N-Hydroxy-4-(4-chlorophenyl)thiazole-2(3*H*)-thione as a Photochemical Hydroxyl-radical Source: Photochemistry and Oxidative Damage of DNA (Strand Breaks) and 2′-Deoxyguanosine (8-oxodG Formation)¶

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N-Hydroxy-4-(4-chlorophenyl)thiazole-2(3*H*)-thione as a Photochemical Hydroxyl-radical Source: Photochemistry and Oxidative Damage of DNA (Strand Breaks) and 2′-Deoxyguanosine (8-oxodG Formation)¶

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ABSTRACT

On irradiation of *N*-hydroxythiazole-2(3*H*)-thione 3 at 300 nm, the photoproducts disulfide 4, bisthiazole 5 and thiazole 6 are formed. During this photolysis, hydroxyl radicals are released, which have been detected by spin trapping with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), coupled with electron paramagnetic resonance spectroscopy. In the presence of supercoiled pBR322 DNA, irradiation of thiazolethione 3 induces strand breaks through the photogenerated hydroxyl-radicals, as confirmed by control experiment with the hydroxyl-radical scavenger isopropanol. Singlet oxygen appears not to be involved, as attested by the lack of a D_2O isotope effect. During the photoreaction of thiazolethione 3 in the presence of 2′-deoxyguanosine (dG), the latter is photooxidized (*ca* 10% conversion after 2 h of irradiation) to the 7,8-dihydro-8-oxo-2′-deoxyguanosine as the main oxidation product. The dG conversion levels off after complete consumption of thiazolethione 3 and is suppressed by the addition of the hydroxyl-radical scavenger 2,6-di-*tert*-butylcresol or DMPO. Since the photoproducts 4–6 are ineffective as sensitizers for the photooxidation of dG and DNA, the hydroxyl radicals released in the photolysis of thiazolethione 3 are the oxidizing species of DNA and dG. These results suggest that the thiazolethione 3 may serve as a novel and effective photochemical hydroxyl-radical source for photobiological studies.

INTRODUCTION

The elucidation of the molecular mechanism of the DNA damage by reactive oxygen species is important to understand the complex cellular events such as carcinogenesis and mutagenesis through oxidative stress (1). Oxyl radicals (hydroxyl or alkoxyl) have been known to damage DNA through base modification and strand breaks (2). Efficient oxyl-radical sources, which generate selectively oxyl radicals under mild conditions, *e.g.* in aqueous media at pH 7 on exposure to UVA light (photo-Fenton reagents [3,4]), are needed to assess radical-induced oxidative damage of biological materials. Some photochemical sources of oxyl radicals, which display DNA oxidizing activity (strand breaks and base modification under relatively mild conditions), have been reported during the last decade (3–13).

N-hydroxy- and *N*-alkoxypyridinethiones **1** (Chart 1) have served as convenient oxyl radical sources to assess oxidative damage (6,7); indeed, the molecular mechanism of their photooxidative DNA damage has been studied thoroughly (14–17). Although pyridinethiones **1** are readily available and serve as efficient oxyl-radical sources, their DNA damage is complicated: not only do the photoreleased oxyl radicals cause strand breaks and base modifications, but the photoproducts of the pyridinethiones display significant photosensitizing DNA damage (14). Recently, *N*-hydroxy-2(1*H*)-pyridone **2** has been reported as a more selective photochemical hydroxyl-radical source for oxidative DNA modification (12,13); however, its efficacy for DNA photooxidation was low (13). Therefore, it is highly desirable to develop a selective and effective photochemical source of oxyl radicals for the elucidation of the DNA damage caused by oxyl radicals, but without the undesirable photosensitizing activity by its photoproducts.

N-hydroxy- or *N*-alkoxythiazole-2(3*H*)-thione derivatives are well known to generate oxyl radicals for synthetic purposes (18–22). In this context, it has recently been reported that the modified thiazolethione chromophore **3** serves as an improved and clean photochemical precursor for oxyl radical (21,22). These facts encouraged us to examine the DNA-photooxidizing activity of the thiazolethione **3**; herein we report the results of this investigation.

