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REVIEW ARTICLE

NUCLEOSIDE-BASED ADENOSINE A_3 RECEPTOR ANTAGONISTS AS DRUG CANDIDATES

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ABSTRACT

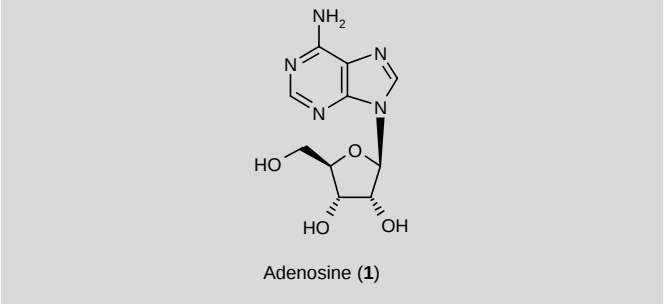
Purine nucleoside derivatives that are selective ligands for the A_3 adenosine receptor (AR) have been structurally modified such that their ability to activate the receptor is lost while retaining high binding affinity. This loss of efficacy in otherwise selectively binding nucleosides has been shown to result in antagonism of the effects of known agonists in functional assays. Modification of the ribose moiety has been the most effective strategy to accomplish this aim. Steric constraints have been introduced, as well as replacement of the various hydrogen bond-donating groups, to achieve a reduction in efficacy. High selectivity has recently been achieved for such nucleoside-based A_3 AR antagonists. Thus, it is now possible to compare nucleoside-based A_3 AR antagonists with well-characterized heterocyclic nonpurine antagonists as clinical candidates for the treatment of glaucoma, asthma and inflammation.

INTRODUCTION

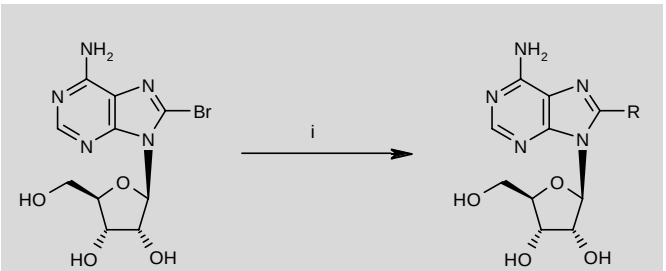
Adenosine (**1**) is a natural mediator that regulates cell signaling through specific interactions with adenosine receptors (ARs). Most of the actions of extracellular adenosine are mediated by four AR subtypes, termed A_1, A_{2A}, A_{2B} and A_3. Agonists and antagonists of each of the subtypes are associated with preclinical activity in various diseases and conditions. For example, the A_3 AR, which is the most recently identified AR subtype, is a promising therapeutic target for the development of clinically efficacious drug candidates for ischemic and inflammatory diseases (1-3). A_3 AR agonists are effective for the treatment of rheumatoid arthritis, cardiac ischemia, cerebral ischemia and cancer (4-6), and A_3 AR antagonists have been investigated as antiasthma, antiglaucoma and antiinflammatory agents (7-9), as described in several recent reviews (10, 11). This review is focused exclusively on the synthetic and medicinal chemistry aspects of recently reported nucleoside-based A_3 AR antagonists. These AR-targeting molecules now complement a large group of heterocyclic nonpurine antagonists that have been extensively explored pharmacologically and tested in preclinical models (12).

NUCLEOSIDE-BASED ADENOSINE A_3 RECEPTOR ANTAGONISTS

Adenine and adenosine derivatives bearing an incomplete or truncated ribose moiety at the 9-position (13) have been explored extensively as antagonists of A_1 receptors. However, modified adenosine derivatives that act as selective A_3 AR antagonists have been reported only recently. The first purine nucleoside derivative to be characterized as an A_3 AR antagonist was a 1,3-dibutylxanthine-7-(3′-deoxyriboside) derivative (14). Cristalli et al. (15) reported the first



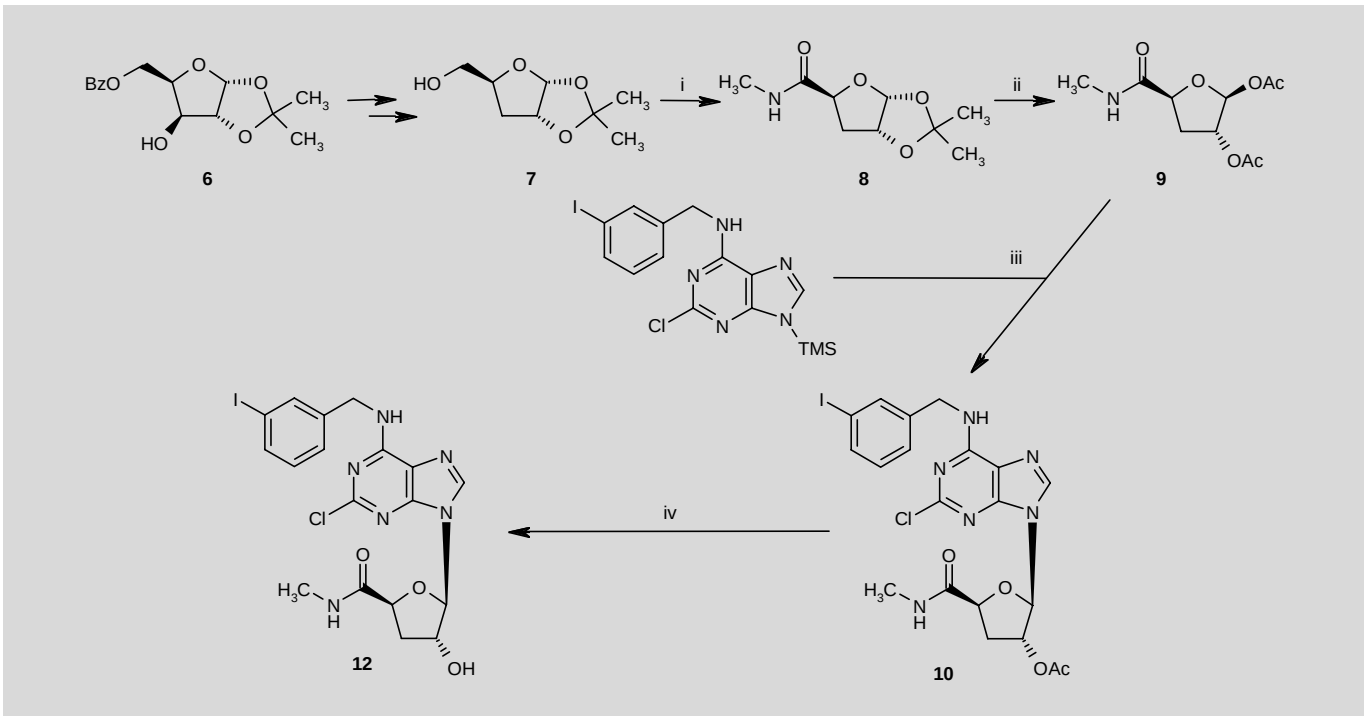
Adenosine (**1**)



2

3 R = 1-hexynyl
4 R = 2-phenylethynyl
5 R = 3-oxo-3-phenyl-1-propynyl

Scheme 1. Reagents: (i) Alkynes, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF.



