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Novel synthetic 2-amino-10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone derivatives as potent DNA-binding antiproliferative agents

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ABSTRACT

A series of novel 9(10*H*)-acridinone derivatives with terminal amino substituents at C2 position on the acridinone ring were synthesized and studied for their antiproliferative activity and underlying mechanisms. These compounds demonstrated promising cytotoxicity to leukemia cells CCRF-CEM, displaying IC_{50} values in the low micromolar range. Structure–activity relationships (SAR) indicated that the compound **6d** bearing a pyrrolidine substituent and **8a** with a methyl ammonium side chain displayed higher cytotoxicity to CCRF-CEM cells and also solid tumor cells A549, HepG2, and MCF7. Furthermore, the compounds **6d** and **8a** had strong binding activity to calf thymus DNA (ct DNA), as detected by UV absorption and fluorescence quenching assays, but limited inhibitory activity to human topoisomerase 1 (topo 1). Taken together, this study discovered a series of new synthetic 9(10*H*)-acridinone derivatives with potent DNA binding and anticancer activity.

1. Introduction

DNA Topoisomerases regulate topological changes between two DNA strands during transcription and replication and thus are important and ubiquitous enzymes required for cell growth and proliferation.[1,2] Tumor-specific up-regulation of the topoisomerases has made them a promising target for the development of chemical intervention agents.[3,4] Topo 1 is one of topoisomerases that can break and reseal a single DNA strand, and topo 1 inhibitors, such as camptothecin and topotecan, can effectively inhibit tumor cell growth.[5–8] Design and development of novel, topo 1 targeted small molecules holds the promising of effective cancer interventions.[6,9–11]

Acridine and acridinone derivatives show potent antitumor activity in vitro/vivo, some of which act as DNA intercalators and topoisomerase inhibitors.[12–18] Previously, we have synthesized a series of structurally related 10-benzyl-9(10*H*)-acridinone derivatives in high yields and tested for their in vitro antitumor activities, among which the compound 10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone (**I**, Table 1) was found to be the most active against CCRF-CEM cell growth.[19] In order to improve their cytotoxicity to cancer cells, two series of 10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone with terminal amino substituents at C2 position on the acridinone ring were developed as potent calf thymus DNA binders and topoisomerase inhibitors. We reasoned that acridinones are aza-analogs of anthrones[20,21] or xanthoneshas,[22] which have a semiplanar structure with the ability to bind DNA by intercalation;[17,23–27] and the flexible basic side chain introduced to the semiplanar chromophore has been in general shown to improve DNA targeting and binding potential through simple electrostatic complementarity to negatively charged DNA.[28] Consequently, the acridinones with 3,5-dimethoxy benzyl and terminal amino substituents may be potent DNA intercalators, which would distort DNA helix and affect the catalytic activity of topoisomerase.[29] In the present paper we describe the synthesis, structure–activity relationships and the interactions with calf thymus DNA and topo 1 of these acridinone derivatives.

2. Results and discussion

2.1. Chemistry

Synthesis of the 2-aminopropionamido-10-(3,5-dimethoxy)-benzyl-9(10*H*)-acridinone derivatives were accomplished as described in Scheme 1. Starting from the commercially available material acridinone **1**, 2-nitro-9(10*H*)-acridinone was obtained in high yields by nitration reaction in the presence of nitric acid and acetic acid, which was then converted to 2-nitro-10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone, following the route described

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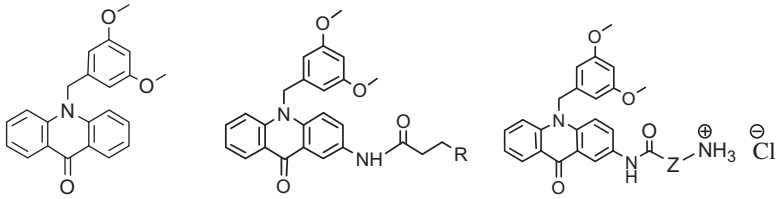
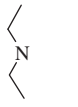
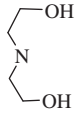
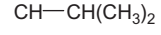
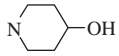
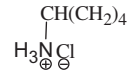
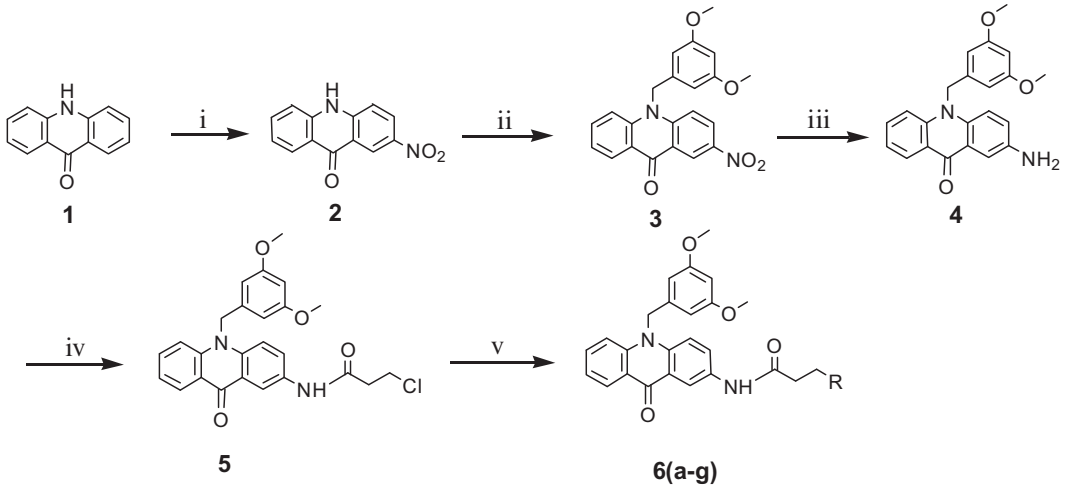
Table 1
Chemical data and antiproliferative activity against CCRF-CEM cells of compound **6a–g** and **8a–h**