MATERIALS AND METHODS

Materials. Thiazolethione **3** was prepared by the method reported previously (20,22) and recrystallized from isopropanol before use. Supercoiled pBR322 DNA was purchased from Pharmasia Biotech Europe GmbH (Freiburg, Germany) and 5,5-dimethyl-1-pyrorrine *N*-oxide (DMPO)† was obtaiend from Fulka Chemie AG (Buchs, Swit-

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†*Abbreviations:* DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; dG, 2′-deoxyguanosine; EPR, electron paramagnetic resonance; GRP, guanidine-releasing products; 4-HO-8-oxodG, 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine; HPLC, high-performance liquid

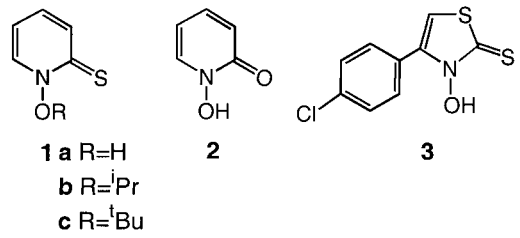
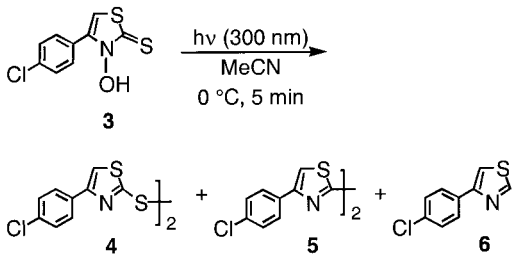


Chart 1.



Scheme 1.

zerland). Ethidium bromide for biochemical use and boric acid were obtained from Merck KGaA (Freiburg, Germany). Tris base was purchased from Sigma–Aldrich Chemie GmbH (Deisenhofen, Germany) and agarose was purchased from Serva Feinbiochemica GmbH (Heiderberg, Germany). Preparative thin-layer chromatography (TLC) separations were performed on Merck PLC plates (20 × 20 cm^2) coated with silica gel 60.

Radiation sources. The irradiation experiments were carried out in a Rayonet photoreactor (Southern New England Ultraviolet Company, Brandford, CT), equipped with 16 RPR-3000 Å (300 nm, 21 W) or RPR-3500 Å (350 nm, 24 W) lamps.

*Photochemistry of thiazolethione **3***. Thiazolethione **3** (100 mg, 0.41 mmol) was dissolved in 100 mL of acetonitrile and the solution was irradiated at 300 nm in a Rayonet photoreactor with 16 RPR-3000 Å lamps (300 nm, 21 W) at 0°C for 10 min. The solvent was evaporated under reduced pressure (20°C, 18 Torr) and the residue was extracted with ethyl acetate (*ca* 3 mL). The extract was concentrated and separated by preparative TLC (silica gel, 5:1 hexane : ethyl acetate) to give disulfone **7** (Scheme 1) (19.0 mg, 40%, R_f 0–0.24) and a mixture of disulfide **4**, bisthiazole **5** and thiazole **6** (R_f 0.22–0.30). Subsequent preparative TLC separation of the latter mixture (silica gel, 1:1 petroleum ether : dichloromethane) gave thiazole **6** (6.0 mg, 18%, R_f 0.080–0.20) and a 3:1 mixture of disulfide **4**: bisthiazole **5** (9.0 mg, **4**: 18%, **5**: 6%, R_f 0.22–0.30). The disulfide **4** was partly separated from the mixture of **4** and **5** by preparative medium-pressure liquid chromatography (MPLC, silica gel, 5:1 dichloromethane : petroleum ether) and directly characterized, while the bisthiazole **5** was characterized in the mixture by ^1H nuclear magnetic resonance (NMR) and mass spectroscopy.

4: Colorless fine prisms, mp 156–158°C (lit. 155°C [24]). ^1H NMR ($CDCl_3$) δ 7.80 (m, 4H), 7.50 (s, 2H), 7.38 (m, 4H); high-resolution mass spectroscopy (HRMS) (EI) *m/e* 451.9107, calcd for $C_{18}H_{10}Cl_2N_2S_4$ 451.9104.

5: ^1H NMR ($CDCl_3$) δ 7.72 (m, 4H), 7.44 (s, 2H), 7.34 (m, 4H); HRMS (EI) *m/e* 387.9660, calcd for $C_{18}H_{10}Cl_2N_2S_2$ 387.9662.

6: Colorless prisms, mp 51–52.5°C (lit. 51–53°C [25]). ^1H NMR ($CDCl_3$) δ 8.89 (d, 1H, J = 2.0 Hz), 7.87 (m, 2H), 7.55 (d, 1H, J = 2.0 Hz), 7.41 (m, 2H); MS (EI) *m/e* 195 (100%) (M^+).

