Construction and Biological Evaluation of Small Libraries Based on the Intermediates within the Total Synthesis of Uvaretin

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Introduction

Natural products (NPs) have played a vital role in the treatment of diseases, and are the cornerstone for modern drug discovery, since 2600 B.C. in which oils from *Cupressus semperviren* and *Commiphora* were used in the treatment of coughs, colds, and inflammation.[1] From individual therapeutic agent discovery to inspiration for the construction of non-natural bioactive agents, their impact is immeasurable.[2] While NPs constituted the major pipeline for therapeutic agent discovery for decades, the introduction of high-throughput screening (HTS) in the late 1980s allowed for the discovery of drug lead scaffolds via chemical screening libraries (CSLs); initially solely populated by NPs, NP fragments, and NPs analogs.[3] To answer the call for the population of CSL in a rapid fashion the industry turned towards the use of sp^2–sp^2 metal catalyzed coupling reactions to gain access to tens to hundreds of thousands of small molecules for HTS, as means of identifying new drug lead scaffolds.[4] While numerous FDA approved drugs have been discovered in this fashion, these compounds tend to lack in the chemical diversity, complexity, and inherent biological activity of natural products.[4] To address the diversity of CSLs, multiple research programs have undertaken various approaches to construct new CSLs that either possess structural complexity, rapid small-molecule construction, biological activity-based design, and others.[5] All of these approaches, as expected, have their pros and cons associated with them. Noting the historical importance of NPs, the development of CSLs based upon NPs should deliver CSLs with not only biological activity, but chemical diversity in terms of structural complexity, biological modulating properties, and rich physicochemical properties.

Highlighting the importance of NPs, both in bioactivity and in chemical complexity, and acknowledging that each NP has been designed by nature to elicit a specific and discrete biological affect, our laboratory proposes that CSLs constructed from the intermediates of total synthesis campaigns will give access to new bioactive compounds with tunable chemical complexity. Previously, we have disclosed a new total synthesis route to the chalcone based uvaretin family of natural products.[6] To explore our concept of CSL construction from intermediates of a total synthesis campaign, this route served as the "proof-of-concept" to test feasibility. A chalcone based natural product was chosen for this given their well reported diverse biological activity. From this total synthesis route, and the intermediates within (**1** and **2**, shown for example), numerous small molecules (**3**–**5** and **6**–**7**, shown for example) were constructed and found to possess diverse and interesting biological activity (Figure 1). Furthermore, many of the various phenolic protected variants (MOM or methyl protected) were elaborated onto the carbon framework of uvaretin as analogs, in which the trimethylated **8** was shown to have moderate activity while its allyl protected *o*-hydroxybenzyl (**6**) precursor possessing single-digit micromolar cytotoxicity.

Over 45 compounds were synthesized through these efforts, which were then investigated for sole agent cytotoxicity and as possible small molecule potentiators. Intermediate **1** was used as a starting point for the construction of a 12-member library differing about the alkyl substitution of the phenol positions in ring A. Sole cytotoxicity of these compounds was variable, ranging from 2.2 ± 0.6 to >20 μM IC_{50} values. Interestingly, it was found that many of these varied alkyl substituted phenolic chalcones possessed mild to good potentiating properties with the clinical prescribed acute lymphocytic leukemia drug 6-thiopurine (6TP, Figure 1). 6TP is not commonly used in the treatment of other cancers, due to limited cytotoxicity. However, it was shown to be active in the pancreatic cancer cell line MIA PaCa-2 when used in combination with **4**. The specific alkylation patterns upon the phenolic positions in ring A did reveal interesting potentiation properties. However the alkyl substitutions explored were limited to methyl and methoxymethyl ethers. Elaboration of **1** onto its benzylated counterpart, ring C inclusion, revealed compounds with single digit μM cytotoxicity, but no potentiation properties were observed. Building above these findings, additional investigations into the role of each of the rings and the alkyl effects upon the phenolic central B-ring was explored and disclosed herein.

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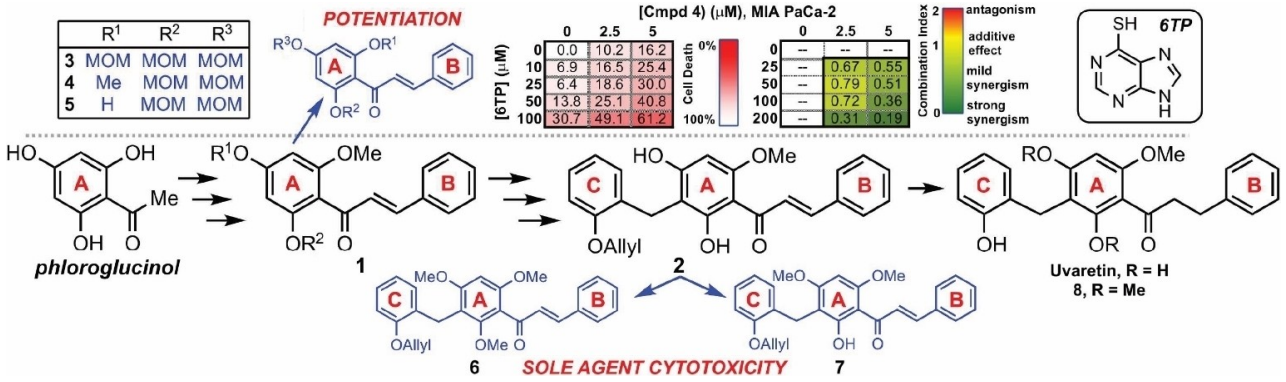
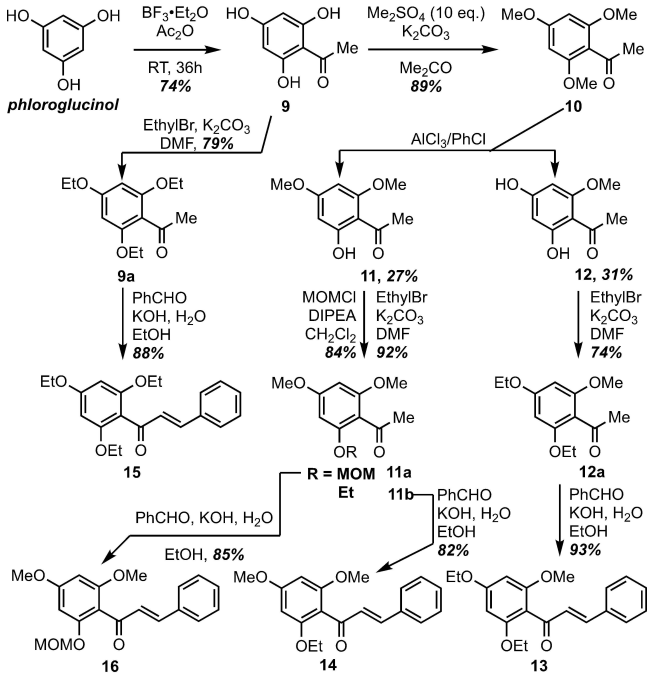


Figure 1. Previously disclosed total synthesis route to uvaretin and its unsaturated enone variant, token examples of small-molecule libraries constructed around intermediates **1** and **2**, and biological assessment.

