# Are Pyridinethiones Reliable Photochemical Oxyl-Radical Sources for Photobiological Studies? The Importance of Secondary Photolysis Products in the Guanine Oxidation of 2′-Deoxyguanosine and Cell-Free DNA

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**Abstract:** The photolysis of the pyridinethiones **1a**−**d** releases oxyl (hydroxyl, isopropyloxyl, *tert*-butoxyl, and benzoyloxyl) radicals, which damage DNA through strand cleavage and guanine oxidation. Unexpectedly, the *N*-hydroxy derivative **1a** is significantly less effective in the oxidation of cell-free DNA than the alkoxy and benzoyloxy ones **1b**−**d**. A similar trend was observed for the photooxidative consumption of 2′-deoxyguanosine (**dG**) by these pyridinethiones. Detailed mechanistic investigations have revealed that the guanine oxidation by the pyridinethiones **1b**−**d** is not caused by oxyl radicals but by a type-I photooxidation process through the novel betain intermediate **8**, which is formed in small amounts (ca. 5%) during the photolysis of these thiones from the intermediary photoproduct disulfide **3**. This photosensitized guanine oxidation is effectively inhibited by the *N*,*N*′-dioxide **7**, which is produced only in the photolysis of the *N*-hydroxypyridine-2-thione (**1a**) and not from the *N*-alkoxy and *N*-benzoyloxy derivatives **1b**−**d**. Thus, for the *N*-hydroxy derivative **1a** hydroxyl radicals are the main DNA-damaging species. The *N*-alkoxy and *N*-benzoyloxy derivatives **1b**−**d** are more effective DNA-photooxidizing reagents than the *N*-hydroxypyridinethione **1a**, because **1b**−**d** oxidize DNA photocatalytically through sensitization by the betain **8**, while **1a** oxidizes DNA mainly through the stoichiometrically photogenerated hydroxyl radicals.

## Introduction

Oxygen radicals belong to the most reactive oxidants and play a key role in the so-called *Oxidative Stress*. Their reactivity has also been utilized therapeutically in several antitumor agents, e.g. bleomycin.[1] Unquestionably, it is relevant and important to assess the efficacy of DNA damage by oxygen-radical sources.

For chemical model studies of such DNA damage, the methods that are available to generate hydroxyl radicals entail the chemical formation through the Fenton reaction[2] or the $\gamma$ radiolysis of water.[3] Such conventional hydroxyl-radical sources are of limited scope for biological investigations, because besides hydroxyl radicals, the Fenton reaction may initiate undesirable redox chemistry, while $\gamma$ radiolysis may cause direct ionization of DNA. For this reason, during the past few years new photochemical equivalents have been developed,[4−7] the so-called *photo-Fenton* reagents. Among these, the *N*-hydroxypyridine-2-thione (**1a**)[4] recently has received much attention. The latter is a commercial substance known as *Omadine*, a versatile antibacterial and antifungal agent employed in antidandruff shampoos for many years. In vitro, thione **1a** exhibits cytotoxic activity in leukemia cells and is, therefore, a potential chemotherapeutic anticancer agent.[8] Indeed, the genotoxicity of *N*-hydroxypyridinethiones has been demonstrated in cellular systems[7b] upon irradiation, for which the observed DNA-strand cleavage and base oxidation has been attributed to the photochemically released hydroxyl radicals.[7]


**1a:** R = H **2**
**1b:** R = isopropyl
**1c:** R = *tert*-butyl
**1d:** R = benzoyl

Recently we have reported[9] that the *N*-alkoxy and *N*-benzoyloxy derivatives **1b**−**d** of *N*-hydroxypyridinethione **1a**

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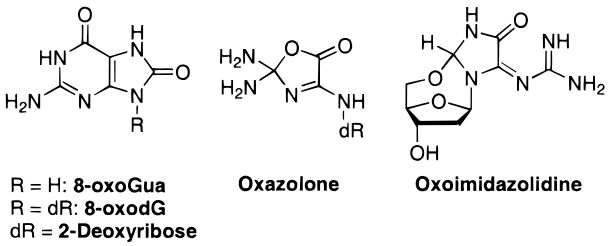
induce strand breaks in supercoiled pBR 322 DNA upon irradiation, due to alkoxyl or benzoyloxyl radicals.[9,10] In the present chemical model studies, we have examined whether *N*-isopropoxy (**1b**), *N*-*tert*-butoxy (**1c**), and *N*-benzoyloxy (**1d**) derivatives may serve as effective photochemical oxyl-radical sources for the investigation of DNA-base modification by alkoxyl radicals because compared to hydroxyl radicals, there is still little known about this for alkoxyl radicals.[11] Herein we report that the pyridinethiones **1b**−**d** are not well suited as photochemical alkoxyl-radical sources to assess the efficacy of alkoxyl radicals for DNA oxidation. Only on prolonged irradiation are the *N*-alkoxy- and *N*-benzoyl-substituted pyridinethiones **1b**−**d** significantly more effective than the *N*-hydroxy derivative **1a** in photooxidizing DNA. To rationalize this unexpected photooxidative activity of pyridinethiones mechanistically, the photooxidation of 2′-deoxyguanosine and *calf-thymus* DNA has been investigated in detail.

## Results

The *N*-substituted pyridinethiones **1** were synthesized under complete light exclusion according to literature procedures.[12−14] As already described, the *N*-alkoxypyridinethiones photodecompose at 350 nm completely within a few minutes, and the formation of alkoxyl radicals in the photolysis was substantiated by EPR-spectroscopic detection of DMPO−alkoxyl−radical adducts.[9] Now, the results of the DNA-oxidizing activity of the radical sources **1** are presented below.

