Molecular modeling approaches for the discovery of adenosine A_{2B} receptor antagonists: current status and future perspectives

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Adenosine receptors (ARs) are classified as A_1, A_{2A}, A_{2B}, and A_3 subtypes belonging to the superfamily of G-protein-coupled receptors (GPCRs). Several molecular modeling approaches have been developed for A_{2B}AR and its antagonists, from the construction of a homology model, molecular docking, molecular dynamics (MD) simulations, and 3D quantitative structure–activity relationship (QSAR) modeling to pharmacophore modeling, each of which has different objectives and outcomes. In this review, we provide a systematic outline of advances in molecular modeling approaches towards A_{2B}AR for deducing its structure and interactions with various types of antagonist. The information, methods and perspectives presented here provides impetus for medicinal chemists to discover potential ligands that can bind selectively with higher affinity to A_{2B}AR.

Introduction

GPCRs are the largest single class of cell membrane proteins encoded in the human genome and also are validated as potential drug targets [1,2]. Most clinical GPCR drugs administered currently exert their effects by modulating GPCR signaling pathways [3]. ARs belong to the purinergic class of GPCRs and are classified as A_1, A_{2A}, A_{2B}, and A_3 subtypes, all of which are activated by an endogenous ligand, adenosine [4]. Among the ARs, A_{2B}AR is the least characterized, primarily because of the lack of suitable and specific ligands [5]. A_{2B} receptor signaling is associated with enhancing tissue adaptations to hypoxia and ischemia [6]. By contrast, selective A_{2B} antagonists decrease inflammation and are promising candidates for the management of chronic obstructive pulmonary disease (COPD), asthma, and cancer [5,7,8]. Moreover, inhibition of A_{2B}AR might be useful in the treatment of cardiac fibrosis, bone homeostasis modulation, and diabetes [9,10]. Thus, there has been increased interest in the search for novel ligands, particularly as antagonists of A_{2B}AR, for the development of potential therapeutic agents.

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Continuous advancements in computational techniques have rendered both structure-based and ligand-based computer-aided drug design (CADD) strategies reliable approaches to gain insight into the physicochemical requirements of ligand–receptor interactions for the design and discovery of novel drugs [11,12]. Ligand-based drug design (LBDD) is based mainly on 2D or 3D QSAR [13] and pharmacophore modeling [14] studies; whereas structure-based drug design (SBDD) is mainly dependent on molecular docking [15] and MD simulations [11,16] studies. The structure of target proteins (i.e., GPCRs) required for SBDD can be obtained from either the Protein Data Bank (PDB) as 3D X-ray crystal structures or can be constructed by using appropriate homologous protein templates via homology modeling [17]. Modern approaches of drug design utilize both SBDD and LBDD techniques either individually or in combination [12,18]. An appropriate hand-to-hand protocol of molecular modeling (virtual and dry) followed by an experimental verification (actual and wet) can provide crucial information facilitating *de novo* drug design and development [19]. In this review, we highlight the various important ligand- and structure-based CADD approaches adopted in the discovery and design of A_{2B}AR antagonists.

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Structural insights into A_{2B}AR

A_{2B}AR comprises 332 amino acids (molecular mass 36–37 KDa) and belongs to class A of the GPCRs, spanning seven transmembrane (TM) domains, each with an α-helix comprising 21–28 amino acids. The N-terminal amino acids of A_{2B}AR orients toward the extracellular region, whereas the C-terminal amino acids are present on the intracellular cytoplasmic region [20]. The 3D arrangement of the α-helical TM domains generates a pocket for the ligand-binding site. Three extracellular loops (ELs) and three cytoplasmic hydrophilic intracellular loops (ILs) of unequal size are coupled together in TM domains. *N*-linked glycosylation occurs on the second EL, which stabilizes the conformation, protects from proteases, and modulates the specific function of protein. TM regions with homology or structural similarity with TM2, TM3, and TM5 are conserved with respect to elongated amino acid residues. Most of the variations in amino acid sequences have been detected in the hypervariable region of the second EL [21].

A_{2B}AR amino acid sequence identity is similar across all the species, from 85% (mouse and human) to 95% (mouse and rat) [22]. Most of the differences occur in the second EL and in the intracellular C terminus. By contrast, the sequence of the TM domains differs only in one (TM1–5) or two (TM6–7) amino acids. The TM domains of hA_{2B}AR are also similar to the pharmacologically different A_{2A}AR subtype. The amino acid identity of which ranges from 58% (TM1) to 87% (TM3), assuming homology between the four aliphatic amino acids (Ala, Val, Leu, and Ile) could be from 80% (TM7) to 100% (TM3) for A_2AR subtypes [23]. In all AR subtypes, two histidine residues are conserved that are considered to have an important role in ligand recognition, with the exception of A_3AR (deficient in histidine) [24]. The development of molecular models for A_{2B}AR and its ligands are discussed here.

Structure-based approaches in A_{2B}AR modeling

In 2002, Ivanov *et al.* reported [25] the first homology model of human A_{2B}AR by using the X-ray structure of bovine rhodopsin (PDB: 1F88) [26]. Their results indicated the significance of the modeling intracellular hydrophilic loops and consequent disulfide bond development between two cysteine residues that are conserved in all receptors and are homologous with rhodopsin. The disulfide linkage, which emerges from cysteine residues (EL1 and EL2), could be indispensable for stabilization of the restricted number of conformations of many GPCRs. The authors further validated the stereochemical quality of the constructed homology model by conducting molecular docking of adenosine (**1**) (Fig. 1a) as a nonselective and native agonist. The binding modes of the agonist **2** (NECA) and antagonist **3** (DPSPX) on the modeled receptor revealed that nonconservative amino acids (Ile239 and Val250) have a crucial role in the binding of selective ligands to A_{2B}AR [25].

