LC-mHTT-AN2 oversees lactate transport, hypoxia, and glucose homeostasis through dual regulation of MCT-1/4 in the tumor microenvironment

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Abstract: Cancer cells have accelerated glycolysis rate, resulting in excessive lactate generation, which is critical in rapidly growing cancerous cells. Lactate is primarily transported by MCT-1/MCT-4, the two H+/lactate transporters that promote cellular proliferation and growth. Through *in-silico* investigation, we aimed to find new dual MCT-1 and MCT-4 inhibitor for therapeutic intervention of breast cancer. A library of 4098 natural product-like compounds (HY-L057L) was retrieved and screened based on structural similarity with Syrosingopine (above70%). Our findings revealed that LC-mHTT-AN2 has good docking score with both proteins (MCT-1 and MCT-4) and favourable drug likeness, ADMET profiling. The overall findings from *in -silico* support the pre-clinical efficacy of LC-mHTT-AN2 in the treatment of breast carcinoma by combined inhibition of MCT-1 and MCT-4. Further *in-vitro* and *in-vivo* research is needed to verify its usefulness in the chemoprevention of breast cancer.

Introduction

Breast cancer is the most frequently diagnosed cancer in females and nearly, 2.3 million cases were reported in the year 2020 accounting for 685,000 deaths worldwide [1]. Of deaths caused by breast cancer approximately 90% are due to metastasis, a condition in which tumor cells invade to organs and blood vessels [2]. Metastatic tumor have a higher rate of mortality and early treatment strategies include surgery, radiotherapy, chemotherapy, and targeted drug therapy [3]. However, the failure of radiotherapy and chemotherapy in malignant solid tumors occurs due to metabolic adaptations. Tumor cells in this situation majorly rely on glycolysis (aerobic mode of energy production) to meet energy demands for growth and development [4].

In 1920, German scientist, Otto Warburg put forward that cancer cells perform repeated glycolysis and produce excess amounts of lactate as an energy source. In past, lactate was considered as a metabolic waste, whereas studies depicted that lactate play a significant role in cancer homeostatic [5] Lactate has the ability to supply energy to oxidative cancer cells, and function as a pro-angiogenic agent. Lactate participates in cellular proliferation, immune suppression, and chemotherapy resistance. Therefore, an appropriate handling of lactate holds

considerable significance in cancer cells. Lactate transport is significantly influenced by Monocarboxylate transporters (MCTs) to prevent intracellular acidification of the tumor microenvironment [6,7].

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 [7,8]. MCT-1 facilitates the bidirectional transport of lactate, whereas MCT-4 exports lactate in hypoxic microenvironment. Lactate transport from MCTs depends on the concentration gradient of the transport membrane. In the hypoxic microenvironment, tumor cells stimulate the expression of HIF-1α (hypoxia-inducible factor) a transcription factor that upregulated the activity of GLUT-1 and MCT-4 for excess production of lactate instead of pyruvate. Lactate further travels to the adjacent cells through the MCT-1 transporter and is involved in ATP generation, which favors processes such as metastasis, angiogenesis, and anti-tumor immune suppression [9]. Considering that both MCT-1 and MCT-4 work in tandem, it was considered worth targeting the regulation of MCT-1 and MCT-4 by selective dual inhibitors. In this regard, we performed *in-silico* screening from the natural based library (HY-L057L). Based on *in-silico* results (Lipinski's rule, ADMET profile, molecular docking) and availability, we found LC- mHTT-AN2 as a novel chemical compound with potential to regulate both MCT-1 and MCT-4. LC- mHTT-AN2(5,7-dihydroxy-4-phenylcoumarin) is a 4-phenyl hydroxycomarins derivative [10].

Material and Methods

Homology modelling of target proteins

The 3D protein structure of MCT-1(6LYY) was retrieved from the RCSB (https://www.rcsb.org/) protein data bank, and MCT-4 was generated by homology modeling using Phyre2 (http: //www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The amino acid sequence of MCT-4(O15374) protein was obtained from the Uniport database (https://www.uniprot.org/) 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 [11,12].

PyRx virtual screening:

A library of 4098 natural product-like compounds (HY-L057L) was retrieved from MedChemExpress (https://www.medchemexpress.com), and screened from data warrior V5.2.1 software based on structural similarity with Syrosingopine (above70%) which was previously reported as dual MCT-1 and MCT-4 inhibitor [13]. The most structurally similar compounds were selected and downloaded in the SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih). Thereafter virtually screened with PyRx (https://pyrx.sourceforge.io/) software. The five best virtually screened ligands were selected based on their molecular interaction and further processed for pharmacokinetic profiling and drug-likeness.

