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Carboxylesterase inhibitors

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Abstract

Introduction—Carboxylesterases play major roles in the hydrolysis of numerous therapeutically active compounds. This is, in part, due to the prevalence of the ester moiety in these small molecules. However, the impact these enzymes may play on drug stability and pharmacokinetics is rarely considered prior to molecule development. Therefore, the application of selective inhibitors of this class of proteins may have utility in modulating the metabolism, distribution and toxicity of agents that are subjected to enzyme hydrolysis.

Areas covered—This review details the development of all such compounds dating back to 1986, but principally focuses on the very recent identification of selective human carboxylesterases inhibitors.

Expert opinion—The implementation of carboxylesterase inhibitors may significantly revolutionize drug discovery. Such molecules may allow for improved efficacy of compounds inactivated by this class of enzymes and/or reduce the toxicity of agents that are activated by these proteins. Furthermore, since lack of carboxylesterase activity appears to have no obvious biological consequence, these compounds could be applied in combination with virtually any esterified drug. Therefore, inhibitors of these proteins may have utility in altering drug hydrolysis and distribution in vivo. The characteristics, chemical and biological properties, and potential uses of such agents, are discussed here.

1. Introduction

Carboxylesterases (CE) are ubiquitous enzymes that are responsible for the hydrolysis of carboxylic acid esters into their corresponding acid and alcohol [1, 2]. To date, no endogenous substrates have been definitively identified for these ubiquitously expressed enzymes, and as a consequence they are generally considered protective, detoxifying proteins [3]. This is in part, born out by their pattern of expression (they tend to be located in the epithelia that are likely to be exposed to xenobiotics) and the plastic nature of the active site that can accommodate substrates of widely differing structure [4]. The reason that these proteins are of importance to the biomedical field, apart from their interesting biochemistry, is that since numerous drugs, pesticides, and veterinary products contain ester moieties, these small molecules are de facto substrates for these enzymes. Hence, molecules as structurally diverse as irinotecan (CPT-11; [5-7]), Tamiflu [8], Ritalin [9], the insecticides trans-permethrin and bioresmethrin [10], as well as cholesteryl esters [11], are all substrates for CEs (Figure 1).

Furthermore, since the majority of new drugs are discovered through synthetic drug discovery programs rather than from natural products, and the pharmaceutical industry

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frequently uses esters groups to improve water solubility of clinical leads, it is likely that the metabolism of many of these agents will be impacted by this class of enzymes. For example, β-flestolol (Figure 1) is an ester that is rapidly degraded in vivo by CEs [12]. Since the half life of this molecule, which acts as a beta blocker, is very short, improvements in drug stability might be apparent if the isoforms and levels of enzyme that inactivate this drug are examined. In addition, while it has not been specifically tested, methoprene (Figure 1), a component of the broad spectrum insecticide Frontline, would be expected to be a substrate for CEs. Therefore understanding the biology, biochemistry, levels of expression in target tissues, and substrate specificity of these proteins should allow better application of small molecule therapies.

It should also be noted however, that the hydrolysis mediated by CEs may act to either activate or inactive a particular molecule. For example, CPT-11 is an anticancer prodrug for which hydrolysis is absolutely required for the generation of SN-38, a potent topoisomerase I poison [7]. Similarly, capecitabine (Figure 1), a 5-fluorouracil derived prodrug requires sequential activation by several enzymes, including CE, to exerts its biological activity [13, 14]. By contrast, compounds such as cocaine, lidocaine, Demerol, etc (Figure 1), are all inactivated by this process [15-18]. Hence, modulation of CE activity may present an opportunity to alter drug metabolism and pharmacokinetics, with the ultimate goal of improving therapy. With this goal in mind, small molecule inhibitors of this class of enzyme have been developed with the specific intention of altering drug-induced toxicity [19-24]. This review details the identification, development, and potential utility of such molecules, and an evaluation of the current status of patents and applications that seek to achieve these goals.

2. Carboxylesterase inhibitors

2.1 Preamble

Recent searches (February 2011) of both Entrez PubMed and the patent databases indicate that very few specific CE inhibitors have been identified. Indeed, while numerous patents report methods and approaches that might use a ‘putative inhibitory compound’, no information concerning the availability of such a molecule is presented. It should be noted that patent applications that detail the development of specific CE inhibitors have been submitted to the United States Patent and Trademark Office (Applications #20080146548 and 20050054691). Hence in this article, I will detail the science behind the development of the compounds identified in the Potter laboratory, evaluate their potential application towards CE inhibition, and discuss why little effort has been expended to isolate and develop such compounds.

