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A COMPARATIVE DOCKING ANALYSIS FOR THE VIRTUAL SCREENING OF INTERLEUKIN-2 INHIBITORS

Sobia Ahsan Halim[1,*], Zaheer-ul-Haq[2] and Sauleha Khan[3]

[1]*Department of Biochemistry, Kinnaird College for Women, 93-Jail Road, Lahore-Pakistan.*
[2]*Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.*
[3]*Department of Chemistry, University of Karachi, Karachi-75270, Pakistan.*
***Corresponding Author: E-mail:** sobia.ahsan@kinnaird.edu.pk

ABSTRACT

Interleukin-2 augments T-cells growth, activation and proliferation. IL-2 has become a promising drug target for several immunological disease conditions. We conducted a comparative analysis of three widely used docking programs: MOE, GOLD and FRED. The docking ability was assessed by the re-docking of known IL-2 inhibitors in their cognate binding site. MOE and FRED were best in accurate pose prediction. Scoring functions were scrutinized by the docking of a large database comprising 3100 drug like compounds and 38 known inhibitors. Scoring functions were tested to identify known actives embedded in dataset. Based on FRED re-docking performance, FRED was used to dock the library. Furthermore, the library was re-scored by GOLD, CScore module of Sybyl and MOE. Chemgauss 2 scoring function of FRED showed 70% enrichment of active inhibitors in top 5% of ranked database. The results suggest that the FRED docking program is significantly better for the virtual screening of IL-2 inhibitors.

Key Words: Interleukin-2, virtual screening, FRED, GOLD, MOE

Abbreviations
ASP: Astex Statistical Potential, EF: Enrichment Factor, FRED: Fast Rigid Exhaustive Docking, GA: Genetic Algorithm, GOLD: Genetic Optimization for Ligand Docking, IL-2: Interleukin-2, IL-2R: IL-2 receptor, MOE: Molecular Operating Environment, PDB: Protein Data Bank, PLP: Piecewise Linear Potential, PMF: Potential of Mean Force, RMSD: Root Mean Square Deviation, VS: Virtual Screening

INTRODUCTION

Interleukin-2 (IL-2) plays an essential role in the activation and maintenance of an immune response, and in lymphocyte development. IL-2 acts as the most powerful growth factor and activator of T cells. Uncontrolled activated T cells are involved in the patho-physiology by stimulating inflammation and autoimmune diseases like Psoriasis and Crohn's disease. T cells activation can be suppressed either by preventing the production of IL-2 or blocking the IL-2 receptor. Two well organized immunosuppressive Drugs: Cyclosporine and Tacrolimus act by preventing the transcription of IL-2 gene. It is reported that patients treated with the Cyclosporin and Tacrolimus are at high risk of developing renal injury and hypertension. Long term studies have confirmed that these drugs invariably produced reduction in the glomerular filtration rate and an increased vulnerability to opportunistic fungal and viral infections. These drugs can also cause dose-dependent nephro-toxicity and hypertension (Arkin and Wells 2004).

Treatment with cyclosporine is associated with a number of potentially serious adverse drug reactions (ADRs) and adverse drug interactions including gum hyperplasia, peptic ulcers, pancreatitis, fever, vomiting, and diarrhea, breathing difficulties, high blood pressure and potassium retention. While the side effects of Tacrolimus can be more severe including blurred vision, seizures, tremors, hypertension, hypomagnesemia, diabetes mellitus, hyperkalemia, itching, insomnia, and loss of appetite, hyperglycemia, weakness, depression, cramps, and neuropathy (Mihatsch *et al.*, 1998). Newly developed monoclonal antibodies including Basiliximab and dacluzimab, blocks IL-2 receptor (IL-2R) on activated T cells, hence offers promising alternative for the treatment of autoimmune diseases. Both Basiliximab and dacluzimab directly prevents T cells activation by binding IL-2 receptor α (IL-2Rα) (Pascual *et al*., 2001). However these antibodies also have serious side effects. In particular high cost-of-goods and lack of oral bioavailability, there may be a potential to induce malignancy through reduced immune surveillance. Limitations of antibodies like high costs, the need for intravenous treatment and the side effects, can be subsided by the small molecule inhibitor of IL-2 (Halim *et al*., 2013). The small inhibitor binds to the same site on IL-2 that binds the IL-

2Rα, thereby inhibit T cells activation. The development of immunosuppressive agents with less toxicity and more specificity has led researchers to explore the role of IL-2 in the immune-suppression cascade (Halim *et al*., 2013).

