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Structure−Activity Relationships of Substituted 1-Pyridyl-2-phenyl-1,2-ethanediones: Potent, Selective Carboxylesterase Inhibitors

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Inhibition of intestinal carboxylesterases may allow modification of the pharmacokinetics/pharmacodynamic profile of existing drugs by altering half-life or toxicity. Since previously identified diarylethane-1,2-dione inhibitors are decidedly hydrophobic, a modified dione scaffold was designed and elaborated into a >300 member library, which was subsequently screened to establish the SAR for esterase inhibition. This allowed the identification of single digit nanomolar hiCE inhibitors that showed improvement in selectivity and measured solubility.

Introduction

Carboxylesterases (CEs[a]) are widely expressed enzymes believed to be responsible for hydrolysis of xenobiotics in the body by catalyzing the hydrolysis of esters to the corresponding alcohols and carboxylic acids.[1] Since it is known that a number of important pharmaceutical agents are substrates for metabolism by CEs, it is apparent that compounds that modulate the activity of these enzymes may be useful in altering the half-life and/or toxicities that are associated with these drugs. The conversion of antitumor prodrug **1** (CPT-11) to **2** (SN-38), a potent topoisomerase I poison, (Figure 1) is an example of drug activation in vivo and is likely mediated by the human intestinal CE (hiCE, CES2), an enzyme primarily expressed in the liver and duodenum.[2,3] A second CE, hCE1 (CES1), is expressed in human liver, but this enzyme demonstrates poor kinetic properties toward **1** and it is unclear whether hCE1 significantly contributes to drug activation in humans.[2,3] The dose limiting toxicity for **1** is diarrhea that likely arises in part from the direct hydrolysis of **1** in the intestine by hiCE. This is due to the drug being excreted into the bile and hence deposited into the duodenum. Developing specific inhibitors of hiCE that could potentially ameliorate toxicity of **1** would have broad clinical utility, improving patient quality of care while potentially allowing for the administration of higher and more effective doses.

We have recently identified several different chemical classes of selective CE inhibitors, including bisbenzene sulfonamides, isatins, trifluoroketones, and ethane-1,2-diones.[4−8] In general, these compounds are potent (K_i as low as 5 nM) and demonstrate no cross reactivity with acetyl- or butyrylcholinesterase. Benzil can inhibit hiCE intracellularly resulting in reduced sensitivity of cells to **1**.[9] However, the active site of CEs is located at the base of a deep hydrophobic pocket, and efficacious inhibitors also tend to be highly lipophilic and water insoluble.[10] We wished to determine if the diarylethane-1,2-dione scaffold could be modified in such a way as to maintain or increase compound potency while simultaneously allowing for improved solubility relative to benzil. Since the introduction of a polar pyridine group would be expected to enhance solubility and bioavailability, we felt the most promising strategy involved replacing one of the phenyl groups of benzil with a pyridine group. Furthermore, we planned to introduce a synthetically accessible functional group for both aryl rings of the 1-phenyl-2-pyridyl-1,2-ethanedione that would also serve as sites for diversification with the appropriate reagents. A survey of available synthons led to the introduction of an amino group onto the 1-phenyl-2-pyridyl-1,2-ethanedione scaffold in our design, which could be elaborated into an amide library through parallel synthesis to maintain potency toward CE inhibition but have improved solubility relative to benzil, as determined by solubility measurements of active compounds.

Chemistry

Our synthetic plan is outlined in Scheme 1 and was based on intermediate preparation step efficiency, with the intermediates subjected to diversification via amide coupling, then oxidation, all in a parallel format. We began by exploring the Sonogashira coupling conditions for **3a** with **4b** to give **6a**. We found that standard conditions (catalytic $Pd(PPh_3)_2Cl_2$ and CuI) did provide the desired product; however, the product was frequently contaminated with unidentified impurities that coeluted during chromatography. In an effort to improve the yield and purity of **6a**, we then examined conditions described by Fu and Buchwald for the Sonogashira coupling of aryl bromides at room temperature using $Pd(PhCN)_2Cl_2$, CuI, and $^tBu_3P$.[11,12] These conditions worked exceptionally well,

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[a] Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CE, carboxylesterase; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; hCE1 (CES1), human carboxylesterase 1; hiCE (CES2), human intestinal carboxylesterase; MAOS, microwave assisted organic synthesis; NaHMDS, sodium bis-(trimethylsilyl)amide; SAR, structure−activity relationship.

