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Novel heterocyclic family of phenyl naphthothiazole carboxamides derived from naphthalimides: synthesis, antitumor evaluation, and DNA photocleavage

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Abstract—A new heterocyclic family of (2-(dimethylamino)ethyl)-2-substituted phenylnaphtho[2,1-*d*]thiazole-5-carboxamides modified from naphthalimides was designed, synthesized, and quantitatively evaluated as antitumor agents and photonucleases. All these compounds were found to be more cytotoxic against P388 than against A549. **B_3** (*m*-NO_2) was found to be the strongest inhibitor for P388 with IC_{50} of 1.49 μM, while **B_2** was the most cytotoxic compound against A549 with IC_{50} of 12 μM. **B_4** (*p*-CH_3), the most efficient DNA photocleaver, showed detectable DNA cleavage at 0.5 μM and total cleavage from form I to 100% form II at 50 μM. The photocleaving mechanism was changed with the modification to be via superoxide anion and radical.

1. Introduction

Naphthalimides form a famous class of intercalating agents that consists of a flat, generally π-deficient aromatic or heteroaromatic system, which binds to DNA by insertion between base pairs of the double helix. Many of them have not only shown high antitumor activities upon a variety of murine and human tumor cells[1] but also capabilities of generating a multitude of reactive intermediates that resulted in DNA photocleavage.[2,3]

Various attempts made to modify the naphthalimide units to promote their DNA antitumor and photocleaving abilities are focused on incorporating substituents or on fusing five or six membered (phenyl or heterocyclic) rings on the naphthalene skeletons.[1–3] The larger aromatic ring system was proved to account for the higher affinity for DNA and consequently for higher antitumor and photocleaving activities. A family of phenyl thiazonaphthalimide intercalators, **A_1**–**A_4** (Fig. 1a), has been reported as efficient photonucleases in our previous research[2f] whose photocleavage was caused by the naphthalimide-thiazole radicals produced via excited triplet state.

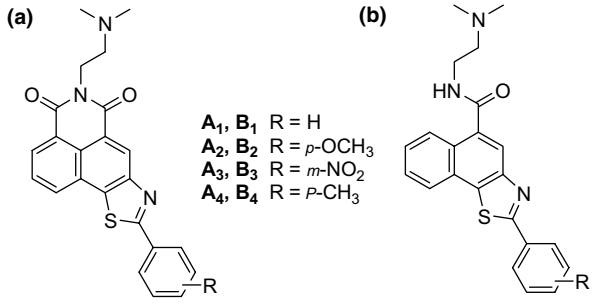

Figure 1. Structures of the reported and novel designed compounds.

In our continued efforts to develop simple but efficient antitumor and photocleaving agents, we proposed a novel molecular design of modifying the reported photonucleases, **A_1**–**A_4**, to corresponding ring-opened models of phenyl naphthothiazole carboxamides, **B_1**–**B_4** (Fig. 1b), whose planar tri-cyclic ring systems may also intercalate into DNA to show medical or biological