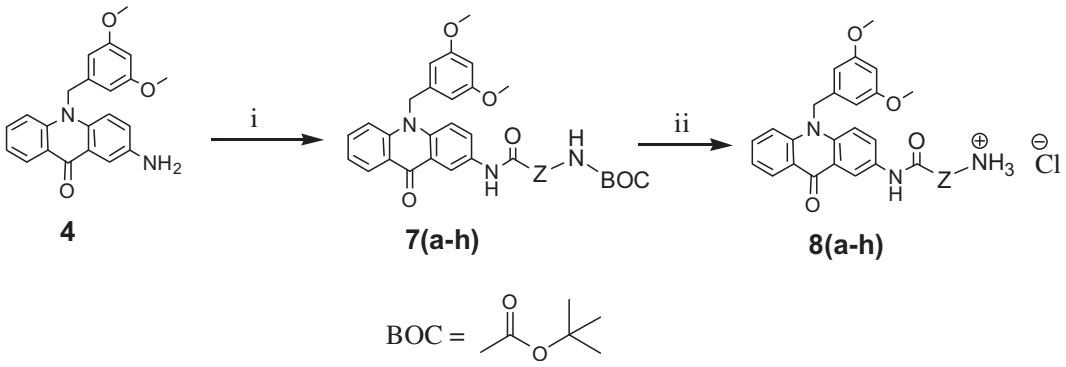
I **6a-6g** **8a-8h**

Compound	R	IC_{50} (μM)	Compound	Z	IC_{50} (μM)
I	–	0.70	**8a**	CH_2	0.39
6a		0.91	**8b**	$(CH_2)_2$	2.55
6b		0.72	**8c**	$(CH_2)_3$	0.67
6c		0.95	**8d**	$(CH_2)_4$	1.19
6d		0.28	**8e**	$(CH_2)_5$	0.75
6e		0.60	**8f**		0.61
6f		3.01	**8g**		0.81
6g		0.64	**8h**		0.75



Scheme 1. Reagents and conditions: (i) HNO_3, CH_3COOH; (ii) 3,5-dimethoxybenzyl chloride, NaH; (iii) $Na_2S{\cdot}9H_2O$, C_2H_5OH; (iv) chloropropionyl chloride, THF; (v) R_2NH, EtOH, KI, reflux.

previously.[19] Subsequent reduction to 2-amino-10-(3,5-dimethoxy)-benzyl-9(10*H*)-acridinone **4** was tried with different methods. We have recently used zinc dust as a reduction reagent in acetic acid and successfully reduced the nitro resveratrol derivative to a corresponding amino resveratrol product.[30] We attempted the same reduction system first, but this reaction led to the formation of many byproducts, with only traces of the desired amino substituted product 2-amino-10-(3,5-dimethoxy)-benzyl-



Scheme 2. Reagents and conditions: (i) HOBt, DIC; (ii) 3 M HCl.

9(10*H*)-acridinone **4** synthesized. It has been reported that $SnCl_2{\cdot}2H_2O$ is a moderate reduction agent for the reduction of nitro acridinone derivatives to the corresponding amino products under acidic conditions.[31] Resulting data showed that the yields were very low and a tedious separation was needed. The low yields may be due to the carbonyl group, which could be readily involved in the reduction reaction under acidic conditions. Thereby, we conducted this reduction under alkaline conditions. In aqueous Na_2S solution, the nitro acridinone derivatives were almost completely converted to the expected amino substituents with no remainders of the starting materials as detected by TLC analysis. This synthetic method is suitable for larger scale preparation of this derivative. The intermediate **5** was obtained in essentially quantitative yield by acylation of **4** with 3-chloropropionyl chloride at room temperature, which was then reacted with the appropriate amine in the presence of KI and gave the desired analogues **6** in good yields.

The preparation of derivatives **8a–8h** was carried out via the routes illustrated in Scheme 2, in a short synthetic procedure starting from **4**. Initial acylation of **4** with appropriate Boc-amino acid in dry tetrahydrofuran in the presence of anhydrous 1-hydroxybenzotriazole (HOBt) and *N*,*N*′-diisopropylcarbodiimide (DIC) provided the intermediate **7**. The removal of the *tert*-butoxycarbonyl (BOC) group was readily accomplished by exposing to 3 M HCl in ethyl acetate, producing **8** hydrochlorides in good yields.

The purity of new compounds synthesized was verified by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrum. The structure features and synthetic yields of these products are listed in Table 1.

2.2. In vitro cell growth inhibition assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT) cell proliferation assay has been widely accepted as a quick and reliable method to measure the cell proliferation rate. In our previous studies, we reported that 10-benzyl-9(10*H*)-acridinone derivatives could inhibit CCRF-CEM leukemia cells growth and the cytotoxic potency was highly dependent on the substitution patterns on the benzene ring.[19] In the present study, two series of 10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone derivatives with a range of side chain substituents at C2 position on the acridinone ring were evaluated for antiproliferative activity against CCRF-CEM leukemia cells using the MTT assay to establish structure–activity relationships for subsequent rational drug development.

The 2-aminopropionamido-10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone derivatives (**6a**–**6g**) described here exhibited promising cytotoxicity with IC_{50} values at a low micromolar range against CCRF-CEM cells, some of which displayed better antiproliferative activity than the parental compound 10-(3, 5-dimethoxy)benzyl-9(10*H*)-acridinone. Compound **6d** containing a pyrrolidine substituent was considered as the most potent agent with an IC_{50} at 0.28 μM, while the morpholine derivative (**6f**) was the most inactive. The piperidine (**6e**) and functionalized piperidine (**6g**) derivatives were slightly more cytotoxic than the parental compound, but approximately twofold less active than the corresponding pyrrolidine derivatives. In addition, compounds bearing extended substituents with a terminal acyclic amino moiety (**6a**–**6c**) were also evaluated and showed similar or slightly less cytotoxic activity compared to the parental compound. Compound **6b** with a diethylamine substituent demonstrated more cytotoxic than compound **6a** (a dimethylamine substituent) and **6c** (a diethanolamine substituent). This data indicated that the cytotoxicity of this series of 10-(3,5-dialkyloxy)benzyl-9(10*H*)-acridinone derivatives is related to the size and types of the terminal amino moiety.[32]

Furthermore, the length of alkyl chains on the amino acid moiety significantly influenced on the cytotoxicity of these compounds listed in Table 1. Analogues **8** with an odd number of CH_2 groups showed a higher antiproliferative activity than those with an even number of CH_2. In the series of **8a**–**8e**, compounds with the odd CH_2 groups resulted in decreased activity as the chain length increased, while those with even number of CH_2 groups led to an increased potency. Moreover, as shown in Table 1, by changing the methylene group (**8a**) to methyl methylene (**8f**) or isopropyl methylene (**8g**), compounds showed decreased inhibitory effect. This data clearly demonstrates that the cytotoxicity of compounds decreases with the increased steric effect. Compound **8e**, when another ammonium chloride was introduced (**8h**), showed similar antitumor efficiency, suggesting that the a CH_2 moiety can be substituted with ammonium group without any loss in potency.

