Use of Virtual Screening, Flexible Docking, and Molecular Interaction Fields To Design Novel HMG-CoA Reductase Inhibitors for the Treatment of Hypercholesterolemia†

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Dietary changes associated with drug therapy can reduce high serum cholesterol levels and dramatically decrease the risk of coronary artery disease, stroke, and overall mortality. Statins are hypolipemic drugs that are effective in the reduction of cholesterol serum levels, attenuating cholesterol synthesis in liver by competitive inhibition regarding the substrate or molecular target HMG-CoA reductase. We have herewith used computer-aided molecular design tools, i.e., flexible docking, virtual screening in large data bases, molecular interaction fields to propose novel potential HMG-CoA reductase inhibitors that are promising for the treatment of hypercholesterolemia.

1. Introduction

Coronary artery disease, together with cancer and AIDS, is one of the most investigated diseases in medicinal chemistry.[1−3] The risk of overall mortality and stroke can be dramatically reduced by dietary changes.[4] The mevalonate pathway is responsible in humans for the endogenous synthesis of cholesterol. The enzyme HMG-CoA reductase, important molecular target of the drugs known as statins, catalyzes the reaction of the mevalonate pathway converting HMG-CoA to mevalonate. Statins are effective in reduction of cholesterol serum levels, attenuating cholesterol synthesis in liver by competitive inhibition regarding the substrate HMG-CoA.[5]

Two classes of HMG-CoA reductase appears to arise during the evolution process from a common ancestor, i.e., the HMG-CoA reductase of eukaryotes (class I) and procaryotes (class II). In humans the enzyme is formed by a highly conserved carboxyl-terminal catalytic domain and a poorly conserved amino-terminal membrane anchor domain consisting of two to eight inferred transmembrane helices.[6] The HMG-CoA reductase enzyme is a single polypeptide chain of 888 amino acids. The first 339 residues are bound to the membrane and they are located at the endoplasmic reticulum. The catalytic domain of the protein (residues 460−888) is found in the citoplasm. A linker region (residues 340−459) plays a role in connecting these two portions of the protein.[7]

Several statins are in late-stage clinical development. Compactin, lovastatin, and pravastatin, which are fungal statins, act as competitive HMG-CoA reductase inhibitors, yielding a market of 15 billion dollars.[8] Compactin was an antibiotic product of *Penicillium brevicompactum*[9] and *Penicillium citrinum*.[10] Endo as well as Alberts et al.[11,12] independently discovered the most active methylated form of compactin (lovastatin) in broths of *Monascus ruber* and *Aspergillus terreus*, respectively. Lovastatin was approved by the FDA in 1987.

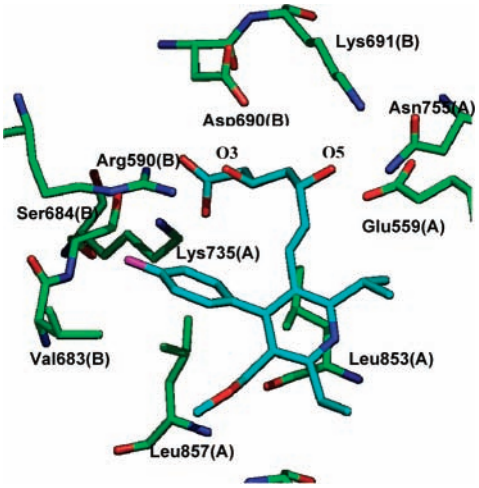

Figure 1. Orientation of cerivastatin in the active site of HMG-Coa reductase showing the residues, from chains A and B, responsible for the main interactions with the ligand.

Symvastatin is a semisynthetic lovastatin derivative. Fluvastatin, derived from indole and atorvastatin derived from pyrrole are fully synthetic statins. Each one shares a HMG-CoA-like moiety, which may be present in the substrate as well.

