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Photo-induced DNA cleavage by (heterocyclo)carbonyl oxime esters of anthraquinone

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Abstract

Various (heterocyclo)carbonyl mono-oxime esters of anthraquinone were synthesized, which exhibited an ability for DNA cleavage upon UV irradiation. Their structure–activity relationship was established, in which the most potent compound was anthraquinone *O*-9-(1,3-benzothiazole-2-carbonyl)oxime (**4j**). It can produce radical species and nick DNA at the concentration as low as 1.0 μM.

Photodynamic,[1,2] photodiagnostic,[3] and photochemotherapy[4] are in the main themes of genetherapy. Development of new chemical leads for photogenetherapy often requires compounds that can cleave DNA or RNA under photolytic conditions.[5] Some 'chemical nucleases' in this category with low toxicity, such as porphyrins[6] and furocoumarins,[7] possess clinical value. 8-Methoxypsoralen represents another prominent example. It has been widely used in dermatology, particularly for the treatment of psoriasis by oral or topical administration of psoralen followed by UV irradiation.[8,9]

We planned to develop a controllable method for DNA cleavages by radical species that came from heterocyclic oxime esters. The weak O–N bond therein may undergo homolytic fission by photolysis with UV light.[10] Some heterocyclic compounds play special roles toward nucleic acids. Examples include Neidle[11] synthesized furan derivatives of berenil to target the minor-groove DNA. Dervan[12] et al. developed pyridine derivatives that can bind double helical DNA. In 2000, Wang et al.[13] synthesized a series of stacked-dimer compounds, in which benzofuran is used as DNA minor-groove binders. Westman,[14] Yarmoluk,[15] and their co-workers used benzothiazole derivatives as DNA groove binders. These heterocycles are therefore merged into our design of new DNA-cleaving agents.

Nishimoto[16] and Kawaguchi[17] applied anthraquinone derivatives as intercalators for DNA scission. Our previous report also indicates that anthraquinone serves as a better intercalator than fluoren-9-one and thioxanthen-9-one 10,10-dioxide in their corresponding benzoyl oxime esters for DNA breaks.[10] Accordingly, we selected anthraquinone to conjugate with heterocycles as the candidates in the new compound library of oxime esters. It is our wish that the outcome resulting from the current systematic study may disclose the clues on: (1) which (heterocyclo)carbonyl oxime esters exhibit great potency; (2) whether the (heterocyclo)carboxyl radical or the anthraquinone iminyl radical generated from oxime esters is primarily responsible for DNA cleavage; and (3) what kinds of the structure–activity relationship can be drawn. We report here that the unprecedented (heterocyclo)carbonyl oxime esters **4b**, **4g**, **4h**, and **4j** were able to nick DNA at the concentrations as low as 11, 5.1, 1.5, and 1.0 μM, individually, upon triggering by UV light.

To obtain various heterocycle-containing mono-oxime esters **4**, we applied an established method[18] by the

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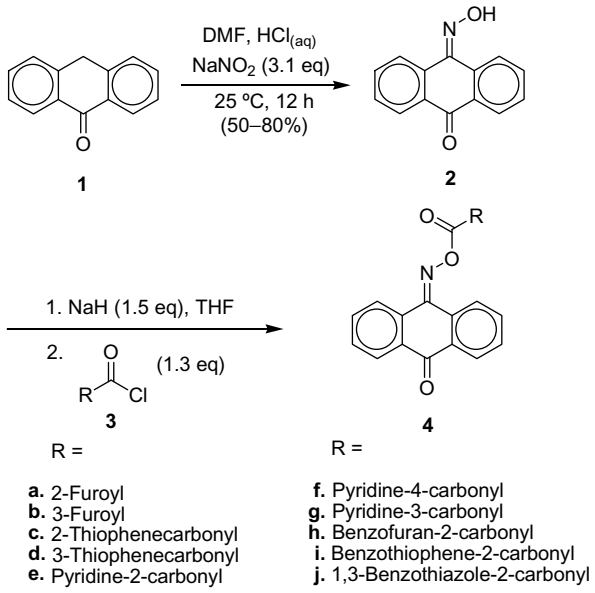


Scheme 1. Synthesis of various (heterocyclo)carbonyl oxime esters from 9-anthrone.

