Journal of Medicinal Chemistry

Article

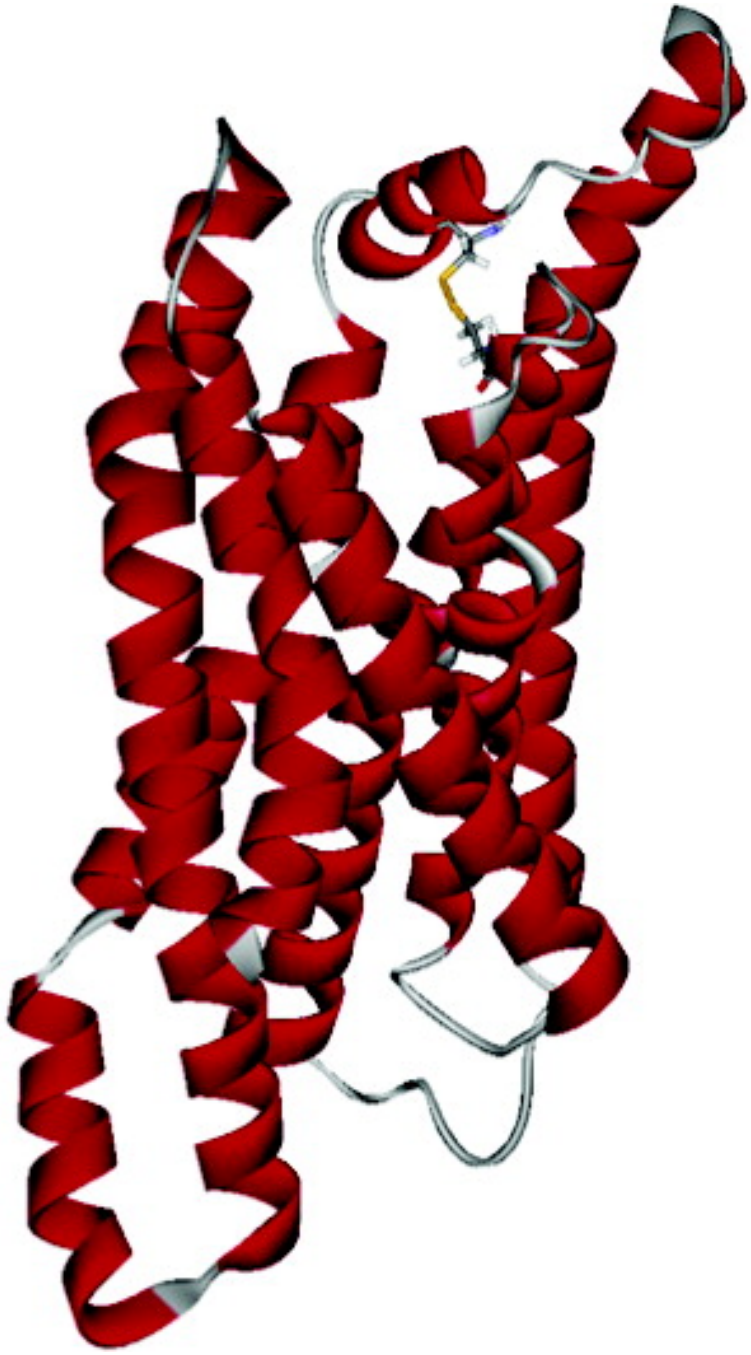
Molecular Modeling and Molecular Dynamics Simulation of the Human A Adenosine Receptor. The Study of the Possible Binding Modes of the A Receptor Antagonists

Andrei A. Ivanov, Igor I. Baskin, Vladimir A. Palyulin, Laura Piccagli, Pier G. Baraldi, and Nikolai S. Zefirov

J. Med. Chem., **2005**, 48 (22), 6813-6820• DOI: 10.1021/jm049418o • Publication Date (Web): 12 October 2005

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of
Medicinal Chemistry



Molecular Modeling and Molecular Dynamics Simulation of the Human A_{2B} Adenosine Receptor. The Study of the Possible Binding Modes of the A_{2B} Receptor Antagonists

Andrei A. Ivanov,[†] Igor I. Baskin,[†] Vladimir A. Palyulin,[†] Laura Piccagli,[‡] Pier G. Baraldi,[‡] and Nikolai S. Zefirov*,[†]

Department of Chemistry, M. V. Lomonosov Moscow State University, 119992 Moscow, Russian Federation, and Dipartimento di Scienze Farmaceutiche, Universita degli Studi di Ferrara, I-44100 Ferrara, Italy

Received July 22, 2004

A molecular model of the human A_{2B} adenosine receptor containing seven transmembrane α helices connected by three intracellular and three extracellular hydrophilic loops had been constructed. A molecular docking of seven structurally diverse xanthine antagonists of the A_{2B} receptor was performed, and the differences in their binding modes were investigated. The 1 ns molecular dynamics (MD) simulations of several obtained ligand−receptor complexes inserted into the phospholipid bilayer were carried out. The conformational changes of the A_{2B} receptor occurring during MD simulations were explored, and the stable binding modes of the studied antagonists were determined. According to the models presented in this work, the involvement of the His251, Asn282, Ser92, Thr89, and some aromatic residues in ligand recognition was determined. The obtained binding modes of the A_{2B} antagonists demonstrate good agreement with the site-directed mutagenesis data.

Introduction

Four subtypes of the adenosine receptors, namely, A_1, A_{2A}, A_{2B}, and A_3, have been cloned and characterized.[1] All of them are rhodopsin-like G-protein-coupled receptors (GPCRs). Adenosine receptors are widely distributed in most mammal and human tissues and mediate many biological effects. It is well-known that activation of A_1 and A_3 receptors results in a decrease of the range of cAMP while activation of A_{2A} and A_{2B} receptors increases it. Moreover, it was established that activation of the A_1 adenosine receptor affects the functioning of K^+ and Ca^{2+} ion channels.[2−4] Therefore, the ligands of these receptors are very important for pharmacology and medicine, in particular for the treatment of the hypoxia, asthma, Parkinson's disease, and other serious disorders.

A large number of synthetic agonists and antagonists of the adenosine receptors have been proposed during the past 10 years.[5−7] Generally, the structure of the adenosine is used as a starting point for the design of new agonists, while the xanthine moiety is used as a common fragment for new antagonists. Additionally, some non-xanthines were proposed as effective antagonists.[8,9]

Like other GPCRs belonging to the rhodopsin family, adenosine receptors are membrane proteins. All of them consist of seven transmembrane α helices connected by three extracellular and three intracellular hydrophilic loops.[10] Such macromolecules are not easily amenable to crystallization and, therefore, to precise structure elucidation via X-ray diffraction. Nowadays the data of X-ray diffraction are published for the rhodopsin structure only.[11,12] For this reason the molecular modeling approach is the most useful way for studying GPCR structure.

