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Absorption Characteristics and Quantum Yields of Singlet Oxygen Generation of Thioguanosine Derivatives

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ABSTRACT

6–Thioguanine (1a) is considered to be photochemotherapeutic agents due to its specific characteristics of photosensitivity to UVA light and singlet molecular oxygen generation. To extend its phototherapeutic ability, two related thioguanines, 8–thioguanine (2a) and 6,8–dithioguanine (3a), have been designed and explored. Since the solubility of these thioguanines in dehydrated organic solvents is too poor to study, their tri–acetyl–protected ribonucleosides, i.e. 2′,3′,5′–tri–O–acetyl–6–thioguanosine (1c), 2′,3′,5′–tri–O–acetyl–8–thioguanosine (2c) and 2′,3′,5′–tri–O–acetyl–6,8–dithioguanosine (3c) were prepared and investigated. The absorption maxima of 1c, 2c and 3c in acetonitrile were found at longer wavelengths than that of un–thiolated guanosine (4c). Especially, 3c has the longest wavelength for absorption maximum and the highest value in terms of molar absorption coefficient among all thio–nucleobases and thio–nucleosides reported. These absorption properties were also well reproduced by quantum chemical calculations. Quantum yields of singlet oxygen generation of 2c and 3c were determined by near–infrared emission measurements to be as large as that of 1c. These results suggest that the newly synthesized thioguanosines, in particular 3c, can be further developed as a potential photosensitive agent for light–induced therapies.

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INTRODUCTION

6-Thioguanine (1a, see Scheme 1) and other thio–analogues of natural nucleobases have high affinity to proliferating cells, and some of them have been prescribed for the treatment of cancers, leukemia and angina among others (1–9). 1a can be converted into 6-thioguanosine (1b) through cellular metabolism, followed by being incorporated into RNA and DNA (1–6,10,11). O’Donovan et al. reported that 1a localizing in tumor cell generated reactive oxygen species (ROS) by its exposure to UV A light and thus cellular apoptosis was induced (8). These findings indicate that 1a and its nucleosides could be used as an effective medical tool for cancer treatment due to their unique properties as photochemotherapeutic drugs like 4-thiothymidine, including a photoactivatable genotoxic agent and a photosensitizer for photodynamic therapy (PDT), in addition to the hitherto known use as an anticancer medicine (5,9,12,13).

>Scheme 1<

As a photochemotherapeutic agent, the photosensitizer must be sensitive to the light penetrating into deep hypodermal tissue and can also effectively generate singlet oxygen (\(1^2O_2\)), one type of ROS. In the ultraviolet and visible light region, the longer wavelength light has higher permeability to subcutaneous tissues (14). However, the absorption maxima of 1a and 1b have been observed at around 350 nm, which is not long enough to allow the light to penetrate into deep subcutaneous tissues (3,15–20). Thus, it is well worth designing and developing alternative thioguanines and their nucleosides with an absorption band at longer wavelengths.

Photophysical and photochemical properties of 2a, 3a and their nucleosides (2b and 3b) have not been documented although their synthetic studies were reported (21–24). 8-Oxoguanine (with a carbonyl group at 8–position of the purine ring and also known as an oxidation photoproduct of guanine (4a)), was reported to exhibit a red–shifted absorption band relative to its 6–oxo–analogues (4a and 4b) (25–27). As well as 1a and 1b, thiocarbonyl–modified pyrimidine bases (such as thiolated–uracil and –thymine), have a strong absorption maximum at longer wavelength than their respective un–thiolated nucleobases (28–32). Thus, the newly–designed thioguanines, especially 3a, could outstrip the thio–analogues of nucleobases examined so far in terms of their absorption properties. Photochemical experiments should be carried out in rigidly dehydrated organic solvents to clarify the intrinsic property of the excited states for the thio–nucleobases, however, purine nucleobases have generally low solubility in most of organic solvents (33). Thus, we prepared tri–acetyl–protected derivatives (1c–4c) for better solubility and easier handlings.

In this article, we report our work on chemical synthesis and photochemical investigation of thiolated guanine derivatives (1c, 2c and 3c). We also present out results on their structural
characterizations by NMR, absorption properties by steady-state absorption spectra and quantum yields of $^{1}\text{O}_2^\bullet$ generation by time-resolved near-infrared emission measurement. It is our view that these thioguanosine derivatives can be further developed as a potential photochemotherapeutic agent.

