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Molecular docking of the highly hypolipidemic agent α-asarone with the catalytic portion of HMG-CoA reductase

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Abstract—Docking experiments using a number of published crystal structures of HMG-CoA reductase with the potent hypocholesterolemic agent α-asarone are described. The results indicate that α-asarone binds in the enzyme's active site. The methoxy groups play a key role in the binding and probably also in its biological activity, as shown by extensive SAR studies reported for analogues of α-asarone. The docking results will be valuable for the structure-based design of novel hypolipidemic agents.
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Research in new lipid-lowering drugs is very active because hypercholesterolemia[1] and high levels of serum LDL-cholesterol[2] have been generally recognized to contribute significantly to the progression of atherosclerosis. Cholesterol biosynthesis in the body is mainly regulated in the liver by the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (HMGR).[3] Inhibition of this enzyme has proven to be the most efficient therapy for hyperlipidemia, since the enzymatic transformation of HMG-CoA to mevalonate represents one of the key steps in the metabolic pathway toward the biosynthesis of isoprenoids and sterols, such as cholesterol.[4] Among the most effective hypocholesterolemic drugs for clinical use today are the statins, which possess an HMG-like moiety linked to a hydrophobic decalin core.[5–7] Synthetic statin-like compounds including an HMG-like moiety have shown significant hypocholesterolemic activity.[8–10]

The structures of the catalytic portion of human HMG-CoA reductase (HMGR) in complex with the substrate and six statins have been recently determined.[11,12] The enzyme forms tetramers and has four actives sites formed by residues of two monomers. The HMG-binding pocket is characterized by the so-called *cis* loop (residues 682–694).[11] A proposed catalytic mechanism suggests that the residues Leu-691 and Glu-559 participate directly in the reduction of the substrate HMG-CoA (Fig. 1).[13]

α-Asarone (Fig. 1) is the main biological active component of the bark extract of *Guatteria gaumeri* Greenman (Annonaceae),[14] a medicinal plant utilized in Mexico to treat hypercholesterolemia and cholelithiasis.[15] In view of its remarkable hypolipidemic activity,[16,17] and more recently of its use as a potential antithrombotic,[18] antimicrobial, insecticidal, nematicidal, and antifeedant agent,[19] α-asarone has attracted widespread interest.

The hypolipidemic action mechanism of α-asarone has recently been established in a rat model as an inhibitory effect on hepatic HMGR.[20] The stimulation of bile secretion was found as an additional mechanism on reducing the serum cholesterol levels and on its associated cholelitholytic activity. Although these results suggest that α-asarone inhibits cholesterol biosynthesis in parallel with the statin mechanism,[12] there is no

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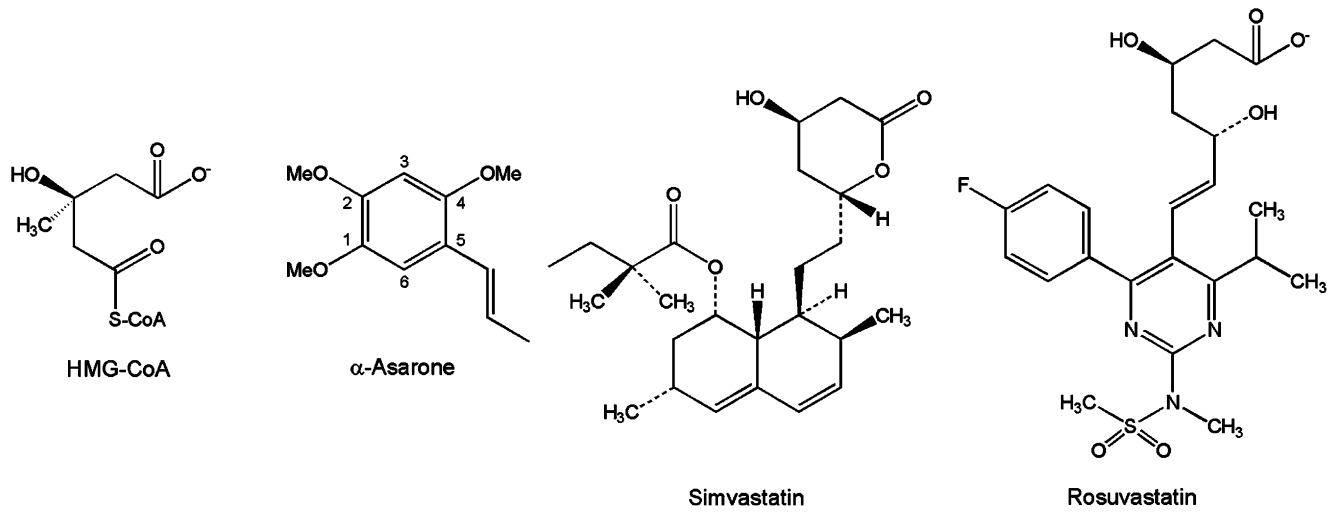

