

Original Article

Inhibitory effect of isothiocyanate derivant targeting AGPS by computer-aid drug design on proliferation of glioma and hepatic carcinoma cells

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Abstract: Lipids metabolism was involved in the process of many types of tumor and alkylglycerone phosphate synthase (AGPS) was considered implicated in tumor process. Benzyl isothiocyanate (BITC) showed the inhibitory effect of tumor and AGPS activity, therefore, we screened a group of small molecular compound based on BITC by computer-aid design targeting AGPS and the results showed that the derivants could suppress the proliferation, the expression of tumor related genes such as survivin and Bcl-2, and the level of ether lipids such as lysophosphatidic acid ether (LPAe) and platelet activating factor ether (PAFe); however, the activity of caspase-3/8 was improved in glioma U87MG and hepatic carcinoma HepG2 cells *in vitro*.

Keywords: Computer-aid drug design, alkylglycerone phosphate synthase, glioma, hepatic carcinoma

Introduction

Previous studies have shown that the lipid metabolism by tumor cells is in a different way from that by normal cells, and the ether lipid level such as lysophosphatidic acid ether (LPAe) and platelet activating factor ether (PAFe) which was considered an oncogenic property is significantly increased in highly invasive tumor cells [1, 3, 8, 11]. Recent studies have found that alkylglycerone phosphate synthase (AGPS) is essential for ether lipid synthesis in tumor cells, the expression of which is increased and that suppression of AGPS can significantly reduce the invasiveness of tumor cells [5].

Computer-aid drug design (CADD) is an important application of computer technology and information technology in drug design and development process, which can identify specific binding ligands based on the structure and properties of the receptor by means of molecular docking, active site analysis and quantitative structure-activity relationship (QSAR).

We found that benzyl isothiocyanate (BITC) could inhibit tumor proliferation and invasion, as well as the activity of AGPS [2]. Therefore, we hypothesized the inhibition of AGPS activity was one of the mechanisms of BITC's anti-tumor effect. In this study, we screened a group of small molecular compound based on BITC by computer aid design targeting AGPS and explored the effect on the proliferation and the expression of tumor related gene in glioma U87MG and hepatic carcinoma HepG2 cells *in vitro*.

Materials and methods

Cell lines and cell culture

Human glioma U87 and hepatic carcinoma HepG2 cell lines were obtained from the American Type Culture Collection. Cells were grown and maintained in DMEM medium (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies) at 37°C with 5% CO₂.

Software and database

The computer-aid design in this study was performed on DELL T5500 workstation and Discovery Studio 3.5 software packages. CDocker was used as docking program. Three-dimensional structural model for AGPS (as 2UUV in PDBID) was obtained through downloading from the Brookhaven Protein Database (<http://www.rcsb.org/>) and small molecular structures were drawn by ChemBio-Office2010 and docking through processing with the Prepare Ligands Tool module of the package, including valence repair, 3D conformation generation and energy minimization regarding the small molecule.

MTS assay

Cells were cultured into a 96 well plate (5×10^3 cells/well) with 37°C, 5% CO₂ for 24 h. Then BITC or derivative compounds are added into medium with the final concentration of 0, 1, 2, 5, 10, 20, 50 and 100 µM respectively for 72 h. Then the medium was removed and MTS was added for another 4 h and OD value was measured at 490 nm. The tumor growth inhibition rate (%) = $(1 - OD_{\text{treatment}} / OD_{\text{control}}) \times 100\%$.

Western blotting

Cells were cultured into a 6 well plate (5×10^5 cells/well) with 37°C, 5% CO₂ for 24 h. Then 2 and 5 µM BITC or derivative compounds was added and cultured another 24 h. Cells were digested, lysed and centrifugation at 4°C to extract the supernatant to obtain the total protein. Electrophoresis was performed in 12% SDS polyacrylamide gel, and transferred the protein samples to a PVDF membrane. The membrane was incubated at 4°C with 5% skimmed milk overnight. AGPS (1:500), Survivin (1:500), Bcl-2 (1:500) and β-actin (1:5000) antibody was added and incubated at 4°C overnight. Then IgG labeled with horseradish peroxidase (1:2000) was added and incubated at room temperature for 1 h. The protein was visualized with the Phototope HRP Western blot detection system. β-actin was as an internal control.