**MATERIALS AND METHODS**

*General.* Reagents were purchased from standard suppliers and used without further purification. Solvents were used after distillation. Reactions were monitored with thin-layer chromatograph (TLC) plate (Silica gel 60, F254). Spots on the TLC plate were monitored with UV, ninhydrin or anisaldehyde. A C–200 Silica gel was used for silica gel flash chromatography. $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra were measured with 500 MHz NMR (JEOL, JNM–ECX 500 MHz), and typical $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra are shown in the Supporting Information (Figures S1–S8). The multiplicity was expressed as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. The chemical shifts are expressed in ppm relative to residual solvent as an internal standard, and coupling constants ($J$ values) were represented in hertz. Mass spectrum was measured by using FAB–MS (JEOL, JMS–700 MStation).

**Synthesis of 2′,3′,5′−tri−O−acetylguanosine (4c).** Acetic anhydride (9 mL, 95.2 mmol) was added to a solution of guanosine (4b, 2.83 g, 10.0 mmol) in pyridine (27 mL), and the mixture was kept for 2 h at 80 °C. The reaction was quenched by addition of H$_2$O at 0 °C after checking the completion of the reaction by TLC. The reaction mixture was dissolved in ethyl acetate and washed with saturated NH$_4$Cl, and the organic layer was dried with anhydrous magnesium sulfate. After evaporation of the solvent and column chromatography (CH$_2$Cl$_2$/MeOH = 9:1), 2′,3′,5′−tri−O−acetylguanosine (4c, 2.21 g, 5.40 mmol, 54%) was obtained as white solid. Rf = 0.40 (CH$_2$Cl$_2$/MeOH = 9:1): $^1\text{H}$ NMR (500 MHz, dimethylsulfoxide–$d_6$) (δ, ppm) 10.76 (1H, s), 7.89 (1H, s), 6.55 (2H, br s), 5.95 (1H, d, $J$ = 6.2 Hz), 5.75 (1H, t, $J$ = 5.5 Hz), 5.46 (1H, dd, $J$ = 5.5, 4.5 Hz), 4.34 (1H, dd, $J$ = 13.8, 4.1 Hz), 4.29–4.26 (1H, m), 4.22 (1H, dd, $J$ = 11.7, 5.5 Hz), 2.07 (3H, s), 2.00 (3H, s), 1.86 (3H, s); $^{13}\text{C}$ NMR (125 MHz, dimethylsulfoxide–$d_6$) (δ, ppm) 170.6, 170.0, 157.2, 154.5, 151.6, 136.2, 117.3, 84.9, 80.1, 72.6, 70.8, 63.6, 21.1, 20.9, 20.7.

**Synthesis of 2′,3′,5′−tri−O−acetyl−6−thioguanosine (1c).** Lawesson’s reagent (1.19 g, 2.93 mmol) was added to a solution of 2′,3′,5′−tri−O−acetylguanosine (4c, 2.01 g, 4.91 mmol) in dioxane (40 mL), and the mixture was kept for 5 h at +100°C. The reaction mixture was dissolved in ethyl acetate and washed with saturated NaHCO$_3$, and the organic layer was dried with anhydrous magnesium sulfate. After evaporation of the solvent and column chromatography (CH$_2$Cl$_2$/MeOH = 97:3), 2′,3′,5′−tri−O−acetyl−6−thioguanosine (1c, 0.999 g, 2.35 mmol, 48%) was obtained as white solid. Rf = 0.51 (CH$_2$Cl$_2$/MeOH = 9:1): $^1\text{H}$ NMR (500 MHz, dimethylsulfoxide–$d_6$) (δ, ppm) 12.03 (1H, s), 8.09 (1H, s), 6.84 (2H, s), 5.95 (1H,
d, J = 6.2 Hz), 5.76 (1H, t, J = 6.2 Hz), 5.45 (1H, dd, J = 6.2, 4.1 Hz), 4.34 (1H, dd, J = 11.3, 3.7 Hz), 4.30 (1H, dd, J = 9.6, 4.1 Hz), 4.23 (1H, dd, J = 11.0, 5.5 Hz), 2.07 (3H, s), 2.00 (3H, s), 1.99 (3H, s); $^{13}$C NMR (125 MHz, dimethylsulfoxide–$d_6$) (δ, ppm) 176.0, 170.0, 169.9, 169.8, 153.7, 148.2, 139.0, 128.9, 85.1, 80.2, 72.5, 70.8, 63.5, 21.1, 20.9, 20.7.

**Synthesis of 8−bromoguanosine (5).** Br$_2$ (3.0 mL) was added to a suspension liquid of guanosine (4b, 5.0 g, 17.7 mmol) in H$_2$O (100 mL), and the vigorous stirring was kept for 24 h at room temperature. Excess Br$_2$ was quenched by addition of saturated sodium thiosulfate solution (3.0 mL), the precipitate was collected by filtration and washed by H$_2$O on a Buchner funnel. After evaporation of the solvent, 8−bromoguanosine (5, 6.3 g, 17.5 mmol, 99%) was obtained as white solid.