Figure 1. Chemical structures of the HMGR substrate, α-asarone and representative statins used in this study.

evidence as to how α-asarone prevents the binding of HMG-CoA to the target enzyme. In order to gain insight into this problem, to explore the interactions to the active site and consequently, to improve the development of more efficient inhibitors associated with structural features of α-asarone, a study of docking of α-asarone into HMGR and its binding pattern is hereby described.

The docking experiments[21] were performed using the structures of HMGR complexed with the substrate[11] and complexed with statins.[12] The HMG structures were retrieved from the RCSB protein data base (PDB entries 1DQ8, 1HW8, 1HW9, 1HWI, 1HWJ, 1HWK, and 1HWL).[29] Before docking α-asarone, the docking protocol was validated. HMG and the statins were removed from the active site and docked back into the binding site. The root mean square deviation (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation led to RMSD values below or near 1 Å. The statins with the lowest RMSD values were simvastatin and rosuvastatin (Fig. 1). Validation results for the substrate and the statins with the lowest RMSD values are summarized in Table 1. The docked energy, the binding free energy (ΔG), and the inhibition constant (K_i) calculated by AutoDock are also reported, including the experimental biological activity.

The interaction energies calculated for simvastatin and rosuvastatin (Table 1) are in agreement with the recent published experimental results, which shows that rosuvastatin is a more potent inhibitor than simvastatin.[30,31] The docked energy and the binding free energy calculated by AutoDock for rosuvastatin are more favorable than that calculated for simvastatin (Table 1). A comparison with the calculated interaction energy for HMG is not representative since the crystallographic structure of HMG corresponds to the hydrolyzed molecule and not to HMG-CoA.[11]

The above results indicate that the parameters used for AutoDock successfully reproduced the X-ray structures. The ability of AutoDock to reproduce the binding conformation of statins, which have several flexible bonds (Fig. 2) is remarkable.

α-Asarone was first docked with the structure of HMGR in complex with the substrate and in complex with simvastatin and rosuvastatin, the statins for which the lowest RMSD values were obtained in the validation of the docking protocol. The docking results are summarized in Table 2.

The docked energy and the binding free energy estimated for α-asarone when docked into the binding site of simvastatin and rosuvasatin are almost identical, which is not surprising since the binding pocket geometries are very similar (Table 2).[12] The slightly lower energy calculated with the binding pocket of the substrate may be explained by favorable interactions with the residue Leu-862. The latter is absent in the pocket geometry of simvastatin and rosuvastatin (cf. Fig. 4).[12] The differences in the conformation of the side chains of some residues of the binding pocket such as Asp-690, Arg-590, Leu-857, and Leu-853 (cf. Fig. 6)

Table 1. Docking of HMG and statins

Compd	Docked energy (kcal/mol)	ΔG (kcal/mol)	K_i (at 298.15 K)	RMSD	Experimental IC_{50} (M)[a]
HMG	−11.27	−10.74	23.5×10^{-9}	1.19	4000×10^{-9}[b]
Simvastatin	−15.29	−11.70	2.6×10^{-9}	0.89	11.2×10^{-9}
Rosuvastatin	−15.81	−12.34	0.9×10^{-9}	0.81	5.4×10^{-9}

[a] Average IC_{50} of inhibition of HMGR.[30]
[b] This is the Michaelis constant for HMG-CoA.[12]