In 2003, Pastorin *et al.* developed an improved homology model of human A_{2B}AR [27] based on the X-ray crystal structure of bovine rhodopsin (PDB: 1F88) [28] and optimized based on an earlier report by Moro *et al.* [29]. Quantum mechanical (QM) calculations followed by docking of ligands were performed in the hypothetical binding site of A_{2B}AR. These authors highlighted the importance of bulky substituents at the N^8 position, and acetyl group at N^8 of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines in inducing potential selective antagonism towards hA_{2B}AR. Their study also revealed that interactions between ligands and receptors are mainly lipophilic, occurring inside a deep cleft near to the TM3, TM5, and TM6 region of the hA_{2B}AR.

In 2005, Ivanov *et al.* [30] constructed an A_{2B}AR homology model and investigated the possible binding modes of seven structurally diverse xanthine-based A_{2B}AR antagonists (Fig. 1a) by molecular docking followed by MD simulations using phospholipid (POPC) bilayer solvated by water molecules. The MD simulation facilitated the construction of a more accurate model with better insights into A_{2B}AR compared with their initially reported model [25]. Their study indicated that amino acid residues His251, Asn282, Ser92, and Thr89 are crucial in ligand recognition and subsequent H-bonding interactions. The amino acid residues of the EL2 hydrophilic loop were deemed to be indispensable for binding with bulky ligands, whereas His251 was important as a H-bond donor for ligand recognition in all AR subtypes. Aromatic residues (Phe243 and Trp247 located in TM4 and Phe187 located in EL2) could be important for the recognition of potential ligands by forming π–π interactions, whereas *n*-propyl group in the ligands were necessary for binding because they showed hydrophobic interactions [30].

Ivanov *et al.* reported a computer-assisted comparative study of AR agonist binding modes towards all ARs, including A_{2B}AR [31]. Initially, the authors constructed homology models for all AR subtypes using bovine rhodopsin as a template. The optimized model exhibiting the best probability density function (PDF) and the highest parameters was subjected for further docking studies of AR agonists. The binding mode of adenosine in A_{2B}AR subtype suggested that Ser165 located in EL2 established a H-bond interaction with the *N*-atom of adenosine at the 3-position. The binding orientation of (*S*)-PHPNECA derived for all ARs (except A_1AR) verified that the secondary hydroxyl group of the (*S*)-phenyl-hydroxypropynyl side chain was involved in H-bonding with a cysteine residue positioned in EL2 (particularly with Cys166 of A_{2B}AR).

Many molecular models of hA_{2B}AR have been proposed, all of which were generated by using comparative (homology) modeling techniques by considering the bovine rhodopsin X-ray structure as a suitable template protein. Given that the sequence identity between rhodopsin and hA_{2B}AR is only ~20%, various protocols have been used to refine the modeled proteins. Given this constraint, the rhodopsin-based A_{2B} homology models were inadequate for further experimental verification for establishing ligand–receptor interactions [32].

In 2008, Ivanov *et al.* reported the binding mode predictions of various derivatives of adenosine and its 5′-*N*-methyluronamides bearing substituents at the N^6 and 2-positions [33]. The molecular docking results indicated that the N^6-amino moiety of adenosine agonists was positioned towards TM5, whereas the adenine ring (2-position) was located in EL2 of the protein. The secondary hydroxyl groups (2′- and 3′-position) of the nucleosides showed H-bond interactions with conserved amino acid residues (His280 and Thr89). Furthermore, the same authors constructed 3D-QSAR (CoMFA and CoMSIA) models based on the docked conformations ($R^2 = 0.960$, $Q^2 = 0.676$, F=158, SEE = 0.175, and $R^2_{test} = 0.782$) [33]. Based on the combined structure- and ligand-based molecular modeling studies, they successfully designed and synthesized four novel derivatives of adenosine that were found to be potent and full agonists of hA_{2B}AR (174–965 nM), validating their use in *in silico* drug design approach. The authors concluded that the ligands can

