Near-visible light generation of a quinone methide from 3-hydroxymethyl-2-anthrol

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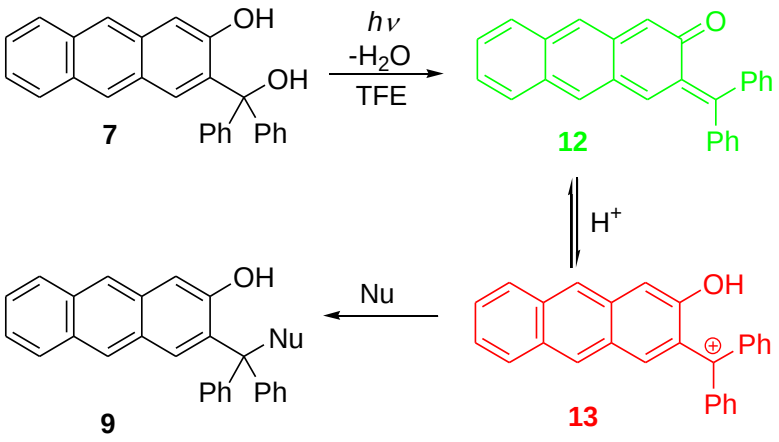
Graphical abstract:



Abstract: Excitation of 2-hydroxy-3-(diphenylhydroxymethyl)anthracene (**7**) to S_1 initiates photodehydration giving the corresponding quinone methide (QM) that was detected by laser flash photolysis (LFP) in 2,2,2-trifluoroethanol, TFE (λ = 580 nm, τ = 690 ± 10 ns). The QM decays by protonation, giving cation (λ = 520 nm, τ = 84 ± 3 μs) which subsequently reacts with nucleophiles. The rate constants in the reactions with nucleophiles were determined by LFP, whereas the adducts were isolated via preparative photolyses. The photogeneration of QMs in the anthrol series is important for potential use in biological systems since the chromophore absorbs at wavelengths > 400 nm. Antiproliferative investigations conducted with 2-anthrol derivative **7** on three human cancer cell lines showed higher activity for irradiated cells.

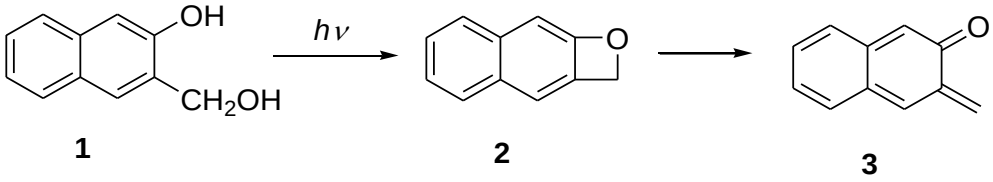
Key Words: antiproliferative activity, laser flash photolysis, anthrols, photodehydration, proton transfer, quinone methides

Introduction

Quinone methides (QM) are common reactive intermediates in chemistry and in the photochemistry of phenols, attracting much attention recently owing to their biological activity.[1] Although the partial zwitterionic character of QMs makes them both electrophilic and nucleophilic, their reactivity with nucleophiles is especially important in biological systems. It has been demonstrated that QMs react with amino acids[2] and proteins,[3] and inhibits the action of some enzymes.[4] Moreover, QMs also react with nucleotides[5] inducing alkylation and the cross-linking of DNA.[6] The ability of QMs to cross-link DNA renders them as potential anticancer therapeutics.[7] Some antineoplastic agents such as mitomycin[8] base their antiproliferative action on the metabolic formation of QMs that alkylate DNA. Moreover, some classes of anthracyclines

such as daunomycin, that base their action on DNA cross-linking, also metabolically form QMs.[9] However, later it was shown that QMs are not responsible reagents that cross-link DNA, but it probably involves metabolic formation of formaldehyde.[10]

QMs can be formed under mild conditions in photochemical reactions of suitably substituted phenols, such as photodehydroxylation of hydroxybenzylphenols,[11] photoelimination of acetic acid,[12] amines,[13] or ammonium salts.[2b] Photodehydration has also been reported in the larger chromophoric systems such as suitably substituted phenylphenols[14] and naphthols.[15] Popik *et al*. studied photodehydration of naphthol **1** which gave benzoxete intermediate **2** that subsequently underwent ring opening to QM **3** (eq.1).[16] The photogeneration of naphthalene QMs was later applied to photo-caging[17] and for surface modification and photolithography.[18]



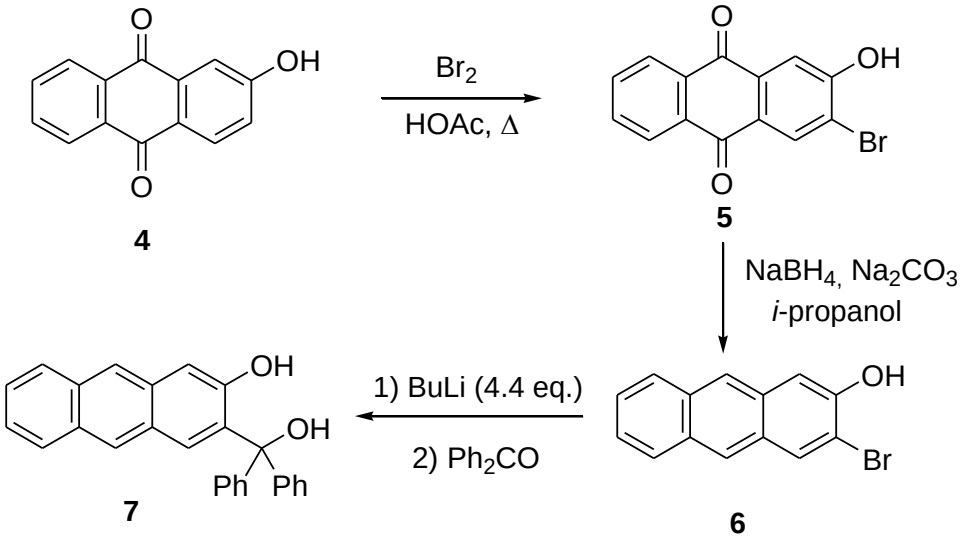
1 **2** **3** (1)

We became interested in the photochemical formation of sterically congested QMs that have potential biological applicability.[19] Photo-cytotoxicity was investigated on three human cancer lines. Photo-induced antiproliferative activity was reported for naphthol,[15b] 1,1'-bi-2-naphthol (BINOL)[20] and naphthalene diimide QM derivatives.[21] For ultimate application *in vivo* it is essential to develop systems that can photogenerate QMs when excited with the light of longer wavelengths (> 350 nm). However, the photogeneration of QMs by photodehydration reaction in 2-anthrols or larger chromophoric systems has never been reported.[9] Herein, we report the first example of photochemical QM formation (via dehydration) in an anthrol derivative **7**. The photoreactivity was studied by preparative irradiations, fluorescence measurements, and by laser

flash photolysis (LFP). Antiproliferative investigation was performed on three human cancer cell lines with and without irradiation.

