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Extended N^6 substitution of rigid C2-arylethynyl nucleosides for exploring the role of extracellular loops in ligand recognition at the A_3 adenosine receptor

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ABSTRACT

2-Arylethynyl-(N)-methanocarba adenosine 5′-methyluronamides containing rigid N^6-(*trans*-2-phenylcyclopropyl) and 2-phenylethynyl groups were synthesized as agonists for probing structural features of the A_3 adenosine receptor (AR). Radioligand binding confirmed A_3AR selectivity and N^6-1*S*,2*R* stereoselectivity for one diastereomeric pair. The environment of receptor-bound, conformationally constrained N^6 groups was explored by docking to an A_3AR homology model, indicating specific hydrophobic interactions with the second extracellular loop able to modulate the affinity profile. 2-Pyridylethynyl derivative **18** was administered orally in mice to reduce chronic neuropathic pain in the chronic constriction injury model.

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X-ray crystallographic structures of the A_{2A} adenosine receptor (A_{2A}AR) in complex with both agonists and antagonists[1–3] can serve as suitable templates for accurately predicting the ligand interactions of other AR subtypes. We now focus on sterically bulky and hydrophobic N^6 substituents on adenosine derivatives that are associated with high affinity at the A_3AR. Adenosine derivatives that selectively activate the A_3AR are in clinical trials for autoimmune inflammatory diseases and liver cancer; thus, there is considerable interest in drug discovery at this receptor.[4]

Many studies have established the effects of diverse substitution of the exocyclic amine of adenosine on the AR subtype selectivity, with predominantly hydrophobic N^6 groups found to be best suited for high affinity.[5–7] For example, the structure activity relationship (SAR) of the N^6-(2-*trans*-phenylcyclopropyl) group and its substituted analogues was studied in monosubstituted adenosine derivatives (e.g., **1**, **2**, Chart 1), which tended toward selectivity at the A_3AR.[8] The steric constraint and stereochemical definition of the N^6-(2-*trans*-phenylcyclopropyl) group, especially in combination with rigid ribose substitutions, make it possible to study the structural implications of this conformationally constrained substituent in A_3AR recognition. Here we use a recent homology model[9] of the human (h) A_3AR to probe the structural basis for the effects on binding affinity in this class of agonists.

We previously explored a class of constrained (N)-methanocarba adenosine-5′-methylamide nucleosides, including 2-chloro derivatives (e.g., **3**)[10] and C2-arylethynyl analogues (e.g., **4**),[9,11] which are very potent and selective full agonists of the A_3AR. N^6-Benzyl-type substitution is often used because of its selective enhancement of A_3AR affinity. Here, we have synthesized new N^6-(2-phenylcyclopropyl)-substituted analogues in this extended methanocarba series and characterized them pharmacologically. We already reported the A_3AR selectivity of one analogue, **5** (R^1 = H) in which a rigid ribose-like (N)-methanocarba scaffold[10] was combined with a sterically restricted N^6-(2-phenylcyclopropyl) substitution, based on our earlier study of SAR in the 9-riboside series.[8] The corresponding 4′-truncated (N)-methanocarba nucleoside containing a racemic N^6-(2-phenylcyclopropyl) substitution was found to be a selective A_3AR antagonist (K_i 1.3 nM).[12] The 1*S*,2*R* diastereoisomer of **2** was ~10-fold more potent in binding to the hA_3AR (K_i 11.2 nM) than the corresponding 1*R*,2*S* isomer. This N^6 substituent was also combined with C2-cyano or carboxamide substitution in a subsequent 9-riboside series resulting in

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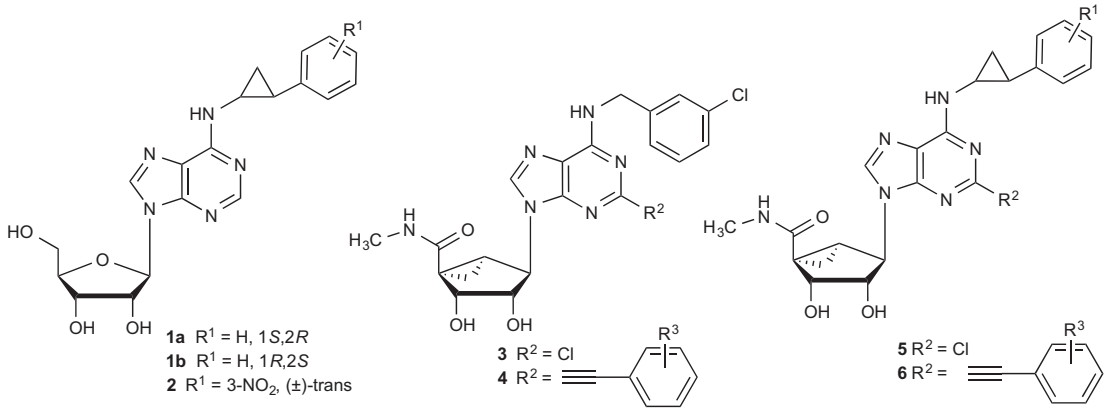

Chart 1. Progression of SAR studies of A_3AR agonists containing bulky N^6 substituents, such as *trans*-2-phenylcyclopropyl, and modification of the ribose as a ring-constrained bicyclic system.

a moderate reduction of A_3AR affinity.[13] In our earlier study of ribosides,[8] there was a 38-fold stereoselectivity of **1a** versus **1b** in A_3AR binding, and a smaller ratio was observed for stereoisomers of a 3-nitro derivative **2**. A large species difference was associated with the N^6-(2-phenylcyclopropyl) group, such that affinity was greatly enhanced in progressing from rat to human.

