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In silico Study of Thioguanine Derivatives As Hemopexin Matrix Metalloproteinase9 (Pex-9) Inhibitors

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Abstract

Matrix Metalloproteinase (MMP) was broadly studied as one of the protein targets in stopping angiogenesis. The most common target in MMP, i.e., the catalytic site, is reported as being the non-selective target for inhibition amongst all MMPs, but its inhibition is associated with adverse side effects. Hemopexin in MMP9 (PEX9) was found to be different to the other domains in the MMP family, which could be the next target for anticancer due to the availability of its crystal structure in the protein data bank (pdb). In this study, we design the analogues of a compound from the ZINC database bearing a pyrimidinone scaffold which previously showed activity as a PEX9 inhibitor using molecular docking. The docking is carried out using Autodock 4.0 with parameters such as 250 run and lammarckian genetic algorithm. The result shows that six designed compounds have a free energy of binding from -7.0 to -11.0 kcal/mol. The important amino acid residues which are involved in the hydrogen bond interaction are ARG106, GLU60, GLU14, and GLN154. In conclusion, the six design compounds could give an insight mechanism to inhibit the PEX-9 activity *in silico*.

Keywords: Docking, Hemopexin, MMP9, PEX9, Thioguanine

Uji Aktivitas Turunan Thioguanine Terhadap Hemopexin Matrix Metalloproteinase9 (Pex-9) *In silico*

Abstrak

Matrix Metalloproteinase (MMP) telah dipelajari sebagai salah satu target protein dalam menghentikan angiogenesis. *Catalytic site* yang merupakan bagian yang banyak ditarget dari MMP dilaporkan bersifat tidak selektif untuk dihambat sehingga menimbulkan efek samping. Hemopexin pada MMP9 (PEX9) ditemukan berbeda dengan domain pada MMP lain sehingga dapat digunakan sebagai target baru untuk antikanker karena ketersediaan struktur kristalnya di protein data bank (pdb). Pada penelitian ini, kami mendesain analog senyawa dari database ZINC yang mempunyai kerangka pyrimidinone yang pada penelitian sebelumnya ditemukan memiliki aktivitas menghambat PEX9 dengan *docking*. *Docking* dilakukan dengan menggunakan Autodock 4.0 dengan parameter seperti 250 run dan algoritma genetik lammarckian. Hasil menunjukkan enam senyawa memiliki energi ikatan sebesar -7.0 sampai -11.0 kcal/mol. Residu asam amino yang penting berinteraksi secara hidrogen dengan ligan antara lain ARG106, GLU60, GLU14, dan GLN154. Dapat disimpulkan bahwa keenam senyawa kemungkinan mempunyai mekanisme menghambat MMP9 pada bagian hemopexin domain *in silico*.

Kata Kunci: *Docking*, Hemopexin, MMP9, PEX9, Tioguanin

1. Introduction

Cancer is the second main cause of mortality in the world. In 2012, there were 14.1 million new cancer cases worldwide. Breast cancer is the most common case in female.[1] In 2013, breast cancer has been reported as one of the cancer diseases with the highest prevalence in Indonesia, i.e. 0,5%.[2] According to the World Health Organization (2014), the death caused by breast cancer in Indonesian female is quite high, i.e. 21.4%.[3]

Cancer is a complicated disease which is indicated by abnormal and uncontrolled cell growth caused by genetic mutation and therefore spread over from one to another organ. The expansion of cancer cells is named by metastasis which is the primary cause of death in cancer patients.[4] Welch, Steeg, & Rinker-schaeffer (2000) defined that metastasis of cancer cells to visceral organs and brain has been identified to danger and shortens the expectation of life up to 5 years with the probability is less than 20%.[5] Cancer metastasis causes death in 90% of people with it.[6]

Matrix metalloproteinases (MMPs) has an important rule in the invasion and metastasis of cancer cells. Instead, MMP also takes a role in angiogenesis.[7] This is an enzyme belonging to zinc-dependent endopeptidase working by degrading extracellular matrix (ECM). In human, there are 23 MMP subfamilies which are categorized in to 6 types based on their substrate specificity.[8]

