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Novel thiazonaphthalimides as efficient antitumor and DNA photocleaving agents: Effects of intercalation, side chains, and substituent groups

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Abstract—A series of novel antitumor and DNA photocleaving agents was designed and synthesized by fusing a (substituted) thiazole ring to the naphthalimide skeletons. $\mathbf{C_1}$, the most active compound against A549, was about 30-fold more cytotoxic than the compound amonafide. $\mathbf{A_1}$, the most active compound against P388, was about 6-fold more cytotoxic than amonafide. $\mathbf{C_2}$, the most efficient DNA intercalator, showed the strongest DNA photocleaving activity via superoxide anion produced under UV light at 360 nm.

1. Introduction

DNA intercalating agents, characterized by the presence of a planar chromophore, generally a π-deficient aromatic system and one or two flexible basic side chains, constitute an important class of drugs in anticancer therapy.[1] Naphthalimides are significant examples, which have not only high antitumor activities on a variety of murine and human tumor cells,[2] but also capabilities of generating various reactive intermediates that result in DNA photocleavage.[3,4]

To broaden the scope of such cytotoxic and DNA photocleaving agents, a series of naphthalimides having larger chromophores, where the naphthalene chromophore was replaced by an anthracene or heterocyclic fused naphthalene chromophore,[4–6] has been reported (Fig. 1, **2**,[5a] **3**,[6c] **4**,[4f] **5**,[4b] as examples). The presence of a larger aromatic chromophore was proved to improve the affinity of the intercalator for the DNA molecule, consequently making it more cytotoxic than the parent compound amonafide (Fig. 1)[5,6] and/or giving it stronger DNA photocleaving activity.[4]

Inspired by the effect of fusing an aromatic ring to the naphthalimide skeleton on the antitumor and/or photocleaving activities, we synthesized a series of novel thiazonaphthalimides, $\mathbf{A_{1-2}}$–$\mathbf{C_{1-2}}$, and evaluated their DNA intercalating, antitumor, and photocleaving activities (Fig. 1). The thiazole ring, having appeared in several known anticancer antibiotics[7] or DNA photocleavers,[4c] has been fused to naphthalimide skeletons. Trifluoromethyl and methyl groups were also introduced to the thiazole ring to study their effects on bioactivities, which is important for future rational molecular design.

2. Results and discussion

2.1. Synthesis and spectra

Compounds, $\mathbf{A_{1-2}}$, $\mathbf{B_{1-2}}$, $\mathbf{C_{1-2}}$, were all synthesized from 4-bromo-3-nitro-1,8-naphthalic anhydride,[8] as shown in Scheme 1. 4-Bromo-3-nitro-1,8-naphthalic anhydride was reacted with sodium disulfide under reflux for 8 h in water to form 4-mercapto-3-amino-1,8-naphthalic anhydride **7** whose solution was dropped into glacial

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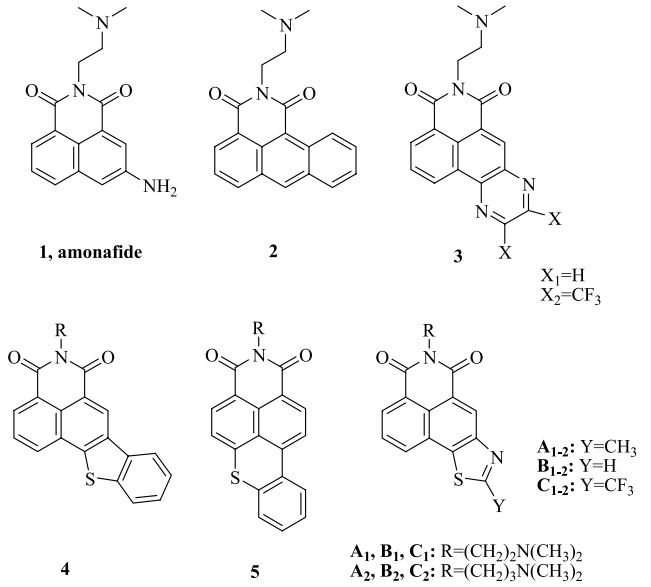


Figure 1. Structures of the reported (**1**–**5**) and novel (**A**–**C**) naphthalimides.

acetic acid containing acetaldehyde or formaldehyde immediately, and then refluxed for 4 h to get **8** or **9** as the most important intermediate. The obtained anhydride was then condensed with the corresponding amine in ethanol under reflux to form the corresponding $\mathbf{A}_{1-2}$ or $\mathbf{B}_{1-2}$. Solution of **7** was acidified by concentrated HCl, filtered under the protection of N_2 to get the solid 4-mercapto-3-amino-1,8-naphthalic anhydride, which was then quickly added into PPA and reacted with $(CF_3CO)_2O$ at 90 °C for 4 h to form **10**. $\mathbf{C}_{1-2}$ was synthesized after **10** was condensed with the corresponding amine in ethanol under reflux. Each pure product was obtained after careful column chromatography and was well identified by ^1H NMR, HRMS, and IR.

