World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Research Article

An insilico study on anti inflammatory compounds from marine system using Molegro Virtual Docker

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Received: 06-03-2014 / Revised: 17-03-2014 / Accepted: 28-03-2014

ABSTRACT

Computer aided drug discovery is an upcoming discipline that uses several bioinformatics tools to find out the 3D structure of target molecules, to manage databases of small compounds and to develop new and improved lead compounds. It is intended to assist the traditional drug discovery approaches, thereby reducing enormous amount of time and money. Molecular docking is a computational method, searching for a ligand that is able to fit both geometrically and energetically the active site of a target. Molegro Virtual Docker (MVD) is a program for determining the most likely conformation of how a ligand will bind to a macromolecule. Here target molecules identified as Phospholipase A2, Nitric oxide synthase, Cox-2, NF Kappa B, Lipooxygenase, which is over expressed in many forms of inflammatory diseases and its inhibitors found to have anti-inflammatory properties. Chemical compounds are got from marine system, which may bind to the active site of above described target proteins.

Keywords: Inflammation, Target, Molegro Virtual Docker (MVD), Phospholipase A2, Nitric oxide synthase, Cox-2, NF Kappa B, Lipooxygenase

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. [1] It is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. [2] In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. [3] Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. [4] During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational adjustments resulting in the overall binding is referred to as "induced-fit". [5] Certain protein-design calculations involve using an experimentally determined high-resolution structure as a template to identify new sequences that can adopt the same fold. This approach has led to the successful design of many novel, well-folded, native-like proteins. Although any atomic-resolution structure can serve as a template in such calculations, most successful designs have used high-resolution crystal structures. Because there are many proteins for which crystal structures are not available, it is of interest whether NMR templates are also appropriate. [6]

ChemBank (http://chembank.broad.harvard.edu/) is a public, web-based informatics environment developed through collaboration between the Chemical Biology Program and Platform at the Broad Institute of Harvard and MIT. This knowledge environment includes freely available data derived from small molecules and small-molecule screens and resources for studying these data. [7] PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). The American Chemical

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Society tried to get the U.S. Congress to restrict the operation of PubChem, because they claim it competes with their Chemical Abstracts Service. More than 80 database vendors contribute to the growing PubChem database. The American Chemical Society has raised concerns about the publicly supported PubChem database, since. It appears to directly compete with their existing Chemical Abstracts Service. [8] Phospholipases A2 (PLA2s) are enzymes that release fatty acids from the second carbon group of glycerol. This particular phospholipase specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids. Upon downstream modification by cyclooxygenases, arachidonic acid is modified into active compounds called eicosanoids. Eicosanoids include prostaglandins and leukotrienes, which are categorized as anti-inflammatory and inflammatory mediators. [9]

As opposed to the critical calcium-dependent regulation of constitutive NOS enzymes (nNOS and eNOS), iNOS has been described as calcium-insensitive, likely due to its tight non-covalent interaction with calmodulin (CaM) and Ca^{2+}. The gene coding for iNOS is located on Chromosome 17. [7] While evidence for 'baseline' iNOS expression has been elusive, IRF1 and NF-κB-dependent activation of the inducible NOS promoter supports an inflammation mediated stimulation of this transcript. [10]

Both COX-1 and -2 (also known as PGHS-1 and -2) also oxygenate two other essential fatty acids – DGLA (ω-6) and EPA (ω-3) – to give the series-1 and series-3 prostanoids, which are less inflammatory than those of series-2. DGLA and EPA are competitive inhibitors with AA for the COX pathways. This inhibition is a major mode of action in the way that dietary sources of DGLA and EPA (e.g., borage, fish oil) reduce inflammation. [11]

Because NF-κB controls many genes involved in inflammation, it is not surprising that NF-κB is found to be chronically active in many inflammatory diseases, such as inflammatory bowel disease, arthritis, sepsis, gastritis, asthma, atherosclerosis [12] and others.

Lipoxygenases are non-heme iron–containing enzymes that catalyze the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids with a 1, 4-*cis*, *cis*-pentadiene motif. [13] With respect to atherosclerosis 2 of the 6 (human)/7 (mice) lipoxygenase family members have received the most attention because of their expression patterns in inflammatory cells and in some settings within endothelial cells; these are the 12/15-lipoxygenase (12/15-LO; also known as the leukocyte-type 12-lipoxygenase and 15-lipoxygenase-1) and 5-lipoxygenase. [14, 15]

In this study we identified Phospho Lipase A2, Nitric Oxide Synthase, Cox-2, Nuclear Factor Kappa B and Lipo Oxygenase as target proteins and use this proteins to dock with chemical compounds got from marine system, which may bind to the active site of above described target proteins.

Molegro: MolDock is based on a new heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. To further improve docking accuracy, a re-ranking scoring function is introduced, which identifies the most promising docking solution from the solutions obtained by the docking algorithm. The docking accuracy of MolDock has been evaluated by docking flexible ligands to 77 protein targets. MolDock was able to identify the correct binding mode of 87% of the complexes. [16] Extensive review of literature concludes that marine system has known to exert effective biological activity. However, when compared with terrestrial plants very little research has been carried out so far on the anti inflammatory studies with marine compounds. Therefore this study aims to evaluate the *in silico* inflammatory inhibition effect of marine compounds.

