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Novel angular furoquinolinones bearing flexible chain as antitumor agent: Design, synthesis, cytotoxic evaluation, and DNA-binding studies

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ABSTRACT

A series of novel N-substituted angular furoquinolinone derivatives were synthesized and evaluated for their antitumor activities against QGY, K562, HeLa, P388, and A549 cell lines in vitro. The derivatives bearing basic amino side chain showed an improved antitumor activity. Compound **5h** *N*-(2-dimethylamino-ethyl)-2-(4,8,9-trimethyl-2-oxo-2H-furo[2,3-*h*]quinolin-1-yl)-acetamide exhibited the highest activities against P388 and A549 cell lines, which are evidenced by the IC_{50} values that are four to five fold lower than that for unsubstituted parent compound. DNA-binding experiments suggested that these derivatives bind to DNA through intercalation.

1. Introduction

DNA-intercalators containing a linear or angular planar chromophore with a polyaromatic ring can influence the structures and physiological functions of DNA.[1–7] Some intercalators such as furocoumarin, acridines, anthraquinones, naphthalimides, and phenanthridine are used in cancer treatment.[2,5,7,8] Angelicin (see Fig. 1), an angular furocoumarin, has been used to treat pain in the loins and knees,[9] and skin diseases in phototherapy.[10] Guiotto et al. have synthesized a series of furoquinolinones,[11–16] in which the O atom of furocoumarin has been substituted by NH (see Fig. 1), and some of them showed strong antiproliferative activity against tumor cell lines upon UVA (Ultraviolet Radiation A) irradiation. However, these furoquinolinones also revealed evident skin phototoxicity and marked clastogenic activity due to the formation of covalent monoadducts (MA) with DNA base and covalent DNA–protein cross-links (DPC) upon UVA activation. In the dark, these furoquinolinones exhibited weak antiproliferative activity with a mechanism of action related to topoisomerase II inhibition. In contrast to normal cell, many tumor cells show high expression levels of topoisomerase II, making this enzyme an ideal drug target.[17–19]

To enhance antitumor activity, generally the DNA-intercalator should carry one or two flexible basic side chains on chromophore.[1–7,20–23] The flexible basic side chains are highly influential in directing the thermodynamic-binding mechanism,

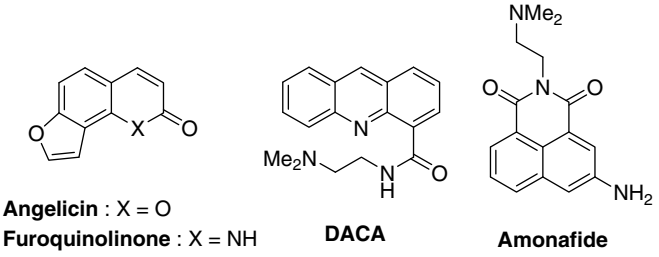

Figure 1.

geometry of the ligand–DNA complex, and sequence selectivities.[2,4,5,7,20–23] For example, the acridine does not have antitumor activity, but the DACA (see Fig. 1),[19] an acridine derivative with a *N*-dimethylamino acetamide side chain, shows high activity against solid tumours and has been used in clinic. Presence of the dimethylamine or pyrrolidine side chain is essential for cytotoxic activity of the naphthalimide derivatives,[2,24] such as in the Amonafide (see Fig. 1). In an attempt to further explore effect of side chain in furoquinolinone, we herein wish to report the design, synthesis, and biological evaluation of a series of N-substituted angular furoquinolinone derivatives with flexible side chains. The introducing flexible amino chain could achieve high activity enough to be used as antitumor agents even under no irradiation. Thus, the side effects of phototherapy can be neglected.

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2. Results and discussion

2.1. Synthesis

The synthetic route of the target compounds is described in Scheme 1. According to the literature,[25] the intermediate **3** (4-methyl-7-hydroxyquinolin-2-one) could be prepared from *m*-phenylenediamine and ethyl acetoacetate by three steps including Pechmann condensation reaction, diazotization, and hydrolysis. Compound **3** reacted with 3-chloro-2-butanone to give intermediate **4** (4-methyl-7-[2′-(3′-oxo)butyloxy]quinolin-2-one). Compound **5a** was obtained by the cyclization of **4** with PPA (polyphosphoric acid). An excess of halogenoalkane was added dropwise into a mixture of **5a**, NaH, and KI in dry DMF in the ice bath to yield the target products **5b**–**j**. Noticeably, the application of PPA to form furan ring in the reaction greatly reduced the reaction time to 4–6 h and increased the yield to 62% compared with 40–60 h and 47% reported before.[14] In addition, the cyclization of furan ring using PPA has been rarely reported.

