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β_3-Adrenergic receptor ligands: insight into structure–activity relationships using Monte-Carlo conformational analysis in water

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Abstract—This paper deals with the application of a Monte-Carlo (MC)-based conformational analysis carried out in water on a set of known β_3-adrenergic ligands. On the basis of their conformation at the global minimum, the molecules under study can be grouped into two clusters: the 'extended' and the 'folded' cluster. Each cluster is identified by well-defined values of torsion angles and distances between the pharmacophoric groups. It is worth noting that a ligand included in the cluster characterized by an extended conformation invariably shows a higher affinity for the human β_3-adrenoreceptor with respect to the corresponding rodent receptor.

1. Introduction

The β_3-adrenergic receptors belong to the wide family of adrenergic receptors, whose endogenous ligand is norepinephrine (**3**) (Chart 1). Following the first general distinction into α- and β-adrenergic receptors, which was based on differential responses to natural and synthetic catecholamines,[1] further studies led to the identification of β_1- and β_2-receptor subtypes[2] and to the design of selective agonists or antagonists suitable for therapeutic applications. A number of these compounds, in fact, proved to be effective as cardiovascular drugs (β_1-blocking agents)[3] or in the treatment of asthma (β_2-adrenergic agonists).[4]

More recently, a careful analysis of the pharmacological profile of a set of β-adrenergic ligands revealed the presence of an atypical receptor with a widespread tissue distribution, which was later defined as β_3-adrenoreceptor.[5] The human, rodent, bovine and many other mammalian β_3-receptor encoding genes have been isolated and characterized from both the structural and pharmacological point of view.[6–15] The β_3-adrenoreceptor is a seven transmembrane G-protein coupled receptor, present in white and brown adipose tissues (WAT and BAT, respectively), gastrointestinal tract, stomach and some heart tissues.[11,16–18] Stimulation of this receptor activates a cAMP dependent lipase, increases the production of uncoupling protein-1 (UCP-1) in BAT and enhances the sensitivity to insulin.[17–18] These activities result in a reduction of body weight and ameliorate diabetic symptoms in various animal models.[11,18–21]

The first promising results have encouraged intense research aimed at the design of β-adrenoceptor agonists and antagonists selectively acting at the β_3 human receptors. Unfortunately, compounds that showed a favorable activity–selectivity profile in rodents gave poorer results when tested in humans. As a matter of fact, no valuable drug candidate has been proposed so far.[11] Nonetheless, a large number of β_3-adrenoreceptor agonists and antagonists have been synthesized and tested, whose structure is characterized by the presence of either the arylethanolamine or aryloxypropanolamine function.[22–26] Based on their β-adrenergic pharmacological profile, these derivatives can be roughly divided into four groups:

1. unselective β-adrenergic agonists (β_1, β_2 and β_3 agonists);
2. selective β_3-agonists (β_3 agonists; β_1 and β_2 antagonists);
3. unselective β-adrenergic antagonists (β_1, β_2 and β_3 antagonists);
4. selective β_3-antagonists.

The key to success in this area is the design of drugs that possess a higher selectivity for human rather than for rodent β_3-receptors. In addition, a prerequisite for their therapeutic potential in the treatment of obesity and diabetes is the absence of cardiovascular or other side effects mediated by the stimulation of β_1- and β_2-adrenoceptors.[11,17]

The goal of this work is to contribute to the comprehension of the structure–activity relationships of agonists and

