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Naphthalimide–thiazoles as novel photonucleases: molecular design, synthesis, and evaluation

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Abstract—A new family of photonucleases, naphthalimide–thiazoles was synthesized and evaluated. These compounds intercalated into DNA efficiently and damaged DNA photochemically at concentrations as low as 5 μM. Mechanistic experiment suggests that a novel naphthalimide–thiazole radical produced via an excited triple state might be involved in the DNA photodamage. Different activity may arise from the impact of substituents at 2-phenyl ring of thiazole on the electron population of excited triple state according to AM1 semi-empirical calculation.

The design of novel photonucleases is of great significance from the standpoints of chemistry, biology, and medicine. Activated by the ultra-violet or visible light, these photocleavers can cause DNA scission via radicals, active oxygen species or electron transfer mechanisms.[1] As for radicals generators, it is of interest to incorporate new functional groups into the design of new types of photonucleases. Hecht's group has reported that halogen and carbon-centered thiazole radicals are produced via photohomolysis of carbon–halogen bonds in halo-bithiazole compounds and that halogen radicals play a major role in the DNA damage event.[2] Other forms of thiazole radicals and their production of DNA damage have not been reported yet. However, the conjugated C=N bond in aromatic heterocycles was reported to generate the photoexcited[3] ($n-\pi^*$) state, which would have radical character and could be able to cleave DNA photochemically.[3] Continuing our research that is focused on the DNA photodamage induced by oxygen-centered radicals,[4] a new family of photonucleases, which might produce a novel naphthalimide–thiazole radical as the photocleaving group via an excited triple state, is designed and evaluated herein.

Thiazole or polythiazole is known as the sequence recognition moiety, which appears in several photonucleases and anti-cancer bleomycin antibiotics.[2b,5] We expected that conjugated thiazole itself might be a novel DNA photocleaving group based on relevant references.[3] Inspired by these reports, a series of novel photonucleases containing a 2-phenyl (or substituted phenyl) thiazole and a naphthalimide group the $\mathbf{A_1}$–$\mathbf{A_6}$ were designed (Fig. 1) in our study. Here, thiazole was introduced as photocleaving group and a conjugated naphthalimide as a potent intercalation moiety, because it was reported that 2+1 unfused tri-cyclic aromatic systems were 'minimal intercalators'.[6] Furthermore, a *N*,*N*-dimethylaminoethyl group was incorporated for enhanced DNA affinity, because it commonly appears in clinically useful anti-cancer drugs that interact with DNA, such as amonafide and mitonafide.[7]

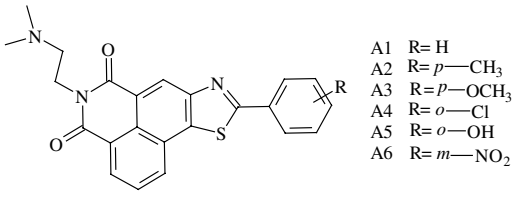

Figure 1. A new family of photonucleases $\mathbf{A_1}$–$\mathbf{A_6}$.

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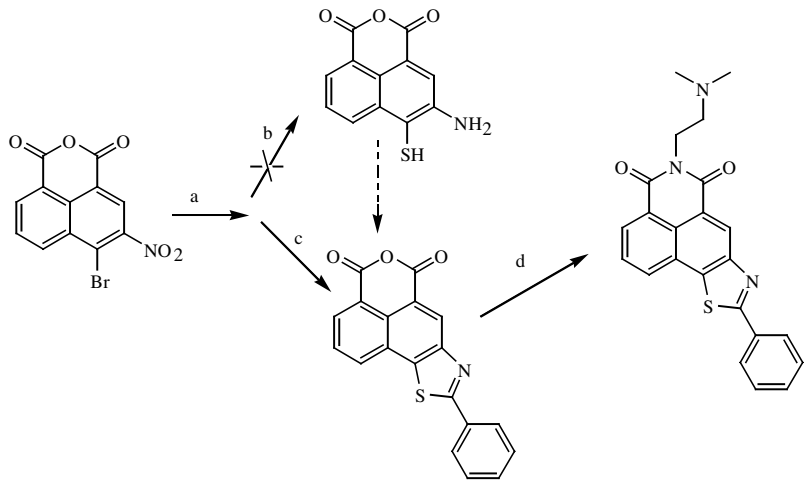

