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The winding road of the uvaretin class of natural products: from total synthesis to bioactive agent discovery†

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Herein, we disclose the development of a synthetic route to gain access to the uvaretin class of chalcone natural products. In this, the construction of a small library was achieved, and the collection was evaluated for cytotoxicity and other biological properties. Uvaretin (**1**) was accessed via a seven-step route in an overall yield of 15.1%. Within this route, the unsaturated enone variant of uvaretin (**2**), also a natural product, was accessed in a 16.7% yield over six steps. This route provides a nearly three-fold increase in yields of **1** and **2** in comparison to the previous synthetic route accessing them in 5.8% and 3.0% overall yields, respectively. Evaluation of **1** and **2** revealed IC₅₀ values between 2.0 and 5.1 μM in the cancerous cell lines HeLa, U937, A549, and MIA PaCa-2. Screening of the whole chalcone library set led to the discovery of over 30 compounds, within six cancerous cell lines, possessing single digit μM IC₅₀ activity as sole agents. Furthermore, multiple library members were found to possess promising potentiating properties with known chemotherapeutic agents.

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1. Introduction

Chalcones, known for their classical α,β -unsaturated ketone moiety flanked by phenyl groups, are a central component to numerous natural products (Fig. 1), such as, but not limited to: butein, cardamonin, derricin, flavokawain, isosalipurposide, isoliquiritigenin, licochalcone, naringenin, xanthohumol, xanthoangelol, and the uvaretin class.¹ They have been reported to possess wide encompassing biological properties including: anticancer,^{2–7} antimalarial,^{8,9} anti-inflammatory,^{10–13} antileishmanial,^{10,13} antituberculous,¹⁴ antimetabolic,¹⁵ analgesic,¹⁶ antibacterial, and antifungal.¹⁷ There are limited reports suggesting that they are also anti-mutagenic, antitumorigenic, and gastric protectant.¹⁸ In addition to these properties, they are considered as the precursor of flavonoids and isoflavonoids, which are highly abundant in edible plants.¹⁹

It has been shown that many chalcone containing natural products and synthetic compounds elicit their biological effects through enone addition. In this, soft nucleophiles such as thiols add into the β carbon of the enone. These properties make the chalcone core biologically important, as hard nucle-

ophiles, such as amines and hydroxyl groups, both ubiquitous in biological system, will not add into chalcones. The mode of action of many chalcones arises from their interaction between multiple proteins related to cell apoptosis and proliferation. Such as, the NF- κ B^{20–22} and Nrf2/ARE^{23,24} pathways in which chalcones are reported to possess inhibitory properties within, leading to their anti-inflammatory effects and induction of phase II detoxifying enzyme expression.²⁵

Our laboratory is interested in identifying new small molecules with interesting and potent biological activity, such as anticancer. Either as sole agents or as potentiating agents to be used alongside current prescribed therapeutics. In these efforts, we aim at utilizing intermediates accessed within total synthesis campaigns, as well as failed routes, for the assembly of small screening libraries. Noting the rich literature in chalcone based compounds and their diverse biological activity, we choose to focus on constructing a synthetic route that would be amendable for library construction for uvaretin (**1**) and its analogous unsaturated enone (*E*)-1-(2,4-dihydroxy-3-(2-hydroxybenzyl)-6-methoxyphenyl)-3-phenylprop-2-en-1-one (**2**) shown in Fig. 2.

Uvaretin (**1**) was first isolated in 1976 by Wiedhopf and Bates from the chloroform extract of *Uvaria acuminata* Oliv (Annonaceae).²⁶ Evaluation of **1** revealed activity *in vivo* against P-388 lymphocytic leukemia in mice and *in vitro* activity against cells derived from human carcinoma of the nasopharynx.²⁷ In 2006, Suttisri and co-workers isolated **2**, the unsaturated enone analog of **1**, from the aerial part of

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Fig. 1 Chalcone core and the representative set of natural products containing said core highlighted in green.

Ellipeiopsis cherreensis (Annonaceae), which was found to possess cytotoxicity towards the human small-cell lung cancer, epidermoid carcinoma, and breast cancer.²⁸ The total synthesis of **1**, as well as **2** which was not isolated to date, was accomplished in 1985 by Malterud and coworkers.²⁹ Malterud's route (Scheme 1) commenced with the condensation of 2,4-dihydroxy-6-methoxyacetophenone (**3**)³⁰ and 2-hydroxyphenyl-1,1-bis-piperidinomethane (**4**)³¹ accessing **5** in 60% yield. This step highlighted their key amination approach, previously disclosed by Kallay and co-workers.^{32,33} Piperidine removal was accomplished *via* hydrogenation in a variable 50–60% yield affording **6**, the A/B ring system. The attempts to perform the aldol condensation upon **6** to access **2** was successful, albeit, in a 9% yield over 15 days. Hydrogenation of **2** afforded **1** in an overall yield of 2.2% in 4-steps from the reported starting materials. Efforts to optimize their route led to the benzyl protection of the phenolic groups, thought to have an adverse effect in the aldol condensation, giving access to the dibenzylated **7a** in 10% yield and a mixture of the monobenzylated **7b/c** in 21%. Aldol condensation upon **7a** and **7b/c** gave access to the A/B/C core in 69% and 79% yields, respectively, and hydrogenation to afford **1** in 74% and 73% yields, respectively. Through this new benzyl protection route, **1** was accessed in an overall yield of 5.8% (combined yields of **7a** and **7b/c**) over five-steps from **3** (requiring three synthetic steps to access), and **4** (requiring one synthetic step to access).

While the route developed by Malterud provided access to both **1** and **2**, it was the late-stage installation of the chalcone core that led us to develop a new route to these natural products. Given the desire to construct a new small library collection for screening to identify new compounds with biological

activity, early installation of the chalcone core seemed more likely to achieve this task. Generally, synthesized natural products and their relatively simple SAR analogs are evaluated for biological activity, which generally failed to identify new small molecules with different bioactive properties. Herein, we plan on screening all synthetic intermediates accessed in the efforts of identifying new bioactive small molecules, in which the chalcone core will prove most useful. Studies will be performed to identify new sole agent compounds, ones that have potentiating properties with known therapeutics, and/or new small molecules with interesting biological properties.

2. Results and discussion

2.1 Synthesis

Efforts towards accessing the chalcone core early rather than late stage commenced with construction of the B-ring. From phloroglucinol (**8**), the 2,4,6-trihydroxy-acetophenone (**9**) was accessed through acylation with acetic anhydride in the presence of boron trifluoride etherate in 88% yield (Scheme 2).³⁴ Treatment of **9** with dimethyl sulfate in the presence of potassium carbonate in acetone afforded trimethoxyacetophenone (**10**) in 89% yield. Accessing **11** was accomplished through a potassium hydroxide catalyzed aldol condensation between **10** and benzaldehyde in 81% yield.¹⁹

Attention was then directed towards the installation of the western *ortho*-hydroxybenzyl moiety (A-ring) *via* a Friedel-Craft alkylation approach. Construction of the requisite material commenced with the allylation of *ortho*-hydroxybenzaldehyde (**12**) with allyl bromide and potassium carbonate in dimethylformamide to access **13** in 95% yield,³⁵ which was then reduced with sodium borohydride in methanol to give access to the desired, protected benzyl alcohol (**14**) in 72% yield (Scheme 3).^{36–38}

Next, efforts were directed towards the incorporation of **14** onto **11**. The Lewis acid mediated reaction between **11** and **14** proved to be more problematic than originally anticipated (Scheme 4). Please see the ESI† for a detail table of all conditions attempted. Two different Lewis acids were explored, boron trifluoride etherate and ferric(III) chloride, in various solvents, temperatures, and orders of addition. It was originally thought that the use of an excess Lewis acid would facilitate

Fig. 2 Chalcone based natural products uravetin (**1**) and its unsaturated enone analog (**2**).

Scheme 1 Malterud's total synthesis route to uvaretin.

the formation of the benzyl carbocation of **14**. This benzyl carbocation would subsequently be attacked by **11** in a Friedel–Craft alkylation mechanism to afford **15** in acceptable yields. However, the formation of **16** was observed in high yields. Finally, it was found through utilizing the step-wise addition of boron trifluoride etherate in dioxane in equal portions over a 10 minute period to a vigorously stirring solution of **11** and **14** in dioxane, followed by heating at 60 °C for 3 h led to a minimized yield of **16**. The formation of the uvaretin carbon core **15** was established in a 58% yield.

