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Selective arylthiolane deprotection by singlet oxygen: a promising tool for sensors and prodrugs†‡

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A routine thioketal protecting group reacts rapidly and selectively with singlet oxygen to reveal ketone products in good (aryl 1,3-dithiolane) to excellent (aryl 1,3-oxathiolane) yields. Arylthiolanes are stable to biologically relevant reactive oxygen species and can be used as a light-activated gating mechanism for activating fluorescent sensors or small molecule prodrugs.

Photoswitches for small molecules are integral components of next-generation pharmaceuticals and biomaterials,[1] biomolecular probes,[2] and even nanomachines. Many common photoswitches are based on derivatives of nitrosoveratryloxycarbonyl groups,[3] *cis–trans* isomerizing azobenzenes,[4] spiro N–O bond cleavage,[5] and two-photon actuation strategies.[6] Despite the dominance of these mostly ultraviolet-actuated photoswitches in many chemical applications, therapeutically relevant switches require visible or infrared light for optimal tissue transparency. This has led to the development and delivery of more efficient infrared-absorbing photosensitizers producing singlet oxygen ($^1O_2$) for photodynamic therapy (PDT).[7,8] Next-generation photosensitizers possess an enhanced combination of near-infrared light absorption, $^1O_2$ production, cell targeting/penetration, and minimal dark toxicity. If combined with a practical, chemoselective and sensitive $^1O_2$-mediated chemical transformation, these developments could lead to new classes of visible/near-infrared photoswitches compatible with methods in PDT and enabling localized small molecule therapies.

Ideally a $^1O_2$-mediated photoswitch should be (a) stable in aqueous or serum-containing solutions, (b) modular for simple incorporation into numerous small molecules, (c) inert to common biological sources of ROS and (d) cleanly reveal a useful functional group in high yields. Thioketal protecting groups are well known for their stability to hydrolysis, simple formation, and unique redox potentials allowing for selective removal and resulting in their ubiquitous use as protecting groups in organic syntheses. For their removal a number of single electron transfer (SET) reagents, and even common name reactions (*e.g.* Corey-Seebach) are available. In organic solvents, photoinduced electron transfer (PET) can provide a catalytic mechanism for the removal of some thioketal containing substrates and there have even been some reports that thioketals are $^1O_2$ reactive in organic solvents.[9,10] Owing to some of these reports and the ubiquitous use of thioketals as protecting groups in organic synthesis, it was sought to ascertain whether thioketals could be tailored as a modular component for $^1O_2$-mediated activation of fluorescent sensors and prodrugs of potential compatibility with methods in PDT.

To ascertain the optimal structural parameters for $^1O_2$-mediated transformation of thioketals into ketone products, numerous thioketal containing small molecules possessing modifications to adjacent R, R′, and R″ positions were synthesized (Fig. 1A). These compounds were irradiated by 25 000 lumen white light in buffered protic solvents with known $^1O_2$ producing photosensitizers. Reactivity and product yields were assessed in pH 7.4 buffered aqueous–ethanolic solutions to maximize the probability that the observed reactions and mechanisms would proceed similarly in an *in vivo* like environment. Good to excellent yields and rapid formation of ketone product were observed with arylthiolanes (Fig. 1, **1a–5a**, **8a**, **14**), especially in the presence of a strong electron donor (**4a**). Aryldithiolanes (**1b–5b**) and aryldithianes (**6a**, **6b**, and **7**) exhibited reduced reaction rates and yields of ketone product. Thioketal reactivity was only observed in the presence of α-thioether protons and yields of ketone product are minimal in the absence of an α-aryl substituent to the thioketal or when a competing α-thioether proton is present (**9–13**). These results differ from SET mechanisms for removal of thioketals in polar aprotic solvents and

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† B. M. L. dedicates this work to the memory of Carlos F. Barbas III, an outstanding mentor and friend.

