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Computer aided comparative analysis of the binding modes of the adenosine receptor agonists for all known subtypes of adenosine receptors

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Abstract

Molecular models of all known subtypes (A_1, A_{2A}, A_{2B}, and A_3) of the human adenosine receptors were built in homology with bovine rhodopsin. These models include the transmembrane domain as well as all extracellular and intracellular hydrophilic loops and terminal domains. The molecular docking of adenosine and 46 selected derivatives was performed for each receptor subtype.

A binding mode common for all studied agonists was proposed, and possible explanations for differences in the ligand activities were suggested.

Keywords: Adenosine receptor; Agonist; Binding mode; Homology modeling; Docking

1. Introduction

The adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been cloned, characterized and classified as G protein-coupled receptors (GPCRs) of the rhodopsin family [1]. These receptors consist of seven transmembrane α-helices (TM) connected by three intracellular and three extracellular hydrophilic loops. Adenosine receptors are widely distributed in most tissues and involved in regulation of various biological processes. It is known that the activation of A_1 and A_3 receptors decreases the cAMP level, while the activation of A_{2A} and A_{2B} receptors increases it [2]. Moreover, stimulation of the A_1 receptor produces an activation of K^+ channels and an inhibition of Ca^{2+} channels [3–5]. Furthermore, it was demonstrated that adenosine A_1 receptor activation induced inhibition of the dopamine D_1 receptors [6], while stimulation of the A_{2A} receptor results in functional inhibition of the D_2 dopamine receptors [7].

Ligands of the adenosine receptors can be used as probes in pharmacology and as experimental therapeutic agents. It was shown that agonists of the A_1 receptor potentially could be used for treating myocardial infarction as well as diabetes [8–10]. Antagonists of the A_{2A} receptor can be applied to increase the effect of dopaminergic neurotransmission in the treatment of Parkinson's disease, and other adenosine receptor ligands maybe useful for the treatment of Alzheimer's disease as well as schizophrenia [7,11]. A_{2A}-agonists can reduce ischemia/reperfusion-induced liver injury [12]. Theophylline – an effective antagonist of the A_{2B} receptor – is a strong antiasthmatic drug [13]. Also, activation of the A_{2B} receptor mediates the relaxation of coronary blood vessels [14]. A_3 adenosine receptor agonists reduce myocardial ischemia/reperfusion injury [15].

In view of the importance of adenosine receptor ligands many different agonists and antagonists of these receptors have been proposed in recent years. In this paper, we focus on adenosine receptor agonists. In general, all known agonists of the adenosine receptors are derivatives of the natural non-selective agonist—adenosine. 5′-*N*-Ethylcarboxamidoadenosine (NECA) and N^6-[2-(4-aminophenyl)ethyl]adenosine (APNEA) are more potent than adenosine, but are not selective ligands. N^6-Cyclopentyladenosine (CPA), 2-Cl-N^6-cyclopentyladenosine (CCPA) and N^6-(*R*)-phenylisopropyladenosine (*R*-PIA) are selective A_1 receptor agonists [1,2].

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Also, 2-Cl-N^6-phenoxyalkyl and 2-Cl-N^6-piperidinyl adenosine derivatives were reported as selective agonists for this subtype [16]. Recently, a series of 2-haloadenosine analogs substituted in the N^6-position by 2(*S*)-endo-norborn-2-yl, 2(*S*)-endo-norborn-5-en-2-yl and 2(*S*)-endo-5,6-epoxynorborn-2-yl moieties were proposed as high-affinity A_1 receptor agonists [17]. In addition, 2′-deoxy- and 1-deazaadenosine derivatives were proposed [18] as agonists of the A_1 receptor.

A_{2A} selective agonists usually contain a bulky substituent in the 2-position of the adenine ring of NECA. For example, 2-([4-(2-carboxyethyl)phenylethyl]amino)adenosine-5′-*N*-ethylcarboxamide (CGS 21680) [19] was proposed as a selective agonist for the A_{2A} adenosine receptor. Also, some analogs of NECA substituted in the 2-position of the adenine ring by an alkyl group as well as an aromatic or heteroaromatic ring conjugated through a triple bond showed high potency at A_3 and A_{2A} receptors [20]. For example, 2-hexyl-NECA (HENECA) showed high affinity at the A_{2A} and A_3 receptors and significant selectivity versus the A_1 subtype. The compounds of this type also were shown to be potent agonists for the A_{2B} adenosine receptor [20]. For example (*R*,*S*)-2-phenylhydroxypropynyl-NECA ((*R*,*S*)-PHPNECA) is one of the most potent (but not selective) A_{2B} receptor agonists. Moreover, it was found that (*S*)-PHPNECA is a more potent A_{2B} agonist ($EC_{50} = 0.22$ μM) than its (*R*)-diastereomer ($EC_{50} = 2.4$ μM) [21]. Recently, five non-adenosine derivatives, namely 4-phenyl-substituted 2-amino-4-phenyl-6-phenylsulfonylpyridine-3,5-dicarbonitriles, were proposed as high-potency agonists for the A_{2B} receptor [22]. However, despite intensive attempts, A_{2B} selective agonists have not been proposed.

Today, N^6-3-iodobenzyl-5′-*N*-methylcarboxamidoadenosine (Cl-IB-MECA) is used as a standard A_3 agonist. Furthermore, the N^6-unsubstituted analog (MECA) is a moderately selective and potent A_3 receptor agonist [1], while NECA is a non-selective ligand. Also, some methanocarba adenosine derivatives were proposed as high-affinity A_3 receptor agonists [23]. Recently, a series of 3′-aminoadenosine-5′-uronamides [24], N^6-substituted D-4′-thioadenosine-5′-methyluronamides [25], N^6-[4-(substituted)sulfonamidophenylcarbamoyl]adenosine-5′-uronamides [26], and 2-pyrazolyl-N^6-substituted adenosine derivatives [27] were synthesized and proposed as potent and selective A_3 adenosine receptor agonists.

The configuration of the binding site of adenosine receptors as well as the mechanisms of the ligand binding are under intensive investigation. The data of mutational analysis of the adenosine receptors was recently reviewed in detail and summarized by Fredholm et al. [1] and Jacobson et al. [28]. Here, we briefly review the mutagenesis data concerning only agonist binding.

It was demonstrated by Olah et al. [29] that two histidine residues, namely His251$^{6.52}$ and His278$^{7.43}$ (here the first number is an absolute number of the residue in the sequence; the superscripted number is the relative number of the residue based on the Ballesteros–Weinstein nomenclature [30,31]) of the bovine A_1 adenosine receptor are essential for the ligand binding. In addition, it was found that His278$^{7.43}$ is crucial for the agonist recognition, while His251$^{6.52}$ is also involved in agonist binding, but it is even more important for antagonist binding. Mutations of Thr277$^{7.42}$ of the human A_1 receptor to serine and alanine [32] suggested that Thr277$^{7.42}$ is very important for 5′-*N*-substituted agonists (such as NECA) and its hydroxyl moiety can interact with the 5′-substituent of NECA. The mutational analysis of several residues located in the first four α-helices of the A_1 receptor [33] demonstrated that Glu16$^{1.39}$, Asp55$^{2.50}$, and Ser94$^{3.39}$ play important roles in the agonist-receptor interactions, while mutations of Ser6 (N-terminal domain), Ser23$^{1.46}$, Ser93$^{3.38}$, Ser135$^{4.53}$, and Thr141$^{4.59}$ produced no changes in radioligand binding. The roles of Gly14$^{1.37}$, Pro25$^{1.48}$, Ile31$^{1.54}$, Leu65$^{2.60}$, Met82$^{3.27}$, Pro86$^{3.31}$, Val87$^{3.32}$, Leu88$^{3.33}$, Thr91$^{3.36}$, Gln92$^{3.37}$, Ala125$^{4.43}$, and Phe144$^{4.62}$, in the interactions of ligands with the human A_1 receptor were investigated by Rivkees et al. [34]. Mutations of Ile31$^{1.52}$, Leu65$^{2.60}$, Met82$^{3.27}$, Val87$^{3.32}$, Ala125$^{4.43}$ and Phe144$^{4.62}$ produced no change in agonist binding. In contrast, the mutation of Gly14$^{1.37}$ to threonine resulted in increased affinity of agonists. Mutations of five other amino acid residues resulted in the reduction of agonist affinity. Recently, Thr270$^{7.35}$, Ala273$^{7.38}$, Ile274$^{7.39}$, Thr277$^{7.42}$, His278$^{7.43}$, Asn284$^{7.49}$, and Tyr288$^{7.53}$ of the A_1 adenosine receptor were determined by Dawson and Wells to be exposed to the ligand-binding crevice [35].

