RESEARCH ARTICLE

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Identification of High-Affinity Small Molecule Targeting *IDH2* for the Clinical Treatment of Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) is symbolized by an increase in the number of myeloid cells in the bone marrow and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia) with or without leukocytosis either by a predominance of immature forms or a loss of normal hematopoiesis. *IDH2* gene encodes for isocitrate dehydrogenase enzyme which is involved in the TCA cycle domino effect and converts isocitrate to alpha-ketoglutarate. In the U.S, the annual incidence of AML progressively increases with age to a peak of 12.6 per 100,000 adults of 65 years or older. Mutations in isocitrate dehydrogenase 2 (arginine 132) have been demonstrated to be recurrent gene alterations in acute myeloid leukemia (AML) by forming 2-Hydroxy alpha ketoglutarate which, instead of participating in TCA cycle, accumulates to form AML. The current study approaches by molecular docking and virtual screening to elucidate inhibitor with superior affinity against *IDH2* and achieve a pharmacological profile. To obtain the best established drug Molegro Virtual Docker algorithm was executed. The compound AG-221 (Pub CID 71299339) having the high affinity score was subjected to similarity search to retrieve the drugs with similar properties. The virtual screened compound SCHEMBL16391748 (PubChem CID-117816179) shows high affinity for the protein. Comparative study and ADMET study for both the above compounds resulted in equivalent chemical properties. Virtual screened compound SCHEMBL16391748 (PubChem CID-117816179) shows the lowest re-rank score. These drugs are identified as high potential *IDH2* inhibitors and can halt AML when validated through further In vitro screening.

Keywords: Acute myeloid leukemia- *IDH2*- molecular docking- virtual Screening- pharmacophore-ADMET

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Introduction

According to the American Cancer Society (ACS), Acute Myeloid Leukemia (AML) is a group of hematological diseases, phenotypic and genetically heterogeneous, characterized by clonal expansion of myeloid with diminished capacity for differentiation. It is characterized by the fast growth of white blood cells, red blood cells, platelets, and it's a build-up in the bone marrow restricting the production of traditional blood cells. Once healthy bone marrow cells are replaced with the leukemic cells, it results in a decline in red blood cells, platelets, and healthy white blood cells. Mainly, symptoms that are seen in patients affected by AML are Weight loss, Fatigue, Fever, Night sweat, Loss of appetite, shortness of breath, natural bruising and bleeding, with lots of body abnormalities and a magnified risk of infection.

According to investigations, 15 to 20% of acute leukemia cases are in children and 80% in adults, 3-4 cases of AML per 100,000 adults are diagnosed annually. Among all reported cases, 42.8% of patients >65 years age are rarely diagnosed before the age of 40 years which concludes that Acute Myeloid Leukemia (AML) is predominantly found in adults (Deschler et al., 2006). AML varies with gender; incidence rate of AML is 4.3 and 3 percent per 100,000 males and females respectively and is more commonly found in blacks than whites with the incidence rate of 4.3 and 3.9 respectively per 100,000 (Redaelli et al., 2003).

Since AML is a genetically heterogeneous clonal disorder, the somatic mutation in many genes is identified such as *FLT3, C-Kit, N-RAS, NPM1, CEBPA, WT1, ASXL1,*

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DNMT3A, MLL, IDH1, IDH2 and *TET-2* (Marcucci et al., 2011). Recent advances in mutational analysis led to the discovery of isocitrate dehydrogenase (*IDH*) mutations in AML. *IDH2* is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate. Its mutated version leads to the accumulation of the oncometabolite (R)-2 hydroxyglutarate, which disrupts several cell processes and leads to a blockage in differentiation. The isocitrate dehydrogenase *IDH2* gene is identified to be frequently mutated in acute myeloid leukemia (AML) patients. The *IDH2* is located in the mitochondrion and are normally involved in citrate metabolism in the tricarboxylic acid cycle. Targeting IDH2 is compelling, as it is an early and stable mutation in AML (Amaya and Pollyea, 2018). However, hematopoieticstem cell disorder also results in the blockage created by hematopoiesis and overproliferation of immature cells or blast cells which leads to Acute myeloid leukemia (AML) (Shipley et al., 2009). This leukemia gets developed in precursors of myeloid cell lineages due to chromosomal rearrangements and mutation in multiple genes.

Mutation in DNA of stem cells of bone marrow, hematopoietic stem cell, which is responsible for the production of red blood cell, platelets, and white blood cells, causes more production of white blood cells than required. The *IDH2* gene also encodes for an enzyme, NADP(+) dependent homodimer isocitrate dehydrogenase-2, found in mitochondria at chromosome position 15q26.1 and participates in producing energy for cell activities. The enzyme converts isocitrate compound to alpha-ketoglutarate (α-KG) and produces a molecule NADPH which protects the molecule from highly reactive oxygen species (ROS) (Lu, Venneti et al., 2013). Somatic mutation of the *IDH2* gene was initially identified in 80% of gliomas and 20% of acute myeloid leukemia (AMLs) (Lu et al., 2013). However, somatic monoallelic point mutation only manipulates some of the residues consequently. It is not non-functional and it produces 2-hydroxyglutarate (2HG) from alpha-ketoglutarate (Ward et al., 2010).Mutation in the gene was identified by analyzing patients with acute myeloid leukemia (AML) at the position of *IDH1R132C*, *IDH2R140* and *IDH2R172K* using whole genome sequencing technique. This mutation decreases the affinity of isocitrate dehydrogenase for isocitrate and enhances it for alpha-KG which leads to oxidative decarboxylation of alpha-ketoglutarate to isocitrate. It converts by the reduction of alpha-ketoglutarate to 2- hydroxyglutarate (D-2HG). Excess accumulation of D-HG causes AML and glioma also. This conversion of the enzyme activity of alpha-KG to 2-DHG, from wild-type to mutant forms the neomorphic activity of enzyme (Ward et al., 2010).

