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Five-member thio-heterocyclic fused naphthalimides with aminoalkyl side chains: intercalation and photocleavage to DNA

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Abstract—Novel five-member thio-heterocyclic fused naphthalimides with aminoalkyl side chains were designed, synthesized and evaluated. These compounds have high Scatchard binding constants. They could damage DNA (supercoiled pBR322) from form I (closed) to II (nicked) at a concentration as low as 10 μM and from form I to form III at a concentration of 50 μM. The results implied that the influence of intercalating ability of chromophores on photocleaving ability of photocleavers depended on the mechanism of photocleavage.

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, are able to photocleave DNA from form I to III (linear). It was known that many naphthalimide derivatives are famous anti-cancer agents or DNA photocleavers, but there are few heterocyclic fused naphthalimide as intercalative photocleavers for DNA.[1g,2] Large planar chromophore might show good DNA intercalative ability, some believe that the high intercalation would increase the photocleaver's affinity to DNA and promote photocleavage. We reported two isomers, six- and five-member heterocyclic fused naphthlimides with hydroperoxyl group (**C** and **D**, Fig. 1).[1m,n] **D** with high intercalating ability (binding constant $8.72\times10^5\ M^{-1}$) could damage DNA from form I to form II at a concentration as low as 1 μM and **C** with lower intercalating ability (binding constant $4.26\times10^3\ M^{-1}$) damaged DNA from form I to form II at a concentration of 5 μM. For these DNA photocleaving agents of radicals, it seemed that the intercalation was the main factor to affect photocleaving abilities.

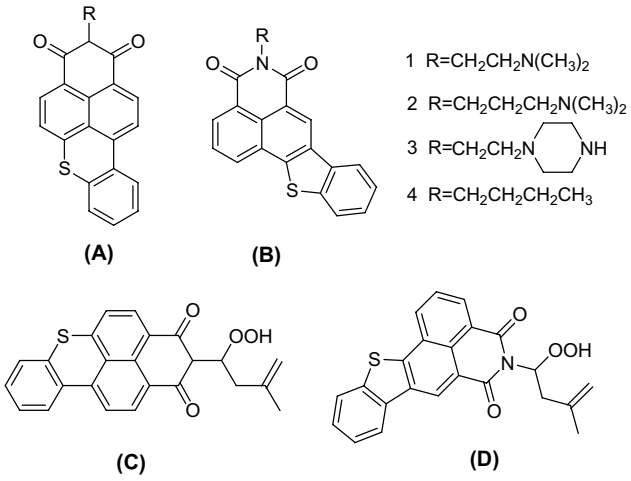

Figure 1. Structures of reported (**A**), (**C**), (**D**) and novel (**B**) photocleavers.

However, we wondered if the case is the same for those of nonradicals, which damaged DNA mainly through electron transfer mechanism. Therefore, we hope to investigate photocleavers of five- and six-member heterocyclic fused naphthlimides with aminoalkyl side chains.

Previously, we already reported that six-member $\mathbf{A_1}$–$\mathbf{A_4}$ could photodamage the circular supercoiled pBR322 DNA from form I to II at a concentration as low as

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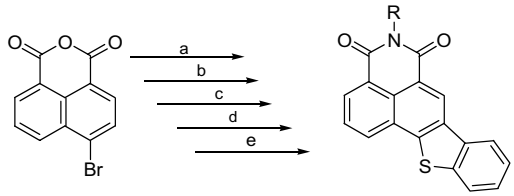


Scheme 1. Synthesis of novel naphthalimide-derived photocleavers. Reagents and conditions: (a) HNO_3/H_2SO_4; (b) benzenethiol, Pyridine, ethanol, reflux, 5 h, 87% yield; (c) $SnCl_2$/HCl, 85 °C, 1 h, 96% yield; (d) $NaNO_2$, HOAc, H_2SO_4; $CuSO_4$, HOAc, H_2O, 86% yield; (e) RNH_2, ethanol, reflux, 2–3 h, 85% yield.

Table 1. Spectra data of compounds $\mathbf{B_1}$–$\mathbf{B_4}$[a,b]

Compd	UV λ_{max}/nm (lg ε)	FL λ_{max}/nm (ϕ)
$\mathbf{B_1}$	384 (4.24)	436 (0.0215)
$\mathbf{B_2}$	383 (4.27)	433 (0.0213)
$\mathbf{B_3}$	382 (4.15)	429 (0.0151)
$\mathbf{B_4}$	380 (4.08)	426 (0.0333)

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

0.5 μM[11,o] and to form III at a concentration of 50 μM. For comparison, we would present here the isomers of **A**, novel photocleavers of five-member thio-heterocyclic fused naphthalimides with aminoalkyl side chains at the imide (**B**), and study the influence of the intercalation on the photocleavage.

