Synthesis and evaluation of caged *Garcinia* xanthones†

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Inspired by the combination of unique structure and potent bioactivities exhibited by several family members of the caged *Garcinia* xanthones, we developed a synthesis of simplified analogues that maintain the overall caged motif. The caged structure of these compounds was constructed *via* a site-selective Claisen/Diels–Alder reaction cascade. We found that the fully substituted caged structure, in which are included the C18 and C23 geminal methyl groups, is necessary to maintain bioactivity. Analogue **17** had comparable activity to the natural products of this family, such as gambogic acid. These compounds exhibit cytotoxicity in a variety of tumor cell lines at low micromolar concentrations and were found to induce apoptosis in HUVE cells. In addition, studies with HL-60 and HL-60/ADR cells indicate that these compounds are not affected by the mechanisms of multidrug resistance, conferred by P glycoprotein expression, typical of relapsed cancers and thus represent a new and potent pharmacophore.

Introduction

The *Garcinia* genus of tropical plants has yielded a structurally intriguing family of caged xanthone-derived natural products that have interesting bioactivities and a documented value in traditional Eastern medicine.[1] The structure of forbesione (**1**, Fig. 1) typifies this unusual architecture in which a 4-oxa-tricyclo[4.3.1.0^{3,7}] dec-8-en-2-one scaffold has been built onto the C-ring of a xanthone backbone.[2] This motif is further customized *via* substitutions at the A-ring and peripheral oxidations to produce a variety of structural subfamilies such as the morellins (**2**,**3**),[3] the gaudichaudiones (**4**,**5**)[4] and the gambogins (**6**,**7**).[5] Recent biological reports attest to the biological and pharmacological potential of these compounds. For instance, desoxymorellin (**3**) and gaudichaudione A (**4**) were found to exhibit potent cytotoxicity against several cancer cell lines.[5b,6] In addition, gambogic acid (**6**) was shown to induce apoptosis in T47D (breast cancer) *via* a mechanism that is independent of cell cycle and may involve binding to the transferring receptor.[7] Related studies in MGC-803 (gastric carcinoma) cells indicated that **6** regulates expression of Bax and Bcl-2 proteins that are known to play a crucial role in apoptosis.[8] In addition to its anticancer activity, gambogic acid was shown to inhibit the growth of Gram positive bacteria.[9]

Inspired by the therapeutic potential of the caged *Garcinia* xanthones, we sought to develop a chemical strategy that would allow synthetic access to these metabolites, and thus facilitate their thorough biological and pharmacological evaluation. Along these lines, we have recently reported a unified strategy for the synthesis of selected caged *Garcinia* xanthones.[10,11] This strategy relies on a biomimetic Claisen/Diels–Alder/Claisen reaction cascade[12] that produces the pentacyclic motif of forbesione (**1**) from a tricyclic prenylated xanthone. Subsequent functionalizations at the periphery of the A ring of **1** produced desoxymorellin (**3**), desoxygaudichaudione A (**5**) and gambogin (**7**).[10,13] Herein we present an application of this strategy to the synthesis of simplified analogues of the parent structures and their biological evaluation.

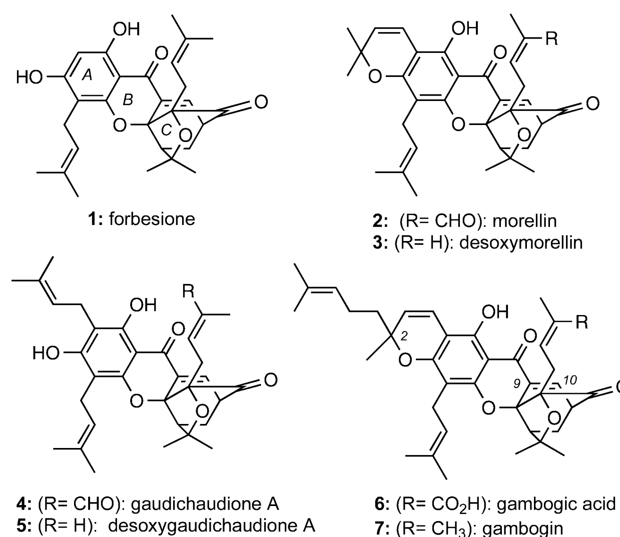

Fig. 1 Chemical structures of selected caged *Garcinia* xanthones.