Hence, inhibition of *IDH2* plays a vital role in treatment of AML. As the protein does not contain an active catalytic site, blocking *IDH2* activation would necessitate an inhibition of dimerization through allosteric interactions. Screening of vast chemical libraries and the use of computational models to evaluate binding ability have revealed a number of compounds that inhibit *IDH2* dimerization and exhibit biologic activity against tumor cell. *IDH2* represents a promising anticancer target for pharmacologic intervention, due to its central position in numerous signaling pathways.

Materials and Methods

Selection of Inhibitors

Pre-existing inhibitors of *IDH2* involved in Acute Myeloid Leukemia (AML) were chosen from a few literary works. Four inhibitors were chosen in this study. The inhibitors having PubChem CID, their molecular weight in gm. /mol, H-bond acceptor, H-bond donor and log p-value is listed in table (Table 1). The 3D structure of each one of those compounds were saved in SDF format. All those inhibitors are indexed accordingly having Pubchem ID with 3D structure (Table 1).

Protein and Ligand preparation

The crystal structure of target protein, Isocitrate Dehydrogenases (*IDH2*), was recuperated from Protein Data Bank (PDB ID: 5SVN)and was fetched for further studies of docking process(Xie et al, 2017) (Figure 1).The 3D conformers of inhibitors having pubchem ID were saved in SDF format. Furthermore, the complete hoard of inhibitors in the form of of 3D structures was prepared using OPLS 2005 force field algorithm embedded in the LegPrep module of Schrödinger suite, 2013 (Schrodinger. LLC, New York, NY) (Babitha et al., 2015; Bandaru et al., 2017a; Basak et al., 2016a, Dunna et al., 2015a). Eventually, in this procedure of preparation, the protein was added with disulfide bonds, missing side chains were filled, water molecules were removed beyond 5 Å from hetero groups, and was saved in the SDF format for further docking studies (Dunna et al., 2015b; Divya et al., 2019; Bandaru et al., 2015a; Kelotra et al., 2014a;Basak et al., 2016b).

Molecular Docking

The molecular docking studies was performed by using Molegro Virtual Docker (MVD) which is unified with high potential Piece Wise Linear Potential (PLP) and MolDock scoring function (Kelotra et al., 2014b; Bandaru et al., 2014; Khandekar et al., 2016; Bandaru et al., 2013). Subsequently, all cavities were detected to get high volume cavity and hence the highest volume cavity (>2000Å) was selected for docking and to target the active of the IDH2 structure (PDB ID: 5SVN) with the pre-prepared 4 ligands. The docking procedure parameters were selected as required, having a maximum population size of 50, maximum iteration of 1,500 and 0.20Å as grid resolution (Khandelwal et al., 2018; Bandaru, 2017b; Majhi et al., 2018; Nasr et al., 2015). Further, the post-docking process carries hydrogen bonds optimization and energy minimization, Simplex Evolution at max steps 300 and neighbor distance technical setting fast at 1.00(Sharda S et al., 2019; Ali MA et al., 2019).

Binding affinity and interactions of ligands with protein was evaluated from the internal electrostatic interaction, internal hydrogen bond interactions, and sp2-sp2 torsions. Later on, the energy of the ligand-receptor complex was minimized using Nelder Mead Simplex Minimization (using non-grid force field and H bond directionality)

(Padmini et al., 2019; Sinha et al., 2019; Palak et al., 2019; Patidar et al., 2016). On the basis of the lowest re-ranking score, the best interacting compound was selected for the further studies (Nayarisseri et al., 2018; Bandaru, 2015b; Praseetha et al., 2016; Mendonça-Junior et al., 2019).

Structure-based virtual Screening

Similarity search was carried out to obtain the best compound having greater affinity other than any established drugs. The filtration properties parameter set by component rule of Lipinski's rule of five at Threshold >=95% against NCBI's Pubchem compound database which is a NIH organized public chemical repository of 93 million chemical compounds (Patidar et al., 2019; Monteiro et al., 2019; Rao et al., 2010; Shaheen et al., 2015). As a result, the complete compounds retrieved were again compared with docking studies to obtain a candidate with a better affinity that can halt target protein *IDH2*.

