Published in final edited form as:
Curr Top Med Chem. 2004 ; 4(8): 855–862.

Partial Agonists for A_3 Adenosine Receptors

Zhan-Guo Gao* and **Kenneth A. Jacobson**
Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA

Abstract

Selective agonists for A_3 adenosine receptors (ARs) could potentially be therapeutic agents for a variety of disorders, including brain and heart ischemic conditions, while partial agonists may have advantages over full agonists as a result of an increased selectivity of action. A number of structural determinants for A_3AR activation have recently been identified, including the N^6-benzyl group, methanocarba substitution of ribose, 2-chloro and 2-fluoro substituents, various 2'- and 3'-substitutions and 4'-thio substitution of oxygen. The 2-chloro substitution of CPA and R-PIA led to A_3 antagonism (CCPA) and partial agonism (Cl-R-PIA). 2-Chloroadenosine was a full agonist, while 2-fluoroadenosine was a partial agonist. Both 2'- and 3'- substitutions have a pronounced effect on its efficacy, although the effect of 2'-substitution was more dramatic. The 4-thio substitution of oxygen may also diminish efficacy, depending on other substitutions. Both N^6-methyl and N^6-benzyl groups may contribute to the A_3 affinity and selectivity; however, an N^6-benzyl group but not an N^6-methyl group diminishes A_3AR efficacy. N^6-benzyl substituted adenosine derivatives have similar potency for human and rat A_3ARS while N^6-methyl substitution was preferable for the human A_3AR. The combination of 2-chloro and N^6-benzyl substitutions appeared to reduce efficacy further than either modification alone. The A_{2A}AR agonist DPMA was shown to be an antagonist for the human A_3AR. Thus, the efficacy of adenosine derivatives at the A_3AR appears to be more sensitive to small structural changes than at other subtypes. Potent and selective partial agonists for the A_3AR could be identified by screening known adenosine derivatives and by modifying adenosine and the adenosine derivatives.

INTRODUCTION

Four adenosine receptor (AR) subtypes have been cloned and pharmacologically characterized; they are termed A_1, A_{2A}, A_{2B} and A_3 [1]. The A_1 and A_3 ARs preferentially couple to the $G_{i/o}$ family of G proteins and A_{2A} and A_{2B} ARs to G_s proteins. Adenosine is an endogenous agonist for all of these receptors. All four subtypes of the AR are attractive targets for drug development. Agonists for A_1 and A_3 ARs could, for example, be used for both brain and heart protection, however, severe cardiovascular side effects may be expected due to the strong hypotensive effects of a series of A_1 AR agonists [2]. The use of full agonists of the A_1 AR as antiarrhythmic agents is limited by their causing of high-grade atrioventricular (AV) block, profound bradycardia, atrial fibrillation, and vasodilation. These side effects were major drawbacks in the clinical application of AR agonists. Partial agonists could, however, produce less pronounced cardiovascular effects and may act more selectively; e.g. the partial agonist 2-deoxyCPA 1 produced a less dramatic effect on heart rate than the full agonist CPA [3]. The A_1 AR partial agonist, 5-{6-[((3*R*)oxolan-3-

*Address correspondence to this author at Molecular Recognition Section, Bldg. 8A, Rm. B1A-23, NIH, NIDDK, LBC, Bethesda, MD 20892-0810,USA; Tel.: 301-402-3776; Fax: 301-480-8422; zhanguog@intra.niddk.nih.gov.

yl)amino]purin-9-yl}(3*S*,2*R*,4*R*,5*R*)-3,4-dihydroxyoxolan-2-yl)methoxy]-*N*-methyl-carboxamide (CVT-2759 2)*, selectively slows AV conduction in guinea pig hearts. Partial agonists may also be useful for slowing AV nodal conduction, and thereby ventricular rate, without causing AV block, bradycardia, atrial arrhythmias, or vasodilation [4]. Another advantage to partial agonists is that they are less likely to cause receptor desensitization and downregulation than full agonists. Partial agonists for β-adrenergic receptors, for example, have met with considerable success as therapeutic agents [5].

Modification of adenosine and its analogues leading to partial agonists for A_1 ARs has been well documented [6–8]. In addition, partial agonists for A_1 ARs have clearly envisioned therapeutic applications. Isolated examples of partial agonists for A_{2A} ARs have been reported [9–11]. Hutchinson *et al.* [12] reported that 2-iodo-N^6-(2-*S*-*endo*-norborn-2-yl)adenosine **3** was a full agonist at the A_1AR but have no detectable agonist activity at the A_{2A}AR. Recent publications from our laboratory and others have made an extensive evaluation of both the known adenosine derivatives and those containing novel modification of both the ribose and adenine moieties [13–18, 10, 19]. A number of structural determinants for A_3 AR activation have been identified, and lead to the conclusion that the efficacy of adenosine derivatives appears to be more dependent on smaller structural changes at A_3 than at other subtypes. For example, 2-chloro-N^6-cyclopentyladenosine (CCPA **4b**) and 2-chloro-N^6-R-phenylisopropyladenosine Cl-R-PIA **5b** are full agonists at A_1 ARs, but at the human A_3AR they are an antagonist and a partial agonist, respectively. N^6-[2-(3.5-dimethoxyphenyl)-2- (2-methylphenylethyl)] adenosine (DPMA **6**), an agonist at A_{2A}ARs, is an antagonist at the human A_3AR [15].

Partial agonists for A_3 ARs could potentially be therapeutic agents for brain and heart ischemic conditions. It may be productive to explore simple and small substitutions that diminish efficacy and enhance affinity leading to selectivity. In this review, we describe the substitutions at the adenosine and adenosine derivatives that led to a number of high affinity and selective partial agonists showing a great variation in degree of agonism.

SUBSTITUTION OF THE ADENINE MOIETY AT 2- AND 8-POSITION

Analogues of the A_1-selective agonist N^6-cyclopentyl-adenosine (CPA **4a**) having 8-alkyl substituents displayed both reduced affinity and efficacy at the A_1AR [20–21]. At the A_3 AR, 8-substitution greatly reduces affinity [22].