**Synthesis of 8−thioguanosine (2b).** Thiourea was added to a suspension liquid of 8−bromoguanosine (5, 4.01 g, 11.1 mmol) in ethanol (40 mL). A small quantity of H$_2$O (5mL) was added until the suspension liquid being dissolved, and kept heated to reflux for 24 h. The reaction mixture was cooled at room temperature, excess ethanol was evaporated and the precipitated solid was filtered. The solid was washed with H$_2$O on a Buchner funnel, and after evaporation of the solvent, 8−thioguanosine (2b, 2.39 g, 7.59 mmol, 68%) was obtained as white solid.

**Synthesis of 2’,3’,5’−tri−O−acetyl−8−thioguanosine (2c).** Acetic anhydride (2 mL, 21.2 mmol) was added to a solution of 8−thioguanosine (2b, 2.10 g, 6.69 mmol) in pyridine (35 mL), and the mixture was kept for 8 h at room temperature. The reaction was quenched by addition of H$_2$O at 0°C after checking the completion of the reaction by TLC. The reaction mixture was dissolved in ethyl acetate and washed with saturated NH$_4$Cl, and the organic layer was dried with anhydrous magnesium sulfate. After evaporation of the solvent and column chromatography (CH$_2$Cl$_2$/MeOH = 95:5), 2’,3’,5’−tri−O−acetyl−8−thioguanosine (2c, 1.76 g, 3.98 mmol, 59%) was obtained as white solid. Rf = 0.46 (CH$_2$Cl$_2$/MeOH = 9:1): $^1$H−NMR (500 MHz, dimethylsulfoxide–$d_6$) 13.02 (1H, br), 11.09 (1H, br), 6.62 (2H, br), 6.38 (1H, s), 6.10 (1H, br), 5.63 (1H, t, J = 6.0 Hz), 4.38 (1H, dd, J = 11.7, 3.4 Hz), 4.20 (1H, m), 4.17 (1H, dd, J = 11.3, 6.5 Hz) 2.06 (3H, s), 2.02 (3H, s), 1.97 (3H, s); $^{13}$C−NMR (125 MHz, dimethylsulfoxide–$d_6$) 170.7, 169.88, 169.82, 165.5, 154.4, 151.4, 149.7,104.4, 86.8, 79.5, 71.3, 70.7, 63.5, 21.0, 20.8, 20.7.

**Synthesis of 2’,3’,5’−tri−O−acetyl−8−bromoguanosine (6).** Acetic anhydride (5 mL, 52.9 mmol) was added to a solution of 8−bromoguanosine (5, 4.76 g, 13.2 mmol) in pyridine (29 mL), and the mixture was kept for 6 h at room temperature. The reaction was quenched by addition of H$_2$O at 0°C after checking the completion of the reaction by TLC. The reaction mixture was dissolved in ethyl acetate and washed with saturated NH$_4$Cl, and the organic layer was dried with anhydrous magnesium sulfate. After evaporation of the solvent and column chromatography (CH$_2$Cl$_2$/MeOH = 95:5), 2’,3’,5’−tri−O−acetyl−8−bromoguanosine (6, 4.79 g, 9.84 mmol, 75%) was obtained as white solid. Rf = 0.48 (CH$_2$Cl$_2$/MeOH = 9:1).
Synthesis of 2′,3′,5′−tri−O−acetyl−6,8−dithioguanosine (3c). Lawesson’s reagent (8.75 g, 21.6 mmol) was added to a solution of 2′,3′,5′−tri−O−acetyl−8−bromoguanosine (6, 5.00 g, 10.3 mmol) in dioxane (100 mL), and the mixture was kept for 4 h at +100°C. The reaction mixture was dissolved in ethyl acetate and washed with saturated NaHCO₃, and the organic layer was dried with anhydrous magnesium sulfate. After evaporation of the solvent and column chromatography (CH₂Cl₂/MeOH = 97:3), 2′,3′,5′−tri−O−acetyl−6,8−thioguanosine (3c, 2.33 g, 5.10 mmol, 50%) was obtained as yellow solid. Rf = 0.54 (CH₂Cl₂/MeOH = 9:1):

1H−NMR (500 MHz, dimethylsulfoxide−d₆) 13.11 (1H, br), 12.21 (1H, br), 6.93 (2H, br), 6.40 (1H, d, J = 4.1 Hz), 6.08 (1H, t, J = 5.5 Hz), 5.60 (1H, t, J = 6.2 Hz), 4.38 (1H, dd, J = 11.7, 6.9 Hz), 4.22 (1H, m), 4.16 (1H, dd, J = 11.7, 6.9 Hz), 2.06 (3H, s), 2.02 (3H, s), 1.97 (3H, s):

13C−NMR (125 MHz, dimethylsulfoxide−d₆) 170.6, 169.88, 169.84, 168.1, 164.1, 154.1, 146.7, 117.8, 86.8, 79.6, 71.1, 70.7, 63.5, 21.1, 20.8, 20.7: MS (FAB+) m/z 458 (MH⁺).