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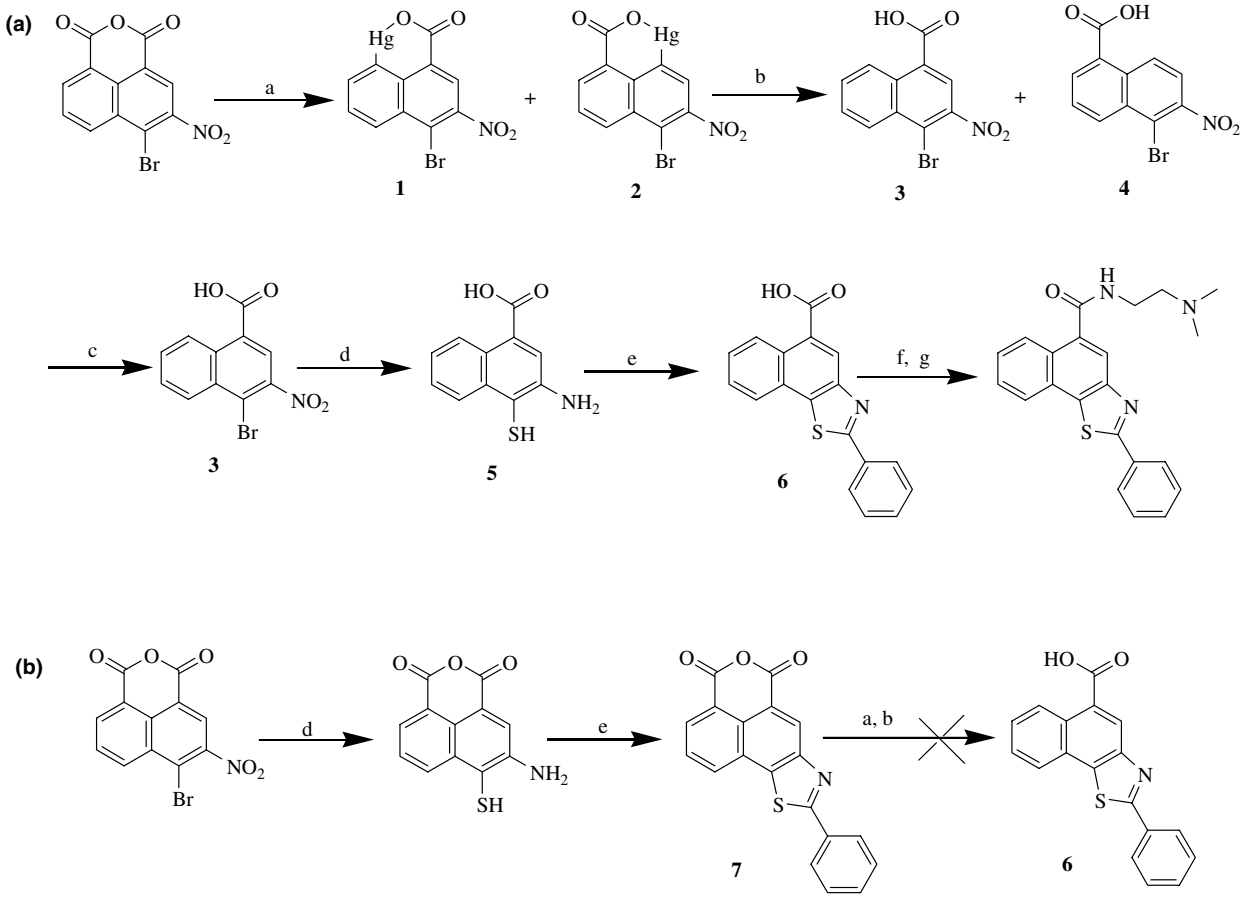
potential. Furthermore, the functional phenyl thiazole groups proven active in DNA photocleavage[2f] were remained. The aminoalkyl side chain serving as DNA groove binder and/or external electrostatic binder was inferred to be more flexible to increase the affinity with DNA.

2. Results and discussion

2.1. Synthesis and spectra

The replacement by mercury of one of the carbonyl groups in naphthalic anhydride derivatives has long been known,[4] but the mercuric oxide mediated decarbonylation of 4-bromo-3-nitro-1,8-naphthalic anhydride[5] has not been reported. When 4-bromo-3-nitro-1,8-naphthalic anhydride reacted with red mercuric oxide and acetic acid according to the methods[4] used in other (substituted) naphthalic anhydrides, two isomers, 4-bromo-3-nitro-1-naphthoic acid **3** and 4-bromo-3-nitro-8-naphthoic acid **4** were obtained with the ratio of 4:1 via ^{1}H NMR, and pure 4-bromo-3-nitro-1-naphthoic acid **3** was obtained after recrystallization from acetic acid. The obtained pure acid **3** refluxed in water with sodium disulfide for 8 h to form 4-mercapto-3-amino-1-naphthoic acid **5** whose solution was dropped into glacial acetic acid containing benzaldehyde (e.g., $\mathbf{B_1}$) immediately, then refluxed for 4 h to get phenyl naphthothiazole-5-carboxylic acid **6**. Finally, the corresponding naphthoyl chloride was prepared from the obtained acid by refluxing for 20 h in a mixture of thionyl chloride and chloroform, then the naphthoyl chloride condensed with *N,N*-dimethylethylenediamine in dichloromethane to form the desired product (Scheme 1a). Structures of the final products were all identified by ^{1}H NMR, HRMS, IR. The above experiments also provided a new way to synthesize (substituted) phenyl naphthothiazole carboxamide systems which may have potential in other fields. However, compared with those of (substituted) naphthalic anhydrides, the reacting ability of phenyl thiazonaphthalic anhydride **7**[2f] decreased which was possibly caused by the introduction of heterocyclic atoms and/or the enlargement of the conjugation area resulting in the decarbonylation reaction mediated by red mercuric oxide not taking place (Scheme 1b).

