BJPS

Brazilian Journal of Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e21394

Article

Identification of *Escherichia coli* β-glucuronidase inhibitors from *Polygonum cuspidatum* Siebold & Zucc.

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Gut bacterial *β*-glucuronidase (GUS) can reactivate xenobiotics that exert enterohepatic circulation-triggered gastrointestinal tract toxicity. GUS inhibitors can alleviate drug-induced enteropathy and improve treatment outcomes. We evaluated the inhibitory effect of *Polygonum cuspidatum* Siebold & Zucc. and its major constituents against *Escherichia coli* GUS (EcGUS), and characterized the inhibitory mechanism of each of the components. *T*rans-resveratrol 4'-O-β-D-glucopyranoside (**HZ-1**) and (-)-epicatechin gallate (**HZ-2**) isolated from *P. cuspidatum* were identified as the key components and potent inhibitors. These two components displayed strong to moderate inhibitory effects on EcGUS, with K_i values of 9.95 and 1.95 μM, respectively. Results from molecular docking indicated that **HZ-1** and **HZ-2** could interact with the key residues Asp163, Ser360, Ile 363, Glu413, Glu504, and Lys 568 of EcGUS via hydrogen bonding. Our findings demonstrate the inhibitory effect of *P. cuspidatum* and its two components on EcGUS, which supported the further evaluation and development of *P. cuspidatum* and its two active components as novel candidates for alleviating drug-induced damage in the mammalian gut.

Keywords: β-glucuronidase. *Polygonum cuspidatum* Siebold & Zucc.. Trans-resveratrol 4'-O-β-D-glucopyranoside. (-)-Epicatechin gallate. Inhibitory mechanism.

INTRODUCTION

Gut bacterial β-glucuronidase (GUS) is an acid glycoside hydrolase residing in the gastrointestinal tract, that catalyzes the hydrolysis of glucuronide conjugates and produces the corresponding aglycone (Pellock, Redinbo, 2017; Pollet *et al.*, 2017; Wang *et al.*, 2019). The released aglycone can be absorbed and accumulate in the intestinal tract, causing dose-limited gastrointestinal toxicities exemplified by the chemotherapy drugs (Clarke *et al.*, 2019; Ervin *et al.*, 2019). Irinotecan (CPT-11), an anti-cancer drug mainly used to treat colorectal cancer, can lead to severe delayed diarrhea and neutropenia, limiting its clinical applications (Bailly, 2019; Hahn *et al.*, 2019; Shi *et al.*, 2021). CPT-11 is hydrolyzed *in vivo* by carboxylesterase in the liver to its active as well as toxic metabolite SN-38 (Hicks *et al.*, 2009; Tobin *et al.*, 2006). After exerting its antitumor effect, SN-38 is primarily metabolized in the liver by uridine diphosphate glucuronyltransferase 1A1 to its inactive form SN-38-glucuronide (SN-38G), which is then excreted through the urine and bile (Iyer *et al.*, 1998). Nevertheless, the highly expressed GUS in the gut can hydrolyze SN-38G to SN-38, leading to an excessive SN-38 present in the gut that

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can cause delayed diarrhea and neutropenia (Jariwala *et al*., 2020). Recently, GUS inhibitors have been shown to alleviate the gastrointestinal toxicity of CPT-11 and other anticancer drugs (Awolade *et al*., 2020; Bhatt *et al*., 2020; Chamseddine *et al*., 2019; Wallace *et al*., 2010).

Based on their source, existing GUS inhibitors can be divided into mainly two types including synthetic and natural inhibitors (Awolade et al., 2020; Cheng *et al*., 2017; Zhou *et al*., 2020). Natural products-based inhibitors of GUS have attracted considerable attention owing to their physiological tolerance, satisfying safety and favorable pharmacodynamic profiles (Li, 2020; Sun*, et al*., 2020; Weng *et al*., 2017; Zhong *et al*., 2020). Therefore, it is important to discover novel GUS inhibitors with improved efficacy and safety to alleviate the side effects caused by CPT-11 and anti-cancer drugs as well as assist the cancer treatment. *Polygonum cuspidatum* Siebold & Zucc.*,* known as Huzhang in China, is often used to treat inflammation, damp-heat jaundice, rheumatoid or rheumatoid arthritis and other diseases (Bralley *et al*., 2008; Hu *et al*., 2018; Liu *et al*., 2014; S. Liu *et al*., 2016; Lu *et al*., 2012). Despite extensive pharmacological studies, there are currently no reports investigating the interactions between *P. cuspidatum* and the gut microbiota. In a preliminary experiment, *P. cuspidatum* displayed strong inhibitory effects on gut bacterial GUS, which elicited our interest in exploring the main components responsible for its inhibitory effects.

The study aimed to identify the main components of the ethanolic extract of *P. cuspidatum* as it exhibited significant inhibitory effects on *Escherichia coli* GUS (EcGUS), and characterize the inhibitory mechanism and determine the inhibition constant of each inhibitory component. The chemical fingerprint of *P. cuspidatum* and EcGUS inhibition profile were combined to identify the components responsible for the inhibitory effect. Inhibition kinetic assays were performed to characterize the inhibitory behavior and obtain the kinetic constants (IC_{50}, K_i) of the components that were active against EcGUS. Furthermore, molecular docking was carried out for evaluating the potential molecular determinants responsible for the potent inhibitory effects of the identified components of *P. cuspidatum* towards EcGUS.

