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Synthesis of Novel Amide Derivatives with In Vitro Antiproliferative and Cytotoxic Activity

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ABSTRACT: *Some amide derivatives are highly valuable products with antiproliferative and cytotoxic bioactivity. In this paper, 12 novel amide compounds were synthesized correspondingly by boc-protection glycine, deprotection, and condensation reactions. The antiproliferative and cytotoxic activity of these compounds was also evaluated by human cervical cancer (Hela) and hepatoma carcinoma (SK-Hep-1) cancer cell lines. The assay revealed that eight compounds (***5a–d***, ***6a–d****) exhibited activity against Hela cancer cells. Four of them (****5a–d****) also showed activity against SK-Hep-1 cancer cells.* © 2012 Wiley Periodicals, Inc. Heteroatom Chem. 24:9–17, 2013; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.21057

INTRODUCTION

Amide compounds, especially macrocyclic compounds, have important biological functions in natural and synthetic anion receptors. Macrocyclic polyamines, which possess properties similar to those of crown ethers, are very important host molecules in supramolecular chemistry [1–3]. Amide compounds are also widely used in medical, industrial, and agricultural areas. The effects of macrocyclic polyamines on the reactive oxygen species level, apoptosis events, cellular viability, and activity of antioxidant enzymes that were assessed in some mammalian cell cultures have been reported [4, 5]. The synthesis of amides and the study on their biological activity have been an area of wide interest and diversity in recent years [6–9]. A number of different strategies involving macrocyclic and nonmacrocyclic amides have been developed for many biological functions [10–13]. The synthesis of structural analogs of amide derivatives with similar pharmacological activity has been a major focus of attention due to various biological activities of amide derivatives [14, 15].

In the present study, we reported the design and the synthesis of 12 novel amide compounds involving four macrocyclic tetraamides, which were synthesized by a condensation reaction [2, 11, 18] and eight linear amides, which were synthesized by coupling of boc-glycine and deprotection reactions [16, 17]. Their antiproliferative and cytotoxicity effects on human cervical cancer (Hela) and hepatoma carcinoma (SK-Hep-1) cancer cell lines were also tested here.

RESULTS AND DISCUSSION

Chemistry

The synthesis methods of compounds **4a–d**, **5a–d**, and **6a–d** were carried out according to Schemes 1 and 2. Compounds **2a** or **2b** were synthesized from 3,4-dichloronitrobenzene **1a** or *p*-chloronitrobenzene **1b**, commercially available,

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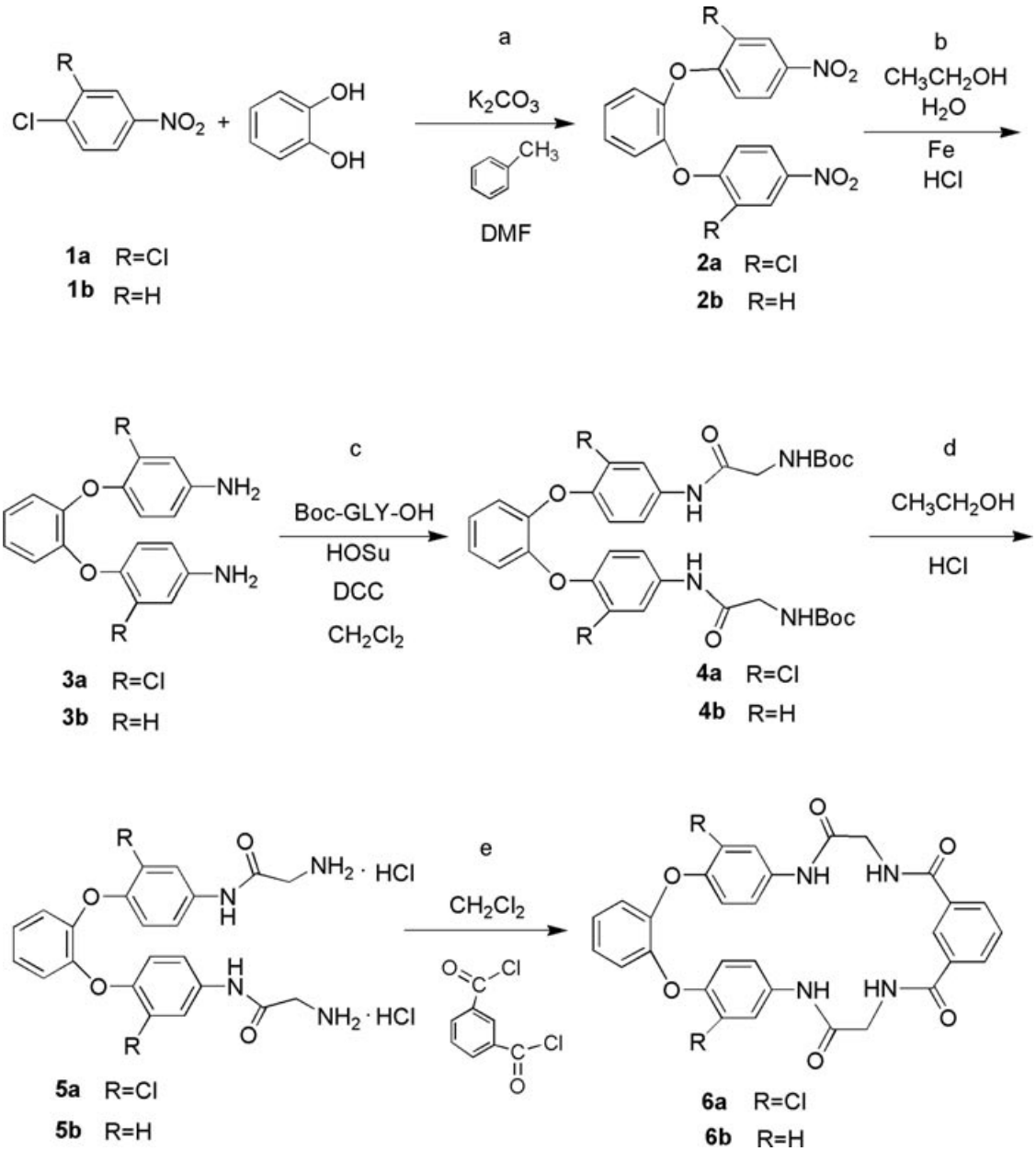
Supporting Information is available in the online issue at wileyonlinelibrary.com.

