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Highly-efficient DNA photocleavers with long wavelength absorptions: thio-heterocyclic fused naphthalimides containing aminoalkyl side chains

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Abstract—Thio-heterocyclic fused naphthalimides with aminoalkyl side chains were designed, synthesized and evaluated. These compounds have long wavelength absorptions and binding affinities to Calf thymus DNA. They could photodamage supercoiled pBR322 DNA from form I (closed) to II (nicked) at a concentration as low as 0.5 μM and to form III (linear) at a concentration of 50 μM. A possible mechanism of superoxide anion was provided.

Synthetic photochemical DNA cleaving reagents are of great interest in chemistry, biology and medicine. These reagents can site selectively or nonselectively cleave DNA triggered by near-UV light.[1] Although many photocleavers can damage DNA (supercoiled pBR322) from form I (closed) to II (nicked), only a few of them at a lower concentration, was able to photocleave DNA from form I to III (linear). It was known that many naphthalimide derivatives are famous anti-cancer drugs or DNA photocleavers, but there was few heterocyclic fused naphthalimide as DNA photocleaver.[1g,2] We ever reported that *N*-dimethylaminoethyl thioxo-naphthalimide could damage DNA from form I to form III at 200 μM,[1m] and the presence of thio-moiety on heterocyclic conjugation promoted the photocleavage.[1n] Herein, we would present novel photocleavers of thio-heterocyclic fused naphthalimides containing aminoalkyl side chains at the imide moiety with long wavelength absorption in the preferred visible area, which could photocleave DNA from form I to form II and III at a very low concentration.

Firstly, we noted that benzo[*k*,*l*]xanthene-3,4-dicarboxylic anhydride had absorption wavelength in the visible region and nearly planar structure.[3] Its absorption could make target compounds activated by visible light to cleave DNA, which was safe for the manipulation. Secondly, *N*,*N*-dimethyl aminoethyl group or analogues were chosen as side chains for its imide moiety because they appeared commonly in clinically useful anti-cancer drugs that bound with DNA, such as amonafide and mitonafide.[4] More importantly, we expected that the anhydride attached with these groups mentioned above would damage DNA photochemically and efficiently. Therefore, several novel photocleavers $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ were designed (Fig. 1). All of these compounds were shown to possess excellent photocleaving abilities to the closed supercoiled pBR322 DNA rather than their counterparts, compound $\mathbf{A_4}$ and $\mathbf{A_5}$ (Fig. 1).

These compounds were synthesized from 4-bromonaphthalic anhydride shown in Scheme 1.[3] After the

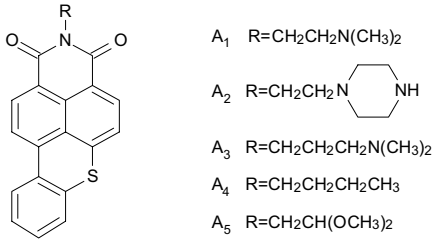

Figure 1. Novel photocleavers and counterparts.

Keywords: DNA photocleaver heterocycle.
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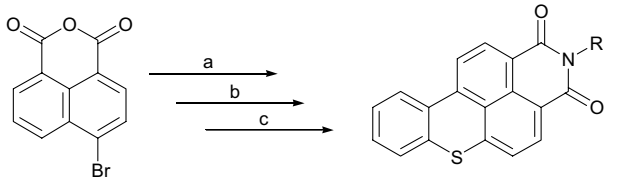

Scheme 1. Synthesis of novel naphthalimide-derived photocleavers. (a) 2-aminobenzenethiol, K_2CO_3, DMF, reflux, 30 min, 77% yield; (b) $NaNO_2$, HOAc, H_2SO_4 ; $CuSO_4$, HOAc, H_2O, 99% yield; (c) RNH_2, ethanol, reflux, 2–4 h, 85% yield.

