Bioorganic Chemistry 87 (2019) 16–22

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Azacoccone E inhibits cancer cell growth by targeting 3-phosphoglycerate dehydrogenase

Jing Guo[a,1], Xiaoxia Gu[a,1], Mengzhu Zheng[a,1], Yonghui Zhang[a,*], Lixia Chen[b,*], Hua Li[a,b,*]

[a] Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

[b] Wuya College of Innovation, Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China

ARTICLE INFO

Keywords:
Azacoccone E
PHGDH inhibitor
Natural product
Cancer

ABSTRACT

Serine plays critically important roles in tumorigenesis. Homo sapiens 3-phosphoglycerate dehydrogenase (PHGDH) catalyzes the first committed step for the synthesis of glucose-derived serine via the phosphoserine pathway and has been associated with a wide variety of cancers, including breast cancer, melanoma, colon cancer, glioma, nasopharyngeal carcinoma, cervical adenocarcinoma, *etc*. Azacoccone E, an aza-epicoccone derivative from the culture of *Aspergillus flavipes*, exhibited effective inhibitory activity against PHGDH *in vitro*. The microscale thermophoresis (MST) method and the cellular thermal shift assay (CETSA) confirmed that azacoccone E directly bound to PHGDH. And the cell-based experiments showed that this compound was selectively toxic to PHGDH-dependent cancer cells and could cause apoptosis. Further biochemical assays revealed that it was a noncompetitive inhibitor with respect to the substrate of 3-PG and exhibited a time-dependent inhibition. Furthermore, molecular docking demonstrated that azacoccone E coordinated in an allosteric site of PHGDH with low binding energy. Therefore, azacoccone E can be considered as a possible drug candidate targeting at PHGDH for treatment of cancers.

1. Introduction

Serine, one of the so-called non-essential amino acids, supports a number of anabolic processes [1]. It is an essential component of proteins and provides the precursors for the synthesis of lipids, such as sphingolipids and phosphatidylserine (PS) [2]. Moreover, serine is the major source of “one-carbon” units carried by tetrahydrofolate and incorporated into nucleotides [3]. Thus, serine plays multiple important roles in cellular metabolism.

It has long been recognized that serine can not only be obtained exogenously via amino acid transporters, but also be synthesized from glucose via the phosphoserine pathway in cells [4], in which the NAD^+-dependent enzyme 3-phosphoglycerate dehydrogenase (PHGDH) catalyzes the first and also the rate-limiting step converting glycolytic intermediate 3-phosphoglycerate (3-PG) to serine via three sequential enzymatic reactions [5]. An analysis of human cancers showed that PHGDH is in a genomic region of recurrent copy number gain most commonly found in breast cancer and melanoma [6]. Suppression of PHGDH in cell lines with high PHGDH expression level causes the reduction of serine synthesis and strong inhibition of cell proliferation, which has triggered great interest in understanding serine synthesis and downstream metabolism including one-carbon unit and alpha-ketoglutarate [7]. Thus, PHGDH inhibitors as a targeted cancer therapy represent an exciting clinical opportunity.

Natural products (NPs) are considered as a rich source of bioactive molecules for drug discovery, and approximately 60% of the small-molecule anti-cancer drugs approved were either NPs or derivatives of NPs from the 1930s to 2014. In NPs, secondary metabolites of endophytic fungi are considered to be rich natural product resources with unique structure and biological activity [8,9]. This study herein is focusing on the identification of small molecule inhibitors of PHGDH. We screened in-house database of NPs which contained more than 600 compounds using the enzymatic assay, and azacoccones C and E, two aza-epicoccone derivatives obtained from *Aspergillus flavipes* were identified as promising candidates (see Fig. 1), showed significant PHGDH inhibitory activity compared with the positive compound CBR-5884 and had almost no effect on other $NAD(P)^+$-dependent enzymes like isocitrate dehydrogenase (IDH1). Meanwhile, azacoccones C and E

* Corresponding authors.
E-mail addresses: zhangyh@mails.tjmu.edu.cn (Y. Zhang), syzyclx@163.com (L. Chen), li_hua@hust.edu.cn (H. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bioorg.2019.02.037
Received 25 November 2018; Received in revised form 14 February 2019; Accepted 17 February 2019
Available online 23 February 2019
0045-2068/

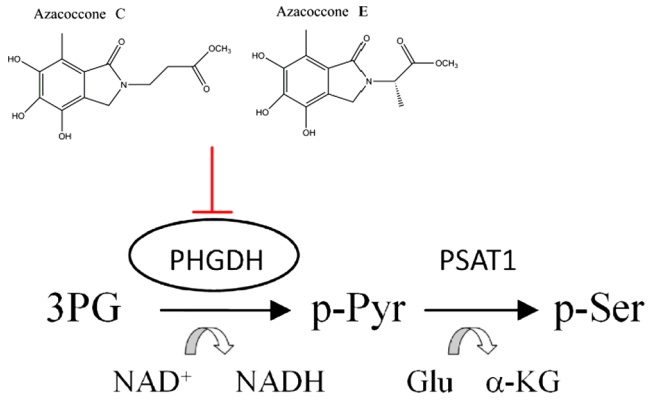

Fig. 1. Biological activity of PHGDH and chemical structures of azacoccones C and E.

treatment selectively inhibited PHGDH-dependent cancer cell proliferation and induced apoptosis. The microscale thermophoresis (MST) method and cellular thermal shift assay (CETSA) showed that azacoccone E was directly binding to PHGDH. Mechanistically, azacoccone E was found to be a noncompetitive inhibitor in a time-dependent manner. Molecular docking demonstrated that azacoccone E coordinated at the allosteric site of PHGDH, which is vital for decreasing the enzyme activity.