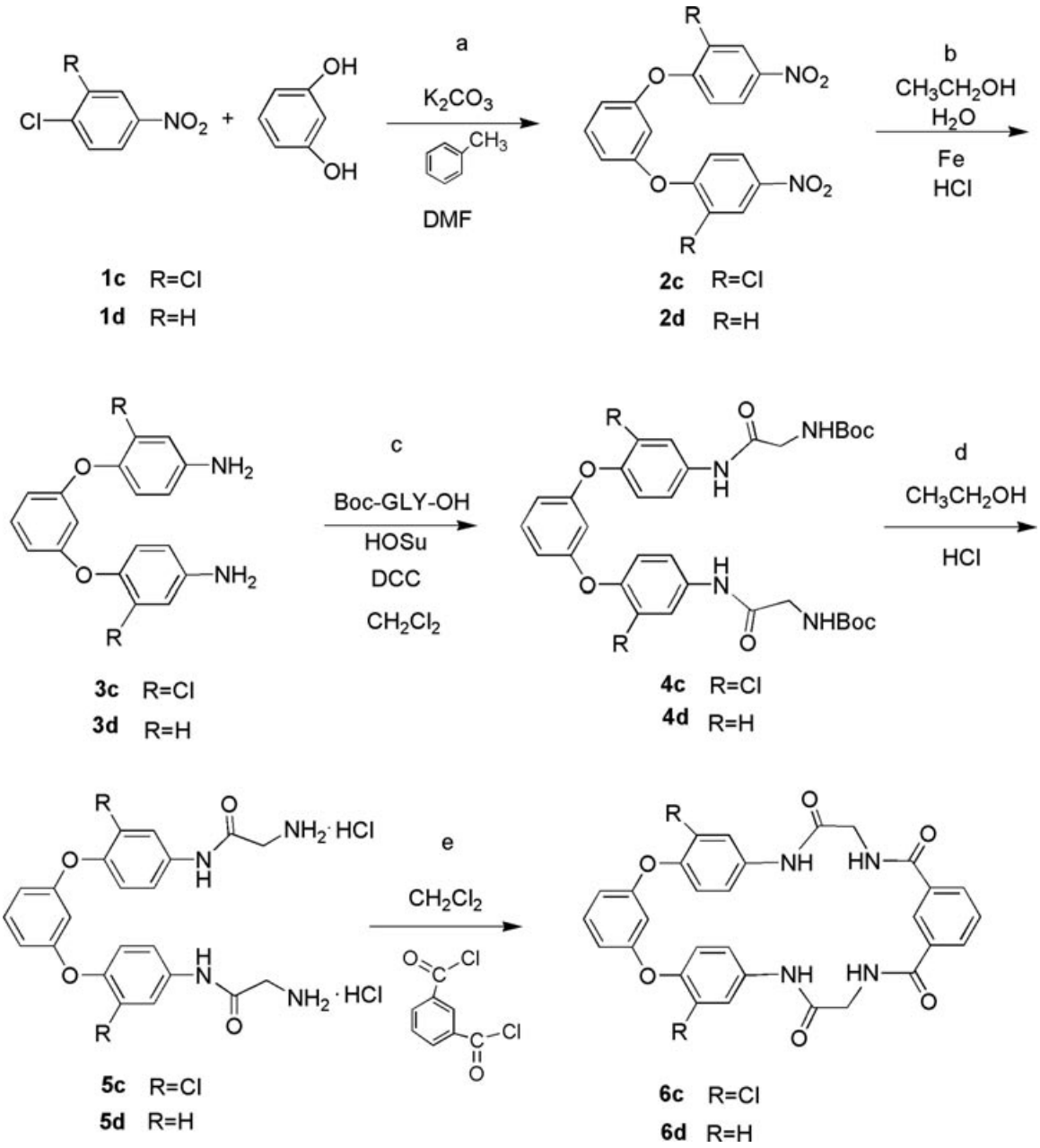


SCHEME 1 Synthesis of compounds **4a**–**b**, **5a**-**b**, and **6a**–**b**.

with catechol, K_2CO_3 in the solution of DMF, and methylbenzene. The **2c** and **2d** were prepared in the similar steps except that resorcinol was used as the reagent to reduce the effect of steric hindrance.

Compounds **3a–d** were prepared by the reduction reaction that the compounds of **2a–d** were treated with Fe and HCl dissolved in ethanol/water. Compounds **4a–d** were obtained by the treatment of diamine **3a–d** with Boc-GLY-OH, HOSu, and dicyclohexylcarbodiimide (DCC) in dry CH_2Cl_2. Compounds **5a–d** were obtained by treating **4a–d** with HCl in ethanol. The final cyclization step between **5a–d** and isophthaloyl dichloride was carried out under high diluted conditions in CH_2Cl_2 and triethylamine, giving macrocyclic polyamides **6a–d** in low yields. The structures of all synthesized compounds were characterized by elemental analyses, ^{1}H NMR (Figs. S1–S20 in Supporting Information), ^{13}C NMR (Figs. S21–S40 in Supporting Information), and MS (Figs. S41–S60 in Supporting Information).