Scheme 2. Reagents: (i) a, RuO_2, $NaIO_4$; b, MeOH, EDAC, DMAP; c, $MeNH_2$, THF; (ii) H_2SO_4, Ac_2O, AcOH; (iii) TMSOTf $C_2H_2Cl_2$; (iv) NH_3/MeOH.

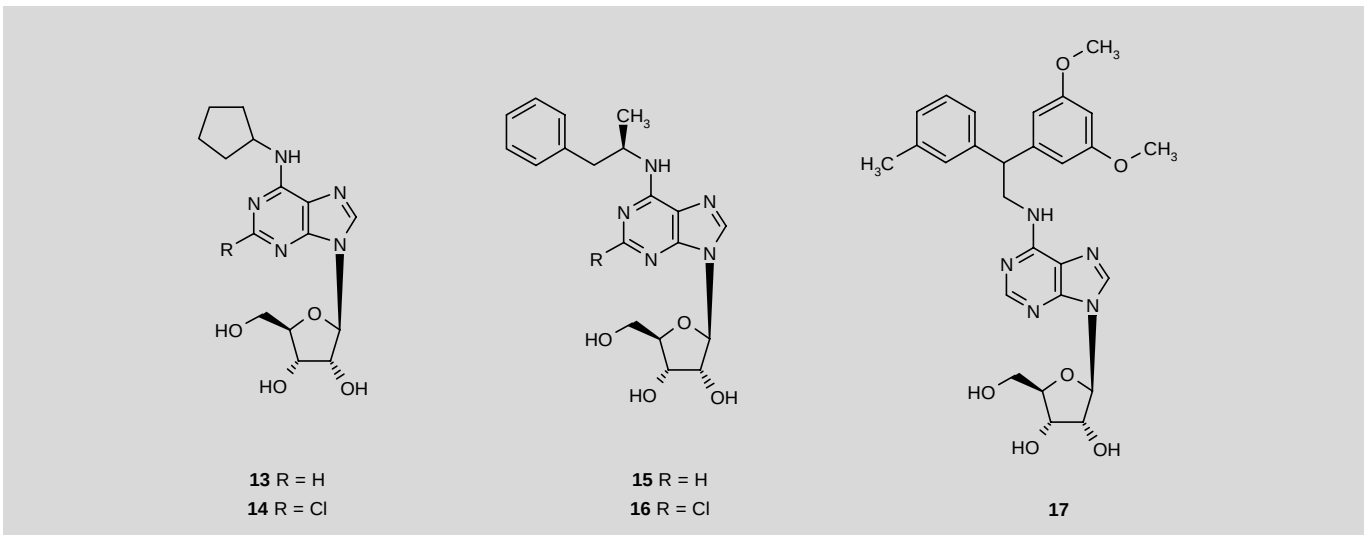


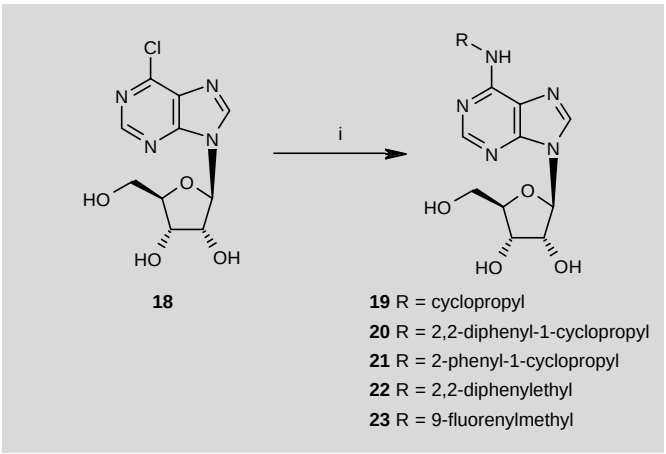
Figure 1.

series of adenosine derivatives having an unmodified ribose moiety as A_3 AR antagonists. They synthesized 8-substituted adenosines from the commercially available 8-bromoadenosine **2** by treatment with various alkynes and alkenes in the presence of bis(triphenylphosphine)palladium dichloride, copper iodide and triethylamine to give compounds **3**-**5** (Scheme 1). These nucleosides were evaluated at human recombinant ARs stably expressed in Chinese hamster ovary (CHO) cells using radioligand binding assays. The binding studies indicated that compounds **3** and **4** exhibited A_3 AR affinity in the high nanomolar range (K_i = 650 and 790 nM, respectively), while they were nearly inactive at other AR subtypes. On the other hand, the phenylketopropynyl analogue **5** showed weak activity in binding to the A_3 AR (K_i = 9.83 μM). The data from the evaluation of compounds **3**-**5** alone or in the presence of the nonselective AR agonist NECA for stimulation or inhibition of guanosine 5′-(γ-thio)triphosphate ([^{35}S]GTPγS) binding in membranes of A_3 AR-expressing CHO cells are even more remarkable. Compounds **3-5** do not stimulate basal [^{35}S]GTPγS binding but inhibit NECA-stimulated

binding to various extents. The 8-phenylethynyl analogue **4** proved to be the most potent A_3 antagonist in the series, with 73% inhibition of NECA-stimulated binding. It was also noted that the presence of the same alkynyl chain at the 8-position of 9-ethyladenine led to a relatively selective antagonist of the A_3 subtype (K_i = 0.086 μM).

Jacobson's group reported that replacement of the 2′- and 3′-hydroxyl groups of known A_3 agonists with hydrogen failed to provide A_3-selective antagonists (16). The formalistic removal of the 3′-hydroxyl group from IB-MECA resulted in compound **12**, which was a full agonist at the rat A_3 receptor and moderately selective for this subtype (13). This nucleoside was prepared by condensation of the 3′-deoxy sugar **9** with silylated adenine by a modified Vorbrüggen method to give compound **10**, which upon deprotection of the acetyl group with methanolic ammonia afforded 3′-deoxy-2-chloro-IB-MECA (**12**; Scheme 2). Replacement of the 2′-hydroxyl group with fluorine was highly detrimental to receptor binding, whereas replacement of the 3′-hydroxyl group with fluorine provided antagonists (17).

