JOC Article

Photochemistry of *N*-Isopropoxy-Substituted 2(1*H*)-Pyridone and 4-*p*-Tolylthiazole-2(3*H*)-thione: Alkoxyl-Radical Release (Spin-Trapping, EPR, and Transient Spectroscopy) and Its Significance in the Photooxidative Induction of DNA Strand Breaks

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UVA-irradiation of the photo-Fenton reagents *N*-isopropoxypyridone **2b** and *N*-isopropoxythiazole-2(3*H*)-thione **3b** releases radicals which induce strand breaks. Transient spectroscopy establishes N−O bond scission [Φ_{N-O} = (75 ± 8)% for **2b** and (65 ± 7)% **3b**] as the dominating primary photochemical process to afford the DNA-damaging radicals. Product studies and laser-flash experiments reveal that the thiazolethione **3b** leads primarily to the disulfide **5**, from which through C−S bond breakage, the bithiazyl **6**, the thiazole **7**, and the isothiocyanate **8** are derived. Upon irradiation of pyridone **2b** (300 nm) in aqueous media, a mixture of isopropoxyl and 2-hydroxyprop-2-yl radicals is formed, as confirmed by trapping with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and EPR spectroscopy. In contrast, the photolysis of the thiazolethione **3b** (350 nm) affords exclusively the DMPO adducts of the isopropoxyl radicals. Control experiments disclose that the thiazolethione-derived photoproduct disulfide **5**, or the intermediary thiyl radicals **B**, scavenge the carbon-centered 2-hydroxyprop-2-yl radicals, which are generated from the isopropoxyl radicals by hydrogen shift. With supercoiled pBR 322 DNA in a 60:40 mixture of H_2O−MeCN, the pyridone **2b** and the thiazolethione **3b** display moderate strand-break activity (17% open-circular DNA for **2b** and 12% for **3b**). In pure water, however, the pyridone **2b** photoinduces substantially more DNA cleavage (32% open-circular DNA), which is attributed to the peroxyl radicals generated from the 2-hydroxyprop-2-yl radicals by oxygen trapping. The lower strand-break activity of the thiazolethione **3b** derives presumably from isopropoxyl radicals, because only these are detected in the photolysis of this photo-Fenton reagent.

Introduction

In the photooxidative damage of biomolecules, the reactive oxygen species, hydroxyl, alkoxyl, and peroxyl radicals as well as singlet oxygen, have been held responsible for the chemical transformations.[1] In particular, oxidative modifications of cellular DNA by such potent oxidants are implicated in aging,[2,3] mutagenesis, and carcinogenesis[4] and have been classified under the general phenomenon of *oxidative stress*.[5] In view of the steady increase of skin cancer incidences during the past decades as a consequence of exposure to the UV radiation in sunlight, it is essential to elucidate the mechanism of these biologically relevant photooxidative events to develop effective methods for prevention. For such studies, the reactive oxygen species need to be generated selectively under as mild conditions as possible to elucidate their chemical behavior and interaction with biomolecules.

In the case of hydroxyl and alkoxyl radicals, the Fenton reaction has been used for their generation. This reaction plays an important role in cellular systems, since lipid hydroperoxides serve as precursors for lipid alkoxyl radicals, which have been shown to induce strand breaks in DNA.[6] However, the Fenton reaction, despite its biological relevance, is not a selective route to oxyl radicals, since alternative redox processes with biomolecules may compete.[7] More recently, photo-Fenton reagents have been developed, which release oxyl radicals

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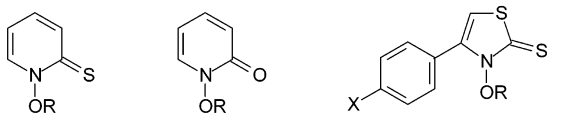
on irradiation with UVA or visible light.[8−12] As a model system for alkoxyl radicals, the *tert*-butoxyl radical has been chosen, for which DNA intercalating[13] and DNA binding[14,15] reagents have been developed. Strand-break formation and base oxidation have been observed as damage of DNA induced by the alkoxyl radicals.

For a photo-Fenton reagent to act selectively, it is indispensable that neither it itself nor its photoproducts display significant oxidative damages on the biological targets through photosensitization (type I and type II photooxidation).[16] Among such photo-Fenton reagents, pyridinethiones **1** have been extensively investigated as photochemical sources of hydroxyl and alkoxyl radicals.[17−19]



1a R = H
1b R = ^{i}Pr

2a R = H
2b R = ^{i}Pr

3a R = H, X = Me
3b R = ^{i}Pr, X = Me
3c R = H, X = Cl

They cause strand breaks and oxidative base modification in DNA upon photolysis;[18] however, the DNA oxidation by the pyridinethiones **1** is complicated by the fact that their photoproducts may possess significant damaging activity by photosensitization.[19,20]

Recently, we have reported that *N*-hydroxypyridone **2a**[21] and *N*-hydroxythiazolthione **3c**[22] serve as selective photochemical hydroxyl-radical sources for photobiological studies: On UVA irradiation, they induce strand breaks and base oxidation in DNA by the released hydroxyl radicals, while their photoproducts display negligible oxidative activity. These advantages motivated us to utilize the chromophores in the *N*-isopropoxy derivatives of the pyridone **2b** and thiazolethione **3b** to generate photochemically isopropoxyl radicals. Of particular interest was to assess the efficacy and selectivity of these alkoxyl radical sources for the oxidative damage of DNA.

