Computer-aided design of temozolomide derivatives based on alkylglycerone phosphate synthase structure with isothiocyanate and their pharmacokinetic/toxicity prediction and anti-tumor activity *in vitro*

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Abstract. Despite the development of temozolomide (TMZ), a novel type of glioma therapeutic drug, malignant glioma remains to cause severe damage to human health. The present study aimed to utilize the molecular biological differences between tumor and normal cells to design TMZ derivatives with improved selectivity and targeting using computer-aided drug design (CADD). Taking alkylglycerone phosphate synthase (AGPS) as a target, a 3D structure-activity relationship model was built using CADD technology; molecular docking of isothiocyanate (ITC) and TMZ compounds was conducted; ITC-TMZ derivatives were designed; and predictions on the absorption, distribution, metabolism and excretion (ADME) processes and toxicity of the ITC-TMZ derivatives were established in order to obtain improved understanding of the structure-activity relationship of the candidate compounds. Using these techniques, it was identified that the docking scores of the structural derivatives S1-9 were higher than that of TMZ. Additionally, S3, -6, -7, -8, -9 and -10 exhibited enhanced ADME and similar toxicity to that of TMZ. The half maximal inhibitory concentrations of the CADD derivatives were also assessed in the glioma U87MG and U251 cell lines, and the activities of S1, -3, -8 and -10 were determined to be greater than that of TMZ, suggesting their potential as anti-cancer drugs with adequate AGPS targeting, ADME/toxicity and anti-tumor activity.

Introduction

Malignant glioma may cause severe damage to human health, and accounts for ~70% of primary malignant brain tumors, with an annual incidence of ~5/100,000 in China (1). Also in China, the number of new cases exceeds 14,000 each year (2). For patients with grade IV glioblastoma according to World Health Organization criteria (3), median survival time is between 14 and 17 months and total annual mortalities may reach 30,000 worldwide (4). Temozolomide (TMZ) is a novel type of glioma therapeutic drug, which is able to penetrate the blood-brain barrier to exert targeted therapeutic effects on brain tumor tissue (5,6). To date, studies have aimed to enhance the efficacy and reduce the toxic side effects of TMZ by reconstructing its chemical structure (7,8). To build on these previous studies, the present study utilized the molecular biological differences between tumor and normal cells to design TMZ derivatives with enhanced selectivity and targeting activities through rational computer-aided drug design (CADD).

Alkylglycerone phosphate synthase (AGPS) is considered to serve as an oncogene through its involvement in lipid metabolic processes associated with cancer pathogenicity (9). A previous study by our group demonstrated through RNA interference technology that silencing of AGPS expression in glioma and liver cancer cells inhibited the proliferation and invasion of the tumor cells, and improved the drug sensitivity of resistant cells (10). Thus, AGPS may be a therapeutic candidate for glioma therapy.

CADD may overcome the disadvantages of traditional drug development processes, including their long clinical trial cycle period, low combinatorial variety and low efficiency, by simulating the interaction between drugs and receptors

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of biological macromolecules (11,12). CADD has identified numerous drugs to date, and is becoming a core technology in the field of novel drug development (13). In particular, it may aid to identify more optimized TMZ derivatives with enhanced therapeutic effect in glioma patients.

On the basis of previous study (14), the current study has taken AGPS as a target, built a 3D structure-activity relationship model through CADD technology, and conducted molecular docking of isothiocyanate (ITC)-TMZ compounds. A total of 10 ITC-TMZ derivatives were identified as novel compounds with adequate targeting of AGPS. Additionally, predictions on the absorption, distribution, metabolism and excretion (ADME) processes and toxicity of the ITC-TMZ derivatives were established, along with anti-tumor activity *in vitro*, in order to elucidate the structure-activity relationship of the candidate compounds, and thus further optimize the structure of the lead compounds.

Materials and methods

Protein structure database. A 3D structure model of AGPS (Fig.1A) was downloaded from the Protein Data Bank (www.rcsb.org; PDB ID: 2UUV) and the protein modules were processed using Discovery Studio 3.5 software (BIOVIA, San Diego, CA, USA) as follows: A single chain (the A chain) in the tetramer was retained; missing atoms to residues were added; the loop area was repaired; combinations and free water molecules were removed; unsaturated atoms were hydrogenated; valence was repaired; protein and original ligands were retained; and six basic pharmacophore elements were selected, namely hydrogen bond acceptor, hydrogen bond donor, hydrophobic, positive electricity center, negative electricity center and aromatic center. This obtained a final 3D AGPS structure for docking. The structure of TMZ used is depicted in Fig. 1B (15).