Both CCPA **4b** and its 2-H analogue, CPA **4a**, are potent agonists for human A_1 ARs while having moderate binding affinities at the human A_3AR. We investigated the activation by CPA and CCPA of the human A_3AR stably transfected in Chinese hamster ovary (CHO) cells [14]. CPA inhibited forskolin-stimulated cyclic AMP production in CHO cells in a dose-dependent manner, corresponding to an EC_{50} value of 240 nM, but CCPA had no such effect, suggesting that CCPA might be an antagonist for the human A_3AR. This was further demonstrated through the antagonism, by CCPA, of the effect of the potent A_3 AR agonist Cl-IB-MECA **7b**. CCPA shifted the Cl-IB-MECA dose-response curve to the right in a concentration-dependent manner. The differential effects of CPA and CCPA on the human A_3AR were further demonstrated in a functional assay of phospholipase C (PLC) activity. CPA, but not CCPA, induced phosphoinositide turnover in intact CHO cells expressing the human A_3AR in a concentration-dependent manner. Thus, the 2-substituted adenosine analogue, CCPA, was a moderately potent antagonist (K_i=38 nM) at the human A_3AR.

*Zablocki *et al.*, this volume.

Similar to CPA, R-PIA **5a** was also a potent and selective agonist for A_1 ARs; the 2-substituted analogue, 2-Cl-R-PIA **5b**, was demonstrated to be less efficacious than its 2-H analogue [15]. 2-Chloroadenosine **9** was fully efficacious, however, 2-fluoroadenosine **8** displayed approximately 30% of the efficacy of the full agonist 2-chloroadenosine. IJzerman and coworkers also reported that the introduction of 2-chloro might decrease the efficacy of both N^6- and N^6,5'-di-substituted adenosine derivatives; this is in line with the above findings [18].

Previously, it was found that 2-fluoroadenosine acted as a partial agonist at the A_{2A} AR [9]. The finding that 2-fluoroadenosine was a partial agonist for A_3 ARs is consistent with, and an extension of that report. It was interesting that the 2-halo substitution was generally thought to enhance only the affinity of the ligands, little attention has been paid to the dramatic effects of 2-substitution to diminish the A_3AR efficacy. However, it remains to be determined if most of the other adenosine derivatives currently used are full agonists for one subtype while at the same time being partial agonists, full agonists or antagonists for other AR subtypes. Also, it should be noted that a compound might be classified as a nearly full agonist, a partial agonist, or an antagonist in different tissues; the classification will also depend on the tissue in which the measurement is made [23].

N^6-SUBSTITUTION OF THE ADENINE MOIETY

Actually, the first partial agonists for A_3 ARs, such as N^6-benzyladenosine, have existed long before they were recognized as such. These compounds might have been regarded as full agonists for the A_3 AR because they are full agonists at other subtypes and N^6-benzyl NECA **11** and other N^6-benzyl-5'-*N*-alkylamide adenosine derivatives are known to be full agonists at A_3 ARs [22, 19].

Recently, the activation of the human A_3AR by a wide range of N^6-substituted adenosine derivatives was studied in intact CHO cells stably expressing this receptor. A number of substitutions at the N^6-position of the adenine moiety, including benzyl and large cycloalkyl groups, were found to decrease the maximum agonist efficacy at the A_3AR. A chloro substituent was demonstrated to either increase or decrease the efficacy, depending on the position of substitution at the benzyl group [15]. Interestingly, DPMA **6**, a potent agonist for the A_{2A}AR, was demonstrated to be a moderately potent antagonist for the human A_3AR (K_i=106 nM).

The N^6-(3-iodobenzyl) group, which enhances the affinity for A_3 ARs [22], may lead to a reduction of efficacy even in the absence of multiple substitutions of adenosine [17, 14, 13]. Thus, MRS 541 **12a** was a low-efficacy, partial agonist. The combination of 2-chloro and N^6-substitution appears to reduce efficacy further than either modification alone. Thus, MRS 542 **12b** was demonstrated to be an antagonist with little, if any, detectable agonist activity.

The N^6-cyclopentyl group alone did not reduce efficacy, however the combination of 2-chloro and N^6-cyclopentyl for **4b** in the present pharmacological system appeared to abolish agonism entirely [14].

The above-mentioned effects of reduction of efficacy are subject to reversal upon, at least certain, flexible 5'-substitutions of the molecule. The 5 '-alkylamide group (either *N*-ethyl as in NECA **10**, or *N*-methyl) appeared to restore efficacy. Comparison of pairs of compounds differing only in the presence of the 5'-alkylamide group demonstrates that the efficacy-preserving effect of this substituent takes precedence over other modifications that may reduce efficacy. Thus, IB-MECA **7a** was more efficacious than MRS 541 **12a** (N^6-(3-iodobenzyl)), Cl-IB-MECA **7b** was more efficacious than MRS 542 **12b** (2-chloro and N^6-

(3-iodobenzyl)), MRS 1939 **15** was more efficacious than MRS 1743 **13** (N^6-(3-iodobenzyl) and (N)-methanocarba), and MRS 1898 **16** was more efficacious than MRS 1760 **14** (2-chloro, N^6-(3-iodobenzyl), and (N)-methanocarba). Also, the full agonism of MRS 1898 was consistent with the above patterns, since although this compound is a 2-chloro-(N)-methanocarba derivative, it also contains the 5'-*N*-methylamide group.

As described above, 2-chloro substitution of the adenine ring may both increase the affinity and decrease the efficacy of the adenosine derivatives for the human A_3AR [18, 14, 13]. A further study demonstrated that a chloro substituent might alternately decrease, increase, or have no effect on the efficacy of the adenosine derivatives, depending on the position of substitution [15]. In contrast, a chloro substituent at the 2- or 4- positions (**17b,c**) of the benzyl group of N^6-benzyladenosine **17a** (itself having 55% of maximal efficacy) significantly increased the efficacy, converting the partial agonist N^6-benzyladenosine into full agonists. Chloro substitution at the 3-position of the benzyl group also significantly increased the efficacy, although to a lesser extent. Similarly, a fluoro substituent at the 2-position of the benzyl ring also induced a modest increase in the efficacy. In contrast to the N^6-benzyl group, which decreased the efficacy, the N^6-phenyl group did not significantly influence the efficacy, thus N^6-phenyladenosine **18** was still a full agonist. Also, similarly to Cl-IB-MECA **7b** [13], the benzyl group did not influence the efficacy of NECA **10**, and thus, N^6-benzyl-NECA **11** [22] was a full agonist.

