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Conformational analysis of fenoterol stereoisomers

Analiza konformacyjna stereoizomerów fenoterolu

Fenoterol (2-(3,5-dihydroxyphenyl)-2-hydroxy-2′-(4-hydroxyphenyl)-1′-methyldiethylamine) is a full selective agonist of β_2-AR. This compound exists as four stereoisomers (Fig.1). The clinically used drug, *rac*-fenoterol, is a racemic mixture of (R,R)- and (S,S)-fenoterol. The medication is used to treat acute asthma and other lung problems such as chronic bronchitis, in which the air passages can become narrowed. This β_2-sympathomimetic drug is routinely used to inhibit uterine contractions (tocolysis) and may also be useful in the treatment of congestive heart failure. In the previous study, four stereoisomers of fenoterol and its 19 derivatives were synthesized [3]. For some of these molecules, submicromolar binding affinities to β_2-AR were determined [3]. The experimentally calculated affinity constants show differences in binding stereoisomers to β_2-AR [3]. (R,R) and (R,S) stereoisomers are usually more active than (S,R) and (S,S) ones.

Molecular interactions of agonists or antagonists with the active site of β_2-AR is relatively well defined [1,2,4,6,7]. In our subsequent study, computational docking simulations was used to understand binding interactions of fenoterol stereoisomers to the model of β_2-AR binding site. All stereoisomers assume a very similar position in the binding cavity of β_2-AR. The molecules also exhibit a very similar pattern of interactions. However, the molecule of each fenoterol stereoisomer has to significantly change its internal conformation to meet requirements for uniform pattern of binding interactions. Thus, a detailed conformational analysis of the four stereoisomers was carried out. We employed the method of stepwise rotation of each dihedral angle of the molecule with potential energy estimation. In addition, molecular dynamics (MD) simulations *in vacuo* and in water were also performed. The aim of this study was to analyze low energy conformations of four stereoisomers of fenoterol molecule (see Fig. 1) in order to compare it with conformations obtained in docking simulations

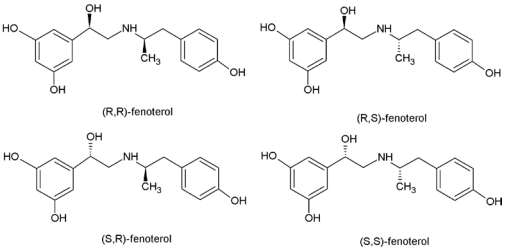


Fig. 1. Structures of the stereoisomers of fenoterol

METHODS

Molecular models of fenoterol stereoisomers were built using the HyperChem 6.0 software. Fenoterol has a very flexible structure as it contains 6 heavy-atom rotatable bonds (defined as A, B, C, D, E and F in Fig. 2). The method based on the gradual rotation of dihedral angles allows to perform a systematic search for different conformations of the studied compounds and to determine the corresponding energies. Each of fenoterol stereoisomers was rotated step by step (along the considered bond) by 10 degrees from 00 to 360˚ and the energy of the conformer was evaluated using the semi-empirical AM1 method (HyperChem 6.0). For each dihedral, the angle corresponding to the lowest energy was determined and the set of these values for dihedrals A-F was used to construct the global minimum conformation. Each stereoisomer in global minimum conformation was subjected to MD simulations *in vacuo* and in water. MD i*n vacuo* were done in HyperChem (AM1 semiempirical method, temperature = 300 K, time of simulation = 5 ps. MD in water were done in Yasara 9.1.25 software by Yasara Biosciences (www.yasara.org) (AMBER 99 force field, temperature = 298 K, time = 30 ns). In both cases the size of the simulation box was 30 Å × 30 Å × 30 Å.

