Validation of in Silico Docking Analysis of Oligophenylpropanoids to Xanthine Oxidase by Correlation with in Vitro Bioassay and Its Application to Phlorotannins

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Abstract: The oligo-phenylpropanoids 1−7, isolated from Hyptis rhombodes, have been found to possess potent inhibitory activity against xanthine oxidase (EC 1.2.3.2, bovine milk). To rationalize such activity, computer assisted docking of these compounds and allopurinol, a positive control, on the xanthine oxidase was undertaken in this study. The docking scores, obtained by London (trimatch)−refinement (Forcefield Affinity ∆G) mode, showed good correlation with the IC₅₀ values. That the compounds possessing 7′-Z configuration had much better inhibitory activity than those 7′-E isomers is well rationalized by this docking study. Virtual screening of eight phlorotannins (8−15) by this refinement mode found good docking scores. The bioassay result of three available ones (9, 12, 13) also indicated the consistency with the docking scores. While refined by Forcefield−London mode, certain inconsistency among the docking score and bioassay result was observed on either phenylpropanoid oligomers or three phlorotannins. Hence the London (trimatch)−refinement (Forcefield−Affinity ∆G) mode is recommended for virtual screening of the related phenolics. Three phlorotannins (11, 14, 15) were found to have better docking score than 6,6’-bieckol (12) and dieckol (13), both showing comparable inhibitory activity against xanthine oxidase to allopurinol, and thus they deserve further study. In addition, as these phlorotannins are rich in the Ecklonia genus, the common edible seaweeds such as E. cava and E. stolonifera are demonstrated to be beneficial to hyperuricemic patients.

Keywords: Xanthine Oxidase Inhibitors, Oligo-phenylpropanoids, Bioassay, Molecular Docking, Phlorotannins

1. Introduction

Xanthine oxidase (XO) is a molybdenum-containing hydroxylase which catalyzes the oxidation of purine substrates, hypoxanthine and xanthine, to yield uric acid and reactive oxygen species [1]. The excessive production or insufficient elimination of uric acid results in hyperuricemia, which is associated with gout [2]. XO inhibitors have been proven their efficacy in lowering blood uric acid in animal models and clinical application [3]. Thus the development of xanthine oxidase inhibitors has become one of the therapeutic approaches for treating hyperuricemia.

Allopurinol, an XO inhibitor, is the most commonly used anti-gout drug in the past decades [3]. However, some incidences caused by allopurinol have been reported, including hypersensitivity reactions, Steven’s Johnson syndrome, hepatitis, nephropathy, and 6-mercaptopurine toxicity [4]. Therefore, alternatives of allopurinol with potent anti-XO efficacy and less or void of side effects are in medical need.

Our recent study found that phenylpropanoid oligomers (Figure 1), isolated from Hyptis rhombodes Mart. & Gal. [5], possessed potent anti-XO activity, comparable to allopurinol (Table 1). That study also indicated that for geometric isomers,
those possessing a cis-styrenyl moiety are more potent than the corresponding trans isomer (e.g. 2 vs. 1; 4 vs. 3) and for those diastereomers, that having (7'R, 8'S)-configuration is more potent than the corresponding (7'S, 8'S)-isomer (e.g. 6 vs. 4); and that with a carboxyl group is more potent than its methyl ester (e.g. 4 vs. 7). The rationale of such structure and activity relationship, however, required further clarification.

As the crystal structure of xanthine oxidase had been documented on the Protein Data Bank (PDB ID: 1F1Q), this current study was aimed to apply computer assisted molecular docking in clarification of this relationship. In addition, the established docking refinement mode was applied as a virtual screening tool on the related polyphenolics, phlorotannins, to find potential xanthine oxidase inhibitors.

Figure 1. Phenylpropanoid oligomers isolated from Hypit s rhombodes [5]: netpetolidin A (1), netpetolidin B (2), hyprhombin A−C (3−5), epihyprhombin B (6), hyprhombin B methyl ester (7).

2. Materials and Methods

2.1. Molecular Dynamic Simulation

2.1.1. Preparing the 3D Structure of Xanthine Oxidase

The crystal structure of xanthine oxidase enzyme (XO) from bovine milk was obtained from the Protein Data Bank (PDB ID: 1F1Q). This enzyme is a homodimer. Each monomer acts independently in the catalysis process and addition, the established docking refinement mode was applied as a virtual screening tool on the related polyphenolics, phlorotannins, to find potential xanthine oxidase inhibitors.