separation through silica gel column chromatography with the eluent of chloroform–acetone (1:1, v/v), their structures were confirmed by IR, ^1H NMR, MS and element analysis.[5] It was found from Table 1 that their absorptions were all in the visible absorption area around 464 nm with the similar intensities. They also had the emission at 520 nm with different fluorescent intensities. It was noticed their fluorescent quantum yields (in absolute ethanol, almost without free protons) seemed to be inversely proportional to their intramolecular photoinduced electron transfer (PET) abilities, which would quench fluorescence. Someone believed that the PET to naphthalene moiety from a lone pair of *N*,*N*-dimethyl aminoethyl group at N-imide of naphthalimide was usually weak,[6] but we ever observed the PET effects and changes in the fluorescence intensities for the similar naphthalimide derivatives.[7]

The fluorescences of compounds **A** upon addition of Calf thymus DNA were quenched, the analyses showed that their affinities to DNA were not so strong. With $\mathbf{A_1}$ as an example, the Scatchard binding constant[8] between $\mathbf{A_1}$ and CT-DNA was determined to be $8.27\times10^3\ M^{-1}$, indicating that $\mathbf{A_1}$ might bind DNA via electrostatic attraction between the ammonium (positive charge) and DNA (negative charge), rather than plausible intercalation exerted by the chromophore of thio-heterocyclic fused naphthalimides.

Photocleaving abilities of compounds $\mathbf{A_1}$–$\mathbf{A_5}$ were then examined with the closed supercoiled pBR322 DNA under the photoirradiation at 450 nm as shown in Figure 2a. The cleaving efficiency was defined by the degree of the relaxation of supercoiled DNA. It was apparent that $\mathbf{A_2}$ exhibited the greatest DNA cleaving ability over $\mathbf{A_1}$ and $\mathbf{A_3}$, as $\mathbf{A_2}$ resulted in more form III than $\mathbf{A_1}$ did, while $\mathbf{A_4}$ and $\mathbf{A_5}$ could hardly damage DNA under the same condition. The order of their photocleaving abilities was as follows: $\mathbf{A_2} > \mathbf{A_1} > \mathbf{A_3} > \mathbf{A_5}\sim\mathbf{A_4}$.

Table 1. Spectra data of compounds $\mathbf{A_1}$–$\mathbf{A_5}$[a,b]

Compound	UV λ_{max}/nm (log ε)	FL λ_{max}/nm (ϕ)
$\mathbf{A_1}$	462 (4.42)	521 (0.51)
$\mathbf{A_2}$	461 (4.20)	521 (0.47)
$\mathbf{A_3}$	462 (4.37)	521 (0.63)
$\mathbf{A_4}$	462 (4.45)	519 (0.89)
$\mathbf{A_5}$	467 (4.37)	520 (0.86)

[a] In absolute ethanol.
[b] With fluorescein as standard (ϕ =0.90).

