Accepted Manuscript

Facile synthesis of 1,2-dione-containing abietane analogues for the generation of human carboxylesterase inhibitors

Randall J. Binder, M. Jason Hatfield, Liying Chi, Philip M. Potter

PII: S0223-5234(18)30188-0

DOI: 10.1016/j.ejmech.2018.02.052

Reference: EJMECH 10229

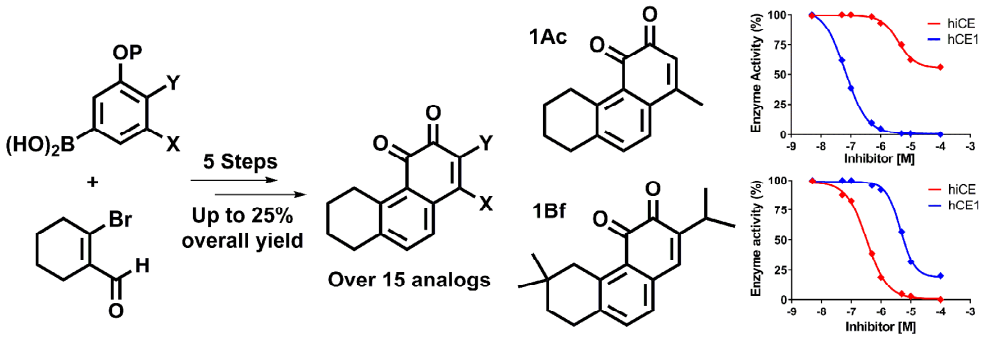
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 20 December 2017

Revised Date: 13 February 2018

Accepted Date: 14 February 2018

Please cite this article as: R.J. Binder, M.J. Hatfield, L. Chi, P.M. Potter, Facile synthesis of 1,2-dione-containing abietane analogues for the generation of human carboxylesterase inhibitors, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.02.052.



Facile synthesis of 1,2-dione-containing abietane analogues for the generation of human carboxylesterase inhibitors

Randall J. Binder, M. Jason Hatfield, Liying Chi and Philip M. Potter

Department of Chemical Biology and Therapeutics,

262 Danny Thomas Place,

St. Jude Children's Research Hospital,

Memphis, TN 38105,

United States

Email addresses:

Randall.binder@stjude.org

Jason.hatfield@stjude.org

Liying.chi@stjude.org

Phil.potter@stjude.org

Corresponding author.

Philip M. Potter

Department of Chemical Biology and Therapeutics,

St. Jude Children's Research Hospital,

262 Danny Thomas Place,

Memphis,

TN 38105, United States

Tel: 901-595-6045

Fax: 901-595-4293

E-mail: phil.potter@stjude.org.

Keywords

Abietane analogues, carboxylesterase; enzyme inhibition; synthesis.

ABSTRACT

Recently, a series of selective human carboxylesterase inhibitors have been identified based upon the tanshinones, with biologically active molecules containing a 1,2-dione group as part of a naphthoquinone core. Unfortunately, the synthesis of such compounds is complex. Here we describe a novel method for the generation of 1,2-dione containing diterpenoids using a unified approach, by which boronic acids are joined to vinyl bromo-cyclohexene derivatives via Suzuki coupling, followed by electrocyclization and oxidation to the *o*-phenanthroquinones. This has allowed the construction of a panel of miltirone analogues containing an array of substituents (methyl, isopropyl, fluorine, methoxy) which have been used to develop preliminary SAR with the two human carboxylesterase isoforms. As a consequence, we have synthesized highly potent inhibitors of these enzymes (K_i <15nM), that maintain the core tanshinone scaffold. Hence, we have developed a facile and reproducible method for the synthesis of abietane analogues that have resulted in a panel of miltirone derivatives that will be useful tool compounds to assess carboxylesterase biology.

INTRODUCTION

Carboxylesterases (CEs[1]) are ubiquitous enzymes that are responsible for the hydrolysis of ester containing molecules, typically xenobiotics.[1-3] This includes natural products such as cocaine and heroin[4, 5], many clinically used drugs (oseltamivir, meperidine, lidocaine, procaine, etc)[6, 7] as well as agents used in the agricultural industry (e.g., malathion, aldicarb).[8, 9] Since CEs are responsible for the activation and inactivation of such compounds, modulating their enzymatic activity may have a significant effect on the biodistribution and toxicity of the small molecules. Indeed, we have demonstrated that compounds such as benzil, a potent inhibitor of human CEs, can significantly modulate the cytotoxicity of CPT-11, an anticancer prodrug that this activated by this class of enzymes.[10, 11] In efforts to try and identify CE inhibitors in natural products, we recently determined that the Chinese herbal medicine Danshen contains significant amounts of such compounds. These molecules (principally, tanshinone IIA and miltirone), share structural features with benzil (1,2-dione moiety), are cell permeable, and can modulate ester-containing prodrug hydrolysis mediated by CEs.[12]

The abietane class of terpenoids have a unique scaffold composed of polycyclic fused rings, and may contain an ortho-quinone moiety (e.g., miltirone and the related tanshinones). While several reports describe the generation of these compounds to produce libraries of analogues[13, 14], until now, these synthetic efforts required

[1] Abbreviations used in this manuscript: CE – carboxylesterase; CPT-11 – irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; hAChE – human acetylcholinesterase; hBChE – human butyrylcholinesterase; hCE1 – human liver CE; hiCE – human intestinal CE; o-NPA – o-nitrophenyl acetate.

upwards of 10 linear steps in order to furnish each compound.[15-17] In an effort to use these molecules to act as probes to understand the architecture of the CE active sites, and potentially design prototypic molecules that could be used to modulate drug disposition in vivo, we sought to generate a panel of tanshinone analogues. However, due to the complexity of the synthesis of such compounds, a novel route was required. The scheme that we have devised takes advantage of a modular approach, suitable for rapid library generation and evaluation of new compounds.

Initially, we attempted to replicate the work by Snyder and coworkers, who employed sonication-enhanced Diels-Alder annulations.[15, 16] However, these intermolecular cyclizations were relatively inefficient, and were compounded by the fact that each of the various dienes were laborious to synthesize. Furthermore, a review of the literature indicated that no truly efficient approach existed by which miltirone-like compounds could be rapidly synthesized and that were amenable to producing defined analogue libraries.[17-24] Therefore, drawing upon inspiration from Harvey and coworkers, we devised a method by which decorated phenanthrols could be converted to o-quinones.[25-27] The methodology that we have developed is relatively facile, can be used to rapidly generate different regioisomers, and requires mild reagents and protection strategies. We describe here, the chemistry associated with this approach and the development of a small library of miltirones that demonstrate potent and selective inhibition of human CEs.

Materials and Methods

Reagents and instrumentation

Unless otherwise noted, all reactions were carried out under nitrogen using anhydrous solvents. All reagents were used as-is from commercial sources and were of ACS grade or better. A detailed description of the synthesis and characterization of all compounds used in this article is reported in the Supporting Information. Compounds were purified using silica cartridge and high pressure chromatography. NMR spectra (^{1}H, ^{13}C and ^{19}F) of purified molecules dissolved in DMSO-d_6, $CDCl_3$ or $CHCl_3$, were taken on either 400 MHz Bruker Avance or 500 MHz Bruker Ascend spectrometers with TMS or fluorobenzene as standards. A Waters Acquity UPLC – Xevo G2 QTOF spectrometer was used to determine purity and collect HRMS data.

Chromatographically pure human CEs, hCE1 and hiCE, were generated as previously described[5, 28] and human acetyl- (hAChE) and butyrylcholinesterase (hBChE) were obtained from Sigma Biochemicals (St., Louis, MO).

Enzyme inhibition

Inhibition of CEs was determined in 96 well plates using 3mM o-nitrophenyl acetate (o-NPA) as a substrate.[10] Briefly, inhibitors (dissolved in DMSO) were aliquoted into individual wells containing 50mM Hepes, pH7.4 and o-NPA, and the reaction was initiated by the addition of enzymes. All data points assays were run in triplicate, at least 8 concentrations of inhibitor were used and samples were compared to DMSO-alone treated controls. Results were fitted to a multifactorial equation[29] and then analyzed using Akaike's information criteria[30, 31] to assess the best model for CE inhibition. Once established, data were plotted using Prism (GraphPad, La Jolla, CA) and K_i values were determined from derived curve fits.

A determination of the inhibition of hAChE or hBChE was undertaken in a similar fashion except that acetylthiocholine or butyrylthiocholine were used as a substrates, respectively.[32, 33] Inhibitors were assayed at a single concentration (5μM).

Computer-assisted docking

Compounds were docked into the active site of hCE1 using ICMPro software (Molsoft, San Diego, CA) and the x-ray coordinates derived from PDB: 1MX1.[34] Small molecules were minimized and docked within the active site using standard program descriptors. To maximize the accuracy of the finals models, the 'Thoroughness' parameter was set to 10. Distances from the serine Oγ atom to the carbonyl carbon atoms present within the 1,2-dione were then measured. Statistical correlations between these datasets and the K_i values for the human CEs were evaluated using Prism software.