It was found that compounds, $\mathbf{B_1}$, $\mathbf{B_2}$, $\mathbf{B_4}$, had slight difference in either emission wavelength (394, 396, 395 nm, respectively) or absorption wavelength (358, 360, 359 nm, respectively). Due to the nitro group's electron-withdrawing effect, the maximal absorption of $\mathbf{B_3}$ was found to decrease to 348 nm, while its emission



Scheme 1. Synthesis of target (2-(dimethylamino)ethyl)-2-phenyl naphtho[2,1-*d*]thiazole-5-carboxamide (e.g., $\mathbf{B_1}$). Reagents and conditions: (a) HgO (red), NaOH, AcOH, H_2O, reflux 4 days, 98% yield; (b) concentrated HCl, reflux 2 h; (c) recrystallized with AcOH, 40% yield; (d) Na_2S_2, H_2O, 4 h; (e) benzaldehyde, AcOH, N_2, reflux 4 h, 65% yield; (f) $SOCl_2$, $CHCl_3$, Et_3N; (g) *N*,*N*-dimethylethylenediamine, CH_2Cl_2, 20 h.

Table 1. Spectra data,[a,b] photocleaving activity[c,d] and cytotoxicity (A-549[e], P388[f]) of compounds

Compd	UV $\lambda_{max/nm}$ (lg ε)	FL $\lambda_{max/nm}$ (Φ)	Photocleaving activity[c,d]		Cytotoxicity (IC_{50}, μM)	
			I%	II%	A549[e]	P388[f]
B_1	358 (3.73)	394 (0.003)	29	71	206	2.36
B_2	360 (3.91)	396 (0.009)	22	78	12	2.16
B_3	348 (3.32)	405 (0.001)	35	65	870	1.49
B_4	359 (4.37)	395 (0.005)	0	100	408	2.66
A_1[2f]	376 (3.85)	425 (0.006)	4	96	NT	NT
A_2[2f]	386 (3.76)	491 (0.017)	53	47	NT	NT
A_3[2f]	384 (3.78)	447 (0.009)	57	43	NT	NT
A_4[2f]	376 (3.90)	412 (0.002)	58	42	NT	NT

NT, not tested.
[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard ($\Phi = 0.55$).
[c,d] The concentration used for **B_1**–**B_4** in photocleaving activity evaluation was 50 μM, while that for **A_1**–**A_4** was 100 μM.
[e] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.[7]
[f] CTX against murine leukemia cells (P388) was measured by microculture tetrazolium-formazan method.[8]

wavelength had a red-shift to 405 nm. Compared with those of their corresponding naphthalimides, **A_1**–**A_4**,[2f] (Table 1), values of both the emission wavelength and the absorption wavelength of **B_1**–**B_4** are blue-shifted due to the reduction of their conjugation areas and electronic pushing–pulling ICT (intramolecular charge transfer) effects caused by the decarbonylation reaction.

2.2. DNA-intercalating property

The Scatchard binding constants for compounds, **B_1**, **B_2** and **B_4**, to calf thymus DNA (buffered in 20 mM Tris–HCl buffer, pH 7.5) were determined to be $6.39 \times 10^4\ M^{-1}$, $1.22 \times 10^5\ M^{-1}$, and $7.36 \times 10^4\ M^{-1}$, respectively, according to the method of fluorescence quenching technique (Fig. 2).[6] A more stable complex of DNA and **B_2** was inferred to be formed by hydrogen bond between the oxygen atom on the phenyl ring and the hydrogen atom in base pairs of DNA molecule which may account for the higher constant value of **B_2** ($1.22 \times 10^5\ M^{-1}$).

The constant of **B_3** was not measured due to its weak fluorescence, however, the intercalation experiment of **B_3** was carried out by using an electronic absorption spectra method (Fig. 3) instead of fluorescence quenching technique. In the process of adding calf thymus DNA into the compound solution, absorption intensities decreased with the increase of DNA concentrations.

