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Identification of the Therapeutic Protein Targets of 2-Hydroxydicarboxylic Acid and 3D Analysis using *Insilico* Protocols

***Maithreyee S.*[1*], *Prabha V.*[2] *and Balaji Munivelan*[3]**
[1]*Research Scholar, Department of Biochemistry,*
DKM College for Women, Vellor (Tamil Nadu), India.
[2]*Assistant Professor & HOD., Department of Biochemistry,*
DKM College for Women, Vellore (Tamil Nadu), India.
[3]*CEO, Department of Drug Designing & Development,*
ABS Geno-informatics, Chennai (Tamil Nadu), India.

(Corresponding author: Maithreyee S.)*
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ABSTRACT: The aim of the *Insilico* study is to efficiently bind the chemical compound,2-hydroxydicarboxylic acid with the respective protein receptor/targets using *Insilico* tools. 2-hydroxydicarboxylic acid acts as a food acidity regulator and a fundamental metabolite. The primary methodology focuses on the analysis of the 2D structure of hydroxydicarboxylic acid along with its molecular properties. Bioactivity test and 3D structure prediction are also done. In the second step, the protein targets are bound to the chemical molecule at its active site using advanced *Insilico* target prediction tool. The results obtained by the study clearly elucidate that based on the various targets identified, the derivatives of 2-hydroxydicarboxylic acid can be discovered for various diseases. The best target molecules which efficiently bind with 2-hydroxydicarboxylic acid were viewed in 3D and molecular dynamics view. The targets identified for this chemical molecule could potentially be used for future drug designing and discovery studies.

Keywords: 2-hydroxydicarboxylic acid, target prediction, 3D structure prediction.

INTRODUCTION

The finding, creation and repurposing of bioactive compounds requires the identification of targeted proteins. Latest bio/chemoinformatics techniques can at present provide effective assistance to estimate the most likely targets of small compounds (Byrne and Schneider 2019; Chaudhari *et al.,* 2017). These target prediction (also known as target fishing) methods can be classified into one of two groups of computer-aided molecular design. The first method makes use of the protein's three-dimensional structure (structure-based) and the second method does not make use of it (ligand-based) (Lavecchia and Cerchia 2016). In the drug development field, ligand-based target prediction has demonstrated to be tremendously effective and rapid in the prediction of the proper protein targets of compounds. Validating the intuitive molecular similarity hypothesis,' which puts forward that common proteins are targeted by similar molecules, was performed by assessing the similarity between compounds using several techniques (Willett and Winterman 1986; Johnson *et al.,* 1989).

Here, we use Malic acid which is a naturally derived compound. Malic acid is a 2-hydroxydicarboxylic acid that is succinic acid where one of the hydrogens attached to a carbon is replaced by a hydroxy group. It plays the role of food acidity regulator and a fundamental metabolite. It is a 2-hydroxydicarboxylic acid and a C4-dicarboxylic acid. It is derived from a succinic acid. It is a conjugate acid of a malate (2-) and a malate.

Several literature works have proved that the source of malic acid is available easily. Carl Wilhelm Scheele in 1785 primarily extracted malic acid from apple juice. In 1787, Antoine Lavoisier suggested the name acidemalique derived from the Latin word for apple, mālum—as is its genus name Malus. In German, it is called as Äpfelsäure (or *Apfelsäure*) which is the plural or singular form of a sour thing from the apple fruit, but the salt(s) are called *Malat*(e). The major acid present in several fruits, such as blackberries, apricots, cherries, blueberries, mirabelles, grapes, pears, peaches, quince and plums is malic acid. This acid is identified in lesser concentrations in fruits such as citrus (Duarte *et al.,* 2012). It is responsible for the sour taste of unripe apples. Malic acid is present in high concentrations in sour apples. It is found in grapes and sometimes in high concentrations up to 5 g/L in most wines (Ough, 1988). It is responsible for the tart taste of wine; as the fruit ripens the concentration of malic acid decreases. The primary flavour of malic acid is very distinct and clear in the herbaceous plant, rhubarb. Malic acid is also accountable for the tart flavour in sumac spice. This acid is added as an ingredient it is also a component of certain artificial vinegar flavors, like "salt and vinegar" flavored potato chips.

Organic farming yields citrus fruits containing higher concentrations of malic acid than fruits produced using conventional agriculture.

