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Identification of novel inhibitors of GLUT1 by virtual screening and cell-based assays

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Abstract: In order to fuel the uncontrolled cell proliferation and division, tumor cells reprogram the energy metabolism to Warburg effect, where glucose is favorite converted by glycolysis even in the presence of oxygen. However, the high energetic demand of tumor cells require upregulating the expression of glucose transporters, notably GLUT1, which substantially increases glucose uptake into cytoplasm. GLUT1 is overexpressed in a variety of tumor cells and is likely to be a potential drug target for the pan-cancer treatment. Although many small molecules were reported to inhibit the glucose uptake function by various measurements, several shortcomings such as weak binding affinity, low specificity of the known inhibitors demand the identification of alternative inhibitors with novel scaffolds. In this study, we performed a virtual screening campaign by docking each compound from Chemdiv database to the glucose binding pocket based on the crystal structure of GLUT1 (PDB ID 4PYP) and four small molecules with novel scaffolds were identified to inhibit the glucose uptake of cancer

cells at the sub-micromole levels. The identified compounds may serve as starting points for the development of anti-cancer drugs by manipulating the energy metabolism.

Keywords: GLUT1, Glucose, Virtual screening, Inhibitors, Cancer

1 INTRODUCTION

ATPs (adenosine-triphosphates) serve as the main energetic molecules for biological cells and the production of ATP can be divided into two pathways: oxidative phosphorylation reaction and glycolysis. However, the energetic efficiency of OP is around 18 fold higher than that of glycolysis. In the presence of oxygen, normal cells acquire ATPs mainly from OP, while cancer cells get ATPs through glycolysis, known as the "Warburg effect"[1,2], although cancer cells require more ATPs for their uncontrolled proliferation and division. In order to compensate the low energetic efficiency of glycolysis, cancer cells need much higher amount of glucose than normal cells to meet their metabolic needs. Since the glucose transport of tumor cells is mainly contributed by GLUT1, up-regulating the expression of GLUT1 protein is the main strategy for tumor cells to increase the glucose uptake, which can be observed in various cancer cells such as colorectal cancer[3-5], lung cancer[6], non-small cell lung cancer[7,8], breast cancer[7,9], renal cell carcinoma[10,11], pancreatic cancer[12], cervical cancer[13], and so on. Therefore, inhibition of the glucose transport function of GLUT1 protein can decrease the energy metabolism of tumor cells and eventually affect the proliferation and division.

GLUT1 has a very broad substrate specificity and can transport a variety of aldoses including pentose and hexose[14,15]. GLUT1 is the most important energy carrier of the brain. It exists in the blood-brain barrier and assures the energy-independent, facilitative transport of glucose into the brain[16]. GLUT1 comprises 492 amino acid

with 12 transmembrane regions, which renders the difficulties in the process of protein purification and the study of its 3D structures until recent[17]. The crystal structure of human GLUT1 reveals the allosteric mode of GLUT1 and the important amino acids in the key region. It points out that GLUT1 has four allosteric modes in the process of glucose transport, and the main region where allosteric occurs is the N-terminal domain, and the main function of the C-terminal is to provide a substrate binding site. This provides a prerequisite for structure-based drug design[18,19]. The research on GLUT1 inhibitors has made great progress in recent years since the energy metabolism of cancer cells was well elucidated. A variety of compounds such as apigenin, naringenin and quercetin were reported to inhibit GLUT1 but the mechanism of action of these compounds are diverse. For example, apigenin directly down-regulates the expression of GLUT1 to affect the glucose uptake level of cells[20]. Naringenin inhibits the glucose uptake function of GLUT1 by affecting the activity of phosphoinositide 3-kinase[21], while quercetin belongs to the tyrosine kinase inhibitor[22]. Therefore, these compounds inhibit the glucose uptake but they did not directly target GLUT1. Theophylline[23], resveratrol[24], BAY-876[25], WZB117[26], STF-31[10] were reported to directly bind to GLUT1 and inhibit the transport of glucose. The discovery of compounds BAY-876 and STF-31 were through high-throughput screening of compound database, while the compound WZB117 was accidentally identified to have GLUT1 inhibitory function during the development of diabetes drugs. Although these compounds have inhibitory effects on GLUT1, they generally have problems such as low inhibition rate, poor specificity, and poor druggability, and that may be the reason why they are still premature for clinical applications. Therefore, the identification of alternative inhibitors of GLUT1 with novel scaffolds are needed. In this study, we performed a virtual screening campaign by FRED package through the entire Chemdiv database to evaluate the binding affinity of each compound to the glucose binding

