Evaluation of the pharmacophoric motif of the caged *Garcinia* xanthones†

Oraphin Chantarasriwong,[a,b] Woo Cheal Cho,[a] Ayse Batova,*[c] Warinthorn Chavasiri,[b] Curtis Moore,[a] Arnold L. Rheingold[a] and Emmanuel A. Theodorakis*[a]

Received 8th July 2009, Accepted 28th August 2009
First published as an Advance Article on the web 24th September 2009
DOI: 10.1039/b913496d

The combination of unique structure and potent bioactivity exhibited by several family members of the caged *Garcinia* xanthones, led us to evaluate their pharmacophore. We have developed a Pd(0)-catalyzed method for the reverse prenylation of catechols that, together with a Claisen/Diels–Alder reaction cascade, provides rapid and efficient access to various caged analogues. Evaluation of the growth inhibitory activity of these compounds leads to the conclusion that the intact ABC ring system containing the C-ring caged structure is essential to the bioactivity. Studies with cluvenone (**7**) also showed that these compounds induce apoptosis and exhibit significant cytotoxicity in multidrug-resistant leukemia cells. As such, the caged *Garcinia* xanthone motif represents a new and potent pharmacophore.

Introduction

The tropical trees of the genus *Garcinia*, found in lowland rainforests of Southeast Asia, are widely known for their use in folk medicines.[1] Efforts to identify the bioactive ingredients from these plants have yielded a family of natural products structurally characterized by a xanthone backbone in which the C ring has been converted into a caged tricyclic structure. Plant-specific substitutions and oxidations of this motif produce several subfamilies, such as the morellins (**1**, **2**),[2] the gaudichaudiones (**3**, **4**)[3] and the gambogins (**5**, **6**)[4] (Fig. 1).

The ability of several caged *Garcinia* xanthones to selectively inhibit tumor cell proliferation and exhibit potent cytotoxicity at low μM concentrations has been well documented.[5] Moreover, gaudichaudione A (**3**) and gambogic acid (**5**) displayed strong growth inhibitory activities against both parental murine leukemic P388 and P388/doxorubicin-resistant cell lines, suggesting that they are not subject to the multidrug-resistance mechanisms that are typical of several relapsed cancers.[6] In addition, **5** displays antitumor activity in animal models and has an appropriate therapeutic window for clinical applications as an anticancer agent.[7]

A recent study reported that gambogic acid binds to the Bcl-2 family of proteins resulting in apoptosis presumably by blocking the antiapoptotic activity of these proteins.[8] Related mode-of-action studies have suggested that this compound binds to the transferrin receptor 1 and that this binding correlates with the induction of apoptosis.[9] Structure–activity relationship studies have proposed that the caged motif plays an essential role in the cytotoxicity.[10]

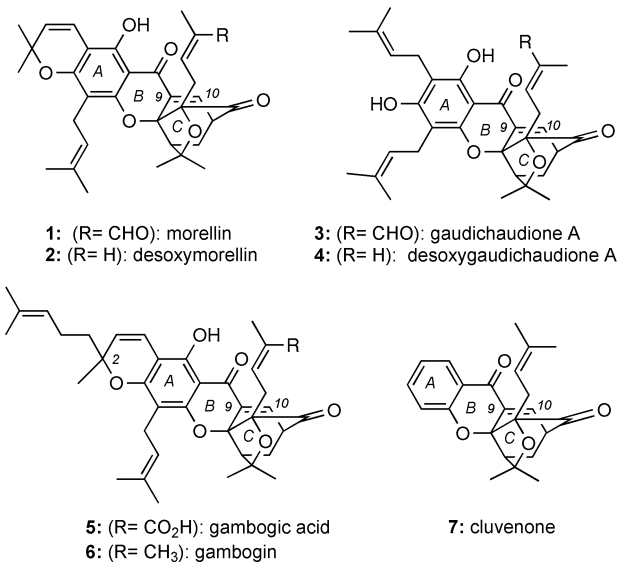


Fig. 1 Chemical structures of selected caged *Garcinia* xanthones.

Inspired by the therapeutic potential of the caged *Garcinia* xanthones, we developed a chemical strategy that allows access to these natural products and related analogues, such as cluvenone (**7**).[11,12] This strategy relies on a biomimetic Claisen/Diels–Alder/Claisen reaction cascade that produces the caged motif from a reverse prenylated xanthone.[13,14] Moreover, we have found that cluvenone (**7**) maintains the activity exhibited by the more structurally complex natural products of this family.[12] We have also shown that this compound is equally cytotoxic to HL-60 cells and the multidrug-resistant clone, HL-60/ADR, at low μM concentrations, attesting to the pharmacologically promising caged *Garcinia* xanthone motif. Herein, we report our studies on the evaluation of the pharmacophoric motif of these compounds. We also

[a]*Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive MC: 0358, La Jolla, CA 92093-0358, USA. E-mail: etheodor@ucsd.edu; Fax: +1-858-822-0386; Tel: +1-858-822-0456*
[b]*Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[c]*Department of Pediatrics/Hematology-Oncology, UC San Diego, 200 West Arbor Drive, San Diego, CA 92103-8447, USA*

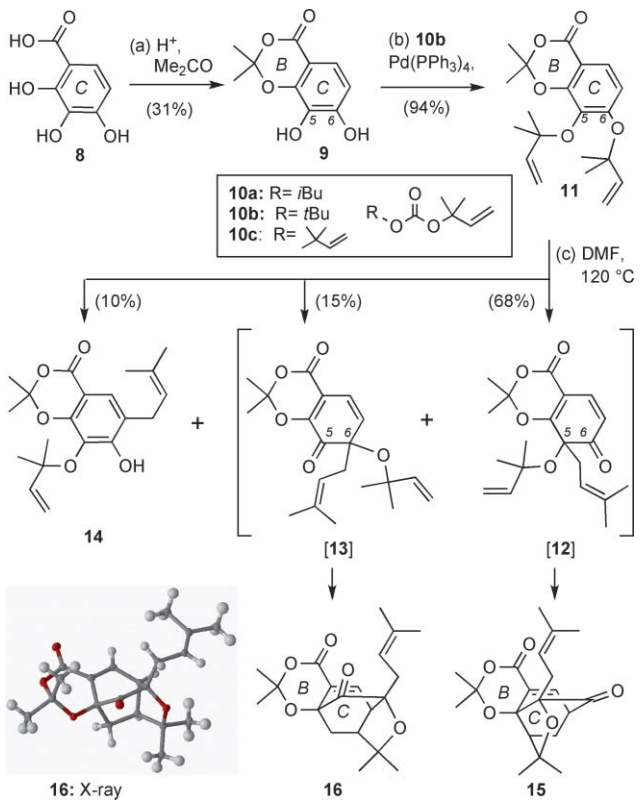
† Electronic supplementary information (ESI) available: Experimental procedures for compounds **9–10**, **18–20**, **22–23**, **34–36**, **38–39** and **42–46**. Crystallographic tables for compounds **7**, **15–16**, **26**, **36**, **42** and **43**. ^{1}H and ^{13}C NMR spectra for compounds **7**, **9**, **10a**, **10b**, **10c**, **11**, **14–24**, **26**, **31–46**. CCDC reference numbers 614936, 737621, 737622, 737623, 737624, 737625 and 737626. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b913496d

present a new Pd(0)-catalyzed method for the reverse prenylation (installation of 1,1-dimethyl-2-propenyl units) of catechols, the synthetic precursors of the caged scaffolds. This reaction led to a synthesis of several caged *Garcinia* analogues and to an optimized synthesis of cluvenone (**7**).