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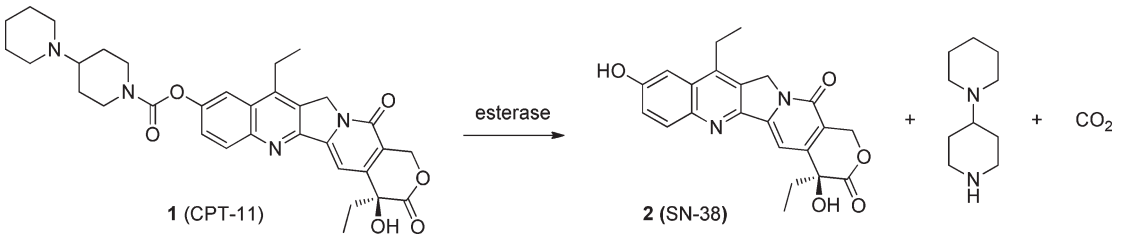
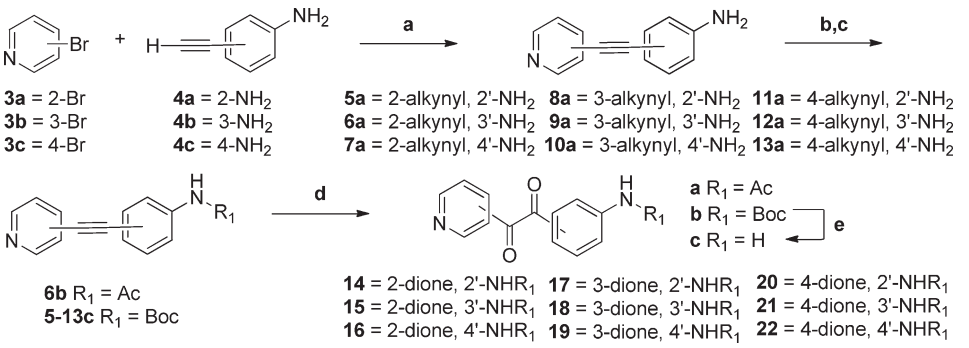


Figure 1. Chemical structure and hydrolysis of **1** resulting in the formation of **2**.

Scheme 1. Synthesis of 1-Pyridyl-2-(aminophenyl)ethane-1,2-diones[a]



[a] Reagents and conditions: (a) 2% CuI, 3% $Pd(PhCN)_2Cl_2$, 6% $^tBu_3P \cdot HBF_4$, 1.2 equiv of $^iPr_2NH$, dioxane, 25 °C, 3 h; (b) 1.5 equiv of AcCl, 2.0 equiv of Et_3N, THF, 0 °C; (c) NaHMDS, Boc_2O, THF, 0−25 °C; (d) $KMnO_4$, Na_2SO_4, $NaHCO_3$, aqueous acetone; (d) AcCl, MeOH, 0 °C.

proceeding rapidly (3 h at 25 °C or within 30 min at 80 °C) and giving **6a** in 86% yield. We also found that these conditions work well on larger scales (the preparation of **6a** could be scaled up to a 60 mmol scale without compromising yield or purity).

With these coupling conditions in hand, we prepared **6b** as a model compound to examine the feasibility of a parallel oxidation reaction. At the time of our investigation, several methods had been published for the oxidation of diarylalkynes into ethane-1,2-diones; however, all required harsh oxidants and/or conditions, and most of these reports did not elucidate the scope and limitations of functional group compatibility with the reported reaction conditions.[13−16] Of these methods, we initially chose conditions using $KMnO_4$ described by Walsh and Mandal to determine suitability for adaptation of these conditions into a parallel format.[17] Though the oxidation successfully produced the desired dione **15a**, there were several concerns. The reaction was exceedingly fast at both 0 and 25 °C with substantial loss of desired product, due to overoxidation, if the reaction was not promptly quenched. Additionally, the quench with sodium bisulfite solution resulted in an exotherm and gas evolution that could create an overpressure resulting in overflow or, in a sealed format, a possible explosion.