Results and discussion

Synthesis of caged *Garcinia* xanthones

The synthesis of simplified analogues of the caged *Garcinia* motif is shown in Scheme 1. $ZnCl_2$-induced condensation[14] of *o*-anisic

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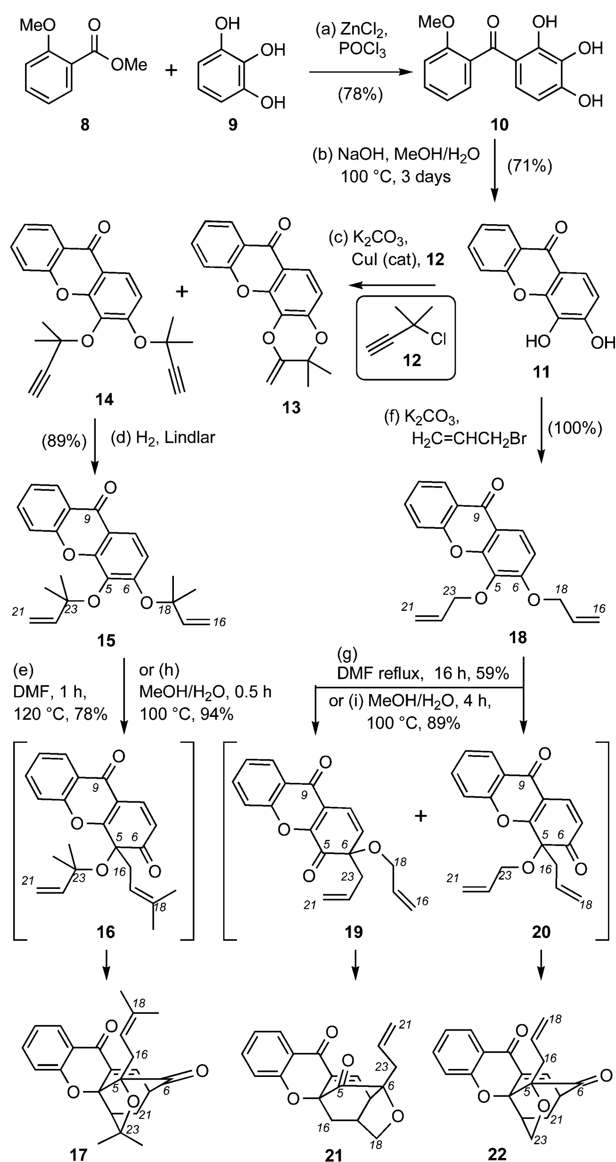

Scheme 1 *Reagents and conditions*: (a) **8** (1.0 equiv.), **9** (1.1 equiv.), $ZnCl_2$ (5.0 equiv.), $POCl_3$, 65 °C, 8 h, 78%; (b) aq. NaOH (30%), MeOH, 100 °C, 3 d, 71%; (c) K_2CO_3 (2.2 equiv.), KI (2.2 equiv.), **12** (2.2 equiv.), CuI (0.2 equiv.), acetone, 45 °C, 3 h, **13** (35%) and **14** (50%); (d) $Pd/BaSO_4$ (10%), quinoline, EtOAc, 25 °C, 6 h, 89%; (e) DMF, 120 °C, 1 h, 78%; (f) K_2CO_3 (2.2 equiv.), $H_2C{=}CHCH_2Br$ (2.4 equiv.), acetone, 45 °C, 2 h, 100%; (g) DMF, reflux (153 °C), 16 h, **22** (32%) and **21** (27%); (h) MeOH : H_2O 1/1, 100 °C, 0.5 h, 94%; (i) MeOH : H_2O 1/1, 100 °C, 4 h, **22** (67%) and 21 (22%).

acid (**8**) with pyrogallol (**9**) in $POCl_3$ produced benzophenone adduct **10** that underwent a base-induced cyclization to form xanthone **11** (55% yield over two steps). Conversion of **11** to bis(dimethylallyloxy) xanthone **15** was accomplished by a two steps procedure that involved propargylation of the C5 and C6 phenols with 2-chloro-2-methyl butyne (**12**) to form **14**[15] followed by Lindlar reduction of the pendant alkynes[16] (45% combined yield). The main side-product of this sequence was alkene **13** formed by concomitant reaction of the C5 phenol at the vinyl organometallic intermediate. Attempts to decrease the amount of **13** by adding more equivalents of chloride **12** proved to be fruitless. Nonetheless, this compound was easily separable from desired product **15** *via* a simple chromatography on silica gel. Heating of **15** in DMF (120 °C, 1 h) gave rise to the caged motif **17** *via* a Claisen-rearranged intermediate **16**. The excellent site-selectivity of this Claisen/Diels–Alder reaction cascade (C5 *versus* C6 allylation) can be rationalized by considering that the electronically deficient C9 carbonyl group polarizes selectively the O–C18 bond facilitating its rupture.[10,17] In addition, recent computational studies by Houk and co-workers on similar substrates have shown that the Claisen rearrangement is reversible and that the rate of the Diels–Alder reaction controls the product selection.[18]

In a similar manner compound **11** was allylated with allyl bromide to afford adduct **18** in quantitative yield. We found that this compound could undergo the Claisen/Diels–Alder reaction only at elevated temperatures and prolonged heating (DMF, reflux, 16 h). In this case we isolated the regular caged structure **22** together with the neo isomer **21** in a ratio of 1.2 : 1 and a combined yield of 59%.