RESULTS AND DISCUSSION

Retrosynthetic strategy

Previous analysis of biologically active 1,2-diones and the tanshinone structures indicated that this chemotype was essential for enzyme inhibition, presumably to act as a target for the active site serine residue in CEs.[12] In addition, the potency and substrate specificity of such compounds was significantly modulated by the groups appended to the core cyclic structure. Using this as a guide, we developed a retrosynthetic approach towards the synthesis of such molecules by joining two fragments that would allow for the introduction of chemical diversity and generate the 1,2-dione chemotype (Scheme 1). This was accomplished by the use of Suzuki reactions to couple vinyl bromo aldehydes (**4**) with phenylboronic acids (**5**) to yield enol ethers (**3**). Subsequent acid-mediated electrocyclization of **3** would then generate the tricyclic compounds (**2**), which following subsequent deprotection and oxidation would yield the corresponding naphthols and *o*-naphthoquinones (**1**). This segmented approach would be amenable to library generation since a myriad of substituted phenylboronic acids and vinyl bromo-aldehydes can be readily obtained and/or synthesized.

Therefore, to accomplish the above scheme, the synthesis of two classes of reactants was required: a panel of bromovinyl aldehydes that allowed for the formation of the central aromatic rings and the introduction of diversity on the left hand side of the molecule; and a series of boronic acids or esters, that allow for the construction of the 1,2-dione and provides sufficient flexibility to permit inclusion of a variety of chemotypes for analogue synthesis.

Development of required reagents

a) Bromovinyl aldehydes

The appropriate vinylbromo-aldehydes **4** were synthesized by subjecting cyclohexanone derivatives to Vilsmeier-Haack conditions[35]. Using this approach, we have generated 18 compounds with yields ranging from 40 – 70%. In general, these reactions proceeded well, and due to their versatility, we have been able to adapt them to a panel of substituted cyclohexanes and benzene derivatives. As indicated in Scheme 2, this allows for significant diversity to be introduced into these molecules, and allows for generation of novel miltirone analogues.

b) Boronic acids/esters

In general, the boronic acids (**5**) used to generate the right hand side of the miltirone analogues were either purchased or synthesized using standard approaches[36, 37]. Typically boronic acids were generated from bromobenzene derivatives with the desired substituents. Treatment of the bromobenzenes with butyllithium followed immediately by triisopropyl borate and quenching with aqueous acid provided the desired boronic acids in good yields. Using this methodology, we acquired or generated 8 building blocks (Scheme 3) that allowed for the inclusion of a variety of substituents at the 2- and 3-positions. This included fluorine, simple alkyl substituents and methoxy groups, and suggests that other chemotypes could be readily incorporated into these molecules.

Synthesis of miltirone analogues

Scheme 4 outlines the synthetic route used to generate miltirone analogues. The vinylbromo-aldehydes **4** were converted into methyl enol ethers **7** in good yields (75-80%) using Wittig reagent **6**. This was then joined to boronic acids (or boronic ester) **5** in high yields by palladium tetrakis(triphenylphosphine)-catalyzed Suzuki coupling.[38] Subsequent acid-catalyzed electrocyclization/aromatization furnished the tricyclic

intermediate **2** and the minor regioisomer **8**, which, following deprotection, gave rise to phenols **9** and **10** over two steps. Subsequent treatment of these phenols with 2-iodoxybenzoic acid (**11**) produces the *o*-quinones **1** and **12**.[26] The route has been optimized such that overall yields are as high as 38%, starting from the vinyl bromo-aldehyde.

Synthetic efforts to generate and use derivatives of enol ether **7** were taken with care as these dienes were relatively unstable and appeared to self-condense (dependent upon the concentration of the solution). Due to their apparent limited shelf life, these compounds were synthesized and typically used within 24 hours of purification. If necessary, they were stored at 4C as dilute solutions in diethyl ether before use. However, the overall yields of miltirone analogues generated from this synthetic route from derivatives of enol ether **7** were up to 40%.

In some instances, the harsh conditions required to unmask the naphthol from a methyl ether were not amenable to analogues with oxygen-containing functionalities, requiring protecting groups that could be removed under milder deprotection conditions, specifically as *tert*-butyldimethyl silyl ethers. In addition, to eliminate potential losses from purification and chromatography, the crude tricyclic intermediates were directly deprotected to yield the resulting naphthols. This step typically increased overall yield by 10-15%.

Introduction of diversity

As shown, the above route allows for a fair degree of chemical diversity on either end of the molecule. On the quinone portion, decorations are nearly limitless, depending upon the commercial availability of boronic acids/esters or their ease of synthesis. Suzuki couplings were conducted in tetrahydrofuran to maximize solubility of both coupling

partners and were adapted from a similar coupling by Frontier and co-workers.[38] In some cases, when yields of the Suzuki coupling were poor, a “ligandless” catalyst method was employed. In a modified approach, vinylbromo aldehydes **4** were directly attached to the boronic acid using a palladium acetate-mediated Suzuki coupling (Scheme 5). The resulting vinyl benzene cyclohexenal products **14** were then converted to the enol ether using the same Wittig reagent as per the normal route. This approach was less desirable from a library-generation perspective as the branch point for adding diversity is added earlier in the reaction sequence. However, this approach also illustrates the broader applicability and overall flexibility of the strategy.

This approach allows for a fair degree of chemical diversity on either end of the molecule by taking advantage of the modular approach (Table 1). Inclusion of a pyran within the scaffold in the left-hand ring demonstrates the utility of this approach towards generating heterocyclic analogues of these compounds. Significant modification also can be introduced into the quinone moiety to provide numerous analogues, dependent upon the commercial availability of boronic acids/esters and/or their ease of synthesis. Occasionally the product quinones were relatively unstable, however this tended to occur only with compounds containing electronegative substituents on the C ring. We believe that compounds bearing electron-donating groups possess longer shelf-lives as they are less prone to the aromatization. Additionally, LCMS data strongly suggested that dimerization via an inverse-demand hetero Diels-Alder cycloaddition could account for decomposition.

Inhibition of human CEs by miltirone analogues

Having generated a panel of miltirone analogues in good yield, we assessed their ability to inhibit both hCE1 and hiCE, using o-NPA as a substrate. As indicated in Table 2, the

majority of the novel compounds were more potent than the natural product towards the former enzyme. For example, molecules **1Ad** and **1Be** yielded K_i values that were ~210- and ~175-fold lower, respectively, than that seen for miltirone. While potency towards hiCE was comparable to that seen for the natural product, several analogues demonstrated increased potency (e.g., **1Bd** and **1Bf**). Indeed, **1Bd** was the most potent non-selective analogue, inhibiting both hCE1 (K_i = 42nM) and hiCE (K_i = 15nM) with similar efficacy. However, selectivity was also observed, and in some instances the relative potencies towards the different CE isozymes differed by ~60-fold, comparable to miltirone. For example, **1Bf** demonstrated K_i values of 31.2nM and 1,871nM for hiCE and hCE1, respectively with a comparable ratio being observed for **1Cf** (K_i values of 4,204nM with hCE1 and 70.7nM for hiCE; Table 2). Interestingly, while the selectively between the different enzymes was highly variable, it was not readily apparent which parts of the molecules contributed to the specificity of inhibition. Overall however, even in this small series of analogues, we have increased the potency of molecules that contain the miltirone scaffold towards hCE1 by 200-fold (see inhibition data for **1Ad**), and to hiCE by ~3-fold (compound **1Bd**).

Gratifyingly, none of the compounds demonstrated any appreciable inhibition of hAChE, with an observed maximum of 30% by **1Cf** at 5µM. Two molecules did inhibit hBChE (54% and 62% for **1Aa** and **1Ad**, respectively at 5µM), however these studies confirm that the synthesized analogs are not promiscuous inhibitors of human esterases, and demonstrate considerable selectivity for CEs.

The compounds synthesized were chosen to evaluate three main properties within the quinone portion: the relative steric bulk; the overall hydrophobicity of the molecule; and the biological effect of modulating the electronegativity of groups

appended adjacent to the 1,2-dione chemotype. Initially, we focused on the quinone portion of the molecule, hypothesizing that altering the electrophilicity of the carbonyl carbon atoms would allow more facile attack by the serine Oγ atom present within the CE active site. Therefore analogues were generated containing both electron-withdrawing and donating groups as substituents, expecting that the more electron-deficient quinones would be more electrophilic, and thus more potent inhibitors. As anticipated, the electron-withdrawing nature of the fluorine on analogues **1Ad**, **1Ae**, and **1Be** results in compounds with significantly greater potency, particularly with regard to hCE1 (Table 2). Indeed, these represent some of the most effective inhibitors of this isozyme. However, with hiCE, the most potent compounds possess either larger alkyl or fluoro substituents at the α-position relative to the dione (e.g., **1Bf**, **1Cf** and **1Bd**). While the former group can be considered to be electronically neutral, they add more steric bulk to the molecules and as such may play a crucial role in the positioning of the compounds within the active site. In addition, the alkyl substituents significantly increase the logP of these molecules, however in this limited series of analogues, the inclusion of these groups did not dramatically increase the biological activity of the 1,2-diones as compared to miltirone.