Results and Discussion

Given the observed potentiation properties of **3**–**5**, and the moderately strong synergistic combination of **4** with 6TP, investigations into the substitution effects with the polyphenolic A ring of the chalcone small library were undertaken. No direct correlation between the number of MOM protected phenol groups was observed in the previously constructed small library. Furthermore, no correlation could be extrapolated on the overall effects of the MOM groups on the observed potentiation activity. In the effort to probe the substituent(s) effects upon this phenolic core, four new chalcones were constructed (Scheme 1). Acetylation of phloroglucinol with acetic anhydride and boron trifluoride etherate gave access to **9** in 74% yield,[7] which was then trimethylated with dimethyl sulfate in acetone affording **10** in 89% yield. Previously, no potentiation properties were observed with either 6TP or doxorubicin with the aldol condensation product of **10**. Demethylation was then performed with $AlCl_3$ in chlorobenzene to give access to demethylated **11** and monomethylated **12** in 27 and 31% yield, respectively. Introduction of diethyl group upon the free phenols of **12** followed by aldol condensation with benzaldehyde afforded chalcone **13** in 68% yield over two-steps.[6,8] In a similar fashion, the free phenol within **11** was ethylated and transformed into its corresponding chalcone **14** in 75% yield over two-steps. The triethylated phenol species was accessed from **9** through ethylation followed by aldol condensation to afford **15** in 70% yield over two-steps. A single MOM group was installed upon **11**, previously no single MOM protected chalcones were constructed and investigated for potentiation properties, in 84% yield and was transformed to its corresponding chalcone **16** in 85% yield.



Scheme 1. Construction of various ethyl, methyl, and MOM chalcones.

With these four analogs of the original chalcone small library set in hand, biological evaluation was then pursued in the same cancerous cell lines as our original study, in which cellular viability was assessed by Alamar Blue quantification:[9] cervical (HeLa), lymphoma (U937), lung (A549), and pancreatic (MIA PaCa-2). Our previously disclosed MOM-chalcone CSL possessed single to double digit μM IC_{50} activity in both HeLa and U937, but showed little to no active in both the A549 and MIA PaCa-2 cancerous cell lines. The diethylated/monomethylated chalcone **13**, possessing greater hydrophobic character than its corresponding di-MOM protected/monomethylated **4** counterpart previous disclosed, possessed 0.9–2.4 μM cytotoxicity in all cell lines screened. Cytotoxicity decreased for the monoethylated/dimethylated chalcone **14**, marginally within HeLa and U937 but drastically in A549 and MIA PaCa-2. The triethylated chalcone **15** had a drastic decrease in cytotoxicity, all values hovering at 20 μM. (Table 1). A common trend observed within the alkyl chalcone compounds **13, 14**, and **16** is that moderate increase in the hydrophobic character translates to decreased cytotoxicity, as observed with higher IC_{50} values in **15**, the triethylated chalcone, to the diethylated/monomethylated chalcone **13**. However, the monoethylated/dimethylated chalcone **14** was shown to possess weaker

Table 1. Evaluation of chalcones **13**–**16** for cytotoxicity and potentiation properties.

Cmpd	Observed IC_{50} [μM][a]				C.I.[b]	
	HeLa	U937	A549	MIA PaCa-2	6TP	Dox
4	9.7 ± 1.3	5.9 ± 1.1	4.2 ± 1.9	2.2 ± 0.6	N.T.	N.T.
13	2.4 ± 0.8	0.9 ± 0.9	1.5 ± 1.0	1.1 ± 3.7	1.29	1.76
14	5.9 ± 2.1	8.2 ± 3.0	> 20	> 20	1.01	1.82
15	18.5 ± 3.2	15.3 ± 4.7	> 20	19.1 ± 2.3	1.84	1.99
16	8.3 ± 3.5	7.1 ± 1.4	16.8 ± 3.6	> 20	0.31	0.98

[a] Cytotoxicity was evaluated in 384-well plates over a 72 h treatment. Adherent cells were seeded at 1500 cells/well, whereas suspension cells at 2200 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. [b] Cytotoxicity evaluation for combinational index (C.I.) studies were the same as for sole agent cytotoxicity, and the C.I. was determined based on the Chou-Talalay protocol.[10] N.T.: not tested and n = three biological replicates.

cytotoxicity than **13**. This suggests that the tuning of the hydrophobic character of the phenolic A ring could alter and enhance the cytotoxic activity of the chalcone system. It was observed that with the inclusion of a single MOM group in the dimethylated chalcone, a general increase in cytotoxicity was observed with respect to the previous MOM-chalcone small library. In terms of small molecule potentiation with 6TP and doxorubicin, it was observed that the presence of at least one MOM group is essential for synergistic activity. Assessing the synergist properties of these compounds, and all other compounds disclosed herein, with either 6TP or doxorubicin was performed in a 3x5 matrix format. Two concentrations of the small molecule screened were used that would induce 10–20% cell death within a 72-h period. Concentrations of either 6TP or doxorubicin were chosen to result in 40–60% cell death within the same time period. Combinational effects of cell death verses concentration were then tabulated, and from these values the effects of potentiation were determined by their combination index (C.I.), a value calculated by employing the Chou-Talalay protocol.[10] C.I. values closer to 0 represent strong synergism, 0.5 mild synergism, 1 an additive effect, and values greater than 1 denote antagonism in this method. Synergy was shown with **16** and both 6TP and doxorubicin with C.I. of 0.31 and 0.98, respectively. Based upon these investigations a general trend of the effects of hydrophobicity of the phenolic core was observed; decreased synergy with increased hydrophobic character.

