Structure–Activity Relationships of New *N*-Acylanthranilic Acid Derivatives as Plasminogen Activator Inhibitor-1 Inhibitors

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Novel anthranilic acid derivatives having substituted *N*-acyl side chains were designed and synthesized for evaluation as plasminogen activator inhibitor-1 (PAI-1) inhibitors. Compounds with a 4-diphenylmethyl-1-piperazinyl moiety on the acyl side chains in general exhibited potent *in vitro* PAI-1 inhibitory activity and good pharmacokinetic profiles after oral administration in rats. Compound 16f (TM5275) was identified as a promising candidate for further pharmacological evaluation.

Key words plasminogen activator inhibitor-1; inhibitor; *N*-acylanthranilic acid derivative; structure–activity relationship; TM5275

Elevated levels of plasminogen activator inhibitor-1 (PAI-1), a serine protease which inhibits tissue-type and urokinase-type plasminogen activators (tPA and uPA, respectively), is thought to be a cause of a variety of diseases,[1] such as thrombotic diseases (*e.g.*, brain infarction, deep vein thrombosis, *etc.*), tissue fibrotic diseases (*e.g.*, lung thrombosis, renal thrombosis, liver thrombosis *etc.*), arteriosclerosis, obesity and cancer, due to insufficient activation of a proteolytic enzyme plasmin. Furthermore, recent reports suggest that PAI-1 inhibitors may be useful to treat Alzheimer's disease,[2] glaucoma,[3] and retinopathy.[4] Thus, a number of synthetic small molecule PAI-1 inhibitors, *e.g.*, PAI-039 (tiplaxtinin),[5] PAI-749 (diaplasinin),[6] PAZ-417[2] have been reported to date targeted towards the development of new drugs to modify above-mentioned diseases, although the clinical benefits of these inhibitors are still not known.

We have also previously reported novel PAI-1 inhibitors TM5001,[7] TM5007[7] and their related compounds such as **1** and **2**[8] (Fig. 1) with an acylaminothiophene-carboxylic acid dimer structure to which lipophilic or/and bulky groups are attached as the aromatic ring substituents. Although these compounds had potent *in vitro* PAI-1 inhibitory activity, they exhibited rather undesirable pharmacokinetic (PK) property (*e.g.*, slow and insufficient absorption, too long duration of the plasma level) when administered orally; plasma C_{max}, T_{max} and $T_{1/2}$ were 32 μM, 18 h, and 54 h for TM5001,[7] 5.8 μM, 18 h, and >174 h for **1**, 2.32 μM, 48 h, and >48 h for **2**, respectively, in rats given 50 mg/kg. We therefore continued our search to identify compounds with both potent PAI-1 inhibitory activity and improved oral PK profiles by designing more drug-like structures than the previously reported thiophene dimers (TM5001, **1**, **2**). Our major efforts were directed toward the syntheses of novel compounds without the dimer structure, possessing as low molecular weight as possible, and with lower ClogP[9] compared to those of TM5001 (5.79), **1** (5.32) and **2** (7.06).

One of the most important findings in the thiophene dimer series which we have reported was that the dicarboxyl compound **1** and the corresponding mono-ester compound **2** were almost equipotent in *in vitro* PAI-1 inhibition, suggesting that the symmetrical dimer structure was not always necessary and one carboxyl moiety was sufficient to elicit PAI-1 inhibitory property.[8] Based upon these findings, we investigated further structure-optimization as briefly illustrated in Fig. 2. This paper describes structure-activity relationship (SAR) studies to identify a novel PAI-1 inhibitor, TM5275[10] (Fig. 3) as an orally bioavailable, promising drug candidate.

Chemistry General synthetic routes to various novel target compounds are outlined in Charts 1 and 2.

N-Acylanthranilic acid derivatives (**8a**, **a′**, **13**) were first prepared as shown in Chart 1 in order to demonstrate if one or both of the thiophene rings of the lead compounds, TM5001, **1** and **2** could be converted to the possibly bioisosteric benzene ring(s). *tert*-Butyl 2-amino-4-phenylthiophene-

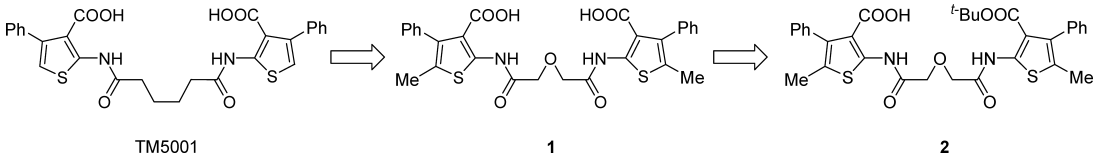

Fig. 1. Modifications of *N*-Acylaminothiophene Dimers

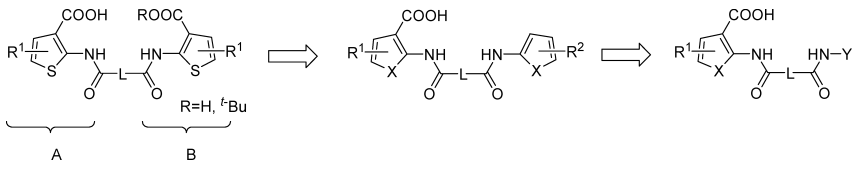

Fig. 2. Schematic Design for Lead Optimization

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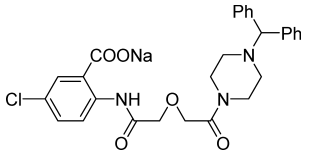
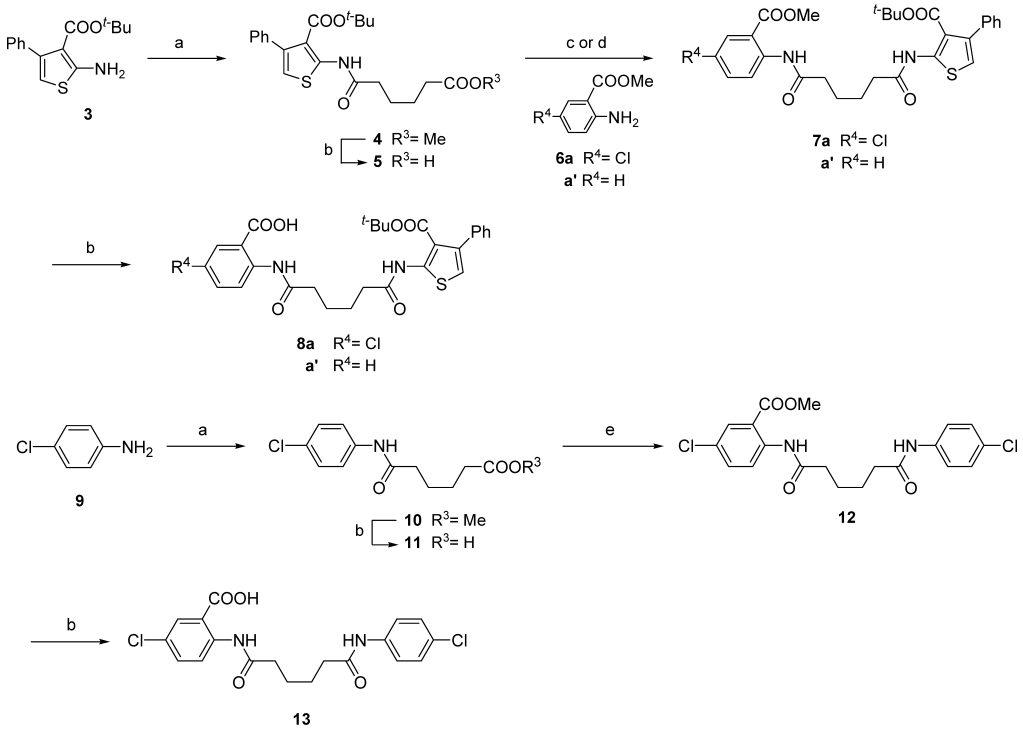


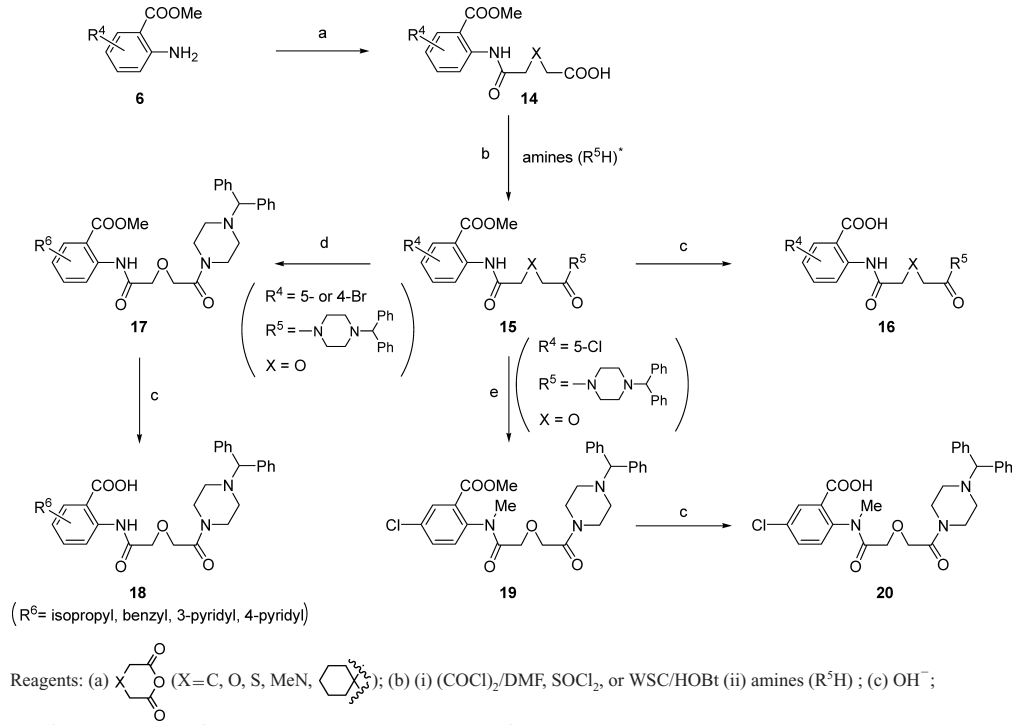
Fig. 3. Structure of TM5275 (**16f**)

3-carboxylate (**3**) and *p*-chloroaniline (**9**) were acylated with methyl adipoyl chloride, followed by alkaline hydrolysis to obtain the corresponding adipic acid mono-amide derivatives **5** and **11**, respectively. Compounds **5** and **11** were condensed with the methyl anthranilates (**6a**, **a′**) to afford **7a**, **a′** and **12**, which were then hydrolyzed to furnish *N*-acylanthranilic acid



Reagents: (a) $MeOCO(CH_2)_4COCl$, (b) OH^-; (c) (i) *i*-BuOCOCl, Et_3N, (ii) **6a**; (d) (i) MsCl, Et_3N, (ii) **6a′**; (e) (i) $SOCl_2$, (ii) **6a**.

