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Synthesis of new amonafide analogues via coupling reaction and their cytotoxic evaluation and DNA-binding studies

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ABSTRACT

A series of 5-alkylamino substituted amonafide analogues were synthesized from naphthalic anhydride by three steps including bromization, amination and CuI/proline catalyzed coupling reaction. The CuI/L-proline catalyzed coupling reaction was first applied to the naphthalimide system. These new amonafide analogues showed potential anticancer activities against HeLa and P388D1 cell lines in vitro, and **4a**, **4b**, and **4h** exhibited better activity than amonafide against HeLa cell under the same experimental conditions. More importantly, the new analogues could avoid the side effect of amonafide due to their structure, in which lacks a primary amine at the 5 position. Moreover, the DNA-binding of the analogues was also investigated.

1. Introduction

In 1973, Braña et al. published four different 5-nitronaphthalimides with activity against HeLa and KB cells.[1] Later, a large series of 4-, 5-, and 6-substituted naphthalimide derivatives were synthesized and revealed that the 5-position substituted derivatives are superior to 4- or 6-substituted derivatives by testing the antitumour activity against tumour cells.[2–7] Amonafide, a 5-amino substituted naphthalimide (see Fig. 1), was the first drug that reached the clinical trial stage in this family and exhibited excellent antitumour activity against advanced breast cancer. However in the clinical studies, it was found that amonafide was easily metabolized to *N*-acetyl-amonafide (Fig. 1) by enzyme *N*-acetyltransferase 2 (NAT2), which caused a high-variable, unpredictable toxicity.[8,9] To reduce the acetylation by NAT2, a series of N-substituted amonafide derivatives were synthesized by Kiss et al.,[10] in which the hydrogen atom at 5-position amino group was substituted by acyl chlorine, aldehyde, isocyanates and isothiocyanates. Although some *N*-substituted derivatives showed similar to or better antitumour activity than amonafide, according to the reported synthetic method, the type and numbers of *N*-substituted derivatives was limited and not meet the need to screen potential antitumour agents.

Based on the research for potential antitumour agents, the introduction of alkylamino groups into some pharmacophore can improve their antitumour activity. For example, introduction of *N*-(2-dimethylamino-ethyl)-acetamide into acridine gave the DACA[11] with high antitumour activity. The acenaphtho[1,2-*b*]pyrrole-carbonitrile[12] and acenaphtho[1,2-*b*]pyrrole-carboxylic acid esters[13] derivatives bearing *N,N*-dimethyl ethylenediamine or *N*-methyl piperazine groups exhibited obvious improved antitumour activities compared with the parent compound. Therefore, introduction of different aliphatic amine groups into the 5-position of naphthalimide would provide a series of new amonafide analogues. The new amonafide analogues could avoid side effects owing to their structure in which lacks a primary amine at the 5-position, and retained biological activity. On the basis of above consideration, we were interesting in synthesis of 5-alkylamino substituted amonafide analogues. There are two synthetic routes to choose for the synthesis, one is above mentioned Kiss's method, but it is necessary to have high active reaction material such as acyl chlorine, and this limited its use. Other is amination of 5-halogenated naphthalimide. However, the nucleophilic substitution reaction at the 5-position of naphthalimide is difficult to occur

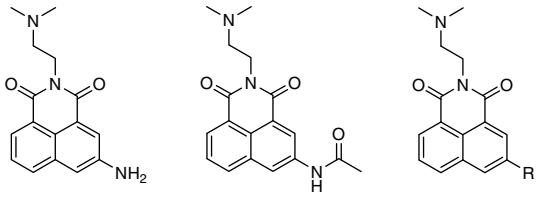

Figure 1.

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by traditional chemical method, due to electron-charge accumulation induced by strong electron-withdrawing ability of carbonyl.

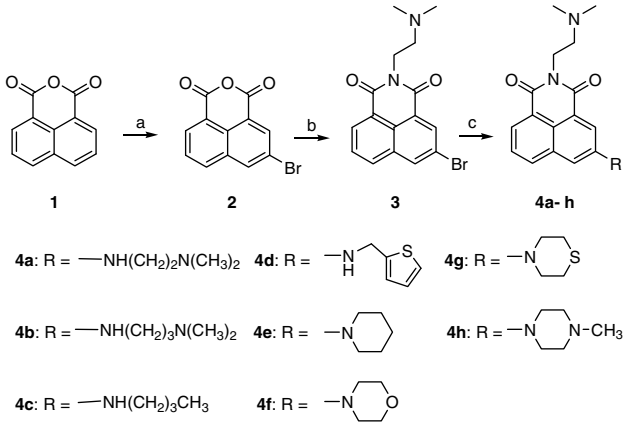
In recent years, transition metal catalyzed C–N bond-forming processes have been greatly developed, and some previously inaccessible compounds were synthesized, nevertheless the scope of substrate was mainly limited to simple aryl.[14–18] Stimulated by these results, we attempted to synthesize the amonafide analogues by coupling reaction of aryl halides with aliphatic amines. In this paper, we first report the synthesis of 5-alkylamino substituted amonafide analogues (Fig. 1), cytotoxic evaluation in vitro, and the DNA-binding.

