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Synthesis and antiproliferative evaluation of certain iminonaphtho[2,3-*b*]furan derivatives

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ABSTRACT

Certain iminonaphtho[2,3-*b*]furan derivatives were synthesized from their respective carbonyl precursors in the regiospecific and the stereospecific manners. These compounds were evaluated for their antiproliferative effects against four human carcinoma cells (MCF7, NCI-H460, SF-268, and K562) and the normal fibroblast cell line (Detroit 551). Among them, (*Z*)-4-(hydroxyimino)naphtho[2,3-*b*]furan-9(4*H*)-one (**8**) and (*Z*)-4-methoxy-iminonaphtho[2,3-*b*]furan-9(4*H*)-one (**9**) exhibited GI_{50} values of 0.82 and 0.60 μM, respectively, against the growth of K562 cells and were inactive against the normal fibroblast Detroit 551. The selectivity index (SI) on K562 cell for **8** and **9** was >121.95 and >166.67, respectively, which is comparable to daunorubicin (SI = 239) and is more favorable than camptothecin (SI = 16.5). The cell cycle analysis on K562 indicated that these compounds arrest the cell cycle at the G2/M phase. The morphological assessment and DNA fragmentation analysis indicated that **9**-induced cell apoptosis in K562 cells. The apoptotic induction may through caspase-3 activity and cleavage of PARP.

1. Introduction

The quinone structure is a common constituent moiety to numerous natural products and is associated with anticancer, antibacterial, antimalarial, and fungicide activities.[1–4] Anthracyclines such as doxorubicin and daunorubicin are examples possessing planar tricyclic quinones. These drugs have been found to act as topoisomerase inhibitors, via DNA intercalation.[5,6] However, the molecular basis of cardiotoxicity associated with anthracyclines is the reduction of quinone moiety to semiquinone radicals which then reduce oxygen to generate superoxide anion radicals. Iminoquinones show less facile redox cycling and radical generation than the corresponding quinones, and in some cases (e.g., 5-iminodaunorubicin) these properties have led to lessened cardiotoxicity than the parent quinone (e.g., daunorubicin).[7] These results encouraged the extensive studies on the synthesis and biological evaluations of iminoquinones. A number of aryliminonaphthoquinone derivatives, **3–6** have been synthesized and proved to exhibit potent anticancer activities (Fig. 1).[8,9]

The condensed quinone derivatives such as furonaphthoquinones have been proved to possess broad anticancer activities (Fig. 1).[10–18] Naphtho[1,2-*b*]furan-4,5-dione (**1**) was reported to inhibit the protein tyrosine phosphatase CD45 and T-cell proliferation.[15] We found that **1** was capable of inhibiting the proliferation of breast cancer MDA-MB-231 cells through the induction of S-phase arrest and apoptosis.[16] Naphtho[2,3-*b*]furan-4,9-dione (avicequinone-B, **2**), the linear isomer of **1**, was isolated from the stem bark of *Avicennia alba*[17] which exhibited a modest cytotoxicity to rat hepatoma cells.[18] We have also reported certain biologically active quinoline derivatives such as furo[2,3-*b*]quinolines, indolo[2,3-*b*]quinolines, and indeno[1,2-*c*]quinolines which possess hydroxyimino, methoxyimino, and aminoalkoxylimino moieties.[19–21] Among them, 9-methoxy-6-(piperazin-1-yl)-11*H*-indeno[1,2-*c*]quinolin-11-one *O*-3-(dimethylamino)propyl oxime (**7**, Fig. 1) was found to exhibit GI_{50} values of 0.37and 0.38 μM against the growth of HeLa and A549 cells, respectively.[21] In continuation of our search for potential anticancer agents, we describe herein the introduction of hydroxyimino, methoxyimino, aminoalkoxylimino, monoarylimino, and diarylimino moieties on the tricyclic avicequinone-B (**2**) for antiproliferative evaluation.

2. Chemistry

Treatment of the known naphtho[2,3-*b*]furan-4,9-dione (**2**)[22,23] with NH_2OH proceeded in the regiospecific and the stereospecific manners to give (*Z*)-4-(hydroxyimino)naphtho[2,3-*b*]furan-9(4*H*)-

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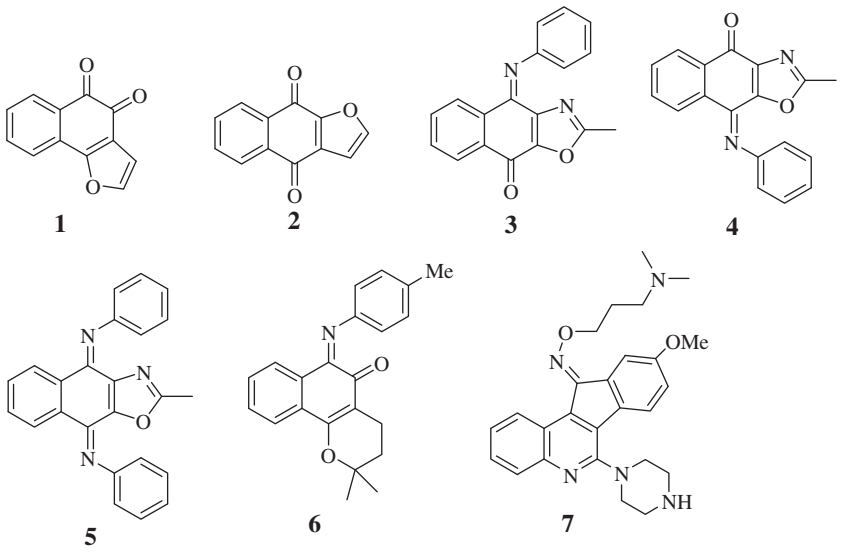
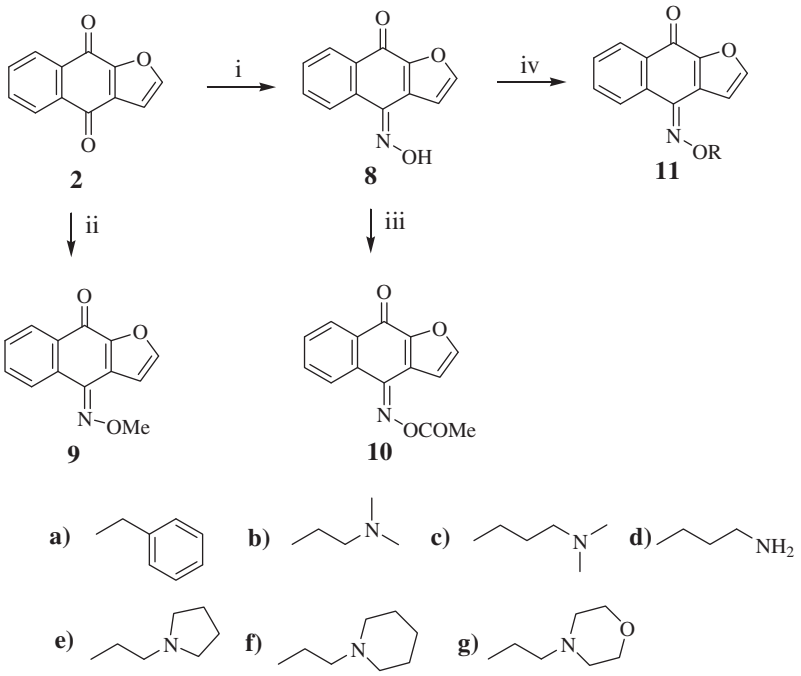


Figure 1.

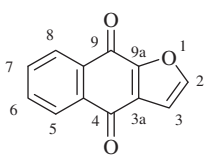
one (**8**) as a sole product as shown in Scheme 1. The regiospecific oximination occurred at C-4 rather than C-9 carbonyl was established based on the ^{13}C NMR in which the more downfield C-4 carbonyl shifted from 180.56[15] to 140.94 ppm while the more upfield C-9 carbonyl shifted from 173.60[15] to 171.97 ppm as shown in Table 1. The stereospecific oximination to give *Z*-form product rather than the *E*-isomer can be realized in which the hydroxyl group proximate the furan ring is less sterically hindered.[17] The correlation of HMBC for C-4 NOH (δ_H 13.34) and C-4 (δ_C 140.94) further confirmed the regiospecific oximination (Table 1). Accordingly, treatment of **2** with NH_2OMe gave the sole product of (*Z*)-4-(methoxyimino)naphtho[2,3-*b*]furan-9(4*H*)-one (**9**). Compound **8** was converted to (*Z*)-4-acetoxyiminonaphtho[2,3-*b*]furan-9(4*H*)-one (**10**) by the treatment with acetic anhydride. Benzylation of **8** with benzyl chloride afforded (*Z*)-4-[(benzoxy)imino]naphtho[2,3-*b*]furan-9(4*H*)-one (**11a**). Accordingly, reaction of **8** with various alkyl halides gave their respective alkylated products **11b–g** as shown in Scheme 1.

