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Dedicated to Professor Ionel Haiduc
on the occasion of his 75th anniversary

CONFORMATIONAL ANALYSIS OF PROSTAGLANDIN E2. II

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The PM3 semiempirical MO method was used to perform a systematic conformational search for prostaglandin E2, PGE2. 2D potential energy profiles were obtained from PM3 single point calculations performed on 1152 conformers generated by simultaneous rotation of two pairs of dihedrals with steps of 15º. Through geometry optimization all the conformers fall in the global minimum and in 72 local minima with energy differences between 0.0 and 7.4 kcal/mol. Among them there is no conformer with a geometry similar with that of a conformer docked in a 3D model of the EP4 receptor.

INTRODUCTION

(Z)-7-[(1R,2R,3R)-3-hydroxy-2-[(E,3S)-3-hydroxyoct-1-enyl]-5-oxocyclohept-5-pentyl]enoic acid, prostaglandin E2, PGE2 or dinprostone is the endogenous ligand of the EP receptor subtypes which belong to the A class of the large family of G protein-coupled receptors (GPCR). The EP 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 regulate secretion and motility in the gastrointestinal tract, as well as ions and water transport from the kidneys. 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] They are involved in apoptosis, cellular differentiation, and oncogenesis. EP prostanoid receptors regulate the activity of blood platelets, both positively and negatively, and are involved in vascular homeostasis and hemostasis.[2] Coleman et al. classified the EP receptors in four subtypes: EP1, EP2, EP3, and EP4, all responding to the endogenous agonist PGE2, but differing in their actions and responses to various analogous compounds.[3,4]

Presently, the interaction of PGE2 with the four EP receptor subtypes is not well understood. A conformational analysis of PGE2 can help to find out the conformational space occupied by possible conformers of PGE2 and also the steric properties important for the PGE2-EP receptor interaction. In the previous paper[5] the results of a conformational analysis of PGE2 performed automatically with the Conformational search module from Hyper Chem7.52 software[6] using the PM3 semiempirical MO method were reported. In this paper are presented the results of a systematic conformational search performed with the PM3 semiempirical MO method on 1152 PGE2 conformers generated by simultaneous rotation of two pairs of dihedrals which have an important role in determining the shape and energy of the

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PGE2 conformers. From the PM3 single point calculations the 2D profiles of the potential energy are obtained, while from the geometry optimization resulted 73 low energy conformers.

METHODS

Starting from the conformer with the lowest PM3 energy obtained from an automatic conformational search with the Conformational Search module from the HyperChem7.52 software, 576 conformers were generated through simultaneous rotation of a pair of dihedrals between 0º and 360° with steps of 15°. Two such pairs of dihedrals, which produce very high sterical hindrance, have been systematically modified and thus 1152 conformers have been generated. The energy of these generated conformers was obtained both by PM3 single point calculations, and by full geometry optimization. The optimization criteria were: an SCF convergence of 10^{-5} and a stop optimization at a RMS gradient of 0.01 kcal/Åmol. By plotting the energy difference between each conformer and the one of the global minimum against the values of each pair of dihedrals, the 2D potential energy profiles of the two pairs of dihedrals were obtained.

RESULTS

PGE2 (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-hydroxy-cyclopentanone ring and one (S) on the 3-(S)-hydroxyoct-1-enyl substituent.

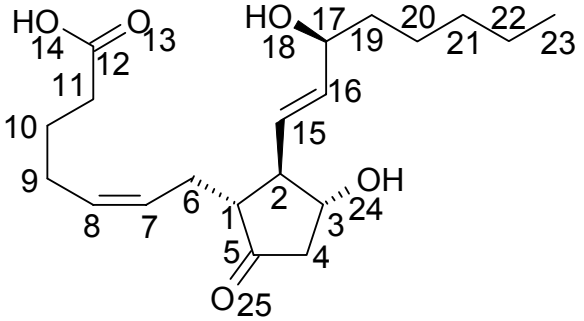

Fig. 1 – Structure and numbering of atoms in PGE2.