Ultraviolet−visible (UV−vis) absorption spectroscopy. The UV−vis absorption spectra were recorded at room temperature on a spectrophotometer (JASCO, U−best V550) using a quartz cuvette of 1 cm optical path length. The sample solution was prepared with acetonitrile as a solvent.

Time−resolved near−infrared emission spectroscopy. Time−resolved near−infrared emission measurement was carried out with a thermoelectric cooled near−infrared photomultiplier tube (Hamamatsu Photonics, H10330−45; InP/InGaAsP, spectral response 950 to 1400 nm) combined with a longpass filter (Thorlabs, FEL1250; cut−on wavelength 1250 nm) and a bandpass filter (Edmund, Hard−coated bandpass filter; 1275 ± 50 nm) (Figure S9). A forth harmonic of a Nd³⁺:YAG laser (Continuum, Surelite II−10, 5 ns pulse duration, 10 Hz, 266 nm) was used as an excitation light source. The sample solution was prepared with acetonitrile as a solvent.

Quantum chemical calculation. Ground− and excited−state calculations for corresponding purine bases (1a−4a) were performed using the Gaussian 09W program package (30). Ground−state geometries of the purine bases were optimized by the density functional theory (DFT) at the B3LYP/6−311+G(d,p) level. Vertical excitation energies were estimated by the time−dependent DFT (TD−DFT) at the TD−B3LYP/6−311+G(d,p) level. Solvent effects were modeled with the polarizable continuum model (PCM) for the ground− and excited−states.

RESULTS AND DISCUSSION

Synthesis of thioguanosine derivatives

Scheme 2 outlines the synthetic route to 1c−4c. The syntheses of 1c, 2c and 4c have been reported previously (35−44). To the best of our knowledge, this is the first report on chemical synthesis of 3c. The structures of all synthesized products were characterized by ¹H NMR and their purities were estimated to be 99% for 1c, 99% for 2c, 99% for 3c, and 97% for 4c.
with a minor amount of impurity being H$_2$O. The concentration of H$_2$O was determined by subtracting the peak area deriving from H$_2$O in dimethylsulfoxide–$d_6$ solvent from that in 1c–4c solutions to remove the intrinsic moisture content of the deuterated solvent. No other impurity was detected by HPLC spectra as shown in Figure S10.

>Scheme 2<

1c was prepared in a 2–step process for the first time. First, 4b was quantitatively converted to 4c, which then was transformed to 1c with Lawesson’s reagent. Following the reported procedure (21,43,44), 2c was synthesized in a 3–step process from 4b. To prepare 3c, 6 is the key intermediate which can be obtained from bromination of 4b followed by acetylation of the resultant 5 in an excellent yield (23,44). In an early report (23), 6 had been treated with phosphoryl chloride and hydrolyzed to afford 2–amino–6,8–dichloro purine, followed by nucleophilic substitution reaction with thiourea to yield 3b. In order to overcome the difficulty in handling the phosphoryl chloride and its low yield of 3b (33%) (23), thus, we developed another synthetic route to 3c. By a simple treatment of 6 with Lawesson’s reagent, 3c was successfully afforded with a higher yield of 50%.

$^1$H and $^{13}$C NMR analysis of thioguanosine derivatives

To ascertain the correct structures of the synthesized products, NMR spectroscopy was used. The $^1$H NMR chemical shifts of 1c–4c are listed in Table 1. The chemical shift values for the proton at 1–position (the imide group) of thioguanosine derivatives were observed at lower magnetic field than that of un–thiolated guanosine. The peaks for the imide group, especially in 1c and 3c, were significantly shifted to a lower magnetic field. This shift can be ascribed to the thiacarbonyl substitution at 6–position of the purine ring. The thiacarbonyl modification at 8–position also causes a significant shift of the peak for the imide group to a lower magnetic field, as observed for 2c. The peaks for the amine protons at 2–position of the purine ring for 1c and 3c also exhibited a slight shift to the low field in comparison with the corresponding peak for 2c and 4c. The chemical shift values deriving from ribose sugar protons, especially for anomeric proton (1’H), were also shifted to a lower magnetic field in both 2c and 3c. These shifts can be ascribed to the thiacarbonyl modification at 8–position, consistent with a recent publication in which the peak for the proton at 3–position (the imide group) in 4–thio–pyrimidines was also found to be at lower magnetic field than that of un–thiolated pyrimidine bases (45).