2. Results and discussion

2.1. Synthesis

The target compounds **4a–h** were synthesized starting from naphthalic anhydride by three steps including bromization, amination and CuI/L-proline catalyzed coupling reaction, and the synthetic route was described in the Scheme 1.

Because the third step reaction was the most crucial and difficult in the synthetic route, the coupling reaction of **3** with *n*-butylamine was choosed as the model reaction to investigate suitable conditions and detailed in Table 1. Although Pd-catalyzed amination of aryl halides was successfully applied,[19,20] a common catalyst $Pd(PPh_3)_4$ failed to give the target product **4c** and large amount of **3** and its debrominated product were found in our experiment (entry 1). According to the literature,[14,15,21–23] copper catalysts were also effective to the coupling of aryl halides with amines. CuI, a common copper resource, was tested, and the 6% yield of **4c** suggested that the reaction can occur and need to optimize (entry 2). It is well known that a ligand can facilitate the copper-catalyzed aryl amination reaction.[24] The *N,N*-diethylsalicylamide[25] was used for the coupling reaction, and the 9% target compound (entry 3) indicated that it was not suitable as a ligand. To our delight, when proline as a ligand was employed, **4c** was obtained in 34% yield, and L- or D-proline afforded the same yield (entries 4 and 5). Because base also play important role in the coupling reaction,[19] Cs_2CO_3 and K_2CO_3 were checked. Cs_2CO_3 as base gave a better yield (entries 5 and 6), it seems likely that Cs_2CO_3 have better basicity and solubility in organic solvent compared with K_2CO_3. To determine the effect of solvent on the yield, the DMF and DMSO were also evaluated, and DMSO provided a better result (entry 5) than those of DMF (entry 7). Considering the slow rate at 90 °C (entry 8), the reaction temperature was raised to 110 °C, and the reaction time greatly was shortened to 9 h (entry 5). On the basis of above studies, it can be concluded that the coupling reaction carry out in an accepted yield using the CuI/L-proline as catalyst and Cs_2CO_3 as the base in DMSO at 110 °C.



Scheme 1. Reagents and conditions: (a) Br_2, concd HNO_3, 70 °C, 2 h, room temperature overnight; (b) $NH_2(CH_2)_2N(CH_3)_2$, C_2H_5OH, reflux, 2 h; (c) RNH_2, CuI–proline, Cs_2CO_3, DMSO, 110 °C, 8–12 h.

Table 1
The optimization of the reaction conditions in coupling reaction of **3** with *n*-butylamine

Entry	Ligand	Base	Solvent	Yield (%)
1[a]	No	K_2CO_3	toluene	0
2[b]	Nno	Cs_2CO_3	DMSO	6
3[b]	*N,N*-Diethylsalicylamide	Cs_2CO_3	DMSO	10
4[b]	D-Proline	Cs_2CO_3	DMSO	34
5[b]	L-Proline	Cs_2CO_3	DMSO	34
6[b]	L-Proline	K_2CO_3	DMSO	28
7[b]	L-Proline	Cs_2CO_3	DMF	29
8[c]	L-Proline	Cs_2CO_3	DMSO	33

[a] Reaction conditions: **3** (0.5 mmol), $Pd(PPh_3)_4$ (0.025 mmol), base (0.75 mmol), solvent (2 mL), 100 °C, 20 h.
[b] Reaction conditions: **3** (0.5 mmol), CuI (0.05 mmol), ligand (0.1 mmol), base (0.75 mmol), solvent (2 mL), 110 °C, 9 h.
[c] Reaction conditions: **3** (0.5 mmol), CuI (0.05 mmol), ligand (0.1 mmol), base (0.75 mmol), solvent (2 mL), 90 °C, 24 h.

