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# Synthesis of adenosine analogues with indole moiety as human adenosine $A_3$ receptor ligands

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Adenosine is an endogenous modulator exerting its functions through the activation of four adenosine receptor (AR) subtypes, termed $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$, which belong to the G-protein-coupled receptor superfamily. The human $A_3$AR ($hA_3$AR) subtype is implicated in several cytoprotective functions. Therefore, $hA_3$AR modulators, and in particular agonists, are sought for their potential application as anti-inflammatory, anti-cancer and cardioprotective agents. Here, we prepared novel adenosine derivatives with indole moiety as $hA_3$AR ligands. According to the biological assay, we found that 2-substituents **11** were critical structural determinants for $A_3$AR ligands ($K_i = 111$ nM). The observed structure–affinity relationships of this class of ligands were also exhaustively rationalized using the molecular modelling approach. This allows the investigation on the binding mode of the potential compound in the ligand-binding pocket of the human $A_3$ receptor. The results demonstrated that **11** can interact with the ASN250, GLN167, PHE168 and VAL178 through hydrogen bonding, which are shown to be important for ligand–receptor interaction.

## 1. Introduction

Adenosine is an endogenous purine nucleoside that modulates many physiological processes, which is composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety

via a β-$N_9$-glycosidic bond [1–3]. Adenosine is widely found in nature and plays an important role in biochemical processes, such as energy transfer as adenosine triphosphate (ATP) and adenosine diphosphate (ADP), as well as in signal transduction as cyclic adenosine monophosphate (cAMP). It is also a neuromodulator, believed to play a role in promoting sleep and suppressing arousal. Adenosine also plays a role in the regulation of blood flow to various organs through vasodilation [4–6]. Cellular signalling by adenosine occurs through four known adenosine receptor (AR) subtypes ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$) [7]. All AR subtypes are G-protein-coupled receptors. The four receptor subtypes are further classified based on their ability to either stimulate or inhibit adenylate cyclase activity. They have long been considered to be promising therapeutic targets in a wide range of conditions, ranging from cerebral diseases to cancer, including inflammatory and immunological disorders [8,9]. Adenosine contributes in a significant manner to the maintenance of tissue integrity by modulating the immune system. Encouraging results have emerged with AR ligands for the management of several physiological conditions in preclinical and clinical settings [7,10].

Among the four AR subtypes, the $A_3$AR, probably the most studied AR subtype, is also ubiquitously expressed [11]. The distribution of $A_3$AR is species-dependent, and in humans, this subtype is expressed in the lungs, liver, heart, kidneys and brain [12–14]. The widespread distribution in different cells and tissues of the $A_3$AR suggests a potential involvement in various pathologies and the possible use as a selective pharmacological target [15]. This subtype of ARs is involved in a variety of important pathophysiological processes, ranging from modulation of cerebral and cardiac ischaemic damage to regulation of immunosuppression and inflammation [16].

The increasing knowledge about $A_3$ARs, in particular regarding the molecular biology of this subtype, has provided important evidence to consider this receptor as a novel therapeutic target. In addition, it enables rational design and the development of potent and selective $A_3$AR ligands as promising therapeutic options for a variety of diseases [17,18]. Therefore, small molecule modulators targeting the $A_3$AR have been sought for their potential application as anti-inflammatory, anti-cancer and cardioprotective agents [19–21].

Indoles probably represent one of the most important structural classes in drug discovery [22]. The indole substructure is a basic element for a number of biologically active natural and synthetic products. Indoles are found in a wide range of therapeutically important drugs [23,24]. Over the years, a considerable amount of effort has been made to find indoles with various biological activities and select certain agents for leads in drug discovery research. On account of their potent biological activities, indoles have continued to attract the interest of chemists and biologists alike in drug discovery.

