Highly Efficient DNA Strand Scission by Photoactivated Chlorobithiazoles

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Molecules that interact with DNA under the influence of light can function by a variety of mechanisms.[1] These include some that alkylate DNA via photocycloaddition[2] and others that form active oxygen species,[3] contain a photoreactive metal center,[4] or function via electron transfer.[5] Photochemical DNA-cleaving agents have been used to probe nucleic acid structure,[4] as prosthetic groups for antisense oligonucleotides,[6] as designed "photonucleases",[7] and as photofootprinting agents.[8]

Of particular interest is the DNA strand scission by photoactivated promazine derivatives, believed to involve three distinct mechanisms.[9] When tested at 80 μM concentration, chlorpromazine was the most active of the derivatives studied, apparently due to a reactive intermediate resulting from C–Cl bond homolysis. Presently, we describe three chlorinated bithiazole derivatives (**3–5**) structurally related to bleomycin A_5 (**1**) which mediate light dependent DNA cleavage at remarkably low concentrations.

Preparation of bithiazoles **3–5** was accomplished starting from the *N-t*-Boc derivative of methyl 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylate;[10] conversion to the chlorinated bithiazoles was then accomplished[11] by lithiation–chlorination[12] or radical chlorination[13] in analogy with known transformations. The requisite C-terminal substituents were then introduced as described previously.[14] Bithiazole **3** was also prepared by a stepwise synthesis to verify the site of chlorination.

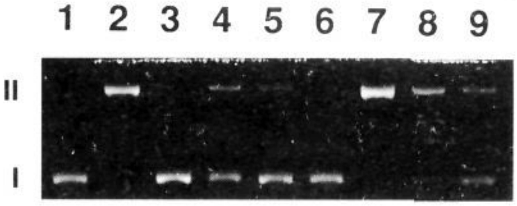
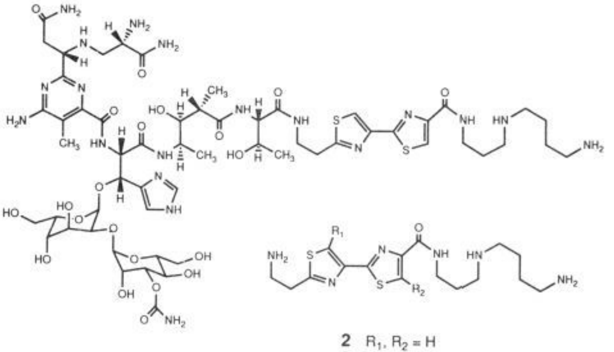


Figure 1. Cleavage of supercoiled plasmid pBR322 DNA by chlorobithiazole derivatives **4** and **5**. Forty-microliter reaction mixtures containing 200 ng of plasmid DNA in 0.5 mM sodium cacodylate, pH 7.2, were either maintained at 25 °C (lanes 1 and 2) or irradiated in ice through a Pyrex filter with a Rayonet RS photochemical reactor (2537-Å lamps). After 30 min, the reaction mixtures were analyzed on a 1.2% agarose gel. Lane 1: DNA alone. Lane 2: 10 μM Fe^{2+} + 0.19% H_2O_2. Lane 3: irradiated DNA. Lanes 4–6: 50, 20, and 10 nM chlorobithiazole **4**, respectively. Lanes 7–9: 50, 20, and 10 nM bis-chlorobithiazole **5**, respectively.



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2 $R_1, R_2 = H$
3 $R_1 = Cl, R_2 = H$
4 $R_1 = H, R_2 = Cl$
5 $R_1, R_2 = Cl$
6 $R_1 = Br, R_2 = H$

The abilities of bithiazoles **2–5** to effect DNA cleavage were determined using supercoiled plasmid pBR322 DNA. As shown in Figure 1, DNA cleavage by chlorobithiazole **4** was observed at 50 and 20 nM concentrations; comparable results were obtained using isomeric chlorobithiazole **3** (Figure 2). Bis-chlorobithiazole **5** had greater potency, producing DNA nicks in a concentration dependent fashion at 50, 20, and 10 nM concentrations. Testing at a lower concentration of **5** demonstrated detectable cleavage at 2 nM concentration (not shown). Since the concentration of DNA plasmid employed was ~3–4 nM, it is clear that the efficiency of DNA cleavage by chlorobithiazoles **3–5** is remarkable! No DNA cleavage was observed for any of the bithiazoles in the absence of irradiation (Figure 2). Likewise, nonchlorinated bithiazole **2** failed to effect plasmid DNA relaxation with or without irradiation (not shown). In contrast with Fe·bleomycin,[15] DNA cleavage by chlorobithiazole **4** was shown not to require O_2, excluding the possible involvement of singlet oxygen in the observed DNA strand scission.[16]

