Pergamon

Tetrahedron Letters 42 (2001) 6175–6178

TETRAHEDRON LETTERS

N-Aroyloxynaphthalimides as novel highly efficient DNA photocleavers: substituent effects

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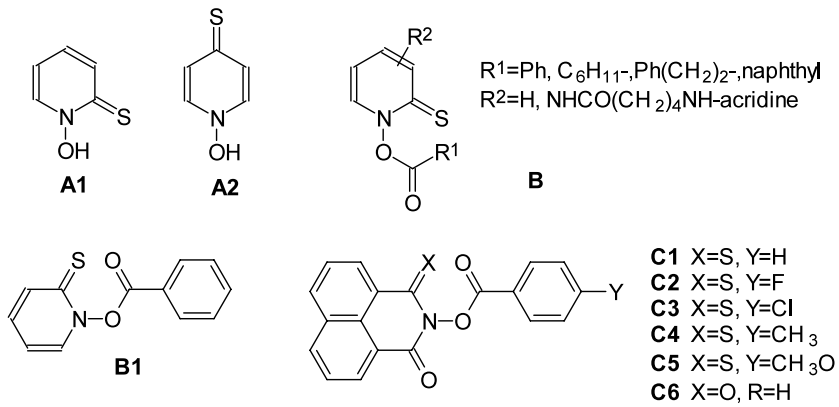
Received 30 April 2001; accepted 5 July 2001

Abstract—A novel DNA photocleaver family of *N*-benzoyloxythioxonaphthalimides, which show improved DNA intercalation and photocleaving activity compared with their oxo- counterparts, were synthesized and evaluated. The substituents on the benzene ring of the benzoyloxy moiety have an important and unusual influence on the DNA photocleaving activity. *N*-(*p*-Methoxylbenzoyloxy)thioxonaphthalimide is able to photocleave DNA at low concentration (5 μM) with 'time-resolved' and pH independent properties. © 2001 Elsevier Science Ltd. All rights reserved.

There are a variety of studies concerning DNA photocleavage by photosensitizers which either initiate a single electron transfer from a base to the triplet state of the chromophore or generate an active oxygen species upon photoirradiation. Oxygen radicals are very important in chemistry, biology and medicine.[1–3] In recent years, the design and study of DNA 'artificial photonucleases' of the oxygen radical type with no need of a metal or external oxidant, have attracted much attention and have led to the development of both specific gene-targeted drugs and artificial restriction enzymes.[4,5] However, there have been few reports about substituent effects on DNA photocleaving activity. We have reported the effects of thioxo- and oxo- groups in aromatic heterocycles as DNA photocleavers of the electron-transfer type, and that of trifluoromethyl- and methyl groups in hydroperoxides as DNA photocleavers of the hydroxyl radical type. Here we report important and unusual substituent effects in *N*-aroyloxynaphthalimides as DNA photocleavers of the aroyloxy radical type.

It is known that *N*-hydroxypyridinethiones (**A1**, **A2**) and *N*-aroyloxypyridine-2-thiones (**B**) are DNA photocleavers through release of hydroxy or aroyloxy radicals[6,7] (Scheme 1). On the other hand, naphthalimides usually exhibit strong, long-wavelength absorption and good DNA intercalative binding abilities,



Scheme 1.

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PII: S0040-4039(01)01214-X

whilst some hydroperoxides and other derivatives have shown DNA photocleaving activity.[8–10] Therefore, by integrating the *N*-aroyloxy-2-thiopyridone photocleaving moiety with the naphthalimide intercalative moiety, a novel highly efficient DNA photocleaving family of reagents of *N*-aroyloxythionaphthalimides (**C1**–**C5**) became our target.

The target *N*-benzoyloxythionaphthalimides **C1**–**C5** were synthesized by condensation of *N*-hydroxy–thionaphthalimide with substituted benzoyl chlorides. The oxo-compound **C6** was synthesized for comparison and all the compounds were identified by using ^{1}H NMR, EI-MS, IR and elemental analysis (Scheme 2).