A great number of molecular models of various GPCRs including adenosine receptors were proposed.[13−16] Most of them were created using molecular modeling by homology with the structure of bovine rhodopsin. However, the proposed models usually consist of transmembrane helices only or several hydrophilic loops. It is a serious restriction for the practical application of these models, and it seems to be insufficient for a complete understanding of the protein structure as well as ligand−receptor interactions. On the other hand, the structure of the A_{2B} adenosine receptor is still practically unstudied.

Recently we have published communications on new molecular models of the A_1, A_{2A}, and A_{2B} human adenosine receptors based on homology with bovine rhodopsin.[17−19] These models include not only transmembrane α helices but also all hydrophilic loops.

The aim of the present paper is the further study of the structure of the human A_{2B} adenosine receptor and characterization of the binding modes of the A_{2B} receptor antagonists using molecular modeling. Additionally, it seems to be possible to obtain more accurate model using molecular dynamics (MD) simulations. The early MD simulations of membrane receptors were performed in a water environment.[20] However, nowadays the commonly accepted way for performing MD simulations of membrane proteins is the use of the phospholipid bilayer solvated by water as the most adequate environment.[21−24]

Results and Discussion

Molecular Modeling. As is noted above, among GPCRs at present the experimental data of X-ray

* To whom correspondence should be addressed. Phone: +7 (095) 939-1620. Fax: +7 (095) 939-0290. E-mail: zefirov@org.chem.msu.su.
[†] M. V. Lomonosov Moscow State University.
[‡] Universita degli Studi di Ferrara.

10.1021/jm049418o CCC: $30.25
Published on Web 10/12/2005

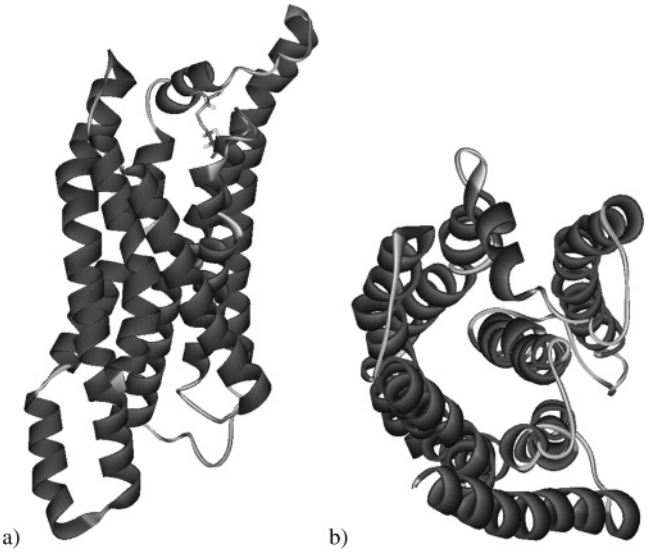


Figure 1. Molecular model of the human A_{2B} adenosine receptor: (a) front view; (b) top view.

diffraction are known for bovine rhodopsin only. It is commonly accepted that all rhodopsin-like receptors consist of seven transmembrane α helices connected by hydrophilic loops.[25] Also, it is accepted that the arrangement of the GPCRs helices is similar to the arrangement of rhodopsin helices. Despite the fact that the homology between amino acid sequences of bovine rhodopsin and some GPCRs including human adenosine receptors is not very significant (about 20% or less), the use of the rhodopsin structure as a template seems to be an applicable way for creating the molecular model.

For these reasons, the molecular modeling of the human A_{2B} adenosine receptor was performed by homology with a structure of bovine rhodopsin.[12] The analysis of amino acid sequences of the A_{2B} receptor and the bovine rhodopsin demonstrates the absence of any significant homology in the hydrophilic loops of these proteins. Moreover, some fragments of the intracellular loops of the rhodopsin are not present in the X-ray structure. For this reason, the configuration of the hydrophilic loops of the A_{2B} receptor was determined using the LOOP SEARCH command of Sybyl 6.9.1.[26] The loops with the highest homology and with the lowest root-mean-squared value were chosen from the Sybyl database as templates for the A_{2B} receptor loops. Additionally, the correspondence of each loop configuration with the common knowledge of the arrangement of the GPCRs hydrophilic loops was taken into account.

One of the most important points of the hydrophilic loops modeling is the formation of the disulfide bond between two cystein residues, which are highly conserved among all rhodopsin-like receptors.[10] In the obtained model of the A_{2B} receptor (Figure 1) these two cystein residues located in the first and second extracellular loops appear to be arranged at a distance suitable for the formation of this bond (2.03 Å). The performed analysis of the Ramachandran plot obtained using the PROCHECK[27] software suggests that less than 1% of amino acids have the disallowed geometry. The obtained results suggest that the created molecular model of the A_{2B} receptor should be reliable and could be used for further studies.

Molecular Docking. Two well-known antagonists of the human A_{2B} adenosine receptor (theophylline and DPSPX[28−30]) as well as some antagonists of this subtype recently proposed by Baraldi[31] were docked to the putative binding site of the A_{2B} adenosine receptor given in Figure 2. It was shown that all of these ligands have a significant affinity for this receptor. On the other hand, all of them are structurally diverse xanthine derivatives. To study the differences between the binding modes of these antagonists and to reveal the most essential amino acid residues involved in ligand recognition, molecular docking was performed. The molecular docking of all studied ligands was carried out using the DOCK command of the Sybyl 6.9.1 package as described in the computational methods section. It was demonstrated by Jacobson and co-workers[41,42] that the use of the Tripos force field with KOLLMAN-ALL charges for a receptor and Gasteiger−Hückel charges for ligands provides good results for adenosine receptors modeling. In this study the same protocol was applied.

The obtained results of the molecular docking of the theophyllin, one of the most potent but not selective A_{2B} antagonist, suggest that three amino acid residues of the receptor directly interact with the ligand: Ser92, Asn282, and Trp247. The Ser92 and Asn282 form hydrogen bonds with a carbonyl group at the 2-position of the xanthine ring, while Trp247 seems to be essential for binding because of a $\pi-\pi$ interaction. These results are in a good agreement with the available data on the site-directed mutagenesis obtained for adenosine receptors.[14,15,32−35] The binding mode of the DPSPX is generally the same as the binding mode of the theophyllin; however, the oxygen atom of the sulfo group of the DPSPX forms a hydrogen bond with His251, which is suggested to be important for the binding of the adenosine receptors ligands. The phenyl ring of the DPSPX is located inside the hydrophobic pocket formed by Thr89, His251, and Val250. The *n*-propyl chains lie inside two hydrophobic pockets formed by (i) Leu195, Met198, and Ala244 and (ii) Leu49, Asp53, Asn286, and Pro287. Additionally, Trp247 and Phe243 are involved in ligand binding via $\pi-\pi$ interactions with the phenylxanthine moiety.

