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In Silico Molecular Modeling and Docking Studies on Luteolin Derivatives as Novel Helicobacter Pylori β-hydroxyacyl-acyl carrier protein dehydratase Inhibitor from Biebersteinia *multifida* DC.

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Abstract: Helicobacter pylori infection is responsible for the majority of peptic ulcer and gastric cancer. Due to the uprising resistance for the suppression of Helicobacter pylori problem through the present and common proton pump inhibitors regimens the investigation of novel candidates is the inevitable issue. Medicinal plants have always been a source of lead compounds for drug discovery. The researches of the related effective enzymes linked with this gram-negative bacterium are critical for the discovery of novel drug targets. Flavonoids are one of the known compounds of many medicinal plants which exhibit different pharmacological effects including anti-Helicobacter activity. Therefore, in this study, we have proposed the use of in silico molecular modeling and docking methods to design some potential active agents based on the most effective flavones, *luteolin*, as a novel Helicobacter pylori β-hydroxyacyl-acyl carrier protein dehydratase (HpFabZ) inhibitors. Then we have predicted probable binding conformation of the compounds, which is experimentally not known, using a computational modeling method. Conformations of the designed compounds were optimized through semi-empirical method followed by PM3 calculation by using the HYPERCHEM software. Among all energy minima conformers, the global minimum was selected. Then the crystal of HpFabZ (3CF9) was obtained from the Protein Data Bank (PDB) server. Finally Docking calculations were carried out using Auto-Dock program. The good interaction of the derivatives and also the Ki (inhibition constant) showed that they can be as potent HpFabZ inhibitors and act as novel anti-Helicobacter agents. We hope this Computational study can offer some useful references in order to understand the inhibition mechanism better so that the molecular designing would be improved and modification of these series of HpFabZ inhibitors would be more practiced.

Keyword: HpFabZ, Natural products, Inhibitory mechanism, Molecular modeling, Docking.

Introduction

A Gram-negative bacterium called Helicobacter pylori is one of the most prevalent infections in human worldwide [1]. Helicobacter pylori infection is directly in connection with the peptic ulcer and gastric cancer and treatment and control of this infection is now suggested as the first line of therapy for patients with peptic ulcer disease [2]. Different medical regimens have been given for the treatment of Helicobacter pylori with several combinations of medicines including antibiotics, bismuth subcitrate, proton pump inhibitors and H2-blockers [3]. The rising resistance to antibacterial agents confines the usage of these compounds in the treatment of the H. Pylori infections in developed and developing countries [4, 5]. Moreover, because of the adverse side effects of the drugs and the considerable expenditure of combination therapy the further studies are required to find the alternative drugs and potent agents [6]. Consequently, many studies have been focused to evaluate the anti-Helicobacter pylori effects of traditional herbal medicines from various parts of the world with noticeable bactericidal activities against Helicobacter pylori [7-11]. Natural products are the most successful source of leads. As a part of our drug discovery program on Iranian medicinal plants, we have been working on the endemic plant Biebersteinia *multifida* DC (Biebersteiniaceae). The root of the plant was used as folk medicine for the treatment of some disease [12]. Also the flavonoids constituents of this plant were reported [13]. The largest parts of the flavonoids belonged to the luteolin and its derivatives. Moreover there is a report about the inhibition of the three flavonoids (quercetin, apigenin, and (S)-sakuranetin) against the HpFabZ by enzymatic assay and crystal structure analysis. All these flavonoids are chemical counterpart of the luteolin [14]. Based on this precedent, we undertook a discovery endeavor to identify novel and potent HpFabZ inhibitors which might be useful in treatment of H. Pylori infection. We carried out of this aim through modification of the luteolin (Figure 1) with a focus on altering the central hydroxyl moieties to increase the hydrophobic interactions. These efforts included replacement of the hydroxyl group with alternative methoxy, ethoxy, nitro, fluorine and chlorine groups as showed in Table 1.