Noting the importance of this alkylation step, efforts were taken to optimize the reaction through modifications of the benzyl alcohol. It was speculated that the Lewis acid activation of the benzyl alcohol tended to accelerate the formation of **16**. To suppress this, mesylate protection of the alcohol was performed with mesyl chloride to afford **17** in 88% yield (Scheme 5). While this material was successfully transformed onto **15**, all conditions attempted failed to provide yields greater than 17%. Transforming the benzyl alcohol into its chloride counterpart (**18**) was also performed in 92% yield, but failed to give **15** in yields above 20%. The addition of sodium iodide to this reaction failed to provide any benefits in the formation of **15**.

With the core skeleton of the uvaretin family in hand, the removal of the allyl group and enone reduction were undertaken to validate the route as a feasible means of accessing the carbon core of the uvaretin family of natural products. Subjecting **15** to $\text{Pd}(\text{PPh}_3)_4$ and potassium carbonate in methanol successfully deprotected the allyl group upon the western phenol to give access to **19** in 76% yield (Scheme 6). Reduction of the enone was accomplished with H_2 and 10% Pd/C in methanol to the corresponding ketone **20** in 82% yield.

With **20** in hand, the general route to the uvaretin core has been established, allowing for the next steps in the construction of these natural products.

Acquiring both **19** and **20** led to efforts to explore if demethylation upon the B-ring could be performed giving access to both **2** and uvaretin directly. Demethylation of methoxy arenes is well preceded in the literature.^{29,39–42} Illustrated within the insert of Scheme 6 highlights the use of aluminum chloride in the demethylation of **10** accessing the di- and monomethylated acetophenone species. Employing the same conditions upon **20** did afford uvaretin, albeit, in a 3% yield along with decomposed material. Furthermore, the reaction did not consistently afford **1**, and in most cases gave decomposition only. Also, in multiple cases the debenzylated chalcone **21** was isolated, which also had a single methyl group removed. The formation of **21** was not consistently obtained; however, when it was obtained, the yields were less than 33%. Attempting the same upon **19** afforded decomposition, however, one attempt did provide a <1% yield of **2**. Similar to **20**, debenzylation of the A-ring was observed affording **21** in yields no greater than 15%. It was suspected that the free phenol could be affecting the demethylation, as such demethylation with AlCl_3 was attempted upon **15**. Unfortunately, only decomposition of the material was observed. The formation of **21** was not observed from **15**, unlike from **19** and **20**.

While both desired natural products, **1** and **2**, were accessed in route described, we were unsatisfied with the yields in the final step and decided to pursue demethylation before the alkylation step. Treating chalcone **11** with aluminum chloride in chlorobenzene afforded the dimethylated chalcone **21** in 68% yield (Scheme 7). Concurrently, it was found that selective methylation, under equivalent control, of **9** gave access to **22** in 86% yield. **22** was then subjected to potassium hydroxide mediated aldol conditions to afford **21** in 99% yield, overall yield of 85% over two-steps. Subjecting **21** to the alkylation conditions employed in Scheme 3, as well as

Scheme 2 Synthetic route to obtain the trimethylated chalone core.

Scheme 3 Constructed the required O-allyl benzyl alcohol for acylation attempts.

Scheme 4 Friedel-Crafts alkylation conditions gaining access to the uvaretin carbon core.

the other conditions presented in the ESI,[†] failed to afford 23. The isolation of the *O*-alkylated compound was observed in numerous trials. As such, the free phenolic group within the B-ring was suspected to be the cause of this failed alkylation. Acetylation of this phenol was attempted, followed by alkylation conditions to afford <5% of 23. Under these conditions, deacetylation occurred rapidly which prevented the desired alkylation. Protection of the phenol with a more robust protecting group was then explored.

Methoxymethyl ether (MOM) protecting group was chosen to protect the free phenol given the strong literature precedence for its application in similar systems.^{1,43–46} Subjecting 22 to MOMCl and potassium carbonate in acetone afforded the desired protected material 24 in 85% yield, which was elaborated onto its corresponding chalcone *via* the standard protocol to access 25 in 68% yield (Scheme 8). Employing the same alkylation conditions outlined in Scheme 4, and all other alkylation techniques outlined in the ESI,[†] failed to afford the desired alkylated material, but rather massive decomposition and the recovery of 25. However, it was found by treating 25 to alkylation conditions followed by immediate removal of the allyl group, with a short silica plug used in between, gave access to the desired material in 39%. Removal of the MOM groups was performed under standard conditions with methanolic HCl to access 26 in 66% yield.¹ Based upon these findings, we decided to explore if the triphenol analog of 26 could be accessed. The full MOM protection of 9 was accomplished under sodium hydride deprotonation followed by MOMCl addition to give access to 27 in 83% yield, which was elaborated onto 28 in 94% yield. However, all attempts at alkylating this material failed, including the sequential allyl deprotection. Curious as to whether the presence of three MOM groups could possibly shut down the alkylation due to chelation of the Lewis acid, 9 was transformed into its di-MOM protected 29 in 79% yield. Methylation of the free phenol with dimethyl sulfate proceeded in an 91% yield that was subsequently subjected to the aldol condensation to afford 30 in 80% yield, 73% yield over two-steps. We were pleasantly surprised to observe

the successful alkylation under standard condition, with immediately allyl deprotection. Removal of the MOM groups gave access to 2 in a 33% yield over two-steps, for an overall yield of 16.7% over six-synthetic steps. Hydrogenation of 2 gave access to uvaretin in 90% yield, with an overall yield of 15.1% over seven-synthetic steps. Subjecting 29 to aldol conditions gave rise to chalcone 31. However, subjecting 31 to multiple alkylation conditions, followed by the conditions used to access 2, failed to afford the desired material.

2.2 Biology

2.2.1 Cytotoxicity evaluation of uvaretin, 2, and compounds accessed. After accessing both natural products as well as a collection of small molecules based upon the chalcone core, we pursued biological evaluation of this library set. Initially, the collection was assessed in the cervical cancerous cell line HeLa to probe if the activity warrants the expansion to screen other cells lines. Cellular viability was assessed *via* Alamar Blue quantification,^{47–49} measure of alive cells as a function of electron transport activity. Summarized in Table 1 are the IC_{50} values, concentration required to inhibit cell proliferation by 50% of the control (Table S2[†] provides all structures and their corresponding IC_{50} values). Several of the compounds screened showed promising cytotoxicity, such as 10, 19, 26, and uvaretin. The low μM IC_{50} 's observed with these four compounds, in addition to the large number of high single and low double digits values promoted us to expand our evaluation. The small library was then assessed in five other cancerous cell lines: lymphoma (U937), lung (A549), pancreatic (MIA PaCa-2), acute lymphocytic leukemia (Reh), and colon (HCT-116). A large percentage of compounds were observed to have promising activity in the U937, A549, and MIA PaCa-2 cell lines. However, there was limited activity within the Reh cell line ranging from 9.2 to 19.4 μM . The activity within HCT-116 was very limited, with only two possessing activities below the 20 μM threshold.

2.2.2 Identification of small molecule potentiators. There has been much effort placed in the discovery of small molecules that act as positive potentiators; a compound that increases the efficacy of a drug without increasing the dosage of the drug. The discovery of such potentiators, when used in parallel with known therapeutics, allow for lower dosages to be administered while retaining the same therapeutic profile. The benefit of this strategy is that the toxicities of the drug are decreased as the drug dosage is lowered. The discovery of positive potentiators is an arduous task. Most commonly, this

Scheme 5 Attempted optimization of the Friedel Crafts alkylation through employing mesylate and chloride benzyl systems.