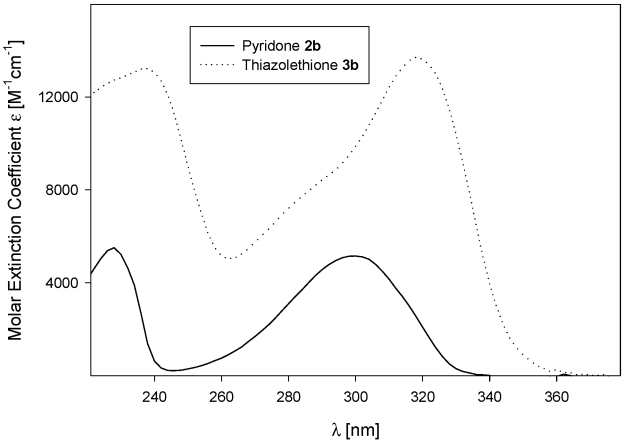
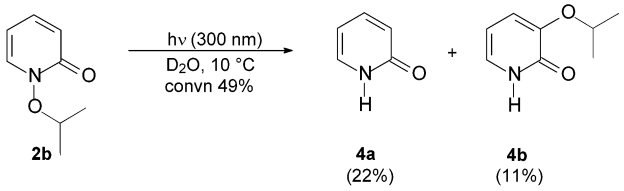


FIGURE 1. Electronic absorption spectra of pyridone **2b** and thiazolethione **3b** in a H_2O−MeCN mixture (60:40).

SCHEME 1. Photoproducts of the Pyridone 2b



In the present work, we report the details of the photochemistry of the *N*-isopropoxy derivatives of pyridone **2b** and the thiazolethione **3b** in terms of product studies. The intermediary radicals have been detected by means of laser-flash spectroscopy and EPR-spectral analysis of the spin-trapped adducts derived from 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). The efficiency of photoinduced strand-break formation in DNA has been determined for these alkoxyl-radical sources.

Results

The pyridone **2b**[23,24] and the thiazolethione **3b**[25] were synthesized according to the literature procedures. Pyridone **2b** is soluble in water, whereas the thiazolethione **3b** requires MeCN as cosolvent (40%) for solubilization in aqueous media. To enable direct comparison, both substrates were investigated in a H_2O−MeCN mixture (60:40).

The absorption spectra are shown in Figure 1. The pyridone **2b** possesses an absorption maximum at λ_{max} 300 nm (log $\epsilon = 3.73$), whereas for the thiazolethione **3b** the λ_{max} appears at 318 nm (log $\epsilon = 4.15$), with tailing to 360 nm. These absorption characteristics indicate that the thiazolethione **3b** absorbs UVA radiation more efficiently and out to a longer wavelength than the pyridone **2b**.

Photodecomposition of the Photo-Fenton Reagents 2b and 3b. Photoproducts of the Pyridone 2b. To elucidate the photochemical behavior of the pyridone **2b**, a product analysis was essential. The photolysis (300 nm) of the pyridone **2b** (Scheme 1) led to the parent pyridone **4a** (22%) and the isopropoxypyridone **4b** (11%); the rest was made up by undefined, intractable

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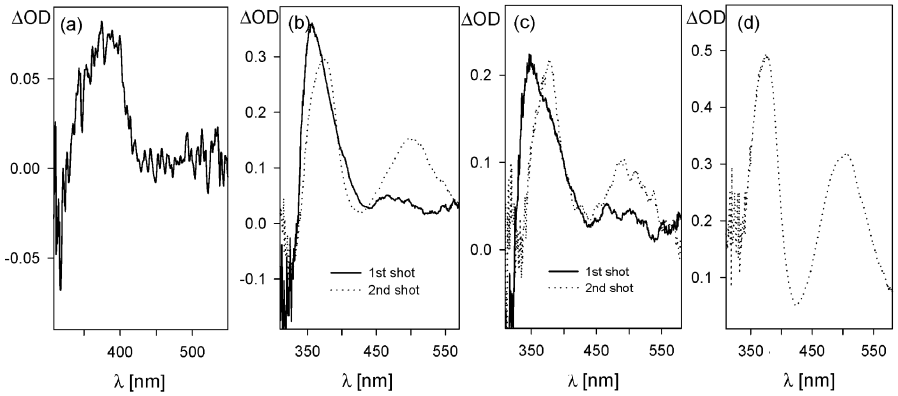
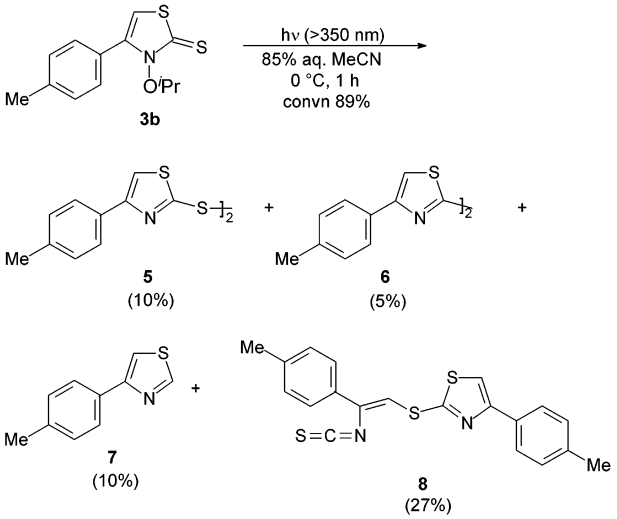


FIGURE 2. Transient spectra observed in the laser-flash photolysis (308 nm) for pyridone **2b** in water (a), and for the thiazolethiones **3b** (b) and **3a** (c) and disulfide **5** (d) in MeCN; for the thiazolethiones **3b** (b) and **3a** (c), the transient spectra display the traces for the first and the second laser shots.

SCHEME 2. Photoproducts of the Thiazolethione 3b

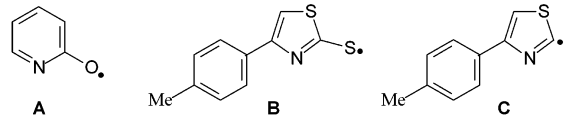


higher-molecular-weight material. The structure of the rearrangement product **4b** was determined by ^{1}H and ^{13}C NMR spectroscopy (especially by HMBC and HMQC methods) and by its mass spectrum.