2.2 Esterase inhibitors

The field of esterase inhibitors is enormous, with the vast majority of agents being targeted towards acetylcholinesterase. This has in part been due to their development by armed forces for use as chemical warfare agents (e.g., Sarin, Soman, etc). This, combined with their ease of synthesis, their incredible potency, their facile large-scale delivery and deployment (Sarin is toxic both by inhalation and transdermal absorption, even in vapor form [25]), and their immediate biological effects, has resulted in significant efforts to understand acetylcholinesterase biology [26, 27]. This has lead, for example, to over 150 structures of this enzyme with associated ligands being deposited in the RCSB database. Obviously, there have been significant benefits for this research towards the medical field, including the development of donepezil (Aricept; [28, 29]) and rivastigmine (Exelon; [29, 30]) for the treatment of Alzheimer's disease [31]. However, it is tempting to speculate that

this would have been much less advanced if military funding for this area of research were not involved.

Since the consequences of CE inhibition are much less catastrophic (indeed plasma esterase-deficient mice are viable, healthy, and live a normal life span [32, 33]) less effort and funds have been directed towards understanding the biology of these enzyme. This is exemplified by the fact that only 15 mammalian CEs structures have been reported and 14 have resulted from collaborative efforts from the Potter and Redinbo laboratories [2, 4, 34-39]. However, when taken in context, it is likely that many more people are affected by the everyday effects of CE-mediated metabolism of drugs, rather than the threat of nerve agent poisoning.

2.3 Carboxylesterase inhibitors

Prior to 2004, essentially no selective, potent mammalian CE inhibitors had been identified. Several had been purported to have very modest activity (e.g., the series of Bomins and bis(4-nitrophenyl)phosphate), however these reagents are organophosphates (compounds that may have activity towards acetyl- and butyrylcholinesterases) and are unlikely to be specific for CEs. Following the development of recombinant expression methodology for two different human CEs [40], efforts to identify inhibitors of these enzymes were initiated using a small scale screening strategy (Telik's Target-Related Affinity Profiling technology [41, 42]). Using this approach, multiple rounds of enzymatic assays were conducted using defined scaffolds, based upon the TRAP criteria. Ultimately, ~250 compounds were screened to provide coverage of ~10,000 small molecules. All CE inhibitors were then screened against human AChE and those demonstrating activity were discarded. This resulted in the isolation of several different chemical classes of inhibitors, which generally fell into two classes: those that were specific for human intestinal CE (hiCE; hCE2), as compared to the human liver isoform (hCE1), and those that were pan CE inhibitors. The following discussion details the properties of these compounds.

2.3.1 Bisbenzene sulfonamides—The bisbenzene sulfonamides (Figure 2) were demonstrated to be specific hiCE and yielded K_i values for CE inhibition in the low nM range [24]. No inhibition of hCE1 was observed at inhibitor concentrations up to 100uM, but more importantly, these molecules demonstrated no activity toward purified human acetyl- or butyrylcholinesterase. QSAR analyses indicated that the potency of these compounds could be enhanced by introducing halogen atom substitutions on the benzene rings [20], although this improvement in activity was likely due to an increase in the clogP of the compounds, rather than altering specific interactions within the catalytic domain. Since the active sites of the human CEs are highly hydrophobic [1, 4, 35], preferred localization of more lipophilic molecules would be apparent in an aqueous environment. Analysis of the kinetic parameters for these compounds indicated that the mode of enzyme inhibition was partially competitive, i.e., that while their structure resembled a bona fide CE substrate, they were unable to completely reduce substrate hydrolysis to 0% [20, 24, 43]. Sulfonamide analogues have also demonstrated inhibition of thrombin (e.g. argatoban and NAPAP; [44]) and matrix metalloproteinases [45], and in each case, the major contributors toward small molecule binding was the presence of hydrogen bonds between the sulfonyl oxygen atoms and exposed protons within the enzyme active site. While no direct evidence exists that this is also true for the interaction of the bisbenzene sulfonamides with hiCE, since the enzymes demonstrate similar catalytic mechanisms, it is likely that this is the case.

Recent studies have indicated that these small molecules can modulate hiCE-mediated hydrolysis of several therapeutically relevant substrates (CPT-11, cocaine, heroin) [18, 20, 24]. However, the sulfonamides demonstrate very poor solubility in both aqueous buffers and organic solvents (they essentially only dissolve in DMSO), and in cell culture-based

assays they weakly inhibit intracellularly expressed hiCE, suggesting that they are poorly membrane permeable [22]. Due to these limitations, further development of these compounds has been limited.

2.3.2 1,2-Diones—The second class of molecules that was identified in the TRAP screening were ethane-1,2-diones, with benzil being the prototypical molecule (Figure 2) [23]. This compound inhibited the hydrolysis of o-nitrophenyl acetate (a generic esterase substrate) by both hiCE and hCE1 with K_i values of 15 and 45nM, respectively [23]. In addition, this molecule also inhibited a rabbit liver CE, hence benzil is a pan CE inhibitor.