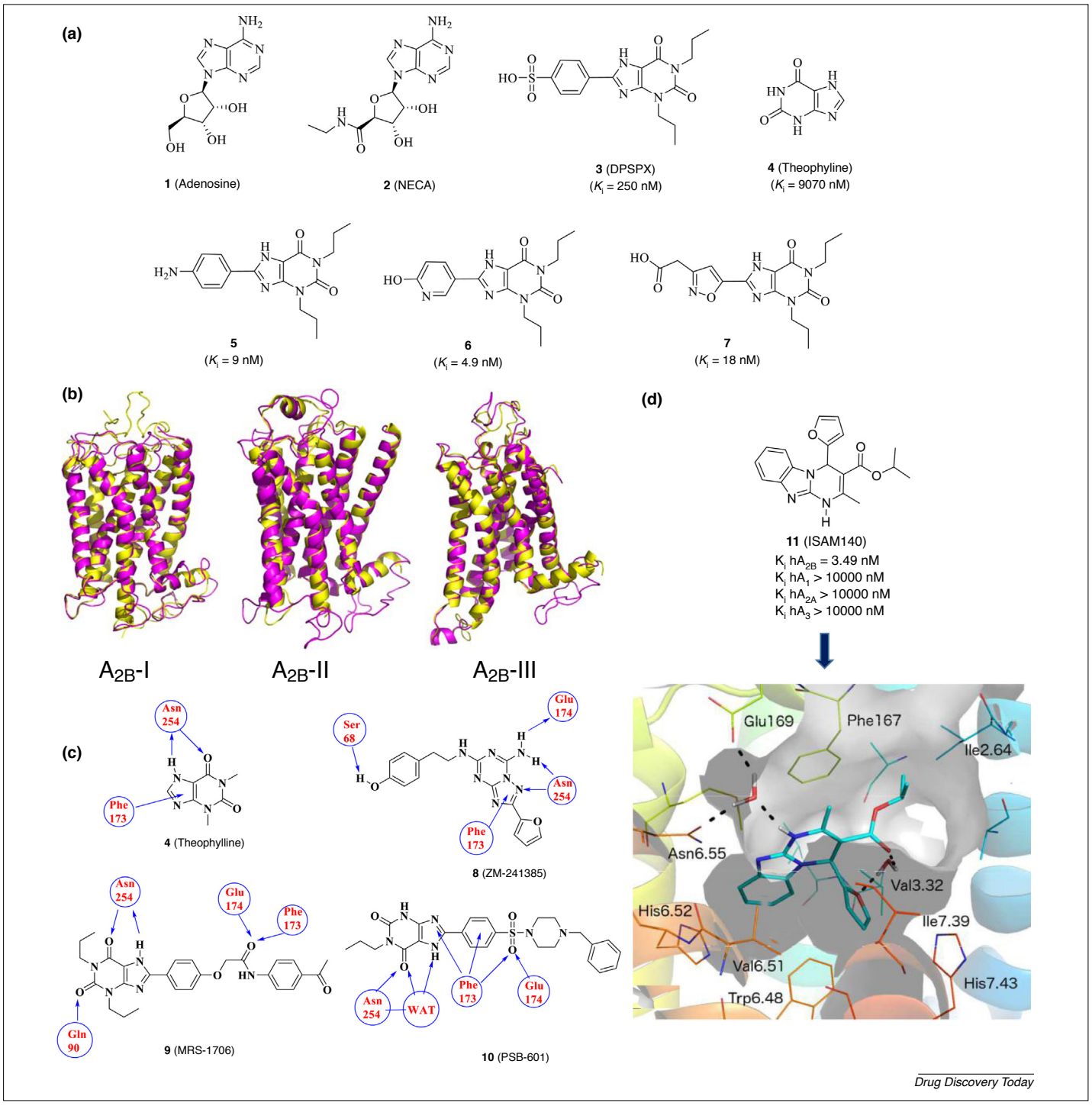

FIG. 1

Structure-based design of xanthine and non-xanthine derivatives for A_{2B} AR. **(a)** Structures of adenosine and xanthine-based derivatives. **(b)** Homology models of human adenosine receptor A_{2B}AR (magenta) along with their templates (yellow): model A_{2B}-I [template bovine rhodopsin, Protein Data Bank (PDB): 1U19), model A_{2B}-II (template β_2-adrenergic receptor, PDB: 2RH1), model A_{2B}-III (template A_{2A} AR, PDB: 3EML). **(c)** The binding orientation and interaction of theophylline in model **A_{2B}-I**, MRS-1706 and PSB-601in model **A_{2B}-II**, and ZM-241385 in model **A_{2B}-III**. **(d)** The binding orientation and interaction of **11** (ISAM140) inside A_{2B}AR. Adapted, with permission, from Refs [37] (b,c) and [46] (d).

bind effectively into the nucleoside-binding site located in the TM region of the receptor model. The validated QSAR models further supported the orientation of the N^6-amino group and substituents at the 2-position of adenine toward the TM5 and EL2 regions, respectively. In 2008, Martinelli and Tuccinardi reviewed the main experimental data and computational homology modeling procedures concerning the construction of the 3D structures of all AR subtypes, along with the relevant validation methods [34].

The discovery of the X-ray crystal structure of A_{2A}AR (PDB: 3EML) [35] has rendered it as the most suitable and reliable template for the construction of homology models of A_{2B}AR, because of its improved sequence homology (56%). Ivanov *et al.*

[36] compared the model quality of the 3D X-ray crystal structure of A_{2A}AR (PDB: 3EML) and their in-house-constructed rhodopsin-based homology model of A_{2A}AR (PDB: 1UPE) and concluded that both yielded similar results when they were used for agonist docking experiments. The authors established computationally that the superimposition of Cα-atoms of certain amino acid residues in the ligand-binding pocket afforded a root-mean-square deviation (RMSD) value of 0.90 Å. At the same time, the proximity of conserved amino acids between Glu13 (TM1) and His278 (TM7) was established and identified to be in agreement with the reported X-ray crystal structure. This study also demonstrated the formation of two prominent H-bond interactions between the carbonyl group (C=O) of Ser281 with Asn24 (amino group) and Asp52 (hydroxyl group), which were found to be in accordance with the previous model predicted by these authors. To support these research findings, the authors studied the significance of side-chain flexibility in the ligand-binding site by using the Induced Fit docking (IFD) protocol of the Schrodinger package. Their study demonstrated homology modeling techniques for GPCRs to be reliable and useful approaches for predicting the binding orientation for ligands in the active site of the target protein as well as for solving various protein structures.

