Biochemical Pharmacology, Vol. 38, No. 15, pp. 2441–2447, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

PHYSICO-CHEMICAL PROPERTIES OF PROSTAGLANDINS AND RELATED PHARMACOLOGICAL COMPOUNDS

A THEORETICAL STUDY ON CONFORMATIONAL RELATED ACTIVITY

MICHEL DELEERS*† and ROBERT BRASSEUR‡

*UCB Pharmaceutical Sector, Bldg B3, B-1420 Braine-l'Alleud, Belgium; and ‡Macromolecules at Interfaces, C.P./206/2, University of Brussels, B-1050 Brussels, Belgium

(*Received* 1 *September* 1988; *accepted* 1 *February* 1989)

Abstract—Thromboxane A_2, prostaglandin H2, a series of chemically stable cyclic endoperoxide analogues (U 46619, U 44069, ONO 11113, 9, 11, diazo PGH_2 and SQ 26655) and different isomers of SQ 26655 were analysed for their spatial configuration by conformational analysis in a simulated membrane–water interface environment with a "structure tree" procedure already described for prostaglandins, leukotrienes and lipoxins. The conformers derived from the structure tree and with a high probability of existence are presented. A new method allows one to visualize the surface charge density of the calculated molecules. The spatial configuration and the surface charge density of each molecule are compared to their known order of competition binding to the putative TXA_2/PGH_2 receptor of platelets. The conformational and charge density analysis merely shows that the different stereochemistry of these molecules lead to spatial conformation, that mimics (agonists), or that are far from (antagonists) the TXA_2/PGH_2 conformation.

Blood platelets and blood vessel endothelial cells play important roles in either the development or the prevention of atherosclerosis and related disorders [1, 2]. Conversion of arachidonic acid obtained by the action of phospholipase A_2 on membrane phospholipids and by subsequent activation of different specific enzymes finally leads to the formation of potent labile pro-aggregatory substances from platelets [3, 4] and to potent labile anti-aggregatory substances from the vessel wall cells [4, 5].

The adversary action of these different molecules and their extreme lability has encouraged the search for stable analogues [6–9] of both types of activity for evident pharmacological and therapeutic purposes [10–17]. Systematic approaches have then been made by different groups to synthesize and develop molecules that mimic or antagonize the effect of prostaglandin H_2 and thromboxane A_2 [6–9, 13, 18–20]. Chemically stable analogues have thus been obtained by modifications of the dioxabicyclo (3,1,1)-heptane ring system of TXA_2 while generally leaving the 5-heptenoic acid and 3-hydroxy 1-octenyl chains unchanged and in the same stereochemistry. Changes in the activity of some stable ring structures generally assessed by studies on platelets systems have also been obtained either by modifications of the side chains and carbinol stereochemistries, or by complete modification of the side chains.

However, despite the considerable amount of work dedicated to the finding of molecules suitable for one of the major health problems, no systematic attempts have been made for the study on conformational structure–activity relationship of these prostaglandins-related compounds. Nevertheless, it should be noted that calculated conformational energy has been undertaken for some prostaglandins and thromboxanes [21–25] and that theoretical analysis has also made hypothetical interaction of calcium with prostaglandins [26] and thromboxane A_2 [27] understandable.

The good agreement between prediction methods and different experimental observations has indeed put forward the analysis of conformations for molecular recognition [28]. Theoretical conformational analysis appears to be a powerful tool for visualizing molecules and for obtaining information on interactions between compounds [29, 30], on structure–activity relationship of pharmacological drugs or biological compounds [23, 25, 31–36], but also on the mode of microorganization of amphiphilic molecules in the vicinity of membranes [37–44]. So, conformational analysis may help at least in part, to understand the biological and pharmacological activities of a wide variety of molecules.

The present paper will focus on conformational analysis as one of the ways to gain insight into the molecular structure of TXA_2, PGH_2 and a series of chemically stable endoperoxide analogues obtained either by modifications of the bicycloheptane ring or by modifications of the side chains stereochemistry on one of these stable ring structures.

These are U 46619, U 44069, 9–11 diazo PGH_2, ONO 11113, SQ 26655 and SQ 26538 and some similar molecules because they appear to be the molecules most studied.

MATERIALS AND METHODS

The method follows a strategy generally used to study the conformation of polypeptides [45]. Our theoretical prediction (molecular structure analysis) of each isolated stable endoperoxide analogue firstly

† To whom correspondence should be sent.