Results and Discussion

Synthesis of the new anthrol **7** was accomplished in 4 steps, starting from the commercially available 2-aminoanthraquinone which was converted to 2-hydroxyanthraquinone (**4**) *via* a known procedure.[22] Bromination of **4** afforded 3-bromo derivative **5** (40%), and 1,3-dibromo derivative (38%) which was separated from **5** by a column chromatography (Scheme 1). Subsequent reduction of **5** gave 2-bromo-3-anthrol (**6**) in 80% yield and a small amount of 2-anthrol (**8**) due to undesired concomitant debromination. Lithiation with an excess of BuLi and quenching with benzophenone furnished **7** in 40% yield.



Scheme 1.

Absorption spectra of **7** and **8** in CH_3CN solution exhibit absorption band of lowest energy centered between 350-400 nm, 50 nm bathochromically shifted compared to that of anthracene.

The pK_a of 2-anthrol (**8**) in CH_3CN-H_2O (1:9) in S_0 and S_1 was determined by UV-vis and fluorescence titration, respectively (pK_a = 9.40 ± 0.03; pK_a* = 2.13 ± 0.01, see the SI). Since it is known that intramolecular H-bonding with the benzyl alcohol increases acidity in 2-naphthol,[16] the anticipated pK_a values for **7** are expected to be somewhat lower.

The photophysical properties of **7** and **8** were investigated in CH_3CN and CH_3CN-H_2O (1:1). Results are compiled in Table 1. Fluorescence in aqueous solution is quenched compared to that in neat CH_3CN. The quenching is due to proton transfer (PT) to solvent. Furthermore, Φ_F for **7** in both solvents is lower than for **8**. Since **8** cannot give QMs, the lower Φ_F of **7** may be due to the photochemical pathway giving QM. Contrary to 2-methoxyanthracene,[23] **7** and **8** are characterized by a single exponential decay of fluorescence in CH_3CN. Addition of H_2O changes the decay. For **8** it becomes double-exponential with a rise component (assigned to phenolate) that contributes more at longer wavelengths. For **7**, the best fit was obtained by a three-exponential function with two rise components. Although a firm elucidation of the decay kinetics cannot be made at this time, the finding is consistent with a scheme that photodissociation of the anthrol OH of **7** in S_1 triggers elimination of OH^- (overall loss of H_2O) that leads to the formation of QM.

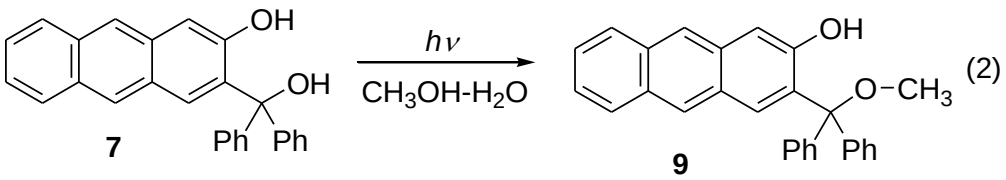
Table 1. Photophysical properties of **7** and **8** in CH_3CN and CH_3CN-H_2O (1:1).

	7	**8**
Φ_F (CH_3CN)[a]	0.86 ± 0.01	0.88 ± 0.05
Φ_F (CH_3CN-H_2O)[a]	0.39 ± 0.01	0.57 ± 0.02
τ (CH_3CN)/ns[b]	17.8 ± 0.1	25.3 ± 0.1
τ (CH_3CN-H_2O)/ns[b]	1.7 ± 0.2 phenolate	15.4 ± 0.1 phenolate

	8.1 ± 0.2 phenolate	25.3 ± 0.1 phenol
	24.5 ± 0.1 phenol	

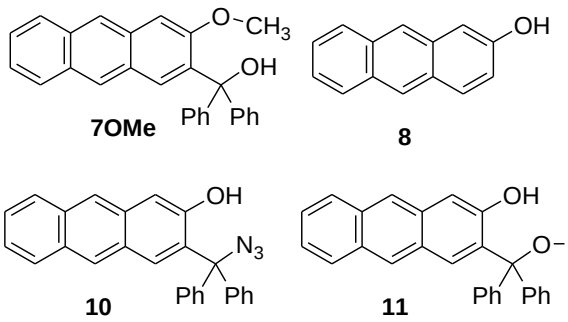
[a] Quantum yields of fluorescence measured by use of quinine sulfate in 0.05 M aqueous H_2SO_4 (Φ_F = 0.53) as a reference.[24] [b] Fluorescence lifetimes measured by time-correlated single photon timing method.

Photochemical solvolysis of **7** was investigated by irradiations in CH_3OH-H_2O (see the SI). Irradiations gave cleanly one product (**9**, Eq. 2). Conversion to **9** after 1 h of irradiation was higher in the presence of 20% H_2O (≈70%) than in neat CH_3OH (≈50%). Such a finding has been reported in systems wherein the phenolic OH is not H-bonded to the benzylic alcohol,[19] and explained by a higher ability of phenols to deprotonate in S_1 to clusters of H_2O than CH_3OH.[25]



7 **9** (2)

Photosolvolysis of trityl derivatives, can in principle, take place via carbocations. To probe if **7** undergoes heterolytic cleavage of OH to give carbocation **13** (Scheme 2), irradiation of **7OMe** was performed. However, in the condition where **7** gave 72% of **9**, **7OMe** remained unchanged. This finding indicates that the free phenolic OH is required for the formation of the solovolysis products. Furthermore, the finding is in accord with the photosolvolysis mechanism involving QM **12** (*vide infra*).