MATERIALS AND METHODS

The X-ray crystallographic structure of Protein's complexed with known ligands retrieved from Protein Data Bank (PDB ID: 1O2E, 1M8E, 1CX2, 2H2N, 1HU9). This structure was saved as a standard PDB file. The ligand and the receptor protein's are separated and saved in two different files using Swiss PDB viewer. The Smiles formula of ligand was retrieved from PDB and used to generate the original three dimensional structure of the ligand. For this the free standing molecular building tool CORINA is used. The active site of the proteins was defined as the residues within 4Å vicinity of the ligand molecule with the help of PDB viewer. MVD (Molegro Virtual Docker), was used for protein ligand docking. The CORINA generated ligand structure was docked into the active site and the MoldockScore was recorded. Appropriate settings were chosen and Molegro was run. Marine compounds obtained through PubChem and smiles formula of these molecules downloaded in SDF format and their three dimensional structure were generated with the help of CORINA. Totally around 24 molecules. The

smiles formula of these hit molecules was retrieved and their three dimensional structure was generated with the help of CORINA. These molecules were then docked independently for MolDockscore and Rerankscore. Molecules which got least scores for MolDockscore were selected for testing validity in the Wet lab.

MolDock Score: 'MolDock Score [Grid]' is a grid based version of the MolDock Score function. It precalculates potential-energy values on an evenly spaced cubic grid in order to speed up calculations. Notice that unlike the standard MolDock Score, the grid version does not take hydrogen bond directionality into account. The energy potential is assessed by using tri-linear interpolation between relevant grid points. The rest of the terms on the MolDock Score [Grid] version (i.e. internal ligand energy contributions and constraint penalties) are identical to the standard version of the scoring function. The docking search algorithm (MolDock Optimizer) used in MVD is based on an evolutionary algorithm.

Rerank Score: The pose Organizer can also automatically rotate hydrogens in both the receptor and the ligand to their optimal position. It can also be used to rerank the ligands (using a rank score). In analyzing docking results the middle panel allows for recalculation of scoring functions together with MolDock Score, Binding Affinity Score, and re-ranking Score. These scoring function values are previously calculated if the poses are imported from mvdresults file. Pressing the Recalculate Energies button will recalculate the scores for each of the three measures (using the coefficients specified in the files for the binding affinity and re-ranking scores). It is possible to rerank poses using a computationally more complex scoring function than the function used during docking. This reranking score function is a sum of several terms (such as Vander Waals forces, electrostatic interactions and solvent terms), which can be manually adapted. The MolDock, MolDock grid, and the rerank score in MVD are not articulated in chemically relevant units. However, Molegro Virtual Docker can provide a rough estimate of the binding affinity.

RESULTS AND DISCUSSION

The proteins identified above are found to be over expressed in many forms of inflammation. As the x-ray crystallographic structure of the target molecules is available it is possible to apply structure based rational drug design approaches to develop new lead compounds based on it. The active site of the target molecules was found out and chembank containing the 3D structures of molecules, those derived from marine system are used for molecular docking. Molegro Virtual Docker 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 ligands. Molegro Virtual Docker offers high-quality docking based on a novel optimization technique combined with a user interface experience focusing on usability and productivity. The computational experiment undertaken has resulted in the identification of six molecules, which docked well into the active site of the target. Careful visual inspection of the top ranked molecules (hits) yielded a list of around 6 small molecules for each protein. The docked poses, along with their corresponding Moldockscore is given. These molecules are suggested to be interesting candidates for further testing in the laboratory. It is interesting that the MolDockscore calculated for "hits" are significantly higher than that calculated for the original ligand-protein docking. Here according to energy concept least score is taken because a molecule is stable in the least energy. So it is worth testing out the actual binding affinities of these small molecules to the target protein's to see whether the computer predictions reflect the biological scenario.

CONCLUSION

The study has identified around 6 small molecule inhibitors for each of these above discussed 5 proteins that might bind well to the active site of the target molecule chosen for the study. These molecules, predicted to "dock" well into the active site of these proteins active site should be considered as "interesting" molecules that need to be further tested in the laboratory. Finally, this purely in silico study strongly underscores the importance of Computational approaches in drug discovery, supplementing classical methods, thus saving enormous amount of time and money. This study concludes that marine micro organisms are expected to serve as lead compounds for potential drug development or pharmaceutical tools for basic research in life science. Much attention has been paid to identify, isolate and purify them for useful bio chemicals and utilize them in human welfare and development. The ocean is a rich source of biological diversity and potential for discovery of novel drugs.

ACKNOWLEDGEMENT

The Authors thank DBT Bioinformatics Centre, Bharathiar University and Kerala University for providing facilities.