pocket of GLUT1 and four small molecules with novel scaffolds were identified to inhibit the glucose uptake of cancer cells at the sub-micromole levels.

2 Materials and methods

2.1 Acquisition and preparation of GLUT1 3D structure

Crystal structures of GLUT1 in complexed with glucose were retrieved from the Protein Data Bank (<http://www.rcsb.org>, PDB ID 4PYP, there are two mutations, N45T and E329Q), which was optimized by removing water molecules, salt and ions, and completing the missing atoms, then protonated at the conditions of pH 7.0, 27°C, Salt concentration 0.1 M, with the force field of Amber10: EHT by using the protonate 3D plug-in, Molecular Operating Environment (MOE) (Version 2019, Chemical Computing Group Inc, Canada).

2.2 Multiple conformation generation for Chemdiv compounds

The Chemdiv compound library (Version 2019, <https://www.chemdiv.com>) contained 1,535,478 compounds. The Chemdiv database was selected to screen potential GLUT1 inhibitors. The compounds were optimized by removing solvates and minimized with a forcefield of Amber10: EHT. Omega2[27] (Version 3.0.1.2, OpenEye Scientific Software Inc.) was used to create multiple conformers for each compound from Chemdiv with default parameters.

2.3 Determination of small molecule binding pockets and molecular docking

MOE-SiteFinder was used to determine all possible pockets on GLUT1 and three potential pockets were detected and evaluated. The first pocket (Pocket1) had a relative

bid volume and contained the glucose binding site (Q283 N288 N317 E380). The second pocket (Pocket2) had a small volume but also contained glucose binding site (Q283 N288 N317 E380). The last pocket (Pocket3) was detected in the ICH domain, which may function as a latch to secure closure of the intracellular gate in the outward-facing conformation. 122 known inhibitors of GLUT1 were retrieved from BindingDB (www.bindingdb.org) and was used as active compounds in the dataset called EvaData, while 7552 decoy compounds based on the active compounds were generated by DUD-E (<http://dude.docking.org>). The constructed dataset EvaData was used to evaluate the screening efficiency for each pocket by calculating the enrichment curve (EC).

The structure of GLUT1 were protonated and optimized by MOE. The processed GLUT1 structure were then converted to the formatted receptor files which defined the docking box by using apopdb2receptor module of OpenEye software (Version 3.2.0.2, OpenEye Scientific Software Inc.). OEDocking[28] distribution (Release 3.0, OpenEye Scientific Software Inc) was used to dock all conformations of compounds to the docking box of GLUT1. Molecular docking was performed through FRED's (Ver3.2.0.2, OpenEye, Scientific Software Inc.) rigid docking algorithm, and Chemgauss4 was used to calculate the binding affinity between compounds and receptor. The parameters for running molecular docking were set as default in rigid docking protocol.

To study the binding modes of four identified inhibitors, a flexible docking approach were applied to simulate the interaction between SMI290, SMI291, SMI428 and SMI433 by using Induced Fit docking protocol in MOE package, where the London delta G scoring function was used for optimization, and the GBVI/WAS scoring function was applied to predict the binding affinity.

