Bioorganic & Medicinal Chemistry 20 (2012) 2558–2563

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Selenium-containing naphthalimides as anticancer agents: Design, synthesis and bioactivity

Liwei Zhao [a,b], Jianfeng Li [a], Yiquan Li [a], Jianwen Liu [a], Thomas Wirth [b], Zhong Li [a,*]

[a] Shanghai Key Lab of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Meilong Road 130, Shanghai 200237, China
[b] School of Chemistry, Cardiff University, Park Place, Cardiff CF10 3AT, UK

ARTICLE INFO

Article history:
Received 29 December 2011
Revised 21 February 2012
Accepted 22 February 2012
Available online 1 March 2012

Keywords:
Anticancer
Naphthalimides
Selenium
K562
DNA

ABSTRACT

Selenium analogues (**4b–4h,** and **4j**) of two known sulfur compounds were synthesized and tested their anticancer activities. The selenium compound **4b** had comparable activity with its sulfur analogue **4a**, while DNA-binding study showed these two compounds had similar interaction with ct-DNA, the K_b was 8.23 and 2.36, respectively. The primary results showed that most compounds had moderate anticancer activities with IC_{50} values between 10^{-6} and 10^{-5} M. Another selenium analogue **4j** showed the highest activity with the IC_{50} values around 5.3 μM against K562 and MCF-7 cell lines. More importantly, the organochalcogen compounds exhibited stronger anticancer activities against K562 cell line than the other cell lines tested.

1. Introduction

In the last decade, naphthalimide analogs have been considered as a promising group of anticancer agents by intercalating DNA. Amonafide and mitonafide (see Fig. 1) were two of the most active naphthalimide-based topo II inhibitors and had been tested in clinical trials.[1] However, on account of the central neurotoxicity and limited efficacy in solid tumors, the clinical development was regrettably terminated, while amonafide was found easily metabolized to *N*-acetyl-amonafide which caused unpredictable toxicity.[2]

To improve the efficacy and toxicological profiles, considerable efforts have been made on the design of more active naphthalimides focusing on the analogues with higher DNA-binding affi nity[3–9] and contacting two naphthalimide moiety together such as bisnafide and elinafide.[10–13]

Recently, Qian co-workers have prepared several novel naphthalimides as potent anticancer reagents.[14–18] Sulfur-containing amine substituted in the position 6 attracted our attention as the secondary amine in this position was difficult to acetylate and the sulfur moiety was part of novel apoptotic inducer.[19] And also, previous study showed that sulfur-containing compound had higher activities than the corresponding oxygen analogue in some cases.[20] This research prompted us to explore the effect of exchanging the sulfur to selenium on anticancer activities.

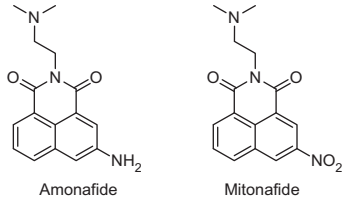


Figure 1. Structural formulas of amonafide and mitonafide.

The application of organoselenium compounds in cancer prevention and treatment is a fascinating field for selenium research. Selenium compounds have been proven as potent anticarcinogenic agents in different models, such as spontaneous, chemically induced, transplanted tumors or in culture.[21,22] Although the bioisosteric replacement of the oxygen or sulfur atom in known bioactive compounds with selenium is usually an easy strategy, it is still efficient and encouraged in medicinal chemistry. Firstly, the structural features were retained. Secondly, selenium is a softer atom which can be served as hydrogen-bond acceptor or electron donor. Several reports and our reaearch[23] indicated that selenium-containing compounds had higher activity than their sulfur analogues or additional properties in some aspects. Se,Se′-1,4-phenylene-bis(1,2-ethanediyl) bisisoselenourea(PBISe), which were designed by Desai et al. demonstrated that substitution of sulfur with selenium in known iNOS inhibitor increased the compound's anticancer potency by several folds.[24] The novel Se-analogue as a potent

* Corresponding author. Tel.: +86 21 64253540; fax: +86 21 64252603.
E-mail address: lizhong@ecust.edu.cn (Z. Li).

0968-0896/$ - see front matter Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2012.02.049

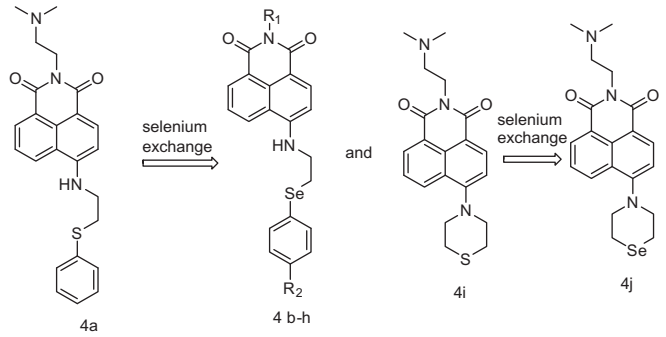


Figure 2. Selenium bioisosteric exchange of sulfur.

