Benzil Based Inhibitors of Carboxylesterases

Elizabeth Parkinson

Department of Chemistry
Rhodes College
Memphis, Tennessee

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Dr. Loretta Jackson-Hayes
Project Advisor

_____

Dr. Kimberly Fisher
Second Reader

_____

Dr. Philip Potter
Extra-Departmental Reader

_____

Dr. Jon Russ
Department Chair

_____

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Lyudmila Tsurkan

Latorya D. Hicks

Jason M. Hatfield

Carol C. Edwards

Monica Weirdl

Bing Yan

Hongyu Zhuo

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ABSTRACT

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by

Elizabeth Ivy Parkinson

Toxicity of chemotherapeutic agents is a major problem which often limits the ability to treat patients. One chemotherapeutic drug, irinotecan (CPT-11), causes diarrhea which is the dose limiting toxicity for this agent. This toxicity is thought to be caused by high levels of the active metabolite (SN-38) produced in the intestine. High levels of SN-38 exist due to the presence of carboxylesterase enzymes (CE) in this tissue that convert CPT-11 to SN-38. Therefore, identification of specific CE inhibitors that could ameliorate the delayed diarrhea associated with this agent, by reducing the amount of SN-38 produced in the intestine, may improve chemotherapy with this drug. Benzil (phenylethane-1,2-dione) was previously found to be a potent inhibitor of human CEs. Recently, two types of benzil derivatives, alkyl diones and molecules with atoms inserted between the phenyl ring and the dione, have demonstrated the necessity of hydrophobicity of these molecules for enzyme inhibition. In this study, we investigated the ability of a new set of compounds, 1-phenyl-1,2-alkyl diones, to inhibit CEs. These compounds consist of a 1,2-dione with a phenyl moiety on one side and an alkyl chain on the other. These compounds further support the link between inhibitor hydrophobicity and CE inhibition.

MAIN TEXT

INTRODUCTION

Carboxylesterases (CE) are ubiquitous enzymes found in a wide range of organisms, from humans to bacteria (Redinbo, 2005). Two main CEs exist in humans, human liver CE (hCE1) and human intestinal CE (hiCE). hCE1 is expressed mostly in the liver, while hiCE is found in the liver, small intestine, kidney, and both heart and skeletal muscle (Redinbo, 2005). CEs catalyze the hydrolysis of ester-containing xenobiotics using a catalytic serine within a Ser-His-Glu triad at the base of a long active site gorge (Potter, 2006). Some of the esters hydrolyzed by CEs include pesticides such as malathion, nerve gases like sarin and tabun, and many clinically used drugs, specifically analgesics like aspirin, narcotics such as cocaine, and cancer chemotherapeutic agents (Redinbo, 2005; Bencharit, 2002). The products that result from this reaction are the respective alcohol and carboxylic acid (Potter, 2006).

One of the CE substrates is the chemotherapy agents, CPT-11 (irinotecan, Camptosar). CPT-11 is a prodrug that is hydrolyzed to its active metabolite (SN-38), a potent topoisomerase I inhibitor (Wadkins, 2004). CPT-11 and SN-38 are both cleared from circulation via the bile duct which drains into the intestine (Wadkins, 2004). Because high levels of hiCE are expressed in the small intestine, high concentrations of SN-38 are produced in this tissue following administration to cancer patients. These high concentrations of SN-38 result in diarrhea, 48-96 hours following treatment, which is the dose limiting toxicity for CPT-11 (Wadkins, 2004). Therefore, identifying CE inhibitors

which could be used in combination with CPT-11 to ameliorate this delayed diarrhea may have clinical utility.

Using Telik's lead technology TRAP (target-related affinity profiling), benzil (Figure 1) was previously found to be a potent selective CE inhibitor (Wadkins, 2005). Additionally, benzil analogues (Figure 1) were studied in order to determine the necessity of the 1,2-dione and the phenyl rings for inhibition (Wadkins, 2005). Compounds without the 1,2-dione such as benzoin (**A,** Figure 1) had a significant reduction or complete loss of enzyme inhibition. The necessity of the dione for CE inhibition likely relates to the fact that the carbonyl carbon-carbon bond mimics an ester (Wadkins, 2005). These compounds likely inhibit in the following way: A deprotonated serine residue present within the active site attacks the carbonyl bond in the dione, resulting in a tetrahedral carbon intermediate similar to that seen following nucleophilic attack of esters. However, the carbon-carbon bond in the inhibitor is significantly less polarized than the carbon-oxygen bond found in esters, and would be unlikely to undergo cleavage. This results in enzyme inhibition due to the repetitive attack and release of the dione within the active site (Wadkins, 2005). The phenyl rings were also found to play an important role in enzyme inhibition. Specifically, compounds lacking the phenyl moiety, such as butane-2,3-dione (Figure 1), did not inhibit CEs. The CE active site gorge is lined with aromatic amino acids, resulting in a highly hydrophobic environment and allowing for preferential localization of like molecules (Redinbo, 2005). The lack of inhibition by butane-2,3-dione is most likely due to the lesser hydrophobicity of this molecule compared to benzil. More recently, the importance of the hydrophobic nature of the inhibitors was explored in benzil analogues containing a spacer atom between the

1,2-dione and the phenyl rings (Figure 1). When the spacer atom was a carbon (**B,** Figure 1), little inhibition was observed. However, when the spacer was a CBrH (**C**, Figure 1), the molecule demonstrated greater potency. Because bromine increases the hydrophobicity of the molecule, this further supports the importance of this parameter towards enzyme inhibition. Finally, a recent study investigated the necessity of the phenyl ring for inhibition. A series of alkyl-1,2-diones were produced and found to inhibit CEs (Figure 1). Additionally, the potency of their inhibition directly related to their hydrophobicity, further supporting the necessity of a hydrophobic group, not necessarily a phenyl moiety, for inhibition.

In this study, we synthesized a set of phenylalkyl-1,2-diones, tested for their ability to inhibit CEs *in vitro* and *in vivo*, and assessed their ability to modulate CPT-11 toxicity in a human glioblastoma cell line (U373MG).

MATERIALS AND METHODS

Chemicals: 1-Phenyl-1-alkynes (1-phenyl-1-octyne, 1-phenyl-1-heptyne, 1-phenyl-1-hexyne, 1-phenyl-1-pentyne, and 1-phenyl-1-butyne) were obtained from TCI America; all other chemicals were obtained from Sigma Aldrich.

