Interaction of naphthyl heterocycles with DNA: effects of thiono and thio groups

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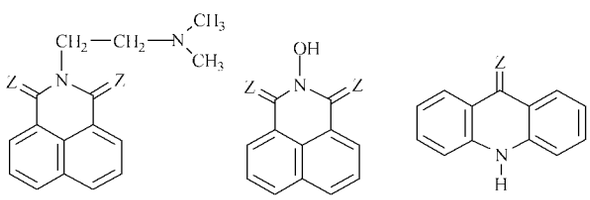
Received (in Cambridge, UK) 4th November 1999, Accepted 14th January 2000

The intercalation and photocleavage of DNA by *N*-[β-(*N*′,*N*′-dimethylamino)ethyl]dithiono-1,8-naphthalimide (**2**) were extremely effective compared to the use of the oxygen-containing counterpart (**1**). Their photocleavage action under 366 nm UV light is proposed to proceed by electron transfer from bases to the triplet state of the naphthalimides. The enhancement of the intercalation of DNA and the photocleavage of DNA were also observed for other compounds possessing a thiono or thio group compared with their oxygen-containing counterparts.

Introduction

There is increasing interest in artificial nucleases,[1–4] small molecules capable of cleaving duplex or single-stranded DNA under controllable conditions. In particular, photonucleases can be triggered by exposure to light. Visible or near-UV light is an attractive and safe cofactor, since it is easy to manipulate and inert to nucleic acid molecules. There are a variety of studies concerning DNA photocleavage[5–12] by photosensitizers which either initiate a single electron transfer from a base to the triplet state of chromophores, which often leads to a selective cleavage at the 5′-G of GG step in duplex DNA, or generates active oxygen species upon photoirradiation, but there have as yet been no reports on comparing the cleaving and intercalating activities of compounds with oxo or oxy groups with those of their thiono or thio counterparts. The sulfur-containing molecule has advantages, such as easy preparation and derivatization, and longer-wavelength absorption. Our results showed that DNA photocleavages and intercalations were significantly enhanced in the case of the compounds possessing a thiono or thio group compared with oxygen-containing counterparts.



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2: Z=S

3: Z=O
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5: Z=O
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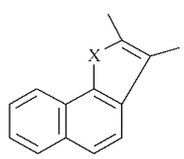

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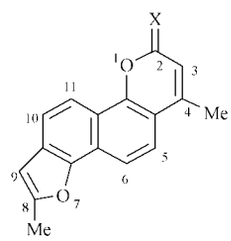

9: Z=O
10: Z=S

Results and discussion

First, we prepared two naphthalimide compounds possessing oxo and thiono units, *i.e.* *N*-[β-(*N*′,*N*′-dimethylamino)ethyl]-1,8-naphthalimide (**1**)[15,16] and *N*-[β-(*N*′,*N*′-dimethylamino)-ethyl]dithiono-1,8-naphthalimide (**2**), as the candidate photoactive compounds, and examined their DNA cleaving activities under photoirradiative conditions quantitatively by measuring the conversion of pUC 19 DNA (form I) to relaxed circular DNA (form II). When pUC 19 DNA (form I) was photoirradiated with a 450 W high-pressure mercury lamp for 2 h at 366 nm using a transilluminator in the presence of **1** and **2**, it was cleaved to afford relaxed circular DNA (form II). Single-strand cleaving abilities exhibited by **1** and **2** are listed in Table 1 and shown in Figs. 1–3. It was found that the photocleavage action of compound **2** with a thiono moiety was much stronger than that of its oxygen-containing counterpart **1**, and at a higher concentration level (200 μmol L^{-1}) the strong photocleavage of compound **2** to pUC 19 DNA (form I) gave not only relaxed circular DNA (form II) but also linear DNA (form III) (shown in Fig. 2).

