2-(3-Methylthiopropyl)-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-dione Derivatives as Novel Photo-induced DNA Cleavers

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The efficient DNA cleavage of φx 174 DNA is observed in the longer wavelength (>350 nm) irradiation of 2-(3-methylthiopropyl)-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones **1a–e**, but not in the cases of 2-isobutyl-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones **1f, g** which is explained by considering the participation of persulfoxirane intermediate **4** in the DNA photocleavage.

DNA cleavage by 'synthetic restriction enzyme' is a recent topic in biology and organic chemistry.[1] The strategy for the DNA cleavage depends on the design of molecules that generate highly active species (involving active oxygen radicals) in the vicinity of the DNA molecule. Much effort has been devoted to the synthesis of highly active ene-diyne molecules which generate the π-radicals triggered by inter- or intra-molecular di- or tri-sulfides.[2] There are also several reports[3] concerning the photocleavage of DNA by hydroxyl radicals generated by the photodecomposition of hydroperoxides. Another approach to DNA photocleavage is the use of photosensitizers that generate active oxygen species upon photoirradiation.[4]

Although there is a variety of studies concerning DNA photocleavage by highly reactive oxygen species, there have been as yet no reports on DNA photocleavage using sulfur–dioxygen complexes which can be obtained by the oxidation of sulfides with singlet molecular oxygen or direct photosensitization of sulfides with oxygen.[5] The reactivity of sulfur–dioxygen complexes have been studied extensively and the accumulated results concerning the highly reactive sulfur–dioxygen complexes imply strong potential DNA cleaving activities. So it is a promising strategy to utilize the chemical properties of sulfur–dioxygen complexes for designing DNA cleaving reagents.

Here, we have prepared a series of 2-(3-methylthiopropyl)-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones **1a–e**, from the condensation reaction of 1,8-naphthalic anhydride derivatives and 3-thiomethylpropylamine, as the candidate sulfur–dioxygen forming compounds, and examined their DNA cleaving activities under photoirradiative conditions.

When the acetonitrile solution of **1** (0.1 mmol dm^{-3}) was photoirradiated with a 100 W high-pressure mercury UV lamp through the Pyrex filter under aerobic conditions, **2** was obtained as the only photoproduct. In the absence of oxygen, **1** did not afford **2** at all even after 12 h photoirradiation. Neither was **2** formed by standing the solution of **1** in aerobic conditions without photoirradiation (7 days). All these results strongly support the mechanism for the photolytic conversion of **1** to **2** shown in Scheme 1, where **1** reacts with molecular oxygen (singlet oxygen) to afford the persulfoxirane-type intermediates **4**, which then react with another equivalent of **1** to afford two moles of **2** quantitatively.

As the highly reactive persulfoxirane **4** was anticipated to intervene in the conversion of **1** to **2**, DNA photocleavage by **1** was quantitatively studied by measuring the conversion of supercoiled circular φx 174 DNA (form I) to relaxed circular φx 174 DNA (form II).

When φx 174 DNA (form I) was photoirradiated for 30 min at 366 nm using a transilluminator in the presence of **1a–e**, it was cleaved to afford relaxed circular DNA (form II). In the case of **1d**, the relative ratio II:I increases up to 6.5, while 2-isobutyl-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones **1f,g**, which lack the S atom in the side chain, did not cause any significant DNA photocleavage under the same reaction conditions; *i.e.* DNA photocleavage by **1d** was extremely effective compared to that by **1g** (*cf.* II:I = 6.52 for **1d**, 0.15 for

Table 1 Cleavage of the supercoiled circular φx 174 DNA (form I) to relaxed circular DNA (form II) by 10 μmol dm^{-3} of 2-(3-methylthiopropyl)-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones **1a–g** upon photo-irradiation[a] under aerobic conditions for 1 h