The molecular docking of compounds **3**−**5** was performed in accordance with the results obtained for the DPSPX and theophyllin. The proposed binding mode of ligand **3** suggests that the hydroxyl group of the Ser92 forms a hydrogen bond with the carbonyl oxygen at the 2-position of the ligand xanthine ring. However, the involvement of the amino group of the Asn282 in the hydrogen bonding with this oxygen atom is not obvious. The distance between the closest hydrogen atom of the Asn282 amino group and the carbonyl oxygen of the **3** is about 3.5 Å. In addition, the amino group of the ligand is located at a distance of more than 4 Å from the nonprotonated nitrogen atom of the His251. On the other hand, this amino group is located at the same distance to a hydroxyl group of the Thr89. Despite the lack of the direct hydrogen bonding of the amino group of compound **3**, the location of this group between two hydrophilic functional groups seems to be very favorable for ligand−receptor interaction. The other amino acid

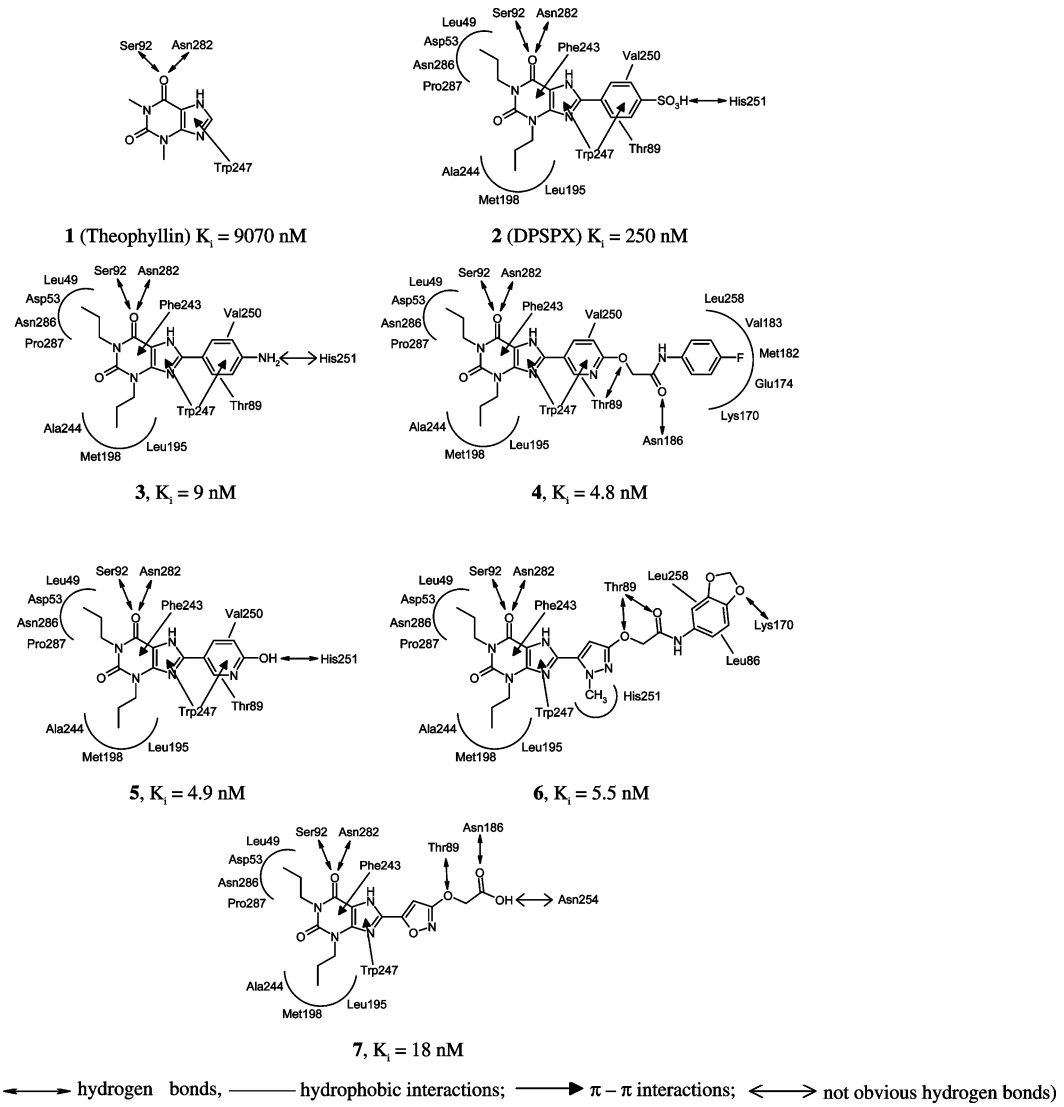


Figure 2. Binding modes of the A_{2B} receptor antagonists.

residues involved in binding the ligand are the same as for DPSPX. In particular, Trp247 and Phe243 seem to be involved in $\pi-\pi$ interactions with the xanthine and pyridine moieties. However, according to the found binding mode, the nitrogen atom of the pyridine ring of ligands **4** and **5** is not involved in hydrogen bonding with any residues.

The obtained results of the molecular docking of **5** and **3** suggest that the binding modes of these compounds are similar to that of DPSPX. The involvement of the His251 and Asn282 in the hydrogen bonding with compound **5** is the main difference between the binding modes of **5** and **3**. The His251 forms a hydrogen bond with the hydroxyl group of **5**, while the Asn282 is hydrogen-bonded to the carbonyl oxygen at the 2-position of the xanthine ring of this compound. In contrast, ligand **3** does not interact with Asn282. Also, the hydrogen bond between this ligand and His251 is not obvious.

According to the model obtained for compound **4**, the arrangement of the phenylxanthine moiety of this ligand inside the binding site is also the same as for DPSPX and **5**. The ester oxygen is located near (3.04 Å) the hydroxyl group of Thr89 and seems to be involved in the hydrogen bonding with this residue. The amino group of the Asn186 is hydrogen-bonded to the carbonyl oxygen of ligand **4**. Leu258, Val183, Glu174, Met182, and Lys170 form a suitable pocket for the fluorophenyl ring of the ligand.

The data obtained for compound **6** suggest that the arrangement of the xanthine moiety of this ligand is similar to that for other above-mentioned ligands and the Trp247 and Phe243 in this case are also involved into the ligand binding. Additionally, the oxygen atoms of the ester and amido groups of **6** are hydrogen-bonded to the Thr89 hydroxyl group while the oxygen of the benzodioxol moiety is hydrogen-bonded to Lys170. The methyl groups of Leu258 and Leu86 are involved in hydrophobic interactions with the benzodioxol ring. The methyl group of the pyrazole ring is located inside the pocket formed by hydrophobic moieties of Leu195, Trp247, and His251.

