DFT study on hydroxy acid–lactone interconversion of statins: the case of fluvastatin†

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Fluvastatin is a member of the HMG-CoA reductase inhibitor family of drugs, commonly referred to as statins. It is generally known that, under physiological conditions, statins are susceptible to pH-dependent interconversion between their active (hydroxy acid) and inactive (lactone) forms. The mechanism of this interconversion, under both acidic and basic conditions, was investigated theoretically using the density functional theory (DFT) method. Regardless of the conditions, the lactone form was always higher in energy by 6–19 kcal mol^{-1}. However, under basic conditions, the activation barrier for the hydrolysis was significantly lower (9 kcal mol^{-1}) than for the reverse reaction (28 kcal mol^{-1}), making the lactone form unstable. The activation barriers under acidic conditions were of comparable height in both directions (22 and 28 kcal mol^{-1}), making the occurrence of both forms equally probable. Due to the high activation barrier (>40 kcal mol^{-1}), a one-step, direct interconversion between the two forms turned out to be unfavourable. Moreover, the potential energy surface of fluvastatin was briefly inspected, revealing relatively small energetic differences (<5 kcal mol^{-1}) between the key conformers.

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are first-line drugs in the treatment of lipid disorders. By inhibiting HMG-CoA reductase—a crucial enzyme in the biosynthesis of cholesterol—they suppress the production of endogenous cholesterol. As the liver synthesises less cholesterol, it in turn stimulates the production of high affinity low-density lipoprotein (LDL) receptors on the surface of liver cells. In consequence, the liver removes more LDL from the blood, resulting in the reduction of blood levels of both LDL and cholesterol.[1,2] Statins have been shown to slow the progression of coronary artery disease[3] and to reduce mortality from cardiovascular disease.[4] They have been suggested to have an anti-inflammatory[5] and anti-cancer[6] activity. Some of them are also being tested against Alzheimer's disease and osteoporosis.[7]

Thanks to their broad spectrum of clinical applications, statins are receiving more and more attention in the medical community. With the expanding treatment of many complicated diseases, however, the probability of diverse metabolic pathways and side-effects increases substantially. It is therefore desirable to gain as much knowledge about statins as possible, in order to provide a rational basis for their safe and effective use.

One of the intriguing issues associated with the pharmacological use of statins is their pH sensitivity *in vivo*. It has been shown that the labile 3,5-diol moiety undergoes reversible lactonisation (schematically presented in Fig. 1) at a rate which is usually pH-dependent.[8,9] At physiological pH and higher, the lactone form is unstable and the equilibrium favours hydrolysis to open the lactone and yield the hydroxy acid form. The latter, under acidic conditions, is susceptible to lactone formation.[10,11] In general, the lactone and acid forms co-exist in equilibrium *in vivo*, and in the case of many statins the lactone form is at least as abundant as the hydroxy acid.[12,13] It should be stressed, however, that only the hydroxy acid form is biologically active. Except for lovastatin (LV) and simvastatin (SV), all currently available statins are administered just in this form. LV and SV are administered as δ-lactone pro-drugs; *in vivo*, they convert both chemically and enzymatically to their respective hydroxy acids.

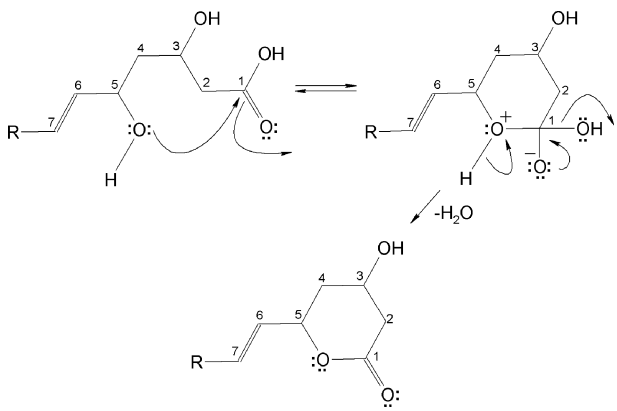

Fig. 1 General mechanism for the δ-lactonisation of hydroxy acids. R represents the remainder of the molecule.

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Despite a great deal of pharmacological evidence for the pH-dependent character of the hydroxy acid–lactone interconversion of statins, there are no detailed thermodynamic data concerning this reaction available at this moment.

Some literature reports indicate that concomitant administration of statins with an acidic carbonated beverage results in decreased bioavailability due to instability of the drug in acidic media.[14–16] It is possible that after disintegration of a dosage form and dissolution of drug particles in the stomach, the reactive moiety will tend to convert to the corresponding inactive lactone structure by the non-enzymatic, acid-catalysed reaction in the stomach before absorption from the gastrointestinal tract. On the other hand, after absorption into the bloodstream (pH = 7.4) the pH-dependent equilibrium should be shifted towards the hydroxy acid form.

