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LETTER

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Type 1 Phototherapeutic Agents, Part I: Preparation and Cancer Cell Viability Studies of Novel Photolabile Sulfenamides

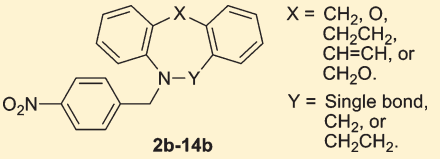
Amolkumar S. Karwa,[†] Amruta R. Poreddy,[†] Bethel Asmelash,[†] Tien-Sung Lin,[‡] Richard B. Dorshow,[†] and Raghavan Rajagopalan*[,†]

[†]Covidien Pharmaceuticals, 675 McDonnell Boulevard, Hazelwood, Missouri 63042, United States

[‡]Department of Chemistry, Washington University, 1 Brookings Drive, St. Louis, Missouri 63130, United States

Supporting Information

ABSTRACT: Novel type 1 phototherapeutic agents based on compounds containing S−N bonds (sulfenamides) were synthesized, assessed for free radical generation, and evaluated in vitro for cell death efficacy in four cancer cell lines (U937, HTC11, KB, and HT29). All of the compounds were found to produce copious free radicals upon photoexcitation with UV-A and/or UV-B light, as determined by electron spin resonance spectroscopy. Among the sulfenamides, the most potent compounds were derived from dibenzazepine **7b** and dihydroacridine **8b** as determined in all of the four cancer cell lines.



KEYWORDS: Sulfenamides, ROS, type 1, type 2, photosensitizers, radicals, ESR

Phototherapeutic agents are classified according to the reaction pathway involved in the cell death process. In general, photosensitizers operate via two main mechanisms, type 1 and type 2,[1−3] and type 2 photosensitzers have been used extensively for the treatment of various lesions.[4−9] For example, commercial photosensitizers such as Visudyne used for the treatment of wet macular degeneration and Photofrin used for the treatment of certain types of cancers, are porphyrin-based type 2 agents. The vast majority of current research is focused on improving the efficacy of such porphyrin-based type 2 agents. Surprisingly, very little attention has been devoted to type 1 agents despite the fact that there are numerous classes of compounds suitable for the development of such photosensitizers.

In type 2 process (referred to as photodynamic therapy or "PDT"), the energy of the excited photosensitizer is transferred to nearby molecular oxygen, creating singlet oxygen and subsequent reactive oxygen species (ROS) such as hydroxyl radical, superoxide radical anion, etc. that are responsible for the cell death.[10−12] This process may be repeated continuously because a substantial proportion of the photosensitizer returns to the ground state without photodegradation, but the depletion of oxygen level (local hypoxia) induces inflammatory response that causes vasodilatation and enables the transport of cancer cells to other regions resulting in tumor metastases.[13] However, the risk of metastases can be mitigated by using combination therapy, where an anti-VEGF (vascular endothelial growth factor) agent, such as Avastin, is coadministered with the photosenstizer.[14]

On the other hand, in type 1 process, the absorption of light causes the photosensitizer itself to undergo bond fragmentation to generate reactive intermediates such as free radicals, which induces the cell death. Whereas the PDT is mediated by singlet oxygen and requires red light for optimal generation of singlet oxygen, the type 1 process does not require oxygen for activity, nor is it restricted by wavelength of light. Hence, type 1 photosensitizers could be useful for ablation of lesions under hypoxic conditions. In the type 1 process, the photosensitzer undergoes irreversible change, and the delivery of an efficacious amount of type 1 agents to a lesion could be a limiting factor. However, the advent of newly developed drug delivery systems, especially the many varieties of nanoparticles, should allow efficacious delivery of these agents with increased safety margins.[15] Thus, we have implemented a research effort on developing type 1 phototherapeutic agents, and in this letter, we present a preliminary study on the synthesis and in vitro evaluation of type 1 compounds containing photolabile S−N bonds[16] **1b**−**14b** (Chart 1).

