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Synthesis, antitumor and DNA photocleaving activities of novel naphthalene carboxamides: effects of different thio-heterocyclic rings and aminoalkyl side chains

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Abstract—Two kinds of thio-heterocyclic fused naphthalene carboxamides, **3a–b**, **4a–b**, were designed, synthesized and quantitatively evaluated as efficient antitumor and DNA photocleaving agents. Compound **3a** or **3b**, having the thiophene ring, intercalated into DNA more strongly than compound **4a** or **4b**, having the thioxanthene ring. Compound **4a** or **4b**, photocleaved DNA more efficiently than **3a** or **3b** via superoxide anion. Compound **4a** was the strongest inhibitor for P388 (murine leukemia cell), while **3a** was the most cytotoxic one against A549 (human lung cancer cell). Each new compound showed stronger DNA photocleaving activity than corresponding naphthalimide.

1. Introduction

DNA intercalating agents form an important class of drugs in anticancer therapy.[1] To study the interaction between such agents and DNA, in particular, effects of structural characters of them on the interaction, could offer novel insights into the design of new DNA targeting antitumor drugs.[2] Naphthalimides are significant examples of DNA-intercalating agents,[1,3–5] many of, which have shown efficient antitumor activities upon a variety of murine and human tumor cells,[4] and/or DNA photocleaving activities.[5] They are generally characterized by the presence of a planar tri- or tetracylic aromatic chromophore and one or two flexible basic side chains.

To promote the antitumor and/or DNA photocleaving activities, previous attempts were focused on incorporating substituents or fusing aromatic rings to the naphthalimide skeletons.[4,5] The presence of a larger aromatic chromophore was proved to improve the affinity of the intercalator for the DNA molecule, consequently to a greater cytotoxic and/or DNA photocleaving activity.[4,5] We have ever reported a series of thio-heterocyclic fused naphthalimides, **1a–d**[5f] and **2a–d**,[5e] as efficient DNA photocleavers (Fig. 1). In our continued efforts for simple but efficient antitumor and/or DNA photocleaving agents, we report here, the molecular design and chemical synthesis of a novel heterocyclic family of **3a–b** and **4a–b** (Fig. 1) by modification of the naphthalimide skeletons via mercuric oxide mediated decarbonylation. Compounds **3a–b** or **4a–b**, which could be considered as ring opened models of **1a–b** or **2a–b**, were expected to intercalate into DNA more strongly to shown

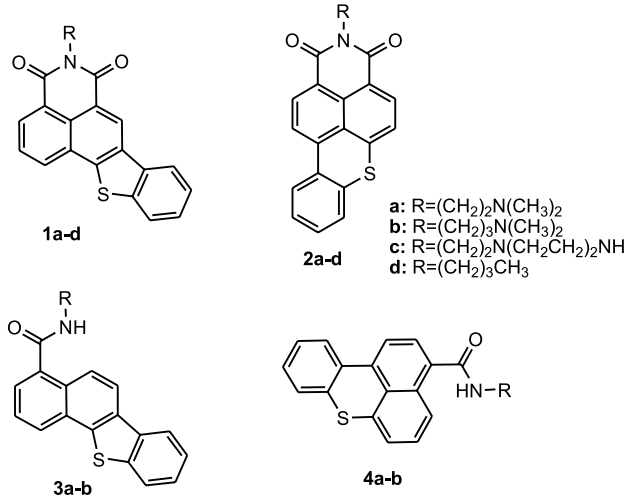

Figure 1. Structures of the reported naphthalimides (**1**, ,**2**) and novel designed naphthalene carboxamides (**3**, **4**).

Keywords: DNA photocleavage; Antitumor; Intercalating.
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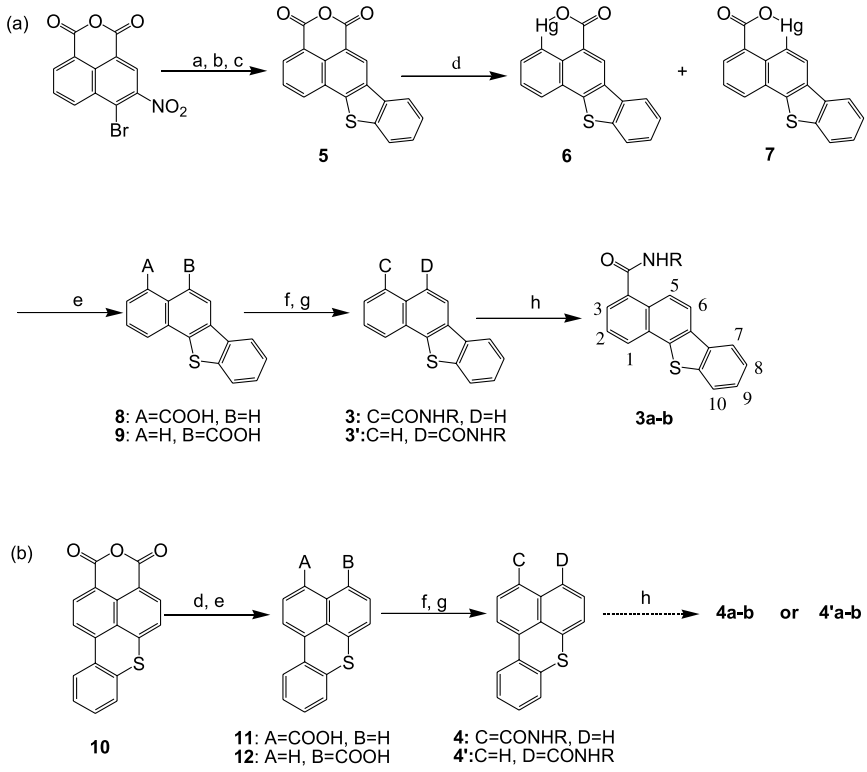