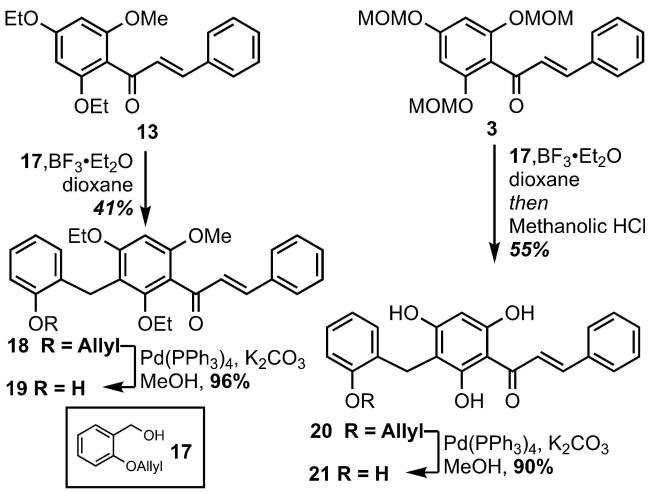
Incorporation of a *ortho*-hydroxybenzyl substituent upon the phenolic ring gives rise to the uvaretin family of natural products. We previously disclosed that **6** and **7** (Figure 1), the trimethylated phenol and allyl protected *o*-hydroxybenzyl group/allyl deprotected free phenol, possessed strong sole agent cytotoxicity (Table 2). From this, it was envisioned to incorporate **13**, the diethyl/monomethyl chalcone into this uvaretin carbon framework for cytotoxicity investigations. Subjecting **13** to our reported Friedel–Crafts alkylation conditions with **17** gave access to **18** in 41% yield, and treatment with $Pd(PPh_3)_4$ and potassium carbonate in methanol deprotected the allyl group to afford **19** in 96% yield (Scheme 2). In parallel, the free phenol analog was prepared from **3** by Friedel–Crafts alkylation with **17**, with subsequent MOM deprotection with methanolic HCl furnished **20** in 55%. Subsequent allyl deprotection with $Pd(PPh_3)_4$ and potassium carbonate in methanol gave access to **21** in 90% yield. Screening of **18**–**21** against the four cancerous cell line panel revealed interesting patterns. In both substitution patterns of the phenolic core, greater cytotoxicity was observed in allyl deprotected **19** and **21**. Overall, the more hydrophobic phenol core showed greater cytotoxicity. No potentiation properties with 6TP and **18**–**21** were observed.

Previously, *o*-allyloxy benzyl alcohol (**17**) was employed in our total synthesis of the natural product uvaretin, as well as in

Table 2. Evaluation of C-benzylated chalcones **18**–**21** for cytotoxicity properties.

Cmpd	Observed IC_{50} [μM][a]			
	HeLa	U937	A549	MIA PaCa-2
6	5.8 ± 0.5	7.5 ± 1.1	8.5 ± 0.6	9.4 ± 1.3
7	2.7 ± 0.6	4.2 ± 1.4	9.3 ± 1.1	4.1 ± 0.3
18	1.2 ± 0.9	0.8 ± 0.5	1.4 ± 1.2	9.7 ± 2.1
19	0.8 ± 0.7	0.9 ± 1.0	1.1 ± 0.7	4.1 ± 1.8
20	9.7 ± 1.1	11.7 ± 2.8	15.4 ± 3.5	> 20
21	2.7 ± 1.9	4.7 ± 1.8	3.1 ± 1.8	10.7 ± 4.3

[a] Cytotoxicity was evaluated in 384-well plates over 72 h of treatment. Adherent cells were seeded at 1500 cells/well, suspension cells at 2200 cells/well. Cellular viability was evaluated with Alamar Blue. Doxorubicin was used as the positive cell death control, and wells with only cells as the alive control (n = three biological replicates).



Scheme 2. Elaboration of chalcones **13** and **3** onto their C-benzylated variants.

the uvaretin framework of chalcone analogs disclosed herein. Keeping in mind the overarching goals of this project, construction of new small chemical screening libraries, efforts were placed towards modifications upon the benzyl (left ring) of the trimethylated chalcone uvaretin system to explore what effects, if any, they had upon cytotoxicity. Initially, *para*-allyloxy benzyl alcohol was attached onto **22** via our Friedel–Crafts alkylation protocol for the construction of its corresponding uvaretin carbon core **23** in 67% yield, which was subsequently transformed into the free phenol under our standard allyl deprotection condition to access **24** in 33% yield (Figure 2). The Friedel–Crafts alkylation worked well for both the *ortho* and *para* substituted allyloxy benzyl alcohols, however, the *meta* system, **25**, failed to give any desired product. Lower yields were obtained with the Friedel–Crafts alkylations of the *ortho* (**26**) and *para* (**27**) chloro benzyl alcohols, 28 and 25% yields, respectively. Treating **22** with 2,3-methylenedioxy benzyl alcohol afforded **28** in 29% yield, further supporting carbocation stabilization. No product was observed when benzyl alcohol (**29**) or 2-(hydroxymethyl)benzaldehyde (**30**) were attempted. Based on these results, it can be concluded that electron donating groups are required in the *ortho* and *para* positions to stabilize the induced carbocation from the boron trifluoride complexation with the benzyl alcohol. Screening of **26** and **27** showed $>20\,\mu M$ cytotoxicity, and no potentiation properties with either 6TP or doxorubicin. Unexpectedly, both the allyl (**23**) and free phenol (**24**) uvaretin carbon core analogs showed cytotoxicity low double digit μM cytotoxicity, with no potentiating properties observed. Limited cytotoxicity was observed from **28**, IC_{50} of $18.5\pm3.8\,\mu M$ in U937, but had strong synergism with doxorubicin possessing a combination index of 0.33. From this, it can be speculated that the substitution pattern upon the left benzyl ring plays a critical role in the cytotoxicity of uvaretin based small libraries.

Previously, we disclosed that the trimethylated chalcone **22** was shown to have 4.3–6.7 μM activity in HeLa, U937, and A549 cancerous cells lines, with no activity observed in MIA PaCa-2.[6] Varying the groups upon the phenolic oxygens with a mixture of MOM, ethyl, and methyl groups gave rise to different small-molecule potentiators with varying micromolar cytotoxicity. Based on this, it could be concluded that the enone functionality is not only responsible for cytotoxicity, but also potentiation. To explore this further, the enone in **22** was reduced to **31** in 99% yield (Scheme 3). Noting the single digit μM cytotoxicity observed with **22**, the complete loss of activity for **31** was unexpected. Uvaretin, the parent natural product that spurred these investigations, is a 2.0–3.7 μM cytotoxic compound that possesses no enone functional group. Given that uvaretin does not possess an enone group, we speculate that the *o*-hydroxyl benzyl group along with the phenolic central ring is responsible for its cytotoxicity. To probe the effects of the reduced enone portion of the chalcone, noting that no sole cytotoxicity was observed, potentiating studies were undertaken. A mild synergism, combinational index of 0.72, was observed with **31** and 6TP in MIA PaCa-2 suggesting that the reduced enone portion could be responsible for potentiation character or transport.

To exploit the possibility that the enone and *o*-hydroxy benzyl group are responsible for cytotoxicity and the reduced enone responsible for potentiation and transport properties, our focus shifted towards the construction of a hybrid trimethylated uvaretin analog possessing all of these attributes. Diacetylation of phloroglucinol was accomplished with acetic anhydride and boron trifluoride etherate accessing **32** in 52% yield, which was subsequently trimethylated with dimethyl sulfate in acetone to afford **33** in quantitative yield (Scheme 4). Aldol condensation upon **33** with excess equivalences of benzylaldehyde and potassium hydroxide furnished chalcone **34** in 82% yield. Subjecting **34** to Friedel–Crafts alkylation conditions with **17** furnished the dienone/*o*-allyloxybenzyl chalcone **35** in 46% yield, followed by allyl deprotection with $Pd(PPh_3)_4$ and potassium carbonate in methanol to afford **36** in 90% yield. Full reduction of the enones within **34** gave access to **37** in quantitative yield, subjection to Friedel–Crafts alkylation conditions with **17** afforded **38** in 29% yield. Allyl deprotection upon **38** was troublesome, as such, reduction of the enones in **36** was accomplished to give access to **39**. Biological assessment of these compounds, discussed below, lead to the construction of a hybrid chalcone possessing both an enone and reduced enone. Accessing the enone/reduced enone system was undertaken by treating **33** to Aldol Condensation with benzaldehyde (0.9 equiv.) to access **40** in 69% yield. Reduction of the enone was accomplished with palladium on carbon in 71% yield followed by Aldol Condensation to afford **41** in 45% yield. Unfortunately, all attempts to include the *o*-allyloxy benzyl alcohol upon **41** failed.