The sulfenamides **1b**, **2b**, and **6b**−**10b** were prepared in one step by the reaction of 4-nitrobenzenesulfenyl chloride with corresponding commercially available arylamines **1a**, **2a**, and **6a**−**10a** (Scheme 1). Compounds **3b**−**5b** and **11b**−**14b** were prepared similarly from the corresponding known tricyclic amines **3a**−**5a** and **11a**−**14a**.[17,18] The reaction of sulfenyl chlorides with diarylamines is known to generate diarlyl sulfide **B** (Scheme 1) resulting from C-sulfenylation of the phenyl ring, but the electronic factors controlling the product distribution of **A** and **B** has not been fully understood.[19] For example, the diarylamines **1a**−**5a**, **7a**, and **14a** gave N-sulfenylated products **A** exclusively, whereas **6a** and **12a** gave the C-sulfenylated isomers **B** exclusively. All other amines gave variable mixtures of **A** and **B**. It may also be noted that the preparation of sulfenamides without the nitro group or with an electron-donating group on the thiophenyl ring was not successful, which is consistent with the observation reported earlier by others for the preparation of sulfenates.[20]

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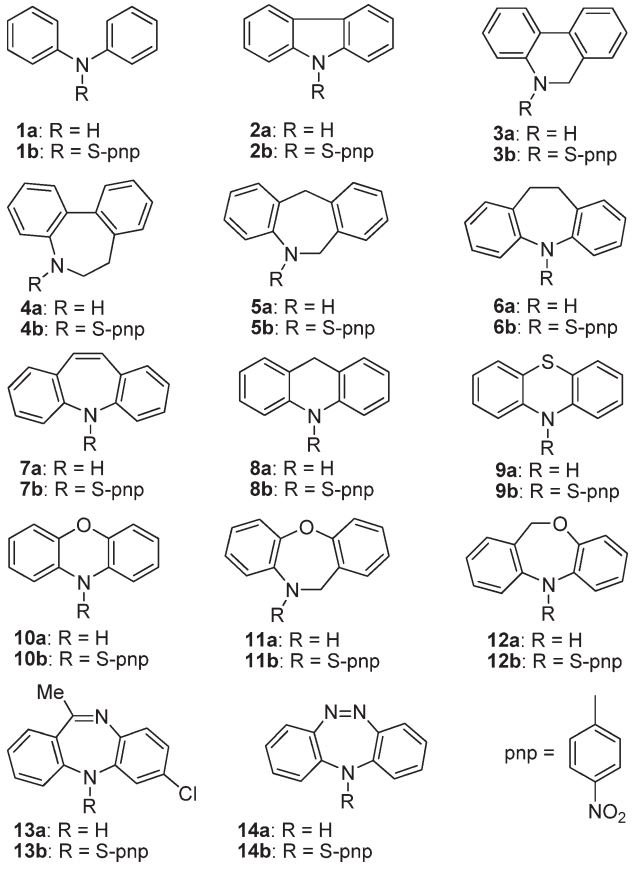
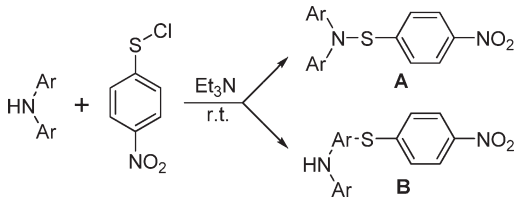
Chart 1. Structures of Sulfenamides



Scheme 1



All of the sulfenamides generated copious free radicals upon photofragmentation of S−N bond as evidenced from the electron spin resonance (ESR) measurements. ESR spectroscopy is a powerful and widely used technique for the detection and characterization of chemical species with unpaired electrons (e.g., free radicals). Representative ESR spectra corresponding to the photolyzed sulfenamides **1b** and **7b** are shown in Figure 1. Because most of the free radicals are short-lived, a spin trapping agent, 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), was used to trap radical species in the ESR studies. The DMPO spin adducts are relatively stable nitroxides with unique ESR spin parameters and spectral patterns depending on the type of free radicals added to the carbon atom at the 2-position (i.e., the β-carbon) in DMPO.[21] All of the compounds generated the same fast-decaying sulfur-centered DMPO spin adducts (DMPO/ArS$^{\bullet}$) upon photolysis as evidenced by the same paramagnetic parameters and decay characteristics (Figure 1a,c). The paramagnetic parameters of all DMPO/ArS$^{\bullet}$ spin adducts are g = 2.0052; $a_N = a_{H\beta}$ = 1.32 mT.

