

Potent histone deacetylase inhibitors: *N*-hydroxybenzamides with antitumor activities

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Abstract—The screening tests of *N*-hydroxybenzamides for their HDAC-inhibitory activities led to the discovery of the promising compounds with a 2-naphthylcarbonyl group and with a 1,4-biphenylcarbonyl group. These compounds were further modified to optimize their physico-chemical profile. As a result, the inhibitor with a 6-amino-2-naphthylcarbonyl was obtained, which showed not only promising growth inhibitions against a panel of tumor cells, but also an improved water solubility. It exhibited the maximal 185% of survival rate (%T/C) in a *in vivo* experiment with P388 cell-inoculated mice.

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1. Introduction

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a crucial role in gene expression through reversible acetylation and deacetylation of histones. HAT-mediated hyperacetylation of positively charged lysine residues in the N-terminal tails of core histones loosens the histone–DNA binding and activates a gene transcription. In contrast, HDAC-catalyzed deacetylation of ϵ -N acetyl group of lysine residues leads to the tight histone–DNA binding, which restricts the access transcription factors. It is known that these enzymes are correlated with cell cycle progression, differentiation and apoptosis, and their deregulation is associated with tumorigenesis.¹ HDAC inhibitors are attractive new antitumor candidates since they are expected to suppress the cell cycle progression and induce terminal differentiation of human tumor cells.² Some HDAC inhibitors proved to have efficacies in clinical phase studies; they include SAHA³ and CI-994.⁴

Recently, we reported that novel HDAC inhibitors: *N*-hydroxybenzamides possessing a terminal bicyclic arylcarbonyl group showed potent inhibitions against HDACs as well as proliferation of HCT 116 colorectal human carcinoma cells.⁵ Especially, **6a** having the 2-naphthylcarbonyl exhibited the most potent activities, and **6b** possessing the 1,4-biphenylcarbonyl followed it. It is conceivable that **6a** adopts a molecular shape close to those of TSA and SAHA, which are adequate to pack in the tube-like HDAC pocket⁶ as shown in Figure 1, since they all have nearly the same molecular length in the extended form. This packing is expected to make multiple contacts to the hydrophobic protein residues at its rim, walls and bottom.⁶ Of importance is the benzyl component of **6a**, which is presumed to make tight contacts with the phenyl groups of Phe150 and Phe205 facing each other in parallel at the walls as does the methyl-substituted vinyl moiety of TSA⁶. Furthermore, the large size of the 2-naphthylcarbonyl component of **6a** as compared with the phenylamino motif of SAHA as a cap of the HDAC pocket might allow it to contact more extensively at the rim of the pocket.^{6,7} We, thus, speculate that these multiple contacts between **6a** and the HDAC pocket, along with the Zn²⁺-chelation by its hydroxamic acid may cause the several tens nanomolar potency of **6a** in inhibiting HDAC activity. Therefore,

Keywords: Histone deacetylase inhibitor; *N*-hydroxybenzamide; HCT116; P388 cell.

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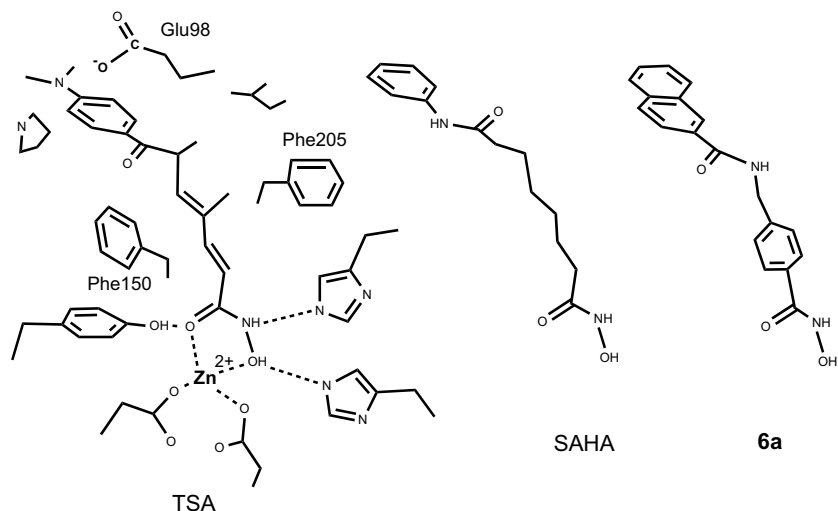


Figure 1. Proposed molecular shapes of TSA, SAHA and **6a** packing in the HDAC pocket, which were prepared by modifying the TSA-HDL (histone deacetylase like protein) binding representation in Figure 3 illustrated in Ref. 6.

the structures of **6a** as well as **6b** were further tried to be modified to optimize their physico-chemical profile through introduction of a nitrogen, fluorine or oxygen atom at their terminal bicyclic arylcarbonyl moieties. Further growth inhibition tests against a panel of tumor cells led to attainment of three promising compounds: **6g**, **6h** and **6i**, which were examined for in vivo activity against murine P388 tumor model.⁸

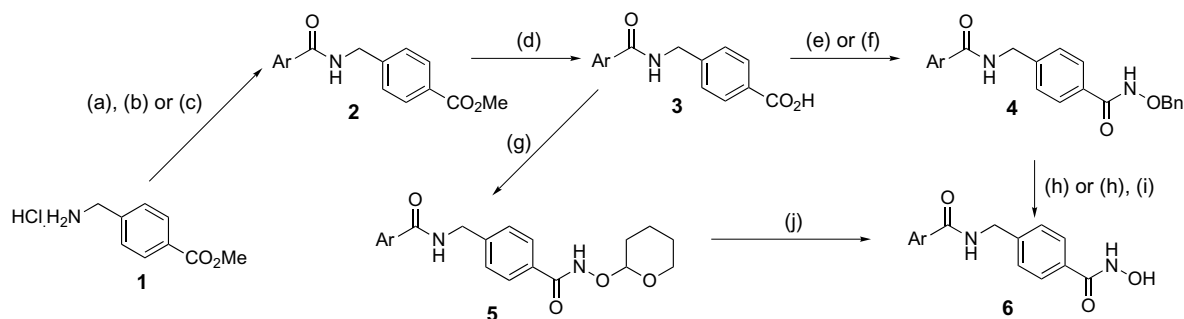
The present paper deals with the synthesis of the HDAC inhibitors, as well as evaluation of their antitumor efficacy.

2. Results and discussion

New series HDAC inhibitors were synthesized according to the procedures in the preceding paper⁵ except for **6i** as shown in Scheme 1. Thus, compounds **6a–i** were prepared starting from condensation of 4-aminomethylbenzoic acid methyl ester hydrochloride (**1**) with aryl acid chloride or with aryl acid to give aryl amides **2a–i**. These compounds, after alkaline hydrolysis to acids **3a–i**, were condensed with benzyloxyamine hydrochloride.

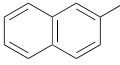
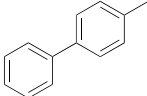
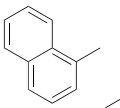
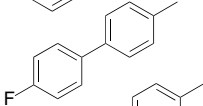
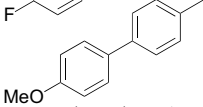
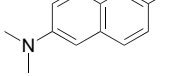
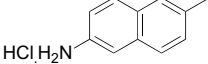
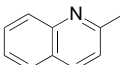
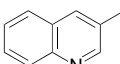
Catalytic hydrogenation of the condensation products **4a–f** and **4h** afforded *N*-hydroxycarboxamides **6a–f** and **6h**. Furthermore, the same treatment of **4g**, having a 6-amino-2-naphthylcarbonyl group, followed by successive deblocking of a Boc-protected 6-amino group with 12 N HCl/MeOH gave rise to the desired **6g**. Compound **6i** was synthesized through the deblocking of tetrahydropyranyl ether in **5i**, but not through catalytic hydrogenation of the corresponding benzyloxy derivative, since the latter reaction led to formation of over-reduced compound. Thus, acid **3i** was condensed with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine to afford **5i**, which was in turn hydrolyzed with aq acetic acid to yield the final compound.