The discovery of an effective and potent inhibitor requires many years and the frequent emergence of wide variety of diseases demand the discoveries to be done actively. This demand has been largely accomplished by the use of Computer-aided drug design (CADD). The latest technological advances (QSAR/QSPR, structure-based design, combinatorial library design, chem-informatics and bioinformatics); the growing number of chemical and biological databases; and an explosion in currently available software tools are providing improved basis for the design of ligands and inhibitors with desired specificity (Ashry *et al.,* 2012; Halim *et al.,* 2013; Halim *et al.,* 2015a; Halim *et al.,* 2015b; Mesaik *et al.,* 2010; Mesaik *et al*., 2012; Saifullah *et al*., 2013).

CADD either relies on the structure of ligand (ligand-based or indirect drug design) or 3D- structure of the biological target (Structure-based or direct drug design). Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist (Ashry *et al.,* 2012; Halim *et al.,* 2013; Halim *et al.,* 2015a; Halim *et al.,* 2015b; Mesaik *et al.,* 2010; Mesaik *et al*., 2012; Saifullah *et al*., 2013). This study mainly focuses on the identification of best docking program to be used for the virtual screening of IL-2 inhibitors. Hence comparative analysis was performed with three docking programs: FRED, MOE and GOLD.

MATERIALS AND METHODS

Protein-Ligand Complexes Used in this study

The X-ray crystal structures of three protein-ligand complexes of IL-2 were retrieved from PDB. The details of used complexes are given in **Table 1**. Structures of the co-crystallized ligand are presented in **Figure 1**. Initially the docking accuracy was assessed by re-docking of co-crystallized ligand into its cognate ligand binding site, RMSD values were calculated between the docked and the original co-ordinates of the ligand. Finally the ability of scoring function was examined by the screening of known inhibitors embedded in a set of decoys collected from our in-house database.

Preparation of Protein and Ligand Files for Docking

The PDB heteroatom records (HETATM) including cofactors and bound ligands, chain B from 1M48 (Arkin *et al.,* 2003) and chain C and D from 1PW6 (Thonas *et al.,* 2003) and 1PY2 (Thonas *et al.,* 2003) were removed from the coordinate files. Hydrogens were added to proteins. Three sets of proteins were used in re-docking and virtual screening steps. The 3D structures of the ligands were extracted from protein structure (Known as reference structure). Atom and bond types were rectified, hydrogens were added, and partial charges were assigned according to the Gasteiger-Hückel method using SYBYL program (Sybyl 2007). Finally a short energy minimization for 1000 steepest descent steps with the Tripos Force Field (Clark and Opdenbosch, 1989) was performed to release the internal strain of ligand.

Molecular docking

Docking experiments were conducted by MOE2006.08 (MOE, 2006), GOLD3.2 (GOLD 2008) and FRED2.2.3 (http://www.eyesopen.com/). MOE with two docking algorithms: Alpha Triangle matcher and Proxy Triangle, GOLD with Genetic Algorithm, and FRED with Exhaustive searching were used in the re-docking protocol. The CPU times were recorded as docking speed is a critical issue in screening large libraries of compounds. The time to dock one ligand was approximately 2-3 min for FRED and 9-10 min for GOLD and MOE.

i. MOE2006.08

Proteins were minimized by MMFF94 force field, keeping all the heavy atoms fixed until a RMSD gradient of 0.05 kcal mol-1 Å-1 was reached (MOE, 2006). The active site was generated for each protein at the position of the bound ligand. Docking was performed using two docking algorithms (i) Triangle Matcher and (ii) Proxy Triangle, in combination with London dG scoring function. Ten top ranked docked poses were saved for further study.

ii. GOLD 3.2

For docking, the active site with a 10Å radius sphere around bound ligand was defined. For each genetic algorithm (GA) run, 10,000 operations were performed on a single population of 100 individuals (GOLD 2008). Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. The maximum distance between hydrogen donors and fitting points was set to 4.0Å, and non-bonded vdW energies were cut-off at 2.5Å. A set of ten top ranked poses were saved.

iii. FRED 2.2.3

Initially multi-conformer libraries of the input ligand database were generated by OMEGA. The receptor files were created by using protein structure with bound ligand (http://www.eyesopen.com/). Eight scoring functions were used for scoring purpose. Ten top ranked poses were saved and analyzed.

