Monatsh Chem (2010) 141:893–899
DOI 10.1007/s00706-010-0337-x

ORIGINAL PAPER

Novel naphthalimide–indomethacin hybrids as potential antitumor agents: effects of linkers on hypoxic/oxic cytotoxicity and apoptosis-inducing activity

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Received: 13 January 2010 / Accepted: 29 May 2010 / Published online: 24 July 2010

Abstract A series of novel naphthalimide–indomethacin hybrids with different linkers were designed and synthesized. Their antitumor activity was evaluated against HeLa, A549, P388, HL-60, MCF-7, HCT-8, and A375 cancer cell lines in vitro. Preliminary results showed that the hybrids had moderate cytotoxic activity with 50% inhibition concentration (IC_{50}) values of $\sim 10^{-5}$ M, and could effectively induce apoptosis in HeLa cells. More importantly, the amide derivatives had better cytotoxic and proapoptotic activity than their ester counterparts, whereas the ester derivatives had hypoxic preferred cytotoxicity and might be used as promising candidates of prodrug in hypoxic tumor cells. This work provides a novel class of naphthalimide–indomethacin hybrids with unique antitumor activity for further optimization.

Keywords Apoptosis · Cytotoxicity · Hybrid · Indomethacin · Naphthalimide

Introduction

The search for novel antitumor agents has been focused on compounds that can induce apoptosis selectively in malignant cells [1, 2], or that could be used as prodrugs that are specifically activated in tumor tissue to improve their therapeutic index [3, 4]. However, the majority of clinically used anticancer drugs are not truly selective for cancer cells, and usually they have side-effects to some extent, which made their therapy less effective, leading ultimately to their failure [5]. It was, therefore, imperative that innovative approaches be employed to circumvent these defects. To fulfill this need, one strategy was the development of hybrids (Fig. 1) composed of two antitumor moieties with different mechanisms of action [6, 7]. These derivatives might carry a significant advantage in cancer therapy because tumor growth is caused by multiple mutations [8]. Therefore, activation of more than one signaling pathway could frequently define cancerous cell phenotype [9] and possibly augment the potency of both compounds or reduce side-effects and drug resistance development [10].

Naphthalimides, first discovered by Braña and co-workers [11, 12], are DNA-targeted chemotherapeutic agents acting primarily by attacking DNA at some level (synthesis, replication, or processing) [13]. Heretofore, plenty of naphthalimide-based anticancer drugs have been synthesized [9, 14–25], and promising results have been obtained. Meanwhile, indomethacin is of particular interest [26], being a member of the nonsteroidal anti-inflammatory drugs, which are widely applied in treatment of arthritis [27] and cardiovascular diseases [28], cancer prevention [29, 30], etc. [31–35]. It has been proven that indomethacin can induce G_1 arrest and apoptosis of human colorectal cancer cells by influencing the Wnt signaling pathway [36], downregulate transcriptional activity of the peroxisome proliferation-activated receptor [37], and inhibit angiogenesis [29]. Based on the characteristic structure of multitargeted drugs [9] and our previously reported results [38], we speculated that

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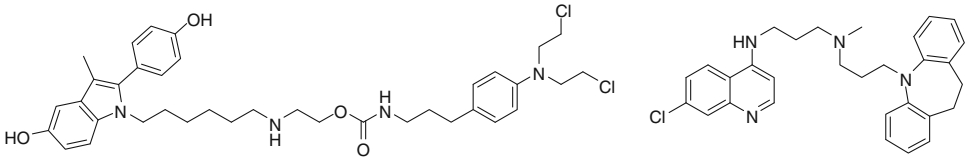
Fig. 1 Structures of some reported hybrids



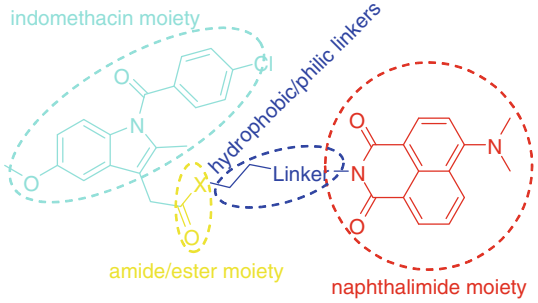


Fig. 2 Design strategy of naphthalimide–indomethacin hybrids

naphthalimide–indomethacin hybrids with different linkers might have improved or different biological activity.

