International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.4, No.2, pp 733-738, April-June 2012

Design and Molecular Docking Studies of luteolin derivatives, from Biebersteinia *multifida* DC., as novel HMG-CoA reductase inhibitors

Alireza Nematollahi[1]*, Noushin Aminimoghadamfarouj[1], Mohammad Reza Jalilvand [1], Seyed Ali Vakili[2]

[1]Research Center of natural products safety and medicinal plants, North Khorasan University of Medical Sciences, Bojnurd, Iran.

[2]Department of Food and Drug, North Khorasan University of Medical Sciences, Bojnurd, Iran.

***Corres.author: alireza_nemat@yahoo.com**
Phone No: +98-584-2248003

Abstract: Natural products and medicinal plants have been broadly used in traditional medicines and are an important source for new drug discovery. On the other hand, widespread molecular modeling studies based on the crystallographic data have been used to help designing novel synthetic analogues of the natural products. Therefore, in this study, we have proposed the use of molecular modeling and docking techniques to design some novel inhibitors of HMG-CoA reductase based on the one of the most effective flavones, *luteolin*, from a traditional Iranian medicinal plant called Biebersteinia *multifida* DC. To achieve this point, 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 HMG-CoA reductase enzyme 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 HMG-CoA reductase inhibitors and act as novel antihyperlipidemic agents. Compound 13 was found to enclose the lowest binding free energy and reasonable pose inside the binding site. 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 HMG-CoA reductase inhibitors would be more practiced.
Keywords: HMG-CoA reductase, Natural products, *flavones*, Molecular modeling, Docking.

Introduction

Coronary heart disease (CHD) is the most important reason of death in the United States [1]. Agreed that hypercholesterolemia is a prominent risk factor for CHD, extensive efforts have been undertaken to lessen this condition [2]. The typical of treatment for hypercholesterolemia is the use of HMG-CoA reductase inhibitors, statins, which block the rate-limiting step of cholesterol biosynthesis [3].

Atorvastatin is the best selling branded lipid lowering drug in the world. Conversely, disorders of muscles, ranging in severity from asymptomatic creatine kinase (CK) elevation to rhabdomyolysis, are the most discussed adverse effects connected with statins [4]. 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 diseases [5]. Also the flavonoids constituents of this plant were reported [6]. The largest parts of the flavonoids belonged to the luteolin and its derivatives. Moreover there is a report that cholesterol synthesis may be blocked by the luteolin through the inhibition of HMG-CoA reductase enzyme, the same action as statin drugs such as atorvastatin [7].Based on this precedent, we undertook a discovery endeavor to recognize the functional groups and main interactions of the potent newly designed HMG-CoA reductase inhibitors which might be useful in helping patients to reach LDL-C reduction goals. We carried out of this aim through modification of the partition coefficient index of the luteolin (Figure 1) and increasing the interaction sites of the luteolin with a focus on altering the hydroxyl moieties. Regarding these issues, the structures of desired inhibitors were built 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 HMG-CoA reductase (Figure 2) was obtained from the Protein Data Bank (PDB) server (PDB entry: 2Q1L). Then Docking calculations were carried out using AutoDock program (Ver4).

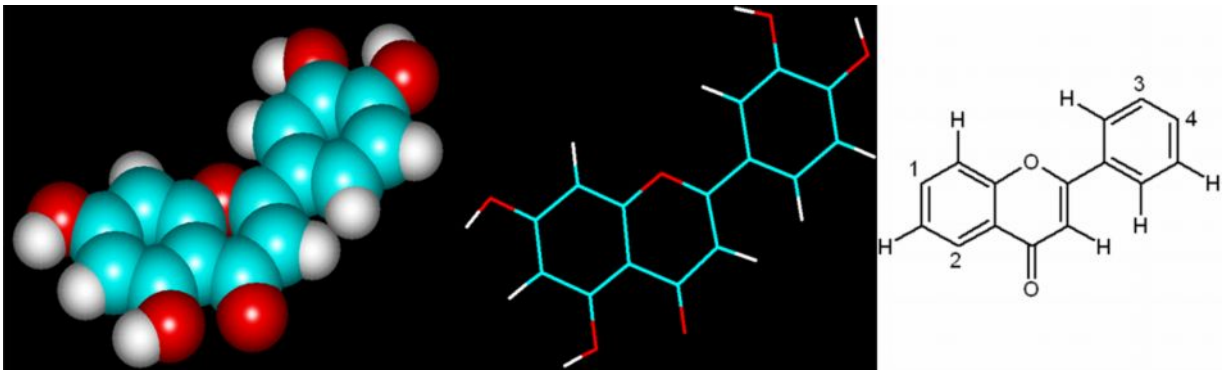

Figure1. Molecular structure of luteolin. The global minimum energy conformation (space-filling and sticks model) is shown.

