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Novel 2-aminothiazonaphthalimides as visible light activatable photonucleases: effects of intercalation, heterocyclic-fused area and side chains

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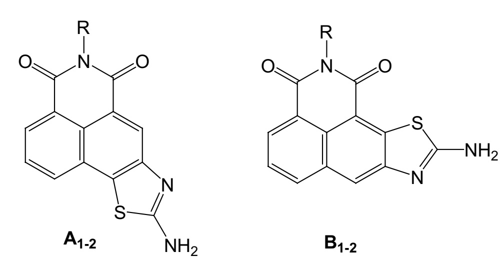
Abstract—A new family of 2-aminothiazonaphthalimides with different side chains as novel intercalative and visible light activatable photonucleases, was designed, synthesized and quantitatively evaluated. The order of their photocleaving abilities was parallel to that of their intercalative properties. The compound with linear heterocyclic-fused chromophore could intercalate into and photocleave DNA more efficiently than the one with angular heterocyclic-fused chromophore. **B$_2$**, the most efficient compound, caused obvious DNA damage at 1 μM. Mechanism experiment showed that superoxide anion was involved.

Photonucleases hold great promise in molecular biology and medicinal chemistry. Some of them have been proven to be valuable tools in biotechnology including therapeutic agents,[1] structural probes and photofootprinting agents as well.[2] Triggered by ultra-violet or visible light, such agents can cause significant damage on DNA with or without site selectivity.[3]

Many naphthalimide derivatives were well-known photonucleases[3] or anticancer drugs.[4] Our previous study has demonstrated that the presence of sulfur rather than its oxo-counterpart promoted the DNA intercalating and photocleaving ability.[3f] Based on this knowledge, we expected fusing novel thio-heterocyclic group into the naphthalimide skeleton would be an efficient strategy.

2-Aminothiazole moiety, proven its value in medicinal chemistry, has been successfully applied in dopamine agonists, such as B-HT920, PD118440 and pramipexole, the widely used anti-Parkinsonian agent.[5] In these cases, 2-aminothiazole group was assumed to interact with nucleic acid to form a stable DNA–drug complex with the aid of hydrogen bonds formed between the amino group and the sugar–phosphate chain. Herein, a new family of 2-aminothiazonaphthalimides, **A$_{1–2}$** and **B$_{1–2}$** (Fig. 1), was synthesized by fusing a 2-aminothiazole group to different positions of the naphthalimide skeleton. Two aminoalkyl side chains varying in length were incorporated into the parent structures not only to increase the affinity interaction with DNA[4,6] but also to elucidate the influence of them on biological activities. Furthermore, the comparison of effects of structural features of such agents on photocleaving activities could offer novel insights for rational molecular design.



A$_1$, B$_1$: R=$CH_2CH_2N(CH_3)_2$
A$_2$, B$_2$: R=$CH_2CH_2CH_2N(CH_3)_2$

Figure 1. Structures of novel 2-aminothiazonaphthalimides.

Keywords: Plasmid DNA; Photocleaver; Intercalation; Heterocycles.

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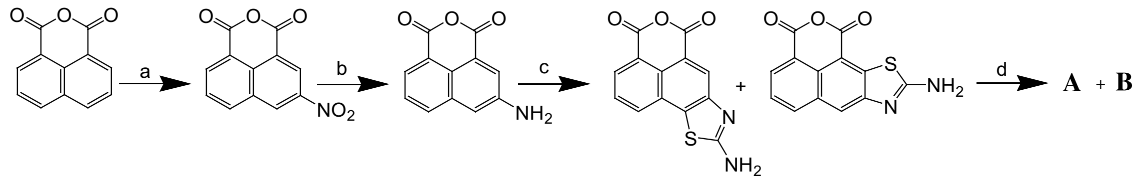

Scheme 1. Synthesis of 2-aminothiazonaphthalimides. Reagents and conditions: (a) $NaNO_3$, H_2SO_4, 0 °C, 1 h, 85% yield; (b) $SnCl_2$, concentrated HCl, 80 °C, 2 h, 80% yield; (c) KSCN, Br_2, AcOH, 48 h, 95% yield; (d) corresponding amine, ethanol, reflux 2 h.