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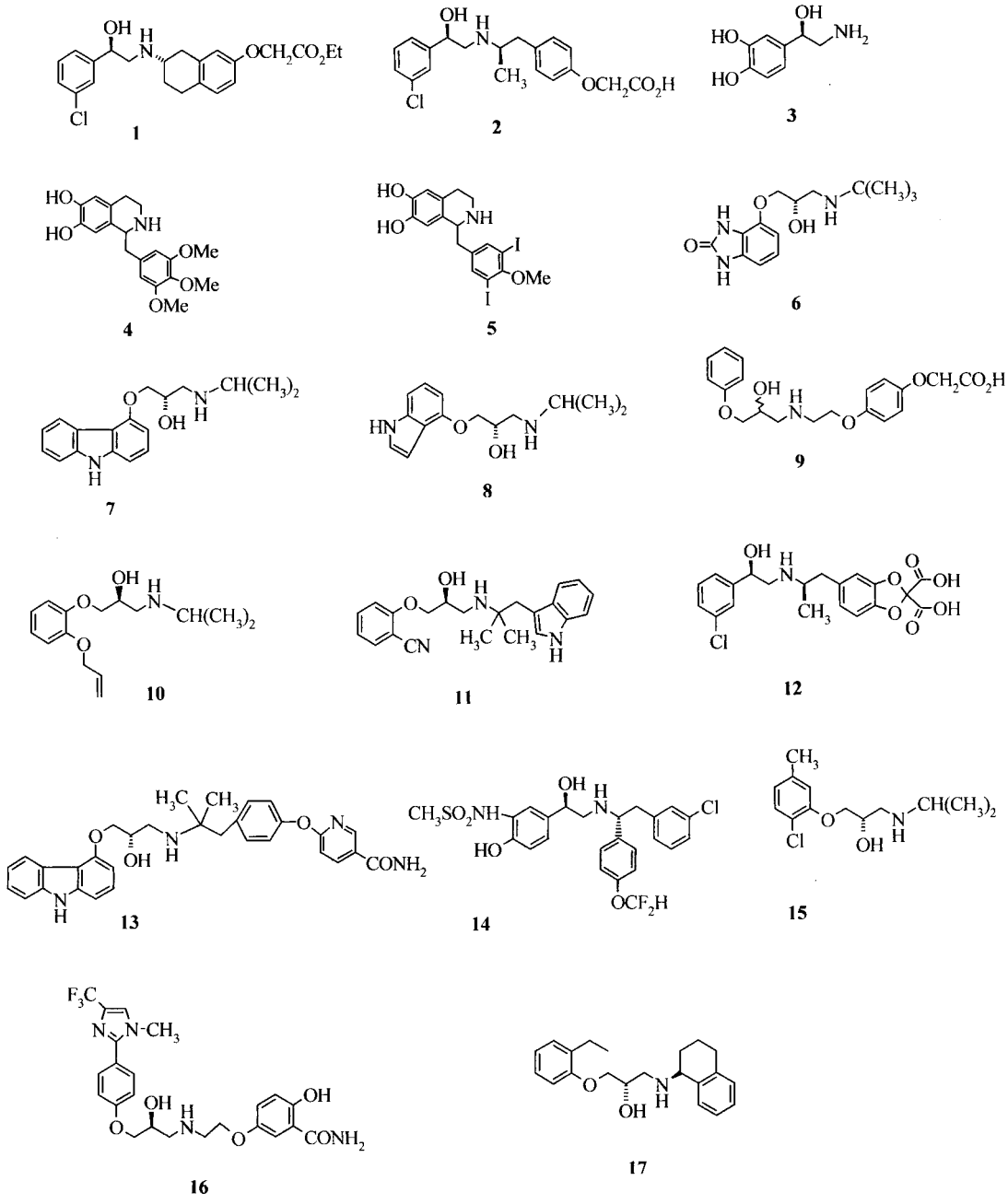

Chart 1. Structures of the investigated compounds.

antagonists active at β_3-adrenergic receptors, by studying their conformational profiles and evaluating the role of conformation in the process of molecular recognition of the ligand by the receptor. We will focus our attention on the conformational analysis of the ligands, since no X-ray structure of the receptor is available yet. Among the huge number of compounds reported in the literature as ligands of the β_3-adrenoceptors we selected those whose selectivity for the different β-adrenoceptors or among the β_3-adrenoceptors subtypes has been clearly defined. Based on our findings, we will provide insights into the features of the pharmacophoric groups, which could be suitable in the design of new drugs active at human β_3 adrenoceptors.

