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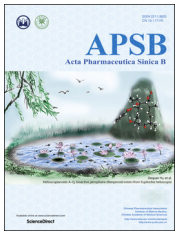

REVIEW

# Human carboxylesterases: a comprehensive review

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**KEYWORDS**

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**Abstract** Mammalian carboxylesterases (CEs) are key enzymes from the serine hydrolase superfamily. In the human body, two predominant carboxylesterases (CES1 and CES2) have been identified and extensively studied over the past decade. These two enzymes play crucial roles in the metabolism of a wide variety of endogenous esters, ester-containing drugs and environmental toxicants. The key roles of CES in both human health and xenobiotic metabolism arouse great interest in the discovery of potent CES modulators to regulate endobiotic metabolism or to improve the efficacy of ester drugs. This review covers the structural and catalytic features of CES, tissue distributions, biological functions, genetic polymorphisms, substrate specificities and inhibitor properties of CES1 and CES2, as well as the significance and recent progress on the discovery of CES modulators. The information presented here will help pharmacologists explore the relevance of CES to human diseases or to assign the contribution of certain CES in xenobiotic metabolism. It will also facilitate medicinal chemistry efforts to design prodrugs activated by a given CES isoform, or to develop potent and selective modulators of CES for potential biomedical applications.



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## 1. Introduction

Mammalian carboxylesterases (CES, E.C. 3.1.1.1) are essential members of the serine hydrolase superfamily, which are localized within the lumen of the endoplasmic reticulum in many tissues[1–3]. As their name implies, CES catalyze the ester cleavage of a large number of structurally diverse ester- or amide-containing substrates into the corresponding alcohol and carboxylic acid[1–3]. Actually, CES can hydrolyze ester, thioester, amide, and carbamate linkages in a wide variety of endo- and xenobiotic compounds, thus playing key roles in both endobiotic metabolism, and in activation and/or detoxification of xenobiotics[3–5]. In the human body, three CES have been identified, although human carboxylesterase 1 (CES1) and human carboxylesterase 2 (CES2) are the two extensively studied isoenzymes involved in xenobiotic metabolism[3–8]. Both CES1 and CES2 play crucial roles in the metabolism of various ester xenobiotics including many ester drugs (such as oseltamivir, clopidogrel, irinotecan and capecitabine) and environmental toxicants (such as pyrethroids)[9–13]. These two enzymes are also known to metabolize endogenous esters including cholesteryl esters, triacylglycerols and other endogenous lipids, thus playing vital physiological functions in lipid homeostasis[14–18].

Over the past twenty years, many studies have provided powerful insight into the roles of CES in metabolic diseases and xenobiotic metabolism[14]. The key roles of CES in both endogenous and xenobiotic metabolism have attracted great interest in the discovery of CES modulators to regulate lipid metabolism or to enhance the activity of ester drugs[19–24]. This review covers the structural and catalytic features of CES, tissue distribution, biological functions, substrate specificities and inhibitor profiles of two predominant CES, as well as the significance and recent progress on the discovery of CES modulators. It will be very helpful for pharmacologists to explore the relevance of CES to human diseases or to confirm the contribution of CES in xenobiotic metabolism. In addition, it will assist medicinal chemists in designing ideal prodrugs which can be activated by a given CES isoform in the human body, or to develop more potent inhibitors/inducers of CES.

## 2. Structural features and catalytic properties of CES

### 2.1. *Structural features of CES*

CES belong to the *α/β*-hydrolase fold superfamily of proteins. The majority of mammalian carboxylesterases are intracellular proteins found predominantly in the microsomal fraction that encompass the endoplasmic reticulum (ER)[3,25,26]. Microsomal CES from human, rabbit, and mouse carry the HXEL motifs of the KDEL consensus ER retrieval sequence at their C-terminal (such as HIEL and HTEL for CES1 and CES2, respectively), which is essential for the localization of these enzymes to the ER lumen in mammalian cells[26]. Following cleavage of the C-terminal signal peptide, microsomal carboxylesterases can be released from their membrane-associated state, suggesting that these enzymes are not transmembrane proteins but soluble proteins that reside in the ER lumen[9].

The three-dimensional (3D) structures of several mammalian CEs including human carboxylesterase 1 (CES1) have been solved by X-ray crystallography with several ligands[9,25–31]. As depicted in Fig. 1, CES1 is composed by a central catalytic domain, an *αβ* domain, and an adjacent regulatory domain which containing the low-affinity surface ligand-binding Z-site[28,30,31]. The X-ray crystal structure of CES1 demonstrated its existence as monomer, trimer, or hexamer, with substrate-dependent equilibrium of homooligomer formation. In contrast, CES2 and CES3 exist as monomers. Both sequence aligments and secondary sequence predictions have suggested that these three CES are members of *α/β* hydrolase family[32–34]. Although the 3D structures of CES2 and CES3 have not been reported, the 3D structure modelling of both CES2 and CES3 can be downloaded from the SWISS-MODEL repository (a database of annotated 3D protein structure models generated by the SWISS-MODEL homology-modelling pipeline).

The molecular properties of CES1 and CES2 are listed in Table 1[6,9,19,25,27,29]. Similar to all reported serine hydrolases, the catalytic domain of human CEs contain a catalytic triad (such as $Ser^{221}$, $Glu^{354}$, and $His^{468}$ in CES1) at the interface of the three domains, which is highly conserved among all mammalian carboxylesterases and is crucial for carboxylesterases-mediated catalysis (Fig. 1B)[9,27]. Mutation of any residue of the catalytic triad will lead to the loss of carboxylesterase activity[18]. Furthermore, the oxyanion hole formed by $Gly^{142}$ and $Gly^{143}$ in the HGGG motif is also highly conserved among all mammalian carboxylesterases and is essential for carboxylesterase activity. Notably, the active cavity of human CES1 is quite large (about 1,300 $Å^3$) and is lined mainly by hydrophobic amino acids, except the residues (such as $Ser^{221}$) of the catalytic triad. The residue $Ser^{221}$ divides the whole ligand-binding pocket of CES1 into two pockets, one is a rigid pocket which makes CES1 selective for those substrates with small acyl group, and another is a flexible pocket which makes CES1 promiscuous towards

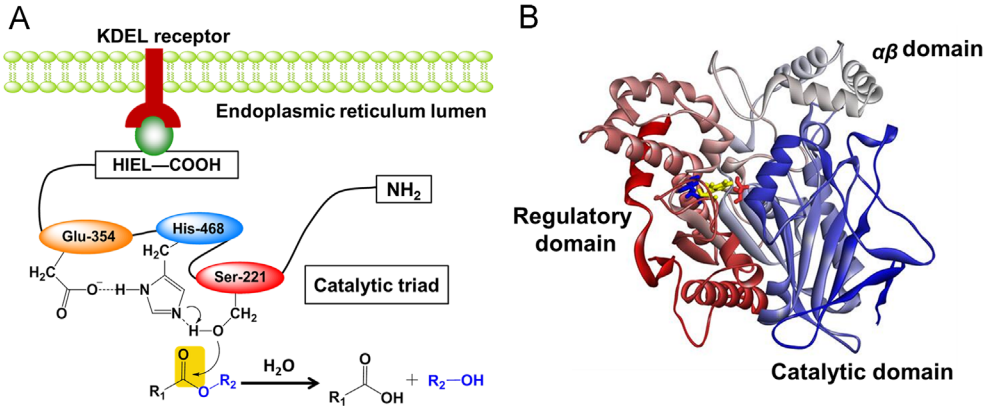


**Figure 1** The structural features of CES1. (A) The scheme for catalyzing (hydrolysis) ester groups; (B) The 3D structure of CES1. The catalytic triad including Ser221, Glu354 and His468 are colored in red, yellow and blue, respectively.

a wide range of esters with various acyl groups[28]. These features make CES1 capable of interacting with a wide variety of chemically diverse ligands[28].