Results and discussion

Synthesis of BC and C ring caged analogues

Our initial studies aimed to produce analogues of the caged *Garcinia* xanthones lacking the A ring. With this in mind, commercially available 2,3,4-trihydroxybenzoic acid (**8**) was treated with acetone in the presence of TFA/TFAA (Scheme 1). To minimize formation of diprotected products, the reaction was performed at 0 °C and produced dioxanone **9** in 31% yield together with starting material **8** (60% yield). Initial efforts to convert **9** to **11** were based on a previously reported two-step sequence that involves propargylation using 2-chloro-2-methyl butyne followed by Lindlar reduction of the resulting alkynes.[13] However, this two-step process proved to be tedious and difficult to streamline. To overcome this problem we decided to develop an alternative method for the one-step introduction of the 1,1-dimethyl-2-propenyl unit (reverse prenyl group) to a catechol motif. Support for this reaction came from a report on the reverse prenylation of a substituted phenol using 1,1-dimethylpropenyl isobutyl carbonate (**10a**) under Pd(0) catalysis.[15] Using 1,1-dimethylpropenyl isobutyl carbonate (**10a**) we obtained the desired compound **11** (62–69% yield) together

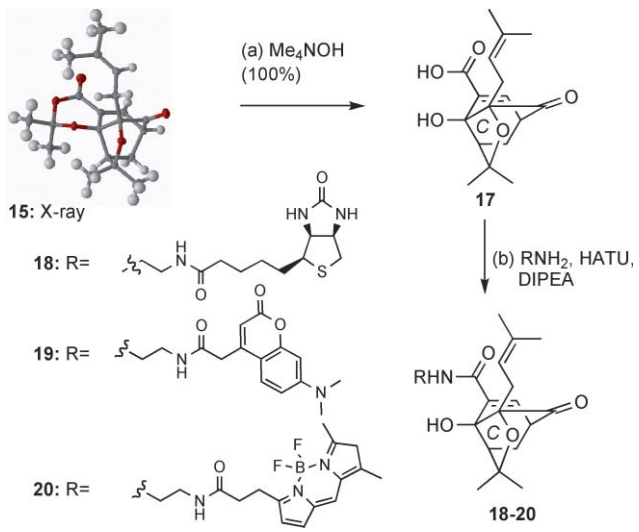


Scheme 1 *Reagents and conditions*: (a) 6.0 equiv. $(CH_3)_2CO$, 20 equiv. TFA, 10 equiv. TFAA, 19 h, 0 °C, 31% of **9**, 60% RSM; (b) 10 equiv. *t*-butyl 2-methylbut-3-en-2-yl carbonate (**10b**), 10 mol% $Pd(PPh_3)_4$, THF, 20 min, 5 °C, 94%; (c) DMF, 1 h, 120 °C, **14**: 10%, **15**: 68%, **16**: 15%.

with significant amounts of isobutyl addition products (5–10%). To minimize formation of the side-products we tested the allylation reaction with 1,1-dimethylpropenyl *t*-butyl carbonate (**10b**). In this case we observed the formation of **11** as the only product (94% isolated yield). Similar yields (90–92%) were obtained using the unexplored bis(1,1-dimethylpropenyl)carbonate (**10c**).[16] We also evaluated this reaction under Rh(I) catalysis but did not observe the formation of any prenylation product.[17]

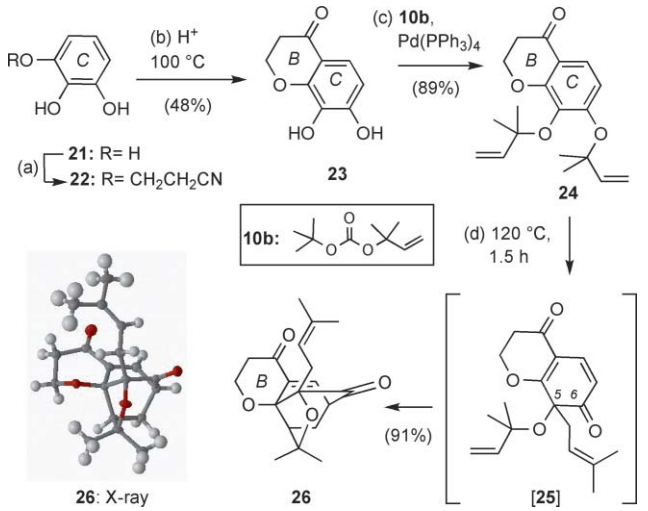
Heating of **11** in DMF (120 °C, 1 h) gave rise to two caged compounds **15** and **16** in 68% and 15% yields, respectively (Scheme 1). The structure and relative stereochemistry of these compounds was unambiguously confirmed *via* a single-crystal X-ray analysis.[18] Under these conditions we also observed the formation of phenol **14** arising from a Claisen rearrangement of **11** (10% yield). The formation of compounds **15** and **16** can be explained by considering an intramolecular Diels–Alder cycloaddition of intermediates **12** and **13** respectively, that have been formed *via* a Claisen rearrangement of **11**. The observed site-selectivity of this reaction cascade (C5 *versus* C6 allylation) parallels our previous observations and favors the formation of the regular caged structure **15** *versus* the neo isomer **16**.[12,13] It is worth noting that, upon additional heating at 120 °C, phenol **14** produced caged compounds **15** and **16**, supporting the reversibility of the Claisen rearrangement.[19,20]

Deprotection of the acetonide unit of compound **15** can open the B ring, producing C-ring caged analogues. To this end, exposure of **15** to 10% aqueous Me_4NOH in MeOH provided the optimum saponification conditions and produced the desired β-hydroxy acid **17** in quantitative yield (Scheme 2). This compound was converted to amides **18–20** in good yields using standard amide coupling protocols.



Scheme 2 *Reagents and conditions*: (a) excess 10% NMe_4OH (aq), MeOH, 24 h, 25 °C, 100%; (b) 2.0 equiv. DIPEA, 1.2 equiv. HATU, CH_2Cl_2, 24 h, 25 °C, **18**: 54%, **19**: 59%, **20**: 68%.

In a similar manner, reaction of pyrrogallol (**21**) with acrylonitrile formed nitrile **22** (Scheme 3). Reflux of **22** in the presence of H_2SO_4 (50% aq.) produced dihydroxychromanone **23** (16% combined yield).[21] The Pd(0)-catalyzed reverse prenylation of **23** with **10b** formed compound **24** (89% yield) which upon heating



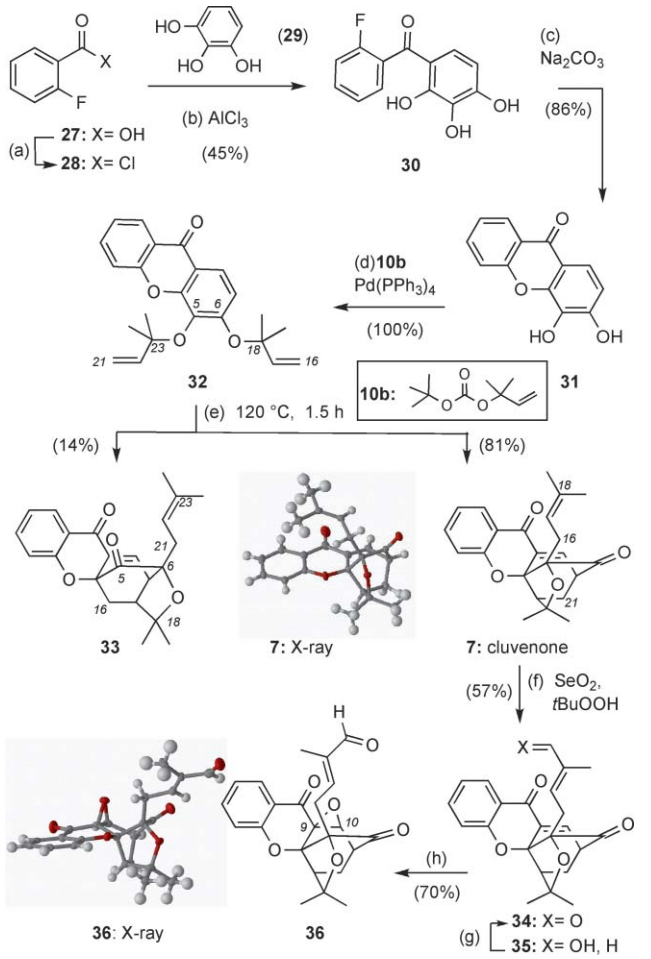
Scheme 3 *Reagents and conditions*: (a) 3.4 equiv. acrylonitrile, 0.3 equiv. NaOMe, 7 h, reflux, 76 °C, 34%; (b) excess 50% (w/w) H_2SO_4 (aq), 3 h, reflux, 100 °C, 48%; (c) 10 equiv. *t*-butyl 2-methylbut-3-en-2-yl carbonate (**10b**), 10 mol% $Pd(PPh_3)_4$, THF, 2 h, 5 °C, 89%; (d) DMF, 1.5 h, 120 °C, 91%.

gave rise to caged motif **26** in 91% yield. Single crystal X-ray analysis of **26** confirmed its chemical structure.[18] In this case we did not detect the formation of the neo caged structure.