Enzymes: Pure hCE1 and rabbit liver CE (rCE) were prepared as described previously (Morton, 2000). Pure hiCE was generated by concentration and purification from baculovirus media obtained from Sf9 cells expressing a secreted form of the protein (Hicks, 2007). Human acetylcholinesterase (hAChE) and butyrylcholinesterase (hBChE) were obtained from Sigma Biochemicals (St. Louis, MO).

Synthesis of phenylalkyl-1,2-diones: Phenylalkyl-1,2-diones were synthesized by the oxidation of the corresponding alkyne using potassium permanganate in an acetone/water phase transfer system (Srinivasan, 1979). Routinely, 10.8 mM 1-phenyl-1-alkyne dissolved in 420 mL acetone was added to 240 mL aqueous 32 mM $NaHCO_3$ and 22 mM $MgSO_4$. Potassium permanganate (6.65g, 42 mmol) was added in one portion and the reaction stirred for 4 hours at rt. Small portions of $NaNO_2$ and 10% H_2SO_4 were added until the reaction was clear (~5 g and 50 mL) and solid NaCl was then added to achieve saturation. The upper organic layer was removed and lower layer extracted with ethyl acetate. The organic phases were pooled, and the solvent was removed in vacuo. Following solution of the residue in 50mL ethyl acetate, the compound was extracted 3 times with 50 mL of 1 M NaOH and once with saturated NaCl. Following drying over Na_2SO_4, the solvent was removed. Compound identity was confirmed by ^1H NMR,

elemental analysis (Microlab Inc., Norcross, GA), and HRMS (UIUC School of Chemical Sciences, Urbana, IL).

Enzyme inhibition assays and determination of K_i values using o-nitrophenol acetate (o-NPA) as a substrate. Inhibition of CEs was determined by a multiwell plate spectrophotometric assay using 3 mM o-NPA as substrate (Wadkins, 2005). Briefly, inhibitors (100 µM) and substrate were aliquoted into wells and enzyme was added. The rate of change in absorbance at 420 nm was recorded at 15 s intervals for 5 min, and data compared to wells lacking inhibitor. Data was expressed as the yield of o-nitrophenol per minute per milligram of protein. Compounds that demonstrated greater than 50% reduction of CE activity were re-screened with dilutions ranging from 10 pM to 100 µM. Data was fitted to the following equation to determine the mode of inhibition (Webb, 1963).

$$\iota = \frac{[I]\{[s](1-\beta) + Ks(\alpha-\beta)\}}{[I]\{[s] + \alpha Ks\} + Ki\{\alpha[s] + \alpha Ks\}}$$

where ι is the fractional inhibition, [*I*] is the inhibitor concentration, [s] is the substrate concentration, α is the change in affinity of the substrate for enzyme, β is the change in the rate of enzyme substrate complex decomposition, K_s is the dissociation constant for the enzyme substrate complex, and K_i is the inhibitor constant. Examination of the curve fits where α ranged from 0 to ∞ and β ranged from 0 to 1 were performed using GraphPad Prism software and Perl data language (Wadkins, 2005). Curves were generated and analyzed using Akaike's information criteria (Akaike, 1973; Akaike,

1974). Using the equation generated by Prism to be the best fit, K_i values were computed (Wadkins, 2005).

Inhibition of acetylcholinesterase and butyrylcholinesterase: Inhibition of hAcChE and hBuChE was determined as previously described using either 1 mM acetylthiocholine (AcTCh) or butyrylthiocholine (BuTCh), respectively, as substrates (Ellman, 1961; Doctor, 1987).

Enzyme inhibition assays and determination of Ki values using CPT-11 as a substrate. CPT-11 (20 μM) and inhibitor concentrations ranging from 0-100 μM were pre-incubated for 2 min at 37 ºC in 50 mM HEPES pH 7.4. Purified hiCE (25 U) was then added and the sample was incubated at 37 ºC for 5 min. The reaction was stopped with 100 μL of acid methanol. Samples were separated, and the amount of SN-38 produced was determined using high performance liquid chromatography. K_is were then determined using Prism software as described earlier.

Intracellular CE inhibition assay. Intracellular CE inhibition was assessed using the substrate 4-methylumbelliferone acetate (4-MUA) as previously described (Hyatt, 2006). Briefly, 10^6 cells were suspended in PBS and cells were pre-incubated with inhibitor (10 μM) for 1 hour. 4-MUA was then added (100 μM) and fluorescence was determined using a Hitachi F-2000 fluorometer, with an excitation wavelength of 365 nm and an emission wavelength of 460 nm. Data was collected every second for 2 minutes and the percentage of inhibition of product formation as compared to cell not treated with inhibitor was determined by assessing the difference in fluorescence intensity at 30 s.

Growth inhibition assay. Cells were plated in 6-well plates (50,000 cells/well) and allowed to adhere overnight. Following pre-incubation with inhibitor (10 μM) for 1 hour, cells were treated for 2 hours with both inhibitor and varying concentrations of CPT-11 (0-100 μM). Routinely, all concentrations were conducted in triplicate. After the requisite time period, compounds were removed and cells were then allowed to grow in drug-free medium for 4 days (~3 cell doublings). Cells were harvested, and the number of cells per well were then determined using a Coulter Z2 counter (Beckman Coulter, Fullerton, CA). The percentage of surviving cells was determined for each treatment condition and data was compared to control cells not exposed to inhibitor. Survival curves were produced and IC_{50} values were calculated using Prism software (GraphPad, San Diego, CA).

clogP Determination: LogP values were calculated using ChemSilico Predict v2.0 software (ChemSilico LLC, Tewksbury, MA).

RESULTS

Synthesis of phenylalkyl-1,2-diones

The synthesis of the phenylalkyl-1,2-diones with alkyl chains ranging from two to six carbon atoms in length was achieved with reasonable yields (typically over 70%). This was accomplished by oxidation of the corresponding phenyl alkyne using potassium permanganate in an acetone/water phase transfer system (Figure 2). Compound identity was confirmed by spectroscopy and physical analysis (Table 1), and the obtained parameters were in good agreement with previous literature reports, when available (Torii, 1983; Chang, 2004). Typically, compounds were pale yellow liquids.

