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Oxazolonaphthalimides and their hydroperoxides: photophysical and photobiological properties

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Abstract

Novel oxazolonaphthalimide hydroperoxides **2** and **3** have been designed, synthesized and shown to be very efficient in photocleavage of DNA by the plazmid nicking assay. The absorption and fluorescence properties of these compounds and their relevant intermediates are discussed. An explanation for the higher DNA-cleavage efficiency of these oxazole-conjugated naphthalimide hydroperoxides than the nonsubstituted counterpart **1** is provided. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Oxazolonaphthalimide; Hydroperoxide; DNA photocleave; Plazmid nicking

1. Introduction

In recent years considerable efforts have been devoted towards the design and synthesis of DNA- cleaving molecules for the use as structural probes and therapeutic agents [1–3], especially, the development of intercalating photocleavers which can recognize specific DNA sequences, and cause efficient photochemical strand cleavage at those sites [4–7]. Generally, both the active species and the binding parts play crucial roles for photocleavers [8]. Among the active species, the hydroxyl radical has proved to be very efficient, and this can easily be produced from hydroperoxides [9–11]. We have previously reported the synthesis and applications of 1,8-naphthalimide hydroperoxides as novel intercalating DNA cleavers [11], and the higher DNA cleavage efficiencies of these reagents than the phthalimide analogue were proposed to be due to the introduction of a fused benzene ring, enhancing the planarity of these molecules. Encouraged by these results, new naphthalimide hydroperoxides **2** and **3** bearing a conjugated oxazole ring were designed and synthesized, and they were shown to be more efficient than the nonsubstituted counterpart **1** in DNA photonicking.

2. Results and discussion

The syntheses of the oxazolonaphthalimide hydroperoxides **2** and **3** are illustrated in Fig. 1.

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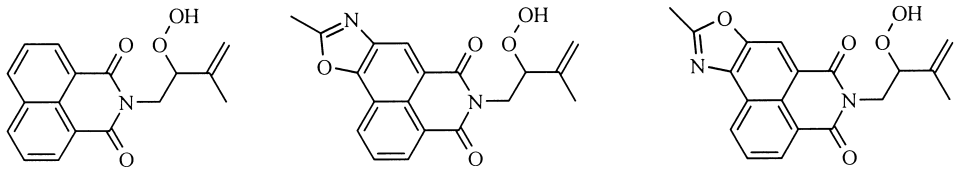


1 2 3

Hydrogenation of 4-hydroxy-3-nitro-1,8-naphthalic anhydride[12] and 3-hydroxy-4-nitro-1,8-naphthalic anhydride[13], which were derived from 4-bromo-1,8-naphthalimide and 1,8-naphthalic anhydride, respectively, gave compounds **8** and **9**, and compounds **8** and **9** were subsequently condensed with acetic acid in the presence of polyphosphoric acid (PPA) to afford the corresponding key intermediates **6** and **7** in approximately 60% yield. Compounds **6** and **7** were further reacted with 3,3-dimethylallylamine to give the oxazolonaphthalimides **4** and **5**, which were photooxidized to obtain the final products **2** and **3**. The structures of the hydroperoxides and their precursors were

