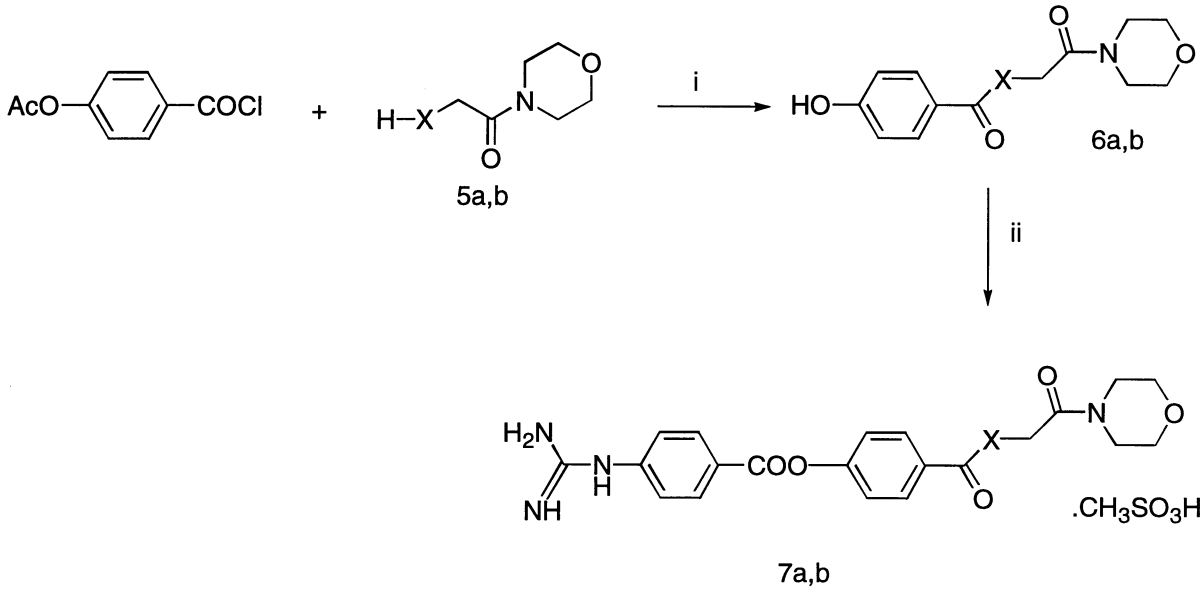
N^1-BOC-$N^{6,8}$ di-Z-Arginine was coupled with compounds **1a** and **1d** to get esters **12a** and **b**. After hydrogenolysis of both Z-protective groups, compounds **13a** and **b** were obtained (*figure 5*).

Isonipecitic acid was protected by the BOC group to get **14** and coupled with compounds **1a** and **1b** via the DCC method to give esters **15a** and **b**. After acidic cleavage of the BOC protective group, the free amines were reacted with cyanamide to give compounds **16a** and **b** (*figure 6*).

3. Results and discussion

All inhibiting activities are presented in *table I*.

There are no remarkable differences in the inhibiting activity on trypsin, thrombin or urokinase in the first group of compounds **2a–h**. After the benzene ring was replaced by the naphthalene ring, the increase of the selectivity for trypsin/thrombin is observable (compare



Cpd.No.	X
5,6,7, a	NH
5,6,7, b	S

Figure 3. i: 1. TEA, DCM, 2. Na_2CO_3, MeOH, H_2O. ii: 4-Guanidinobenzoic acid mesylate/DCC/pyridine.

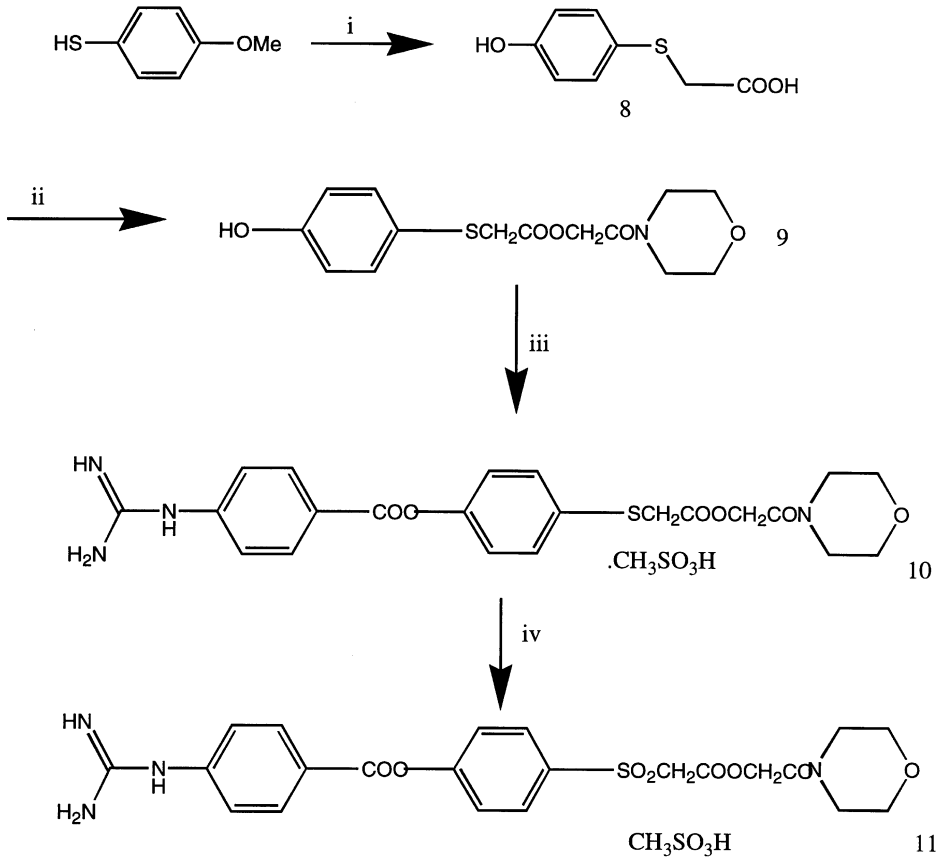


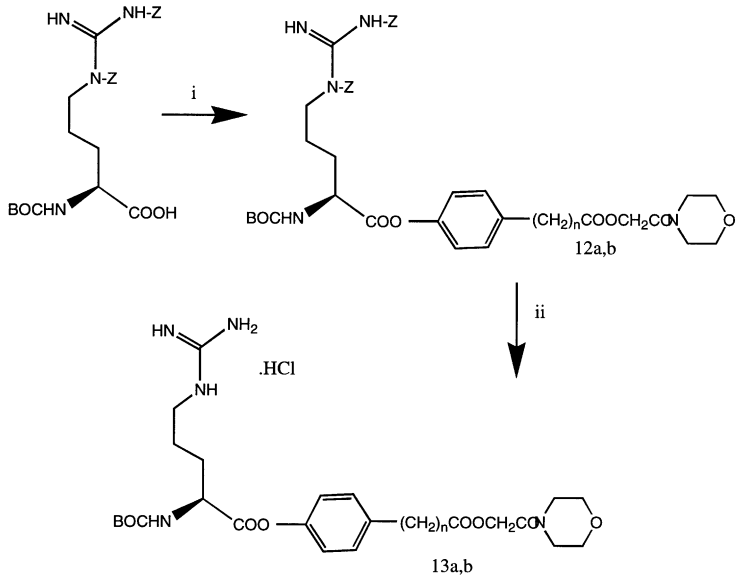
Figure 4. i: 1. ClCH2COOEt/NaOET/EtOH, 2. HBr/AcOH. ii: N-Chloroacetyl morpholine/TEA/MeCN. iii: 4-Guanidinobenzoic acid mesylate/DCC/pyridine. iv: H_2O_2/AcOH.

