MedChemComm

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Journal:	*MedChemComm*
Manuscript ID	MD-RES-01-2019-000052.R1
Article Type:	Research Article
Date Submitted by the Author:	15-May-2019
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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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ABSTRACT

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 varient 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 IC_{50} 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 IC_{50} activity as sole agents. Furthermore, multiple library members were found to possess promising potentiating properties with known chemotherapeutic agents.

1. Introduction

Chalcones, known for their classical α,β-unsaturated ketone moiety flanked by phenyl groups, are a central component to numerous natural products (Figure 1), such as, but not limited to: butein, cardamonin, derricin, flavokawain, isoalipurposide, 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] antituberculotic,[14] antimitotic,[15] analgesic,[16] antibacterial, and antifungal.[17] There are limited reports suggesting that they are also antimutagenic, 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]

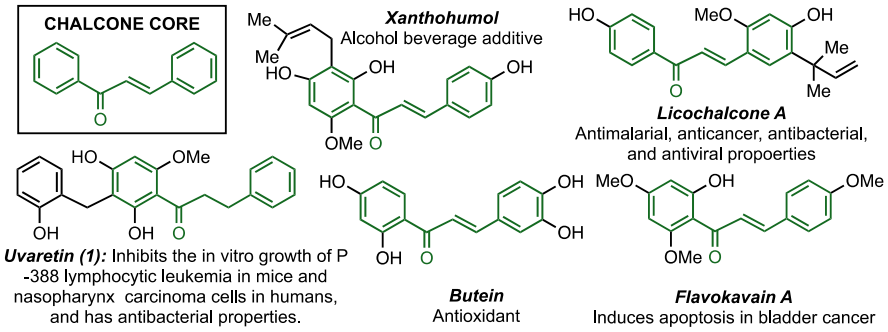
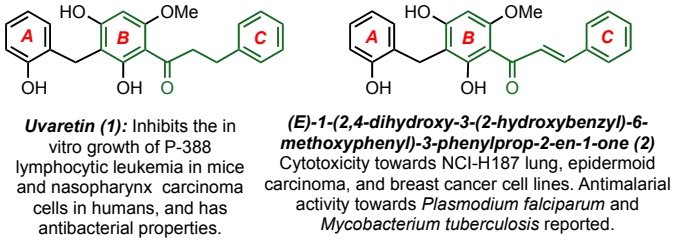


Figure 1 Chalcone core and the representative set of natural products containing said core highlighted in green.

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 nucleophiles, 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. The NF-κB[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 Figure 2.



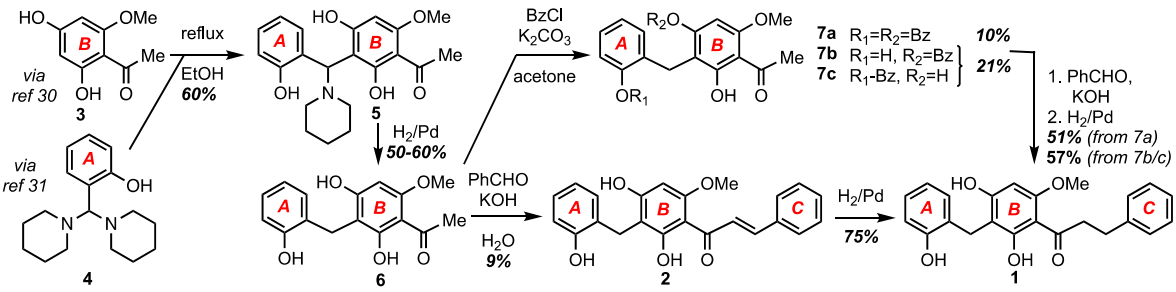
Uvaretin (1): Inhibits the in vitro growth of P-388 lymphocytic leukemia in mice and nasopharynx carcinoma cells in humans, and has antibacterial properties.

(E)-1-(2,4-dihydroxy-3-(2-hydroxybenzyl)-6-methoxyphenyl)-3-phenylprop-2-en-1-one (2) Cytotoxicity towards NCI-H187 lung, epidermoid carcinoma, and breast cancer cell lines. Antimalarial activity towards *Plasmodium falciparum* and *Mycobacterium tuberculosis* reported.