Therefore, several novel photocleavers $\mathbf{B_{1-4}}$ were designed (Fig. 1). These compounds were synthesized from 4-bromonaphthalic anhydride shown in Scheme 1.[3] After the separation through silica gel column chromatography with the eluent of chloroform–acetone (1:1, v/v), their structures were confirmed by IR, ^{1}H NMR, MS and element analysis.[4] It was obvious from Table 1 that their absorptions were around 382 nm with the similar intensities. They also had the emission at 430 nm with weak fluorescent intensities. All of these compounds were shown to possess high intercalating abilities rather than their isomers, compound **A** (Fig. 1).

The affinities to DNA of these compounds were very strong, binding constants were about $\sim 10^5\ M^{-1}$. With $\mathbf{B_1}$ as an example, the Scatchard binding constant[5] between $\mathbf{B_1}$ and CT-DNA was determined to be $2.8 \times 10^5\ M^{-1}$, which is very similar to that of **D**. It indicated that $\mathbf{B_{1-4}}$ bind DNA mainly via intercalation exerted by the chromophore of thio-heterocyclic fused naphthalimides.

Photocleaving abilities of compounds $\mathbf{B_1}$–$\mathbf{B_4}$ were then examined with the closed supercoiled pBR322 DNA under the photoirradiation at 366 nm as shown in Figure 2a. The cleaving efficiency was defined by the degree of the relaxation of supercoiled DNA. It was apparent that $\mathbf{B_3}$ exhibited the greatest DNA cleaving ability over $\mathbf{B_1}$ and $\mathbf{B_4}$, while $\mathbf{B_4}$ could hardly damage DNA under the same condition. The order of their photocleaving abilities was as follows: $\mathbf{B_3} > \mathbf{B_2} > \mathbf{B_1} > \mathbf{B_4}$.

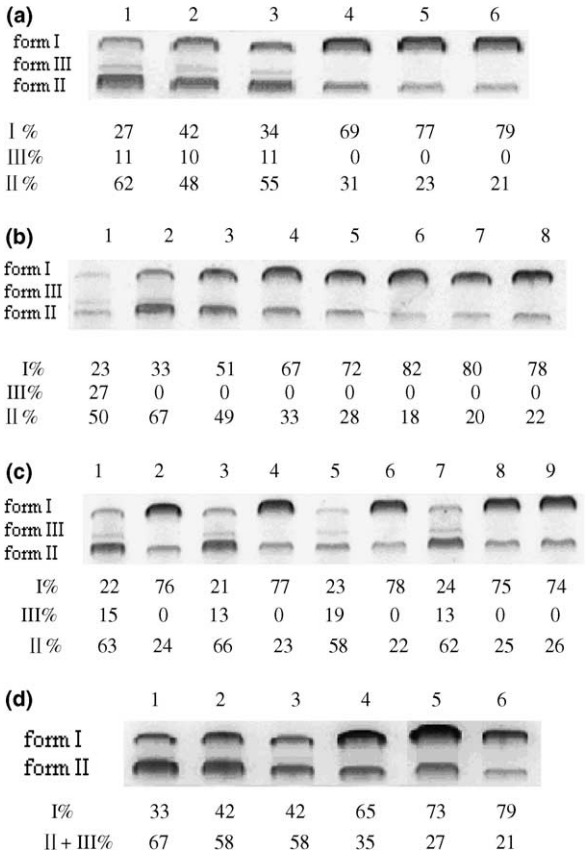
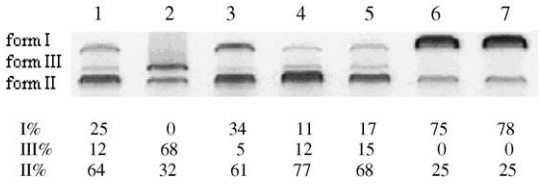


Figure 2. Photocleavage of the supercoiled pBR322 DNA. The cleavage activities were evaluated using the supercoiled circular pBR322 DNA (form I) (30 ng/μL) with a compound in the buffer Tris–HCl (pH 7.5) under photoirradiation (2300 W/cm^2) through a transluminator (366 nm) in the distance of 20 cm at 0 °C and then analyzed on a 1% agarose gel. (a) Photocleavage of compounds $\mathbf{B_1}$–$\mathbf{B_4}$. Photoirradiation: 2 h; lane 1–4: DNA and compounds $\mathbf{B_3}$, $\mathbf{B_1}$, $\mathbf{B_2}$, $\mathbf{B_4}$ at the concentration of 50 μM, respectively; lane 5: DNA alone; lane 6: DNA alone (no hν). (b) Concentration dependent of $\mathbf{B_3}$'s photocleavage. Lane 1–7: DNA and $\mathbf{B_3}$ at the concentration of 100, 50, 20, 10, 5, 2, 0.5 μM, respectively; lane 8: 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 $\mathbf{B_3}$ (50 μM), pH = 8.5, 8.0, 7.5, 7.0, respectively. Lane 9: DNA alone (no hν). (d) Time dependence of photocleavage for $\mathbf{B_3}$ lane 1–4: DNA and $\mathbf{B_3}$ (50 μM) (hν, 120, 90, 60, 30 min), respectively; lane 5: DNA alone (hν, 75 min); lane 6: DNA alone (no hν).