2.1.2. Ligands for Docking

Seven phenylpropanoid oligomers (1−7) (Figure 1), XO inhibitors isolated from H. rhombodes [5], were chosen for the establishment of an appropriate refinement mode during molecular docking study. While for virtual screening against XO, eight representative phlorotannins (8−15) [10, 11] were chosen. Two-dimensional (2D) structures of the selective ligands were depicted using ChemBioDraw and their Mol files which were subjected to energy minimization and molecular docking study. While for virtual screening against XO, eight representative phlorotannins (8−15) [10, 11] were chosen. Two-dimensional (2D) structures of the selective ligands were depicted using ChemBioDraw and their Mol files which were subjected to energy minimization and molecular docking study.
3. Results and Discussion

3.1. Interaction of XO with Oligo-phenylpropanoids 1–7

Molecular docking of oligo-phenylpropanoids 1–7 on xanthines oxidase template was undertaken initially via London (trimatch)–refinement (Forcefield–London) mode and the results are shown in Table 1. The correlation between docking score and bioassay result (IC_{50}) is generally consistent except for two potent compounds, 5 and 6, where a little disagreement was observed.

Netpetoidin A (1) and netpetoidin B (2) are geometric isomers at C-7'. The caffeoyl residue of both 1 and 2 is oriented toward the binding pocket interior of XO, leading to the formation of H-bond with the side chain of Arg880 and Thr1010. Relative to 1, netpetoidin B (2) formed three additional H-bonds from the styrenyl catechol residue to Ser876, His875, and Glu879, of which Ser876 is located within and the other two close to the pocket site. Such interaction is due to different orientation of the geometrical constraint, in which 2 showed better shape complementarity as depicted in Figure 2a, resulting better binding affinity (\Delta G = -19.32 Kcal/mol) vs. -15.33 Kcal/mol). This correlates well with the bioassay result (2, IC_{50} = 11.7 \mu M; 1, IC_{50} > 159.2 \mu M). Similarly, for the geometric isomers 3 and 4, the 7'-Z isomer (4) had better binding affinity than the 7'-E one (3), ascribable to one additional H-bond and better shape complementarity as depicted in Figure 2b. In addition, the residue in 3 which interacts with the binding pocket interior is the styrenyl catechol and that in 4 is the phenylpropenoic acid.

As indicated above, the C-9" carboxylic acid residue played important role in docking affinity to XO. Compound 7, the methyl ester of 4, should possess weaker binding affinity. Indeed, the favorite docking of 7 to XO showed relatively poor shape and geometric complementarity (\Delta G = -17.87 Kcal/mol) within the binding site, consistent with the inhibitory activity (IC_{50} = 30.9 \mu M vs. 5.2 \mu M, 4).

As for C-7"/C-8" positional isomers 5 and 6, similar docking orientation (Figure 2d) was also observed. The adjustment of the catechol and the carboxyl group to nearly overlap in both compounds makes the styrenyl catechol residue oriented quite differently. Such difference, however, affects the binding score only a little (\Delta G = -21.95 Kcal/mol) since the phenylpropenoic acid residue dominates the binding affinity. The bioassay result (IC_{50} = 0.6 \mu M, 5 vs. 2.0 \mu M, 6), however, is not so consistent with the docking score.

![Figure 2. Molecular docking of 1 versus 2 (a), 3 versus 4 (b), 4 versus 6 (c), and 5 versus 6 (d) to XO template using the refinement.](image-url)
Attempts to verify this inconsistency were made and it was found that using the refinement (Forcefield−Affinity $\Delta G$) mode, not only the docking scores of 5 and 6 (Table 1) but also those of the rest compounds were in good agreement with the bioassay results. Thus this refinement mode is adopted for later on study.