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

Uvaretin (**1**) was first isolated in 1976 by Wiedhopf and Bates from the chloroform extract of *Uvaria acuminate* 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 *Ellipeiopsis cherrevenis* (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-methoxyacetophone (**3**)[30] and 2-hydroxyphenyl-bis-1,1-piperidinomethane (**4**)[31] accessing **5** in 60% yield. This step highlighted their key aminal 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 condenation, 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 acccess), and **4** (requiring one synthetic step to access).



Scheme 1 Malterud's total synthesis route to uvaretin.

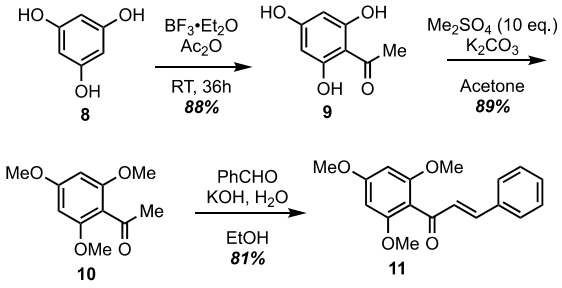
While the route developed by Malterud provided access to both **1** and **2**, it was the late-stage installation of the chalcone core that led us to develop a new route to these natural products. Given the desire to construct a new small library collection for screening to identify new compounds with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

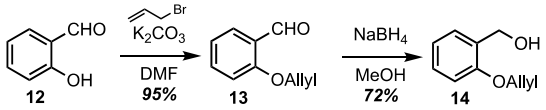
Efforts towards accessing the chalcone core early rather than late stage commenced with construction of the B-ring. From phloroglucinol (**8**), the 2,4,6-trihydroxy-acetophenone (**9**) was accessed through acylation with acetic anhydride in the

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 trimethoxy-acetophenone (**10**) in 89% yield. Accessing **11** was accomplished through a potassium hydroxide catalyzed aldol condensation between **10** and benzaldehyde in 81% yield.[19]



Scheme 2 Synthetic route to obtain the trimethylated chalone core.

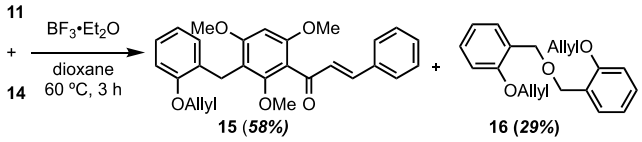
Attention was then directed towards the installation of the western *ortho*-hydroxybenzyl moiety (A-ring) via a Friedel-Craft alkylation approach. Construction of the requisite material commenced with the allylation of *ortho*-hydroxybenzaldehyde (**12**) with allyl bromide and potassium carbonate in 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]



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

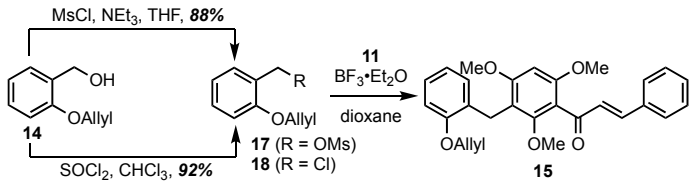
Next, efforts were directed towards the incorporation of **14** onto **11**. The Lewis acid mediated reaction between **11** and **14** proved to be more problematic than originally anticipated (Scheme 4). Please see the supporting information for a detail table of all conditions attempted. Two different 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 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.



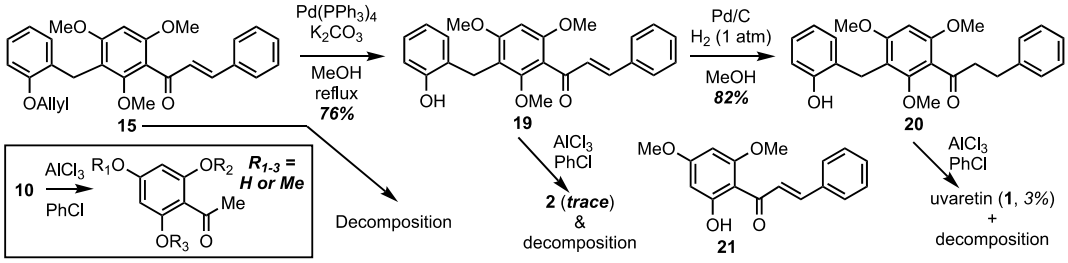
Scheme 4 Friedel-Crafts alkylation conditions gaining access to the uvaretin carbon core.