Table 1. Spectra data,[a,b] Scatchard binding constants of these compounds

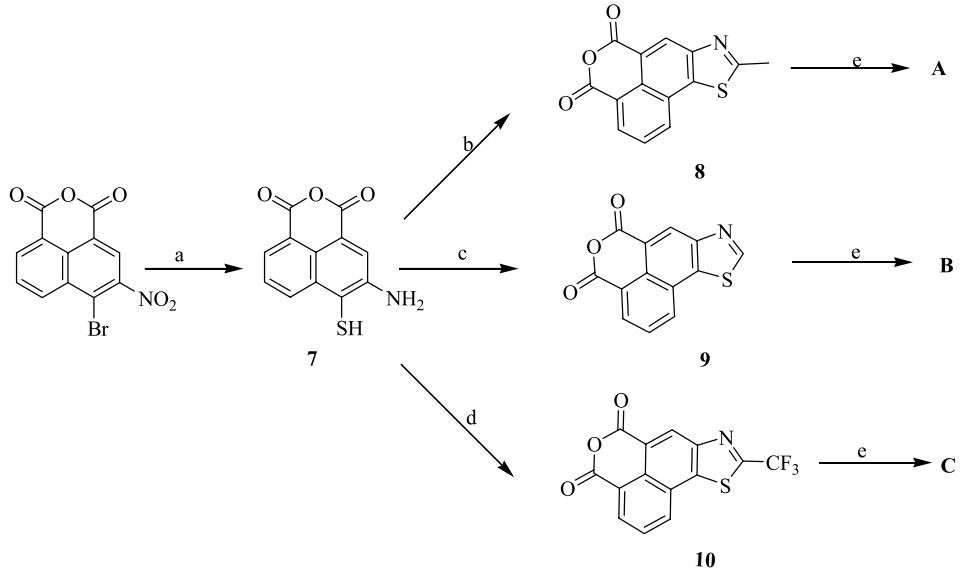
Compound	UV λ_{max} ($\log\varepsilon$)	FL λ_{max} (Φ)	Scatchard binding constants ($\times10^5$ M^{-1})
$\mathbf{A}_1$	351 (4.1)	425 (0.00015)	2.31
$\mathbf{A}_2$	353 (3.92)	423 (0.00048)	6.66
$\mathbf{B}_1$	348 (3.99)	426 (0.00019)	2.08
$\mathbf{B}_2$	347 (3.98)	426 (0.00015)	3.64
$\mathbf{C}_1$	353 (3.99)	429 (0.00025)	4.13
$\mathbf{C}_2$	353 (3.88)	426 (0.00012)	8.87

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

The UV–vis and fluorescent data for these thiazonaphthalimides were measured and are summarized in Table 1. Their maximum absorptions were all around 350 nm with similar intensities around 4.0 ($\log\varepsilon$). They also had similar emissions at about 425 nm with weak fluorescent intensities. The length of the aminoalkyl side chain and substituent groups had no obvious effects on the spectral data of these compounds.

3. DNA intercalating property

The affinities of these compounds for calf-thymus DNA were very strong, with the Scatchard binding constants as all above $\sim10^5$ M^{-1} (Table 1), determined by the fluorescence quenching technique[9] (Figs. 2 and 3), indicating that their binding abilities via intercalation by the thiazonaphthalimide chromophores were very efficient. It is obvious that the binding constants $\mathbf{X}_2$ are larger than those of $\mathbf{X}_1$ ($\mathbf{X}$ = **A**, **B**, and **C**), 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



Scheme 1. Synthesis of thiazonaphthalimides, $\mathbf{A}_{1-2}$, $\mathbf{B}_{1-2}$, and $\mathbf{C}_{1-2}$. Reagents and conditions: (a) Na_2S_2, H_2O, 8 h; (b) acetaldehyde, AcOH, N_2, reflux 4 h, 65% yield; (c) formaldehyde, AcOH, N_2, reflux 4 h, 69% yield; (d) $(CF_3CO)_2O$, PPA, 90 °C, 4 h, 60% yield; (e) corresponding amine, ethanol, reflux 3 h.