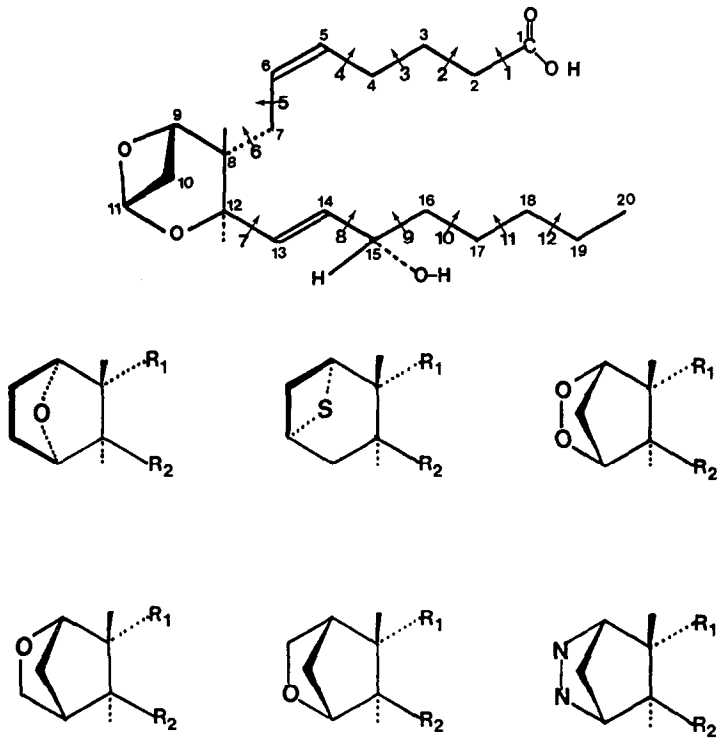

Fig. 1. (a) Initial conformation of TXA_2 and numbering of atoms f atoms and angles in the side chains taken as reference for all the molecules investigated. (b) Structure of prostanoids investigated in this work. R_1 and R_2 indicate that the heptenoic acid and octenyl chains respectively, are identical to those found in natural prostanoids. Ring structures of SQ 26655, ONO 11113, PGH_2, U 44069, U 46619 and diazo-PGH_2 are respectively shown from upper left to lower right.

evaluates the total conformational energy calculated as the sum of three equations of energy:

(1) The London–van der Waals energy of interaction between all pairs of non-mutually bonded atoms. This is well suited by the atom–atom interaction function of Buckingam:

$$E_{vdw} = \sum_{ij}[A_{ij} \exp(-B_{ij} \cdot r_{ij}) - C_{ij} \cdot r_{ij}^{-6}], \quad (1)$$

where $i, j, = 1, 2, 3, \ldots$ are the non-bonded atoms, r_{ij} their distances from each other in a given structure, and A_{ij}, B_{ij} and C_{ij} coefficient assigned to atom pairs. These coefficients have been reported by Liquori and coworkers [46, 47] and like other quantum-mechanical results, they emerge in part as the solution of the Schrödinger equation and in part as heuristic variables. They have been applied with success to molecular structure analysis of polypeptides, proteins and amphiphilic molecules. An imposed repulsive cut-off value of 100 kcal/mol is applied where r_{ij} is smaller than 0.1 nm.

(2) The generalized equation of Coulomb's electrostatic interaction between atomic point charge in which the values of discrete atomic point charge are similar to those currently used in conformational analysis [31, 45, 48, 49]. Discrete charges of the COOH group also correspond to those used, the sum of the charges being equal to 0.

$$E_{cb} = 332\left(\sum \frac{e_i \cdot e_j}{r_{ij}\varepsilon_{ij}}\right). \quad (2)$$

The dielectric constant ε_{ij} is the determinant for the importance of this electrostatic term. In the first calculation, the dielectric constant is assumed to be 16.5 since it has been determined experimentally and theoretically that the dielectric constant value in a membrane–water interface is about 10–30 and that the dielectric constant of the inner lipid matrix is about 3 [50, 51].

(3) The potential energy of rotation of torsional angles. The rotation around the C—C or C—O bond was calculated by

$$E_{tor} = \frac{U_{ij}}{2}(1 + \cos \Phi_{ij}), \quad (3)$$

where U_{ij} correspond to the energy barrier in the eclipsed conformation during the rotation. U_{ij} is equal to 2.8 kcal/mol for the C—C bond and 1.8 kcal/mol for the C—O bond.