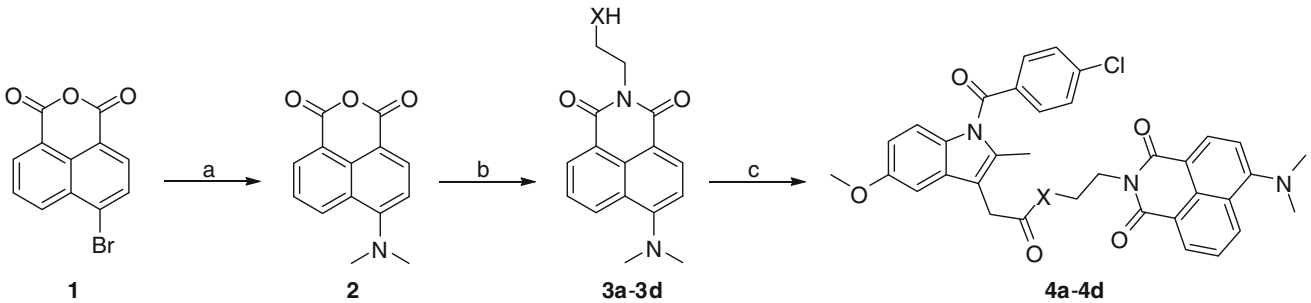
As shown in Fig. 2, the naphthalimide scaffold was utilized as the key prototype structural unit, and dimethylamine and indomethacin functional groups were conjugated to the naphthalene ring. The dimethylamino substituent introduced at position 6 of naphthalimide is difficult to acetylate [39] and might be involved in DNA synthesis arrest [40]. Various (hydrophobic or hydrophilic) linkers were designed between naphthalimide and indomethacin in order to investigate their effects on biological activity, which might lead to concomitant increase in cytotoxicity against tumor cell lines [41–44]. Introduction of an amide/ester moiety might result in different pharmacologic profiles and optimal therapeutic window for hybrids [45–48]. Therefore, four naphthalimide–indomethacin hybrids **4a**–**4d** were prepared and their antitumor activity evaluated against a variety of cancer cell lines. Their hypoxic cytotoxicity and apoptosis-inducing activity were also studied in this work.

Results and discussion

The synthetic routes of the designed compounds **4a**–**4d** are shown in Scheme 1. 6-Bromobenzo[*de*]isochromene-1,3-dione (**1**) was treated with dimethylamine in *N*,*N*-dimethylformamide (DMF) catalyzed by $CuSO_4{\cdot}5H_2O$ to afford intermediate **2** [49] with satisfactory yield of 85%. Subsequent nucleophilic substitution of **2** with corresponding amines in EtOH led to key intermediates **3a**–**3d**, which were subjected to condensation with indomethacin, affording the target compounds **4a**–**4d** with moderate yields of 70–80%. The structures of all newly synthesized compounds were confirmed by using ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, high-resolution mass spectroscopy (HRMS), infrared (IR), and elemental analysis.

The in vitro antitumor activity of the target compounds was evaluated by examining their cytotoxic effects using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium dye assay [50]. IC_{50} represents the drug concentration (μM) required to inhibit cell growth by 50%, as summarized in Table 1.

As shown in Table 1, the hybrids had cytotoxicity better than indomethacin and **3a** against the cancer cell lines tested. Under oxic condition, IC_{50} values were $\sim 10^{-5}$ M against HeLa, HL-60, HCT-8, and A375 cell lines. For A549, P388, and MCF-7 cell lines, there was no activity. It was interesting that amide derivatives had better cytotoxic activity than their ester counterparts, which indicated that different kinds of compounds had important effects on cytotoxicity. Furthermore, the length of the linker also influenced its bioactivity. It was obvious that compounds



Reagents and conditions: (a) dimethylamine, $CuSO_4{\cdot}5H_2O$, DMF, reflux 1.5 h, 85% yield; (b) corresponding amine, EtOH, reflux 1.5 h, 85-90% yield; (c) indomethacin, EDCI, DMAP, $CHCl_3$, r.t. 48 h, 70-80% yield

4a: X=-O-; **4b:** X=-O$(CH_2)_2$O-;
4c: X=-NH-; **4d:** X=-NH$(CH_2)_4$-;

Scheme 1

Table 1 Cytotoxicity of naphthalimide–indomethacin hybrids against HeLa, A549, P388, HL-60, MCF-7, HCT-8, and A375 cell lines

