

Computational Docking Experiments to Find a Ligand that Will Bind to Xanthine Oxidase

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Abstract

This project was to seek a uric acid derivative compound that will be able to bind XOD with high affinity, which could possibly be used as an inhibitor to the XOD activity. A computer was used to design several compounds and the program Autodock Vina was used to perform docking of those compounds to see if they can bind XOD. The uric acid structure contains a six-membered and a five-membered rings fused together with three carbonyl groups on the periphery. We sequentially replaced each peripheral carbonyl group by a sulfur atom (a less polar atom), followed by an aldehyde and a carboxylic acid groups (more polar groups) to obtain different derivatives of uric acid. The values of affinity energies for the three derivatives with all three peripheral carbonyls were replaced by either three sulfur atoms, three aldehydes or three carboxylic acids are -3.6 kcal/mol, -1.2 kcal/mol and 0.70 kcal/mol, respectively. The results obtained showed that the sulfur derivative (#2/#6/#8) had higher affinity than the other ones. However, the carboxylic acid derivatives (most polar group) generally should have higher affinity than the aldehydes and sulfur derivatives because sulfur atoms usually are not as polar as oxygen atoms.

Introduction

Xanthine Oxidase (XOD) is an enzyme that converts purines to hypoxanthine and then to uric acid, a metabolic waste product (see Fig. 1). Its structure contains two subunits, each containing a molybdopterin group and one flavin adenine dinucleotide group (1). When the activity of XOD is accelerated, the level of uric acid increases and high level of this can lead to a build up of urate crystals in a person's joint, leading to the cause of gout disease.

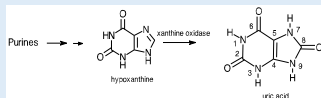


Figure 1. Oxidation of purines to produce uric acid (4).

When uric acid is newly produced by the XOD, it binds to the active side of the enzyme via its peripheral oxygen atoms and the hydrogen atoms at the five member ring. The C2 double bond oxygen atom forms hydrogen bonds to the side chain of Arginine-880 and to the NH of threonine-1010. The C8 double bond oxygen atom forms a hydrogen bond to the NH of Alanine-1079. The hydrogen atoms of N7 and N9 form hydrogen bonds to the side chains of glutamate-802 and glutamate-1261, respectively. In addition, the purine ring of uric acid has hydrophobic interactions with the side chain of phenylalanine-914, for having its ring being bound in parallel to that of the aromatic ring of phenylalanine-914 (see Fig. 2).

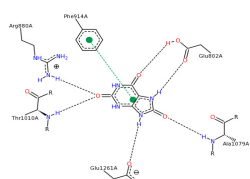


Figure 2. Interactions of uric acid with various groups of XOD lining the active site (4).

This study examines the different derivatives of uric acid to determine which compound can bind to the active site with the same or greater strength as the original uric acid ligand. This was done by computational docking using the Autodock Vina software (2). Also this project aims to predict which derivative compound has the highest affinity, best mode of bonding and similar or same kinds of interactions as uric acid.

Objective

The purpose of this study is to use computational technique to perform "docking" of multiple compounds with structures similar to Uric Acid that could bind to XOD and possibly be an inhibitor. Table 1 shows the substituent groups at position C2, C6 and C8 on the uric acid ring that were used in this project.

C2	C6	C8
=S	=O	=O
=O	=S	=O
=O	=O	=S
=S	=S	=S
-CO2	=O	=O
=O	-CO2	=O
=O	=O	-CO2
-CO2	-CO2	-CO2
-COH	=O	=O
=O	-COH	=O
=O	=O	-COH
-COH	-COH	-COH

Methods

All derivatives for this study were manually created with the Spartan software, using the PDB file of XOD with Uric Acid downloaded from Protein Data Bank. Only one subunit containing one active site was used for docking. The other subunits and groups associated with the XOD structure were deleted. The native uric acid structure was obtained by deleting everything but the uric acid atoms. Uric acid derivatives results were obtained by using Autodock Vina software (2). Docking techniques was used to determine mode of binding, interactions, root-mean-square (RMSD) and the binding energy value.

Size	Center
x=12	x=-70.094
y=12	y=-25.821
z=12	z=-39.822

Figure 5. Grid box size used for running Autodock Vina for results.