The internal energy of the molecule under investigation is calculated for a large number of conformations in a systematic analysis structure tree where six changes of 60° each were succesively imposed to n torsional angles chosen yielding 6^n conformers in each branch of the structure tree [39]. The internal energy is calculated for each conformer and the most probable configuration was taken following Boltzmann's equation of probability of existence. The effect of entropy was considered at this stage of the calculation process as negligible and hence, the selec-

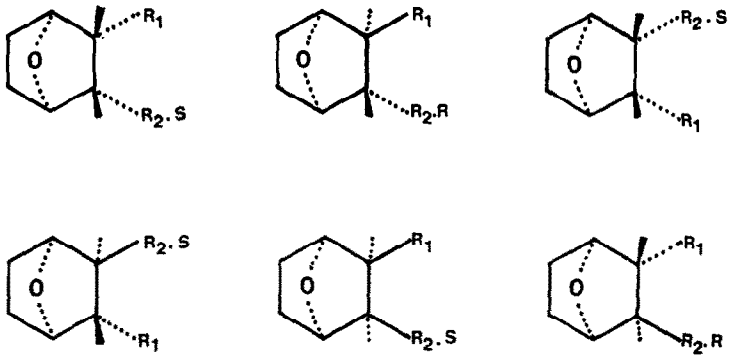

Fig. 2. Structure of the six isomers of SQ 26655 investigated in this study, respectively from upper left to lower right; compounds 3a (SQ 26538), 4a, 3c, 5c, 2a and 5a (SQ 26655) taken from Ref. 9. R_1 and R_2 indicate that the position of the heptenoic acid and octenyl chains are different in compounds 3c and 5c. (R) and (S) indicate the hydroxy stereochemistry.

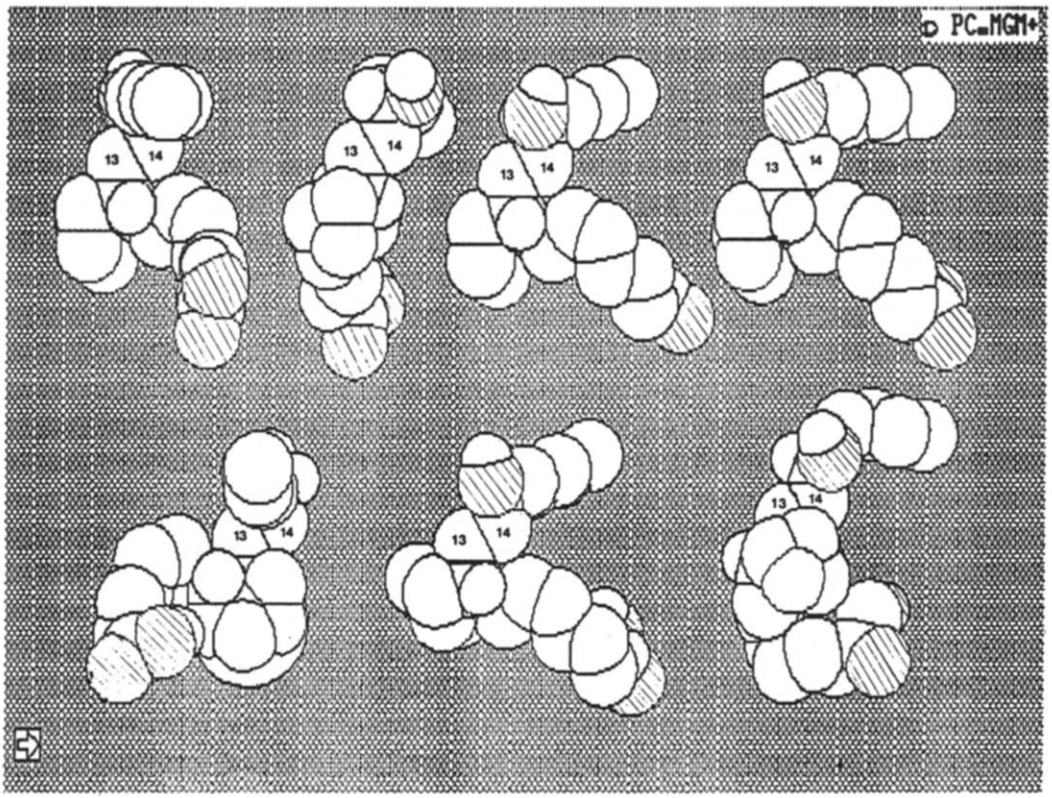

Fig. 3. Full van der Waals volume representation of the most probable conformations of respectively PGH_2, diazo-PGH_2, U 44069, U 46619, ONO 11113, SQ 26655 and TXA_2 from upper left to lower right. For each molecule, the double bond C_{13}=C_{14} is in the plane of the figure. Oxygen atoms are shaded in order to have a better insight into the molecules.

tion of conformers was based on their energy rather than free energy. To limit the number of possible degrees of freedom, bond lengths and bond angles were assumed to be constant. The conformations derived from the systematic study and yielding a low internal energy were submitted to a second analysis using a simplex minimization procedure [52], in order to further reduce their total energy in a variable dielectric field and by taking the transfer energy of each part of the molecule [53] through a simulated interface [37–39, 41]. The simplex minimization procedure is made with a resolution of less than 10° on each torsional angle. We have assumed a dielectric constant equal to 3 above the interface while the atom most deeply immersed in the aqueous phase delineates a plane parallel to the first where the dielectric constant was assumed to be 30 [37, 39, 41, 50, 51]. Between these two planes, the dielectric constant was allowed to

