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Reverse Docking on Five Original PPO Structures: Plant, Bacterial, and Human

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Abstract

Protoporphyrinogen oxidase has known remarkable interest in biochemical studies, it is considered a perfect target for the development of new herbicides. PPO herbicides have been developed for more than forty years, and research on this enzyme remains until today, to find new more effective herbicides. In this work, we investigated the inhibitory activity of a compound derived from N-phenylphthalimide with the highest inhibitory activity among a series of 29 molecules, on five PPO structures from various origins, including Plant origin, bacterial, and human, we have based on Reverse Docking, to know the affinity between the inhibitor and the five targets, and the different ligand-receptor interactions. As well as Molecular Dynamics. Interesting results have been obtained, which may help us to discover new targets concerning herbicides.

Keywords: *PPO, N-phenylphthalimide, Reverse Docking, MD simulations.*

1. Introduction

Protoporphyrinogen oxidases (PPO: EC 1.3.3.4) have been of interest in biochemical research. PPO enzymes containing type III copper. They catalyze the oxidation of phenols to o-quinones, this oxidation changes the color of several food products which modifies the quality of agricultural products. During oxidation, protoporphyrinogen IX transforms into protoporphyrin-X [1.2]. Protoporphyrinogen IX is the common intermediate leading to heme and chlorophyll which gives plants their green color, this phenomenon is called enzymatic browning, it is not always undesirable, sometimes desirable, in the case for example of tea, harvest dates, it improves the taste, but generally, this phenomenon is to be avoided, the reason why the PPO enzyme is, on the one hand, an important target for several herbicides and on the flip side, it is among the targets of most interesting actions to treat many diseases, notably cancer, and variegata porphin (VP) [3.4], the latter causing because of defects in the PPO gene, it is common in the white African population, South Africa [5.6]. PPO enzymes are found in bacteria, fungi, plants, insects, animals, and humans.

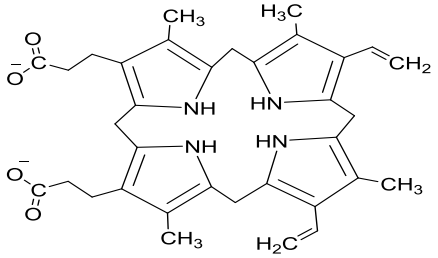


Figure 1. Protoporphyrinogene oxidase

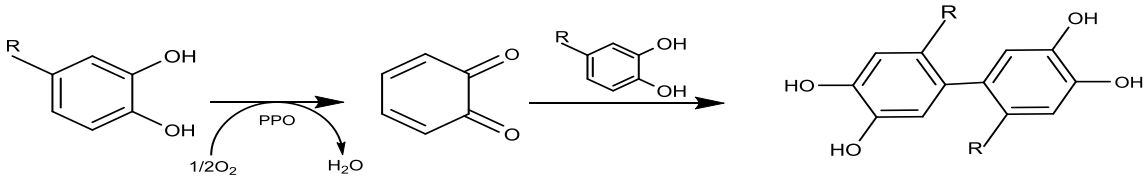


Figure 2. Oxidation of phenols to o-quinones

There are two isoforms of PPO in plants [7.8], plastid PPO (PPO1) and mitochondrial PPO (PPO2), the first is found in the thylakoid and envelope membranes of chloroplasts, and the second is located on the outer surface of the internal mitochondrial membrane. Regarding the structural biology of PPO, according to literature, five structures have been defined, one in the plant Nicotiana tobacco, two in bacteria Myxococcus Xanthus, and Bacillus subtilis, and two in humans. The objective of our work is to carry out a theoretical study on the five structures of PPO, by Reverse Docking, to know the different interactions, as well as the affinity between the different targets of PPO, and the ligand as an inhibitor of this enzyme. The technique of molecular simulation MD is thus carried out to study the stability of the complex having more important interactions. This study will thus allow us to define the best targets among the different structures for the most active inhibitor, which leads to the development of new more suitable, and effective inhibitors.