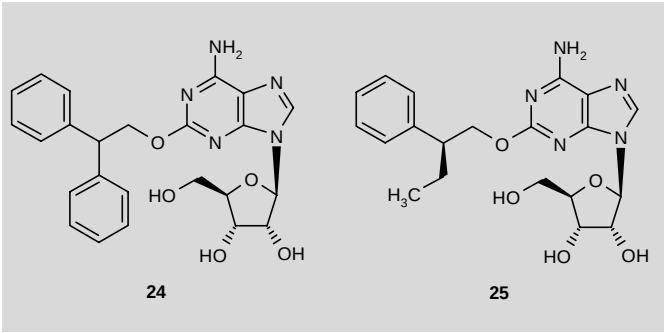
A successful approach consisted of either adding substituents to adenosine derivatives or rigidifying the nucleosides to reduce their intrinsic efficacy. Gradually, with more systematic studies of structure–activity relationships (SAR) upon substitution of adenosine at the N^6-, ribose (18) and C2 adenine (19) positions, it became apparent that efficacy at A_3 receptors is more easily reduced upon structural modification than efficacy at other subtypes. In some cases, N^6-substitution of adenine having 5′-OH with larger groups (e.g., substituted benzyl groups or a larger cycloalkyl ring) reduced maximal efficacy, leading to partial agonist activity at the A_3 receptor. For example, Cl-R-PIA (**16**) is a partial agonist and N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl] (DPMA, **17**) is an antagonist at the human A_3 AR (Fig. 1) (18). It is interesting to note that 2-chloroadenosine is a full agonist while 2-fluoroadenosine is a partial agonist (16).



18

19 R = cyclopropyl
20 R = 2,2-diphenyl-1-cyclopropyl
21 R = 2-phenyl-1-cyclopropyl
22 R = 2,2-diphenylethyl
23 R = 9-fluorenylmethyl

Scheme 3. Reagents and conditions: (i) RNH_2, EtOH, Et_3N, reflux.



24 **25**

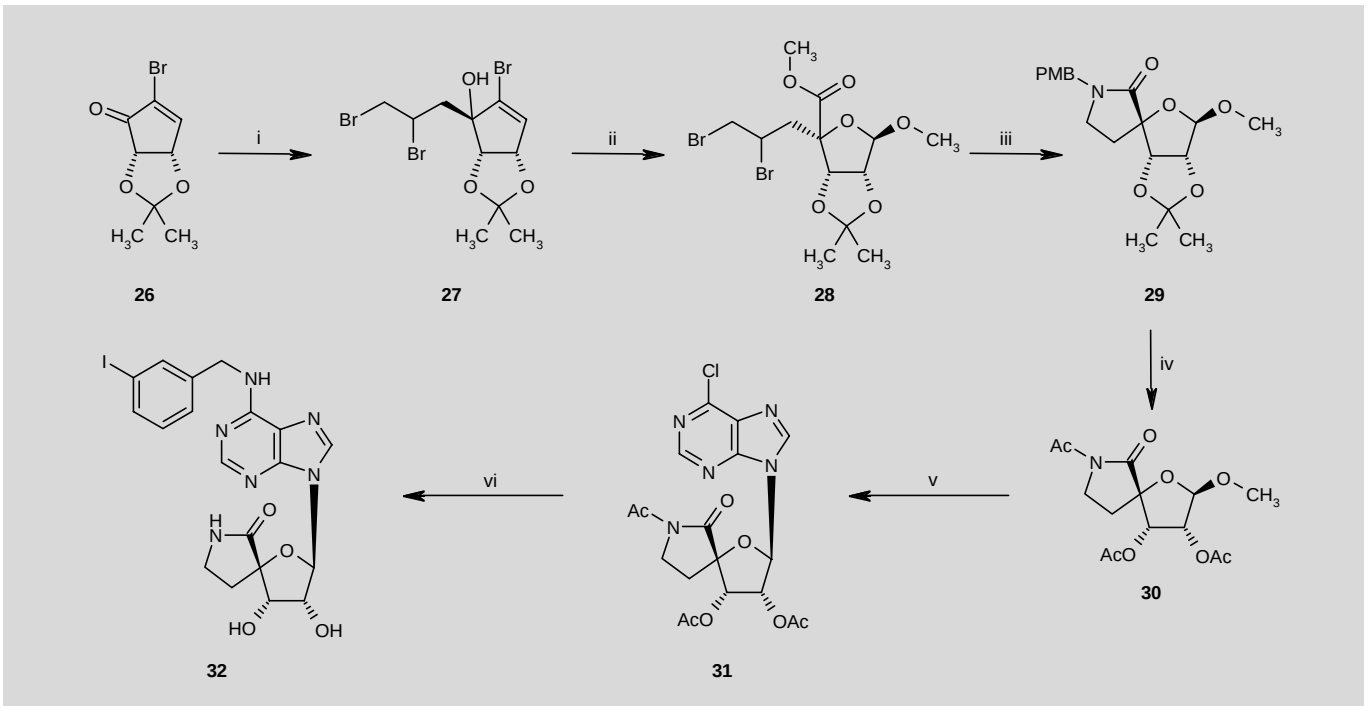
Figure 2.

Based on SAR data, over the years it was concluded that the efficacy of adenosine derivatives at the A_3 AR, in contrast to other AR subtypes, appears to be more dependent on subtle structural changes. For example, the chloro substitution at the 2-position of the known A_1 receptor agonists CPA (**13**) and R-PIA (**15**) (Fig. 1) led to A_3 antagonism and partial agonism, respectively, by their chloro counterparts CCPA (**14**) and **16** (16). Both 2′- and 3′-nucleoside substitution had pronounced effects on the efficacy of A_3 receptor ligands, although the effect of 2′-substitution was more dramatic. The 4′-thio substitution of oxygen also reduced efficacy, depending on other substitutions. The 4′-thio substitution is discussed further below. Both the N^6-methyl and N^6-benzyl groups may contribute to A_3 AR affinity and selectivity; however, an N^6-benzyl group but not an N^6-methyl group reduces A_3 AR efficacy (19). The combination of 2-chloro and N^6-benzyl substitutions appeared to reduce efficacy further than either modification alone. Other examples of simple derivatives of adenosine that have fully reduced efficacy at A_3 AR while perhaps remaining full agonists at other subtypes are CCPA (**14**) (also a potent A_1 agonist) and DPMA (**17**) (also a moderately selective A_{2A} agonist) (18, 20). While steric constraint of the ribose moiety, especially near the 5′-position, is associated with loss of A_3 efficacy, for hydrophobic N^6-substitutions there are examples in which steric rigidification is associated with the restoration of agonist efficacy.