RESULTS ANALYSIS

Initially re-docking was performed to identify the most accurate docking method for the screening of IL-2 Inhibitors. The re-docking results were quantified by RMSD which indicates the difference between the predicted docked and the co-crystallized conformation of the ligand. FRED was used in the virtual screening. A set of 3100 compounds was selected from our *In-house* database according to the Lipinski rule of five (Lipinski *et al.,* 1997). 38 known inhibitors of IL-2 were collected from literature (Braisted et al., 2003; Geng *et al.,* 2002; Tilley *et al.,* 1997; Waal *et al.*, 2005) and included in a set of 3100 compounds (http://www.iccs.edu). The ability of the scoring functions to identify and rank the known actives in the top of the screening library was quantified. Eight scoring functions from FRED including Chemgauss2, Chemgauss3, ChemScore, PLP, Consensus, Oechemscore, Screenscore and Shapegauss were used in VS. Furthermore, the top-ranked FRED docked poses were re-scored using GOLD and CScore implemented in SYBYL7.3. GOLD was used with GOLD score, ChemScore and ASP while CScore was used with five scoring functions i.e. G_Score, D_Score, ChemScore, PMF and CScore in rigid and relaxed modes. The VS results were quantified in terms of enrichment factors which is a common metric used when comparing virtual screening results. EF is defined as:

$$\mathbf{EF = (HITS_{sampled}/HITS_{total}) / \mathit{N}_{sampled}/\mathit{N}_{total}}$$

Here, N_{total} is the number of ligands in the docked database, $N_{sampled}$ is the number of ligands in the docked database to be examined, $HITS_{total}$ is the total number of the known active ligands, and $HIT_{sampled}$ is the number of known active ligands found in the top $N_{sampled}$ ligands of docked database.

RESULTS AND DISCUSSION

Re-Docking Analysis

Three docking methods MOE, GOLD and FRED were used to predict bound conformations of three IL-2/ligand complex structures (**Table 1**). Each docking protocol returned with ten docked poses for each ligand; RMSD values were computed for all returned poses. **Figure 2-4** shows the top ranked docking poses predicted by GOLD, MOE and FRED, respectively. RMSD values of the top ranked docking poses are tabulated in **Table 2**. Among all the docking methods used in this study, MOE_TM was the most efficient in generating poses closest to co-crystallized ligands in the binding pocket of IL-2, followed by FRED. GOLD identified correct pose of two ligands out of three. **Figure 2** shows the top ranked posses of GOLD. The top ranked docked pose of 1M48 (**Figure 2a**) is found to be inverted since biaryl alkyne fragment is placed in the hydrophilic binding site instead of being in hydrophobic site, and piperidyl guanidine fragment is placed in highly mobile hydrophobic binding site instead of hydrophilic site, thus GOLD failed in reproducing the correct interactions with important contact residues. The top ranked docked poses of 1PW6 and 1PY2 are displayed in **Figure 2b** and **2c**, respectively. It shows that two molecules adopt the conformation matches to the reference molecule with slight variation. **Table 2** shows the RMSD>4Å for 1M48 and >2Å for 1PY2 and the fitness scores for each ligand. For all scoring functions, higher GOLD score indicate more favorable poses. The GOLD score was not correlated well with the IC_{50} values of the ligands.

MOE was used with two docking placement methods: Triangle Matcher (MOE_TM) which is the default method and Proxy Triangle (MOE_PT) method. MOE_TM generated good pose (RMSD <2Å). Comparatively, the performance of MOE_TM was better than MOE_PT method (Table 2). MOE_TM is a method in which Poses are generated by aligning ligand triplets of atoms on triplets of alpha spheres in a more systematic way [19]. MOE_TM not only took less time but also found to be feasible for the placement of ligand into the binding site. The top ranked docking poses of MOE in **Figure 3**. In MOE, highly negative scores indicate more favorable poses, London dG was appropriate in correlating binding scores with IC_{50} values. Thus the performance of MOE in combination with Triangle matcher method and London dG scoring function was found to be good.

Top ranked docked poses of FRED is shown in **Figure 4**. FRED offers various options for the efficient docking include rejection of the crude docking solutions and orientations that clash with the protein as well as number of scoring functions (Chemgauss3, Chemgauss2, ChemScore, PLP, Consensus, OeChemScore, ScreenScore and

Shapegauss). FRED is able to return a subset of the conformers with their scores (ranking of compounds in dataset) within a short period of time (seconds to minutes), that is the main demand of virtual screening.

Table 1. Three protein-ligand complexes of IL-2 used in this study.

S#	PDB	Ligand ID	Resolution (Å)	IC_{50} (µM)	References
1	1M48	FRG	1.9	3.0	[18]
2	1PW6	FRB	2.60	6.0	[19]
3	1PY2	FRH	2.80	0.6	[19]

Table 2. Docking performance of MOE*, GOLD and FRED.