Addition of fluorine, either in the α or β position with respect to the 1,2-dione group for the less complex molecules, resulted in significant increases in inhibitory potency for both human CEs. For example, **1Ad** and **1Ae** are 10- and 5-fold more potent than the unsubstituted analogue (**1Aa**) with regard to hCE1 (Table 2). This is likely due to an increase in electropositivity of the carbonyl carbon atom allowing for more facile attack by the serine Oγ atom, the initial step in the interaction between the enzyme and the small molecule inhibitors. However, this was not obviously apparent for

the gem dimethyl substituted compounds, as exemplified by molecules **1Ba**, **1Bd**, **1Be** and **1Bg**. Essentially, no large changes in the K_i values were observed by inclusion of the fluorine atoms in these compounds when assayed against hCE1. Interestingly, the α-fluoro-substituted analogue (**1Bd**) was ~23-fold more potent towards hiCE than **1Ba**, and the disubstituted molecule **1Bg** also demonstrated increased potency (~8-fold). These results argue, that for this enzyme, the group appended immediately adjacent to the carbonyl carbon atom significantly impacts the activity of the compound. This was also observed in the α-methyl-substituted derivative (**1Bb**) which is ~4-fold more potent towards hiCE, but ~75-fold less active against hCE1 (Table 2). Our data indicate that the substitutions in this region play a significant role both in inhibitor potency and isozyme selectivity.

Similarly, effects of substituent on the A ring have been considered from three perspectives: hydrophobicity; the steric bulk associated with this domain; and the planarity of the overall molecule. Specifically, we compared the biological activity of compounds containing a either a benzene or cyclohexyl ring, or those containing the geminal-dimethyl groups at various positions within the latter. A direct comparison of the effect of aromaticity was demonstrated by compounds **1Ad** and **1Dd**. The latter compound (A ring = benzene) demonstrated reduced potency towards hCE1 (4-fold) than the former molecule (A ring = cyclohexyl), but was ~2-fold more potent towards hiCE (Table 2). Due to the similarity in the clogP values of these two small molecules, it would appear that a change in conformation (**1Dd** would be more planar, whereas **1Ad** possesses more bulk) likely influences enzyme inhibition. Interestingly, an analogue containing geminal-dimethyl groups within the cyclohexyl ring (**1Bd**), was considerably more potent than the parent compound (**1Ad**) towards hiCE. It should be noted

however, that **1Bd** is likely to be much more hydrophobic (clogP values for **1Ad**, **1Dd**, and **1Bd** were calculated to be 2.85, 2.45 and 3.89, respectively using ChemBioDraw Ultra) than **1Ad**. Overall these results indicate that it is a combination of factors (steric bulk, hydrophobicity, etc) that impact inhibitor potency with this class of molecules.

This was further demonstrated by comparing compounds **1Ab**, **1Bb**, and **1Cb**. As can be seen from Table 2, the addition of the geminal dimethyl groups does not drastically impact the potency of these molecules toward hCE1 with **1Cb** being the most effective (although still in the mid nM range). Additionally, with hiCE, the 3-substituted compound (**1Bb**) was more effective than the molecule that maintains the 2-substitution (**1Cb**). This is potentially due to steric clashes within the hiCE active site, since there was no statistically significant correlation observed between the hiCE K_i values and the clogP of the molecules. In past studies with other 1,2-dione-containing structures, we have observed good correlations between these parameters[39].

Molecular docking of miltirone analogues

Having generated a panel of miltirone-derived CE inhibitors that demonstrated biological activity, we sought to determine whether computer docking studies might allow for a determination of the potency of such molecules. Therefore, we used ICM Pro to dock the respective compounds into the active site of hCE1 using the crystal structure coordinates of this protein (1MX1). Following docking, the distances from the serine Oγ atom to the carbonyl carbon atoms (the point at which this amino acid would attack the 1,2-diones) was determined (Table 3). As indicated in Table 4, there were weak linear correlations between the potency of hCE1 inhibition and the docking of the molecules within the active site when assessing either the interatomic distances, or the clogP values of the small molecules (r^2 values of ~ 0.5). However, when correlation tests were

performed using these same datasets, highly significant Pearson r values were obtained ($P<0.001$; Table 4). Unfortunately, using this information to design more potent inhibitors is unlikely to be fruitful. However, these analyses do allow a gross analysis of inhibitor binding and, as can be seen in Figure 1, **1Be** docks in a different conformation to miltirone. Since the difference in potency of these two molecules towards hCE1 is ~180-fold, these results argue that the more efficacious analogues localize within the active site in an alternative manner to the natural product. Presently, the crystal structure of hiCE has not been determined, and hence we have been unable to perform similar studies with this enzyme.

Advantages of the developed methodology

The described Suzuki coupling approach should work well with most β-bromovinyl α-β unsaturated aldehydes and aromatic boronic acids. Due to the robustness of the reaction, the building-block strategy we have developed towards generating polycyclic compounds, allows for the installation of diverse functionality within either portion of the molecule. Additionally, we have demonstrated the ability to generate heterocyclic precursors for incorporation into this class of compounds as exemplified by compound **1Eb** (Table 1). However, it is likely that the elaboration of more complex functional groups may require forethought before developing syntheses adhering to this route. While the methods originally described by Harvey and co-workers used exclusively aromatic building blocks[26], we have demonstrated that more saturated starting materials can be coupled with considerable success. In doing so, we have demonstrated a facile route towards diversely-substituted polycyclic aromatic compounds, a scaffold common to many terpenoid natural products and derivatives. Furthermore, due to the commercial availability, and ease of synthesis of boronic acids,

this approach also requires minimal synthetic manipulations to yield analogues that can be used to develop structure-activity relationships.

Other approaches to this class of compounds have used Diels-Alder annulations or involved cascade cyclizations to generate polycyclic molecules.[15-17, 21] The major drawbacks to these strategies is the laborious synthetic work required to yield the key intermediates, typically requiring multiple synthetic steps. As a consequence, this results in generally lower yields of the final products and reduced cost effectiveness. Additionally, these routes tend to be linear and do not offer many opportunities for the introduction of diversity without significant modification of the chemistry. The approach described here minimizes the material requirements to generate each analogue, results in high overall yields, and is suitable for small scale library generation.

Synthetic efforts by Snieckus and coworkers have employed a similar strategy by which key bonds are formed using Suzuki couplings, followed by annulations via ortho-metalation/condensation onto pendant amides.[40, 41] These approaches have been employed towards the synthesis of fully aromatic phenanthrenes and β-lapachone. However, there are functional group incompatibilities that exist in the former, and the generation of the natural product requires at least 8 steps. Hence there are potential limitations of these methods. Our route provides access to compounds containing a saturated A-ring in a far more efficient manner.

It is also worth noting the regioselectivity of the annulation, as two regioisomers are typically generated using this route (compounds **9** and **10** in Scheme 4). Scheme 6 illustrates how the selectivity between these isomers is dictated by competing steric and electronic forces within the benzene portion of intermediates **3a** and **3b**. Since free rotation can occur around the carbon-carbon bond between the benzene and

cyclohexenyl rings, such movement juxtaposes either the Y or the OP groups adjacent to the diene. It can be postulated that an electron-withdrawing group would reduce the nucleophilicity of the benzene at the 2' position allowing the 6' position to act as the intramolecular nucleophile. As a result, those compounds bearing a large or electron-withdrawing group at the 3' position would overwhelm the steric interactions imposed by the protective group (P) on the 5' oxygen, thereby producing the "undesired" isomer (**10**). Thus, in these cases, larger protective groups were used to favor an equilibrium that ensured the formation of the desired regioisomer (**9**). For example, with **3Ae** and **3Be**, where X and Y represent H and F, respectively (Schemes 1 and 4, Table 2), the yield of the desired isomer was relatively low (~35:65, **2**:**8**) when the 5' oxygen was protected as a methyl ether. However, when these cyclization reactions were undertaken with a larger protective group (e.g., *tert*-butyldimethylsilyl ether), the selectivity was inverted and improved to ~65:35 (**2**:**8**).'

Our primary future interests lie in generating members of the tanshinone subclass of terpenoids, based upon our successful implementation of this approach, specifically towards miltirone analogues. Given their known biological activity, we have generated several analogues of these compounds. Furthermore, the wide range of biological activities exhibited by similar terpenoids, including those isolated from *Salvia* species, may make them attractive synthetic targets.[42-47] We anticipate that such compounds could be readily synthesized, using in the described approaches, with minimal modification. Consequently, most of our synthetic efforts are focused on generating the Suzuki coupling precursors i.e., building in substitutions and functionality prior to the key carbon-carbon bond forming step. Following this methodology, we can develop more complex boronic acids with relative ease and apply them towards the

generation of more complex polycyclic compounds. Given the relative ease with which the necessary bromovinyl aldehydes are generated, a wide array of precursor cyclic ketones can be used, provided they tolerate of the conditions used to install the β-bromovinyl α-β unsaturated aldehyde functionality. Due to the flexibility and efficiency of this approach, such studies are currently underway.