As outlined in Schemes 1 and 2, compounds **4a–d** and **5a–d** were synthesized in high yields but compounds **6a–d** were obtained in low yields due



SCHEME 2 Synthesis of compounds **4c–d**, **5c–d**, and **6c--d**.

to side reactions resulted from the formation of oligomers or polymers.

Biological Evaluation

The antiproliferative and cytotoxic bioactivities of compounds **4a–d**, **5a–d**, and **6a–d** were evaluated in vitro against human cervical cancer Hela cells and hepatoma carcinoma SK-Hep-1 cells by colorimetric MTT assay (cisplatin was used as positive control and expressed as IC_{50} values. IC_{50} is the concentration (μg/mL) that was determined to inhibit tumor cell proliferation by 50% after 24 h of the exposure of the cells to a tested compound.) The measured IC_{50} values for the **4a–d**, **5a–d**, and **6a–d** are summarized in Table 1.

As shown in Table 1, compounds **4a–d** did not exhibit antiproliferative and cytotoxic bioactivity against the Hela cells and SK-Hep-1 cells. It may be due to the steric structure of the Boc group hindering the interaction **4a–d** with tumor cell, which reduced compounds' bioactivity. Compound **6d** exhibited higher cytotoxicity against Hela cells compared with cisplatin.

TABLE 1 Cytotoxicity Activity In Vitro of the **4a–d**, **5a–d**, and **6a–d** against Human Cervical Cancer (HeLa) and Hepatoma Carcinoma (SK-Hep-1) Cells

	IC_{50} (μg/mL)	
Compounds	*Hela*[a]	*SK-Hep-1*[b]
4a	–	–
4b	–	–
4c	–	–
4d	–	–
5a	29	15
5b	35	30
5c	20	30
5d	33	35
6a	28	–
6b	22	–
6c	41	–
6d	13	–
Cisplatin	17	5

[a]Human cervical cancer cells.
[b]Hepatoma carcinoma cells.

The cytotoxicity of **4a–d**, **5a–d**, and **6a–d** in Hela cells and SK-Hep-1 cells was assayed using the MTT test over a range of doses (0–100 μg/mL). As evident from Figs. 1 and 2, the compounds **4a–d** did not reveal antiproliferative and cytotoxic bioactivity against the Hela cells and SK-Hep-1 cells. Compounds **5a–d** were found to have antiproliferative and cytotoxic bioactivity against Hela cells and SK-Hep-1 cells. Cyclic compounds **6a–d** also had antiproliferative and cytotoxic bioactivity against Hela cells but had no antiproliferative and cytotoxic bioactivity against SK-Hep-1 cells.

The cell viability of 0.76%, 10.24%, 9.46%, and 11.36% was observed after incubation of SK-Hep-1 cells with 100 μg/mL of **5a–d**, respectively. The same situation occurred after the treatment of Hela cells with 100 μg/mL of **5a–d** and **6a–d**.

Analyzing the structures of compounds and their activity, it was found that the presence of a chlorine atom substituent in the benzene ring of compounds **5a** and **5c** exhibited higher antiproliferative activity compared with compounds **5b** and **5d**. The lower antiproliferative activity was exhibited in the series of compounds **6a** and **6c**, which possessed chlorine atoms as the substituent. This suggests that the substituents in the benzene ring may be a key factor affecting their biological activity and they have different effects on macrocyclic and nonmacrocyclic amides.

CONCLUSIONS

In this paper, 12 novel amide compounds were synthesized. The structures of all synthesized compounds were characterized by elemental analyses, ^{1}H NMR, ^{13}C NMR, and MS. The antiproliferative and cytotoxic bioactivity of these compounds was demonstrated by Hela and SK-Hep-1 cancer cell lines. Compounds (**5a–d**, **6a–d**) exhibited activity against Hela cancer cells. Moreover, four of them (**5a–d**) showed activity against SK-Hep-1 cancer cells. Among the compounds having antiproliferative and cytotoxic bioactivity, the compound **6d** was the most notable in view of cytotoxicity against Hela cells.

EXPERIMENTAL

General

Melting points of the compounds were determined using a hot stage microscope without calibration.

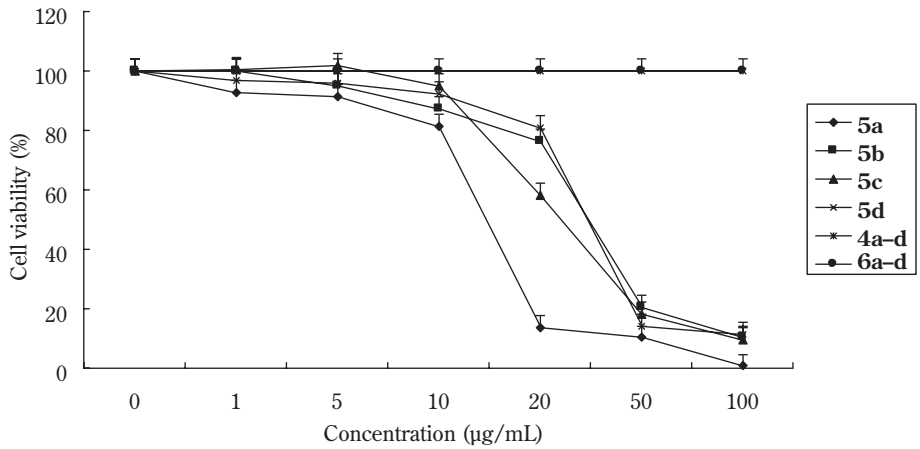


FIGURE 1 Dose–response curves for the treatment of SK-Hep-1 cells with **4a–d**, **5a–d**, and **6a–d**. Cytotoxicity was measured using the MMT assay as cell viability 48 h after incubation with the indicated substances for 24 h and is shown by mean + SD ($n = 3$).