2a versus **2e**). The extremely high activity (picomolar range) of **2c** on plasmin is remarkable. Tert. butyl esters **2g** and **2h** didn't show any remarkable activity. But after removal of the ester group, compounds **2i**, **2j** and **2k** show remarkable selectivity for trypsin over thrombin. Introduction of the pyridine ring instead of the benzene ring to increase the ester function reactivity didn't lead to a considerable change in the activity, similarly to the compound **2l**. Introduction of the thioester moiety was intended to increase the ester reactivity towards nucleophiles. No effect of the change of sulfide (**10**, no M^+) and sulfone **11** (M^- effect and proposed enhancement of the nucleophile reactivity) has been observed.

Mild effects of conjugation on compounds of group **4** were observed (compare compounds **4a** and **4b** with **4d** and **4e**). Reduced activity was observed in the compound **7a** (compare e.g. with **2a**) but the thioester **7b** shows the similar activity to the oxygen analogue **2a**. The compounds with an arginine moiety **13a**, **13b** and with an N-formamidinoisonipecotic acid moiety **16a** and **16b** are inactive. It is possible to conclude that the activity is not dependent on the ester function reactivity in nucleophilic substitution but only on the rate of hydrolysis of the 4-guanidinobenzoyl-trypsine complex which is the commonly accepted mechanism of the inhibiting mechanism of serine proteinases [4].

4. Experimental protocols

Melting points were measured on a Boetius microscope and are uncorrected. NMR spectra were run on a Varian 200 (200 MHz) using TMS as internal standard. All chemicals were supplied from Merck and Aldrich, solvents from Microchem (Slovakia) and enzymes and substrates from Sigma. HPLC was performed on a Pye-Unicam system using Tessek C-18 columns (25 ×



Cpds.No.	n
12,13 a	0
12,13 b	2

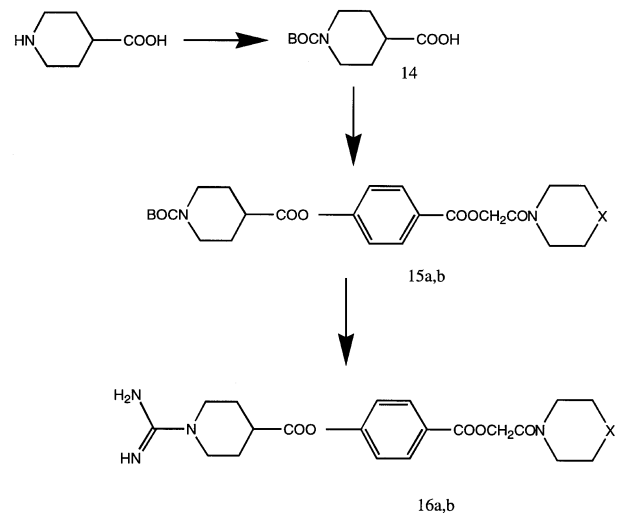
Figure 5. i: 1a or 1d/DCC, DCM/MeCN. ii: H_2/Pd-C/EtOH/HCl.

0.25 cm) for analytical purposes and Labio C-18 (25 × 5 cm) for semipreparations. The gradient MeCN-H_2O (each contained 0.05% of trifluoroacetic acid) 0–90% was used at a flow rate of 1 mL/min (analytical) over 50 min, or 10 mL/min (semipreparations). GC-MS analyses were run on Carlo Erba 1106.

N-Chloroacetyl-N´-methylpiperazine hydrochloride and 4-guanidinobenzoic acid mesylate were prepared according to the described procedure [3], as well as N^2BOC-N^6, N^8-di-Z-Arginine [5].

4.1. N-chloroacetyl glycine benzyl ester

Glycine benzylester tosylate (16.85 g, 0.05 mol) were dissolved in 250 mL of dry dichloromethane and 26 mL (0.1 mol) of dry triethylamine were added. The mixture was cooled to 0 °C, and 3.8 mL of chloroacetyl chloride in 15 mL of dry dichloromethane were added dropwise. The mixture was stirred for 1 h at room temperature, extracted with water, sat. $NaHCO_3$ and brine, dried over Na_2SO_4, evaporated and used without further purification. The analytical sample was purified by high vacuum (0.0005 torr) molecular distillation (bath temperature, 80 °C), to give the solidifying oil. Yield: 11.3 g, 74% (M.p. after solidification, 33–35 °C). MS: M^+-H^+ = 241,



Cpd.No.	X
15a,b	O
16a,b	NMe

Figure 6. i: BOC_2O/Na_2CO_3/wt. dioxane. ii: 1a or 1b/DCC/DMAP/MeCN-DCM. iii: 1. EtOAc/HCl, 2. cyanamide/concd./HCl/EtOH.

Table I. Inhibiting activities. IC_{50} (nM) of compounds **2a-l**, **7a**, **7b**, **10**, **11**, **13a**, **13b**, **16a**, **16b** on trypsin, thrombin, plasmin and urokinase.

Compound	Trypsin	Thrombin	Plasmin	Urokinase
2a	10	35	13	75
2b	10	94	28	55
2c	8	12	0.05	32
2d	65	475	1 163	130
2e	26	1 030	446	715
2f	5	40	8	106
2g	32	78	336	710
2h	375	435	5 025	1 005
2i	10	726	38	43
2j	19	2 557	381	213
2k	700	8 310	8 015	2 235
2l	8	34	36	11
4a	32	447	343	117
4b	140	1 018	1 450	130
4c	30	192	79	NT
4d	13	249	76	45
4e	13	218	49	21
7a	75	321	129	384
7b	17	44	8	22
10	15	22	507	31
11	15	64	291	33
13a	4.4×10^5	NT	NT	NT
13b	4.72×10^5	NT	NT	NT
16a	1.3×10^4	NT	NT	NT
16b	1.7×10^4	NT	NT	NT

NT = not tested.

other peaks: 192 (M^+-CH_2Cl), 164 (M^+-$ClCH_2CO$), 150 (M^+-tropylium), 91 (tropylium). ^{1}H-NMR ($CDCl_3$): 2.38 s (2H, $ClCH_2$), 3.68 d (2H, CH_2NH), 3.88 s (2H, CH_2O), 5.85 bs (1.3 H, NH), 6.49–6.56 m (5H, H arom).

4.2. N-chloroacetyl morpholine

Chloroacetyl chloride (7.5 mL, 0.1 mol), dissolved in 50 mL of dry ether, was added dropwise to the stirred solution of morpholine (22 mL, 0.2 mol) in 300 mL of dry ether at –20 °C. The mixture was stirred for 30 min at room temperature, the morpholinium chloride was filtered and washed with ether, the filtrate was evaporated and the oily residue distilled in vacuo. B.p. 67–69 °C/0.6 torr. Yield: 15.2 g, 93%. MS: 162 (M^+-H^+), other peaks: 114 (M^+-$ClCH_2$), 86 (morpholinyl). ^{1}H-NMR: 2.22 s (2H, $ClCH_2$), 3.65 m (4H), 4.44 m (2H) and 4.56 m (2H).

4.3. Typical procedure for preparation of hydroxy-derivatives **1a–h** *was described in [3]*

Physical-analytical data for each:

1a: m.p. 185–186 °C (iPr_2O/EtOAc), yield: 1.48 g, 56%. Elemental anal. for $C_{13}H_{15}NO_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 3.43–3.46 (2H), 3.55–3.64 m (6H), 4.95 s (2H), 6.74 d (2H), J = 7.56 Hz), 7.81 d (2H, J = 7.55 Hz).

