Sciences & Technologie **C – N°26,** décembre (2007), pp.69-73.

NOVEL HIGH AFFINITY INHIBITORS BASED ON THE CHEMICAL MODIFICATIONS OF 3, 4 DIHYDROXY BENZOIC ACID : DOCKING SIMULATIONS ON LIPOXYGENASE

Reçu le 21/05/2007– Accepté le 14/11/2007

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Résumé

La modélisation des interactions enzyme-inhibiteur utilisant le programme FlexX permet de concevoir de nouveaux inhibiteurs à haute affinité de la lipoxygénase de soja (LOX-3). Les résultats du docking montrent que l'énergie de liaison de l'enzyme avec son inhibiteur, l'acide 3,4 dihydroxy benzoique (DHB), est ΔG = -10.564 Kj/mol. Cette énergie de liaison augmente lorsqu'un groupement hydroxyle est rajouté sur le DHB (ΔG = -16.959 Kj /mol) ou bien sa fonction carboxylique est remplacée par d'autres fonctions, le groupement hydroxy méthyl en particulier (ΔG = - 21.127 Kj/mol). L'étude de docking peut être utilisée pour améliorer l'activité biologique d'un composé pour une cible donnée.

***Mots clés**: Energie de liaison, Docking; Modélisation; Complexe Enzyme-Inhibitor; Lipoxygenase; Interaction ligand-protein*

Abstract

Modeling enzyme-inhiubitor interactions using FlexX program allowed to design novel high affinity inhibitors of soybean lipoxygenase (LOX-3). Docking results show that the binding energy of the enzyme with its inhibitor 3,4 dihydroxy benzoic acid (DHB) is ΔG = -10.564 Kj/mol. .This binding energy enhances when a hydroxyl group is added on the ring of DHB (ΔG = -16.959 Kj /mol) or its carboxylic group is replaced by other functional groups, particularly a methyl hydroxyl group (ΔG = - 21.127 Kj/mol). Docking study may be useful to improve the biological activity of a compound for a given target.

***Keywords**: Binding Energy; Docking; Modeling; Enzyme-Inhibitor Complex; Lipoxygenase; Interaction protein-ligand*

ملخص

تسمح نمذجة التفاعلات إنزيم-مثبط باستعمال البرنامج Flex-X بتصور مثبطات جديدة ذات جاذبية مرتفعة لإنزيم Lipoxygénaseلنبات الصويا (LOX3)

تبين نتائج Doking أن طاقة ارتباط الأنزيم بالمثبط (حامض DHB هي .ΔG = -10,564Kj/mol).

و ترتفع طاقة الأرتباط هذه عند إضافة مجموعة OH إلى DHB (ΔG=-16,959Kj/mol.) أو عند استبدال وظيفته الكربوكسيلية بوظائف أخرى و خاصة مجموعة الهيدروكسي ميثيل (.ΔG = -21,127 Kj/mol)

يمكن استعمال هذه الدراسة لتحسين النشاط الحيوي لمركب ما.

***الكلمات المفتاحية**: طاقة ارتباط Docking نمذجة مركب إنزيم-مثبط Lipoxygenase التفاعلات إنزيم-رابط*

Drug discovery has traditionally made progress by a combination of random screening and rational drug design with the help of experimental high-throughput strategies, NMR, X-ray structures of biological macromolecules, combinatorial chemistry, molecular modelling [1,2].

On the other hand, computer graphics and fast computational programs have been developed to provide valuable structural information on electrostatic potentials and hydrophobicity of binding site [3].

The latter approaches try to predict, via molecular docking, the binding mode of a ligand at the active site which aid the drug discovery process [4-7].With the aim of improving pharmacological properties of a compound, modification of the structure of protocatechuic acid (3,4-dihydroxybenzoic acid), a LOX-3 inhibitor, was systematically investigated in an effort to enhance its activity.

The structural modifications consist in either replacing the carboxylic function by other functional groups or to change the position of hydroxyl groups carried initially in 3 and 4 by the inhibitor.

Lipoxygenases (LOXs), which are widely distributed in both the plant and animal kingdoms, [8, 9] belong to a class of non-heme iron-containing enzymes which catalyze dioxygen incorporation into polyunsaturated fatty acids (PUFA), such as linoleic and arachidonic acid, to form hydroperoxide products [10]. In humans, PUFA metabolites are involved in cancer, asthma, atherosclerosis and a variety of inflammatory conditions.

Blocking their production by inhibiting lipid peroxidation pathway might be a successful way to control and relieve such problems.

At first, LOX was used as target for a set of three inhibitors (compounds 1-3) (Tab.1). Their inhibitory effect differ depending on their chemical structure. Their IC_{50} value could be at or below several ten of micromoles which make them feasible therapeutic agents[11].

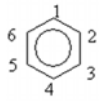
This study on LOX-3 contributes to the understanding of biocatalytic properties involved in the interactions between this enzyme and its ligand. Based on the modelling and docking simulation, the binding energies of three enzyme-inhibitor complexes were determined. Their X-ray structure were taken from the Brookhaven Protein Data Bank [12] (PDB entries 1N8Q, 1NO3,1HU9).

