In-silico screening and validation of small molecules as dual MCT-1 and MCT-4 inhibitor

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Abstract

Cancer cells have accelerated glycolysis rate, leading to the overproduction of lactate. This excess lactate plays a crucial role in the rapid growth of tumor cells. The transportation of lactate is primarily facilitated by two proton-coupled lactate transporters, MCT-1 and MCT-4 that promotes cell proliferation and growth.. Through *in-silico* investigation, we aimed to find new dual MCT-1 and MCT-4 inhibitor for therapeutic intervention in breast cancer. A natural based library NPASS (Natural product activity and species source base) containg 1042 compounds were screened based on structural similarity to Syrosingopine (Syro). Further screened compounds were virtually docked with MCT-1 and MCT-4 protein using PyRx software. The five best virtually screened ligands were selected based on their molecular interaction and further processed for ADMET profiling using SwissADME and pkCSM. The pharmacokinetic profiling suggested that Ginkgolide B as the most promising lead molecule which inhibits both MCT-1 and MCT-4. Our results strongly supports the utility of developing a dual MCT-1 and MCT-4 inhibitor for breast cancer chemoprevention.

Keywords: Breast cancer, Screening, Lactate, MCT-1, MCT-4

Introduction

Breast cancer is the most often diagnosed cancer and the leading cause of cancer-related death among women globally. The prevalence and mortality of this illness have increased; GLOBICON 2022 statistics indicate that 2.3 million new cases of breast cancer were identified, resulting in 685,000 deaths [1]. Many factors increased the risk of developing breast cancer, including behavioral, chemical, genetic and environmental factors. Numerous treatments like radiation therapy, chemotherapy, hormone therapy, or targeted therapy were used but unfortunately they are not responding well due to metabolic reprogramming. To overcome this there is considerable interest to develop therapy that specifically target the metabolic alteration [2].

In 1920, German scientist, Otto Warburg proposed that cancer cells run a high rate of glycolysis and rely on pyruvate reduction to lactate for adenosine triphosphate (ATP) generation, irrespective of oxygen available to support their metabolic proliferation, invasiveness, and resistance to apoptosis under hypoxia [3,4]. The repeated glycolysis perpetuates the accumulation of lactate in the cells as an end product of glycolysis [5].The accumulated lactate in the hypoxic cells is transported to the extracellular region through proton linked monocarboxylate transporters which are present in the plasma membrane and favor processes such as metastasis, angiogenesis, and anti-tumor immune suppression which are associated with the worst clinical prognosis [6].

MCTs belong to the solute carrier (SLC16) family of which MCT-1 to MCT-4 are characterized biochemically and overexpressed in different types of cancer including breast cancer [7]. MCT-1 facilitates the bidirectional transport of lactate, whereas MCT-4 exports lactate in hypoxic microenvironment. Further, lactate favors processes such as metastasis, angiogenesis, and anti-tumor immune suppression[8].Considering that both MCT-1 and MCT-4 work in tandem, it was considered worth to screen and validate small molecules as dual MCT-1 and MCT-4 inhibitors for breast cancer chemopreventation using in-silico approaches.

Material and Methods

Screening of library

Natural based library(NPASS) were downloaded and screened from data warrior V5.2.1 software based on structural similarity with Syro (above70%) which was previously reported as dual MCT-1 and MCT-4 inhibitor.The most structurally similar compounds were selected and downloaded in the SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih) and further virtually screened using PyRx softwar [9].

Target proteins prepration

The 3D protein structure of MCT-1(6LYY) and was retrieved from the RCSB (https://www.rcsb.org/) protein data bank, and MCT-4 was generated by homology modeling using Phyre2. The amino acid sequence of MCT-4(O15374) protein was obtained from the Uniport database and submitted to Phyre2 in order to model protein structure. The stereochemical quality of target proteins was analyzed using Saves v6 web Server

(Ramachandran plot) (htt://mordred.bioc.cam.ac.uk /rapper/rampage).Proteins were visualized using Pymol 2.1.0 [10].

Virtual screening

Virtual screening is a computational approach used in drug discovery and other fields to efficiently scan large databases of molecules for potential candidates with desired properties. A high-throughput virtual screening analysis of the selected natural compounds against the prepared MCT1 amd MCT4 proteins was carried out using the PyRx software, thus obtaining a ranking of the ligands based on their binding score. A computer grid was constructed around the active site of the target proteins. The grid measured 20 × 20 × 20 Å in dimensions and was centered at the following coordinates: X: 77.3 Å, Y: 16.36 Å, and Z: 111.16 Å [11].

Drug Likeness and ADMET profiling:

The drug-likeliness and ADMETof top 10 virtually screnned compounds were assessed using web servers SwissADME (http://www.swissadme.ch/) and pkCSM (http://structure.bioc.cam.ac.uk/pkcsm). The SMILES strings of selected ligands were provided to these servers to evaluate various pharmacokinetic properties [12].