Results of site-directed mutagenesis of the human A_{2A} adenosine receptor [36] suggested that Phe182$^{5.43}$, His250$^{6.52}$, Asn253$^{6.55}$, His278$^{7.43}$, and Ser281$^{7.46}$ located in TMs 5–7 are important for binding of both agonists and antagonists. It was demonstrated than both histidine residues are very important for agonist and antagonist binding, but His250$^{6.52}$ is not involved in hydrogen bonding with ligands. The involvement of His278$^{7.43}$ (as well as Glu13$^{1.39}$) in agonist recognition was also determined by Gao et al. [37]. Mutations of Phe182$^{5.43}$ resulted in a reduction of the affinity of *R*-PIA and NECA. The substitution of Ser281$^{7.46}$ with asparagine or threonine resulted in minor changes in binding affinities of ligands, while the mutation of Asn253$^{6.55}$ resulted in the loss of agonist and antagonist binding. Furthermore, it was demonstrated that Ser277$^{7.42}$ is essential for agonist binding. The mutation of Phe180$^{5.41}$, Asn181$^{5.42}$, Cys254$^{6.56}$, Phe257$^{6.57}$, and Ile274$^{7.39}$ was also performed by Kim et al. [36] and it was found that substitutions of Phe180$^{5.41}$ and Cys254$^{6.56}$ resulted in no change in ligand affinity. Replacement of Asn181$^{5.42}$ with serine mostly affected the affinities of *R*-PIA and 2-chloroadenosine, while the affinity of NECA was only slightly changed. In contrast, mutation of Phe257$^{6.57}$ and Ile274$^{7.39}$ resulted in the total loss of agonist and antagonist binding. The roles of several A_{2A} receptor residues located in the third and seventh α-helices were examined by Jiang et al. [38], and it was shown that Thr88$^{3.36}$ is important for agonists binding while Gln89$^{3.37}$ can indirectly influence ligand binding. It was also found that replacement of Ser90$^{3.38}$ and Ser91$^{3.39}$ with alanine as well as replacement of Ser277$^{7.42}$ with cysteine produced moderate changes in ligand-binding affinity and replacement of Ser281$^{7.46}$ with Asn increased agonist affinity. The involvement

of several amino acid residues in the second and third extracellular loops (EL2 and EL3) of the human A_{2A} adenosine receptor in ligand recognition was also analyzed [39]. The results obtained suggested that Glu151 and Glu169 located in EL2 are essential for ligand recognition, while Glu161 is not involved in receptor–ligand interactions. Furthermore, Glu169 is important for binding of adenosine derivatives substituted at the N^6-position, but not at C2- and C5′-positions. In contrast, mutations of Asp170, Pro173 (EL2), and Cys262 located in EL3 resulted in no change in both agonist and antagonist binding.

The mutational analysis of the human A_{2B} adenosine receptor was performed by Beukers et al. [40,41]. It was reported that a replacements of Val11$^{1.36}$, Ala12$^{1.37}$, Leu258$^{6.59}$, and Phe259$^{6.60}$ resulted in no change in ligand binding [40]. In contrast, replacement of Asn273$^{7.36}$ with Tyr increased the affinity of 2-substituted adenosines. Single mutations of Asn36 (IL1), Thr42$^{2.39}$, Val54$^{2.51}$, Phe84$^{3.31}$, and Ser91$^{3.38}$ as well as combined mutations of N36S, V87A$^{3.34}$; N36S, T42A$^{2.39}$, T66A$^{2.63}$; T42A$^{2.39}$, V54A$^{2.51}$; F84L$^{3.31}$, S95G$^{3.42}$; A18T$^{1.43}$, A23V$^{1.48}$, C83Y$^{3.30}$, A106V (IL2), R112S (IL2); I242T$^{6.43}$, K269R (EL3), V284A$^{7.47}$, H302Q$^{7.65}$; and Y299C$^{7.62}$, F301C$^{7.64}$, L310P (C-terminal domain) resulted in the decreased value of the EC_{50} for NECA [41]. At receptors with Q214L (IL3), I230N$^{6.31}$, V240M$^{6.41}$, V250M$^{6.51}$, N254Y$^{6.55}$, T257S$^{6.59}$, K269stop (EL3) mutations NECA was inactive.

The results of site-directed mutagenesis obtained for the human A_3 adenosine receptor were described by Gao et al. [42]. Several amino acid residues located in TM3, TM6 and in the second extracellular loop were mutated. The obtained results suggest that replacements of Trp243$^{6.48}$ and Lys152 located in EL2 are not essential for agonists binding. On the other hand, it was shown that Trp243$^{6.48}$ is important for the receptor activation. Mutation of His95$^{3.37}$, Leu244$^{6.49}$, and Ser247$^{6.52}$ decreased agonist affinity. Furthermore, it was found that Asn250$^{6.55}$ (conserved among all adenosine receptors) is crucial for both agonist and antagonist binding. The replacement of this asparagine residue with alanine resulted in the loss of ligand binding. The role of the highly conserved His272$^{7.43}$ of the A_3 receptor in the ligand recognition was studied by Jacobson et al. [43] and it was found that H272E$^{7.43}$ mutation decreased the affinity for uncharged adenosine derivatives.

Site-directed mutagenesis data can provide information about the involvement of particular amino acid residues in ligand recognition. In some cases it is also possible to presume which part or functional group of the ligand interacts with the mutated residue and what is the nature of this interaction. However, a mutational analysis cannot provide precise information about the orientation of the ligand inside the binding site. X-ray analysis could provide such information, however, among all GPCRs of the rhodopsin family an X-ray analysis has been reported only for bovine rhodopsin [44–46]. For this reason, one of the widely and effectively used approaches to the investigation of ligand-binding modes as well as protein structure prediction is molecular modeling.

The aim of this study is a further investigation of the structure of adenosine receptors and a comparative analysis of the binding modes of their known agonists using a molecular modeling approach.