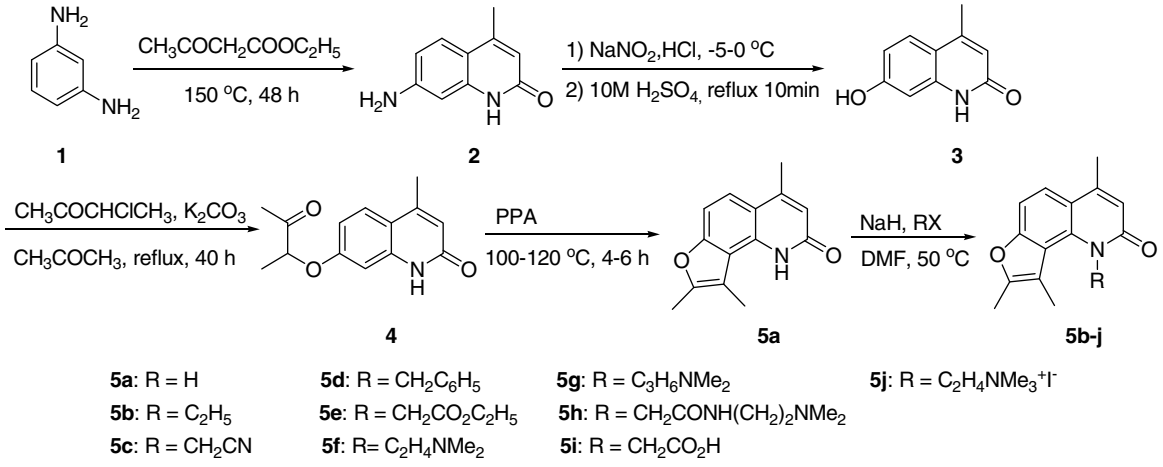
2.2. Cytotoxic evaluation in vitro and in the dark

The in vitro antitumor activities of these compounds were evaluated by examining their cytotoxic effects using sulforhodamine B (SRB) assay[26] against A549 (human lung cancer cells) and MTT tetrazolium dye assay[27] against P388 (murine leukemia cells), QGY (human liver cancer cells), K562 (human leukemia cells), and HeLa (human cervical carcinoma cells) (Table 1) under no UVA irradiation. The IC_{50} represents the drug concentration (μM) required to inhibit cell growth by 50%.

These compounds with basic amino chain (**5f**, **5g**, **5h**, and **5j**) exhibited improved cytotoxicity compared with parent compound **5a** and other non-amino chain compounds, especially the compound **5h** showed the highest activities against P388, A549, and HeLa cell lines with IC_{50} values being four to five fold lower than those for the **5a** under the same experimental conditions. Therefore, it is suggested that the *N* position of furoquinolinone is a crucial point, the introduction of appropriate group to the position can obviously improve the antitumor activity. The cytotoxicity of **5j** was weaker than that of **5f** against HeLa cell. The possible reason is that **5j**, as quaternized product of **5f**, has more difficulties to penetrate the cell membrane. Moreover, **5j** can not form hydrogen bonding with DNA bases. Compounds **5f**, **5g**, and **5h** exhibited antitumor activities, but **5e** and **5i** were inactive. The nitrogen atom at amino chain of **5f**, **5g**, and **5h** could form hydrogen bonding with DNA bases, which may contribute to the biological activity.[28,29]

2.3. DNA-binding studies

It has been pointed out that only a combination of selected methods provides sufficient information to determine the binding mode.[3–7,30,31] In order to reveal the influence of introducing side



Scheme 1.

Table 1
Cytotoxic evaluation against QGY, K562, HeLa, P388, and A549 cell lines under no irradiation and Scatchard-binding constants

Compound	Cytotoxicity (IC_{50}, μM)					K_b[c] (10^5 M^{-1})
	QGY[a]	K562[a]	HeLa[a]	P388[a]	A549[b]	
5a	98.94 ± 8.52	>100	89.25 ± 9.31	>100	99.29 ± 10.90	2.57 ± 0.09
5b	>100	>100	>100	>100	>100	2.79 ± 0.08
5c	>100	>100	>100	>100	>100	2.06 ± 0.09
5d	>100	>100	>100	>100	>100	3.15 ± 0.02
5e	>100	>100	>100	>100	>100	2.54 ± 0.07
5f	72.68 ± 9.81	55.73 ± 5.37	39.09 ± 6.04	51.54 ± 4.92	30.40 ± 4.57	2.66 ± 0.06
5g	74.87 ± 6.45	71.09 ± 7.91	35 ± 1.67	45.32 ± 3.25	29.62 ± 3.81	2.53 ± 0.10
5h	ND[d]	ND[d]	20.7 ± 2.72	14.45 ± 2.12	20.45 ± 2.13	1.58 ± 0.07
5i	ND[d]	ND[d]	>100	ND[d]	ND[d]	2.69 ± 0.05
5j	ND[d]	ND[d]	49.52 ± 7.06	ND[d]	ND[d]	3.18 ± 0.06

[a] CTX (cytotoxicity) against tumor cells (P388, QGY, K562, and HeLa) was measured by microculture tetrazolium–formazan method.
[b] CTX against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.
[c] K_b: Scatchard-binding constants
[d] ND, not determined.

chain on mechanism of antitumor action of these furoquinolinone derivatives, the DNA-binding studies were investigated by spectroscopic techniques, electrophoresis and viscosity measurement.