Molecular docking

The molecular docking analysis was conducted with AutoDock Vina Tools version 1.5.7. Ligands and proteins are generated by eliminating water molecules, incorporating polar hydrogen atoms, applying Kollman charges to the protein, and assigning Gasteiger charges to the ligands, respectively. Proteins and ligands were converted into PDBQT format. To enhance drug binding inside the anticipated binding pocket, all residues involved in the substrate binding pocket were included into a grid box with 4Å spacing for MCT-1 and MCT-4, followed by docking procedures. Following docking, several ligand conformations have been created based on amino acid interactions and binding affinities, which were shown using LigPlot+ [13].

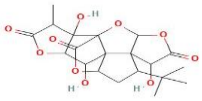
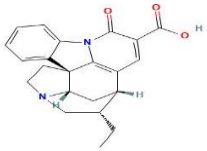
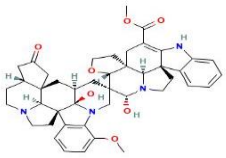
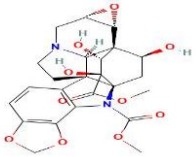
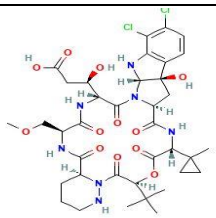
Result and Discussion

The study aimed to find new MCT-1 and MCT-4 inhibitor in breast cancer and also sought to optimize the compound's potency as an inhibitor to contribute to better therapeutic interventions for this cancer type. In the computational analysis, the Ramachandran plot was employed to evaluate the stereochemical quality of protein structures[14]. Ramachandran plot analysis of MCT-1 and MCT-4 proteins revealed that 89.9% of residues in MCT-1 and 90.2% of residues in MCT-4 were located in the allowed region, indicating that these proteins are situated in the most favorable conformational region.

After that, a structure based screening was performed which reveals that about 102 compounds showed above 60% structural similar to Syro, indicating promising prospects for

novel inhibitors. Additionally best inhibtors were analysed using PyRx software, and binding score were recorde (Table 1).

Table 1: List of top dual MCT-1 and MCT-4 inhibitors, with their structural similarity, molecular weight and binding affinitiy analyzed using Data Warrior and PyRx software.

Compound Name/ID	Molecular Weight(g/mol)	Structure	Structural similarity with Syro (%)	PyRx binding energy(kcal/mol) With MCT-1/MCT-4
Ginkgolide B	424.4		87	-10.2/8.89
Melodinine C	362.4		82	-8.51/-7.1
Biscarpamontamine B	746.9		79	-6.9/-6.8
KopsimalineD	502.5		78	-8.8/-7.4
Kutzneride 3	857.3		75	-6.21/-9.2

Virtual screening, a widely-used computational method in drug design, is employed to identify potential lead compounds by screening large databases [15]. A drug candidate's success depends not only on its efficacy but also on its favorable ADMET characteristics. Various ADMET tools are available to predict the pharmacokinetic. Computational ADMET is now advised for early-stage drug development to assess safety. In this study, further ADMET analysis was conducted using free online webserver, SwissADME and pkCSM. The pharmacokinetic findings revealed no violations of Lipinski's rule and no blood-brain barrier (BBB) penetration. Moreover, all ten ligand molecules showed positive absorption and logP values between 1.02 and 3.53, indicating their complexes can be easily absorbed through the human intestine. To assess active drug efflux, which involves the return of absorbed drugs to the intestinal lumen, Caco-2 cells (human colon carcinoma cells) were cultured. The positive

results for Caco-2 cells suggest good permeability, implying these ligands can effectively traverse the intestinal barrier. Notably, none of the ligands inhibited CYP2D6 activity, suggesting they do not disrupt its normal metabolic functions (Table 2).

Table 2: Drug likeness and ADMET properties of top five selected screened compounds using SwissADME and PkCSM

Parameters	Ginkgolide B	Melodinine C	Biscarpamontamine B	Kopsimaline D	Kutzneride 3
Drug likeness					
LogP	-1.36	2.73	3.67	-1.07	-0.26
No. of Lipinski rule violation	0	1	0	0	0
Absorption					
Water solubility	-3.85	-3.22	-2.90	-2.41	-3.01
$Caco_2$permeability	0.94	0.98	1.33	1.81	-0.31
Intestinal absorption (% A)	57.92	97.48	100	100	18.86
Skin permeability (log Kp)	-2.73	-2.74	-2.73	-2.73	-2.73
P – glycoprotein inhibitor	No	No	Yes	No	No
Distribution					
VDs	0.45	0.08	0.49	0.48	-0.70
Fraction unbound	0.395	0.37	0.29	0.39	0.42
BBB permeability (log BB)	-0.57	0.42	-0.92	-0.98	-1.48
CNS permeability (log PS)	-3.041	-2.17	-3.26	-3.36	-4.06
Metabolism					
CYP2D6 prediction	No	No	No	No	No
Excretion					
Total clearance (log ml/min/kg)	0.308	0.576	0.107	0.758	0.199