peroxisome proliferator-activated receptors(PPARs) β/δ ligand developed by Sharma et al. also might possess additional anticancer properties.[25] A series of 4′-selenonucleosides were prepared by Jeong et al. as the 3rd generation nucleosides, some of which were found exhibit high potent anticancer activity.[26,27] The improved photosensitizers were used as antimicrobial drugs in photodynamic therapy of localized infections, while the replacement of oxygen with selenium slightly enhanced corresponding effectiveness.[28] In DNA crystal structure studies, Huang et al replaced oxygen on the nucleobases in DNA with selenium and observed new Se-mediated hydrogen bond formation in base-paring selectivity.[29]

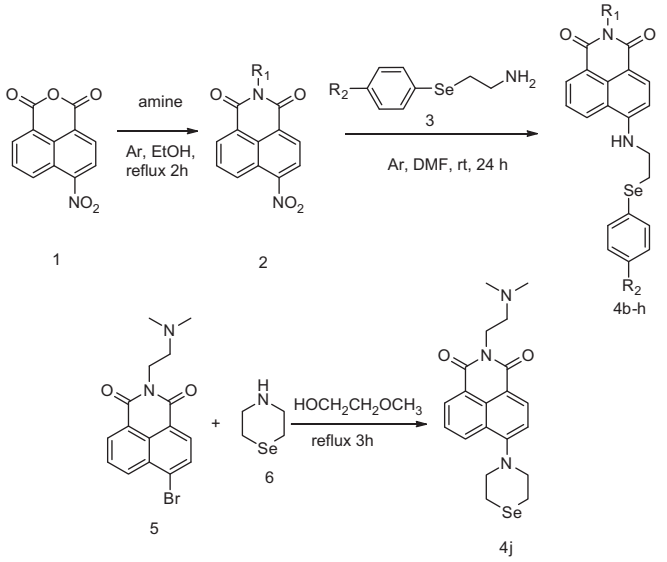
Therefore we prepared the selenium analogues of the former reported compounds **4a** and **4i** (see Fig. 2). After the successful bioisosteric exchange of phenylthio to phenylseleno, more attention was focused on additional modification of the phenyl ring and naphthalimide to improve anticancer activity or selectivity.

2. Results and discussion

2.1. Synthesis

The intermediates 2-aryl-selanyl-ethylamine **3** were prepared from 2-chloroethylamine with organylselenolate anions which generated in situ by treatment of the corresponding diselenides with $NaBH_4$ in ethnaol.[30] Selenomorpholine was prepared according to published procedure.[31] The target compounds **4b**–**4h** were synthesized similar to the sulfur analogues, starting from 6-nitro-naphthalimides **2**. The nitro group was easily substituted by amine **3** in DMF at room temperature after 24 h. Compound **4j** was prepared by the substitution of bromine with selenomorpholine (see Scheme 1). After completion, the reaction mixture was concentrated under vacuum to give crude oil, which was further purified by silica gel column chromatography using CH_2Cl_2/CH_3OH 50:1 (v/v) as eluent. The product was then recrystallized from dichloromethane and petroleum ether, affording the product as bright yellow solid. The structures and yields of these compounds were shown in Table 1. All newly synthesized compounds were well identified by ^1H NMR, ^{13}C NMR, HRMS and IR spectra.

Naphthalimides were known chromophoric group,[32] and the UV–vis and fluorescent data of the target compounds was shown in Table 2. The different substituents of **4b–4h** had a slight effect on absorption and emission, which convinced us to believe that the electronic property of aryl–selanyl moiety was not the dominant factor. The absorption and emission maxima of these compounds were around 438 and 528 nm, respectively. But compound **4j** with a quite different substitution had blue shift of both absorption and emission maxima, the values were 393 and 519 nm respectively, while its fluorescence quantum yield was dramatically deceased.



Scheme 1. The synthesis of **4b**–**h** and **4j**.

Table 1
Structures and yields of target compounds

Compound	R_1	R_2	Yield (%)
4b	$CH_2CH_2N(CH_3)_2$	H	56
4c	$CH_2CH_2N(CH_3)_2$	CH_3	51
4d	$CH_2CH_2N(CH_3)_2$	OCH_3	65
4e	$CH_2CH_2N(CH_3)_2$	OCF_3	38
4f	$CH_2CH_2N(CH_2CH_3)_2$	H	48
4g	C_4H_9	H	60
4h	C_8H_{17}	H	57
4j	—	—	35

Table 2
Absorption and emission data[a,b] of selenium-containing naphthalimide derivatives

Compound	UV λ_{max} (log ε)	FL λ_{max} (Φ)
4b	437 (4.20)	528 (0.2548)
4c	438 (4.26)	528 (0.2936)
4d	441 (4.25)	527 (0.2499)
4e	437 (4.29)	527 (0.2635)
4f	439 (4.31)	523 (0.2805)
4g	438 (4.23)	529 (0.2418)
4h	437 (4.17)	527 (0.2726)
4j	393 (4.18)	519 (0.0491)

[a] In absolute ethanol.
[b] With quinine sulfate in 0.1 mol/L sulfuric acid as quantum yield standard (Φ = 0.58).

2.2. Cytotoxic evaluation in vitro

The in vitro antitumor activities of the target compounds were evaluated by examining their cytotoxic effects using MTT tetrazolium dye assay against Hela (human cervical carcinoma cell line), HCT116 (human colon cancer cell line), K562 (chronic myelogenous leukemia cell line), A549 (human lung cancer cell line) and MCF-7 (Human Caucasian breast adenocarcinoma cell line), respectively. The IC_{50} values representing the drug concentration (μM) required to inhibit cell growth by 50% were summarized in Table 3.