The current expanded series (general formula **6**) combined rigid C2-arylethynyl and N^6-(2-phenylcyclopropyl) groups. This extended, sterically defined N^6 substitution allows for the exploration of the outer regions of the A_3AR by docking of the ligands to homology models of the receptor. Thus, the effects of the interaction of different N^6 substituents with the normally flexible extracellular loops (ELs) were analyzed by detailed modeling.

Chronic neuropathic pain (NP) is an important unsolved medical need, which is associated with nerve injury and often with diseases such as cancer and diabetes.[14,15] We recently reported that A_3AR agonists have unanticipated efficacy in vivo in various models of chronic but not acute pain.[1] Although N^6-(2-phenylcyclopropyl) substitution in the riboside series greatly reduces the affinity at the murine A_3ARs,[24] we studied one of the new analogues in a mouse model of chronic pain resulting from constriction injury (CCI).[16]

The nucleoside derivatives **9**–**19** (Table 1) were synthesized, characterized and examined in AR binding assays. Compounds **1**, **4**, **5**, **7** and **8**, prepared earlier,[8–11] were included as reference compounds. To synthesize N^6-(2-phenylcyclopropyl) derivatives, protected intermediate **20**[9] was sequentially treated with the appropriate 2-phenylcyclopropylamine to yield **21a**–**h** followed by methylamine to provide **22a**–**h** (Scheme 1). Then, intermediates **22a**–**h** were subjected to Sonogashira coupling with the appropriate arylacetylene in the presence of $PdCl_2(Ph_3P)_2$, CuI and triethylamine to give protected intermediates **23a**–**j**, which upon acid hydrolysis gave the target compounds **10**–**19**. The synthesis of 6-NH_2 derivative **9** will be reported elsewhere. Although most of the entries in Table 1 contain a mixture of stereoisomers, compounds **16**–**19** correspond to products with well-defined (1*R*,2*S* or 1*S*,2*R*) stereochemistry of the N^6-(2-phenylcyclopropyl) group. Compound **15** is the racemic form of pure diastereoisomers **16** and **17**.

The binding assays were based on widely used radioligands using membranes of CHO cells expressing the hA_1AR ([^{3}H]**24**) or hA_3AR ([^{125}I]**26**) or HEK293 cells expressing the hA_{2A}AR ([^{3}H]**25**).[17–19] Most of the nucleosides bound to the hA_3AR with low nanomolar affinity and with minimal binding to the hA_1AR and hA_{2A}AR. Although the A_3AR affinity range of the newly synthesized 2-arylethynyl compounds was somewhat less than the reference 2-chloro-(N)-methanocarba nucleoside **5**, the selectivity remained high, with the significant reduction in affinity at the A_1 and A_{2A}ARs. The unsubstituted, racemic N^6-(2-phenylcyclopropyl) derivative **10** retained a higher A_3AR affinity than the ring-substituted analogues **11**–**15**. However, the 3,4-difluorophenyl analogue **15** was very selective for the A_3AR, and the fluoro substitution was intended to diminish aromatic oxidation in vivo.[25] A comparison of the A_3AR affinity of diastereomeric pairs showed a 4-fold preference for the 1*S*,2*R* diastereoisomer in the pair of 2-phenylethynyl derivatives **16** and **17**, but there was no difference in A_3AR affinities of the 2-(2-pyridylethynyl) diastereomeric pair **18** and **19**.

Therefore, we have used a combination of adenine substitutions and a ribose-like scaffold, all of greatly reduced conformational freedom, to help analyzing ligand recognition in the outer regions of the A_3AR. The environment of the receptor-bound N^6-(2-phenylcyclopropyl) group was explored by docking to an A_3AR homology model. We used our previously-reported homology model of the hA_3AR,[9,11] which was based on a hybrid A_{2A}AR-β_2 adrenergic receptor template. The general methodology used for homology modeling (MOE homology modeling tool)[20] and ligand docking (Glide module of the Schrödinger Suite)[17] has been described before,[9,11] and specific methodological details are reported in the Supporting Information. In the present study, after the first round of docking to the initial A_3AR homology model and selection of the best docking pose for derivative **7**, we performed refinement of the portion of the second EL in contact with the ligand (from Gln167 to Arg173), using the Prime module of the Schrödinger Suite,[21] to optimize its conformation around the N^6 substituent. This step was followed by a second round of docking of all derivatives to the optimized model, to identify the final proposed docking poses. All of the final nucleosides were also subjected to docking simulations at a hA_{2A}AR crystal structure (PDB ID: 2YDV)[3] to explore the reasons for ligand selectivity.

As expected, docking results at the hA_3AR for all the analyzed derivatives showed the pseudo-sugar moiety, adenine N7 and exocyclic NH participating in highly conserved H-bonding interactions with key residues of the binding site (Fig. 1), such as Thr94 (3.36), Asn250 (6.55), Ser271 (7.42) and His272 (7.43) (numbers in parenthesis follow the Ballesteros–Weinstein notation).[22] The same H-bonding network has been observed in the agonist-bound hA_{2A}AR crystal structures and is supposed to be important for receptor activation.[2,3] Another important binding feature for AR ligands, both agonists and antagonists, is the π–π stacking interaction with an aromatic residue in EL2 (Phe168 at the hA_3AR). Thus, considering that our quite rigid compounds showed all these critical interactions when docked to the A_3AR model, we could analyze in more detail the possible interactions with the less defined EL regions of the receptor. Docking results showed the rigid C2-arylethynyl