MATERIAL AND METHODS

Chemicals and reagents

Dried rhizomes of *P. cuspidatum* were purchased from Beijing Tongrentang Co., Ltd. (Dalian, China) in January 2020, and identified by Prof. Jing-Ming Jia (Shenyang Pharmaceutical University). A voucher specimen (MO202001) has been deposited in the Department of Medicinal Chemistry, Dalian Medical University. DDAO was chemically synthesized and DDAOG was biosynthesized in our laboratory as reported previously (Feng *et al*., 2018). GUS from *E. coli* was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical or HPLC grade.

Preparation of *P. cuspidatum* ethanol extract

The dried plant materials (500 g) were crushed, powered, and then extracted by 95% ethanol (100 mL) for three times in total 90 min. *P. cuspidatum* ethanol extract was afforded after removing the solvent. Subsequently, the extract (5 mg) was suspended in DMSO (100 μL), and stored at 4 °C.

Bioactivity-guided isolation and identification of active compounds of *P. cuspidatum*

Chemical fingerprinting and fraction collection were performed using a Waters Prominence HPLC system, equipped with a Waters 2767 sample manager, Waters 2545 binary gradient module, and Waters 2489 UV/visible detector. A Waters XBridge C18 (19 mm × 250 mm, 5 μm) chromatographic column was used. The mobile phase consisted of methanol (A) and water (B), and the following gradient condition was used: 0.0-10.0 min, 20% B; 10.0-15.0 min, 20%-40% B; 15.0-45.00 min, 40%-100% B; 45.0-46.0 min, 100% B; 46.0-60.0 min, 100% B; 60.0-65.0 min, 100%-20% B; 65.0-80.0 min, 20% B. The flow rate was set to 10 mL/min and the injection volume of the crude extract (50 mg/mL) was 300 μL. The effluent was monitored at 270 and 220 nm with LC-UV detection. After establishing the

analytical method, the fractions were automatically collected into sample tubes based on chromatographic peaks or retention time. After rotary evaporation, all dried fractions were weighed and used subsequently for EcGUS inhibition assays. The fractions displaying potent inhibitory effects on EcGUS were further isolated and purified using semi-preparative high-performance liquid chromatography (HPLC) (Singh *et al.*, 2020). Finally, the structures of the purified compounds were elucidated using nuclear magnetic resonance spectroscopy.

DDAOG hydrolysis-based inhibition assays

The inhibitory effects of *P. cuspidatum* extract and its constituents on EcGUS were investigated using DDAOG as a specific fluorescent probe for EcGUS (Feng *et al.,* 2018). The incubation system consisted of PBS buffer (pH 6.5, 100 mM), EcGUS (0.2 U/mL), DDAOG (10 μM for inhibition screening; 5-40 μM to determine the inhibition constants) in the presence or absence of the inhibitor. Each reaction was started by the addition of 10 μL DDAOG. After incubation at 37 °C for 30 min, the reaction was terminated by addition of an equal volume of ice cold acetonitrile. Then, 200 μL aliquots of the supernatants were diverted into a 96-well plate for fluorescence detection, at excitation and emission wavelengths of 600 and 660 nm, respectively.

Molecular Docking

The 3D crystal structure of EcGUS (PDB code: 3K4D) was downloaded from Protein Data Bank (http://www.rcsb.org/, code: 3K4D). PyMOL 2.4 was used to add non-polar hydrogen and remove water molecules. The cavity of EcGUS was set based on the site of the intrinsic ligand in 3D crystal structure of EcGUS, and the interactions of **HZ-1** and **HZ-2** with EcGUS were analyzed by AutoDock 4.2 with default parameters.

Data analysis

IC_{50} (half maximal inhibitory concentration) and K_i values were calculated by the nonlinear regression analysis of Graphpad Prism 7.0 (San Diego, CA, USA) (He *et al.*, 2020; Song *et al.*, 2019; Sun *et al.*, 2020). The inhibition kinetic types including competitive inhibition, noncompetitive inhibition, uncompetitive type, or mixed inhibition were determined based on the goodness-of-fit parameters (Hou *et al.*, 2020; Yi *et al.*, 2019; Zhang *et al.*, 2018).

RESULTS AND DISCUSSION

Inhibitory effects of *P. cuspidatum* extract on DDAOG hydrolysis

As shown in Figure 1a, at 10 μg/mL ethanolic extract of *P. cuspidatum*, the residual activity of DDAOG hydrolysis was markedly reduced to be less than 5% of the negative control. Moreover, the crude ethanolic extract of *P. cuspidatum* inhibited EcGUS-mediated DDAOG hydrolysis in a dose-dependent manner with an IC_{50} value as low as 0.79 μg/mL, as depicted in Figure 1b. These results demonstrate that the ethanolic extract of *P. cuspidatum* exhibited a strong inhibitory effect on EcGUS, indicating that the herb may contain potent inhibitors of EcGUS.