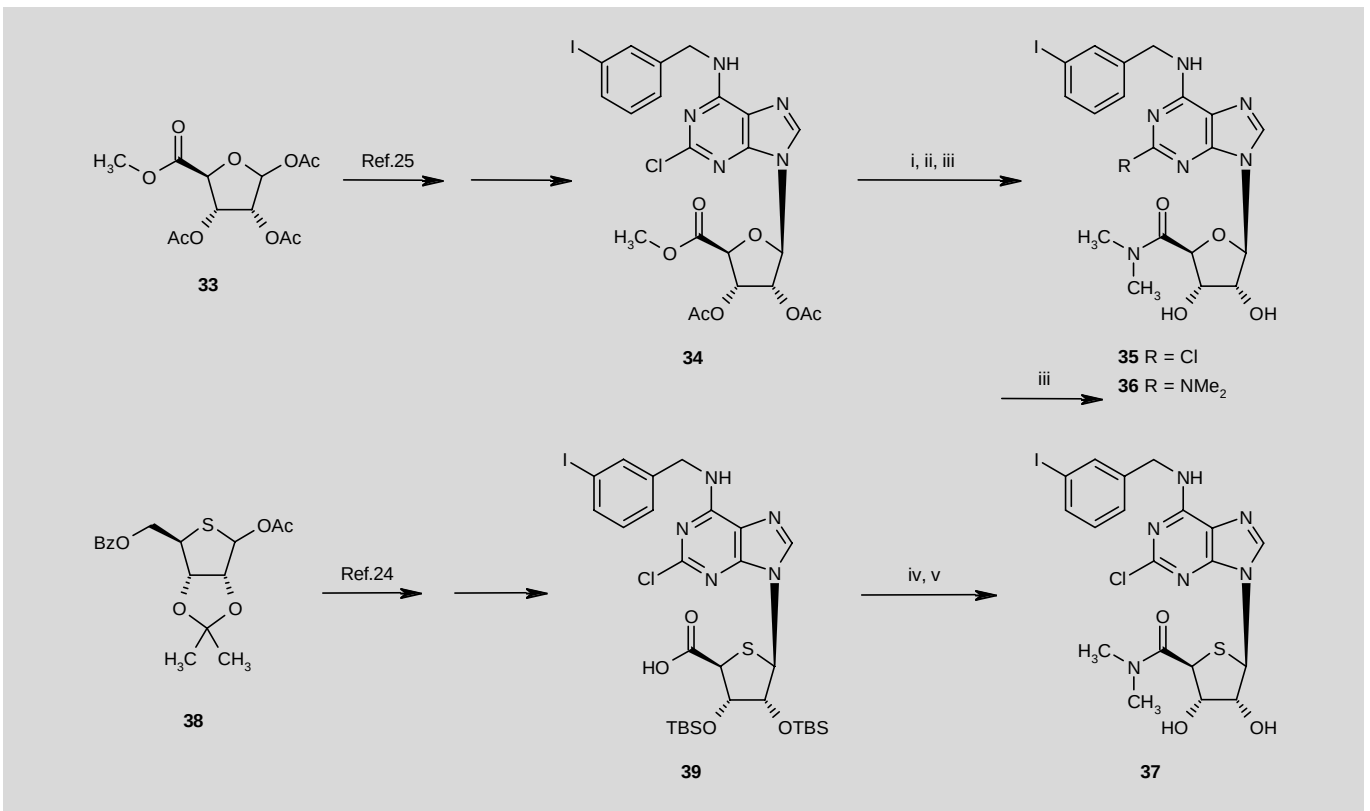
The SAR at the human A_3 receptor for the N^6-phenylethyl derivatives, including sterically constrained N^6-(2-phenylcyclopropyl) analogues, has been thoroughly explored from this perspective by Tchilibon et al. (Scheme 3) (20). In their studies, the N^6-cyclopropyl analogue **19** was found to be an A_3 AR antagonist, and adding one or two phenyl rings at the 2-position of the cyclopropyl moiety restored efficacy. Also N^6-(2,2-diphenylethyl)adenosine (**22**) was found to be an antagonist at the human A_3 AR, while the corresponding N^6-fluorenylmethyl analogue **23** was a full agonist. They also found that the affinity and selectivity of these nucleosides are highly dependent on the species examined and on the distal aryl substitution. In their synthetic route, the appropriately substituted amines were treated directly with compound **18** to give the desired adenosine derivatives.

Jacobson et al. (19) found that adenosine derivatives **24** and **25**, having a sterically bulky substitution of a 2,2-diphenylethyloxy or an (*S*)-2-phenylbutyloxy group at the 2-position of adenosine, were antagonists of A_3 AR (Fig. 2).

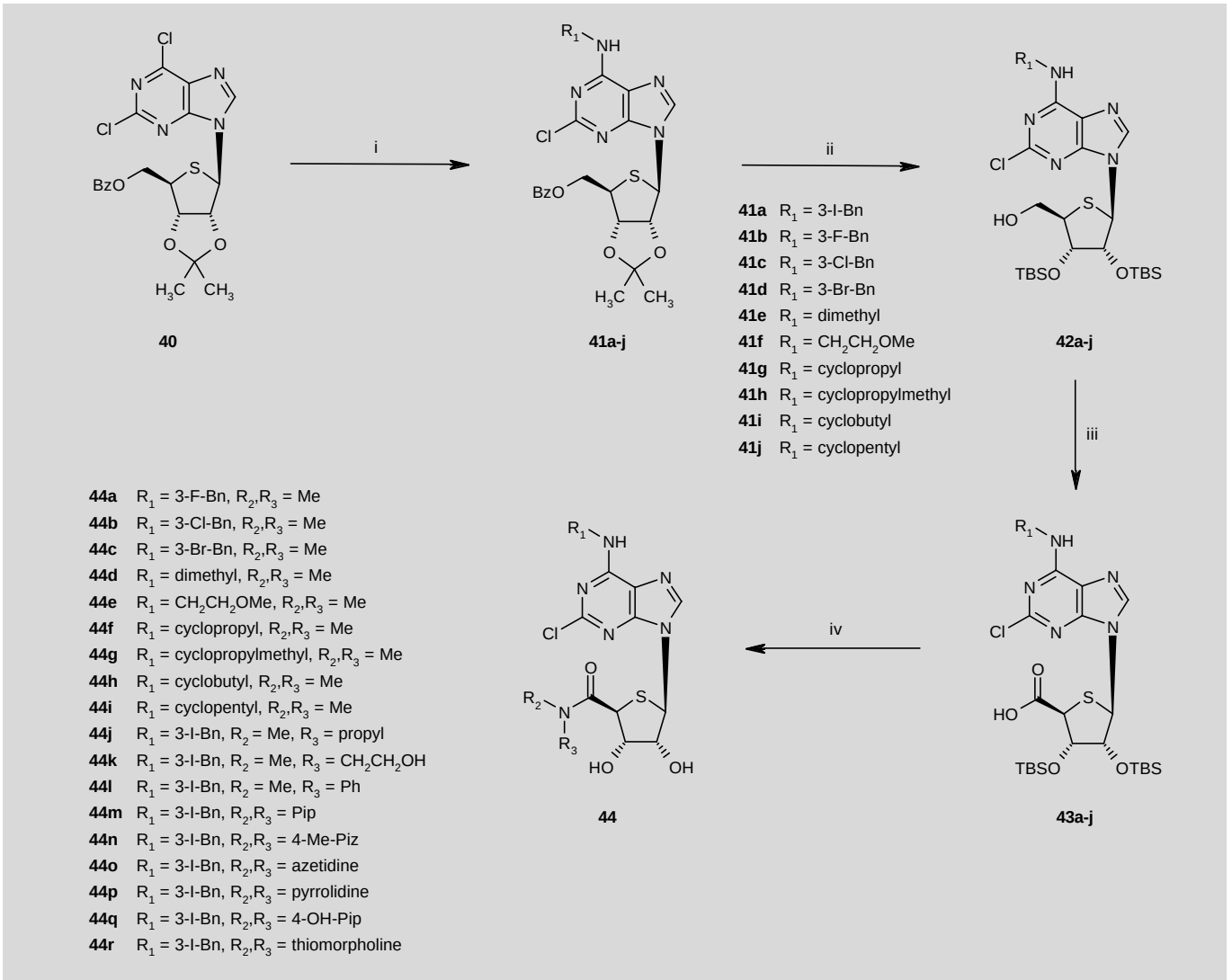
After several modifications on the nucleobase moiety of adenosine and its 5′-uronamide derivatives, Jacobson's group found that rigidification of the ribose ring moiety in the region of a 5′-uronamide group reduced A_3 efficacy (20). They reported that the spirolactam derivative MRS 1292 (**32**) is a potent and highly selective nucleoside-based A_3 receptor antagonist. This spiro analogue was prepared as described by Gao et al. starting from the bromocyclopentenone