Inhibition of mammalian CEs by 1,2-diones

All of the phenylalkyl-1,2-diones were found to inhibit the mammalian CEs *in vitro* using *o*-NPA substrate (Table 2), with K_i values ranging from 5,270nM to 2.2nM. For each enzyme (hCE1, hiCE and a rabbit liver CE, rCE), a similar trend was observed, with inhibitory potency increasing as the alkyl chain increased in length. Therefore we compared the enzyme inhibitory constants with the clogP values for these small molecules and this yielded direct correlations (Figure 3a), demonstrating the importance of the hydrophobicity of these molecules towards inhibition. Gratifyingly, none of these compounds inhibited hAcChE or hBuChE, demonstrating the selectivity of these molecules for CEs.

Similar to that observed above, the K_i values for the inhibition of hiCE when using CPT-11 as a substrate were comparable to those obtained for o-NPA (Table 3). In

general, the K_i values decreased as the inhibitors became more hydrophobic, but only a weak correlation was observed with the clogP (Figure 3b).

Intracellular inhibition of hiCE and modulation of CPT-11 toxicity

While the *in vitro* assays are informative with regard to enzyme specificity and potency, they do not indicate the ability of the inhibitors to modulate CE activity intracellulary. Therefore, we determined the inhibition of 4-MUA metabolism in cells that were designed to express high levels of hiCE. This would provide a measure of the cell permeability of the inhibitor and whether inhibition would result in modulation of CPT-11 toxicity. The levels of CE inhibition in U373MG cells increased with increasing hydrophobicity (compounds **2-6)**. Indeed, for phenyl-1,2-octanedione, intracellular inhibition was similar to that seen for benzil, the first compound demonstrated to be active in these assays (Table 3).

Having demonstrated that the phenylalkyl-1,2-dione could inhibit hiCE within cells, we sought to evaluate whether this would result in a reduction in the toxicity of CPT-11. Therefore, cultured cells were pretreated with inhibitor and the dose response to CPT-11 was determined. A cell line, U373MG, that had been engineered to express high levels of hiCE was used. As indicated in Figure 4 panel b, following administration of compound **6**, cells were ~3-fold less sensitive to CPT-11. While not as impressive as benzil, where a ~6-fold change was observed (Figure A, panel a), these assays indicate that the phenyl alkyl diones can modulate hydrolysis of the chemotherapeutic agent intracellularly. By comparison, the alkyl-1,2-diones were unable to modulate cytotoxicity

of CPT-11 (Figure A, panel c). In this instance, compound **12** yielded no change in the IC_{50} value for this drug.

DISCUSSION

In this study, we successfully synthesized a series of phenylalkyl-1,2-diones and demonstrated their ability to inhibit mammalian CEs both *in vitro* and *in vivo*. When the potency of inhibition of phenylalkyl-1,2-diones, with o-NPA as a substrate, was compared to a series of alkyl-1,2-diones, it was found that potency of inhibition was directly associated with the hydrophobicity of the molecules (Table 2; Figure 3a). Results from our studies presented here, validate this finding indicating that this parameter represents an important factor towards enzyme inhibition. Additionally, the gradients of the lines for all of these datasets were similar, suggesting that the contribution of clogP towards inhibition is similar for each enzyme (Figure 3a). This also indicates that the small molecules likely interact with the each protein in a similar fashion, and potentially, react with the active site amino acids in an identical manner. A similar trend was found when CPT-11 was used as a substrate, demonstrating that these molecules are most likely acting as inhibitors of the enzymes and not interacting with the substrate in the assay (Table 3; Figure 3b).

It should be noted that molecules with similar chain lengths, such as **1** and **7**, had drastically different potencies of inhibition (1840 nM and >100,000 nM respectively for hiCE). This suggests that the ability to inhibit CEs does not depend on the length of the alkyl chain. However, comparison of compounds with similar clogP values, such as **6** and **11**, reveals nearly identical K_i values (28.7and 57.7 nM respectively for hiCE). This suggests that it is the hydrophobicity of the molecules that is directly related to inhibitor potency. In fact, a direct correlation between hydrophobicity and inhibitor potency exists, regardless of the presence of a phenyl ring (Figure 3a). This is in agreement with

past data, in which the benzil analogue **C** (Figure 1) was more potent than compound **B** due to presence of the more hydrophobic bromine atom. Additionally, this correlation is, in part, validated by the structure of the CEs which are known to contain long active site gorges lined with aromatic amino acids which would limit the ability of more hydrophilic molecules to enter the protein. Such a domain also limits that ability of molecules to interact with the catalytic residues. Potentially, this may explain why a weaker correlation was observed between the inhibition constants for hiCE when using CPT-11 as a substrate, and the clogP values of the inhibitors. Since CPT-11 is a large and bulky molecule, the ability of the drug to access the active site is not only dependent upon its hydrophobicity, but also the steric interactions that occur within the gorge.

The active site of CEs is also known to contain an electrostatic gradient which draws the molecules into the active site (Redinbo, 2005). For this reason, the electronic effects of the phenyl ring were believed to possibly affect inhibitor potency. However, the fact that alkyl and phenylalkyl-1,2-diones demonstrate similar inhibition suggest that this is not the case. Instead, the phenyl rings in benzil and the phenylalkyl-1,2-diones appear to be necessary, primarily for their hydrophobic effect.

The phenylalkyl-1,2-diones were also found to inhibit intracellular CEs. Their high hydrophobicity suggests that they may be able to cross the hydrophobic cell membrane, but further studies would need to be completed to confirm this. However, one consequence of increasing hydrophobicity is reduced water solubility. Hence drug development for these compounds may require some elegant medicinal chemistry to ensure potent enzyme inhibition, and the ability to formulate the molecule for administration to humans.

It should be noted that intracellular inhibition generally increased with increasing chain length and hydrophobicity, further supporting the link between hydrophobicity and inhibition (Table 3). When compared to the alkyl-1,2-diones, the phenylalkyl-1,2-diones consistently inhibited CEs slightly better. The reason for this difference in intracellular inhibition is unclear. It is possible that this was simply a difference in the activity of the cells used for the assay, but since these results were observed for all compounds, it may represent more efficient localization of the phenyl group within the active site. Since the alkyl chain is very flexible, numerous conformations can be adapted for these molecules; many of which may be relatively weak at inhibiting the CEs. Thus the inhibitory potency of the mixture of confomers is likely to be reduced, as compared to the phenyl derivatives, which would demonstrate significantly less physical conformations.