The synthesis of these compounds was outlined in Scheme 1. Nitration of the starting material, 1,8-naphthalic anhydride with sodium nitrate in concentrated H_2SO_4 at 0 °C for 1 h, 3-nitro-1,8-naphthalic anhydride was obtained and then reduced by Tin(II) chloride dehydrate in concentrated hydrochloric acid to give 3-amino-1,8-naphthalic anhydride. Upon cyclization of 3-amino-1,8-naphthalic anhydride by a reported procedure,[7] in which 3-amino-1,8-naphthalic anhydride was treated with potassium thiocyanate and bromine in acetic acid, two 2-aminothiazonaphthalic anhydride isomers were obtained by filtration in high yield. Without further purification, the obtained anhydride mixtures were condensed with the corresponding amine in ethanol to form the corresponding naphthalimide mixtures, $\mathbf{A_{1-2}}$ and $\mathbf{B_{1-2}}$, with the ratio of 1:10. After separation with careful column chromatography, each pure targeted product was obtained. All of their structures were confirmed by ^{1}H NMR, HRMS and IR.[8]

The UV–vis and fluorescent data for these compounds were measured and shown in Table 1. It was found that they had slight differences in both absorption wavelength (around 420 nm) and emission wavelength (around 475 nm). The activating factor was visible light (420 nm) rather than UV light, indicating the safe manipulation towards them and thus in turn their prevalent advantage as biological tools.

The Scatchard binding constants between compounds, $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{B_1}$, $\mathbf{B_2}$ and CT-DNA (in 30 mM Tris–HCl buffer, pH 7.5) were determined using fluorescence technique method (Fig. 2)[9] and were shown in Table 1. The intercalating abilities of $\mathbf{B}_n$ were stronger than those of their isomers, $\mathbf{A}_n$ ($n = 1,2$), indicating that the compound with linear heterocyclic-fused chromophore was more easily intercalated into DNA than the one with angular chromophore. Furthermore, the intercalating abilities of $\mathbf{X_2}$ were stronger than those of $\mathbf{X_1}$ ($\mathbf{X} = \mathbf{A}, \mathbf{B}$), suggesting the importance of the length of aminoalkyl chain serving as DNA groove binder and/or external electrostatic binder. In our cases, the chain with three methylene units between two nitrogen atoms significantly enhanced the intercalating abilities of the aminothiazonaphthalimides. That might be the reason accounting for the order of intercalating ability: $\mathbf{A_2} > \mathbf{A_1}$, $\mathbf{B_2} > \mathbf{B_1}$.

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

Compds	UV λ_{max} (lg ε)	FL λ_{max} (Φ)	Scatchard binding constants ($\times 10^4$ M^{-1})
$\mathbf{A_1}$	421 (3.50)	475.5 (0.003)	1.98
$\mathbf{A_2}$	422 (3.73)	478.3 (0.004)	2.87
$\mathbf{B_1}$	421 (3.72)	475.5 (0.003)	2.56
$\mathbf{B_2}$	421 (3.81)	478.4 (0.004)	4.73

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

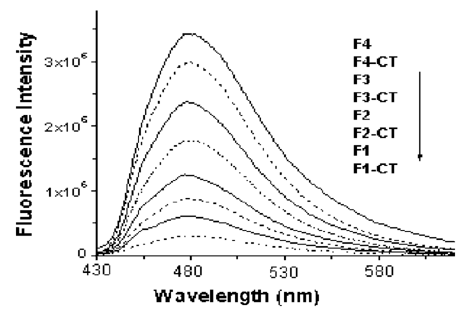

Figure 2. Fluorescence spectra before and after interaction of compound $\mathbf{B_2}$ and calf thymus DNA. Curves F and F-CT corresponded to compound $\mathbf{B_2}$ before and after being mixed with DNA. Numbers 1–4 indicated the concentration of $\mathbf{B_2}$, 5, 10, 20, 40 µM, respectively. DNA applied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