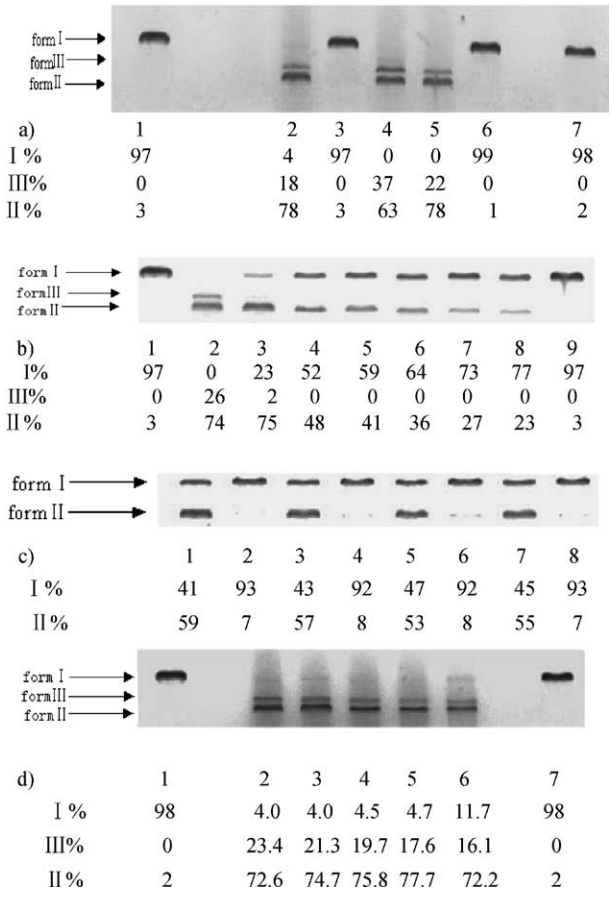

Figure 2. Photocleavage of the supercoiled pBR322 DNA. The cleavage activities were evaluated using the supercoiled circular pBR322 DNA (form I) (30 ng/μL) with a compound in the buffer Tris–HCl (pH 7.5) under photo-irradiation (2300 W/cm^2) though a transluminator (450 nm) in the distance of 20 cm at 0 °C and then analyzed on a 1% agarose gel. (a) Photocleavage of compounds **A1**–**A5**. Photoirradiation: 2 h; lane 1: DNA alone; lane 2–6: DNA and compounds **A3**,**A5**,**A2**,**A1**,**A4** at the concentration of 100 μM, respectively; lane 7: DNA alone (no hν). (b) Concentration-dependent of **A2**'s photocleavage. Lane 1: DNA alone (hν, 90 min); lane 2–8: DNA and **A2** at the concentration of 50, 20, 10, 5, 2, 1, 0.5 μM, respectively; lane 9: DNA alone (no hν). (c) pH-dependence of photocleavage lane 2, 4, 6, 8: DNA alone (hν, 60 min), pH=8.5, 8.0, 7.5, 7.0; lane 1, 3, 5, 7: DNA and **A2** (20/μM), pH=8.5, 8.0, 7.5, 7.0, respectively. (d) Time-dependence of photocleavage for **A2** lane 1: DNA alone (hν, 75 min); lane 2–7: DNA and C3 (100 μM) (hν, 75, 60, 45, 30, 15, 0 min, respectively.

In fact, the nitrogen atom of *N*,*N*-dimethyl aminoethyl group or analogues at imide moiety was easily protonated in aqueous solution at the physiological pH, in this case the increasing of compound's fluorescence intensity should be proportional to the protonation extent of *N*,*N*-dimethyl aminoethyl group or analogues at the imide moiety, as intramolecular PET to quench fluorescence was inhibited in this case. Our H^+ addition experiments showed that the fluorescence intensities of $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ were obviously increased due to totally protonation. However, the counterpart $\mathbf{A_4}$ without the similar side chains at N-imide only had small changes in fluorescence intensity when acidified ($\mathbf{A_5}$ was not involved because of its instability under an acidified condition). It was noticed that there seemed a parallel

Table 2. Change of fluorescence quantum yield before and after H^+ addition[a]

	A1	**A1** (H^+)	**A2**	**A2** (H^+)	**A3**	**A3** (H^+)	**A4**	**A4** (H^+)
ϕ	0.32	0.45	0.34	0.53	0.37	0.42	0.48	0.51
$\Delta\phi/\phi$	+41%		+58%		+11%		+6%	

[a] Measured in ethanol–H_2O (4:1) using fluorescein as standard ($\phi = 0.90$).

relationship between the order ($\mathbf{A_2} > \mathbf{A_1} > \mathbf{A_3} > \mathbf{A_4}$) of their fluorescence enhancements in the presence of protons and that of their photocleavages (Table 2). It implied that charge–charge interactions between these ammoniums and DNA, probably controlled the photocleavage profile.

These above results clearly indicated that the existence of the *N*,*N*-dimethyl aminoethyl group or analogues was very important for their photo-cleaving abilities. Especially, $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ could cleave the closed supercoiled DNA almost completely to the nicked and linear DNA at the concentration 100 μM. This fact implied that the chromophore of thio-heterocyclic fused naphthalimides also played an important role in the photocleavage by comparison with that of the similar *N*-aminoalkyl thioxo-naphthalimide, which could damage DNA from form I to form II and III at about 200 μM.[1m]

Further experiment indicated that $\mathbf{A_2}$ could cleave the closed supercoiled DNA to the nicked form at a concentration as low as 0.5 μM and to the linear form at a concentration of 50 μM. However, no cleavage was observed in the control reactions run in the dark or without compounds (Fig. 2b). In addition, the buffer's pH value did not obviously affect its DNA cleaving actions (Fig. 2c), and it exhibited better DNA damage abilities under prolonged photo-irradiation. (Fig. 2d).