To further investigate the antiproliferative potential, we measured the antiproliferative activity of the active compounds **6d** and **8a** against three different solid tumor cell lines. As shown in Table 2, both compounds possess potent activities against the growth of A549 (human lung adenocarcinoma cells), HepG2 (human hepatocellular carcinoma cells), and MCF7 (human breast adenocarcinoma cells). However, the human leukemic CCRF-CEM cells seem more sensitive to these new compounds than the solid tumor cells.

Table 2
Antiproliferative activity against solid tumor cells of compounds **6d** and **8a**

Compound	IC_{50} (μM)		
	A549	HepG2	MCF-7
6d	25.9	4.0	5.7
8a	3.4	6.5	11.7

2.3. UV–vis spectral absorbance

Absorption spectroscopy is one of the most convenient tools to investigate the interaction between drugs and DNA. In general, the binding of a drug to DNA results in hypochromism and red-shift in the drug absorption band due to the intercalation mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA.[15,33] In order to address the DNA binding ability of this new series of 2-amino-10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone derivatives, the UV–vis absorption spectra titration of the compounds with cattle thymus (ct) DNA in Tris–HCl buffer was recorded. Two representative spectra of **6d** and **8a** in the absence and presence of ct DNA were presented in Figure 1. Compound **8a** presented here showed remarkable decrease of the peak intensity in the presence of DNA, while DNA did not absorb light in this region. In addition to the hypochromic phenomenon, a small bathochromic shift of the chromophore was also observable in the spectra. In compound **6d**, the changes in the absorption spectrum in the presence of DNA were quite smaller compared with those of compound **8a**. This hypochromic and bathochromic effect indicated that these two compounds may bind to DNA and form stable complexes by intercalation mode through the stacking of the acridinone chromophore and DNA bases.[15,34]

In order to quantitatively assess the binding capability of the compounds with DNA, the values of equilibrium binding constant K_b was determined using Eq. (1).

$$[\mathrm{DNA}]/(\epsilon_a - \epsilon_f) = [\mathrm{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f) \quad (1)$$

where [DNA], ε_a, ε_f and ε_b are the DNA concentrations in base pairs, the extinction coefficient observed for the compound absorption band at a given DNA concentration, the extinction coefficient of the complex free in solution and the extinction coefficient of the complex when fully bound to DNA, respectively. These binding constants were obtained as the ratio of slope to the intercept (Fig. 1). The binding constant of **8a** (K_b, $1.5 \times 10^5\ M^{-1}$) was higher than that of **6d** (K_b, $5.0 \times 10^4\ M^{-1}$), which indicated that the binding of **8a** with ct DNA was stronger than that of **6d**.

2.4. Fluorescence emission spectra

In order to confirm the interaction of this new series of compounds with ct DNA, the binding properties were also investigated by the fluorescence spectroscopy. Approximately, ct DNA (about 20 or 40 μM at final) was added to solutions of the synthesized acridinone derivatives (about 20 μM at final) in 50 mM sodium chloride/10 mM Tris–HCl at pH 7.2. Fluorescence emission was detected after equilibrium was reached to an optimum level. As shown in Figure 2, upon binding to DNA the fluorescence from compound **8a** was efficiently quenched by the DNA bases, but the decrease in fluorescence emission of compound **6d** upon binding to DNA was much smaller. This emission-quenching phenomenon also reflected the interaction between the compounds and ct DNA, consistent with the results from electronic absorption spectroscopy.

2.5. DNA topo 1 inhibition assay

Some of Acridine and acridinone derivatives demonstrate topoisomerase 1 inhibitory activity.[16,35] To test whether these novel synthetic compounds possess activity to inhibit topoisomerase 1 activity, the DNA relaxation assay induced by topoisomerase 1 was performed. As shown in Figure 3, compounds **8a** showed moderate topo 1 inhibitory activity at 50 μM whereas others had very low or undetectable activities. However, most of the compounds displayed certain topo 1 inhibitory activity at 250 μM, a concentration far higher than the IC_{50}. This data suggest that these novel compounds exert the antiproliferative activity unlikely through topoisomerase 1 inhibition, but they may be potent lead compounds for the development of topoisomerase 1 inhibitors.

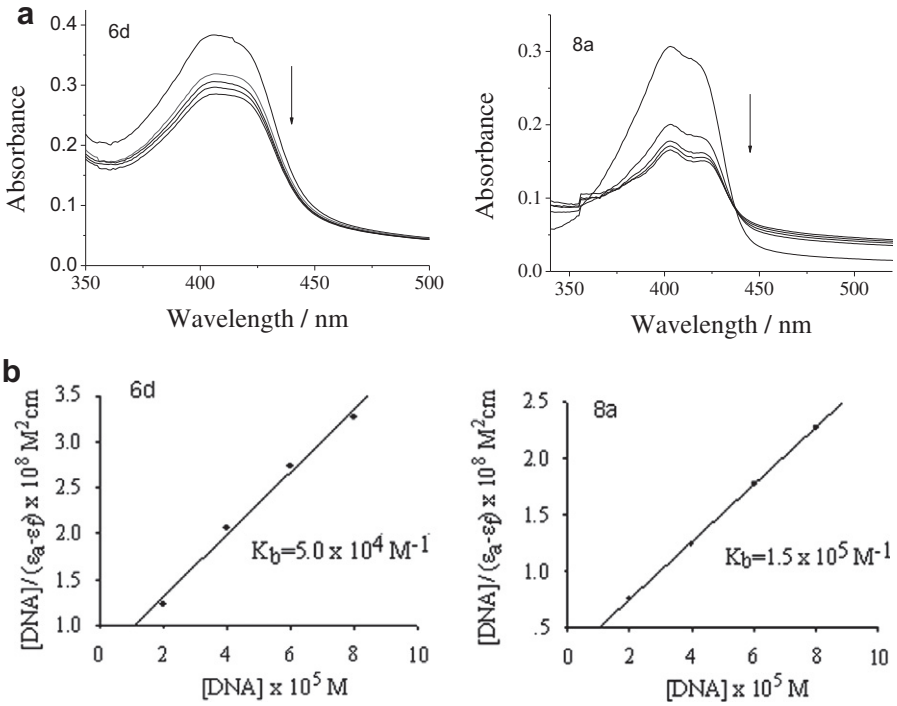


Figure 1. (a) UV–vis absorption spectra of acridinone derivatives **6d** (40 μM) and **8a** (40 μM) in the presence of increasing amounts of ct DNA; [DNA] = 0, 20, 40, 60, 80 μM. DNA titration of the compounds were performed in 10 mM Tris–Hcl buffer containing 50 mM NaCl at pH 7.2. Vertical arrows indicate the increase of the absorbance change upon increasing DNA concentration. (b) The plot of $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ as a function of DNA concentration as determined from the absorption spectral data.