The degree of steric bulk present on the N^6-substituent was correlated with loss of A_3 efficacy. The appending of an additional cyclopropyl group to N^6-cyclopropylmethyl adenosine **20** caused a slight change of its affinity and dramatically diminished its maximal A_3AR efficacy. Similarly, bridging the methylene group in N^6-(2-methylbenzyl) adenosine (the nonselective, full agonist metrifudil) to give N^6-R-l-indanyl-adenosine **19**, thus introducing a ring constraint, also reduced A_3 efficacy. A comparison of the compounds N^6-cyclopropylmethyl **20** and N^6-cyclohexylmethyl **21** suggested that the enlargement of a ring attached to the N^6-methyl group appeared to lower both affinity and efficacy at the human A_3AR, while lengthening the chain between N^6 and the phenyl group seemed to mainly decrease efficacy, with minimum efficacy observed with N^6-benzyladenosine **17a**. Compared with N^6-(2-phenylethyl) adenosine, the introduction of an oxygen (hydroxylamine linkage; 2-phenylethoxy **22**) seemed to induce a larger decrease in its affinity for all three AR subtypes and a smaller decrease of its maximal A_3AR efficacy. This was in contrast to N^6-methoxyadenosine **23**, which showed enhanced potency at the rat A_3AR as a result of the oxygen inclusion [24]. Branching al the terminal alkyl position (R-1-phenyl-isopentyl **24** versus R-1-phenyl-2-pentyl **25**) did not change the A_3AR affinity but diminished the A_3AR efficacy.

Hence, within a narrow series of adenosine derivatives, careful examination of the structure activity relationship (SAR) of the N^6-substituted group shows that a great variation in degree of agonism may be produced. From our studies, this series of adenosine derivatives displayed the entire range from 0% to 100% agonism.

SUBSTITUTIONS OF THE RIBOSE MOIETY

A number of strategies have been pursued to develop partial agonists for ARs by modifying the ribose moiety. A 5'-chloro-5'-deoxy substitution **26**, already reported for adenosine agonists [24, 10], greatly reduced A_3AR efficacy. Modifications at the ribose moiety of adenosine resulted in a number of A_1-selective compounds with reduced A_1AR agonist activity. Removal of the 2'- or 3'-hydroxyl group of full agonists resulted in a reduction of both affinity and efficacy [25]. The 2'-and 3'-hydroxy groups of adenosine and its derivatives are required for agonist activity and high affinity binding to the A_1AR [26]. The

respective 5’-modifications (including 5’-thioethers), in combination with N^6-benzyl substituents known to increase A_3AR affinity, resulted in partial agonists with high affinity at the human A_3AR [17, 10]. Increasing the size of the 5’-substituents reduced the efficacy at A_3 ARs [18, 10].

Consistent with earlier findings, it has recently been found that both 2'- and 3'-hydroxyl groups in the ribose moiety are essential for agonist binding and activation, with the 2'-hydroxyl being more critical [27, 16]. Thus, the 2'-fluoro substitution, as in **27**, eliminated both binding and activation, while a 3'-fluoro substitution, as in **28**, led to only a partial reduction of potency and efficacy at the A_3AR. The diminished efficacy caused by 3'-fluoro could not be overcome with a 5'-amide [16]. The 4’-thio substitution generally enhanced potency [28]. The combined 4'-thio and 5’-amide substitution led to **29**, the most potent and selective A_3AR agonist (N^6-methyl) yet reported [28]. The 4’-thio substitution enhanced or diminished the efficacy, depending on other substitutions. Interestingly, the shifting of the N^6-(3-iodobenzyl)adenine moiety from the 1'- to 4'-position **30** had only a minor influence on its selectivity for the A_3AR, but transformed the potent agonist 4’-thio substituted Cl-IB-MECA into a potent antagonist **31** (K_i=4.3 nM). This unusual nucleoside analogue antagonized agonist-induced inhibition of cyclic AMP production in A_3AR-expressing CHO cells (K_B=3.0 nM). However, a 3’-deoxyadenosine derivative **32** was still a full agonist for the A_3AR with no affinity change [29].

The stereochemistry and substitution of the ribose hydroxyl groups greatly affected the intrinsic efficacy [16]. The arabino adenosine derivative **33** displayed greatly reduced A_3AR binding affinity due to inversion of stereochemistry at the 3’-carbon. Like its stereoisomer, MRS 542 **12b**, it did not display agonist action at the human A_3AR. The 2’-fluoro substitution of MRS 542 eliminated human A_3AR binding. The 3’-fluoro substitution of MRS 542 allowed a greater degree of agonism than at the 2’-position. The binding affinity of the corresponding 3’-fluoro analogue was weaker than for MRS 542 **12b** itself, although it remained an antagonist. The partial agonism of 3’-fluoro analogues could not be overcome (i.e., full agonism restored) with the 5’-amide modification, unlike previous findings with partial agonists having both 2’- and 3’-hydroxyl groups [13]. The effect of unsurmountable diminished agonism at the A_3AR in 3’-fluoro analogues was even greater when the N^6-(3-iodobenzyl) group was present, as in the antagonist 2-chloro-N^6-(3-iodobenzyl)-5'-*N*-methylcarbamoyl-3’-fluoro-3’-deoxyadenosine [16].

The 4’-thio modification generally enhanced potency, yet decreased efficacy. The N^6-methyl group counteracted the potency-reducing effect of a 2-chloro group; thus, 2-chloro-4’-thio-N^6-methyl adenosine **34** was a very potent full agonist. The 4’-thio group increased efficacy in comparison to its oxygen analogue, MRS 542. Consistent with the ability of a flexible 5’-amide group to overcome various efficacy-reducing structural changes [13], the 4’-thio substituted Cl-IB-MECA **31** was still a full agonist. 2’,3’-Epoxide derivatives, in both riboside **35** and (N)-methanocarba **36** series, and a cyclized 4’,5’-uronamide derivative MRS 1292 **37** were antagonists [13]. Although the 5’-uronamide structure is present in MRS 1292, it is cyclic and, therefore, sterically rigid and does not restore efficacy.