Compound DHB reveals the most inhibitory effect. It has been identified by X-ray analysis as the major product of oxidative degradation quercetin by LOX-3 [11].

We have also designed several novel compounds and their relative inhibitory capacities were calculated. Enhancing binding energy indicates which inhibitor is likely to be most active.

Table 1: Structure formulas of ligands

Compound	Substitutions other than H in the following position of the reference structure (see footnotes)					
	1	2	3	4	5	6
1	COO^-		OH	OH		
2	$NO2^-$		OH	OH		
3	O-OH		OCH3	OH		
4	CH2OH		OH	OH		
5	$CH2COO^-$		OH	OH		
6	NH2		OH	OH		
7	CONH2		OH	OH		
8	CH2NH2		OH	OH		
9	COO^-		OH	OH	OH	
10	COO^-	OH			OH	
11	COO^-		OCH3	OH		

Footnotes: 

MATERIALS AND METHODS

Docking of ligands into the active site of LOX-3 was performed with FlexX program [13].

FlexX is a docking procedure aimed at detecting energetically favourable binding modes of a ligand into the protein active site. The protein is considered rigid, whereas the ligand conformation is flexible by allowing rotations around acyclic single bonds and multiple conformers for ring structures.

The docking algorithm is based on hydrophobic interactions and hydrogen bonds in particular. The ligand is placed into the binding site by an incremental fragment placing technique [14, 15]. The first placement step is carried out with the base fragment selected by docking algorithm.

The goal of the base placement algorithm is to find position of the base fragment in the active site such that a sufficient number of favourable interactions between the fragments and the protein can occur simultaneously. In the following step, the ligand is build up fragment by fragment by a rotatable single bond.

The binding free energy is calculated with an empirically derived scoring function [16]. Docking produced a set of conformational solutions for each ligand. The optimal conformational pose is indicated by the best FlexX score displaying the most negative value of the binding free energy [17] and the lowest rmsd (root-mean square deviation) [18].

The structural ligand optimization is performed with the MM+ force field mechanics method prior to use as input files for docking calculations.

RESULTS AND DISCUSSION

The minimized structure of each inhibitor was docked with LOX-3. By comparing the calculated protein-ligand complex geometries to geometries of the cocrystallized ligands, it appears that docking solutions superimpose well with the coordinates from the X-ray structure (Fig.1).

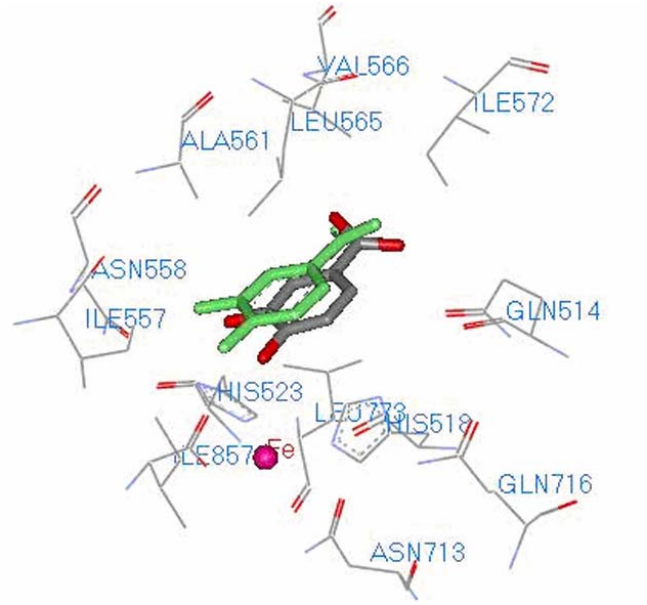


Figure 1 Geometrical comparison between X-ray structure of inhibitor DHB (colored by atom type) and docked (colored green) by FlexX. Hydrogen atoms of the amino acid residues and ligand are not shown for clarity.

The FlexX program has been shown to successfully reproduce experimentally observed binding modes (rmsd ≤ 2 Å).The optimal conformational pose corresponded to the lowest rmsd value and the highest ranked solution are listed in table 2.

Table 2: Binding energy and rmsd values of three lipoxygenase-inhibitor complexes

Inhibitor	1	2	3
rmsd (Å)	0.922	0.911	0.563
Binding energy (Kj/mol.)	-10.564	-8.337	-5.539

The compound 1 (DHB) showed better binding energy (ΔG = - 10.564 Kj/mol) compared to other inhibitors. Its C3-OH group forms a hydrogen bond with ND1 His523 residue (2.91 Å), C4-OH group establishes a hydrogen bonds with C=O from C-terminus Ile857 residue (2.36Å) and metal Fe (3.48 Å), carboxylic group is incorporated into the hydrogen bonding network of the active site neighbourhood via Gln514 residue (3.22Å) [11].

In the second part of this study, two groups of compounds are proposed. In the first group (compounds 4-8), the carboxylic function of DHB is replaced by other functional groups as methyl hydroxyl group (4), methyl carboxylic group (5), amine group (6), amide group (7) and methyl amine group (8).