Table 1 indicated the inhibitory activities against the growth of the HCT 116 cells as well as the HDACs. The HDAC inhibition was estimated according to method⁹ by Yoshida et al., but using partially purified HDACs from human T cell leukemia Jurkat FM3A.¹⁰ The *N*-hydroxybenzamides **6a** with the 2-naphthylcarbonyl moiety and **6b** with the 1,4-biphenylcarbonyl moiety showed potent activities in these in vitro tests. Introduction of a dimethylamino group (**6f**) and a



Scheme 1. Conditions: (a) ArCOCl, Et₃N/CH₂Cl₂; (b) ArCO₂H, BOP-Cl, Et₃N/DMF; (c) ArCO₂H, HONB, WSCI, Et₃N/DMF; (d) 1 M LiOH/H₂O–THF; (e) H₂N–OBn, BOP-Cl, Et₃N/CH₂Cl₂ or DMF; (f) H₂N–OBn, HONB, WSCI, Et₃N/DMF; (g) NH₂OTHP, HONB, WSCI, Et₃N/CH₂Cl₂–DMF; (h) H₂/5% Pd–C/MeOH or H₂/10% Pd–C/MeOH; (i) 12 N HCl/MeOH; (j) CH₃CO₂H–THF–H₂O.

Table 1. Inhibition against HCT116 cell growth and HDACs

Compd	Ar	HCT116	HDACs
		IC ₅₀ (M) ^{a,b}	IC ₅₀ (nM) ^b
6a		0.7	44
6b		3.2	240
6c		9.7	830
6d		8.2	180
6e		36.1	>10,000
6f		80.4	>10,000
6g		3.9	39
6h		3.8	26
6i		3.6	43
TSA		0.5	3
SAHA		6.3	263

^a Measured after 2 day incubation of a test compound with cells.^b Assays were performed in triplicate.

methoxy group (**6e**) at **6a** and **6b** caused the significant decrease of in vitro activities, respectively, suggesting that a further addition of a bulky group at the C-6 of their terminal bicyclic arylcarbonyl group would hinder them from capping snugly the HDAC catalytic pocket. On the contrary, introduction of a nitrogen atom (**6g–i**) at the 2-naphthylcarbonyl group of **6a** led not only to retention of in vitro activities, but also to improvement in water solubility (cf. solubility in 10% HCO-60/PBS: **6a** ≤ 1 mg/mL; **6g** and **6h** ≥ 8 mg/mL; **6i** ≥ 4 mg/mL). Replacement of H (**6b**) with F (**6d**) on C-4' of the 1,4-biphenylcarbonyl group did not increase the activities.

Compounds **6g–i** were evaluated further for growth inhibition against a panel of tumor cells including examples of breast (T-47D and MDA-MB-231), renal (A498), nonsmall cell lung (NCI-H460), colon (HCT116), melanoma (MALME-3M) and murine leukemia (P388 and P388/ADR). As shown in Table 2, **6g** exhibited the most promising inhibition profile against these cells. The reason for this profile is not clear at present, but the electronic interaction between the Glu98 carboxylic acid of a HDAC pocket and the amino group of **6g** might enhance its cell growth inhibitory activity. These three compounds were further assessed for the in vivo assay against murine P388 leukemia

Table 2. Growth inhibition of **6g–i** and SAHA against tumor cells^a

Cell	IC ₅₀ value (M) ^b			
	6g	6h	6i	SAHA
T-47D	0.6	1.8	1.4	1.4
MDA-MB-231	1.1	1.5	3.1	2.9
NCI-H460	1.3	1.1	5.5	1.7
A498	0.8	1.3	3.1	3.0
HCT116	0.3	1.0	1.4	0.6
MALME-3M	0.6	1.1	2.3	1.8
P388	0.3	0.5	1.6	1.2
P388/ADR	0.8	1.4	4.2	1.1

^a Assays were performed in triplicate.^b Measured after 3 day incubation of a test compound with cells.**Table 3.** Antitumor activity of **6g–i** against murine P388 model^a

Compd	Dose (mg/kg/day, qld × 7)	T/C ^b (%)	D/U ^c
Control	0	100	0/6
6g	40	144	0/5
	80	185	0/5
	160	153	2/5
6h	40	104	0/5
	80	102	0/5
	160	100	0/5
6i	40	111	0/4
	80	118	0/4
	160	140	0/3

^a P388 cells (2 × 10⁴/mouse) were implanted i.p. on day 0 in CDF1 mice. Compound **6g–i** dissolved in 10% HCO-60/PBS was injected i.p. on days 1–7 (qld × 7).^b Survival time (%) of treated animals versus controls.^c Number of mice that died of toxicity/number of mice used.

model by intraperitoneal injection. Table 3 indicated that **6g** and **6i** had an anti-tumor activity (%T/C > 125), whereas **6h** did not. The treatment with **6g** at 80 mg/kg/day resulted in 185%T/C along with one of six cure. However, the treatment of **6g** at 160 mg/kg/day caused the diarrhea from day 5 as well as 2 mice toxic deaths although the rest animals survived longer than control mice (%T/C: 153). For **6i**, the best %T/C was obtained only at 160 mg/kg/day (%T/C: 140).

3. Conclusion

N-Hydroxybenzamides **6a** and **6b** exhibiting the promising in vitro activities were further optimized to improve their physico-chemical profile. As a result, compound **6g** with the 6-amino-2-naphthylcarbonyl group was obtained, which showed not only the in vitro activities comparable with **6a**, but also the improvement in water solubility. The in vivo murine P388 experiment presents the probability that **6g** will be a potent anti-tumor agent. Further study with **6g** and related compounds is underway to investigate therapeutic efficacy against human cancers.

4. Experimental

Melting points were determined on a Yanagimoto MP-32 micromelting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu FTIR-8400 infrared spectrophotometer. Low resolution (LR)-FAB-MS spectra measured on a JEOL JMS-HX 100 instrument, whereas high resolution (HR)-and LR-electron impact (EI)-MS spectra, on JEOL The Tandem MStation JMS-700. ^1H NMR spectra were recorded on JEOL EX-270 (270 MHz), JEOL EX-400 (400 MHz) instruments using tetramethylsilane (TMS) as an internal standard. Analytical TLC and PLC were performed using Silica gel 60 F₂₅₄ (Merck, 0.25 and 0.5 mm, respectively) glass plates. Column chromatography was performed using Silica Gel 60 (70–230 mesh ASTM). All extracted solvents were dried over Na_2SO_4 , followed by evaporation in vacuo. The human cancer cell lines, HCT116 (colon cancer), MDA-MB-231 (breast cancer), T-47D (breast cancer), NCI-H460 (nonsmall cell lung cancer), A498 (renal cancer) and MALME-3M (melanoma), were purchased from American Type Culture Collection. P388 and P388/ADR were provided by the Cancer Chemotherapy Center of the Japanese Foundation for Cancer Research (Tokyo 170-8455, Japan). TSA was purchased from Sigma-Aldrich Co. (lot No. 083K4028, USA). Female CDF1 mice (6-week age) were obtained from Charles River Japan, Inc. (Kanagawa 222-0033, Japan). All procedures involving animals were in accord with guidelines for the care and use of laboratory animals in Nippon Kayaku.

4.1. Evaluation of histone deacetylase inhibitory activities

According to the procedure⁹ by M. Yoshida, et al., [^3H]-labeled histones were prepared from Human T cell leukemia Jurkat cells (2×10^8), which were pretreated with [^3H]acetic acid. Partial purification of HDACs and measurement of inhibitory activities of test compounds against the HDACs using [^3H]-acetylated histones were performed according the procedure¹⁰ by H. Mori et al. IC_{50} values in Tables represent the molar concentrations (nM) required to inhibit the HDACs by 50%.