The scope of the photosolvolysis was further investigated by performing irradiations in the presence of other nucleophiles. Thus, azide adduct **10**, and 2,2,2-trifluoroethanol (TFE) adduct **11** were isolated after irradiation of **7** in CH_3CN-H_2O, and CH_3CN-TFE, respectively, in ≈35-40% yield. The structures of the photoproducts **9**, **10**, and **11** were confirmed by NMR analysis. Additionally, all photosolvolyses were achieved by use of light > 350 nm (350 nm, 420 nm, and vis-lamps, see the SI).

The efficiency of the photosolvolysis in CH_3OH-H_2O (4:1) was determined by simultaneous use of three actinometers, valerophenone, KI/KIO_3 and ferioxalate.[26] Irradiation was performed by use of monochromatic light at 254 nm, and the composition of the irradiated solution of **7** was analyzed by HPLC. All actinometers gave $\Phi_R = 0.023 \pm 0.001$ for reaction.

To probe for QM and other plausible long-lived intermediates in the photochemistry of **7**, LFP measurements were performed. The samples were excited by use of a Nd:YAG laser at 354 nm. The measurements were performed in CH_3CN and CH_3CN-H_2O (1:1), and the difference was anticipated due to PT in the aqueous solution. In CH_3CN solution (N_2- and O_2-purged), a band centered at 700 nm can be seen ($\tau = 1.0 \pm 0.1$ μs) that in the aqueous solution decays during the laser pulse. The transient is tentatively assigned to anthrol radical-cation which in the aqueous

solution decays by deprotonation.[27] In addition, in both solvents, more persistent transients were detected absorbing at shorter wavelengths 400-600 nm. The decay is multi-exponential revealing several species. The major contribution to the band more centered at shorter wavelengths (400-500 nm) decays slower ($\tau \approx$ 10-100 μs and $\tau \approx$ 0.1-2 s) and can also be seen in the transient spectra of **8** (see the supporting info). The transients are not affected by the presence of O_2 and H_2O. The shorter-lived species is tentatively assigned to phenoxyl radical, according to the comparison with the published spectra and decay kinetics.[28] In addition, a transient absorption was detected at 500-600 nm that decays multi-exponentially that could be quenched by nucleophiles (and not by O_2). Therefore, this transient could tentatively be assigned to QMs or other electrophilic species (see the SI). However, due to complex decay kinetics and overlapping of the transient absorption spectra with several other species, no firm assignment to a QM can be made at this time.

A cleaner picture was obtained for LFP experiments carried out in TFE. TFE is a polar non-nucleophilic solvent in which electrophilic species such as QMs[14,15,19] and carbocations[29] exhibit longer lifetimes. LFP measurement for **7** in O_2-purged TFE gave rise to a strong transient absorption centered at 580 nm that is formed within the laser pulse. It decayed ($k_{12\rightarrow13} = 1.4\times10^6$ s^{-1}, $\tau = 690 \pm 10$ ns) giving a new species absorbing at 520 nm (Fig.1) that also decays ($k_{13\rightarrow9} = 1.4\times10^4$ s^{-1}, $\tau = 84 \pm 3$ μs). Addition of ethanolamine, an ubiquitous quencher of QMs,[14,19] changed the appearance of the spectra. However, the decay of the absorption at 580 nm was slower, and faster at 520 nm, giving one band in the spectra. This finding suggests that two species are in equilibrium that is influenced by pH (ethanolamine is a base). Quenching with nucleophiles was successful for species absorbing at 520 nm (not affecting the faster decay at 580 nm). The quenching rate constants are compiled in Table 2. According to the quenching data and

the position of the maximum in the absorption spectra (comparison with the known spectra of trityl cation),[30] the long-lived transient was assigned to carbocation **13**. Since the short-lived transient is sensitive to acidity and in equilibrium with the cation, it is tentatively assigned to QM **12**. Consequently, the first detected species in TFE by LFP is QM **12** which decays by protonation giving cation **13** (Scheme 2). Subsequent reaction with nucleophiles furnishes adducts. Interestingly, ethanolamine did not quench QM **12** in the tested concentration. The finding was explained by the ethanolamine basicity (pK_a = 9.5) which prevented fast protonation of QM **12** to cation **13**.