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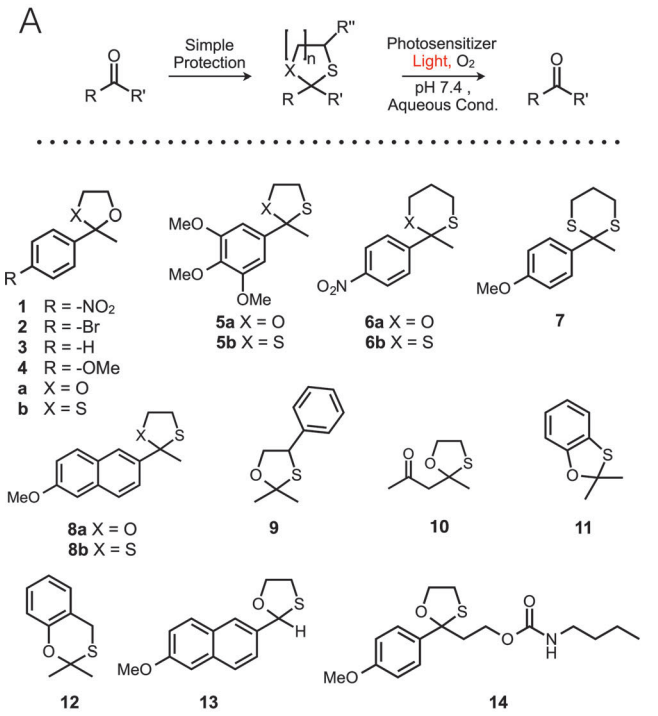

B

Substrate	Photosensitizer	Ketone Formation Rate (μM/min)	SM $t_{1/2}$ (min)	Ketone Yield
1a	RB	1.4	723	50%
1b	RB	2.0	484	48%
2a	RB	6.1	231	70%
2b	RB	4.3	297	64%
3a	RB	7.1	210	75%
3b	RB	5.9	213	63%
4a	RB/MB/mPPa	11.6/9.5/15.3	166/201/127	96/95/97%*
4a[a]	RB	88.1	22	97%*
4b	RB	7.2	195	70%
5a	RB	7.8	182	71%
5b	RB	5.7	185	53%
6a	RB	0.2	7449	90%
6b	RB	0.1	1643	12%
7	RB	0.7	300	10%
8a	RB/MB/mPPa	7.4/4.8/14.3	250/381/130	93/90/93%*
8b	RB/MB/mPPa	5.0/3.3/8.9	285/417/160	71/69/71%*
9	RB	-	N/A	0
10	RB	-	N/A	27%
11	RB	-	-	0
12	RB	-	-	0
13	RB	-	-	0
14[a]	RB	41.1	45	94%

Fig. 1 Optimization of thioketal removal with $^1O_2$. (A) General synthetic scheme for producing thioketals and $^1O_2$-mediated revealing of ketone substrates. (B) Degradation rates and yields of ketone product derived from photo-oxidation of selected thioketal substrates. *Standard conditions*: 2 mM thioketal, 7 : 3 EtOH/H_2O + 50 mM TrisHCl pH 7.4 and 5 mol% photosensitizer (MB = methylene blue, RB = rose bengal, mPPa = methylpyropheophorbide *a*), exposed to 25 000 lumen compact fluorescent light. Reported yields from HPLC. * NMR yields. [a] Substrate was oxidized under an oxygen atmosphere.

are more consistent with mechanistic observations associated with the reactivity of ethyl disulfide, 1,3-dithianes, and various thioethers with $^1O_2$.[9] Specifically, $^1O_2$ is expected to produce a peroxysulfonium ylide intermediate with reactivity strongly dependent on an aprotic or protic environment. Generally, $^1O_2$ oxidation yields sulfone or sulfoxide products in aprotic solvents, whereas protic conditions tend to quench the ylide, leading to alternative reactivity. In agreement with these reactive proclivities, photo-oxidation with rose bengal (RB), methylene blue (MB), or methyl pyropheophorbide *a* (mPPa) in polar aprotic solvents exclusively provided sulfoxide products, whereas the same reaction in protic solvent led exclusively to ketone product. Under protic conditions an oxidized sulfide byproduct was characterized by NMR and MS analysis suggestive of an elimination mechanism (ESI,‡ Fig. S1). From the data in Fig. 1, it was determined that an optimal thioketal structure for $^1O_2$-mediated transformation of thioketals into ketone products in buffered aqueous solvents possessed (1) activated α-aryl groups, (2) a single pair of α-thioether protons, and an 1,3-oxathiolane thioketal (exemplified by **4a**).