In 2009, Sherbiny *et al*. [37] developed three human A_{2B}AR homology models (I–III) (Fig. 1b), based on three different crystal structures as protein templates: bacteriorhodopsin (PDB: 1U19) [38], human β_2-adrenergic receptor (PDB: 2RH1) [39] and hA_{2A}AR (PDB: 3EML) [35]. The modeling validation showed consistent matching of the modeling output with results reported through pharmacological characterization and biochemical experiments (ligand-binding assays and site-directed mutagenesis). In the generation of an acceptable ligand-binding site for a modeled protein, a sequence similarity of 30% is generally considered a minimum requirement in most comparative modeling procedures [40]. The sequence similarity of hA_{2B}AR with other GPCR templates, such as bovine rhodopsin and β_2-adrenergic receptor, are reported to be 23% and 31%, respectively. Thus, a 56% sequence similarity between hA_{2A} and A_{2B} ARs explains the advantage of hA_{2B}AR homology model derived from the 3D-crystal structure of hA_{2A}AR as a template.

An automated docking study was carried out for various ligands into the active-site of A_{2A} and A_{2B} ARs followed by successive MD simulations [41]. Quantitatively, all of the derived models performed equally well in terms of predicting the binding affinities, but differed in predicting the binding modes qualitatively [37]. The results indicated Model A_{2B}-III to be the best model, exhibiting high sequence similarity (56%) with an ideal gap ratio and low RMSD compared with A_{2A}AR; subsequently, the computational results were also found to be in agreement with the experimental reports [30]. The predicted binding modes and interactions of the studied ligands with active-site amino acids are presented in Fig. 1c.

By utilizing an optimized and reliable model (A_{2B}-III), the authors predicted the binding modes of different ligands, including antagonists, and identified that amino acid residues in the active site, such as Leu86, Trp247, Asn254, Ile276, and His280, have crucial role in ligand binding [37]. They also proposed a general ligand-binding site and recommended a research focus on the nonconserved amino acid residues (Leu81, Val250, Val256, Asn266, Lys267, Lys269, Ala271, and Asn273), which might be crucial for the recognition of potential ligands as well as AR subtype selectivity [37].

In 2011, Rodríguez *et al*. explored key structural elements of hA_2ARs (hA_{2A}AR and hA_{2B}AR) by MD simulation techniques utilizing the 3D X-ray structure of hA_{2A}AR for characterization of the dormant conformation of hA_2ARs. This study involved the initial generation of hA_{2B}AR homology models followed by MD simulations of apo-forms of A_2ARs. The simulation results explained the significance of the conserved amino acid residues of ARs with conformational equilibrium of receptors. In particular, the combined rotameric transition of an aromatic amino acid Trp6.48 ('toggle switch') and concerted rotation of His6.52 (conserved amino acid) initiated the activation of the GPCRs together with stabilization of the new conformation by an interaction network involving Gln3.37 (TM3) and Asn5.42 (TM5) residues. Among all ARs, weaker interactions were observed between conserved residues (i.e., His7.43 and Glu1.39), which had an impact on the dynamics and structure of the protein. Thus, the authors examined this interaction and concluded that the presence of specific protein residues in ARs influences its conformational equilibrium and stability [42]. Later, this homology model (hA_{2B}AR) was implemented by Crespo *et al*. to gain insights into the binding interactions of a novel series of 3,4-dihydropyrimidin-2(1*H*)-ones as potent and selective antagonists of hA_{2B}AR [43]. This *in silico* study revealed the important role of $Val250^{6.51}$ for selective interactions with antagonists at the TM region of hA_{2B}AR.

In 2012, Mansourian *et al*. characterized A_{2B}AR in its native environment by conducting homology modeling using hA_{2A}AR (PDB: 3EML) as a template, followed by molecular docking and MD simulation experiments. The major aspect of their work described the influence of the lipid bilayer on A_{2B}AR structure and subsequent post-translational modifications (PTMs) on its folding. EL2 residues, such as Glu174 and Phe173, exhibited H-bonding and π-π stacking interactions, whereas Asn254 was required for the formation of an additional H-bond. In particular, Glu174 and Phe173 in EL2 shifted slightly away from the bilayer toward the aqueous phase. The authors showed that Asn254 and Thr257 in TM6 moved toward the lipid–water interface, which demonstrated that the conformations of EL2 in the unmodified structure are not well optimized to accommodate a ligand. Furthermore, the authors suggested the association of the membrane–water interface with EL2 and the adjacent (Asn-X-Ser/Thr) sequence motif of A_{2B}AR places stress and/or strain on the protein, and concluded that MD models can serve as a platform for structure–function studies on A_{2B}AR and other homologous GPCRs [44].

In 2013, Thimm *et al*. [45] studied the implication of ligand binding on the activation of hA_{2B}AR and revealed differences between A_{2B} and A_{2A} ARs. Initially, the authors developed three different homology models of A_{2B}AR using different X-ray crystal structures of human A_{2A}AR templates (PDB: 3EML, 3QAK, and 2YDV). For the docking of antagonists, an inactive model (PDB: 3EML) was used, as well as to predict mutagenesis studies. For agonist docking, the authors used two A_{2B}AR homology models, constructed based on 2YDV and 3QAK, respectively. Their results suggested that A_{2B}AR had only one disulfide bond in the EL region, whereas A_{2A}AR exhibited four disulfide linkages (in the EL region), suggested that the amino acid residues Trp247, Val250, and Ser279

mediated ligand binding by forming different types of direct interaction. The significance of various crucial amino acids in ligand recognition and their interaction is shown in Fig. 2a. Furthermore, the authors deduced that Ser279 is vital for specific nucleoside binding, whereas Asn186 is necessary for the binding of xanthine antagonists. Thus, these results contributed significantly to our understanding of GPCR function and to the design of highly selective and potential ligands for A_{2B}AR.