Improved synthesis of cluvenone and synthesis of related allylic oxidation products

An improved synthesis of cluvenone (**7**), featuring the Pd(0)-catalyzed reverse prenylation reaction, is shown in Scheme 4. The tricyclic xanthone **31** was prepared in two steps: (a) a Friedel–Crafts acylation of pyrrogallol (**29**) with 2-fluorobenzoyl chloride (**28**) in the presence of $AlCl_3$ and (b) a base-induced cyclization of the resulting benzophenone **30** (2 steps, 34% combined yield). Pd(0)-catalyzed reverse prenylation of **31** using 1,1-dimethylpropenyl *t*-butyl carbonate (**10b**) gave rise to compound **32** in quantitative yield. Importantly, during scale-up of this reaction to gram amounts we were able to decrease the amount of the Pd(0) to 3 mol% and the reaction time to 10 min at 25 °C. The heat-induced Claisen/Diels–Alder reaction cascade furnished cluvenone (**7**) in 81% yield, along with small amounts of the neo caged xanthone **33** (14% yield).

With compound **7** in hand we explored an allylic oxidation reaction (Scheme 4). Treatment of **7** with SeO_2 and *t*-BuOOH produced aldehyde **34** as the main product, isolated in 57% yield, along with alcohol **35** (21%). The latter compound was converted to **34** *via* PCC oxidation in 95% yield. However, all attempts to oxidize aldehyde **34** to the corresponding acid met with failure. In all these cases the characteristic signal corresponding to the C10 proton disappeared, indicating a conjugate addition reaction of the enone bond. Under relatively mild oxidation conditions ($NaClO_2$) we were able to isolate epoxide **36** in 70% yield, the structure of which was confirmed *via* a single-crystal X-ray analysis.[18] This observation supports the expected reactivity of the C9–C10 enone motif as a conjugate electrophile.

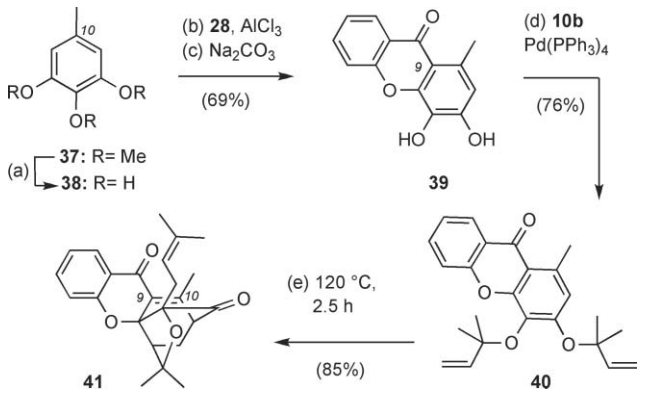


Scheme 4 *Reagents and conditions*: (a) 1.2 equiv. ClCOCOCl (2.0 M in DCM), DCM, DMF (cat.), 1.5 h, 0 to 25 °C, 87%; (b) 2.0 equiv. **29**, 2.9 equiv. $AlCl_3$, chloroform, DCM, 12 h, 25 °C; then 4 h, reflux, 60 °C, 45%; (c) 1.5 equiv. Na_2CO_3, DMF, 3.5 h, 90 °C, 86%; (d) 10.0 equiv. *t*-butyl 2-methylbut-3-en-2-yl carbonate **10b**, 3 mol% $Pd(PPh_3)_4$, THF, 10 min, 25 °C, 100%; (e) DMF, 1.5 h, 120 °C, **7**: 81%, **33**: 14%; (f) 5 mol% SeO_2, 1.8 equiv. *t*-BuOOH, DCM, 19 h, 25 °C, **34**: 57%, **35**: 21%; (g) 1.5 equiv. PCC, DCM, 30 min, 25 °C, 95% (h) 3.0 equiv. $NaClO_2$, 3.0 equiv. $NaH_2PO_4 \cdot H_2O$, 8.0 equiv. 2-methyl-2-butene, *t*-BuOH/H_2O (2:1), 4 h, 0 °C, 70%.

Synthesis of caged *Garcinia* xanthone analogues modified at the C9–C10 enone bond

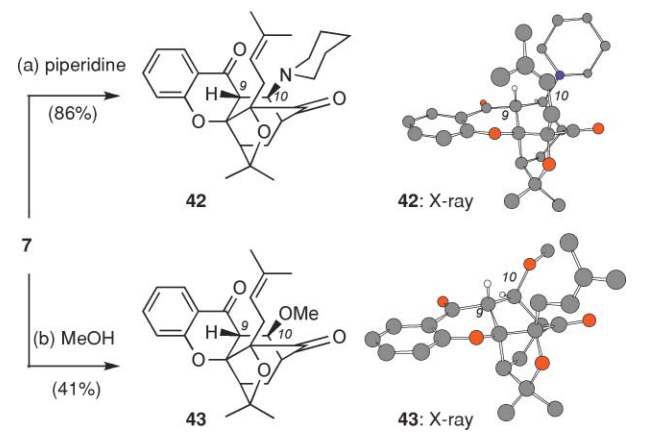
It has been suggested that the C9–C10 double bond of the caged *Garcinia* xanthones plays an essential role in the bioactivity of these molecules.[10a] For instance, conjugate reduction of the enone motif has yielded compounds that have reduced cytotoxicity. This has been tentatively attributed to their decreased ability to act as conjugate electrophiles. However, the C9–C10 conjugate reduction affects the chemical structure of the caged xanthone motif, which in turn could be responsible for the lack of activity. To further test this hypothesis, we sought to evaluate the bioactivity of the C10 methylated analogue **41**. This compound was prepared as shown in Scheme 5. Commercially available 1,2,3-trimethoxy-5-methylbenzene (**37**) was demethylated with excess BBr_3 to form polyphenol **38** in 59% yield.[22] Friedel–Crafts acylation of **38** with 2-fluorobenzoyl chloride (**28**) in the presence of $AlCl_3$ followed

by Na_2CO_3-induced cyclization of the resulting benzophenone produced xanthone **39** in 69% combined yield. The Pd(0)-catalyzed reverse prenylation with carbonate **10b** gave rise to compound **40** which, upon heating, underwent the Claisen/Diels–Alder reaction cascade to form caged xanthone **41** in 85% yield (Scheme 5).



Scheme 5 *Reagents and conditions*: (a) excess BBr_3, DCM, 3 h, 0 to 25 °C, 59%; (b) 1.5 equiv. **28**, 2.0 equiv. $AlCl_3$, chloroform, DCM, 1.5 h, 25 °C; then 6 h, 60 °C; (c) 1.5 equiv. Na_2CO_3, DMF, 69% (over two steps); (d) 10.0 equiv. *t*-butyl 2-methylbut-3-en-2-yl carbonate **10b**, 10 mol% $Pd(PPh_3)_4$, THF, 2 h, 5 °C, 76%; (e) DMF, 2.5 h, 120 °C, 85%.

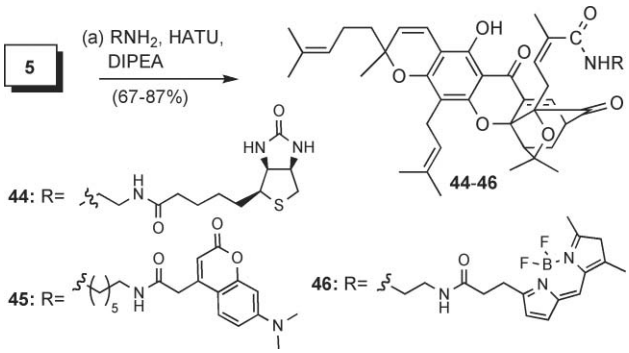
We have also treated cluvenone (**7**) with piperidine and MeOH under basic conditions. These reactions led to the formation of the conjugate addition products **42** and **43** which were isolated in 86% and 41% yield, respectively (Scheme 6). The chemical structure of these compounds was determined *via* a single crystal X-ray analysis[18] and indicated that the conjugate addition proceeded in a syn fashion across the C9–C10 enone bond.



Scheme 6 *Reagents and conditions*: (a) 4.0 equiv. piperidine, DCM, 6 h, 60 °C, 86%; (b) MeOH, 3 d, 65 °C, 41%.

Synthesis of amide analogues of gambogic acid

To evaluate the bioactivity of the carboxylic acid functionality of gambogic acid (**5**) we prepared the amide derivatives **44–46**. These compounds contain affinity and fluorescent probes and can be used for studies related to receptor binding assays and subcellular localization of the caged *Garcinia* xanthones. The amide coupling reaction proceeded in high yields using the DIPEA and HATU protocol (Scheme 7).