The molecular docking performed for compound **7** suggests that there are no hydrogen bonds between the isoxazole ring of the ligand and A_{2B} receptor. Also, the hydroxyl group of the carboxylic acid moiety seems to be not involved in interactions with the receptor. The amino acid residue located near this group is Asn254; however the distance between the hydroxyl group of **7** and Asn254 is 4.15 Å. On the other hand, the carboxylic

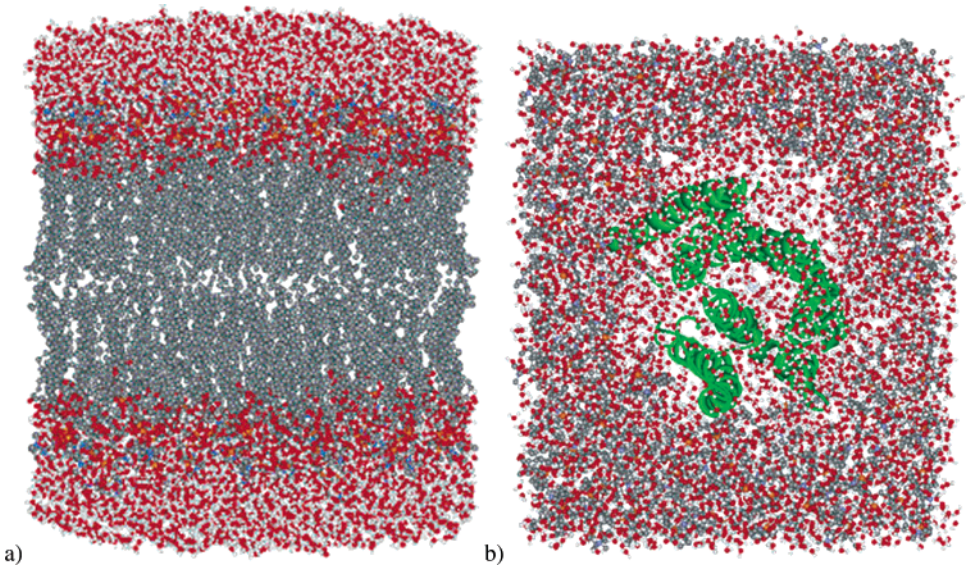


Figure 3. (a) Model of POPC bilayer proposed by Heller. (b) A_{2B} adenosine receptor inserted into bilayer (top view).

oxygen forms a strong hydrogen bond with the amino group of the Asn186 and the ester oxygen is hydrogen-bonded to Thr89.

The obtained results of molecular docking of the studied A_{2B} receptor antagonists allowed us to propose a general binding mode of these ligands and to determine residues involved in the ligand recognition. The obtained models demonstrate that Ser92 and Asn282 could be essential for hydrogen bonding with the carbonyl group at the 2-position of the xanthine ring of the ligands; the Thr89 could form hydrogen bonds with the ester and amido group oxygens, while Trp247 and Phe243 could be involved in the ligand binding because of $\pi-\pi$ interactions with the ligands.

In the next stage of the study, the MD simulations were performed. The aim of the MD simulations was to obtain more precise ligand−receptor models in the state close to natural conditions and to further explore the binding modes of the ligands.

Molecular Dynamics Simulation. It is commonly accepted that the most realistic and useful way for performing MD simulations of membrane proteins is the use of a phospholipid bilayer solvated by water molecules. Nowadays, a large number of phospholipid bilayer models are obtained using MD simulations. However, most of them include no more than 128 molecules of phospholipids. This seems to be enough for exploring physicochemical properties of the phospholipid bilayer; however, it is insufficient for simulating the receptor in the phospholipid bilayer environment. For this reason, we have used the model of the POPC bilayer proposed by Heller.[38] This bilayer contains 200 molecules of POPC solvated by TIP3 water molecules. Such an amount of phospholipids seems to be enough for inserting the A_{2B} receptor model into the bilayer. The complexes of the A_{2B} receptor with the three most structurally diverse xanthine antagonists (theophyllin, DPSPX, and compound **6**) with initial geometry obtained after the docking studies were selected for MD simulations. These complexes were separately placed into the phospholipid bilayer as described in the computational methods section.

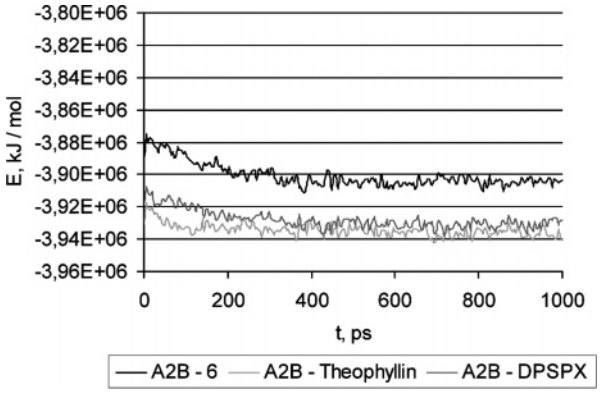


Figure 4. Total energy fluctuations obtained for the studied complexes during 1 ns of MD.

On the average, each system contains 130 POPC molecules, 4220 water molecules, an A_{2B} receptor, and the ligand (overall, about 22 350 atoms) (Figure 3).

The stability of the models was evaluated by calculating of the total energy of the systems. As is shown in Figure 4, all systems under study become stable after about 400 ps of the MD simulation.

To examine temporal changes in the receptor structure during 1 ns of the MD simulation, the root-mean-square deviation (rmsd) with respect to the starting structure was calculated for each complex (Figure 5). The obtained rmsd values are about 4 nm for each complex and stabilize after 400 ps, being in good agreement with the results of total energy calculations.

To determine the most deviated residues, the rms fluctuations (rmsf) of the Cα atoms of the A_{2B} receptor were calculated and plotted as a function of the residue number for each complex. As is shown in Figure 6, the residues located in the hydrophilic loops have the greatest rmsf values, while the rmsf of the transmembrane region is less than 0.15 nm. The greatest rmsf values were obtained for residues located in the longest hydrophilic loop (E2); in particular, a maximum value of 0.4 nm was obtained for Thr155 of the A_{2B}−**6** complex.