2. Computational Methods

All calculations were performed using the Macromodel/Batchmin 6.5 package,[27] with the AMBER force field included therein. The reason for the choice of this set of parameters was that they were implemented to describe also the behavior of small molecules in the GB/SA aqueous environment.[27] Moreover, docking experiments into the protein receptor model are going to be undertaken and, since AMBER was developed for the description of peptide and protein systems, it seemed to be the most appropriate force field in the analysis of the investigated molecules. The calculations were carried out using the GB/SA solvation model of Macromodel for water.[28] In such a way, we

Table 1. Pharmacological properties of the four groups of β_3-adrenergic receptor ligands

Compound	Class of activity	Rodent β_3AR		Human β_3AR	
		Binding K_i (nM)	Effect (K_{act})	Binding K_i (nM)	Effect (K_{act})
β_1, β_2, β_3 Agonists					
1[a]	(*R*,*S*)-SR58611A	1350±270	19±4	6640±960	25.0±5
2[b]	(*R*,*R*)-BRL37344	290±136	0.4±0.1	287±92	15.0±3
3[b]	(*R*)-Norepinefrine	1840±600	13±4	475±75	6.3±0.7
4[c]	TMQ			3715±2	2.5±0.2
5[c]				21.3±0.15	1.7±0.2
β_1, β_2 Antagonists, β_3 Agonists					
6[b]	(*S*)-CGP12177	152±19	41±9	88±22	139±44
7[b]	(*S*)-Carazolol	18	25	2.0±0.2	11.3±1.2
8[b]	(*S*)-Pindolol	315±40	999±187	11±2	153±12
9[b]	(±)-ICI201651	239±104	15±1	85±12	20±9
10[a]	(*S*)-Oxprenolol	147±31	535±79	70±10	77±13
11[b]	(*S*)-Bucindolol	21±5	40±14	23±10	7.0±1.2
12[b]	(*R*,*R*)-CL316,243	1000±200	0.71±0.2	14,000	68
13[d]	LY377604			4.3[e]	
14[f]	BMS210285			9.0	
β_1, β_2, β_3 Antagonists					
15[b]	(*S*)-Bupranolol	42±19		50±14	
16[b]	(*S*)-CGP20712A	13,000±7100		2300±450	
β_3 Selective antagonists					
17[g]	(*S*,*S*)-SR 59230A			40[e]	

[a] Ref. 35.
[b] Ref. 11.
[c] Ref. 36.
[d] Ref. 37.
[e] The data refers to the value of the inhibition constant (IC_{50}).
[f] Ref. 17.
[g] Ref. 26.

simulated a water environment and its influence on the conformations of our lead compounds. All calculations were run with a Van der Waals cutoff of 7.0 Å, and an electrostatic cutoff of 12.0 Å.

All the studied compounds, whose structure is depicted in Chart 1, were simulated with a +1 positive charge on the nitrogen atom to mimic the situation at physiological pH.[29–31] In addition, the compounds functionalized with a carboxylic acid group were analyzed in the ionized form which is the dominant species at physiological pH, as well as in the unionized form to mimic the acidic condition characteristic of the receptor environment. The ionized or the undissociated form of the carboxylic acid group does not affect the conformation of the global minimum. All the molecules were subjected to an initial minimization with the truncated Newton conjugated gradient (TNCG) method. The conformational searches were carried out using 40,000 steps of the pseudosystematic variant of the Monte-Carlo/energy minimization (MC/EM) searching procedure.[32,33] Each step of this procedure consists of an MC pseudo-systematic move of torsion angles followed by an energy minimization step.[32] All the bonds that could undergo free rotation were used as torsion variables in the MC steps. The energy minimization at this stage of the conformational search is used simply to eliminate as many duplicate conformations as possible. Energy minimization at this stage was performed using the TNCG method, and was terminated either after 500 iterations or when the energy gradient rms fell below 0.01 kJ/mol Å. All the conformers that differed from the global minimum energy conformation by no more than 100 kJ/mol were saved. At the end of the 40,000 steps of MC/EM, all the conformers of each compound within the first 100 kJ/mol were fully re-minimized (TNCG method, gradient less than 0.001 kJ/mol Å) to allow a more accurate determination of the relative energies. For each compound, the structure-ensemble emerging from this procedure was used in our analysis.