2. Experiment

2.1. General

Azacoccones C and E were obtained in our previous work [10]. Anti-PHGDH antibody (14719-1-AP) was purchased from Proteintech Group Inc (China). And D-(-)-3-phosphoglyceric acid disodium salt (sc-214793) was brought from Santa Cruz Biotechnology (U.S.). All of them were stored at −20 °C. MDA-MB-231, MDA-MB-468 and Hela cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences.

2.2. Molecular docking

The crystal structure of human PHGDH (PDB code: 2G76) was obtained from the Protein Data Bank (http://www.rcsb.org). Molecular docking was implemented in ICM-Pro 3.8.2 modeling software on an Intel i7 4960 processor (MolSoft LLC, San Diego, CA). Ligand binding pocket residue was selected by graphical tools in the ICM software to create the boundaries of the docking search. Chemical structures of compounds were input as mol2 files for docking. In the docking calculation, default parameters were applied to calculate the potential energy maps of the receptor. Compounds were imported into the ICM filed as an index project. Conformational sampling was based on the Monte Carlo procedure, and finally the ligand with the lowest energy and the most favorable orientation was selected [11].

2.3. The expression and purification of PHGDH and PSAT1

The gene of PHGDH (Gene ID: 26227) and PSAT1 (Gene ID: 29968) were synthesized chemically and cloned into the pET-28a vector (Novagen), respectively. Protein was produced in *E. coli* BL21 (DE3) (Invitrogen). Cells were cultivated in LuriaBertani medium (LB) containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl at 37 °C to an OD_{600} of 0.8–1.0, then induced with 0.4 mM isopropyl-Dthiogalactopyranoside (IPTG) at 19 °C for 16 h. Cells were harvested by centrifugation and lysed in a buffer containing 20 mM Tris-HCl pH 8.5, 200 mM NaCl, 5% glycerol, 5 mM mercaptoethanol and 0.1% TritonX-100. After centrifugation (21,500*g* 40 min, 4 °C), the soluble fraction was loaded onto the Ni-NTA resin (Qiagen), washed with a buffer containing 20 mM Tris pH 8.5, 10 mM imidazole and 200 mM NaCl, then eluted with a buffer containing 20 mM Tris-HCl pH 8.5, 300 mM imidazole and 200 mM NaCl. Finally, protein was further purified on S200 gel filtration chromatography in 200 mM NaCl and 20 mM Tris-HCl pH 8.5 [12].

2.4. Enzyme inhibition assays

To assay the inhibitory effects of azacoccones C and E on PHGDH, NADH which was generated through a coupled reaction with phosphoserine amino transferase (PSAT1) was monitored by fluorescence (340/460 nm; Ex/Em) every minute for 20 min on a SpectraMax M5e plate reader. Briefly, 40 μL PHGDH protein (880 nM) was incubated with 10 μL compound solution at room temperature for 30 min. Then 50 μL assay mix containing 50 mM Tris-HCl, pH 8.5, 1 mM EDTA, 2 μM PSAT1, 0.81 mM NAD^+, 0.73 mM 3-phosphoglycerate (3-PG) and 30 mM glutamate was added. For IC_{50} assays, twofold serial dilutions of compound solution ranging from 100 μM to 0.3 μM were preincubated with enzyme for 30 min before initiating the enzyme reaction with substrate mix. For time-dependent IC_{50} assays, compound preincubation time was 4, 1, or 0.5 h. For inhibition pattern measurements, compound (0, 5, 10, 20 μM) and enzyme were preincubated for 30 min. Initial rate plots were fit using Prism [5].

2.5. Microscale thermophoresis (MST) assay

The binding capacity of PHGDH to azacoccones C and E was determined on Monolith NT.115 (NanoTemper Technologies, München, Germany). The protein was diluted to 10 μM in standard buffer (20 mM HEPES pH 7.5, 0.05 (v/v) % Tween-20) and incubated for 30 min with an equal volume of 20 μM NT647 at room temperature. The labeled protein was separated from the excess dye by gravity flow column. The compounds were diluted into 12 standard dilutions with standard buffer in the appropriate concentration range. After incubation at 22 °C

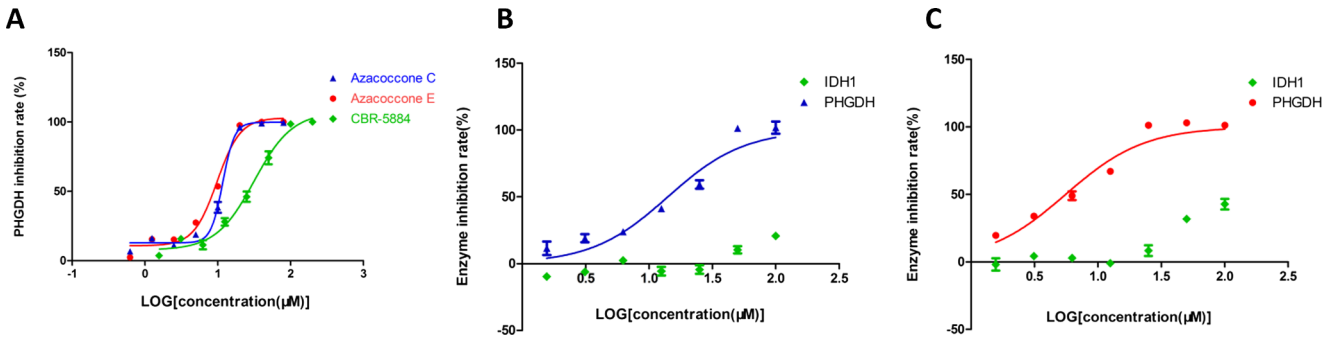

Fig. 2. *In vitro* enzyme inhibition assay. (A) Dose-dependent inhibition of PHGDH by azacoccones C and E. (B) IC_{50} of azacoccone C against PHGDH and IDH1. (C) IC_{50} of azacoccone E against PHGDH and IDH1.

