

Article

Tricyclic Analogs of Thioguanine as Photosensitizers of Reactive Oxygen Species-Induced DNA and RNA Damage

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Abstract

Analogues of tricyclic thiopurine nucleosides combine structural features of endogenous DNA adducts with efficient photosensitizing chromophores, making them valuable models for studying nucleic acid damage induced by reactive oxygen species (ROS). In this work, we investigate the photochemical properties of two tricyclic guanosine derivatives, 9-thio-1,N²-ethenoguanosine and 6-methyl-9-thio-1,N²-ethenoguanosine, under UVA irradiation. We characterize their excited-state behavior, their ability to generate singlet oxygen (¹O₂) and superoxide radicals (O₂^{•−}), and the resulting oxidative transformation pathways. Both compounds are photochemically stable under anaerobic conditions but undergo efficient oxygen-dependent phototransformation, yielding a diverse set of oxidative and dimeric photoproducts. Product analysis reveals that singlet oxygen mediates desulfurization, ring opening, and extensive sulfur oxidation, whereas radical pathways involving superoxide lead exclusively to dimer formation. Importantly, the triplet excited states of these tricyclic thiopurines are not quenched by natural nucleosides, allowing both Type I and Type II photosensitizing pathways to operate in nucleic-acid-like environments. These results provide molecular-level insight into ROS-induced purine damage and highlight tricyclic thiopurines as effective photosensitizers of oxidative DNA and RNA damage.

Keywords: photosensitizer of reactive oxygen species; singlet oxygen; tricyclic thiopurine derivatives

1. Introduction

Reactive oxygen species (ROS) play a key role in the chemical modification of nucleic acids under oxidative stress [1,2]. Among them, singlet oxygen (¹O₂), peroxide (O₂^{•−}), and other radicals generated by UVA-induced photosensitization are particularly important [3,4]. These species cause DNA/RNA damage, induce inflammation, and are responsible for the phototoxic side effects of drugs that can act as photosensitizers when exposed to light [5,6]. Oxidative transformations of purine bases lead to a wide range of DNA damage. The products of these reactions include oxopurines, etheno adducts, and cross-links formed between two nucleosides through covalent bonds [7–9]. Many of these lesions interfere with replication and repair processes, thereby contributing to disease progression [10,11].

Purines with sulfur instead of oxygen at position 6 of the purine ring constitute a biologically important class of modified nucleic acids [12,13]. Clinically used thiopurine



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drugs, such as 6-thioguanine and its nucleoside derivatives, can be incorporated into DNA and RNA, where they act as endogenous UVA-absorbing chromophores [14]. Upon photoexcitation, these compounds generate long-lived triplet states that sensitize ROS formation, leading to DNA/RNA damage [15]. Significantly, the photochemical behavior of thiopurines is strongly influenced by their molecular environment, including natural nucleosides, which effectively quench the triplet state of classical thiopurines, thereby limiting their photosensitizing effect in biological systems [16].

Guanine 1, N^2 -etheno adducts constitute another critical class of DNA damage and have been detected in human DNA [17]. They form when 2'-deoxyguanosine in DNA reacts with α,β -unsaturated aldehydes, which can originate from both endogenous and exogenous sources. These tricyclic modifications arise endogenously as a result of lipid peroxidation and oxidative stress and are considered mutagenic due to their altered base-pairing properties and limited repair efficiency [18,19]. For this reason, DNA adducts are considered potential biomarkers of diseases caused by oxidative stress. Furthermore, monitoring their levels in the body can be used to predict disease progression [20,21].

In this study, we focused on a new class of guanosine analogs containing an additional five-membered ring and a sulfur atom at the C-(9) position, namely 9-thio-1, N^2 -ethenoguanosine (TEGuo) and 6-methyl-9-thio-1, N^2 -ethenoguanosine (6MeTEGuo) (Figure 1). These compounds combine two independently significant structural motifs: a thiocarbonyl group, responsible for efficient formation of the triplet state and, consequently, for the generation of ROS, and a tricyclic etheno structure characteristic of endogenous DNA adducts. This combination is expected to have a profound effect on the dynamics of the excited state, the efficiency of ROS generation, and oxidative reactions.

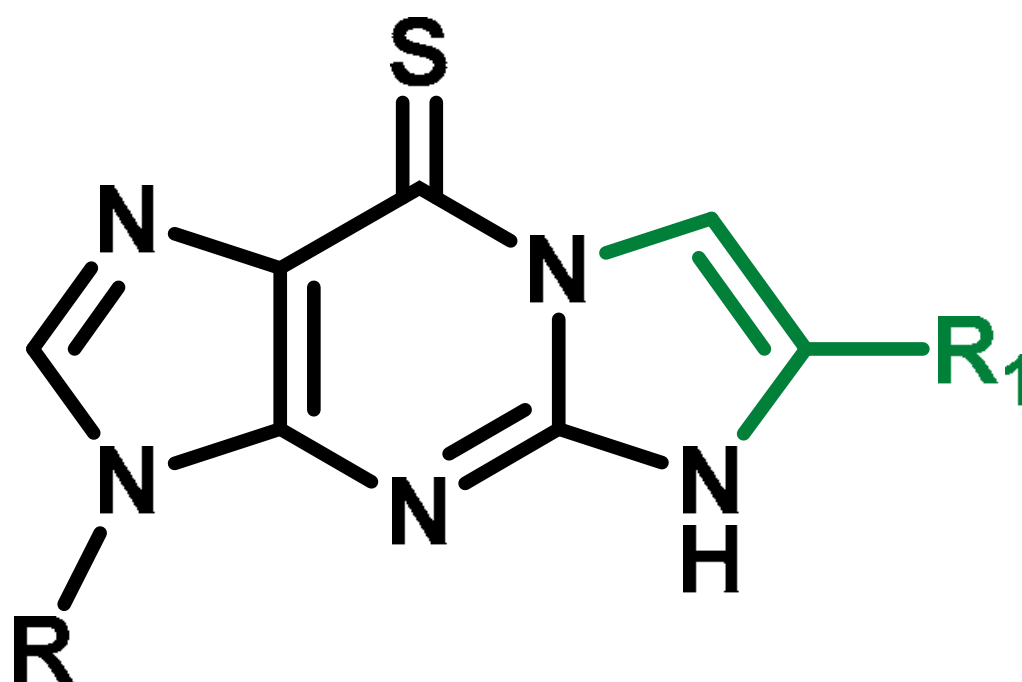


Figure 1. Structures of guanosine analogs containing an additional five-membered ring and a sulfur atom at the C-(9) position: (1) 9-thio-1, N^2 -ethenoguanosine (TEGuo), where R = β -D-ribose, R_1 = H; (2) 6-methyl-9-thio-1, N^2 -ethenoguanosine (6MeTEGuo), where R = 2',3',5'-tri-O-acetyl- β -D-ribose, R_1 = CH_3 .