The K_b (Scatchard-binding constants) were calculated according to the fluorescence quenching technique (Table 1).[32] Typical-binding constant between organic compound and DNA usually ranges from 10^4–10^6 M^{-1}, and these compounds are actually moderate DNA-intercalators. In most cases, compounds strongly binding to DNA are high cytotoxic agent. However, the results (Table 1) indicated that there is no obvious relationship between DNA-binding and cytotoxicity. The fluorescence and UV–vis spectra of **5h** are shown in Figures 2 and 3, respectively. The hypochromicity (300–340 nm) and isosbestic points were observed in the absorption titration spectrum of **5h**, and no significant shift was observed (maximum absorption peak at 301 nm). The hypochromism and the appearance of isosbestic points are characteristics of intercalative binding.[33] It was found that the fluorescence of **5h** was quenched with CT-DNA (calf-thymus DNA) concentration increasing as most of the intercalators did.

CD (circular dichroism) is a very powerful technique to monitor the conformational state of the DNA double helix in solution. The CD spectrum of free CT-DNA exhibited a negative band at 247 nm due to the helicity, and a positive band at 279 nm due to the base stacking, which is the characteristic of DNA in the right-hand B form. The increases in the intensity both of the positive band and the negative band were observed (Fig. 4) when the compounds were incubated with CT-DNA. The changes in CD signals of DNA after adding **5f** and **5h** were consistent with Scatchard-binding constants, **5f** > **5h**, which indicated that DNA-binding ability of **5f** is stronger than that of **5h**. The appearance of small negative ICD signal (300–310 nm) suggested that **5f** and **5h** intercalated into DNA with its long axis parallel to the base-pair long axis.[34]

Viscosity measurement is regarded as a reliable tool to determine the binding model in solution.[3–7,30,31] Intercalation of a molecule into DNA results in a lengthening, unwinding, and stiffening of the helix which increases the viscosity of the solution.[30,31,33] The increase in viscosity of DNA solution was observed versus the increase in concentration of **5f** and **5h** (Fig. 5).

The gel mobility assay is sensitive to changes in DNA length or conformation, since the electrophoretic mobility of nucleic acid is proportional to the length of the nucleic acid molecules.[33] Because furoquinolinone could induce damage to DNA upon irradiation, the

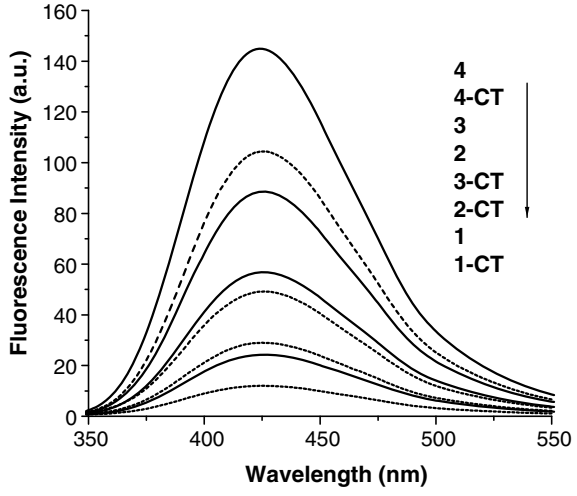


Figure 2. Fluorescence spectrum before and after interaction of **5h** and CT-DNA in Tris–HCl buffer (30 mM, pH 7.5). Numbers 1–4 indicat the concentration of **5h**, 10, 25, 50, and 100 μM, respectively. DNA applied was 50 μM (bp).

