



CONFORMATIONAL ANALYSIS FOR PROSTAGLANDIN E2. I.**

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Results of a conformational analysis using the PM3 semiempirical MO method performed for prostaglandin E2, PGE2, the endogenous ligand of the four EP1 – EP4 receptor subtypes are reported. The PM3 conformational analysis gave around 500 conformers in a range of 5 kcal/mol and 82 conformers in a range of 3 kcal/mol above the energy of the global minimum conformer. Potential energy profiles show that flexible bonds attached to the double bonds or 1- and 2- positions of the cyclopentyl ring can be rotated in only narrow intervals. Rotating these bonds leads to only few low energy conformers that can be interconverted in other low energy conformers. The carboxyl group which is supposedly implicated in the interaction of PGE2 with the four EP receptor subtypes can be easily rotated. This group has in different conformers a large spectrum of values, suggesting that its flexibility favours the PGE2 - EP receptor interaction.

INTRODUCTION

Prostanoids are metabolites of the arachidonic acid resulted through cyclooxygenases. They include prostaglandin (PG) PGD2, PGE2, PGF2 α , PGI2, and thromboxane A2 (Tx). Their receptors, known as prostanoid receptors, belong to the A class of the large family of G protein-coupled receptors (GPCR). The prostanoid receptors exert a variety of actions in certain cells and tissues. Their most typical actions are relaxing or contracting various types of smooth muscles. They also modulate neuronal activity by either inhibiting or stimulating the release of neurotransmitters, sensitizing nervous fibres to nociceptive stimuli, or inducing central actions such as fever generation and induction of sleep.¹ Prostanoid receptors also regulate secretion and motility in the gastrointestinal tract, as well as ions and water transport from the kidneys. They are involved in apoptosis, cellular differentiation, and oncogenesis.

Prostanoid receptors regulate the activity of blood platelets, both positively and negatively, and are involved in vascular homeostasis and hemostasis.²

Coleman *et al.* proposed a classification of the prostanoid receptors, namely the specific receptors for Tx, PGI, PGE, PGF, and PGD, and named them TP, IP, EP, FP and DP prostanoid receptors, respectively.³ They further classified the EP receptors in three subtypes: EP1, EP2, and EP3, all responding to the endogenous agonist PGE2, but differing in their actions and responses to various analogous compounds. They reported later a fourth subtype, the EP4 receptor.^{3,4}

Presently, experimental and theoretical data on the interaction of the EP receptor subtypes with PGE2 is missing. This prompted us to perform a conformational analysis of PGE2, to get information on the conformational space of PGE2 and its steric properties which could influence its interaction with the EP receptor subtypes. Some results are presented in this paper.

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** Supplementary information on <http://web.icf.ro/rrch>

METHODS

Initially, for conformational analysis were used the PM3 hamiltonian and the Conformational Search module from the HyperChem7.52 package.⁵ The conformational analysis was performed by modifying randomly, with steps of 45°, the dihedral angles corresponding to all flexible bonds of PGE2. The option to preserve the chirality of the conformers during the conformational analysis, an energy criterion of 100 kcal/mol above the lowest found conformer, and the method “usage directed” were used for generating the conformers. To perform the geometry optimization, the Polak-Robiere conjugated gradient algorithm was used and the stop criterion was set to a RMS gradient ≤ 0.01 kcal/Åmol. The number of optimization cycles for each conformer was set to 10000 and the number of optimized conformers to 1500.

Using the PM3 method several molecular dynamics simulations have been performed on the conformer's geometry from the global minimum. Simulated annealing was carried out using following conditions: Heat time 0.5 ps; Run time 5 ps; Cool time 0.05 ps; Step size 0.5 fs; Starting temp 0 K; Simulation temp 400 K, 600 K, 800 K or 1000 K; Final temp 0 K; Temp step 4 K, 6 K, 8 K, or 10 K respectively; Bath relaxation time 0.1 ps.

Starting from the geometry of the PM3 conformer with the lowest energy the potential energy profiles were obtained by rotating all flexible bonds of the PGE2 with steps of 10°. For the resulted conformers PM3 single point calculations were performed and the difference between the energy of each conformer and the one of the global minimum conformer was plotted against the values of each dihedral.

RESULTS

(Z)-7-[(1R,2R,3R)-3-hydroxy-2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclo hept-5-pentyl]enoic acid, PGE2 or dinprostone (see structure and numbering of atoms in Fig. 1) has 15 flexible

bonds, two double bonds, one Z and the other E, and four chiral atoms: three (R) on the 3-hydroxycyclopentanone ring and one (S) on the (E,3S)-3-hydroxyoct-1-enyl substituent.