The photocleavage activities of $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{B_1}$, $\mathbf{B_2}$, were evaluated using closed supercoiled pBR322 DNA under photo-irradiation with a transluminator (400 nm) at a distance of 20 cm at 0 °C for 3 h under aerobic conditions and analyzed on a 1% agarose gel. The cleavage efficiency was defined by the degree of conversion of the supercoiled pBR322 DNA (form I) to relaxed circular DNA (form II). It was apparent that these compounds photocleaved the supercoiled DNA with different activities by the order: $\mathbf{B_2} > \mathbf{A_2} > \mathbf{B_1} > \mathbf{A_1}$ (Fig. 3a), which was parallel to that of their intercalative properties. Further the experiment showed that $\mathbf{B_2}$, the most efficient compound, nicked supercoiled DNA (form I, DNA in 200 µM base pair) to give a 29% relaxed circular form (form II) at a concentration as low as 1 µM, while the concentration for giving 100% form II (Fig. 3b) was 100 µM under identical conditions. Also the cleaving activity of $\mathbf{B_2}$ increased remarkably with prolonging photo-irradiation time (Fig. 3c), indicating it was a time-dependent process. As no damage was observed in the absence of light (lane 3), it was thus believed that the visible light functioned as a trigger to initiate the DNA strand scission. These newly designed naphthalimides possessing 2-aminothiazole showed much high photobiological activities by comparison with their parent compound without 2-aminothiazole.[3f]

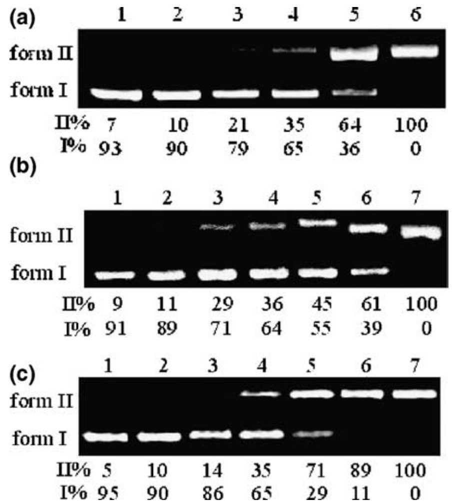

Figure 3. Photocleavage of supercoiled pBR322 DNA (200 μM base pair) in the buffer of Tris–HCl (20 mM, pH 7.5). (a) Photocleavage of pBR322 DNA by different compounds (100 μM) for 3 h. Lane 1, DNA alone (no h*ν*); lane 2, DNA alone (h*ν* 3 h); lanes 3–6, DNA and compound $\mathbf{A_1}$, $\mathbf{B_1}$, $\mathbf{A_2}$, $\mathbf{B_2}$ (h*ν* 3 h), respectively. (b) Photocleavage of pBR322 DNA by $\mathbf{B_2}$ at various concentrations for 3 h. Lane 1, DNA alone (no h*ν*); lane 2, DNA alone (h*ν* 3 h); lanes 3–7, $\mathbf{B_2}$ at concentration of 1, 5, 10, 50, 100 μM, respectively. (c) Photocleavage of pBR322 DNA by $\mathbf{B_2}$ (100 μM) at various time intervals. Lane 1, DNA alone (no h*ν*); lane 2, DNA alone (h*ν* 3 h); lanes 3–7, 0, 30, 60, 120, 180 min, respectively.

In order to establish the reactive species responsible for cleavage of the plasmid DNA, $\mathbf{B_2}$ was chosen as an example to perform mechanistic experiments by addition of histidine (singlet oxygen $^1O_2$ quencher), dithiothreitol (DTT, superoxide anion $O_2^{\cdot -}$ scavenger), ethanol (hydroxyl free radical $OH^{\cdot}$ scavenger) and superoxide dismutase (SOD, superoxide anion $O_2^{\cdot -}$ killer), respectively (Fig. 4). It was clear that histidine and ethanol had no obvious effects on the cleavage reaction, indicating that singlet oxygen and hydroxyl free radical were not likely to be the cleaving agents. The DNA-cleaving activity of $\mathbf{B_2}$ decreased greatly in the presence of DTT (Fig. 4, lane 5), indicating the superoxide anion was likely to be involved. In our cases, compounds possibly bound with DNA by aminoalkyl side chains which then transfer electrons from nucleobases to the chromophores, then to oxygen to generate superoxide anions