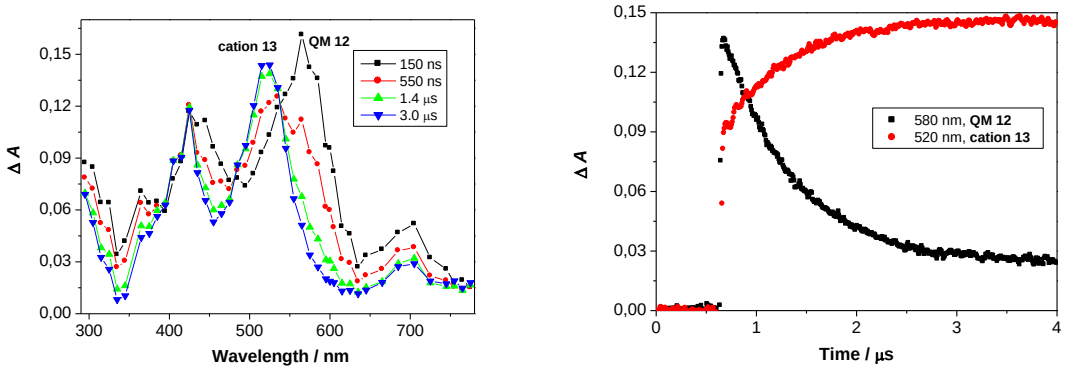


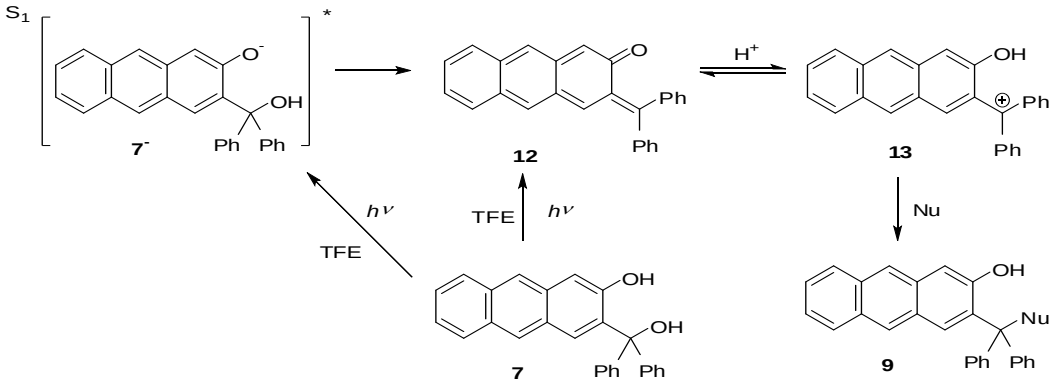
Figure 1. Transient absorption spectra of **7** in O_2-purged 2,2,2-trifluoroethanol (TFE) (left), and growth and decay of the transient absorption at 520 nm (corresponding to cation **13**) and 580 nm (corresponding to QM **12**), respectively (right).

Table 2. Rate constants for the quenching of cation **13** with nucleophiles (k_q or $k_{13\rightarrow9}$ / s^{-1} M^{-1}).[a]

Nucleophile	k_q / s^{-1} M^{-1}
CH_3OH	9.8×10^3
H_2O	1.6×10^3

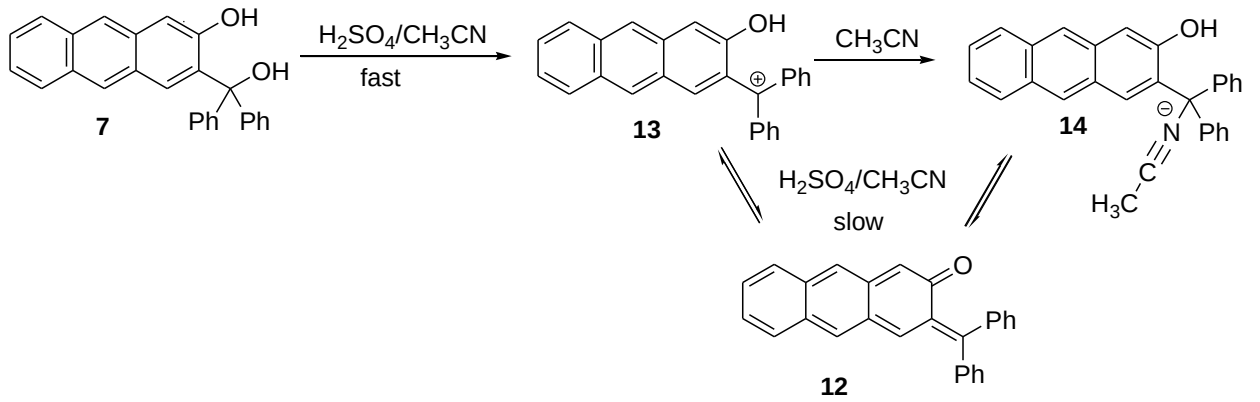
NaN_3 2.1×10^8

[a] Measurements performed in air-saturated TFE.



Scheme 2.

Cation **13** can also be formed in the CH_3CN solution in a thermal acid-catalyzed reaction and detected by UV-vis spectroscopy. To the CH_3CN solution was added H_2SO_4, (c = 0.25 M) resulting in an immediate color change to red. The difference absorption spectrum has a maximum at 520 nm (see the Supporting info) with another band at longer wavelengths (≈700 nm) and resembles the transient absorption of **7** measured in TFE and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) after decay of the short-lived species assigned to QM **12**. The transient in CH_3CN decays (τ = 0.63 ± 0.02 min) probably giving CH_3CN adduct **14**, resulting in a colorless solution. However, within 45-60 min, a new species is formed absorbing at 500-700 nm, resulting in green color of the solution. The latter is tentatively assigned to QM **12** formed in a thermal acid-catalyzed reaction (Scheme 3).



Scheme 3.

Antiproliferative investigation was conducted with **7** on three human cancer cell lines HCT 116 (colon), MCF-7 (breast), and H 460 (lung) with and without exposure to irradiation (350 nm and 420 nm). In addition, the cells were irradiated in the absence of compounds to check for the cytotoxic effect of irradiations. The results are compiled in Table 3. Irradiation of the cells at 350 nm that were not treated with **7** induced up to maximum 25% of inhibition of tumor cell growth, whereas 420 nm did not cause any significant inhibition of tumor cell growth. On the other hand, exposure of the cells treated with **7** to irradiation induced higher cytotoxic effect than cells that were kept in dark. Consequently, higher photocytotoxicity of the compound allows for potential biological applications. However, for the actual photo-chemotherapeutic applications (except for maybe some forms of skin cancer) the compounds should bear chromophores absorbing at > 600 nm. Photochemicaly induced higher cytotoxicity of **7** suggests that the enhanced antiproliferative activity is due to the photo-generation of QMs. Although the exact mechanism of the enhanced antiproliferative activity was not determined, it is presumed that it is due to the reactivity of **12** or **13** with DNA,[5,6] as well as some particular proteins (e.g. enzymes).[2-4] Since the LFP experiments did not indicate formation of the triplet excited state, singlet oxygen probably did not induce

antiproliferative activity. The investigation of biological action of different anthrol derivatives is currently under way and will be published separately.

Table 3. IC_{50} values (in μM) for **7**.[a]

Cell lines	**HCT 116**	**MCF-7**	**H 460**
Not irradiated	21±0.3	20±0.5	19±1
3 × 5 min 350 nm	2±0.4	4±3	3±1
3 × 5 min 420 nm	2±0.4	2±0.1	2±0.2

[a] IC_{50}; the concentration that causes 50% growth inhibition. Irradiation of the cells at 350 nm that were not treated with 7 induced up to maximum 25% inhibition of tumor cell growth, whereas irradiation of untreated cells at 420 nm showed no effect. For the calculation of IC_{50} see the experimental.