There has been recent elucidation[4,13] of structures of the catalytic domain of human HMG-CoA reductase with ligands. Complexes of this enzyme with several statins, such as mevastatin, cerivastatin, and rosuvastatin are available in the Protein Data Bank. We note that compactin is no longer commercially available (as cerivastatin). It is, however, a classic and potent inhibitor of the HMG-CoA reductase. The protein is a tightly associated tetramer with bipartite active sites formed by residues of two neighboring monomers.[4]

The HMG-CoA reductase monomer structure is composed of three domains: an N-terminal helical domain (N-domain), a large domain (L-domain) whose architecture is close to a prism, and a small domain (S-domain) that is inserted into the

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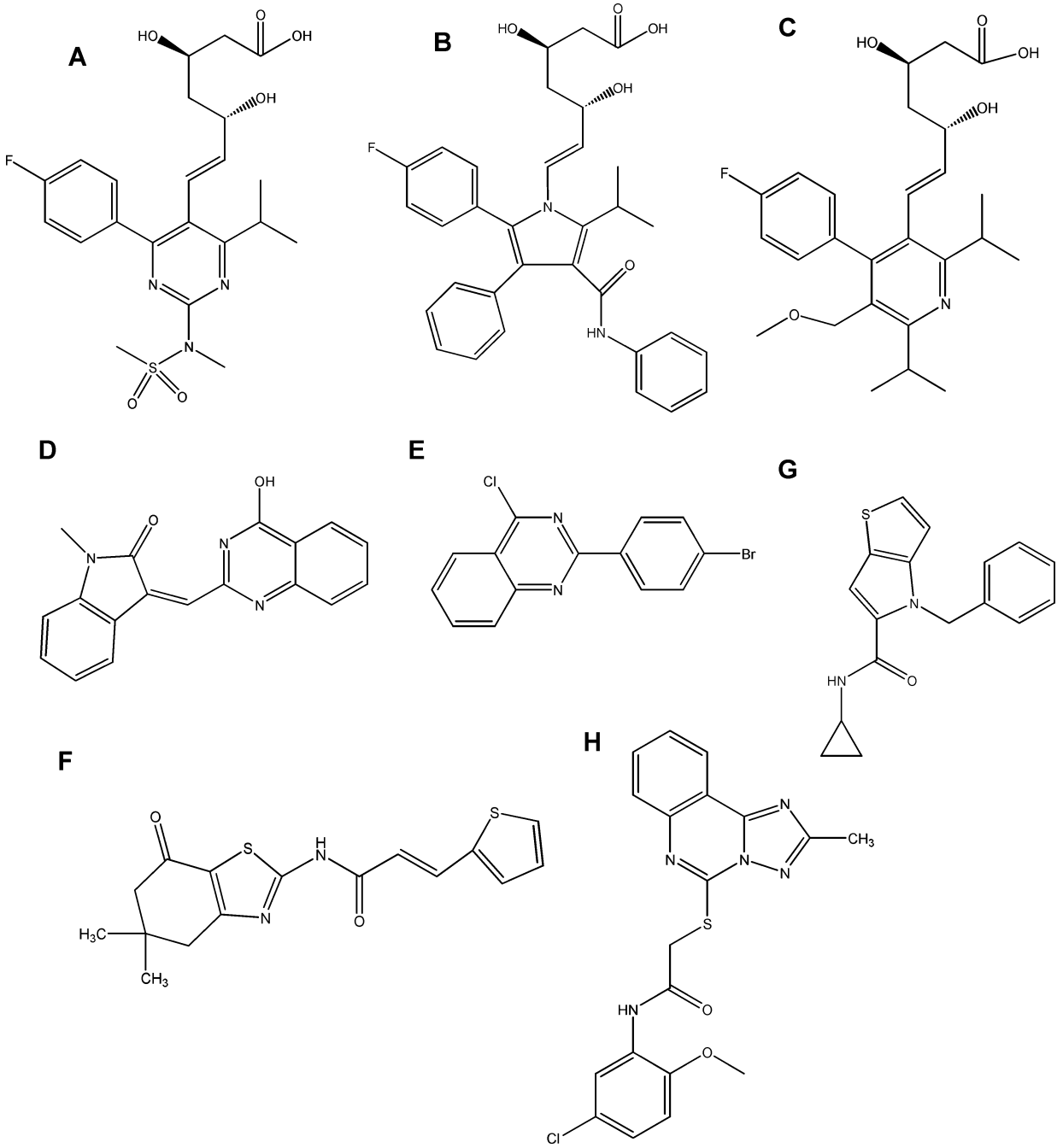