2. Methods

2.1. Molecular modeling

The primary sequences of adenosine receptors and bovine rhodopsin were taken from the SWISSPROT protein data bank [47]. The sequence alignment of the subsequences corresponding to the TM1–7 for all four subtypes of adenosine receptors and bovine rhodopsin was published previously [48–51]. Here, we supplemented that alignment with subsequences of all extracellular and intracellular loops as well as terminal domains using ClustalX [52]. Then, the obtained alignment was manually refined. The alignment is shown below:

```
                                                    TM1                 ↓
 AA1R   ----------------------------MP---PSISAFQAAYIGIEVLIALVSVPGNVL  29
AA2AR   ----------------------------MP------IMGSSVYITVELAIAVLAILGNVL  26
AA2BR   ----------------------------M-----LLETQDALYVALELVIAALSVAGNVL  27
 AA3R   ----------------------------MPNNSTALSLANVTYITMEIFIGLCAIVGNVL  32
 OPSD   ---MNGTEGPNFYVPFSNKTGVVRSPFEAPQYYLAEPWQFSMLAAYMFLLIMLGFPINFL  57

        TM2                 ↓                                TM3
 AA1R   VIWAVKVNQALRDATFCFIVSLAVADVAVGALVIPLAIL--INIGPQTYFHTCLMVACPV  87
AA2AR   VCWAVWLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAIT--ISTGFCAACHGCLFIACFV  84
AA2BR   VCAAVGTANTLQTPTNYFLVSLAAADVAVGLFAIPFAIT--ISLGFCTDFYGCLFLACFV  85
 AA3R   VICVVKLNPSLQTTTFYFIVSLALADIAVGVLVMPLAIV--VSLGITIHFYSCLFMTCLL  90
 OPSD   TLYVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTTLYTSLHGYFVFGPTGCNLEGFFA 117
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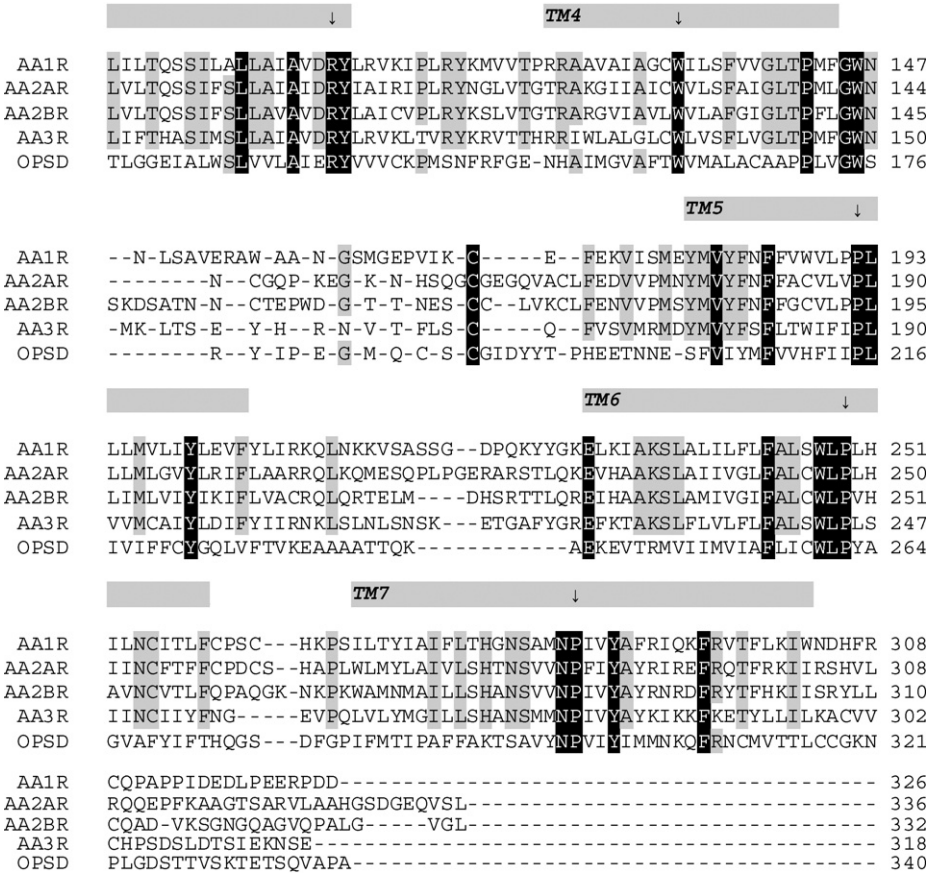


(transmembrane α-helices are highlighted and the most conserved residues (X.50) in each α-helix are indicated by arrows, receptor names are abbreviated based on the SWISSPROT entry names [47]).

The molecular models of the adenosine receptors were generated using the program Modeller [53,54]. Initially, for each subtype of adenosine receptor 10 independent models with different conformations of the loop regions were built. The geometrical parameters of the models were evaluated using the program Procheck [55,56]. The model with the best parameters and with the optimal value of the probability density function was selected for further improvement. Based on the selected model twenty additional models with the refined configuration of the loops were built using the Modeller loop routine, and the one with the best parameters was selected.

Then, all hydrogen atoms were added to the model and Kollman-All atomic charges [57] were assigned. The Powell energy minimization was performed using the Sybyl 6.9.1 package [57] in the Tripos force field [57] until the root mean square (RMS) gradient of the potential energy was less than 0.05 kcal/mol $Å^{-1}$. The verification of the optimized models was performed using the ProTable procedure of the Sybyl package as well as the Procheck software [55,56]. The analyzed parameters of the Ramachandran plots obtained for the models were compared with the RMS values as well as with parameters obtained for the native structure of rhodopsin.

2.2. Molecular docking

The molecular docking of the set of the adenosine receptor agonists was performed manually using the DOCK command of the Sybyl 6.9.1 package. In the beginning, the structures of the ligands containing all hydrogen atoms were sketched using Sybyl. Gasteiger–Hückel atomic charges [57] were calculated and assigned for each ligand. The geometry optimization of the agonists was performed using Sybyl in the Tripos force field. Then, optimized ligands were manually placed into the putative binding site of the receptor according to the available data from site-directed mutagenesis [29,32–43]. The functional groups of the ligands that were apparently involved in ligand–receptor interactions were manually prearranged at distances suitable for interacting with the corresponding functional groups of the receptor.

Initially, the positions of the backbone and side chains atoms of the receptors were fixed during the docking process and only the ligand geometry was made flexible. When the most energetically favorable location of the ligand was found the procedure of the molecular docking was repeated with the flexible ligand and receptor geometry. After that, the energy

minimization of the whole protein–ligand complexes was performed.

3. Discussion

3.1. Molecular models

As was mentioned above, the adenosine receptors are transmembrane GPCRs belonging to the rhodopsin family. The data of X-ray analysis have not been published for such proteins with the one exception of bovine rhodopsin. For this reason precise data of the 3D structure of the rhodopsin-like receptors are unavailable yet and molecular modeling is one of the widely used methods providing us some knowledge of GPCR structures. In the present study the molecular modeling of all four known subtypes of the human adenosine receptors was performed using a *molecular modeling with homology* approach, which is commonly accepted for prediction of 3D structures of the rhodopsin family of GPCRs [58]. The X-ray structure of the transmembrane domain of the bovine rhodopsin [45] as well as its hydrophilic loops and terminal domains were used as a template for the modeling.

It should be noted here that adenosine receptors and bovine rhodopsin are related but different proteins and the configuration of their transmembrane domains, hydrophilic loops and terminal domains also could be significantly different. Moreover, there is no significant homology in the sequences of adenosine receptors and rhodopsin. However, since only the data of X-ray analysis can provide the precise structure of the receptor and such data are not available for adenosine receptors, in this study we presume the hypothesis of the high-structural similarity between rhodopsin and the studied receptors. The second point which has to be noted is that the available structure of rhodopsin was obtained for its ground state, which is probably not the best starting point for a molecular modeling study of the binding modes of adenosine receptor agonists. On the other hand, several examples of the successful use of rhodopsin-based models of GPCRs in studies of agonist binding modes have been published [59–61]. Since in the present study we are considering the *static* agonist binding mode (not the *process* of the receptor activation), and taking into account a great amount of experimental data of site-directed mutagenesis of the adenosine receptors we presume it to be acceptable to use the structure of rhodopsin as a template for model construction. In the future, for further investigation of the differences between active and inactive states of the receptor, these models could be subjected to molecular dynamics simulation studies.

