N-Aroyloxy-2-thiopyridones as efficient oxygen-radical generators: novel time-controlled DNA photocleaving reagents

Emmanuel A. Theodorakis* and Keith M. Wilcoxen

Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0358

N-Aroyloxy-2-thiopyridones efficiently cleave DNA upon visible light illumination *via* the formation of aroyloxyl radicals.

Cell damaging effects by oxygen radicals are well recognized in several biological events, including carcinogenesis and cell apoptosis.[1] On the other hand, the DNA strand cleavage induced by oxygen-centred radicals has found major applications in both chemistry[2] and molecular biology.[3] From this perspective, the design of organic molecules that can induce DNA cleavage is of great interest and constitutes a timely and challenging research topic.[4,5]

Our strategy for DNA cleavage is based on the design of molecules that upon visible light photolysis ($\lambda > 350$ nm) generate oxygen-centred radicals in the vicinity of nucleic acid strands. To this extent, *N*-hydroxy-2-thiopyridone **1**[6] and its aroyloxy derivatives (such as **3**) are ideal candidates since they possess the following characteristics: purely organic structure, facile one-step synthesis and efficient isolation, prolonged stability in the absence of light, and well documented radical chemistry.[7] In general, visible-light irradiation of **1** and **3**, results in the formation of the hydroxyl radical **2**[8] and the aroyloxyl radical **4**[9] respectively (through homolytic cleavage of the N–O bond). Aroyloxyl radicals **4** are known to be persistent radicals ($k > 10^5$ s^{-1}), undergoing efficient decarboxylation only above 120 °C.[9] We therefore envisioned that these oxygen-centred radicals could induce DNA strand cleavage, similarly for the hydroxyl radical (Fig. 1).

Our initial experiments were performed with the yellow crystalline benzoyloxy derivative **5**, formed *via* benzoylation of the parent compound **1** (87% yield) (Scheme 1).† The generation of benzoyloxyl radicals **6** was unequivocally confirmed by photolysis of **5** in the presence of 5.0 equiv. of Bu^{t}SH in degassed methylene chloride, which resulted in the isolation of benzoic acid **7** and disulfide **8** in 92 and 89% yields respectively, in accordance to a radical-chain reaction process (Scheme 1).

We further examined the visible light photolysis of **5** and **1** in the presence of supercoiled circular φX174 DNA.‡ Control experiments indicated that both **5** and light are neccessary for the observed relaxation of the DNA, thus strongly suggesting the formation of benzoyloxyl radicals **6** (Fig. 2). Formation of radicals **6** was further confirmed by inhibition of DNA cleavage, when the same experiment was conducted in the presence of glutathione as radical scavenger (Fig. 2, lanes 10, 11). On the other hand, the concomitant generation of the sulfur-centred thiopyridyl radical is known not to contribute to the DNA damage.[6] It is interesting to compare the DNA-cleavage efficiencies of **5** and **1**, under otherwise identical experimental conditions (Fig. 2, lanes 10, 12). The enhanced reactivity of **5** is a result of the increased absorbance of **5**, relative to **1**, in the emission region of the light source, during the same photolysis time (30 min).§ This accelerated DNA-cleaving ability of **5** indicates that **5** undergoes homolytic N–O bond cleavage prior to any saponification (affording **1**) and further supports the notion that benzoyloxyl radicals **6** are involved in the photocleavage of DNA, induced by **5**.

We then examined the time-dependent DNA-scission induced by **5** and **1** (Fig. 3).§ Our data indicate that benzoyloxyl radicals **6** are generated from compound **5**, at a relatively linear rate. This continuous generation of reactive oxygen-centred radicals provides an alternative to the Fenton-based chemistry, where oxygen radicals are formed as a rapid burst. In addition, the DNA cleavage with **5** does not occur in the absence of visible light. This method is therefore attractive for 'time-

