FINAL

Send Orders for Reprints to reprints@benthamscience.ae

Current Medicinal Chemistry, **2018**, *25*, **1-24**

REVIEW ARTICLE

Carboxylesterase Inhibitors: An Update

Li-Wei Zou[a,#], Qiang Jin[a,b,#], Dan-Dan Wang[a,b], Qing-Kai Qian[a], Da-Cheng Hao[c], Guang-Bo Ge[a,b,*] and Ling Yang[a]

[a]*Institute of Interdisciplinary Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, China;* [b]*Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, China;* [c]*School of Environment and Chemical Engineering, Dalian Jiaotong University, Dalian, 116028, China*

ARTICLE HISTORY

Received: September 14, 2017
Revised: November 10, 2017
Accepted: November 29, 2017

DOI:
10.2174/0929867325666171204155558

Abstract: Mammalian carboxylesterases are key serine hydrolases that catalyze the hydrolysis of a wide variety of ester compounds in the corresponding carboxylic acids and alcohols. In human, two major carboxylesterases, CES1 and CES2, have been identified and well-studied over the past decade. CES1 inhibitors have potential applications in the treatment of hypertriglyceridaemia, obesity and type 2 diabetes, owing to that this enzyme plays prominent role in the metabolism of cholesteryl esters. CES2 plays crucial roles in the metabolic activation of many prodrugs including anticancer agents capecitabine and CPT-11. Co-administration with CES2 inhibitors may ameliorate CPT-11 associated life-threatening diarrhea or improve the half-lives of CES2-substrate drugs. The important roles of carboxylesterases in both endogenous and xenobiotic metabolism arouse great interest in the discovery and development of potent and selective inhibitors against these enzymes. This review is focused on the application potentials and recent advances in the discovery and development of carboxylesterases inhibitors. The inhibitory capacities and inhibition mechanism of a variety of carboxylesterases inhibitors including synthetic, semi-synthetic and natural compounds are comprehensively summarized. Furthermore, the key structural features and structure-activity relationships (SARs) of different classes of CES1 and CES2 inhibitors are discussed. All information and knowledge summarized in this review will be very helpful for the medicinal chemists to design and develop more potent and highly selective carboxylesterases inhibitors for potential biomedical applications.

Keywords: Carboxylesterases, CES1, CES2, Inhibitors, Structure-activity relationships (SARs).

1. INTRODUCTION

Mammalian carboxylesterases (CES) are important members of the serine hydrolase superfamily (E.C. 3.1.1.1), which can catalyze the ester cleavage of many structurally diverse compounds into the corresponding carboxylic acid and alcohol [1-5]. Actually, carboxylesterases can hydrolyse ester, thioester, amide-ester and carbamate linkages in a broad spectrum of endogenous and xenobiotic substrates, such as cholesteryl esters, therapeutic drugs and pyrethroids [2, 3, 6-11]. At present, five families of mammalian carboxylesterases have been found, but most carboxylesterases are segregated into the CES1 and CES2 families [1]. In human, CES1 and CES2 are two major carboxylesterase isoforms involved in endogenous and xenobiotic metabolism, which have been extensively investigated over the past decade [12, 13].

The crystal structures of mammalian carboxylesterases including human carboxylesterase 1 (CES1) have been reported [14-16]. It is found that an *N*-terminal hydrophobic signal peptide is responsible for the localization of mammalian carboxylesterases to the endoplasmic reticulum (ER), while a C-terminal domain

*Address correspondence to this author at the Institute of Interdisciplinary Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, 201203, China; Tel: +86 021-51323184;
E-mails: geguangbo@dicp.ac.cn, geguangbo@shutcm.edu.cn

[#]These authors contributed equally to this work.

0929-8673/18 $58.00+.00

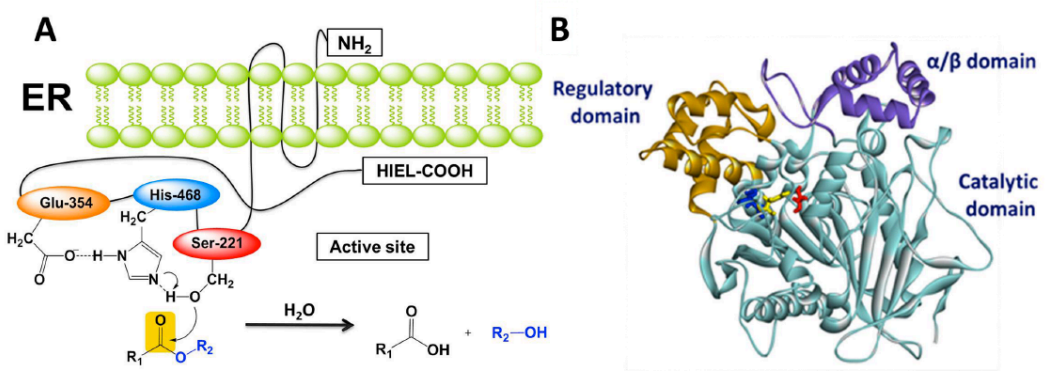


Fig. (1). The structure features of CES1. **A**) CES1 is localized at the ER membrane and the scheme for catalyzing the ester cleavage of various esters; **B**) The 3D structure of CES1. The catalytic triad including Ser-221, His-468 and Glu-354 are colored in red, yellow and blue, respectively.

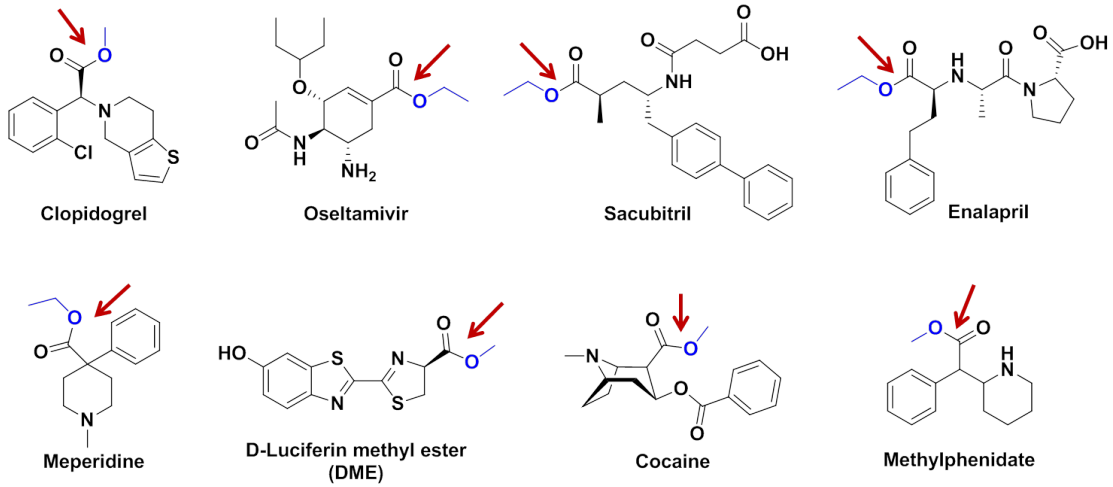


Fig. (2). Substrate specificity of CES1. All representative substrates for CES1 containa small alcoholic group and a bulkyacyl group.

(HIEL and HTEL for CES1 and CES2 respectively) prevents secretion of these proteins from the ER in mammalian cells [17]. All mammalian carboxylesterases contain a catalytic triad (such as Ser221, Glu354 and His468 in CES1) at the interface of the three domains, and an oxyanion hole (such as Gly141-Gly142) which is highly conserved among all mammalian carboxylesterases and is very crucial for carboxylesterases-mediated catalysis (Fig. **1**). As one of the most extensively studied mammalian carboxylesterases, CES1 is composed of a central catalytic domain, a αβ domain and a regulatory domain containing the low-affinity surface ligand-binding Z-site [11, 14, 18]. Notably, the active cavity of CES1 is quite large (~1,300 $Å^3$ in volume) and is lined predominantly by hydrophobic amino acids, with the exception of residues (such as Ser221) of the catalytic triad. The substrate binding gorge of CES1 contains a large, flexible pocket on one side of Ser221 and a small, rigid pocket on the opposite side. These features make CES1 to be promiscuous and capable of interacting with a variety of chemically diverse ligands [15, 16].

The two human carboxylesterase isoforms (CES1 and CES2) share 47% amino acid sequence identity, but these two enzymes exhibit differential tissue distribution, as well as distinct substrate and inhibitor specificities [3, 19]. Generally, CES1 is mainly expressed in the liver, with lesser amounts in the intestine, lung, kidney, heart, testis, adipocyte, monocyte and macrophage [20]. CES1 prefers to hydrolyse the ester substrates containing a bulky acyl group and small alcoholic group (Fig. **2**), such as clopidogrel, cocaine, enalapril, imidapril, oseltamivir and meperidine [8, 11, 21-23]. In contrast, CES2 is expressed at relatively high levels in the small intestine and colon, while this enzyme prefers to hydrolyse the esters with a relatively small acyl group and a large alcohol group (Fig. **3**), such as irinotecan, capecitabine, flutamide and

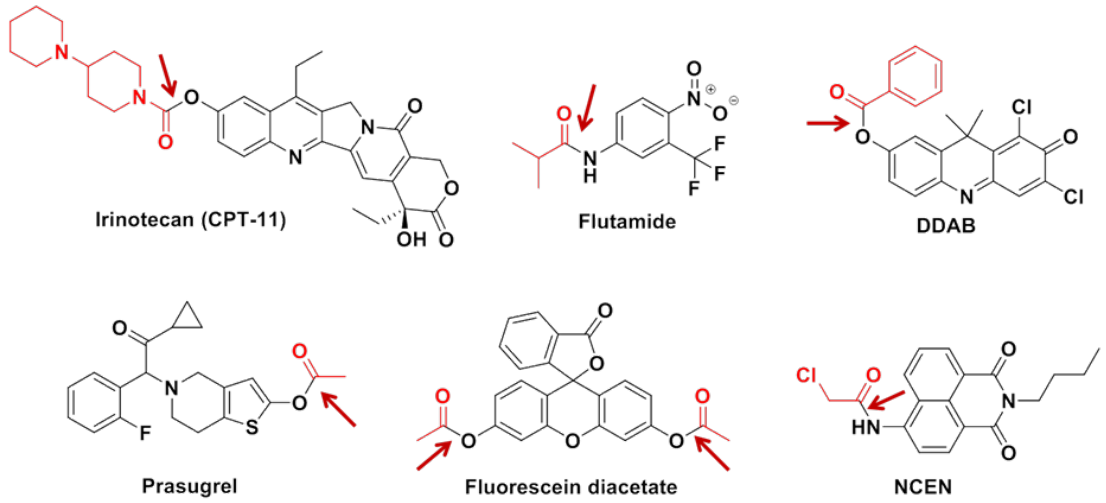


Fig. (3). Substrate specificity of CES2. CES2 prefers to hydrolyze the esters with a relatively large alcohol group and a small acyl group.

procaine [5, 24-30]. Over the past decade, these known CES substrates are always used to screen potential CES inhibitors, but the detection of these substrates and the corresponding hydrolytic metabolites require expensive instruments (such as LC-MS/MS), tedious operation process and cannot be used for high-throughput screening (HTS). In recent years, several optical probe substrates have been designed and developed for highly selective sensing the real activities of CES1 and CES2 in complex biological samples, on the basis of the substrate specificity of carboxylesterases. These optical probes exhibit excellent selectivity and ultra-high sensitivity (Table **S1**), and provide practical and efficient tools for high-throughput screening of carboxylesterases inhibitors using cell or tissue preparations as enzyme sources [31-52].

As one of the most abundant serine hydrolases distributed in human liver and adipocytes, CES1 is responsible for the hydrolysis of a wide variety of endogenous esters (such as cholesteryl esters and triacylglycerols) and thus plays key roles in some physiological or pathological processes, such as cholesterol homeostasis [53-56]. Notably, it has been reported that the enzymatic activities of CES1 are markedly elevated in obese individuals and patients with type 2 diabetes, while treatment with CES1 inhibitors will bring multiple beneficial effects on both lipid and glucose homeostasis *in vivo* [22]. Thus, CES1 has been recognized as a key target to modulate cholesterol and lipid metabolism, and some CES1 inhibitors have been developed for this purpose [57-58]. On other hand, CES1 also participates in the inactivation or metabolic activation of various ester xenobiotics including many prodrugs or ester drugs such as clopidogrel and oseltamivir [23, 59]. Inhibition of CES1 *in vivo* could slow down the metabolic clearance of these ester drugs, and thus improve their bioavailability, half-lives and modulate their treatment outcomes. The key roles of CES1 in both endogenous and xenobiotic metabolism make the discovery of selective inhibitors of CES1 as drug candidates to be of immense significance in basic researches and clinical applications. Unfortunately, the highly selective and potent inhibitors against CES1 are rarely reported.

Although the endogenous substrates of CES2 have not been reported, CES2 plays crucial roles in the metabolic activation of anticancer drugs including irinotecan (CPT-11), capecitabine, flutamide and LY2334737 (the prodrug of gemcitabine) [60]. Notably, CPT-11 could trigger severe delayed diarrhea due to the overproduction of SN-38 (the hydrolytic metabolite of CPT-11) in the small intestine. The hydrolysis of SN-38G (the glucuronide of SN-38) by bacterial β-glucuronidases (GUS) combined with CES2-mediated hydrolysis of in the small intestine is thought to be two major reasons for irinotecan-induced diarrhea [30, 61]. Co-administration with potent GUS and CES2 inhibitors may ameliorate CPT-11 associated life-threatening diarrhea in patients, and thus improve the patient's quality of life [62, 63]. In addition, as the major carboxylesterases distributed in human intestine, CES2 also plays important roles in the first-pass metabolism of some ester-containing drugs. Potent CES2 inhibitors may modulate the oral bioavailability and metabolism of CES2-substrate drugs. Thus, CES2 inhibitors may present an opportunity to slow down the catalytic activity of CES2 *in vivo*, and then to modulate the pharmacokinetic profiles or to alleviate the toxicity of CES2-

substrate drugs [64, 65]. With this goal in mind, small molecule inhibitors of CES2 have been developed with the specific intention of altering irinotecan-induced toxicity or prolonging the half-lives of CES2-substrate drugs.

