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Original article

Synthesis and cancer antiproliferative activity of new histone deacetylase inhibitors: hydrophilic hydroxamates and 2-aminobenzamide-containing derivatives

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Abstract

New series histone deacetylase inhibitors comprising a hydroxamic acid or 2-aminobenzamide group as a zinc-chelating function were synthesized and evaluated for antiproliferative activities against a panel of human cancer cells. The 2-aminobenzamide series inhibitors generally had the potency in cell growth inhibitions comparable to that of MS-275. Among them, the compound having a (3,4-difluorobenzyl)(2-hydroxyethyl) amino group at one end and a 2-aminobenzamide group at the other of molecule showed the most promising profile as an anticancer drug candidate, since it had a comparatively low toxicity as did MS-275 against a normal fibroblast cell CCD-1059SK. Additionally, the derivative exhibited a high recovery in human plasma stability test.

Keywords: Histone deacetylase inhibitor; Anticancer drug; Hydrophilic hydroxamate HDAC inhibitor; 2-Aminobenzamide-containing HDAC inhibitor; Tandem mass spectrometry; Plasma stability

1. Introduction

Acetylation of the N-terminal region of histone proteins promotes gene expression [1,2]. The aberrant recruitment of transcription corepressors, histone deacetylases (HDACs), results in hypoacetylation of the histone proteins and suppression of gene transcriptional activities, consequently, leading to malignant cell proliferation. Thus, inhibition of HDACs, which induces histone hyperacetylation, provides a potential target for the development of synthetic anticancer drugs [3–7]. A variety of HDAC inhibitors possessing a hydroxamic acid or non-hydroxamic acid moiety such as a 2-aminobenzamide group as a zinc-binding function have so far been reported. Among them, suberoylanilide hydroxamic acid (SAHA) [8,9], FK228 [10–12], MS-275 [13,14], CI-994 [15,16], LAQ824 [17,18] and PDX101 [7] are under clinical trials (Fig. 1).

In the course of our studies to synthesize new HDAC inhibitors, we attained to the hydroxamate **1**, which displayed inhibitory activities stronger than those of SAHA against a panel of cancer cells [19,20]. It was disclosed that **1** arrests the p53-mutated MG 63 human osteosarcoma cells in the G_2/M phase by stimulating p21/WAF1 gene promoter activity [21]. This finding suggested that the increment of the p21/WAF1 protein level by **1** is deeply associated with its antiproliferative activities against cancer cells. Compound **1** showed the survival effect (T/C 185%) at a dosage of 80 mg kg^{-1} in the P388 cell-inoculated mice experiments. However, it declined to T/C 153% at 160 mg kg^{-1} with diarrhea and two mice deaths [20]. This toxicity may be due to the metabolic instability of the 6-amino-2-naphthylcarbonyl or hydroxamic acid group in **1** or to its insufficient solubility in a resolvent (10% HCO60/PBS). Some hydroxamates are prone to hydrolysis giving hydroxylamine which has potent mutagenic properties [22].

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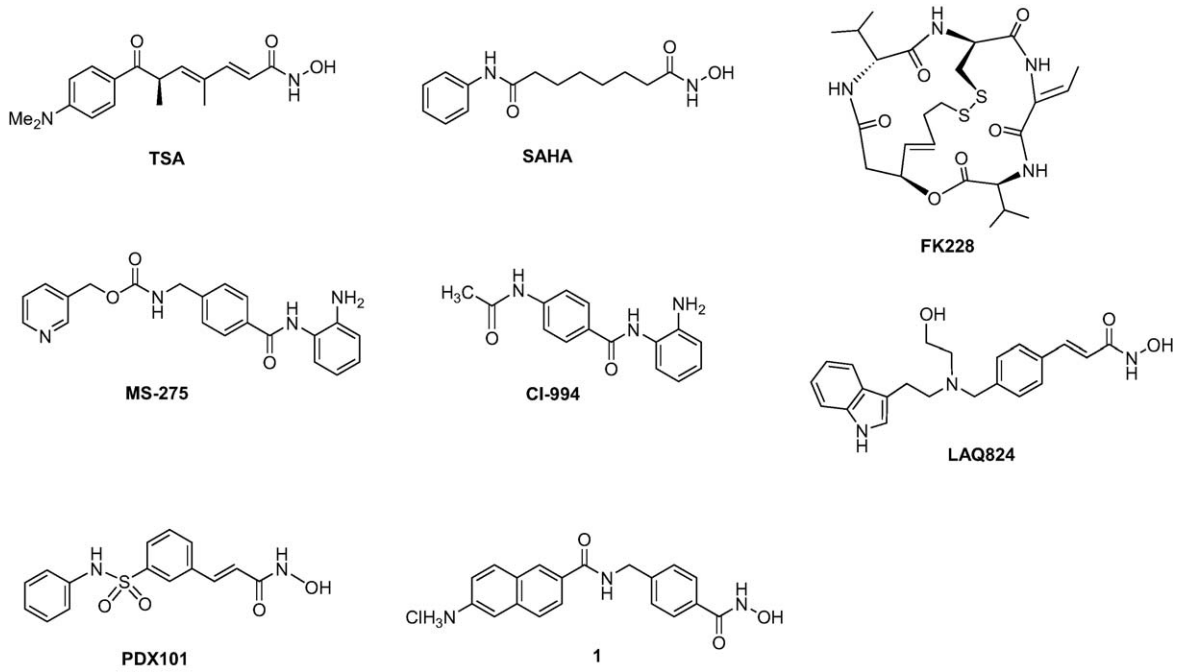


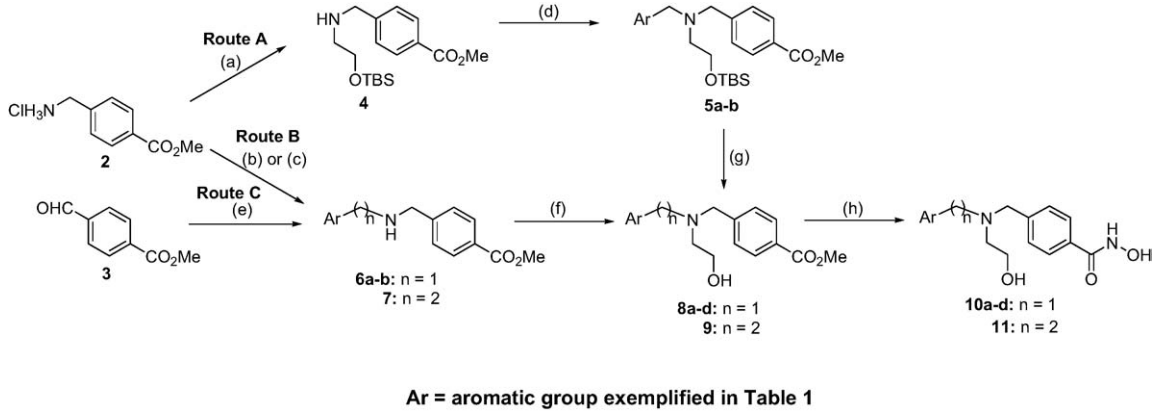
Fig. 1. Structures of HDAC inhibitors.

In the present study, aiming at more potent HDAC inhibitors, we have tired to design not only more hydrophilic analogs of **1**, but also those in which the hydroxamic acid is replaced by another chelating group, a 2-aminobenzamide such as in MS-275 or CI-994.

2. Chemistry

New hydroxamate (hydroxamic acid series) HDAC inhibitors **10a**–**d** and **11** having a (2-hydroxyethyl)(arylalkyl)amino group [17] were synthesized in expectation of improvement in water solubility. Additionally, non-hydroxamate (2-aminobenzamide series) inhibitors **21a**–**f** possessing a (2-hydroxyethyl)(arylalkyl)amino group were synthesized, since MS-275 and CI-994 comprising the 2-aminophenyl moiety in place of the hydroxamic acid has an excellent bioavailability in the in vivo test [13–16].

Scheme 1 exhibited the synthetic routes of the new hydroxamic acid series compounds **10a**–**d** and **11**. Route A: Reductive amination of methyl 4-(aminomethyl)benzoate hydrochloride (**2**) with (*tert*-butyldimethylsilyloxy)acetaldehyde using $NaBH_4$ gave secondary amine **4**. Reductive amination of **4** with 3-quinolinecarboxaldehyde and 3-pyridinecarboxaldehyde using $NaBH_3CN$ gave rise to tertiary amines **5a** and **5b**, respectively. Deblocking of TBS in **5a** and **5b** with TFA afforded **8a** and **8b**, which were then reacted with hydroxylamine in the presence of KOH, yielding the desired **10a** and **10b**, respectively. Route B: Reductive amination of **2** with 2-naphthaldehyde and 1,3-benzodioxol-5-carboxaldehyde using $NaBH_4$ or $NaBH_3CN$ furnished arylamines **6a** and **6b**, which were in turn reacted with 2-bromoethanol in the presence of K_2CO_3 to give **8c** and **8d**, respectively. These compounds were converted to **10c** and **10d**, respectively, in the same way as for **10a** and **10b**. Route C: Reductive amination of methyl 4-for-