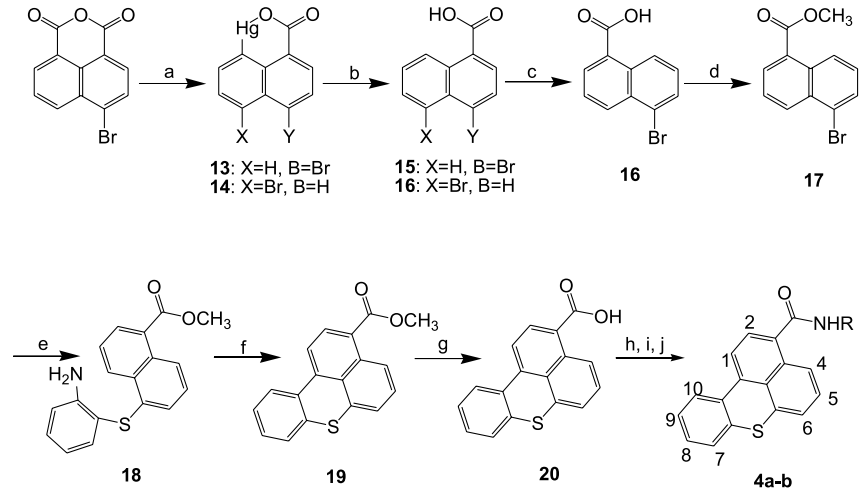
Scheme 1. (a) PhSH, EtOH, reflux, 4 h, 59% yield; (b) $SnCl_2$/concentrated HCl, 65% yirld; (c) Pochorr cyclization: $NaNO_2$, H_2O–HCl–HOAc, 0–5 °C, 2 h; $CuSO_4$, HOAc, reflux, 2 h; (d) HgO (yellow), NaOH, AcOH, H_2O, reflux, 4 days, 95% yield; (e) concentrated HCl, reflux, 2 h, 75% yield; (f) $SOCl_2$, $CHCl_3$, Et_3N, reflux, 20 h; (g) propitiate amine, CH_2Cl_2, room temperature, 24 h; (h) careful column chromatography.

efficient medical and biological use due to the reduction of the steric effect of carbonyl anchor and the flexibility of the aminoalkyl side chains.

2. Results and discussion

2.1. Synthesis and spectra

The mercuric oxide (yellow or red) mediated decarbonylation of (substituted) naphthalic anhydrides has been reported.[6] In our efforts to synthesize target compounds **3a**–**b**, we found that only yellow mercuric oxide successfully mediated the decarbonylation of benzothiophenonaphthalic anhydride **5**, synthesized from 4-bromo-3-nitro anhydride through Pochorr intramolecular cyclization.[5f,7] Two benzothiophenonaphthoic acid isomers, **8** and **9**, were obtained after the reaction, which were then converted to a pair of isomers, **3a**, **3a′** or **3b**, **3b′**. However, only pure **3a** or **3b** was obtained after careful column chromatography due to the slight amount of **3a′** or **3b′** (Scheme 1a).



Scheme 2. (a) HgO (yellow or red), NaOH, AcOH, H_2O, reflux, 4 days, 94% yield; (b) concentrated HCl, reflux, 2 h, 70% yield; (c) recrystalized with AcOH, 40% yield; (d) $SOCl_2$, reflux, 5 h; methanol, reflux, 2 h; (e) 2-aminobenzenethiol, K_2CO_3, DMF, reflux, 80 h; (f) Pochorr cyclization: $NaNO_2$, HOAc H_2SO_4, 0–5 °C, 12 h; $CuSO_4$, HOAc, reflux, 2 h; (g) NaOH, Methanol, reflux, 2 h; HCl; (h) $SOCl_2$, $CHCl_3$, Et_3N, reflux 20 h; (i) propitiate amine, CH_2Cl_2, room temperature, 24 h; (j) careful column chromatography.

