2,4-Dithiothymine as a Potent UVA Chemotherapeutic Agent

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Supporting Information

I. Materials and Methods

1. Materials and Steady-State Spectroscopy

2-thiothymine (2tThy, 98% purity) was obtained from AK Scientific, 4-thiothymidine (4tThd, 99% purity) was obtained from Carbosynth Ltd., and 2-thiothymidine (2tThd) and 2,4-dithiothymine (2,4dtThy) were obtained from Sigma Aldrich. All compounds were used as received. The purity of these compounds was ensured by using fluorescence spectroscopy and by comparison with the published absorption maxima and their corresponding extinction coefficients.[1,2] Phosphate buffer solutions were freshly prepared using 0.12 g of sodium dihydrogen phosphate and 0.089 g of disodium hydrogen phosphate dissolved in 100 mL of ultrapure water and adjusted to pH 7.4 using a 2 M NaOH solution. Steady-state absorption spectra were measured using a Cary 100 Bio spectrometer. All absorption spectra were corrected for the solvent background by subtraction of the absorption of the solvent.

2. Transient Absorption Spectroscopy

Femtosecond transient absorption spectroscopy was performed using a Ti-Sapphire regenerative amplifier laser system (Libra-HE, Coherent, Inc.; 800 nm, 100 fs, 4.0 W at 1 kHz). 2.6 W of the 800 nm beam were used to pump an optical parametric amplifier (TOPAS, Quantronix/Light Conversion) to generate the excitation beam at selected wavelengths of 320, 335, and 360 nm. Contributions to the excitation beam from

other wavelengths and polarizations were removed by a reflective wavelength filter (λ-filter) and a Glan-Taylor polarizer, respectively, and the polarization was randomized using a depolarizing plate in order to prevent rotational effects from contributing to the dynamics, as described in details elsewhere.[3]

A Helios spectrometer (Ultrafast Systems, LLC.) was used for pump-probe measurements. A fraction of the fundamental (800 nm) beam from the Libra-HE was used to generate white light continuum (WLC). The Helios spectrometer uses an optical delay line with a maximum delay of ~3 ns for the 800 nm beam, which was used to pump a continuously-moving 2 mm CaF_2 plate for generation of the WLC broadband probe pulse covering the spectral range from ~320-700 nm. The probe light was then passed through a 730 nm low-pass dichroic optical filter, so as to block residual fundamental beam and to avoid saturating the complementary metal-oxide-semiconductor (CMOS) detectors. The probe pulse was then split into two beams, both of which were re-collimated and focused into the optical fibers leading to their respective CMOS detectors. In this setup, one of the beams serves as reference, while the other is used to probe the sample of interest. A synchronized chopper wheel was used to block every other pump pulse, such that the difference in absorption between two consecutive measurements gives the transient spectrum. The excitation beam was attenuated to 1 μJ/pulse at the sample using a neutral density optical filter before entering the spectrometer. The excitation pulse was focused to a beam diameter three times larger than that of the probe beam at the sample position. Detection time windows of 20 and 200 ps were used in this work.

All solutions were prepared using pH 7.4 aqueous phosphate buffer, with a total phosphate concentration of 16 mM. The solution was continuously stirred in a 2 mm optical path length quartz cell (Starna Cells, Inc.) using a Teflon-coated magnetic stirring bar so as to bring fresh sample to the pump-probe region for each measurement. The solution in the cuvette was replaced with a fresh sample, from the same stock solution, every 5-7 scans, so that all scans had no more than 5% decrease in absorbance at the lowest-energy absorption-band maximum as determined by steady-state absorption

spectroscopy. Importantly, individual scans showed no evidence of photoproduct(s) contamination under the experimental conditions used.

3. Transient Absorption Data Analysis

All broadband transient absorption data were corrected for group velocity dispersion (GVD)[4] of the WLC using a home-made LabView program (National Instruments, Inc.) as previously described.[3] Analysis of transient absorption kinetics was performed in Igor Pro 6.32A (WaveMetrics, Inc.) using the target analysis method, as described previously,[5] and a sequential kinetic model convoluted with an instrument response function of ~200 fs (FWHM). The instrument response was determined from the coherence signal of neat methanol generated at the sample cell. Global fitting analysis was performed using 15 to 30 representative kinetic traces covering the full range of probe wavelengths. At least three independent datasets (i.e., recorded on three different days) were used in the analysis and all uncertainties are reported as twice the standard deviation of the average lifetime. Decay associated spectra shown in Figure 2a were extracted from the global fitting analysis of the transient decay traces taken every third probe wavelength across the entire WLC range.

4. Singlet Oxygen Quantum Yields

Singlet oxygen phosphorescence measurements were performed using a modified Fluorolog-3 spectrometer (HORIBA, Jobin Yvon) in conjunction with a NIR sensitive photomultiplier tube (H10330A-45, Hamamatsu). A 450 W Xe lamp was used for steady-state excitation to record singlet oxygen phosphorescence spectra and a Spectra Physics GCR-150-30 Nd:YAG laser (355 nm, 7 ns pulse width) was used for pulsed excitation to collect singlet oxygen phosphorescence decay traces at 1270 nm, which were stored on a digital oscilloscope (TDS 360, Tektronics).