From the conformational analysis performed with the Conformational Search module and PM3 method resulted over 500 conformers with energies in a range of 5 kcal/mol. Of these, 97 conformers were in a range of 3 kcal/mol. By removing the duplicates or triplicates only 82 conformers remained.

Conformational analysis led to some structures with abnormal bond lengths and angles. The existence of conformers with unrealistic geometry suggests that certain bonds need for their rotation high energy. This also suggests that not all low energy conformers can interconvert one into another.

An analysis of the distribution of the dihedral values among the 82 conformers (resulted from conformational search) was performed by plotting the histograms and also by plotting the values of each dihedral against the number of each conformer. All these plots are given in Figs. 1-32 from Supplementary information. In this paper are presented the results for the dihedrals supposed to be important for the interaction between PGE2 and each of the four EP1-EP4 receptor subtypes.

The C₁₁-C₁₂ bond rotation is supposed to play an important role in the PGE2 – receptor binding. The carboxylate group can form hydrogen bonds or can act by electrostatic interactions with residues containing protonated nitrogen atoms from the binding sites of the EP receptor subtypes.⁶ The histogram in Fig 2 shows that in the 82 conformers the C₁₀C₁₁C₁₂O₁₄ dihedral has the majority of values in a range between 60 and 290°. This large domain of values suggests that the flexibility of this bond could allow PGE2 to adopt an optimal conformation for an efficient interaction with the protonated residues from the ligand binding domain (LBD). In addition, the easy rotation of the C₁₁-C₁₂ bond can lead to many possible conformers and this has a positive effect on the interaction PGE2 – EP receptor.

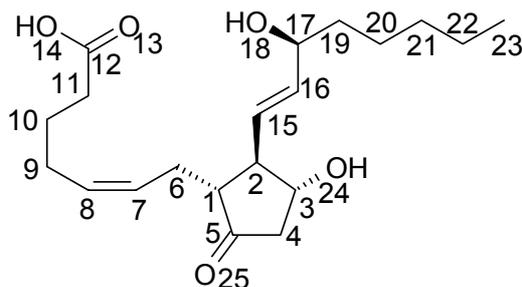


Fig. 1 – Structure and numbering of atoms in PGE2.

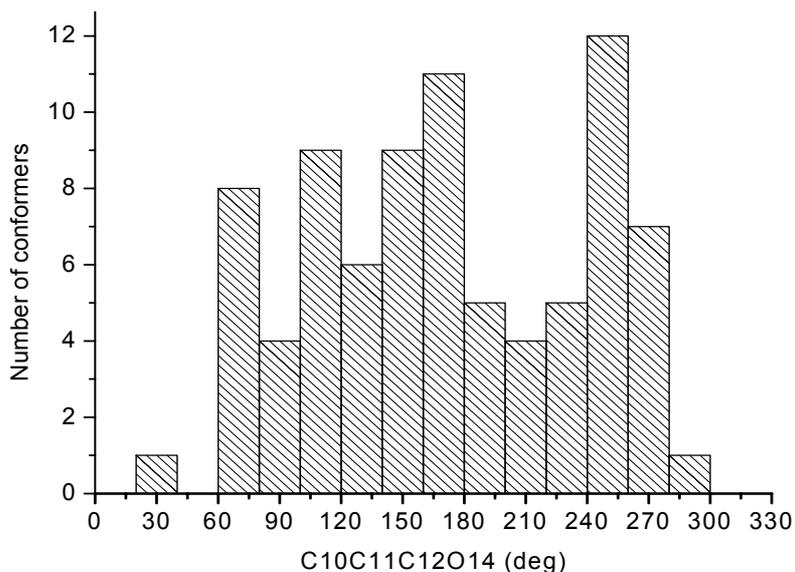


Fig. 2 – Histogram of the $C_{10}C_{11}C_{12}O_{14}$ values in 82 conformers of PGE2 in an energy range of 3 kcal/mol resulted from PM3 conformational search.