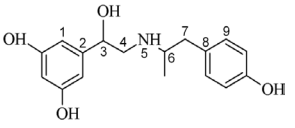


Fig. 2. Structure of fenoterol contains six rotatable bonds (A, B, C, D, E and F) corresponding to six dihedral angles located between atoms: 1–4; 25; 3–6; 4–7; 5–8 and 6–9, respectively

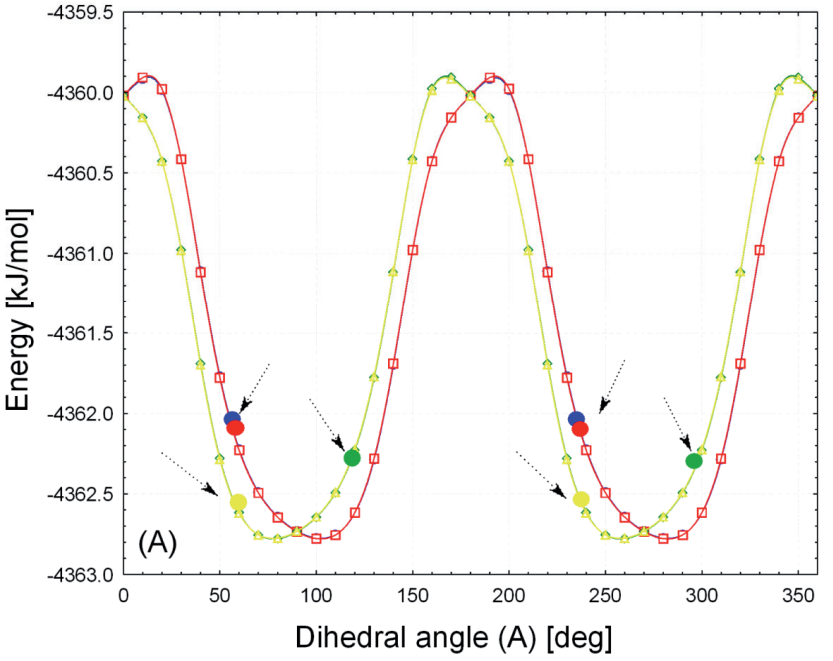
RESULTS

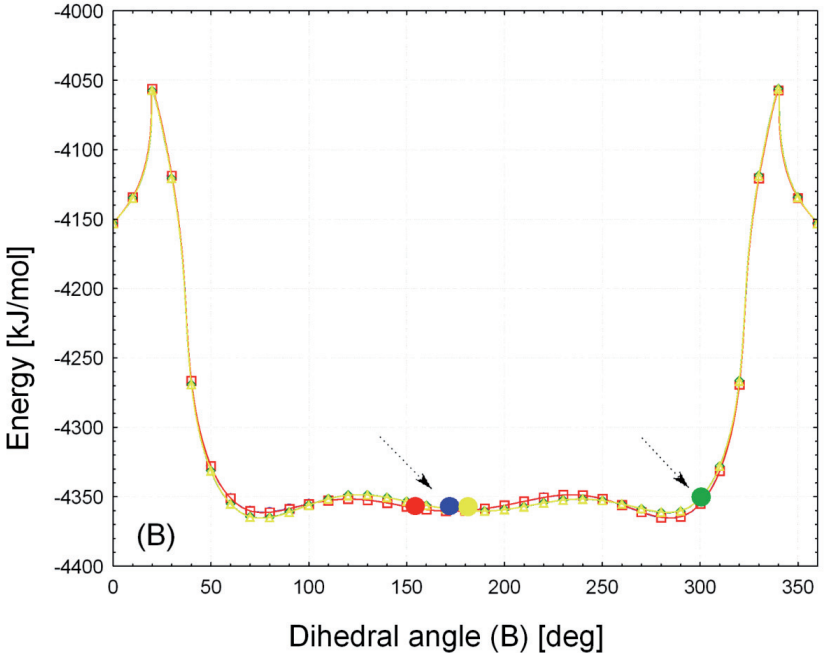
The aim of the molecular dynamics simulations was to find the most stable conformations of the studied stereoisomers. Changes of dihedral angles (A–F), potential energy and root mean square deviation (RMSD) values were monitored during MD. The results of these studies allowed to determine stable and unstable conformers which are represented by the data shown in Fig. 3. The optimal conformations of fenoterol stereoisomers were compared to those found in docking simulations obtained by using the model of β_2-AR [5]. This comparative analysis was performed in terms of the potential energy profiles (Fig. 3). Optimal values of dihedral angles for the lowest energy conformations for all fenoterol stereoisomers are shown in Table 1. The conformations corresponding