It is interesting to note the difference in the Claisen/Diels–Alder reaction between substrates **15** and **18**. Compound **18**, lacking the geminal methyl groups at the C18 and C23 centers, requires forcing conditions for the dearomatization thus producing both constitutional isomers of the Claisen/Diels–Alder reaction cascade. In contrast, substrate **15** undergoes a faster and smoother dearomatization leading selectively to caged structure **17**. This finding may be attributed to the presence of the geminal methyl group at C18 of **15** that stabilizes the partial positive charge, formed at this carbon, during the transition state of the Claisen rearrangement. Such polar transition states have been supported by both computational studies and kinetic isotope effects in related structures.[19]

Encouraged by recent reports on the solvent-induced acceleration of the Claisen/Diels–Alder reaction in similar substrates,[13d,20] we studied the effect of solvent and temperature for the conversion of **18** to **21** and **22**. Table 1 summarizes our findings. No reaction was observed upon heating of **18** in deuterated toluene at 100 °C for 4 hours (entry 1) and either longer reaction times (16 hours, entry 2) or higher temperatures (120 °C, entry 3) led to only a small improvement in product formation. Switching the solvent from toluene to DMF led to an acceleration of the Claisen/Diels–Alder reaction that started to proceed even at 100 °C after 4 hours (compare entries 1 and 4). Increase of both temperature and reaction time led to a substantial increase in product conversion that reached 79% after heating at 150 °C for 16 hours (entry 7). However, increase of the reaction temperature from 100 to 150 °C led to a decrease in the selectivity of product formation (compare ratios of **22** : **21** in entries 5 and 7). In deuterated methanol, this reaction cascade started proceeding even at 60 °C, but the overall product conversion was only 14% even after 16 hours (entry 8). At 100 °C after only 4 hours of heating, this reaction proceeded at 65% conversion (entry 9) and was completed after 16 hours (entry 10). The rate acceleration of this reaction was even more dramatic when a mixture of CD_3OD : D_2O 1 : 1 was used as the solvent. In this case the cyclization proceeded in 73% yield after heating at 60 °C for 4 hours (entry 11), while it was completed after heating at 100 °C for 4 hours (entry 12).

In short, the findings presented in Table 1 demonstrate clearly that polar solvents can accelerate significantly the Claisen/Diels–Alder reaction. In turn, this leads to an efficient product

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Table 1 Effect of solvent and temperature on the conversion of **18** to **21** and **22**

Entry	Solvent[a]	T/°C	t/h	Conversion **21** + **22** (%)	Ratio **22** : **21**
1	Toluene-d_8	100	4	<5	ND
2	Toluene-d_8	100	16	12	2.1 : 1
3	Toluene-d_8	120	16	23	1.8 : 1
4	DMF-d_7	100	4	18	2.4 : 1
5	DMF-d_7	100	16	25	2.3 : 1
6	DMF-d_7	150	4	26	1.3 : 1
7	DMF-d_7	150	16	79	1.2 : 1
8	CD_3OD	60	16	14	2.9 : 1
9	CD_3OD	100	4	65	2.5 : 1
10	CD_3OD	100	16	100	2.3 : 1
11	CD_3OD/D_2O	60	4	73	3.0 : 1
12	CD_3OD/D_2O	100	4	100	2.8 : 1

[a] The reactions were carried out in sealed NMR tubes and monitored by ^{1}H NMR spectrometry. In all cases the concentration of starting material **18** was between 0.04–0.05 mmol.

conversion at relatively low temperatures (between 60–100 °C) and an enhanced product selectivity. Similar observations have also been made by the Nicolaou group[13d] and an explanation of this effect, based upon previous theoretical and experimental work, has been proposed.[20] On a preparative scale, we found that heating of **18** in a mixture of MeOH : H_2O (1 : 1) at 100 °C for 4 h led to the isolation of a 3.1 : 1 mixture of **22** and **21** in a combined yield of 89%. Under these optimized conditions the conversion of **15** to **17** proceeded in 94% isolated yield after heating for 0.5 h (Scheme 1).

Cell proliferation studies

The ability of the synthesized caged *Garcinia* xanthones to inhibit cancer cell growth was evaluated in promyelocytic leukemia cell line, HL-60, using a ^{3}H-thymidine incorporation assay. Cells were incubated with increasing concentrations of the compounds for 48 h, and then pulsed with ^{3}H-thymidine for 6 h. Gambogic acid (**6**) and gambogin (**7**) were the most active among all compounds tested and exhibited an IC_{50} value of 0.3 and 0.8 μM respectively (Table 2). Their comparable activity suggests that the carboxylic acid functionality of **6** does not contribute significantly to its bioactivity. Interestingly, analogue **17** showed similar activity to that of the natural products (IC_{50} = 1.5 μM), while the related structures **21** and **22** were found to be relatively inactive, inducing less than 10% growth inhibition at the highest concentrations tested (2.0 μM). These findings suggest that the geminal methyl groups at the C18 and C23 centers play an important role in the bioactivity of the caged *Garcinia* xanthone system. In addition, we found that none of the acyclic molecules tested (**11**, and **13–16**) showed any growth inhibition at the concentrations tested (data not shown). This supports the notion that the caged motif of this family is essential for bioactivity.