Table 1
Inhibition activities of the compounds.

Compound	K_d (μM)[a]	IC_{50} for PHGDH activities (μM)		IC_{50} for cancer cell activities (μM)		
		IDH1	PHGDH	MDA-MB-231	MDA-MB-468	Hela
azacoccone C	61.30 ± 5.62	> 100	11.71 ± 2.65	> 200	100.40 ± 0.08	67.47 ± 0.17
azacoccone E	15.20 ± 2.94	> 100	9.76 ± 4.32	> 200	59.91 ± 0.07	68.95 ± 0.16

[a] The K_d value was automatically calculated by the curve fitting, and presented as means ± SD for three experiments.

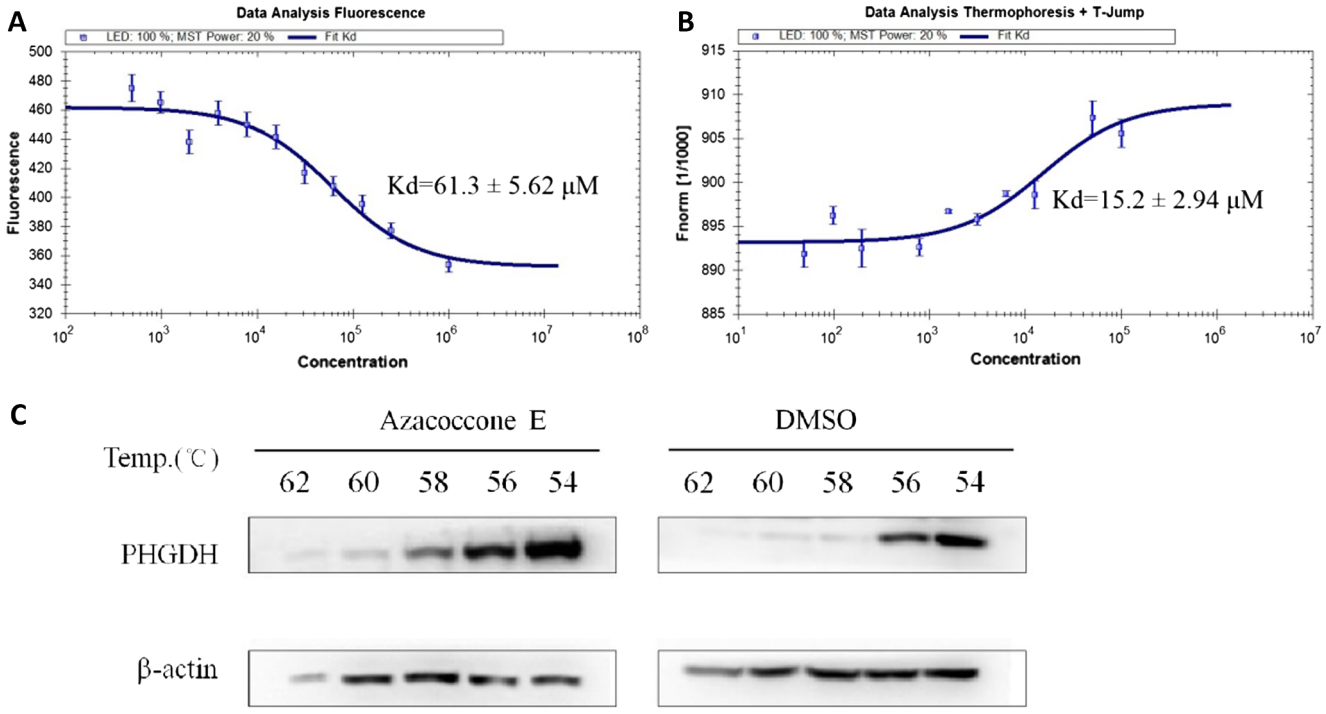

Fig. 3. The inhibition activities of *azacoccones C and E*. (A and B) Measurement of affinity of azacoccones C and E to PHGDH by MST with K_d values of 61.30 ± 5.62 and 15.20 ± 2.94 μM, respectively. (C) Cellular thermal shift assay (CETSA) in Hela cells treated with 50 μM azacoccone E confirmed its binding capacity with PHGDH at the cellular level.

for 15 min, the samples were loaded into NT hydrophobic capillaries and measured with 20% laser power and 100% LED power. The dissociation constant K_d was fitted by NTA analysis software [13,14].

2.6. Cellular thermal shift assay (CETSA)

The cellular thermal shift assay (CETSA) was performed to confirm the interaction between PHGDH and azacoccone E *in vitro* by Western blot assay, and the technique was based on ligand-induced stabilization of target proteins. Briefly, Hela cells cultured with 90% confluent in 100 × 20 mm tissue culture dishes were treated with media containing DMSO (equal volume with compound) or azacoccone E (50 μM) for 8 h. After treatment, the cells were isolated with trypsin, collected by centrifugation, and then resuspended in PBS. The cell suspension was divided equally into 5 PCR tubes and heated at gradient temperature from 54 °C to 62 °C for 5 min. Subsequently, cells were analyzed by Western blot assay [15].

2.7. Cell culture

Cells were routinely grown in high Glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin in 5% CO_2 at 37 °C with 100% humidity.