2. PPO structures

Mitochondrial PPO is localized on the external surface of the mitochondrial brain [9]. This enzyme is involved in the oxidation reaction, it is considered an essential parameter in the level of enzymatic browning when it encounters its substrate (phenolic compounds) in the presence of oxygen. First of all, during an enzymatic browning [10] the phenolic compounds oxidize into quinones [11] under the responsibility of oxygen and the enzyme PPO [12], after the quinones also oxidize without the intervention of any particular enzyme and polymerize to give brown compounds. enzymatic browning will only take place if the tissues of the plant organs are damaged. That is, in healthy cells, the

phenolic compounds are localized in the vacuole, while the enzymes responsible for oxidation are located in the cytoplasm, between the vacuole and the cytoplasm there is a membrane that separates them, this explains the prevention of contact between the oxidation enzymes and their substrates, this leads to the prevention of browning, on the other hand in the case where the cells are damaged all their constituents are mixed, and consequently, the brownings appear.

2.1. PPO structure from Nicotiana tabaccum

The plant Nicotiana tabaccum, called Nicotiana because its active principle is Nicotine, of American origin, but at the moment it is found all over the world, the plants of the genus Nicotiana belong to the nightshade family is called Tobacco. Cigarettes are made from the dried leaves of tobacco plants, so they are used in bioengineering as ornamentals. Nicotiana tabaccum contains a great nicotine promoter which leads to the release of acetylcholine, norepinephrine, dopamine, serotonin, vasopressin, and growth hormone by stimulation of Nicotine receptors, the reason for which it is known for their various pharmacological activities, on several organs including the peripheral nervous system, the central nervous system, the cardiovascular system, the gastrointestinal tract, the exocrine glands, the hematopoietic system. So according to the literature, we found the crystalline structure of mitochondrial PPO in Nicotiana tabaccum, in a complex form with the inhibitor phenyl-pyrazole (PDB code: 1SEZ).

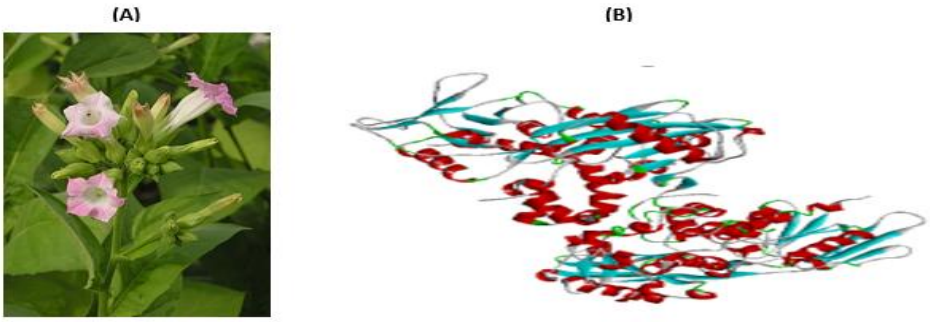


Figure 3. **(A)** Nicotianna-tabaccum. **(B)** PPO structure from Nicotiana-tabaccum (*PDB:1SEZ; Resolution 2.90 A°)*

2.2. PPO from Baccillus Subtilis

Bacillus subtilis is a very interesting microorganism gram-positive, it is involved in several metabolite mechanisms such as the production of enzymes, cell division, the secretion of proteins, the exchange of cytoplasm via intercellular nanotubes, and the formation of spores, it is usable for biological control of plants concerning food fermentation. There is not much difference between Bacillus subtilis enzyme [13.14] and eukaryotic enzyme, both use molecular oxygen as a terminal electron acceptor. The specific substrate of the enzyme Bacillus subtilis is broader than that of the eukaryotic enzyme, as it thus oxidizes the intermediate of the coproporphyrinogen III pathway. The enzyme of Bacillus subtilis is resistant to the inhibition of Acifluorfen. The inhibitor Acifluorfen effectively inhibits the eukaryotic enzyme.

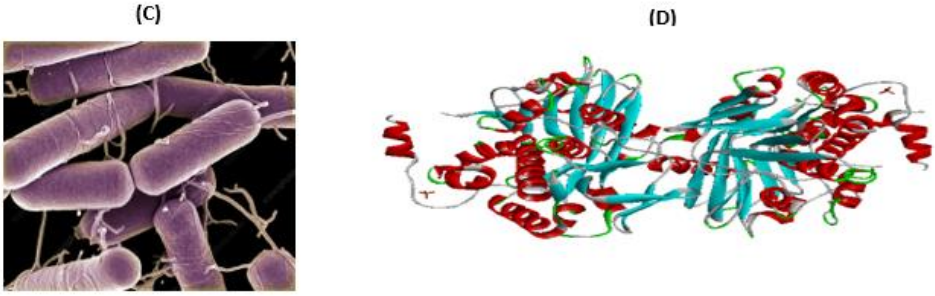


Figure 4. (C) Bacillus subtilis**. (D)** PPO structure from Baccillus Subtilis (*PDB: **2IVE**; Resolution 2.90 A°)*