In Table 3, it can be seen that most compounds showed moderate cytotoxicities against all tested cancer cell lines except **4g**. The selenium analogue **4b** and the sulfur compound **4a** nearly exhibited the same cytotoxicities. Our preliminary modification showed that

Table 3
Cytotoxicity of the target compounds against Hela, HCT116, K562, A549 and MCF-7 cell lines

Compound	Cytotoxicity (IC_{50} μM)				
	Hela	HCT116	K562	A549	MCF-7
4a[18]	15.11	16.4	11.2	18.0	19.11
4b	16.8	19.5	11.7	17.4	18.2
4c	>25	20	21.3	21	19.3
4d	17.9	17.6	16.6	22.3	18.3
4e	>25	9.9	9.4	14.4	16.4
4f	15.5	14.6	8.9	10.7	11.9
4g	>25	>25	>25	>25	>25
4h	16.7	18.1	18.7	20.6	11.6
4i[18]	6.06	ND	ND	1.27	16.04
4j	5.6	5.4	5.3	8.7	5.3

ND: not determined.

the substitution of benzene ring with 4-CH_3-phenyl (**4c**), 4-OCH_3-phenyl (**4d**), 4-OCF_3-phenyl (**4e**) group was less successful compared with **4b**. Meanwhile **4e** had a good result against K562 cell line. The modification position and functional group selection need to be further optimized. *N*,*N*-dimethylamine was a classic basic chain for DNA-binding, while compound **4f** with *N*,*N*-diethylamine had a slightly better activity than **4b**. For compounds **4g** and **4h**, the length of the alkyl chain significantly affected the cytotoxic activity, and this may due to the long chain can induce apoptosis via a mitochondrial pathway according to a former report.[16] Compound **4j**, selenium analogue of **4i**, showed the best bioactivity against all cell lines, although no better selectivity was observed.

The K562 cell lines attracted our attention as a previous report revealed that amonafide was less cytotoxic against K562 cells with IC_{50} >50 μM.[33] However, in our study these compounds showed stronger cytotoxic activity against K562 than other cell lines. Though several phenylaminoethyl selenides were served protective effects against oxidant-induced DNA damage,[34] selenium compounds could play multi-roles such as cancer prevention or DNA damage.[35] Some chalcogen-based agents could turn the oxidizing environment in certain cells over a critical redox threshold and at last induce apoptosis with high selectivity.[36,37] Selenium compound $Na_5SeV_5O_{18}{\cdot}3H_2O$ could significantly inhibit the proliferation of K562 cells in vitro in a time and concentration-dependent manner, and its mechanism of apoptosis was partially due to elevation of intracellular reactive oxygen species (ROS) concentration.[38] This selective anticancer activity may related to the chalcogen atom sulfur or selenium, which can act as a redox catalyst center, modulating the intracellular redox balance.

2.3. DNA binding properties

The interactions of the target compounds between ct-DNA were determined by spectroscopic techniques. The UV–vis spectra and

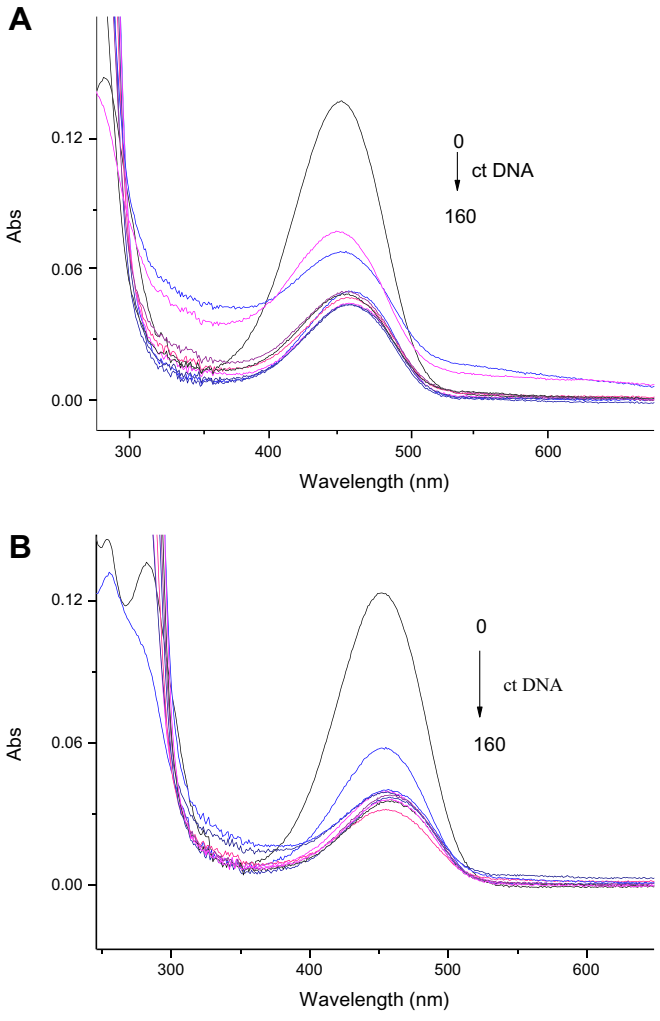


Figure 3. UV–vis absorption spectra of compound **4a** (10 μM) and **4b** (10 μM) in 20 mM Tris–HCl buffer (pH 7.5, 25 °C) containing different concentrations of ct-DNA from 0 to 160 μM. (A) **4a**, (B) **4b**.