2.8. Cell viability assay

Cells were seeded in 96-well plates at a density of 3000 cells/well in DMEM media. On the next day, the media were aspirated, cells were washed with PBS, and serine-replete or -deplete media containing compound (0, 50 μM) or DMEM media containing compound (0–200 μM) were added and incubated for 3–5 days, followed by adding 100 μL FBS-free medium containing 10% CCK8 into each well and incubated for an additional 30 min at 37 °C. The serine-replete or serine-deplete media were made from serine/glycine-free DMEM supplemented with either serine (400 μM) or PBS. And the serine/glycine-free DMEM were purchased from BOSTER (Wuhan, China). The absorbance was measured at 450 nm using an enzyme-linked immunosorbent assay reader. IC_{50} was calculated by comparing the absorbance of cells untreated and compound-treated groups using GraphPad Prism software. The standard deviation for each compound against each cell line was obtained by at least three independent experiments [5,13].

2.9. Cell apoptosis analysis

Hela cells were seeded in 6-well plates at a density of 2×10^4 cells/well and treated with azacoccone E at 75 and 150 μM for 72 h. Then the cells were harvested, washed twice with ice-cold PBS, and mixed in 100 μL of 1 × binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). After culturing for 15 min at room temperature in Annexin-V/PI double staining liquid (Nanjing KeyGen Biotech Inc.), the

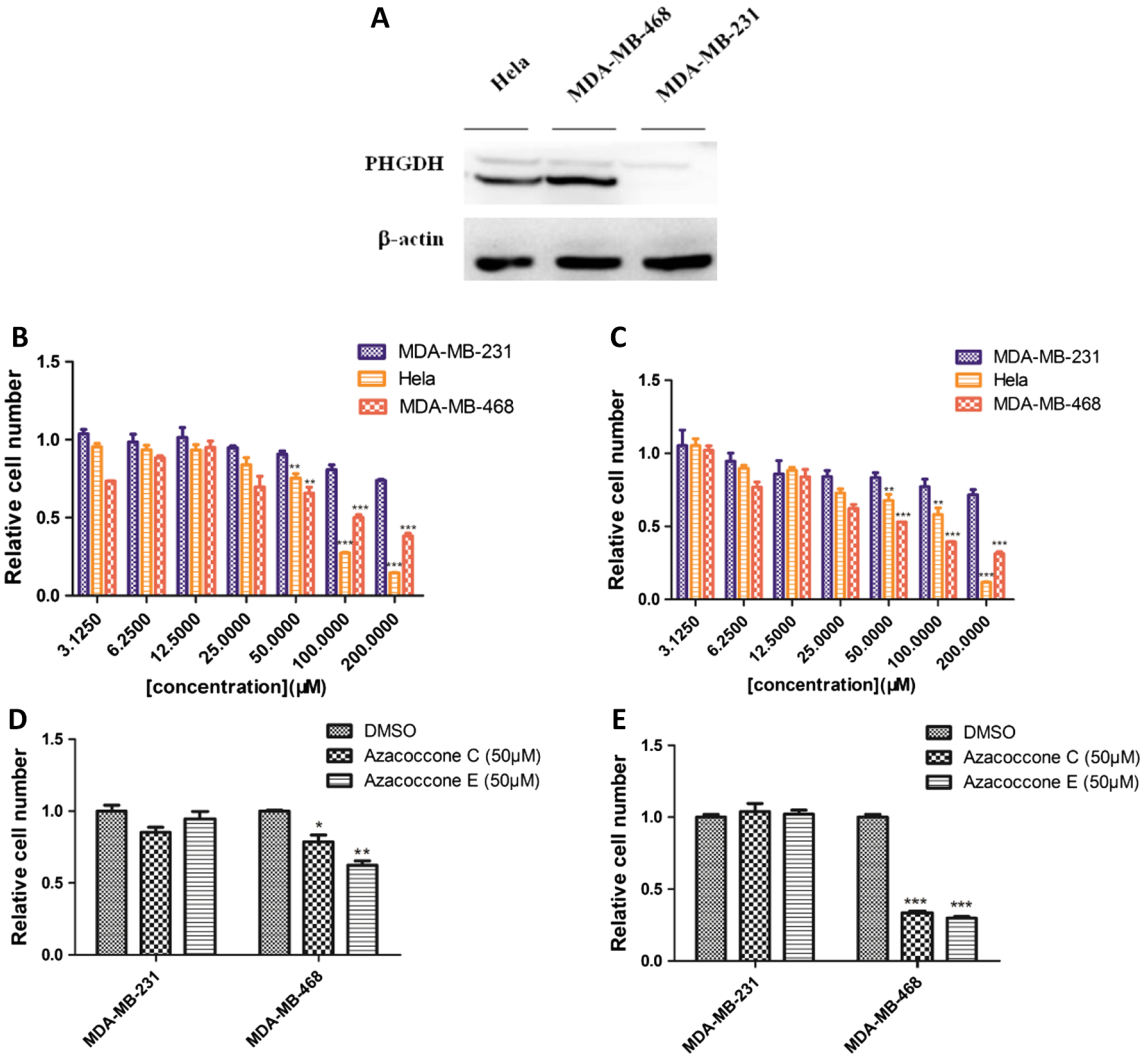

Fig. 4. Cancer cells with high expression level of PHGDH were more sensitive to azacoccones C and E. (A) PHGDH expression level in three cancer cell lines MDA-MB-231, MDA-MB-468 and Hela were determined by Western blot analysis. (B and C) Cancer cells were exposed to varying concentrations of azacoccones C and E for 72 h to investigate its cytotoxic activity. Proliferation assay for lines treated with azacoccone compounds in (D) serine-replete media (+SER) or (E) serine-deplete media (−SER). Azacoccones C and E functioned at the concentration of 50 μM. The number of cells was determined and normalized to that of DMSO group. *P < 0.05, **P < 0.01, ***P < 0.001.

cells were examined by flow cytometry (BD Biosciences, FACSCalibur) [16].