Conclusion

The presented results show that dehydration of 2-anthrol derivative **7** to QM **12** can be initiated in a photochemical reaction, and probably involves deprotonation of the phenol OH as the first step, followed by expulsion of the alcohol OH^-. However, we could not time-resolve the formation of QM **12** (it is formed within the laser pulse). Therefore, we cannot rule out mechanisms that involve (i) excited state intramolecular proton transfer (ESIPT) from the phenol OH to the benzyl alcohol that is coupled with dehydration, or (ii) formation of a benzoxete intermediate that has a lifetime of < 50 ns and subsequently ring opens to form the QM.

Photogeneration of QMs in the anthrol series that can be initiated by near-visible light is of particular importance in biological systems. Preliminary results of antiproliferative investigations

conducted for **7** on three human cancer cell lines showed higher activity for the cells that were irradiated. Consequently, we believe that anthrol derivatives have potential as a new class of photo-chemotherapeutic reagents, or as photo-labeling markers for biological systems.

Experimental Section

Experimental procedure, general

^{1}H and ^{13}C NMR spectra were recorded at 300, or 600 MHz at rt using TMS as a reference and chemical shifts were reported in ppm. Melting points were determined using a Mikroheiztisch apparatus and were not corrected. IR spectra were recorded on a spectrophotometer in KBr and the characteristic peak values were given in cm^{-1}. HRMS were obtained on a MALDI TOF/TOF instrument. For the sample analysis a HPLC was used with C18 (1.8 μm, 4.6×50 mm) column. HPLC runs were conducted at rt (~25 °C) and chromatograms were recorded using UV detector at 254 nm. Other HPLC data were given in the supporting information, since the parameters vary. For the chromatographic separations silica gel (0.05–0.2mm) was used. Irradiation experiments were performed in a reactor equipped with 16 lamps with the output at 350 nm or a reactor equipped with 8 lamps. During the irradiations, the irradiated solutions were continuously purged with Ar and cooled by a tap-water finger-condenser. Solvents for irradiations were of HPLC purity. Chemicals were purchased from the usual commercial sources and were used as received. 2-Hydroxyanthraquinone (**4**) was prepared from commercially available 2-aminoanthraquinone according to the known procedure.[22] Solvents for chromatographic separations were used as they are delivered from supplier (p.a. grade) or purified by distillation (CH_2Cl_2). Diethyl ether used for the reaction with BuLi was previously refluxed over Na and freshly distilled.

3-Bromo-2-hydroxyanthraquinone (**5**)

The reaction was carried out in a two-neck round bottom flask (250 mL) equipped with a condenser and a dropping funnel. 2-Hydroxyanthraquinone (**4**, 4.54 g, 20.2 mmol) was dissolved in glacial acetic acid (50 mL) by heating using an oil bath. Bromine (5 mL, 97.2 mmol) in glacial acetic acid (50 mL) was added to the refluxing reaction mixture over 4 h, and the refluxing was continued overnight (16 h). The progress of the reaction was monitored by HPLC (see the SI). The next day the reaction mixture is allowed to cool to rt and poured on water (300 mL). A saturated solution of Na_2SO_3 was added to destroy the excess of bromine. The yellow precipitate was filtered of, washed with water until neutral (tested with universal indicator paper) and dried in a desiccator over KOH overnight. The crude product contained 3-bromo-2-hydroxyanthraquinone (**5**) and 1,3-dibromohydroxyanthraquinone in ratio 1:1. These two compounds were separated using column chromatography (SiO_2, CH_2Cl_2/EtOAc/HOAc 760:40:1 to 720:80:1) to obtain 1,3-dibromo-2-hydroxyanthraquinone **5** (2.93 g, 7. 68 mmol, 38% yield) and 3-bromo-2-hydroxyanthraquinone (2.45 g, 8.08 mmol, 40% yield) as yellow solid substances.

1,3-Dibromo-2-hydroxyanthraquinone, ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 8.34 (s, 1H), 8.18-8.10 (m, 2H), 7.93-7.87 (m, 2H); IR (KBr) ν_{max}/cm^{-1} 3412 (O-H), 3105 (C-H), 3070 (C-H), 1672 (C=O).

3-Bromo-2-hydroxyanthraquinone (**5**),[31] yellow solid, mp 235-245 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 11.97 (s, 1H), 8.22 (s, 1H), 8.17-8.11 (m, 2H), 7.95-7.86 (m, 2H), 7.64 (s, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ/ppm 182.0 (s), 180.3 (s), 159.6 (s), 134.5 (d), 134.2 (d), 134.0 (s), 132.9 (s), 132.8 (s), 132.1 (d), 126.6 (d, 2C), 125.8 (s), 116.6 (s), 112.8 (d); IR (KBr) ν_{max}/cm^{-}

[1] 3350 (O-H), 1668 (C=O), 1570 (C=O), 1274 (C-H), 719 (C-H); HRMS (MALDI) calculated for $C_{14}H_7BrKO_3^+$ 340.9210, found 340.9204.

3-Bromo-2-hydroxyanthracene (6)

$NaBH_4$ (454 mg, 12 mmol) was dissolved in 1M Na_2CO_3 (aq) (30 mL), *i*-propanol (5 mL) was added (foaming suppressor) and the mixture was heated until boiling point was achieved. 3-Bromo-2-hydroxyanthraquinone (**5**, 910 mg, 3 mmol) was added in three portions. The reaction mixture was refluxed for 15 min (longer reflux time usually produced more debromination product, 2-hydroxyanthracene). The reaction was quenched by careful addition of ice-cooled water (30 mL) followed by the addition of 3M HCl until acidic reaction was achieved (tested by universal indicator paper). The product was collected by filtration, washed with water (until neutral reaction of the filtrate was achieved) and dried in evacuated desiccator (10 mbar) over KOH overnight. The crude product was purified on a column of silica gel using dichloromethane as eluent to give pure product **6** (656 mg, 2.4 mmol, 80% yield over two steps) as yellow powder: mp 225-232 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm 8.27 (s, 1H), 8.24 (s, 1H), 8.22 (s, 1H), 7.93 (t, J = 7.2 Hz, 2H), 7.50 (s, 1H), 7.48-7.38 (m, 2H), 5.64 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ/ppm 150.9 (s), 132.0 (d), 131.7 (s), 131.5 (s), 129.7 (s), 128.0 (d), 127.7 (s), 127.4 (d), 125.7 (d), 125.1 (d), 124.6 (d), 123.1 (d), 114.7 (s), 107.8 (d); IR (KBr) ν_{max}/cm^{-1} 3512 (O-H), 3049 (C-H); HRMS (MALDI) calculated for $C_{14}H_9BrO^+$ 271.9831, found 271.9829.