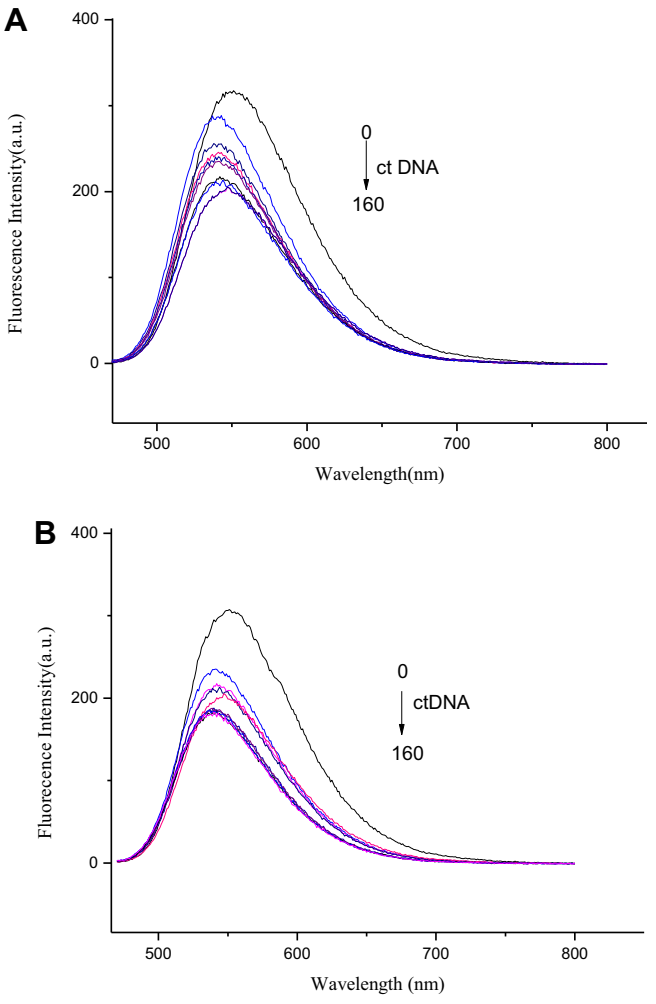


Figure 4. Fluorescence emission spectra of compound **4a** (10 μM) and **4b** (10 μM) in 20 mM Tris–HCl buffer (pH 7.5, 25 °C) containing different concentrations of ct-DNA from 0 to 160 μM. (A) **4a**, (B) **4b**.

fluorescence spectra for compounds **4a** and **4b** were illustrated in Figures 3 and 4, respectively.

It was obvious that UV–vis absorption spectra of compounds **4a** and **4b** exhibited dramatic hypochromic effects with increasing ct-DNA concentration from 0 to 160 μM, while no significant wavelength shift was observed (Fig. 3). These spectral characteristics suggested that there were similar interactions between compounds **4a** and **4b** with ct-DNA. To further investigate the interactions of **4a** and **4b** with DNA, the fluorescence quenching technique was employed to measure the Scatchard binding constants (K_b). The typical binding constant between organic compound and DNA usually range from 10^4 to 10^6 M^{-1}.[14] As shown in Fig. 4, the Scatchard binding constant (K_b) to ct-DNA for **4a** and **4b** was 8.26×10^4, and 2.43×10^4 M^{-1}, respectively. As the two compounds had same scaffold, we assume this difference can be attributed to the bigger size of selenium atom compared to sulfur atom, which lead to more steric demand when binding DNA. The emission intensities of **4a** and **4b** decreased with increasing the amount of ct-DNA as most of the intercalators did, and the wavelength showed slight blue shift.[39]

3. Conclusion

In this paper, the selenium analogues of former reported sulfur compounds were designed and evaluated their antitumor activities. The cytotoxictives of the two classic compounds **4a** and **4b** were nearly the same and spectroscopic technique confirmed that **4a** and **4b** had similar interactions with ct-DNA. Compound **4f** with a basic side chain *N*,*N*-diethylamine showed a slight higher activity than **4b**. The *para*-substituted derivatives were also prepared, while only the compound with trifluromethoxyl group (OCF_3) in this position showed good activity. Importantly, most of the target compounds exhibited selective cytotoxicty against K562 cell than other cell lines tested. Further structural optimization and structure–antitumor activity relationships are currently in progress.

4. Experimental

4.1. Material and methods

All chemical reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel plate. Column chromatography was performed using silica gel 200–300 mesh. ^1H and ^{13}C NMR were obtained with a Bruker AV-400 spectrometer with chemical shifts 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 a HPLC-Q-Tof MS (Micro) spectrometer. Melting points were determined using a Büchi melting point B-540 apparatus and were uncorrected.

4.2. Evaluation of in vitro cell proliferation by means of the MTT colorimetric assay

The target compounds were submitted to School of Pharmacy in East China University of Science and Technology for in vitro antitumor activity assays. Growth inhibitory effects on the cell lines (Hela, HCT116, K562, A549 and MCF-7) were measured by using MTT assay.

