

## DOCKING OF 1-PHENYLSULFONAMIDE-3-TRIFLUOROMETHYL-5-PARABROMOPHENYL-PYRAZOLE TO CYCLOOXYGENASE-2 USING PLANTS

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### ABSTRACT

The docking protocols to virtually screen selective cyclooxygenase-2 (COX-2) ligands using PLANTS docking software were developed and validated. The crystal structure of 1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenyl-pyrazole (S58) binds to cyclooxygenase-2 (COX-2) was used as the reference structure. The developed protocols could predict the binding pose of S58 to COX-2 accurately (RMSD is 1.2 Å).

**Keywords:** docking, PLANTS, cyclooxygenase-2 (COX-2)

### INTRODUCTION

Molecular modeling plays an important role in reducing cost of the drug discovery processes [1]. In contrast, the prices of commercial and fully supported molecular modeling software (e.g. MOE developed by Chemical Computing Group Inc. (<http://www.chemcomp.com>), SYBIL developed by Tripos (<http://www.tripos.com>), and Discovery Studio developed by Accelrys (<http://www.accelrys.com>)) are considered as expensive [1-2]. Free molecular modeling softwares, fortunately, are available and can be downloaded freely from internet [1-2]. These softwares are helpful and powerful although most of them are hardly supported by the developer [2]. A concise review on free molecular modeling softwares can be found in [2].

Docking, as one of the popular molecular modeling approaches [1], is used to perform simulations for predicting binding poses of ligands to proteins [3-6]. Furthermore, docking can be used to predict the affinity of ligands to proteins [7]. This predictive feature are widely used to virtually screen libraries of compounds in order to find lead compounds and, even more, drug candidates [7]. The discoveries of some HIV-protease inhibitors and the discovery of the first clinically-approved HIV Integrase inhibitor ISENTRESS<sup>TM</sup> (raltegravir) are some classic examples of the docking-based drug discovery success stories [8-10]. More recent success stories are reviewed in [11], while some

challenges are concisely reviewed in [7]. Both reviews underline suggestion to perform rigid and robust validations on the development of docking-based virtual screening protocols [7,11].

PLANTS is a free docking software that can be obtained from the developer website (<http://www.tcd.uni-konstanz.de/research/plants.php>) and has been internally validated and benchmarked [12]. In this article, docking procedures of 1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenyl-pyrazole (S58; Fig. 1) to cyclooxygenase-2 (COX-2) by employing PLANTS are validated. The use of some other free softwares (i.e. ChemAxon (<http://www.chemaxon.com>) [13], YASARA (<http://www.yasara.org>) [14-17], and PyMol (<http://www.pymol.org>) [18-20]) for file preparations and results analysis are presented. The results can be developed and validated further to perform virtual screening campaigns in order to design selective COX-2 inhibitors. Moreover, the results indicate the applicability to perform an efficient drug discovery research project by employing free softwares.

### METHOD

#### Materials

Structure of COX-2 which was submitted by Kurumbail et al. to the protein data bank website

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(<http://www.pdb.org/>; PDB code: 6COX (6cox.pdb)) [21] was obtained and employed as the virtual target.

### Instrumentation

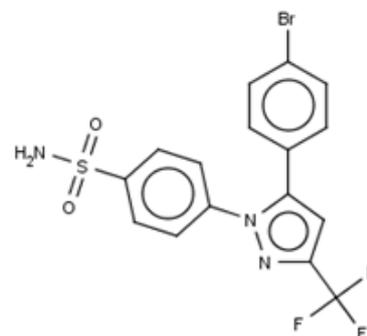
PLANTS (<http://www.tcd.uni-konstanz.de/research/plants.php>) [12] was employed to perform the docking simulations. MarvinSketch 5.2.5.1, 2009, ChemAxon (<http://www.chemaxon.com>) was used for drawing chemical structure of S58 (Fig. 1), and performing conformational searches for preparing the ligand input file in the docking simulations. YASARA 9.10.14 (<http://www.yasara.org>) [14-17] was employed to prepare the virtual COX-2 as the virtual target in the docking simulations and to analyze the docking simulation results. PyMol release 0.99 (developed by DeLano Scientific LLC, USA) was used to visually analyze the docking simulation results files and to produce pictures [19]. As long as no further explanation, the default settings of each application were used. All computational simulations were performed on a Windows-XP machine with Intel Pentium Dual Core T2080 (@ 1.73 GHz) as the processors and 512 MB of RAM. Since PLANTS can only run under Linux environment, Pendrivelinux 2009 (<http://www.pendrivelinux.com>) was employed to have Linux environment on the Windows-XP operating system [22]. For comparison purposes, the docking simulations were also performed in a Linux server (Dell™ PowerEdge™ 1900 installed with Ubuntu Server Edition).