CONCLUSIONS

A facile synthetic route towards abietane analogues has been developed which can be applied to incorporate a variety of functional groups. These syntheses are the shortest routes to date of this class of molecule. Furthermore, the flexibility inherent in the modular approach makes it ideally suited towards generating libraries of this class of compounds. Using this synthetic approach, we have synthesized a growing library of abietane analogues which have demonstrated varying degrees of inhibition of human CEs, some of which are highly potent, with K_i values in single-digit nanomolar concentrations. Furthermore, these compounds are not promiscuous inhibitors of human esterases, indicated by their selectivity for CEs. As more molecules are generated, a more complete structure-activity relationship will become apparent and will guide the development of biological tools that can be used to assess CE activity and function, both in vitro and in vivo.

Acknowledgements

This work was supported in part by an NIH grant AT007531, a Cancer Center Core grant CA21765, and by ALSAC.

REFERENCES

[1] B. Testa, J.M. Mayer, The Hydrolysis of Carboxylic Acid Esters, in: Hydrolysis in Drug and Prodrug Metabolism, Verlag Helvetica Chimica Acta, Zurich, Switzerland, 2003.

[2] J. Cashman, B. Perroti, C. Berkman, J. Lin, Pharmacokinetics and molecular detoxification, Environ. Health Perspect., 104 (1996) 23-40.

[3] M.R. Redinbo, P.M. Potter, Mammalian Carboxylesterases: From drug targets to protein therapeutics., Drug Discov. Today, 10 (2005) 313-325.

[4] M.R. Brzezinski, B.J. Spink, R.A. Dean, C.E. Berkman, J.R. Cashman, W.F. Bosron, Human liver carboxylesterase hCE-1: binding specificity for cocaine, heroin, and their metabolites and analogs, Drug Metab. Dispos., 25 (1997) 1089-1096.

[5] M.J. Hatfield, L. Tsurkan, J.L. Hyatt, X. Yu, C.C. Edwards, L.D. Hicks, R.M. Wadkins, P.M. Potter, Biochemical and molecular analysis of carboxylesterase-mediated hydrolysis of cocaine and heroin, Br. J. Pharmacol., 160 (2010) 1916-1928.

[6] J. Zhang, J.C. Burnell, N. Dumaual, W.F. Bosron, Binding and hydrolysis of meperidine by human liver carboxylesterase hCE-1, J. Pharmacol. Exp. Ther., 290 (1999) 314-318.

[7] D. Shi, J. Yang, D. Yang, E.L. LeCluyse, C. Black, L. You, F. Akhlaghi, B. Yan, Anti-influenza prodrug oseltamivir is activated by carboxylesterase human carboxylesterase 1, and the activation is inhibited by antiplatelet agent clopidogrel, J. Pharmacol. Exp. Ther., 319 (2006) 1477-1484.

[8] S. Whyard, R.J. Russell, V.K. Walker, Insecticide resistance and malathion carboxylesterase in the sheep blowfly, Lucilia cuprina, Biochem. Genet., 32 (1994) 9-24.

[9] P.J. O'Brien, Y.A. Abdel-Aal, J.A. Ottea, J.B. Graves, Relationship of insecticide resistance to carboxylesterases In Aphis gossypii (Homoptera: Aphididae) from Midsouth cotton, J. Econ. Entomol., 85 (1992) 651-657.

[10] R.M. Wadkins, J.L. Hyatt, X. Wei, K.J. Yoon, M. Wierdl, C.C. Edwards, C.L. Morton, J.C. Obenauer, K. Damodaran, P. Beroza, M.K. Danks, P.M. Potter, Identification and characterization of novel benzil (diphenylethane-1,2-dione) analogues as inhibitors of mammalian carboxylesterases, J. Med. Chem., 48 (2005) 2905-2915.

[11] J.L. Hyatt, L. Tsurkan, M. Wierdl, C.C. Edwards, M.K. Danks, P.M. Potter, Intracellular inhibition of carboxylesterases by benzil: Modulation of CPT-11 cytotoxicity, Mol. Cancer Ther., 5 (2006) 2281-2288.

[12] M.J. Hatfield, L.G. Tsurkan, J.L. Hyatt, C.C. Edwards, A. Lemoff, C. Jeffries, B. Yan, P.M. Potter, Modulation of Esterified Drug Metabolism by Tanshinones from Salvia miltiorrhiza ("Danshen"), J. Nat. Prod., 76 (2013) 36-44.

[13] Y. Dong, S.L. Morris-Natschke, K.H. Lee, Biosynthesis, total syntheses, and antitumor activity of tanshinones and their analogs as potential therapeutic agents, Nat. Prod. Rep., 28 (2011) 529-542.

[14] M.A. Gonzalez, Aromatic abietane diterpenoids: total syntheses and synthetic studies, Tetrahedron, 71 (2015) 1883-1908.

[15] J. Lee, Snyder, J. K., Ultrasound-promoted cycloadditions in the synthesis of *Salvia miltiorrhiza* abietanoid *o*-quinones, J. Org. Chem., 55 (1990) 4995-5008.

[16] J. Lee, Mei, H. S., and Snyder, J. K., Synthesis of miltirone by an ultrasound-promoted cyclization, J. Org. Chem., 55 (1990) 5013-5016.

[17] H.M. Chang, K.Y. Chui, F.W. Tan, Y. Yang, Z.P. Zhong, C.M. Lee, S.H. L., H.N. Wong, Structure-activity relationship of miltirone, and active central benzodiazepine

receptor ligand isolated from *Salvia miltiorrhiza* Bunge (Danshen), J. Med. Chem., 34 (1991) 1675-1692.

[18] D. Nasipuri, A.K. Mitra, Synthetic studies in the diterpene series. Part VIII. Synthesis of miltirone, a diterpenoid quinone, J. Chem. Soc.: Perkin Trans. 1, (1973) 285-287.

[19] S. Knapp, Sharma, S., Synthesis of the naturally occuring antioxidant Rosmariquinone, J. Org. Chem., 50 (1985) 4996-4998.

[20] J.G. Luis, L.S. Andres, W.Q. Fletcher, E.H. Lahlou, P. Aurea, Rearrangement of methyl 11,12-di-*O*-methyl-6,7-didehydro-caronsate in basic medium. Easy hemisynthesis of miltirone, J. Chem. Soc.: Perkin Trans. 1, m (1996) 2207-2211.

[21] G. Majetich, S. Liu, J. Fang, D. Siesel, Y. Zhang, Use of conjugated dienones in cyclialkylations: Total syntheses of arucadiol, 1,2-didehydromiltirone, (+/-)-hinokione, (+/-)-nimbdiol, sageone, and miltirone, J. Org. Chem., 62 (1997) 6928-6951.

[22] C. Hall, Cuppett, S., and Dussault, P., Synthesis and antioxidant activity of Rosmariquinone and several analogues, J. Agricul. Food. Chem., 46 (1998) 1303-1310.

[23] A.K. Banerjee, J.A. Azocar, M.C. Sulbaran de Carrasco, M. Laya Mimo, Potential intermediates for diterpenoid quinones: Cryptotanshinone, tanshinone IIA, and miltirone, Synth. Comm., 31 (2001) 2471-2478.

[24] W. Huang, J. Li, W. Zhang, Y. Zhou, C. Xie, Y. Luo, Y. Li, J. Wang, J. Li, W. Lu, Synthesis of miltirone analogues as inhibitors of Cdc25 phosphates, Bioorg. Med. Chem. Lett., 16 (2006) 1905-1908.

[25] D. Xu, T.M. Penning, I.A. Blair, R.G. Harvey, Synthesis of phenol and quinone metabolites of benzo[a]pyrene, a carcinogenic component of tobacco smoke implicated in lung cancer, J. Org. Chem., 74 (2009) 597-604.

[26] A. Wu, Y. Duan, D. Xu, T.M. Penning, R.G. Harvey, Regiospecific oxidation of polycyclic aromatic phenols to quinones by hypervalent iodine reagents, Tetrahedron, 66 (2010) 2111-2118.

[27] A. Wu, D. Xu, D. Lu, T.M. Penning, I.A. Blair, R.G. Harvey, Synthesis of $^{13}C_4$-labelled oxidized metabolites of the carcinogenic polycyclic aromatic hydrocarbon benzo[a]pyrene, Tetrahedron, 68 (2012) 7217-7233.

[28] S. Bencharit, C.L. Morton, Y. Xue, P.M. Potter, M.R. Redinbo, Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme., Nat. Struct. Biol., 10 (2003) 349-356.

[29] R.M. Wadkins, J.L. Hyatt, K.J. Yoon, C.L. Morton, R.E. Lee, K. Damodaran, P. Beroza, M.K. Danks, P.M. Potter, Discovery of novel selective inhibitors of human intestinal carboxylesterase for the amelioration of irinotecan-induced diarrhea: synthesis, quantitative structure-activity relationship analysis, and biological activity, Mol. Pharmacol., 65 (2004) 1336-1343.

[30] H. Akaike, Information theory and an extension of the maximum likelihood principle, in: B.N. Petrov, F. Csaki (Eds.) Second International Symposium on Information Theory, Akademiai Kiado, Budapest, 1973, pp. 267-281.