2. 4 Compound selection by structural diversity and drug properties

Stardrop (Release 6.5.0, Optibrium Ltd, UK) was used to calculate the structural

diversity based on a maximum common substructure algorithm to group together compounds containing a significant common substructure. The oral noncentral neural system drug profile (non-CNS drug score) considered molecular properties such as logS, HIA category, logP, hERG, 2D6, 2C9 affinity, P-gp category, PPB90, and BBB as a scoring function to evaluate the drug properties of each molecule.

2.5 Acquisition of small molecules and cell lines

All small molecules used in the experiment were purchased from TargetMol, and they were diluted to the concentration of 10 mM in DMSO, and stored at -80°C. Human colorectal cancer cell line (HT29) was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and grown in a humid environment at 37°C and 5% CO₂.

2.6 In vitro glucose uptake

HT29 cells were seeded in a 96-well plate at 1.5×10^5 per well and the original medium was replaced with sugar-free medium after 12 hours. After 2 hours of starvation, the cells were treated with different inhibitors for 30 min, then 20 μM 2-NBDG (2-NBDG is the fluorescence analogue of glucose, APExBIO, item number: B6035) was added for 30 min incubation. The HT29 cells were washed with buffer PBS (135 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄ and 8 mM K₂HPO₄, pH 7.2) and the fluorescence intensity of HT29 cells was measured by the microplate reader (Ex/Em 488/542 nm using SpectraMax iD5, MD Inc.).

2.7 In vitro lactate production

HT29 cells were seeded in a 48-well plate at 2×10^5 per well for 12 hours incubation and then the purchased compounds with a concentration of 50 μM were added. After 24 hours of incubation, the cell culture supernatant was collected for determination of lactate production by using the lactic acid content assay kit (Solarbio, China, item

number: BC2235). The cells were collected *via* trypsinization for protein concentration measurement by pierce bicinchoninic acid (BCA) protein assay (Pulilai Gene Technology Co., Ltd., Beijing, China, item number: P1513).

3 results

3.1 Performance of the virtual screening campaign

MOE-SiteFinder was used to determine all possible pockets on GLUT1 and three potential pockets (Pocket1, Pocket2, Pocket3) were detected (Fig.2a). Pocket1 had a relatively big volume accommodating about 352 atoms in size. The pocket contains 89 hydrophobic atoms which may contribute to the ligand binding. The PLB score of the pocket was 3.58 (the value greater than 0 is acceptable, and the larger the better[29]). The amino acids comprising the pocket are F26 T30 F72 S73 S80 V83 N88 R92 T137 G138 P141 M142 G145 E146 R153 G154 A155 G157 T158 H160 Q161 I164 V165 I168 R212 I216 N217 E247 S248 M251 M252 E254 K255 K256 Q282 Q283 I287 N288 F291 Y292 N317 T321 S324 L325 F326 V328 Q329 F379 E380 G384 P385 W388 F389 A392 Q397 G398 R400 P401 I404 A405 G408 N411 W412 N415(Fig.2c and Table.1). Pocket2 is not obtained through MOE-SiteFinder. We use the binding mode of glucose and GLUT1 in the crystal structure as a template to dock glucose and GLUT1 to obtain Pocket2 containing all the amino acids that interact with glucose. The amino acids comprising the pocket are F26 T30 F72 Q161 I164 V165 I168 Q282 Q283 I287 N288 F291 Y292 T321 S324 L325 V328 F379 E380 G384 P385 W388 F389 N411 W412 N415 (Fig.2c and Table.1). Pocket3 had a volume accommodating about 85 atoms in size. The pocket contains 24 hydrophobic atoms which may contribute to the ligand binding. The PLB score of the pocket was 0.94. The amino acids comprising the pocket are N34 A35 P36 Q37 K38 V39 E41 P58 T59 L61 T62 G175 L176 D177 G181

T295 S296 E299 K300 V307 Q423 E426 Q427(Fig.2c and Table.1).