Studies have been reporting the function of MMP in breast cancer using the ELISA method, that the level of MMP-9 has been indicated at a higher concentration than normal breast cells. In breast cancer, MMP-9 is found at the stage of development and metastasis associated with cancer cell proliferation, tumor invasion and mesenchymal epithelial transformation.[9] Overexpression of MMP-9 is also a marker of breast cancer with triple-negative and HER2 positive type.[10]

Naturally, MMP is controlled by tissue inhibitor for metalloproteinases (TIMP), however at breast cancer, the level of TIMP is insufficient to balance the MMPs activity.[7] Many MMP inhibitors such as marimastat, batimastat, periostat, etc. have been studied, unfortunately, none of them has successfully passed the third clinical trial. This is due to its unselective target leading to adverse side effect such as musculoskeletal syndrome.[11] The unselectivity is caused by a high homology among all MMPs particularly, in the catalytic domain by sharing more than 45% homology.[12] Interestingly there is one domain call as hemopexin which shares only 25% homology to all others.[12] Therefore hemopexin could be a more selective target than catalytic domain in the drug discovery of cancer by targeting MMP.

The previous study has found MMP hemopexin-9 (PEX-9) inhibitor based on pyrimidine and arylamide scaffold with Kd = 2,2 μM. Pyrimidine ring provides planar conformation that fits the deep pocket near blade four at the active site of PEX-9. On the other hand, arylamide with a methylene chain facilitates flexible conformation on the surface of the PEX-9 active site. In this study, we adopt pharmacophores mimics Dufour's compound by modifying the pyrimidine ring which is changed to purine ring. The purine ring we utilized is thioguanine which also provides planar conformation as well as Sulphur atom at Dufour's compound. In this present study, we perform computational study via molecular docking of thioguanine derivatives against the crystal structure of hemopexin MMP-9 (PEX-9) i.e. PDB 1ITV. The docking result will be analysed according to the energy of binding and molecular interactions. We hope that this study will contribute to the drug design for breast cancer by inhibiting PEX-9.

2. Methods

2.1. Equipment

ASUS Laptop with a 7th generation i7 intel processor, 8 GB RAM, and 1 TB harddisk; Discovery Studio 3.5 software; Autodocktools 1.5.6 software; Autodock 4.0 software; and Marvin Sketch 16.2.15 software

2.2. Materials

Crystal structure of PEX-9 (PDB 1TV) downloaded from protein data bank website

(www.rcsb.com)

2.3. Method

2.3.1. Validation with control docking

The crystal structure of PEX-9 (PDB 1ITV) is downloaded from protein data bank (www.rcsb.com) and then uploaded in Discovery Studio 3.5 (www.accelerys.com). The PEX-9 crystal structure is dimer therefore, one monomer is removed. The water is also removed and the sulphate ion is separated from the protein. Either protein or sulphate ion is saved in the pdb file. Both ligand and protein are prepared using Autodocktools 1.5.6 (www.scripps.edu) by adding Gasteiger and Kolmann for ligand and protein, respectively. The grid box is set to number of points 70 x 70 x 70 with spacing 0.375 Å and coordinate x = -42.053, y = -30.855 and z = -1.804. The docking is run using Autodock 4.0 with 250 iterations and lammarckian genetic algorithm. Other parameters use the default set in the software. The control docking is defined as good when the RMSD value toward the initial pose is less than 2.0 Å.

2.3.2. Docking of thioguanine ligand

On the other hand, the 2D structure of ligands is sketched using Marvin Sketch 16.2.15 and saved in mol file. The mol file ligands are prepared further in Discovery Studio 3.5 and then saved in pdb file. Both ligand and protein are prepared using the same parameters with the control docking above. The docking result is analysed by selecting the docking pose according to cluster, free energy of binding (ΔGbind) and molecular interaction with important amino acid residues.