Mechanism experiment was performed with the addition of histidine, dithiothreitol, superoxide dismutase and ethanol (Fig. 3). It was found that histidine (singlet oxygen quencher) had no effect on the cleavage reaction, ethanol (radical quencher) strangely accelerated the reaction somewhat. However, dithiothreitol (DTT, superoxide anion radical scavenger) retarded the reaction very efficiently. It also should be pointed out that superoxide dismutase (SOD, superoxide radical killer) accelerated the rate of DNA-cleaving reaction, because the hydrogen peroxide produced by SOD from superoxide anion radical, could decompose to produce hydroxyl radicals in the presence of UV light or after reduction by the trace of metal ions and at last lead to DNA damage.[1b] Although the excited naphthalimide might be able to directly oxidize the guanine in DNA, guanine oxidation would be detected as strand cleavage only after piperidine treatment. Obviously, in our case without using piperidine, superoxide anion radical was involved in the DNA cleavage at least.

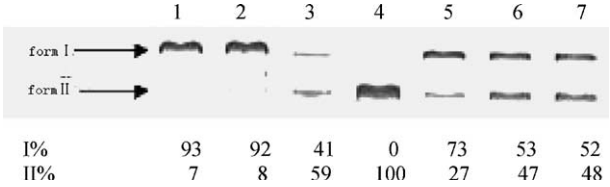

Figure 3. The effect of different additives on the photocleavage of supercoiled pBR322 DNA (30 ng/μL) in the buffer Tris–HCl (pH 7.5) under photo-irradiation (2300 W/cm^2) with a transluminator (450 nm) for 2 h in the distance of 20 cm at 0 °C. Lane 1: DNA alone (no hν); lane 2: DNA alone; lane 3: DNA and **A2** in the presence of ethanol (1.7 M); lane 4: DNA and **A2** in the presence of superoxide dismutase (SOD, 100 μg/mL); lane 5: DNA and **A2** in the presence of dithiothreitol (DTT, 30 mM); lane 6: DNA and **A2** in the presence of histidine (6 mM); lane 7: DNA and **A2** at the concentration of 10 μM.

The above experimental results showed that compounds $\mathbf{A_1}$–$\mathbf{A_3}$ possibly bound with DNA through ammonium's action and mainly generated superoxide anion through intermolecular electron transfer from the chromophore to oxygen, which at last damaged DNA. Both of aminoalkyl side chain at the imide and thio-heterocyclic fused naphthalimide were important for the photo-bioactivities of $\mathbf{A_1}$–$\mathbf{A_3}$, while $\mathbf{A_4}$ and $\mathbf{A_5}$ could not damage the DNA photochemically due to the absence of amino side chain at their imide moieties.

In summary, the present work demonstrated the design and evaluation of novel and highly-efficient photocleavers with long wavelength absorption, thio-heterocyclic fused naphthalimides containing aminoalkyl side chains at the imide $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$. They could photodamage the circular supercoiled pBR322 DNA from form I (closed) to II (nicked) at a concentration as low as 0.5 μM and to form III (linear) at a concentration of 50–100 μM under irradiation of visible light at 450 nm. Mechanism experiment showed that superoxide anion possibly were responsible for the DNA photodamage. The anti-cancer studies on these photocleavers are in progress.

Acknowledgements

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References and notes

1. (a) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1151; (b) Amitage, B. *Chem. Rev.* **1998**, *98*, 1171–1200; (c) Saito, I.; Nakatani, K. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3007–3019; (d) For selected recent example, see Lee, A. H. F.; Chen, J.; Liu, D.; Leung, T. Y. C.; Chan, A. S. C.; Li, T.