Scheme 4. Reagents and conditions: (i) a, Allylmagnesium bromide; b, Br_2, Et_3N; (ii) a, O_3, MeOH, pyridine; b, MeOH, *p*-TsOH; (iii) a, Zn, MeOH; b, O_3, CH_2Cl_2; c, *p*-MeO-$PhCH_2NH_2$, $NaCNBH_3$; (iv) a, Ceric ammonium nitrate, MeCN; b, K_2CO_3, 18-crown-6, MeOH; c, HCl, MeOH; 65 °C; d, Ac_2O, Et_3N; (v) HMDS, 6-chloropurine, TMSOTf, MeCN, 85 °C; (vi) a, NH_3/MeOH; b, 3-I-$PhCH_2NH_2$.HCl, Et_3N, *t*-BuOH, 85 °C.



Scheme 5. Reagents and conditions: (i) K_2CO_3, MeOH, rt; (ii) AcOH; (iii) 40% aq. Me_2NH, rt; (iv) Me_2NH, EDC, HOBT, DIPEA; (v) TBAF, THF.



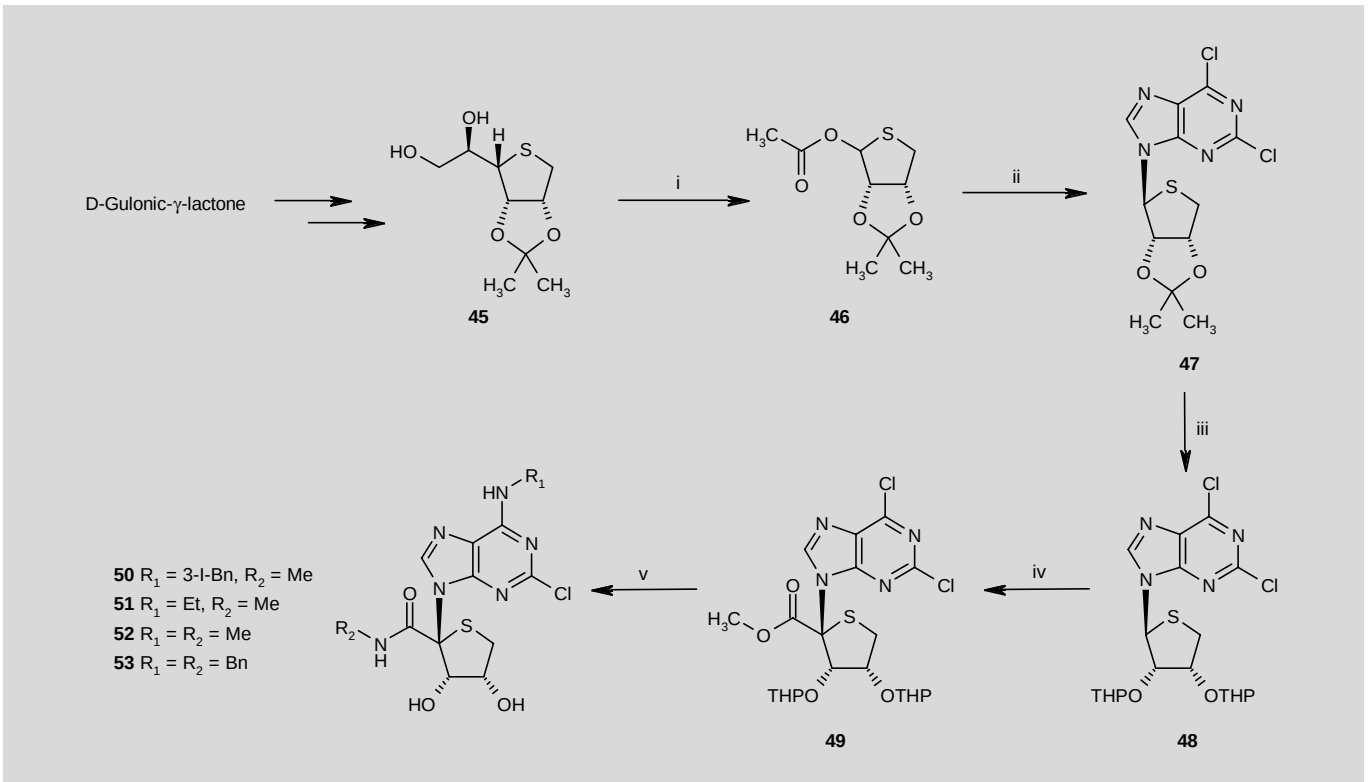
Scheme 6. Reagents and conditions: (i) R_1NH_2, Et_3N, EtOH, rt; (ii) a, 80% AcOH, 70 °C; b, TBSOTf, pyridine, 50 °C; c, NaOMe, MeOH; (iii) PDC, DMF; (iv) a, R_2R_3NH, EDC, HOBT, DIPEA, CH_2Cl_2; b, TBAF, THF.

derivative **26** (Scheme 4). The key step in the synthesis was intramolecular cyclization. Thus, treatment of compound **26** with allyl magnesium bromide followed by bromination gave the intermediate **27**. Ozonolysis of **27** and subsequent acid-catalyzed cyclization in the presence of *p*-TsOH generated the ribose moiety, leading to compound **28**. Reductive elimination of bromine in **28** followed by ozonolysis and amination with *p*-methoxybenzylamine (PMB) provided the spirolactam **29**. Deprotection of the PMB and isopropylidene group followed by reprotection with an acetyl group in the presence of Ac_2O and triethylamine gave the glycosyl donor **30**. Condensation of **30** with silylated 6-chloropurine in the presence of TMSOTf provided the condensed product **31**. Deprotection of the acetyl group upon treatment with methanolic ammonia and treatment of the resulting compound with 3-iodobenzylamine hydrochloride in the presence of triethylamine furnished MRS 1292 (**32**).