Scheme 7 *Reagents and conditions*: (a) 2.0 equiv. DIPEA, 1.2 equiv. HATU, CH_2Cl_2, 24 h, 25 °C, **44**: 67%, **45**: 87%, **46**: 77%.

Cell proliferation studies

The ability of the synthesized caged *Garcinia* xanthones to inhibit cancer cell growth was evaluated in a multidrug-resistant 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 (**5**) and cluvenone (**7**) were the most active among all the compounds tested and exhibited IC_{50} values of 0.5 and 0.4 μM respectively (Table 1). Similar activity was observed for the amide analogues of gambogic acid (compounds **44**, **45** and **46**, entries 18–20 respectively) as well as for the oxidized analogues of cluvenone (compounds **34** and **35**). Gambogin (**6**) also exhibited a low μM activity. These results suggest that: (a) the dihydropyran motif of **5** and **6** is not needed for the bioactivity; (b) the carboxylic acid of **5** can be functionalized without loss of function and (c) oxidation and derivatization of the prenyl group of these compounds is well tolerated during binding

Table 1 Inhibition of cell proliferation by caged *Garcinia* xanthones and analogues in multi-drug resistant promyelocytic leukemia cells

Compound	IC_{50}/μM HL-60/ADR
gambogic acid (**5**)	0.5
gambogin (**6**)	1.1
cluvenone (**7**)	0.4
15	ND[a]
16	ND[a]
17	ND[a]
18	20.1
19	20.7
20	27.3
26	10.4
33	1.3
34	0.7
35	0.8
36	2.5
41	5.1
42	2.8
43	2.5
44	1.1
45	0.3
46	0.6

[a] Less than 10% inhibition at 10.0 μM.

to the putative receptor. In contrast, the intact ABC caged ring structure is important for the bioactivity. For instance, compounds **15**, **16** and **17** induce less than 10% growth inhibition at up to 10 μM concentrations. Similarly, the C-ring amide analogues **18–20** have IC_{50} values greater than 20 μM, while compound **26**, which lacks the A ring, has an IC_{50} value of 10 μM. Compound **33**, containing the neo caged structure, has a low micromolar activity (IC_{50} value of 1.3 μM) but is about 3 times less potent than cluvenone and related compounds with the regular caged motif. On the other hand, substitution of the C9–C10 enone functionality decreases substantially the bioactivity. For instance, compound **41** is almost 10 times less active than cluvenone (**7**), while compounds **36**, **42**, and **43** are about 5 times less active than **7**. A similar decrease in cytotoxicity has been reported for gambogoic acid, the conjugate addition product of **5** with methanol.[23] These results demonstrate the significance of the C9–C10 enone functionality for the bioactivity of these compounds. This may be due to its reactivity as a conjugate electrophile.

Apoptosis studies

To determine whether the mechanism of cytotoxicity of these compounds involves the induction of apoptosis, a cell death detection ELISA which measures histone-associated DNA fragments was performed. These studies were performed with cluvenone (**7**) and are shown in Fig. 2. Compound **7** induced apoptosis, after 7 h of treatment of HL-60 and HL-60/ADR cells, in a dose-dependent manner with EC_{50} values of 0.25 and 0.32 μM respectively. These results are comparable to the apoptotic effect of gambogic acid and related caged *Garcinia* natural products. Specifically, the EC_{50} values of **5** in human breast cancer cells T47D, human colon cancer cells HCT116, and hepatocellular carcinoma cancer cells SNU398 are reported to be about 0.7 μM.[10b] More importantly, the similar EC_{50} values observed for cluvenone (**7**) in the HL-60 and HL-60/ADR cells parallels our previous observations[12] and confirms that its cytotoxicity is not affected by the expression of P-glycoprotein which renders the HL-60/ADR cell lines multidrug-resistant.[24]

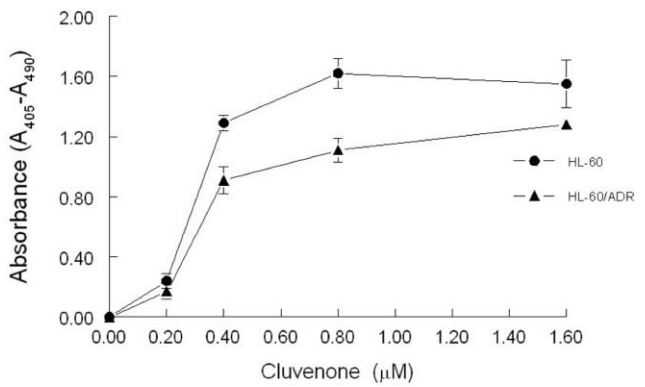


Fig. 2 Induction of apoptosis by cluvenone (**7**) in promyelocytic leukemia cells.

Apoptosis induced in HL60/ADR cells by cluvenone (**7**) was also visualized by fluorescence microscopy after staining with Alexa Fluor® 488 annexin V and propidium iodide (PI) (Fig. 3). The green-fluorescent Alexa Fluor® 488 annexin V detects the externalization of phosphatidylserine, a hallmark of apoptosis

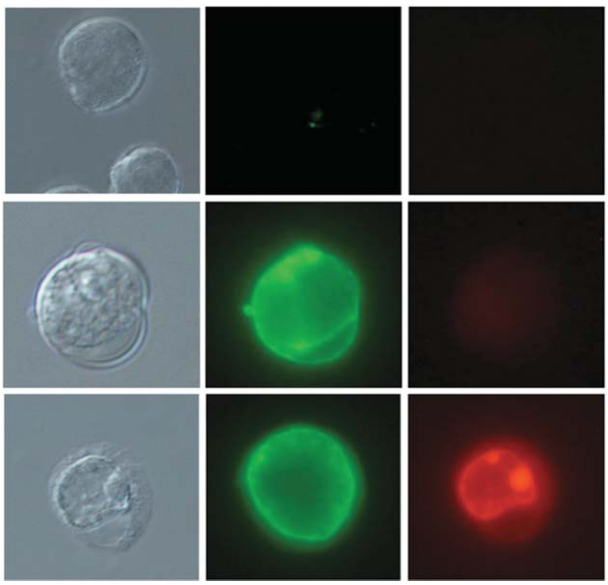


Fig. 3 Induction of apoptosis in HL-60/ADR cells by cluvenone (**7**) visualized by differential interference contrast microscopy (left column) and fluorescence microscopy (middle and right columns). Control untreated cells are shown in the top row. Treated cells undergoing early and late stage apoptosis are shown in the middle and bottom rows respectively.

(Fig. 3, middle column).[25] The red-fluorescent propidium iodide stains DNA during advanced stages of apoptosis and necrosis (Fig. 3, right column). Cells in the left column of Fig. 3 have been visualized by differential interference contrast (DIC) microscopy. While untreated live cells show little or no fluorescence (Fig. 3, top row), cluvenone-treated cells undergoing early stage apoptosis show only green fluorescence after staining with both probes (Fig. 3, middle row). In the middle row is also evident the membrane blebbing, which is characteristic of apoptosis.[25] Cluvenone-treated cells undergoing late stage apoptosis, at which point DNA becomes accessible to staining by PI, display both green and red fluorescence (Fig. 3, bottom row). In this row the chromatin fragmentation is also evident.

Conclusions

We present herein a study aiming to identify the pharmacophoric motif of the caged *Garcinia* xanthones. Our results indicate that the minimum bioactive motif of these compounds is represented by the intact ABC ring containing the C-ring caged structure. Structural changes to this motif result in substantial loss of activity. The C9–C10 enone functionality is also important to the activity, while the C5 prenyl group can be oxidized and functionalized without loss of bioactivity. In fact, this site could be used for modifications that will improve the solubility and pharmacology of these compounds. We have also developed a method for the reverse prenylation (installation of 1,1-dimethyl-2-propenyl units) of catechols. This reaction proceeds in excellent yield under Pd(0)-catalysis using 1,1-dimethylpropenyl *t*-butyl carbonate (**10b**) or bis(1,1-dimethylpropenyl)carbonate (**10c**) as the prenylation reagents. Application of this reaction led to an improved synthesis of lead analogue cluvenone (**7**). In this study, we have also demonstrated that cluvenone induces cell death *via* apoptosis and has similar cytotoxicity in multidrug-resistant and sensitive

leukemia cells. These results support our previous findings on the pharmacological potential of the caged *Garcinia* xanthones, enhance our understanding of the structure–activity relationship, and pave the way for the preparation of therapeutically relevant agents.