2.3.2.1 Benzils: Benzil is a very simple compound (structurally speaking; Figure 2), and this allowed for the rapid interrogation of the requirement of specific domains within the molecule towards biological activity. For example, the related molecules benzophenone, butane-2,3-dione, benzoin, 1-phenyl-propane-1,2-dione, and 1,3-diphenylpropane-1,3-dione all demonstrated very weak or no activity against CEs, indicating that the 1,2-dione moiety was essential for enzyme inhibition [23]. In addition, these studies also indicated that the aromaticity of the benzene rings may play a role in potency [46]. Further detailed examination of a panel of benzil derivatives confirmed these results and led to the development of pseudoreceptor site models for the active sites of the CEs. These models were generated using only the K_i values for enzyme inhibition yet gratifyingly, overlays of these structures onto the x-ray coordinates for the active site of hCE1 confirmed the electronic architecture of residues within this domain, essentially validating this computational approach for inhibitor analysis [23]. In addition, these models have formed the basis for in silico design of novel CE inhibitors that may have practical utility [47]. Benzil and its derivatives have been used as initiators of polymerization of resins in the electronics industry and hence numerous analogues have been reported [48]. To date, over 100 of these compounds have been assayed for their ability to inhibit CEs and cholinesterases and results from these analyses have resulted in a generalized series of parameters that detail potency and selectivity towards the former class of enzymes. For example, molecules with higher clogP values tend to be more potent inhibitors [23, 46]; the planarity of the oxygen atoms within the 1,2-dione moiety, in part, determines selectivity between hiCE and hCE1 [49]; and aromaticity, while important, is not essential for inhibitory activity. Indeed, alkyl diones also demonstrate significant potency, although this only occurs when the clogP values for these compounds exceeds 4 (Parkinson & Potter, unpublished results).

While the mode of enzyme inhibition by the ethane-1,2-diones has been demonstrated to be partially competitive [46], the mechanism by which these agents act has not been definitively identified. It has been postulated that nucleophilic attack by the catalytic serine Oγ atom occurs on one of the carbonyl carbon atoms within this dione, resulting in a transiently stable intermediate (Figure 3). Since the C–O bond that would normally be present in an ester is replaced by a C–C bond in the 1,2-dione, and the latter is stronger and less polarized than the former, cleavage to generate the corresponding product does not occur. Therefore in the presence of inhibitor, repeated attack by the serine nucleophile and subsequent release of the small molecule occurs, effectively resulting in inhibition of the enzyme.

Structural examination of the complex formed between hCE1 and benzil indicates that under prolonged incubation conditions, benzoic acid and potentially benzaldehyde can be detected within the active site of the enzyme [37]. This suggest that under these conditions, hCE1 can cleave the C–C bond present within the 1,2-dione. However, it should be noted that in these crystallographic studies, the enzyme had been allowed to react with benzil for several months prior to analysis of the crystals. Since biochemical studies are routinely performed

on much shorter time scales (minutes), it is unlikely that similar products would be detected in these assays. Indeed, in prolonged incubation of benzil with mammalian CEs, we have been unable to detect any such hydrolysis products (Hatfield and Potter, unpublished results), suggesting that cleavage of the 1,2-dione would not occur in time frames where these compounds would be used in therapeutic studies.

It should also be noted that benzil is essentially non-toxic, demonstrating an LD_{50} for rats of 3g/kg. Furthermore, since the benzoin condensation, and the subsequent oxidation of the latter to benzil, is regularly used in undergraduate synthetic chemistry courses, it is unlikely that these compounds would have any significant acute or delayed toxicity. This portends that inhibition of CE is unlikely to be deleterious, consistent with the observation that esterase-deficient mice are viable and healthy. While specific molecules that may be developed as clinical candidates will obviously require detailed toxicological evaluation, since the benzils lack overt toxicity, and transient CE inhibition (or loss) does not result in side effects, the development of these molecules for modulating drug hydrolysis is unlikely to be hindered by these parameters.

Potential toxicological concerns that may relate to the benzils include the possibility that these compounds may act as radical initiators in mammalian cells [48]. These reactions have been known to occur in the presence of uv light, yielding radicals such as Ph-CO• or (Ph-CO-CO-Ph)•- [50]. The generation of these in cells would likely be deleterious generating both protein and DNA adducts with unknown consequences. However, in gene expression microarray studies using cultured cells, no significant changes in the profiles of transcribed RNAs were observed following treatment with 10μM benzil for 24 hours (Wierdl and Potter, unpublished data). In addition, no toxicity has been observed with this compound in cell cytotoxicity assays [22]. While these studies are obviously not definitive and conclusive, they suggest that benzil may lack any inherent liabilities that might preclude preclinical development.

2.3.2.2 Isatins: Upon isolation of benzil, and the confirmation that the 1,2-dione group was essential for enzymatic activity, we searched chemical databases for molecules that would contain this chemotype. This identified the isatins (indole-2,3-diones; Figure 2) as potential CE inhibitors [21]. After biochemical assays using a panel of 60 or so compounds with both hiCE and hCE1, it was realized that these analogues could indeed be potent inhibitors of this class of proteins. QSAR analyses indicated the potency of the isatin analogues towards these enzymes was significantly increased if the parent molecule was heavily substituted and more hydrophobic. Indeed, reasonable linear correlation coefficients (0.64 and 0.39 for hiCE and hCE1, respectively) were established for the K_i values of these compounds with their clogP constants [21]. This resulted in cutoff limits where molecules with clogP <1.25 were not inhibitors and those >5 were very potent (in the nM range). As indicated above, the likely reason for this correlation is due to the highly hydrophobic nature of the active sites of these proteins. This would allow localization of more lipophilic compounds within this domain, especially in a aqueous solution. While the activity of this class of compounds has not been examined in cell culture systems or animal models, due to the relative ease of synthesis of the isatins, the availability of numerous sites within the molecule for chemical modification, and the previous use of similar compounds as clinical agents (e.g. indomethacin, indoramin), development of these 1,2-diones as CE inhibitors is highly likely.