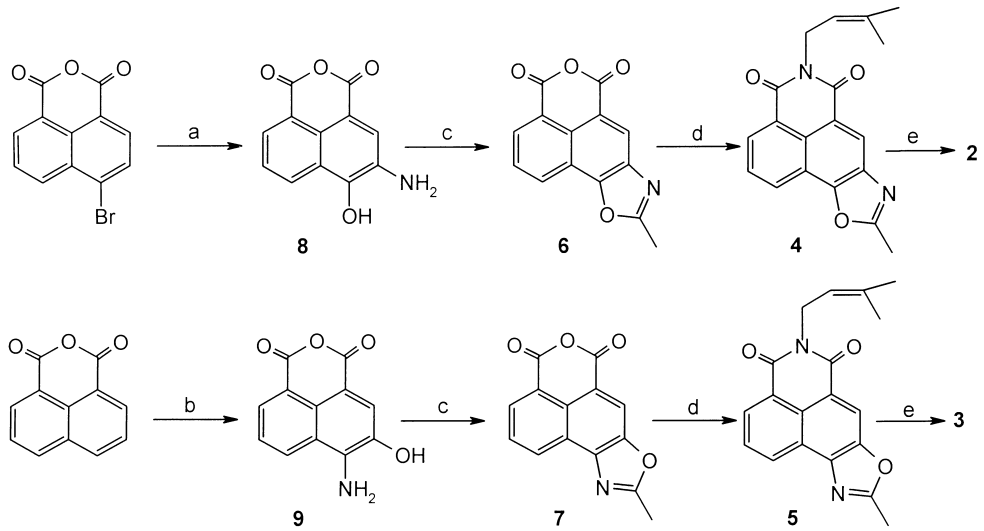


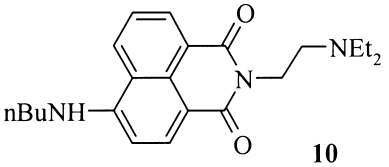
Fig. 1. (a): i. $NaNO_3$/conc. H_2SO_4, 0–5°C; ii. 20% aq. NaOH, 85°C, 8 h; iii. H_2, Pd/C, r.t. (b) i. 30% fuming sulfuric acid, 120°C, 1 h; ii. KOH, 200–300°C, 45 min; iii. HNO_3/H_2SO_4, <5°C 1.5 h; iv. H_2, Pd/C. (c) AcOH/PPA. (d) $H_2NCH_2CH{=}C(CH_3)_2$, EtOH. (e) $h\nu/O_2$, TPP/CH_2Cl_2, -10°C.

Table 1
UV and fluorescence spectra data for oxazolonaphthalimides and their precursors

Compound	UV λ_{max} (nm) (log ε)			Ex λ (nm)	FL λ_{max} (nm) (ϕ)	Stokes shift (nm)
1	235 (4.81)	332 (4.25)		235	372 (0.002)	40
2	250 (4.57)	330 (4.04)	361 (4.03)	250	412 (0.061)	51
3	240 (4.75)	362 (4.38)		240	416 (0.096)	54
4	248 (4.38)	335 (3.66)	362 (3.67)	248	412 (0.011)	50
5	239 (4.57)	362 (4.19)		239	415 (0.011)	53
6	258 (4.61)	335 (3.84)	362 (3.76)	258	412 (0.10)	50
7	241 (4.86)	342 (4.39)	360 (4.38)	241	418 (0.073)	58

identified by ^{1}H NMR, IR, MS and elemental analysis. Both the FT-IR spectra of **2** and **3** displayed the characteristic absorption peaks of the O–O bond vibration around 800 cm^{-1}, which further confirmed the presence of the hydroperoxy groups.

It can be seen in Table 1 that the fluorescence intensities of compounds **4** and **5** are approximately 10 times lower than the corresponding anhydrides **6** and **7** without the allyl moieties. This quenching was presumably due to the photo-induced intramolecular electron transfer (PET) between the excited fluorophore and the allyl group. Such a process was quite unexpected as it has been reported that PET does not occur with compound **10** because the lone pair of the tertiary amine nitrogen atom cannot transfer to the fluorophore through the imide moiety due to its repulsive electric field [14]. However, when compounds **4** and **5** were photooxidized to give **2** and **3**, respectively, the PET process seemed to be dramatically suppressed, resulting in the recovery of fluorescence.



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It is also shown in Table 1 that, because of the extended conjugated systems of **2**–**7**, the UV-absorption and fluorescence maxima of these compounds are at longer wavelength than those of compound **1**, which does not have an oxazole moiety. As a result, the long-UV absorption maxima of **2** and **3** are located around 362 nm, much closer to the photoirradiation wavelength 365 nm comparing to that of **1**. The coincidence of the absorption and irradiation wavelengths should greatly facilitate the excitation of these DNA-cleaving reagents, which would lead to efficient release of hydroxyl radicals under mild conditions. That is, hydroperoxides **2** and **3** could be expected to exhibit higher DNA photonicking abilities than **1**.

The DNA-binding capabilities of compound **1**, **2** were evaluated by a fluorescence quenching technique [15,16]. The apparent association constant K_a values for the interaction of DNA with the hydroperoxides **1**, **2** were 2.67×10^4 M^{-1} and 8.31×10^4 M^{-1} respectively, which revealed that the oxazole-conjugated naphthalimide **2** intercalated more efficiently into DNA than its non-substituted counterpart **1**. This indicates that the enhancement of the molecular planarity by the introduction of the fused oxazole ring plays an important role in the improvement of the DNA-intercalating capabilities of oxazolonaphthalimides **2** and **3**.

The DNA-cleaving activities of **2** and **3** were evaluated and defined as the degree of relaxation of supercoiled pBR322 DNA under photoirradiation. Fig. 2 shows the DNA cleavage profiles of hydroperoxides **1**–**3** at 10, 20, 50, 100 μM, respectively. As expected, compounds **2** and **3** gave better DNA cleavage results as shown by the relative ratio of the Form II/Form I band, in comparison with **1**. Both the two new hydroperoxides behaved similarly at a concentration of 100 μM and exhibited effective cleavage at concentrations below 20 μM, at which concentration the cleavage by **1** could not be detected.