Figure 2. Structures of rosuvastatin (A), atorvastatin (B), cerivastatin (C), and other statin derivatives: proposal 1 (D), proposal 2 (E), proposal 3 (F), proposal 4 (G), proposal 5 (H). The IUPAC names are as follows: rosuvastatin, (*E*,3*R*,5*S*)-7-(2-(*N*-methyl-(*N*-methyl)sulfonamide)-4-(4-fluorophenyl)-6-isopropypyrimidin-5-yl)-3,5-dihydroxyhept-6-enoic acid; atorvastatin, (3*R*,5*R*)-7-(3-(phenylcarbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4-phenyl-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid; cerivastatin, (*E*,3*R*,5*S*)-7-(4-(4-fluorophenyl)-2,6-diisopropyl-5-(methoxymethyl)pyridin-3-yl)-3,5-dihydroxyhept-6-enoic acid; proposal 1, 3-(4-hydroxy-quinazolin-2-ylmethylene)-1-methyl-1,3-dihydro-indol-2-one; proposal 2, 2-(4-bromophenyl)-4-chloroquinazoline; proposal 3, *N*-(5,5-dimethyl-7-oxo-4,5,6,7-tetrahydrobenzothiazol-2-yl)-3-thiophen-2-ylacrylamide; proposal 4, 4-benzyl-4*H*-thieno[3,2-*b*]pyrrole-5-carboxylic acid cyclopropylamide; proposal 5, *N*-(5-chloro-2-methoxyphenyl)-2-(2-methyl[1,2,4]triazolo[1,5-*c*]quinazolin-5-ylsulfanyl)acetamide.

L-domain. The binding site is located in the L-domain. NADP-(H) binds predominantly to the S-domain. S- and L-domains are connected by a loop stabilized by interactions with residues from the neighboring monomer. This loop is formed by the residues 682−694 and is called the "*cis-loop*" because it contains a *cis*-peptide between residues C688 and T689. The *cis-loop* is essential in the formation of the binding site of the enzyme.

In this work we explore virtual screening, flexible docking, and molecular interaction fields to perform computer-aided molecular design of novel potential HMG-CoA reductase inhibitors for the treatment of hypercholesterolemia.

2. Methodology

The complexes of HMG-CoA with inhibitors were analyzed and computer-aided designed using the Insight II[14] package. Drug-like and physical-chemical properties were calculated using DS ViewerPro 5.0 software.[15] Docking simulations were performed with the GOLD 3.1.1[16] software, which performs

TABLE 1: Goldscores for the 3 Statins Investigated and 5 Molecules Selected by Virtual Screening with HMG-CoA Reductase Active Site, as Well as Reported IC50 Values for Three Statins

	ligands			proposals				
	rosuva-statin	atorva-statin	ceriva-statin	1	2	3	4	5
score	75.2	51.8	37.6	57.5	50.6	43.6	56.7	45.2
IC50	5nM	8nM	10nM					