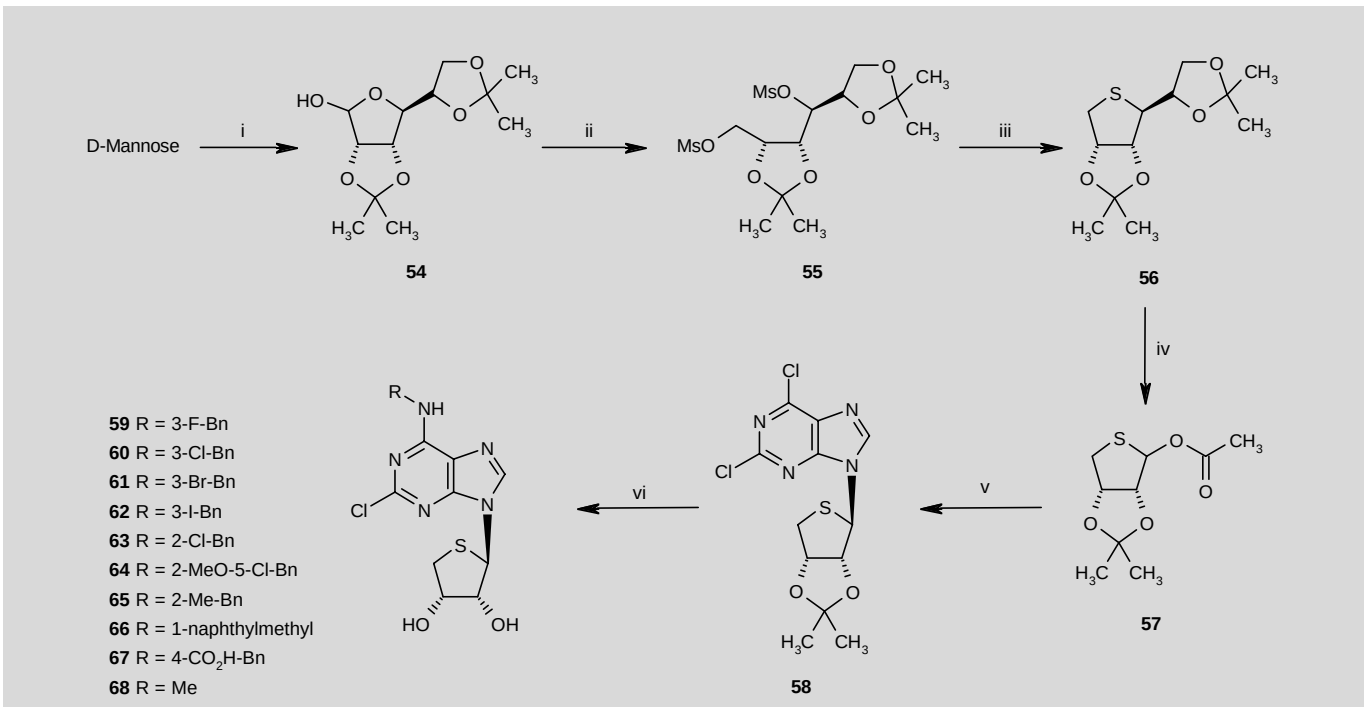
Jacobson's group recently reported (22) a novel method to convert the highly selective agonists of the A_3 AR Cl-IB-MECA (23) and its 4′-thio analogue (24) into selective antagonists simply by appending a second *N*-methyl group on the 5′-uronamide position. Synthetic routes to the *N*,*N*-dimethylamide derivatives **35-37** are depicted in Scheme 5. Deacetylation of compound **34** (25) with K_2CO_3 followed by aminolysis with dimethylamine gave compound **35** and the side product **36**. The 4′-thio analogue **37** was prepared from the acid derivative **39** (24) by condensation with dimethylamine in the presence of EDC, HOBT and DIPEA followed by desilylation with TBAF.

Binding assays indicated high affinities for **35** and **37** for the human A_3 AR (K_i = 29 and 15 nM, respectively), and the compounds were at least 100-fold selective for binding to the human A_3 AR compared to other subtypes. Compound **36** also showed A_3 AR selectivity but lower affinity. Compounds **35** and **37** exhibited pure antagonist activity at the A_3 AR.

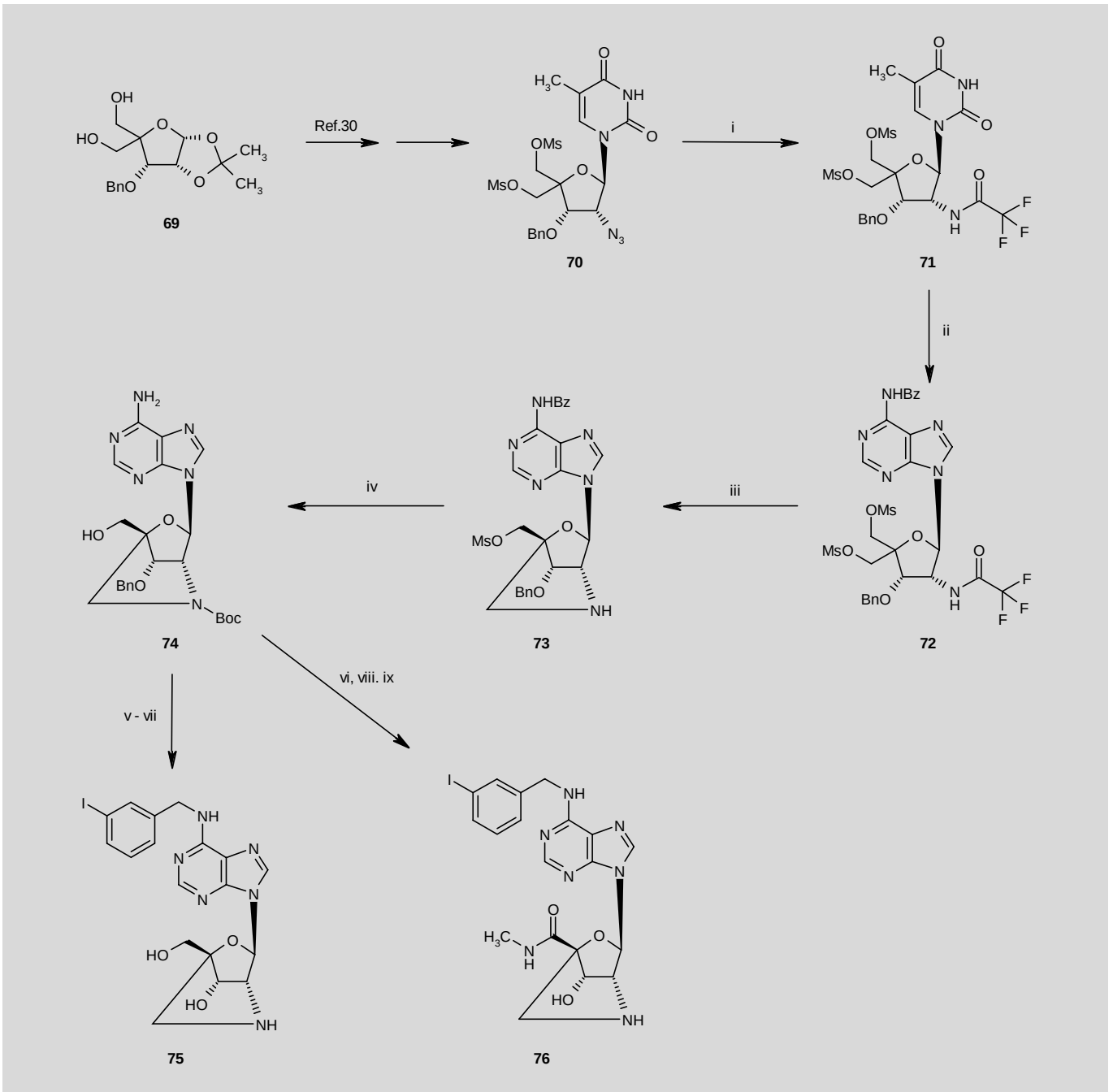
Based on the antagonist activity of compounds **35** and **37** (22), Jeong's group carried out a systematic SAR study (26) on a series of