7: Colorless fine needles, mp 227–229°C. ^1H NMR ($CDCl_3$) δ 7.78 (m, 4H), 7.41 (m, 4H), 6.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 107.7, 127.7, 129.1, 130.0, 136.2, 142.6, 161.4; IR (KBr) 1105 (SO_2), 1240 (NO), 1335 (SO_2) cm^{-1}; MS (EI) *m/e* 548 (37%) (M^+). Anal calcd $C_{18}H_{10}Cl_2N_2O_6S_4$ (549.5): C, 39.35; H, 1.83; N, 5.10; S, 23.34. Found: C, 39.26; H, 1.65; N, 4.93; S, 22.48.

*Photoreaction of disulfide **4***. A solution of disulfide **4** (27.8 mg, 0.0633 mmol) in a mixture of dioxane (1 mL) and water (0.3 mL) was irradiated at 350 nm in a Rayonet photoreactor at 20°C for 1 h. The solvent was evaporated under reduced pressure (20°C, 18 Torr) and the residue was chromatographed on silica gel by using a 1:1 mixture of ether : petroleum ether as eluent to give thiazole **6** (5.3 mg, 43%) and 2-mercaptothiazole **8** (2.3 mg, 16%).

8: Pale yellow prisms, mp 207–210°C (lit. 210–212°C [26]). ^1H NMR (acetone-d_6) δ 7.83 (m, 2H), 7.53 (m, 2H), 7.18 (s, 1H); MS (EI) *m/e* 227 (100%) (M^+).

chromatography; HRMS, high-resolution mass spectroscopy; MPLC, medium-pressure liquid chromatograpy; NMR, nuclear magnetic resonance; 8-oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine; R_f, retention factor; TLC, thin-layer chromatography; t_r, retention time; tris base, tris(hydroxymethyl)aminomethane.

Electron paramagnetic resonance studies. A solution of thiazolethione **3** (5 m*M*), and DMPO (90 m*M*) in water and 20% acetonitrile as cosolvent, was irradiated at 300 nm with 16 RPR-3000 Å lamps (300 nm, 21 W) in a Rayonet photoreactor at 0°C for 5 min in a Pyrex vial, and the resulting mixture was analyzed by electron paramagnetic resonance (EPR) spectroscopy (Bruker EPR 420 spectrophotometer).

Modification of pBR322 DNA. The reactions were carried out in Eppendorf tubes with supercoiled pBR322 DNA (1 μg/mL) and thiazolethione **3** (0.509 m*M*) in 5.0 m*M* KH_2PO_4 buffer at pH 7.4 and 10% acetonitrile as cosolvent. The samples (10 μL final volume) were prepared from stock solutions of pBR322 DNA (33.3 μg/mL in 15.7 m*M* KH_2PO_4 buffer, pH 7.4) and thiazolethione **3** (5.07 m*M* in acetonitrile). The samples were irradiated at 350 nm with 12 RPR-3500 Å lamps (350 nm, 24 W) in a Rayonet photoreactor for 5 min at 0°C. The control experiments were carried out in the presence of isopropanol (10 vol%) or D_2O (60 vol%) to assess the involvement of hydroxyl radicals or singlet oxygen in the photolysis, respectively.

Detection of strand breaks. To the irradiated solutions was added 2.5 μL of bromophenol gel-loading solution. An 8 μL aliquot of the resulting mixture was transferred onto a 1% agarose gel, stained with 0.5 μg/mL ethidium bromide. Electrophoresis was carried out in Tris buffer (18.0 m*M* Tris base, 18.0 m*M* boric acid and 10.0 m*M* ethylenediaminetetraacetic acid at pH 8.0) at 78 V for *ca* 2 h in a Pharmacia horizontal apparatus (GNA 100), equipped with a power supply (GPS 200/400). The fluorescent spots of the DNA were detected by exposure to a UV transilluminator at 254 nm. Photographs of the gels were taken with a Herolab camera E.A.S.Y. 429 K, which was connected to a personal computer, equipped with Herolab E.A.S.Y. software. The ratio of open-circular to the supercoiled DNA was determined from the fluorescence intensities of the spots.