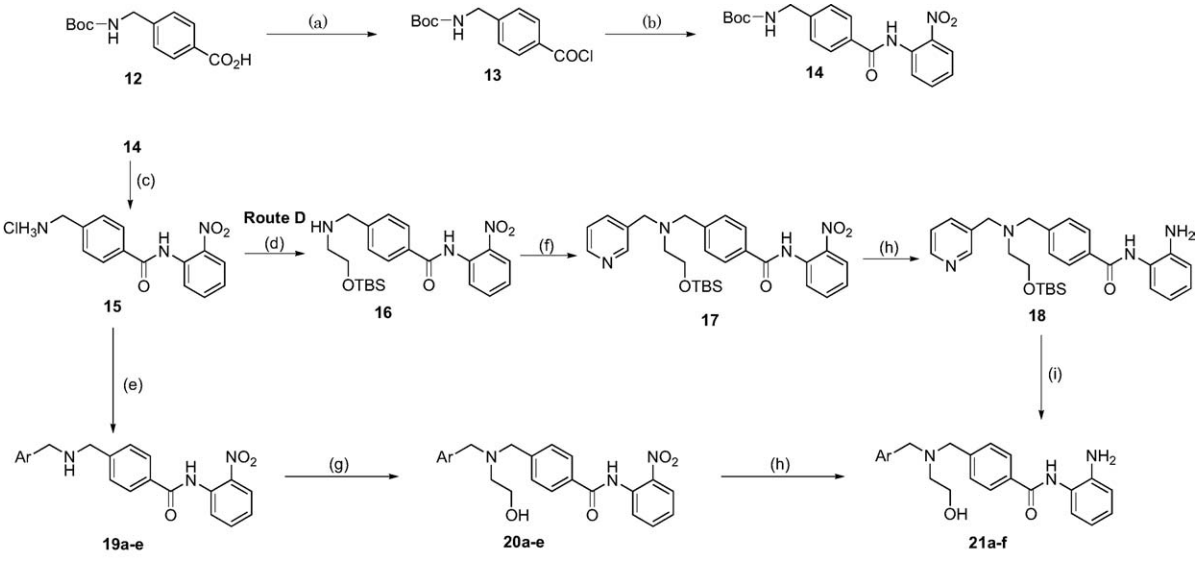
Scheme 1. Conditions: (a) Et_3N, OHC–CH_2–OTBS, $NaBH_4/CH_2Cl_2$; (b) Et_3N, ArCHO, $NaBH_4/CH_2Cl_2$; (c) Et_3N, ArCHO, AcOH, $NaBH_3CN$/MeOH; (d) ArCHO, AcOH, $NaBH_3CN$/MeOH; (e) 3-(2-aminoethyl)indole, AcOH, $NaBH_3CN$/MeOH; (f) K_2CO_3, $Br(CH_2)_2OH$/MeCN; (g) 95% TFA; (h) KOH, NH_2OH/MeOH.

mylbenzoate **3** with 3-(2-aminoethyl)indole using $NaBH_3CN$ yielded amino ester **7**, which was converted to **11** via **9** by the Route B. Furthermore, Scheme 2 showed the synthetic routes of 2-aminobenzamide series compounds **21a**–**f**. Carboxylic acid **12** was treated with oxalyl chloride to give acyl chloride **13**, which was condensed with *o*-nitroaniline to yield amide **14**. Removal of Boc in **14** with 12 N HCl/MeOH provided hydrochloride **15**, which was in turn treated through Route A to give compound **17**. The nitro group in **17** was reduced with $SnCl_2{\cdot}2H_2O$ and NH_4OAc to furnish compound **18**, which was then converted to **21a** in the same way as for **8a** and **8b** (Route D). Compound **15** was converted, via **19a**–**e**, to compounds **20a**–**e** according to the synthetic procedure of **8a**–**d** and **9**. These compounds were then converted to **21b**–**f**, respectively, by reduction of the nitro group as previously mentioned.

3. Results and discussion

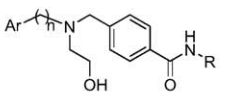
As the exploratory screening for the synthesized compounds, we first evaluated the solubility in 10% HCO60/H_2O, HDACs inhibitory activities and HCT116 (colon carcinoma cells) antiproliferative activities. Table 1 indicates the data for newly synthesized HDAC inhibitors as well as for the known inhibitors **1**, SAHA and MS-275 [23] instead of positive references. Of the hydroxamic acid series compounds, **10a** and **10c** having a bicyclic arylmethyl group retained the potency of **1** in the HDACs and HCT116 inhibitory activities, whereas **10b** and **10d** having a monocyclic arylmethyl group and **11** having an indolylethyl group exhibited rather lower activities in concordance with the previously reported results [19,20]. As expected, introduction of a 2-hydroxyethylamino group at molecule led to a marked improvement of water solubility (more than 20 mg ml^{-1} 10% HCO60/H_2O) except for an oily **10b** as compared with those of **1** and SAHA (each 8 mg ml^{-1} 10% HCO60/H_2O). All the 2-aminobenzamide series compounds had more or less the same HCT116 cell growth inhibition and water solubility as did MS-275 except for **21a**.

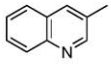
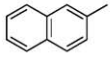
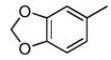
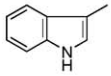
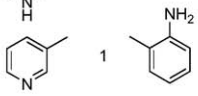
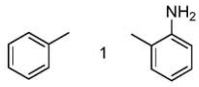
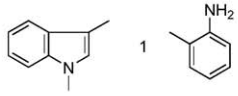
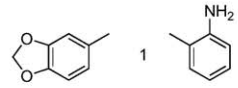
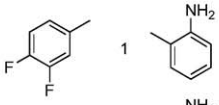
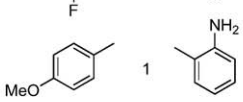
Compounds **10a** and **10c** as well as **21b**–**f** showing a high potency in the first screening tests were assessed further for growth inhibitions against a panel of human cancer cells: hepatoma (HepG2), colon (HCT116 and SW620), breast (SKBR3, MDA-MB-231 and MCF-7) and non-small cell lung (A549). Additionally, they were examined for the toxicity to a normal fibroblast cell (CCD-1059SK). As shown in Table 2, compounds **10a** and **10c**, as well as **1,** inhibited the cell growth to the same extent as did SAHA, but showed more toxicity than SAHA to the CCD-1059SK cell. The 2-aminobenzamide series compounds **21b**, **21d**, **21e** and **21f** had the potency in cell growth inhibitions comparable to that of MS-275, and **21c** was a little inferior to the others. Furthermore, these compounds had much lower IC_{50} values (ranging from 3.8–10.4 μM) against MCF-7 than the hydroxamic acid series compounds. The reason for this fact remains elusive, but their 2 -aminobenzamide moiety could contributes to metabolic stability and bioavailability as well as to the specific binding with the active site of the enzyme as specified in the literatures [13,24], and, thus, enhanced the inhibition against MCF-7. Notably, replacement of the terminal phenyl group (**21b**) with the 3,4-difluorophenyl group (**21e**) led to a significant decrease in the toxicity (from IC_{50} 19.4–94.8 μM) to the CCD-1059SK cell in spite of no particularly conspicuous alteration for the growth inhibition of the cancer cells. The toxicity of **21e** to this cell was even lower than those (IC_{50} 43.5 and 65.0 μM) of SAHA and MS-275, whereas the toxicity (IC_{50} 41.7 μM) of **21d** was almost the same as that of SAHA. Thus, **21d** and **21e** seem to be potent candidates as a cancer therapeutic agent. At present, an uncertainty remains to be solved over how



Scheme 2. Conditions: (a) DMF, Py, $(COCl)_2$/toluene; (b) *o*-nitroaniline/Py; (c) 12 N HCl/MeOH; (d) Et_3N, OHC–CH_2–OTBS, AcOH, $NaBH_3CN$/MeOH; (e) Et_3N, ArCHO, $NaBH_3CN$, AcOH/MeOH; (f) PyCHO, $NaBH_3CN$, AcOH/MeOH; (g) K_2CO_3, $Br(CH_2)_2OH$/MeCN; (h) $SnCl_2{\cdot}2H_2O$, NH_4OAc/MeOH; (i) 95% TFA.

Table 1
Water solubility and inhibition of HDAC inhibitors against HCT 116 cell growth and HDACs



Compd	Ar	n	R	Solubility in 10% HCO60/H_2O (mg/ml)	HCT 116 IC_{50} (μM)[a,b]	HDACs IC_{50} (nM)[c]
10a		1	-OH	>20	4.9	35
10b		1	-OH	-	61.9	420
10c		1	-OH	>20	3.3	20
10d		1	-OH	>20	8.5	110
11		2	-OH	>20	31.8	520
21a		1		-	45.9	-
21b		1		8	4.7	3700
21c		1		8	4.5	2400
21d		1		10	5.7	1800
21e		1		7	4.6	3132
21f		1		8	7.3	4987
SAHA				8	6.3	263
MS-275				8	4.4	2700
1				8	3.9	39

[a]Measured after 2 day incubation of test compounds with cells.
[b]Assays were performed in triplicate.
[c]Assays were performed in duplicate.

fluorinated phenyl group contributed to the reduction of the toxicity to the fibroblast cell.

In the final experiment, we tried to assess the plasma stability of the new HDAC inhibitors **21d** and **21e**, along with **1**, TSA, SAHA and MS-275 using the API 3000 LC-MS/MS system by modifying the procedure of Khan et al. [25]. The transitions for these compounds were 420.0→134.6 (**21d**), 412.0→303.6 (**21e**), 336.3→131.4 (**1**), 303.0→147.6 (TSA), 264.9→231.6 (SAHA) and 377.3→268.6 (MS-275), respectively. As shown in Fig. 2, the hydroxamic acid series compounds were generally less stable than the 2-aminobenzamide series compounds. Especially, TSA was vulnerable to plasma metabolism and decomposed up to 52.5 ± 5.2% of the original concentration in 24 h. On the other hand, compounds **21d** and **21e** were no less stable (103.6 ± 4.9% and 96.1 ± 3.6% recovery) to the plasma enzyme than MS-275 (91.9 ± 4.1% recovery), respectively.

4. Conclusion

New series histone deacetylase inhibitors comprising a hydroxamic acid or 2-amino-benzamide group as a zinc-chelating function were synthesized and assessed for inhibitory efficacies against HDACs and several human cancer cells. Among them, the 2-aminobenzamide series compounds **21d** and **21e** showed the potency comparable with MS-275 in terms of the cancer cell growth inhibitions, the normal fibroblast cell toxicity and the plasma stability. Further study on **21d** and **21e** is underway to investigate the therapeutic efficacy against human cancers.

5. 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 were measured on a JEOL JMS-HX 100 instrument, whereas high-resolution (HR)- and LR-electron impact (EI)-MS spectra were measured on a JEOL The Tandem MStation JMS-700. The plasma stability tests of HDAC inhibitors were carried out on an API-3000 mass spectrometer (Applied Biosystems) fitted with a Turboionspray^{TM} interface. Samples were introduced into the mass spectrometer with an Agilent (Agilent Technologies) 1100 series HPLC system equipped with a degasser, a binary pump, an auto-sampler and a diode-array detector. ^{1}H NMR spectra is recorded on JEOL EX-400 (400 MHz) instruments using tetramethylsilane as an internal standard. Analytical TLC and PLC were performed using Silica gel 60 F_{254} (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, HCT 116 (colon carcinoma), SKBR3 (breast carcinoma), HepG2 (hepatoma), SW620 (colon carcinoma), MDA-MB-231 (breast carcinoma), MCF-7 (breast carcinoma) and A549 (non-small cell lung carcinoma) as well as the human normal cell line, CCD-1059SK (fibroblast) were purchased from American Type Culture Collection. TSA was purchased from Wako Pure Chemical Industries, Ltd.