O_2-saturated acetonitrile solutions of the thiobases and standard (phenalenone) with matching absorbance at 355 nm (O.D. = 0.3 at 1 cm optical path length) were prepared. The measurements were performed in a 1×1 cm quartz cell. Singlet oxygen

quantum yields were determined from the phosphorescence intensity at 1270 nm at the end of the laser pulse using phenalenone as standard ($\Phi_\Delta = 0.98$).[6]

II. Supporting Results

1. Singlet Oxygen Phosphorescence Spectrum

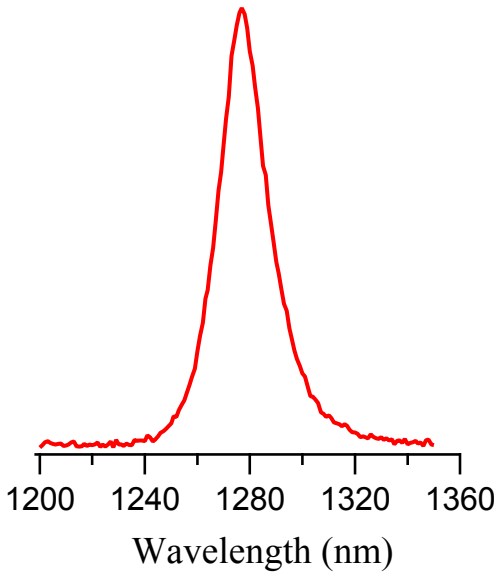

Figure S1. Singlet oxygen phosphorescence spectrum generated by photo-excitation at 355 nm of 4tThd in O_2-saturated acetonitrile solution.

2. Estimation of Effective UVA Treatment Depths

Longer wavelengths of light are able to penetrate deeper into tissues due to less scattering and reduced absorption by natural biomolecules (Fig. S2).[7] Therefore, the redshifted absorption of 2,4dtThy as compared to 4tThd opens up the possibility for it to be effectively used as a photosensitizer in deeper tissue UVA-activated therapies. The maximum treatment depth for these two compounds was estimated by defining an arbitrary absorption cutoff of $\varepsilon = 1000\ M^{-1}cm^{-1}$ (i.e., 371 nm for 4tThd and 395 nm for 2,4dtThy; Fig. S2). This revealed treatment depths of 71 and 87 μm, respectively, corresponding to a lower bound of approximately 23% increase in the maximum

treatment depth of 2,4dtThy as compared to 4tThd. This simple method, however, does not provide the total relative increase in treatment depth because it does not take into account the wavelength dependency of the penetration depth across the entire UVA range (315 – 400 nm). In order to account for this, the area under the penetration depth curve (Fig. S2) was integrated up to the respective absorption cutoff for each compound. Using this integration method the total increase in treatment depth of 2,4dtThy relative to 4tThd was determined to be about 67%. This increase likely serves as an upper bound limit for the actual relative increase because the molar absorptivity coefficients of the compounds will no doubt play a role. However, because the absorption of radiation at a given wavelength is not only dependent on a photosensitizer's molar absorptivity coefficients but also on its concentration in the tissue, our best estimation is that 2,4dtThy could facilitate UVA-activated treatment in tissues 23 to 67% deeper than those that can be effectively treated by 4tThd.

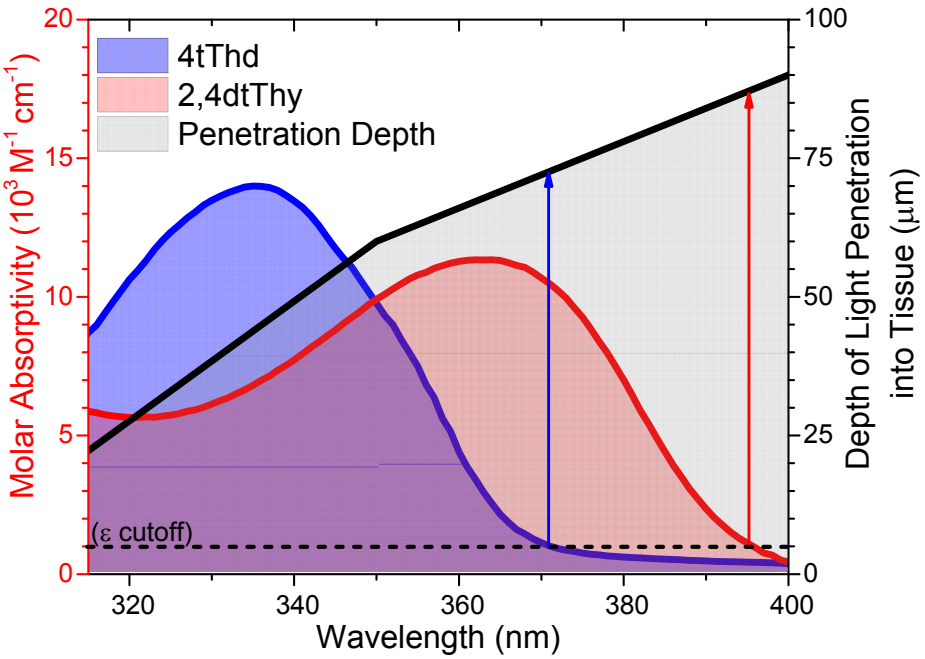

Figure S2. Molar absorptivity coefficients of 4tThd and 2,4dtThy in the UVA region of the spectrum overlaid with the wavelength-dependent penetration depth of UVA light into tissue.

III. References for Supporting Information

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