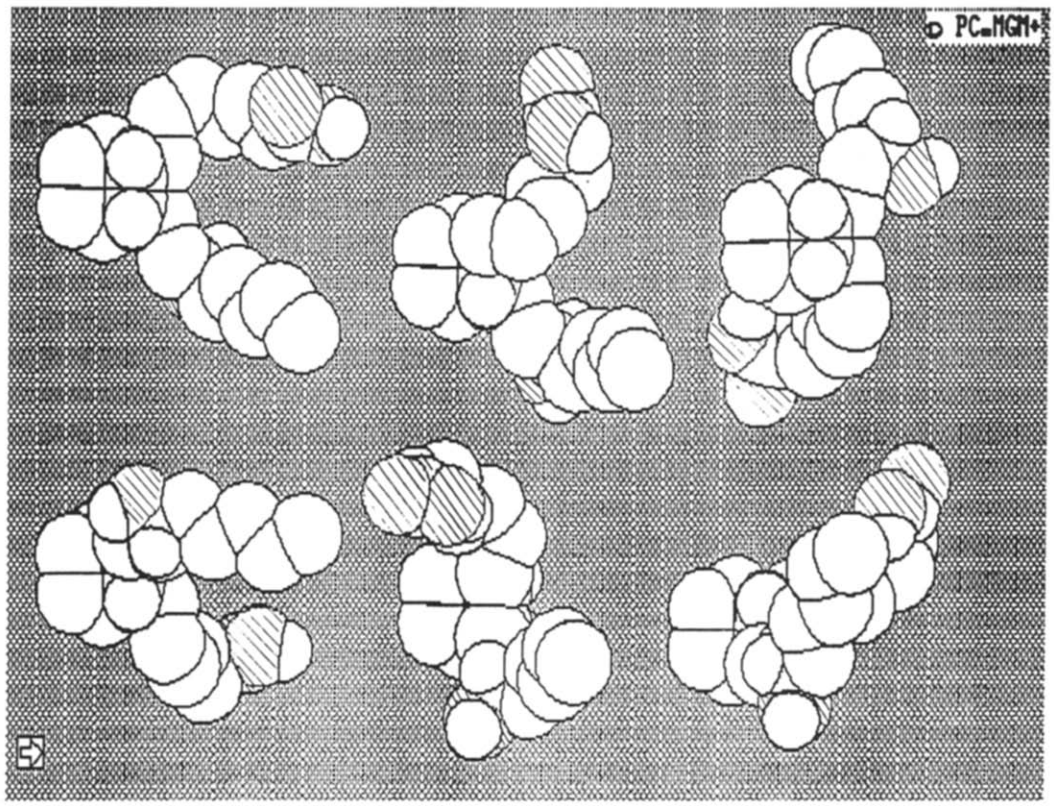

Fig. 4. Full van der Waals volume representation of the most probable conformations of the six isomeric molecules 3a, 4a, 3c, 5c, 2a and 5a. Same position of molecules as in Fig. 2. For each molecule, the ring structure is in the plane of the figure.

increase linearly along the axis perpendicular to the interface.

To better compare the two series of molecules, they were identically oriented in each series. The first series of molecules were oriented with the atom system C_{12}—C_{13}=C_{14}—C_{15} in a plane perpendicular to the view axis while the second series is oriented with the four carbon atoms non-bonded to the oxygen of the 7 oxabicyclo(2,2,1)heptyl ring system in a plane perpendicular to the view axis with the oxygen behind the plane.

Finally, an isoelectrostatic energy contour map showing attractive and repulsive regions was calculated in these defined planes. The electrostatic potential field around one molecule is calculated as follows: a plane is defined by three or four atoms, a virtual charge is translated by steps of 0.05 nm in all directions (x, y) and the electrostatic energy is calculated in each position taking into account a low dielectric constant and all atomic discrete charges of the molecule. A contour to contour interval by steps of 1 kcal/mol is then drawn.

Calculations were made on an Olivetti M380 computer equipped with an Intel 80387 arithmetic co-processor of 32 bits with the aid of the PC-MSA+® program (molecular structure analysis) and the PC-MGM+® program (molecular graphic manipulation). The PC is coupled to an Olivetti laser plotter. With this configuration each conformer is calculated in approximately 0.3 sec.