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[1] For a general review, see: *Bioorganic Photochemistry, Photochemistry and the Nucleic Acids*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1.

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[8] Nielsen, P. E.; Jeppesen, C.; Buchardt, O. *FEBS Lett.* **1988**, *235*, 122–124.

[9] (a) Decuyper, J.; Piette, J.; Lopez, M.; Merville, M.-P.; van de Vorst, A. *Biochem. Pharmacol.* **1984**, *33*, 4025–4031. (b) Chignell, C. F.; Motten, A. G.; Buettner, G. R. *Environ. Health Perspect.* **1985**, *64*, 103–110. (c) Motten, A. G.; Buettner, G. R.; Chignell, C. F. *Photochem. Photobiol.* **1985**, *42*, 9–15. (d) Ciulla, T. A.; Epling, G. A.; Kochevar, I. E. *Photochem. Photobiol.* **1986**, *43*, 607–613. Photoinduced DNA cleavage by other halogenated heterocycles has also been reported. See: Matsumoto, T.; Utsumi, Y.; Sakai, Y.; Toyooka, K.; Shibuya, M. *Heterocycles* **1992**, *34*, 1697–1702.

[10] (a) Zee-Cheng, K. Y.; Cheng, C. C. *J. Heterocycl. Chem.* **1970**, *7*, 1439–1440. (b) McGowan, D. A.; Jordis, U.; Minster, D. K.; Hecht, S. M. *J. Am. Chem. Soc.* **1977**, *99*, 8078–8079.

[11] Chlorination with LDA/Cl_3CCCl_3 in dry THF[12] afforded the precursors to bithiazoles **4** and **5** in 25% and 11% yields, respectively. Radical halogenation[13] afforded the precursor to bithiazole **3** in 74% yield. Full experimental details are provided as supplementary material.

[12] (a) Dondoni, A.; Dall'Occo, T.; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P. *J. Chem. Soc., Chem. Commun.* **1984**, 258–260. (b) Dondoni, A.; Mastellari, A. R.; Medici, A.; Negrini, E.; Pedrini, P. *Synthesis* **1986**, 757–760. (c) Gronowitz, S.; Hörnfeldt, A.-B.; Pettersson, K. *Synth. Commun.* **1973**, *3*, 213–218.

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[16] Irradiation of DNA with 100 or 50 nM **4** under argon (under conditions comparable to those described in the caption below Figure 1) produced slightly more cleavage than in the presence of O_2. The reaction conditions were shown to be anaerobic by the use of Fe^{II}·BLM control reactions.

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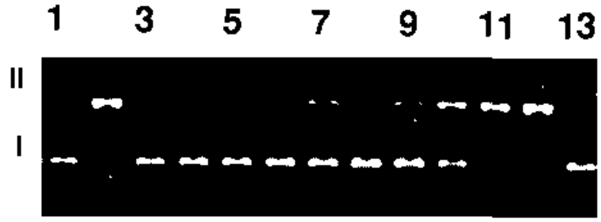
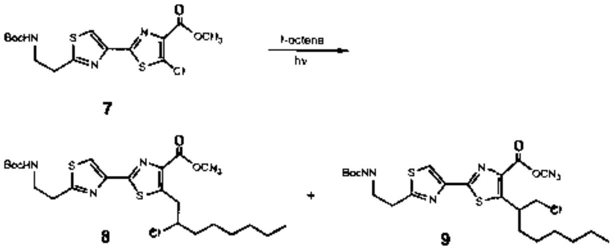


Figure 2. Cleavage of supercoiled plasmid pBR322 DNA by chlorobithiazole 3 and bromobithiazole 6. Reactions were carried out for 15 min as described in the caption below Figure 1. Lane 1: DNA alone. Lane 2: 10 μM Fe^{2+} + 0.19% H_2O_2. Lane 3: irradiated DNA. Lanes 4–7: 20, 50, 100, and 200 nM bromobithiazole **6**, respectively, that were irradiated. Lane 8: 200 nM bromobithiazole 6 maintained in the dark. Lanes 9–12: 20, 50, 100, and 200 nM chlorobithiazole 3, respectively, that were irradiated. Lane 13, 200 nM chlorobithiazole 3 maintained in the dark.