3. Results

There have been six thioguanine ligands designed to be docked into PEX-9 binding site. Figure 1 illustrates the structure of the PEX-9 inhibitor and the thioguanine ligand adapted from it. The ligands have arylamide with a modification of diverse functional groups including sulphonamide, an ester of ethyl carboxylate, 3,4,5-trimethoxy, diazine, merazine, and methazine. Table 1 presents the result of docking based on ΔGbind and hydrogen bonds (H-bonds) interactions with important amino acid residues.

The control docking is evaluated by checking the RMSD value which is observed as 0.98 Å. The free energy of binding of sulphate ion is -5.0 kcal/mol. The amino acid residues have been observed in the molecular interaction with the sulphate ion is ARG10. Figure 2 illustrates the superposition of control docking pose and initial pose of sulphate ion into the PEX-9 binding site.

Ligand 1 has sulphonamide at the

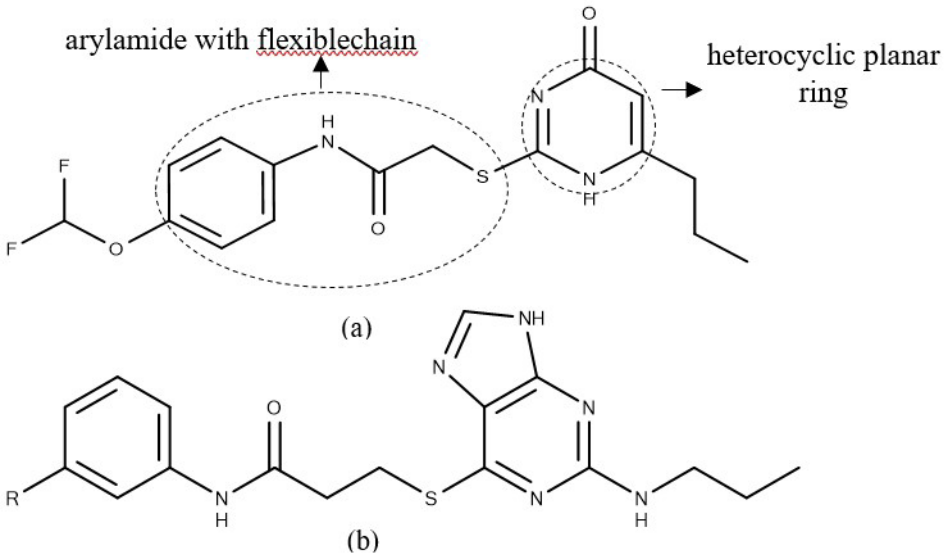
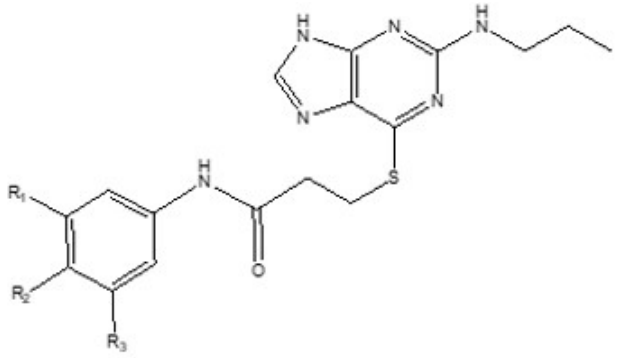
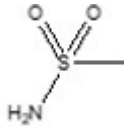
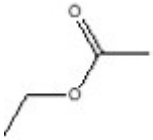
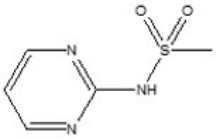
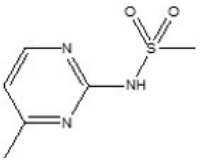
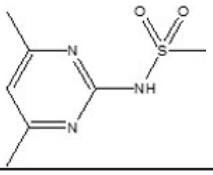


Figure 1. The structure of (a) PEX-9 inhibitor by Dufour et al., (2011) and (b) the thioguanine ligand adapted from (a) with R: (1) sulphonamide, (2) esther of ethyl carboxylate, (3) 3,4,5-trimethoxy, (4) diazine, (5) merazine, and (6) methazine