Principal molecules

ONO 11113: 9, 11-epithio-11,12 methano-TXA_2, U 46619: 15 *S*-hydroxyl-11 α, 9α (epoxymethano) prosta-5Z,13E dienoic acid, U 44069: 15 *S*-hydroxy-11α, 9α (methanoepoxy) prosta-5Z,13E dienoic acid, 9,11-diazo PGH_2: 9,11-diazo prostaglandin H_2, SQ 26655 [1S-(1α,2β)(5Z), 3α (1E,3S),4α]-7-[3-3-hydroxy-1-octenyl, -7-oxabicyclo [2.2.1] hept-2-yl] -5 heptenoic acid, TXA_2: thromboxane A_2, and isomers of SQ 26655 [9].

RESULTS

The molecular structure of TXA_2 has been taken as our initial conformation and is illustrated in Fig. 1a together with the atom numbering and the torsional angles. The molecule is in the full extended configuration at the beginning of the computation procedure. The other molecules we have called "series no. 1" and they were submitted to conformational analysis and are shown in Fig. 1b. The "series no. 2" that contains SQ 26655 and five isomers is shown in Fig. 2. Each molecule has 12 important rotational angles. If these angles were affected by systematic changes of 60°, more than 2.10^9 conformers could be calculated. In order to avoid this large number, the "structure tree" procedure was used [37, 39]. The systematic analysis was carried out on proximal tor-

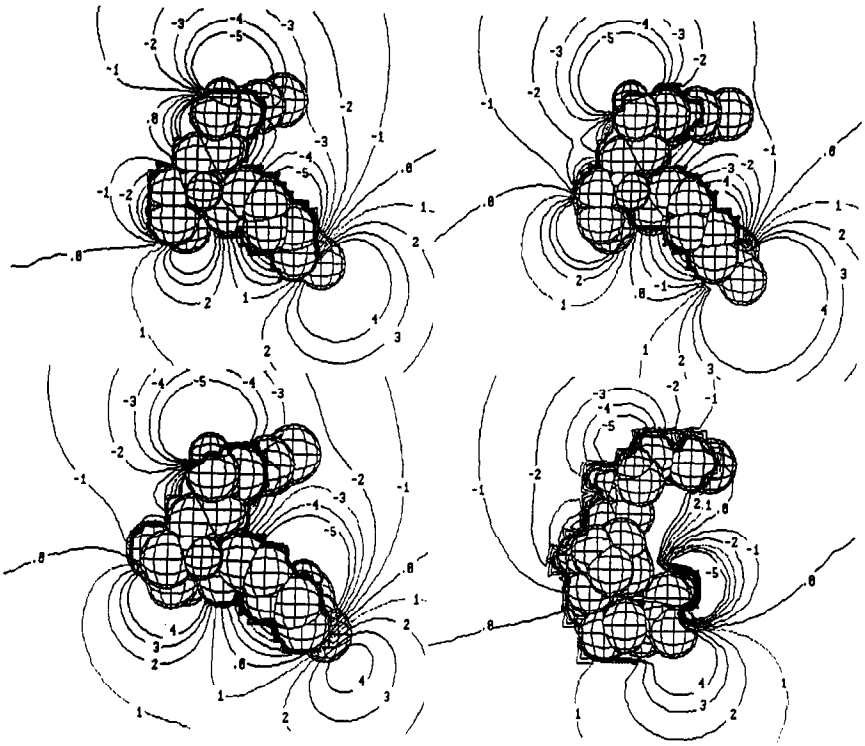

Fig. 5. Electrostatic field contour to contour representation (expressed in kcal/mol) of U 44069, U 46619, SQ 26655 and TXA_2 respectively.

sional angles to the bicyclic ring system by two sequences on five rotational angles (angles 4, 5, 6, 7 and 8 followed by angles 2, 3, 4, 8 and 9) yielding for each molecule 7776 + x.7776 different conformers, x being the number of the most probable case after the first sequence.

After the energy minimization procedure [52] at the simulated interface [37, 38, 41] and that takes all angles into account, the structure tree degenerates into one stable structure for all molecules investigated. These stable conformations have high probabilities of existence (between 88 and 98% of probability).

Figure 3 summarizes the most probable conformations of the seven isolated molecules of the first series. For comparison, the molecules were all oriented with the double C=C bond (C_{13}=C_{14}) in the plane of the figure. As can be seen in this figure, the conformations of U 44069, U 46619 and SQ 26655 are identical. The conformation of TXA_2 is not very far except that the dioxabicyclo ring system is tilted closer to the side chains. Relative to the three similar molecules, PGH_2 possess its ring system in the same position but the side-chains are both rotated in the same direction. The ONO 11113 and diazo-PGH_2 molecules behave differently. This may be due for the former, to the biggest sulphur atom and, for the latter, to a different electrostatic charge at the place of the two nitrogen that replace the two oxygen of PGH_2. However, if one considers a rotation of 90° along the z axis (z and x being in the plane of the figure, while y is perpendicular), the ONO molecule has its two chains in a similar position and configuration than for the three identical molecules. This is also true for PGH_2. So, except for the diazo-PGH_2 analogue, all the molecules presented in Fig. 3 are similar if one considers the van der Waals volume and the general structure.