Taking into account the importance of carboxylesterases in modulating of cholesterol homeostasis, drug metabolism and toxicity, there is a great deal of interest in the discovery and development of potent and selective inhibitors against carboxylesterases for potential use. In the past decade, a variety of compounds with diverse scaffolds have been found with potent inhibitory effects against carboxylesterases, which arouse great interest in medicinal chemists to develop safer and more effective lead compounds or drug candidates. This review has focused on the recent advances in the discovery and development of chemical inhibitors against two human carboxylesterase isoforms, including the reported CES inhibitors and their inhibition potency, as well as the structural features of carboxylesterases inhibitors and the structure-activity relationships (SARs). The knowledge and information presented in this review will be very helpful for the discovery and development of highly selective and potent inhibitors against human carboxylesterases.

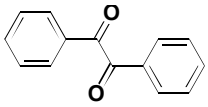
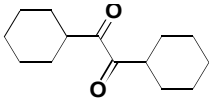
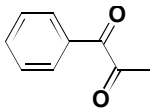
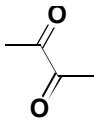
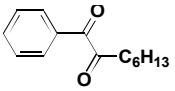
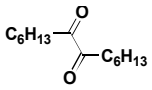
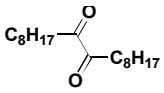
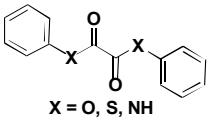
2. REVERSIBLE INHIBITORS OF HUMAN CARBOXYLESTERASES

2.1. 1,2-Diones

1,2-Diones have been identified as the most important chemical compounds for carboxylesterases inhibition [65-67] including benzils [68], alkyl-1,2-diones [69], isatins [70], and 1,2-quinones [71], which demonstrate potent and selective inhibitory effects toward CES1 or CES2, and they do not exhibit inhibitory effects on human acetylcholinesterase (AchE) or butyrylcholinesterase (BchE). SAR studies indicate that the 1,2-dione moiety is very beneficial for carboxylesterases inhibition [66]. When the dione oxygen atoms are *trans*-coplanar or not planar, the compounds are more potent in CES2 inhibition, such as benzils (Table **1**). However, replacement of the benzene rings with alkyl substituents results in an increase of selectivity towards CES1 [67]. Furthermore, the introduction of different atoms (N, O, S) vicinal to the benzene ring and the carbonyl carbon atom results in the loss of inhibitory effects on these two carboxylesterases. Potter *et al.* suggest that the benzils show the excellent inhibitory activity against carboxylesterases, partially because the 1,2-dione moiety mimics the ester chemical character allowing for the initiation of the nucleophilic addition-elimination reactions with serine in the active site to one of the carbonyl groups (Fig. **4**) [68]. However, abortive nucleophilic attack would result in an intermediate that would be unlikely to generate the C-C cleavage, therefore, resulting in enzyme inhibition for the repetitious attack and release by the serine oxygen atom (such as Ser221 of CES1) on the carbonyl groups. This supposition suggests that decreasing the electron density around the carbonyl carbon atom, such as substituted ethane-1,2-diones, would make the carbonyl carbon atom more susceptible to react with the serine oxygen. Taking into account that the catalytic residues of carboxylesterases are buried at the bottom of deep hydrophobic gorges in these enzymes [72-74], it is likely that this is area would favor the localization of more lipophilic compounds. Potter *et al.* assumed that the inhibitory potency of 1,2-diones depends primarily on the electrophilicity of the carbonyl groups, but also on the hydrophobicity of the substituent groups [67, 70].

When the two carbonyl oxygen atoms of 1,2-dione moiety are *cis*-coplanar or fused within a ring structure, such compounds, *e.g.*, 1,2-quinones and isatins, demonstrate more specificity for CES1 as compared to CES2 (Table **2**). Tanshinones, phenanthrene-quinone derivatives isolated from traditional Chinese herb Danshen [75], are found to be potent inhibitors of both CES1 and CES2, but with poor selectivity and specificity toward CES1 [66]. Potter *et al.* point out that the entrance to the active site gorge in CES1 is considerably smaller, and any substitution introduced to the benzene ring of the 1,2-quinone or isatin should be small enough such that it does not hinder access of the inhibitors to the enzyme active cavity [70]. *β*-Lapachone with 1,2-dione moiety in *cis*-coplanar is a potent and reversible carboxylesterases inhibitor, and demonstrates more specificity toward CES2 over CES1 [71]. Its phenoxy analogues (4-phenoxynaphthalene-1,2-diones) demonstrated relatively high selectivity toward CES1 as compared with *β*-lapachone. The amino analogues (4-phenyl- aminonaphthalene-1,2-diones) with the imino forms were inactive toward both human carboxylesterases when compared with phenoxy analogues, suggesting that the imino group at the C-4 site was unbeneficial for carboxylesterases inhibition. The *N*-methylated amino analogues (4-phenyl(methyl) aminonaphthalene-1,2-diones) were equal in potency to the phenoxy analogues and demonstrated selectivity for CES1. Both inhibition analyses and docking simulations demonstrated that the tautomerism of the amino derivatives to the imino forms likely accounts for their loss in biological activity.

Table 1. The inhibition potency of 1,2-diones towards carboxylesterases.

Inhibitors	Structures	K_i (CES1 [a]) μM	K_i (CES2 [a]) μM	References
Benzil (diphenylethane-1,2- dione)		0.0451	0.0147	[68]
1,2-Dicyclohexylethane-1,2-dione		0.072	0.005	[67]
1-Phenylpropane-1,2- dione		1.84	5.27	[68]
Butan-2,3-dione		>100	>100	[68]
Phenyl-1,2-octanedione		0.0022	0.0287	[69]
Tetradecane-7,8-dione		0.0048	0.0764	[69]
Octadecane-9,10-dione		0.00084	0.0057	[69]
Substituted ethane-1,2-diones	 X = O, S, NH	>100	>100	[69]

The type of inhibition: [a] Partially competitive inhibition. O-Nitrophenyl acetate as a substrate.

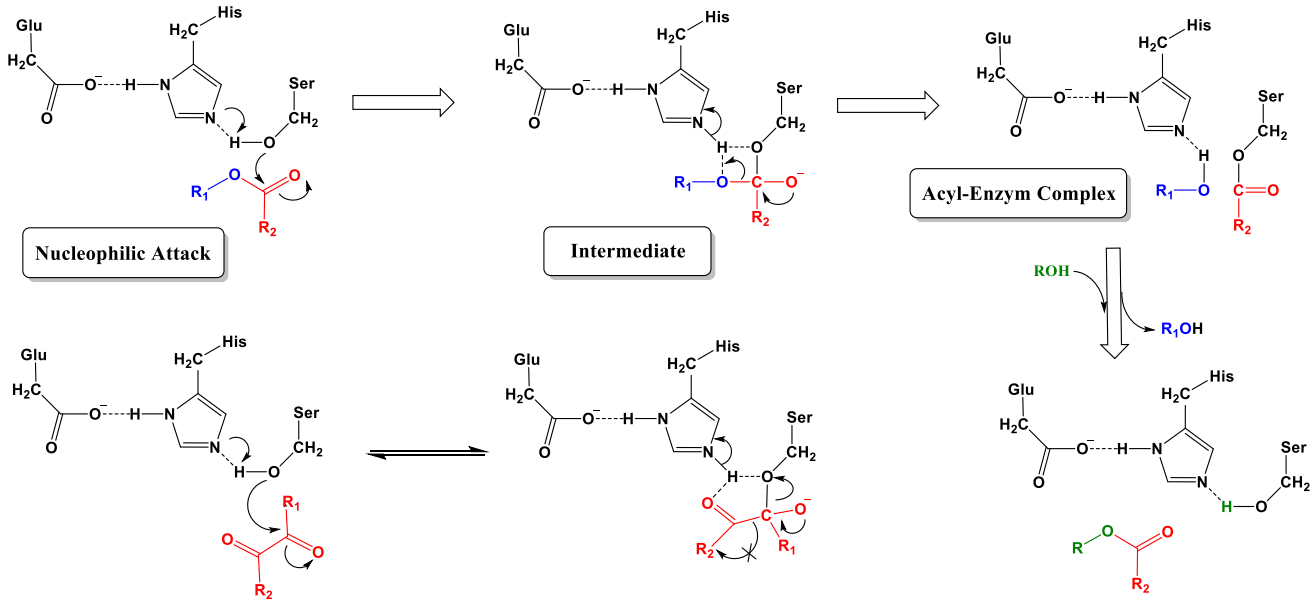
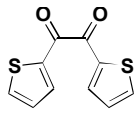
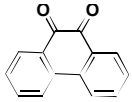
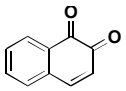
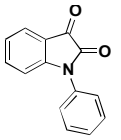
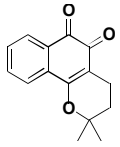
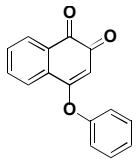
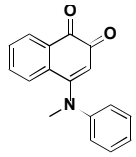
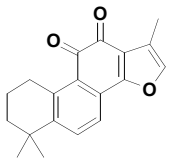
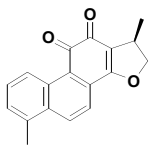


Fig. (4). Proposed mechanism of carboxylesterases inhibition by benzils.

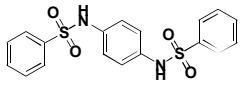
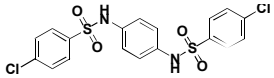
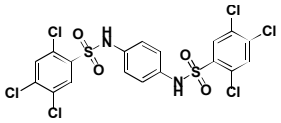
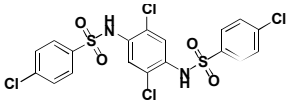
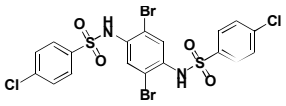
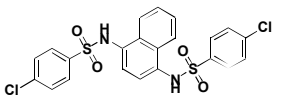
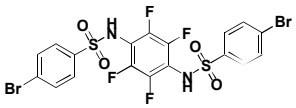
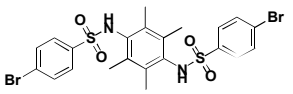
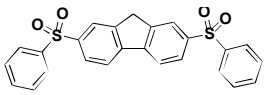
Table 2. The inhibition potency of 1,2-diones towards carboxylesterases: More potent towards CES1.

Inhibitors	Structures	K_i (CES1 [a]) μM	K_i (CES2 [a]) μM	References
2,2'-Thenil (1,2-dithien-2-ylethane-1,2-dione)		0.212	1.04	[76]
Phenanthrene-9,2-dione		0.0025	0.052	[67]
1,2-Naphthoquinone		0.93	2.40	[67]
Cyclohexane-1,2-dione		>100	>100	[68]
1-Phenyl-1*H*-indole-2,3-dione		0.023	0.95	[70]
β-Lapachone		1.220	0.109	[71]
4-Phenoxynaphthalene-1,2-dione		0.0238	0.1998	[71]
4-(Methyl(phenyl)amino)naphthalene-1,2-dione		0.032	>10	[71]
Tanshinone IIA		6.89	2.45	[66]
Dihydrotanshinone		0.40	0.12	[66]

The type of inhibition: [a] Partially competitive inhibition. O-Nitrophenyl acetate as a substrate.

In summary, the SAR analysis of these 1,2-diones revealed that the 1,2-dione moiety, aromatic rings or increasing hydrophobicity, and the substitution which should not hinder access of the inhibitor to the active cavity of the enzyme, were indispensable for carboxylesterases inhibition. Meanwhile, the specificity for CES1 could be achieved by allowing the 1,2-dione in a *cis*-coplanar, the specificity towards CES2 could be accomplished by constraining the 1,2-dione in a *trans*-coplanar or non-planar configuration.

Table 3. The inhibition potency of sulfonamides towards carboxylesterases.

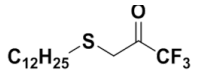
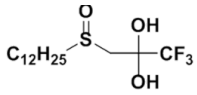
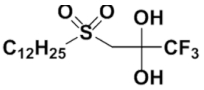
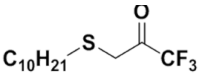
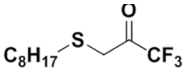
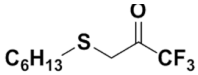
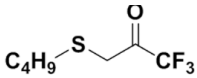
Inhibitors	Structures	K_i (CES1 [a]) μM	K_i (CES2 [a]) μM	References
N,N′-(1,4-phenylene) dibenzene sulfonamide		>100	1.06	[61]
N,N′-(1,4-phenylene)bis(4-chloro-benzene sulfonamide)		13.7	0.053	[61]
N,N′-(1,4-phenylene)bis(2,4,5-trichloro-benzene sulfonamide)		>100	>100	[77]
N,N′-(2,5-dichloro-1,4-phenylene)bis(4-chloro-benzene sulfonamide)		>100	0.45	[77]
N,N′-(2,5-dibromo-1,4-phenylene)bis(4-chloro-benzene sulfonamide)		>100	>100	[77]
N,N′-(naphthalene-1,4-diyl)bis(4-chloro-benzene sulfonamide)		>100	0.20	[61]
N,N'-(perfluoro-1,4-phenylene)bis(4-bromo-benzenesulfonamide)		>100	0.023	[77]
N,N′-(2,3,5,6-tetramethyl-1,4-phenylene)bis(4-bromo-benzenesulfonamide)		>100	3.24	[77]
2,7-Bis(phenyl-sulfonyl)-9*H*-fluorene		>100	0.091	[77]

The type of inhibition: [a] Partially competitive inhibition. O-Nitrophenyl acetate as a substrate.