The desired compounds were synthesized by the optimized conditions, and the results were showed in Table 2. The yield of the primary amines was higher than those of second amines. A possible reason is attributed to steric hindrance of second amines. To our surprise, the yield of **4a** was most high and arrived 52%, which can be associated with *N,N*-dimethylethyldiamine participation in CuI coordination so as to promoted the reaction as the reported.[24]

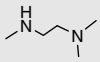
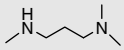
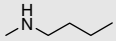
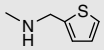
2.2. Cytotoxic evaluation in vitro

The in vitro cytotoxic potencies of the new amonafide analogues against **P388D1** (murine lymphoid neoplasm cells) and **HeLa** (human cervical carcinoma cells) were evaluated by **MTT** tetrazolium dye assay.[26] The results were summarized in Table 2 and compared with the activities of amonafide. The new amonafide analogues exhibited stronger cytotoxicities, especially, **4a**, **4b** and **4h** against HeLa cell, presenting values that are 10-fold, 8.57-fold, and 3-fold lower than the values found for amonafide under the same experimental conditions, respectively. Based on the report,[2] the stabilization of DNA-amonafide complex may be caused by the formation of hydrogen bonds between the amino group and the sugar phosphate chain. Compounds **4a**, **4b**, **4c** and **4d** could form the similar hydrogen bonds with DNA, thus they indicated the higher biological activity. A phenomenon of excellent antitumour agents for target DNA, containing dimethylamino-ethylamino or dimethylamino-propylamino groups, was frequently encountered.[27–30] Similar to the phenomenon, **4a** and **4b** showed the most activity among the amonafide analogues. A reason could be that the protonation of the terminal nitrogen in the substituted group in the experimental situation greatly facilitate the interaction with the anionic DNA polymer. Although **4h** possess the similar protonated terminal nitrogen, it had weaker activity compared with **4a** and **4b**. The different activities could be contributed to the spatial distance between the protonated terminal nitrogen and chromophore in **4h** and the refined structure among **4a, 4b** and **4h**. Moreover, these derivatives displayed stronger cytotoxicity against P388D1 cell than that against HeLa.

2.3. DNA-binding studies

To evaluate the DNA-binding properties of the amonafide analogues, fluorescent spectra, UV–vis absorption spectra, circular dichroism spectra (CD), and viscosity titration of the most active

Table 2
Preparation of **4** by CuI/proline catalyzed amination of **3** with various aliphatic amines[a] and the cytotoxic evaluation against HeLa and P388D1 cell lines

Compound	R	Time (h)	Yield (%)	Cytotoxicity (IC_{50}, μM) HeLa	P388D1	K_b[b] (10^5 M^{-1})
4a		8	52	0.62 ± 0.07	0.83 ± 0.08	1.85 ± 0.09
4b		8	50	0.71 ± 0.05	0.23 ± 0.07	1.75 ± 0.08
4c		9	34	9.42 ± 0.07	3.24 ± 0.11	1.23 ± 0.07
4d		12	31	9.46 ± 0.12	3.54 ± 0.08	1.06 ± 0.06
4e		11	25	21.62 ± 0.19	2.64 ± 0.09	0.85 ± 0.07
4f		10	30	9.44 ± 0.08	0.53 ± 0.07	0.97 ± 0.08
4g		11	27	36.34 ± 0.28	2.34 ± 0.12	0.79 ± 0.07
4h		12	22	1.93 ± 0.06	0.43 ± 0.09	2.48 ± 0.10
Amonafide	—NH_2			6.02 ± 0.09	0.68 ± 0.08	1.05 ± 0.07

[a] Reaction conditions: compound **3** (0.5 mmol), CuI (0.05 mmol), ligand (0.1 mmol), base (0.75 mmol), solvent (2 mL).
[b] K_b Scatchard binding constants which was calculated according to the fluorescence quenching technique.

4a and **4h** were investigated with calf thymus DNA (CT-DNA) and compared with amonafide.

2.3.1. UV–vis absorption spectra studies

A compound binds to DNA through intercalation usually results in hypochromism and bathchromism,[31–34] due to the intercalation mode involving a strong stacking interaction between an aromatic chromophore and the DNA base pairs.[35] The absorption spectra of **4h** in the absence and presence of CT-DNA were given in Figure 2. As the concentration of DNA increased, for **4h**, the hypochromicity was only observed and no significant shift. In order to compare the DNA-binding mode, the plots of absorbance intensity versus the [DNA]/[compound] ratio for **4a**, **4h**, and amonafide were showed in Figure 3. These spectral characteristics suggest that there are some similar interactions between the compounds and DNA.

