Pergamon

Tetrahedron Letters 41 (2000) 7711–7715

TETRAHEDRON LETTERS

# Novel and highly efficient DNA photocleavers: hydroperoxides of heterocyclic-fused naphthalimides

Wei Yao,[a] Xuhong Qian[b,*] and Qingyan Hu[a]

[a]*Institute of Pesticides & Pharmaceuticals, East China University of Science and Technology, Shanghai 200237, China*

[b]*State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China*

Received 21 February 2000; revised 27 July 2000; accepted 28 July 2000

**Abstract**

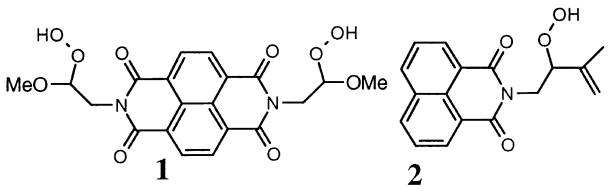
A series of novel hydroperoxides of heterocyclic-fused naphthalimides were designed, synthesized and exhibited excellent DNA photonicking activities. Among these compounds, the benzothiophenonaphthalimide-type reagent proved to be the most efficient, it can induce single-strand nicks in duplex DNA at micromolar concentration upon illumination. The relationship between the molecular structure and cleaving activities was discussed. 

DNA cleavage by synthetic nucleases is of great interest in biology and bio-organic chemistry. During recent decades, many DNA cleavage reagents have been developed as potential antitumor agents or prothetic groups for antisensoligonucleotides.[1–4] One of the most well-known DNA cleavage species is the hydroxyl radical, and much effort has been devoted to the development of efficient methods for $^{\bullet}OH$ generation using organic precursors by low-energy irradiation, such as long-wavelength UV-light ($\lambda$>350 nm) or more preferably by visible light irradiation.[5,6] Among the exploited photochemical DNA-cleavers, hydroperoxides of naphthalimides, such as **1** and **2**, have been proved to be very efficient and could effectively generate a hydroxyl radical under the irradiation of long-wavelength UV-light, and exhibit promising DNA photo-cleavage capabilities.[6–8] The high efficiency of these reagents may also be attributed to their high DNA-intercalating capability due to the large conjugated planarity of these compounds. Encouraged by the excellent DNA photonicking capabilities of this type of reagent and based upon our previous studies,[8–10] we further explored a series of novel DNA cleaving reagents bearing a larger conjugated planar, heterocyclic (oxazole and benzothiophene) fused naphthalene ring and a hydroxyl radical generating group (hydroperoxide). The former functions as the binding part, and the latter as the photonicking functionality. All of them

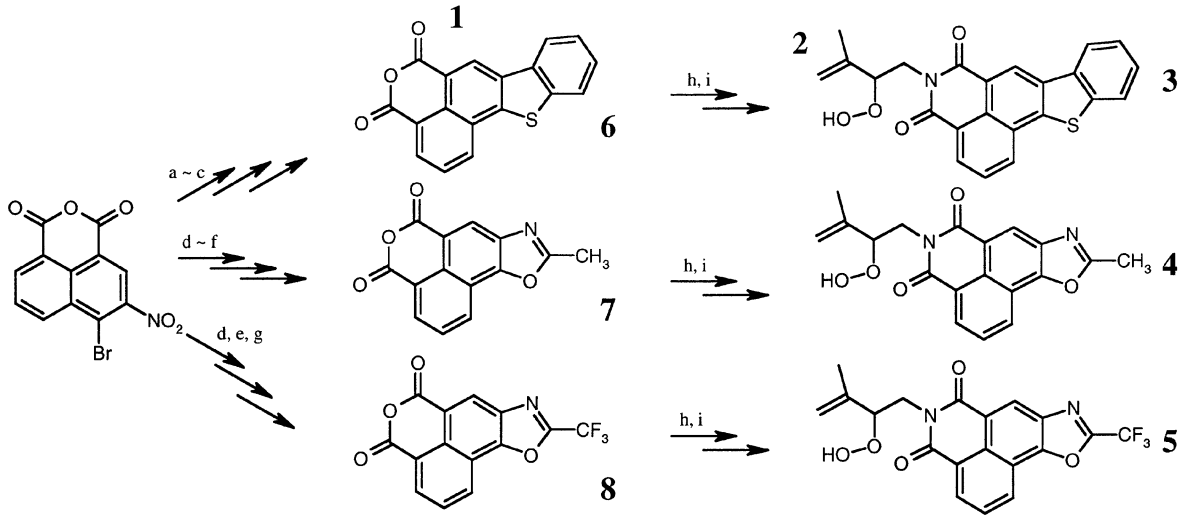
* Corresponding author.