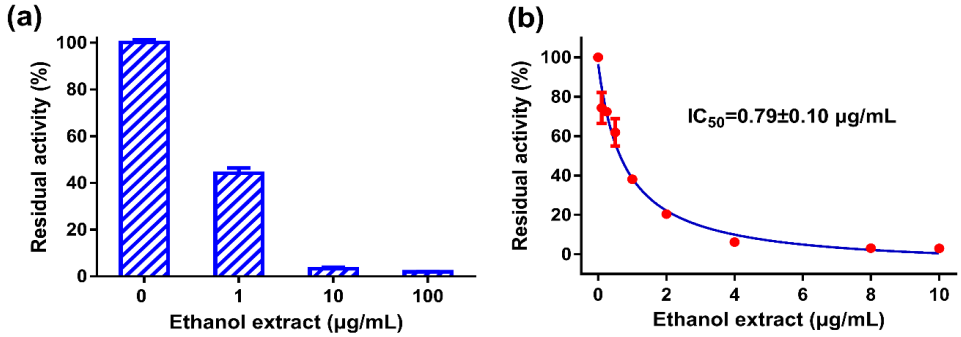

FIGURE 1 - (a) Inhibitory effects of *P. cuspidatum* ethanol extract (0, 1, 10, 100 μg/mL) on EcGUS-mediated DDAOG hydrolysis, and (b) the corresponding dose-dependent inhibition curve of different concentrations of *P. cuspidatum* ethanol extract against EcGUS-mediated DDAOG hydrolysis. All data represent the mean of triplicate determinations.

Identification of the major EcGUS inhibitors in *P. cuspidatum*

After establishing the chemical fingerprint of *P. cuspidatum*, the 20 eluted fractions were automatically collected into sample tubes and evaporated to dryness (Figure 2a). Then, the dried fractions were evaluated for their inhibitory effects on EcGUS based on the high-throughput screening method. As shown in Figure 2b, two fractions (No. 4 and 5) significantly inhibited EcGUS with the residual activities less than 25%, while the other fractions displayed moderate or negligible inhibitory effect on EcGUS. Subsequently, fractions 4 and 5 were isolated using preparative HPLC, and two compounds were purified and identified as trans-resveratrol 4'-O-β-D-glucopyranoside (**HZ-1**) and (-)-epicatechin gallate (**HZ-2**) with purity > 95% (Figure S1-S4).

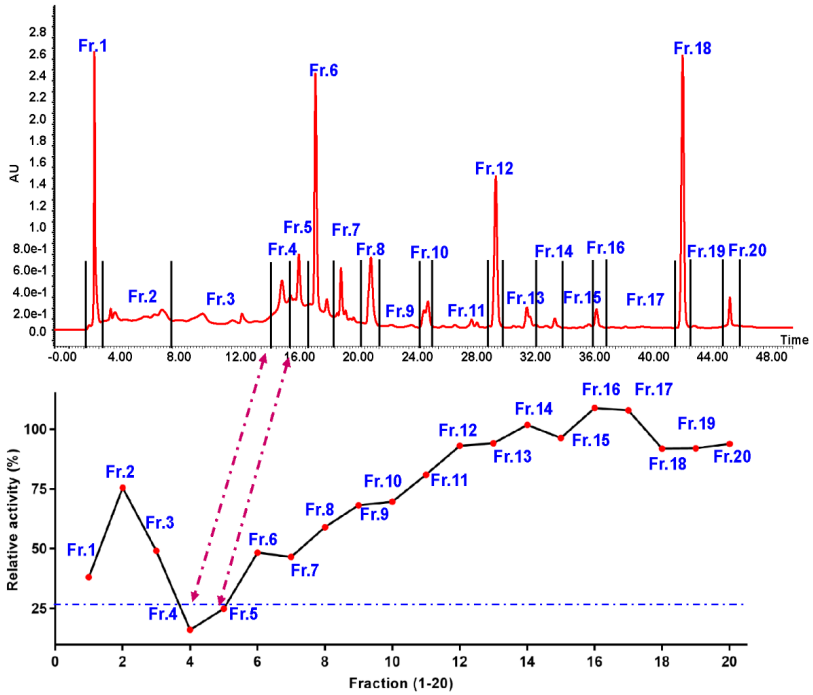

FIGURE 2 - (a) HPLC-UV fingerprint of *P. cuspidatum* ethanol extract (50 mg/mL) monitored at 270 nm, Fr.1-Fr.20 represent eluted fraction numbered from 1 to 20, and (b) the corresponding EcGUS inhibition profile of each eluted fraction (2 μg/mL) towards EcGUS-mediated DDAOG hydrolysis. Note: trans-resveratrol 4'-O-β-D-glucopyranoside (**HZ-1**) and (-)-Epicatechin gallate (**HZ-2**) were purified from Fr. 4 and Fr. 5, respectively.