*Photooxidation of 2′-deoxyguanosine (dG) by thiazolethione **3***. Typical procedure: the samples (final volume 200 μL) contained 0.509 m*M* of dG (25 μL of a 4.07 m*M* aqueous solution) and 2.13 m*M* of thiazolethione **3** (30 μL of a 14.2 m*M* solution in acetonitrile) in 5 m*M* phosphate buffer (pH 7.0) and 15% acetonitrile as cosolvent. The solutions were irradiated in Pyrex vials at 350 nm with 16 RPR-3500 Å lamps (350 nm, 24 W) in a Rayonet photoreactor. An 80 μL aliquot of the photolysate was extracted with ethyl acetate (2 × 200 μL) and the aqueous phase was freeze-dried. The residue was redissolved in 80 μL of water and submitted to high-performance liquid chromatography (HPLC) analysis.

HPLC analysis. The HPLC analytical system consisted of a Bischof HPLC pump model 2200 (Bischof GmbH, Leonberg, Germany), equipped with a Rheodyne loop injector, model 7125 (Berkley, CA). For the detection of 4-hydroxy-8-oxo-4,8-dihydro-2′-deoxyguanosine (4-HO-8-oxodG) and the photoconversion of thiazolethione **3**, Waters 994 photodiode array detector (Waters GmbH, Eschborn, Germany) was used. Guanidine-releasing products (GRP) were, after fluorescent derivatization (27), analyzed with Shimadzu RF-551 spectrofluorometric detector (Bischoff GmBH, Leonberg, Germany). In the determination of the dG conversion and 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxodG) formation, a SunChrom SpectraFlow 600 photodiode array detector (SunChrom GmbH, Friedrichsdorf, Germany) was connected in series with an ESA coulochem model 5100A electrochemical detector, equipped with a high-sensitive analytical cell model 5011 (ESA Inc. Bedford, MA). Conversion of thiazolethione **3** was followed by using a 250 × 4.6 (i.d.) mm Eurospher 100-C18 5 μm column (Knauer GmbH, Berlin, Ger-

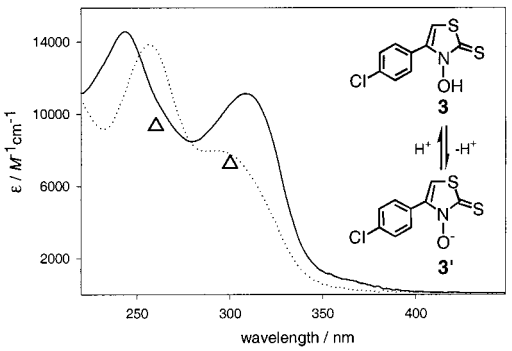
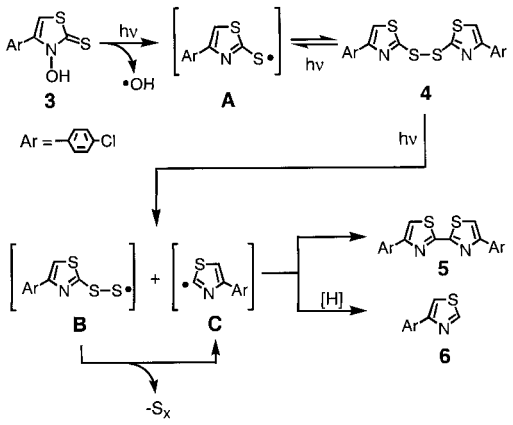


Figure 1. Absorption spectra of thiazolethione **3** in acetonitrile (—) and in 5 m*M* phosphate buffer (pH 7.0), 10% acetonitrile (· · ·); the open triangle (△) denotes the reported absorption maxima of the tetraethylammonium salt of **3** (23).



Scheme 2.

many), with a mixture of methanol and water (95:5) as eluent at a flow rate 1 mL/min, detected at 310 nm (t_R 3 min).

The quantification of dG and 8-oxodG was achieved on a 250 × 4.6 (i.d.) mm Eurospher 100-C18, 7 μm column, by elution with a mixture of 50 m*M* sodium citrate buffer (pH 5.0) and methanol (80:20) at a flow rate of 1 mL/min. For the electrochemical detection of 8-oxodG, oxidation potential was set at +450 mV.