1b: m.p. 201–203 °C (iPr_2O/EtOAc), yield: 1.33 g, 48%. Elemental anal. for $C_{14}H_{18}N_2O_4$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.54 s (3H), 2.55 m (2H), 3.50 m (4H), 3.65 m (2H), 6.78 d (2H), 7.68 d (2H).

1c: m.p. 65 °C (MeOH/iPr_2O), yield: 1.12 g, 46%. Elemental anal. for $C_{18}H_{17}NO_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 3.5 bs (NH), 4.04 d (2H), 4.75 s (2H), 5.22 s (2H), 6.88 d (2H, J = 7.45 Hz), 7.43 m (5H), 7.97 d (2H, J = 7.44 Hz).

1d: m.p. 79–80 °C (iPr_2O/EtOAc), yield: 1.36 g, 48%. Elemental anal. for $C_{16}H_{22}N_2O_4$ (C, H, N). ^{1}H-NMR: 2.54 s (3H, NCH_3), 2.63 t (2H, J = 5.79 Hz), 2.89 m (2H), 3.00 t (2H, J = 5.81 Hz), 3.45 m (6H), 6.75 d (2H, J = 7.36 Hz), 7.43 d (2H, J = 7.38 Hz).

1e: m.p. 165–167 °C (hexane/EtOAc), yield: 1.61 g, 51%. Elemental anal. for $C_{17}H_{17}NO_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 3.65 m (8H), 4.89 s (2H), 7.24–7.31 m (broad, 4H), 7.56 dd (1H), 7.80–7.86 m (4H).

1f: m.p. 212–214 °C (iPr_2O/MeOH), yield: 1.75 g, 63%. Elemental anal. for $C_{15}H_{18}O_5$ (C, H). ^{1}H-NMR (DMSO): 3.75 m (4H), 4.28 m (4H), 4.97 m (2H), 6.53 d (1H), 8.91 dd (1H), 8.24 d (1H, J = 6.56 Hz).

1g: m.p. 134 °C (hexane/EtOAc), yield: 1.80 g, 65%. Elemental anal. for $C_{15}H_{18}O_5$ (C, H). ^{1}H-NMR ($CDCl_3$): 1.52 s (9H), 4.66 s (2H), 6.19 d (1H, J = 9.78 Hz), 6.27 d,

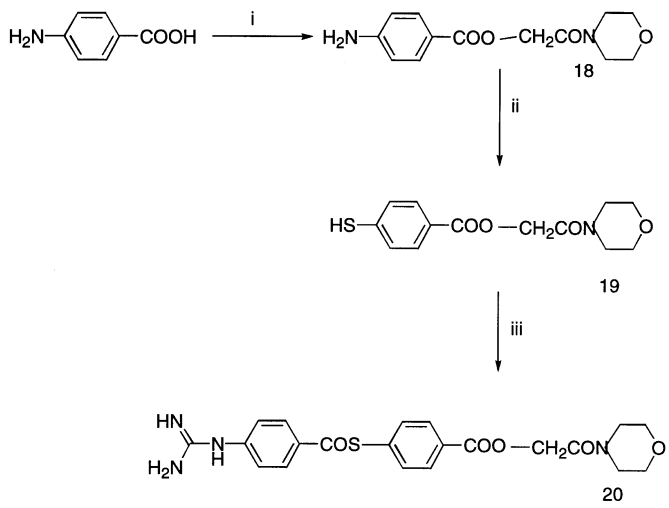


Figure 7. i. N–chloroacetyl morpholin, MeCN, TEA, ii. 1. $NaNO_2$, HCL, 2. KSCS (OEt), 3. wt. NH_3, EtOH, iii. 4–guanidinobenzoic acid/DCC/pyridine.

6.84 d (2H, *J* = 7.55 Hz), 6.99 bs (1H), 7.33 d (2H, *J* = 7.56 Hz) 7.61 d (1H, *J* = 7.78 Hz).

1h: m.p. 129 °C (iPr_2O/EtOAc), yield: 1.19 g, 39%. Elemental anal. for $C_{16}H_{19}NO_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.59 s (9H), 2.28 s (2H), 4.28 s (2H, OCH_2), 6.18 m (1H), 6.29 dd (1H), 7.36 dd (1H), 7.77 m (1H), 8.65 bs (1H, NH).

4.4. Typical procedure for preparation of hydroxyamides **3a–e**

4-hydroxyaromatic acid (0.01 mol) was suspended in acetonitrile (25 mL) and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (1.92 g, 0.01 mol) were added at once. The acid dissolved immediately. Then, triethylamine (1.34 mL 0.01 mol) and the amine (0.01 mol) were added and the mixture was stirred for 18 h. After evaporation of volatile compounds in vacuo, the residue was distributed between water (50 mL) and ethyl acetate (20 mL) and the water phase was twice more extracted with ethyl acetate. Joint organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo. The residue was recrystallised.

3a: m.p. 130 °C (iPr_2O/EtOAc), yield: 1.79 g, 76%. Elemental anal. for $C_{13}H_{17}NO_3$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.56 t (2H, *J* = 5.55 Hz), 2.87 t (2H, *J* = 5.56 Hz), 3.33 m (2H), 3.50 m (2H), 6.75 d (2H, *J* = 7.56 Hz,) 7.03 d (2H, *J* = 7.55 Hz), 9.9 bs (1H).

3b: Syrup. Data for fumarate: m.p. 141–142 °C (THF), yield: 2.88 g 79%. Elemental anal. for $C_{18}H_{24}N_2O_6$ (C, H, N). ^{1}H-NMR (DMSO): 2.18 s (3H), 2.25 t (2H), 2.52 t (2H), 2.66 m (4H), 3.40 m (4H), 6.59 s (2H, fumarate), 6.45 d (2H, *J* = 7.59 Hz), 7.01 d (2H, *J* = 7.60Hz).

3c: m.p. 65–67 °C (iPr_2O/Hexane), yield: 2.72 g, 84%. Elemental anal. for $C_{15}H_{20}N_2O_6$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 2.36 t (2H, *J* = 5.55 Hz), 3.83 t (2H, *J* = 5.57 Hz), 3.36 m (2H), 3.76 m (6H), 5.01 s (2H), 6.75 d (2H, *J* = 7.28 Hz), 7.18 d (2H, *J* = 7.26 Hz).

3d: m.p. 213 °C (hexane/EtOAc), yield: 1.51 g, 65%. Elemental anal. for $C_{13}H_{15}NO_3$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 3.70 m (8H), 6.63 (1H, *J* = 10.27 Hz), 6.86 d (2H *J* = 7.45 Hz), 7.41 d (2H, *J* = 7.44 Hz), 7.67 d (1H, *J* = 10.26 Hz).

3e: m.p. 230–235 °C decomp. (hexane/EtOAc), yield: 1.79 g, 71%. Elemental anal. for $C_{16}H_{15}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 4.56 s (2H), 6.31 d (1H, *J* = 11.0 Hz), 7.1 d (2H, *J* = 7.67 Hz), 7.26–7.29 m (5H), 7.52 d (2H, *J* = 7.67 Hz), 7.69 d (1H, *J* = 10.9 Hz).