Further experiment indicated that $\mathbf{B_3}$ could cleave the closed supercoiled DNA to the form II at a concentration as low as 10 μM and to the form III 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 affected its DNA cleaving actions (Fig. 2c), and it exhibited better DNA damage abilities under prolonged photoirradiation (Fig. 2d). It was observed previously that $\mathbf{A_3}$, the isomer of $\mathbf{B_3}$, 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. It means that **A** (six-member heterocyclic isomer) have a slight higher DNA photocleavage than **B** (five-member heterocyclic isomer), which is much different from the case for **C** and **D**.



	1	2	3	4	5	6	7
I%	25	0	34	11	17	75	78
III%	12	68	5	12	15	0	0
II%	64	32	61	77	68	25	25

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 photoirradiation (2300 W/cm^2) with a transluminator (366 nm) for 2 h in the distance of 20 cm at 0 °C; lane 1: DNA and **B_3** in the presence of ethanol (1.7 M); lane 2: DNA and **B_3** in the presence of superoxide dismutase (SOD, 100 μg/mL); lane 3: DNA and **B_3** in the presence of dithiothreitol (DTT, 30 mM); lane 4: DNA and **B_3** in the presence of histidine (6 mM); lane 5: DNA and **B_3** at the concentration of 50 μM; lane 6: DNA alone; lane 7: DNA alone (no h*v*).

Mechanism experiment was also performed with the addition of histidine, dithiothreitol, superoxide dismutase and ethanol (Fig. 3). It was found that histidine (singlet oxygen quencher) ethanol (radical quencher) had no effect on the cleavage reaction, However, dithiothreitol (DTT, superoxide anion radical scavenger) retarded the reaction efficiently. It 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 lead to DNA damage in the presence of UV light or after reduction by the trace of metal ions. Obviously, superoxide anion radical was involved in the DNA cleavage. It was supposed by Eriksson and co-workers[6] that the reactive superoxide anion radicals formed in the photoirradation had two pathways. The most common way was reduction of the excited triplet by an electron donor (e.g., one of the DNA bases), followed by electron transfer from the reduced photosensitizer to molecular oxygen. Saito's research work[2d] has proved this way. The other way was a direct ionization of the photosensitizer by radiation, and electron uptake by molecular oxygen ('direct electron transfer'). In our experiment, without using piperidine treatment, we could exclude DNA damage by G oxidation and we found that compounds **A4**, **B4** had no any cleaving ability in the absence of aminoalkyl side chain. It implied that the superoxide anion radical was possibly formed through the electron transfer from nitrogen in aminoalkyl side chain to oxygen.

Compared these two types of isomers (**A** and **B**, **C** and **D**), it could be found that the binding abilities of five-member heterocyclic isomers (binding constants $\sim 10^5\ M^{-1}$ for **D** and **B**) were much higher than that of six-member heterocyclic isomers (binding constants $\sim 10^3\ M^{-1}$ for **C** and **A**). Although the photocleaving abilities of **C** and **D** were parallel to their intercalation abilities, it could be found that those of **A** and **B** were almost anti-parallel to their intercalation abilities. We thought that these might be caused by the differences in DNA damage mechanisms.

The intercalation means that the photocleaver is located in the hydrophobic inner of duplex DNA. For case of **C** and **D**, the hydroperoxides damaged DNA through radical mechanism and high intercalating ability promoted the approximation of the photocleaver to DNA and facilitated the radicals to damage DNA. However, in the case of **A** and **B**, which photodamaged DNA in electron transfer mechanism, the high intercalating ability resulted in the isolation of the photocleaver within the hydrophobic inner of DNA from oxygen in surrounding solution might prevent them from undergoing electron transfer[7] to some extent between the amino groups and oxygen. These two cases implied that the intercalation did not always promote photocleavage and the influence of intercalating ability of chromophores on the cleaving ability of photocleavers depended on their damage mechanisms.