Inhibitory effects of the two constituents isolated from *P. cuspidatum* against EcGUS

In order to further validate and explore the inhibitory effects of the two isolated constituents against EcGUS, the preliminarily screening experiments were performed. The residual activities of EcGUS-mediated DDAOG hydrolysis were reduced to be 65.7% and 16.9% of the negative control, respectively, when 10 μM **HZ-1** or **HZ-2** were used. (Figure 3a and 3c). Moreover, their dose-dependent inhibition curves against EcGUS were also depicted. As shown in Figures 3b and 3d, **HZ-1** and **HZ-2** displayed evident concentration-dependent inhibition of EcGUS mediated DDAOG hydrolysis. The IC_{50} values of **HZ-1** and **HZ-2** against EcGUS-mediated DDAOG hydrolysis were evaluated to be 25.88 μM and 2.24 μM, respectively (Table I). These results demonstrated that both **HZ-1** and **HZ-2** exhibited inhibitory effects towards EcGUS, among which, **HZ-2** was a more potent inhibitor of EcGUS. Notably, the IC_{50} of **HZ-2** determined in our study was in agreement with that reported previously (Feng et al., 2018), wherein it was identified to be one of the main constituents of *Rheum palmatum L* responsible for the strong inhibitions of EcGUS.

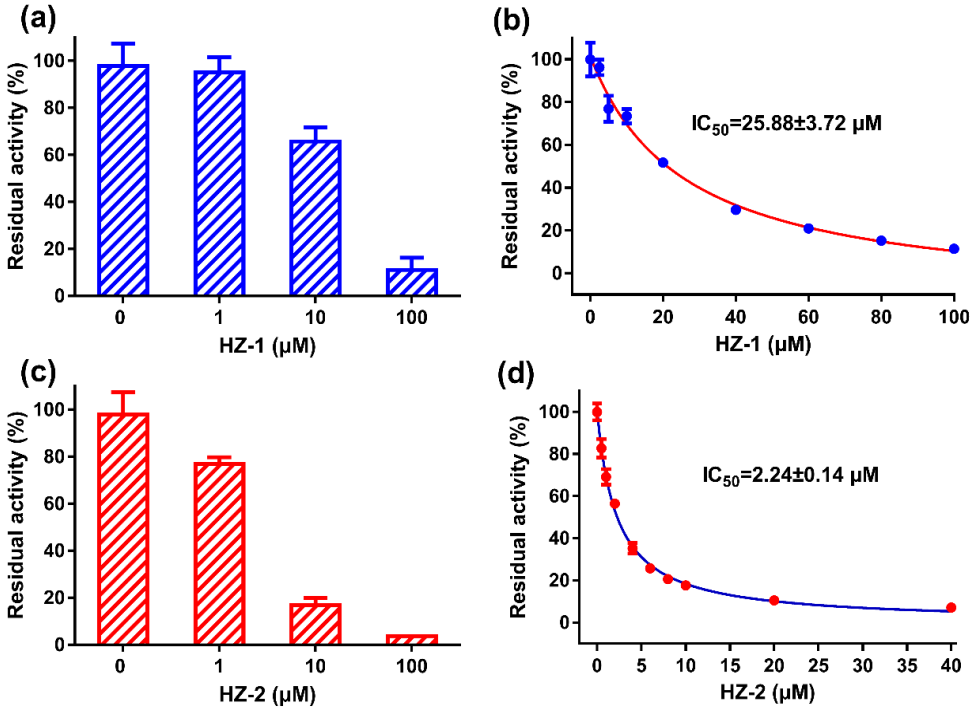

FIGURE 3 - (a) Inhibitory effects of **HZ-1** and **HZ-2** at the concentrations of 0, 1, 10, 100 μg/mL on EcGUS-mediated DDAOG hydrolysis, and (b) the concentration dependent inhibition of EcGUS-catalyzed DDAOG hydrolysis by **HZ-1** and **HZ-2**. Each data point corresponds to the average values calculated from triplicate measurements.

TABLE I - IC_{50} and K_i values of **HZ-1** and **HZ-2** against GUS

Compound name	IC_{50} (μM)	K_i (μM)	Inhibition type
HZ-1	25.88	9.95	Mixed
HZ-2	2.24	1.95	Mixed

Inhibition kinetics of the two identified constituents against EcGUS

Inhibition kinetics experiments were further carried out to calculate the K_i values of the identified constituents for EcGUS and to characterize their corresponding inhibition behaviors. As shown in Figure 4a-4f, both

Lineweaver-Burk and Dixon plots indicated that **HZ-1** and **HZ-2** followed the mixed inhibition behavior against EcGUS-mediated DDAOG hydrolysis. The K_i values for **HZ-1** and **HZ-2** were evaluated as 9.95, and 1.95 μM, respectively. These results further demonstrated that **HZ-1** and **HZ-2** were potential inhibitors towards EcGUS and responsible for the strong inhibitory effects of *P. cuspidatum* towards EcGUS. In addition, **HZ-2** displayed much stronger inhibition potency towards EcGUS compared with **HZ-1**.