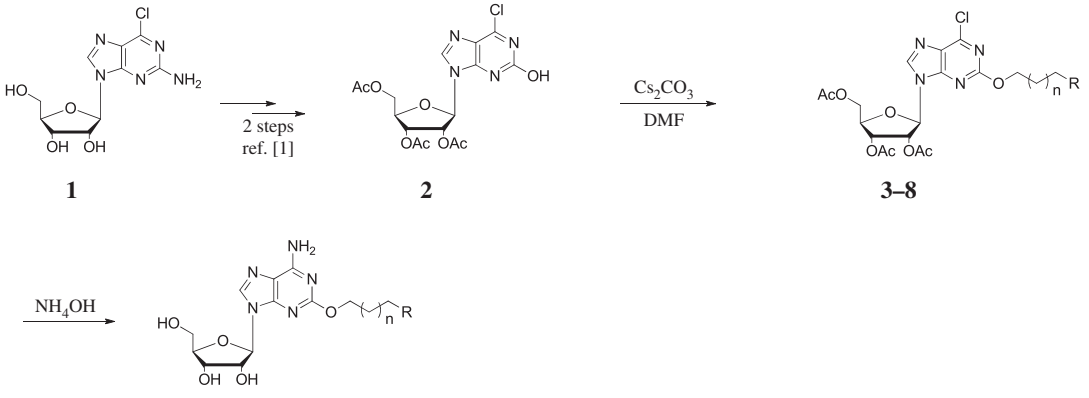
In addition, the scientific community is making intensive efforts to design AR ligands endowed with greater selectivity or to develop innovative compounds acting as receptor modulators. To further explore the importance of indole framework as the basis for AR ligands, we investigate the receptor subdomain that binds the purine moiety by the study of 2-*O*-alkyl derivatives of 2-chloroadenosine. The present paper reports on the synthesis and binding studies of these compounds as well as of adenosine. We describe the lengths of alkyl group in terms of potency at the $A_3$ receptor as well as receptor subtype selectivity. Here, we use the conversion of the 2-alkynyl group into flexible 2-*O*-alkyl between purine and indole moiety.

# 2. Results and discussion

## 2.1. Synthesis

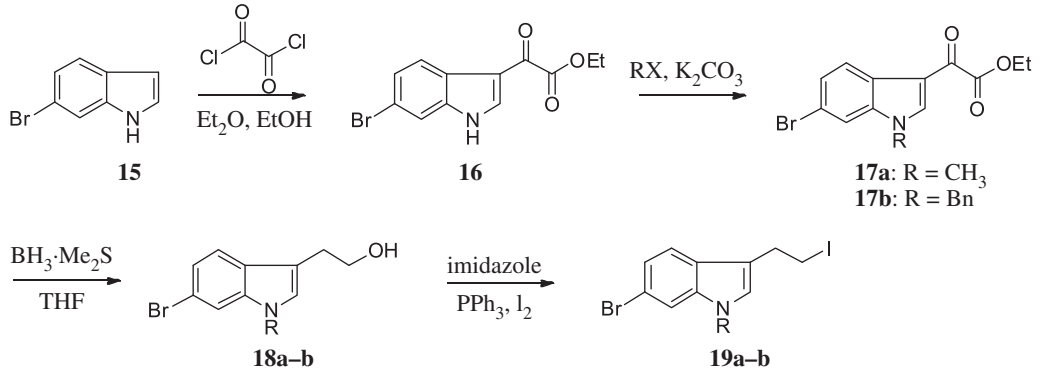
In an effort to discover new nucleoside analogues as potential $A_3$AR ligands, we pursued the 2-oxypurine nucleoside using indole alkyl iodides as shown in scheme 1. The synthesis of the 5′-$CH_2$OH analogues started from 2-amino-6-chloropurine riboside **1**, which was converted into 6-chloro-2-hydroxy-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine **2**, as reported [25]. The reaction of the hydroxyl group at the 2-position of **2** with various indole iodides was conducted in the presence of caesium carbonate to affect compounds **3–8**. Simultaneous removal of the acetyl group and amination at the 6-position of **3–8** using ammonium hydroxide solution yielded compounds **9–14**.