Regarding this issues, the structure of desired inhibitors were built by using HYPERCHEM program. Conformations of the designed compounds were optimized through semi-empirical method followed by PM3 calculation by using the HYPERCHEM software. Among all energy minima conformers, the global minimum was selected. The crystal structure of HpFabZ (Figure 2) was obtained from the Protein Data Bank (PDB) server (PDB entry: 3CF9). Then Docking calculations were carried out using AutoDock program (Ver4).

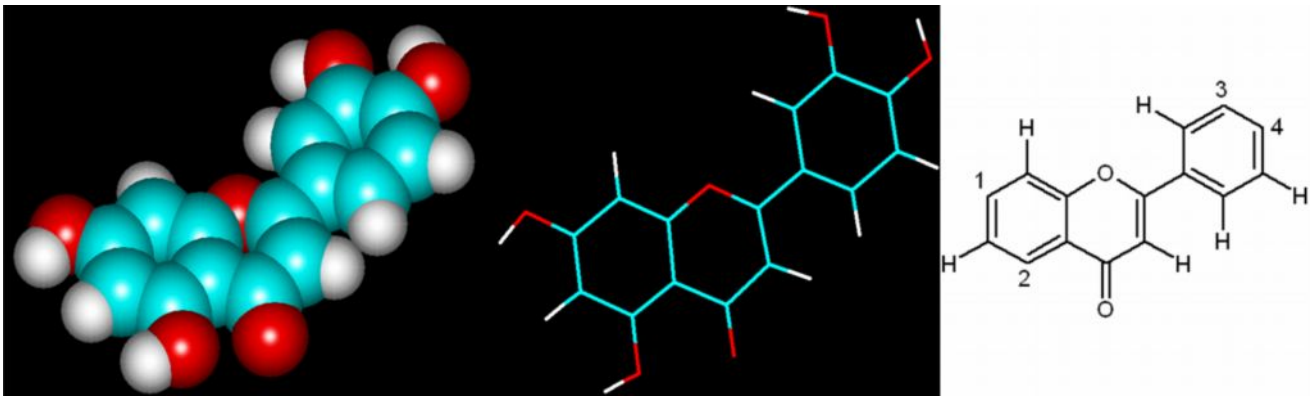


Figure 1. Molecular structure of luteolin .The global minimum energy conformation (space-filing and sticks model) is shown.

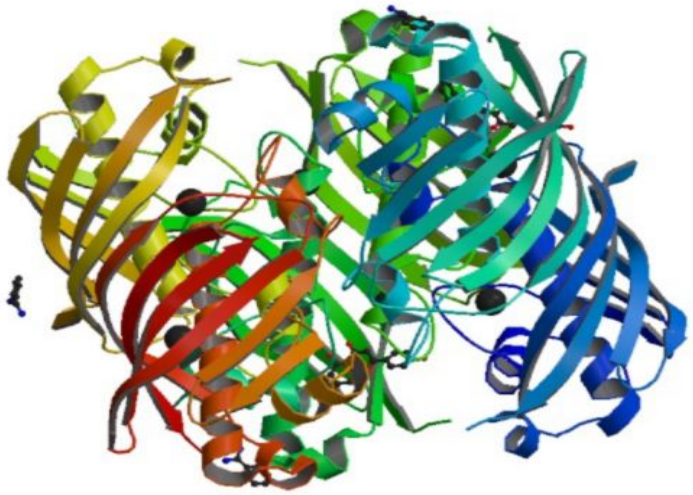


Figure2. The structure of HpFabZ enzyme (3CF9).

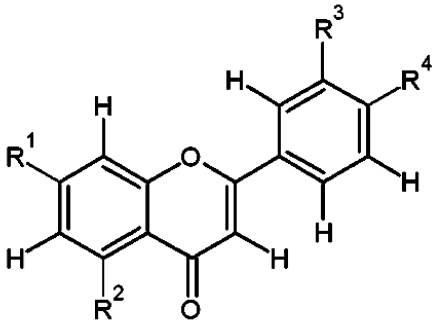
Materials and Methods

a) Molecular Modeling

The chemical structures of *luteolin* (Table.1) were constructed using Hyperchem software (version 7, Hypercube Inc.) .Semi-empirical molecular orbital calculations (PM3) of the structure were performed using the Hyperchem program and the among all energy minima conformers, the global minimum of compounds were consider in docking calculations. And also, Superimposition main pharmacophores of recognized HpFabZ inhibitor compounds and our potent compound was performed.