### 2.2. *The catalytic properties of CES*

The CES hydrolyze substrates using a classic base-catalysed mechanism *via* a two-step reaction which is conserved in all serine hydrolases, including proteases, peptidases and lipases (Fig. 2)[3,5,6]. This process is dependent on an essential catalytic triad which is generally composed by three residues (Ser, His, and Glu) within the active cavity of mammalian carboxylesterases[3,5,6]. First, a nucleophilic attack by the base-activated serine oxygen atom (such as $Ser^{221}$ in human CES1) on the carbonyl carbon of the substrate lead to the formation of an acyl-enzyme intermediate and the release of an alcohol, thiol, or amine product (Fig. 2). Second, the acyl-enzyme intermediate is attacked in an identical fashion with water acting as the nucleophile, leading to release of the carboxylic acid metabolite, which regenerates the carboxylesterase to its original state with the free serine residue[5,6].

Notably, several mammalian CES (especially CES1) can perform transesterification reactions. When ethyl alcohol is present in the CES1 reaction system, alcohol replaces water to attack the acyl-enzyme intermediate to generate an ethyl ester product. One of the well-studied examples of this reaction is the formation of cocaethylene in individuals abusing both cocaine and alcohol[32–36]. Under these conditions, the ethyl group from ethanol replaces the methyl group of cocaine to produce a more toxic metabolite cocaethylene[34,35]. Furthermore, CES1 can catalyze the creation of cholesteryl esters from cholesterol and fatty acids, as well as to generate fatty acid ethyl esters (FAEEs) from fatty acyl-Coenzyme A (CoA) and ethanol, using a transesterification reaction[36,37]. Because CES have cholesteryl esters hydrolysis and FAEE hydrolysis activities, the formation of these endogenous esters is the result of the balance between typical hydrolysis reactions and transesterification reactions.

**Table 1** Molecular properties of CES1 and CES2[6,9,19,25,27,29].

| Property | CES1 | CES2 |
|---|---|---|
| Molecular weight | 60 kD (monomer), 180 kD (trimer) | 60 kD (monomer) |
| Isoelectric point | 5.6–5.8 | 4.8–5.0 |
| Optimal pH | 6.5 | 7.5–8.0 |
| C-terminal signal peptide | HIEL | HTEL |
| Catalytic triad | $Ser^{221}$, $Glu^{354}$ and $His^{468}$ | $Ser^{228}$, $Glu^{345}$ and $His^{457}$ |
| Glycosylation site | Asn-X-Thr and $Asn^{79}$ | Asn-X-Ser/Thr, $Asn^{103}$and $Asn^{267}$ |

## 3. Tissue distribution and substrate specificity of CES

Although CES1 and CES2 share 47% amino acid sequence identity, these two enzymes exhibit extremely different substrate distribution and specificity[5,6]. Typically, CES1 and CES2