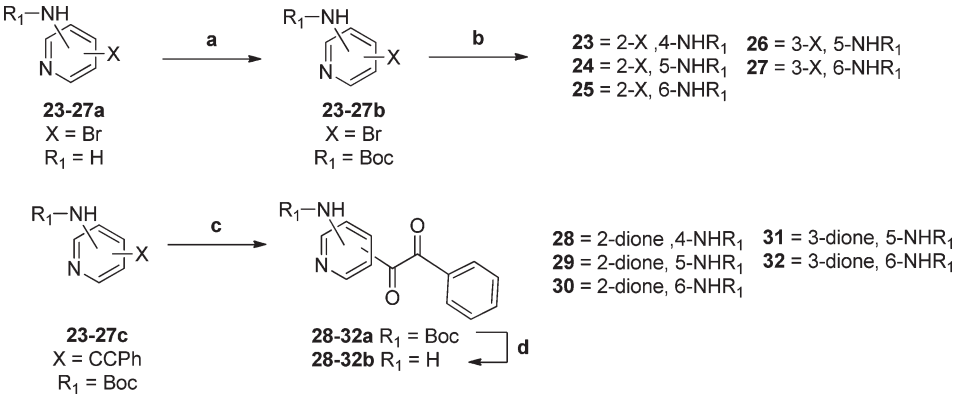
At this initial stage, the parallel oxidation under these conditions appeared impractical, so the synthesis was modified to produce intermediates **14**−**22b** that would then be diversified in the final step. This required the use of an amino protecting group that was stable to the $KMnO_4$ oxidation, yet easily removed in a subsequent step. For this purpose we chose to introduce the Boc protecting group by treating **6a** with Boc anhydride and NaHMDS (1 M in THF) to give **6c** in 78% yield.[18] The Boc derivative **6c** was then oxidized to give the desired Boc protected ethane-1,2-dione **15b** in 60% yield. Though TFA mediated deprotection (even in the presence of triethylsilane)[19] failed to deliver **15c** (due to decomposition of product under these conditions), we found that the use of anhydrous HCl resulted in the production of **15c**, cleanly in 60% yield.[19] **15c** was fully characterized as derivatives, since this aminoketone was not found to be stable to concentration and storage (presumably because of polymerization). We found that **15c** could be used as an intermediate if the CH_2Cl_2 extracts were routinely rapidly concentrated following aqueous workup and stored as a 1 M DMF solution.

With this general experimental protocol available, we then began the synthesis of the remaining aminophenyl positional isomers (**14c**, **16**−**19c**) in order to broaden the scope of our SAR studies. We did find a limitation on the scope of this scheme in that these conditions did not afford the desired dione intermediate in the case of 4-bromopyridine **3c**. This reaction sequence produced **12a**, **12c**, and **21b**, but attempts to deprotect **21b** failed to yield useful amounts of **21c**, presumably because of accelerated polymerization. Recognizing this scope limitation, we excluded **20**−**22c** from our plans for library design. We also excluded 2′-amino scaffolds **14c** and **17c** after a screen of Boc protected compounds **14**−**19b** showed that compounds **14b** and **17b** were inactive at 30 μM when screened against hiCE.

We then turned our attention to the scale-up of the required 1-(aminopyridyl)-2-phenylethane-1,2-dione intermediates (Scheme 2). The details of this approach take advantage of insights gained during the preceding synthetic scope and limitation study. Commercially available aminobromopyridines **23**−**27a** were protected as their Boc derivatives **23**−**27b**, then coupled using the Buchwald−Fu Sonogashira coupling to give **23**−**27c** in good yield (68−85%). The Sonogashira products were then cleanly oxidized to the give the Boc protected diketones **28**−**32a** in 40−65% yield. In general the Boc protecting group removal for **28**−**32a** was slow at room temperature and required several hours at reflux to generate the desired products **28**−**32b** that were then stored as 1 M solutions in DMF.