2.2. Benzene Sulfonamides

Benzene sulfonamides displayed potent inhibitory effects against CES2 with K_i values at micromolar to nanomolar level and relative high selectivity over CES1 (Table **3**), and had no inhibitory effects on either human AchE or BchE [61, 77]. Inhibition kinetic analyses demonstrated that these compounds were partially competitive inhibitors against human carboxylesterases. Quantitative SAR (QSAR) analyses and the clogP correlations indicated that the inclusion of an aromatic, planar, hydrophobic domain within the center of the compound greatly favored the CES2 inhibition [77]. The introduction of halogen in terminal benzene rings was beneficial for the inhibitory property of these inhibitors toward carboxylesterases. However, these compounds containing large bulky substituent within either the central domain or the terminal benzene rings of the benzene sulfonamide analogues demonstrated less potency on CES2. It might be due to the fact that the active site of CES2 is within the hydrophobic environment, and is thought to exist as a long deep gorge in the interior of the enzyme [78, 79]. Because these compounds demonstrate very similar or different clogP values, the change in biological activity may be attributed to the changes in the electron distribution afforded by substitutions within the terminal and central phenyl rings. However, whether the loss of enzyme inhibition is due to electronic effects, or a steric interaction that forces the inhibitor into a conformation such that it could not interact with amino acids of the active site, or a combination of both, is unclear. But these findings suggest that the relative hydrophobicity of these sulfonamides is a key factor for affecting the inhibitory effects on CES2.

Table 4. The inhibition potency of trifluoroketones towards carboxylesterases.

Inhibitors	Structures	K_i (CES1 [a]) μM	K_i (CES2 [a]) μM	References
3-(Dodecylthio)-1,1,1-trifluoropropan-2-one		0.071 [b]	0.015 [a]	[81]
3-(Dodecylsulfinyl)-1,1,1-trifluoropropane-2,2-diol		1.90 [b]	0.054 [a]	[81]
3-(Dodecylsulfonyl)-1,1,1-trifluoropropane-2,2-diol		0.74 [b]	0.032 [c]	[81]
3-(Decylthio)-1,1,1-trifluoropropan-2-one		0.13 [b]	0.15 [c]	[81]
1,1,1-Trifluoro-3-(octylthio)propan-2-one		0.19 [b]	0.22 [b]	[81]
1,1,1-Trifluoro-3-(hexylthio)propan-2-one		0.33 [b]	0.56 [b]	[81]
3-(Butylthio)-1,1,1-trifluoropropan-2-one		0.80 [b]	1.70 [b]	[81]

The type of inhibition: [a] Competitive inhibition, [b] Partially noncompetitive inhibition, [c] Partially competitive inhibition. O-Nitrophenyl acetate as substrate.

2.3. Trifluoroketones

Trifluoroketones are known to be potent inhibitors of a variety of hydrolytic enzymes, such as juvenile hormone esterase [80], acetylcholinesterase [81, 82], chymotrypsin [83], angiotensin converting enzyme [84] and fatty acid amidohydrolase [85]. As potent inhibitors of carboxylesterases, trifluoroketones displayed strong inhibitory effects on carboxylesterases with K_i values in the low nanomolar range, and mostly demonstrated poor specificity toward CES1 or CES2 (Table **4**) [81]. The trifluoro moiety of these compounds could be easily hydrated to give the *gem*-diol (Fig. **5**) [81, 86]. However, the equilibrium between the ketone and *gem*-diol forms must be sufficiently dynamic such that adequate concentrations of the ketone are available for enzyme inhibition [87]. Therefore, the rates of the ketone/*gem*-diol equilibrium could affect the interactions between trifluoroketones and the amino acid residues within the carboxylesterases active site, and thus affect their inhibitor potency. However, this process was poorly understood and would require further investigation to elucidate the mechanism of trifluoroketones mediated carboxylesterases inhibition. In most cases, thioether-containing compounds were more potent inhibitors than sulfinyl or sulfonyl analogs, and these compounds containing longer, more hydrophobic alkyl chains were more potent inhibitors of carboxylesterases (Table **4**).

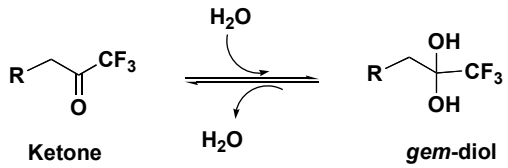
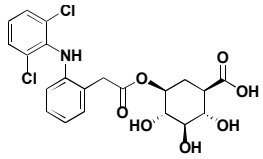
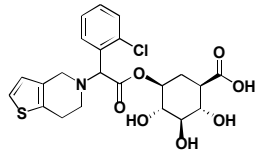
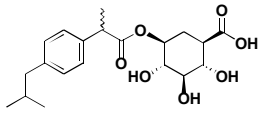


Fig. (5). Interconversion of the ketone and the *gem*-diol forms of the trifluoroketones.

2.4. Acyl Glucuronides

Acyl glucuronides are a class of phase II metabolites where carboxylic acids are bound to a glucuronic acid *via* an ester bond [88, 89]. Commonly, theacyl glucuronides from the phase-II metabolites of drugs were anticipated to be rapidly excreted into urine or bile without exhibiting significant pharmacological activities. However, Williams *et al.* found that some of acyl glucuronides were recognized as the potential carboxylesterases inhibitors including diclofenac-*β*-D-glucuronide, and clopidogrel-*β*-D-glucuronide (Table **5**) [90]. These two compounds could inhibit CES1-mediated 4-nitrophenyl acetate hydrolysis with the

Table 5. The inhibition potency of acyl glucuronides towards carboxylesterases.

Inhibitors	Structures	K_i (CES1) μM	References
diclofenac-*β*-D-glucuronide		4.32	[90]
clopidogrel-*β*-D- glucuronide		24.8	[90]
ibuprofen-*β*-D- glucuronide		355	[90]

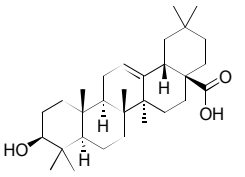
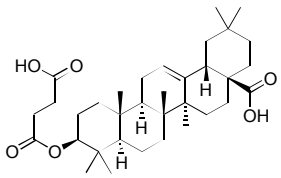
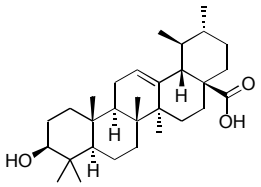
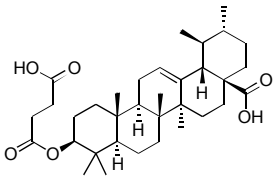
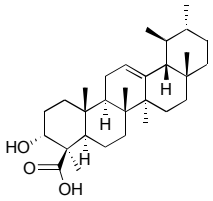
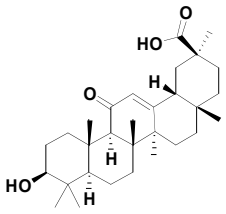
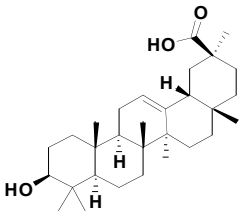
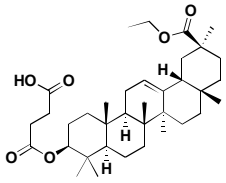
K_i values of 4.32 and 24.8 μM respectively, but did not significantly inhibit CES2, indicating that the glucuronide group introduced in such a position was not beneficial for CES2 inhibition.

2.5. Triterpenoids

Triterpenoids are a diverse group of natural products with wide distribution, high chemical diversity and important pharmacological properties [91, 92]. In the past decades, many researchers in phytochemistry and medicinal chemistry focused on the discovery and development of bioactive compounds from triterpenoids as specific and potent inhibitors to a given enzyme *via* efficient chemical modifications [93, 94]. Ge and Yang *et al.* collected a series of natural triterpenoids and tested their inhibitory effects against carboxylesterases using specific optical substrate for CES1 and CES2, respectively [95]. Two pentacyclic triterpenoids including oleanolic acid (OA) and ursolic acid (UA), displayed strong inhibitory effects on CES1, and thus selected as the lead compounds for the development of potent and selective inhibitors against CES1 (Table **6**). The SAR analysis revealed that the carboxyl group at the C-28 site of OA and UA was essential for CES1 inhibition, and any modifications on this group with amide, ester or alcohol are unbeneficial for CES1 inhibition. In contrast, the replacement of C-3 hydroxyl group with a ketone or ester could improve the inhibitory effects on CES1 and high selectivity over CES2. Guided by these SAR, the C-3 hydroxyl group of OA or UA was modified to 3-O-*β*-carboxypropionyl, the two derivatives including 3*β*-O-(*β*-carboxypropionyl)-olean-12-en-28-oic acid and 3*β*-O-(*β*-carboxy-propionyl)-urs-12-en-28-oic acid displayed very potent inhibitory effects on CES1 and high selectivity over CES2 (3296-fold and 6919-fold over CES2, respectively) [95]. Furthermore, inhibition kinetic analyses and docking simulations demonstrated that 3*β*-O-(*β*-carboxypropionyl)-urs-12-en-28-oic acid functioned as a competitive inhibitor against CES1-mediated DME hydrolysis, with the K_i value of 12.6 nM. To the best of our knowledge, this compound is the most potent and selective inhibitor against CES1, which can serve as a practical tool for exploring the real functions of CES1 in complex biological systems, as well as a promising lead compound for the development of CES1 inhibitor as drug candidate.

Other natural pentacyclic triterpenoids, such as *β*-boswellic acid with carboxyl group at the C-23 site, demonstrated strong inhibitory effects on CES2 and high selectivity over CES1 [95]. Glycyrrhetinic acid (GA), the major bioactive ingredient of the roots and rhizomes of licorice (*Glycyrrhiza* species), which displays satisfying safety during long history of medicinal use, was selected as a reference compound for the development of potent and specific inhibitors against CES2 [96]. Guided by the structure-CES2 inhibition relationships of a series of GA derivatives, Ge and Yang *et al.* designed and developed a more potent compound 3-O-(*β*-carboxypropionyl)- 11-deoxo-glycyrrhetinic acid-30-ethyl ester as a novel and highly selective inhibitor against CES2, with the IC_{50} value of 20 nM and very high selectivity over CES1 (1000-fold), which is 3463-fold more potent than the parent compound GA. The SARs of these pentacyclic triterpenoids as CES1 or CES2 inhibitors are summarized in Fig. (**6**),

Table 6. The inhibition potency of pentacyclic triterpenoids towards carboxylesterases.

Inhibitors	Structures	IC_{50} (CES1) μM	IC_{50} (CES2) μM	References
Oleanolic acid (OA)		0.28	5.49	[95]
3*β*-O-(*β*-Carboxypropionyl)-olean-12-en-28-oic acid		0.017	56.04	[95]
Ursolic acid (UA)		0.24	6.05	[95]
3*β*-O-(*β*-Carboxypropionyl)-urs-12-en-28-oic acid		0.012	83.03	[95]
β-Boswellic acid		>400	2.12	[95]
Glycyrrhetinic acid		20.97	69.26	[96]
11-Deoxo-glycyrrhetinic acid		10.53	6.95	[96]
3-O-(*β*-Carboxypropionyl)-11-deoxo -glycyrrhetinic acid-30-ethyl ester		20.41	0.02	[96]

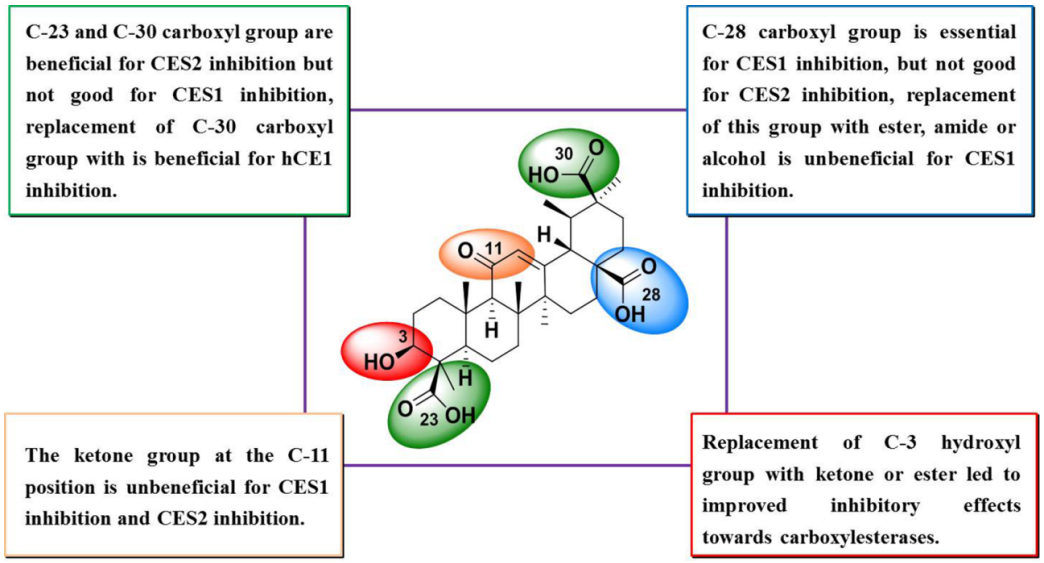


Fig. (6). SAR summaries of pentacyclic triterpenoid derivatives.

which are very helpful for medical chemists to design and develop more potent and highly selective CES1 or CES2 inhibitors for biomedical applications.

Natural protostane triterpenoids, such as protopanaxadiol and protopanaxatriol, exhibit less potency and poor selectivity on CES1, but display strong inhibitory effects on CES2, suggesting that the long alkyl chain at the C-20 site is unbeneficial for CES1 inhibition [95]. Recently, 22 protostane triterpenoids have been isolated from the rhizome of *Alismaorientale* [97], five of which, including alismanol B, 25-O-ethylalisol A, alismanol D, alismanol F, and 11-deoxyalisol A, strongly inhibited CES2 with the IC_{50} values between 2.02 μM and 8.68 μM (Table **7**) [97]. Alisol G (25-anhydroalisol A) is a major protostane triterpene obtained from dried rhizomes of *Alismaorientalis*. Following the biotransformation mediated by *P. janthinellumAS 3.510*, the metabolites of alisol G, including 25*S*-25,26-epoxy-alisol G, alisol G (23,24)-diol acetonide and 25, 26-dihydroxy-alisol G (23,24)-diol acetonide were obtained, which showed significant CES2 inhibitory effects, with the IC_{50} values of 6.81 μM, 3.38 μM, and 6.33 μM, respectively [98].