Interestingly, a subset of the substituted isatins demonstrated weak activity towards human BChE, but little if any towards AChE [21]. While detailed physical studies to identify the interactions between these compounds and both the CE and cholinesterase active sites have not been undertaken, potentially, selective BChE inhibitors might be obtained using this scaffold. While unlikely to have therapeutic use, such molecules may be informative as

biochemical reagents, and as tools for understanding the biology of this enzyme in animal models.

2.3.2.3 Natural products containing 1,2-diones: Recently, two indole alkaloids have been identified in extracts of marine organisms that contain the ethane-1,2-dione chemotype [51, 52]. These compounds, 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione and hyrtiosin B (1-(5-hydroxy-1H-indol-2-yl)-2-(5-hydroxy-1H-indol-3-yl)ethane-1,2-dione) (Figure 2), were isolated from the *Smenospongia* and *Hyrtios* genus of sponges, respectively. While these compounds have not been assessed for their ability to inhibit CEs, since they have readily accessible 1,2-dione moieties, and clogP values of 3.31 and 2.0, respectively, it is likely that they would have biological activity towards these proteins. The role of these compounds in these organisms therefore raises an interesting issue. Potentially, these molecules may have cytotoxic activity alone, providing defense from other organisms. Alternatively, 1,2-bis(1*H*-indol-3-yl)ethane-1,2-dione or hyrtiosin B might inhibit detoxifying CEs present in the predator, thereby potentiating the toxicity of other endogenous toxins within the sponge. While this is clearly conjecture, the development of mechanisms to evade protection systems might be a highly effective method of increasing the potency of cytotoxic agents.

2.3.2.4 1-Phenyl-2-pyridinylethane-1,2-diones: While the ethane-1,2-diones exemplified by benzil are very potent human CE inhibitors, they are very poorly water soluble, mitigating their development as therapeutic agents. To overcome these problems, a series of 1-phenyl-2-pyridinylethane-1,2-diones have been developed [53]. These compounds demonstrate similar potencies towards the human enzymes, and are predicted to be at least 10-fold more water soluble that the benzil analogues. Furthermore, facile synthetic approaches have been developed to allow for the production of both diverse libraries of compounds, as well as large scale preparations of desired analogues [53]. In addition, the asymmetric nature of these molecules (as compared to the symmetric benzils) allows for maintenance of the inhibitory core (the ethane-1,2-dione), while providing considerable flexibility with regard to analogue design and synthesis. To date, nearly 200 compounds have been generated and these compounds are currently being assessed for inhibition of hiCE and hCE1, as well as human acetyl- and butyrylcholinesterase.

2.3.3 Trifluoroketones—The trifluoroketones (Figure 2) were original identified as inhibitors of juvenile hormone esterases. Indeed a patent (US #4,562,292) has been published that described the potential use of these compounds as modulators of insect development. However, these molecules have subsequently been demonstrated to have activity towards rat, pig and human CEs [54, 55]. While these agents were mostly selective for CEs, weak inhibition of human acetyl- and butyrylcholinesterase has been observed with some of these analogues [55]. These compounds are effective inhibitors because the trifluoro moiety generates a highly polarized carbonyl group that is subject to hydration to yield the *gem*-diol [56]. This change in structure results in the formation of a tetrahedral complex that mimics the transition state present within the esterase active site during catalysis [57, 58]. While the trifluoroketones are very potent, reversible inhibitors of human CEs with K_i values as low as 300pM, this class of compounds has also demonstrated activity towards serine protease inhibitors [59-61]. Hence, it is not entirely clear whether sufficient specificity could be engineered into these molecules to ensure selectivity for CEs. However, it should also be noted that the trifluoromethyl group is frequently used to modulate activity and metabolism of clinically used drugs, (e.g., celecoxib [62], fluoxetine [63], sitagliptin [64], etc) and hence is not a liability in these molecules. Therefore, assuming a panel of esterases can be assessed for inhibition by these trifluoroketones, potentially one or more of these agents may act as lead compounds for subsequent drug development.

2.3.4 Miscellaneous carboxylesterase inhibitors

2.3.4.1 Loperamide: The anti-diarrheal medicine loperamide (Figure 2) has been demonstrated to be a selective inhibitor of hiCE. This compound is moderately potent, exhibiting a K_i value of 1.5μM when using 4-methylumbelliferone as a substrate [14]. The mode of hiCE inhibition was determined to be competitive; however, apart from a N,N′-dimethylamide group present within the structure of loperamide, it is not apparent why this compound would be expected to be an inhibitor of this protein. Since the crystal structure for hiCE has not been determined, and no docking studies using homology models of this enzyme have been undertaken, the configuration loperamide adopts within the active site of this protein is not known.