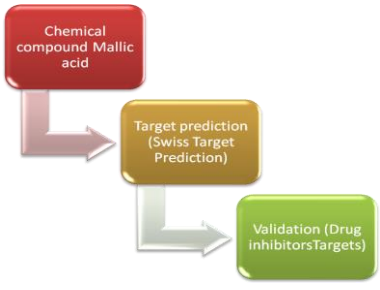
Malolactic fermentation is the process which converts malic acid to much milder lactic acid. Malic acid is naturally present in many vegetables and in almost all fruits. It is generated during fruit metabolism.

When supplemented to food products, Malic acid is denoted by E number E296. It is occasionally used with or in place of the less sour citric acid in sour sweets. These sweets are occasionally accompanied by a warning label mentioning that consuming it excessively may give rise to mouth irritation. It has received approval as food additive in the European Union, United States and Australia and New Zealand (where it is enumerated by its INS number 296). Malic acid consists of 10 kJ (2.39 kilocalories) of energy per gram.

The problem statement of this insilico research investigation is to find out the potential target for malic acid and its molecular binding information. This information would play a key role in computer-aided drug designing of therapeutic agents for various diseases (Greenfield and Southgate 2003; Cereto-Massagué *et al.,* 2015).

METHODOLOGY

Swiss Target Prediction is an online web-based tool developed in 2014 used to carry out ligand-based target prediction for any bioactive small molecule. It is a user-friendly graphical interface which protects non-experts from methodological pitfalls and specialists from exhausting technical efforts. This permits anyone to achieve reverse screening towards chemical libraries which were prepared previously in a careful manner. Ligand-based target prediction has proved to be very effective and quick in predicting the right protein targets of compounds in the context of drug discovery (Ding *et al.,* 2014). Quantifying similarity between compounds by various methods has facilitated validating the instinctive 'molecular similarity hypothesis' which postulates common proteins targeted by similar molecules (Willett and Winterman 1986; Johnson *et al.,* 1989).



Flow Chart: Methodology

At first, Malic acid (Hydroxydicarboxylic acid : CID 525) is retrieved from NCBI PubChem compound database (https://www.ncbi.nlm.nih.gov/pccompound) .The selected compound's SMILES was applied into Swiss Target prediction tool (http://www.swisstargetprediction.ch/) (Daina *et al.,* 2019) in order to identify the potential protein human targets according to the chemical structure of malic acid. The results were represented statistically.

The above schematic representation shows the steps involved in the identification of potential protein compounds for the selected chemical compound. Fig. 1-6 represent the 3D structure of Malic acid viewed using Discovery Studio software.

RESULTS AND DISCUSSION

The results obtained from SWISS target prediction (Fig. 7) shows 3 potential targets (CHRNA7) (Fig. 8-13). Interestingly, CHRNA7 is a gene which is susceptible to various channel issues. Nicotinic acetylcholine receptors (nAChRs) belong to a ligand-gated ion channel super family that mediate rapid synaptic signal transmission. The hetero-pentamers that are part of nAChRs are considered to consist of homologous subunits. According to the proposed structure, each subunit has a conserved N-terminal extracellular domain, three conserved transmembrane domains, a variable cytoplasmic loop, a fourth conserved transmembrane domain, and a short C-terminal extracellular region.

This gene codes for a homo-oligomeric channel that possesses an increased permeability to calcium ions and is a predominant component of brain nicotinic receptors that are blocked by alpha-bungarotoxin and are considerably sensitive to it. When this receptor connects to acetylcholine, it underfoes a predominant conformational shift that affects all subunits and causes the opening of an ion-conducting channel across the plasma membrane. This gene is present in a chromosomal region which is responsible for the hereditary transmission of schizophrenia and has been found out to be an important susceptibility locus for juvenile myoclonic epilepsy. A hybrid consisting of sequences from this gene and a new FAM7A gene has resulted from an evolutionarily recent partial duplication event in this region. Multiple transcript variations occur as a result of alternative splicing.

CHRNA7 (Cholinergic Receptor Nicotinic Alpha 7 Subunit) is a gene which codes for protein. Idiopathic Generalized 7, epilepsy and Chromosome 15Q13.3 Deletion Syndrome are all diseases connected with CHRNA7 (Bondarenko *et al.,* 2022; Özaltun *et al.,* 2021; Meganathan *et al.,* 2021). The related processes include Transmission through Alzheimer's disease, miRNA impacts and Chemical Synapses. The two Gene Ontology (GO) annotations for this gene include Protein *homodimerization activity* and *amyloid-beta binding*. A major paralog of this gene is CHRFAM7A.Following the binding of acetylcholine, the AChR undergoes a large conformational shift that acts on all subunits and results in the opening of an ion-conducting channel presence across the plasma membrane. The channel is obstructed by Alpha-bungarotoxin. There are numerous research works which substantiate our current investigation (Hezinglila Grace *et al.,* 2022; Priyadharshini and Leelavathi 2022; Zashumo *et al*., 2023).