2.3. Antitumor evaluation

The antitumor activities in vitro (under scattered light) of these compounds were evaluated by sulforhodamine B (SRB) assay[7] against A549 (human lung cancer cell) and MTT tetrazolium dye assay[8] against P388 (murine leukemia cell), respectively (Table 1). The IC_{50} represents the drug concentration (micromolar) required to inhibit cell growth by 50%. All these compounds were found to be more cytotoxic against P388 than against A549 with IC_{50} ranging from 1.49 μM to 870 μM. No obvious correlation was found between their DNA affinity or photocleaving abilities (shown as follows) and antitumor activities. **B_3**, the most cytotoxic compound against P388 with IC_{50} of 1.49 μM, showed the weakest photocleaving ability. **B_2** was found to be the strongest growth inhibitor for A549 with IC_{50} of 12 μM. However, the modification of such agents for potent antitumor drugs seems to be not very successful in that many other reported efficient naphthalimides inhibited the tumor cells in nanomolar orders.[1] However, these data indicated

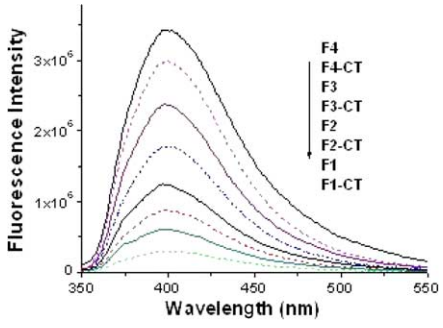


Figure 2. Fluorescence spectra before and after interaction of compound **B_2** and calf thymus DNA. Curves F and F–CT corresponded to compound **B_2** before and after being mixed with DNA. Numbers 1–4 indicated the concentration of **B_2**, 5, 10, 20, 40 μM, respectively. DNA applied was 50 μM (bp).

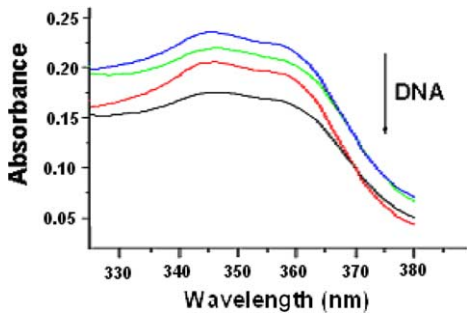


Figure 3. Interaction of **B_3** (50 μM) and calf thymus DNA. Absorption changes of **B_3** during addition of calf thymus DNA (0, 50, 100, 200 μM) (bp) in 20 mM Tris–HCl (pH 7.5) solution.

the importance of the six membered piperidinedione ring in naphthalimides on their antitumor activities.

2.4. DNA photocleavage

The cleavage abilities of compounds, $\mathbf{B_1}$–$\mathbf{B_4}$, were evaluated using closed supercoiled pBR322 DNA as substrate under photo-irradiation with a transluminator (360 nm) at a distance of 20 cm at 0 °C for 3 h under aerobic conditions, and no cleavage was observed without the light irradiation. The photocleaving efficiency of plasmid DNA was defined by the percentage of obtained nicked circular form (form II) converted from supercoiled form (form I) which could be visible on 1% agarose gel by electrophoresis. As shown in Figure 4a, all these compounds photocleaved plasmid DNA from form I to form II efficiently. $\mathbf{B_4}$ was found to be more efficient (100% form II) than its analogues under identical conditions. The concentration-dependent experiment of $\mathbf{B_4}$ exhibited detectable cleavage (21% form II) at concentration of 0.5 μM and total cleavage from form I to 100% form II at concentration of 50 μM (Fig. 4b). Also at concentration of 100 μM, $\mathbf{B_4}$ cleaved plasmid DNA completely to generate much smaller linear fragments which were invisible on the agarose gel (Fig. 4b, lane 8). It was obvious that $\mathbf{B_1}$–$\mathbf{B_4}$ photocleaved DNA more efficiently than their corresponding naphthalimides, $\mathbf{A_1}$–$\mathbf{A_4}$,[2f] through comparison of the obtained percentage of form II, even though the concentration used for $\mathbf{A_1}$–$\mathbf{A_4}$ was 100 μM, two times as high as that for $\mathbf{B_1}$–$\mathbf{B_4}$ (Table 1). The most efficient naphthalimide $\mathbf{A_1}$ showed detectable (19% form II) and total photocleavage (100% form II) of supercoiled pBR322 DNA at 5 and 100 M, respectively. Only 67% form II DNA was obtained at 50 μM by $\mathbf{A_1}$.[2f] These data proved our rational design for efficient DNA photocleaver. Additionally, the photocleaving ability of $\mathbf{B_4}$ increased remarkably with the prolongation of photo-irradiation time (Fig. 4c), showing it was a time-dependent cleavage process. No damage was observed in the absence of light (lane 3), proving UV light's function as trigger to initiate the DNA strand scission.