REPLACEMENT OF THE RIBOSE RING SYSTEM

A carbocyclic modification of the ribose moiety incorporating ring constraints is a general approach for the design of A_1 and A_3 AR agonists having favorable pharmacodynamic properties. While simple carbocyclic substitution of adenosine agonists greatly diminishes potency, methanocarba-adenosine analogues (e.g. **13 – 16** and **38**, tend to have greater potency. Such analogues having conformationally constrained bicyclic rings) have defined

the role of sugar puckering in stabilizing the active AR-bound conformation and thereby have allowed identification of a favored isomer. In such analogues a fused cyclopropane moiety constrains the pseudosugar ring of the nucleoside to either a Northern (N) or Southern (S) conformation, as defined in the pseudorotational cycle. In binding assays at A_1, A_{2A}, and A_3 ARs, (N)-methanocarba-adenosine was of higher affinity than the (S)-analogue, particularly at the human A_3 AR (N/S affinity ratio of 150). We have studied the affinity and efficacy of (N)-methanocarba analogues of various N^6-substituted adenosine derivatives, including cyclopentyl and 3-iodobenzyl, in which the parent compounds are potent agonists at either A_1 or A_3 ARs, respectively [30].

The (N)-methanocarba substitution of the ribose ring, which results in a rigidification of an A_3-AR preferred conformation of this moiety [31], while preserving and/or enhancing binding selectivity, also appears to reduce efficacy except when a 5'-uronamide was also present. Thus, the 2-H analogue, (N)-methanocarba-N^6-(3-iodobenzyl) adenosine (MRS 1743 **13**), was less efficacious than the corresponding riboside, MRS 541 **12a**. Similarly, in the simple 2-chloro case, MRS 1873 **38** was less efficacious than CADO **9**. The 5'-uronamides MRS 1898 **16** and MRS 1939 **15** are fully efficacious. MRS 1743 **13** and its 2-chloro derivative **14** had K_i values of 9.2 and 1.9 nM at the human A_3AR, respectively, and were highly selective partial agonists [31,13]. The 5'-uronamide modification preserved, i.e. N^6-(3-iodobenzyl), or enhanced, i.e. N^6-methyl, affinity at the human A_3AR, while the 2'-deoxy modification reduced affinity and efficacy in a functional assay [32].

SUBSTITUTION OF ADENINE MOIETY WITH XANTHINE

Adenosine is the endogenous agonist for all four subtypes of ARs, and ribose is the basic structure of the adenosine and adenosine derivatives. In an effort to design partial agonists for ARs, several xanthine derivatives (antagonists) have been used to replace the adenine moiety of adenosine and adenosine derivatives.

Theophylline-7-riboside was one of the first partial agonists identified for ARs [6]. Later, 7-β-D-ribofuranosylxanthine was found to have higher affinity and greater selectivity for the A_1 AR than previously reported xanthine nucleosides, and to be a partial agonist [33].

1,3-Dibutylxanthine-7-riboside **39** has been found to be a partial agonist at rat A_3 ARs [22] with moderate potency (K_i=6 μM). l,3-Dialkylxanthine-7-riboside analogues modified at the 1-, 3-, and 8-purine positions and at the ribose 5'-position were synthesized and examined for affinity at A_3 ARs stably expressed in CHO cells [34]. The affinity of xanthine 7-ribosides at A_3 ARs depended on the 1,3-dialkyl substituents in the order: Pent > or = Bu ≫ Hx > Pr, Me. 1,3-Dipentylxanthine 7-riboside was slightly selective for A_3 ARs (2-fold versus A_1 and 10-fold versus A_{2A}). 8-Methoxy substitution was tolerated at A_3 ARs. 2-Thio versus 2-oxo substitution increased potency at all three subtypes and slightly increased A_3 versus A_1 selectivity. The 5'-uronamide modification, which was previously found to enhance A_3 selectivity in N^6-benzyladenosine derivatives, was also incorporated into the xanthine 7-ribosides, with similar results. The affinity of 1,3-dialkylxanthine 7-riboside 5'-uronamides at A_3 ARs depended on the *N*-alkyluronamide substituent in the order: MeNH > EtNH ≫ NH_2 ≫ Me_2N. Thus, 1,3-dibutylxanthine 7-riboside 5'-*N*-methylcarboxa-mide (DBXRM **40**) was the most potent and A_3-selective in the series. Affinity of the 5'-uronamides at A_3 ARs was dependent on the 1,3-dialkyl substitution in the order: Bu > Pent > Hex. DBXRM, with a K_i, value of 229 nM at A_3 ARs, was 160-fold selective for rat A_3 versus A_1 ARs and > 400-fold selective versus A_{2A} ARs. This derivative acted as a full agonist in the rat A_3AR-mediated inhibition of adenylate cyclase [34]. Consistent with this study, it was later demonstrated that DBXRM was a full agonist for the human A_3AR [13], thus there was no species difference in its observed efficacy.

CONCLUSION

The identification of critical structural determinants for A_3AR activation should prove useful for further understanding the mechanism of receptor activation and development of more potent and selective full agonists, partial agonists and antagonists for A_3ARS. Novel and unique ligands may be identified by optimizing the selectivity, efficacy and binding affinity of the adenosine derivatives at different AR subtypes.

Acknowledgments

We thank Joshua Blaustein (NIDDK) for carefully reading and correcting this manuscript.

Biography

CHARTS 1 – 5: CHEMICAL STRUCTURES OF ADENOSINE DERIVATIVES DESCRIBED IN THE TEXT

After each structure number are given pharmacological parameters at the human A_3AR, i.e. the K_i value (nM) in binding experiments (italics) and the % maximal efficacy observed at a concentration of 10 μM.