By the phytochemical investigation of *A. orientalis* and the biotransformation of two major triterpenoids alisol A and alisol B 23-acetate (13) of *A. orientalis*, Liu *et al.* obtained 22 protostane triterpenoids including 10 new protostane-type triterpenoids [99]. Among them, 14 potently inhibited CES2, with the IC_{50} values lower than 10 μM. The inhibition kinetics demonstrated that 25-O-butyl alisol A could inhibit the CES2-catalyzed 4-benzoyl-*N*-butyl-1,8-naphthalimide (MPN) hydrolysis, with the K_i value of 0.57 μM.

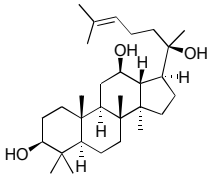
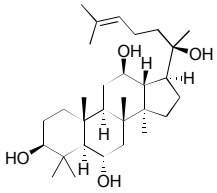
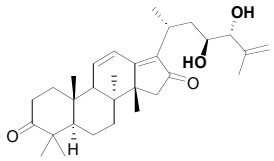
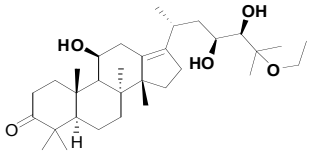
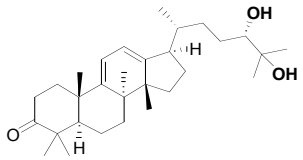
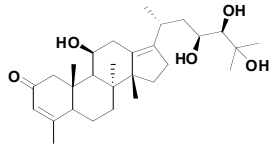
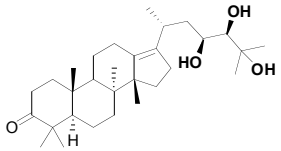
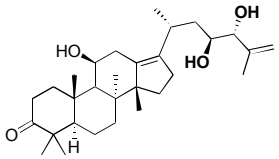
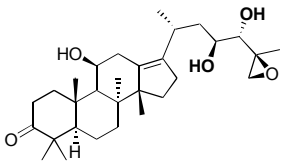
2.6. Flavonoids

Flavonoids are polyphenolic products widely distributed in vegetables, fruits, and beverages such as tea and wine [100, 101]. Recent studies have demonstrated that some natural flavonoids, including bavachinin, coryfolin, corylin, neobavaisoflavone, corylifol A and corylifolinin, potently inhibit both CES1 and CES2 (Table **8**) [102, 103]. Coryfolin, corylin and neobavaisoflavone are noncompetitive inhibitors against CES1-mediated BMBT hydrolysis, while bavachinin is a competitive inhibitor against CES1-mediated BMBT hydrolysis [102]. Neobavaisoflavone, isobavachalcone, bavachinin and corylifol A, also found in *Fructus Psoraleae* (a famous Chinese medicinal herb), are potent inhibitors of CES2 [103], with the K_i values ranging from 0.62 μM to 3.89 μM. The ethanolic extract of the root-bark of white mulberry strongly inhibited CES2, and its three major constituents, *i.e.*, sanggenone D, kuwanon G, andsanggenone C, could non-competitively inhibit CES2-mediated FD hydrolysis in HLM (Table **8**) [104]. These groundbreaking studies suggest that the flavonoids and related phenylpropanoids represent a gold mine, from which carboxylesterase modulators could be developed.

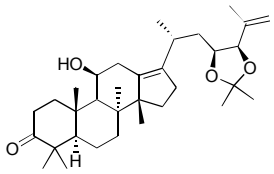
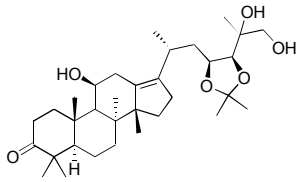
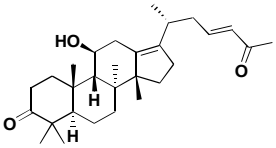
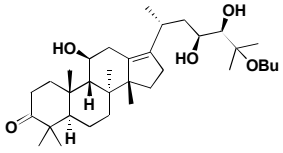
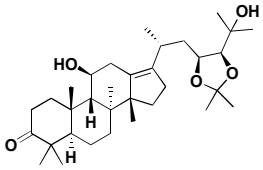
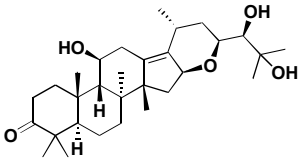
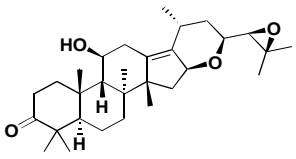
2.7. Other Compounds with Carboxylesterase Inhibition Capacity

Besides the above mentioned compounds, other compounds including fatty acids, sterol, pyrethroids and therapeutic drugs also displayed strong inhibitory effects against carboxylesterase [105-107]. Crow *et al.* found that most naturally occurring fatty acids strongly inhibited the hydrolytic activities of recombinant

Table 7. The inhibition potency of protostane triterpenoids towards carboxylesterases.

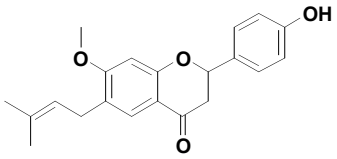
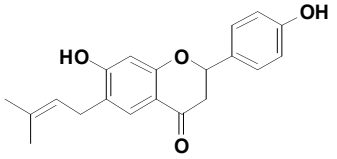
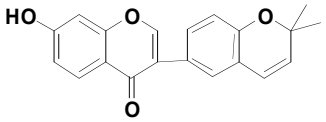
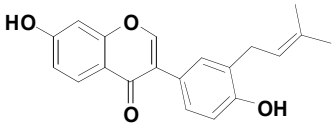
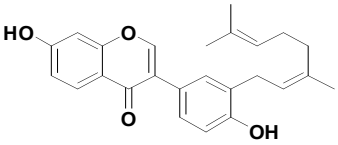
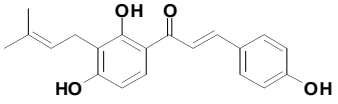
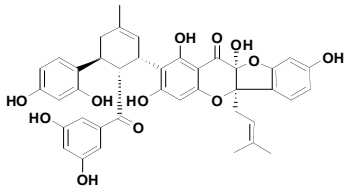
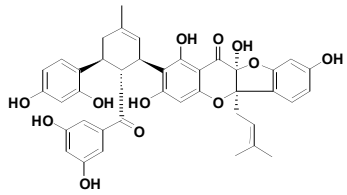
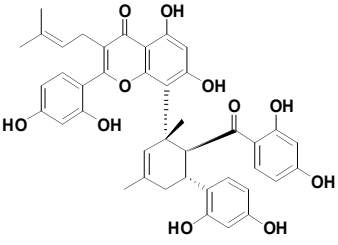
Inhibitors	Structures	IC_{50} (CES1) μM	IC_{50} (CES2) μM	References
Protopanaxadiol		>500	2.658	[95]
Protopanaxatriol		>500	9.800	[95]
Alismanol B		-	8.68	[97]
25-O-Ethylalisol A		-	4.72	[97]
Alismanol D		-	4.58	[97]
Alismanol F		-	2.02	[97]
11-Deoxyalisol A		-	4.63	[97]
25-Anhydroalisol A (alisol G)		-	3.85	[98]
25*S*-25,26-Epoxy-alisol G		-	6.81	[98]

(Table 7) contd….

Inhibitors	Structures	IC_{50} (CES1) μM	IC_{50} (CES2) μM	References
Alisol G (23,24)-diol acetonide		-	3.38	[98]
25,26-Dihydroxy-alisol G (23,24)-diol acetonide		-	6.33	[98]
Alismanol H		-	2.67	[99]
25-O-butyl alisol A		-	0.51	[99]
Alisol A 23,24-acetonide		-	2.99	[99]
24-Deacetyl alisol O		-	2.58	[99]
16,23-Oxido alisol B		-	2.66	[99]

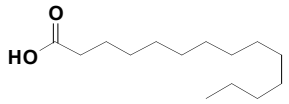
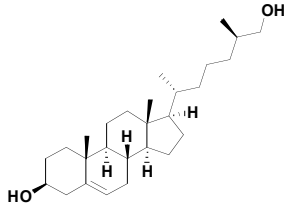
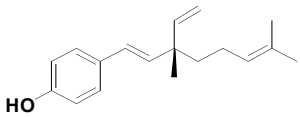
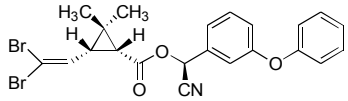
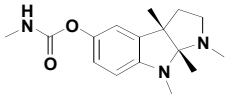
CES1, with the IC_{50} values within the micromolar range [108]. Unsaturated fatty acids displayed better inhibitory effects on CES1 than saturated ones, but they did not display strong inhibition towards CES2. 27-Hydroxycholesterol (27-HC), an oxidized form of cholesterol, also showed promising inhibitory activity against recombinant CES1 (IC_{50} 46 nM) and high selectivity over CES2. 27-HC was a noncompetitive inhibitor against CES1, with the very low K_i value (10 nM) [108]. Bakuchiol, a natural phenolic compound isolated from *Fructus Psoraleae* (Bu-gu-zhi in Chinese), strongly inhibited CES2 [109]. Pyrethroids are a class of organic compounds similar to the natural pyrethrins produced by the flowers of pyrethrums (*Chrysanthemum cinerariaefolium*). Pyrethroids are popular household insecticides for their relatively low toxicity to mammals in contrast to organophosphorus insecticides. Recently, Ge *et al.* found that six commonly used pyrethroids including deltamethrin showed moderate inhibitory effects against both CES1 and CES2 (Table **9**) [107]. Deltamethrin strongly inhibited CES1-mediated DME hydrolysis in HLM, with the IC_{50} value of 2.39 μM. It was a competitive inhibitor against CES1-mediated BMBT hydrolysis, but it acted as a noncompetitive inhibitor against CES1-mediated DME or DMCB hydrolysis in HLM. Both inhibition kinetic

Table 8. The inhibition potency of flavonoids towards carboxylesterases.

Inhibitors	Structures	K_i (CES1) μM	K_i (CES2) μM	References
Bavachinin		0.5 [a]	1.12 [b]	[102, 103]
Coryfolin		9 [a]	-	[102]
Corylin		0.7 [a]	-	[102]
Neobavaisoflavone		5.3 [a]	3.89 [a]	[102, 103]
Corylifol A		-	0.62 [a]	[103]
Corylifolinin		9.4 [a]	1.61 [a]	[102, 103]
Sanggenone C		-	0.76 [a]	[104]
Sanggenone D		-	0.97 [a]	[104]
Kuwanon G		-	1.09 [a]	[104]

The type of inhibition: [a] Noncompetitive inhibition, [b] Competitive inhibition.

Table 9. The inhibition potency of natural compounds towards carboxylesterases.

Inhibitors	Structures	IC_{50} or K_i (CES1) μM	IC_{50} or K_i (CES2) μM	References
Myristic acid		9	>100	[108]
27-Hydroxy cholesterol		0.046	>100	[108]
Bakuchiol		-	2.12	[109]
Deltamethrin (DMT)		2.39	25.16	[107]
Physostigmine		1198	0.358	[110]

analyses and docking simulations suggested that CES1 had at least two ligand-binding sites, and deltamethrin could bind with the same ligand binding site as BMBT. Physostigmine, a natural alkaloid, was a highly specific CES2 inhibitor with the K_i value of 0.358 μM [110]. Physostigmine potently inhibited the hydrolysis of irinotecan but did not affect the hydrolysis of clopidogrel in human liver S9.

Some antihyperlipidemic and antidiabetic drugs, such as simvastatin and troglitazone, significantly inhibited the activity of carboxylesterases (Table **10**) [111]. Simvastatin and troglitazone strongly inhibited CES1, with the K_i values of 0.76 μM and 5.64 μM, respectively. Simvastatin and fenofibrate showed excellent inhibitory effects against CES2 with lower K_i value of 1.85 μM and 0.04 μM, respectively. Antihypertensive drugs nitrendipine and telmisartan inhibited the CES1-mediated imidapril hydrolysis in HLM (Table **10**) [112]. Diltiazem and verapamil showed excellent inhibitory effect against CES2 with lower K_i value as 0.25 and 3.84 μM, respectively [112]. Antiviral drug nelfinavir exhibited strong inhibition on CES1 *via* acompetitive mechanism with the K_i value of 3.7 μM [113]. Other protease inhibitors, *e.g.*, atazanavir, amprenavir, ritonavir and saquinavir, displayed less potent inhibition when compared with nelfinavir. Loperamide is often required to treat chemotherapy-associated diarrhea, and it was a potentand selective CES2 inhibitor with the IC_{50} value of 1.5 μM [114]. Among 25 pharmaceutical excipients, sodium lauryl sulfate (SLS) and polyoxyl 40 hydrogenated castor oil (RH40) could inhibit CES1-mediated imidapril hydrolysis in recombinant CES1A1 and HLM, and Tween 20 and Polyoxyl 35 castor oil (EL35) could inhibit CES2-mediated CPT-11 hydrolysis in recombinant CES1 and HLM (Table **11**) [115]. These results demonstrate that surfactants such as SLS, RH40, Tween 20 and EL35 may attenuate the carboxylesterases activity, therefore such inhibitions should be taken into consideration during drug administration.

3. IRREVERSIBLE INHIBITORS OF HUMAN CARBOXYLESTERASES

3.1. Carbamates

Carbamate compounds were developed to inhibit almost all members of the serine hydrolase family *via* covalent modification of serine at the active site [116-118]. Carbamates have been identified as potent inhibitors of AchE and these compounds have been widely used for the pest control in several crops, as well as in poultry and domestic animals [119, 120]. Recently, several cholinesterase inhibitors containing the

Table 10. The inhibition potency of therapeutic drugs towards carboxylesterases.