The constructed dataset EvaData was used to evaluate the compound screening efficiency of the virtual screening performance based on three pockets (Table.2). The compounds of EvaData was docked to each pocket by using the FRED based rigid docking protocol and the docking scores of each compound to different pockets were different (Fig.2b). The regions in the Pocket1 and Pocket2 are suitable for ligand binding but the region in the Pocket3 area is probably not for ligand binding, because most of the compounds at this area had positive scores of the binding energy (Fig.2b). The distribution of the docking scores in Pocket1 had a wide range indicating that the Pocket1 area had a better distinguish capability than that in the Pocket2 area (Fig.2b). The enrichment curves were then calculated for comparing the performance of the Pocket1 based virtual screening and Pocket2 based virtual screening (Fig.2d). The comparable results indicated both pockets are potential ligand binding sites and either of the regions is acceptable for a docking area for virtual screening. Therefore, in order to maximize the hit rate, we merged both Pocket1 and Pocket2 regions as the docking area for the virtual screening campaign against Chemdiv compounds (Fig.2c).

FRED was used to perform the virtual screening campaign, where the multiple conformations of each compound from Chemdiv were docking to the selected region of GLUT1. The top five thousand compounds with the best docking scores were retained (Fig.3a). Their scoring values are distributed between -21.8 kcal/mol to -17.0 kcal/mol (Fig.3a), which basically performed better than that in the EvaData (Fig.2b). Amongst, 1019 compounds had a docking score < -18 kcal/mol, 127 compounds had a docking score < -19 kcal/mol, 29 compounds had a docking score < -20 kcal/mol, 3 compounds had a docking score < -21 kcal/mol.

3.2 Analysis of the drug properties and structural diversity of screened compounds

The solubility (log S value) and the molecular weights of the top 5000 compounds were calculated by Stardrop (Version 6.5.0), which were further used to screen the potential inhibitors. Based on the docking score and solubility, 135 compounds were kept from the top 5000 compounds (Fig.3a). 18 out of 135 compounds had higher molecular weight and relative weak binding affinity were filtered out (Fig.3b)

Stardrop software (Version 6.5.0) was used to calculate and analyze the chemical properties such as molecular weight, flexibility, hydrogen bond properties, surface accessible area (TPSA), metabolic degradation (CYP2C9), hERG inhibition, intestine absorption (HIA), blood-brain barrier penetration, and the oral noncentral neural system drug profile (non-CNS drug score) was used to evaluate the drug properties of the screened compounds (Fig.3c). Drug score is a mathematical statistics of the above mentioned properties. The drug score ranges from 0 to 1, where the higher of the value indicates higher chance of a compound becoming the potential drug. Drug score was then used to evaluate the drug properties of 117 compounds, and the value distribution range was 0.4-0.7 (Fig.3c). In order to further analyze the structural diversity of these 117 compounds, the compounds were clustered into 14 groups based on the common substructure, where the similarity threshold was set to 0.7 (Fig.3c). Based on the structural diversity and drug score, 23 compounds with structural diversity and good drug properties were selected. These compounds are not structurally similar with the known GLUT inhibitors, indicating the novelty of these compounds structurally (Fig.3d).

3.3 Glucose uptake assays

In order to evaluate the glucose transport inhibitory of 23 small molecules obtained by

virtual screening and drug characteristic analysis, cell based glucose uptake assays were performed (In this experiment, we used 2-NBDG, a fluorescent analog of glucose, to replace glucose for cell uptake, and the glucose transport level was evaluated by detecting the fluorescence intensity of 2-NBDG uptake by cells.), where a known GLUT1 inhibitor WZB117 was set as positive control. We firstly checked the expression level of GLUT1 in different cancer cell types (Colorectal cancer cell lines HT29 and RKO) by western blot, and the results revealed the human colorectal cancer cell line (HT29) expressed GLUT1 in a very high level (Fig.S1). Therefore, HT29 cells were subsequently selected to perform the glucose uptake assays and measure the inhibitory capability of 23 molecules to GLUT1 based glucose uptake. The results showed that 14 out of 23 small molecules inhibited glucose uptake in HT29 cells, indicating a 60% hit rate of the virtual screening (Fig.4a, Table.3). Among them, SMI290, SMI291, SMI428, and SMI433 had comparable inhibitory activity to the positive control WZB117 (Fig.4a). The structures of the four compounds are different (Fig.4c), indicating they may have different binding modes. Subsequently, glucose uptake assays were performed at different concentrations for SMI290, SMI291, SMI428, and SMI433, and these compounds showed a dose-dependent manner (Fig.4b).