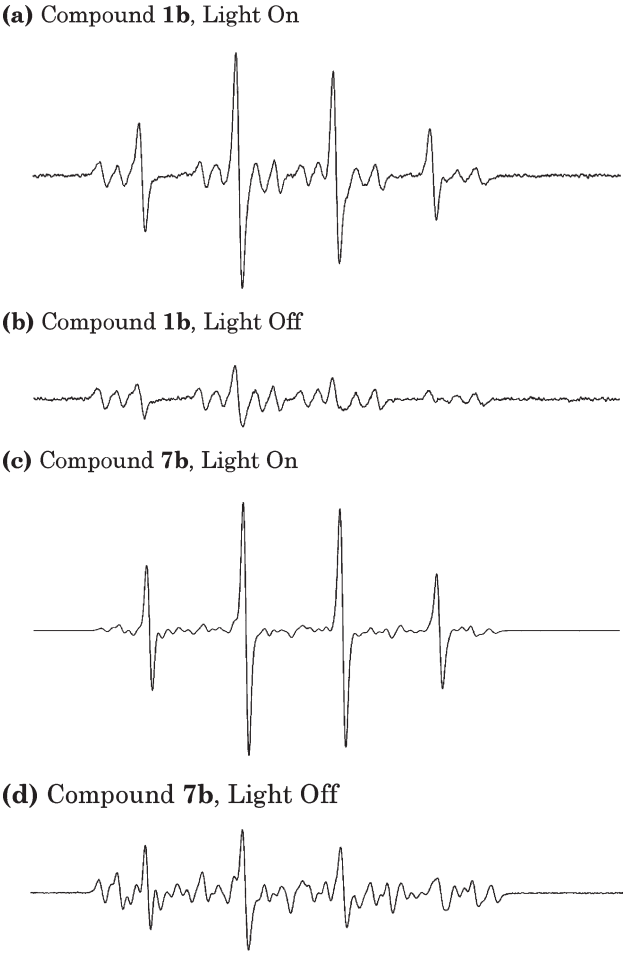


Figure 1. ESR spectra of sulfenamides **1b** and **7b** in benzene with DMPO spin trapping agent: (a) compound **1b** during light exposure, (b) compound **1b** after irradiation in light-off period, (c) compound **7b** during light exposure, and (d) compound **7b** during light-off period. The fast-decaying spin adducts DMPO/ArS$^{\bullet}$ become much weaker, and the weak signals of spectra b and d are assigned to the nitrogen-centered DMPO/Ar$_2$N$^{\bullet}$ spin adducts.

The decay half-life ($t_{1/2}$) of DMPO/ArS$^{\bullet}$ spin adducts from **1b** and **7b** is about 10 s. On the other hand, the nitrogen-centered DMPO spin adducts (DMPO/Ar$_2$N$^{\bullet}$) (Figure 1b,d) are substantially different because the aromatic portion of the N-centered spin adducts are different and may account for the differences in the cell death properties. The typical parameters for DMPO/Ar$_2$N$^{\bullet}$ spin adducts are g = 2.0050; $a_{N\alpha}$ = 1.77 mT; $a_{H\beta}$ = 1.77 mT; and $a_{N\beta}$ = 0.26 mT for compound **1b**. Similar parameters are obtained for compounds **7b** except that more proton splittings ($a_{H\gamma}$) are observed due to extended conjugation of the π system.

