Pergamon

Tetrahedron Letters 39 (1998) 3383–3386

TETRAHEDRON LETTERS

On the Mechanism of Photo-induced Nucleic Acid Cleavage Using *N*-Aroyloxy-2-thiopyridones

Emmanuel A. Theodorakis,* Xin Xiang, Mindo Lee and Tony Gibson

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

Received 21 November 1997; revised 23 February 1998; accepted 25 February 1998

Abstract: Benzoyloxyl radicals react with thymidine, in a mechanism reminiscent of hydroxyl radical mediated thymidine damage. This mode of reactivity may explain the observed DNA cleavage induced by photoirradiation of *N*-aroyloxy-2-thiopyridones.

Over the past twenty years, *N*-hydroxy-2-thiopyridone (**1**) and its *O*-acyl derivatives have been extensively used as a convenient photochemical source of carbon-, nitrogen- and oxygen-centered radicals.[1] Generation of such radicals has been proven to occur via an initial photoexcitation of the thiocarbonyl group ($\lambda \approx 350$ nm), resulting ultimately in homolytic cleavage of the N-O bond (Figure 1).[2] The synthetic efficiency of this process has been clearly demonstrated by the subsequent trapping of the radicals in a variety of ways, allowing the introduction of new functionalities into organic molecules.[1,3]

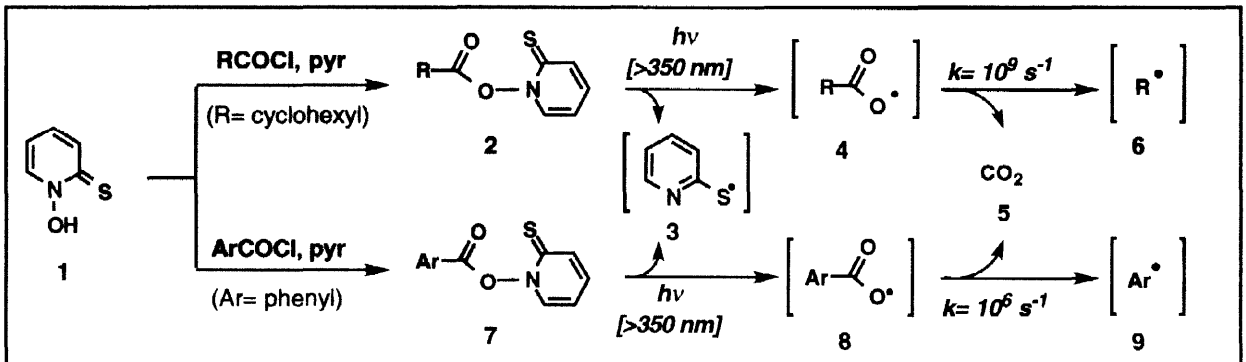


Figure 1. Photoinduced generation of radicals with *N*-acyloxy-(**2**) and *N*-aroyloxy-2-thiopyridones (**7**).

Based on the above data, we envisioned that *N*-hydroxy-2-thiopyridone (**1**) and its carboxylate esters (such as **2** and **7**, Figure 1) could be used as novel photoactivated nucleic acid-cleaving reagents.[4] Our reported studies indicate that, indeed, the *N*-aroyloxy-2-thiopyridones (such as **7**) cleave supercoiled circular and linear duplex DNA.[5,6] However, the identity of the reactive radical species during this event remained unknown. Herein we would like to present evidence that implicates aroyloxyl radicals (**8**) in the observed nucleic acid damage and comment on the mechanism for the observed DNA scission.

In our initial explorations we compared the DNA-cleaving properties of several acyloxyl and aroyloxyl derivatives of **1**. These compounds were photolyzed in the presence of plasmid ϕX174 DNA under identical conditions, as shown in Figure 2.[7] In all experiments performed, only the aroyloxyl derivatives (such as **7**) were shown to cleave the plasmid, while the acyloxyl adducts (such as **2**) did not produce any measurable strand cleavage.