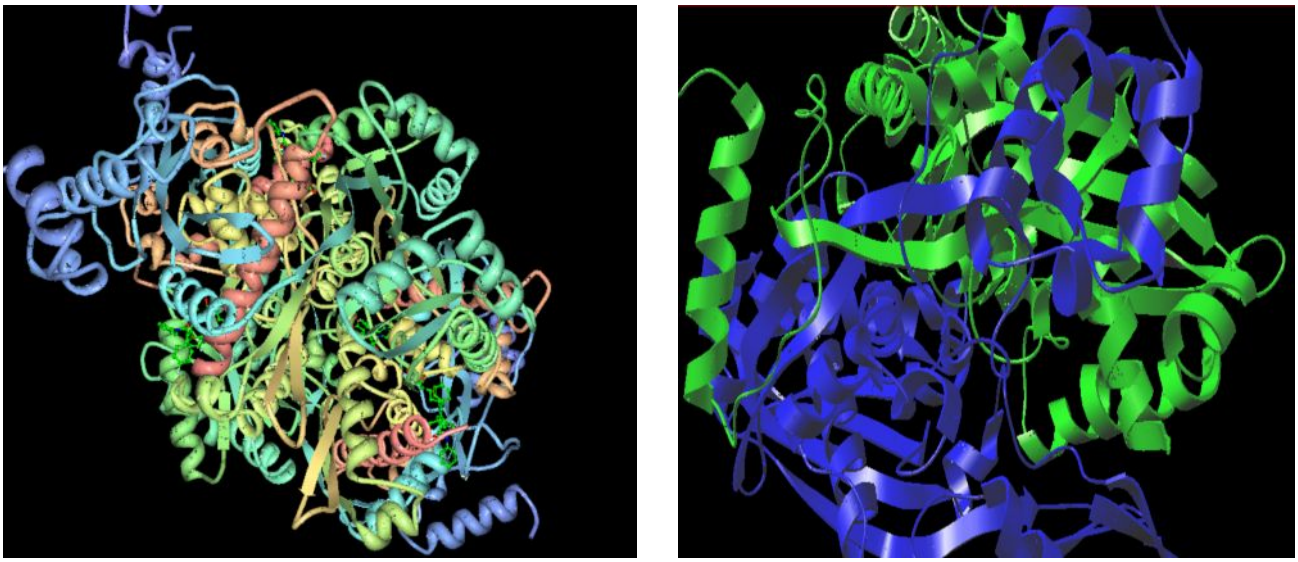

Figure2. The structure of HMG-CoA Reductase enzyme (2Q1L).

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 HMG-CoA reductase inhibitor compounds and our potent compound was performed.

b) Docking study:

Docking studies were carried out by using the program AUTODOCK 4 [8]. 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 HMG-CoA Reductase (PDB entry: 2Q1L), a tetramer resolved at 2.3 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 C and D chains were kept whereas the A, B chains were unmerged and deleted because the tetramer was symmetrical. It should be mentioned that the binding site is composed of C and D chains together.

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 center was placed on the active site which was obtained by trial and error and the previous study done by JA Pfefferkorn et al (Bioorganic & Medicinal Chemistry Letters 2007) and 50x50x50 points with grid spacing of 0.375 were calculated (Figure 3).

Table1. Structures of derivatives

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

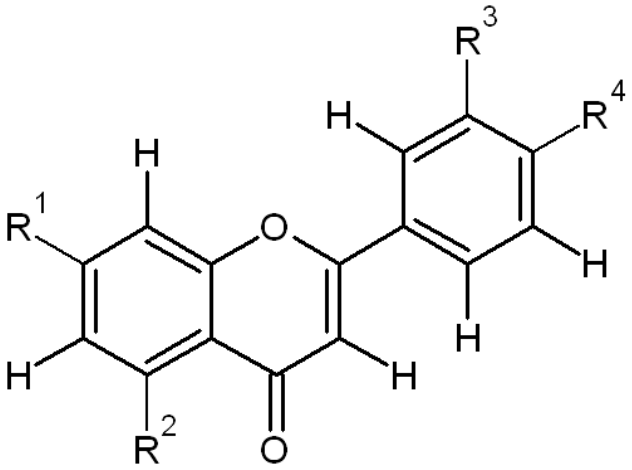

Figure. The active site and grid box of the enzyme.