Treatment of **2** with aniline and a catalytic amount of $TiCl_4$ for 10 min, a mixture of (*Z*)-4-(phenylimino)naphtho[2,3-*b*]furan-9(4*H*)-one (**12a**) and (*Z*)-*N*-((*Z*)-4-(phenylimino)naphtho[2,3-*b*]furan-9(4*H*)-ylidene)benzenamine (**13a**) was obtained in the regiospecific and the stereospecific manners as shown in Scheme 2. Structural assignment of 4-arylimino derivative **12a** was based on the ^{13}C NMR in which the more downfield C-4 shifted from 180.54[15] to 151.72 ppm while the more upfield C-9 remains almost unchange (shifted from 173.59 to 173.78). Both C-4 and C-9 carbon peaks shifted upfield from 180.54 to 152.23 ppm and 173.59 to 148.48 ppm, respectively, in the case of diarylimino derivative **13a**. When the reaction time was extended to 30 min, the sole



Scheme 1. Reagents: (i) $HONH_2HCl$, K_2CO_3; (ii) $MeONH_2HCl$, K_2CO_3; (iii) Ac_2O, pyridine; (iv) NaH, alkyl halides.

Table 1
2D NMR correlations for naphtho[2,3-*b*]furan-4,9-dione (**2**) and (*Z*)-4-(hydroxyimino)naphtho[2,3-*b*]furan-9(4*H*)-one (**8**)



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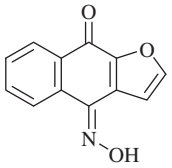
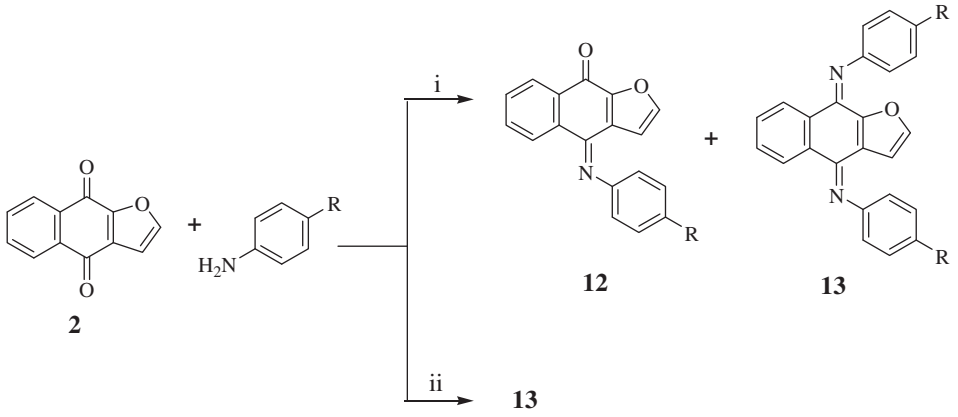


8

Position	**2**[a] (ppm)			**8**[b] (ppm)		
	δ_C	δ_H, mult. (*J* in Hz)	HMBC	δ_C	δ_H, mult. (*J* in Hz)	HMBC
1	—	—	—	—	—	—
2	148.62	7.77, d (1.6)	3, 3a, 9a	149.47	7.53, d (1.6)	3, 3a, 9a
3	108.65	7.01, d (1.6)	2, 3a, 9a	113.10	8.29, d (1.6)	2, 3a, 9a
3a	130.49	—	—	123.22	—	—
4	180.56	—	—	140.94	—	—
4a	132.47	—	—	130.53	—	—
5	126.95	8.22, m	4, 4a, 6	123.42	8.33, dd (8.0, 1.6)	4, 4a, 6
6	133.87	7.77, m	4a, 5, 8, 8a	129.53	7.68, m	4a, 5, 8, 8a
7	133.95	7.77, m	4a, 5, 8, 8a	132.82	7.76, m	4a, 5, 8, 8a
8	127.07	8.22, m	7, 8a, 9	125.96	8.17, dd (8.0, 1.6)	7, 8a, 9
8a	133.23	—	—	133.46	—	—
9	173.60	—	—	171.97	—	—
9a	152.72	—	—	147.58	—	—
NOH	—	—		—	13.34, s	4

[a] In $CDCl_3$.
[b] In DMSO-d_6.



a) R = H; **b**) R = F; **c**) R = Cl; **d**) R = Me; **e**) R = OMe; **f**) R = CF_3;
g) R = NO_2 ; **h**) R = $COOCH_2CH_2NEt_2$

Scheme 2. Reagents and conditions: (i) $TiCl_4$, CH_2Cl_2, 10 min; (ii) $TiCl_4$, CH_2Cl_2, 30 min.

product of **13a** was obtained. Accordingly, a mixture of **12b** and **13b** was obtained by the reaction of compound **2**, 4-fluoroaniline, and a catalytic amount of $TiCl_4$ for 10 min. A sole product of diarylimino derivative **13b** was obtained when the reaction time was extended to 30 min.

Structure of **13a** was unambiguously determined by X-ray crystallographical analysis (Fig. 2). The structure was solved and refined by direct methods Shelx 97[24] suite of programs. Red single crystal ($0.69 \times 0.52 \times 0.47$ mm^3) of **13a** was obtained by slow evaporation from methanol/CH_2Cl_2 (30:70) solution: triclinic, space group *P*1, $a = 8.9667(10)$ Å, $b = 10.1404(11)$ Å, $c = 11.2361(12)$ Å, $\alpha = 69.918(2)°$, $\beta = 69.340(2)°$, $\gamma = 85.021(2)°$, $V = 897.15(17)$ Å^3, $Z = 2$, δ(calcd) = 1.29 mg m^{-3}, FW = 348.39 for $C_{24}H_{16}N_2O$, $F(0\,0\,0) = 364$. Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 753135 for compound **13a**. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk or via www.ccdc.cam.ac.uk).

The diarylimino derivatives **13c–i**, respectively, was synthesized by the reaction of **2**, the respective substituted anilines, and a catalytic amount of $TiCl_4$ for 30 min as shown in Scheme 2.

3. Results and discussion

All compounds were evaluated in vitro against a 3-cell line panel consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). In this protocol, each cell line was inoculated and preincubated on a

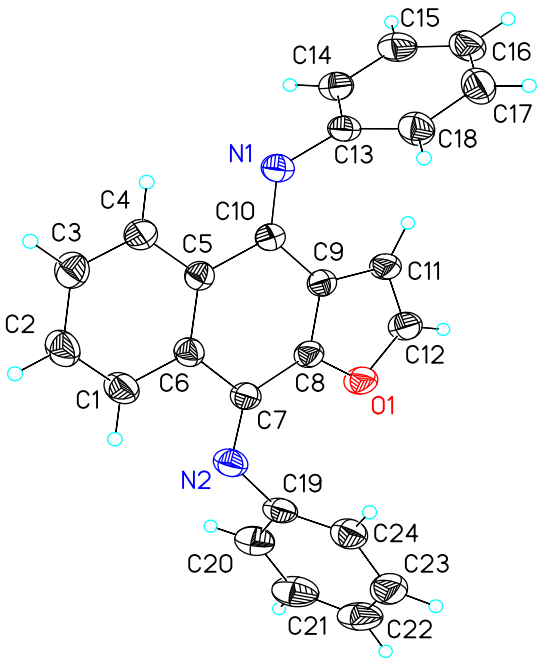


Figure 2. ORTEP view of (14*Z*)-*N*-[(*Z*)-9-(phenylimino)naphtho[2,3-*b*]furan-4(9*H*)-ylidene]-benzenamine (**13a**).

microtiter plate. Each of the tested compounds was then added at a concentration of 4.0 μg/mL and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells against the untreated control cells. Results from Table 2 indicated that both naphtho[1,2-*b*]furan-4,5-dione (**1**) and naphtho[2,3-*b*]furan-4,9-dione (**2**) were able to inhibit the growth of all three cells completely at a concentration of 4.0 μg/mL. The antiproliferative activities were maintained by the derivatization of **2** with hydroxylamine or methyloxime to give the respective hydroximino derivative **8** and methoxyimino derivative **9**. Further derivatization of **8** with acetic anhydride gave acetoxyimino derivative **10** which exhibited a comparable antiproliferative activity with that of the parent. However, the benzylated derivative **11a** was inactive indicated that a bulky substituent is unfavorable. Therefore, compounds **11b** and **11c** which bear more bulky acyclic *N,N*-dimethylaminoalkyl were inactive while compound **11d** which bears a less bulky primary aminopropyl side chain was fairly active. Compounds **11e**–**g** which bear bulky cyclic aminoalkyl side chains were also inactive. For the monophenylimino derivatives, compound **12a** was inactive although its structure is in resemblance to that of compounds **3** and **4** indicated that the oxazole moiety is crucial for antiproliferative activity. Substitution of the phenyl moiety with a fluoro group did not enhance antiproliferative activity in which **12b** was also inactive. For the bisphenylimino derivatives, **13a**–**g** were inactive while the 2-(diethylamino)ethoxycarbonyl derivative **13h** was found to be active against the growth of MCF7, NCI-H460, and SF-268 cancer cells.