REFERENCES

1. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of ARs. Pharmacol. Rev. 2001; 53:527–552. [PubMed: 11734617]
2. Mathot RA, van Schaick EA, Langemeijer MW, Soudijn W, Breimer DD, IJzerman AP, Danhof M. Pharmacokinetic-pharmacodynamic relationship of the cardiovascular effects of adenosine A_1 receptor agonist N^6-cyclopentyladenosine in the rat. J. Pharmacol. Exp. Ther. 1994; 268:616–624. [PubMed: 8113972]
3. Danhof M, Mandema JW, Hoogerkamp A, Mathot RA. Pharmacokinetic-pharmacodynamic modeling in pre-clinical investigations: principles and perspectives. Eur. J. Drug. Metab. Pharmacokinet. 1993; 18:41–47. [PubMed: 8335038]
4. Wu L, Belardinelli L, Zablocki JA, Palle V, Shryock JC. A partial agonist of the A_1-AR selectively slows AV conduction in guinea pig hearts. Am. J. Physiol. Heart Circ. Physiol. 2001; 280:H334–H343. [PubMed: 11123249]
5. Barlow JJ, Main BG, Snow HM. Beta-adrenoceptor stimulant properties of amidoalkylamino-substituted l-aryl-2-ethanols and 1-(aryloxy)-2-propanols. J. Med. Chem. 1981; 24:315–322. [PubMed: 6115058]
6. IJzerman AP, van der Wenden EM, von Frijtag Drabbe Künzel JK, Mathot RA, Danhof M, Borea PA, Varani K. Partial agonism of theophylline-7-riboside on adenosine receptors. Naunyn Schmiedebergs Arch. Pharmacol. 1994; 350:638–645. [PubMed: 7708121]
7. Mathot RA, Van der Wenden EM, Soudijn W, IJzerman AP, Danhof M. Deoxyribose analogues of N^6-cyclopentyladenosine (CPA): partial agonists at the adenosine A1 receptor *in vivo*. Br. J. Pharmacol. 1995; 116:1957–1964. [PubMed: 8640332]
8. van der Wenden EM, Carnielli M, Roelen HC, Lorenzen A, von Frijtag Drabbe Künzel JK, IJzerman AP. 5'-substituted adenosine analogs as new high-affinity partial agonists for the adenosine A_1 receptor. J. Med. Chem. 1998; 41:102–108. [PubMed: 9438026]

9. Daly JW, Padgett W. Agonist activity of 2- and 5'-substituted adenosine analogs and their N^6-cycloalkyl derivatives at A_1 and A_2-ARs coupled to adenylate cyclase. Biochem. Pharmacol. 1992; 43:1089–1093. [PubMed: 1554381]
10. van Tilburg EW, Gremmen M, von Frijtag Drabbe Künzel JK, de Groote M, IJzerman AP. 2,5'-Disubstituted adenosine derivatives: evaluation of selectivity and efficacy for the adenosine A_1, A_{2A}, and A_3 receptor. J. Med. Chem. 2002; 45:420–429. [PubMed: 11784146]
11. van Tilburg EW, Gremmen M, von Frijtag Drabbe Künzel JK, de Groote M, IJzerman AP. 2,8-Disubstituted adenosine derivatives as partial agonists for the adenosine A_{2A} receptor. Bioorg. Med. Chem. 2003; 11:2183–2192. [PubMed: 12713828]
12. Hutchinson SA, Baker SP, Scammells PJ. New 2, N^6-disubstituted adenosines: potent and selective A_1 adenosine receptor agonists. Bioorg. Med. Chem. 2002; 10:1115–1122. [PubMed: 11836122]
13. Gao ZG, Kim SK, Biadatti T, Chen W, Lee K, Barak D, Kim SG, Johnson CR, Jacobson KA. Structural determinants of A_3 adenosine receptor activation: Nucleoside ligands at the agonist/antagonist boundary. J. Med. Chem. 2002; 45:4471–4484. [PubMed: 12238926]
14. Gao ZG, Jacobson KA. 2-Chloro-N^6-cyclopentyladenosine, adenosine A_1 receptor agonist, antagonizes the adenosine A_3 receptor. Eur. J. Pharmacol. 2002; 443:39–42. [PubMed: 12044789]
15. Gao ZG, Blaustein JB, Gross AS, Melman N, Jacobson KA. N^6-Substituted adenosine derivatives: selectivity, efficacy, and species differences at A_3 adenosine receptors. Biochem. Pharmacol. 2003; 55:1675–1684. [PubMed: 12754103]
16. Gao ZG, Jeong LS, Moon HR, Kim HO, Choi WJ, Shin DH, Elhalem E, Comin MJ, Melman N, Mamedova L, Gross AS, Rodriguez JB, Jacobson KA. Structural determinants of efficacy at A_3 adenosine receptors: Modification of the ribose moiety. Biochem. Pharmacol. 2004; 67:893–901. [PubMed: 15104242]
17. van Tilburg EW, von Frijtag Drabbe Künzel J, de Groote M, Vollinga RC, Lorenzen A, IJzerman AP. N^6,5'-Disubstituted adenosine derivatives as partial agonists for the human adenosine A_3 receptor. J. Med. Chem. 1999; 42:1393–1400. [PubMed: 10212125]
18. van Tilburg EW, van der Klein PA, von Frijtag Drabbe Künzel JK, de Groote M, Stannek C, Lorenzen A, IJzerman AP. 5'-O-alkyl ethers of *N*,2-substituted adenosine derivatives: partial agonists for the adenosine A_1 and A_3 receptors. J. Med. Chem. 2001; 44:2966–2975. [PubMed: 11520205]
19. Kim HO, Ji XD, Siddiqi SM, Olah ME, Stiles GL, Jacobson KA. 2-Substitution of N^6-benzyladenosine-5'-uronamides enhances selectivity for A_3- adenosine receptors. J. Med. Chem. 1994; 37:3614–3621. [PubMed: 7932588]
20. Roelen H, Veldman N, Spek AL, von Frijtag Drabbe Künzel JK, Mathot RA, IJzerman AP. N^6,C^8-distributed adenosine derivatives as partial agonists for adenosine A_1 receptors. J. Med. Chem. 1996; 39:1463–1471. [PubMed: 8691477]
21. van Schaick EA, Tukker HE, Roelen HC, IJzerman AP, Danhof M. Selectivity of action of 8-alkylamino analogues of N^6-cyclopentyladenosine *in vivo*: haemodynamic versus anti-lipolytic responses in rats. Br. J. Pharmacol. 1998; 124:607–618. [PubMed: 9647488]
22. Van Galen PJM, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, IJzerman AP, Stiles GL, Jacobson KA. A binding site model and structure-activity relationships for the rat A_3 adenosine receptor. Mol. Pharmacol. 1994; 45:1101–1111. [PubMed: 8022403]
23. Kenakin, TP. The Pharmacologic Analysis of Drug-Receptor Interactions. Raven, Philadelphia: LIPPINCOTT; 1997.
24. Mogensen JP, Roberts SM, Bowler AN, Thomsen C, Knutsen LJ. The synthesis of new adenosine A_3 selective ligands containing bioisosteric isoxazoles. Bioorg. Med. Chem. Lett. 1998; 8:1767–1770. [PubMed: 9873431]
25. van der Wenden EM, von Frijtag Drabbe Künzel JK, Mathot RA, Danhof M, IJzerman AP, Soudijn W. Robose-modified adenosine analogues as potential partial agonists for the AR. J. Med. Chem. 1995; 38:4000–4006. [PubMed: 7562934]
26. Lohse MJ, Klotz KN, Schwabe U, Cristalli G, Vittori S, Grifantini M. 2-Chloro- N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. Naunyn Schmiedebergs Arch. Pharmacol. 1988; 337:687–689. [PubMed: 3216901]