To obtain the 2D-profiles of the potential energy (2D-PPE) two pairs of dihedrals have been chosen: 1) $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ and 2) $C_1C_6C_7C_8$ and $C_{15}C_{16}C_{17}C_{18}$. In Fig. 2 are shown the minima of the 2D-PPE resulted by simultaneous rotation of the first pair of diedrals. One can note there are only a few minima and they have energies with at least 3.5 kcal/mol higher than the energy of the global minimum conformer. The diheral values of the global minimum conformer are missing from the plot. This is not quite unexpected because the sterical hindrance is very high at the rotation of the C_1-C_6 and C_2-C_{15} bonds. The rotation of these two bonds is limited to only narrow intervals between 50º-60° and 240-250º, respectively. In the global minimum conformer the values of the $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ dihedrals are 55.08º and 248.03º, respectively.

Through geometry optimization, the 576 conformers fall in 39 low minima with energies between 0.2 and 7.38 kcal/mol higher than the energy of the global minimum conformer (Table 1 and Fig. 3) and in a minimum of a duplicate of the global minimum. This duplicate is 0.18 kcal/mol higher than the global minimum and it is not counted in Table 1. The two conformers can be considered as duplicates for two reasons: their geometries are very similar (by superposition there is a RMS error of only 0.167 Å) and their energies are very close (see supporting information).

Comparing the energy values in Table 1, one can estimate the distribution of energy differences between the conformers: 12 conformers (excluding the duplicate with the 0.18 kcal/mol difference to the global minimum) are in a range of 3 kcal/mol, while the majority of conformers are in a range of 3-7.4 kcal/mol (27 conformers). In Table 1, one can see that the $C_2C_1C_6C_7$ dihedral has values in the intervals 40-70º (18 conformers), 180-200º (4 conformers) and 280-300º (13 conformers), while in each of the intervals 70-80º, 260-270º, 270-280º and 320-330º there is a single conformer. In the intervals 0-40º, 80-180º, 200-280º, 300-320º and 330-360º there are no conformers.

From the data in Table 1, one can also estimate the distribution of the $C_1C_2C_{15}C_{16}$ dihedral values among the conformers found in the local minima: in intervals 230-250º (15 conformers), 260-270º (3 conformers), 280-310º (5 conformers) and 330-360º (5 conformers). Also in the intervals 110-150º and 10-20º there are 8, and 3 conformers, respectively. The intervals of the $C_1C_2C_{15}C_{16}$ dihedral in which there is no conformer are: 0-10º, 20-110º, 150-230º, 270-280º and 310-330º.