4.5. N-(Mercaptoacetyl)-morpholine (**5b**)

Chloroacetyl morpholin (2.95 g, 0.02 mol) was dissolved in 35 mL of absolute ethanol and potassium ethyldithiocarbonate (4 g, 0.025 mol) was added at once. The mixture was stirred under N_2 and refluxed for 6 h, cooled and 35 mL of water ammonia were added and stirred overnight. The mixture was extracted three times with dichloromethane, dried (Na_2SO_4) and evaporated in vacuo. After distillation under reduced pressure, the pale yellow oil was obtained. B.p. 72–74 °C/0.45 torr, yield: 2.7 g, 86%. GC-MS: 161 (M^+), others: 114 (N-morpholinylcarbonyl), 86 (morpholinyl), 46 (CH_2=S^+). ^{1}H-NMR ($CDCl_3$): 1.11 s (1H), 2.36 s (2H), 3.75–3.79 m (8H).

4.6. Typical procedure for the preparation of compounds **6a** *and* **b**

In 35 mL of dry dichloromethane, N-(aminoacetyl)-morpholin or N-(mercaptoacetyl)-morpholin **5b** (0.02 mol) were dissolved, triethylamine (2.7 mL, 0.02 mol) were added and cooled to –10 °C. Then, the solution of 4-acetoxybenzoyl chloride **2** (3.95 g, 0.02 mol) in 35 mL of dry DCM were added dropwise at –10 °C. The mixture was stirred for 2 h at room temperature, evaporated in vacuo and the residue was dissolved in 30 mL of methanol. Then, 30 mL of sat. Na_2CO_3 were added and the mixture was stirred for an additional 1 h. It was partly evaporated in vacuo, extracted three times with ethyl acetate, washed with brine, dried (Na_2SO_4), evaporated in vacuo and recrystallised.

6a: m.p. 196–197 °C (EtOAc/hexane), yield: 4.1 g, 77%. Elemental anal. for $C_{13}H_{16}N_2O_4$ (C, H, N). ^{1}H-NMR: 3.45–3.59 m (8H), 4.26 d (2H, *J* = 6.96 Hz, CH_2NH), 6.79 d (2H, *J* = 7.56 Hz), 7.10 d (2H, *J* = 7.56 Hz).

6b: m.p. 153–154 °C (EtOAc/hexane), yield: 3.8 g, 72%. Elemental anal. for $C_{13}H_{15}NO_3S$ (C, H, N, S, % S calcd. 12.08, found 12.49). ^{1}H-NMR: 3.38–3.63 m (8H), 3.79 s (2H, CH_2S), 6.83 d (2H, *J* = 7.49 Hz), 7.21 d (2H, *J* = 7.50 Hz).

4.7. Ethyl methoxyphenyl thioacetate

Sodium (2.3 g, 0.1 mol) was dissolved in 250 mL of absolute ethanol and 4-mercaptoanisole (14 g, 0.1 mol) were added. After being stirred for 1 h at room temperature under N_2, 11.4 g (0.1 mol) of ethyl chloroacetate was added. The mixture was stirred and refluxed under N_2 for 6 h, evaporated in vacuo and the residue was distributed between water (100 mL) and ether (50 mL). The water phase was twice more extracted with ether, washed with brine, dried (Na_2SO_4), evaporated in vacuo and distilled in vacuo. B.p. 72–74 °C/0.5 torr, yield: 13.9 g, 88%. GC-MS: 226 (M^+), other peaks: 153 (MeO-C_6H_4-S=CH_2), 107 (MeO-Ph^+), 46 (CH_2=SH^+). ^{1}H-NMR

($CDCl_3$): 1.11 t (3H, ethyl), 3.43 d (2H, SCH_2), 4.11 s (3H, CH_3O), 4.76 q (2H, ethyl), 6.66 d (2H, *J* = 7.34 Hz), 7.24 d (2H, *J* = 7.34 Hz).

4.8. 4-hydroxyphenyl-1-thioacetic acid (**8**)

Ethyl-4-methoxyphenyl thioacetate (12.9 g, 0.057 mol) was dissolved in 50 mL of glacial acetic acid and 25 mL of conc. hydrobromic acid was added. The mixture was gently refluxed under N_2 for 25 min, cooled and evaporated to dryness. The residue was crystallised from water-ethanol. M.p. 266–267 °C. Yield: 5.8 g, 56%. Elemental anal. for $C_8H_8O_3S$ (C, H, N, S, % S calc. 17.40%, found 17.86%). ^{1}H-NMR (DMSO): 3.76 s (2H, CH_2), 6.87 d (2H, *J* = 7.88 Hz), 7.87 d (2H, *J* = 7.87 Hz), 10.11 bs, (1.1H, COOH).

4.9. 4-aminobenzoyloxy-acetylmorpholin (**1l**)

The compound was prepared according to the procedure described for compounds **1a–h**, starting from 1.25 g (0.01 mol) of 4-aminobenzoic acid. M.p. 177 °C (MeOH/diisopropyl ether), yield: 0.9 g, 36%. Elemental anal. $C_{13}H_{16}NO_4$ (C, H, N). ^{1}H-NMR (DMSO): 3.73 m (2H), 3.83 m (6H), 4.76 s (2H), 6.55 d (2H, *J* = 7.23 Hz), 7.43 d (2H, *J* = 7.34 Hz).

4.10. N-(4-Mercaptobenzoyloxy)-acetyl morpholine (**1m**)

N-(4-aminobenzoyl)-oxyacety morpholine **1l** (1.77 g, 0.0072 mol) was dissolved in 20 mL of water and 1.5 mL of conc. HCl was added. The mixture was cooled to 0 °C, and 0.55 g (0.008 mol) of sodium nitrite in 5 mL water was added dropwise at 0 °C. Then, the mixture was stirred for 15 min at the same temperature and 1.28 g (0.008 mol) of potassium ethyl dithiocarbonate in 10 mL of water was added portionwise. The mixture was stirred for 10 min and heated gradually to 60 °C, and then stirred for 2 h at room temperature. The precipitated compound was dissolved by addition of 20 mL of dioxane and 20 mL of water ammonia was added and stirred for 2 h more. The mixture was evaporated partly in vacuo, extracted with four portions of ethyl acetate, washed with sat. $NaHCO_3$, brine, dried (Na_2SO_4), evaporated in vacuo and recrystallised from diisopropyl ether and methanol. M.p. 165–166 °C. Yield: 0.97 g, 48%. Elemental anal. $C_{13}H_{15}NO_4S$ (C, H, N, S, % S calc. 11.39, found 11.10). ^{1}H-NMR ($CDCl_3$): 1.17 s (1.3H, SH), 3.56 m (2H), 3.93 (6H), 4.55 s (2H, CH_2O), 6.14 d (2H, *J* = 6.93 Hz), 7.25 d (2H, *J* = 7.01 Hz).

4.11. Preparation of compounds **2a–h**, **2l**, **4a–e**, **7a**, **7b** *and* **10** *(typical procedure)*

4-Guanidinobenzoic acid mesylate (1.38 g, 0.005 mol) was dissolved in 40 mL of dry pyridine and 0.005 mol of the hydroxycompound were added. The mixture was cooled to 0 °C, and DCC (1.03 g 0.005 mol) were added at once. The mixture was stirred overnight under chlorcalcium tube at room temperature, precipitated dicyclohexyl urea was filtered, washed with two small portions of pyridine and the filtrate was poured into 350 mL of ether. The precipitated product was dissolved in methanol (30–40 mL) and passed through the column of 50 g of neutral Al_2O_3, which was washed with methanol (ca. 50 mL). The solution was evaporated in vacuo to ca. 10 mL volume, and it was poured into 50 mL of dry ethyl acetate. The product was filtered, washed with ethyl acetate, dried in vacuo and the purity was monitored by HPLC.