Chart 1. Synthesis of *N*-Acylanthranilic Acid Derivatives (**8a**, **8a′**, **13**)



Reagents: (a) (X=C, O, S, MeN,); (b) (i) $(COCl)_2$/DMF, $SOCl_2$, or WSC/HOBt (ii) amines (R^5H) ; (c) OH^-;

(d) R^6-boronic acids or R^6-boronic acid esters/base/$Pd(PPh_3)_4$ or R^6ZnBr; (e) NaH, MeI.

* For substituents R^5 see Table 2.

Chart 2. Synthesis of *N*-Acylanthranilic Acid Derivatives (**16**, **18**, **20**)

derivatives, with additional thiophenamide (**8a**, **a′**) and anilide (**13**) structures incorporated in the other side of the molecule, respectively.

Chart 2 illustrates the syntheses of a variety of *N*-acylanthranilic acid derivatives (**16**, **18**, **20**) with hydrophobic carbamoyl moieties as well as various $–CH_2–X–CH_2–$ groups (X=CH_2, O, S, MeN, 1,1-cyclohexylene) as the linker L (Fig. 2). The ester-carboxylic acids (**14**) were prepared from **6** by reaction with the corresponding acid anhydrides. Then compounds **14** were condensed with hydrophobic amines (R^5H) *via* the acid chlorides or the activated esters to give the corresponding amides (**15**).

Compounds **15** where R^4=Br were successfully converted to the alkyl-, aralkyl- or aryl-substituted anthranilic acid ester derivatives (**17**) under Suzuki–Miyaura or Negishi cross-coupling reaction conditions.[11,12] The target *N*-acylanthranilic acid derivatives (**16a**—**q**, **18a**—**d**, Table 2) were obtained from these esters (**15**, **17**) by alkaline hydrolysis in good yields.

The *N*-methyl derivative of **16f** was also prepared to investigate the effect of the *N*-hydrogen atom at this position on the PAI-1 inhibitory activity. Methylation of **15f** with methyl iodide in the presence of sodium hydride followed by hydrolysis furnished **20**.

Results and Discussion

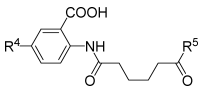
PAI-1 inhibitory activities of newly synthesized compounds are shown in Tables 1 and 2 together with those of previously reported compounds (TM5001, **1**, **2**). The *in vitro* PAI-1 activity is expressed as the remaining activity in percent (%) after incubation of PAI-1 with test compounds as described in the experimental section. Method A and Method B differ with the relative molar ratio of PAI-1 and tPA added to the medium (Method A; tPA : PAI-1 = 1 : 6.9, Method B; tPA : PAI-1 = 1 : 2.5). Method B provides a more sensitive assay than Method A to evaluate the PAI-1 activity at lower concentrations.

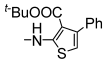
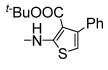
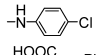
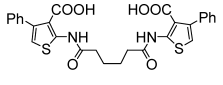
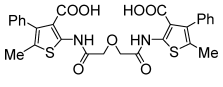
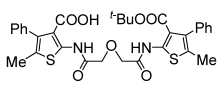
The biological property of compounds with a benzoic acid moiety in place of a thiophene-carboxylic acid structure in the compound **2** was first evaluated utilizing compounds **8a**, **a′** (Table 1). Although compound **8a′** without any substituent on the benzene ring showed considerably decreased PAI-1 inhibitory activity, **8a** with a chlorobenzoic acid structure showed activity comparable to TM5001. The results obtained here suggest that a phenyl-substituted thiophene-carboxylic acid moiety as in **2** can be replaced by a chlorobenzoic acid moiety without significant loss of activity. In addition, it was to be noted that compound **13** possessing anthranilic acid structure with an anilide moiety retained PAI-1 inhibitory property comparable to **8a′**, despite the facts that no thiophene ring was incorporated in the molecule of **13**, and there was a considerable difference between the ClogP values of the two compounds **8a′** and **13**. While appropriate lipophilic or/and bulky substituents may be necessary for these compounds to elicit potent *in vitro* effects, as reported previously,[8] ClogP value of the whole molecule does not seem to affect much the activity. However, it was considered worthwhile to decrease ClogP to improve oral PK profile of this series.

Based upon these observations and concepts, our efforts were then focused, as shown in Table 2, on the syntheses of compounds possessing a substituted-anthranilic acid structure as A part illustrated in Fig. 2, with a variety of hydrophobic amide moieties R^5 on the other end (B part) of the molecule and with different linkers L, taking ClogP of the molecule in consideration as well.

Next we focused on the structure–activity relationships (SAR) of the B part (Fig. 2, Table 2). While substituents such

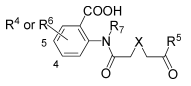
Table 1. PAI-1 Inhibitory Activity of Anthranilic Acid Derivatives

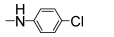
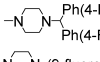
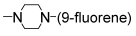


Compounds	R^4	R^5	ClogP[a]	PAI-1 activity (%) (method A)[b]		Rat PK (50 mg/kg, *p.o.*)		
				100 μM	50 μM	C_{max} (μM)	T_{max} (h)	$T_{1/2}$ (h)
8a[c]	Cl		7.48[d]	12.5±7.6	42.5±5.4	ND	ND	ND
8a′[c]	H		6.65[d]	12.1±5.3	97.8±1.2	29.8±9.7	2	9
13	Cl		5.16	37.7±11.3	99.9±0.1	ND	ND	ND
TM5001			5.79	15.9±8.3	51.4±5.9	31.9±9.8	18	54
1			5.32	7.9±3.9[e]	15.5±3.6	5.8±1.2	18	>174
2[f]			7.06	ND	12.7±4.0	2.32[g]	48	>48

Data are expressed as mean±S.D., ND: no data, *a*) calculated by ChemDraw 10.0, *b*) see Experimental, *c*) evaluated as sodium salt, *d*) calculated for the free carboxilic acid, *e*) tPA is slightly inhibited in the test, *f*) tested by method B, *g*) data of *N*=1.

Table 2. PAI-1 Inhibitory Activity and Rat PK of Anthranilic Acid Derivatives



Compounds	R^4 or R^6 (position)	R^5	R^7	X	ClogP[a]	PAI-1 activity (%) (method B)[b]		Rat PK (50 mg/kg, *p.o.*)		
						50 μM	20 μM	C_{max} (μM)	T_{max} (h)	$T_{1/2}$ (h)
16a[c]	5-Cl		H	O	4.29	53.7±7.7	100.0±0.5	ND	ND	ND
16b[d]	5-Cl		H	O	4.50	77.8±7.8	99.8±0.3	ND	ND	ND
16c	5-Cl		H	O	4.69	47.5±7.1	98.4±2.0	89.2±17.0	1.0	1.0
16d	5-Cl		H	O	4.22	89.1±8.7	98.8±1.0	ND	ND	ND
16e[d]	5-Cl		H	O	5.77	7.6±2.8	81.0±13.7	11.6[e]	2.0	2.6
16f[d,f]	5-Cl		H	O	3.37	6.0±1.4	79.0±8.4	34.2±2.6	2.0	2.5
16g	5-Cl		H	O	3.66	15.5±0.4	98.8±1.8	ND	ND	ND
16h	5-Cl		H	O	4.27	26.8±7.1	86.1±5.4	ND	ND	ND
16i	5-F		H	O	2.80	50.4±2.3	92.3±5.7	ND	ND	ND
16j[c,d]	5-Br		H	O	3.52	12.2±6.2	88.9±0.7	22.5±4.0	2.0	7.0
16k[d]	4-Br		H	O	3.52	10.2±3.5	79.8±11.7	ND	ND	ND
16l[c]	5-Ph		H	O	4.43	16.8±2.7	83.8±5.2	4.5±0.4	1.0	1.5
16m[d]	4-Cl		H	O	3.37	13.3±2.5	91.3±9.7	8.3±2.0	1.0	0.8
16n	5-Cl		H	CH_2	4.07	16.4±4.5	85.1±8.7	69.7±23.1	2.0	2.4
16o[g]	5-Cl		H	S	3.75	15.9±3.8	71.2±13.0	97.4±14.5	1.0	2.3
16p[c]	5-Cl		H	MeN	3.93	29.2±3.8	92.8±7.0	49.4±15.8	2.0	1.5
16q[g]	5-Cl		H		6.18	6.5±4.6	35.1±4.2	1.8±0.4	1.0	1.6
18a[d]	5-*i*-Pr		H	O	3.97	9.4±1.7	91.6±10.6	15.4±4.4	2.0	1.6
18b[d]	5-Bn		H	O	4.61	9.5±3.2	98.9±1.4	2.5±0.8	1.0	0.5
18c	5- 		H	O	3.06	11.8±1.6	86.1±0.2	2.9±0.2	1.0	1.3
18d	4- 		H	O	3.06	9.6±4.0	48.6±14.2	0.2±0.1	1.0	0.9
20	5-Cl		Me	O	4.08		27.3±0.5[h]	ND	ND	ND

Data are expressed as mean±S.D., ND: no data, *a*) ClogP was calculated for free carboxylic acid/amine by ChemDraw 10.0, *b*) see Experimental, *c*) tested by method A, *d*) evaluated as sodium salt, *e*) data of $N=1$, *f*) PAI-1 activities (%) of **16f** tested by method A are 23.3±3.2 and 89.6±6.5 at 50 μM and 20 μM, respectively, *g*) evaluated as hydrochloride salt, *h*) PAI-1 activity (%) of 150 μM is shown.

as diphenylamino (**16b**), 1,1-diphenylmethylamino (**16c**), and 4-phenyl-1-piperidinyl group (**16d**) were not satisfactory to potentiate the activity, compounds with 4,4-diphenyl-1-piperidinyl (**16e**) and 4-(diphenylmethyl)-1-piperazinyl groups (**16f**) showed activities superior to **16a**. Although there was little difference between **16e** and **16f** in *in vitro* activity, we decided to concentrate in **16f** type of compounds, because **16f** showed a smaller ClogP and a better PK profile than **16e**, and also diphenylmethylpiperazinyl derivatives could be prepared relatively easily compared to diphenylpiperidinyl ones. Introduction of *N*-bis(4-fluorophenyl)-methyl (**16g**) and *N*-9*H*-fluorenyl (**16h**) substituents in piperazine moiety also resulted in a slight decrease in the *in vitro* activity compared to **16f**. Therefore, we fixed the B part (Fig. 2) as the 4-diphenylmethyl-1-piperazinyl group to conduct further SAR studies.