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).

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 HMG-CoA reductase 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 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 HMG-CoA reductase enzyme. To verify and compare the model we docked *luteolin* as known HMG-CoA reductase inhibitor as well as the designed agents. The free binding energy of the fourteen selected inhibitors scored by Autodock ranged from –5.19 kcal·mol^{-1} to –6.58 kcal·mol^{-1}. The inhibition constant of these inhibitors 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.13 and Comp.14), in the active site of HMG-CoA reductase enzyme were shown by ADT software (Figure5) [6]. This molecular modeling shows that in Comp.13 (Figure 5a), the oxygen of the hydroxyl group in R4 position makes noticeable hydrogen bonding interactions with the NH_2 of LYS692 (distance=1.785A^0). Also the hydrogen binding interaction between oxygen of the carbonyl of the GLY560 and hydrogen of the hydroxyl group in R2 position (distance= 1.944A^0) is shown. In compound 14, there are three hydrogen bonds. The hydrogen bonding of NH_2 groups of LYS691 and ASN755 with the oxygen of the hydroxyl in R1 position (distance= 1.812 and 1.976 A^0) are mentioned (Figure 5a). In addition the hydrogen of the hydroxyl in R1 position interacts with the oxygen of the carbonyl of the GLU559 (distance= 2.039A^0). The predicted binding energies and inhibitory constant of these inhibitors are listed in Table 2.

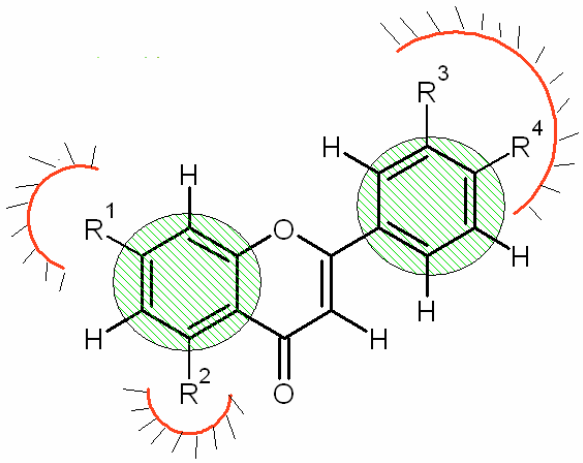

Figure 4. General structure of designed compounds (Green circles show charge transfer sites and red lines present effective in LogP balance and H-bonds.