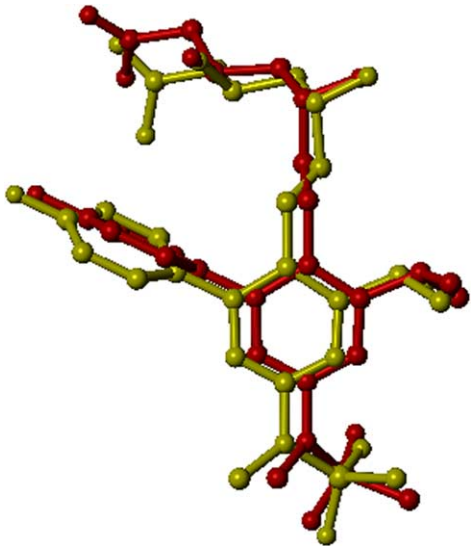

Figure 2. Comparison between the binding position of rosuvastatin found within the crystal structure (yellow) and the conformation predicted by AutoDock (red).

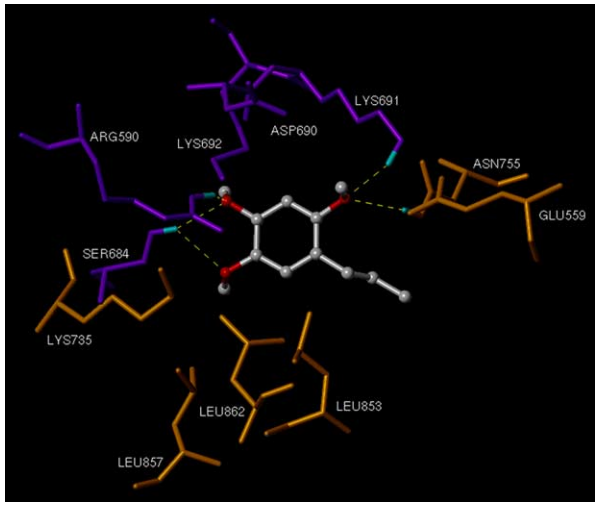

Figure 3. Docking model derived for α-asarone with the catalytic portion of HMGR. The optimized complex of α-asarone with the binding pocket of the substrate is shown. Hydrogen bonds are displayed as yellow dashes. Representative residues within 3.6 Å of α-asarone are shown. Residues from one monomer are orange and those from the other monomer are purple. Nonpolar hydrogens are omitted for clarity except the hydrogens involved in hydrogen bonding.

Table 2. Results of docking experiments of α-asarone with the catalytic portion of human HMGR

Binding pocket	Docked energy (kcal/mol)	ΔG (kcal/mol)	K_i (at 298.15 K)
HMG-CoA	−6.60	−6.02	3.9×10^{-5}
Simvastatin	−6.29	−5.68	6.8×10^{-5}
Rosuvastatin	−6.24	−5.66	7.1×10^{-5}

may also contribute to this difference. The much less favorable binding free energy calculated for α-asarone compared with the free energy of binding calculated for simvastatin and rosuvastatin (cf. Tables 1 and 2) is quite noteworthy. This is in good agreement with the experimental HMGR inhibition trend determined for α-asarone and the statins that show inhibition constants (K_i) and IC_{50} in the nanomolar range for rosuvastatin and simvastatin[30] and IC_{50} in the millimolar range for α-asarone.[20] These observations suggest that the binding mode predicted for α-asarone is reasonable.

AutoDock found one main binding mode for α-asarone in all binding pockets used. α-Asarone binds in the active site of HMGR occupying the region where the 3-hydroxymethylglutaryl moiety of the substrate binds (Fig. 3).

According to the derived docking model, α-asarone interacts with two monomers of the HMGR tetramer. Polar interactions with residues Ser-684, Asp-690, Lys-691, and Lys-692 of the *cis* loop and with Arg-590 of one monomer are observed (Fig. 3). The C-4 methoxy group of α-asarone makes polar contacts with Glu-559 of the second monomer and hydrogen bonds were formed with the side chains of Lys-691 and Asn-755. Additional hydrogen bonds were formed between C-1 and C-2 methoxy groups and Ser-684 and Arg-590 (Fig. 3). The propenyl side chain of α-asarone is close to Glu-559, Leu-562, His-752, Asn-755, and Leu-853, all residues being of one monomer. The aromatic ring is close to Leu-853 and Leu-862, with which van der Waals contacts are made. These interactions seem to be responsible for the affinity of α-asarone with HMGR, thus sterically preventing the substrate from binding. Similar conclusions were obtained when the docking experiments were conducted using the structures of HMGR in complex with compactin, fluvastatin, cerivastatin, and atorvastatin.