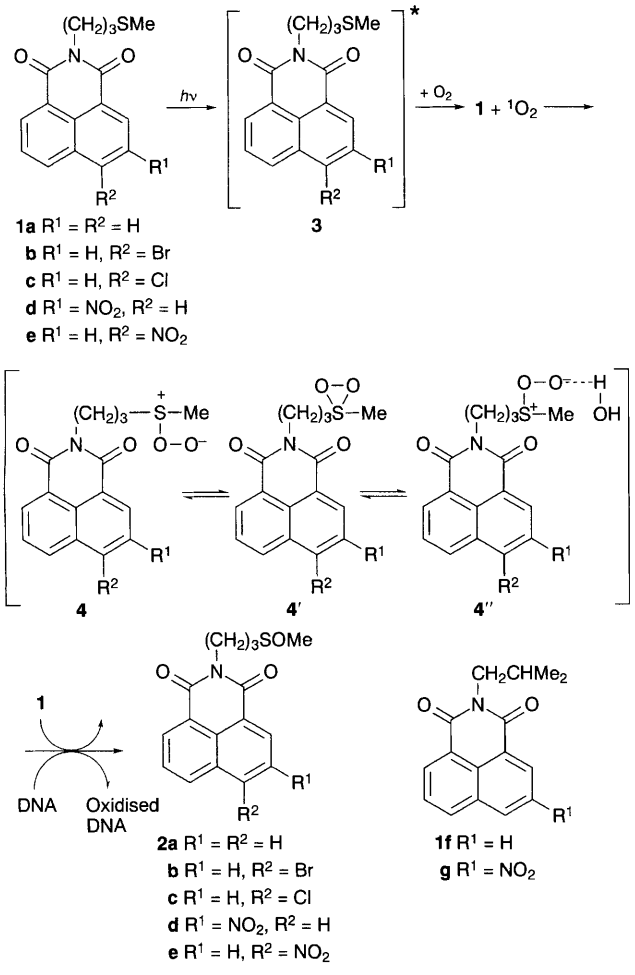
Naphthalimide	Form I (%)[b]	Form II (%)[b]	Form II:Form I
None	94.1	5.9[c]	0.06
1a	84.4	15.6	0.18
1b	74.7	25.3	0.34
1c	79.6	20.4	0.26
1d	13.3	86.7	6.52
1e	38.6	61.4	1.59
1f	91.5	8.5	0.09
1g	86.7	13.3	0.15

[a] Photoirradiation of the reaction mixture containing **1** and φx 174 DNA (30 μg ml^{-1} concentration) was carried out for 1 h at 0 °C at a distance of 10 cm from the transilluminator (366 nm, light intensity 1700 μW cm^{-2} at 10 cm distance). [b] The yields (%) of form I and form II DNA were determined by a computer imaging system. [c] The φx 174 DNA (form I) contains a small amount of relaxed circular DNA (form II).

Table 2 Cleavage of the supercoiled circular φx 174 DNA (form I) to relaxed circular DNA (form II) by 50 μmol dm^{-3} of 2-(3-methylthiopropyl)-1*H*-benz[*d,e*]isoquinoline-1,3(2*H*)-diones (**1a–e**) upon photo-irradiation[a] under aerobic conditions for 0.5 h in H_2O and D_2O solvents[a]

Naphthalimide	Solvent	Form I (%)[b]	Form II (%)[b]	Form II/Form I
none	H_2O	91.9	8.1[c]	0.09
1a	H_2O	69.9	30.1	0.43
1	D_2O	44.8	55.2	1.23
1b	H_2O	56.0	44.0	0.79
1	D_2O	42.4	57.6	1.36
1c	H_2O	61.3	38.7	0.63
1	D_2O	43.2	56.8	1.31
1d	H_2O	31.1	68.9	2.22
1	D_2O	23.9	76.1	3.18
1e	H_2O	49.3	50.7	1.03
1	D_2O	36.3	63.7	1.75
1a[d]	H_2O	84.2	15.8	0.19
1b[d]	H_2O	84.1	15.9	0.19
1c[d]	H_2O	84.2	15.8	0.19
1d[d]	H_2O	63.5	36.5	0.57
1e[d]	H_2O	70.3	29.7	0.42
1a[e]	H_2O	84.2	15.8	0.19
1b[e]	H_2O	84.1	15.9	0.19
1c[e]	H_2O	84.2	15.8	0.19
1d[e]	H_2O	63.5	36.5	0.57
1e[e]	H_2O	70.3	29.7	0.42