Importantly, the compounds shown in Table 2 were also evaluated in HL-60/ADR cells, a multidrug resistant clone obtained by transfection of HL-60 cells with *mdr-1*.[21] The results of parallel experiments indicated that HL-60/ADR had similar sensitivity to the anti-proliferative effects of the caged *Garcinia* xanthones as the parental HL-60 cell line. This significant finding suggests that the caged *Garcinia* xanthones are not subject to the mechanism of chemo-resistance resulting from the expression of *mdr* characteristic of many relapsed cancers.[22]

Active compounds having IC_{50} values less than 2 μM (compounds **3**,**6**,**7** and **17**) were selected for further evaluation in a panel of solid and non-solid tumor cell lines (Table 3). The T-cell acute lymphoblastic leukemia cell line (CEM) was the most sensitive among all the cell lines tested. The IC_{50} values recorded in these cells were in the submicromolar range (0.15–0.35 μM). The solid tumor cell lines were slightly less sensitive than CEM cells with IC_{50} values of the compounds ranging from 0.4 to 3.1 μM, with the exception of compound **17** in A549 cells (IC_{50} > 4 μM). Although, gambogic acid (**6**) was the most active among the compounds evaluated, the differences in activities among them were small (≤3-fold). This supports the notion that the caged structure of these compounds is the major contributor for their activity.

Table 2 Inhibition of cell proliferation by caged *Garcinia* xanthones in adriamycin sensitive and resistant promyelocytic leukemia cells

Compound	IC_{50}/μM	
	HL-60	HL-60/ADR
Forbesione (**1**)	2.2	2.0
Desoxymorellin (**3**)	1.0	1.1
Gambogic acid (**6**)	0.3	0.5
Gambogin (**7**)	0.8	1.1
17	1.5	1.4
21	Inactive[a]	Inactive[a]
22	Inactive[a]	Inactive[a]

[a] Less than 10% inhibition at 2.0 μM.

Cell viability and apoptosis studies

In order to distinguish between cytostatic and cytotoxic effects, two independent cell viability studies were performed. In the first,

Table 3 Inhibition of cell proliferation by caged Garcinia xanthones in solid and non-solid tumor cells

Compound		**3**	**6**	**7**	**17**
Cell line	Tissue type	IC_{50}/μM			
A549	Lung	2.1	1.8	1.8	>4
HT29	Colon	1.2	0.7	1.0	3.1
MCF-7	Breast	0.9	0.4	1.1	ND[a]
M21	Melanoma	1.6	1.2	1.0	2.2
PC3	Prostate	1.2	0.4	1.1	ND[a]
CEM	Leukemia	ND[a]	0.15	0.35	0.3

[a] ND: not determined.

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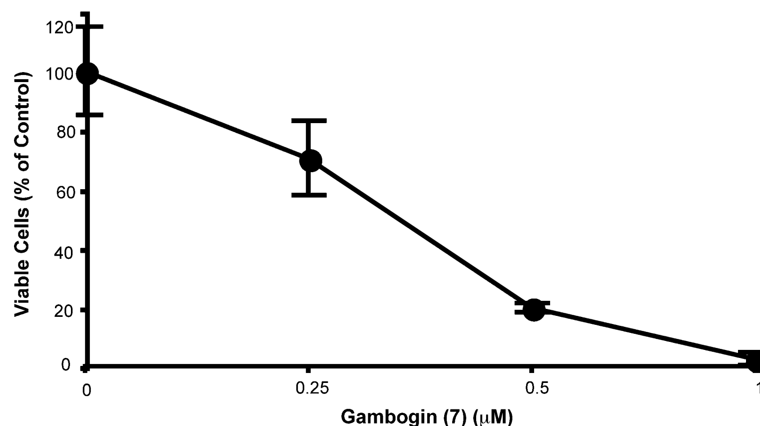

Fig. 2 Effect of gambogin (**7**) on CEM cell viability. CEM cells were treated with increasing concentrations of 7 for 4 days. Cell viability was determined by counting cells excluding trypan blue dye. Error bars represent standard deviation.

viability was determined in CEM cells treated with compound **7** for 4 days using the trypan blue exclusion assay. The results, shown in Fig. 2, demonstrated that CEM cells were very sensitive to **7** with an IC_{50} of 0.3 μM in agreement with that found in the cell proliferation studies (Table 3).