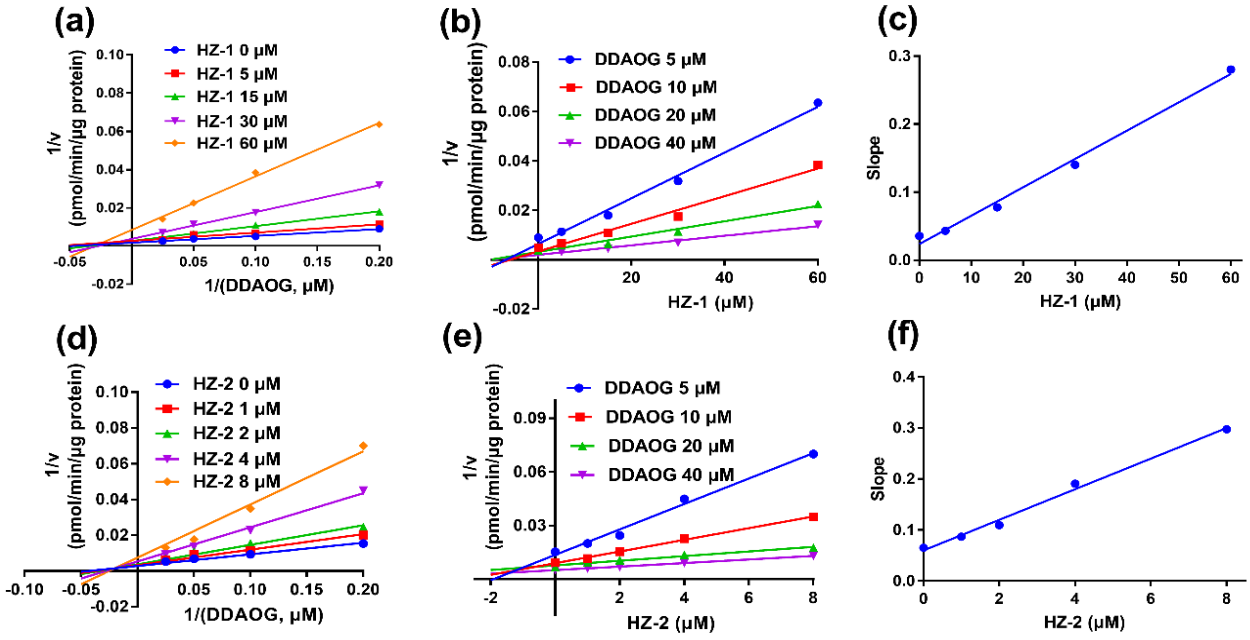

FIGURE 4 - (a) Lineweaver-Burk plot of **HZ-1**'s inhibition towards DDAOG hydrolysis in EcGUS, (b) Dixon plot of **HZ-1**'s inhibition towards DDAOG hydrolysis in EcGUS, (c) Second plot with the slopes from Lineweaver–Burk plot towards the concentrations of **HZ-1**, (d) Lineweaver-Burk plot of the inhibition of **HZ-2** towards EcGUS-catalyzed DDAOG hydrolysis, (e) Dixon plot of the inhibition of **HZ-2** towards EcGUS-catalyzed DDAOG hydrolysis, and (f) Second plot with the slopes from Lineweaver–Burk plot towards the concentrations of **HZ-2**. The results shown are the means of duplicate experiments.

P. cuspidatum and its constituents are known for their wide spectrum of pharmacological activities, such as anti-asthmatic, anti-oxidant, anti-inflammatory, and anti-cancer effects (Peng *et al.,* 2013). In this study, **HZ-1** and **HZ-2** from *P. cuspidatum* were identified as a new class of naturally occurring EcGUS inhibitors. Inhibitory effects and molecular mechanism of the identified EcGUS inhibitors were systemically characterized using a selective fluorescent probe substrate for EcGUS. Compared with synthetic GUS inhibitors, these two naturally occurring dietary compounds might be readily available and safe owing to their natural, nontoxic and multiple pharmacological effects.

Notably, **HZ-1** could also be found in grapes and several traditional medicinal plants, including *Rheum tanguticum*, *R. rhaponticum*, and *P. multiflorum* (Zhao *et al.,* 2019), whereas **HZ-2** is abundantly distributed in teas including green, oolong, and black tea (Tao *et al.,* 2016). It is readily conceivable that these food plants and herbal medicines may also display strong inhibitory effects towards EcGUS due to the presence of **HZ-1** or **HZ-2**. Furthermore, it is admitted that whether these inhibitors can selectively inhibit human gut GUS needs to be evaluated both *in vitro* and *in vivo*. Therefore, further studies are warranted to determine the inhibitory potency of these two inhibitors against human β-glucuronidases and other bacteria strains.

Molecular docking

Molecular docking was used to evaluate the potential determinants responsible for the inhibitory effects of **HZ-1** and **HZ-2** toward EcGUS. The crystal structure of EcGUS was downloaded from Protein Data Bank (http://www.rcsb.org). As depicted in Figures 5a and 5c, **HZ-1** and **HZ-2** could enter the active site of EcGUS and occupy the active pocket to prevent DDAOG hydrolysis. As shown in Figures 5b and 5d, **HZ-1** and **HZ-2** could form hydrogen bonds and have, van der Waals, π-π stacked, T-shaped, and π-alkyl interactions with the amino acid residues Asp163, Ser360, Leu361, Ile363, Glu413, Val446, Met447, Tyr468, Tyr472, Val473, Glu504, Trp549, Leu561, and Lys568. More importantly, the amino acid residues Asp163, Ser360, Ile363, Glu413, Glu504, and Lys568 could interact with the hydroxy and carbonyl groups of **HZ-1** and **HZ-2**, Glu413 and Glu504, especially, in the active site of GUS were responsible for the hydrolysis of the glucuronide glycoside bond. Notably, same bonds with the enzyme are formed with amentoflavone, demethylbellidifolin, and gentisin, which were reported to be inhibitors of EcGUS in the previous researches (Tian *et al.*, 2021; Sun *et al.*, 2012). These findings explain the molecular mechanism of the inhibition of EcGUS by **HZ-1** and **HZ-2**.