The values for $a_{N\beta}$ and $a_{H\gamma}$ parameters varied slightly among compounds depending on the delocalization of the unpaired electron in the aromatic π system. Note that these parameters refer to the spin adducts and not the original nascent radical upon irradiation. Some of these parameters are similar to those of reported values.[22−25] We further observed that the N-centered radicals form molecular complexes with oxygen in the ESR spectra of nondegassed samples (unpublished results). The oxygen complex of nitrogen-centered radical may further undergo electron transfer to form superoxide anion radicals ($^{\bullet}O_2^-$), which is detrimental to cell survival.

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The viability of cancer cells exposed to the sulfenamides in the absence and presence of light was assessed by the standard WST-1 assay[26] using U937 leukemia cell lines. For the two most active sulfenamides **7b** and **8b**, three additional cell lines (HTC11, KB, and HT29) were also used. All of the compounds were dissolved in DMSO at an initial concentration of about 8 mM and were diluted with cell culture media such that amount of DMSO exposed to the cells were below 0.5% (64 mM). The cells were incubated with various concentrations of the photosensitizer for 2 h prior to light exposure. The corresponding control conditions were (a) DMSO only (no photosensitizer), no light; (b) DMSO only (no photosensitizer) light; and (c) photosensitizer, no light (dark toxicity). The cells were irradiated for 20 min with 100 W UV lamp (model B-100A, UVP, Upland, CA) with peak output at 365 nm and a bandwidth of about 50 nm. The light source was not optimized for each compound with respect to power and wavelength. The total power near the surface of the microtiter plate is about 8 mW. The temperature at the surface of the microtiter plates was kept below 37 °C with external air cooling. The viability of cells was assessed after 24 h following light exposure.

Table 1. Absorption and Cell Viability (IC_{50}) Data

photosensitizer	λ_{max} (nm)	IC_{50} (μM)[a]
none (DMSO)		>100000
1b	307	6.8 ± 1.3
2b	325	>20
3b	325	7.3 ± 1.1
4b	341	6.0 ± 1.2
5b	325	5.7 ± 1.2
7b	350	1.2 ± 1.1
8b	321	1.3 ± 1.2
9b	321	12.4 ± 1.2
10b	327	>20
11b	345	17.2 ± 1.1
13b	335	2.0 ± 1.1
14b	420	>20

[a] Average of at least three independent runs.

The λ_{max} and IC_{50} values (defined as the concentration at which 50% decrease in cell viability is observed when the cells are exposed to light and the photosensitizer for 20 min) are given in Table 1. The absorption maxima for all of the sulfenamides fall in the range of 305−420 nm. The sulfenamides can be modified by fusing additional aromatic rings into their structures for extended conjugation to enable them to be activated with visible light. All of the sulfenamides except **6b** displayed dose-dependent and light exposure time-dependent decrease in cell viability. It should be noted that DMSO itself exhibited cytotoxicity only at high concentration and at long exposure time to light. In fact, the cell viability of DMSO was reduced by only about 15% at 100 mM concentration when irradiated for 20 min. The concentration of DMSO in the viability studies is less than 64 mM. Exemplary cell viability dose−response graphs for the sulfenamides **7b** and **8b** in four cancer cell lines (HTC116, HT29, KB, and U937) are shown in Figures 2 and 3.

The parent sulfenamide **1b** exhibits moderate activity with an IC_{50} of about 9 μM. Connecting the two phenyl groups with a single bond essentially abrogates the activity. Insertion of a methylene or an ethylene bridge between the nitrogen and the phenyl ring (compounds **3b** and **4b**, respectively) or insertion of one methylene between the nitrogen and the phenyl ring and the other between the two phenyl rings (compound **5b**) restores the

