Photophysical Studies of the Thiobase Derivative 2,6-Dithiopurine
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Introduction
Thiobases are sulfur derivatives of DNA bases, and are of high interest in the scientific community
because of their potential use as photosensitizers to target and eliminate cancer cells.1-3 This is due to their
photophysical and photochemical properties, which unlike the regular DNA bases found in the human body,
thiobases can absorb the UVB and UVA rays emitted from the sun.4

![Figure 1. Jablonski diagram with photosensitization mechanisms.](image)

In a photosensitization reaction, a molecule that absorbs light of a certain wavelength induces a
physical or chemical change into a different molecule by transferring its absorbed electronic energy (Figure 1). This other molecule can be another DNA base, an amino acid, or even molecular oxygen. Photosensitization can occur through different mechanisms, including photocycloaddition and photocrosslinking reactions, in which light absorption by one molecule induces the formation of a chemical bond between this and another molecule (or between two regions of the same molecule), and Type I and Type II reactions, which often result in oxidized products.5 In a Type I reaction, the molecule that absorbs the light (called the photosensitizer) can react directly with another molecule. In a Type II reaction, the photosensitizer transfers its excess energy or an electron to molecular oxygen, resulting in the formation of reactive oxygen species. Type II reactions have been proposed to play a significant role in the oxidative damage caused by 6-thioguanosine (6tGuo), a thiobase derivative of the DNA base guanine6-7 and the metabolite of the thiopurine prodrugs (Figure 2).

Thiopurines are commonly known as effective immunosuppressants, anti-cancer agents, and help treat inflammatory diseases.8 Although they are highly potent medications, treatment of patients with thiopurine derivatives such as 6-mercaptopurine (6MP), azathioprine (Aza), and 6-thioguanine (6tGua)
(Figure 2) has been associated with an increased risk of skin cancer following sunlight exposure.\textsuperscript{9-12} This side effect has been primarily attributed to the ability of the thiopurines to absorb UVA light and to be incorporated into the patient’s DNA as 6tGuo.\textsuperscript{13} 6tGuo absorbs strongly the UVA radiation from the sun, and can act as a photosensitizer.\textsuperscript{14-15}

\begin{figure}
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\includegraphics[width=\textwidth]{figure2.png}
\caption{From left to right: 6tGua, 6tGuo, 6MP, and Aza.}
\end{figure}

Other thiopurine derivatives, particularly 2,6-dithiopurine (DTP) do not have the toxic effect of the thiopurine prodrugs. This is attributed to the fact that DTP (Figure 3) is not incorporated into the DNA of mammalian cells.\textsuperscript{16} Previous studies show that DTP can act as a nucleophilic trapping agent for electrophilic carcinogens, resulting in a reduced tumor formation in skin carcinogenesis mouse models.\textsuperscript{17} Also, it has been shown to block the cytotoxic and mutagenic effects of two analogues of sulfur mustard in human skin cells.\textsuperscript{16} A recent study shows that DTP can decrease the proliferation of human epidermoid carcinoma cells by up to 63% in vitro, after being activated by a low dose of UVA light.\textsuperscript{3} The same study also compares DTP with two other thiopyrimidine derivatives, and reveals that DTP is more effective in eliminating the skin cancer cells, even though the three thiobase derivatives generate reactive oxygen species in comparable yields. The authors say that the increased efficacy of DTP is likely due to the longer lifetime of the triplet state that forms when the DTP is activated by UVA light, compared to the other two thiobases.\textsuperscript{3} These observations highlight the potential application of DTP as a photodynamic therapy agent against skin cancer cells and suggests that Type I photoreactions can play a significant role in the increased efficacy of this compound.

In this project, the photophysical properties of DTP were studied using steady-state absorption and emission spectroscopies, along with electronic structure calculations to help explain the physical and chemical processes induced by UVA light absorption of DTP.

\begin{figure}
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Chemical structure of DTP.}
\end{figure}
Methods

Steady-state absorption was measured at room temperature using a Cary 100 spectrometer (Varian, Inc.). The molar extinction coefficient for DTP (TCI America) in phosphate buffered saline (PBS) pH 7.4 was determined by preparing a 90 µM solution of DTP and measuring its absorbance. Four dilutions were formulated and the absorbance values for each dilution were recorded. The absorption spectra for DTP were corrected for the solvent by subtraction of the corresponding spectrum obtained. The molar extinction coefficient, $\varepsilon(\lambda)$, was determined from the slope of an absorbance versus concentration plot, by using the Beer-Lambert Law: $A(\lambda)=\varepsilon(\lambda)bC$, where A is the absorption of the sample, b is the width of the cuvette, and C is the concentration of the sample.

Emission and excitation spectra of DTP in PBS pH 7.4 were recorded using a Cary Eclipse spectrofluorometer (Varian, Inc.). For this, a 13 µM solution of DTP in PBS pH 7.4 was used. The excitation wavelength was set to 342 nm, and the emission and excitation data was collected every 0.5 nm, with a 2 s averaging time, excitation and emission slit widths set to 5 nm, and PMT set to 800 V. The nitrogen-saturated solution was purged for 20 minutes before collecting the emission spectrum.

The electronic structure calculations were performed using the Gaussian 16 program. The effect of the solvent on the ground state structure and the vertical excitation energies was incorporated by using the self-consistent reaction field (SCRF) with the integral equation formalism of the polarized continuum model (IEF-PCM). The ground state geometries of DTP were optimized in water by using the density functional theory (DFT) with the B3LYP functional and the 6-311++G(d,p) basis set. These optimized geometries were then used with the time dependent variant of DFT (TD-DFT) to calculate the vertical excitation energies in water using the PBE0 functional and the 6-311++G(d,p) basis set. The character of each electronic transition was assigned by looking at the electron density of the corresponding Kohn-Sham orbitals.