2.3. PPO from Myxococcus Xanthus

Myxococcus Xanthus is a gram-negative soil bacterium, ie appears pink under a microscope, it is involved in the formation of a structure called "Fruiting organ" by multicellular development **[15.16]**. Fruiting is the transformation of flowers into fruit by fertilization. The control of M. Xanthus is done by two genetic systems: the adventurous system (A), and social motility (S). The adventurous system allows cells to move independently, and when cells are near each other **[17]** social motility is activated **[18.19]**. The PPO structure of Myxococcus Xanthus is similar to the human enzyme in catalytic properties, it is also linked to the inhibitor Acifluorfen at a resolution of 2.7 A °.

Figure 5. **(E)** Myxococcus Xanthus. **(F)** PPO structure from Myxococcus Xanthus (*PDB: **3I6D**; Resolution 2.90 A°)*

2.4. Human PPO structure

In humans, mutations linked to the enzyme protoporphyrinogen oxidase can cause many diseases including variegated prophyria, this disease is rare, mainly linked to abnormal production of hem, as well as certain types of cancer. For this reason, several studies have been interested in studying the inhibitory activity of herbicides as inhibitors of PPO to find new compounds targeting PPO to fight against several diseases. According to "Protein Data Bank", we found two structures of human PPO, a crystal structure (R59Q), and a mutant structure (R59G), the two structures form a complex with acifluorfen at a resolution of 2.6A ° and 2.8 A °.

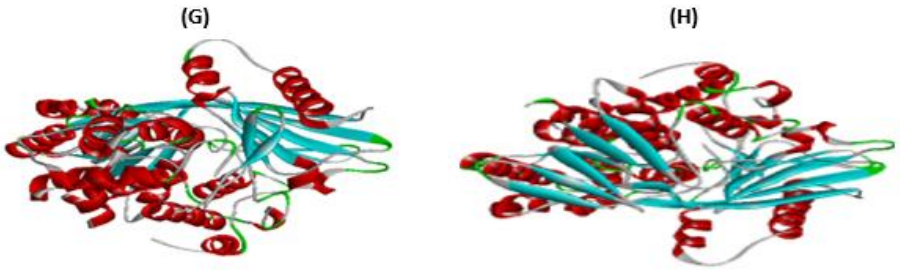


Figure 6. (G) crystal structure (R59Q) *(PDB: **4IVM**; Resolution 2.6 A°).* **(H)** Mutant structure (R59G) (*PDB: **4IVO**; Resolution 2.8 A°).*

3. PPO inhibitors

1.1. N-phenyl-phthalimide inhibitor

N-phenylphthalimide and its derivatives are often used in the field of pharmacology, it is known for these anticonvulsant, anti-inflammatory, hypolipidemic effects, about their preparation, it is prepared by a nucleophilic substitution reaction between the anhydride- phthalic acid and amine in glacial acid, the yields of this reaction ranging from 28% up to 87%, the good yield was obtained when the reaction proceeds in glacial acetic acid. at temperature 110 °.

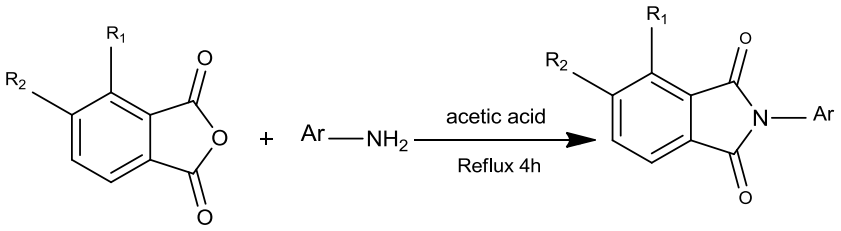
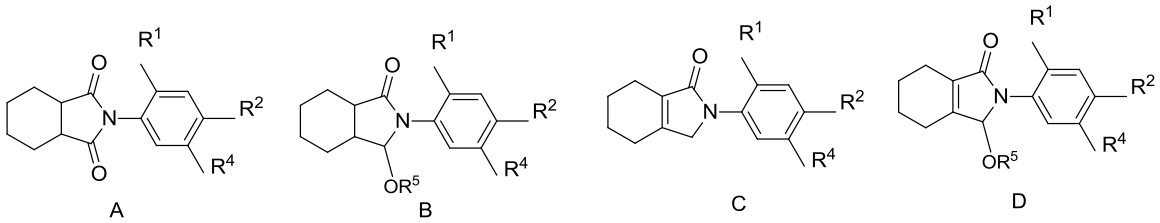