To translate the molecular interaction between A_{2B}AR and the C terminus of α-subunits (G-protein), Liu *et al.* reported a one-GPCR–one-G-protein yeast screening methodology in conjunction with mutagenesis experiments [46]. The authors selected

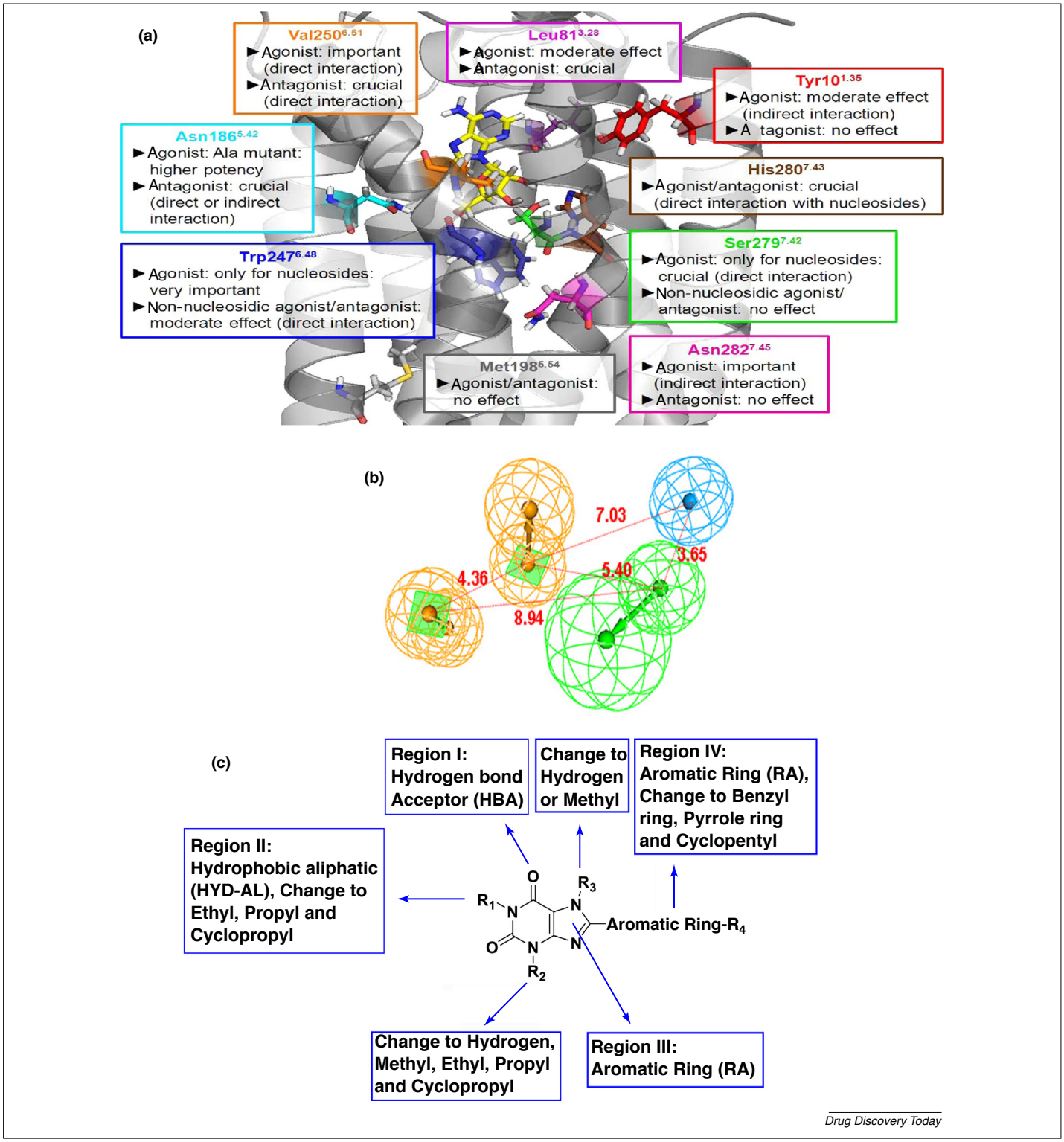

FIG. 2

Pharmacophore model of A_{2B} AR. **(a)** Effects of crucial amino acids in human adenosine receptor A_{2B}AR. **(b)** Best pharmacophore model of Hypo1 [HBA (green), RA (orange), and HYD-AL (cyan)]. **(c)** Proposed modifications of the xanthine moiety. Adapted, with permission, from Refs [45] (a) and [63] (b,c).

A_{2B}AR as a hypothesis and class-A GPCR exhibiting indiscriminate performance in G-protein coupling (yeast system).

The developed model predicted 16 amino acids, of which the authors selected five (Leu236$^{6.37}$, Ser235$^{6.36}$, Leu213^{IL3}, Ilu107$^{3.54}$, and Arg103$^{3.50}$) for mutation experiments and converted them to alanine. The authors also studied the G-protein-coupling contours of mutated A_{2B}ARs and reported that the C terminus tyrosine of the G_{α}-subunit is essential for GPCR activation. The authors concluded that the selective coupling of the receptor with G-protein could contribute in deriving a clear facet on GPCR signaling [46].