3. Results and discussion

In Chart 1 are shown the structures of the derivatives (**1**–**17**) selected for the present investigation, as representative terms of the above reported four groups of activity. In Table 1, compounds **1**–**17** have been ordered on the basis of their pharmacological profile and the corresponding binding (K_i), and activation (K_{act}) constants have been introduced.

The conformations of the tested molecules falling in an energy range of 50 kJ/mol were analyzed and compared.

Our conformational search carried out on each compound is very extensive and the low energy parts of the potential energy surface are visited multiple times, assuring that the lowest energy conformational states have been sampled. The results of our analysis provide evidence that the compounds under study adopt either an extended or a folded conformation and, consequently, they can be grouped into two distinct families (Fig. 1). We found that the energy difference between the two conformations spans the range

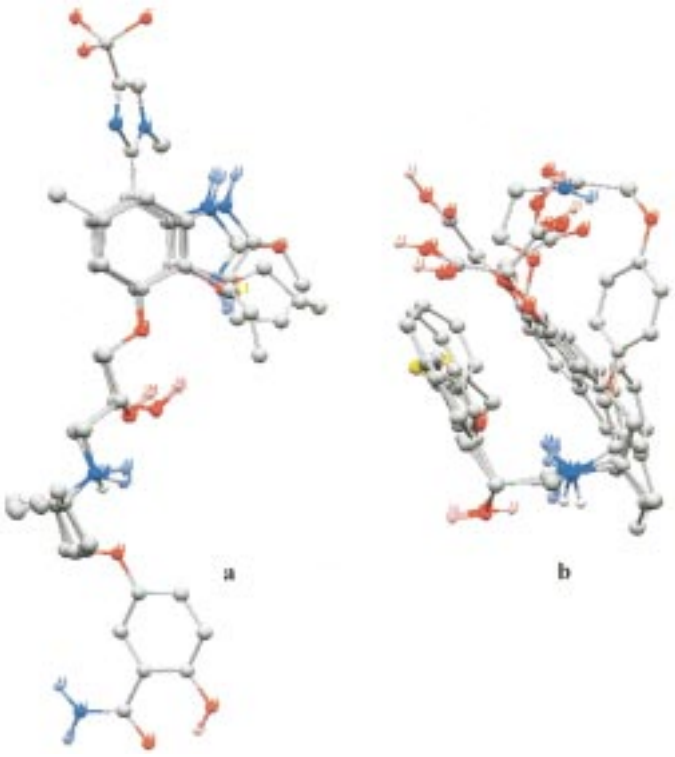

Figure 1. (a) Overlap between the compounds with an extended conformation (cluster 1; rms 0.23 Å); (b) overlap between the compounds with a folded conformation (cluster 2; rms 0.67 Å).

9.9–24.1 kJ/mol (Table 2). Such an energy difference affects the relative population of the two conformers. As a consequence, each molecule adopts preferentially the conformation corresponding to the lower energy state. Bupranolol (**15**) is the only derivative characterized by a low energy difference between the two conformations (0.80 kJ/mol). It is thus conceivable to assume that both the conformational states (folded and extended) of Bupranolol are similarly populated. This aspect of the conformational behavior of Bupranolol had already been reported by other authors through molecular dynamics simulations in vacuo.[29] The present study demonstrates that such a property can also be observed in water.

An interesting result of our investigation comes from the observation of the global minima of all the molecules under study as well as from the comparison among them. Two molecular clusters can be defined by superimposing atoms 1–5 (Fig. 2) of the global minimum of all the molecules. Cluster 1 (extended conformations, Fig. 1(a)) includes all the compounds whose global minimum refers to an extended conformation, e.g. CGP12177A (**6**), Carazolol

Table 2. Energy of the extended and folded conformations of derivatives **1**–**17**