The decarbonylation of benzothioxanthenenaphthalic anhydride **10**[5e] was also only mediated by yellow mercuric oxide. The pair of isomer mixtures, **4a**, **4a′** or **4b**, **4b′** was obtained. However, neither pure product could be separated after careful column chromatography due to the similar molecular polarities between **4a**, **4a′** or **4b**, **4b′** (Scheme 1b). Then we tried the other way outlined in Scheme 2. Either yellow or red mercuric oxide was found to mediate the decarbonylation of 4-bromo-1,8-naphthalic anhydride to obtain a pair of isomers of **15** and **16** with the ratio of 2:3 via ^{1}H NMR. Pure 5-bromo-1-naphthaloic acid **16** was obtained after recrystallization from acetic acid. It was transformed to methyl-5-bromo-1-naphthoate **17**, which was then condensed with 2-aminobenzenthiol to obtain **18**. Followed by the Pochorr intramolecular cyclization, methyl benzothioxanthenenaphthoate **19** was obtained, which was converted to corresponding acid **20**, then to target product **4a** or **4b**, which was purified by careful column chromatography.

Structures of all the final products were well confirmed by ^{1}H NMR, HRMS and IR. Furthermore, the above experiments provided two different ways to synthesize side-armed heterocyclic fused naphthalene derivatives, which may have potentials in other fields.

Spectra data of these new compounds were measured and summarized in Table 1. Compared with those of their corresponding naphthalimides,[5e–f] values of both the emission wavelength and the absorption wavelength of **3a–b**, **4a–b**, were blue-shifted due to the reduction of their conjugation areas and electronic pushing–pulling ICT (intramolecular charge transfer) effects caused by the decarbonylation reaction. Slight effect of the side chains was found on spectra data of these compounds.

Table 1. Spectra data,[a,b] and Scatchard binding constants of compounds **1–4**

Compd	UV λ_{max}/nm (log ε)	FL λ_{max}/nm (Φ)	Scatchard binding constants (M^{-1})
3a	350 (3.32)	423 (0.009)	4.46×10^5
3b	350 (3.42)	421 (0.011)	6.86×10^5
4a	389 (3.73)	455 (0.013)	1.95×10^4
4b	388 (3.91)	454 (0.014)	4.02×10^4
1a[4f]	384 (4.23)	437 (0.0215)	2.8×10^5
2a[4e]	462 (4.42)	521 (0.51)	8.27×10^3

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard ($\phi=0.55$).

2.2. DNA intercalation

The fluorescences of these compounds were quenched upon addition of Calf thymus DNA. The Scatchard binding constants[8] were calculated and summarized in Table 1 with the orders of **3b**>**3a**, **4b**>**4a**, clearly indicating the importance of the aminoalkyl side chains serving as DNA groove binders and/or external electrostatic binders. The chain length is important in placing the protonated side chain nitrogen in proximity to functional groups suitable for hydrogen bonding on the DNA double helix after the thioheterocyclic fused naphthalene chromophore has intercalated. In our cases, the side chain with three methylene units between two nitrogen atoms can significantly enhance the intercalating abilities of the thiazonaphthalimides. As to the intercalation orders of **3a**>**4a**, **3b**>**4b**, it is obvious that the chromophore of **3a** or **3b**, having the thiophene ring could intercalated more strongly than that of **4a** or **4b**, having the thioxanthene ring, in agreement with the comparison of **1a** ($2.8\times10^5\ M^{-1}$)[5f] and **2a** ($8.27\times10^3\ M^{-1}$).[5e] As expected, **3a** or **4a**, exhibited greater affinity for DNA than **1a** or **2a**, indicating that the reduction of the steric effect of carbonyl anchor and/or the higher flexibility of the side chain facilitated its intercalation with DNA (Fig. 2).

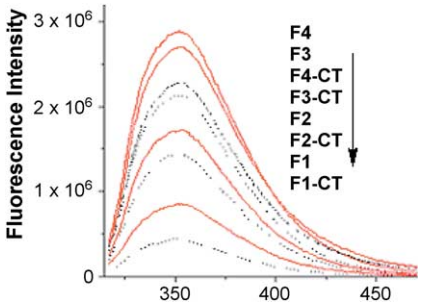


Figure 2. Fluorescence spectra before and after interaction of compound **3b** and CT-DNA. Curves F and F-CT corresponded to compound **3b** before and after mixed with DNA. Numbers 1–4 indicated the concentration of **3b**, 5, 10, 20, 40 μM, respectively. DNA applied was 50 μM (bp).