3.4 Lactate production assay

Tumors were described as being “glucose-avid” and “lactate-producing” by Warburg effect in normoxia. Therefore, the production of lactate of HT29 cells was measured. In this study, compound SMI290, with the highest inhibitory effect on glucose uptake, was selected to measure its influence on lactate production. The results showed that SMI290 at a concentration of 50 μ M, significantly inhibited the production of lactate in HT29 cells (Fig.5), which shows potential inhibitory effect on energy metabolism and acidification of tumor microenvironment.

3.5 Possible binding modes of small molecules and receptor proteins

In order to study the binding mode of the identified compound to GLUT1, 14 novel identified inhibitors were docked to the binding area of GLUT1 in the flexible docking algorithm as described in “Materials and methods” section. Each small molecule retained 30 binding modes (The positive control WZB117 is also docked with binding area of GLUT1, but it did not form any interaction with GLUT1). The binding energies were highly correlated to the inhibition rate with a R^2 of 0.84 (Fig.6a). PLIF (protein ligand interaction fingerprints) analysis revealed that hydrophilic residues Q161, Q282, Q283, N288, E380, P385, W388, W412 W388 and Q283 were involved into the ligand binding and W388, Q283 were served as the hotspots for interactions (Fig.6b). The ligand interaction between four inhibitors (SMI290, SMI291, SMI428, SMI433) and GLUT1 were analyzed (Fig.7). The hydrogen bond between the ethylamino group of SMI290 and the aromatic ring of W412 was formed (Fig.7a). Two hydrogen bonds between SMI291 and GLUT1 were formed, where residues E380 and W388 were involved (Fig.7b). The two connected aromatic rings and the benzene ring at the trifluoromethyl group on SMI428 interacted with the aromatic hydrocarbon group and hydrogen bond on W388 respectively(Fig.7c). The benzene ring, the carbonyl group adjacent to the six-member ring and the amino group near the five-membered ring on SMI433 have hydrogen bond interactions with P385, W388, and N411, respectively. At the same time, the five-membered ring of SMI433 also interacts with the aromatic ring of W412(Fig.7d).

Discussion

Tumor cells are highly dependent on GLUT1 to transport glucose for glycolysis to energetic metabolism. Inhibition of GLUT1 gated glucose transport is reasonably to damage the energy supply of tumor cells and affect their proliferation and survival.

Therefore, GLUT1 inhibitors could be used to interrupt the survival of tumor cells.

GLUT1 related inhibitors are currently premature for clinical application, although several inhibitors such as WZB117, BAY-876 were proved to have anti-tumor activity in vivo. The molecular docking results of BAY-876 and WZB117 to the structure of GLUT1 showed that no interaction between BAY-876 and WZB117 at the binding area of GLUT1, indicates a different binding mode of the known inhibitors. The binding mode may imply our identified compounds showed a different mechanism of action to the known inhibitors BAY-876 and WZB117.

In this study, the area of Pocket3 was discarded because the active compounds could not be distinguished from inactive compounds by Pocket3 based virtual screening. However, this Pocket contains ICH domain of GLUT1 and ICH domain controls the opening of GLUT1 for glucose transport, indicating Pocket3 being an important site for drug design (Fig.2a). Therefore, a virtual screening based on ICH domain is another direction for drug discovery.

