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Molecular Docking Studies of BCl2 towards Chronic Lymphocyctic Leukemia

Nisha Kanwar,

Assistant Professor,

Institute of Biological Science,

SAGE University, Indore

50-A, Shiv Dham Colony, Khandwa Road, Indore (M.P.)

nishakanwar010270@gmail.com

8889505995,7987385524

1. **Abstract:** Leukemia is a cancer that involves the blood-forming tissues of the bone marrow, spleen and lymph nodes. It is characterized by an uncontrolled production of abnormal, immature blood cells. When a person has leukemia, the bone marrow does not work properly. The bone marrow produces abnormal, immature cells, called leukemia cells. Bcl-2 also called as B cell lymphoma 2 is a protein encoded by BCL-2 genes in humans.Bcl-2 is an anti-apoptotic protein that inhibits apoptosis & thus it promotes cell growth. The most mutated gene in CLL is BCL-2 .The current study aimed to identify high affinity small molecules which can block BCL-2 using Molecular Docking studies. Through Molecular Docking studies, Pubchem ID: 45028345, Survivin found to be the most effective compound bounding BCL-2 with a lowest rerank score of -168.022. Hence the same was used for the pharmacophore studies such as H-bond interaction, elector static interaction, Aromatic interaction, Hydrophilic interaction etc. Further studies can continue with Virtual screening, similarity searching for survivin inhibitor showed 312 compounds. The docking result of entire 312 virtual screened compounds showed PubChem CID101725649 found to be the most effective compound bounding BCL-2 with a lowest rerank score of -159.516.

Keywords: Molecular Docking, Virtual Screening, ADMET, BCL2, Chronic Lymphocytic Leukemia

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2. Introduction

Cancer is uncontrolled division of cells that leads to malignancy,but another important reason of cancer is inhibition of programmed cell death.[1] Bcl-2 also called as B cell lymphoma 2 is a protein encoded by BCL-2 genes in humans.Bcl-2 is an anti-apoptotic protein that inhibits apoptosis & thus it promotes cell growth.It contains four BH domains,BH-4 domain is only present in anti-apoptotic proteins,not present in pro-apoptotic proteins that induces apoptosis.[2]

Bcl-2 protein induces cancers, as cancers are also caused by mutations in tumor suppressor genes like P53, over production of growth promoting factors & under production of growth inhibiting factors.[3] Bcl-2 protein belongs to a family of apoptosis regulatory gene products that may be antagonists of death or agonists of death. Over expression of Bcl-2 is observed in patients suffering from chronic lymphocyctic leukemia in which clonal propagation of B cells is seen in blood ,lymph nodes.[4]

Bcl-2 is located in outer membrane of mitochondria, nucleus & endoplasmic reticulum, it inhibits release of cytochrome c from mitochondria & inhibit caspases, a protease that induces apoptosis so that intrinsic apoptotic pathway is inhibited.[5] Expression of Bcl2 mRNA in chronic lymphocytic leukemia is high, therefore antisense RNA technological approaches have been used to down-regulate its expression.[6]

Micro-RNA named as miR-15a and miR-16-1negatively regulate the expression of anti-apoptotic protein Bcl-2.[7] Bcl-2 protein is resistant to chemotherapy treatment also. Antisense oligonucleotide down-regulates the expression of Bcl-2 gene .[8]In the present study structure and ligand based approaches have been implemented to identify drug, which have better inhibitory potential for anti-apoptotic protein Bcl2.