Table 1. The result of docking based on ΔGbind and molecular interactions with important amino acid residues



Ligands	R1	R2	R3	ΔGbind (kcal/mol)	H Bond
1	H		H	-11.40	GLU14, GLU154, GLU157
2	H		H	-10.04	GLU60, GLU14
3	OCH_3	OCH_3	OCH_3	-8.37	ARG106, GLU157, GLU14, GLU60
4	H		H	-8.79	ARG106, ASN17, GLN154, GLU157, GLY16
5	H		H	-8.21	GLU14, ASN17
6	H		H	-8.83	GLU157, GLU 154, GLU14

p-position of arylamide possessing H-bond interaction with GLU14 at its N-sulphonamide, GLN154 at H-sulphonamide and H-imidazole ring, whereas the N-guanine interacts with GLU157. Ligand 2 bearing ester ethyl benzoate at its arylamide p-postion by exhibiting similar binding mode at GLU14 with H-N propyl. One extra H-bond which is different from ligand 1 is at GLU60 interacting with at O-carbonyl of the ester group. Figure 3 illustrates the molecular interactions of ligand 1 and 2 at the binding site of PEX9.

Ligand 3 employs 3,4,5-trimethoxy at the R1, R2, and R3 at the arylamide p-position demonstrating extremely different binding pose with the previous two ligands. The thioguanine ring which is commonly attracted to the site nearby GLN154 and GLU157 flips away from there to bind to the site surrounded by GLU14 and GLU60. Ligand 4 as it has sulphonamide attachment at its arylamide p-position, also has an extra pyrimidine ring

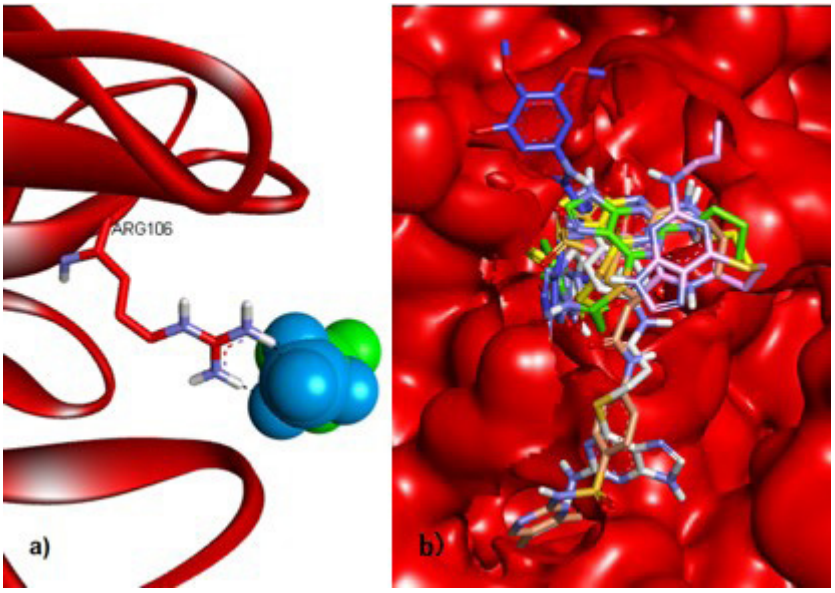


Figure 2. The superposition of a) control docking pose and initial pose of sulphate ion and b) the six thioguanine ligands into PEX-9 binding site

which gives additional H-bond interactions with GLY16, ASN17 and GLU157, instead of ARG106 and GLN154. Figure 4 illustrates the binding pose of ligand 3 and 4 at the binding site of PEX9.

Ligand 5 and 6 have a similar structure with ligand 4, but ligand 5 has an extra methyl group attached at its pyrimidine ring 4-position. Ligand 5 has H-bond interaction with GLU14 and ASN17. Ligand 6 has two extra methyl groups attached at 4,6-position. Ligand 6 has H-bond interaction with GLU157, GLN154, and GLU14. Figure 5 illustrates the binding pose of ligand 3 and 4 at the binding site of PEX9.