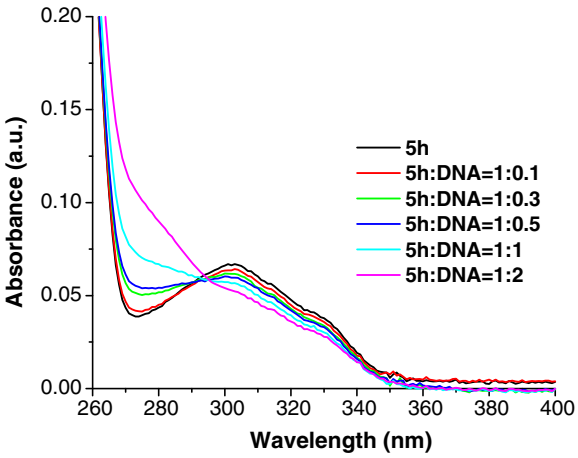


Figure 3. Absorption spectral changes of **5h** (10 μM) in the presence and absence of CT-DNA in Tris–HCl buffer (30 mM, pH 7.5).

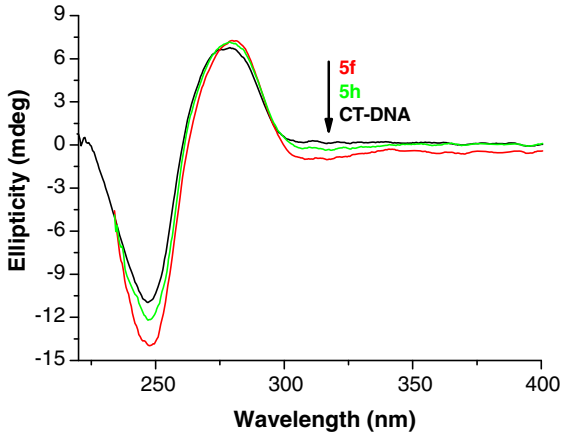


Figure 4. CD spectra of CT-DNA in the absence and presence of **5f** and **5h** at concentration of DNA 100 μM, the concentration of **5f** or **5h** is 10 μM, in Tris–HCl buffer (pH 7.0).

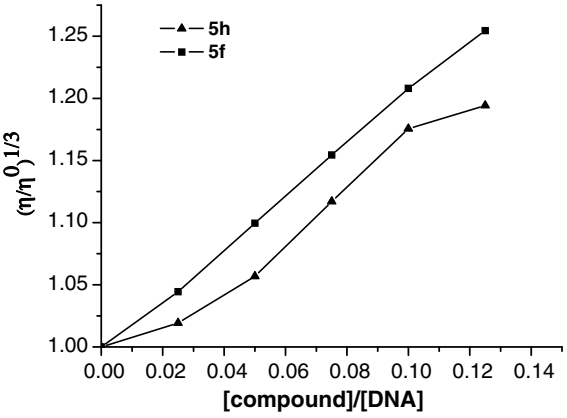


Figure 5. Effect of increasing amounts of compounds **5f** (■) and **5h** (▲) on the relative viscosities of CT-DNA at 25 (± 0.1) °C. [DNA] = 100 μM in Tris–HCl (30 mM, pH 7.5). η is the viscosity of DNA in the presence of the compounds and η^0.

cleavages to DNA were investigated using furoquinolinone derivatives under dark and irradiation. No DNA cleavages by compounds **5a**, **5f**, **5g**, and **5h** in the absence of irradiation (Fig. 6), indicated that these compounds have no ability to modify tertiary DNA structure in the dark. The compounds **5a, 5f**, and **5h** caused signif-

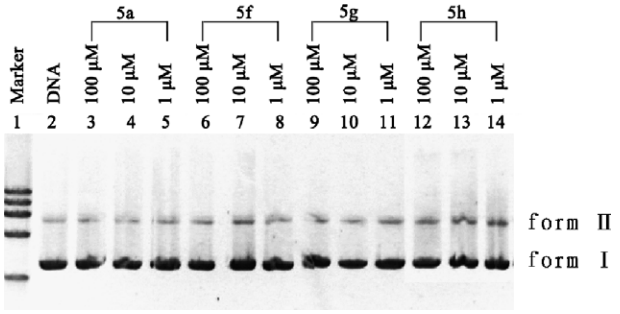


Figure 6. The cleavage of plasmid pBR322 DNA (25 ng/μL) by compounds **5a**, **5f**, **5g**, and **5h** at various concentration in the buffer of Tris–HCl (20 mM, pH 7.5) at 25 °C for 12 h under no irradiation. Lane 1, DNA marker; lanes 2, DNA alone (no irradiation), lanes 3–5, DNA + **5a**; lanes 6–8, DNA + **5f**; lanes 9–11, DNA + **5g**; lanes 12–14, DNA + **5h**.

