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Photoswitching off the Antiproliferative Activity of Combretastatin A-4 Analogues

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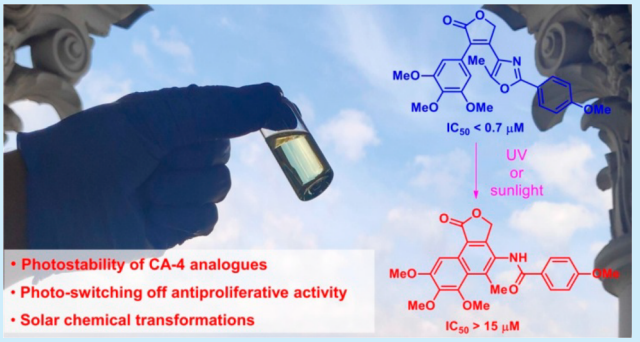
Supporting Information

ABSTRACT: The photostability and antiproliferative activity of combretastatin A-4 (CA-4) analogues against human epidermoid carcinoma cells A-431 were studied. For the first time, it was shown that UV or sunlight irradiation of furanone analogues of CA-4 results in a photorearrangement giving products with relatively low antiproliferative activity. The observed ability of this series CA-4 to the photodegradation can be used for the design of a new class of drug candidates with high selectivity to cancer cells.



Diarylethenes occupy an important place among the photoswitchable compounds, which are extensively studied for the design of smart materials and devices.[1,2] On the other hand, CA-4 analogues, being diarylethene derivatives, are of interest as biologically active compounds acting as inhibitors of tubulin polymerization and suppressing microtubule formation.[3−5] CA-4 analogues occupy a special place among the ligands capable of binding to the colchicine-binding site of tubulin and inhibiting proliferation of cancer cells.[6−8] Due to their simple structures and high biological activity, these compounds are extensively studied as effective anticancer drug candidates.[9−11] Some CA-4 derivatives, such as CA-4P, ombrabulin, and podophyllotoxin, and also several compounds of the colchicine and allocolchicine series, are currently in clinical trials.[4,12]

Despite considerable research on the anticancer activity of CA-4 analogues,[13−15] the photostability of these compounds (resistance to UV light or sunlight) is poorly known,[16,17] although this property is an important factor in drug design. Since heterocyclic analogues of CA-4 have attracted great interest and are potential drug candidates, the aim of this work is to study the photodegradation of new heterocyclic CA-4 analogues of the furanone series[18−20] and to evaluate their antiproliferative activity before and after exposure to UV light or sunlight.

Furanone analogues of CA-4 (**1a−g**) comprising a 3,4,5-trimethoxyphenyl moiety as the ring A and various five-membered heterocycles or 4-methoxyphenyl residue as the ring B were synthesized according to the known method[21] in good yields (Scheme S1, Table 1). Photochemical studies of these compounds showed that as opposed to photochromic diarylethenes,[22] they undergo an irreversible photorearrangement giving naphthalene derivatives (Scheme 1).[23] The photoirradiation of CA-4 analogues was carried out in *N*-methyl-2-pyrrolidone (NMP) (see Scheme S2). The photorearrangement of these diarylethenes provides naphthalene derivatives with different functional groups (Table 1).

Evaluation of the effectiveness of this phototransformation was carried out using UV and NMR spectroscopy. The spectral properties of the initial analogues of CA-4 and the products of photochemical reactions were studied in acetonitrile upon irradiation at 365 nm (for UV−vis spectra of **1a−g** and **2a−g**, see Figures S1−S15). The dynamics of spectral alterations under irradiation of diarylethenes solutions are characterized by the presence of two isosbestic points that is indicative of a high rate of a 1,9-sigmatropic rearrangement with subsequent oxazole ring opening and the absence of the contribution of intermediates to the measured stationary absorption spectra.[24] Therefore, the absorption spectra measured under irradiation of diarylethenes **1a−g** are superpositions of solely two forms, namely, initial **1** and the product **2** (Scheme 1).

The high efficiency of the photorearrangement was demonstrated by ^{1}H NMR monitoring. Figure 1 presents the results of monitoring for diarylethene **1c**. The formation of other byproducts associated with the photocyclization/