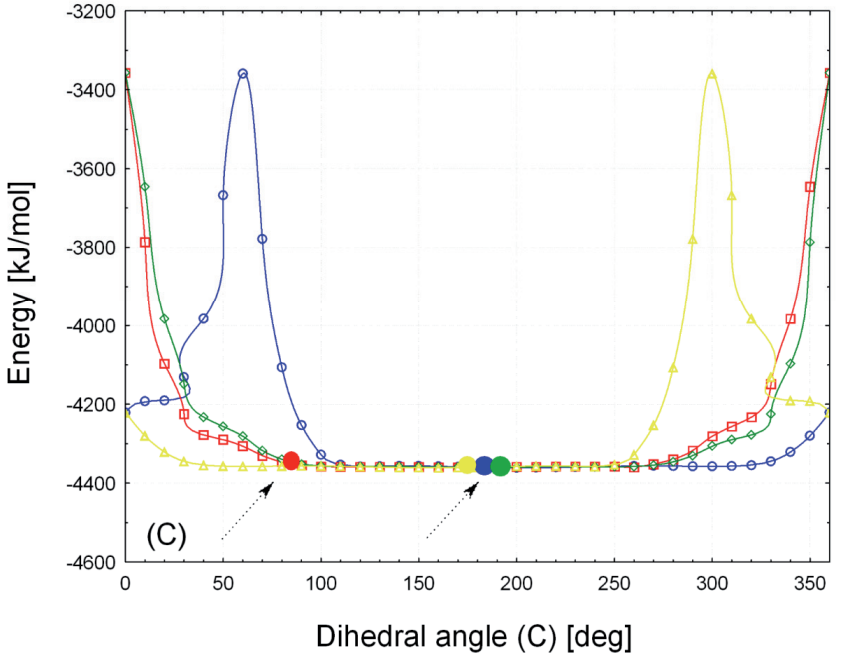
to the energetic global minimum of (R,R)- and (S,S)-fenoterol and (S,R)- and (R,S)-fenoterol are presented in Figure 4 and 5, respectively. In particular, the structures of (R,S)- and (S,R)-fenoterol are very similar to each other; 2,4-dihydroxyphenyl, hydroxyphenyl and NH_3^+ moieties of both stereoisomers are overlapped to each other. In the case of (R,R)- and (S,S)-fenoterol, their structures are much less similar, especially in the 2,4-dihydroxyphenyl region. In both cases, hydroxyl group at the chiral centre can take different orientations but the methyl group takes approximately the same place in the case of each stereoisomer. This results from a very flexible structure of fenoterol molecule. Subsequently, MD of four global minimum conformations of fenoterol stereoisomers in water and *in vacuo* was performed to determine the stability of the obtained conformations. Applying semi-empirical methods allows for obtaining the results within a short period of time. Calculations were performed by using the PC-class computer (3 GB RAM and 2GHz processor). In particular, simulations performed *in vacuo* (5 ps) and in water (30 ns) took 2 minutes and 3.5 hours, respectively. MD in water resulted in conformations characterized by the reversed configuration (i.e. having the protonated amine moiety exposed out of the rest of the molecule) which are presented in Fig. 6. These new conformations are still in the low potential energy region. The stable conformation obtained during *in vacuo* MD has the protonated nitrogen atom located between two aromatic moieties of fenoterol (Fig. 6, structure A). The conformation in which the protonated amine is exposed out of the rest of the molecule (Fig. 6, structure B) is the stable conformation of fenoterol and was obtained during MD simulation performed in water. In the case of the A structure the amine moiety is shielded by the aromatic moieties of fenoterol, while the exposed $–NH_3^+$ group in the B structure interacts with water molecules. During MD simulations in water the appearance frequency of the B structure is larger (64.4% and 61.3%) than that of the A structure (35.6% and 38.7%) for (R,R)- and (R,S)-fenoterol, respectively. Thus, it can be concluded that the B structure is preferred in the aqueous environment.Table 1. The optimal values of dihedral angles corresponding to the lowest energy conformations for all fenoterol stereoisomers