2.3. Antitumor evaluation

The antitumor activities of these compounds were evaluated in vitro (under scattered light) against A549 (human lung cancer cell) and P388 (murine leukemia cell) cell lines, respectively. As shown in Table 2, all these compounds exhibited efficient antitumor activities with IC_{50} ranging from 0.176 to 29 μM. Also, all of them were more cytotoxic against P388 than against A549, reflecting an excellent selectivity for a special murine or leukemia cell type. Cytotoxic potencies of these compounds against two tumor cells were highly dependent on the length of side chains. The compound with two methylene units in the side chain between two nitrogen atoms was more cytotoxic than corresponding homologue with one more methylene unit, as indicated by the cytotoxicity orders of **3a**>**3b**, **4a**>**4b**. Compound **4a** was found to be the strongest inhibitor for P388 with IC_{50} of 0.176 μM, while **3a** was the most cytotoxic one against A549 with IC_{50} of 1.16 μM.

Table 2. Cytotoxicity of these compounds against A549 [a] and P388 [b] cells

Compounds	Cytotoxicity (IC_{50}, μM)	
	A549[a]	P388[b]
3a	1.16	0.428
3b	19.1	0.89
4a	20.5	0.176
4b	29	0.295

[a] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.[9]
[b] CTX against murine leukemia cells (P388) was measured by microculture tetrazolium–formazan method.[10]

2.4. DNA photocleavage

The photocleavage of these compounds to supercoiled plasmid pBR322 DNA were evaluated by 1% agarose gel electrophoresis. The reaction mixture containing each

compound and plasmid DNA was put under photo-irradiation (2300 W/cm^2) through a transluminator (360 nm) at a distance of 20 cm at 0 °C for 3 h under aerobic conditions. The photocleavage efficiency was defined by the conversion ratio from supercoiled pBR322 DNA (form I) to relaxed circular DNA (form II) and linear DNA (form III).

As shown in Figure 3a, all these compounds efficiently cleaved DNA from form I to form II and form III at the concentration of 100 μM with an order of **4b** > **4a** > **3b** > **3a**. Compound **4b** was more active than its analogues in that it could produce more percentage of form III (36%), generally, the result from double-strand cuts or proximal single-strand cuts on opposite strands. The concentration-dependent experiment showed that **4b** exhibited detectable cleavage (21% form II) even at 0.5 μM (Fig. 3b). No damage was observed in the absence of either compound (Fig. 3c, lane 2) or light (lane 3), indicating that they were obligate factors for DNA strand scission. In this case, UV light actually functioned as a trigger to initiate the strand scission.

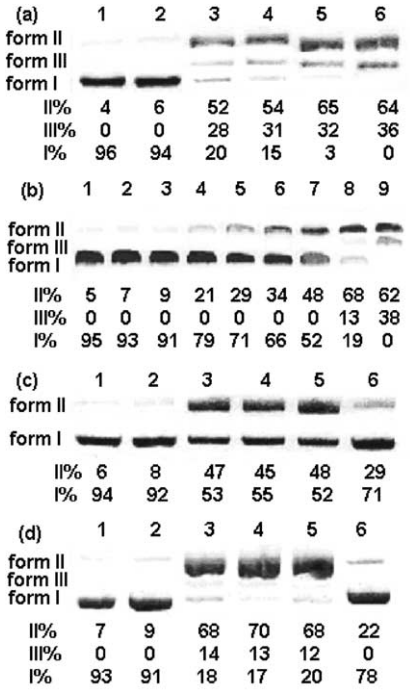


Figure 3. Photocleavage of closed supercoiled pBR322 DNA (200 μM/bp) in the buffer of Tris–HCl (20 mM, pH 7.5). (a) Photocleavage of plasmid DNA by different compounds (100 μM) for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lane 3–6, each compound of **3a**, **3b**, **4a**, **4b**, and DNA, respectively. (b) Photocleavage of plasmid DNA by **4b** at various concentrations for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA with **4b** (100 μM) without UV; Lane 4–9, DNA with **4b** at concentration of 0.5, 5, 10, 25, 50, 100 μM, respectively, following UV irradiation. (c) and (d) Effect of additives on the photocleavage by compound **3b** or **4b** (50 μM) for 3 h. Lane 1, DNA alone; lane 2, DNA with UV; lanes 3 DNA and compound **3b** (c), **4b** (d) with UV; lane 4–6, DNA and compound in the presence of histidine (6 mM), ethanol (1.7 M), dithiothreitol (DTT, 30 mM), respectively, following UV irradiation.

Photocleavage can be via a variety of mechanisms involving free radical, electron transfer and singlet oxygen.[11] In order to establish the reactive species responsible for cleavage of the plasmid, mechanistic experiments were performed by addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide anion scavenger) and ethanol (hydroxyl radical scavenger), respectively. As shown in Figure 3c or d, with **3b**, **4b** as examples, histidine, and ethanol clearly had no obvious effects on the photocleavage, indicating that singlet oxygen and hydroxyl radical were not likely to be cleavage contributors. However, the DNA cleaving activities both decreased greatly in the presence of DTT (lane 6), indicating superoxide anion was most likely to be the reactive specie responsible for plasmid cleavage. Under identical conditions, DTT inhibited the photocleavage activity of **4b** (form II: 68% to 22%) more efficiently than that of **3b** (form II: 47% to 29%), indicating superoxide anion was more easily produced in the photocleaving reaction of **4b**.

