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An attempt to screen top colorectal cancer drugs by using Molegro Virtual Docker

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ABSTRACT

Colorectal cancer is a malignant tumor arising from the inner wall of the large intestine. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. Colorectal cancer is one of the main types of cancer causing overall cancer mortality each year. Thus the best drugs are preferred. The main objective of this work is to utilize the various bioinformatics tools and software such as TSAR software, to perform docking of the drugs obtained from the Drug Bank Database against the selected proteins. In this work, we have selected 6 structure hits of proteins overly expressed in colon cancer as well as other cancer and 8 apoptotic related proteins from Protein Data Bank. Screening studies of 3500 drugs obtained from Drug Bank Database were performed. The drugs obtained were subjected to docking against the selected proteins using Molegro Virtual Docker and the top drugs were obtained. Further to filter the number of drugs obtained against proteins in colon cancer and apoptosis, a Consensus Scoring methodology can be applied such that the top five drugs can be revealed. Then MTT assay protocol can be utilized for evaluating percent inhibition of top compounds against HCT-15 cell lines. Finally this study states that novel compounds can be screened with high affinity against specific target with few computational efforts.

Key Words: Drug Bank Database, Molecular Docking, Molegro Virtual Docker, RMSD, TSAR software.

INTRODUCTION

Colorectal cancer [1,2] is a worldwide problem having global increase in the number of cases and deaths because of the expanding and aging of the population in both developing and developed countries. Virtual screening of chemical databases is an emerging approach in drug

discovery that uses computers to dock chemicals into the active site of a drug target to identify leads through evaluation of binding affinities of the chemicals.

Drug Bank Database : The Drug Bank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains nearly 4800 drug entries including >1,350 FDA-approved small molecule drugs, 123 FDA-approved biotech (protein/peptide) drugs, 71 nutraceuticals and >3,243 experimental drugs. Additionally, more than 2,500 non-redundant protein (i.e. drug target) sequences are linked to these FDA approved drug entries[3,4]. Each Drug Card entry contains more than 100 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data. An image showing the home page of Drug bank database is given in **Fig 1.**

Fig 1: Image Showing the Home Page of Drug Bang Database

Molecular Docking : Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand—protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking[5] can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as important as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear.

DOCK works in 5 steps:

- **Step 1:** Starting with crystal coordinates of target receptor.
- **Step 2:** Generating molecular surface for receptor.
- **Step 3:** Generating spheres to fill the active site of the receptor: The spheres become potential locations for ligand atoms.
- **Step 4:** MATCHING: Sphere centers are then matched to the ligand atoms, to determine possible orientations for the ligand.
- **Step 5:** SCORING: Finding the top scoring orientation.

The various steps of Docking are:
I. LIGAND PREPARATION: It includes the following steps:
1) 2D-3D Conversion
2) Addition of Hydrogen's, Lone pairs
3) Addition of charges
4) Identification of rotatable bonds

II. RECEPTOR PREPARATION: It includes the following steps:
1. Identification of binding site
2. Biochemical information
3. Various geometric criteria

III. BINDING SITE PREPARATION: There are Various Docking algorithms that require prior knowledge of Active site or Binding site so as to limit the search space. It can be determined by various packages like Ac site or PASS.