The analysis of the binding mode of the theophyllin obtained after MD simulation (Figure 7) suggests that

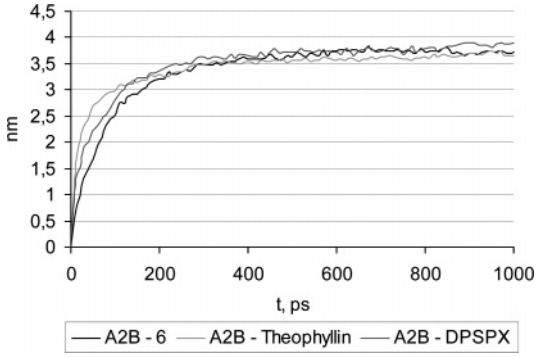


Figure 5. The rmsd of the studied complexes obtained during 1 ns of MD simulations.

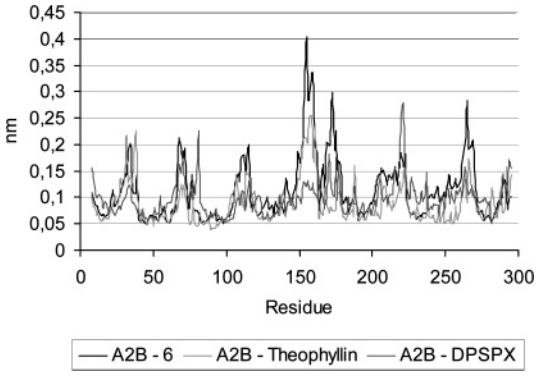


Figure 6. The rms fluctuations of the amino acid residues of the A_{2B} adenosine receptor obtained after MD simulations.

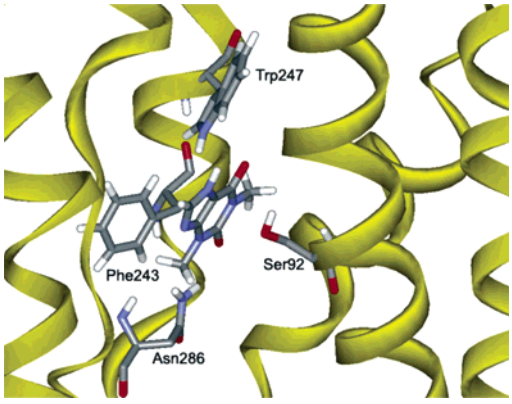


Figure 7. Binding mode of theophyllin obtained after MD simulations.

the ligand is hydrogen-bonded to Asn286 and Trp247. Also, the distance between hydroxyl group of the Ser92 and carbonyl oxygen of the ligand (2.96 Å) seems to be enough for hydrogen bonding. Additionally, the phenyl ring of the Phe243 is involved in $\pi-\pi$ interactions with theophyllin. The differences between binding modes of theophyllin obtained after the molecular docking and after the MD simulation could be explained by the small size of this ligand and the big free space around it. For this reason, on one hand, it is difficult to determine the exact location of theophyllin inside the binding site. On the other hand, it seems to be possible for theophyllin to adopt several reasonable binding modes.

The binding mode of the DPSPX established after MD simulation (Figure 8) is nearly the same as that obtained after the molecular docking. The His251 is involved in hydrogen bonding with the sulfo group of the ligand. The residue Asn282 forms hydrogen bonds

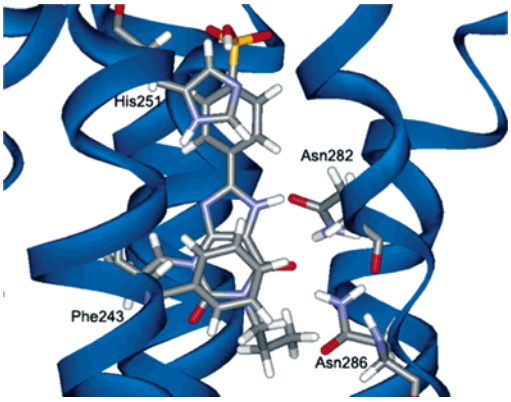


Figure 8. Binding mode of DPSPX obtained after MD simulations.

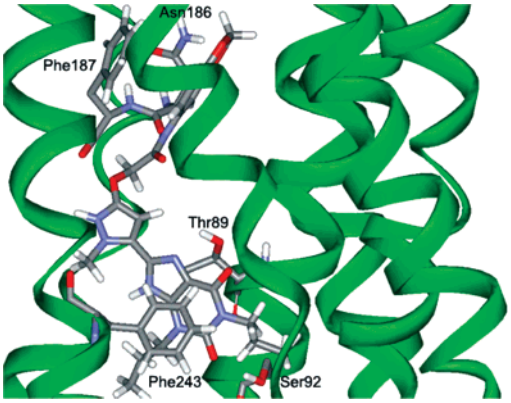


Figure 9. Binding mode of the compound **6** obtained after MD simulations.

with the protonated nitrogen and carbonyl oxygen at the 2-position of the xanthine moiety. Additionally, this oxygen is hydrogen-bonded to Asn286. Also, residue Phe243 is involved in ligand recognition through $\pi-\pi$ interactions. The *n*-propyl chains of the DPSPX are located in the same way as was obtained after the molecular docking study.

The results of the MD simulation obtained for compound **6** (Figure 9) suggest that the carbonyl oxygen at the 2-position of the xanthine ring of the ligand is hydrogen-bonded to the hydroxyl group of Ser92. The same result was obtained using molecular docking. The hydroxyl group of Thr89 seems to be involved in the hydrogen bonding with the nonprotonated nitrogen of the xanthine moiety. The analysis of this model suggests that the carboxyl group of the Asn186 forms a hydrogen bond with the amido nitrogen, while the amino group of this residue is hydrogen-bonded to the benzodioxol oxygen. The distance between Lys170 and the oxygen atom exceeds 4 Å, so the involvement of the Lys170 in ligand recognition is not obvious. Also, the phenyl ring of the Phe187 is located near the benzodioxol moiety of ligand **6** and probably interacts with the ligand. Despite the fact that His251 is not directly bonded to **6**, it is reasonable to suggest that a hydrogen bond could be formed between the nonprotonated nitrogen of this residue and the ester oxygen atom of the ligand.