[31] H. Akaike, A new look at the statistical model identification, IEEE Trans. Automatic Control, AC-19 (1974) 716-723.

[32] G.L. Ellman, K.D. Courtney, V. Anders, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol., 7 (1961) 88-95.

[33] B.P. Doctor, L. Toker, E. Roth, I. Silman, Microtiter assay for acetylcholinesterase, Anal. Biochem., 166 (1987) 399-403.

[34] S. Bencharit, C.L. Morton, J.L. Hyatt, P. Kuhn, M.K. Danks, P.M. Potter, M.R. Redinbo, Crystal structure of human carboxylesterase 1 complexed with the Alzheimer's drug tacrine. From binding promiscuity to selective inhibition., Chem. & Biol., 10 (2003) 341-349.

[35] J.J. Lian, P.C. Chen, Y.P. Lin, H.C. Ting, R.S. Liu, Gold-catalyzed intramolecular [3+2]-cycloaddition of arenyne-yne functionalities, J. Am. Chem. Soc., 128 (2006) 11372-11373.

[36] M.H. Norman, H.D. Smith, C.W. Andresw, F.L. Tang, C.L. Cowan, R.P. Steffen, 4-(Heteroarythio)-2-biphenylyltetrazoles as Nonpeptide Angiotensin II Antagonists, J. Med. Chem., 38 (1995) 4670-4678.

[37] J.V. Mello, N.S. Finney, Convenient synthesis and transformation of 2,6-dichloro-4-iodopyridine, Org. Lett., 3 (2001) 4263-4265.

[38] W.T. Spencer, Frontier, A. J., Cycloaromatization protocol for synthesis of polysubstituted phenol derivatives: Method development and mechanistic studies, J. Org. Chem., 77 (2012) 7730-7736.

[39] J.L. Hyatt, T. Moak, J.M. Hatfield, L. Tsurkan, C.C. Edwards, M. Wierdl, M.K. Danks, R.M. Wadkins, P.M. Potter, Selective inhibition of carboxylesterases by isatins, indole-2,3-diones., J. Med. Chem., 50 (2007) 1876-1885.

[40] M.A.F. Brandao, A.B. de Oliveira, V. Snieckus, Combined directed metalation-cross coupling strategies. A regiospecific route to heteroring-annelated *ortho*-naphthoquinones and a short synthesis of b-lapachone, Tet. Lett., 34 (1993) 2437-2440.

[41] K.B. Jorgensen, Rantanen, T., Dorfler, T., and Snieckus, V., Directed metalation-Suzuki-Miyaura cross-coupling strategies: regioselective synthesis of hydroxylated 1-methylphenanthrenes, J. Org. Chem., 80 (2015) 9410-9424.

[42] Y.B. Wu, Z.Y. Ni, Q.W. Shiu, M. Dong, H. Kiyota, Y.C. Gu, B. Cong, Constituents from *Salvia* species and their biological activities, Chem. Rev., 112 (2012) 5967-6026.

[43] M.A. Gonzalez, Aromatic abietane diterpenoids: their biological activity and synthesis, Nat. Prod. Rep., 32 (2015) 684-704.

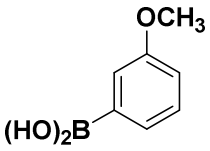
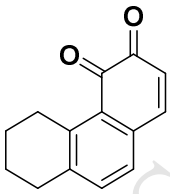
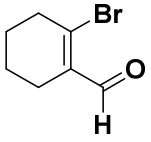
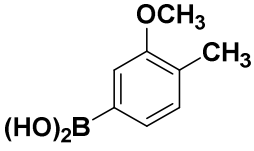
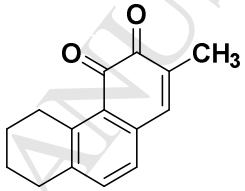
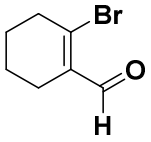
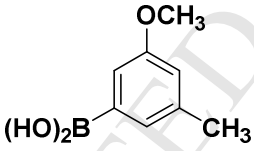
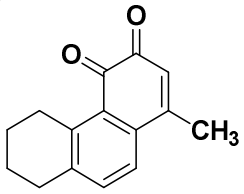
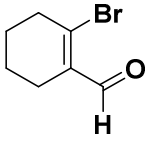
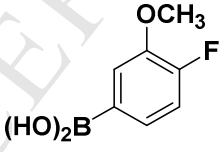
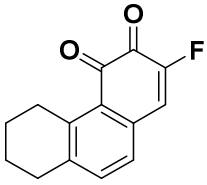
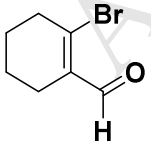
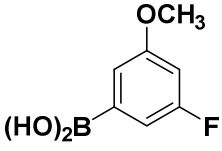
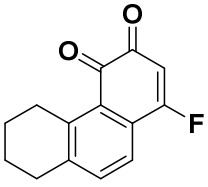
[44] M.A. Gonzalez, Synthetic derivatives of aromatic abietane diterpenoids and their biological activities, Eur. J. Med. Chem., 87 (2014) 834-842.

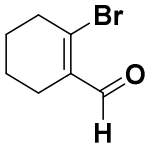
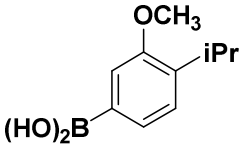
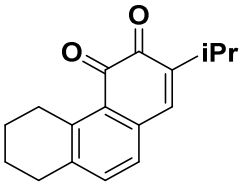
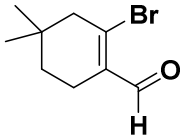
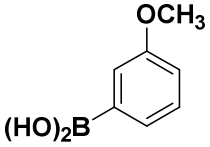
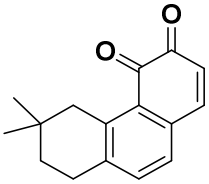
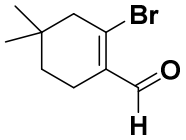
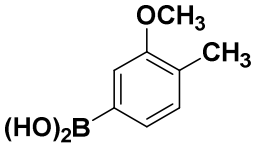
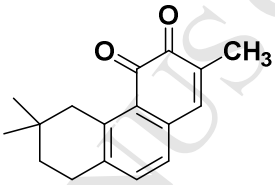
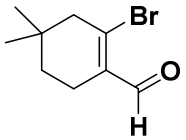
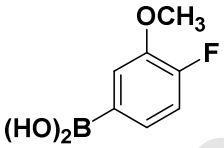
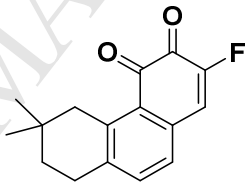
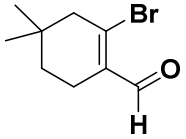
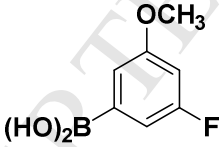
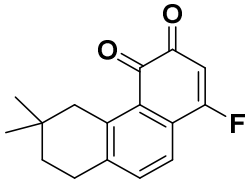
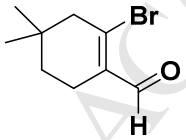
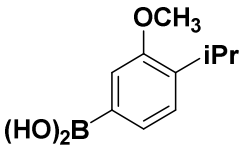
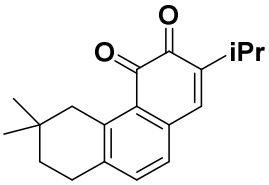
[45] A.K.S. Wan, S.W.S. Leung, D.-Y. Zhu, R.Y.K. Man, Vascular effects of different lipophilic components of "Danshen", a traditional Chinese medicine, in the isolated porcine coronary Artery, J. Nat. Prod., 71 (2008) 1825-1828.