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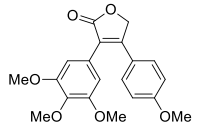
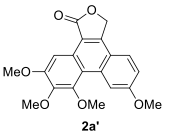
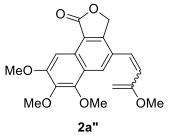
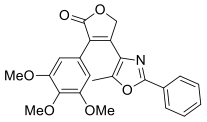
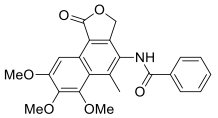
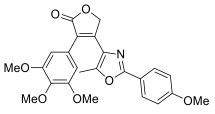
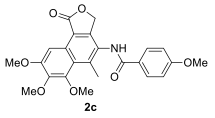
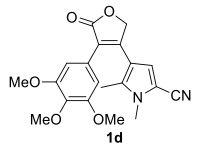
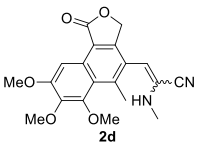
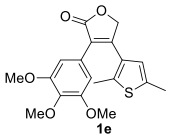
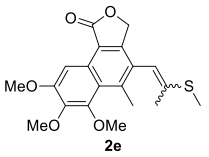
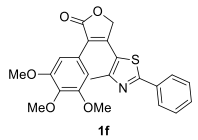
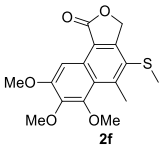
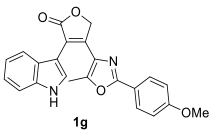
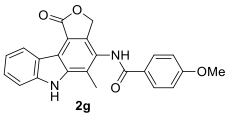
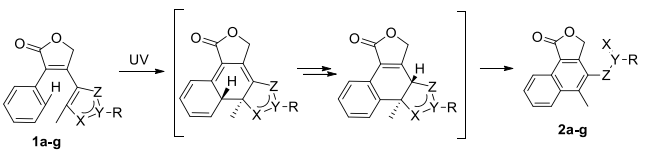
Table 1. Structures and Yields of CA-4 Analogues and Their Photoproducts

#	CA-4 analogues	Yields, %	Photoproducts	Yields, %
1	 1a	75	 2a' +  2a''	12 + 14
2	 1b	68	 2b	95
3	 1c	71	 2c	92
4	 1d	62	 2d	81
5	 1e	64	 2e	70
6	 1f	58	 2f	72
7	 1g	45	 2g	86

Scheme 1. Photorearrangement of CA-4 Analogues



oxidation or photocyclization/elimination was virtually not observed, as can be clearly seen by following the changes in the position of a signal of methoxy groups. As can be seen in Figure 1, the signal of methyl group in the naphthalene system

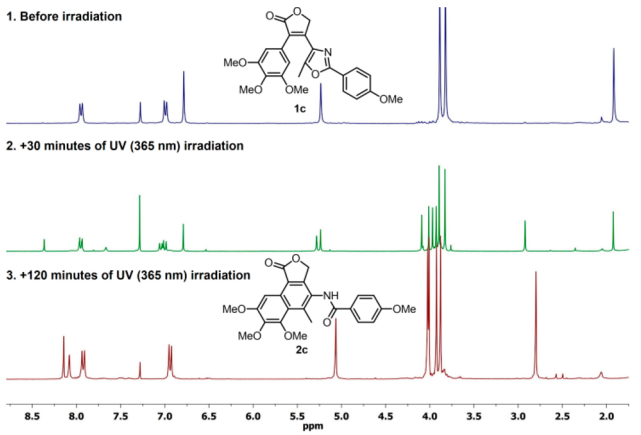
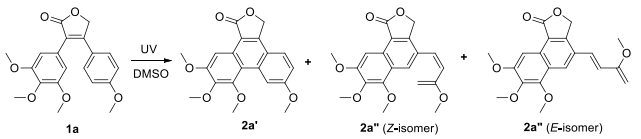


Figure 1. ^{1}H NMR monitoring of the photoirradiation of diarylethene **1c**.

appears at δ 2.80 ppm with the disappearance of the starting diarylethene at δ 1.92 ppm.

The ^{1}H NMR monitoring of **1a** upon irradiation with UV light was also carried out (see Figure S16). As opposed to the heterocyclic analogues, furanone **1a**, comprising a 4-methoxyphenyl residue, gives three photocyclization products upon UV irradiation: phenanthrene derivative **2a′** and two *E*/*Z* isomers of photorearrangement product **2a′′** (Scheme 2).[25]

Scheme 2. Photoreaction of Diarylethene 1a



The photoreaction affords phenanthrene derivative **2a′** as the major product that is formed via the Mallory reaction (tandem photocyclization/oxidation reaction, Figure S16). The formation of the phenanthrene derivative is confirmed by the presence of a characteristic doublet at δ 9.03 ppm with a small spin−spin coupling constant (2.4 Hz). Two isomeric products *E*/*Z*-**2a′′** are generated via a tandem reaction involving the photocyclization of the hexatriene system followed by a rearrangement and benzene ring opening. These compounds are characterized by spin−spin coupling constants of olefinic protons (16.0 and 12.5 Hz for the *E* and *Z* isomers, respectively, Figure 3). It should be noted that the maximum formation of butadiene derivatives **2a′′** (*Z*- and *E*-isomers) is observed when the reaction mixture is irradiated for 2 h; further irradiation leads to the disappearance of these compounds and only phenanthrene **2a′** remains (Figure S16).