4.2. Exploratory screening of HDAC inhibitors for cell growth inhibition

Cells, being maintained in McCoy's 5a medium with 10% fetal bovine serum (lot No. 1183455, Invitrogen, U.S.A.), were plated in 96-well plates at the densities of $1.0 \times 10^5/\text{mL}$. On the same day, compounds were dosed, and the cells were cultured for 2 days. WST-1 and 1-methoxy-5-methyl-phenazinium methylsulfate were added into each well at the final concentration of 0.47 mg/mL and 1.8 $\mu\text{g}/\text{mL}$, respectively.¹¹ After additional 4 h incubation, OD450 and OD630 as reference were measured, and the IC_{50} values were calculated.

4.3. Cell growth inhibition against a panel of tumor cells

All cell lines were cultured in RPMI 1640 medium containing 10% fetal bovine serum (lot No. 49300604,

Moregate Bio Tech, Australia). Some supplements were added into the medium properly. The proliferation assay against human solid-tumor cell lines was assessed by methyleneblue staining method. Briefly, appropriate numbers of cells were inoculated into 96-well microplates. Following overnight culture, serially diluted samples were added into the wells. After a 3-day culture, cells were stained with 0.05% methyleneblue dissolved in 10 mM Tris buffer (pH 8.5) for 30 min, and then thoroughly washed with distilled water. The stained dye was extracted with 3% HCl, and OD660 was measured with microplate reader Benchmark Plus (Bio-Rad, USA) to determine cell growth inhibition. In the case of murine leukemia (P388 and P388/ADR), cells were incubated with compounds for 2 days, and stained by WST-1. WST-1 and 1-methoxy-5-methylphenazinium methylsulfate were added into each well at the final concentration of 0.16 mg/mL and 3.3 $\mu\text{g}/\text{mL}$, respectively. After additional 4 h incubation, OD450 and OD660 as reference were measured.

4.4. Evaluation of therapeutic effect in vivo

Female CDF1 mice (6-week old) were intraperitoneally inoculated with P388 leukemia cells (2×10^4 cells suspended in 0.2 mL of saline) on day 0. Each of **6g** and **6h** was dissolved in 10% HCO-60/PBS at 8 mg/mL, whereas **6i** was dissolved in the solution at 4 mg/mL. The prepared drug solutions were intraperitoneally injected at doses of 40, 80, or 160 mg/kg/day from day 1 to day 7 (q1d $\times$ 7). The control mice were treated with vehicle according to the same schedule. Survival rate of each group was determined by day 30 according to the following equation.

Survival rate (T/C%) = (Median of survival days in each treatment group)/(Median of survival days in the control group) $\times$ 100.

4.5. Methyl 4-[(2-naphthoylamino)methyl]benzoate (2a)

To a suspension of **1** (1.7 g, 8.3 mmol) in CH_2Cl_2 (42 mL) were added Et_3N (4.7 mL, 33 mmol) and 2-naphthoyl chloride (1.6 g, 8.3 mmol). The mixture was stirred at room temperature overnight. After being evaporated, the residue was dissolved in CHCl_3 (200 mL) and washed successively with 1 N HCl (3×50 mL), satd NaHCO_3 (3×50 mL) and brine (3×50 mL). The organic layer was dried, and evaporated to dryness. The resulting residue was recrystallized from CHCl_3 -*n*-hexane to give the pure compound **2a** (2.6 g, 7.8 mmol, 94% yield): mp 133.0–134 °C; IR (KBr) cm^{-1} : 3278, 1717, 1624, 1543, 1280, 1114, 749, 483; ^1H NMR (CDCl_3) δ : 3.91 (3H, s, CO_2Me), 4.76 (2H, d, $J = 5.9$ Hz, PhCH_2), 6.69 (1H, br s, CH_2NH), 7.43–8.32 (11H, m, arom. H_{11}); EI-MS m/z : 319 (M^+); HR-EI-MS m/z : (M^+) calcd for $\text{C}_{20}\text{H}_{17}\text{NO}_3$, 319.1208; found, 319.1183.

4.6. 4-[(2-Naphthoylamino)methyl]benzoic acid (3a)

2a (3.3 g, 9.9 mmol) was hydrolyzed with 1 M LiOH (21.8 mL, 21.8 mmol) in a mixture of water (0.8 mL) and

THF (21 mL), and the mixture was stirred at room temperature overnight. After evaporation of THF, a residual water solution was adjusted at pH 3 with 1 N HCl. The resulting precipitate was filtered off and washed with water to give the pure compound **3a** (2.9 g, 9.4 mmol, 97% yield): mp 215.0–216.0 °C; IR (KBr) cm^{-1} : 3285, 1685, 1636, 1528, 1290, 945, 777; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.76 (2H, d, $J = 5.9$ Hz, PhCH_2), 6.69 (1H, br s, CH_2NH), 7.43–8.32 (11H, m, arom. H_{11}); EI-MS m/z 305 (M^+); HR-EI-MS m/z : (M^+)⁺ calcd for $\text{C}_{19}\text{H}_{15}\text{NO}_3$, 305.1052; found, 305.1066.

4.7. *N*-(4-[(Benzyloxy)amino]carbonyl)benzyl-2-naphthamide (**4a**)

To a suspension of *O*-benzylhydroxylamine hydrochloride (0.52 g, 3.3 mmol) in DMF (16 mL) were added **3a** (1.0 g, 3.3 mmol), BOP-Cl (1.5 g, 5.94 mmol) and Et_3N (1.8 mL, 13.0 mmol). The mixture was stirred at room temperature overnight. After being evaporated, the residue was dissolved in CHCl_3 (200 mL) and washed successively with 1 N HCl (3×30 mL), satd NaHCO_3 (3×30 mL) and brine (3×30 mL). The organic layer was dried and evaporated to dryness. The resulting residue was recrystallized from CHCl_3 –*n*-hexane to give the pure compound **4a** (0.45 g, 1.1 mmol, 34% yield): mp 177.0–178.8 °C; IR (KBr) cm^{-1} : 3271, 1654, 1543, 1308, 1239, 1049, 830, 693; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.56 (2H, d, $J = 5.9$ Hz, PhCH_2), 4.91 (2H, s, OCH_2), 7.33–8.30 (16H, m, arom. H_{16}), 9.25 (1H, t, $J = 5.9$ Hz, CH_2NH), 11.71 (1H, br s, BnONH); EI-MS m/z : 410 (M^+); HR-EI-MS m/z : (M^+)⁺ calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_3$, 410.1630; found, 410.1611.

4.8. *N*-{4-[(Hydroxyamino)carbonyl]benzyl}-2-naphthamide (**6a**)

10% Pd–C (77 mg) was added to a solution of **4a** (0.37 g, 0.9 mmol) in MeOH (30 mL), and the mixture was stirred in a stream of H_2 overnight. The catalyst was removed by filtration and washed with MeOH and CHCl_3 . The combined filtrate was evaporated to give the pure compound **6a** (0.31 g, 9.7 mmol, 100% yield): mp 184.1–184.7 °C; IR (KBr) cm^{-1} : 3290, 1637, 1542, 1309, 1038, 1037, 900, 838, 781, 738; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.56 (2H, d, $J = 5.9$ Hz, PhCH_2), 7.32–8.50 (11H, m, arom. H_{11}), 9.25 (1H, t, $J = 5.9$ Hz, CH_2NH), 11.16 (1H, br s, HONH); FAB-MS m/z : 321 ($\text{M}+\text{H}^+$); HR-FAB-MS m/z : ($\text{M}+\text{H}^+$)⁺ calcd for $\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_3$, 321.1239; found, 321.1255.