0040-4039/98/$19.00
PII: S0040-4039(98)00509-7

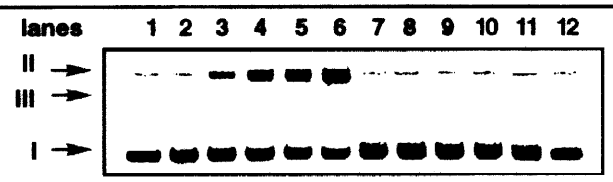


Figure 2. Photocleavage of DNA using **2** and **7**.[7]
The irradiation (all samples except those in lanes 2 and 7) was performed for one hour at 4°C with two lamps (GE, 300W), placed at about 20 cm from the samples. Lane 1: ϕX174 DNA (control); lane 2: DNA and 1.0 mM of **7** (no hv); lanes 3-6: DNA and **7** at concentrations of **7**: 0.1, 0.3, 0.6 and 1.0 mM respectively. Lane 7: DNA and 1.0 mM of **2** (no hv); Lanes 8-11: DNA and **2** at concentrations of **2**: 0.1, 0.3, 0.6 and 1.0 mM respectively; lane 12: ϕX174 DNA (control).

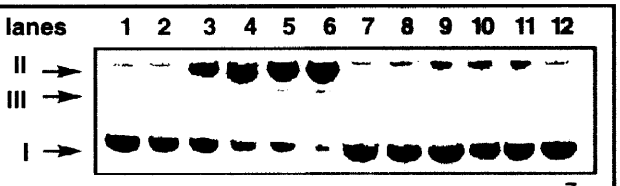


Figure 3. Photocleavage of DNA using **7** and **1**.[7]
The irradiation (all samples except those in lanes 2 and 7) was performed for one hour at 4°C with two lamps (GE, 300W), placed at about 20 cm from the samples. Lane 1: ϕX174 DNA (control); lane 2: DNA and 1.0 mM of **7** (no hv); lanes 3-6: DNA and **7** at concentrations of **7**: 0.5, 1.0, 1.5 and 2.0 mM respectively. Lane 7: DNA and 1.0 mM of **1** (no hv); Lanes 8-11: DNA and **1** at concentrations of **1**: 0.5, 1.0, 1.5 and 2.0 mM respectively; lane 12: ϕX174 DNA (control).

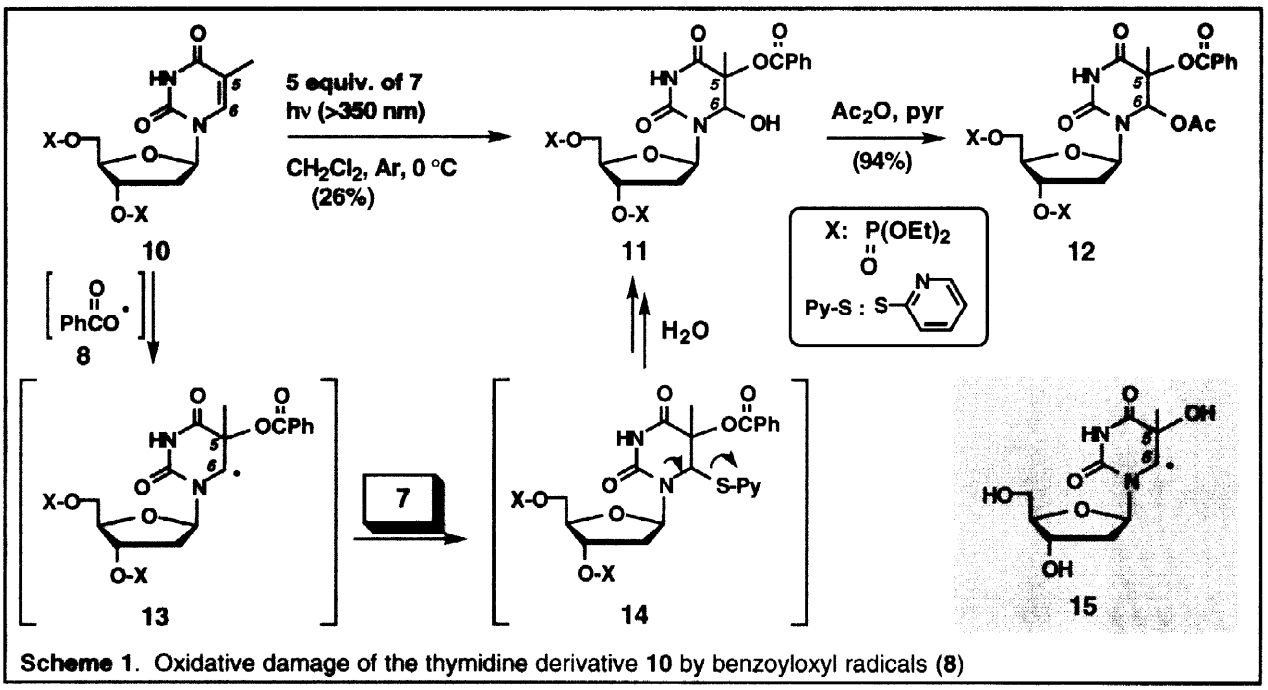
Comparison of **7** with the free *N*-hydroxy-2-thiopyridone (**1**) was also performed (Figure 3).[5] The results showed an increased efficiency of DNA-cleavage using **7**, thus indicating that the generation of reactive radical species happens prior to any saponification (that could afford **1** from **7**). The combination of the above experiments eliminated the involvement of the thiopyridyl radical **3** in the DNA-cleaving reaction and provided indirect evidence that aroyloxyl radicals (such as **8**) may account for the observed strand scission.