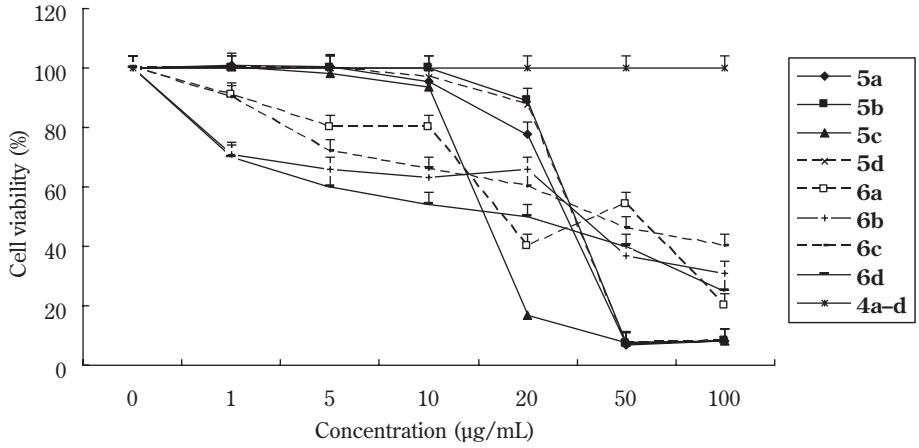


FIGURE 2 Dose–response curves for the treatment of HeLa cells with **4a–d**, **5a–d**, and **6a–d**. Cytotoxicity was measured using the MMT assay as cell viability 48 h after incubation with the indicated substances for 24 h and is shown by mean + SD ($n = 3$).

^{1}H NMR and ^{13}C NMR spectra were recorded in a Bruker Avance-400 spectrometer, Bruker ARX-300 spectrometers with TMS as the internal reference (Bruker BioSciences, Billerica, MA). MS spectra were obtained using an ABI Q-Trap mass spectrometer (Applied Biosystems, USA), Bruker esquire HCT spectrometer (Bruker, Germany). Elemental analyses (CHN) were carried out on a Vario El Cube elemental analyzer (Elementar, Germany). Column chromatography was performed using silica gel (200–300 mesh) purchased from Qingdao Ocean Chemical Reagent Co. (Shandong, China). All reagents were of analytical reagent grade and were used without further purification.

1,2-Bis(2-chloro-4-nitrophenoxy)benzene **2a**

Potassium Carbonate (19.2 g, 0.13 mol) was dissolved in DMF (30 mL) followed by addition of catechol (5.5 g, 0.05 mmol) and methylbenzene (10 mL) at room temperature. The resulting suspension was stirred at 50°C for 2 h. The mixture was cooled down to room temperature, and 3,4-dichloronitrobenzene **1a** (16.6 g, 0.08 mol) was added followed by heating under reflux for 12 h. The reaction mixture was cooled down to room temperature. 2% NaOH solution was added to the resultant solution, and the mixture was stirred for 20 min at room temperature. The solution was filtrated, and the precipitate was washed with dry methanol. The formed solid was filtered off and recrystallized from CH_2Cl_2 and EtOAc to yield 7.7g **2a** (74.3%) as a yellow powder: mp 184.6–186.4°C; ^{1}H NMR (300 MHz,$CDCl_3$) δ: 8.288 (2H, d, $J = 2.7$ Hz), 8.060 (2H, dd, $J = 9.1$, 2.7 Hz), 7.422 (2H, m), 7.310 (2H, m), 6.845 (2H, d, $J = 9.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.78 (2C), 145.25 (2C), 143.10 (2C),127.57 (2C), 126.42 (2C), 124.01 (2C), 123.55 (2C), 123.13 (2C), 115.98 (2C); MS(ESI) *m/z*: 420.7 $[M + H]^+$; Anal. Calcd. for $C_{18}H_{10}Cl_2N_2O_6$: C, 51.33; H, 2.39; N, 6.65. Found: C, 51.30; H, 2.34: N, 6.64.

1,2-Bis(4-nitrophenoxy)benzene **2b**

Compound **2b** was prepared from **1b** following the general procedure described above for **2a** and obtained in yield 72.3%; mp: 134.9–136.3°C; ^{1}H NMR (300 MHz,$CDCl_3$) δ: 8.089(4H, d, $J = 9.2$ Hz), 7.300 (2H, m), 7.288 (2H, m), 6.783 (4H, d, $J = 9.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.25 (2C), 145.96 (2C), 143.06 (2C), 127.23 (2C), 125.81 (2C), 123.38 (4C), 116.28 (4C); MS (ESI) *m/z*: 352.8 $[M + H]^+$; Anal. Calcd. for $C_{18}H_{12}N_2O_6$: C, 61.37; H, 3.43; N, 7.95. Found: C, 61.34; H, 3.39; N, 7.90.

1,3-Bis(2-chloro-4-nitrophenoxy)benzene **2c**

Compound **2c** was prepared from **1c** following the general procedure described above for **2a** and obtained in yield 74.0%; mp: 107.8–109.1°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.394 (2H, d, $J = 2.7$ Hz), 8.116 (2H, dd, $J = 9.1$, 2.7 Hz), 7.484 (1H, t, $J = 8.3$ Hz), 7.022 (2H, d, $J = 9.1$ Hz), 6.953 (2H, dd, $J = 8.3$, 2.4 Hz), 6.815 (1H, t, $J = 2.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ:157.80 (2C), 156.30 (2C), 143.36 (2C), 131.67 (1C), 126.67 (2C), 125.46 (2C), 123.68 (2C), 118.04 (2C), 116.11 (2C), 111.13 (1C); MS (ESI) *m/z*: 420.8 $[M + H]^+$; Anal. Calcd. for $C_{18}H_{10}Cl_2N_2O_6$: C, 51.33; H, 2.39; N, 6.65. Found: C, 51.31; H, 2.29; N, 6.62.