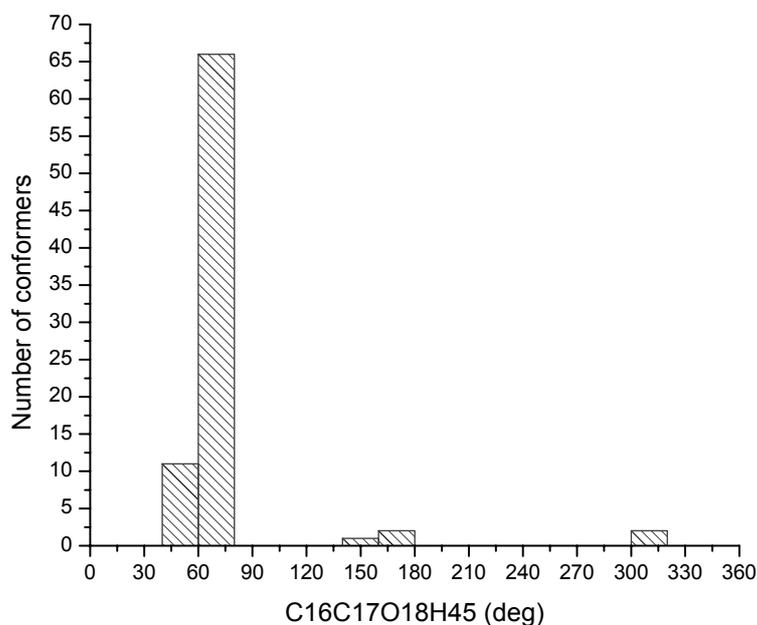


Fig. 3 – Histogram of the $C_{16}C_{17}O_{18}H_{45}$ dihedral values in 82 conformers of PGE2 in an energy range of 3 kcal/mol resulted from PM3 conformational search.

The rotation of the hydroxyl group, $O_{18}H_{45}$, bound to the (S)- C_{17} atom is also important for the interaction of PGE2 through H bonds with serine residues from the LBD of the four EP receptor subtypes. The histogram in Fig. 3 shows that this OH group has predominantly a single position retrieved in the majority of the conformers in which the $C_{16}C_{17}O_{18}H_{45}$ dihedral has values around 60° - 75° .

While the $C_{16}C_{17}O_{18}H_{45}$ dihedral values are around 60° - 75° , in majority of the conformers the values of the $C_2C_3O_{24}H_{57}$ dihedral are around 290° - 310° (Fig. 4). The histograms from Figs. 3 and 4

show that the two hydroxyl groups of PGE2 prefer different orientations. These two groups can be rotated in very narrow limits, while the carboxyl group can have many possible orientations and therefore many chances to interact with residues containing protonated N atoms from LBD.

The PM3 conformer with the lowest energy is displayed in Fig. 5. It has a very packed structure, due to the internal H bond between the H atom of the $O_{18}H$ group and the O_{13} atom of the carboxyl group. The conformer has $v_0 > 0$, thus is in a stable fundamental state.

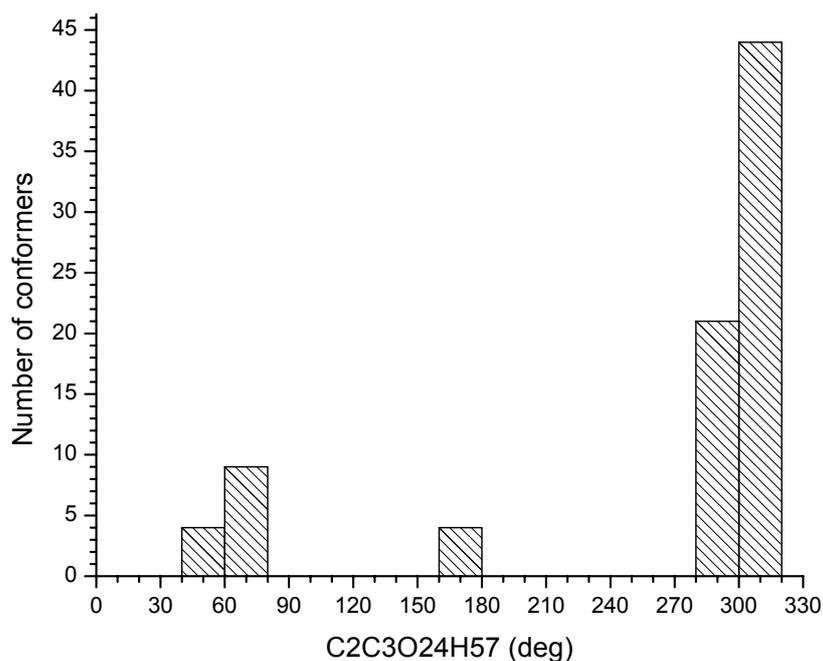


Fig. 4 – Histogram of the $C_2C_3O_{24}H_{57}$ values in 82 conformers of PGE2 in an energy range of 3 kcal/mol resulted from PM3 conformational search.

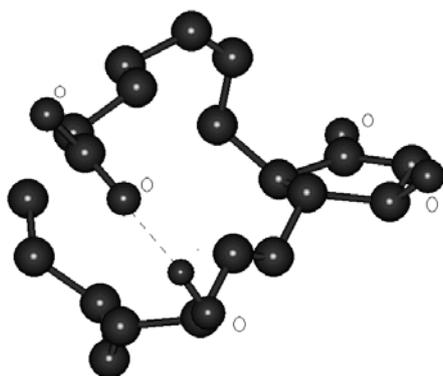


Fig. 5 – The geometry of the PM3 global minimum conformer. Except the H_{45} atom of the $O_{18}H_{45}$ group the rest of hydrogen atoms are hidden for clarity. The disposal of the two substituents (*Z*)-hept-5-enoic acid and (*E*)-(*S*)-oct-1-en-3-ol above and under the 3-hydroxy-5-oxo-cyclopentyl ring respectively is displayed. The intramolecular H bond between the H_{45} atom of the $O_{18}H_{45}$ group and O_{13} atom of the carboxyl group is marked by a dashed line.