Compound	Cytotoxicity [IC_{50} (μM)][a]								
	Oxic							Hypoxic	HCR[b] (O_2/N_2)
	HeLa	A549	P388	HL-60	MCF-7	HCT-8	A375	HeLa	
Indomethacin	74.5 ± 10.1	NA[c]	NA[c]	63.2 ± 9.5	NA[c]	58.7 ± 7.9	92.6 ± 13.9	NA[c]	–
3a	87.3 ± 10.5	NA[c]	NA[c]	50.5 ± 7.7	NA[c]	39.2 ± 4.9	82.1 ± 12.6	NA[c]	–
4a	57.3 ± 6.9	NA[c]	NA[c]	47.4 ± 6.6	NA[c]	27.4 ± 3.3	64.3 ± 7.7	44.3 ± 5.3	1.3
4b	76.3 ± 11.5	NA[c]	NA[c]	57.4 ± 8.6	NA[c]	37.2 ± 5.6	68.7 ± 10.3	19.7 ± 3.0	3.9
4c	13.9 ± 2.5	NA[c]	NA[c]	20.3 ± 3.1	NA[c]	47.4 ± 7.1	44.2 ± 6.6	NA[c]	–
4d	24.4 ± 2.9	NA[c]	NA[c]	24.7 ± 3.0	NA[c]	29.6 ± 3.6	26.9 ± 3.2	NA[c]	–

Cancer cell lines: *A549* human lung cancer cell line, *HeLa* human cervical carcinoma cell line, *P388* murine leukemia cell line, *HL-60* human promyelocytic leukemia cell line, *MCF-7* human Caucasian breast adenocarcinoma cell line, *HCT-8* human ileocecal adenocarcinoma cell line, *A375* human melanoma cell line

[a] Cytotoxicity values are means of three experiments

[b] HCR, hypoxic/oxic cytotoxicity ratio

[c] NA, not active, $IC_{50} > 100$ μM

Table 2 Cell-cycle distribution of HeLa cells in the presence of IC_{50} concentration of the hybrids

Compound	IC_{50} conc. (μM)	Cell cycle distribution (%)[a]			
		G_1	S	G_2/M	Sub-G_1
Control	0	52.86 ± 7.93	33.86 ± 5.08	13.29 ± 1.99	0.72 ± 0.11
Indomethacin	50	55.20 ± 6.63	30.17 ± 3.62	14.63 ± 2.87	8.92 ± 1.78
4a	57	52.62 ± 6.31	35.78 ± 4.29	11.59 ± 1.39	11.88 ± 1.43
4b	76	52.67 ± 6.32	29.66 ± 3.56	17.67 ± 2.12	16.11 ± 1.93
4c	14	47.71 ± 7.16	31.93 ± 4.79	20.36 ± 3.05	38.43 ± 5.76
4d	24	43.12 ± 5.17	43.03 ± 5.16	13.85 ± 1.66	49.84 ± 5.98

[a] Values are means of three experiments

4a and **4c** with CH_2CH_2 linker had relatively lower activity than compounds **4b** and **4d** with $CH_2CH_2OCH_2CH_2$ and hexyl linker. More importantly, ester derivatives demonstrated unique hypoxic preferred cytotoxicity compared with their amide counterparts against HeLa cells. For compounds **4a** and **4b**, the hypoxic/oxic cytotoxicity ratio (HCR) was 1.3 and 3.0, respectively, whereas compounds **4c** and **4d** exhibited no hypoxic cytotoxicity. The results indicated that the ester bond easily underwent a bioreduction process and that the molecule presented better hypoxic cytotoxicity than with an amide bond [48]. It was also demonstrated that different kinds of linkers could significantly impact the bioactivity of the hybrids [42, 43]. Compound **4b** had the best hypoxic cytotoxicity and might be used as a promising candidate prodrug against hypoxic tumor cells for further optimization.

To investigate the possible mechanism of action responsible for antitumor activity of the hybrids, we tested their effects on cell cycle by fluorescence-activated cell sorting (FACS) [51]. The HeLa cell line was used in this assay, and the results are summarized in Table 2.

As shown in Table 2, the hybrids were found to induce apoptosis effectively in the HeLa cell line, better than indomethacin. After incubation with the IC_{50} concentration of these hybrids, the sub-G_1 portions were increased from 0.72% in the control to 11.88–49.84% in cells treated with the target compounds. Compound **4d** was the most potent apoptosis-inducing agent among the hybrids and could induce 49.84% cell apoptosis. It was obvious that amide derivatives **4c** and **4d** had better proapoptotic activity than ester derivatives **4a** and **4b**, which might be attributed to their better partitioning into the lipid phase [47] and different absorption, bioconversion, biodistribution, and pharmacodynamic profiles [35]. Moreover, length of the linker also influenced proapoptotic activity; for example, compound **4d** with a hexyl linker had better activity than compound **4c** with an ethyl linker. In addition, the results in Tables 1 and 2 indicate that there was no obvious relationship between cytotoxicity and apoptosis-inducing activity.