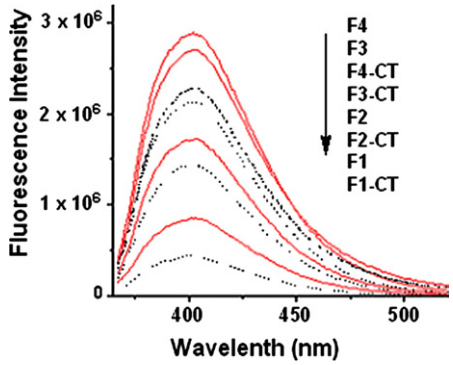


Figure 2. Fluorescence spectra before and after interaction of compound $\mathbf{A_2}$ and CT-DNA. Curves F and F-CT correspond to compound $\mathbf{A_2}$ before and after being mixed with DNA. Numbers 1–4 indicate the concentration of $\mathbf{A_2}$, 5, 10, 20, and 40 μM, respectively. DNA applied was 50 μM (bp).

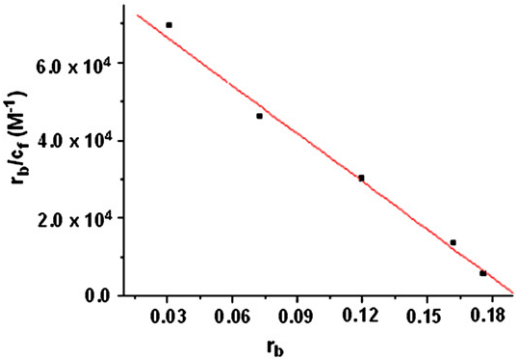


Figure 3. Scatchard curve between $\mathbf{A_2}$ and calf-thymus DNA.

nitrogen in proximity to functional groups suitable for hydrogen bonding on the DNA double helix after the thiazonaphthalimide 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. The intercalation of $\mathbf{C_2}$ with DNA was favored by the highly electron deficient chromophore caused by the two electron-withdrawing trifluoromethyl groups. The hydrogen bonds formed between fluorine atoms and bases in DNA may also play roles.

4. Antitumor evaluation

The antitumor activities of these novel thiazonaphthalimides were evaluated in vitro (under scattered light) against A549 (human lung cancer cell) and P388 (murine leukemia cell) cell lines, respectively. The results are summarized in Table 2 and are compared with the activity of the parent compound amonafide.

According to the data in Table 2, cytotoxic potencies of these compounds against these two tumor cell lines were highly dependent on the length of side chain. The compound with two methylene units in the side chain between two nitrogen atoms was more cytotoxic than the corresponding homologue with one more methylene unit, a result similar to that found for the homologue of amonafide.[10]

Table 2. Cytotoxicity of these compounds against the A549,[a] P388[b]

Compound	Cytotoxicity (IC_{50}, nM)	
	A549[a]	P388[b]
$\mathbf{A_1}$	82.8	31
$\mathbf{A_2}$	1804	1703
$\mathbf{B_1}$	273	120
$\mathbf{B_2}$	319	1511
$\mathbf{C_1}$	31.7	252
$\mathbf{C_2}$	659	1106
Amonafide	1100	200

[a] Cytotoxicity (CTX) against human lung cancer cell (A549) was measured by sulforhodamine B dye-staining method.[11]
[b] CTX against murine leukemia cells (P388) was measured by microculture tetrazolium–formazan method.[12]

Among these efficient analogues, different substituent effects were exhibited by the following cytotoxicity orders: $\mathbf{C_1}$ (–CF_3) > $\mathbf{A_1}$ (–CH_3) > $\mathbf{B_1}$ (–H) against A549, $\mathbf{A_1}$ (–CH_3) > $\mathbf{C_1}$ (–CF_3) > $\mathbf{B_1}$ (–H) against P388. $\mathbf{C_1}$, the most active one against A549, was about 30-fold more cytotoxic than the parent compound amonafide. $\mathbf{A_1}$, the most active one against P388, was about 6-fold more active than amonafide. These results are in agreement with our hypothesis that the introduction of a thiazole ring to the naphthalimide skeleton would increase the affinity of the chromophore for the target DNA, and as a consequence leading to greater cytotoxic potency.