The second viability study was performed in HUVE (human umbilical vein endothelial) cells treated with compound **17** for 24 h. Cell viability was determined using the WST assay that measures metabolic activity of cells alive. Compound **17** was found to be cytotoxic with an IC_{50} value of 1.38 μM. A proliferation assay confirmed the WST assay (data not shown). This finding suggests that compound **17** and related caged xanthones may have a therapeutic potential as inhibitors of angiogenesis.[23]

The mechanism of cytotoxicity in HUVE cells was investigated using an ELISA-based assay that distinguishes between apoptosis and necrosis. This photometric assay allows the determination of histone-associated DNA fragments that are released during cell death. We found that the majority (>90%) of HUVE cells treated with **17** underwent rapid apoptosis after 10 h in a dose-dependent manner (Fig. 3). Cell necrosis was not detected at concentrations lower than 1.5 μM and was only observed in a small subset of cells (ca 10%) at much higher concentrations (>3 μM), indicating that apoptosis is the predominant mechanism of cell death.

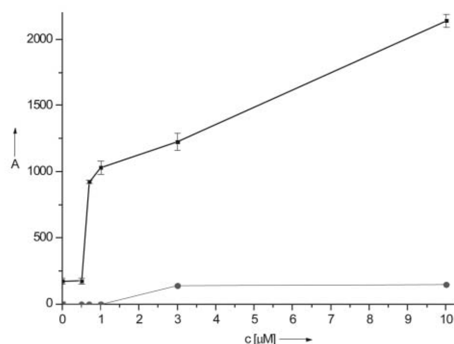

Fig. 3 Induction of HUVE cell apoptosis (black line) and necrosis (gray line) by **17** at different concentrations after 10 h incubation time; $A = A_{405\ nm} - A_{490\ nm}$; c = concentration of **17**.

Conclusions

In conclusion, we present herein a study toward the design of simplified structural analogues of the caged *Garcinia* natural products and preliminary evaluation of their biologic activity in a variety of tumor cell lines and HUVE cells. We found that analogue **17**, which maintains the basic structural motif of the caged *Garcinia* xanthones but is devoid of any functionalization of the xanthone A-ring, maintains the activity exhibited by the more structurally complex natural products of this family. In contrast, compounds **21** and **22** that lack the C18 and C23 geminal methyl groups have substantially reduced activities, suggesting that maintaining intact the caged C-ring is essential for bioactivity. The active compounds had cytotoxicity at low to sub-micromolar concentrations in solid and non-solid tumor cell lines respectively and induced apoptosis in HUVE cells. Remarkably, similar IC_{50} values were obtained for the compounds tested in HL-60 and HL-60/ADR cell lines, suggesting that these compounds are not subject to the mechanism of drug resistance resulting from expression of *mdr*. Therefore, members of this family of compounds may have therapeutic potential in relapsed cancers typically resistant to standard chemotherapeutic agents. In addition, the cytotoxicity observed in HUVE cells suggests that these compounds are interesting leads for the development of new inhibitors of angiogenesis. Future work is aimed at determining tumor cell selectivity *in vitro and in vivo* with the possibility of identifying more potent and more selective analogues.

Experimental

General notes

o-Anisic acid (**8**), pyrogallol (**9**) and 2-chloro-2-methyl butyne (**12**) were purchased from Aldrich. Gambogic acid (**7**) was purchased from Gaia Chemical Corporation (CT, USA). The synthesis and spectroscopic characterization of compounds **1**, **3** and **7** have been reported in reference 10. All reagents were obtained (Aldrich, Acros) at highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred *via* syringe or stainless steel cannula. Organic solutions were concentrated by

rotary evaporation below 45 °C at approximately 20 mmHg. All non-aqueous reactions were carried out under anhydrous conditions, *i.e.* using flame-dried glassware, under an argon atmosphere and in dry, freshly distilled solvents, unless otherwise noted. Dimethylformamide (DMF) and quinoline were distilled from calcium hydride under reduced pressure (20 mmHg) and stored over 4 Å molecular sieves until needed. Yields refer to chromatographically and spectroscopically (^{1}H NMR, ^{13}C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) and visualized under UV light and/or developed by dipping in solutions of 10% ethanolic phosphomolybdic acid (PMA) or *p*-anisaldehyde and applying heat. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash chromatography. Preparative thin-layer chromatography separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Varian Mercury 400 and/or Unity 500 MHz instruments and calibrated using the residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were recorded on a Nicolet 320 Avatar FT-IR spectrometer and values are reported in cm^{-1} units. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under chemical ionization (CI) conditions or on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions.