Assessing the synergist properties of 28, 30, and 31 with 6TP was performed in a matrix format, consisting of a 3×5 matrix. In this, two concentrations of the chalcone were employed that would induce 10 to 20% cell death within a 72 hour period. Four concentrations of 6TP were chosen so that 50% cell death would be observed within the same time period. The combinational effects on cell death is shown in Fig. 3 (within the red tables). There was no significant change in cell death at various concentrations of 28 and 6TP, with

Table 1 Evaluation of natural products uvaetin (1) and 2, and the chalcone small library set for cytotoxicity

Cmpd	Observed IC ₅₀ ^a (μM)					
	HeLa	U937	A549	MIA PaCa-2	Reh	HCT-116
9	>20	>20	>20	>20	>20	>20
10	3.8 ± 1.0	7.2 ± 0.9	9.8 ± 1.3	14.3 ± 1.0	>20	>20
11	5.2 ± 1.4	6.7 ± 0.8	4.3 ± 0.9	>20	>20	>20
15	5.8 ± 0.5	7.5 ± 1.1	8.5 ± 0.6	9.4 ± 1.3	12.6 ± 2.3	>20
19	2.7 ± 0.6	4.2 ± 1.4	9.3 ± 1.1	4.1 ± 0.3	>20	18.6 ± 2.5
20	>20	>20	>20	>20	>20	>20
21	>20	>20	19.7 ± 2.2	18.2 ± 1.7	>20	>20
22	18.7 ± 3.1	>20	>20	>20	>20	>20
24	14.0 ± 1.0	13.3 ± 2.1	16.9 ± 1.6	>20	>20	>20
25	4.8 ± 0.6	2.5 ± 0.5	3.5 ± 0.8	5.6 ± 0.7	19.4 ± 3.2	>20
26	3.1 ± 0.5	6.8 ± 1.7	5.8 ± 1.8	>20	9.7 ± 2.6	>20
27	>20	11.7 ± 1.3	>20	6.1 ± 1.6	>20	>20
28	11.5 ± 1.1	8.1 ± 2.0	8.4 ± 1.3	11.8 ± 2.6	>20	>20
29	12.2 ± 0.9	7.4 ± 0.8	>20	>20	>20	>20
30	9.7 ± 1.3	5.9 ± 1.1	4.2 ± 1.9	2.2 ± 0.6	9.2 ± 0.9	>20
31	16.7 ± 0.6	>20	>20	7.8 ± 0.4	>20	>20
2	4.6 ± 1.4	2.3 ± 1.0	4.3 ± 2.1	5.1 ± 1.8	>20	19.5 ± 3.1
Uvaetin (1)	3.7 ± 0.8	3.6 ± 1.1	2.2 ± 1.0	2.0 ± 1.1	11.3 ± 1.5	>20

^a Cytotoxicity was evaluated in 384-well plates over a 72 h treatment. Adherent cells were seeded at 1500 cells per well, whereas suspension cells at 2200 cells per well. Cellular viability was evaluated *via* Alamar Blue. Doxorubicin was used as the positive cell death control, and wells with only cells as the alive control.

Fig. 3 Evaluation of chalcones 28, 30, and 31 potentiation properties with 6-thiopurine in the cancerous cells line Mia PaCa-2 (pancreatic) and Reh (acute lymphocytic leukemia). Potentiation performed in a 3 × 5 matrix, and synergy assessed (*n* = biological replicates).

percentages remaining between 14–21%. While an overall increase in cell death was observed with chalcone 31, the overall change in cell death did not exceed the cellular death observed by 6TP alone. There was a noted increase in cellular death in the combination of 30 and 6TP, resulting in a 100% increase in death as compared to 6TP alone. The effects of these potentiating studies were assessed by determination of their combination index (CI), a value determined through employing the Chou–Talalay protocol.⁵⁰ CI values closer to 0 represented strong synergism, 1 an additive effect, and values greater than 1 denote antagonism in this method. CI values for 28 and 31 show mild synergism to antagonism and strong antagonism, respectively. However, 30 was shown to possess a CI value of 0.19, at the higher concentrations of both compounds, corresponding to strong synergism. 6TP is not prescribed to treat pancreatic cancer, as such, the syner-

gism observed between 30 and 6TP within Mia PaCa-2 effectively illustrates the importance in full biological evaluation of all small molecules accessed within total synthesis routes. To our surprise, similar strong synergism between 30 and 6TP was not observed within the acute lymphocytic leukemia cancerous cell line Reh, in which 6TP is prescribed for. This illustrates the difficulty in discovering positive potentiating agents for specific drugs in varying cell lines.

With the discovery of 30 possessing strong synergy with 6TP in a cell line that it is not currently prescribed in, we decided to expand our small molecule potentiation screens. Noting that these investigations are both time and financially intensive, we limited further studies to the two natural products (1 and 2) accessed in this work and their trimethoxy (B-ring) analogs 20 and 19, respectively. Employing the same procedures previously described, 6TP potentiation studies

outlined in Fig. 2, these four compounds were evaluated within the same cell lines used in our general IC₅₀ screens (shown in Table 1) towards doxorubicin, etoposide, paclitaxel, and 6TP. Fig. 4 illustrates the median CI index value obtained from each potentiating screen for each of the four compounds with the four cytotoxic agents in five cell lines.

Uvaretin (**1**), possessing the most potent cytotoxicity as a sole agent, showed mild synergist to additive effects in HeLa, U937, A549, and Reh. Within HCT-116, uvaretin was shown to possess antagonism properties with doxorubicin, 6-thiopurine, and etoposide (greater to lower values). A similar pattern of potentiation was observed with **2**, thus showing that these two natural products fail to give significant synergistic properties. Interestingly, it was found that **20**, the trimethoxy analog of uvaretin, while possessing no IC₅₀ values below 20 μ M possesses greater synergism with all four drugs. Most notably, **20** was found to have a CI value of 0.11 with doxorubicin in HeLa, and 0.22 with 6-thiopurine in U937. In contrast, uvaretin possesses a low single μ M IC₅₀ sole agent, and mild synergy to additive effects. It was also shown that low synergism was observed with **20** in combination with doxorubicin, paclitaxel, and 6-thiopurine. A property not observed with either natural product. Evaluation of **19**, the trimethoxy analog of **2**, also showed an increase in overall combinational (additive) and synergistic properties, with a median CI value of 0.12 between **19** and doxorubicin within HeLa. Low synergistic effects were observed in HCT-116 between **19** and etoposide, paclitaxel and 6-thiopurine.

3. Conclusion

In summary, the total syntheses of the natural products uvaretin (**1**) and its unsaturated enone analog (**2**) has been accomplished in 15.1% over seven steps and 16.7% over six steps, respectively. While the route delineated gains access to both natural products, in this body of work, the yields are 3–

5.5 folds greater than the route developed by Malterud. However, the previous route does gain access to **1** and **2** in 1–2 shorter synthetic steps, but, reported starting materials were pre-synthesized and not disclosed in the original manuscript. The route developed, highlighting an early stage chalcone core installation, allowed for the construction of a small chalcone library that was evaluated for sole agent cytotoxicity and select members further investigated for potentiating properties with known chemotherapeutics. Natural products **1** and **2** were found to have low single μ M IC₅₀ values in HeLa, U937, A549 and MIA PaCa-2 cancerous cell lines and moderate double digit μ M in Reh and HCT-116. Also, 31 other compounds from this library were found to possess single digit μ M IC₅₀ activity as sole agents. Chalcone **30** was shown to possess moderate synergy with 6TP in the pancreatic cancer cell line MIA PaCa-2. Compound **19** and **20**, analogs of **2** and **1**, respectively, were observed to have promising potentiating properties with doxorubicin, etoposide, paclitaxel, and 6TP. Further investigations are needed to explore the mode of action for this potentiation, as well as the evaluation of the other library members.

4. Experimental Section

4.1 Chemistry

All reagents were commercially available and used without purification unless otherwise stated. NMR spectra were recorded with a Varian 400 MHz instrument. The chemical shifts are given in parts per million (ppm) relative to residual CHCl₃ at δ 7.26 ppm or DMSO at δ 2.50 ppm for proton spectra and relative to CDCl₃ at δ 77.23 ppm or DMSO at δ 39.52 ppm for carbon spectra, unless otherwise noted. Low-resolution mass spectra were obtained using a Waters Xevo-TQD *via* direct injection; samples were dissolved in methanol, filtered, and the supernatant injected. Flash column chromatography was performed with silica gel grade 60 (230–400 mesh). Dichloromethane (CH₂Cl₂), methanol (CH₃OH), and *N,N*-dimethylformamide (DMF) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves. All commercially available reagents were used as received. All procedures including anhydrous solvents were performed with rigorously dried glassware under inert atmosphere.