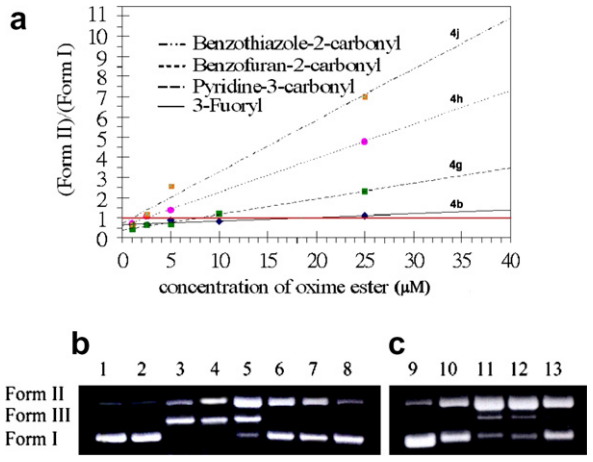


Fig. 2. (a) Dose measurement results from DNA cleavage by mono-oxime esters **4b**, **4g**, **4h**, and **4j** in a sodium phosphate buffer (pH 6.0, 0.10 M) upon irradiation with 312-nm UV light at 25 °C for 2.0 h. (b) The gel electrophoresis data: Lane 1, DNA only; Lane 2, DNA + **4j** (50 μM) in the dark; Lanes 3–8, DNA + **4j** of 50, 25, 5.0, 2.5, 1.0, and 0.50 μM, individually, with UV light. (c) Lane 9, DNA only; Lane 10–13, DNA + **4j** (10 μM) with UV light for 2.0 h in a sodium phosphate buffer with pH 5.0, 6.0, 7.0, and 8.0 in order.

oxidative oximation of 9-anthrone (**1**) with sodium nitrite in DMF (see Scheme 1). The corresponding oxime **2** was then condensed with different (heterocyclo)acyl chlorides **3a**–**j** to produce the desired oxime esters **4a**–**j** in 50–83% yields. The heterocyclic moieties included furan, thiophene, pyridine, benzofuran, benzothiophene, and benzothiazole.

To investigate the ability of DNA-nicking, we dissolved (heterocyclo)carbonyl mono-oxime esters **4a**–**j** (10.0 μM) individually in a sodium phosphate buffer (0.20 M, pH 6.0) and 10% DMSO containing the supercoiled circular *ϕX174* RFI DNA (form I; 50 μM/base pair). These solutions were irradiated with UV light (312 nm, 1.43 mW/cm^2) under aerobic conditions at room temperature for 2.0 h. By analyzing the results from gel electrophoresis on 1.0% agarose gel with ethidium bromide staining, we found that the relaxed circular (i.e., Form II) DNA was generated in all of these experiments (see Fig. 1).

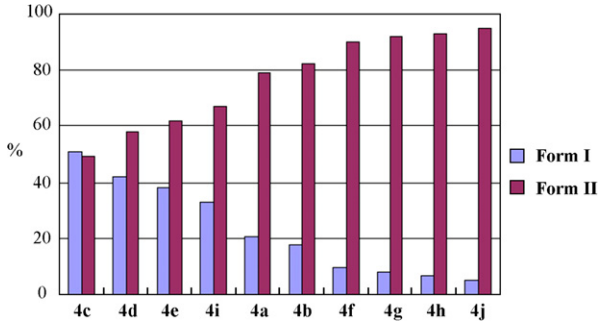


Fig. 1. Heterocyclic mono-oxime esters **4a**–**j** (10.0 μM) in the cleavage of supercoiled circular *ϕX174* RFI DNA (Form I) to relaxed circular DNA (Form II) in a sodium phosphate buffer (pH 6.0) and 10% DMSO under aerobic conditions and photolysis with 312-nm UV light at room temperature for 2.0 h. The order is on the basis of their potency.

Among these compounds, mono-oxime esters **4b**, **4g**, **4h**, and **4j** exhibited greater DNA-cleaving potency than most of the other compounds. Results from dose measurements (0–50.0 μM) reveal that their cleavage of DNA was concentration dependent (Fig. 2a and b). These four mono-oxime esters were able to nick DNA with (Form II)/(Form I) = 1.0 at the concentrations 18.4 μM for **4b**, 8.1 μM for **4g**, 2.6 μM for **4h**, and 1.1 μM for **4j**. Gel electrophoresis data for the most potent compound **4j** are shown in Figure 2b, in which we found that DNA cleavage did not occur in the dark as shown in Lane 1. Thus the UV light functioned as a 'trigger' to initiate the DNA scission process (cf. Lanes 3–7). Moreover, being able to nick DNA under various pH conditions between 5.0 and 8.0 shown in Figure 2(c), compound **4j** exhibited the greatest potency at pH 6.0 (see Lane 11). On the other hand, we found that the isosbestic point of **4j** in its UV spectra showed up at $\lambda = 387$ nm in various buffer solutions with pH 5.0–8.0.

For obtaining the apparent equilibrium binding constants (K_{app}) of **4b**, **4g**, **4h**, and **4j**, we used these oxime esters to inhibit the binding of ethidium bromide to calf thymus DNA. Their K_{app} values were measured as $7.17 \times 10^5\ M^{-1}$ for **4b**, $7.57 \times 10^5\ M^{-1}$ for **4g**, $4.65 \times 10^5\ M^{-1}$ for **4h**, and $6.21 \times 10^5\ M^{-1}$ for **4j** (see Fig. 3).