The nicking efficiency was not reduced whether the photoirradiation was conducted under aerobic conditions or under a nitrogen atmosphere. Clearly, O_2 was not required for efficient cleavage of DNA. It was established through control experiments that active oxygen species were not involved in the reactions of DNA with **1** and **2**. None of the additives, *e.g.* histidine (well known singlet oxygen quencher), superoxide dismutase (SOD, superoxide radicals killer), catalase (H_2O_2 killer), ethanol (hydroxyl radical quencher), showed any significant effects. This suggested that neither singlet oxygen, superoxide anions, hydrogen peroxide nor hydroxyl radicals were involved. We postulated that DNA photocleavage by 1,8-naphthalimide **1** and dithiono-1,8-naphthalimide **2** might be an electron transfer

DOI: 10.1039/a908787g

Table 1 Single-strand cleavage of supercoiled circular pUC 19 DNA (form I) to relaxed circular DNA (form II) by irradiation of oxygen- and sulfur-containing fused heterocycles at 0 °C for 2 h with 366 nm UV light[a]

Heterocycle	% Form I[b]	% Form II[b]	Form II/ Form I	Ratio[c]	LUMO[d]	HOMO[d]	ΔE_{L-H}	$\Delta\Delta E$
1	73	27	0.37		−1.0539	−9.2995	8.2466	2.2510
2	02	98	49.00	**2**:**1** = 132.5	−2.8842	−8.8809	5.9956	
3	74	26	0.35		−1.7380	−9.1950	7.4571	0.2020
4	62	38	0.61	**4**:**3** = 1.75	−1.6514	−8.9065	7.2551	
5	68	32	0.47		−0.5491	−8.4215	7.8721	1.4077
6	43	57	1.33	**6**:**5** = 2.83	−1.8063	−8.2707	6.4644	
7	46	54	1.17		−0.2733	−8.6804	8.4071	0.4782
8	41	59	1.90	**8**:**7** = 1.62	−0.4443	−8.3733	7.9289	
None	96	04	0.04					

[a] Photoirradiation of the reaction mixture containing **1**–**8** (50 μmol L^{-1}) and pUC 19 (4 μmol l^{-1} concentration) was carried out for 2 h at 0 °C at a distance of 15 cm from the transilluminator (366 nm, 1500 μW cm^{-2} at 15 cm). [b] Yields of form I and form II DNA were determined by a computer imaging system. [c] The sulfur-containing compounds to the oxygen counterparts. [d] The frontier orbital energies (eV) were obtained from PM3 MO calculations.

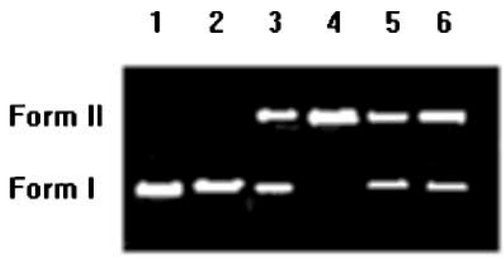

Fig. 1 Effect of UV-light irradiation on single-strand cleavage by compounds **1** and **2**. Electrophoresis of mixtures of pUC19 plasmid DNA (4 μmol L^{-1}) kept in the dark with 50 μmol L^{-1} **2** (lane 3), or 50 μmol L^{-1} **1** (lane 5) in aqueous buffered (20 mmol L^{-1} Tris-HCl, pH 7.6) methyl cyanide solution, compared to samples irradiated (lanes 4, 6 respectively), and with unirradiated (lane 1) and irradiated DNA (lane 2) as control.

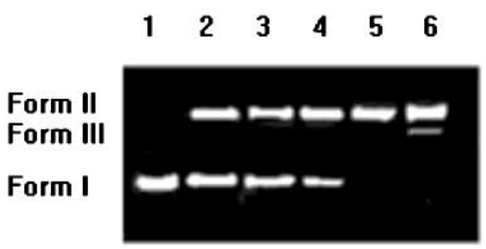

Fig. 2 Effect of concentrations of compound **2** on the photocleavage of DNA. DNA control is in lane 1, the concentrations of **2** were 0.05, 0.5, 5, 50, 200 μmol L^{-1} (lanes 2–6).

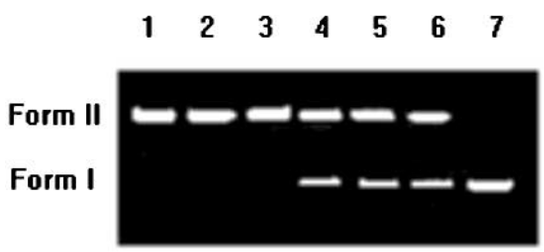

Fig. 3 Effect of radical scavengers on the photocleavage of DNA. 50 μmol L^{-1} **2** (lanes 1–3) or **1** (lanes 4–6) were in aqueous buffered (20 mmol L^{-1} Tris-HCl, pH 7.6) methyl cyanide solution. Lanes 1, 4 were in the presence of ethanol (1.7 mmol L^{-1}); lanes 2, 5 were in the presence of SOD (1.0 μmol L^{-1}); lanes 3, 6 were in the presence of histidine (1.2 μmol L^{-1}); lane 7 was the DNA as control.

process from bases to the triplet state of the naphthalimide, *i.e.* a photodynamic type I reaction. Significantly, the DNA cleavage efficiency for **2** was enhanced approximately 200% upon treatment with piperidine at 90 °C, suggesting that **2** might react preferentially with the DNA base.