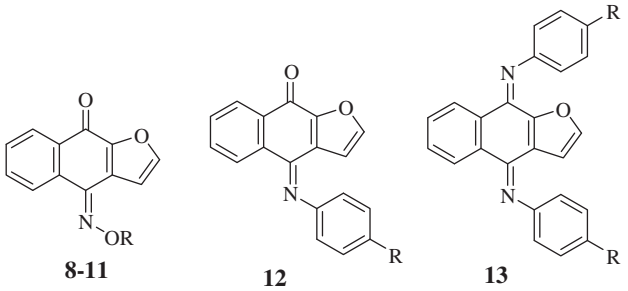
Selected compounds were further evaluated for GI_{50} values against a panel of cancer cell lines including four human carcinoma cells (MCF7, NCI-H460, SF-268, and K562) using XTT (2,3-bis-[2-methyloxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide) assay. The normal fibroblast cell line (Detroit 551) was also evaluated since a potential anticancer drug candidate should selectively affect only tumor cells and not somatic cells. The concentration that inhibited the growth of 50% of cells (GI_{50}) was determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. The GI_{50} results of substituted iminonaphtho[2,3-*b*]furan derivatives are summarized in Table 3. Both the angular naphtho[1,2-*b*]furan-4,5-dione (**1**) and its linear isomer **2** are comparable in the inhibitory activity against the growth of cancer cells. However, compound **2** was less cytotoxic than **1** against the growth of the Detroit 551 cells. Derivatization of **2** with NH_2OH and NH_2OMe gave the respective hydroxyimino derivative **8** and methoxyimino derivative **9** which exhibited comparable antiproliferative activities with the carbonyl precursor **2** against the growth of cancer cells and were not cytotoxic against the Detroit 551 cells (GI_{50} >100 μM). Compounds **1**, **2**, **8**, and **9** are especially active against the growth of K562 cell with GI_{50} values in a range of 0.60–0.82 μM. Both acetoxyimino derivative **10** and aminoalkoxyimino derivative **11d** possess comparable antiproliferative activity with the hydroxyimino precursor **8** against the growth of MCF7, NCI-H460, and SF-268, but were less active against K562 with GI_{50} values of 8.59 and 6.62 μM, respectively. Compound **13h** was found to be not only active against the growth of four cancer cells tested with GI_{50} values of less than 4.10 μM in each case, but also active against the growth of Detroit 551 with a GI_{50} value of 1.97 μM. Among these iminonaphtho[2,3-*b*]furan-9(4*H*)-one derivatives, compounds **8** and **9** emerged as lead molecules based on their selective cytotoxicities between malignant and the normal cells. The selectivity index (SI)[25] on K562 cell for **8** and **9** was >121.95 and >166.67, respectively, which is comparable to daunorubicin (SI = 239) and is more favorable than camptothecin (SI = 16.5).

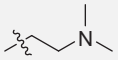
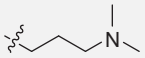
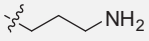
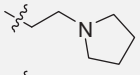
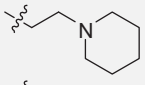
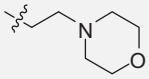
The cell cycle analysis on K562 cell line indicated that these compounds arrest the cell cycle at the G2/M phase, and didn't induce apoptosis at 1.0 μM as shown in Figure 3 and Table 4. The morphological changes of apoptosis include membrane blebbing, cell shrinkage, chromatin condensation, and formation of apoptotic bodies.[26] Stain of **9**-treated K562 cells for 48 h with DAPI clearly showed apoptotic bodies as shown in Figure 4. Cleavage of DNA at the internucleosomal linker sites yielding DNA fragments in multiple fragments (180–200 bp) was regarded as a biochemical hallmark of apoptosis.[27] The appearance of such fragments resulted in a ladder formation evidently when fragmented DNA from **9**-treated cells for 48 h was separated by agarose gel electrophoresis (Fig. 5). To understand the mechanism of **9**-induced apoptosis, we examined the changes of the intracellular proteins related to apoptosis, such as caspase-3, and PARP in cells treated with compound **9**. Caspases, a family of aspartate-specific cysteine proteases, play a crucial role in apoptotic cell death by cleaving a specific site of numerous cellular targets in the execution phase; in particular, caspase-3 could be activated by the proteolytic processing of pro-caspase-3 in response to exogenous apoptosis inducers.[28] Caspase-3 is an executioner caspase whose activation leads to the cleavage of key cellular proteins including DNA repair enzyme poly-(ADP-ribose) polymerase (PARP). PARP is involved in DNA repair predominantly in response to environmental stress, and is important for the maintenance of cell viability.[29] Our results have shown that caspase-3 activity and PARP was cleaved from 116 kDa intact form into 85 kDa fragment after the treatment of **9** for 48 h in a concentration-dependent manner (Fig. 6). Thus, compound **9** induces cell cycle arrest at G2/M phase followed by DNA fragmentation via activation of caspase-3, cleavage of PARP and consequently to cause the cell death (Fig. 7).

4. Conclusion

We report herein the regiospecific and the stereospecific synthesis of certain iminonaphtho[2,3-*b*]furan derivatives for antiproliferative evaluation. Among them, (*Z*)-4-methoxyiminonaphtho[2,3-*b*]furan-9(4*H*)-one (**9**) exhibited a GI_{50} value of 0.60 μM against the growth of K562 cells and was inactive against the normal fibroblast Detroit 551. The selectivity index (SI) on K562 cell for **9** was >166.67 which

Table 2
Preliminary cytotoxicity of substituted iminonaphtho[2,3-*b*]furan derivatives



Compound	R	Growth percentages[a]		
		MCF7 (breast cancer)	NCI-H460 (lung cancer)	SF-268 (CNS)
1	—	−1	−1	0
2	—	1	2	16
8	H	10	5	16
9	Me	36	4	22
10	COMe	10	5	18
11a	$CH_2C_6H_5$	93	85	103
11b		78	51	53
11c		74	51	54
11d		10	44	15
11e		72	51	58
11f		87	79	64
11g		97	68	90
12a	H	74	65	74
12b	F	75	67	77
13a	H	111	111	102
13b	F	102	103	118
13c	Cl	109	112	116
13d	Me	104	104	118
13e	OMe	86	90	85
13f	CF_3	111	115	105
13g	NO_2	111	117	118
13h	$COOCH_2CH_2NEt_2$	−1	−1	18
CPT[b]	—	33	1	25
DAR[c]	—	30	15	16

[a] In this protocol, each cell line was inoculated and preincubated on a microtiter plate. Each of the tested compounds was then added at a concentration of 4.0 μg/mL and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells against the untreated control cells.
[b] CPT, camptothecin.
[c] DAR, daunorubicin.

Table 3
Antiproliferative activity of substituted iminonaphtho[2,3-*b*]furan-4,9-dione derivatives [GI_{50} (μM)][a]

Compound	MCF7 (SI)[b]	NCI-H460 (SI)	SF-268 (SI)	K562 (SI)	Detroit 551
1	6.42 ± 0.07 (0.90)	6.38 ± 0.42 (0.90)	6.38 ± 0.06 (0.90)	0.64 ± 0.05 (9.02)	5.77 ± 0.05
2	5.92 ± 0.07 (2.50)	5.07 ± 0.63 (2.92)	5.65 ± 0.32 (2.62)	0.65 ± 0.01 (22.82)	14.81 ± 2.09
8	12.30 ± 1.15 (>8.13)	7.71 ± 0.47 (>12.97)	8.85 ± 0.83 (>11.30)	0.82 ± 0.02 (>121.95)	>100
9	10.15 ± 1.28 (>9.85)	6.76 ± 1.46 (>14.79)	16.54 ± 1.22 (>6.05)	0.60 ± 0.01 (>166.67)	>100
10	13.32 ± 0.89 (>7.51)	7.46 ± 0.72 (>13.40)	7.90 ± 0.67 (>12.66)	8.59 ± 0.18 (>11.64)	>100
11d	12.61 ± 1.49 (3.24)	7.19 ± 0.48 (5.69)	9.02 ± 0.93 (4.53)	6.62 ± 0.02 (6.18)	40.90 ± 1.92
13h	2.60 ± 0.07 (0.76)	2.23 ± 0.08 (0.88)	4.10 ± 0.42 (0.49)	0.63 ± 0.02 (3.13)	1.97 ± 0.09
CPT	0.57 ± 0.03 (1.74)	0.03 ± 0.003 (33)	0.19 ± 0.006 (5.21)	0.06 ± 0.003 (16.5)	0.99 ± 0.09
DAR	0.86 ± 0.16 (2.06)	0.38 ± 0.04 (4.66)	0.60 ± 0.02 (2.95)	0.0074 ± 0.0002 (239)	1.77 ± 0.10

[a] Values are average of three separate determinations.
[b] SI, selectivity index = (GI_{50} of Detroit 551)/(GI_{50} of cancer ell line).