Interestingly, thioxo-compound **C1** behaved differently from oxo-compound **C6** in DNA photocleavage. Fig. 1a shows that **C1** could cleave the DNA plasmid effectively under irradiation, while its oxo-analog had little effect. It is widely accepted that the DNA photocleaving activities of *N*-hydroxy- and *N*-aroyloxypyridine-2-thione are attributable to the formation of persistent hydroxyl or aroyloxyl radicals during the photolysis, and that they are able to give ESR signals through spin trapping methods, whilst a thiyl radical signal is not detected under similar conditions.[6,11,12] In our case, PBN (*N*-*tert*-butyl-α-phenylnitrone) trapping ESR spectroscopy identified that **C1**[19] in benzene could generate a free radical signal under irradiation (Fig. 2), but not in the case of **C6**.[21] This suggested that free benzoyloxy radicals might be responsible for the DNA damage caused by **C1**.

It was found that compared with **C6** all thioxo-compounds (**C1**–**C5**) had a long-wavelength of absorption and a very weak fluorescence (Table 1). This implies that for the latter, their excitation energy is more easily transferred from the singlet excited state to cleave the N–O bond,[11] besides being dissipated as heat in the

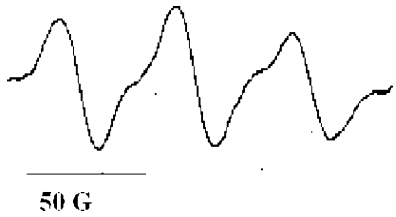


Figure 2. ESR spectroscopy of the radical released by **C1**. ESR with PBN (*N*-*tert*-butyl-α-phenylnitrone) trapping in benzene under light. Microwave frequency 9.782 GHz, mid range 3430 G, scan range 100 G.

system by internal conversion processes. Meanwhile, a study of the intercalation of **C1** and **C6** to calf thymus DNA was carried out using an electronic absorption spectra technique instead of the fluorescence quenching method,[13] as **C1**–**C5** have only very weak fluorescence. During addition of calf thymus DNA the absorption of **C1** became weaker in intensity with a red shift for the maxima with an increase in the concentration of DNA (Fig. 3). **C1** had obvious DNA intercalating activity

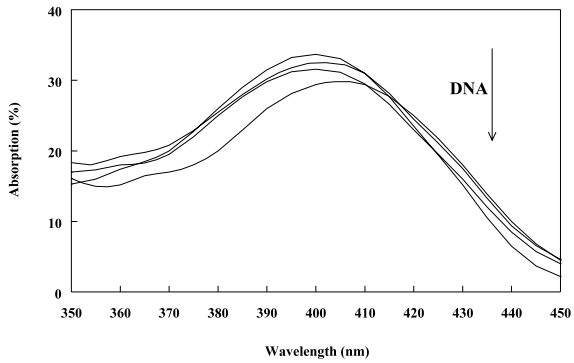
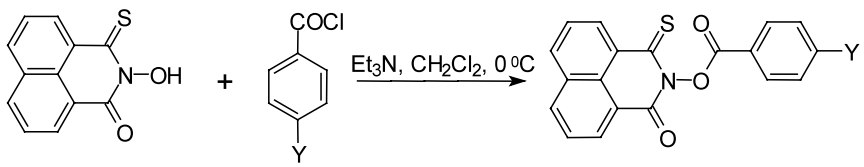


Figure 3. Interaction of **C5** and DNA. Absorption changes from 300–500 nm of compound **C5** (20 μM) during addition of calf thymus DNA (0, 50, 100, 200 μM) in THF, 10 mM Tris·HCl (pH 7.4) solution (3:10, v/v).



Scheme 2.