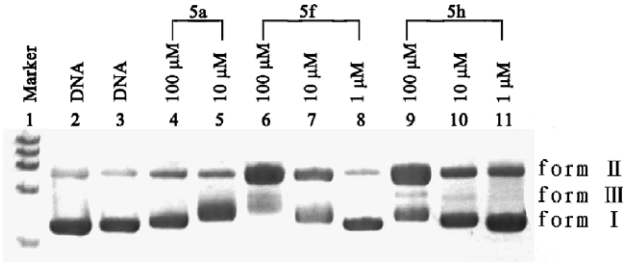


Figure 7. The photocleavage of pBR322 DNA (25 ng/μL) by compound **5a**, **5f**, and **5h** at various concentration in the buffer of Tris–HCl (20 mM, pH 7.5) upon irradiation for 2 h. Lane1, DNA marker; lane 2, DNA alone (no irradiation); lane 3, DNA alone (irradiation); lanes 4,-5, DNA + **5a**; lanes 6–8, DNA + **5f**; lanes 9–11, DNA + **5h**.

icant cleavages to DNA (form II- and form III-linear) (Fig. 7) upon irradiation, which showed that these compounds are photoactive depending on concentration.

3. Conclusions

In summary, we developed a series of novel DNA-intercalators based on furoquinolinone system. The introduction of amino side chain is an effective way to improve the antitumor activity of DNA-intercalator containing planar chromophore. Further studies on action mechanism of furoquinolinone and their structural modification are currently under way. This research may provide some new suggestions for the design of novel antitumor agent based on furoquinolinone.

4. Experimental

All the solvents are of analytic grade. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard). Melting points were determined by using an X-6 micro-melting point apparatus and are uncorrected. Column chromatography was performed using silica gel 200–300 mesh. IR spectra were obtained using a Nicolet 470 FT-IR instrument. High-resolution mass spectra were obtained on a Micromass company GCT CA156 (TOF MS) spectrometer and Micromass LCT (ESI) spectrometer.

The intermediate **3** was prepared according to the published methods[25] and the **4** was prepared according to the literature method.[13] The structures of these compounds were characterized by ^{1}H NMR and electron ionization mass spectra.

4.1. Synthesis

4.1.1. 4, 8, 9-Trimethylfuro [2,3-*h*]quinolin-2(1*H*)-one (5a)[14]

Compound **4** (4.9 g, 20 mmol) was mixed with PPA (20 mL) and stirred at 120–140 °C for 5 h, then cooled to room temperature, washed with water, filtered and dried. The crude product was purified by chromatography on silica gel (CH_2Cl_2/C_2H_5OH = 40:1, v/v) to get **5a** as a white solid in 62% yield, mp 230–232 °C (reported 233 °C). ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 8.94 (s, 1H) 7.49 (d, 1H, J = 8.8 Hz) 7.28–7.25 (m, 1H) 6.48 (s, 1H) 2.53 (s, 3H) 2.49 (s, 3H) 2.42 (s, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 162.2, 155.0, 151.1, 150.3, 132.6, 120.2, 118.1, 115.7, 115.3, 108.5, 106.8, 20.2 11.6, 10.4; IR (KBr, cm^{-1}) 3255, 2952, 1653, 841.

4.1.2. General procedure for N-substituted furoquinolinone derivatives (5b–5j)

Compound **5a** (114 mg, 0.5 mmol), NaH (100 mg, 4.17 mmol) and KI (100 mg, 0.6 mmol) in dry DMF (10 mL) were mixed in ice bath and under nitrogen. After stirring for 15 min, a solution of halogenoalkanes (3 mmol) in dry DMF (10 mL), was added dropwise for over a period of 30 min, and the reaction mixture was stirred for 2 h at 0 °C and for 48 h at 50–60 °C under nitrogen (TLC detection). The solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel with a solution of CH_2Cl_2 and EtOH as eluent to give desired product.

4.1.3. 1-Ethyl-4,8,9-trimethyl-1*H*-furo[2,3-*h*]quinolin-2-one (5b)

Compound **5a** (114 mg, 0.5 mmol) and iodoethane (0.24 mL, 3.0 mmol) were reacted according to the general procedure and gave **5b** after chromatography (CH_2Cl_2/C_2H_5OH = 50:1, v/v), in 45% yield as a white solid, mp 190–191.8 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.64 (d, 1H, J = 9.2 Hz) 7.43 (d, 1H, J = 9.2 Hz) 6.70 (s, 1H) 4.58–4.56 (m, 2H) 2.63 (d, 3H, J = 7.6 Hz) 2.45 (s, 3H) 2.18 (s, 3H) 1.47 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 161.5, 153.9 149.7, 147.0, 142.5, 124.1, 121.0, 118.7, 112.3, 110.5, 108.8, 61.4, 19.6, 14.7, 11.7, 10.4; IR (KBr, cm^{-1}) 3239, 2978, 1648, 840, 785; TOF MS (EI$^+$) calcd $C_{16}H_{17}NO_2$ 255.0926; found: 255.0929.