The use of the entire rhodopsin structure as a template provides the rhodopsin-like arrangement of the extracellular and intracellular loops of the adenosine receptors. Also, in this case two highly conserved cysteine residues (located in EL2 and on the border of TM3 and EL1) are arranged at a distance suitable for the formation of the disulfide bridge.

The molecular modeling of the adenosine receptors was performed using a protocol described in Section 2. The models obtained are shown in Fig. 1. The ProTable procedure of the Sybyl 6.9.1 [57] and the Procheck software [55,56] were used for verification of the geometry of the models. The results of the verification were compared with the RMS values of the analyzed parameters as well as with parameters obtained for the structure of the native bovine rhodopsin (Table 1). As shown in Table 1, no amino acid residues of the A_1, A_{2A}, and A_{2B} receptors and only one residue of the A_3 receptor were located in the disallowed region of the Ramachandran plot. This was in good agreement with the results obtained for the rhodopsin structure as well as with the standard values calculated for the experimentally determined protein structures with a similar resolution. The evaluated values of the G-factors corresponded to the optimal range. In addition, the amount of the bad contacts of the non-bonded atoms per 100 amino acid residues was found to be significantly less than the typical value and it is in good agreement with the corresponding values obtained for the rhodopsin. Thus, the performed analysis of the models' geometry demonstrated the good quality of the models and confirmed the possibility of their further application.

3.2. Agonists binding modes

With the aim of investigating the ligand–receptor interactions of the adenosine receptors agonists, the molecular docking of adenosine and a set of adenosine derivatives (Table 2) was performed manually using DOCK command of the Sybyl 6.9.1, as was described in Section 2.

3.2.1. Adenosine

Initially, with the purpose of understanding the general characteristics of the agonist-binding mode for all four receptor subtypes, the binding mode of adenosine (a non-selective native agonist) was examined. The obtained results suggested that each adenosine receptor subtype could bind adenosine almost identically. Molecular docking has detected that the amino group at the 6-position of adenosine was hydrogen bonded to $Asn^{6.55}$ conserved among all adenosine receptor subtypes (Fig. 2). The 3′-hydroxyl group of the ligand was involved in the interaction with the non-protonated nitrogen atom of $His^{7.43}$. Previously, Kim and co-workers proposed that there are no interactions between the adenosine receptor agonists and $Ile^{7.39}$ [28], however molecular docking results of the current study showed that in all adenosine receptor subtypes this amino acid residue can be hydrogen bonded to the 2′-hydroxyl group of adenosine. Also, as follows from our results, the ring oxygen atom of the ribose moiety of adenosine as well as its 5′-hydroxyl group can form the hydrogen bonds with the $Thr^{3.36}$.

The binding mode of adenosine found for the A_{2B} adenosine receptor subtype allowed us to propose that Ser165 located in EL2 of this subtype is also involved in ligand–receptor interactions through hydrogen bonding with the nitrogen atom at the 3-position of adenosine. This hydrogen bond does not exist for the A_{2A} subtype, however in the case of the A_{2A} receptor the nitrogen atom at the 5-position of adenosine interacts with Lys150 (EL2). Also, the nitrogen atom at the 5-position of

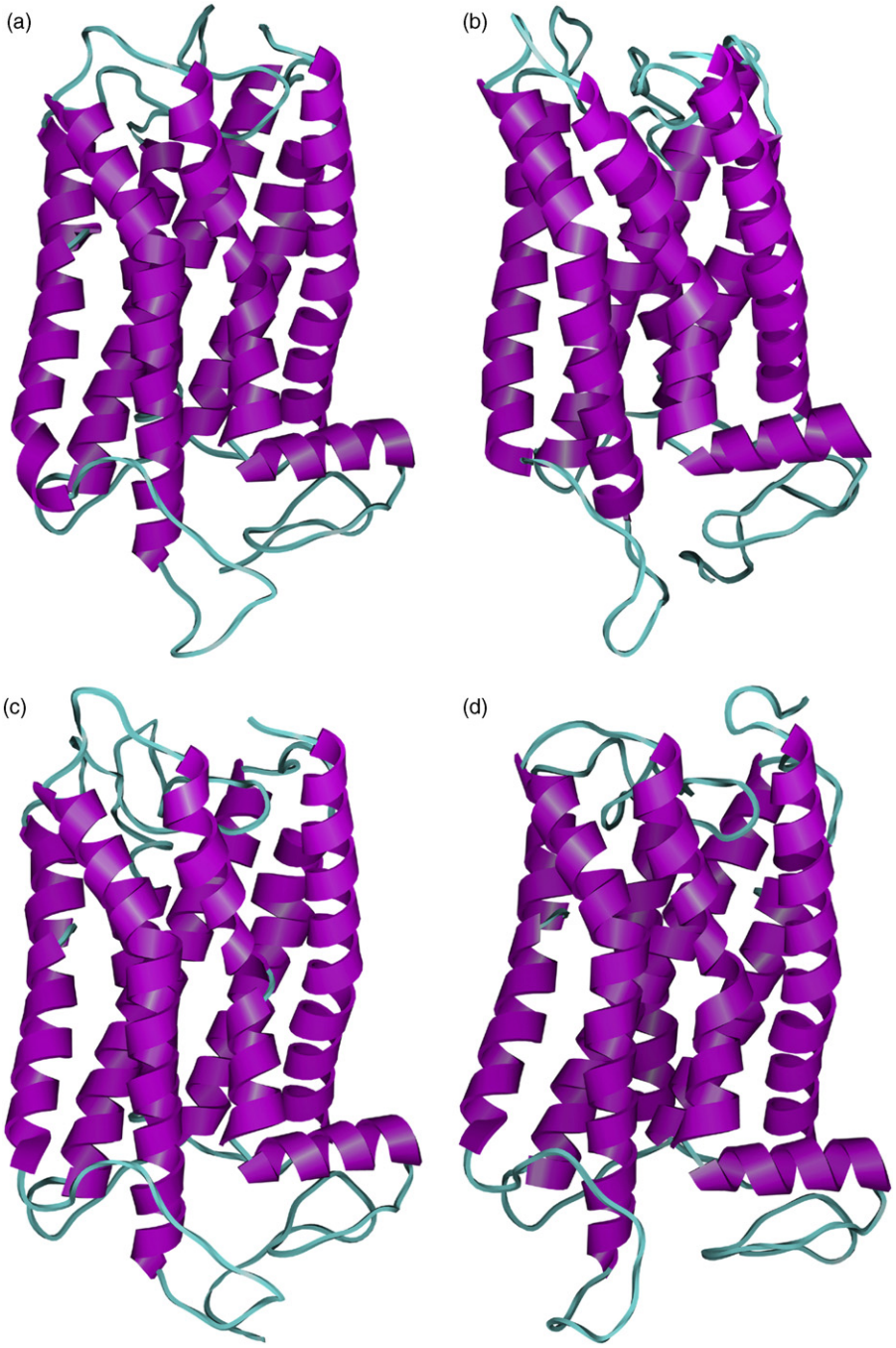


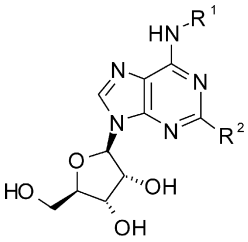
Fig. 1. Molecular models of the human adenosine receptors: (a) A_1; (b) A_{2A}; (c) A_{2B}; (d) A_3.

Table 1
The geometrical parameters of the molecular models of adenosine receptors

	A_1	A_{2A}	A_{2B}	A_3	Rhodopsin
Residues in the MF (tv = 74.6%)	262 (90.3%)	247 (82.6%)	264 (88.6%)	259 (87.5%)	232 (79.7%)
Residues in the AA	24 (8.3%)	43 (14.4%)	26 (8.7%)	26 (8.8%)	50 (17.2%)
Residues in the GA	4 (1.4%)	9 (3.0%)	8 (2.7%)	10 (3.4%)	7 (2.4%)
Residues in the DA	0	0	0	1 (0.3%)	2 (0.7%)
Bad contacts/100 residues (tv = 12.2)	2.8	2.1	4.2	4.4	3.5
Total G-factor (ov > −0.5)	−0.14	−0.12	−0.04	−0.04	0.1

MF, most favored region; AA, additional allowed region; GA, generously allowed region; DA, disallowed region; tv, typical value; ov, optimal value.

Table 2
Adenosine receptors agonists used for molecular docking studies



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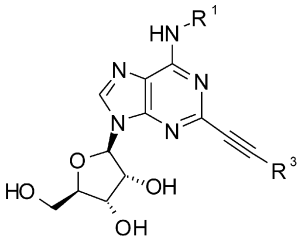

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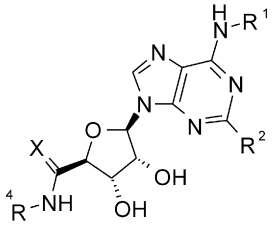

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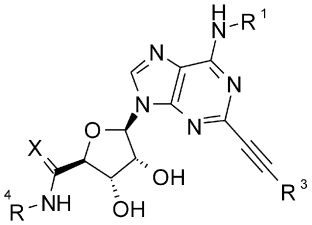


4

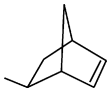
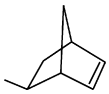
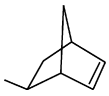
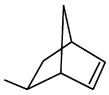
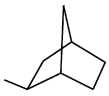
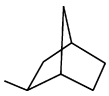
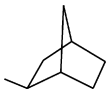
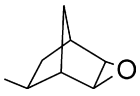
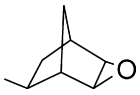
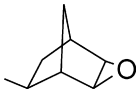
No.	St	R^1	R^2	R^3	R^4	X	K_i (nM)		EC_{50} (μM)	K_i (nM)
							A_1	A_{2A}	A_{2B}	A_3
1 Adenosine	**1**	H	H	–	–	–	0.31[a] [69]	0.73[a] [67]	23.5 [67]	0.29[a] [67]
2 NECA	**3**	H	H	–	Et	O	14 [65]	20 [65]	2.4 [63], 6.2 [72]	10.6 [62], 0.76 [71]
3 NCPCA	**3**	H	H	–	*c*-Pr	O	63[c] [62]	12[c] [62]	5.3 [70]	108 [62]
4 MECA	**3**	H	H	–	Me	O	1084[c] [62]	330[c] [62]	45 [62]	6.4 [62]
5	**3**	H	H	–	*c*-Bu	O	43[c] [62]	130[c] [62]	33 [62]	23 [62]
6	**3**	H	H	–	Et	S	141[c] [62]	130[c] [62]	16 [62]	569 [62]
7	**3**	H	H	–	*c*-Pr	S	137[c] [62]	130[c] [62]	13 [62]	2280 [62]