Figure 7. Chemical reaction to obtain N-phenylphthalimide

4. Materials and methods

We have based on a series of 29 compounds derived from N-phenylphthalimide **[20]**, to obtain the most active molecule among the compounds. Reverse Docking is performed on the compound **C6** with the highest activity in the complex with the five PPO targets. Reverse Docking is carried out using the following software: Sybyl to identify the most relevant ligand position on the PPO target [21]. Pymol binds ligand-receptor, and according to Discovery studio, we have removed water and inhibitors located in a complex form with the resulting PPO structures [22].

All PPO crystal structures were obtained from "Protein Data Bank"



N	R^1	R^2	R^4	R^5	Pki
A_1	F	Cl	OCH_3	-	5.08
A_2	F	Cl	$OCH_2CO_2C_2H_5$	-	4.81
A_3	F	Cl	$OCH(CH_3)CO_2C_2H_5$	-	5.48
B_1	F	Cl		H	4.6
B_2	F	Cl	$OCH(CH_3)_2$	H	4.32
B_3	F	Cl	OCH_3	H	3.88
B_4	F	Cl	$OCH_2CO_2C_2H_5$	H	3.91
B_5	F	Cl	$OCH(CH_3)CO_2C_2H_5$	H	4.31
B_6	F	Cl	OCH_2CCH	H	4.2
B_7	F	Cl	OCH_2CHCH_2	H	4.35
C_1	F	Cl	$OCH(CH_3)_2$	-	6.16
C_2	H	Cl	NH_2	-	4.56
C_3	Cl	Cl	OCH_2CH_3	-	6.7
C_4	F	Cl	OCH_2CH_3	-	6.17
C_5	Cl	Cl	$OCH_2CH_2CH_3$	-	6.52
***C_6**	**F**	**Cl**	**$OCH_2CH_2CH_3$**	**-**	**7**
C_7	F	Cl	OCH_2CHCH_2	-	6.92
D_1	F	Br	OH	H	4.71
D_2	F	Cl	3-F-$C_6H_4CH_2O$	H	6.11
D_3	F	Cl	3-Cl-$C_6H_4CH_2O$	H	6.27
D_4	Cl	Cl	OCH_2CHCH_2	H	5.13
D_5	F	Cl	OCH_2CHCH_2	H	5.99
D_6	OCH_2CH_3	Cl	NO_2	H	3.44
D_7	F	Br	OH	CH_2CHCH_2	4.38
D_8	F	Cl	3-Cl-$C_6H_4CH_2O$	CH_3	5.99
D_9	F	Cl	OCH_3	CH_3	5.35
D_{10}	F	Cl	3-Cl-$C_6H_4CH_2O$	CH_2CHCH_2	4.18
D_{11}	F	Cl	OCH_2CHCH_2	CH_2CHCH_2	4.48
D_{12}	F	Cl	OCH_2CH_3	CH_2CHCH_2	5.15