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 provided 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**.



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

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 $Pd(PPh_3)_4$ 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.



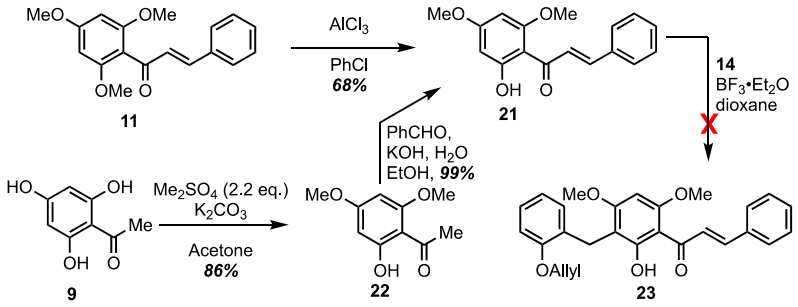
Scheme 6 Allyl deprotection, construction of analogs **19** and **20**, and attempts at accessing natural products **1** and **2** via selective demethylation.

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 precedented 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 the other conditions presented in the SI, failed to afford **23**. The isolation of the *O*-alkylated compound was observed in numerous trials. As such, the free phenolic group within the

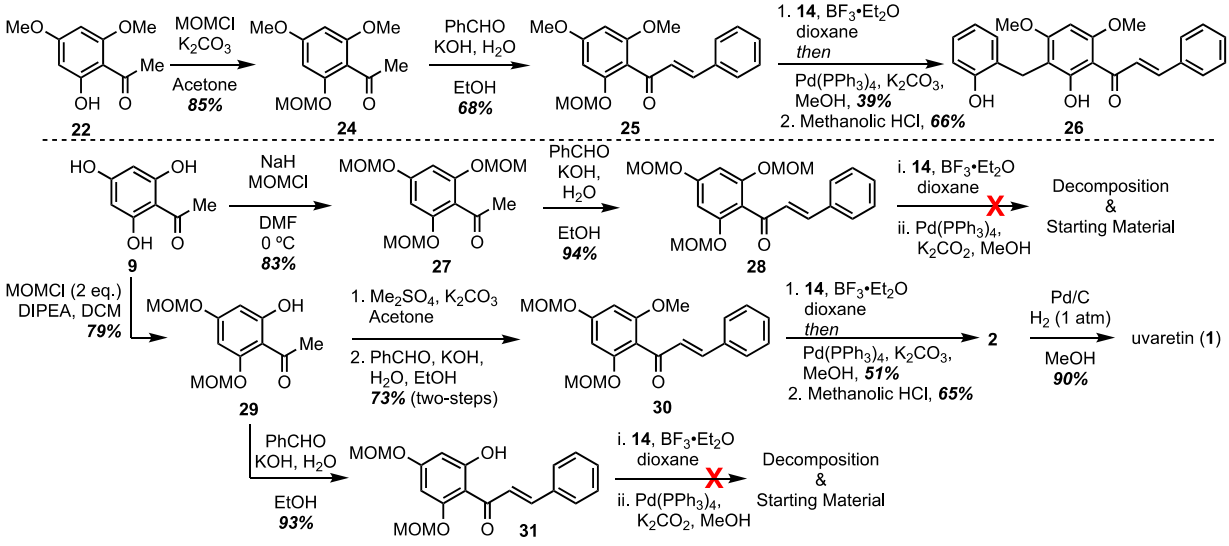
B-ring was suspected to be the cause of this failed alkylation. Acetylation of this phenol was attempted, followed by alkylation conditions to afford <5% of **23**. Under these conditions, deacetylation occurred rapidly which prevented the desired alkylation. Protection of the phenol with a more robust protecting group was then explored.



Scheme 7 Attempted conditions at gaining accessing to the monophenol variant chalcone.

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 SI, 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.



Scheme 8 Elaboration of **22** onto the natural products uvaretin (**1**) and **2**.

2.3 Biology

2.3.1 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 accessed 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.

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

Cmpd	Observed IC_{50} (μM)[a]					
	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	12.2 ±0.9	7.4 ±0.8	>20	>20	>20	>20
29	11.5 ±1.1	8.1 ±2.0	8.4 ±1.3	11.8 ±2.6	>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
Uvaretin (**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 1,500 cells/well, whereas suspension cells at 2,200 cells/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.