Drug – Drug comparative study

Hereof, the unnamed complex structure of both the outcomes i.e. established compound and virtual screened compound was further imported in Molecular Docker for comparative study. Both the compounds were cleaned by removing constraints. Both these compounds were first exported as the best-posed compounds from the docking results. The comparative result was imported in excel sheet to analyze the affinities, hydrogen interaction, steric energy and high rerank score to identify the best inhibitor.

ADMET studies

The admetSAR database with ADMET properties of a compound deal with its absorption, distribution, metabolism, excretion, and toxicity in the human body which play the key role in the discovery of a compound. Owing to the superior affinity of the best-established compound and virtual screened compound, the bioactivity properties and toxicity was predicted by using admetSAR (Shameer et al., 2017; Sharda et al., 2017; Sharma et al., 2018; Nayarisseri et al., 2019). The admetSAR database which constitutes the pharmacokinetic profile of a drug molecule is essential in evaluating its pharmacodynamic activities. This database owes free interface interpellation having a distinct biological and chemical contour. The admetSAR database considers on the 5 quantitative regression and 22 qualitative classification models which provide a more established precise result. The free interface of admetSAR tool was used (http://lmmd.ecust.edu.cn:8000/)(Natchimuthu et al., 2016; Chandrakar et al., 2013; Pandey et al., 2013).The compounds which carried greater affinity score from established compounds and virtual screened compounds after docking were used to analyze the ADMET competency (Sinha et al., 2015; Sinha et al., 2014; Trishang et al., 2019; Vuree et al., 2013; Gudala et al., 2015).

Software and web server used

NCBI PubChem database were used toretrieve the 3D structure of compounds in SDF format to perform virtual screening against the PubChem database. Ligand preparation was optimized by the LegPrep module of Schrodinger suite 2013. Protein was processed and refined with the protein preparation wizard of Schrodinger suite 2013 (Schrodinger. LLC, 2009, New York, NY). The Flexible molecular docking of the compounds with the target was completed using Molegro Virtual Docker 2010.4.0.0. Accelrys Discovery Studio® Visualizer 3.5.0.12158 (Copyright© 2005-12, Accelrys Software Inc.). ADMET profiles were calculated using admetSAR online tool (Laboratory of Molecular Modeling and Design. Copyright@ 2012, East China University of Science and Technology, Shanghai Key Laboratory for New Drug Design).

BOILED EGG PLOT

A Boiled Egg plot, endows a supportive guidance, intuitive, accommodatingly reproducible and an incomparable statistical plot to foreshow the two passive prediction of small molecules, i.e., gastrointestinal absorption and brain-permeant. This model supplies a superior optimization method. Gastrointestinal absorption and Brain access are two crucial pharmacokinetic parameters necessary in estimating the stages of the drug discovery processes. The Brain and IntestinaL EstimateD permeation method (BOILED-Egg) is proposed as an accurate predictive model that works by computing the lipophilicity and polarity of small molecules. This computational model also confers parameters; MW, TPSA, MLOGP, GI and BBB. Herein, if the compound falls on the white ellipse of the plot, the probability of a good intestinal absorption is high. Whereas if the compound falls on the yellow ellipse (i.e. the egg yolk), the probability of Blood–Brain Barrier (BBB) crossing is high. Apart from both the white area and yellow ellipse, the compounds found on the grey area are considered to be out of range of the plot as the molecules are exclusively non-absorptive by GI and BBB non-permeate. Furthermore, among the established compound the two best drugs based on the lower re-rank score was retrieved and the same procedure with the compounds of the screened compound (Tables 3 and 4).

Results

Docking results Established Compound Docking Results

The molecular docking studies of 4 pre-established inhibitorswas performed using Molegro Virtual Docker (Table.1). It showed the compound AG-221(PubChem ID-89683805) to be the best established compound among the all pre-established compounds.This compound has the lowest energy with -97.4,976 rerank score and shows the higher affinity score directed towards our target protein *IDH2* (Table 2). General properties are: molecular weight 473.383 g/mol, 3 hydrogen bond donor, 14 hydrogen bond acceptor , topological polar surface area 109 A^2 and the logP value is 3.5.

Virtual Screening result

Further similarity search for AG-221inhibitor resulted in 66 virtual screened compounds (Table 3). The compound with SCHEMBL16391748 (PubChem CID-117816179) has the highest affinity among others.

Table 1. Potential IDH2 Inhibitors with PubChem ID

Sl.	Inhibitors	Pub ID	MW (Gm./Mol)	H-Bond Acceptor	H-Bond Donor	Log p	Ref
1	Enasidenib, AG-221	89683805	473.383	14	3	3.5	Sjöblom T et al., 2006
2	AGI-6780	71299339	481.508	8	3	4.2	Sjöblom T et al., 2006
3	7-Ketocholesterol	91474	400.647	2	1	7.5	Katharine Yen et al, 2013
4	AG-120	89699486	582.968	9	1	3.4	(Xiaodong Fu et al., 2014; Stein EM et al., 2016)