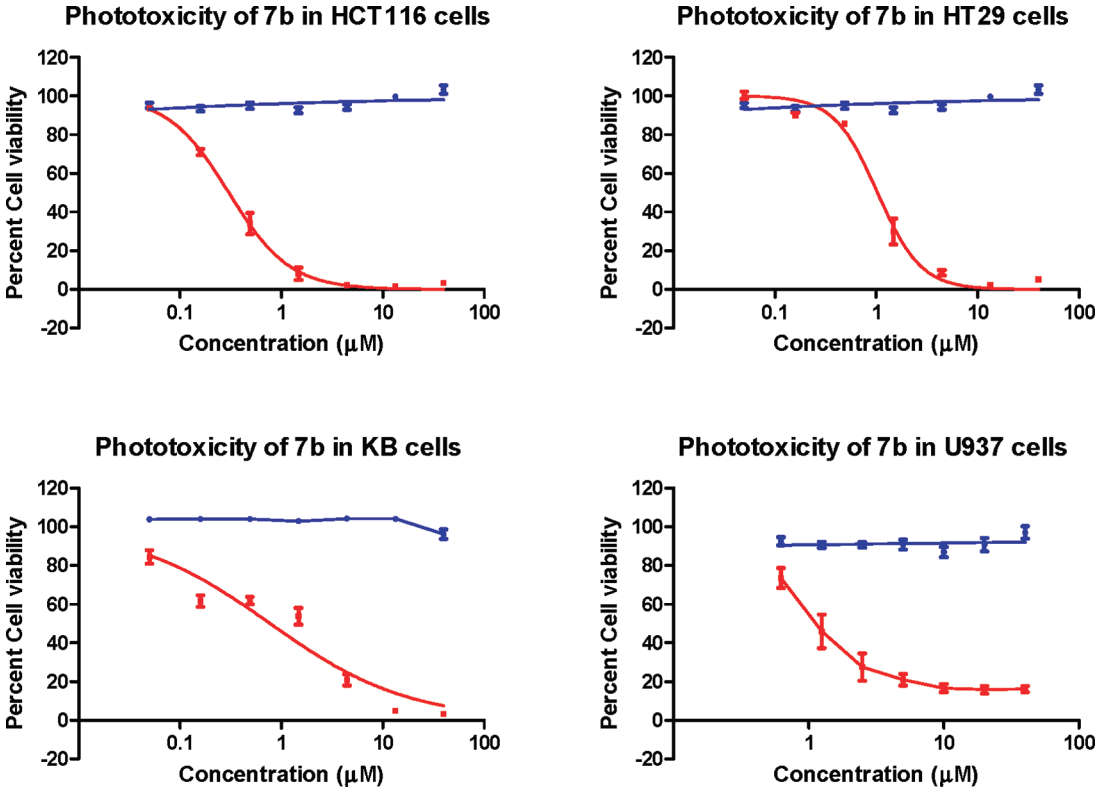

Figure 2. Phototoxicity of **7b**. Blue, no light exposure (dark toxicity); red, 20 min of light exposure (phototoxicity).