Toxicity					
AMES toxicity	No	No	No	No	No
Max. tolerated dose (log mg/kg/day)	-0.94	0.41	-0.146	-1.38	0.41
Oral rate acute toxicity (LD_{50})	2.63	2.39	2.79	3.87	2.73

Further, on the basis of pharmacokinetic profiling ginkgolide B was identified as most promising compound and further revalidation was done using Autodock Vina. The docking result reveled that ginkgolide B shows maximal binding score with MCT(-11.2 Kcal/mol) and with MCT-4 (-12.3 Kcal/mol) respectively .The key amino acid residues Thr395 interact with MCT-1 protein and Ser156,Arg 278 and Tyr 30 interact with MCT-4 protein, respectively (Figure 1).

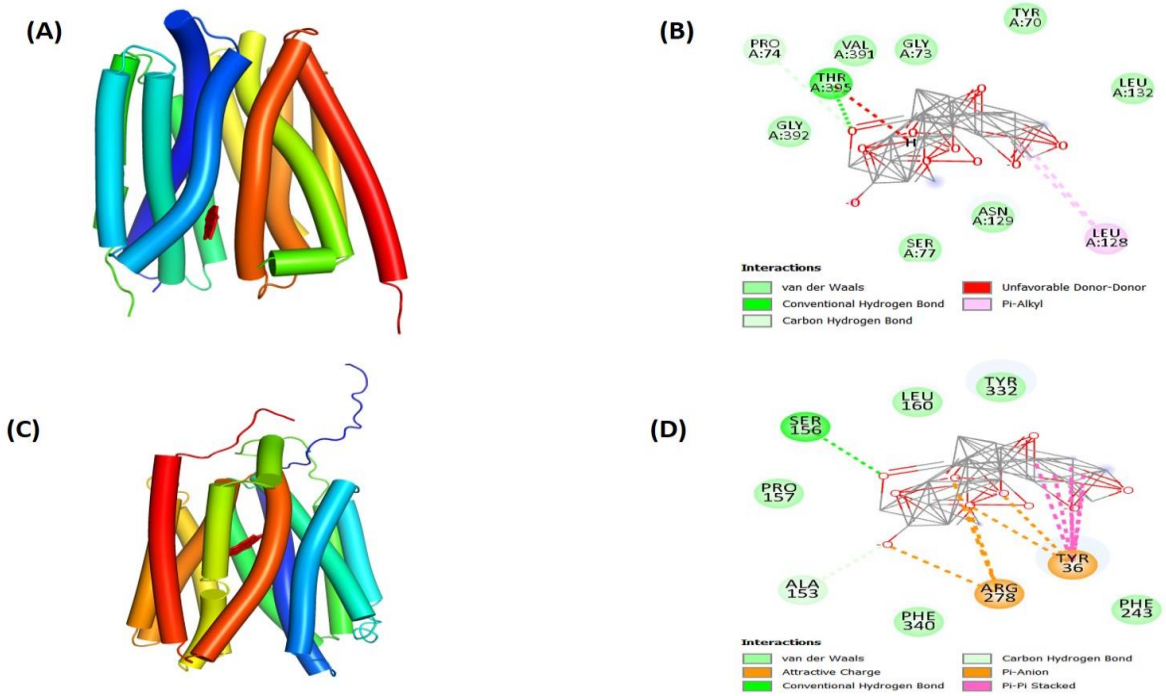


Figure 1: Molecular docking of Ginkgolide B with MCT-1 and MCT-4.

The figure illustrates the most favourable 3D docking pose with MCT-1 and MCT-4. **(A)** represent ribbon structure of MCT1 docked with Ginkgolide B **(B)** indicating interaction of ginkgolide B with amino acid residues of MCT-1. **(C)** represent ribbon structure of MCT -4 docked with Ginkgolide B **(D)** indicating binding interaction of Ginkgolide B with amino acid residues of MCT-4.

Conclusion

The research aims to identify novel MCT-1 and MCT-4 inhibitors for breast cancer chemoprevention through a in-silico structure based screening.Sophisticated computational techniques, including virtual screening, were employed to refine the selection process.

Pharmacokinetics analysis were conducted to identify promising compounds. Based on virtual screening and ADMET profiling , ginkgolide B emerged as a potential candidate, which was subsequently validated using Autodock Vina. In the future, it is essential to conduct *in- vitro* and *in- vivo* investigations to validate their biological activity.

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Nil.

Declaration of competing interest

There are no conflicts of interest.

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