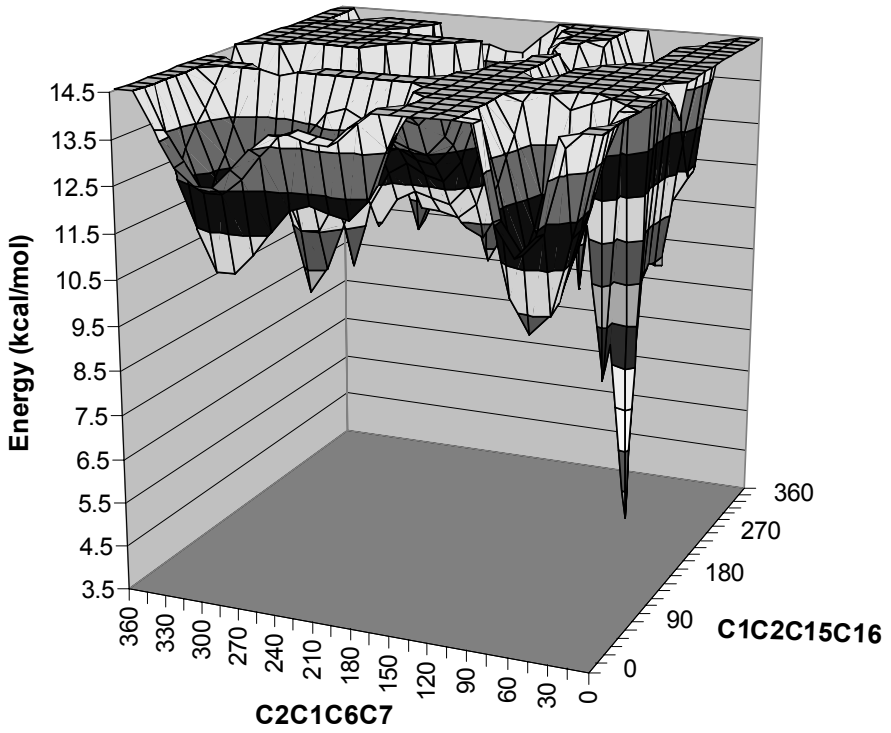

Fig. 2 – Plot of the 2D-profile of the potential energy obtained by PM3 single point calculations for the 576 conformers generated from the lowest PM3 conformer of PGE2 by simultaneous rotation of the $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ dihedrals.

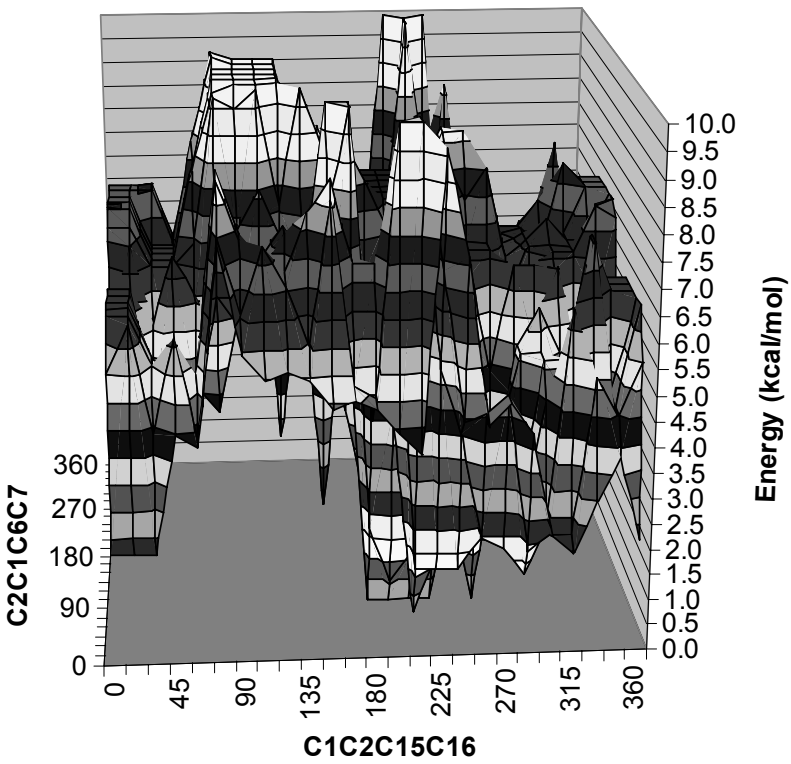

Fig. 3 – Plot of the 2D-profile of the potential energy obtained by PM3 optimization of the geometries of 576 conformers generated from the lowest PM3 conformer of PGE2 by simultaneous rotation of the $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ dihedrals.

Table 1

Energy differences and dihedrals in conformers in low energy minima resulted by PM3 optimization of geometry of the 576 conformers generated by rotation of the $C_2C_1C_6C_7$ and $C_1C_2C_{15}C_{16}$ dihedrals