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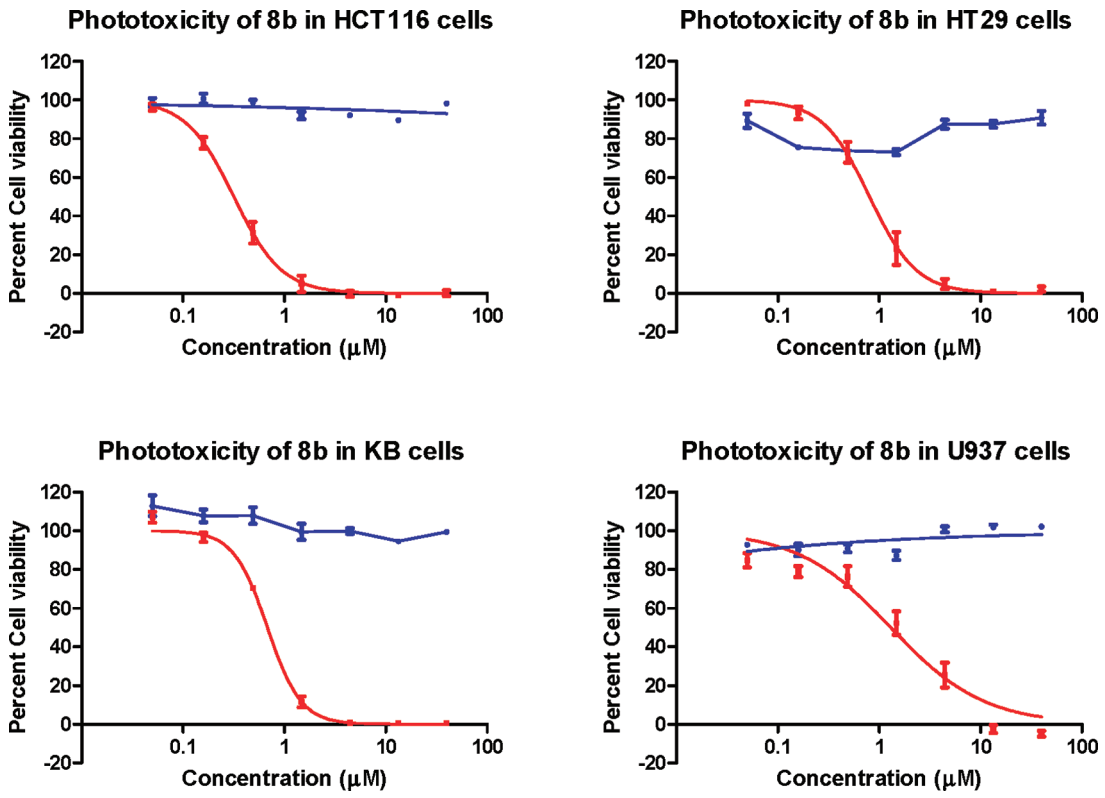

Figure 3. Phototoxicity of **8b**. Blue, no light exposure (dark toxicity); red, 20 min of light exposure (phototoxicity).

activity. The activity of compound **6a**, which contains an ethylene bridge between the two phenyl groups, could not be determined due to the exclusive formation of the C-sulfenylated thioether product. In contrast, inserting a vinylene bridge between the two phenyl rings in **1** (compound **7b**) or transposing one of the phenyl groups in **2** from an angular to a linear tricyclic arrangement (compound **8b**) greatly increases the activity. Indeed, the dibenzazepine **7b** and dihydroacridine **8b** are the most potent among all of the compounds with IC_{50} of about 1.3 and 1.2 μM, respectively. Replacing the methylene bridge in **8b** with oxygen or sulfur atom substantially reduces or even obviates the activity (compounds **9b** and **10b**). Likewise, replacing a methylene unit in **5b** or **6b** (compounds **11b** and **12b**) also eliminated the activity. Introduction of imino bridge between the two phenyl groups in **1** resulted in a potent compound **13b**, but surprisingly, introduction of an azo bridge (compound **14b**) completely abrogated the activity.

The difference in activity between the sulfenamides **2b** and **7b** could be rationalized on the basis of transition states leading to the formation of reactive species as illustrated in Scheme 2. If the transition state is dipolar in nature or if homolytic cleavage of S−N bond followed by electron transfer from nitrogen to sulfur leads to ionic species such as **15** and **16**, then the transition state for the photofragmentation of compound **2b** should resemble cyclopentadienyl cation **15**, which is antiaromatic, whereas for **7b** the transition state should resemble azatropylium cation[27] **16**, which is aromatic. The high resolution mass spectrum of **7b** clearly indicates the formation of two fragments: the azatropylium cation (exact mass, 193.0886) and the *p*-nitrophenylsulfide anion (exact mass, 153.9967). Furthermore, cyclic voltammetry studies with **7a** indicates that the formation of cation **16** from the radical **17** is a highly favored process with $E_{1/2}$ of 0.88 V.[28] Thus, it could be expected that the formation of reactive intermediate from **2b** would be considerably suppressed as compared to **7b** under the same photolytic conditions. Although the contribution, if any, of *p*-nitrophenylsulfide anion on cytotoxicity is not determined, it should be the same for all of the sulfenamides and should not affect the relative cell viabilities among these photosensitzers.