Table 1: DOCKING SCORE

TARGET	MOLDOCKSCORE	MOLECULE DESCRIPTION		
		ORIGINAL LIGAND	MOLECULAR WEIGHT	MOLECULAR FORMULA
PHOSPHO LIPASE A2	19292	ANN	151.139	$C_8H_8O_3$
NITRIC OXIDE SYNTHASE	18871	HEM	614.471	$C_{34}H_{32}N_4O_4Fe$
COX-2	25.05	HEM	614.471	$C_{34}H_{32}N_4O_4Fe$
NUCLEAR FACTOR KAPPA B	99.9931	ACT	58.036	$C_2H_3O_2$
LIPO OXYGENASE	-33.62	4HM	137.113	$C_7H_8O_4$

1. PHOSPHO LIPASE A2 "HIT" MOLECULES

SI NO	MOLECULE NAME	MOLDOCKSCORE
1	Manoalide-1	-122.435
2	Irgarol-1	-91.626
3	Topsentin-1	-126.67
4	Avarol-2	-82.368
5	Cytarabine-2	-73.759
6	Manoalide-2	-78.225
7	Avarol-3	-65.133
8	Cytarabine-3	-74.377
9	Dithranol-3	-96.82
10	Avarol-4	-113.282
11	Conotoxin-4	-108.46
12	Dithranol-4	-94.154
13	Avarol-5	-76.367
14	Cacospongionolide B-5	-82.758
15	Cytarabine-5	-78.092

2. NITRIC OXIDE SYNTHASE "HIT" MOLECULES

SI NO	MOLECULE NAME	MOLDOCKSCORE
1	LB-18-1	-94.984
2	Petrosaspongiolide M-1	-95.60
3	Topsentin-1	-130.25
4	Cacospongionolide B-2	-100.823
5	Ecteinascidin-743-2	-95.571
6	Yondellis-2	-95.472
7	Ecteinascidin-743-3	-94.197
8	Glycoconjugates-3	-89.677
9	Manoalide-3	-80.187
10	Cacospongionolide B-4	-77.651
11	Conotoxin-4	-104.436
12	Dithranol-4	-85.417
13	Bryostatin-5	-63.867
14	Cytarabine-5	-69.845
15	Dithranol-5	-80.412

3. COX-2 "HIT" MOLECULES

SI NO	MOLECULE NAME	MOLDOCKSCORE
1	Avarol-1	-75.26
2	Conotoxin-1	-112.33
3	Dolastatin 10-1	-92.5
4	Cacospongionolide B-2	-82.10
5	Conotoxin-2	-93.41
6	Manoalide-2	-76.75
7	Ecteinascidin-743-3	-82.06
8	Halichondrin-B-3	-108.19

9	Trabectedin-3	-85.17
10	Avarol-4	-73.04
11	Benzethonium chloride-4	-92.20
12	Dithranol-4	-70.91
13	Avarol-5	-57.12
14	Cacospongionolide B-5	-79.75
15	Dithranol-5	-74.63

4. NUCLEAR FACTOR KAPPA B "HIT" MOLECULES

SI NO	MOLECULE NAME	MOLDOCKSCORE
1	Avarol-1	62.428
2	Dithiocyanate-1	99.085
3	Dithiocyanate-2	99.63
4	Dithiocyanate-3	98.29
5	Dithiocyanate-4	88.37
6	Dithiocyanate-5	98.97
7	Bryostatin-5	-6.102

5. LIPO OXYGENASE "HIT" MOLECULES

SI NO	MOLECULE NAME	MOLDOCKSCORE
1	Dolastatin-10-1	-65.44
2	Petrosaspongiolide M-1	-50.79
3	Topsentin-1	-52.54
4	Cytarabine-2	-40.47
5	Discodermolide-2	-55.89
6	Glycoconjugates-3	-81.36
7	Petrosaspongiolide M-3	-44.12
8	Trabectedin-3	-36.79
9	Benzethonium chloride-4	-54.45
10	Petrosaspongiolide M-4	-58.93
11	Cacospongionolide B-5	-58.32
12	Cytarabine-5	-36.78
13	Petrosaspongiolide M-5	-44.61