Screening **34**–**41** for sole agent cytotoxicity against the four panel cancerous cell lines used in this work was then undertaken (Table 3). The di-enone chalone (**34**) possessed similar IC_{50} values in comparison to its mono-enone chalcone counterpart **22**, suggesting that the presence of a second enone does not

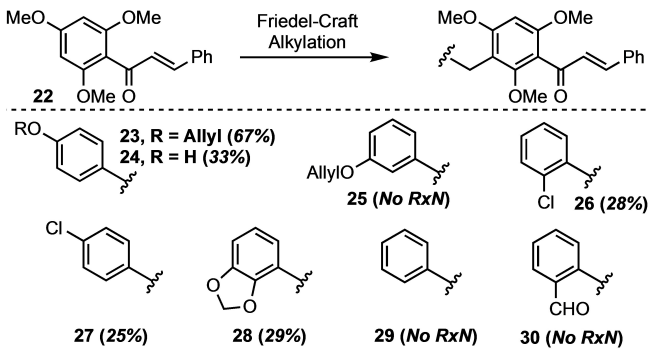
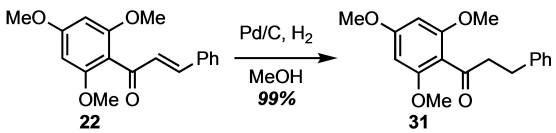
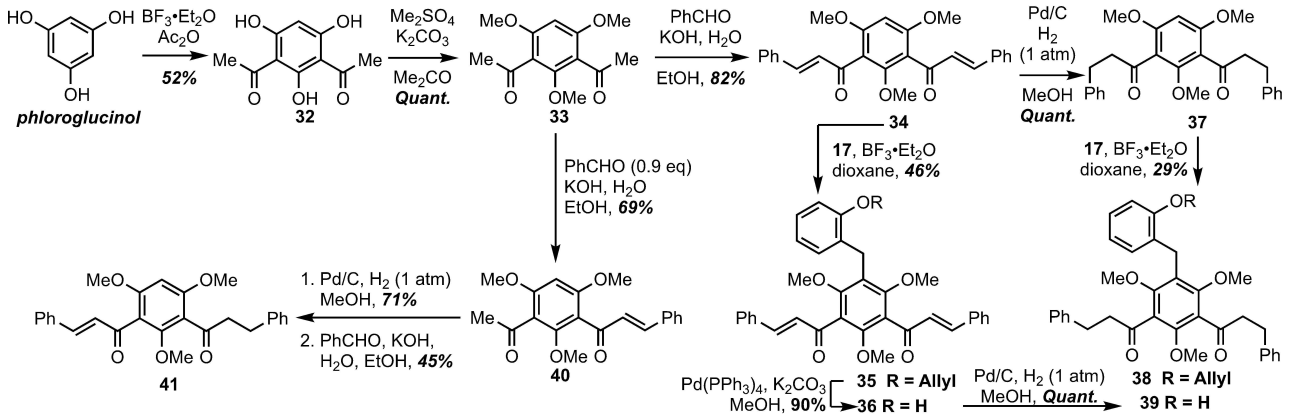


Figure 2. Friedel–Crafts alkylation attempts upon **22** with varied substituted benzyl alcohol systems.



Scheme 3. Enone reduction for potentiation and cytotoxicity comparisons.



Scheme 4. Construction of hybrid chalcone and reduced enone chalcones.

Table 3. Evaluation of chalcones **34**–**41** for cytotoxicity and potentiation properties.

Cmpd	Observed IC_{50} [μM][a]				C.I.[b]
	HeLa	U937	A549	MIA PaCa-2	**6TP**
22	5.2 ± 1.4	6.7 ± 0.8	4.3 ± 0.9	> 20	N.T.
34	4.7 ± 2.4	5.4 ± 0.7	3.7 ± 1.1	18.4 ± 5.8	1.51
35	1.1 ± 0.5	1.7 ± 1.2	0.8 ± 0.9	12.4 ± 8.2	1.78
36	1.0 ± 0.9	2.0 ± 0.8	1.5 ± 1.5	13.5 ± 4.7	1.67
37	19.7 ± 5.1	> 20	18.0 ± 2.4	> 20	N.T.
38	13.4 ± 5.0	16.7 ± 6.2	11.8 ± 1.9	13.9 ± 2.5	1.18
39	11.8 ± 2.1	15.8 ± 4.8	12.0 ± 3.7	15.0 ± 3.2	1.04
41	4.7 ± 2.0	4.8 ± 3.1	3.7 ± 2.9	19.2 ± 7.3	0.42

[a] Cytotoxicity was evaluated in 384-well plates over a 72 h treatment. Adherent cells were seeded at 1500 cells/well, whereas suspension cells at 2200 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. [b] Cytotoxicity evaluation for combinational index (C.I.) studies were the same as for sole agent cytotoxicity, and the C.I. was determined based on the Chou-Talalay protocol.[10] N.T.: not tested and n = three biological replicates.

increase the cytotoxic character of the chalcone. Incorporation of the *o*-allyloxy benzyl group did translate into an across-the-board increase in cytotoxicity. However, deprotection of the allyl group did not significantly affect cytotoxic properties. A near complete loss of cytotoxicity was observed in the reduced di-enone **37**, *o*-allyloxy benzyl incorporation (**38**) did show a marginal increase in cytotoxicity relative to **37**. Like with **35** and **36**, de-allylated **39** showed non-significant changes in cytotoxicity relative to allylated **38**. Investigating possible potentiating properties of **34**–**39** with 6TP in MIA PaCa-2 were concluded to investigate, and elucidate, the possible role of the enone and reduced enone. All enone containing compounds (**34**–**36**) were found to possess antagonism, non-synergist relationships. In contrast, the reduced enones (**38** and **39**) were shown to possess borderline mild synergism. These biological investigations led support to our initial theory that both the enone and *o*-allyloxy and hydroxy benzyl groups tend to be responsible for sole agent cytotoxicity and the reduced enone supports potentiation properties. Evaluation of the enone/reduced enone hybrid **41** for sole agent cytotoxicity revealed cytotoxicity similar to **34** and **22**. Most excitingly, potentiation of **34** with 6TP in MIA Paca-2 was shown to have mild to strong synergism, while also possessing sole cytotoxic properties.