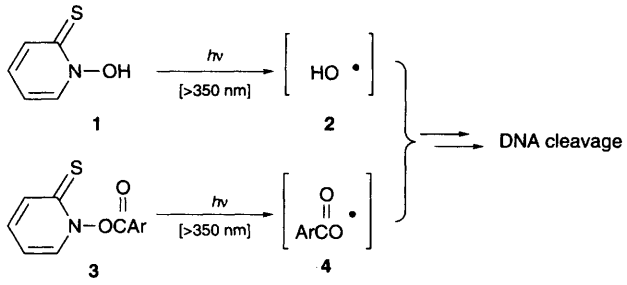
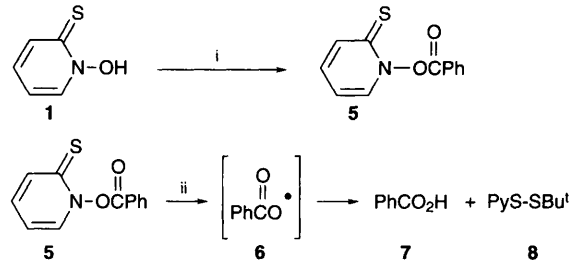


Fig. 1 Photoinduced DNA cleavage with thiohydroxamic acid **1** and aryloxy derivatives **3**



Scheme 1 *Reagents and conditions*: i, 1.1 equiv. of PhCOCl, 1.4 equiv. of pyridine, CH_2Cl_2, 0 °C, 1 h, 92%; ii, 5.0 equiv. of Bu^{t}SH, CH_2Cl_2, *hν* (GE, 300 W, $\lambda > 350$ nm), 0 °C, 1 h, 92% (for **7**) and 89% (for **8**)

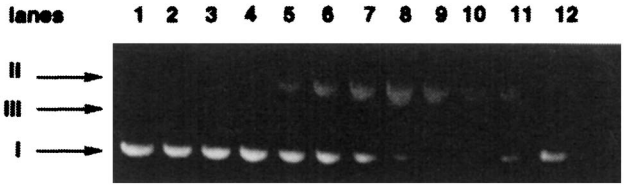


Fig. 2 Concentration-dependent photocleavage of φX174 DNA induced by **5** and **1**. The DNA cleavage was analysed on 1% agarose gel (Tris-acetate buffer) stained with ethidium bromide. The photolysis was performed at 5 °C for 30 min, with one lamp (GE300W) placed at approximately 20 cm from the samples (lanes 1 and 3–12). Lane 1: φX174DNA (control); lane 2: DNA and 3 mmol dm^{-3} of **5** in the absence of light. Lanes 3–10: DNA and varying concentrations of **5**: lane 3: 0.1 mmol dm^{-3}; lane 4: 0.3 mmol dm^{-3}; lane 5: 0.5 mmol dm^{-3}; lane 6: 0.7 mmol dm^{-3}; lane 7: 1.0 mmol dm^{-3}; lane 8: 1.5 mmol dm^{-3}; lane 9: 2.0 mmol dm^{-3}; lane 10: 3.0 mmol dm^{-3}. Lane 11: DNA, 3.0 mmol dm^{-3} of **5**, and 3.0 mmol dm^{-3} of glutathione. Lane 12: DNA and 3.0 mmol dm^{-3} of **1**.

resolved' DNA cleavage studies, and for *in vivo* biomedical applications involving 'photodynamic therapy'.[10]

The versatility and efficiency of the aroyloxy derivatives of the *N*-hydroxy-2-thiopyridone as DNA photocleaving reagents can be demonstrated by the one-step synthesis of a variety of compounds (Fig. 4); all of these derivatives exhibited similar DNA-cleaving profiles, under visible light photolysis, thus establishing the generality of our method.

Furthermore our data demonstrate indisputably and for the first time that aroyloxy radicals can induce significant DNA cleavage[11] and that derivatives such as **5** and **9–13** can be used as time-controlled DNA cleaving reagents. The development of new photofootprinting reagents and artificial photonucleases, based on the *N*-hydroxy-2-thiopyridone chemistry, is now under investigation in our laboratories.