The GRP analysis (27) was performed as follows: a 100 μL aliquot of the photolysate was extracted with ethyl acetate (2 × 200 μL), and the aqueous phase was freeze-dried. The residue was redissolved in 100 μL of water, and to the solution was added 38 μL of 1*N* NaOH and 20 μL of an aqueous solution of 1,2-naphthoquinone-4-sulfonic acid (5 mg/mL). The mixture was kept at 65°C for 10 min in the dark and then acidified with 42 μL of 1*N* HCl. The resulting mixture was separated on a 250 × 46 mm (i.d.) Eurospher 100-C18, 5 μm column with a mixture of methanol and 25 m*M* ammonium formate (20:80) as eluent, at a flow rate of 1 mL/min and detected spectrofluorometrically (λ_{ex} = 355 nm, λ_{em} = 405 nm).

RESULTS AND DISCUSSION

Absorption spectra of thiazolethione 3

The absorption properties of thiazolethione **3** were measured in aqueous buffer and, for comparison, in acetonitrile. As shown in Fig. 1, thiazolethione **3** shows absorption maxima at 244 and 310 nm in acetonitrile, while in 5 m*M* phosphate buffer (10% acetonitrile as cosolvent, pH 7.0) the first absorption band shifts to 300 nm. The absorption characteristics of thiazolethione **3** in the phosphate buffer are similar to those reported for tetraethylammonium salt **3**′-NEt_4 (22). Therefore, in aqueous phosphate buffer (pH 7.0), the conjugate base **3**′ of the thiazolethione **3** dominates in the acid–base equilibrium.

Photochemistry of thiazolethione 3

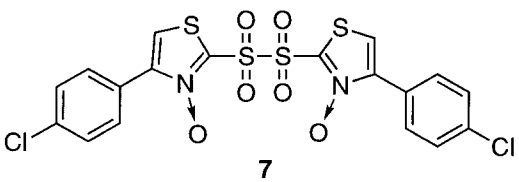
Since it is important to know the photoproducts of the thiazolethione **3** in order to assess their individual photooxidation activity (14), a detailed product study of the thiazolethione **3** photochemistry was conducted. Thiazolthione **3** (100 mg, 0.41 mmol) was irradiated in a Rayonet photoreactor (300 nm) at 0°C for 10 min in 100 mL of acetonitrile. After separation of the photolysate by preparative TLC on silica gel and medium-pressure liquid chromatography (MPLC, silica gel), disulfide **4** and thiazole **6** (18%) were isolated and identified (23,24) (Scheme 1)‡.

Although disulfide **4** was partly separated by MPLC from a mixture of disulfide **4** and bisthiazole **5**, the latter was not separated from the disulfide **4**. Thus, bisthiazole **5** was characterized by ^{1}H NMR and mass spectroscopy, and the yields of disulfide **4** (18%) and bisthiazole **5** (6%) were determined by ^{1}H NMR. When thiazolethione **3** was irradiated at 350 nm, the same products were observed as in the photolysis at 300 nm (data not shown). For the photolysis of thiazolethione **3** in an aqueous medium (3:1 mixture of dioxane : water), the disulfide **4** (6%), bisthiazole **5** (1%) and thiazole **6** (18%) were observed as photoproducts.

A mechanism is proposed for the formation of the photoproducts derived from the thiazolethione **3** in Scheme 2. On irradiation, thiazolethione **3** releases hydroxyl radicals (*vide infra*) to give the thiyl radical **A**, and subsequent dimerization of the thiyl radical **A** affords the disulfide **4** (22,28). Photodissociation of the C–S bond (28,29) in the disulfide **4** may give the radicals **B** and **C**. Such a C–S bond cleavage has been suggested in the photolysis of bis(2-benzothiazolyl) disulfide (28). C–S bond fission may compete efficiently with the expected S–S bond homolysis in disulfide **4**, which produces the carbon-centered radical **C**; the latter dimerizes to the bisthiazole **5** or abstracts a hydrogen atom to afford thiazole **6**. The disulfanyl radical **B** may also generate the thiazolyl radical **C** by sulfur extrusion.

When the disulfide **4** was irradiated at 350 nm in a 3:1

‡Disulfone **7** was isolated after thin-layer chromatographic (TLC) (silica gel) separation of the crude photolysate and characterized by spectroscopic data and elemental analysis. However, the disulfone **7** was not detected in the photolysate of thiazolethione **3** before work-up and, thus, it is not a direct photoproduct. A control experiment showed that when the thiazolethione **3** was placed onto a preparative TLC plate (silica gel) and eluted with 2:5 ethyl acetate : hexane without irradiation and even in the dark, the disulfone **7** was observed.