To further confirm their proapoptotic function, FACS analysis was carried out after double-staining cells with

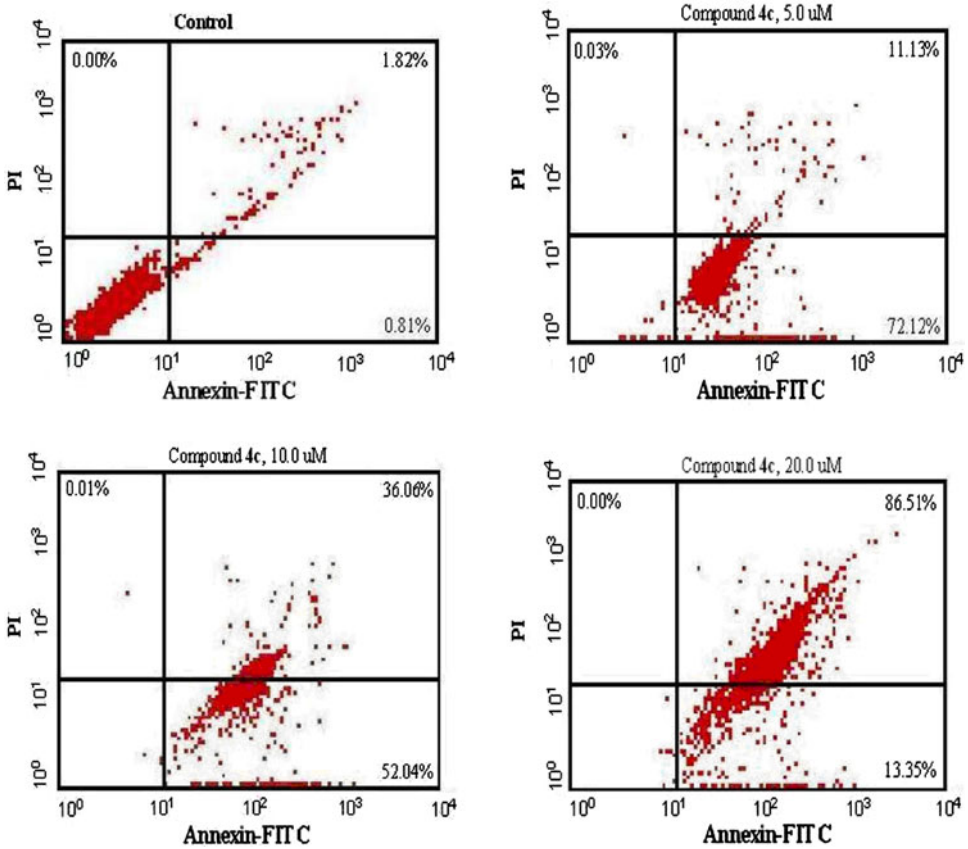


Fig. 3 Compound **4c** induced apoptosis of HeLa cell line. HeLa cells were treated with 5.0, 10.0, and 20.0 μM **4c** or with vehicle solvent [0.1% dimethyl sulfoxide (DMSO)] for 36 h and stained with annexin V-FITC and propidium iodide. Stained cells then were subjected to FACScalibur analysis to determine the distribution of cells

propidium iodide and annexin V-fluorescein isothiocyanate (FITC) [51]. Early apoptosis corresponded to annexin V single-positive cells and late apoptosis/necrosis corresponded to annexin V double-positive cells. As shown in Fig. 3, the representative compound **4c** was very effective in inducing apoptosis in a dose-dependent manner. Treatment of HeLa cells by 5, 10, and 20 μM **4c** for 36 h resulted in 72.12%, 52.04%, and 13.35% cell apoptosis, as compared with 0.81% in an untreated control. Obviously, necrosis increased as the concentration of **4c** increasing. Also, the treatment could notably induce morphological changes of HeLa cells into round form (data not listed), which was a primary indication of apoptosis and cell death. These results collectively suggested that the target compounds might inhibit growth of the HeLa cell line by induction of apoptosis.

In conclusion, a series of novel naphthalimide–indomethacin hybrids with different linkers were designed and synthesized, and their antitumor activity was evaluated against a variety of cancer cell lines in vitro. Preliminary results showed that the hybrids had moderate cytotoxic activity and could effectively induce apoptosis in HeLa cells. More importantly, the amide derivatives had better cytotoxic and proapoptotic activity than their ester analogues, whereas the ester derivatives exhibited hypoxic preferred cytotoxicity and might be used as promising candidate prodrug against hypoxic tumor cells. The results indicated that linkers played important roles in biological activity. This work provides a novel class of hybrid lead compounds with improved bioactivity for further optimization. Detailed biological studies on the molecular mechanism of action of the hybrids are in progress.