**The most active compound*

5. Results and discussion

1.2. Reverse Docking

To study the inhibitory activity of compound **C6** derived from N-phenylphthalimide on the five targets of protoporphyrinogen oxidase, we based on Reverse Docking **(Table 1)**. The results found to relate to the original PPO target of the Nicotiana tabaccum plant reveal a low score, which indicates that the PPO enzyme has a high affinity for the inhibitor N-phenylphthalimide. Regarding the ligand-receptor interactions, we obtain the interactions: Van der Waals, Carbon Hydrogen Bond (between the hydrogens of substituent R4 and the residues LYS: 127; SER: 126), the interactions Alkyl and Pi-Alkyl (between the chlorine and the PHE residue: 160, and between the R4 substituent terminal carbon and the LEU residues: 116,122; ILE: 117) **(Figure 8)**. It is observed that there are various interactions distributed over the compound, which stabilizes the complex. For the PPO target derived from the bacterium Bacillus subtilis, it is observed that the score is a little high, which leads to a decrease in the level of affinity, apartment one sees diversity in the level of interactions. We find interactions of Van der Waals, Carbon Hydrogen Bond (between the hydrogen of substituent R4 and the residue SER: 64), Halogen bond (between the fluorine and the residue PRO: 62), Alkyl and Pi-Alkyl (between the phenylphthalimide, benzene, and residues ILE: 412; ALA: 47; PRO: 62) **(Figure 9)**. The affinity of PPO of the bacterium Myxococcus Xanthus is low, since the score is high, we compare it with the PPO of Bacillus subtilis on the one hand, on the other hand, we see that the interactions are more interesting than those of Bacillus subtilis. We obtain the interactions of van der Waals, conventional Hydrogen Bond (between fluorine and the residue ARG: 364), Carbon Hydrogen Bond (between the five-ring hydrogen and the residues GLY: 63; TYR: 366), the bond Halogen (between benzene and ASP: 65), and finally Alkyl and Pi-Alkyl interactions (between phenylphthalimide, the R4 substituent terminal carbon, and PRO residues: 64; TRP: 409; PRO: 451 respectively) **(Figure 10)**.

Table 1. ***Scoring results of the different targets and the types of interactions obtained.***

Origin	Target	Score	Interactions
Nicotiana-tabaccum	(PDB:1SEZ)	-1.858	Van der Waals Carbonn Hydrogen Bond Pi-sigma Alkyl; Pi-Alkyl
Bacillus Subtilis	(PDB:2IVE)	4.316	Van der Waals Carbonn Hydrogen Bond Halogen Alkyl; Pi-Alkyl
Myxococcus Xanthus	(PDB :3I6D)	6.067	Van der Waals Conventional Hydrogen Bond Carbonn Hydrogen Bond Halogen Pi-Donor Hydrogen Bond Alkyl; Pi-Alkyl
Human	(PDB:4IVM)	3.182	Van der Waals Conventional Hydrogen Bond Halogen Pi-Donor Hydrogen Bond Alkyl; Pi-Alkyl
Human	(PDB:4IVO)	4.249	Van der Waals Carbon Hydrogen Bond Alkyl; Pi-Alkyl

In the case of human PPO we find two structures, the crystal structure R59Q (PDB: 4IVM), and mutants R59G which are among the mutations that cause Variegated Porphyria (VP) disease-causing a 50% decrease in activity. from PPO.

According to the results obtained for the human PPO target, it is observed that the affinity between PPO (R59Q) and the inhibitor is high (Score = 3.182) compared to that of PPO (R59G) (Score = 4.249). In the case of PPO (R59Q), we find the interactions of Van der Waals, the Conventional Hydrogen Bond (between fluorine and ARG: 62), the Halogen bond (between chlorine, the terminal carbon of the R4 substituent, benzene, and the residues: ALA: 162; LEU: 166; ARG: 97) **(Figure 11)**.

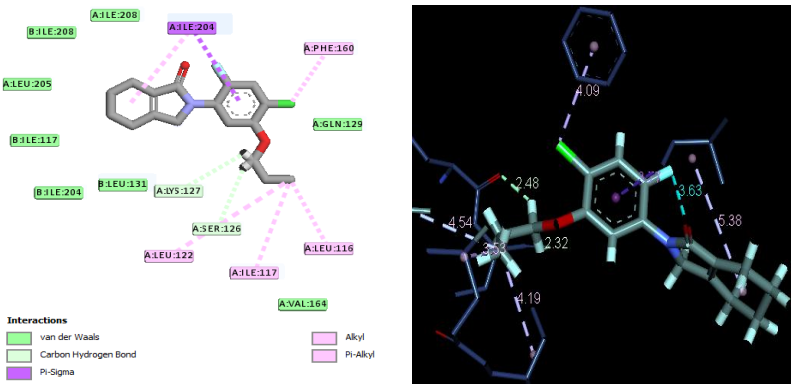


Figure 8. Interactions obtained concerning PPO of Nicotiana-tabaccum (PDB: 1SEZ)