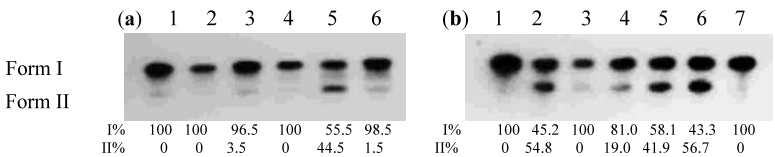


Figure 1. Photocleavage on pBR 322 DNA. The cleavage activities were evaluated using supercoiled circular pBR322 (form I) DNA (50 M/bp) with the compound in the buffer Tris·HCl (pH 7.6) under photoirradiation (2300 W/cm^2) with a transluminator (366 nm) at a distance of 20 cm at 0°C and then analyzed on a 1% agarose gel. DNA photocleavage efficiency was defined as the degree of relaxation of the supercoiled DNA, relaxed circular DNA (single-stranded cleavage) as form II. **(a) Comparison of photocleavage on pBR 322 DNA by C1 and C6** (100 μM; *hv*, 1 h). Lane 1: DNA alone (no *hv*); lane 2: DNA and **C6** (no *hv*); lane 3: DNA and **C6** (*hv*); lane 4: DNA and **C1** (no *hv*); lane 5: DNA and **C1** (*hv*); lane 6: DNA alone (*hv*). **(b) Effects of different substituents on photocleavage** (100 μM; *hv*, 45 min). Lane 1: DNA alone (no *hv*); lane 2: DNA and **C1**; lane 3: DNA and **C 2**; lane 4: DNA and **C3**; lane 5: DNA and **C4**; lane 6: DNA and**C5**; lane 7: DNA alone (*hv*).

Table 1. UV–vis and FL spectra data of *N*-benzoyloxynaphthalimides[a,b]

Compound	UV λ_{max}/nm (log ε)	FL λ_{max}/nm (ϕ)	Compound	UV λ_{max}/nm (log ε)	FL λ_{max}/nm (ϕ)
C1	392 (4.29)	491 (<0.001)	**C5**	390 (4.21)	454 (<0.001)
C2	400 (4.37)	454 (<0.001)	**C6**	350 (4.04)	378 (0.10)
C3	390 (4.34)	466 (<0.001)	**B1**	365 (3.69)	409.6 (0.047)
C4	390 (4.23)	466 (<0.001)			

[a] In absolute ethanol.
[b] With quinine sulfate in sulfuric acid as quantum yield standard.

while **C6** did not, which would also make them differ in their photobiology as DNA binding is helpful for the photocleavage. In addition, we found that thioxo-compounds as DNA photocleavers of the electron transfer type, had better photocleaving and intercalative activity than the corresponding oxo-compounds,[14,15] but the difference was not great.

The photocleaving ability for DNA double strand-scission was evaluated for all the novel *N*-benzoyloxythionaphthalimides (Fig. 4b). It is known that in radical chemistry, electron donating substituents can improve the stability of oxygen- and heteroatom-centered radicals, while electron withdrawing substituents do not.[16] The more stable the oxygen-centered radicals, the stronger the radical DNA photocleaving ability should be. In our case, it was found that the DNA photocleaving abilities of these substituents were in the order: CH_3O>H>CH_3>Cl>F; the compound with the methoxy group (**C6**) showed the highest activity, while that with fluoro- group was the worst. This observation in the DNA system under light irradiation was very different from the case of *O*-type radicals in a chemical system,[16] and the photocleaving abilities of **C1** and **C5** were at the same level (54.8 and 56.8%), so the electron donating effect was not so important. This at least means that there might be some other unknown factor playing a very important role rather than an electron effect, and that the substituent effects in DNA photocleavage are unusual and very complicated. Similarly, we never observed that the trifluoromethyl group had a negative effect on the DNA intercalative and DNA photocleaving activity for DNA photocleavers of the hydroxyl radical type in comparison with a methyl group.[17]

Fig. 4a indicates that the benzoyloxyl radicals were generated from **C5**[20] at a relatively linear rate. This continuous generation of reactive oxygen-centered radicals provides an alternative to Fenton-based chemistry, where oxygen radicals are formed in a rapid burst. This kind of reagent is attractive for 'time-resolved' DNA cleavage studies.[18] Fig. 4b demonstrates that **C5** could damage DNA effectively at a concentration of 5 μM. While it has been reported and has also been found in our experiments that **B1** photocleaves DNA at a concentration as high as 500 μM.[10] This further confirmed the rational design of the new molecules through integrating the photocleaving moiety and the intercalative moiety through a covalent linkage. Fig. 4c shows that pH had little influence on the photocleaving activity of **C5**, which means that the benzoyloxyl radicals are quite stable over a wide range of pH values. In addition, preliminary experimentals in polyacrylamide gel electrophoresis showed that **C5** could give different photocleaving results to double strands of 5′-GCGCGCGC and 5′-ATATATAT and a detailed study of its specific photocleavage of duplex DNA will be carried out.