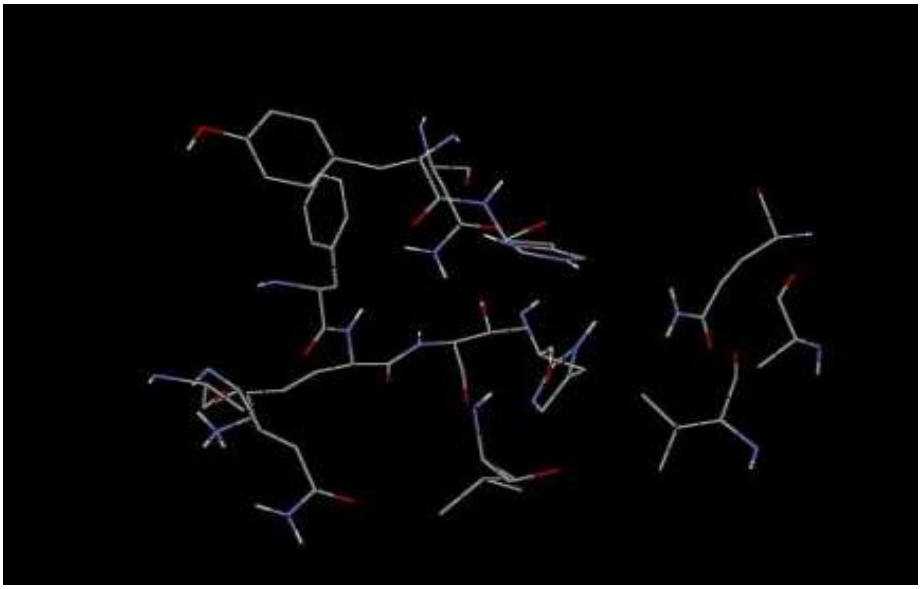


Figure 1: 1cx2 Active Site

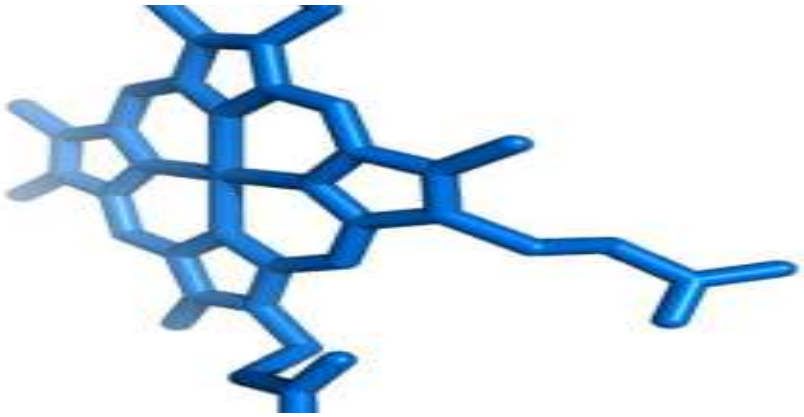
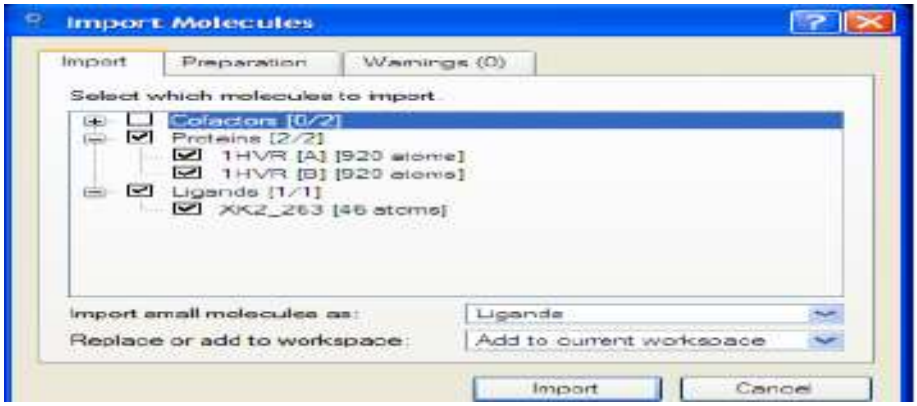


Figure 2: Ligand of Cox-2 (1cx2)