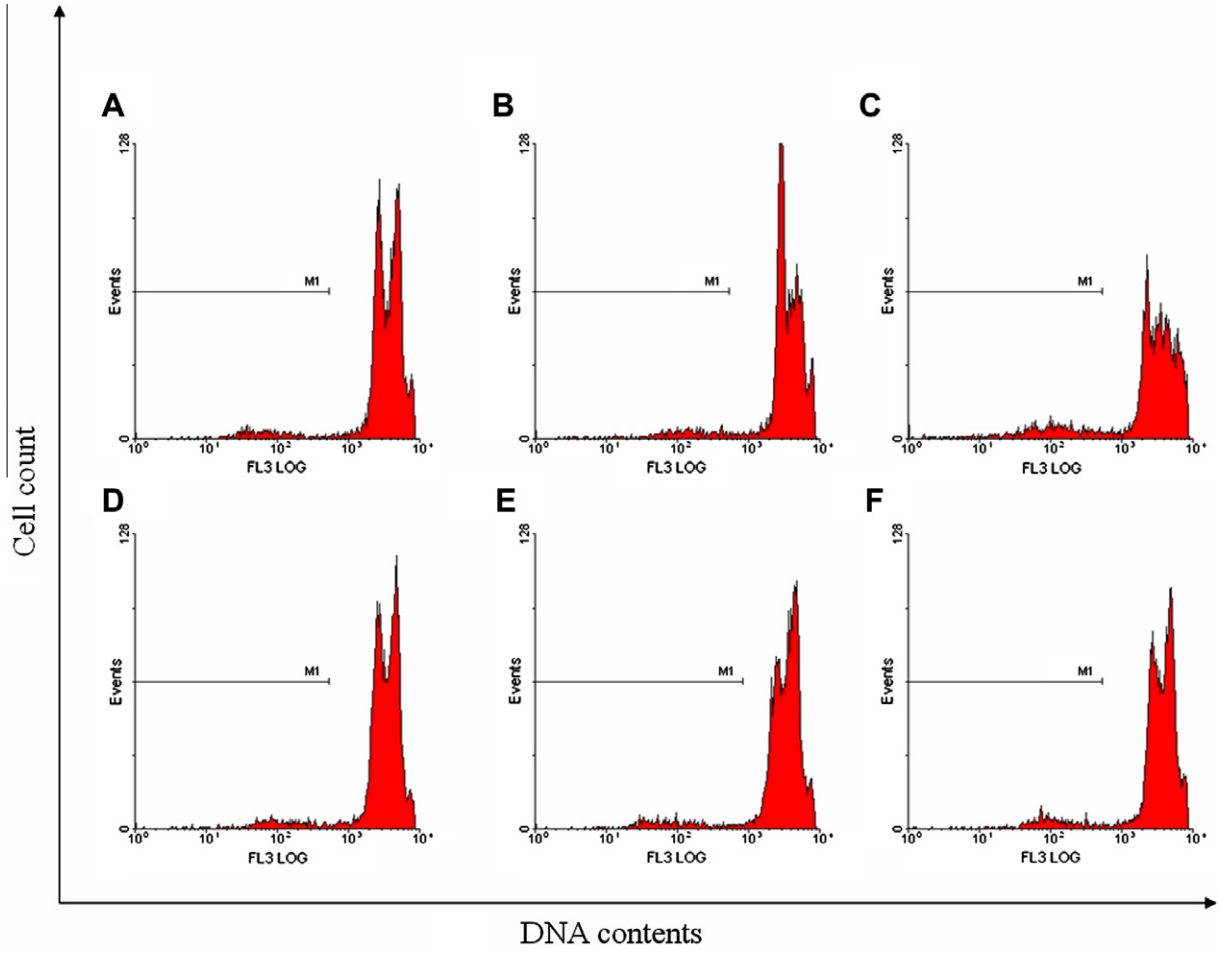


Figure 3. Flow cytometric analysis of compounds **1**, **2**, **8**, **9**, and **10** on K562 cells. Cells were treated with DMSO (A), compound **1** (B), compound **2** (C), compound **8** (D), compound **9** (E), and compound **10** (F) at 1.0 μM for 24 h. Cells were harvested, fixed, and stained with propidium iodide as described in Section 5 prior to analysis by flow cytometry. The percentage of cells in each cell cycle phase was quantified in Table 4.

Table 4
Cell cycle effect of naphtho[2,3-*b*]furan-4,9-dione derivatives on K562 cell

Compound	G0/G1 (%)	S (%)	G2/M (%)	Sub G1 (%)
Control	32.4	37.8	29.8	3.88
1	36.1	51.2	12.7	3.75
2	27.4	29.4	43.2	8.24
8	37.7	4.4	58.0	4.41
9	26.1	35.7	38.2	4.8
10	29.0	30.2	41.9	5.82
11d	33.5	3.2	63.3	4.19
13h	40.4	7.1	52.5	5.56

Cells were treated with 1.0 μM of each compound for 24 h and then the cell cycle was analyzed by flow cytometry analysis.

is comparable to daunorubicin (SI = 239) and is more favorable than camptothecin (SI = 16.5). Therefore, compound **9** has been identified as a lead molecule for further structural optimization.

5. Experimental

5.1. General

TLC: precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories, Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). Mp: Yamato MP-21 melting-point apparatus;

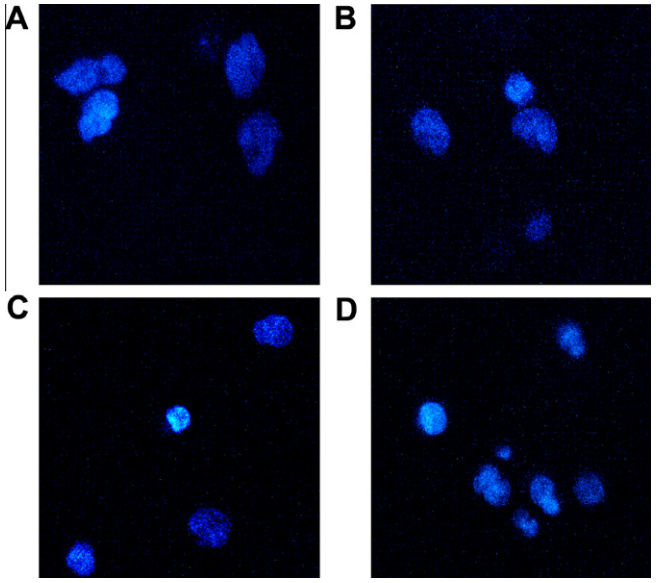


Figure 4. Morphologic representation of apoptosis by nuclear staining with DAPI in K562 cells. Cells were treated with DMSO (A), compound **9** at 1 μM (B), 5 μM (C), or 10 μM (D) for 48 h at 37 °C and photographed (600×) under a fluorescence microscope.

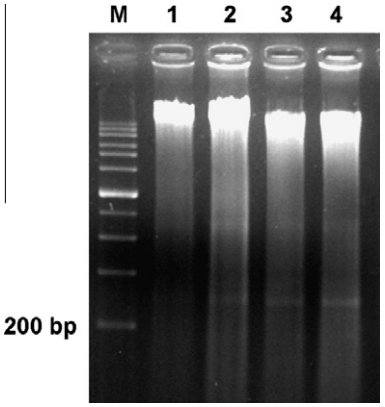


Figure 5. Agarose gel electrophoresis for detecting DNA fragmentation in K562 cells treated with compound **9** for 48 h. Lane M, DNA size marker; lane 1, DMSO; lane 2, 1.0 μM; lane 3, 5.0 μM; lane 4, 10.0 μM

uncorrected. ^{1}H, ^{13}C, and 2D HMBC NMR spectra: Varian-Unity-400 spectrometer at 400 and 100 MHz, chemical shifts in ppm with $SiMe_4$ as an internal standard (=0 ppm), coupling constants *J* in Hz. Mass spectra were recorded on Finnigan/Thermo Quest MAT 95XL (ESIMS) and Thermo-Finnigan PolarisQ (70 eV, EIMS). Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.1.1. (*Z*)-4-(Hydroxyimino)naphtho[2,3-*b*]furan-9(4*H*)-one (8)

To a suspension of **2** (0.20 g, 1.0 mmol) in 2-ethoxyethanol (30 mL) was added $NH_2OH{\cdot}HCl$ (0.20 g, 3.0 mmol) and K_2CO_3. The reaction mixture was heated at reflux for 8 h (TLC monitoring). The solvent was removed in vacuo and the residue was poured into H_2O (20 mL). The crude product was purified by column chromatography ($MeOH/CH_2Cl_2$ 1:20) and recrystallized from MeOH to give **8** (0.16 g, 75%). Mp: 185–186 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.53 (d, 1H, *J* = 1.6 Hz, 2-H), 7.66–7.78 (m, 2H, 6- and 7-H), 8.17 (dd, 1H, *J* = 8.0, 1.6 Hz, 8-H), 8.29 (d, 1H, *J* = 1.6 Hz, 3-H), 8.33 (dd, 1H, *J* = 8.0, 1.6 Hz, 5-H), 13.34 (s, 1H, NOH). ^{13}C NMR (100 MHz, $CDCl_3$): 113.10 (3-C), 123.22 (3a-C), 123.42 (5-C), 125.96 (8-C), 129.53 (6-C), 130.53 (4a-C), 132.82 (7-C), 133.46 (8a-C), 140.94 (4-C), 147.58 (9a-C), 149.47 (2-C), 171.97 (9-C). ESIMS (*m*/*z*): 214 $[M+H]^+$. Anal. Calcd for $C_{12}H_7NO_3{\cdot}0.3H_2O$: C, 65.93; H, 3.50; N, 6.41. Found: C, 65.67; H, 3.55; N, 6.54.

5.1.2. (*Z*)-4-Methoxyiminonaphtho[2,3-*b*]furan-9(4*H*)-one (9)

Compound **9** was prepared from **2** and NH_2OMe HCl as described for **8**. Yield: 33%. Mp: 150–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 4.28 (s, 3H, NOMe), 7.33 (d, 1H, *J* = 2.0 Hz, 2-H), 7.87–7.65 (m, 2H, 6- and 7-H), 7.76 (d, 1H, *J* = 2.0 Hz, 3-H), 8.29–8.37 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.98 (OMe), 113.21 (3-C), 123.38 (3a-C), 123.94 (5-C), 126.61 (8-C), 129.58 (6-C), 131.06 (4a-C), 132.47 (7-C), 133.20 (8a-C), 141.32 (4-C), 147.55 (2-C), 148.66 (9a-C), 173.09 (9-C). ESIMS (*m*/*z*): 228 $[M+H]^+$. Anal. Calcd for $C_{13}H_9NO_3{\cdot}0.3H_2O$: C, 67.12; H, 4.16; N, 6.02. Found: C, 67.24; H, 3.81; N, 5.91.