Results and Discussion

A. Steady-state absorption

The absorption spectrum of DTP in PBS pH 7.4 (Figure 4) shows three bands in the UV-Visible region of the electromagnetic spectrum. The UVA band is centered at 348 nm, while three other higher energy bands are centered at 286 nm, 244 nm, and 215 nm.
Figure 4. Absorption versus wavelength spectra for DTP in PBS pH 7.4.

The molar absorptivity coefficient for DTP in PBS pH 7.4 was calculated using Beer-Lambert’s Law, in which we see that absorbance and concentration are linearly related. Figure 5 shows the linear fit applied to the absorbance versus concentration plot, from which we obtained \( \varepsilon_{348} = (16,451 \pm 67) \ \text{M}^{-1}\text{cm}^{-1} \). Figure 6 shows the molar absorptivity spectrum obtained by normalizing the absorption spectrum for the 90 \( \mu \text{M} \) DTP solution to the UVA absorption peak. This lets us know the \( \varepsilon \) values for DTP at wavelengths other than 348 nm in PBS pH 7.4, a solvent that simulates physiological conditions.

Figure 6 also has the vertical excitation energies plotted with the corresponding oscillator strengths (green), both obtained from the electronic structure calculations (see below). From this graph, we can see that the electronic transitions predicted by the TD-DFT calculations match the absorption spectrum that we collected in the laboratory.
Figure 5. Absorption (at 348 nm) versus concentration plot to determine $\varepsilon_{348}$ for DTP in PBS pH 7.4.

Figure 6. Left: Molar absorptivity spectrum for DTP (red), and vertical electronic transitions with their oscillator strengths (green) calculated at the TD-PBE0/IEF-PCM/6-311++G(d,p) level of theory in water. Right: Optimized structure of DTP in water, calculated at the B3LYP/IEF-PCM/6-311++G(d,p) level of theory.

The chemical structure of DTP is very similar to that of the thiopurine prodrug 6MP (Figure 2), with the addition of an extra S group in C2 (Figure 6). According to the scientific literature, DTP has one negative charge under physiological conditions.\textsuperscript{25-26} Twelve different tautomers of anionic DTP were considered, and the data shown corresponds to the lowest-energy structure obtained, shown in Figure 6.
B. Electronic structure calculations

![Diagram of electronic structure calculations](image)

Figure 7. Vertical excitation energies (left) and Kohn-Sham orbitals (right) for DTP calculated at the TD-PBE0/IEF-PCM/6-311++G(d,p) level of theory in water

Figure 7 (left) shows the accessible singlet and triplet electronic states following excitation of DTP with UVA light (≈2.8 to ≈4.1 eV). There are two singlets available for excitation, \( S_1 (n\pi^*) \) and \( S_2 (\pi\pi^*) \), and three triplets lower in energy than the \( S_2 (\pi\pi^*) \) singlet available for intersystem crossing: \( T_3 (\pi\pi^*) \), \( T_2 (n\pi^*) \), and \( T_1 (\pi\pi^*) \). The character of each state was assigned by looking at the electron density of the Kohn-Sham orbitals contributing to each vertical transition. Figure 7 (right) shows the orbitals considered to assign the character of the first two singlets, and shows the difference between a \( n\pi^* \) and a \( \pi\pi^* \) transition.

C. Steady-state emission

Figure 8 shows the steady-state emission spectra obtained for DTP solutions using 342 nm as the excitation wavelength. Two low-intensity emission bands were observed: one around 425 nm and the other one around 475 nm. The intensity of the 425 nm bands is about the same for both solutions. The only difference between the two samples was that one was purged with nitrogen for 20 minutes before recording the spectrum (blue), while the other one was not (red). Purging with nitrogen removes oxygen from the cuvette, and we know from Type II photosensitization reactions that oxygen can react with excited triplet states, but not singlet states. Since the 475 nm band shows a higher intensity when purged with nitrogen...
compared to the air sample, we can say that this emission comes from a triplet state (phosphorescence) while the 425 nm emission comes from a singlet state (fluorescence).

Figure 8. Emission spectra for air-saturated (red) and nitrogen-purged solutions of DTP in PBS pH 7.4.

Figure 9 shows the normalized excitation spectrum recorded at 475 nm (red) plotted with the normalized absorption spectrum (blue) taken from Figure 4. The wavelength maxima and the shape of the excitation spectra matches well with the absorption spectra, indicating that the emission observed in Figure 8 comes from DTP.

Figure 9. Normalized absorption (blue) and excitation (red) spectra for DTP in PBS pH 7.4.
Conclusions

The results obtained from this project will be used in combination with more advanced spectroscopic techniques to help understand the interaction between DTP and UVA light. Future work on this research may provide valuable information on the photosensitization mechanisms of DTP and its possible use in photodynamic therapy.

Personal Experience

During this 8 week SEED Program, I learned a ton. My first week here I was nervous because I didn’t really know what to expect but it turned out great! I had the opportunity to do many procedures in the lab such as weighing the mass of compounds to lowering pH balances. I’m so glad I got to work alongside Glesmarie during this summer, she was ready to answer any questions I had.

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References

8. 20th Who model list of essential medicines