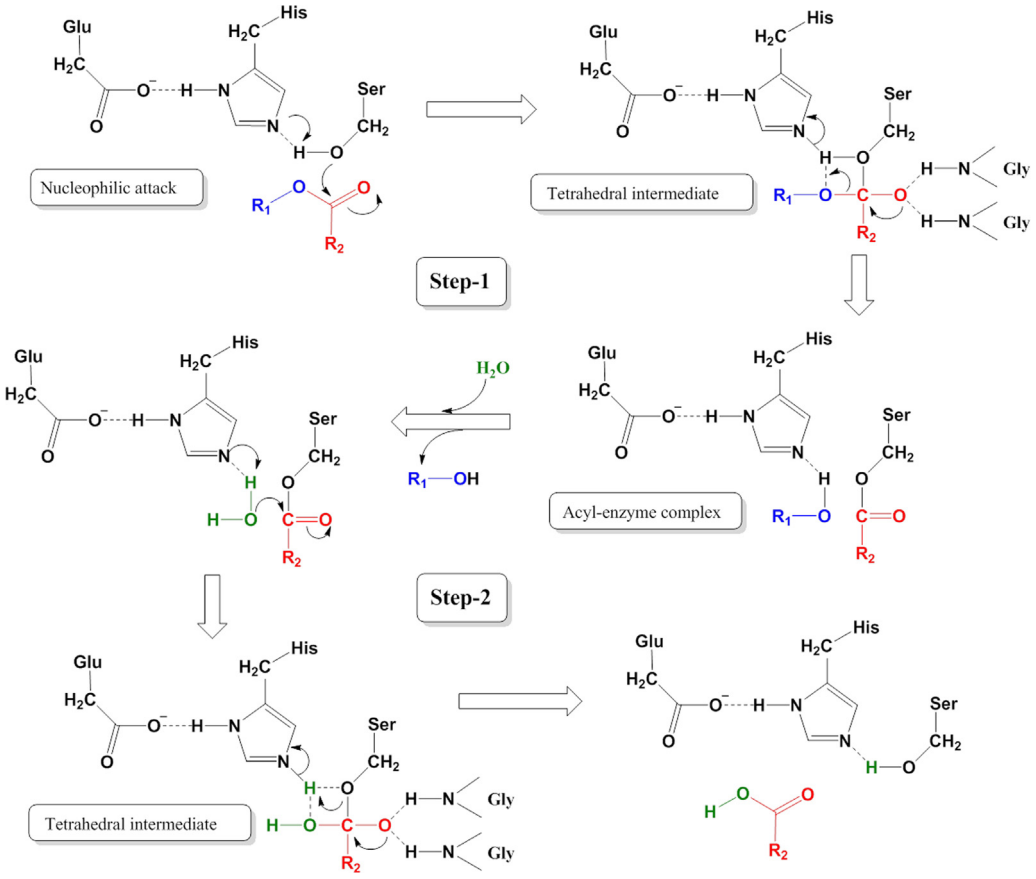


**Figure 2** The two-step catalytic mechanism of mammalian carboxylesterases.

are highly expressed in the epithelia of most metabolic organs including liver, intestine and kidney, indicating that these two enzymes play a protective role against xenobiotics. CES1 is abundantly expressed in the liver and adipocyte, with lesser amounts in the kidney, monocytes, lung, intestine, testis, heart, and macrophages[1,8,38–45]. In contrast, CES2 is expressed mainly in the small intestine and colon, but also observed in kidney, liver, heart, brain and testis[1,8,42]. Quantitative data on CES abundance in the human liver microsomes (HLMs) and liver cytosol (HLC) have been reported. Protein levels of CES1 and CES2 in 16 individual HLMs were 402 and 29.8 pmol/mg, respectively, while in HLC were 54.5 and 2.76 pmol/mg, respectively[39]. Furthermore, the secreted forms of CES and very high activity levels of CES were detected in rodent blood, yet CES1 and CES2 activities were barely detected in human blood[43,44]. Notably, the expression profiles of CES1 and CES2 in tumour tissues or cancer cell lines differ markedly from those of healthy cells. For example, human Caco-2 cells mainly express the CES1 isoenzyme, yet protein levels of CES1 in the human intestine are extremely low[42]. In addition, CES2 is overexpressed in several types of cancer or cancer cell lines, including multiple myeloma, thyroid papillary carcinoma, esophageal squamous carcinoma, and kidney adenocarcinoma[46]. CES2 expression in cancer cell lines and tumour tissue significantly correlate with bioactivation of several cancer prodrugs (such as irinotecan and LY2334737, the prodrug of gemcitabine), in agreement with the anticancer effects of these drugs[46–48]. These results suggest that the rational design of CES2-bioactivated prodrugs will be very useful for cancer therapy.

Human carboxylesterases have a broad substrate specificity, which can hydrolyze a vast number of endo- and xenobiotic substrates with ester, thioester, carbamate, and amide bonds (Fig. 3, Fig. 4, and Supplementary Information Table S1)[4–6,9–11,24,49–57]. Over the past decade, many studies have reported that CES1 and CES2 exhibit distinct substrate specificities. The representative substrates for CES1 and CES2 have been depicted in Fig. 3, and Fig. 4, respectively[4–6,9–11,49–57]. In general, CES1 prefers to metabolize the ester substrates that contain a small alcohol group and a bulky acyl group, such as enalapril, oseltamivir, imidapril, clopidogrel, meperidine, D-luciferin methyl ester, and the illegal drugs heroin and cocaine[5,6,49]. In contrast, CES2 prefers to hydrolyse esters with a relatively large alcohol group and a small acyl group, such as irinotecan, prasugrel, capecitabine, flutamide, and fluorescein diacetate[9,50–52,57]. However, several substrates with a small acyl group, such as *R*-propionyl propranolol, can also be hydrolyzed by CES1. As mentioned above, CES1 has two ligand-binding pockets, one is a rigid pocket and another is a flexible pocket, which makes CES1 promiscuous towards a vast number of substrates[32]. The substrate specificity of CES3 has not been extensively studied, but CES3 has been found with irinotecan hydrolysis activity but exhibits much lower hydrolysis activity, compared with CES2[57]. Based on the substrate specificities of both CES1 and CES2, some optical probe substrates have been recently developed for assessing the real activities of CES1 or CES2 in complex biological systems (Supplementary Information Table S2)[49,51,58–64]. These optical probes provide practical and efficient tools for high-throughput screening (HTS) of CES modulators in cell/tissue preparations or even in living cells, due to the inherent advantages including non-destructive, highly sensitive, easily managed, and applicable to HTS assay[49,58–69].

## 4. Biological functions of CES

The primary physiological function of CES appears to be xenobiotic metabolism. Over the past twenty years, CES have been regarded as the classic xenobiotic-metabolizing enzymes which are responsible for in the metabolism of a variety of ester-containing drugs, prodrugs, and environmental toxins. Many clinical drugs with ester moieties can be readily hydrolyzed by CES. Such compounds include the anticancer prodrugs (such as irinotecan, capecitabine), opioids and stimulants (cocaine, heroin, and meperidine), angiotensin-converting enzyme inhibitors (enalapril, temocapril, imidapril and quinapril), and other drugs with ester moieties (oseltamivir, clopidogrel, flumazenil, procaine, oxybutynin, delapril, flutamide, and prasugrel)[3–6,70]. In addition to drug metabolism, CES also participate in the detoxification and metabolic clearance of many ester-containing toxicants, such as the pyrethroid insecticides (deltamethrin and permethrin)[12,71]. Notably, strong inhibition of CES may slow down the hydrolysis of CES substrates, which may affect their pharmacokinetic properties and thus modulate their activities *in vivo*[13,22]. For example,

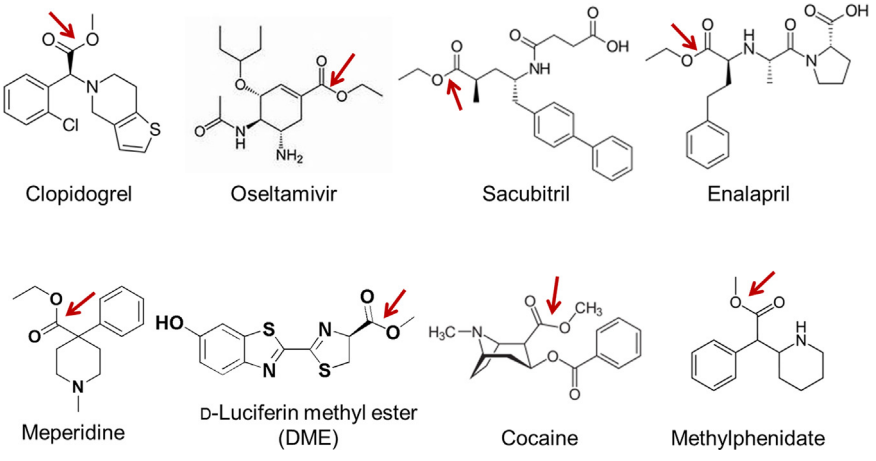


**Figure 3** Substrate specificity of CES1. Generally, CES1 prefers to hydrolyze the substrates containing a small alcohol group and a bulky acyl group.