4.3. DNA-binding studies

4.3.1. Preparation of ct-DNA solution

Ct-DNA (highly polymerized) was purchased from Sigma–Aldrich. Solutions of ct-DNA in 20 mM Tris–HCl, 1 mM EDTA buffer (pH 7.5) gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the ct-DNA was sufficiently free of protein. The concentration of ct-DNA determined by the molar absorption was 6600 M^{-1} cm^{-1} (260 nm).[40] The solution was stored at 4 °C and used within 4 days.

4.3.2. DNA titration experiments

The concentrations of compounds (**4a** and **4b**) were 10 μM and ct-DNA were increased from 0 to 10, 20, 40, 60, 80, 100, 120, 140, 160 μM respectively, in 20 mM Tris–HCl (pH 7.5). The final solution volume was 10 mL for fluorescence titration experiments. All the solutions were stored at 25 °C in dark after 1 day for equilibrium. The scatchard binding constants (K_b) were calculated according to the equation $I = I_0 + \{(I_\infty - I_0)/2[Q]_0\} \times \{([DNA]_0 + [Q]_0 + 1/K_b) - \{([DNA]_0 + [Q]_0 + 1/K_b)^2 - 4[DNA]_0[Q]_0\}^{1/2}\}$, wherein I_0, I, and I_∞ represented the fluorescence intensities of compounds alone, the sample, and DNA totally bound, respectively. $[DNA]_0$ and $[Q]_0$ were the initial analytical concentrations of ct-DNA and the agents, respectively.[17,41]

4.4. Synthesis

4.4.1. 2-(2-(Dimethylamino)ethyl)-6(2(phenylseleno) ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4b)

Yield 56%, orange solid, mp 142.3–143.4 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.57 (d, 1H, *J* = 7.3 Hz), 8.41 (d, 1H, *J* = 8.4 Hz), 7.83 (d, 1H, *J* = 8.3 Hz), 7.62–7.54 (m, 3H), 7.36–7.28 (m, 3H), 6.59 (d, 1H, *J* = 8.4 Hz), 5.72 (s, br, 1H), 4.39 (t, 2H, *J* = 6.8 Hz), 3.68 (dd, 2H, J_1 = 6.1 Hz, J_2 = 11.8 Hz), 3.30 (t, 2H, *J* = 6.4 Hz), 2.85 (s, br, 2H), 2.52 (s, 6H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$): δ 164.70, 164.07, 134.40, 133.71, 131.22, 129.81, 129.53, 128.21, 127.88, 126.19, 124.79, 122.91, 120.39, 110.58, 104.37, 56.77, 45.29, 43.16, 37.28, 29.69 ppm; IR (KBr, cm^{-1}): 3451.46, 2988.32, 2902.17, 1639.55, 1586.99, 1394.71, 1344.24, 1251.02, 1066.63, 824.31; HRMS (EI) *m/z* $(M)^+$ calcd for $C_{24}H_{25}N_3O_2{}^{80}Se$ 467.1112, found 467.1111.

4.4.2. 2-(2-(Dimethylamino)ethyl)-6-(2-(4-methyl-phenylse leno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4c)

Yield 51%, yellow solid, mp 127.9–129.0 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 8.55 (d, 1H, *J* = 7.0 Hz), 8.38 (d, 1H, *J* = 8.4 Hz), 7.82 (d, 1H, *J* = 8.3 Hz), 7.54 (t, 1H, *J* = 7.8 Hz), 7.49 (d, 2H, *J* = 8.0 Hz), 7.10 (d, 2H, *J* = 7.8 Hz), 6.57 (d, 1H, *J* = 8.4 Hz), 5.74 (s, br, 1H), 4.35 (t, 2H, *J* = 7.1 Hz), 3.66 (dd, 2H, J_1 = 6.0 Hz, J_2=11.8 Hz), 3.25 (t, 2H, *J* = 6.4 Hz), 2.74 (t, 1H, *J* = 7.0 Hz), 2.44 (s, 6H), 2.35 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.67, 164.07, 148.93, 138.15, 134.35, 134.09, 131.15, 130.32, 129.81, 126.15, 124.69, 124.30, 122.96, 120.37, 110.58, 104.36, 56.93, 45.50, 43.19, 37.56, 26.88, 21.13 ppm; IR (KBr, cm^{-1}): 3382.02, 3264.04, 2933.20, 2758.43, 1679.44, 1637.26, 1586.64, 1344.82, 1386.99, 1243.59, 852.18, 693.25; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{25}H_{28}N_3O_2{}^{80}Se$ 482.1347, found 482.1346.

4.4.3. 2-(2-(Dimethylamino)ethyl)-6-(2-(4-methoxyl-phenylse leno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4d)

Yield 65%, yellow solid, mp 134.9-135.6 °C; ^1H NMR (400 MHz, CDCl3): δ 8.57 (d, 1H, *J* = 6.6 Hz), 8.40 (d, 1H, *J* = 8.4 Hz), 7.86 (d, 1H, *J* = 7.8 Hz), 7.59–7.51(m,3H), 6.83 (d, 2H, *J* = 8.8 Hz), 6.57 (d, 1H, *J* = 8.4 Hz), 5.72 (t, 1H, *J* = 4.8 Hz), 4.34 (t, 2H, *J* = 7.2 Hz), 3.81 (s, 3H), 3.64 (dd, 2H, J_1=6.0 Hz, J_2 = 11.8 Hz), 3.21 (t, 2H, *J* = 6.3 Hz), 2.71 (t, 2H, *J* = 7.1 Hz), 2.42 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.67, 164.08, 159.96, 148.87, 136.35, 134.36, 131.18, 129.78, 126.04, 124.74, 123.03, 120.36, 117.76, 115.20, 110.66, 104.39, 57.00, 55.32, 45.61, 43.03, 37.68, 27.42 ppm; IR (KBr, cm^{-1}): 3387.64, 3280.90, 2843.82, 2814.61, 2758.43, 1682.25, 1645.15, 1583.33, 1541.65, 1545.41, 1384.19, 1240.77, 1117.05, 1024.25, 765.55; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{25}H_{28}N_3O_3{}^{80}Se$ 498.1296, found 498.1297.