Scheme 1. Synthesis of thiazole–naphthalimide. (a) Na_2S_2, H_2O, 8 h; (b) HCl; (c) benzaldehyde, HOAc, Ar, reflux, 4 h, 61% yield; (d) *N*,*N*-dimethylethylenediamine, ethanol, reflux, 2.5 h, 83% yield.

These compounds were synthesized from 4-bromo-3-nitro-1, 8-naphthalic anhydride[3c] as shown in Scheme 1 (with compound $\mathbf{A_1}$ as an example):[8] 4-bromo-3-nitro-1, 8-naphthalic anhydride was reacted with sodium disulfide, by refluxing for 8 h in water. Since it was difficult to acidify the reaction mixture to form 4-mercapto-3-aminonaphthalic anhydride, due to its instability in air, was dropped in situ into glacial acetic acid containing benzaldehyde, and then refluxed for 4 h. This 'one pot' synthesis gave the 2-phenyl thiazole conjugated naphthalic anhydride. The obtained anhydride was condensed with *N*,*N*-dimethylethylenediamine in ethanol to form the designed product. Structures were identified by IR, ^{1}H NMR, MS, and element analysis.[9]

The UV–vis and fluorescence data for $\mathbf{A_1}$–$\mathbf{A_6}$ are shown in Table 1. The maximal absorptions of $\mathbf{A_2}$, $\mathbf{A_3}$, and $\mathbf{A_5}$, with electron-donating groups on 2-phenyl ring, are around 386 nm, and the others around 376 nm. The maximal emission wavelengths were obviously different from each other (from 412 to 491 nm) by comparison of their absorptions. The red-shift tendency in fluorescence for $\mathbf{A_2}$, $\mathbf{A_3}$, and $\mathbf{A_5}$ is the same as that in absorption, which is caused by that the presence of electron-donating groups on the 2-phenyl ring that enhance the electronic pushing–pulling effects for this ICT molecule. The great changes in fluorescence wavelength implies that there are great differences in the photophysical properties of their excited states due to the different pushing–pulling effects of the substituents. In addition, their fluorescence quantum yields are very low, which might be caused by excitation energy being transferred from the singlet excited state to the triplet excited state, or dissipated as heat through the internal conversion system.

Table 1. UV–vis and fluorescence data of $\mathbf{A_1}$–$\mathbf{A_6}$[a,b]

Compound	UVλmax/nm (lgε)	FLλmax/nm (Φ)
$\mathbf{A_1}$	376 (3.85)	425 (0.006)
$\mathbf{A_2}$	384 (3.78)	447 (0.009)
$\mathbf{A_3}$	386 (3.76)	491 (0.017)
$\mathbf{A_4}$	376 (4.13)	417 (0.003)
$\mathbf{A_5}$	387 (3.85)	436 (<0.001)
$\mathbf{A_6}$	376 (3.90)	412 (0.002)

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard.

Using $\mathbf{A_2}$ as an example, the Scatchard binding constant[10] between it and CT-DNA (in 30 mM Tris–HCl buffer, pH 7.5) was determined to be $1.19\times10^5\ M^{-1}$, indicating that efficient DNA binding.