4.1.4. (4,8,9-Trimethyl-2-oxo-2*H*-furo[2,3-*h*]quinolin-1-yl)-acetonitrile (5c)

Compound **5a** (114 mg, 0.5 mmol) and bromoacetonitrile (0.21 mL, 3 mmol) were reacted according to the general procedure and gave **5c** after chromatography (CH_2Cl_2/C_2H_5OH = 50:1, v/v), in 38% yield as a white solid, mp 193.2–194.8 °C; 1H NMR (400 MHz $CDCl_3$) δ (ppm) 7.68 (d, 1H, J = 8.8 Hz) 7.27 (d, 1H, J = 8.8 Hz) 6.79 (s, 1H) 5.15 (s, 2H) 2.69 (s, 3H) 2.63 (s, 3H) 2.47 (s, 3H). 13C NMR (100 MHz $CDCl_3$) δ (ppm) 158.7, 154.1, 150.4, 148.7, 141.4, 124.4, 121.9, 118.7, 116.2, 112.3, 110.2, 109.3, 49.9, 19.7, 11.8, 10.4; IR (KBr, cm^{-1}) 2952, 2361, 1617; TOF MS (EI$^+$) calcd for $C_{16}H_{14}N_2O_2$ 266.1055; found: 266.1054.

4.1.5. 1-Benzyl-4,8,9-trimethyl-1*H*-furo[2,3-*h*]quinolin-2-one (5d)

Compound **5a** (114 mg, 0.5 mmol) and benzyl bromide (0.36 mL, 3 mmol) were reacted according to the general procedure and gave **5d** after chromatography (CH_2Cl_2/C_2H_5OH = 40:1, v/v) in 62% yield as a white solid, mp 192.3–194.1 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.65 (d, 1H, J = 8.8 Hz) 7.51 (d, 2H, J = 7.2 Hz) 7.45 (d, 1H, J = 8.8 Hz) 7.39 (t, 2H, J = 7.2 Hz) 7.33–7.31 (m, 1H) 6.79 (s, 1H) 5.60 (s, 2H) 2.65 (s, 3H) 2.62 (s, 3H) 2.45 (s, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 161.2, 154.0, 149.8, 147.3, 142.3, 137.8, 128.4, 127.9, 127.7, 124.2, 121.3, 118.7, 112.3, 110.5, 109.1, 67.2, 19.6, 11.7, 10.5; IR (KBr, cm^{-1}) 3039, 2952, 2921, 1601; TOF MS (EI$^+$) calcd for $C_{21}H_{19}NO_2$ 317.1416; found: 317.1411.

4.1.6. (4,8,9-Trimethyl-2-oxo-2*H*-furo[2,3-*h*]quinolin-1-yl)-acetic acid ethyl ester (5e)

Compound **5a** (114 mg, 0.5 mmol) and ethyl bromoacetate (0.33 mL, 3 mmol) were reacted according to the general procedure and gave **5e** after chromatography (CH_2Cl_2/C_2H_5OH = 50:1, v/v), in 51.4% yield as a white solid, mp 92.5–93.4 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.65 (d, 1H, J = 8.8 Hz) 7.46 (d, 1H, J = 8.8 Hz) 6.86 (s, 1H) 5.05 (s, 2H) 4.25–4.23 (m, 2H) 2.67 (s, 3H) 2.55 (s, 3H) 2.44 (s, 3H) 1.26 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 169.6, 160.1, 154.0, 149.9, 147.9, 141.9, 124.1, 121.6, 118.8, 112.3, 110.0, 109.5, 62.4, 61.0, 19.7, 14.2, 11.7, 10.3; IR (KBr, cm^{-1}) 2973, 2755, 2612, 1463, 1350; TOF MS (EI^+) calcd for $C_{18}H_{19}NO_4$ 313.1314; found: 313.1312.

4.1.7. 1-(2-Dimethylamino-ethyl)-4,8,9-trimethyl-1*H*-furo[2,3-*h*]quinolin-2-one (5f)

Compound **5a** (114 mg, 0.5 mmol) and 2-chloro-*N*,*N*-dimethylethanamine (323 mg, 3 mmol) were reacted according to the general procedure and gave **5f** after chromatography (CH_2Cl_2/C_2H_5OH = 30:1, v/v), in 67% yield as a white solid, mp 197.4–199.2 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.67 (d, 1H, J = 8.8 Hz) 7.50 (d, 1H, J = 8.8 Hz) 6.80 (s, 1H) 5.05 (t, 2H, J = 4.8 Hz) 3.61 (t, 2H, J = 4.8 Hz) 2.94 (s, 6H) 2.68 (s, 3H) 2.58 (s, 3H) 2.46 (s, 3H). ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 159.6, 154.1, 150.4, 148.5, 141.8, 124.1, 121.6, 118.8, 112.0, 109.9, 59.3, 56.5, 43.6, 19.7, 11.8, 10.7; IR (KBr, cm^{-1}) 3291, 3245, 2942, 1606; TOF MS (EI^+) calcd for $C_{18}H_{22}N_2O_2$ 298.1681; found: 298.1674.