For synthesizing the key compounds, we should synthesize intermediates for the 2-ether component depicted in schemes 2–4. Scheme 2 illustrates the synthesis of two carbons as linker indole iodides. The indole 2-oxoacetate **16** was accessed by the treatment of 6-bromoindole **15** to oxalyl chloride, followed by a reaction with ethanol. Alkylation of indole nitrogen by $CH_3$I and BnBr provided *N*-alkyl **17a** and **17b**, respectively. The following exposure to $BH_3$-$SMe_2$ provided corresponding alcohols **18a** and

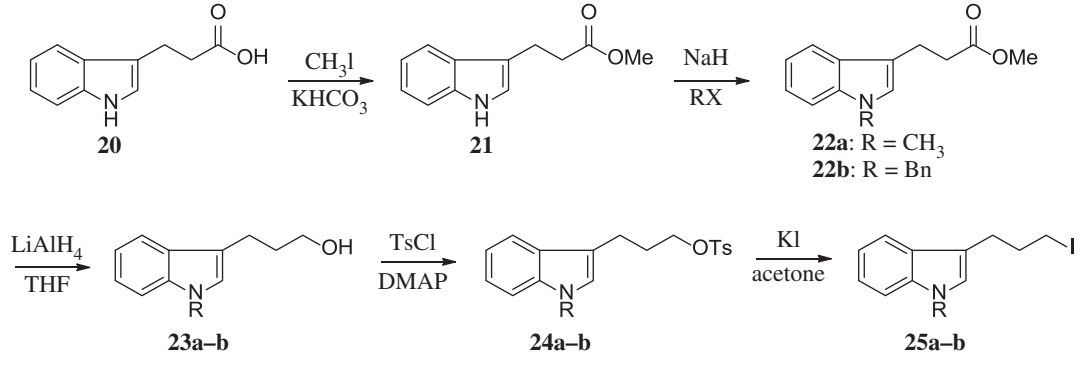



**3** ⟶ **9** R = 3″-(6″-Br-*N*-Me-indoly-), *n* = 0
**4** ⟶ **10** R = 3″-(6″-Br-*N*-Bn-indoly-), *n* = 0
**5** ⟶ **11** R = 3″-(*N*-Me-indoly-), *n* = 1
**6** ⟶ **12** R = 3″-(*N*-Bn-indoly-), *n* = 1
**7** ⟶ **13** R = 3″-(6″-Br-*N*-Me-indoly-), *n* = 1
**8** ⟶ **14** R = 3″-(6″-Br-*N*-Bn-indoly-), *n* = 1