Conclusion

The construction of new small-molecule chemical libraries based upon the intermediates of our total synthesis route towards the uvaretin class of natural products is disclosed. Building from our previous efforts, further structure–activity relationship (SAR) studies were performed upon the sole chalcone core, possessing no benzylated system. Previously, we found interesting potentiation properties for the MOM protected chalcone system **3**–**5**, of which **4** was shown to have good synergistic properties towards 6TP in the pancreatic cell line MIA PaCa-2; 6TP has no reported activity in this cell line. In the efforts to optimize the potentiation properties of these chalcones, we constructed **13**–**16** possessing various states of methyl, ethyl, and single MOM group inclusion upon the triphenol ring. It was found that increasing the hydrophobic character upon the triphenol ring led to an overall decrease in cytotoxicity. Unexpectedly, the dimethylated mono-MOM protected **16**, while showing moderating cytotoxicity, was found to possess strong synergistic properties with both 6TP and doxorubicin in comparison to its more hydrophobic counterparts **13**–**15**. Through our previous efforts, and the ones disclosed herein, the role of the benzyl group was explored. The triethylated chalcone (**15**) was observed to have little to no cytotoxicity, however when incorporation of the *o*-hydroxybenzyl to form **18**/**19** gave access to cytotoxic activity. Thus, suggesting that the *o*-hydroxybenzyl group possess cytotoxicity in its own right, thus helping to explain the cytotoxic activity of uvaretin. Through these efforts, the identification of the enone as the bioactive portion of the chalcone was further confirmed, and through the libraries constructed and evaluated it was observed that the reduced enone systems, while possessing marginal cytotoxicity, possess potentiation properties. In the efforts to merge these two characteristics, we set forth to construct our final library set of eight compounds, **34**–**41**. Sole

agent cytotoxicity was observed with dienones **34**–**37**, and potentiation properties with the dual reduced enones **37**–**39**. Construction of the hybrid enone/reduced enone **41** was accomplished and was shown to possess single digit μM cytotoxicity in all cell lines screened, except MIA PaCa-2, and retained strong synergistic properties with 6TP in MIA PaCa-2. We can conclude that small chemical library construction, as well as SAR studies upon said libraries, has led to the identification of new and interesting small molecules with diverse biological activities.

Experimental Section

Chemistry and general methods

All reagents were commercially available and used without purification unless otherwise stated. NMR spectra were recorded with a Varian and Bruker 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), and methanol (CH_3OH) 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.

General procedures

Aldol condensation. To a round bottom flask (RBF) was added the acetophenone species (1.0 equiv.), benzaldehyde (1.0 equiv., unless otherwise noted), and EtOH (20 mL) and brought to 0 °C. Once at temperature, a solution of KOH (17 equiv.) 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 (×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.

Friedel–Crafts alkylation. To a flamed dried RBF under an argon atmosphere was added chalcone (1.0 equiv.) and dry dioxane (final concentration of 46 mM) and stirred at RT. In a second flamed dried RBF was added **17**[6] (1.2 equiv.) in dry dioxane (final concentration of 0.22 M), which after stirring for 5 min at RT was added to the chalcone mixture. To a separate flamed dried RBF under an argon atmosphere was added $BF_3 \cdot Et_2O$ (3.05 equiv.) 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 (×2), and extracted with EtOAc (×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.

Allyl deprotection. To a flamed dried RBF under an argon atmosphere was added chalcone species (1.0 equiv.), K_2CO_3 (6 equiv.), and $Pd(PPh_3)_4$ (0.02 equiv.) 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. equiv. to initial MeOH), and concentrated to afford the desired product.

Enone reduction. To a flamed dried RBF under an argon atmosphere was added the enone material (1.0 equiv.) 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 at 25 °C for 2 h, filtered through Celite, and rinsed with MeOH or $CHCl_3$. The filtrate was concentration to afford the ketone species.

Compound synthesis

*2,4,6-Trihydroxyacetophenone (**9**):*[11] $BF_3 \cdot OEt_2$ (7.5 mL, 59.4 mmol) and acetic anhydride (1.9 mL, 19.8 mmol) followed by phloroglucinol (2.5 g, 19.8 mmol) was added to a RBF under argon, and the mixture was 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, CD_3OD) δ 5.79 (s, 2H), 2.59 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 204.56, 166.32, 165.90, 105.60, 95.59, 32.71.

*2,4,6-Trimethyoxyacetophenone (**10**):*[12] Compound **9** (0.5 g, 2.98 mmol), K_2CO_3 (2.0 g, 14.9 mmol), and anhydrous acetone (10 mL) were added to a flamed RBF under argon. The reaction mixture was stirred 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 the mixture was extracted with EtOAc (×3). The organic layers were combined, washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatograph (Hex/EtOAc 1:1) 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): 202.1, 162.5, 158.6, 113.8, 90.7, 56.0, 55.7, 32.8.

*2,4-Dimethoxy-6-hydroxy-acetephone (**11**) and 2-Methoxy-4,6-dihydroxy-acetophenone (**12**):* To a flamed-dried RBF was added **10** (0.5 g, 2.38 mmol) and PhCl (24 mL) followed by $AlCl_3$ (0.64 g, 4.8 mmol), and the mixture was left to stir o/n at RT. The mixture was then brought to 70 °C and stirred for an additional 24 h. The reaction was quenched with saturated Rochelle Salt, and the mixture was stirred for 30 min, then extracted with EtOAc (×3). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to afford **11** (126 mg) in 27% yield and **12** (134 mg) in 31% yield. Compound **8**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.90 (1H, d, J = 2.1 Hz), 3.84 (3H, s), 3.80 (3H, s), 2.60 (3H, s). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 203.97, 166.83, 166.41, 162.85, 105.60, 92.14, 32.98. Compound **9**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 8.30 (s, 1H), 6.03 (d, J = 2.0 Hz, 1H), 5.99 (d, J = 2.0 Hz, 1H), 3.91 (s, 3H), 2.59 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 204.0, 166.7, 165.2, 164.6, 106.5, 96.8, 92.0, 56.0, 33.0.

*(E)-1-(2,4-Diethoxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one (**13**):* The general procedure for aldol condensation was followed using **12a** (0.3 g, 1.26 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **13** (382 mg, pale-white solid) in 93% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67–7.58 (m, 3H), 7.55–7.44 (m, 3H), 7.30 (d, J = 15.0 Hz, 1H), 6.56 (dd, J = 15.6, 2.0 Hz, 2H), 4.39 (q, J = 5.9 Hz, 2H), 4.06 (q, J = 5.9 Hz, 2H), 3.91 (s, 3H), 1.65 (t, J = 5.9 Hz, 3H), 1.34 (t, J = 5.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.6, 162.4, 162.1, 161.9, 142.9,

135.5, 129.8, 129.0, 128.6, 126.1, 112.1, 95.4, 92.8, 65.2, 63.9, 56.0, 14.7, 14.2. LRMS (ESI): *m/z* calcd for $C_{20}H_{23}O_4^+$: $[M+H]^+$ 327.15, found 327.16.