The analysis of the results of molecular docking and molecular dynamics simulations of several protein–ligand systems confirmed the existence of a suitable binding site located inside the transmembrane domain. Also, amino acid residues located in the E2 hydrophilic

Table 1. Comparison of Molecular Modeling Results with Experimental Data

residue	mutagenesis data
L49[c]	
D53[c]	A_1 D55A increase in Ag affinity, no change in Ant affinity[33]
L86[c]	A_1 L88A substantial reduction of Ag and Ant binding[15]
T89[a]	A_1 T91A substantial reduction of Ag and Ant binding[15] A_{2A} T88A/S/R/D/E substantial reduction of Ag and Ant binding[32]
S92[a]	A_1 S94A no detectable Ag and Ant binding; S94T minor changes in ligand binding[33] A_{2A} S91A marginal changes in ligand binding[32]
K170[a]	
N186[a]	
F187[b]	A_{2A} F182A loss Ag and Ant radioligand binding 14; F182Y/W modest reduction of Ag binding[14]
L195[c]	
M198[c]	
F243[b]	
W247[b]	A_3 W243A/F substantial reduction of Ant, no change in Ag binding[35]
A244[c]	
V250[c]	
H251[a]	A_1 H251L Ant affinity reduced 4-fold, no change in Ag affinity[34] A_{2A} H250A loss of Ag and Ant radioligand binding, no Ag activity in functional assays; H250F/Y modest reduction of Ag binding, no effect on Ant binding[14]
N254[a]	A_{2A} N253A loss of Ag and Ant radioligand binding[14] A_3 N250A loss Ag and Ant binding[35]
L258[c]	
N282[a]	
N286[c]	
P287[c]	

[a] Hydrogen bonding. [b] Hydrophobic interactions. [c] $\pi-\pi$ interactions. Ag: agonist. Ant: antagonist.

loop seem to be essential for binding with large ligands. It was found that His251, proposed to be essential for the recognition of the ligands of all adenosine receptor subtypes, is involved in the hydrogen bonding with A_{2B} antagonists as a donor of the hydrogen bond and not as an acceptor. Another important finding is that some aromatic residues, in particular Phe243, Trp247 located in TM VI, and Phe187 located in E2, could be essential for ligand recognition through $\pi-\pi$ interactions. Additionally, it was established that *n*-propyl chains of the ligands are essential for binding because of strong hydrophobic interactions with residues located in TM II, TM V, TM VI, and TM VII. The results of molecular docking and molecular dynamics simulations are summarized and compared with available experimental data of site-directed mutagenesis in Table 1.

Conclusions

The molecular modeling and MD simulations presented in this work provide the first detailed study of the human A_{2B} adenosine receptor structure inserted into the phospholipid bilayer. A molecular model of the human A_{2B} adenosine receptor containing seven transmembrane α helices connected by three extracellular and three intracellular hydrophilic loops was created and described in this work. The molecular docking of the differently substituted xanthine antagonists of the A_{2B} receptor was performed. The obtained results allowed us to explore the main differences of binding modes of these ligands and to determine the amino acid residues involved in the recognition of the A_{2B} receptor antagonists.

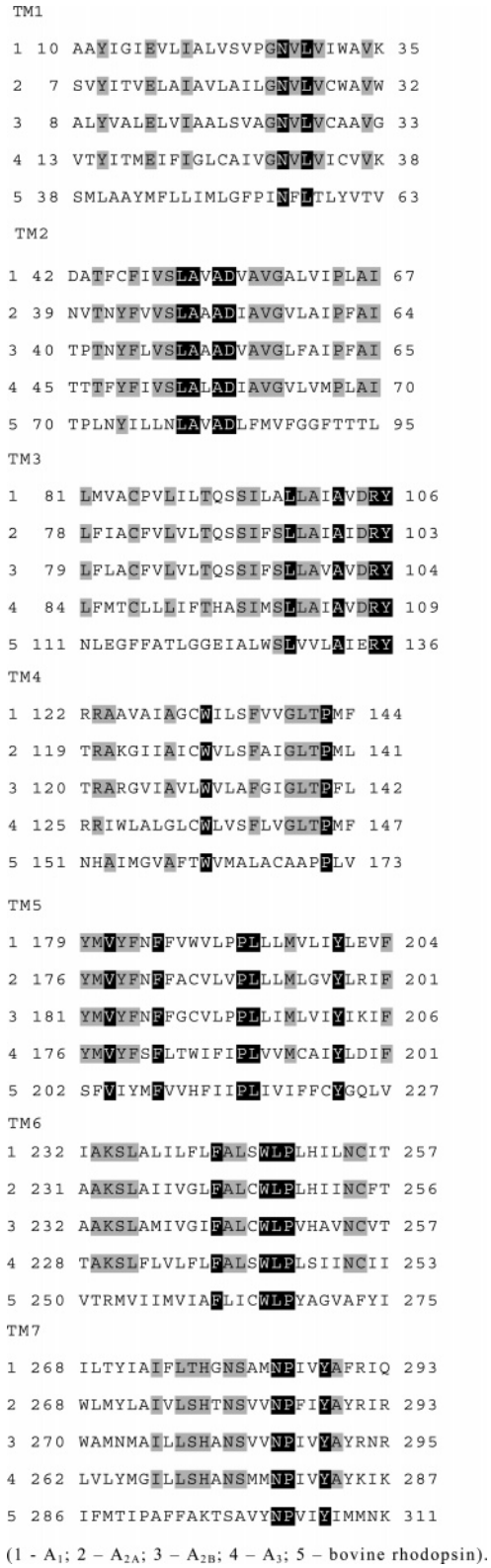
The molecular dynamics simulations of the complexes of the A_{2B} receptor with different antagonists inserted into the phospholipid bilayer were performed. The simulations were analyzed in terms of average structures and energies.

According to the models presented in this work, residues His251, Asn282, Ser92, and Thr89 as well as some aromatic residues are involved in ligand recognition. Additionally, it was suggested that in some cases the residues located in the hydrophilic loops could interact with the ligands. The obtained binding modes of the A_{2B} antagonists are in good agreement with known site-directed mutagenesis data.

Methods

Molecular Modeling. A sequence alignment of four known subtypes of the human adenosine receptors and bovine rhodopsin was performed to reveal amino acid residues forming the transmembrane α-helical domain (see Chart 1). The primary sequences were taken from the SWISSPROT protein data bank.[25] Then the amino acid residues of rhodopsin were manually replaced by the residues of the A_{2B} receptor using the COMPOSER module of Sybyl 6.9.1.[26] The obtained model of the A_{2B} receptor was optimized with the Tripos force field (KOLLMAN-ALL atomic charges[26]). The extracellular and intracellular hydrophilic loops were inserted into the model with the LOOP SEARCH command of the Sybyl 6.9.1 package. The loops with the highest homology and the best value of the root-mean square (rms) deviation were chosen from the Sybyl database. In addition, the theoretical data on the loops arrangement were taken into account. The geometry of the created model containing all transmembrane α helices and hydrophilic loops was optimized with the protocol described above and checked with the PROCHECK software.[27]

Chart 1



(1 - A_1; 2 – A_{2A}; 3 – A_{2B}; 4 – A_3; 5 – bovine rhodopsin).

Molecular Docking. The structures of the two well-known (theophylline, DPSPX[28–30]) and four recently proposed[31] antagonists of the human A_{2B} adenosine receptor containing all hydrogen atoms were optimized with the Tripos force field using Gasteiger–Hückel charges.[26] Then, according to the available data on the site directed mutagenesis,[14,15,32–35] the optimized ligands were manually placed into the putative binding site of the A_{2B} receptor and the best location and conformation of each ligand were determined using the DOCK command of Sybyl.[26] Finally, the energy minimization of the obtained protein–ligand complexes was performed.