Figure 4 shows the most probable conformations of six isomeric molecules of SQ 26655. This series is oriented with the four carbon atoms non-bonded to the oxygen of the ring system in the plane of the figure (same orientation for the ring system and same place for the molecule than Fig. 2). The position of the heptenoic acid and 3-hydroxy octenyl chain on the ring system and the chain inversion in the two weak agonist analogues [9] are clearly shown here (upper right and lower left). It is obvious that these isomeric molecules have very different spatial conformations and van der Waals volume.

Figure 5 demonstrates that the electrostatic field taken around molecules U 44069, U 46619, SQ 26655 and TXA_2 in the plane in the C_{13}=C_{14} double bond are quasi-identical. On the contrary, Fig. 6 shows that the electrostatic field around the six isomeric molecules in the plane of the ring system does not present any similarities between them.

DISCUSSION

It has been reported that the five molecules analogues of TXA_2 or PGH_2 have effective concentrations for half maximal response for platelet aggregation that are very similar [13, 14], the more

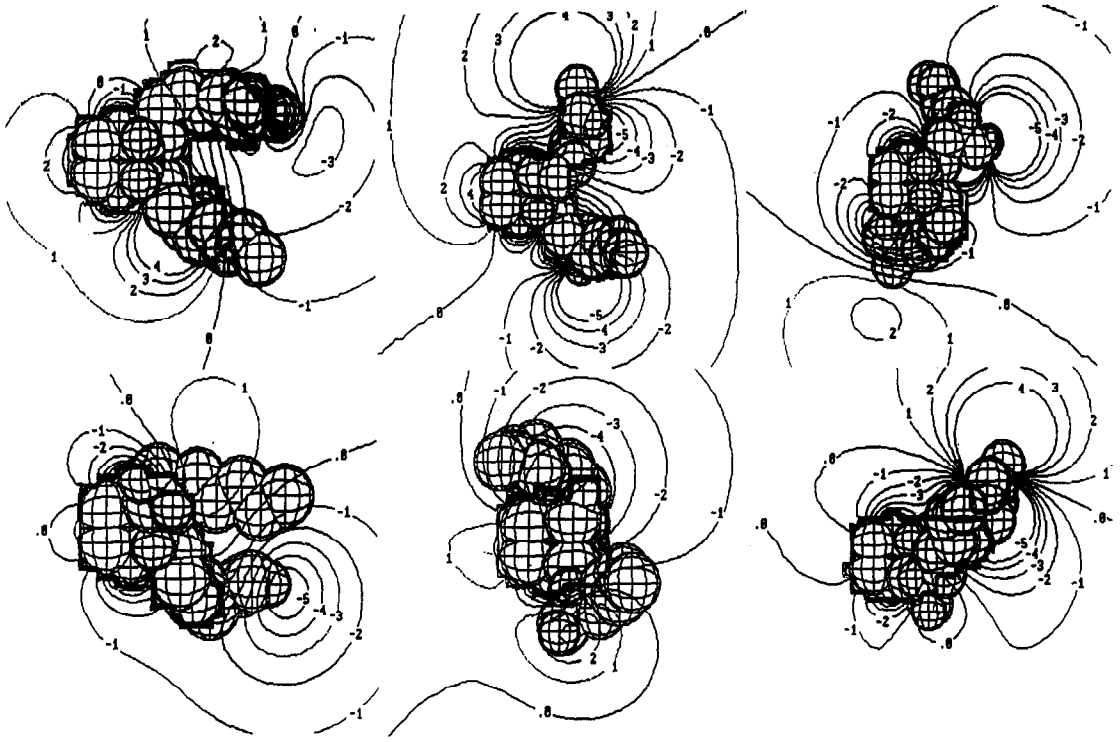

Fig. 6. Electrostatic field contour to contour representation (expressed in kcal/mol) of the six isomers of SQ 26655 presented in the same position as in Fig. 2.

potent (ONO 11113) being only 4 times more potent than the diazo derivative. The cellular response evoked by the three other molecules (U 44069, U 46619 and SQ 26655) are quasi-identical [14]. This could be well reflected by the most probable conformation of these molecules and by their global van der Waals volumes seen in Fig. 3. The similarities between these molecules are also well reflected by the electrostatic field taken around the plane chosen (Fig. 4). When one considers PGH_2 and to a lesser extent ONO 11113, this is also true. However the electrostatic field around the diazo-PGH_2 derivative may not be compared to the other molecules due to the presence of the nitrogen atoms.