Table1. Structures of derivatives

Compounds	R^1	R^2	R^3	R^4
1	OH	OH	OH	OH
2	OCH_3	OCH_3	OCH_3	OH
3	OH	OH	OH	OCH_3
4	OC_2H_5	OH	OC_2H_5	OH
5	OH	OC_2H_5	OH	OC_2H_5
6	F	F	OH	OH
7	OH	OH	OH	F
8	COOH	COOH	OH	OH
9	OH	OH	OH	COOH
10	NO_2	NO_2	OH	OH
11	OH	OH	OH	NO_2
12	Cl	Cl	OH	OH
13	OH	OH	OH	Cl



b) Docking study:
Docking studies were carried out by using the program AUTODOCK 4 [15]. This program starts with a ligand molecule in an arbitrary conformation, orientation and position and finds favorable dockings in a protein-binding site using both simulating annealing and genetic algorithms. The program AutoDockTools (ADT), which has been released as an extension suite to the Python Molecular Viewer, was used to prepare the protein and the ligands. For the macromolecule the 3D crystal structure of HpFabZ (PDB entry: 3CF9), resolved at 2.6 Å, was chosen as the docking pattern from the Protein Data Bank. Earlier than docking studies, the macromolecular structure was modified to get more logical and precise outcomes. The A, B and C chains were kept whereas the D, E and F chains were unmerged and deleted because the hexamer was symmetrical. There are two active sites in this macromolecule. It should be mentioned that the interaction and binding sites are composed of C side chain plus the tunnel between A and B chains. The existed ligands in the crystal were all omitted so that other molecules could be docked and also crystallographic water molecules in the structure were eliminated. Polar hydrogens were added, and then Kollman United Atom charges and atomic solvation parameters were assigned. The grid maps of docking studies were computed using the AutoGrid4 included in the Autodock4 distribution. Grid centers were placed on the active sites which were obtained by trial and error and the previous study done by L Zhang et al (Protein Sci. 2008) and 50x50x50 points with grid spacing of 0.375 were calculated (Figure 3).

The GA-LS method was adopted to perform the molecular docking. The parameters for GA were defined as follows: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; mutation and crossover rates of 0.02 and 0.8, respectively. Pseudo-Solis & Wets parameters were used for local search and 300 iterations of Solis & Wets local search were imposed. The number of docking runs was set to 50. Both Autogrid and Autodock computations were performed on Cygwin. After docking, all structures generated were assigned to clusters based on a tolerance of 1 A ° all-atom RMSD from the lowest-energy structure. Hydrogen bonding and hydrophobic interactions between docked potent agents and macromolecule were analyzed using ADT (Version 1.50).