1,3-Bis(4-nitrophenoxy)benzene **2d**

Compound **2d** was prepared from **1d** following the general procedure described above for **2a** and obtained in yield 74.1%; mp 108.9–110.5°C; ^{1}H NMR (300 MHz,$CDCl_3$) δ: 8.170 (4H, d, J = 9.3 Hz), 7.404 (1H, t, J = 8.2 Hz), 7.010 (4H, d, J = 9.3 Hz), 6.900 (2H, dd, J = 8.2,2.3 Hz), 6.776 (1H, t, J = 2.3 Hz); ^{13}C NMR(75 MHz, $CDCl_3$) δ: 162.30 (2C), 156.41 (2C), 143.13 (2C), 131.53 (1C), 126.00 (4C), 117.63 (4C), 116.70 (2C), 112.38 (1C); MS (ESI) *m/z*: 352.8 [M + H]$^+$; Anal. Calcd. for $C_{18}H_{12}N_2O_6$: C, 61.37; H, 3.43; N, 7.95. Found: C, 61.33; H, 3.35; N, 7.91.

1,2-Bis(4-amino-2-chlorophenoxy)benzene **3a**

Under nitrogen atmosphere, a mixture of compound **2a** (5.5 g, 3.5 mmol) and powder of Fe (5 g, 80 mmol) was stirred in the solution of H_2O (20 mL) and ethanol (20 mL). After that, 37.5% HCl (1.0 mL) was added dropwise. The mixture was heated under reflux for 3 h and was cooled down to room temperature. The solid was removed by filtration, the filtrate was extracted with CH_2Cl_2, and the organic layer was washed with water, saturated brine, dried in vacuo over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography (*n*-hexane: EtOAc = 3:1) to give **3a** (0.87 g, 69.0%) as a brown solid: mp 137.7–140.9°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.929 (2H, d, J = 2.6 Hz), 6.892 (2H, d, J = 8.6 Hz), 6.762 (4H, m), 6.538 (2H, dd, J = 8.6, 2.7 Hz), 3.609 (NH, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 147.70 (2C), 144.07 (2C), 143.57 (2C), 126.46 (2C), 123.04 (2C), 122.29 (2C), 117.63 (2C), 116.57 (2C), 114.46 (2C); MS (ESI) *m/z*: 360.8 [M + H]$^+$; Anal. Calcd. for $C_{18}H_{14}Cl_2N_2O_2$: C, 59.85; H, 3.91; N, 7.76. Found: C, 59.80; H, 3.84; N, 7.72.

1,2-Bis(4-aminophenoxy)benzene **3b**

Compound **3b** was prepared from **2b** following the general procedure described above for **3a** and obtained in 62.0% yield as a yellow solid: mp 137.2–139.3°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.948 (2H, m), 6.887 (2H, m), 6.831(4H, d, J = 8.6 Hz), 6.638 (4H, d, J = 8.6 Hz), 3.483 (NH, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.21 (2C), 145.81 (2C), 142.88 (2C), 127.25 (2C), 125.80 (4C). 123.39 (2C), 116.19 (4C); MS (ESI) *m/z*: 292.9 [M + H]$^+$; Anal. Calcd. for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.92; H, 5.48; N, 9.57.

1,3-Bis(4-amino-2-chlorophenoxy)benzene **3c**

Compound **3c** was prepared from **2c** following the general procedure described above for **3a** and obtained in 63.6% yield as a yellow solid: mp 141.2–145.9°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.180 (1H, t, J = 8.1 Hz), 6.950 (2H, d, J = 8.6 Hz), 6.810 (2H, d, J = 2.7 Hz), 6.600 (2H, dd, J = 8.6, 2.7 Hz), 6.543 (3H, m), 3.698 (NH, s); ^{13}C NMR (75 MHz,$CDCl_3$) δ: 159.60 (2C), 144.12 (2C), 143.32 (2C), 129.94 (1C), 127.31 (2C), 123.35 (2C), 116.58 (2C), 114.55 (2C), 109.95 (2C), 105.25 (1C); MS (ESI) *m/z*: 360.8 [M + H]$^+$; Anal. Calcd. for $C_{18}H_{14}Cl_2N_2O_2$: C, 59.85; H, 3.91; N, 7.76. Found: C, 59.81; H, 3.83; N, 7.74.

1,3-Bis(4-aminophenoxy)benzene **3d**

Compound **3d** was prepared from **2d** following the general procedure described above for **3a** and obtained in 63.0% yield as a yellow solid: mp 116.5–119.0°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.176 (1H, t, J = 8.0 Hz), 6.909 (4H, d, J = 8.6 Hz), 6.707 (4H, d, J = 8.6 Hz), 6.607 (2H, m),6.569 (1H, d, J = 2.5 Hz), 3.698 (NH, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.17 (2C), 148.36 (2C), 142.81 (2C), 129.89 (1C), 121.15 (4C), 116.20 (4C), 110.71 (2C),106.74 (1C); MS (ESI) *m/z*: 292.9 [M + H]$^+$; Anal. Calcd. for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.91; H, 5.46; N, 9.55.