4.1.1 Aldol condensation general procedure. To a round bottom flask (RBF) was added the acetophenone species (1.0 eq.), benzaldehyde (1.0 eq.), and EtOH (20 mL) and brought to 0 °C. Once at temperature, a solution of KOH (17 eq.) in water (25 mM final concentration) was added dropwise, after which the mixture was brought to 25 °C and left to stir for 14 h. The reaction was diluted with EtOAc, washed with water, acidified with 1 M HCl, and extracted with EtOAc ($\times$ 2). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated to afford the crude material. Specific purification protocols are given with each specific reaction.

4.1.2 General alkylation procedure. To a flamed dried RBF under an argon atmosphere was added chalcone (1.0 eq.) and

Fig. 4 Median CI values for each chalcone/chemotherapeutic combination for each cell line. CI values <1 are synergistic, lower values representative of higher levels of synergy. CI values of 1 denote additive effects of the two compounds, and values >1 antagonistic effects (n = three biological replicates).

dry dioxane (final concentration of 46 mM) and stirred at RT. In a separate flamed dried RBF under an argon atmosphere was added $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (3.05 eq.) and dioxane (final concentration of 0.56 M), this solution was added to the previously prepared RBF in four portions 10 min apart. The reaction was then brought to 60 °C and stirred for 3 h. The reaction was cooled, diluted with EtOAc, washed with water ($\times 2$), and extracted with EtOAc ($\times 2$). The combined organic layers were combined, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Specific purification protocols are given with each specific reaction.

4.1.3 General allyl deprotection conditions. To a flamed dried RBF under an argon atmosphere was added chalcone species (1.0 eq.), K_2CO_3 (6 eq.), and $\text{Pd}(\text{PPh}_3)_4$ (0.02 eq.) in MeOH (final concentration relative to chalcone 16 mM) and allowed to stir at reflux for 2 h. The reaction mixture was filtered through a short silica plug, rinsed with MeOH (1 vol. eq. to initial MeOH), and concentrated to afford the desired product.

4.1.4 General enone reduction procedure. To a flamed dried RBF under an argon atmosphere was added the enone material (1.0 eq.) and 10% palladium over carbon in MeOH (2.3 M final concentration relative to the enone). A hydrogen balloon was introduced and the mixture was left to stir for 25 °C for 2 h, filtered through Celite, and rinsed with MeOH or CHCl_3 . The filtrate was concentration to afford the ketone species.

4.1.5 2,4,6-Trihydroxyacetophenone (9).⁵¹ To a RBF under an argon atmosphere was added $\text{BF}_3 \cdot \text{OEt}_2$ (7.5 mL, 59.4 mmol) and acetic anhydride (1.9 mL, 19.8 mmol) followed by phloroglucinol (2.5 g, 19.8 mmol) and allowed stirred at room temperature (RT). After 15 h, the reaction mixture was poured onto a 10% NaOAc solution (70 mL) and left to stir overnight (O/N) under ambient atmosphere. The precipitate was filtered off and washed with water to obtain **9** (2.9 g) in 88% yield. ^1H NMR (400 MHz, methanol- d_4) δ 5.79 (s, 2H), 2.59 (m, 3H). ^{13}C NMR (101 MHz, methanol- d_4) δ 204.56, 166.32, 165.90, 105.60, 95.59, 32.71.

4.1.6 2,4,6-Trimethoxyacetophenone (10).^{52,53} To a flamed RBF under an argon atmosphere was added **9** (0.5 g, 2.98 mmol), K_2CO_3 (2.0 g, 14.9 mmol), and anhydrous acetone (10 mL). The reaction was allowed to stir at 25 °C for 15 h, and then brought to reflux for an additional 4 h. The reaction was quenched by the addition of saturated ammonium chloride and extracted with EtOAc ($\times 3$). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude material was purified *via* flash silica gel chromatograph (1:1, Hex:EtOAc) to afford the **10** (556 mg) in 89% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 6.10 (s, 2H), 3.82 (s, 3H), 3.79 (s, 6H), 2.46 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 202.14, 162.54, 158.57, 113.78, 90.67, 56.04, 55.66, 32.81.

4.1.7 (E)-3-Phenyl-1-(2,4,6-trimethoxyphenyl)prop-2-en-1-one (11).²⁵ The aldol condensation general procedure was followed using **10** (0.62 g, 2.95 mmol) and 0.31 mL of benzaldehyde. The crude material was purified by flash silica gel

chromatography (2:1, Hex:EtOAc) to give **11** (0.7 g, yellow crystals) in 80% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.54–7.52 (m, 2H), 7.39–7.35 (m, 5H), 6.97 (d, 1H, J = 12 Hz), 3.87 (s, 3H), 3.78 (s, 6H). ^{13}C NMR (DMSO, 101 MHz) δ (ppm): 193.23, 161.94, 158.06, 143.50, 134.34, 130.44, 128.99, 128.94, 128.45, 110.98, 91.07, 55.80, 55.45. LRMS (ESI): m/z calcd for $\text{C}_{18}\text{H}_{19}\text{O}_4^+$: $[\text{M} + \text{H}]^+$ 299.12, found 299.12.

4.1.8 2-(Allyloxy)benzaldehyde (13). To a flamed dried RBF under an argon atmosphere was added salicylaldehyde (**12**, 10 g, 82.0 mmol), K_2CO_3 (17 g, 123.0 mmol) and DMF (30 mL) and left to stir. Once homogenous allyl bromide (10 mL, 123.0 mmol) was added and the reaction was left to stir at 25 °C for 2 h. The reaction mixture was diluted with EtOAc, washed with saturated ammonium chloride, water ($\times 4$), and brine. The organic layers were dried over sodium sulfate and concentrated under reduced pressure to furnish **13** (12 g, colorless oil) in 90% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 10.54 (s, 1H), 7.84 (dd, J = 7.7, 1.8 Hz, 1H), 7.53 (ddd, J = 8.9, 7.4, 1.9 Hz, 1H), 7.06–6.95 (m, 2H), 6.08 (ddt, J = 17.1, 10.4, 5.1 Hz, 1H), 5.45 (dd, J = 17.3, 1.6 Hz, 1H), 5.34 (dd, J = 10.6, 1.4 Hz, 1H), 4.66 (dt, J = 5.2, 1.6 Hz, 2H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 189.96, 161.15, 136.04, 132.60, 128.66, 125.33, 121.08, 118.29, 113.07, 69.40. LRMS (ESI): m/z calcd for $\text{C}_{10}\text{H}_{10}\text{O}_2\text{Na}^+$: $[\text{M} + \text{Na}]^+$ 185.06, found 185.06.

4.1.9 2-(Allyloxy)benzylalcohol (14). To a flamed dried RBF under an argon atmosphere was added **13** (12 g, 73.8 mmol) and methanol (100 mL), which was stirred and cooled to 0 °C. Once at temperature sodium borohydride (5.6 g, 147.6 mmol) was added in three equal portions over 10 min, then left to stir at 0 °C for 1 h. The reaction mixture was diluted with EtOAc, quenched with the addition of 1 M HCl, stirred for 2 h, and extracted with EtOAc ($\times 2$). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude material was purified *via* flash silica gel chromatography to afford **14** (10 g, colorless oil) in 83% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.32 (dd, J = 7.4, 1.6 Hz, 1H), 7.29–7.22 (m, 1H), 6.96 (dd, J = 8.1, 6.8 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.13–6.01 (m, 1H), 5.43 (dd, J = 17.3, 1.6 Hz, 1H), 5.30 (dd, J = 10.5, 1.5 Hz, 1H), 4.72 (s, 2H), 4.59–4.51 (m, 2H), 2.84 (bs, 1H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 156.48, 133.38, 129.78, 128.90, 128.81, 121.08, 117.73, 111.68, 68.94, 61.83. LRMS (ESI): m/z calcd for $\text{C}_{10}\text{H}_{13}\text{O}_2^+$: $[\text{M} + \text{H}]^+$ 165.09, found 165.09.