2.3.2 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 is done through screening campaigns of newly accessed small molecules with therapeutic agents. Investigations are currently underway with the small molecules accessed in this work as potentiators against known therapeutic agents. One agent, 6-thiopurine (6TP), a currently a prescribed drug in the treatment of acute lymphocyte leukemia, acts as a nucleotide mimic which upon DNA incorporation causes apoptosis via base pair mis-matching. Chalcones **28**, **30**, and **31** were shown to have IC_{50} values of 11.8, 2.2, and 7.8 μM, respectively, in MIA PaCa-2. There are no reported therapeutic properties of 6TP within this pancreatic cell line. Investigations into the possible potentiating properties of these three chalcones with 6TP in MIA PaCa-2 were conducted to probe the possible usefulness in the small molecule chalcone collection assembled.

Assessing the synergist properties of **28**, **30**, and **31** with 6TP was performed in a matrix format, consisting of a 3x5 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 Figure 3 (within the red tables). There was no significant change in cell death at various concentrations of **28** and 6TP, with 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 increased 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 synergism 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.

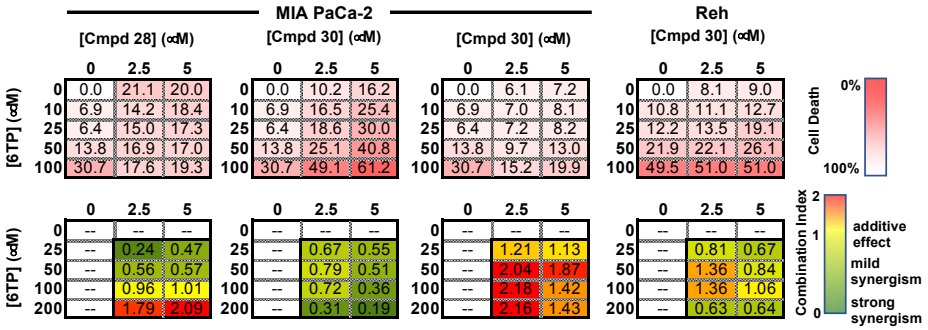


Figure 3 Evaluation of chalcones **28**, **30**, and **31** potentiation properties with 6-thiopurine in the cancerous cells line Mia PaCa-2 (pancreatic) and Reh (acute lymphocytic leukemia). Potentiation performed in a 3x5 matrix, and synergy assessed. *(n = biological replicates)*.

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 Figure 2, these four compounds were evaluated within the same cell lines used in our general IC_{50} screens (shown in Table 1) towards doxorubicin, etoposide, paclitaxel, and 6TP. Figure 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.

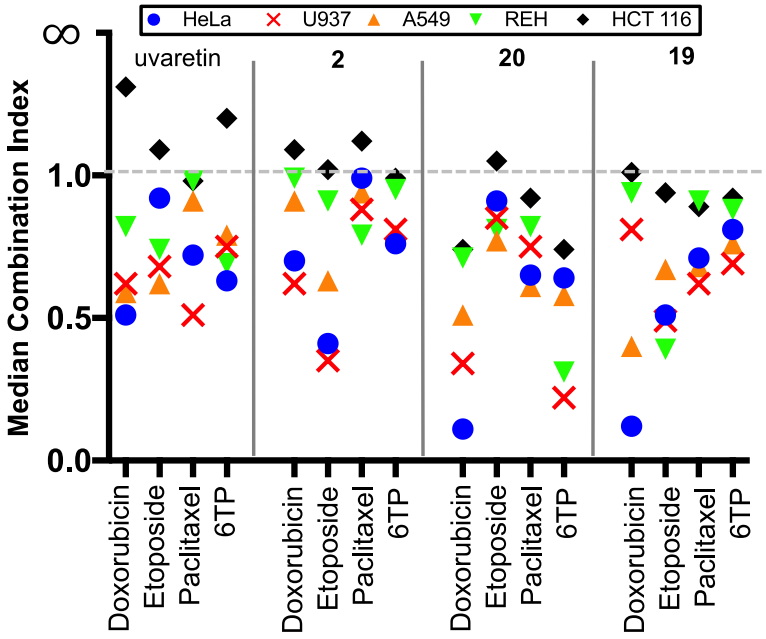


Figure 4 Median CI values for each chalcone/chemotherapeutic combination for each cell line. CI values < 1 are synergistic, lower values representative of higher levels of synergy. CI values of 1 denote additive effects of the two compounds, and values > 1 antagonistic effects *(n = three biological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