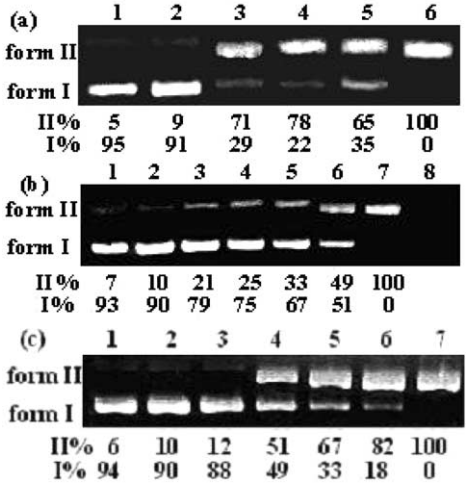


Figure 4. Photocleavage of closed supercoiled pBR322 DNA in the buffer of Tris–HCl (20 mM, pH 7.5). (a) Photocleavage of pBR322 DNA by different compounds (50 μM) for 3 h; lane 1, DNA alone (no hν); lane 2, DNA alone; lane 3–6, compounds, $\mathbf{B_1}$–$\mathbf{B_4}$, individually and DNA. (b) Photocleavage of DNA by $\mathbf{B_2}$ at various concentrations for 3 h. lane 1, DNA alone (no hν); lane 2, DNA alone; lane 3–7, $\mathbf{B_2}$ at concentration of 0.5, 1, 5, 10, 50, 100 μM, respectively. (c) Photocleavage of DNA by $\mathbf{B_2}$ (50 μM) at various time intervals. lane 1, DNA alone (no hν); lane 2, DNA alone; lane 3–7, 0, 30, 60, 120, 180 min, respectively.

Photocleavage can be via a variety of mechanisms involving free radical, electron transfer and singlet oxygen. In order to establish the reactive species responsible for cleavage of the plasmid DNA, $\mathbf{B_4}$ and $\mathbf{B_3}$ were chosen as example compounds to perform mechanistic experiments by addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide anion scavenger), ethanol (radical scavenger), respectively (Fig. 5a and b). It was clear that DTT and ethanol retarded the photocleavage process as for both $\mathbf{B_3}$ and $\mathbf{B_4}$ (lane 5, 6), indicating superoxide anion and radical were most possibly responsible for the DNA cleavage. In our cases, the damage to DNA by these compounds was possibly through both superoxide anions generated through electron transfer from the chromophores to oxygen, and radicals produced by the C=N bond in phenyl thiazole group and/or the C=O bond via photo-excited[3] (n–π*) state under photo-irradiation. The mechanism was found to be different from that of $\mathbf{A_1}$–$\mathbf{A_4}$, whose photocleavage was only via radicals produced via excited triplet state.[2f]

Electron densities of the phenyl thiazonaphthalene chromophores were relatively higher than those of the corresponding naphthalimides due to the reduction of one strong electron-withdrawing group, carbonyl group. The higher chromophores' electron densities were inferred to grant $\mathbf{B_1}$–$\mathbf{B_4}$ stronger abilities of transferring electrons, which were from their chromophores to oxygen to form superoxide anions responsible for photocleavage under photo-activation, consequently, $\mathbf{B_1}$–$\mathbf{B_4}$ exhibited higher photocleavage abilities with the emergence of superoxide anion species than $\mathbf{A_1}$–$\mathbf{A_4}$.

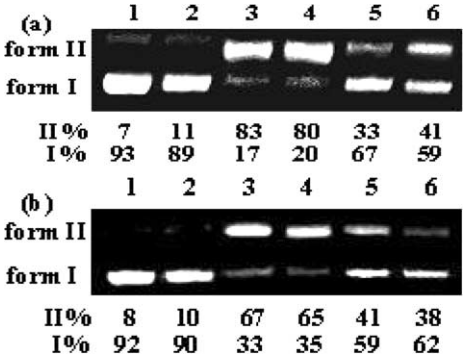


Figure 5. Effect of additives on the photocleavage of closed supercoiled pBR322 DNA by: (a) compound $\mathbf{B_4}$ (30 μM) and (b) $\mathbf{B_3}$ (50 μM) in the buffer of Tris–HCl (20 mM, pH 7.5) for 3 h. lane 1, DNA alone (no hν); lane 2, DNA alone; lane 3, DNA, and compound $\mathbf{B_4}$ or $\mathbf{B_3}$; lane 4–6, DNA and compound in the presence of histidine (12 mM), dithiothreitol (DTT, 60 mM), ethanol (3.4 M), respectively.