Compound	Conformation of the global minimum	Energy of the folded conformation (kJ/mol)	Energy of the extended conformation (kJ/mol)	ΔE^{a} (kJ/mol)
1	Folded	−254.15	−237.75	−16.4
2	Folded	−279.10	−265.3	−13.8
3	Extended	n.d	−410.34	
4	Folded	−205.57	−195.63	−9.9
5	Folded	−196.04	−172.19	−23.9
6	Extended	−228.58	−240.70	+12.1
7	Extended	−105.08	−116.25	+11.2
8	Extended	−279.75	−290.62	+10.9
9	Folded	−381.22	−369.97	−11.2
10	Extended	−245.11	−255.04	+9.9
11	Folded	−185.96	−161.90	−24.1
12	Folded	−110.47	−96.96	−13.5
13	Extended	−471.80	−481.88	+10.0
14	Extended	−356.13	−369.44	+13.1
15		−230.58	−231.36	+0.8
16	Extended	−405.75	−418.23	+12.5
17	Folded	−175.08	−163.38	−11.7

[a] Energy difference between folded and extended conformations.

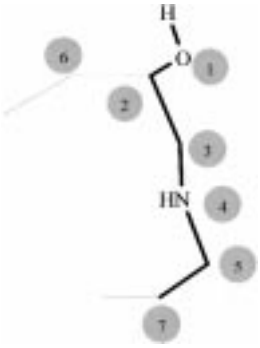

Figure 2. Atoms used to superimpose the molecules.

(**7**), Pindolol (**8**), Oxprenolol (**10**), LY377604 (**13**), and CGP20712A (**16**) (Chart 1 and Table 2). A common feature of the molecules of this cluster is the presence of a bulky aliphatic moiety appended at the NH group of the ethanolamine side chain. The sole exception is represented by CGP20712A (**16**), which contains an aromatic moiety. Norepinephrine (**3**) was inserted in this cluster based on its values of torsion angles θ_1, θ_2, and distance d_3. The sterically demanding aliphatic group present in this set of compounds would force the molecules to adopt an extended conformation. In all the derivatives the OH group of the ethanolamine moiety points in the same direction in space, giving rise to an orientation that could be favorable for the interaction with the Ser and Asp residues of the β_3-receptor, which have been proven to be crucial for the activity.[29,30] The total superimposition rms value of this cluster is 0.23 Å.

On the other hand, cluster 2 (Fig. 1(b)) comprises the molecules, e.g. SR58611A (**1**), BRL37344 (**2**), TMQ (**4**), (**5**), ICI201651 (**9**), and SR59230A (**17**) whose global minimum corresponds to a folded, U-shaped, conformation. The total superimposition rms value of this cluster is 0.67 Å. All the molecules are characterized by the presence of an aromatic substituent on the amino group, which is responsible for a stacking interaction with the other aromatic ring. This π-stacking interaction can be considered as the major factor in inducing a folded conformation, since this effect would minimize the surface area accessible to water by reducing the unfavorable contact between hydrophobic groups and the surrounding molecules of water. Moreover, this conformation would force the OH group, present in each molecule of this cluster, to point towards either the bulk water or groups capable of yielding a hydrogen bond, e.g. a Ser or an Asp residue. The first conformation of Bupranolol (**15**) above the global minimum can fit well into this second cluster and we will show that this observation may have consequences for the pharmacological profile of this ligand. Hence, from the comparison of the two clusters, we can infer that mainly steric, hydrophobic and π-stacking interactions are responsible for the