Homomeric receptors are produced by the joining of the neuronal nicotinic receptor subunit alpha 7 (alpha 7). The alpha7 nAChR is identified on GABAergic interneurons in the stratum oriens and stratum radiatum,

as well as pyramidal neurones, in the hippocampus. Our chemical compound, Malic acid, binds well with CHRNA7. Hence malic acid can be used in future studies for research purposes. Our findings play a major role in curing complicated neurological disorders such GTCs (Generalized tonic clonic seizures) and various associated seizures.

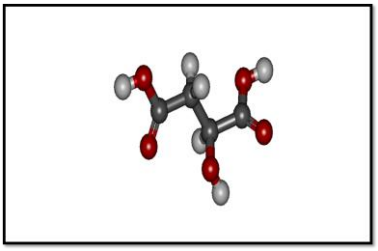


Fig. 1. The above picture represents the 3D structure of Malic acid with respective coloured atoms viewed using Discovery studio software (Model 1).

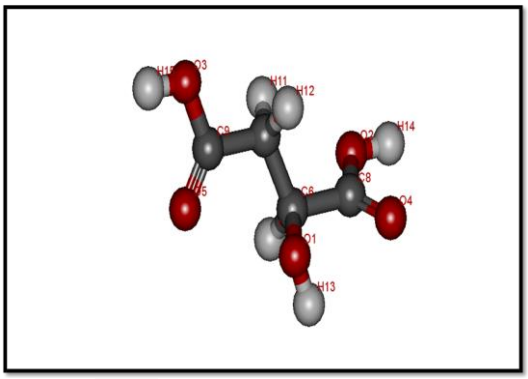


Fig. 2. The above picture represents the 3D structure of Malic acid with respective coloured and labelled atoms viewed using Discovery studio software (Model 2).

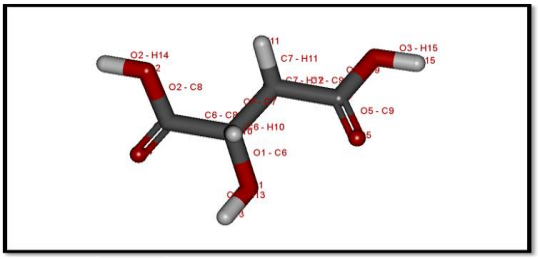


Fig. 3. The above picture represents the 3D structure of Malic acid with respective coloured and bonded atoms viewed using Discovery studio software (Model 3).

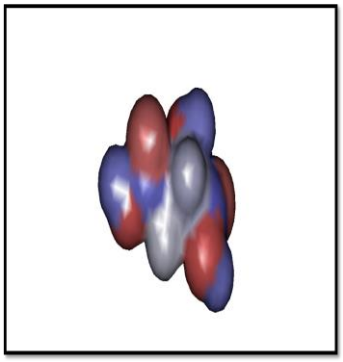


Fig. 4. The above picture represents the 3D structure of Malic acid in Van der Waals-surface model viewed using Discovery studio software (Model 4).

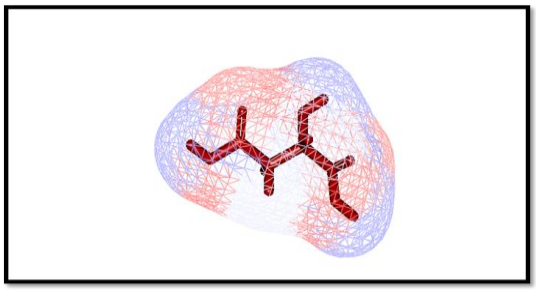


Fig. 5. The above picture represents the 3D structure of Malic acid in Electrostatic Force view viewed using Discovery studio software (Model 5).

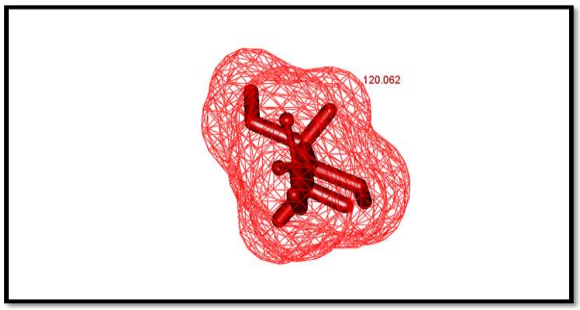


Fig. 6. The above picture represents the 3D structure of Malic acid in Wire mesh distance model viewed using Discovery studio software (Model 6).