The photoinduced relaxation of plasmid DNA by compounds $\mathbf{A_1}$–$\mathbf{A_6}$ was assayed with supercoiled pBR322DNA with a transluminator (366 nm) at a distance of 20 cm at 0 °C for 3 h and analyzed on a 1% agarose gel. The photocleavage efficiency was defined by the degree of the relaxation of supercoiled DNA to the relaxed circular form (form II, single-strand cleavage). It was apparent from Figure 2 that the photocleaving activity of $\mathbf{A_1}$, without a substituent (R=H) on phenyl moiety, was stronger than those of analogues $\mathbf{A_2}$–$\mathbf{A_6}$,

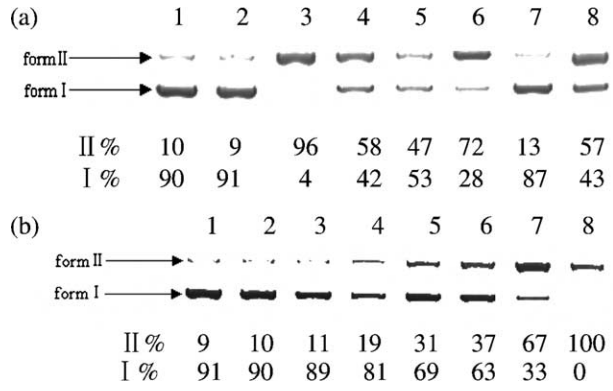

Figure 2. Photocleavage of closed supercoiled DNA in 10% acetonitrile in Tris–HCl buffer. (a) Photoirradiation: 3 h; lane 1: DNA alone (no *hv*); lane 2: DNA alone; lanes 3–8: DNA and compound $\mathbf{A_1}$–$\mathbf{A_6}$ at concentration of 100 μM, respectively. (b) Lane 1: DNA alone (no *hv*); lane 2: DNA alone (*hv*, 3 h); lanes 3–8: DNA and $\mathbf{A_1}$ at concentration of 2, 5, 10, 20, 50, 100 μM, respectively.

with a strong electron-donating or electron-withdrawing group. $\mathbf{A_1}$ caused the obvious single-strand cleavage at concentration as low as 5 μM and led to total single-strand cleavage at a concentration of 100 μM (Fig. 2). It was more active than its parent compound, 1,8-naphthalimide, which only caused 27% of single-strand cleavage of pUC19 plasmid DNA under irradiation at a concentration of 50 μM.[11] Further experiments showed that the photocleaving activity of $\mathbf{A_1}$ was sensitive to pH. With the elongation of irradiation time, it photonicked DNA more intensely. However, no DNA damage was observed in the absence of light.

Mechanistic experiments were also performed with the addition of histidine (singlet oxygen quencher), dithiothreitol (DTT, superoxide radical scavenger), superoxide dismutase (SOD, superoxide radical killer), and dimethyl sulfoxide (radical scavenger), respectively (Fig. 3). It is clear that the DNA-cleaving activity of $\mathbf{A_1}$ decreases greatly in the presence of dimethyl sulfoxide (lane 7 and 8) and that a radical is involved in the photodamage of pBR322DNA. Organic triple states have been demonstrated to react with DNA and individual nucleotides via electron transfer or hydrogen abstraction pathways.[12] The excited triple state of naphthalimide or thioxo-naphthalimide (without substituents on the naphthalene ring), which can damage DNA only via electron transfer, have also been reported by Saito, Kelly, and our group.[11,13] However, in our case, the DNA cleavage ability mainly comes from the formation of naphthalimide–thiazole radical under photoirradiation, which might damage DNA by hydrogen abstraction.

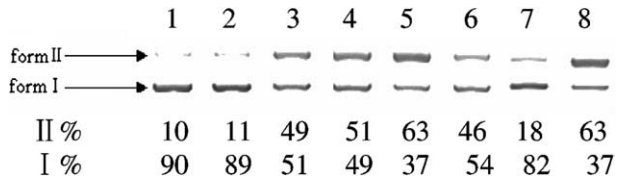

Figure 3. Mechanistic experiments of $\mathbf{A_1}$ on the photocleavage of DNA. Photoirradiation: 3 h; lane 1: DNA alone (no *hv*); lane 2: DNA alone; lane 3: DNA and compound $\mathbf{A_1}$ (30 μM); lane 4: DNA and $\mathbf{A_1}$ (30 μM) in the presence of histidine (6 mM); lane 5: DNA and $\mathbf{A_1}$ (30 μM) in the presence of dithiothreitol (DTT, 30 mM); lane 6: DNA and $\mathbf{A_1}$ (30 μM) in the presence of superoxide dismutase (SOD, 100 μg/mL). Photoirradiation: 2 h; lane 7: DNA and compound $\mathbf{A_1}$ at concentration of 100 μM in 10% DMSO in Tris–HCl buffer, respectively; lane 8: DNA and $\mathbf{A_1}$ at concentration of 100 μM in 10% acetonitrile in Tris–HCl buffer.