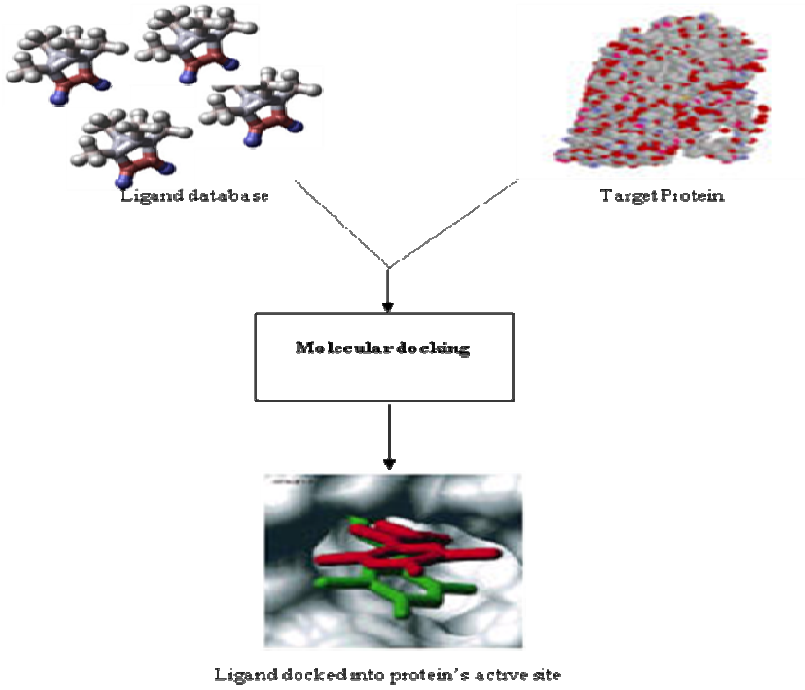

Fig 2: Image showing MolecularDocking

IV. SCORING/ENERGY EVALUATION:
1. Deriving a "fitness" or "energy" of the docked complex.
2. Useful for distinguishing "binders" from "non-binders".
3. Useful for ranking the binders in order of fitness/energy.

V. RUNNING SEARCH ALGORITHM:
There are Various Search Algorithms that can be used:
1. Monte Carlo Simulated Annealing
2. Genetic Algorithms
3. Tabu search
4. Hybrid Global-Local Search
e.g., Lamarckian Genetic Algorithm

Molegro Virtual Docker : Molegro Virtual Docker (MVD) was used to perform docking. MVD is an integrated platform for predicting protein - ligand interactions[6,7]. It handles all aspects of the docking process from preparation of the molecules to determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligand. It provides the user with high-quality docking based on a novel optimization technique combined with a user interface experience focusing on productivity and usability. MVD has been shown to yield higher docking accuracy than other state-of-the-art docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%).

RMSD (Root Mean Square Deviation): The Root Mean Square Deviation (RMSD) is the measure of the average distance between the backbones of superimposed proteins. A widely used way to compare the structures of biomolecules or solid bodies is to translate and rotate one structure with respect to the other to minimize the RMSD. Coutsias, *et al*. presented a simple derivation, based on quaternion's, for the optimal solid body transformation [rotation-translation] that minimizes the RMSD between two sets of vectors.

The equation: [8]

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{i=N}\delta_i^2}$$

Where δ is the distance between N pairs of equivalent atoms [usually *Cα* and sometimes *C,N,O,Cβ*].

Normally a rigid superposition which minimizes the RMSD is performed, and this minimum is returned. Given two sets of *n* points $\mathbf{v}$and $\mathbf{w}$, the RMSD is defined as follows:

$$\begin{aligned}\mathrm{RMSD}(\mathbf{v},\mathbf{w}) &= \sqrt{\frac{1}{n}\sum_{i=1}^{n}\|v_i - w_i\|^2} \\ &= \sqrt{\frac{1}{n}\sum_{i=1}^{n}(v_{ix}-w_{ix})^2 + (v_{iy}-w_{iy})^2 + (v_{iz}-w_{iz})^2}\end{aligned}$$

An RMSD value is expressed in length units. The most commonly used unit in structural biology is the Ångström (Å) which is equal to 10^{-10}m.

MATERIALS AND METHODS

Materials

List of insilico tools used in dry lab:

- Molegro Virtual Docker
- TSAR software
- Protein Data Bank
- Drug Bank Data Base

Methodology:

Molegro Virtual Docker was used to perform docking. The Molegro Virtual Docker window is shown in **Fig 3.**

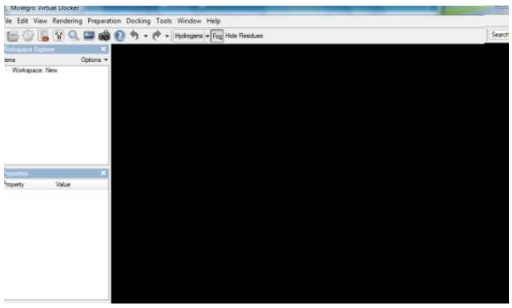

Fig 3: Image showing the Molegro Virtual Docker Window

Initially, the 6 structure hits of proteins expressing in common and colon cancer were selected based on specific criteria and downloaded from Protein Data Bank [9.10].