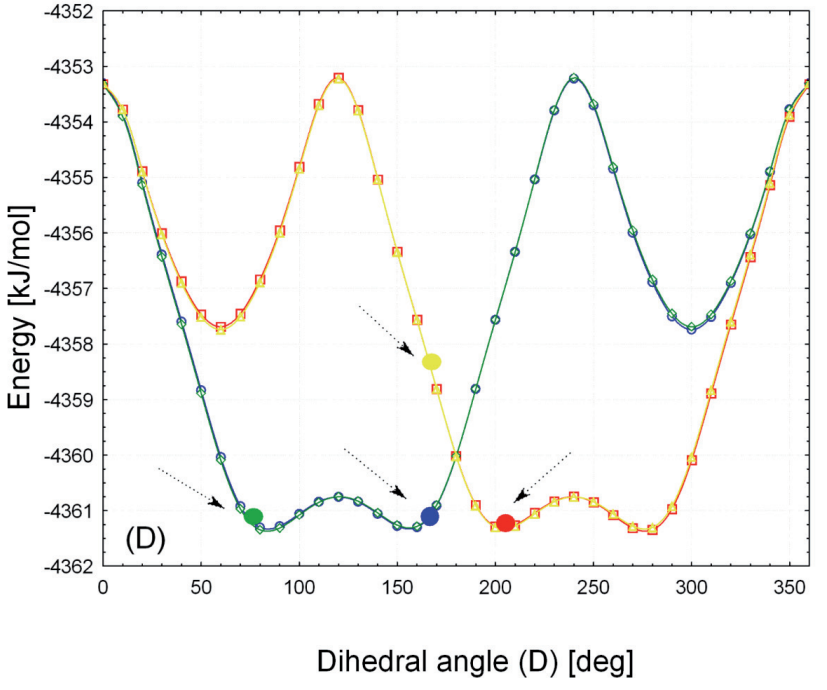
Table 1. The optimal values of dihedral angles corresponding to the lowest energy conformations for all fenoterol stereoisomers

Angle	(R,R)	(R,S)	(S,R)	(S,S)
A	280	280	80	80
B	280	280	80	80
C	200	180	180	160
D	80	280	80	280
E	280	80	280	80
F	260	280	80	100
The lowest energy (kJ/mol); MD in water	-4411.2	-4411.0	-4410.9	-4411.2
The lowest energy (kJ/mol); MD in vacuo	-4383.7	-4381.0	-4379.1	-4380.9


Energy [kJ/mol]
-4359.5
-4360.0
-4360.5
-4361.0
-4361.5
-4362.0
-4362.5
-4363.0
(A)
0
50
100
150
200
250
300
350
Dihedral angle (A) [deg]


Energy [kJ/mol]
-4000
-4050
-4100
-4150
-4200
-4250
-4300
-4350
-4400
(B)
0
50
100
150
200
250
300
350
Dihedral angle (B) [deg]


-3200
-3400
-3600
-3800
-4000
-4200
-4400
-4600
Energy [kJ/mol]
(C)
0
50
100
150
200
250
300
350
Dihedral angle (C) [deg]


-4352
-4353
-4354
-4355
-4356
-4357
-4358
-4359
-4360
-4361
-4362
Energy [kJ/mol]
(D)
0
50
100
150
200
250
300
350
Dihedral angle (D) [deg]