Interestingly however, loperamide is frequently administered to patients suffering from CPT-11-associated diarrhea [65-67]. Since hiCE, in part, may contribute to activation of the anticancer prodrug in the gut, and hence the toxicity associated with this agent, the amelioration of diarrhea by loperamide may arise from two distinct mechanisms. Firstly, it binds to the μ-opioid receptors in the large intestine and via effects on the smooth muscle in the gut, decreases motility of its contents; and secondly, it may directly inhibit SN-38 formation by inhibition of hiCE. Both processes therefore would limit diarrhea in this patient population. At present, it is unknown if the concentrations of loperamide that are achieved in the gut are sufficient to account for hiCE inhibition in vivo.

2.3.4.2 Cholesterol analogues: Recent reports have indicated that 27-hydroxycholesterol (Figure 2) can act as a potent inhibitor of CE in mammalian cells, demonstrating a K_i of 33nM [68]. However, other cholesterol analogues (e.g., cholesterol, 25-hydroxycholesterol) were essentially inactive in these assays. Biochemical experiments validated results seen in cell extracts and 27-hydroxycholesterol was demonstrated to be a nM inhibitor of recombinant hCE1. Interestingly, this compound lacked activity towards hiCE. The significance of these observations are not fully understood, but since CEs can hydrolyze cholesteryl esters, potentially, 27-hydroxycholesterol may act to regulate enzymatic activity in the cholesteryl biosynthesis/recycling pathway.

2.3.4.3 Bis(4-nitrophenyl)phosphate): As indicated above (**2.3**), bis(4-nitrophenyl)phosphate (BNPP; Figure 4) has been demonstrated to be a CE inhibitor [69]. This organophosphate acts as an irreversible inhibitor of these proteins, resulting in the generation of a stable phosphate ester covalently attached to the catalytic serine reside present within the enzyme active site. BNPP has been used both in vitro and in vivo to modulate enzymatic activity and alter the pharmacokinetic profiles of CE hydrolyzed drugs [7, 70, 71]. These studies underscore the potential that inhibitors of this class of proteins may have, although it is not clear whether irreversible enzyme inhibition, as opposed to a reversible mechanism, would be desirable. The potential issue with the former, is that prolonged irreversible inactivation of CEs, may potentiate individuals to toxicity for other agents (for example, exogenous natural xenobiotics), that would then require enzyme re-synthesis to afford protection. A reversible inhibitor, with promising pharmacokinetic properties, would likely be more desirable.

2.3.4.4 Benzodioxaphosphorines: A series of 2-alkoxy-2-oxo-3H-1,4,2-benzodioxaphosphorines (Bomin-1, -2 and -3; Figure 4; [72, 73]) have been described as selective CE inhibitors. These compounds were initially described in a Russian patent (#1187444; [74]) and the chemical properties of these compounds have been described [72, 73], however there are no literature reports of these compounds being used for CE inhibition. The Potter lab has evaluated these as selective inhibitors of mammalian CEs, and we have observed essentially no inhibition of recombinant purified enzymes (hiCE, hCE1

and a rabbit liver CE, rCE) at concentrations up to 1mM (Hatfield and Potter, unpublished results).

The structurally related analogues, 2-substituted-2-oxo-4H-1,3,2-benzodioxaphosphorines, (exemplified by 2-octyl-2-oxo-4H-1,3,2-benzodioxaphosphorine and 2-(o-cresyl)-2-oxo-4H-1,3,2-benzodioxaphosphorine (CBDP); Figure 4) have also been demonstrated to be CE, and neuropathy target esterase, inhibitors, respectively [75, 76]. However, since the latter is a phospholipase/lysophospholipase, this suggests that these benzodioxaphosphorines are not specific for CEs. While the selectivity of these analogues for the different mammalian CEs has not been tested in a rigorous and systematic fashion, it is unlikely that these molecules could be considered for in vivo use due to their cross reactivity with other classes of esterases.

2.3.4.5 Pesticides: Many pesticides contain organophosphate or carbamate moieties that can inhibit esterases, including chlorpyrifos oxon, paraoxon, or methyl paraoxon (Figure 4), that have all been demonstrated to inhibit human CEs with K_i values in the nM range [11]. In addition, numerous reports exist of the inhibition of esterases from lower organisms by such compounds. For example, carbaryl (Sevin; 1-naphthyl methylcarbamate), parathion and aziphos-methyl (Guthion; *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl]dithiophosphate; Figure 4) have all been demonstrated to be inhibitors of CEs [77-79]. However, these molecules also tend to inhibit acetylcholinesterase and hence their applicability for use in therapeutic applications is minimal. Due to the vast array of literature related to insecticides and pesticides that is too broad to cover here, the reader is referred to the excellent reviews (and references therein) by others more accomplished in this field [80, 81].