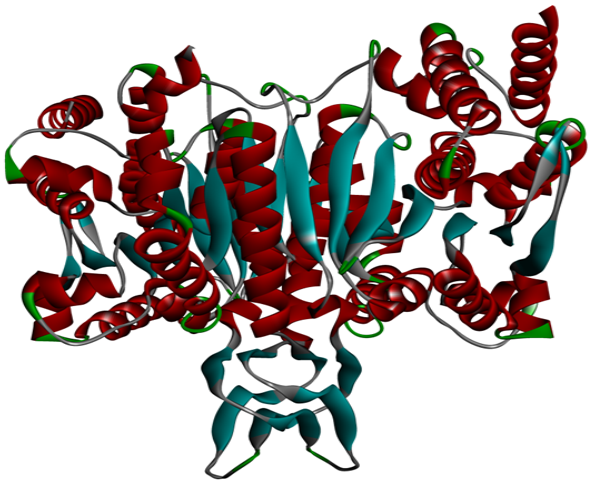

Figure 1. Protein 3D Structure of *IDH-2* Obtained from PDB (PDBID: 5SVN) Visualization in Accelrys Discovery Studio

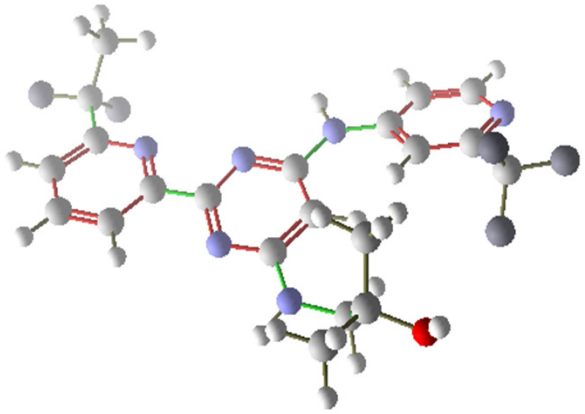

Figure 2. SCHEMBL16391748, PubChem CID: 117816179 Show the Most Effective Compound Binding with *IDH2*

This compound has a molecular weight of 468.432 g/mol 3 hydrogen bond donor and 12 hydrogen bond acceptor, a topological surface area of 95.8 A^2 and a log P value is 3.7. Among all these 66 virtual screened compounds and 4 pre- established compounds, the drug SCHEMBL16391748 (PubChem CID-117816179) has much potential inhibition against Acute Myeloid Leukemia(AML) over the target protein *IDH2*.

Pharmacophore studies

Pharmacophore mapping is a depicting model which provides the finest accuracy of binding site as well as in the discovery of the novel drug. Pharmacophore studies provide accurate query on the optimum interaction with suitable target annotations and represent the aligned poses of the molecule and help us to find the high interaction mode between target protein and compound. This mapping model was employed to identify targets and the compound interaction in its cavity. Owing to the predicted higher affinity of virtual screened compound SCHEMBL16391748 (PubChem CID: 117816179) with the high-affinity score it was moved ahead for pharmacophore mapping studies. Pharmacophore mapping also results in the positive intensities of electrostatics and there is a variation in intensities of the aromatic compounds and the charges respectively.

The H-bond interactions of the virtual screened compound SCHEMBL16391748 (PubChem CID: 117816179) with lowest rerank score possessing immense affinity at the active sites of *IDH2* cavity is shown in (Figure 3). The small green dotted lines are hydrogen bond interaction where SCHEMBL16391748 forms three hydrogen bond interactions with residue Gln316.

(PubChem CID: 117816179) with protein residues involved Gln 316

(Figure 4) shows the residue interaction of virtual screened compound SCHEMBL16391748 PubChem

Table 2.Docking Results for *IDH2* Inhibitors

Name	MolDock Score	Rerank Score	Interaction	HBond	MW
[02] 89683805	-131.767	-97.4976	-165.709	-14.7664	473.375
[03] 89683805	-126.856	-94.9008	-156.297	-8.18506	473.375
[01] 89699486	-151.317	-92.2886	-140.589	-9.25299	582.961
[01] 89683805	-127.392	-91.7372	-156.855	-9.9447	473.375
[03] 91474	-114.25	-91.1626	-121.211	-1.92038	400.637
[02] 91474	-115.811	-91.0259	-124.627	-1.51956	400.637
[00] 91474	-117.687	-90.8601	-125.468	-3.84462	400.637
[04] 89683805	-124.328	-90.6903	-158.038	-4.36196	473.375
[00] 71299339	-132.593	-88.8993	-146.046	-6.19172	481.511

Table 3.Docking Results for Virtual Screened Compounds

Name	MolDock Score	Rerank Score	Interaction	HBond	MW
[00] 117816179	-158.131	-130.314	-180.051	-6.16134	468.423
[00] 129060692	-142.052	-123.882	-166.809	-4.61276	419.404
[00] 129060700	-145.482	-120.91	-163.557	-6.71656	419.404
[00] 129066642	-137.537	-120.591	-176.01	-4.57791	491.365
[00] 117847478	-146.414	-117.861	-167.946	-4.3994	473.375
[00] 89684023	-148.648	-117.753	-178.445	-4.87312	473.375
[00] 121453832	-147.688	-117.692	-178.178	-5	473.375
[00] 129060704	-143.096	-117.384	-162.743	-10.1233	419.404