Table 2 lists $^{13}$C NMR chemical shifts of all the carbon atoms in compounds 1c–4c. The peak at 157.2 ppm in un–thiolated 4c was found to shift to 176.0 ppm (18.8 ppm lower magnetic field) in the 6–thiolated analogue (1c). Similarly, the peak at 136.2 ppm in the
un–thiolated 4c was also found to shift substantially to 165.5 ppm in the 8–thiolated analogue (2c), resulting 29.3 ppm lower magnetic field shift. Compound 3c is a doubly thiolated analogue, both of the thio–carbonyl carbons are shifted to a lower field (i.e.168.1 and 164.1 ppm) compared with those of un–thiolated 4c. These shifts should be due to the thiolation at their respective positions (6–position and 8–position). The similar effect was also reported for the carbon atoms of thio–carbonyl carbons in 2–thiouracil and 4–thiouracil (45,46).

**UV–vis absorption spectroscopy**

The absorption spectra of 1c, 2c, 3c and 4c are shown in Figure 1a. Absorption spectrum of 4c, appeared in the spectral range less than 295 nm, was almost identical to that of guanosine (4b), revealing that the acetylation of three hydroxyl groups in the sugar component had little effect on electronic states concerning with transitions in this spectral range. Since the solubility of 4c (tri–acyl protected guanosine) in acetonitrile was over 300 times larger than that of 4b (un–protected guanosine) in units of molarity (16.5 mM for 4c and 41.6 μM for 4b), thus corresponding tri–acyl protected analogues instead of the un–protected analogue were used to study their UV properties. The absorption spectrum for 2c (8–thiolated analogy) exhibited an intense absorption band centered at 302 nm with a high molar absorption coefficient [ε\textsubscript{302} = (2.39 ± 0.01) × 10^4 M\textsuperscript{-1} cm\textsuperscript{-1}]. In comparison with 4c, this red–shift of the band (48 nm) observed from 2c is likely to result from the extension of the π – conjugation due to the thiocarbonyl modification at 8–position of the purine ring. 1c (6–thiolated analogy) has a much large red–shifted band (91 nm) with a higher molar absorption coefficient [ε\textsubscript{346} = (2.82 ± 0.01) × 10^4 M\textsuperscript{-1} cm\textsuperscript{-1}]. The absorption maximum of 1c appeared in the longer wavelength region than that of 2c, indicating that thio–carbonyl modification at 6–position has more contribution to its electronic transition than that at 8–position. Our findings offer a solid support to an early report (17) that the replacement of an oxygen atom by a sulfur atom in a carbonyl group is expected to shift the absorption band to the red. The absorption maximum of 3c (di–thiolated analogue) was observed at 381 nm, which surprisingly results in a red–shift up to 126 nm in comparison with 4c. In addition, the absorption intensity at the maximum [ε\textsubscript{381} = (3.76 ± 0.02) × 10^4 M\textsuperscript{-1} cm\textsuperscript{-1}] was remarkably higher than any other thio–analogues of nucleobases examined so far (3,15–19,28–32), revealing 3c can be irradiated with a very low dose of UV light. These desirable UV properties suggest that 3c would be much sensitive to the light penetrating into the human skin and could be used as a powerful photosensitive agent for light–induced therapies, including PDT. Thioguanosines (1c, 2c and 3c) are not regarded as suitable agents for PDT because of the absence of one–photon absorption at visible light. However, in our recent works (47,48), 1b and 3c were successfully excited at red light by multi–photon excitation. Thus, with the multi–photon approach, thioguanosines have offered a potential as a PDT sensitizer.
Steady–state emission measurements were also carried out on 1c–3c, but no emission was observed at room temperature, indicating a fluorescence quantum yield of virtually zero. On the other hand, emission was clearly observed at 77 K in glassy ethanol matrix, as described in the supporting materials (Figure S11). Those emission bands exhibited significantly large Stokes shift from those absorption maxima (beyond 100 nm, as listed in Table 3) with long life times, over a microsecond. The emission spectrum of 1c was identical to the reported phosphorescence spectrum of 1b (49,50). Although the quantum yields of triplet formation for the thioguanosines (1c, 2c and 3c) have not been obtained yet, the triplet–triplet absorption spectra were observed at room temperature, which will be described in detail in the next paper. In addition, these thioguanosines have relatively high quantum yields of singlet molecular oxygen generation (see below), indicating high triplet formation of these compounds. Therefore, those emissions can be confidently assigned to their respective phosphorescence of 1c–3c. Thus, 2c and 3c as well as 1a–1c will form the excited triplet manifold through intersystem crossing from the singlet excited states.