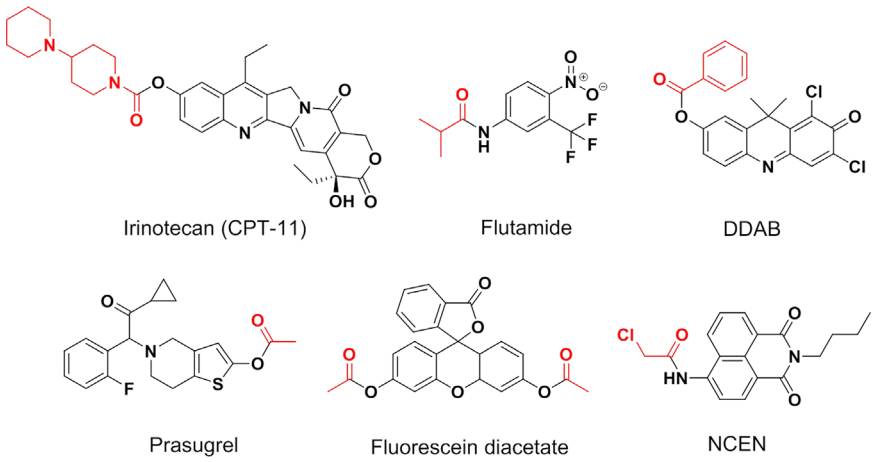


**Figure 4** Substrate specificity of CES2. CES2 prefers to hydrolyze the esters with a relatively large alcohol group and a small acyl group.

irinotecan (CES2 substrate) can trigger severe delayed diarrhea due to the overproduction of SN-38 (the hydrolytic product of irinotecan) in the small intestine, but co-administration with potent CES2 inhibitors may ameliorate CPT-11 caused severe diarrhea in patients and thus improve the therapeutic effect[72,73]. Besides the well-known roles of CES in xenobiotic metabolism, these enzymes have recently been studied for their participation in endogenous metabolism. As one of the most abundant serine hydrolases found in human hepatocytes and adipocytes, CES1 is responsible for the hydrolysis of a vast number of endogenous esters (such as cholesteryl esters and triacylglycerols) thereby participating in physiological and pathological processes, such as lipid metabolism, cholesterol homeostasis, and fatty liver disease[14,74–76]. Notably, it has been shown that the protein expression and enzymatic activities of CES1 in adipose tissues from obese and type 2 diabetic patients are markedly elevated compared to lean subjects, yet treatment with CE1 inhibitors has multiple beneficial effects on both glucose and lipid homeostasis in mice[20]. More recently, CES2 was shown to exhibit triacylglycerol and diacylglycerol hydrolase activity, and its expression and function in liver are strongly related to several metabolic diseases, such as obesity and non-alcoholic steatohepatitis[16,20,77].

Besides key roles in both endo- and xenobiotic metabolism, CES also have other biological roles, such as trafficking and retention of proteins in the endoplasmic reticulum (ER). In the ER, CES1 and CES2 appear to regulate protein trafficking, including release of proteins. For instance, CES1 can directly bind to the C-reactive protein (CRP) and retain this small protein before its release into the plasma[78]. Both CES use a region of amino acid sequence adjacent to the 'side door', which is comprised of the loop between $\alpha$15 and $\beta$18, to contact CRP. These CEs could hold a small reservoir of CRP within the ER, and then release it during the stage of tissue injury. It has also been reported that CES can directly interact with $\beta$-glucuronidases, the enzymes responsible for the removal of glucuronic acid moieties which are typically conjugated to drugs and endobiotics by the UDP-glucuronosyltransferase (UGT) enzymes, in the ER[79]. Although the interactions between $\beta$-glucuronidases with CEs have not been extensively investigated, several studies have demonstrated that some compounds (such as organophosphate) are capable of inducing the release of $\beta$-glucuronidases from the ER by disrupting the $\beta$-glucuronidase-CES1 complex[79].

## 5. Genetic polymorphisms of *CES*

The genomic structures of *CES1* and *CES2* has been ascertained, and both are located on 16q13-q22.1[80]. Over the past decade, a vast number of single-nucleotide polymorphisms (SNPs) have been reported in the NCBI SNP database. It is worth note that the allele and haplotype frequencies of known SNPs showed significant differences among ethnic groups. For instance, the G143E and the D260fs variants were two important functional SNPs in Caucasian populations, while these two *CES1* polymorphisms were not found in a Korean population[80]. To date, a number of functional genetic variants of CES1 and CES2 have been reported, which may be associated with substantial individual variations in the responses to pharmacologic therapies (Table 2[10,38,70,81,82]). Clopidogrel pharmacotherapy has been associated with substantial inter-individual variability in clinical responses. Zhu et al.[10] found the *CES1* variants G143E and D260fs showed diminished enzymatic activity, which impaired the hydrolysis of clopidogrel and 2-oxo-clopidogrel. Tang et al.[38] reported the antiplatelet agent aspirin could be hydrolyzed by CES2 and the CES2 variant A139T showed decreased CES activity accounting for the decreased aspirin hydrolysis. The association between SNPs in the human CES2 gene and CPT-11 hydrolysis has also been investigated[81,83]. Among Japanese subjects, the CES2 variants rs72547531 and rs72547532 were associated with decreased CES2 activity and reduced CPT-11 hydrolysis activity *in vivo*[81]. Taking into account that both CES1 and CES2 are the major enzymes responsible for the hydrolysis of many clinically-used ester drugs, the SNPs of *CES* are therefore recognized as important pharmacogenetic regulators of xenobiotic ester metabolism and therefore treatment outcomes with these drugs. Functional analysis of the novel genetic polymorphisms of *CES* that can lead to clinically significant alterations in pharmacokinetics and drug responses of their substrates has become essential.

## 6. CES inhibitors

The key roles of CES in both human health and xenobiotic metabolism arouse great interest in the discovery of potent modulators to regulate enzyme expression in order to modulate endogenous metabolism or to improve patient responses to

**Table 2** Significant genetic variants of *CES* and the associated effects on drug metabolism[10,38,70,81,82].

| SNP | Drug | Function | |
|---|---|---|---|
| | | *In vitro* | *In vivo* |
| *CES1* rs2244613 | Dabigatran etexilate | - | Decrease in trough concentrations of dabigatran etexilate |
| *CES1* rs71647871 | Methylphenidate | Decrease the catalytic function of CES1 | Required lower doses of methylphenidate for symptom reduction |
| *CES1* rs71647871 | Oseltamivir | Decrease the catalytic function of CES1 | - |
| *CES1* rs71647871 | Clopidogrel | Decrease the catalytic function of CES1 | Significantly higher levels of active clopidogrel metabolite ($P = 0.001$) and better clopidogrel response |
| *CES1* rs121912777 | Oseltamivir | - | Increased oseltamivir AUC and 23% smaller carboxylatetooseltamivir AUC |
| *CES1* rs3785161 | Imidapril | - | The responder rate was significantly higher |
| *CES2* A139T | Aspirin | 40% Maximal decrease in CES2 functioning and, thus, decreased aspirin hydrolysis | - |
| *CES2* rs72547531 | Irinotecan | Associated with low *in vitro* expression and function of CES2 | Reduced *in vivo* CES2 activity in irinotecantreated patients |
| *CES2* rs72547532 | Irinotecan | Associated with low *in vitro* expression and function of CES2 | Reduced *in vivo* CES2 activity in irinotecantreated patients |

-Not assessed.

ester drugs. With this goal in mind, many small molecule inhibitors or inducers of CES have been identified with the specific intention of altering enzyme activity for therapeutic purposes.