To verify if other conformers might have lower energy than the one of the global minimum conformer, starting from this conformer and using the PM3 method, molecular dynamics simulations were carried out at different simulation temperatures. Simulating annealing conditions are presented in Methods. The form of the potential energy variation resulted from the simulating annealing performed at 400 K is shown in Fig. 6. One can see that the potential energy of the structure resulted at the end of simulation run is higher than that of the global minimum conformer. By optimization the geometry of this structure, a conformer with an energy of -5633.32 resulted at times of data collection of 0.016 ps. The $C_2C_1C_6C_7$ dihedral values vary in time between 50.36° , the starting value from the global minimum conformer and 325.96° in a conformer at 1.872 ps. Starting from the global minimum conformer and rerunning

the simulation in the same conditions and optimizing the geometries of the resulted conformers an energy of -5629.74 kcal/mol was obtained at times of data collection of 0.001 ps. Raising the simulation temperature from 400 K to 600, 800 or 1000 K the resulted conformers at the end of simulation run had also higher energies than the energy of the global minimum conformer. The potential energy variation in time as well that of the dihedrals $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ are given in Figs. 33-44 from Supplementary information. These results confirm that the found global minimum is a true global minimum.

An important aspect in conformational analysis is to find the intervals in which the flexible bonds can rotate. To determine these intervals, the PM3 potential energy profiles were calculated using the conformers generated by the rotation of all flexible bonds of PGE2 with steps of 10° . The values of

dihedrals in the global minimum conformer and in conformers from minima and maxima and the difference between the energy of each conformer and the energy of the global minimum conformer are collected in Table 1.

In Fig. 6 the potential energy profiles resulted through the rotation of the OH groups are plotted. The rotation of the OH group from the carboxyl function leads to the conformer in the global minimum which has the $C_{11}C_{12}O_{14}H_{41}$ dihedral of 180° and to a conformer at 0° which is with 4.21 kcal/mol higher than the global minimum (squares in Fig 6). A minimum with a value of 60° for the $C_{16}C_{17}O_{18}H_{45}$ dihedral is obtained to the rotation of the $C_{17}-O_{18}$ bond. The rotation of the C_3-O_{24} bond gives a value of 300° for the $C_2C_3O_{24}H_{57}$ dihedral from the global minimum conformer. Possibly, due to the H bond and internal packed structure of the global minimum conformer, the rotation of the OH groups needs energies higher than 3 kcal/mol.

By rotating the dihedrals $C_2C_1C_6O_7$, $C_1C_6C_7C_8$, $C_7C_8C_9C_{10}$, $C_8C_9C_{10}C_{11}$, and $C_9C_{10}C_{11}C_{12}$ from 0° to 360° with steps of 10° the potential energy profiles from Figs. 45-49 from Supplementary information are obtained. The values in Table 1 show that the rotation of the C_1-C_6 bond gives two minima and two maxima at values of the $C_2C_1C_6C_7$ dihedral of 50° , 180° , 120° , and in interval $[230-360^\circ$ and $0-40^\circ]$. The energy profile shows that the

rotation of the C_1-C_6 bond is limited to very narrow limits between $50^\circ-60^\circ$. By rotating the C_6-C_7 bond two minima and two maxima are obtained at the values of the $C_1C_6C_7C_8$ dihedral of 130° , 230° , 160° and in interval $[250-360^\circ$ and $0-70^\circ]$, respectively (Fig. 46, Supplementary information). As expected, similarly to the precedent bond, by rotating the C_8-C_9 bond two minima and two maxima are obtained at the values of the $C_7C_8C_9C_{10}$ dihedral of 110° , 220° , $160-170^\circ$, and in interval $[280-360$ and $0-90^\circ]$, respectively. The potential energy profiles obtained through the rotation of the C_6-C_7 and C_8-C_9 bonds show that these bonds can rotate only in narrow intervals between $220-250^\circ$, and $100-120^\circ$, respectively. The rotation of the C_9-C_{10} bond leads to two minima and two maxima at values of the $C_8C_9C_{10}C_{11}$ dihedral of 80° , 190° , 110° and $220-330^\circ$, respectively. The potential energy profile shows that interconversions of certain low energy conformers in other low energy conformers including the global minimum conformer are possible by rotation of the C_9-C_{10} bond between 40 and 200° . Rotation of the $C_{10}-C_{11}$ bond (the $C_9C_{10}C_{11}O_{12}$ dihedral) gives two minima and two maxima at 180° , 290° , in interval $[340-360^\circ$ and $0-160^\circ]$, and 240° .