Quantum Chemical Calculations

Optimized ground–state geometries of 1a, 2a and 3a are shown in Figure S12. These molecules belong to the C₃ symmetry, and all atoms lie in a plane of the purine ring. The bond lengths and angles were comparable to each other except for the C₆=S and C₆=O bond lengths. The C₆=S bond lengths of 1a and 3a (1.69 Å) were significantly larger than the C₆=O bond lengths of 2a and 4a (1.23 Å). This clearly reveals that the strength of the C₆=S bond is weaker than that of the C₆=O bond.

Computational vertical transition energies and oscillator strengths of 1a–3a are shown in Figure 1b, and listed in Table 3. The calculated vertical transition energies and oscillator strengths of 1a–3a well reproduce the red–shifted absorptions of 1c–3c in comparison with 4c.

Molecular orbitals involved in the transitions to first and second excited singlet states of 1a–3a are shown in Figure 2. In all compounds, HOMO–1 has n character with electronic density localized around the sulfur atom perpendicularly to the molecular plane whereas both HOMO and LUMO have π and π* character with extended electronic density throughout the molecular plane, respectively. For 1a, the first excited singlet (S₁) state arises from the transition from the n orbital localized on the sulfur atom (HOMO–1) to the π* orbital (LUMO), and the calculated small oscillator strength (f < 0.0001) indicates the forbidden S₁(nπ*)←S₀ transition. On the other hand, the transition to the second excited singlet (S₂) state is the allowed ππ* transition (HOMO → LUMO) (f = 0.571). Thus, the intense absorption band of 1c around 346 nm would be attributed to the S₂(ππ*)←S₀ transition. For 2a and 3a, the transition to the S₁ and S₂ states arise from the allowed ππ* transition (HOMO
→ LUMO) and forbidden \( n\pi^* \) transition (HOMO–1 → LUMO). Thus, the absorption peak at the longest wavelength of \( 2c \) and \( 3c \) would be attributed to the \( S_1(\pi\pi^*) \) ← \( S_0 \) transition. The \( \pi \) and \( \pi^* \) orbitals of \( 2a \) and \( 3a \) were widely extended to the 8–position of the purine ring as shown in Figure 2, resulting in the red–shifted absorption of \( 2c \) and \( 3c \) with respect to \( 4c \) and \( 1c \), respectively.

For \( 1a–3a \), the \( T_1 \) state was also optimized and shown in Figure S13. The optimized structures for \( 1a–3a \) at the \( T_1 \) state are also comparable to that at ground–state except for the \( S^6 \) atom of \( 1a \) at the \( T_1 \) state (only the \( S^6 \) atom is out of molecular plane). The optimized structures for \( 1a–3a \) at the \( T_1 \) state can be assigned to the \( \pi\pi^* \) state, as shown in Figure S14. The \( T_1 \) state energies of \( 1a–3a \) are listed in Table 3. The calculated \( T_1 \) energies well agree with the experimental values, estimated from the maximum emission wavelength in the phosphorescence spectra of \( 1c–3c \).

**Time–resolved near–infrared emission spectroscopy**

The quantum yield of singlet oxygen generation for the thioguanosines was determined for exploration of these potential drugs in photochemotherapy. Figure 3a shows the decay profiles of singlet oxygen phosphorescence measured at around 1275 nm by photosensitization with \( 1c \), \( 2c \), and \( 3c \) in \( O_2 \) saturated acetonitrile solutions. All signals decayed mono–exponentially and their lifetimes were about 65 µs, well agreeing to the lifetime of \( ^1O_2^\ast \) in acetonitrile (51,52). Since this signal was not detectable in Ar saturated solutions, the emission should be due to \( ^1O_2^\ast \), generated by photosensitization with thioguanosines.

The quantum yields of \( ^1O_2^\ast \) generation of thioguanosines were determined in \( O_2 \) saturated acetonitrile solutions relative to optically matched phenalenone (PN) solution (\( \Phi_\Delta = 1.00 \pm 0.03 \)) (53). Individual phosphorescence traces were fitted by using a single–exponential function to estimate the emission intensity maxima immediately after laser irradiation (\( I_{S^0}^\ast \)). The \( I_{S^0}^\ast \) value was plotted against the laser fluence (\( I_L \)) (Figure 3b). A good linear relationship was observed between \( I_{S^0}^\ast \) and \( I_L \). This finding reveals that \( ^1O_2^\ast \) was generated by one–photon process through photosensitization by thioguanosines. The values of the slope obtained from these plots (\( I_{S^0}^\ast / I_L \)) were plotted against the ground–state absorptance at excitation wavelength (1–10\(^\text{–4}\)), as shown in Figure 3c. These plots also show good linear relationships. By comparing the slopes of thioguanosines with that of PN, we were able to determine \( \Phi_\Delta \) values, with a high degree of accuracy, as 0.37 ± 0.01 for \( 1c \), 0.28 ± 0.01 for \( 2c \) and 0.33 ± 0.01 for \( 3c \). The \( \Phi_\Delta \) value for \( 1c \) was close to those for \( 1a \) and \( 1b \) in the previous report (18,20). These results further confirm that those thioguanosines generate \( ^1O_2^\ast \) effectively through photosensitization.