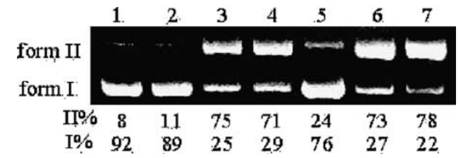

Figure 4. Effect of additives on the photocleavage of closed supercoiled pBR322 DNA (200 μM per base pair) by compound $\mathbf{B_2}$ (70 μM) in the buffer of Tris–HCl (20 mM, pH 7.5) for 3 h. Lane 1, DNA alone (no h*ν*); lane 2, DNA alone (h*ν* 3 h); lane 3 DNA and compound $\mathbf{B_2}$; lanes 4–7, DNA and compound $\mathbf{B_2}$ in the presence of histidine (6 mM), dithiothreitol (DTT, 30 mM), ethanol (1.7 M), superoxide dismutase (SOD, 100 mg/mL), respectively.

under photo-irradiation. However, it should be pointed out that addition of SOD to the reaction mixture did not slow the rate of DNA-cleaving reaction. This observation does not rule out the possibility that superoxide anion is involved in the DNA-cleaving process. It is because the hydrogen peroxide produced by SOD from superoxide anion under photo-irradiation or in the presence of trace amount of metallic cations, could itself lead to DNA cleavage which then contradicts the inhibition of superoxide anion by SOD.

With AM1-semi-empirical quantum calculations (Hyperchem 7.0), the electron densities of these compounds were calculated, showing slight difference between the ground state (figure not shown) and excited (singlet or triplet) state for the same compound. However, the electron densities between these compounds were very different at either the ground state or excited state. Electron densities of these compounds at excited triplet state were shown in Figure 5 as examples: both nitrogen and sulfur atoms in the thiazole groups of $\mathbf{B}_n$ ($n = 1$, 2) were 'naked', while $\mathbf{A}_n$ ($n = 1, 2$) had obviously higher electron densities in these two atoms. It was inferred that the nitrogen and sulfur atoms in the thiazole groups of $\mathbf{B}_n$ could attract the electron more strongly, then the electron might be easier to transfer from the side chains to the thiazole cycles and then to oxygen to form superoxide anions. The mechanism experiment also implied that electron transfer might play an important role in the course of photocleavage. Therefore, compared to those of $\mathbf{A}_n$, the higher electron transfer abilities of $\mathbf{B}_n$ were inferred to confer them higher activities.

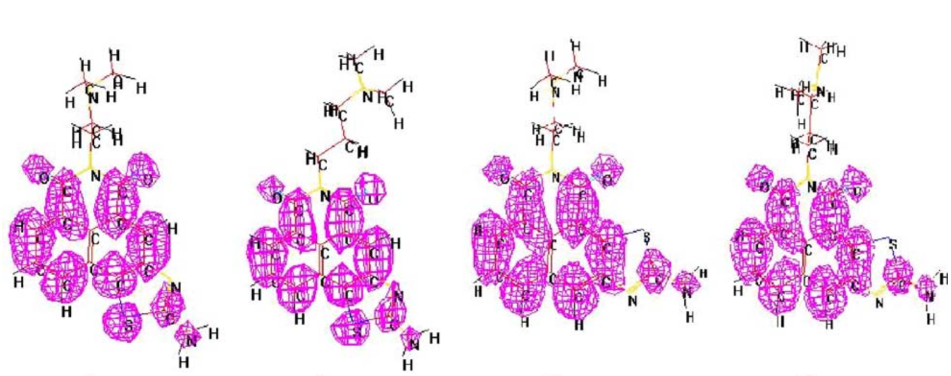

Figure 5. Electron densities at the excited triplet state of $\mathbf{A_1}$, $\mathbf{A_2}$, $\mathbf{B_1}$, $\mathbf{B_2}$ simulated with AM1 semi-empirical calculation.