Substituent effects on photocleavage were described by the photocleavage ability order: $\mathbf{B_4}$ (p-CH_3) > $\mathbf{B_2}$ (p-OCH_3) > $\mathbf{B_1}$(H) > $\mathbf{B_3}$ (m-NO_2). Obviously, the compounds with electron-donating groups ($\mathbf{B_2}$, $\mathbf{B_4}$) photocleaved DNA more efficiently than $\mathbf{B_3}$ (m-NO_2) with electron-withdrawing group. The electron-donating groups were believed to have stronger abilities to push electrons so as to facilitate the electron transferring to form superoxide anions. DTT inhibited the photocleavage reaction of $\mathbf{B_4}$ more efficiently (form II: from 83% to 33%) than that of $\mathbf{B_3}$ (form II: from 67% to 41%) proving that superoxide anion was more easily produced in the photocleavage course of $\mathbf{B_4}$ (lane 5). As for $\mathbf{B_2}$, the hydrogen bond formed between the oxygen atom on phenyl ring and base pairs of DNA probably inhibit the electron-donating ability of the methyloxyl group, which might explain why $\mathbf{B_2}$ had a stronger electron-donating group but had lower efficiency in photocleavage than $\mathbf{B_4}$. By comparison, the reported photocleavage order of $\mathbf{A_1}$ (H) > $\mathbf{A_4}$ (p-CH_3) > $\mathbf{A_3}$ (m-NO_2) > $\mathbf{A_2}$ (p-OCH_3)[2f] could partly prove that superoxide anions might be not involved in photocleavage reactions of $\mathbf{A_1}$–$\mathbf{A_4}$.

3. Conclusion

The present work demonstrated the design and synthesis of compounds, $\mathbf{B_1}$–$\mathbf{B_4}$, as novel antitumor agents and DNA photocleavers. All these compounds were found to be more cytotoxic against P388 than against A549 with IC_{50} ranging from 1.49 to 870 μM. $\mathbf{B_3}$ (m-NO_2) was found to be the strongest inhibitor for P388 with IC_{50} of 1.49 μM, while $\mathbf{B_2}$ was the most cytotoxic one against A549 with IC_{50} of 12 μM. Compared with their corresponding naphthalimide derivatives, these compounds photocleaved DNA more efficiently. $\mathbf{B_4}$ (p-CH_3), the most efficient cleaver, showed detectable DNA cleavage at 0.5 μM and total cleavage from form I to form II at 50 μM. The mechanism experiments showed the cleavage occurred to DNA might be via superoxide anion and radical.

4. Experimental

4.1. Materials

All the solvents were of analytic grade. ^{1}H NMR was measured on a Bruker AV-400 spectrometer with chemical shifts reported as parts per million (in DMSO/$CDCl_3$-d_6, TMS as an 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. Anhydro-8-hydroxylmercuri-1-naphthoic acids (1 and 2). 4-Bromo-3-nitro-1,8-naphthalic anhydride (9.66 g, 30 mmol) was suspend in 100 mL aqueous sodium hydroxide (3.75 g, 94 mmol) and refluxed until the solid material dissolved, a solution of HgO (red) (6.6 g) in a mixture of H_2O (10 mL) and AcOH (6 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 overnight to give the mixture of **1** and **2** (15 g, 98% yield). Attempts to purify and separate the anhydro compounds were unsuccessful. They are insoluble in organic solvents.

4.2.2. 4-Bromo-3-nitro-1-naphthoic acid (3). The obtained precipitates, **1** and **2** were suspended in 80 mL concentrated HCl, stirred, heated under reflux for 3 h. Hot filtration gave the mixture of 4-bromo-3-nitro-1-naphthoic acid **3** and 4-bromo-3-nitro-8-naphthoic acid **4** with ratio of 4:1 via ^{1}H NMR. Pure 4-bromo-3-nitro-1-naphthoic acid **3** was obtained after recrystallization from acetic acid (3.4 g, 40% yield). Mp: 225.1–226 °C. ^{1}H NMR (DMSO-d_6) δ (ppm) 7.94 (m, J_1 = 7.8 Hz, J_2 = 7.2 Hz, J_2 = 7.6 Hz, 2H, 5-H, 8-H), 8.50 (J_1 = 6.0 Hz, J_2 = 7.2 Hz, J_2 = 6.8 Hz, 2H, 6-H, 7-H), 8.94 (s, 1H, 2-H). ESI-MS (negative) m/z: 293.9 $(M-H)^+$.