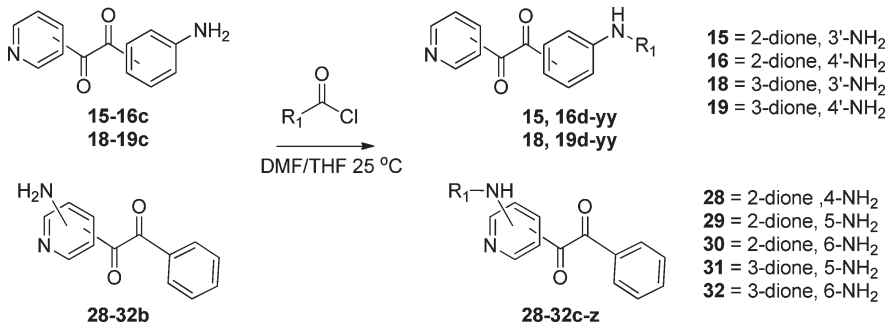
Having prepared the desired intermediates, we then designed a library that used a selection of diverse acid chloride building blocks for the generation of new amides to probe the

Scheme 2. Synthesis of 1-(Aminopyridyl)-2-phenylethane-1,2-diones[a]



[a] Reagents and conditions: (a) Boc_2O, NaHMDS, THF 0−25 °C, 16 h; (b) 2% CuI, 3% $Pd(PhCN)_2Cl_2$, 6% $^tBu_3P\cdot HBF_4$, 1.2 equiv of $^iPr_2NH$, 1.1 equiv of phenylacetylene, dioxane 25 °C, 3 h; (c) $KMnO_4$, Na_2SO_4, $NaHCO_3$, aqueous acetone; (d) AcCl, MeOH, 80 °C.

Scheme 3. Preparation of the CE Inhibitor Library[a]



[a] R_1 comprised a diverse selection of alkyl, aryl, and heteroaryl groups. See Supporting Information for data on individual library members.

effect of substitution on CE potency (see Scheme 3). The parallel synthesis was undertaken in 48 position XT Miniblock reaction synthesizer blocks. A range of primary, secondary, and tertiary aliphatic compounds and aromatic and heteroaromatic acid chlorides were successfully utilized with this protocol with the exception of the acid chlorides of nicotinic and isonicotinic acid. Compounds were isolated by preparative reverse phase HPLC purification and submitted for screening following completion of our in-house quality control protocols. Eighty-two percent of the targeted compounds in the 308-member library were obtained with ≥85% purity in ≥1 mg quantity.

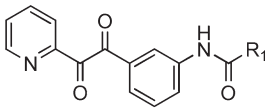
Results and Discussion

We then assessed the ability of these compounds to inhibit hiCE and hCE1 using a series of spectrophotometric assays.[20−22] In the initial screen IC_{50} values were measured (Table 1), and on the basis of these results, a set of the most potent compounds were then subjected to K_i determination (Table 2). Analysis of the resulting IC_{50} values revealed a defined SAR selectivity pattern. No library members utilizing the 1-(aminopyridyl)-2-phenylethanedione scaffolds **28**−**32** exhibited an IC_{50} potency below 2.5 μM, with the majority of these analogues being poorly active or inactive in our assay. Similar results were seen from library members derived from **18c** and **19c**. Fortunately, better results were observed with scaffolds **15c** and **16c**. Compounds based on **16c** incorporating a thiophene based amide yielded several submicromolar inhibitors. The most potent compounds that we identified at this point were obtained from scaffold **15c**. Ten substituted aromatic amide analogues were identified with IC_{50} values below 350 nM (Table 1) and nine additional analogues with sub-600 nM IC_{50} values. Overall, aliphatic amides of **15c** were much less potent than aromatic amides, although an increase in chain length (and therefore hydrophobicity) was associated with increased potency, as shown in Table 1. (Assay data for all library members can be found in the Supporting Information.)

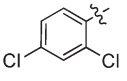
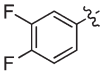
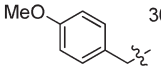
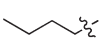
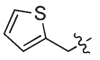
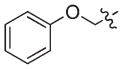
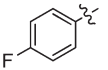
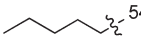
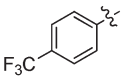
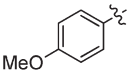
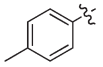
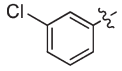
At this point in the project we became aware of a report of a diarylalkyne oxidation protocol mediated by catalytic amounts of $PdBr_2$ and PdI_2 at elevated temperature in DMSO.[23] This report was especially intriguing to us, since the reaction conditions appeared compatible with several functional groups on the diarylalkynes, including amides. The authors also noted 1-pyridyl-2-arylalkynes oxidized under these conditions but required increased reaction time to effect conversion. We believed this procedure would allow us to affect our original experimental design, a parallel oxidation following intermediate diversification, so we chose to examine this potentially efficient alternative scheme.

