Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 2957–2960

TETRAHEDRON LETTERS

Oxime esters of anthraquinone as photo-induced DNA-cleaving agents for single- and double-strand scissions

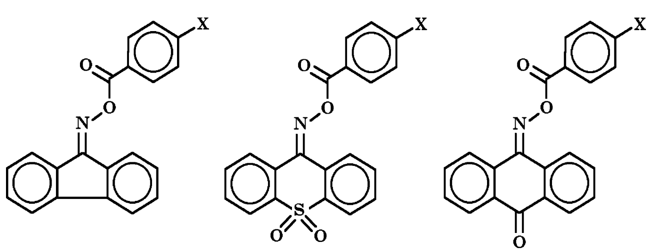
Jih Ru Hwu,[a,*] Shwu-Chen Tsay,[a] Shih Chin Hong,[a] Yi-Jing Leu,[a] Chih-Fen Liu[b] and Shang-Shing P. Chou[b,*]

[a]*Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30013, ROC*
[b]*Department of Chemistry, Fu Jen Catholic University, Taiwan 24205, ROC*

Received 15 October 2002; revised 6 February 2003; accepted 10 February 2003

Abstract—Anthraquinone-*O*-9-(4-cyanobenzoyl)oxime (**13**) with binding constant of 4.49×10^4 M^{-1} exhibited single-strand scission of DNA at the concentration of 10 μM and double-strand scission at 50 μM upon UV irradiation. © 2003 Published by Elsevier Science Ltd.

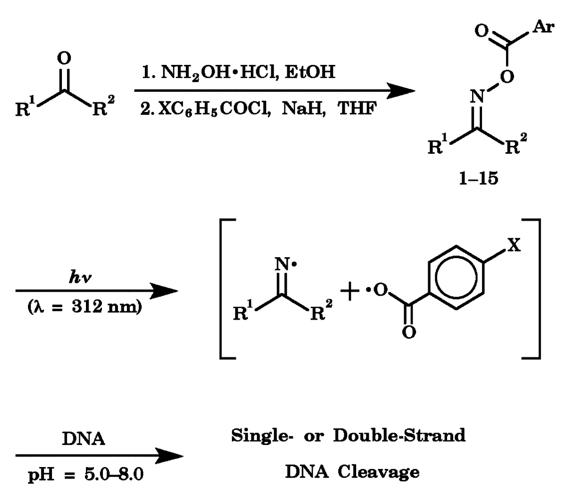
Organic molecules with DNA-cleaving ability are of great potential in the development of biotechnology and gene therapy.[1] Most of the newly developed agents in this category nick one strand of double-helical DNA.[2] It is our plan to obtain double-strand scission by a molecule upon activation by UV light. Herein we report our findings that the new compound *p*-cyanobenzoyl oxime of anthraquinone (**13**) reached this goal.



X =	X =	X =
1. H	**6.** H	**11.** H
2. Me	**7.** Me	**12.** Me
3. CN	**8.** CN	**13.** CN
4. F	**9.** F	**14.** F
5. NO_2	**10.** NO_2	**15.** NO_2

We incorporated the oxime ester functionality onto DNA intercalators, including anthraquinone,[3] fluoren-9-one,[4] and thioxanthen-9-one 10,10-dioxide by oximation with hydroxylamine followed by benzoylation with *p*-substituted benzoyl chlorides (Scheme 1).

The resultant oxime esters **1–15** were irradiated with UV light (312 nm, 16 W) at the concentration of 500 μM in a sodium phosphate buffer (pH 5.0, 6.0, 7.0, or 8.0) containing the supercoiled circular *ϕX174* RFI DNA (form I; 50 μM/base pair) at room temperature for 2.0 h.[5] Results from gel electrophoresis on 1% agarose with ethidium bromide staining showed that agents **1–5** and **10–15** possessed significant DNA-cleaving activity and gave the relaxed circular (i.e. form II)



Scheme 1. Synthesis of oxime esters **1–15** as DNA cleaving agents.

Keywords: anthraquinone; DNA cleavage; photolysis; radicals.