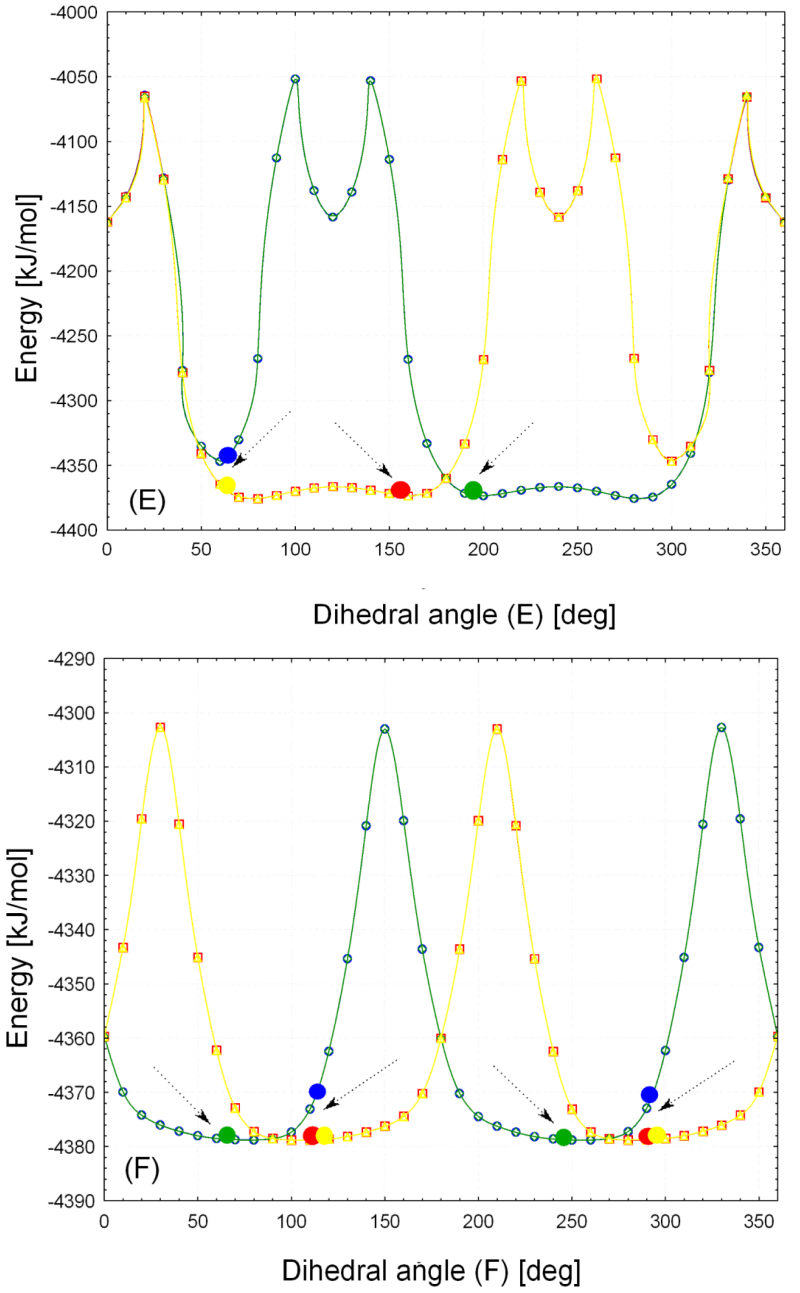


Fig. 3. The potential energy profile presented as the function of the value of dihedral angles A, B, C, D, E and F. Measurements were performed for fenoterol stereoisomers with NH_3^+ protonated amine moiety. The following notation was used: (R,R): blue circles; (R,S): red squares; (S,R): green diamonds; (S,S): yellow triangles. Filled circles of respective colors denote actual dihedrals obtained during the docking simulations [5]. The abscissa and coordinate axis represent the value of the torsional angles A–F (in degrees) and the energy (in kJ/mol), respectively