5.1.3. (*Z*)-4-Acetoxyiminonaphtho[2,3-*b*]furan-9(4*H*)-one (10)

To a suspension of **8** (0.21 g, 1.0 mmol) in Ac_2O (10 mL) was added pyridine (1.0 mL). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the residue was triturated with H_2O (20 mL), filtered, and washed with H_2O. The crude product was recrystallized from EtOH to give **10** (0.20 g, 78%). Mp: 213–214 °C. ^{1}H NMR (200 MHz, DMSO-d_6): 2.45 (s, 3H, COMe), 7.46 (d, 1H, *J* = 1.8 Hz, 2-H), 7.72–7.84 (m, 2H, 3- and 6-H), 8.13–8.17 (m, 1H, 7-H), 8.32–8.36 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, DMSO-d_6): 19.47 (Me), 113.40 (3-C), 122.34 (3a-C), 124.62 (5-C), 126.44 (8-C), 131.11 (4a-C), 131.55 (6-C), 133.47 (7-C), 133.81 (8a-C), 146.27 (4-C), 148.87 (9a-C), 149.78 (2-C), 167.55 (C=O), 171.95 (9-C). ESIMS (*m*/*z*): 256 $[M+H]^+$. Anal. Calcd for $C_{14}H_9NO_4$: C, 65.88; H, 3.55; N, 5.49. Found: C, 65.77; H, 3.56; N, 5.46.

5.1.4. (*Z*)-4-[(Benzoxy)imino]naphtho[2,3-*b*]furan-9(4*H*)-one (11a)

To a solution of **8** (0.35 g, 1 mmol) in dry DMF (20 mL) was added sodium hydride (60% in oil, 0.5 g) at 0 °C for 1 h. Benzyl chloride (0.42 g, 4.0 mmol) was added and the mixture was heated at 80 °C for 60 min. The reaction mixture was partitioned between H_2O and CH_2Cl_2. The organic layer was collected, dried over $MgSO_4$, and concentrated. The resulting residue was purified by column

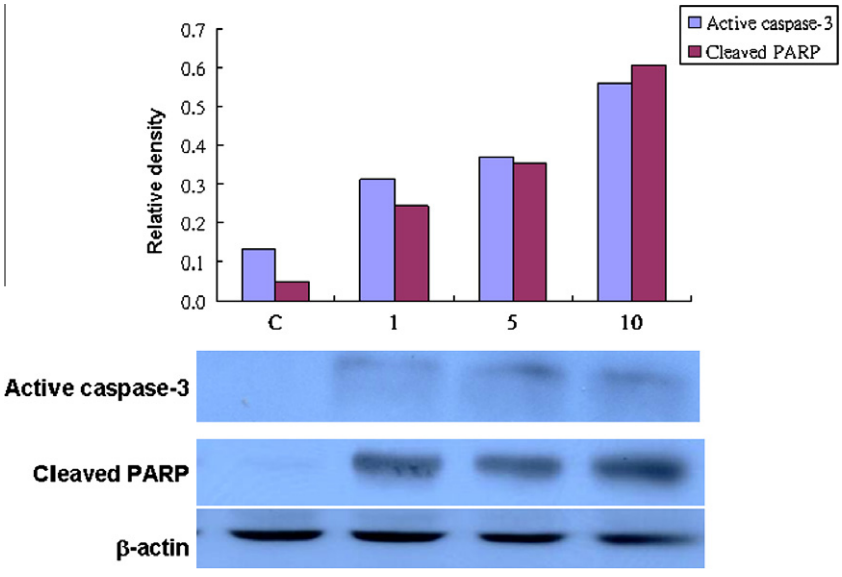


Figure 6. Immunoblot analysis of activation caspase-3 and cleaved PARP protein expression in compound **9**-treated K562 cells. Cells were lysed after incubation with various concentrations of compound **9** (1.0, 5.0, and 10.0 μM) for 48 h. The cellular proteins were separated by SDS–PAGE and transferred onto PVDF membranes. The membranes were probed with the indicated primary antibodies then with horseradish peroxidase-conjugated goat anti-rabbit IgG. β-Actin was used as an internal control.

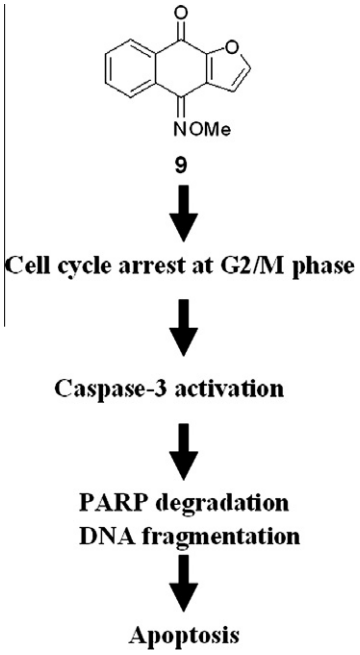


Figure 7. Proposed apoptotic pathway induced by compound **9** in K562 cells.

chromatography (MeOH/CH_2Cl_2 1:50) and recrystallized from MeOH to give **11a** (0.08 g, 25%). Mp: 124–125 °C. 1H NMR (400 MHz, $CDCl_3$): 5.50 (s, 2H, CH_2), 7.31 (d, 1H, J = 2.0 Hz, 2-H), 7.37–7.44 (m, 3H, Ar-H), 7.48–7.51 (m, 2H, Ar-H), 7.56–7.65 (m, 2H, 6- and 7-H), 7.73 (d, 1H, J = 2.0 Hz, 3-H), 8.29–8.32 (m, 1H, 8-H), 8.36–8.38 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 78.57 (OCH_2), 113.38 (3-C), 123.40 (3a-C), 124.04 (5-C), 126.64 (8-C), 128.43 (2 Ar-C), 128.54 (Ar-C), 128.66 (2 Ar-C), 129.66 (6-C), 131.09 (4a-C), 132.51 (7-C), 133.22 (8a-C), 141.75 (4-C), 146.19 (Ar-C), 147.62 (2-C), 148.67 (9a-C), 173.11 (9-C). ESIMS (m/z): 304 $[M+H]^+$. Anal. Calcd for $C_{19}H_{13}NO_3$: C, 75.24; H, 4.32; N, 4.62. Found: C, 75.46; H, 4.46; N, 4.50.

5.1.5. (*Z*)-4-{[2-(Dimethylamino)ethoxy]imino}naphtho[2,3-*b*]furan-9(4*H*)-one (11b)

Compound **11b** was prepared from **8** and 2-dimethylaminoethyl chloride hydrochloride by the same procedures as described for **11a**. The resulting residue was purified by column chromatography (MeOH/CH_2Cl_2 1:10) to give **11b** (0.08 g, 53%) as a beige liquid. 1H NMR (400 MHz, $CDCl_3$): 2.36 (s, 6H, NMe_2), 2.82 (t, 2H, J = 6.0 Hz, CH_2N), 4.59 (t, 2H, J = 6.0 Hz, OCH_2), 7.38 (d, 1H, J = 2.0 Hz, 2-H), 7.55–7.65 (m, 2H, 6- and 7-H), 7.76 (d, 1H, J = 2.0 Hz, 3-H), 8.29–8.31 (m, 1H, 8-H), 8.34–8.36 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 45.93 (NMe_2), 58.13 (CH_2N), 74.64 (OCH_2), 113.30 (3-C), 123.39 (3a-C), 123.94 (5-C), 126.57 (8-C), 129.55 (6-C), 131.03 (4a-C), 132.43 (7-C), 133.22 (8a-C), 141.49 (4-C), 147.56 (2-C), 148.61 (9a-C), 173.05 (9-C). ESIMS $[M+H]^+$: 285. HRMS (ESI+): m/z calcd for $C_{16}H_{17}N_2O_3$ $[M+H]^+$ 285.1239, found 285.1237.

5.1.6. (*Z*)-4-{[3-(Dimethylamino)propoxy]imino}naphtho[2,3-*b*]furan-9(4*H*)-one (11c)

Compound **11c** was prepared from **8** and 3-dimethylaminopropyl chloride hydrochloride by the same procedures as described for **11a**. Yield: 28% as a beige liquid. 1H NMR (400 MHz, $CDCl_3$): 2.05 (quint, 2H, J = 6.4 Hz, $CH_2CH_2CH_2$), 2.28 (s, 6H, NMe_2), 2.47 (t, 2H, J = 6.4 Hz, CH_2N), 4.54 (t, 2H, J = 6.4 Hz, OCH_2), 7.35 (d, 1H, J = 2.0 Hz, 2-H), 7.56–7.65 (m, 2H, 6- and 7-H), 7.77 (d, 1H, J = 2.0 Hz, 3-H), 8.31 (m, 1H, 8-H), 8.36 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 27.56 (CH_2), 45.49 (NMe_2), 56.23 (CH_2N), 74.81 (OCH_2), 113.17 (3-C), 123.40 (3a-C), 123.94 (5-C), 126.58 (8-C), 129.51 (6-C), 131.04 (4a-C), 132.43 (7-C), 133.34 (8a-C), 141.27 (4-C), 147.57 (2-C), 148.66 (9a-C), 173.10 (9-C). ESIMS $[M+H]^+$: 299. Anal. Calcd for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.28; H, 6.18; N, 9.20.