The proteins overly expressed in colon cancer include:
1. Cyclooxygenase-2 (COX-2)
2. Focal Adhesion Kinase (FAK)
3. Thymidylate Synthase (TS)

The proteins expressed in other cancer include:
1. B-cell lymphoma/leukaemia-2 (BCL-2)
2. Dihydrofolate Reductase (DHFR)
3. Transforming Growth Factor beta (TGF-β)

These proteins were docked using Molegro Virtual Docker for three times to obtain stability . Similarly, 8 Apoptotic proteins were selected from Protein Data Bank and were docked using Molegro Virtual Docker.

The apoptotic related proteins include:

1. Caspase-3 (Apopain, P20) [11]
2. Cell division protein kinase-7 [12]
3. Cyclin-Dependent Kinase-5
4. Glycogen Synthase Kinase-3 Beta
5. Tumor necrosis factor
6. Cell Division Protein Kinase-2
7. Cell Division Protein Kinase-9
8. Mast/Stem cell growth factor receptor

The steps involved in docking were:

1. *IMPORTING THE MOLECULES OR LIGANDS*: The protein and ligand molecules present in the PDB or Mol2 formats were imported into the workspace of the Molegro Virtual Docker software. An image showing the importing of molecule is given in **Fig 4**. An image showing the imported molecule is given in **Fig 5.**

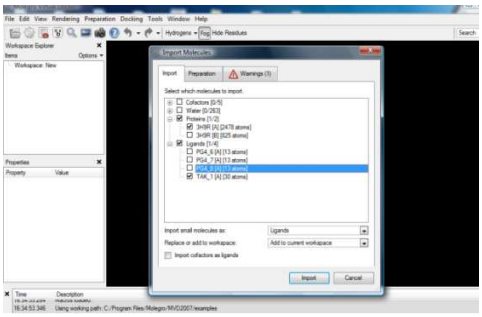

Fig 4: Image showing the importing of molecule

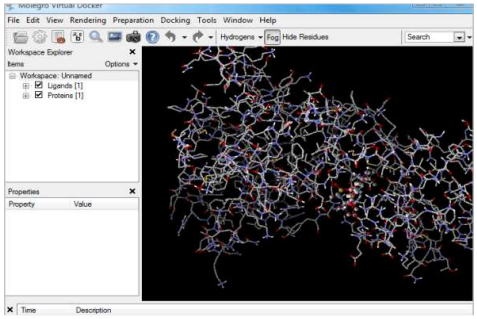

Fig 5: Image showing the imported molecule

2. *PREPARING THE MOLECULES:* The molecules were prepared after imported into the workspace of MVD.

3. *CREATING TEMPLATE*: The cavities present in the protein can be detected by the Detect Cavities option and the large cavity was selected as the binding site for the ligand while performing docking. An image showing creating the template is given in **Fig 6**. An image showing the created template is given in **Fig 7**.