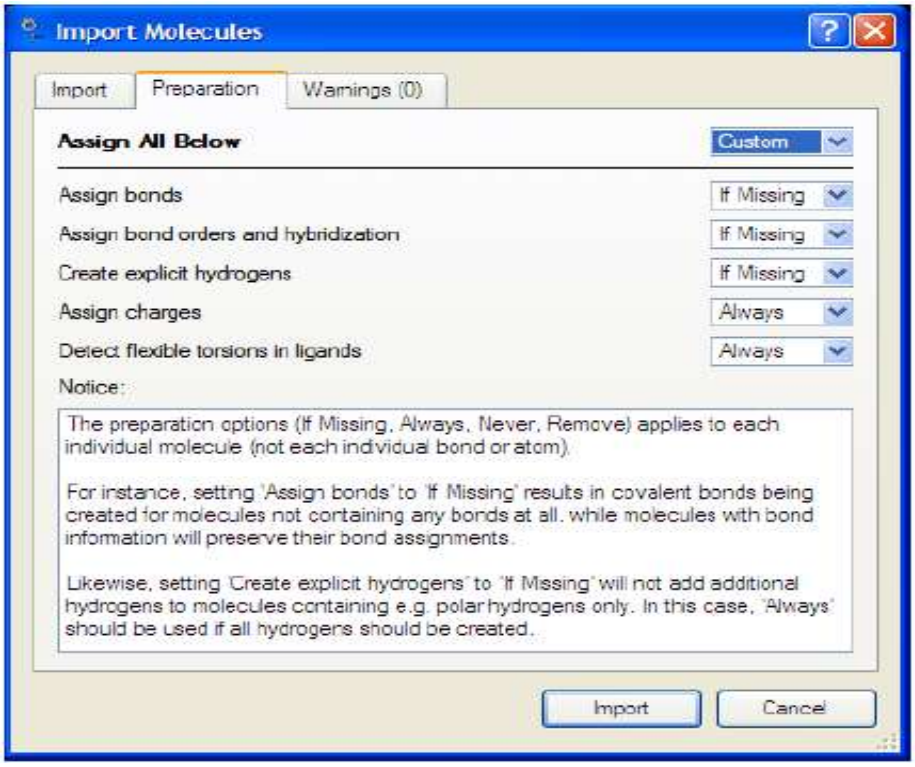


Figure 3: Preparing pdb File

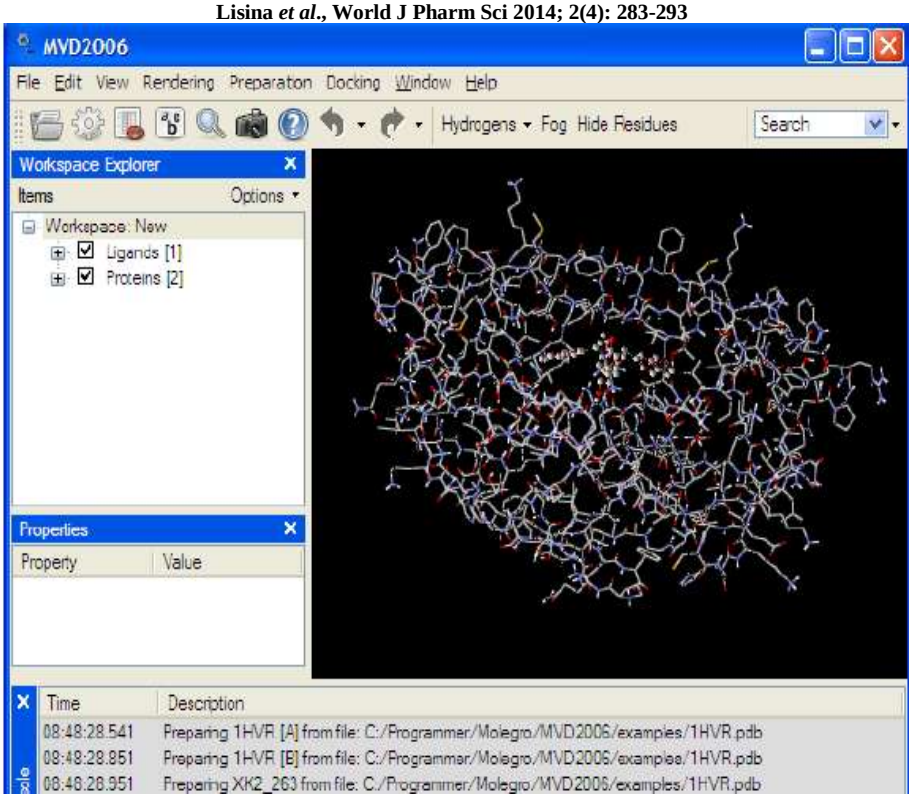


Figure 4: The Imported Structure

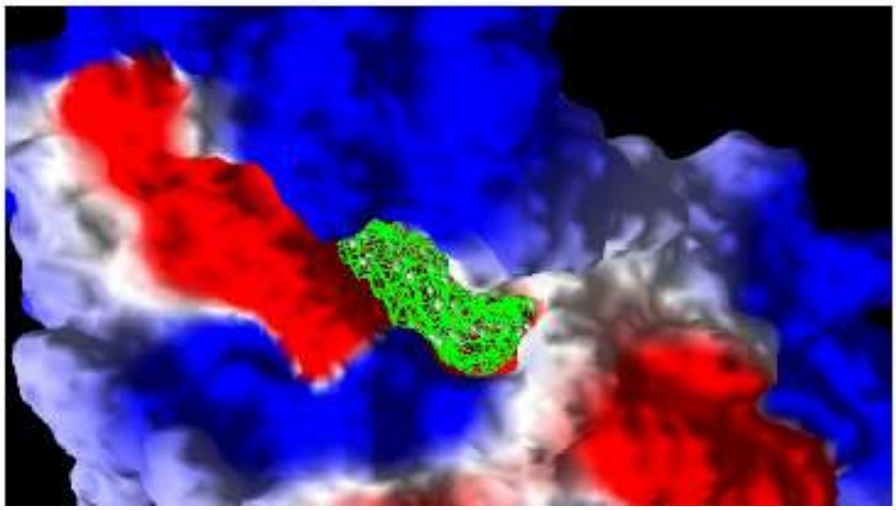
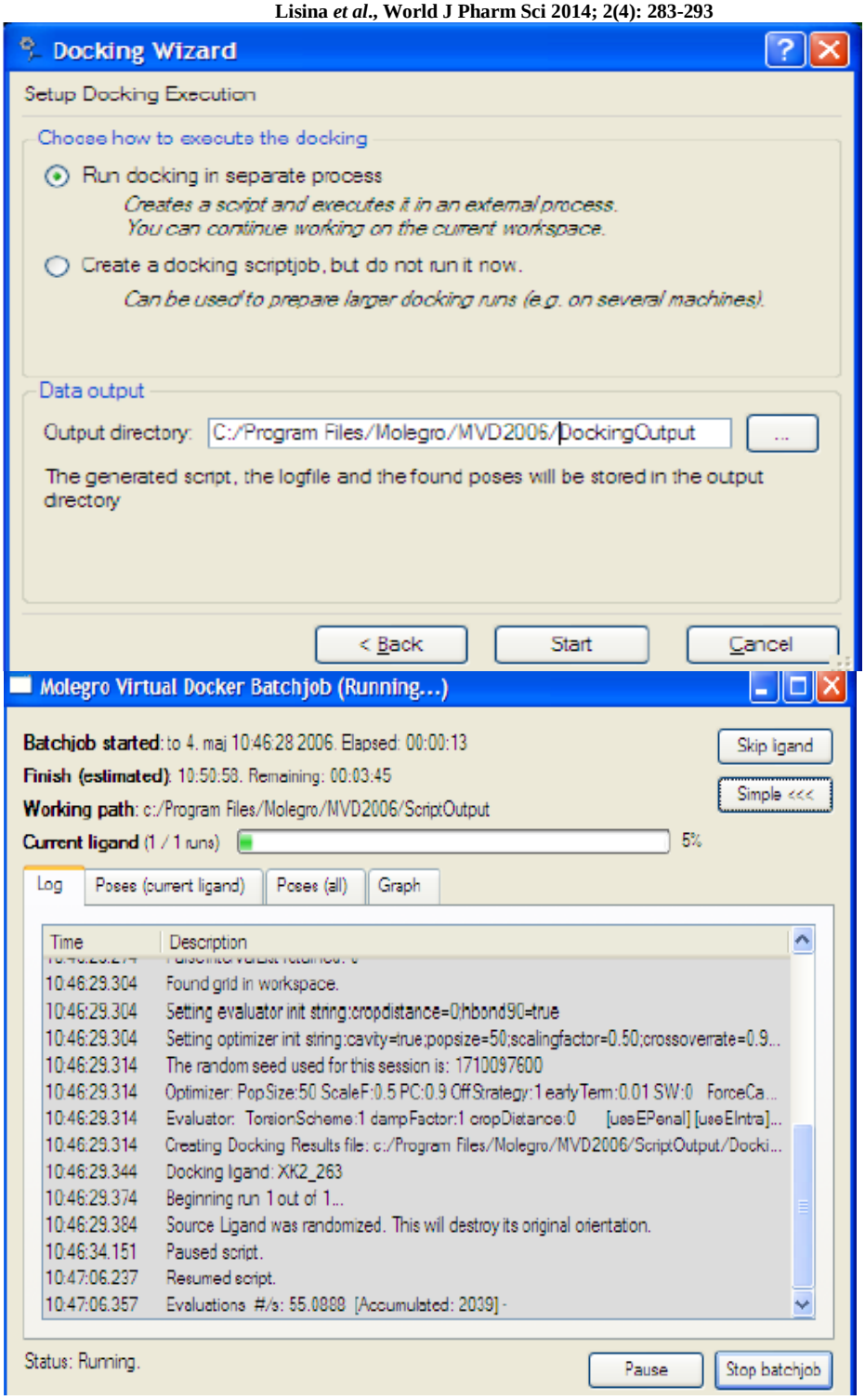