Experimental

All reagents were of commercial quality and used without purification. ^{1}H and ^{13}C NMR were obtained with a Bruker AV-400 spectrometer; chemical shifts are reported as ppm (in $CDCl_3$/DMSO-d_6, TMS as internal standard). IR spectra were obtained using a Perkin-Elmer 2000 Fourier-transform IR (FTIR) instrument. High-resolution mass spectra (HRMS) were obtained on a HPLC-Q-Tof MS (Micro) spectrometer. Melting points were determined with an X-6 micro-melting point apparatus and were corrected using standard compounds. Elemental analysis was obtained on a PE-2400 analyzer. Column chromatography was performed on 200–300 mesh silica gel. 6-(Dimethylamino)benzo[*de*]-

isochromene-1,3-dione (**2**) was prepared by using a previously reported method [49].

The target compounds were submitted to the Chinese National Center for Drug Screening and School of Pharmacy in East China University of Science and Technology for in vitro antitumor activity assays. Growth inhibitory effects on the cancer cell lines were measured by MTT assay [50]. Hypoxic cytotoxicity was tested according to our reported procedure [52].

Cell cycle analysis was analyzed on FACScalibur (Becton–Dickinson, San Jose, CA). HeLa cells were incubated with different concentration of the compounds. After centrifugation at 1,000 rpm for 5 min at room temperature, the supernatant was removed. Then the cells were washed twice with phosphate-buffered saline (PBS) solution and fixed with 0.3 cm^3 PBS and 0.7 cm^3 ice-cold 75% EtOH overnight. Fixed cells were harvested by centrifugation at 1,000 rpm for 10 min and washed twice with PBS. Collected cells were resuspended in 1 cm^3 PBS and treated with 5×10^{-4} cm^3 RNase A at 37 °C for 30 min. Propidium iodide was then added to final concentration of 50 μg cm^{-3} for DNA staining, and 20,000 fixed cells were analyzed using the Modifit program.

Extent of apoptosis was measured through annexin V-FITC apoptosis detection kit as described by the manufacturer's instructions. Briefly, HeLa cells were collected 36 h after target compound treatment, and washed twice with PBS, then resuspended in 0.4 cm^3 1× binding buffer. Cells were transferred to a 5-cm^3 culture tube containing 5×10^{-3} cm^3 annexin V-FITC and 0.01 cm^3 propidium iodide, and then incubated for 15 min at room temperature in the dark. After 1× binding buffer was added to each tube, stained cells were analyzed by flow cytometry.

6-(N,N-Dimethylamino)-2-(2-hydroxyethyl)-1H-benzo[de]-isoquinoline-1,3(2H)-dione (**3a**, $C_{16}H_{16}N_2O_3$)
Compound **2** (500 mg, 2.07 mmol) was dissolved in 10 cm^3 EtOH, then 190 mg ethanolamine (3.11 mmol) was added. The solution was stirred and refluxed under nitrogen for 1.5 h, and then cooled and filtered. The residue was subjected to column chromatography on silica gel by using CH_2Cl_2/CH_3OH 30:1 (v/v) as eluent, affording **3a** (529 mg, 1.86 mmol, 90%) as orange solid. M.p.: 130.6–132.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.57$ (dd, $J_1 = 0.7$ Hz, $J_2 = 3.8$ Hz, 9-*Ar*–H), 8.48 (d, $J = 8.3$ Hz, 4-*Ar*–H), 8.55 (dd, $J_1 = 0.7$ Hz, $J_2 = 3.8$ Hz, 7-*Ar*–H), 7.66 (t, $J = 7.6$ Hz, 8-*Ar*–H), 7.11 (d, $J = 8.3$ Hz, 5-*Ar*–H), 4.45 (t, $J = 5.1$ Hz, N$CH_2$$CH_2$OH), 3.97 (t, $J = 5.2$ Hz, NCH$_2$$CH_2$OH), 3.13 (s, N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.4$, 159.0, 158.0, 139.6, 139.0, 135.6, 129.0, 125.3, 123.5, 115.0, 57.2, 44.9, 39.9 ppm; HRMS (ES+): $m/z = 285.1233$ $(M + H)^+$, required 285.1239.