Based upon these considerations, we set out to gain a theoretical insight into the mechanism of interconversion of statins using fluvastatin (FLV) as a model compound, due to the fact that other statins have even greater conformational freedom.

FLV is the first totally synthetic HMG-CoA reductase inhibitor and is administered orally as its monosodium salt. Compared to other statins, it is a relatively hydrophilic (octanol/water partition coefficient of 20 at pH = 7) weak acid with an ionisation constant (pK_a) of 5.5. Chemically, the FLV molecule is composed of a bulky lipophilic indole moiety and a heptanoic acid side chain bearing a 3,5-diol (see Fig. 2). The latter is very similar to the HMG portion of HMG-CoA, and is necessary for pharmacological action.[17–19]

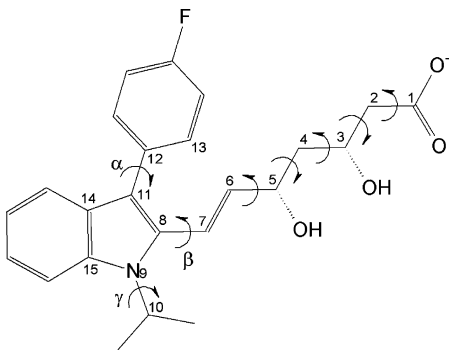

Fig. 2 Chemical formula and selected atom numbering of fluvastatin in its ionic form. Torsion angles varied during the initial scan are marked with Greek letters: α: C(14)–C(11)–C(12)–C(13); β: H–C(7)–C(8)–C(11) and γ: C(15)–N(9)–C(10)–H.

Computational details

All calculations presented in this paper were performed with the Gaussian 03 program.[20] Geometries of all conformers were fully optimised within the density functional theory (DFT) framework. The B3LYP[21,22] hybrid functional combined with the medium-size basis set 6-31G[23] augmented with diffuse and polarisation functions was used. At least a single set of diffuse functions should be used for the proper description of the ionic system.[24] For the structure generated by molecular construction methods and optimised at the B3LYP/6-31 + G(d) level, conformational energy maps were obtained through the discrete rotation of selected torsional angles, in 15° increments. At each point the energy was calculated at the HF/6-31 + G(d) level of theory.

All optimised structures were vibrationally characterised, *i.e.*, checked for the absence of imaginary frequencies in the minima and for the presence of only one imaginary frequency in the transition states. The complete reaction pathways for all the mechanisms were verified using intrinsic reaction coordinate (IRC) analysis of all transition states. For each transition state, geometries of the last IRC points were optimised to identify the reactant and product to which they were related.

In addition, the non-iterative COSMO-based PCM method,[25,26] as implemented in Gaussian 03, was used to estimate the effect of protein environment on the energy of key conformers of FLV. Instead of the default UA0 model, the cavity was built using the Pauling atomic radii with the dielectric constant (ε) set to 2.

Results and discussion

Conformational freedom of FLV

Prior to the hydroxy acid–lactone interconversion analysis, we decided to roughly examine the conformational space of FLV and locate the structurally most important minima on its potential energy surface (PES). Based on collected results, we aimed to determine the minimum energy pathway connecting the crystallographically observed structure[27] (pdb code: 1HWI) with the other low energy minima of FLV. Finally, through the incorporation of solvation effects to gas-phase calculations, we were able to estimate the energetic cost of this transition in the protein environment. Accurate prediction of protein–ligand interactions is decidedly non-trivial.[28–30] However, in the case of statins, crystal structures clearly indicate that the linear forms are predisposed to binding to the enzyme, as the terminal carboxylate group forms salt bridges with Lys692 and Lys735, while the δ-hydroxy group serves as a charge-assisted hydrogen bond donor to Glu559, and as a hydrogen bond acceptor from Lys691.[27] In the case of the lactone form of FLV, these strong directional interactions would be absent, so the lactone form could not be a strong inhibitor.

Fluvastatin exhibits an extreme conformational flexibility due to many rotational degrees of freedom associated with single bonds (see Fig. 2). Even a very simple conformational analysis based on 6 torsion angles (0°, 60°, 120°...) for each bond gives more than 1 million possible conformations. To cover such a complex space would be not only a very demanding and time-consuming task, but first of all not really necessary from the viewpoint of the discussed reaction. Hence, we decided to initially inspect only torsions associated with α, β, and γ (see Fig. 2), in order to find the energetically most favourable orientation of three main subunits of FLV, and then to focus our entire attention solely on the hydroxy acid side chain.