0040-4039/00/$ - see front matter 
PII: S0040-4039(00)01304-6

(compounds **3**–**5**) were observed to exhibit significantly higher cleaving abilities to the super-coiled circular pBR322 DNA rather than the non-substituted counterpart, DNA cleaver **2**, upon photoirradiation.



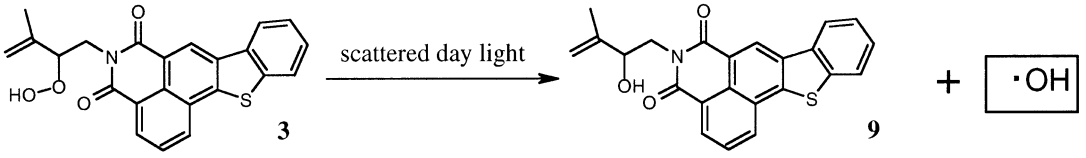
These reagents were synthesized from 4-bromo-3-nitro-1,8-naphthalic anhydride[11] as shown in Scheme 1. Photooxidation of the corresponding *N*-isopentenyl-1,8-naphthalimides resulted in the formation of compounds **3**, **4** and **5** as shelf-stable crystals.[12] All of these hydroperoxides were identified via $^1$H NMR, EI-MS, IR and elemental analysis and displayed the characteristic absorption peak of the O–O bond vibration around 800 cm$^{-1}$ in FTIR or FT-Raman spectra.



Scheme 1. Synthesis of novel naphthalimide-derived DNA cleaving reagents. (a) PhSH, EtOH, reflux, 4 h, 51% yield; (b) $SnCl_2$/HCl, 92.6% yield; (c) Pschorr cyclization,[13] 83% yield; (d) 20% NaOH, 85°C, 8 h, 83.3% yield; (e) $H_2$, Pd/C, 97% yield; (f) $CH_3COOH$, PPA, 120°C, 3.5 h, 62% yield; (g) $(CF_3CO)_2O$, PPA, 90°C, 3 h, 75.9% yield; (h) $H_2NCH_2CH{=}C(CH_3)_2$, EtOH, 3 h, >90% yields; (i) h$\nu$, $O_2$, TPP/$CH_2Cl_2$, −10°C, 3.5 h, 32∼40% yields

Interestingly, upon exposure of the chloroform solution of **3** to scattered daylight at room temperature for about 3 h, the corresponding hydroxyl derivative **9**[14] was generated. The characteristic absorption peak of the O–O bond of **3** at 799 cm$^{-1}$ gradually disappeared, accompanied by the emergence of the broad absorption peak of the hydroxyl group at 3460 cm$^{-1}$ by FT-Raman and IR monitoring. The sensitivity of this compound might be due to the prolonged conjugated system of **3**, which leads to stronger UV absorption than **2** at long-wave-length UV-light even in daylight and more effectively absorbing energy to overcome the energy barrier between the ground and the excited state. The formation of this hydroxyl compound was proposed to be involved in intramolecular energy transfer from the photoexcited chromophore to the hydroperoxy group and consequently homolysis of the O–O bond with the release of the active species $^{\bullet}$OH and the substituted allyloxy radical, which was quenched in a hydrogen-

donating solvent to give compound **9**. However, it is known that most organic hydroperoxides are converted into the corresponding ketones derivatives via an γ-H abstraction mechanism under the irradiation of UV-light.[6,15,16] The difference between the two pathways was probably because the enlarged conjugated system decreases the energy gap between the LUMO and HOMO, thus the released and transferred energy from the photoexicited chromophore can only selectively cleave the O–O bond, due to its weaker bond energy, rather than the γ-C–H bond. The probable generation of a hydroxyl radical also suggested that compound **3** might exhibit high DNA photocleaving activity.




The cleavage activities of **3**, **4** and **5** were evaluated using supercoiled circular pBR322 (form I) DNA (50 μM/base pair) under photoirradiation with a transluminator (366 nm) at a distance of 20 cm at 0°C for 0.5 h and analyzed on a 1% agarose gel. DNA photocleavage efficiency was defined as the degree of relaxation of the supercoiled DNA. Comparing to hydroperoxide **2** at the same conditions, reagents **3**, **4** and **5** demonstrated higher DNA cleavage ability as shown in Figs. 1 and 2. All the three new hydroperoxides exhibited cleaving capabilities at the concentrations below 50 μM, while no obvious cleavage was observed for **2** even at as high as 100 μM concentrations. As speculated above, compound **3** showed excellent cleavage activity which can effectively photonick form I DNA into form II DNA at a concentration as low as 1 μM (Fig. 1).