### Procedure

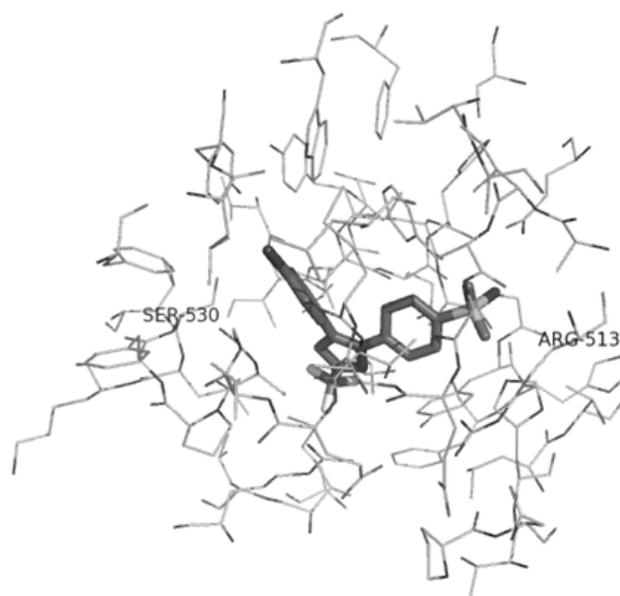
The two dimensions (2D) structure of S58 (Fig. 1) was built in MarvinSketch. The “major species” module in MarvinSketch was employed to have the major species of compound S58 in the pH of 7.4. Subsequently, the “conformers” module in MarvinSketch was used to perform conformational search. The results (*i.e.*, S58 conformers in three dimensions (3D)) were stored in a mol2 file, which was used as the ligand input file in the docking simulations.

The virtual target was prepared using YASARA. Only the protein atoms from chain A were extracted from the downloaded pdb file. Hydrogens were added to the protein using “edit” module in YASARA. The result was then exported as a mol2 file, which was subsequently used as the virtual target in the docking simulations.

The ligand input file and the virtual target file were employed together in the docking simulation using PLANTS directed by a prepared configuration file, which was a modification from the default configuration file the basic example in the PLANTS website ([http://www.tcd.uni-konstanz.de/research/PLANTS/simple\\_dock.zip](http://www.tcd.uni-konstanz.de/research/PLANTS/simple_dock.zip)). The binding site definition in the configuration file was changed



**Fig 1.** Structure of 1-phenylsulfonamide-3-trifluoromethyl-5-parabromophenyl-pyrazole (S58).



**Fig 2.** S58 in the binding pocket of COX-2 [21]. The residues are presented as lines and the ligand is presented as sticks. Only residues within 5 Å from the ligand are presented here. Carbon atoms are presented in black, hydrogen atoms are not shown, fluorine atoms are presented in white, nitrogen atoms are presented in dark grey, and oxygen atoms are presented in grey, and sulphur atoms are presented in light grey. SER 530 and ARG 513 are labelled since these residues are suggested as important residues in the binding of the ligand to COX-2 and the COX-2/COX-1 selectivity [21].