8 CPA	**1**	*c*-C_5H_9	H	–	–	–	2.2 [63]	794 [63]	18.6 [63]	43 [63]
9	**1**	Ph	H	–	–	–	30[c] [71]	5%[b] [71]	6.3 [71]	140 [71]
10 *R*-PIA	**1**	CH(Me)CH_2Ph	H	–	–	–	2.0 [63]	859 [63]	11.2 [63]	16.3 [63]
11 SPA	**1**	Ph-*p*-SO_3H	H	–	–	–	1400[c] [63]	4700[c] [63]	14 [63]	340 [63]
12	**1**	CH_2Ph	H	–	–	–	–	–	96 [73]	–
13	**1**	Ph-*p*-Cl	H	–	–	–	40[c] [71]	570[c] [71]	1.1 [71]	37 [71]
14	**1**	Ph-*p*-Br	H	–	–	–	18[c] [71]	480[c] [71]	1.3 [71]	56 [71]
15	**1**	Ph-*p*-I	H	–	–	–	20[c] [71]	710[c] [71]	0.37 [71]	31 [71]
16	**1**	Ph-*p*-COOH	H	–	–	–	270[c] [71]	2500[c] [71]	8.0 [71]	210 [71]
17	**1**	Ph-*p*-SO_2NH_2	H	–	–	–	30[c] [71]	50%[b,c] [71]	0.44 [74]	37 [71]
18	**1**	Ph-*p*-$CONH_2$	H	–	–	–	20[c] [71]	65%[b,c] [71]	1.4 [74]	84 [71]
19	**3**	Me	H	–	Et	O	–	–	19 [63]	–
20	**3**	Ph	H	–	Et	O	32[c] [71]	634[c] [71]	2.4 [71]	41 [71]
21	**3**	Ph-*p*-Cl	H	–	Et	O	30[c] [71]	183[c] [71]	0.73 [71]	64 [71]
22	**3**	Ph-*p*-I	H	–	Et	O	17[c] [71]	46%[b,c] [71]	1.9 [71]	7.6 [71]
23 2-ClAdo	**1**	H	Cl	–	–	–	9.3[c] [75]	63[c] [75]	24 [63], 8.9 [40]	1890[c] [75]
24 HEAdo	**2**	H	–	Bu	–	–	18 [20]	5.7 [20]	100 [20]	4.7[20]
25 (*R*) PHPAdo	**2**	H	–	CH(OH)Ph	–	–	0.44 [20]	29 [20]	6.2 [20]	5.0 [20]
26 (*S*) PHPAdo	**2**	H	–	CH(OH)Ph	–	–	0.67 [20]	1.8 [20]	0.92 [20]	1.4 [20]
27 (*R*,*S*)	**2**	Me	–	CH(OH)Ph	–	–	8.4 [20]	273 [20]	2.7 [20]	0.76 [20]
28 (*R*,*S*)	**2**	Et	–	CH(OH)Ph	–	–	2.7 [20]	94 [20]	1.7 [20]	0.97 [20]
29 (*R*,*S*)	**2**	*i*-Pr	–	CH(OH)Ph	–	–	1.5 [20]	96 [65]	6.1 [20]	2.3 [20]
30 HENECA	**4**	H	–	Bu	Et	O	60 [64]	6.4 [64]	6.1 [64]	2.4 [64]
31	**4**	H	–	4-thiazolyl	Et	O	12 [65]	84 [65]	4.6 [65]	3.7 [65]
32 (*R*) PHPNECA	**4**	H	–	CH(OH)Ph	Et	O	1.9 [65]	39 [65]	2.4 [20]	5.5 [65]
33 (*S*) PHPNECA	**4**	H	–	CH(OH)Ph	Et	O	2.1 [65]	2.0 [65]	0.22 [20]	0.75 [65]
34 (*R*,*S*)	**4**	H	–	C(OH)MePh	Et	O	9.4 [65]	56 [65]	2.3 [65]	2.4 [65]
35 (*R*,*S*)	**4**	H	–	CH_2CH(OH)Me	Et	O	40 [65]	14 [65]	13.3 [68]	4.1 [68]
36 (*R*,*S*)	**4**	H	–	CH(OH)Et	Et	O	4.1 [68]	3.1 [68]	1.3 [68]	1.0 [68]
37 (*R*,*S*) PHPMECA	**4**	H	–	CH(OH)Ph	Me	O	14 [68]	3.0 [68]	5.0 [68]	1.7 [68]
38	**1**		F	–	–	–	0.9 [17]	71.9 [17]	–	–

Table 2 (*Continued*)

No.	St	R^1	R^2	R^3	R^4	X	K_i (nM)		EC_{50} (μM)	K_i (nM)
							A_1	A_{2A}	A_{2B}	A_3
39	**1**		Cl	–	–	–	3.9 [17]	49.3 [17]	–	–
40	**1**		Br	–	–	–	7.0 [17]	26.9 [17]	–	–
41	**1**		I	–	–	–	21.8 [17]	11.1 [17]	–	–
42	**1**		F	–	–	–	0.6 [17]	10.1 [17]	–	–
43	**1**		Cl	–	–	–	2.2 [17]	10.7 [17]	–	–
44	**1**		I	–	–	–	49 [17]	6.4 [17]	–	–
45	**1**		F	–	–	–	1.3 [17]	19.4 [17]	–	–
46	**1**		Cl	–	–	–	4.4 [17]	101.5 [17]	–	–
47	**1**		Br	–	–	–	14.9 [17]	129.3 [17]	–	–

St: type of structure (**1**–**4**).
[a] EC_{50} values in μM.
[b] Percentage displacement of [^{3}H]CGS 21680 from rat striatal membranes at 1 mM concentration.
[c] Rat.

adenosine can interact with Lys152 and Glu167 both located in EL2 of the A_3 receptor, while in the case of the A_1 adenosine receptor both 3- and 5-nitrogen atoms of adenosine were not involved in ligand–receptor interactions.

The analysis of obtained results of the molecular docking of adenosine demonstrated that binding modes found for the ligand are in a good agreement with the available literature data and have confirmed a good quality of the modeled complexes as well as determined configurations of the putative receptors binding sites.

3.2.2. 5′-*N*-alkylcarboxamidoadenosines

In general, all interactions determined for adenosine were also conserved for studied 5′-*N*-alkylcarboxamidoadenosine derivatives (**2**–**7**) (Fig. 3). However, the hydroxyl group of Thr$^{3.36}$ was found to be hydrogen bonded with the NH-group of the ligand amide moiety, while the oxygen atom of this moiety interacted with either Thr277$^{7.42}$ of the A_1 receptor or corresponding serine residue of the A_{2A}, A_{2B}, and A_3 receptors. These interactions were not observed for the 5′-*N*-alkylthio-analogues and this is a possible reason for the experimentally detected decreasing of affinities of 5′-*N*-alkylthiocarboxamido derivatives. The molecular docking studies suggested that the *N*-alkyl chains of the ligands can be located inside the pocket formed by Ser$^{3.39}$, Asn$^{7.45}$, and Trp$^{6.48}$ conserved among all adenosine receptor subtypes (Fig. 4). Cys246$^{6.47}$ (conserved among A_2 subtypes) of the A_{2B} receptor is also involved in the formation of this pocket. Furthermore, in the models obtained for the A_1, A_{2B}, and A_3 receptors Phe$^{6.44}$ was found near the 5′-*N*-alkyl chains, while in the A_{2A} receptor this amino acid residue was located far from these alkyl groups. Additionally, it was observed that in the case of A_1, A_{2A} and A_{2B} receptors

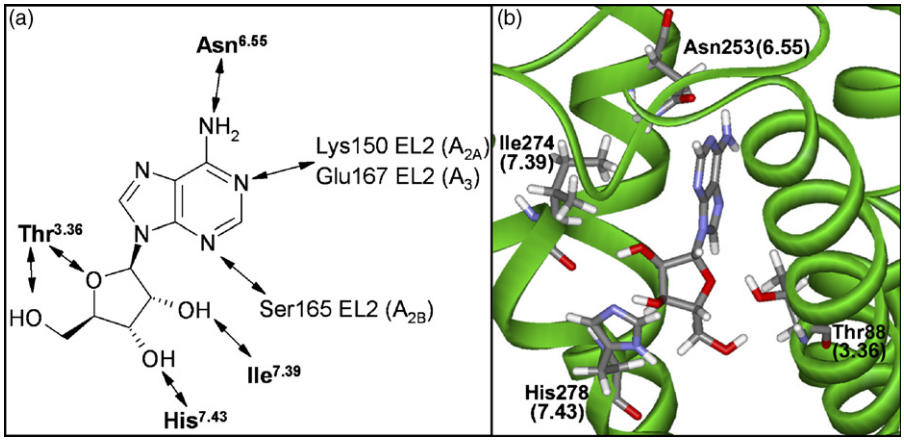


Fig. 2. (a) The putative binding mode of adenosine with adenosine receptors (↔, hydrogen bonds) and (b) the binding site of the A_{2A} adenosine receptor with adenosine.

Leu$^{3.35}$ is near the 5′-*N*-alkyl chains of the ligands. Interestingly, the A_3 receptor shows a phenylalanine residue at the 3.35-position. This particular feature of the A_3 adenosine receptor combined with the obtained binding modes allows us to propose an explanation of the highest affinity of MECA at this subtype in comparison to the other adenosine receptor subtypes. In the case of the A_3 receptor, shortening of the *N*-ethyl chain of NECA to the *N*-methyl (MECA) can reduce the unfavorable interactions between the ligand and the aromatic ring of Phe93$^{3.35}$ ($K_{i(NECA)}$ = 10.6 nM [62], $K_{i(MECA)}$ = 6.4 nM [62]). In contrast, such change of the alkyl chain produces the decreasing of the favorable hydrophobic interactions between the ligand and other three subtypes of receptors (for A_{2B} $EC_{50(NECA)}$ = 2.4 μM [63], $EC_{50(MECA)}$ = 45.0 μM [62]).

Also, obtained orientations of ligands inside the putative binding sites of the adenosine receptors suggested that, in general, the A_{2A} and A_{2B} subtypes have a smaller volume of the hydrophobic pocket than the A_1 and A_3 receptors. That is a possible reason for the decreased affinity of a 5′-*N*-cyclobutylcarboxamido derivative **5** for the A_{2B} receptor in comparison with the other ligands affinities for this receptor.

3.2.3. N^6-alkyl-, N^6-cycloalkyl-, and N^6-phenyl-substituted adenosine and NECA derivatives