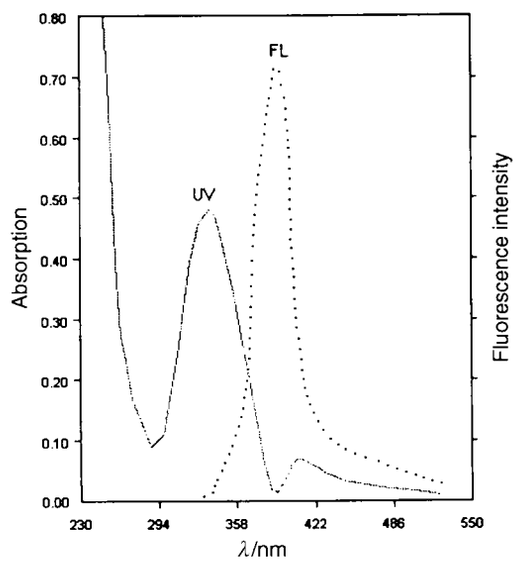

Fig. 4 The UV–Vis absorption and fluorescence spectra of compound **2** in ethanol.

In order to examine the general situation that thiono or thio groups promote efficient reaction for cleavage of DNA, we also prepared some other compounds, *e.g. N*-hydroxy-1,8-naphthalimide (**3**), *N*-hydroxydithiono-1,8-naphthalimide (**4**), acridone (**5**), thionoacridone (**6**), naphtho[1,2-*b*]furan (**7**), and naphtho[1,2-*b*]thiophene (**8**), and examined their DNA cleaving activities. Inspection of the data of relative ratios of form II to form I and sulfur to oxygen in Table 1 revealed that DNA photocleavages were significantly enhanced in the case of the compounds possessing a thiono or thio group (**4**, **6**, **8**) compared with their oxygen-containing counterparts (**3**, **5**, **7**). Compound **2** was the most active DNA-nicking agent among the eight compounds.

One of the reasons for thiono or thio compounds having high DNA cleaving activity might be that they usually have more efficient intersystem crossing efficiency, and much higher photosensitizing activity.[24–26]

The LUMO and HOMO orbital energies for the eight compounds were obtained from PM3 calculations and are listed in Table 1. The calculated results show that the energy differences between LUMO and HOMO (ΔE_{L-H}) are roughly reversed parallel to their DNA cleaving abilities (form II/form I) and that the value of ΔE_{L-H} ($E_{LUMO} - E_{HOMO}$) for **2** is the smallest. The MO calculations also provide insight into the character of the LUMO and the HOMO. For **2**, LUMO is mainly located at the C=S bond, the lowest singlet state (S_1) is mainly $n\pi^*$ in

nature for **2**. Therefore it is reasonable to conclude that the smallest ΔE_{L-H} for **2** is responsible for its prominent DNA photocleaving ability. This proposal tallies with the single electronic mechanism described above.

As oxo-naphthalimide **1** was a known DNA intercalator, we supposed that compound **2** should have much higher DNA intercalative action, which might account for one factor to promote its photocleaving action.

We ran the dark interaction of **2** with calf thymus DNA in Tris-HCl (pH 7.4) containing 10% DMSO (v/v) by a fluorescence quenching technique,[13,14,20] which assumed that the amount of fluorescence quenching is proportional to that of chemicals bound to DNA. The UV and fluorescence (EtOH) spectra for compound **2** (shown in Fig. 4) gave maximum absorption at 345 nm (4.85×10^4) with a shoulder absorption at 410 nm (0.72×10^4), fluorescence at λ_x 395 nm, φfl 0.28 (quinine sulfate as standard) with excitation at λ_x 340 nm. It was found that in the dark thiono-naphthalimide **2** had an apparent Scatchard binding constant K_a 3.0×10^5 M^{-1} compared with oxo-naphthalimide **1** with 1.7×10^4 M^{-1}.[15] It means at least that the thiono moiety would also promote efficient interaction with DNA. We have found that oxygen-containing dimethylfuronaphthopyrone **9** is a good DNA intercalator, so we prepared its thiono derivative **10**, carried out the interaction of **10** with calf thymus DNA by using a fluorescence quenching technique and also found that thiono derivative **10** with K_a 1.37×10^6 M^{-1} has a higher affinity to DNA than oxo-compound **9** with K_a 4.74×10^5 M^{-1}.[20]

In summary, the present studies primarily revealed that taking sulfur instead of oxygen is a useful strategy for constructing novel photocleaving agents and intercalators of DNA. The present observations are also of interest from the standpoint of naphthalimide anti-tumour drugs.