4.9. Methyl 4-[(1,1'-biphenyl)-4-ylcarbonyl]amino]benzoate (**2b**)

[1,1'-Biphenyl]-4-carbonyl chloride (4.7 g, 31.1 mmol) was reacted with **1** (5.0 g, 24.9 mmol) in the presence of Et_3N (17.2 mL, 124 mmol) in CH_2Cl_2 (134 mL) in the same way as for **2a**, yielding **2b** (6.8 g, 20.0 mmol, 77% yield): mp 219.9 °C; IR (KBr) cm^{-1} : 3276, 3059, 2947, 1714, 1631, 1609, 1551, 1439, 1281, 1113, 846, 739, 500;

^1H NMR ($\text{DMSO}-d_6$) δ : 3.87 (3H, s, CO_2Me), 4.60 (2H, d, $J = 5.9$ Hz, PhCH_2), 7.38–7.53 (5H, m, arom. H_5), 7.73–8.24 (4H, m, arom. H_4), 8.26–8.34 (4H, m, arom. H_4), 9.21 (1H, t, $J = 5.9$ Hz, CH_2NH); EI-MS m/z : 345 (M^+); HR-EI-MS m/z : (M^+)⁺ calcd for $\text{C}_{22}\text{H}_{19}\text{NO}_3$, 345.1365; found, 345.1360.

4.10. 4-[(1,1'-Biphenyl)-4-ylcarbonyl]amino]methyl]-benzoic acid (**3b**)

2b (6.8 g, 20.0 mmol) was hydrolyzed with 1 M LiOH (60.0 mL, 60.0 mmol) in a mixture of water (2 mL) and THF (68 mL) in the same way as for **3a**, yielding **3b** (5.9 g, 18.0 mmol, 85% yield): mp 394.2 °C; IR (KBr) cm^{-1} : 3323, 1632, 1601, 1553, 1430, 1316, 854, 776, 744, 689, 503; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.57 (2H, d, $J = 5.4$ Hz, PhCH_2), 7.40–7.52 (5H, m, arom. H_5), 7.72–7.90 (4H, m, arom. H_4), 7.98 (2H, d, $J = 8.1$ Hz, arom. H_2), 8.05 (2H, d, $J = 8.1$ Hz, arom. H_2), 9.33 (1H, br s, CH_2NH); EI-MS m/z : 331 (M^+); HR-EI-MS m/z : (M^+)⁺ calcd for $\text{C}_{21}\text{H}_{17}\text{NO}_3$, 331.1208; found, 331.1214.

4.11. *N*-(4-[(Benzyloxy)amino]carbonyl)benzyl)[1,1'-biphenyl]-4-carboxamide (**4b**)

3b (5.7 g, 17.0 mmol), BOP-Cl (7.8 g, 30.6 mmol) and Et_3N (9.5 mL, 68.0 mmol) were added to a suspension of *O*-benzylhydroxylamine hydrochloride (2.7 g, 17.0 mmol) in DMF (85 mL). The mixture was treated in the same way as for **4a**, giving rise to the pure compound **4b** (8.6 g, 19.7 mmol, 97% yield): mp 233.4–234.7 °C; IR (KBr) cm^{-1} : 3306, 3227, 3030, 1634, 1539, 1317, 1256, 849, 743; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.53–4.55 (2H, d, $J = 5.7$ Hz, PhCH_2), 4.91 (2H, s, OCH_2), 7.31–7.51 (9H, m, arom. H_9), 7.69–7.92 (7H, m, arom. H_7), 7.99 (2H, d, $J = 8.4$ Hz, arom. H_2), 9.14 (1H, t, $J = 5.7$ Hz, CH_2NH); EI-MS m/z : 436 (M^+); HR-EI-MS m/z : (M^+)⁺ calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_3$, 436.1787; found, 436.1763.

4.12. *N*-{4-[(Hydroxyamino)carbonyl]benzyl}[1,1'-biphenyl]-4-carboxamide (**6b**)

4b (0.10 g, 0.23 mmol) was subjected to hydrogenolysis with 10% Pd–C (50 mg) in MeOH (30 mL) in the same way as for **6a**, yielding **6b** (78 mg, 0.22 mmol, 98% yield): mp 326.3 °C; IR (KBr) cm^{-1} : 3294, 1699, 1684, 1636, 1506, 1292, 853; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.62 (2H, d, $J = 5.4$ Hz, PhCH_2), 7.46–7.56 (5H, m, arom. H_5), 7.78–8.08 (8H, m, arom. H_8), 9.28 (1H, t, $J = 5.4$ Hz, CH_2NH); FAB-MS m/z : 369 ($\text{M}+\text{Na}^+$), 347 ($\text{M}+\text{H}^+$); HR-FAB-MS m/z : ($\text{M}+\text{H}^+$)⁺ calcd for $\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_3$, 347.1396; found, 347.1417.

4.13. Methyl 4-[(1-naphthoylamino)methyl]benzoate (**2c**)

1-Naphthoyl chloride (1.8 g, 9.5 mmol) was reacted with **1** (2.0 g, 10.0 mmol) in the presence of Et_3N (3.4 mL, 24.5 mmol) in CH_2Cl_2 (134 mL) in the usual way, yielding

2c (3.7 g, 11.6 mmol, 81.5% yield): mp 162.8 °C; IR (KBr) cm^{-1} : 3267, 3047, 2951, 1729, 1632, 1536, 1434, 1281, 962, 771; ^1H NMR (CDCl_3) δ : 3.93 (3H, s, CO_2Me), 4.78 (2H, d, $J = 6.0$ Hz, PhCH_2), 7.42–8.38 (11H, m, arom. H_{11}); EI-MS m/z : 319.12 (M^+); HR-EI-MS m/z : (M^+) calcd for $\text{C}_{20}\text{H}_{17}\text{NO}_3$, 319.1208; found, 319.1206.

4.14. 4-[(1-Naphthoylamino)methyl]benzoic acid (**3c**)

2c (3.0 g, 9.4 mmol) was hydrolyzed with 1 M LiOH (27 mL, 27 mmol) in a mixture of water (0.5 mL) and THF (16.5 mL), affording **3c** (2.3 g, 7.5 mmol, 78.8% yield): mp 255.1 °C; IR (KBr) cm^{-1} : 3267, 2562, 1692, 1632, 1578, 1536, 1433, 1286, 950, 751; ^1H NMR (CDCl_3) δ : 4.61 (2H, d, $J = 6.0$ Hz, PhCH_2), 7.50–8.04 (11H, m, arom. H_{11}), 9.16 (1H, t, $J = 6.0$ Hz, CH_2NH), 12.95 (1H, br s, CO_2H); EI-MS m/z : 305.11 (M^+); HR-EI-MS m/z : (M^+) calcd for $\text{C}_{19}\text{H}_{15}\text{NO}_3$, 305.1052; found, 305.1066.

4.15. *N*-{4-[(Benzyloxyamino)carbonyl]benzyl}methyl-1-naphthamide (**4c**)

3c (1.0 g, 3.3 mmol) was condensed with *O*-benzylhydroxylamine hydrochloride (0.5 g, 3.1 mmol) in the presence of BOP-Cl (0.9 g, 3.5 mmol) and Et_3N (0.5 mL, 3.6 mmol) in CH_2Cl_2 (45 mL), yielding **4c** (8.6 g, 19.7 mmol, 39.3% yield): mp 198.0 °C; IR (KBr) cm^{-1} : 3270, 3035, 1643, 1518, 1424, 1304, 1256, 1016, 785, 696; ^1H NMR (CDCl_3) δ : 4.58 (2H, d, $J = 6.0$ Hz, PhCH_2), 4.94 (2H, s, OCH_2), 7.33–8.23 (16H, m, arom. H_{16}), 9.14 (1H, t, $J = 6.0$ Hz, CH_2NH), 11.74 (1H, br s, BnONH); EI-MS m/z : 410.16 (M^+); HR-EI-MS m/z : (M^+) calcd for $\text{C}_{26}\text{H}_{22}\text{N}_2\text{O}_3$, 410.1630; found, 410.1611.

4.16. *N*-{4-[(Hydroxyamino)carbonyl]benzyl}methyl-1-naphthamide (**6c**)

4c (0.31 g, 0.73 mmol) was subjected to hydrogenolysis with 10% Pd–C (80 mg) in MeOH (23 mL), furnishing **6c** (0.12 g, 0.37 mmol, 56.0% yield): mp 169.5 °C; IR (KBr) cm^{-1} : 3302, 1637, 1530, 1388, 1303, 1245, 1154, 1031, 900, 783; ^1H NMR (CDCl_3) δ : 4.58 (2H, d, $J = 6.0$ Hz, PhCH_2), 7.46–8.22 (11H, m, arom. H_{11}), 9.00 (1H, s, HONH), 9.13 (1H, t, $J = 6.0$ Hz, CH_2NH), 11.19 (1H, br s, OH); FAB-MS m/z : 321 ($\text{M}+\text{H}^+$); HR-FAB-MS m/z : ($\text{M}+\text{H}^+$) calcd for $\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_3$, 321.1239; found, 321.1219.