The molecular docking of several adenosine analogs substituted at the N^6-position (ligands **8**–**18**) as well as N^6-substituted NECA derivatives (ligands **19**–**22**) was performed.

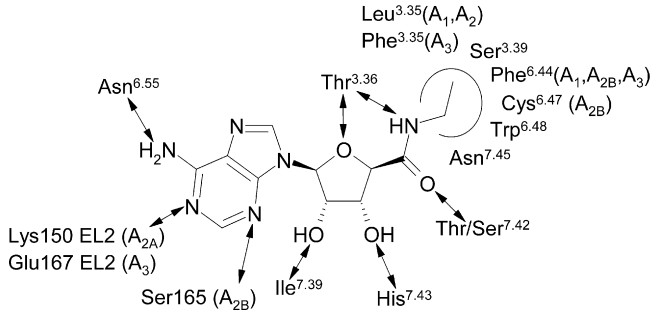


Fig. 3. The putative binding mode of 5′-*N*-alkylcarboxamidoadenosines: (↔) hydrogen bonds and (–) hydrophobic interactions.

The obtained binding modes of the ligands allowed us to propose that substituents at the N^6-position of the ligands are located inside the pocket formed by several amino acid residues. In particular, it was observed that Thr$^{3.36}$, Phe$^{5.43}$, Trp$^{6.48}$, and Asn$^{6.55}$ conserved among all four subtypes are arranged within 3 Å around the cyclopentyl ring of CPA (Fig. 5). In addition, Gln$^{3.37}$ of the A_1, A_{2A}, and A_{2B} receptors were located within 3 Å, while the corresponding His95$^{3.37}$ of the A_3 receptor was found within 4 Å from ligands. Furthermore, His$^{6.52}$ of the A_1, A_{2A}, and A_{2B} receptors as well as corresponding Ser247$^{6.52}$ of the A_3 subtype were also arranged near the N^6-position of the ligands.

Some differences in the binding modes of the N^6-substituted adenosine and NECA derivatives with different adenosine receptors were observed based on the results obtained. Leu$^{3.33}$ and Asn$^{5.42}$ of the A_1, A_{2A} and A_{2B} receptors were found to be involved in interactions with the N^6-substituent. In contrast, Leu88$^{3.33}$ of the A_3 receptor and Ser181$^{5.42}$ were located within 5 Å from the N^6-group of the ligands. Val138$^{4.56}$ of the A_1 receptor and corresponding Ile135$^{4.56}$ of the A_{2A} subtype were found within 3 Å from the cyclopentyl group of CPA. The Ile136$^{4.56}$ residue of the A_{2B} receptor was located within 4 Å, while the distance between the cyclopentyl group of CPA and Val141$^{4.56}$ of the A_3 adenosine receptor was 5 Å. The results of the molecular docking of the A_1 agonist *R*-PIA suggested that the methyl group of the phenylisopropyl moiety is arranged between Thr91$^{3.36}$ and Trp247$^{6.48}$ of the A_1 receptor, while Gln92$^{3.37}$, Val138$^{4.56}$, Asn184$^{5.42}$, and Phe185$^{5.43}$ are clustered around the ligand phenyl ring. As a result, π–π interactions between the phenyl ring of *R*-PIA and Phe185$^{5.43}$ are possible, but questionable. Based on the observed arrangement of *R*-PIA at the A_{2A} receptor-binding site some differences of its binding mode in comparison with the A_1 receptor were identified. Initially, we tried to place the ligand into the putative A_{2A} binding site similarly to its placement in the A_1 receptor. However, undesirable steric effects, resulting from the absence of sufficient free space to accommodate the ligand phenyl ring, were observed. The search for a more favorable location of *R*-PIA inside the A_{2A} binding site was performed with the assistance of an iterative molecular docking procedure. In the most favorable

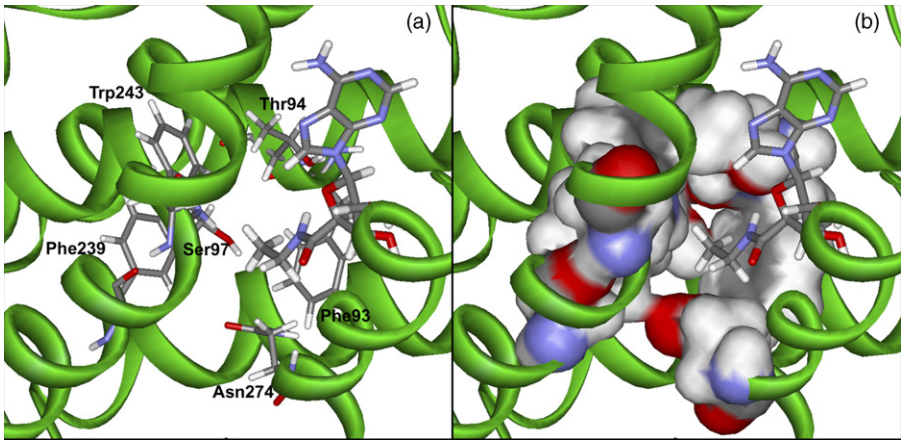


Fig. 4. (a) NECA inside the binding site of the A_3 adenosine receptor and (b) the arrangement of the ethyl group of NECA inside the binding site of the A_3 adenosine receptor represented as van der Waals surfaces.

position the phenyl ring of *R*-PIA was surrounded with Leu85$^{3.33}$, Ile135$^{4.56}$, Asn181$^{5.42}$, and Val186$^{5.47}$ of the A_{2A} receptor, while the methyl group of the ligand was oriented in proximity to Leu249$^{6.51}$. However, this most favorable arrangement of *R*-PIA inside the A_{2A} receptor has two serious disadvantages in comparison to the binding mode obtained for the A_1 subtype. Firstly, in the A_{2A} receptor the *R*-PIA methyl group was located near the carbonyl group of Asn253$^{6.56}$, which resulted in unfavorable interactions. Second, because of a large distance, Asn253$^{6.56}$ was unable to form the very important hydrogen bond with the N^6-amino group of the ligand. These results provide an explanation of the considerable difference between the K_i values of *R*-PIA determined for the A_1 (K_i = 2.0 nM [63]) and A_{2A} (K_i = 859 nM [63]) receptors.

The binding modes obtained for the agonists containing the phenyl ring at the N^6-position proposed that in general the phenyl ring of the ligand is surrounded with the same amino acid residues as alkyl groups of the N^6-alkyl-adenosine and NECA derivatives. For example, in the models obtained the *p*-halo-substituted phenyl groups of ligands **13**–**15**, **21** and **22** were surrounded by Thr91$^{3.36}$, Gln92$^{3.37}$, Val138$^{4.56}$, Asn184$^{5.42}$, Phe185$^{5.43}$, Val190$^{5.47}$, and His251$^{6.52}$ of the A_1 receptor. However, mechanisms of the ligand–receptor interactions obtained for the ligands containing functional groups capable of forming hydrogen bonds appeared to be different for different receptor subtypes. According to the generated models, the carbonyl group of the ligands **16** and **18** can interact with the A_1 receptor due to the hydrogen bonding with the amino group of Gln92$^{3.37}$, while the hydroxyl group of **16** and the amino group of **18** can form a hydrogen bond with the carbonyl group of Asn184$^{5.42}$. The similar binding modes were observed for ligands **11** and **17**, however, in that case the hydroxyl group of **11** (corresponding to the amino group of **17**) and one oxygen atom of the sulfo group of the ligand were involved in the binding with the receptor.

In contrast, hydrogen bonding between the sulfo group of **11** and the A_{2A} adenosine receptor was not detected. Moreover, the distances between S=O groups of the ligand and alkyl chains of Ile135$^{4.56}$ (2.0 Å) and Val136$^{5.47}$ (1.4 Å) were found to be very short. The observed differences in the binding modes of **11** with the A_1 and A_{2A} receptors can provide an explanation for the considerable difference in the ligand affinity for these receptors. On the other hand, according to the molecular modeling results, the hydroxyl group of compound **16** as well as the amino group of **18** was hydrogen bonded to the Asn181$^{5.42}$ of the A_{2A} subtype.

As follows from the molecular models obtained for the complexes of the A_{2B} receptor with N^6-substituted agonists, the

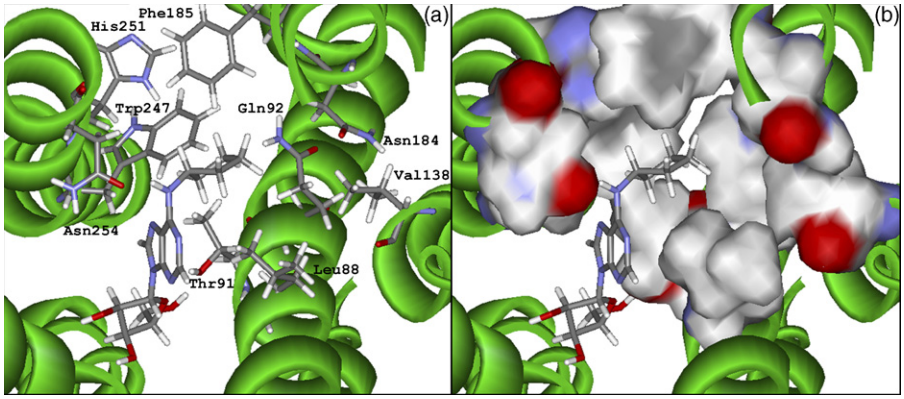