Thus, the photochemical studies of CA-4 analogues of the furanone series showed that the compounds containing five-membered heterocycles as the ring B are very sensitive to UV light and undergo a rearrangement giving naphthalene derivatives. Diarylethenes comprising the 4-methoxyphenyl moiety as the ring B undergo either a tandem photocyclization/oxidation reaction (Mallory reaction) giving a phenanthrene **2a′** or a tandem photocyclization/rearrangement reaction accompanied by the benzene ring opening to form an *E*/*Z* isomer mixture of butadienes **2a′′**. For a comparative evaluation of the antiproliferative activity of CA-4

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analogues and their photoproducts, analytically pure samples were prepared.

Combretastatin A-4 analogues and their photoproducts were examined for their effect on viability of human epidermoid carcinoma cells A-431. The activity was evaluated in the micromolar concentration range from 0.7 to 15 μM. Six pairs of compounds (CA-4 analogues and their photoproducts) were tested (Table 2). Two CA-4 analogues, compounds **1a** and **1c**, exhibited high activity against A-431 cells (IC_{50} of these compounds were less than 0.7 μM). These compounds were selected for more detailed investigation. Both compounds contain the 4-methoxyphenyl moiety as the ring B, which is apparently responsible for their high activity.[26] The antiproliferative properties of **1a** and **1c** are much better compared to the reference cisplatin, and the latter shows an IC_{50} of about 6 μM. Naphthalene **2c** obtained from compound **1c** via a photoreaction exerted a weak effect on cell viability and, when used at a concentration of 15 μM, insignificantly inhibited the cell growth. A similar, but less pronounced, decrease in activity was observed for photoproduct **2d** as compared to starting **1d**.

Table 2. Effect of the Synthesized Compounds on A-431 Cell Viability (72 h Growth in the Presence of Compounds)

entry	compd	IC_{50} (μM)	cell viability (15 μM treatment) (%)
	DMSO (vehicle control)		99
1	**1a**	<0.7	9
2	**1b**	>15	60
3	**2b**	>15	81
4	**1c**	<0.7	10
5	**2c**	>15	77
6	**1d**	>15	41
7	**2d**	>15	77
8	**1e**	>15	44
9	**2e**	>15	11
10	**1f**	>15	22
11	**2f**	>15	82
12	**1g**	>15	74
13	**2g**	>15	57
14	cisplatin	6.3 ± 0.9	25

On the contrary, photoproduct **2e** proved to be more active that the starting compound. CA-4 analogue **1f** exhibited high activity, and the cell viability in the presence of this compound at a concentration of 15 μM was about 20%. Its photoproduct **2f** showed much lower antiproliferative activity. Interesting results were obtained for **1b**. Thus, the replacement of the peripheral 4-methoxyphenyl substituent in compound **1c** by the phenyl group led to a significant loss in the activity of both diarylethene **1b** and its photoproduct **2b**. These compounds did not show a pronounced antiproliferative effect on A-431 cells; at a concentration of 15 μM, they induced less than 40% cell death. Compounds **1g** and **2g** also did not show significant antiproliferative effects. In this pair, photoproduct **2g** is more active than the starting diarylethene **2g**. Additional assays were performed for more active compounds.

Thus, the photobiological studies demonstrated that CA-4 analogues containing the 3,4,5-trimethylphenyl group as the ring A and different five-membered heterocycles as the ring B are highly susceptible to UV irradiation and undergo photodegradation. Most photodegradation products exhibit relatively low activity compared to the starting diarylethenes.

For a more detailed study and dynamic evaluation of biological activity, we selected two compounds (**1a** and **1c**) displaying activity in the submicromolar concentration range. To determine the dependence of antiproliferative activity of these compounds on UV irradiation, we evaluated the activity before and after irradiation for 2 and 4 h. Irradiation was performed before treatment of A-431 cells. As can be seen in Figure 2, in the concentration range under study, these compounds did not exhibit inhibitory activity against A-431 cell growth after 2 h irradiation. The UV irradiation of the compounds **1a** and **1c** for 4 h also led to the loss of antiproliferative activity. As can be seen in Figure 2 and Table S1, these compounds did not exhibit inhibitory activity against A-431 cell growth after 2 h irradiation. The UV irradiation of the compounds **1a** and **1c** for 4 h also led to the loss of antiproliferative activity.