Drug Likeness and ADMET profiling:

The *in-silico* web server SwissADME (http://www.swissadme.ch/) from the Swiss Institute of Bioinformatics and pkCSM (http://structure.bioc.cam.ac.uk/pkcsm) was used to evaluate the drug-likeness and pharmacokinetic (ADMET) profiling. The

SMILES string of selected ligands were submitted to the web server to determine the nature of lipophilicity, including water solubility, log P, molecular weight, and the capacity of molecules to give and absorb hydrogen among the physicochemical factors. Pharmacokinetic parameters like absorption, BBB permeability, volume of distribution, metabolism, excretion, and toxicity characteristics were also examined**.** On the basis of pharmacokinetic profiling one compound were selected and further redocking was done to validated binding affinity with target proteins [14]**.**

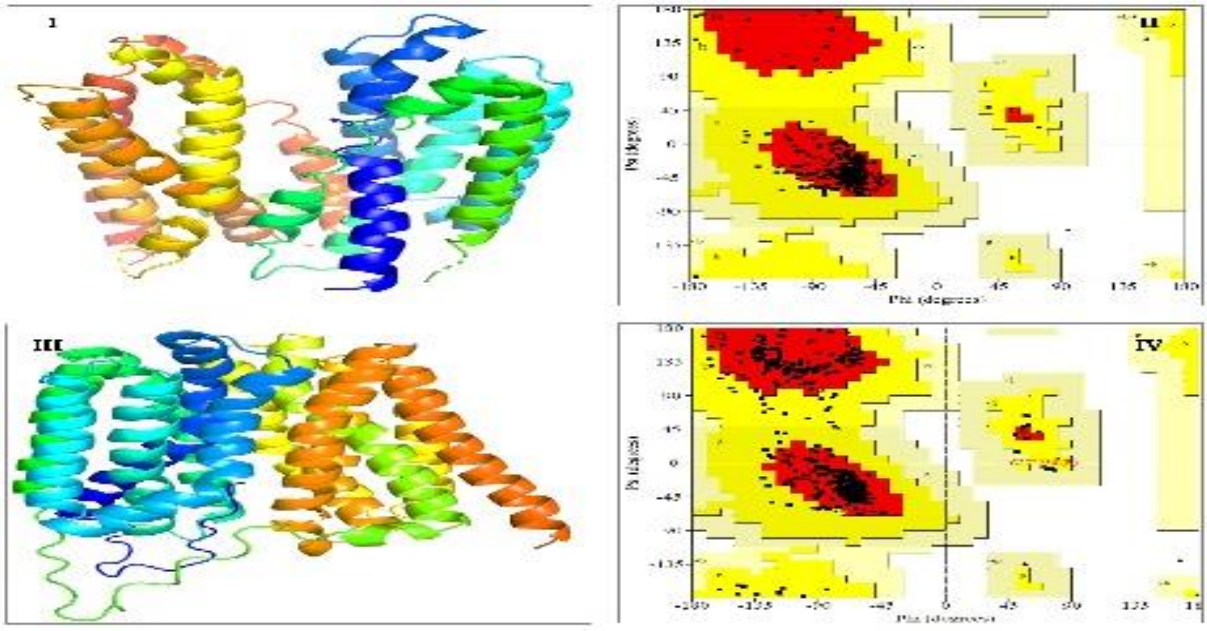
Docking study

The molecular docking study was carried out using AutoDock Vina Tools 1.5.7. Ligands and proteins were prepared by removing water molecules, adding polar hydrogen atoms, Kollman charge on the protein, and Gesteiger charge on the ligands, respectively. Prepared proteins and ligands were converted into PDBQT format. The grid box coordinates were 104.64, 116.27, and 110.00 for MCT-1 and 12.79,0.60, and 0.26 for MCT-4, respectively. The grid box size was set to 72, 36, and 64 for MCT-1 and 44, 38, and 40 for MCT-4. To optimize drug binding in the predicted binding pocket, all residues implicated in the substrate binding pocket were included in grid box with 4A spaces for MCT-1 and MCT-4 and docking was performed. After docking, several ligand states were generated based on amino acid interactions and binding affinities, which were visualized using LigPlot+ [15]**.**

Results and Discussion

The Ramachandran plot, used in the computational study to assess the stereochemical quality of protein structure, revealed that the most residues (MCT-1 89.5% and MCT-4 90.2%) were sterically allowed region, suggesting that proteins are found in the most preferred locations **(Figure1)**.

Figure 1: 3D structure of MCT-1 and MCT-4 proteins with their Ramachandran plot. (**I, III)** represent the 3Dstructure of MCT-1 and MCT-4 proteins and figure **(II, IV)** shows the quality of MCT-1 and MCT-4 proteins which revealed that the majority of residues (MCT-1 89.5% and MCT-4 90.2%) were present in sterically allowed region.



After target protein validation, LC-mHTT-AN2 was analyzed for drug likeliness and ADMET profiling which reveals that there is no violation of the Lipinski rule and has good intestinal absorption (93.69%) and Vd (-0.0034). Moreover, showed no CYP2D6

prediction, and total renal clearance was found to be (0.824 log ml/min/kg). Toxicity data shows that there is no AMES toxicity, and predicated oral rat acute toxicity (LD50) was 2.329 **(Table 2)**.