3. Methodology

3.1 Selection of inhibitors:

In the present study, 57 established inhibitors of BCL2 that target CLL were selected. Some inhibitors were lacking 3D structures, so their 3D structures was created using Marvin Sketch and was saved in SDF format. Table[1] shows all those inhibitors that are having Pubchem ID with 3D structure and Pubchem ID with prepared 3D structure :

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Table 1: Inhibitors selected for the study

Sl no	Inhibitors	Pubchem ID	Molecular weight(g/mol)	H-Bond donor	H-Bond acceptor	logpvalue	Ref
01	Venetoclax	49846579	868.447	3	11	8.2	[9]
02	Obatoclax mesylate	16727411	413.492	3	6		[10]
03	Navitoclax	24978538	974.611	2	14	9.6	[11]
04	Flavopiridol	5287969	401.843	3	6	3.3	[12]
05	7-hydroxy-staurosporine	72271	482.54	3	5	2.7	[12]
06	Ibrutinib	24821094	440.507	1	6	3.6	[13],[14]
07	Ofatumumab	6918251	242.238	1	6	-0.3	[14]
08	Survivin	45028345	1063.367	12	15	-1.4	[15]
09	MDM2 Inhibitor	10022508	377.972	2	3		[16]
10	SNS-032	3025986	380.525	2	7	3	[17]
11	Duvelisib	50905713	416.869	2	5	4.1	[18]
12	Fludarabine	657237	285.235	4	9	-0.6	[19]
13	Homoharringtonine	285033	545.629	2	10	0.8	[20]
14	Nutlin-3A	11433190	581.494	1	5	5.2	[21]
15	Isosorbide	12597	146.142	2	4	-1.4	[22]
16	Sabutoclax	46236925	700.788	8	8	8.9	[23]
17	Idelalisib	11625818	415.432	2	7	3.7	[24]
18	Antimycin A	14957	548.633	3	9	5.3	[25]
19	Salinomycin	3085092	751.011	4	11	5.7	[26]
20	Roscovitine	160355	354.458	3	6	3.2	[27]
21	Zolpidem	5732	307.397	0	2	2.5	[28]
22	Ha14-1	35490	409.236	1	7	3	[29]
23	Entinostat	4261	376.416	3	5	2	[30]
24	SGI-1776	24795070	405.425	1	8	4.3	[31]
25	Bendamustine	65628	358.263	1	4	2.9	[32]
26	Cyclophosphamide	2907	261.083	1	4	0.6	[33]
27	Dasatinib	3062316	488.007	3	9	3.6	[34]
28	Entinostat	4261	376.416				[35]
29	Romidepsin	121595947	540.694	4	8	2.2	[36]
30	Silvestrol	11787114	654.665	4	13	1.6	[37]
31	Salinosporamide A	11347535	313.778	2	4	1.8	[38]
32	Mitoxantrone	4212	444.488	8	10	1	[39]
33	JQ1 compound	46907787	456.989	0	0	4.9	[40]
34	Nucleozin	2863945	426.857	0	6	3.9	[41]

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35	Gefitinib	123631	446.907	1	8	4.1	[42]
36	TW-37	11455910	573.704	4	6	7.8	[43]
37	Hyaluronidase	91820602	354.288	2	3		[44]
38	Midostaurin	9829523	570.649	1	4	4.8	[45]
39	Valproic Acid	3121	144.214	1	2	2.8	[46]
40	2-Naphthoxyacetic acid	8422	202.209	1	3	2.5	[47]
41	Edaravone	4021	174.203	0	2	1.3	[47]
42	Wnt-C59	57519544	379.463	1	3	4.2	[48]
43	Frakefamide	5493563	563.63	6	8	1.4	[48]
44	Beta-Catenin	3463933	361.193	1	5	3.9	[48]
45	CPP32	1992	675.648	8	13	-1.3	[49]
46	Levomenthol	16666	156.269	1	1	3	[50]
47	Cephradine	38103	349.405	3	6	0.4	[50]
48	Chlorambucil	2708	304.211	1	3	1.7	[51],[54]
49	Lenalidomide	216326	259.265	2	4	-0.5	[52]
50	Pentostatin	439693	268.273	4	6	-2.1	[53]
51	Cladribine	20279	285.688	3	7	0.8	[54]
52	Doxorubicin	31703	543.525	6	12	1.3	[54]
53	Vincristine	5978	824.972	3	12	2.8	[55]
54	Prednisone	5865	358.434	2	5	1.5	[55]
55	GA101	91747743	520.813	0	6		[56]
56	Fostamatinib	11671467	580.466	4	15	1.6	[57]
57	Gossypol	3503	518.562	6	8	6.9	[58]