4.1.8. 1-(3-Dimethylamino-propyl)-4,8,9-trimethyl-1*H*-furo[2,3-*h*]quinolin-2-one (5g)

Compound **5a** (114 mg, 0.5 mmol) and 3-chloro-*N*,*N*-dimethylpropan-1-amine (364.83 mg, 3 mmol) were reacted according to the general procedure and gave **5g** after chromatography (CH_2Cl_2/C_2H_5OH = 30:1, v/v), in 62% yield as a white solid, mp 198.2–200 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.64 (d, 1H, J = 8.8 Hz) 7.47 (d, 1H, J = 8.8 Hz) 6.70 (s, 1H) 4.64 (t, 2H, J = 5.6 Hz) 3.36 (t, 2H, J = 7.6 Hz) 2.86 (s, 6H) 2.66 (s, 3H) 2.59 (s, 3H) 2.53 (br, 2H) 2.45 (s, 3H). ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 160.6, 154.0, 150.2, 147.8, 142.1, 124.1, 121.3, 118.7, 112.1, 109.9, 109.5, 62.0, 56.2, 43.2, 43.1, 24.1, 19.7, 11.8, 10.6; IR (KBr, cm^{-1}) 3409, 3003, 2936, 1596; TOF MS (EI^+) calcd for $C_{19}H_{24}N_2O_2$ 312.1838; found: 312.1844.

4.1.9. *N*-(2-Dimethylamino-ethyl)-2-(4,8,9-trimethyl-2-oxo-2*H*-furo[2,3-*h*]quinolin-1-yl)-acetamide (5h)

Compound **5e** (156.5 mg, 0.5 mmol) was dissolved in *N*,*N*-dimethyl ethylenediamine (10 mL), the mixture was refluxed under nitrogen for 24 h. The solvent was removed under reduced pressure, the residue was purified by chromatography on silica gel (CH_2Cl_2/C_2H_5OH = 25:1, v/v) to give title product in 51% yield as a white solid, mp 200.2–201 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.65 (d, 1H, J = 8.8 Hz) 7.48 (d, 1H, J = 8.8 Hz) 6.82 (s, 1H) 5.03 (s, 2H) 3.43–3.39 (m, 2H) 2.69 (s, 3H) 2.59 (s, 3H) 2.45 (s, 3H) 2.41 (t, 2H, J = 6.0 Hz) 2.15 (s, 6H); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 169.1, 159.7, 154.0, 150.1, 148.0, 142.0, 125.0, 124.3, 121.5, 118.6, 112.5, 109.8, 109.7, 64.7, 57.8, 45.0, 36.3, 29.4, 19.7, 11.7, 10.5; IR (KBr, cm^{-1}) 3286, 3101, 1653, 1606; HMRS (ESI) m/z $(M + H)^+$ calcd for $C_{20}H_{25}N_3O_3$ 356.1974; found: 356.1956.

4.1.10. (4,8,9-Trimethyl-2-oxo-2*H*-furo[2,3-*h*]quinolin-1-yl)-acetic acid (5i)

Compound **5e** (156.5 mg, 0.5 mmol) was mixed with aqueous NaOH (10 mL, 20%), the mixture was refluxed for 30 h. After concentration, cooling and acidification with concd HCl, the residue was collected and crystallized from EtOH to give target compound in 35% yield as a white solid, mp 208.7–209.6 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 8.04 (s, 1H) 7.68 (d, 1H, J = 8.8 Hz) 7.50 (d, 1H, J = 8.8 Hz) 6.88 (s, 1H) 5.11 (s, 2H) 2.70 (s, 3H) 2.53 (s, 3H) 2.41 (s, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 160.1, 154.1, 150.2, 148.6, 141.6, 124.1, 121.8, 118.8, 112.2, 109.9, 109.8, 62.6, 19.7, 11.6, 10.3; IR (KBr, cm^{-1}) 3409, 2998, 1607, 1339; TOF MS (EI^+) calcd for $C_{16}H_{15}NO_4$ 285.1001; found: 285.1001.