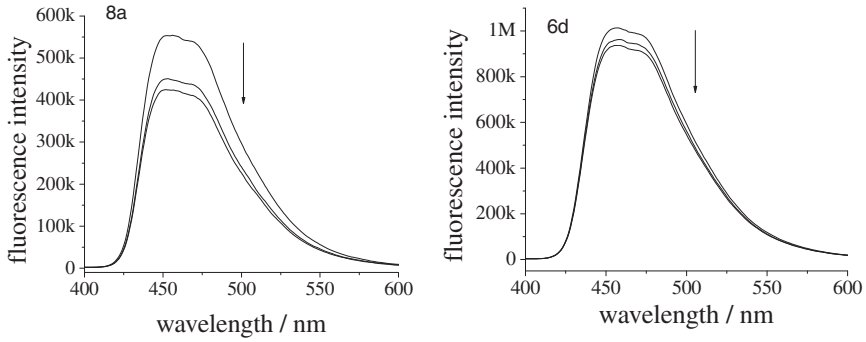


Figure 2. Fluorescence emission spectra of acridinone derivatives **6d** (20 μM) and **8a** (20 μM) in the absence or presence of ct DNA ([DNA] = 0, 20, 40 μM) after excitation at λ_{ex} = 380 nm. Vertical arrows indicate the increase of the fluorescence emission change upon increasing DNA concentration.

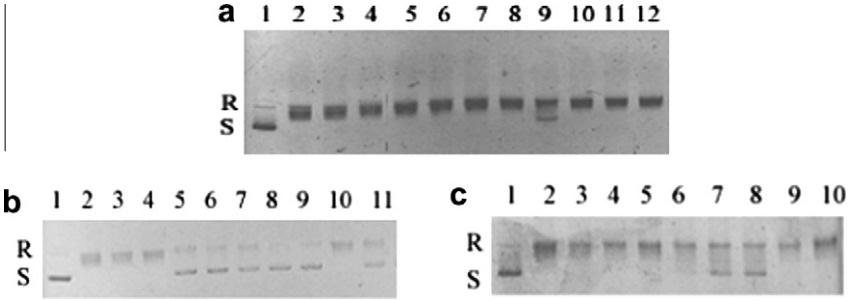


Figure 3. Effect of the compounds on the relaxation of plasmid DNA by human topoisomerase 1. (a) Lane 1, DNA pBR322, Lane 2, topo 1+ DNA pBR322, Lane 3, topo 1+ DNA pBR322+DMSO, Lanes 4–6, DNA pBR322 relaxation by topo 1 and **6d** at concentrations of 10, 20, and 50 μM, respectively, Lanes 7–9, DNA pBR322 relaxation by topo 1 and **8a** at concentrations of 10, 20, and 50 μM, respectively, Lanes 10–12, DNA pBR322 relaxation by topo 1 and **I** at concentrations of 10, 20, and 50 μM, respectively. (b) Lane 1, DNA pBR322, Lane 2, topo 1+ DNA pBR322, Lane 3, topo 1+ DNA pBR322+DMSO, Lanes 4–11, DNA pBR322 relaxation by topo 1 and **I**, **8b**, **8c**, **8d**, **8e**, **8h**, **8g**, and **8f** at concentrations of 250 μM, respectively. (c) Lane 1, DNA pBR322, Lane 2, topo 1+ DNA pBR322, Lane 3, topo 1+ DNA pBR322+DMSO, Lanes 4–10, DNA pBR322 relaxation by topo 1 and **6e**, **6f**, **6c**, **6a**, **6b**, **6d**, and **6g** at concentrations of 250 μM, respectively.

3. Conclusion

A series of new 9(10*H*)-acridinone derivatives were synthesized and demonstrated promising cytotoxicity against CCRF-CEM cells. The most active compounds **6d** and **8a** also exhibited activity to human solid tumor cells (A549, HepG2, and MCF7) at a less extent compared to CCRF-CEM. Structure–activity relationship studies revealed that the terminal amino substituents at C2 position on the acridinone ring played an important role in cellular growth inhibition. The hit compounds **6d** and **8a** could bind to DNA and form stable complexes, being potential genotoxic agents for cancer therapy. The topo 1 inhibitory activity of these compounds was limited, but they may be potential lead compounds.

4. Experimental procedures

4.1. Synthesis and characterization

See Supplementary data for general methods and the preparation of compounds **2**–**5**.

4.1.1. General aminolysis procedure of compound 6

To a stirred refluxing suspension of **5** (225 mg, 0.5 mmol) and KI (83 mg, 0.5 mmol) in EtOH (5 mL) was added dropwise the corresponding secondary amines (3.0 mL). The mixture was stirred at reflux until TLC indicated completion of reaction. After cooling to room temperature, water was added with rapid stirring under ice-water bath. Yellow crystals (solids) were obtained after filtration. The products were purified by recrystallization from ethanol.

4.1.1.1. 2-[(Dimethylamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6a). Yield 191 mg, 83%; yellow crystals; mp 260–261 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.79 (s, 6H), 2.97 (t, ^3J = 7.3 Hz, 2H), 3.40 (t, 2H, ^3J = 7.3 Hz), 3.67 (s, 3H), 5.72 (s, 2H), 6.29 (m, 2H), 6.42 (br s, 1H), 7.33 (m, 1H), 7.61–7.66 (m, 2H), 7.76 (m, 1H), 7.98 (m, 1H), 8.37 (m, 1H), 8.71 (br s, 1H), 10.72 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 31.4, 42.8, 49.5, 53.1, 55.7, 98.8, 104.6, 116.1, 116.6, 117.4, 121.7, 121.9, 122.2, 127.1, 127.2, 133.7, 134.7, 138.9, 139.4, 142.3, 161.4, 168.5, 176.9; IR (KBr, cm^{-1}) v: 686, 763, 814, 1050, 1069, 1161, 1201, 1276, 1325, 1486, 1544, 1593, 1686, 2472, 2573, 2960, 3071, 3102, 3211, 3241, 3436; HRMS calcd for $C_{27}H_{30}N_3O_4$ [M+H]$^+$ 460.2236, found 460.2237.