### 6.1. Clinical drugs and pharmaceutical excipients

The crucial roles of CES in the metabolism of many ester-containing drugs suggest that some drugs might serve as CES inhibitors with the potential to cause significant drug–drug interactions[38,84,85]. Some antihyperlipidemic drugs, such as simvastatin and fenofibrate, could significantly inhibit the catalytic activities of CES[86]. It was reported that simvastatin was a potent inhibitor against imidapril hydrolysis in recombinant CES1 with the $K_i$ value of 0.11 μmol/L, while CES2-mediated irinotecan (CPT-11) hydrolysis could be strongly inhibited by both fenofibrate and simvastatin (Fig. 5)[72,87–91]. The antihypertensive drugs telmisartan and nitrendipine, displayed strong inhibitory effects on CES1 with the $K_i$ values of 1.69 and 1.24 μmol/L, respectively[87]. Carvedilol and diltiazem showed excellent inhibitory effects against CES2 with the $K_i$ values of 1.60 and 0.25 μmol/L, respectively[72]. Physostigmine, an anticholinesterase drug, was reported to be a strong CES2 inhibitor with the $K_i$ value of 0.20 μmol/L[88]. Loperamide was often used to treat CPT-11 associated diarrhea, and it was a potent and selective CES2 inhibitor ($IC_{50} = 1.5$ μmol/L)[72]. Pharmaceutical excipients are applied to obtained appropriate biopharmaceutical and physicochemical properties[89,90]. But this has been neglected as evidenced by the lack of mechanisms to evaluate excipient safety outside the new drug application process. Zhang et al.[91] found that sodium lauryl sulfate (SLS) and polyoxyl 40 hydrogenated castor oil (RH40) could significantly inhibit CES1-mediated imidapril hydrolysis, and Tween 20 could dramatically inhibit CES2-mediated CPT-11 hydrolysis. These results indicate that some pharmaceutical excipients, such as SLS, RH40 and Tween 20, may attenuate carboxylesterases activity, therefore such inhibitions should be regarded with some care during drug administration.

### 6.2. Natural products

Natural products have been an important source of potential drug leads and inspiration for medicinal chemists to develop more potent modulators for a given enzyme *via* efficient chemical modifications[92–94]. However, their use against molecular targets has diminished over the past two decades, due to the technical barriers to screening natural products[95]. Zou et al. [96,97] have collected a series of natural triterpenoids and characterized their inhibitory effects against CES using DME (D-luciferin methyl ester, a probe for CES1) and DDAB (6,8-dichloro-9,9-dimethyl-7-oxo-7,9-dihydroacridin-2-yl benzoate, a probe for CES2) as the specific substrates for high-throughput screening of inhibitors against CES1 and CES2, respectively. Two pentacyclic triterpenoids, ursolic acid (UA) and oleanolic acid (OA), exhibited strong inhibitory effects on CES1 (Fig. 6)[97–100]. By structural modifications on OA and UA, two derivatives including 3*β*-*O*-(*β*-carboxy-propionyl)-urs-12-en-28-oic acid and 3*β*-*O*-(*β*-carboxypropionyl)-olean-12-en-28-oic acid were obtained, which displayed very strong inhibitory effects against CES1 ($K_i$ value as 0.012 μmol/L and 0.017 μmol/L, respectively) and high selectivity over CES2 (6919-fold and 3296-fold against CES2, respectively). Guided by the structure-CES2 inhibition relationships of a series of glycyrrhetinic acid (GA) derivatives, Zou et al.[98] designed and developed a novel compound 3-*O*-(*β*-carboxypropionyl)-11-deoxo-glycyrrhetinic acid-30-ethyl ester as the most potent inhibitor against CES2 ($IC_{50}$ = 20 nmol/L). This compound showed high selectivity over CES1 (>1000-fold), which is 3463-fold more potent than the parent compound GA. Recently, 22 protostane triterpenoids have been isolated from the rhizome of *Alismaorientale*[99]. Among them, five could potently inhibit CES2, with $IC_{50}$ values less than 10 μmol/L. The inhibition kinetics demonstrated that alismanol F could inhibit the CES2-catalyzed 4-benzoyl-*N*-butyl-1,8-naphthalimide hydrolysis with the $K_i$ value of 1.76 μmol/L *via* mixed inhibition. Zhang et al.[100] investigated the inhibitory effects of 22 protostane triterpenoids including 10 new protostane-type triterpenoids from the phytochemical investigation of *A. orientalis*, on CES2. Among them, five compounds,