5.1.7. (*Z*)-4-[(3-Aminopropoxy)imino]naphtho[2,3-*b*]furan-9(4*H*)-one hydrobromide (11d)

Compound **11d** was prepared from **8** and 3-bromopropylamine hydrobromide by the same procedures as described for **11a**. Yield: 31% as a beige liquid. 1H NMR (400 MHz, DMSO-d_6): 2.14 (m, 2H, $CH_2CH_2CH_2$), 2.98 (t, 2H, J = 7.4 Hz, CH_2N), 4.55 (t, 2H, J = 6.0 Hz, OCH_2), 7.46 (d, 1H, J = 2.0 Hz, 2-H), 7.69–7.84 (m, 4H, NH_2, 6- and 7-H), 8.17 (dd, 1H, J = 8.0, 1.4 Hz, 8-H), 8.29 (dd, 1H, J = 8.0, 0.8 Hz, 5-H), 8.36 (d, 1H, J = 2.0 Hz, 3-H). ^{13}C NMR (100 MHz, DMSO-d_6): 27.01 (CH_2), 36.19 (CH_2N), 72.93 (OCH_2), 113.08 (3-C), 122.86 (3a-C), 123.72 (5-C), 126.12 (8-C), 130.19 (6-C), 130.55 (4a-C), 132.39 (8a-C), 132.99 (7-C), 141.25 (4-C), 148.00 (9a-C), 149.65 (2-C), 171.88 (9-C). ESIMS $[M+H]^+$: 271. Anal. Calcd for $C_{15}H_{14}N_2O_3 \cdot 1.5H_2O \cdot 1.0HBr$: C, 47.63; H, 4.53; N, 7.41. Found: C, 47.71; H, 4.86; N, 7.38.

5.1.8. (*Z*)-4-{[2-(Pyrrolidin-1-yl)ethoxy]imino}naphtho[2,3-*b*]furan-9(4*H*)-one (11e)

Compound **11e** was prepared from **8** and 1-(2-chloroethyl)pyrrolidine hydrochloride by the same procedures as described for **11a**. Yield: 28% as a beige liquid. 1H NMR (400 MHz, $CDCl_3$): 1.81 (m, 4H, pyrrolidinyl-H), 2.65 (m, 4H, pyrrolidinyl-H), 3.00 (t, 2H, J = 6.0 Hz, CH_2N), 4.64 (t, 2H, J = 6.0 Hz, OCH_2), 7.40 (d, 1H, J = 2.0 Hz, 2-H), 7.56–7.65 (m, 2H, 6- and 7-H), 7.77 (d, 1H, J = 2.0 Hz, 3-H), 8.31 (m, 1H, 8-H), 8.37 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 23.52 (pyrrolidinyl-C), 54.76 (C-pyrrolidinyl-C), 54.84 (CH_2N), 75.70 (OCH_2), 113.30 (3-C), 123.43 (3a-C), 123.97 (5-C), 126.61 (8-C), 129.58 (6-C), 131.05 (4a-C), 132.46 (7-C), 133.27 (8a-C), 141.47 (4-C), 147.59 (2-C), 148.64 (9a-C), 173.10 (9-C). ESIMS $[M+H]^+$: 311. Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.60; H, 6.04; N, 8.81.

5.1.9. (*Z*)-4-{[2-(Piperidin-1-yl)ethoxy]imino}naphtho[2,3-*b*]furan-9(4*H*)-one (11f)

Compound **11f** was prepared from **8** and 1-(2-chloroethyl)piperdine hydrochloride by the same procedures as described for **11a**. Yield: 26% as a beige liquid. 1H NMR (400 MHz, $CDCl_3$): 1.45 (m, 2H, piperidinyl-H), 1.62 (m, 4H, piperidinyl-H), 2.52 (m, 4H, piperidinyl-H), 2.85 (t, 2H, J = 6.0 Hz, CH_2N), 4.62 (t, 2H, J = 6.0 Hz, OCH_2), 7.41 (d, 1H, J = 2.0 Hz, 2-H), 7.56–7.65 (m, 2H, 6- and 7-H), 7.77 (d, 1H, J = 2.0 Hz, 3-H), 8.31 (m, 1H, 8-H), 8.36 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 24.16 (piperidinyl-C), 26.00 (2 piperidinyl-C), 54.96 (2 piperidinyl-C), 57.76 (CH_2N), 74.44 (OCH_2), 113.34 (3-C), 123.47 (3a-C), 123.97 (5-C), 126.62 (8-C), 129.57 (6-C), 131.06 (4a-C), 132.46 (7-C), 133.28 (8a-C), 141.50 (4-C), 147.57 (2-C), 148.65 (9a-C), 173.12 (9-C). ESIMS $[M+H]^+$: 325. Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.49; H, 6.29; N, 8.56.

5.1.10. (*Z*)-4-[(2-Morpholinoethoxy)imino]naphtho[2,3-*b*]furan-9(4*H*)-one (11g)

Compound **11g** was prepared from **8** and 1-(2-chloroethyl)morpholine hydrochloride by the same procedures as described for **11a**. Yield: 25% as a beige liquid. 1H NMR (400 MHz, $CDCl_3$): 2.59 (m, 4H, morpholinyl-H), 2.88 (t, 2H, J = 6.0 Hz, CH_2N), 3.73 (m, 4H, morpholinyl-H), 4.60 (t, 2H, J = 6.0 Hz, OCH_2), 7.40 (d, 1H, J = 1.6 Hz, 2-H), 7.57–7.66 (m, 2H, 6- and 7-H), 7.78 (d, 1H, J = 2.0 Hz, 3-H), 8.31 (m, 1H, 8-H), 8.35 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 53.94 (2 morpholinyl-C), 57.44 (CH_2N), 66.97

(2 morpholinyl-C), 73.83 (OCH_2), 113.27 (3-C), 123.38 (3a-C), 123.94 (5-C), 126.67 (8-C), 129.66 (6-C), 131.09 (4a-C), 132.49 (7-C), 133.18 (8a-C), 141.68 (4-C), 147.58 (2-C), 148.71 (9a-C), 173.07 (9-C). ESIMS $[M+H]^+$: 327. Anal. Calcd for $C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58. Found: C, 66.42; H, 5.77; N, 8.35.

5.1.11. (*Z*)-4-(Phenylimino)naphtho[2,3-*b*]furan-9(4*H*)-one (12a) and (*Z*)-*N*-((*Z*)-4-(phenylimino)naphtho[2,3-*b*]furan-9(4*H*)-ylidene)benzenamine (13a)

Method A. To a stirred solution of **2** (0.2 g, 1.0 mmol) in dry CH_2Cl_2 (20 mL) were added 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (1.0 mL, 1.0 mmol), a solution of aniline (2.0 mmol) in CH_2Cl_2 (10 mL), and dry triethylamine (1.78 mL, 12.4 mmol) successively. After 10 min, more 1.0 M solution of $TiCl_4$ in CH_2Cl_2 (1.0 mL, 1.0 mmol) was added and then the reaction mixture was poured into 100 mL of cold water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The crude product was chromatographed on a column of silica gel using CH_2Cl_2/MeOH = 100:1 and recrystallized from MeOH to give **12a** (25%) and **13a** (30%).

Method B. The procedures were the same with that of Method A except the reaction time was extended from 10 to 30 min. The sole product of **13a** was obtained in 69% yield.

*Compound **12a**:* Mp: 171–172 °C. 1H NMR (400 MHz, $CDCl_3$): 5.45 (d, 1H, *J* = 2.0 Hz, 2-H), 6.88–6.90 (m, 2H, Ar-H), 7.22–7.26 (m, 1H, Ar-H), 7.42–7.46 (m, 3H, Ar-H and 3-H), 7.68–7.77 (m, 2H, 6- and 7-H), 8.31 (m, 1H, 8-H), 8.58 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.90 (3-C), 117.82 (2 Ar-C), 124.47 (Ar-H), 125.13 (3a-C), 126.41 (5-C), 126.63 (8-C), 129.47 (2 Ar-C), 131.29 (6-C), 131.83 (4a-C), 133.14 (7-C), 135.66 (8a-C), 146.78 (2-C), 150.68 (Ar-C), 151.18 (9a-C), 151.72 (4-C), 173.78 (9-C). EIMS (*m*/*z*): 273 $[M]^+$. Anal. Calcd for $C_{18}H_{11}NO_2$: C, 79.11; H, 4.06; N, 5.13. Found: C, 78.87; H, 4.09; N, 5.08.