	GOLD			MOE_TM			MOE_PT			FRED		
	RMSD	**Score**	**Rank**	**RMSD**	**Score**	**Rank**	**RMSD**	**Score**	**Rank**	**RMSD**	**Score**	**Rank**
1PY2	2.9	69.39	**2**	**1.2**	-34.57	**1**	**2.8**	-38.10	**1**	**1.3**	-45.44	**3**
1M48	4.9	63.64	**3**	**1.02**	-29.91	**2**	**0.80**	-28.14	**2**	**0.85**	-52.88	**1**
1PW6	1.8	84.97	**1**	**1.7**	-28.13	**3**	**3.2**	-26.51	**3**	**1.2**	-50.49	**2**

**MOE was used with two methods Triangle Matcher (MOE_TM) and Proxy Triangle (MOE_PT)*

Table 3. Standard enrichment factor of different scoring functions at 5% and 10% of ranked database.

Scoring Functions	1M48		1PW6		1PY2	
	5%	**10%**	**5%**	**10%**	**5%**	**10%**
Chemgauss2 (CG2)	14.75	8.95	14.75	8.14	14.22	8.95
PLP	6.85	6.24	8.43	4.88	10.53	6.78
Shapegauss (SG)	4.74	7.05	8.43	6.51	0	0
ASP	5.79	4.33	11.59	6.78	12.64	8.14
Gold Score	7.37	4.61	13.17	7.05	10.00	5.97
CS_Gold	1.57	1.90	7.37	5.15	0.00	1.89
Oechem Score	3.16	4.34	5.27	4.6	6.32	6.51
Chemscore-FRED	1.05	0.54	0.53	0.27	0	0.54
Chemgauss3 (CG3)	0.53	0.54	1.05	1.36	10.53	6.78
Consensus	0.53	0.27	4.21	3.79	7.89	5.69
Screenscore	0	1.08	4.74	3.79	0	0
G-score	0	0	0.52	0.54	0	0
PMF score	9.48	6.78	3.16	4.88	0	0.54
D-score	4.71	4.61	0.52	4.61	0.53	0.81
ChemScore-Cscore	0	0	0	0	0	0.27
Cscore	8.95	4.61	0.52	5.42	7.37	3.79
R-G score	2.11	1.9	2.11	1.63	0	0.54
R-pmf score	5.79	5.42	4.74	3.52	3.16	2.71
R-D score	4.74	2.44	0.52	3.52	0.53	2.17
R-ChemScore_Cscore	0	0.27	0	0.54	0	1.35
R-Cscore	7.37	3.79	6.84	3.52	3.16	6.51
MOE	5.27	6.78	8.95	7.05	4.74	4.34
	Identified known binders and consistent among all three protein setup					
	Identified known binders but not consistent among all three protein					
	Identified known binders but not consistent with 1PY2					

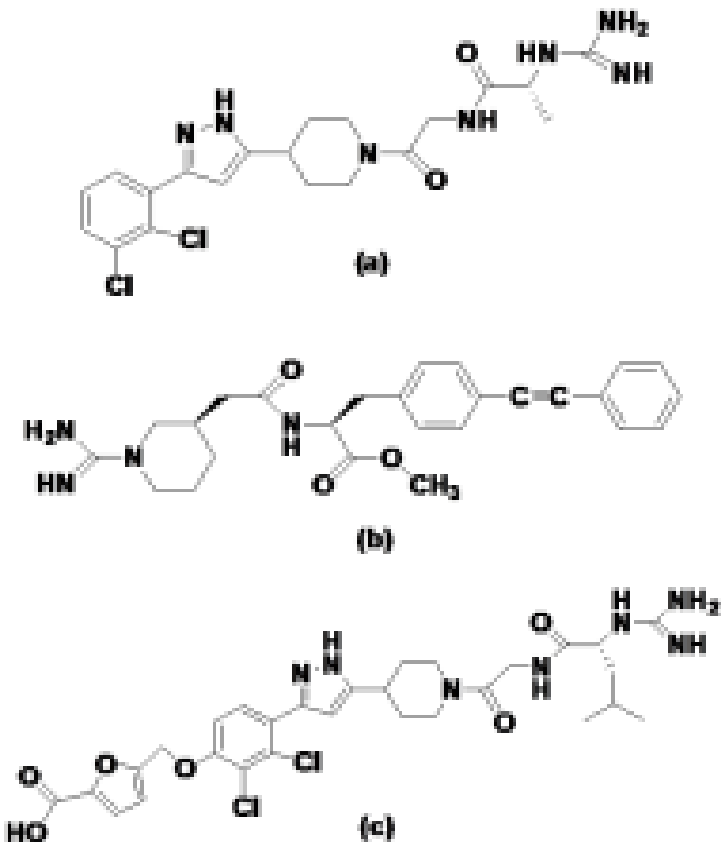

Fig. 1. Structure of the reference ligands used in this study for the evaluation of docking accuracy.