Singlet oxygen is one of several classes of ROS, which includes superoxide, peroxides, nitric oxide, and various oxygen radical substituents. To assess the specificity of the 1,3-dithiolane and 1,3-oxathiolanes to degradation by $^1O_2$ in real time, **8a** and **8b** were subjected to $^1O_2$ producing photosensitizers and light or representative alternative ROS. The ketone product, 6-methoxy-2-acetonapthhone, is fluorescent UV irradiation, whereas the thioketal and sulfoxide oxidation products lack fluorescence.

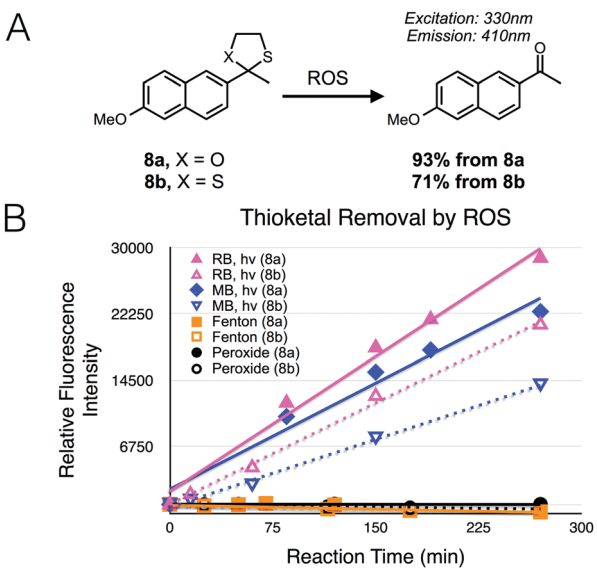

Fig. 2 Assessment of arylthiolane reactivity with ROS. (A) General reaction for the production of the fluorescent ketone product. (B) Arylthiolane degradation by ROS. *Standard conditions*: 0.5 mM arylthiolane **8a/b**, 7 : 3 EtOH/H_2O + 50 mM TrisHCl pH 7.4. *ROS conditions*: 5 mol% RB or MB photosensitizer + 25 000 lumen compact fluorescent light, 100 mM H_2O_2, or 10 mM $FeCl_3$ and 100 mM H_2O_2 (Fenton).

In the presence of RB, MB, or mPPa, (mPPa not shown in Fig. 2 due to fluorescence overlap), deprotection could be monitored in

real time *via* Tecan Fluorimeter (Fig. 2A). Among the fluorescent sensors, the 1,3-oxathiolane sensor **8a** gave the best yields of ketone product and showed over 20% enhanced reactivity compared to the 1,3-dithiolane sensor **8b** (Fig. 2B). Peroxide, superoxide, and Fenton conditions (iron^{3+}/peroxide – hydroxyl radical and superoxide mixtures) were unreactive to the arylthiolanes over the analysis time. Deprotection of either **8a**/**b** was not observed even with >100-fold excess superoxide reagent, either derived *in situ* (as part of the Fenton reaction) or in organic solvent (50-fold excess potassium superoxide and 18-crown-6 ether in refluxing THF, not shown), even over prolonged periods of time (days). The unreactivity of the thioketal was further confirmed by HPLC analysis at the end of assay, to ensure the absence of non-fluorescent oxidation products. Although the lability of thioketal polymer-based systems to superoxide is precedented in the literature, we hypothesize that the cyclic rather than acyclic structure results in differential reactivity of these thioketals for superoxide or other ROS.[11] Finally, since cellular compartments contain a range of acidic and basic conditions, the stability of the thioketal protecting group in pH 1, 7.4, and 13 solutions was investigated. It was observed in all pH ranges that **8a** and **8b** were unreactive and stable, with only slow hydrolysis of **8a** observed in strongly acidic solution (ESI,‡ Fig. S2).