In this work, we present the photochemical characterization of TEGuo and 6MeTEGuo in an aqueous environment. We investigated the ability of these compounds to generate singlet oxygen, the susceptibility of their triplet excited states to quenching by natural nucleosides, and proposed mechanisms for the formation of photoproducts generated

by singlet oxygen or radicals. By correlating the properties of the excited state with ROS-specific reaction pathways, this study aims to provide molecular-level information on ROS-induced purine damage and to evaluate the potential of tricyclic thiopurines as photosensitizers for DNA/RNA modification.

The thiopurine nucleoside analogs studied constitute a unique class of DNA-associated chromophores in which photoinduced ROS generation can aid in elucidating the mechanisms underlying processes that lead to DNA/RNA damage. We will discuss our results on two modified thiopurine nucleosides that can serve as models for studying oxidative DNA damage and lesion formation under controlled conditions.

2. Materials and Methods

2.1. Chemicals

All reagents and solvents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Synthesis

The compounds described in this work were synthesized in a two-step procedure. First, a tricyclic linear structure was obtained by reacting guanosine with chloroacetaldehyde or bromoacetone. Depending on the reagent, tricyclic guanosine analogs with different substituents at the C-(6) position were formed. Second, thionation of the synthesized compounds afforded the final tricyclic thiopurine analogs: TEGuo (1, N²-etheno-9-thioguanosine) and 6MeTEGuo (6-methyl-1, N²-etheno-9-thioguanosine) (see Figure 1). The detailed synthesis and identification of the compounds were reported in a previous work [22].

2.3. Steady-State Spectroscopy

UV-Vis absorption spectra were recorded using a Cary 100 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) in 1 cm × 1 cm quartz cells. The spectra were recorded with 1 nm resolution over the 220 to 550 nm interval to obtain spectra around the maximum λ for each compound studied.

2.4. Steady-State Irradiation

All experiments were carried out in a 1 × 1 cm rectangular cell at room temperature. For irradiation in the absence of oxygen, solutions were deaerated for approximately 30 min using an argon flow to remove traces of O₂; otherwise, the solutions were irradiated in an open-air cell (air-equilibrated). Two irradiation systems were used: a Genesis CX355STM OPSL laser from Coherent (Coherent—Rheinland-Pfalz, Germany) with a 355 nm emission wavelength and a ThorLabs LED (ThorLabs Sweden AB, Mölndal, Sweden) with 365 or 505 nm emission (output power was set to 20 mW). The course of the photoreaction was monitored by measuring the absorption spectrum and by HPLC analysis. The concentrations of substrate and photoproducts were determined by HPLC analysis. The photoproducts were identified by comparing their HPLC retention times and UV-Vis absorption spectra with those of a standard reference, and MS spectra.

2.5. HPLC Method

The purity of all samples was monitored by an HPLC method. Irradiation mixtures were analyzed on an Agilent 1200 Rapid Resolution UHPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a reversed-phase column (Eclipse Plus C18, 5 mm,

4.6 × 250 mm). A gradient of acetonitrile in water served as the eluent. The water was doubly distilled and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.6. Analysis of UPLC-MS Data

The irradiated products were characterized and quantified by UPLC-MS. The prepared samples (10 µL) were injected into a reversed-phase C18 column (Zorbax ODS 5 µm; 3.0 × 250 mm; Agilent Technologies (Santa Clara, CA, USA)) maintained at 30 °C and eluted with a gradient of buffers A (0.1% *v/v* formic acid in water) and B (80% *v/v* acetonitrile with 0.1% formic acid in water) at a flow rate of 0.2 mL min^{−1} for a total duration of 25 min. The gradient consisted of: 2% B for 2 min, 20% B for 3 min, an increase to 95% B over 7 min, and a return to 2% B over 1 min, followed by equilibration at 5% B for 5 min until the end of the run. The eluted materials were then introduced into a Bruker Impact II Q-TOF equipped with an electrospray ionization (ESI) source operating in positive-ion mode. Mass spectra were collected at 1 Hz over the range of 50–1500 *m/z*. The ESI source operating conditions were as follows: capillary voltage 4.5 kV; temperature 250 °C; and dry N₂ gas 5 L min^{−1}. LC-MS data were collected using Bruker Compass/Hystar software (version 1.4) and processed using Bruker Quant Analysis software (version 1.4, Bruker Bremen, Germany).

2.7. Quantum Yield of the Photochemical Reaction

To determine the quantum yield of the photochemical reaction, a sample of the investigated substance (*c* ≈ 0.1 mM in water) was irradiated in a quartz cuvette with an optical path length of 1 cm using a Coherent Genesis CX355STM OPSL laser (Coherent—Rheinland-Pfalz, Germany) diode operating at a wavelength of 355 nm (power: 40 mW). Irradiation was carried out in a cuvette under continuous stirring. For experiments conducted in the absence of oxygen, the solution was deoxygenated for 20 min before irradiation by bubbling with high-purity argon. The samples were irradiated until approximately 10% (30 s) of the substrate was consumed; for the deoxygenated sample, irradiation was continued for two hours. The progress of the reaction was monitored by measuring the absorption spectrum. The substrate concentration before and after irradiation was determined by HPLC analysis.