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 IC_{50} values below 20 μM 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 μM IC_{50} 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 unsaturuated 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 μM IC_{50} values in HeLa, U937, A549 and MIA PaCa-2 cancerous cell lines and moderate double digit μM in Reh and HCT-116. Also, 31 other compounds from this library were found to possess single digit μM IC_{50} 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, pacilitaxel, 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 $CHCl_3$ at δ 7.26 ppm or DMSO at δ 2.50 ppm for proton spectra and relative to $CDCl_3$ 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 (CH_2Cl_2), methanol (CH_3OH), 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 (x2). 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 dry dioxane (final concentration of 46 mM) and stirred at RT. In a separate flamed dried RBF under an argon atmosphere was added BF_3•Et_2O (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 (x2), and extracted with EtOAc (x2). 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 $Pd(PPh_3)_4$ (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 $BF_3{\cdot}OEt_2$ (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. ^{1}H 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-Trimethyoxyacetophenone (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 (x3). 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. ^{1}H 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. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.54-7.52 (m, 2H), 7.39-7.35 (m, 5H), 6.97 (d, 1H, J = 12Hz), 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 $C_{18}H_{19}O_4^+$: $[M+H]^+$ 299.12, found 299.12.

4.1.8 2-(Allyloxy)benzaldehyde (13): To a flamed dried RBF under an argon atmosphere was added salicyaldehyde (**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 (x4), 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. ^{1}H 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 $C_{10}H_{10}O_2Na^+$: $[M+ Na]^+$ 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 (x2). 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. ^{1}H 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 $C_{10}H_{13}O_2^+$: $[M+ H]^+$ 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. ^{1}H 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. ^{1}H 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. ^{1}H 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): **Demethylation 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 (x3). 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. ^{1}H 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 (x3). 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. ^{1}H 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 (x2). 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. ^{1}H 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. ^{1}H 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-3-(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. ^{1}H 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 $C_{24}H_{23}O_5^+$: $[M+H]^+$ 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 (x2). 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. ^{1}H 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 $C_{14}H_{20}O_7Na^+$: $[M+Na]^+$ 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. ^{1}H 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 $C_{21}H_{24}O_7Na^+$: $[M+Na]^+$ 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 (x2). 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). ^{1}H 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 $C_{12}H_{16}ONa^{+}$: $[M+Na]^{+}$ 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 (.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 (x2). 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. ^{1}H 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*d*) 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 $C_{20}H_{23}O_6^{+}$: $[M+H]^{+}$ 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 uvaretin 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. ^{1}H 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 $C_{23}H_{21}O_5^+$: $[M+H]^+$ 377.13, found 377.13.

4.1.23 Uravetin (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. ^{1}H 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 $C_{23}H_{23}O_5^+$: $[M+H]^+$ 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. ^{1}H 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 $C_{19}H_{21}O_6^+$: $[M+H]^+$ 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 µg/mL penicillin and 100 U/mL 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 (1,500 cells/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 (2,200 cell/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 4,000 cells/well (adherent cells) and 7,000 cells/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.

Acknowledgements

This work could not have been undertaken without the gracious financial support from the Johnson Cancer Research Center of Kansas State University and Startup Capital from Kansas State University. Funding for this research was also provided by the NSF REU program under grant number *CHE-1460898*. J.D. was supported by the NSF-REU program. This paper is warmly dedicated to Professor Robert M. Williams on the occasion of his 66th birthday.

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, *Anticancer 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. Dominquez, 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, *Biorg. 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.
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, *The Journal of organic chemistry*, 1977, **42**, 1295-1302.
28. L. Wirasathien, T. Pengsuparp, M. Moriyasu, K. Kawanishi and R. Suttisri, *Arch. Pharmacal 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, *Berichte der deutschen chemischen Gesellschaft*, 1904, **37**, 4496-4502.

32. F. Kallay, G. Janzso, I. Egyed and E. Baitz-Gacs, 1977.
33. F. Kallay, G. Janzso, I. Egyed, E. Baitzgacs and J. Tamas, *Acta Chimica Academiae Scientarium Hungaricae*, 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-Stuttgart*, 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, 1951.
40. N. Anand, R. Iyer and K. Venkataraman, 1949.
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, **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.