As seen in Figure 2, the photocleaving activities were in the following order: $\mathbf{A_1}$(H) > $\mathbf{A_4}$(*o*-Cl) > $\mathbf{A_2}$(*p*-Me) > $\mathbf{A_6}$(*m*-NO_2) > $\mathbf{A_3}$(*p*-OMe) > $\mathbf{A_5}$(*o*-OH). $\mathbf{A_1}$, without a substituent on phenyl moiety, was the most effective photocleaver. This contradicts the idea that a strong electron-donating group would improve the stability of a radical and the 'heavy atom effect', which would be favorable to the presence of the triplet-excited state in a chemical system.

We did not find any steric effects on photocleaving abilities of substituents on 2-phenyl ring by molecular modeling (Pcmodel 6.0). With AM1 semi-empirical quantum calculations (Hyperchem 5.5), no obvious relationship was observed between the electron densities in the ground state or excited singlet state of those substituents and their photocleaving abilities. However, it was found from AM1 calculations that the electron clouds in the triplet state of these compounds (except $\mathbf{A_6}$) were mainly concentrated on the thiazole ring, and the electron clouds density on C=N bond of $\mathbf{A_1}$ was higher than those of $\mathbf{A_2}$–$\mathbf{A_5}$ (with $\mathbf{A_1}$ and $\mathbf{A_3}$ as examples in Figure 4). In addition, only $\mathbf{A_1}$ has obvious electron density on the nitrogen atom of thiazole ring. The other compounds have almost 'naked' nitrogen atoms. It was inferred that concentrated electronic clouds on the thiazole ring and the C=N bond in the triplet state possibly granted it high reactivity. $\mathbf{A_6}$ was an exception, the electron clouds were populated mostly on the nitro group in the triple state. Thus, the reactivity of $\mathbf{A_6}$ possibly arose from the nitro group in the triple state,[14] although we cannot exclude the participation of thiazole ring.

In conclusion, the present work demonstrates the design and evaluation of novel photonucleases, thiazole–naphthalimides $\mathbf{A_1}$–$\mathbf{A_6}$, using conjugated thiazole ring as a novel photocleaving group. These compounds possibly cleave circular supercoiled pBR322 DNA efficiently via non-diffusible radical mechanism under the irradiation of long-wavelength UV light (366 nm). Unsubstituented $\mathbf{A_1}$ showed stronger DNA photocleaving ability than $\mathbf{A_2}$–$\mathbf{A_6}$, with electron-withdrawing or electron-pushing substituents on the phenyl ring. These compounds may have anti-cancer activities and cytotoxicity studies are in progress.

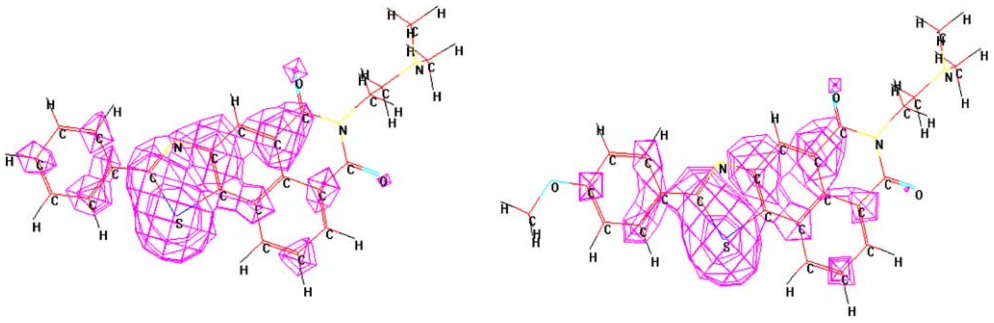

Figure 4. Electron clouds population of $\mathbf{A_1}$ and $\mathbf{A_3}$ simulated with AM1 semi-empirical calculation.