Conformational energy maps for the rotation around the C(11)–C(12) (α), C(7)–C(8) (β), and N(9)–C(10) (γ) bonds are presented in Fig. 3. As expected, the fluorophenyl rotation around the C–C bond resulted in a relatively flat potential. In fact, apart from the eclipsed and nearly-eclipsed orientations of the rotated subunits, the energy differences between particular conformations ranged from 2 to 8 kcal mol^{-1}. Such a structural flexibility should translate into an increased capability of FLV to best fit into the HMG-binding pocket. In the case of the isopropyl group, the situation was quite the opposite, as rotation around the C–N bond

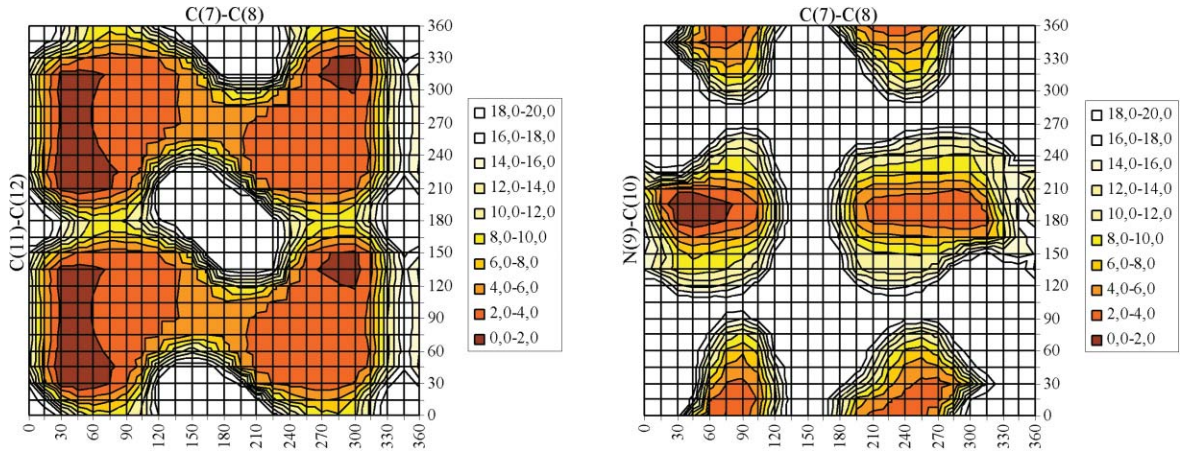

Fig. 3 Conformational energy maps for the rotation around C(11)–C(12) and C(7)–C(8) (left panel), and N(9)–C(10) and C(7)–C(8) (right panel). The C_{2v} symmetry of the fluorophenyl residue is easily visible. The energy cutoff value was set at 20 kcal mol^{-1}. For the definition of angles see Fig. 2.

Table 1 Comparison of DFT energies obtained for selected conformers of FLV. α and β correspond to torsional angles depicted in Fig. 1. X-Ray values are shown for comparison

			ΔE/kcal mol^{-1} [a]	
Conformer	α/°	β/°	DFT	DFT + C-PCM
Flv_1	124.1	34.5	1.9	3.8
Flv_2	227.0	36.2	0.9	0.6
Flv_3	133.1	324.4	0.9	0.6
Flv_4	125.3	139.4	0.5	0.4
Flv_5	232.9	225.9	0.0	0.0
X-Ray	238.2	44.1	—	—

[a] Calculated at the B3LYP/6-31 + G(d) level of theory.

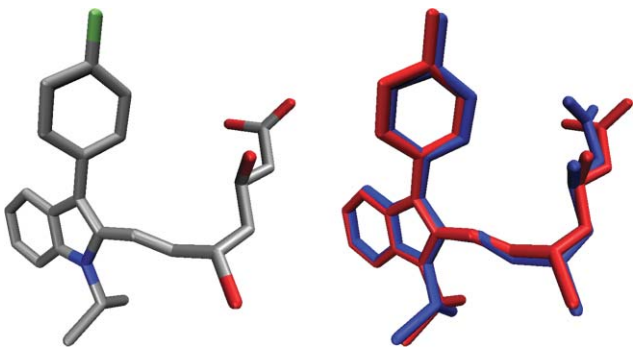

Fig. 4 Left: Fluvastatin, as observed in the crystal structure of human HMGR complex with FLV. Right: The same structure (red) superimposed on the calculated minimum (blue).

was almost completely hindered by the steric repulsion with the hydroxy acid chain.