3.2 Retrieval of Ligand Structure, Optimization and Protein Preparation: The three dimensional structures of 57 BCL2 inhibitors were retrieved from NCBI's PubChem database. The structures of selected Bcl-2 inhibitors were optimized and cleaned in 3d format using Marvin View (MarvinView 5.6.0.2, 1998-2011, Copyright © ChemAxon Ltd) (Csizmadia, 2000).[59] Some inhibitors have no 3D structures available, so their 3D structures was made by using Marvin View. Further ligand preparation was done by taking the 3D structure of all those inhibitors embedded in LigPrep module of Schrodinger suite, 2013 (Schrodinger. LLC, New York, NY) and were optimized through OPLS 2005 force field algorithm.[60]The prepared ligands were saved in single SDF file for further docking studies.[61] The three-dimensional structure of Bcl-2 [PDB: 2XAO][62] was retrieved from the Protein Data Bank. The protein was prepared by removing all bound water molecules and ligands.

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3.3 Molecular Docking studies:

Molecular docking program Molegro Virtual Docker (MVD) which incorporates highly efficient PLP (Piece wise Linear Potential) and MolDock scoring function provided a flexible docking platform.[63] The pre-prepared 57 ligands were saved in one single SDF file. PDB file of target protein consist preexisting ligands were removed and prepared by detecting cavities were found in which the first cavity bear highest volume and was targeted for further procedure of docking with ligands. The 3D structures of inhibitors (ligands) were docked in binding site of Bcl-2 receptor . Docking parameters were set to 0.20 Å as grid resolution, maximum iteration of 1500 and maximum population size of 50.

Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. Binding affinity and interactions of similar compounds with protein were evaluated on the basis of the internal ES (Internal electrostatic Interaction), internal hydrogen bond interactions and sp2-sp2 torsions. On the basis of MolDock rerank score, best interacting compound was selected from each class.

3.4 Virtual screening parameters:

On the basis of lowest rerank score of an established inhibitor obtained after filtered docking result, similarity searching was done against NCBI's Pubchem compound database to obtain best compound having greater affinity than this established inhibitor. The filtrations properties parameter set by component rule of Lipinski's rule of five at threshold >=95%.

3.5 Drug – Drug comparative study: The unnamed complex structure was retrieved from established drug docking filtered result and was imported in Molecular docking program Molegro Virtual Docker (MVD). It was cleaned by removing all the ligands, constraints, and cavities except protein, then best posed drug with lowest rerank score was imported and exported as best drug docked file in PDB format. Then unnamed complex structure was retrieved from virtual screnning docking result and the procedure was repeated. The excel sheet was prepared to check all the affinities, hydrogen interaction, steric energy and lowest rerank score to indentify the best drug.

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3.6 ADMET studies: ADMET studies of best established drug with pubchem CID-45028345 and best virtually screened drug with PubChem CID101725649 was carried out by admetSAR server.[64]

3.7 Pharmacophore Mapping: Pharmacophric mapping which involves ligand interactionpatterns, hydrogen bond interaction, hydrophobic interactionsand solvent accessible surface area upon ligand binding was evaluated using Accelrys Discovery Studio 3.5 DS Visualizer.[65]

3.8 Softwares, Suites and Webservers used:

Pubchem database from NCBI was used to search and prepare library of similar chemical compounds for virtual screening. All the chemical structures were optimized in MarvinSketch 5.6.0.2, (1998-2011, Copyright © ChemAxon Ltd). Flexible Molecular docking of the compounds with target was completed using Molegro Virtual Docker 2010.4.0.0. Accelrys Discovery Studio® Visualizer 3.5.0.12158 (Copyright© 2005-12, Accelyrys Software Inc.) was used for visualization.