27. Lim MH, Kim HO, Moon HR, Lee SJ, Chun MW, Gao ZG, Melman N, Jacobson KA, Kim JH, Jeong LS. Design, synthesis and binding affinity of 3'-fluoro analogues of Cl-IB-MECA as adenosine A_3 receptor ligands. Bioorg. Med. Chem. Lett. 2003; 13:817–820. [PubMed: 12617898]

28. Jeong LS, Jin DZ, Moon HR, Kim HO, Chun MW, Melman N, Gao ZG, Jacobson KA. N^6-Substituted D-4'-thioadenosine-5'-methyluronamides: Potent and selective agonists at the human A_3 adenosine receptor. J. Med. Chem. in press.

29. Jacobson KA, Siddiqi SM, Olah ME, Ji XD, Melman N, Bellamkonda K, Meshulam Y, Stiles GL, Kim HO. Structure-activity relationships of 9-alkyladenine and ribose-modified adenosine derivatives at rat A_3 adenosine receptors. J. Med. Chem. 1995; 35:1720–1735. [PubMed: 7752196]

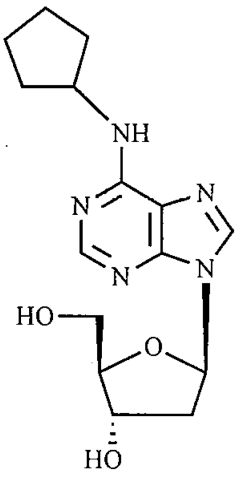
30. Jacobson KA, Ji X, Li AH, Melman N, Siddiqui MA, Shin KJ, Marquez VE, Ravi RG. Methanocarba analogues of purine nucleosides as potent and selective AR agonists. J. Med. Chem. 2000; 43:2196–2203. [PubMed: 10841798]

31. Jacobson KA, Ji X, Li AH, Melman N, Siddiqui MA, Shin KJ, Marquez VE, Ravi RG. Methanocarba analogues of purine nucleosides as potent and selective AR agonists. J. Med. Chem. 2000; 43:2196–2203. [PubMed: 10841798]

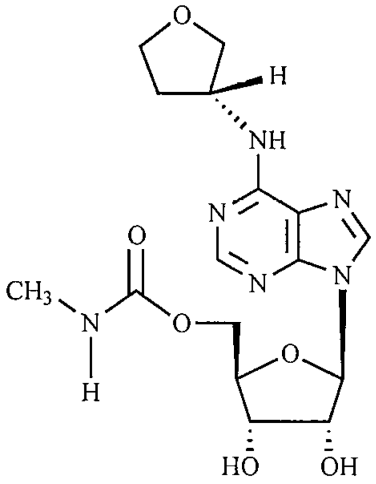
32. Lee K, Ravi G, Ji XD, Marquez VE, Jacobson KA. Ring-Constrained (*N*)-methanocarba nucleosides as adenosine receptor agonists: independent 5'-uronamide and 2'-deoxy modifications. Bioorg. Med. Chem. Lett. 2001; 11:1333–1337. [PubMed: 11392549]

33. Bridson PK, Lin X, Melman N, Ji XD, Jacobson KA. Synthesis and AR affinity of 7-β-D-ribofuranosylxanthine. Nucleosides Nucleotides. 1998; 17:759–768. [PubMed: 9708335]

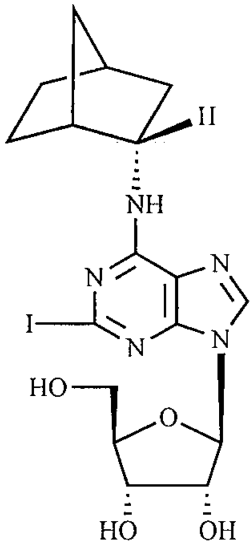
34. Kim HO, Ji XD, Melman N, Olah ME, Stiles GL, Jacobson KA. Selective ligands for rat A_3 adenosine receptors: structure-activity relationships of 1,3-dialkylxanthine 7-riboside derivatives. J. Med. Chem. 1994; 37:4020–4030. [PubMed: 7966162]



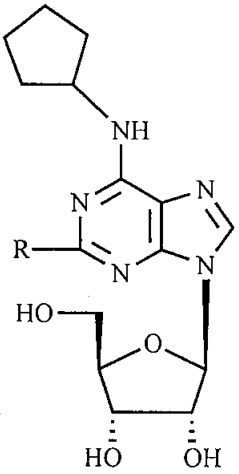
1



2

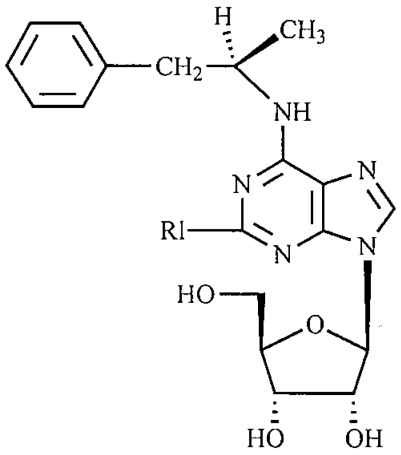


3



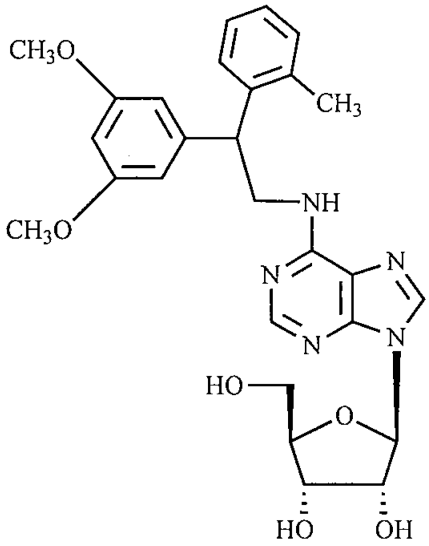
4a R = H, *72*, 97%

4b R = Cl, *38*, 0%



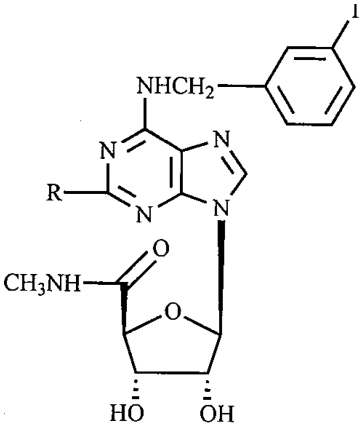
5a R = H, *8.7*, 100%

5b R = Cl, *13.1*, 76%



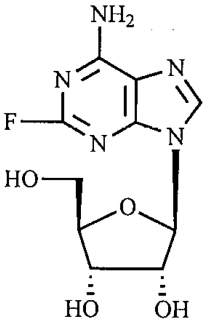
6, *46* 0%

Chart 1.