Experimental

General methods

Melting points were taken on a digital melting point apparatus, WRS-1, made in Shanghai and are uncorrected. Infrared spectra were recorded on a Nicolet FT IR-20SX, mass spectra on a Hitachi M80, ^{1}H NMR spectra on a Bruker AM-300 using $CDCl_3$ or TMS as an internal standard. Combustion analysis for elemental composition was done on an Italy MOD. 1106 analyser. Absorption spectra were measured on a Shimadzu UV-265, fluorescence spectra on a Perkin Elmer LS50 with quinine sulfate in sulfuric acid as the quantum yield standard. Commercial reagents and solvents were purchased from a standard chemical supplier and used without further purification.

Compounds **1**,[15,16] **2**,[21–23] **3**,[17] **5**[27] and **6**,[19] **7**,[18] **9**[20] were synthesized and characterized according to the literature.

Synthesis of *N*-hydroxydithiono-1,8-naphthalimide (4)

246 mg (1 mmol) of trithio-1,8-naphthalic anhydride and 100 mg (1.5 mmol) of hydroxylamine were refluxed in 1 ml pyridine for 2.5 h. After removal of pyridine in vacuum and addition of methylene chloride, the separation was carried out on silica gel with a mixture (petroleum ether–ethyl acetate = 1 : 1, v/v) as eluent; 137 mg of a deep brown solid were obtained in 56% yield with mp 102 ~ 104 °C (dec), ν_{max} (KBr)/cm^{-1} 3500–3000 (br), 1600, 1500 (C=C), 1260 (C=S), 1150 (br), 1050, 840, 720. *m*/*z* 245 (M^+, 2.2%), 229 ($M^+ - O$, 12.9%), 213 ($M^+ - S$, 9.6%), 196 ($M^+ - HO - S$, 25.4%), 126 (9.5%), 63 (100%)

Synthesis of 2,3-dimethylnaphthothiophene (8)

3.2 g 1-mercaptonaphthalene, which was prepared from the reduction of naphthalenesulfonyl chloride with zinc powder in a mixture of water and sulfuric acid at −5–0 °C, 4.0 ml (40.0 mmol) 3-chlorobutan-2-one and 3.29 g (23.8 mmol) potassium carbonate in 50 ml butan-2-one were refluxed for 2 days. After removal of the solvent in vacuum and addition of water (50 ml), extraction of the reaction mixture with a solvent mixture (3 × 50 ml, ether–petroleum ether = 16 : 1, v/v), washing the organic phase with water, drying over $MgSO_4$, and removal of solvent, a brown yellow liquid of 1-[(2′-oxobut-3′-yl)thio]-naphthalene as an intermediate was obtained in 80.4% yield. 3.7 g intermediate and 28.0 g polyphosphoric acid were heated at 140 °C and stirred for 4 h; after cooling, adding ice water (150 ml) and removal of solvent, the separation was carried out on silica gel with a mixture (petroleum ether–ethyl acetate = 7 : 1, v/v) as eluent to give a brown oily liquid in 71% yield. ν_{max}(film)/cm^{-1} 3050, 2940, 1630, 1430, 1380, 1350, 800, 770, 700, 680. *m*/*z* (%) 212 (M^+, 100%), 197 (36%), 119 (27%).