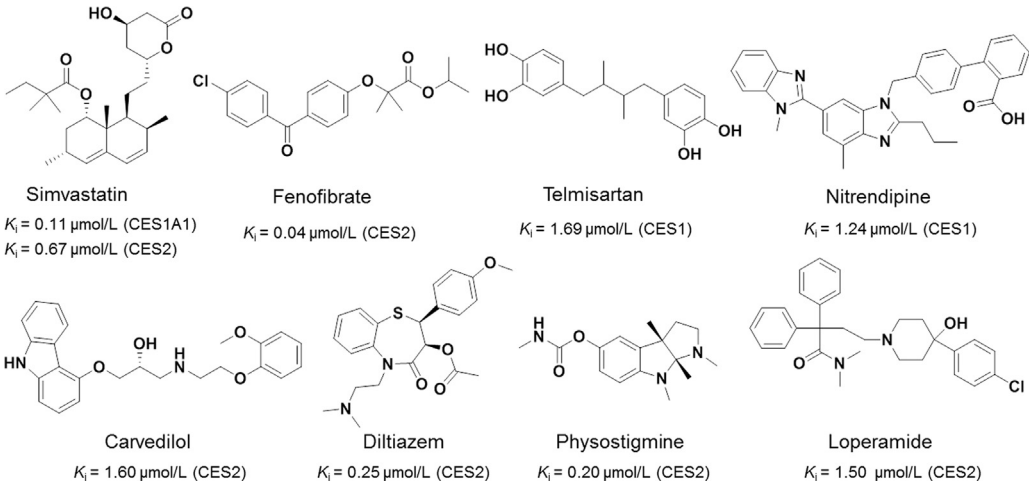


**Figure 5** Clinical drugs as inhibitors of CES.

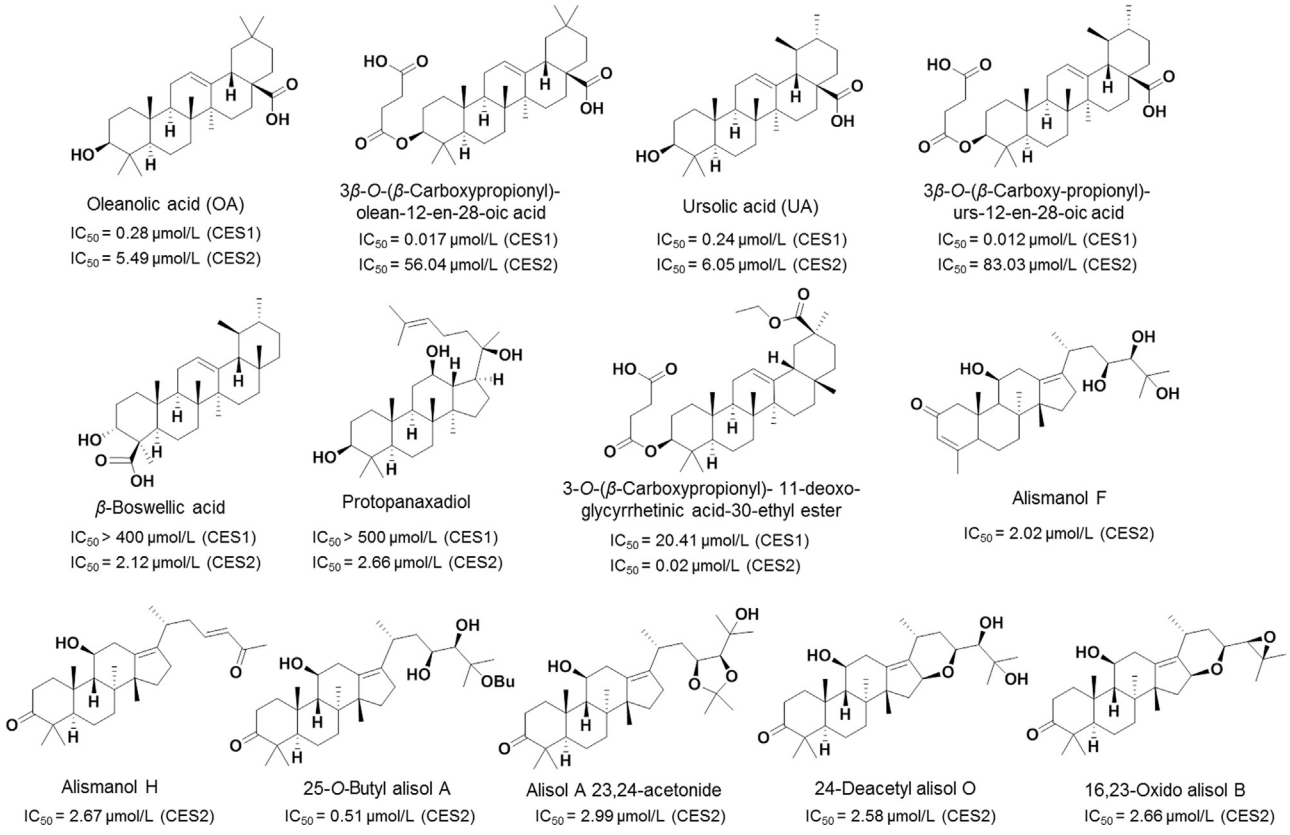


**Figure 6** Triterpenoids as inhibitors of CES.

including alismanol H, 25-*O*-butyl alisol A, alisol A 23,24-acetonide, 24-deacetyl alisol O, 16,23-oxido alisol B displayed strong inhibitofry effects on CES2, with the $IC_{50}$ values less than 3.0 μmol/L (Fig. 6)[97–101].

Flavonoids are a large group of polyphenolic products widely distributed in vegetables, fruits, and beverages such as wine and tea[102,103]. Recent research has revealed that some natural flavonoids are strong inhibitor against both CES1 and CES2 (Fig. 7)[98–100]. Bavachinin and corylin significantly inhibited the CES1-mediated BMBT hydrolysis with low $K_i$ values as 0.5 μmol/L and 0.7 μmol/L, respectively, while corylifol A found in Fructus Psoraleae (also named Bu-gui-shi), is a potent inhibitor against CES2 with the $K_i$ value of 0.62 μmol/L[100,101,104–106]. More recently, three major constituent from the root-bark of white mulberry (also named Sang-bai-pi) including sanggenone D, kuwanon G, and sanggenone C, could strongly inhibit CES2-mediated FD hydrolysis in HLM *via* non-competitive manner[107]. Furthermore, some naturally occurring fatty acids displayed potential inhibitory effects on the hydrolytic activities of CES1[17]. In contrast to saturated fatty acids, unsaturated fatty acids displayed more potent inhibitory effects on CES1, and arachidonic acid demonstrated strong inhibitory effects on CES1 with the $K_i$ value of 1.7 μmol/L. 27-Hydroxycholesterol (27-HC), an oxidized form of cholesterol, also showed promising inhibitory activity against CES1 and high selectivity over CES2[17]. Further investigation on the inhibitory behavior of

27-HC demonstrated that 27-HC functioned as a noncompetitive inhibitor against CES1, with the very low $K_i$ value (10 nmol/L).

Bakuchiol, a natural phenolic compound isolated from *Fructus Psoraleae*, displayed strong inhibitory effects against CES2[101]. The $K_i$ value of bakuchiol against CES2-mediated FD hydrolysis is 2.12 μmol/L and the inhibition type was non-competitive inhibition. Bysspectin A, a polyketide-derived octaketide dimer, displayed selective inhibitory effect on CES2-mediated FD hydrolysis[108]. Further investigation suggested that bysspectin A functioned as a competitive inhibitor against CES2, and the *O*-atom of the C-3′ phenolic or the *O*-atom at the funan ring could strongly interact with the Ser-288 (the key amino acid of the catalytic triad) of CES2 *via* hydrogen bonding. Hatfield et al.[109,110] found that *Salvia miltiorrhiza* root extracts demonstrated strong inhibitory effects on CES, due to the presence of tanshinones. These bioactive compounds have been found to be potent inhibitors of both CES1 and CES2, while most of these tanshinone-type compounds could inhibit neither human acetylcholinesterase (AchE) nor human butyrylcholinesterase (BchE). Furthermore, both tanshinones and *S. miltiorrhiza* root extracts could inhibit the hydrolysis of CPT-11 by the cell-based assays[109,110].

### 6.3. *Other compounds*

1,2-Diones including benzils, alkyl-1,2-diones, isatins, and 1,2-quinones (Fig. 8)[109–111] have been identified as the most important chemical compounds for CEs inhibition with $K_i$ values in the nanomolar range, which demonstrate potent and selective inhibitory effects toward CES1 or CES2; these agents do not exhibit inhibitory effects on human AchE and BchE. Benzene sulfonamides usually displayed potent inhibitory effects against CES2 and relative high selectivity over CES1, and had no inhibitory effects on either human AchE and BchE[24,112]. The SAR analysis of benzene sulfonamides revealed that the relative hydrophobicity of these sulfonamides is an important factor affecting the inhibitory potency to CES2. Trifluoroketones were generally inhibitors to CES with $K_i$ values at the nanomolar range, and mostly demonstrated poor specificity toward CES1 or CES2, but these compounds displayed weak inhibition towards human AchE and BchE[113,114]. Clopidogrel acyl-β-D-glucuronide, the phase-II metabolites of clopidogrel carboxylic acid by uridine diphosphate glucuronosyltransferases (UGTs), could inhibit CES1-mediated 4-nitrophenyl acetate hydrolysis with the $K_i$ values of 4.32 μmol/L, but did not significantly inhibit CES2[115,116]. Pyrethroids are popular household insecticides for their relatively low toxicity to mammals in contrast to organophosphorus insecticides[117,118]. Recently, Lei et al.[119] found that six commonly used pyrethroids showed moderate inhibitory effects on CES. Among them, deltamethrin demonstrated strong inhibitory effects toward CES1 with the $IC_{50}$ value of 2.39 μmol/L. Further investigation demonstrated that deltamethrin was a competitive inhibitor toward CES1-mediated BMBT hydrolysis, but acted as a noncompetitive inhibitor against CES1-mediated DME or DMCB hydrolysis in HLM.