Can aroyloxyl radicals cleave DNA? An alternative proposal could attribute this damage to the presence of aryl radicals (**9**), arising through slow decarboxylation of **8** (Figure 1). Literature data indicate that the rate constant for this decarboxylative process is approximately 10^6 s^{-1} at 25 °C, while the decarboxylation of aliphatic derivatives (such as **4**) under the same conditions is at least 1000 times faster (10^9 s^{-1}).[8,2] Although relatively slow, such a process could hence generate aryl radicals (**9**) that are known to damage DNA.[9]

In an attempt to "shine light" on the above question, we examined the reaction of the thymidine derivative **10** with the *N*-benzoyloxy-2-thiopyridone (**7**) (Scheme 1). Thus, 5.0 equivalents of **7** were introduced portionwise at 0 °C to a solution of **10** under constant irradiation with two lamps (tungsten, 300 W). At the end of the reaction we were able to isolate the thymidine adduct **11**[10] in 26% yield, easily separated from the unreacted starting material **10** (recovered in 68% yield). The somewhat surprising formation of **11** can be explained if we consider an addition of the *N*-benzoyloxy-2-thiopyridone (**7**) across the C5-C6 double bond of the nucleobase. Our proposed mechanism involves initial attack of the benzoyloxyl radical (**8**) on the C5 carbon of the thymine, which could produce the C6 radical **13** (Scheme 1). Subsequent trapping of **13** with the thiocarbonyl entity of unreacted **7** is expected to form adduct **14**, thus propagating the radical chain reaction.[3] Hydrolysis of the unstable thioaminal of **14**, during isolation, could then lead to **11**. Additional characterization of **11** was obtained by its conversion to acetate **12** (94% yield).[10] In none of the above reactions were we able to detect either the addition of phenyl radicals on the thymidine, or the formation of benzene (GCMS analysis of the crude mixture). Furthermore, no reaction occurred between **2** (5-10 equivalents) and **10** upon irradiation; the starting thymidine (**10**) and the 1-cyclohexyl-2-thiopyridine (Cy-SPy) were recovered in quantitative yield.

Radical **13** is reminiscent of thymidinyl radical **15**, the major intermediate formed upon exposure of thymidine to hydroxyl radicals.[11,12] This resemblance between **11** and **15** provides strong evidence that both benzoyloxyl and hydroxyl radicals have comparable electrophilic character and react similarly with nucleic acids.

Interestingly, the rate of addition of hydroxyl radicals on the nucleobases is ca 10^9 $M^{-1}•s^{-1}$.[11] A similar addition rate can be expected for the benzoyloxyl radicals, which under standard conditions is much faster than the unimolecular decarboxylation event (ca 10^6 s^{-1} at 25°C).



Scheme 1. Oxidative damage of the thymidine derivative **10** by benzoyloxyl radicals (**8**)

Hydroxyl radicals are known to cleave DNA in a variety of ways including: direct hydrogen atom abstraction from the backbone and nucleobase modifications.[11] When such modifications occur within the duplex, they result in weakening the Watson-Crick hydrogen bonds and produce alkali-labile sites on the DNA backbone. Our data indicate that benzoyloxyl radicals (**8**) behave comparably to hydroxyl radicals and could cleave DNA by similar pathways, including induction of frank strand cleavage and formation of alkali-labile sites. For example, the observed oxidative damage of thymidine by **8** (Scheme 1) could lead to DNA cleavage *via* the generation of alkali-labile sites.[12] This hypothesis is further justified by our observation that piperidine treatment after photoirradiation enhances the DNA strand scission.[6]

The above results do not imply that thymidine (or any other base) modification is the only mechanism accounting for the observed DNA cleavage. In fact, in the duplex DNA the bases are somewhat protected in the interior of the double strand and hydrogen atom abstraction from the phosphodiester backbone should play a major role for the cleavage. *However they do show that aroyloxyl radicals are able to react with DNA in a mode of action reminiscent of the hydroxyl radical-mediated damage.* The previously mentioned kinetic data are also in favor of this mode of action.