Molecular Dynamics Simulation. Molecular dynamics (MD) simulations were performed using the Gromacs 3.1.4 package.[36,37] The obtained complexes of the human A_{2B} adenosine receptor with three antagonists (theophyllin, DPSPX, and compound **6**) were used for performing MD simulations. The model of the POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine) bilayer proposed by Heller[38] was used for simulation of the phospholipid environment around the receptor. The lipid parameters were taken from literature.[39,40] The ligand–receptor complexes were manually inserted into the center of the POPC bilayer in such a way that the α helices of the receptor were oriented approximately parallel to the hydrocarbon chains of the phospholipids, and the hydrophilic loops of the receptor were placed into water layers. After that, all water molecules and phospholipids within a radius of 2.3 Å around the protein were deleted and a suitable hole for the A_{2B} receptor was obtained. Therefore, three complexes of the A_{2B} receptor with its antagonists were formed. The energy of these complexes was minimized using the steepest descent approach realized in the Gromacs package. After the minimization, 1 ns of MD simulations were performed at the NPT and the periodic boundary conditions in all three dimensions with the standard parameters of the GROMACS force field.[36,37] Phospholipids, water molecules, receptor, and ligand were coupled separately to a temperature bath at 300 K, with a coupling constant $\tau_t = 0.1$ ps. Each studied system was simulated at a constant pressure of 1 bar, with a coupling constant $\tau_p = 1$ ps. A cutoff of 1.8 nm was used for the long-range Coulomb interactions with updates every 10 steps. A twin-range cutoff of 0.1 nm was used for the Lennard-Jones and short-range Coulomb interactions.

Acknowledgment. This work was supported by the Russian Foundation for Basic Research (Project No. 03-03-32630).

References

(1) Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors. *Pharmacol. Rev.* **2001**, *53*, 527–552.
(2) Song, W. J.; Tkatch, T.; Surmeier, D. J. Adenosine Receptor Expression and Modulation of Ca^{2+} Channels in Rat Striatal Cholinergic Interneurons. *J. Neurophysiol.* **2000**, *83*, 322–332.
(3) Heurteaux, C.; Lauritzen, I.; Windmann, C.; Lazdunski, M. Essential Role of Adenosine, Adenosine A_1 Receptors, and ATP-Sensitive K^+ Channels in Cerebral Ischemic Preconditioning. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4666–4670.
(4) Mark, M. D.; Herlitze, S. G-Protein Mediated Gating of Inward-Rectifier K^+ Channels. *Eur. J. Biochem.* **2000**, *267*, 5830–5836.
(5) van Tilburg, E. W.; von Frijtag Drabbe Künzel, J.; de Groote, M.; IJzerman, A. P. 2,5′-Disubstituted Adenosine Derivatives: Evaluation of Selectivity and Efficacy for the Adenosine A_1, A_{2A}, and A_3 Receptor. *J. Med. Chem.* **2002**, *45*, 420–429.
(6) Volpini, R.; Costanzi, S.; Lambertucci, C.; Taffi, S.; Vittori, S.; Klotz, K. N.; Cristalli, G. J. *N*(6)-Alkyl-2-alkynyl Derivatives of Adenosine as Potent and Selective Agonists at the Human Adenosine A_3 Receptor and a Starting Point for Searching A_{2B} Ligands. *J. Med. Chem.* **2002**, *45*, 3271–3279.
(7) Kim, Y. C.; Ji, X.; Melman, N.; Linden, J.; Jacobson, K. A. Anilide Derivatives of an 8-Phenylxanthine Carboxylic Congener Are Highly Potent and Selective Antagonists at Human A_{2B} Adenosine Receptors. *J. Med. Chem.* **2000**, *43*, 1165–1172.
(8) Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Pineda de las Infantos y Villatoro, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. Pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]-pyrimidine Derivatives: Potent and Selective A_{2A} Adenosine Antagonists. *J. Med. Chem.* **1996**, *39*, 1164–1171.
(9) van Muijlwijk-Koezen, J. E.; Timmerman, H.; van der Goot, H.; Menge, W. M.; von Frijtag Drabbe Künzel, J. F.; de Groote, M.; IJzerman, A. P. Isoquinoline and Quinazoline Urea Analogues as Antagonists for the Human Adenosine A_3 Receptor. *J. Med. Chem.* **2000**, *43*, 2227–2238.
(10) Bockaert, J.; Pin, J. P. Molecular Tinkering of G Protein-Coupled Receptors: An Evolutionary Success. *EMBO J.* **1999**, *18*, 1723–1729.