Fig. 5. (a) CPA inside the binding site of the A_1 adenosine receptor and (b) the cyclopentyl moiety of CPA inside the hydrophobic pocket of the binding site of the A_1 adenosine receptor.

amino group of Gln90$^{3.37}$ and the NH-group of the imidazole moiety of His251$^{6.52}$ are also involved in hydrogen bonding with the studied ligands.

A serious difference in the ligand-binding mode obtained for the A_3 receptor is the involvement of Lys152 located in EL2 in ligand–receptor interactions. As follows from the molecular models, Lys152 can form a hydrogen bond with the hydroxyl group of **16** and **18** as well as with one of the oxygen atoms of the **11** and **17** sulfo groups. Furthermore, His95$^{3.37}$ (located at the site of Gln$^{3.37}$ in other subtypes) was found to be hydrogen bonded to the hydroxyl group of the ligands, while Ser247$^{6.52}$, corresponding to His$^{6.52}$ of A_1 and A_2 receptors, can interact with the oxygen atom of carbonyl groups of **16** and **18** and with the sulfur atoms of **11** and **17**. Thus, three additional hydrogen bonds between a ligand and the A_3 receptor can be formed, while at the A_1 subtypes only two hydrogen bonds were observed. Moreover, the hydrophobic part of Lys152 of the A_3 receptor can provide additional favorable interactions with the ligand's phenyl ring, which could be the reason of the higher affinity of the agonist at the A_3 receptor than at the A_1.

3.2.4. N^6-norbornenyl, N^6-norbornyl, and N^6-epoxynorbornyl-substituted derivatives of adenosine

Adenosine derivatives containing norbornenyl, norbornyl, and epoxynorbornyl moieties at the N^6-position were recently proposed as effective A_1 receptor agonists [17]. Here we describe the molecular modeling results obtained for several agonists (**38**–**47**) of this type. The experimental pK_i values of these ligands have been published for A_1 and A_{2A} receptors only. For this reason, in this study we also focused on these two subtypes of adenosine receptors. The results of the molecular docking of these ligands to the A_1 receptor suggest that in the most favorable position the bicyclic fragment of the agonists was surrounded within 3 Å by Thr91$^{3.36}$, Gln92$^{3.37}$, Phe185$^{5.43}$, Val189$^{5.47}$, Trp247$^{6.48}$, Leu250$^{6.51}$, and His251$^{6.52}$. Unfavorable interactions between amino acid residues and the bulky fragment of the ligand were not observed. Moreover, the oxygen atom of the epoxy group of agonists **45**–**47** was located near His251$^{6.52}$ and could form a hydrogen bond with this residue (Fig. 6). In contrast, in the A_{2A} receptor arrangement of the bicyclic fragment of the agonists was considerably different in comparison to the A_1 receptor. The double bond of the norbornenyl-substituted derivatives was found to be oriented towards Leu250$^{6.51}$ and His251$^{6.52}$ of the A_1 receptor, while in the case of the A_{2A} subtype, a similar orientation of this bond is not desirable due to the overlapping with the amino acid residues mentioned above. For this reason, the bicyclic groups of the ligands inside the A_{2A} receptor-binding site were oriented on the opposite side, namely, toward Glu163 (EL2) and Asn181$^{5.42}$. Furthermore, the amino group of Asn181$^{5.42}$ appeared at a distance of 3.7 Å from the oxygen atom of the epoxy group of ligands **45**–**47**, however, the hydrogen bond between Asn181$^{5.42}$ and this oxygen atom was not observed. These findings can provide a possible explanation for the higher affinity of these agonists at the A_1 receptor in comparison to the A_{2A} receptor.

3.2.5. 2-Substituted derivatives of the adenosine and NECA

Adenosine and NECA derivatives containing various alkynyl substituents at the 2-position of the adenine ring were recently proposed as effective agonists for the adenosine receptors [20,64–68]. With the purpose of understanding the reasons of specific ligand–receptor interactions for such agonists we have performed a molecular docking of ligands **24**–**37** to each adenosine receptor subtype.

It was observed that the substituent at the 2-position of the ligands is surrounded by amino acid residues located not only in the transmembarane domain of the receptors, but also in EL1 and EL2. In particular, the hexynyl fragment of HENECA was found to be surrounded with Val$_{A1}$/Ile$_{A2A}$/Leu$_{A2B}$$^{3.28}$, Ala$^{3.29}$, Val$^{3.32}$ and Leu$^{3.33}$ of the A_1 and A_2 receptors, while in case of the A_3 subtype only Thr87$^{3.29}$ could interact with the alkynyl group of the agonists. On the other hand, in contrast to the A_1 and A_2 subtypes, Ile268$^{7.39}$ and Lys287$^{7.58}$ of the A_3 receptor were involved in ligand–receptor interactions. Also, Lys168 of the A_1 receptor, Gly158 of the A_{2A} as well as Ser165 of the A_{2B} and A_3 receptors, all located in EL2, seem to be involved in ligand recognition via hydrophobic interactions. Interestingly, according to the models obtained for each receptor subtype, both cysteine residues involved in the disulfide bridge formation were located in proximity to the alkynyl moiety of the ligands. Furthermore, the cysteine residue located in EL2 could be hydrogen bonded to the hydroxyl group of the hydroxypropynyl derivatives.

The analysis of the binding modes of the thiazolyl derivative **31** demonstrated the absence of additional hydrogen bonds between the thiazolyl group of the ligand and any amino acid residue of the receptors. The most favorable arrangement of this group in **31** was found in the ligand–receptor complexes obtained for the A_{2B} and A_3 receptors. In addition to the amino acid residues mentioned above, three cysteine residues of the A_{2B} receptor (Cys78 (EL1), Cys154 (EL2), and Cys166 (EL2))

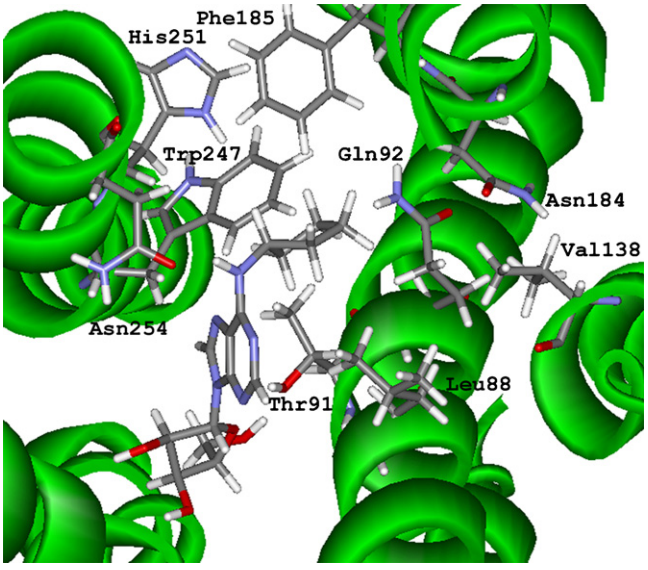


Fig. 6. Ligand **46** inside the binding site of the A_1 adenosine receptor.

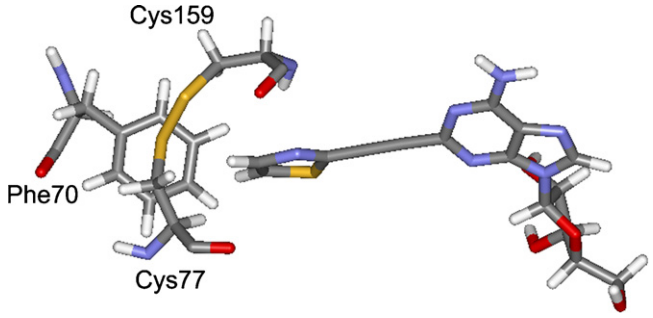


Fig. 7. The arrangement of the thiazolyl moiety of ligand **31** inside the A_{2A} receptor-binding site.