* Corresponding authors. Tel.: +886-3-572-5813; fax: +886-3-572-1594 (J.R.H.); tel.: +886-2-2901-9367; fax: +886-2-2902-8073 (S.-S.P.C.); e-mail: jrhwu@mx.nthu.edu.tw; chem1004@mails.fju.edu.tw

0040-4039/03/$ - see front matter © 2003 Published by Elsevier Science Ltd.
doi:10.1016/S0040-4039(03)00375-7

DNA. Oxime esters **13–15** were found to nick DNA efficiently even at the concentration as low as 10, 16, and 30 μm, individually. Their apparent equilibrium binding constants were measured as 4.49, 3.64, and 3.31×10^{4} M^{-1} for **13–15**, respectively.[6] More importantly, oxime ester **13** exhibited a unique character by cleaving *ϕX174* RFI DNA in a phosphate buffer (pH 6.0) to give the linear (i.e. form III) DNA at the concentration of 50 μM or higher (Fig. 1).

In control experiments, we removed molecular oxygen from the buffer solution of **13** by bubbling argon gas or removed singlet oxygen by adding sodium azide.[7] The cleaving potency of **13** remained the same. Thus oxime esters were able to cleave DNA under anaerobic conditions and the nicking process did not involve singlet oxygen. Furthermore, we found that DNA cleavage did not occur in the dark, as shown in lane 1 of Figure 1. Thus the UV light functioned as a 'trigger' to initiate the DNA scission process.[8–10]

To understand the mechanism of cleavage, we tried to detect the intermediates generated in the photolysis of oxime esters. In the first step, we irradiated with UV light a phosphate buffer solution (pH 6.0, 0.10 M) containing oxime ester **13** or **14** in the presence of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). Both experiments gave a set of EPR signal with intensities 1:2:2:1, in which the g value was 2.0061 and hyperfine splitting constants $a_N = a_H =$ 14.50 G. We believe that the radicals detected came from DMPO and *p*-substituted benzoyloxy adducts (Fig. 2).

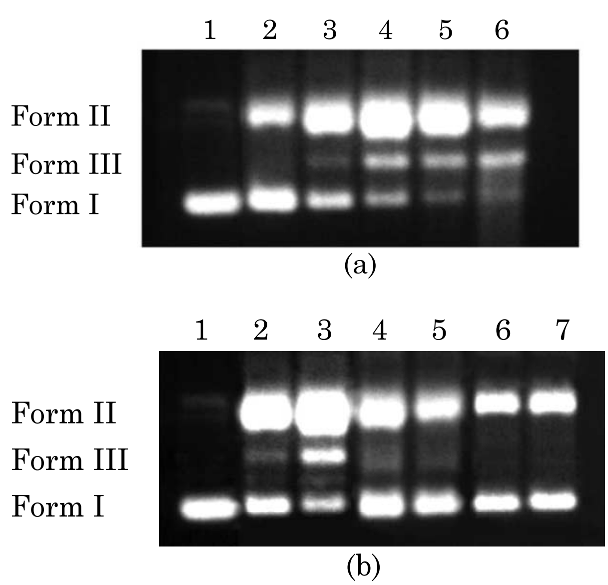


Figure 1. (a) Dose measurement of compound **13** for its DNA cleaving ability in a sodium phosphate buffer (pH 6.0) upon irradiation with UV light (312 nm, 16 W) for 2.0 h at 25°C; Lane 1, DNA with **13** (500 μM) in the dark; Lanes 2–6, 10, 50, 100, 250, 500 μM, individually. (b) Comparison of DNA cleaving ability of compounds **13–15** in a sodium phosphate buffer (pH 6.0) upon irradiation with UV light (312 nm, 16 W) for 2.0 h at 25°C; Lane 1, DNA with **13** (500 μM) in the dark; Lanes 2–7, **13** (50 μM), **13** (100 μM), **14** (50 μM), **14** (100 μM), **15** (50 μM), **15** (100 μM).