5.1. Evaluation of histone deacetylase inhibitory activities

The IC_{50} values of **1** and SAHA were measured by using partially purified HDACs and [^{3}H]-acetylated histones according to the procedure by Mori et al. [26], whereas those of other compounds were determined utilizing CycLex HDAC Assay

Table 2
Growth inhibition of HDAC inhibitors against a panel of cancer cells and cytotoxicity to CCD-1059SK cell (IC_{50} in μM)

cell line	SAHA	MS-275	1	10a	10c	21b	21c	21d	21e	21f
cancer cell										
Hep G2	0.6	0.9	0.1	0.7	0.1	1.2	3.5	0.9	1.0	0.9
HCT 116	0.9	0.7	0.5	0.8	0.7	0.9	5.7	0.8	0.8	0.8
SW 620	0.6	3.2	0.6	0.6	0.5	3.3	4.5	2.2	3.6	2.1
SKBR3	0.8	3.7	0.6	0.9	0.5	3.9	6.9	3.5	3.6	3.4
MDA-MB-231	4.4	4.8	2.3	4.3	4.0	9.2	18.4	9.6	8.3	7.4
MCF-7	>100	4.3	78.1	>100	>100	10.0	8.4	3.8	10.4	4.9
A549	12.6	23.3	6.2	16.2	7.9	20.5	20.8	20.0	24.2	17.3
normal cell										
CCD-1059SK	43.5	65.0	6.4	1.5	15.0	19.4	28.3	41.7	94.8	18.4

[a]Measured after 3 day incubation of test compounds with cells.
[b]Assays were performed in triplicate.

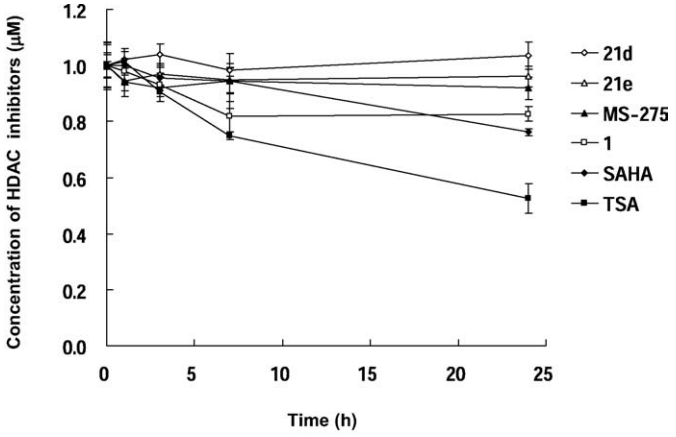


Fig. 2. Plasma concentration–time profile of HDAC inhibitors.
The HDAC inhibitors in the plasma stability assay were tested in triplicate for each time. The concentrations at each time present the mean ± S.D.

kit Protocol. The IC_{50} values in Tables 1 and 2 represent the molar concentrations (nM) required to inhibit the HDACs by 50%.

5.2. Exploratory screening of HDAC inhibitors for cell growth inhibition

HCT 116 cells, being maintained in McCoy's 5a medium with 10% fetal bovine serum, were plated in 96-well plates at densities of 1.0×10^5 cells ml^{-1}. On the same day, testing compounds were added, and their IC_{50} values were measured by the conventional method.

5.3. Cell growth inhibition against human tumor cells and a normal fibroblast cell

HepG2, SW620, MDA-MB-231, MCF-7 and A549 were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). HCT116 and SKBR3 were cultured in McCoy's 5a medium with 10% FBS. CCD-1059SK was cultured in MEMR medium with 10% FBS. Appropriate numbers of cells (1×10^4 cells ml^{-1} for SW620, HepG2, HCT116 and CCD-1059SK; 2.0×10^4 cells ml^{-1} for SKBR3; 2.2×10^4 cells ml^{-1} for MDA-MB-231, MCF-7 and A549) were inoculated onto standard 96-well microtiter plates. Following overnight culture, serially diluted samples were added into the wells. After a 3-day culture, the cell growth rate was evaluated by performing the WST-1 assay, and the IC_{50} values were calculated.

5.3.1. Methyl 4-{[(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl) amino]methyl}benzoate (4)

To a solution of **2** (9.26 g, 45.9 mmol) in CH_2Cl_2 (92 ml) were successively added Et_3N (7.4 ml, 53.3 mmol), (*tert*-butyldimethylsilyloxy)acetaldehyde (8.7 ml, 45.9 mmol) and $NaBH_4$ (1.7 g, 45.0 mmol). The mixture was stirred at room temperature for 6 h. After being evaporated, the resulting residue was dissolved in $CHCl_3$ (400 ml) and washed successively with 10% K_2CO_3 (3 × 50 ml) and brine (3 × 50 ml). The organic layer was dried. Evaporation and purification by silica gel column chromatography (AcOEt/*n*-hexane/$CHCl_3$/Et_3N 100:200:700:9) gave **4** as a yellowish oil (5.0 g, 15.5 mmol, 33.6% yield): IR (KBr): ν (cm^{-1}) 3325, 1726, 1612, 1435, 1360, 1258, 1107, 1020, 939, 756; ^1H NMR ($CDCl_3$): δ 0.02 (6H, s, $Si(CH_3)_2$), 0.76 (9H, s, Si^tBu), 2.67 (2H, t, $J = 5.2$ Hz, NCH_2CH_2OSi), 3.68 (2H, t, $J = 5.2$ Hz, NCH_2CH_2OSi), 3.81 (2H, s, CH_2PhenyleneCO), 3.85 (3H, s, CO_2Me), 7.34 (2H, d, $J = 8.0$ Hz, arom. H_2), 7.94 (2H, d, $J = 8.4$ Hz, arom. H_2); FAB-MS *m/z*: 324 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{17}H_{30}NO_3Si$, 324.1995; found, 324.2025.

5.3.2. Methyl-4-{[(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)(3-quinolinylmethyl)amino]methyl}benzoate (5a)

To a solution of **4** (0.2 g, 0.62 mmol) in MeOH (1.2 ml) were successively added 3-quinolinecarboxaldehyde (97.4 mg, 0.62 mmol), 1% methanolic AcOH (12 μl, 0.21 mmol) and $NaBH_3CN$ (40.2 mg, 0.64 mmol). The mixture was stirred at room temperature for 6 h. After being evaporated, the resulting residue was dissolved in $CHCl_3$ (80 ml) and washed successively with 10% K_2CO_3 (3 × 10 ml) and brine (3 × 10 ml). The organic layer was dried. Evaporation and purification by silica gel column chromatography

($CHCl_3$/MeOH 19:1) gave **5a** as a brownish oil. (0.11 g, 0.24 mmol, 38.4% yield): IR (KBr): ν (cm^{-1}) 2951, 2955, 2330, 1722, 1611, 1497, 1387, 1279, 1192, 958, 812; ^{1}H NMR ($CDCl_3$): δ 0.02 (6H, s, $Si(CH_3)_2$), 0.85 (9H, s, Si^tBu), 2.67 (2H, t, J = 6.0 Hz, NCH_2CH_2OSi), 3.73 (2H, t, J = 6.0 Hz, NCH_2CH_2OSi), 3.75 (2H, s, quinoline-CH_2), 3.86 (2H, s, CH_2PhenyleneCO), 3.89 (3H, s, CO_2Me), 7.34 (2H, d, J = 8.0 Hz, arom. H_2), 7.94 (2H, d, J = 8.4 Hz, arom. H_2), 7.25–8.09 (9H, m, arom. H_9), 8.93 (1H, m, arom. H_1); FAB-MS m/z: 465 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{27}H_{37}N_2O_3Si$, 465.2573; found, 465.2563.

5.3.3. Methyl 4-{[(2-{[tert-butyl(dimethyl)silyl]oxy}ethyl)(3-pyridinylmethyl)amino]methyl}benzoate (5b)

3-Pyridinecarboxaldehyde (0.39 ml, 3.2 mmol), **4** (1.3 g, 4.02 mmol), 1% methanolic AcOH (0.4 ml, 7.0 mmol) and $NaBH_3CN$ (0.26 g, 4.1 mmol) were reacted in MeOH (41 ml) in the same way as for **5a**, affording the pure compound **5b** as a brownish oil (0.82 g, 1.98 mmol, 61.9% yield): IR (KBr): ν (cm^{-1}) 3186, 2829, 2361, 1638, 1458, 1431, 1136, 1032, 1016, 895; ^{1}H NMR ($CDCl_3$): δ 0.02 (6H, s, $Si(CH_3)_2$), 0.86 (9H, s, Si^tBu), 2.64 (2H, t, J = 6.0 Hz, NCH_2CH_2OSi), 3.68 (2H, s, pyridine-CH_2), 3.71 (2H, s, CH_2PhenyleneCO), 3.73 (2H, t, J = 4.4 Hz, NCH_2CH_2OSi), 3.89 (3H, s, CO_2Me), 7.22–7.25 (1H, m, arom. H_1), 7.47 (2H, d, J = 8.4 Hz, arom. H_2), 7.72 (1H, m, arom. H_1), 8.00 (2H, d, J = 8.4 Hz, arom. H_2), 8.49 (1H, dd, J = 1.6, 4.6 Hz, arom. H_1), 8.59 (1H, d, J = 1.2 Hz, arom. H_1); FAB-MS m/z: 415 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{23}H_{35}N_2O_3Si$, 415.2417; found, 415.2443.

5.3.4. Methyl 4-{[(2-naphthylmethyl)amino]methyl}benzoate (6a)

2-Naphthaldehyde (3.88 g, 24.8 mmol), **2** (5.0 g, 24.8 mmol), Et_3N (4.1 ml, 29.6 mmol) and $NaBH_4$ (0.94 g, 24.8 mmol) were reacted in CH_2Cl_2 (50 ml) in the same way as for **4**, affording the pure compound **6a** as a colorless solid. (2.2 g, 7.2 mmol, 29.6% yield): m.p. 56.3–59.2 °C; IR (KBr): ν (cm^{-1}) 2951, 2824, 1715, 1508, 1437, 1308, 1275, 1196, 1016, 860, 756; ^{1}H NMR ($CDCl_3$): δ 3.90 (2H, s, NaphCH_2), 3.91 (3H, s, CO_2Me), 3.97 (2H, s, PhenyleneCH_2), 7.42–7.49 (5H, m, arom. H_5), 7.75–7.83 (4H, m, arom. H_4), 7.99 (1H, m, J = 2.2 Hz, arom. H_1), 8.01 (1H, m, arom. H_1); EI-MS m/z: 305 $(M)^+$; HR-EI-MS m/z: $(M)^+$ calcd for $C_{20}H_{19}NO_2$, 305.1416; found, 305.1415.