With **6d** as a test substrate, a pilot study was performed using 10 mol % $PdBr_2$ at 140 °C for 9 h. Despite producing the desired dione **15d**, a thick emulsion formed during the aqueous workup requiring filtration through Celite to facilitate extraction. Though this difficulty was easily handled on a preparative scale; the emulsion posed a serious barrier for library purification by reverse phase HPLC. In an attempt to circumvent the emulsion problem, we turned to microwave assisted organic synthesis (MAOS).[24,25] The application of MAOS has steadily increased over the past decade because of its increased heating efficiency that allows for rapid reactions

Table 1. Assay IC_{50} Results for hiCE of Selected Aminopyridylphenylethane-1,2-dioneamides[a]



15

entry	IC_{50}	entry	IC_{50}	entry	IC_{50}	entry	IC_{50}	entry	IC_{50}
1 	54 nM **15d**	5 	135 nM **15h**	9 	302 nM **15l**	13 	3.3 μM **15p**	17 	868 nM **15t**
2 	55 nM **15e**	6 	177 nM **15i**	10 	326 nM **15m**	14 	2.4 μM **15q**	18 	540 nM **15u**
3 	59 nM **15f**	7 	213 nM **15j**	11 	8 μM **15n**	15 	2.3 μM **15r**	19 	354 nM **15v**
4 	132 nM **15g**	8 	250 nM **15k**	12 	2.5 μM **15o**	16 	1.1 μM **15s**		

[a] Data obtained from primary screen.

Table 2. Selectivity Profile of **15d**−**m** against hiCE, hCE1, AChE, and BChE Including Measured Solubility and cLogP

	K_i (nM)[a]					
compd	hiCE	hCE1	AChE (nM)	BChE (nM)	solubility (μg/mL)[b]	cLogP
15d	1.48 ± 0.15	279 ± 45	> 100000	> 100000	3.3	4.51
15e	4.65 ± 0.4	> 100000	> 100000	> 100000	19.6	3.17
15f	1.17 ± 0.12	437 ± 61	> 100000	> 100000	0.5	4.12
15g	2.86 ± 0.19	1034 ± 145	> 100000	> 100000	8.4	3.67
15h	2.73 ± 0.14	1244 ± 155	> 100000	> 100000	0.7	3.59
15i	3.68 ± 0.4	> 100000	> 100000	> 100000	16	3.02
15j	4.67 ± 0.39	> 100000	> 100000	> 100000	13.7	3.17
15k	1.55 ± 0.16	756 ± 92	> 100000	> 100000	3.1	3.88
15l	3.23 ± 0.17	> 100000	> 100000	> 100000	14.1	3.20
15m	3.6 ± 0.23	381 ± 90	> 100000	> 100000	5.1	3.39

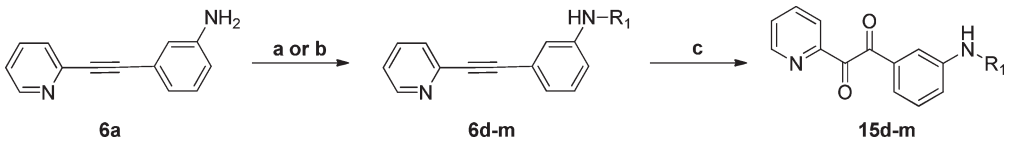
[a] Measured K_i ± standard error obtained from resynthesized, fully characterized compounds. [b] Solubility measured at pH 4.0.

with the potential for fewer side reactions. Since the alkyne oxidation required elevated temperatures for good conversion, we explored the use of microwave irradiation to shorten the reaction time and suppress formation of the (unknown) emulsifying agents. We were gratified to find that the oxidation of **6d** was complete within 3 min at 200 °C and that the emulsion formation was significantly reduced during aqueous workup. The emulsion was further minimized by switching from ethyl acetate to chloroform for the extractive workups. By use of this protocol, **15d** was isolated in 58% yield, following purification on silica gel.