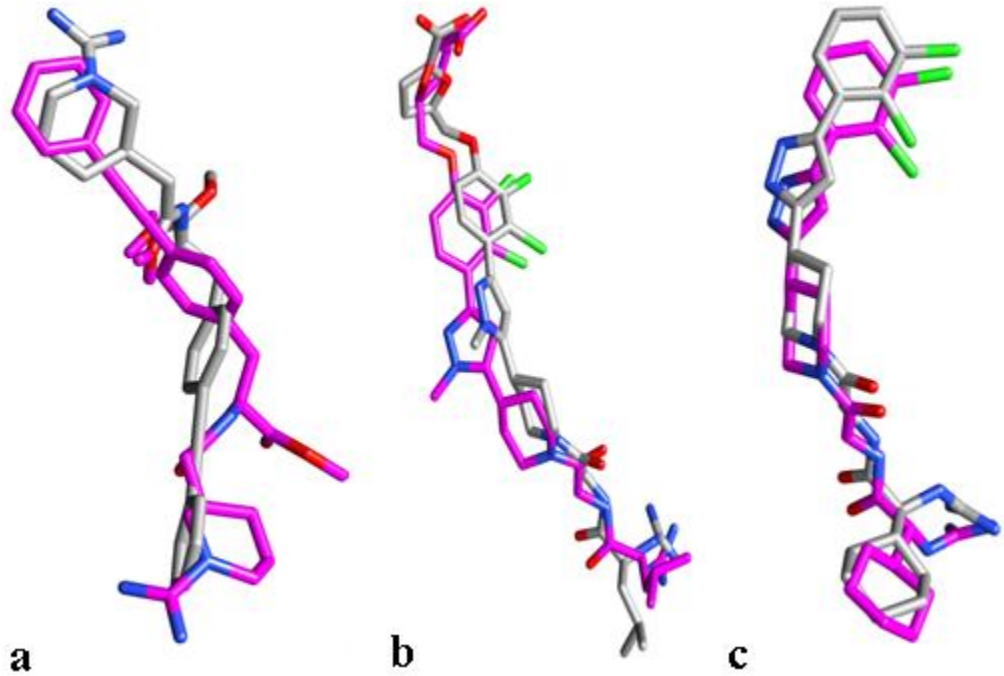

Fig. 2. Superimposed view of docked poses of 1M48 (a), 1PY2 (b) and 1PW6 (c), obtained by GOLD docking program. Reference ligand is shown in pink (sticks) and docked poses are shown in gray (sticks).

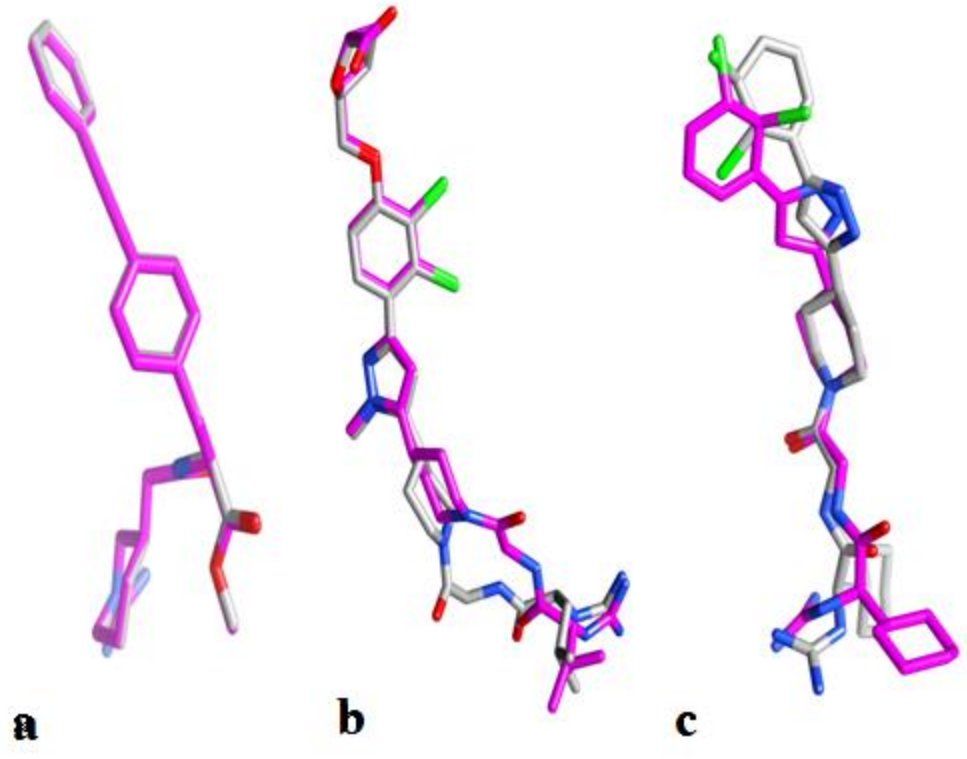

Fig. 3. Superimposed view of docked poses of 1M48 (a), 1PY2 (b) and 1PW6 (c), obtained by MOE docking program. Reference ligand is shown in pink (sticks) and docked poses are shown in gray (sticks).