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It was noted that there are only very small differences in the $\Phi_\Delta$ values among the thioguanosines (see Table 3). $^1\text{O}_2^*$ is considered to generate through energy transfer from the $T_1$ state of donor molecule to an oxygen molecule ($X^3\Sigma_g^-$) as an energy acceptor by collision each other, thus the $\Phi_\Delta$ value should depend on the following factors: the intersystem crossing quantum yield, the triplet lifetime of the sensitizer and the $S_\Delta$ value (a fraction of the triplet states quenched by dissolved oxygen which gives rise to singlet oxygen formation). Generally, triplet states of $\pi\pi^*$ have been reported to give a $S_\Delta$ value of a range of 0.7–1.0, whereas it is ~0.3 for $n\pi^*$ triplet states (54). All the $T_1$ state of 1c, 2c and 3c have $\pi\pi^*$ character in the Franck–Condon region obtained by the TD–DFT calculation, and its $T_1$ energies are large enough to surpass vertical transition energy of oxygen molecules (0.97 eV; $a^1\Delta_g \leftarrow X^3\Sigma_g^-$), as discussed above. Therefore, the differences in $\Phi_\Delta$ will depend on the lifetime of each $T_1$ state and/or quantum yields of intersystem crossing to triplet manifolds. To gain the more detailed information on the triplet state such as lifetime and quantum yield, time–resolved spectroscopy are under way.

CONCLUSION

Three novel thioguanosine derivatives (1c–3c) have been successfully synthesized and characterized by various spectroscopies. The absorption bands of these thioguanosines are found to be at longer wavelengths than those of un–thiolated guanosines (4b and 4c). Especially, 3c has the most red–shifted band with a large molar absorption coefficient, indicating that 3c is much sensitive to the light penetrating into the human skin. The red–shifted spectra for thio–analogues were well reproduced with the quantum chemical calculations. The $T_1$ character was found to be a $\pi\pi^*$ character. In addition, the thioguanosines generated $^1\text{O}_2^*$ effectively through photosensitization ($\Phi_\Delta = 0.28$–0.37). Taken together, these results clearly show that our reported thioguanosines have some potential for photochemotherapy, as a photoactivatable genotoxic agent and/or a photosensitizer for PDT. Although the $S_\Delta$ value for the $T_1$ ($\pi\pi^*$) was known to be high, the small difference in $\Phi_\Delta$ values among the thioguanosines is likely to be dependent on the intersystem crossing quantum yield and/or $T_1$ state lifetime of each individual thioguanosine.

ACKNOWLEDGEMENTS

[Ask author if they wish to add an acknowledgement statement]

SUPPORTING INFORMATION

Additional Supporting Information is available in the online version of this article:

Figure S1. $^1$H NMR spectrum of 1c in dimethylsulfoxide–$d_6$ solution.
Figure S2. $^{13}$C NMR spectrum of 1c in dimethylsulfoxide–$d_6$ solution.
Figure S3. $^1$H NMR spectrum of 2c in dimethylsulfoxide–$d_6$ solution.

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Figure S4. $^{13}$C NMR spectrum of 2c in dimethylsulfoxide–$d_6$ solution.

Figure S5. $^1$H NMR spectrum of 3c in dimethylsulfoxide–$d_6$ solution.

Figure S6. $^{13}$C NMR spectrum of 3c in dimethylsulfoxide–$d_6$ solution.

Figure S7. $^1$H NMR spectrum of 4c in dimethylsulfoxide–$d_6$ solution.

Figure S8. $^{13}$C NMR spectrum of 4c in dimethylsulfoxide–$d_6$ solution.

Figure S9. Schematic diagram of the experimental setup for the time–resolved near IR emission measurement.

Figure S10. HPLC chart for (a) 1c, (b) 2c, (c) 3c and (d) 4c. The ratio of eluents (water and acetonitrile) was kept comparable. The signal was monitored by absorption at 340 nm for 1c, 300 nm for 2c, 370 nm for 3c and 260 nm for 4c. Retention times: 1c 8.6 min; 2c 10.73 min; 3c 5.18 min; 4c 7.45 min.