Being able to nick DNA, radical species may initiate the strand scission by trapping a C-3′ or C-5′ hydrogen of deoxyribose therein.[19] Given these established mechanisms, we hypothesized that heterocyclic mono-oxime esters could nick DNA by generating radicals through photolysis. To obtain evidence in support of this hypothesis, we irradiated **4j** in a phosphate buffer solution (pH 6.0, 0.10 M) with 312-nm UV light. Although not being able to detect any EPR signal directly, we successfully trapped some radical species

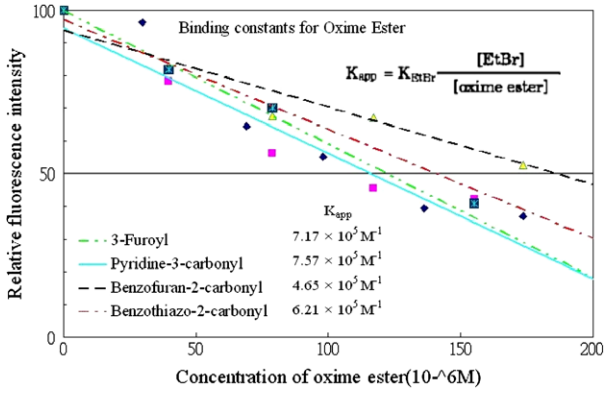


Fig. 3. Measurement of apparent equilibrium binding constants of heterocyclic mono-oxime esters **4b**, **4g**, **4h**, and **4j**.

by adding 5,5-dimethyl-1-pyrroline N-oxide (DMPO) in situ. Figure 4a–e shows our results from a series of control experiments.

In the first step, we photolyzed **4j** in the presence of an excess amount (3.0 equiv) of DMPO to give mixed signals as shown in Figure 4c. These signals were not resolvable. In the second step, the same experiment was repeated to give the spectrum shown in Figure 4d by the addition of DMPO at less than a stoichiometric amount (i.e., 0.80 equiv). In comparison with Figure 4d, the extra three peaks with a high intensity in Figure 4c came from the intact DMPO, which exhibited a triplet as those shown in Figure 4a. We also obtained the same type of signals shown in Figure 4b from a mixture of **4j** and DMPO (3.0 equiv) in the dark.

On the other hand, we generated a simulated spectrum (i.e., Fig. 4e) for radical **7** with peak intensities 1:1:1:1:1:1, in which the g value was 2.0061 and hyperfine splitting constants $a_N = 12.01$ G and $a_H = 9.10$ G (cf. Fig. 4g). Radical **7** is the adduct of DMPO and radical **6**, which may be generated through homolytic fission of the N–O bond in **4j** as shown in Scheme 2. Figure 4f shows that the simulated spectrum of Figure 4e can be impeccably superimposed over Figure 4d. These outcomes convince us to believe that the radical detected by EPR experiment came from the adduct **7**. Furthermore, we tried a different method to trap the radical intermediate **5**, which was not detected by EPR spectroscopy. Thus **4j** in a benzene solution (0.10 M) containing 1,4-cyclohexadiene (15 equiv) was irradiated with 312-nm UV light under aerobic conditions for 2.0 h. After aqueous workup, the reaction products were separated by the use of HPLC with a silica gel column. We obtained anthraquinone in 81% yield, which came from the corresponding unstable imine through hydrolysis. This imine intermediate was generated by the transfer of a hydrogen atom from 1,4-cyclohexadiene to imine radical **5**. Results from these control experiments provide direct evidence on our successful generation of a radical intermediate from (heterocyclo)carbonyl mono-oxime esters of anthraquinone by photolysis.