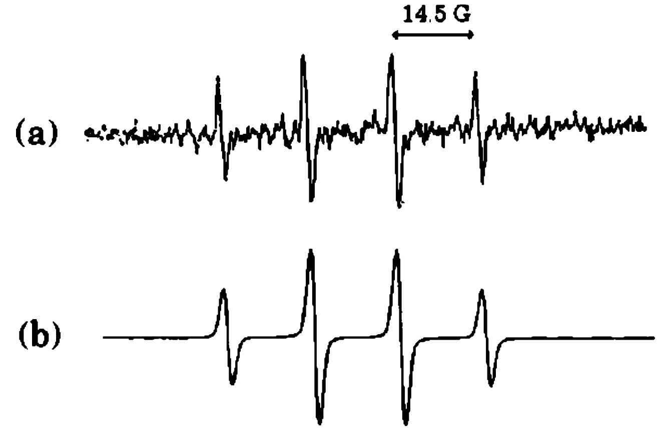
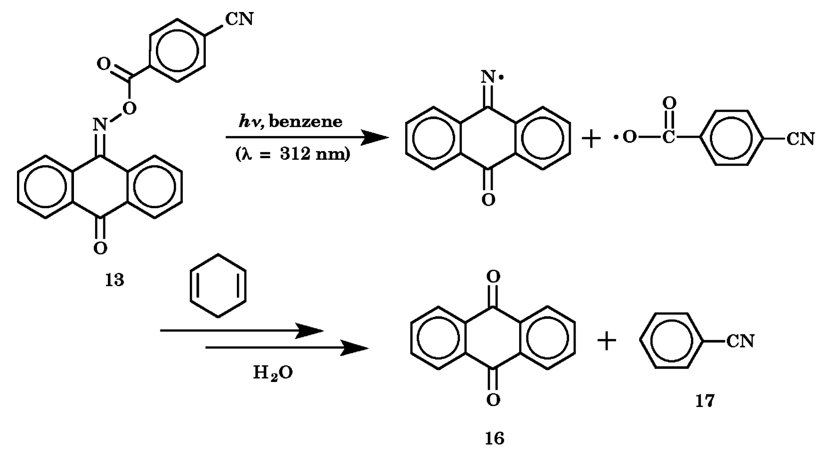


Figure 2. The ESR spectra of radicals obtained by (a) photolysis of oxime ester **13** (10 mM) in a phosphate buffer (pH 6.0), and (b) computer simulation. These two spectra are very superimposable.

In the second step, we planned to trap the radical intermediates with 1,4-cyclohexadiene[11] and then identified the final products. Accordingly, a benzene solution containing oxime ester **13** and 1,4-cyclohexadiene was irradiated with UV light at room temperature for 12 h (Scheme 2). The product mixtures were separated by column chromatography packed with wet silica gel. Anthraquinone (**16**) and benzonitrile (**17**) were isolated in 78 and 80% yields, respectively.

Photolysis of *O*-benzoyl oxime can produce iminyl and benzoyloxy radicals through homolytic fission.[12–14] In 1996, Theodorakis and Wilcoxen[15] reported a way to generate benzoyloxy radicals by illuminating *N*-benzoyloxy-2-thiopyridones with visible light. Their data indicate for the first time that aroyloxy radicals can induce significant DNA cleavage. Benzoyloxy radical [$PhCOO^{\bullet}$] can also undergo rapid decarboxylation to yield phenyl radical ($Ph^{\bullet}$).[16] It is unclear which of these two species is more responsible for DNA damaging though there is considerable evidence to support that the latter species plays a significant role on DNA-strand breakage.[17] Our results shown in Scheme 2 indicate that three types of radicals were generated in the media of photochemical reactions: anthraquinone iminyl radical, *p*-cyanobenzoyloxy radical, and *p*-cyanophenyl radical. These radicals are responsible for DNA scissions.

Among oxime esters **13–15**, **13** was the only molecule that performed double-strand scission. In comparison with single-strand cleavers **14** and **15**, stronger potency for compound **13** could be due to the great reactivity associated with the resultant *p*-cyanobenzoyloxy and *p*-cyanophenyl radicals upon its photo-dissociation. In 1988, Ingold et al.[18] reported the kinetic characteristics of various substituted aroyloxy radicals. Their data indicate that reactivity is greater for p-NC–$C_6H_4COO^{\bullet}$ than p-F–$C_6H_4COO^{\bullet}$. The latter radical species was generated from **14**. Therefore we believe that highly



Scheme 2. Irradiation with UV light of a benzene solution an oxime ester **13** and 1,4-cyclohexadiene.

reactive *p*-cyano radical species contribute significantly to the ability of **13** for its double-strand scission.

Acknowledgements

For financial support, we thank the Ministry of Education, the National Science Council of Republic of China, and Academia Sinica.