Figure 5: The Predicted Binding Site


Docking Wizard
Setup Docking Execution
Choose how to execute the docking
Run docking in separate process
Creates a script and executes it in an external process.
You can continue working on the current workspace.
Create a docking scriptjob, but do not run it now.
Can be used to prepare larger docking runs (e.g. on several machines).
Data output
Output directory:
C:/Program Files/Molegro/MVD2006/DockingOutput
...
The generated script, the logfile and the found poses will be stored in the output directory
< Back
Start
Cancel
Molegro Virtual Docker Batchjob (Running...)
Batchjob started: to 4. maj 10:46:28 2006. Elapsed: 00:00:13
Finish (estimated): 10:50:58. Remaining: 00:03:45
Working path: c:/Program Files/Molegro/MVD2006/ScriptOutput
Current ligand (1 / 1 runs)
5%
Skip ligand
Simple <<<
Log
Poses (current ligand)
Poses (all)
Graph
Time
Description
10:46:29.304 Found grid in workspace.
10:46:29.304 Setting evaluator init string:cropdistance=0;hbond90=true
10:46:29.304 Setting optimizer init string:cavity=true;popsize=50;scalingfactor=0.50;crossoverrate=0.9...
10:46:29.314 The random seed used for this session is: 1710097600
10:46:29.314 Optimizer: PopSize:50 ScaleF:0.5 PC:0.9 OffStrategy:1 earlyTerm:0.01 SW:0 ForceCa...
10:46:29.314 Evaluator: TorsionScheme:1 dampFactor:1 cropDistance:0 [useEPenal] [useEIntra]...
10:46:29.314 Creating Docking Results file: c:/Program Files/Molegro/MVD2006/ScriptOutput/Docki...
10:46:29.344 Docking ligand: XK2_263
10:46:29.374 Beginning run 1 out of 1...
10:46:29.384 Source Ligand was randomized. This will destroy its original orientation.
10:46:34.151 Paused script.
10:47:06.237 Resumed script.
10:47:06.357 Evaluations #/s: 55.0888 [Accumulated: 2039] -
Status: Running.
Pause
Stop batchjob