The quantum yield of the photochemical reaction was calculated using the following Equation (1):

$$\Phi_{PR} = \Delta c / (I_a t) \quad (1)$$

where Φ_{PR} is the quantum yield of the photochemical reaction; Δc is the difference in substrate concentration before and after irradiation; I_a is the intensity of absorbed radiation, equal to 7.5×10^{-6} Einst dm^{−3} s^{−1} for a power of 20 mW and 15.0×10^{-6} Einst dm^{−3} s^{−1} for a power of 40 mW; and *t* is the irradiation time. The intensity of absorbed radiation was determined using Equation (2):

$$I_a = I_0 (1 - 10^{-A_\lambda}) \quad (2)$$

where I_a is the intensity of absorbed radiation [Einst dm^{−3} s^{−1}], and I_0 is the intensity of incident light [Einst dm^{−3} s^{−1}], A_λ is the absorbance at the wavelength of the incident radiation.

2.8. Laser Flash Photolysis (LFP) Experiments

The setup for the nanosecond laser flash photolysis (LFP) experiments and its data acquisition system have previously been described in detail [23]. The excitation source for LFP measurements was a pulsed Nd:YAG laser ($\lambda = 355$ nm, 3 mJ). Transient decays (averaged over 10 pulses) were measured at different wavelengths using the step-scan

method with a 10 nm step size across the range 320–800 nm. Measurements were performed on both argon-saturated and air-equilibrated H₂O solutions.

2.9. The Triplet-State Quenching in the Presence of Nucleosides

For a series of tested compounds, measurements of triplet-state quenching in the presence of purine and pyrimidine nucleosides were performed. A series of aqueous solutions of the tested compounds (TEGuo, 6MeTEGuo) at 10^{−5} M concentration, containing selected acetylated nucleosides (2',3',5'-tri-O-acetyl-adenosine (taAde), 2',3',5'-tri-O-acetyl-thymidine (taThy), 2',3',5'-tri-O-acetyl-uridine (taUrd), and 2',3',5'-tri-O-acetyl-guanosine (taGuo)) at varying concentrations (taAde: 0.000–0.001 M; taThy: 0.00–0.02 M; taUrd: 0.00–0.02 M; taGuo: 0.000–0.005 M) were prepared. The prepared samples were deoxygenated for 20 min before measurement. Transient absorption decay ($\lambda_{exc} = 355$ nm) was measured at wavelengths corresponding to the transient absorption maxima of the tested compounds. To determine the quenching rate constants of the excited triplet state (T_1), the relationship between the triplet lifetime ($1/\tau_T$) of the tested compounds and the concentration of quenching nucleosides was used.

2.10. Synthesis and Characterization of Sulfonic Acid

The sulfonic acids TEGuo and 6MeTEGuo were synthesized by reacting with KMnO₄ according to the procedure described in [24]. The substrate was dissolved in 3 mL of PBS (pH = 7.4) ($c = 9.1 \times 10^{-5}$ M), and 30 μ L of an aqueous KMnO₄ solution ($c = 1.82 \times 10^{-4}$ M) was added. After 10 min of reagent mixing, the product was isolated by HPLC. The synthesized sulfonic acid was isolated from the reaction mixture and was analyzed by LC-MS. The sulfonic acid was characterized by measuring the emission quantum yield, which was 0.1 in water ($\lambda_{exc} = 390$ nm, $\lambda_{em} = 510$ nm; fluorescence quantum yield determined relative to perylene in cyclohexane). The absorption range of ϵ GuoSO₃H (260–450 nm) allows it to be excited by the radiation source used in TEGuo/6MeTEGuo irradiation; therefore, its reactivity was assessed under the same conditions. Sulfonic acid ($A_{355} = 0.5$) was irradiated in water in the presence of oxygen ($\lambda_{exc} = 355$ nm, excitation source power 40 mW). The course of the reaction was monitored spectrophotometrically at 5 min intervals. No loss of substrate was observed even after 45 min of irradiation (HPLC analysis). A sample of ϵ GuoSO₃H left at room temperature for 24 h also showed stability. We also carried out irradiation of ϵ GuoSO₃H in the presence of RB (Rose Bengal) in water, selectively exciting only the singlet oxygen sensitizer ($\lambda_{exc} = 505$ nm, 45 min irradiation). No product formation was observed during irradiation, indicating that the sulfonic acid does not react with singlet oxygen under the experimental conditions employed.

3. Results

3.1. Photochemical Stability of Thiopurine Etheno Adducts Under Anaerobic Conditions

The photochemical reactivity of TEGuo and 6MeTEGuo was first examined under anaerobic conditions in H₂O and PBS over the concentration range 2.0×10^{-5} – 1.0×10^{-4} M. Samples were irradiated with either a 355 nm laser or a 365 nm LED. Monitoring the reaction progress by UV-Vis spectroscopy revealed no detectable changes in the absorption spectra, even after prolonged irradiation times of up to 4 h. HPLC analysis indicated a minor substrate loss of approximately 5% (4 h irradiation), corresponding to very low quantum yields of photochemical transformation ($\Phi \approx 1.1 \times 10^{-5}$) for both compounds. Importantly, no new peaks attributable to the photoproducts were observed. Furthermore, irradiation experiments performed in the presence of natural purine and pyrimidine nucleosides (adenosine, guanosine, thymidine, and uridine) under identical anaerobic conditions showed no evidence of photocross-linking or intermolecular photoadduct formation. This behavior clearly distin-

guishes TEGuo and 6MeTEGuo from classical thionucleobases, such as 6-thiopurine, which readily undergo [2 + 2] photocycloaddition reactions with nucleosides under oxygen-free conditions [25]. Previous studies have demonstrated that thiopurine reactions proceed from the lowest excited triplet state (T_1) [17,26]. Therefore, we examined whether the triplet states of the tricyclic nucleosides investigated in this study were quenched by naturally occurring components of nucleic acids. Owing to their better solubility, acetylated analogs of uridine (taUrd), adenosine (taAde), thymidine (taThy), and guanosine (taGuo) were used in this study.