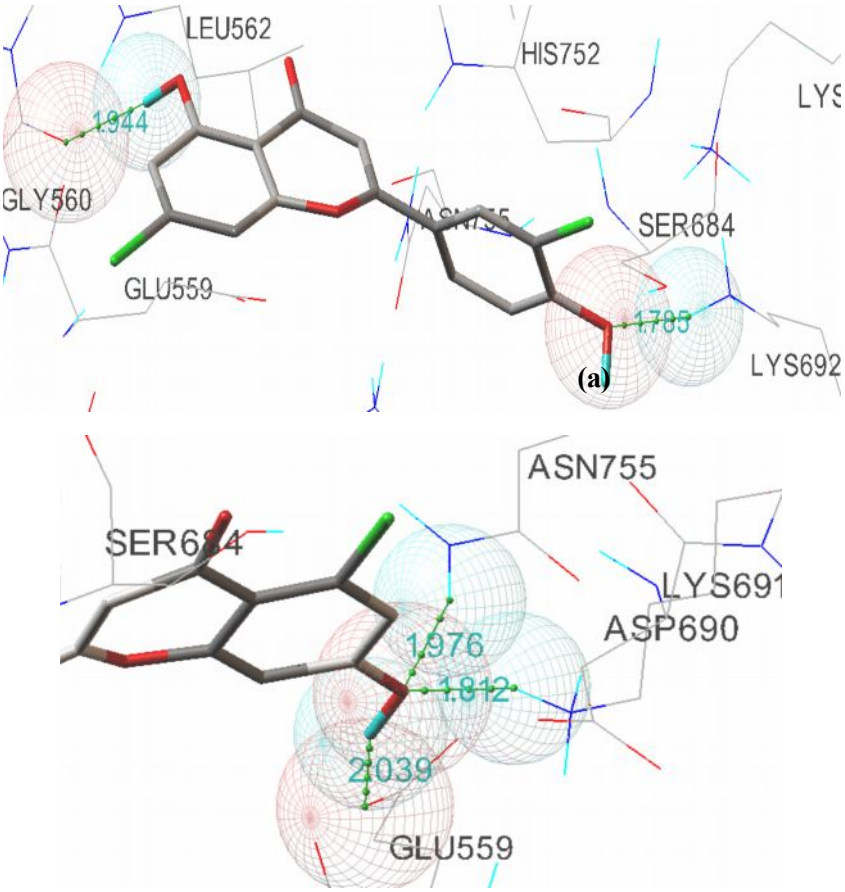

Figure5. Docked structures of Comp 13(a), Comp 14 (b) in Model of HMG-CoA reductase enzyme. Our designed compounds are displayed as sticks, and Hydrogen bonds are represented with dashed green lines. (Docking study by using ADT program and HMG-CoA reductase enzyme obtained from PDB server).

Table2. Docking results by using AutoDock4 software

Compounds	Binding Energy[1]	Ki[2]
1	-6.39	2.1
2	-6.29	2.4
3	-5.78	5.7
4	-5.95	4.3
5	-5.19	15
6	-6.06	3.6
7	-6.27	2.5
8	-6.03	3.7
9	-6.33	2.2
10	-6.02	3.8
11	-5.28	13.3
12	-5.42	10.7
13	-6.58	1.5
14	-6.47	1.8

1) The predicted binding energy (Kcal/mol)
2) The predicted inhibitory constant (10^{-6} molar)

Based on the results we come to these conclusions:
1- Regarding that hydroxyl moieties have H-bond site and they have the best inhibitory effects.
2- The lipophilic moieties due to their charge transfer interaction have better Ki than *luteolin*. (comp. 13, 14)
3- The results of comp.11 and 12 show that there is no enough space in active site pocket due to the existence of both moieties. (H-bond site and lipophilic)
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 each side plus results exhibit that the H-bond role is more crucial than the charge transfer interaction.
By considering the obtained results and also focusing on these observations we can expect the designed compounds to be as novel HMG-CoA reductase inhibitors which have the power to act as antihyper lipidemic agents.

Conclusion

To sum up, in this study a luteolin compound collection was built in order to study whether there are inhibitive compounds against HMG-CoA Reductase 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 antihyperlipidemic effects of the traditional Iranian medicinal plant called Biebersteinia *multifida* DC, which are currently being conducted by our research team.

Acknowledgment
We thank Professor Arthur J. Olson for his kindness in offering us the Auto Dock 4 program. The financial support of North Khorasan University of Medical Sciences in performing the computational studies is gratefully acknowledged.

References

[1] American Heart Association Statistics Committee and Stroke Statistics Subcommittee, 2007 Update. Dallas, TX: American Heart Association, 2007.
[2] J.C. LaRosa, S.M. Grundy, D.D. Walters, C. Shear, P. Barter, J.-C. Fruchart, A.M. Gotto, H. Greten, J.J.P. Kastelein, J. Shepard and N.K. Wenger. N. Engl. J. Med., 352 (2005), p. 1425.
[3] Schwartz G.G., Olsson A.G., Ezekowitz M.D., Ganz P., Oliver M.F., Waters D., Zeiher A., Stern T. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes the MIRACL study: A randomized controlled trial (2001) Journal of the American Medical Association, 285 (13), pp. 1711-1718.
[4] D.J. Graham, J.A. Staffa, D. Shatin, S.E. Andrade, S.D. Schech, L. La Grenade, J.H. Gurwitz, K.A. Chan, M.J. Goodman and R. Platt. J. Am. Med. Assos, 292 (2004), pp. 2585–2590
[5] Hassan Farsam , Massoud Amanlou, Ahmad Reza Dehpour, Fereshteh Jahaniania, Anti-inflammatory and analgesic activity of Biebersteinia multifida DC. root extract. Journal of Ethnopharmacology Volume 71, Issue 3, August 2000, Pages 443-447
[6] V.B Omurkamzinova, N.D Maurel and T.N Bikbulatova, Flavonoids from Biebersteinia multifida. Khimiya Prirodnykh Soedinenii, 5 (1991), pp. 720–721
[7] Gebhardt R., Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (Cynara scolymus L.) extracts., J Pharmacol Exp Ther. 1998 Sep;286(3):1122-8.
[8] Docking studies were performed using Autodock softwareVersion 4.0.

*****