The interactions of α-asarone with the catalytic site of HMGR resemble the interactions observed in the substrate and statins complexes. The C-4 methoxy group of α-asarone occupies a region similar to the C-5 carbonyl oxygen of HMG-CoA and to the C-5 hydroxyl group of statins, the latter interacting with the side chains of Lys-691 and Glu-559, which seem to participate directly in the catalysis (Fig. 4).[13] According to the mechanism of catalysis proposed for the reduction of HMG-CoA,[13] which suggests a Glu-559 protonated under physiological conditions, the C-4 oxygen of the methoxy group in α-asarone may also form a hydrogen bond with the carboxylic group of Glu-559.

The C-1 and C-2 methoxy groups of α-asarone have also similar interactions to those of the C-1 carboxylate group of both the substrate and the statins, including hydrogen bonds with Ser-684 and interactions with the polar side chains of Lys-692 and Lys-735.[11,12] The C-1 methoxy group is located in the binding region of the butyryl group of simvastatin and the fluorophenyl group of rosuvastatin (Fig. 4b and c, respectively). The methyl group of the C-1 methoxy group makes van der Waals contacts with the side chain of Leu-857. The propenyl side chain of α-asarone is located near the hydrophobic group of the statins.

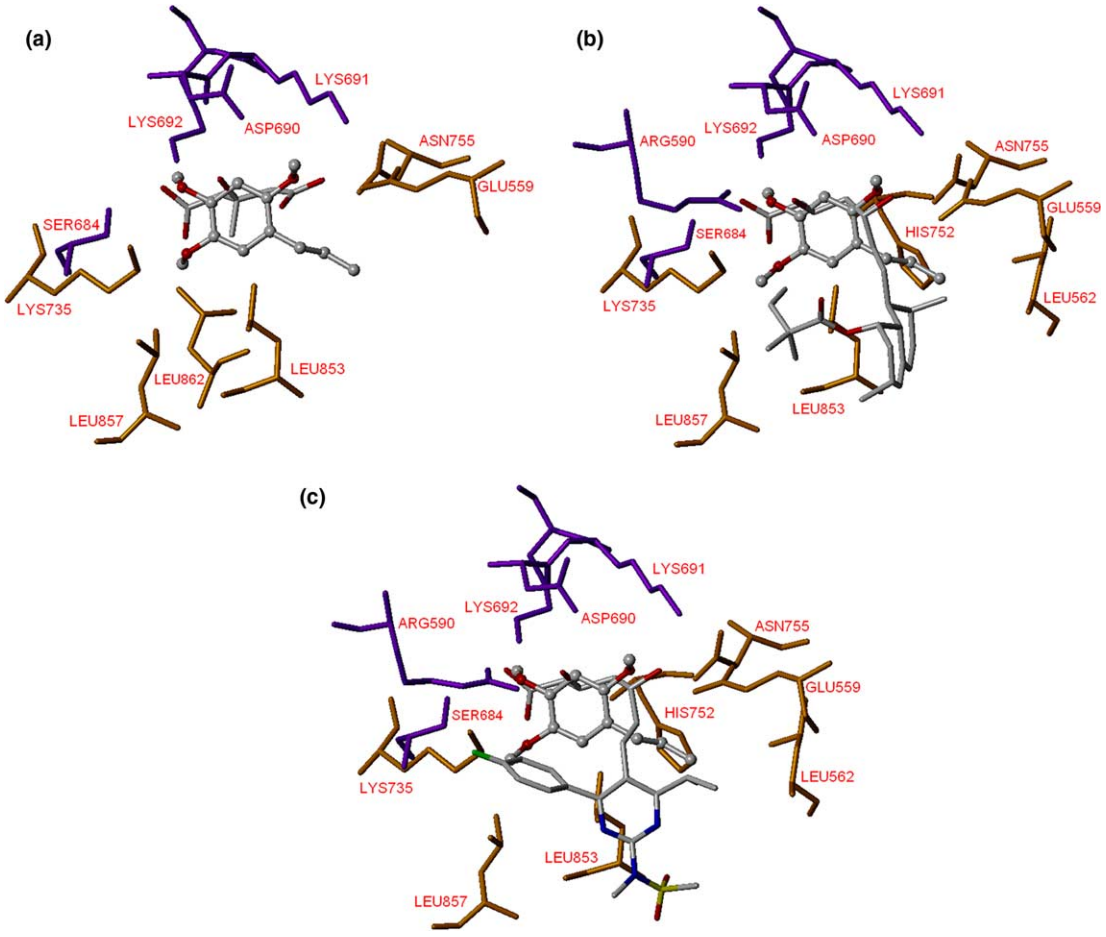

Figure 4. Comparison of the predicted binding mode of α-asarone with the actual binding mode of (a) the substrate; (b) simvastatin, and (c) rosuvastatin. Representative residues within 3.6 Å of α-asarone are shown. Residues are colored as in Figure 3. Nonpolar hydrogens are omitted for clarity.

The above observations suggest that the interactions of the C-1, C-2, and C-4 oxygen atoms on α-asarone are very important in the α-asarone binding to HMGR and play a similar role to that of the HMG-like moiety present in the substrate and the statins (Fig. 5). This is in agreement with the experimental observation that the trimethoxybenzene core seems to be fundamental for the hypocholesterolemic activity of α-asarone analogues.[32] The docking model also helps to visualize, at the molecular level, the experimental results that indicate that the side chain of α-asarone should be at the *meta* and *para* positions of the C-1 and C-2 methoxy groups, respectively.[33]