5. DNA photocleavage

The photocleavage of these compounds to supercoiled plasmid pBR322 DNA was evaluated by 1% agarose gel electrophoresis. The reaction mixture containing each compound and plasmid DNA was placed 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 revealed in Figure 4a, all these compounds showed efficient photocleaving activities in the order as follows: $\mathbf{C_2}$ > $\mathbf{A_2}$>$\mathbf{C_1}$ > $\mathbf{A_1}$ > $\mathbf{B_2}$ > $\mathbf{B_1}$. The photocleaving activity of $\mathbf{C_2}$ was stronger than those of their analogues in that it could totally convert supercoiled pBR322 DNA (form I) to form II (85%) and even form III (15%), the double-strand cuts or proximal single-strand cuts on opposite strands under identical conditions. Obvious DNA damage (22% form II) was found by $\mathbf{C_2}$ at a concentration of as low as 1 μM in a concentration-dependent experiment (Fig. 4b). Moreover, $\mathbf{C_2}$ photocleaved DNA more efficiently with the prolongation of photo-irradiation, indicating that it was a time-controlled photocleaving process. No damage was observed in the absence of either light or compound (Fig. 4c), proving that they

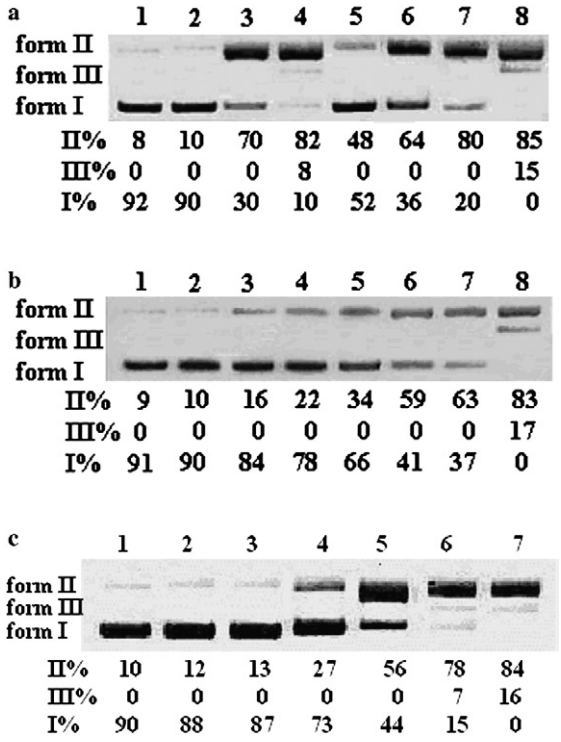


Figure 4. Photocleavage of closed supercoiled pBR322 DNA (200 μM/bp) in the buffer of Tris–HCl (20 mM, pH 7.5). (a) Photocleavage of plasmid pBR322 DNA by compounds (100 μM) for 3 h. Lane 1, DNA alone (without UV); lane 2, DNA alone (with UV); lanes 3–8, compounds $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{B_1}$, $\mathbf{B_2}$, $\mathbf{C_1}$, $\mathbf{C_2}$, individually, and DNA (with UV). (b) Photocleavage of plasmid pBR322 DNA by $\mathbf{C_2}$ at various concentrations for 3 h. Lane 1, DNA alone (without UV); lane 2, DNA alone (with UV); lanes 3–7, $\mathbf{C_2}$ at concentrations of 1, 5, 10, 50, and 100 μM, respectively (with UV). (c) Photocleavage of plasmid pBR322 DNA by $\mathbf{C_2}$ (100 μM) at various time intervals. Lane 1, DNA alone (without UV); lane 2, DNA alone (with UV); lanes 3–7, 0, 30, 60, 120, and 180 min (with UV), respectively.

were obligate factors for DNA strand scission. In our cases, UV light actually functioned as trigger to initiate the strand scission.

The photocleavage order of these compounds was almost parallel to that of their DNA intercalating abilities. However, there seemed to be no obvious correlation between DNA photocleaving/intercalating and antitumor activities. For example, $\mathbf{C_2}$ was the most efficient DNA intercalator/photocleaver, while it exhibited the weakest antitumor activity against A549. The reason accounting for it is probably 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 the different basicities of the corresponding nitrogen atoms. The protonation extent significantly affected the ability of the molecule to pass through lipophilic membranes to bind to DNA. A lower degree of protonation is desirable for cell penetration, but a higher degree of protonation favors DNA binding. Once these two factors are effectively balanced, higher antitumor potency is possible, as with $\mathbf{A_1}$ and $\mathbf{C_1}$. Poor balance of these two factors may have occured with $\mathbf{C_2}$, which resulted in its weak antitumor activity in the end.