5.3.5. Methyl 4-{[(1,3-benzodioxol-5-ylmethyl)amino]methyl}benzoate (6b)

1,3-Benzodioxol-5-carboxaldehyde (1.0 g, 6.7 mmol), **2** (1.3 g, 6.5 mmol), Et_3N (0.92 ml, 6.6 mmol), 1% methanolic AcOH (0.67 ml, 11.7 mmol) and $NaBH_3CN$ (0.94 g, 15.0 mmol) were reacted in MeOH (67 ml) in the usual way, yielding the pure compound **6b** as a colorless solid (0.65 g, 2.2 mmol, 32.6% yield): m.p. 41.2–43.9 °C; IR (KBr): ν (cm^{-1}) 3308, 2839, 1947, 1703, 1240, 1092, 928, 802; ^{1}H NMR ($CDCl_3$): δ 1.66 (1H, s, NHCH$_2$Phenylene), 3.69 (2H, s, 1,3-benzodioxol-CH_2), 3.82 (2H, s, CH_2Phenylene), 3.90 (3H, s, CO_2Me), 5.95 (2H, s, OCH_2), 6.75 (2H, d, J = 0.8 Hz, arom. H_2), 6.85 (1H, s, arom. H_1), 7.40 (2H, d, J = 8.8 Hz, arom. H_2), 8.00 (2H, dd, J = 1.6, 6.6 Hz, arom. H_2); FAB-MS m/z: 300 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{17}H_{18}NO_4$, 300.1236; found, 300.1238.

5.3.6. Methyl 4-({[2-(1H-indole-3-yl)ethyl]amino}methyl) benzoate (7)

3-(2-Aminoethyl)indole (1.0 g, 6.24 mmol), **3** (1.0 g, 6.1 mmol), 1% methanolic AcOH (0.11 ml, 1.9 mmol) and $NaBH_3CN$ (0.39 g, 6.21 mmol) were reacted in MeOH (11 ml) in the usual way, furnishing the pure compound **7** as a brownish oil. (1.2 g, 3.9 mmol, 63.0% yield): IR (KBr): ν (cm^{-1}) 3408, 2843, 2361, 1715, 1612, 1435, 1416, 1109, 966, 743; ^{1}H NMR ($CDCl_3$): δ 2.95–3.02 (4H, m, indole-C_2H_4), 3.84 (2H, s, PhenyleneCH_2), 3.89 (3H, s, CO_2Me), 6.98 (1H, s, indole-NH), 7.08–7.33 (6H, m, arom. H_6), 7.60 (1H, d, J = 8.0 Hz, arom. H_1), 7.95 (2H, d, J = 8.0 Hz, arom. H_2), 8.24 (1H, s, NHCH$_2$Phenylene); EI-MS m/z: 308 $(M)^+$; HR-EI-MS m/z: $(M)^+$ calcd for $C_{19}H_{20}N_2O_2$, 308.1525; found, 308.1495.

5.3.7. Methyl 4-{[(2-hydroxyethyl)(3-quinolinylmethyl)amino]methyl}benzoate (8a)

A solution of **5a** (70 mg, 0.15 mmol) in 95% TFA (0.67 ml) was stirred at 50 °C for 2 h. Evaporation and purification by PLC ($CHCl_3$/MeOH 9:1) gave **8a** as a brownish oil. (40.8 mg, 0.12 mmol, 77.4% yield): IR (KBr): ν (cm^{-1}) 3300, 2800, 1717, 1609, 1499, 1277, 1252, 1105, 982, 835; ^{1}H NMR ($CDCl_3$): δ 2.75 (2H, t, J = 5.2 Hz, NCH_2CH_2OH), 3.68 (2H, t, J = 5.2 Hz, NCH_2CH_2OH), 3.71 (2H, s, quinoline-CH_2), 3.84 (2H, s, CH_2PhenyleneCO), 3.88 (3H, s, CO_2Me), 7.23–8.12 (9H, m, arom. H_9), 8.89 (1H, s, arom. H_1); FAB-MS m/z: 351 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{21}H_{23}N_2O_3$, 351.1706; found, 351.1716.

5.3.8. Methyl 4-{[(2-hydroxyethyl)(3-pyridinylmethyl)amino]methyl}benzoate (8b)

5b (0.82 g, 1.98 mmol) was reacted in 95% TFA (8.8 ml) in the same way for **9a**, yielding the pure compound **8b** as a yellowish oil (0.33 g, 1.1 mmol, 54.2% yield): IR (KBr): ν (cm^{-1}) 3252, 2824, 1717, 1611, 1435, 1281, 1175, 1111, 1020, 964, 756; ^{1}H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH_2OH), 3.64–3.69 (6H, m, NCH_2CH_2OH, pyridine-CH_2, CH_2PhenyleneCO), 3.91 (3H, s, CO_2Me), 3.89 (3H, s, CO_2Me), 7.27 (1H, t, J = 4.8 Hz, arom. H_1), 7.40 (2H, d, J = 8.0 Hz, arom. H_2), 7.66 (1H, d, J = 7.6 Hz, arom. H_1), 8.00 (2H, d, J = 8.0 Hz, arom. H_2), 8.50 (1H, d, J = 4.8 Hz, arom. H_1), 8.53 (1H, s, arom. H_1); FAB-MS m/z: 301 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{17}H_{21}N_2O_3$, 301.1552; found, 301.1563.

5.3.9. Methyl 4-{[(2-hydroxyethyl)(2-naphthylmethyl)amino]methyl}benzoate (8c)

To a solution of **6a** (0.5 g, 1.6 mmol) in MeCN (16 ml) were added K_2CO_3 (0.45 g, 3.26 mmol) and 2-bromoethanol

(2.3 ml, 32.4 mmol). The mixture was stirred at 60 °C for overnight. The resulting precipitate was removed by filtration and washed with MeCN. After evaporation of the combined filtrates, the resulting residue was purified by silica gel column chromatography (AcOEt/*n*-hexane 1:2), affording **8c** as a colorless solid (0.23 g, 0.6 mmol, 39.7% yield): m.p. 93.5–96.1 °C; IR (KBr): ν (cm^{-1}) 3528, 2827, 2708, 1715, 1609, 1508, 1360, 1173, 986, 854; ^1H NMR ($CDCl_3$): δ 2.71 (2H, t, $J = 5.2$ Hz, NCH_2CH_2OH), 3.61 (2H, t, $J = 6.4$ Hz, NCH_2CH_2OH), 3.71 (2H, s, $NaphCH_2$), 3.77 (2H, s, $PhenyleneCH_2$), 3.90 (3H, s, CO_2Me), 7.37–7.50 (5H, m, arom. H_5), 7.71 (1H, s, arom. H_1), 7.77–7.83 (3H, m, arom. H_3), 8.00 (1H, d, $J = 1.6$ Hz, arom. H_1), 8.01 (1H, d, $J = 1.6$ Hz, arom. H_1); EI-MS *m/z*: 349 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{22}H_{23}NO_3$, 349.1678; found, 349.1691.

5.3.10. Methyl 4-{[(1,3-benzodioxol-5-ylmethyl)(2-hydroxyethyl)amino]methyl}benzoate (8d)

6b (0.38 g, 1.26 mmol) was reacted with 2-bromoethanol (0.98 ml, 13.8 mmol) in the presence of K_2CO_3 (0.70 g, 5.1 mmol) in MeCN (16 ml) in the same way as for **8c** to give the pure compound **8d** as a brownish oil (0.13 g, 0.38 mmol, 31.0%): IR (KBr): ν (cm^{-1}) 3433, 2951, 1720, 1489, 1281, 1040, 930, 756; ^1H NMR ($CDCl_3$): δ 2.65 (2H, t, $J = 5.6$ Hz, NCH_2CH_2OH), 3.52 (2H, s, 1,3-benzodioxol-CH_2), 3.59 (2H, t, $J = 5.6$ Hz, NCH_2CH_2OH), 5.94 (2H, s, OCH_2), 6.70–6.80 (3H, m, arom. H_3), 7.39 (2H, d, $J = 8.4$ Hz, arom. H_2), 8.00 (2H, dd, $J = 1.6$, 7.2 Hz, arom. H_2); FAB-MS *m/z*: 344 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{19}H_{22}NO_5$, 344.1498; found, 344.1511.

5.3.11. Methyl 4-({(2-hydroxyethyl)[2-(1H-indole-3-yl)ethyl]amino}methyl)benzoate (9)

7 (0.62 g, 2.0 mmol) was reacted with 2-bromoethanol (0.71 ml, 10.0 mmol) in the presence of K_2CO_3 (1.1 g, 8.0 mmol) in MeCN (20 ml) in the usual way to give the pure compound **9** as a brownish oil (0.47 g, 1.34 mmol, 66.2%): IR (KBr): ν (cm^{-1}) 3410, 2949, 1715, 1611, 1574, 1456, 1283, 1113, 1018, 964; ^1H NMR ($CDCl_3$): δ 2.74 (2H, t, $J = 6.0$ Hz, NCH_2CH_2OH), 2.84–2.95 (4H, m, Indole-C_2H_4), 3.56 (2H, t, $J = 6.0$ Hz, NCH_2CH_2OH), 3.75 (2H, s, $PhenyleneCH_2$), 3.91 (3H, s, CO_2Me), 6.93 (1H, s, indole-N*H*), 7.02–7.43 (7H, m, $J = 8.0$ Hz, arom. H_7), 7.92 (2H, d, $J = 8.4$ Hz, arom. H_2), 8.06 (1H, s, $NHCH_2Phenylene$); EI-MS *m/z*: 352 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{21}H_{24}N_2O_3$, 352.1787; found, 352.1780.