As shown in Table 1, fully-relaxed energy optimisations of selected conformers revealed the existence of a few distinct minima on the PES of FLV. The average difference in energy, however, did not exceed 2 kcal mol^{-1}. In this situation, for the purpose of a side-chain geometry analysis we arbitrarily chose the conformer **Flv_2** (see Table 1), characterised by the smallest deviation of α, β, and γ torsions compared to the crystal structure of FLV complexed with HMGR.

The hydroxy acid side chain is definitely the most flexible part of the entire molecule. Due to the structural similarity to the HMG moiety, it is mainly responsible for the competitive inhibition of human HMGR. The only available crystallographic structure of FLV is presented in Fig. 4, while a set of 8 representative conformers revealed by our calculations is presented in Fig. 5. It seems that in the absence of surrounding residues, intramolecular hydrogen bonds (HB) between hydroxyl and carboxyl groups provide a major structural basis for the formation of stable conformers. Five possible arrangements of such interactions were observed. Without a doubt, the most stable conformation is the linear one with a system of two cooperative HBs (see Fig. 5, **Flv_a**). Conformations **Flv_b** and **Flv_c**, with only one HB, are 10 and 5 kcal mol^{-1} less stable than **Flv_a**. Interestingly, the conformer **Flv_c** shares the highest geometrical similarity with the structure observed in the enzyme, and deserves special attention. Conformations **Flv_d** and **Flv_e** are other examples with two HBs, but this time both hydroxyl groups interact with the same carboxyl moiety simultaneously. In both cases, the relative energy values with respect to **Flv_a** are about 2 kcal mol^{-1}. With its low energy, conformer **Flv_e** turned out to be an ideal substrate for FLV lactonisation. In the case of the structure devoid of hydrogen bonds (**Flv_f**), the energy substantially rises to more than 13 kcal mol^{-1}.

During the study we observed evidence for the existence of an additional stabilising interaction between the oxygen(s) from the terminal carboxyl group and the *m*- and *p*-hydrogens of the fluorophenyl ring. Two local minima of that type were indeed found (**Flv_g** and **Flv_h**), but their relative energies were higher than **Flv_a** by 10 and 15 kcal mol^{-1}, respectively. All energy values discussed so far are summarised in Table 2. It should be noted that there are many other potential energy minima separated by torsional barriers which we do not report here for the sake of brevity. Two examples of such minima, however, are depicted in Fig. 7 (**MIN1** and **MIN2**) as they appeared during the analysis of the conformational pathway (*vide infra*).

Since the conformer **Flv_c** differs by 0.604 Å RMSD from the crystal structure of FLV it is very likely that these structures are linked together. Geometry optimisation of the X-ray structure supported this assumption, showing that **Flv_c** is the nearest gas-phase minimum. Starting from this point, it was possible to