**Scheme 1.** Synthesis of compounds **9–14**.



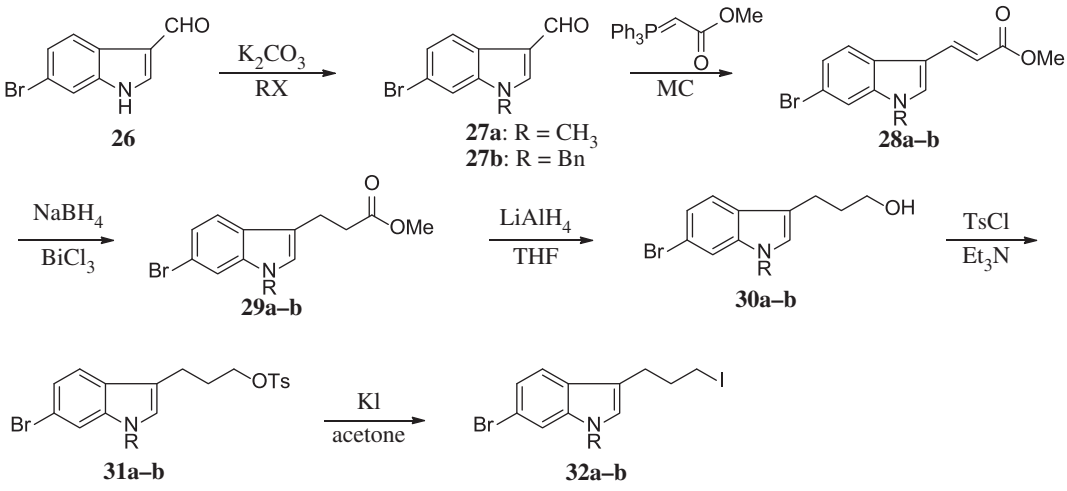

**Scheme 2.** Synthesis of intermediates **19a–b**.




**Scheme 3.** Synthesis of intermediates **25a–b**.

**18b**. The alcohols were transformed to corresponding iodides **19a–b** by iodine, triphenylphosphine and imidazole.

The three carbons used as the linker of indole iodides shown in scheme 3 were obtained from the commercially available 3-indolepropionic acid **20**. Propionate **21** was prepared by the conversion of carboxylic acid into methyl ester by treatment with $CH_3I$ and $KHCO_3$. Subsequent N-alkylation of the indole nitrogen provided **22a** and **22b** followed by reduction of carboxylic methyl ester to produce the




**Scheme 4.** Synthesis of intermediates **32a–b**.

corresponding alcohols **23a** and **23b**. Finally, iodides **25a** and **25b** were derived from **23a–b** via tosylate alcohol, which underwent substitution with iodine.

The synthetic strategy for the preparation of substituted indole derivatives is described in scheme 4. The commercially available 4-bromoindole-3-carboxaldehyde **26** was reacted with $CH_3I$ and BnBr to give rise to N-alkylation **27a–b**, respectively. The Wittig reaction of aldehyde provided *trans*-indole acrylate **28a–b**. The following treatment with $NaBH_4$ and $BiCl_3$ yielded propionates **29a–b**. $LiAlH_4$ reduction of the carboxylic ester produced intermediates **30a–b** followed by treatment with tosyl chloride to afford the corresponding **31a–b**, and the tosylate moiety was displaced with good yield with iodine to incorporate the iodo functionality. Detailed synthetic procedures including the yield of reactions and characteristic data can be found in the electronic supplementary material.

## 2.2. Binding affinity studies

The aim of the present study is to expand knowledge of the structure–activity relationships at the $A_3AR$ and at other subtypes, both in relation to binding affinity and intrinsic efficacy, of adenosine derivatives modified in the 2-position. A screening campaign to discover new scaffolds for $A_3AR$ inhibition yielded the moderate-potency lead **11** (table 1). Among 2-substituted derivatives, 2-ethers were more potent than the corresponding amines or thioethers [26]. The effect of bromine substitution of the phenyl ring was evaluated. This series of 5′-brom analogue showed a tendency towards increased $K_i$ values with $A_1$ and $A_3ARs$, depending on the bulkiness of the bromine atom. Of these analogues, compound **10** was equipotent to **13** with $A_1AR$. However, its selectivity to binding $A_3AR$ was improved. Unexpectedly, **14** was threefold less potent than **13** in binding to $A_3AR$ and was tolerated by $A_{2A}AR$. Although the 5′-bromo derivative **13** was somewhat equipotent to **11** with $A_3AR$, its selectivity was reduced.

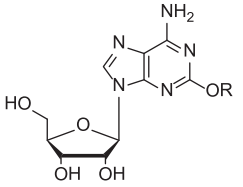
Interestingly, the 3-indolyl analogue **11** was sevenfold more potent than **12** with $A_3AR$. The increased size or steric hindrance of the *N*-substituent markedly decreases $A_3AR$ potency. Nevertheless, **12** was less potent than **13** in binding to $A_{2A}AR$. The effect of the space of the alkyl chain between the 2-ethers and the indole moiety was tested. Elongation increased the affinity for $A_3AR$. Compound **11** showed a fivefold increased potency with $A_3AR$, while it was somewhat tolerated by $A_{2A}AR$. The affinity of **9** to all three AR subtypes showed low potency compared with that of **13**, similar to the results with **10**.