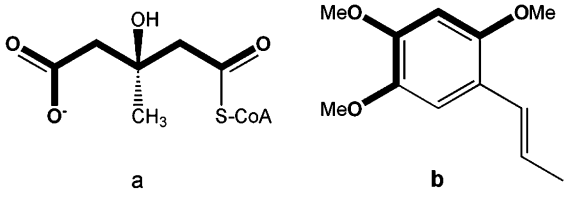

Figure 5. (a) HMG-like moiety of the substrate (in bold). (b) Structure fragment of α-asarone (in bold) that play a similar role to that of the HMG-like moiety in the binding to HMGR.

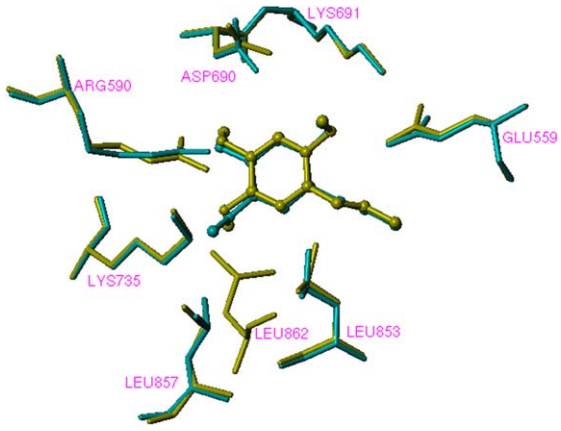

Figure 6. Predicted binding conformations for the C-1 methoxy group of α-asarone when docked into the binding pocket of the substrate (yellow) and into the binding pocket of rosuvastatin (cyan). Representative residues are shown. Nonpolar hydrogens are omitted for clarity.

AutoDock found two possible binding conformations for the C-1 methoxy group depending on the binding site. In the binding pocket of the substrate, the methoxy group was in a *cis* position relative to the vicinal C-2

methoxy group (dihedral angle of 281.6°) while, in the binding pocket of the statins the relative position was *trans* (dihedral angle of 72.6°). According to the ab initio (HF/6-31G**) energy calculations, the conformation of α-asarone with the C-1 methoxy group in a *cis* position was more stable by ca. 2 kcal/mol than the conformation with the C-2 methoxy group in the *trans* position. The different predicted conformation for the C-1 methoxy group might be explained by the presence of the residue Leu-862 in the binding pocket of the substrate that is lacking in the binding geometry of the statins (Fig. 6).[12] Thus, in the binding site of the substrate, the C-1 methoxy group, possibly having steric hindrance with the side chain of Leu-862, prefers the *cis* position. The slightly different conformation of the side chains of residues Leu-857 and Arg-590 in the pocket geometries may also contribute to the different orientation of the methoxy group. Despite the different conformations predicted for the C-1 methoxy group, the orientation of the benzene ring of α-asarone and the conformation of both C-2 and C-4 methoxy groups and the propenyl side chain were predicted to be the same in all binding geometries (Fig. 6).