Experimental

General notes

Gambogic acid (**5**), $Pd(PPh_3)_4$, and 2-fluorobenzoic acid (**27**) were purchased from Gaia Chemical Corporation (Gaylordsville, CT), Strem Chemicals, Inc. (Newburyport, MA), and TCI America (Portland, OR), respectively. Biotin ethylenediamine hydrobromide and BODIPY FL EDA were purchased from Invitrogen (Carlsbad, CA). The rest of the 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. 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. X-ray data were recorded on a Bruker SMART APEX 3 kW Sealed Tube X-ray diffraction system.

2,2-Dimethyl-7,8-bis(2-methylbut-3-en-2-yloxy)-4*H*-benzo[*d*]-[1,3]dioxin-4-one 11. To a 25 mL round-bottomed flask was added acetonide **9** (95 mg, 0.45 mmol) followed by THF (0.65 mL). The reaction vessel was degassed by argon and was placed in an ice bath. To the clear homogeneous solution was added *tert*-butyl 2-methylbut-3-en-2-yl carbonate **10b** (0.89 mL, 4.50 mmol) *via* syringe, followed by $Pd(PPh_3)_4$ (52.0 mg, 45.0 μmol). The reaction vessel was stirred under argon at 5 °C for 20 min. The onset of a blue suspension indicated the formation of the desired product **11**. The solvent was removed by rotary evaporation and the crude material was purified through flash column chromatography (silica, 10% EtOAc-hexane) to yield the desired product **11** (146.5 mg, 94%). **11**: colorless oil; $R_f = 0.61$ (25% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 8.8 Hz, 1H), 6.81 (d, J = 8.9 Hz, 1H), 6.16 (m, 2H), 5.20 (m, 3H), 5.02 (d, J = 10.9 Hz, 1H), 1.72 (s, 3H), 1.55 (s, 3H), 1.47 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 158.6, 152.1, 143.9, 143.8, 135.6, 124.5, 114.3, 113.7, 113.0, 107.6, 106.4, 106.4, 83.3, 82.1, 27.4, 27.1, 26.1; HRMS calc. for $C_{20}H_{26}O_5$ $(M + H)^+$ 369.1672, found 369.1674.

Caged bicycle 15 and neo-caged bicycle 16. Alkene **11** (99 mg, 0.28 mmol) was dissolved in dry DMF (1.8 mL) and the solution was stirred under argon at 120 °C. After 1 hour, the reaction mixture was concentrated under reduced pressure. The crude material was purified by flash column chromatography (silica, 10-17% EtOAc-hexane) to yield the caged product **15** (67 mg, 68%), neo-caged product **16** (15 mg, 15%), and compound **14** (10 mg, 10%), respectively. **Caged product 15**: white solid; R_f = 0.10 (25% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 6.9 Hz, 1H), 4.41 (m, 1H), 3.42 (t, J = 4.3 Hz, 1H), 2.72 (dd, J = 13.8, 10.4 Hz, 1H), 2.63 (m, 1H), 2.50 (d, J = 9.7 Hz, 1H), 2.31 (dd, J = 13.6, 4.7 Hz, 1H), 1.69 (s, 3H), 1.67 (s, 3H), 1.62 (s, 3H), 1.54 (s, 3H), 1.53 (s, 3H), 1.44 (dd, J = 13.6, 9.3 Hz, 1H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.2, 159.9, 139.0, 135.6, 128.1, 118.1, 105.1, 85.0, 84.9, 82.9, 48.6, 46.8, 30.2, 29.1, 28.8, 28.5, 28.0, 26.9, 25.9, 18.4; HRMS calc. for $C_{20}H_{26}O_5$ $(M + Na)^+$ 369.1672, found 369.1675. **Neo-caged product 16**: white solid; R_f = 0.30 (25% EtOAc-hexane);^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 7.0 Hz, 1H), 4.95 (t, J = 7.0 Hz, 1H), 3.63 (dd, J = 7.0, 4.5 Hz, 1H), 2.36-2.25 (m, 4H), 1.69 (s, 3H), 1.68 (s, 3H), 1.64-1.61 (m, 1H), 1.59 (s, 3H), 1.43 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 205.0, 159.2, 140.1, 136.7, 127.7, 117.2, 106.5, 83.6, 81.2, 80.1, 46.0, 45.5, 34.1, 30.7, 30.2, 28.6, 27.8, 27.0, 26.2, 18.2; HRMS calc. for $C_{20}H_{26}O_5$ $(M + Na)^+$ 369.1672, found 369.1686. **Phenol 14**: colorless oil; R_f = 0.52 (25% EtOAc-hexane);^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 6.27 (s, 1H), 6.15 (dd, J = 17.5, 10.8 Hz, 1H), 5.35-5.25 (m, 2H), 5.17 (d, J = 10.9 Hz, 1H), 3.26 (d, J = 7.1 Hz, 1H), 1.74 (s, 3H), 1.72 (s, 6H), 1.69 (s, 3H) 1.48 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.6, 156.0, 149.1, 143.3, 133.9, 130.0, 125.6, 123.1, 121.3, 114.6, 106.4, 105.8, 83.6, 27.9, 26.9, 26.1, 18.0; HRMS calc. for $C_{20}H_{26}O_5$ $(M + Na)^+$ 369.1672, found 369.1680.

Carboxylic acid 17. To a 25 mL round-bottomed flask was added caged product **15** (41 mg, 0.12 mmol) followed by methanol (1.5 mL). The flask was placed in an ice bath and the solution was stirred at 0 °C. To the stirring solution was then added 10% NMe_4OH (aq) (1.7 mL, 159 mmol) dropwise *via* syringe. The light yellow reaction mixture was allowed to warm to room temperature and further stirred at 25 °C for another 24 hours. Acetic acid (10 mL) was then added to neutralize the reaction mixture. The reaction mixture was partitioned between ethyl acetate (2 × 25 mL) and water (25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The crude material was purified by recrystallization (DCM-hexane) to yield the acid **17** (37 mg, 100%). **17**: white solid; R_f = 0.11 (67% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 7.1 Hz, 1H), 4.64 (t, J = 6.4 Hz, 1H), 3.33 (t, J = 5.3 Hz, 1H), 2.69 (dd, J = 13.8, 9.8 Hz, 1H), 2.58 (dd, J = 13.8, 5.3 Hz, 1H), 2.28 (d, J = 4.5 Hz, 1H), 2.24 (d, J = 10.0 Hz, 1H), 1.60 (s, 6H), 1.57 (s, 3H), 1.40 (dd, J = 13.4, 9.6 Hz, 1H), 1.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.7, 168.4, 142.0, 135.5, 130.7, 118.8, 85.0,

84.0, 83.5, 49.5, 47.1, 30.2, 29.2, 28.7, 27.1, 26.1, 18.0; HRMS calc. for $C_{17}H_{22}O_5$ $(M + Na)^+$ 329.1359, found 329.1362.

7,8-Bis(2-methylbut-3-en-2-yloxy)chroman-4-one 24. To a 25 mL round-bottomed flask was added 7,8-dihydroxychroman-4-one **23** (50 mg, 0.28 mmol) followed by dry THF (1.5 mL). The flask was degassed by argon and was placed in an ice water bath. To the yellow homogeneous solution was added *tert*-butyl 2-methylbut-3-en-2-yl carbonate **10b** (522 mg, 2.80 mmol), *via* syringe, followed by $Pd(PPh_3)_4$ (32 mg, 0.028 mmol). The reaction vessel was stirred under argon at 5 °C for 2 hours. The onset of a yellow suspension indicated the formation of the alkene **24**. The solvent was removed by rotary evaporation and the crude material was purified through flash column chromatography (silica, 30-40% EtOAc-hexane) to yield 7,8-bis(2-methylbut-3-en-2-yloxy)choman-4-one **24** (79 mg, 89%). **24**: yellow oil; R_f = 0.52 (30 % EtOAC-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 9.0 Hz, 1H), 6.72 (d, J = 9.0, 1H), 6.19 (dd, J = 17.4, 10.6 Hz, 1H), 6.12 (dd, J = 17.6, 10.9 Hz, 1H), 5.13 (m, 3H), 4.98 (dd, J = 10.9, 1.1 Hz, 1H), 4.47 (t, J = 6.5 Hz, 2H), 2.71 (t, J = 6.6 Hz, 2H), 1.51 (s, 6H), 1.47 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.2, 157.8, 143.7, 135.8, 121.7, 116.7, 113.8, 113.6, 112.5, 82.8, 81.8, 67.1, 37.4, 27.1, 26.7; HRMS calc. for $C_{19}H_{24}O_4$ $(M + Na)^+$ 339.1567, found 339.1569.