Thus, our study provides a novel 'time-resolved' and highly efficient DNA photocleaving family of *N*-aroyloxythionaphthalimides, which have more efficient DNA binding and photocleaving activity than their oxo-counterparts, and which generate benzoyloxyl radicals upon visible light illumination and which are pH independent (pH range: 7.0–8.5). It was found that substitutents on the benzene ring of the benzoyloxy group had an important and unusual influence on DNA photocleaving activity, the methoxy compound **C5** showed optimum photocleaving activity.

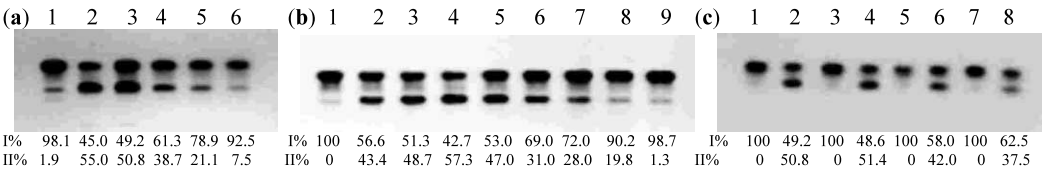


Figure 4. (a) Time-dependant photocleavage of DNA using C5 (100 μM). Lane 1: DNA alone (no *hv*); lane 2: **C5** and DNA (*hv*, 90 min); lane 3: **C5** and DNA (*hv*, 75 min); lane 4: **C5** and DNA (*hv*, 60 min); lane 5: **C5** and DNA (*hv*, 30 min); lane 6: DNA alone (*hv*, 1.5 h). **(b) Concentration-dependant photocleavage of DNA using C5** (*hv*, 1 h). Lane 1: DNA alone (no *hv*); lane 2–8: DNA and **C5** at concentrations of 200, 100, 50, 20, 10, 5 and 1 μM, respectively. Lane 9: DNA alone (*hv*). **(c) pH effect on DNA photocleavage of C5** (100 μM; *hv*, 1 h). Lane 1, 3, 5, 7: DNA alone, pH 8.5, 8.0, 7.5, 7.0, respectively; lane 2, 4, 6, 8: DNA and **C5**, pH 8.5, 8.0, 7.5, 7.0, respectively.

Acknowledgements

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

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19. **C1**: mp: 189–190°C EI–MS (m/z, %): 333 (M^+, 1.9), 213 ([M+H$^+$–PhCOO$^\bullet$], 2.8), 105 (PhCO$^+$, 100); IR (KBr): 3030, 1780, 1715, 1610, 1580, 1270, 780, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 9.01 (dd, $J_1=7.6$ Hz, $J_2=0.9$ Hz, 1H, 7-H), 8.67 (dd, $J_1=7.2$ Hz, $J_2=1.1$ Hz, 1H, 2-H), 8.27 (m, 4H, 3∼6H), 7.72 (m, 3H, 2′-,4′-,6′-H), 7.57 (m, 2H, 3′-,5′-H); $C_{19}H_{11}NO_3S$ requires: C, 68.46, H, 3.32, N, 4.20; Found: C, 68.51, H, 3.49, N, 4.22%.
20. **C5**: mp: 200–201°C. EI–MS (m/z, %): 363 (M+, 1.6), 213 (1.5), 135 (p-MeOPhCO$^+$, 100); IR (KBr): 3040, 1780. 1710, 1600, 1580, 1510, 1270, 845 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 8.92 (dd, $J_1=7.6$ Hz, $J_2=1.0$ Hz, 1H, 2-H), 8.63 (m, 3H, 7-,4-,5-H), 8.16 (m, 2H, 3-,6-H), 7.96 (m, 2H, 2′-,6′-H), 7.22 (m, 2H, 3′-,5′-H), 3.92 (s, 3H, -OCH_3). $C_{20}H_{13}NO_4S$ requires: C, 66.10; H, 3.61; N, 3.85. Found: C, 66.57; H, 3.78; N, 3.65%.
21. **C6**: mp 260∼262°C. IR (KBr): 3030, 1780, 1610, 1600, 1580, 790, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.65 (d, $J=7.3$ Hz, 2H, 2-, 7-H), 8.29 (m, 4H, 3-∼6-H), 7.52–7.83 (m, 5H, Ph). EIMS (m/z, %): 317 (M^+, 4.0), 105 (PhCO$^+$, 100).