Deoxygenated solutions of TEGuo/6MeTEGuo ($c = 1.3 \times 10^{-5}$ M) containing quenchers at concentrations from 0 to 0.02 M were prepared. Transient absorption decays at 600 nm were recorded for a series of solutions using laser flash photolysis. None of the nucleosides affected the excited triplet-state lifetime of the tricyclic thiopurine derivatives. This indicates that under anaerobic conditions, nucleosides do not quench the triplet excited state of the investigated compounds and therefore do not influence processes involving the triplet excited state (Figure 2a). For comparison, analogous measurements were performed under identical experimental conditions for 6-thioguanosine. In this case, quenching was observed; for example, the quenching rate constant, $k_q = 1.8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, was determined for taThy (Figure 2b).

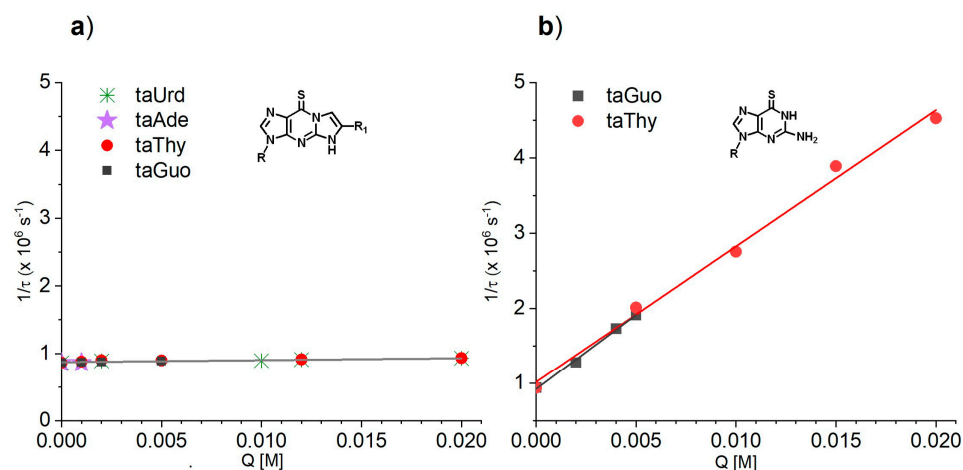


Figure 2. Stern–Volmer plots of the reciprocal of the triplet lifetime ($1/\tau_T$) of TEGuo/6MeTEGuo (a) and 6-thioguanosine (b) versus quencher concentration.

Canonical nucleosides do not quench the triplet excited state T_1 of TEGuo/6MeTEGuo via a triplet–triplet energy transfer mechanism because the T_1 energies of natural nucleosides are estimated to be higher than those of TEGuo and 6MeTEGuo. In addition, DNA/RNA nucleosides do not quench the triplet excited state T_1 of TEGuo/6MeTEGuo through a photochemical reaction, unlike 6-thioguanosine (Figure 2b).

3.2. Oxygen-Dependent Phototransformation and ROS Generation

Published research on thiopurines and etheno analogs of guanosine indicates that reactive oxygen species can react with both the thiocarbonyl group present in purine thioanalogs and the $1N^2$ -etheno bridge [27–29].

Despite their high stability under anaerobic conditions, both TEGuo and 6MeTEGuo undergo efficient photochemical transformation under UVA irradiation in the presence of molecular oxygen. Irradiation in water or PBS (pH 7.4) caused a progressive decrease in the characteristic thiocarbonyl absorption band at $\lambda \approx 352$ nm, accompanied by the appearance of new absorption features in the 280–330 nm range and near 410 nm (Figure S1 in the Supplementary Materials (SM)). These spectral changes were independent of the excita-

tion source and solvent, indicating similar photochemical pathways under all conditions tested. The progress of irradiation ($\lambda_{\text{exc}} = 355 \text{ nm}$) was monitored by HPLC analysis. The first analysis after 30 s showed about 10% substrate loss and the formation of six major photoproducts for TEGuo and five for 6MeTEGuo. Radiation was applied until 70% of the substrate had disappeared. With increasing irradiation time, the concentrations of all previously recorded products increased (Figure 3). It is important to note that no new products were generated during prolonged irradiation.

The quantum yields of the photochemical transformation, as determined by substrate depletion monitored by HPLC, were $\Phi = 0.014$ for TEGuo and $\Phi = 0.012$ for 6MeTEGuo.

Relative product yields were estimated based on substrate decomposition and relative HPLC peak areas measured at a wavelength of 280 nm, taking into account differences in molar absorption coefficients. The individual photoproducts were estimated to form with the following chemical yields (by HPLC): **1** (7%), **2** (22%), **3** (8%), **4** (17%), **5** (7%), and **6** (28%), collectively accounting for approximately 89% of the substrate consumption. Thus, the Type I and Type II photosensitizing pathways are estimated to occur with comparable contributions. Irradiation of TEGuo and 6TEGuo yields similar products; therefore, we will focus our discussion on the results obtained using TEGuo as an example, with the data for 6TEGuo provided in the Supplementary Materials (HPLC analysis of 6MeTEGuo and absorption spectra of products are presented in Figure S2 in the Supplementary Materials (SM)).