## 7. CES inactivators

Carbamate compounds were developed as pharmaceutical agents specifically targeting members of the serine hydrolase superfamily *via* covalent binding and modification of serine at the active site[120] These compounds, as potent inhibitors of AchE, have been widely used for the pest control in domestic animals and agriculture. However, several cholinesterase inhibitors containing the carbamate moiety, such as JZL184 and phenethylcymserine (Fig. 9)[120–129],

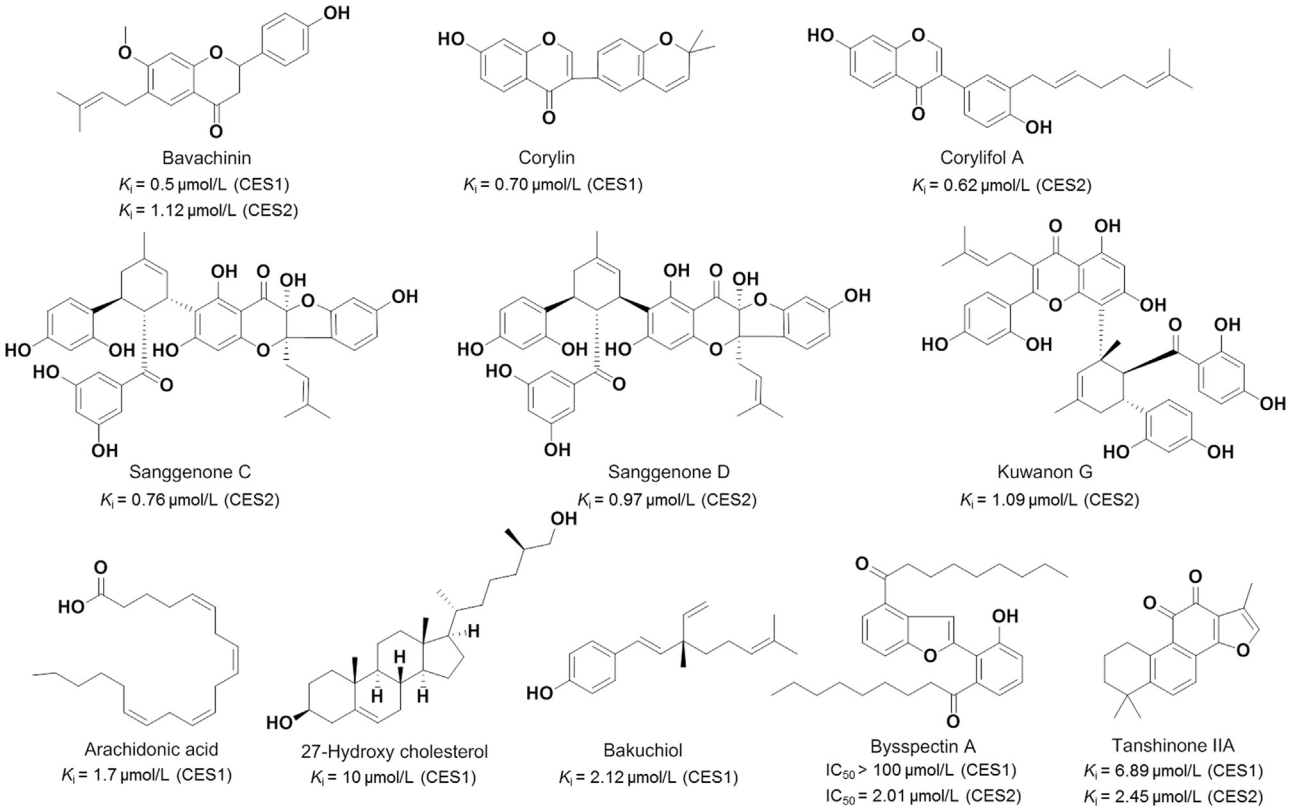


**Figure 7** Natural products as inhibitors of CES.

were found to be CES inhibitors[120–122]. But all these compounds displayed poor isoform selectivity towards various CES. Organophosphate (OP) insecticides are inhibitors to AChE, which exert their toxicity through the termination of nerve impulses by metabolism of the neurotransmitter acetylcholine[123,124]. A number of serine hydrolases including cholinesterases and carboxylesterases (CES1 and CES2) could be significantly inhibited following exposure to OPs[125,126]. OPs could react with CES and generate a stable phosphate ester that is covalently linked to the catalytic residue (such as Ser-221 of CES1) of CES. Several OPs, including chlorpyrifos oxon, paraoxon, and bis(4-nitrophenyl)phosphate (BNPP), are potent irreversible inhibitors of CES with $IC_{50}$ values at the nanomolar level[127–129].

## 8. CES inducers

Over the past two decades, most researchers have focused on the discovery of CES inhibitors. In contrast, only a few studies were conducted to explore the regulation of CES expression. Recent studies have reported the relevance of CES to some metabolic disorders (such as diabetes and obesity), indicating these enzymes might be potential targets for treatment of these metabolic diseases. Especially, the key roles of CES1 and CES2 in lipid metabolism in which the protein expression in liver and adipose tissues are strongly related with several human diseases such as non-alcoholic steatohepatitis, and obesity[14]. Thus, it is necessary to discover more potent CES modulators to regulate the expression or function of CES, and then to modulate endogenous metabolism or to improve the therapeutic effect of patients administrated with ester drugs.

Generally, microsomal enzyme inducers (MEIs) exert their effects on target genes through constitutive androstane receptor (CAR), pregnane X receptor (PXR), nuclear factor erythroid 2-related factor 2 (Nrf2), and peroxisome proliferator-activated receptors (PPARs)[130–135]. Recent studies have demonstrated that the expression of mammalian CES could be regulated *via* activation of CAR, AhR, PPARs, LXR, PXR, hepatocyte nuclear factor-4$\alpha$ (HNF-4$\alpha$), and/or Nrf2 transcriptional pathways[130–132]. It is worth noting that most of these investigations focus on the transcriptional regulation of rodent *Ces* genes. A few studies have indicated Nrf2 can be activated by MEIs and induced CES1 in human tumour cells[133]. Moreover, a PXR-activating agent, rifampicin, caused moderate induction of both CES1 and CES2 gene expression in human hepatocytes[134]. Future investigations should be conducted to explore whether these signalling pathways associated with CES expression in rodent animals are conserved in humans.