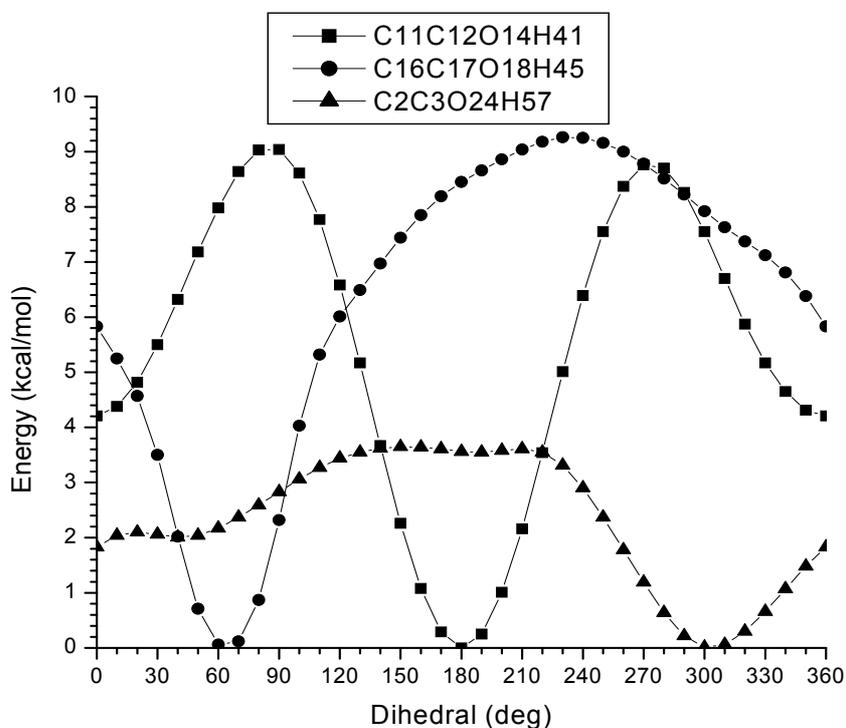


Fig. 6 – PM3 potential energy profiles for the PGE2 conformer interconversion against the energy of the global minimum conformer taken as zero.

Table 1

Values of the dihedrals and potential energy differences for the minima and maxima from the potential energy profiles resulted to rotation of all flexible bonds of PGE2

Bond	Dihedral	Minima (°)		Maxima (°)	
		(Energy differences) (kcal/mol)		(Energy differences) (kcal/mol)	
C ₁ -C ₆	C ₂ C ₁ C ₆ C ₇	50; 180		120; 230÷40 ^b	
		(0.04) ^a ; (10.16)		(14.20); (27.82÷3525) ^c	
C ₆ -C ₇	C ₁ C ₆ C ₇ C ₈	130; [230 236.8] ^d		160; 250÷70	
		(7.46); [(2.96) (0)]		(8.10); (38.60÷5393)	
C ₈ -C ₉	C ₇ C ₈ C ₉ C ₁₀	[110 114.6]; 220		160 ÷ 170; 280÷90	
		[(2.07) (0)]; (6.22)		(7.81); (60.14÷5638)	
C ₉ -C ₁₀	C ₈ C ₉ C ₁₀ C ₁₁	80; [188.7 190]		110; 220÷330	
		(4.95); [(0) 0.10]		(6.69); (36.56÷268.3)	
C ₁₀ -C ₁₁	C ₉ C ₁₀ C ₁₁ C ₁₂	[180 181.6]; 290		340÷160; 240	
		[(0.25) (0)]; (2.86)		(40.38÷1295); (4.60)	
C ₁₁ -C ₁₂	C ₁₀ C ₁₁ C ₁₂ O ₁₄	100; 250; 310		0; 230÷240; 280	
		(0); (2.99); (2.65)		(3.78); (3.19) (9.12)	
C ₂ -C ₁₅	C ₁ C ₂ C ₁₅ C ₁₆	40; [240 242.5]		130÷230; 280	
		(10.14); [(2.03) (0)]		(45.13÷4978); (13.96)	
C ₁₆ -C ₁₇	C ₁₅ C ₁₆ C ₁₇ C ₁₉	40; 80; 260		50; 110÷240; 310÷350	
		(9.53); (9.18); (0.07)		(10.38); (39.06÷6300); (34.44÷72.81)	
C ₁₇ -C ₁₉	C ₁₆ C ₁₇ C ₁₉ C ₂₀	[78.5 80]; 180		260÷60; 130	
		[(0) (0.195)]; (5.47)		(26.14÷630.2); (7.06)	
C ₁₉ -C ₂₀	C ₁₇ C ₁₉ C ₂₀ C ₂₁	170; [260 265.7]		280÷50; 220	
		(4.23); [(1.70) (0)]		(42.29÷865); (5.32)	
C ₂₀ -C ₂₁	C ₁₉ C ₂₀ C ₂₁ C ₂₂	170; [298. 300]		330÷100; 240	
		(2.56); [(0) (0.20)]		(36.42÷571); (5.62)	
C ₂₁ -C ₂₂	C ₂₀ C ₂₁ C ₂₂ C ₂₃	[175.8 180]; 290		0-140; 230-240	
		[(0) (0.53)]; (2.96)		(26.98÷103); (5.3)	
C ₁₂ -O ₁₄	C ₁₁ C ₁₂ O ₁₄ H	0; 180		90; 270	
		(4.21); (0)		(9.04); (8.76)	
C ₁₇ -O ₁₈	C ₁₆ C ₁₇ O ₁₈ H	[60 64.1]		230	
		[(0.06) (0)]		(9.26)	
C ₃ -O ₂₄	C ₂ C ₃ O ₂₄ H	[300 303.0]		150	
		[(0.01) (0)]		(3.65)	