Photocleavage can be effected via a variety of mechanisms involving free radicals, electron transfer, and singlet oxygen.[13] To establish the reactive species responsible for cleavage of the plasmid, mechanistic experiments were performed by the addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide anion scavenger), and ethanol (hydroxyl radical scavenger), respectively. The result for $\mathbf{C_2}$ is shown in Figure 5 as an example. It is clear that histidine and ethanol had no obvious effect on the cleavage reaction, indicating that singlet oxygen and hydroxyl radical were not likely to be the cleaving agents. However, the DNA cleaving activity of $\mathbf{C_2}$ decreased greatly in the presence of DTT (lane 6), suggesting that superoxide anion was most likely to be the reactive species responsible for the cleavage of plasmid. In our cases, compounds possibly were bound to DNA by aminoalkyl side chains, which also transfer electrons from nucleobases to the chromophores, and then to oxygen to generate superoxide anions responsible for DNA cleavage under photo-activation.

To illustrate the difference in photocleaving activity, the electron densities of these compounds were calculated with AM1 semi-empirical quantum calculation (Hyperchem7.0). No obvious difference was found between electron densities of these compounds at ground state, which were all concentrated on the aminoalkyl side chains (Fig. 7, $\mathbf{C_2}$ as an example). Also, electron densities of the same compound at either excited singlet state (figure not shown) or excited triplet state (Fig. 6) were almost the same. As shown in Figure 6, electron densities of $\mathbf{A_{1-2}}$ and $\mathbf{B_{1-2}}$ at excited state were almost equally diffused in their chromophores, while those of $\mathbf{C_{1-2}}$ were mainly concentrated on the trifluoromethyl thiazole rings, possibly caused by the strong electron-withdrawing trifluoromethyl groups. By comparing electron densities of these analogues at ground and excited states, we can see that the electron density differences between two states were in the following order: $\mathbf{C_{1-2}} > \mathbf{A_{1-2}}$–$\mathbf{B_{1-2}}$. The electrons were inferred to be more easily transferred from the side chains to the trifluoromethyl thiazole rings of $\mathbf{C_{1-2}}$, then to oxygen to generate the reactive superoxide anions under photo-irradiation, which could account for their higher DNA photocleaving activities in the end.

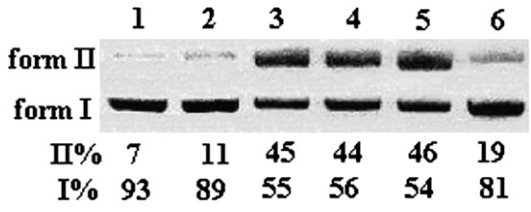


Figure 5. Effect of additives on the photocleavage of closed supercoiled pBR322 DNA (200 μM/bp) by compound $\mathbf{C_2}$ (15 μM) in the buffer of Tris–HCl (20 mM, pH 7.5) for 3 h. Lane 1, DNA alone (without UV); lane 2, DNA alone (with UV); lanes 3 DNA and compound; lanes 4–6, DNA and compound in the presence of histidine (6 mM), ethanol (1.7 M), dithiothreitol (DTT, 30 mM) (with UV), respectively.