Conformer ID	Energy*	$C_2C_1C_6C_7$	$C_1C_2C_{15}C_{16}$	$C_1C_6C_7C_8$	$C_{15}C_{16}C_{17}C_{19}$
	0.18	50.4	-117.6	-123.2	-99.4
1	0.20	48.7	-118.9	-112.9	-119.0
2	0.30	51.6	-118.8	-122.0	-109.8
3	0.78	48.7	-114.9	-121.4	-113.7
4	1.36	52.3	-118.3	-120.1	-116.5
5	1.40	55.7	-119.1	-124.4	-93.8
6	1.87	57.4	-123.2	-117.1	-123.8
7	2.04	54.3	-120.5	-124.5	-97.2
8	2.13	56.2	-110.7	-125.2	-115.3
9	2.37	-66.8	-20.2	-86.3	-80.9
10	2.37	-65.6	10.4	-85.9	-99.7
11	2.50	51.0	-126.8	-119.7	-95.8
12	2.52	-65.6	10.4	-85.9	-99.7
13	3.16	52.9	-124.3	-121.9	-88.5
14	3.20	-73.3	-120.1	-124.1	-81.2
15	3.33	47.6	-116.3	-141.1	-125.2
16	3.46	-73.9	-75.1	-136.0	-111.3
17	3.66	71.1	-114.5	-109.3	-83.8
18	4.01	52.92	-124.3	-121.9	-88.5
19	4.17	50.3	-55.4	-132.3	102.6
20	4.31	59.7	13.2	-114.6	-95.3
21	4.41	61.7	-10.6	-114.1	-78.3
22	4.42	-72.4	-91.7	-118.0	-106.2
23	4.44	-39.3	-68.7	-108.8	-109.3
24	4.48	60.4	-93.1	-121.0	-88.2
25	4.58	-68.7	-116.2	-90.5	-130.2
26	4.63	-82.2	148.6	-137.3	-68.1
27	4.95	62.9	127.6	-114.5	125.1
28	5.05	-62.8	-20.0	-134.0	-80.0
29	5.07	-68.1	115.5	-89.7	-39.4
30	5.50	-77.3	-52.4	-145.7	-67.9
31	5.50	-96.8	-8.2	-145.5	-109.3
32	5.61	-75.6	133.1	-135.9	-79.0
33	5.62	-71.1	119.7	-134.9	-72.2
34	5.64	-69.9	122.1	-90.5	-76.2
35	6.04	-171.9	-95.2	-139.2	-103.1
36	6.07	57.0	122.0	-141.6	-91.4
37	6.23	-178.1	-20.2	-139.3	-79.9
38	6.25	-164.7	-58.9	-141.9	-112.8
39	7.38	-163.2	131.7	-139.1	-66.4

* - difference between the energy of each conformer and the one of the global minimum conformer (-5638.6 kcal/mol).

In Figs. 4 and 5 the 2D profiles of the potential energy resulted by the rotation of the second pair of dihedrals ($C_1C_6C_7C_8$ and $C_{15}C_{16}C_{17}C_{19}$) are shown.

For the second pair of dihedrals the results of PM3 single point calculations are similar to those for the first pair. Again, the global minimum is missing and the local minima are higher than the usually obtained values. This is due to the high sterical hindrance to the rotation of the second pair of dihedrals. The potential energy corresponding to the C_6-C_7 and C_{16}-C_{17} bond rotation increases sharply in certain intervals. The rotation of these bonds is limited to only narrow intervals: between 220º-240° and 250-270º, respectively. In the global minimum conformer, the $C_1C_6C_7C_8$ and

$C_{15}C_{16}C_{17}C_{19}$ dihedral values are 231.23º and 259.56º, respectively. By geometry optimization, the 576 conformers fall in 34 low minima (including the global minimum conformer) with energies between 0 and 7.23 kcal/mol higher than the energy of the global minimum conformer (Fig. 5 and Table 2).