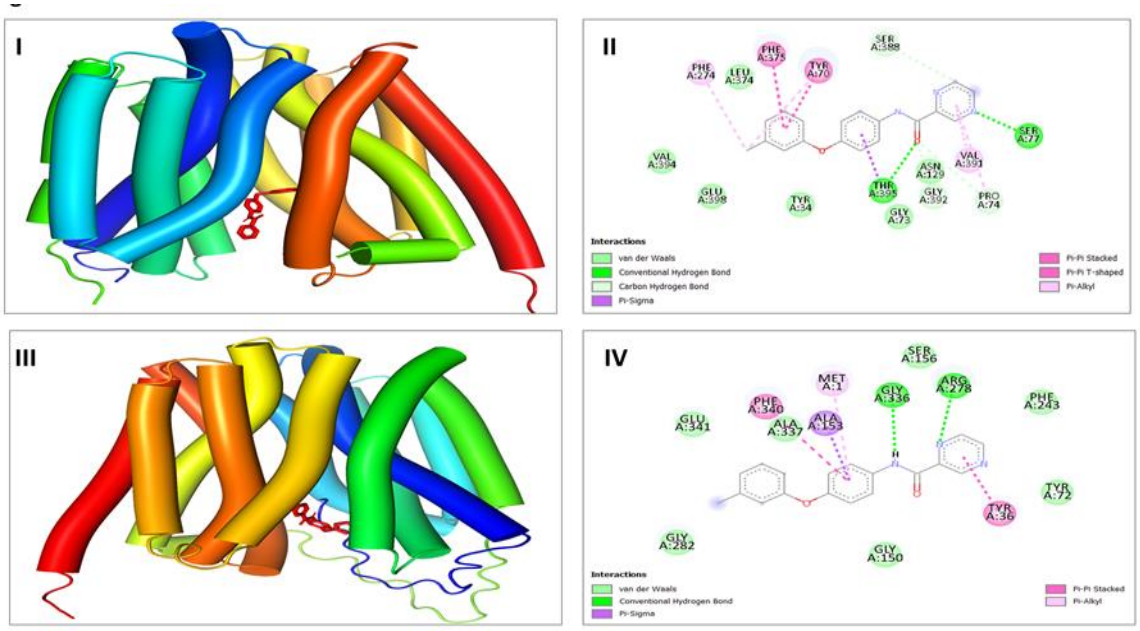
Table 1: Drug likeness and ADMET profiling of top five screened compounds.

Parameters	Ginkgoic acid	Octa-hydrocurcumin	2-Hydroxy-4-methoxybenzoic acid	LC-mHTT--AN2	Hispidin
Drug likeness					
LogP	3.89	3.53	0.89	2.0	1.61
No. of Lipinski rule violation	1	0	0	0	0
Absorption					
Water solubility	-3.33	-3.72	-1.82	-3.6	-2.91
$Caco_2$permeability	1.04	-0.22	0.295		0.9
Intestinal absorption (% A)	94.7	72.38	88.09	93	89.00
Skin permeability (log Kp)	-2.7	-2.74	-2.73	-2.7	-2.7
P – glycoprotein inhibitor	No	No	No	No	No
Distribution					
VDs	-1.2	0.26	-1.8	-0.3	0.57
Fraction unbound	0.05	0.01	0.51	0.7	0.40
BBB permeability (log BB)	-0.2	-1.0	-0.3	0.1	-0.70
CNS permeability (log PS)	-2.3	-3.24	-2.62	-2.2	-2.33
Metabolism					
CYP2D6 prediction	No	No	No	No	No
Excretion					
Total clearance (log ml/min/ kg)	1.51	0.68	0.67	0.4	0.45
Toxicity					
AMES toxicity	No	No	No	No	No
Max. tolerated dose (log mg/kg/day)	0.24	0.47	0.72	0.3	-0.00
Oral rat acute toxicity (LD_{50})	2.43	2.63	2.05	1.1	1.7

After that, the proteins MCT-1 and MCT-4 dock with LC-mHTT-AN2, and the maximal binding affinity was found to be -7.2 kcal/mol and -6.5 Kcal/mol. Further, Ligplot analysis was done to depict the hydrophilic interaction of LC-mHTT-AN2 with MCT-1 and MCT-4 protein. The key amino acid residues Asp309, and Arg313 interact with MCT-1 protein and Lys40, Ser156, and Arg278 interact with MCT-4 protein, respectively **Figure 2.**

Figure 2: Molecular docking of LC-mHTT-AN2 with MCT-1 and MCT-4 proteins. Figure **(I, III)** depict 3D dock pose of LC-mHTT-AN2 with MCT-1 and MCT-4 target proteins and 2D representations of LC-mHTT-AN2 with MCT-1 and MCT-4 using

LigPlot **(II, IV)**. Hydrogen bonds are represented as green dotted lines, while the spoked arcs represent residues forming hydrophobic interaction with ligand.



Conclusion

The present study demonstrates a newer approach for achieving anticancer potential by regulating lactate transport through the MCT-1 and MCT-4 transporters. The overall findings from *in –silico* support the efficacy of LC-mHTT-AN2 as dual MCT-1and MCT-4 inhibitor for breast cancer chemoprevention. Further, in-vitro and in-vivo research is needed to verify its usefulness in breast cancer chemoprevention.

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