4. Discussion

The idea to create thioguanine as the ligand is inspired by the study of Dufour et al., (2011) and Alford et al., (2017).[12,13] Dufour reported earlier that compound bearing pyrimidine and arylamide connected by flexible ethylene chain contributes to the binding into PEX-9 binding site. The pyrimidine is reported for its binding into the PEX-9 due to the six-member heterocycle ring which provides a planar conformation that fits into the cavity of PEX9 domain blades. Besides, the arylamide moiety facilitates a more flexible conformation, which bound to the surface near to the cavity.

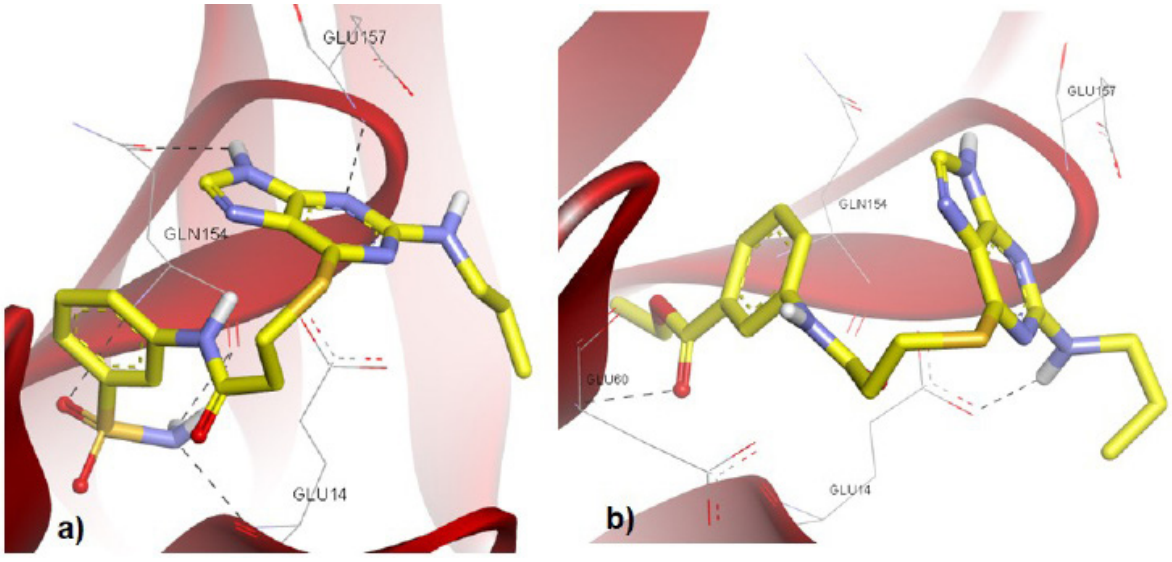


Figure 3. The molecular interactions of a) ligand 1 and b) 2 at the binding site of PEX9