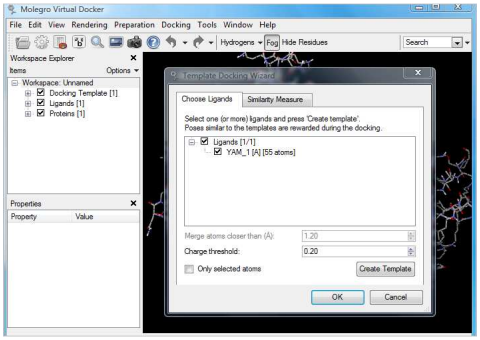

Fig 6: Image showing creating the template

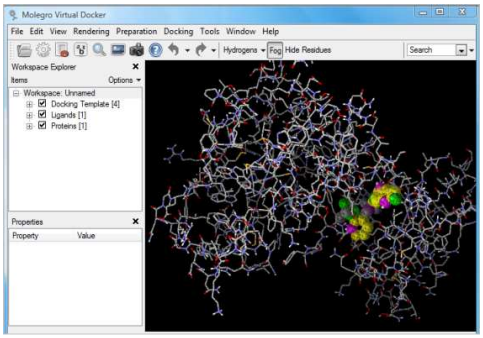

Fig 7: Image showing the created template

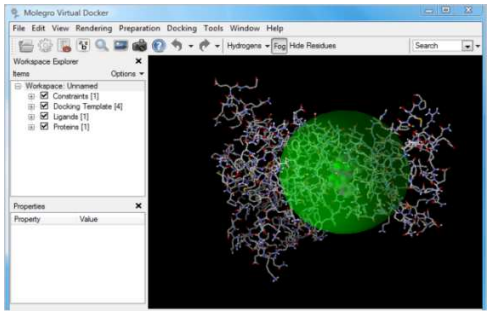

Fig 8: Image showing the docking wizard

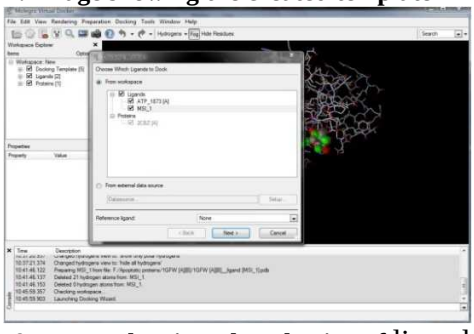

Fig 9: Image showing the selection of ligand

DOCKING: The docking was performed using the docking wizard. An image showing the Docking wizard is given in **Fig 8.**