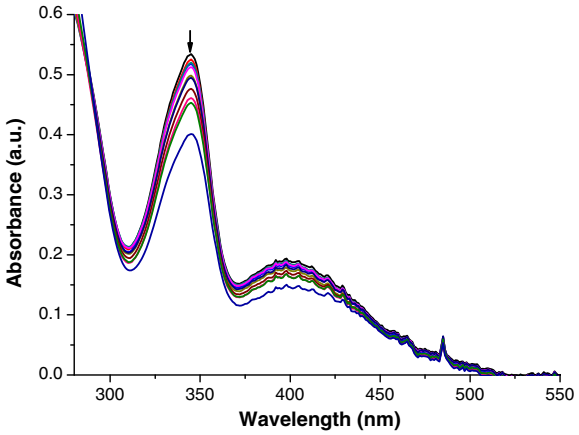


Figure 2. Absorption spectral changes of **4h** (50 μM) in the presence of CT-DNA ([DNA] = 0–50 μM) in Tris–HCl buffer (30 mM, pH 7.5).

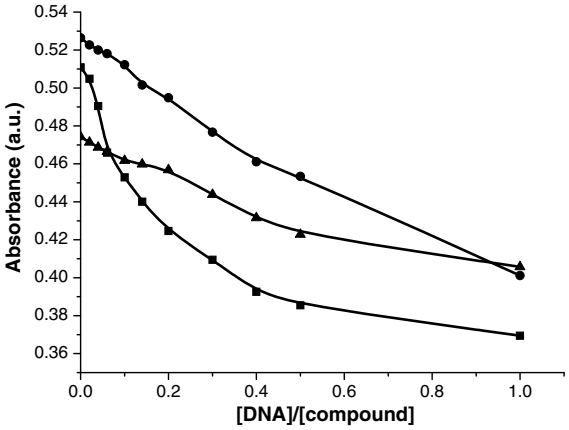


Figure 3. Plots of absorbance intensity (346 nm) versus the [DNA]/[compound] ratio for **4a** (▲), **4h** (●) and amonafide (■) in Tris–HCl buffer (30 mM, pH 7.5), [compound] = 50 μM.

2.3.2. Fluorescent spectra studies

The fluorescent properties were tested and Scatchard binding constants (K_b) were calculated according to the fluorescence quenching technique[36] (Table 2). The emission intensities of **4a** decreased with increasing the amount of CT-DNA as most of the intercalators did[31–34] and the wavelength showed blue shift (Fig. 4). The blue shift would tend to indicating that the **4a** enters CT-DNA-stacking region with lower polarity rather than the bulk solution of CT-DNA.[37] Upon the addition of the compounds, the relative emission intensity (F/F_o) versus ratio of [compound]/[DNA] for **4a**, **4h** and amonafide displayed same trend (Fig. 5), implying the compounds could interact with DNA in the same way. The K_b data are bigger and the cytotoxicities of the compounds are stronger except **4h** (Table 2). In most cases, compounds strongly binding to DNA are high cytotoxic agent. However, the results indicated

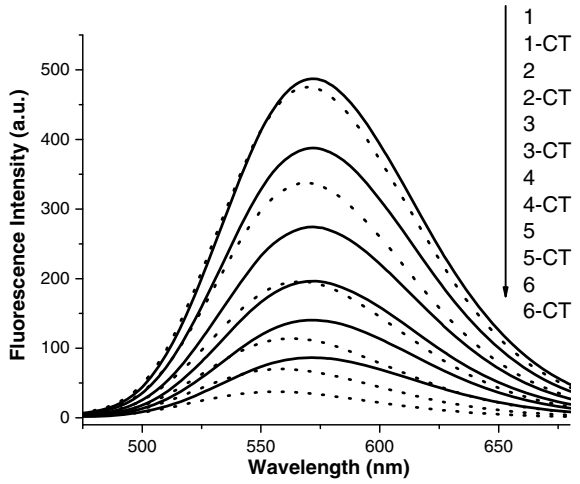


Figure 4. Fluorescence spectrum before and after interaction of **4a** and CT-DNA (calf thymus DNA) in Tris–HCl buffer (30 mM, pH 7.5). Numbers 1–6 indicated the concentration of **4a**, 20, 15, 10, 7, 5, 3 μM, respectively. Numbers 1–6 CT indicated the concentration of **4a**, 20, 15, 10, 7, 5, 3 μM contained 50 μM DNA, respectively.