4.17. Methyl 4-({[(4'-fluoro[1,1'-biphenyl]-4-yl)carbonyl]amino}methyl)benzoate (**2d**)

4'-Fluoro[1,1'-biphenyl]-4-carboxylic acid (2.8 g, 13 mmol) was condensed with **1** (2.9 g, 14 mmol) in the presence of BOP-Cl (4.18 g, 15.6 mmol) and Et_3N (14.4 mL, 104 mmol) in absolute DMF (80 mL) in the usual way, affording the pure compound **2d** (3.6 g, 9.9 mmol, 76% yield): mp 194.6–196.5 °C; IR (KBr) cm^{-1} : 1720, 1495, 1283, 1240, 1103, 831, 760, 708, 598; ^1H NMR (CDCl_3) δ : 3.91 (3H, s, CO_2Me), 4.73 (2H, d,

$J = 6.4$ Hz, PhCH_2), 6.60 (1H, br s, CH_2NH), 7.15 (2H, t, $J = 8.4$ Hz, arom. H_2), 7.43 (2H, d, $J = 8.4$ Hz, arom. H_2), 7.57 (2H, dd, $J = 1.6, 5.6$ Hz, arom. H_2), 7.61 (2H, d, $J = 8.4$ Hz, arom. H_2), 7.88 (2H, d, $J = 8.4$ Hz, arom. H_2), 8.02 (2H, d, $J = 8.4$ Hz, arom. H_2); EI-MS m/z : 363 (M^+); HR-EI MS m/z : (M^+) calcd for $\text{C}_{22}\text{H}_{18}\text{NO}_3\text{F}$, 363.1271; found, 363.1274.

4.18. 4-({[(4'-Fluoro[1,1'-biphenyl]-4-yl)carbonyl]amino}methyl)benzoic acid (**3d**)

2d (1.1 g, 3.1 mmol) was hydrolyzed with 1 M LiOH (13.0 mL, 13.0 mmol) in a mixture of water (0.5 mL) and THF (17.5 mL), affording **3d** (0.9 g, 2.6 mmol, 83% yield): mp 267.3–269.4 °C; IR (KBr) cm^{-1} : 3292, 3099, 2542, 2341, 1686, 820, 756, 669; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.50 (2H, d, $J = 5.6$ Hz, PhCH_2), 7.23 (2H, d, $J = 7.2$ Hz, arom. H_2), 7.32 (2H, t, $J = 8.8$ Hz, arom. H_2), 7.75–7.82 (6H, m, arom. H_6), 7.99 (2H, d, $J = 8.0$ Hz, arom. H_2), 9.09 (1H, br s, CH_2NH); EI-MS m/z : 349 (M^+); HR-EI-MS m/z : (M^+) calcd for $\text{C}_{21}\text{H}_{16}\text{NO}_3\text{F}$, 349.1114; found, 349.1118.

4.19. *N*-(4-({[(Benzyloxy)amino]carbonyl}benzyl)-4'-fluoro[1,1'-biphenyl]-4-carboxamide (**4d**))

3d (1.0 g, 2.9 mmol) was condensed with *O*-benzylhydroxylamine hydrochloride (0.54 g, 3.4 mmol) in the presence of BOP-Cl (1.3 g, 5.2 mmol) and Et_3N (1.6 mL, 11.4 mmol) and DMF (15 mL) in the usual way, giving rise to **4d** (0.41 g, 0.9 mmol, 40% yield): mp 241.4–243.7 °C; IR (KBr) cm^{-1} : 3321, 3206, 1634, 1495, 1279, 893, 860, 772; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.55 (2H, d, $J = 5.7$ Hz, PhCH_2), 4.92 (2H, s, OCH_2), 7.29–7.60 (8H, m, arom. H_8), 7.71–7.98 (6H, m, arom. H_6), 8.01–8.12 (3H, m, arom. H_3), 9.16 (1H, t, $J = 5.7$ Hz, CH_2NH), 11.7 (1H, s, BnONH); FAB-MS m/z : 455 ($\text{M}+\text{H}^+$); HR-FAB-MS m/z : ($\text{M}+\text{H}^+$) calcd for $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_3\text{F}$, 455.1771; found, 455.1747.

4.20. 4'-Fluoro-*N*-{4-[(hydroxyamino)carbonyl]benzyl}-[1,1'-biphenyl]-4-carboxamide (**6d**)

4d (0.10 g, 0.22 mmol) was subjected to hydrogenolysis with 10% Pd–C (70 mg) in MeOH (30 mL), yielding **6d** (57 mg, 0.16 mmol, 71% yield): mp (dec) 218.7 °C; IR (KBr) cm^{-1} : 3267, 3049, 2806, 1880, 1641, 1612, 1537, 1514, 1470, 829, 770, 739, 719, 692, 594, 523, 436; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.54 (2H, d, $J = 5.7$ Hz, PhCH_2), 7.29–7.44 (4H, m, arom. H_4), 7.70–8.00 (8H, m, arom. H_8), 9.14 (1H, br s, CH_2NH); FAB-MS m/z : 365 ($\text{M}+\text{H}^+$); HR-FAB-MS m/z : ($\text{M}+\text{H}^+$) calcd for $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}_3\text{F}$, 365.1301; found, 365.1308.

4.21. Methyl 4-({[(4'-methoxy[1,1'-biphenyl]-4-yl)carbonyl]amino}methyl)benzoate (**2e**)

4'-Methoxy[1,1'-biphenyl]-4-carboxylic acid (1.9 g, 8.3 mmol) was condensed with **1** (2.2 g, 10.9 mmol) in

the presence of BOP-Cl (3.82 g, 15.0 mmol) and Et₃N (4.6 mL, 33.0 mmol) in absolute DMF (51 mL) in the usual way, affording the pure compound **2e** (2.1 g, 5.6 mmol, 67% yield): mp 216.9–219.1 °C; IR (KBr) cm⁻¹: 3271, 1715, 1607, 1498, 1246, 1109, 1034, 818, 750; ¹H NMR (DMSO-*d*₆) δ: 3.77 (3H, s, OMe), 3.85 (3H, s, -CO₂Me), 4.60 (2H, d, *J* = 5.6 Hz, PhCH₂), 7.04–7.47 (2H, m, arom.H₂), 7.69–8.00 (10H, m, arom.H₁₀), 9.14 (1H, t, *J* = 5.6 Hz, CH₂NH); EI-MS *m/z*: 375 (M)⁺, 211; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₃H₂₁NO₄, 375.1471; found, 375.1451.

4.22. Methyl 4-({[4'-methoxy[1,1'-biphenyl]-4-yl]carbonyl}amino)methylbenzoic acid (**3e**)

2e (2.0 g, 5.3 mmol) was hydrolyzed with 1 M LiOH (21.3 mL, 21.3 mmol) in a mixture of water (0.8 mL) and THF (29 mL), affording **3e** (1.5 g, 4.2 mmol, 80% yield): mp 270.3–273.3 °C; IR (KBr) cm⁻¹: 3288, 2544, 1686, 1630, 1429, 1288, 827, 770; ¹H NMR (DMSO-*d*₆) δ: 3.81 (3H, s, -OMe), 4.57 (2H, d, *J* = 5.7 Hz, PhCH₂), 7.03–8.00 (12H, m, arom.H₁₂), 9.16 (1H, t, *J* = 5.7 Hz, CH₂NH); EI-MS *m/z*: 361 (M)⁺, 211; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₃H₁₉NO₄, 361.1314; found, 361.1318.