The positions of the substituents on the benzoic acid moiety (A part) were evaluated with compounds **16i**—**18d**. Although introduction of a fluorine atom to the 5-position resulted in a slight decrease in activity, compounds with bulkier or more hydrophobic substituents such as bromine atom (**16j**), isopropyl (**18a**) and benzyl (**18b**) groups tended to retain the activity. 4-Bromo, 5-(3-pyridyl) and 4-(4-pyridyl) derivatives (**16k**, **18c**, **18d**) also exhibited potent inhibitory activity. The difference in the position of these substituents (4- or 5-position) did not seem to significantly affect the potency of *in vitro* activity, suggesting that steric bulkiness adjacent to the phenyl ring appears to be more important than the position of the substitution as has been observed with the thiophene-carboxylic acid series.[8] Variation of the linker L by introducing a methylene group, sulfur and nitrogen atoms as X (Fig. 2) as in **16n**—**16q** (Table 2), indicated that the activity observed with **16f** was retained or improved.

N-Methylation of **16f**, *i.e.*, compound **20**, resulted in a

drastic loss in activity indicating that the NH group at this position seems to play a very important role in the *N*-acylanthranilic acid derivatives to elicit potent PAI-1 inhibitory activity (Table 2).

In order to identify a good orally-bioavailable compound for further *in vivo* studies, pharmacokinetic (PK) profiles of the representative compounds were compared in rats by oral administration (Table 2; see Experimental in detail). Although no direct correlation was seen between ClogP and PK, compounds showing good PK had ClogP$<$5.0 as shown in Table 2. Compounds **18c** and **18d** with a pyridyl group at the 4- or 5-position had a low ClogP (3.06) but exhibited only poor PK profiles, while the reason is not known at present. From these results compound **16f** (TM5275)[10] possessing both potent *in vitro* PAI-1 inhibitory activity and a good PK profile, *e.g.*, high maximum plasma concentration (C_{max}), was selected as a candidate for future development.

Conclusion

Based upon the basic structure of 2-acylamino-3-thiophenecarboxylic acids (TM5001, **1**, **2**) which we have previously reported,[7,8] novel *N*-acylanthranilic acid derivatives, in particular *N*-acyl-5-chloroanthranilic acid derivatives were designed, synthesized and evaluated for *in vitro* PAI-1 inhibitory activity and oral PK profiles in rats. It was found that both PAI-1 inhibition and oral bioavailability were much improved when a 4-diphenylmethyl-1-piperazinyl group was introduced on the acyl side chain. Thus compound **16f** (TM5275)[10] was finally identified as a promising candidate for further pharmacological evaluation.

TM5275 may provide not only a useful tool for a better understanding of the pathophysiology of PAI-1 but also a potential new treatment for diseases where PAI-1 has been implicated.

Experimental

General Melting points (mp) were determined on METTLER TOLEDO MP70 Melting Point Systems and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer, with tetramethylsilane as an internal standard. TLC analyses were carried out on MERCK Silica gel 60 F_{254} plates. Elemental analyses were carried out by Takeda Analytical Laboratories, Ltd., and are within ±0.4% of the theoretical values unless otherwise noted. Chromatographic purification was carried out on silica gel columns (MERCK Silica gel 60 0.040—0.063 mm) unless otherwise noted. The yields reported are not optimized. Analytical HPLC was conducted on a Puresil C_{18} column (5 μm, 4.4×150 mm i.d.) eluted with 0.1% (v/v) trifluoroacetic acid (TFA) in water (solvent A) and 0.08% (v/v) TFA in acetonitrile (MeCN)/water (80%/20% (v/v)) (solvent B), according to the following elution gradient: 0—20 min, 50—80% B; 20—45 min, 80% B at a flow rate of 0.8 ml/min. LC/MS spectra were recorded on an Alliance HPLC system using a ULTORON VX-ODS C_{18} column (5 μm, 4.6×150 mm i.d.) coupled with an micromass ZQ as MS detector in the mode of electrospray ionization (ESI) and a gradient of 5—100% B over 20 min was used with a flow rate of 1.0 ml/min. Formic acid 0.1% was added to solvents A and B (water (A) and MeCN (B)).

***tert*-Butyl 2-[(6-Methoxy-6-oxohexanoyl)amino]-4-phenylthiophene-3-carboxylate (4)** Methyl adipoyl chloride (15.8 g, 88.2 mmol) was added to a solution of *tert*-butyl 2-amino-4-phenylthiophene-3-carboxylate (**3**) (24.3 g, 88.2 mmol) in dimethylacetamide (DMA) (70 ml) at 0 °C, and the mixture was stirred at room temperature for 1 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the resulting precipitate was collected by suction filtration, washed with water and *n*-hexane, and dried *in vacuo* to afford 30.3 g (yield 82%) of **4** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 1.19 (9H, s), 1.64—1.92 (4H, m), 2.39 (2H, t, J=7.1 Hz), 2.55 (2H, t, J=7.1 Hz), 3.68 (3H, s), 6.55 (1H, d, J=0.7 Hz), 7.21—7.39 (5H, m), 11.3 (1H, s).

6-{[3-(*tert*-Butoxycarbonyl)-4-phenylthiophen-2-yl]amino}-6-oxohexanoic Acid (5) To a solution of **4** (29.0 g, 69.4 mmol) in tetrahydrofuran (THF) (100 ml) was added 1 N aqueous NaOH solution (104 ml) and the mixture was stirred for 2.5 h at 60 °C. After evaporation of organic solvent, the aqueous solution was washed with ethyl acetate (EtOAc). After 1 N aqueous HCl was added to the aqueous solution, the mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by recrystallization from EtOAc/*n*-hexane to afford 20.2 g (yield 72%) of **5** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 1.19 (9H, s), 1.66—1.94 (4H, m), 2.43 (2H, t, J=7.1 Hz), 2.56 (2H, t, J=7.1 Hz), 6.55 (1H, s), 7.19—7.40 (5H, m), 11.3 (1H, s).

***tert*-Butyl 2-[(6-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-6-oxohexanoyl)amino]-4-phenylthiophene-3-carboxylate (7a)** To a mixture of **5** (1.0 g, 2.47 mmol) and triethylamine (Et_3N) (326 mg, 3.22 mmol) in THF (19 ml) was added isobutyl chloroformate (406 mg, 2.97 mmol) at 0 °C and the mixture was stirred for 1 h. A solution of methyl 2-amino-5-chlorobenzoate (**6a**) (460 mg, 2.47 mmol) in THF (1 ml) was added to the reaction mixture at 0 °C. The mixture was stirred at room temperature for 16 h. Water was added to the reaction mixture and the whole mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane 1/6) to afford 650 mg (yield 46%) of **7a** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 1.19 (9H, s), 1.81—1.93 (4H, m), 2.46—2.64 (4H, m), 3.94 (3H, s), 6.54 (1H, d, J=0.9 Hz), 7.20—7.39 (5H, m), 7.48 (1H, dd, J=9.2, 2.6 Hz), 7.99 (1H, d, J=2.6 Hz), 8.72 (1H, d, J=9.2 Hz), 11.0 (1H, s), 11.3 (1H, s).

***tert*-Butyl 2-[(6-{[2-(Methoxycarbonyl)phenyl]amino}-6-oxohexanoyl)-amino]-4-phenylthiophene-3-carboxylate (7a′)** To a mixture of **5** (1.0 g, 2.47 mmol) and Et_3N (326 mg, 3.22 mmol) in THF (9 ml) was added methanesulfonyl chloride (369 mg, 322 mmol) at 0 °C and the mixture was stirred for 1 h. A solution of methyl anthranilate (**6a′**) (487 mg, 3.22 mmol) in THF (1 ml) was added to the reaction mixture at 0 °C. The mixture was stirred at room temperature for 16 h. Water was added to the reaction mixture and the whole mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (acetone/*n*-hexane 1/5) to afford 730 mg (yield 55%) of **7a′** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 1.19 (9H, s), 1.80—1.94 (4H, m), 2.43—2.66 (4H, m), 3.93 (3H, s), 6.54 (1H, s), 7.03—7.13 (1H, m), 7.20—7.40 (5H, m), 7.49—7.58 (1H, m), 8.00—8.06 (1H, m), 8.69—8.76 (1H, m), 11.1 (1H, s), 11.3 (1H, s).

Sodium 2-[(6-{[3-(*tert*-Butoxycarbonyl)-4-phenylthiophen-2-yl]amino}-6-oxohexanoyl)amino]-5-chlorobenzoate (8a) To a solution of **7a** (650 mg, 1.13 mmol) in THF (10 ml) was added 1 N aqueous NaOH solution (2.3 ml) and the mixture was stirred for 2 h at 60 °C. After cooling, the reaction mixture was evaporated *in vacuo* to remove THF and water was added. The precipitate was collected by filtration, washed with water and diisopropyl ether (IPE), and dried *in vacuo* to afford 507 mg (yield 77%) of **8a** as an amorphous solid, ^{1}H-NMR (DMSO-d_6) δ: 1.17 (9H, s), 1.60—1.75 (4H, m), 2.25—2.40 (2H, m), 2.51—2.64 (2H, m), 6.83 (1H, s), 7.20—7.44 (6H, m), 7.92 (1H, d, J=2.7 Hz), 8.49 (1H, d, J=8.8 Hz), 11.0 (1H, s), 14.3 (1H, s), HPLC (250 nm) 99.4% (t_R=42.0 min), LC/MS (ESI) m/z: 557 $(M+H)^+$, 555 $(M-H)^-$.