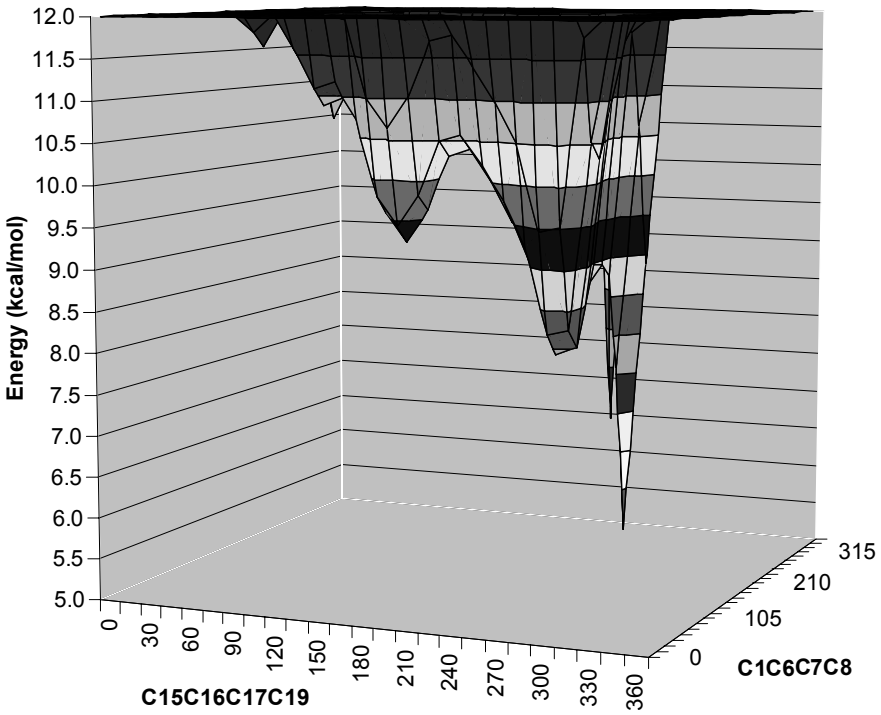

Fig. 4 – Plot of the 2D- profile of the potential energy obtained by PM3 single point calculations for the 576 conformers generated from the lowest PM3 conformer of PGE2 by simultaneous rotation of the $C_1C_6C_7C_8$ and $C_{15}C_{16}C_{17}C_{19}$ dihedrals.

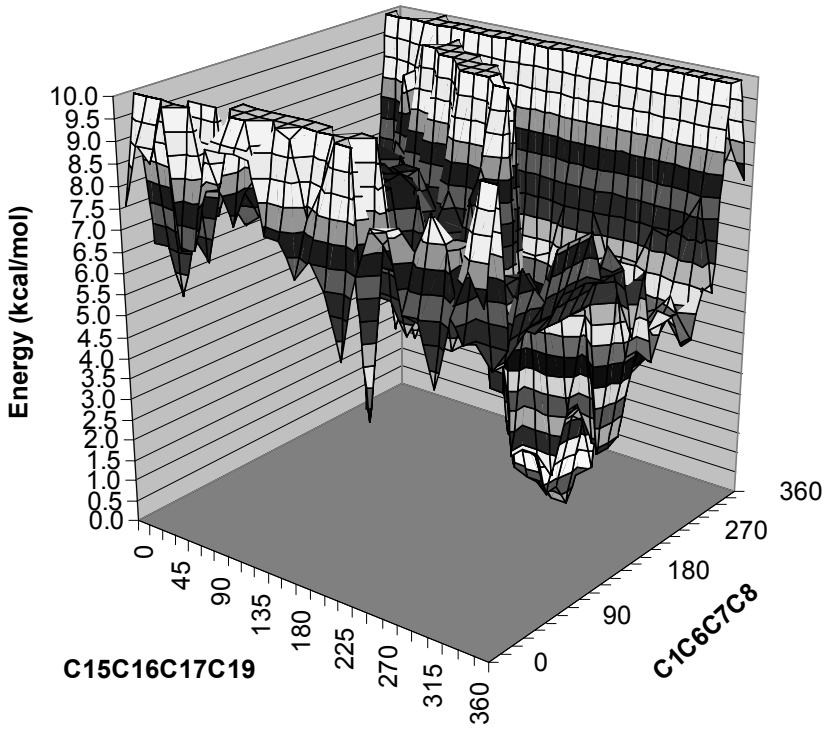

Fig. 5 – Plot of the 2D-profile of the potential energy obtained by PM3 optimization of the geometries of 576 conformers generated from the lowest PM3 conformer of PGE2 by simultaneous rotation of the $C_1C_6C_7C_8$ and $C_{15}C_{16}C_{17}C_{19}$ dihedrals.

