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COMMENTARY

Advances in selective targeting of serine hydrolases: A targeted covalent approach against hCES2A mitigates irinotecan toxicity *in vivo*



KEY WORDS

Human carboxylesterase 2 (hCES2A);
Structure-based drug design (SBDD);
Donepezil derivatives;
Structure–activity relationship (SAR)

The serine hydrolase (SH) superfamily, one of the largest enzyme groups in mammals with over 200 members, is characterized by a serine-containing catalytic triad within its active site¹. These enzymes hydrolyze amide and/or ester bonds through a nucleophilic attack mediated by the catalytic serine residue. SHs are expressed across various mammalian tissues and play critical roles in diverse physiological and pathological processes, including the regulation of lipid signaling, maintenance of metabolic homeostasis, and drug activation and detoxification. Aberrant SH activity has been implicated in numerous diseases, such as metabolic syndromes, neurodegeneration, cancer, and drug-induced organ injury, making these enzymes attractive targets for therapeutic intervention. Indeed, many approved drugs function through the inhibition of key SHs (*e.g.*, acetylcholinesterase inhibitors for Alzheimer's disease; thrombin inhibitors for thrombosis, etc.)². Nonetheless, developing potent and specific SH inhibitors with favorable drug-like properties remains a major challenge, primarily due to the high degree of active site homology across this superfamily.

In this issue, Ge et al.³ present a comprehensive research framework that led to the successful development of drug-like targeted covalent inhibitors against a specific intestinal serine hydrolase-human carboxylesterase 2A (hCES2A). Predominantly expressed in epithelial cells of the small intestine and colon, hCES2A metabolizes endogenous esters, as well as ester-containing drugs and environmental toxins^{4–6}. Increasing evidence indicates that hCES2A plays a key role in first-pass drug metabolism, which can result in the premature formation of toxic metabolites, as exemplified by the anticancer prodrug irinotecan, used in the treatment of various solid tumors. Specifically, SN-38, a highly toxic metabolite of irinotecan, can accumulate in the intestinal tract and induce severe diarrhea^{7,8}. Hence, target inhibition of hCES2A represents a viable strategy to ameliorate irinotecan-triggered gastrointestinal toxicity (ITGT)^{9,10}.

Ge and colleagues addressed this challenge by performing an *in silico* high-throughput screening of 2000 FDA-approved drugs to identify drug-like ligands of hCES2A, followed by *in vitro* validation. Through multiple rounds of structure-based drug design and optimization, the authors progressed from the initial hit, donepezil, to develop a highly potent covalent hCES2A inhibitor, **C3**. Comprehensive pharmacological characterization of **C3** demonstrated anti-ITGT efficacy across multiple *in vivo* models, along with favorable drug-like properties. Thorough characterization of the pharmacological effects of **C3** was performed, showing anti-ITGT activity in different *in vivo* models of the gut, coupled with desirable drug-like properties. Collectively, this work demonstrates a structure-guided paradigm for precision drug discovery *via* targeted covalent inhibition, providing a scalable blueprint for targeting other pharmacologically relevant SHs.

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Both compound C3 and two additional hCES2A covalent inhibitors recently reported by the same team^{9,10} feature a carbamate warhead, introduced through modification of a phenolic group on the lead compound to enable covalent engagement of the catalytic serine. These carbamate-based inhibitors represent a class of substrate-mimicking molecules that readily access the enzyme's catalytic cavity and are recognized as native substrates. Following nucleophilic attack by the catalytic serine on the carbamate group, the alcohol moiety is released, resulting in permanent inactivation of hCES2A *via* carbamylation of the catalytic serine. Interestingly, an additional serine residue within the active site was also found to be susceptible to carbamylation by C3.

This mechanism cleverly co-opts the enzyme's catalytic machinery to modify the nucleophilic serine *via* a mild electrophile, while leveraging substrate-like interactions specific to the architecture of the hCES2A active site to achieve selectivity. This approach effectively reduces off-target activity against other homologous SHs, such as hCES1A, BuChE, and AChE, which were also tested in this study. Overall, these findings establish a broadly applicable framework that can be adapted for targeting other pharmacologically important enzymes with tailored covalent inhibitors in the future.

References

- Hedstrom L. Serine protease mechanism and specificity. *Chem Rev* 2002;**102**:4501–24.
- Bachovchin DA, Cravatt BF. The pharmacological landscape and therapeutic potential of serine hydrolases. *Nat Rev Drug Discov* 2012; **11**:52–68.
- Zhang Y, Fan YF, Song YQ, Zhu GH, Li XJ, Huang J, et al. Discovery of orally active and serine-targeting covalent inhibitors against hCES2A for ameliorating irinotecan-triggered gut toxicity. *Acta Pharm Sin B* 2025;**15**:5312–26.
- Wang DD, Zou LW, Jin Q, Hou J, Ge GB, Yang L. Human carboxylesterases: a comprehensive review. *Acta Pharm Sin B* 2018;**8**: 699–712.
- Jin Q, Song LL, Ding LL, Zhang J, Wang DD, Song YQ, et al. High-throughput optical assays for sensing serine hydrolases in living systems and their applications. *Trends Analyt Chem* 2022;**152**:116620.
- Sanghani SP, Sanghani PC, Schiel MA, Bosron WF. Human carboxylesterases: an update on CES1, CES2 and CES3. *Protein Pept Lett* 2009;**16**:1207–14.
- Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G, et al. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 2001;**7**:2182–94.
- Gao YQ, Li W, Chen JQ, Wang X, Lv YT, Huang Y, et al. Pharmacometabolomic prediction of individual differences of gastrointestinal toxicity complicating myelosuppression in rats induced by irinotecan. *Acta Pharm Sin B* 2019;**9**:157–66.
- Sang ZP, Zhang Y, Fan YF, Luan CH, Liu ZW, Zhang QY, et al. AI-driven discovery of highly specific and efficacious hCES2A inhibitors for ameliorating irinotecan-triggered gut toxicity. *J Med Chem* 2025; **68**:6252–69.
- Hu DY, Zeng HR, Li WX, Zhang Y, Chi XQ, Liu XY, et al. Discovery of a novel serine-targeting covalent inhibitor against hCES2A for treating drug-induced diarrhea and ulcerative colitis. *J Med Chem* 2025;**68**:11663–78.

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