4.23. *N*-(4-({[Benzyloxy]amino}carbonyl}benzyl)-4'-methoxy[1,1'-biphenyl]-4-carboxamide (**4e**)

3e (1.4 g, 3.9 mmol) was condensed with *O*-benzylhydroxylamine hydrochloride (0.74 g, 4.6 mmol) in the presence of BOP-Cl (1.9 g, 7.5 mmol) and Et₃N (3.5 mL, 2.6 mmol) and DMF (23 mL) in the usual way, giving rise to **4e** (0.19 g, 0.41 mmol, 11.0% yield): mp 179.5–182.6 °C; IR (KBr) cm⁻¹: 3287, 3036, 2924, 1713, 1628, 1256, 1103, 827; ¹H NMR (CDCl₃) δ: 3.81 (3H, s, OMe), 4.72 (2H, d, *J* = 5.9 Hz, PhCH₂), 5.36 (2H, s, OCH₂), 6.55 (1H, br s, CH₂NH), 6.97–8.07 (17H, m, arom.H₁₇); EI-MS *m/z*: 466 (M)⁺, 375, 211; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₉H₂₆N₂O₄, 466.1893; found, 466.1912.

4.24. *N*-(4-({[Hydroxy]amino}carbonyl}benzyl)-4'-methoxy[1,1'-biphenyl]-4-carboxamide (**6e**)

4e (0.1 g, 0.21 mmol) was subjected to hydrogenolysis with 10% Pd–C (70 mg) in MeOH (30 mL), yielding **6e** (46.7 mg, 0.12 mmol, 60.0% yield): mp 261.6 °C (dec); IR (KBr) cm⁻¹: 3265, 2924, 1636, 1697, 1182, 1036, 829; ¹H NMR (CDCl₃) δ: 3.81 (3H, s, OMe), 4.57 (2H, d, *J* = 5.1 Hz, PhCH₂), 6.87–8.00 (12H, m, arom.H₁₂), 9.17 (1H, s, CH₂NH); EI-MS *m/z*: 376 (M)⁺, 307, 273; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₂H₂₀N₂O₄, 376.1423; found, 376.1431.

4.25. Methyl 4-({[6-[(dimethylamino)-2-naphthoyl]amino]-methyl}benzoate) (**2f**)

6-(Dimethylamino)-2-naphthoic acid (4.3 g, 20.0 mmol) was condensed with **1** (3.3 g, 20.0 mmol) in the presence of BOP-Cl (5.6 g, 22.0 mmol) and Et₃N (11.2 mL,

80.7 mmol) in absolute DMF (100 mL) in the usual way, affording the pure compound **2f** (2.5 g, 11.4 mmol, 57.2% yield). mp 188.0–190.5 °C; IR (KBr) cm⁻¹: 3288, 1703, 1632, 1603, 1547, 1319, 1111, 806, 685, 487; ¹H NMR (CDCl₃) δ: 3.08 (6H, s, N(CH₃)₂), 3.91 (3H, s, -CO₂Me), 4.74 (2H, d, *J* = 5.7 Hz, PhCH₂), 6.90 (1H, s, CH₂NH), 7.10–8.10 (10H, m, arom.H₁₀); EI-MS *m/z*: 362 (M)⁺, 198, 170; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₂H₂₂N₂O₃, 362.1630; found, 362.1623.

4.26. 4-({[6-[(Dimethylamino)-2-naphthoyl]amino]-methyl}benzoic acid (**3f**)

2f (0.90 g, 2.5 mmol) was hydrolyzed with 1 M LiOH (5.8 mL, 5.8 mmol) in a mixture of water (0.3 mL) and THF (11.2 mL), affording **3f** (0.50 g, 1.4 mmol, 55.3% yield). mp 167.0–171.2 °C; IR (KBr) cm⁻¹: 3288, 1692, 1632, 1547, 1283, 1111, 856, 806, 469; ¹H NMR (DMSO-*d*₆) δ: 3.04 (6H, s, N(CH₃)₂), 4.57 (2H, d, *J* = 4.3 Hz, PhCH₂), 6.9–8.3 (10H, m, arom.H₁₀), 9.07 (1H, br s, CH₂NH), 12.84 (1H, s, CO₂H); EI-MS *m/z*: 348 (M)⁺, 198, 170; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₁H₂₀N₂O₃, 348.1474; found, 348.1497.

4.27. *N*-{4-[(Benzyloxyamino)carbonyl]benzyl}-6-(dimethylamino)-2-naphthamide (**4f**)

3f (0.37 g, 1.06 mmol) was condensed with *O*-benzylhydroxylamine hydrochloride (0.17 g, 1.06 mmol) in the presence of BOP-Cl (0.30 g, 1.2 mmol) and Et₃N (0.59 mL, 4.2 mmol) in DMF (5.3 mL) in the usual way, giving rise to **4f** (0.12 g, 0.27 mmol, 25% yield): mp 128.6–129.8 °C; IR (KBr) cm⁻¹: 3285, 1701, 1632, 1504, 1321, 1113, 907, 854, 808, 750, 469; ¹H NMR (CDCl₃) δ: 3.08 (6H, s, N(CH₃)₂), 4.74 (2H, d, *J* = 5.9 Hz, PhCH₂), 4.87 (2H, s, OCH₂), 6.58 (1H, br s, CH₂NH), 6.89 (1H, s, BnONH), 7.19–8.18 (10H, m, arom.H₁₀); EI-MS *m/z*: 453 (M)⁺, HR-EI-MS *m/z*: (M)⁺ calcd for C₂₈H₂₇N₃O₃, 453.2052; found, 453.2065.

4.28. 6-Dimethylamino-*N*-{4-[(hydroxyamino)carbonyl]benzyl}-2-naphthamide (**6f**)

4f (0.1 g, 0.22 mmol) was subjected to hydrogenolysis with 10% Pd–C (20 mg) in MeOH (20 mL), yielding **6f** (88 mg, 0.19 mmol, 88% yield): mp 212.5–216.0 °C; IR (KBr) cm⁻¹: 3296, 1692, 1626, 1531, 1288, 853, 808, 750; ¹H NMR (CD₃OD) δ: 2.97 (6H, s, N(CH₃)₂), 4.56 (2H, s, PhCH₂), 6.8–8.1 (10H, m, arom.H₁₀); FAB-MS *m/z*: 364 (M+H)⁺, HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₂₁H₂₂N₃O₃, 364.1661; found, 364.1670.

4.29. Methyl 4-({[6-[(*tert*-butoxycarbonyl)amino]-2-naphthoyl]amino)methylbenzoate (**2g**)

To a suspension of (20.0 g, 70.0 mmol), 4-[(6-[(*tert*-butoxycarbonyl)amino]-2-naphthoic acid, HONB (15.6 g, 84.0 mmol), and WSCI (13.0 mL, 84.0 mmol) in absolute DMF (300 mL) was added a suspension of **1** (14.1 g,

70.0 mmol) and Et₃N (10.7 mL, 77.0 mmol) in absolute DMF (300 mL). The mixture was stirred at room temperature for 7 h. After being evaporated, the residue was dissolved in CHCl₃ (1000 mL) and washed successively with satd NaHCO₃ (3 × 100 mL), brine (3 × 100 mL). The organic layer was dried, and evaporated to dryness. The resulting residue was purified by silica gel column chromatography, eluting with CHCl₃ to give **2g** (7.5 g, 17.3 mmol, 24.6% yield): mp 165.1–165.6 °C; IR (KBr) cm⁻¹: 3328, 3300, 1720, 1693, 1631, 1523, 1244, 1168, 1055, 881, 852, 680, 636, 570, 476; ¹H NMR (DMSO-*d*₆ δ: 1.51 (9H, s, ^tBu), 3.83 (3H, s, CO₂Me), 4.58 (2H, d, *J* = 5.7 Hz, PhCH₂), 7.4–8.4 (10H, m, arom.H₁₀), 9.20 (1H, t, *J* = 5.7 Hz, CH₂NH), 9.68 (1H, br s, BocNH); EI-MS *m/z*: 434 (M)⁺; HR-EI-MS *m/z*: (M)⁺ calcd for C₂₅H₂₆N₂O₅, 434.1842; found, 434.1857.