Phenylalkyl-1,2-dione **6** was able to modulate the toxicity of CPT-11 with U373MG cells expressing hiCE (Figure 4b). The ability to reduce cell death suggests that the phenylalkyl-1,2-diones are able to enter the cell, where they then inhibit the conversion of CPT-11 to its active metabolite SN-38, resulting in reduced toxicity. Comparison of **6** with benzil and the **12**, an alkyl dione with the same chain length and similar hydrophobicity, demonstrated an interesting trend. Although all three compounds inhibit hiCE similarly *in vitro,* and demonstrate similar intracellular inhibition with the 4-MUA assay, their abilities to reduce sensitivity to CPT-11 in the growth inhibition assay differ drastically (Figure 4). Benzil results in an IC_{50} of 22.9 μM for CPT-11, while pretreatment of cells with **6** yields an IC_{50} approximately half of that (10.2 μM). Compound **12** actually demonstrates no inhibition activity with an IC_{50} nearly identical to that of the control (3.98 nM and 3.55 nM respectively). Hence while the *in vitro* assays

provide information regarding the ability of molecules to inhibit CPT-11 hydrolysis, they cannot predict their efficacy in cytotoxicity assays with the drug.

The inability of these different compounds to inhibit CEs in the growth inhibition assay was puzzling, since the 4-MUA assay demonstrated similar efficacies towards intracellular enzyme inhibition. However, the growth inhibition assay was performed on a plastic (polystyrene) plate with media containing serum, while the 4-MUA assay is performed in quartz cuvettes in PBS. It is possible that the long hydrophobic alkyl chains are able to interact with either the plastic of the plate, or the media, or the serum. Assays performed on glass demonstrated little difference from those on plastic, suggesting that this was not the issue (Appendix 2). We also performed growth inhibition assays using PBS in place of media with serum. However, the growth inhibition assay takes significantly more time than the 4-MUA assay and resulted in poor survival of the cells. Finally, growth inhibition assays were performed in media lacking serum, but little difference in the IC_{50} values were observed suggesting that the molecules are not interacting with serum. We hypothesize therefore that that these alkyl diones may interact with a component of the medium, but further studies would need to be performed to confirm this.

Overall, this study demonstrated the necessity of hydrophobic side groups for CE inhibition by 1,2-diones. Comparison of K_i and clogP values of phenylalkyl-1,2-diones with alkyl-1,2-diones and benzil, suggest that the phenyl ring enhances inhibition *in vitro* primarily by its hydrophobicity interactions and not its electronic effects. Additionally, phenylalkyl-1,2-diones are able to inhibit intracellular CEs and reduce sensitivity to CPT-11. Therefore, we envisage that these compounds may be lead molecules for the

development of clinical candidates for modulating the delayed diarrhea associated with CPT-11 administration.

TABLES AND FIGURES

Figure 1. Structures of benzil and benzil analogues.

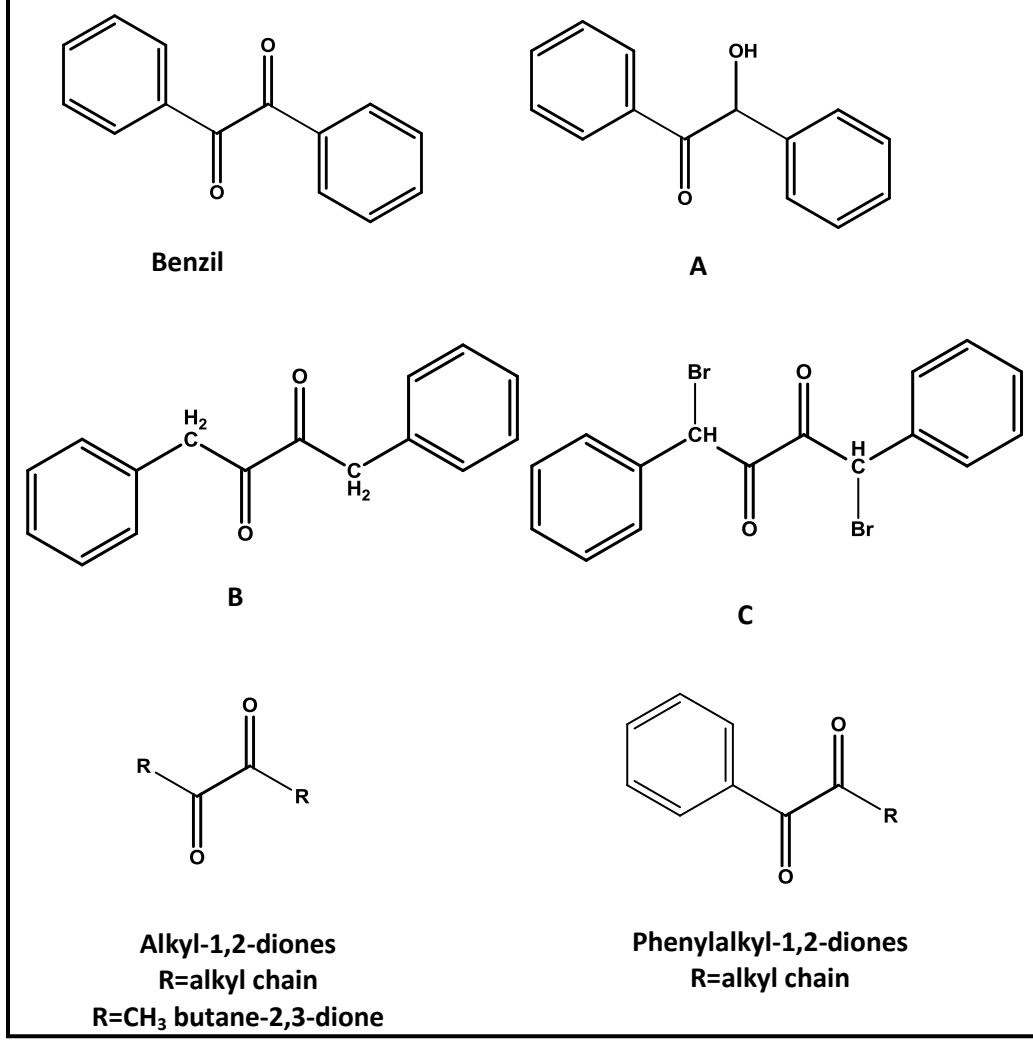


Figure 2: Synthesis of phenylalkyl-1,2-diones from 1-phenylalkynes.

