Computational Docking of Uric Acid and Allopurinol Derivatives That Can Bind to Xanthine Oxidase

Lysengkeng Her and Thao Yang
Department of Chemistry, University Wisconsin-Eau Claire, Eau Claire WI
Celebration of Excellence in Research and Creative Activity (CERCA) May 3rd to 4th 2017

Abstract

This project is to seek a uric acid and allopurinol derivative compound that will be able to bind XOD with high affinity, which could possibly be used as an inhibitor against the XOD activity. A computer was used to design several compounds and employed the program Autodock Vina to perform dockings 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. The allopurinol structure also contains the two membered rings as uric acid but instead only has a single carbonyl group attached to it. We sequentially replaced each peripheral carbonyl group by a sulfur atom (a less polar atom), follow by an aldehyde, a fluorine and carboxylic acid groups (more polar groups) to obtain different derivatives of uric acid. Oxygen atoms and fluorine atoms were also added to allopurinol on the peripheries. The results showed that the most polar groups, carboxylic acid and fluorine should have a higher affinity than the other groups. After the analysis of data and results we concluded that for the uric acid derivative the carboxylic#8' and the allopurinol derivative fluorine#2',6' could be potential inhibitors for XOD.

Introduction

Xanthine Oxidase (XOD) is an enzyme that converts purines to hypoxanthine 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. Gout disease may be treated with allopurinol but may cause serious side effects.

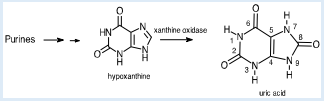

Figure 1. Oxidation of purines to produce uric acid (4).

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 membered 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 C6 double bond oxygen atom forms hydrogen bonds to the side chain of Glutamate-802. 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)**.

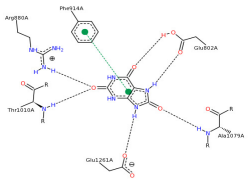

Figure 2. Interactions of uric acid with various groups of XOD lining the active site (4).

This study examines the different derivatives of uric acid and allopurinol compared to the original docked structures to determine which compound can bind at the active site similar to the native ligand with either the same strength or greater strength. 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 and Allopurinol that could bind to XOD and possibly be potential inhibitors. Docked derivatives with high affinity, low RMSD values, similar mode of bonding and similar interactions were examined by using Pymol (3).

Molecule	Affinity	Kd (μM)
Docked urate	-5.4	116.4
Docked Allopurinol	-5.7	70.41
-CO_2#8'	-6.7	13.16
-F#2',6'	-6.4	21.76

Table 1: Kd value was calculated using $\Delta G = RTlnKd$, R=.001987kcal/K*mole, and T=300K.

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 is used for docking. The other subunits and groups associated with the XOD structure were deleted. The native uric acid structure is obtained by deleting everything but the uric acid atoms (e.g. atom coordinates). The structures of docked Uric acid or Allopurinol derivatives were obtained by using Autodock Vina software (2). Docking techniques was used to determine mode of binding, interactions, root-mean-square deviation (RMSD) and the binding energy value.

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

Table 2. Grid box size used for running Autodock Vina for results.

Results

Molecule	Energy (kcal/mole)	Structure (best fit)	RMSD
Docked Urate	-5.4	Mode #7	1.974, 4.007
Docked Allopurinol	-5.7	Mode #4	2.160, 2917

Table 6. Affinity, best fit, and RMSD values of docked Urate and docked Allopurinol.

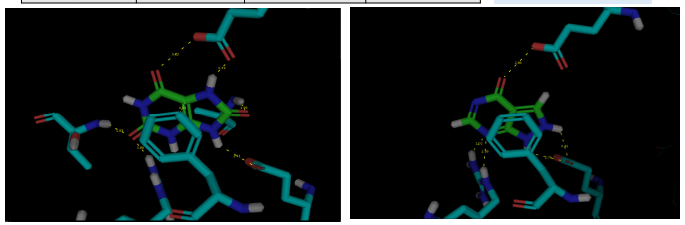

Figure 4A.B. (A). The interactions of original docked uric acid with hydrogen bond lengths shown (best fit). **(B).** Interactions of docked Allopurinol with hydrogen bond lengths displayed (best fit).