4.30. 4-[(6-[(*tert*-Butoxycarbonyl)amino]-2-naphthyl)amino]methylbenzoic acid (**3g**)

2g (7.0 g, 16.1 mmol) was hydrolyzed with 1 M LiOH (64.0 mL, 64.0 mmol) in a mixture of water (2 mL) and THF (73 mL), to give the pure compound **3g** (6.5 g, 15.5 mmol, 96% yield): mp 194.2–194.4 °C; IR (KBr) cm⁻¹: 3328, 1697, 1631, 1531, 1427, 1170, 1055, 804, 478; ¹H NMR (DMSO-*d*₆ δ: 1.51 (9H, s, ^tBu), 4.55 (2H, d, *J* = 5.7 Hz, PhCH₂), 7.3–8.4 (10H, m, arom.H₁₀), 9.15 (1H, t, *J* = 5.9 Hz, CH₂NH), 9.69 (1H, s, BocNH); FAB-MS *m/z*: 421 (M+H)⁺; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₂₄H₂₅N₂O₅, 421.1763; found, 421.1744.

4.31. *tert*-Butyl-6-[(4-[(benzyloxy)amino]carbonyl]benzyl)amino]carbonyl-2-naphthylcarbamate (**4g**)

To a suspension of **3g** (6.5 g, 15.4 mmol), HONB (2.8 g, 15.4 mmol), and WSCI (2.4 mL, 15.4 mmol) in absolute DMF (130 mL) was added a suspension of *O*-benzylhydroxylamine hydrochloride (2.5 g, 15.4 mmol) and Et₃N (2.1 mL, 15.4 mmol) in absolute DMF (160 mL). The mixture was stirred at room temperature for 8 h. After being evaporated, the residue was dissolved in CHCl₃ (500 mL) and washed successively with satd NaHCO₃ solution (3 × 100 mL), brine (3 × 100 mL). The organic layer was dried, and evaporated to dryness. The resulting residue was recrystallized from AcOEt–MeOH to give **4g** (1.8 g, 3.4 mmol, 22% yield): mp 171.0–171.8 °C; IR (KBr) cm⁻¹: 3252, 1666, 1631, 1542, 1496, 1163, 1070, 914, 883, 744, 617; ¹H NMR (DMSO-*d*₆ δ: 1.51 (9H, s, ^tBu), 4.56 (2H, d, *J* = 5.7 Hz, PhCH₂), 4.91 (2H, s, OCH₂), 7.3–8.4 (15H, m, arom.H₁₅), 9.19 (1H, t, *J* = 5.7 Hz, CH₂NH), 9.70 (1H, s, BocNH), 11.74 (1H, s, BnONH); FAB-MS *m/z*: 526 (M+H)⁺, 347; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₃₁H₃₂N₃O₅, 526.2342; found, 526.2368.

4.32. 6-Amino-*N*-{4-[(hydroxyamino)carbonyl]benzyl}-2-naphthamide hydrochloride (**6g**)

5% Pd–C (150 mg) was added to a solution of **4g** (1.5 g, 2.9 mmol) in MeOH (300 mL), and the mixture was

stirred in a stream of H₂ overnight. The catalyst was removed by filtration and washed with MeOH. The filtrate was evaporated and the resulting residue was dissolved in MeOH (500 mL). To this solution was added 12 N HCl (50 mL), and the mixture was stirred for 1 h at room temperature. After being evaporated, the resulting residue was recrystallized from Et₂O to give the pure compound **6g** (0.7 g, 1.9 mmol, 65% yield): mp 190.0–190.8 °C; IR (KBr) cm⁻¹: 3300, 1612, 1305, 1211, 895, 815; ¹H NMR (DMSO-*d*₆ δ: 4.55 (2H, d, *J* = 4.5 Hz, PhCH₂), 7.4–8.5 (10H, m, arom.H₁₀), 9.30 (1H, t, *J* = 4.5 Hz, CH₂NH), 10.33 (1H, s, HONH); FAB-MS *m/z*: 335 (M)⁺; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₁₉H₁₈N₃O₃, 336.1348; found, 336.1362.

4.33. Methyl 4-[(2-quinolinylcarbonyl)amino]methylbenzoate (**2h**)

Quinaldic acid (13.3 g, 77.0 mmol) was condensed with **1** (15.5 g, 77.0 mmol) in the presence of HONB (13.8 g, 77.0 mmol), WSCI (12.0 mL, 77.0 mmol) and Et₃N (10.7 mL, 77.0 mmol) in absolute DMF (400 mL) in the usual way, giving the pure compound **2h** (8.5 g, 26.6 mmol, 34.4% yield): mp 117.0–118.1 °C; IR (KBr) cm⁻¹: 3325, 1719, 1666, 1528, 1277, 1107, 777; ¹H NMR (CDCl₃) δ: 3.90 (3H, s, CO₂Me), 4.79 (2H, d, *J* = 6.2 Hz, PhCH₂), 7.45–8.36 (10H, m, arom.H₁₀); FAB-MS *m/z*: 321 (M+H)⁺, 307, 289, 232, 225, 154, 137, 79; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₁₉H₁₇N₂O₃, 321.1239; found 321.1244.

4.34. 4-[(2-Quinolinylcarbonyl)amino]methylbenzoic acid (**3h**)

2h (8.5 g, 26.5 mmol) was hydrolyzed with 1 M LiOH (107 mL, 107 mmol) in a mixture of water (5 mL) and THF (155 mL), giving the pure compound **3h** (0.9 g, 2.6 mmol, 83% yield): mp 223.4–226.6 °C; IR (KBr) cm⁻¹: 3329, 1709, 1649, 1537, 1236, 775; ¹H NMR (DMSO-*d*₆ δ: 4.63 (2H, d, *J* = 6.5 Hz, PhCH₂), 7.45–8.60 (10H, m, arom.H₁₀); FAB-MS *m/z*: 307 (M+H)⁺, 289, 232, 225, 154, 137, 79; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₁₈H₁₅N₂O₃, 307.1083; found, 307.1091.

4.35. *N*-(4-[(Benzyloxy)amino]carbonylbenzyl)-2-quinolinecarboxamide (**4h**)

3h (7.2 g, 23.6 mmol) was condensed with *O*-benzylhydroxylamine hydrochloride (3.8 g, 23.6 mmol) in the presence HONB (4.2 g, 23.6 mmol), WSCI (3.7 mL, 23.6 mmol) and Et₃N (3.3 mL, 23.6 mmol) in absolute DMF (260 mL) in the usual way, giving the pure compound **4h** (3.5 g, 10.9 mmol, 35.7% yield): mp 153.6–155.0 °C; IR (KBr) cm⁻¹: 3325, 1680, 1625, 1574, 1533, 1242, 654; ¹H NMR (CDCl₃) δ: 4.75 (2H, s, PhCH₂), 5.03 (2H, s, OCH₂), 7.25–8.09 (15H, m, arom.H₁₅); FAB-MS *m/z*: 412 (M+H)⁺, 330, 307, 289, 232, 157, 137, 79; HR-FAB-MS *m/z*: (M+H)⁺ calcd for C₂₅H₂₂N₃O₃, 412.1661; found, 412.1669.

4.36. *N*-{4-[(Hydroxyamino)carbonyl]benzyl}-2-quinoline-carboxamide (6h)

4h (3.5 g, 8.5 mmol) was subjected to hydrogenolysis with 5% Pd–C (350 mg) in MeOH (300 mL), yielding the pure compound **6h** (2.6 g, 8.1 mmol, 96% yield); mp 90.4–94.3 °C; IR (KBr) cm^{-1} : 3320, 3210, 1632, 1497, 1332, 750; ^1H NMR (CD_3OD) δ : 4.63 (2H, s, PhCH_2), 7.20–8.12 (10H, m, arom. H_{10}); FAB-MS m/z : 322 ($\text{M}+\text{H}$) $^+$; HR-FAB-MS m/z : ($\text{M}+\text{H}$) $^+$ calcd for $\text{C}_{18}\text{H}_{16}\text{N}_3\text{O}_3$, 322.1192; found, 322.1219.