6-(N,N-Dimethylamino)-2-[2-(2-hydroxyethoxy)ethyl]-1H-benzo[de]isoquinoline-1,3(2H)-dione (**3b**, $C_{18}H_{20}N_2O_4$)
3b was prepared from **2** and 2-(2-aminoethoxy)ethanol by using the procedure described for preparation of **3a**; yield 85%, orange solid. M.p.: 100.1–102.1 °C; ^{1}H NMR (500 MHz, DMSO-d_6): $\delta = 8.49$ (d, $J = 8.5$ Hz, 9-*Ar*–H), 8.44 (d, $J = 7.2$ Hz, 4-*Ar*–H), 8.32 (d, $J = 8.2$ Hz, 7-*Ar*–H), 7.74 (t, $J = 7.6$ Hz, 8-*Ar*–H), 7.19 (d, $J = 8.2$ Hz, 5-*Ar*–H), 4.20 (t, $J = 6.6$ Hz, N$CH_2$$CH_2$O), 3.61 (t, $J = 6.6$ Hz, NCH$_2$$CH_2$O), 3.63–3.42 (m, O$CH_2$$CH_2$OH), 3.07 (s, N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 163.8$, 157.4, 156.2, 139.6, 136.9, 134.5, 129.1, 125.3, 124.9, 123.0, 115.9, 68.9, 64.3, 62.5, 44.8, 38.7 ppm; HRMS (ES+): $m/z = 329.1503$ $(M + H)^+$, required 329.1501.

2-(2-Aminoethyl)-6-(N,N-dimethylamino)-1H-benzo[de]-isoquinoline-1,3(2H)-dione (**3c**, $C_{16}H_{17}N_3O_2$)
3c was prepared from **2** and ethane-1,2-diamine by using the procedure described for preparation of **3a**; yield 85%, orange-red solid. M.p.: 153.3–155.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.57$ (d, $J = 7.3$ Hz, 9-*Ar*–H), 8.47 (d, $J = 8.2$ Hz, 4-*Ar*–H), 8.44 (d, $J = 8.5$ Hz, 7-*Ar*–H), 7.66 (t, $J = 7.9$ Hz, 8-*Ar*–H), 7.11 (d, $J = 8.2$ Hz, 5-*Ar*–H), 4.28 (t, $J = 6.5$ Hz, N$CH_2$$CH_2$$NH_2$), 3.12 (s, N$(CH_3)_2$), 3.08 (t, $J = 6.5$ Hz, NCH$_2$$CH_2$$NH_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.6$, 158.8, 136.3, 133.0, 129.5, 124.8, 122.9, 115.8, 55.4, 44.7, 38.5 ppm; HRMS (ES+): $m/z = 284.1395$ $(M + H)^+$, required 284.1399.

2-(6-Aminohexyl)-6-(N,N-dimethylamino)-1H-benzo[de]-isoquinoline-1,3(2H)-dione (**3d**, $C_{20}H_{25}N_3O_2$)
3d was prepared from **2** and hexane-1,2-diamine by using the procedure described for preparation of **3a**; yield 90%, orange solid. M.p.: 100.6–102.7 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.56$ (d, $J = 6.9$ Hz, 9-*Ar*–H), 8.47 (d, $J = 8.2$ Hz, 4-*Ar*–H), 8.43 (d, $J = 8.4$ Hz, 7-*Ar*–H), 7.65 (t, $J = 7.8$ Hz, 8-*Ar*–H), 7.11 (d, $J = 8.2$ Hz, 5-*Ar*–H), 4.16 (t, $J = 7.6$ Hz, NCH$_2$), 3.10 (s, N$(CH_3)_2$), 2.68 (t, $J = 6.7$ Hz, $CH_2$$NH_2$), 1.77–1.68 (m, NCH$_2$$CH_2$), 1.53–1.40 (m, NCH$_2CH_2$$CH_2$$CH_2$$CH_2CH_2$$NH_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 164.6$, 157.9, 157.3, 139.4, 136.3, 133.7, 129.1, 125.5, 124.7, 123.0, 115.0, 44.8, 41.6, 38.3, 31.9, 29.1, 27.3, 26.5 ppm; HRMS (ES+): $m/z = 340.2029$ $(M + H)^+$, required 340.2025.

2-[6-(Dimethylamino)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl]ethyl 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetate (**4a**, $C_{35}H_{30}ClN_3O_6$)
To a solution of 100 mg **3a** (0.352 mmol) in 5 cm^3 $CHCl_3$, 151 mg indomethacin (0.422 mmol), 135 mg 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.704 mmol), and 86 mg 4-dimethylaminopyridine (DMAP) (0.704 mmol) were added. The solution was