4.2.3. 2-Phenylnaphtho[2,1-*d*]thiazole-5-carboxylic acid (6). $Na_2S{\cdot}9H_2O$ (4.32 g, 18 mmol) and S (1.152 g, 36 mmol) were refluxed in 50 mL H_2O for 0.5 h till S was all dissolved. Pure 4-bromo-3-nitro-1-naphthoic acid **3** (1.18 g, 4 mmol) was added within 0.5 h and refluxed for 8 h, the reaction mixture cooled and filtered to get the dark red solution of 4-mercapto-3-amino-1-naphthoic acid **5**. Due to its instability in the air, it was dropped into glacial acetic acid containing benzaldehyde (0.448 mL) immediately under the protection of N_2 and refluxed for 4 h, cooled, and poured into 1000 mL ice water, filtered, washed with water, and dried under vacuum at 40 °C over night to obtain the brown solid of 2-phenylnaphtho[2,1-*d*]thiazole-5-carboxylic acid **6**. (0.75 g, 65% yield). Mp: 232–235 °C. ESI-MS (negative) m/z: 306.0 $(M-H)^+$.

4.2.4. *N*-(2-(Dimethylamino)ethyl)-2-phenylnaphtho[2,1-*d*]-thiazole-5-carboxamide ($\mathbf{B_1}$). 2-Phenylnaphtho[2,1-*d*]thiazole-5-carboxylic acid **6** (0.75 g) was treated with thionyl chloride (15 mL) and DMF (1 drop) in $CHCl_3$ (15 mL) at reflux temperature for 20 h. After removal of the solvent and excess thionyl chloride, the crude solid, and *N*,*N*-dimethyl ethyl diamine (0.5 mL) were combined in 25 mL CH_2Cl_2. The mixture cooled in an ice bath while Et_3N (0.55 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 afford the pure product $\mathbf{B_1}$ (0.45 g, 51% yield). Mp: 186–187 °C. ^{1}H NMR ($CDCl_3$-d_6) δ (ppm): 2.33 (s, H, NCH_3), 2.66 (t, J_1 = 6 Hz, J_2 = 5.2 Hz, 2H, NCH_2), 3.69 (d, J_1 = 4 Hz, 2H, $CONHCH_2$), 7.02 (s, 1H, CONH), 7.52 (d, J = 5.2 Hz, 3H, 3′-H, 4′-H, 5′-H), 7.62 (m, J_1 = 7.6 Hz, J_2 = 8 Hz, J_3 = 7.6 Hz, J_4 = 7.6 Hz, 2H, 7-H, 8-H), 8.05 (d, J_1 = 7.6 Hz, 9-H), 8.13 (t, J_1 = 2 Hz, J_2 = 3.2 Hz, 2H, 1′-H, 6′-H), 8.26 (s, 1H,

4-H), 8.50 (d, $J = 8$ Hz, 1H, 6-H). HRMS (ESI): calcd for $C_{22}H_{22}N_3OS$ $(M+H)^+$: 376.1484. Found: 376.1475. IR (KBr): 3285, 2921, 2850, 1635, 1536, 761 cm^{-1}.

4.2.5. *N*-(2-(Dimethylamino)ethyl)-2-(4-methoxyphenyl) naphtha[2,1-*d*]thiazole-5-carboxamide (B_2). Prepared in a similar manner as that in B_1, 4-methoxybenzaldehyde was used here instead of benzaldehyde and separated on silica gel chromatography (CH_2Cl_2/MeOH, 4:1, v/v). Mp: 194–195 °C. ^1H NMR ($CDCl_3$-d_6) δ (ppm): 2.92 (s, 6H, NCH_3), 3.44 (s, 2H, NCH_2), 3.86 (s, 3H, OCH_3), 3.98 (s, 2H, $CONHCH_2$), 6.92 (d, $J = 8.8$ Hz, 2H, 3′-H, 5′-H), 7.46 (m, $J_1 = 7.6$ Hz, $J_2 = 8.8$ Hz, $J_3 = 8.8$ Hz, $J_4 = 6.4$ Hz, 2H, 7-H, 8-H), 7.82 (d, $J = 7.6$ Hz, 6-H), 7.89 (d, $J = 8.4$ Hz, 2H, 2′-H, 6′-H), 8.30 (s, 1H, 4-H), 8.33 (d, $J = 8$ Hz, 1H, 9-H), 8.57 (s, 1H, CONH). HRMS (ESI): calcd for $C_{23}H_{24}N_3O_2S$ $(M+H)^+$: 406.1545. Found: 406.1554. IR (KBr): 3285, 2921, 2850, 1635, 1606, 827 cm^{-1}.