In summary, a new family of 2-aminothiazonaphthalimides as visible light activatable photonucleases was synthesized and determined to have different DNA photocleaving activities. The order of their photocleaving abilities was parallel to that of their intercalative properties. The compound with linear heterocyclic-fused chromophore exhibited higher DNA intercalating and DNA photocleaving activities than the one with angular chromophore. **B_2** showed the highest activity. Mechanism experiment showed that superoxide anion was involved.

Acknowledgments

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8. Compound **A_1**: mp >300 °C. ^1H NMR (DMSO-d_6) δ (ppm): 2.64 (s, 6H, NCH_3), 3.10 (s, 2H, NCH_2), 4.32 (t, J_1 = 6.0 Hz, J_2 = 6.0 Hz, 2H, $CONCH_2$), 7.85 (t, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1H, 2-H), 8.48 (t, J = 7.6 Hz, 3H, 1-H, NH_2), 8.72 (d, J = 8 Hz, 1H, 3-H), 8.87 (s, 1H, 7-H). HRMS: calcd for $C_{17}H_{17}N_4O_2S$ $(M+H)^+$: 341.1072. Found: 341.1070. IR (KBr): 2927, 2857, 1730, 1646, 1351, 779 cm^{-1}. Compound **A_2**: mp 260–261 °C. ^1H NMR (DMSO-d_6) δ (ppm): 1.62 (s, 2H, CH_2), 2.59 (s, 6H, NCH_3), 2.97 (s, 2H, NCH_2), 4.11 (s, 2H, $CONCH_2$), 7.84 (t, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 1H, 2-H), 8.46 (d, J = 7.8 Hz, 1H, 1-H), 8.50 (s, 2H, NH_2), 8.73 (d, J = 8.4 Hz, 1H, 3-H), 8.85 (s, 1H, 7-H). HRMS: calcd for $C_{18}H_{19}N_4O_2S$ $(M+H)^+$: 355.1229. Found: 355.1211. IR (KBr): 2920, 2850, 1720, 1650, 1330, 779 cm^{-1}. Compound **B_1**: mp 290–291 °C ^1H NMR (DMSO-d_6) δ (ppm): 2.50 (s, 6H, NCH_3), 2.91 (s, 2H, NCH_2), 4.26 (s, 2H, $CONCH_2$), 7.84 (t, J_1 = 8 Hz, J_2 = 8 Hz, 1H, 2-H), 8.03 (s, 2H, NH_2), 8.28 (d, J = 8.2 Hz, 1-H), 8.36 (d, J = 6 Hz, 1H, 3-H), 8.40 (s, 1H, 10-H). HRMS: calcd for $C_{17}H_{17}N_4O_2S$ $(M+H)^+$: 341.1072. Found: 341.1073. IR (KBr): 2921, 2850, 1696, 1654, 1330, 777 cm^{-1}. Compound **B_2**: mp 240–241 °C. ^1H NMR (DMSO-d_6) δ (ppm): 1.82 (t, J_1 = 6.4 Hz, J_2 = 7.2 Hz, 2H, CH_2), 2.28 (s, 6H, NCH_3), 3.16 (s, 2H, NCH_2), 4.06 (t, J_1 = 7.2 Hz, J_2 = 7.2 Hz, 2H, $CONCH_2$, 7.79 (t, J_1 = 7.8 Hz, J_2 = 8 Hz, 1H, 2-H), 8.01 (s, 2H, NH_2), 8.20 (d, J = 8.4 Hz, 1-H), 8.31 (d, J = 7.2 Hz, 1H, 3-H), 8.34 (s, 1H, 10-H). HRMS: calcd for $C_{18}H_{19}N_4O_2S$ $(M+H^+)$: 355.1229. Found: 355.1219. IR (KBr): 2960, 2823, 1695, 1651, 1335, 779 cm^{-1}.
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