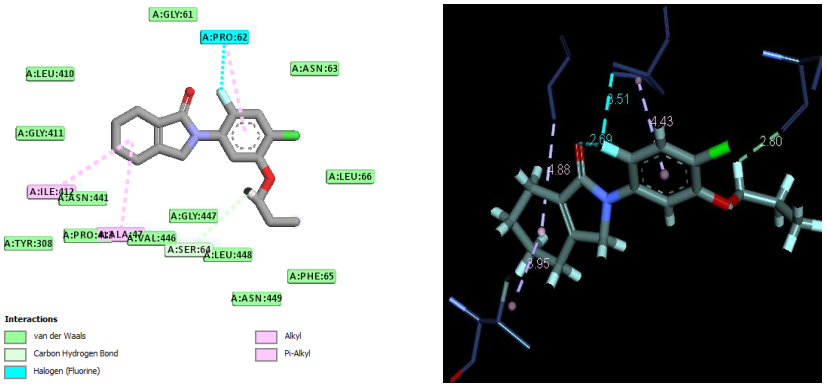


Figure 9. Interactions obtained concerning PPO of Bacillus Subtilis (PDB: 2IVE)

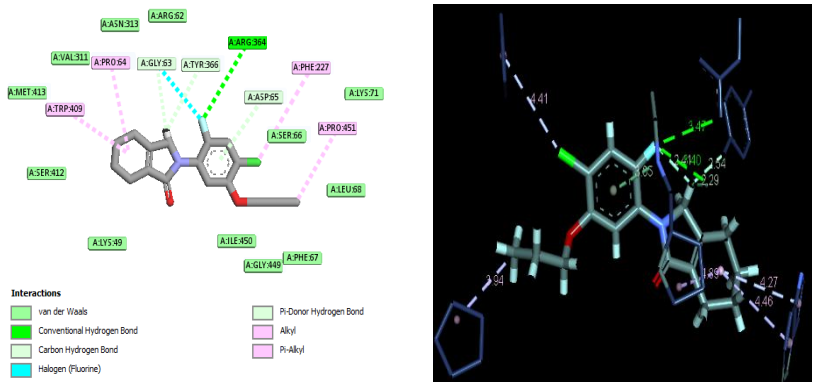


Figure 10. Interactions obtained concerning PPO of Myxococcus Xanthus (PDB: 3I6D)

While in the case of mutant PPO (R59G) we obtain the interactions Van der Waals, Carbon Hydrogen Bond (between the five-ring hydrogen and GLY: 169), Alkyl and Pi-Alkyl (between phenylphthalimide, benzene, chlorine, terminal carbon and residues: VAL (347,170); PHE: 331; LES (334,166); ILE: 419; ALA: 172) **(Figure 12)**. It is observed that the interactions of the crystalline structure are more important than those of mutants, due to the presence of hydrogen bond concerning the complex of PPO (R59Q).

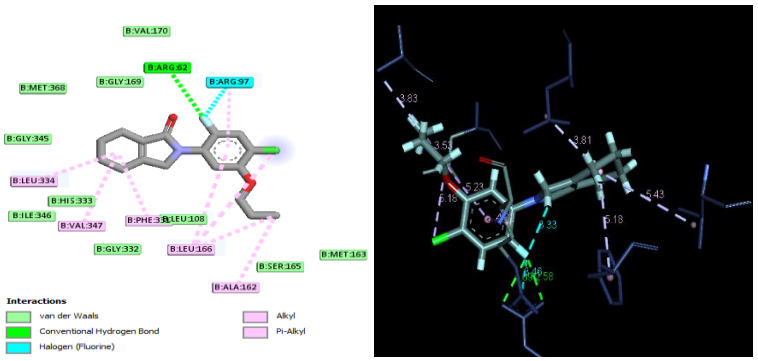


Figure 11. Interactions obtained concerning crystal structure (R59Q) (PDB: 4IVM)