Table 2

Energy differences and dihedrals in conformers in low energy minima resulted by PM3 optimization of the geometries of 576 conformers generated by rotation of the $C_1C_6C_7C_8$ and $C_{15}C_{16}C_{17}C_{19}$ dihedrals

Nr	Energy*	$C_2C_1C_6C_7$	$C_1C_2C_{15}C_{16}$	$C_1C_6C_7C_8$	$C_{15}C_{16}C_{17}C_{19}$
1	0.00	55.1	-112.0	-128.8	-100.4
2	0.18	50.6	-117.7	-123.0	-99.9
3	1.23	63.5	-62.4	-108.0	158.1
4	1.26	68.0	-66.1	-106.6	143.6
5	1.48	29.1	-99.3	-115.5	-115.4
6	2.35	54.4	-123.1	-123.1	-83.9
7	3.24	63.9	-100.2	-126.7	-176.1
8	3.26	73.2	-105.9	-116.2	151.1
9	4.25	-73.4	-97.1	124.1	-113.4
10	4.45	65.7	-66.3	-152.5	122.6
11	4.46	70.6	-115.0	-109.2	-20.6
12	4.96	52.5	-36.0	142.2	146.0
13	5.02	62.0	-91.3	-143.5	141.0
14	5.14	59.2	-140.0	-113.7	67.8
15	5.17	70.5	-99.5	-104.9	-22.1
16	5.18	91.6	-110.5	-104.8	166.6
17	5.24	57.5	-117.2	-119.3	-16.1
18	5.31	50.4	-117.6	128.7	145.7
19	5.44	64.9	-89.6	-114.9	66.3
20	5.46	50.4	-117.6	104.8	77.6
21	5.51	54.8	-41.9	139.0	142.1
22	5.62	63.0	-117.6	-102.0	106.1
23	5.63	75.9	-101.4	-97.7	-85.3
24	5.64	52.7	-35.6	138.2	133.5
25	5.7	42.0	-101.5	126.2	-111.3
26	5.93	64.4	-175.4	125.3	80.1
27	6.09	52.4	-110.3	-125.4	100.1
28	6.3	50.0	147.2	129.6	115.1
29	6.33	54.8	-112.7	-134.1	131.8
30	6.8	48.6	-143.5	128.2	33.6
31	6.96	82.5	-107.4	-98.5	114.5
32	6.96	64.7	-141.8	127.3	67.6
33	7.22	65.3	-174. 5	119.3	79.8
34	7.23	64.8	-130.7	127.8	67.6

* - difference between the energy of each conformer and the one of the global minimum conformer (-5638.6 kcal/mol).

In Table 2 the distribution of energy differences between the low energy conformers and the global minimum conformer can be estimated. Rotation of this pair of dihedrals gives only five conformers with energy differences in a range of 0-3 kcal/mol above the global minimum conformer, while the majority of conformers have energy differences in a range of 3-7 kcal/mol (28 conformers) above the global minimum. In Table 2 one can see that the $C_1C_6C_7C_8$ dihedral has values in intervals 100-150º (14 conformers) and 200-270º (20 conformers), while in intervals 0-100º, 150-200º, and 270-360º there is no conformer. Also, in Table 2 the distribution of the $C_{15}C_{16}C_{17}C_{19}$ dihedral values among the conformers found in the low minima can be estimated. In intervals 60-80º, 100-170º, 240-280º, and 330-350º there are 7, 15, 7 and 3 conformers, respectively, while in each of the intervals 30-40º and 180-190º there is one conformer.

In the previous paper[5], the geometry of a conformer docked in a 3D model of the EP4

receptor[7] was presented and this geometry was compared to the geometries resulted from an automatic conformational search performed by modifying randomly, with steps of 45°, of the dihedral angles corresponding to all flexible bonds of PGE2 and using the PM3 method for geometry optimization. In the best docked conformer the values of the four dihedrals ($C_2C_1C_6C_7$, $C_1C_6C_7C_8$, $C_1C_2C_{15}C_{16}$, and $C_{15}C_{16}C_{17}C_{19}$) which have a major influence on the geometry of the conformer in the binding site are: 196.5º, 87.1º, 238.2º, and 236.5º, respectively. From the automatic conformational search resulted that none of the 82 conformers in a range of 3 kcal/mol higher than the global minimum has values close to the values of dihedrals from the docked conformer. Searching among all conformers resulted from automatic conformational analysis, there have been found 58 conformers having similar trend values. They had energies with 4-11 kcal/mol higher than the energy of the global minimum conformer. The conformer which gave the lowest RMS error was a conformer with an energy with 8.8 kcal/mol higher than the one of the global minimum conformer. Compared to the global minimum conformer which has a very packed geometry, this conformer has a much more extended geometry.