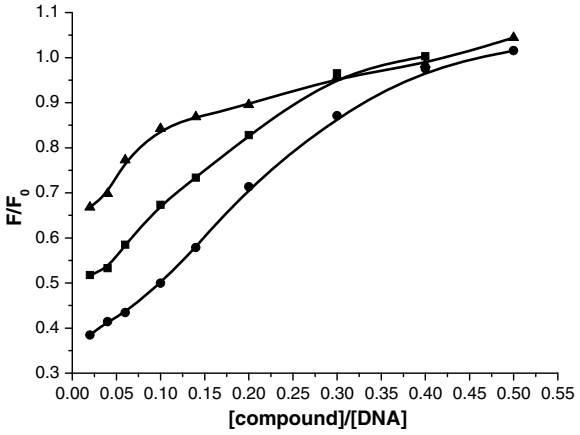


Figure 5. Plots of relative emission intensity (F/F_0) versus the [compound]/[DNA] ratio for **4a** (■), **4h** (●), and amonafide (▲) in Tris–HCl buffer (30 mM, pH 7.5), [DNA] = 50 μM.

that there is no same relationship between DNA-binding and cytotoxicity.

2.3.3. Circular dichroism spectra studies

CD (circular dichroism) is a very powerful technique to monitor the conformational state of the DNA double helix in solution. When the compounds were incubated with CT-DNA, the increases in the intensity of the positive band and the decrease in the intensity of the negative band were observed, which was consistent with the B to A-like conformational change (Fig. 6).[38] The changes in CD signals of DNA after adding **4a** and **4h** were consistent with the K_b, **4h** > **4a** (Table 2), which indicated that DNA-binding ability of **4h** is stronger than that of **4a**. Generally, a positive ICD indicates a perpendicular orientation of the transition dipole moment of the intercalated chromophore relative to the long axis of the intercalation pocket, and a negative ICD indicates a parallel orientation of the transition dipole moment of the intercalated chromophore relative to the long axis of the intercalation pocket.[38,39] The positive and negative ICD signal in the 330–500 nm region were also observed in Figure 6. It seems that some of the both compounds could intercalate into DNA in the perpendicular and others could intercalate into DNA in the parallel fashion.

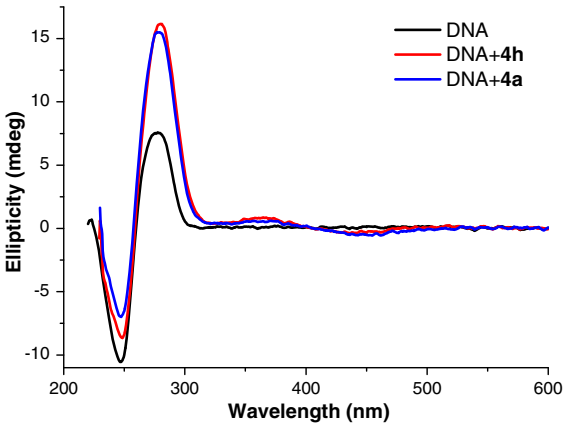


Figure 6. CD spectra of CT-DNA in the absence and presence of **4a**, **4h** at concentration of DNA 100 μM, the concentration of **4a** or **4h** is 10 μM, in Tris–HCl buffer (pH 7.0).

2.3.4. Viscosity measurement

Viscosity measurement is regarded as a reliable tool to determine the binding model in solution in the absence of crystallographic structural data and NMR data.[31] Intercalation of a molecule into DNA could result in a lengthening, unwinding and stiffening of the helix and usually accompany by an increases in solution viscosity.[33,34,39] The increase in viscosity of DNA solution was observed versus the increase in concentration of **4a** and **4h** (Fig. 7), implying the lengthening and unwinding of CT-DNA. The result is contrary to the phenomenon of CD, where CT-DNA should be compressed along the helix axis and accompany by B to A-like conformational change. Because many factors such as DNA itself, DNA-drug complex and environment could affect the CD spectra and viscosity when the compounds bind to CT-DNA, exact change of the conformation require further study.

3. Conclusion

The study demonstrated that the CuI/ proline catalyzed coupling reaction can serve as a valuable tool for the functionalization of naphthalimide system and provided amonafide analogues, potential antitumour agents, for clinical screen. The amonafide analogues not only showed improved antitumour activity over amonafide, but also can avoid the side effects. The DNA-binding studies revealed that they bind DNA via similar mode of amonafide.