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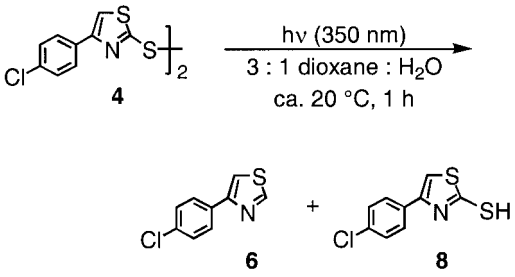

Scheme 3.

mixture of dioxane : water, the thiazole **6** (43%) and 2-mercaptothiazole **8** (16%) were obtained (Scheme 3). These results support the proposed mechanism in Scheme 2, in that the observed photoproducts of thiazolethione **3** are formed through the cleavage of the S–S and C–S bonds of its primary photoproduct, namely the disulfide **4**.

Detection of hydroxyl radicals on photolysis of thiazolethione 3

In order to assess the propensity of thiazolethione **3** to generate hydroxyl radical on photolysis, a spin-trapping experiment with DMPO was conducted. When a solution of **3** (1 m*M*) in a 80:20 mixture of water and acetonitrile was irradiated at 300 nm in the presence of DMPO (89.5 m*M*), EPR spectroscopy revealed a characteristic 1:2:2:1 pattern (g = 2.0061, $\alpha_N = \alpha_H$ = 14.6 G) for the DMPO adduct of hydroxyl radical (30) (Fig. 2). On irradiation of thiazolethione **3** at 350 nm, the same hydroxyl-radical spin adduct of DMPO was detected as in the case of 300 nm. This confirms unambiguously that thiazolethione **3** generates hydroxyl radicals on photoexcitation in aqueous medium; however, the expected sulfur-centered radical **A** (*cf* Scheme 2) was not detected as a spin adduct of DMPO. It has been reported (31) that the spin adduct of DMPO with 2-pyridylthiyl radical, formed in the photolysis of *N*-hydroxypyridinethione **1a**, is too short-lived for EPR-spectral detection. By analogy, we suspect that the spin adduct of DMPO with the thiyl radical **A** also does not persist to be observed under the present conditions.

Strand-break formation in pBR322 DNA on photolysis of thiazolethione 3

The photolysis of supercoiled pBR322 DNA (10 μg/mL in 5 m*M* KH_2PO_4 buffer at pH 7.4, 10% acetonitrile as cosolvent) was conducted in the presence of thiazolethione **3**. As shown in Fig. 3 (lane 1), this photolysis produced 30(±3)% of the open-circular form of DNA. The blank (lane 4), *i.e.* the absence of thiazolethione **3**, was ineffective (for convenience of comparison, the blank value [30% open-circular form] was set to zero). Addition of isopropanol, an efficient hydroxyl-radical scavenger (32), inhibited efficiently strand-break formation (3[±2]%, Fig. 3 [lane 2]). To assess whether DNA strand breaks are induced by sensitized formation of singlet oxygen, generated through photosensitization by thiazolethione **3** or its photoproducts, the photolysis was carried out in D_2O, in which the lifetime of singlet oxygen is about 10 times longer than in H_2O (33). As shown in Fig. 3 (lane 3), D_2O exhibited no significant effect in the strand-break

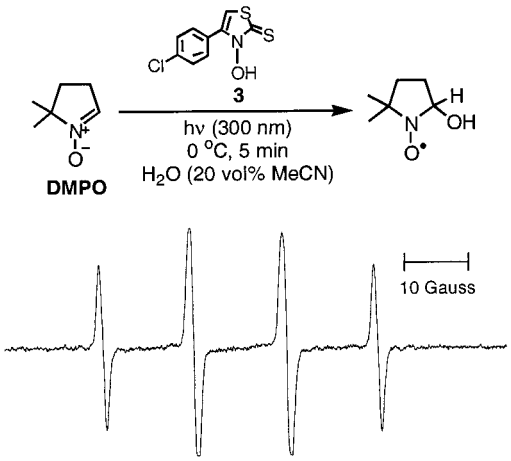

Figure 2. EPR spectrum of the DMPO (89.5 m*M*) spin-trapping adduct with the hydroxyl radical, formed in the photolysis of thiazolethione **3** (1 m*M*, irradiated at 300 nm) in H_2O and 20 vol% acetonitrile as cosolvent.