*(E)-1-(2-Ethoxy-4,6-dimethoxyphenyl)-3-phenylprop-2-en-1-one (**14**):* The general procedure for aldol condensation was followed using **11 a** (0.37 g, 1.65 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **14** (423 mg, white solid) in 82% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67–7.60 (m, 3H), 7.54–7.43 (m, 6H), 7.29 (d, $J=15.1$ Hz, 1H), 6.50 (s, 2H), 4.39 (q, $J=5.9$ Hz, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 1.65 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.6, 165.4, 162.6, 162.0, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 111.2, 95.9, 93.8, 65.2, 56.0, 14.2. LRMS (ESI): *m/z* calcd for $C_{20}H_{21}O_4^+$: $[M+H]^+$ 313.14, found 313.14.

(E)-3-Phenyl-1-(2,4,6-triethoxyphenyl)prop-2-en-1-one (): The general procedure for aldol condensation was followed using **9 a** (0.28 g, 1.11 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **15** (332 mg, white solid) in 88% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.68–7.59 (m, 3H), 7.56–7.42 (m, 3H), 7.30 (d, $J=15.1$ Hz, 1H), 6.57 (s, 2H), 4.39 (q, $J=5.9$ Hz, 4H), 4.06 (q, $J=5.9$ Hz, 2H), 1.65 (t, $J=5.9$ Hz, 6H), 1.34 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.7, 162.5, 162.0, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 113.9, 95.2, 65.2, 63.9, 14.6, 14.2. LRMS (ESI): *m/z* calcd for $C_{21}H_{24}O_4^+$: $[M+H]^+$ 340.17, found 340.17.

*(E)-1-(2,4-Dimethoxy-6-(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one (**16**):* The general procedure for aldol condensation was followed using **11 b** (0.3 g, 1.25 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **16** (348 mg, white solid) in 85% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65–7.59 (m, 3H), 7.54–7.44 (m, 3H), 7.31 (d, $J=15.1$ Hz, 1H), 6.52 (d, $J=2.0$ Hz, 1H), 6.51 (d, $J=1.8$ Hz, 1H), 6.02 (s, 2H), 3.91 (s, 3H), 3.80 (s, 3H), 3.24 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.6, 165.3, 161.4, 142.9, 135.5, 129.8, 129.0, 128.6, 126.1, 110.8, 96.0, 95.2, 94.4, 56.4. LRMS (ESI): *m/z* calcd for $C_{19}H_{21}O_5^+$: $[M+H]^+$ 329.14, found 329.14.

*(E)-1-(3-(2-(Allyloxy)benzyl)-2,4-diethoxy-6-methoxyphenyl)-3-phenylprop-2-en-1-one (**18**):* The general procedure for Friedel–Crafts alkylation was followed using **13** (0.2 g, 0.61 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **18** (118 mg, white solid) in 41% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.65–7.60 (m, 3H), 7.53–7.44 (m, 3H), 7.26 (d, $J=15.1$ Hz, 1H), 7.22 (ddt, $J=7.4$, 2.0, 1.0 Hz, 1H), 7.00 (td, $J=7.5$, 2.0 Hz, 1H), 6.90 (td, $J=7.4$, 2.1 Hz, 1H), 6.81 (dd, $J=7.4$, 2.0 Hz, 1H), 6.77 (s, 1H), 6.05 (ddt, $J=16.3$, 10.1, 6.2 Hz, 1H), 5.58 (ddt, $J=13.9$, 10.1, 1.1 Hz, 1H), 5.33 (ddt, $J=16.7$, 13.7, 0.9 Hz, 1H), 4.57 (dt, $J=6.2$, 1.1 Hz, 2H), 4.34 (q, $J=8.0$ Hz, 2H), 4.08 (q, $J=8.0$ Hz, 2H), 3.91 (s, 3H), 3.90 (d, $J=1.1$ Hz, 2H), 1.64–1.58 (m, 3H), 1.43 (t, $J=8.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.1, 162.1, 161.5, 160.3, 156.2, 142.1, 135.5, 133.0, 131.0, 129.8, 129.3, 129.0, 128.6, 128.2, 16.0, 121.6, 117.8, 114.0, 113.8, 113.6, 97.4, 69.2, 68.5, 65.0, 56.2, 23.0, 15.0, 14.5. LRMS (ESI): *m/z* calcd for $C_{30}H_{33}O_5^+$: $[M+H]^+$ 473.23, found 473.22.

*(E)-1-(2,4-Diethoxy-3-(2-hydroxybenzyl)-6-methoxyphenyl)-3-phenylprop-2-en-1-one (**19**):* The general procedure for allyl deprotection was followed using **18** (93 mg, 0.19 mmol) to give **19** (82 mg, pale yellow oil) in 96% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.68–7.63 (m, 3H), 7.54–7.44 (m, 3H), 7.35 (d, $J=15.1$ Hz, 1H), 7.28 (ddt, $J=7.4$, 2.0, 1.0 Hz, 1H), 7.14 (td, $J=7.5$, 2.0 Hz, 1H), 6.93 (td, $J=7.5$, 2.0 Hz, 1H), 6.85 (dd, $J=7.5$, 2.0 Hz, 1H), 6.71 (s, 1H), 4.34 (q, $J=5.9$ Hz, 2H), 4.08 (q, $J=5.9$ Hz, 2H), 3.91 (s, 3H), 3.90 (d, $J=1.1$ Hz, 2H), 1.61 (t, $J=5.9$ Hz, 3H), 1.43 (t, $J=5.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 192.1, 162.1, 161.5, 160.3, 154.2, 142.1, 135.5, 130.2, 129.8, 129.0, 128.6, 128.3, 126.9, 126.0, 120.9, 115.8, 97.4, 68.5, 65.0, 56.2, 22.9, 15.0, 14.5. LRMS (ESI): *m/z* calcd for $C_{27}H_{29}O_5^+$: $[M+H]^+$ 433.12, found 433.12.

*(E)-1-(3-(2-(Allyloxy)benzyl)-2,4,6-trihydroxyphenyl)-3-phenylprop-2-en-1-one (**20**)*: The general procedure for Friedel–Crafts alkylation was followed using **3** (0.3 g, 0.77 mmol). The crude material was purified by flash silica gel chromatography (Hex/EtOAc 7:3) to give **20** (171 mg, white solid) in 55% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.70–7.65 (m, 3H), 7.63 (dt, $J=15.0$, 0.9 Hz, 1H), 7.54–7.35 (m, 5H), 7.00 (td, $J=7.5$, 2.1 Hz, 1H), 6.95 (td, $J=7.4$, 2.2 Hz, 1H), 6.83 (dd, $J=7.4$, 2.2 Hz, 1H), 6.27 (s, 1H), 6.05 (ddt, $J=16.3$, 10.1, 6.2 Hz, 1H), 5.58 (ddt, $J=13.9$, 10.1, 1.1 Hz, 1H), 5.33 (ddt, $J=16.7$, 13.7, 0.9 Hz, 1H), 4.57 (dt, $J=6.2$, 1.1 Hz, 2H), 3.90 (d, $J=1.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 193.5, 164.1, 162.4, 160.6, 156.2, 142.1, 135.5, 133.0, 131.0, 129.8, 129.3, 129.0, 128.6, 128.2, 126.0, 121.6, 117.8, 114.0, 107.1, 105.3, 98.2, 69.2, 23.1. LRMS (ESI): *m/z* calcd for $C_{25}H_{23}O_5^+$: $[M+H]^+$ 403.15, found 403.14.