Sodium 2-[(6-{[3-(*tert*-Butoxycarbonyl)-4-phenylthiophen-2-yl]amino}-6-oxohexanoyl)amino]benzoate (8a′) This compound was prepared in 33% yield as an amorphous solid from **7a′** and 1 N aqueous NaOH solution following a procedure similar to that described for the preparation of **8a**, ^{1}H-NMR (DMSO-d_6) δ: 1.17 (9H, s), 1.53—1.80 (4H, m), 2.25—2.40 (2H, m), 2.55—2.67 (2H, m), 6.84 (1H, s), 6.86—6.96 (1H, m), 7.17—7.43 (6H, m), 7.94—8.00 (1H, m), 8.42—8.50 (1H, m), 11.0 (1H, s), 14.3 (1H, s), HPLC (250 nm) 98.7% (t_R=32.1 min), LC/MS (ESI) m/z: 523 $(M+H)^+$, 521 $(M-H)^-$.

Methyl 6-[(4-Chlorophenyl)amino]-6-oxohexanoate (10) Methyl adipoyl chloride (8.6 g, 48 mmol) was added to a solution of 4-chloroaniline (**9**) (6.7 g, 53 mmol) in DMA at 0 °C, and the mixture was stirred at room temperature for 17 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the resulting precipitate was collected by filtration, washed with water and IPE, and dried *in vacuo* to afford 11.0 g (yield 85%) of **10** as a solid, ^{1}H-NMR (DMSO-d_6) δ: 1.51—1.59 (4H, m), 2.28—2.37 (4H, m), 3.58 (3H, s), 7.30—7.38 (2H, m), 7.58—7.65 (2H, m), 10.0 (1H, s).

6-[(4-Chlorophenyl)amino]-6-oxohexanoic Acid (11) To a solution of **10** (12.0 g, 44 mmol) in THF (445 ml) was added 1 N aqueous NaOH solution (61.2 ml) and the mixture was stirred for 2 h at 60 °C. After cooling, THF was removed *in vacuo* and 1 N aqueous HCl solution was added to the residue. The precipitate was collected by filtration, washed with EtOAc,

water and IPE, and dried *in vacuo* to afford 10.3 g (yield 86%) of **11** as a solid, ^{1}H-NMR (DMSO-d_6) δ: 1.50—1.67 (4H, m), 2.21—2.35 (4H, m), 7.30—7.38 (2H, m), 7.59—7.66 (2H, m), 10.0 (1H, s), 12.0 (1H, bs).

Methyl 5-Chloro-2-({6-[(4-chlorophenyl)amino]-6-oxohexanoyl}amino)benzoate (12) Thionyl chloride (2.2 ml, 30 mmol) was added to **11** (770 mg, 3.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo*. A solution of **6a** (610 mg, 3.3 mmol) in DMA (10 ml) was add to the acid chloride at room temperature for 17 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the whole mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo.* IPE was added to the residue and the resulting crystal was collected by filtration, and dried *in vacuo* to afford 610 mg (yield 48%) of **12** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 1.80—1.87 (4H, m), 2.39—2.56 (4H, m), 3.93 (3H, s), 7.23—7.30 (2H, m), 7.47—7.54 (3H, m), 7.69 (1H, s), 8.00 (1H, d, J=2.7 Hz), 8.70 (1H, d, J=9.2 Hz), 11.0 (1H, s).

5-Chloro-2-({6-[(4-chlorophenyl)amino]-6-oxohexanoyl}amino)benzoic Acid (13) To a solution of **12** (570 mg, 1.3 mmol) in THF (13 ml) was added 1 N aqueous NaOH solution (2.0 ml) and the mixture was stirred for 3 h at 60 °C. After cooling, THF was removed *in vacuo* and 1 N aqueous HCl solution was added to the residue. The precipitate was collected by filtration, washed with water and IPE, and dried *in vacuo* to afford 490 mg (yield 91%) of **13** as a solid, mp 232—235 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.53—1.72 (4H, m), 2.27—2.38 (4H, m), 7.27—7.40 (2H, m), 7.53—7.67 (2H, m), 7.60 (1H, dd, J=9.0, 2.7 Hz), 7.91, (1H, d, J=2.7 Hz), 8.50 (1H, d, J=9.0 Hz), 10.1 (1H, s), 11.5 (1H, s), HPLC (250 nm) 99.4% (t_R=16.1 min), LC/MS (ESI) m/z: 409 $(M+H)^+$, 407 $(M-H)^-$.

(2-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethoxy)acetic Acid (14a) To a solution of **6a** (10 g, 53.9 mmol) in THF (135 ml) was added diglycolic anhydride (6.9 g, 59.3 mmol) and the mixture was refluxed for 7.5 h. After cooling, solvent was removed *in vacuo* and IPE was added to the residue. The resulting precipitate was collected by filtration, washed with IPE, and dried *in vacuo* to afford 15.7 g (yield 97%) of **14a** as a solid, ^{1}H-NMR (DMSO-d_6) δ: 3.90 (3H, s), 4.22 (2H, s), 4.28 (2H, s), 7.71 (1H, dd, J=9.0, 2.7 Hz), 7.93 (1H, d, J=2.7 Hz), 8.62 (1H, d, J=8.8 Hz), 11.4 (1H, s) 12.9 (1H, br).

(2-{[4-Fluoro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethoxy)acetic Acid (14b) This compound was prepared in 97% yield as a solid from methyl 2-amino-5-fluorobenzoate (**6b**) and diglycolic anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s), 4.20 (2H, s), 4.27 (2H, s), 7.55 (1H, dddd, J=14.6, 5.1, 3.1, 1.4 Hz), 7.73 (1H, dd, J=9.3, 3.1 Hz), 8.60 (1H, dddd, J=14.6, 9.3, 5.1, 3.1 Hz), 11.3 (1H, s).

(2-{[4-Bromo-2-(methoxycarbonyl)phenyl]amino}-2-oxoethoxy)acetic Acid (14c) This compound was prepared in 92% yield as a solid from methyl 2-amino-5-bromobenzoate (**6c**) and diglycolic anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s), 4.20 (2H, s), 4.26 (2H, s), 7.85 (1H, dd, J=9.0, 3.0 Hz), 8.08 (1H, d, J=3.0 Hz), 8.57 (1H, d, J=9.0 Hz), 11.4 (1H, s), 12.9 (1H, br).

(2-{[3-(Methoxycarbonyl)biphenyl-4-yl]amino}-2-oxoethoxy)acetic Acid (14d) This compound was prepared in 89% yield as a solid from methyl 4-aminobiphenyl-3-carboxylate (**6d**)[13] and diglycolic anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.93 (3H, s), 4.23 (2H, s), 4.29 (2H, s), 7.35—7.71 (5H, m), 7.98 (1H, dd, J=8.8, 2.4 Hz), 8.24 (1H, d, J=2.4 Hz), 8.71 (1H, d, J=8.8 Hz), 11.5 (1H, s), 12.9 (1H, br).

(2-{[5-Bromo-2-(methoxycarbonyl)phenyl]amino}-2-oxoethoxy)acetic Acid (14e) This compound was prepared in 89% yield as a solid from methyl 2-amino-4-bromobenzoate (**6e**) and diglycolic anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s), 4.22 (2H, s), 4.28 (2H, s), 7.42 (1H, dd, J=8.6, 2.2 Hz), 7.92 (1H, d, J=8.6 Hz), 8.86 (1H, d, J=2.2 Hz), 11.5 (1H, s), 12.9 (1H, br).

(2-{[5-Chloro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethoxy)acetic Acid (14f) This compound was prepared in 94% yield as a solid from methyl 2-amino-4-chlorobenzoate (**6f**) and diglycolic anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.89 (3H, s), 4.23 (2H, s), 4.28 (2H, s), 7.29 (1H, dd, J=8.6, 2.0 Hz), 8.00 (1H, d, J=8.6 Hz), 8.71 (1H, d, J=2.0 Hz), 11.5 (1H, s), 12.9 (1H, br).

5-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-5-oxopentanoic Acid (14g) This compound was prepared in 61% yield as a solid from **6a** and glutaric anhydride following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 1.82 (2H, q, J=7.3 Hz), 2.30 (2H, t, J=7.3 Hz), 2.42 (2H, t, J=7.3 Hz), 3.85 (3H, s), 7.66 (1H, dd, J=9.0, 2.6 Hz), 7.83 (1H, d, J=2.6 Hz), 8.18 (1H, d, J=9.0 Hz), 10.5 (1H, s), 12.1 (1H, br).

[(2-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethyl)sulfanyl]acetic Acid (14h) This compound was prepared in 84% yield as a solid from **6a** and 1,4-oxathiane-2,6-dione following a procedure similar to that described for the preparation of **14a**, ^{1}H-NMR (DMSO-d_6) δ: 3.38 (2H, s), 3.56 (2H, s), 3.88 (3H, s), 7.68 (1H, dd, J=9.0, 2.7 Hz), 7.87 (1H, d, J=2.7 Hz), 8.33 (1H, d, J=9.0 Hz), 11.1 (1H, s), 12.7 (1H, br).

[(2-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethyl)-(methyl)amino]acetic Acid (14i) A mixture of *N*-methyliminodiacetic acid (3.2 g, 21.5 mmol) and acetic anhydride (16 ml) was refluxed for 0.5 h. After evaporation of acetic anhydride, a solution of **6a** (4.0 g, 21.6 mmol) in THF (20 ml) was added to this residue and the mixture was refluxed for 1.5 h. After evaporation of organic solvent, the residue was purified by column chromatography on silica gel ($CHCl_3$/methanol (MeOH)=5/1) to afford 5.0 g (yield 73%) of **14i** as a solid, ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s), 3.38 (2H, s), 3.46 (2H, s), 3.89 (3H, s), 7.68 (1H, dd, J=9.0, 2.6 Hz), 7.91 (1H, d, J=2.4 Hz), 8.64 (1H, d, J=9.0 Hz), 11.6 (1H, s).