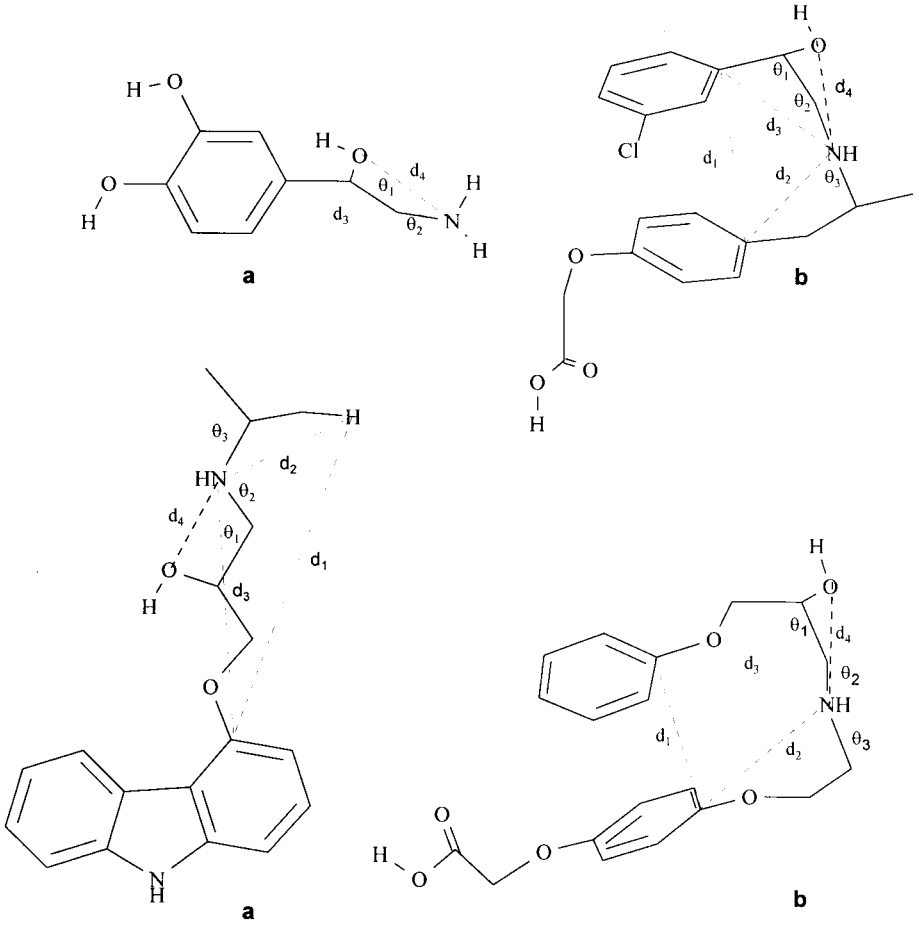

Figure 3. Distances and torsion angles for cluster 1 (**a**) and cluster 2 (**b**).

Table 3. Average distances and torsion angles of clusters 1 and 2 found by molecular modeling

		Cluster 1	Cluster 2
Distances (Å)	d_1	7.4±0.1	4.2±0.3
	d_2	2.7±0.1	3.3±0.5
	d_3	6.1±0.2	3.6±0.5
	d_4	2.7±0.1	2.7±0.1
Torsions (degrees)	$\lvert\theta_1\rvert$	178±3	76±3
	$\lvert\theta_2\rvert$	176±1	174±4
	$\lvert\theta_3\rvert$	164±2	82±36

The results are the mean±standard deviation.

observed conformations.[34] Since we were also interested in studying the conformational factors involved in the recognition of the ligands by the different β_3-adrenoreceptors, we analyzed the distance and torsion parameters (Fig. 3) to gain a better insight into the characteristics of a hypothetical pharmacophore. In Table 3, we report the average values of distances d_1, d_2, d_3, d_4, as well as torsion angles θ_1, θ_2, and θ_3 of the molecules belonging to both clusters. In cluster 1 (extended conformation) the low values of the standard deviation for each distance and torsion angle indicate that the parameters taken into account are practically constant over the set of compounds. On the other hand, inspection of the data of cluster 2 (folded conformation) suggests that, whereas the distance values d_1–d_4 and torsion angles θ_1 and θ_2 are very similar for all the molecules of the cluster, the standard deviation of torsion angle θ_3 is quite high due to the presence in the set of SR59230A (**17**), which possesses a value of θ_3 (155°) significantly different from the average value (82°). By comparing the values of the above-reported parameters for the two clusters, we corroborated the already evidenced structural diversity. The conformational profile of the molecules belonging to cluster 1 can be described by the high value of the torsion angles and by the values of d_1 and d_3 higher than those calculated for cluster 2.