**1. Photooxidation of dG and *CT* DNA. (a) Photooxidation of dG by the *N*-Alkoxypyridinethiones 1b,c.** On irradiation of the *N*-alkoxypyridinethiones **1b** and **1c** (350 nm, 3 h), the catalytic decomposition of 0.50 mM 2′-deoxyguanosine (**dG**) was observed since <1 equiv of **1b,c** induced the complete conversion of **dG** (Figure 1 shows the results for **1b**). Alkaline treatment of the reaction samples released guanidine from the formed **oxazolone** (also **oxoimidazolidine**, but for convenience we refer here only to the former) in about 30% yield (Figure 2), which was quantified by HPLC analysis by means of the fluorescence-labeling assay with 1,2-naphthoquinone-4-sulfonic acid.[15] Neither the 4*R** and 4*S** diastereomers of 4-hydroxy-




8-oxo-4,8-dihydro-2′-deoxyguanosine (4-HO-8-oxodGuo), which are characteristic type-II photooxidation products of dGuo,[15,16] nor 7,8-dihydro-8-oxo-2′-deoxyguanosine (**8-oxodG**) was detected (HPLC, UV detection for 4-HO-8-oxodGuo; HPLC, electrochemical detection at 450 mV for 8-oxodG) as photooxidation product. The addition of the radical scavenger di-*tert*-butylcresol (1 or 10 equiv referred to **dG**) significantly decreased the **dG** conversion and the formation of the guanidine-releasing product **oxazolone** (Figure 2). However, the oxyl-radical scavenger *tert*-butyl alcohol,[6a] which usually effectively lowers the formation of strand breaks by *N*-alkoxypyridinethiones,[9] did not influence appreciably the conversion of **dG** caused by the radical sources **1b,c** (data not shown).

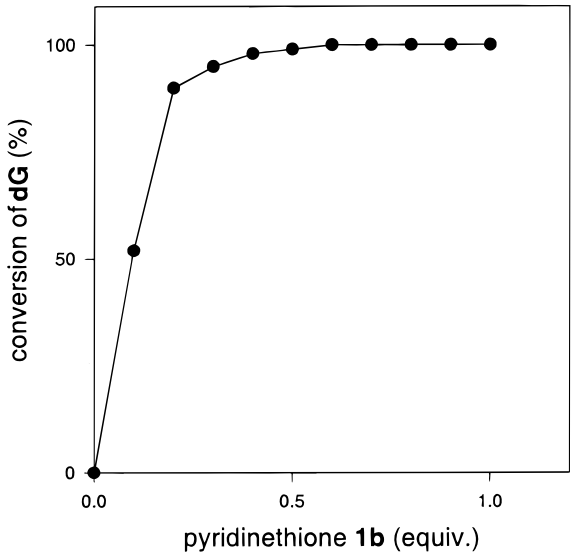



**Figure 1.** Conversion of **dG** (0.50 mM) upon irradiation (350 nm) of *N*-isopropoxypyridinethione **1b** (50 $\mu$M to 0.50 mM, 0.1−1.0 equiv) in phosphate buffer (5.0 mM, pH 7.0)/$CH_3CN$ (10%) at 10 °C for 3 h; without irradiation no **dG** conversion was observed.

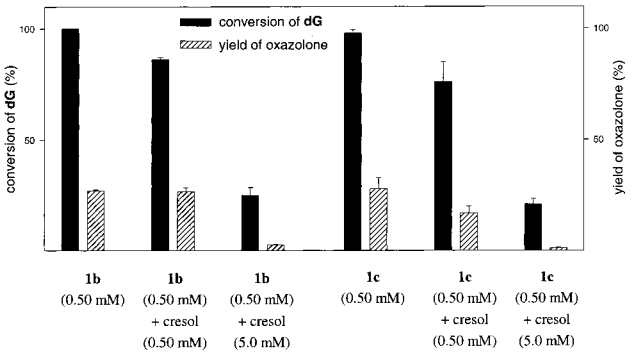



**Figure 2.** Conversion of **dG** and yield of **oxazolone** upon irradiation (350 nm) of **dG** (0.50 mM) in the presence of thiones **1b** or **1c** with and without di-*tert*-butylcresol in phosphate buffer (5.0 mM, pH 7.0)/$CH_3CN$ (10%) at 10 °C for 3 h; without irradiation no **dG** conversion was observed; **8-oxoGua** was not formed in significant yields.

*N*-Methylpyridine-2-thione[14] (**2**), a substance that contains the same chromophore but cannot generate alkoxyl radicals, did not promote any notable (4%) conversion of **dG** nor did it lead to any appreciable (1%) amount of **oxazolone** under the same conditions as above. Thus, the pyridinethione chromophore itself does not possess any significant activity as type-I photosensitizer.

To gain further insight into the mode of action of the *N*-alkoxypyridinethiones **1b** and **1c**, the time dependence of the **dG** conversion compared to that of *N*-alkoxypyridinethiones **1b** and **1c** was monitored in detail. For comparison, the *N*-benzoyloxypyridinethione **1d** was employed as well. The results (Figure 3) demonstrate clearly that the **dG** conversion starts