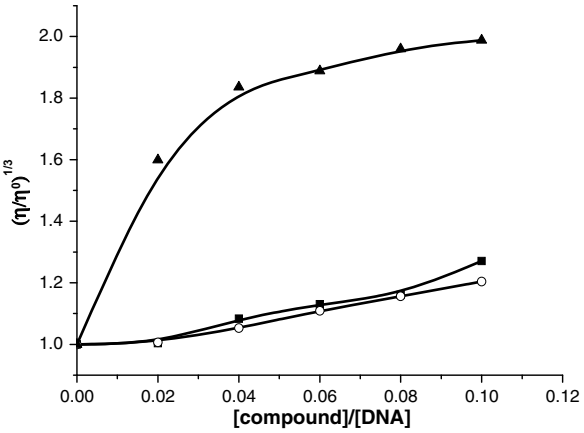


Figure 7. Effect of **4a** (■), **4h** (▲), and amonafide (○) on the relative viscosities of CT-DNA at 25 (±0.1) °C. [DNA] = 100 μM. η is the viscosity of DNA in the presence of the compounds and η^0 is the viscosity of DNA in the absence of the compounds.

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 HP 1100 LC–MS spectrometer.

4.1. Synthesis

4.1.1. 5-Bromo-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (3)

The compound **2** was prepared according the report.[40] The compound **2** (277 mg, 1.0 mmol) and *N*,*N*-dimethylethyldiamine (92 mg, 1.0 mmol) were refluxed in EtOH (15 mL) for 2 h to give intermediate **3**.

Yield 92%, an light brown solid, mp 77.2–78.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.65 (s, 1H) 8.59 (dd, 1H, J = 1.0 and 7.2 Hz) 8.35 (s, 1H) 8.11 (dd, 1H, J = 0.7 and 8.3 Hz) 7.77 (t, 1H, J = 7.8 Hz) 4.34 (t, 2H, J = 6.9 Hz) 2.70 (t, 2H, J = 6.9 Hz) 2.39 (s, 6H); MS (APCI) m/z 348 (M+1).

4.1.2. General procedure for the amonafide analogues (4a-h)

The intermediate **3** (174 mg, 0.5 mmol), CuI (9 mg, 0.05 mmol), proline (11 mg, 0.1 mmol), Cs_2CO_3 (244 mg, 0.75 mmol) and aliphatic amine (0.75 mmol) in dry DMSO (2 mL) were mixed and stirred at 110 °C for 8–12 h under nitrogen. The crude products were purified by chromatography on silica gel with a solution of CH_2Cl_2 and MeOH as eluent to give desired product.

4.1.3. 5-(Dimethylamino-ethylamino)-2-[2-(dimethylamino) ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4a)

Yield 52%, an orange solid, mp 97.2–98.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.27 (dd, 1H, J = 0.8 and 7.2 Hz) 8.02 (s, 1H) 7.93 (d, 1H, J = 7.6 Hz) 7.58 (t, 1H, J = 7.2 Hz) 7.11 (s, 1H) 4.94 (t, 1H, J = 4.4 Hz) 4.32 (t, 2H, J = 7.2 Hz) 3.32–3.28 (m, 2H) 2.67–2.64 (m, 4H) 2.37(s, 6H) 2.30 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.6, 164.4, 147.1, 133.8, 131.6, 127.1, 126.6, 123.3, 122.4, 122.0, 121.9, 110.0, 57.4, 57.0, 45.7, 45.1, 41.0, 38.1;IR (KBr cm^{-1}) 3377, 2955, 1684 1654; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{20}H_{26}N_4O_2$ 355.2134; found: 355.2169.

4.1.4. 5-(Dimethylamino-propylamino)-2-[2-(dimethylamino) ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4b)

Yield 50%, an orange solid, mp 115.8–116.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.25 (d, 1H, J = 0.8 and 7.2 Hz) 7.97 (s, 1H) 7.92 (d, 1H, J = 8.4 Hz) 7.57 (t, 1H, J = 7.2 Hz) 7.08 (s, 1H) 5.53 (s, 1H) 4.34–4.30 (m, 2H) 3.36 (s, 2H) 2.67–2.64 (m, 2H) 2.52–2.49 (m, 2H) 2.37 (s, 6H) 2.31 (s, 6H) 1.90–1.87 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.7, 164.5, 147.2, 133.9, 131.6, 127.0, 126.4, 123.2, 122.4, 122.2, 121.9, 109.3, 58.6, 57.0, 45.7, 43.5, 38.1, 25.9; IR (KBr cm^{-1}) 3370, 1691, 1654; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{28}N_4O_2$ 369.2291; found: 369.2294.