To examine the suitability of these new conditions for parallel synthesis, we applied this new approach to the resynthesis (see Scheme 4) of the 10 most potent compounds identified in our original screen. Thus, **6a** was acylated under standard conditions with the required acid chlorides to produce **6d**−**m** in excellent yield (≥85%). In one case (**6f**) it was necessary to use the corresponding carboxylic acid in the presence of HBTU in DMF to give the desired compound, which proceeded in excellent (92%) yield. This result is notable in that **15c** failed to acylate using this type of coupling conditions, which necessitated the use of more reactive acid chlorides for our library production. Though widely available, the number of commercially available acid chlorides is significantly smaller than that of commercially available carboxylic acids. This substantially expands the scope of this scheme. Following purification, **6d**−**m** were taken up in DMSO, treated with $PdBr_2$, and sequentially brought to 200 °C, using microwave heating in a Biotage Initiator. Upon cooling, the compounds were then subjected to a parallel aqueous workup. Following $CHCl_3$ extraction, the crude products were concentrated and purified on silica to give the desired products **15d**−**m** in 46−71% yield. Subsequent determination of K_i for these compounds closely recapitulated the results of our original screen and increased our confidence in the SAR trends observed.

In the previous examination of diarylethane-1,2-diones as CE inhibitors, it was noted that inhibitory potency increased with cLogP measurements. After performing cLogP calculations for the library members derived from scaffold **15**,[26] we measured the solubility of compounds **15d**−**m** at pH 4.0 and 7.4 and noted some interesting correlations (Table 2 and Supporting Information Figure 1). While the scaffold **15** library did generally follow the same trend of increased potency with higher cLogP values that has also been reported with other scaffolds,[27] several important differences to this

Scheme 4. Parallel Synthesis of 1-Pyridyl-2-(aminophenyl)ethane-1,2-diones via Microwave Irradiation[a]



[a] Reagents and conditions: (a) 0.125 mmol of **6b**, 1.3 equiv of acid chloride, 1.5 equiv of Et_3N, THF; (b) 1.2 equiv of carboxylic acid, 1.3 equiv of HBTU, 1.5 equiv of Et_3N, DMF, 25 °C; (c) 10 mol % $PdBr_2$, DMSO, 200 °C, microwave heating. See Table 1 for R_1 groups.

correlation were observed. Interestingly these diketones show a pronounced difference in slope of log K_i versus cLogP between the two esterases (hiCE and hCE), whereas the previously reported sulfonamide inhibitors show an essentially identical correlation slope (see Supporting Information Figure 1). It is also fortuitous that the slopes of log K_i versus measured solubility show a favorable difference in slope (see Supporting Information Figure 1). The "outliers" were also of interest, such as **15e**, which has a cLogP of 3.17 and 6 times the measured solubility (19.6 μg/mL vs 3.3 μg/mL) of our most potent compound **15d** which has a cLogP of 4.51. Compounds **15i** and **15l** were also identified as potent analogues with cLogP values of 3 with good measured solubilities of 16.0 and 14.1 μg/mL, respectively. Thus, we established that it is possible to maintain potent activity and high selectivity while improving solubility with this particular scaffold.

We further determined the selectivity of **15d**−**m** versus hCE1, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). As shown in Table 2, all 10 compounds were inactive against AChE and BChE. Compounds **15d** and **15f** were shown to be the most potent inhibitors of hCE1 among our 10 most active analogues and showed the desired selectivity (100-fold or more) in favor of hiCE inhibition. It is also of interest to note that all four analogues with measured solubilities greater than 10 μg/mL were inactive against hCE1. Graphical analysis of the K_i for the 1,2-diones vs either their cLogP or aqueous solubilities indicated good correlation coefficients (see Supporting Information Figure 1). Interestingly, better correlations were observed with the former parameter with hiCE, and the latter with hCE1. We were surprised by the r^2 values that were obtained by these analyses (as high as 0.83), considering the relatively small data sets that were analyzed. While it is apparent that the active sites for CEs are highly hydrophobic, the reasons for the differences observed between the two human enzymes are unclear. More importantly, however, these analyses could provide parameters that will guide the design and synthesis of novel inhibitors with additional improvements in selectivity and potency.