5.3.12. N-hydroxy-4-{[(2-hydroxyethyl)(3-quinolinylmethyl)amino]methyl}benzamide (10a)

A solution of 2 M NH_2OH (72.6 mg, 2.2 mmol) in MeOH (1.1 ml) was added to a solution of **8a** (40 mg, 0.11 mmol) and 1 M KOH (11.0 mg, 0.2 mmol) in MeOH (0.2 ml). The mixture was stirred at room temperature for 5 h. When the reaction was completed, a small amount of dry ice was added to the mixture. The resulting precipitate was removed by filtration and washed with MeOH. After evaporation of the combined filtrates, the resulting residue was purified by PLC ($CHCl_3$/MeOH 9:1), affording **10a** as a brownish solid (20.1 mg, 0.06 mmol, 51.9% yield): m.p. 62.9–65.2 °C; IR (KBr): ν (cm^{-1}) 2924, 2851, 1715, 1697, 1557, 1499, 1362, 1202, 1016, 897; ^1H NMR (CD_3OD): δ 2.61 (2H, t, $J = 6.0$ Hz, NCH_2CH_2OH), 3.59 (2H, t, $J = 6.0$ Hz, NCH_2CH_2OH), 3.69 (2H, s, quinoline-CH_2), 3.79 (2H, s, $CH_2PhenyleneCO$), 7.40–7.91 (8H, m, arom. H_8), 8.19 (1H, s, arom. H_1), 8.77 (1H, s, arom. H_1); FAB-MS *m/z*: 352 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{20}H_{22}N_3O_3$, 352.1661; found, 352.1644.

5.3.13. N-hydroxy-4-{[(2-hydroxyethyl)(3-pyridiinylmethyl)amino]methyl}benzamide (10b)

A solution of 2 M NH_2OH (224.4 mg, 6.8 mmol) in MeOH (3.4 ml) was added to a solution of **8b** (140 mg, 0.47 mmol) and 1 M KOH (29.7 mg, 0.54 mmol) in MeOH (0.54 ml) in the same way as for **10a**, affording the pure compound **10b** as a brownish oil (16.8 mg, 0.06 mmol, 16.4% yield): IR (KBr): ν (cm^{-1}) 3186, 2829, 2361, 1638, 1458, 1431, 1313, 1136, 1032, 850; ^1H NMR ($CDCl_3$): δ 2.69 (2H, t, $J = 5.6$ Hz, NCH_2CH_2OH), 3.64–3.72 (6H, m, NCH_2CH_2OH, pyridine-CH_2, CH_2PhCO), 3.91 (3H, s, CO_2Me), 7.27 (1H, t, $J = 4.8$ Hz, arom. H_1), 7.40 (2H, d, $J = 8.0$ Hz, arom. H_2), 7.66 (1H, d, $J = 7.6$ Hz, arom. H_1), 8.00 (2H, d, $J = 8.0$ Hz, arom. H_2), 8.50 (1H, d, $J = 4.8$ Hz, arom. H_1), 8.53 (1H, s, arom. H_1); FAB-MS *m/z*: 302 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{16}H_{20}N_3O_3$, 302.1505; found, 302.1511.

5.3.14. N-hydroxy-4-{[(2-hydroxyethyl)(2-naphthylmethyl)amino]methyl}benzamide (10c)

A solution of 2 M NH_2OH (92.4 mg, 2.8 mmol) in MeOH (1.4 ml) was added to a solution of **8c** (0.1 g, 0.29 mmol) and 1 M KOH (13.8 mg, 0.25 mmol) in MeOH (0.25 ml) in the usual way, affording the pure compound **10c** as a brownish solid (27.9 mg, 0.08 mmol, 27.6% yield): m.p. 61.1–63.5 °C; IR (KBr): ν (cm^{-1}) 3647, 3529, 2826, 2360, 2340, 1616, 1124, 1016, 897, 856; ^1H NMR (CD_3OD): δ 2.62 (2H, t, $J = 6.0$ Hz, NCH_2CH_2OH), 3.56 (2H, t, $J = 6.4$ Hz, NCH_2CH_2OH), 3.70 (2H, s, $NaphCH_2$), 3.77 (2H, s, $PhenyleneCH_2$), 7.30–7.44 (5H, m, arom. H_5), 7.70 (4H, d, $J = 8.4$ Hz, arom. H_4), 7.87 (2H, d, $J = 8.0$ Hz, arom. H_2); FAB-MS *m/z*: 351 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{21}H_{23}N_2O_3$, 351.1709; found, 351.1692.

5.3.15. 4-{[(1,3-Benzodioxol-5-ylmethyl)(2-hydroxyethyl)amino]methyl}-N-hydroxybenzamide (10d)

A solution of 2 M NH_2OH (0.49 g, 14.8 mmol) in MeOH (7.4 ml) was added to a solution of **8d** (0.1 g, 0.29 mmol) and 1 M KOH (70 mg, 1.2 mmol) in MeOH (1.2 ml) in the usual way, furnishing the pure compound **10d** as a colorless solid (74.1 mg, 0.21 mmol, 72.9% yield): m.p. 90.0–92.3 °C; IR (KBr): ν (cm^{-1}) 2822, 2359, 1489, 1246, 1038, 930, 812, 754; ^1H NMR ($CDCl_3$): δ 2.66 (2H, t, $J = 5.6$ Hz, NCH_2CH_2OH), 3.48 (2H, s, 1,3-benzodioxol-CH_2), 3.52–3.58 (4H, m, $CH_2Phenylene$, NCH_2CH_2OH), 5.95 (2H, s, OCH_2), 6.70–6.79 (3H, m, arom. H_3), 7.39 (2H, d, $J = 8.4$ Hz, arom. H_2), 7.65 (2H, brs, arom. H_2); FAB-MS *m/z*: 345 $(M + H)^+$; HR-FAB-

MS *m/z*: $(M + H)^+$ calcd for $C_{18}H_{21}N_2O_5$, 345.1450; found, 345.1440.

*5.3.16. N-hydroxy-4-({(2-hydroxyethyl)[2-(1H-indole-3-yl) ethyl]amino}methyl)benzamide (**11**)*

A solution of 2 M NH_2OH (0.55 g, 16.6 mmol) in MeOH (8.3 ml) was added to a solution of **9** (0.46 g, 1.3 mmol) and 1 M KOH (80 mg, 1.4 mmol) in MeOH (1.4 ml) in the usual way, giving rise to the pure compound **11** as a brownish solid (0.1 g, 0.28 mmol, 22.1% yield): m.p. 93.4–95.8 °C; IR (KBr): ν (cm^{-1}) 3532, 2851, 1715, 1611, 1574, 1456, 1283, 1113, 1018, 964; ^1H NMR (CD_3OD): δ 2.73–2.95 (6H, m, NC H_2CH_2OH, indole-C_2H_4), 3.63 (2H, t, $J = 5.6$ Hz, NCH_2CH_2OH), 3.80 (2H, s, PhenyleneCH_2), 6.92 (1H, t, $J = 7.6$ Hz, arom. H_1), 6.98 (1H, s, arom. H_1), 7.04 (1H, t, $J = 7.6$ Hz, arom. H_1), 7.29 (1H, d, $J = 8.0$ Hz, arom. H_1), 7.37 (1H, d, $J = 7.6$ Hz, arom. H_1), 7.44 (2H, d, $J = 7.6$ Hz, arom. H_2), 7.67 (2H, d, $J = 7.2$ Hz, arom. H_2); FAB-MS *m/z*: 354 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{20}H_{24}N_3O_3$, 354.1818; found, 354.1832.

*5.3.17. tert-Butyl 4-(chlorocarbonyl)benzylcarbamate (**13**)*

To a suspension of **12** (14.2 g, 56.5 mmol) in toluene (240 ml) were added successively DMF (0.16 ml), pyridine (27 ml) and oxalyl chloride (9.7 ml, 113 mmol). The mixture was stirred at room temperature for 6 h, and then the resulting precipitate was removed by filtration and washed with toluene. The combined filtrates were evaporated to give **13** (15.1 g, 100% yield), which was used without further purification due to hygroscopicity.

*5.3.18. tert-Butyl 4-[(2-nitroanilino)carbonyl]benzylcarbamate (**14**)*

To a solution of **13** (21.6 g, 80.0 mmol) in pyridine (240 ml) was added *o*-nitroaniline (12.2 g, 88.3 mmol). The mixture was stirred at room temperature for 13 h. After being evaporated, the resulting residue was dissolved in $CHCl_3$ (600 ml) and washed successively with 10% HCl (3 × 150 ml), satd. $NaHCO_3$ (3 × 150 ml) and brine (3 × 150 ml). The organic layer was dried. Evaporation and purification by silica gel column chromatography ($CHCl_3$) gave **14** as a yellowish solid. (17.3 g, 46.6 mmol, 58% yield): m.p. 129.4–131.6 °C; IR (KBr): ν (cm^{-1}) 3356, 1678, 1607, 1583, 1433, 1366, 1171, 970, 899, 870; ^1H NMR (CD_3OD): δ 1.48 (9H, s, ^{t}Bu), 4.42 (2H, d, $J = 5.6$ Hz, PhenyleneCH_2), 7.23 (1H, t, $J = 7.6$ Hz, arom. H_1), 7.46 (2H, d, $J = 8.0$ Hz, arom. H_2), 7.72 (1H, t, $J = 7.2$ Hz, arom. H_1), 7.97 (2H, d, $J = 8.4$ Hz, arom. H_2), 8.29 (1H, dd, $J = 1.2$, 8.2 Hz, arom. H_1), 9.00 (1H, dd, $J = 1.6$, 8.6 Hz, arom. H_1); EI-MS *m/z*: 371 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{19}H_{21}N_3O_5$, 371.1481; found, 371.1487.

*5.3.19. 4-(Aminomethyl)-N-(2-nitrophenyl)benzamide hydrochloride (**15**)*

To a solution of **14** (17.1 g, 45.8 mmol) in MeOH (1700 ml) was added 12 N HCl (60 ml). The mixture was stirred at room temperature for 13 h. After being evaporated, the resulting residue was dried to give the pure compound **15** as a yellowish solid (14.0 g, 45.6 mmol, 99% yield): m.p. 223.3–226.7 °C; IR (KBr): ν (cm^{-1}) 3373, 2905, 1688, 1609, 1585, 1454, 1387, 1198, 959, 887; ^1H NMR ($CDCl_3$): δ 4.23 (2H, s, PhenyleneC H_2), 7.39 (1H, t, $J = 7.2$ Hz, arom. H_1), 7.66 (2H, d, $J = 8.4$ Hz, arom. H_2), 7.76 (1H, t, $J = 6.8$ Hz, arom. H_1), 8.06 (2H, d, $J = 8.0$ Hz, arom. H_2), 8.20 (1H, dd, $J = 1.6$, 8.2 Hz, arom. H_1), 8.35 (1H, d, $J = 8.4$ Hz, arom. H_1); EI-MS *m/z*: 271 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{14}H_{13}N_3O_3$, 271.0957; found, 271.0959.