Although GLUT1 was highly expressed on HT29 cell line, other GLUT members such as GLUT2-4 were also expressed in different levels. Therefore, four compounds SMI290, SMI291, SMI428 and SMI433, which inhibit glucose uptake, may also impair the function of GLUT 2, 3, 4. Therefore, SMI290, SMI291, SMI428, and SMI433 are probably not specific inhibitors of GLUT1, and may also be inhibitors of other members of the GLUT family.

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Author contributions

Xiaotong Chen conceived and performed the experiments and analysed the data. The manuscript was written through contributions of all authors and Jiangfeng Du overall supervised the experiments and revised the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

No animal model was used in the study and all the researches were performed under the approval of the Ethics Committee of Zhengzhou University

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Figure.1 The flow chart of virtual screening (VS). The chart contains the results of each screening step, including molecular docking, drug properties analysis and experimental verification.

Figure.2 Detection of drug pockets. a Three pockets obtained in the inwardly open

conformation of GLUT1. **b** Binding energies of known inhibitors based on different pockets of GLUT1. **c** The residues of each pocket in GLUT1 sequence (Pocket 1 in blue, Pocket 2 in red and Pocket 3 in green). **d** The enrichment curve of the virtual screening of EvaData based on Pocket 1 and Pocket 2 respectively.

Figure.3 Top 5000 compounds were screened by drug properties and structural diversity. The optimal compounds were shown in blue. **a** According to solubility ($\log S > 0.5$) and binding energy (Docking Score < -20 kcal/mol), 135 small molecules were selected. **b** According to molecular weight ($MW < 600$) or binding energy (Docking Score < -21 kcal/mol), 117 small molecules were retained. **c** According to drug score and structural diversity, 23 small molecules were retained. **d** 23 screened compounds (blue) and 122 known inhibitors (red) were clustered.

Figure.4 Inhibitory rate of screened compounds for glucose uptake in HT29 cells. **a** Quantitative analysis of the inhibition of glucose uptake of HT29 by small molecules of 100 μ M. Data are shown as means $\pm$ s.d. ($n = 3$). **b** Quantitative analysis of the inhibition of glucose uptake of HT29 by WZB117, SMI290, SMI291, SMI428, SMI433 of 25 μ M, 100 μ M, 200 μ M. Data are shown as means $\pm$ s.d. ($n = 3$), $**p < 0.01$, $***p < 0.001$. **c** Structure of SMI290, SMI291, SMI428, and SMI433.

Figure.5 Inhibitory rate of compound SMI290 for lactate production in HT29. Data are shown as means $\pm$ s.d. ($n = 3$), $**p < 0.01$, $***p < 0.001$.

Figure.6 Binding modes of 14 identified inhibitors. **a** Correlation plot between the docking scores and inhibitory rate (100 μ M) of 14 compounds. **b** the contact residues of GLUT1 in binding 14 small molecules.

Figure.7 Protein-ligand interaction of four inhibitors (a: SMI290, b: SMI291, c: SMI428, d: SMI433).

Table 1. The amino acid and the pocket properties.

	Size	PLB	Hyd	Identity
Pocket 1	352	3.58	89	F26 T30 F72 S73 S80 V83 N88 R92 T137 G138 P141 M142
				G145 E146 R153 G154 A155 G157 T158 H160 Q161 I164
				V165 I168 R212 I216 N217 E247 S248 M251 M252 E254
				K255 K256 Q282 Q283 I287 N288 F291 Y292 N317 T321
				S324 L325 F326 V328 Q329 F379 E380 G384 P385 W388
				F389 A392 Q397 G398 R400 P401 I404 A405 G408 N411
Pocket 2	-	-	-	W412 N415
				F26 T30 F72 Q161 I164 V165 I168 Q282 Q283 I287 N288
				F291 Y292 T321 S324 L325 V328 F379 E380 G384 P385
				W388 F389 N411 W412 N415
Pocket 3	85	0.94	24	N34 A35 P36 Q37 K38 V39 E41 P58 T59 L61 T62 G175
				L176 D177 G181 T295 S296 E299 K300 V307 Q423 E426
				Q427