J. Am. Chem. Soc. **2002**, *124*, 13972–13973; (e) Teulade-Fichou, M.; Perrin, D.; Boutorine, A.; Polverari, D.; Vigneron, J.; Lehn, J.; Sun, J. S.; Garestier, T.; Helene, C. *J. Am. Chem. Soc.* **2001**, *123*, 9283–9292; (f) Hwu, J. R.; Lu, K. L.; Yu, S. F.; Yu, L. J.; Kumaresan, S.; Lin, K. J.; Tsay, S. C. *Photochem. Photobiol.* **2002**, *75*, 457–461; (g) Rogers, J. E.; Le, T. P.; Kelly, L. A. *Photochem. Photobiol.* **2001**, *73*, 223–229; (h) Zhang, Y.; Yang, S.; Liu, C.; Dai, X.; Cao, W.; Xu, J.; Li, Y. *New J. Chem.* **2002**, *26*, 617–620; (i) Hurley, A. L.; Maddox, M. P., III; Scott, T. L.; Flood, M. R.; Mohler, D. L. *Org. Lett.* **2001**, *3*, 2761–2764; (j) Tsai, F. Y.; Lin, S. B.; Tsay, S. C.; Lin, W. C.; Hsieh, C. L.; Chuang, S. H.; Kan, L. S.; Hwu, J. R. *Tetrahedron Lett.* **2001**, *42*, 5733–5735; (k) Fernandez, M. J.; Grant, K. B.; Herraiz, F.; Yang, X.; Lorente, A. *Tetrahedron Lett.* **2001**, *42*, 5701–5704; (l) Cornia, M.; Menozzi, M.; Ragg, E.; Mazzini, S.; Scarafoni, A.; Zanardi, F.; Casiraghi, G. *Tetrahedron* **2000**, *56*, 3977–3983; (m) Qian, X.; Huang, T., et al. *J. Chem. Soc., Perkin. Trans.* **2000**, *2*, 715; (n) Qian, X.; Yao, W. *Tetrahedron Lett.* **2001**, *42*, 6175.