As depicted in Fig. 10[132–139] and (Supplementary Information Table S3)[133,136], some endogenous and exogenous substances have been confirmed with regulatory effects on the expression of mammalian CES. Treatment of mice with glucose could induce hepatic CE1 expression *in vivo*, due to glucose significantly activated the promoter activity of CES1 and increased acetylation of histone 3 and 4 in the CES1 chromatin[137]. Xu et al.[130] suggested that cholic acid or FXR agonist induced the expression of hepatic CES1, then reduced the levels of plasma cholesterol, hepatic TG, and plasma TG. CES1 could also be highly induced by sensitizers and antioxidants in several cell lines. Recently, Chen et al.[133] reported that sensitizer trinitrobenzene sulfonate (TNBS) and antioxidant sulforaphane induce CES1 through a novel element, nuclear factor-E2 related factor-2, in primary hepatocytes and cell lines (human fibrosarcoma cell line HT1080 and Huh7). Another report showed that NO1886 (Ibrolipim), a lipoprotein lipase-promoting agent, could slightly induce CES1 and CES2 in primary cultures of cryopreserved human hepatocytes[136]. In addition, urethane dimethacrylate (UDMA) was proved to induce the mRNA expression of *CES2* in human dental pulp cells, without regulating the *CES1* or *CES3* mRNA expression[138]. In addition, gambogic acid decreased the protein expression of CES1 and CES2 *via* a dose-dependent manner, and the hydrolytic activities of CES1 and CES2 were also significantly decreased upon addition of gambogic acid[139].

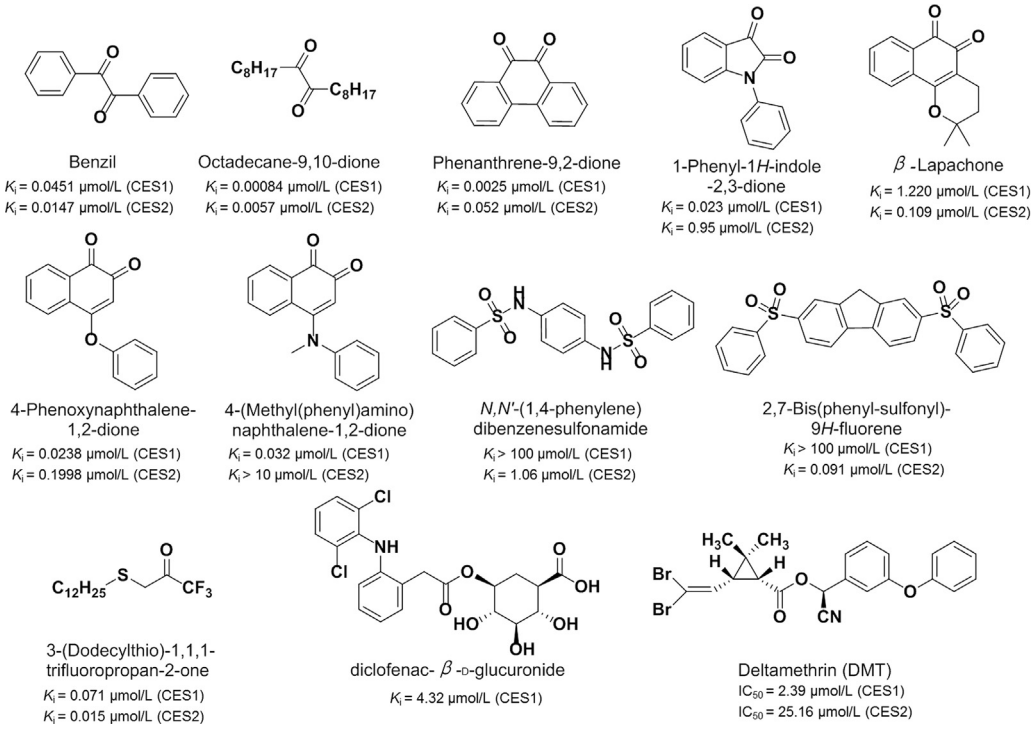


**Figure 8** Inhibitors of CES.

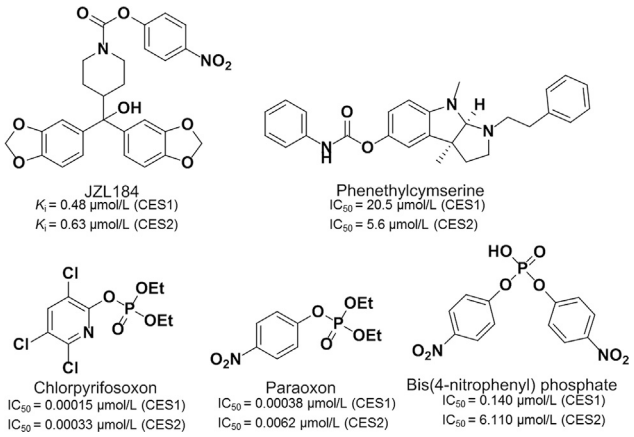


**Figure 9** Inactivators of CES.

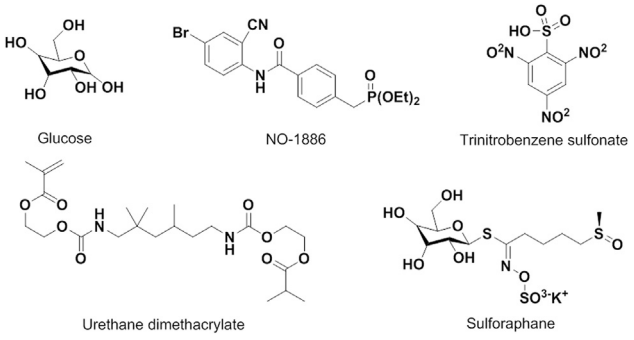


**Figure 10** Inducers of CES.

## 9. Summary

Over the past twenty years, the molecular properties, substrate specificities and the biological roles of human carboxylesterases (including CES1 and CES2) in both endo- and xenobiotic metabolism have been extensively studied. Recent studies have suggested that CES participate in the hydrolysis of a vast number of endogenous esters and thus serve as therapeutic targets for the treatment of a variety of human metabolic disorders. The importance of CES in both human health and xenobiotic metabolism arouse great interest in the discovery of potent CES modulators. The development of new optical substrates for CES1 and CES2 has made the screening of CES modulators more convenient and efficient. Although many CES inhibitors with various scaffolds have been reported, their ability to target intracellular CES combined with their *in vivo* efficacy and safety profiles have not been well-studied. In contrast to the structurally diverse CES inhibitors, the inducers of CES are rarely reported and most studies focus on the transcriptional regulation of rodent *Ces* genes. Thus, it is necessary to find and develop more potent CES inducers by using cell-based assays. In addition, more in-depth investigations on the physiological functions of CES, the relevance of CES to human diseases, the species differences between human CES and other mammalian CES, as well as the interactions between CES and ligands, should be conducted in near future. These studies will be very helpful for revealing the crucial roles of CES in human health and diseases, as well as for the discovery and development of CES modulators with potential biomedical applications.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apsb.2018.05.005.

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