2.10. Hoechst 33258 staining

Hela cells were seeded in 6-well plates at a density of 2×10^4 cells/well and treated with azacoccone E at 50 and 100 μM for 72 h. After treatment, cells were fixed with 4% paraformaldehyde for 15 min and then incubated with Hoechst 33258 at 37 °C for 30 min. The apoptotic morphological changes were observed by Hoechst 33258 under a fluorescence microscope (Zeiss, OBSERVER D1/AX10 cam HRC) [17].

2.11. Western blot analysis

The supernatants and intact cells in a 6-well plate after treatment with or without compound were collected and the expression level of PHGDH was detected by western blot. The cells were cleaved with RIPA lysate and the protein concentration was determined by BCA kit and equalized before loading. Equal amounts of protein from each group were denatured and subjected to electrophoresis in 12% SDS-PAGE gels followed by transfered to PVDF membrane and probed with PHGDH andβ-actin antibodies. Blot bands were visualized using the horseradish peroxidase-conjugated secondary antibodies combined with chemiluminescent substrate [18].

3. Results and discussion

3.1. Inhibitory effects of azacoccones C and E on PHGDH

To explore the inhibitory activity of azacoccones C and E on PHGDH enzyme *in vitro*, recombinant human PHGDH and PSAT1 protein were expressed and purified (Fig. S1). And the enzymatic activity was evaluated by detecting the increase of NADH monitored by fluorescence (340/460 nm; Ex/Em). Azacoccones C and E showed inhibitory effects against PHGDH with IC_{50} values of 11.71 ± 2.65 and 9.76 ± 4.32 μM, which were better effect than the positive control CBR-5884 (31.58 ± 7.18 μM) (Fig. 2A). And at the same inhibitor concentrations, azacoccones C and E had no effect on another $NAD(P)^+$-dependent dehydrogenases, isocitrate dehydrogenase (IDH1) (Figs. 2B, 2C and Table 1).

3.2. Specific binding of azacoccones C and E with PHGDH in vitro

To further validate the effects of azacoccones C and E binding to

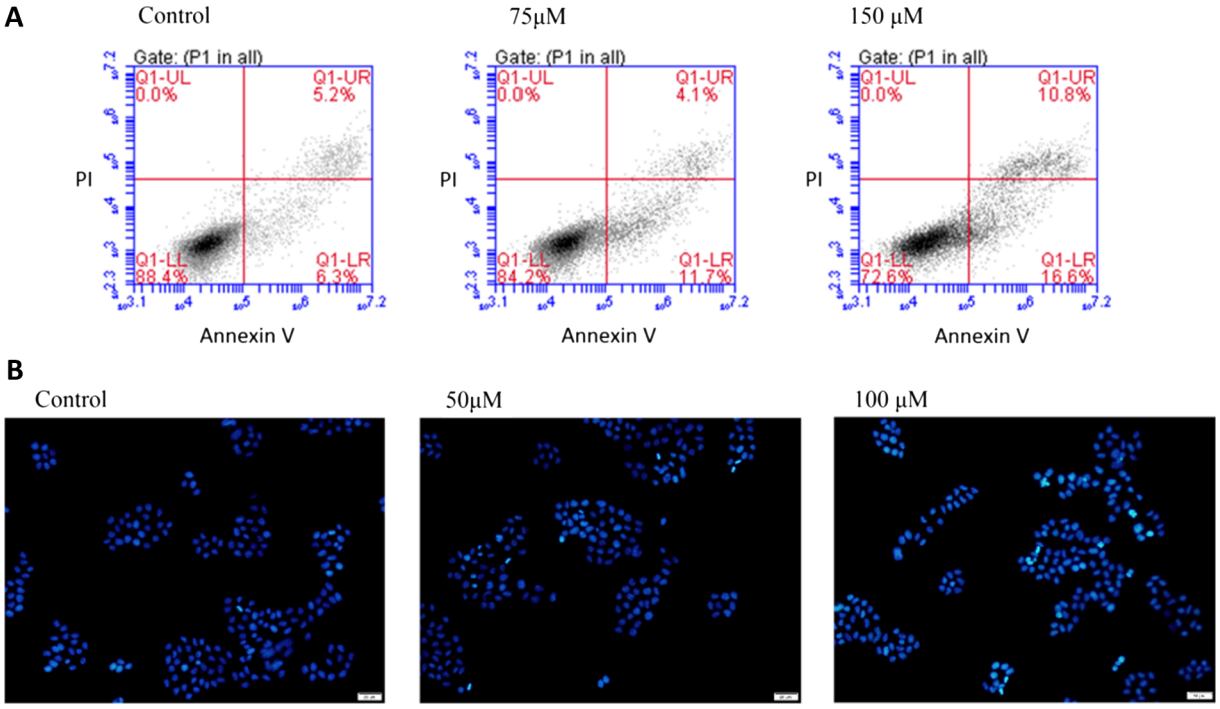

Fig. 5. Apoptotic-driven effects of azacoccone E against Hela cell line. (A) Apoptosis ratio of azacoccone E detected by flow cytometry. Cells were treated with compound azacoccone E for 72 h. (B) Effect of azacoccone E on the nuclear morphological changes of Hela cells. Images were acquired using a fluorescence microscope (200× magnification). Bar = 20 μm.