Formation of the thymidine adduct **11** deserves an additional comment. This type of base modification within genomic DNA may lead to enzymatic misreading giving rise to side-mutations. Since benzoyl peroxide is known to be mutagenic,[13] our approach may be used to explain the chemical origins of the reported mutagenesis. Further studies along these lines are now in progress in our laboratory.

Acknowledgments: This work was supported by the Cancer Research Coordinating Committee, the UCSD Academic Senate and the donors of the Petroleum Research Funds administered by the ACS.

References and Notes

1. Barton, D. H. R.; Crich, D.; Motherwell, W. *J. Chem. Soc. Chem. Commun.* **1983**, 939-941. Barton, D. H. R.; Togo, H.; Zard, S. Z. *Tetrahedron Lett.* **1985**, *26*, 6349-6352. Barton, D. H. R.; Tachdjian, C. *Tetrahedron*, **1992**, *48*, 7091-7108. Barton, D. H. R.; Chern, C.-Y.; Jaszberenyi, J. Cs. *Tetrahedron Lett.* **1992**, *33*, 5017-5020. Newcomb, M.; Deeb, T. M. *J. Am. Chem. Soc.* **1987**, *109*, 3163-3165. Newcomb, M.; Kumar, M. U. *Tetrahedron Lett.* **1990**, *31*, 1675-1678. Barton, D. H. R.; Jaszberenyi, J. Cs.; Morrell, A. I. *Tetrahedron Lett.* **1991**, *32*, 311-314.
2. Bohne, C.; Boch, R.; Scaiano, J. C. *J. Org. Chem.* **1990**, *55*, 5414. Newcomb, M.; Park, S. U. *J. Am. Chem. Soc.* **1986**, *108*, 4132. Barton, D. H. R.; Zard, S. Z. *Pure & Appl. Chem.* **1986**, *58*, 675-684.
3. For selected reviews on this topic see: Crich, D. *Aldrichimica Acta* **1987**, *20*, 35-42. Crich, D.; Quintero, L. *Chem. Rev.* **1989**, *89*, 1413-1432. Barton, D. H. R. *Aldrichimica Acta* **1990**, *23*, 3-10. Barton, D. H. R. *Tetrahedron* **1992**, *55*, 5414-5418.
4. For similar studies from other laboratories see: Hess, K. M.; Dix, T. A. *Anal. Biochem.* **1992**, *206*, 309-314. Adam, W.; Ballmaier, D.; Epe, B.; Grimm, G. N.; Saha-Moller, C. R. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2156-2158. Boivin, J.; Crepon, E.; Zard, S. Z. *Tetrahedron*, **1990**, *31*, 6869-6872. Aveline, B. M.; Kochevar, I. E.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 289-290. Aveline, B. M.; Kochevar, I. E.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *117*, 9699-9708. Aveline, B. M.; Kochevar, I. E.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 10113-10123. Epe, B.; Ballmaier, D.; Adam, W.; Grimm, G. N.; Saha-Moller, C. R. *Nucl. Acids Res.* **1996**, *24*, 1625-1631.
5. Theodorakis, E. A.; Wilcoxen, K. M. *Chem. Commun.* **1996**, 1927-1928.
6. Theodorakis, E. A.; Xiang, X.; Blom, P. *Chem. Commun.* **1997**, 1463-1464.
7. ϕX174 (50 μM/base pair) plasmid DNA was incubated for 1 hr at 25°C with the indicated amounts of **1**, **2**, or **7** (30mM Tris-HCl, 20 mM NaCl), then placed at 4 °C and subjected to irradiation as indicated in Figures 2,3. The results were analyzed on 1% agarose gel (Tris-acetate buffer) stained with ethidium bromide. The photo-cleavage efficiency was determined as the degree of conversion of supercoiled DNA (form I) to circular nicked (form II) and linear (form III).