[a] Photoirradiation of the reaction mixture containing **1** and φx 174 DNA (30 μg ml^{-1} concentration) was carried out for 1 h at 0 °C at a distance of 10 cm from the transilluminator (366 nm, light intensity 1700 μW cm^{-2} at 10 cm distance). [b] Yields of form I and form II DNA were determined by a computer imaging system. [c] The φx 174 DNA (form I, 30 μg m^{-1}) contains a small amount of relaxed circular DNA (form II). [d] Sodium azide (1 mmol dm^{-3}) was added to the reaction mixture. [e] Diazabicyclo[2.2.2]octane (1 mmol dm^{-3}) was added to the reaction mixture.



Scheme 1

1g). If the singlet oxygen produced from the photoreaction of **1** is the sole species for DNA photocleavage, such a big difference in the DNA photocleavage should not be observed in these cases. These results clearly indicate the importance of the thiomethyl group in the side chain; *i.e.* the sulfide atom present in the alkyl side chain plays a crucial role in the DNA photocleavage (Table 1).

To clarify the role of singlet oxygen in the DNA photocleavage reaction, the effect of D_2O on the DNA photocleavage was studied. The photocleavage was significantly enhanced in D_2O compared with H_2O for all cases. For example, the ratio II : I for **1a** was 0.43 in H_2O, while in D_2O it became 1.23 (*ca.* 3× greater). In addition, sodium azide (1 mmol dm^{-3}) or diazabicyclo[2.2.2]octane (1 mmol dm^{-3}), well-known singlet oxygen quenchers, suppressed the DNA photocleavage significantly in every case, as shown in Table 2. The concentration of sodium azide required for 70% inhibition of the reaction was approximately 1 mmol dm^{-3} when **1b** at 50 μmol dm^{-3} was irradiated with φx 174 DNA for 30 min. All these results clearly indicate that singlet oxygen is involved in the DNA photocleavage by **1**. If the singlet oxygen reacts directly with DNA to cleave it, the D_2O effect should be much greater (several to ten times) than that observed in H_2O because the lifetime of singlet oxygen in D_2O is more than 20 times greater than in H_2O.[6] Furthermore, the effect of D_2O on the **1d** and **1g** mediated DNA cleavage differed greatly; the activity of **1g** was far less than that of **1d**. These results strongly suggest that the DNA photocleavage by **1** is not a simple photodynamic type II reaction. From the present experimental results, we can draw a reaction mechanism for DNA photocleavage by **1** (Scheme 1), where the persulfoxirane intermediate **4**, produced from the reaction of **1a–e** and singlet oxygen, acts as an ultimate reactive species in the DNA photocleavage.

The present studies revealed that **1**, although having quite a simple chemical structure carries the two essential requirements for efficient DNA chain cleavers: the strong DNA binding ability of 1*H*-benz[*d*,*e*]isoquinoline-1,3(2*H*)-dione,[7] and the formation of highly reactive persulfoxirane. Since **1** has several advantages such as easy preparation and derivatization, the introduction of various functional groups can be easily attained as an intercalator or a groove binder (or protein). Thus, **1** provides an alternative new approach for developing efficient photomediated DNA modifying reagents. The present observations are also of interest from the standpoint of the physiological importance of protein damage in DNA cleavage. It might be possible that the DNA binding protein is damaged by active oxygen species to form the persulfoxirane in the vicinity of DNA.