Photoproducts of the Thiazolethione 3b. When the thiazolethione **3b** was irradiated (>350 nm) in a H_2O−MeCN mixture (15:85), the disulfide **5** (10%), the bithiazyl **6** (5%), and the thiazole **7** (10%) were isolated and characterized (Scheme 2), as previously described for the photolysis of the *N*-hydroxythiazole-2(3*H*)-thione derivative (**3c**).[22] The disulfide **5** and the bithiazyl **6** resisted all attempts of chromatographic separation, and consequently they were characterized directly in their mixture by ^{1}H NMR and mass spectra and are in close analogy to their *p*-chlorophenyl derivatives.[22]

Additionally, the unknown isothiocyanate **8** was formed in the photoreaction of the *N*-isopropoxythiazolethione **3b** as the main photoproduct (27%). With only 52% of the products accounted for, presumably much material was lost on workup due to the hydrolysis of such labile ring-opened products as the isothiocyanate **8**. The presence of the isothiocyanate functionality was confirmed by a strong IR absorption band (2074 cm^{-1}), the full structure was established by ^{1}H and ^{13}C NMR spectra on the basis of HMQC, HMBC, and NOESY techniques. Also a high-resolution mass spectrum supports the assigned structure **8**.

Transient Spectroscopy. The transient spectrum of the laser-flash photolysis (308 nm) of the pyridone **2b** is shown in Figure 2a. It displays a transient UV band with λ_{max} 390 nm, which corresponds to the pyridyloxyl radical **A**, since it matches the reported spectrum for the authentic species.[26]



The flash photolysis of the thiazolethione **3b** afforded a transient absorption with maxima at 355 and 470 nm (Figure 2b, solid curve). The same transient spectrum was observed for the hydroxy derivative **3a** (Figure 2c, solid curve). In view of these facts, the transient spectra (Figures 2b and 2c, solid curves) have been assigned to the thiyl radical **B**, generated through photochemical N−O bond scission. The absorption band at 470 nm was not affected by molecular oxygen, but was diminished by 2-propanol and 1,3-cyclohexadiene (1,3-CHD). Such chemical behavior is characteristic for aromatic thiyl radicals,[20,27,28] which possess absorption maxima at 300−390 and 460−500 nm, as reported for the thiyl radicals derived from thiophenols and thionaphthols.[29]

On further flash photolysis (second laser shot) of the thiazolethione **3b**, a new transient was observed with absorption maxima at 375 and 500 nm (Figure 2b, dotted curve). The same transient was detected on photolysis of the photoproduct of the thiazolethione **3b**, namely the disulfide **5** (Figure 2d, dotted curve). Since both absorption bands decayed faster in the presence of molecular oxygen, this new radical species cannot be sulfur-centered and is presumably the carbon-centered radical **C**, formed by C−S bond breakage in the disulfide **5**. On one hand,

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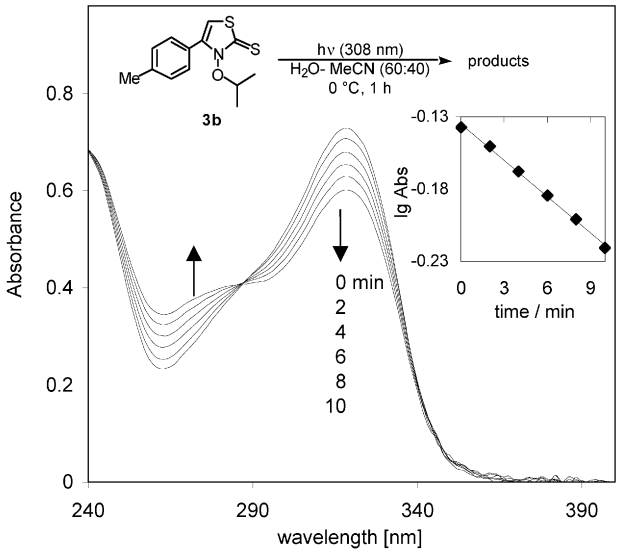

FIGURE 3. Time profile of the UV spectra during the irradiation (308 nm, 20 °C) of the thiazolethione**3b**; the inset displays the decrease of the absorbance at λ_{max} 318 nm.

carbon-centered radicals are efficiently trapped by molecular oxygen;[30] on the other hand, products **6** and **7** (Scheme 2) demand that such a heteroaryl radical intervenes.

Quantum Yields for the Photodecomposition. The efficiencies of the photochemical degradation of pyridone **2b** and thiazolethione **3b** reflect their photoreactivity and the upper-limit efficiency of radical generation from these photo-Fenton reagents. For this purpose, the quantum yields of photodecomposition were determined for the compounds **2b** and **3b** in order to compare their photoreactivity. The pyridone **2b** and the thiazolethione **3b** were irradiated at 308 or 312 nm, potassium ferrioxalate was used as actinometer.[31] For the quantification of the substrate conversion, a detailed examination of their absorption spectra as a function of time was necessary. As shown in Figure 3, the absorbance of the thiazolethione **3b** at λ_{max} 318 nm decreased during irradiation; the change displays a first-order dependence on the irradiation time up to 10 min (Figure 3, inset). Although the photoproducts of the thiazolethione **3b** may absorb light at 318 nm and possibly serve as photosensitizer, the linear logarithmic diminution of the absorbance and the isosbestic point at 290 nm indicate that the absorption at 318 nm is not falsified by photoproduct absorption. Therefore, the time profile reflects reliably the consumption of the starting thiazolethione **3b** during short irradiation times and this was used to quantify its conversion upon irradiation.[32] In contrast, in the case of the pyridone **2b**, the absorption by its photoproducts

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(32) While this clean photochemical behavior may contradict the results of the laser-flash experiments (Figure 2), in which substantial secondary photolysis of the disulfide **5** product has been observed (see Scheme 5), it should be kept in mind that in the laser-flash experiments a much higher photon density applies, such that significantly more disulfide **5** is formed through recombination of the thiyl radical **B**, and hence photocleavage of the latter is much more pronounced than in the steady-state photolysis (Figure 3).