2-Hydroxy-3-(diphenylhydroxymethyl)anthracene (7)

The reaction was carried out in a two-necked round bottom flask (50 mL) under a N_2 inert atmosphere, equipped with a N_2-balloon and a septum. The flask was charged with 3-bromo-2-hydroxyanthracene (**6**, 286 mg, 1.00 mmol) and dry Et_2O (10 mL) and cooled in an ice-methanol

bath (-15 to -10 °C). BuLi (2.5 M in hexanes, 1.2 mL, 3.00 mmol) was added dropwise over 15 min changing the color to brown. The reaction mixture was then emerged from the ice bath and stirred for 15 min at rt whereby all solid compound was dissolved giving a clear brown solution. The reaction mixture was then again cooled to -10 °C, and benzophenone (900 mg, 4.94 mmol) in dry Et_2O (4 mL) was added. Stirring was continued 1 h at -10 °C, then the reaction mixture was allowed to reach rt and the stirring was continued overnight. The reaction was quenched by careful addition of water (15 mL) and transferred to the separation funnel. A solution of 1M NaOH (20 mL) and water (100 mL) was added and the aqueous layer was extracted with hexane (2×20 mL) in order to remove unreacted benzophenone and the product of the reaction of benzophenone with BuLi. The aqueous layer was then acidified with 10% acetic acid and extracted with Et_2O (3×25 mL). The ether extracts were combined and dried over anhydrous $MgSO_4$. After filtration and removal of the solvent, the crude product was purified on a column of silica gel using dichloromethane as eluent to give pure **7** (763 mg, 2.03 mmol, 40% yield) in the form of yellow-orange solid. mp 199-200 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 10.22 (s, 1H), 8.28 (s, 1H), 8.26 (s, 1H), 7.95 (t, J = 7.0 Hz, 2H), 7.44-7.24 (m, 14 H), 6.70 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ/ppm 153.5, 145.9, 145.7, 136.5, 132.1, 131.8, 129.8, 128.6, 128.1, 128.0, 127.7, 127.6, 127.3, 127.1, 126.7, 126.6, 126.4, 126.2, 125.6, 124.2, 122.3, 108.5, 81.6, 74.2, 54.9; IR (KBr) ν_{max}/cm^{-1} 3367 (O-H), 3051 (C-H), 3024 (C-H), 1447 (C-H); HRMS (MALDI) calculated for $C_{27}H_{19}O^+$ 359.1430, found 359.1433.

2-Methoxy-3-(diphenylhydroxymethyl)anthracene (7OMe)

The reaction was carried out in a two-neck round bottom flask (25 mL) under N_2 atmosphere. 2-Hydroxy-3-(diphenylhydroxymethyl)anthracene (**7**, 18 mg, 48 μmol) was dissolved in acetone (8 mL) and K_2CO_3 (50 mg, 362 μmol) was added. The resulting suspension was heated at reflux

resulting in a change of the color from pale yellow to yellow. MeI (50 μL, 803 μmol) was then added and the reaction mixture was stirred 5h at rt (the color returns to pale yellow). The reaction mixture was then filtered and the filtrate evaporated on a rotary evaporator. The residue product was purified on short column of silica gel (10×1 cm) using CH_2Cl_2 as eluent to give pure 2-methoxy-3-(diphenylhydroxymethyl)anthracene (**7OMe,** 11 mg, 31 μmol, 64% yield) in the form of pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 8.25 (s, 1H), 8.12 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.44-7.40 (m, 2H), 7.34-7.28 (m, 11H), 7.11 (s, 1H), 5.19 (s, 1H), 3.79 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ/ppm 155.9 (s), 146.3 (s), 136.8 (s), 132.3 (s), 131.8 (s), 130.7 (s), 130.1 (d), 128.1 (d), 128.0 (d), 127.8 (d), 127.7 (d), 127.5 (d), 127.3 (s), 127.0 (d), 125.6 (d), 124.6 (d), 123.5 (d), 105.7 (d), 82.1 (s), 55.6 (q); IR (KBr) ν_{max}/cm^{-1} 3448 (O-H), 2920 (C-H), 2851 (C-H), 1458 (C-H); HRMS (MALDI) calculated for $C_{28}H_{22}O_2^+$ 390.1614, found 390.1624.

Irradiation experiments

Preparative photomethanolysis

In a quartz vessel was placed a CH_3OH–H_2O (4:1) solution of compound **7** (100 mL, c ~ 4×10^{-5} M) and irradiated in a Rayonet reactor using 16 lamps at 350 nm for 30-120 min. Prior to, and during the irradiation, the solution was continuously purged with a stream of Ar and cooled by a coldfinger condenser. After 90 min of irradiation, the solvent was removed on a rotary evaporator and the residue was dried (water was removed as azeotrope with toluene). The photoproduct was purified on a column of silica gel (4×1 cm) using CH_2Cl_2 as eluent to obtain pure **9** (13 mg, 33 μmol, 84% yield) in the form of pale yellow film on the walls of the flask. ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 8.68 (s, 1H), 8.20 (s, 1H), 8.19 (s, 1H), 7.90 (d, J = 8.4 Hz, 1H,), 7.86 (d, J = 8.4

Hz, 1H), 7.68 (s, 1H), 7.46-7.42 (m, 4H), 7.41-7.31 (m, 8H), 3.35 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ/ppm 153.9 (s), 140.6 (s), 132.6 (s), 132.4 (s), 132.1 (s), 130.5 (d), 129.0 (d), 128.1 (d), 128.0 (d), 127.9 (d), 127.5 (d), 126.9 (s), 126.7 (d), 125.5 (d), 124.2 (d), 123.0 (d), 110.1 (d), 53.1 (q); IR (KBr) ν_{max}/cm^{-1} 3421 (O-H), 3055 (C-H), 2970 (C-H), 2924 (C-H), 2953 (C-H), 1448 (C-H); HRMS (MALDI) calculated for $C_{27}H_{20}O^+$ 360.1509, found 360.1506.