Results

Below are results for Urate derivatives with Sulfur, Carboxylic Acid, Aldehyde and Fluorine substituents that replaced one double bond oxygen atom on either C2, C6, or C8, and replacing all three double bond oxygen atoms at three carbon positions, denoted as C2-6-8. Also for Allopurinol derivatives with Oxygen, and Fluorine substituents replacing the double bond Oxygen on C2, or added on the C6, and C7 position. The best fit derivative does not necessarily mean it is the ligand that will have the exact interactions as the native ligand, but is the best structure for which it is positioned relative to the original docked structure.

Table 3A: Urate Derivatives (refer to Figure 1)

Molecules	Affinity Energy (kcal/mole)	Structure (best fit)	RMSD
=S#2'	-3.7	Mode #8	2.166, 2.715
=S#6'	-4.9	Mode #3	2.671, 4.400
=S#8'	-3.4	Mode #9	1.400, 2.876
=S#2'-#6'-#8'	-1.1	Mode #7	1.621, 4.027
-CHO#2'	-4.6	Mode #6	2.145, 2.906
-CHO#6'	-4.9	Mode #2	1.916, 4.420
-CHO#8'	-3.2	Mode #9	1.654, 3.067
-CHO#2'-#6'-#8'	-1.2	Mode #9	1.966, 3.839
-CO_2#2'	-3.4	Mode #5	2.038, 4.873
-CO_2#6'	-4.8	Mode #1	0.000, 0.000
-CO_2#8'	-6.7	Mode #1	0.000, 0.000
-CO_2#2'-#6'-#8'	-0.7	Mode #4	2.369, 4.360
-F#2'	-4.8	Mode #7	2.285, 2.928
-F#6'	-5.7	Mode #3	1.999, 4.012
-F#8'	-4.5	Mode #8	1.819, 3.328
-F#2'-#6'-#8'	-5.1	Mode #8	1.897, 3.962

Table 3B: Allopurinol Derivatives (refer to Figure 5)

Molecule	Affinity Energy (kcal/mole)	Structures (Best fit)	RMSD
=O#7'	-5.6	Mode #6	2.576, 4.329
=O#6',7'	-5.8	Mode #7	2.255, 2.896
=O#2',6',7'	-6.1	Mode #8	1.641,4.324
-F#6'	-6.0	Mode #3	2.250, 2.908
-F#2' =O#6'	-5.7	Mode #6	2.463, 3.122
-F#7' =O#6'	-5.5	Mode #8	2.377, 3.002
-F#7'	-5.8	Mode #6	1.799, 2.583
-F#2',7'	-6.1	Mode #5	1.755, 2.457
-O#1,3	-5.9	Mode #3	1.868, 4.491
-O#1,3 -F#6'	-6.2	Mode #2	2.312,2.999
-O#1,3, =O#7' -F#2',6'	-6.2	Mode #6	3.066,4.654
-O#1,3, =O#6 -F#2',7'	-6.1	Mode #7	2.238,3.094
=O#7', -F#2',6'	-6.2	Mode #6	2.614,4.505
=O#2',7' -F#2'	-5.7	Mode #7	2.893,4.476
-F#2',6'	-6.4	Mode #4	1.995,3.148

Table 1A-B: (A) Data of best fit derivatives of different groups for Urate (Sulfur, Carboxylic Acid, Aldehyde) replaced on C2, C6, C8 and C2-6-8. **(B)** Best fit derivatives for Allopurinol derivatives (Oxygen, Fluorine) replaced on C2, C6 and C7. Prime symbol (') on each number represents the peripheral atom that is bonded to the specified numbered carbon atom on the ring (e.g. =O#7', a double bond Oxygen bonded to C#7 on the five membered ring).

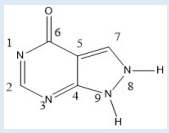

Figure 3: Allopurinol structure with atom positions numbered.