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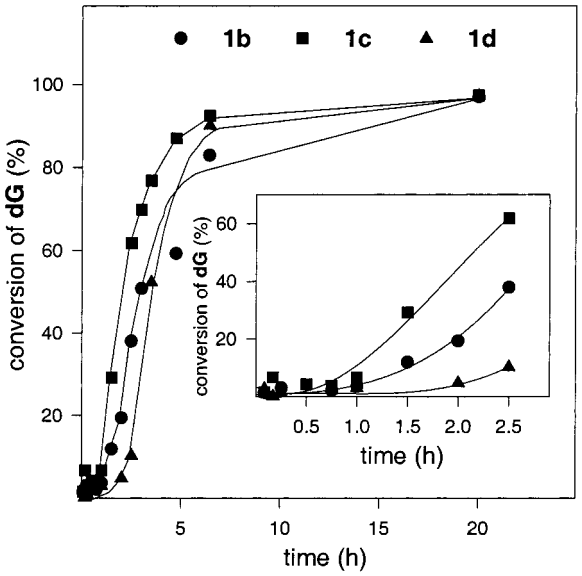


**Figure 3.** Time dependence of the photochemical (350 nm) conversion of **dG** (0.50 mM) in the presence of the pyridinethiones **1b** (50.0 $\mu$M), **1c** (50.0 $\mu$M), or **1d** (0.150 mM) in phosphate buffer (5 mM, pH 7.0)/$CH_3CN$ (20%) at 10 °C; without irradiation no **dG** conversion was observed.

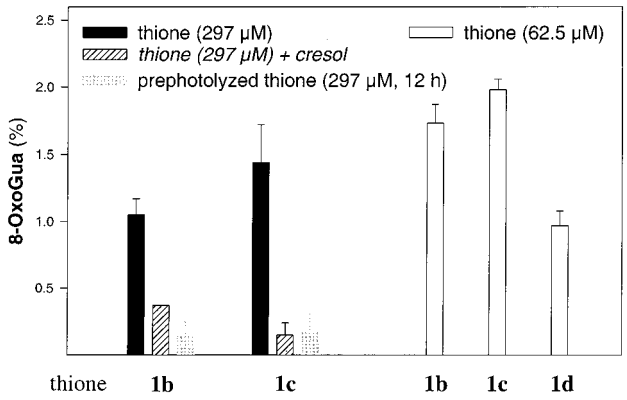


**Figure 4.** Formation of **8-oxoGua** during the photolysis (350 nm, 3.0 h) of *N*-isopropoxy- (**1b**), *N*-*tert*-butoxy- (**1c**), and *N*-benzoyloxypyridine-2-thione (**1d**) in the presence of *calf-thymus* DNA in phosphate buffer (5 mM, pH 7.0)/$CH_3CN$ (7%); inhibition by the radical scavenger di-*tert*-butylcresol (5.33 mM) and the efficiency of the photooxidation by the photoproducts of the thiones **1b** and **1c** after 12 h of preirradiation; without irradiation no DNA oxidation was observed; guanidine-releasing products were not formed in significant amounts (<0.2%).

only after ca. 1 h of irradiation time (see inset), whereas the decomposition of the pyridinethiones **1b**−**d** is complete within the **first** minute of irradiation (detected by HPLC analysis). When the pyridinethiones **1b**−**d** were irradiated until their complete consumption and subsequently **dG** was added to the photolyzate, its full conversion was observed on further photolysis.

**(b) Photooxidation of *Calf-Thymus* DNA by the Pyridinethiones 1b**−**d.** Irradiation of the pyridinethiones **1b**−**d** afforded mainly 7,8-dihydro-8-oxoguanine (**8-oxoGua**) by oxidative damage of *calf-thymus* DNA (Figure 4); guanidine-releasing products were formed in insignificant amounts (<0.1%). The time dependence of the **8-oxoGua** formation from *calf-thymus* DNA (data not shown) revealed that the maximum yield of **8-oxoGua** was reached after 2 to 3 h of irradiation for the pyridinethiones **1b,c**. As to the concentration dependence,

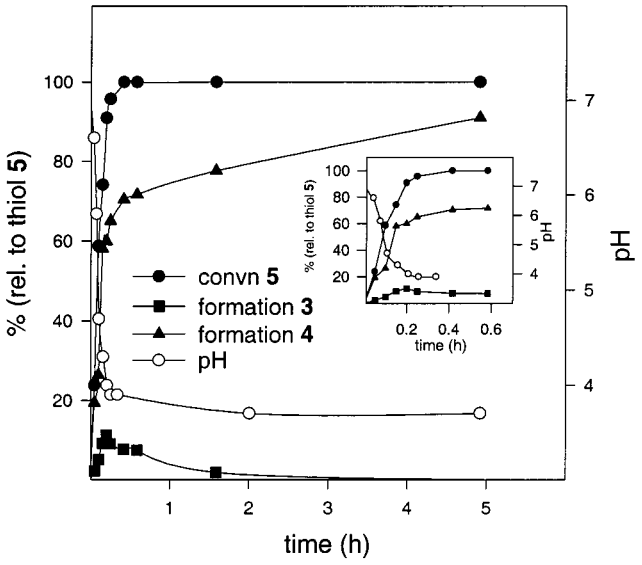


**Figure 5.** Time dependence for the photochemical (350 nm) conversion of pyridine-2-thiol (**5**; 0.50 mM) in water/$CH_3CN$ (20%), the formation of the disulfide **3** and sulfonic acid **4**, and the pH profile.

smaller amounts of the *N*-alkoxypyridinethiones **1b** and **1c** (62.5 versus 295 $\mu$M) led to higher yields of **8-oxoGua** (Figure 4).

As observed for **dG**, also for *calf-thymus* DNA the addition of the radical scavenger di-*tert*-butylcresol decreased the oxidation activity significantly, as is evident from the reduced amount of **8-oxoGua** (Figure 4). The photolyzate of the *N*-alkoxypyridinethiones **1b** and **1c** after 12 h of preirradiation at 350 nm (pyridine-2-sulfonic acid **4** is the main photolysis product) did not lead to any **8-oxoGua** upon further irradiation in the presence of *CT* DNA. The pyridinethione chromophore was inactive, because the photostable *N*-methylpyridine-2-thione (**2**) gave only 0.15 ± 0.1% **8-oxoGua**, which is small compared to that of the *N*-alkoxypyridinethiones **1b** and **1c** (1.0−1.5%).