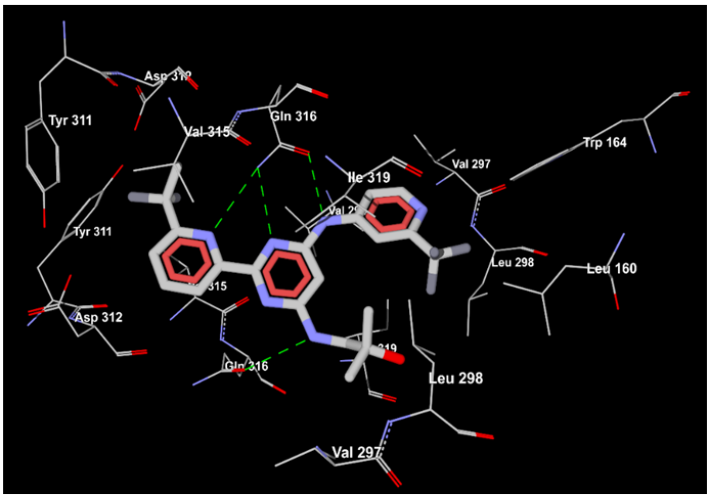

Figure 3. Showing Three H-bond Interaction of Ligand SCHEMBL16391748 (PubChem CID: 117816179) with Protein Residues Involved Gln 316.

CID: 117816179 in the cavity of protein *IDH-2*. The interaction of the residues with ligands which are green circled shows van der Waals interaction and the residues which are pink circled show Electrostatic interactions. A green arrow between the residues and ligands show Hydrogen bond interaction. This figure shows two hydrogen bond interactions with Gln 316 (Figure 4).

Electrostatic interaction of virtual screened compound SCHEMBL 16391748 (PubChem CID: 117816179) with protein *IDH2* having high affinity embedded in the protein cavity is shown in (Figure 5). Electrostatic interaction manifests that clusters of charged and polar residues that are detected on protein–protein interfaces and intensify complex stability, although the total effect of electrostatics is generally net destabilizing. The white surface is electrically neutral. The protein carries two types of variant colors for demonstrating positive and negative area, red color revealing electro-negativity zone whereas

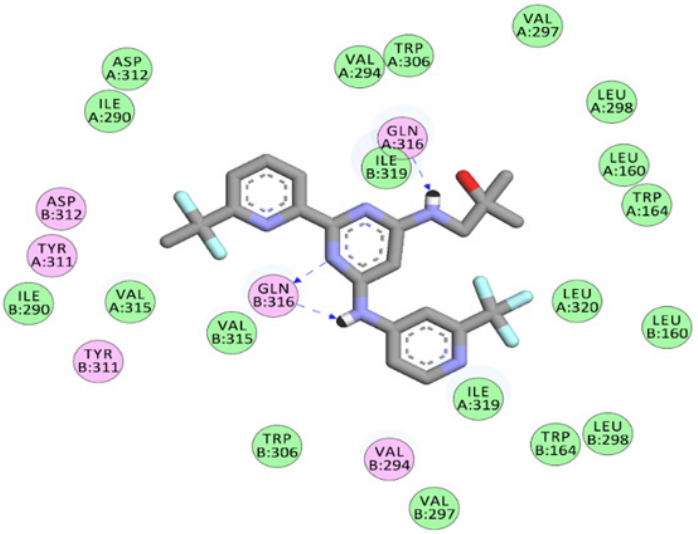

Figure 4. The High Affinity Compound SCHEMBL16391748 (PubChem CID: 117816179) Showing van der Waals Interaction

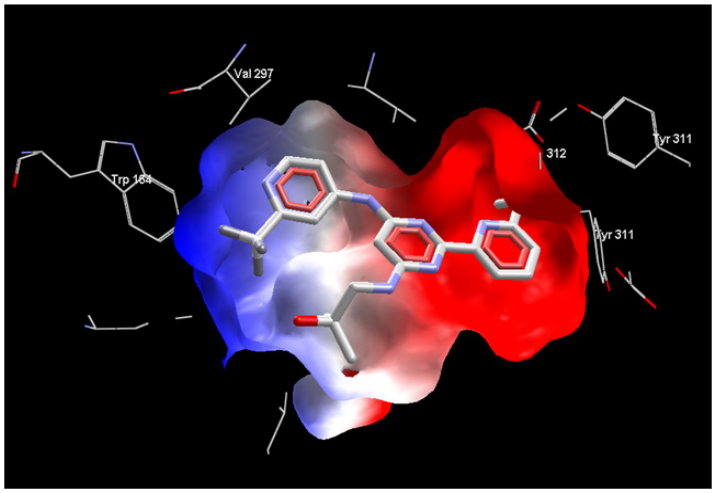

Figure 5. The High Affinity Compound SCHEMBL16391748 (PubChem CID: 117816179) Revealing Electrostatic Interaction

blue color revealing electropositive zone (Figure 5). The target compound SCHEMBL16391748 (PubChem CID: 117816179) is surrounded by electronegative residues in red color (Figure 5).