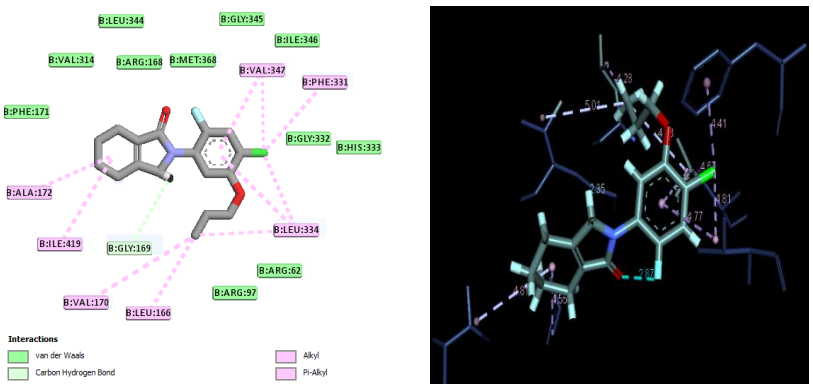


Figure 12. Interactions obtained concerning Mutant structure (R59G) (PDB: 4IVO)

1.3. MD simulations

RMSD measures the average variation of the displacement of the atoms containing the ligand relative to the receptor, the RMSD graph shows the evolution of the complex (compound C6 and Protoporphirinogen oxidase of human origin of the crystal structure R56Q (PDB: 4IVM)) during the time **(Figure 13)**. From the results obtained, it is observed that the ligand and protein values become close to 13 nsec.

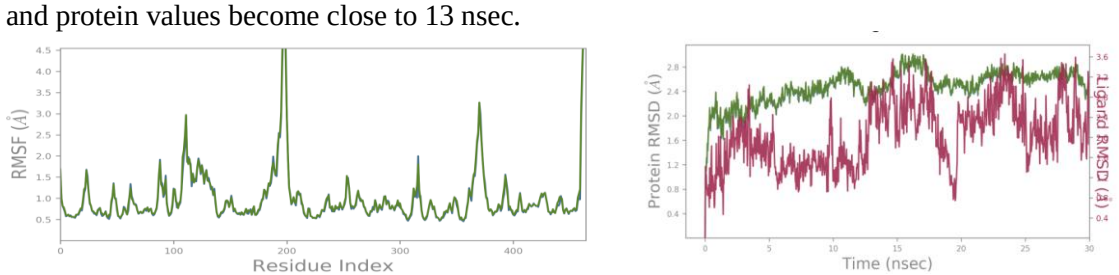


Figure 13. Molecular dynamics simulation (RMSD evolution of complex and the average square root of fluctuations of protein).

The complex fluctuates up to 20 n sec, after the dynamics of the complex become stable, indicating that the complex reaches equilibrium at this level, the ligand reaches equilibrium at 3.2 A°, while the protein reaches equilibrium at 2 A°, which shows the good stability of the complex [23]. From the fluctuation profile of the protein, we observe that there is not a great movement with an average RMSF of 2 A °, which shows the stability of the interactions between the C6 compound and the PPO protein of human origin (crystal structure R59Q).

Conclusion

In this work we used the inverse docking method, to study the inhibitory activity of the compound with the highest activity among 29 molecules, derived from N-phenylphthalimide. Thus, molecular dynamics makes it possible to validate or refine a position for reverse docking. The study is carried out on five PPO targets, from different origins: plant, bacterial and human origin. The results obtained generally show a good inhibitory capacity for the five targets.
A high affinity is obtained for the PPO of the plant Nicotiana tabaccum. Interesting interactions were obtained for the PPO of the bacterium Myxococcus Xanthus, and the crystalline structure of human PPO (R59Q), due to the existence of the hydrogen bond which stabilizes the complex. Molecular Dynamics simulation results confirm the stability of the complex, which may indicate that the results are satisfactory. We can state that human PPO (R59Q) is an ideal target to fight against several diseases, by the use of inhibitors derived from N-phenylphthalimide.

References

[1] R. J. Porra, J. E. Falk, "The enzymatic conversion of coproporphyrinogen III into protoporphyrin IX", *Biochem. J*, 90(1) (1964) 69–72. DOI: 10.1042/bj0900069.

[2] S. M. Gerald, "The biosynthesis of heme and chlorophyll", *Bot. ReV*, 32 (1966) 56–94.

[3] D. A. Brenner, J. R. Bloomer, "The enzymatic defect in variegate prophyria. Stuies with human cultured skin fibroblasts". *N Engl J Med*., 302(14) (1980) 765–769.

[4] Y. Nordmann, H. Puy," Human hereditary hepatic porphyrias". *Clin. Chim. Acta*., 325(2002) 17–37.

