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Extended rhodamine photosensitizers for photodynamic therapy of cancer cells

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ABSTRACT

Extended thio- and selenorhodamines with a linear or angular fused benzo group were prepared. The absorption maxima for these compounds fell between 640 and 700 nm. The extended rhodamines were evaluated for their potential as photosensitizers for photodynamic therapy in Colo-26 cells. These compounds were examined for their photophysical properties (absorption, fluorescence, and ability to generate singlet oxygen), for their dark and phototoxicity toward Colo-26 cells, and for their co-localization with mitochondrial-specific agents in Colo-26 and HUT-78 cells. The angular extended rhodamines were effective photosensitizers toward Colo-26 cells with 1.0 J cm^{-2} laser light delivered at $\lambda_{max} \pm 2$ nm with values of EC_{50} of $(2.8 \pm 0.4) \times 10^{-7}$ M for sulfur-containing analogue **6-S** and $(6.4 \pm 0.4) \times 10^{-8}$ M for selenium-containing analogue **6-Se**. The linear extended rhodamines were effective photosensitizers toward Colo-26 cells with 5 and 10 J cm^{-2} of broad-band light (EC_{50}'s $\leqslant 2.4 \times 10^{-7}$ M).

1. Introduction

Photodynamic therapy (PDT) is a treatment for cancer that utilizes a photosensitizer that is targeted to the tumor and light directed to the tumor-localized photosensitizer to generate cytotoxic reactive oxygen species.[1] Ideally, the photosensitizer absorbs light in the 600–800-nm window where penetration of light in tissue is optimal. In addition, the photosensitizer should display minimal toxicity in the absence of light and should not photosensitize the patient to light in general.

The rhodamine dyes are cationic dyes based on the xanthylium core. The rhodamines and other cationic dyes have been examined as photosensitizers for PDT due to their ability to target the mitochondria of transformed cells and to absorb light.[2,3] Rhodamine-123, in particular, targeted the mitochondria and was also a chemotherapeutic agent in the dark.[4,5] Rhodamine-123 and other members of the rhodamine/rosamine family absorb wavelengths of light too short for effective penetration of tissue (λ_{max} 500–550 nm). Furthermore, these molecules generate singlet oxygen ($^1O_2$) and other reactive oxygen species inefficiently due to the lack of a heavy atom in the chromophore.

Heavy atoms have been introduced to the rhodamine core in two different ways. Bromination of the xanthylium nucleus gave brominated rhodamines that generated $^1O_2$ more efficiently, but still absorbed light in the 500–550-nm window.[6–9] We have prepared rhodamine derivatives with selenium replacing oxygen in the xanthylium core. Not only did this substitution give quantum yields for the generation of $^1O_2$ [$\Phi(^1O_2)$] of up to 0.87 for the selenorosamine **TMR-Se** (Chart 1), but also gave a longer-wavelength absorption maximum (λ_{max}) of 580 nm.[10] We have recently described the synthesis of selenorhodamines **1–3** (Chart 1) as the first rhodamine photosensitizers with values of $\lambda_{max} > 600$ nm that maintain their ability to target mitochondria in cancer cells and are useful as photosensitizers for PDT.[11,12]

Extending values of λ_{max} to even longer wavelengths while still maintaining high values of $\Phi(^1O_2)$ and the ability to localize in the mitochondria of cancer cells should provide even better photosensitizers for PDT. One possible approach to longer-wavelength-absorbing rhodamine-related dyes is the incorporation of additional fused benzene rings. This approach has been successful with fluorescein-related molecules[13–15] and has given chromophores with longer wavelengths of absorption and emission with such as examples **4** [λ_{max} (H_2O) 542 nm, λ_{EM} 629 nm][14] and **5** [λ_{max} (H_2O) 536 nm, λ_{EM} 733 nm][14] shown in Chart 2. It should be possible to prepare rhodamine-related structures with similar additional fused rings as well as derivatives that substitute sulfur and selenium for the xanthene oxygen atom. Herein, we report our initial biological evaluation of extended chalcogenorhodamine chromophores **6-E** and **7-E** (E = S or Se, Chart 2) as photosensitizers for PDT.

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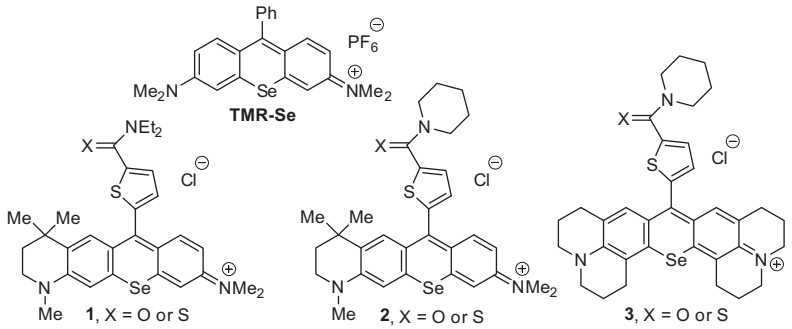

Chart 1. Structures of **TMR-Se** and selenorhodamines **1**–**3**.

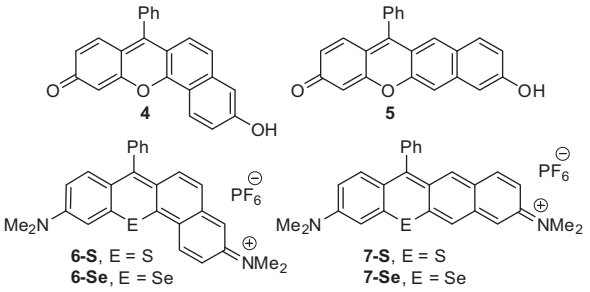

Chart 2. Extended fluorescein molecules **4** and **5** and extended rhodamine analogues **6-E** and **7-E**.