El Maatougui *et al*. utilized the previously developed homology model of hA_{2B}AR [42] and performed ligand–receptor docking and MD studies based on their earlier reported *in silico* protocol [43], to demonstrate the antagonistic activity of novel non-xanthine 3,4-dihydropyrimidin-2(1*H*)-ones [47]. One of the compounds, ISAM140 (**11**), showed remarkable potency and selectivity for hA_{2B}AR (K_i hA_{2B} = 3.49 nM, K_i hA_1 > 10 000 nM, K_i hA_{2A} > 10 000 nM, K_i hA_3 > 10 000 nM), which is now available on the market (SML1902, www.sigmaaldrich.com) for further exploration. The binding mode of ISAM140 with hA_{2B}AR is depicted in Fig. 1d. Molecular docking was conducted with GOLD software by using Chemscore as the scoring function, while keeping two water molecules at the binding site of the receptor. The study showed the formation of an important water-mediated H-bond between the most potent compounds of the series with Glu169 at EL2. The interaction of Val250 with the novel antagonists was found to be the reason for their specificity towards hA_{2B}AR, as observed in their previous study [43]. The conserved polar interaction with Asn254 as well as the interaction of aromatic moieties within the aromatic cavity formed by Trp247 and His251 was also found to have crucial role in binding with potential antagonists. Carbajales *et al*. carried out docking studies for novel 2-cyanoimino-4-substituted-6-methyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate derivatives by using a similar computational protocol [43,47] and reported the importance of the A_{2B}AR-specific residues Val250 and Phe173 (aromatic interaction) required for binding with antagonists [48]. Various structure-based approaches pertaining to the modeling of A_{2B}AR and its ligands are presented in Table 1.

Ligand-based approaches in A_{2B}AR modeling

In the absence of a 3D X-ray crystal structure of a target protein and/or coordinate of a molecular model, the ligand-based approach (Table 2) can offer crucial information to support new drug design strategies. Song *et al*. predicted the affinity of an A_{2B}AR antagonist by using molecular field analysis (MFA). Various xanthine and nonxanthine derivatives were utilized as a suitable data set and constructed 3D-QSAR, which revealed the equal contribution of electrostatic and steric interactions to the antagonistic properties of A_{2B}AR. A SAR study described that the H-bond donor (HBD) on 7-position and negative charge on N^1 of xanthine could be the parameters determining the perceived higher affinity. An alkyl substituent (1-position) and the aromatic substitutions (8-position) of alloxazine further enhanced the activity [49]. SARs of A_{2B}AR ligands and the use of GPCRs as potential drug targets have been reviewed elsewhere [50,51].

In 2007, Wei *et al*. [52] developed 3D-pharmacophore models (ligand-based) for selective A_{2B} AR antagonists. The best pharmacophore hypothesis, Hypo-A_{2B}, was deduced from a training set of selective A_{2B}AR antagonists reported previously by Hayallah *et al*. [53]. Hypo-A_{2B} had four features: two H-bond acceptors (L), one hydrophobic-aliphatic (Z), and one ring aromatic (R), and was validated by utilizing an internal test set bearing structurally diversified ligands [54]. The generated model was able to efficiently identify and differentiate highly potent antagonists between AR subtypes. The results of this study suggested that the ligand-based model was in good agreement with the structure-based model reported previously by Ivanov *et al*. [30].

In 2008, Joseph *et al*. [55] developed a 3D-QSAR model from which they constructed a pharmacophore model based on known A_{2B}AR antagonists [56,57]. The QSAR model derived from genetic algorithm-partial least squares (G/PLS) recognized the electrophilicity, conformational flexibility shape and size of the molecules as imperative molecular descriptors. The best predictive model derived with satisfactory statistical parameters was Equation 1:

[1] Antagonistic activity = 5.5424–0.000985 (Hf) + 6.693 (Shadow-YZ-frac) + 0.00074 (Jurs-PPSA-2) – 0.2542 (LUMO) + 0.08516 (logZ) – 0.0445 (Fh2o) + 0.2936 (SC-3_C).

[n = 40; R^2 = 0.921; Q^2 = 0.862; PRESS = 3.339; N = 5; R^2_{pred} = 0.791; R^2_{BS} = 0.913],

where, Hf is the heat of formation; Jurs-PPSA-2 is the partial positive solvent-accessible surface area; LUMO is the lowest unoccupied molecular orbital energy; logZ is a coefficient; Fh2o is the aqueous desolvation free energy; and SC-3_C is a topological descriptor].

Papers also published in 2008 reviewed the identification of new ligands for ARs using QSAR and updated the trends in identifying such receptor-selective ligands [34,58]. Gonzalez and co-workers focused mainly on the applications of QSAR methodologies to develop new AR antagonists [58]. According to their review, the QSAR model developed by Doichinova *et al*. [59] showed that the selectivity and affinity for A_2AR subtypes depends on the electronic and hydrophobic properties of substituents present in the xanthine moiety. The authors also presented the results of various aspects of QSAR studies carried out by different research groups [49,60,61] and discussed the limitations incurred with ligand selectivity towards ARs while using different QSAR models.

To classify and predict the binding affinity of A_{2B}AR antagonists, Bonet *et al*. developed a novel ensemble machine learning algorithm [62]. A total of 381 ligands (antagonists) were classified as potent (204), moderate (95), and weak (82) based on their binding affinity (K_i) values. The authors reported the fully automatic algorithm development protocol involving the training of various classifier models simultaneously considering neural networks, support vector machines, and k-nearest neighbor (kNN) as single classifiers. A group of diversity measures was applied for the selection of base classifiers and subsequent combining into an ensemble. Given that multiclassifiers can provide more accurate and consistent results, the authors constructed several multiclassifiers (Vote, Bagging, and Boosting). Pairwise and nonpairwise diversity measures were used to select the base classifiers. For each database, the authors determined 20 ensembles and compared their performance measures. After generating the prediction results of the final multiclassifier, the authors conducted external and tenfold cross validations for the algorithm.