*(E)-3-Phenyl-1-(2,4,6-trihydroxy-3-(2-hydroxybenzyl)phenyl)prop-2-en-1-one (**21**):* The general procedure for allyl deprotection was followed using **20** (88 mg, 0.22 mmol) to give **21** (71 mg, pale oil) in 90% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.67–7.43 (m, 7H), 7.20 (ddt, $J=7.5$, 2.1, 1.1 Hz, 1H), 7.12 (td, $J=7.4$, 2.0 Hz, 1H), 6.91 (td, $J=7.5$, 2.0 Hz, 1H), 6.78 (dd, $J=7.4$, 2.0 Hz, 1H), 6.22 (s, 1H), 3.90 (d, $J=1.1$ Hz, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 193.5, 164.1, 162.4, 160.6, 154.2, 142.1, 135.5, 130.2, 129.8, 129.0, 128.6, 128.3, 126.8, 126.0, 120.9, 115.8, 107.1, 105.3, 98.2, 22.8. LRMS (ESI): *m/z* calcd for $C_{22}H_{19}O_5^+$: $[M+H]^+$ 363.12, found 363.13.

*1,1′-(2,4,6-Trihydroxy-1,3-phenylene)bis(ethan-1-one) (**32**):* To a flame dried RBF under an argon atmosphere was added $BF_3{\cdot}OEt_2$ (0.44 mL, 3.56 mmol) and acetic anhydride (0.12 mL, 1.19 mmol) under argon and left to stir for 30 min at RT. The solution was added dropwise over 10 min to a flame dried RBF charged with phloroglucinol (0.1 g, 0.59 mmol) under argon and left to stir at RT overnight. The reaction was quenched with a 10% sodium acetate (2.8 mL) solution and left to stir overnight. The mixture was filtered, rinsed with water and air dried to obtain **32** (64 mg, white solid) in 52% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 5.82 (s, 1H), 2.73 (s, 8H). LRMS (ESI): *m/z* calcd for $C_{10}H_{10}O_5Na^+$: $[M+Na]^+$ 233.04, found 233.03.

*1,1′-(2,4,6-Trimethoxy-1,3-phenylene)bis(ethan-1-one) (**33**):* To a flamed dried RBF under argon was added acetone (3.2 mL), **32** (0.2 g, 0.95 mmol) and potassium carbonate (0.66 g, 4.76 mmol) followed by dimethyl sulfate (0.9 mL, 9.52 mmol) and left to stir at RT for 12 h and then brought to reflux for 4 h. The mixture was allowed to cool to RT and then was quenched with $NH_4Cl_{(satd)}$ and extracted with EtOAc (x2). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 3:1) to afford **33** (239 mg, white solid)in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.19 (s, 1H), 3.78 (s, 6H), 3.64 (s, 3H), 2.41 (s, 6H). LRMS (ESI): *m/z* calcd for $C_{13}H_{17}O_5^+$: $[M+H]^+$ 253.10, found 253.10.

*(2E,2′E)-1,1′-(2,4,6-Trimethoxy-1,3-phenylene)bis(3-phenylprop-2-en-1-one) (**34**):* To a RBF was added **33** (0.20 g, 0.79 mmol), benzylaldehyde (0.16 mL, 1.58 mmol), and ethanol (10.5 mL). The mixture was cooled to 0 °C to which a solution of KOH (1.51 g, 2.55 mmol) in water (3.16 mL) was added dropwise and left to stir overnight while warming to RT. The reaction mixture was quenched by acidification with 1 M HCl and extracted with EtOAc (x3). The organic layers were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified by flash silica gel chromatography (Hex/EtOAc 3:1) to afford **34** (277 mg, yellow solid) in 82% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.61–7.53 (m,

4H), 7.50–7.38 (m, 7H), 7.05 (d, $J=16.1$ Hz, 2H), 6.37 (s, 2H), 3.89 (s, 6H), 3.71 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 193.6, 159.6, 159.5, 157.1, 145.2, 134.6, 130.6, 128.9, 128.6, 128.5, 116.2, 91.1, 63.4, 56.1. LRMS (ESI): *m/z* calcd for $C_{27}H_{25}O_5^+$: $[M+H]^+$ 429.17, found 429.18.

*(2E,2′E)-1,1′-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis (3-phenylprop-2-en-1-one) (**35**):* The general procedure for Friedel–Crafts alkylation was followed using **34** (0.1 g, 0.23 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 9:1, 5:1, to 7:3) to afford **35** (60 mg, white solid) in 46% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.47–7.42 (m, 6H), 7.33 (d, $J=16.1$ Hz, 2H), 7.30–7.26 (m, 4H), 6.96 (d, $J=16.0$ Hz, 1H), 6.83–6.62 (m, 3H), 6.07–5.97 (m, 1H), 5.40 (dq, $J=17.3$, 1.7 Hz, 1H), 5.20 (dt, $J=10.6$, 1.6 Hz, 1H), 4.53 (dt, $J=5.0$, 1.7 Hz, 2H), 3.94 (s, 2H), 3.76 (s, 3H), 3.72 (s, 3H), 3.44 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 194.6, 160.6, 158.0, 157.1, 156.3, 144.7, 134.9, 133.8, 133.0, 130.3, 129.8, 129.6, 128.89, 128.84, 127.8, 126.5, 120.9, 120.4, 117.7, 116.8, 116.5, 116.4, 114.2, 114.2, 111.5, 111.2, 91.5, 68.8, 68.7, 62.9, 62.2, 56.0, 55.8, 22.8. LRMS (ESI): *m/z* calcd for $C_{37}H_{35}O_6^+$: $[M+H]^+$ 575.24, found 575.24.

*(2E,2′E)-1,1′-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis (3-phenylprop-2-en-1-one) (**36**):* The general procedure for allyl deprotection was followed using **35** (26 mg, 0.05 mmol) to afford **36** (60 mg, white solid) in 46% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.72–7.63 (m, 3H), 7.59–7.42 (m, 6H), 7.41–7.30 (m, 6H), 7.08 (td, $J=7.7$, 1.8 Hz, 2H), 6.99 (d, $J=16.1$ Hz, 2H), 6.86–6.75 (m, 2H), 3.91 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 194.1, 159.6, 157.3, 157.2, 155.8, 154.9, 145.5, 134.7, 13.0, 132.0, 131.98, 131.96, 131.6, 130.5, 128.9, 128.5, 128.33, 128.29, 127.8, 125.7, 119.7, 116.3, 116.10, 116.08, 114.02, 114.00, 92.1, 63.6, 56.1, 55.9, 29.7, 24.4. LRMS (ESI): *m/z* calcd for $C_{34}H_{30}O_6Na^+$: $[M+Na]^+$ 557.19, found 557.19.