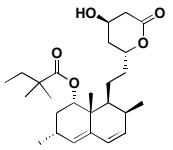
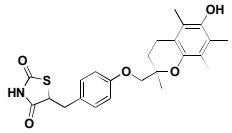
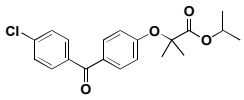
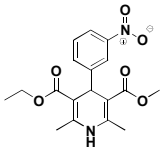
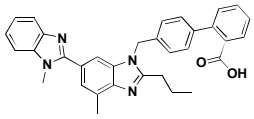
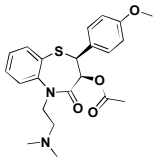
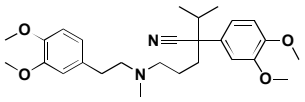
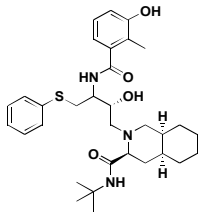
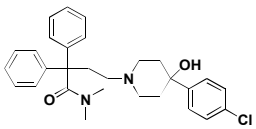
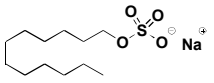
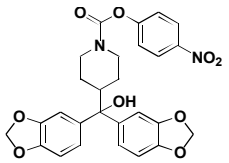
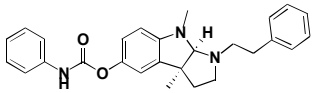
Inhibitors	Structures	K_i (CES1) μM	K_i (CES2) μM	References
Simvastatin		0.76	1.85	[111]
Troglitazone		5.64	-	[111]
Fenofibrate		-	0.04	[111]
Nitrendipine		1.24	-	[112]
Telmisartan		1.69	-	[112]
Diltiazem		-	0.25	[112]
Verapamil		-	3.84	[112]
Nelfinavir		3.7	-	[113]
Loperamide		-	1.5 (IC_{50})	[114]
Sodium lauryl sulfate		0.12 (μg/mL)	-	[115]
Polyoxyl 40 hydrogenated castor oil	-	0.76 (μg/mL)	-	[115]
Tween 20	-	-	0.93 (μg/mL)	[115]
Polyoxyl 35 castor oil	-	-	4.40 (μg/mL)	[115]

Table 11. The inhibition potency of carbamates towards carboxylesterases.

Inhibitors	Structures	IC_{50} or K_i (CES1) μM	IC_{50}or K_i (CES2) μM	References
JZL184		0.48	0.63	[121]
Phenethylcymserine		20.5	5.6	[122]

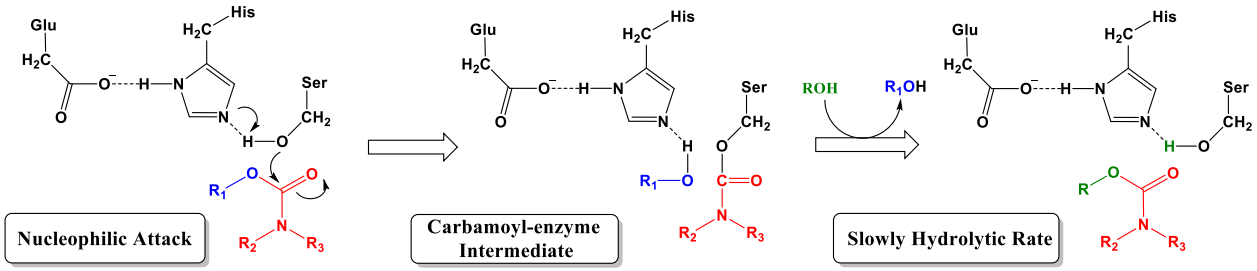
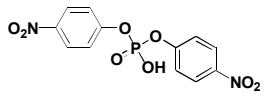
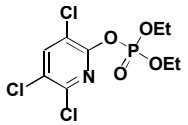
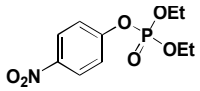


Fig. (7). Proposed mechanism of carboxylesterases inhibition by carbamates.

Table 12. The inhibition potency of organophosphates towards carboxylesterases.

Inhibitors	Structures	IC_{50} (CES1) nM	IC_{50} (CES2) nM	References
Bis(4-nitrophenyl) phosphate (BNPP)		140	6110	[96]
Chlorpyrifosoxon		0.15	0.33	[136]
Paraoxon		0.38	6.2	[136]

carbamate moiety, such as JZL184 and phenethylcymserine, were found to be carboxylesterases inhibitors (Table **11**) [121, 122]. But all these compounds displayed poor isoform selectivity towards carboxylesterases. The strong inhibition of carbamates against carboxylesterases could be attributed to the very slow hydrolysis rates of the carbamoyl-enzyme intermediate, which was much slower than that of the acetyl-enzyme intermediate (Fig. **7**) [123].

3.2. Organophosphates

Organophosphates (OPs) exert their toxicity through the inhibition of synaptic AchE [124-127]. OPs have been utilized extensively as insecticides since 1940s and also been developed as nerve gas agents [128, 129]. A number of serine hydrolases including cholinesterases and carboxylesterases including CES1 and CES2 have been reported to react with OPs and their catalytic activities could be significantly inhibited following exposure to OPs (Table **12**) [130, 131]. OPs could react with carboxylesterases and generate a stable phosphate ester that was covalently linked to the catalytic residue (such as Ser221 of CES1) of carboxylesterases. Several Ops, including bis(4-nitrophenyl)- phosphate (BNPP) , chlorpyrifos oxon, and paraoxon, are potent irreversible inhibitors of human

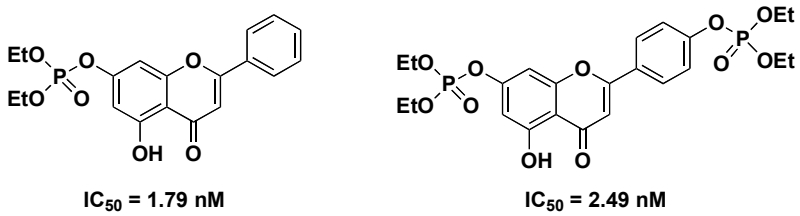


Fig. (8). The inhibition potency of phosphorylated flavonoids towards porcine liver carboxylesterases.

carboxylesterases with the IC_{50} values within the nM range [96, 132, 133]. The SAR analysis revealed that the phosphate group of these compounds was essential for carboxylesterases inhibition, while the introduction of phosphate group to target compounds were likely beneficial for carboxylesterases inhibition. A series of synthesized phosphorylated flavonoids exhibited significantly improved inhibition potency towards carboxylesterases when compared with their parent compounds [134, 135], and their IC_{50} values were as low as 1.79 nM (Fig. **8**). These phosphorylated flavonoids were irreversible competitive inhibitors against mammalian carboxylesterases.

CONCLUSION

Over the past decade, the medicinal chemists have made significant breakthrough on the discovery and development of highly selective and potent inhibitors against carboxylesterases. Thanks to the newly developed optical substrates for CES1 and CES2, the present screening of carboxylesterases inhibitors is more convenient and efficient. So far, many potent carboxylesterases inhibitors with distinct scaffolds (such as 1,2-diones and triterpenoid) have been discovered, some of which displayed very high efficacy with the IC_{50} values at ten nanomolar level. However, the potent and highly selective carboxylesterases inhibitors are rarely reported, while the key structural features and the SAR of CES1 or CES2 inhibitors have not been well-characterized. Thus, it is necessary to explore the key structural determinant and SAR for CES1 or CES2 inhibition, by using a series of compounds with good structural connectivity. Apart from the optimization of potency and selectivity, the ADME/Tox properties of carboxylesterases inhibitors should also be evaluated and optimized, which will be very helpful for the development of carboxylesterases inhibitors as drug candidates. Currently, most of the reported carboxylesterases inhibitors were assayed *in vitro*, and their ability of targeting intracellular carboxylesterases and their potency against carboxylesterases in living systems have not been well investigated. Therefore, it is highly desirable to find more efficacious carboxylesterases inhibitors and to test their safety and efficacy *in vivo*. Prior to the whole body test in animals, it is necessary to characterize the species differences in the function and the responses towards inhibitor(s) of mammalian carboxylesterases among different species [137]. Additionally, given that carboxylesterases have multiple ligand-binding sites, it is necessary to characterize the binding site(s) and the inhibition types for the carboxylesterases inhibitors of interest, which will be very helpful for the deeper understanding of the interactions between ligands and carboxylesterases. Taken together, current data call for more in-depth studies including the biological functions of carboxylesterases, the relationships between carboxylesterases and human diseases, the species differences of mammalian carboxylesterases, as well as the interactions between carboxylesterases and ligands, which will be obliging for the discovery and development of carboxylesterases inhibitors for potential biomedical applications.

LIST OF ABBREVIATIONS

27-HC	=	27-Hydroxycholesterol
AchE	=	Acetylcholinesterase
ADME/Tox	=	Absorption, distribution, metabolism and excretion/toxicity
BchE	=	Butyrylcholinesterase
BMBT	=	2-(2-Hydroxy-3-methoxyphenyl) benzothiazole
BNPP	=	Bis(4-nitrophenyl)phosphate
CES1	=	Human carboxylesterase 1
CES2	=	Human carboxylesterase 2
CPT-11	=	Irinotecan
DDAB	=	6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl benzoate
DME	=	D-luciferin methyl ester
EBHF	=	4-oxo-2-phenyl-4*H*-chromen-3-yl 4-ethylbenzoate
EL35	=	Polyoxyl 35 castor oil

ER = Endoplasmic reticulum

FD = Fluorescein diacetate

GA = Glycyrrhetinic acid

HLM = Human liver microsomes

NCEN = *N*-(2- butyl- 1,3-dioxo- 2,3- dihydro- 1H-benzo[de]isoquinolin- 6-yl)- 2- chloroacetamide

OA = Oleanolic acid

OPs = Organophosphates

RH40 = Polyoxyl 40 hydrogenated castor oil

SAR = Structure-activity relationships

SLS = Sodium lauryl sulfate

SN38 = 7-Ethyl-10-hydroxy-camptothecin

TCFB = (*E*)-4-(2- (4-cyano-5- (dicyanomethylene)- 2,2-dimethyl- 2,5-dihydro- furan-3-yl)vinyl) phenyl benzoate

UA = Ursolic acid

UGTs = Uridine diphosphateglucuronosyltransferases

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work was supported by the NSF of China (21602219, 81703604, 81773687, 81672961, 81573501 and 81473181), the First Affiliated Hospital of Zhengzhou University (201613), innovative entrepreneurship program of high-level talents in Dalian (2016RQ025), and the National S&T Major Projects of China (2017ZX09101004).

REFERENCES

[1] Sanghani, S.P.; Sanghani, P.C.; Schiel, M.A.; Bosron, W.F., Human Carboxylesterases: An Update on CES1, CES2 and CES3. *Protein and Peptide Letters,* **2009**, *16*, (10), 1207-1214.

[2] Satoh, T.; Hosokawa, M., Structure, function and regulation of carboxylesterases. *Chemico-Biological Interactions,* **2006**, *162*, (3), 195-211.

[3] Satoh, T.; Taylor, P.; Bosron, W.F.; Sanghani, S.P.; Hosokawa, M.; La Du, B.N., Current progress on esterases: From molecular structure to function. *Drug Metabolism and Disposition,* **2002**, *30*, (5), 488-493.

[4] Satoh, T.; Hosokawa, M., The mammalian carboxylesterases: From molecules to functions. *Annual Review of Pharmacology and Toxicology,* **1998**, *38*, 257-288.

[5] Sanghani, S.P.; Quinney, S.K.; Fredenburg, T.B.; Sun, Z.J.; Davis, W.I.; Murry, D.J.; Cummings, O.W.; Seitz, D.E.; Bosron, W.F., Carboxylesterases expressed in human colon tumor tissue and their role in CPT-11 hydrolysis. *Clinical Cancer Research,* **2003**, *9*, (13), 4983-4991.

[6] Merali, Z.; Ross, S.; Pare, G., The pharmacogenetics of carboxylesterases: CES1 and CES2 genetic variants and their clinical effect. *Drug metabolism and drug interactions,* **2014**, *29*, (3), 143-151.

[7] Holmes, R.S.; Wright, M.W.; Laulederkind, S.J.F.; Cox, L.A.; Hosokawa, M.; Imai, T.; Ishibashi, S.; Lehner, R.; Miyazaki, M.; Perkins, E.J.; Potter, P.M.; Redinbo, M.R.; Robert, J.; Satoh, T.; Yamashita, T.; Yan, B.; Yokoi, T.; Zechner, R.; Maltais, L.J., Recommended nomenclature for five mammalian carboxylesterase gene families: human, mouse, and rat genes and proteins. *Mammalian Genome,* **2010**, *21*, (9-10), 427-441.

[8] Hosokawa, M., Structure and catalytic properties of carboxylesterase isozymes involved in metabolic activation of prodrugs. *Molecules,* **2008**, *13*, (2), 412-431.

[9] Laizure, S.C.; Herring, V.; Hu, Z.; Witbrodt, K.; Parker, R.B., The Role of Human Carboxylesterases in Drug Metabolism: Have We Overlooked Their Importance? *Pharmacotherapy,* **2013**, *33*, (2), 210-222.

[10] Ross, M.K.; Crow, J.A., Human Carboxylesterases and their role in xenobiotic and endobiotic metabolism. *Journal of Biochemical and Molecular Toxicology,* **2007**, *21*, (4), 187-196.

[11] Bencharit, S.; Edwards, C.C.; Morton, C.L.; Howard-Williams, E.L.; Kuhn, P.; Potter, P.M.; Redinbo, M.R., Multisite promiscuity in the processing of endogenous substrates by human carboxylesterase 1. *Journal of Molecular Biology,* **2006**, *363*, (1), 201-214.

[12] Yang, D.; Pearce, R.E.; Wang, X.; Gaedigk, R.; Wan, Y.-J.Y.; Yan, B., Human carboxylesterases HCE1 and HCE2: Ontogenic expression, inter-individual variability and differential hydrolysis of oseltamivir, aspirin, deltamethrin and permethrin. *Biochemical Pharmacology,* **2009**, *77*, (2), 238-247.