1,2-Bis(2-chloro-4-(N-boc-aminoacetamido) phenoxy)benzene **4a**

Boc-GLY-OH (175.0 mg, 2.3 mmol) and HOSu (63.5 mg, 0.55 mmol) was dissolved in dry CH_2Cl_2 (5 mL). After the addition over a 10 min period of a solution of **3a** (166.7 mg, 0.46 mmol) in dry CH_2Cl_2 (5 mL), the mixture was stirred at 0°C under nitrogen atmosphere for 20 min. Then a suspension of DCC (113.5 mg, 0.55 mmol) dissolved in dry CH_2Cl_2 (2.5 mL) was dropped into the stirred resultant solution at 0°C. The reaction mixture was stirred at room temperature for 12 h and then concentrated in vacuo to give the residue that dissolved in EtOAc (100 mL), then washed with water, dried in vacuo over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography (*n*-hexane: EtOAc = 1:1) to give **4a** (0.14 g, 46.7%) as a yellow powder: mp 109.0–111.2°C; ^{1}H NMR (300 MHz, acetone-d_6) δ: 9.355 (NH, s), 7.923 (2H, s), 7.430 (2H, d, J = 8.8 Hz), 7.144 (2H, dd, J = 6.0, 3.6 Hz), 6.979 (2H, dd, J = 6.0, 3.6 Hz), 6.906 (2H, d, J = 8.8 Hz), 6.308 (NH, s), 3.859 (4H, m), 1.390 (18H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.93 (2C), 148.95 (2C), 147.80 (2C), 136.28 (2C), 125.63 (2C), 124.93 (2C), 121.87 (2C), 120.83 (2C),120.32 (2C), 119.80 (2C), 79.39 (2C), 45.02 (2C), 28.49 (6C); MS (ESI) *m/z*: 675.0 [M + H]$^+$; Anal. Calcd. for $C_{32}H_{36}Cl_2N_4O_8$:

C, 56.89; H, 5.37; N, 8.29. Found: C, 56.90; H, 5.33; N, 8.27.

1,2-Bis(4-(N-boc-aminoacetamido) phenoxy)benzene **4b**

Compound **4b** was prepared from **3b** following the general procedure described above for **4a** and obtained in 56.0% yield as a yellow powder: mp 99.1–99.4°C; ^{1}H NMR (300 MHz, acetone-d_6) δ: 9.427 (NH, s), 7.557 (4H, d, J = 8.1 Hz), 7.102 (2H, dd, J = 5.9, 3.7 Hz),7.007 (2H, dd, J = 5.9, 3.6 Hz), 6.801 (4H, d, J = 8.4 Hz), 6.450 (NH, s), 3.975 (4H, s), 1.385 (18H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ:, 168.56 (2C), 156.82 (2C), 154.26 (2C), 148.76 (2C), 135.00 (2C), 125.57 (2C), 122.16 (4C), 121.65 (4C), 118.42 (2C), 79.38 (2C), 45.20 (2C), 28.49 (6C); MS (ESI) *m/z*: 607.0 $[M + H]^+$; Anal. Calcd. for $C_{32}H_{38}N_4O_8$: C, 63.35; H, 6.31; N, 9.24. Found: C, 63.32; H, 6.25; N, 9.20.

1,3-Bis(2-chloro-4-(N-boc-aminoacetamido) phenoxy)benzene **4c**

Compound **4c** was prepared from **3c** following the general procedure described above for **4a** and obtained in 48.2% yield as a white powder: mp 186.8–188.2°C; ^{1}H NMR (300 MHz, acetone-d_6) δ: 9.254 (NH, s), 7.886 (2H, s), 7.405 (2H, d, J = 8.9 Hz), 7.181 (1H, t, J = 8.3 Hz), 6.993 (2H, d, J = ,8.8 Hz), 6.504 (1H, dd, J = 8.3, 2.4 Hz), 6.476 (1H, dd, J = 8.3, 2.4 Hz), 6.344 (1H, s), 6.170 (NH, s), 3.766 (4H, m), 1.292 (18H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ: 169.10 (2C), 159.83 (2C), 156.87 (2C),147.76 (2C), 137.38 (2C), 131.47 (1C), 126.67 (2C), 123.03 (2C), 122.06 (2C), 120.15 (2C), 111.84 (2C), 106.54 (1C), 79.48 (2C), 45.27 (2C), 28.48 (6C); MS (ESI) *m/z*: 697.0 $[M + Na]^{+;}$ Anal. Calcd. for $C_{32}H_{36}Cl_2N_4O_8$: C, 56.89; H, 5.37; N, 8.29. Found: C, 56.85; H, 5.34; N, 8.26.

1,3-Bis(4-(N-boc-aminoacetamido) phenoxy)benzene **4d**

Compound **4d** was prepared from **3d** following the general procedure described above for **4a** and obtained in 45.2% yield as a gray powder: mp 116.1–117.4°C; ^{1}H NMR (300 MHz, acetone-d_6) δ: 9.160 (NH, s), 7.540 (4H, d, J = 8.8 Hz), 7.186 (1H, t, J = 8.2 Hz), 6.875 (4H, d, J = 8.8 Hz), 6.562 (1H, dd, J = 8.2, 2.1 Hz), 6.535 (1H, dd, J = 8.2, 2.1 Hz), 6.428 (1H, s), 6.190 (NH, s), 3.747 (4H, s), 1.300 (18H, s); ^{13}C NMR (75 MHz, acetone-d_6) δ: 168.71 (2C), 160.19 (2C), 156.92 (2C), 152.90 (2C), 136.01 (2C), 131.46 (1C), 121.75 (4C), 120.56 (4C), 112.94 (2C), 108.48 (1C), 79.38 (2C), 45.15 (2C), 28.50 (6C); MS (ESI) *m/z*: 629.3 $[M + Na]^+$; Anal. Calcd. for $C_{32}H_{38}N_4O_8$: C, 63.35; H, 6.31; N, 9.24. Found: C, 63.32; H, 6.27; N, 9.24.