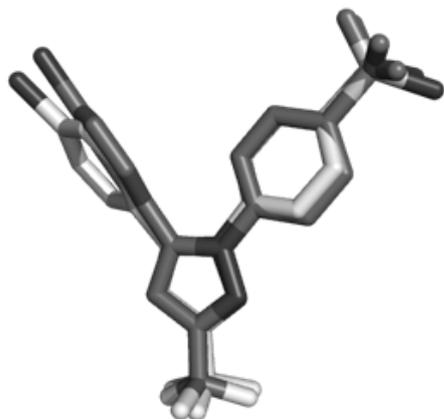
to 5 Å from the coordinates of the location where S58 bound to COX-2 (Fig. 2). The pose that gives the best score was selected as the best predicted binding pose. The RMSD values were calculated in YASARA.

### RESULT AND DISCUSSION

The ligand input file consists of 10 different conformers of S58, as the results of the “conformers”

**Table 1.** The scores of the best pose of each input conformer of the molecular docking results

No.	Entry	PLANTS <sub>CHEMPLP</sub> Score
1.	Conformer 01	-71.1549
2.	Conformer 02	-71.3034
3.	<b>Conformer 03</b>	<b>-71.4036</b>
4.	Conformer 04	-70.6239
5.	Conformer 05	-70.7118
6.	Conformer 06	-70.7903
7.	Conformer 07	-71.2090
8.	Conformer 08	-70.9063
9.	Conformer 09	-71.2408
10.	Conformer 10	-70.7563

**Fig 3.** Overlay of the original S58 pose obtained from the crystal structure (carbon atoms are presented in white) [21] and the predicted docking pose obtained from the docking simulations (carbon atoms are presented in dark grey). Nitrogen atoms are presented in dark grey, and oxygen atoms are presented in grey, and sulphur atoms are presented in light grey.

module in MarvinSketch. Since, by default in PLANTS, each S58 conformer produces 10 possible binding poses, the docking simulations provide 100 binding poses. The PLANTS<sub>CHEMPLP</sub> scores (the default scoring function used in PLANTS) of the best pose of each input conformer are presented in Table 1. The different starting points give different results in these simulations, which are related to the stochastic algorithm used in PLANTS [12]. Due to stochastic nature of the scoring functions used in PLANTS [12] and to avoid bias by using the “active” conformer (conformer obtain from the reference crystal structure), this multi conformers approach is suggested. The procedures will be developed to screen libraries of compounds with no information of the “active” conformers. Therefore, this approach is considered more suitable compared to the use of the “active” conformation as the input conformation for the docking simulations.

Based on Table 1, the best PLANTS<sub>CHEMPLP</sub> score comes from the 3<sup>rd</sup> conformer. The RMSD calculations of

the best predicted binding pose using YASARA gives value of 1.2 Å. The similar study using e-HiTS virtual screening software reports a better RMSD value (0.5 Å) [23]. Since the RMSD values less than 2.0 Å are considered as valid and appropriate [24], both protocols developed in this research and in the research conducted by [23] are promising to be developed further. This means that the docking simulations using PLANTS can also remarkably reproduce the binding pose of S58 to COX-2 as presented in the crystal structure [21] (Fig. 3). By visual analysis using PyMol, the best predicted binding pose of S59 to COX-2 shows that the pose can maintain the important protein-ligand interactions [21]. Notably, instead of the “active” conformer, the 2D structure was used as the starting point. Moreover, all computational preparations and simulations were performed in the free softwares (*i.e.*, MarvinSketch, YASARA, and PLANTS). The very similar simulations performed in the Linux server provide a similar result (RMSD value of 1.2 Å).

These results open opportunities to develop efficient virtual screening protocols to discover new selective COX-2 inhibitors. The benchmark databases collected by Huang et al. are available for further virtual screening validations [25]. Together with the in-house availability of the pharmacological experimental systems to test compounds as COX-2 inhibitors *in vitro* [26], the validated virtual screening protocols can serve as a significant complementary tool to design new COX-2 inhibitors as well as to explain the molecular determinants of newly pharmacologically proven COX-2 inhibitors.

## CONCLUSION

Performing docking simulations using free softwares are promising to efficiently run a drug discovery research project. Combine applications of ChemAxon for ligand file preparation, YASARA for virtual target preparation, and PLANTS for running the docking simulations give accurate binding pose of S58 to COX-2. Further extensive virtual screening validations based on the developed protocols in this research are encouraged as well as experimental validations.

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