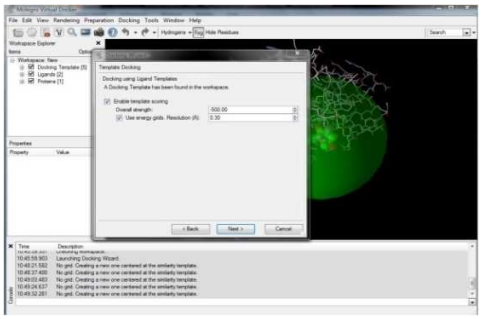

Fig 10: Image showing the resolution value

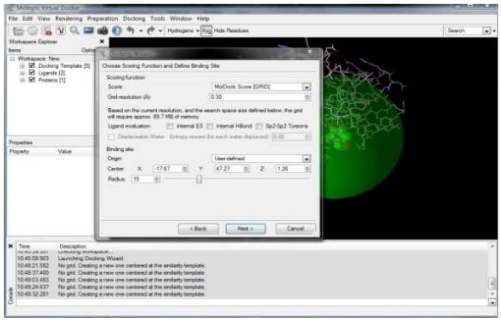

Fig 11: Image showing the default values of user defined ligand

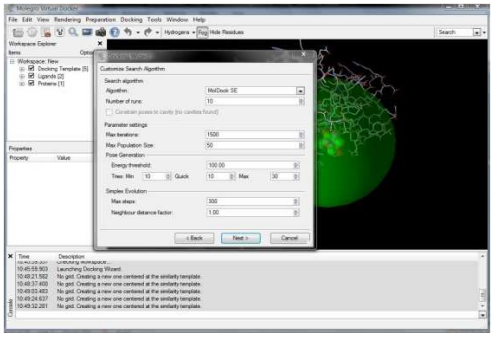

Fig 12: Image showing the default value

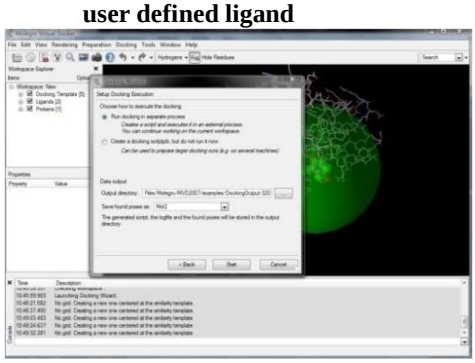

Fig 13: Image showing the data output

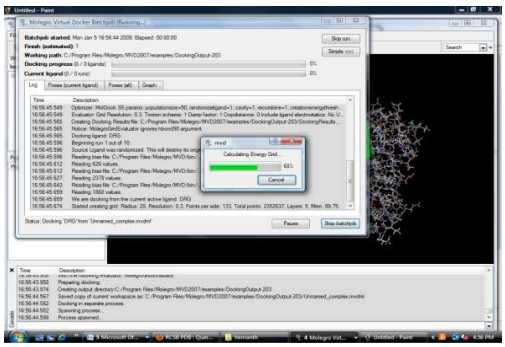

Fig 14: Image showing the grid calculation

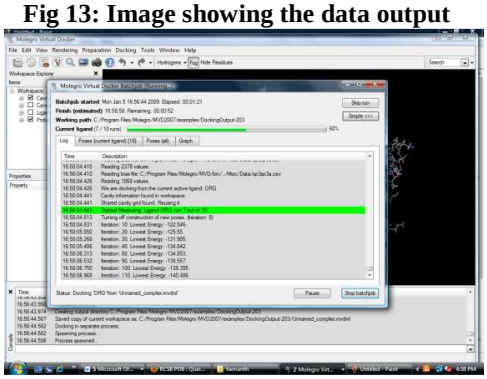

Fig 15: Image showing the docking process

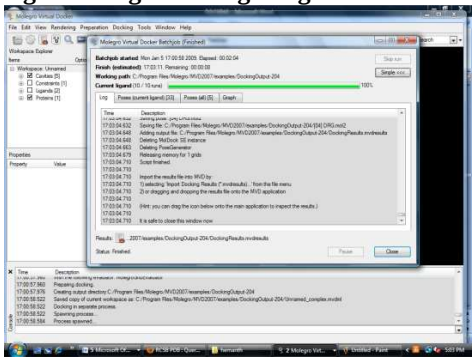

Fig 16: Image showing the result page

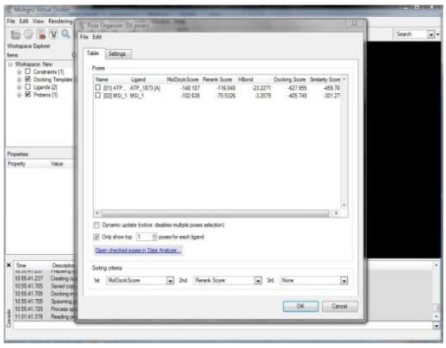

Fig17: Image showing the final results

Nearly 3500 drug compounds available in market, were taken from Drug Bank, and these drug compounds were segregated into four different sets in order to perform better analysis in various pc's and 12 proteins expressing in common and colon cancer were docked with these four sets of drug compounds. Molecular dock scores of common and colon cancer expressing proteins were compared with the molecular dock scores of drug compounds.

RESULTS AND DISCUSSION

The 6 structure hits of proteins expressing in common and colon cancer and 8 apoptotic proteins were selected based on specific criteria and downloaded from Protein Data Bank , these proteins were docked using Molegro Virtual Docker for three times to obtain stability and average of three molecular dock scores along with average RMSD are given in Table 1 and Table 2. The Molecular dock scores of common and colon cancer expressing proteins were compared with the molecular dock scores of drug compounds, top 5 molecular dock scores of each protein were taken based upon greater score than the original ligand-protein complex scores and are tabulated in Tables 3, Table 4, Table 5, Table 6, Table 6, Table 7 and Table 8. Only top 5 drugs in each case are shown. From this, it has been observed that few drugs are represented in more than one case. Few drugs are active only against few targets and therefore to study the affinity of binding, molecular docking was carried out with 12 proteins versus top 15 drugs.