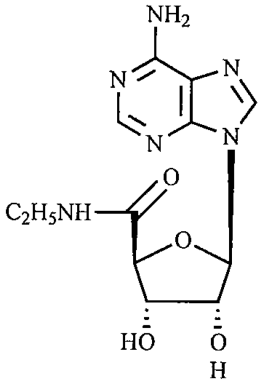
7a R = H, *1.2* 100%

7b R = Cl, *1.4* 99%

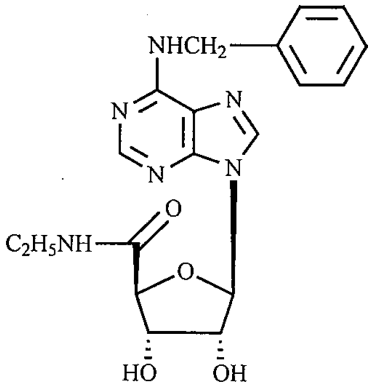


8 R = F, *99* 31%

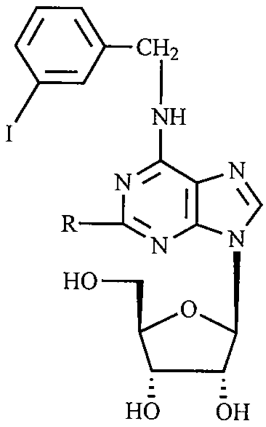
9 R = Cl, *87* 100%



10, *35* 103%

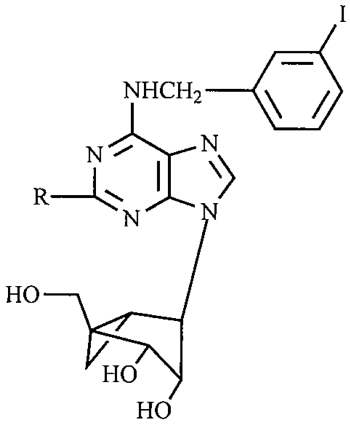


11, *10.8* 107%



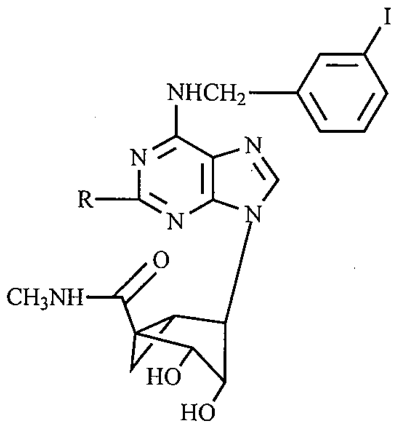
12a R = H, *5.8* 46%

12b R = Cl, *1.8* 0%



13, R = H, *9.2* 13%

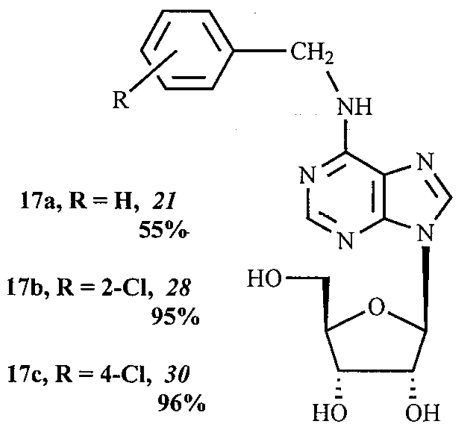
14, R = Cl, *1.9* 3%



15, R = H, *2.4* 100%

16, R = Cl, *2.1* 100%

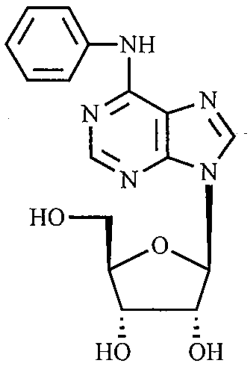
Chart 2.



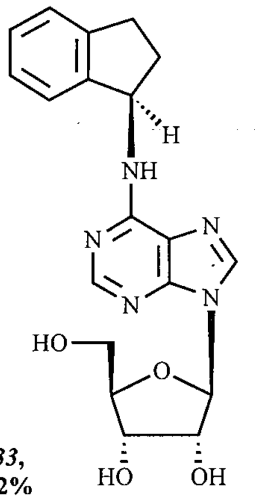
17a, R = H, *21*
55%

17b, R = 2-Cl, *28*
95%

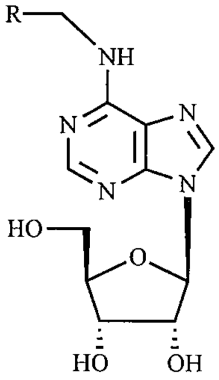
17c, R = 4-Cl, *30*
96%



18, *20*,
102%

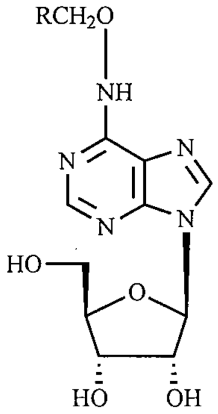


19, *233*,
82%



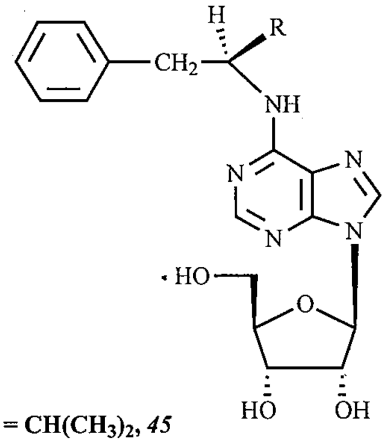
20, R = cyclopropyl, *17*
108%

21, R = cyclohexyl , *263*
38%



22 R = $CH_2C_6H_5$, *88.7*
73%

23 R = H, *28.6*
107%



24 R = $CH(CH_3)_2$, *45*
77%

25 R = $(CH_2)_2CH_3$, *43*
92%

Chart 3.