2.4 Choice of compounds for preclinical development

As discussed above, there are numerous potential candidates for preclinical development of CE inhibitors. However, based upon the discussion of the different properties of these compounds, it would seem likely that the most reasonable choices would either come from the benzils (or a derivative thereof) or the isatins. This is principally based upon the potency of these molecules toward human CEs, their ability to modulate CE activity intracellularly, their lack of apparent cross reactivity to other esterases, and the absence of toxicity of these agents in biological systems. While of course, it is impossible to predict the issues that may be encountered during both preclinical and clinical development of any agent, since the 1,2-diones do not contain any inherent liabilities that have been identified to date, potentially, the medicinal chemistry approaches to design such molecules should be facile.

3. Expert opinion

While the synthesis of molecules that lack problematic chemotypes (e.g., groups that may be subject to oxidation by cytochrome P450s) is frequently considered in drug design, until recently, the generation of such molecules that do not contain ester moieties has not received such attention. This is probably due to a variety of factors including the lack of expression of CE in human plasma [82], the versatility of these chemical modifications in enhancing bioavailability and water solubility, and the ease with which such compounds can be formulated. However, with the recent identification of compounds that can efficiently inhibit CEs (see above), such molecules may have significant promise in extending the utility of currently available drugs. By way of comparison, such an approach would be similar to the development of Augmentin. The latter is a combination of amoxicillin, an antibiotic hydrolyzed by β-lactamase, and potassium clavulanate, a specific inhibitor of this enzyme [83]. Hence, clinical resistance to amoxicillin that frequently occurs via overexpression of β-lactamase, is mitigated by the clavulanate inhibitor. Applying this methodology to an

esterified drug and a CE inhibitor, should result in an increased half-life of the former molecule. Alternatively for an agent such as CPT-11, whose toxicity (delayed diarrhea) is mediated in part by expression of hiCE within the intestinal epithelia [32], selective application of a CE inhibitor at the appropriate time should moderate these effects.

Similarly, heroin is a prodrug that is hydrolyzed by CEs to yield morphine [15, 18, 84]. For individuals who have overdosed on this agent, it may be possible to administer a CE inhibitor to reduce the circulating levels of morphine, potentially providing additional time for emergency personnel to treat such patients. Hence, CE inhibitors potentially have dual roles in modulating drug action, by both reducing induced toxicity and/or increasing molecule half life.

While this review has naturally concentrated on the therapeutic consequences of CE inhibition, these molecules may also have utility in other biological systems. For example, resistance to pesticides in insects is frequently achieved by overexpression of endogenous CEs that can inactivate these compounds [85-88]. Potentially therefore, combining a selective inhibitor of this enzyme with the pesticide, should result in increased toxicity of the latter to the organism. This situation is analogous to that described above for Augmentin, where resistance is overcome by selective inhibition of the inactivating enzyme. Furthermore, as indicated above, numerous patents detail the potential use of selective CE inhibitors when combined with other molecules. While this review cannot address these in detail, it is clear that the development of such compounds will be highly useful, not only as biochemical reagents to assess enzyme structure and function, but also as tools to evaluate the effects of esterified small molecules in biological systems. It is anticipated that within the next 5 years, a panel of CE inhibitors will be developed, with both selective and general inhibitory properties, that can be used for such purposes.

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Article Highlights

- The application of selective inhibitors of this class of proteins may have utility in modulating the metabolism, distribution and toxicity of agents that are subjected to enzyme hydrolysis.
- When taken in context, it is likely that many more people are affected by the everyday effects of CE-mediated metabolism of drugs, rather than the threat of nerve agent poisoning.
- This portends that inhibition of CE is unlikely to be deleterious, consistent with the observation that esterase-deficient mice are viable and healthy.
- CE inhibitors potentially have dual roles in modulating drug action, by both reducing induced toxicity and/or increasing molecule half life.