TABLE 1

Structure-based approaches for molecular modeling of A_{2B}AR and its ligands

Year	Modeling task	Template/Method	Energy minimization	Software used	Refs
2002	Homology modeling and docking	Bovine rhodopsin (PDB: 1F88)	Tripos force field	Sybyl 6.7.2 and DOCK-Sybyl 6.7.2	[25]
2003	Homology modeling and docking	Bovine rhodopsin (PDB: 1F88)	Amber94 force field and MMFF94	Molecular Operating Environment (MOE-v2002.03) and MOE-DOCK	[27]
2005	Docking and MD simulations	Model of POPC	Tripos force field and GROMACS force field	DOCK-Sybyl 6.9.1 and Gromacs 3.1.4	[30]
2007	Homology modeling and docking	Bovine rhodopsin (PDB: 1F88)	Tripos force field	Modeller and DOCK-Sybyl 6.9.1	[31]
2008	Docking	Flexible docking	MMFF	MacroModel	[33]
2009	Homology modeling and molecular docking	β_2 adrenoceptor (PDB: 2RH1) and A_{2A} AR (PDB: 3EML)	AMBER99 and MMFF94	MOE software modules and Schrodinger Macromodel package (Glide XP and InducedFit of Schrodinger) and Tabu search docking (MOE)	[36]
	Homology modeling, molecular docking, MD simulations and IFD protocol	Bovine rhodopsin (PDB: 1F88); β_2 adrenoceptor (PDB: 2RH1) and A_{2A} AR (PDB: 3EML)	Tripos force field	homology modeling server: Homer FlexX software, MD-package Amber, Prime 2.0, IFD module and Glide5.0	[37]
2010	Homology modeling, molecular docking and mapping of pharmacophore onto homology model	A_{2A} AR (PDB: 3EML) as template and A_{2B} AR homology model as target	Amber 10 package	SYBYL7.0 package (loop search), Maestro 8.5 (protein structure alignment protocol- Schrödinger software package), IFD, Glide-SP, Glide-XP-Maestro 8.5 and Discovery Studio 2.1 package	[63]
2011	Homology modeling of A_2 adenosine receptors and MD simulations	A_{2A} AR (PDB: 3EML) as template and using half-ε double-pairlist method	MMFF	Modeller version 9.4 (loop building), Macromodel-Schrodinger (energy minimization) and GROMACS	[42]
2012	Homology modeling, molecular docking and MD simulations	A_{2A} AR (PDB: 3EML) and docked complex	ffgmx force field	MODELLER (9v5), AutoDock software version 4.2 and GROMACS 4	[44]
2013	Homology modeling, molecular docking and MD simulations	3EML, 3QAK, and 2YDV homology models; docked complex	Tripos force field	Modeler (Accelrys), FlexX (BioSolveIT) and Amber (MD package)	[45]
2014	Molecular modeling and mutagenesis, homology model (hA_{2B} receptor/G-protein)	β_2-adrenergic receptor (PDB: 3SN6)	ICM force field	ICM Homology tool (Molsoft V3.7-2)	[46]
2016 and 2017	Molecular docking	Homology model based on A_{2A} AR (PDB: 3EML)	MMFF	Modeler (homology modeling), Schrödinger software (LigPrep) and GOLD (docking)	[47,48]

The authors proposed a strategy that suggested the following: (i) overall accuracy is derived for single classifiers coupled with feature selections; (ii) based on the reported ensemble, combinations in multiclassifier models result in a better performance compared with single classifiers; and (iii) the performance of multiclassifier models is better than that of earlier models reported in the literature. Furthermore, the authors claimed that the multiclassifier approach can be used to predict the affinity of A_{2B} AR antagonists as well as further assisting in the development of other QSAR models.

In 2010, Cheng *et al.* [63] proposed a complementary study involving both the ligand- and structure-based methods to gain insights into the binding modes of A_{2B}AR antagonists. The best pharmacophore model Hypo1 (Fig. 2b) had four features: two ring aromatics (RA), one hydrophobic-aliphatic (HYD-AL) and one hydrophobic-aromatic (HBA). In terms of the correlation of experimental data with predicted affinity (K_i) values, the authors found the reliability of the model to be >90% [63]. Cheng *et al.* also developed a A_{2A}-based homology model for A_{2B}AR for docking