4.1.1.2. 2-[(Diethylamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6b). Yield 200 mg, 82%; yellow crystals; mp 192–193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 0.99 (t, ^3J = 7.1 Hz, 6H), 2.45 (t, ^3J = 7.0 Hz, 2H), 2.51 (q, 4H, ^3J = 7.1 Hz), 2.77 (t, ^3J = 7.0 Hz, 2H), 3.67 (s, 3H), 5.71 (s, 2H), 6.29 (m, 2H), 6.42 (br s, 1H), 7.33 (m, 1H), 7.60–7.64 (m, 2H), 7.75 (m, 1H), 7.98 (m, 1H), 8.37 (m, 1H), 8.61 (br s, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 7.6, 29.9, 41.9, 44.1, 44.8, 50.9, 94.1, 99.8, 110.9, 111.8, 112.6, 116.9, 117.1, 117.6, 122.3, 122.5, 129.4, 129.9, 133.9, 134.7, 137.6, 156.7, 166.3, 172.2; HRMS calcd for $C_{29}H_{34}N_3O_4$ [M+H]$^+$ 488.2549, found 488.2535.

4.1.1.3. 2-[(Diethanolamino)propionamido]-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6c). Yield 221 mg, 86%; yellow solids; mp 132–134 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ:

2.51 (m, 2H), 2.59 (m, 4H), 2.85 (t, 2H, ^3J = 6.3 Hz), 3.47 (m, 4H), 3.66 (s, 6H), 4.43 (t, 2H, ^3J = 6.3 Hz), 5.71 (s, 2H), 6.28 (br s, 2H), 6.42 (br s, 1H), 7.31 (m, 1H), 7.61–7.64 (m, 2H), 7.76 (m, 1H), 7.96 (m, 1H), 8.37 (m, 1H), 8.65 (br s, 1H), 10.40 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 35.0, 49.5, 51.2, 55.6, 56.7, 59.7, 98.8, 104.6, 115.9, 116.5, 117.2, 121.6, 121.9, 122.3, 127.2, 134.1, 134.6, 138.7, 139.4, 142.3, 161.5, 171.1, 176.9; HRMS calcd for $C_{29}H_{34}N_3O_6$ $[M+H]^+$ 520.2448, found 520.2427.

4.1.1.4. 2-(Pyrrolidinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6d). Yield 218 mg, 90%; yellow crystals; mp 236–238 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.68 (br s, 2H), 2.48–2.54 (m, 6H), 2.74 (t, 2H, ^3J = 6.8 Hz), 3.67 (s, 6H), 5.71 (s, 2H), 6.29 (br s, 2H), 6.43 (br s, 1H), 7.33 (m, 1H), 7.60–7.65 (m, 2H), 7.75 (m, 1H), 7.98 (m, 1H), 8.38 (m, 1H), 8.65 (m, 1H), 10.33 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.7, 36.7, 49.6, 52.1, 54.0, 55.6, 98.8, 104.6, 115.7, 116.5, 117.3, 121.6, 121.8, 122.3, 127.0, 127.2, 134.2, 134.6, 138.7, 139.4, 142.3, 161.4, 170.7, 176.9; HRMS calcd for $C_{29}H_{32}N_3O_4$ $[M+H]^+$ 486.2393, found 486.2386.

4.1.1.5. 2-(Piperidinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6e). Yield 220 mg, 88%; yellow crystals; mp 226–227 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.39 (m, 2H), 1.50–1.51 (m, 4H), 2.40–2.51 (m, 6H), 2.63 (m, 2H), 3.66 (s, 6H), 5.71 (s, 2H), 6.28 (m, 2H), 6.42 (br s, 1H), 7.33 (m, 1H), 7.59–7.65 (m, 2H), 7.75 (m, 1H), 7.96 (m, 1H), 8.37 (m, 1H), 8.61 (m, 1H), 10.40 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 19.1, 24.5, 26.1, 34.5, 49.5, 54.2, 54.9, 55.6, 56.6, 98.8, 104.6, 115.7, 116.5, 117.4, 121.6, 121.9, 122.3, 127.0, 127.2, 134.1, 134.7, 138.7, 139.4, 142.3, 161.4, 170.9, 176.9; HRMS calcd for $C_{30}H_{34}N_3O_4$ $[M+H]^+$ 500.2549, found 500.2527.

4.1.1.6. 2-(Morpholinopropionamido)-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone (6f). Yield 216 mg, 86%; yellow crystals; mp 199–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.41 (m, 4H), 2.65 (m, 2H), 3.57 (m, 4H), 3.66 (s, 6H), 5.70 (s, 2H), 6.27 (m, 2H), 6.41 (br s, 1H), 7.32 (m, 1H), 7.62–7.64 (m, 2H), 7.75 (m, 1H), 7.96 (m, 1H), 8.37 (m, 1H), 8.61 (m, 1H), 10.30 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.4, 49.5, 53.5, 54.7, 55.6, 66.7, 98.8, 104.5, 115.7, 116.5, 117.3, 121.6, 121.8, 122.2, 127.0, 127.2, 134.1, 134.6, 138.7, 139.4, 142.3, 161.4, 170.6, 176.8; HRMS calcd for $C_{29}H_{32}N_3O_5$ $[M+H]^+$ 502.2342, found 502.2334.

4.1.1.7. 2-[(4-Hydroxyl-piperidino)propionamido]-10-(3,5-dimethoxybenzyl) 9,10-dihydro-acridinone (6g). Yield 227 mg, 88%; yellow crystals; mp 222–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.40 (m, 2H), 1.73 (m, 2H), 2.09 (m, 2H), 2.49 (m, 2H), 2.64 (m, 2H), 2.77 (m, 2H), 3.46 (m, 1H), 3.67 (s, 6H), 4.58 (br s, 1H), 5.71 (s, 2H), 6.28 (br s, 2H), 6.42 (br s, 1H), 7.33 (m, 1H), 7.59–7.65 (m, 2H), 7.75 (m, 1H), 7.96 (m, 1H), 8.38 (m, 1H), 8.63 (m, 1H), 10.39 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 34.9, 35.0, 49.6, 51.2, 54.3, 55.6, 66.8, 98.8, 104.6, 115.7, 116.5, 117.3, 121.6, 121.9, 122.3, 127.0, 127.2, 134.1, 134.6, 138.7, 139.4, 142.3, 161.5, 170.8, 176.9; HRMS calcd for $C_{30}H_{34}N_3O_5$ $[M+H]^+$ 516.2499, found 516.2488.