4.1.10 (E)-1-(3-(2-(Allyloxy)benzyl)-2,4,6-trimethoxyphenyl)-3-phenylprop-2-en-1-one (15). The general alkylation procedure was followed using **11** (0.11 g, 0.37 mmol) and **14** (72 mg, 0.44 mmol). The crude material was purified by flash silica gel chromatography (2:1, Hex:EtOAc) to give **15** (52 mg, light yellow solid) in 58% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.55–7.50 (m, 2H), 7.43–7.39 (m, 1H), 7.38–7.35 (m, 2H), 7.12 (ddd, J = 8.8, 6.9, 2.2 Hz, 1H), 7.03 (d, J = 16.0 Hz, 1H), 6.86–6.77 (m, 3H), 6.38 (s, 1H), 6.12 (ddt, J = 17.3, 10.2, 5.0 Hz, 2H), 5.48 (dq, J = 17.3, 1.8 Hz, 1H), 5.28 (dq, J = 10.6, 1.6 Hz, 1H), 4.61 (dq, J = 5.3, 1.8 Hz, 2H), 4.01 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.52 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz)

δ (ppm): 194.78, 160.84, 158.24, 157.28, 156.46, 144.83, 135.11, 133.95, 130.51, 130.08, 129.02, 128.97, 128.70, 128.29, 126.67, 120.62, 117.03, 116.70, 114.48, 111.42, 91.72, 69.00, 63.07, 56.26, 56.04, 22.98. LRMS (ESI): m/z calcd for $C_{28}H_{29}O_5^+$: $[M + H]^+$ 445.20, found 445.20.

4.1.11 (*E*)-1-(3-(2-Hydroxybenzyl)-2,4,6-trimethoxyphenyl)-3-phenylprop-2-en-1-one (19). The allyl deprotection procedure was followed using 15 (29 mg, 0.065 mmol). The product 19 (20 mg, white solid) was obtained in 76% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.52 (dd, $J = 6.7, 3.0$ Hz, 2H), 7.41–7.33 (m, 6H), 7.11 (td, $J = 8.4, 7.9, 1.7$ Hz, 1H), 6.98 (d, $J = 16.1$ Hz, 1H), 6.87–6.80 (m, 2H), 6.33 (s, 1H), 3.95 (s, 3H), 3.86 (s, 2H), 3.84 (s, 3H), 3.78 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 194.24, 159.71, 157.47, 155.92, 155.01, 145.76, 134.91, 131.87, 130.71, 129.07, 128.79, 128.50, 128.10, 125.90, 120.06, 116.52, 116.36, 114.16, 92.38, 63.86, 56.33, 56.15, 24.58 LRMS (ESI): m/z calcd for $C_{25}H_{25}O_5^+$: $[M + H]^+$ 405.17, found 405.17.

4.1.12 1-(3-(2-Hydroxybenzyl)-2,4,6-trimethoxyphenyl)-3-phenylpropan-1-one (20). The enone reduction procedure was followed using 19 (3 mg, 0.007 mmol) and 10% Pd/C (1 mg) to afford 20 (3 mg) in quantitative yields. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.34 (dd, $J = 7.5, 1.7$ Hz, 1H), 7.28–7.15 (m, 6H), 7.11–7.07 (m, 1H), 6.85–6.78 (m, 2H), 6.25 (s, 1H), 3.90 (s, 3H), 3.80 (s, 2H), 3.76 (s, 3H), 3.74 (s, 3H), 3.09 (t, $J = 7.5$ Hz, 2H), 3.00 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 203.58, 159.61, 156.73, 155.19, 154.90, 141.38, 131.77, 128.74, 128.53, 128.12, 126.11, 125.81, 120.08, 118.42, 116.53, 114.35, 92.31, 64.38, 56.12, 46.51, 30.04, 24.45. LRMS (ESI): m/z calcd for $C_{25}H_{27}O_5^+$: $[M + H]^+$ 407.18, found 407.18.

4.1.13 (*E*)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one (21). **De-methylation approach.** To a flamed dried RBF was added 11 (0.129 mg, 0.40 mmol) and PhCl (4 mL) followed by $AlCl_3$ (0.11 g, 0.8 mmol) and left to stir O/N at RT. The reaction was then brought to 70 °C and allowed to stir for an additional 24 h. The reaction was quenched with saturated Rochelle salt and stirred for 30 min, and then extracted with EtOAc ($\times 3$). The organic layers were combined, dried over sodium sulfate, and concentrated under reduce pressure. The crude material was purified *via* flash silica gel chromatography (7 : 3, Hex:EtOAc) to afford 21 (77 mg) with inconsistent yields. **Aldol approach.** The aldol condensation general procedure was followed using 22 (0.4 g, 2.0 mmol) and 0.24 mL of benzaldehyde. The crude material was purified by flash silica gel chromatography (7 : 3, Hex:EtOAc) to give 21 (0.7 g, yellow crystals) in 99% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.88 (d, $J = 15.6$ Hz, 1H), 7.76 (d, $J = 15.5$ Hz, 1H), 7.62–7.60 (m, 2H), 7.42–7.38 (m, 3H), 6.11 (d, $J = 2.4$ Hz, 1H), 5.97 (d, $J = 2.4$ Hz, 1H), 3.92 (s, 3H), 3.84 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.83, 168.64, 166.48, 162.74, 142.50, 135.79, 130.28, 129.10, 128.57, 127.76, 106.54, 94.05, 91.47, 56.06, 55.79. LRMS (ESI): m/z calcd for $C_{17}H_{17}O_4^+$: $[M + H]^+$ 285.11, found 285.11.

4.1.14 1-(2-Hydroxy-4,6-dimethoxyphenyl)ethan-1-one (22). To a flamed dried RBF under an argon atmosphere was added 9 (0.5 g, 2.98 mmol), K_2CO_3 (2 g, 14.9 mmol), Me_2SO_4

(0.85 mL, 8.94 mmol) in anhydrous acetone (10 mL) and stirred at 25 °C for 15 h. The reaction was quenched with the addition of saturated ammonium chloride and extracted with EtOAc ($\times 3$). The organic layers were combined, washed with brine, dried over sodium sulfate, and concentrated under reduce pressure. The crude material was purified *via* flash silica gel chromatography (2 : 1, Hex:EtOAc) to afford 22 (501 mg) in 86% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.05 (d, $J = 1.6$ Hz, 1H), 5.91 (d, $J = 2.4$ Hz, 1H), 3.85 (d, $J = 0.7$ Hz, 3H), 3.81 (d, $J = 0.7$ Hz, 3H), 2.60 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 203.34, 167.80, 166.29, 163.12, 104.99, 93.70, 90.94, 55.75, 33.13. LRMS (ESI): m/z calcd for $C_{10}H_{13}O_4^+$: $[M + H]^+$ 197.08, found 197.08.

4.1.15 1-(2,4-Dimethoxy-6-(methoxymethoxy)phenyl)ethan-1-one (24). To a RBF was added 22 (0.58 g, 2.95 mmol) to acetone (10 mL) followed by MOMCl (0.68 mL, 8.84 mmol) and K_2CO_3 (2.0 g, 14.8 mmol). The reaction was brought to reflux for 4 h, quenched with saturated ammonium chloride, and extracted with EtOAc ($\times 2$). The organic layers were combined, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified *via* flash silica gel chromatography (7 : 3, Hex:EtOAc) to afford 24 (0.6 g, pale-yellow oil) in 58% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.32 (s, 1H), 6.14 (s, 1H), 5.14 (s, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 3.46 (s, 3H), 2.46 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 201.79, 162.34, 158.28, 155.95, 115.10, 95.06, 93.57, 92.47, 56.50, 56.01, 55.68, 32.77. LRMS (ESI): m/z calcd for $C_{12}H_{17}O_5^+$: $[M + H]^+$ 241.10, found 241.10.