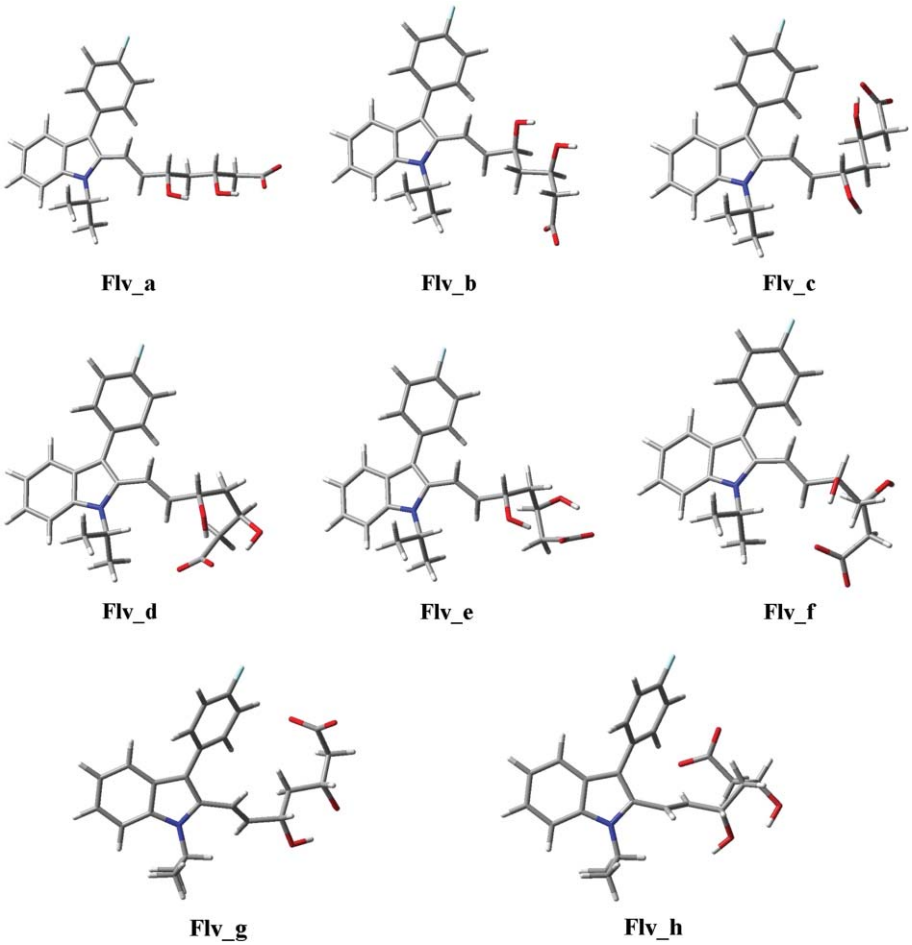

Fig. 5 Selected conformers of FLV optimised at the B3LYP/6-31 + G(d) level of theory.

Table 2 Comparison of DFT energies obtained for selected conformers of FLV showed in Fig. 4. α and β correspond to torsional angles depicted in Fig. 1

			ΔE/kcal mol^{-1} [a]	
Conformer	α/°	β/°	DFT	DFT + C-PCM
Flv_a	227.2	36.2	0.0	0.0
Flv_b	225.0	34.9	9.9	9.2
Flv_c	228.7	33.1	5.1	4.6
Flv_d	226.3	37.1	2.1	5.1
Flv_e	226.2	38.9	1.9	3.2
Flv_f	228.4	29.6	13.7	11.9
Flv_g	225.8	35.4	10.3	9.0
Flv_h	235.8	105.4	15.2	15.0

[a] Calculated at the B3LYP/6-31 + G(d) level of theory.

establish the minimum energy pathways leading to both global and pre-reaction minimum structures. Thermodynamic properties for these pathways are listed in Table 3. It turned out that the conversion to the global minimum (**MIN_global**) structure proceeds through only one transition state (**TS**) located less than 4 kcal mol^{-1} higher, while the conversion to the pre-reaction (**MIN_prereact**) conformation is a three-step process with a highest energy barrier of 5 kcal mol^{-1} (**TS1**). All energy barriers are associated with internal rotations around C–C bonds. A complete pathway together with geometries of the corresponding minima and transition states are presented in Fig. 6 and Fig. 7, respectively. As can be clearly seen, the inclusion of solvent effects did, in fact, affect the overall reaction profile. While some energy barriers were visibly lowered (**TS** especially), the relative energy of the last transition state (**TS3**) increased. This might suggest that, in the protein environment, the eventual conformational rearrangement of FLV would be directed towards the linear hydroxy acid form.

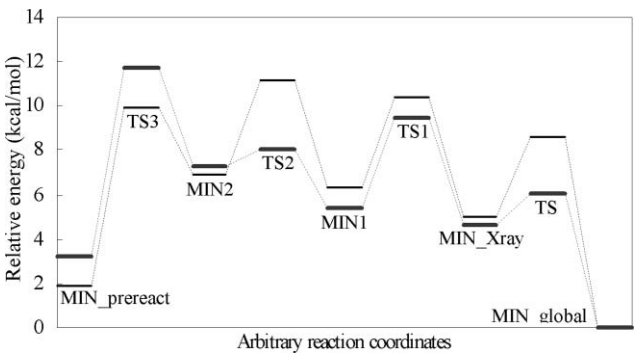

Fig. 6 Conformational pathway of FLV; gas phase (plain line), C-PCM corrected (bold line).

Table 3 Activation energies, enthalpies of activation and free energies of activation for the fluvastatin rearrangement between structurally important conformations: crystallographically nearest minimum (**MIN_Xray**), pre-reaction minimum (**MIN_prereact**) and global minimum (**MIN_global**) at 298.15 K

Direction		ΔE/kcal mol^{-1} [a]	$\Delta H^{\ddagger}$/kcal mol^{-1} [a]	$\Delta G^{\ddagger}$/kcal mol^{-1} [a]
MIN_Xray → MIN_global	TS	2.8	2.4	1.6
MIN_Xray → MIN_prereact	TS1	3.9	4.2	3.8
	TS2	5.0	4.7	4.3
	TS3	4.3	3.5	2.8

[a] Calculated at the B3LYP/6-31 + G(d) level of theory.