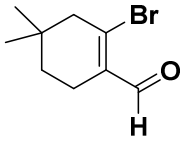
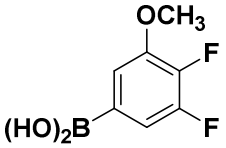
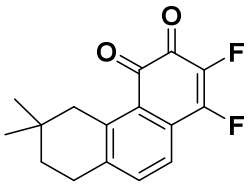
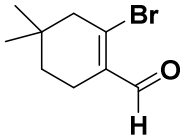
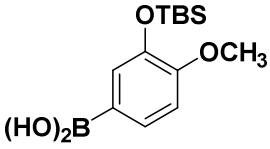
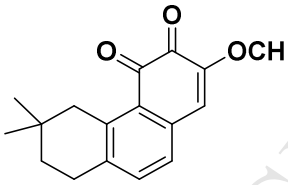
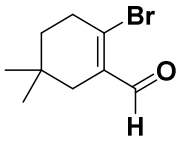
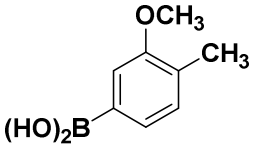
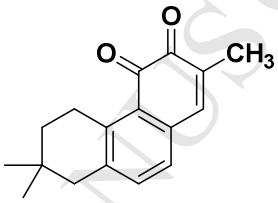
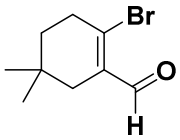
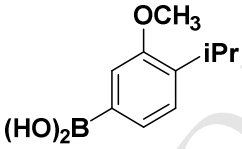
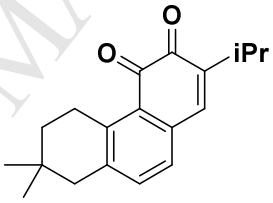
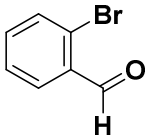
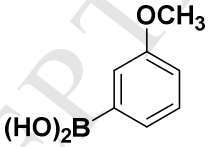
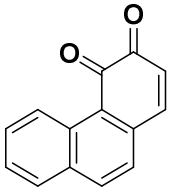
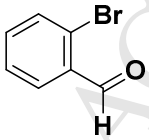
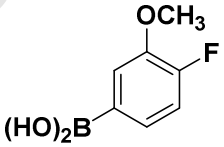
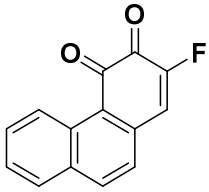
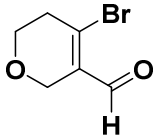
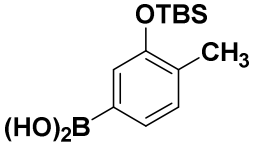
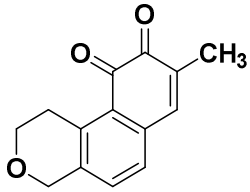
[46] Y.M. Cui, E. Yasutomi, Y. Otani, T. Yoshinaga, K. Ido, T. Ohwada, Design, synthesis, and characterization of podocarpate derivatives as openers of BK channels, Bioorg. Med. Chem. Lett., 18 (2008) 5197-5200.

[47] Z. Dang, K. Jung, L. Zhu, H. Xie, K.H. Lee, C.H. Chen, L. Huang, Phenolic diterpenoid derivatives as anti-influenza A virus agents, ACS Med. Chem. Lett., 6 (2015) 355-358.

Table 1. Reactants, products and yields of 1,2-diones synthesized using the strategy described herein.

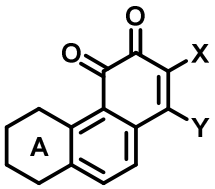
Vinyl bromoaldehyde	Boronic acid	Quinone product	Overall yield
 **4A**	 **5a**	 **1Aa**	22%
 **4A**	 **5b**	 **1Ab**	21%
 **4A**	 **5c**	 **1Ac**	21%
 **4A**	 **5d**	 **1Ad**	17%
 **4A**	 **5e**	 **1Ae**	21%

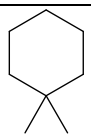
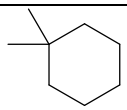
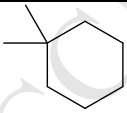
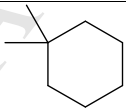
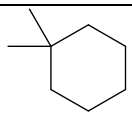
 **4A**	 **5f**	 **1Af**	16%
 **4B**	 **5a**	 **1Ba**	18%
 **4B**	 **5b**	 **1Bb**	24%
 **4B**	 **5d**	 **1Bd**	23%
 **4B**	 **5e**	 **1Be**	19%
 **4B**	 **5f**	 **1Bf**	22%

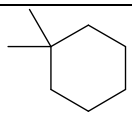
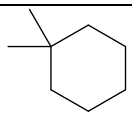
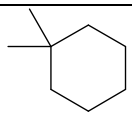
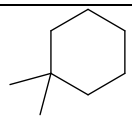
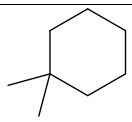
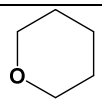
 **4B**	 **5g**	 **1Bg**	14%
 **4B**	 **5h**	 **1Bh**	26%
 **4C**	 **5b**	 **1Cb**	21%
 **4C**	 **5f**	 **1Cf**	21%
 **4D**	 **5a**	 **1Da**	30%
 **4D**	 **5d**	 **1Dd**	29%
			28%

4E	**14b**	**1Eb**

Table 2. Inhibition of human CEs by miltirone analogues



ID	X, Y	A ring	hCE1 K_i (nM)	hiCE K_i (nM)	K_i selectivity hCE1/hiCE	hAChE inhibition (%) at 5μM	hBChE inhibition (%) at 5μM
1Aa	H, H	Chx[a]	131 ± 14	2178 ± 133	0.06	0	54
1Ab	CH_3, H	Chx	1223 ± 107	423 ± 9.4	2.9	5	4
1Ac	H, CH_3	Chx	25.1 ± 2.4	≥10000	<0.01	13	30
1Ad	F, H	Chx	12.2 ± 1.1	112 ± 9.7	0.11	16	62
1Ae	H, F	Chx	25.9 ± 5.6	171 ± 8.3	0.15	4	33
1Af	iPr, H	Chx	1054 ± 262	111 ± 19	39	16	1
Miltirone	iPr, H		2530 ± 1,040	40 ± 3.4	63	22	6
1Ba	H, H		31.9 ± 2.3	341 ± 30	0.09	2	13
1Bb	CH_3, H		2425 ± 146	87.9 ± 5.4	27.6	0	1
1Bd	F, H		42.0 ± 7.9	14.6 ± 1.7	2.9	3	23
1Be	H, F		14.4 ± 3.8	386 ± 72	0.04	10	15

1Bg	F, F		102 ± 24	43.3 ± 16	2.3	4	1
1Bf	iPr, H		1871 ± 386	31.2 ± 2.9	60	20	4
1Bh	OCH_3, H		42.5 ± 2.4	159 ± 24	0.3	2	1
1Cb	CH_3, H		495 ± 49	182 ± 32	2.7	1	2
1Cf	iPr, H		4204 ± 607	70.7 ± 2.4	59.5	30	3
1Da	H, H	Benzene	942 ± 89	≥10000	<0.06	16	13
1Dd	F, H	Benzene	48.7 ± 12	51.3 ± 9.7	0.48	8	3
1Eb	CH_3, H		≥10000	≥10000	N/A	27	0

[a] Chx – Cyclohexane

Table 3. Molecular docking parameters with hCE1, and clogP values for synthesized compounds.

ID	hCE1 Ser221 Oγ - carbonyl distances (Å) (total distance)	hCE1 Ser221 Oγ - carbonyl distances (Å); serine charged (total distance)	ClogP
1Aa	3.41, 3.49 (6.90)	3.36, 3.67 (7.03)	2.47
1Ab	3.22, 3.24 (6.46)	5.1, 5.4 (10.5)	2.99
1Ac	3.11, 3.12 (6.23)	3.12, 3.47 (6.59)	2.99
1Ad	3.31, 3.37 (6.68)	3.04, 3.34 (6.38)	2.85
1Ae	3.15, 3.23 (6.38)	3.10, 3.47 (6.57)	2.45
1Af	3.20, 3.42 (6.62)	5.67, 5.91 (11.58)	3.92
Miltirone	7.31, 7.86 (15.17)	5.79, 5.99 (11.78)	4.96
1Ba	3.12, 3.15 (6.27)	3.08, 3.08 (6.16)	3.51
1Bb	3.45, 3.63 (7.08)	7.02, 7.94 (14.96)	4.03
1Bd	3.16, 3.26 (6.42)	7.08, 8.04 (15.12)	3.89
1Be	3.02, 3.07	2.99, 3.02	3.49

	(6.09)	(6.01)	
1Bg	3.15, 3.26 (6.41)	3.47, 3.75 (7.22)	3.86
1Bf	3.66, 3.88 (7.54)	3.69, 3.86 (7.55)	4.96
1Bh	3.07, 3.12 (6.19)	4.47, 5.53 (10.00)	3.44
1Cb	2.64, 2.86 (5.50)	7.06, 7.94 (15.00)	4.03
1Cf	5.95, 6.15 (12.1)	5.80, 6.01 (11.81)	4.96
1Da	3.45, 3.48 (6.93)	4.77, 5.64 (10.41)	2.08
1Dd	3.39, 3.41 (6.80)	4.46, 5.62 (10.08)	2.45
1Eb	3.2, 3.24 (6.44)	4.16, 5.16 (9.32)	0.74

Table 4. Statistical tests for correlations of physical parameters with enzyme inhibition data

Comparator 1[a]	Comparator 2	Linear regression (r^2)	Pearson r	P value
hCE1 K_i	Smallest Oγ - carbonyl distance	0.55	**0.743**	**<0.001**
	Total Oγ - carbonyl distances	0.55	**0.745**	**<0.001**
	Smallest Oγ - carbonyl distance; charged serine	0.24	**0.486**	**0.041**
	Total Oγ - carbonyl distances; charged serine	0.19	0.435	0.071
	clogP	0.44	**0.666**	**0.003**
hiCE K_i	clogP	0.20	-0.442	0.086

[a] – Compounds that demonstrated no inhibition for the respective enzyme (i.e., $K_i \geq$ 10,000nM) were excluded from these analyses.

Legends

Scheme 1. Retrosynthetic strategy for the synthesis of 1,2-dione containing abietane diterpinoids.

Scheme 2. Synthesis of vinyl bromoaldehydes and their application towards the generation of different tanshinone analogues.