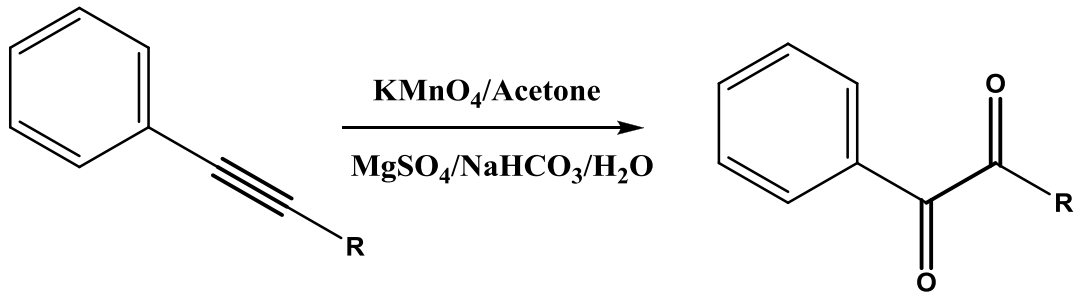


Table 1: Compound Yield and Identification by ^{1}H NMR, GC-MS, and Elemental Analysis

ID	Compound	Yield	^{1}H NMR	MS *m/z*	Elemental analysis
2	1-phenyl-1,2-butanedione $C_{10}H_{10}O_2$ MW=162.19	74%		Calcd. 162.06808 Found. 162.06712	Anal. ($C_{10}H_{10}O_2$); Calcd (%): C, 74.07; H, 6.21; O, 19.73; N, 0.00. Found (%) C, 73.77; H, 6.28; O, 19.88; N, 0.0
3	1-phenyl-1,2-pentanedione $C_{11}H_{12}O_2$ MW=176.21	70%	1.01 (m, 3H, CH_3); 1.73 (m, 2H, CH_2); 2.85 (m, 2H, CH_2); 7.50 (m, 3H, aromatic); 7.97 (m, 2H, aromatic)	Calcd. 176.08373 Found. 176.08401	Anal. ($C_{11}H_{12}O_2$); Calcd (%): C, 74.98; H, 6.86; O, 18.16; N, 0.00. Found (%) C, 75.15; H, 7.11; O, 17.94; N, 0.0
4	1-phenyl-1,2-hexanedione $C_{12}H_{14}O_2$ MW=190.24	78%	0.96 (m, 3H, CH_3); 1.40 (m, 2H, CH_2); 1.69 (m, 2H, CH_2); 2.88 (t, 2H, CH_2); 7.50 (m, 3H, aromatic); 7.97 (d, 2H, aromatic)	Calcd. 190.09938 Found. 190.09911	Anal. ($C_{12}H_{14}O_2$); Calcd (%): C, 75.76; H, 7.42; O, 16.82; N, 0.00. Found (%) C, 75.59; H, 7.60; O, 16.95; N, 0.0
5	1-phenyl-1,2-heptanedione $C_{13}H_{16}O_2$ MW=204.26	84%	0.940 (t, 3H, CH_3); 1.375-1.402 (m, 4H, CH_2); 1.756-1.719 (m, 2H, CH_2); 2.905 (t, 2H, CH_2); 7.533 (t, 2H, aromatic); 7.65 (t, 1H, aromatic); 8.02 (d, 2H, aromatic)	Calcd. 204.11503 Found. 204.11407	Anal. ($C_{13}H_{16}O_2$); Calcd (%): C, 76.44; H, 7.90; O, 15.67; N, 0.00. Found (%) C, 76.34; H, 8.02; O, 15.81; N, 0.0
6	1-phenyl-1,2-octanedione $C_{14}H_{18}O_2$ MW=218.29	72%	0.89 (m, 3H, CH_3); 1.31 (m, 6H, CH_2); 2.89 (t, 2H, CH_2); 7.50-7.96 (m, 3H, aromatic); 7.99 (m, 2H, aromatic)	Calcd. 218.13068 Found. 218.13125	Anal. ($C_{14}H_{18}O_2$); Calcd (%): C, 77.03; H, 8.31; O, 14.66; N, 0.00. Found (%) C, 76.76; H, 8.44; O, 14.91; N, 0.0

Table 2. Ki values for the inhibition of mammalian CEs by alkyl-1,2-diones with o-NPA. Phenyl alkyl diones are above the bold line. Alkyl diones and benzil are below the bold line.

ID	R_1	R_2	Chain length	clogP	hiCE (oNPA) (nM)	hCE1 (oNPA) (nM)	rCE (oNPA) (nM)	AcChE (nM)
1	CH_3	C_6H_5	1	1.07	1840 ± 260	5270 ± 1730	4930 ± 1320	>100,000
2	CH_2CH_3	C_6H_5	2	1.66	2423 ± 152.2	1551 ± 379.9	12500 ± 1984	>100,000
3	$(CH_2)_2CH_3$	C_6H_5	3	2.25	400.5 ± 20.94	102.2± 12.10	2712 ± 171.1	>100,000
4	$(CH_2)_3CH_3$	C_6H_5	4	2.83	72.71 ± 5.054	26.10 ± 2.191	337.6 ± 22.50	>100,000
5	$(CH_2)_4CH_3$	C_6H_5	5	3.42	45.54 ± 6.106	6.653 ± 0.2241	109.5 ± 10.03	>100,000
6	$(CH_2)_5CH_3$	C_6H_5	6	4.01	28.74 ± 2.329	2.191 ± 0.0765	28.15 ± 1.826	>100,000
7	CH_3	CH_3	1	-0.47	>100,000	>100,000	>100,000	>100,000
8	CH_2CH_3	CH_2CH_3	2	0.75	>100,000	>100,000	>100,000	>100,000
9	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	3	1.89	8062+329.1	4665+257.6	17785+1287	>100,000
10	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	4	2.92	189 ± 15	46.6 ± 9.3	944 ± 75	>100,000
11	$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	5	3.94	57.7 ± 2.2	2.95 ± 0.39	38.6 ± 1.6	>100,000
12	$(CH_2)_5CH_3$	$(CH_2)_5CH_3$	6	4.89	76.4 ± 9.4	4.8 ± 0.3	15.1 ± 0.9	>100,000
13	$(CH_2)_7CH_3$	$(CH_2)_7CH_3$	7	5.68	25.6 ± 2.3	7.6 ± 1.6	6.5 ± 1.9	>100,000
14	$(CH_2)_8CH_3$	$(CH_2)_8CH_3$	8	6.03	5.71 ± 1.37	2.97 ± 0.50	9.01 ± 3.80	>100,000
benzil	C_6H_5	C_6H_5			20.5 ± 0.8	73.4 ± 7.3	169 ± 20	>100,000

Table 3. Ki values for the inhibition of mammalian CEs by alkyl-1,2-diones with CPT-11 and percent intracellular inhibition using 4-MUA. Phenyl alkyl diones are above the bold line. Below are the alkyl diones and benzil.