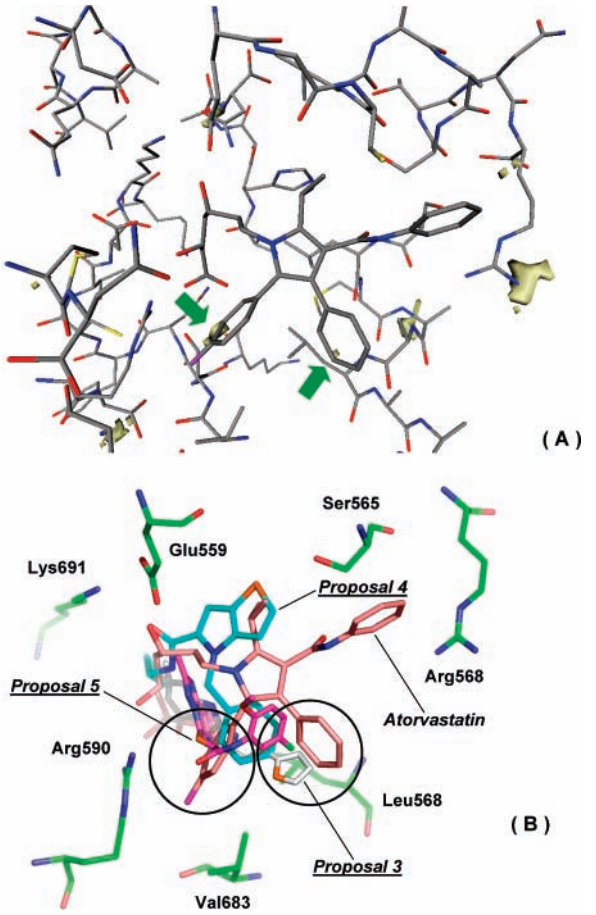

Figure 3. (A) Molecular interaction fields generated using a DRY probe with the HMG-CoA reductase active site. Arrows indicate the most favorable hydrophobic binding sites. Energy contours are shown in phase with the crystallographic structure of atorvastatin (PDB code 1HWK). (B) GOLD solutions obtained for proposals 3, 4, and 5, superimposed with the crystallographic orientation of atorvastatin inside the HMG-CoA reductase active site. Aromatic rings of proposals 3 and 4 fit with the MIFs shown in (A), whose regions are marked by circles. Selected residues of the enzyme active site are shown.

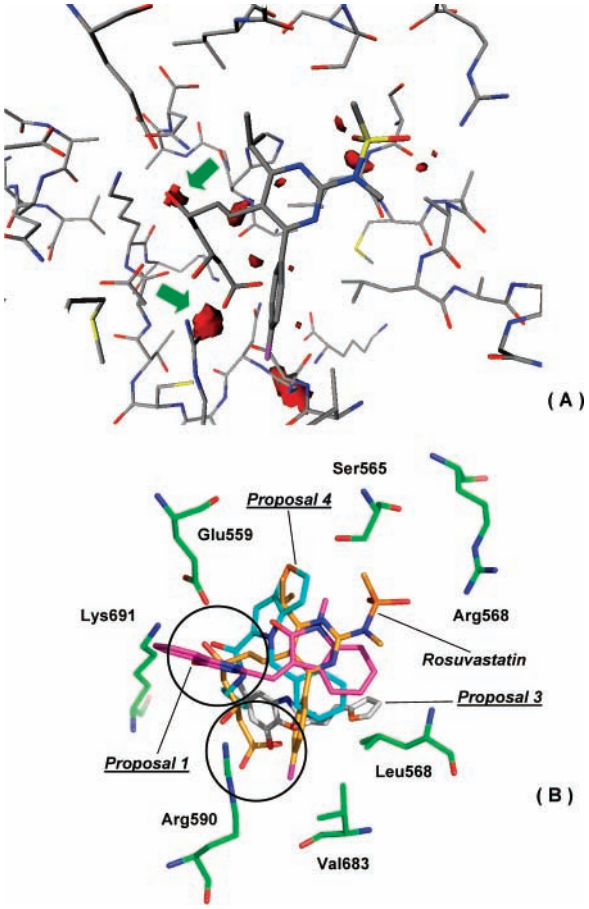

Figure 4. (A) Molecular interaction fields generated using a carbonyl probe with the HMG-CoA reductase active site. Arrows indicate the most favorable carbonyl binding sites. Energy contours are shown in phase with the crystallographic structure of rosuvastatin (PDB code 1HWL). (B) GOLD solutions obtained for proposals 1, 3, and 4, superimposed with the crystallographic orientation of rosuvastatin inside the HMG-CoA reductase active site. Carbonyl groups of proposals 3 and 4 fit with the MIFs shown in (A), whose regions are marked by circles. Selected residues of the enzyme active site are shown.

flexible docking using a genetic algorithm. The parameters used in this algorithm were originally optimized from a set of 305 complex structures with coordinates deposited in the Protein Data Bank (PDB). Among the parameters available in the program, we used a population equivalent to 100, 100000 operations, 95 mutations, and 95 crossovers. These calculations were performed inside a sphere of 15 Å radius centered at carbon ξ of the R590 side chain of chain B inside the catalytic domain of HMG-CoA reductase. We have used as a training set the structures in complex with cerivastatin, rosuvastatin, and atorvastatin inhibitors (PDB codes 1HWJ, 1HWL and 1HWK, respectively), which are molecules of a same series of statins.[4] Previous to the docking calculations, hydrogen atoms were added and oriented after the removal of cerivastatin and crystallographic waters of the complex structure, and atomic charges and potentials were assigned to the protein using Insight II. Five orientations of highest score for each compound were then selected using the score function denominated GoldScore. On the basis of this function, GOLD classifies the orientations of the molecules in a decreasing order of affinity (score or fitness) with the ligand site of the receptor. The top-ranked solution for each proposal was used for further analysis. The iResearch Library was used for screening 100000 drug-like compounds. Molecular interaction field analyses were performed using the Almond module of the Sybyl 7.3 package.[17]