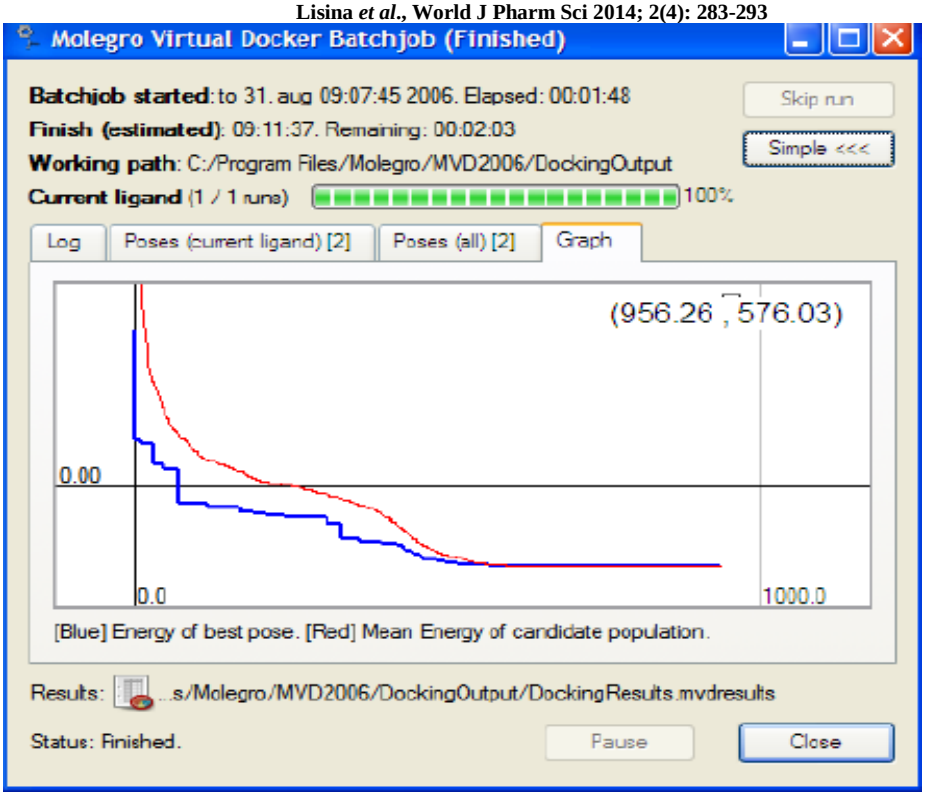


Figure 6: Docking progress Dialog with Convergence Graph

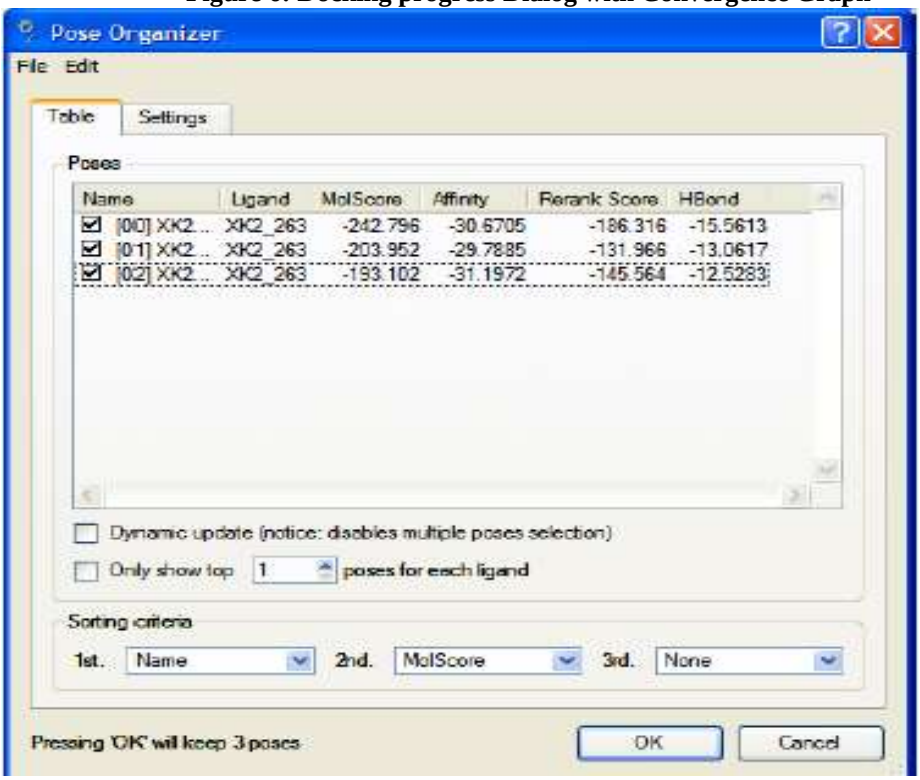


Figure 7: The Pose Organizer Dialog

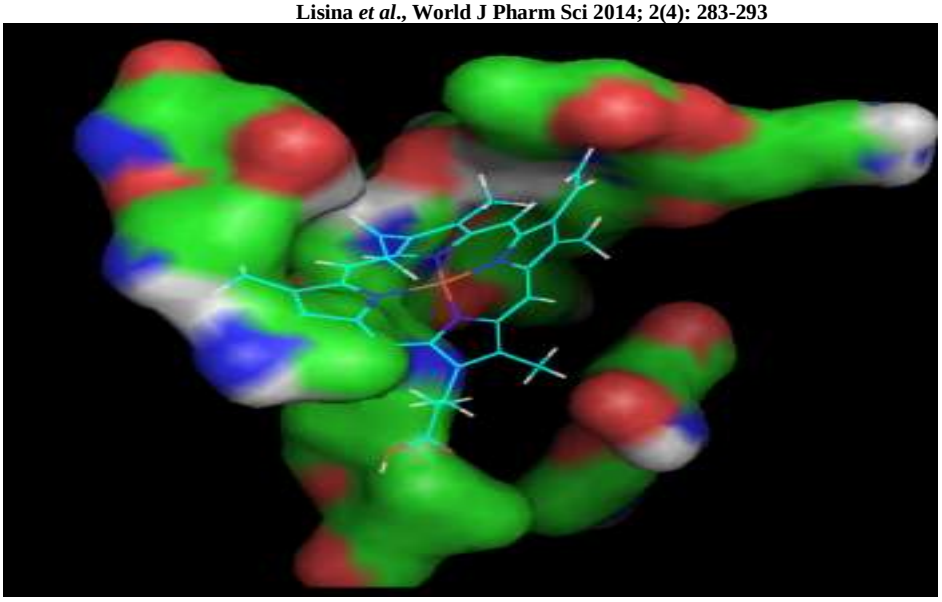


Figure 8: Active site of cox-2 and its original ligand docked in molegro

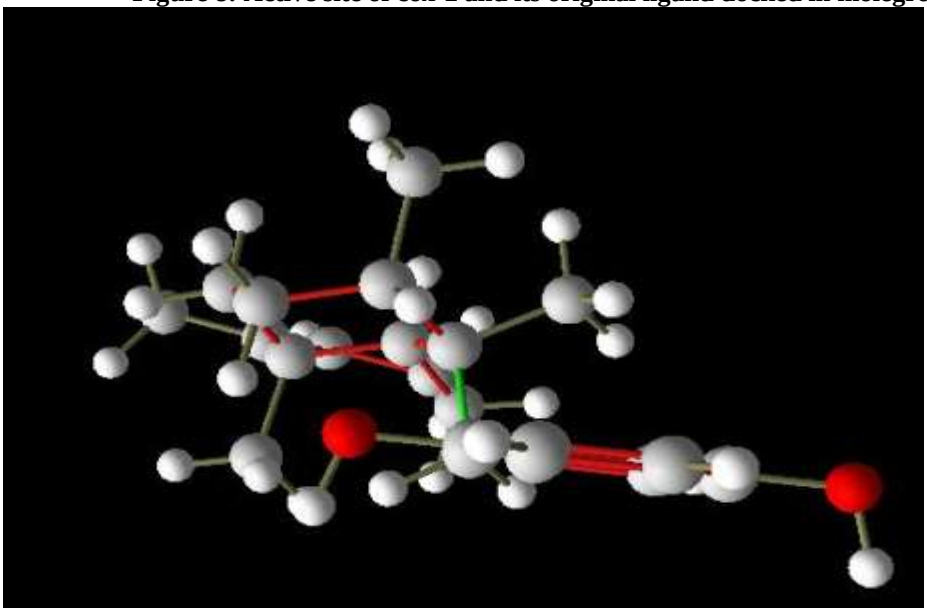