(2-Methoxyphenyl)(2,3,4-trihydroxyphenyl)methanone (10). To a 250 ml round-bottomed flask containing flame-dried under vacuum $ZnCl_2$ (10.2 g, 75 mmol) was added *o*-anisic acid (**8**) (2.49 g, 15 mmol) and pyrogallol (**9**) (2.07 g, 16.5 mmol) followed by $POCl_3$ (20 mL). The reaction vessel was then equipped with a reflux condenser and stirred under argon at 65 °C for 8 h. The red colored reaction mixture was then cooled to 25 °C and poured into a beaker of about 500 g of ice. The mixture was extracted with ethyl ether (3 × 100 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was purified through column chromatography (30% Et_2O–hexane) to yield benzophenone **10** (3.04 g, 78%); yellow solid; $R_f = 0.65$ (90% Et_2O–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (ddd, J = 8.4, 7.2, 1.6 Hz, 1H), 7.26 (dd, J = 7.2, 2 Hz, 1H), 7.04–6.98 (m, 2H), 6.87 (d, J = 9.2 Hz, 1H), 6.42 (d, J = 9.2 Hz, 1H), 3.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.5, 156.2, 151, 150.1, 131.5, 130.8, 128.7, 127.4, 126.5, 120.2, 114.2, 111.3, 107.1, 55.7; HRMS calc. for $C_{14}H_{12}O_5$ (M + H$^+$) 261.0763, found 261.0741.

3,4-Dihydroxy-9*H*-xanthen-9-one (11). To a solution of benzophenone **10** (1.6 g, 6.15 mmol) in methanol (20 mL) was added a solution of aqueous NaOH (30% w/w, 20 mL) and water (10 mL). The green colored reaction mixture was then refluxed at 100 °C for 3 days. The red colored reaction mixture was cooled to 25 °C and acidified with aqueous HCl (10%, 600 mL). The reaction mixture was partitioned between water and ethyl ether (50 mL). The aqueous layer was then back extracted with ethyl ether (2 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was purified through column chromatography (20–40% Et_2O in hexanes) to yield xanthone **11** (0.99 g, 71%); yellow solid; $R_f = 0.4$ (90% Et_2O–hexane); ^{1}H NMR (400 MHz, DMSO) δ 8.14 (dd, J = 8.0, 1.6 Hz, 1H), 7.82–7.78 (m, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 8.8 Hz, 1H), 7.43–7.39 (m, 1H), 7.0 (d, J = 8.8 Hz, 1 H); ^{13}C NMR (100 MHz, DMSO) δ 175.1, 155.3, 151.4, 146.2, 134.6, 132.5, 125.7, 123.8, 120.7, 117.9, 116.4, 114.6, 113.2; HRMS calc. for $C_{13}H_8O_4$ (M + H$^+$) 229.0501, found 229.0509.

3,4-Bis(2-methylbut-3-yn-2-yloxy)-9*H*-xanthen-9-one (14). To a round-bottomed flask containing xanthone **11** (500 mg, 2.19 mmol), KI (800 mg, 4.82 mmol), K_2CO_3 (666.2 mg, 4.82 mmol), and CuI (42 mg, 0.22 mmol) was added dry acetone (20 mL). The reaction vessel was then equipped with a reflux condenser, and the reaction was heated at 45 °C under argon. After 20 minutes, 2-chloro-2-methylbut-3-yne (**12**) (0.55 ml, 4.82 mmol) was added, and the reaction was heated for two more hours. The reaction was then cooled to 25 °C and acidified with 10% HCl solution. The reaction mixture was partitioned between ethyl ether (30 mL) and water. The aqueous layer was back-extracted (2 × 30 mL), and the combined ethyl ether layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was purified through column chromatography (5–20% Et_2O in hexane) to yield compound **14** (394.8 mg, 50%); yellow solid; R_f = 0.45 (50% Et_2O–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (dd, J = 8.0, 1.2 Hz, 1H), 8.06 (d, J = 9.2 Hz, 1H), 7.71–7.67 (m, 1H), 7.65 (d, J = 9.2 Hz, 1H), 7.51 (d, J = 8.4 Hz, 1H), 7.36 (dd, J = 8.0, 7.2 Hz, 1H), 2.66 (s, 1H), 2.29 (s, 1H), 1.83 (s, 6H), 1.76 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 155.8, 155.7, 152.2, 134.2, 126.4, 123.7, 121.7, 117.9, 115.8, 74.8, 73.6, 30.6, 29.7; HRMS calc. for $C_{23}H_{20}O_4$ (M + H$^+$) 361.1440, found 361.1464.