Thus, it is suggested that the enlargement of the molecular planarity of compounds **2** and **3** by introducing the conjugated oxazole ring plays an

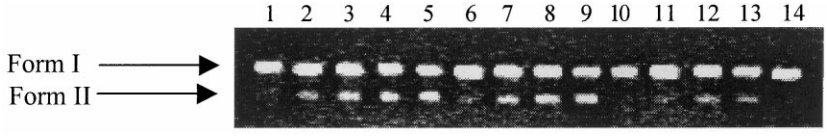

Fig. 2. DNA cleavage by hydroperoxides **2** and **3** compared with **1**. pBR322 DNA (50 μM/bp) was incubated in 10 μl of 1×TEA with drugs for 0.5 h in the dark at room temperature, then subjected to irradiation with a transluminator (366 nm) at a distance of 20 cm at 0°C for 0.5 h and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). Lanes 1: DNA alone; Lanes 2–5: DNA and 10, 20, 50 and 100 μM of **2** respectively; Lanes 6–9: DNA and 10, 20, 50 and 100 μM of **3** respectively; Lane 10–13: DNA and 10, 20, 50 and 100 μM of **1** respectively; Lane 14: DNA alone (without hν).

important role in the improvement of their DNA-intercalating capabilities, as well as in causing a bathochromic shift of their UV-absorption band, both factors, contributing to their enhanced DNA cleaving capabilities.

3. Experimental

3.1. General

Melting points were taken on a digital melting point apparatus made in Shanghai of China and are uncorrected. Infrared spectra were recorded on an IR-7650 spectrometer made in Shanghai of China, mass spectra were recorded on a Hitachi M 80, ^{1}H NMR on a Brucker AM-500 (500 MHz) using TMS as internal standard. Combustion analysis for elemental composition was carried out on an Italy MOD 1106 analyzer. Absorption spectra were measured in absolute ethanol on a Shimadzu UV-265 and fluorescence spectra on a Perkin Elmer LS 50 using quinine sulphate in sulphuric acid as quantum yield standard. Commercial reagents and solvents were purchased from standard chemical supplier.

3.2. Synthesis of 4H, 6H-9-methyl-benz[de]oxazolo[5,4-g]benzopyran- 4,6-dione (6)

A mixture of compound **8** (800 mg), acetic acid (1.2 ml) and polyphosphoric acid (35 ml) was stirred for 3.5 h at 120°C. The hot reaction mixture was poured into ice water (350 ml). The resulting pale yellow precipitate was collected and washed with water and recrystallized from THF to give **6** as yellow needles in 61.9 % yield. mp 278–279°C. EI-MS (*m*/*z*, %): 253 (M^+, 93.2), 209 (M^+-CO_2, 100), 181 (M^+-CO_2-CO, 25.0)

3.3. Synthesis of 4H,6H-5-(3′,3′-dimethylallyl)-9-methyl-benz[de]oxazolo[5,4-g] isoquinolone-4,6-dione (4)

Compound **6** (300 mg) and 3,3- dimethylallylamine (120 mg) in 10 ml of ethanol was refluxed for 3 h. After removal of solvent and recrystallization from a mixture of ethyl acetate and petroleum ether, white needles of **4** were obtained in 86% yield. mp 202–203°C (Found: C, 71.07; H, 4.99; N, 8.83. $C_{19}H_{16}N_2O_3$ requires: C, 71.24; H, 5.03; N, 8.74%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.73 (s, 3H, 3′-CH_3), 1.93 (s, 3H, 3′-CH_3), 2.82 (s, 3H, 9-CH_3), 4.81 (d, *J* 7.0, 2H, 1′-CH_2), 5.35 (t, *J* 7.0, 1H, 2′-CH), 7.87 (dd, J 7.5, *J′* 8.1, 1H, 2-H), 8.51 (dd, *J* 1.1, *J′* 8.1, 1H, 1-H), 8.65 (dd, *J* 1.1, *J′* 7.5, 1H, 3-H), 8.91 (s, 1H, 7-H). EI-MS (*m*/*z*, %): 320 (M^+, 39.5), 252 (100). ν(KBr)$_{max}$ (cm^{-1}) 3078, 1700, 1660, 1360 and 790.