^a The values in round parentheses are the differences between the energy of a conformer and the energy (-5638.6 kcal/mol) of the global minimum conformer.

^b Intervals noted such as 230 ÷ 40 are continuum intervals; in this example the interval is between 230-360° and 0-40°.

^c Potential energy values such as 27.82 ÷ 3525 kcal/mol are the values of the minimum and the maximum in a continuum interval of a large maximum.

^d For minima which are different from zero, in square brackets the dihedral value of the global minimum is also given.

From the potential energy profile it results that the C₁₀-C₁₁ bond can rotate between 180° and 300°.

Rotating the C₁₁-C₁₂ bond (C₁₀C₁₁C₁₂O₁₄ dihedral) three minima and three maxima are obtained (see Table 1 and Fig. 7). The low values of the maxima show that the rotation of the carboxyl group can give many low energy conformers in which this group can have different orientations. The potential energy profile of the carboxyl group rotation is in accord with the results obtained from the histogram of the values of the C₁₀C₁₁C₁₂O₁₄ dihedral among the 82 PM3 conformers in a range of 3 kcal/mol.

By rotating the dihedrals C₁C₂C₁₅C₁₆, C₁₅C₁₆C₁₇C₁₉, C₁₆C₁₇C₁₉C₂₀, C₁₇C₁₉C₂₀C₂₁, C₁₉C₂₀C₂₁C₂₂, and C₂₀C₂₁C₂₂C₂₃ the profiles of the

potential energy from Figs. 50-55 from Supplementary information are obtained. Rotating the C₂-C₁₅ bond two minima and two maxima resulted at values of the C₁C₂C₁₅C₁₆ dihedral of 40°, 240°, 130-230°, and 280°, respectively. The rotation of the C₁₆-C₁₇ bond gives three minima and three maxima at the values of the C₁₅C₁₆C₁₇C₁₉ dihedral of 40°, 80°, 260°, 50°, 110-240°, and 310-350°.

Both the potential energy profile of the C₂-C₁₅ bond and C₁₆-C₁₇ bond show that these bonds can be rotated only in narrow intervals between 240-250°, and 260-270°, respectively, in which low energy conformers can be found.