3. Results and Discussion

3.1. Statins−HMG-CoA Reductase Interactions. Statins show a common scaffold necessary to bind HMG-CoA reducatse, and one of them could be selected to discuss the main interactions with this enzyme. When the complex structure of cerivastatin with HMG-CoA reductase (PDB code 1HWJ),[4] one of the most representative complexes of this class of enzyme, is analyzed, the molecule is placed into a narrow pocket that corresponds to the binding site of the substrate (HMG-CoA) in the catalytic domain of the enzyme (Figure 1). The HMG-CoA-like moiety of cerivastatin is responsible for several polar

TABLE 2: ADME Properties Related to the "Lipinski's Rule of Five" for Atorvastatin, Rosuvastain, Cerivastatin, and the Five Proposals

	ligands			proposal				
ADME properties	atorvastatin	rosuvastatin	cerivastatin	1	2	3	4	5
mol wt	558.25	481.16	459.24	303.3	318.0	332.5	296.1	413.9
no. of H bond acceptors	6	9	7	4	4	5	2	7
no. of H bond donors	4	3	3	1	0	1	1	1
log *P*	5.05	2.4	4.18	3.62	4.6	3.98	3.02	4.76

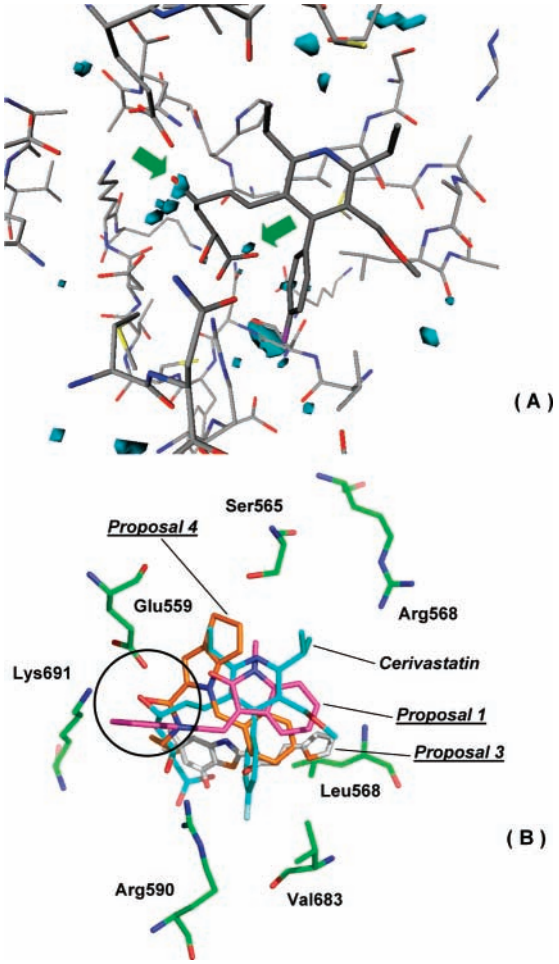

Figure 5. (A) Molecular interaction fields generated using a water probe with the HMG-CoA reductase active site. Arrows indicate the most favorable hydroxyl binding sites. Energy contours are shown in phase with the crystallographic structure of cerivastatin (PDB code 1HWJ). (B) GOLD solutions obtained for the proposals 1, 3, and 4, superimposed with the crystallographic orientation of cerivastatin inside the HMG-CoA reductase active site. The hydroxyl group of proposal 4 fit with the MIFs shown in (A), whose region is marked by a circle. Selected residues of the enzyme active site are shown.

interactions with the enzyme, especially O5 and O3 hydroxyl groups. The most important residues seem to be Glu559 (chain A), Lys691 (chain B), and Asn755 (chain A) that form a hydrogen-bonding network with the O5 hydroxyl group as well as R590 (chain B) and D690 (chain B) that interact with O3 hydroxyl group. The carboxy group shows polar contacts with residues Ser684 (chain B) and Lys735 (chain A). The hydrophobic ring structures shows van der Waals contacts with side chains of Leu853 (A), Leu857 (A), and Val683 (B) residues.