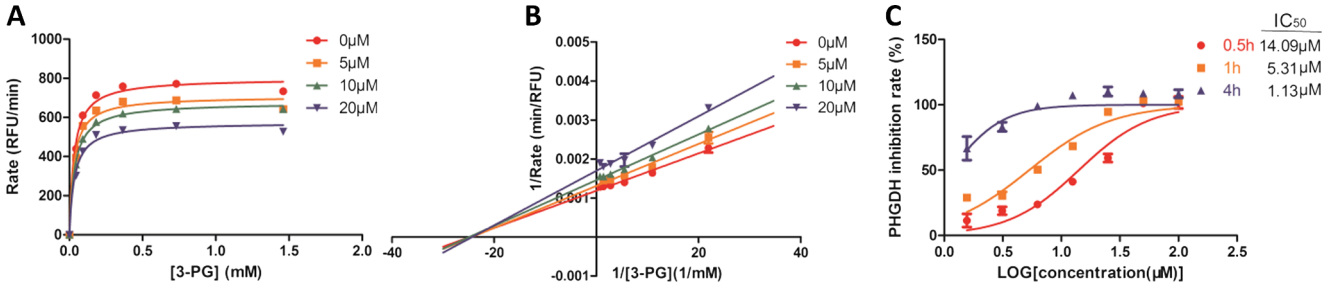

Fig. 6. The inhibition mechanism of azacoccone E. (A) 3-PG saturation profiles for PHGDH at a range of concentrations of azacoccone E. (B) Line weaver–Burk double-reciprocal representation of the 3-PG saturation profiles for PHGDH at a range of concentrations of azacoccone E. (C) Time-dependent inhibition was measured by preincubating azacoccone E and PHGDH for 0.5, 1, or 4 h.

Table 2
Kinetic parameters with azacoccone E treatment on PHGDH.[a]

Azacoccone E (μM)	Km (μM)	Vmax (△RFU/min)
0	31.42 ± 4.37	798.60 ± 18.24
5	29.56 ± 4.81	707.80 ± 18.33
10	36.55 ± 2.37	675.20 ± 7.75
20	34.55 ± 5.26	573.80 ± 15.04

[a] K_m and V_{max} values were obtained from Lineweaver-Burk plots. The values were the means of results of three experiments.

PHGDH, microscale thermophoresis (MST) method which had previously been used to investigate protein-protein, small organic molecule-protein and antibody-protein interactions was performed. Measuring the thermophoretic behavior of a protein in the presence of different ligand concentrations by MST allows quantitative analysis of molecular interactions in solution. In this study, MST was utilized for the confirmation of the dissociation constant (K_d) of azacoccones C and E to PHGDH. As shown in Fig. 3A and B, the K_d values of azacoccones C and E to PHGDH were 61.30 ± 5.62 and 15.20 ± 2.94 μM, respectively. Therefore, the results further confirmed the specific binding of azacoccone E to PHGDH.

To quantitatively measure the interaction between PHGDH and azacoccone E, the cellular thermal shift assay (CETSA), a recently developed method that allows rapid and simple assessment of target binding of compounds in a cellular context was employed. The thermal stability of PHGDH in Hela cells was tested at the temperature range of 54–62 °C. The results implied that azacoccone E specifically targeted PHGDH proteins in cells, showing the high sensitivity of azacoccone E in Hela cancer cells (Fig. 3C).

3.3. Azacoccones C and E selectively inhibited the proliferation of high PHGDH-expressing cell lines

Human breast cancer cell lines MDA-MB-231 and MDA-MB-468, and cervical cancer cell line Hela were initially selected to examine the PHGDH expression level by Western blot assay. PHGDH protein content in these samples was normalized by the signal intensity of structure protein β-actin. As is shown in Fig. 4A, MDA-MB-468 cells and Hela cells exhibited higher expression level of PHGDH than that in MDA-MB-231 cells by analyzing the quantitation of band intensity. After exposure to azacoccones C and E for 3 days, MDA-MB-468 and Hela cells