ID	Chain length	clogP	hiCE (CPT-11) (nM)	Intracellular hiCE inhibition (%)
2	2	1.66	2012 ± 189.1	70.34
3	3	2.25	495.4 ± 39.0	86.10
4	4	2.83	167.9 ± 16.56	92.95
5	5	3.42	98.16 ± 20.58	93.58
6	6	4.01	130.3 ± 10.96	95.08
10	4	2.92	139.5 ± 18.0	85.00
11	5	3.94	193.2 ± 26.8	90.00
12	6	4.89	270.5 ± 42.7	91.20
13	7	5.68	66.78 ± 9.75	92.91
14	8	6.03	24.47 ± 2.35	80.36
Benzil			175 ± 29 (Wadkins, 2004)	95.11

Figure 3a. Correlation of logP with $logK_i$ of the alkyl and phenylalkyl-1,2-diones with o-NPA.

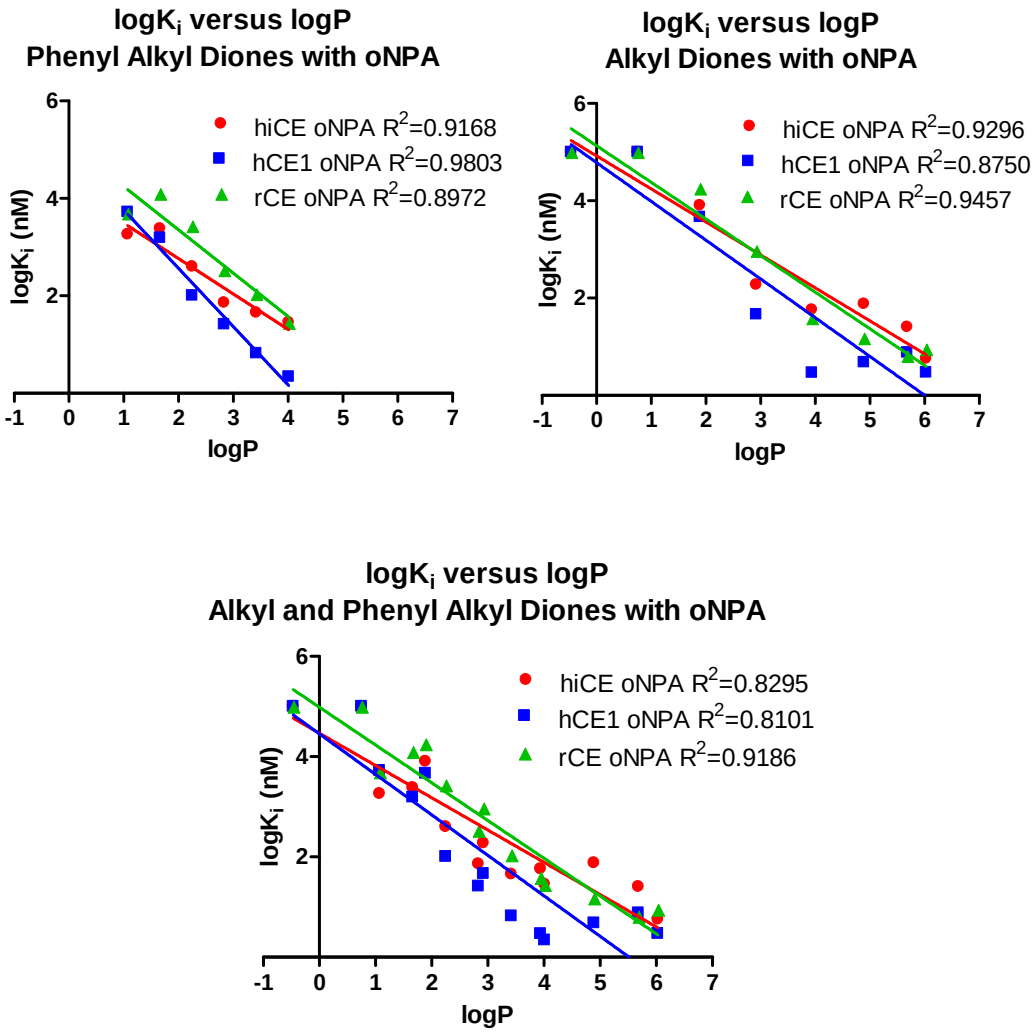


Figure 3b. Correlation of logP with $logK_i$ of the alkyl and phenylalkyl-1,2-diones with CPT-11.