In summary, we have prepared a number of amino-substituted 1-phenyl-2-pyridylethane-1,2-dione scaffolds, which were readily diversified using commercially available acid chlorides. This library produced a clear SAR pointing to a single scaffold (**15**) that when properly modified gave potent (single digit nanomolar), selective, and relatively soluble hiCE inhibitors. The top 10 active analogues identified in the primary screen were synthesized on a preparative scale using an efficient microwave assisted protocol that reduces the number of required intermediate preparatory steps and allows the introduction of carboxylic acids as a point of diversity for future libraries. The measured K_i for these compounds confirmed our initial screening results, and further profiling showed that they have significant selectivity for hiCE versus hCE1, AChE, and BChE. Several of these compounds also gave notable improvements in measured solubility relative to benzil, confirming that the diarylethane-1,2-dione scaffold can be elaborated into a highly potent and selective leadlike compound class. Additionally we have uncovered a trend in the SAR that allows us to couple improvements in solubility and selectivity over hCE1. With aromatic amides of scaffold **15** as a starting point, we plan to use these observations to prepare compounds for eventual in vivo experiments and will report these results in due course.

Experimental Section

General Methods. All reagents and anhydrous solvents were purchased from Sigma-Aldrich or Acros Organics and used as received. Reactions were set up in air and carried out under nitrogen atmosphere. Parallel synthesis was accomplished using MiniBlock XT synthesizers (purchased from Mettler-Toledo AutoChem) placed on a stirring hot plate. Intermediates were prepared using standard glassware purchased from Chemglass or in glass microwave vials with inert septa-aluminum crimp caps purchased from Biotage or Chemglass. Flash chromatography was carried out on prepacked silica cartridges using a Biotage SP4 or Biotage Isolera chromatography system. ^1H and ^{13}C NMR spectra were recorded on a Bruker-400 MHz spectrometer at 400 and 101 MHz respectively. Prepurification and QC analysis was performed on a Waters Acquity UPLC/PDA/ELSD/MS system using a BEH C18 2.1 mm × 50 mm column with a 90:10 to 5:95 0.1% aqueous formic acid/acetonitrile 2 min gradient elution method. Library purification was performed using a Dionex mass directed HPLC purification system using a Phenomenex Gemini Aixia packed C18 30 mm × 50 mm, 5 μm column using either 0.1% aqueous formic acid/acetonitrile or 0.1% aqueous ammonium bicarbonate/acetonitrile gradient adjusted based on prepurification results. Fully characterized compounds have purities of ≥95%; purity values for library members are contained in the Supporting Information.

3-(Pyridine-2-ylethynyl)aniline (6a). In a 20 mL glass microwave vial, CuI (0.019 g, 0.1 mmol, 0.02 equiv), $Pd(PhCN)_2Cl_2$ (0.057 g, 0.15 mmol, 0.03 equiv), and $^tBu_3PHBF_4$ (0.087 g, 0.3 mmol, 0.06 equiv) were combined; the vial was sealed with an aluminum crimp cap, evacuated, and placed under a nitrogen atmosphere. The mixture was diluted with 15 mL of anhydrous 1,4-dioxane followed by 2-bromopyridine (0.5 mL, 5.00 mmol, 1 equiv), 3-ethynylaniline (0.70 g, 6.00 mmol, 1.2 equiv), and $^iPr_2NH$ (1.4 mL, 10 mmol, 2 equiv). The mixture was then stirred at 25 °C until the starting materials were consumed as observed by TLC. This was visually indicated by the precipitation of Pr_2NH-HBr. The crimp cap was removed and the thick slurry diluted with EtOAc and transferred to a fritted funnel containing Celite and filtered. The filtrate was concentrated and the residue purified by chromatography on silica gel using a 0−60% EtOAc/hexane gradient to give 0.72 g of **6a** in 74% yield as an off white solid.

***tert*-Butyl (3-(Pyridin-2-ylethynyl)phenyl)carbamate (6c).** In a 100 mL round-bottom flask under nitrogen, **6a** (0.971 g, 5.0 mmol, 1 equiv) and di-*tert*-butyl dicarbonate (1.20 g, 5.5 mmol, 1.1 equiv) were combined and dissolved in 10 mL of anhydrous THF. The mixture was cooled to 0 °C and treated with NaHMDS (1 M in THF, 10.5 mmol, 2.1 equiv) dropwise over 20 min. The mixture was allowed to slowly warm to 25 °C overnight, then treated with 30 mL of saturated NH_4Cl. The mixture was extracted with

EtOAc (3 × 20 mL), and the combined organics were washed with saturated NaCl, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography on silica gel using a 0−40% EtOAc/hexane gradient to give 1.14 g of **6c** in 78% yield as an off white solid.