Caged chromanone 26. A solution of compound **24** (36 mg, 0.11 mmol) in DMF (1.5 mL) was heated at 120 °C for 1.5 hours. The onset of a brown color indicated the formation of the caged xanthone **26**. The reaction mixture was then cooled to 25 °C and the solvent was removed by rotary evaporation. The crude material was purified through flash column chromatography (silica, 50-55 % EtOAc-hexane) to yield the caged product **26** (33 mg, 91%). **26**: white solid; R_f = 0.21 (30 % EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 6.6 Hz, 1H), 4.41 (m, 1H), 4.17 (ddd, J = 12.1, 6.5, 1.4 Hz, 1H), 3.94 (dt, J = 12.3, 2.9 Hz, 1H), 3.34 (m, 1H), 2.63 (d, J = 8.6 Hz, 1H), 2.50 (dd, J = 12.4, 6.5 Hz, 1H), 2.42 (dd, J = 2.9, 1.4 Hz, 1H), 2.37 (m, 1H), 2.31 (dd, J = 13.6, 4.5 Hz, 1H), 1.59 (s, 3H), 1.53 (s, 3H), 1.48 (s, 3H), 1.32 (m, 1H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.9, 192.0, 136.9, 135.4, 133.8, 119.2, 87.4, 84.2, 82.9, 60.0, 46.2, 44.5, 38.1, 30.1, 28.8, 27.7, 25.5, 17.9; HRMS calc. for $C_{19}H_{24}O_4$ $(M + Na)^+$ 339.1567, found 339.1571.

3,4-Dihydroxy-9*H*-xanthen-9-one 31. To a clean, dried 250 mL round-bottomed flask was added 2-fluorobenzoic acid **27** (5.09 g, 36.3 mmol). The flask containing 2-fluorobenzoic acid **27** and a magnetic stir bar was placed under high vacuum for about 10 min. The flask was carefully sealed and DCM (100 mL) was added by using a syringe under argon. The flask was then placed in an ice bath and the reaction mixture was stirred at 0 °C. To the stirring solution of 2-fluorobenzoic acid **27** and DCM was added a solution of oxalyl chloride (2.0 M in dichloromethane, 21.0 mL, 42.0 mmol) dropwise, *via* syringe, followed by a catalytic amount of DMF. The ice bath was removed and the reaction mixture was stirred at room temperature for 1.5 hours. The solution was concentrated by rotary evaporation under argon to yield a colorless oil, 2-fluorobenzoyl chloride **28** (5.01 g, 87%). To a mixture of pyrogallol **29** (6.48 g, 51.3 mmol), aluminium chloride (14.6 g, 110 mmol), chloroform (80 mL), and DCM (200 mL) in a 1 L round-bottomed flask was added a solution of 2-fluorobenzoyl chloride **28** in DCM (10 mL) dropwise *via* syringe. The reaction mixture was stirred at room temperature under argon for 12 hours. The reaction vessel was then equipped with a reflux condenser and stirred under argon at 80 °C for another 4 hours. The cooled, red homogeneous solution was acidified with 1 N HCl (300 mL). The reaction mixture was then partitioned between water and ethyl acetate (3 × 200 mL). The aqueous layer was back-extracted with ethyl acetate (2 × 200 mL) until the color of the aqueous layer was almost clear. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to yield (2-fluorophenyl)(2,3,4-trihydroxy-phenyl)methanone **30** (3.54 g, 45%). To a 500 mL round-bottomed flask containing sodium carbonate (2.27 g, 21.4 mmol) and DMF (100 mL) was added (2-fluorophenyl)(2,3,4-trihydroxyphenyl)methanone **30**. The reaction vessel was then equipped with a reflux condenser and stirred under argon at 90 °C for 3.5 hours. The dark reaction mixture was cooled to room temperature and acidified with 1 N HCl (300 mL). The reaction mixture was then partitioned between water and ethyl acetate (3 × 150 mL). The aqueous layer was back extracted with ethyl acetate (5 × 150 mL). The combined brown organic layers were dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The crude material was purified through flash column chromatography (silica, 50-60% EtOAc-hexane) to yield 3,4-dihydroxy-9*H*-xanthen-9-one **31** (2.79 g, 86%). **31**: pale yellow solid; R_f = 0.42 (90% Et_2O-hexane); ^{1}H NMR (400 MHz, DMSO-d6) δ 10.49 (s, 1H), 9.43 (s, 1H), 8.15 (dd, J = 7.9, 1.7 Hz, 1H), 7.83 (ddd, J = 8.6, 7.2, 1.7 Hz,1H), 7.63 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 8.7 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d6) 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-en-2-yloxy)-9*H*-xanthen-9-one 32. To a 50 mL round-bottomed flask was added 3,4-dihydroxy-9*H*-xanthen-9-one **31** (1.0 g, 4.39 mmol) followed by dry THF (15 mL). To the yellow homogeneous solution was added *tert*-butyl 2-methylbut-3-en-2-yl carbonate **10b** (8.18 g, 43.9 mmol), *via* syringe, followed by $Pd(PPh_3)_4$ (0.15 g, 0.13 mmol). The reaction vessel was stirred under argon at 25 °C for 10 minutes. The onset of a yellow suspension indicated the formation of the alkene **32**. The solvent was removed by rotary evaporation and the crude material was purified through flash column chromatography (silica, 10-15% EtOAc-hexane) to yield 3,4-bis(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one **32** (1.59 g, 100%). **32**: yellow solid; R_f = 0.67 (30 % EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (dd, J = 8.0, 1.7 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.68 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.36 (ddd, J = 8.0, 7.2, 0.9 Hz, 1H), 7.12 (d, J = 8.9 Hz, 1H), 6.28 (dd, J = 17.5, 10.8 Hz, 1H), 6.18 (dd, J = 17.6, 10.9 Hz, 1H), 5.19 (m, 3H), 5.01 (dd, J = 10.9, 1.0 Hz, 1H), 1.58 (s, 6H), 1.56 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 156.9, 155.9, 152.4, 143.5, 143.4, 135.7, 134.3, 126.5, 123.7, 121.5, 121.0, 117.8, 117.1, 116.8, 114.1, 113.0, 83.5, 82.1, 27.1, 26.9; HRMS calc. for $C_{23}H_{24}O_4$ $(M + H)^+$ 365.1753, found 365.1740.

Caged xanthones 7 and 33. A solution of compound **31** (350 mg, 0.96 mmol) in DMF (6 mL) was heated at 120 °C for 1.5 hours. The onset of a brown color indicated the formation of the xanthones **33** and **7**. The brown reaction mixture was then cooled to room temperature and the solvent was removed

by rotary evaporation. The crude material was then purified by column chromatography (silica, 20-30% Et_2O-hexane) to yield a mixture of caged products **7** (285 mg, 81%) and **33** (50 mg, 14%). **7**: white solid; $R_f = 0.28$ (25% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, $J = 8.0$ Hz, 1.7 Hz, 1H), 7.51 (ddd, $J = 8.9$, 7.3, 1.7 Hz, 1H), 7.42 (d, $J = 6.9$ Hz, 1H), 7.05 (m, 2H), 4.39 (m, 1H), 3.48 (dd, $J = 6.7$ Hz, 4.6 Hz, 1H), 2.64 (m, 2H), 2.45 (d, $J = 9.6$ Hz, 1H), 2.33 (dd, $J = 13.5$ Hz, 4.6 Hz, 1H), 1.71 (s, 3H), 1.29 (m, 1H), 1.29 (s, 6H), 0.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 176.4, 159.5, 136.1, 134.8, 134.7, 133.7, 126.8, 121.8, 118.9, 118.9, 118.0, 90.2, 84.5, 83.4, 48.7, 46.7, 30.2, 29.0, 25.2, 25.0, 16.6; HRMS calc. for $C_{23}H_{24}O_4$ $(M + H)^+$ 365.1753, found 365.1765. **33**: yellow solid; $R_f = 0.34$ (25% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, $J = 7.9$, 1.7 Hz, 1H), 7.54 (m, 1H), 7.25 (d, $J = 7.1$ Hz, 1H), 7.18 (d, $J = 8.5$ Hz, 1H), 7.05 (ddd, $J = 8.0$, 7.3, 1.0 Hz, 1H), 5.02 (m, 1H), 3.76 (dd, $J = 6.9$, 4.6 Hz, 1H), 2.50 (m, 2H), 2.13 (m, 2H), 1.87 (dd, $J = 13.2$, 10.0 Hz, 1H), 1.71 (s, 3H), 1.59 (s, 3H), 1.38 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7, 175.4, 160.2, 136.5, 136.1, 135.9, 134.9, 127.0, 122.0, 119.2, 118.3, 117.3, 84.1, 83.7, 78.8, 44.8, 42.1, 33.1, 30.2, 29.7, 26.8, 26.0, 18.2; HRMS calc. for $C_{23}H_{24}O_4$ $(M + H)^+$ 365.1753, found 365.1766.