[1-(2-{[4-Chloro-2-(methoxycarbonyl)phenyl]amino}-2-oxoethyl)cyclohexyl]acetic Acid (14j) To a solution of **6a** (9.3 g, 49.9 mmol) in THF (60 ml) was added 3-oxaspiro[5.5]undecane-2,4-dione (10.0 g, 49.9 mmol) and the mixture was refluxed for 35 h. After cooling, solvent was removed *in vacuo* to give a crude product which was recrystallized from EtOAc to afford 10.9 g (yield 59%) of **14j** as a solid, ^{1}H-NMR (DMSO-d_6) δ: 1.30—1.40 (10H, m), 2.43 (2H, s), 2.56 (2H, s), 3.85 (3H, s), 7.65 (1H, dd, J=9.0, 2.6 Hz), 7.83 (1H, d, J=2.6 Hz), 8.23 (1H, d, J=9.0 Hz), 10.5 (1H, s), 12.1 (1H, br).

Methyl 5-Chloro-2-[({2-[(4-chlorophenyl)amino]-2-oxoethoxy}acetyl)-amino]benzoate (15a) To a mixture of **14a** (500 mg 1.66 mmol) and *N*,*N*-dimethylformamide (DMF) (cat. amount) in THF (5 ml) was added oxalyl dichloride (414 mg,1.99 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. After evaporation of organic solvent, a solution of 4-chloroaniline (211 mg, 1.66 mmol) in DMA (5 ml) was added to the residue at 0 °C, and the mixture was stirred at room temperature for 1 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the resulting precipitate was collected by suction filtration and washed with water to afford 345 mg (yield 51%) of **15a** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 3.85 (3H, s), 4.26 (2H, s), 4.27 (2H, s), 7.26—7.35 (2H, m), 7.55 (1H, dd, J=9.2, 2.6 Hz), 7.65—7.75 (2H, m), 8.05 (1H, d, J=2.6 Hz), 8.78 (1H, d, J=9.2 Hz), 8.83 (1H, s), 11.9 (1H, s).

Methyl 5-Chloro-2-({[2-(diphenylamino)-2-oxoethoxy]acetyl}amino)-benzoate (15b) To a mixture of **14a** (1.0 g, 3.31 mmol) and DMF (cat. amount) in THF (10 ml) was added oxalyl dichloride (505 mg, 3.98 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. After evaporation of organic solvent, a solution of diphenylamine (617 mg, 3.65 mmol) in DMA (10 ml) was added to the residue at 0 °C, and the mixture was stirred at room temperature for 1.5 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane=1/1) to afford 717 mg (yield 48%) of **15b** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 3.92 (3H, s), 4.22 (2H, s), 4.25 (2H, s), 7.24—7.48 (10H, m), 7.48 (1H, dd, J=9.0, 2.6 Hz), 8.00 (1H, d, J=2.6 Hz), 8.72 (1H, d, J=9.0 Hz), 11.6 (1H, s).

Methyl 5-Chloro-2-[({2-[(diphenylmethyl)amino]-2-oxoethoxy}acetyl)-amino]benzoate (15c) To a mixture of **14a** (0.81 g, 2.68 mmol) and DMF (cat. amount) in THF (15 ml) was added oxalyl dichloride (411 mg, 3.24 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. After evaporation of organic solvent, a solution of benzhydrylamine (594 mg, 3.24 mmol) in DMA (15 ml) was added to the residue at 0 °C, and the mixture was stirred at room temperature for 14 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. IPE was added to the residue and the resulting crystal was collected by filtration, and dried *in vacuo* to afford 610 mg (yield 49%) of **15c** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 3.32 (3H, s), 4.21 (2H, s), 4.25 (2H, s), 6.48 (1H, d, J=9.2 Hz), 7.25—7.36 (10H, m), 7.52 (1H, dd, J=9.0, 2.6 Hz), 7.90 (1H, d, J=9.2 Hz), 7.98 (1H, d, J=2.6 Hz), 8.76 (1H, d, J=9.0 Hz), 11.9 (1H, s).

Methyl 5-Chloro-2-({[2-oxo-2-(4-phenylpiperidin-1-yl)ethoxy]acetyl}-amino)benzoate (15d) This compound was prepared in 85% yield as a solid from **14a**, oxalyl dichloride, DMF and 4-phenylpiperidine following a

procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.80 (2H, m), 1.80—2.00 (2H, m), 2.60—2.90 (2H, m), 3.10—3.30 (1H, m), 3.85—3.05 (1H, m), 3.92 (3H, s), 4.28 (2H, s), 4.44 (2H, d, J=2.0 Hz), 4.70—4.85 (1H, m), 7.15—7.40 (5H, m), 7.45 (1H, dd, J=9.1, 2.7 Hz), 8.02 (1H, d, J=2.7 Hz), 8.77 (1H, d, J=9.1 Hz), 11.7 (1H, br).

Methyl 5-Chloro-2-({[2-(4,4-diphenylpiperidin-1-yl)-2-oxoethoxy]-acetyl}amino)benzoate (15e) To a mixture of **14a** (1.0 g, 3.31 mmol) and DMF (cat. amount) in THF (20 ml) was added oxalyl dichloride (505 mg, 3.98 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. After evaporation of organic solvent, a mixture of 4,4-diphenylpiperidine hydrochloride (998 mg, 3.65 mg) and Et_3N (369 mg, 3.65 mmol) in DMA (20 ml) was added to the residue at 0 °C, and the mixture was stirred at room temperature for 1.5 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane=1/1) to afford 850 mg (yield 49%) of **15e** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.40—2.52 (4H, m), 3.46—3.77 (4H, m), 3.81 (3H, s), 4.23 (2H, s), 4.38 (2H, s), 7.10—7.37 (10H, m), 7.49 (1H, dd, J=9.2, 2.6 Hz), 8.00 (1H, d, J=2.6 Hz), 8.75 (1H, d, J=9.2 Hz), 11.7 (1H, s).

Methyl 5-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (15f) This compound was prepared in 81% yield as a solid from **14a**, oxalyl dichloride, DMF and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 2.40—2.50 (4H, m), 3.50—3.66 (4H, m), 3.81 (3H, s), 4.21 (2H, s), 4.21 (1H, s), 4.34 (2H, s), 7.14—7.41 (10H, m), 7.49 (1H, dd, J=9.0, 2.7 Hz), 7.99 (1H, d, J=2.7 Hz), 8.75 (1H, d, J=9.0 Hz), 11.7 (1H, s).

Methyl 2-{[(2-{4-[Bis(4-fluorophenyl)methyl]piperazin-1-yl}-2-oxo-ethoxy)acetyl]amino}-5-chlorobenzoate (15g) This compound was prepared in 97% yield as a solid from **14a**, oxalyl dichloride, DMF and 1-[bis(4-fluorophenyl)methyl]piperazine following a procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 2.35—2.40 (4H, m), 3.50—3.66 (4H, m), 3.83 (3H, s), 4.22 (2H, s), 4.22 (1H, s), 4.35 (2H, s), 6.93—7.02 (4H, m), 7.26—7.36 (4H, m), 7.51 (1H, dd, J=9.1, 2.6 Hz), 8.00 (1H, d, J=2.6 Hz), 8.75 (1H, d, J=9.1 Hz), 11.7 (1H, s).

Methyl 5-Chloro-2-[({2-[4-(9*H*-fluoren-9-yl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (15h) This compound was prepared in 95% yield as a solid from **14a**, oxalyl dichloride, DMF and 1-[bis(4-fluorophenyl)methyl]piperazine following a procedure similar to that described for the preparation of **15a**, ^{1}H-NMR ($CDCl_3$) δ: 2.45—2.47 (4H, m), 2.75—2.80 (2H, m), 3.41—3.43 (2H, m), 3.62—3.67 (2H, m), 3.80 (3H, s), 4.19 (2H, s), 4.31 (2H, s), 4.85 (1H, s), 7.21—7.68 (9H, m), 7.98 (1H, d, J=2.6 Hz), 8.73 (1H, d, J=9.1 Hz), 11.6 (1H, s).

Methyl 2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)-amino]-5-fluorobenzoate (15i) This compound was prepared in 90% yield as a solid from **14b**, oxalyl dichloride, DMF and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 2.28—2.42 (4H, m), 3.51—3.66 (4H, m), 3.79 (3H, s), 4.22 (2H, s), 4.22 (1H, s), 4.35 (2H, s), 7.15—7.42 (11H, m), 7.70 (1H, dd, J=9.2, 3.3 Hz), 8.76 (1H, dd, J=9.2, 5.2 Hz), 11.6 (1H, s).

Methyl 5-Bromo-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (15j) This compound was prepared in 91% yield as a solid from **14c**, oxalyl dichloride, DMF and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 2.31—2.48 (4H, m), 3.50—3.66 (4H, m), 3.81 (3H, s), 4.21 (2H, s), 4.22 (1H, s), 4.34 (2H, s), 7.14—7.41 (10H, m), 7.64 (1H, dd, J=9.2, 2.6 Hz), 8.15 (1H, d, J=2.6 Hz), 8.68 (1H, d, J=9.2 Hz), 11.6 (1H, s).

Methyl 4-Bromo-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (15k) To a mixture of **14e** (4.0 g, 11.6 mmol) in DMA (28 ml) were added 1-benzhydrylpiperazine (2.93 g, 11.6 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) (2.67 g, 13.9 mmol) and 1-hydroxy-1*H*-benzotriazole (HOBt) (1.88 g, 13.9 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the resulting precipitate was collected by filtration and washed with water to afford 'quantitative' yield of **15k** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.37—2.45 (4H, m), 3.50—3.68 (4H, m), 3.80 (3H, s), 4.21 (2H, s), 4.34 (1H, s), 4.34 (2H, s), 7.14—7.41 (11H, m), 7.87 (1H, d, J=8.6 Hz), 9.02 (1H, d, J=2.0 Hz), 11.7 (1H, s).