***tert*-Butyl (3-(2-Oxo-2-(pyridin-2-yl)acetyl)phenyl)carbamate (15b).** In a 250 mL round-bottom, **6c** (1.47 g, 5 mmol, 1 equiv) was dissolved in acetone (60 mL) at 25 °C and treated with a 0.22% $NaHCO_3$/2.2% $MgSO_4$ aqueous solution (30 mL). $KMnO_4$ (1.97 g, 12.5 mmol, 2.5 equiv) was added portionwise over 5 min to ensure dissolution in the vigorously stirred solution. After the indicated period, the reaction was quenched by dropwise addition of 50% aqueous $NaHSO_3$ (30 mL) followed by stirring for 1 h. The milky suspension was filtered through a fritted filter containing Celite. The filtrate was extracted with EtOAc (3 × 25 mL), and the combined organics were washed with saturated NaCl, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by chromatography on silica gel using a 0−40% EtOAc/hexane gradient to give 0.98 g of **15b** in 60% yield as a yellow solid.

1-(3-Aminophenyl)-2-(pyridin-2-yl)ethane-1,2-dione (15c). In a 100 mL round-bottom flask under nitrogen, anhydrous MeOH (20 mL) was cooled to 0 °C and treated with acetyl chloride (7.96 mL, 112 mmol, 10 equiv) over 30 min. After 15 min, the resulting solution was transferred (via cannula or syringe) to a separate 250 mL round-bottom flask that contained a solution of **15b** (3.63 g, 11.15 mmol, 1 equiv) in anhydrous MeOH under nitrogen at 25 °C. The mixture was stirred until the starting material was consumed as judged by TLC or LCMS. The reaction was quenched by the slow addition of saturated $NaHCO_3$ (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the combined extracts were washed with saturated NaCl, dried with $MgSO_4$, filtered, and concentrated to give 1.51 g of **15c** as an orange oil in 60% yield. The oil was immediately taken up in 6.7 mL of DMF to prepare a 1 M stock solution.

Procedure for the Acylation of Amino(phenyl, pyridyl)-1,2-ethane Dione Library. A 48 position Mettler-Toldeo Miniblock XT containing 11.5 mm × 110 mm reaction tubes with stir bars was charged with 75 μL of the desired amino(phenyl, pyridyl)-1,2-ethanedione (0.075 mmol), 1 M DMF stock solution, followed by Et_3N (16 μL, 0.113 mmol, 1.5 equiv). The reaction block was topped with an inert atmosphere manifold and purged with nitrogen. The vessels were then treated with 100 μL of the appropriate acid chloride stock solution (1 M in THF) and allowed to stir overnight at 25 °C. 2.5 mL of saturated NH_4Cl solution was added to each vessel and mixture extracted with EtOAc (2.5 mL). The organic extracts were transferred to a Genevac 16 mm × 100 mm test tube rack and concentrated. The residue was dissolved in DMSO (1.75 mL) and transferred to a 2 mL Thomson filter plate (25 μm), prepacked with Celite, connected to a Waters 96-well 2 mL collection plate. Upon filtration, the collection plate was analyzed by UPLC and product bearing wells purified via reverse phase chromatography. Final purity was measured by the total wavelength current (TWC) from λ = 210−400 nm on UPLC.

Determination of Inhibition Constants. For CEs, K_i values were determined with 3 mM *o*-nitrophenyl acetate as a substrate using a spectrophotometric approach as previously reported.[7,8] Inhibition of cholinesterases was determined using acetylthiocholine or butyrylthiocholine as substrates.[20,28] In all cases, data were analyzed using a multifactorial equation[29] and K_i values calculated using GraphPad Prism software.

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Supporting Information Available: General synthetic methods, characterization data for all intermediates and potent analogues, analytical data for amide library, and assay description. This material is available free of charge via the Internet at http://pubs.acs.org.

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