*Compound **13a**:* Mp: 211–212 °C. 1H NMR (400 MHz, $CDCl_3$): 5.34 (d, 1H, *J* = 1.6 Hz, 2-H), 6.87–6.94 (m, 4H, Ar-H), 6.98 (d, 1H, *J* = 2.0 Hz, 3-H), 7.10–7.19 (m, 2H, Ar-H), 7.33–7.41 (m, 4H, Ar-H), 7.64–7.67 (m, 2H, 6- and 7-H), 8.58–8.62 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 109.74 (3-C), 118.27 (2 Ar-C), 118.59 (2 Ar-C), 121.96 (3a-C), 123.53 (Ar-C), 123.87 (Ar-C), 126.02 (5-C), 126.13 (8-C), 128.54 (2 Ar-C), 129.34 (2 Ar-C), 130.60 (6-C), 130.80 (7-C), 133.18 (4a-C), 133.71 (8a-C), 144.54 (2-C), 145.00 (9a-C), 148.48 (9-C), 151.87 (Ar-C), 152.04 (Ar-C), 152.23 (4-C). EIMS (*m*/*z*): 348 $[M]^+$. Anal. Calcd for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04. Found: C, 82.81; H, 4.62; N, 8.01.

5.1.12. (*Z*)-4-(4-fluorophenylimino)naphtho[2,3-*b*]furan-9(4*H*)-one (12b) and (14*Z*)-*N*-[(*Z*)-9-(4-fluorophenylimino)naphtho[2,3-*b*]furan-4(9*H*)-ylidene]-4-fluorobenzenamine (13b)

Compounds **12b** (35%) and **13b** (30%) were prepared from **2** and 4-fluoroaniline by the same procedures as described in Method A. The sole product of compound **13b** (63%) was obtained from **2** and 4-fluoroaniline by the same procedures as described in Method B.

*Compound **12b**:* Yield: 35%. Mp: 189–190 °C. ^1H NMR (400 MHz, $CDCl_3$): 5.55 (d, 1H, *J* = 2.0 Hz, 2-H), 6.84–6.88 (m, 2H, Ar-H), 7.13–7.26 (m, 2H, Ar-H), 7.47 (d, 1H, *J* = 2.0 Hz, 3-H), 7.68–7.76 (m, 2H, 6- and 7-H), 8.31 (m, 1H, 8-H), 8.55 (m, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.73, 116.34 2 Ar-C, *J* = 22.8 Hz), 119.40 (2 Ar-C, *J* = 7.6 Hz), 124.85 (3a-C), 126.46 (5-C), 126.61 (8-C), 131.42 (6-C), 131.80 (4a-C), 133.18 (7-C), 135.56 (8a-C), 146.88 (2-C), 147.64 (Ar-C, *J* = 2.3 Hz), 150.70 (9a-C), 151.93 (4-C, *J* = 1.5 Hz), 160.13 (Ar-H, *J* = 241.7 Hz), 173.72 (9-C). ESIMS $[M+H]^+$: 292. Anal. Calcd for $C_{18}H_{10}FNO_2$: C, 74.22; H, 3.46; N, 4.81. Found: C, 74.01; H, 3.57; N, 4.75.

*Compound **13b**:* Yield: 30%. Mp: 201–202 °C. ^1H NMR (400 MHz, $CDCl_3$): 5.44(d, 1H, *J* = 2.0 Hz, 2-H), 6.83–6.92 (m, 4H, Ar-H), 7.02–7.14 (m, 5H, Ar-H and 3-H), 7.64–7.69 (m, 2H, 6- and 7-H), 8.57–8.61 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 109.71 (3-C), 115.31 (*J* = 22.0 Hz, 2 Ar-C), 116.19 (*J* = 22.0 Hz, 2 Ar-C), 119.74 (*J* = 7.6 Hz, 2 Ar-C), 120.12 (*J* = 7.6 Hz, 2 Ar-C), 121.86 (3a-C), 126.04 (5-C), 126.16 (8-C), 130.76 (6-C), 130.97 (7-C), 133.06 (4a-C), 133.61 (8a-C), 144.62 (2-C), 145.66 (9a-C), 147.92 (Ar-H), 148.09 (Ar-H), 148.50 (9-C), 152.59 (4-C), 159.80 (*J* = 239.5 Hz, Ar-C), 159.87 (*J* = 241.1 Hz, Ar-C). EIMS (*m*/*z*): 384 $[M]^+$. Anal. Calcd for $C_{24}H_{14}F_2N_2O$: C, 74.99; H, 3.67; N, 7.29. Found: C, 75.01; H, 3.86; N, 7.10.

5.1.13. (14*Z*)-*N*-[(*Z*)-9-(4-Chlorophenylimino)naphtho[2,3-*b*]furan-4(9*H*)-ylidene]-4-chlorobenzenamine (13c)

Compound **13c** (65%) was obtained from **2** and 4-chloroaniline by the same procedures as described in Method B. Mp: 223–224 °C. ^1H NMR (400 MHz, $CDCl_3$): 5.49 (d, 1H, J = 2.0 Hz, 2-H), 6.81–6.88 (m, 4H, Ar-H), 7.08 (d, 1H, J = 2.0 Hz, 3-H), 7.29–7.38 (m, 4H, Ar-H), 7.64–7.68 (m, 2H, 6- and 7-H), 8.54–8.58 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 109.80 (3-C), 119.78 (2 Ar-C), 120.63 (2 Ar-C), 121.94 (3a-C), 126.09 (5-C), 126.23 (8-C), 128.67 (2 Ar-C), 128.92 (Ar-C), 129.31 (Ar-C), 129.49 (2 Ar-C), 130.83 (6-C), 131.04 (7-C), 132.96 (4a-C), 133.55 (8a-C), 144.90 (2-C), 145.46 (9a-C), 148.45 (9-C), 150.48 (Ar-C), 150.56 (Ar-C), 152.25 (4-C). ESIMS $[M+H]^+$: 417. Anal. Calcd for $C_{24}H_{14}Cl_2N_2O$: C, 69.08; H, 3.38; N, 6.71. Found: C, 68.85; H, 3.34; N, 6.64.

5.1.14. (14*Z*)-*N*-[(*Z*)-9-(*p*-Tolylimino)naphtho[2,3-*b*]furan-4(9*H*)-ylidene]-4-methyl-benzenamine (13d)

Compound **13d** (61%) was obtained from **2** and 4-methylaniline by the same procedures as described in Method B. Mp: 198–199 °C. ^1H NMR (400 MHz, $CDCl_3$): 2.37 (s, 3H, Me), 2.39 (s, 3H, Me), 5.42 (d, 1H, *J* = 2.0 Hz, 2-H), 6.76–6.86 (m, 4H, Ar-H), 7.01 (d, 1H, *J* = 2.0 Hz, 3-H) 7.14–7.20 (m, 4H, Ar-H), 7.62–7.65 (m, 2H, 6- and 7-H), 8.57–8.61 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 20.96 (Me), 21.02 (Me), 109.83 (3-C), 118.25 (2 Ar-C), 118.79 (2 Ar-C), 121.88 (3a-C), 125.98 (5-C), 126.04 (8-C), 129.11 (2 Ar-C), 129.86 (2 Ar-C), 130.43 (6-C), 130.66 (7-C), 133.11 (4a-C), 133.30 (8a-C), 133.38 (Ar-C), 133.73 (Ar-C), 144.25 (2-C), 144.90 (9a-C), 148.50 (9-C), 149.35 (Ar-C), 149.67 (Ar-C), 152.04 (4-C). ESIMS $[M+H]^+$: 377. Anal. Calcd for $C_{26}H_{20}N_2O$: C, 82.95; H, 5.35; N, 7.44. Found: C, 83.20; H, 5.38; N, 7.41.

5.1.15. (14*Z*)-*N*-[(*Z*)-9-(4-Methoxyphenylimino)naphtho[2,3-*b*]furan-4(9*H*)-ylidene]-4-methoxybenzenamine (13e)

Compound **13e** (75%) was obtained from **2** and 4-methoxyaniline by the same procedures as described in Method B. Mp: 187–188 °C. 1H NMR (400 MHz, $CDCl_3$): 3.84 (s, 3H, OMe), 3.85 (s, 3H, OMe), 5.48 (*d*, 1H, *J* = 2.0 Hz, 2-H), 6.82–6.96 (m, 8H, Ar-H), 7.03 (d, 1H, *J* = 2.0 Hz, 3-H), 7.62–7.65 (m, 2H, 6- and 7-H), 8.58–8.62 (m, 2H, 54- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.43 (OMe), 55.51 (OMe), 109.78 (3-C), 113.81 (2 Ar-C), 114.63 (2 Ar-C), 119.72 (2 Ar-C), 120.54 (2 Ar-C), 121.70 (3a-C), 125.92 (5-C), 125.98 (8-C), 130.34 (6-C), 130.61 (7-C), 133.42 (4a-C), 133.75 (8a-C), 144.07 (2-C), 145.01 (Ar-C), 145.13 (Ar-C), 145.49 (9a-C), 148.54 (9-C), 152.48 (4-C), 156.55 (Ar-C), 156.60 (Ar-C). Anal. Calcd for $C_{26}H_{20}N_2O_3$: C, 76.45; H, 4.94; N, 6.86. Found: C, 76.45; H, 4.88; N, 6.81.