were located near the thiazolyl group and could form a pocket suitable for the placement of this group. In the case of the A_1 receptor, Cys80 (EL1), Asn148, Leu149, Cys169, and Glu170 located in EL2 could interact with the thiazolyl moiety; however, Asn148 appeared near the double bond of the thiazolyl ring and provided some unfavorable interactions with the ligand. The most undesirable arrangement of the compound **31** was obtained in its complex with the A_{2A} receptor due to the presence of Phe70 (EL1) (Fig. 7). In the model, the Phe70 residue was located under the thiazolyl ring of the ligand, which resulted in the lifting of this thiazolyl group. For this reason the CH-groups of the double bond of the ring became unfavorably located between the carbonyl groups of Cys159 and Cys77. These findings are in good agreement with the experimental data. As shown in Table 2 the highest value of K_i for **31** corresponds to the A_{2A} adenosine receptor.

The different binding modes of optical isomers of the 2-hydroxypropynyl-substituted agonists were compared. The hydroxyl group of (*S*)-PHPNECA was found to be arranged between the hydrophilic groups of Lys168, Cys169, and Glu170 located in EL2 of the A_1 receptor. In the case of the (*R*)-isomer, this hydroxyl group was found at a distance of 4 Å from and oriented toward Leu88$^{3.33}$, Ala84$^{3.29}$, and Cys80 (EL1). However, the existence of additional hydrogen bonds between phenylhydroxypropynyl group of PHPNECA and the A_1 receptor was not observed for either (*R*)- or (*S*)-isomer, and no undesirable interactions were detected for this ligand–receptor pair. This arrangement of PHPNECA inside the A_1 receptor can explain almost the identical activity experimentally determined for both (*S*)- and (*R*)-isomers.

The binding modes of PHPNECA obtained for the A_{2A}, A_{2B}, and A_3 receptors (Fig. 8) demonstrated that the hydroxyl group of the (*S*)-phenylhydroxypropynyl fragment could be hydrogen bonded to the cysteine residue, which is located in EL2 and forms the disulfide bridge. Moreover, Gly160 (EL2) of the A_{2A} receptor and Gln167 (EL2) of the A_3 receptor were located at a distance of 3 Å from this hydroxyl group and also can be involved in the hydrogen bonding. In contrast with (*S*)-PHPNECA, the hydroxyl group of the (*R*)-phenylhydroxypropynyl fragment was surrounded by the hydrophobic moieties of Leu$^{3.33}$ and Ala$^{3.29}$ of the A_{2A} and A_{2B} receptors. In the case of the A_3 subtype, the distances between the hydroxyl group of the (*R*)-isomer and Ser165, Cys166, and Gln167 located in EL2 were less than

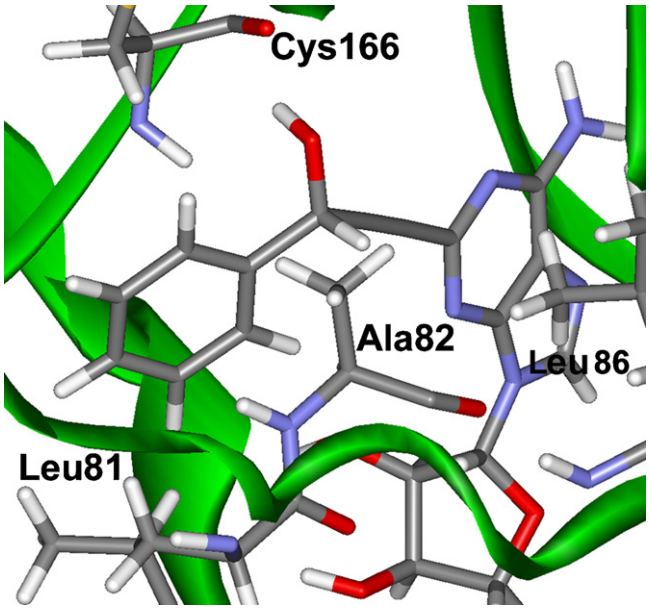


Fig. 8. The putative binding mode of (*S*)-PHPNECA with the A_{2B} adenosine receptor.

2 Å, thus resulting in unfavorable ligand–receptor interactions and in an increased K_i value.

Unfortunately, the experimental K_i values measured for ligands **34** and **35** have been published only for the isomeric mixtures. It makes it difficult to evaluate the proposed binding modes of these agonists. However, the results of the molecular docking performed for both (*R*)- and (*S*)-isomers of **34** and **35** allow us to draw the following conclusions. At each subtype of the adenosine receptors the hydroxyl group of the (*R*)-phenylhydroxymethyl substituent of **34** was found to be hydrogen bonded to the highly conserved cysteine residue located in EL2, while the methyl substituent was located inside the hydrophobic pocket formed, in the case of A_1 receptor, by Ala84$^{3.29}$, Val87$^{3.32}$, and Leu88$^{3.33}$. The phenyl ring of the ligand was surrounded with Cys80 (EL1), Val83$^{3.28}$, and Ala84$^{3.29}$. Phe70/71 residue located in EL2 of the A_{2A}/A_{2B} receptors also appeared near the phenyl ring of the ligand. However, according to the results obtained the involvement of the phenylalanine residue in π–π interactions with the ligand phenyl ring seems to be unlikely for both subtypes. In contrast with (*R*)-**34**, the arrangement of the (*S*)-phenylhydroxymethyl moiety of the ligand inside the putative binding site of receptors was less favorable. In this case, the hydroxyl group was surrounded by hydrophobic groups of non-polar amino acid residues. Moreover, there was a short distance (1.5 Å for A_{2B} receptor) between the carbonyl group of the cysteine residue and the methyl group of the ligand. Based on these findings, it can be proposed that (*R*)-isomers of the 2-phenylhydroxymethylpropynyl derivatives of adenosine and NECA could be more effective than the corresponding (*S*)-isomers.

The binding modes derived for **35** allowed us to conclude that ligand–receptor interactions for this ligand can be similar to the interactions of **34**. However, the binding modes of (*S*)-**35** correspond to the binding modes of (*R*)-**34**. In particular, the hydroxyl group of the pentynyl fragment of (*S*)-**35** was

hydrogen bonded to the corresponding cysteine residue, while the methyl group was located inside the hydrophobic pocket. In contrast, the hydroxyl group of the (*R*)-isomer of **35** was surrounded by hydrophobic residues, while its methyl group was located near the carbonyl group of the cysteine residue. These results make it possible to propose that the (*S*)-isomers of 2-(4-hydroxy-1-pentynyl)-substituted derivatives of adenosine and NECA could be more effective than their (*R*)-analogs.

In conclusion, the molecular models of the complete structure of each known subtype of the adenosine receptors were built and described in this study. Using these models computational molecular docking of the native agonist adenosine and 46 adenosine derivatives was performed. It should be mentioned here, that molecular models and ligand–receptor complexes for each receptor subtype in this study were built using one common protocol so that they could be easily compared. The comparison of the binding modes as well as configurations of the binding site obtained for each receptor–ligand complex allowed us to characterize a general agonist-binding mode. Also, based on the results obtained, possible explanations for the differences in agonist activities were described. We hope that these findings can be applied to a better understanding of the ligand–receptor interactions as well as for the further design of new, effective and selective adenosine receptor agonists.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmgm.2006.06.004.

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