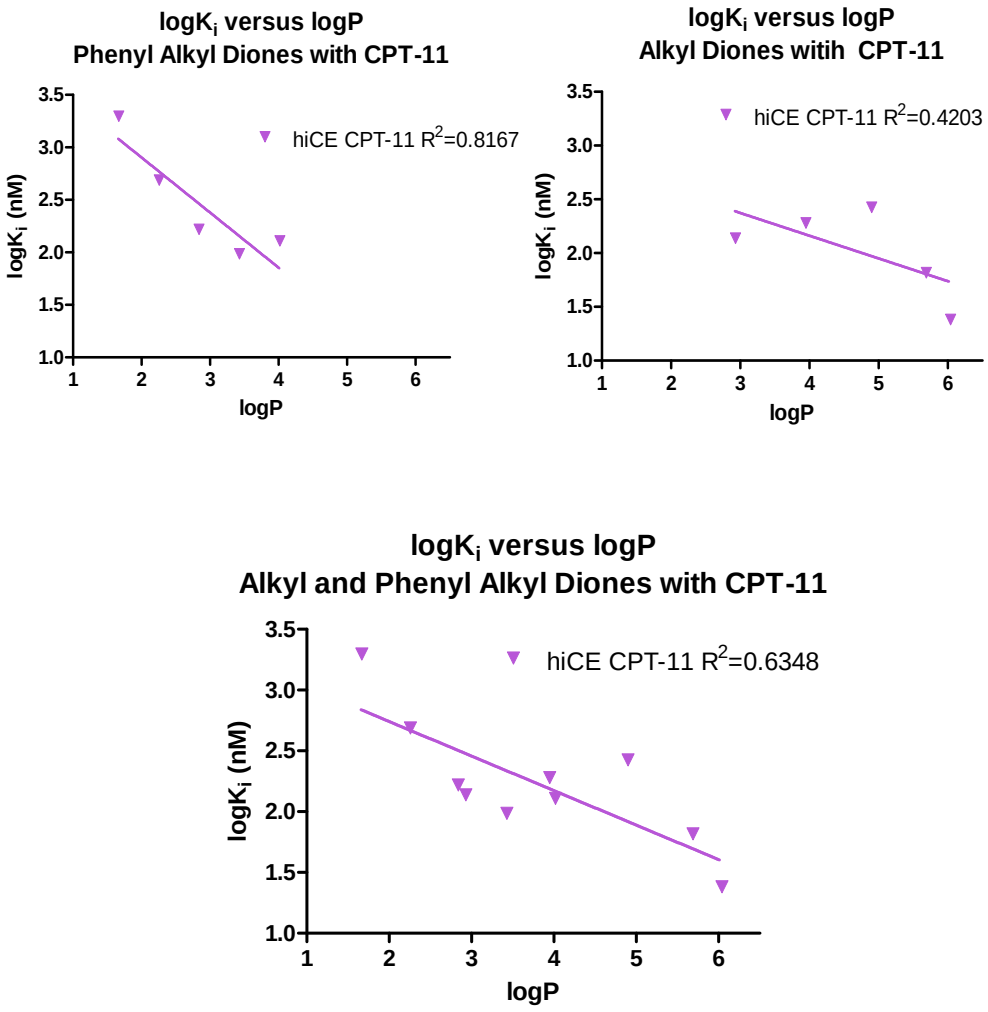
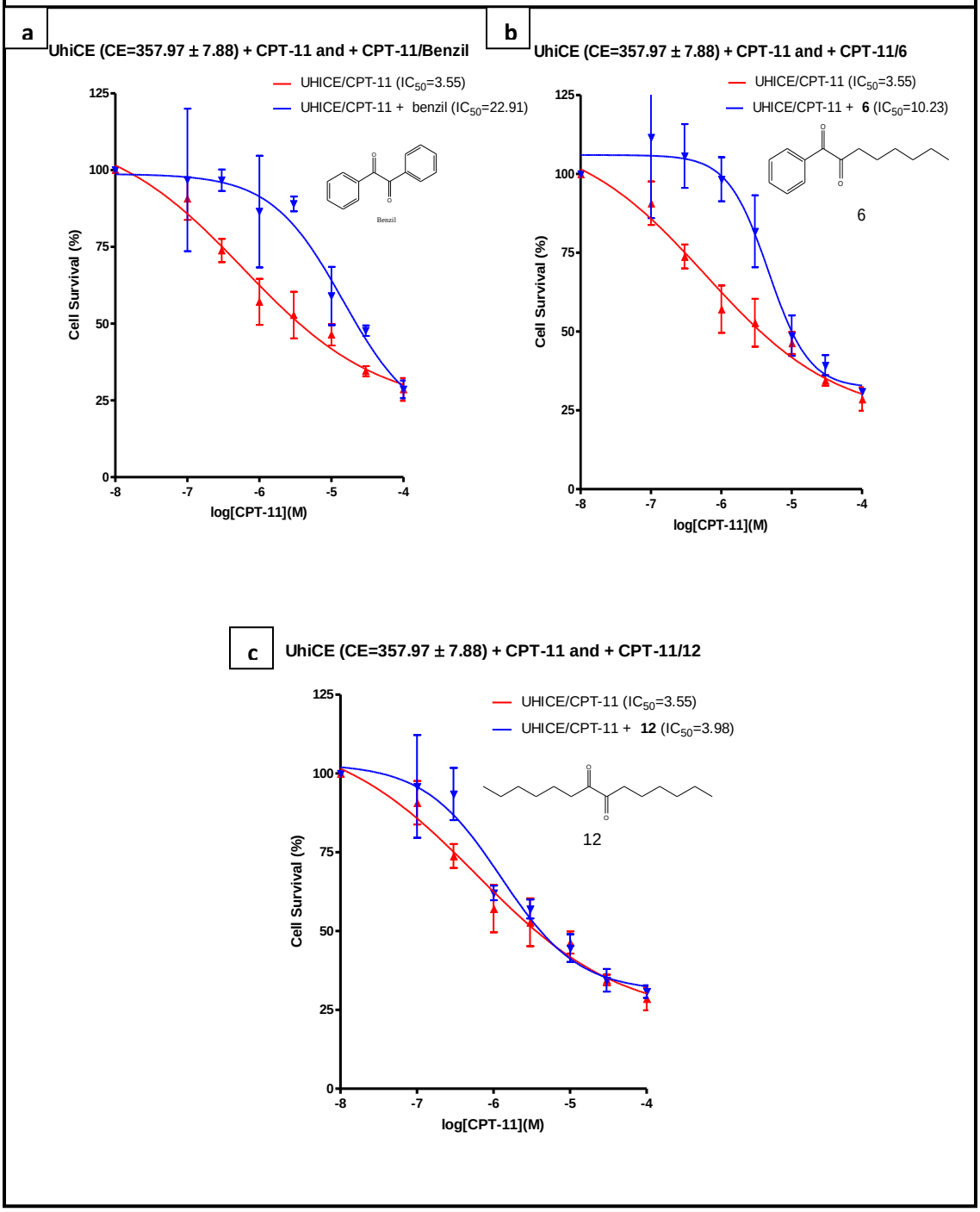


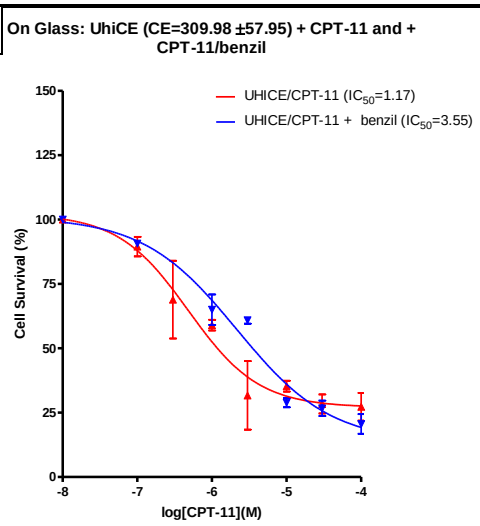
FIGURE 4: The growth inhibition assays of cells treated with CPT-11. Red lines correspond to cells treated with CPT-11 alone, and blue lines refer to cells pre-incubated with inhibitor. IC_{50} values are indicated in μM.