The ligands were optimized by using the software Schrodinger suite 2013 (Schrodinger.LLC, 2009, New York, NY). ADMET profiles were studied and calculated using admetSAR (Laboratory of Molecular Modeling and Design. Copyright© 2012 East China University of Science and Technology, Shanghai Key Laboratory for New Drug Drug Design). Accelrys Discovery Studio® Visualizer 3.5.0.12158 (Copyright© 2005-12, Accelyrys Software Inc.) was used for visualization.

4. Results and Discussions

4.1 Docking results

The docking studies of complete pre-established 57 drugs resulted as compound 08 is best established compound. Table [2] as the compound 08 is Survivin has pubchem CID-45028345 shows the higher affinity score direct toward our target protein and has the great affinity properties as molecular weight 1063.367 g/mol, hydrogen bond donor count 12 and hydrogen

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bond acceptor count 15, topological polar surface area 385 A^2 and log value is -1.4. Thus the compound reveals the superior inhibitory affinity over protein BCL2.

Table 2: Docking scores of inhibitors arranged in descending order

S.No.	Ligand	Filename	MolDock Score	Rerank Score	Interaction	HBond
1	45028345	[00] 45028345.mol2	-261.513	-168.022	-241.93	-11.4443
2	45028345	[01] 45028345.mol2	-248.088	-151.08	-197.702	-9.59171
3	45028345	[02] 45028345.mol2	-231.855	-140.357	-200.256	-6.84983
4	1992	[02] 1992.mol2	-198.317	-133.767	-196.681	-8.24646
5	5493563	[00] 5493563.mol2	-164.718	-125.183	-179.608	-2.25351
6	1992	[03] 1992.mol2	-197.229	-124.922	-192.271	-6.09625
7	5493563	[04] 5493563.mol2	-152.809	-123.427	-184.456	-4.87982
8	24795070	[00] 24795070.mol2	-160.186	-122.675	-182.571	-1.79242
9	49846579	[00] 49846579.mol2	-182.686	-122.171	-216.697	-8.18314
10	5493563	[01] 5493563.mol2	-153.193	-118.845	-170.396	-2.88687

4.2 Virtual Screening:

Further similarity searching for survivin inhibitor showed 312 compounds. Table [3] shows the docking result of entire 312 virtual screened compounds showed PubChem CID101725649 as a compound with high affinity. This compound has a molecular weight of 968.09 g/mol, 13 hydrogen bond donor, 18 hydrogen bond acceptor, a topological surface area of 430 A^2 and a log P value is -6. Suchwise, among all 312 compounds the drug with pubchem CID-101725649 has much potential inhibition against CLL cancer over the target protein BCL2.

Table 3: Virtual screened drugs docking result with reference to high affinity Survivin

S.No.	Ligand	Filename	MolDock Score	Rerank Score	Interaction	HBond
1	101725649	[00]101725649.mvdml	-228.959	-159.516	-231.403	-13.6032
2	101725649	[00]101725649.mvdml	-228.959	-159.516	-231.403	-13.6032
3	101725649	[00]101725649.mvdml	-228.959	-159.516	-231.403	-13.6032
4	101725649	[00]101725649.mvdml	-228.959	-159.516	-231.403	-13.6032
5	23496768	[00]23496768.mvdml	-227.357	-159.013	-211.57	-10.7869
6	23496768	[00]23496768.mvdml	-227.357	-159.013	-211.57	-10.7869
7	23496768	[00]23496768.mvdml	-227.357	-159.013	-211.57	-10.7869
8	23496768	[00]23496768.mvdml	-227.357	-159.013	-211.57	-10.7869
9	101129057	[01]101129057.mvdml	-214.502	-158.611	-224.738	-8.41583
10	101129057	[01]101129057.mvdml	-214.502	-158.611	-224.738	-8.41583

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4.3 Drug – Drug comparative study:

Table [4] shows the rerank scores of the established drug & virtually screened drug against the target protein BCL2 on CLL cancer. Hence, total energy of established inhibitor with pubchem CID 45028345 is lowest among the entire virtual screened compound with preferable affinity. Surprisingly, the other interaction of both the compounds displaying the virtual screened compound with pubchem CID 101725649 has more affinity interaction properties according to steric energy of PLP (Piecewise Linear Potential) and steric energy of LJ12-6(Leonard-Jones approximation) than the established drug, whereas the stability of hydrogen bonds is more in virtually screened drug than in established drug. So it demonstrates that the both the compounds have equivalent potential inhibition towards the target protein BCL2.