An interesting feature stemming from our conformational analysis is related to the pharmacological behavior of our model compounds reported in Table 1. The comparison of the pharmacological data of derivatives **1**–**17** with their conformational features shows that each cluster includes compounds belonging to the first two groups of β_3-adrenergic ligands (β_1, β_2, β_3 agonists, or β_1, β_2 antagonist and β_3 agonists). Conversely, the derivatives provided with an unselective β_1, β_2, β_3 antagonist activity, classified as the third group, belong solely to cluster 1 (extended), whereas selective β_3 antagonists are included into cluster 2 (folded). Bupranolol (**15**) with a low energy difference between the two conformations (0.80 kJ/mol) fits well into both clusters. Interestingly, compounds belonging to each cluster give quite different pharmacological responses to the rodent and human β_3-receptor. All the molecules with an extended conformation show an affinity higher for the cloned human β_3-adrenoceptor than for the rodent one, while molecules belonging to cluster 2 display higher affinity for the rodent receptor, or possess comparable affinity for both receptors. It is worth pointing out that the global minimum of BMS 210285 (**14**) shows both an extended and a folded conformation due to the presence in its structure of a side chain with two aromatic rings. From the high affinity (K_i=9 nM) of **14** for the human β_3-adrenoreceptor we can infer the involvement of the extended conformation. As shown before, due to the small energy difference between its extended and folded conformation, Bupranolol (**15**) can fit well into both conformational clusters, and this is reflected by its very similar K_i values for the rodent and human β_3-adrenoreceptor.

The different pharmacological profile of the two clusters could be related to structural differences between the human and the rodent receptor, even though their homology is more than 80%.[11] As a matter of fact, the major differences between the human and the rodent β_3-adrenergic receptor are localized in the first transmembrane domain (TM1). The sequence Val^{48}–Leu^{49}–Ala^{50}, present in the human β_3-adrenoceptor, is deleted in the rodent receptor and, in addition, a cysteine of TM4 and TM6 is replaced by an arginine.[11]

According to the literature,[11,17,20,26,36] some of the molecules reported in Table 1 also bind to human β_1- and β_2-adrenoreceptors. Hence, the existence of a ligand in an extended or folded conformation does not seem to be the reason for a discrimination between these receptors. As a consequence, it is not possible to correlate the inclusion of a compound into a cluster either with its selectivity for the human β_3-adrenergic receptor with respect to human β_1- and β_2-adrenoreceptors or with its mode of action (agonist or antagonist).

4. Conclusions

In conclusion, we have shown that, by conducting thorough conformational searches in water, a connection between the conformational features of a ligand and its pharmacological profile can be shown. All the derivatives investigated can be grouped into two clusters based on the structures of their global energy minima. The molecules belonging to each cluster show different pharmacological behavior. In particular, compounds characterized by an extended conformation seem to be selective for the human β_3-adrenergic receptor with respect to the corresponding rodent receptor. New ligands characterized by either an extended or a folded conformation have been designed and from the evaluation of their selectivity for the human or rodent β_3-adrenoreceptor we will challenge our hypothesis and contribute to the development of novel drug candidates useful in the treatment of obesity and diabetes.

References

1. Ahlquist, R. P. *Am. J. Physiol.* **1948**, *153*, 586–600.
2. Lands, A. M.; Arnold, A.; MacAuliff, J. P.; Luduena, F. P.; Brown, T. G. *Nature* **1967**, *214*, 597–598.
3. Cases, A. *Drug News Perspect.* **1999**, *12*, 372–377.
4. Nelson, H. S. *New Engl. J. Med.* **1995**, *333*, 499–506.
5. Tan, S.; Curtis-Prior, P. B. *Int. J. Obesity* **1983**, *7*, 409–414.
6. Wilson, C.; Wilson, S.; Piercy, V.; Sennitt, M. V.; Arch, J. R. S. *Eur. J. Pharmacol.* **1984**, *100*, 309–319.
7. Emorine, L. J.; Marullo, S.; Briend-Sutren, M. M.; Patey, G.;