4.1.16 (*E*)-1-(2,4-Dimethoxy-6-(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (25). The aldol condensation general procedure was followed using 24 (0.4 g, 1.66 mmol) and 0.19 mL of benzaldehyde. The crude material was purified by flash silica gel chromatography (7 : 3, Hex:EtOAc) to give 25 (372 mg, pale-yellow solid) in 68% yield. 1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.53–7.50 (m, 2H), 7.36–7.34 (m, 4H), 6.97 (d, 1H, $J = 12$ Hz), 6.39 (s, 1H), 6.22 (s, 1H), 5.12 (s, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.39 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 194.52, 162.48, 158.40, 156.45, 144.71, 135.15, 130.49, 129.29, 129.08, 128.56, 113.00, 94.87, 93.59, 92.58, 56.47, 56.11, 55.73. LRMS (ESI): m/z calcd for $C_{19}H_{21}O_5^+$: $[M + H]^+$ 329.13, found 329.13.

4.1.17 (*E*)-1-(6-Hydroxy-5-(2-hydroxybenzyl)-2,4-dimethoxyphenyl)-3-phenylprop-2-en-1-one (26). The general acylation procedure was followed using 25 (310 mg, 1.09 mmol) and 14 (215 mg, 1.31 mmol). The crude material was not columned, but rather subjected to the allyl deprotection procedure to afford the carbon core of the uvaretin family (25b, 183 mg) in 39% yield. The material was dissolved into MeOH (final concentration 0.05 M) and a 10% HCl solution (1/2 vol. eq. of the MeOH) was added.¹ The solution was refluxed for 30 min, the mixture poured onto cold water (2 vol. eq. to MeOH) and extracted with EtOAc. The organic layers were combined, dried over sodium sulfate, and concentrated under reduce pressure. The crude material was purified *via* flash silica gel chromatography (1 : 1, Hex:EtOAc) to afford 26 in 66% yield.

^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.92–7.79 (m, 4H), 7.66–7.58 (m, 3H), 7.42 (dt, J = 5.2, 2.9 Hz, 4H), 6.87 (dd, J = 8.1, 1.3 Hz, 1H), 6.81 (td, J = 7.4, 1.3 Hz, 1H), 6.04 (s, 1H), 3.99 (s, 3H), 3.96 (s, 3H), 3.89 (s, 2H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 193.38, 163.90, 163.02, 162.14, 155.07, 143.38, 135.62, 131.91, 130.48, 129.12, 128.67, 127.93, 127.37, 126.47, 119.96, 116.71, 109.29, 106.39, 87.43, 56.18, 55.96, 23.35. LRMS (ESI): m/z calcd for $\text{C}_{24}\text{H}_{23}\text{O}_5^+$: $[\text{M} + \text{H}]^+$ 391.15, found 391.15.

4.1.18 1-(2,4,6-Tris(methoxymethoxy)phenyl)ethan-1-one (27). To a flamed dried RBF under an argon atmosphere was added **9** (0.5 g, 2.97 mmol) in DMF (15 mL) and cooled to 0 °C. NaH (60% dispersion, 0.53 g, 13.3 mmol) was then added slowly and allowed to stir for 5 min. MOMCl (1.01 mL, 13.3 mmol) was added dropwise over 15 min. The reaction was stirred, while being warmed to RT, for 30 min, quenched with water, and then extracted with EtOAc ($\times 2$). The organic layers were combined, washed with water and brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (7 : 3, Hex:EtOAc) to give **27** (0.7 g) in 83% yield. ^1H NMR (CDCl_3 , 400 MHz) δ 6.50 (s, 2H), 5.13 (s, 2H), 5.13 (s, 4H), 3.46 (s, 3H), 3.45 (s, 6H), 2.48 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ 201.65, 159.68, 155.41, 117.16, 97.34, 94.99, 94.69, 56.55, 56.44, 56.34, 32.76. LRMS (ESI): m/z for $\text{C}_{14}\text{H}_{20}\text{O}_7\text{Na}^+$: $[\text{M} + \text{Na}]^+$ 323.11; found 323.11.

4.1.19 (E)-3-Phenyl-1-(2,4,6-tris(methoxymethoxy)phenyl)prop-2-en-1-one (28). The aldol condensation general procedure was followed using **27** (170 mg, 0.5 mmol) and 0.05 mL of benzaldehyde. The crude material was purified by flash silica gel chromatography (4 : 1, Hex:EtOAc) to give **28** (195 mg) in a quantitative yield. ^1H NMR (CDCl_3 , 400 MHz) δ 7.54–7.49 (m, 2H), 7.39–7.33 (m, 4H), 6.97 (d, J = 16.2 Hz, 1H), 6.57 (s, 2H), 5.18 (s, 3H), 5.11 (s, 4H), 3.50 (s, 3H), 3.38 (s, 6H). ^{13}C NMR (CDCl_3 , 101 MHz) δ 194.50, 159.88, 156.06, 145.22, 135.02, 130.61, 129.22, 129.12, 128.55, 114.99, 97.36, 94.99, 94.81, 94.79, 56.51. LRMS (ESI): m/z calcd. for $\text{C}_{21}\text{H}_{24}\text{O}_7\text{Na}^+$: $[\text{M} + \text{Na}]^+$ 411.14; found 411.14.

4.1.20 1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)ethan-1-one (29). To a RBF under an argon atmosphere was added **9** (500 mg, 2.97 mmol) and CH_2Cl_2 (15 mL), which was then cooled to 0 °C and then diisopropylethylamine (1.6 mL, 8.91 mmol) and MOMCl (0.5 mL, 6.5 mmol) was added. The solution was then stirred O/N, allowed to warm to RT, and then quenched with methanol and washed with brine ($\times 2$). The combined organic layers were dried over anhydrous sodium sulfate, concentrated, and purified *via* flash silica gel chromatography (4 : 1, Hex:EtOAc) to afford **29** in 79% yield (601 mg). ^1H NMR (CDCl_3 , 400 MHz) δ 13.70 (s, 1H), 6.24 (1H), 6.22 (s, 1H), 5.24 (s, 2H), 5.15 (s, 2H), 3.50 (s, 3H), 3.45 (s, 3H), 2.63 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ 203.40, 167.02, 163.66, 160.57, 107.12, 97.32, 94.69, 94.21, 56.91, 56.64, 33.21. LRMS (ESI): m/z calcd. for $\text{C}_{12}\text{H}_{16}\text{O}_6\text{Na}^+$: $[\text{M} + \text{Na}]^+$ 279.08; found 279.08.

4.1.21 (E)-1-(2-Methoxy-4,6-bis(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (30). To a flame dried RBF under an argon atmosphere was added dry acetone (15 mL) to which

was added **29** (400 mg, 1.56 mmol) under vigorous stirring. Once a homogenous solution was obtained, K_2CO_3 (1.07 g, 7.8 mmol) and Me_2SO_4 (0.74 mL, 7.8 mmol, 5 eq.) were added. The reaction mixture was refluxed for 4 h, quenched with saturated ammonium chloride, and extracted with EtOAc ($\times 2$). The organic layers were combined, dried over sodium sulfate, concentrated, and purified *via* flash silica gel chromatography (7 : 3 Hex:EtOAc) to obtain the desired methylated acetophenone material (**29b**). The aldol condensation general procedure was followed using **29b** (350 mg, 1.29 mmol) and 0.16 mL of benzaldehyde. The crude material was purified by flash silica gel chromatography (7 : 3, Hex:EtOAc) to afford **30** (408 mg) in a 73% overall yield. ^1H NMR (CDCl_3 , 400 MHz) δ 7.52 (dq, J = 6.5, 4.0, 3.0 Hz, 2H), 7.38–7.34 (m, 4H), 6.96 (d, J = 16.1 Hz, 1H), 6.50 (dd, J = 6.2, 2.0 Hz, 1H), 6.37 (d, J = 2.0 Hz, 1H), 5.20 (s, 1H), 5.11 (s, 1H), 3.76 (s, 3H), 3.51 (s, 3H), 3.39 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ 194.56, 160.10, 158.77, 156.16, 144.91, 135.13, 130.52, 129.19, 129.07, 128.58, 96.15, 94.84, 94.80, 94.21, 56.53, 56.48, 56.16. LRMS (ESI) m/z calcd. for $\text{C}_{20}\text{H}_{23}\text{O}_6^+$: $[\text{M} + \text{H}]^+$ 359.15; found 359.15.