stirred at room temperature under nitrogen for 48 h and then concentrated. The residue was purified on silica gel column chromatography by using CH_2Cl_2/CH_3OH 60:1 (v/v) as eluent, affording **4a** as orange-yellow solid. Yield 80%; m.p.: 189.5–191.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.49$ (d, $J = 7.1$ Hz, 9-*naphthalene*-H), 8.43 (d, $J = 8.3$ Hz, 7-*naphthalene*-H), 8.40 (d, $J = 8.2$ Hz, 4-*naphthalene*-H), 7.65–7.62 (m, 8-*naphthalene*-H and 2,6-*Ph*-H), 7.44 (d, $J = 8.5$ Hz, 3,5-*Ph*-H), 7.09 (d, $J = 8.2$ Hz, 5-*naphthalene*-H), 6.94 (d, $J = 2.5$ Hz, 4-*indole*-H), 6.81 (d, $J = 9.0$ Hz, 7-*indole*-H), 6.53 (dd, $J_1 = 2.5$ Hz, $J_2 = 9.0$ Hz, 6-*indole*-H), 4.49 (s, COOCH_2CH_2N), 3.74 (s, CH_3O), 3.63 (s, CH_2COO), 3.11 (s, $N(CH_3)_2$), 2.28 (s, 2-indole-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.8, 168.2, 164.6, 164.0, 157.0, 156.0, 139.0, 135.8, 134.0, 132.7, 131.3, 131.2, 131.1, 130.7, 130.3, 129.0, 125.3, 124.9, 122.7, 114.8, 114.6, 113.3, 112.6, 111.7, 101.0, 62.4, 55.6, 44.8, 38.8, 30.2, 13.3$ ppm; FTIR (KBr): $\bar{\nu} = 2{,}948, 1{,}736, 1{,}688, 1{,}651, 1{,}584, 1{,}472, 1{,}376, 1{,}354, 1{,}324, 1{,}231, 1{,}172, 1{,}071, 912, 834, 778$ cm^{-1}; HRMS (ES+): $m/z = 624.1898$ $(M + H)^+$, required 624.1901.

2-[2-[6-(Dimethylamino)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl]ethoxy]ethyl 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetate
(**4b**, $C_{37}H_{34}ClN_3O_7$)

4b was prepared from **3b** and indomethacin by using the procedure described for preparation of **4a**; yield 70%, orange-yellow solid. M.p.: 155.3–157.3 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.57$ (d, $J = 7.2$ Hz, 9-*naphthalene*-H), 8.48–8.45 (m, 4,7-*naphthalene*-H), 7.68–7.65 (m, 8-*naphthalene*-H and 2,6-*Ph*-H), 7.46 (d, $J = 8.4$ Hz, 3,5-*Ph*-H), 7.12 (d, $J = 8.2$ Hz, 5-*naphthalene*-H), 6.95 (d, $J = 2.3$ Hz, 4-*indole*-H), 6.85 (d, $J = 9.0$ Hz, 7-*indole*-H), 6.63 (dd, $J_1 = 2.4$ Hz, $J_2 = 9.0$ Hz, 6-*indole*-H), 4.39 (t, $J = 6.2$ Hz, NCH_2CH_2O), 4.24 (t, $J = 4.5$ Hz, $COOCH_2$), 3.82 (s, CH_3O), 3.79 (t, $J = 6.2$ Hz, NCH_2CH_2O), 3.76 (t, $J = 4.7$ Hz, $CH_2CH_2OCH_2CH_2N$), 3.62 (s, CH_2COO), 3.11 (s, $N(CH_3)_2$), 2.33 (s, 2-indole-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.8, 168.3, 164.6, 164.0, 157.0, 156.0, 139.2, 135.9, 134.0, 132.7, 131.3, 131.2, 131.1, 130.8, 130.7, 130.3, 129.1, 125.3, 124.9, 123.0, 114.9, 113.3, 112.6, 111.7, 101.2, 68.5, 68.0, 64.3, 55.7, 44.8, 38.7, 30.1, 29.7, 13.3$ ppm; FTIR (KBr): $\bar{\nu} = 2{,}948, 2{,}851, 1{,}721, 1{,}688, 1{,}654, 1{,}584, 1{,}476, 1{,}368, 1{,}316, 1{,}231, 1{,}094, 782, 752$ cm^{-1}; HRMS (ES+): $m/z = 668.2135$ $(M + H)^+$, required 668.2164.