TABLE 1. Quantum Yields for the Photodecomposition (Φ_{dec}[a]) and for the N−O Bond Scission (Φ_{N-O}[b]) of the Pyridone 2b and the Thiazolethione 3b

substrate	solvent	Φ_{dec}	Φ_{N-O}
2b	H_2O−MeCN (60:40)	0.36 ± 0.02	-
2b	H_2O	0.32 ± 0.01	0.75 ± 0.08[c]
3b	H_2O−MeCN (60:40)	0.60 ± 0.05	0.65 ± 0.07[d]

[a] Potassium ferrioxalate actinometer was used to determine the photon fluence of the light sources. The error limits are based on at least two independent runs; the pyridone **2b** was irradiated with a black-light lamp (312 nm), the thiazolethione **3b** with the Xe lamp of a fluorescence photometer (308 nm). [b] Determined by transient spectroscopy through actinometry with the triplet−triplet absorption of benzophenone as reference (see Supporting Information). [c] Calculated with $\epsilon_{\mathbf{A}} = 680\ M^{-1}\ cm^{-1}$ at 390 nm (ref 26). [d] Estimated by assuming that the extinction coefficient of the transient **B** ($\epsilon_{\mathbf{B}}$) is that of the pyridyl-2-thiyl radical [$\Phi_{PyS} = 600\ M^{-1}\ cm^{-1}$ at 460 nm (ref 20)].

significantly overlap with that of the starting pyridone **2b**. Hence, the conversion of pyridone **2b** was followed by HPLC analysis, coupled with UV detection.

The quantum yields for the photodecomposition of pyridone **2b** and thiazolethione **3b** are listed in Table 1. These data disclose that the thiazolethione **3b** (Φ_{dec} = 0.60 ± 0.05) is about twice more photolabile than the pyridone **2b** (Φ_{dec} = 0.36 ± 0.02). For the pyridone **2b**, the change of the solvent from the H_2O−MeCN mixture (60:40) to water did not significantly influence the quantum yield of photodecomposition.

Since the quantum yield of the decomposition is only an upper-limit of the effective radical release, it was of interest to determine the quantum yield of the N−O bond scission Φ_{N-O}, which is equal to $\Phi_O i_{Pr}$, the efficiency of the production of isopropoxyl radicals. By means of transient absorption spectroscopy (see Supporting Information),[26,33] the quantum yield of radical release from the pyridone **2b** in aqueous media was determined to be (75 ± 8)%, for which the reported value for $\epsilon_{\mathbf{A}}$ (680 M^{-1} cm^{-1})[26] at 390 nm in water was used.

For the thiazolethione **3b**, the quantum yield of the N−O bond scission could only be estimated, because the extinction coefficient $\epsilon_{\mathbf{B}}$ of the thiyl radical **B** is not known. With the assumption that its extinction coefficient is the same as for the related pyridyl-2-thiyl radical (ϵ_{PyS} = 600 M^{-1} cm^{-1}),[20] the quantum yield of radical release from the thiazolethione **3b** was estimated to be (65 ± 7)%.

Radical Trapping by DMPO in the Photolysis of the Photo-Fenton Reagents 2b and 3b. Dependence of Radical Trapping on the Radical Source and the Solvent. To assess whether the pyridone **2b** and the thiazolethione **3b** release alkoxyl radicals upon irradiation, spin-trapping experiments with DMPO were performed, followed by EPR-spectral detection (Figure 4).

Indeed, the photolysis in benzene led to isopropoxyl radicals for both photo-Fenton reagents, as manifested by their detection in the form of the spin-trapping adduct with DMPO (Figures 4a and 4b). The *g* values and the hyperfine coupling constants are consistent with the reported ones for the isopropoxyl-radical spin adduct DMPO−O^{i}Pr.[18] In a mixture of H_2O−MeCN (60:40), again only the DMPO−O^{i}Pr was observed for the photolysis of the thiazolethione **3b** (Figure 4c). In contrast, in the case of the pyridone **2b**, also EPR signals assign-

