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ORIGINAL PAPER

Derivatives of 5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione: design, synthesis, and biological activity

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Abstract A series of mono and bis-2-(2-(dimethylamino)-ethyl)-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-diones with different amino side chains, a novel family of antitumor agents, has been 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 most of the derivatives had antitumor activity comparable with that of mitonafide, with IC_{50} values of 10^{-6}–10^{-5} M. More importantly, the derivatives had distinct antitumor selectivity against different cancer cell lines. This work provided a novel class of mitonafide-based lead compounds with improved antitumor selectivity against cancer cell lines for further optimization.

Keywords 5-Nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione · Antitumor · Cytotoxicity · Selectivity · Synthesis

Introduction

In organic and medicinal chemistry, the design and synthesis of highly efficient antitumor agents remain of significant importance [1, 2]. 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione-based anticancer drugs have been an indispensable part in the development of antitumor agents [3]. Since the first lead compounds, amonafide and mitonafide (Scheme 1), were discovered by Braña et al. [4, 5], many of their analogues have been prepared and their antitumor activity has been evaluated with a variety of cancer cell lines. Although promising results have been obtained in this way, most of the compounds were abandoned because of poor therapeutic index, unpredictable toxicity, insolubility, and complicated synthesis [6–9]. Therefore, it was necessary for us to attempt alternative structural modifications of 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione.

In recent years, various anticancer drugs modified by a polyamine side chain or other functional groups have been reported, for example anthraquinone [10], doxorubicin [11], naphthalimide [12], 1*H*-benzo[*c,d*]indol-2-one [13], and camptothecin [14], and significant results have been obtained. It is well known that basic amino side chains are important in their chemical and biological functions. Molecular flexibility, also, is attracting increasing attention in drug discovery and design [15]. Furthermore, the biological activity of dimers, for example elinafide (Scheme 1), is usually distinctly different from that of the monomers [16–18]. On the basis of this consideration, we decided to focus on mitonafide analogues with substitution at position 6, because such compounds have not previously been well investigated for antitumor activity. Our objective was to identify novel mitonafide-based leads with potent anticancer effects, hopefully with antitumor selectivity.

In molecular design, the amino substituents introduced at position 6 of mitonafide were difficult to acetylate [19] and might involve arrest of the cell cycle [20], which contributed to improving their biological activity [3]. Different linkers, semi-rigid piperazine and flexible 1,6-hexanediamine, were fused between the bis-mitonafide scaffold in order to investigate their effects on biological activity. As shown in Scheme 2, the target compounds

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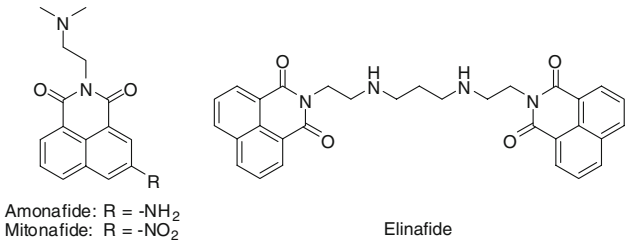


Scheme 1 The structures of some reported compounds

4a–**4e** were prepared. Their in vitro cytotoxicity was evaluated against HeLa, A549, P388, HL-60, MCF-7, HCT-8, and A375 cancer cell lines.

Table 1 Cytotoxicity of the target compounds against HeLa, A549, P388, HL-60, MCF-7, HCT-8, and A375 cancer cell lines

Compound	Cytotoxicity (IC_{50}/μM)						
	HeLa[a]	A549[b]	P388[a]	HL-60[a]	MCF-7[a]	HCT-8[a]	A375[a]
Mitonafide	2.8	26.0	9.1	35.9	23.8	18.5	15.0
4a	6.4	0.8	65.5	18.4	7.2	33.4	82.4
4b	45.3	10.3	29.0	25.0	68.5	17.4	20.1
4c	12.3	0.5	15.1	5.7	4.9	11.1	75.6
4d	71.7	86.5	92.6	93.1	17.1	90.4	73.8
4e	29.8	41.2	49.6	95.0	37.0	69.3	79.7