Scheme 3. General approaches used for the synthesis of boronic acids.

Scheme 4. Overall reaction scheme for synthesis of the compounds described here.

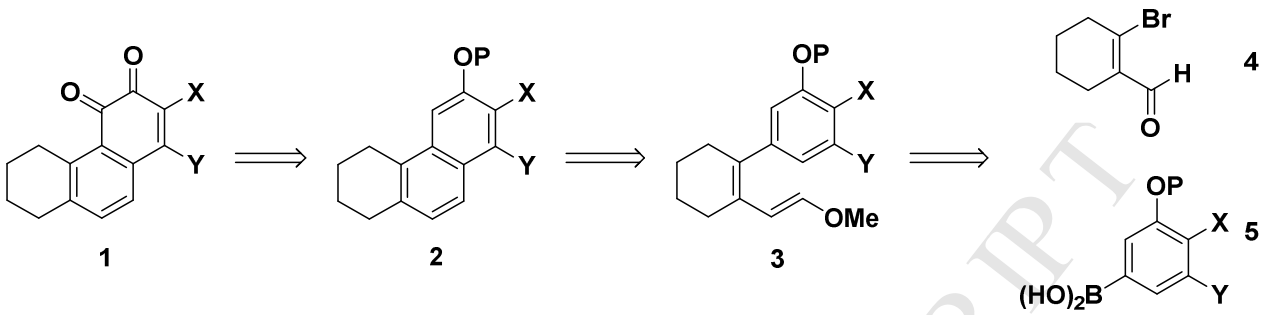
Scheme 5. Palladium acetate-mediated coupling of vinyl bromoaldehydes to boronic acids.

Scheme 6. Regioselectivity of formation of **2** and **8** is dictated by steric and electronic effects of benzene ring substituents.

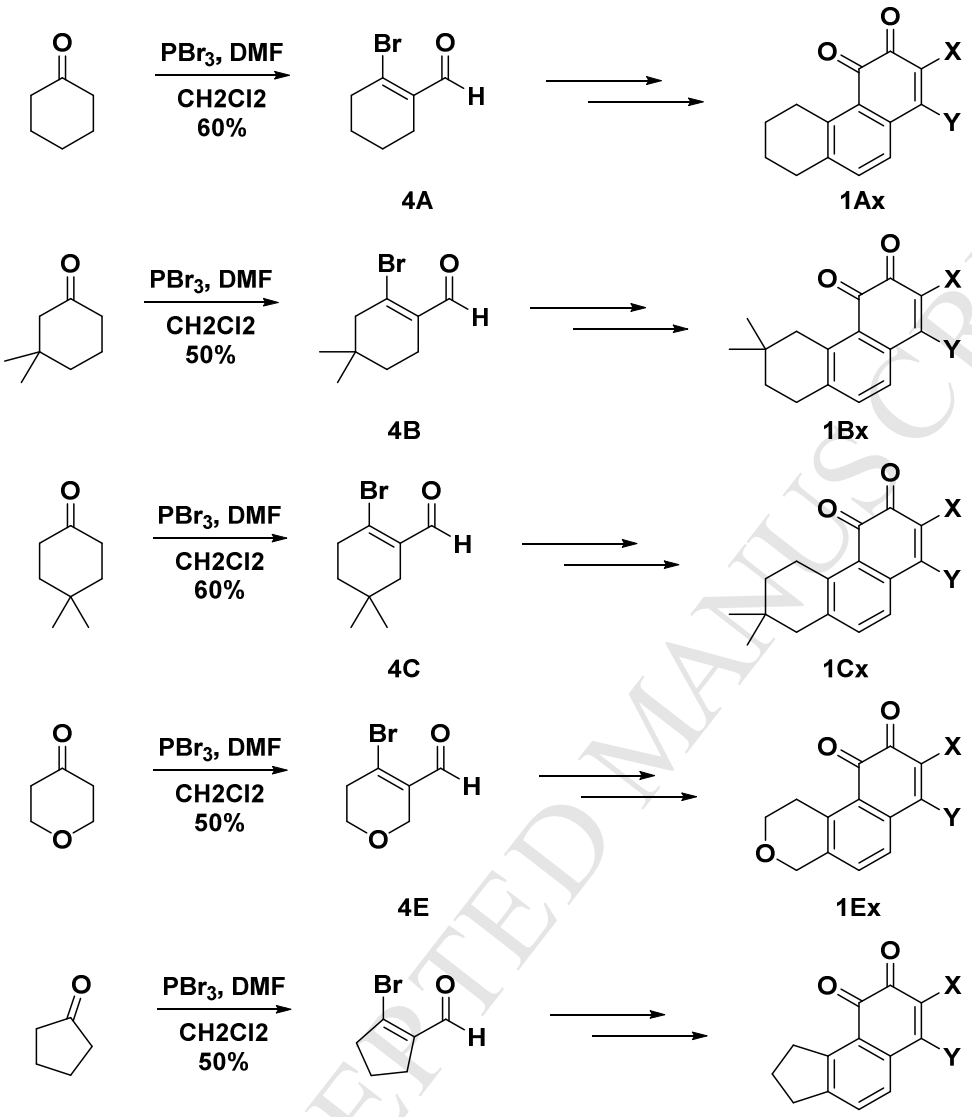
Figure 1. Computer-aided docking of miltirone (grey) and **1Be** (yellow) into the active site of hCE1.

Distances from the active site serine Oγ atom to the carbonyl carbon atoms of the 1,2-diones are indicated in Ångtroms.

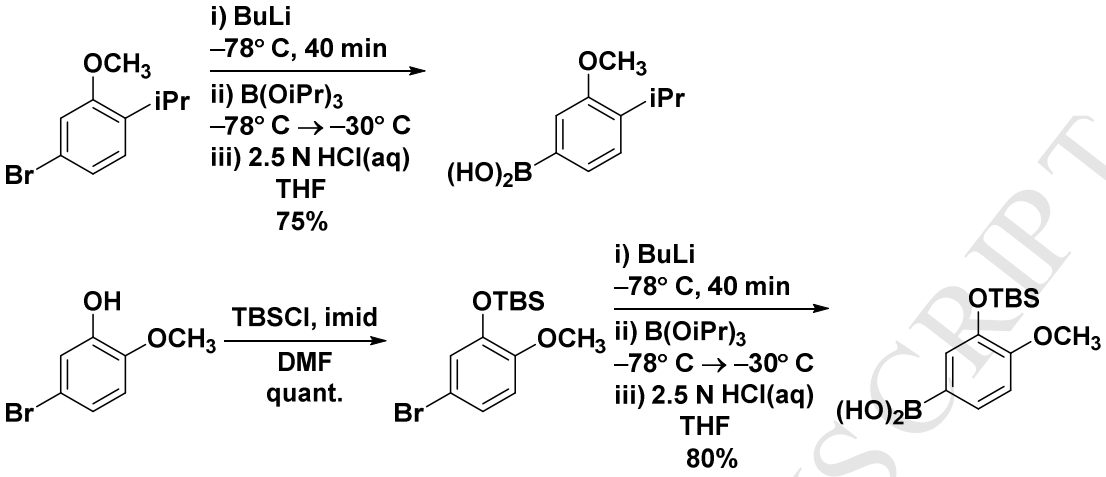
Scheme 1.