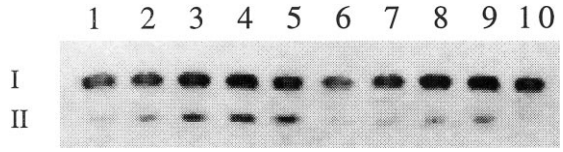



Figure 1. Cleavage of supercoiled circular pBR322 DNA by hydroperoxides **3** and **2**. Lane 1: DNA alone; lanes 2–5: DNA and **3** at concentrations of **3**: 0.5, 1, 5 and 10 μM, respectively; lanes 6–9: DNA and **2** at concentrations of **2**: 1, 10, 50 and 100 μM, respectively; lane 10: DNA alone (no h$\nu$)

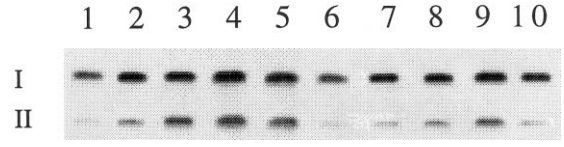



Figure 2. Cleavage of supercoiled circular pBR322 DNA by hydroperoxides **4** and **5**. Lane 1: DNA alone (no h$\nu$); lanes 2–5: DNA and **4** at concentrations of **4**: 10, 20, 50 and 100 μM, respectively; lanes 6–9: DNA and **5** at concentrations of **5**: 10, 20, 50 and 100 μM, respectively; lane 10: DNA alone

The significantly enhanced cleavage activities of compounds **3**, **4** and **5** also confirmed the rationality of design of the molecular structures. UV spectra showed the wavelengths of the maximum absorption of reagents **3**, **4** and **5** were 379 nm (4.05), 335 nm (4.04), 328 nm (4.04), respectively. It was obvious that the $\lambda_{max}$ of compound **3** was the longest and the closest to the

Table 1
Growth inhibitory activities against human tumor cell lines for **2**, **3**, **4** and **5**

| Tumor type | BEL-7404 Human liver cancer[a] | | | HL-60 Human leukemia[b] | | |
|---|---|---|---|---|---|---|
| Conc. (mol/L)[c] | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| **2** | 90.7 | 92.8 | 22.7 | 100 | 100 | 33.3 |
| **3** | 95.9 | 95.4 | 18.5 | 99.2 | 96.2 | 51.1 |
| **4** | 94.2 | 91.4 | 0.5 | 99.0 | 100.0 | 9.7 |
| **5** | 95.3 | 46.1 | 18.1 | 88.3 | 66.7 | 5.7 |

[a] Experiments were carried out with incubation for 72 h using an SRB assay.
[b] Experiments were carried out with incubation for 48 h using an MIT assay.
[c] The concentration of tested reagents **2**, **3**, **4**, **5**.

photoirradiation wavelength 365 nm, which may be responsible for the improvement of cleaving activity since the increased absorption of energy would speed up the O–O bond homolysis to form the active species, a hydroxyl radical. On the other hand, the enlargement of the molecular plane of these compounds with the conjugated heterocycles enhanced their DNA-intercalating capabilities. The binding constant of compounds **2**, **3** and **4** to calf thymus DNA measured using the fluorescence quenching method[17] are $2.67\times10^4$, $8.72\times10^5$ and $8.31\times10^4$, respectively. The higher intercalating capabilities of these compounds may be another contribution to their plausible DNA cleaving capabilities.

The antitumor activities of **3**, **4** and **5** were further examined by the growth inhibition against human tumor cells, HL-60 and BEP-7404 tumor cell lines. The results were parallel to their DNA cleavage activities, as shown in Table 1, hydroperoxide **3** also exhibited the highest efficiency of inhibition.

In general, novel hydroperoxides of heterocyclic-fused naphthalimides **3**, **4** and **5**, which can be synthesized from substituted 1,8-naphthalimides as shelf-stable crystals, were shown to be very efficient DNA cleavage reagents, and were further studied as valuable leading compounds of anti-tumor drugs.

## Acknowledgements

This work was supported by the Fuk Ying Tung Foundation and the National Natural Science Foundation of China.

## References

1. Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1991**, *113*, 5116.
2. Saito, I.; Takayama, M.; Sugiyama, H.; Nakamura, T. In *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*; Menunier, B., Ed. Kluwer: The Netherlands, 1996; pp. 163–176.
3. Breslin, D. T.; Coury, J. E.; Anderson, J. R.; McFailIsom, L.; Kan, Y. Z.; Williams, L. D.; Bottomley, L. A.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 5043.
4. Armitage, B. *Chem. Rev.* **1998**, *98*, 1171–1200.