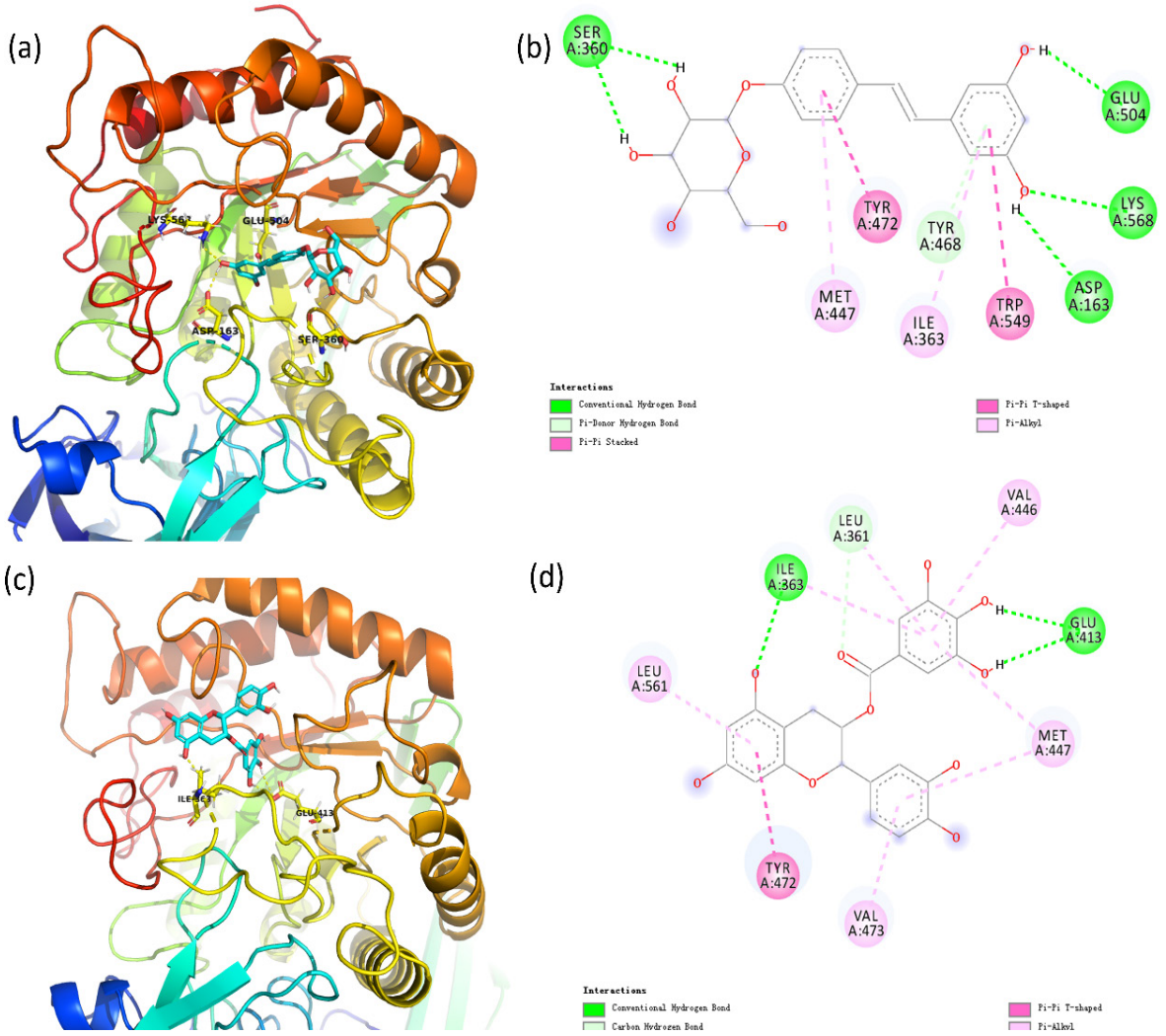

FIGURE 5 - A stereo view of the docking conformation of **HZ-1** (a) and **HZ-2** (c) (stick model) in the active site of EcGUS. The carbon atoms in these two molecules (**HZ-1** and **HZ-2**) were colored in cyan. Residues in EcGUS interacting with **HZ-1** (b) and **HZ-2** (d) are shown (conventional hydrogen bond, green; π-donor hydrogen bond, light green; π-π stacked, magenta).