In the second group, compounds 9-11 are the similar compounds obtained from the PDB database: the carboxylic function of the DHB is maintained, Only the position or nature of the hydroxyl groups is changed. The aim of these structural modifications is to enhance interaction energy of the complex.

Docking calculations revealed that the binding energy increased with all docked ligands (Tab.3). The best result was obtained with compound 4 followed by compound 9. Replacement of the carboxylic functional group of DHB with methyl hydroxyl group in the compound 4 provides the best interaction energy (ΔG = - 21.127 Kj/mol).

Table 3: Binding energies values for complexes of the LOX-3 with compounds 4-11

Compound	Binding energy (Kj/mol)
4	-21.13
5	-15.36
6	-14.99
7	-14.10
8	-12.68
9	-16.96
10	-12.05
11	-11.93

The new functional group develops two novel hydrogen bonds with Gln514 (CH_2O–H…O=C–CA 1.94 Å) and His518 (CH_2O-H…ND1 3.19 Å). Compound 4 is also stabilized by aromatic interactions with His518, Trp519, His523 and van der Walls interactions with residues Leu565 and Leu773 (distance < 4 Å).

The distance between the C4-OH of compound 1 and iron atom was about 4.90 Å. Since the binding pocket of LOX-3 is rather hydrophobic, the predicted binding mode of compound 4 may be reasoned by its lipophilicity which is expressed by a good log *P* value of the docked conformer : 0.82 [19].

The enhanced binding of compound 9 (ΔG = -16.959 Kj/mol) might be attributed to the formation of a new hydrogen bond between. C3-OH and NE2 of His523 residue (3.34 Å). Hydrophobic interactions are also observed with His518, Trp519, His523 Leu565, Leu773 and Ile572. The distance between C3-OH and iron is 4.72 Å (Fig.2). The calculated log *P* value is 0.62 (http://bioserv.rpbs.jussieu.fr/Help/FAFDrugs.html).

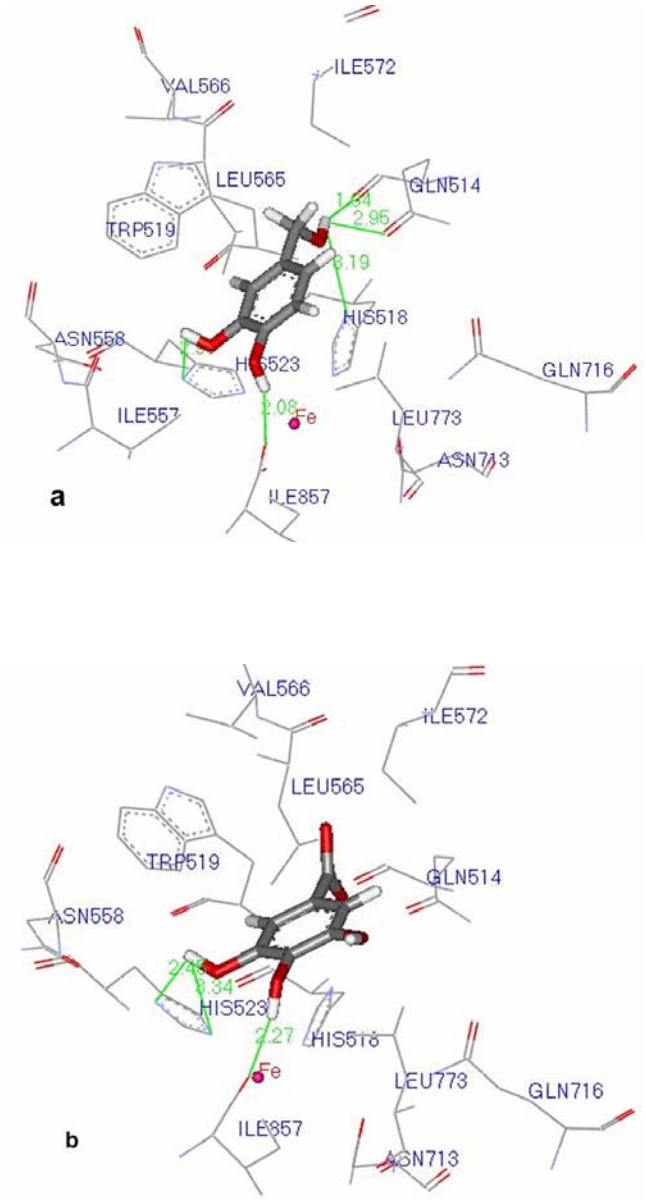


Figure 2 Binding modes of ligands 4 (a) and 9 (b) with the active site of LOX-3. The green line represents hydrogen bond.

The inhibitory activity against soybean lipoxygenase of compounds 4-11 was examined and docking studies on the active site of the enzyme were performed. Among the eight compounds used in this study, compound 4 constitutes the best inhibitor of LOX.

Replacement of the carboxylic group of the LOX-3 inhibitor DHB with a methyl hydroxyl (compound 4) enhances the binding energy to -21.127 kJ/mol. Docking results show that the compounds 4-11 may aid the development of more potent inhibitors of lipoxygenase.

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