4.4.4. 2-(2-(Dimethylamino)ethyl)-6-(2-(4-trifluoromethoxylphenylseleno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4e)

Yield 38%, orange solid, mp 112.5–114.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 7.2 Hz), 8.42 (d, 1H, J = 8.3 Hz), 7.97 (d, 1H, J = 8.4 Hz), 7.62–7.58(m, 3H), 7.14 (d, 2H, J = 8.2 Hz), 6.63 (d, 1H, J = 8.4 Hz), 5.73 (s, br, 1H), 4.39 (t, 2H, J = 6.8 Hz), 3.70 (dd, 2H, J_1 = 5.6 Hz, J_2 = 11.3 Hz), 3.31 (t, 2H, J = 6.6 Hz), 2.50 (s, br, 3H), 1.66 (s, br, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.25, 156.17, 147.79, 139.18, 134.13, 133.09, 131.54, 130.20, 128.97, 127.60, 122.17, 116.55, 114.88, 112.15, 108.60, 44.43, 25.88 ppm; IR (KBr, cm^{-1}): 3396.46, 3310.95, 2936.87, 2849.67, 2800.13, 1697.29, 1625.95, 1597.34, 1384.19, 1120.68, 872.73; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{25}H_{25}F_3N_3O_3{}^{80}Se$ 552.1013, found 552.1015.

4.4.5. 2-(2-(Diethylamino)ethyl)-6-(2-(phenylseleno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4f)

Yield 48%, yellow solid, mp 136.5–137.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (d, 1H, J = 7.2 Hz), 8.41 (d, 1H, J = 8.4 Hz), 7.83 (d, 1H, J = 8.3 Hz), 7.61–7.55 (m, 3H), 7.35–7.28 (m, 3H), 6.60 (d, 1H, J = 8.4 Hz), 5.67 (t, 1H, J = 4.9 Hz), 4.31–4.27 (m, 2H), 3.69 (dd, 2H, J_1 = 6.0 Hz, J_2=11.8 Hz), 3.31 (t, 2H, J = 6.4 Hz), 2.83–2.79 (m, 2H), 2.71 (q, 4H, J = 7.1 Hz), 1.14 (t, 6H, J = 7.1 Hz) ppm; ^{13}C NMR 164.59, 164.02, 148.74, 134.23, 133.71, 131.10, 129.73, 129.54, 128.17, 127.90, 125.93, 124.84, 123.09, 120.41, 110.85, 104.42, 49.87, 47.70, 43.15, 37.60, 26.80, 12.2 ppm; IR (KBr, cm^{-1}): 3337.64, 2960.67, 2921.35, 1727.24, 1679.44, 1626.01, 1575.40, 1538.84, 1392.62, 1361.69, 1243.59, 768.37; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{26}H_{30}N_3O_2{}^{80}Se$ 496.1503, found 496.1505.

4.4.6. 2-*n*-Butyl-6-(2-(phenylseleno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4g)

Yield 60%, orange solid, mp 121.1–121.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 6.7 Hz), 8.42 (d, 1H, J = 8.4 Hz), 7.83 (d, 1H, J = 8.3 Hz), 7.61–7.56 (m, 3H), 7.33–7.28 (m, 3H), 6.62 (d, 1H, J = 8.4 Hz), 5.52 (br, s, 1H), 4.17–4.11 (m, 2H), 3.70 (t, 2H, J = 6.3 Hz), 3.31 (t, 2H, J = 6.3 Hz), 1.74–1.68 (m, 2H), 1.48–1.43 (m, 2H), 1.00–0.97 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.61, 164.08, 148.71, 134.20, 133.66, 131.06, 128.62, 127.86, 124.79, 123.13, 120.38, 110.90, 104.36, 43.16, 39.99, 30.32, 26.76, 20.44, 13.90 ppm; IR (KBr, cm^{-1}): 3426.83, 3168.89, 2953.30, 1686.04, 1648.09, 1587.79, 1399.42, 1277.25, 785.87; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{24}H_{25}N_2O_2{}^{80}Se$ 453.1081, found 453.1084.