On the other hand, the results of the second series study emphasize the obvious existence of great differences in the spatial configuration of the isomeric molecules of SQ 26655 that were chosen here for their vastly different activities on platelets [9]. Indeed, the six molecules have a broad spectrum of activity on platelets; a strong agonist (SQ 26655), a ten times less strong agonist (SQ 26538), two weeks agonists, a weak and a strong antagonist. This is also evident when one looks at the electrostatic field around these molecules. It could however be argued that the plane through the ring system is less representative than the plane taken through the C_{13}=C_{14} bond chosen for the analogues of TXA_2/PGH_2. However, if this last picture convention is taken for the six isomeric molecules (data not shown except for SQ 26655 in Fig. 4), it appears that the six isomers have no similarities between them for either their global van der Waals volumes or for their electrostatic environment. More, it even appears that SQ 26538 whose activity in platelets is relatively high, does not resemble SQ 26655 as far as the two parameters are considered.

The present work aimed at characterizing the most probable conformations of TXA_2, PGH_2 and five strong agonist analogues. The aim was also to characterize isomers with vastly different activities. The most probable conformation obtained for these molecules appears to have a link and even to parallel their biological activities.

It should be noted here that if conformational analysis of TXA_2, PGH_2 and their five analogues is conducted in a homogenous dielectric environment, several structures with high probabilities (>5%) are found. On the contrary, the analysis of these seven molecules each time leads to only one structure with a high probability when the simulated interface is introduced in the computation method.

This work on molecular structure analysis and on the environmental electrostatic field of some prostanoid analogues may thus help in understanding differences in the biological potency of the molecules studied. However, we have already shown that the action of certain molecules on presumed and hypothetical membrane receptors may require an equilibrium between two different forms with high probabilities of existence [39, 42, 43]. The high probability of existence of only one conformation suggests here the very specific action of these compounds.

This work tends to prove the usefulness of conformational analysis made in a simulated membrane–water interface. This type of conformational study

may indeed provide the basis for work on receptor recognition and for interactions between receptors and PGH_2/TXA_2 analogues and/or antagonists involved in signal transduction in blood platelets.

The polymorphism in solution of these molecules seems to degenerate into one stable structure in the vicinity of a membrane or in the membrane interface.

If the assumption is made that a very specific and unique conformation of agonists is needed for recognition, then the similarities in conformations obtained in the simulated membrane–water interface for the first series of molecules may explain the binding to the putative TXA_2/PGH_2 receptor [11, 17].