**2. Photoproducts of the Pyridinethiones 1b**−**d and Their DNA-Oxidizing Activity.** Since the time profile (Figure 3) exposed that the photoproducts of the pyridinethiones **1b**−**d** are responsible for the DNA photooxidation, their photochemistry was studied in detail. The photolysis of **1b**−**d** led to 2,2′-dipyridine disulfide (**3**), which upon exhaustive photolysis was converted to pyridine-2-sulfonic acid (**4**) as final product (Scheme 1, Figure 5). In contrast to a recent report,[17] in which the photolysis of disulfide **3** was performed in acetonitrile or 2-propanol, we did not detect pyridine-2-thiol (**5**). Instead, under our photolysis conditions, thiol **5** was oxidized to its sulfonic acid **4** (Scheme 1), which was also manifested by the pH decrease during the photolysis (Figure 5).

**(a) Photooxidation of dG and DNA by Disulfide 3 and Sulfonic Acid 4.** Like the pyridinethiones **1b**−**d**, the disulfide **3** also converted **dG** catalytically upon irradiation (350 nm). Again this took place only after about 30 min of irradiation, whereas the consumption of the disulfide **3** and the formation of its final oxidation product, the sulfonic acid **4**, started immediately on irradiation (Figure 6). Control experiments showed that the sulfonic acid **4**, produced from the pyridine derivatives **1**, **3** or **5**, did not photooxidize **dG**. For comparison, on long preirradiation (after 12 h), the pyridinethiones **1b**−**d** also did not significantly consume **dG** upon further irradiation. Furthermore, in the photooxidation of *calf-thymus* DNA, the photoproduct of disulfide **3** was highly active, since 5.5% of

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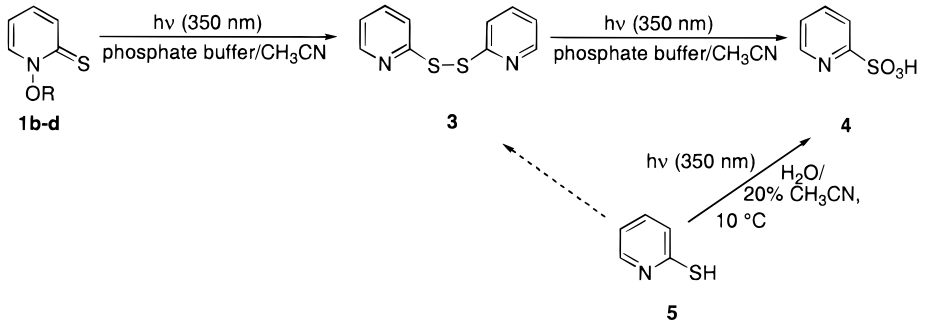
**Scheme 1:** Photolysis of the pyridinethiones **1b**−**d** and pyridine-2-thiol (**5**)



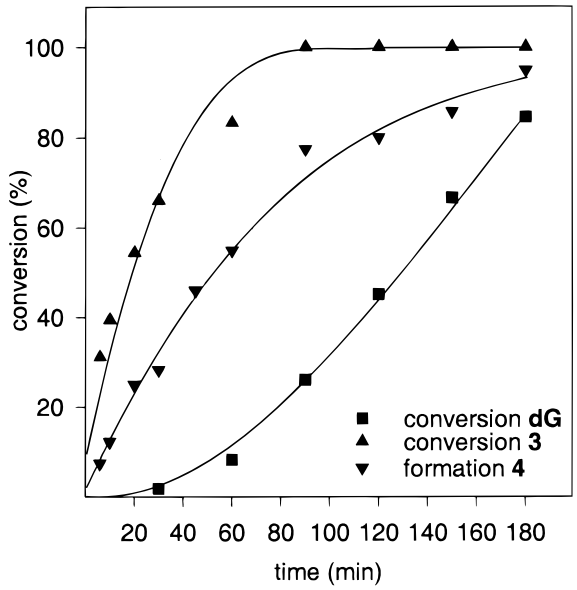




**Figure 6.** Time dependence of the photochemical (350 nm) conversion of **dG** [0.50 mM, in phosphate buffer (5.0 mM, pH 7.0)/$CH_3CN$ (6%)] in the presence of the disulfide **3** (0.250 mM) and conversion of the latter, as well as the formation of the sulfonic acid **4**; without irradiation no **dG** conversion was observed.