CONCLUSIONS

Taken together, our results demonstrated that **HZ-1** and **HZ-2** from *P. cuspidatum* were the key components responsible for EcGUS inhibition. Inhibition kinetic analysis demonstrated that both **HZ-1** and **HZ-2** were mixed-type inhibitors of EcGUS-mediated DDAOG hydrolysis. Molecular docking results elucidated the importance of amino acid residues Asp163, Ser360, Ile 363, Glu413, Glu504, and Lys 568 in EcGUS inhibition by **HZ-1** and **HZ-2**. These findings demonstrated the inhibitory effects of *P. cuspidatum* and its two components toward EcGUS, thereby supporting their further evaluation and development as novel candidates for alleviating drug-induced intestinal damage.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (No 82174064), National Key R&D Program of China (2018YFC1705900), Distinguished professor of Liaoning Province, Open Fund of Key Laboratory of Biotechnology and Bioresources Utilization, Ministry of Education, the Innovative Entrepreneurship Program of High-level Talents in Dalian (2016RQ025 & 2017RQ121), Dalian Young Star of Science and Technology Project (2020RQ068), "1+X "program for Clinical Competency enhancement–Interdisciplinary Innovation Project, Second Hospital of Dalian Medical University and National Training Program for College Students' Innovation and Entrepreneurship (S202010161009).

REFERENCES

Awolade P, Cele N, Kerru N, Gummidi L, Oluwakemi E, Singh P. Therapeutic significance of beta-glucuronidase activity and its inhibitors: A review. Eur J Med Chem. 2020;187:111921

Bailly C. Irinotecan: 25 years of cancer treatment. Pharmacol Res. 2019;148:104398.

Bhatt AP, Pellock SJ, Biernat KA, Walton WG, Wallace BD, Creekmore BC, et al. Targeted inhibition of gut bacterial beta-glucuronidase activity enhances anticancer drug efficacy. Proc Natl Acad Sci U S A. 2020;117(13):7374-7381.

Bralley EE, Greenspan P, Hargrove JL, Wicker L, Hartle DK. Topical anti-inflammatory activity of Polygonum cuspidatum extract in the TPA model of mouse ear inflammation. J Inflamm-Lond. 2008;5:1.

Chamseddine AN, Ducreux M, Armand JP, Paoletti X, Satar T, Paci A, et al. Intestinal bacterial beta-glucuronidase as a possible predictive biomarker of irinotecan-induced diarrhea severity. Pharmacol Ther. 2019;199:1-15.

Cheng KW, Tseng CH, Yang CN, Tzeng CC, Cheng TC, Leu YL, et al. Specific inhibition of bacterial beta-glucuronidase by pyrazolo[4,3-c]quinoline derivatives via a pH-dependent manner to suppress chemotherapy-induced intestinal toxicity. J Med Chem. 2017;60(22):9222-9238.

Clarke G, Sandhu KV, Griffin BT, Dinan TG, Cryan JF, Hyland NP. Gut reactions: Breaking down xenobiotic-microbiome interactions. Pharmacol Rev. 2019;71(2):198-224.

Ervin SM, Hanley RP, Lim L, Walton WG, Pearce KH, Bhatt AP, et al. Targeting regorafenib-induced toxicity through inhibition of gut microbial beta-glucuronidases. ACS Chem Biol. 2019;14(12):2737-2744.

Feng L, Yang YL, Huo XK, Tian XG, Feng YJ, Yuan HW, et al. Highly selective NIR probe for intestinal beta-glucuronidase and high-throughput screening inhibitors to therapy intestinal damage. Acs Sensors. 2018;3(9):1727-1734.

Hahn RZ, Antunes MV, Verza SG, Perassolo MS, Suyenaga ES, Schwartsmann G, et al. Pharmacokinetic and pharmacogenetic markers of irinotecan toxicity. Curr Med Chem. 2019;26(12):2085-2107.

He X, Zhao WY, Shao B, Zhang BJ, Liu TT, Sun CP, et al. Natural soluble epoxide hydrolase inhibitors from Inula helenium and their interactions with soluble epoxide hydrolase. Int J Biol Macromol. 2020;158:1362-1368.

Hicks LD, Hyatt JL, Stoddard S, Tsurkan L, Edwards CC, Wadkins RM, et al. 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.

Hou XD, Song LL, Cao YF, Wang YN, Zhou Q, Fang SQ, et al. Pancreatic lipase inhibitory constituents from Fructus Psoraleae. Chin J Nat Med. 2020;18(5):369-378.

Hu WH, Chan GKL, Lou JS, Wu QY, Wang HY, Duan R, et al. The extract of Polygoni Cuspidati Rhizoma et Radix suppresses the vascular endothelial growth factor-induced angiogenesis. Phytomedicine. 2018;42:135-143.

Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, et al. Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest. 1998;101(4):847-854.

Jariwala PB, Pellock SJ, Goldfarb D, Cloer EW, Artola M, Simpson JB, et al. Discovering the microbial enzymes driving drug toxicity with activity-based protein profiling. ACS Chem Biol. 2020;15(1):217-225.

Li XW. Chemical ecology-driven discovery of bioactive marine natural products as potential drug leads. Chin J Nat Med. 2020;18(11):837-838.

Liu LT, Zheng G-J, Zhang WG, Guo G, Wu M. Clinical study on treatment of carotid atherosclerosis with extraction of polygoni cuspidati rhizoma et radix and crataegi fructus: a randomized controlled trial. Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China J Chin Mater Med. 2014;39(6):1115-1119.

Liu S, Zhang X-x, Zhuang S, Li C-h, Li Y-b. Effect of polygoni cuspidati rhizoma et radix and its ingredient resveratrol on experimental autoimmune myasthenia gravis by suppressing immune response. Chin Herbal Med. 2016;8(3):251-258.