(11) Palzewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A.; Trong, I. L.; Teller, D. C.; Okada, T.; Stenkamp, R. E.; Yamamoto, M.; Miyano, M. Crystal Structure of Rhodopsin: A G Protein-Coupled Receptor. *Science* **2000**, *289,* 739−745.
(12) Okada, T.; Fujiyoshi, Y.; Silow, M.; Navarro, J.; Landau, E. M.; Shichida, Y. Functional Role of Internal Water Molecules in Rhodopsin Revealed by X-Ray Crystallography. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5982−5987.
(13) IJzerman, A. P.; van der Wenden, E. M.; van Galen, P. J.; Jacobson, K. A. Molecular Modeling of Adenosine Receptors. The Ligand Binding Site on the Rat Adenosine A_{2A} Receptor. *Eur. J. Pharmacol*. **1994**, *268*, 95−104.
(14) Kim, J.; Wess, J.; van Rhee, A. M.; Schöneberg, T.; Jacobson, K. A. Site-Directed Mutagenesis Identifies Residues Involved into Ligand Recognition in the Human A_{2A} Adenosine Receptor. *J. Biol. Chem*. **1995**, *270*, 13987−13997.
(15) Rivkees, S. A.; Barbhaiya, H.; IJzerman, A. P. Identification of the Adenine Binding Site of the Human A_1 Adenosine Receptor. *J. Biol. Chem*. **1999**, *274*, 3617−3621.
(16) Jacobson, K. A.; Gao, Z. G.; Chen, A.; Barak, D.; Kim, S. A.; Lee, K.; Link, A.; Rompaey, P. V.; van Calenbergh, S.; Liang, B. T. Neoceptor Concept Based on Molecular Complementarity in GPCRs: A Mutant Adenosine A_3 Receptor with Selectively Enhanced Affinity for Amine-Modified Nucleosides. *J. Med. Chem*. **2001**, *44*, 4125−4136.
(17) Ivanov, A. A.; Baskin, I. I.; Palyulin, V. A.; Zefirov, N. S. Molecular Modeling the Human A_1 Adenosine Receptor and Study of the Mechanisms of Its Selective Ligand Binding. *Dokl. Biochem. Biophys*. **2002**, *386*, 271−274.
(18) Ivanov, A. A.; Baskin, I. I.; Palyulin, V. A.; Zefirov, N. S. Molecular Modeling of the Human A_{2A} Adenosine Receptor. *Dokl. Biochem. Biophys*. **2003**, *389*, 94−97.
(19) Ivanov, A. A.; Baskin, I. I.; Palyulin, V. A.; Baraldi, P. G.; Zefirov, N. S. Molecular Modelling of the Human A_{2B} Adenosine Receptor and an Analysis of the Binding Modes of Its Selective Ligands. *Mendeleev Commun*. **2002**, *6*, 211−212.
(20) Dahl, S. G.; Edvardsen, O.; Sylte, I. Molecular Dynamics of Dopamine at the D_2 Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 8111−8115.
(21) Tieleman, D. P.; Berendsen, H. J.; Sansom, M. S. An Alamethicin Channel in a Lipid Bilayer: Molecular Dynamics Simulations. *Biophys. J*. **1999**, *76*, 1757−1769.
(22) Bachar, M.; Becker, O. M. Protein-Induced Membrane Disorder: A Molecular Dynamics Study of Melittin in a Dipalmitoylphosphatidylcholine Bilayer. *Biophys. J*. **2000**, *78*, 1359−1375.
(23) Capener, C. E.; Shrivastava, I. H.; Ranatunga, K. M.; Forrest, L. R.; Smith, G. R.; Sansom, M. S. Homology Modeling and Molecular Dynamics Simulation Studies of an Inward Rectifier Potassium Channel. *Biophys. J*. **2000**, *78*, 2929−2942.
(24) Elmore, D. E.; Dougherty, D. A. Molecular Dynamics Simulations of Wild-Type and Mutant Forms of the Mycobacterium Tuberculosis MscL Channel. *Biophys. J*. **2001**, *81*, 1345−1359.
(25) Bairoch, A.; Apweiler, R. The SWISS-PROT Protein Sequence Database and Its Supplement TrEMBL in 2000. *Nucleic Acids Res*. **2000**, *28*, 45−48.
(26) *Sybyl Molecular Modeling System*, version 6.9.1; Tripos, Inc., St. Louis, MO, 2003.
(27) Laskowski, R. A.; MacArthur, M. W.; Moss, D. S.; Thornton, J. M. PROCHECK: A Program To Check the Stereochemical Quality of Protein Structures. *J. Appl. Crystallogr*. **1993**, *26*, 283−291.
(28) Feoktistov, I.; Biaggioni, I. Adenosine A_{2B} Receptors. *Pharmacol. Rev*. **1997**, *49*, 381−402.
(29) Jacobson, K. A.; IJzerman, A. P.; Linden, J. 1,3-Dialkylxanthine Derivatives Having High Potency as Antagonists at Human A_{2B} Adenosine Receptors. *Drug. Dev. Res.* **1999**, *47*, 45−53.
(30) Feoktistov, I.; Biaggioni, I. Characterization of Adenosine Receptors in Human Erythroleukemia-Cells and Platelets. Further Evidence for Heterogeneity of Adenosine-A_2 Receptor Subtypes. *Mol. Pharmacol.* **1993**, *43*, 909−914.
(31) Baraldi, P. G.; Tabrizi, M. A.; Preti, D.; Bovero, A.; Romagnoli, R.; Fruttarolo, F.; Zaid, N. A.; Moorman, A. R.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Design, Synthesis, and Biological Evaluation of New 8-Heterocyclic Xanthine Derivatives as Highly Potent and Selective Human A_{2B} Adenosine Receptor Antagonists. *J. Med. Chem*. **2004**, *47*, 1434−1447.
(32) Jiang, Q.; van Rhee, A. M.; Kim, J.; Yehle, S.; Wess, J.; Jacobson, K. A. Hydrophilic Side Chains in the Third and Seventh Transmembrane Helical Domains of Human A_{2A} Adenosine Receptors Are Required for Ligand Recognition. *Mol. Pharmacol*. **1996**, *50*, 512−521.
(33) Barbhaiya, H.; McClain, R.; IJzerman, A.; Rivkees, S. A. Site-Directed Mutagenesis of the Human A_1 Adenosine Receptor: Influences of Acidic and Hydroxy Residues in the First Four Transmembrane Domains on Ligand Binding. *Mol. Pharmacol*. **1996**, *50*, 1635−1642.
(34) Olah, M. E.; Ren, H.; Ostrowski, J.; Jacobson, K. A.; Stiles, G. L. Cloning, Expression, and Characterization of the Unique Bovine A_1 Adenosine Receptor. *J. Biol. Chem.* **1992**, *267*, 10764−10770.
(35) Gao, Z.-G.; Chen, A.; Barak, D.; Kim, S.-K.; Müller, C. E.; Jacobson, K. A. Identification by Site-Directed Mutagenesis of Residues Involved in Ligand Recognition and Activation of the Human A_3 Adenosine Receptor. *J. Biol. Chem*. **2002**, *277*, 19056−19063.
(36) Berendsen, H. J. C.; van der Spoel, D.; van Drunen, R. GROMACS: A Message-Passing Parallel Molecular Dynamics Implementation. *Comput. Phys. Commun*. **1995**, *91*, 43−56.
(37) Lindahl, E.; Hess, B.; van der Spoel, D. GROMACS 3.0: A Package for Molecular Simulation and Trajectory Analysis. *J. Mol. Model*. **2001**, *7*, 306−317.
(38) Heller, H.; Schaefer, M.; Schulten, K. Molecular Dynamics Simulation of a Bilayer of 200 Lipids in the Gel and in the Liquid Crystal Phase. *J. Phys. Chem.* **1993**, *97*, 8343−8360.
(39) Berger, O.; Edholm, O.; Jahnig, F. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. *Biophys. J*. **1997**, *72*, 2002−2013.
(40) URL: http://moose.bio.ucalgary.ca.
(41) Gao, Z.-G.; Kim, S.-K.; Biadatti, T.; Chen, W.; Lee, K.; Barak, D.; Kim, S. G.; Johnson, C. R.; Jacobson, K. A. Structural Determination of A_3 Adenosine Receptor Activation: Nucleoside Ligands at the Agonist/Antagonist Boundary. *J. Med. Chem.* **2002**, *45*, 4471−4484.
(42) Kim, S.-K.; Gao, Z.-G.; Van Rompaey, P.; Gross, A. S.; Chen, A.; Van Calenbergh, S.; Jacobson, K. A. Modeling the Adenosine Receptor: Comparison of the Binding Domains of A_{2A} Agonists and Antagonists. *J. Med. Chem.* **2003**, *46*, 4847−4859.

JM049418O