4.1.2. General preparation of compound 8

To a solution of Boc-amino acid in dry THF (10 mL) was added HOBt (81 mg, 0.6 mmol), DIC (76 mg, 0.6 mmol) and 2-amino-10-(3,5-dimethoxybenzyl)-9,10-dihydro-acridinone 4 (180 mg, 0.5 mmol). The reaction suspension was stirred under nitrogen overnight at room temperature. The volatile parts were removed under reduced pressure and compound **7** was obtained by column chromatography. Compound **7** was suspended and stirred in ethyl acetate (15 mL), and then hydrochloride (3 M, 8 mL) was added dropwise. The suspension was stirred at room temperature until TLC indicated completion of reaction. The volatile parts were removed under reduced pressure and water was added with rapid stirring under ice-water bath. Yellow solids were obtained after filtration. The products were purified by recrystallization from ethanol and ethyl acetate.

4.1.2.1. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-2-amino-acetamide hydrochloride (8a). Yield 136 mg, 60%; yellow solids; mp 297–299 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.62 (s, 6H), 3.83 (m, 2H), 5.72 (s, 2H), 6.28 (m, 2H), 6.42 (m, 1H), 7.36 (m, 1H), 7.63 (m, 1H), 7.68 (m, 1H), 7.78 (m, 1H), 7.94 (m, 1H), 8.23 (br s, 3H), 8.38 (m, 1H), 8.68 (m, 1H), 10.82 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 41.5, 49.6, 55.6, 98.9, 104.6, 116.3, 116.5, 117.5, 121.7, 122.0, 122.3, 127.0, 127.1, 132.9, 134.8, 139.2, 139.3, 142.4, 161.5, 165.2, 176.9; IR (KBr, cm^{-1}) ν: 687, 756, 814, 1070, 1164, 1208, 1276, 1353, 1506, 1548, 1588, 1696, 2343, 2842, 2963, 3005, 3105, 3229, 3320; HRMS calcd for $C_{24}H_{24}N_3O_4Cl$ $[M-Cl]^+$ 418.1767, found 418.1758.

4.1.2.2. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-3-amino-propionamide hydrochloride (8b). Yield 145 mg, 62%; yellow solids; mp 229–231 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.80 (t, 2H, ^3J = 6.8 Hz), 3.12 (m, 2H), 3.66 (s, 6H), 5.71 (s, 2H), 6.28 (m, 2H), 6.42 (m, 1H), 7.33 (m, 1H), 7.60–7.66 (m, 2H), 7.76 (m, 1H), 7.97 (m, 1H), 8.04 (br s, 3H), 8.37 (m, 1H), 8.71 (m, 1H), 10.56 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 33.7, 35.5, 49.5, 55.6, 98.8, 104.6, 116.1, 116.6, 117.3, 121.6, 121.9, 122.2, 127.1, 127.2, 133.8, 134.7, 138.8, 139.4, 142.3, 161.4, 168.9, 176.9; HRMS calcd for $C_{25}H_{26}N_3O_4Cl$ $[M-Cl]^+$ 432.1923, found 432.1913.

4.1.2.3. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-4-amino-butyramide hydrochloride (8c). Yield 145 mg, 60%; yellow solids; mp 270–271 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (m, 2H), 2.48 (m, 2H, ^3J = 6.8 Hz), 2.87 (m, 2H), 3.66 (s, 6H), 5.72 (s, 2H), 6.27 (m, 2H), 6.42 (m, 1H), 7.33 (m, 1H), 7.60–7.66 (m, 2H), 7.76 (m, 1H), 7.86 (br s, 3H), 7.96 (m, 1H), 8.37 (m, 1H), 8.66 (m, 1H), 10.34 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 23.5, 33.4, 38.9, 49.5, 55.6, 98.7, 104.6, 115.9, 116.6, 117.3, 121.6, 121.9, 122.2, 127.2, 134.0, 134.7, 138.8, 139.4, 142.3, 161.4, 170.9, 176.9; HRMS calcd for $C_{26}H_{28}N_3O_4$ $[M-Cl]^+$ 446.2080, found 446.2079.

4.1.2.4. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-5-amino-pentylamide hydrochloride (8d). Yield 161 mg, 65%; yellow solids; mp 242–243 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.61–1.70 (m, 4H), 2.40 (m, 2H, ^3J = 6.8 Hz), 2.82 (m, 2H), 3.66 (s, 6H), 5.71 (s, 2H), 6.28 (m, 2H), 6.42 (m, 1H), 7.32 (m, 1H), 7.60–7.64 (m, 2H), 7.75 (m, 1H), 7.91 (br s, 3H), 7.99 (m, 1H), 8.36 (m, 1H), 8.67 (m, 1H), 10.31 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 22.5, 26.9, 36.0, 39.2, 49.4, 55.6, 98.7, 104.6, 116.0, 116.4, 117.2, 121.5, 122.0, 122.1, 127.2, 127.3, 133.9, 134.8, 138.7, 139.2, 142.2, 161.4, 171.7, 177.1; HRMS calcd for $C_{27}H_{30}N_3O_4$ $[M-Cl]^+$ 460.2236, found 460.2238.

4.1.2.5. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-6-amino-hexylamide hydrochloride (8e). Yield 178 mg, 70%; yellow solids; mp 195–197 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.38 (m, 2H), 1.60–1.64 (m, 4H), 2.37 (m, 2H), 2.79 (m, 2H), 3.66 (s, 6H), 5.71 (s, 2H), 6.28 (m, 2H), 6.42 (m, 1H), 7.33 (m, 1H), 7.60–7.65 (m, 2H), 7.75 (m, 1H), 7.91 (br s, 3H), 7.99 (m, 1H), 8.37 (m, 1H), 8.67 (m, 1H), 10.28 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 25.1, 26.0, 27.2, 36.5, 39.2, 49.5, 55.7, 98.9, 104.6, 115.9, 116.4, 117.1, 121.6, 121.8, 122.3, 127.2, 134.2, 134.5, 138.7, 139.4, 142.3, 161.4, 171.6, 176.9; HRMS calcd for $C_{28}H_{32}N_3O_4$ $[M-Cl]^+$ 474.2393, found 474.2393.