TABLE 2

Ligand-based approaches for molecular modeling of $A_{2B}AR$ and its ligands

Year	Approach	Modeling task	Type of ligands	Number of ligands in training set	Software employed	Statistical protocol	Validation results of the best model	Refs
2005	3D-QSAR	CoMFA Model	Xanthines	85	SYBYL 6.6	PLS [Leave One Out (LOO)] regression	$Q^2 = 0.752$; $R^2 = 0.982$	[49]
2007	Pharmacophore modeling	Qualitative model-HipHop	Xanthines	5	Catalyst v4.10	External test set prediction	Best fit values = 3.20–4.00	[52]
2008	3D-QSAR and Pharmacophore modeling	CoMFA model and quantitative model (HypoGen)	Xanthines	63 and 23	CERIUS2 and Catalyst v4.11	Genetic partial least square (G/PLS) and pharmacophore modeling	$Q^2 = 0.752$; $R^2 = 0.982$; best fit value = 2.79	[55]
	3D-QSAR	CoMFA and CoMSIA Model	Adenosine and its 5′-*N*-methyluro-namide with diverse substitutions	72	SYBYL 7.3	PLS components	$R^2 = 0.960$; $Q^2 = 0.676$; F=158; SEE = 0.175; $R^2_{test} = 0.782$	[33]
2010	Pharmacophore modeling	Quantitative model: HypoGen	Xanthines and nonxanthines	31	Catalyst4.10.	Pharmacophore modeling	R = 0.865	[63]
2013	Machine-learning algorithm	Joining Tree Clustering (JTC), k-means cluster analysis and molecular descriptor calculations		381	DRAGON software (version 5.4)	Principal component analysis (PCA); JTC; DRAGON, MOE, MODESLA, MOPAC software packages, and STATISTICA software version 8.0.	Internal accuracy = 83.7%; external accuracy = 86.9%	[62]
2014	2D-QSAR and 3D-QSAR	HQSAR (model) and CoMFA model	9-Deaza-xanthines	141	DRAGON	MOBYDIGS 1.1 Software (multiple linear regression); PIROUETTE 4.0 software (PCA); Surflex–Sim software (3D chemical similarity – SIM, alignment)	$R^2 = 0.85$, $Q^2_{LOO} = 0.77$; and $R^2 = 0.86$, $Q^2 = 0.70$	[64]
2015	QSAR model	Physicochemical parameter calculations, calculations of quantum chemical descriptors; partial least squares (PLS) method	Nonxanthines	30	Gaussian 98W	HyperChem and DRAGON software	$R^2 = 0.936$; $Q^2 = 0.867$	[65]
		k-means clustering	Xanthines and deazaxanthines	130	DRAGON software (ver. 6), MOE (ver. 2008.10)	STATISTICA software version 8.0.	Statistical significance of equation >0.05% and >0.02% for variables	[66]

various ligands and observed the significance of Phe173 and Glu174 in antagonist binding. Based on these combined approaches, Cheng *et al*. suggested modifications of xanthine-based compounds to obtain potent antagonists for A_{2B}AR (Fig. 2c).

In 2014, Paz *et al*. reported steric and electronic requirements for molecules with a deazaxanthine scaffold to impart A_{2B}AR antagonism quantitatively by using 2D-QSAR (HQSAR) and 3D-QSAR (CoMFA) approaches. A data set of 195 deazaxanthine derivatives was used for the generation of multiple linear regression (MLR) models. Paz *et al*. derived statistically reliable and a reasonably good model (HQSAR: $R^2 = 0.85$, $Q^2_{LOO} = 0.77$; CoMFA: $R^2 = 0.86$, $Q^2 = 0.70$) with satisfactory predictive ability ($R^2_{pred1} = 0.78$; and $R^2_{pred2} = 0.70$ respectively) to provide useful information for the development of more potent 9-deazaxanthine derivatives [64].

Similarly, Mansourian *et al*. reported a ligand-based QSAR model and subsequent validation by structure-based (docking) methods for a diverse set of substituted-aminobenzothiazole amide (non-xanthine) A_{2B}AR antagonists. Mansourian *et al*. developed a valid and reliable QSAR model ($R^2 = 0.936$ and $Q^2 = 0.867$) and performed docking studies to identify the ideal structural features essential for antagonists in conjunction with the verification of the potential binding site of A_{2B}AR. Mansourian *et al*. identified His280, Ile276 (TM7), Lys269 (EL3), Asn254, His251 (TM6), and Glu174, Phe173 (EL2) as crucial amino acid residues implicated in ligand–receptor interactions [65].

Alfonso *et al*. developed a QSAR model (using 419 ligands) for A_{2B}AR to predict and interpret A_{2B}AR antagonist activity of xanthine and deazaxanthine analogs. The authors highlighted the significance of the presence of long hydrophobic substituents in the N^1 position for higher A_{2B}AR antagonistic potency, whereas opposite effect was observed if the same type of substituent was present in the N^3 position of xanthines/deazaxanthines [66].

Currently, several nonxanthine scaffolds have been identified, which opens new prospects in QSAR and docking analyses of A_{2B}AR antagonists [67,68]. Positive or negative allosteric modulators of human A_{2B}AR have also been recently developed as new pharmacological tools to treat pathological conditions related to the altered functionality of A_{2B}AR. Specifically, positive A_{2B}AR allosteric modulators are useful in the formation of new bones, differentiating osteoblasts, and show antiproliferative effects on cancer stem cells [69,70].

Concluding remarks and future perspectives

In pursuit of the design and discovery of ligands for A_{2B}AR, xanthine derivatives have been explored for the construction of LBDD and SBDD. The discovery of the X-ray crystal structure of A_{2A}AR enabled computational medicinal chemists to gain further insights into the structure of ARs and to construct improved homology models of A_{2B}AR, determining the amino acid residues responsible for selective ligand binding towards A_{2B}AR. The SBDD approach involves homology modeling followed by molecular docking, and MD simulation studies to design and derive SAR for A_{2B}AR ligands. Recent strategies have utilized a docked complex and/or bioactive conformation of ligands inside A_{2B}AR for building QSARs or pharmacophore models, which has been shown to be a more successful approach compared with single LBDD approaches [71].

More studies involving nonxanthine structures should be encouraged to identify potential drug candidates with desired pharmacodynamic and pharmacokinetic properties. Furthermore, allosteric modulators of A_{2B}AR could offer clinical advantages over agonists or antagonists of this receptor in terms of improving receptor selectivity. More modeling work is anticipated for these allosteric modulators of A_{2B}AR in the future.

The detailed analysis of various methodologies pertaining to homology modeling along with various insightful CADD approaches discussed in this review provide an impetus to medicinal chemists to build new strategies to discover potential A_{2B}AR selective antagonists with higher affinity as novel therapeutic agents.

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