*1,1′-(2,4,6-Trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**37**):* The general procedure enone reduction was followed using **34** (25 mg, 0.058 mmol) to give afford **37** (25 mg, white solid) in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.25–7.04 (m, 10H), 6.12 (s, 1H), 3.71 (s, 6H), 3.46 (s, 3H), 3.01 (ddd, $J=8.4$, 6.9, 1.7 Hz, 4H), 2.93 (ddd, $J=8.6$, 6.8, 1.7 Hz, 4H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 202.7, 158.70, 158.67, 155.8, 141.2, 128.50, 128.45, 128.37, 128.32, 128.2, 126.0, 118.29, 118.28, 91.1, 64.4, 55.9, 46.4, 29.8. LRMS (ESI): *m/z* calcd for $C_{27}H_{29}O_5^+$: $[M+H]^+$ 433.20, found 433.20.

*1,1′-(5-(2-(Allyloxy)benzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**38**):* The general procedure for Friedel–Crafts alkylation was followed using **37** (83 mg, 0.19 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 9:1, 5:1, 7:3, to 1:1) to afford **38** (32 mg, white solid) in 29% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.24–6.94 (m, 8H), 6.92–6.57 (m, 4H), 6.11–5.90 (m, 3H), 5.41–5.33 (m, 1H), 5.26–5.10 (m, 1H), 4.64 (s, 2H), 4.52 (ddt, $J=5.3$, 3.4, 1.6 Hz, 4H), 3.88 (s, 2H), 3.72 (d, $J=2.0$ Hz, 3H), 3.67 (s, 3H), 3.37 (s, 3H), 3.09–3.01 (m, 2H), 2.97–2.90 (m, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 204.0, 160.5, 157.3, 156.5, 154.5, 141.5, 134.0, 133.5, 133.0, 132.6, 131.2, 129.7, 129.4, 129.0, 128.5, 127.4, 127.0, 120.9, 120.6, 118.3, 117.7, 116.9, 114.3, 111.7, 111.5, 111.4, 91.4, 68.9, 63.2, 55.8, 46.4, 35.1, 30.0. LRMS (ESI): *m/z* calcd for $C_{37}H_{39}O_6^+$: $[M+H]^+$ 579.27, found 579.27.

*1,1′-(5-(2-Hydroxybenzyl)-2,4,6-trimethoxy-1,3-phenylene)bis(3-phenylpropan-1-one) (**39**):* The general procedure for enone reduction was followed using **36** (8 mg, 0.015 mmol) to afford **39** (7 mg, white solid) in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.86–7.76 (m, 1H), 7.75–7.65 (m, 2H), 7.63–7.52 (m, 2H), 7.49–7.31 (m, 2H), 7.25–7.02 (m, 4H), 7.29–6.98 (m, 4H), 6.88–6.74 (m, 4H), 3.92 (s, 3H), 3.81 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.11 (t, $J=8.2$ Hz, 2H), 3.02 (t, $J=7.6$ Hz, 2H). LRMS (ESI): *m/z* calcd for $C_{34}H_{34}O_6Na^+$: $[M+Na]^+$ 561.23, found 561.23.

*(E)-3-Phenyl-1-(2,4,6-trimethoxy-3-(3-phenylpropanoyl)phenyl)prop-2-en-1-one (**41**):* The general procedure for enone reduction was followed using **40** (70 mg, 0.21 mmol) to give afford the reduced enone in 71% yield. The general procedure for aldol condensation was followed using the formed reduced enone (50 mg, 0.19 mmol). The crude material was purified by flash silica gel chromatography (gradient Hex/EtOAc: 100:0, 5:1, 7:3, 3:2, to 1:1) to give **38** in 45% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.50–7.44 (m, 2H), 7.33–7.29 (m, 4H), 7.23–7.14 (m, 3H), 7.14–7.06 (m, 2H), 6.92 (d, $J=16.0$ Hz, 1H), 6.20 (s, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.55 (s, 3H), 3.09–3.01 (m, 2H), 3.00–2.92 (m, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ (ppm): 202.8, 193.4, 159.4, 158.8, 156.5, 145.2, 141.2, 134.6, 130.6, 129.6, 128.9, 128.6, 128.50, 128.46, 128.4, 128.3, 126.0, 118.2, 116.3, 91.1, 63.8, 56.1, 56.0, 46.4, 29.8. LRMS (ESI): *m/z* calcd for $C_{27}H_{27}O_5^+$: $[M+H]^+$ 431.18, found 431.18.

Biological

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.

IC_{50} value determination for adherent cells using Alamar Blue. Adherent cells were added to 384-well plated (1500 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. Doxorubicin 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)

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/well) in media (10 μL) were added after 40 μL of compound in medium were added to the 384-well plate. No time was given to allow cells to adhere.

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 independently. To each well on the plate was added two different concentration of each compound and five concentrations of 6TP or doxorubicin (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/well (adherent cells) and 7000 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.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords: library construction · medicinal chemistry · natural products · small-molecule potentiating agents

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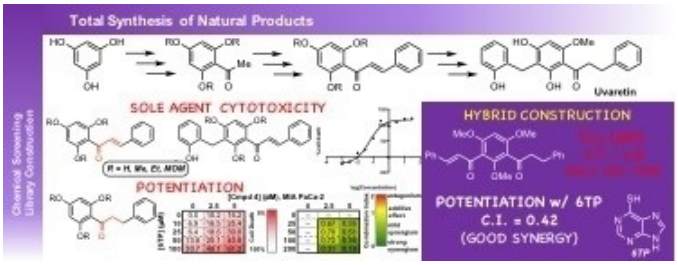

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Discovering therapeutic agents: New bioactive agents, either as sole or combinational agents, have been constructed through the synthetic manipulation of the intermediates within the total synthesis of the uvaretin class of natural products. It was found that increasing the hydrophobic character of the phenolic core correlates to a decrease in sole agent cytotoxicity. The synthesis of new, small chemical screening libraries (CSL) constructed from the intermediates of our total synthesis route of the uvaretin class of natural products is demonstrated herein. Numerous chalcone-based CSLs with various substitution on the phenolic groups within the chalcone core were assembled. Through cytotoxicity investigations, it was found that the level of hydrophobicity of the phenolic core of the chalcones gives biases: less cytotoxicity with more hydrophobic cores. In addition, it was observed that the potentiation, evaluated with 6-thiopurine in the pancreatic cancer cell line MIA PaCa-2, is tunable by the inclusion of less-hydrophobic character on the phenolic core. The role of the *o*-hydroxybenzyl group, present within the uvaretin family, was revealed to be cytotoxic in character. Merging all of the structure–activity relationship studies performed on the CSLs constructed in this effort led to the construction of a new chalcone hybrid possessing both a cytotoxic enone group and a small-molecule-potentiating, reduced enone group.