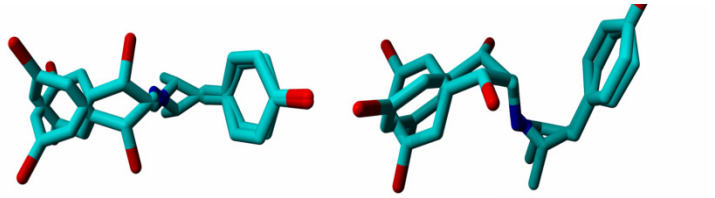


Fig. 4. The conformations corresponding to the global minimum of energy for (R,R)- and (S,S)-fenoterol. Molecules were overlaid based on minimal RMSD criterion

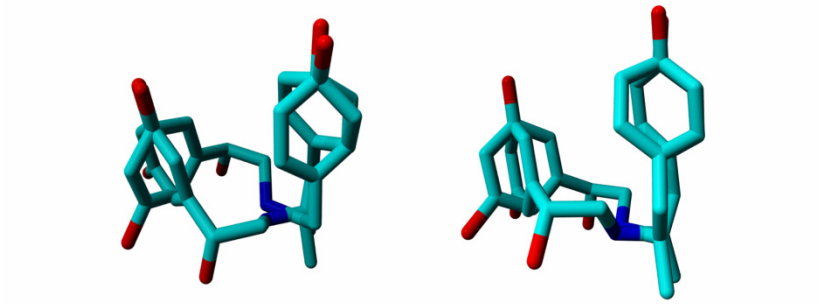


Fig. 5. The conformations corresponding to the global minimum of energy for (S,R)- and (R,S)-fenoterol. Molecules were overlaid based on minimal RMSD criterion

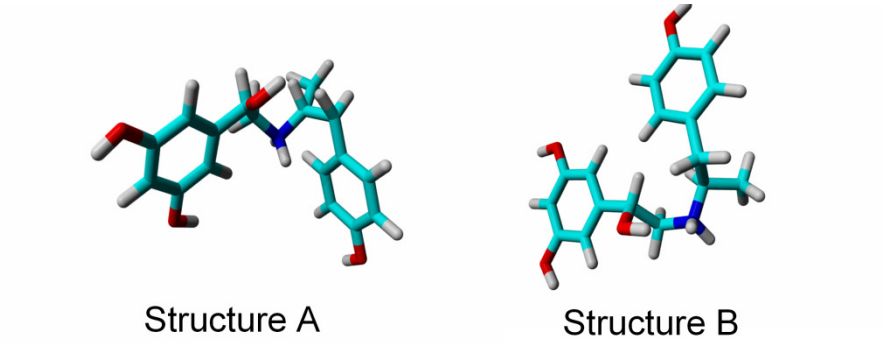


Fig. 6. The results of the MD simulation for (R,R)-fenoterol conformations obtained for simulations performed *in vacuo* (structure A) and in water (structure B)

CONCLUSIONS

During the molecular modeling the four conformations of fenoterol stereoisomers corresponding to the global minimum of energy were obtained. These results let us determine both stable and unstable conformations of stereoisomers of fenoterol. The values of energy calculated for structures of fenoterol stereoisomers docked to the β2-AR are close to the values of the global minimum of energy obtained in the present study. The potential energy profiles are symmetrical for enantiomers. Molecular dynamics simulations in water generated the preferred conformation in which the protonated amine group is exposed out of the rest of the molecule. The potential energy values are

very similar for each stereoisomer and they vary from -4385 to -4350 kJ/mol in the case of simulations performed in vacuo and in water, respectively. The lowest potential energy was obtained for (R,R)-fenoterol; however, the values of the potential energy for each stable configuration are similar (Table 1). Moreover, conformations of fenoterol stereoisomers obtained in docking simulations are similar to the minimal conformation obtained in the MD simulations in water. The binding of (R,R)-fenoterol, in comparison to other isomers, is additionally promoted by the lowest increase of internal energy associated with adopting to the receptor-induced conformation upon binding. The conformation analysis is very useful for comparison of ligand conformations obtained by docking studies. Fenoterol has a very flexible structure. It can demonstrate various conformations during docking simulations. This research lets us verify if the conformation of the ligand docked to the cavity of the receptor corresponds to the global or local minimum of energy.