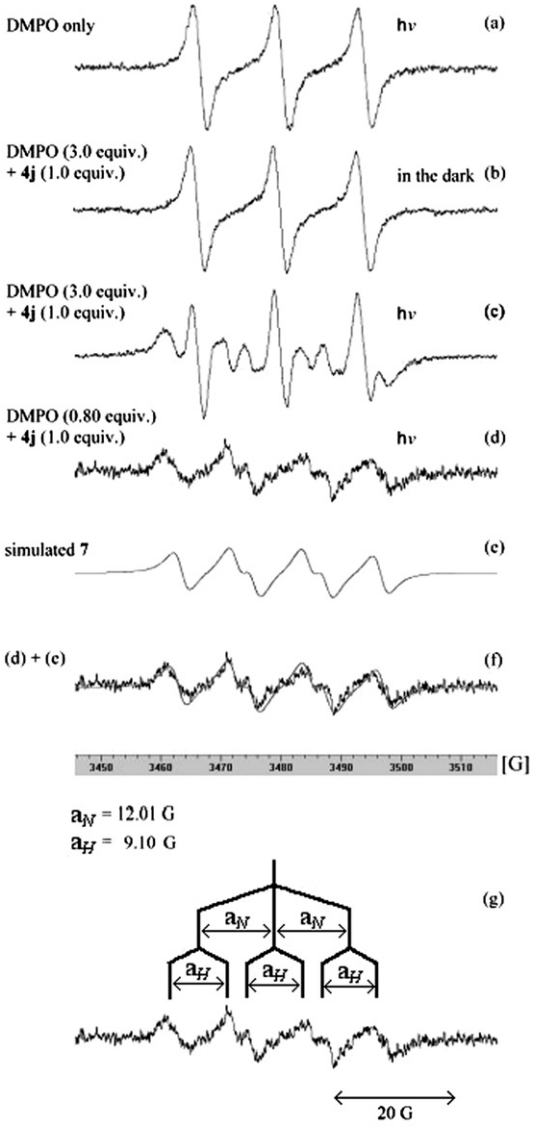
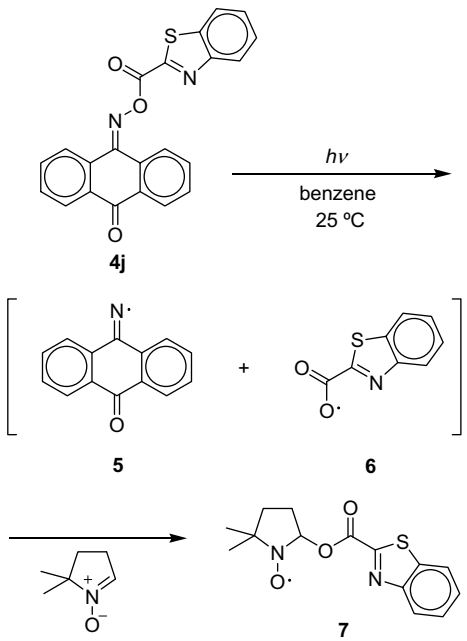


Fig. 4. EPR spectra and their analysis of DMPO (alone) and the DMPO–**6** adduct (i.e., **7**).

DNA scissions by **4j** may be contributed to both the iminyl radical **5** and the heterocyclocarboxyl radical **6**. After examining the results from **4a** to **4j** shown in Figure 1, we found that their potency varied to a great extent. All of these mono-oxime esters would generate the common anthraquinone iminyl radical **5**. Therefore the difference of DNA-cleaving potency must be due to the different heterocyclic radical moieties resulting from the homolytic fission of **4a**–**j**. These analyses lead us to believe that the (heterocyclo)carboxyl radicals functioned as the major DNA-nicking species. On the other hand, the possibility cannot be excluded that a small amount of their decarboxylated species might be generated[20] and played a role on DNA scission.

Among compounds **4a**–**j**, different heterocycles were attached to the same anthraquinone mono-oxime moiety. By analyzing the ratios of (Form II DNA)/(Form



Scheme 2. Trapping of the carboxyl radical by DMPO during photolysis of *O*-9-(1,3-benzothiazole-2-carbonyl)oxime (**4j**).

I DNA) shown in Figure 1, we deduce the following structure–activity relationships:

(a) Introduction of a heteroatom, including O, S, and N atoms, into cyclopentadiene and cyclohexadiene rings (i.e., **4a**–**j**) enables the resultant heterocycle–containing oxime esters of anthraquinone to possess appealing DNA-cleaving ability;
(b) the DNA-cleaving potency was greater for fused bicyclic than monocyclic heterocyclic compounds (cf. **4h** > **4a** and **4i** > **4c**);
(c) bicyclic heterocyclic compounds containing two heteroatoms possess greater potency than those containing a single heteroatom (cf. **4j** > **4h** and **4j** > **4i**); and
(d) the DNA-cleaving potency resulting from the heterocyclic rings of anthraquinone oximes followed the order benzothiazole > benzofuran > pyridine ~ furan > thiophene.

In conclusion, a series of heterocycle–containing mono-oxime esters of anthraquinone were synthesized and found to possess DNA-cleaving ability. Upon UV irradiation, the most potent compound **4j** nicked DNA at the concentration as low as 1.0 μM. The (benzothiazole)carboxyl radical, generated from **4j** through homolytic fission, was trapped by DMPO and the resultant adduct was detected by EPR spectrometer. Our results indicate that heterocycle-containing radicals, instead of the anthraquinone iminyl radical, were primarily responsible for DNA cleavage. Finally, the structure–activity relationship is illustrated on the basis of their DNA-cleaving ability.

Acknowledgments

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Supplementary data

Synthetic and experimental procedures, and spectroscopic data of compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.03.056.

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