3,4-Bis(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one (15). To a solution of xanthone **14** (230 mg, 0.64 mmol) in EtOAc (10 mL) was added 10% Pd/$BaSO_4$ (23 mg) and quinoline (0.66 mL, 0.56 mmol). The reaction mixture was degassed using argon and stirred under an atmosphere of hydrogen for 3 hours. Spectroscopic analysis (^{1}H NMR) of the crude mixture revealed that the reaction had proceeded by *ca*. 35%. During that time, an additional amount of 10% Pd/$BaSO_4$ (10 mg) was added to accelerate the reaction. After stirring for an additional 3 hours under a hydrogen atmosphere, the reaction was stopped and the reduction, estimated by ^{1}H NMR of the crude, was found to be quantitative. The reaction mixture was filtered through a plug of silica gel, and the residue concentrated and purified through a column chromatography (3–10% Et_2O–hexane) to yield **15** (207.3 mg, 89%); yellow solid; R_f = 0.66 (70% Et_2O–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, J = 6.0, 1.6 Hz, 1H), 7.90 (d, J = 8.8, 0.8 Hz, 1H), 7.65–7.61 (m, 1H), 7.46–7.43 (m, 1H), 7.31–7.27 (m, 1H), 7.09 (dd, J = 9.2, 2.0 Hz, 1H), 6.29–6.10 (m, 2H), 5.18–5.11 (m, 3H), 4.99–4.96 (m, 1H), 1.54 (d, J = 2 Hz, 6H), 1.52 (d, J = 2.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 156.5, 155.5, 152.1, 143.0, 135.4, 134.0, 126.2, 123.8, 123.5, 121.2, 120.8, 120.7, 117.7, 117.6, 116.9, 116.4, 113.8, 112.8, 83.3, 81.9, 27.1, 26.9; HRMS calc. for $C_{23}H_{24}O_4$ (M + H$^+$) 365.1753, found 365.1740.

Caged xanthone 17. A solution of **15** (55 mg, 0.15 mmol) in DMF (2.0 mL) was heated at 120 °C for 1 hour. The yellow reaction mixture was cooled 25 °C and the mixture purified by column chromatography (15–20% Et_2O–hexane) to yield the caged xanthone **17** (42.5 mg, 78%). Alternatively, a solution of **15** (39 mg,

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0.11 mmol) in MeOH–H_2O 1 : 1 (2.0 mL) was heated at 100 °C for 0.5 hours. Purification as indicated above yielded caged xanthone **17** (36.6 mg, 94%); white solid; R_f = 0.55 (70% Et_2O–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J = 8.0, 1.6 Hz, 1H), 7.53–7.49 (m, 1H), 7.42 (d, J = 6.8 Hz, 1H), 7.07–7.03 (m, 2H), 4.42–4.38 (m, 1H), 3.49 (dd, J = 6.8, 4.8 Hz, 1H), 2.67–2.61 (m, 2H), 2.45 (d, J = 9.6 Hz, 1H), 2.33 (dd, J = 13.6, 4.8 Hz, 1H), 1.72 (s, 3H), 1.30 (s, 6H), 0.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.7, 176.2, 159.4, 136.0, 134.7, 134.6, 133.5, 126.8, 121.7, 118.9, 118.8, 117.9, 90.2, 84.5, 83.5, 48.8, 46.8, 30.4, 29.8, 29.2, 25.4, 25.2, 16.8; HRMS calc. for $C_{23}H_{24}O_4$ (M + H^+) 365.1753, found 365.1765.

3,4-Bis(allyloxy)-9*H*-xanthen-9-one (18). To a flask containing xanthone **11** (500 mg, 2.19 mmol) and K_2CO_3 (666.2 mg, 4.82 mmol) was added dry acetone (20 mL), followed by allylbromide (0.42 mL, 4.82 mmol). The reaction vessel was then equipped with a reflux condenser, and the reaction was heated at 45 °C under argon for two hours. The mixture was then cooled to room temperature and acidified with 10% aqueous HCl solution. The reaction mixture was partitioned between ethyl ether (20 mL) and water (20 mL). The aqueous layer was back-extracted (2 × 20 mL), and the combined ethyl ether layers were dried over $MgSO_4$, filtered, and concentrated. The crude material was purified through a column chromatography (5–20% Et_2O–hexane) to yield allylated xanthone **18** (675.2 mg, 100%); white solid; R_f = 0.45 (50% Et_2O–hexane); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.3 (dd, J = 8.0, 1.6 Hz, 1H), 8.04 (d, J = 8.8 Hz, 1H), 7.69 (dt, J = 8.4, 1.2 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 8.0, 7.2 Hz), 6.97 (d, J = 8.8 Hz), 6.23–6.03 (m, 2H), 5.49–5.32 (m, 3H), 5.38 (dd, J = 10.4, 0.8 Hz, 1H), 4.71 (dd, J = 6.4, 5.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 156.5, 155.9, 150.6, 134.3, 133.6, 132.1, 126.4, 123.7, 122.1, 121.4, 118.3, 118.1, 117.9, 116.6, 109.8, 74.7, 69.8; HRMS calc. for $C_{19}H_{16}O_4$ (M + H^+) 309.1127, found 309.1134.