Figure 9: Avarol-1 (A Marine Compound)

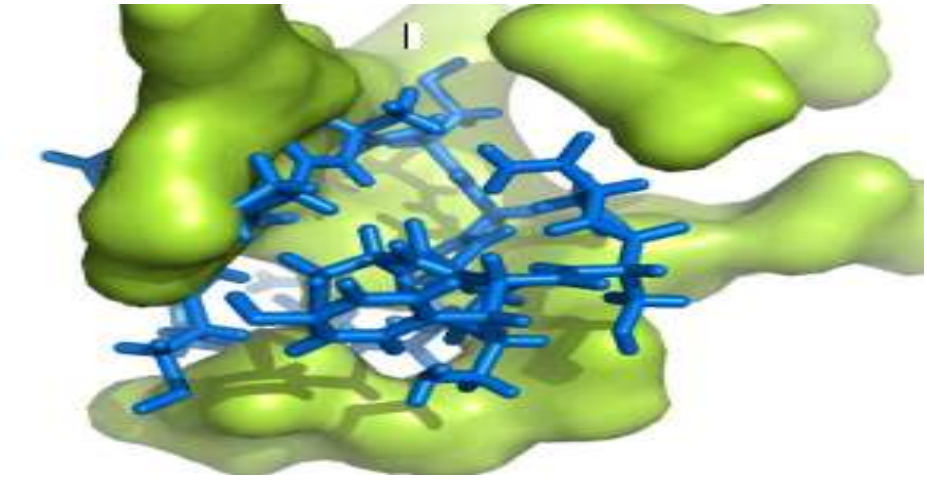


Figure 10: Active site of cox-2 (1cx2) with its Docked Molecule Avarol-1

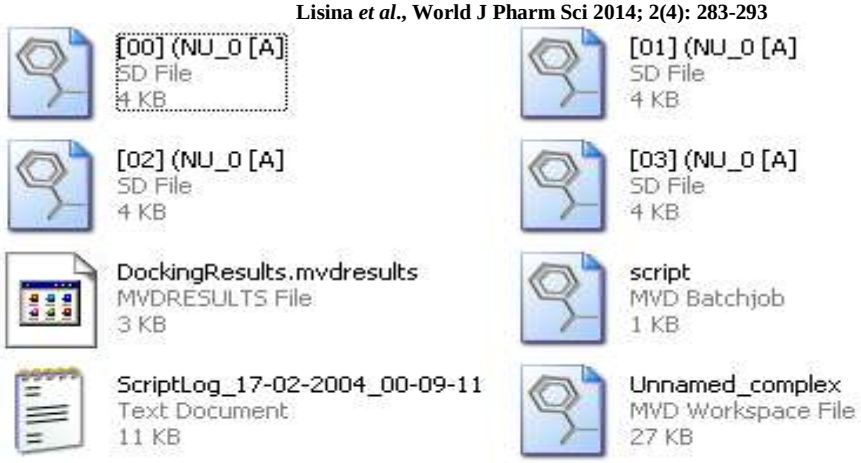


Figure 11: Docked Poses of 1cx-2 with Avarol

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