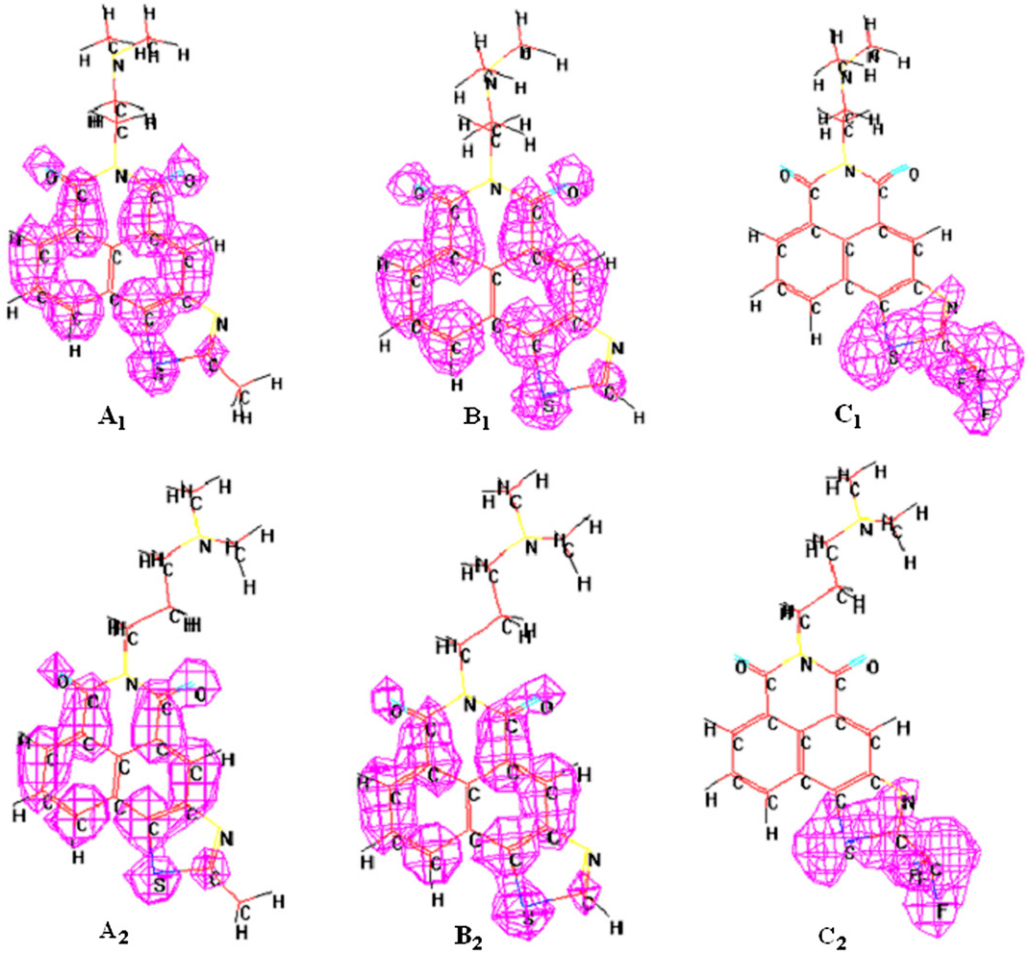


Figure 6. The simulated electron densities of these compounds at excited triplet state by AM1 semi-empirical calculation.

Figure 7. The simulated electron densities of $\mathbf{C_2}$ at ground state by AM1.

6. Conclusion

In conclusion, the presence of a thiazole ring fused to the naphthalimide skeleton leads to a greater cytotoxic potency with respect to the unfused compound amonafide against A549 and P388 cell lines. $\mathbf{C_1}$, the most active one against A549, was about 30-fold more cytotoxic than compound amonafide. $\mathbf{A_1}$, the most active one against P388, was about 6-fold more active than amonafide. All these compounds exhibited efficient DNA photocleaving activities. $\mathbf{C_2}$, the strongest DNA intercalator, was proved to be the most efficient DNA photocleaver via superoxide anion produced under UV light at 360 nm.

7. Experimental

7.1. Materials and methods

All the solvents were of analytic grade. The closed supercoiled pBR322 DNA was a gift of Takara Biotech Co. Ltd (Dalian). ^{1}H NMR was 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 with an X-6 micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Nexus 770 spectrometer. Fluorescence spectra were determined on a Hitachi F-4500. Absorption spectra were determined on a PGENERAL TU-1901 UV–vis spectrophotometer.

7.2. Synthesis

7.2.1. 4-Mercapto-3-amino-1,8-naphthalic anhydride (7). $Na_2S{\cdot}9H_2O$ (4.32 g, 18 mmol) and S (1.152 g, 36 mmol) were refluxed in H_2O (50 mL) for 0.5 h until S was entirely dissolved. 4-Bromo-3-nitro-1,8-naphthalic anhydride (1.288 g, 4 mmol) was added within 0.5 h and refluxed for 8 h, and the reaction mixture was cooled and filtered to get the dark red solution of **7**.

7.2.2. Methylthiazonaphthalic anhydride (8). The solution of **7** was added into glacial acid (60 mL) containing acetaldehyde (0.48 g 40% aq, 4.8 mmol) under the protection of N_2, the mixture was refluxed for 4 h, cooled and poured into 1000 mL ice water, and then filtered and dried to get the red product of **8** (0.78 g, 2.6 mmol, 65% yield), mp: 220–223 °C.

7.2.3. *N*-(2-(Dimethylamino)ethyl)-2-methylthiazo-naphthalimide (A_1). The above-obtained solid (0.3 g) was refluxed with *N*,*N*-dimethylethyldiamine (0.17 mL) in ethanol (20 mL) for 3 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2/MeOH = 6:1, v/v) to afford the pure product $\mathbf{A_1}$: mp: 176–177 °C. ^{1}H NMR ($CDCl_3$-d_6) δ (ppm): 2.49 (s, H, NCH_3), 2.84 (t, 2H, NCH_2), 2.99 (s, 3H, 9-CH_3), 4.42 (t, J_1 = 6.8 Hz, J_2 = 7.2 Hz, 2H, $CONCH_2$), 7.83 (t, J_1 = 7.6 Hz, J_2 = 8 Hz, 1H, 2-H), 8.31 (d, J = 7.6 Hz, 1H, 1-H), 8.64 (d, J = 6.4 Hz, 1H, 3-H), 9.08 (s, 1H, 7-H). HRMS (ESI): Calcd for $C_{18}H_{18}N_3O_2S$ $[M+H]^+$: 340.1120. Found: 340.1130. IR (KBr): 2920, 2850, 1700, 1660, 1330, 780 cm^{-1}.