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References

1 For the application of artificial restriction enzyme see the following reviews and references cited therein: P. A. Beal and P. B. Dervan, *Science*, 1991, **251**, 1360; J.-C. S. Francois, T. Saison-Behmoaras, C. Barbier, M. Chassignol, N. T. Thuong and C. Helene, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 9702; P. E. Nielsen, C. Hiort, S. H. Sonnichsen, O. Buchardt, O. Dahl and B. Norden, *J. Am. Chem. Soc.*, 1992, **114**, 4967; N. T. Thuong and C. Helene, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 666; D. S. Sigman, A. Mazumder and D. M. Perin, *Chem. Rev.*, 1993, **93**, 2295.

2 For example see the following reviews and references cited therein: K. C. Nicolaou and A. L. Smith, *Pure Appl. Chem.*, 1993, **65**, 1271; K. C. Nicolaou, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1377; P. C. Dedon and I. H. Goldberg, *Chem. Res. Toxicol.*, 1992, **5**, 311; K. C. Nicolaou and A. L. Smith, *Acc. Chem. Res.*, 1992, **25**, 497; K. C. Nicolaou and W.-M. Dai, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1387; I. H. Goldberg, *Acc. Chem. Res.*, 1991, **24**, 191.

3 S. Matsugo, M. Takayama, T. Matsuura and I. Saito, *Photomed. Photobiol.*, 1989, **11**, 9; I. Saito, M. Takayama, T. Matsuura, S. Matsugo and S. Kawanishi, *J. Am. Chem. Soc.*, 1990, **112**, 883; S. Matsugo, S. Kawanishi, K. Yamamoto, H. Sugiyama, T. Matsuura and I. Saito, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1351; G. N. Liang, P. Gannett, X. L. Shi, Y. Zhang, F. X. Chen and B. Gold, *J. Am. Chem. Soc.*, 1994, **116**, 1131.

4 For example see the following papers and references cited therein: O. Buchardt, M. Egholm, G. Karup and P. E. Nielsen, *J. Chem. Soc., Chem. Commun.*, 1987, 1696; P. E. Nielsen, C. Jeppesen, M. Egholm and O. Buchardt, *Biochemistry*, 1988, **27**, 6338; E. Nishiwaki, H. Lee, T. Matsumoto, K. Toyooka, H. Sakurai and M. Shibuya, *Tetrahedron Lett.*, 1990, **31**, 1299; S. Takenaka, T. Ihara and M. Takagi, *Chem. Lett.*, 1992, 1; D. M. Perrin, P. A. King, V. E. Anderson, J. O. Edwards, G. Gustafson, R. C. Plumb and J. W. Suggs, *J. Am. Chem. Soc.* 1992, **114**, 5430; R. Kuroda and M. Shinomiya, *Biochem. Biophys. Res. Commun.*, 1991, **181**, 1266; T. Koch, J. D. Ropp, S. G. Sligar and G. B. Schuster, *Photochem. Photobiol.*, 1993, **58** 554; H. Tokuyama, S. Yamago, E. Nakamura, T. Shiraki and Y. Sugiura, *J. Am. Chem. Soc.*, 1993, **115**, 7918; J. Mehrotra, K. Misra and R. K. Mishra, *Nucleosides, Nucleotides*, 1994, **14**, 963; X. S. Chen and J. Kagan, *J. Photochem. Photobiol., B-Biol.*, 1994, **23**, 27.

5 For example see the following reviews and references cited therein: K. Nahm, Y. Li, D. Evanseck, K. N. Houk and C. S. Foote, *J. Am. Chem. Soc.*, 1993, **113**, 4879; T. Akasaka and W. Ando, *Organic Peroxides*, ed. W. Ando, Wiley, New York, 1992, pp. 599–659; Y. Watanabe, N. Kuriki, K. Ishiguro and Y. Sawaki, *J. Am. Chem. Soc.*, 1991, **113**, 2677; F. Jensen and C. S. Foote, *J. Am. Chem. Soc.*, 1988, **110**, 2368.

6 D. Bellus, *Adv. Photochem.*, 1979, **11**, 105; M. A. J. Rodjers, *J. Am. Chem. Soc.*, 1983, **105**, 4129.

7 W. D. Waring, A. Gonzalez, A. Jimenez and D. Vazquex, *Nucleic Acid Res.*, 1979, **7**, 217.