Synthesis of 2*H*-4,8-dimethylfuro[2′,3′ : 5,6]naphtho[1,2-*b*]-pyran-2-thione (10)

0.450 g (1.02 mmol) of Lawesson's reagent and 0.500 g (1.89 mmol) of 2*H*-2,4-dimethylfuro[2′,3′ : 5,6]naphtho[1,2-*b*]pyran-2-one in 3 ml toluene were refluxed for 2 h; after cooling, removal of solvent and recrystallization in a solvent mixture (methylene chloride–petroleum ether = 1 : 1, v/v), 0.402 g yellow needles were obtained in 76% yield with mp 288.4–289.2 °C, ν_{max}(KBr)/cm^{-1} 2920, 1640, 1605, 1582, 1548, 1470, 1434, 1414, 1378, 1320, 1296, 1170, 1090, 1036. δ_H ($CDCl_3$)/ppm 2.50 (3H, s, 4-CH_3), 2.62 (3H, s, 8-CH_3), 6.60 (1H, s, 9-H), 7.31 (1H, s, 3-H), 7.72 (1H, d, J = 8.85 Hz, 5-H), 7.77 (1H, d, J = 8.74 Hz, 10-H), 8.19 (1H, d, J = 8.85 Hz, 6-H), 8.53 (1H, d, J = 8.74 Hz, 11-H). *m*/*z* 282 ($M^+ + 2$, 6.2%), 280 (M^+, 100%), 236 ($M^+ -$ CS, 91.1%), 235 (20.5%) (Found: C, 72.28; H, 4.24. $C_{17}H_{12}O_2S$ requires C, 72.83; H, 4.31%).

Acknowledgements

This work was supported by the Fuk Ying Tung Foundation. T. Huang expresses his appreciation for support from the China and Shanghai Postdoctoral Science Foundation.

References

1 S. Steenken, *Chem. Rev.*, 1989, **89**, 503.
2 A. Sitlani, E. C. Long, A. M. Pyle and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 2303.
3 D. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295.
4 G. Pratviel, J. Bernadou and B. Meunier, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 746.
5 S. Matsugo, S, Nakano, K. Adachi and T. Konishi, *J. Chem. Soc., Chem. Commun.*, 1995, 311.
6 I. Saito and M. Takayama, *J. Am. Chem. Soc.*, 1995, **117**, 5590.
7 I. Saito, M. Takvama, H. Sugiyama and K. Nakatani, *J. Am. Chem. Soc.*, 1995, **117**, 6406.
8 H. Sugiyama and I. Saito, *J. Am. Chem. Soc.*, l996, **118**, 7063.
9 D. T. Breslin and G. B. Schuster, *J. Am. Chem. Soc.*, 1996, **118**, 2311.
10 D. Ly, Y. Kan, B. Armitage and G. B. Schuster, *J. Am. Chem. Soc.*, 1996, **118**, 8747.
11 J. L. Sessler, P. I. Sansom, V. Kral, D. O'Connor and B. L. Iverson, *J. Am. Chem. Soc.*, 1996, **118**, 12 322.
12 B. M. Aveline, S. Matsugo and R. W. Redmond, *J. Am. Chem. Soc.*, 1997, **119**, 1785.
13 G. Scatchard, *Ann. N. Y. Acad. Sci.*, 1949, **51**, 660.
14 M. Gupta and R. Ali, *J. Biochem.*, 1984, **95**, 1253.
15 S. Yen, E. J. Gabbay and W. D. Wilson, *Biochemistry*, 1992, **21**, 2070.
16 J. Mehratra, K. J. Misra and R. K. Mishra, *Nucleosides, Nucleotides*, 1994, **13**, 963.
17 J. Suszko and S. Kinastowski, *Rocz. Chem.*, 1967, **41**, 319.
18 G. Pratviel, J. Beradou and B. Meunier, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 746.
19 J. Chen and M. S. Gibson, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1138.
20 X. Qian, Z. Tao and D. Wei, *Monatsh. Chem.*, 1996, **127**, 569.
21 W. John, *Bull. Soc. Chim. Fr.*, 1996, **2**, 566.

22 T. Huang, X. Qian, Z. Tao, K. Wang, G. Song and L. Liu, *Heteroatom Chem.*, 1999, **10**, 141.
23 T. Huang, J. Zhang, W. Yao and X. Qian, *Synthesis* (*Stuttgart*), 1999, 1109.
24 A. Jakobs and J. Piette, *J. Photochem. Photobiol.*, *B*, *Biol.*, 1994, **22**, 9.
25 A. E. Jakobs and J. Piette, *J. Med. Chem.*, 1995, **38**, 869.
26 D. Vedaldi, S. Caffieri, S. Frank, F. Dall'Aqua, A. Jakobs and J. Piette, *Il Farmaco*, 1995, **50**, 527.
27 A. De Leenheer, Y. Y. Shum, J. E. Sinsheimer and J. H. Burckhalter, *J. Pharm. Sci.*, 1971, **60**, 1238.

Paper a908787g