Results

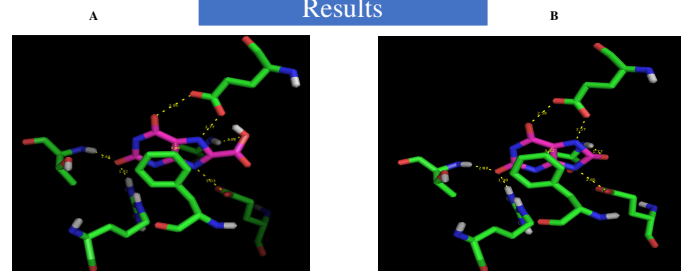


Figure 5A,B. (A). The interactions of Carboxylic Acid derivative #8 (best derivative) compared to those of the original ligand, bound Uric Acid (B) (see Fig 2). Very similar bond lengths (angstrom) and types of interactions resulted, but Carboxylic Acid structure #8 has slightly different values than those of the original ligand.

Results

Below are results for best fit derivative ligands of Sulfur, Carboxylic Acid and Aldehyde substituents that replaced the double bond Oxygen atom on C2, C6, C8 and C2-6-8. Although some results show a significant difference of atomic positions from the original ligand. The best fit derivative does not necessarily mean it is the ligand that will have the exact interactions as the native ligand, but it is the best structure for where it is positioned relative to the bound uric acid.

Figure 4A

Molecules	Energy (kcal/mol)	Structure (best fit)	RMSD
Sulfur #2	-3.7	Mode #8	2.166, 2.715
Sulfur #6	-4.9	Mode #3	2.671, 4.400
Sulfur #8	-3.4	Mode #9	1.400, 2.876
Sulfur #2-#6-#8	-3.6	Mode #1	0, 0

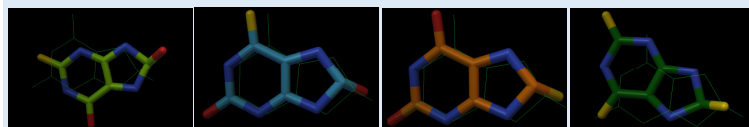


Figure 4B

Molecules	Energy (kcal/mol)	Structure (best fit)	RMSD
Carboxylic Acid #2	-3.4	Mode #5	2.038, 4.873
Carboxylic Acid #6	-4.8	Mode #1	0, 0
Carboxylic Acid #8	-6.7	Mode #1	0, 0
Carboxylic Acid #2-#6-#8	-0.7	Mode #4	2.369, 4.360

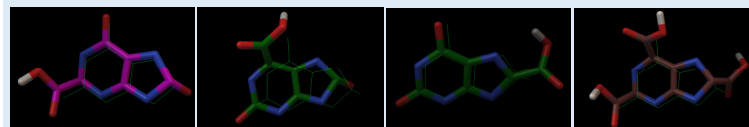


Figure 4C

Molecules	Energy (kcal/mol)	Structure (best fit)	RMSD
Aldehyde #2	-4.6	Mode #6	2.145, 2.906
Aldehyde #6	-4.9	Mode #2	1.916, 4.420
Aldehyde #8	-3.2	Mode #9	1.654, 3.067
Aldehyde #2-#6-#8	-1.2	Mode #9	1.966, 3.839

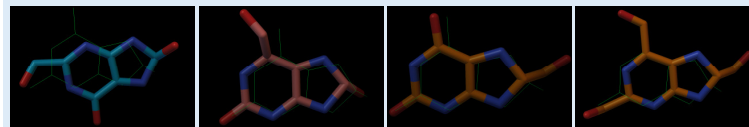


Figure 4A-B-C: Displays of best fit derivatives of different functional groups (Sulfur, Carboxylic Acid, Aldehyde) at C2, C6 and C8 separately, and the single structure with the same functional group at all three positions at C2-6-8. Thick bonds is the best fit derivative overlaid with original ligand Uric Acid (line bonds).

Figure 4D

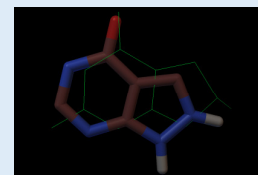


Figure 4D. Docking results of known inhibitor, allopurinol, of Xanthine Oxidase. Energy, best fit mode and RMSD are shown below.

Molecule	Energy	Best fit	RMSD
Allopurinol	-5.7	Mode #4	2.160, 2.917

Conclusions

It is concluded that possibly Carboxylic Acid #8 has the best ability to bind to XOD, but is positioned slightly different from the original ligand. Allopurinol, which is a known inhibitor of XOD, has an energy value of -5.7 kcal/mol and is positioned significantly different than the original ligand (Fig. 4D). However, the Carboxylic Acid #8 (derivative) has a significantly higher affinity value (-6.7 kcal/mol) than the allopurinol and is positioned just slightly different from the original ligand. This suggests that Carboxylic Acid #8 derivative might possibly be an inhibitor of XOD. Further testing and docking will be required in the future to prove this hypothesis.

References

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