4.1.5. 5-Butylamino-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (4c)

Yield 34%, an orange solid, mp 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.25 (d, 1H, J = 7.2 Hz) 7.95–7.91 (m, 2H) 7.58 (t, 1H, J = 7.2 Hz) 7.07 (s, 1H) 4.33 (t, 2H, J = 6.8 Hz) 4.19 (s, 1H) 3.29–3.24 (m, 2H) 2.68 (t, 2H, J = 7.2 Hz) 2.39 (s, 6H) 1.74–1.67 (m, 2H) 1.55–1.45 (m, 2H) 1.01 (t, 3H, J = 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.6, 164.4, 146.9, 133.8, 131.7, 127.1, 126.6, 123.2, 122.3, 122.0, 121.8, 109.6, 56.9, 45.6, 43.6, 38.0, 31.3, 20.3, 13.9; IR (KBr cm^{-1}) 3237, 2948, 1704, 1648; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{20}H_{25}N_3O_2$ 340.2025; found: 340.2037.

4.1.6. 5-[(Thiophen-2-yimethyl)-amino]-2-[2-(dimethylamino) ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4d)

Yield 31%, an orange solid, mp 144.7–146.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.29 (d, 1H, J = 7.2 Hz) 8.04 (s, 1H) 7.94 (d, 1H, J = 8.0 Hz) 7.60 (t, 1H, J = 8.0 Hz) 7.22–7.18 (m, 1H) 7.18 (s, 1H) 7.09 (s, 1H) 7.02–7.00 (m, 1H) 4.70 (s, 3H) 4.38 (t, 2H, J = 6.8 Hz) 2.84 (t, 2H, J = 6.0 Hz) 2.50 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 164.6, 164.3, 146.0, 141.5, 133.6, 132.1, 127.2, 127.1, 125.5, 125.0, 123.3, 122.4, 122.3, 121.9, 110.7, 56.5, 45.1, 43.2, 37.3, 29.7; IR (KBr cm^{-1}) 3377, 2918, 1695, 1651; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{21}N_3O_2S$ 380.1433; found: 380.1451.

4.1.7. 5-Piperidin-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (4e)

Yield 25%, an orange solid, mp 130.3–131.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38 (s, 1H) 8.32 (d, 1H, J = 7.2 Hz) 7.98 (d, 1H, J = 8.0 Hz) 7.61 (t, 1H, J = 8.0 Hz) 7.41 (s, 1H) 4.34 (t, 2H, J = 7.6 Hz) 3.39 (t, 4H, J = 5.6 Hz) 2.68 (t, 2H, J = 7.2 Hz) 2.38 (s, 6H) 1.79–1.76 (m, 4H) 1.70–1.67 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.6, 150.4, 133.5, 132.3, 131.3, 127.6, 127.0, 123.8, 123.1, 122.6, 122.3, 115.1, 56.9, 50.1, 45.6, 38.0, 25.5, 24.2; IR (KBr cm^{-1}) 2926, 1695, 1654; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{25}N_3O_2$ 352.2025; found: 352.2030.

4.1.8. 5-Morpholine-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*] isoquinoline-1,3(2*H*)-dione (4f)

Yield 30%, an orange solid, mp 147.8–149.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38–8.35 (m, 2H) 8.02 (d, 1H, J = 8.0 Hz) 7.65 (t, 1H, J = 8.0 Hz) 7.43 (s, 1H) 4.37 (t, 2H, J = 6.8 Hz) 3.94 (t, 4H, J = 4.8 Hz) 3.38 (t, 4H, J = 4.8 Hz) 2.78 (t, 2H, J = 6.8 Hz) 2.46 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.4, 149.8, 134.0, 133.2, 132.6, 131.3, 128.2, 127.3, 126.9, 123.3, 123.1, 122.8, 122.3, 115.1, 66.7, 56.7, 49.0, 45.3, 37.6; IR (KBr cm^{-1}) 3414, 2955, 1691, 1651, 1261; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{20}H_{23}N_3O_3$ 354.1818; found: 354.1829.

4.1.9. 5-Thiomorpholin-2-[2-(dimethylamino)ethyl]-1*H*-benzo [*de*]isoquinoline-1,3(2*H*)-dione (4g)

Yield 27%, an orange solid, mp 167.5–168.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.35 (d, 1H, J = 7.6 Hz) 8.31 (s, 1H) 8.00 (d, 1H, J = 8.4 Hz) 7.64 (t, 1H, J = 7.6 Hz) 7.40 (s, 1H) 4.37 (t, 2H, J = 6.8 Hz) 3.78 (t, 4H, J = 5.2 Hz) 2.81 (t, 4H, J = 5.2 Hz) 2.73 (t, 2H, J = 6.8 Hz) 2.43 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.4, 149.3, 133.4, 132.3, 128.0, 127.3, 126.9, 123.8, 123.6, 122.8, 122.2, 115.9, 57.0, 51.6, 45.7, 38.2, 26.5; IR (KBr cm^{-1}) 2948, 1691, 1654; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{20}H_{23}N_3O_2S$ 370.2349; found: 370.2358.