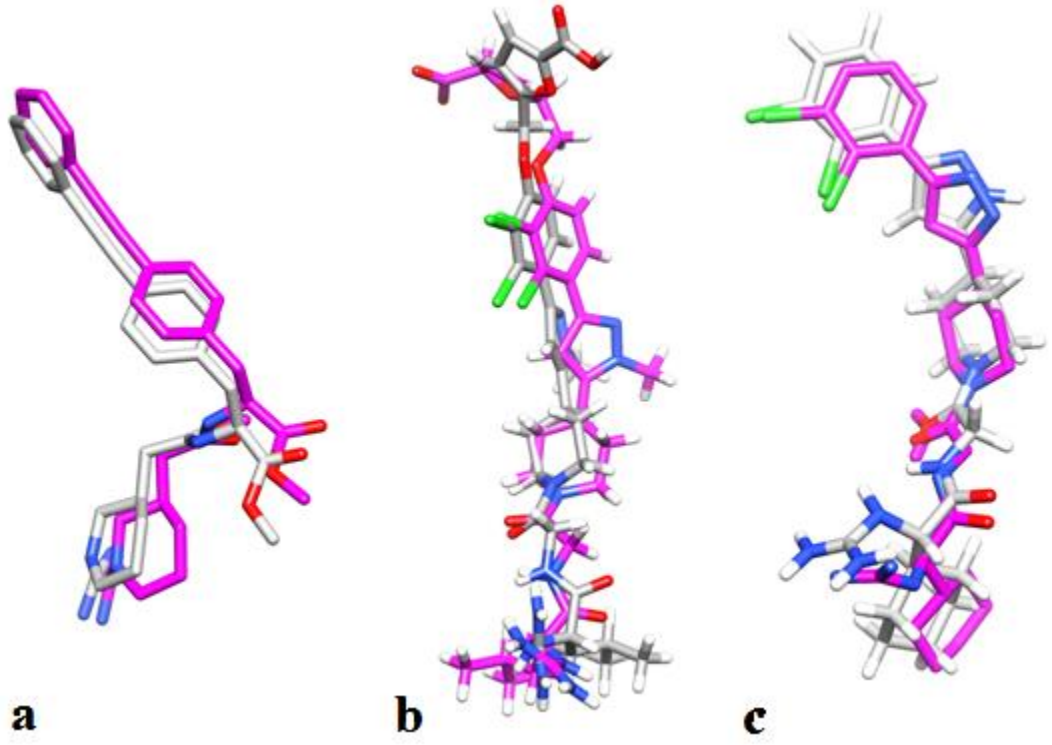

Fig. 4. Superimposed view of docked poses of 1M48 (a), 1PY2 (b) and 1PW6 (c), obtained by FRED docking program. Reference ligand is shown in pink (sticks) and docked poses are shown in gray (sticks).