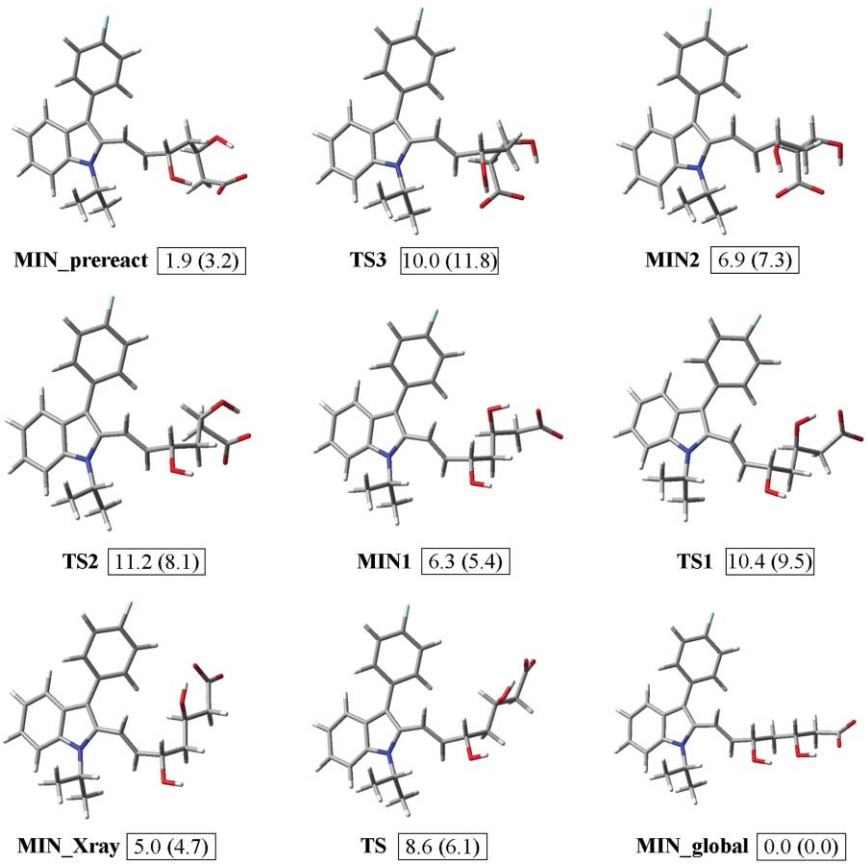

Fig. 7 Geometries of selected stationary points along the conformational pathway of FLV. Relative energy values (in kcal mol^{-1}) calculated at the B3LYP/6-31 + G(d) level of theory are given in boxes (C-PCM corrected values are given in parentheses).

Mechanism for the transition from the acid form (A) to the lactone form (L) of FLV under acidic conditions

Under acidic conditions, experimental measurements indicate that conversion between the lactone and acid forms of FLV occurs, and that both species are in an equilibrium depending on the pH of the solution.[10,11] In our calculations the reaction leading from the acid form of FLV to the lactone form starts in a conformation in which the 5-OH group is in the vicinity of the COOH moiety. It is well known that the reaction mechanisms studied computationally with OH^- or H_3O^+ (not mentioning those using an isolated proton) show very low energy barriers for isolated species.[31,32] Our previous studies indicate that the use of uncharged groups as the source of protons results in more realistic interconversion barriers.[33,34] Thus, we decided to include an additional carboxylic acid moiety (formic acid) as a source of protons, because it better mimics mild acidic conditions than the H_3O^+ cation.

In our initial pre-reaction complex, the formic acid forms hydrogen bonds with the acid form of FLV, as it accepts a hydrogen bond from the 5-OH group of FLV and donates another hydrogen bond to the carboxylic oxygen atom of FLV (see Fig. 9A). The reaction leading from **A** to **L** may proceed directly *via* transition states **AL1** or **AL2** (see Fig. 8 and 9). These states differ from each other by protonation patterns *i.e.* in **AL1** the leaving water molecule is in the axial position with respect to the forming lactone ring, while in **AL2** it is in the equatorial position. The energy barriers are 43 kcal mol^{-1} and 46 kcal mol^{-1}, above the lowest energy conformation of FLV in the acid form, for **AL1**, and **AL2**, respectively. Such high energy barriers are not likely to be overcome under physiological conditions, therefore the reaction