4.1.10. 5-(4′-Methyl-piperazin)-2-[2-(dimethylamino)ethyl]-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4h)

Yield 22%, an orange solid, mp 121.2–122.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.38 (s, 1H) 8.35 (d, 1H, J = 6.8 Hz) 8.01 (d, 1H, J = 8.0 Hz) 7.64 (t, 1H, J = 8.0 Hz) 7.43 (s, 1H) 4.34 (t, 2H, J = 6.8 Hz) 3.44 (t, 4H, J = 4.8 Hz) 2.68–2.64 (m, 6H) 2.40 (s, 3H) 2.37 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 164.5, 149.7, 133.3, 132.4, 127.9, 127.2, 123.2, 122.3, 115.1, 57.0, 54.8, 48.8, 46.1, 45.7, 38.1; IR (KBr cm^{-1}) 3414, 1688, 1647; HRMS (ESI) m/z (M+H)$^+$ calcd for $C_{21}H_{26}N_4O_2$ 367.2134; found: 367.2142.

4.2. Cytotoxic evaluation in vitro

HeLa and P388D1 cells were seeded into 96-well microculture plates and allowed to adhere for 24 h (8 h for HeLa). After cells were exposed to compounds at concentrations from 100 to 0.01 μM for 48 h, medium was aspirated and replenished with complete medium. IC_{50} was evaluated by MTT tetrazolium dye assay.[26] Each experiment was performed three times.

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 (CT) DNA was purchased from the Sino–American Biotechnology Company. Solution 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 CT-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. UV–vis absorption spectra studies

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 200–600 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.

4.3.2. Fluorescent spectra studies

The two groups of samples for experiments were prepared, one at a constant DNA concentration of 50 μM and at concentrations compounds ranging from 1 μM to 20 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 ultrasonic shaken for 1 day at 25 °C in the dark. Fluorescence wavelength and intensity area of the samples were measured by the excitation wavelength at 350 nm. Based on the Scatchard method,[36,41] two following Eqs. 1 and 2 are generated, where C_o is the total concentration of bond and free drug, C_{DNA} is the free DNA concentration, and F_o and F_1 are the fluorescence intensity before and after the intercalation of the drug into DNA.

$$r_b = C_o(F_0 - F_1)/(C_{DNA}F_0) \quad (1)$$

$$r_b/r_f = (F_0 - F_1)/(C_{DNA}F_1) \quad (2)$$

A plot of r_b/r_f versus r_b was constructed using linear regression at the origin as showed in Figure 8 and the Scatchard binding constant was evaluated from the slope of the plot.

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. Viscosity experiments

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 concentration of CT-DNA was 100 μM.[39] Viscometric titrations were performed with an Ubbelodhe viscometer immersed in a thermostated bath maintained 25 (±0.1) °C. The flow times were measured with a digital stopwatch, each sample was measured three times, and an average flow time was calculated. Data are presented as $(\eta/\eta^0)^{1/3}$ versus [complex]/[DNA], where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flowing time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta = (t - t_0)$.[39,42]

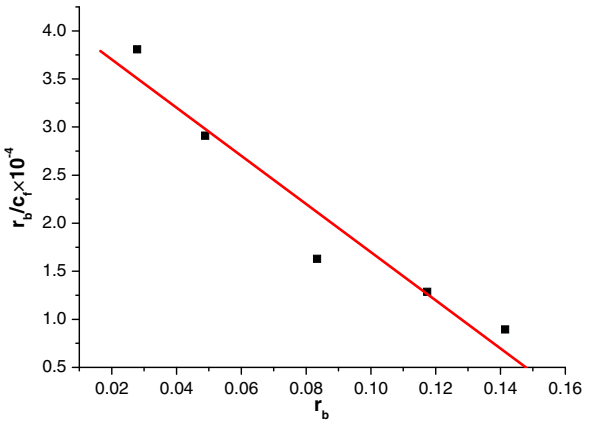


Figure 8. Scatchard plot of binding of **4h** to CT-DNA. Measurement was made in 30 mM Tris–HCl buffer, pH 7.5.

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