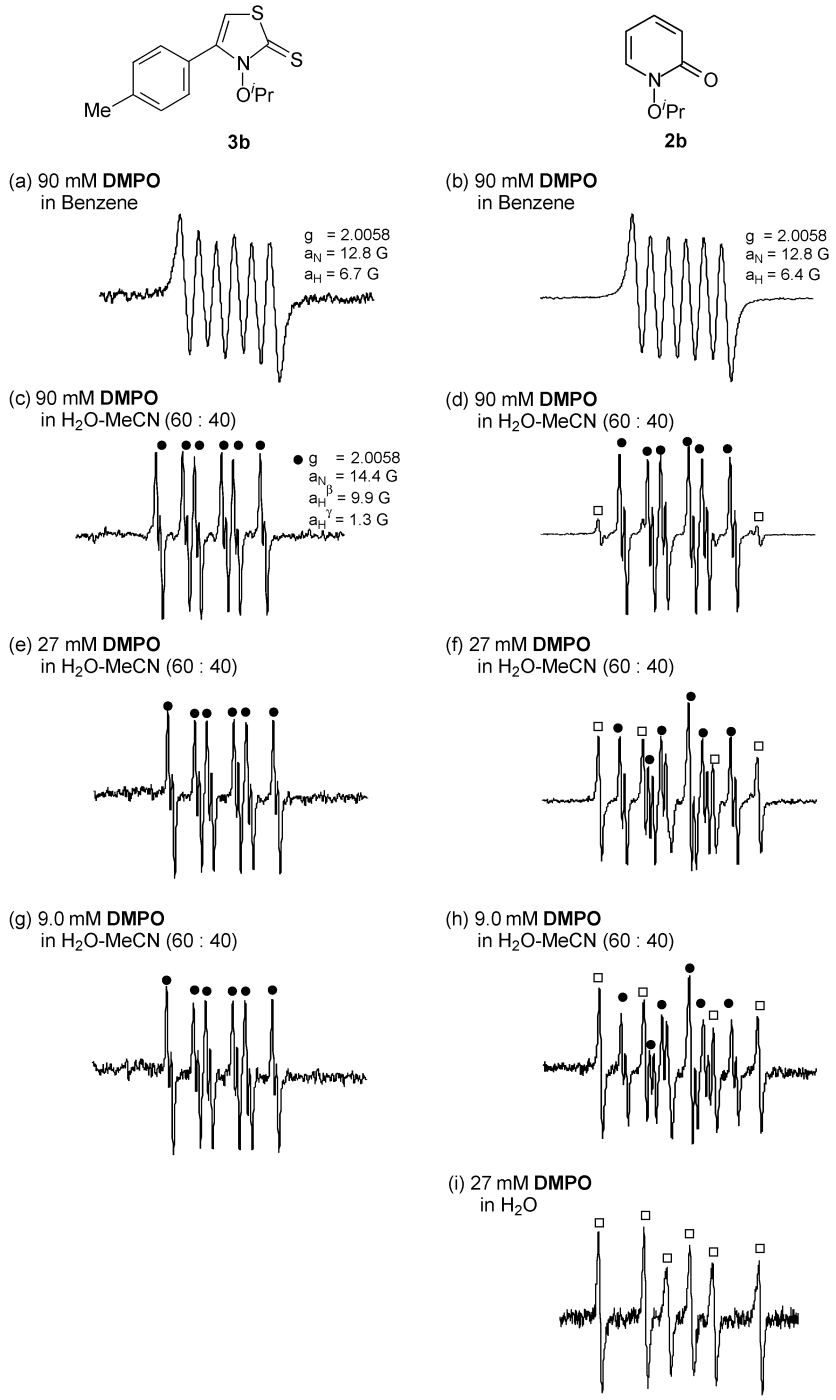


FIGURE 4. EPR spectra recorded immediately after irradiation of the thiazolethione **3b** (350 nm, 10 °C, 5 min) or the pyridone **2b** (300 nm, 10 °C, 5 min) in various solvents and at different DMPO concentrations; DMPO−O^{i}Pr (●), DMPO−CMe_2OH (□).

able to a carbon-centered radical appeared in addition to those of DMPO−O^{i}Pr (Figure 4d). When the pyridone **2b** was irradiated in pure water instead of a H_2O−MeCN mixture (60:40), the carbon-centered radical was detected exclusively (Figure 4i).

Dependence of the Radical Trapping on the DMPO Concentration. To assess how effective the radical trapping by DMPO may compete with the transformation of the isopropoxyl radicals generated in the photoreactions of the substrates **2b** and **3b**, their photolysis was conducted as a function of the DMPO concentration in a 60:40 H_2O−MeCN mixture. In the case of the thiazolethione **3b**, its photolysis afforded exclusively isopropoxyl radicals (Figure 4g) even at 9 mM of DMPO. In contrast, in the photolysis of the pyridone **2b**, a decrease of the DMPO concentration increased substantially the fraction of the carbon-centered radicals in the EPR spectra (Figure 4d, 4f, 4h).

Analysis of the EPR Spectra. Generally, for primary and secondary alkoxyl radicals, hydrogen shift dominates β scission.[34−36] For example, it is known that in aqueous solution, the isopropoxyl radical readily undergoes a formal 1,2-H shift to form the 2-hydroxyprop-2-yl radical

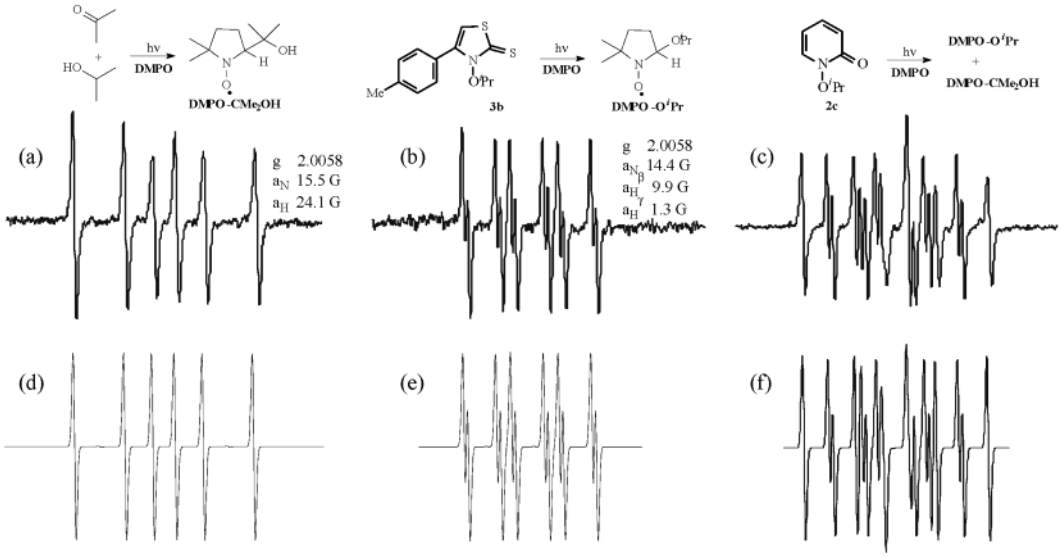
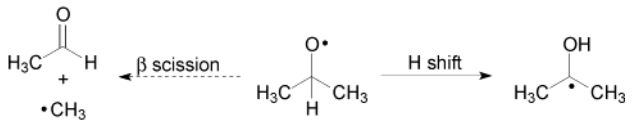


FIGURE 5. Experimental and simulated EPR spectra of DMPO spin adducts with the isopropoxyl and 2-hydroxypropyl-2-yl radicals: (a) DMPO−CMe_2OH detected in the photolysis of acetone (480 mM) in 2-propanol (600 mM) and DMPO (90 mM) in a H_2O−MeCN mixture (60:40); (b) DMPO−O^{i}Pr detected in the photolysis of thiazolethione **3b** (3.00 mM) and DMPO (27 mM); (c) a mixture of DMPO−CMe_2OH and DMPO−O^{i}Pr detected in the photolysis of pyridone **2b** (3.00 mM) and DMPO (27 mM); Figs.-(d) and (e) are the simulated spectra of DMPO−CMe_2OH and DMPO−O^{i}Pr computed with the parameters acquired from the experimental spectra (a and b); (f) the superposition of the simulated spectra (d and e) in the ratio of 1:3.