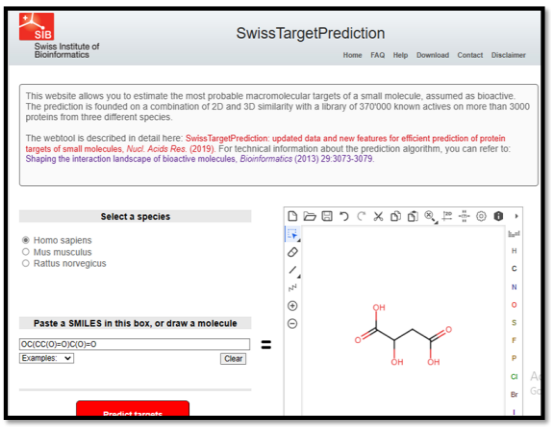


Fig. 7. The above picture shows the respective chemical compounds where the overall results were obtained from Swiss target prediction tool.

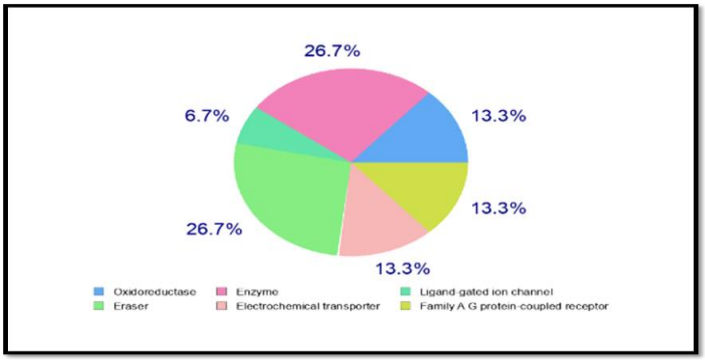


Fig. 8. The above pie diagram clearly shows the total number of drug binding regions occupied by Malic acid.

SwissTargetPrediction

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Egl nine homolog 1	EGLN1	Q9GZT9	CHEMBL5697	Oxidoreductase	0.0649510872641	0 / 2
ATP-citrate synthase	ACLY	P53396	CHEMBL3720	Enzyme	0.0544879468076	0 / 1
Neuronal acetylcholine receptor protein alpha-7 subunit (by homology)	CHRNA7	P36544	CHEMBL2492	Ligand-gated ion channel	0.0238327432783	0 / 2
Squalene synthetase (by homology)	FDFT1	P37268	CHEMBL3338	Enzyme	0.0238327432783	0 / 1
Histone deacetylase 3	HDAC3	O15379	CHEMBL1829	Eraser	0.0	0 / 1
Aldose reductase	AKR1B1	P15121	CHEMBL1900	Enzyme	0.0	0 / 1
HMG-CoA reductase	HMGCR	P04035	CHEMBL402	Oxidoreductase	0.0	0 / 1
Excitatory amino acid transporter 3	SLC1A1	P43005	CHEMBL2721	Electrochemical transporter	0.0	0 / 1
Excitatory amino acid transporter 2	SLC1A2	P43004	CHEMBL4973	Electrochemical transporter	0.0	0 / 1
Hydroxyacid oxidase 1	HAO1	Q9UJM8	CHEMBL4229	Enzyme	0.0	0 / 1
Lysine-specific demethylase 2A	KDM2A	Q9Y2K7	CHEMBL1938210	Eraser	0.0	0 / 1
Histone lysine demethylase PHF8	PHF8	Q9UPP1	CHEMBL1938212	Eraser	0.0	0 / 1
Lysine-specific demethylase 5C	KDM5C	P41229	CHEMBL2163176	Eraser	0.0	0 / 1
Prostanoid EP2 receptor	PTGER2	P43116	CHEMBL1881	Family A G protein-coupled receptor	0.0	0 / 1
Prostanoid FP receptor	PTGFR	P43088	CHEMBL1987	Family A G protein-coupled	0.0	0 / 1

Fig. 9. The above graphical picture explains the Bioactivities and Assays. The graphs display the molecular dynamics of Malic acid.