Acknowledgements

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9. **A1**: mp 220–221 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.25 (s, 8H, NCH_2, CH_3), 4.20 (s, 2H, $CONCH_2$), 7.66 (d, $J = 4.54$ Hz, 3H, 3′-H, 4′-H, 5′-H), 8.03 (t, $J_1 = 7.79$, $J_2 = 7.57$, 1H, 2-H), 8.17–8.25 (m, 2H, 2′-H, 6′-H), 8.56 (d, $J = 6.97$, 1H, 1-H), 8.67 (d, $J = 49$, 1H, 3-H), 8.95 (s, 1H, 7-H). HR-MS: $C_{23}H_{19}N_3O_2S$ calculated: 401.1198; Found: 401.1186; m/z (%) 401 (M^+) (5.46), 343 (5.73), 313 (8.58), 285 (9.97), 157 (7.56), 71 (41.4), 58 (100). IR (KBr): 2960, 2800, 1700, 1660, 1320, 780 cm^{-1}. Elemental analysis: $C_{23}H_{19}N_3O_2S$: Calcd: C 68.81, H 4.77, N 10.47; Found: C 68.54, H 4.89, N 10.51.
A2: mp 202–203 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.31 (s, 6H, NCH_3), 2.43 (s, 5H, NCH_2, 4′-CH_3), 4.22 (s, 2H, $CONCH_2$), 7.46 (d, $J = 7.91$ Hz, 2H, 3′-H, 5′-H), 8.03 (t, $J_1 = 7.81$, $J_2 = 7.83$, 1H, 2-H), 8.10 (d, $J = 7.85$ Hz, 2H, 2′-H, 4′-H), 8.57 (d, $J = 7.19$, 1H, 1-H), 8.66 (d, $J = 8.11$, 1H, 3-H), 8.94 (s, 1H, 7-H). EIMS: m/z (%) 415 (M^+) (15.48), 371 (3.51), 327 (5.66), 299 (6.45), 157 (6.50), 71 (71.0), 58 (100). IR (KBr): 2960, 2820, 1700, 1660, 1325, 790 cm^{-1}. Elemental analysis: $C_{24}H_{21}N_3O_2S$: Calcd: C 69.38, H 5.09, N 10.11; Found: C 69.30, H 4.71, N 10.25.
A3: mp 216–217 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.27 (s, 6H, CH_3), 2.61 (s, 2H, NCH_2), 3.89 (s, 3H, 4′-OCH_3), 4.21 (t, 2H, $J_1 = 6.56$, $J_2 = 6.64$, $CONCH_2$), 7.19 (d, $J = 8.66$ Hz, 2H, 3′-H, 5′-H), 8.01 (t, $J_1 = 7.77$, $J_2 = 7.77$, 1H, 2-H), 8.15 (d, $J = 8.50$ Hz, 2H, 2′-H, 4′-H), 8.55 (d, $J = 7.28$, 1H, 1-H), 8.63 (d, $J = 8.18$, 1H, 3-H), 8.91 (s, 1H, 7-H). HRMS; $C_{24}H_{21}N_3O_3S$: Calcd: 431.1304; Found: 431.1311; m/z (%) 431(M^+) (3.39), 386 (2.49), 343 (1.70), 246 (1.69), 157 (1.75), 71 (73.67), 58 (100). IR (KBr): 2970, 2810, 1700, 1665, 1330, 780 cm^{-1}. Elemental analysis: $C_{24}H_{21}N_3O_3S$: Calcd: C 66.80, H 4.91, N 9.74; Found: C 66.94, H 4.73, N 10.03.