Methyl 4-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)-amino]biphenyl-3-carboxylate (15l) This compound was prepared in 61% yield as a solid from **14d**, oxalyl dichloride, DMF and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15b**, ^{1}H-NMR ($CDCl_3$) δ: 2.38—2.44 (4H, m), 3.54—3.64 (4H, m), 3.83 (3H, s), 4.22 (1H, s), 4.24 (2H, s), 4.37 (2H, s), 7.14—7.62 (15H, m), 7.79 (1H, dd, J=8.7, 2.2 Hz), 8.27 (1H, d, J=2.2 Hz), 8.83 (1H, d, J=8.7 Hz), 11.7 (1H, s).

Methyl 4-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (15m) This compound was prepared in 96% yield as a solid from **14f**, WSC, HOBt and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15k**, ^{1}H-NMR ($CDCl_3$) δ: 2.39—2.48 (4H, m), 3.52—3.65 (4H, m), 3.80 (3H, s), 4.22 (2H, s), 4.22 (1H, s), 4.34 (2H, s), 7.08 (1H, dd, J=8.6, 2.0 Hz), 7.14—7.42 (10H, m), 7.95 (1H, d, J=8.6 Hz), 8.86 (1H, d, J=2.0 Hz), 11.8 (1H, s).

Methyl 5-Chloro-2-({5-[4-(diphenylmethyl)piperazin-1-yl]-5-oxopentanoyl}amino)benzoate (15n) This compound was prepared in 88% yield as a solid from **14g**, WSC, HOBt and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15k**, ^{1}H-NMR ($CDCl_3$) δ: 2.06 (2H, q, J=7.1 Hz), 2.34—2.56 (8H, m), 3.45—3.64 (4H, m), 3.92 (3H, s), 4.21 (1H, s), 7.14—7.43 (10H, m), 7.47 (1H, dd, J=9.0, 2.6 Hz), 7.99 (1H, d, J=2.6 Hz), 8.67 (1H, d, J=9.0 Hz), 11.0 (1H, s).

Methyl 5-Chloro-2-{[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethyl}sulfanyl)acetyl]amino}benzoate (15o) To a mixture of **14h** (4.88 g, 15.4 mmol) in DMA (66 ml) were added 1-benzhydrylpiperazine (3.88 g, 15.4 mmol), WSC (3.53 g, 18.4 mmol) and HOBt (2.49 g, 18.4 mmol) at 0 °C, and the mixture was stirred at room temperature for 6 h. Aqueous $NaHCO_3$ solution was added to the reaction mixture and the solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane=1/1) to afford 6.55 g (yield 77%) of **15o** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.28—2.46 (4H, m), 3.43—3.63 (4H, m), 3.46 (2H, s), 3.52 (2H, s), 3.90 (3H, s), 4.18 (1H, s), 7.13—7.43 (10H, m), 7.49 (1H, dd, J=9.0, 2.7 Hz), 8.01 (1H, d, J=2.7 Hz), 8.69 (1H, J=9.0 Hz), 11.5 (1H, s).

Methyl 5-Chloro-2-({[{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethyl}(methyl)amino]acetyl}amino)benzoate (15p) This compound was prepared in 44% yield as a solid from **14i**, methanesulfonyl chloride and Et_3N following a procedure similar to that described for the preparation of **6a**, ^{1}H-NMR ($CDCl_3$) δ: 2.29—2.43 (4H, m), 2.48 (3H, s), 3.35 (2H, s), 3.44 (2H, s), 3.54—3.66 (4H, m), 3.71 (3H, s), 4.15 (1H, s), 7.10—7.40 (10H, m), 7.50 (1H, dd, J=9.2, 2.6 Hz), 7.99 (1H, d, J=2.6 Hz), 8.78 (1H, d, J=9.2 Hz), 11.8 (1H, s).

Methyl 5-Chloro-2-{[(1-{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethyl}cyclohexyl)acetyl]amino}benzoate (15q) This compound was prepared quantitatively as a solid from **14j**, WSC, HOBt and 1-benzhydrylpiperazine following a procedure similar to that described for the preparation of **15k**, ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.60 (10H, m), 2.20—2.40 (4H, m), 2.49 (2H, s), 2.73 (2H, s), 3.45—3.70 (4H, m), 3.92 (3H, s), 4.08 (1H, s), 7.12—7.45 (10H, m), 7.46 (1H, dd, J=9.0, 2.7 Hz), 7.99 (1H, d, J=2.7 Hz), 8.62 (1H, d, J=9.0 Hz), 10.9 (1H, s).

5-Chloro-2-[({2-[(4-chlorophenyl)amino]-2-oxoethoxy}acetyl)amino]-benzoic Acid (16a) To a solution of **15a** (345 mg, 0.84 mmol) in THF (5 ml) was added 1 N aqueous NaOH solution (1.3 ml) and the mixture was stirred for 0.5 h at 60 °C. The reaction solution was acidified with 1 N aqueous HCl solution and the organic solvent was removed *in vacuo*. The resulting precipitate was collected by filtration and washed with water and IPE, and dried *in vacuo* to afford 309 mg (yield 93%) of **16a** as a solid, mp 128—130 °C, ^{1}H-NMR (DMSO-d_6) δ: 4.28 (2H, s), 4.31 (2H, s), 7.32—7.43 (2H, m), 7.68—7.78 (2H, m), 7.70 (1H, dd, J=9.0, 2.7 Hz), 7.98 (1H, d, J=9.0 Hz), 8.69 (1H, d, J=9.0 Hz), 9.88 (1H, s), 11.9 (1H, s), HPLC (250 nm): 97.2% (t_R=16.8 min), LC/MS (ESI) *m/z*: 397 $(M+H)^+$, 395 $(M-H)^-$.

Sodium 5-Chloro-2-({[2-(diphenylamino)-2-oxoethoxy]acetyl}amino)-benzoate (16b) To a solution of **15b** (717 mg, 1.6 mmol) in THF (13 ml) was added 1 N aqueous NaOH solution (4.3 ml) and the mixture was stirred at 60 °C for 7 h. After cooling and evaporation of THF *in vacuo*, the resulting precipitate was collected by suction filtration and washed with water to give a crude product which was recrystallized from a mixed solvent of MeOH and water to afford 508 mg (yield 70%) of **16b** as a solid, mp >300 °C (MeOH/H_2O), ^{1}H-NMR (DMSO-d_6) δ: 4.12 (2H, s), 4.14 (2H, s), 7.15—7.60 (10H, m), 7.29 (1H, dd, J=8.9, 2.8 Hz), 7.92 (1H, d, J=2.8 Hz), 8.51 (1H, d, J=8.9 Hz), 14.3 (1H, s), HPLC (250 nm): >99.9% (t_R=16.6 min), LC/MS (ESI) *m/z*: 439 $(M+H)^+$, 437 $(M-H)^-$.

5-Chloro-2-[({2-[(diphenylmethyl)amino]-2-oxoethoxy}acetyl)amino]-

benzoic Acid (16c) This compound was prepared in 92% yield as a solid from **15c** following a procedure similar to that described for the preparation of **16a**, mp 208—210 °C, ^{1}H-NMR (DMSO-d_6) δ: 4.23 (2H, s), 4.25 (2H, s), 6.20 (1H, d, J=8.5 Hz), 7.22—7.35 (10H, m), 7.67 (1H, dd, J=9.0, 2.6 Hz), 7.96 (1H, d, J=2.6 Hz), 8.66 (1H, d, J=9.0 Hz), 8.81 (1H, d, J=8.5 Hz), 12.0 (1H, s), HPLC (250 nm): 99.4% (t_R=18.9 min), LC/MS (ESI) m/z: 453 $(M+H)^+$, 451 $(M-H)^-$.

5-Chloro-2-({[2-oxo-2-(4-phenylpiperidin-1-yl)ethoxy]acetyl}amino)-benzoic Acid (16d) This compound was prepared in 85% yield as a solid from **15d** following a procedure similar to that described for the preparation of **16a**, mp 178—179 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.40—1.90 (4H, m), 2.60—2.85 (2H, m), 2.95—3.10 (1H, m), 3.70—3.90 (1H, m), 4.19 (2H, s), 4.40—4.60 (1H, m), 4.43 (2H, d, J=2.7 Hz), 7.10—7.35 (5H, m), 7.67 (1H, dd, J=8.9, 2.7 Hz), 7.96 (1H, d, J=2.7 Hz), 8.69 (1H, d, J=8.9 Hz), 12.1 (1H, s), HPLC (250 nm): 97.7% (t_R=15.0 min), LC/MS (ESI) m/z: 431 $(M+H)^+$, 429 $(M-H)^-$.

Sodium 5-Chloro-2-({[2-(4,4-diphenylpiperidin-1-yl)-2-oxoethoxy]-acetyl}amino)benzoate (16e) This compound was prepared in 88% yield as an amorphous solid from **15e** following a procedure similar to that described for the preparation of **16b**, ^{1}H-NMR (DMSO-d_6) δ: 2.16—2.50 (4H, m), 3.30—3.52 (4H, m), 4.08 (2H, s), 4.35 (2H, s), 7.00—7.40 (11H, m), 7.96 (1H, d, J=2.7 Hz), 8.54 (1H, d, J=8.8 Hz), 14.3 (1H, s), HPLC (250 nm): 98.9% (t_R=20.2 min), LC/MS (ESI) m/z: 507 $(M+H)^+$, 505 $(M-H)^-$.

Sodium 5-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (16f) This compound was prepared in 83% yield as a solid from **15f** following a procedure similar to that described for the preparation of **16b**, mp 240 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.28—2.48 (4H, m), 3.34—3.44 (4H, m), 4.06 (2H, s), 4.29 (2H, s), 4.32 (1H, s), 7.14—7.45 (11H, m), 7.93 (1H, d, J=2.7 Hz), 8.52 (1H, d, J=8.8 Hz), 14.4 (1H, s), *Anal.* Calcd for $C_{28}H_{27}ClN_3NaO_5$: C, 61.82; H, 5.00; N, 7.72. Found: C, 61.64; H, 5.01; N, 7.68, HPLC (250 nm): 99.5% (t_R=6.3 min), LC/MS (ESI) m/z: 522 $(M+H)^+$, 520 $(M-H)^-$.