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SUMMARY

Fenoterol is a relatively long-acting selective agonist of β_2 adrenergic receptor (β_2-AR) used in the treatment of asthma. Its molecule contains two stereogenic centers, thus, the compound exists as four stereoisomers. The clinically used drug, *rac*-fenoterol, is a racemic mixture of (R,R)-fenoterol and (S,S)-fenoterol. Our previous research [3] indicated that fenoterol stereoisomers significantly differ in affinity, selectivity and functional activity with respect to β_2-AR. Radioligand displacement studies indicated that (R,R)-isomer shows the strongest affinity and selectivity followed by (R,S)- and (S,R)-forms. Molecular modeling and docking simulations suggest that stereochemistry significantly influences the orientation of the molecule within the binding site and its internal conformation. Conformational search analysis of all four isomers was performed by monitoring the potential

energy change with gradual rotation of each rotatable dihedral angle. Local minimum was selected for each dihedral and used to constructed optimized molecule. The molecule was further subjected to energy minimization and molecular dynamics simulations *in vacuo* and in water. The results allowed to define conformations representing the global minimum of energy for each stereoisomer. These conformations can be compared to the internal conformation of each stereoisomer in complex with β_2-AR. Among stereoisomers, the conformation of (R,R)-fenoterol in complex with β_2-AR shows the closest correspondence with the conformation obtained in molecular dynamics simulation in water. Thus, binding of (R,R)-isomer is additionally promoted by the lowest increase of internal energy associated with adopting the receptor-induced conformation upon binding.

STRESZCZENIE

Fenoterol jest względnie długodziałającym, selektywnym agonistą receptora β2 adrenergicznego (β2-AR), stosowanym w leczeniu astmy. Cząsteczka fenoterolu posiadająca dwa centra chiralne występuje w postaci czterech stereoizomerów. W medycynie stosowana jest mieszanina racemiczna (R,R)- i (S,S)-fenoterolu. Przeprowadzone wcześniej badania [3] dowiodły, że stereoizomery fenoterolu wykazują różną aktywność w stosunku do β2-AR. Testy wypierania radioliganda wykazały, że stereoizomer (R,R), w porównaniu z pozostałymi stereoizomerami wykazuje większe powinowactwo i selektywność w wiązaniu do β2-AR względem β1-AR. Modelowanie molekularne i dokowanie wskazują na to, iż stereochemia związków wpływa znacząco na sposób umiejscowienia cząsteczki liganda w kieszeni wiążącej receptora oraz na jego konformację. Analiza konformacyjna przeprowadzona została dla czterech stereoizomerów fenoterolu. Podczas stopniowych zmian kątów torsyjnych w cząsteczce monitorowana była jej energia potencjalna. Struktury badanych stereoizomerów poddane były minimalizacji energii, następnie przeprowadzona została dynamika molekularna w próżni oraz w wodzie. Rezultaty przeprowadzonych symulacji pozwoliły na wskazanie konformacji odpowiadających globalnemu minimum energii potencjalnej. Następnie konformacje te porównane zostały z konformacjami przyjmowanymi przez stereoizomery fenoterolu w kompleksach z β2-AR. Konformacja (R,R)-fenoterolu w kompleksie z β2-AR wykazała największe podobieństwo do niskoenergetycznej konformacji cząsteczki, otrzymanej podczas symulacji dynamiki molekularnej w wodzie. Wiązanie (R,R)-fenoterolu do β2-AR jest dodatkowo preferowane przez najniższy przyrost energii wewnętrznej związanej ze zmianą konformacji przyjmowanej przez ligand.