Table 4: Drug – Drug comparative study

Energy overview:Descriptors	Established MolDock Score	Rerank Score	Virtual screened MolDock Score	Rerank Score
Total Energy	-263.842	-169.88	-229.497	-159.935
External Ligand interactions	-244.255	-202.261	-231.938	-196.908
Protein - Ligand interactions	-244.255	-202.261	-231.938	-196.908
Steric (by PLP)	-196.256	-134.632	-217.797	-149.408
Steric (by LJ12-6)		-33.008		-36.299
Hydrogen bonds	-13.758	-10.896	-14.141	-11.2
Hydrogen bonds (no directionality)		0		0
Electrostatic (short range)	-24.978	-22.28	0	0
Electrostatic (long range)	-9.263	-1.445	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
Water - Ligand interactions	0	0	0	0
Internal Ligand interactions	-19.587	32.381	2.441	36.973
Torsional strain	36.26	34.012	31.332	29.39
Torsional strain (sp2-sp2)		0		0
Hydrogen bonds		0		0
Steric (by PLP)	-35.873	-6.17	-2.395	-0.412

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Steric (by LJ12-6)		5.081		7.995
Electrostatic	-1.242	-0.543	0	0
Soft Constraint Penalty	0		0	
Search Space Penalty	0		0	

4.4 ADMET studies:

Table 5: ADMET profiles of Established drug and Virtual screened compound by AdmetSAR

Properties		Established drug	Virtual screened compound
Absorption Probability	**Result**	**Probability**	
Blood-Brain Barrier	BBB-	0.7522	0.8447
Human Intestinal Absorption	HIA+	0.8327	0.6152
Caco-2 Permeability	Caco2-	0.7121	0.7117
P-glycoprotein Substrate	Substrate	0.8212	0.8203
	Non-inhibitor	0.8558	0.8614
P-glycoprotein Inhibitor	Non-inhibitor	0.9927	0.9903
Renal Organic Cation Transporter	Non-inhibitor	0.9293	0.9443
Distribution			
Subcellular localization	Mitochondria	0.6209	0.5485
Metabolism			
CYP450 2C9 Substrate	Non-substrate	0.8547	0.8138
CYP450 2D6 Substrate	Non-substrate	0.7651	0.738
CYP450 3A4 Substrate	Non-substrate	0.5806	0.5759
CYP450 1A2 Inhibitor	Non-inhibitor	0.9335	0.8983
CYP450 2C9 Inhibitor	Non-inhibitor	0.8844	0.8843
CYP450 2D6 Inhibitor	Non-inhibitor	0.8957	0.9093
CYP450 2C19 Inhibitor	Non-inhibitor	0.8406	0.8509
CYP450 3A4 Inhibitor	Non-inhibitor	0.9061	0.9585
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	0.9936	0.9894
Excretion			
Toxicity			
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9836	0.986
	Non-inhibitor	0.8819	0.8398
AMES Toxicity	Non AMES toxic	0.855	0.8535
Carcinogens	Non-carcinogens	0.9342	0.9386
Fish Toxicity	High FHMT	0.9575	0.9171