Scheme 7. Reagents and conditions: (i) $Pb(OAc)_4$, EtOAc, rt; (ii) HMDS, 2,6-dichloropurine, TMSOTf, $C_2H_2Cl_2$, 70 °C; (iii) a, 2M HCl, THF; b, DHP, *p*-TsOH, CH_2Cl_2; (iv) LiHMDS, $ClCO_2Me$, −78 °C, THF; (v) a, R_1NH_2/(then $MeNH_2$, THF for **50** and **51**), EtOH; b, *p*-TsOH.



Scheme 8. Reagents and conditions: (i) 2,2-Dimethoxypropane, CSA, acetone, rt; (ii) a, $NaBH_4$, EtOH, rt; b, MsCl, Et_3N, CH_2Cl_2, rt; (iii) Na_2S, DMF, 80 °C; (iv) a, 60% AcOH, rt; b, $Pb(OAc)_4$, EtOAc; (v) HMDS, 2,6-dichloropurine, 170 °C; (vi) a, 2N HCl, THF, rt; b, RNH_2, Et_3N, EtOH, rt.



Scheme 9. Reagents and conditions: (i) a, Me_3P, THF; b, TFA; (ii) N^6-benzoyladenine, BSA, $C_2H_2Cl_2$; (iii) LiOH, THF/H_2O; (iv) a, Boc_2O, Et_3N, CH_2Cl_2; b, NaOBz, DMSO; c, NH_3/MeOH; (v) HCO_2NH_4, $Pd(OH)_2$/C, MeOH; (vi) a, Iodobenzyl bromide, DMF; b, NH_4OH, MeOH, 50 °C; (vii) TFA, rt; (viii) a, $PhI(OAc)_2$, TEMPO, $MeCN/H_2O$; b, SO_2Cl, EtOH; c, $MeNH_2$, MeOH; (ix) MsOH, CH_2Cl_2, rt.

5′-*N*,*N*-dialkyluronamide derivatives in which the N^6- and 4′-hydroxymethyl groups were modified. Compound **40** was prepared by this method (24), which upon treatment with various alkyl or arylalkylamines gave the N^6-substituted analogues **41a-j** (Scheme 6). Compounds **41a-j** were converted to the TBS derivatives by deprotection with 80% acetic acid followed by reprotection with TBSOTf. The resulting compounds were treated with sodium methoxide in methanol to give the key intermediates **42a-j**. The primary hydroxyl group of **42a-j** was oxidized with PDC in DMF to give the corresponding acids **43a-j**, which were coupled with various dialkyl- or cycloalkylamines in the presence of EDC and HOBt to give amides **44a-r**. The radioligand binding results indicated that, among all the synthesized nucleosides, compound **44c** exhibited the highest affinity (K_i = 9.32 nM) for the human A_3 AR and very low affinities for other AR subtypes.

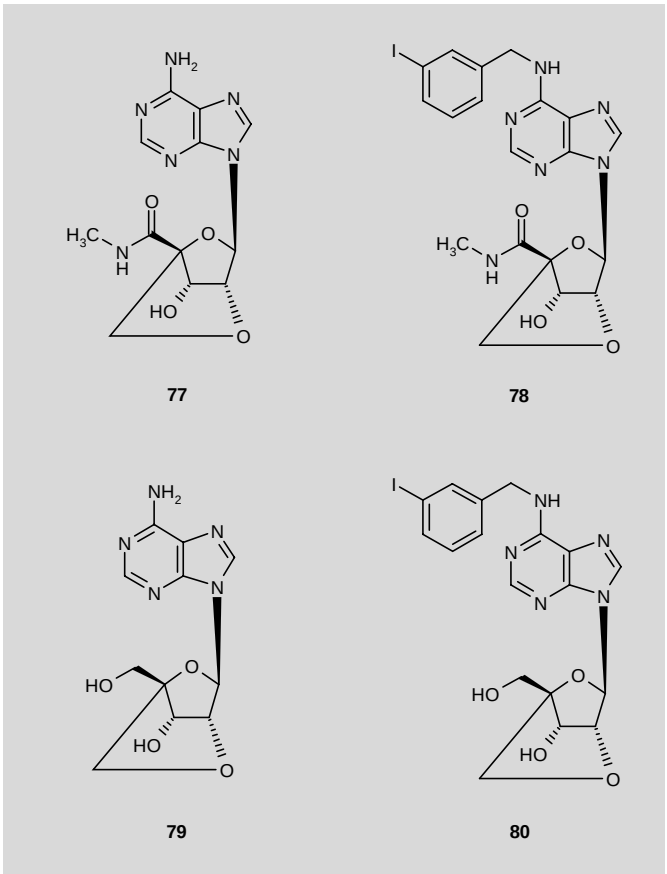


Figure 3.

Functional assays indicated that all of these analogues completely lack agonist activity at the A_3 AR and display pure A_3 AR-antagonist activity. It was evident that removal of the hydrogen bond-donating ability of the 5′-uronamide of such derivatives was essential for the pure A_3 AR antagonism. Steric factors at the 5′-region also play an important role in the binding of the nucleosides to the human A_3 AR.