2a: amorphous, yield: 1.06 g, 43%. Elemental anal. for $C_{22}H_{26}N_4O_9S$ (C, H, N, S) % S calc. 6.14, found 5.79. ^{1}H-NMR (DMSO): 2.98 s (3H), 3.73 m (8H), 5.06 s (2H), 7.21 d (2H), 7.24 d (2H), 7.86 d (2H), 7.99 d (2H). ^{13}C-NMR (DMSO): 41.67, 44.36, 62.09, 65.96 (OCH_2), 122.43, 122.57, 124.89, 127.20, 129.47, 131.52, 131.14, 155.45, 162.33, 163.57, 169.65.

2b: amorphous, yield: 1.04 g, 39%. Elemental anal. for $C_{23}H_{29}N_5O_8S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.96 s (3H), 3.34 m (2H), 3.36 s (3H), 3.55 m (6H), 4.99 s (2H), 7.11 d (2H), 7.23 d (2H), 7.76 d (2H), 8.06 d (2H). ^{13}C-NMR (DMSO): 38.46, 45.36, 45.76, 47.31, 47.26, 51.31, 58.42, 122.49, 124.8, 127.1, 151.1, 131.3, 151.6, 141.5, 155.6, 163.5, 164.6, 171.3.

2c: after semi-preparative HPLC purification, 250 mg of the compound was purified.

Amorphous, Elemental anal. for $C_{27}H_{29}N_4O_{10}S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.43 s (3H), 3.46 s (2H, OCH_2Ar), 3.84 s (2H, $NHCH_2$), 5.53 s (2H, OCH_2CO), 6.90 d (2H, *J* = 7.55 Hz), 7.29 m (7H), 7.96 d (2H, *J* = 7.56 Hz), 8.25 d (2H, *J* = 8.91 Hz), 9.72 bs (3H). ^{13}C-NMR (DMSO): 39.50 (mesylate), 40.97 (OCH_2Ar), 61.31 (OCH_2CO), 115.4, 117.0, 120.1, 122.6, 125.3, 126.6, 127.6, 128.5, 128.2, 129.7, 130.3, 134.9, 155.4, 163.2, 164.9, 165.3, 170.6.

2d: amorphous, yield: 1.40 g, 45%. Elemental anal. for $C_{27}H_{30}N_5O_8S$. ^{1}H-NMR (DMSO): 2.37 s (3H, mesylate), 2.39 s (3H, CH_3N), 2.75 t (2H, *J* = 4.78 Hz, CH_2), 2.90 t (2H, *J* = 4.78 Hz, CH_2), 3.66 m (8H), 4.78 s (2H, OCH_2), 6.90 d (2H, *J* = 8.76 Hz), 7.10 d (2H, *J* = 7.56 Hz), 7.32 d (2H, *J* = 8.77 Hz), 7.90 d (2H, *J* = 8.76 Hz). ^{13}C-NMR (DMSO): 29.4 (CH_2), 34.7 (CH_2), 39.4 (mesylate), 45.6, 54.11, 54.2, 61.3 (OCH_2), 119.0, 121.7,

121.8, 122.46, 129.18, 130.87, 137.8, 149.1, 153.7, 156.5, 164.6, 171.7.

2e: amorphous, yield: 1.17 g, 45%. Elemental anal. for $C_{24}H_{28}N_4O_9S$ (C, H, N, S) % S calc. 5.84, found 5.41. ^{1}H-NMR (DMSO): 2.44 (3H, mesylate), 3.45 m (8H), 5.05 s (1H), 7.2–7.32 m (5H), 7.51–7.53 m (2H), 7.77–7.79 m (1H), 8.64 m (1H). ^{13}C-NMR (DMSO): 38.9 (mesylate), 40.7, 43.7, 60.4 (OCH_2), 66.3, 118.3, 119.2, 124.6, 126.2, 126.9, 129.5, 129.9, 130.5, 131.6, 133.7, 136.48, 143.8, 146.3, 155.8, 165.8, 1265.9, 167.4.

2f: amorphous, yield: 1.19 g, 45%. Elemental anal. for $C_{21}H_{25}N_5O_9S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.36 s (3H, mesylate), 3.55–3.74 2×d (8H), 5.05 s (2H), 6.57 d (1H), 7.36 d (2H, *J* = 8.76 Hz), 7.65 d (2H, *J* = 8.77 Hz), 8.04 dd (1H), 8.29 d (1H). ^{13}C-NMR (DMSO): 39.5 (mesylate), 43.43, 46.15, 62.68 (OCH_2), 67.5, 67.6, 111.3, 120.4, 121.5, 124.3, 130.34, 131.3, 135.4, 141.8, 141.9, 155.7, 160.3, 165.8, 174.6.

2g: amorphous, yield: 1.03 g, 39%. Elemental anal. for $C_{25}H_{29}N_3O_9S$ (C, H, N, S). ^{1}H-NMR, (DMSO): 1.49 s (9H, tBuO), 2.46 (3H, mesylate), 4.66 s (2H, OCH_2), 6.69 d (1H, *J* = 11.35 Hz, CH=), 7.19 d (2H, *J* = 7.45 Hz), 7.45 d (2H, *J* = 8.1 Hz), 7.71 d (1H, *J* = 11.36 Hz), 7.89 d (2H, *J* = 7.98 Hz), 8.17 d (2H, *J* = 7.44 Hz). ^{13}C-NMR (DMSO): 27.65, 39.65 (MsOH), 61.14 (OCH_2), 81.58, 117.3, 122.43, 122.6, 124.7, 130.5, 131.5, 131.9, 142.1, 143.3, 152.2, 155.6, 163.8, 165.6, 166.8.

2h: amorphous, yield: 1.12 g, 40%. Elemental anal. for $C_{25}H_{30}N_4O_9S$ (C, H, N, S) % S calc. 5.70, found 5.39. ^{1}H-NMR (DMSO): 1.42 s (9H, tBuO), 2.38 s (3H, MsOH), 4.58 s (2H, OCH_2), 6.90 d (2H, *J* = 7.34 Hz), 7.12 dd (1H), 7.36–7.40 m (2H), 7.86 d (2H, *J* = 7.83 Hz), 8.88 s (1H, NH ind.). ^{13}C-NMR (DMSO): 27.5 (tBu), 36.6 (MsOH), 37.2 (CH_2Ar), 56.6 (OCH_2), 111.1, 118.69, 119.3, 121.6, 121.8, 121.9, 123.5, 123.9, 125.8, 131.6, 136.3, 141.4, 142.3, 155.1, 161.3, 163.8, 170.9.

2l: amorphous, yield: 1.29 g, 48%. Elemental anal. for $C_{22}H_{26}N_4O_8S_2$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.34 s (3H, MsOH), 3.56 m (2H), 3.87 m (6H), 7.23 d (2H, *J* = 7.56 Hz), 7.36 d (2H, *J* = 7.80 Hz), 7.85 d (2H, *J* = 7.55 Hz), 7.98 d (2H, *J* = 7.58 Hz). ^{13}C-NMR (DMSO): 36.7 (MsOH), 45.5, 46.1, 59.1 (OCH_2), 65.5, 116.3, 117.5, 117.9, 118.0, 118.2, 121.3, 138.3, 139.8, 155.8, 160.2, 162.1, 165.3.

4a: amorphous, yield: 1.19 g, 46%. Elemental anal. for $C_{24}H_{28}N_4O_7S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.36 s (3H, MsOH), 2.64 t (2H, *J* = 8.96 Hz), 2.86 t (2H, *J* = 7.77 Hz), 3.43 m (8H), 7.11 d (2H, *J* = 6.98 Hz), 7.17 d (2H, *J* = 7.85 Hz), 7.37 d (2H, *J* = 7.00 Hz), 8.04 d (2H, *J* = 7.85 Hz). ^{13}C-NMR (DMSO): 33.3 (CH_2), 33.7 (CH_2), 39.7 (MsOH), 40.4, 66.1 (OCH_2), 121.6, 122.0, 122.7, 129.4, 131.2, 138.9, 148.9, 149.5, 154.4, 164.2, 170.0.