4.1.11. Trimethyl-[2-(4,8,9-trimethyl-2-oxo-2*H*-furo[2,3-*h*]quinolin-1-yl)-ethyl]ammonium iodide (5j)

Compound **5f** (149 mg, 0.5 mmol) and excess of 8–10 times iodomethane (0.50–0.62 mL, 4–5 mmol) were dissolved in EtOH (10 mL) and stirred at 30–40 °C under nitrogen for 24 h. The solvent was removed under reduced pressure, the residue was purified by chromatography on silica gel (CH_2Cl_2/C_2H_5OH = 15:1, v/v) to give target product in 38% yield as a white solid, mp 239.1–240.5 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ (ppm) 7.66 (d, 1H, J = 8.8 Hz) 7.51 (d, 1H, J = 8.8 Hz) 6.79 (s, 1H) 5.06 (s, 2H) 4.30 (s, 2H) 3.64 (s, 9H) 2.69 (s, 3H) 2.60 (s, 3H) 2.46 (s, 3H); ^{13}C NMR (100 MHz $CDCl_3$) δ (ppm) 159.2, 154.3, 150.7, 121.7, 118.7, 112.0, 110.3, 109.5, 55.2, 19.7, 11.8, 11.0; IR (KBr, cm^{-1}) 3378, 2921, 1735; TOF MS (EI^+) calcd for $C_{19}H_{25}N_2O_2I$ 227.0946; found: 227.0946; HMRS (ESI) m/z $(M+H)^+$ calcd for $C_{19}H_{25}N_2O_2I$ 313.1916; found: 313.1911.

4.2. Cytotoxic evaluation in vitro

The prepared compounds were submitted to Shanghai Institute of Materia Medica Chinese Academy of Sciences and Dalian institute of chemical physics Chinese Academy of Sciences to test their cytotoxicities. Growth inhibitory effect on the cell lines (P388, QGY, K562, and HeLa) was measured by using the MTT assay.[27] For A-549 cell lines, the growth inhibition was tested by the sulforhodamine B (SRB) assay.[26]

4.3. DNA-binding studies

UV–vis absorption spectra were recorded on a PGENERAL TU-1901 UV–vis spectrophotometer and fluorescent spectra were measured on a Hitachi F-4500 luminescence spectrophotometer. Calf-thymus DNA was purchased from the Sino-American Biotechnology Company. Solutions of CT-DNA in Tris–HCl buffer (30 mM, pH 7.5) gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free from protein. The concentration of calf-thymus DNA was determined by its absorption intensity at 260 nm with a known molar absorption coefficient value of 6600 M^{-1} cm^{-1}.

4.3.1. Fluorescence spectrum study

The two groups of samples for experiments were prepared, one at a constant DNA concentration of 50 μM and at compound concentration ranging from 1 to 10 μM in Tris–HCl (30 mM, pH 7.5), and the other having the same concentration of compound but absence of DNA as control. All the above solutions were ultrasonically shaken for 1 day at 25 °C in the dark. Fluorescence wavelength and intensity area of samples were measured.

4.3.2. UV–vis absorption spectra study

The titration absorption spectra studies were performed by keeping constant the concentration of compound, while varying the DNA concentration at room temperature. Initially, solutions of the blank buffer were placed in the reference and sample cuvettes (1 cm path length), respectively, and then the first spectrum was recorded in the range 200–400 nm. During the titration, aliquots of buffered DNA solution were added and the solutions were mixed by repeated inversion. After mixing for 10 min, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for at least four titrations indicating binding saturation had been achieved.

4.3.3. Circular dichroism spectra studies

The CD (circular dichroism) spectra were scanned with a J-810 spectrophotometer (Jasco, Japan) using a 1-cm path quartz cell and subtracted from the spectrum of Tris–HCl buffer alone. The CD spectra were recorded at the compound concentration of 10 μM and DNA concentration of 100 μM, in the region 200–600 nm.

4.3.4. Viscometry studies

Calf-thymus DNA was dissolved in Tris–HCl buffer (30 mM, pH 7.5) and left at 4 °C overnight. It was treated in an ultrasonic bath for 10 min, and the solution was filtered through a PVDF membrane filter (pore size of 0.45 μm) to remove insoluble material, the DNA samples concentration is 100 μM.[35] Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature at 25 (±0.1) °C in a thermostated bath. Flow times were measured with a digital stopwatch and each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ versus the ratio of the concentration of compounds to that of DNA, where η is the viscosity of DNA in the presence of the compound and η^0 is the viscosity of DNA in the absence of the compound.[33,36]

4.3.5. DNA cleavage studies

The solution containing pBR322 DNA (form I, 250 ng, 1 μL) and compound in DMSO (1 μL) was diluted by Tris–HCl buffer (pH 7.5, 20 mM) to 10 μL, then irradiated for 2 h with light (300 nm) using lamp (50 W High Pressure Mercury Lamp) placed at 20 cm from the sample. The samples were analyzed by gel electrophoresis in 1% agarose gel, which was stained with ethidium bromide. Supercoiled DNA runs at position I, nicked DNA at position II, and linear DNA at position III. Experiments were repeated in triplicate.

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