Irradiation in the presence of NaN_3

In a quartz vessel was placed CH_3CN solution (70 mL) of compound **7** (15 mg, 40 μmol), and solution of NaN_3 (1 g, 15 mmol) in water (30 mL) was added. Upon addition of NaN_3 solution changed color to yellow, probably due to deprotonation of the anthrol OH (caused by hydrolysis of the azide). The solution was irradiated in a reactor using 12 lamps at 350 nm for 2 h. Prior to, and during the irradiation, the solution was continuously purged with a stream of Ar and cooled using a coldfinger condenser. After the irradiation, the reaction mixture was poured on water (150 mL) and extracted with diethyl ether (3×20 mL). The organic extracts were combined and washed with water (2×100 mL). The organic phase was separated and dried over anhydrous $MgSO_4$, filtered and the solvent was removed on a rotary evaporator. The crude product was chromatographed on a short column of silica gel (10×1 cm) using CH_2Cl_2 as an eluent to obtain pure product **10** (6 mg, 15 mmol, 38% yield) in the form of thin yellowish film on the walls of the flask. ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 8.23 (s, 1H), 8.18 (s, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.44-7.41 (m, 8H), 7.39 (s, 1H), 7.30 (m, 4H), 6.92 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ /ppm 152.4 (s), 140.9 (s), 132.54 (s), 132.46 (s), 131.4 (s), 130.9 (d), 130.4 (s), 128.6 (d), 128.4 (d), 128.3 (d), 128.2 (d), 128.1 (d), 127.6 (d), 127.1 (d), 126.8 (s), 125.6 (d), 124.5 (d) 123.2 (d), 111.3 (d), 29.6 (s); IR (KBr) ν_{max}/cm^{-1} 3421 (O-H), 3057 (C-H), 2924 (C-H), 2853 (C-H), 2104 (N≡N); HRMS (MALDI) calculated for $C_{27}H_{20}O^+$ 360.1509, found 360.1523.

Irradiation in the presence of 2,2,2-trifluoroethanol (TFE)

In a quartz vessel was placed a CH_3CN solution (90 mL) of compound **7** (13 mg, 35 μmol) and 2,2,2-trifluoroethanol (10 mL) was added. The solution was irradiated in a reactor using 12 lamps at 350 nm for 30 min. Prior to, and during the irradiation, the solution was continuously purged with a stream of Ar and cooled using a cold finger condenser. After the irradiation, the solvent was evaporated on rotary evaporator. The crude product was chromatographed on a short column of silica gel (10×1 cm) using CH_2Cl_2 as an eluent to obtain the pure product **11** (6 mg, 13 μmol, 37% yield) in the form of thin yellowish film on the walls of the flask. ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 8.22 (2s, 2H), 7.92 (d, J = 8.8 Hz, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.72 (s, 1H), 7.49-7.45 (m, 4H), 7.43-7.35 (m, 9H), 3.77 (q, $^3J_{H,F}$ = 8.1 Hz, 2H), ^{13}C NMR (150 MHz, $CDCl_3$) δ/ppm 152.9 (s), 139.7 (s), 132.64 (s), 132.58 (s), 132.4 (s), 130.9 (d), 130.4 (s), 128.6 (d), 128.5 (d), 128.3 (d), 128.1 (d), 127.6 (d), 127.0 (d), 126.9 (s), 125.8 (d), 125.5 (q, $^1J_{C,F}$ = 251 Hz), 124.5 (d), 123.2 (d), 111.0 (d), 62.7 (q, $^3J_{C,H}$ = 34 Hz), 29.6 (s); IR (KBr) ν_{max}/cm^{-1} 3435 (O-H), 3059 (C-H), 2924 (C-H), 2953 (C-H), 1281 (C-F), 1165 (C-F); HRMS (MALDI) calculated for $C_{27}H_{20}O^+$ 360.1509, found 360.1504.

Quantum yield of the photomethanolysis reaction

Quantum yield of the photomethanolysis reaction was determined by use of three actinometers simultaneously: valerophenone,[24] ferrioxalate[25] and KI/KIO_3.[32] A solution of ferrioxalate actinometer was handled in dark. The measurement was performed in five quartz cells with the same dimensions (square, for UV-vis measurement, ca. 3 mL), that were during the irradiations wrapped in black paper, except from the front side to ensure the controlled absorption of the light

from one side only (no absorption of the radiation reflected from the walls of the reactor). The solution of compound **7** in CH_3OH-H_2O (4:1), as well as the solution of valerophenone in CH_3CN-H_2O (4:1) were freshly prepared and their concentrations adjusted to have absorbances 0.4–0.8 at 254 nm. After adjustment of the concentrations and measurement of the corresponding UV-vis spectra, 2.5 mL of the solution was transferred to the quartz cell and the solutions were purged with a stream of N_2 (20 min each), and then, sealed with a cap. Freshly prepared solution of potassium trioxalatoferrate(III) (0.012 M $K_3[Fe(C_2O_4)_3]\times 3H_2O$ in 0.05 M H_2SO_4) (2.5 mL) and the solution containing potassium iodate (0.1 M) and potassium iodide (0.6 M) in borate buffer (pH = 9.25) (2.5 mL) were placed in the third and the forth quartz cell, respectively. Potassium trioxalatoferrate(III) (2.5 mL) was placed in the fifth cell which was not irradiated (blank sample). Before the irradiation, A_{352} for the solution of KI/KIO_3 was measured. The cells were placed in a holder which ensured equal distance of all samples from the lamp and irradiated at the same time in the reactor with 1 lamp at 254 nm for 30 s. Before and after the irradiation, the samples were taken from the cells by use of a syringe and analyzed by HPLC to determine conversion of **7** to **9** and valerophenone to acetophenone. The conversion did not exceed 30% to avoid change of the absorbance, or filtering of the light by the product. A_{352} of KI/KIO_3 actinometer solution was measured to determine the concentration of I_3^- using $\varepsilon_{352} = 27600\ M^{-1}cm^{-1}$.[32] To both solutions of ferrioxalate actinometer (irradiated and blank) a solution of phenantroline was added (0.5 mL, 0.1% phenanthroline in buffer containing 1.65 M NaOAc and 0.5 M H_2SO_4) and A_{510} was measured. The concentration of Fe^{II} was determined using $\varepsilon_{510} = 11100\ M^{-1}cm^{-1}$.[25] From the conversion (valerophenone) and concentration of the photoproducts (in ferrioxalate and KI/KIO_3) irradiance was calculated. The similar values were obtained for all three actinometers. These values and the value of conversion **7** → **9** were used to calculate the

quantum yield of photomethanolysis for compound **7**. The mean value of three measurements was reported. All equations for the calculation of quantum yields are given in the supporting info.