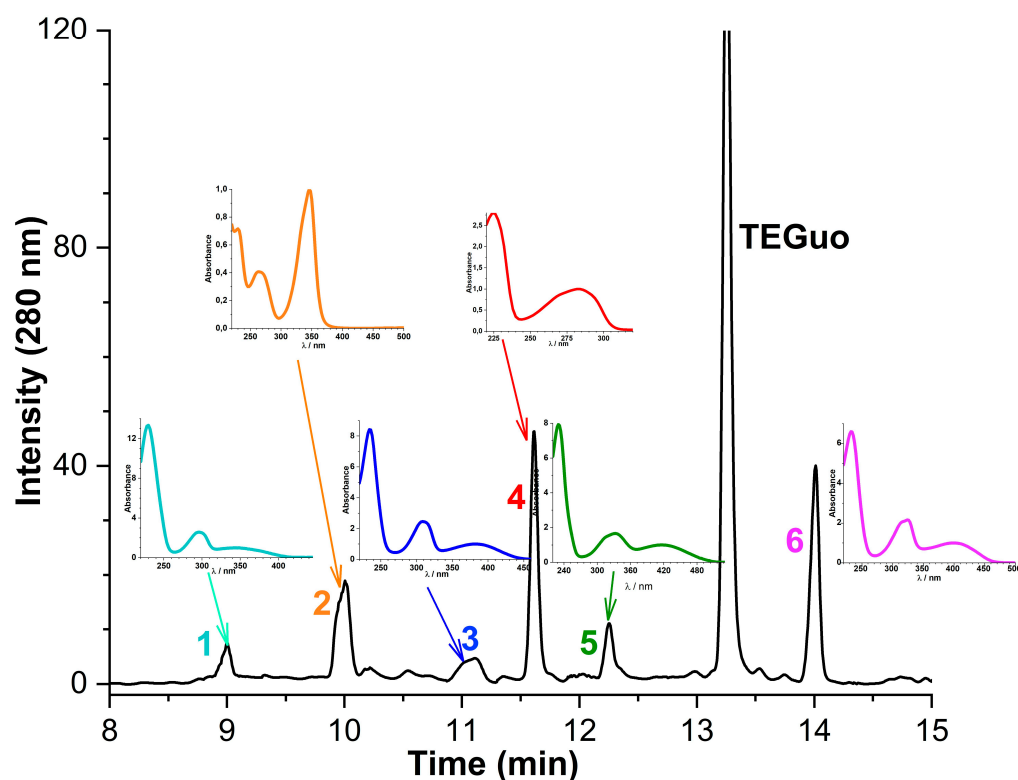


Figure 3. HPLC chromatogram of TEGuo phototransformation under UVA irradiation (in the presence of oxygen, recorded at 280 nm, irradiation time 200 s). Major photoproducts (1–6) and the remaining substrate are indicated; insets show normalized UV–Vis absorption spectra of the products.

For clarity and consistency, the numbering and color coding of photoproducts introduced in Figure 3 are maintained throughout the subsequent discussion.

3.2.1. Identification of Photoproducts Generated Under Aerobic Conditions

All photoproducts formed upon irradiation of TEGuo and 6MeTEGuo in the presence of oxygen were identified by LC–MS analysis, with results supported by comparison with available reference compounds. The experimentally determined m/z values showed excellent agreement with calculated masses, confirming the proposed molecular formulas and structures.

- Product 1: TEGuo/6MeTEGuo Desulfurization Product

Photoproduct 1 was identified as the desulfurized oxo analog, 3-(β -D-ribofuranosyl)-imidazo[1,2- α]purine (Figure 4 and Table 1). Although a commercial standard was unavailable, the UV–Vis absorption spectrum closely matched literature data for the analogous oxo-compound, supporting this identification [30]. The desulfurization product is an unexpected photochemical product, as it is not formed by photooxidation of most heteroaromatic thiocarbonyl compounds [13,31]. In our earlier work on the reactivity of the purine derivative 8-thioguanosine with reactive oxygen species (ROS) and free radicals, we discussed in detail the possibility of their formation [32].

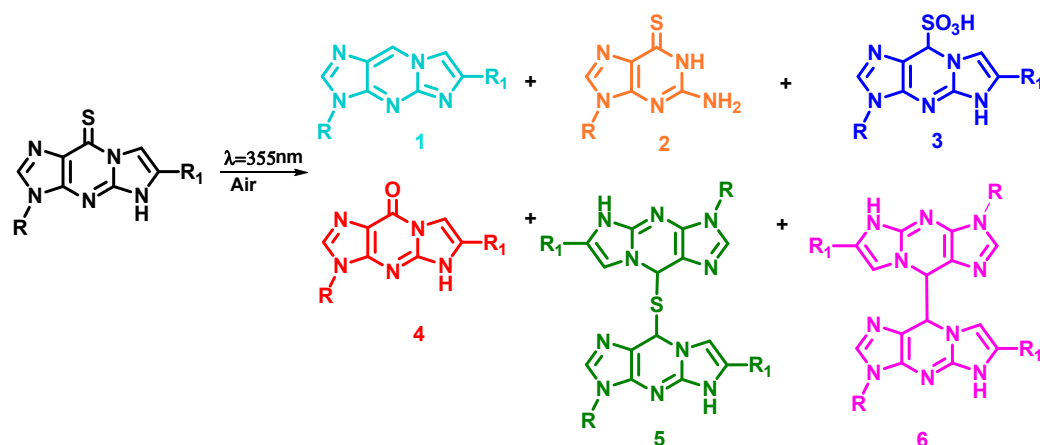


Figure 4. ROS-mediated photochemical transformation pathways of TEGuo under UVA irradiation, yielding oxidative and cross-link-type lesion analogs.

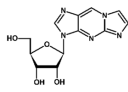
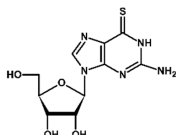
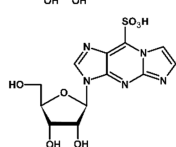
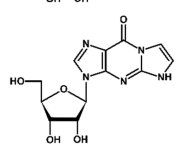
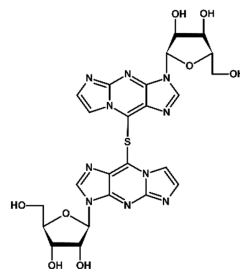
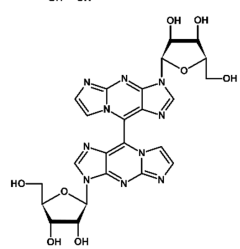
- Photoproduct 2: 6-Thioguanosine

Photoproduct 2 was identified as 6-thioguanosine by LC–MS analysis (Figure 4 and Table 1) and confirmed by co-elution and spectral comparison with a standard (see Supplementary Materials, Figure S3). The formation of this product indicates oxidative cleavage of the 1, N²-etheno bridge and represents a transformation that links tricyclic thiopurines to well-established thiopurine DNA lesions.