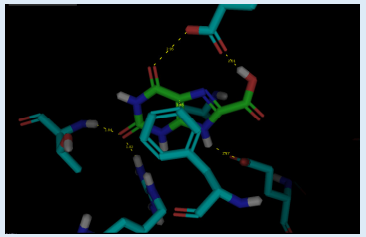

Figure 5: Docked result of Carboxylic #8' (Pink color), with hydrogen bond interactions and lengths displayed (angstroms). This docked derivative has very similar interactions compared to the original urate structure (see Figure 6A). It only has one different interaction which involves the hydrogen bond of the carboxyl group to the Glu-802A residue.

Table 4. hydrogen bonds with distances for carboxylic#8' in angstrom.

Hydrogen bond	Å
=O#6'- Glu802	2.95
=O#2'- Thr1010	1.84
=O#2'- Arg880	1.82
-NH#9'- Glu1261	2.27
-CO_2#8'- Glu802	2.01
distances between the rings =C#5- Phe914	3.46

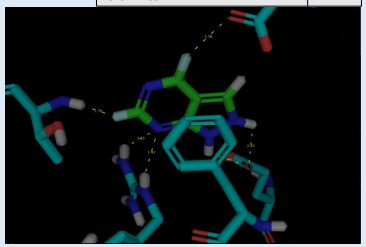

Figure 6: Docked result of Allopurinol F#2',6' (Green color) with hydrogen interactions and lengths displayed (angstroms). The docked Allopurinol derivative has similar interactions compared to the original docked Allopurinol (Figure 6B). It only has one different interaction, which is the hydrogen bond of the Fluorine#2' to the NH of Thr-1010A residue.

Table 5. hydrogen bonds with distances for Allopurinol F#2',6' in angstrom.

Hydrogen Bond	Å
-F#6'- Glu802	2.76
-F#2'- Thr1010	1.76
-N#3- Arg880	2.41
-N#3- Arg880	2.16
-NH#8'- Glu1261	2.43
-NH#9'- Glu1261	2.25

Table 7. Urate hydrogen bonds and distances between the rings in angstrom

Urate	
Hydrogen bond	Å
=O#6'-Glu802	3.12
=O#2'-Thr1010	1.83
=O#2'-Arg880	1.80
-NH#9'-Glu1261	2.41
-NH#7'-Glu802	1.73
=O#8'-Ala1079	1.59
distances between the rings =C#5-Phe914	3.44

Table 8. Allopurinol hydrogen bonds with distances in angstrom.

Allopurinol	
Hydrogen bond	Å
=O#6'-Glu802	2.86
-N#3-Arg880	2.01
-N#3-Arg880	2.39
-NH#8'-Glu1261	2.41
-NH#9'-Glu1261	2.76

Conclusions

It is concluded that possibly the uric acid derivative carboxylic #8' could have the ability to bind to XOD, because of it's similar interactions and higher affinity than the original uric acid docked structure. The derivative fluorine#2',6' for allopurinol also shows potential binding to XOD because of it's similar interactions to the docked allopurinol structure, and higher affinity. This suggests that the uric acid Carboxylic Acid #8 derivative and allopurinol derivative fluorine#2',6' may possibly be good inhibitors of XOD.

References

1. Okamoto, K., Kawaguchi, Y., Eger, B.T., Pai, E. F., and Nishino, T. Crystal Structures of Urate Bound Form of Xanthine Oxidoreductase: Substrate Orientation and Structure of the Key Reaction Intermediate. J. Am. Chem. Soc. 132, 17080-3 (2010). **DOI**: 10.1021/ja1077574
2. O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry* 31, 455-461, (2010).
3. The PyMol Molecular Graphics System, Version 1.8, Schrodinger, LLC. http://pymol.org/educational
4. Image from the RCSB PDB website (www.rcsb.org), Bovine Xanthine Oxidoreductase Urate Bound Form, PDB ID: 3AMZ. Authors are those of reference #1.

Acknowledgements

I would like to thank the UWEC Office of Research and Sponsored Programs (ORSP) for providing the Diversity Mentoring Program grant to support this research, the UWEC Chemistry Department for use of the Autodock Vina software, and also the Blugold Commitment for printing this poster.