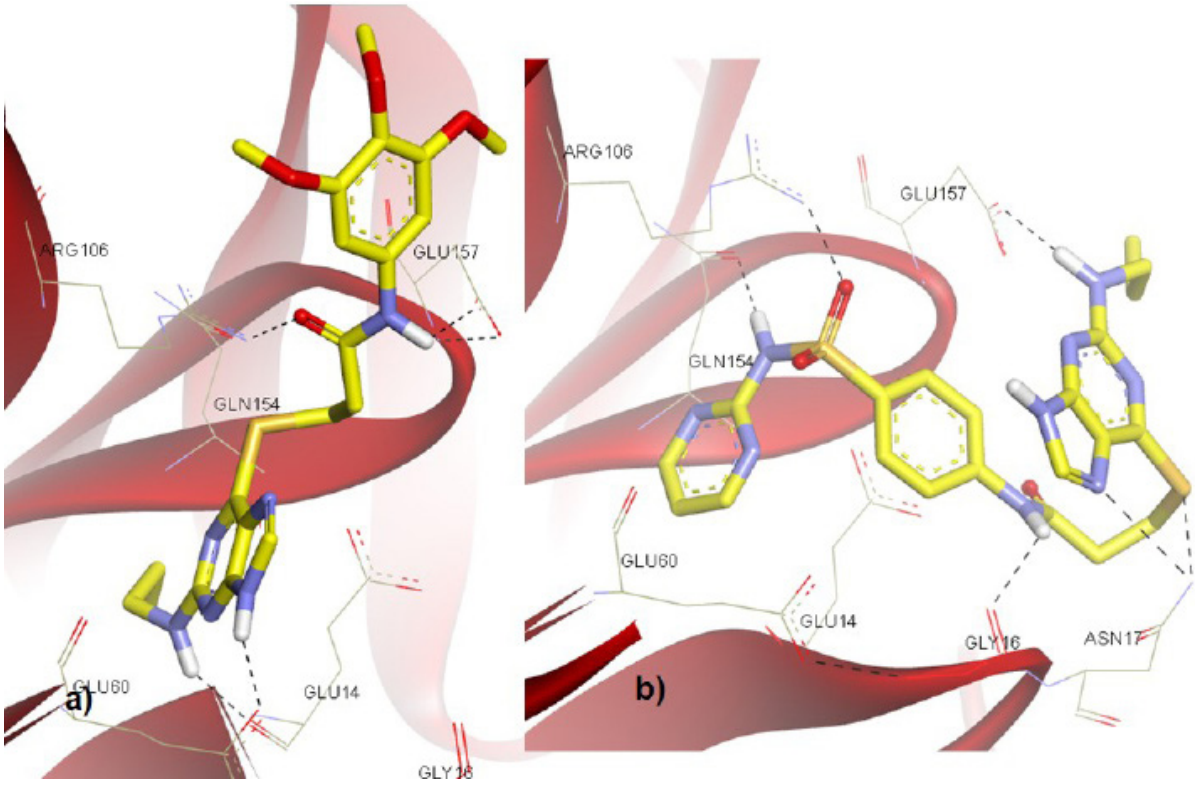


Figure 4. The molecular interactions of a) ligand 3 and b) 4 at the binding site of PEX9

The parameter of docking as well as the quality of crystal structure are defined as valid because it is less than 2 Å (0,98 Å). The free energy of binding of sulphate ion is -5.0 kcal/mol defining the affinity of the ligand with the protein. The amino acid residues have been observed in the molecular interaction with the sulphate ion is ARG10 indicating the importance of this amino acid residue in the PEX9 binding site. This describes the capability of the crystal structure to be used as the protein model to predict the new ligand proposed for the PEX-9 inhibitor.

Thioguanine is used for the planar ring as it is similar to the character of pyrimidine by Dufour's hit compound. Among the six