Lu Y, Suh SJ, Li X, Hwang SL, Li Y, Hwangbo K, et al. Citreorosein, a naturally occurring anthraquinone derivative isolated from Polygoni cuspidati radix, attenuates cyclooxygenase-2-dependent prostaglandin D-2 generation by blocking Akt and JNK pathways in mouse bone marrow-derived mast cells. Food Chem Toxicol. 2012;50(3-4):913-919.

Pellock SJ, Redinbo MR. Glucuronides in the gut: Sugar-driven symbioses between microbe and host. J Biol Chem. 2017;292(21):8569-8576.

Peng W, Qin RX, Li XL, Zhou H. Botany, phytochemistry, pharmacology, and potential application of Polygonum cuspidatum Sieb.et Zucc. A review. J Ethnopharmacol. 2013;148(3):729-745.

Pollet RM, D'Agostino EH, Walton WG, Xu YM, Little MS, Biernat KA, et al. An atlas of beta-glucuronidases in the human intestinal microbiome. Structure. 2017;25(7): 967-977.

Shi JW, Li ZZ, Wu JS, Jin WY, Chang XY, Sun H, et al. Identification of the bioactive components of Banxia Xiexin Decoction that protect against CPT-11-induced intestinal toxicity via UPLC-based spectrum-effect relationship analyses. J Ethnopharmacol. 2021;266:113421.

Singh A, Raju R, Mrad M, Reddell P, Munch G. The reciprocal EC50 value as a convenient measure of the potency of a compound in bioactivity-guided purification of natural products. Fitoterapia. 2020;143:104598.

Song PF, Zhu YD, Ma HY, Wang YN, Wang DD, Zou LW, et al. Discovery of natural pentacyclic triterpenoids as potent and selective inhibitors against human carboxylesterase 1. Fitoterapia. 2019;137:104199.

Sun CP, Yan JK, Yi J, Zhang XY, Yu ZL, Huo XK, et al. The study of inhibitory effect of natural flavonoids toward beta-glucuronidase and interaction of flavonoids with beta-glucuronidase. Int J Biol Macromol. 2020;143:349-358.

Sun CP, Zhang J, Zhao WY, Yi J, Yan JK, Wang YL, et al. Protostane-type triterpenoids as natural soluble epoxide hydrolase inhibitors: Inhibition potentials and molecular dynamics. Bioorg Chem. 2020;96:103637.

Tao WQ, Zhou ZG, Zhao B, Wei TY. Simultaneous determination of eight catechins and four theaflavins in green, black and oolong tea using new HPLC-MS-MS method. J Pharm Biomed Anal. 2016;131:140-145.

Tian XG, Yan JK, Sun CP, Li JX, Ning J, Wang C, et al. Amentoflavone from Selaginella tamariscina as a potent inhibitor of gut bacterial β-glucuronidase: Inhibition kinetics and molecular dynamics stimulation. Chem Biol Interact. 2021;340:109453.

Tobin P, Clarke S, Seale JP, Lee S, Solomon M, Aulds S, et al. The in vitro metabolism of irinotecan (CPT-11) by carboxylesterase and beta-glucuronidase in human colorectal tumours. Br J Clin Pharmacol. 2006;62(1):122-129.

Wallace BD, Wang HW, Lane KT, Scott JE, Orans J, Koo JS, et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science. 2010;330(6005):831-835.

Wang J, Feng WW, Tang F, Ao H, Peng C. Gut microbial transformation, a potential improving factor in the therapeutic activities of four groups of natural compounds isolated from herbal medicines. Fitoterapia. 2019;138:104293.

Weng ZM, Wang P, Ge GB, Dai ZR, Wu DC, Zou LW, et al. Structure-activity relationships of flavonoids as natural inhibitors against E. coli beta-glucuronidase. Food Chem Toxicol. 2017;109:975-983.

Yi J, Bai R, An Y, Liu TT, Liang JH, Tian XG, et al. A natural inhibitor from Alisma orientale against human carboxylesterase 2: Kinetics, circular dichroism spectroscopic analysis, and docking simulation. Int J Biol Macromol. 2019;133:184-189.

Zhao XH, Tao JH, Zhang T, Jiang SR, Wei W, Han HP, et al. Resveratroloside alleviates postprandial hyperglycemia in

diabetic mice by competitively inhibiting alpha-glucosidase. J Agric Food Chem. 2019;67(10):2886-2893.

Zhang J, Lian JH, Zhao JC, Wang YL, Dong PP, Liu XG, et al. Xylarianins A-D from the endophytic fungus Xylaria sp SYPF 8246 as natural inhibitors of human carboxylesterase 2. Bioorg Chem. 2018;81:350-355.

Zhong JC, Li XB, Lyu WY, Ye WC, Zhang DM. Natural products as potent inhibitors of hypoxia-inducible factor-1 alpha in cancer therapy. Chin J Nat Med. 2020;18(9):696-703.

Zhou TS, Wei B, He M, Li YS, Wang YK, Wang SJ, et al. Thiazolidin-2-cyanamides derivatives as novel potent Escherichia coli beta-glucuronidase inhibitors and their structure-inhibitory activity relationships. J Enzyme Inhib Med Chem. 2020;35(1):1736-1742.

Received for publication on 05th August 2021
Accepted for publication on 07th December 2021