2-{[(2-{4-[Bis(4-fluorophenyl)methyl]piperazin-1-yl}-2-oxoethoxy)-acetyl]amino}-5-chlorobenzoic Acid (16g) To a solution of **15g** (1.47 g, 2.56 mmol) in THF (26 ml) was added 1 N aqueous NaOH solution (3.8 ml) and the mixture was stirred at 60 °C for 1 h. The reaction solution was neutralized with 1 N aqueous HCl solution and the organic solvent was removed *in vacuo*. The resulting precipitate was collected by filtration and washed with water and IPE, and dried *in vacuo* to afford 1.33 g (yield 93%) of **16g** as an amorphous solid, ^{1}H-NMR (DMSO-d_6) δ: 2.20—2.38 (4H, m), 3.35—3.57 (4H, m), 4.13 (2H, s), 4.35 (2H, s), 4.40 (1H, s), 7.08—7.46 (8H, m), 7.65 (1H, dd, J=9.0, 2.4 Hz), 7.95 (1H, d, J=2.4 Hz), 8.67 (1H, d, J=9.0 Hz), 12.1 (1H, s), HPLC (250 nm): 95.5% (t_R=8.6 min), LC/MS (ESI) m/z: 558 $(M+H)^+$, 556 $(M-H)^-$.

5-Chloro-2-[({2-[4-(9*H*-fluoren-9-yl)piperazin-1-yl]-2-oxoethoxy}-acetyl)amino]benzoic Acid (16h) This compound was prepared in 96% yield as a solid from **15h** following a procedure similar to that described for the preparation of **16g**, mp 224 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.42—2.53 (2H, m), 3.24—3.57 (4H, m), 4.11 (2H, s), 4.34 (2H, s), 4.97 (1H, s), 7.25—7.80 (8H, m), 7.67 (1H, dd, J=9.1, 2.6 Hz), 7.94 (1H, d, J=2.6 Hz), 8.66 (1H, d, J=9.1 Hz), 11.9 (1H, s), HPLC (250 nm): 95.0% (t_R=5.9 min), LC/MS (ESI) m/z: 520 $(M+H)^+$, 518 $(M-H)^-$.

2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)amino]-5-fluorobenzoic Acid (16i) This compound was prepared in 93% yield as an amorphous solid from **15i** following a procedure similar to that described for the preparation of **16g**, ^{1}H-NMR (DMSO-d_6) δ: 2.35—2.59 (4H, m), 3.49—3.60 (4H, m), 4.14 (2H, s), 4.37 (2H, s), 4.53 (1H, s), 7.19—7.60 (11H, m), 7.73 (1H, dd, J=9.3, 2.9 Hz), 8.68 (1H, dd, J=9.3, 5.1 Hz), 11.7 (1H, s), HPLC (250 nm) >99.9% (t_R=2.3 min), LC/MS (ESI) m/z: 506 $(M+H)^+$, 504 $(M-H)^-$.

Sodium 5-Bromo-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (16j) This compound was prepared in 77% yield as a solid from **15j** following a procedure similar to that described for the preparation of **16b**, mp 240 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.20—2.39 (4H, m), 3.30—3.59 (4H, m), 4.08 (2H, s), 4.30 (1H, s), 4.30 (2H, s), 7.14—7.44 (10H, m), 7.53 (1H, dd, J=9.0, 2.7 Hz), 8.09 (1H, d, J=2.7 Hz), 8.51 (1H, d, J=9.0 Hz), 13.7 (1H, s), HPLC (250 nm): 97.5% (t_R=6.2 min), LC/MS (ESI) m/z: 566 $(M+H)^+$, 564 $(M-H)^-$.

Sodium 4-Bromo-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (16k) This compound was prepared in 79% yield as a solid from **15k** following a procedure similar to that described for the preparation of **16b**, mp 240 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.27—2.39 (4H, m), 3.30—3.51 (4H, m), 4.08 (2H, s), 4.30 (1H, s), 4.30 (2H, s), 7.13—7.44 (11H, m), 7.92 (1H, d, J=8.3 Hz), 8.74 (1H, d, J=2.2 Hz), 14.6 (1H, s), HPLC (250 nm): >99.9% (t_R=6.9 min), LC/MS (ESI) m/z: 566 $(M+H)^+$, 564 $(M-H)^-$.

4-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)amino]-biphenyl-3-carboxylic Acid (16l) This compound was prepared in 88% yield as an amorphous solid from **15l** following a procedure similar to that described for the preparation of **16g**, ^{1}H-NMR (DMSO-d_6) δ: 2.23—2.40 (4H, m), 3.30—3.58 (4H, m), 4.14 (2H, s), 4.28 (1H, s), 4.36 (2H, s), 7.13—7.66 (15H, m), 7.85 (1H, dd, J=8.5, 2.2 Hz), 8.30 (1H, d, J=2.2 Hz), 8.72 (1H, d, J=8.5 Hz), 12.6 (1H, s), HPLC (250 nm): 99.2% (t_R=10.6 min), LC/MS (ESI) m/z: 564 $(M+H)^+$, 562 $(M-H)^-$.

Sodium 4-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (16m) This compound was prepared in 68% yield as a solid from **15m** following a procedure similar to that described for the preparation of **16b**, mp 240 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.28—2.38 (4H, m), 3.37—3.52 (4H, m), 4.08 (2H, s), 4.30 (2H, s), 4.30 (1H, s), 7.00 (1H, dd, J=8.3, 2.1 Hz), 7.18—7.44 (10H, m), 7.98 (1H, d, J=8.3 Hz), 8.59 (1H, d, J=2.1 Hz), 14.6 (1H, s), HPLC (250 nm): 98.6% (t_R=7.2 min), LC/MS (ESI) m/z: 522 $(M+H)^+$, 520 $(M-H)^-$.

5-Chloro-2-({5-[4-(diphenylmethyl)piperazin-1-yl]-5-oxopentanoyl}-amino)benzoic Acid (16n) This compound was prepared in 93% yield as an amorphous solid from **15n** following a procedure similar to that described for the preparation of **16g**, ^{1}H-NMR (DMSO-d_6) δ: 1.76—1.92 (2H, m), 2.34—2.54 (8H, m), 3.41—3.62 (4H, m), 4.43 (1H, s), 7.17—7.48 (10H, m), 7.63 (1H, dd, J=9.0, 2.7 Hz), 7.92 (1H, d, J=2.7 Hz), 8.48 (1H, d, J=9.0 Hz), 11.1 (1H, s), HPLC (250 nm): 97.0% (t_R=6.5 min), LC/MS (ESI) m/z: 520 $(M+H)^+$, 518 $(M-H)^-$.

5-Chloro-2-{[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethyl}sul-fanyl)acetyl]amino}benzoic Acid Hydrochloride (16o) This compound was prepared in 90% yield as a solid from **15o** following a procedure similar to that described for the preparation of **16a**, mp 205 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.60—4.20 (8H, m), 3.56 (2H, s), 3.60 (2H, s), 5.30 (1H, br), 7.26—7.90 (10H, m), 7.68 (1H, dd, J=9.0, 2.7 Hz), 7.94 (1H, d, J=2.7 Hz), 8.51 (1H, d, J=9.0 Hz), 11.5 (1H, s), HPLC (250 nm): 94.2% (t_R=7.1 min), MS (ESI) m/z 538 $(M+H)^+$, 536 $(M-H)^-$.

5-Chloro-2-({[{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethyl}-(methyl)amino]acetyl}amino)benzoic Acid (16p) To a solution of **15p** (760 mg, 1.38 mmol) in THF (10 ml) was added 1 N aqueous NaOH solution (2.1 ml) and the mixture was stirred for 1 h at 40 °C. The mixture was neutralized with 1 N aqueous HCl solution, and the mixture was concentrated *in vacuo*. The residue was dissolved in IPE and the solution was dried (Na_2SO_4) and concentrated to give oil. EtOAc and *n*-hexane was added to residue and the resulting precipitate was collected by filtration to afford 517 mg (yield 70%) of **16p** as a solid, mp 179—181 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.18—2.32 (4H, m), 2.42 (3H, s), 3.34 (2H, s), 3.49 (2H, s), 3.35—3.60 (4H, m), 4.25 (1H, s), 7.10—7.45 (10H, m), 7.66 (1H, dd, J=9.0, 2.7 Hz), 7.94 (1H, d, J=2.6 Hz), 8.64 (1H, d, J=9.0 Hz), 12.1 (1H, s), HPLC (250 nm): 97.3% (t_R=2.9 min), LC/MS (ESI) m/z: 535 $(M+H)^+$, 533 $(M-H)^-$.

5-Chloro-2-{[(1-{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethyl}cy-clohexyl)acetyl]amino}benzoic Acid Hydrochloride (16q) This compound was prepared in 59% yield as a solid from **15q** following a procedure similar to that described for the preparation of **16a**, mp 190—192 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.70 (10H, m), 2.04—2.20 (4H, m), 2.40 (2H, s), 2.60 (2H, s), 3.35—3.52 (4H, m), 4.03 (1H, s), 7.13—7.43 (10H, m), 7.67 (1H, dd, J=9.0, 2.6 Hz), 7.96 (1H, d, J=2.6 Hz), 8.49 (1H, d, J=9.0 Hz), 11.0 (1H, s), HPLC (250 nm): >99.9% (t_R=13.4 min), LC/MS (ESI) m/z: 588 $(M+H)^+$, 586 $(M-H)^-$.

Methyl 2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)-amino]-5-(2-methylpropyl)benzoate (17a) Under argon atmosphere, a solution of 0.5 M isobutylzinc bromide in THF (16 ml, 8.0 mmol) was added to a solution of **15j** (1.16 g, 2.0 mmol) in THF (20 ml) and tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) (0.23 g, 0.2 mmol), and the mixture was refluxed for 9 h. Aqueous NH_4Cl solution was added to the reaction mixture and the solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane=1/3) to afford 0.32 g (yield 25%) of **17a** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s), 0.92 (3H, s), 1.74—1.98 (1H, m), 2.34—2.41 (4H, m), 2.46 (2H, d, J=7.1 Hz), 3.53—3.67 (4H, m), 3.78 (3H, s), 4.21 (2H, s), 4.22 (1H, s), 4.35 (2H, s), 7.14—7.23 (11H, m), 7.79 (1H, d, J=2.2 Hz), 8.64 (1H, d, J=8.6 Hz), 11.6 (1H, s).