Real-time PCR assay

Cells were cultured into a 6-well plate (5×10^5 cells/well) with 37°C, 5% CO₂ for 24 h. Then 2 and 5 µM BITC or derivative compounds were added and cultured another 24 h. The total mRNA was extracted and the mRNA expression

was detected by real-time PCR as following: decontamination at 60°C for 60 s, denaturation at 95°C for 65 s, followed by 40 cycles at 95°C for 30 s and at hybridization 65°C for 40 s. Primer Sequences were AGPS, Forward: 5'-ACCAGATTCCCTGGAGTTCA-3', Reverse: 5'-GAACCACCAGGTCCTCGATA-3'; Survivin, Forward: 5'-GCATGGGTGCCCCGACGTTG-3', Reverse: 5'-GCTCCGGCCAGAGGCCTCAA-3'; Bcl-2, Forward: 5'-ACGGGGTGAAGTGGGGGAGGA-3', Reverse: 5'-TGTTTGGGGCAGGCATGTTGACT-3'; β-actin, Forward: 5'-TGAGCGCGCTACAGCTT-3', Reverse: 5'-TCCTTAATGTCACGCACGATTT-3'. β-actin was as an internal control.

ELIASA assay

Cells were cultured into a 6 well plate (5×10^5 cells/well) with 37°C, 5% CO₂ for 24 h. Then 2 and 5 µM BITC or derivative compounds were added and cultured another 24 h. The activity of caspase-3/8 was measured in accordance with operating manuals (Promega, USA).

LC-MS/MS assay

Cells were cultured into a 6-well plate (5×10^5 cells/well) with 37°C, 5% CO₂ for 24 h. Then 2 and 5 µM BITC or derivative compounds was added and cultured another 24 h. Cells were collected and flash frozen at -80°C. LPA and PAFe were measured by Agilent 6430 LC-MS/MS in accordance with operating manuals.

Statistical analysis

The data is analyzed by SPSS11.0. One-way ANOVA is adopted to compare the data, with considering $P < 0.05$ as the standard of statistically significant. Each experiment is repeated for three times.

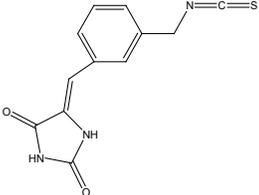
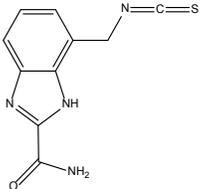
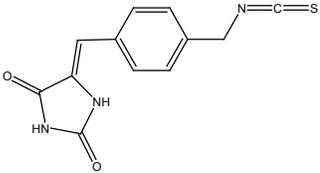
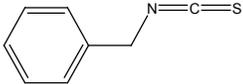
Results

Effects of BITC derivants on the proliferation of human glioma U87MG and hepatic carcinoma HepG2 cells

We designed 43 compounds and virtual screening was performed. There are three compounds were selected with better affinity to target point AGPS than that with parent compound BITC. Further, we found that there was an anti-tumor activity of above compound by MTS assay. The ranking of scores of docking between the small molecule and protein active sites and the IC₅₀ of above mentioned compounds are shown in **Table 1**.

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Table 1. Evaluation of small molecule compound binding AGPS

Compound structure	Score	IC50 (μM)	
		U87MG	HepG2
	19.8472	12.6	18.5
	17.3121	14.3	19.3
	16.3696	14.7	21.4
	15.7856	15.2	23.7

Effect of BITC derivants on the expression of AGPS and proliferation related gene of human glioma U87MG and hepatic carcinoma HepG2 cells

Western blotting and real-time PCR assay showed that there was a decreased protein and mRNA expression of AGPS, survivin and Bcl-2, however, ELISA assay showed that there was an increased activity of caspase-3/8 in human glioma U87MG and hepatic carcinoma HepG2 cells with 2 and 5 μM BITC derivants (compound 1-3) treatment as showed in **Figure 1**.

Effect of BITC derivants on the expression of proliferation related lipids of human glioma U87MG and hepatic carcinoma HepG2 cells

LC-MS/MS assay showed that there was a decreased expression of LPA and PAFe in human glioma U87MG and hepatic carcinoma HepG2 cells with 2 and 5 μM BITC derivants (compound 1-3) treatment as showed in **Figure 2**.