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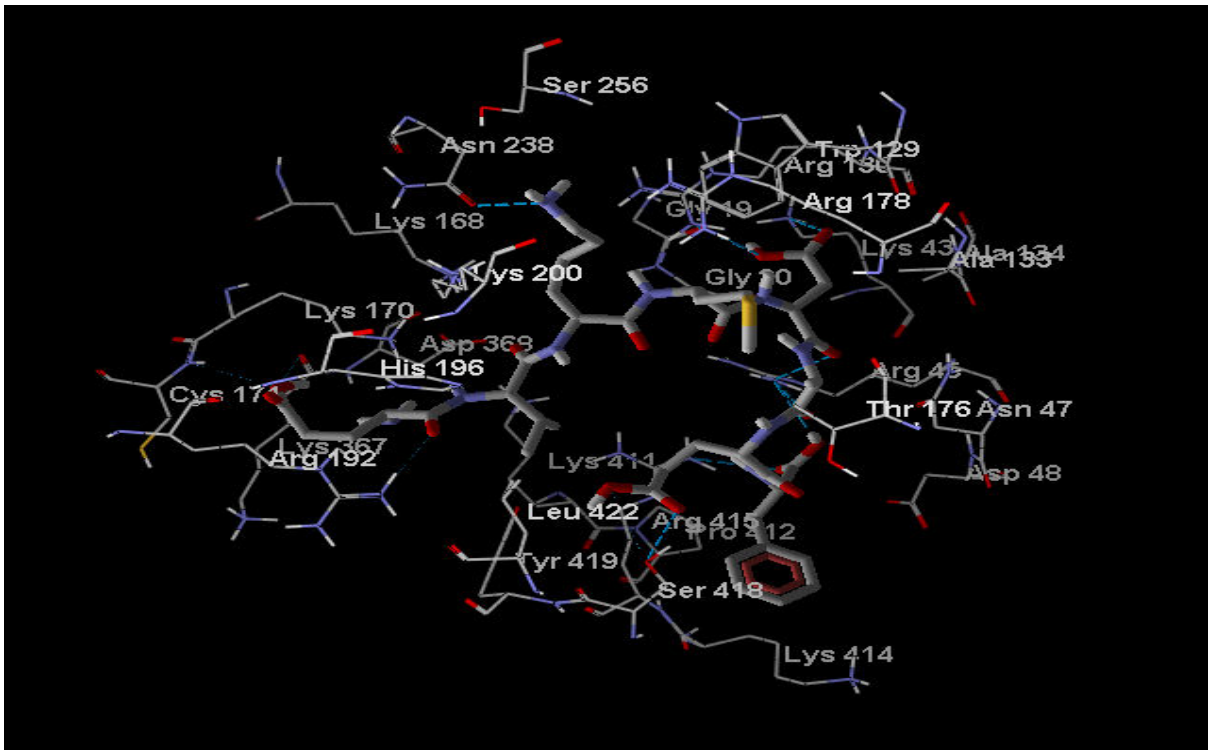
Tetrahymena Pyriformis Toxicity	High TPT	0.9853	0.9561
Honey Bee Toxicity	Low HBT	0.7953	0.7802
Biodegradation	Not ready biodegradable	0.9476	0.9266
Acute Oral Toxicity	III	0.7257	0.6933
Carcinogenicity (Three-class)	Non-required	0.7008	0.7195

ADMET Predicted Profile Regression

	Model	Unit	Established drug Values	Virtual screened drug Values
Absorption				
	Aqueous solubility	LogS	-3.1573	-2.6622
	Caco-2 Permeability	LogPapp, cm/s	0.5011	0.2829
Distribution				
Metabolism				
Excretion				
Toxicity				
	Rat Acute Toxicity	LD50, mol/kg	2.1486	2.2399
	Fish Toxicity	pLC50, mg/L	1.8413	1.9241
	Tetrahymena Pyriformis Toxicity	pIGC50, ug/L	0.2731	0.2037

4.5 Pharmacophore Study:



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Most effective Virtual screened compound(PubID: 101725649) showing H-Bond interactions

5. Conclusion: Inhibition of BCl2 protein has now been as an important target of drug against Chronic Lymphocyctic Leukemia. Established drug Survivin with pubchem CID-45028345 & virtually screened drug with pubchem CID-101725649 are established inhibitors which are in clinical trials and promise to be potent drug in the near future. Our study utilizing in silico approaches has indicated that compounds 45028345 and 101725649 had better inhibitory potential for BCL2 anti-apoptotic protein.

6. Acknowledgement: Declared none.

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