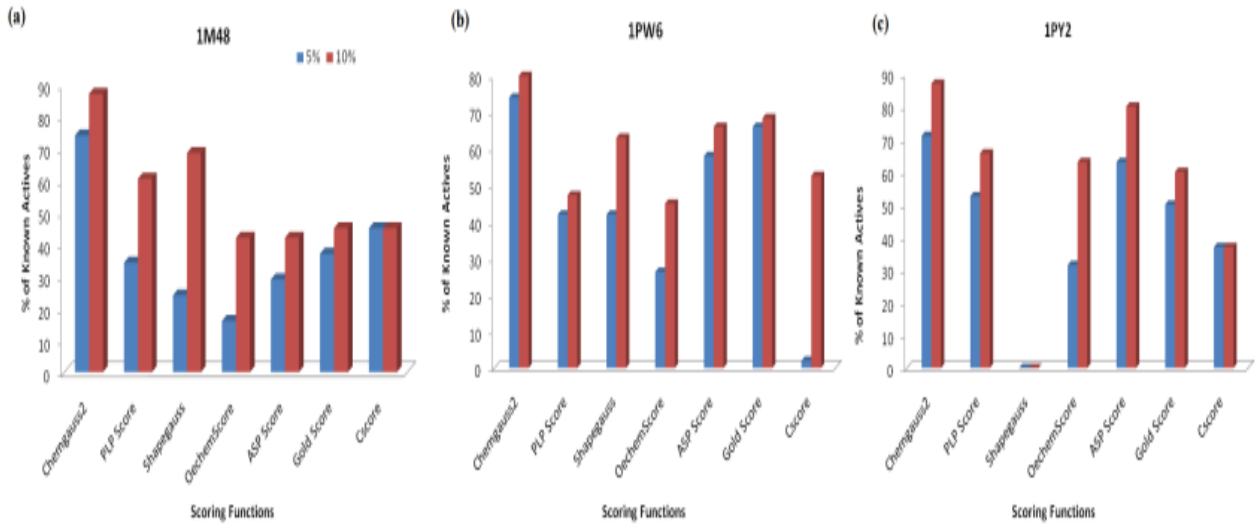

Fig. 5. Percentage (%) of active compounds identified in (a) 1M48, (b) 1M49, (c) 1PW6, at 5% and 10% of screened database.

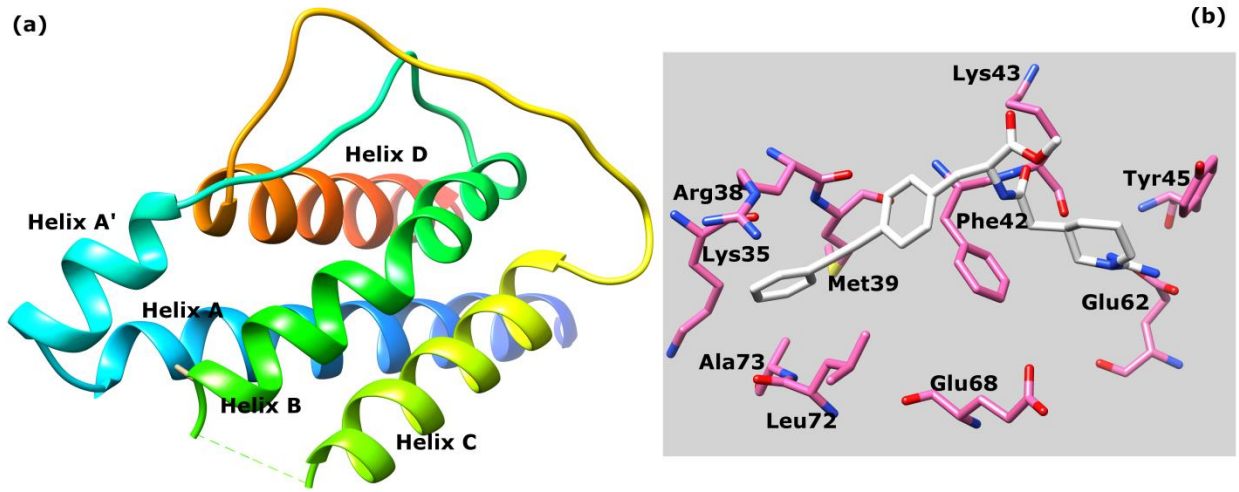

Fig. 6 (a). Ligand/IL-2 complex determined by X-ray crystallography (ligand is not shown). Four α helices, helix A in blue, helix B in dark green, helix C in light green and helix D in yellow orange. Helix A in loop appear in front to give helix A' shown in light blue color.
(b) Important residues of active site of IL-2 (in pink sticks) and ligand (in white sticks) taken from X-ray coordinates. All residues are labeled; Leu 72, Met 39, Lys 35, Arg 38 form a groove for hydrophobic interactions and Glu 62, Lys 43, Tyr 45 primarily give hydrophilic interactions.

In our study, FRED's performance was comparatively similar to MOE_TM in the generation of good orientation of poses (RMSD <2Å) (Table 2). Hence MOE_TM and FRED were found to be the most suitable programs in the generation of poses with lower RMSD (<2Å). Since the FRED is faster than all the used docking programs hence FRED was chosen for subsequent virtual screening studies.

Correlation of Biological Activities with the Docking Scores

The results tabulated in table 2, clearly indicates that only MOE (both MOE_TM and MOE_PT) significantly correlate the biological activities of the selected ligands with the docking scores. However the docking scores of GOLD and FRED did not correlate with IC_{50} values of the ligand.

Virtual Screening Performance

Scoring functions are used to estimate the free energy of binding of molecule to evaluate the binding affinities of molecules in the active site of protein in a given conformation. Thus scoring function must be able to identify the correct binding mode of a ligand out of alternative docking solutions on the crude pre-filtering of millions of compounds (3100 in our study) during virtual screening. Hence computational chemists rely on the evaluation of best scoring functions in order to select the compounds that are the best inhibitors of their protein.