4b: amorphous, yield: 1.14 g, 44%. Elemental anal. for $C_{24}H_{31}N_5O_6S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.37 s (3H, Me-N), 2.46 s (3H, MsOH), 2.73 t (2H, *J* = 5.58 Hz), 2.85 t (2H, *J* = 5.53 Hz), 3.32 m (6H), 3.64 m (2H), 7.19 d (2H, *J* = 7.45 Hz), 7.36 d (2H, *J* = 8.24 Hz), 7.45 d (2H, *J* = 7.46 Hz), 8.17 d (2H, *J* = 8.22 Hz). ^{13}C-NMR (DMSO): 29.9 (CH_2), 33.6 (CH_2), 39.3 (MsOH), 43.1 (MeN), 44.1, 52.9, 53.1, 121.5, 122.5, 122.7, 125.3, 129.5, 138.1, 148.7, 155.5, 164.1, 170.1.

4c: amorphous, yield: 1.17 g, 44%. Elemental anal. for $C_{24}H_{31}N_4O_8S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.38 s (3H, MsOH), 2.56 t (2H, *J* = 5.87 Hz), 2.89 t (2H, *J* = 5.88 Hz), 3.28 bs (2H), 3.87 m (6H), 4.22 d (2H, CH_2NH), 6.66 bs (1H, NH), 6.87 d (2H, *J* = 7.81 Hz, 7.12 d (2H, *J* = 7.56 Hz), 7.87 d (2H, *J* = 7.87 Hz), 7.99 d (2H, *J* = 7.53 Hz), 10.11 bs (2H, NH), 11.1 bs (1.5H, NH). ^{13}C-NMR (DMSO): 30.1 (CH_2), 31.7 (CH_2), 38.9 (MsOH), 40.0, 45.7, 50.2, 53.3, 61.1 (OCH_2), 120.1, 122.21, 123.1, 123.8, 125.7, 126.6, 129.3, 138.3, 141.5, 151.3, 158.7, 161.6, 170.0.

4d: amorphous, yield: 0.88 g, 38%. Elemental anal. for $C_{20}H_{26}N_4O_7S$ (C, H, N, S), % S calc. 6.87, found 6.45. ^{1}H-NMR (DMSO): 2.38 s (3H, MsOH), 3.60 m (8H), 6.67 d (1H, *J* = 10.26 Hz), 7.23 d (2H, *J* = 7.83 Hz), 7.36 d (2H, *J* = 7.22 Hz), 7.66 d (1H, *J* = 10.3 Hz), 8.06 d (2H, *J* = 7.28 Hz), 8.11 d (2H, *J* = 7.81 Hz). ^{13}C-NMR (DMSO): 39.91 (MsOH), 42.5, 45.7, 66.2, 66.3, 118.13, 122.3, 122.5, 122.7, 122.8, 129.3, 131.4, 132.9, 140.7, 147.1, 151.6, 154.7, 164.1, 164.5.

4e: amorphous, yield: 1.22 g, 48%. Elemental anal. for $C_{25}H_{26}N_4O_6S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.39 s (3H, MsOH), 2.75 s (2H, CH_2NH), 6.72 d (1H, *J* = 10.3 Hz), 7.27 m (5H), 7.62 d (2H, *J* = 7.80 Hz), 7.71 d (2H, *J* = 7.21 Hz), 8.08 d (1H, *J* = 10.4 Hz), 8.17 d (1H, *J* = 7.85 Hz), 8.24 d, (2H, *J* = 7.23 Hz). ^{13}C-NMR (DMSO): 39.5 (MsOH), 44.37, 116.7, 122.1, 122.3, 123.3, 123.4, 125.1, 128.3, 128.44, 129.3, 129.8, 130.2, 140.9, 145.3, 155.8, 168.8, 178.2.

7a: amorphous, yield: 1.28 g, 49%. Elemental anal. for $C_{22}H_{27}N_5O_8S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.41 s (3H, MsOH), 3.48 m (2H), 3.46 m (6H), 5.62 d (2H, CH_2NH), 7.11 d (2H, *J* = 7.77 Hz), 7.49 d (2H, *J* = 7.42 Hz), 7.58 d (2H, *J* = 7.77 Hz), 8.11 d (2H, *J* = 7.39 Hz). ^{13}C-NMR (DMSO): 33.3 (MsOH), 40.1, 41.8, 47.5, ($NHCH_2$), 66.1, 114.8, 122.7, 124.8, 129.1, 131.5, 141.3, 148.5, 155.4, 160.2, 165.1, 167.9.

7b: amorphous, yield: 1.00 g, 38%. Elemental anal. for $C_{21}H_{26}N_4O_8S_2$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.41 s (3H, MsOH), 3.40 m (2H), 3.57 m (6H), 4.14 s (2H, SCH_2), 7.39 d (2H, *J* = 7.33 Hz), 7.59 d (2H, *J* = 7.87 Hz), 8.05 d (2H, *J* = 7.34 Hz), 8.19 d (2H, *J* = 7.87 Hz). ^{13}C-NMR (DMSO): 39.5 (MsOH), 42.1, 45.9, 52.7

(SCH_2), 66.4, 115.3, 122.4, 128.5, 129.2, 130.7, 131.6, 133.4, 155.9, 162.0, 163.6, 165.2.

10: amorphous, yield: 1.01 g, 37%. Elemental anal. for $C_{23}H_{28}N_4O_9S_2$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.42 s (3H, MsOH), 3.37 m (2H), 3.63 m (6H), 4.02 s (2H, SCH_2), 4.87 s (2H, OCH_2), 7.21 d (2H, *J* = 7.45 Hz), 7.32 d (2H, *J* = 8.00 Hz), 7.51 d (2H, *J* = 7.56 Hz), 8.13 d (2H, *J* = 8.02 Hz). ^{13}C-NMR (DMSO): 39.7 (MsOH), 41.5, 44.18, 44.27 (SCH_2), 62.2 (OCH_2), 65.8, 116.1, 122.6, 122.9, 129.7, 130.9, 133.3, 134.4, 149.1, 155.3, 164.0, 164.6, 168.9.

4.12. Preparation of compound **2i**

In the common apparatus for hydrogenation under normal pressure, was dissolved 850 mg (1.12 mmol) of the compound **2c** in 10 mL of methanol, and 21 mg of 10% Pd on charcoal were added. The hydrogenation proceeded for 4 h, the catalyst was filtered, washed with methanol, the methanolic solution was partly evaporated, and the product was precipitated with an excess of ethyl acetate. After purification by semi-preparative HPLC and lyophilisation, 420 mg of the product was gained as an amorphous powder (yield: 74%). Elemental anal. for $C_{20}H_{22}N_4O_{10}S$. (C, H, N, S). ^{1}H-NMR (DMSO): 2.80 s (3H, MsOH), 3.61 d (2H, $NHCH_2$), 4.75 s (2H, OCH_2), 7.33 d (2H, *J* = 7.11 Hz), 7.45 d (2H, *J* = 7.34 Hz), 8.06 d (2H, *J* = 7.09 Hz), 8.21 d (2H, *J* = 7.14 Hz), 11.01 bs (3.5H, NH and COOH). ^{13}C-NMR (DMSO): 20.4 (MsOH), 41.4 ($NHCH_2$), 62.4 (OCH_2), 114.4, 122.1, 122.3, 124.2, 126.8, 130.9, 131.1, 131.4, 132.9, 142.2, 155.9, 163.5, 164.2, 165.9, 172.7.