Table 1: Molecular Dock Scores of 3 colon cancer proteins and 3 other cancer proteins

S.NO	PDB ID	Mol Dock Score(kcal/mol)			Average Mol Dock Score (kcal/mol)	Average RMSD value(kcal/mol)
		1 Run	2 Run	3 Run		
1	1CX2	-156. 237	-156.255	-156.019	-156.170	0.710441
2	3BZ3	-176.088	-173.622	-178.676	-176.128	0.376894
3	1JU6	-141.585	-145.102	-142.961	-143.216	0.300471
4	3INQ	-181.222	-180.78	-178.686	-180.229	0.677587
5	3GI2	-190.084	-188.997	-190.345	-189.808	0.268168
6	1PY5	-125.063	-124.705	-124.891	-124.886	0.171633

All the above top 5 ligands in each case are pooled up and the total 25 drugs from the above are selected and were docked against 8 apoptotic proteins. The resultant top 3 compounds were tabulated below for each apoptotic protein. Drug compounds screened and compared from both cancer proteins and Apoptotic proteins were listed below. Table 11 shows the Final list of 21 compounds. These final listed 21 drugs are docked and their molecular dock scores were used to perform ranking using TSAR software. All compound dock scores are ranked hierarchically into 3 ranks and the top rank receives a value of 3, similarly the second rank received a value of 2 and the last rank receives a value of 1. And the compounds with ranks are mentioned in Fig 18, Fig 19 and Fig 20.

Table 2: Molecular Dock Scores of 8 Apoptotic proteins

S.NO	PDB ID	Mol Dock Score(kcal/mol)			Average Mol Dock Score (kcal/mol)	Average RMSD value(kcal/mol)
		1 Run	2 Run	3 Run		
1	1GFW	-35.672	-36.853	-34.662	-35.729	0.278322
2	1UA2	-153.34	-155.236	-151.632	-153.402	0.345875
3	1UNH	-113.913	-111.663	-114.002	-113.192	0.117078
4	1UV5	-117.858	-115.632	-114.32	-115.936	0.170907
5	2AZ5	-107.613	-105.332	-102.652	-105.199	0.35177
6	2UZ0	-126.54	-123.25	-125.663	-125.151	1.06333
7	3BLR	-114.244	-112.32	-115.632	-114.065	0.172786
8	3GOE	-132.706	-133.20	-135.690	-133.865	0.186848

Top drugs obtained when docked with colon cancer proteins

Table 3: Top 5 drugs obtained when docked with COX-2

1CX2(Cyclooxygenase-2)(COX-2) ***Original Ligand Score****: -156.170 kcal/mol*

S.NO	LIGAND NO.	Mol Dock Score(kcal/mol)
1	Linidamine	-216.612
2	Glimepiride	-187.165
3	Latanoprost	-183.218
4	Prannlukast	-180.681
5	Fosamprenavir	-174.242

Table 4: Top 3 drugs obtained when docked with FAK

3BZ3 (Focal Adhesion Kinase)(FAK): ***Original Ligand Score:*** *-176.128 kcal/mol*

1	Olmesartan	-210.65
2	Ritonavir	-180.325
3	Lapatinib	-180.36

Table 5: Top 4 drugs obtained when docked with TS

1JU6 Thymidylate Synthase(TS) ***Original Ligand Score:*** *-143.216 kcal/mol*

1	Cefpiramide	-179.464
2	Pentagastrin	-169.466
3	Verteporfin	-167.771
4	Reserpine	-165.236

Table 6: Top drug obtained when docked withBCL-2

3INQ(B-cell lymphoma-2) (BCL-2) ***Original Ligand Score:*** *-180.229 kcal/mol*

1	Olmesartan	-184.697

Table 7: Top drugs obtained when docked with DHFR

3GI2 (Dihyrofolate reductase)(DHFR) ***Original Ligand Score: -189.808 kcal/mol***

1	Olmesartan	-210.237
2	Montelukast	-198.796
3	Saprisartan	-196.569

Table 8: Top drugs obtained when docked with TGF-beta

1PY5 (Transforming growth factor beta) (TGF-beta) Original Ligand Score: -124.886 kcal/mol

1	Telmisartan	-198.395
2	Atorvastin	-198.44
3	Nebivolol	-181.81
4	Verteporfin	-180.538
5	Pyrimethamine	-177.214

Table 9: Top drugs obtained when docked with Apoptotic proteins:

