Computational Docking of Caffeine Derivatives and Binding to Xanthine Oxidase

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Abstract

Building off of previous research on XOD ligand docking, the purpose of this project is to seek a caffeine derivative compound structurally similar to Urate and Allopurinol that would exhibit a similar binding mechanism to XOD with high affinity. These modified caffeine molecules could potentially serve as an alternative drug compounds to Allopurinol, an effective inhibitor for XOD. Standard caffeine structure consists of a six-membered and five-membered ring with two carbonyl and three methyl groups directed externally. Our derived caffeine ligands consisted of aldehyde and carboxylic acid side groups replacing the standard methyl groups. Carboxylic and aldehyde side groups were chosen to promote greater polar interactions within the XOD active site. Computational ligand docking to XOD was performed via the Autodock Vina program and ligand analysis focused on ligand binding affinity in comparison to standard Urate values. Our findings indicate that a standard caffeine molecule exhibits poor binding and low affinity to XOD in comparison to Urate. However, a caffeine derivative with a lone carboxylic acid side group and no methyl groups displays a high affinity value comparable to that of Urate and Allopurinol values.

Introduction

Xanthine Oxidase (XOD) is a two-subunit enzyme that is involved in a multitude of metabolic reactions in many mammals, including humans. The structure of XOD is a 290 kDa homodimer, consisting of a molybdenum ion cofactor and an FAD coenzyme.[1] Most notably, XOD is a perpetrator of the development of Gout, a type of arthritis which is characterized by an overproduction of Uric Acid (**Fig. 2**) in the bloodstream. This reaction is facilitated by a low tissue pH and leads to the formation of Uric Acid crystals in inflamed tissues surrounding the joints, giving the typical swollen, red symptom. The mechanism for Uric Acid production is catalyzed by a purine degradation reaction within the XOD active site, in which food-derived purines are converted to hypoxanthine and then Uric Acid.[1] In the XOD active site, there are several key amino acid side chains that participate in the Uric Acid production reaction. Glutamate-802, Glutamate-1261, Threonine-1010, Arginine-880, and Alanine-1079 all have a role in the Uric Acid mechanism through hydrogen bond interactions (**Fig. 1**).

This condition can be treated with the reducing drug molecule, Allopurinol, which inhibits XOD activity and subsequently decreases Uric Acid production. Allopurinol is an analog of the intermediate hypoxanthine, which most likely contributes to its inhibition mechanism within the XOD active site.[2] However, while Allopurinol is an effective drug molecule to combat Uric Acid overproduction, there are some serious side effects. For this reason, we are searching for an alternative molecule that could potentially inhibit XOD action. Interestingly, Caffeine shares a similar dicyclic ring structure with Uric Acid (**Fig. 3**). Native Caffeine has a 6-membered and 5-membered ring with two carbonyl and three peripheral methyl groups bonded to nitrogen at positions 1, 3, and 7. Furthermore, we can derive alternative Caffeine molecules by replacing the methyl groups with more polar substituents, such as carboxylic acids, aldehydes, and lone hydrogens to facilitate more interactions within the XOD active site. Thus, we suspect that due to the structural similarity to Uric Acid, Caffeine and subsequent Caffeine Derivatives could display a similar binding mechanism with comparable affinity and could serve as a possible inhibitor to XOD as with Allopurinol.

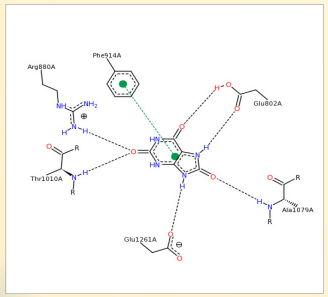

Figure 1. Uric Acid interaction mechanism with amino acid side chains of the XOD active site.[5]

Purpose

This purpose of this study is to use a computational docking approach to evaluate several modified caffeine ligands as potential inhibitors to XOD. Caffeine derivatives that display a similar binding mechanism to Urate and Allopurinol were analyzed for XOD active site interactions, low RMSD values, similar bonding orientation, and high affinity.

Methods

For this study, Xanthine Oxidase (XOD - 3AMZ)[4] and standard uric acid PDB files were obtained from Research Collaboratory for Structural Bioinformatics: Protein Data Bank (RCSB: PDB)[1]. Allopurinol, caffeine, and caffeine derivative ligand PDB files were built using Spartan '18 V1.2.0. To prepare for data collection, a single XOD subunit structure was isolated, and the associated functional group and coenzymes were removed. Computational docking to a native XOD subunit was conducted using the AutodockTools-1.5.6 program[4], and docking visualization was performed using PyMOL + Tcl-Tuk GUI + Console.[3] To calibrate the AutodockTools software, ligand docking coordinates were calculated (**Table 2**). These programs were used to determine binding mode, XOD active site interactions, and binding affinity values (kcal/mole). Root-mean-square deviation (RMSD), which served as a confidence interval, was also determined.

Table 2.

Size	Center
X = 12	X = -70.094
Y = 12	Y = -25.821
Z = 12	Z = -39.822

Table 2. Calculations of in-software grid box size and ligand docking coordinates for AutodockTools ligand analysis.