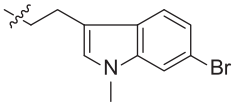
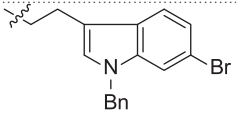
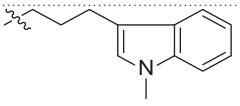
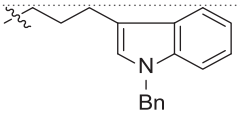
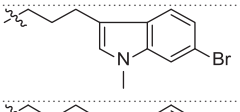
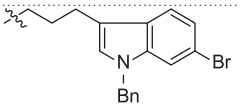
However, the corresponding 2-indolyl derivative **13** was more potent than compound **11** in affinity for $A_1$ and $A_{2A}Rs$. The bulkiness of the *N*-substituent may be related to the increased affinity. Compound **14** was more potent than **12** in binding to all three ARs. Meanwhile, compound **11** displayed a fivefold potency enhancement over **9** with $A_3AR$. The *N*-Bn derivative **10** with potency close to that of **9** was invariant in affinity for $A_1$ and $A_{2A}Rs$.

## 2.3. Molecular docking analysis

Driven by docking of several derivatives with $hA_3R$, we performed the molecular modelling studies [27] to explore the binding modes of all six aforementioned compounds as shown in table 2. Among

**Table 1.** Potency of 2-alkoxyadenosine derivatives to bind human $A_1$, $A_{2A}$ and $A_3$ARs expressed in CHO cells.[a]




| | | $K_i$ (nM ± SEM) or % inhibition at 10 μM | | |
|---|---|---|---|---|
| entry | R | $hA_1AR$[b] | $hA_{2A}AR$[b] | $hA_3AR$[b] |
| 9 |  | (47 ± 5%) | 2770 ± 500 | 679 ± 149 |
| 10 |  | 217 ± 57 | 380 ± 81 | 532 ± 144 |
| 11 |  | 300 ± 70 | 880 ± 200 | 111 ± 30 |
| 12 |  | (34 ± 2%) | (56 ± 5%) | 731 ± 209 |
| 13 |  | 230 ± 26 | 262 ± 192 | 177 ± 43 |
| 14 |  | 152 ± 49 | 371 ± 79 | 491 ± 143 |
| | adenosine | — | — | 290 |

[a]All experiments were performed on CHO cells stably expressing one of three subtypes of human ARs. The binding affinities for $A_1$, $A_{2A}$ and $A_3$ARs were expressed as $K_i$ values and were determined using agonist radioligands ([$^3$H]CCPA), ([$^3$H]CGS21680) and [$^{125}$I]I-AB-MECA, respectively. Values in parentheses are for weak binding, corresponding to an $IC_{50} \geq 10$ μM. Data are expressed as mean ± s.e.

[b]$K_i$ in binding, unless noted.

**Table 2.** The predicted free energy and affinity compared with experimental values.

| entry | experimental $K_i$ ($hA_3AR$, nM) | predicted free energy (kcal mol$^{-1}$) | predicted affinity (nM) |
|---|---|---|---|
| 11 | 111 ± 30 | −9.08 | 222.18 |
| 13 | 177 ± 43 | −8.73 | 398.82 |
| 9 | 679 ± 149 | −8.38 | 719.10 |

these compounds, the compounds cpd**10**, **12** and **14** with the *N*-Bn substituent failed to dock into the ligand-binding site of the protein. The current docking results are consistent with the biological studies mentioned above and serve as an explanation for the sharply reduced affinity. On the contrary, all other three compounds (cpd**9**, **11** and **13**) with *N*-methyl substituent were found to dock into the binding pocket of the protein of interest and further interact with the amino acids GLN167, PHE168, ASN250, etc., through hydrogen bonding or hydrophobic interactions (figures 1 and 2). Detailed information for docking experiment can be found in the electronic supplementary material. In particular, the common interactions for these three compounds were the hydrogen bonding with the amino acid ASN250 of the protein, which is shown to be closely relevant to antagonist interaction [28,29]. Furthermore, the docking