*5.3.20. 4-{[(2-{[tert-Butyl(dimethyl)silyl]oxy}ethyl)amino] methyl}-N-(2-nitrophenyl)benzamide (**16**)*

(*tert*-Butyldimethylsilyloxy)acetaldehyde (5.0 ml, 26.4 mmol), **15** (8.0 g, 26.1 mmol), Et_3N (3.6 ml, 26.1 mmol), 1% methanolic AcOH (0.65 ml, 11.2 mmol) and $NaBH_3CN$ (1.6 g, 26.1 mmol) were reacted in MeOH (65 ml) in the usual way, giving the pure compound **16** as a greenish oil (3.75 g, 8.7 mmol, 33.5% yield): IR (KBr): ν (cm^{-1}) 3360, 2928, 2855, 1692, 1607, 1502, 1454, 1339, 1256, 1074, 899; ^1H NMR (CD_3OD): δ 0.02 (6H, s, $Si(CH_3)_2$), 0.86 (9H, s, Si^{t}Bu), 2.64 (2H, t, $J = 5.2$ Hz, NCH_2CH_2OSi), 3.70 (2H, t, $J = 5.2$ Hz, NCH_2CH_2OSi), 3.80 (2H, s, PhenyleneCH_2), 7.24 (1H, t, $J = 6.4$ Hz, arom. H_1), 7.43 (2H, d, $J = 8.4$ Hz, arom. H_2), 7.63 (1H, t, $J = 6.0$ Hz, arom. H_1), 7.85 (1H, t, $J = 8.4$ Hz, arom. H_1), 8.09 (1H, dd, $J = 1.6$, 8.2 Hz), 8.38 (1H, dd, $J = 1.2$, 8.8 Hz); FAB-MS *m/z*: 430 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{22}H_{32}N_3O_4Si$, 430.2162; found, 430.2176.

*5.3.21. 4-{[(2-{[tert-Butyl(dimethyl)silyl]oxy}ethyl)(3-pyridynylmethyl)amino]methyl}-N-(2-nitropheny)-benzamide (**17**)*

3-Pyridinecarboxaldehyde (0.33 ml, 0.38 g, 3.5 mmol), **16** (1.5 g, 3.5 mmol), 1% methanolic AcOH (0.2 ml, 3.5 mmol) and $NaBH_3CN$ (0.22 g, 3.5 mmol) were reacted in MeOH (20 ml) in the usual way, yielding the pure compound **17** as a greenish oil (0.67 g, 1.3 mmol, 36.6% yield): IR (KBr): ν (cm^{-1}) 3360, 2928, 2855, 1692, 1607, 1501, 1433, 1339, 1146, 1016, 937; ^1H NMR (CD_3OD): δ 0.02 (6H, s, $Si(CH_3)_2$), 0.87 (9H, s, Si^{t}Bu), 2.59 (2H, t, $J = 6.0$ Hz, NC H_2CH_2OSi), 3.68 (2H, t, $J = 4.0$ Hz, NCH_2CH_2OSi), 3.72 (2H, s, CH_2PhenyleneCO), 3.75 (2H, s, pyridine-CH_2), 7.18–8.53 (12H, m, arom. H_{12}); FAB-MS *m/z*: 521 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{28}H_{37}N_4O_4Si$, 521.2584; found, 521.2580.

*5.3.22. N-(2-aminophenyl)-4-{[(2-{[tert-butyl(dimethyl)silyl] oxy}ethyl)(3-pyridinylmethyl)amino]-methyl}benzamide (**18**)*

To a solution of **17** (0.67 g, 1.3 mmol) in MeOH (14 ml) were added $SnCl_2{\cdot}2H_2O$ (1.76 g, 7.8 mmol) and NH_4OAc (1.04 g, 13.5 mmol). The mixture was stirred at 60 °C for 1 h. The resulting precipitate was removed by filtration and washed with MeOH. After concentration of the combined filtrates, the resulting residue was dissolved in $CHCl_3$ (80 ml) and washed successively with satd. $NaHCO_3$ (3 × 10 ml) and brine (3 × 10 ml). The organic layer was dried. Evaporation

gave the pure compound **18** as a brownish oil (0.38 g, 0.78 mmol, 59% yield): IR (KBr): ν (cm^{-1}) 2928, 2855, 2822, 1649, 1502, 1452, 1315, 1099, 937, 835; ^{1}H NMR (CD_3OD): δ 0.03 (6H, s, $Si(CH_3)_2$), 0.87 (9H, s, Si^tBu), 2.62 (2H, t, J = 5.6 Hz, NCH_2CH_2OSi), 3.71(2H, t, J = 4.4 Hz, NCH_2CH_2OSi), 3.73 (2H, s, CH_2PhenyleneCO), 3.74 (2H, s, pyridine-CH_2), 6.74–8.51 (12H, m, arom. H_{12}); FAB-MS *m/z*: 491 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{28}H_{39}N_4O_2Si$, 491.2842; found, 491.2827.

5.3.23. 4-[(Benzylamino)methyl]-N-(2-nitrophenyl)benzamide (19a)

Benzaldehyde (1.8 ml, 17.9 mmol), Et_3N (2.5 ml, 17.9 mmol), **15** (5.5 g, 17.9 mmol), 1% methanolic AcOH (4.4 ml, 77 mmol) and $NaBH_3CN$ (1.13 g, 17.9 mmol) were reacted in MeOH (440 ml) in the usual way, giving the pure compound **19a** as a yellowish solid (3.34 g, 9.25 mmol, 51.6% yield): m.p. 91.0–92.8 °C; IR (KBr): ν (cm^{-1}) 3377, 3328, 2939, 2332, 1682, 1607, 1583, 1435, 1273; ^{1}H NMR ($CDCl_3$): δ 3.81 (2H, s, PhCH_2NH), 3.90 (2H, s, CH_2PhenyleneCO), 7.19 –7.35 (6H, m, arom. H_6), 7.53 (2H, d, J = 8.4 Hz, arom. H_2), 7.73 (1H, t, J = 7.2 Hz, arom. H_1), 7.96 (2H, d, J = 8.0 Hz, arom. H_2), 8.28 (1H, dd, J = 1.6, 8.4 Hz, arom. H_1), 9.00 (1H, dd, J = 1.2, 8.6 Hz, arom. H_1), 11.3 (1H, s, CON*H*); EI-MS *m/z*: 361 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{21}H_{19}N_3O_3$, 361.1426; found, 361.1450.

5.3.24. 4-({[(1-Methyl-1H-indole-3-yl)methyl]amino}methyl)-N-(2-nitrophenyl)benzamide (19b)

1-Methylindole-3-carboxaldehyde (1.56 g, 9.8 mmol), Et_3N (1.5 ml, 9.8 mmol), **15** (3.0 g, 9.8 mmol), 1% methanolic AcOH (3.3 ml, 58 mmol) and $NaBH_3CN$ (0.61 g, 9.8 mmol) were reacted in MeOH (330 ml) in the usual way, giving the pure compound **19b** as a yellowish solid (1.65 g, 4.0 mmol, 40.6% yield): m.p. 178.5–181.3 °C; IR (KBr): ν (cm^{-1}) 2878, 2773, 2704, 1674, 1585, 1506, 1339, 1251, 1150, 972, 739; ^{1}H NMR (DMSO-d_6): δ 3.83 (3H, s, NMe), 4.27 (2H, s, 1-methyl-1*H*-indol-CH_2), 4.34 (2H, s, CH_2Phenylene), 7.12–8.03 (13H, m, arom. H_{13}), 9.52 (1H, brs, CH_2NH), 10.88 (1H, s, CON*H*); FAB-MS *m/z*: 415 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{24}H_{23}N_4O_3$, 415.1770; found, 415.1786.

5.3.25. 4-{[(1,3-Benzodioxol-5-ylmethyl)amino]methyl}-N-(2-nitrophenyl)benzamide (19c)

1,3-Benzodioxol-5-carboxaldehyde (0.5 g, 3.3 mmol), Et_3N (0.5 ml, 3.3 mmol), **15** (1.0 g, 3.3 mmol), 1 % methanolic AcOH (0.88 ml, 15.5 mmol) and $NaBH_3CN$ (0.20 g, 3.2 mmol) were reacted in MeOH (88 ml) in the usual way, giving the pure compound **19c** as a yellowish solid (0.33 g, 0.8 mmol, 24.8% yield): m.p. 74.6–76.8 °C; IR (KBr): ν (cm^{-1}); 3371, 2885, 2800, 2340, 1684, 1604, 1450, 1377, 1192, 988, 899; ^{1}H NMR (CD_3OD): δ 3.68 (2H, s, 1,3-benzodioxol-CH_2), 3.82 (2H, s, PhenyleneCH_2), 5.92 (2H, s, OCH_2), 6.75–6.81 (2H, m, arom. H_2), 6.88 (1H, d, J = 1.2 Hz, arom. H_1), 7.36–8.43 (8H, m, arom. H_8); FAB-MS *m/z*: 406 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{22}H_{20}N_3O_5$, 406.1403; found, 406.1388.

5.3.26. 4-{[(3,4-Difluorobenzyl)amino]methyl}-N-(2-nitrophenyl)benzamide (19d)

3,4-Difluorobenzaldehyde (1.27 ml, 11.6 mmol), Et_3N (1.62 ml, 11.6 mmol), **15** (3.6 g, 11.6 mmol), 1% methanolic AcOH (3.17 ml, 55.8 mmol) and $NaBH_3CN$ (0.73 g, 11.6 mmol) were reacted in MeOH (317 ml) in the usual way, giving the pure compound **19d** a yellowish solid (1.49 g, 3.75 mmol, 32.0% yield): m.p. 65.6–67.8 °C; IR (KBr): ν (cm^{-1}) 3346, 3261, 2835, 1923, 1678, 1607, 1433, 1344, 1271, 899; ^{1}H NMR ($CDCl_3$): δ 3.77 (2H, s, 3,4-difluorophenyl-CH_2), 3.89 (2H, s, PhenyleneCH_2,), 7.06–7.26 (5H, m, arom. H_4), 7.52 (2H, d, J = 8.0 Hz, arom. H_2), 7.74 (1H, t, J = 7.2 Hz, arom. H_1), 7.97 (2H, dd, J = 2.0, 8.4 Hz, arom. H_2), 8.29 (1H, dd, J = 1.6, 8.4 Hz, arom. H_1); EI-MS *m/z*: 397 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{21}H_{17}F_2N_3O_3$, 397.1238; found, 397.1259.