[13] Wong, C.C.; Cheng, K.-W.; Xie, G.; Zhou, D.; Zhu, C.-H.; Constantinides, P.P.; Rigas, B., Carboxylesterases 1 and 2 Hydrolyze Phospho-Nonsteroidal Anti-Inflammatory Drugs: Relevance to Their Pharmacological Activity. *Journal of Pharmacology and Experimental Therapeutics,* **2012**, *340*, (2), 422-432.

[14] Fleming, C.D.; Bencharit, S.; Edwards, C.C.; Hyatt, J.L.; Tsurkan, L.; Bai, F.; Fraga, C.; Morton, C.L.; Howard-Williams, E.L.; Potter, P.M.; Redinbo, M.R., Structural insights into drug processing by human carboxylesterase 1: Tamoxifen, mevastatin, and inhibition by benzil. *Journal of Molecular Biology,* **2005**, *352*, (1), 165-177.

[15] Bencharit, S.; Morton, C.L.; Hyatt, J.L.; Kuhn, P.; Danks, M.K.; Potter, P.M.; Redinbo, M.R., Crystal structure of human carboxylesterase 1 complexed with the Alzheimer's drug tacrine: From binding promiscuity to selective inhibition. *Chemistry & Biology,* **2003**, *10*, (4), 341-349.

[16] Fleming, C.D.; Edwards, C.C.; Kirby, S.D.; Maxwell, D.M.; Potter, P.M.; Cerasoli, D.M.; Redinbo, M.R., Crystal structures of human carboxylesterase 1 in covalent complexes with the chemical warfare agents soman and tabun. *Biochemistry,* **2007**, *46*, (17), 5063-5071.

[17] Potter, P.M.; Wolverton, J.S.; Morton, C.L.; Wierdl, M.; Danks, M.K., Cellular localization domains of a rabbit and a human carboxylesterase: Influence on irinotecan (CPT-

11) metabolism by the rabbit enzyme. *Cancer Research,* **1998**, *58*, (16), 3627-3632.

[18] Vistoli, G.; Pedretti, A.; Mazzolari, A.; Testa, B., In silico prediction of human carboxylesterase-1 (hCES1) metabolism combining docking analyses and MD simulations. *Bioorganic & Medicinal Chemistry,* **2010**, *18*, (1), 320-329.

[19] Imai, T., Human carboxylesterase isozymes: Catalytic properties and rational drug design. *Drug Metabolism and Pharmacokinetics,* **2006**, *21*, (3), 173-185.

[20] Crow, J.A.; Middleton, B.L.; Borazjani, A.; Hatfield, M.J.; Potter, P.M.; Ross, M.K., Inhibition of carboxylesterase 1 is associated with cholesteryl ester retention in human THP-1 monocyte/macrophages. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids,* **2008**, *1781*, (10), 643-654.

[21] Bencharit, S.; Morton, C.L.; Xue, Y.; Potter, P.M.; Redinbo, M.R., Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme (vol 10, pg 349, 2003). *Nature Structural Biology,* **2003**, *10*, (7), 577-577.

[22] Dominguez, E.; Galmozzi, A.; Chang, J.W.; Hsu, K.-L.; Pawlak, J.; Li, W.; Godio, C.; Thomas, J.; Partida, D.; Niessen, S.; O'Brien, P.E.; Russell, A.P.; Watt, M.J.; Nomura, D.K.; Cravatt, B.F.; Saez, E., integrated phenotypic and activity-based profiling links Ces3 to obesity and diabetes. *Nature Chemical Biology,* **2014**, *10*, (2), 113-121.

[23] Zhu, H.-J.; Markowitz, J.S., Activation of the Antiviral Prodrug Oseltamivir Is Impaired by Two Newly Identified Carboxylesterase 1 Variants. *Drug Metabolism and Disposition,* **2009**, *37*, (2), 264-267.

[24] Xu, G.; Zhang, W.; Ma, M.K.; McLeod, H., Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. *Clinical Cancer Research,* **2001**, *7*, (11), 3736S-3736S.

[25] Kobayashi, Y.; Fukami, T.; Shimizu, M.; Nakajima, M.; Yokoi, T., Contributions of Arylacetamide Deacetylase and Carboxylesterase 2 to Flutamide Hydrolysis in Human Liver. *Drug Metabolism and Disposition,* **2012**, *40*, (6), 1080-1084.

[26] Williams, E.T.; Jones, K.O.; Ponsler, G.D.; Lowery, S.M.; Perkins, E.J.; Wrighton, S.A.; Ruterbories, K.J.; Kazui, M.; Farid, N.A., The biotransformation of prasugrel, a new thienopyridine prodrug, by the human carboxylesterases 1 and 2. *Drug Metabolism and Disposition,* **2008**, *36*, (7), 1227-1232.

[27] Bencharit, S.; Morton, C.L.; Howard-Williams, E.L.; Danks, M.K.; Potter, P.M.; Redinbo, M.R., Structural insights into CPT-11 activation by mammalian carboxylesterases. *Nature Structural Biology,* **2002**, *9*, (5), 337-342.

[28] Ribelles, N.; Lopez-Siles, J.; Sanchez, A.; Gonzalez, E.; Sanchez, M.J.; Carabantes, F.; Sanchez-Rovira, P.; Marquez, A.; Duenas, R.; Sevilla, I.; Alba, E., A carboxylesterase 2 gene polymorphism as predictor of capecitabine on response and time to progression. *Current Drug Metabolism,* **2008**, *9*, (4), 336-343.

[29] Takasuna, K.; Hagiwara, T.; Hirohashi, M.; Kato, M.; Nomura, M.; Nagai, E.; Yokoi, T.; Kamataki, T., Involvement of beta-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Research,* **1996**, *56*, (16), 3752-3757.

[30] Ma, M.K.; McLeod, H.L., Lessons learned from the irinotecan metabolic pathway. *Current Medicinal Chemistry,* **2003**, *10*, (1), 41-49.

[31] Bencharit, S.; Morton, C.L.; Xue, Y.; Potter, P.M.; Redinbo, M.R., Structural basis of heroin and cocaine metabolism by a promiscuous human drug-processing enzyme. *Nature Structural Biology*, **2003**, 10, (5), 349-356.

[32] Dai, Z.-R.; Ge, G.-B.; Feng, L.; Ning, J.; Hu, L.-H.; Jin, Q.; Wang, D.-D.; Lv, X.; Dou, T.-Y.; Cui, J.-N.; Yang, L., A Highly Selective Ratiometric Two-Photon Fluorescent Probe for Human Cytochrome P450 1A. *Journal of the American Chemical Society*, **2015**, 137, (45), 14488-14495.

[33] Liu, Z.-M.; Feng, L.; Ge, G.-B.; Lv, X.; Hou, J.; Cao, Y.-F.; Cui, J.-N.; Yang, L., A highly selective ratiometric fluorescent probe for *in vitro* monitoring and cellular imaging of human carboxylesterase 1. *Biosensors & Bioelectronics*, **2014**, 57, 30-35.

[34] Lv, X.; Wang, D.-D.; Feng, L.; Wang, P.; Zou, L.-W.; Hao, D.-C.; Hou, J.; Cui, J.-N.; Ge, G.-B.; Yang, L., A highly selective marker reaction for measuring the activity of human carboxylesterase 1 in complex biological samples. *Rsc Advances*, **2016**, 6, (6), 4302-4309.

[35] Wang, D.-D.; Jin, Q.; Zou, L.-W.; Hou, J.; Lv, X.; Lei, W.; Cheng, H.-L.; Ge, G.-B.; Yang, L., A bioluminescent sensor for highly selective and sensitive detection of human carboxylesterase 1 in complex biological samples. *Chemical Communications*, **2016**, 52, (15), 3183-3186.

[36] Wang, J.; Williams, E.T.; Bourgea, J.; Wong, Y.N.; Patten, C.J., Characterization of Recombinant Human Carboxylesterases: Fluorescein Diacetate as a Probe Substrate for Human Carboxylesterase 2. *Drug Metabolism and Disposition*, **2011**, 39, (8), 1329-1333.

[37] Feng, L.; Liu, Z.-M.; Hou, J.; Lv, X.; Ning, J.; Ge, G.-B.; Cui, J.-N.; Yang, L., A highly selective fluorescent ESIPT probe for the detection of Human carboxylesterase 2 and its biological applications. *Biosensors & Bioelectronics*, **2015**, 65, 9-15.

[38] Liu, Z.-M.; Feng, L.; Hou, J.; Lv, X.; Ning, J.; Ge, G.-B.; Wang, K.-W.; Cui, J.-N.; Yang, L., A ratiometric fluorescent sensor for highly selective detection of human carboxylesterase 2 and its application in living cells. *Sensors and Actuators B-Chemical*, **2014**, 205, 151-157.

[39] Jin, Q.; Feng, L.; Wang, D.-D.; Dai, Z.-R.; Wang, P.; Zou, L.-W.; Liu, Z.-H.; Wang, J.-Y.; Yu, Y.; Ge, G.-B.; Cui, J.-N.; Yang, L., A Two-Photon Ratiometric Fluorescent Probe for Imaging Carboxylesterase 2 in Living Cells and Tissues. *Acs Applied Materials & Interfaces*, **2015**, 7, (51), 28474-28481.

[40] Wang, D.-D.; Jin, Q.; Hou, J.; Feng, L.; Li, N.; Li, S.-Y.; Zhou, Q.; Zou, L.-W.; Ge, G.-B.; Wang, J.-G.; Yang, L., Highly sensitive and selective detection of human carboxylesterase 1 activity by liquid chromatography with fluorescence detection. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*, **2016**, 1008, 212-218.

[41] Lv, X.; Ge, G.B.; Feng, L.; Troberg, J.; Hu, L.H.; Hou, J.; Cheng, H.L.; Wang, P.; Liu, Z.M.; Finel, M.; Cui, J.N.; Yang, L., An optimized ratiometric fluorescent probe for sensing human UDP-glucuronosyltransferase 1A1 and its biological applications. *Biosens Bioelectron*, **2015**, 72, 261-267.

[42] Wang, Y.R.; Feng, L.; Xu, L.; Li, Y.; Wang, D.D.; Hou, J.; Zhou, K.; Jin, Q.; Ge, G.B.; Cui, J.N.; Yang, L., A rapid-response fluorescent probe for the sensitive and selective detection of human albumin in plasma and cell culture supernatants. *Chem Commun*, **2016**, 52, (36), 6064-6067.

[43] Dai, Z.R.; Feng, L.; Jin, Q.; Cheng, H.L.; Li, Y.; Ning, J.; Yu, Y.; Ge, G.B.; Cui, J.N.; Yang, L., A practical strategy to design and develop an isoform-specific fluorescent probe for a target enzyme: CYP1A1 as a case study. *Chem Sci*, **2017**, 8, (4), 2795-2803.

[44] Zou, L.W.; Wang, P.; Qian, X.K.; Feng, L.; Yu, Y.; Wang, D.D.; Jin, Q.; Hou, J.; Liu, Z.H.; Ge, G.B.; Yang, L., A highly specific ratiometric two-photon fluorescent probe to

detect dipeptidyl peptidase IV in plasma and living systems. *Biosens Bioelectron*, **2017**, 90, 283-289.

[45] Jin, Q.; Feng, L.; Wang, D.D.; Wu, J.J.; Hou, J.; Dai, Z.R.; Sun, S.G.; Wang, J.Y.; Ge, G.B.; Cui, J.N.; Yang, L., A highly selective near-infrared fluorescent probe for carboxylesterase 2 and its bioimaging applications in living cells and animals. *Biosens Bioelectron*, **2016**, 83, 193-199.

[46] Feng, L.; Liu, Z.M.; Xu, L.; Lv, X.; Ning, J.; Hou, J.; Ge, G.B.; Cui, J.N.; Yang, L., A highly selective long-wavelength fluorescent probe for the detection of human carboxylesterase 2 and its biomedical applications. *Chem Commun*, **2014**, 50, (93), 14519-14522.

[47] Feng, L.; Liu, Z.M.; Hou, J.; Lv, X.; Ning, J.; Ge, G.B.; Cui, J.N.; Yang, L., A highly selective fluorescent ESIPT probe for the detection of Human carboxylesterase 2 and its biological applications. *Biosens Bioelectron*, **2015**, 65, 9-15.

[48] Wang, P.; Xia, Y.L.; Zou, L.W.; Qian, X.K.; Dou, T.Y.; Jin, Q.; Li, S.Y.; Yu, Y.; Wang, D.D.; Luo, Q.; Ge, G.B.; Yang, L., An Optimized Two-Photon Fluorescent Probe for Biological Sensing and Imaging of Catechol-O-Methyltransferase. *Chem-Eur J*, **2017**, 23, (45), 10800-10807.

[49] Kodani, S.D.; Barthelemy, M.; Kamita, S.G.; Hammock, B.; Morisseau, C., Development of amide-based fluorescent probes for selective measurement of carboxylesterase 1 activity in tissue extracts. *Analytical biochemistry*, **2017**, 539, 81-89.

[50] Huang, H.; Stok, J.E.; Stoutamire, D.W.; Gee, S.J.; Hammock, B.D., Development of optically pure pyrethroid-like fluorescent substrates for carboxylesterases. *Chemical research in toxicology*, **2005**, 18, (3), 516-527.

[51] Park, S.J.; Lee, H.W.; Kim, H.R.; Kang, C.; Kim, H.M., A carboxylesterase-selective ratiometric fluorescent two-photon probe and its application to hepatocytes and liver tissues. *Chem Sci*, **2016**, 7, (6), 3703-3709.

[52] Wang, J.; Williams, E.T.; Bourgea, J.; Wong, Y.N.; Patten, C.J., Characterization of recombinant human carboxylesterases: fluorescein diacetate as a probe substrate for human carboxylesterase 2. *Drug Metab Dispos*, **2011**, 39, (8), 1329-1333.