- Photoproduct 3: Sulfonic Acid Derivative

Photoproduct 3 was identified as the sulfonic acid derivative (ϵ GuoSO₃H) (Figure 4 and Table 1), formed by extensive oxidation of the thiocarbonyl group. This assignment was confirmed by LC–MS and by comparison of retention time in HPLC analysis and UV-vis spectrum with a synthetically prepared reference compound (see Methods and Materials, Section 2.9). The sulfonic acid exhibited strong fluorescence ($\Phi_F = 0.15$) in H₂O ($\lambda_{\text{max,em}} \approx 510$ nm) and exceptional photostability; it did not undergo further photochemical reactions (see Supplementary Data, Figure S4), suggesting that such highly oxidized sulfur-containing lesions may persist under oxidative conditions. Sulfonic acid formation represents extensive oxidation of the thio group and corresponds to highly polar, structurally disruptive DNA lesion analogs. The high photostability of ϵ GuoSO₃H suggests that this lesion could accumulate under oxidative conditions.

Table 1. Photoproducts identified by LC–MS analysis following UVA irradiation of TEGuo in the presence of oxygen, with corresponding m/z values, calculated masses, and proposed structures.

Photoproduct	m/z (ESI-MS)	Calculated Mass	Structure
1	292.1047	292.1046 ($C_{12}H_{13}N_5O_4 + H^+$)	
2	322.0584	322.0586 ($C_{10}H_{13}N_5O_4S + Na^+$)	
3	372.0611	372.0614 ($C_{12}H_{13}N_5O_7S + H^+$)	
4	308.0993	308.0995 ($C_{12}H_{13}N_5O_5 + H^+$)	
5	613.1586	613.1578 ($C_{24}H_{24}N_{10}O_8S + H^+$)	
6	581.1865	581.1857 ($C_{24}H_{24}N_{10}O_8 + H^+$)	

- Photoproduct 4: Oxygen Analog (1, N²-Ethenoguanosine)

Photoproduct 4 was identified as 1, N²-ethenoguanosine, the oxygen analog of TEGuo/6MeTEGuo (Figure 4 and Table 1). Its identity was confirmed by comparison with an independently synthesized reference compound (Supplementary Materials, Figure S5). Notably, 1, N²-ethenoguanosine is a well-known endogenous DNA adduct formed during lipid peroxidation, providing a direct mechanistic link between photoinduced oxidation and biologically relevant DNA damage [21,33].

- Products 5 and 6: radical reaction products

Photoproduct 5 was assigned as a sulfur-bridged dimer and photoproduct 6 as a C(9)–C(9) coupled purine dimer, based on LC–MS data (Figure 4 and Table 1). Although reference standards were unavailable, the observed masses and proposed structures are consistent with radical–radical coupling. These dimeric products structurally resemble cross-link-type and clustered DNA lesions, which are among the most cytotoxic and repair-resistant forms of DNA damage [15,34].

The identified products differ significantly in structure (Figure 4 and Table 1), which supports our observation that different reactive oxygen species may be involved in their formation. In the case of 6MeTEGuo, five products were formed, and we did not observe

sulfur-bridged dimer formation (Supplementary Data Figure S4 and Table S1). We cannot rule out the possibility that this product formed during irradiation, but its concentration was too low to be detected in the mixture analyzed.

3.2.2. Discrimination Between Singlet Oxygen and Superoxide-Mediated Pathways

To clarify the roles of specific reactive oxygen species, selective photosensitization experiments were conducted using Rose Bengal (RB) as a singlet oxygen generator. Under these conditions, photoproducts 1–4 were readily formed (Figure 5), whereas products 5 and 6 were not detected. These results demonstrate that desulfurization, ring opening, and sulfur oxidation are mediated predominantly by singlet oxygen (Type II photosensitization). As shown in Figure 5b, the absorption spectra of products 1–4 are identical in both TEGuo reactions, with and without RB.

In contrast, dimer formation proceeds exclusively via radical pathways involving superoxide radical. Upon UVA irradiation (355–400 nm), TEGuo/6MeTEGuo generated both singlet oxygen ($^1\text{O}_2$) (Type II photosensitization) and superoxide anion radicals ($\text{O}_2^{\bullet-}$) (Type I photosensitization). Consequently, the reaction pathway, product selectivity, and overall efficiency of photoproduct formation are strongly dependent on the nature of the ROS involved. Understanding how photosensitizers control $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$ generation is essential for elucidating mechanisms of DNA and RNA damage. The present results provide a mechanistic framework for the formation of the identified photoproducts. It is important to note that the lack of quenching of the excited triplet T_1 state TEGuo/6MeTEGuo by natural nucleosides means that both ROS formation pathways are possible in the presence of a sensitizer and nucleic acids.