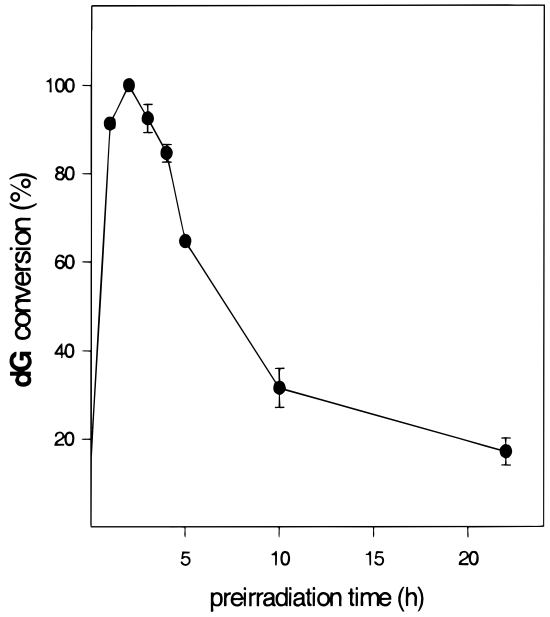


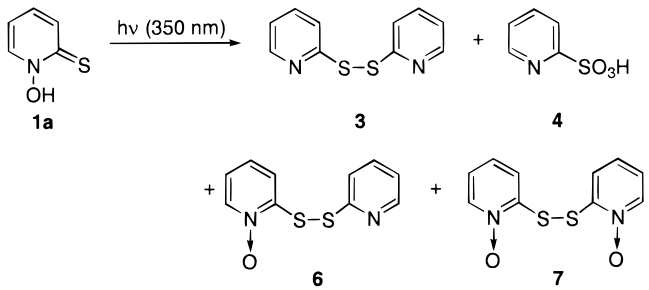

**Figure 7.** Conversion of **dG** (0.50 mM) upon irradiation (350 nm) in the presence of the photolyzate of disulfide **3** as a function of preirradiation time.

**8-oxoGua** were formed. Again, the sulfonic acid **4** as well as preirradiated (12 h) solutions of the pyridinethiones **1b**−**d** were inactive.

Since the time dependence in Figure 6 demonstrates convincingly that the disulfide **3** is not directly responsible for the **dG** photooxidation (**dG** consumption lags about 30 min behind that of **3**), an intermediary product was suspected. Thus, efforts were expended to detect an active species and characterize it. For this purpose, preirradiated solutions of the disulfide **3** were employed in the photoreactions with **dG**. Indeed, the data in Figure 7 clearly manifest that after about 2-h preirradiation of the disulfide **3**, a maximum of photooxidative activity was reached.

**(b) Isolation and Characterization of the Active Intermediate in the Photooxidation of dG by Disulfide 3.** Photooxidation on the semipreparative scale afforded a highly polar DNA-damaging intermediary product **8** (for structure cf. Scheme 3), which was isolated by silica-gel chromatography in ca. 5% yield. The structure of this new betain **8** rests on $^1H$ NMR and $^{13}C$ NMR, HH-COSY, HMQC, and HMBC data, as well as its mass spectrum and IR absorptions. Control experiments confirmed that the photoproduct **8** oxidized **dG** upon irradiation at 350 nm. The time profile (Figure 8) displays that the **dG** conversion started immediately on irradiation, whereas the active species **8** was consumed only half as fast as the **dG**. Irradiation of *calf-thymus* DNA (62.5 $\mu$M guanine) in the presence of the

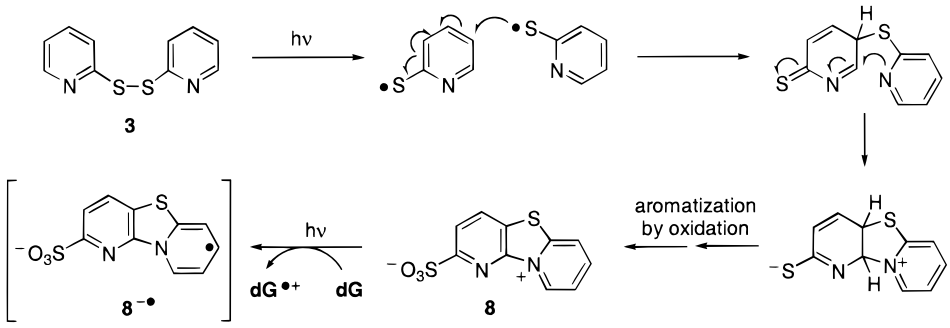
**Scheme 2:** Products of the photolysis of *N*-hydroxypyridine-2-thione (**1a**)




betain **8** (5.25 $\mu$M) led to 3% of **8-oxoGua**; no oxazolone was detected.

**3. Photooxidation of dG and DNA by *N*-Hydroxypyridine-2-thione (1a).** Mechanistically significant is the fact that besides disulfide **3** (7%) and sulfonic acid **4** (22%), the mono-*N*-oxide **6** (15%) and the *N*,*N*′-dioxide **7** (38%) also are formed in the photolysis of *N*-hydroxypyridinethione **1a** at 350 nm (Scheme 2).[4a,b,18] Control experiments disclosed that the *N*,*N*′-dioxide **7** inhibited significantly the **dG** conversion by the pyridinethiones **1b**−**d** and their photoproducts, as demonstrated by the fourth, sixth, eighth, and eleventh columns in Figure 9. Also the DNA oxidation to **8-oxoGua** by the pyridinethiones **1b**−**d** (only derivative **1c** is shown) or by the photoproduct of disulfide **3** was suppressed substantially, which is displayed in Figure 10

**Scheme 3:** Mechanism for the formation of the betain **8** and photosensitized electron-transfer oxidation of dG



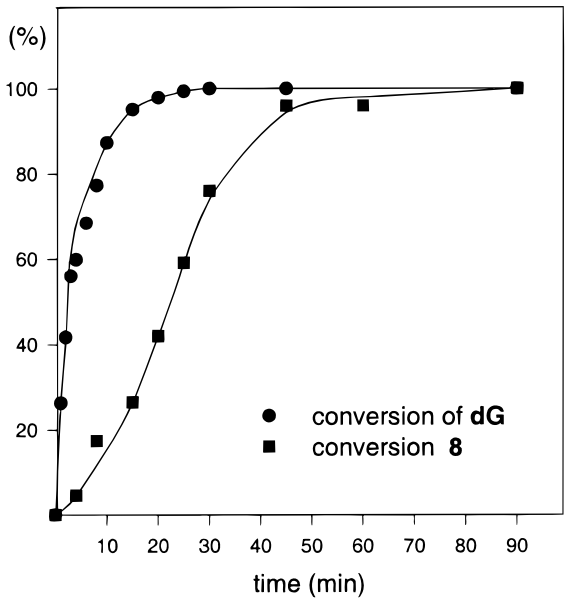




**Figure 8.** Time dependence of the photochemical (350 nm) conversion of **dG** [0.50 mM in 5 mM phosphate buffer (pH 7.0)] in the presence of the isolated active intermediary product **8** (0.084 mM) and conversion of the latter; without irradiation no **dG** conversion was observed.