Discussion

Lipids metabolism was attributed to an important factor for tumor cells proliferation and

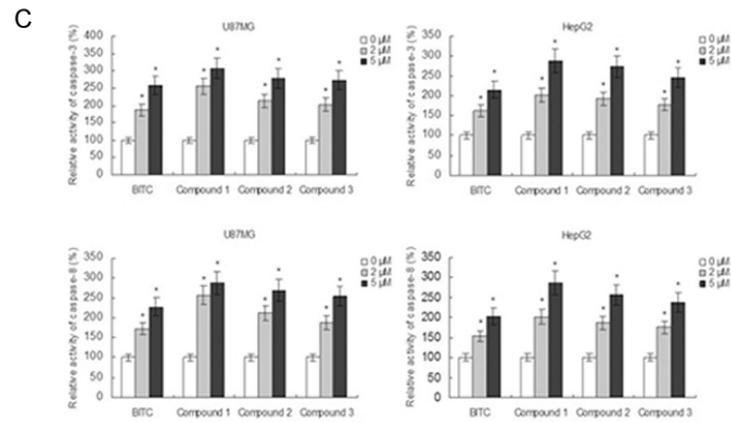
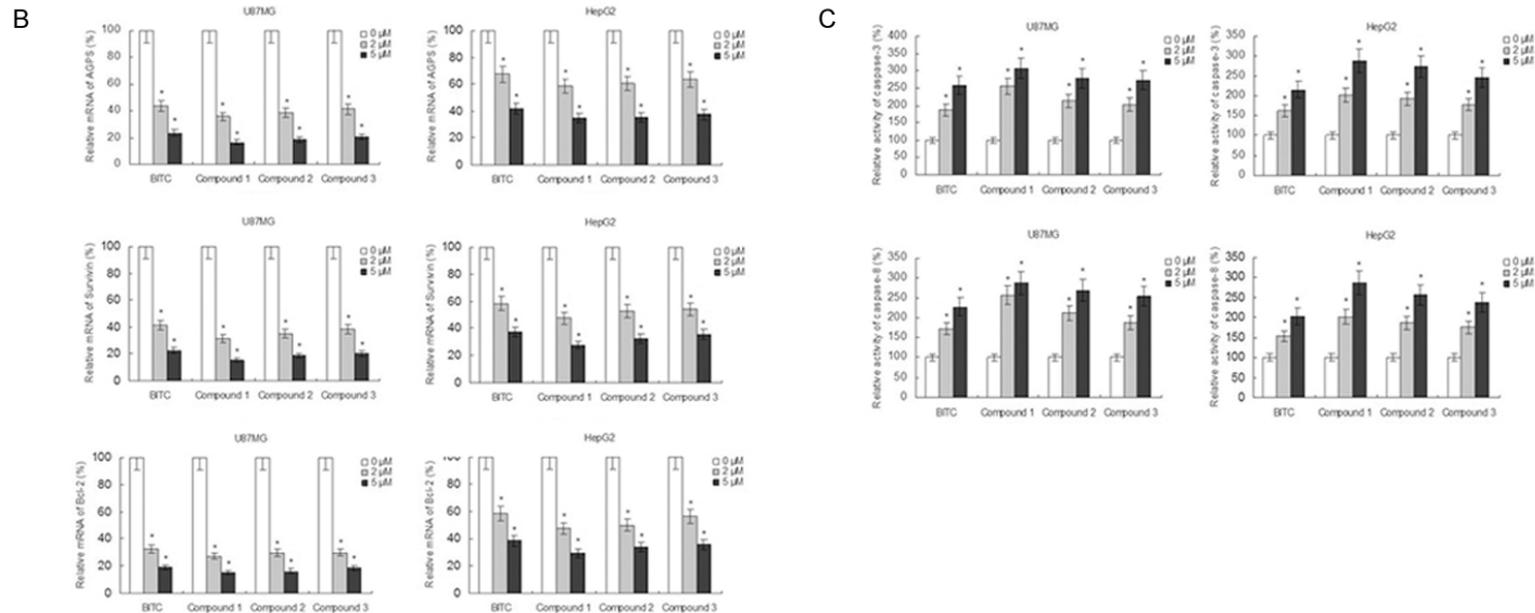
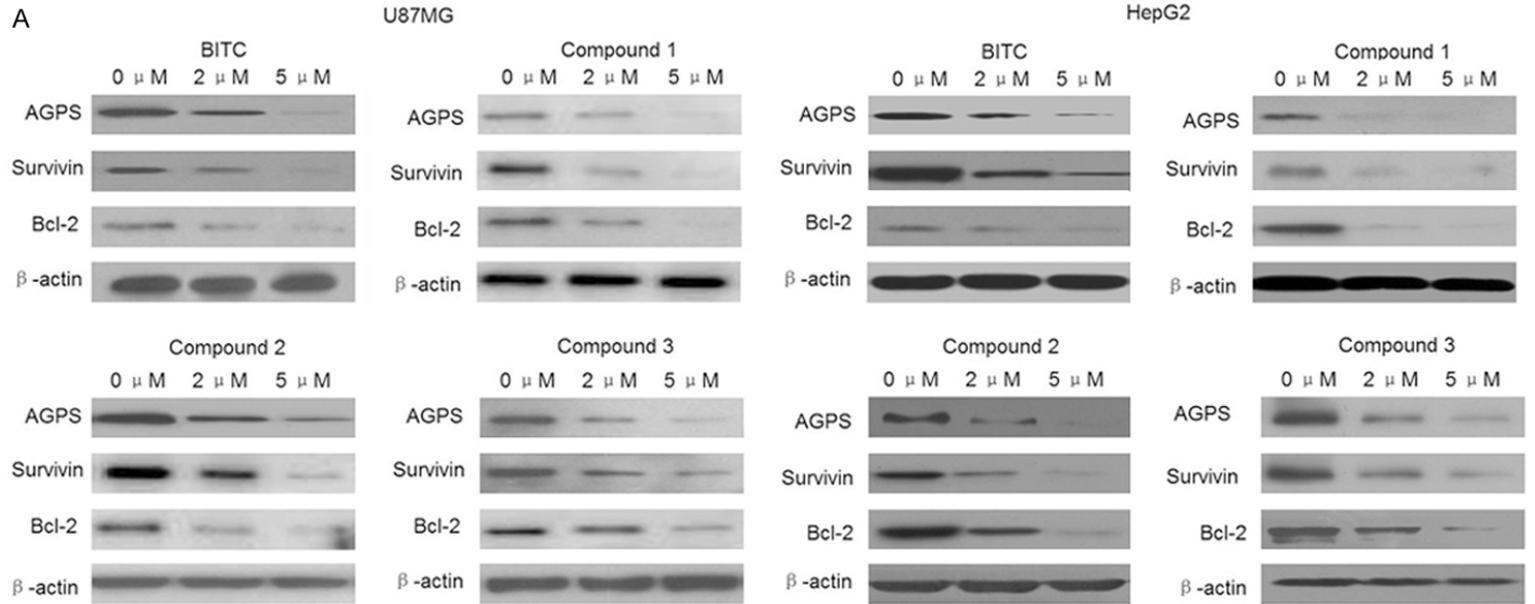
some studies have been showed that there was a higher level of ether lipids, for example lysophosphatidic acid-ether (LPAe) or platelet-activating factor-ether (PAFe), compared with normal cells. The alkylglycerone phosphate synthase (AGPS) which overexpressing in tumor cells was a critical enzyme for ether lipid synthesis. Therefore, it is considered that AGPS was a potential oncogene and drug target.