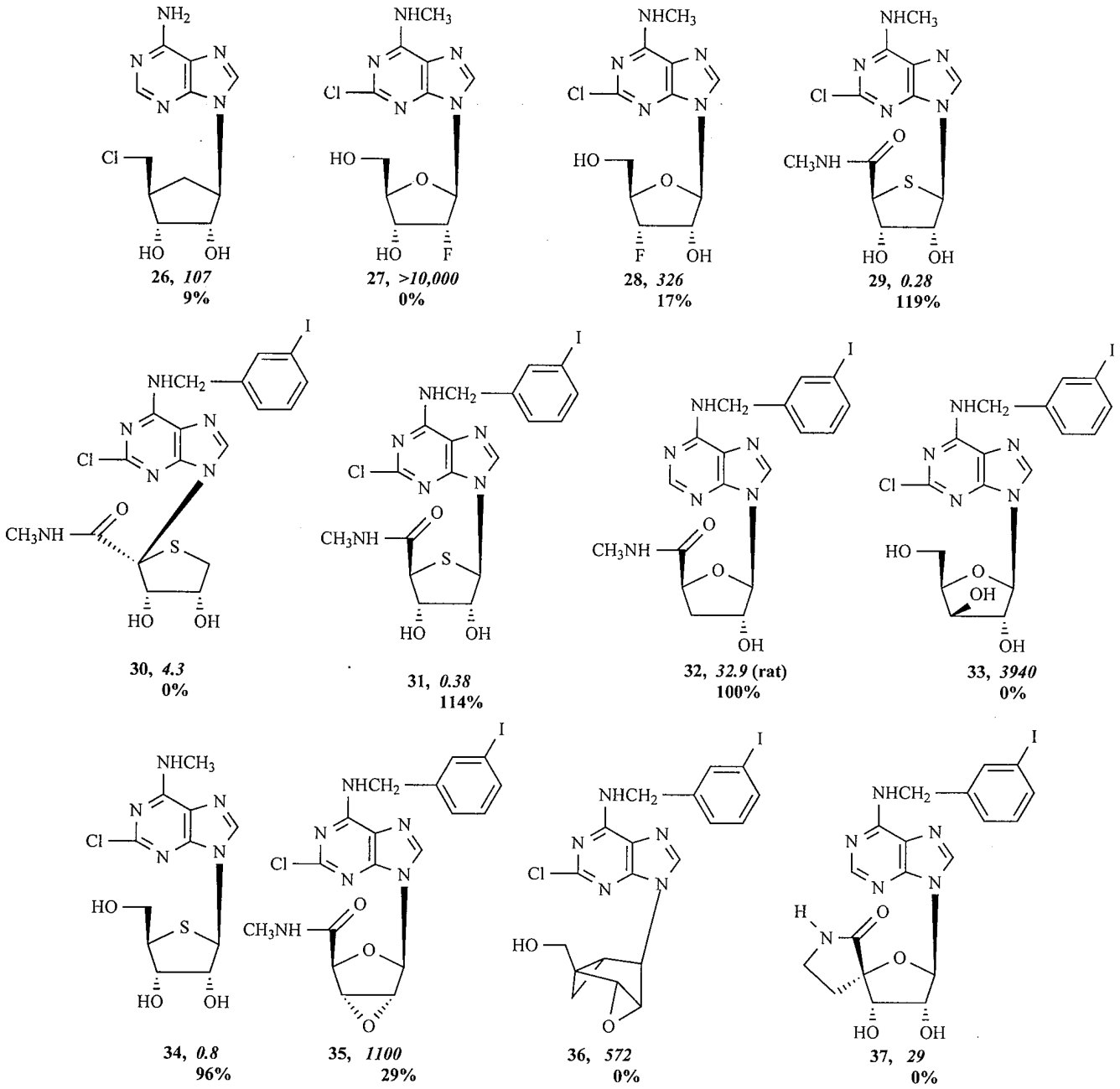
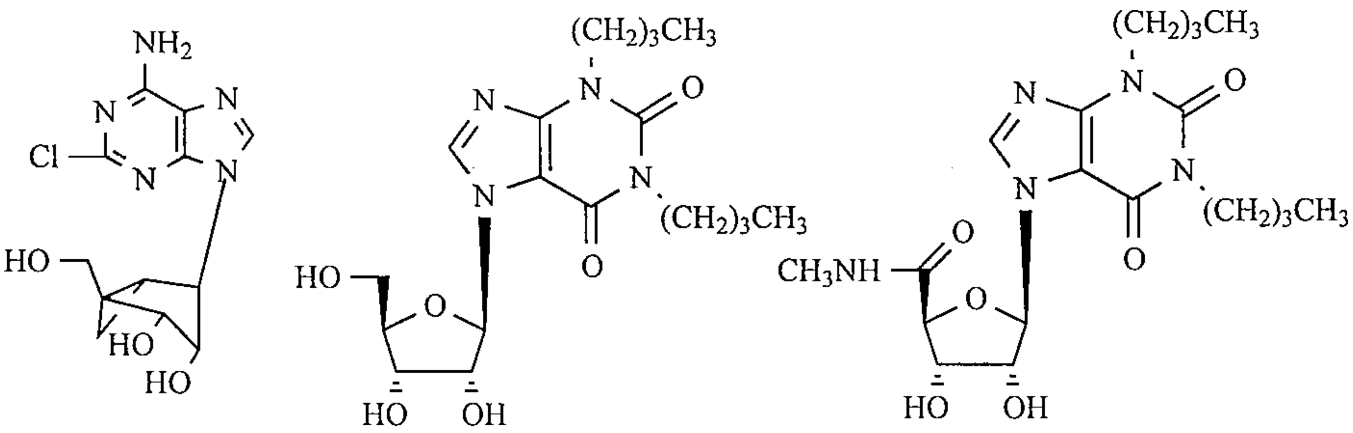


Chart 4.



38, ***353*** **41%**

39, ***6600*** **(rat) 0%**

40, ***2830*** **96%**

Chart 5.