3.4. Synthesis of hydroperoxide (2)

A dichloromethane (40 ml) solution of 200 mg of **4**, which contained 5 mg of tetraphenylporphine (TPP) as a sensitizer, was irradiated externally with a 150 W sodium lamp at −15°C, while a gentle stream of dry oxygen gas was continuously passed through the reaction mixture for 3.5 h. After removal of the solvent, the residue was chromatographed on a silica gel column using a 4:1 mixture of petroleum ether and dichloromethane as eluent to give **2** as white solid in 32% yield, mp 180–182°C (Found: C, 64.28; H, 4.44; N, 8.08. $C_{19}H_{16}N_2O_5$ requires: C, 64.77; H, 4.57; N, 7.95%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00 (s, 3H, 3′-CH_3), 2.87 (s, 3H, 9-CH_3), 4.56, 4.59 (2d, J_1 3.0, J_2 5.9, 2H, 1′-CH_2), 4.73–4.78 (dd, *J* 9.4, *J′* 14.5, 1H, 2′-CH), 5.07, 5.12 (2d, J_1 0.9, J_2 0.9, 2H, 4′-CH_2=), 7.94 (dd, *J* 7.4, *J′* 8.2, 1H, 2-H), 8.59 (dd, *J* 1.2, *J′* 8.2, 1H, 1-H), 8.71 (dd, *J* 1.2, *J′* 7.4, 1H, 3-H), 8.97 (s, 1H, 7-H), 9.76(s, 1H, 2′-OOH). EI-MS (*m*/*z*, %): 352 (M^+, 0.9), 291 (22.0), 264 (100), 266 (71.7), 252 (52.3). ν(KBr)$_{max}$ (cm^{-1}): 3210, 3088, 1700, 1680, 1360, 800.3, 790.

3.5. Synthesis of 4H,6H-9-methyl-benz[de]oxazolo[4,5-g]benzopyran-4,6-dione (7)

Compound **7** was prepared as **6** from **9** in 60% yield. mp 224–226°C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.90 (S, 3H, 9-CH_3), 8.04–8.07 (dd, *J* 8.1, *J′* 7.4, 1H, 2-H), 8.59 (dd, *J* 1.1, *J′* 7.4, 1H, 1-H), 8.84 (dd, *J* 1.1, *J′* 8.1, 1H, 3-H), 8.92 (s, 1 H, 7-H). EI-MS (*m*/*z*, %): 253 (M^+, 73.1); 209 (M^+-CO_2, 100); 181 (45.7).

3.6. Synthesis of 4H,6H-5-(3′,3′-dimethylallyl)-9-methyl-benz[de]oxazolo[4,5-g] isoquinolone-4,6-dione (5)

Compound **5** was obtained as the same procedure as **4** from **7** in 92% yield, mp 204°C (Found: C, 71.17; H, 5.00; N, 8.75. $C_{19}H_{16}N_2O_3$ requires: C, 71.24; H, 5.03; N, 8.74%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.75 (s, 3H, 3′-CH_3), 1.99 (s, 3H, 3′-CH_3), 2.88 (s, 3H, 9-CH_3), 4.83 (d, *J* 7.0, 2H, 1′-CH_2), 5.83 (t, *J* 7.0, 2′-CH), 7.90 (dd, *J* 8.0, *J′* 7.6, 1H, 2-H), 8.68 (d, *J* 7.6, 1H, 1-H), 8.78 (m, 2H, 3-H, 7-H). EI-MS (*m*/*z*, %): 320 (M^+, 100%), 276 (66.2), 252 (10.5). ν(KBr)$_{max}$ (cm^{-1}): 3080, 1690, 1655, 1600, 1560, 1360, 1250, 785.

3.7. Synthesis of hydroperoxide (3)

Compound **3** was photo-oxidized from **5** as **2** from **4** in 36% yield, mp 176–178°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.89 (s, 3H, 3′-CH_3), 2.86(s, 3H, 9-CH_3), 4.54, 4.57 (2d, J_1 2.0, J_2 6.3, 2H, 1′-CH_2), 4.72–4.76 (dd, *J* 14.6, *J′* 9.43, 1H, 2′-CH), 5.06, 5.10 (2s, 2H, 4′-CH_2=), 7.92 (dd, *J* 8.0, *J′* 7.8, 1H, 2-H), 8.69 (dd, *J* 1.1, *J′* 7.8, 1H, 1-H), 8.81 (s, 1H, 7-H). 8.82 (dd, *J* 1.1, *J′* 8.0, 1H, 3-H), 10.12 (bs., 1H, 2′-OOH). EI-MS (*m*/*z*, %): 352 (M^+, 1.2%), 334 ($M^+ + 1\text{-}H_2O$, 8.3), 320 ($M^+ + 1$-OOH, 4.9), 264 (100), 252 (49.6). ν(KBr)$_{max}$ (cm^{-1}): 3190, 3080, 1700, 1665, 1600, 1366, 800, 780.

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