(third and fifth columns). Furthermore, the *N*,*N*′-dioxide **7** inhibited not only the photooxidation of **dG** by the photoproducts of the pyridinethiones **1b**−**d** but also the type-I photooxidation of benzophenone and menadione (Figure 9).

## Discussion

As reported earlier,[9] the observed DNA-strand cleavage is caused by the oxyl (hydroxyl, alkoxyl, benzoyloxyl) radicals, generated in the photolysis of the pyridinethione derivatives **1a**−**d**. However, the finding that at prolonged irradiation times (2−3 h) the *N*-alkoxy- and *N*-benzoyloxy-substituted pyridinethiones **1b**−**d** are more effective photooxidants of the guanine base in **dG** and DNA than the *N*-hydroxy derivative **1a** is unexpected because generally alkoxyl and acyloxyl radicals are less reactive than hydroxyl ones.[19] Since the hydroxyl-radical activity in oxidative DNA damage has been previously demonstrated for the hydroxy-substituted **1a**,[16] this implies that other than oxyl radicals are responsible for the DNA-base oxidation by the pyridinethiones **1b**−**d**. Indeed, the lack of inhibition of the **dG** conversion by the oxyl-radical scavenger *tert*-butyl alcohol[6a] supports that the base oxidation by the substrates **1b**−**d** is not induced by alkoxyl radicals. However, di-*tert*-butylcresol inhibited significantly guanine oxidation in **dG** (Figure 2) and DNA (Figure 4). Control experiments with the type-I photosensitizer benzophenone revealed that the radical scavenger di-*tert*-butylcresol also inhibits the electron-transfer-mediated type-I photooxidation of **dG** (data not shown). Therefore, we conclude that the decreased extent of guanine oxidation induced by the pyridinethiones **1b**−**d** in the presence of the cresol is due to the inhibition of the type-I photooxidation process. Presumably, the electron-rich cresol competes with dG as electron donor in this photooxidation reaction.

That oxyl radicals are not responsible for the observed guanine oxidation by the pyridinethiones **1b**−**d** is further convincingly proven by the time-dependence study (Figure 3) of the **dG** oxidation. Thus, the **dG** conversion starts only after 1 h of irradiation, when all of the pyridinethione has been completely consumed and, thus, oxyl-radical production must have already ceased. Also the maximum DNA-base oxidation was reached only after ca. 2 h of irradiation. Relevant is the fact that the main photoproduct of the pyridinethiones **1b**−**d**, namely the disulfide **3**, was shown to consume **dG** catalytically on irradiation and oxidize highly efficiently the guanine base in DNA (Figure 6). However, the disulfide **3** is not the ultimate oxidant of DNA, since these time-dependence studies demonstrate clearly that the **dG** conversion starts quite late (only after 30 min), whereas the disulfide consumption commences immediately upon irradiation (Figure 6). Even more convincing is the fact that preirradiated solutions of the disulfide **3** are more reactive in the photooxidation of DNA and of **dG** than the pure, not-preirradiated compound **3** (Figure 7). With increasing preirradiation time, the activity increases at first and afterward drops again, an indication that a photooxidizing intermediate is formed, which itself is photolabile. Indeed, the novel betain **8** was isolated as DNA-photooxidizing species, which is generated in the photolysis of disulfide **3** in small amounts (ca. 5%).

Earlier it was reported that the irradiation of disulfide **3** leads to S−S bond cleavage,[17] while presently we have shown that the resulting thiyl-radical products are further oxidized to afford finally the pyridinesulfonic acid **4** (Scheme 1). The latter was observed as the end-product in the photolysis of the pyridinethiones **1a**−**d**, of the disulfide **3**, and of the thiol **5**. Furthermore, the present data show unequivocally that the photolysis of the pyridinethiones **1b**−**d** yields, besides the disulfide **3** and the sulfonic acid **4**, the highly active photooxidizing intermediary product **8**, an unusual and novel tricyclic betain. A similar photolabile compound with such a tricyclic betain structure has been reported previously.[20] The formation of the zwitterionic species **8** is mechanistically rationalized in Scheme 3 in terms of the hitherto unprecedented head-to-tail coupling of two thiyl radicals, subsequent cyclization by intramolecular nucleophilic