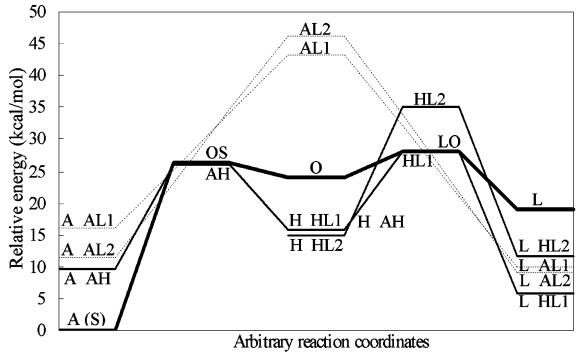

Fig. 8 Reaction pathways for the interconversion between the acid (**A**) and lactone (**L**) forms of FLV under mildly acidic conditions (plain and dashed lines), and between the carboxylate salt (**S**) and the lactone (**L**) under basic conditions (bold line). The lowest energy forms of **A** and **S** were chosen as the reference points, so their relative energy is 0 kcal mol^{-1}.

involving the lactone and the acid form of FLV proceeds in a different way. Firstly, the 5-OH hydroxyl group attacks the carboxylic carbon atom, as it does in **AL1** and **AL2**. This time, however, the carbonyl oxygen is protonated. The transition state **AH** has a relative energy of 26 kcal mol^{-1}.

Next, the hydrated lactone (**H**) of FLV is formed. In fact there are several potential energy minima corresponding to the **H** structures (**H_AH**, **H_HL1**, **H_HL2**), interacting in our model, with the second carboxylic acid moiety. These structures have relative energies in the range from 12 to 14.5 kcal mol^{-1}, and differ from each other by different protonation patterns. Due to various modes of interaction with the second carboxylic acid moiety, the interchanges between various **H** structures are easy and relative energies of these transitions, with respect to the lowest energy **H** structure, are lower than 5 kcal mol^{-1}(see Fig. 8). On the way to the lactone form, the hydrated lactone eliminates water molecule which may leave the newly formed lactone ring in the axial or equatorial positions *via* transition states **HL1** and **HL2**, respectively. These transition states have relative energies of 28 kcal mol^{-1} (**HL1**) and 35 kcal mol^{-1} (**HL2**) with respect to the lowest energy (**A**) form. Among the created lactone forms **L**, the one with the lowest potential energy is still 6 kcal mol^{-1} higher in energy than the **A** form. Therefore, the reaction leading from the acid form of FLV (**A**) to the lactone form (**L**) is endoergic and requires slightly more than 6 kcal mol^{-1}. The energy barrier for this conversion is about 28 kcal mol^{-1}, and the highest energy point corresponds to the reaction eliminating the water molecule from the hydrated lactone form of FLV, as the reaction of lactonisation of FLV proceeds in two steps *via* a hydrated lactone form. In the case of the hydrolysis of the lactone form of FLV under mildly acidic conditions, the reaction is exoergic, as the acid form is 6 kcal mol^{-1} lower in energy. Moreover, the activation energy for hydrolysis is 6 kcal mol^{-1} lower than for the lactonisation reaction, and amounts to 22 kcal mol^{-1}.

Mechanism for the transition from the lactone form (L) to the carboxylate salt (S) of FLV under basic conditions

The hydrolysis of the lactone form of FLV occurs experimentally under basic conditions.[11] In our calculations, the lowest energy structure of FLV in the lactone form with the hydroxyl anion is