Figure S11. Phosphorescence spectra in optically matched ($\lambda_{ex} = 266$ nm, $A_{266 \text{ nm}} = 0.4$) 1c, 2c, and 3c glassy ethanol matrix measured at 77 K. The spectrum of 2c was 10 times multiplied.

Figure S12. Optimized structures at the ground state of 1a, 2a, and 3a.

Figure S13. Optimized structures at the triplet state of 1a, 2a, and 3a.

Figure S14. Molecular orbitals at the T$_1$ state of 1a, 2a and 3a.

Table S1. Cartesian coordinates for optimized structure at the ground state of 1a.

Table S2. Cartesian coordinates for optimized structure at the ground state of 2a.

Table S3. Cartesian coordinates for optimized structure at the ground state of 3a.

Table S4. Cartesian coordinates for optimized structure at the T$_1$ state of 1a.

Table S5. Cartesian coordinates for optimized structure at the T$_1$ state of 2a.

Table S6. Cartesian coordinates for optimized structure at the T$_1$ state of 3a.

REFERENCES


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**Table 1.** $^1$H NMR Chemical Shifts of 1c–4c.

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<th>N1H</th>
<th>N2H</th>
<th>N3H2</th>
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<th>2'</th>
<th>3'</th>
<th>4'</th>
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<tr>
<td>1c</td>
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<td>–</td>
<td>6.84</td>
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<td>5.76</td>
<td>5.45</td>
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<tr>
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<td>13.02</td>
<td>6.62</td>
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<td>6.40</td>
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<td>5.75</td>
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Table 2. $^{13}$C NMR Chemical Shifts of 1c–4c.

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<th>C⁶</th>
<th>C⁸</th>
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<th>2'</th>
<th>3'</th>
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<td>70.8</td>
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<tr>
<td>2c</td>
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<td>154.4</td>
<td>104.4</td>
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<td><strong>165.5</strong></td>
<td>86.8</td>
<td>79.5</td>
<td>71.3</td>
<td>70.7</td>
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<td>3c</td>
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<td>154.1</td>
<td>117.8</td>
<td><strong>168.1</strong></td>
<td><strong>164.1</strong></td>
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<td>79.6</td>
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<tr>
<td>4c</td>
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<td>151.6</td>
<td>117.3</td>
<td>157.2</td>
<td>136.2</td>
<td>84.9</td>
<td>80.1</td>
<td>72.6</td>
<td>70.8</td>
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Table 3. Photophysical Properties of 1c, 2c and 3c in Acetonitrile Solution.

<table>
<thead>
<tr>
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<th>Computational‡‡</th>
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<tr>
<td></td>
<td>λ_{max}</td>
<td>ε_{max} (^{\dagger})</td>
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<tr>
<td>1c</td>
<td>346</td>
<td>2.82 ± 0.01</td>
</tr>
<tr>
<td>2c</td>
<td>302</td>
<td>2.39 ± 0.01</td>
</tr>
<tr>
<td>3c</td>
<td><strong>381</strong></td>
<td><strong>3.76 ± 0.02</strong></td>
</tr>
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\(^{†}\) Wavelength at absorption maximum. \(^{‡}\) Molar absorption coefficient at absorption maximum. \(^{†}\) Triplet state energy obtained from emission peak of phosphorescence spectrum. \(^{§}\) Quantum yield of singlet oxygen generation. \(^{¶}\) Vertical transition energy. \(^{**}\) Oscillator strength. \(^{††}\) Triplet state energy. \(^{‡‡}\) Calculated at the PCM/TD–B3LYP/6–311+G(d,p) level.
Scheme 1. Structures of guanine, thioguanines, and their nucleosides.

Scheme 2. The synthesis route for thioguanosine and guanosine derivatives.
FIGURE CAPTIONS

Figure 1. (a) Absorption spectra of 1c–4c in acetonitrile solution, and (b) Computational vertical transition energy and oscillator strength of 1a–4a at PCM/TD–B3LYP/6–311G+(d,p) level.

Figure 2. Molecular orbitals involved in transitions to the first and second excited single states of 1a, 2a and 3a.

Figure 3. (a) Decay profiles of singlet oxygen phosphorescence measured at around 1275 nm of thioguanosines and PN in acetonitrile solution. Signals are corrected for absorptance at excitation wavelength (266 nm) and incident laser power. (b) Plots of the emission intensity maxima ($I_{S0}^0$) immediately after laser irradiation in 3c solutions against incident laser power ($I_L$), and (c) plots of the $I_{S0}^0/I_L$ value of 1c, 2c, 3c, and PN against the absorptance (1–10$^{-4}$) at excitation wavelength (266 nm).
Figure 1. S. Miyata et al.
Figure 2. S. Miyata et al.
Figures 3. S. Miyata et al.