References

1. (a) Prost, C. L.; Perun, T. J. *In Nucleic Acid Targeted Drug Design*; Dekker, Ed.; New York, 1992; (b) Hurley, L. H.; Chaires, J. B. In *Advances in DNA Sequence Specific Agents*; JAI Press: London, 1996; Vol. 2; (c) Dai, W.-M.; Lai, K. W.; Wu, A.; Hamaguchi, W.; Lee, M. Y. H.; Zhou, L.; Ishii, A.; Nishimoto, S.-I. *J. Med. Chem.* **2002**, *45*, 758; (d) Wipf, P.; Hopkins, C. R.; Phillips, E. O.; Lazo, J. S. *Tetrahedron* **2002**, *58*, 6367; (e) Warner, P. M.; Qi, J.; Meng, B.; Li, G.; Xie, L.; El-Shafey, A.; Jones, G. B. *Bio. Med. Chem. Lett.* **2002**, *12*, 1; (f) Thomas, C. J.; McCormick, M. M.; Vialas, C.; Tao, Z.-F.; Leitheiser, C. J.; Rishel, M. J.; Wu, X.; Hecht, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 3875; (g) Kuzuya, A.; Machida, K.; Mizoguchi, R.; Komiyama, M. *Bioconjugate Chem.* **2002**, *13*, 365.
2. For recent articles, see: (a) Miyashita, K.; Park, M.; Adachi, S.; Seki, S.; Obika, S.; Imanishi, T. *Bio. Med. Chem. Lett.* **2002**, *12*, 1075; (b) Tao, Z.-F.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **2000**, *122*, 1602; (c) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.
3. Gasper, S. M.; Armitage, B.; Shui, X.; Hu, G. G.; Yu, C.; Schuster, G. B.; Williams, L. D. *J. Am. Chem. Soc.* **1998**, *120*, 12402.
4. Feigon, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
5. **General procedures for DNA-cleavage by use of oxime esters**. A reaction mixture (10 μL) containing supercoiled circular *ϕX174* RFI DNA stock solution (form I, 50 μM/base pair), an oxime ester and the phosphate buffers (pH 5.0–8.0, 0.10 M) in a Pyrex vial was preincubated at 37°C for 30 min. It was then irradiated with UV light (312 nm, 16 W) under aerobic condition at room temperature for 2.0 h. After addition of gel-loading buffer (2.5 μL containing 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol), the reaction mixture was loaded on a 1% agarose gel with ethidium bromide staining. The electrophoresis tank was attached to a power supply at a constant current (∼100 mA). The gel was visualized by 312 nm UV transilluminator and photographed by FB-PDC-34 camera. Quantitation of DNA cleavage was performed by integration of the optical density as a function of the band area by use of a Microtek scanner and NIH 1.60 image program.
6. **Measurement of apparent binding constants by fluorescence spectrometer**. The maximum fluorescence intensity of a solution containing ethidium bromide (1.0 μM) and calf thymus DNA (1.35 μM) in a phosphate buffer (pH 6.0) was measured by use of a spectrofluorometer excitation at 522 nm and emission at 596 nm. The aliquot oxime ester solutions (∼10 mM) in DMSO was added serially into the DNA–ethidium solution. Then the fluorescent intensity was measured after the equilibration between an oxime ester and the DNA–ethidium solution for 20 min. The fluorescence was measured after each addition until a 50% reduction of fluorescence had occurred. The apparent binding constants (K) were then obtained by the equation $K_{\mathrm{app}} = K_{\mathrm{EtBr}} \times$[EtBr]/[oxime esters]. The term [oxime esters] represents the concentration of the oxime esters causing a 50% reduction of the fluorescent intensity of the DNA–ethidium solution. The K_{EtBr} value is 1.0×10^{7} M^{-1}.
7. (a) Sharpatyi, V. A.; Kralji'c, I. *Photochem. Photobiol.* **1978**, *28*, 583; (b) Kuroda, R.; Shinomiya, M. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 1266.
8. Toshima, K.; Okuno, Y.; Nakajima, Y.; Matsumura, S. *Bio. Med. Chem. Lett.* **2002**, *12*, 671.
9. Dong, S.; Fu, P. P.; Shirsta, R. N.; Hwang, H.-M.; Leszczynski, J.; Yu, H. *Chem. Res. Toxicol.* **2002**, *15*, 400.
10. Hwu, J. R.; Yau, C. S.; Tsai, F. Y.; Tsay, S.-C. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2451.
11. Grissom, J. W.; Klingberg, D. *J. Org. Chem.* **1993**, *58*, 6559.

12. Okada, T.; Kawanisi, M.; Nozaki, H. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 2981.
13. Ohta, H.; Tokumaru, K. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2393.
14. Sakuragi, H.; Ishikawa, S.-I.; Nishimura, T.; Yoshida, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1949.
15. Theodorakis, E. A.; Wilcoxen, K. M. *Chem. Commun.* **1996**, 1927.
16. Grossi, L.; Lusztyk, J.; Ingold, K. U. *J. Org. Chem.* **1985**, *50*, 5882.
17. Hazlewood, C.; Davis, M. J.; Gilbert, B. C.; Packer, J. E. *J. Chem. Soc., Perkin Trans. 2* **1995**, 2167.
18. Chateauneuf, J.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1988**, *110*, 2886.