5.3.27. 4-{[(4-Methoxybenzyl)amino]methyl}-N-(2-nitrophenyl)benzamide (19e)

4-Methoxybenzaldehyde (0.4 ml, 3.25 mmol), Et_3N (0.45 ml, 3.25 mmol), **15** (1.0 g, 3.25 mmol), 1% methanolic AcOH (1.0 ml, 17.6 mmol) and $NaBH_3CN$ (0.20 g, 3.25 mmol) were reacted in MeOH (100 ml) in the usual way, giving the pure compound **19e** a greenish solid (0.25 g, 0.64 mmol, 20.0% yield): m.p. 58.0–60.3 °C; IR (KBr): ν (cm^{-1}) 3369, 2822, 1682, 1607, 1551, 1508, 1437, 1339, 986, 935; ^{1}H NMR ($CDCl_3$): δ 3.71 (2H, s, 4-methoxyphenyl-CH_2), 3.78 (3H, s, OMe), 3.88 (2H, s, PhenyleneCH_2,), 6.86 (2H, d, J = 6.4 Hz, arom. H_2), 7.17 (1H, t, J = 7.2 Hz, arom. H_1), 7.25 (2H, d, J = 8.4 Hz, arom. H_2), 7.49 (2H, d, J = 8.0 Hz, arom. H_2), 7.66 (1H, t, J = 6.0 Hz, arom. H_1), 7.93 (2H, d, J = 8.4 Hz, arom. H_2), 8.23 (1H, dd, J = 1.6, 8.6 Hz, arom. H_1), 8.97 (1H, dd, J = 1.2, 8.4 Hz, arom. H_1), 11.30 (1H, s, CONH); EI-MS *m/z*: 391 $(M)^+$; HR-EI-MS *m/z*: $(M)^+$ calcd for $C_{22}H_{21}N_3O_4$, 391.1532; found, 391.1534.

5.3.28. 4-{[Benzyl(2-hydroxyethyl)amino]methyl}-N-(2-nitrophenyl)benzamide (20a)

19a (3.2 g, 8.9 mmol) was reacted with 2-bromoethanol (12.5 ml, 176 mmol) in the presence of K_2CO_3 (4.9 g, 35.5 mmol) in MeCN (85 ml) in the usual way, to give the pure compound **20a** as a yellowish oil (1.47 g, 3.63 mmol, 41.0% yield): IR (KBr): ν (cm^{-1}) 3362, 2806, 1688, 1607, 1502, 1454, 1433, 1275, 1146, 980, 862; ^{1}H NMR (CD_3OD): δ 2.54 (2H, t, J = 6.4 Hz, NCH_2CH_2OH), 3.52 (2H, s, PhCH_2NH), 3.55 (2H, s, CH_2PhenyleneCO), 3.58 (2H, t, J = 6.4 Hz, NCH_2CH_2OH), 7.08–7.75 (9H, m, arom. H_9), 7.76 (2H, t, J = 8.8 Hz, arom. H_2), 8.00 (1H, dd, J = 1.6, 8.4 Hz, arom. H_1), 8.49 (1H, dd, J = 1.2, 8.4 Hz, arom. H_1); FAB-MS *m/z*: 406 $(M + H)^+$; HR-FAB-MS *m/z*: $(M + H)^+$ calcd for $C_{23}H_{24}N_3O_4$, 406.1767; found, 407.1765.

5.3.29. 4-({(2-Hydroxyethyl)[(1-methyl-1H-indol-3-yl)methyl]amino}methyl)-N-(2-nitrophenyl)benzamide (20b)

19b (0.7 g, 1.7 mmol) was reacted with 2-bromoethanol (2.4 ml, 33.8 mmol) in the presence of K_2CO_3 (0.93 g, 6.7 mmol) in MeCN (17 ml) in the usual way to give the pure

compound **20b** as a yellowish oil (0.48 g, 1.05 mmol, 62.7% yield): IR (KBr): ν (cm^{-1}) 3356, 2930, 2822, 1805, 1774, 1688, 1607, 1454, 1271, 1146, 1072, 899; ^{1}H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH$_2$OH), 3.61 (2H, t, J = 5.6 Hz, NCH$_2$CH_2OH), 3.68 (2H, s, 1-methyl-1*H*-indol-CH_2), 3.70 (3H, s, NMe), 3.80 (2H, s, CH_2Phenylene), 6.97–8.92 (13H, m, arom. H_{13}), 11.23 (1H, s, CONH); FAB-MS *m*/*z*: 459 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{26}H_{27}N_4O_4$, 459.2032; found, 459.2017.

5.3.30. 4-{[(1,3-Benzodioxol-5-ylmethyl)(2-hydroxyethyl) amino]methyl}-N-(2-nitrophenyl)benzamide (20c)

19c (0.19 g, 0.47 mmol) was reacted with 2-bromoethanol (0.67 ml, 9.4 mmol) in the presence of K_2CO_3 (0.13 g, 0.94 mmol) in MeCN (47 ml) in the usual way to give the pure compound **20c** as a yellowish oil (0.2 g, 0.45 mmol, 94.1% yield): IR (KBr): ν (cm^{-1}) 3362, 2887, 2316, 1805, 1774, 1688, 1587, 1248, 1148, 1074, 972, 773; ^{1}H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH$_2$OH), 3.55 (2H, s, 1,3-benzodioxol-CH_2), 3.62 (2H, t, J = 5.6 Hz, NCH$_2$CH_2OH), 3.69 (2H, s, PhenyleneCH_2), 5.95 (2H, s, OCH$_2$), 6.73–6.82 (3H, m, arom. H_3), 7.22 (1H, t, J = 6.0 Hz, arom. H_1), 7.49 (2H, d, J = 8.4 Hz, arom. H_2), 7.72 (1H, t, J = 8.4 Hz, arom. H_1), 7.97 (2H, d, J = 8.0 Hz, arom. H_2), 8.28 (1H, dd, J = 1.6, 8.6 Hz, arom. H_1), 9.00 (1H, dd, J = 1.2 Hz, 8.6 Hz, arom. H_1), 11.34 (1H, s, PhenyleneCON*H*); FAB-MS *m*/*z*: 450 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{24}H_{24}N_3O_6$, 450.1665; found, 450.1692.

5.3.31. 4-{[(3,4-Difluorobenzyl)(2-hydroxyethyl)amino] methyl}-N-(2-nitrophenyl)benzamide (20d)

19d (0.92 g, 2.3 mmol) was reacted with 2-bromoethanol (0.82 ml, 11.5 mmol) in the presence of K_2CO_3 (0.64 g, 4.6 mmol) in MeCN (23 ml) in the usual way to give the pure compound **20d** as a yellowish oil (0.94 g, 2.13 mmol, 92.3% yield): IR (KBr): ν (cm^{-1}) 3481, 3352, 2922, 1684, 1607, 1499, 1456, 1340, 1205, 955; ^{1}H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH$_2$OH), 3.57–3.65 (4H, m, 3,4-difluorophenyl-CH_2, NCH$_2$CH_2OH), 3.66 (2H, s, PhenyleneCH_2), 7.02–7.50 (6H, m, arom. H_6), 7.72 (1H, t, J = 7.2 Hz, arom. H_1), 7.96–8.29 (2H, m, arom. H_2), 8.99 (1H, d, J = 1.3 Hz, arom. H_1), 9.01 (1H, d, J = 1.2 Hz, arom. H_1); FAB-MS *m*/*z*: 442 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{23}H_{22}F_2N_3O_4$, 442.1578; found, 442.1558.

5.3.32. 4-{[(2-Hydroxyethyl)(4-methoxybenzyl)amino]methyl}-N-(2-nitrophenyl)benzamide (20e)

19e (0.21 g, 0.5 mmol) was reacted with 2-bromoethanol (0.75 ml, 10.6 mmol) in the presence of K_2CO_3 (0.29 g, 2.1 mmol) in MeCN (10 ml) in the usual way to give the pure compound **20e** as a greenish oil (0.16 g, 0.38 mmol, 75.3% yield): IR (KBr): ν (cm^{-1}) 3360, 2930, 1805, 1774, 1688, 1607, 1454, 1340, 1074, 899; ^{1}H NMR ($CDCl_3$): δ 2.67 (2H, t, J = 5.6 Hz, NCH_2CH$_2$OH), 3.56–3.62 (4H, m, 4-methoxyphenyl-CH_2, NCH$_2$CH_2OH), 3.69 (2H, s, PhenyleneCH_2), 3.79 (3H, s, OMe), 6.87 (2H, d, J = 6.8 Hz, arom. H_2), 7.22–7.24 (3H, m, arom. H_3), 7.50 (2H, d, J = 8.4 Hz, arom. H_2), 7.71 (1H, t, J = 5.6 Hz, arom. H_1), 7.93 (2H, d, J = 1.6 Hz, arom. H_2), 8.25 (1H, dd J = 1.2, 8.4 Hz, arom. H_1), 8.94 (1H, d, J = 7.6 Hz, arom. H_1), 11.27 (1H, s, CONH); FAB-MS *m*/*z*: 436 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{24}H_{26}N_3O_5$, 436.1872; found, 436.1855.

5.3.33. N-(2-aminophenyl)-4-{[(2-hydroxyethyl)(3-pyridinylmethyl)amino]methyl}benzamide (21a)

18 (0.32 g, 0.65 mmol) was reacted in 95% TFA (3.8 ml) in the usual way, yielding the pure compound **21a** as a greenish oil (0.24 g, 0.64 mmol, 98% yield): IR (KBr): ν (cm^{-1}) 3624, 2851, 2611, 1730, 1680, 1632, 1454, 1315, 1198, 1134, 837; ^{1}H NMR (CD_3OD): δ 3.89 (2H, t, J = 5.2 Hz, NCH_2CH$_2$OH), 3.92 (2H, s, pyridine-CH_2), 4.62 (2H, t, J = 7.8 Hz, NCH$_2$CH_2OH), 4.79 (2H, s, CH_2PhenyleneCO), 7.34–9.06 (12H, m, arom. H_{12}); FAB-MS *m*/*z*: 377 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{22}H_{25}N_4O_2$, 377.1978; found, 377.1987.