Phlorotannins, rich in the seaweeds of the Ecklonia genus, such as *E. cava* and *E. stolonifera*, are polyphenolics like compounds 1−7. Thus they might possess anti-XO activity. To support this assumption, molecular docking of some phlorotannins (8−15) (Figure 3) [6, 7] to XO was undertaken via the London−refinement (Forcefield−Affinity $\Delta G$) mode. The result indicated that phlorofucofuroeckol B (11), 2,7-phloroglucinol-6,6-bieckol (14), and pyrogallol-phloroglucinol-6,6-bieckol (15) showed better affinity with the docking score in the range from $-23.23$ to $-24.07$ Kcal/mol. This result correlated well with the bioassay data of three available phlorotannins, 2-phloroeckol (9), 6,6′-bieckol (12) [14], and dieckol (13) [15], against xanthine oxidase (Table 2). The IC$_{50}$ values of these three compounds ranged from 5.6 $\mu$M (13) to 18.2 $\mu$M (9), comparable to that of the positive control allopurinol (IC$_{50}$ 8.5 $\mu$M). While using the refinement (Forcefield−London) mode, the docking and the bioassay results of these three compounds (Table 2) were not consistent as that described in 5 and 6.

Since the pocket of the active site is another choice for molecular docking and the alpha mode is another refinement method, they were applied to virtually screen these three available phlorotannins. The result indicated the corresponding conformation for the best docking score was seriously distorted for the planar phenyl group. Thus the alpha refinement mode and the docking on the pocket are not suitable as virtual screening tool for this type compounds.

### 3.2. Interaction of XO with Phlorotannins 8−15

Compound 9 is the 2-$O$-phloroglucinolated derivative of eckol (8). This additional substitution in 9 caused steric hindrance to the binding site, leading to two H-bonds less than 8 and hence weaker binding affinity than 8 ($\Delta G$ -18.16 vs. -20.99 Kcal/mol) (Figure 4a).

For the structural isomers of di-phloroglucinolated eckols 10 and 11, the conformation of 11 is linear-like while that of 10 contains an L-shape skeleton to give a skew conformation with two phloroglucinol residues. Such difference allowed 11 to have higher flexibility to let it adapt much better to the binding pocket than 10, reflected by stronger binding affinity ($\Delta G$ -24.07 vs. -20.31 Kcal/mol).

![Figure 3. Structures of phlorotannins 8-15.](image-url)
bulky phloroglucinol residue of 14 into the binding site bioassay result (IC50/ Forcefield−Affinity ∆G). µ relatively bulky than that of 15. While insertion of the bottom comparison showed that the structure conformation of 14 was 6,6′-bieckols and are positional isomers. The visual ∆G and thus had stronger binding affinity (µM, 13 vs. 7.4 M, 12). Similarly, for the isomeric dieckols 12 and 13, the isomeric dieckols 12 and 13, the interaction associated strongly with further investigation if they are available. They might be more potent XO inhibitors and are worthy of further investigation if they are available.

### 4. Conclusion

The binding affinity of compounds (1−15) toward xanthine oxidase showed that the interaction associated strongly with blocked the binding pocket entrance, accounting for a little higher binding score (∆G -23.75 Kcal/mol, 14 vs. -23.23 Kcal/mol, 15).

As phlorofucofuroeckol B (11), 6,6-bieckol of 2,7-phloroglucinol (14), and pyrogallol-phloroglucinol (15) showed better affinity to xanthine oxidase than dieckol (13), they might be more potent XO inhibitors and are worthy of further investigation if they are available.

### 4. Conclusion

The binding affinity of compounds (1−15) toward xanthine oxidase showed that the interaction associated strongly with...
certain amino acid residues in the active site such as Arg880, Thr1010, and Glu1261. These amino acid residues form H-bond or/and ionic interaction with the active ligands, which play critical roles against XO. Using the refinement (Forcefield−Affinity $\Delta G$) mode, the docking results of either phenylpropanoids 1−7 or three phlorotannins (9, 12, 13) were more consistent with the bioassay results than those obtained by the refinement (Forcefield−London) mode. Thus the London (trimatch)−refinement (Forcefield−Affinity $\Delta G$) docking method is recommended for virtual screening of the related phenolic compounds. As 6,6′-bieckol (12) and dieckol (13) showed inhibitory activity against XO comparable to the positive control allopurinol, three phlorotannins (11, 14, 15) having better docking score than 12 and 13 deserve further study. Since these phlorotannins are rich in *Ecklonia cava* and *E. stolonifera*, these common edible seaweeds should be beneficial to persons suffering hyperuricemia.

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