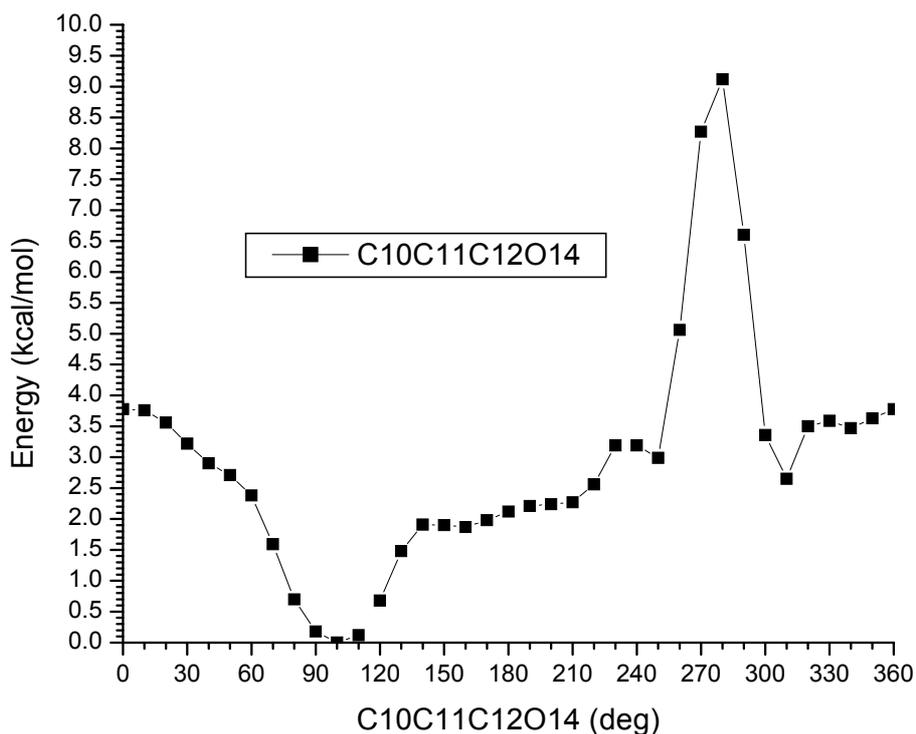


Fig. 7 – Potential energy profile of the C₁₁-C₁₂ bond rotation.

Rotating the C₁₇-C₁₉ bond two minima and two maxima are obtained at values of the C₁₆C₁₇C₁₉C₂₀ dihedral of 80°, 180°, [260-360° and 0-60°] and 130°. The profile of the potential energy shows that the C₁₇-C₁₉ bond can rotate between 70 and 100°. The rotation of the C₁₉-C₂₀ bond gives two minima and two maxima at values of the C₁₇C₁₉C₂₀C₂₁ dihedral of 170°, 260°, [280-360 and 0-50°], and 220°. From the potential energy profile for rotating the C₁₉-C₂₀ bond it results that this bond can rotate between 150° and 270°, where low energy conformers can be found. Rotating the C₂₀-C₂₁ bond again two minima and two maxima are obtained at values of the C₁₉C₂₀C₂₁C₂₂ dihedral of 170°, 300°, [330-360° and 0-100°] and 240°. The profile of the C₂₀-C₂₁ bond rotation shows that this bond can rotate between 100 and 310°. By rotating the C₂₁-C₂₂ bond two minima and two maxima are obtained at values of the C₂₀C₂₁C₂₂C₂₃ dihedral of 180°, 290°, 0-140°, and 230-240°. This potential energy profile suggests that each of the low energy conformers could interconvert in any other.

Although the experimental structures of the EP receptor subtypes are not determined, a 3D model of the EP4 receptor⁷ shows that the PGE2 conformer docked in the LBD has a shape different to the supposed hairpin shape (Fig. 8). In this figure four H bonds can be observed. The first is formed between the O₁₃ atom of the carboxyl

group of PGE2 and the H atom from the OH group of the serine, Ser285 residue. The second H bond is formed between the H₅₇ atom from the O₂₄H₅₇ group of PGE2 and the O atom of the Thr64 C=O group from the C α chain. The third H bond is formed between the H₄₅ atom of the O₁₈H₄₅ group of PGE2 and the O atom of the Phe103 C=O group from the C α chain. The H₄₅ atom is implicated also in the fourth H bond with the O atom from the OH group of the Thr64 residue.

In the docked conformer the most important dihedrals which determine the binding conformation of PGE2, namely C₂C₁C₆C₇, C₁C₆C₇C₈, C₁C₂C₁₅C₁₆, and C₁₅C₁₆C₁₇C₁₉ have the following values: 196.5°, 87.1°, 238.2°, and 236.5°, respectively. Among the 82 conformers in a range of 3 kcal/mol no one has values of all these dihedrals close to the values of dihedrals from the docked conformer. Searching among all conformers resulted from conformational analysis, 58 conformers having similar trend values of these dihedrals have been found. They have energies with 4-11 kcal/mol higher than the energy of the global minimum conformer. The conformer which gives the lowest RMS fit is a conformer with an energy with 8.8 kcal/mol higher than the one of the global minimum conformer. The superposition of this conformer on the docked conformer is shown in Fig. 9.