[53] Wei, E.; Ali, Y.B.; Lyon, J.; Wang, H.; Nelson, R.; Dolinsky, V.W.; Dyck, J.R.B.; Mitchell, G.; Korbutt, G.S.; Lehner, R., Loss of TGH/Ces3 in Mice Decreases Blood Lipids, Improves Glucose Tolerance, and Increases Energy Expenditure. *Cell Metabolism*, **2010**, 11, (3), 183-193.

[54] Bie, J.; Wang, J.; Marqueen, K.E.; Osborne, R.; Kakiyama, G.; Korzun, W.; Ghosh, S.S.; Ghosh, S., Liver-Specific Cholesteryl Ester Hydrolase Deficiency Attenuates Sterol Elimination in the Feces and Increases Atherosclerosis in Ldlr(-/-) Mice. *Arteriosclerosis Thrombosis and Vascular Biology*, **2013**, 33, (8), 1795-1802.

[55] Quiroga, A.D.; Li, L.; Troetzmueller, M.; Nelson, R.; Proctor, S.D.; Koefeler, H.; Lehner, R., Deficiency of Carboxylesterase 1/Esterase-x Results in Obesity, Hepatic Steatosis, and Hyperlipidemia. *Hepatology*, **2012**, 56, (6), 2188-2198.

[56] Ghosh, S., Cholesteryl ester hydrolase in human monocyte/macrophage: cloning, sequencing, and expression of full-length cDNA. *Physiological Genomics*, **2000**, 2, (1), 1-8.

[57] Dominguez, E.; Galmozzi, A.; Chang, J.W.; Hsu, K.L.; Pawlak, J.; Li, W.; Godio, C.; Thomas, J.; Partida, D.; Niessen, S.; O'Brien, P.E.; Russell, A.P.; Watt, M.J.; Nomura, D.K.; Cravatt, B.F.; Saez, E., Integrated phenotypic and activity-based profiling links Ces3 to obesity and diabetes. *Nature chemical biology*, **2014**, 10, (2), 113-121.

[58] Nagashima, S.; Yagyu, H.; Takahashi, N.; Kurashina, T.; Takahashi, M.; Tsuchita, T.; Tazoe, F.; Wang, X.L.; Bayasgalan, T.; Sato, N.; Okada, K.; Nagasaka, S.; Gotoh, T.; Kojima, M.; Hyodo, M.; Horie, H.; Hosoya, Y.; Okada, M.; Yasuda, Y.; Fujiwara, H.; Ohwada, M.; Iwamoto, S.; Suzuki, M.; Nagai, H.; Ishibashi, S., Depot-Specific Expression of Lipolytic Genes in Human Adipose Tissues - Association Among CES1 Expression, Triglyceride Lipase Activity and Adiposity. *Journal of Atherosclerosis and Thrombosis*, **2011**, 18, (3), 190-199.

[59] Zhu, H.-J.; Wang, X.; Gawronski, B.E.; Brinda, B.J.; Angiolillo, D.J.; Markowitz, J.S., Carboxylesterase 1 as a Determinant of Clopidogrel Metabolism and Activation. *Journal of Pharmacology and Experimental Therapeutics*, **2013**, 344, (3), 665-672.

[60] Pratt, S.E.; Durland-Busbice, S.; Shepard, R.L.; Heinz-Taheny, K.; Iversen, P.W.; Dantzig, A.H., Human Carboxylesterase-2 Hydrolyzes the Prodrug of Gemcitabine (LY2334737) and Confers Prodrug Sensitivity to Cancer Cells. *Clinical Cancer Research*, **2013**, 19, (5), 1159-1168.

[61] Wadkins, R.M.; Hyatt, J.L.; Yoon, K.J.P.; Morton, C.L.; Lee, R.E.; Damodaran, K.; Beroza, P.; Danks, M.K.; Potter, P.M., 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*, **2004**, 65, (6), 1336-1343.

[62] Hicks, L.D.; Hyatt, J.L.; Stoddard, S.; Tsurkan, L.; Edwards, C.C.; Wadkins, R.M.; Potter, P.M., Improved, selective, human intestinal carboxylesterase inhibitors designed to modulate 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (Irinotecan; CPT-11) toxicity. *J Med Chem*, **2009**, 52, (12), 3742-3752.

[63] Wadkins, R.M.; Hyatt, J.L.; Yoon, K.J.; Morton, C.L.; Lee, R.E.; Damodaran, K.; Beroza, P.; Danks, M.K.; Potter, P.M., 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*, **2004**, 65, (6), 1336-1343.

[64] Yoon, K.J.P.; Hyatt, J.L.; Morton, C.L.; Lee, R.E.; Potter, P.M.; Danks, M.K., Characterization of inhibitors of specific carboxylesterases: Development of carboxylesterase inhibitors for translational application. *Molecular Cancer Therapeutics*, **2004**, 3, (8), 903-909.

[65] Hatfield, M.J.; Potter, P.M., Carboxylesterase inhibitors. *Expert Opinion on Therapeutic Patents*, **2011**, 21, (8), 1159-1171.

[66] Hatfield, M.J.; Tsurkan, L.G.; Hyatt, J.L.; Edwards, C.C.; Lemoff, A.; Jeffries, C.; Yan, B.; Potter, P.M., Modulation of Esterified Drug Metabolism by Tanshinones from Salvia miltiorrhiza ("Danshen"). *J Nat Prod*, **2013**, 76, (1), 36-44.

[67] Hyatt, J.L.; Wadkins, R.M.; Tsurkan, L.; Hicks, L.D.; Hatfield, M.J.; Edwards, C.C.; Ross, C.R.; Cantalupo, S.A.; Crundwell, G.; Danks, M.K.; Guy, R.K.; Potter, P.M., Planarity and constraint of the carbonyl groups in 1,2-diones are determinants for selective inhibition of human carboxylesterase 1. *J Med Chem*, **2007**, 50, (23), 5727-5734.

[68] Wadkins, R.M.; Hyatt, J.L.; Wei, X.; Yoon, K.J.P.; Wierdl, M.; Edwards, C.C.; Morton, C.L.; Obenauer, J.C.; Damodaran, K.; Beroza, P.; Danks, M.K.; Potter, P.M., Identification and characterization of novel benzil (diphenylethane-1,2-dione) analogues as inhibitors of mammalian carboxylesterases. *J Med Chem*, **2005**, 48, (8), 2906-2915.

[69] Parkinson, E.I.; Hatfield, M.J.; Tsurkan, L.; Hyatt, J.L.; Edwards, C.C.; Hicks, L.D.; Yan, B.; Potter, P.M., Requirements for mammalian carboxylesterase inhibition by substituted ethane-1,2-diones. *Bioorgan Med Chem*, **2011**, 19, (15), 4635-4643.

[70] Hyatt, J.L.; Moak, T.; Hatfield, M.J.; Tsurkan, L.; Edwards, C.C.; Wierdl, M.; Danks, M.K.; Wadkins, R.M.; Potter, P.M., Selective inhibition of carboxylesterases by isatins, indole-2,3-diones. *J Med Chem*, **2007**, 50, (8), 1876-1885.

[71] Hatfield, M.J.; Chen, J.W.; Fratt, E.M.; Chi, L.Y.; Bollinger, J.C.; Binder, R.J.; Bowling, J.; Hyatt, J.L.; Scarborough, J.; Jeffries, C.; Potter, P.M., Selective Inhibitors of Human Liver Carboxylesterase Based on a beta-Lapachone Scaffold: Novel Reagents for Reaction Profiling. *J Med Chem*, **2017**, 60, (4), 1568-1579.

[72] Wadkins, R.M.; Morton, C.L.; Weeks, J.K.; Oliver, L.; Wierdl, M.; Danks, M.K.; Potter, P.M., Structural constraints affect the metabolism of 7-ethyl-10-[4-(1-piperidino)-1- piperidino] carbonyloxycamptothecin (CPT-11) by carboxylesterases. *Mol Pharmacol*, **2001**, 60, (2), 355-362.

[73] Imai, T.; Hosokawa, M., Prodrug approach using carboxylesterases activity: catalytic properties and gene regulation of carboxylesterase in mammalian tissue. *J Pestic Sci*, **2010**, 35, (3), 229-239.

[74] Redinbo, M.R.; Potter, P.M., Mammalian carboxylesterases: from drug targets to protein therapeutics. *Drug Discov Today*, **2005**, 10, (5), 313-+.

[75] De Palma, A.; Rossi, R.; Carai, M.; Cabras, C.; Colombo, G.; Arnoldi, L.; Fuzzati, N.; Riva, A.; Morazzoni, P.; Mauri, P.L., Pharmaceutical and Biomedical Analysis of Terpene Constituents in Salvia miltiorrhiza. *Curr Pharm Anal*, **2008**, 4, (4), 249-257.

[76] Hyatt, J.L.; Stacy, V.; Wadkins, R.M.; Yoon, K.J.P.; Wierdl, M.; Edwards, C.C.; Zeller, M.; Hunter, A.D.; Danks, M.K.; Crundwell, G.; Potter, P.M., Inhibition of carboxylesterases by benzil (diphenylethane-1,2-dione) and heterocyclic analogues is dependent upon the aromaticity of the ring and the flexibility of the dione moiety. *J Med Chem*, **2005**, 48, (17), 5543-5550.

[77] Hicks, L.D.; Hyatt, J.L.; Stoddard, S.; Tsurkan, L.; Edwards, C.C.; Wadkins, R.M.; Potter, P.M., Improved, Selective, Human Intestinal Carboxylesterase Inhibitors Designed to Modulate 7-Ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (Irinotecan; CPT-11) Toxicity. *J Med Chem*, **2009**, 52, (12), 3742-3752.

[78] Wu, M.H.; Chen, P.X.; Remo, B.F.; Cook, E.H.; Das, S.; Dolan, M.E., Characterization of multiple promoters in the human carboxylesterase 2 gene. *Pharmacogenetics*, **2003**, 13, (7), 425-435.

[79] Lamego, J.; Cunha, B.; Peixoto, C.; Sousa, M.F.; Alves, P.M.; Simplicio, A.L.; Coroadinha, A.S., Carboxylesterase 2 production and characterization in human cells: new insights into enzyme oligomerization and activity. *Appl Microbiol Biot*, **2013**, 97, (3), 1161-1173.

[80] Prestwich, G.D.; Eng, W.S.; Roe, R.M.; Hammock, B.D., Synthesis and bioassay of isoprenoid 3-alkylthio-1,1,1-trifluoro-2-propanones: potent, selective inhibitors of juvenile hormone esterase. *Archives of biochemistry and biophysics*, **1984**, 228, (2), 639-645.

[81] Wadkins, R.M.; Hyatt, J.L.; Edwards, C.C.; Tsurkan, L.; Redinbo, M.R.; Wheelock, C.E.; Jones, P.D.; Hammock, B.D.; Potter, P.M., Analysis of mammalian carboxylesterase inhibition by trifluoromethylketone-containing compounds. *Mol Pharmacol*, **2007**, 71, (3), 713-723.

[82] Ashour, M.B.; Hammock, B.D., Substituted trifluoroketones as potent, selective inhibitors of mammalian carboxylesterases. *Biochem Pharmacol*, **1987**, 36, (12), 1869-1879.

[83] Brady, K.; Abeles, R.H., Inhibition of chymotrypsin by peptidyl trifluoromethyl ketones: determinants of slow-binding kinetics. *Biochemistry*, **1990**, 29, (33), 7608-7617.

[84] Ojima, I.; Jameison, F.A.; Pete, B.; Radunz, H.; Schittenhelm, C.; Lindner, H.J.; Emith, A.E., Design, synthesis and enzyme inhibitory activities of new trifluoromethyl-containing inhibitors for angiotensin converting enzyme. *Drug design and discovery*, **1994**, 11, (2), 91-113.

[85] Boger, D.L.; Sato, H.; Lerner, A.E.; Austin, B.J.; Patterson, J.E.; Patricelli, M.P.; Cravatt, B.F., Trifluoromethyl ketone inhibitors of fatty acid amide hydrolase: a probe of structural and conformational features contributing to inhibition. *Bioorganic & medicinal chemistry letters*, **1999**, 9, (2), 265-270.

[86] Gelb, M.H.; Svaren, J.P.; Abeles, R.H., Fluoro ketone inhibitors of hydrolytic enzymes. *Biochemistry,* **1985**, *24*, (8), 1813-1817.

[87] Rosell, G.; Herrero, S.; Guerrero, A., New trifluoromethyl ketones as potent inhibitors of esterases: F-19 NMR spectroscopy of transition state analog complexes and structure-activity relationships. *Biochem Bioph Res Co,* **1996**, *226*, (1), 287-292.

[88] Di Meo, F.; Steel, M.; Nicolas, P.; Marquet, P.; Duroux, J.L.; Trouillas, P., Acylglucuronide in alkaline conditions: migration vs. hydrolysis. *J Mol Model,* **2013**, *19*, (6), 2423-2432.

[89] Buchheit, D.; Dragan, C.A.; Schmitt, E.I.; Bureik, M., Production of Ibuprofen Acyl Glucosides by Human UGT2B7. *Drug Metab Dispos,* **2011**, *39*, (12), 2174-2181.

[90] Inoue, N.R.; Hall, A.; Lai, W.G.; Williams, E.T., Reversible Inhibition of Human Carboxylesterases by Acyl Glucuronides. *Drug Metab Dispos,* **2013**, *41*, (4), 698-703.

[91] Zhou, M.; Zhang, R.H.; Wang, M.; Xu, G.B.; Liao, S.G., Prodrugs of triterpenoids and their derivatives. *Eur J Med Chem,* **2017**, *131*, 222-236.

[92] Zebiri, I.; Haddad, M.; Duca, L.; Sauvain, M.; Paloque, L.; Cabanillas, B.; Rengifo, E.; Behr, J.B.; Voutquenne-Nazabadioko, L., Biological activities of triterpenoids from Poraqueiba sericea stems. *Nat Prod Res,* **2017**, *31*, (11), 1333-1338.

[93] Kazakova, O.B.; Giniyatullina, G.V.; Tolstikov, G.A.; Kataev, V.E.; Musin, R.Z., Synthesis and modification of triterpenoids with two lupan backbones. *Russ J Bioorg Chem+,* **2009**, *35*, (5), 645-650.