Benzyl isothiocyanate (BITC) showed the inhibitive effect of proliferation of human glioma U87MG and hepatic carcinoma HepG2 cells, meanwhile, we found that BITC could suppress the expression of AGPS in tumor cells our previous study [12]. Therefore, we screened a group of small molecular compound based on BITC by computer aid design targeting AGPS to explore potential therapeutic drug.

In this study, 43 compounds were designed based on the structure of BITC, and were selected 3 small molecule compounds with better ligand affinity better than BITC via computer simulation of compound-AGPS molecular dynamics. Then, we synthesized above 3 compounds and we found that there was a significant proliferation inhibitive potential and AGPS expression in human glioma U87MG and hepatic carcinoma HepG2 cells.

In order to explore the potential mechanism, we study the effect of derivants on proliferation related gene in human glioma U87MG and hepatic carcinoma HepG2 cells. We selected 2 μM (no toxicity concentration, inhibition ratio < 5%) and 5 μM (low toxicity concentration, inhibition ratio < 15%) to following study. We found that there was a decreased expression of Survivin and Bcl-2, however, there was an increased activity of caspase-3/8 which was the important proliferation related gene in human glioma U87MG and hepatic carcinoma HepG2 cells with BITC derivants treatment [7, 10]. LPA and PAFe were involved tumor process and LC-MS/MS assay showed that there was a decreased expression of LPA and PAFe with BITC derivants treatment, indicating above

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Figure 1. Effect of BITC derivants on the expression of AGPS and proliferation related gene of human glioma U87MG and hepatic carcinoma HepG2 cells. A. The western blotting assay showed that there was a decreased protein expression of AGPS, Survivin and Bcl-2 in human glioma U87MG and hepatic carcinoma HepG2 cells with BITC derivants treatment. β -actin was used for normalization. B. The real-time PCR assay showed that there was a decreased mRNA expression of AGPS, Survivin and Bcl-2 in human glioma U87MG and hepatic carcinoma HepG2 cells with BITC derivants treatment. β -actin was used for normalization. C. ELIASA assay showed that there was an increased activity of caspase-3/8 in human glioma U87MG and hepatic carcinoma HepG2 cells with BITC derivants treatment.

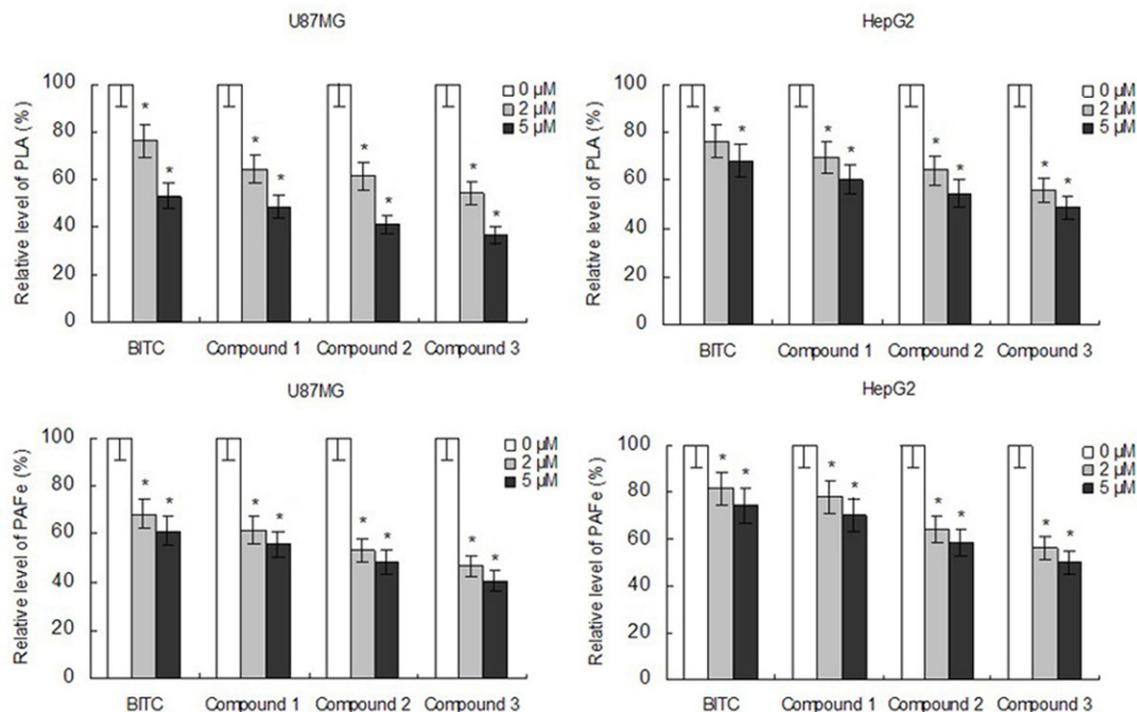


Figure 2. Effect of BITC derivants on the expression of proliferation related lipids of human glioma U87MG and hepatic carcinoma HepG2 cells. LC-MS/MS assay showed that there was a decreased expression of LPA and PAFe in human glioma U87MG and hepatic carcinoma HepG2 cells with BITC derivants treatment.

derivants could regulate metabolism of LPA and PAFe which may be due to the regulated expression of AGPS [4, 6].

Computer-Aided Drug Design (CADD) could conduce to compound design and optimization by simulating, calculating and estimating the relationship between the drug and biological macromolecular receptor via computer. We also found 3 potential anti-tumor drugs by CADD. However, BITC was restricted to clinical therapeutic due to the higher toxicity, therefore, we would tried to explore less toxicity derivant based on AGPS target to head into clinical application [9].

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Disclosure of conflict of interest

None.

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