With the objective of obtaining better compounds for use as A_3 AR antagonists, Jeong's group (27) designed a new class of compounds, **50-53** (Scheme 7), in which they functionalized the 1′-position by placing the 5′-uronamide group at the 1′-position. The thiosugar derivative **45** was prepared from D-gulonic-γ-lactone following their previously reported method (24). Oxidative cleavage of diol **45** in the presence of lead tetraacetate provided the acetate derivative **46**, in which a three-step conversion (cleavage, oxidation and decarboxylation) occurred in a single step. Condensation of compound **46** with the silylated 2,6-dichloropurine intermediate in the presence of TMSOTf provided the desired β-anomer **47** exclusively. Due to a problem in removing the isopropylidene group at the final stage, this group was deprotected with 2M HCl followed by reprotection as THP ether to give compound **48**. In order to introduce the amide group at the 1′-position of the nucleoside, compound **48** was treated with LiHMDS in the presence of methyl chloroacetate, providing the desired compound **49** stereoselectively. The lithiated intermediate may be coordinated with the N^3-nitrogen of the purine ring and force the electrophile to come from the a face to give exclusively the α ester derivative **49**. Aminolysis of **49** with various amines followed by deprotection of the THP group furnished the target nucleosides **50-53**. Radioligand binding studies indicated that all of the synthesized nucleosides were devoid of measurable binding affinity for all AR subtypes at up to 10 μM. It appears that the 1′-uronamide group may form strong intramolecular hydrogen bonding with the 2′-hydroxyl group, hindering a proper binding interaction of the synthesized molecules with the binding site of the receptor.

Recently, Jeong et al. (28) have also reported a novel class of 4′-thionucleoside templates that were highly potent and selective A_3 AR antagonists. The hypothesis underlying their strategy was that completely removing the 5′-hydroxymethyl group might impede the conformational change required for receptor activation. Truncation of 4′-thioadenosine analogues at the 4′-carbon as a means of converting full A_3 AR agonists to antagonists was recently explored in greater detail by Jeong's group (32). The 2-H series and 2-Cl series were found to have comparable affinities and A_3 AR selectivity. The synthetic route for the truncated nucleosides is represented in Scheme 8. Thus, D-mannose was protected with an isopropylidene group in the presence of 2,2-dimethoxypropane and camphorsulfonic acid (CSA) in acetone to give **54**, which upon sodium borohydride reduction followed by mesylation gave the dimesylate **55**. Cyclization of **55** with anhydrous sodium sulfide in DMF yielded the thiosugar **56**, which underwent a selective acid hydrolysis followed by oxidative cleavage of the resulting diol in the presence of lead tetraacetate to give the glycosyl donor **57**. Base condensation of **57** with 2,6-dichloropurine in the presence of TMSOTf provided the β-anomer **58** as a single stereoisomer. Deprotection of the isopropylidene group with 2N HCl and amination of the resulting compound with various alkyl or arylalkylamines afforded the N^6-substituted D-4′-thionucleosides **59-68**. Most of the synthesized compounds exhibited high binding affinity for the A_3 AR, with extremely high selectivity over other AR subtypes. Among the compounds tested, compound **60** (R = 3-chlorobenzyl) showed the highest binding affinity (K_i = 1.66 ± 0.9 nM) at the human A_3 AR, with extremely low affinities for other ARs. Although compounds **68** (R = Me; K_i = 3.69 ± 0.25 nM) and **62** (R = 3-iodobenzyl; K_i = 4.16 ± 0.50 nM) exhibited high affinities for the human A_3 AR, these compounds were less selective than **60**. Compound **62** also bound with high affinity to the rat A_3 AR. Compound **67** substituted with a polar carboxylic acid on the aromatic ring was totally devoid of binding affinity for the human A_3 AR, indicating that hydrophobic N^6-substitution was essential for the binding interaction. All compounds tested were found to be full antagonists in a cAMP functional assay at the human A_3 AR.

Ravn et al. (29) reported antagonist activity for a type of adenosine analogues based on the bicyclo[2.2.1]heptane scaffold of locked nucleic acid (LNA). The synthetic route for the 2′-amino-LNA adenosine analogue is depicted in Scheme 9. The intermediate **70**, prepared from compound **69** by a known method (30, 31), was reduced in the presence of trimethyl phosphine followed by *N*-acylation with trifluoroacetic anhydride to give compound **71**. Transnucleosidation of **71** with N^6-benzoyladenine under Vorbrüggen conditions provided the corresponding β-purine nucleoside derivative **72**. Hydrolysis of the trifluoroacetyl group in **72** led to subsequent ring closure to give the fully protected 2′-amino-LNA-substituted adenosine nucleoside **73**. Protection of the secondary amino group with Boc_2O followed by deprotection of the mesyl and benzoyl group gave the intermediate

74. For the synthesis of the 5′-hydroxyl-LNA adenosine analogue **75**, compound **74** was debenzylated followed by *N*-benzylation with 3-iodobenzyl bromide and subsequent Boc deprotection with trifluoroacetic acid to furnish the final nucleoside **75**. Furthermore, for the synthesis of the 5′-uronamide analogue, intermediate **74** was first *N*-benzylated with 3-iodobenzyl bromide and the resulting compound was converted to 5′-uronamide followed by deprotection of the Boc group with methanesulfonic acid to give compound **76**.

This group also synthesized a series of 2′-*O*-LNA-substituted adenosine derivatives **77-80** (Fig. 3). All of the compounds synthesized were tested for activity at the human A_3 AR. It was observed that among the 2′-*O*-LNA adenosine analogues **77-80**, all compounds completely lacked agonist activity, and compound **80** had only moderate antagonist activity (150 nM) at the A_3 AR. In the 2′-amino-LNA adenosine analogues, it was worth noting that compounds **75** (8.2 nM) and **76** (12 nM) exhibited superior antagonist activity at the A_3 AR compared to compound **80**.

The approach of truncation of adenine nucleosides for converting human A_3 AR agonists to antagonists was recently extended by Jacobson's group to a series of (*N*)-methanocarba nucleosides, which contain a bicyclo[3.1.0]hexane ring system in place of the ribose moiety (33). N^6-3-Halobenzyl and related arylalkyl derivatives in this series were potent human A_3 AR antagonists, with binding K_i values of 0.7-1.4 nM and selectivity typically of > 1,000-fold in comparison to the human A_1 and A_{2A} ARs. Functional studies using rat and mouse A_3 ARs were not conducted.

CONCLUSIONS

Modification of the ribose moiety has been the most fruitful strategy for accomplishing the aim of turning A_3 AR agonists into antagonists. Steric constraints have been introduced, as well as replacement of the various hydrogen bond-donating groups, to achieve a reduction in efficacy. High selectivity has recently been achieved for such nucleoside-based A_3 AR antagonists, e.g., 5′-truncated 4′-thio analogues. Thus, it is now possible to compare nucleoside-based A_3 AR antagonists with well-characterized heterocyclic nonpurine antagonists as clinical candidates for the treatment of glaucoma, asthma and inflammation.

ACKNOWLEDGEMENTS

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