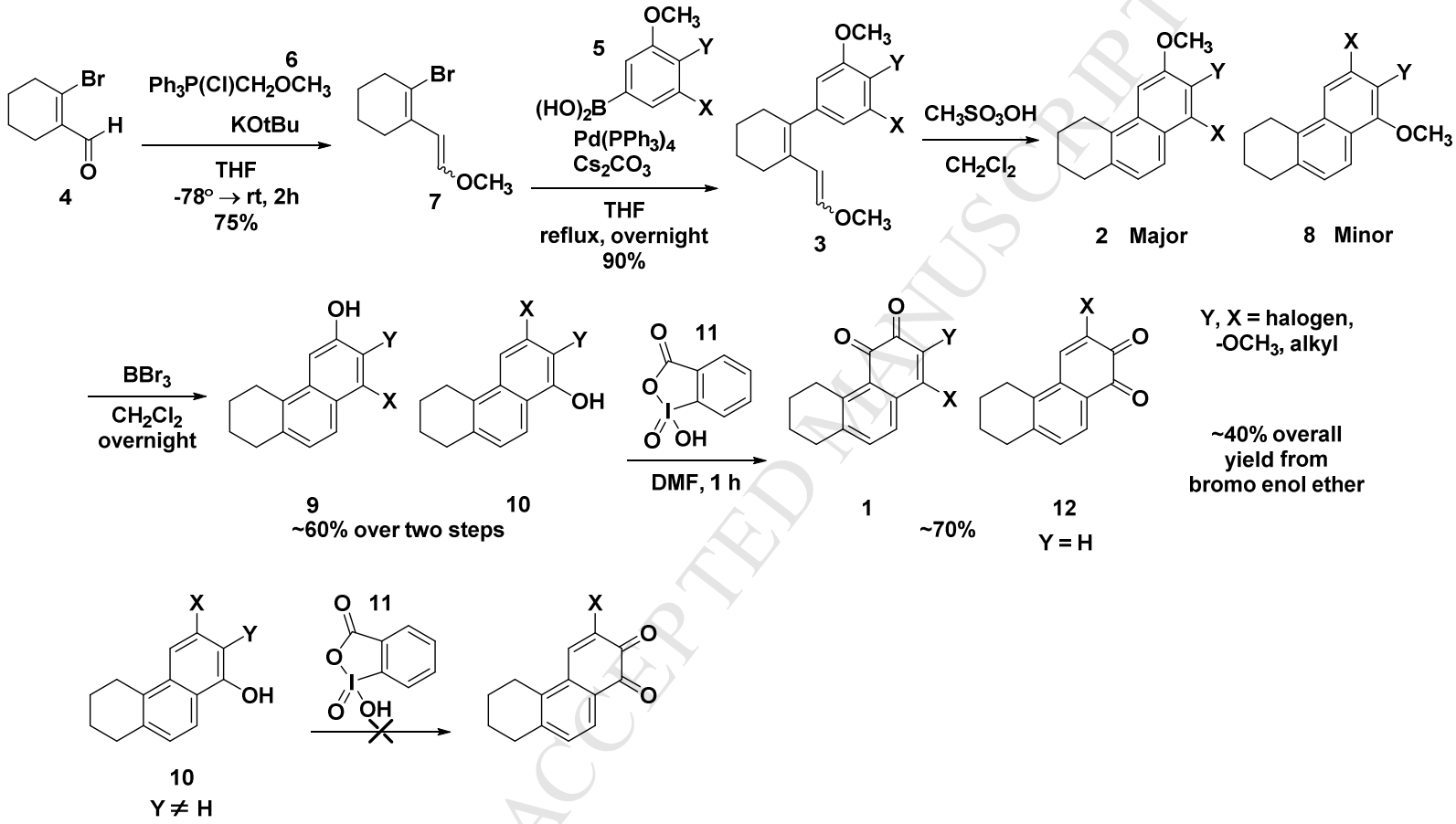
Scheme 2.



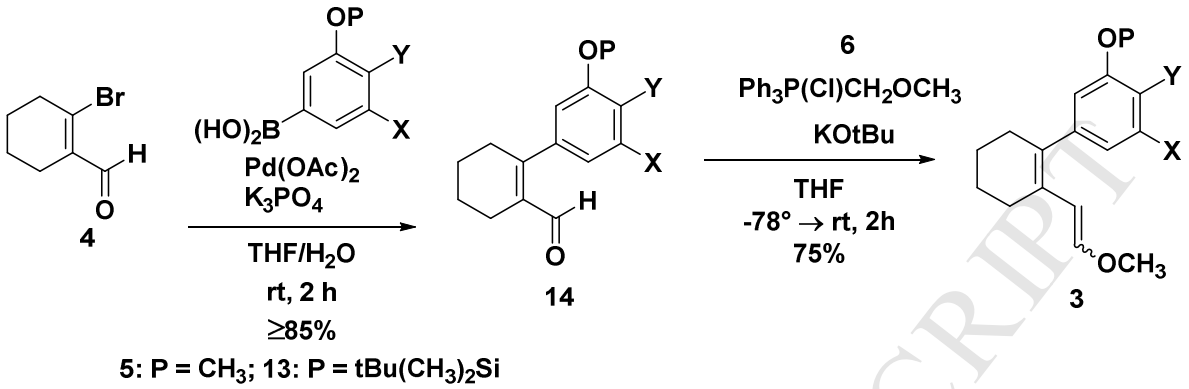
Scheme 3.



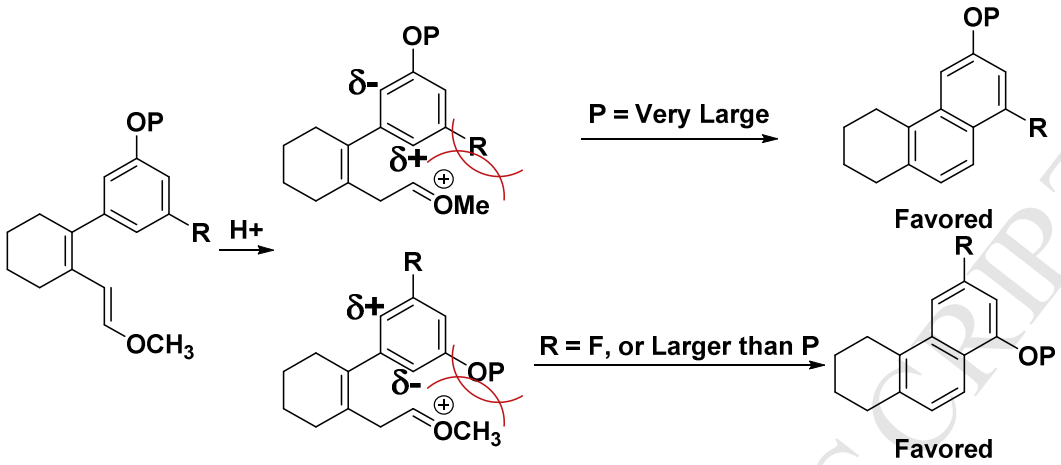
Scheme 4.

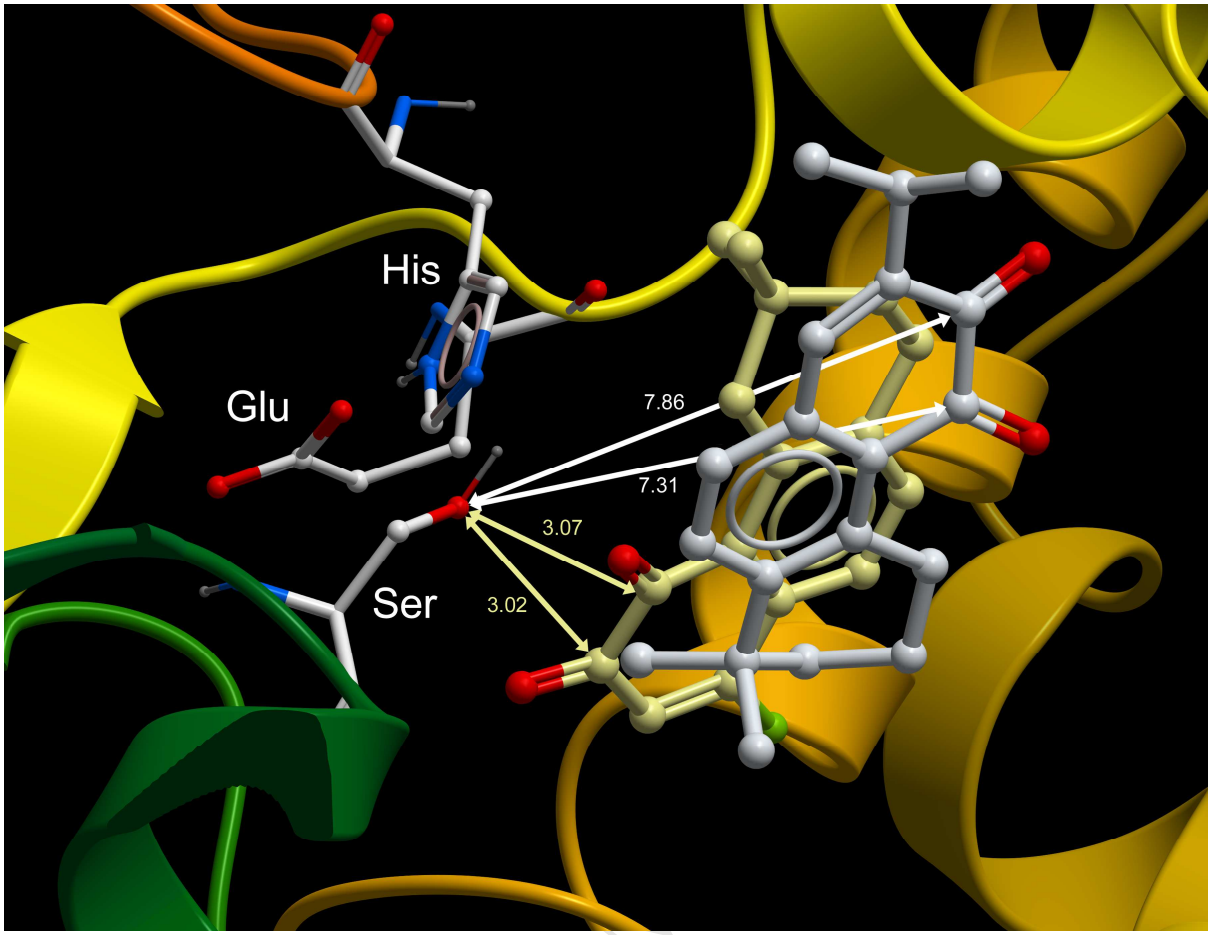


Scheme 5.



Scheme 6.





Highlights

- Novel, facile methods for the synthesis of miltirone analogues
- Allows for extensive analog generation and SAR
- Selective, non-promiscuous, human carboxylesterase inhibitors have been generated