4.1.22 2',4'-Dihydroxy-3'-(2-hydroxybenzyl)-6'-methoxychalcone (2, *c*-benzylated chalcone). The general alkylation procedure was followed using **30** (247 mg, 0.69 mmol) and **14** (215 mg, 0.83 mmol). The crude material was not purified by column chromatography, but rather subjected to the allyl deprotection procedure to afford the carbon core of the uvaetin family (**30b**) in 51% yield. The material was dissolved into MeOH (final concentration 0.05 M) and a 10% HCl solution (1/2 vol. eq. of the MeOH) was added.¹ The solution was refluxed for 30 min, the mixture poured onto cold water (2 vol. eq. to MeOH) and extracted with EtOAc. The organic layers were combined, dried over sodium sulfate, and concentrated under reduce pressure. The crude material was purified *via* flash silica gel chromatography (1 : 1, Hex:EtOAc) to afford **2** (86 mg) in 65% yield, with an overall yield of 33% over two-steps. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 15.06 (s, 1H), 7.87 (d, J = 15.6 Hz, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.62–7.54 (m, 3H), 7.39 (d, J = 6.0 Hz, 3H), 7.07 (t, J = 7.7 Hz, 1H), 6.85 (t, J = 7.9 Hz, 2H), 5.96 (s, 1H), 3.91 (s, 2H), 3.87 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 192.89, 165.40, 161.97, 161.52, 153.52, 142.37, 135.79, 132.15, 130.17, 129.02, 128.53, 127.83, 127.74, 127.15, 120.85, 115.86, 108.05, 106.12, 91.80, 55.91, 22.81. LRMS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{21}\text{O}_5^+$: $[\text{M} + \text{H}]^+$ 377.13, found 377.13.

4.1.23 Uvaetin (1). The enone reduction procedure was followed using **2** (5 mg, 0.13 mmol) and 10% Pd/C (3 mg) to afford **1** (4.5 mg) in 90% yield. ^1H NMR (CDCl_3 , 400 MHz) δ (ppm): 7.54 (d, J = 7.6 Hz, 1H), 7.33–7.28 (m, 3H), 7.23 (d, J = 8.2 Hz, 2H), 7.08 (t, J = 7.8 Hz, 1H), 6.84 (dd, J = 13.2, 7.6 Hz, 2H), 5.91 (s, 1H), 3.90 (s, 2H), 3.77 (s, 3H), 3.30 (t, J = 7.8 Hz, 2H), 2.98 (t, J = 7.8 Hz, 2H). ^{13}C NMR (CDCl_3 , 101 MHz) δ (ppm): 205.41, 163.63, 162.31, 155.02, 141.73, 131.91, 128.67, 128.65, 127.92, 126.44, 126.21, 119.97, 116.67, 109.05, 105.75, 104.99, 86.94, 55.92, 46.04, 30.86, 23.25. LRMS (ESI): m/z calcd for $\text{C}_{23}\text{H}_{23}\text{O}_5^+$: $[\text{M} + \text{H}]^+$ 379.15, found 379.15.

4.1.24 (E)-1-(2-Hydroxy-4,6-bis(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (31). The aldol condensation general procedure was followed using **29** (360 mg, 1.4 mmol) and 0.14 mL of benzaldehyde. The crude material was purified by flash silica gel chromatography (4:1, Hex:EtOAc) to give **31** (449 mg) in 93% yield. ^1H NMR (CDCl_3 , 400 MHz) δ 13.81 (s, 1H), 7.93 (d, J = 15.6 Hz, 1H), 7.79 (d, J = 15.7 Hz, 1H), 7.68–7.57 (m, 3H), 7.46–7.35 (m, 4H), 6.26 (d, J = 2.4 Hz, 1H), 5.29 (s, 2H), 5.19 (s, 2H), 3.51 (s, 3H), 3.50 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz) δ 193.15, 167.57, 163.74, 160.12, 142.72, 135.69, 130.40, 129.16, 128.54, 127.61, 107.76, 97.73, 95.38, 94.99, 94.30, 57.11, 56.70. LRMS (ESI): m/z calcd for $\text{C}_{19}\text{H}_{21}\text{O}_6$: $[\text{M} + \text{H}]^+$ 345.13; found 345.14.

4.2 Biological

4.2.1 Cell culture information. The cancerous cell lines used in these investigations were purchased directly from the American Type Culture Collection. Cell were grown in media supplemented with fetal bovine serum (FBS) and antibiotics (100 $\mu\text{g mL}^{-1}$ penicillin and 100 U mL^{-1} streptomycin). Specifically, experiments were performed using the following cell lines and media compositions: HeLa, U937, Reh (RPMI-1640 + 10% FBS), Mia PaCa-2 (DMEM + 10% FBS), A549 (F-12K + 10% FBS), and HCT-116 (McCoy 5A + 10% FBS). Cells were incubated at 37 °C in a 5% CO_2 , 95% humidity atmosphere for all experiments.

4.2.2 IC_{50} value determination for adherent cells using Alamar Blue. Adherent cells were added to 384-well plated (1500 cells per well) in 10 μL of media and were allowed to adhere to 2–3 h. Compounds were solubilized in DMSO (10 μM stock solutions), added to a 96-well plate over a range of concentrations (31.6 nM to 200 μM) with media, 40 μL was added to the 384-well plate in triplicate for each concentration of compound. After 69 h of continuous exposure, 5 μL of Alamar Blue was added to each well and the cells were allowed to incubate for an additional 3 h. The plates were then read for fluorescence intensity with an excitation of 560 nm and emission of 590 nm on a BioTek Synergy H1 plate reader. Doxorubin and etoposide were both used as positive death controls and wells with no compounds added as negative death controls. IC_{50} values were determined from three or more independent experiments using GraphPad Prism 7.0. (LaJolla California).

4.2.3 IC_{50} value determination for non-adherent cells using Alamar Blue. The same procedure for adherent cells was used, with the following modifications. Cells (2200 cell per well) in media (10 μL) were added after 40 μL of compound in media were added to the 384-well plate. No time was given to allow cells to adhere.

4.2.4 Combination studies. All combinational cell death experiments were performed in 96-well plates with a total volume of 100 μL . To each well was added 49 μL of cell media, either 2.5 or 5 μM of 6TP (from a DMSO stock solution), and compounds **28**, **30**, or **31**, independently. To each well on the plate was added two different concentration of **28**, **30**, or **31**

and five concentrations of 6TP (both prepared from 10 mM stock solutions in DMSO), cell media (adjusted to reach 50 μL volume) and 0.5 μL of DMSO to achieve a 1% DMSO concentration. To each well was then added 50 μL of a suspension of cells to obtain a final cell density of 4000 cells per well (adherent cells) and 7000 cells per well (suspension cells). Doxorubicin and etoposide were both used as positive death controls, and wells with no compounds added as negative death controls. Plates were incubated at 37 °C with 5% CO_2 for 72 h, at which time they were assessed by Alamar Blue. IC_{50} values were determined from three or more independent experiments using GraphPad Prism 7.0.

Conflicts of interest

The authors declare no conflict of interest.

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References

- 1 B. X. Zhang, D. Z. Duan, C. P. Ge, J. Yao, Y. P. Liu, X. M. Li and J. G. Fang, *J. Med. Chem.*, 2015, **58**, 1795–1805.
- 2 E. J. Park, H. R. Park, J. S. Lee and J. Kim, *Planta Med.*, 1998, **64**, 464–466.
- 3 R. De Vincenzo, G. Scambia, P. Benedetti Panici, F. O. Ranelletti, G. Bonanno, A. Ercoli, F. Delle Monache, F. Ferrari, M. Piantelli and S. Mancuso, *Anti-Cancer Drug Des.*, 1995, **10**, 481–490.
- 4 A. C. Claude, C. L. Jean, T. Patric, P. Christelle, H. Gerard, J. C. Albert and L. D. Jean, *Anticancer Res.*, 2001, **21**.
- 5 S. K. Kumar, E. Hager, C. Pettit, H. Gurulingappa, N. E. Davidson and S. R. Khan, *J. Med. Chem.*, 2003, **46**, 2813–2815.
- 6 Y. Xia, Z. Y. Yang, P. Xia, K. F. Bastow, Y. Nakanishi and K. H. Lee, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 699–701.
- 7 F. Bois, C. Beney, A. Boumendjel, A. M. Mariotte, G. Conseil and A. Di Pietro, *J. Med. Chem.*, 1998, **41**, 4161–4164.
- 8 M. Liu, P. Wilairat and M. L. Go, *J. Med. Chem.*, 2001, **44**, 4443–4452.
- 9 J. Domínguez, J. E. Charris, G. Lobo, N. Domínguez, E. Sanchez and P. J. Rosenthal, *Eur. J. Med. Chem.*, 2001, **36**, 555–560.
- 10 H. K. Hsieh, L. T. Tsao, J. P. Wang and C. N. Lin, *J. Pharm. Pharmacol.*, 2000, **52**, 163–171.
- 11 H. Matsuda, T. Morikawa, S. Ando, I. Toguchida and M. Yoshikawa, *Bioorg. Med. Chem.*, 2003, **11**, 1995–2000.