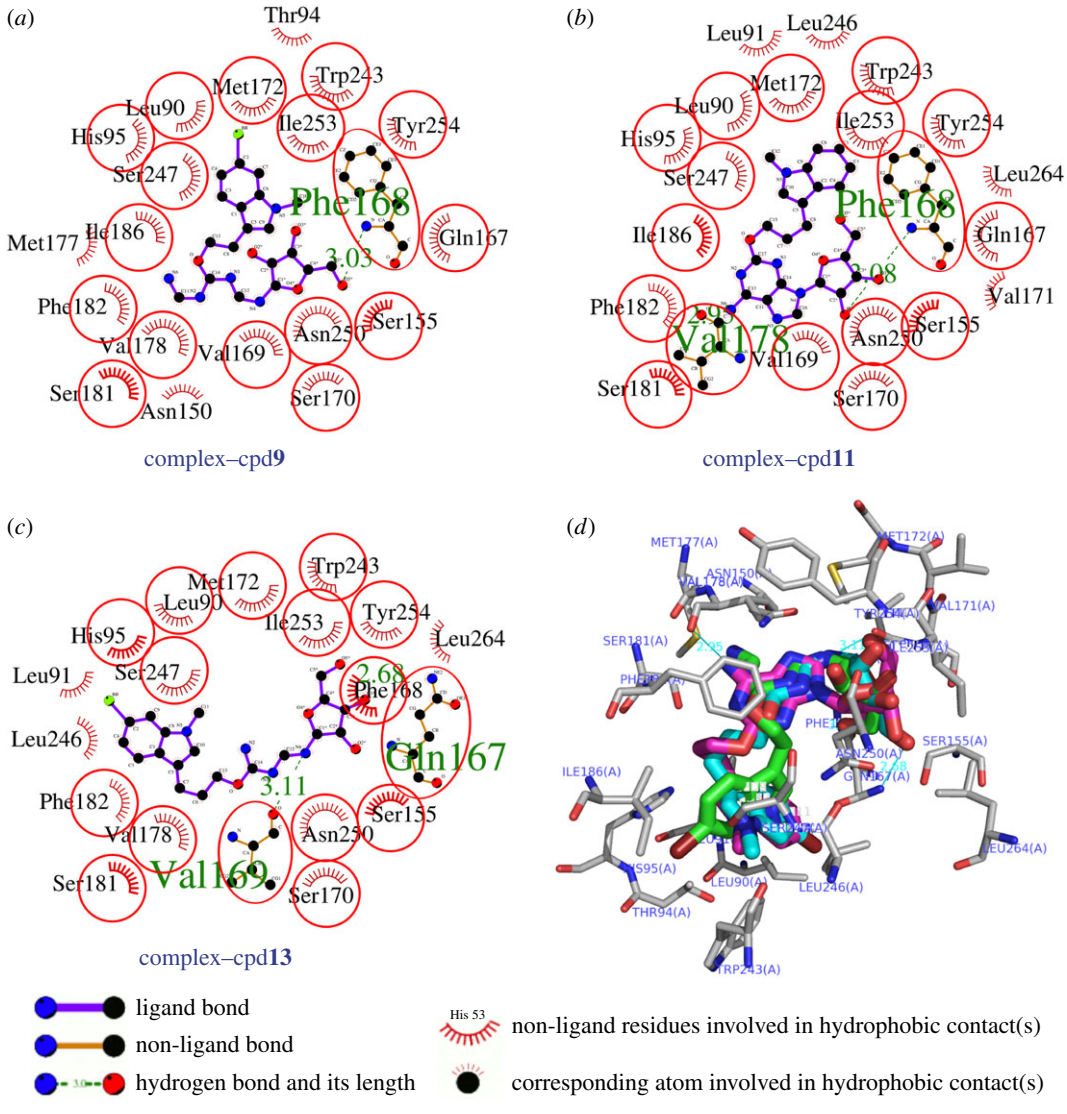



**Figure 1.** The two-dimensional image of ligand–h$A_3$AR interactions with the hydrogen bonds and hydrophobic interactions (*a–c*); (*d*) the cpd**9** is coloured green, the cpd**11** is coloured magenta, the cpd**13** is coloured cyan, respectively. The surrounding residues interacting with these ligands are also shown and labelled. The meaning of the items on the two-dimensional plot above is listed in the bottom panel.

results showed that the compound cpd**11** with the highest $K_i$ value has the highest predicted affinity ($-9.08$ kcal mol$^{-1}$, table 2).