4.1.2.6. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-2-methyl-2-aminoacetamide hydrochloride (8f). Yield 147 mg, 63%; yellow solids; mp 209–211 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.53 (d, 3H, ^3J = 6.9 Hz), 3.67 (s, 6H), 4.15 (q, 1H, ^3J = 6.9 Hz), 5.74 (s, 2H), 6.29 (m, 2H), 6.43 (m, 1H), 7.35 (m, 1H), 7.62–7.80 (m, 3H), 8.02 (m, 1H), 8.38 (m, 1H), 8.47 (br s, 3H), 8.74 (m, 1H), 11.14 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 17.7, 49.5. 49.6, 55.7, 98.8, 104.6, 116.4, 116.6, 117.6, 121.7, 122.0, 122.2, 127.1, 127.2, 133.1, 134.8, 139.2, 139.3, 142.3, 161.4, 168.8, 176.8; HRMS calcd for $C_{25}H_{26}N_3O_4$ $[M-Cl]^+$ 432.1923, found 432.1917.

4.1.2.7. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-2-isopropyl-2-aminoacetamide hydrochloride (8g). Yield 158 mg, 64%; yellow solids; mp 197–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.04 (d, 3H, ^3J = 6.8 Hz), 2.27 (m, 1H), 3.67 (s, 6H), 3.94 (m, 1H), 5.74 (s, 2H), 6.30 (m, 2H), 6.43 (m, 1H), 7.35 (m, 1H), 7.62–7.80 (m, 3H), 8.03 (m, 1H), 8.39 (m, 1H), 8.47 (br s, 3H), 8.75 (m, 1H), 11.20 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 18.5, 19.0, 30.5, 49.6, 55.7, 58.6, 98.8, 104.6, 116.5, 116.6, 117.6, 121.7, 122.1, 122.2, 127.2, 132.9, 134.8, 139.3, 139.3, 142.4, 161.5, 167.4, 176.9; HRMS calcd for $C_{27}H_{30}N_3O_4$ $[M-Cl]^+$ 460.2236, found 460.2227.

4.1.2.8. *N*-(10-(3,5-Dimethoxybenzyl)-9,10-dihydro-9-oxoacridin-2-yl)-2,6-diaminohexylamide dihydrochloride (8h). Yield 158 mg, 60%; yellow solids; mp 193–195 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.47 (m, 2H), 1.63 (m, 2H), 1.92 (m, 2H), 2.79 (m, 2H), 3.67 (s, 6H), 4.11 (m, 1H), 5.74 (s, 2H), 6.28 (m, 2H), 6.43 (m, 1H), 7.35 (m, 1H), 7.62–7.77 (m, 3H), 8.02–8.03 (m, 4H), 8.38 (m, 1H), 8.53 (br s, 3H), 8.76 (m, 1H), 11.29 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 21.7, 26.8, 31.0, 38.7, 49.5, 53.1, 55.7, 98.8, 104.6, 116.5, 116.6, 117.6, 121.7, 122.1, 122.2, 127.2, 127.3, 133.0, 134.8, 139.2, 139.3, 142.3, 161.4, 167.9, 176.9; MS calcd for $C_{28}H_{33}N_4O_4$ $[M-HCl_2]^+$ 489.3, found 489.3.

4.2. Bioassay

4.2.1. Cell culture

CCRF-CEM leukemia cells were cultured in RPIM 1640 medium (Cibco), containing 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc.), 100 units/mL penicillin, and 100 μg/mL streptomycin in a 5% CO_2-humidified atmosphere at 37 °C. Other cell lines including A549, HepG2 and MCF7 were cultured in DMEM, with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin in humidified air at 37 °C with 5% CO_2.

4.2.2. Cell growth inhibition

The cells were suspended at a concentration of 2×10^5 cells/mL and seeded in 96-well microtiter plates. Various concentrations of compound dissolved in DMSO were added to each well in quintuplet followed by incubation for the 48 h. After treatment, the cells were incubated with 10 μL of MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide from Sigma) solution (5 mg/mL) for 4 h. The formazan precipitate was dissolved in 100 μL DMSO and the absorbance at 490 nm was measured by a Benchmark microplate reader (Molecular Devices Corporation). IC_{50} values are the concentration at which cell growth was inhibited by 50%.

4.2.3. DNA topo 1 inhibition assay

The mixture of 100 ng of plasmid DNA pBR322 (from Takara) and 1.0 units of recombinant human DNA topoisomerase I (from Takara) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 0.01% bovine serum albumin, 2 mM spermidine, 5 mM dithiothreitol). DNA samples were then electrophoresed on a 1% agarose gel at 100 V for 25 min with a running buffer of TAE. Gels were visualized by ethidium bromide staining under ultraviolet light.

4.3. Biophysical evaluation

4.3.1. Materials

The measurements involving the interactions of the compounds with CT DNA were carried out in Ultra-pure MilliQ water (18.2 mΩ) buffer containing 10 mM Tris and 50 mM NaCl, and adjusted to pH 7.2 with hydrochloric acid. UV–vis spectrometer was employed to check a solution of CT DNA purity (A260:A280 = 1.87) and the concentration of DNA per mole phosphate was determined optically using a molar extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm in the buffer.

4.3.2. Absorption spectra

UV–vis absorption spectra were all recorded on a computer-controlled Beckman Coulter DU 800 spectrophotometer by using a quartz cell having 1.0 cm pathway. Two microliters of 2 mM tested compounds DMSO solution were transferred to the quartz cell with 50 μL above buffer solution, and then a known volume (0–4) μL of 2 mM ct DNA was also added. The above solutions were mixed and then diluted to 100 μL with the Tris–HCl buffer (pH 7.2) solution. The solution was incubated for 5 min and then tested.

4.3.3. Fluorescence emission spectra

All the emission spectra were measured on Fluorolog spectrometer. Xenon arc lamp is used as the excitation light source in the measurements of emission spectra. Twenty micrometers of the various tested compounds (diluted from 5 mM DMSO solution) were incubated in Tris–HCl buffer (pH 7.2) with 50 mM NaCl in the presence or absence of 20 or 40 μM of ct DNA. The excitation wavelength was set at 380 nm.

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Supplementary data

Supplementary data (general methods and the preparation of compounds **2**–**5**; ^{1}H NMR and ^{13}C NMR spectrum) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.058.