Protein farnesyltransferase	FNTA FNTB	P49354 P49356	CHEMBL2094108	Enzyme	0.0	0 / 3
Niemann-Pick C1-like protein 1	NPC1L1	Q9UHC9	CHEMBL2027	Other membrane protein	0.0	0 / 1
UDP-glucuronosyltransferase 2B7	UGT2B7	P16662	CHEMBL4370	Enzyme	0.0	0 / 2
GABA transporter 1	SLC6A1	P30531	CHEMBL1903	Electrochemical transporter	0.0	0 / 1
GABA-A receptor; alpha-1/beta-2/ gamma-2	GABRA1 GABRB2 GABRG2	P14867 P47870 P18507	CHEMBL2095172	Ligand-gated ion channel	0.0	0 / 1
GABA A receptor alpha-3/beta-2/ gamma-2	GABRA3 GABRB2 GABRG2	P34903 P47870 P18507	CHEMBL2111339	Ligand-gated ion channel	0.0	0 / 1
GABA A receptor alpha-2/beta-2/ gamma-2	GABRA2 GABRB2 GABRG2	P47869 P47870 P18507	CHEMBL2111413	Ligand-gated ion channel	0.0	0 / 1
GABA-B receptor	GABBR2 GABBR1	O75899 Q9UBS5	CHEMBL2111463	Family C G protein-coupled receptor	0.0	0 / 1
GABA receptor rho-1	GABRR1	P24046	CHEMBL3561	Ligand-gated	0.0	0 / 1
subunit				ion channel		
GABA transporter 3	SLC6A11	P48066	CHEMBL5208	Electrochemical transporter	0.0	0 / 1
Solute carrier family 22 member 6 (by homology)	SLC22A6	Q4U2R8	CHEMBL1641347	Electrochemical transporter	0.0	0 / 2
GABA transporter 2 (by homology)	SLC6A13	Q9NSD5	CHEMBL4535	Electrochemical transporter	0.0	0 / 1
Carnitine O-palmitoyltransferase 1, liver isoform (by homology)	CPT1A	P50416	CHEMBL1293194	Enzyme	0.0	0 / 2
Glucose-6-phosphate 1-dehydrogenase	G6PD	P11413	CHEMBL5347	Enzyme	0.0	0 / 1
Fatty acid binding protein adipocyte	FABP4	P15090	CHEMBL2083	Fatty acid binding protein family	0.0	0 / 1
Peroxisome proliferator-activated receptor alpha	PPARA	Q07869	CHEMBL239	Nuclear receptor	0.0	0 / 3
Fatty acid binding protein muscle	FABP3	P05413	CHEMBL3344	Fatty acid binding protein family	0.0	0 / 1
Fatty acid binding protein epidermal	FABP5	Q01469	CHEMBL3674	Fatty acid binding protein family	0.0	0 / 1
Peroxisome proliferator-activated receptor delta	PPARD	Q03181	CHEMBL3979	Nuclear receptor	0.0	0 / 2
Free fatty acid receptor 1	FFAR1	O14842	CHEMBL4422	Family A G protein-coupled receptor	0.0	0 / 1
Fatty acid binding protein intestinal	FABP2	P12104	CHEMBL4879	Fatty acid binding protein family	0.0	0 / 1

Fig. 10. The above graphical picture explains the Bioactivities and Assays. The graphs display the molecular dynamics of Malic acid.

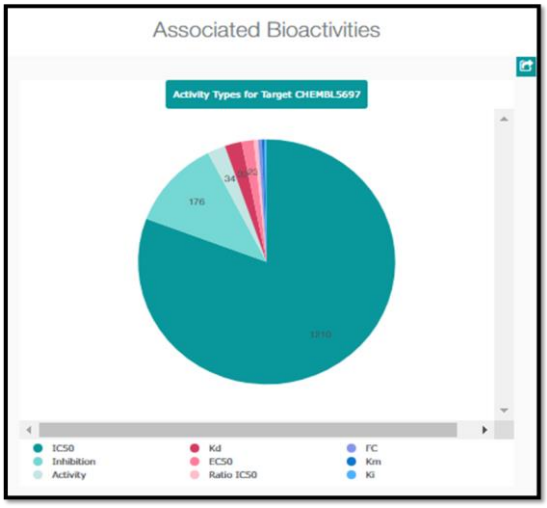


Fig. 11. The above Pie Diagram explains the Bioactivities and Assays. Both the graphs display the molecular dynamics of Malic acid.

Fig. 12. The above Pie diagram explains the Associated Assays. Both the graphs display the molecular dynamics of Malic acid.

Fig. 13. The above graphical picture explains the Ligand efficiency.

CONCLUSIONS

In pharmaceutical industries, the identification of human diseases targets which can efficiently bind with chemical compounds is a challenge. Overall, our results clearly show that Malic acid can be efficiently used to correct ion-conducting channel issues. Hence, we conclude that Malic acid acts as a potential therapeutic agent for Epilepsy disorder associated to CHRNA7 gene.

FUTURE SCOPE

This work can be extended to molecular drug docking studies and computer-aided drug designing in order to develop novel drug candidates for various human diseases.

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Conflict of Interest. None.

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