A4: mp 235–237 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.28 (s, 6H, CH_3), 2.62 (d, 2H, $J = 1.44$, NCH_2), 4.19 (t, 2H, $J_1 = 6.78$, $J_2 = 6.81$, $CONCH_2$), 7.59–7.67 (m, 2H, 3′-H, 5′-H), 7.74–7.78 (m, 1H, 4′-H), 7.99 (t, $J_1 = 7.74$, $J_2 = 7.75$, 1H, 2-H), 8.38 (dd, $J_1 = 1.78$, $J_2 = 7.50$, 1H, 6′-H), 8.54 (d, $J = 7.32$, 1H, 1-H), 8.70 (d, $J = 8.17$, 1H, 3-H), 8.94 (s, 1H, 7-H). HRMS: $C_{23}H_{18}ClN_3O_2S$ Calcd: 435.0808; Found: 435.0792; m/z (%) 435 (M^+) (5.06), 377 (2.45), 347 (4.14), 319 (4.03), 259 (1.14), 157 (7.16), 71 (67.76), 58 (100). IR (KBr): 2980, 2800, 1700, 1660, 1340, 780 cm^{-1}. Elemental analysis: $C_{23}H_{18}ClN_3O_2S$: Calcd: C 63.37, H 4.16, N 9.64; Found: C 63.42, H 4.43, N 9.35.
A5: mp 240–241 °C. ^{1}H NMR(d_6-DMSO) δ (ppm): 2.27 (s, 6H, CH_3), 2.60 (s, 2H, NCH_2), 4.21 (t, 2H, $J_1 = 6.88$, $J_2 = 6.95$, $CONCH_2$), 7.07 (t, 1H, $J_1 = 7.39$, $J_2 = 7.32$, 5′-H), 7.15 (d, 1H, $J = 8.21$, 3′-H), 7.44–7.48 (m, 1H, 4′-H), 7.99 (t, $J_1 = 7.76$, $J_2 = 7.81$, 1H, 2-H), 8.30 (dd, $J_1 = 1.46$, $J_2 = 8.11$, 1H, 6′-H), 8.53 (d, $J = 7.25$, 1H, 1-H), 8.71 (d, $J = 8.10$, 1H, 3-H), 8.94 (s, 1H, 7-H). HRMS; $C_{23}H_{19}N_3O_3S$ Calcd: 417.1147; Found: 417.0342; m/z (%) 417 (M^+) (6.20), 372 (2.65), 358 (3.38), 300 (4.03), 259 (4.62), 156 (8.19), 71 (100), 58 (88.55). IR (KBr): 2980, 2800, 2500 (Br), 1700, 1660, 1330, 780 cm^{-1}. Elemental analysis $C_{23}H_{19}N_3O_3S$: Calcd: C 66.17, H 4.59, N 10.07; Found: C 66.34, H 4.78, N 9.89.
A6: mp 229–230 °C. ^{1}H NMR (d_6-DMSO) δ (ppm): 2.27 (s, 6H, CH_3), 2.59 (s, 2H, NCH_2), 4.21 (s, 2H, $CONCH_2$), 7.91 (d, 1H, $J = 5.55$, 5′-H), 7.96–8.05 (m, 1H, 4′-H), 8.39–8.47 (m, 1H, 6′-H), 8.49–8.53 (m, 2H, 2-H, 2′-H), 8.54–8.70 (m, 1H, 1-H), 8.76–8.84 (m, 1H, 3-H), 8.85–8.95 (m, 1H, 7-H). EI-MS; m/z (%) 446 (M^+) (1.47), 388 (1.05), 358.0 (1.79), 342 (1.10), 258 (1.62), 157 (1.98), 71 (41.03), 58 (100). IR (KBr): 2970, 2800, 2750, 1700, 1660, 1525, 1340, 780 cm^{-1}. Elemental analysis $C_{23}H_{18}N_4O_4S$: Calcd: C 61.87, H 4.06, N 12.55; Found: C 62.03, H 4.31, N 12.79.
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