[94] Callies, O.; Bedoya, L.M.; Beltran, M.; Munoz, A.; Calderon, P.O.; Osorio, A.A.; Jimenez, I.A.; Alcami, J.; Bazzocchi, I.L., Isolation, Structural Modification, and HIV Inhibition of Pentacyclic Lupane-Type Triterpenoids from Cassine xylocarpa and Maytenus cuzcoina. *J Nat Prod,* **2015**, *78*, (5), 1045-1055.

[95] Zou, L.W.; Dou, T.Y.; Wang, P.; Lei, W.; Weng, Z.M.; Hou, J.; Wang, D.D.; Fan, Y.M.; Zhang, W.D.; Ge, G.B.; Yang, L., Structure-Activity Relationships of Pentacyclic Triterpenoids as Potent and Selective Inhibitors against Human Carboxylesterase 1. *Front Pharmacol,* **2017**, *8*, 438.

[96] Zou, L.W.; Li, Y.G.; Wang, P.; Zhou, K.; Hou, J.; Jin, Q.; Hao, D.C.; Ge, G.B.; Yang, L., Design, synthesis, and structure-activity relationship study of glycyrrhetinic acid derivatives as potent and selective inhibitors against human carboxylesterase 2. *Eur J Med Chem,* **2016**, *112*, 280-288.

[97] Mai, Z.P.; Zhou, K.; Ge, G.B.; Wang, C.; Huo, X.K.; Dong, P.P.; Deng, S.; Zhang, B.J.; Zhang, H.L.; Huang, S.S.; Ma, X.C., Protostane Triterpenoids from the Rhizome of Alisma orientale Exhibit Inhibitory Effects on Human Carboxylesterase 2. *J Nat Prod,* **2015**, *78*, (10), 2372-2380.

[98] Mai, Z.P.; Xin, X.L.; Sun, Z.; Zhang, N.; Huang, S.S.; Wang, C.; Chen, L.; Li, Y.; Huo, X.K.; Fan, G.J., Biotransformation of alisol G by Penicillium janthinellum and the hCE2 inhibitory effects of its metabolites. *Phytochem Lett,* **2015**, *13*, 228-233.

[99] Zhang, Z.J.; Huo, X.K.; Tian, X.G.; Feng, L.; Ning, J.; Zhao, X.Y.; Sun, C.P.; Wang, C.; Deng, S.; Zhang, B.J.; Zhang, H.L.; Liu, Y., Novel protostane-type triterpenoids with inhibitory human carboxylesterase 2 activities. *Rsc Adv,* **2017**, *7*, (46), 28702-28710.

[100] Wen, L.R.; Jiang, Y.M.; Yang, J.L.; Zhao, Y.P.; Tian, M.M.; Yang, B., Structure, bioactivity, and synthesis of methylated flavonoids. *Ann Ny Acad Sci,* **2017**, *1398*, (1), 120-129.

[101] Santos-Buelga, C.; Feliciano, A.S., Flavonoids: From Structure to Health Issues. *Molecules,* **2017**, *22*, (3), 447.

[102] Sun, D.X.; Ge, G.B.; Dong, P.P.; Cao, Y.F.; Fu, Z.W.; Ran, R.X.; Wu, X.; Zhang, Y.Y.; Hua, H.M.; Zhao, Z.Y.; Fang, Z.Z., Inhibition behavior of fructus psoraleae's ingredients towards human carboxylesterase 1 (hCES1). *Xenobiotica,* **2016**, *46*, (6), 503-510.

[103] Li, Y.G.; Hou, J.; Li, S.Y.; Lv, X.; Ning, J.; Wang, P.; Liu, Z.M.; Ge, G.B.; Ren, J.Y.; Yang, L., Fructus Psoraleae contains natural compounds with potent inhibitory effects towards human carboxylesterase 2. *Fitoterapia,* **2015**, *101*, 99-106.

[104] Liu, Y.J.; Li, S.Y.; Hou, J.; Liu, Y.F.; Wang, D.D.; Jiang, Y.S.; Ge, G.B.; Liang, X.M.; Yang, L., Identification and characterization of naturally occurring inhibitors against human carboxylesterase 2 in White Mulberry Root-bark. *Fitoterapia,* **2016**, *115*, 57-63.

[105] Wang, D.D.; Zou, L.W.; Jin, Q.; Hou, J.; Ge, G.B.; Yang, L., Recent progress in the discovery of natural inhibitors against human carboxylesterases. *Fitoterapia,* **2017**, *117*, 84-95.

[106] Xu, Y.J.; Zhang, C.L.; He, W.X.; Liu, D., Regulations of Xenobiotics and Endobiotics on Carboxylesterases: A Comprehensive Review. *Eur J Drug Metab Ph,* **2016**, *41*, (4), 321-330.

[107] Lei, W.; Wang, D.D.; Dou, T.Y.; Hou, J.E.; Feng, L.; Yin, H.; Luo, Q.; Sun, J.E.; Ge, G.B.; Yang, L., Assessment of the inhibitory effects of pyrethroids against human carboxylesterases. *Toxicol Appl Pharm,* **2017**, *321*, 48-56.

[108] Crow, J.A.; Herring, K.L.; Xie, S.; Borazjani, A.; Potter, P.M.; Ross, M.K., Inhibition of carboxylesterase activity of THP1 monocytes/macrophages and recombinant human carboxylesterase 1 by oxysterols and fatty acids. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids,* **2010**, *1801*, (1), 31-41.

[109] Li, Y.-G.; Hou, J.; Li, S.-Y.; Lv, X.; Ning, J.; Wang, P.; Liu, Z.-M.; Ge, G.-B.; Ren, J.-Y.; Yang, L., Fructus Psoraleae contains natural compounds with potent inhibitory effects towards human carboxylesterase 2. *Fitoterapia,* **2015**, *101*, 99-106.

[110] Umehara, K.I.; Zollinger, M.; Kigondu, E.; Witschi, M.; Juif, C.; Huth, F.; Schiller, H.; Chibale, K.; Camenisch, G., Esterase phenotyping in human liver *in vitro*: specificity of carboxylesterase inhibitors. *Xenobiotica,* **2016**, *46*, (10), 862-867.

[111] Fukami, T.; Takahashi, S.; Nakagawa, N.; Maruichi, T.; Nakajima, M.; Yokoi, T., *In Vitro* Evaluation of Inhibitory Effects of Antidiabetic and Antihyperlipidemic Drugs on Human Carboxylesterase Activities. *Drug Metab Dispos,* **2010**, *38*, (12), 2173-2178.

[112] Xu, Y.J.; Zhang, C.L.; Li, X.P.; Wu, T.; Ren, X.H.; Liu, D., Evaluation of the Inhibitory Effects of Antihypertensive Drugs on Human Carboxylesterase *In Vitro*. *Drug Metab Pharmacok,* **2013**, *28*, (6), 468-474.

[113] Rhoades, J.A.; Peterson, Y.K.; Zhu, H.J.; Appel, D.I.; Peloquin, C.A.; Markowitz, J.S., Prediction and *In Vitro* Evaluation of Selected Protease Inhibitor Antiviral Drugs as Inhibitors of Carboxylesterase 1: A Potential Source of Drug-Drug Interactions. *Pharm Res-Dordr,* **2012**, *29*, (4), 972-982.

[114] Quinney, S.K.; Sanghani, S.P.; Davis, W.I.; Hurley, T.D.; Sun, Z.; Murry, D.J.; Bosron, W.F., Hydrolysis of capecitabine to 5 '-deoxy-5-fluorocytidine by human carboxylesterases and inhibition by loperamide. *J Pharmacol Exp Ther,* **2005**, *313*, (3), 1011-1016.

[115] Zhang, C.L.; Xu, Y.J.; Zhong, Q.N.; Li, X.P.; Gao, P.; Feng, C.Y.; Chu, Q.; Chen, Y.; Liu, D., *In Vitro* Evaluation of the Inhibitory Potential of Pharmaceutical Excipients on Human Carboxylesterase 1A and 2. *Plos One,* **2014**, *9*, (4), e93819.

[116] Chang, J.W.; Cognetta, A.B., III; Niphakis, M.J.; Cravatt, B.F., Proteome-Wide Reactivity Profiling Identifies Diverse Carbamate Chemotypes Tuned for Serine Hydrolase Inhibition. *Acs Chemical Biology,* **2013**, *8*, (7), 1590-1599.

[117] Long, J.Z.; Li, W.; Booker, L.; Burston, J.J.; Kinsey, S.G.; Schlosburg, J.E.; Pavon, F.J.; Serrano, A.M.; Selley, D.E.; Parsons, L.H.; Lichtman, A.H.; Cravatt, B.F., Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nature chemical biology,* **2009**, *5*, (1), 37-44.

[118] Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D., Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med,* **2003**, *9*, (1), 76-81.

[119] Roy, K.K.; Tota, S.; Tripathi, T.; Chander, S.; Nath, C.; Saxena, A.K., Lead optimization studies towards the discovery of novel carbamates as potent AChE inhibitors for the potential treatment of Alzheimer's disease. *Bioorg Med Chem,* 2012, *20*, (21), 6313-6320.

[120] Casida, J.E.; Durkin, K.A., Anticholinesterase insecticide retrospective. *Chem Biol Interact,* 2013, *203*, (1), 221-225.

[121] Crow, J.A.; Bittles, V.; Borazjani, A.; Potter, P.M.; Ross, M.K., Covalent inhibition of recombinant human carboxylesterase 1 and 2 and monoacylglycerol lipase by the carbamates JZL184 and URB597. *Biochem Pharmacol,* **2012**, *84*, (9), 1215-1222.

[122] Tsurkan, L.G.; Hatfield, M.J.; Edwards, C.C.; Hyatt, J.L.; Potter, P.M., Inhibition of human carboxylesterases hCE1 and hiCE by cholinesterase inhibitors. *Chem-Biol Interact,* **2013**, *203*, (1), 226-230.

[123] Scaloni, A.; Barra, D.; Jones, W.M.; Manning, J.M., Human acylpeptide hydrolase. Studies on its thiol groups and mechanism of action. *The Journal of biological chemistry,* **1994**, *269*, (21), 15076-15084.

[124] Lee, I.; Eriksson, P.; Fredriksson, A.; Buratovic, S.; Viberg, H., Developmental neurotoxic effects of two pesticides: Behavior and biomolecular studies on chlorpyrifos and carbaryl. *Toxicol Appl Pharm,* **2015**, *288*, (3), 429-438.

[125] Scholz, N.L.; Truelove, N.K.; Labenia, J.S.; Baldwin, D.H.; Collier, T.K., Dose-additive inhibition of chinook salmon acetylcholinesterase activity by mixtures of organophosphate and carbamate insecticides. *Environ Toxicol Chem,* **2006**, *25*, (5), 1200-1207.

[126] Bloch-Shilderman, E.; Rabinovitz, I.; Egoz, I.; Raveh, L.; Allon, N.; Grauer, E.; Gilat, E.; Weissman, B.A., Subchronic-exposure to low-doses of the nerve agent VX: Physiological, behavioral, histopathological and neurochemical studies. *Toxicol Appl Pharm,* **2008**, *231*, (1), 17-23.

[127] Bocquene, G.; Roig, A.; Fournier, D., Cholinesterases from the common oyster (Crassostrea gigas) - Evidence for the presence of a soluble acetylcholinesterase insensitive to organophosphate and carbamate inhibitors. *Febs Lett,* **1997**, *407*, (3), 261-266.

[128] Qadri, S.S.; Kashi, K.P., Assaying the oral toxicity of organophosphates to house flies without body contact with

the insecticides. *Journal of economic entomology,* **1971**, *64*, (5), 1015-1018.

[129] Tong, H.; Su, Q.; Zhou, X.; Bai, L., Field resistance of Spodoptera litura (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *Journal of pest science,* **2013**, *86*, 599-609.

[130] Kovach, I.M., Structure and dynamics of serine hydrolase-organophosphate adducts. *Journal of enzyme inhibition,* **1988**, *2*, (3), 199-208.

[131] Nomura, D.K.; Durkin, K.A.; Chiang, K.P.; Quistad, G.B.; Cravatt, B.F.; Casida, J.E., Serine hydrolase KIAA1363: toxicological and structural features with emphasis on organophosphate interactions. *Chemical research in toxicology,* **2006**, *19*, (9), 1142-1150.

[132] Heymann, E.; Mentlein, R.; Schmalz, R.; Schwabe, C.; Wagenmann, F., A method for the estimation of esterase synthesis and degradation and its application to evaluate the influence of insulin and glucagon. *European journal of biochemistry,* **1979**, *102*, (2), 509-519.

[133] Casida, J.E.; Quistad, G.B., Serine hydrolase targets of organophosphorus toxicants. *Chem Biol Interact,* **2005**, *157-158*, 277-283.

[134] Wei, Y.L.; Peng, A.Y.; Wang, B.; Ma, L.; Peng, G.P.; Du, Y.D.; Tang, J.M., Synthesis and biological evaluation of phosphorylated flavonoids as potent and selective inhibitors of cholesterol esterase. *Eur J Med Chem,* **2014**, *74*, 751-758.

[135] Wei, Y.L.; Peng, A.Y.; Huang, J., Inhibition of porcine liver carboxylesterase by phosphorylated flavonoids. *Chem-Biol Interact,* **2013**, *204*, (2), 75-79.

[136] Crow, J.A.; Bittles, V.; Herring, K.L.; Borazjani, A.; Potter, P.M.; Ross, M.K., Inhibition of recombinant human carboxylesterase 1 and 2 and monoacylglycerol lipase by chlorpyrifos oxon, paraoxon and methyl paraoxon. *Toxicol Appl Pharm,* **2012**, *258*, (1), 145-150.

[137] Ma, H.Y.; Yang. J.D.; Hou, J.;, Zou, L.W.; Jin, Q.; Hao, D.C.; Ning, J.; Ge, G.B.; Yang, L., Comparative metabolism of DDAO benzoate in liver microsomes from various species. *Toxicol Vitro* **2017,** 44, 280-286.

PMID: 29210644