The effect of side chains on the order of photocleavage abilities was parallel to that of their DNA intercalating abilities (**3b** > **3a**, **4b** > **4a**), but anti-parallel to that of their antitumor activities (**3a** > **3b**, **4a** > **4b**). The reason accounting for it probably lies on that: the cytotoxicity of one compound is determined by two conflicting factors including cell membrane crossing ability and DNA binding ability. The side chains could be protonated to different extent under physiological pH due to their different basicity of corresponding nitrogen atoms. The protonation extent significantly affected the ability of the molecule to pass through lipophilic memebranes to bind to DNA. Lower degree of protonation is desirable for cell penetration, but higher degree of protonation favored DNA binding. Once these two factors effectively compromise, the higher antitumor potency is possible, such as **3a**, **4a**. Also, the selective cytotoxicities of these compounds were possibly determined by their different memebrane crossing abilities for different cell lines.

The effect of heterocyclic rings on the order of photocleavage abilities (**4a** > **3a**, **4b** > **3b**) was anti-parallel to that of their DNA intercalating abilities (**3a** > **4a**, **3b** > **4b**). In our case, the five-membered thiophene ring was better conjugated to the naphthalene skeleton than the six-membered thioxanthene ring, resulting in the better planar chromophore of **3a** or **3b**. The better planar chromophore of **3** in turn accounted for the more efficient intercalating activity of **3**. As to **4a** or **4b**, its worse conjugating structure made it less stable under photo-irradiation. Then electrons were more easily transferred from the chromophore of **4** to oxygen to generate superoxide anions responsible for DNA cleavage to lead to its stronger photocleaving activity.

Furthermore, **3a–b** or **4a–b** photocleaved DNA more efficiently than corresponding napthalimides **1a–b** or **2a–b**.[5e–f] Electron densities of the thio-heterocyclic fused naphthalene chromophores were relatively higher than those of their corresponding naphthalimides due to the reduction of one strong electron-withdrawing carbonyl group. The higher chromophores' electron densities were inferred to grant **3a–b** or **4a–b** stronger abilities of transferring electrons, which were from their chromophores to oxygen to form more superoxide anions for photocleavage under photo-activation, consequently to higher photocleavage abilities than **1a–b** or **2a–b**.

3. Conclusion

In summary, the present work demonstrated the design, synthesis and quantitative evaluation of two kinds of thio-heterocyclic fused naphthalene carboxamides as efficient antitumor and DNA photocleaving agents. All these compounds were found to be more cytotoxic to P388 than to A549. Compounds with the five-membered thiophene rings were proved to be more efficient DNA intercalators while those with the six-membered thioxanthene rings were more efficient DNA photocleavers. The side chains played different roles in intercalating/photocleaving and antitumor activities.

4. Experimental

4.1. Materials

All the solvents were of analytic grade. ^{1}H NMR were measured on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO/$CDCl_3$-d_6, TMS as internal standard). Mass spectra were measured on a HP 1100 LC–MS spectrometer. Melting points were determined by an X-6 micro-melting point apparatus and uncorrected. Absorption spectra were determined on PGENERAL TU-1901 UV–VIS Spectrophotometer.