4.2.6. *N*-(2-(Dimethylamino)ethyl)-2-(3-nitrophenyl) naphtho[2,1-*d*]thiazole-5-carboxamide (B_3). Prepared in a similar manner as that in B_1, 3-nitrobenzaldehyde was used here instead of benzaldehyde and separated on silica gel chromatography (CH_2Cl_2/MeOH, 3:1, v/v). Mp: 225.1–226 °C. ^1H NMR (DMSO-d_6) δ (ppm): 2.50 (s, H, NCH_3), 2.58 (s, 2H, NCH_2), 3.50 (s, 2H, $CONHCH_2$), 7.69 (t, $J_1 = 8.1$ Hz, $J_2 = 7.2$ Hz, 1H, 8-H), 7.753 (t, $J_1 = 7.8$ Hz, $J_2 = 7.2$ Hz, 1H, 7-H), 7.90 (t, $J_1 = 8.1$ Hz, $J_2 = 7.2$ Hz, 1H, 5′-H), 8.22 (d, $J = 6$ Hz, 2H, 2′-H, 9-H), 8.39 (d, $J = 8.2$ Hz, 2H, 4′-H, 6′-H), 8.54 (d, $J = 7.9$ Hz 1H, 6-H), 8.86 (s, 1H, 4-H), HRMS (ESI): calcd for $C_{22}H_{21}N_4O_3S$ $(M+H)^+$): 421.1334. Found: 421.1332. IR (KBr): 3272, 2947, 2859, 1639, 1532, 1344, 760 cm^{-1}.

4.2.7. *N*-(2-(Dimethylamino)ethyl)-2-*p*-tolylnaphtho[2,1-*d*]-thiazole-5-carboxamide (B_4). Prepared in a similar manner as that in B_1, 4-methylbenzaldehyde was used here instead of benzaldehyde and separated on silica gel chromatography (CH_2Cl_2/MeOH, 6:1, v/v). Mp: 189–190 °C. ^1H NMR ($CDCl_3$-d_6) δ (ppm): 2.39 (s, H, NCH_3), 2.43 (s, 3H, CH_3), 2.73 (t, $J = 5.6$ Hz, 2H, NCH_2), 3.70 (d, $J = 5.6$ Hz, 2H, $CONHCH_2$), 7.16 (s, 1H, CONH), 7.30 (d, $J = 8$ Hz, 2H, 3′-H, 5′-H), 7.58 (m, $J_1 = 5.6$ Hz, $J_2 = 8.4$ Hz, $J_3 = 8$ Hz, $J_4 = 7.2$ Hz, 2H, 7-H, 8-H), 7.98 (d, $J = 8$ Hz, 3H, 1-H, 2′-H, 6′-H), 8.24 (s, 1H, 4-H), 8.459 (d, $J = 8$ Hz, 1H, 6-H). HRMS (ESI): calcd for $C_{23}H_{24}N_3OS$ $(M+H^+)$: 390.1640. Found: 390.1618. IR (KBr): 3273, 2922, 2852, 1634, 1537, 820 cm^{-1}.

4.2.8. 2-Phenyl thiazonaphthalic anhydride (7). Prepared in a similar manner as that in 2-phenylnaphtho[2,1-*d*]-thiazole-5-carboxylic acid **6**, 4-bromo-3-nitro-1,8-naphthalic anhydride was used here instead of 4-bromo-3-nitro-1-naphthoic acid **3**.

4.2.9. Intercalation studies of compounds to CT-DNA

(a) Solution of compound B_1, B_2, B_4 (0.1 mL) 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.

(b) Solution of B_3 (0.25 mL), in DMSO (10^{-3} M) mixed with 20 mM Tris–HCl (pH 7.5) to 5 mL. Then, one group of samples were prepared in the concentration of chemical at 50 μM, one did not contain calf thymus DNA as control, the others contained DNA with the concentration of 50, 100, 200 μM, respectively. All the above solution was shaken for 3 days at 25 °C in the dark. Absorption spectra of samples were measured.

4.2.10. Cytotoxic activity in vitro. The prepared compounds have been submitted to Shanghai Institute of Materia Medica for testing their cytotoxicities in vitro.

4.2.11. Photocleavage of supercoiled pBR322 DNA. Two hundred and fifty nanograms of pBR322 DNA (form I), 1 μL solution of chemical in organic solvent 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. 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.

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