Scheme I. Photoinduced Reaction of Bithiazole **7** with 1-Octene



Bithiazole derivative **2** has been shown to bind efficiently to DNA,[14b] a property that is undoubtedly also shared by derivatives **3–6**. In the belief that DNA cleavage must be mediated by some species formed upon irradiation of the chlorobithiazoles, chlorobithiazole derivative **7** was dissolved in 1-octene under an argon atmosphere and irradiated for 1 h at 30 °C (Scheme I).[17] Isomeric octene adducts **8** and **9** were obtained in comparable amounts (overall yield 70%), consistent with a mechanism involving initial homolysis of the bithiazole C–Cl bond to produce Cl and bithiazole radicals.[18] Because either chlorine or aryl radicals could in principle produce the observed DNA damage,[19] the mechanism of plasmid relaxation by the chlorobithiazoles was studied further. Supercoiled plasmid DNA was irradiated in the presence of chlorobithiazole **3** and bromobithiazole **6** at each of four concentrations. As shown in Figure 2, the irradiated chlorobithiazole produced much more DNA strand scission than the bromobithiazole. On the assumption that **3** and **6** both undergo carbon–halogen bond homolysis upon irradiation,[20] this result suggests that Cl• is the primary mediator of DNA scission by **3**. Analysis of the DNA cleavage products of both 3'- and 5'-^{32}P end labeled DNA duplexes by polyacrylamide gel electrophoresis indicated the presence of bands that comigrated with Maxam–Gilbert sequencing bands. These types of products have been observed for other radical-generating species that mediate DNA strand scission via initial abstraction of H• from DNA sugars,[15,19,22] suggesting that the chlorobithiazoles may also function in this fashion.[23]

These findings establish a potent, mechanistically novel method for DNA photocleavage that can be utilized as part of the ongoing development of photonucleases and photofootprinting agents. Conceivably, chlorobithiazoles may also facilitate an analysis of the mode of binding of the bithiazole moiety of BLM to DNA.

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Supplementary Material Available: Experimental details for the synthesis of chlorinated bithiazoles and the photolysis of chlorinated bithiazole in 1-octene (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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[17] Organic halides are known to add to olefins in a fashion typical of radical species. See: (a) Poutsma, M. L. In *Methods in Free-Radical Chemistry*; Huyser, E. S., Ed.; Academic Press: New York, 1969; Vol. 1, pp 163–186. (b) Thaler, W. A. In *Methods in Free-Radical Chemistry*; Huyser, E. S., Ed.; Academic Press: New York, 1969; Vol. 2, pp 214–218.

[18] No reaction occurred in the absence of light, even at reflux over a period of 12 h. Irradiation of bithiazole **4** in the presence of calf thymus DNA resulted in dechlorination of **4** to afford apparently stoichiometric conversion to bithiazole **2** as judged by HPLC analysis. Dehalogenation occurred on the same time scale as the relaxation of supercoiled plasmid DNA by this species (Figure 1).

[19] For examples of aryl radical mediated DNA cleavage, see: Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191–198 and references therein.

[20] In fact irradiation of the bromobithiazole derivative analogous to **7** in the presence of 1-octene also resulted in olefin addition in good yield. Nonetheless, we cannot exclude the possibility that the bromobithiazole reacted with the olefin via an ionic mechanism. See, e.g.: Kropp, P. J. *Acc. Chem. Res.* **1984**, *17*, 131–137.

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[22] Hertzberg, R. P.; Dervan, P. B. *Biochemistry* **1984**, *23*, 3934–3945.

[23] As also found using plasmid DNA as a substrate, no DNA cleavage was observed following irradiation unless chlorobithiazole was present during the irradiation.