The docking time of FRED is less; hence FRED was employed for the screening of 3100 drug like compounds collected from our in-house database [24]. For virtual screening, FRED was used with eight scoring functions: CG2, CG3, OCS, SS, CS, PLP, consensus and SG. Subsequently the ranked docked poses of compounds obtained by were re-scored by GOLD, MOE and CScore. GOLD was used in combination with GOLD score, ChemScore (CS_GOLD) and ASP, while Cscore was utilized in combination with G_Score, D_Score, Cscore, PMF and ChemScore (CS_Cscore). Cscore was used in rigid and relax modes. The reason of using large number of scoring functions (**Table 3**) is that so far there is no universal tool available that offers reliable scoring for pharmaceutically relevant targets. Therefore, it is crucial for the scoring functions to rank the known actives top of nearly all of the decoy ligands.

The performance of different scoring functions was evaluated on three protein setup. The scoring functions were evaluated based on enrichment factor (EF) achieved in top 5% and 10% of the screened database. **Figure 5** displays the percentage of known actives (total thirty eight) recovered as a function of the percent of ranked database sampled for CG3, CG2, CS, PLP, SG, SS, OCS, Consensus, GOLD score, CS_GOLD, ASP, G_Score, D_Score, PMF and CS_Cscore, for all three proteins setup (1M48, 1PW6 and 1PY2).

Enrichment Factor for 1M48, 1PY2 and 1PW6:

Enrichment factor was calculated to select most appropriate scoring function. **Table 3** shows the EF calculated at 5% and 10% of screened library for each scoring function when used with each of the three protein sets (1M48, 1PW6 and 1PY2 used in virtual screening). As indicated by the name "Enrichment factor" calculate that how much of thirty eight known binders are identified by any of particular scoring function in its Top ranked solutions. For example: 5% of total decoy set give us the total number of active compounds identified by a particular scoring function in top 151 compounds of 3102 database. Similarly, 10% of total decoy set give us the total number of active compounds identified by any scoring function in top 310 compounds of 3102 database and so on.

On 1M48 protein, CG2 identified 74% and 87% of known active compounds in 5% and 10% of screened dataset, respectively (**Figure 5a**). SG, PLP and OCS identified 68.5%, 60.5% and 42% active hits in 10% screened library, respectively. While CS, Consensus, CG3, SS were failed in correct ranking of active compounds in the top of the screened database. In Cscore, the PMF and Cscore identified 66% and 45% of known binders in rigid mode in top 10% library, respectively. In relaxed mode, all the scoring functions of Cscore failed to produce any significant result. In GOLD, GoldScore identified 45% known binders in top 10% of database screened. Hence on protein 1M48, CG2, PLP and SG performed successfully in virtual screening of the active hits (**Figure 5a**). However on 1PW6, CG2 retrieved 74% and 79% of active compounds in top 5% and 10% of screened database, respectively (F**igure 5b**). The performance of SG, PLP and OCS was similar as observed in case of 1M48; these scoring functions recognized 63.1%, 47.3% and 45% active compounds in 10% library, respectively. On the other hand, CS, Consensus, CG3 and SS did not succeed to find out active compounds in top 10% of database. The overall performance of GOLD and Cscore were similar as observed in case of 1M48 in top 5% of screened library (**Figure 5b**). PLP and SG identified 47.3% and 63.1% known actives, respectively. G_Score, D_Score, Cscore, PMF and CS_Cscore, in rigid and relaxed modes were unsuccessful in identification of active compounds within 10%. ASP and GoldScore identified 66% and 68% of known actives, respectively. However the results were not consistent on proteins 1M48 and 1PY2. On 1PY2, the performance of FRED was similar as observed in case of 1M48 and 1PW6. CG2 identified 71% and 87% of known actives in 5% and 10% of library, respectively. PLP identified 66% known binders in top 10% of ranked database however the performance of SG decreased drastically (**Figure 5c**) and failed to rank any known binder. Among all three set of proteins used in this study, the scoring functions did not performed well on 1PY2, this may be due to lower resolution of this protein. MOE did not significantly discriminated binders and non-binders during virtual screening experiments. Hence the results shows that the CG2 scoring function outperformed all the scoring functions and can efficiently use for the virtual screening of IL-2 inhibitors. The three dimensional structure of IL-2 is displayed in Figure 6, which will further guide us to develop novel inhibitors of IL-2.

Conclusion

A comparative docking analysis was conducted to set an efficient docking protocol against the drug target, IL-2. The docking method produced consistent results in reproducing the binding modes of the known binders. The docking methods were used to calculate the enrichment factors of a compound's dataset consisting of 3100 compounds with 38 known binders. The results showed that the computational tools accurately predicted the binding modes of the known binders. The enrichment factors further indicated that the scoring functions were able to discriminate between the decoy compounds and the known binders. Among all scoring functions, ChemGauss2 was best. The method provides useful insights into the setting up of a suitable structure-based docking protocol against IL-2.

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