5.1.16. (13*Z*)-*N*-{(*Z*)-4-[4-(Trifluoromethyl)phenylimino]-naphtho[2,3-*b*]furan-9(4*H*)-ylidene}-4-(trifluoromethyl)-benzenamine (13f)

Compound **13f** (52%) was obtained from **2** and 4-trifluoromethylaniline by the same procedures as described in Method B. Mp: 193–194 °C. ^1H NMR (400 MHz, $CDCl_3$): 5.40 (*d*, 1H, *J* = 2.0 Hz, 2-H), 6.86–6.94 (m, 4H, Ar-H), 7.06 (d, 1H, *J* = 2.0 Hz, 3-H), 7.60–7.72 (m, 6H, Ar-H, 6- and 7-H), 8.55–8.59 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 109.68 (3-C), 118.39 (2 Ar-H), 118.50

(2 Ar-H), 122.10 (3a-C), 125.93 (2 Ar-C, J = 3.8 Hz), 126.22 (5-C), 126.40 (8-C), 126.68 (2 Ar-C, J = 3.1 Hz), 131.12 (6-C), 131.30 (7-C), 132.71 (4a-C), 133.39 (8a-C), 145.28 (9a-C), 145.44 (2-C), 148.40 (9-C), 151.82 (4-C), 154.98 (2 Ar-C). ESIMS $[M+H]^+$: 485. Anal. Calcd for $C_{26}H_{14}F_6N_2O$: C, 64.47; H, 2.91; N, 5.78. Found: C, 64.61; H, 2.92; N, 5.80.

5.1.17. (13*Z*)-*N*-[(*Z*)-4-(4-Nitrophenylimino)naphtho[2,3-*b*]furan-9(4*H*)-ylidene]-4-nitro benzenamine (13g)

Compound **13g** (71%) was obtained from **2** and 4-nitroaniline by the same procedures as described in Method B. Mp: 281–282 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 5.50 (d, 1H, J = 2.0 Hz, 2-H), 6.98–7.02 (m, 4H, Ar-H), 7.12 (d, 1H, J = 2.0 Hz, 3-H), 7.71–7.74 (m, 2H, 6- and 7-H), 8.24–8.33 (m, 4H, Ar-H), 8.50–8.58 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 109.78 (3-C), 118.60 (2 Ar-C), 118.67 (2 Ar-C), 122.39 (3a-C), 124.90 (2 Ar-C), 125.53 (2 Ar-C), 126.46 (5-C), 126.69 (8-C), 131.54 (6-C), 131.72 (7-C), 132.18 (4a-C), 132.93 (8a-C), 143.96 (Ar-C), 144.16 (Ar-C), 145.22 (9a-C), 146.08 (2-C), 151.59 (9-C), 157.68 (Ar-C), 158.02 (Ar-C). ESIMS $[M+H]^+$: 439. Anal. Calcd for $C_{24}H_{14}N_4O_5$: C, 65.78; H, 3.22; N, 12.78. Found: C, 66.62; H, 3.32; N, 12.81.

5.1.18. Bis[2-(diethylamino)ethyl]-4,4′-(1*Z*,1′*Z*)-naphtho[2,3-*b*]furan-4,9-diylidenebis (azan-1-yl-1-ylidene)dibenzoate (13h)

Compound **13h** (22%) was obtained from **2** and 2-(diethylamino)ethyl 4-aminobenzoate by the same procedures as described in Method B. Mp: 177–178 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08–1.13 (m, 12H, 4× NCH_2CH_3), 2.66–2.72 (m, 8H, 4× NCH_2CH_3), 2.89–2.93 (m, 4H, 2× CH_2N), 4.42 (t, 4H, J = 6.0 Hz, 2× OCH_2), 5.40 (d, 1H, J = 1.8 Hz, 2-H), 6.92–6.95 (m, 4H, Ar-H), 7.01 (d, 1H, J = 1.8 Hz, 3-H), 7.68–7.70 (m, 2H, 6- and 7-H), 8.03 (m, 4H, Ar-H), 8.56–8.59 (m, 2H, 5- and 8-H). ^{13}C NMR (100 MHz, $CDCl_3$): 11.78 (2 NCH_2CH_3), 11.82 (2 NCH_2CH_3), 47.77 (2 NCH_2CH_3), 47.80 (2 NCH_2CH_3), 51.02 (2 CH_2N), 62.89 (OCH_2), 63.12 (OCH_2), 109.85 (3-C), 118.09 (2 Ar-C), 118.21 (2 Ar-C), 125.29 (Ar-C), 125.77 (Ar-C), 126.23 (5-C), 126.40 (8-C), 130.52 (2 Ar-C), 131.05 (6-C), 131.21 (2 Ar-C and 7-C), 132.72 (4a-C), 133.41 (8a-C), 145.00 (9a-C), 145.34 (2-C), 151.51 (9-C), 156.35 (Ar-C), 156.50 (Ar-C), 166.29 (C═O), 166.55 (C═O). Anal. Calcd for $C_{38}H_{42}N_4O_5{\cdot}0.3H_2O$: C, 71.28; H, 6.72; N, 8.75. Found: C, 70.97; H, 6.64; N, 8.60.

5.2. Pharmacological methods

5.2.1. Antiproliferative assay

These naphtho[2,3-*b*]furan-4,9-dione derivatives were also evaluated in vitro against a panel of cell lines consisting of MCF7 (breast), NCI-H460 (lung), K562 (leukemia), and SF-268 (CNS) using sulforhodamine B assay as described previously.[30] Compounds which reduced the growth of any one of the cell lines to 50% or less at the concentration of 4 μg/mL were subjected to further evaluation for their dose–response effects and IC_{50} measurement. The cells were treated with at least five different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the tetrazolium dye reduction assay (XTT assay),[31] and the experiment was performed as recommended by the manufacturer (Promega, Madison, WI). The absorbance was measured at 490 nm on a Wallac 1420 VICTOR2 Multilabel counter (Perkin-Elmer, Boston, MA). The results of these assays were used to obtain the dose–response curves from which IC_{50} (μM) values were determined. An IC_{50} value represents the concentration of the tested compound at which a 50% cell growth inhibition after 3 days of incubation was produced.

5.2.2. Cell cycle analysis

Flow cytometry was used to measure cell cycle profile. For cell cycle analysis, K562 cell treated with compounds (1.0 μM) for 24 h was harvested by centrifugation. After being washed with PBS, the cell was fixed with ice-cold 70% ethanol for 30 min, washed with PBS, and then treated with 1 mL of 1 mg/mL of RNase A solution at 37 °C for 30 min. Cells were harvested by centrifugation at 1000 rpm for 5 min and further stained with 250 μL DNA staining solution (10 mg propidium iodide [PI], 0.1 mg trisodium citrate, and 0.03 mL Triton X-100 dissolved in 100 mL H_2O) at room temperature for 30 min in the dark. After loading 500 μL PBS, the DNA contents of 10,000 events were measured by FACScan (Elite ESP, Beckman Coulter, Brea, CA) and the cell cycle profile was analyzed from the DNA content histograms by using WinCycle software.

5.2.3. Nuclear staining with DAPI

Leukemia K562 Cells were treated with DMSO or compound **9** for 48 h were used for 4′-6-diamidino-2-phenylindole (DAPI) staining. After incubation, cells were collected and washed with 1× PBS twice and fixed in 4% paraformaldehyde for 1 h. Finally, washed with PBS and stained with DAPI (0.1 mg/mL) for 5 min at room temperature in the dark. Removed the excess DAPI solution and washed with PBS twice. The stained cells were examined under a fluorescent microscope. Chromatin fluorescence was observed under a UV-light microscope, and apoptotic cells were morphologically defined by cytoplasmic and nuclear shrinkage and chromatin condensation.[32]

5.2.4. DNA fragmentation assay

DNA fragmentation was determined by agarose gel electrophoresis. Cells were treated with various concentrations of compound **9** (1.0, 5.0, and 10.0 μM) for 48 h and then washed twice with PBS. Total DNA was isolated using a commercial kit (Genomic DNA Purification Kit, Fermentas Life Sciences). DNA agarose electrophoresis was executed at 100 V on a 2.0% agarose gel in 1× TAE buffer (40 mmol/L of Tris, 2 mmol/L of EDTA, and 20 mmol/L of acetic acid). DNA ladder marker (0.2–14.0 kb; GeneMark) was added to gel as a reference for the analysis of internucleosomal DNA fragmentation. The gel was stained with ethidium bromide (20 μg/mL) and photographed under ultraviolet illumination.

5.2.5. Immunoblot analysis

After treatment of compound **9** for 48 h, cells were collected and washed twice with cold PBS and then lysed in lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM EGTA, 1 mM $NaVO_3$, 10 mM NaF, 1 mM DTT, 1 mM PMSF, 25 μg/mL aprotinin, and 25 μg/mL leupeptin) and kept on ice for 30 min. The lysates were centrifuged at 12,000g at 4 °C for 20 min and the supernatants were stored at −70 °C. The protein concentration was determined by the Bradford method. 20 μg protein were separated by 10–15% SDS–PAGE and transferred onto a PVDF membrane using a glycine transfer buffer (192 mM glycine, 25 mM Tris–HCl, pH 8.8, and 20% methanol [v/v]). After blocking with 5% non-fat dried milk, the membrane was incubated for 2 h with primary antibodies, followed by 30 min with secondary antibodies in milk containing Tris-buffered saline (TBS) and 0.5% Tween. Anti-caspase-3 and anti-human-PARP antibodies were used at a 1:1000 dilution as the primary antibodies, while horseradish peroxidase-conjugated horse anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used at a 1:5000 dilution as the secondary antibody. The membrane was then exposed to X-ray film. Protein bands were detected using the enhanced chemiluminescence blotting detection system (Amersham, USA).

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