SCHEME 3. Chemical Transformation of the Isopropoxyl Radical



(Scheme 3).[34] Thus, the observed carbon-centered radical trapped during the photolysis of pyridone **2b** was suspected to be the 2-hydroxyprop-2-yl radical. To confirm the identity of the carbon-centered radical as the corresponding radical adduct of DMPO (DMPO−CMe_2OH), it was independently prepared by the photolysis of acetone in the presence of 2-propanol and DMPO. Figure 5(a) shows the resulting EPR spectrum of the DMPO−CMe_2-OH adduct, which is consistent with the reported one.[37] Both EPR spectra, that of DMPO−CMe_2OH generated authentically (Figure 5a) and that of DMPO−O^{i}Pr observed during the photolysis of the thiazolethione **3b** (Figure 5b), were simulated as shown in Figures 5d and 5e. The superposition of these two simulated spectra in a 1:3 ratio (Figure 5f) reproduced exactly the experimental spectrum measured for the photolysis of pyridone **2b** and DMPO in a 60:40 mixture of H_2O and MeCN (Figure 5c). Thus, the carbon-centered radical trapped by DMPO during the irradiation of pyridone **2b** was unequivocally assigned to the 2-hydroxyprop-2-yl radical.

Radical Scavenging by the Thiazolethione 3b and the Disulfide 9. To clarify why the 2-hydroxyprop-2-yl radicals derived from the isopropoxyl radicals were observed only in the photolysis of the pyridone **2b**, but not in the case of the thiazolethione **3b**, possible radical scavenging by the thiazolethione **3b** and/or its disulfide **5** photoproduct was investigated by means of control experiments. When the pyridone **2b** was irradiated (300 nm) in the presence of the thiazolethione **3b** or the disulfide **9**, exclusively isopropoxyl radicals were detected as the spin-trapping adduct with DMPO (Scheme 4). Therefore, it is concluded that the formation of the

DMPO adduct of the 2-hydroxyprop-2-yl radical was strongly supressed by the photoproducts of the thiazolethione **3b**, namely by the disulfide **5**, as inferred from the disulfide **9** as surrogate.

Strand-Break Formation in DNA. To assess the DNA-photocleaving efficacy of the pyridone **2b** and the thiazolethione **3b**, their photoinduced strand-break formation in supercoiled pBR 322 DNA was examined. As shown in Figure 6, both the pyridone **2b** and the thiazolethione **3b** display moderate DNA cleavage, that is, (17 ± 1)% open-circular DNA for **2b** and (12 ± 1)% for **3b** (for convenience the blank was set to zero). Addition of 2-propanol (10 vol %) suppressed almost completely the strand-break formation by pyridone **2b** and thiazolethione **3b** (Figure 6), which signifies that radicals are responsible for the DNA damage. Also the solvent effect on the DNA-cleaving activity was examined for pyridone **2b** in a H_2O−MeCN mixture (60:40) and in H_2O. As shown in Figure 6, the irradiation of pyridone **2b** in H_2O gave about twice more open-circular DNA [(32 ± 2)%] than in the 60:40 H_2O−MeCN mixture [(17 ± 1)%].

Discussion

The EPR experiments (Figures 4 and 5) provide unequivocal evidence that both the pyridone **2b** and the thiazolethione **3b** generate isopropoxyl radicals upon photoexcitation.

The most significant difference in the photochemical properties between these two photo-Fenton reagents is that in addition to the isopropoxyl radicals, the pyridone

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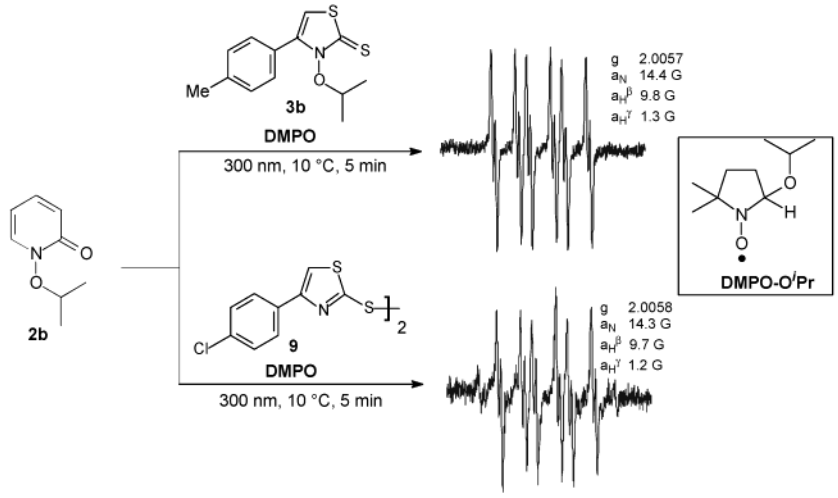
SCHEME 4. Effect of the Thiazolethione 3b (1.0 mM) and the Disulfide 9 (<0.5 mM) on the EPR Spectra of Pyridone 2b (1.0 mM) upon Photolysis in the Presence of DMPO (27 mM)



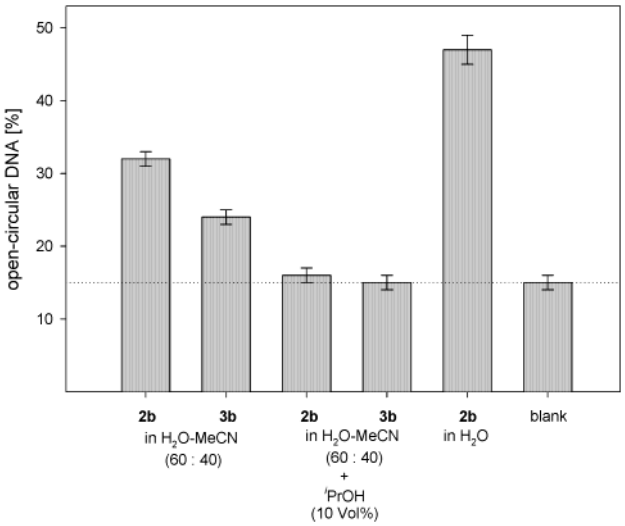


FIGURE 6. Strand-break formation for the photolysis (312 nm, 0 °C, 30 min) of thiazolethione **3b** (4.00 mM) or pyridone **2b** (4.00 mM) in the presence of pBR 322 DNA (10 μg/mL) in KH_2PO_4 buffer (5 mM, pH 7.4) and MeCN (60:40).