Table 2. 122 known inhibitors were clustered into 38 clusters with a similarity of 0.5 by StarDrop (Release 6.5.0, Optibrium Ltd, UK)

Skeleton	Count	Reference	Skeleton	Count	Reference
45	[30]		4	[30]
12	[31]		8	[32,33]
3	[26,34]		4	[30]
2	[22]		2	[32]
5	[32]		2	[30]

5 [35,36]

2 [38,22]

1 [30]

1 [38]

1 [38]

1 [38]

3 [37,32]

1 [30]

1 [30]

1 [38]

1 [38]

1 [38]

1

[38]

1

[23]

1

[32]

1

[39]

1

[22]

1

[40]

1

[41,25]

1

[30]

1

[30]

1

[42]

1

[43]

1

[44]

1

[45]

1

[46]

1

[47]

1

[48]

Table 3. Information of 23 screened compounds. logS was calculated by Stardrop (Release 6.5.0, Optibrium Ltd, UK), Binding energy was calculated by FRED. MolID272 indicated the known inhibitor WZB117.

MolID	Structure	Inhibition%(100μM)	logS	MW	Binding energy (kcal/mol)
272		66.0±3.0	0.658	368.3	-12.89
275		-153.0±10.6	1.25	759.1	-13.16
279		-7395.0±380.1	1.79	490.6	-13.31
280		-1231.0±63.1	0.5	577.6	-19.13
285		-4448.0±156.8	1.37	441.5	-13.4

286		-68.0 ± 32.8	0.89	494.5	-15.33
290		71.0 ± 11.0	4.37	412.6	-12.02
291		55.0 ± 21.0	3.86	510.8	-13.07
421		45.0 ± 5.9	1.04	396.4	-19.35
422		27.0 ± 4.7	-0.57	475.5	-20.23
423		49.0 ± 12.1	-1.71	574.6	-20.63
424		-16.0 ± 7.6	-1.18	561.6	-21.11

425		30.0 ± 0.0	-0.66	536.5	-20.32
426		56.0 ± 3.1	-1.88	588.7	-20.43
427		8.0 ± 9.1	1.21	525.6	-18.84
428		62.0 ± 7.5	0.71	574.6	-19.05
429		-434.0 ± 84.5	1.23	461.6	-19.19
430		28.0 ± 3.6	0.66	426.6	-18.99

431		-40.0 ± 10.4	0.44	495.6	-20.09
432		-1380.0 ± 136.3	-2.65	517.4	-20.59
433		68.0 ± 2.9	0.87	495	-20.19
434		19.0 ± 4.4	0.79	531.1	-19.36
435		42.0 ± 12.1	1.48	529.1	-20.74
436		41.0 ± 14.1	1.00	431.5	-18.95

Figures

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The flow chart of virtual screening (VS).The chart contains the results of each screening step, including molecular docking, drug properties analysis and experimental verification.

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Detection of drug pockets. **a** Three pockets obtained in the inwardly open conformation of GLUT1. **b** Binding energies of known inhibitors based on different pockets of GLUT1. **c** The residues of each pocket in GLUT1 sequence (Pocket 1 in blue, Pocket 2 in red and Pocket 3 in green). **d** The enrichment curve of the virtual screening of EvaData based on Pocket 1 and Pocket 2 respectively.

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Inhibitory rate of screened compounds for glucose uptake in HT29 cells. a Quantitative analysis of the inhibition of glucose uptake of HT29 by small molecules of 100μM. Data are shown as means $\pm$ s.d. (n = 3). b Quantitative analysis of the inhibition of glucose uptake of HT29 by WZB117,SMI290,SMI291,SMI428,SMI433 of 25μM–100μM–200μM. Data are shown as means $\pm$ s.d. (n = 3), **p < 0.01,***p < 0.001.c Structure of SMI290, SMI291, SMI428, and SMI433.

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Figure 7

Protein-ligand interaction of four inhibitors (a: SMI290, b: SMI291, c: SMI428, d: SMI433).