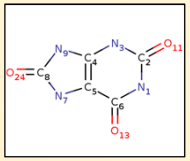

Figure 2. Uric Acid structure.

Results

Below are results of Autodock Vina computations of docked Uric Acid, Allopurinol, Caffeine, and Caffeine Derivatives in XOD. Results consist of binding affinity (kcal/mol), RMSD, binding model (orientation in regards to Uric Acid), and K_d calculations (μM). Caffeine derivatives were created with Aldehyde, Carboxylic Acid, and Hydrogen substituents replacing the three Methyl groups present on native Caffeine (Positions N1, N3, N7). These are denoted CHO', COOH', and H' respectively with their positions on the Caffeine structure. Key derivative structures are COOH#7-H#1,#3' and COOH#7' (**Table 3.**) and their binding models are shown (**Table 7.**, **Table 8.**). COOH#7' derivative served as a control for COOH#7-H#1,#3' against the hydrogen replaced methyl groups (N1, N3).

Table 3.

Ligand	Affinity (kcal/mol)	RMSD	Binding Model
CHO#7'	-3.7	0.000, 0.000	1
CHO#1'	-3.2	1.836, 3.932	2
CHO#1'	-2.1	2.232, 4.972	4
CHO#3'	-0.6	1.832, 3.763	7
CHO#1,#7'	-3.9	0.000, 0.000	1
CHO#3,#7'	-3.4	0.000, 0.000	1
CHO#1,#3'	-2.9	1.588, 3.813	3
CHO#1,#3,#7'	-1.3	1.121, 3.394	7
CHO#1,#3 - COOH#1'	-3	0.000, 0.000	1
CHO#1,#3 - COOH#1'	-2.4	1.509, 4.469	2
CHO#3,#7 - COOH#1'	-2.6	0.000, 0.000	1
CHO#1,#7 - COOH#3'	-2.9	0.000, 0.000	1
CHO#3 - COOH#1,#7'	-1.7	0.000, 0.000	1
CHO#1 - COOH#3,#7'	-1.9	0.000, 0.000	1
CHO#7 - COOH#1,#3'	0.0	0.000, 0.000	1
COOH#1,#3,#7'	0.9	0.000, 0.000	1
COOH#1 - H#3,#7'	-7.3	0.000, 0.000	1
COOH#3 - H#1,#7'	-6.5	0.000, 0.000	1
COOH#7 - H#1,#3'	-7.4	0.000, 0.000	1
COOH#1,#7 - H#3'	-4.8	1.572, 2.912	2
COOH#3,#7 - H#1'	-5.9	0.000, 0.000	1
COOH#1,#3 - H#7'	-4.1	0.000, 0.000	1
COOH#7'	-0.6	1.390, 2.962	7

Table 3. Caffeine derivatives data, consisting of different variations of Aldehyde, Carboxylic Acid, and Hydrogen groups replacing Methyl groups on N1, N3, N7. Affinity values, RMSD, and Binding model results were determined. Binding model refers to 'best fit' orientation with regards to Uric Acid; lower values do not necessarily mean best binding mechanism.

Ligand	Affinity (kcal/mol)	K_d (μM)
Uric Acid	-5.4	99.79
Allopurinol	-5.8	50.43
Native Caffeine	-1.1	1531
COOH#7 - H#1,#3'	-7.4	3.291
COOH#7'	-0.6	359300

Table 1. Affinity and K_d values for key results. K_d values were calculated using $\Delta G = RTlnKd$, R=.001987kcal/K*mole, and T=295K.

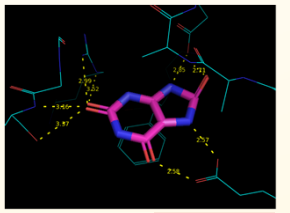

Table 4.

Hydrogen bond	Å
Glu802 - #NH7	1.57
Glu802 - #O13	1.58
Thr1010 - #O11	2.16
Arg880 - #O11	1.99
Glu1261 - #NH9	1.85
Ala1079 - #O24	1.71

Figure 4. Best fit binding orientation of Uric Acid with hydrogen bonds to XOD active site AA side chains shown. **Table 4.** Uric Acid hydrogen bond lengths in Angstroms (Å).

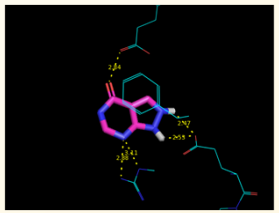

Table 5.

Hydrogen bond	Å
Glu802 - #O6	2.84
Arg880 - #N3	1.88
Arg880 - #N3	2.11
Glu1261 - #NH8	2.47
Glu1261 - #NH9	2.53

Figure 5. Best fit binding orientation of Allopurinol with hydrogen bonds to XOD active site AA side chains shown. **Table 5.** Allopurinol hydrogen bond lengths in Angstroms (Å).

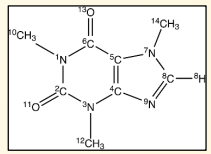

Figure 3. Native Caffeine structure with atomic numbering.