5. Bielski, B. H.; Gehicki, J. M. *Free Radic. Biol.* **1977**, *1*, 1.
6. Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305–1310.
7. Matsugo, S.; Saito, I. *Tetrahedron Lett.* **1991**, *25*, 2949–2950.
8. Tao, Z.; Qian, X.; Wei, D. *Dyes Pigments* **1996**, *31*, 245–251.
9. Qian, X.; Tang, J.; Zhang, J.; Zhang, Y. *Dyes Pigments* **1994**, *25*, 109.
10. Tao, Z.; Qian, X.; Tang, J. *Dyes Pigments* **1996**, *30*, 247–252.
11. Kadhim, A. M.; Peters, A. T. *J. Soc. Dyers Colourists* **1974**, *90*, 153.
12. Compound **3**: $\delta_H$ (500 M, $CDCl_3$) 2.01 (s, 3H), 4.70 (m, 3H), 5.08 (s, 1H), 5.13 (s, 1H), 7.56 (m, 2H), 7.75 (m, 1H), 7.92 (d, $J=7.6$ Hz, 1H), 8.19 (d, $J=7.6$ Hz, 1H), 8.32 (d, $J=8.0$ Hz, 1H), 8.53 (d, $J=7.1$ Hz, 1H), 9.04 (s, 1H), 10.17 (br, 1H); EI-MS $m/z$ 386 $(M-OH)^+$; IR (KBr): 3258, 1688, 1640, 1587, 1335, 906, 799, 780 $cm^{-1}$; FT-Raman: 3065, 1689, 1587, 1400, 1377, 799 $cm^{-1}$. Compound **4**: $\delta_H$ (500 M, $CDCl_3$): $\delta$ 2.00 (s, 3H), 2.87 (s, 3H), 4.56, 4.59 (2d, $J_1=3.0$, $J_2=5.9$, 2H), 4.73–4.78 (dd, $J=9.4$, $J'=14.5$, 1H), 5.07, 5.12 (2d, $J_1=0.9$, $J_2=0.9$, 2H), 7.94 (dd, $J=7.4$, $J'=8.2$, 1H), 8.59 (dd, $J=1.2$, $J'=8.2$, 1H), 8.71 (dd, $J=1.2$, $J'=7.4$, 1H), 8.97 (s, 1H), 9.76 (br, 1H); EI-MS $m/z$ 352 $M^+$; IR (KBr): 3200, 1700, 1670, 1600, 1450, 1380, 910, 800 $cm^{-1}$; anal. calcd for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.57; N, 7.95. Found: C, 64.28; H, 4.44; N, 8.08. Compound **5**: $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ 2.00 (s, 3H), 4.57–4.60 (m, 2H), 4.74–4.79 (dd, $J=9.1$ Hz, $J'=14.4$ Hz, 1H), 5.06 (s, 1H), 5.12 (s, 1H), 8.05 (dd, $J=7.9$ Hz, $J'=7.8$ Hz, 1H), 8.72 (dd, $J=0.8$ Hz, $J'=7.9$ Hz, 1H), 8.82 (dd, $J=0.7$ Hz, $J'=7.8$ Hz, 1H), 9.12 (s, 1H), 9.79 (br, 1H); EI-MS $m/z$: 407 $M^+$+1; IR (KBr): 3360, 3090, 1700, 1665, 910, 800, 790 $cm^{-1}$. $C_{19}H_{13}F_3N_2O_5$ requires: C, 56.17; H, 3.22; N, 6.89. Found: C, 55.92; H, 3.03; N, 6.56.
13. Peters, A. T.; Bide, M. J. *Dyes Pigments* **1985**, *6*, 267–275.
14. Compound **9**: $\delta_H$ (500 M, $CDCl_3$) 1.96 (s, 3H), 4.42 (d, $J=5.9$ Hz, 2H), 4.53 (t, $J=5.9$ Hz, 1H), 4.98 (s, 1H), 5.18 (s, 1H), 7.56 (m, 2H), 7.78 (m, 1H), 7.94 (m, 1H), 8.23 (m, 1H), 8.35 (d, $J=7.8$ Hz, 1H), 8.56 (d, $J=7.3$ Hz, 1H), 9.11 (s, 1H), 9.78 (br, 1H); EI-MS $m/z$ 387 $M^+$; IR (KBr): 3458, 1699, 1652, 1587, 1334, 913, 780 $cm^{-1}$; FT-Raman: 3061, 1700, 1588, 1401, 1377 $cm^{-1}$.
15. Howard, J. A.; Chenier, J. H. B. *Can. J. Chem.* **1980**, *58*, 2808–2812.
16. Saito, I.; Takayama, M.; Matsuura, T. *J. Am. Chem. Soc.* **1990**, *112*, 883–884.
17. Gupta, M.; Ali, R. *J. Biochem.* **1984**, *95*, 1253–1257.