4.2. Synthesis

4.2.1. Benzothiophenonaphthalic anhydride 5. 4-Bromo-3-nitro-1,8-naphthalic anhydride (6.44 g) was stirred under reflux in ethanol (60 mL) with thiophenol (2.6 mL) for 5 h. The liquor was reduced in volume to 30 mL and filtered, washed with some ethanol, dried, giving 6.83 g (97.3%) of 3-nitro-4-phenylthio-1,8-naphthalic anhydride. The recrystallization from glycol monomethyl ether gave long golden needles. Mp: 177–178 °C. The above nitro compound (2.7 g) was stirred into a mixture of stannous chloride (8.81 g) and concentrated hydrochloric acid (12 mL). After warming to 40 °C, the temperature rose spontaneously to 85 °C and was maintained at 85 °C for 1 h (color change from orange to olive-green). The suspension was cooled and filtered to give 2.4 g crude product. Recrystallization from pyridine gave greenish-yellow needles of 3-amino-4-phenylthio-1,8-naphthalic anhydride. Mp: 224–225 °C ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 8.69 (d, J=8.5 Hz, 1H, 7-H), 8.37 (d, J=7.6 Hz, 1H, 5-H), 8.15 (s, 1H, 2-H), 8.15 (t, J_1=7.6 Hz, J_2=8.5 Hz, 1H, 6-H), 7.25–7.20 (m, 2H, 3′-H, 5′-H), 7.17–7.13 (m, 1H, 4′-H), 7.06–7.02 (m, 2H, 2′-H, 6′-H), 5.08 (s, 2H, NH_2). EI-MS: (m/z): 321.3 (M^+). Sodium nitrite (0.7 g) and glacial acid (2 mL) were added dropwise to concentrated sulfuric acid (10 mL). The mixture was cooled to 0–5 °C and 3-amino-4-phenylthio-1,8-naphthalic anhydride (3.2 g) was slowly added over 1 h. After stirring for 1 h, the dark red viscous liquor was added over 90 min to a boiling solution of copper sulfate (70 g) in water (1000 mL) and glacial acetic acid (120 mL). After the addition was complete, the liquor was refluxed for 30 min, cooled, filtered, dried and an orange solid (2.63 g, 85%) was collected. Recrystallization from DMF gave deep red needles. Mp: 284–286 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 9.4 (s, 1H, 7-H), 8.77–8.73 (m, 2H, 3-H, 1-H), 8.59 (d, J_1=1.0 Hz, J_2=7.4 Hz, 1H, 11-H), 8.30–8.26 (m, 1H, 2-H), 8.04 (d, J_1=8.2 Hz, J_2=7.4 Hz, 1H, 8-H), 7.70–7.66 (m, 2H, 9-H, 10-H). HRMS (ESI): Calcd for $C_{18}H_9O_3S$ $(M+H)^+$: 305.0272. Found: 305.0281.

4.2.2. Anhydro-8-hydroxylmercuri-1-benzothiophenonaphthoic acids 8 and 9. The obtained precipitate **5** (2.63 g) was suspend in 60 mL aqueous sodium hydroxide (1 g) and refluxed until the solid material dissolved, a solution of HgO (yellow) (2.34 g) in a mixture of H_2O (10 mL) and AcOH (7 mL) was added with stirring to result in slow evolution of carbon dioxide. The reaction mixture refluxed for 96 h, then cooled and filtered. The highly insoluble yellow solid was washed with water and dried under vacuum at 50 °C over night to give the mixture of **6** and **7** (4.11 g, 95% yield). Attempts to purify and separate the anhydro compounds were unsuccessful. It is insoluble in organic solvents. The above obtained solid (4.11 g) were suspended in 30 mL concentrated HCl, stirred, heated under reflux for 3 h. Hot filtration gave the mixture thio-heterocyclic fused naphthoic acids **8** and **9** (1.86 g, 76% yield). HRMS (ESI): Calcd for $C_{17}H_{12}O_2S$ $(M+H)^+$: 279.0480. Found: 279.0472.

4.2.3. *N*-(2-(Dimethylamino)ethyl)benzothiophenonaphalene-4-carboxamide 3a. The acids **9** and **10** (0.6 g) were treated with thionyl chloride (10 mL) and DMF (1 drop) in $CHCl_3$ (10 mL) under reflux for 20 h. After removal of the solvent and excess thionyl chloride, the crude solid and *N*,*N*-dimethyl ethyl diamine (0.4 mL) were combined in 25 mL CH_2Cl_2. The mixture cooled in an ice bath while Et_3N (0.45 mL) was added dropwise with stirring. The stirring continued for 20 h at room temperature. Removal of solvent and separated on silica gel chromatography (CH_2Cl_2:MeOH=6:1, v/v) to get pure **3a** (0.32 g, 42% yield). Mp: 194–195 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.42 (s, 6H, NCH_3), 2.75 (t, J_1=J_2=6.4 Hz, 2H, NCH_2), 3.55 (s, 2H, J_1=4 Hz, J_2=6.8 Hz, $CONHCH_2$), 7.58 (t, J_1=6.8 Hz, J_2=6.4 Hz, 2H, 8-H, 9-H), 7.72 (t, J_1=6.0 Hz, J_2=7.2 Hz, 2H, 2-H, 3-H), 8.13 (t, J_1=5.2 Hz, J_2=3.6 Hz, 1H, 10-H), 8.22 (t, J_1=4 Hz, J_2=5.2 Hz, 1H, 1-H), 8.30 (d, J=8 Hz, 1H, 6-H), 8.42 (s, 1H, 7-H), 8.44 (s, 1H, 5-H). HRMS (ESI): Calcd for $C_{21}H_{21}N_2OS$ $(M+H)^+$: 349.1375. Found: 349.1368. IR (KBr): 3268, 2921, 2815, 1634, 1555, 768 cm^{-1}.