The binding pattern of ligand SCHEMBL16391748 (PubChem CID: 117816179) in the cavity of *IDH2* is shown in (Figure 6).The pink lines represent various interactions like electrostatic, van der Waals, stearic, hydrogen bonding and hydrophobic interactions that enable energetically favorable binding of the ligand in the cavity.

The hydrophobic interaction in (Figure 7) imparts an entropic effect and can account for several biophysical events in the protein-ligand binding that are of immense importance in drug design (Figure 7).At the binding site of protein IDH2, virtual screened compound SCHEMBL16391748 CID :(117816179) is interpolated with hydrophobic intensities (Figure 7). As according to figure the hydrophobicity ranges its intensity from 3.00 (maximum hydrophobic zones) to -3.00 (minimum hydrophobic zones). The minimum intensity areas are with blues shaded region and maximum intensity areas are with dull brownish color. Target inhibitor interpolated in dull brown color surfaces show high hydrophobic intensity whereas the pre-established inhibitor AG-120 (PubChem CID-89699486) has very low hydrophobic intensity.

ADMET studies

The ADMET studies of two compounds i.e. the best pre-established compound AG-221(PubChem ID-89683805) and the virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) revealed slight difference demonstrating equivalent properties (Table 4). The parameter of BBB-Blood-Brain Barrier in ADMET study revealed positive absorption for the candidate drug SCHEMBL16391748 (PubChem ID-117816179). The HIA-Human Intestinal Absorption probability for both the compounds is nearly equal. The values are nearly the samefor P-glycoprotein Substrate and P-glycoprotein Inhibitor for the virtual screened compound. Thereafter, in terms of distribution of subcellular localization in mitochondria, SCHEMBL16391748 (PubChem

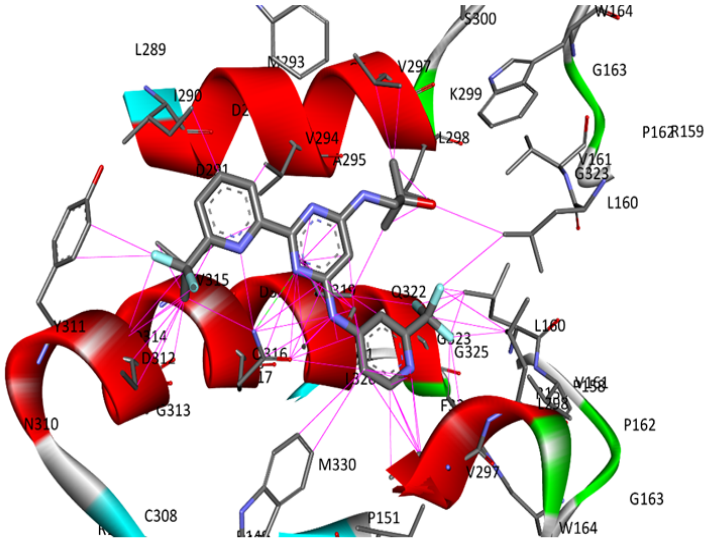

Figure 6. The High Affinity Compound SCHEMBL16391748 (PubChem CID:117816179) Shows Binding Pattern of Ligands

Table 4. ADMET Predicted Profile- Classification

Model	Result	Compound AG-221 CID-:89683805 Probability	SCHEMBL16391748 CID-:(117816179) Probability
Absorption			
Blood-Brain Barrier	BBB+	0.8041	0.836
Human Intestinal Absorption	HIA+	1	0.9965
Caco-2 Permeability	Caco2-	0.5734	0.5806
P-glycoprotein Substrate	Substrate	0.6798	0.6585
P-glycoprotein Inhibitor	Non-inhibitor	0.6358	0.5714
	Non-inhibitor	0.6694	0.525
Renal Organic Cation Transporter	Non-inhibitor	0.9045	0.9283
Distribution			
Subcellular localization	Mitochondria	0.5694	0.5083
Metabolism			
CYP450 2C9 Substrate	Non-substrate	0.8424	0.815
CYP450 2D6 Substrate	Non-substrate	0.7993	0.7935
CYP450 3A4 Substrate	Non-substrate	0.5651	0.5352
CYP450 1A2 Inhibitor	Non-inhibitor	0.5	0.518
CYP450 2C9 Inhibitor	Non-inhibitor	0.7458	0.7473
CYP450 2D6 Inhibitor	Non-inhibitor	0.7483	0.758
CYP450 2C19 Inhibitor	Non-inhibitor	0.6534	0.6195
CYP450 3A4 Inhibitor	Inhibitor	0.511	0.6774
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.7468	0.5913
Excretion Toxicity			
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9772	0.9765
	Non-inhibitor	0.5824	0.5645
AMES Toxicity	Non-AMES toxic	0.7258	0.7308
Carcinogens	Non-carcinogens	0.7461	0.7
Fish Toxicity	Low FHMT	0.5992	0.5599
Tetrahymena Pyriformis Toxicity	High TPT	0.9851	0.9889
Honey Bee Toxicity	Low HBT	0.8319	0.8197
Biodegradation	Not ready biodegradable	1	1
Acute Oral Toxicity	III	0.6553	0.6361
Carcinogenicity (Three-class)	Non-required	0.5086	0.4697

ID-117816179) displayed slightly higher probability values with an established best compound. Later on, in terms of metabolism prediction both the compounds show the probability values nearly equal to each other. In case of CYP450 3A4 Inhibitor, virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) has a higher value than the established compound AG-221 (PubChem ID-89683805) whereas in terms of CYP Inhibitory Promiscuity it is the opposite, showing the established compound result having a higher value than the virtual screened compound. Moving forward to excretion and toxicity, both the candidates have equivalent values.