Original Ligand Scores:	**1GFW(Caspase-3)** -35.729kcal/mol	**1UA2(CDPK-7)** -113.192kcal/mol	**1UNH(CDK-5)** -153.402kcal/mol	**1UV5 (GSK-3)** 115.936kcal/mol

S. N0.	**PROT-EINS**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**
1	1COX2	Fosino- pril	-84.27	Epro-sartan	-160.622	Epro-sartan	-155.569	Epro-sartan	-147.274
		Epro-sartan	-72.7739			Prann-lukast	-145.686	Glime-piride	-132.493
		Latano-prost	-59.9832			Fosino-pril	-140.031	Fosino-pril	-129.888
2	FAK	Olme-sartan	-121.95	Olme-artan	-178.141	Olme-sartan	-174.703	Olme-sartan	-163.241
		Rito-navir	-55.34	Rito-navir	-158.362	Lapa-tinib	-160.701	Rito-navir	-147.723
						Rito-avir	-120.47	Lapa-tinib	-135.237
3	TS	Verte-porfin	-90.039	Vrete-porfin	-197.296	Penta-gastrin	-151.917	Verte-porfin	-166.856
		Penta-gastrin	-88.068			Verte-porfin	-140.286	Penta-gastrin	-158.353
		Cefpi-ramide	-79.3615					Cefpi-ramide	-137.825
4	BCL-2	Olme-sartan	-98.9084	Olme-sartan	-170.765	Olme-sartan	-181.509	Olme-sartan	-173.278
5	DHFR	Olme-sartan	-97.3985	Olme-sartan	-186.43	Monte-lukast	-168.258	Nebi-volol	-172.45
		Monte-lukast	-51.0611	Sapri-sartan	-165.495	Olmesartan	-162.414	Monte-lukast	-165.000

								Olme-sartan	-154.921
6	TGF	Atorva-stin	-93.9692	Verte-porfin	-193.898	Verte-porfin	-157.145	Telmi-sartan	-157.86
		Loni-damine	-90.8518	Nebi-volol	-168.54	Azelaic acid	-155.106	Azelaic acid	-144.881
		Alis-kiren	-89.2216	Atorva-stin	-165.468	Telmi- artan	-154.463	Verte-orfin	-137.295

Table 10: Top drugs obtained when docked with Apoptotic proteins

Original Ligand Scores:	**2AZ5 (TNF)** -105.199 kcal/mol	**2UZO (CDPK-2)** -125.151kcal/mol	**3BLR(CDPK-9)** -114.065kcal/mol	**3GOE (M\S CGFR)** -133.865kcal/mol

S.NO	**PROT-EINS**	**Drug Molecule**	**Mol Dock Score (cal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**	**Drug Molecule**	**Mol Dock Score (kcal/mol)**
1	1COX2	Epro-sartan	-102.018	Epro-sartan	-152.742	Epro-sartan	-167.589	Glime-piride	-138.652
				Latano-prost	-141.819	Glime-piride	-144.705	Epro-sartan	-132.879
				Fosino-pril	-137.067	Fosino-pril	-143.477		
2	FAK	Olme-sartan	-108.425	Olme-sartan	-163.241	Olme-sartan	-202.212	Olme-sartan	-161.209
		Rito-navir	-105.276	Rito-navir	-147.723	Lapa-tinib	-165.586	Lapa-tinib	-158.435
				Lapa-tinib	-135.237	Rito-navir	-153.458		
3	TS	Verte-porfin	-113.867	Verte-porfin	-180.097	Verte-porfin	-191.222	Verte-porfin	-141.959
		Penta-gastrin	-110.294	Penta-gastrin	-158.754	Penta-gastrin	-163.915		
4	BCL-2	Olme-sartan	-114.236	Olme-sartan	-168.339	Olme-sartan	-187.788	Olme-sartan	-179.705
5	DHFR	Olme-sartan	-123.634	Olme-sartan	-170.019	Olme-sartan	-174.711	Olme-sartan	-170.311
		Nebi-volol	-108.953	Montel-ukast	-145.356	Montel-ukast	-150.835	Montel-ukast	-155.074
				Sapri-sartan	-142.662			Sapri-sartan	-155.332
6	TGF	Verte-porfin	-125.661	Verte-porfin	-175.915	Verte-porfin	200.259	Nebi-volol	-159.495
		Eprosartan	-102.977	Telmi-sartan	-161.761	Telmi-sartan	-192.742	Dihydro-ergotamine	-148.103
		Atorva-stin	-103.491	Azelaic-acid	-134.227	Atorva-stin	-182.87	Azelaic-acid	-135.03