4.2.4. *N*-(2-(Dimethylamino)propyl)benzothiophenonaphthalene-4-carboxamide 3b. Prepared and purified in a similar manner as that in **3a**, *N*,*N*-dimethylpropyl diamine was used here, instead of *N*,*N*-dimethylethyl diamine and separated on silica gel chromatography (CH_2Cl_2:MeOH=5:1, v/v) to get pure **3b** (39% yield). Mp: 183–184 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 1.82 (t, J_1=6.4 Hz, J_2=7.2 Hz, 2H, CH_2), 2.42 (s, 6H, NCH_3), 3.16 (s, 2H, NCH_2), 4.06 (t, J_1=7.2 Hz, J_2=7.2 Hz, 2H, $CONCH_2$), 7.56 (t, J_1=4.4 Hz, J_2=4 Hz, 2H, 8-H, 9-H), 7.69 (d, J=7.8 Hz, 1H, 3-H), 7.74 (t, J_1=J_2=8 Hz, 1H, 2-H), 8.17 (t, J_1=4 Hz, J_2=4.8 Hz, 1H, 1-H), 8.23 (d, J=8.8 Hz, 2H, 7-H, 10-H), 8.47 (t, J_1=8.8 Hz, J_2=7.8 Hz, 2H, 5-H, 6-H). HRMS (ESI): calcd for $C_{22}H_{23}N_2OS$ $(M+H)^+$: 363.1531, Found: 363.1523. IR (KBr): 3257, 2922, 2853, 1632, 1544, 767 cm^{-1}.

4.2.5. 5-Bromo-1-naphthoic acid 16. 4-Bromo-1,8-naphthalic anhydride (2.77 g) was suspend in 50 mL aqueous sodium hydroxide (1.25 g) and refluxed until the solid material dissolved, a solution of HgO (red or yellow) (2.2 g) in a mixture of H_2O (2 mL) and AcOH (2 mL) was added with stirring to result in slow evolution of carbon dioxide. The reaction mixture refluxed for 96 h, then cooled and filtered. The highly insoluble yellow solid was washed with water and dried under vacuum at 100 °C over night to give the mixture of **13** and **14** (4.9 g, 96% yield). The obtained precipitates were suspended in 25 mL concentrated HCl, stirred, heated under reflux for 3 h. Hot filtration gave the mixture of 4-bromo-1-naphthoic acid **15** and 5-bromo-1-naphthoic acid **16** with ratio of 2:3 via ^{1}H NMR. Pure 5-bromo-1-naphthoic acid **16** was obtained after recrystallization from acetic acid (1.1 g, 42% yield), mp: 205–206 °C, ^{1}H NMR (d_6-DMSO) δ (ppm): 7.57 (t, J_1= 8 Hz, J_2=7.6 Hz, 1H, 7-H), 7.77 (t, J_1=8 Hz, J_2=8.4 Hz, 1H, 3-H), 8.00 (d, J=7.6 Hz, 1H, 6-H), 8.23 (d, J=7.2 Hz, 1H, 4-H), 8.43 (d, J=8.4 Hz, 1H, 2-H), 8.88 (d, J=8.8 Hz, 1H, 8-H). HRMS (ESI): Calcd for $C_{11}H_8BrO_2$ $(M+H)^+$: 250.9708. Found: 250.9715.

4.3. Methyl-5-bromo-1-naphthoate 17

The pure 5-bromo-1-naphthoic acid **16** (4.16 g) was combined with thionyl chloride (30 mL) and DMF (1 drop) under reflux for 20 h. After removal of the excess thionyl chloride, 30 mL methanol was added, reacted for 1 h under reflux. The crude methyl-5-bromo-1-naphthoate was obtained after removal of the excess methanol. ESI-MS (positive) *m/z*: 266.5 $(M+H)^+$.

4.3.1. Benzothioxanthenenaphthoic acid 20. Methyl-5-bromo-1-naphthoate **17** (3.75 g) was stirred under reflux in DMF (40 mL) with 2-aminobenzenethiol (2.12 g) and K_2CO_3 (1.035 g) for 80 h. Cooled and poured into 100 mL water, filtered and dried under vacuum to obtain the crude solid of **18** (3.56 g, 74%). ESI-MS (positive) *m/z*: 310.1 $(M+H)^+$. 11 mL aqueous solution of sodium nitrite (8.35 g) was slowly added into mixture of **18** (3.56 g), HCl (3 mL) glacial acetic acid (30 mL) and H_2O (5 mL) within 1 h at 0–5 °C. After stirring for 12 h, the dark red viscous liquor was added over 90 min to a boiling solution of copper sulfate (8.17 g) in water (120 mL) and glacial acetic acid (7 mL). After the addition was complete, the liquor was refluxed for 30 min, cooled, filtered, dried and an dark-orange solid **19** (3.23 g, 95%) was collected. ESI-MS (positive) *m/z*: 293.1 $(M+H)^+$. The above obtained solid **19** was added into the mixture of 20%NaOH (50 mL) and methanol (50 mL), then the mixture was refluxed for 1 h, removal of excess methanol, cooled, filtered, dried under vacuum to obtain **20** (1.95 g, 62%), mp: >300 °C. HRMS (ESI): Calcd for $C_{17}H_{12}O_2S$ $(M+H)^+$: 279.0480. Found: 279.0489.