4.4.7. 2-Octyl-6-(2-(phenylseleno)ethylamino)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4h)

Yield 57%, yellow solid, mp 116.7–117.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 7.3 Hz), 8.42 (d, 1H, J = 8.4 Hz), 7.83 (d, 1H, J = 8.3 Hz), 7.61–7.55 (m, 3H), 7.33–7.28 (m, 3H), 6.61 (d, 1H, J = 8.4 Hz), 5.57 (s, br, 1H), 4.16 (t, 2H, J = 6.0 Hz), 3.69 (t, 2H, J = 6.4 Hz), 3.31 (t, 2H, J = 6.4 Hz), 1.73 (td, 2H, J_1 = 7.5 Hz, J_2 = 15.3 Hz), 1.45–1.39 (m, 2H), 1.35–1.25 (m, 7H), 0.98–0.87 (m. 4H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$): δ 164.59, 164.07, 148.64, 134.20, 133.70, 131.08, 129.70, 129.54, 128.18, 127.89, 125.82, 124.82, 123.21, 120.39, 111.03, 104.41, 43.13, 40.26, 31.83, 29.39, 29.24, 28.22, 27.21, 26.82, 22.64, 14.09 ppm; IR (KBr, cm^{-1}): 3426.97, 2960.67, 2915.73, 2853.93, 1679.44, 1645.60, 1536.03, 1578.21, 1384.50, 1237.96, 1100.18, 779.61; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{28}H_{33}N_2O_2{}^{80}Se$ 509.1707, found 509.1707.

4.4.8. 2-(2-(Dimethylamino)ethyl)-6selenomorpholino-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4j)

Yield 35%, orange solid, mp 119.2–121.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (d, 1H, J = 7.2 Hz), 8.54 (d, 1H, J = 8.2 Hz), 8.41 (d, 1H, J = 8.4 Hz), 7.74 (t, 1H, J = 7.9 Hz), 7.27 (d, 1H, J = 10.1 Hz), 4.40 (t, 2H, J = 6.2 Hz), 3.67–3.64 (m, 4H), 3.04–3.01 (m, 4H), 2.83 (s, br, 2H), 2.50 (s, br, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.50, 164.00, 157.40, 132.60, 132.55, 131.33, 130.12, 130.08, 129.98, 126.78, 125.97, 116.24, 77.10, 56.29, 18.53 ppm; IR (KBr, cm^{-1}): 2952.25, 2917.32, 2765.35, 1724.55, 1654.46, 1583.71, 1415.29, 1326.15, 1290.49, 1152.11, 1127.47, 959.62, 785.38; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{20}H_{24}N_3O_2{}^{80}Se$ 418.1034, found 418.1035.

Acknowledgments

This work was financially supported by the National Basic Research Program of China (973 Program, 2010CB126100), National High Technology Research, the Development Program of China (863 Program, 2011AA10A207), and the National Key Technology R&D Program of China (2011BAE06B01). This work was also partly supported by the Fundamental Research Funds for the Central Universities. The authors would like to thank Professors Jianwen Liu and Yiquan Li from School of Pharmacy, East China University and Science and Technology, for anticancer evaluation and valuable suggestions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.049. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Braña, M. F.; Ramos, A. *Curr. Med. Chem. Anticancer Agents* **2001**, *1*, 237.
2. Ratain, M. J.; Mick, R.; Berezin, F.; Janisch, L.; Schilsky, R. L.; Vogelzang, N. J.; Lane, L. B. *Cancer Res.* **1993**, *53*, 2304.
3. Braña, M. F.; Gradillas, A.; Gómez, A.; Acero, N.; Llinares, F.; Muñoz-Mingarro, D.; Abradelo, G.; Rey-Stolle, F.; Yuste, M.; Campos, J.; Gallo, M. Á.; Espinosa, A. *J. Med. Chem.* **2004**, *47*, 2236.
4. Braña, M. F.; Cacho, M.; García, M. A.; Pascual-Tereas, B.; Ramos, A.; Domínguez, M. T.; Pozuelo, J. M.; Abradelo, C.; Rey-Stolle, M. F.; Yuste, M.; Báñez-Corone, M.; Lacal, J. C. *J. Med. Chem.* **2004**, *47*, 1391.
5. Braña, M. F.; Cacho, M.; García, M. A.; Pascual-Tereas, B.; Ramos, A.; Acero, N.; Llinares, F.; Muñoz-Mingarro, D.; Abadelo, C.; Rey-Stolle, M. F.; Yuste, M. *J. Med. Chem.* **2002**, *45*, 5813.
6. Li, Z.; Yang, Q.; Qian, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1769.
7. Qian, X.; Li, Z.; Yang, Q. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 6846.
8. Lv, M.; Xu, H. *Curr. Med. Chem.* **2009**, *16*, 4797.
9. Ingrassia, L.; Lefranc, F.; Kiss, R.; Mijatovic, T. *Curr. Med. Chem.* **2009**, *16*, 1192.
10. Braña, M. F.; Castellano, J. M.; Perron, D.; Conlon, D.; Bousquet, P. F.; George, J.; Qian, X.-D.; Robinson, S. P. *J. Med. Chem.* **1997**, *40*, 449.
11. Carrasco, C.; Joubert, A.; Tardy, C.; Maestre, N.; Cacho, M.; Braña, M. F.; Bailly, C. *Biochemistry* **2003**, *42*, 11751.
12. González-Bulnes, L.; Gallego, J. *J. Am. Chem. Soc.* **2009**, *131*, 7781.
13. Tumiatti, V.; Milelli, A.; Minarini, A.; Micco, M.; Campani, A. G.; Roncuzzi, L.; Baiocchi, D.; Marinello, J.; Capranico, G.; Zini, M.; Stefanelli, C.; Melchiorre, C. *J. Med. Chem.* **2009**, *52*, 7873.
14. Xie, L.; Cui, J.; Qian, X.; Xu, Y.; Liu, J.; Xu, R. *Bioorg. Med. Chem.* **2011**, *19*, 961.
15. Li, X.; Lin, Y.; Wang, Q.; Yuan, Y.; Zhang, H.; Qian, X. *Eur. J. Med. Chem.* **2011**, *46*, 1274.
16. Chen, Z.; Liang, X.; Zhang, H.; Xie, H.; Liu, J.; Xu, Y.; Zhu, W.; Wang, Y.; Wang, X.; Tan, S.; Kuang, D.; Qian, X. *J. Med. Chem.* **2010**, *53*, 2589.
17. Wu, A.; Xu, Y.; Qian, X. *Bioorg. Med. Chem.* **2009**, *17*, 592.
18. Wu, A.; Xu, Y.; Qian, X.; Wang, J.; Liu, J. *Eur. J. Med. Chem.* **2009**, *44*, 4674.
19. Park, C.; Oie, T.; Petros, A. M.; Zhang, H.; Nimmer, P. M.; Henry, R. F.; Elmore, S. W. *J. Am. Chem. Soc.* **2006**, *128*, 16206.
20. Qian, X.; Huang, T.; Wei, D. *J. Chem. Soc., Perkin Trans. 2* **2000**, 715.
21. Mugesh, G.; Mont, M.-M.; Sies, H. *Chem. Rev.* **2001**, *101*, 2125.
22. Nogueira, C. W.; Zeni, G.; Rocha, J. B. T. *Chem. Rev.* **2004**, *104*, 6255.
23. Ma, Y.; Liu, R.; Gong, X.; Li, Z.; Huang, Q.; Wang, H.; Song, G. *J. Agric. Food. Chem.* **2006**, *54*, 7724.
24. Desai, D.; Madhunapantula, S. V.; Gowdahalli, K.; Sharma, A.; Chandagaludoreswamy, R.; El-Bayoumy, K.; Robertson, G. P.; Amin, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2038.
25. Sharma, A. K.; Sk, U. H.; He, P.; Peters, J. M.; Amin, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4050.
26. Jeong, L. S.; Tosh, D. K.; Kim, H. O.; Wang, T.; Hou, X.; Yun, H. S.; Kwon, Y.; Lee, S. K.; Choi, J.; Zhao, L. X. *Org. Lett.* **2008**, *10*, 209.
27. Jeong, L. S.; Tosh, D. K.; Choi, W. J.; Lee, S. K.; Kang, Y.-J.; Choi, S.; Lee, J. H.; Lee, H.; Lee, H. W.; Kim, H. O. *J. Med. Chem.* **2009**, *52*, 5303.