formation by thiazolethione **3**. As the photolysate of thiazolethione **3** (preirradiated for 10 min) induced insignificant (<8% open-circular form) strand breaks on irradiation (data not shown), the photoproducts of thiazolethione **3** have only a minor effect on the induction of strand breaks. Furthermore, the expected thiyl radicals **A** (Scheme 2) are not responsible for the strand-break formation, since it has been reported that sulfur-centered radicals do not cause significant DNA damage (34). Therefore, it is concluded that the strand breaks have resulted from the hydroxyl radicals, which have been generated in the photolysis of thiazolethione **3**.

Oxidation of dG in the photolysis of thiazolethione 3

In the photooxidation of dG (Chart 2) by thiazolethione **3**, moderate conversion of dG (up to 10% after 1 h of irradiation at 350 nm in phosphate buffer [pH 7.0] and 15% acetonitrile as cosolvent) was observed when a four-fold excess of thiazolethione **3** *versus* dG was used. In this photoreaction, 8-oxodG was obtained as the major oxidation product; 4-HO-8-oxodG, the typical Type-II product (27), was not observed. Only trace amounts of oxazolone, the GRP, a Type-I product (27), was detected. The product distribution

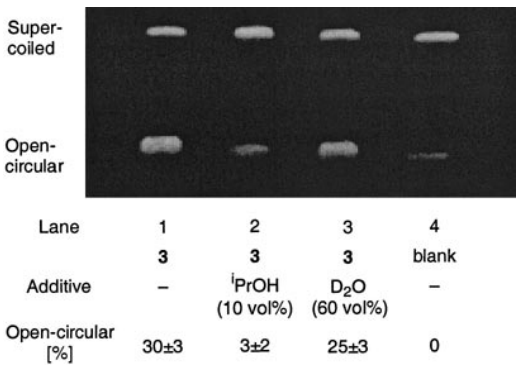

Figure 3. Gel-electrophoretic detection of photoinduced strand breaks with pBR322 DNA (10 μg/mL) in KH_2PO_4 buffer (5 m*M*, pH 7.4) and 10 vol% acetonitrile as cosolvent, irradiated at 350 nm and 0°C in the presence of thiazolethione **3**.

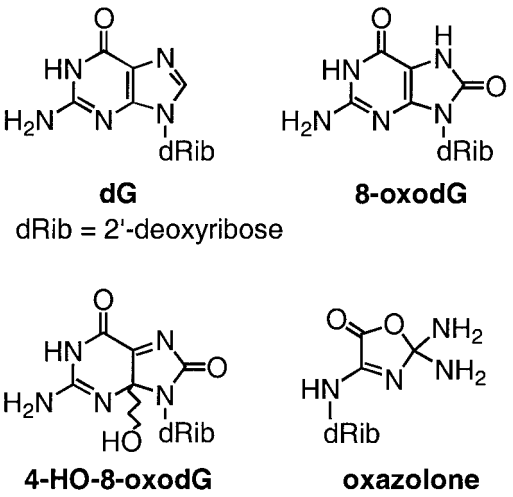

Chart 2.

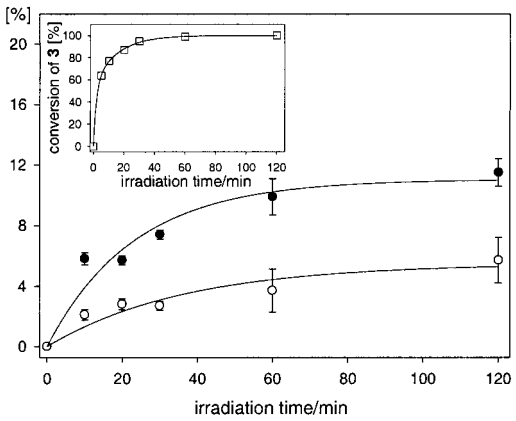

Figure 4. Conversion (%) of dG (●) and yield (%) of 8-oxodG (○) in the photooxidation of dG (0.509 m*M*) by thiazolethione **3** (2.13 m*M*), irradiated at 350 nm in phosphate buffer (pH 7.0) and 15% acetonitrile as cosolvent; the error bars represent at least two independent experiments; the inset displays conversion of thiazolethione **3** (2.13 m*M*) during the irradiation at 350 nm in phosphate buffer (pH 7.0) and 15% acetonitrile as cosolvent.

suggests that neither the oxidation by singlet oxygen nor an electron-transfer process is important in the photooxidation of dG.