4.3.2. *N*-(2-(Dimethylamino)ethyl) benzothioxanthenenaphthalene-3-carboxamide 4a. The acid **20** (0.6 g) was treated with thionyl chloride (10 mL) and DMF (1 drop) in $CHCl_3$ (10 mL) under reflux for 20 h. After removal of the solvent and excess thionyl chloride, the crude solid and *N*,*N*-dimethyl ethyl diamine (0.4 mL) were combined in 25 mL CH_2Cl_2. The mixture cooled in an ice bath while Et_3N (0.45 mL) was added dropwise with stirring. The stirring continued for 20 h at room temperature. Removal of solvent and separated on silica gel chromatography to afford the pure products. Separated on silica gel chromatography (CH_2Cl_2:MeOH=6:1, v/v) to get pure **4a** (0.30 g, 42% yield). Mp: 223–224 °C. ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 2.88 (s, 6H, NCH_3), 3.05 (t, J_1=J_2=3.6 Hz, 2H, NCH_2), 3.67 (d, 2H, J=5.6 Hz, $CONHCH_2$), 7.40 (t, J_1=J_2=3.6 Hz, 2H, 8-H, 9-H), 7.49 (d, J=3.2 Hz, 1H, 1-H), 7.61 (t, J_1=J_2=6.4 Hz, 1H, 5-H), 7.85 (s, 1H, 2-H), 8.17 (d, J=8 Hz, 1H, 10-H), 8.23 (d, J=6.8 Hz, 1H, 6-H). 8.30 (d, J=8 Hz, 1H, 7-H), 8.94 (t, J_1=J_2=3.6 Hz, 1H, 4-H). HRMS (ESI): Calcd for $C_{21}H_{21}SN_2O$ $(M+H)^+$: 349.1375. Found: 349.1378. IR (KBr): 3266, 2918, 2849, 1638, 1548, 762 cm^{-1}.

4.3.3. *N*-(2-(Dimethylamino)propyl) benzothioxanthenenaphthalene-3-carboxamide 4b. Prepared and purified in a similar manner as that in **4a**, *N*,*N*-dimethylpropyl diamine was used here, instead of *N*,*N*-dimethylethyl diamine and separated on silica gel chromatography (CH_2Cl_2: MeOH= 4:1, v/v) to get pure **4b** (36% yield). Mp: 217–218 °C. ^{1}H NMR (d_6-$CDCl_3$) δ (ppm): 1.89 (t, J_1=6.4 Hz, J_2=7.2 Hz, 2H, CH_2), 2.58 (s, 6H, NCH_3), 2.62 (s, 2H, NCH_2), 3.03 (t, J_1=7.2 Hz, J_2=7.2 Hz, 2H, $CONCH_2$), 7.42 (t, J_1=J_2= 6.4 Hz, 2H, 8-H, 9-H), 7.48 (d, J=3.2 Hz, 1H, 1-H), 7.65 (t, J_1=J_2=6.8 Hz, 1H, 5-H), 7.81 (s, 1H, 2-H), 8.16 (d, J= 7.8 Hz, 2H, 7-H, 10-H), 8.247 (d, J=6.4 Hz, 1H, 6-H). 8.29 (d, J=6.4 Hz, 1H, 4-H). HRMS (ESI): Calcd for $C_{22}H_{23}N_2OS$ $(M+H)^+$: 363.1531. Found: 363.1535. IR (KBr): 3274, 2940, 2856, 1634, 1541, 763 cm^{-1}.

4.4. Intercalation studies of compounds to CT-DNA

0.1 mL solution of a compound in DMSO (10^{-3}–10^{-4} M) mixed with 20 mM Tris–HCl (pH=7.5) to 5 mL. Then, two groups of samples were prepared in the concentration of chemical at 5, 10, 20, 40 μM, one contained Calf-thymus DNA 50 μM, the other contained no DNA but had the same concentration of chemical as control. All the above solution was shaken for 3 days at 25 °C in the dark. Fluorescence wavelength and intensity area of samples were measured.

4.5. Cytocoxicity in vitro evaluation

The prepared compounds have been submitted to Shanghai Institute of Materia Medica for testing their cytotoxicities.

4.6. Photocleavage of supercoiled pBR322 DNA

250 mg pBR322DNA (form I), 1 μL solution of chemical and 20 mM Tris–HCl (pH=7.5) were mixed to 10 μL, then irradiated for 3 h with light (360 nm) using lamp placed at 20 cm from sample. Supercoiled DNA runs at position I, nicked DNA at position II, linear DNA at position III. The samples were analyzed by gel electrophoresis in 1% Agarose and gel was stained with ethidium bromide.

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