Table 11: Table showing the final list of 21 compounds

S.NO	**DRUG COMPOUNDS**	**S.NO**	**DRUG COMPOUNDS**
1	Pentagastrin	2	Glimepiride
3	Dihydroergotamine	4	Cefpiramide
5	Montelukast	6	Fosinopril

7	Azelaic acid	8	Latanoprost
9	Eprosartan	10	Telmisartan
11	Atorvastin	12	Prannlukast
13	Aliskiren	14	Lapatinib
15	Saprisartan	16	Olmesartan
17	Ritonavir	18	Verteporfin
19	Lonidamine	20	Nebivolol
21	Temsirolimus		

Fig 18, 19, 20: Images showing the compounds with ranks

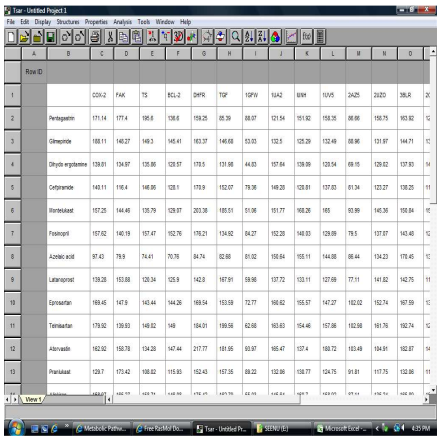

Fig 18

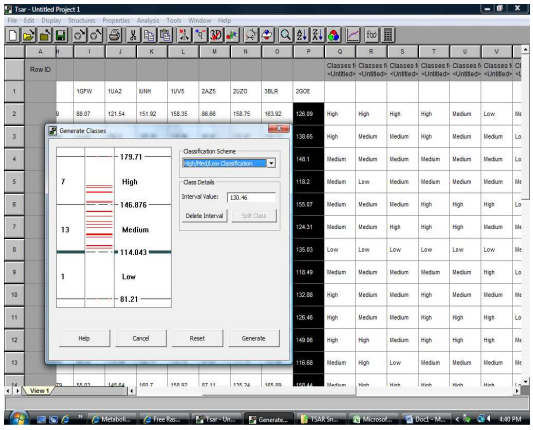

Fig 19

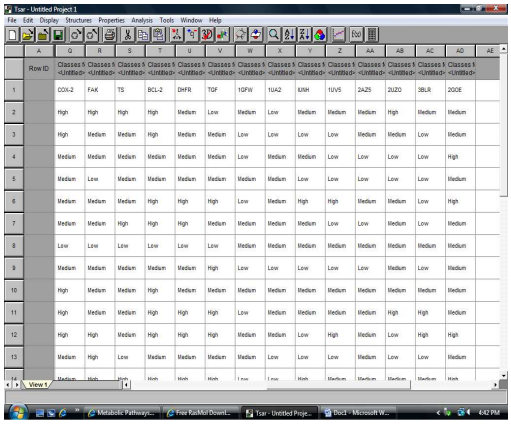

Fig 20

CONCLUSION

Screening studies of 3500 drugs obtained from drug bank database are docked against six proteins which are highly expressed in colon cancer and common cancer and eight apoptosis proteins using Molegro Virtual Docker (MVD) software resulted in 21 drugs with few drugs

such as Olmesartan, Verteporfin, Ritonavir, Telmisartan, Eprosartan obtained as best compounds in more than one case. Similar observation was also obtained in apoptosis related proteins. Further to filter the number of drugs obtained against proteins in colon cancer and apoptosis, a consensus docking and scoring can be employed such that the top five drugs can be revealed. Finally this study states that novel compounds can be screened with high affinity against specific target with few computational efforts.

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