2b affords also the carbon-centered 2-hydroxyprop-2-yl radical, especially in aqueous media. The 2-hydroxyprop-2-yl radicals results from H shift of the isopropoxyl radicals (Scheme 3); such transformation is known for secondary alkoxyl radicals and is facilitated in water as reaction medium.[34,36] However, the 2-hydroxyprop-2-yl radical was not detected in the photolysis of the thiazolethione **3b** even at low DMPO concentration (Figure 4g), although isopropoxyl radicals have been generated. Presumably, the 2-hydroxyprop-2-yl radical, if formed through the transformation of the released isopropoxyl radical, are scavenged in the photoreaction of the thiazolethione **3b**. Indeed, control experiments with the pyridone **2b** confirmed (Scheme 4) that for the photolysis in the presence of the thiazolethione **3b** or the disulfide **9**, the 2-hydroxyprop-2-yl radical was not detected. Evidently, in the photolysis of the thiazolethione **3b**, the carbon-centered radical is efficiently trapped presumably by the disulfide product **5**, but also intermediary thiyl radicals may serve this purpose. Consequently, to provide mechanistic insight on this point, the photochemistry of the two photo-Fenton reagents **2b** and **3b** needs to be considered.

The photochemical pathways shown in Scheme 5 are proposed for the photoproducts derived from the thiazolethione **3b**. The formation of the disulfide **5**, the bithiazyl **6**, and the thiazole **7** may be explained analogously to the reported photoreaction of *N*-hydroxythiazolethione **3c**.[22] Dimerization of the thiyl radical **B** gives the disulfide **5** (Scheme 5, path a), which on further photolysis affords radicals **C** and **D** through the C−S bond cleavage, a photoreactivity which was confirmed by the transient spectrum of the disulfide **5** upon laser flash photolysis (Figure 2d).[38,39] The radical **C**, which may also result from sulfur extrusion of the radical **D**,[38] abstracts a hydrogen atom to give the thiazole **7** or dimerizes to the bithiazyl **6**. The unexpected isothiocyanate **8** may result from ring-opening of the thiyl radical **B** to the thiyl radical **E** (Scheme 5, path b) and subsequent radical coupling between the thiyl radicals **C** and **E**.

In regard to the trapping of the 2-hydroxyprop-2-yl radical, formed by transformation of the released isopropoxyl radical, a likely scavenger is the disulfide **5** which may afford hemithioacetal **10** upon S_H2 reaction.[40] Furthermore, also the thiyl radical **B** or the perthiyl radical **D** may serve as trapping agent.

The photoproducts of the pyridone **2b** are accounted for on the basis of the reported photoreactions of *N*-oxyl pyridones (Scheme 6).[41] Upon photoexcitation, N−O homolysis and subsequent hydrogen-atom abstraction by the pyridyloxyl radical **A**, followed by combination of the radical pair, provides the observed products **4a**,**b**. The high value of 75% for the quantum yield of the N−O bond scission Φ_{N-O} reveals that this cleavage is the dominant

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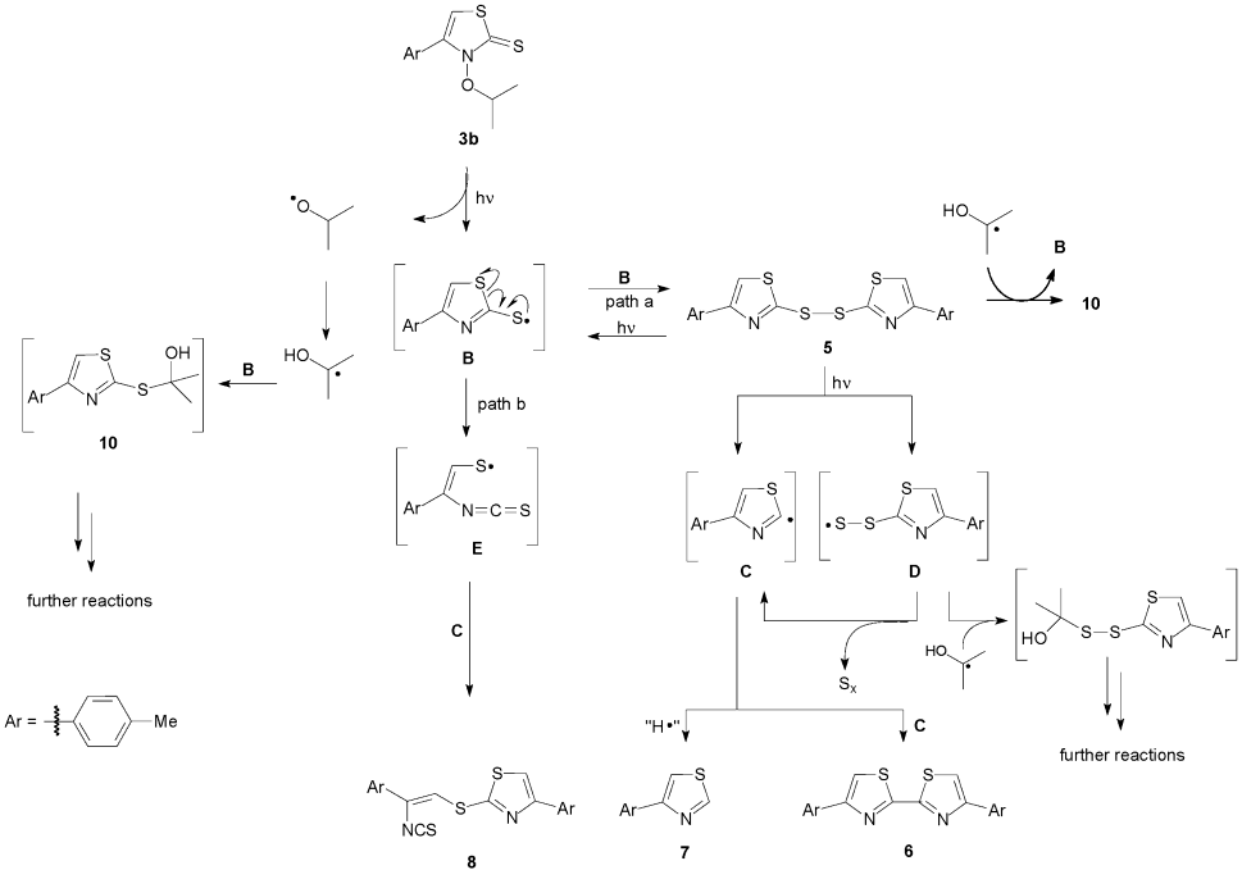
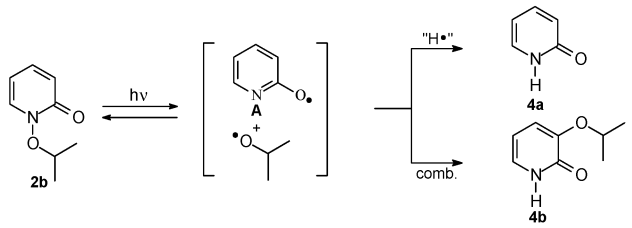
SCHEME 5. Proposed Mechanism for the Photoproducts of the Thiazolethione 3b Photolysis