The high activities of **7b** and **8b** could be explained on the basis of two different pathways as illustrated in Scheme 2. In the case of **7b**, the N-centered radical **17** could also react with molecular oxygen to produce, via electron transfer, an ion pair **18**, where superoxide anion radical is known to be detrimental to cell viability. In the photolysis of **8b**, the dihydroacridine radical **19** could itself cause cell death directly or react with oxygen to form a stable nitroxide radical **20**,[29] which could cause cell death. Alternatively, the radicals **19** or **20** could undergo oxidation to the nitrone **21**, which may form an ion pair with superoxide anion radical ($^{\bullet}O_2^-$). Indeed, the ESR spectrum of **8b** as well as previous ESR studies with phenoxazines and phenothiazines[24,25] supports the formation of such nitroxide radicals.

In this preliminary communication, we have demonstrated that incorporation of S−N moiety into the photosensitizer structure is a viable approach for the design of suitable type 1 phototherapeutic agents. The results clearly support the hypothesis that the cell death occurs through initial formation of nitrogen-centered radicals. Whether these radicals cause cell death directly or through secondary ROS as well as the precise mechanism of action can only be ascertained with additional ESR and ultrafast spectroscopic studies and rigorous theoretical

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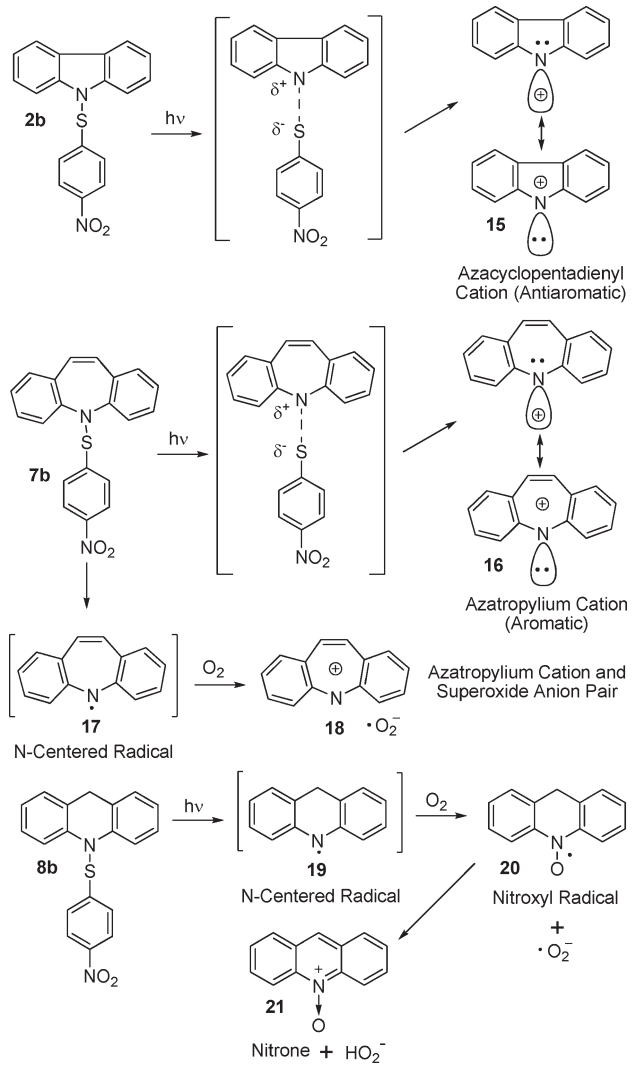
Scheme 2



calculations. These are currently being investigated and will be reported subsequently.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for the synthesis of compounds **1b**−**14b** and cell viability assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 314-654-3800. Fax: 314-654-8900. E-mail: Raghavan.rajagopalan@covidien.com.

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