The docking model proposed for α-asarone prompts for modifications that could improve its affinity for HMGR and increase the hypercholesterolemic activity. The C-4 methoxy group may be replaced by a hydroxyl group to increase the polar interactions with the side chains of the catalytic important residues Lys-691 and Glu-559. Also, a cavity formed by the residues of the *cis* loop may be occupied by a larger group attached to the C-2 oxygen atom of α-asarone that could make polar interactions with these residues.

A further possible modification is to increase the size of the side chain (hydrophobic group) of α-asarone to resemble the hydrophobic group of statins. It has been suggested that the contacts associated with this hydrophobic group of statins, such as rosuvastatin, are predominately responsible for their nanomolar K_i values.[12]

In conclusion, we have developed a docking model for α-asarone with HMGR, using an automated docking approach. To the best of our knowledge, this is the first automated docking study reported with the recently resolved structure of HMGR. The docking program used, AutoDock, successfully reproduced the binding modes of crystal structures. The docking model suggests that, similar to statins of type 1 (simvastatin) and type 2 (rosuvastatin), α-asarone inhibits HMGR by binding to the enzyme's active site, thus sterically preventing the substrate from binding. The binding energies calculated by the docking program are in good agreement with the observed inhibitory abilities of α-asarone, simvasatin, and rosuvastatin. According to the docking model, the C-1, C-2, and C-4 oxygen atoms make polar contacts with residues of the binding pocket and may form several hydrogen bonds. Their interaction with the catalytic important residues Lys-691 and Glu-559 is remarkable. The aromatic ring and the propenyl side chain of α-asarone makes van der Waals contacts with residues of the binding pocket. The present results help to explain, at the molecular level, several previous SAR studies for α-asarone analogues which indicate the importance of the C-1 and C-2 oxygens of the methoxy groups and a hydrocarbon side chain in the *meta* and *para* positions to these groups. The docking results suggest modifications to the α-asarone structure to improve its HMGR inhibitory activity, which will be valuable for the structure-based design of further α-asarone analogues.

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21. Automated docking was used to locate the appropriate binding orientation and conformation of α-asarone with HMGR. The powerful genetic algorithm method implemented in the program AutoDock 3.0[22] was employed. The structures of α-asarone and the proteins were prepared using Sybyl 6.8.[23] All water, adenosine-5′-diphosphate, and 2,3-dihydroxy-1,4-dithiobutane molecules when present were removed from the original RCSB protein data base files. Polar hydrogen atoms were added and Kollman charges,[24] atomic solvation parameters and fragmental volumes were assigned to the protein using AutoDock tools (ADT). For docking calculations, Gasteiger–Marsili partial charges[25] were assigned to the ligands and nonpolar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The auxiliary program AutoGrid generated the grid maps. Each grid was centered at the crystal structure of the substrate or the corresponding statin. The grid dimensions were $23 \times 23 \times 23$ Å^3 with points separated by 0.375 Å. The grid dimensions were large enough to cover the substrate, the statins and the NADP(H) binding sites. Lennard–Jones parameters 12–10 and 12–6, supplied with the program, were used for modeling H-bonds and van der Waals interactions, respectively. The distance-dependent dielectric permittivity of Mehler and Solmajer[26] was used for the calculation of the electrostatic grid maps. For all ligands, random starting positions, random orientations, and torsions were used. The translation, quaternion, and torsion steps were taken from default values in AutoDock. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters. The number of docking runs was 100. The population in the genetic algorithm was 50, the energy evaluations were 250,000, and the maximum number of iterations 27,000. The complexes of α-asarone with HMGR resulting from molecular docking were further structurally optimized with the Tripos force field.[27] During minimization, atoms within 6 Å from the ligand were free to move (other atoms were fixed). For complex optimization, α-asarone with Mülliken charges obtained from ab initio (HF/6-31G**) single-point energy calculation was used. For the protein, Kollman all charges encoded in Sybyl 6.8 were employed. The ab initio calculations were conducted with Spartan'02.[28]
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