[a] Cytotoxicity (CTX) against cancer cell lines was measured by the microculture tetrazolium–formazan method after 48 h

[b] CTX against cancer cell line was measured by the sulforhodamine B dye-staining method after 72 h

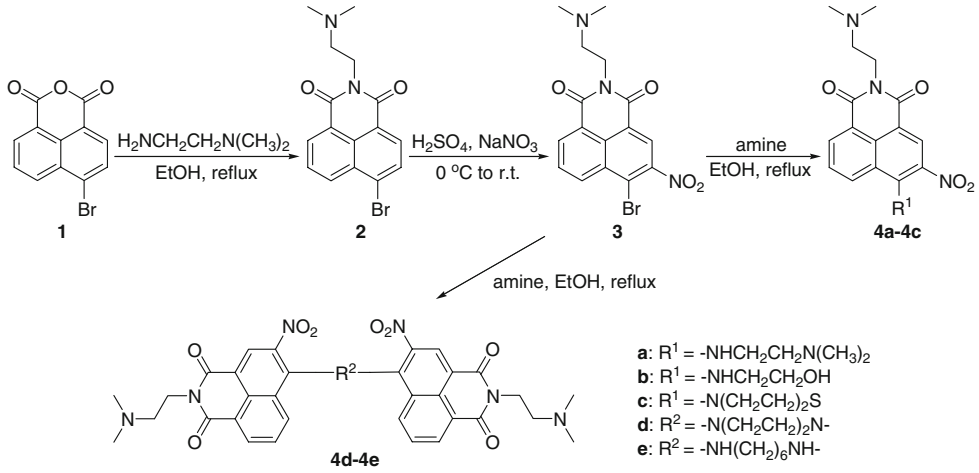
Results and discussion

The synthetic routes of the designed 5-nitro-1*H*-benzo-[*de*]isoquinoline-1,3(2*H*)-dione derivatives are shown in Scheme 2. 6-Bromobenzo[*de*]isochromene-1,3-dione **1** was treated with *N*,*N*-dimethylethane-1,2-diamine in EtOH, affording the intermediate **2** with satisfactory yield of 95% [21]. Subsequent nitration of **2** with $NaNO_3/H_2SO_4$ from 0 °C to room temperature led to the key intermediate **3** with moderate yield of 70%. Nucleophilic substitution of **3** with the corresponding amines then afforded the target compounds **4a**–**4e** with yields of 50–65%. The structures of all the newly synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, HRMS, EI, and IR.

The in-vitro antitumor activity of the target compounds was evaluated by examining their cytotoxic effects using the sulforhodamine B (SRB) assay [22] against A549 and the MTT tetrazolium dye assay [23] against HeLa, P388, HL-60, MCF-7, HCT-8, and A375. The IC_{50} represented the drug concentration (μM) required to inhibit cell growth by 50%; the results are summarized in Table 1.

As shown in Table 1, the target compounds had cytotoxicity comparable with that of mitonafide against the cancer cell lines tested, with IC_{50} values of 10^{-6}–10^{-5} M. Compound **4c** had the highest cytotoxicity against A549, P388, HL-60, MCF-7, and HCT-8 cell lines among each group with the IC_{50} values of 0.5, 15.1, 5.7, 4.9, and 11.1 μM. For the HeLa and A375 cell lines, the most cytotoxic compounds were **4a** and **4b**. Among these, the IC_{50} values of compound **4c** were factors of 52, 6.3, 4.9, and 1.6 lower than that of mitonafide against A549, HL-60, MCF-7, and HCT-8 cell lines. In most cases, cytotoxicity increased in the sequence of **4d**, **4e**, **4b**, **4a**, and **4c**, which indicated that the magnitude and conformation of the alkyl/aryl substituents had a substantial effect on the cytotoxic activity and selectivity of these compounds. For example, compound **4a** was more cytotoxic than compound **4b** but less cytotoxic than compound **4c** against A549, and they all had better selectivity for A549 than mitonafide. Moreover, the linkers in position 6 of mitonafide also affected their cytotoxicity. We found that compound **4d** bearing a semi-

Scheme 2 The synthetic routes of the target compounds



rigid piperazine linker was strikingly different from compound **4e** bearing a flexible alkyl linker. It was interesting that compound **4d** had exclusive antitumor activity against MCF-7 ($IC_{50} = 17.1$ μM), whereas for the other cancer cell lines tested it had no activity ($IC_{50} > 50$ μM). Additionally, the dimers **4d** and **4e** were less cytotoxic than the monomers, which indicated there was no obvious relationship between cytotoxicity and the quantity of active units of mitonafide.