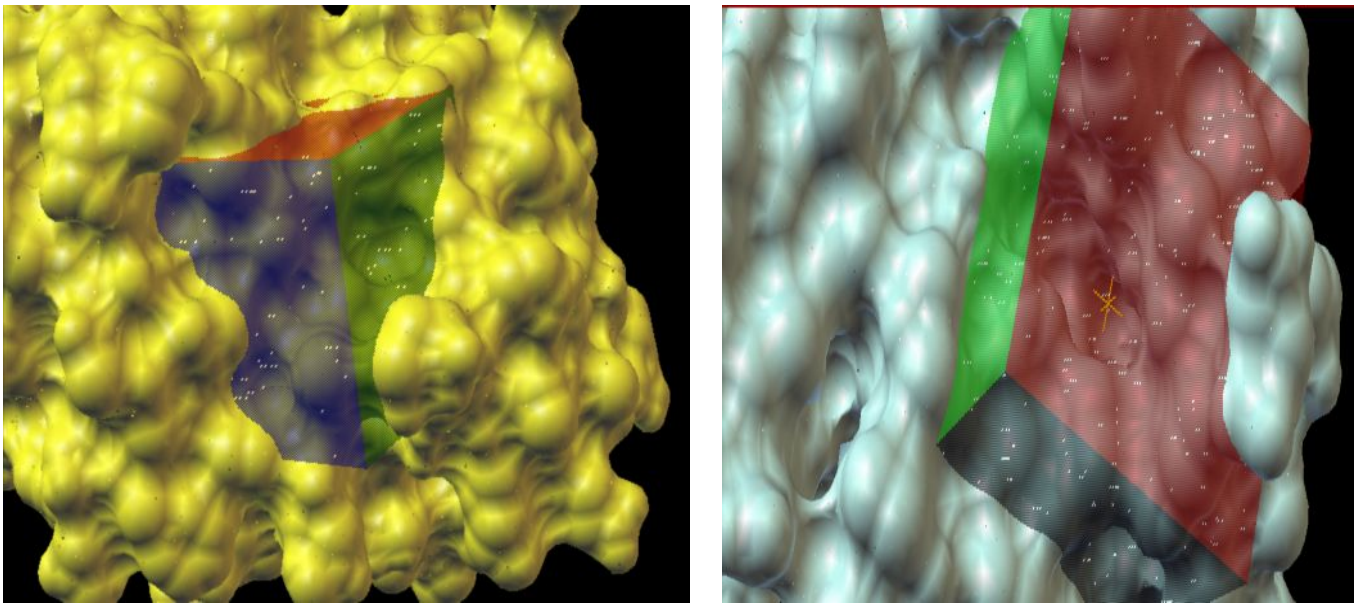


Figure 3.The active sites and grid boxes of the enzyme (Right C side Chain –Left Tunnel between A and B chains).

Results

Molecular geometry of the designed compounds has been calculated by the semi-empirical method using PM3. Now, Based on the results obtained from the superimposition of the designed compounds on the main pharmacophore belonged to HpFabZ inhibitor (*luteolin*), we expect that the potential H-bond acceptor sites created by the oxygen of hydroxyl groups have a prominent responsibility. Moreover aromatic groups in these compounds play key role in charge transfer interaction. And also, we inserted the lipophilic moiety (chlorine) and methoxy, nitro, fluorine and ethoxy in 1, 2, 3 and 4 positions to optimize LogP and improve H-bond sites (Figure 4).

Flexible docking of all data sets used for the computational study was carried out on the active site of HpFabZ enzyme. To verify and compare the model we docked *luteolin* as known HpFabZ inhibitor as well as the designed agents. In these complex structures, the HpFabZ hexamer molecule, as mentioned above there is two active sites. In the tunnel between A and B chains whose entrance is covered by Tyr100, the free binding energy of the thirteen selected inhibitors scored by Autodock ranged from –3.95 kcal·mol^{-1} to –5.47 kcal·mol^{-1}. For the second binding position the free binding energy of the thirteen selected inhibitors scored by Autodock ranged from –5.94 kcal·mol^{-1} to –8.62 kcal·mol^{-1}. The inhibition constant of these inhibitors for both interaction sites were converted into the unified unit micromol·L^{-1} (10^{-6} mol·L^{-1}) and are illustrated in table2. The interactions are shown as follow. The orientation of the most potent compounds (Comp.11 and Comp.12), in the active sites of HpFabZ enzyme were shown by ADT software (Figure5) [6]. For tunnel site, this molecular modeling shows that in Comp.11 (Figure 5a), the oxygen of the nitro group in R4 position makes noticeable hydrogen bonding interactions with the NH_2 of LYS62 (distance=1.872A^0). In connection with chain C in Compound 12, there are two hydrogen bonds. The hydrogen bonding of OH groups of ALA94 and ILE20 with the hydrogen of the hydroxyl groups in R3 and R4 positions (distance= 2.170 and 2.012 A^0 Respectively) are mentioned (Figure 5b). The predicted binding energies and inhibitory constant of these inhibitors are listed in Table 2.