[5] G. Dean, "The porphyrias: A story of inheritance and environment"*2nd ed. Pitman Medical*: London, (1971) 1-70.

[6] T. Jenkins," The South African malady". *Nat. Genet.*,13 (1996) 7–9.

[7] J. M. Jacobs, N. J. Jacobs," Protoporphyrinogen oxidation, an enzymatic step in heme and chlorophyll synthesis: partial characterization of the reaction in plant organelles and comparison with mammalian and bacterial systems". *Arch. Biochem. Biophys*., 229 (1984) 312–319.

[8] S. Jung, Y. Lee, K. Yang, S. B. Lee, S. M. Jang, S. B. Ha, K. Back, "Dual targeting of Myxococcus Xanthus protoporphyrinogen oxidase into chloroplasts and mitochondria and high-level oxyfluorfen resistance" *Plant Cell and EnVironment*., 27 (2004) 1436–1446.

[9] G.C. Ferreira, H.A. Dailey," Mouse protoporphyrinogen oxidase—kinetic-parameters and demonstration of inhibition by bilirubin" *Biochem. J.*, 250 (1988) 597–603

[10] J. R. L. Walker, P. H. Ferrar, "Diphenol Oxidases, Enzyme catalyzed Browning and Plant Disease Resistance" *Biotechnol. Genetic Engg. Rev.*, 15 (1998) 457-498.

[11] V. Pilizota, D. Subaric, "Control of Enzymatic Browning of Foods, Food Technol" *Biotechnol.*, 36 (1998) 219.

[12] K. Kato, R. Tanaka, S. Sano, A. Tanaka," Hosaka, H. Identification of a gene essential for protoporphyrinogen IX oxidase activity in the cyanobacterium Synechocystis" *Natl. Acad. Sci.*, 107 (2010) 16649–16654.

[13] M. Hansson, L. Hederstedt, "Cloning and characterization of the Bacillus subtilis hemEHY gene cluster, which encodes protoheme IX biosynthetic enzymes" *J. Bacteriol.*, 174 (1992) 8081– 8093

[14] Dailey, T. A., Meissner, P., and Dailey, H. A. (1994) J. Biol. Chem. 269, 813– 815

[15] J. P. Rodriguez-Soto, D. Kaiser, "Identification and localization of the Tgl protein, required for Myxococcus Xanthus social motility" *J. Bacteriol*., 179 (1997) 4372–4381.
[16] J. P. Rodriguez-Soto, D. Kaiser, "The tgl gene: social motility and stimulation in Myxococcus Xanthus" *J. Bacteriol*. 179 (1997) 4361–4371.
[17] J. Hodgkin, D. Kaiser, "Genetics of gliding motility in Myxococcus Xanthus (Myxobacterales): genes controlling the movement of single cells" *Mol. Gen. Genet.* 171(1979) 167–176.
[18] S. S. Wu, D. Kaiser," Genetic and functional evidence that type IV pili are required for social gliding motility in Myxococcus Xanthus" *Mol. Microbiol*. 18 (1995) 547–558.
[19] S. S. Wu, J. Wu, D. Kaiser," The Myxococcus Xanthus pilT locus is required for social gliding motility although pili are still produced" *Mol Microbiol*. 23(1997) 109–21.
[20] O. Abdessadak et al, "2D-QSAR analysis on compounds (N-phenylpyrolidin-2-ones and N-phenyl-1H-pyrrol-2-ones) whose inhibitory activity of PPO (protoporphyrinogen oxidase)" *RHAZES: Green and Applied Chemistry.*, 9 (2020) 92-101
[21] H.Halima *et al.* Assessment of asthma treatment against SARS CoV-2 by using a computer approach. In: *E3S Web of Conferences*. EDP Sciences, 2021. p. 01024.
[22] H. Halima, et al. Antiproliferative Activity: Discovery of new Benzoxanthenes derivatives by Using Various Statistical Methods 2D/3D-QSAR and Molecular Docking. *RHAZES: Green and Applied Chemistry*, 12 (2021 40-59.
[23] H. Halima, T. Kamal, E. Fatima, et al. In Silico Investigation on the Beneficial Effects of Medicinal Plants on Diabetes and Obesity: Molecular Docking, Molecular Dynamic Simulations, and ADMET Studies. 2021.

(2022) ; https://revues.imist.ma/index.php/morjchem