1,2-Bis(2-chloro-4-(aminoacetamido)phenoxy) benzene Hydrochloride **5a**

A mixture of 37.5% HCl and ethanol (v:v = 1:1) was added dropwise over 10 min at 0°C to the suspension of compound **4a** (0.3663 g, 0.54 mmol) in ethanol (10 mL). The mixture was stirred at room temperature for 4 h. The solid was removed by filtration and washed with dry ethanol to give a product **5a** (0.16 g, 62.2%) as a white solid: mp 245.4–247.1°C; ^{1}H NMR (300 MHz, D_2O) δ: 7.387 (2H, d, J = 2.1 Hz), 6.993 (4H, m), 6.837 (2H, m), 6.622 (2H, d, J = 8.9 Hz), 3.746 (4H, s); ^{13}C NMR (75 MHz, D_2O) δ: 163.73 (2C), 147.55 (2C), 144.49 (2C), 131.56 (2C), 124.19 (2C), 122.52 (2C), 121.32 (2C), 119.12 (2C), 117.72 (2C), 89.16 (2C), 39.70 (2C); MS (ESI) *m/z*: 474.9 $[M - 2Cl - H]^+$; Anal. Calcd. for $C_{22}H_{22}Cl_4N_4O_4$: C, 48.20; H, 4.04; N, 10.22. Found: C, 48.16; H, 4.00; N, 10.20.

1,2-Bis(4-(aminoacetamido)phenoxy)benzene Hydrochloride **5b**

Compound 5**b** was prepared from 4**b** following the general procedure described above for 5**a** and obtained in 66.2% yield as a pink powder: mp 263.2–265.4°C; ^{1}H NMR (300 MHz, D_2O) δ: 7.147 (4H, m), 7.033 (2H, m), 6.958 (2H, m), 6.707 (4H, m), 3.743 (4H, s); ^{13}C NMR (75 MHz, D_2O) δ:167.61 (2C), 156.61 (2C), 149.16 (2C), 133.99 (2C), 128.06 (2C), 125.29 (4C), 124.11 (4C), 120.17 (2C), 43.28 (2C); MS (ESI) *m/z*: 407.0 $[M - 2Cl - H]^+$; Anal. Calcd. for $C_{22}H_{24}Cl_2N_4O_4$: C, 55.12; H, 5.05; N, 11.69. Found: C, 55.10; H, 5.00; N, 11.63.

1,3-Bis(2-chloro-4-(aminoacetamido)phenoxy) benzene Hydrochloride **5c**

Compound **5c** was prepared from **4c** following the general procedure described above for **5a** and obtained in 52.0% yield as a white solid: mp 261.0–262.5°C; ^{1}H NMR (300 MHz, D_2O) δ: 7.547 (2H, s), 7.177 (3H, m), 6.915 (2H, d, J = 8.8 Hz), 6.563 (2H, d, J = 8.3 Hz), 6.396 (1H, s), 3.850 (4H, s); ^{13}C NMR (75 MHz, D_2O) δ: 164.14 (2C), 156.80 (2C), 147.01 (2C), 132.63 (2C), 129.87 (1C), 124.44 (2C), 121.83 (2C), 120.63 (2C), 119.81 (2C), 110.97 (2C), 105.58 (1C), 39.90 (2C); MS (ESI) *m/z*: 475.0 $[M - 2Cl - H]^+$; Anal. Calcd. for $C_{22}H_{22}Cl_4N_4O_4$: C, 48.20; H, 4.04; N, 10.22. Found: C, 48.18; H, 4.01; N, 10.21.

1,3-Bis(4-(aminoacetamido)phenoxy)benzene Hydrochloride **5d**

Compound **5d** was prepared from **4d** following the general procedure described above for **5a** and obtained in 36.6% yield as a white solid: mp 258.9–261.4°C; ^{1}H NMR (300 MHz, D_2O) δ: 7.253 (4H, d, J = 8.4 Hz), 7.149 (1H, t, J = 8.0 Hz), 6.822 (4H, d, J = 8.4 Hz), 6.583 (2H, d, J = 8.0 Hz), 6.426 (1H, s), 3.830 (4H, s); ^{13}C NMR(75 MHz, D_2O) δ:164.04 (2C), 156.85 (2C), 152.24 (2C), 130.85 (2C), 129.68 (1C), 121.87 (4C), 118.37 (4C), 112.21 (2C), 107.51 (1C), 39.56 (2C); MS (ESI) *m/z*: 407.2 $[M - 2Cl - H]^+$; Anal. Calcd. for $C_{22}H_{24}Cl_2N_4O_4$: C, 55.12; H, 5.05; N, 11.69. Found: C, 55.09; H, 5.02; N, 11.65.

11,31-Dichloro-2,9-dioxa-15,18,24,27-tetraoxo-14,17,25,28-tetraazapentacyclo [27.2.2.2^{10,13}.1^{19,23}.0^{3,8}] pentatriacont-3,5,7,10,12,19,21,23,29,31,33,34-dodecaene **6a**

The cyclization was carried out under high dilution condition. A suspension of compound **5a** (24.3 mg, 0.05 mmol) and Et_3N (1.0 mL) in dry CH_2Cl_2 (30 mL) was added dropwise over 30 min at 0°C to a solution of iso-phthaloyl dichloride (12 mg, 0.06 mmol) in dry CH_2Cl_2 (50 mL). The mixture was stirred for 12 h at 30°C. After filtration, the mixture was concentrated in vacuo to give the residue that was dissolved in dry CH_2Cl_2, then washed with water, dried in vacuo over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography (methanol: EtOAc = 1:10) to give **6a** (0.012 g, 40%) as a yellow powder: mp 323.8–326.1°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.897 (NH, s), 8.674 (NH, s), 8.201 (1H, s), 7.875(2H, d, J = 7.6 Hz), 7.685 (2H, s), 7.543 (1H, t, J = 7.6 Hz), 7.317 (4H, s), 7.091 (2H, d, J = 9.0 Hz), 6.570 (2H, d, J = 9.0 Hz), 3.960 (4H, m); ^{13}C NMR (75 MHz DMSO-d_6) δ: 167.77 (2C), 166.56 (2C), 148.90 (2C), 146.62 (2C), 134.51 (2C), 134.28 (2C), 130.01 (2C), 128.72 (1C), 126.93 (2C), 126.34 (1C), 123.84 (2C), 121.64 (2C), 120.84 (2C), 119.18 (2C), 116.76 (2C), 45.66 (2C); MS (ESI) *m/z*: 604.9 $[M + H]^+$; Anal. Calcd. for $C_{30}H_{22}Cl_2N_4O_6$: C, 59.52; H, 3.66; N, 9.25. Found: C, 59.50; H, 3.60; N, 9.22.