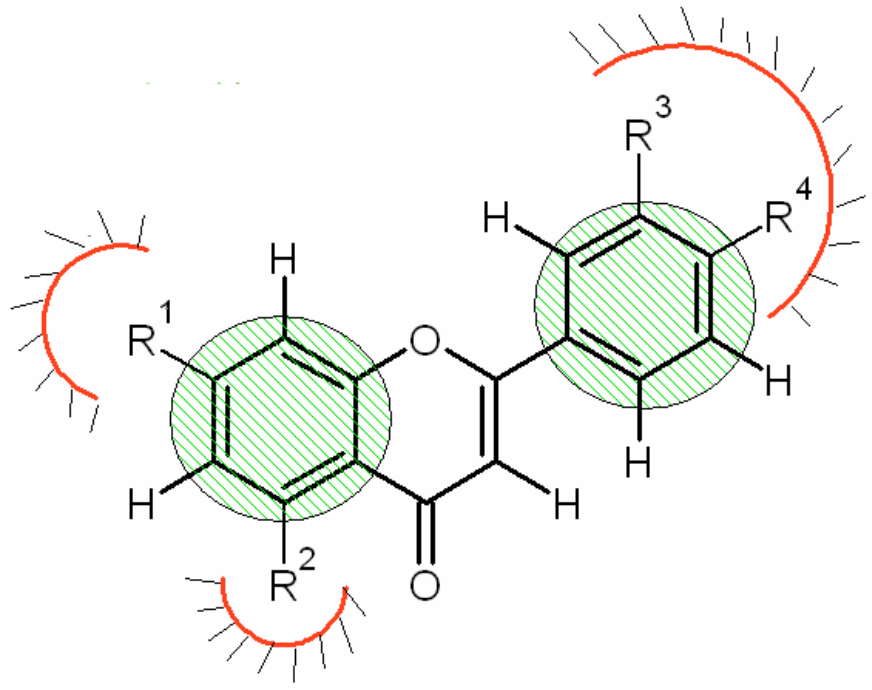
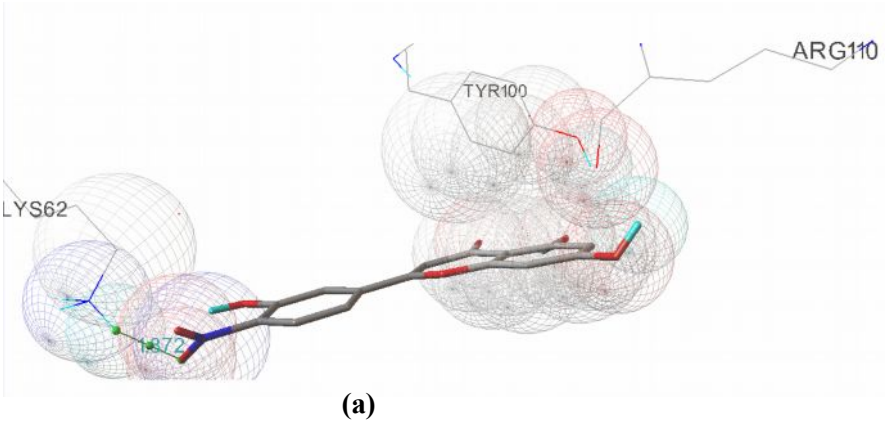
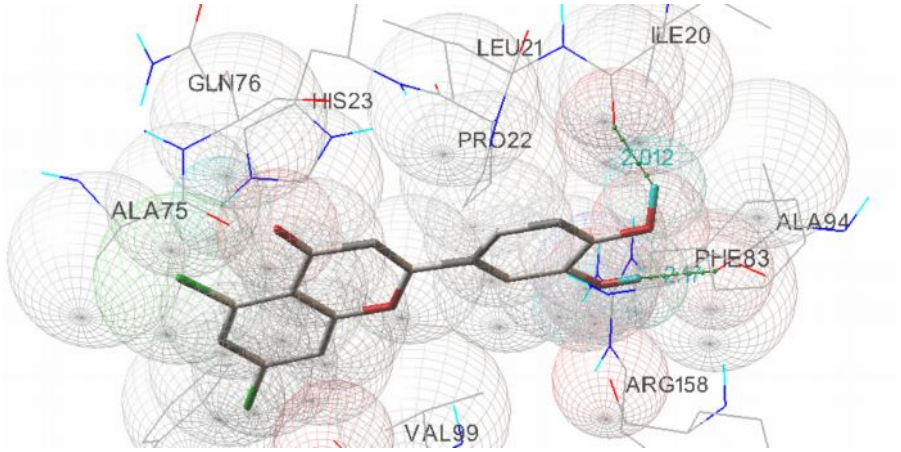