5.3.34. N-(2-aminophenyl)-4-{[benzyl(2-hydroxyethyl)amino] methyl}benzamide (21b)

20a (1.4 g, 3.45 mmol), $SnCl_2{\cdot}2H_2O$ (4.87 g, 21.6 mmol) and NH_4OAc (2.87 g, 37.2 mmol) were reacted in MeOH (120 ml) in the same way as for **18**, yielding the pure compound **21b** as a yellowish solid (0.53 g, 1.4 mmol, 40% yield): m.p. 119.2–121.9 °C; IR (KBr): ν (cm^{-1}) 3360, 2795, 2714, 1632, 1611, 1531, 1362, 1232, 1161, 885; ^{1}H NMR (CD_3OD): δ 2.64 (2H, t, J = 6.0 Hz, NCH_2CH$_2$OH), 3.62 (2H, t, J = 7.6 Hz, NCH$_2$CH_2OH), 3.68 (2H, s, PhCH_2NH), 3.74 (2H, s, CH_2PhenyleneCO), 6.73–7.37 (9H, m, arom. H_9), 7.51 (2H, d, J = 8.4 Hz, arom. H_2), 7.93 (2H, d, J = 8.0 Hz, arom. H_2); EI-MS *m*/*z*: 375 $(M)^+$; HR-EI-MS *m*/*z*: $(M)^+$ calcd for $C_{23}H_{25}N_3O_2$, 375.1947; found, 375.1973.

5.3.35. N-(2-aminophenyl)-4-({(2-hydroxyethyl)[(1-methyl-1H-indol-3-yl)methyl]amino}methyl)benzamide (21c)

20b (0.22 g, 0.48 mmol), $SnCl_2{\cdot}2H_2O$ (0.66 g, 2.92 mmol) and NH_4OAc (0.39 g, 5.05 mmol) were reacted in MeOH (18 ml) in the usual way, yielding the pure compound **21c** as a brownish solid (26.4 mg, 0.062 mmol, 12.6% yield): m.p. 154.1–156.7 °C; IR (KBr): ν (cm^{-1}) 3725, 2924, 2820, 1651, 1612, 1506, 1454, 1327, 1051, 976, 856; ^{1}H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH$_2$OH), 3.58 (2H, t, J = 5.6 Hz, NCH$_2$CH_2OH), 3.63 (2H, s, 1-methyl-1*H*-indol-CH_2), 3.75 (3H, s, NMe), 3.82 (2H, s, CH_2Phenylene), 6.79–8.02 (13H, m, arom. H_{13}); FAB-MS *m*/*z*: 429 $(M + H)^+$; HR-FAB-MS *m*/*z*: $(M + H)^+$ calcd for $C_{26}H_{29}N_4O_2$, 429.2291; found, 429.2263.

5.3.36. N-(2-aminophenyl)-4-{[(1,3-benzodioxol-5-ylmethyl)(2-hydroxyethyl)amino]methyl}benzamide (21d)

20c (73.7 mg, 0.16 mmol), $SnCl_2{\cdot}2H_2O$ (0.22 g, 0.97 mmol) and NH_4OAc (0.13 g, 1.71 mmol) were reacted in MeOH (4.8 ml) in the usual way, yielding the pure compound **21d** as a yellowish solid (22.0 mg, 0.052 mmol, 32.5% yield): m.p. 135.2–137.3 °C; IR (KBr): ν (cm^{-1}) 3389, 3310, 2939, 2833, 1638, 1524, 1439, 1325, 1248, 1057, 860; ^{1}H NMR

(CD_3OD): δ 2.52 (2H, t, J = 6.0 Hz, NCH_2CH_2OH), 3.45 (2H, s, 1,3-benzodioxol-CH_2), 3.53 (2H, t, J = 6.0 Hz, NCH_2CH_2OH), 3.59 (2H, s, PhenyleneCH_2), 5.80 (2H, s, OCH_2), 6.63–7.21 (7H, m, arom. H_7), 7.43 (2H, d, J = 8.0 Hz, arom. H_2), 7.84 (2H, d, J = 8.0 Hz, arom. H_2); FAB-MS m/z: 420 $(M + H)^+$; HR-FAB-MS m/z: $(M + H)^+$ calcd for $C_{24}H_{26}N_3O_4$, 420.1923; found, 420.1919.

*5.3.37. N-(2-aminophenyl)-4-{[(3,4-difluorobenzyl)(2-hydroxyethyl)amino]methyl}benzamide (**21e**)*

20d (0.46 g, 1.04 mmol), $SnCl_2{\cdot}2H_2O$ (1.38 g, 6.11 mmol) and NH_4OAc (0.83 g, 10.8 mmol) were reacted in MeOH (30 ml) in the usual way, yielding the pure compound **21e** as a yellowish solid (0.13 g, 0.3 mmol, 29.2% yield): m.p. 137.6–140.1 °C; IR (KBr): ν (cm^{-1}) 3356, 2959, 2357, 1611, 1516, 1458, 1325, 1203, 970, 940; ^1H NMR (CD_3OD): δ 2.68 (2H, t, J = 5.2 Hz, NCH_2CH_2OH), 3.48 (2H, s, 3,4-difluorophenyl-CH_2), 3.60–3.69 (4H, m, NCH_2CH_2OH, PhenyleneCH_2), 6.85–7.45 (9H, m, arom. H_9), 7.89 (2H, d, J = 7.6 Hz, arom. H_2); EI-MS m/z: 411 $(M)^+$; HR-EI-MS m/z: $(M)^+$ calcd for $C_{23}H_{23}F_2N_3O_2$, 411.1758; found, 411.1787.

*5.3.38. N-(2-aminophenyl)-4-{[(2-hydroxyethyl)(4-methoxybenzyl)amino]methyl}benzamide (**21f**)*

20e (0.15 g, 3.44 mmol), $SnCl_2{\cdot}2H_2O$ (0.47 g, 2.08 mmol) and NH_4OAc (0.28 g, 3.63 mmol) were reacted in MeOH (13 ml) in the usual way, yielding the pure compound **21f** as a yellowish solid (17 mg, 0.042 mmol, 12% yield): m.p. 127.0–129.1 °C; IR (KBr): ν (cm^{-1}) 3360, 2924, 2359, 1611, 1506, 1454, 1321, 1240, 939 ^1H NMR ($CDCl_3$): δ 2.68 (2H, t, J = 5.6 Hz, NCH_2CH_2OH), 3.59–3.62 (4H, m, 4-methoxyphenyl–CH_2, NCH_2CH_2OH), 3.68 (2H, s, Phenylene CH_2), 3.81 (3H, s, OMe), 6.85–6.88 (4H, m, arom. H_4), 7.10 (1H, t, J = 6.0 Hz, arom. H_1), 7.22 (2H, d, J = 8.8 Hz, arom. H_2), 7.34 (1H, d, J = 8.0 Hz, arom. H_1), 7.44 (2H, d, J = 8.4 Hz, arom. H_2), 7.81 (1H, s, CONH), 7.87 (2H, d, J = 8.4 Hz, arom. H_2); EI-MS m/z: 405 $(M)^+$; HR-EI-MS m/z: $(M)^+$ calcd for $C_{24}H_{27}N_3O_3$, 405.2052; found, 405.2057.

5.4. Sample preparation of plasma stability test

Stock solutions (2 mM) of **21d**, **21e**, **1**, TSA, SAHA and MS-275 in DMSO were prepared, respectively. An aliquot (10 μl) of each stock solution was diluted with plasma (1990 μl), resulting in an incubation solution of 10 μM. Incubation was carried out at 37 °C under gentle shaking for 24 h. Samples (100 μl) were taken immediately from each incubation solution at the beginning and after 1, 3, 7, 24 h, and were transferred to a 1.5 ml microtube respectively. Acetonitrile (400 μl) was added to precipitate plasma proteins, and the mixture was vortex-mixed for 15 s and centrifuged for 5 min at 10,000 rpm. An aliquot (400 μl) of the resulting supernatant was taken and transferred to a new 1.5 ml microtube, and a mixture of acetonitrile/Milli-Q (50:50, v/v) with 0.1% formic acid (400 μl) were added to dilute at 1.0 μM. For calibration curves, above-mentioned each incubation solution was serially diluted in acetonitrile and a mixture of acetonitrile/Milli-Q (50:50, v/v) with 0.1% formic acid at 0.125, 0.25, 0.5, 1.0, 2.0 μM. Aliquots (5 μl each) of these prepared samples were injected onto the LC-MS/MS system.

Table 3
Ionization condition and transition for **21d, 21e, 1,** TSA, SAHA and MS-275

Compd	DP[b] (V)	CAD[c] gas (psi)	Collision energy (eV)	Transition
21d	35	4	15	420.0→134.6
21e	80	4	30	412.0→303.6
1	65	4	24	336.3→131.4
SAHA	30	4	20	264.9→231.6
MS-275	39	4	30	377.3→268.6

[a] Ionization mode is electrospray; [b] Declustering potential; [c] collision-activated dissociation.

5.5. LC conditions

Sample solutions of 10 μl aliquots were injected and introduced directly into mass spectrometer from the HPLC system without the separation with column chromatography. The mobile phase consisted of acetonitrile/water (1:1) with 0.1% formic acid and the flow rate was 200 μl min^{-1}.

5.6. Mass spectrometric conditions

Sample analysis was achieved in multiple reaction monitoring (MRM), monitoring the specific transition from a protonated precursor ion to product ion for (m/z 420.0–134.6), **21e** (m/z 412.0–303.6), **1** (m/z 336.3–131.4), TSA (m/z 303.0–147.6), SAHA (m/z 264.9–231.6) and MS-275 (m/z 377.3–268.6)" respectively. The Turbo gas temperature was 500 °C and ionspray probe voltage was 5000 V. Other details for quantification are described in Table 3. The Analyst 1.3 software was used to control LC-MS/MS system and to perform sample and data analysis. The ratio of the total ion numbers were used to construct a linear calibration curve using weighted regression analysis.

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