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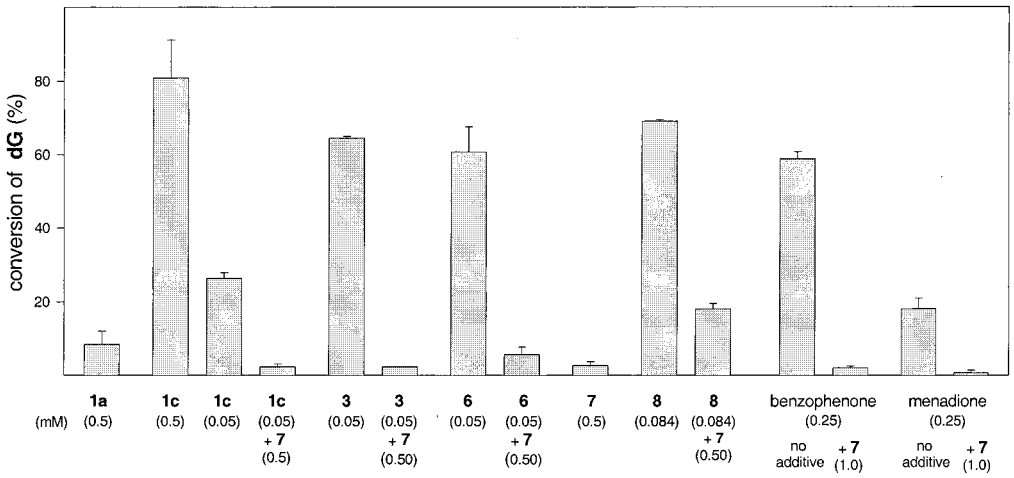


**Figure 9.** Photochemical (350 nm) conversion of **dG** [0.50 mM in phosphate buffer (5.0 mM, pH 7.0)] in the presence of *N*-hydroxypyridine-2-thione (**1a**, 1.5 h), *N*-*tert*-butoxypyridine-2-thione (**1c**, 3.0 h), 2,2′-dipyridine disulfide (**3**, 3.0 h), 2,2′-dipyridine disulfide mono-*N*-oxide (**6**, 3.0 h), 2,2′-dipyridine disulfide di-*N*-oxide (**7**, 3.0 h), betain **8** (12 min), benzophenone (1.25 h), or menadione (1.25 h); inhibitory effect of di-*N*-oxide **7** in the photooxidation of **dG**.

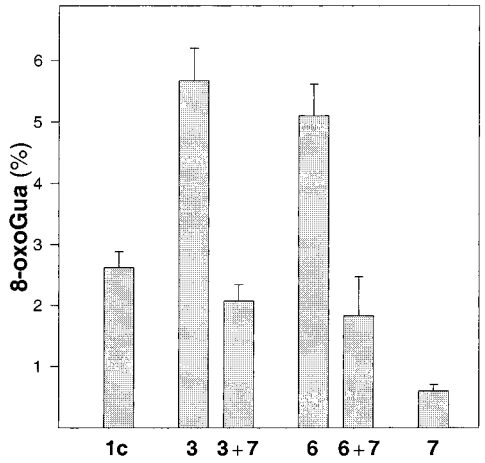


**Figure 10.** Formation of **8-oxoGua** in the irradiation (350 nm) of *CT* DNA (62.5 $\mu$M Gua in 5 mM phosphate buffer, pH 7.0) in the presence of *N*-*tert*-butoxypyridinethione **1c** (62.5 $\mu$M, 1.0 equiv), of 2,2′-dipyridine disulfide **3** (31.3 $\mu$M, 0.50 equiv), of 2,2′-dipyridine disulfide mono-*N*-oxide **6** (31.3 $\mu$M, 0.50 equiv), and of *N*,*N*′-dioxide **7** (62.5 $\mu$M, 1.0 equiv); inhibitory effect of *N*,*N*′-dioxide **7** (125 $\mu$M, 2.0 equiv); without irradiation no DNA oxidation was observed.

attack and final oxidative aromatization, followed by the conversion of the sulfhydryl to the sulfonic acid functionality.

The betain **8** exhibits photooxidizing propensity, as confirmed by the **dG** conversion (Figure 8) and the formation of **8-oxoGua** in *CT* DNA. This is not astonishing, since aromatic iminium salts are in general excellent electron acceptors and their photochemical electron-transfer reactions have been frequently used for synthetic purposes.[21] Therefore, we propose (Scheme 3) that the betain **8** oxidizes the guanine base in DNA by a type-I photooxidation process (photochemical electron transfer), but does not efficiently induce DNA strand breaks. The product distribution in the photooxidation of **dG** by the pyridinethiones **1b**−**d** supports this mechanistic rationale: In the **dG** photooxidation by **1b**−**d**, high amounts (25%) of guanidine-releasing products (**oxazolone** and **oxoimidazolidin**e) but no **8-oxoGua** were observed, a characteristic feature of type-I sensitizers.[15,22,23] However, in DNA, significant amounts of **8-oxoGua** (3%) but no notable quantities of guanidine-releasing products were formed. In contrast to dG, in DNA the guanyl radical cation, formed by one-electron oxidation, is stabilized and its hydration leads to 8-oxodG.[22,24] Therefore, in the photooxidation of DNA by the pyridinethiones **1b**−**d**, the 8-oxodG is formed by the type-I mechanism. Recently it was reported that the one-electron oxidation of *calf thymus* DNA[25] and oligonucleotides[26] leads mainly to oxazolone and its precursor imidazolone. The observation that at lower concentration of *N*-alkoxypyridinethiones higher yields of **8-oxoGua** are obtained (Figure 4) also supports the mechanistic rationale that a type-I photooxidation process is involved in the DNA-base oxidation by **1b**−**d**, since it has been reported that the guanine photooxidation product **8-oxoGua** is efficiently further oxidized by type-I photosensitizers.[27]

The question that remains to be answered is, why the *N*-hydroxy derivative **1a** is much less active in the photooxidation of guanine in dG and DNA than the alkoxy (**1b**,**c**) and benzoyloxy (**1d**) ones. Intensive product and mechanistic studies provide a convincing answer to this query: Definitive is the fact that in the photolysis of the *N*-hydroxy derivative **1a** the *N*,*N*′-dioxide **7** is formed[28] in significant amounts (yield 38%), but not at all from **1b**−**d**. Control experiments showed that the *N*,*N*′-dioxide **7** is an efficient quencher of the type-I photooxidation of **dG** and DNA by the photoproduct of disulfide **3** (Figures 9 and 10) and also by the authentic betain **8**. In fact, the *N*,*N*′-dioxide **7** also inhibits **dG** consumption by the established type-I photooxidants benzophenone[29] and menadione[30] (Figure 9), which convert guanine to its radical cation by