Figure 4. General structure of designed compounds (Green circles show charge transfer sites and red lines present effective sites in LogP balance and H-bonds).



(a)



(b)

Figure5. Docked structures of Comp 11(a), Comp 12 (b) in Model of HpFabZ enzyme. Our designed compounds are displayed as sticks, and Hydrogen bonds are represented with dashed green lines. (Docking study by using ADT program and HpFabZ enzyme obtained from PDB server).

Table2. Docking results by using AutoDock4 software

Compounds	Binding Energy Site 1[(1)]	Ki Site 1[(2)]	Binding Energy Site 2[(3)]	Ki Site 2[(4)]
1	-5.24	145	-7.47	3.32
2	-4.90	258	-7.61	2.66
3	-4.76	321	-7.78	1.98
4	-4.77	320	-7.47	3.34
5	-3.95	998	-7.63	2.56
6	-4.64	397	-7.15	5.75
7	-4.85	277	-7.47	3.37
8	-4.10	988	-5.94	44.38
9	-4.32	675	-6.77	10.88
10	-4.79	307	-7.05	6.83
11	-5.47	97	-7.75	2.08
12	-5.28	135	-8.62	0.478
13	-5.01	212	-7.65	2.45

1) The predicted binding energy (Tunnel between A and B chains) (Kcal/mol)
2) The predicted inhibitory constant (Tunnel between A and B chains) (10^{-6} molar)
3) The predicted binding energy (C side chain) (Kcal/mol)
4) The predicted inhibitory constant (C side chain) (10^{-6} molar)

Based on the results we come to these conclusions:
1- Regarding that hydroxyl and nitro moieties have H-bond site and they have the best inhibitory effects (Comp. 11, 12).
2- The lipophilic moieties due to their charge transfer interaction have better Ki than *luteolin*. (comp. 12)
3- The results of comp.5 and 8 show that there is no enough space in R2 position in both active site pockets due to the existence of large moieties.
All in all the results anticipate that the optimized structure that can be tolerated by the active site should only have small moiety (hydroxyl and chlorine) in R2 position plus results exhibit that the H-bond role is more crucial than the charge transfer interaction in tunnel A and B active site. Meanwhile, the designed agents bind into a C chain pocket through hydrophobic interactions. The compounds stay in the right place via the hydrophobic and charge transfer interactions between their phenol rings and residues Leu21, Pro22, HIS23, GLN76, Val99 and Phe83. Besides the hydrophobic and charge transfer interactions, the binding of compound 12 is also assisted by some hydrogen bonds formed directly via ALA94 and Ile20. It should be mentioned that totally the C side chain has more significant role than the tunnel between A and B chains as interaction site. By considering the obtained results and also focusing on these observations we can expect the designed compounds to be as novel HpFabZ inhibitors which have the power to act as anti helicobacter pylori agents.

Conclusion

To sum up, in this study a luteolin compound collection was built in order to study whether there are inhibitive compounds against HpFabZ enzyme by using the molecular docking method. In addition, the performance of Autodock was evaluated in this study to make sure that the result is reasonable and reliable. In conclusion, luteolin derivatives are potential inhibitors. The present study warrants further phytochemical investigations and in vitro studies in connection with anti H.pylori effects of the traditional Iranian medicinal plant called Biebersteinia *multifida* DC, which are currently being conducted by our research team.

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[15] Docking studies were performed using Autodock software Version 4.0.

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