The geometry of the best docked conformer was optimized with the PM3 method. The resulted conformer has the energy with around 7.8 kcal/mol higher than the one of the PM3 global minimum conformer. By superposing the initial geometry of the best docked conformer and the one of a conformer resulted through PM3 optimization of the docked conformer geometry, a RMS error of 1.151 Å (see supporting information) results, while the distances between oxygen atoms from the two conformers implicated in H bonds with residues from the binding site of the EP4 receptor are: O_{13}÷O_{13} 1.834 Å, O_{18}÷O_{18} 2.084 Å and O_{24}÷O_{24} 2.267 Å. These distances are much larger than those obtained by superposing the geometries of the best docked conformer and a conformer resulted from a conformational search using the PM3 method and the Conformational Search module from HyperChem7.52 software. In this case the distances are O_{13}÷O_{13} 0.442 Å, O_{18}÷O_{18} 0.639 Å and O_{24}÷O_{24} 0.619 Å.

In Tables 1 and 2 one can see that there is no conformer whith similar values of the four dihedrals with the dihedral values of the best docked conformer. Thus, starting from the PM3 geometry of the global minimum conformer, by systematic search of the variation of two pairs of dihedrals a geometry similar with that of the best docked conformer cannot be found. This may be due to the starting geometry from which only certain conformers can be obtained.

Another important reason for this result is that, generally, the semiempirical MO methods give global minima with very packed geometries and the majority of conformers with extended geometries have energies with more than 3 kcal/mol higher than the global minimum. The range of 3 kcal/mol, accepted by the scientific community for searching conformers with geometries similar to those bound in the active sites of proteins is satisfactory for molecular mechanics force fields, because the molecular mechanics metods give many structures in extended conformations in this range of 0-3 kcal/mol above the global minimum. From our data it results that for semiempirical MO methods this interval should be increased up to around 10 kcal/mol.

Maybe the large disimilarity between the geometry of the global minimum conformer and the one of the conformer bound in the binding site of the EP4 receptor is one of the reasons for which statistically significant QSARs could not be obtained for the series of the PGE2 derivatives active on the four EP receptor subtypes.

The same result could be also obtained for many compounds with similar geometries having 1-, 2-, 3- substituted cyclopentane rings in which on two neighbor large substituents double bonds are placed near to the ring. In these cases the PM3 systematic search can be a suitable method only for searching the global minimum, or for verifying if the global minimum is the true one.

CONCLUSIONS

The systematic search of low energy PM3 conformers performed for two pairs of dihedrals (which produce very high sterical hindrance) led to the global minimum conformer and other 72 local minima in a range of 0.2 – 7.4 kcal/mol above the global minimum conformer.

On the 2D-profiles of the potential energy certain low minima could be evidenced with the exception of the global minimum conformer. These energy profiles correspond to compounds with high sterical hindrance to the rotation of certain flexible bonds.

The search of minima through the PM3 optimization of the geometries of conformers generated systematically starting from the lowest energy conformer did not lead to PGE2 conformers with a geometry similar to the one of the conformer bound in the active site of a 3D model of the EP4 receptor. For compounds with a molecular structure similar to that of PGE2 the systematic search starting from very low energy conformers should be performed only to find the global minimum conformer.

Due to the fact that the geometry of the global minimum conformer given by semiempirical MO methods is very packed, to have succesful QSAR studies the geometries in a range of around 10 kcal/mol should be considered. One or more geometries in extended conformations should be chosen as starting geometries for calculation of descriptors.

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