We thank Professors Y. Tor and K. C. Nicolaou of this Department for useful discussions. Financial support by the University of California, San Diego, in the form of startup grant is greatly appreciated.

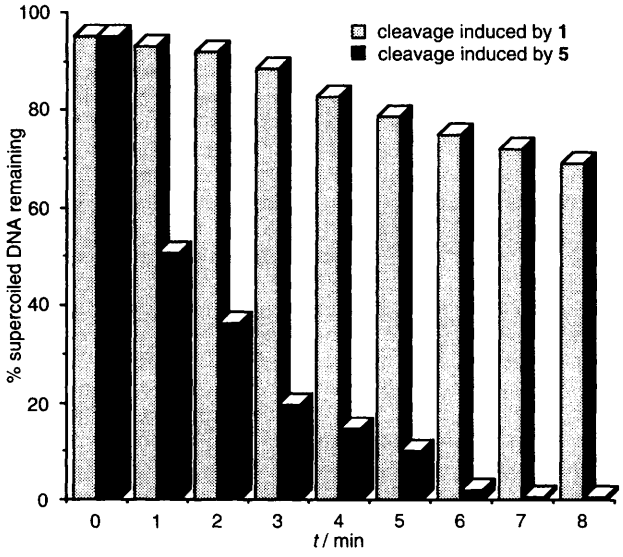


Fig. 3 Time-controlled photocleavage of φX174 DNA induced by **1** and **5**, *via* hydroxy **2** and benzoyloxy radicals **6** respectively. The reaction mixtures containing 500 ng of DNA and 1.0 mmol dm^{-3} of **5** or **1** were photolysed, at 5 °C, at a distance of 20 cm from a 300W GE lamp for the time given below and then placed in the dark for the remainder of 20 min. The data were obtained from densitometry reading of the DNA-scission.

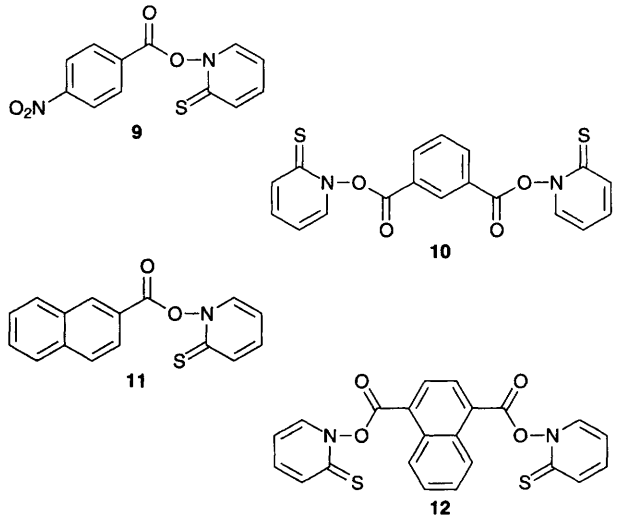


Fig. 4 Designed thiohydroxamic acid derivatives as time-controlled DNA photocleaving reagents

Footnotes

† Although **5** can be stored in the dark without any appreciable decomposition, it smoothly decolourizes upon visible light photolysis (GE, 300 W) presumably *via* the intermediacy of the transient benzoyloxyl radical **6**.

‡ The photocleavage efficiency was determined as the degree of conversion of supercoiled DNA (form I) to circular nicked (form II) and linear (form III). The photolysis was performed simultaneously with all samples at 5 °C for 30 min, with one lamp (GE 300 W, λ ca. 350 nm).

§ The difference in absorbance is reflected in the colour of **5**, which is bright yellow, while **1** is colourless and the UV–VIS absorptions of the thiocarbonyl moieties of **5** (λ_{max} = 368 nm) and **1** (λ_{max} = 349 nm). This difference accounts for the faster formation of benzoyloxyl radicals **6** and may justify the 100-fold accelerated DNA-scission observed with **6** as compared to **2** (Fig. 3).

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Received, 29th March 1996; Com. 6/02193J