The large number of hydrogen bonds and salt bridges results in both charge and shape complementarity between the enzyme active site and the HMG-CoA-like moiety of statins. Identical bonding interactions are observed between the enzyme and the substrate, and presumably with the reaction product mevalonate as well. Because mevalonate is released from the active site, it is likely that only several of their interactions with HMG-CoA reductase are stabilized. These observations suggest that the hydrophobic groups of the inhibitors are responsible for the nanomolar inhibition constant (K_i) values observed for them. The surface complementarity between the active site of HMG-CoA reductase and the hydrophobic ring structures of statins is observed in general in the enzyme−inhibitor complexes.[4]

3.2. Docking Simulations and Molecular Interaction Field Analyses. Flexible docking simulations were performed to investigate binding modes of the molecules identified in this work, as well as to predict the efficiency of novel compounds for inhibition of HMG-CoA reductase enzyme activity. These novel potential HMG-CoA reductase inhibitors were obtained from virtual screening of 100000 drug-like compunds of the IResearch Library database. For docking simulations, a dimeric structure was used, which includes chains A and B from HMG-CoA reductase, because this enzyme forms a bipartite active site, where two neighboring momomers are relevant for making interactions with statins. We have selected five ligands with the highest Gold scores. Structures of all the ligands here investigated are shown in Figure 2. Docking simulations were also performed with the structures of cerivastatin, atorvastatin and rosuvastatin to compare the orientations of highest scores obtained using GOLD with the crystallographic data and the IC50 values reported from the literature.[4] Table 1 shows the GoldScores for these statins and the five selected potential HMG-CoA reductase inhibitors. Table 1 also reveals a good agreement between the high ranking gold score of the three statins here investigated and their respective IC50 values. Figures 3−5 show the orientations of atorvastatin, rosuvastatin and cerivastatin, respectively, and the five highest ranked ligands obtained from virtual screening of our large database iResearch Library using GOLD.

Molecular interaction field (MIF) studies were performed for all the systems investigated, using three prototypical, probes: hydrophobic (DRY), carbonyl and water probes, which should yield the principal intermolecular interactions, and the results are shown in Figures 3−5. The energy contours are superimposed with three important statins, considering the statin with best interaction profile for each probe analyzed, whose complexes have been extracted from PDB: DRY contours in phase with atorvastatin (PDB code 1HWK), carbonyl contours are shown in phase with rosuvastatin (PDB code 1HWL), water contours in phase with cerivastatin (PDB code 1HWJ). The top-ranked orientations for the best five ligands selected from the database are superimposed to investigate their structural moieties that fit with the MIFs.

The results obtained point out the thiazol moiety of proposal 3 as well as the phenyl ring of proposal 4 fits the hydrophobic binding site generated by the DRY probe (Figure 3). In Figure 4, it can also be observed that the cetonic carbonyl of proposal 3 as well as the carbonyl group of proposal 4 fits the carbonyl MIF (Figure 4). Results also point out that only one carbonyl group of proposal 4 fits the MIF generated using the water probe, which also represents potential H bond donor site (Figure

5). Lys691 is responsible for generating one of the carbonyl binding sites observed in Figure 4, due to its hydrogen bond donor group, whereas Glu559 is responsible for generating one of the hydroxyl virtual receptor sites observed in Figure 5, at the same position observed for the carbonyl binding site discussed above for the Figure 4. Thus, proposals 3 and 4 are the most promising HMG-CoA reductase inhibitors, analyzing only the MIFs and comparing with the orientation of known potent inhibitors of this enzyme (statins). Analyzing the GoldScore values obtained for rosuvastatin, atorvastatin and cerivastatin shows that this function is able to rank these statins according to their activity levels. In this way, all the proposals presented are promising, where proposal 1 shows the highest score value with the HMG-CoA reductase active site (Table 1).

For all of these five proposals, as well as cerivastatin, rosuvastatin, and atorvastatin, the parameters of the "Lipinski's Rule of Five" were calculated,[18] which are followed in most of the oral drugs available, such as molecular weight lower than 500, log *P* lower than 5, number of hydrogen bond acceptors equal or less than 10, and number of hydrogen bond donors equal or less than 5. None of these proposals here investigated violated any of these four parameters of the "Rule of Five" (Table 2).

4. Conclusions

In the present work, we have proposed three novel HMG-CoA reductase inhibitors as potential hypolipemic drugs. We have used virtual screening in a large database, flexible docking, and molecular field interactions which yield 5 proposals of which proposal 4 is the most promising and could be a novel candidate as a pharmaceutical for the treatment of hypercholesterolemia.

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