SCHEME 6. Proposed Mechanism for the Formation of the Photoproducts in the Photolysis of the Pyridone 2b



primary photochemical step in the decomposition pathway of the pyridone **2b**. The Norrish-Type II reaction is of minor importance. Neither the pyridone **2b** itself nor its photoproducts **4a**,**b** are expected to trap the 2-hydroxyprop-2-yl radical, and therefore the latter carbon-centered radicals may be observed in the photolysis of the pyridone **2b**, in addition to the isopropoxyl radical, by DMPO trapping and EPR-spectral detection.

Since the quantum yield of radical release (Φ_{N-O}) is about twice (Table 1) that of the pyridone conversion (Φ_{dec}), it is concluded that recombination of the pyridyloxyl radical **A** with the isopropoxyl radical back to the pyridone **2b** is an efficient pathway. Consequently, the Φ_{dec} quantum yield is a better measure of alkoxyl radicals effective for oxidation purposes.

With the difference in the photochemical behavior of the photo-Fenton reagents **2b** and **3b** mechanistically rationalized, its consequence on the DNA-cleaving activity was of relevance. In a mixture of $H_2O-MeCN$ (60:40), both reagents display moderate DNA-damaging activity (Figure 6). In the presence of 2-propanol (10 vol %), the strand-break formation is suppressed, which confirms that radical species are generated in the photolysis of both photo-Fenton reagents and are responsible for the DNA damage.

The pertinent question relates to which radical is responsible for the DNA damage. For the photolysis of pyridone **2b** in aqueous medium, the 2-hydroxyprop-2-yl radical was exclusively detected as the spin-trapping adduct with DMPO (Figure 4i). Under these conditions, a 2-fold higher DNA-cleaving activity was observed compared to that in a 60:40 $H_2O-MeCN$ mixture (Figure 6). Since these DNA oxidations are run under atmospheric conditions, presumably the 2-hydroxyprop-2-yl radicals couple with molecular oxygen to afford the more active alkylperoxyl radicals as oxidant.[42] Therefore, the photoinduced DNA-damaging activity of the pyridone **2b** in water may be attributed to the damaging species formed in the radical cascade of the isopropoxyl radicals, with the involvement of molecular oxygen. Also in a mixture of $H_2O-MeCN$ (60:40), the radical cascade is operative because the 2-hydroxyprop-2-yl radical was detected in the photolysis of the pyridone **2b** under these conditions (Figures 4d, 4f, 4h).

In the case of the photolysis of thiazolethione **3b**, in contrast to the pyridone **2b**, only isopropoxyl radicals were detected under all conditions (Figures 4c, 4e, 4g), since the 2-hydroxyprop-2-yl radical is efficiently scavenged in situ (Scheme 4). The moderate DNA-damaging activity of the thiazolethione **3b** is, thus, due to the isopropoxyl radical that is generated in the photolysis of

this photo-Fenton reagent. Clearly, the thiazolethione **3b** is a less effective photochemical DNA-cleaving agent than the pyridone **2b**, despite the fact that the thiazolethione **3b** ($\Phi_{dec} = 0.60$) is about twice more efficiently photolyzed than the pyridone **2b** ($\Phi_{dec} = 0.36$), see Table 1.

In summary, the isopropoxyl radicals themselves, generated initially from the photo-Fenton reagents **2b** and **3b** on photolysis, rearrange readily in aqueous media by H shift to the 2-hydroxyprop-2-yl radicals, which on coupling with molecular oxygen provide the peroxyl radicals as DNA-damaging species.[42] Most significant for the purpose of assessing the potential deleterious effects of alkoxyl radicals in photobiological applications, of the two photo-Fenton reagents examined herein, the thiazolethione **3b** serves as a clean source of isopropoxyl radicals. The reason for this advantage over the pyridone **2b** is in-situ trapping of any potentially generated carbon-centered radicals by *N*-alkoxythiazolethione-derived photoproducts such as the disulfide **5**.

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Supporting Information Available: Experimental details for the synthetic work, for the EPR and transient-spectroscopy, and for the determination of strand breaks in pBR 322 DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

JO025856W

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