4.13. Preparation of compounds **2j** *and* **2k** *(general procedure)*

The tert. butyl ester **2g** or **2h** (0.005 mol) was dissolved in 40% trifluoroacetic acid in dry DCM (10 mL), containing 2 mL of anisole, and stirred for 15 min. The volatile compounds were removed in vacuo and the oily residue was treated with ethyl acetate. The precipitate was filtered and purified by semi-preparative HPLC. After lyophilisation of the appropriate fraction, amorphous product was obtained.

2j: yield: 354 mg, 56%. Elemental anal. for $C_{22}H_{20}N_3O_9S$ (C, H, N, S) % S calc. 6.38, found 6.80. ^{1}H-NMR (DMSO): 2.42 s (3H, MsOH), 4.70 s (2H, OCH_2), 6.78 d (1H, *J* = 10.3 Hz), 7.36 d (2H, *J* = 6.91 Hz), 7.71 d (2H, *J* = 7.87 Hz), 7.84 d (1H, *J* = 10.1Hz), 7.94 d (2H, *J* = 6.92 Hz) 8.28 d (2H, *J* = 7.87 Hz), 9.9 bs (4H, COOH and NH). ^{13}C-NMR: 39.9 (MsOH), 60.6 (OCH_2), 117.5, 122.5, 122.7, 122.8, 129.8, 130.8, 130.9, 131.0, 131.5, 131.8, 140.9, 144.5, 155.3, 161.7, 165.6, 169.1.

2k: amorphous, yield: 372 mg, 59%. Elemental anal. for $C_{21}H_{22}N_4O_9S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.38 s (3H, MsOH), 2.56 t (2H, *J* = 5.87 Hz), 2.89 t (2H, *J* = 5.88 Hz), 3.28 bs (2H), 3.87 m (6H), 4.22 d (2H, CH_2N), 6.66 bs (1H, NH), 6.87 d (2H, *J* = 7.84 Hz), 7.12 d (2H, *J* = 7.56 Hz), 7.87 d (2H, *J* = 7.87 Hz), 7.99 d (2H, *J* = 7.54 Hz), 10.11 bs (2H), 11.10 bs (1H). ^{13}C-NMR (DMSO): 30.1 (CH_2), 31.7 (CH_2), 38.9 (MsOH), 40.0, 45.7, 50.2, 53.3, 61.11 (OCH_2), 120.1, 122.2, 123.1, 123.8, 125.7, 126.6, 129.3, 138.3, 141.5, 151.3, 158.7, 161.6, 170.00.

4.14. Preparation of compound **11**

Compound **10** (1.45 g, 2.55 mmol) were dissolved in 15 mL of glacial acetic acid and cooled to 0 °C. Then, 4.5 mL of 30% water hydrogen peroxide were added and the mixture was stirred overnight at room temperature. The mixture was evaporated in vacuo (bath temp. max. 30 °C), the gummy residue dissolved in methanol and precipitated with ethyl acetate. 1.22 g (87%) of the crude compound was gained. 350 mg of this compound were purified by semi-preparative HPLC and proceeded to the analyses and inhibiting activities. Elemental anal. $C_{23}H_{28}N_3O_{11}S$ (C, H, N, S). ^{1}H-NMR (DMSO): 2.42 s (3H, MsOH), 3.36 m (4H), 3.56 m (4H), 4.87 s (2H), 4.87 s (2H), 7.23 d (2H, *J* = 7.86 Hz), 7.36 d (2H, *J* = 8.11 Hz), 7.87 d (2H, *J* = 7.88 Hz), 8.01 d (2H, *J* = 8.09 Hz). ^{13}C-NMR (DMSO): 25.4 (MsOH), 41.6, 60.1 (OCH_2), 62.4 (SO_2CH_2), 65.8, 122.4, 122.6, 122.8, 122.9, 124.9, 126.1, 133.9, 141.9, 155.5, 162.4, 163.7, 164.8.

4.15. 1-BOC-isonipecotic acid **14**

Isonipecotic acid (12.9 g, 0.1 mol) was dissolved in water (200 mL) containing 10.6 g (0.1 mol) of sodium carbonate and the mixture was cooled to 0 °C with stirring. Then, the solution of di-tert. butyl dicarbonate in 200 mL of dioxane was slowly added at the same temperature. The mixture was stirred overnight at room temperature, evaporated in vacuo to ca. 200 mL of the volume, diluted with 200 mL of water, acidified with an excess of the solid citric acid and extracted with three portions of ethyl acetate. The ethyl acetate solution was washed with sat. sodium carbonate, brine, dried (NA_2SO_4) and evaporated in vacuo.The product was recrystallised from hexane-EtOAc. M.p. 146–147 °C, yield: 19.9 g, 86%. Elemental anal. for $C_{11}H_{19}NO_4$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.03–1.45 m (4H), 2.79 s (9H, BOC), 3.11 m (4H), 4.33 m (1H, CH-N).

4.16. Preparation of compounds **12a** *and* **b** *and* **15a** *and* **b** *(typical procedure)*

The corresponding acid (0.001 mol) was dissolved in the mixture of dichloromethane-acetonitrile (both dry) (1:1 v/v, 20 mL) and the corresponding hydroxy derivatives (0.001 mol) were added. After both compounds were dissolved, the solution was cooled to –20 °C under chlorcalcium cover and then DCC (2.01 g, 0.001 mol) was added at once. The mixture was stirred overnight and it was allowed to reach room temperature. The dicyclohexyl urea was removed by filtration, washed with two small portions of dichloromethane, the filtrate was evaporated and the product was crystallised.

12a: m.p. 145–146 °C (iPr_2O-EtOAc). Yield: 490 mg, 63%. Elemental anal. for $C_{40}H_{47}N_5O_{12}$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.48 s (9H, BOC), 1.77 m (2H, CH_2 Arg), 3.87 t (2H, CH_2 Arg.), 3.91–4.01 m (12H), 4.13 m (1H, CH-N), 4.9 s (2H, CH_2O), 5.13 s (2H, CH_2O), 5.25 s (2H, CH_2O), 7.23 d (2H, *J* = 7.46 Hz), 7.25–7.36 m (10H, C_6H_5), 8.11 d (2H, *J* = 7.50 Hz). ^{13}C-NMR (DMSO): 24.8 (CH_2 Arg), 28.2 (BOC), 33.9 (CH_2N Arg), 42.1 (CH_2 Arg), 44.1 and 45.1 (OCH_2Ph), 49.1 (CH-N), 53.5 (OCH_2), 61.7, 66.32, 69.0, (morpholine), 80.1 (BOC), 115.43, 121.48, 127.82, 128.1, 128.3, 128.6, 129.1, 129.2, 129.3, 129.4, 131.5, 132.1, 134.3, 136.7, 154.4, 155.6, 155.7, 160.4, 161.6, 165.9, 170.7.