Figure 2 depicts the binding mode of the compound cpd**11** in the ligand-binding pocket of the protein in details. Cpd**11** can be well docked into the binding site of interest. In the most potent molecule cpd**11**, the adenosine core contributed strongly to the binding affinity, which also demonstrates the rationality of the core as a key scaffold for the further chemical modifications. The hydroxyl oxygen of furan ring and the 6-postion *N* atom of adenyl group interact with the ASN250, GLN167, PHE168 and VAL178 through hydrogen bonding. On the other hand, the high affinity also requires the presence of the 2-*O*-alkyl-substituted groups for producing the strong hydrophobic interactions.

From these results, we have illustrated the interactions of our newly synthesized 2-*O*-alkyl-substituted adenosine analogues with a ligand-binding site of h$A_3$AR from the molecular modelling point of view. The results also showed the different roles of the substitutions which are strongly linked to the increased or decreased affinity. The exploration for these interactions can provide us the guidance for the future chemical modifications.

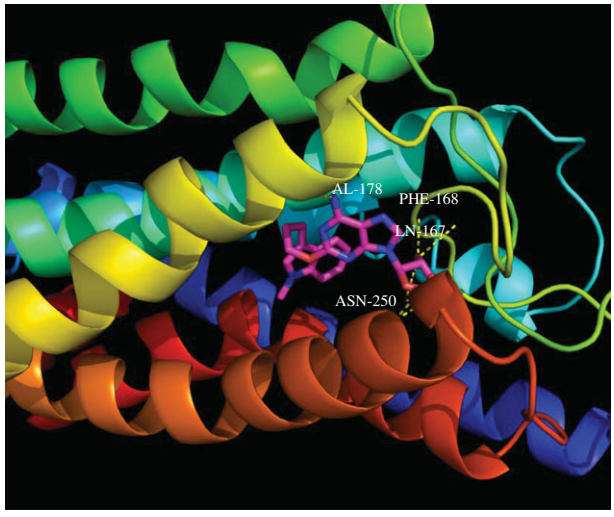



**Figure 2.** The most active compound cpd**11** (magenta colour) in the active site of human adenosine $A_3$ receptor showing hydrogen bonding interactions.

# 3. Conclusion

In this work, we designed and synthesized a series of 2-*O*-alkyl-substituted adenosine analogues with indole moiety. The 2-substituents **11** was the most potent among the series, and it was confirmed to be a modulator in a functional assay measuring its capacity to bind receptors in CHO cells expressing the $hA_3A$ receptor. We found that 2-substituents **11** were critical structural determinant for $A_3AR$ ligands ($K_i = 111$ nM). The promising compound can be considered a valuable seed for the design and development of new and even more selective and potent compounds. The molecular modelling studies have also been performed to investigate the binding mode of the potential compound in the ligand-binding pocket of human $A_3$ receptor. Here, this study provides useful foundations for the attainment of a detailed pharmacological and physiological characterization of the adenosine $A_3$ receptor.

Data accessibility. The detailed experimental synthetic procedures and spectra of the final compounds are provided in the electronic supplementary material.

Authors' contributions. Y.X. carried out the synthetic work and performed the NMR experiments. X.Z. carried out the biological screening. R.H. helped analyse and interpret the data. J.W. conceived the study, designed it and drafted the manuscript. All authors gave final approval for publication.

Competing interests. The authors declare that they have no competing interests.

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