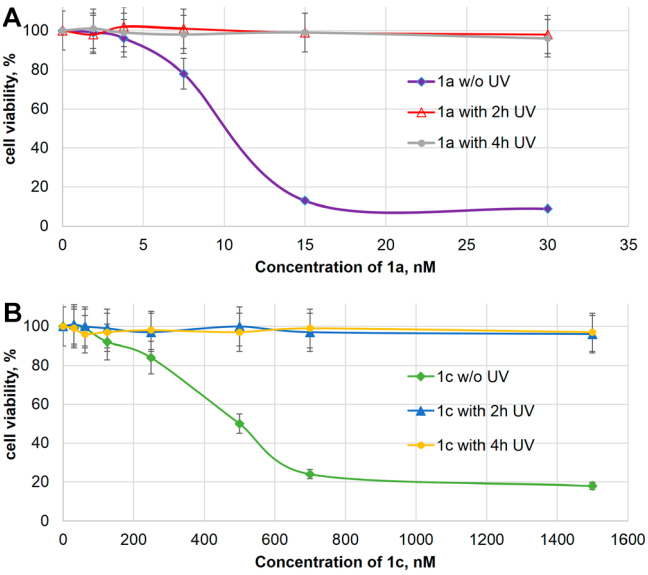

Figure 2. Effect of compounds **1a** (A) and **1c** (B) on the viability of the A-431 cells (72 h cell growth in the presence of compounds), w/o UV, with UV (λ = 365 nm) for 2 or 4 h.

Since light sensitivity of biologically active compounds is an important factor in the efficient drug design,[16,27,28] we have studied the sunlight-induced photodegradation of two diarylethenes. Solutions of CA-4 analogues in DMSO-d_6 were monitored by ^{1}H NMR spectroscopy upon exposure to sunlight (Figure 3, the experiment was performed at the latitude of Moscow at room temperature in April 2019).

The exposure to sunlight was found to induce processes similar to those caused by UV irradiation. Furanone **1a** gives three products, including the phenanthrene analogue. The only difference is the reaction time. As expected, upon exposure to sunlight, the reaction is not completed even within 4 days, whereas under UV irradiation, the reaction proceeds within 4 h (compare Figure 3 with Figure S16). A similar pattern was observed for diarylethene **1c** (Figure S17). The complete conversion giving a photocyclization/rearrangement product was achieved also after irradiation for 4 days.

To summarize, we have synthesized combretastatin A-4 analogues of the furanone series comprising a 3,4,5-trimethoxyphenyl derivative as the ring A and various five-membered heterocycles as the ring B. Photochemical and antiproliferative properties of the synthesized compounds and

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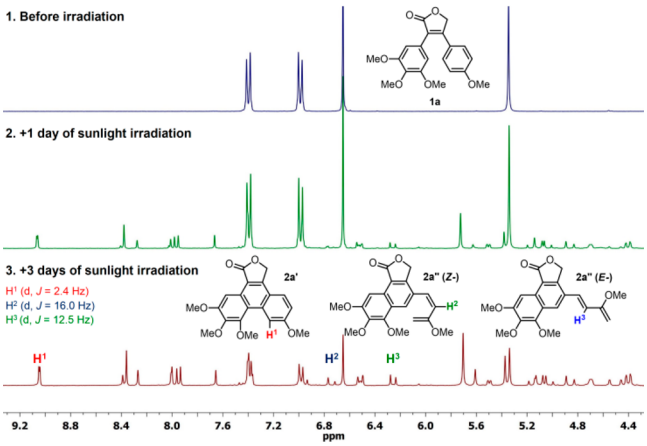

Figure 3. ^{1}H NMR monitoring of diarylethene **1a** upon sunlight irradiation.

their photoproducts were studied. When exposed to UV light, these compounds undergo a photorearrangement giving photoproducts with relatively low antiproliferative activity. The antiproliferative activity of the most active compounds (**1a** and **1c**) against A-431 cells was evaluated before and after UV irradiation at λ = 365 nm. The irradiation was found to cause a significant decrease in antiproliferative activity. For the first time, it was demonstrated that these compounds undergo similar sunlight-induced photodegradation. To conclude, it can be noted that, despite relatively high antiproliferative activity, CA-4 analogues undergo photodegradation, with loss of activity, under exposure to sunlight (UV light). Investigations of the effect of sunlight (UV light) on the photodegradation and antiproliferative activity of other CA-4 analogues will be continued. The observed ability of this series compounds to undergo photodegradation can be used for the design of a new class of drug candidates with high selectivity to cancer cells, which are particularly interesting for the treatment of skin tumors. The neutralization of toxic (adverse) effects on the surrounding normal tissues by means of irradiation will help in designing new drugs with improved therapeutic profiles (high selectivity/low overall and organ-specific toxicity).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b03780.

Detailed experimental procedures, compound characterization data, UV−vis, HRMS, and NMR spectra, and biological assays (PDF)

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Notes

The authors declare no competing financial interest.

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