2,9-Dioxa-15,18,24,27-tetraoxo-14,17,25,28-tetraazapentacyclo[27.2.2^{10,13}.1^{19,23}.0^{3,8}] pentatriacont-3,5,7,10,12,19,21,23,29,31,33,34-dodecaene **6b**

Compound **6b** was prepared from **5b** following the general procedure described above for **6a** and obtained in 36.6% yield as a white powder: mp 308.4–310.8°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.783 (NH, s), 8.769 (NH, s), 8.257 (1H, s), 7.859 (2H, d, J = 7.6 Hz), 7.532 (1H, t, J = 7.6 Hz), 7.303 (4H, d, J = 8.4 Hz) 7.266 (4H, m), 7.649 (4H, d, J = 8.4 Hz), 3.954 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 167.30 (2C), 166.62 (2C), 153.73 (2C), 147.36 (2C), 134.58 (2C), 133.36 (2C), 129.80 (2C), 128.54 (1C), 126.76 (1C), 125.72 (2C), 123.90 (4C), 120.61 (4C), 116.19 (2C), 45.53 (2C); MS (ESI) *m/z*: 537.0 $[M + H]^+$; Anal. Calcd. for $C_{30}H_{24}N_4O_6$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.13; H, 4.46; N, 10.40.

10,30-Dichloro-2,8-dioxa-14,17,23,26-tetraoxo-13,16,24,27-tetraazapentacyclo[26.2.2.2^{9,12}.1^{3,7}.1^{18,22}] pentatriacont -3,5,7,9,11,18,20,22,28,30,31,33-dodecaene **6c**

Compound **6c** was prepared form **5c** following the general procedure described above for **6a** and obtained in 51.6% yield as a white solid: mp 312.3–314.5°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.167 (NH, s), 8.824 (NH, s), 8.292 (1H, s), 7.929 (2H, d, J = 6.6 Hz),7.863 (2H, d, J = 2.3 Hz), 7.566 (1H, t, J = 7.5 Hz), 7.459 (1H, d, J = 8.3 Hz),7.403 (2H, d, J = 9.0 Hz), 7.084 (2H, d, J = 9.0 Hz), 6.972 (2H, d, J = 2.3 Hz),5.785 (1H, s), 3.989–3.974 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 168.10 (2C), 166.49 (2C), 157.66 (2C), 145.56 (2C), 136.28 (2C), 134.34 (2C), 131.45 (2C), 129.86 (1C), 128.50 (1C), 126.78 (2C), 124.47 (1C), 121.48 (2C), 120.98 (2C), 119.52 (2C), 112.85 (2C), 101.50 (1C), 44.71 (2C); MS (ESI) *m/z*: 603.2 $[M - H]^-$; Anal. Calcd. for $C_{30}H_{22}Cl_2N_4O_6$: C, 59.52; H, 3.66; N, 9.25. Found: C, 59.49; H, 3.64; N, 9.21.

2,8-Dioxa-14,17,23,26-tetraoxo-13,16,24,27-tetraazapentacyclo[26.2.2.2^{9,12}.1^{3,7}.1^{18,22}] pentatriacont -3,5,7,9,11,18,20,22,28,30,31,33-dodecaene **6d**

Compound **6c** was prepared from **5c** following the general procedure described above for **6a** and obtained in 48.6% yield as a yellow solid: mp 310.2–312.5°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.991 (NH, s), 8.822 (NH, s), 8.334 (1H, s), 7.915 (2H, d, J = 7.6 Hz), 7.582 (1H, d, J = 7.6 Hz), 7.531 (4H, d, J = 8.7 Hz), 7.388 (1H, t, J = 8.1 Hz), 6.961 (4H, d, J = 8.5 Hz), 6.883 (2H, d, J = 8.1 Hz), 6.050 (1H, s), 3.977 (4H, s); ^{13}C NMR(75 MHz, DMSO-d_6) δ: 167.70 (2C), 166.63 (2C), 158.21 (2C), 150.89 (2C), 134.97 (2C), 134.54 (2C), 131.38 (2C), 129.98 (1C), 128.58 (1C), 126.72 (1C), 121.21 (4C), 119.31 (4C), 113.21 (2C), 103.80 (1C), 44.68 (2C); MS (ESI) *m/z*: 535.5 $[M - 1]^-$; Anal. Calcd. for $C_{30}H_{24}N_4O_6$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.11; H, 4.44; N, 10.41.

Cytotoxicity

Cytotoxicity of compounds was assessed by a standard 3-(4,5- dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described [19]. Cells were plated on 96-well plates at a density of 110 cells per well 24 h prior to the addition of the MTT reagent. Since compounds **4a–d**, **5a–d**, and **6a–d** had low aqueous solubility, all dilutions involving these compounds were performed in DMSO prior to the addition of 0.5 μL aliquots to each well. After 24 h of incubation, the substances were removed. Then the cells was washed and incubated in complete medium for another 48 h. All of the compounds were initially tested once in each of the cell lines. The active compounds (IC_{50} < 100 μg/mL) were tested three times, and the listed value for each cytotoxic substance was the average value of three experiments. Cell viability was expressed as percentage reduction of incorporated MTT.

SUPPORTING INFORMATION

Supporting Information is available in the online issue at wileyonlinelibrary.com.

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