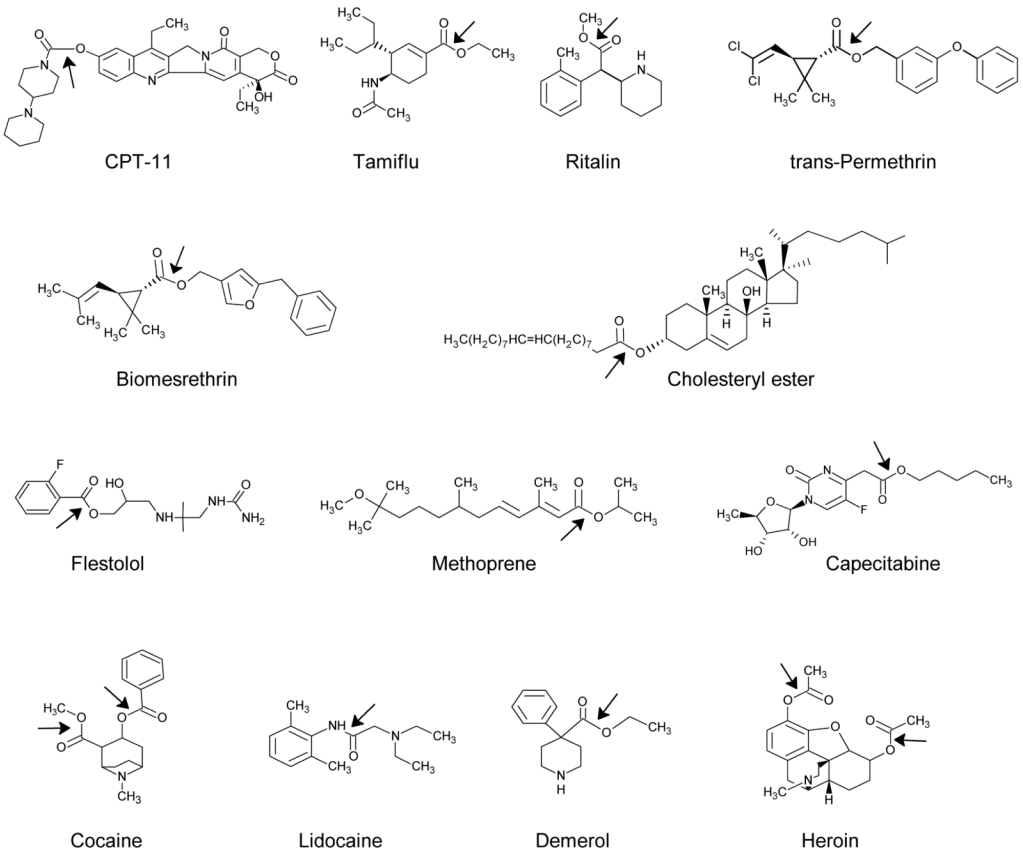

Figure 1.
Carboxylesterase substrates. The site(s) of enzymatic cleavage is(are) indicated by the arrow(s).

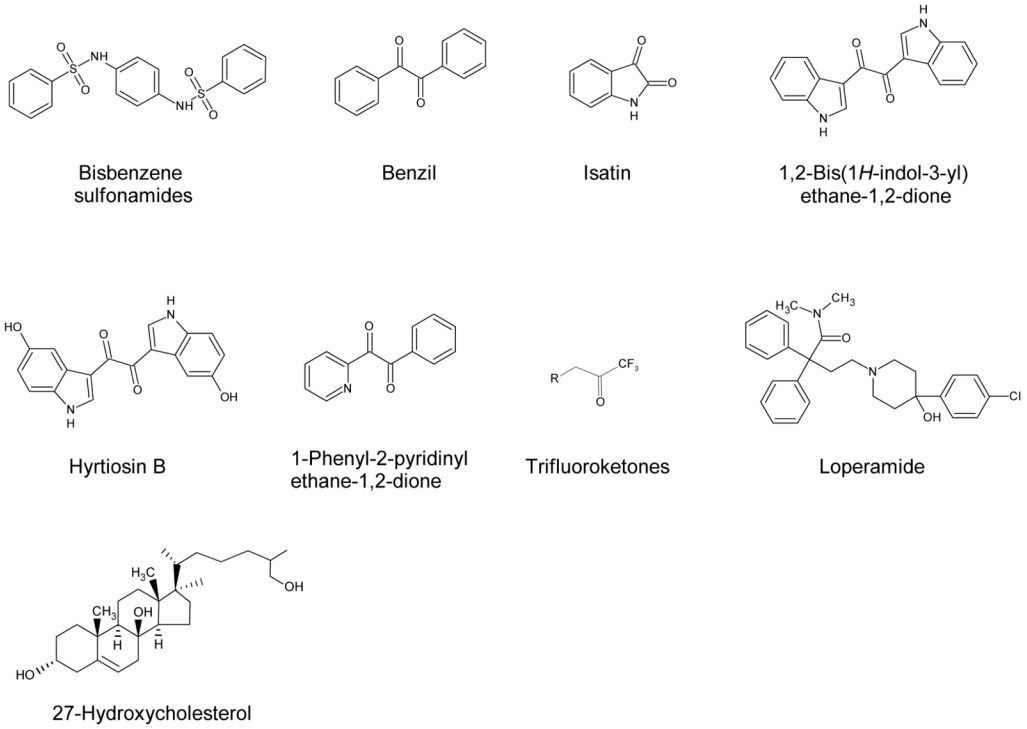

Figure 2.
Chemical structures of carboxylesterase inhibitors.