Table 5.ADMET Predicted Profile-Regression

Model	Unit	Compound AG-221 CID: 89683805 Value	SCHEMBL16391748 CID-(117816179) Value
Absorption			
Aqueous solubility	LogS	-3.1818	-3.243
Caco-2 Permeability	LogPapp, cm/s	0.6503	0.6056
Toxicity			
Rat Acute Toxicity	LD50, mol/kg	2.5366	2.5411
Fish Toxicity	pLC50, mg/L	1.7437	1.6543
Tetrahymena Pyriformis Toxicity	pIGC50, ug/L	0.3212	0.398

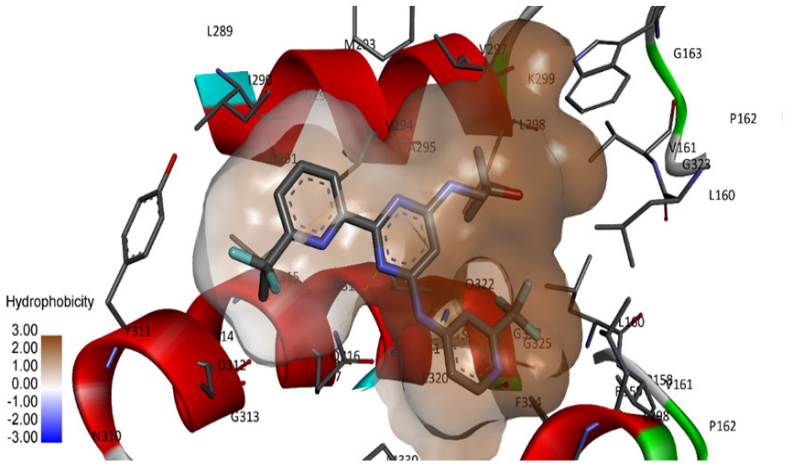

Figure 7. The High Affinity Compound SCHEMBL16391748 (PubChem CID: 117816179) Depicting Hydrophobic Interaction

Furthermore, the ADMET profile prediction on the basis of profile regression and in terms of absorption and toxicity, show that both the compounds are almost equivalent to each other (Table 5).

Drug-Drug comparison studies

The drug-drug comparative study of two compounds, AG-221 (PubChem ID-89683805) and SCHEMBL16391748 (PubChem ID-117816179) revealed the affinity and interaction respectively (Table 6). The best virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) is represented to have its total energy superior to established docked compound AG-221(PubChem ID-89683805). The steric energy of PLP (Piecewise Linear Potential) and steric energy of LJ12-6 (Leonard-Jones approximation) of these two compound represents that the virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) has

Table 6. Drug-Drug Comparison

	Established drug		Virtual Screened drug	
Energy overview: Descriptors	MolDock Score	Rerank Score	MolDock Score	Rerank Score
Total Energy	-131.764	-97.495	-158.118	-130.317
External Ligand interactions	-165.713	-131.037	-180.054	-149.953
Protein - Ligand interactions	-165.713	-131.037	-180.054	-149.953
Steric (by PLP)	-150.945	-103.549	-173.891	-119.289
Steric (by LJ12-6)		-15.793		-25.783
Hydrogen bonds	-14.768	-11.696	-6.163	-4.881
Hydrogen bonds (no directionality)		0		0
Electrostatic (short range)	0	0	0	0
Electrostatic (long range)	0	0	0	0
Cofactor – Ligand	0	0	0	0
Steric (by PLP)	0		0	
Steric (by LJ12-6)		0		0
Hydrogen bonds	0	0	0	0
Electrostatic	0	0	0	0
Displaceable Water interactions	0	0	0	0
Internal Ligand interactions	33.949	33.543	21.936	19.637
Torsional strain	14.259	13.375	1.919	1.8
Torsional strain (sp2-sp2)		6.979		0.499
Hydrogen bonds		0		0
Steric (by PLP)	22.191	3.817	20.017	3.443
Steric (by LJ12-6)		9.372		13.895
Electrostatic	0	0	0	0
Soft Constraint Penalty	0		0	
Search Space Penalty	0		0	