2. Results and discussion

2.1. Synthesis of selenorhodamines 6-E and 7-E

The key intermediates to the extended rhodamine dyes **6-E** and **7-E** are the corresponding extended xanthones **8-E** and **9-E**. As shown in Scheme 1, xanthones **8-E** and **9-E** can be prepared by the cyclization of diaryl chalcogenides **10-E** and **11-E**, respectively. The addition of bis-3-dimethylaminophenyl dichalcogenides **12-E**[16] to 1-lithio-6-dimethylamino-2-naphthamide **13** or 3-lithio-6-dimethylamino-2-naphthamide **14**, respectively, in turn gives the diaryl chalcogenides **10-E** and **11-E**. The regioselectivity of deprotonation of **15** to give **13** and **14** will determine the efficiency of the synthesis.

In our synthetic approach to **TMR-Se** and selenorhodamines **1** and **2**, directed metalations of 4-dimethylamino-substituted benzamide derivatives with *s*-BuLi/TMEDA gave 2-lithio-4-dimethylamino-benzamides in excellent yield, which then reacted with diaryl dichalcogenides to give xanthone precursors.[16] A similar approach with naphthamide **15** leads to precursors to xanthones **8-E** from 1-lithio-2-naphthamides **13** and to **9-E** from 3-lithio-2-naphthamides **14**. However, the regiochemical preference for metalation with *s*-BuLi/TMEDA is not clear. The directed metalation of 2-substituted naphthalenes in the literature has varying regioselectivity depending upon the specific substrate, directing group, base, and solvent conditions.[17–21]

Amide **15** was subjected to directed metalation with *s*-BuLi and TMEDA at −78 °C and the resulting mixture of anions was quenched with dichalcogenides **12-E** (Scheme 2). Lithiation at the 1-position appeared to be favored by a ratio of 2.5:1 as determined by ^{1}H NMR spectroscopy following quenching of the anion mixture with either disulfide **12-S** or diselenide **12-Se** to give a mixture of **10-S** and **11-S** in 76% isolated yield and a mixture of **10-Se** and **11-Se** in 60% isolated yield (Scheme 2). At this stage, the mixture of regioisomers **10-E/11-E** was not readily separable by chromatography and the mixture was treated with $POCl_3$ and Et_3N in refluxing acetonitrile to give the extended xanthones **8-E** and **9-E**. From the mixture of regioisomers **10-E/11-E**, the two isomeric xanthones were separable by silica chromatography and **8-S** was isolated in 41% yield and **9-S** in 20% isolated yield while **8-Se** was isolated in 45% yield and **9-Se** was isolated in 21% yield (Scheme 2).

The ^{1}H NMR spectra of the extended xanthones **8-E** and **9-E** (Supplementary data) allowed the unambiguous assignment of structure. The number of aromatic protons with coupling constants consistent with an *ortho*-coupling partner (~7–9 Hz) define the structure. Xanthones **8-E** have six protons with *ortho*-coupling partners while xanthones **9-E** have four.

The addition of phenylmagnesium bromide to a stirred suspension of **8-E** or **9-E** in THF (16 h at reflux) followed by work up with 10% aqueous HPF_6 gave dyes **6-E** and **7-E**, respectively, in 84–95% isolated yield (Scheme 3). The dyes **6-E** and **7-E** were sparingly soluble in most organic solvents, which made the acquisition of high-quality ^{13}C and any ^{77}Se NMR spectra difficult. However, the ^{1}H and ^{13}C NMR spectra (Supplementary data) were consistent with expected structures and the extended rhodamine dyes all passed combustion analysis and high-resolution mass spectral analysis. The solubility of the dyes **6-E** and **7-E** also limited an accurate experimental determination of values of the *n*-octanol/water partition coefficient (log*P*).

2.2. Photophysical properties

Absorption spectra for **6-E** and **7-E** are shown in Figure 1 and values of λ_{max} and the molar extinction coefficient (ε) in MeOH and in CH_2Cl_2 are compiled in Table 1. The extended rhodamines have values of λ_{max} in the 630–700-nm range. Fluorescence emission maxima (λ_{FL}) with excitation at 532 nm are also compiled in Table 1. Fluorescence quantum yields (Φ_{FL}) were determined from the steady-state fluorescence spectra for **6-E** and **7-E** (**TMR-Se** was used as a reference standard with known fluorescence quantum yield, Φ_{FL} = 0.009 in MeOH).[10] Dyes **6-S** and **7-S** had values of Φ_{FL} of 0.47 and 0.18, respectively, while the dyes **6-Se** and **7-Se** were weakly fluorescent with identical values of Φ_{FL} of 0.009. Quantum yields for the generation of $^1O_2$ [$\Phi(^1O_2)$] by **6-E** and **7-E** were measured by time-resolved spectroscopy at 1270 nm of $^1O_2$ luminescence in air-saturated MeOH.[22] While values of $\Phi(^1O_2)$ could be quantified for **6-S** and **6-Se** with excitation at 532 nm, only a weak 1270-nm signal was detected from **7-S** and **7-Se**, which could not be resolved to an accurate value of $\Phi(^1O_2)$. Values of $\Phi(^1O_2)$ for **7-S** and **7-Se** are listed as <0.05 to place an upper boundary on the value and to acknowledge that weak, but detectable, amounts of $^1O_2$ are being produced.