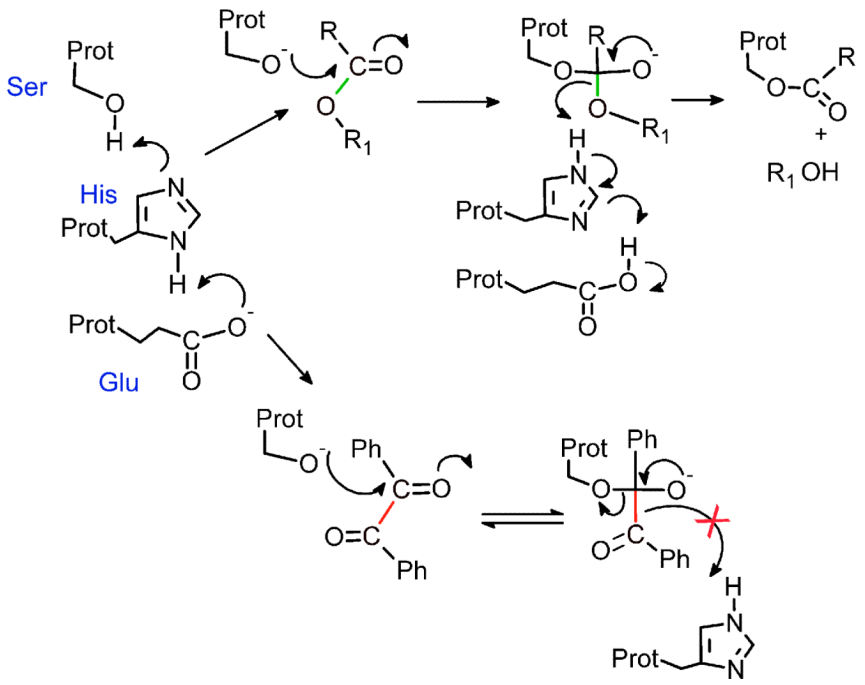

Figure 3.

Proposed mechanism of CE inhibition by benzil. The essential catalytic residues in CEs (Ser, His and Glu) are depicted, and the upper half of the figure demonstrates the initial steps in the hydrolysis of an ester ($RCOOR_1$). The serine nucleophile attacks the carbonyl carbon atom to yield a tetrahedral complex that collapses (cleavage of green C–O bond) to release the alcohol and generate a serine ester. When the enzyme interacts with benzil (lower half of the figure), it is presumed that the same attack occurs, but now the complex cannot degrade since the C–O bond is now replaced by the stronger C–C bond (red). Due to the lack of polarization and increased strength of the latter, cleavage does not occur. Hence, an equilibrium is likely established between the enzyme, and free and complexed inhibitor, that essential results in an inactivation of the protein. Adapted from [23] with permission.

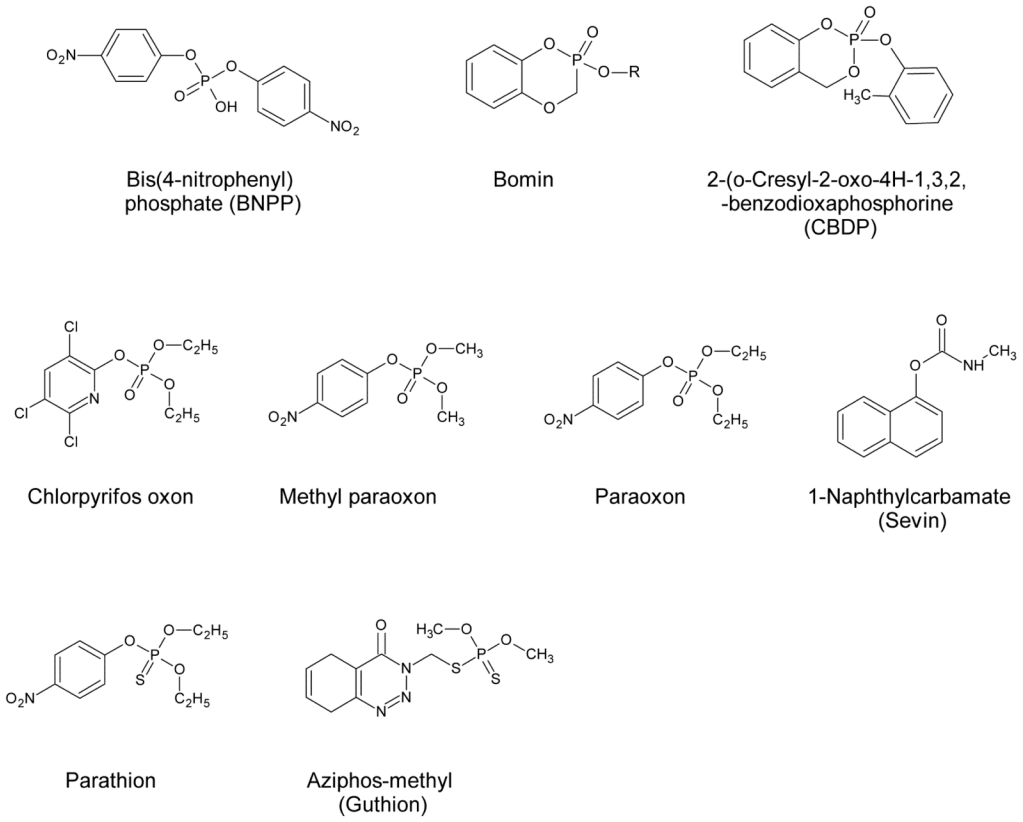

Figure 4.
Organophosphate and carbamate inhibitors of carboxylesterases