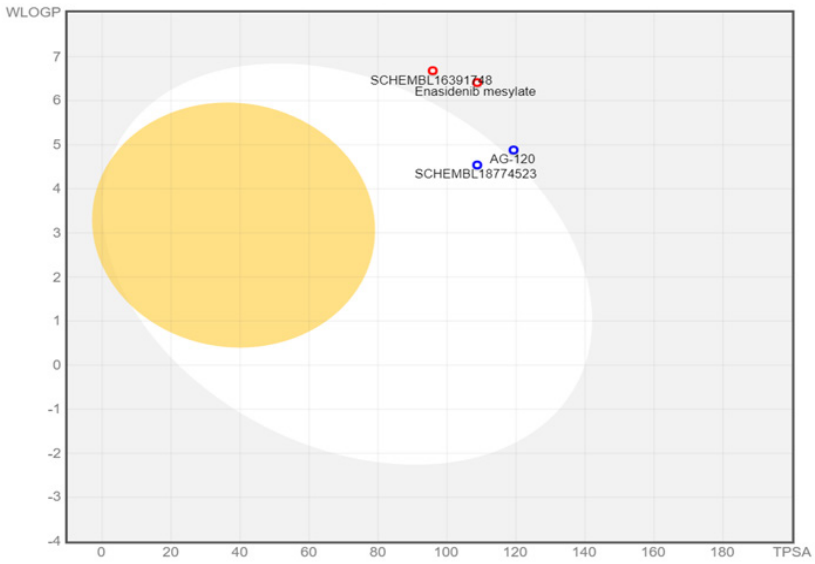

Figure 8. Predictive Model Brain Or IntestinaL EstimateD Permeation Method (BOILED-Egg)

Table 7. Best 6 Compounds from Established Dock Result and Virtual Screened Docked Result Used for Boiled Egg

Molecule	Pub Id	MW	TPSA	WLOGP	MLOGP	GI absorption	BBB permeant
Enasidenib mesylate	89683805	473.37	108.74	6.41	1.41	Low	No
AG-120	89699486	582.96	119.29	4.88	2.07	Low	No
SCHEMBL16391748	117816179	468.42	95.85	6.68	2.11	Low	No
SCHEMBL18774523	129060692	419.4	108.74	4.54	0.68	High	No

high affinity. The hydrogen bond interaction of these two drugs shows high affinity for established drug AG-221(PubChem ID-89683805). As per the complete estimation of comparative study it shows that virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) has high probability to inhibit protein *IDH2*.

Boiled Egg Plot Analysis

The BOILED-Egg plot of 4 candidates having two favorable properties: Blood brain barrier and gastrointestinal absorption bestow an efficient pharmacokinetics profile (Padmini et al., 2019; Sinha et al., 2019; Palak et al., 2019; Patidar et al., 2019; Khandelwal et al., 2018; Majhi et al., 2018). Two compounds were selected from established docked results: Enasidenib mesylate (PubChem ID-89683805) and AG-120(PubChem CID-89699486). Two compounds were selected from virtual docked results: SCHEMBL16391748 (PubChem ID-117816179) and SCHEMBL18774523 (PubChem CID-129060692 (Table 7) SCHEMBL16391748 (PubChem ID-117816179), AG-120(PubChem CID-89699486) and Enasidenib mesylate display low GI-Gastrointestinal absorption and low brain permeation lying under the grey zone of egg plot. AG-120 (PubChem CID-89699486) find its place in the white zone of egg Figure 9, which explains high GI absorption.No compound lie on the yellow ellipse (yolk) and hence are impermeable to the brain. The compound SCHEMBL18774523 (PubChem CID-129060692) is neither absorbed nor brain penetrable (Table 6).

Discussion

The study is an attempt to illustrate inhibitors from various literature which inhibits the protein *IDH2* for finding new leads in the treatment of acute myeloid leukemia (AML). This study intends to demonstrate inhibitory action2- hydroxyglutarate (D-2HG), by blocking the action of isocitrate dehydrogenase protein. The execution of computational prediction of molecular docking studies shows AG-120 (PubChem CID-89699486) as the best established inhibitor. With reference to this compound, virtual screening and subsequent docking shows a compound SCHEMBL16391748 (PubChem ID-117816179). Comparative prediction and docking studies for these candidates show that virtual screened docked drug SCHEMBL16391748 has high-affinity score and potential ability to inhibit the activity of *IDH2*. In this research, we show the pharmacophore mapping of the virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) which depicts optimal binding in the cavity of IDH2 protein with inhibitory ability. Established compound AG-120 and virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) were examined for ADMET study which shows the compounds as non-carcinogenic and non-mutagenic having analogous properties. According to the results, the virtual screened compound was found having a high probability of HIA-Human Intestinal Absorption and high BBB-Blood Brain Barrier probability besides showing non AMES toxicity. Prediction with BOILED-Egg plot shows

that SCHEMBL16391748 (PubChem ID-117816179) represents high bioavailable optimization as compared to other drugs: PubChem CID-129060692, pre-established compound AG-120(PubChem CID-89699486) and Enasidenib mesylate(PubChem ID-89683805). Virtually screened inhibitor SCHEMBL16391748 (PubChem ID-117816179) also showed a very low rerank score describing its stability with the protein. On consideration of all the above properties, the virtual screened compound SCHEMBL16391748 (PubChem ID-117816179) can be a potential inhibitor for the protein and can emerge as an important drug in the treatment of AML in future ahead.

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Author contributions

All authors have been personally and actively involved in substantive work leading to the manuscript, and will hold themselves jointly and individually responsible for its content.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval and informed consent not required for this manuscript.

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