Virtual screening. The structures of TMZ derivatives were combined with benzyl isothiocyanate (BITC) and the ITC-TMZ derivatives were drawn using ChemBioOffice 2010 (PerkinElmer, Waltham, MA, USA), and each structure was saved in mol2 format. The structures were imported into Discovery Studio 3.5 for virtual screening. The CDOCKER protocol in Discovery Studio 3.5 was used for molecular docking; 'CDOCKER energy' was used as a scoring index, with higher scores indicating greater docking affinity.

The experiment screened 50 molecules. Firstly, it used the pharmacophore model generated by the HipHop algorithm to perform fit value sorting of the compounds based on the characteristics of the pharmacophore model, with small molecular compounds with the highest scores retained (16). The pharmacophore model generated by the Counting Belief Propagation algorithm was then used on reserved compounds to obtain preferable compounds that conformed to the characteristics of the two pharmacophore types (17). BITC was used as a control for the virtual screening.

ADME and toxicity prediction. Protein structure processing was completed with the QikProp module of Schrodinger Suite 2009 software (Schrodinger, LLC, Cambridge, MA, USA). The downloaded AGPS target crystal structure (PDB ID: 2UUV)
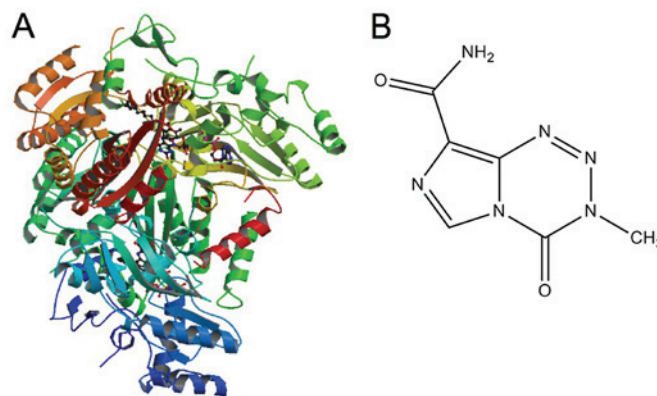

Figure 1. Structures of AGPS and TMZ. (A) 3D structural model of AGPS; (B) structure of TMZ. TMZ, temozolomide; AGPS, alkylglycerone phosphate synthase.

was imported into the Schrodinger software and processed as follows: Metal ions were removed; unsaturated atoms were hydrogenated; amino acid residues at the N and C termini were made neutral; and protein and original ligands were retained to remove atom and water molecule impurities. The OPLS_2005 position was selected to optimize protein structure and utilize sample water orientations as parameters to optimize hydrogen bonding.

Ligand preparation utilized ChemBioDraw Ultra 11.0 in the ChemBioOffice 2010 to draw planar structures of the micromolecules, which were imported into ChemBio3D Ultra 11.0 for the generation of 3D structures, which were saved in mol2 format. The mol2fileswere then imported into Schrodinger Suite 2009 software and processed with the LigPrep module, complete desalting, add charge and produce ionization consistent with human body pH. The OPLS_2005 position was also selected for the optimized molecules to match that of the optimized acceptor. ADME parameters, namely oil-water partition coefficient (logPo/w), polarization surface area (PSA), water solubility (logS) and apparent Maden Darby Canine Kidney cell permeability (PMDCK), and toxicity factors, namely rodent carcinogenicity, mutagenicity, skin irritancy, ocular irritation and aerobic biodegradability, were subsequently simulated in the Schrodinger Suite 2009 software.

Cell lines and culture. The human glioma cell lines U87MG and U251 were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. It is important to note that U87MG has been identified to differ from the original cell line, and is probably an authentic human glioblastoma cell line of unknown origin according to DNA profiling (18).

MTS assay. A total of 5x103 cells/well were cultured in 96-well plates at 37˚C with 5% CO_2 for 24 h. ITC-TMZ derivatives were designed and synthesized via CADD (14) and added to the medium at final concentrations of 0 (negative control), 1, 2, 5, 10, 20, 50 and 100 μM, respectively. Subsequently, the cells were cultured for 72 h at 37˚C, after which the medium was

Figure 2. Structures, combination models with alkylglycerone phosphate synthase target and docking scores of isothiocyanate-temozolomide derivatives (S1-10). S, structural derivative; BITC, benzyl isothiocyanate.