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(28) The formation of the *N*,*N*′-dioxide **7** is mechanistically rationalized in the literature.[4a,b] The key step is H abstraction from the *N*-hydroxypyridinethione **1a** or its thiol tautomer, a reaction that cannot occur for the *N*-alkoxy or acyloxy derivatives **1b**−**d**.
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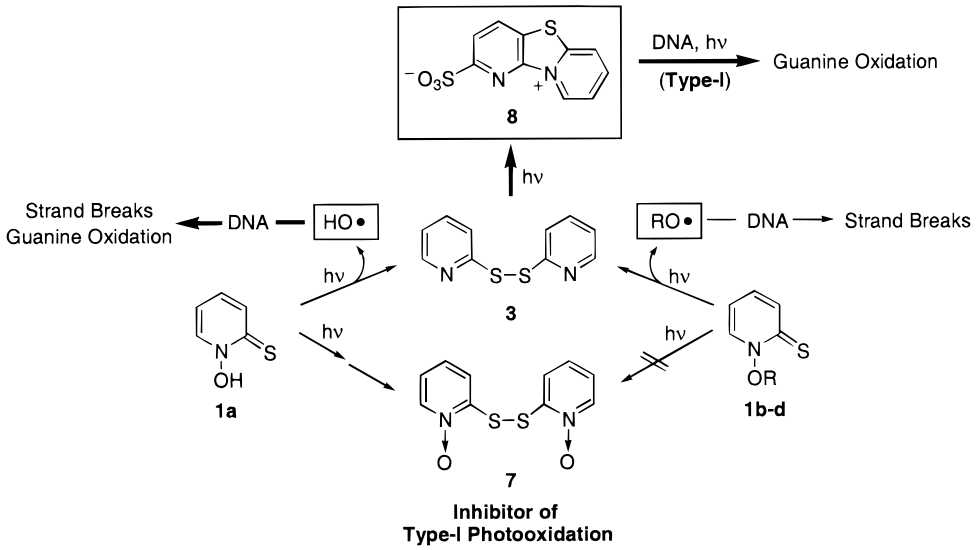
**Scheme 4:** Comparative DNA photooxidation of the pyridinethiones **1a**−**d** by oxyl radicals and betain **8**, and the inhibitory effect of the *N*,*N*′-dioxide **7**




electron transfer. Therefore, we propose that the *N*,*N*′-dioxide **7** serves as a competitive electron donor and protects **dG** and DNA against the type-I photooxidation by the betain **8,** and in turn by the disulfide **3**, since the latter is the photochemical source for the betain **8**. Consequently, for the *N*-hydroxypyridine-2-thione (**1a**), the photoproduct *N*,*N*′-dioxide **7** suppresses the catalytic type-I photooxidation of DNA and the dominant process is the less efficient stoichiometric oxidation by the released hydroxyl radicals. In contrast, for the pyridinethiones **1b**−**d**, for which the *N*,*N*′-dioxide **7** is not formed, the catalytic photosensitized guanine oxidation by the betain **8** generated in the photolysis of **1b**−**d** overshadows the oxyl-radical activity. In contrast, as reported earlier,[17] for the formation of strand breaks, oxyl radicals manifest themselves as the active species in the photolysis of all the pyridinethiones **1a**−**d** because type-I-photooxidation reactions do not significantly induce strand breaks.[31] Furthermore, the short irradiation period (25 min) employed for the DNA-cleavage experiments[9] does not form any detectable amount of the betain **8**.

The above-mentioned complex mechanistic features of the photooxidative DNA damage by the pyridinethiones **1a** versus **1b**−**d** are compared in Scheme 4. Two photochemical processes run alongside one another, namely the release of oxyl (hydroxyl, alkoxyl, acyloxyl) radicals[4,9,13] and the formation of the betain **8** from the disulfide **3**, and the latter is a primary photolysis product of the pyridinethiones. DNA damage (base oxidation and strand breaks) by oxyl radicals is a stoichiometric process, i.e., for every photooxidative event at least one pyridinethione molecule is consumed. In contrast, the betain **8**, produced in the photolysis of the pyridinethiones, acts through a photocatalytic process (type-I photooxidation), which is inhibited in the case of *N*-hydroxypyridinethione **1a** by the *N*,*N*′-dioxide **7**. Consequently, the stoichiometrically formed hydroxyl radicals in the photolysis of the hydroxy-substituted **1a** dominate the photooxidation of dG and DNA. The photocatalytic efficiency of the betain **8** is rather poor, since only six times more dG is converted by this photosensitizer, and the latter is decomposed on prolonged irradiation (cf. Figure 8).

Our present results establish that the pyridinethiones **1** not only release oxyl radicals[4,7,9,13] but also generate disulfide-derived secondary photoproducts such as the betain **8** (electron-transfer photooxidant) and the *N*,*N*′-dioxide **7** (electron-transfer inhibitor), which have a profound effect on the photooxidation of **dG** and DNA. Caution should be exercised when these photochemical oxyl-radical sources are employed to assess the efficiency of oxyl radicals in the oxidative damage of biological substrates.

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**Supporting Information Available:** Experimental details for the photolysis studies, the oxidation of *calf thymus* DNA, the modification of 2′-deoxyguanosine and the HPLC procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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