1-Methyl-3,4-bis(2-methylbut-3-en-2-yloxy)-9*H*-xanthen-9-one 40. To a 25 mL round-bottomed flask was added methyl xanthone **39** (46 mg, 0.19 mmol) followed by dry THF (1.5 mL). The flask was degassed by argon and was placed in an ice water bath. To the yellow homogeneous solution was added *tert*-butyl 2-methylbut-3-en-2-yl carbonate **10b** (354 mg, 1.9 mmol), *via* syringe, followed by $Pd(PPh_3)_4$ (22 mg, 0.019 mmol). The reaction vessel was stirred under argon at 5 °C for 2 hours. The onset of a yellow suspension indicated the formation of the desired product **40**. The solvent was removed by rotary evaporation and the crude material was purified through flash column chromatography (silica, 10-15% EtOAc-hexane) to yield 1-methyl-3,4-bis(2-methylbut-3-en-2-yloxy)-9*H* - xanthen - 9 - one **40** (55 mg, 76%). **40**: yellow oil; $R_f = 0.66$ (30% EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (dd, $J = 7.9$, 1.5 Hz, 1H), 7.64 (ddd, $J = 8.6$, 7.2, 1.7 Hz, 1H), 7.43 (d, $J = 8.4$ Hz, 1H), 7.31 (t, $J = 7.5$ Hz, 1H), 6.86 (s, 1H), 6.28 (dd, $J = 17.5$, 10.9 Hz, 1H), 6.19 (dd, $J = 17.6$, 10.8 Hz, 1H), 5.18 (m, 3H), 5.01 (dd, $J = 10.9$, 1.0 Hz, 1H), 2.80 (s, 3H), 1.56 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.4, 155.3, 155.0, 153.5, 143.8, 143.6, 136.3, 133.9, 126.5, 123.5, 122.5, 119.1, 117.3, 115.5, 113.9, 112.8, 83.1, 82.0, 27.2, 26.9, 23.5; HRMS calc. for $C_{24}H_{26}O_4$ $(M + H)^+$ 379.1904, found 379.1911.

Caged xanthone 41. A solution of compound **40** (35 mg, 0.092 mmol) in DMF (1.5 mL) was heated at 120 °C under argon for 2.5 hours. The onset of a yellow color indicated the formation of the methyl caged xanthone **41**. The reaction mixture was then cooled to room temperature and the solvent was removed by rotary evaporation. The crude material was purified through flash column chromatography (silica, 15-20% EtOAc-hexane) to yield the methyl caged xanthone **41** (30 mg, 85%). **41**: white solid; $R_f = 0.56$ (30 % EtOAc-hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, $J = 7.7$ Hz, 1H), 7.47 (m, 1H), 7.03 (m, 2H), 4.42 (t, $J = 7.0$ Hz, 1H), 3.18 (d, $J = 4.4$ Hz, 1H), 2.61 (m, 1H), 2.51 (s, 3H), 2.45 (d, $J = 9.5$ Hz, 1H), 2.28 (dd, $J = 13.5$, 4.7 Hz, 1H), 1.70 (s, 3H), 1.35 (s, 3H), 1.34 (m, 1H), 1.27 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.6, 179.3, 158.3, 150.7, 135.5, 134.8, 126.8, 121.7, 120.6, 118.3, 117.6, 90.5, 84.8, 83.2, 55.8, 49.2, 30.3, 29.0, 28.6, 25.6, 24.9, 19.7, 16.7; HRMS calc. for $C_{24}H_{26}O_4$ $(M + H)^+$ 379.1904, found 379.1909.

^{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).

Apoptosis assays

ELISA assay. The compounds were dissolved in DMSO and further diluted in complete medium to obtain final concentrations as indicated. HL-60 and HL-60/ADR cells were seeded into each well of a 96-well cell culture plate at 10,000 cells per well and incubated at 37 °C for 7 h with the indicated concentrations of each compound. Control samples were incubated in 0.1% DMSO. Each condition was in triplicate. The proapoptotic effect was detected by using the Cell Death Detection ELISA^{PLUS} kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer instructions. This 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. The absorption values A ($A_{405nm} - A_{490nm}$) measured give a quantitative indication of the induced amount of apoptosis.

Fluorescence microscopy of annexin V/PI stained cells. HL-60/ADR cells were plated in a 6-well plate at 1 × 10^6 cell/ml (4 ml) and treated with 0.5 μM **7** while control cells received 0.1% DMSO. Cells were incubated overnight and then stained with Alexa Fluor 488 annexin V and propidium iodide using the Vybrant Apoptosis Assay Kit (Molecular Probes, Eugene, OR) according to manufacturer's recommendations. Cells were then viewed on an E800 Nikon (New York City, NY) research microscope equipped with an EXFO (Vanier, Canada) X-cite fluorescent 120 W metal halide illuminator and imaged with a DMX 1200F Nikon fluorescence sensitive digital camera.

Acknowledgements

Financial support from the National Institutes of Health (CA 133002) is gratefully acknowledged. We also acknowledge partial support to O.C. and W.C. from the Thailand Research Fund for a Royal Golden Jubilee Ph.D. Fellowship (Grant No. PHD/0223/2548) and Center for Petroleum, Petrochemistry and Advanced Materials. We thank the National Science Foundation for instrumentation grants CHE-9709183 and CHE-0741968. We gratefully acknowledge Dr Barbara Davids at UCSD, Department of Pathology, Division of Infectious Diseases, for performing the fluorescence microscopy. We also thank Dr A. Mrse and Dr

Y. Su for NMR spectroscopic and mass spectrometric assistance respectively.

Notes and references

1 For selected general references on this topic see: (*a*) W. D. Ollis, B. T. Redman, I. O. Sutherland and K. Jewers, *J. Chem. Soc., Chem. Commun.*, 1969, 879–880; (*b*) P. Kumar and R. K. Baslas, *Herba Hungarica*, 1980, **19**, 81–91; (*c*) J. Gruenwald, T. Brendler and C. Jaenicke, Eds. *PDR for Herbal Medicines*, 2nd Ed, Medical Economics Co. Montvale, NJ, 2000, 325–326.

2 (*a*) B. S. Rao, *J. Chem. Soc. (C)*, 1937, 853–855; (*b*) G. Kartha, G. N. Ramachandran, H. B. Bhat, P. M. Nair, V. K. V. Raghavan and K. Venkataraman, *Tetrahedron Lett.*, 1963, **4**, 459–462; (*c*) H. B. Bhat, P. M. Nair and K. Venkataraman, *Indian J. Chem.*, 1964, **2**, 405–410; (*d*) C. G. Karanjgaonkar, P. M. Nair and K. Venkataraman, *Tetrahedron Lett.*, 1966, **7**, 687–691.

3 (*a*) S.-G. Cao, X.-H. Wu, K.-Y. Sim, B. K. H. Tan, J. T. Pereira, W. H. Wong, N. F. Hew and S. H. Goh, *Tetrahedron Lett.*, 1998, **39**, 3353–3356; (*b*) J. Wu, Y. J. Xu, X. F. Cheng, L. J. Harrison, K. Y. Sim and S. H. Goh, *Tetrahedron Lett.*, 2001, **42**, 727–729; (*c*) Y. J. Xu, S. C. Yip, S. Kosela, E. Fitri, M. Hana, S. H. Goh and K. Y. Sim, *Org. Lett.*, 2000, **2**, 3945–3948; (*d*) X.-H. Wu, B. K. H. Tan, S.-G. Cao, K. Y. Sim and S. H. Goh, *Nat. Prod. Lett.*, 2000, **14**, 453–458.

4 (*a*) W. D. Ollis, M. V. J. Ramsay, I. O. Sutherland and S. Mongkolsuk, *Tetrahedron*, 1965, **21**, 1453–1470; (*b*) G. Cardillo and L. Merlini, *Tetrahedron Lett.*, 1967, **8**, 2529–2530; (*c*) T. J. R. Weakley, S.-X. Cai, H.-Z. Zhang and J. F. W. Keana, *J. Chem. Crystallogr.*, 2001, **31**, 501–505.