12b: m.p. 69–70 °C (iPr_2O/EtOAc). Yield: 555 mg, 68%. Elemental anal. for $C_{42}H_{51}N_5O_{12}$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.47 s (9H, BOC), 2.70 t (2H, *J* = 5.7 Hz, Ph-CH_2CH_2-), 2.91 t (2H, *J* = 5.6 Hz, $PhCH_2CH_2$-), 5.53 m (2H, CH_2 Arg), 3.65–3.75 m (8H), 4.03 m (1H), 4.70 s (2H, OCH_2), 5.11 s (2H, OCH_2), 5.25 s (2H, OCH_2), 6.90 d (2H, *J* = 7.9 Hz), 7.18 d (2H, *J* = 7.8 Hz), 7.22–7.26 m (10 H). ^{13}C-NMR (DMSO): 24.9 (CH_2Arg), 28.2 (BOC), 30.1, 33.9, 35.4, 42.1, 44.1, 44.9, 49.1, 53.4, 61.1, 66.6, 66.9, 70.1, 79.9, 115.4, 121.3, 127.7, 127.9, 128.1, 128.2, 128.3, 128.4, 128.6, 128.7, 129.1, 129.2, 134.6, 136.8, 138.1, 148.8, 155.4, 155.7, 160.4, 163.7, 163.8, 165.1.

15a: m.p. 111–112 °C (Hexane/EtOAC). Yield: 280 mg, 58%. Elemental anal. for $C_{24}H_{32}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.08 and 1.09 dd (1H), 1.4 s (9H, BOC), 2.00 and 2.03 dd (1H), 2.92 dd (1H), 3.45 bs (2H), 3.58 m (3H), 3.70 bs (6H), 4.04 d (1H, *J* = 5.45 Hz), 4.90 s (2H, CH_2O), 7.15 d (2H, *J* = 7.62 Hz), 8.16 d (2H, J = 7.66 Hz). ^{13}C-NMR (DMSO): 27.8, 28.4, 41.5, 48.9, 81.1, 127.8, 128.1, 128.5, 132.3, 133.1, 138.7, 154.2, 165.3, 166.2, 172.4.

15b: m.p. 126–127 °C (iPr_2O-EtOAc). Yield: 273 mg, 56%. Elemental anal. for $C_{25}H_{35}N_3O_7$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 1.43 s (9H), 1.72 dd (1H), 1.89 dd (1H), 2.0 dd (1H), 2.50 bs (2H), 3.33 s (3H, CH_3N), 3.38 m (3H), 3.65 m (6H), 4.11 dd (1H), 4.96 s (2H, OCH_2), 7.14 d (2H, *J* = 7.41 Hz), 8.10 d (2H, *J* = 7.41 Hz). ^{13}C-NMR (DMSO): 28.3 (BOC), 34.7, 41.5, 41.8, 42.9, 43.1, 54.5, 61.8, 79.7 (BOC), 121.4, 121.6, 127.0, 131.5, 154.5, 164.7, 165.3, 172.5.

4.17. Preparation of compounds **13a** *and* **b** *(typical procedure)*

In the standard apparatus for hydrogenation under normal pressure, were placed, 0.0005 mol of the compound **16a** or **16b** dissolved in 10 mL of methanol, and 5 μl of the 99% $MeSO_3H$ were added, followed by 50 mg of the 10% Pd on charcoal, and the hydrogenation proceeded over 2 h. The catalyst was removed by filtration, washed with methanol, the methanolic solution was partly evaporated in vacuo (to ca. 4–5 mL) and the product was precipitated with an excess of diisopropyl ether, filtered and purified via semi-preparative HPLC.

13a: m.p. amorphous. Yield: 121 mg (after HPLC purification). Elemental anal. for $C_{25}H_{39}N_5O_{11}S$ (CHNS). ^{1}H-NMR (DMSO): 1.81 m (2H, CH_2 Arg), 2.87 s (9H, BOC), 3.25 t (2H, CH_2 Arg), 3.37 m (2H, CH_2N Arg), 3.59 bs (3H), 3.76 m (5H), 4.78 m (1H, CH-N), 4.87 s (2H, OCH_2), 6.71 d (2H, *J* = 7.46 Hz), 7.82 d (2H, *J* = 7.49 Hz). ^{13}C-NMR (DMSO): 25.7, 26.12 (BOC), 35.7, 39.4, 42.1, 43.4, 46.4, 47.1, 48.1 (MsOH), 50.4, 62.6, 81.1, 115.3, 118.4, 133.7, 142.5, 160.3, 168.4, 170.1, 176.9.

13b: amorphous. Yield: 134 mg (after HPLC purification). Elemental anal. for $C_{27}H_{43}N_5O_{11}S$ (C, H, N, S). ^{1}H-NMR (DMSO): 1.18 m (3H), 1.51 s (9H), 2.36 t (2H, *J* = 6.1 Hz), 2.71 t (2H, *J* = 6.0 Hz), 3.33 m (2H), 3.781–3.75 m (6H), 3.91 m (2H), 4.2 m (1H), 4.81 s (2H), 7.11 d (2H, *J* = 7.11 Hz), 7.26 d (2H, *J* = 7.13 Hz), 10.11 bs (3H). ^{13}C-NMR (DMSO): 24.9, 29.7 (BOC), 31.3, 33.9, 35.6, 42.1, 44.1, 44.9, 50.1, 56.4, 60.3 (OCH_2), 81.3 (BOC), 118.3, 121.6, 127.3, 136.6, 151.1, 155.6, 161.4, 168.6, 171.2.

4.18. Preparation of compounds **16a** *and* **b** *(typical procedure)*

The BOC protected compound **16a** or **16b** (0.005 mol) was dissolved in 10 mL of ethyl acetate and cooled to 0 °C. Then, 5 mL of 5 M HCl in EtOAc was added dropwise over 5 min. The precipitate appeared immediately. The mixture was standing for another 30 min, the precipitate was filtered, dried in vacuo and dissolved in 10 mL of ethanol. Cyanamide (0.27 g, 0.0065 mol) and conc. HCl (2 mL) were added and the mixture was stirred overnight. The product was precipitated after the reaction

mixture was kept at –20 °C for 24 h. The precipitate was filtered, washed with isopropanol, dried and purified by semi-preparative HPLC.

16a: amorphous. Yield: 127 mg (after HPLC purification). Elemental anal. for $C_{20}H_{26}N_4O_6Cl$ (C, H, N, Cl, % Cl calc. 7.83, found 8.11). ^{1}H-NMR (DMSO): 1.92 m (2H), 2.15 m (2H), 2.93 m (2H), 3.28–3.61 m (9H), 5.44 m (1H), 5.05 s (2H, CH_2O), 7.39 d (2H, J = 7.75 Hz), 8.09 d (2H, J = 7.73 Hz), 9.10–9.19 bs (4H). ^{13}C-NMR (DMSO): 22.7, 40.7, 41.6, 44.3, 44.4, 56.6, 61.1, 61.4, 65.9, 110.5, 115.3, 120.1, 126.2, 131.8, 157.3, 160.1, 160.4, 162.12, 165.2.

16b: amorphous. Yield: 111 mg (after HPLC purification). Elemental anal. for $C_{20}H_{32}N_5O_5Cl_2$ (C, H, N, Cl, % Cl calc. 14.38, found 14.79). ^{1}H-NMR (DMSO): 2.61 m (2H), 2.81 m (2H), 2.81–3.51 m (10H), 3.51s (3H), 4.51 m (1H), 5.11 s (2H, OCH_2), 7.53 d (2H, J = 7.63 Hz), 8.04 d (2H, J = 7.38 Hz). ^{13}C-NMR (DMSO): 16.19, 24.3, 37.7 (MsOH), 38.6, 40.7, 41.9, 46.5, 46.6, 46.9, 57.0, 61.9, 122.2, 127.1, 130.9, 131.6, 154.2, 164.1, 164.7, 171.6.

4.19. Inhibiting activity assays

Inhibiting activities were measured according to the described procedure [3] using the following substrates: Bz-Arg-pNa for trypsin, Bz-Phe-Val-Arg-pNa for thrombin, D-Val-Leu-Lys-pNa for plasmin and Z-Val-Gly-Arg-pNa for urokinase.

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