In conclusion, a series of novel 5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione derivatives were designed and synthesized, and their antitumor activity was evaluated against a variety of cancer cell lines in vitro. Preliminary results showed that most of the derivatives had antitumor activity comparable with that of mitonafide, with IC_{50} values of 10^{-6}–10^{-5} M. More importantly, these derivatives had distinct antitumor selectivity against different cancer cell lines, which might be contributed by the introduction of different amino side chains. This work provided a novel class of mitonafide-based lead compounds with improved antitumor selectivity for further optimization. Detailed biological studies on the molecular mechanism of action of these derivatives are in progress.

Experimental

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

The target compounds were submitted to the Chinese National Center for Drug Screening for in vitro antitumor activity assays. Growth inhibitory effects on the cell lines HeLa, P388, HL-60, MCF-7, HCT-8, and A375 were measured by use of the MTT assay [23]. For A549 cell line, the growth inhibition effect was tested by use of the sulforhodamine B (SRB) assay [22].

6-Bromo-2-(2-(dimethylamino)ethyl)-1H-benzo[de]-isoquinoline-1,3(2H)-dione (**2**, $C_{16}H_{15}BrN_2O_2$)
Compound **1** (500 mg, 1.805 mmol) was dissolved in 5 cm^3 EtOH, then 191 mg *N*,*N*-dimethylethane-1,2-diamine (2.166 mmol) was added. The solution was stirred and heated under reflux under nitrogen for 60 min, then cooled and filtered. The residue was subjected to recrystallization twice from EtOH, affording **2** (595 mg, 1.715 mmol, 95%) as a pale-yellow solid. M.p.: 149.0–151.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.658$ (d, $J = 7.2$ Hz, 9-*Ar*-H), 8.570 (d, $J = 8.4$ Hz, 7-*Ar*-H), 8.414 (d, $J = 8$ Hz, 4-*Ar*-H), 8.040 (d, $J = 7.6$ Hz, 5-*Ar*-H), 7.847 (t, $J = 8$ Hz, 8-*Ar*-H), 4.360 (t, $J = 6.8$ Hz, NCH_2), 2.748 (t, $J = 6.8$ Hz, CH_2N$(CH_3)_2$), 2.423 (s, N$(CH_3)_2$) ppm; MS (70 eV): $m/z = 346.0$ (M^+-1).

6-Bromo-2-(2-(dimethylamino)ethyl)-5-nitro-1H-benzo-[de]isoquinoline-1,3(2H)-dione (**3**, $C_{16}H_{14}BrN_3O_4$)
Compound **2** (500 mg, 1.440 mmol) was dissolved in 5 cm^3 ice-cold concentrated sulfuric acid, then 184 mg sodium nitrate (2.160 mmol) was added in batches within 30 min. The solution was stirred at 0 °C for 3 h, then for another 1 h at room temperature, and then poured into 50 cm^3 ice-cold water. The solid precipitate obtained was purified by silica gel column chromatography, using CH_2Cl_2/CH_3OH 30:1 (*v*/*v*) as eluent, affording **3** (395 mg, 1.008 mmol, 70%) as a khaki solid. M.p.: 158.9–160.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.804$–8.784 (m, 3H, *Ar*-H), 8.011 (t, $J = 8.0$ Hz, 8-*Ar*-H), 4.360 (t, $J = 4.0$ Hz, NCH_2), 2.715 (br s, CH_2N$(CH_3)_2$), 2.379 (s, N$(CH_3)_2$) ppm; MS (70 eV): $m/z = 391.0$ (M^+-1).