7.2.4. *N*-(2-(Dimethylamino)propyl)-2-methylthiazo-naphthalimide (A_2). Prepared and purified in a similar manner as that in $\mathbf{A_1}$, *N*,*N*-dimethylpropyl diamine was used here instead of *N*,*N*-dimethylethyl diamine. Separated on silica gel chromatography (CH_2Cl_2/MeOH = 4:1, v/v) to afford pure $\mathbf{A_2}$: mp: 173–175 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.82 (m, 2H, NCH_2), 2.70 (s, 6H, NCH_3), 2.96 (s, 3H, 9-CH_3), 3.14 (t, J_1 = 7.2 Hz, J_2 = 8.4 Hz, 2H, CH_2), 4.13 (t, 2H, $CONCH_2$), 7.91 (t, J_1 = 8 Hz, J_2 = 7.2 Hz, 1H, 2-H), 8.51 (d, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 2H, 1-H, 3-H), 8.77 (s, 1H, 7-H). HRMS (ESI): Calcd for $C_{19}H_{20}N_3O_2S$ $[M+H]^+$: 354.1276. Found: 354.1292. IR (KBr): 2920, 2850, 2640, 1700, 1660, 1330, 780 cm^{-1}.

7.2.5. Thiazonaphthalic anhydride (9). Prepared and purified in a similar manner as that in **8**, formaldehyde was used here instead of acetaldehyde (69% yield), mp: 260–263 °C.

7.2.6. *N*-(2-(Dimethylamino)ethyl)-2-thiazonaphthalimide (B_1). Prepared and purified in a similar manner as that in $\mathbf{A_1}$, **9** was used here instead of **8**. Separated on silica gel chromatography (CH_2Cl_2/MeOH = 9:1, v/v) to afford the pure product $\mathbf{B_1}$: mp: 250–251 °C. ^{1}H NMR ($CDCl_3$-d_6) δ (ppm): 2.40 (s, 6H, NCH_3), 2.73 (t, 2H, NCH_2), 4.38 (t, J_1 = 6 Hz, J_2 = 6 Hz, 2H, $CONCH_2$), 7.87 (t, J_1 = 7.6 Hz, J_2 = 7.6 Hz, 1H, 2-H), 8.38 (d, J = 7.6 Hz, 1H, 1-H), 8.64 (d, J = 7.6 Hz, 1H, 3-H), 9.21 (s, 1H, 7-H), 9.24 (s, 1H, 9-H). HRMS (ESI): Calcd for $C_{17}H_{16}N_3O_2S$ $[M+H^+]$: 326.0963. Found: 326.0960. IR (KBr): 2920, 2850, 1700, 1660, 1330, 780 cm^{-1}.

7.2.7. *N*-(2-(Dimethylamino)propyl)-2-thiazonaphthalimide (B_2). Prepared and purified in a similar manner as that in $\mathbf{A_2}$, **9** was used here instead of **8**. Separated on silica gel chromatography (CH_2Cl_2/MeOH = 5:1, v/v) to afford the pure product $\mathbf{B_2}$: mp: 239–240 °C. ^{1}H NMR ($CDCl_3$-d_6) δ (ppm): 2.01 (m, J_1 = 6.4 Hz, J_2 = 7.8 Hz, J_3 = 6.8 Hz, 2H, CH_2), 2.36 (s, 6H, NCH_3), 2.59 (t, J_1 = 6.8 Hz, J_2 = 8 Hz, 2H, NCH_2), 4.29 (t, J_1 = 6.8 Hz, J_2 = 8 Hz, 2H, $CONCH_2$), 7.88 (t, J_1 = 8 Hz, J_2 = 8 Hz, 1H, 2-H), 8.389 (d, J = 8 Hz, 1H, 3-H), 8.65 (d, J = 8 Hz, 1H, 1-H), 9.22 (s, 1H, 7-H), 9.24 (s, 1H, 9-H). HRMS (ESI): Calcd for $C_{18}H_{18}N_3O_2S$ $[M+H^+]$: 340.1120. Found: 340.1125. IR (KBr): 2920, 2850, 2640, 1700, 1660, 1330, 780 cm^{-1}.