replenished with 200 μl plasma-free medium and 40 μl MTS, followed by incubation at 37˚C for another 4 h. The optical density (OD) value was measured using an enzyme-labeled meter at 490 nm. Inhibition rate (%) was calculated as follows: (1-OD value of the medicine-treated group/OD value of the negative control group) x 100%. Half maximal inhibitory concentrations (IC50s) were subsequently determined using GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Virtual screening and core hopping. Visual screening of 50 small molecular compounds was performed using Schrodinger Suite 2009. A total of 10 ITC-TMZ derivatives and the compound BITC exhibited micromolecular structures with improved combination with the target protein AGPS compared with TMZ, determined by referring to the combination mode of the micromolecules and surrounding amino acids, and evaluation of scoring function from CDOCKER energy. The scoring of the docking between these micromolecules [structures (S)1-10 and BITC] and the AGPS active site is presented in Fig. 2. The 11 candidate compounds exhibited marked interactions with the protein cavity, with key amino acid residues forming hydrogen bonds and the hydrophobic effect occurring with nitrogen and sulfur atoms and carbon-sulfur covalent bonds of the ITC-TMZ derivative molecules. The other substituent residues in the micromolecules formed certain hydrogen bonding interactions with surrounding residues to an extent. As a result, as evident in the pattern diagrams (Fig. 2), the main ITC active group and small regions of substitute

Table I. ADME predictions of isothiocyanate-TMZ derivatives.

Candidate	ADME parameter			
	PSA[a]	logPo/w[b]	logS[c]	PMDCK[d]
S1	204.93	0.596	-4.40485	13.345
S2	205.35	1.243	-5.29240	10.342
S3	147.15	2.015	-4.10003	111.705
S4	162.39	0.869	-4.41749	46.410
S5	171.62	0.896	-4.14306	50.200
S6	167.32	1.560	-4.32601	70.105
S7	147.15	2.536	-4.94407	183.872
S8	147.15	2.99	-5.45929	481.063
S9	147.15	3.623	-6.24712	121.998
S10	147.15	3.293	-5.66361	875.571
BITC	44.45	3.321	-2.5125	10,000.000
TMZ	79.92	-1.810	-1.1118	66.744

Parameter reference ranges: [a]7.0-200.0; [b]-2.0-6.5; [c]-6.5-0.5; [d]<25 indicates poor suitability and >500 indicates high suitability for drug development (16). ADME, absorption, distribution, metabolism and excretion; PSA, polarization surface area; logPo/w, oil-water partition coefficient; logS, water solubility; PMDCK, apparent Maden Darby Canine Kidney cell permeability; TMZ, temozolomide; S, structural derivative; BITC, benzyl isothiocyanate.

residues in the TMZ derivatives with some impurity atoms were respectively connected to both sides of the hydrophobic and rigid ring planes. These structural data suggest improved interactions and combination of the ITC-TMZ derivatives with the AGPS target compared with TMZ. Indeed, the docking scores of S1-9 and BITC were increased compared with that of TMZ, suggesting enhanced AGPS targeting.

ADME and toxicity predictions. ADME properties, including logPo/w, PSA, logS and PMDCK, and toxicity properties, including rodent carcinogenicity, mutagenicity, skin irritancy, ocular irritation and aerobic biodegradability, aid to determine whether therapeutic candidates are of clinical standard (16). The present results on ADME (Table I) and toxicity (Table II) parameters indicated the 10 ITC-TMZ derivatives to have improved interaction with AGPS. Firstly, the results suggested adequate ADME parameters for BITC regarding physico-chemical properties. In turn, the ADME parameters of S3, -6, -7, -8, -9 and -10 were optimized compared with TMZ, particularly regarding logPo/w, logS and PMDCK, suggesting the structure of BITC may improve TMZ physico-chemical properties. Meanwhile, the general toxicity parameters of the above compounds were not increased according to the predictions.

Anti-tumor activity of ITC-TMZ derivatives. MTS assay was performed to observe the effect of the ITC-TMZ derivatives on the viability of human glioma U87MG and U251 cells *in vitro*. On determination of IC50 values, S1, -3, -8 and -10 were identified to exert greater inhibitory effect on glioma cell viability compared with TMZ (Table III).

Discussion

In 1920, cancer was described as a metabolic disease by Otto Warburg, due to his identification of the high glycolytic rate

of tumor cells (19). A subsequent study demonstrated that the process of tumor development is accompanied by abnormal lipid metabolism (20). Notably, inactivation of the key enzyme for ether lipid synthesis, AGPS, has been reported to reduce ether lipid levels in tumor cells and thus decrease cancer pathogenicity (10). Additionally, its overexpression may enhance the ether lipid levels of tumor cells, increase the survival and motility of tumor cells, and promote the growth and invasion of tumors (21). Therefore, AGPS is considered to be a novel target of antitumor drugs, with specific inhibitors expected to have marked advantages over traditional chemotherapy methods. The characteristic elements of an effective inhibitor include the following: A hydrogen bond acceptor, a hydrogen bond donor, hydrophobicity, a positive electricity center, a negative electricity center and an aromatic center (22,23). The present study identified elevated docking scores for S1-9 compared with TMZ, suggesting enhanced AGPS targeting over TMZ.