2-(2-(Dimethylamino)ethyl)-6-(2-(dimethylamino)ethyl-amino)-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4a**, $C_{20}H_{25}N_5O_4$)
Compound **3** (100 mg, 0.255 mmol) was dissolved in 3 cm^3 EtOH, then 27 mg *N*,*N*-dimethylethane-1,2-diamine (0.306 mmol) was added. The solution was stirred and heated under reflux under nitrogen for 60 min, then cooled and concentrated. The residue was purified by silica gel column chromatography, using CH_2Cl_2/CH_3OH 30:1 (*v*/*v*) as eluent, affording **4a** (66 mg, 0.166 mmol, 65%) as an orange–yellow solid. M.p.: 216.2–218.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 10.272$ (s, 6-Ar-*NH*), 9.248 (s, 4-*Ar*-H), 8.658–8.636 (m, 2H, *Ar*-H), 7.667 (t, $J = 8.0$ Hz, 8-*Ar*-H), 4.324 (t, $J = 6.4$ Hz, 2-Ar-NCH_2), 3.960 (dd, $J_1 = 4.8$ Hz, $J_2 = 5.4$ Hz, 6-Ar-NHCH_2), 2.742 (t, $J = 6.4$ Hz, 2-Ar-NCH$_2CH_2$), 2.637 (t, $J = 5.6$ Hz, 6-Ar-NHCH$_2CH_2$), 2.426 (s, 2-Ar-NCH$_2$CH$_2$N$(CH_3)_2$), 2.394 (s, 6-Ar-NHCH$_2$CH$_2$N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.65$, 162.71, 150.30, 133.68, 133.06, 132.58, 130.23, 129.99, 125.22, 123.53, 123.19, 110.51, 58.42, 56.75, 47.66, 45.45, 45.00, 37.74 ppm; FT-IR (KBr): $\bar{\nu} = 3{,}192$, 2,970, 2,940, 2,822, 2,770, 1,688, 1,647, 1,599, 1,561, 1,539, 1,454, 1,409, 1,339, 1,272, 1,209, 1,127, 1,071, 752 cm^{-1}; HRMS (ES+): $m/z = 400.1981$ $(M+H)^+$, required 400.1985.

2-(2-(Dimethylamino)ethyl)-6-(2-hydroxyethylamino)-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4b**, $C_{18}H_{20}N_4O_5$)

4b was prepared from **3** and 2-aminoethanol by using the procedure described for preparation of **4a**; yield 65%, bright orange solid. M.p.: 232.8–234.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.948$ (s, 6-Ar-*NH*), 9.271 (s, 4-*Ar*-H), 8.685 (d, $J = 7.2$ Hz, 9-*Ar*-H), 8.614 (d, $J = 8.4$ Hz, 7-*Ar*-H), 7.699 (t, $J = 7.6$ Hz, 8-*Ar*-H), 4.371 (t, $J = 6.8$ Hz, 2-Ar-NCH_2), 4.055 (t, $J = 6.8$ Hz, 6-Ar-NHCH$_2CH_2$), 3.961 (t, $J = 6.8$ Hz, 6-Ar-NHCH_2), 2.674 (t, $J = 6.8$ Hz, $(CH_3)_2$ NCH_2), 2.365 (s, N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.05$, 162.21, 150.10, 133.48, 132.86, 132.38, 130.03, 129.79, 125.02, 123.33, 122.99, 110.31, 58.39, 56.76, 48.45, 45.71, 45.58, 36.13 ppm; FT-IR (KBr): $\bar{\nu} = 3{,}365$, 2,927, 2,910, 2,790, 1,680, 1,642, 1,599, 1,541, 1,429, 1,329, 1,252, 1,201, 1,123, 1,051, 750 cm^{-1}; MS (ES+): *m/z* = 373.1 $(M+H)^+$, required 373.4.