Tate, K.; Delavier-Klutchko, C.; Strosberg, A. D. *Science* **1989**, *245*, 1118–1121.
8. Zaagsma, J.; Nahrosky, S. R. *Trends Pharmacol. Sci.* **1990**, *11*, 3–7.
9. Arch, J. R. S.; Kaumann, A. *J. Med. Res. Rev.* **1993**, *13*, 373–381.
10. Granneman, J. G.; Lahners, K. N.; Chaudry, A. *Mol. Pharmacol.* **1991**, *40*, 895–899.
11. Strosberg, A. D. *Ann. Rev. Pharmacol. Toxicol.* **1997**, *37*, 421–450.
12. Granneman, J. G.; Lanhners, K. N.; Zhai, Y. *Mol. Pharmacol.* **1998**, *53*, 856–861.
13. Gros, J.; Manning, S. B.; Petri-Rouxel, F.; Guillaume, J.-L.; Drumare, M.-F.; Strosberg, A. D. *Eur. J. Biochem.* **1998**, *251*, 590–596.
14. Muzzin, P.; Revelli, J.; Kuhne, F.; Gocayne, J. D.; McConbie, W. R.; Venter, J. C.; Giacobino, J. P.; Fraser, C. M. *J. Biol. Chem.* **1991**, *266*, 24053–24058.
15. Lelias, J. M.; Kaghad, M.; Rodriguez, M.; Chalon, P.; Bonnin, J. *FEBS Lett.* **1993**, *324*, 127–130.
16. Gauthier, C.; Tavernier, G.; Charpentier, F.; Langin, D.; Lemarec, H. *J. Clin. Invest.* **1996**, *98*, 56–62.
17. Kordik, C. P.; Reitz, A. B. *J. Med. Chem.* **1999**, *42*, 181–201.
18. Cinti, S. L'obesità. *Il Diabete* **1998**, 25–46.
19. Arch, J.; Wilson, S. *Int. J. Obesity* **1996**, *20*, 191–199.
20. Dow, R. L. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1811–1825.
21. Arch, J. R. S.; Ainworth, A. T.; Cawthorne, M. A.; Piercy, V.; Sennitt, M. V.; Thody, V. E.; Wilson, C.; Wilson, S. *Nature* **1984**, *309*, 163–165.
22. Oriowo, M. A.; Chapman, H.; Kirkham, D. M.; Sennitt, M. V.; Ruffolo, R. R.; Cawthorne, M. A. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 22–27.
23. Manara, L.; Bianchetti, A. *Trends Pharmacol. Sci.* **1990**, *11*, 229–230.
24. Howe, R.; Rao, B. S.; Holloway, B. R.; Stribbling, D. *J. Med. Chem.* **1992**, *35*, 1751–1759.
25. Howe, R.; Rao, B. S.; Holloway, B. R.; Stribbling, D. *J. Med. Chem.* **1992**, *35*, 1759–1764.
26. Manara, L.; Badone, D.; Baroni, M.; Boccardi, G.; Cecchi, R.; Croci, T.; Giudice, A.; Guzzi, U.; Landi, M.; Le Fur, G. *Br. J. Pharmacol.* **1996**, *117*, 435–442.
27. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caulfield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
28. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
29. Blin, N.; Camoin, L.; Maigret, B.; Strosberg, A. D. *Mol. Pharmacol.* **1993**, *44*, 1094–1104.
30. Kontoyianni, M.; DeWeese, C.; Penzotti, J. E.; Lybrand, T. P. *J. Med. Chem.* **1996**, *39*, 4406–4420.
31. Klein, C. D. P.; Klingmuller, M.; Schellinski, C.; Landmann, S.; Hauschild, S.; Heber, D.; Mohr, K.; Hopfinger, A. J. *J. Med. Chem.* **1999**, *42*, 3874–3888.
32. Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110–1117.
33. Colombo, G.; Toba, S.; Merz, K. M. *J. Am. Chem. Soc.* **1999**, *121*, 3486–3493.
34. Strosberg, A. D.; Camoin, L.; Blin, N.; Maigret, B. *Drug Des. Disc.* **1993**, *9*, 199–211.
35. Blin, N.; Nahmias, C.; Drumare, F.; Strosberg, A. D. *Br. J. Pharmacol.* **1994**, *112*, 911–919.
36. Zheng, W.; Nikulin, I. V.; Konkar, A. A.; Vansal, S. S.; Shams, G.; Feller, D. R.; Miller, D. D. *J. Med. Chem.* **1999**, *42*, 2287–2294.
37. Shuker, A. J. *21st ACS National Meet* 21–25 March **1999**, Anaheim, Abst MEDI 159