References and notes

1. Chhatriwala, H.; Jafri, N.; Salgia, R. *Cancer Biol. Ther.* **2006**, *5*, 1600.
2. Rothenberg, M. L. *Ann. Oncol.* **1997**, *8*, 837.
3. Rasheed, Z. A.; Rubin, E. H. *Oncogene* **2003**, *22*, 7296.
4. Cho, W. J.; Le, Q. M.; Van, H. T. M.; Lee, K. Y.; Kang, B. Y.; Lee, E. S.; Lee, S. K.; Kwon, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3531.
5. Liew, S. T.; Yang, L. X. *Curr. Pharm. Des.* **2008**, *14*, 1078.
6. Pommier, Y. *Chem. Rev.* **2009**, *109*, 2894.
7. You, Q. D.; Li, Z. Y.; Huang, C. H.; Yang, Q.; Wang, X. J.; Guo, Q. L.; Chen, X. G.; He, X. G.; Li, T. K.; Chern, J. W. *J. Med. Chem.* **2009**, *52*, 5649.
8. Hochster, H.; Liebes, L.; Buckley, M.; Sorich, J.; Fry, D.; Hamilton, A.; Wright, J.; Muggia, F. *Clin. Cancer Res.* **2001**, *7*, 3710s.
9. Teicher, B.; Kurtzberg, L.; Schmid, S.; Pommier, Y.; Lavoie, E.; Ewesuedo, R.; Roth, S.; Krumbholz, R.; Rouleau, C.; Bagley, R. *Ann. Oncol.* **2010**, *21*, 35.
10. Breslin, D.; Sato, Y.; Karki, S. B. *Eur. J. Pharm. Sci.* **2010**, *39*, 291.
11. Cao, Y. Z.; Yang, J. *Cancer Res.* **2009**, *69*, 199s.
12. Spicer, J. A.; Gamage, S. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1919.

13. Dittrich, C.; Coudert, B.; Paz-Ares, L.; Caponigro, F.; Salzberg, M.; Gamucci, T.; Paoletti, X.; Hermans, C.; Lacombe, D.; Fumoleau, P.; Canc, E. O. R. T. *Eur. J. Cancer* **2003**, *39*, 330.
14. Goodell, J. R.; Madhok, A. A.; Hiasa, H.; Ferguson, D. M. *Bioorg. Med. Chem.* **2006**, *14*, 5467.
15. David-Cordonnier, M. H.; Hildebrand, M. P.; Baldeyrou, B.; Lansiaux, A.; Keuser, C.; Benzschawel, K.; Lemster, T.; Pindur, U. *Eur. J. Med. Chem.* **2007**, *42*, 752.
16. Galvez-Peralta, M.; Hackbarth, J. S.; Flatten, K. S.; Kaufmann, S. H.; Hiasa, H.; Xing, C. G.; Ferguson, D. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4459.
17. Manfroni, G.; Paeshuyse, J.; Massari, S.; Zanoli, S.; Gatto, B.; Maga, G.; Tabarrini, O.; Cecchetti, V.; Fravolini, A.; Neyts, J. *J. Med. Chem.* **2009**, *52*, 3354.
18. Sabisz, M.; Wesierska-Gadek, J.; Skladanowski, A. *Biochem. Pharmacol.* **2010**, *79*, 1387.
19. Gao, C. M.; Jiang, Y. Y.; Tan, C. Y.; Zu, X. Y.; Liu, H. C.; Cao, D. R. *Bioorg. Med. Chem.* **2008**, *16*, 8670.
20. Cao, D. R.; Gao, C. M.; Meier, H. *Synlett* **2005**, 3166.
21. Gao, C. M.; Cao, D. R.; Xu, S. Y.; Meier, H. *J. Org. Chem.* **2006**, *71*, 3071.
22. Liu, Y.; Zou, L.; Ma, L.; Chen, W. H.; Wang, B.; Xu, Z. L. *Bioorg. Med. Chem.* **2006**, *14*, 5683.
23. Stankiewicz-Drogon, A.; Dorner, B.; Erker, T.; Boguszewska-Chachulska, A. M. *J. Med. Chem.* **2010**, *53*, 3117.
24. Qiu, B.; Guo, L. H.; Chen, Z. T.; Chi, Y. W.; Zhang, L.; Chen, G. N. *Biosens. Bioelectron.* **2009**, *24*, 1281.
25. Prasad, V. V. S. R.; Rao, J. V.; Giri, R. S.; Sathish, N. K.; Kumar, S. M. S.; Mayur, Y. C. *Chem. Biol. Interact.* **2008**, *176*, 212.
26. Chen, J. H.; Zhang, J.; Huang, L. Y.; Lin, X. H.; Chen, G. N. *Biosens. Bioelectron.* **2008**, *24*, 349.
27. Stankiewicz-Drogon, A.; Palchykovska, L. G.; Kostina, V. G.; Alexeeva, I. V.; Shved, A. D.; Boguszewska-Chachulska, A. M. *Bioorg. Med. Chem.* **2008**, *16*, 8846.
28. Antonini, I.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Menta, E.; Pescalli, N.; Stefanska, B.; Mazerski, J.; Martelli, S. *J. Med. Chem.* **1997**, *40*, 3749.
29. Via, L. D.; Magno, S. M.; Gia, O.; Marini, A. M.; Da Settimo, F.; Salerno, S.; La Motta, C.; Simorini, F.; Taliani, S.; Lavecchia, A.; Di Giovanni, C.; Brancato, G.; Barone, V.; Novellino, E. *J. Med. Chem.* **2009**, *52*, 5429.
30. Liu, H. C.; Dong, A. J.; Gao, C. M.; Tan, C. Y.; Liu, H. X.; Zu, X. Y.; Jiang, Y. Y. *Bioorg. Med. Chem.* **2008**, *16*, 10013.
31. Tabarrini, O.; Manfroni, G.; Fravolini, A.; Cecchetti, V.; Sabatini, S.; De Clercq, E.; Rozenski, J.; Canard, B.; Dutartre, H. N.; Paeshuyse, J.; Neyts, J. *J. Med. Chem.* **2006**, *49*, 2621.
32. Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins, T. C.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3253.
33. Foxon, S. P.; Phillips, T.; Gill, M. R.; Towrie, M.; Parker, A. W.; Webb, M.; Thomas, J. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 3686.
34. Zhang, L. Z.; Tang, G. Q. *J. Photochem. Photobiol., B* **2004**, *74*, 119.
35. Cho, H. J.; Jung, M. J.; Kwon, Y.; Na, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6766.