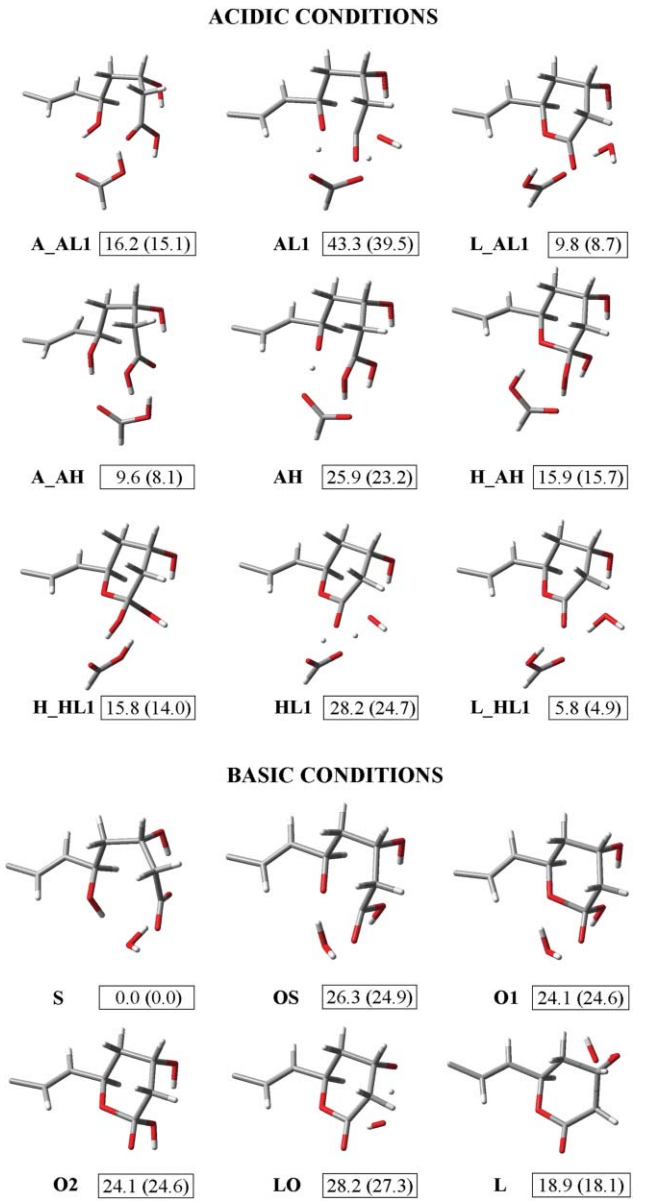

Fig. 9 Geometries of selected stationary points along FLV interconversion pathways. Relative energy values (in kcal mol^{-1}) calculated at the B3LYP/6-31 + G(d) level of theory are presented in boxes (C-PCM-corrected values are given in parentheses). For the sake of clarity only the reactive moiety is presented.

19 kcal mol^{-1} higher in energy than the corresponding carboxylate salt of FLV. The activation energy for lactone hydrolysis under basic conditions is about 9 kcal mol^{-1}—much lower than the 22 kcal mol^{-1} under mildly acidic conditions (see Fig. 8). The transition state **LO**, in which the hydroxyl anion attacks the lactone ring, has a relative energy of 28 kcal mol^{-1} with respect to the carboxylate salt formed as the result of the lactone hydrolysis. The structure **O** is a local and shallow minimum in the potential energy, and its relative energy is 24 kcal mol^{-1}. However, the following transition state, **OS**, has a relative energy of only 26 kcal mol^{-1}, and on the other side of this potential energy saddle is the global minimum **S**. All in all, the hydrolysis of the lactone form of FLV under basic conditions is an exoergic reaction, as

under acidic conditions. However, the energy gain under basic conditions is much higher, and amounts to about 19 kcal mol^{-1}, while under mildly acidic conditions it is about 6 kcal mol^{-1}. Moreover, the activation barrier for the hydrolysis reaction is only 9 kcal mol^{-1}. Together this renders the lactone form unstable under basic conditions, and the reaction proceeds towards the carboxylate salt of FLV. For the reverse reaction leading from the carboxylate salt to the lactone, the energy barrier would be as high as 28 kcal mol^{-1}, and the lactone form would be much higher in energy (19 kcal mol^{-1}) than the carboxylate salt.

Conclusions

The DFT-based study of the hydroxy acid–lactone interconversion of fluvastatin presented in this paper fully supports previously reported findings on the pH-dependent character of this reaction. Major effort was put into the accurate calculation of energy barriers associated with the formation of corresponding forms, and four possible pathways were analysed. Due to high activation barriers (>40 kcal mol^{-1}), the two first pathways (one-step, direct interconversion) were of little interest. The other two, however, gave more interesting results. Because of a comparable height of energy barriers (22 and 28 kcal mol^{-1}) in an acidic environment, the hydroxy acid–lactone system exists an the equilibrium state, while under basic conditions, this equilibrium is shifted towards the hydroxy acid form, since the activation barrier for the hydrolysis drops significantly to 9 kcal mol^{-1}. It is worth noticing that, regardless of the conditions, the hydroxy acid form of FLV is more stable than the lactone form.

Due to a high structural resemblance between statins, and based on the fact that the reactive moiety is a simple, unhindered, dihydroxycarboxylic acid, one might expect that the results obtained in this paper for FLV should be, at least qualitatively, similar in the case of all statins. Nevertheless, the reader should not assume this to be true for all lactonic compounds. In the case of irinotecan, another anti-cancer drug, the hydroxy acid–lactone equilibrium is reversed: acid conditions promote the formation of the lactone, while more basic conditions favour the hydroxy acid form.[35]

In addition, based on the potential energy surface scan performed in the initial stages of this study, a set of 8 representative conformers of FLV was selected. One of the obtained conformers was structurally very close to the one recently observed in the crystal of the human HMGR complex with FLV. The analysis of possible conformational rearrangements of this conformer revealed a very low rotational barrier (1.4 kcal mol^{-1} in the protein environment) on the way to the global minimum (the stranded hydroxy acid form).

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