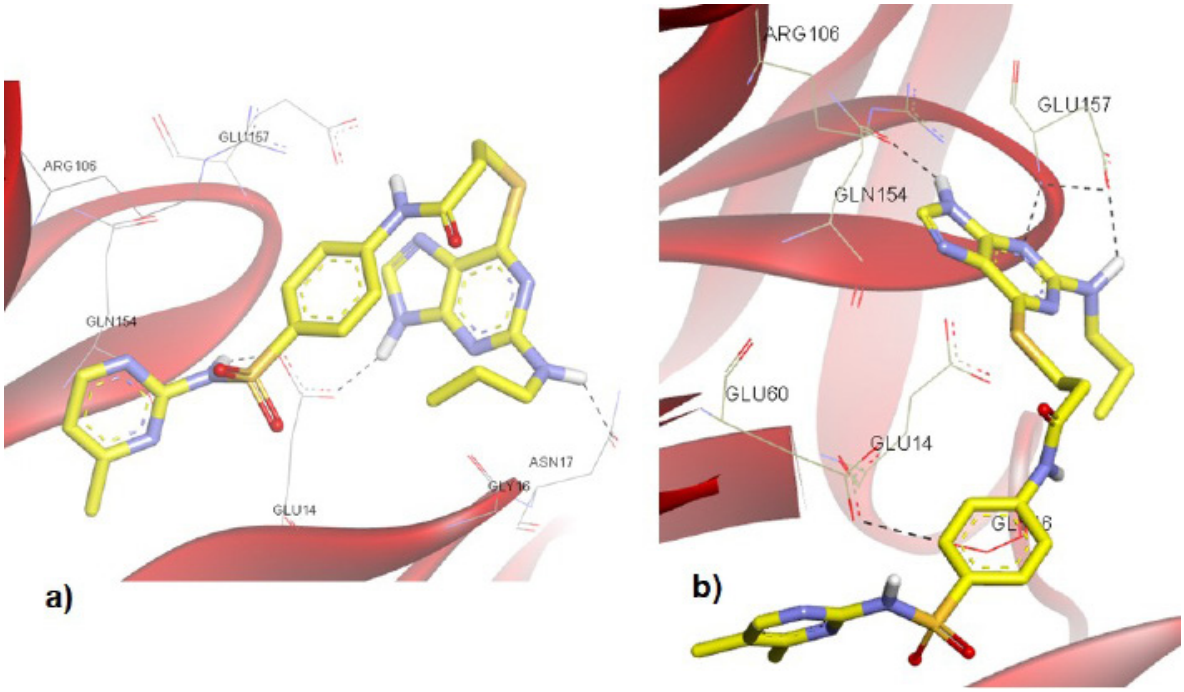


Figure 5. The docking poses of a) ligand 5 and b) ligand 6 into the binding site of PEX-9

ligands, the one having the lowest ΔGbind is ligand 1 associating with its highest capacity of the ligand to bind the PEX-9 binding site. The second lowest ΔGbind goes to ligand 2. Ligand 3 attracted to the site nearby GLN154 and GLU157 flips away from there to bind to the site surrounded by GLU14 and GLU60. These interactions contribute to the ΔGbind of ligand 3 become higher than five other ligands. This could be affected by the different character of R groups being attached in arylamide. Methoxy group is totally electron donating group (EDG) which is different from two previous ligand, which employs dominantly electron withdrawing group (EWG). However, one of similar interaction with that of control docking is the interaction between O-carbonyl amide with ARG106 associating with potential interaction with the corresponding protein.

Ligand 4 does a similar pose with that of ligand 1. An extra pyrimidine ring here gives additional H-bond interactions with GLY16, ASN17 and GLU157, instead of ARG106 and GLN154 are assigned as important ones. The ΔGbind of ligand 4 is lower than ligand 3, however this is still higher than ligand 1 and 2. Interestingly, the pose of thioguanine ring, and arylamide combined with sulphonamide pyrimidine is similar to that ligand 1. The last two ligands (5 and 6) are the extention of ligand 4 by attaching 4-methyl group at ligand 5 and 4,6-dimethyl group at ligand 6. The pose of thioguanine ring is roughly similar where GLN154 and GLU157 being surrounded and the arylamide poses surround GLU14 and GLU60. The most difference between them is the absence of interaction with ARG106 in ligand 6 associating with its higher ΔGbind because this kind of interaction is predicted to have the contribution in protein-ligand binding.

5. Conclusion

There have been six ligands bearing thioguanine as the scaffold designed as PEX-9 inhibitor. The thioguanine having planar character is expected to interact with a deep pocket of PEX-9 surrounded by GLU154 and GLN 157, whereas the aryamide ring connected by ethylene chain to the thioguanine is expected to bind to the shallow pocket near the surface surrounded by GLU14 and GLU60 of PEX-9. In addition, the extention of the diverse functional group at the p-position of the arylamide ring is aimed to give extra binding to the enzyme. The free energy of binding for all six ligands are at a range -11.10 to -8.21 associating with its capability to strongly bind to PEX-9. Further studies should chemically synthesize these six ligands to be proven its in silico prediction by in vitro evaluation.

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