5 (*a*) W. Liu, Q.-L. Guo, Q.-D. You, L. Zhao, H.-Y. Gu and S. T. Yuan, *World J. Gastroenterol.*, 2005, **11**, 3655–3659; (*b*) J. Asano, K. Chiba, M. Tada and T. Yoshii, *Phytochemistry*, 1996, **41**, 815–820; (*c*) S.-G. Gao, V. H. L. Sng, X.-H. Wu, K.-Y. Sim, B. H. K. Tan, J. T. Pereira and S. H. Goh, *Tetrahedron*, 1998, **54**, 10915–10924; (*d*) O. Thoison, J. Fahy, V. Dumontet, A. Chiaroni, C. Riche, M. V. Tri and T. Sevenet, *J. Nat. Prod.*, 2000, **63**, 441–446.

6 X. Wu, S. Cao, S. Goh, A. Hsu and B. K. H. Tan, *Planta Med.*, 2002, **68**, 198–203.

7 (*a*) Q. Guo, Q. Qi, Q. You, H. Gu, L. Zhao and Z. Wu, *Basic & Clin. Pharmacol. & Toxicol.*, 2001, **31**, 178–184; (*b*) Q. Qi, Q. You, H. Gu, L. Zhao, W. Liu, N. Lu and Q. Guo, *J. Ethnopharmacol.*, 2008, **117**, 433–438; (*c*) H. Gu, Q. You, W. Liu, Y. Yang, L. Zhao, Q. Qi, J. Zhao, J. Wang, N. Lu, H. Ling, Q. Guo and X. Wang, *Int. Immunopharmacol.*, 2008, **8**, 1493–1502.

8 (*a*) D. Zhai, C. Jin, C.-w. Shiau, S. Kitada, A. C. Satterthwait and J. C. Reed, *Mol. Cancer Ther.*, 2008, **7**, 1639–1646; (*b*) L. Zhao, Q.-L. Guo, Q.-D. You, Z.-Q. Wu, H.-Y. Gu and Biol, *Biol. Pharm. Bull.*, 2004, **27**, 998–1003.

9 (*a*) S. Kasibhatla, K. A. Jessen, S. Maliartchouk, J. Y. Wang, N. M. English, J. Drewe, L. Qiu, S. P. Archer, A. E. Ponce, N. Sirisoma, S. Jiang, H. Zhang, K. R. Gehisen, S. X. Cai, D. R. Green and B. Tseng, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 12095–12100; (*b*) E. Ortiz-Sanchez, T. R. Daniels, G. Helguera, O. Martinez-Maza, B. Bonavida and M. L. Penichet, *Leukemia*, 2009, **23**, 59–70.

10 (*a*) H.-Z. Zhang, S. Kasibhatla, Y. Wang, J. Herich, J. Guastella, B. Tseng, J. Drewe and S.-X. Cai, *Bioorg. Med. Chem.*, 2004, **12**, 309–317; (*b*) J. Kuemmerle, S. Jiang, B. Tseng, S. Kasibhatla, J. Drewe and S. X. Cai, *Bioorg. Med. Chem.*, 2008, **16**, 4233–4241; (*c*) N. G. Li, Q. D. You, X. F. Huang, J. X. Wang, Q. L. Guo, X. G. Chen, Y. Li and H. Y. Li, *Chin. Chem. Lett.*, 2007, **18**, 659–662; (*d*) J. Wang, L. Zhao, Y. Hu, Q. Guo, L. Zhang, X. Wang, N. Li and Q. You, *Eur. J. Med. Chem.*, 2009, **44**, 2611–2620.

11 (*a*) E. J. Tisdale, C. Chowdhury, B. G. Vong, H. Li and E. A. Theodorakis, *Org. Lett.*, 2002, **4**, 909–912; (*b*) E. J. Tisdale, H. Li, B. G. Vong, S. H. Kim and E. A. Theodorakis, *Org. Lett.*, 2003, **5**, 1491–1494; (*c*) E. J. Tisdale, B. G. Vong, H. Li, S. H. Kim, C. Chowdhury and E. A. Theodorakis, *Tetrahedron*, 2003, **59**, 6873–6887; (*d*) E. J. Tisdale, I. Slobodov and E. A. Theodorakis, *Org. Biomol. Chem.*, 2003, **1**, 4418–4422.

12 A. Batova, T. Lam, V. Waschol owski, A. L. Yu, A. Giannis and E. A. Theodorakis, *Org. Biomol. Chem.*, 2007, **5**, 494–500.

13 E. J. Tisdale, I. Slobodov and E. A. Theodorakis, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 12030–12035.

14 For related synthetic studies see: (*a*) K. C. Nicolaou and J. Li, *Angew. Chem., Int. Ed.*, 2001, **40**, 4264–4268; (*b*) K. C. Nicolaou, P. K. Sasmal, H. Xu, K. Namoto and A. Ritzen, *Angew. Chem., Int. Ed.*, 2003, **42**, 4225–4229; (*c*) K. C. Nicolaou, P. K. Sasmal and H. Xu, *J. Am. Chem. Soc.*, 2004, **126**, 5493–5501; (*d*) K. C. Nicolaou, H. Xu and M. Wartmann, *Angew. Chem., Int. Ed.*, 2005, **44**, 756–761.

15 T. Kamei, M. Shindo and K. Shishido, *Tetrahedron Lett.*, 2003, **44**, 8505–8507.

16 N. Le Gall, D. Luart, J.-Y. Salaun, J. Talarmin and H. Des Abbayes, *Organometallics*, 2002, **21**, 1775–1781.

17 P. A. Evans and J. D. Nelson, *Tetrahedron Lett.*, 1998, **39**, 1725–1728.

18 CCDC-737621 (**7**), CCDC-737622 (**15**), CCDC-737623 (**16**), CCDC-737624 (**26**), CCDC-737625 (**36**), CCDC-737626 (**42**) and CCDC-614936 (**43**) contain the supplementary crystallographic data for this paper.† Since these compounds have been synthesized as racemic mixtures, the X-rays establish in each case the relative stereochemistry. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

19 For relevant studies on the Claisen rearrangement see: (*a*) F. C. Gozzo, S. A. Fernandes, D. C. Rodrigues, M. N. Eberlin and A. J. Marsaioli, *J. Org. Chem.*, 2003, **68**, 5493–5499; (*b*) T. R. Pettus, X.-T. Chen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1998, **120**, 12684–12685; (*c*) T. R. Pettus, M. Inoue, X.-T. Chen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2000, **122**, 6160–6168.

20 A. E. Hayden, H. Xu, K. C. Nicolaou and K. N. Houk, *Org. Lett.*, 2006, **8**, 2989–2992.

21 V. Siddaiah, M. Maheswara, C. V. Rao, S. Venkateswarlu and G. V. Subbaraju, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1288–1290.

22 C. Paizs, U. Bartlewski-Hof and J. Petey, *Chem.–Eur. J.*, 2007, **13**, 2805–2811.

23 Q.-B. Han, S. Cheung, J. Tai, C.-F. Qiao, J.-Z. Song and H.-X. Xu, *Biol. Pharm. Bull.*, 2005, **28**, 2335–2337.

24 For selected references see: (*a*) M. M. Gottesman and I. Pastan, *Annu. Rev. Biochem.*, 1993, **62**, 385–427; (*b*) S. Simon, D. Roy and M. Schindler, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 1128–1132; (*c*) A. K. Larsen, A. E. Escargeuil and A. Skladanowski, *Pharmacol. Ther.*, 2000, **85**, 217–229.

25 For selected references on this topic see: (*a*) D. Hanahan and R. A. Weinberg, *Cell*, 2000, **100**, 57–70; (*b*) J. C. Reed and K. J. Tomaselli, *Curr. Opin. Biotechnol.*, 2000, **11**, 586–592; (*c*) S. L. Mckenna and T. G. Cotter, *Apoptosis and Cancer*, 1997, 192–221; (*d*) R. Kim, K. Tanabe, Y. Uchida, M. Emi, H. Inoue and T. Toge, *Cancer Chemother. Pharmacol.*, 2002, **50**, 343–352; K. Vermeulen, Zwi N. Berneman and D. R. Van Bockstaele, *Cell Proliferation*, 2003, **36**, 165–175.