7.2.8. Trifluoromethylthiazonaphthalic anhydride (10). The solution of **7** was acidified by concentrated HCl, filtered under the protection of N_2. The obtained solid was added into PPA (40 mL) containing $(CF_3CO)_2O$ (1.5 mL) as soon as possible. The mixture was stirred mechanically, heated to 90 °C, and reacted for 4 h, then cooled and poured into 500 mL ice water, and then filtered and dried to get the crude product of **10** (0.35 g, 60% yield), mp: 240–244 °C.

7.2.9. *N*-(2-(Dimethylamino)ethyl)-2-trifluoromethylthiazo-naphthalimide (C_1). The above-obtained solid was refluxed with *N*-dimethylethyldiamine (0.2 mL) in ethanol (20 mL) for 2 h, cooled, the solvent removed, and separated on silica gel chromatography (CH_2Cl_2/MeOH = 7:1, v/v) to afford the pure product $\mathbf{C_1}$: mp: 215–216 °C. ^{1}H NMR ($CDCl_3$-d_6) δ (ppm): 2.64 (s, H, NCH_3), 3.04 (t, 2H, NCH_2), 4.49 (t, J_1 = 6.8 Hz, J_2 = 6.4 Hz, 2H, $CONCH_2$), 7.83 (t, J_1 = 8 Hz, J_2 = 7.6 Hz, 1H, 2-H), 8.31 (d, J = 8 Hz, 1H, 1-H), 8.64 (d, J = 7.2 Hz, 1H, 3-H), 9.08 (s, 1H, 7-H). HRMS (ESI): Calcd for $C_{18}H_{16}N_3O_2SF_3$ $[M+H^+]$: 394.0837. Found: 394.0837. IR (KBr): 2920, 2850, 1700, 1660, 1330, 780 cm^{-1}.

7.2.10. *N*-(2-(Dimethylamino)propyl)-2-trifluoromethylthiazo-naphthalimide (C_2). Prepared and purified in a similar manner as that in $\mathbf{C_1}$, *N*,*N*-dimethylpropyl diamine was used here instead of *N*,*N*-dimethylethyl diamine. Separated on silica gel chromatography (CH_2Cl_2/MeOH = 4:1, v/v) to afford the pure product $\mathbf{C_2}$: mp: 203–204 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.36 (t, J_1 = 8 Hz, J_2 = 7.6 Hz, 2H, CH_2), 2.80 (s, 6H, NCH_3), 3.15 (t, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 2H, NCH_2), 4.35 (t, J_1 = 6.4 Hz, J_2 = 6.8 Hz, 2 H, $CONCH_2$), 7.96 (t, J_1 = 8 Hz, J_2 = 7.6 Hz, 1H, 2-H), 8.42 (d, J = 8 Hz, 1H, 1-H), 8.72 (d, J = 7.2 Hz, 1H, 3-H), 9.24 (s, 1H, 7-H). HRMS (ESI): Calcd for $C_{19}H_{17}SO_2N_3F_3$ $[M+H]^+$: 408.0994. Found: 408.0990. IR (KBr): 2920, 2850, 2640, 1700, 1660, 1330, 780 cm^{-1}.

7.2.11. Spectroscopic measurements. The compounds were dissolved in absolute DMSO to give 10^{-5} M solutions, which were read with Shimadzu UV for absorption spectra and with Perkin-Elmer LS 50 using

quinine sulfate in sulfuric acid as quantum yield for fluorescence spectra.

7.2.12. Intercalation studies of compounds to CT-DNA. 0.1 mL solution of a compound (10^{-3}–10^{-4} M) was mixed with 20 mM Tris–HCl (pH 7.5) to 10 mL. Then, the two groups of samples were prepared at a concentration of chemical of 10^{-4}–10^{-5} M, one containing calf-thymus DNA of 50 μM, the other containing no DNA but having 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.

7.2.13. Cytotoxicity in vitro evaluation. The prepared compounds have been submitted to the Shanghai Institute of Materia Medica for testing their cytotoxicities.

7.2.14. Photocleavage of supercoiled pBR322 DNA. Two hundred and fifty nanograms of pBR322 DNA (form I), 1 μL solution of one compound, and 20 mM Tris–HCl (pH 7.5) were mixed to 10 μL, and then irradiated for 3 h with light (360 nm) using a lamp placed at 20 cm from the sample. Supercoiled DNA runs at position I, nicked DNA at position II, and linear DNA at position III. The samples were analyzed by gel electrophoresis in 1% agarose and the gel was stained with ethidium bromide.

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