4.37. Methyl 4-[(3-quinolinylcarbonyl)amino]methyl]-benzoate (2i)

3-Quinoline carboxylic acid (5.2 g, 29.7 mmol) was condensed with **1** (6.0 g, 29.7 mmol) in the presence of HONB (5.3 g, 29.7 mmol), WSCI (5.2 mL, 29.7 mmol) and Et_3N (4.0 mL, 29.7 mmol) in absolute DMF (110 mL) in the usual way, giving the pure compound **2i** (7.8 g, 24.3 mmol, 82% yield); mp 117.0–118.1 °C; IR (KBr) cm^{-1} : 3325, 1719, 1666, 1528, 1277, 1107, 777; ^1H NMR (CDCl_3) δ : 3.90 (3H, s, CO_2Me), 4.79 (2H, d, $J = 6.2$ Hz, PhCH_2), 7.45–8.36 (10H, m, arom. H_{10}); FAB-MS m/z : 321 ($\text{M}+\text{H}$) $^+$, 307, 289, 232, 225, 154, 137, 79; HR-FAB-MS m/z : ($\text{M}+\text{H}$) $^+$ calcd for $\text{C}_{19}\text{H}_{17}\text{N}_2\text{O}_3$, 321.1239; found 321.1244.

4.38. 4-[(3-Quinolinylcarbonyl)amino]methyl]benzoic acid (3i)

2i (3.2 g, 10.0 mmol) was hydrolyzed with 1 M LiOH (40.0 mL, 40.0 mmol) in a mixture of water (2 mL) and THF (62 mL), affording the pure compound **3i** (3.0 g, 9.8 mmol, 98% yield); mp 273.8–276.3 °C; IR (KBr) cm^{-1} : 3331, 1688, 1651, 1502, 1319, 1290, 1242, 789; ^1H NMR ($\text{DMSO}-d_6$) δ : 4.64 (2H, d, $J = 6.0$ Hz, PhCH_2), 7.49–8.11 (8H, m, arom. H_8), 8.89 (1H, s, arom. H_1), 9.33 (1H, s, arom. H_1); FAB-MS m/z : 307 ($\text{M}+\text{H}$) $^+$, 232, 225, 157, 137, 136, 79; HR-FAB-MS m/z : 307 ($\text{M}+\text{H}$) $^+$ calcd for $\text{C}_{18}\text{H}_{15}\text{N}_2\text{O}_3$, 307.1083; found, 307.1095.

4.39. *N*-(4-[(Tetrahydro-2H-pyran-2-yloxy)amino]carbonyl]benzyl)-3-quinolinecarboxamide (5i)

HONB (0.61 g, 3.4 mmol) and WSCI (0.60 mL, 3.4 mmol) were added to a solution of **3i** (4.0 g, 13.1 mmol) in CH_2Cl_2 (30 mL) and absolute DMF (6 mL), and the mixture was stirred at room temperature for 30 min. To this solution was added a solution of *O*-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.4 g, 3.4 mmol) and Et_3N (0.34 mL, 3.4 mmol) in CH_2Cl_2 (60 mL) and absolute DMF (6 mL). After stirring at room temperature for 7 h, the reaction mixture was diluted with CHCl_3 (70 mL), washed with satd NaHCO_3 (3 $\times$ 20 mL) and satd NaCl (3 $\times$ 20 mL) and dried. Evaporation of the solvent gave a residue, which was purified with silica gel column chromatography with EtOAc, yielding **5i** (0.72 g, 2.3 mmol, 68% yield). mp 163.7–167.3 °C; IR (KBr) cm^{-1} : 2932, 1632, 1545, 1479,

1313, 1296, 1281, 1115, 900; ^1H NMR (CD_3OD) δ : 1.60–1.87 (6H, m, $\text{CH}_2 \times 6$), 3.60–4.12 (2H, m, CH_2), 4.67 (2H, s, PhCH_2), 5.05 (1H, s, CH), 7.50–8.08 (8H, m, arom. H_8), 8.83 (1H, d, $J = 2.0$ Hz, arom. H_1), 9.28 (1H, d, $J = 2.0$ Hz, arom. H_1); FAB-MS m/z : 406 ($\text{M}+\text{H}$) $^+$, 322, 306, 262; HR-FAB-MS m/z : ($\text{M}+\text{H}$) $^+$ calcd for $\text{C}_{23}\text{H}_{24}\text{N}_3\text{O}_4$, 406.1767; found, 406.1791.

4.40. *N*-{4-[(Hydroxyamino)carbonyl]benzyl}-3-quinolinecarboxamide (6i)

A solution of **5i** (0.58 g, 1.4 mmol) in AcOH (8 mL), THF (2 mL), and H_2O (1 mL) was heated at reflux overnight. After being evaporated, the resulting residue was recrystallized from EtOH to give the pure compound **6i** (0.33 g, 1.0 mmol, 71.4% yield); mp 153.8–159.4 °C; IR (KBr) cm^{-1} : 3070, 1700, 1649, 1535, 1308, 1016, 899; ^1H NMR (CD_3OD) δ : 4.60 (2H, s, PhCH_2), 7.24–8.00 (8H, m, arom. H_8), 8.73 (1H, s, arom. H_1), 9.18 (1H, s, arom. H_1); FAB-MS m/z : 322 ($\text{M}+\text{H}$) $^+$, 391, 307, 289, 232, 154, 137, 79; HR-FAB-MS m/z : ($\text{M}+\text{H}$) $^+$ calcd for $\text{C}_{18}\text{H}_{16}\text{N}_3\text{O}_3$, 322.1192; found, 322.1181.

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References and notes

- (a) Wade, P. A. *Hum. Mol. Genet.* **2001**, *10*, 693–698; (b) Cress, W. D.; Seto, E. *J. Cell. Physiol.* **2000**, *184*, 1–16; (c) Marks, P.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K. *Nat. Rev. Cancer* **2001**, *1*, 194–202.
- (a) Jung, M. *Curr. Med. Chem.* **2001**, *8*, 1505–1511; (b) Grozinger, C. M.; Schreiber, S. L. *Chem. Biol.* **2002**, *9*, 3–16; (c) Johnstone, R. W. *Nat. Rev. Drug. Discov.* **2002**, *1*, 287–299; (d) Kramer, O. H.; Gottlicher, M.; Heinzl, T. *Trends Endocrinol. Metab.* **2001**, *12*, 294–300.
- Kelly, W. K.; Richon, V. M.; O'Connor, O.; Curley, T.; MacGregor-Curtelli, B.; Tong, W.; Klang, M.; Schwartz, L.; Richardson, S.; Rosa, E.; Drobnjak, M.; Cordon-Cordo, C.; Chiao, J. H.; Rifkind, R.; Marks, P. A.; Scher, H. *Clin. Cancer Res.* **2003**, *9*, 3578–3588.
- Nemunaitis, J. J.; Orr, D.; Eager, R.; Cunningham, C. C.; Williams, A.; Mennel, R.; Grove, W.; Olson, S. *Cancer J.* **2003**, *9*, 58–66.
- Uesato, S.; Kitagawa, M.; Nagaoka, Y.; Maeda, T.; Kuwajima, H.; Yamori, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1347–1349.
- Finnin, M. S.; Donigian, J. R.; Gohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188–193.
- Lu, Q.; Yang, Y.-T.; Chen, C.-S.; Davis, M.; Byrd, J. C.; Etherton, M. R.; Umar, A.; Chen, C.-S. *J. Med. Chem.* **2004**, *47*, 467–474.

8. Venditti, J. M. *Cancer Treat. Rep.* **1983**, 67, 767–772.
9. Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, 265, 17174–17179.
10. Mori, H.; Sakamoto, K.; Tsurumi, Y.; Takase, S.; Hino, M. WO 00/21979, 2000; *Chem. Abstr.* 2000, 132, 288796.
11. Takenouchi, T.; Muneakata, E. *Life Sci.* **1995**, 56, 479–484.