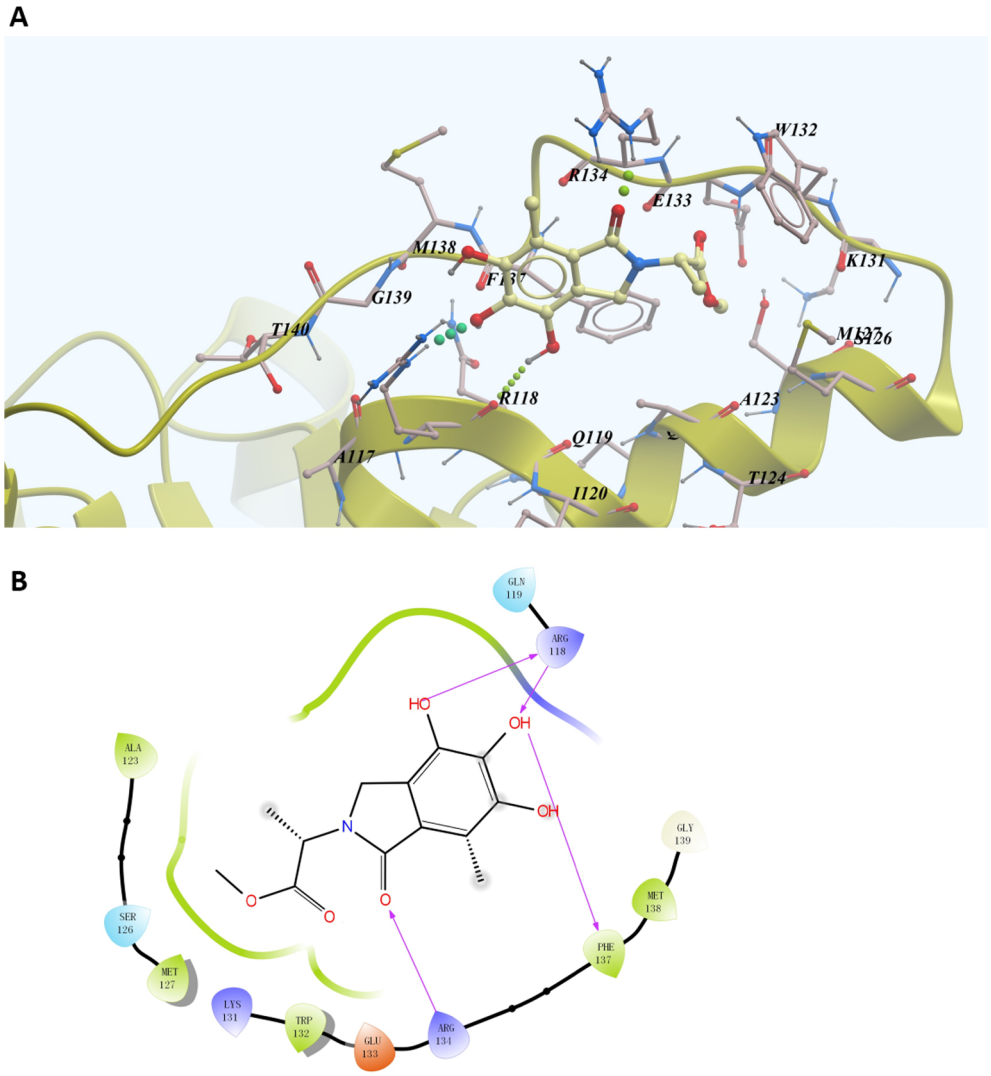

Fig. 7. Low-energy binding conformations of azacoccone E bound to PHGDH generated by molecular docking. (A) Detailed view of azacoccone E binding in the allosteric site of the enzyme. (B) Ligand interaction diagram of azacoccone E with PHGDH.

with higher expression of PHGDH showed more sensitive to azacoccones C and E than MDA-MB-231 cells (Fig. 4B and C), indicating that azacoccones C and E induced specific cancer cells to death through a PHGDH-dependent manner. To test whether tumor cells were more sensitive to azacoccone compounds in a serine-deplete media, low PHGDH-expressing lines MDA-MB-231 and high PHGDH-expressing lines MDA-MB-468 were cultured respectively in serine-replete or serine-deplete media. The results showed that azacoccone E (50 μM) inhibited the proliferation of MDA-MB-468 cells by 37.57% in serine-replete medium. When the serine in the medium was withdrawn, the inhibition effect of compound on MDA-MB-468 proliferation can be improved by 70.13%. However, the azacoccone compounds had no effect on MDA-MB-231, futher confirming that azacoccones C and E were selectively toxic to cells with high serine synthesis activity (Fig. 4D and E).

3.4. Azacoccone E-induced apoptotic effect detected by flow cytometry analysis and Hoechst 33258 staining

The apoptosis effects of azacoccone E were monitored by flow cytometry analysis. Followed by an exposure to 75 and 150 μM of azacoccone E for 72 h, the apoptotic cells positive for annexin-V and PI increased from 11.5% (vehicle) to 15.8% and 27.4% at 75 and 150 μM, respectively (Fig. 5A), which disclosed that azacoccone E showed a dose-dependent proapoptotic activity.

To determine the morphological changes induced by azacoccone E in Hela cells, Hoechst 33258 staining was carried out. Hela cells were treated with a variety of concentrations (0, 50, 100 μM) of azacoccone E for 72 h. Then the cells were stained with Hoechst 33258 and the typical morphological features of later stage apoptosis increased condensation of chromatin material and fragmentation of the nuclei were observed. The number of apoptotic nuclei significantly increased with the increase of the concentration of azacoccone E to 100 μM (Fig. 5B).

3.5. Manner of azacoccone E inhibition

We sought to more deeply characterize the inhibitory mechanism of azacoccone E on PHGDH. As displayed in Fig. 6A, B and Table 2, in the presence of azacoccone E, the maximal reaction rate (V_{max}) values of 3-PG was decreased in a concentration-dependent manner while the kinetic constants (K_m) value of 3-PG was not obviously affected, suggesting a non-competitive inhibition manner of azacoccone E against PHGDH. Furthermore, in order to assess whether the inhibition was time-dependent, azacoccone E and PHGDH were preincubated before initiating the enzymatic reaction. As expected, azacoccone E was progressively more potent with increasing preincubation time (Fig. 6C).

3.6. Molecular docking revealed the possible binding mode of azacoccone E with PHGDH

In order to further elucidate the binding mode of azacoccone E with PHGDH, molecular docking was performed by using ICM-Pro 3.8.2 modeling software (MolSoft LLC, San Diego, CA). The lowest-energy binding conformation of azacoccone E was shown as Fig. 7A. From the generated docking model, azacoccone E was well fitted an allosteric site of the enzyme, where it was away from the active site. This ligand binding pocket was relatively hydrophilic in the entrance, with two arginines protruded from the shallow pocket. Hydrogen binding was predicted between Arg118 with two phenol group, Arg134 with lactam carbonyl (Fig. 7B). The predicted binding of azacoccone E in the allosteric site of the enzyme was consistent with observed enzyme inhibition kinetics.

4. Discussion and conclusions

Cancer cells can reprogram metabolism to support their proliferation and growth. The PHGDH gene involved in cancer metabolic reprogramming is located at chromosome 1p12, which shows copy-number gain in 16% of all cancers including 40% of melanoma and some triple-negative breast cancers [19]. Currently, the discovery of drugs targeting at PHGDH is mainly concentrated on breast cancer, more studies on other tumor types remain to be carried out.