8. Chateauneuf, J.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1988**, *110*, 2886-2893.
9. Griffiths, J.; Murphy, J. A. *J. Chem. Soc. Chem. Commun.* **1992**, 24-26. Nicolaou, K. C.; Dai, W. M.; Tsay, S. C.; Estevez, V. A.; Wrasidlo, W. *Science*, **1992**, *256*, 1172-1178.
10. Selected data for compound **10**: IR (film) ν_{max} 3483, 2986, 1690, 1468, 1266, 1159, 1038; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.45 (s, 1H), 6.39 (dd, 1H, J= 5.5, 7.5 Hz), 5.01 (t, 1H, J= 6.0 Hz), 4.32 (bs, 1H), 4.24 (m, 2H), 4.13 (m, 8H), 2.51 (m, 1H), 2.15 (m, 1H), 1.92 (s, 3H), 1.32 (m, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 164.0, 150.5, 134.8, 111.4, 83.2, 83.1, 66.2, 64.0, 64.0, 53.2, 38.2, 15.7, 15.7, 12.0; HRMS, cacd for $C_{18}H_{32}N_2O_{11}P_2$ ($M+H^+$) 515.1560, found 515.1581. Selected data for compound **11**: IR (film) ν_{max} 3230, 2988, 1721, 1600, 1250, 1030; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, 2H, J= 7.5 Hz), 7.66 (s, 1H), 7.62 (t, 1H, J= 7.5 Hz), 7.46 (t, 2H, J= 7.5 Hz), 6.62 (s, 1H), 6.48 (t, 1H, J= 7.5 Hz), 4.90 (m, 1H), 4.30 (m, 2H), 4.24-4.0 (m, 9H), 2.24 (m, 2H), 1.48 (s, 3H), 1.33-1.30 (m, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 168.8, 164.8, 151.8, 134.2, 130.1, 128.7, 128.3, 84.4, 83.6, 78.0, 70.1, 67.1, 64.2, 62.1, 35.5, 18.2, 15.9, 15.9; HRMS, cacd for $C_{25}H_{38}N_2O_{14}P_2$ ($M+Cs^+$) 785.0853, found 785.0831. Selected data for compound **12**: IR (film) ν_{max} 2990, 1722, 1451, 1245, 1025; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, 2H, J= 7.5 Hz), 7.79 (s, 1H), 7.63 (t, 1H, J= 7.5 Hz), 7.47 (t, 2H, J= 7.5 Hz), 7.03 (s, 1H), 6.23 (dd, 1H, J= 5.5, 7.5 Hz), 4.93 (m, 1H), 4.21-4.0 (m, 11H), 2.24 (m, 2H), 2.07 (s, 3H), 1.68 (s, 3H) 1.33 (m, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 169.1, 165.7, 164.2, 151.7, 134.5, 130.1, 128.9, 127.9, 84.1, 82.1, 76.7, 75.8, 65.7, 64.1, 64.1, 53.4, 35.1, 21.3, 16.1, 16.0, 15.6; HRMS, cacd for $C_{27}H_{40}N_2O_{15}P_2$ ($M+Cs^+$) 827.0950, found 827.0980.
11. For recent reviews on this topic see: Breen, A. P.; Murphy, J. A. *Free Radical Biol. & Med.* **1995**, *18*, 1033-1077. von Sonntag, C. "The Chemical Basis of Radiation Biology" Taylor & Francis, N.Y. 1987. "Bioorganic Photochemistry", Vol. 1, Morrison H. (Ed.), J. Wiley & Sons, N.Y. **1990**.
12. Matray, T. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 6931-6932. Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Goodman, B. K.; Matray, T. J.; Tronche, C.; Vencatesan, H. *J. Am. Chem. Soc.* **1997**, *119*, 1828-1839. Barvian, M. R.; Greenberg, M. M. *J. Org. Chem.* **1995**, *60*, 1916.
13. Swauger, J. E.; Dolan, P. M.; Zweier, J. L.; Kuppusamy, P.; Kensler, T. W. *Chem. Res. Toxicol.*, **1991**, *4*, 223-228. Akman, S. A.; Kensler, T. W.; Doroshow, J. H.; Dizdaroglu, M. *Carcinogenesis*, **1993**, *14*, 1971-1974.