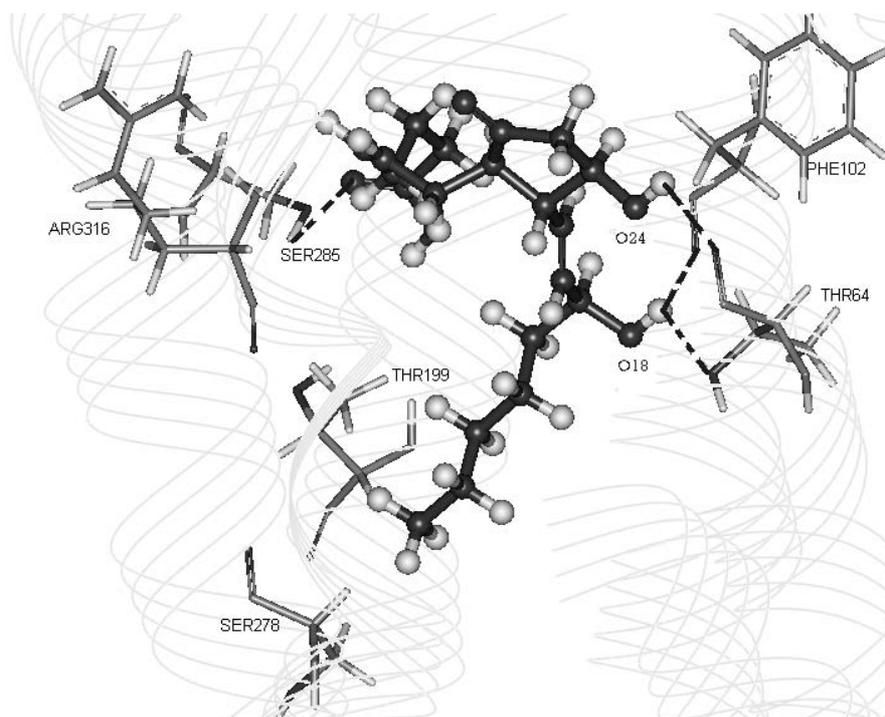


Fig. 8 – PGE2 conformer docked in the 3D model of the EP4 receptor. Dashed lines mark the H bonds formed between the PGE2 and the Ser285, Thr64 and Phe102 residues from the LBD of the EP4 receptor subtype.

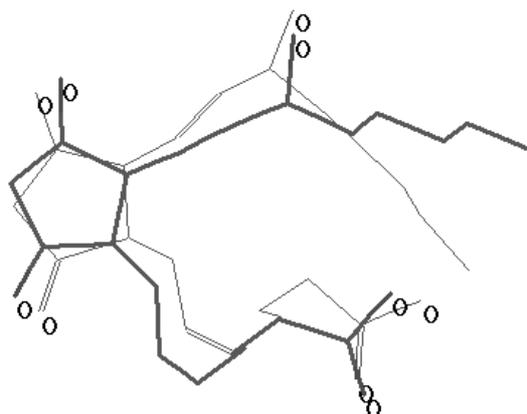


Fig. 9 – Superposition of the docked conformer (thick lines) and a conformer resulted using the Conformational search module and PM3 method. H atoms are hidden for clarity.

The RMS fit is 1.185 Å and distances between oxygen atoms implicated in H bonds from the two conformers are: $O_{24} \div O_{24}$ 0.619 Å, $O_{18} \div O_{18}$ 0.639 Å and $O_{13} \div O_{13}$ 0.442 Å. Due to the small distances between oxygen atoms from the two conformers, both conformers may interact efficiently with the EP receptor subtype by H bond formation. Thus the PM3 method can give conformers with similar trend of dihedrals which determine the geometry of the conformer in the binding site. These conformers which can interact by H bonds with the residues from the LBD are found in a large energy range between 4 and 11 kcal/mol above the global minimum conformer.

CONCLUSIONS

From the conformational analysis of the PGE2 performed automatically using the Conformational Search module from the Hyperchem7.52 package for generating conformers and the PM3 method for optimizing them, resulted 82 conformers in a range of 3 kcal/mol and around 500 conformers in a range of 5 kcal/mol. Some structures are optimized with abnormal geometries, suggesting that not all low energy conformers can be interconverted in other low energy conformers. Histograms representing the distribution of dihedral values among the 82 PM3 conformers in a range of

3 kcal/mol show that the two hydroxyl groups in PGE2 prefer different orientation and have a very narrow domain in which they can be rotated. In contrast, the carboxyl group has a large spectrum of possible orientations suggesting that this group can easily modify its orientation to be able to interact with residues (from the binding site) containing protonated nitrogen atoms.

Simulated annealing runs at different simulation temperatures between 400 and 1000 K gave different final structures which by optimization had energies higher than the energy of the global minimum, confirming this way that the global minimum found is a true global minimum.

The energy profiles resulted from the PM3 single point calculations show that the flexible bonds attached to the double bonds or the 3-hydroxy-5-cyclopentanone ring can be rotated in only narrow domains, while the rest of the bonds can be rotated in larger intervals where certain interconversions of the low energy conformers one in another is possible. All PM3 data suggest that ex-LBD PGE2 can have a lot of low energy conformations which could interact with residues from the LBD of the EP receptor subtypes.

The PM3 method gives many conformers with similar geometries to that of the best docked conformer. These conformers have energies with around 4-11 kcal/mol higher than the energy of the global minimum conformer.

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