2. (a) Brana, M.; Ramos, A. *Curr. Med. Chem.: Anti-cancer Agents* **2001**, *1*, 237–255; (b) Rogers, J. E.; Weiss, S. J.; Kelly, L. A. *J. Am. Chem. Soc.* **2000**, *122*, 427–436; (c) Rogers, J. E.; Kelly, L. A. *J. Am. Chem. Soc.* **1999**, *121*, 3854–3861; (d) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K. *J. Am. Chem. Soc.* **1995**, *117*, 6406–6407; (e) Qian, X.; Huang, T.-B.; Wei, D.-Z.; Zhu, D.-H.; Fan, M.-C.; Yao, W. *J. Chem. Soc., Perkin. Trans.* **2000**, *2*, 715–718.
3. Grayshan, P. H.; Kadhim, A. M.; Peters, A. T. *J. Heterocycl. Chem.* **1974**, *11*, 33–38.
4. (a) Malviya, V. K.; Liu, P. Y.; Alberts, D. S.; Surwit, E. A.; Craig, J. B.; Hanningan, E. V. *Am. J. Clin. Oncol.* **1992**, *15*, 41–44; (b) Rosell, R.; Carles, J.; Abad, A.; Ribelles, N.; Barnadas, A.; Benavides, A.; Martin, M. *Invest. New Drugs* **1992**, *10*, 171–175.
5. **A1**: Mp 212–213 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.20–1.34 (8H, m, NCH_3, NCH_2), 4.42 (2H, t, *J* 6.78 and 6.65, $CONCH_2$), 7.34–7.42 (m, 3H, 8-H, 9-H, 10-H), 7.48 (1H, d, *J* 7.99, 7-H), 8.14–8.20 (2H, m, 1-H, 6-H), 8.40 (1H, d, *J* 7.98, 2-H), 8.59 (1H, d, *J* 8.12, 5-H); *m*/*z* 374 (M^+, 1.62%), 304 (23.30), 303 (29.64), 71 (29.58), 58 (100), 56 (9.81), 43 (12.49); ν_{max} (KBr)/cm^{-1} 2950, 2870, 1695, 1660, 1560 and 1380; $C_{22}H_{18}N_2O_2S$ requires C, 70.57; H, 4.85; N, 7.48; found C, 70.46; H, 4.98; N, 7.35. **A2**: Mp 286–287 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 2.80 (2H, t, *J* 5.96 and 6.00, NCH_2), 2.89 (4H, s, $NHCH_2$ (cyclo)), 3.14 (4H, s, NCH_2 (cyclo)), 4.32 (2H, t, *J* 6.08 and 5.88, $CONCH_2$), 7.38–7.46 (3H, m, 8-H, 9-H, 10-H), 7.54 (1H, d, *J* 8.06, 7-H), 8.19–8.25 (2H, m, 1-H, 6-H), 8.42 (1H, d, *J* 8.01, 2-H), 8.62 (1H, d, *J* 8.18, 5-H); *m*/*z* 415 (M^+, 5.45%), 373 (39.42), 330 (35.98), 303 (27.95), 99 (100.0), 70 (27.95), 56 (42.09), 42 (21.07); ν_{max} (KBr)/cm^{-1} 3410, 2970, 2840, 1690, 1640 and 1330; $C_{24}H_{21}N_3O_2S$ requires C, 69.38; H, 5.09; N, 10.11; found C, 69.56; H, 5.23; N, 9.96. **A3**: Mp 202–203 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 2.46 (8H, s, NCH_3, NCH_2), 2.70 (2H, t, *J* 7.39 and 7.49, CH_2), 4.25 (2H, t, *J* 7.24 and 7.24, $CONCH_2$), 7.40 (3H, m, 8-H, 9-H, 10-H), 7.49 (1H, d, *J* 7.98, 7-H), 8.20 (2H, m, 1-H, 6-H), 8.39 (1H, d, *J* 7.98, 2-H), 8.59 (1H, d, *J* 8.10, 5-H). **A4**: Mp 185–186 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 0.98 (3H, t, *J* 7.37 and 7.37, CH_3), 1.43–1.50 (2H, m, (Me)-CH_2), 1.68–1.78 (2H, m, (C_2H_5)-CH_2), 4.19 (2H, t, *J* 7.55 and 7.65, $CONCH_2$), 7.38–7.43 (3H, m, 8-H, 9-H, 10-H), 7.52 (1H, d, *J* 7.98, 7-H), 8.20–8.27 (2H, m, 1-H, 6-H), 8.44 (1H, d, *J* 7.98, 2-H), 8.64 (1H, d, *J* 8.13, 5-H). **A5**: Mp 185–186 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 3.42 (6H, s, O-CH_3), 4.39 (2H, d, *J* 5.74, $CONCH_2$), 4.93 (1H, t, *J* 5.68 and 5.68, CH), 7.38–7.44 (3H, m, 8-H, 9-H, 10-H), 7.52 (1H, d, *J* 7.98, 7-H), 8.21 (2H, m, 1-H, 6-H), 8.44 (1H, d, *J* 7.99, 2-H), 8.64 (1H, d, *J* 8.13, 5-H); *m*/*z* 391 (M^+, 13.01%), 360 (23.51), 328 (16.07), 303 (35.21), 232 (14.91), 75 (100), 57 (16.54), 43 (17.20); ν_{max} (KBr)/cm^{-1} 2960, 2850, 1690, 1650 and 1330; $C_{22}H_{17}NO_4S$ requires C, 67.50; H, 4.38; N, 3.58; found C, 67.24; H, 4.76; N, 3.73.
6. de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J.-L.; McCoy, C. P.; Rice, T. E.; Soumillion, J.-P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1728–1729.
7. Yao, W.; Qian, X. *Dyes Pigments* **2001**, *48*, 43–47.
8. Gupta, M.; Ali, R. *J. Biochem.* **1984**, *95*, 1253–1257.