Figure 4 shows the time profiles for the dG conversion and the yield of 8-oxodG in the photolysis of thiazolethione **3**, as well as the time dependence of the photodegradation of thiazolethione **3** (Fig. 4, inset) for comparison. The conversion of dG starts immediately (no induction period) and levels off after 1 h of irradiation. The yield of 8-oxodG, given relative to consumed dG, shows the same time dependence as the dG conversion; as much as 5% (based on consumed dG) was obtained after exposure for 2 h.

Both time profiles, the one for the dG conversion and the one for the 8-oxodG formation, run almost parallel to the profile for the photoconsumption of thiazolethione **3** (see Fig. 4, inset). Thus, after about 30 min, essentially all thiazolethione **3** is photolyzed, while beyond this time the conversion of dG and the formation of 8-oxodG have leveled off. This correspondence suggests that the photoproducts of the thiazolethione **3** do not sensitize the photooxidation of dG. To confirm this unequivocally, the authentic photoproducts of the thiazolethione **3** were tested for their efficacy of dG photooxidation. When dG was irradiated (300 nm) with the individual photoproducts (**4** [0.05 m*M*], 3:1 mixture of **4** and **5** [**4**: 0.038 m*M*, **5**: 0.013 m*M*] or **6** [0.05 m*M*]) in phosphate buffer (pH 7.0) and 10% acetonitrile as cosolvent, insignificant dG consumption and only a trace amount of 8-oxodG were detected (0.1% for **4**, 0.2% for **4** and **5**, and none for **6**, based on the initial amount of dG). These results confirm unequivocally that the photoproducts of thiazolethione **3** are all ineffective in sensitizing the photooxidation of dG. That sulfur-centered radicals are not involved in the dG oxidation is substantiated by the fact that disulfide **4**, which generates thiyl radicals **A** on photoexcitation (Schemes 2 and 3), does not cause any significant dG conversion. Consequently, the photooxidation of dG is caused by the hydroxyl radicals released in the photolysis of thiazolethione **3**.

To substantiate the involvement of hydroxyl radicals in the dG photooxidation by thiazolethione **3**, inhibition experiments with the established radical scavenger 2,6-di-*tert*-butylcresol (35) and with the efficient radical trap DMPO (30) were conducted. The conversion of dG was suppressed moderately in the presence of 5 m*M* of 2,6-di-*tert*-butylcresol after 30 min of irradiation (2.7%, *cf* 4.2% in the absence of any additive), while an excess amount of DMPO (895 m*M*) inhibited, essentially completely, the dG conversion (<0.4%) and 8-oxodG formation (0%). Consequently, these scavenging data corroborate that hydroxyl radicals are also the main active species in the photooxidation of dG by thiazolethione **3**.

CONCLUSIONS

The photooxidative damage of DNA (strand-break formation) and dG (8-oxodG formation) by thiazolethione **3**, examined herein for the first time, is caused by the hydroxyl radicals released upon irradiation. Spin-trapping and radical-scavenging experiments confirm the generation of the hydroxyl radicals and their involvement in the photooxidation of DNA and dG. The photoproducts of thiazolethione **3** are ineffective in causing strand breaks and base oxidation, as attested by control experiments.

Although the *N*-hydroxypyridinethione **1a** has been established as effective and convenient photochemical hydroxyl-radical source (6,7), its photoproducts significantly sensitize the photooxidation of DNA (14). Evidently, thiazolethione **3** may serve as an advantageous alternative to *N*-hydroxypyridinethione **1a** for the photobiological studies, since it generates hydroxyl radicals more selectively on photolysis, without the complications of photosensitization. Also, thiazolethione **3** is more advantageous than pyridone **2** for photobiological studies because the former absorbs UVA light (λ = 350 nm) sufficiently to be photoreactive at this wavelength, while the latter essentially does not absorb light at $\lambda > 330$ nm (12). Indeed, the thiazolethione **3** is about twofold more efficient in dG photooxidation than pyridone **2**, since 4 equiv. of thiazolethione **3** afforded 0.6% 8-oxodG (based on starting amount of dG), while 10 equiv. of pyridone **2** gave maximally 0.8% of 8-oxodG (13).

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