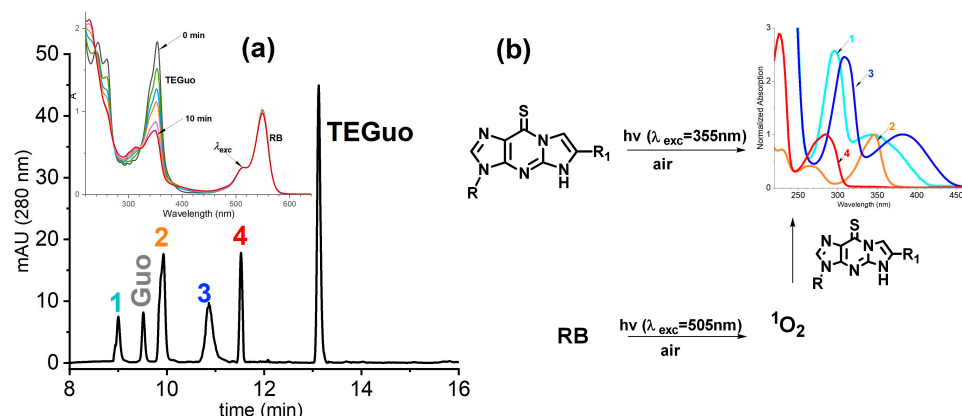


Figure 5. (a) Changes in the absorption spectra of a TEGuo solution during irradiation in the presence of Rose Bengal (RB), along with HPLC analysis of TEGuo phototransformation under selective singlet oxygen photosensitization. Chromatogram recorded at 280 nm after 10 min of UVA irradiation ($\lambda_{\text{exc}} = 505 \text{ nm}$). (b) The reaction mechanism showing the formation of the same products 1–4 during irradiation of TEGuo ($\lambda_{\text{exc}} = 355 \text{ nm}$) and of TEGuo in the presence of RB ($\lambda_{\text{exc}} = 505 \text{ nm}$), resulting from reaction with $^1\text{O}_2$. In the inset, the absorption spectra of the products obtained upon irradiation of TEGuo in the presence and absence of Rose Bengal were compared. The absorption spectra of photoproducts 1–4 formed via both pathways fully overlap in spectral range and shape, indicating that the products are identical.

4. Discussion

Mechanistic Implications and Relevance to Oxidative DNA Damage Chemistry

Upon UVA excitation, nucleosides modified with a thiocarbonyl group efficiently populate long-lived triplet excited states and sensitize singlet oxygen via triplet–triplet energy transfer. Mechanistically, oxidative pathways in nucleic acid systems involve both Type II reactions mediated by singlet oxygen ($^1\text{O}_2$) and Type I electron-transfer processes

that generate radical and peroxide intermediates [35]. Singlet oxygen is a key mediator of oxidative DNA damage, with well-established mechanisms of guanine oxidation and the formation of associated lesions. In agreement with this framework, the tricyclic thiopurine TEGuo/6MeTEGuo was found to generate both $^1\text{O}_2$ and $\text{O}_2^{\bullet-}$, as evidenced by the nature of the photoproducts identified in this study.

Singlet oxygen reacts preferentially with the electron-rich thiocarbonyl group ($\text{C}=\text{S}$), initiating oxidation. This reaction proceeds via concerted or stepwise addition, yielding transient sulfur–oxygen peroxide intermediates. Fragmentation or rearrangement of these unstable intermediates leads to desulfurization and the formation of oxo-purine derivatives, as well as ring-opened products resulting from cleavage of the 1, N^2 -etheno moiety (Figure 6) [12,29,32]. These reactions constitute a form of direct oxidative base damage and are mechanistically analogous to canonical singlet oxygen-mediated oxidation of guanine.

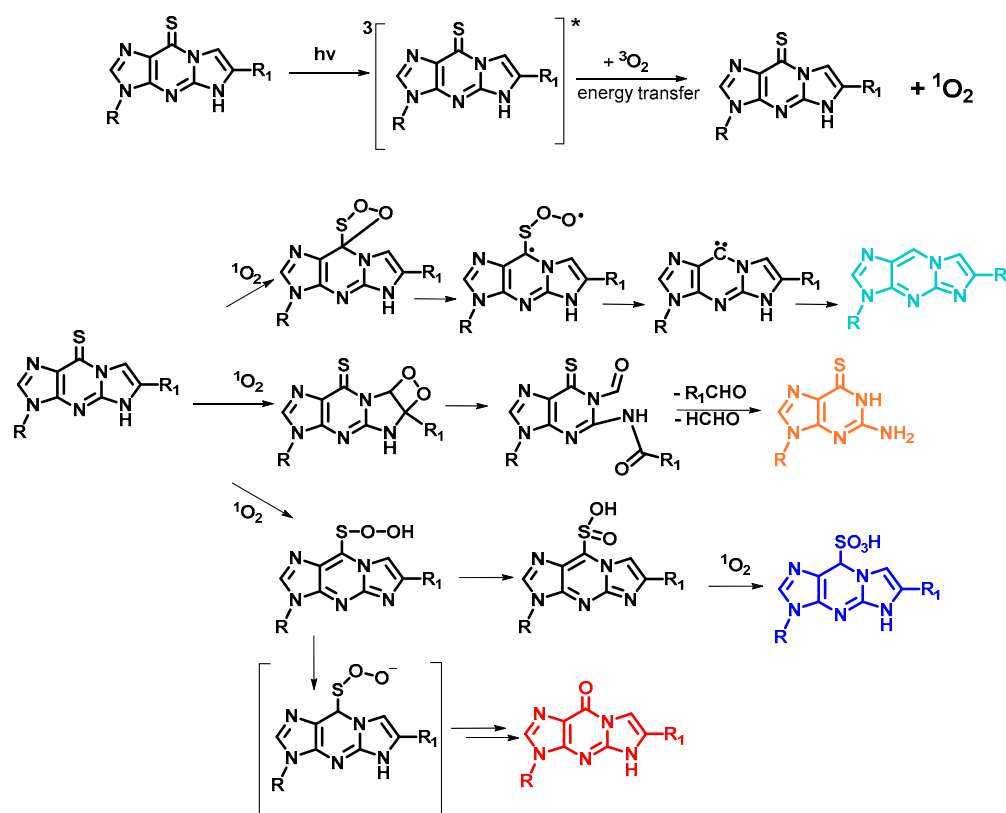


Figure 6. Proposed mechanisms for the formation of products from the interaction of $^1\text{O}_2$ with TEGuo/6MeTEGuo. Singlet oxygen reacts with the thiocarbonyl group and the 1, N^2 -etheno bridge, leading to desulfurization, ring opening, and sulfur oxidation through short-lived peroxide and hydroperoxide intermediates (* indicates the excited triplet state T_1).

Singlet oxygen can induce stepwise oxidation of the sulfur atom. These transformations proceed through transient peroxide intermediates, yielding sulfonated nucleosides that are highly polar, photochemically stable, and structurally disruptive, thereby strongly perturbing hydrogen bonding and base-stacking interactions.

These features suggest that sulfonic acid derivatives may represent persistent DNA lesion analogs that accumulate under oxidative stress conditions.