Three studies have reported some compounds with anti-PHGDH activity recently. The first PHGDH inhibitor CBR-5884 was found by screening 800,000-compound library and showed enzymatic inhibitory activity with an IC_{50} of 33.0 ± 12.0 μM in a time-dependent manner. It was speculated that CBR-5884 was a noncompetitive inhibitor and disrupted the oligomerization state of PHGDH. Serine metabolism was detected by gas chromatography-mass spectrometry, and CBR-5884 specifically inhibited 30% of de novo serine synthesis. Furthermore, CBR-5884 had been shown to inhibit cell proliferation in PHGDH-overexpressing cancer cell lines. However, the binding of CBR-5884 with PHGDH and its targeting to PHGDH remain to be studied [5]. Another reported non-competitive inhibitor NCT-503 exhibited the best IC_{50} value of 2.5 ± 0.6 μM by screening 400,000-compound of NIH molecular libraries and showed some selectivity in PHGDH-amplified breast cancer cell lines. Although NCT-503 was found to be non-competitive, its specific binding site remains unknown [3]. The third study reported two allosteric inhibitors for PHGDH using a structure-based design approach with an optimal IC_{50} of 28.1 ± 1.3 μM. And it was confirmed that PKUMDL-WQ-2101 and PKUMDL-WQ-2201 specifically bind to PHGDH in PHGDH-amplified breast cancer cells [13].

In our research, azacoccone E was found by screening in-house database of NPs and found to have an optimal IC_{50} of 9.8 ± 4.3 μM. The enzyme activity kinetics study confirmed that the azacoccone E was a non-competitive inhibitor in a time-dependent manner. The further MST method and the cell level CETSA assay were performed to verify that azacoccone E directly bound to PHGDH. Meanwhile, azacoccone E selectively inhibited PHGDH-amplified cancer cell proliferation and showed a dose-dependent proapoptotic activity in Hela cells. Molecular docking demonstrated that azacoccone E coordinated at the allosteric site of PHGDH, which is essential for the ability of diminishing the enzyme activity. Moreover, azacoccone E is the first PHGDH inhibitor derived from natural products and provides a new scaffold for the study of anti-tumor drug research targeting PHGDH. In our future research, more detailed anti-tumor mechanisms of azacoccone E will be studied, and more pre-clinical evaluations will be performed.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC) (grant numbers 81773637, U1703111, U1803122, and 81773594), the Fundamental Research Fund for the Central Universities (grant number 2017KFYXJJ151), Liaoning Province Natural Science Foundation (grant number 201602689). We acknowledge support from Schrödinger Release 2018-4: Maestro, Schrödinger, LLC, New York, NY, 2018.

Competing interests

The authors have declared that no competing interest exists.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.02.037.

References

[1] J.S. Metcalf, et al., L-serine: a naturally-occurring amino acid with therapeutic potential, Neurotox. Res. 33 (1) (2018) 213–221.
[2] C.K. Zogg, Phosphoglycerate dehydrogenase: potential therapeutic target and putative metabolic oncogene, J. Oncol. 2014 (2014) 524101.
[3] M.E. Pacold, et al., A PHGDH inhibitor reveals coordination of serine synthesis and one-carbon unit fate, Nat. Chem. Biol. 12 (6) (2016) 452–458.
[4] T.J. de Koning, et al., L-serine in disease and development, Biochem. J. 371 (Pt 3) (2003) 653–661.
[5] E. Mullarky, et al., Identification of a small molecule inhibitor of 3-phosphoglycerate dehydrogenase to target serine biosynthesis in cancers, Proc. Natl. Acad. Sci. USA 113 (7) (2016) 1778–1783.
[6] J.W. Locasale, et al., Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis, Nat. Genet. 43 (9) (2011) 869–874.
[7] R. Possemato, et al., Functional genomics reveal that the serine synthesis pathway is essential in breast cancer, Nature 476 (7360) (2011) 346–350.
[8] R.N. Kharwar, et al., Anticancer compounds derived from fungal endophytes: their importance and future challenges, Nat. Prod. Rep. 28 (7) (2011) 1208–1228.
[9] R.X. Tan, W.X. Zou, Endophytes: a rich source of functional metabolites, Nat. Prod. Rep. 18 (4) (2001) 448–459.
[10] S. Zou, et al., Azacoccones A-E, five new aza-epicoccone derivatives from Aspergillus flavipes, Fitoterapia 124 (2018) 127–131.
[11] M.T. Islam, et al., Anticancer activity of thymol: a literature-based review and docking study emphasized on its anticancer mechanisms, IUBMB Life (2018).
[12] J.E. Unterlass, et al., Structural insights into the enzymatic activity and potential substrate promiscuity of human 3-phosphoglycerate dehydrogenase (PHGDH), Oncotarget 8 (61) (2017) 104478–104491.
[13] Q. Wang, et al., Rational design of selective allosteric inhibitors of PHGDH and serine synthesis with anti-tumor activity, Cell Chem. Biol. 24 (1) (2017) 55–65.
[14] W. Sun, et al., Novel small molecule 11beta-HSD1 inhibitor from the endophytic fungus Penicillium commune, Sci. Rep. 6 (2016) 26418.
[15] F. Bao, et al., New natural inhibitors of hexokinase 2 (HK2): Steroids from Ganoderma sinense, Fitoterapia 125 (2018) 123–129.
[16] Z. Li, et al., The dietary compound luteolin inhibits pancreatic cancer growth by targeting BCL-2, Food Funct. 9 (5) (2018) 3018–3027.
[17] D. Mahendiran, et al., In vitro and in vivo anti-proliferative evaluation of bis(4'-(4-tolyl)-2,2':6',2"-terpyridine)copper(II) complex against Ehrlich ascites carcinoma tumors, J. Biol. Inorg. Chem. 22 (7) (2017) 1109–1122.
[18] Y. Gao, et al., Farnesyl phenolic enantiomers as natural MTH1 inhibitors from Ganoderma sinense, Oncotarget 8 (56) (2017) 95865–95879.
[19] S. Ravez, et al., Challenges and opportunities in the development of serine synthetic pathway inhibitors for cancer therapy, J. Med. Chem. 60 (4) (2017) 1227–1237.