- 12 H. H. Ko, L. T. Tsao, K. L. Yu, C. T. Liu, J. P. Wang and C. N. Lin, *Bioorg. Med. Chem.*, 2003, **11**, 105–111.
- 13 F. Herencia, M. L. Ferrandiz, A. Ubeda, J. N. Dominguez, J. E. Charris, G. M. Lobo and M. J. Alcaraz, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1169–1174.
- 14 Y. M. Lin, Y. S. Zhou, M. T. Flavin, L. M. Zhou, W. G. Nie and F. C. Chen, *Bioorg. Med. Chem.*, 2002, **10**, 2795–2802.
- 15 S. Ducki, R. Forrest, J. A. Hadfield, A. Kendall, N. J. Lawrence, A. T. McGown and D. Rennison, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1051–1056.
- 16 K. Maria, H. L. Dimitra and G. Maria, *Med. Chem.*, 2008, **4**, 586–596.
- 17 S. N. Lopez, M. V. Castelli, S. A. Zacchino, J. N. Dominguez, G. Lobo, J. Charris-Charris, J. C. Cortes, J. C. Ribas, C. Devia, A. M. Rodriguez and R. D. Enriz, *Bioorg. Med. Chem.*, 2001, **9**, 1999–2013.
- 18 S. Shibata, *Stem Cells*, 1994, **12**, 44–52.
- 19 Y. K. Rao, S. H. Fang and Y. M. Tzeng, *Bioorg. Med. Chem.*, 2004, **12**, 2679–2686.
- 20 Y. C. Liu, C. W. Hsieh, C. C. Wu and B. S. Wung, *Life Sci.*, 2007, **80**, 1420–1430.
- 21 J. H. Lee, H. S. Jung, P. M. Giang, X. Jin, S. Lee, P. T. Son, D. Lee, Y. S. Hong, K. Lee and J. J. Lee, *J. Pharmacol. Exp. Ther.*, 2006, **316**, 271–278.
- 22 H. S. Ban, K. Suzuki, S. S. Lim, S. H. Jung, S. Lee, J. Ji, H. S. Lee, Y. S. Lee, K. H. Shin and K. Ohuchi, *Biochem. Pharmacol.*, 2004, **67**, 1549–1557.
- 23 E. Heiss, C. Herhaus, K. Klimo, H. Bartsch and C. Gerhauser, *J. Biol. Chem.*, 2001, **276**, 32008–32015.
- 24 R. Foresti, M. Hoque, D. Monti, C. J. Green and R. Motterlini, *J. Pharmacol. Exp. Ther.*, 2005, **312**, 686–693.
- 25 V. Kumar, S. Kumar, M. Hassan, H. Wu, R. K. Thimmulappa, A. Kumar, S. K. Sharma, V. S. Parmar, S. Biswal and S. V. Malhotra, *J. Med. Chem.*, 2011, **54**, 4147–4159.
- 26 J. R. Cole, S. J. Torrance, R. M. Wiedhopf, S. K. Arora and R. B. Bates, *J. Org. Chem.*, 1976, **41**, 1852–1855.
- 27 W. L. Lasswell Jr and C. D. Hufford, *J. Org. Chem.*, 1977, **42**, 1295–1302.
- 28 L. Wirasathien, T. Pengsuparp, M. Moriyasu, K. Kawanishi and R. Suttisri, *Arch. Pharmacol. Res.*, 2006, **29**, 497–502.
- 29 K. E. Malterud, J. Undheim and J. E. Erdal, *Tetrahedron Lett.*, 1985, **26**, 4807–4810.
- 30 K. C. Gulati and K. Venkataraman, *J. Chem. Soc.*, 1936, 267–269.
- 31 E. Knoevenagel and R. Arnot, *Ber. Dtsch. Chem. Ges.*, 1904, **37**, 4496–4502.
- 32 F. Kallay, G. Janzso, I. Egyed and E. Baitz-Gacs, *Flavonoids, Bioflavonoids, Proc. 5th Hung. Bioflavonoid Symp.*, 1977, p. 235.
- 33 F. Kallay, G. Janzso, I. Egyed, E. Baitzgacs and J. Tamas, *Acta Chim. Acad. Sci. Hung.*, 1979, **100**, 311–317.
- 34 T. Liu and Y. Z. Hu, *Synth. Commun.*, 2004, **34**, 3209–3218.
- 35 D. Gärtner, H. Konnerth and A. J. von Wangelin, *Catal. Sci. Technol.*, 2013, **3**, 2541–2545.
- 36 R. N. Malhas and Y. A. Ibrahim, *Synthesis*, 2006, **2006**, 3261–3269.
- 37 B. L. Ryland, S. D. McCann, T. C. Brunold and S. S. Stahl, *J. Am. Chem. Soc.*, 2014, **136**, 12166–12173.
- 38 H. S. P. Rao, S. Rafi, P. R. Kumar, C. Guravaiah and N. Muthanna, *Tetrahedron Lett.*, 2012, **53**, 6877–6880.
- 39 R. Iyer, K. Shah and K. Venkataraman, *Proc. Indian Acad. Sci. (Math. Sci.)*, 1951, **33**, 116–119.
- 40 N. Anand, R. Iyer and K. Venkataraman, *Proc. Indian Acad. Sci. (Math. Sci.)*, 1949, **29**, 203–209.
- 41 W. J. Zhang, J. F. Wu, P. F. Zhou, Y. Wang and A. J. Hou, *Tetrahedron*, 2013, **69**, 5850–5858.
- 42 M. Mondal, V. G. Puranik and N. P. Argade, *J. Org. Chem.*, 2007, **72**, 2068–2076.
- 43 E. M. Jung and Y. R. Lee, *Bull. Korean Chem. Soc.*, 2008, **29**, 1199–1204.
- 44 J. C. Aponte, D. Castillo, Y. Estevez, G. Gonzalez, J. Arevalo, G. B. Hammond and M. Sauvain, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 100–103.
- 45 Y. Tang, C. Jiang, X. Zhang, C. Liu, J. Lin, Y. Wang, C. Du, X. Peng, W. Li, Y. Liu and M. Cheng, *J. Org. Chem.*, 2017, **82**, 11102–11109.
- 46 A. Yamagami, H. Ishimura, A. Katori, K. Kuramochi and K. Tsubaki, *Org. Biomol. Chem.*, 2016, **14**, 10963–10972.
- 47 A. Fatino, C. Weese, G. Baca, A. Lansakara and R. Rafferty, *Abstracts of Papers of the American Chemical Society*, 2017, vol. 253.
- 48 V. L. Hoang, Y. C. Zhang and R. J. Rafferty, *Tetrahedron Lett.*, 2017, **58**, 4432–4435.
- 49 A. X. Torres Hernandez, C. J. Weeramange, P. Desman, A. Fatino, O. Haney and R. J. Rafferty, *MedChemComm*, 2019, **10**, 169–179.
- 50 T. C. Chou and P. Talalay, *Adv. Enzyme Regul.*, 1984, **22**, 27–55.
- 51 G. Wei and B. Yu, *Eur. J. Org. Chem.*, 2008, **2008**, 3156–3163.
- 52 R. S. Khupse and P. W. Erhardt, *J. Nat. Prod.*, 2007, **70**, 1507–1509.
- 53 S. Vogel, S. Ohmayer, G. Brunner and J. Heilmann, *Bioorg. Med. Chem.*, 2008, **16**, 4286–4293.