Steady State and Time-Resolved Fluorescence Measurements

The steady state measurements were performed a luminescence spectrometer. The samples were dissolved in CH_3CN, or CH_3CN-H_2O (1:1) and the concentrations were adjusted to have absorbances at the excitation wavelength (330, 350, or 370 nm) < 0.1. Solutions were purged with nitrogen for 30 min prior to analysis. The measurements were performed at 20 °C. Fluorescence quantum yields were determined by comparison of the integral of the emission bands with the one of quinine sulfate in 0.05 M aqueous H_2SO_4 (Φ_F = 0.53). The measurements were performed in triplicate and the mean value reported. Typically, three absorption traces were recorded (and averaged) and three fluorescence emission traces, exciting at three different wavelengths. Three quantum yields were calculated and the mean value reported.

Fluorescence decays were obtained on an instrument equipped with a light emitting diode (excitation wavelength 375 nm), using time-correlated single photon counting technique in 1023 channels. Histograms of the instrument response functions (using LUDOX scatterer), and sample decays were recorded at 410, 420, 450 and 550 nm until they reached 2×10^3 counts in the peak channel. The half width of the instrument response function was ≈ 0.2 ns. The time increment per channel was 0.02441 ns. Obtained histograms were fitted as sums of exponential using Gaussian-weighted non-linear least-squares fitting based on Marquardt-Levenberg minimization implemented in the software package of the instrument. The fitting parameters (decay times and pre-exponential factors) were determined by minimizing the global reduced chi-square χ^2.

Additional graphical method was used to judge the quality of the fit that included plots of surfaces ("carpets") of the weighted residuals vs. channel number.

Determination of pK_a and pK_a * for 2-anthrol (8)

UV-vis titration

A stock solution of the compound **8** was prepared by dissolving 0.50 mg of **8** in 500 μL CH_3CN. 195 μL of the stock solution was diluted to 200 mL with CH_3CN-H_2O (1:9) to adjust the concentration of the compound to 5.0×10^{-6} M (for titration I). For titration II, 7.93 mg of **8** was dissolved in 200 mL CH_3CN/H_2O (2:8). 150.0 mL of each solution was titrated with a diluted solution of NaOH (until pH 12.7 was reached). The pH was measured by a pH-meter and UV-vis spectra were recorded on an instrument. The measurement was performed at 25 °C. The resulting UV-vis spectra were processed by multivariate nonlinear regression analysis using Specfit program.

Fluorescence titration

A stock solution of the compound **8** was prepared by dissolving 1 mg of **8** in 1000 μL CH_3CN. 855 μL of the stock solution was diluted to 200 mL with CH_3CN-H_2O (1:9) to adjust the concentration of the compound to 2.2×10^{-5} M. 150.0 mL of the solution was basified with a diluted solution of NaOH (until pH 12.3 was reached) and titrated with a diluted solution of H_2SO_4 (until pH 0.5 was reached). The pH was measured by a pH-meter and fluorescence spectra were recorded on an instrument (slits 2×2.5 nm). The measurement was performed at 25 °C. The resulting fluorescence spectra were processed by multivariate nonlinear regression analysis using Specfit program.

Laser Flash Photolysis (LFP)

All LFP studies were conducted at the University of Victoria LFP facility employing a YAG laser, with a pulse width of 10 ns and excitation wavelength 355 nm. Static cells (0.7 cm) were used and solutions were purged with nitrogen or oxygen for 20 min prior to measurements. Absorbances at 355 nm were ~ 0.4.

Antiproliferative investigation

The experiments were carried out on three human carcinoma cell lines HCT 116, MCF-7 and H 460. Cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

The cells were inoculated in parallel on two 96-well microtiter plates on day 0, at 1.5×10^4 cells/mL. Test agents were added in ten-fold dilutions (10^{-8} to 10^{-4} M) on the next day and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. One of the plates was left in the dark, while the other was irradiated in a reactor (6 lamps 350 nm or 420 nm, 5 min) 4, 24, and 48 hours after the addition of the compounds. After 72 h of incubation the cell growth rate was evaluated by performing the MTT assay[33] (for the irradiated and non-irradiated cells) which detects dehydrogenase activity in viable cells. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean A_{test} – mean A_{tzero}) ≥ 0, then PG = 100 × (mean A_{test} – mean A_{tzero}) / (mean A_{ctrl} – mean A_{tzero}).

If (mean A_{test} – mean A_{tzero}) < 0, then: PG = 100 × (mean A_{test} – mean A_{tzero}) / A_{tzero}, where the mean A_{tzero} is the average of absorbance measurements before exposure of cells to the test compound, the mean A_{test} is the average of absorbance measurements after the desired period of time and the mean A_{ctrl} is the average of absorbance measurements after the desired period of time with no exposure of cells to the test compound. In the experiments where the cells were irradiated, A_{ctrl} represents irradiated control cells. After irradiation at 350 nm 25% growth inhibition compared to A_{ctrl} without irradiation was observed, while after the irradiation at 420 nm no significant growth inhibition was observed.

The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values are calculated from concentration-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (*i.e.* 50%). Each test was performed in quadruplicate in at least two individual experiments.

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Supporting information The contents of Supporting Information includes copies of the ^{1}H and ^{13}C NMR spectra, UV-vis, fluorescence, and LFP data. This material is available free of charge via the Internet at http://pubs.acs.org.

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