ADME and toxicity are considered as important drug properties (24). In previous years, drug discovery principally focused on the discovery of active compounds. However, prior to the development stage, this method encountered problems regarding pharmacokinetics (PK), toxicity, solubility and stability (25). A previous study reported that drug failure in the developmental stage typically occurred due to poor biopharmaceutical properties (PK and bioavailability) (26). Due to high development costs, this failure results in major economic loss in the field of drug development. Therefore, it may be concluded that drug ADME and toxicity are now key considerations in the process of drug discovery, and may be used to guide the selection and optimization of lead compounds.

The present study identified that while the ADME parameters of TMZ were suitable, those of BITC were enhanced regarding physico-chemical properties, thus implicating BITC as a favorable modification for TMZ. Additionally,

Table II. Toxicity prediction of isothiocyanate-TMZ derivatives.

Candidate	Mouse female NTP prediction	Mouse male NTP prediction	Rat female NTP prediction	Rat male NTP prediction	Ames test prediction	Skin irritancy	Ocular irritancy	Aerobic biodegradability prediction
S1	NC	NC	NC	NC	NM	Mild	Moderate	ND
S2	NC	NC	NC	NC	NM	Mild	Moderate	ND
S3	NC	NC	NC	NC	NM	Mild	Moderate	ND
S4	NC	NC	NC	NC	NM	Mild	Moderate	ND
S5	NC	NC	NC	NC	NM	Mild	Moderate	ND
S6	NC	NC	NC	NC	NM	Mild	Moderate	ND
S7	NC	NC	NC	NC	NM	Mild	Moderate	ND
S8	NC	NC	NC	NC	NM	Mild	Moderate	ND
S9	NC	NC	NC	NC	NM	Mild	Moderate	ND
S10	NC	NC	NC	NC	NM	Mild	Mild Severe	ND
TMZ	NC	NC	NC	NC	NM	None	Mild	ND
BITC	NC	NC	C	C	NM	Mild	Severe	ND

NTP, National Toxicology Program; NC, non-carcinogen; C, carcinogen; NM, non-mutagen; ND, non-degradable; TMZ, temozolomide; S, structural derivative; BITC, benzyl isothiocyanate.

Table III. IC50 values of isothiocyanate-TMZ derivatives in human glioma cell lines.

Candidate	IC50 (μM)	
	U87MG	U251
S1	21.7	28.3
S2	35.8	47.4
S3	24.5	31.2
S4	115.2	168.5
S5	76.7	62.5
S6	98.6	87.1
S7	87.4	88.5
S8	41.8	32.2
S9	157.6	91.5
S10	45.5	35.8
BITC	15.2	18.7
TMZ	54.5	37.5

IC50, half maximal inhibitory concentration; TMZ, temozolomide; S, structural derivative; BITC, benzyl isothiocyanate.

the physical and chemical ADME parameters of S3, -6, -7, -8, -9 and -10 were optimized compared with TMZ, particularly regarding logPo/w, logS and PMDCK. The thiocyanate group may serve a key role in these improvements. However, all of the derivatives exhibited similar toxicity to TMZ, suggesting the thiocyanate group is unable to decrease the toxicity of TMZ.

Nevertheless, the ADME parameters of S3, -6, -7, -8, -9 and -10 indicated the suitability of these candidates as potential clinical drugs, which thus require further study. Additionally, the present study investigated the activities of S1-10 *in vitro* following their synthesis, and identified that the ITC-TMZ derivatives S1, -8 and -10 suppressed the proliferation of U87MG and U251 cells to a greater extent than TMZ, again indicating their potential as anti-cancer drugs. Due to the established anti-tumor activity of TMZ, the thiocyanate group may also be key to proliferation inhibition.

In conclusion, the present study identified the ITC-TMZ derivatives S1, -8 and -10 to be potential anti-cancer drugs. The results for S8 and -10 were in accordance with the predictions by CADD, while S1 exhibited more optimal activity than predicted from docking score. Thus, the thiocyanate group may be involved in a complex mechanism influenced by differences in simulated and in situ environments, compound stability and cell membrane interference, among other factors, which warrants further investigation. Additionally, although U87MG was implicated to be of unknown origin, albeit an authentic human glioblastoma cell line, the results indicated the anti-tumor activity of S1, -3, -8 and -10 against glioblastoma.

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Availability of data and materials

All data and materials relevant to this study are described in this published article or available from the corresponding author on reasonable request.

Authors' contributions

BY was responsible for cell experiments, XL for computer-aided drug design, LH for the MTS assay and YZ for the study design.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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