Caged xanthones 21 and 22. A solution of **18** (46.2 mg, 0.15 mmol) in DMF (2.0 mL) was refluxed (153 °C) for 16 h. The yellow reaction mixture was then cooled to 25 °C and the residue was column chromatographed (10–30% Et_2O–hexane) to yield a mixture of caged xanthones **21** (12.4 mg, 27%) and **22** (14.7 mg, 32%). Alternatively, a solution of **18** (25.1 mg, 0.08 mmol) in MeOH–H_2O 1 : 1 (2.0 mL) was heated at 100 °C for 4 hours. Purification as indicated above yielded caged xanthones **21** (5.2 mg, 21%) and **22** (16.7 mg, 67%); **21**: white solid; R_f = 0.55 (70% Et_2O–hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 7.8 Hz, 1H), 7.59–7.54 (m, 1H), 7.34 (d, J = 7.2 Hz, 1H), 7.11–6.98 (m, 2H), 5.29–5.15 (m, 1H), 4.68 (d, J = 9.9 Hz, 1H), 4.56–4.50 (m, 2H), 3.91 (d, J = 7.5 Hz, 1H), 3.54–3.44 (m, 1H), 2.61 (m, 2H), 2.23 (m, 3H); HRMS calc. for $C_{19}H_{16}O_4$ (M + H^+) 309.1127, found 309.1132. **22**: white solid; R_f = 0.54 (70%, Et_2O–hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.0, 2.0 Hz, 1H), 7.58–7.53 (m, 1H), 7.30 (d, J = 6.8 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 7.09–7.05 (m, 1H), 5.65–5.54 (m, 1H), 5.14–5.09 (m, 2H), 4.08 (dd, J = 8.4, 3.6 Hz, 1H), 3.94 (m, 1H), 3.47 (dd, J = 6.4, 4.4 Hz, 1H), 2.63 (dd, J = 14, 6 Hz, 1H), 2.57–2,48 (m, 1H), 2.28 (dd, J = 14, 7.6 Hz, 1H), 2.21 (d, J = 12.8, 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.1, 175.4, 160.2, 136.6, 136.5, 134.5, 134.3, 134.2, 131.6, 131.4, 127.2, 126.9, 122.3, 121.9, 119.6, 119,1, 118.5, 118.2, 83.7, 76.2, 75.9, 45.7, 45.4; HRMS calc. for $C_{19}H_{16}O_4$ (M + H^+) 309.1127, found 309.1133.

^{3}H-Thymidine incorporation assay. Cells were plated in a 96-well plate at 10–20 × 10^3 cells/well in RPMI supplemented with 10% fetal bovine serum, 2 mM glutamine, 1% penicillin/streptomycin (complete medium). The caged *Garcinia* xanthones were added to the cells at increasing concentrations and 0.1% DMSO was added to control cells. Cells were incubated for 48 h and then pulsed with ^{3}H-thymidine for 6 h. Incorporation of ^{3}H-thymidine was determined in a scintillation counter (Beckman Coulter Inc., Fullerton, CA) after cells were washed and deposited onto glass microfiber filters using a cell harvester M-24 (Brandel, Gaithersbur, MD).

Trypan-blue exclusion assay. CEM cells were plated in a 24-well plate in complete media at 50 × 10^3 cells/well. Cells were treated with **7** at concentrations of 0.25, 0.5, 1.0 μM or with 0.1% DMSO (control cells). Cells were incubated for 4 days and then the number of viable cells was determined after the addition of trypan-blue dye by counting the cells which exclude trypan-blue in a hemocytometer.

WST assay. Compound **17** was dissolved in DMSO and further diluted with Endothelial Cell Growth Medium (PromoCell, Heidelberg, Germany) to obtain a final concentration as indicated. HUVE cells (PromoCell, Heidelberg, Germany) were seeded into each well of a 96-well cell culture plate at 7000 cells per well and incubated at 37 °C for 24 h with the indicated concentrations of each compound. The final volume was 100 μL per well. Control samples were incubated with the solvent alone. Each experiment was repeated in triplicate. Afterwards the WST-1 reagent was added to the cells at 10 μL per well and the cells further incubated at 37 °C for additional 3 h. Then the cell culture plate was agitated thoroughly for 1 minute on a shaker at 200 U/min. The absorbance of each sample was measured using a microplate reader at 440 nm. The reference wavelength was 690 nm.

Apoptosis assay. The compounds were dissolved in DMSO and further diluted with Endothelial Cell Growth Medium (PromoCell, Heidelberg, Germany) to obtain final concentrations as indicated. HUVE cells were seeded into each well of a 96-well cell culture plate at 10000 cells per well and incubated at 37 °C for 10 h with the indicated concentrations of each compound. The final volume was 100 μL per well. Control samples were incubated with the solvent alone. Each sample was repeated three times. The proapoptotic effect was detected by using the Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufactor's instructions. The kit constitutes a photometric enzyme-immunoassay for the qualitative and quantitative *in vitro* determination of cytoplasmic histone-associated-DNA-fragments (mono- and oligo-nucleosomes) after induced cell death. Due to the working procedure the kind of cell death (apoptosis or necrosis) can be determined. The absorption values A ($A_{405\,nm} - A_{490\,nm}$) measured give a quantitative indication of the induced amount of apoptosis/necrosis. The higher the absorption A, the higher the induction of apoptosis/necrosis at the corresponding concentrations of the compounds.

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