28. Foley, J. W.; Song, X.; Demidova, T. N.; Jilal, F.; Hamblin, M. R. *J. Med. Chem.* **2006**, *49*, 5291.
29. Salon, J.; Sheng, J.; Jiang, J.; Chen, G.; Caton-Williams, J.; Huang, Z. *J. Am. Chem. Soc.* **2007**, *129*, 4862.
30. Amosova, S. V.; Makhaeva, N. A.; Martynov, A. V.; Potapov, V. A.; Steele, B. R.; Kostas, I. D. *Synthesis* **2005**, *10*, 1641.
31. Hu, L.; Chen, Z.; Lu, S.; Li, X.; Liu, Z.; Xu, H. *Phosphorus, Sulfur, Silicon* **2004**, *179*, 1065.
32. Qian, X.; Xiao, Y.; Xu, Y.; Guo, X.; Qian, J.; Zhu, W. *Chem. Commun.* **2010**, *46*, 6418.
33. Tian, Z.; Xie, S.; Mei, Z.; Zhao, J.; Gao, W.; Wang, C. *Org. Biomol. Chem.* **2009**, *7*, 4651.
34. Silva, V. D.; Woznichak, M. M.; Burns, K. L.; Grant, K. B.; May, S. W. *J. Am. Chem. Soc.* **2004**, *126*, 2409.
35. Letavayova, L.; Vlckova, V.; Brozmanov, J. *Toxicology* **2006**, *227*, 1.
36. Doering, M.; Ba, L. A.; Lilienthal, N.; Nicco, C.; Scherer, C.; Abbas, M.; Zada, A. A. P.; Coriat, R.; Burkholz, T.; Wessjohann, L.; Diederich, M.; Batteux, F.; Herling, M.; Jacob, C. *J. Med. Chem.* **2010**, *53*, 6954.
37. Jamier, V.; Ba, L. A.; Claus Jaco, C. *Chem. Eur. J.* **2010**, *16*, 10920.
38. Yang, J.; Wang, Z. *Arch. Pharm. Res.* **2006**, *29*, 859.
39. Yang, X.; Liu, W.; Jin, W.; Shen, G.; Yu, R. *Spectrochim. Acta, Part A* **1999**, *55*, 2719.
40. Reichmann, M. E.; Rice, S. A.; Thomas, C. A.; Doty, P. *J. Am. Chem. Soc.* **1954**, *76*, 3047.
41. Xi, Z.; Jones, G. B.; Qabaja, G.; Wright, J.; Johnson, F.; Goldberg, I. H. *Org. Lett.* **1999**, *1*, 1375.