Alongside these singlet oxygen-driven processes, a competing Type I pathway operates via photoinduced electron transfer from the triplet excited state to molecular oxygen. This reaction generates superoxide anion radicals ($\text{O}_2^{\bullet-}$) together with sulfur-centered or carbon-centered radicals localized on the purine scaffold, particularly at the C(9) position (Figure 7). Sulfur-centered radicals are highly reactive and rapidly add molecular oxygen to form short-

lived sulfur-peroxyl radicals (RSOO•) [36,37]. These intermediates can undergo further oxidation, fragment with extrusion of sulfur dioxide, or participate in radical–radical coupling reactions.

Radical coupling between carbon- or sulfur-centered species leads to the formation of dimer photoproducts, including C(9)–C(9) linked purine dimers and sulfur-bridged dimers (Figure 7). Such products are characteristic of radical-mediated chemistry and are structurally analogous to clustered and cross-linked DNA lesions. These lesion types are among the most cytotoxic forms of oxidative DNA damage, as they severely distort DNA structure and pose significant challenges to cellular repair machinery.

Both TEGuo and 6MeTEGuo effectively generated singlet oxygen and superoxide radicals upon UVA excitation, indicating that the introduction of a methyl substituent at the C(6) position did not significantly alter the overall photosensitizing behavior of these tricyclic thiopurine derivatives. The similarity in the nature and distribution of the photoproducts formed from both compounds suggests that their excited-state dynamics, including the intersystem crossing (ISC) efficiency and triplet-state reactivity, are primarily governed by the thiocarbonyl group. Although minor quantitative differences in photoreactivity cannot be excluded, no qualitative differences in the reactive oxygen species (ROS) generation pathways were observed under the experimental conditions. Differential mechanistic pathways in photosensitized oxidation by TEGuo/6MeTEGuo have been attributed to Type II singlet oxygen versus Type I radical processes, leading to superoxide and related ROS.

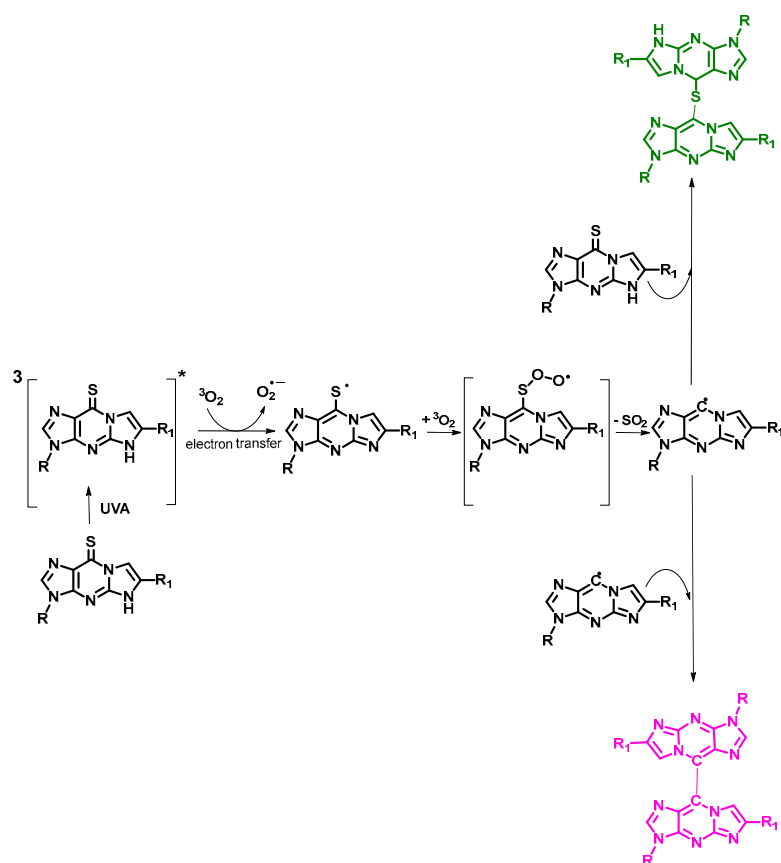


Figure 7. Proposed mechanisms of TEGuo degradation that lead to the formation of dimers induced by the peroxyl anion radical $\text{O}_2^{\bullet-}$, (* indicates the excited triplet state T_1)

5. Conclusions

The diversity of the identified products highlights the dual Type I/Type II photosensitizing action of tricyclic thiopurines. This provides molecular-level insight into how

photoactivated thiopurine analogs may contribute to oxidative DNA damage under UVA irradiation. Although the experiments were performed on isolated nucleoside models, the identified photoproducts closely resemble oxidative and cross-linking DNA lesions induced by ROS and previously described. Given the importance of both thiopurines and 1, N²-etheno adducts for DNA/RNA damage studies, the results provide a better understanding of ROS-induced purine damage and extend our knowledge of the photochemistry of nucleic acids under oxidative stress conditions. The photoproducts identified in the presence of singlet oxygen and superoxide radicals highlight fundamentally distinct oxidative pathways that lead to purine base damage. The findings notably indicate that tricyclic thiopurine analogs, representing a novel class of modified nucleosides, effectively generate reactive oxygen species upon exposure to UVA irradiation. Given prior study underscoring the clinical significance of thiopurine and other nucleoside-based photosensitizers, the compounds investigated in this study exhibit potential for use in photodynamic or photoinduced therapeutic strategies that employ controlled oxidative damage mechanisms.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biom16020275/s1>, Figure S1: Changes in the absorption spectra of (a) TEGuo and (b) 6MeTEGuo during irradiation; Figure S2: HPLC chromatogram of 6MeTEGuo phototransformation under UVA irradiation in the presence of oxygen; Table S1. Photoproducts identified by LC–MS analysis after UVA irradiation of 6MeTEGuo; Figure S3: Comparison of HPLC retention times and UV–Vis absorption spectra of standard and Photoproduct 2; Figure S4: Comparison of HPLC retention times and UV–Vis absorption spectra of standard and Photoproduct 3; Figure S5: Comparison of HPLC retention times and UV–Vis absorption spectra of standard and Photoproduct 4.

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