2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-N-[2-[6-(dimethylamino)-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl]ethyl]acetamide (**4c**, $C_{35}H_{31}ClN_4O_5$)

4c was prepared from **3c** and indomethacin by using the procedure described for preparation of **4a**; yield 80%, orange solid. M.p.: 91.8–93.8 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.41$ (d, $J = 8.4$ Hz, 9-*naphthalene*-H), 8.32 (d, $J = 7.2$ Hz, 7-*naphthalene*-H), 8.20 (d, $J = 8.2$ Hz, 4-*naphthalene*-H), 7.84 (d, $J = 8.3$ Hz, 2,6-*Ph*-H), 7.59 (t, $J = 7.8$ Hz, 8-*naphthalene*-H), 7.49 (d, $J = 8.3$ Hz, 3,5-*Ph*-H), 7.02 (d, $J = 8.2$ Hz, 5-*naphthalene*-H), 6.82 (d, $J = 9.0$ Hz, 7-*indole*-H), 6.70 (d, $J = 1.6$ Hz, 4-*indole*-H), 6.54 (br s, CONH), 6.46 (dd, $J_1 = 1.8$ Hz, $J_2 = 8.8$ Hz, 6-*indole*-H), 4.28 (t, $J = 4.8$ Hz, NCH_2CH_2), 3.63 (d, $J = 4.1$ Hz, NCH_2CH_2), 3.57 (s, CH_3O), 3.54 (s, CH_2CONH), 3.11 (s, $N(CH_3)_2$), 2.31 (s, 2-indole-CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.6, 156.0, 134.1, 133.0, 131.5, 131.4, 131.3, 131.0, 130.4, 129.1, 124.8, 122.3, 115.0, 113.2, 112.7, 112.3, 100.2, 55.4, 44.7, 38.5, 32.1, 13.3$ ppm; FTIR (KBr): $\bar{\nu} = 3{,}325, 2{,}925, 1{,}680, 1{,}647, 1{,}513, 1{,}472, 1{,}450, 1{,}350, 1{,}316, 1{,}224, 1{,}083, 1{,}057, 778, 752$ cm^{-1}; HRMS (ES+): $m/z = 645.1864$ $(M + Na)^+$, required 645.1881.

2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-N-[6-[6-(dimethylamino)-1,3-dioxo-1H-benzo[de]-isoquinolin-2(3H)-yl]hexyl]acetamide
(**4d**, $C_{39}H_{39}ClN_4O_5$)

4d was prepared from **3d** and indomethacin by using the procedure described for preparation of **4a**; yield 70%, orange-yellow solid. M.p.: 164.6–166.6 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.49$ (d, $J = 7.2$ Hz, 9-*naphthalene*-H), 8.43 (d, $J = 8.4$ Hz, 7-*naphthalene*-H), 8.39 (d, $J = 8.2$ Hz, 4-*naphthalene*-H), 7.65 (d, $J = 8.2$ Hz, 8-*naphthalene*-H), 7.61 (d, $J = 8.5$ Hz, 2,6-*Ph*-H), 7.44 (d, $J = 8.5$ Hz, 3,5-*Ph*-H), 7.10 (d, $J = 8.2$ Hz, 5-*naphthalene*-H), 6.91 (d, $J = 2.3$ Hz, 4-*indole*-H), 6.83 (d, $J = 9.0$ Hz, 7-*indole*-H), 6.66 (dd, $J_1 = 2.4$ Hz, $J_2 = 9.0$ Hz, 6-*indole*-H), 5.89 (br s, CONH), 4.06 (t, $J = 7.3$ Hz, NCH_2), 3.81 (s, CH_3O), 3.65 (s, CH_2CONH), 3.19 (dd, $J_1 = 6.7$ Hz, $J_2 = 13.0$ Hz, $CONHCH_2$), 3.10 (s, $N(CH_3)_2$), 2.39 (s, 2-indole-CH_3), 1.68–1.62 (m, NCH_2CH_2), 1.46–1.41 (m, $NHCH_2CH_2$), 1.33–1.29 (m, $CH_2CH_2CH_2CH_2CH_2CH_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 169.8, 168.2, 164.6, 164.1, 156.9, 156.3, 139.4, 136.3, 133.7, 132.6, 131.1, 131.0, 130.9, 130.4, 130.2, 129.1, 125.3, 124.9, 123.0, 115.1, 115.0, 113.3, 113.1, 112.3, 100.8, 55.7, 44.8, 39.6, 39.3, 32.3, 29.1, 27.7, 26.1, 25.9, 13.3$ ppm; FTIR (KBr): $\bar{\nu} = 3{,}281, 3{,}074, 2{,}925, 2{,}844, 2{,}770, 1{,}688, 1{,}651, 1{,}584, 1{,}472, 1{,}450, 1{,}354, 1{,}316, 1{,}220, 1{,}142, 1{,}083, 1{,}060, 845, 778, 752$ cm^{-1}; HRMS (ES+): $m/z = 701.2497$ $(M + Na)^+$, required 701.2507.

Acknowledgments This work is supported by the National Natural Science Foundation of China (20536010, 20746003) and Program of Shanghai Subject Chief Scientist and Shanghai Leading Academic Discipline Project (B507) and the National High Technology Research and Development Program of China (863 Program 2006AA10A201).

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