Methyl 5-Benzyl-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (17b) This compound was prepared in

47% yield as a solid from **15j**, $Pd(PPh_3)_4$ and benzylzinc bromide following a procedure similar to that described for the preparation of **17a**, ^{1}H-NMR ($CDCl_3$) δ: 2.34—2.52 (4H, m), 3.55—3.73 (4H, m), 3.76 (3H, s), 3.96 (2H, s), 4.20 (2H, s), 4.21 (1H, s), 4.33 (2H, s), 7.12—7.40 (16H, m), 7.86 (1H, d, J=2.2 Hz), 8.65 (1H, d, J=8.6 Hz), 11.6 (1H, s).

Methyl 2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)-amino]-5-(pyridin-3-yl)benzoate (17c) Under argon atmosphere, 3-pyridineboronic acid (0.34 g, 3.8 mmol) was added to a mixture of **15j** (1.16 g, 2.0 mmol), $Pd(PPh_3)_4$ (69.3 mg, 0.06 mmol) in a mixture of toluene (6.7 ml), MeOH (1.6 ml), and 2 N Na_2CO_3 aqueous solution (5.6 ml), and the resulting mixture was refluxed for 15 h. Water was added to the reaction mixture and the mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane/MeOH=2/2/0.3 to 2/2/0.5) to afford 0.92 g (yield 80%) of **17c** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.38—2.43 (4H, m), 3.53—3.67 (4H, m), 3.85 (3H, s), 4.25 (1H, s), 4.29 (2H, s), 4.38 (2H, s), 7.15—7.42 (11H, m), 7.78 (1H, dd, J=8.6, 2.4 Hz), 7.86—7.91 (1H, m), 8.27 (1H, d, J=2.4 Hz), 8.61 (1H, dd, J=5.0, 1.7 Hz), 8.85—8.91 (2H, m), 11.8 (1H, s).

Methyl 2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)-amino]-4-(pyridin-4-yl)benzoate (17d) Under argon atmosphere, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (0.62 g, 3.0 mmol) was added to a mixture of **15k** (1.16 g, 2.0 mmol), Cs_2CO_3 (0.98 g, 3.0 mmol) and $Pd(PPh_3)_4$ (0.23 g, 0.2 mmol) in THF (20 ml) and the mixture was refluxed for 8 h. After evaporation of organic solvent, water was added to the mixture and the aqueous solution was extracted with EtOAc three times. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/MeOH=4/1) to afford 'quantitative' yield of **17d** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.38—2.43 (4H, m), 3.41—3.65 (4H, m), 3.85 (3H, s), 4.23 (1H, s), 4.26 (2H, s), 4.38 (2H, s), 7.15—7.73 (13H, m), 8.13 (1H, d, J=8.2 Hz), 8.67—8.71 (2H, m), 9.14 (1H, d, J=1.9 Hz), 11.8 (1H, s).

Sodium 2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}-acetyl)amino]-5-(2-methylpropyl)benzoate (18a) This compound was prepared in 54% yield as a solid from **17a** following a procedure similar to that described for the preparation of **16b**, mp 230 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, s), 0.86 (3H, s), 1.71—1.86 (1H, m), 2.18—2.38 (4H, m), 2.39 (2H, d, J=6.8 Hz), 3.21—3.63 (4H, m), 4.06 (2H, s), 4.27 (1H, s), 4.34 (2H, s), 7.16—7.42 (11H, m), 7.79 (1H, d, J=1.9 Hz), 8.45 (1H, d, J=8.5 Hz), 13.6 (1H, s), HPLC (250 nm): 98.9% (t_R=11.6 min), LC/MS (ESI) m/z: 544 $(M+H)^+$, 542 $(M-H)^-$.

Sodium 5-Benzyl-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)amino]benzoate (18b) This compound was prepared in 54% yield as an amorphous solid from **17b** following a procedure similar to that described for the preparation of **16b**, ^{1}H-NMR (DMSO-d_6) δ: 2.18—2.36 (4H, m), 3.21—3.53 (4H, m), 3.90 (2H, s), 4.07 (2H, s), 4.26 (1H, s), 4.32 (2H, s), 7.14—7.41 (16H, m), 7.86 (1H, d, J=2.2 Hz), 8.49 (1H, d, J=8.3 Hz), 13.0 (1H, s), HPLC (250 nm): >99.9% (t_R=10.8 min), LC/MS (ESI) m/z: 578 $(M+H)^+$, 576 $(M-H)^-$.

2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)amino]-5-(pyridin-3-yl)benzoic Acid (18c) This compound was prepared in 66% yield as an amorphous solid from **17c** following a procedure similar to that described for the preparation of **16g**, ^{1}H-NMR (DMSO-d_6) δ: 2.28—2.40 (4H, m), 3.38—3.59 (4H, m), 4.17 (2H, s), 4.34 (1H, s), 4.38 (2H, s), 7.15—7.54 (11H, m), 8.00—8.13 (2H, m), 8.30 (1H, d, J=2.4 Hz), 8.59 (1H, d, J=4.9 Hz), 8.79 (1H, d, J=8.8 Hz), 8.92 (1H, d, J=2.4 Hz), 11.9 (1H, s), HPLC (250 nm): 98.0% (t_R=1.9 min), LC/MS (ESI) m/z: 565 $(M+H)^+$, 563 $(M-H)^-$.

2-[({2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}acetyl)amino]-4-(pyridin-4-yl)benzoic Acid (18d) This compound was prepared in 67% yield as a solid from **17d** following a procedure similar to that described for the preparation of **16g**, mp 190 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 2.29—2.38 (4H, m), 3.38—3.57 (4H, m), 4.17 (2H, s), 4.31 (1H, s), 4.38 (2H, s), 7.14—7.43 (10H, m), 7.58 (1H, dd, J=8.3, 1.7 Hz), 7.68 (2H, d, J=6.1 Hz), 8.13 (1H, d, J=8.3 Hz), 8.70 (1H, d, J=6.1 Hz), 9.07 (1H, d, J=1.7 Hz), 12.1 (1H, s), HPLC (250 nm): 96.4% (t_R=2.0min), LC/MS (ESI) m/z: 565 $(M+H)^+$, 563 $(M-H)^-$.

Methyl 5-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxo-ethoxy}acetyl)(methyl)amino]benzoate (19) To a suspension of sodium hydride (60% purity, 230 mg, 6.0 mmol) in THF (35 ml) was added **15f** (2.14 g, 4.0 mmol) at 0 °C, and stirred at 0 °C for 0.5 h under an argon atmosphere. Iodomethane (2.48 ml, 40 mmol) was then added and the mixture was refluxed for 7 h. After cooling and evaporation of THF *in vacuo*, aqueous sodium thiosulfate solution was added to the residue and the whole mixture was extracted with EtOAc three times. The combined organic layers were washed with brine, water, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/*n*-hexane/MeOH=2/2/0.2 to 2/3/0.3) to afford 1.10 g (yield 50%) of **19** as a solid, ^{1}H-NMR ($CDCl_3$) δ: 2.30—2.45 (4H, m), 3.14 (3H, s), 3.50—3.65 (4H, m), 3.78 (1H, d, J=15.2 Hz), 3.88 (3H, s), 3.89 (1H, d, J=15.2 Hz), 4.14 (1H, s), 4.22 (2H, s), 7.10—7.45 (11H, m), 7.55 (1H, dd, J=8.2, 2.6 Hz), 7.97 (1H, d, J=2.6 Hz).

5-Chloro-2-[({2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethoxy}-acetyl)(methyl)amino]benzoic Acid (20) To a solution of **19** (1.08 g, 1.96 mmol) in THF (20 ml) was added 1 N aqueous NaOH solution (2.9 ml) and the mixture was stirred at 40 °C for 1 h. The mixture was neutralized with 1 N aqueous HCl solution. The precipitate was collected by suction filtration and washed with water. The resulting residue was purified by column chromatography ($CHCl_3$/MeOH=9/1) and recrystallized form EtOAc/*n*-hexane to afford 510 mg (yield 49%) of **20** as an amorphous solid, ^{1}H-NMR (DMSO-d_6) δ: 2.10—2.35 (4H, brs), 3.01 (3H, s), 3.25—3.55 (4H, br s), 3.79 (2H, s), 4.05 (2H, s), 4.30 (1H, s), 7.10—7.55 (12H, m), 7.66 (1H, d, J=2.4 Hz), HPLC (250 nm): >99.9% (t_R=4.0 min), LC/MS (ESI) m/z: 536 $(M+H)^+$, 534 $(M-H)^-$.

Biological Procedures. Method A and Method B PAI-1 activity was evaluated by two different methods. For the screening of newly synthesized compounds for their PAI-1 inhibitory activities, we used a biological assay utilizing a synthetic substrate for tPA. In brief, human PAI-1 (Molecular Innovations, Southfield, MI, U.S.A.) was incubated at 37 °C for 15 min in the reaction buffer containing 100 mM Tris HCl, pH 8, 0.1% Tween 80 in the presence or absence of the tested compounds in a 96-well polystyrene plate. The mixture was subsequently incubated for 15 min with human tPA (American Diagnostica Inc., Stanford, CT, U.S.A.), and eventually fortified with a chromogenic substrate, S-2288 (Chromogenix, Milano, Italy). The final mixture contained 100 mM Tris–HCl, pH 8, 30 mM NaCl, 1% DMSO, 0.1% Tween 80, 67 nM PAI-1 (Method A) or 24.5 nM (Method B), 9.8 nM tPA, 1 mM S-2288, and tested compounds at various concentrations (20, 50, 100 μM). Kinetics of *p*-nitroaniline release during peptide cleavage was monitored with a spectrophotometer at 405 nm. The residual inhibitory activity of PAI-1 was expressed as the percentage of the initial activity.

Pharmacokinetics Compounds (50 mg/kg) were given by gavage to male Wistar rats. Heparinized blood samples were collected from the vein before (0 h) and at 1, 2, 6, 18, 24 48 h after administration. Drug concentration was determined in the plasma by reverse-phase HPLC. Maximum drug concentration (C_{max}) and drug half-life ($T_{1/2}$) were calculated.

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