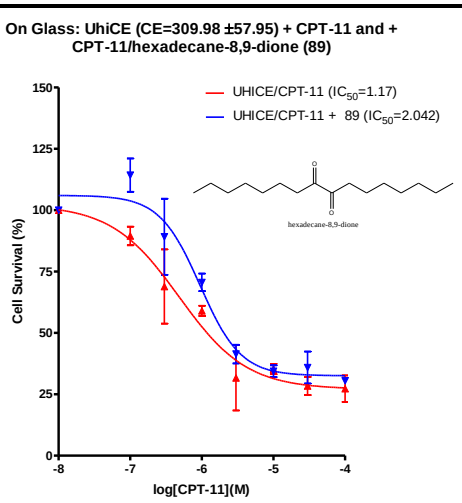


Appendices

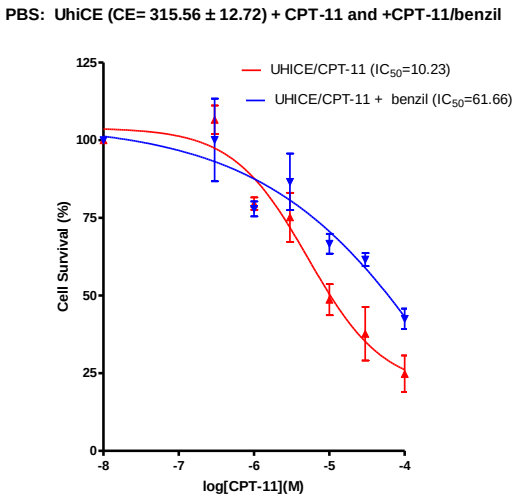
Appendix 1a-b: Growth inhibition assays under differing culture conditions. a) These graphs correspond to the assays performed on glass slides, as opposed to plastic tissue culture plates. Cells treated with benzil have reduced toxicity. However, the IC_{50} for benzil is low, suggesting that cell viability on glass may not be optimum. b) These graphs correspond to assays performed in PBS, instead of media. Benzil does show reduced toxicity but the alkyl dione 910 does not. Additionally, cell counts with this assay were extremely low, suggesting that this data may not be reliable and that the cells do not survive the longer incubation in PBS. IC_{50} values are indicated in µM.

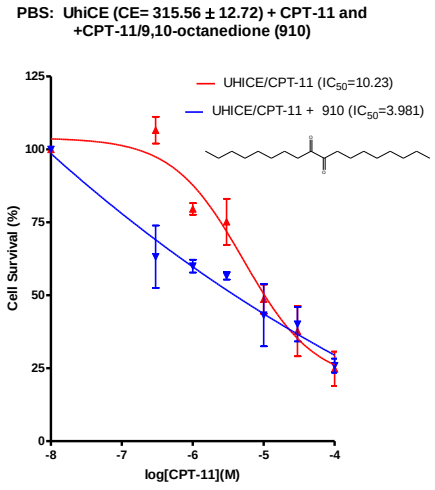
a



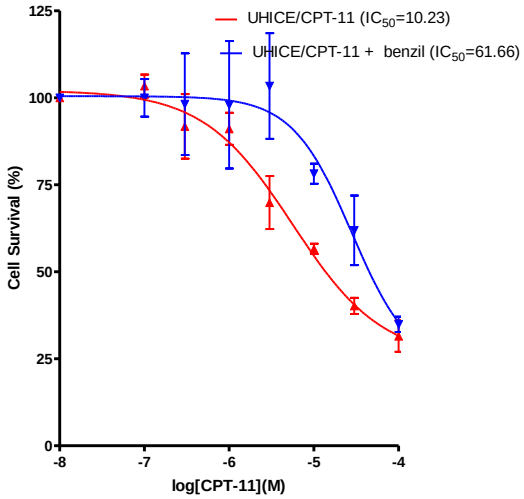


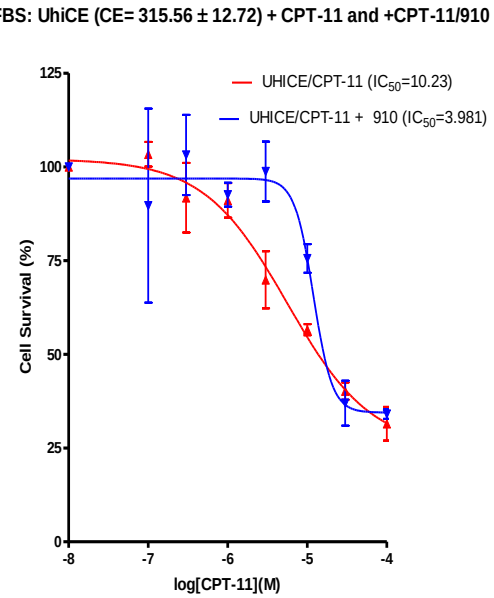
b





Appendix 1: c) These graphs correspond to the growth inhibition assays performed in media without FBS. Little difference from the normal assay is observed. As can be seen, benzil still decreases sensitivity to CPT-11 while 910 (an alkyl dione) does not.





List of Symbols/Abbreviations

CE=Carboxylesterase

hiCE=human intestinal Carboxylesterase

hCE1=human liver Carboxylesterase

rCE=rabbit liver Carboxylesterase

CPT-11=irinotecan

o-NPA=o-Nitrophenol acetate

4-MUA= 4-methylumbelliferone acetate

K_i=inhibitor constant

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