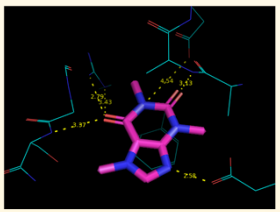

Table 6.

Hydrogen bond	Å
Ala1079 - #O11	2.13
Glu1261 - #N1	3.54
Glu802 - #N9	1.58
Arg880 - #O13	1.79
Arg880 - #O13	2.43
Thr1010 - #O13	2.37

Table 6. Native Caffeine hydrogen bond lengths in Angstroms (Å).

Figure 6. Best fit binding of native Caffeine docked to XOD active site. Native Caffeine exhibits a similar binding interaction to Uric Acid, however there are noticeable differences. First, there is only one hydrogen bond to Glu802 compared to Uric Acid's two. Secondly, the bond length to Glu1261 is much longer than in Uric Acid (3.54 Å: 1.85 Å).

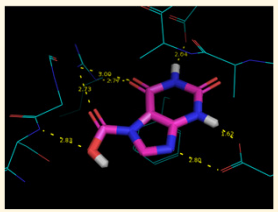

Table 7.

Hydrogen bond	Å
Glu1261 - #NH1	2.04
Glu802 - #NH3	1.62
Glu802 - #N9	1.8
Thr1010 - #O15	1.83
Arg880 - #O16	1.73
Arg880 - #O13	1.77
Arg880 - #O13	2
Ala1079 - #O	NONE

Table 7. Hydrogen bond lengths in Angstroms (Å) for Caffeine derivative, COOH#7- H#1,#3'.

Figure 7. Best fit binding of Caffeine derivative (COOH#7 - H#1,#3'), which has a carboxylic acid substituent on N7, and hydrogens replacing the remaining methyl groups on positions 1 and 3 (N1, N3). With all methyl groups replaced, this derivative displays the most similar hydrogen bond interaction within the XOD active site to Uric Acid's (**Figure 4.**). Important to note that a hydrogen bond to Ala1079 – O is not evident.

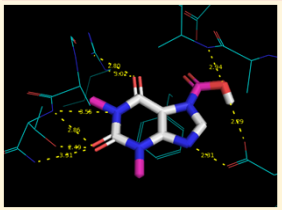

Table 8.

Hydrogen bond	Å
Ala1079 - #O15	1.94
Glu802 - #O15	2.29
Glu802 - #N9	1.31
Arg880 - #O13	1.8
Arg880 - #O13	2.02
Thr1010 - #N1	2.55
Thr1010 - #O11	1.86
Thr1010 - #O11	1.49
Val1011 - #O11	2.31
Glu1261 - #O	NONE

Table 8. Hydrogen bond lengths in Angstroms (Å) for control Caffeine derivative, COOH#7'.

Figure 8. Best fit binding of control Caffeine derivative (COOH#7') in XOD. This molecule has a carboxylic acid substituent on N7 only, with methyl groups on N1 and N3 remaining. With the remaining two methyl groups intact, this molecule exhibits a subpar bonding interaction and in a flipped orientation in comparison with COOH#7 - H#1,#3' (**Figure 7.**). Important to note that this molecule shows a hydrogen interaction with Val1011 and an absence of a Glu1261 – O interaction.

Conclusion

In comparison with Urate and Allopurinol, native Caffeine exhibits a poor binding interaction with XOD. Although there are some similarities in the model, the standard Caffeine molecule does not have an affinity comparable (-1.1 kcal/mol) with Uric Acid and Allopurinol, the standard inhibitor of XOD. We suspect this to be due to the nonpolar and bulky nature of the methyl groups, which must hinder its reaction with the side chains within XOD's active site. However, when we replace these methyl groups with reactive substituents, the affinity seems to improve. This change is especially seen in the derivative COOH#7 - H#1,#3' , where we see a dramatic change in affinity (-7.4 kcal/mol). This value alone surpasses Uric Acid and Allopurinol's, which hints at this derivative being a possible inhibitor to XOD. This caffeine derivative also exhibits comparable binding within the XOD site. Moreover, when we keep the methyl groups on positions N1,3 in derivative COOH#7', we see a dramatic drop in affinity and bond interaction/orientation (-0.6 kcal/mol), suggesting that the methyl groups in Caffeine play a substantial role in hindering the bond interaction within the XOD active site. Nevertheless, our carboxylic acid-hydrogen Caffeine derivative shows promise as a potential inhibitor in the XOD mechanism.

References

1. Okamoto, K., Kawaguchi, Y., Eger, B.T., Pai, E. F., 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. Massey, V., Komai, H., Palmer, G., Elion, G. On the Mechanism of Inactivation of Xanthine Oxidase by Allopurinol and Other Pyrazolo[3,4-*d*]pyrimidines. J. Biol. Chem. Soc. 245:2837-2844 (1970).
3. The PyMol Molecular Graphics System, Version 1.8, Schrodinger, LLC. http://pymol.org/educational
4. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009) Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility, *J. Computational Chemistry* 2009, **16**: 2785-91
5. Image from the RCSB PDB website (www.rcsb.org), Bovine Xanthine Oxidoreductase Urate Bound Form, PDB ID: 3AMZ.

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