2-(2-(Dimethylamino)ethyl)-5-nitro-6-(thiomorpholin-4-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (**4c**, $C_{20}H_{22}N_4O_4S$)

4c was prepared from **3** and thiomorpholine by using the procedure described for preparation of **4a**; yield 60%, orange solid. M.p.: 172.1–174.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.717$ (s, 4-*Ar*-H), 8.708 (d, $J = 7.6$ Hz, 9-*Ar*-H), 8.574 (d, $J = 8.4$ Hz, 7-*Ar*-H), 7.872 (t, $J = 8.0$ Hz, 8-*Ar*-H), 4.370 (t, $J = 6.4$ Hz, 2-Ar-NCH_2), 3.574 (t, $J = 4.4$ Hz, 6-Ar-N$(CH_2CH_2)_2$S), 2.934 (br s, 6-Ar-N$(CH_2CH_2)_2$S), 2.811 (br s, 2-Ar-NCH$_2CH_2$), 2.459 (s, N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.68$, 162.53, 148.35, 143.57, 133.30, 130.81, 130.39, 130.09, 128.02, 127.27, 123.72, 118.78, 56.65, 53.85, 45.34, 27.97 ppm; FT-IR (KBr): $\bar{\nu} = 2{,}962$, 2,762, 1,691, 1,636, 1,521, 1,391, 1,335, 1,138, 960, 778 cm^{-1}; HRMS (ES+): *m/z* = 415.1404 $(M+H)^+$, required 415.1440.

6,6′-(Piperazine-1,4-diyl)bis(2-(2-(dimethylamino)ethyl)-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione) (**4d**, $C_{36}H_{36}N_8O_8$)

4d was prepared from **3** and piperazine by using the procedure described for preparation of **4a**; yield 50%, golden solid. M.p.: 272.7–274.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.775$–8.753 (m, 4H, *Ar*-H), 8.723 (d, $J = 8.4$ Hz, 7-*Ar*-H), 7.976 (t, $J = 8.4$ Hz, 8-*Ar*-H), 4.342 (t, $J = 6.8$ Hz, 2-Ar-NCH_2), 3.634 (br s, 6-Ar-N$(CH_2CH_2)_2$N), 2.665 (t, $J = 6.8$ Hz, CH_2N$(CH_3)_2$), 2.374 (s, N$(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 163.45$, 162.34, 146.82, 143.74, 133.54, 130.72, 130.40, 129.91, 128.55, 126.98, 123.83, 119.49, 56.96, 51.60, 45.77, 38.41 ppm; FT-IR (KBr): $\bar{\nu} = 2{,}955$, 2,851, 2,814, 2,762, 1,699, 1,658, 1,587, 1,573, 1,528, 1,446, 1,387, 1,342, 1,302, 1,235, 1,205, 1,138, 1,034, 942, 782, 748 cm^{-1}; HRMS (ES+): *m/z* = 709.2710 $(M+H)^+$, required 709.2734.

6,6′-((Hexane-1,6-diyl)diimino)bis(2-(2-(dimethylamino)ethyl)-5-nitro-1H-benzo[de]isoquinoline-1,3(2H)-dione) (**4e**, $C_{38}H_{42}N_8O_8$)

4e was prepared from **3** and hexane-1,6-diamine by using the procedure described for preparation of **4a**; yield 50%, bright orange solid. M.p.: 230.4–232.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.874$ (br s, 6-Ar-*NH*), 9.283 (s, 4-*Ar*-H), 8.690 (d, $J = 7.2$ Hz, 9-*Ar*-H), 8.636 (d, $J = 8.4$ Hz, 7-*Ar*-H), 7.714 (t, $J = 8.0$ Hz, 8-*Ar*-H), 4.320 (t, $J = 6.8$ Hz, 2-Ar-NCH_2), 3.994 (t, $J = 6.4$ Hz, 6-Ar-NHCH_2), 2.685 (br s, CH_2N$(CH_3)_2$), 2.378 (s, N$(CH_3)_2$), 1.900 (br s, CH_2CH_2), 1.578 (br s, CH_2CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 162.62$, 150.83, 134.06, 133.40, 129.85, 125.56, 123.30, 56.90, 50.52, 45.72, 30.96, 26.10 ppm; FT-IR (KBr): $\bar{\nu} = 3{,}096$, 2,940, 2,814, 2,770, 1,699, 1,654, 1,599, 1,532, 1,461, 1,432, 1,376, 1,331, 1,279, 1,194, 1,123, 1,064, 760 cm^{-1}; HRMS (ES+): *m/z* = 739.3223 $(M+H)^+$, required 739.3204.

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