It has recently been reported that photosensitizer–prodrug conjugates joined by an aminoacrylate $^1O_2$-labile linker can be activated with visible/near-infrared light to site-specifically eliminate tumors *in vitro* and *in vivo*.[12,13] It was hypothesized that an aryl-1,3-oxathiolane linker, based on a serum-induced β-elimination mechanism employed by catalytic antibody activated prodrugs and derived from optimal thioketal **4a**, could be used as a general scaffold for facile creation of $^1O_2$-activated prodrugs from small molecules possessing free amines, hydroxyls, or carboxylate functional handles.[13] This contrasts with aminoacrylate linkers which are presently limited to the release of phenol products.[12] This strategy could significantly expand the utility of $^1O_2$-mediated transformations to cover most small molecule therapeutics and even enable simple bioconjugations with oxyamines, hydrazines, or hydrazones.[14] A short synthetic effort provided prodrug **15** (ESI,‡ Scheme S1), doxorubicin conjugated to an optimized $^1O_2$-sensitive arylthiolane linker. Substrate **15** was oxidized to **16** in PBS–Tris–DMF solution and both the arylthiolane degradation and appearance of ketone product was observed by HPLC and LCMS. Rose bengal and methylene blue photosensitizers and 25 000 lumen compact fluorescent white light (Fig. 3B) exposure cleanly removed the 1,3-oxathiolane without modification to doxorubicin. In the absence of light or photosensitizer, arylthiolane degradation was not observed and approximately 12% hydrolysis was observed over 3 days, as measured by the appearance of doxorubicin on HPLC and the disappearance of **15**, indicating that the arylthiolane moiety remains stable in a biological environment. Although the ketone-containing product is slow to eliminate in PBS or pH 7.4 buffered solutions, upon dilution in serum-containing media, the β-elimination reaction is almost instantaneous.[15]

In summary, a $^1O_2$-mediated transformation of arylthiolanes into aryl ketones was structurally optimized for buffered aqueous

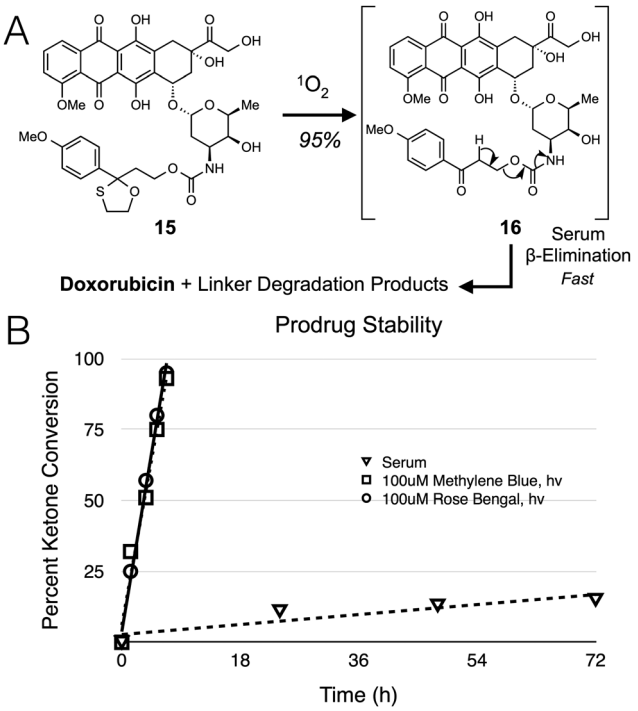

Fig. 3 (A) Schematic for oxidation of **15** to **16** and release of doxorubicin. (B) Stability of **15** in the presence of photosensitizer/light and fetal bovine serum. Conditions: 0.5 mM **15** 2 : 1 : 1 DMF/PBS/50 mM Tris pH 7.4 or serum.

conditions and shows promise as a modular component in light-activated sensors and prodrugs. The thioketal moiety reacts selectively with $^1O_2$ and is stable to other ROS commonly found in biological settings. When incorporated into a $^1O_2$-labile linker and conjugated to doxorubicin, photooxidation exclusively led to linker degradation. The simplicity of the synthesis of the thioketals, their stability in solution and during synthetic preparation, and the compatibility with photosensitizers across multiple wavelengths make arylthiolane-based linkers attractive for incorporation into fluorescent sensors for *in vivo* imaging and localized drug activation.[12,16] We anticipate many applications of arylthiolanes as an important intramolecular component of light-activated small molecules, bioconjugated proteins, and key reactive components of $^1O_2$-activated liposomal drug delivery vehicles.

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