REFERENCES

1. S. Moncada and J. R. Vane, *Pharmac. Rev.* **30**, 293 (1979).
2. G. De Gaetano, *Clinics in Haematol.* **10**, 297 (1981).
3. M. Hamberg, J. Svensson and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
4. B. Samuelsson, M. Goldyne, E. Granstrom, M. Hamberg, S. Hammarstrom and C. Malmsten, *Ann. Rev. Biochem.* **47**, 997 (1978).
5. S. Moncada, R. Gryglewski, S. Bunting and J. R. Vane, *Nature* **263**, 663 (1976).
6. G. Bundy, *Tetrahedron Lett.* **24**, 1957 (1975).
7. E. Corey, K. Nicolaou, Y. Machedra, C. Malmsten and B. Samuelsson, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3355 (1975).
8. N. H. Wilson, V. Peesapati, R. L. Jones and K. Hamilton, *J. med. Chem.* **25**, 495 (1982).
9. P. W. Sprague, J. E. Heikes, J. Z. Gougoutas, M. F. Malley, D. N. Harris and R. Greenberg, *J. med. Chem.* **28**, 1580 (1985).
10. R. A. Armstrong, R. L. Jones and N. H. Wilson, *Br. J. Pharmacol* **79**, 953 (1983).
11. Y. Kawahara, J. Yamanishi, Y. Furuta, K. Kaibuchi, Y. Takai and H. Fukuzaki, *Biochem. biophys. Res. Commun.* **117**, 663 (1983).
12. D. Saussy, D. Mais, R. Burch and P. Halushka, *J. biol. Chem.* **261**, 3025 (1985).
13. R. A. Armstrong, R. L. Jones, V. Peesapati, S. G. Wiel and N. H. Wilson, *Br. J. Pharmac.* **84**, 595 (1985).
14. P. V. Halushka, P. J. Kochel and D. E. Mais, *Br. J. Pharmac.* **91**, 223 (1987).
15. B. Vargftig, D. Joseph and M. Hatmi, *Thrombosis Res.* **46**, 509 (1987).
16. N. Liel, D. E. Mais and P. V. Halushka, *Prostaglandins* **33**, 789 (1987).
17. H. Zehender, E.-C. Witte, H.-P. Wolff and H. Patscheke, *Biochem. Pharmac.* **37**, 491 (1988).
18. S. Ohuchida, N. Hamaka and M. Hayashi, *Tetrahedron* **39**, 4269 (1983).
19. R. L. Jones, D. E. MacIntyre, W. K. Pollock, A. M. Shaw and N. H. Wilson, *Br. J. Pharmac.* **84**, 148P (1985).
20. D. N. Harris, S. E. Hall, A. Hedberg and M. L. Ogletree, *Drugs of the future* **13**, 153 (1988).
21. A. Murakami and Y. Akahori, *Chem. Pharmac. Bull.* **27**, 548 (1979).
22. S. P. Simonyan, M. D. Shenderovich and J. Freimanis, *Bioorg. Khim.* **13**, 113 (1987).
23. A. Kalia and V. Kothekar, *FEBS Lett.* **193**, 99 (1985).
24. H. Beierbeck, G. Kotovych and M. Sugiura, *Can. J. Chem.* **63**, 1143 (1985).
25. V. Kothekar, *J. Theor. Biol.* **101**, 233 (1983).
26. M. Deleers, P. Grognet and R. Brasseur, *Biochem. Pharmac.* **34**, 3831 (1985).
27. R. Brasseur and J. M. Ruysschaert, *Res. Commun. Chem. Path. Pharmac.* **46**, 175 (1984).
28. J. Rebek, *Science* **235**, 1478 (1987).
29. R. Brasseur, M. Deleers and W. J. Malaisse, *Biochem. Pharmac.* **32**, 437 (1983).
30. R. Brasseur, M. Deleers, J. M. Ruysschaert and W. J. Malaisse, *Biochem. Int.* **7**, 71 (1983).
31. E. Ralston, J. L. Decoen and R. Walter, *Proc. natn. Acad. Sci. U.S.A.* **71**, 1142 (1974).
32. S. C. Basak, D. P. Gierchen, V. R. Magnusson and D. K. Harris, *IRCS Med. Sci.* **10**, 619 (1982).
33. W. L. Duax, G. D. Smith, J. F. Griffin and P. S. Portoghese, *Science* **220**, 417 (1983).
34. M. Deleers, R. Brasseur, J. M. Ruysschaert and W. J. Malaisse, *Biophys. Chem.* **17**, 313 (1983).
35. M. Deleers, R. Brasseur and W. J. Malaisse, *Res. Commun. Chem. Path. Pharmac.* **42**, 181 (1983).
36. M. Deleers, R. Brasseur and W. J. Malaisse, *Chem. Phys. Lipids* **33**, 11 (1983).
37. R. Brasseur, M. Deleers, W. J. Malaisse and J. M. Ruysschaert, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2895 (1982).
38. R. Brasseur, M. Deleers and J. M. Ruysschaert, *Biosc. Rpts.* **4**, 651 (1984).
39. R. Brasseur and M. Deleers, *Proc. natn. Acad. Sci. U.S.A.* **81**, 3370 (1984).
40. R. Brasseur, *J. molec. Graphics* **4**, 117 (1986).
41. R. Brasseur, M. Deleers and J. M. Ruysschaert, *J. Colloid Interface Sci.* **114**, 277 (1986).
42. R. Brasseur, C. N. Serhan and M. Deleers, in *Lipoxins: Biosynthesis, Chemistry and Biological Activities* (Eds. P. Y. Wong and C. N. Serhan), p. 93. Plenum Publishing Corporation, New York (1987).
43. R. Brasseur, M. Deleers, J. M. Ruysschaert, B. Samuelsson and C. N. Serhan, *Biochim. Biophys. Acta* **960**, 245 (1988).
44. M. P. Mingeot-Leclercq, A. Schank, M. Ronveau-Dupal, M. Deleers, R. Brasseur, J. M. Ruysschaert, G. Laurent and P. M. Tulkens, *Biochem. Pharmac.* **38**, 729 (1989).
45. E. Ralston and J. L. Decoen, *J. Molec. Biol.* **83**, 393 (1974).
46. A. M. Liquori, E. Ciglio and L. Mozzarella, *Nuovo Cimento* **55**, 475 (1968).
47. A. M. Liquori, *Quat. Rev. Biophys.* **2**, 65 (1969).
48. J. L. DeCoen and E. Ralston, *Biopol.* **73**, 38 (1977).
49. A. J. Hopfinger, in *Conformational Properties of Macromolecules.* Academic Press, New York (1973).
50. F. Bellemare and M. Fragata, *J. Colloid Interface Sci.* **77**, 243 (1980).
51. M. Fragata and F. Bellemare, *Chem. Phys. Lipids* **27**, 93 (1980).
52. J. A. Nelder and R. Mead, *Computer J.* **7**, 308 (1965).
53. C. Tanford, in *The Hydrophobic Effect: Formation of Micelles and Biological Membranes.* John Wiley, New York (1973).