In summary, the present work demonstrated the design and evaluation of novel and high intercalating photocleavers with five-member thio-heterocyclic fused naphthalimides containing aminoalkyl side chains at the imide. They could damage DNA (supercoiled pBR322) from form I (closed) to II (nicked) at a concentration as low as 5 μM and from form I to form III at a concentration of 50 μM. The comparison of these two types of isomers implied that the influence of intercalating abilities of chromophores on the cleaving abilities of photocleavers depended on their damage mechanisms. The experimental results suggested that the aminoalkyl side chain connected with chromophore is also very important for photodamage in electron transfer mechanism. The anti-cancer studies on these photocleavers are also in progress.

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4. **B_1**: mp: 171–172 °C. δ_H (500 MHz; DMSO-*d*; Me_4Si) 2.30 (s, 6H, NCH_3), 2.58–2.62 (m, 2H, NCH_2), 4.21 (t, 2H, *J* 6.55 and 6.62, $CONCH_2$), 7.61–7.69 (m, 2H, 9-H, 10-H), 7.96 (dd, 1H, *J* 7.86 and 7.82, 8-H), 8.18–8.22 (m, 1H, 2-H), 8.54 (d, 1H, *J* 7.20, 11-H), 8.62–8.70 (m, 2H, 1-H, 3-H), 9.28 (s, 1H, 7-H); HRMS: $C_{22}H_{18}N_2O_2S$ calcd: 374.1089, found: 374.1081; ν_{max} (KBr)/cm^{-1} 1660; element analysis: $C_{22}H_{18}N_2O_2S$ requires C, 70.57; H, 4.85; N, 7.48; Found C, 70.45; H, 4.75; N, 7.43. **B_2**: mp 168–169 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.92–1.96 (m, 2H, CH_2), 2.24 (s, 6H, $NHCH_2$), 2.45 (t, 2H, *J* 6.63 and 6.63), 4.23 (t, 2H, *J* 7.38 and 7.69, $CONCH_2$), 7.50–7.58 (m, 2H, 9-H, 10-H), 7.81 (dd, 1H, *J* 7.86 and 7.82, 8-H), 7.92–8.00 (m, 1H, 2H), 8.33 (d, 1H, *J* 7.33, 11-H), 8.44 (d, 1H, *J* 8.00, 3-H), 8.59 (d, 1H, *J* 7.00, 1-H), 9.35 (s, 1H, 7-H); HRMS: $C_{23}H_{20}N_2O_2S$ calcd: 388.1245, found: 388.1253; ν_{max} (KBr)/cm^{-1} 1660; element analysis: $C_{23}H_{20}N_2O_2S$ requires C, 71.11; H, 5.19; N, 7.21; Found C, 70.87; H, 5.10; N, 7.11. **B_3**: mp 226–227 °C. δ_H (500 MHz; DMSO-*d*; Me_4Si) 2.56–2.64 (m, 6H, NCH_2), 3.34 (s, 4H, $NH(CH_2)$), 4.20 (t, 2H, *J* 6.47 and 6.47, $CONCH_2$), 7.61–7.67 (m, 2H, 9-H, 10-H), 7.96 (t, 1H, *J* 7.75 and 7.82, 8-H), 8.16–8.24 (m, 1H, 2-H), 8.54 (d, 1H, *J* 7.27, 11-H), 8.64 (d, 1H, *J* 8.13, 3-H), 8.66 (d, 1H, *J* 7.96, 1-H), 8.80 (br, 1H, NH), 9.25 (s, 1H, 7-H). HRMS: $C_{24}H_{21}N_3O_2S$ calcd: 415.1354, found: 415.1349; ν_{max} (KBr)/cm^{-1} 1660; element analysis: $C_{24}H_{21}N_3O_2S$: requires C, 69.37; H, 5.09; N, 10.11; Found C, 69.23; H, 5.01; N, 10.11. **B_4**: mp 170–171 °C. δ_H (500 MHz; $CDCl_3$; Me_4Si) 0.92 (t, 3H, *J* 7.36 and 7.36, CH_3), 1.41–1.47(m, 2H, Me–CH_2), 1.65–1.71 (m, 2H, Et–CH_2), 4.13 (t, 2H, *J* 7.57 and 7.62, $CONCH_2$), 7.50–7.58 (m, 2H, 9-H, 10-H), 7.79 (t, *J* 7.78 and 7.66, 1H, 8-H), 7.92 (dd, 1H, *J* 7.14 and 1.42, 1H, 2-H), 8.27–8.35 (m, 1H, 11-H), 8.40 (d, 1H, *J* 7.67, 3-H), 8.60 (dd, 1H, *J* 7.32 and 0.90, 1-H), 9.20 (s, 1H, 7-H). HRMS: $C_{22}H_{17}NO_2S$ calcd: 359.0980, found: 359.0992; ν_{max} (KBr)/cm^{-1} 1660; element analysis: $C_{22}H_{17}NO_2S$ requires C, 73.51; H, 4.77; N, 3.90; Found C, 73.47; H, 4.96; N, 3.84.
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