Articles

2,6-Disubstituted and 2,6,8-Trisubstituted Purines as Adenosine Receptor Antagonists

Lisa C. W. Chang, Ronald F. Spanjersberg, Jacobien K. von Frijtag Drabbe Künzel, Thea Mulder-Krieger, Johannes Brussee, and Adriaan P. IJzerman*

Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, P.O. Box 9502, 2300 RA Leiden, The Netherlands

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Purines have long been exploited as adenosine receptor antagonists. The substitution pattern about the purine ring has been well investigated, and certain criteria have become almost a prerequisite for good affinity at the adenosine A_1 receptor. The adaptation of the pharmacophore and the initial series of pyrimidines developed in an earlier publication resulted in a series of purines with an entirely new substitution pattern. One compound in particular, 8-cyclopentyl-2,6-diphenylpurine (**31**, LUF 5962) has been shown to be very promising with an affinity of 0.29 nM at the human adenosine A_1 receptor.

Introduction

The adenosine receptors belong to the superfamily of G-protein-coupled receptors. There are four subclasses of adenosine receptors that have been identified to date, and these are A_1, A_{2A}, A_{2B}, and A_3 receptors. The adenosine receptors are distributed around many different tissue types in the human body in varying levels of expression.[1] This has led to intense research into the therapeutic potential of the ability to control the activity of adenosine receptors. For example, BG-9928 (bicyclo[2.2.2]-octane-1-propanoic acid, 4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-) is an A_1 receptor antagonist in phase II clinical development for renal chronic heart failure.

We have recently shown that a simple pharmacophore can lead to new types of adenosine A_1 receptor antagonists.[2] The resulting 2,4,6-trisubstituted pyrimidines were shown to be both potent at and selective for the human A_1 receptor. In an attempt to judge the relative location of the hydrogen-bond acceptor close to the top of a central aromatic group and a more precise orientation of the L2 lipophilic group in relation to the central ring, the fixation of this group was considered. Figure 1a−c shows the logical development of the 4-amido-2,6-diphenylpyrimidines into the purines explored in this article. Fixing the hydrogen-bond-accepting group at the top of the central group prompted the change of the heteroatom for both synthetic ease and the preservation of the C═heteroatom unsaturated bond.

Purines have been explored at length as adenosine receptor antagonists.[3] However, these have mainly been direct analogues of adenine, in the sense that the N^6 group has always been preserved. This N^6 amino group has usually been substituted with a cyclopentyl group to attain good affinity for the A_1 receptor.[4−9] Further exploration of this central core also deems the necessity of N9 substitution for potency, with both large benzyl derivatives[7] and small methyl[9] or ethyl substituents[8] showing good affinity for the A_1 receptor. De Ligt et al. postulated certain features to enhance the selectivity of the basic adenine ring (Figure 2).[9] The resulting series of compounds displayed good affinity for the human adenosine A_1 receptor, with N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine showing the best K_i value at 7.7 nM. These types of compounds with the presence of the N^6-group and with N9 substitution show good affinity at the A_1 receptor, corroborating

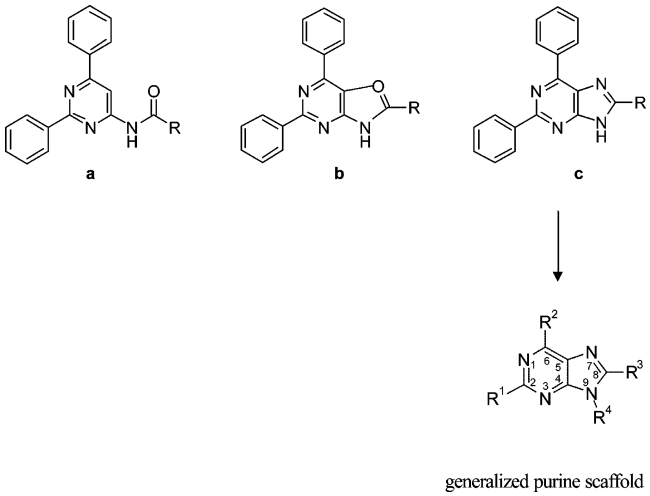

Figure 1. (a) 2,6-Diphenyl-4-amidopyrimidine, a high-affinity antagonist at adenosine A_1 receptors (ref 2). (b) Fixation of the hydrogen-bond acceptor at the top of the molecule. (c) Change of the heteroatom to accomplish this fixation. From the latter molecule, a more generalized purine scaffold is proposed, which we used as a starting point in our synthetic program.

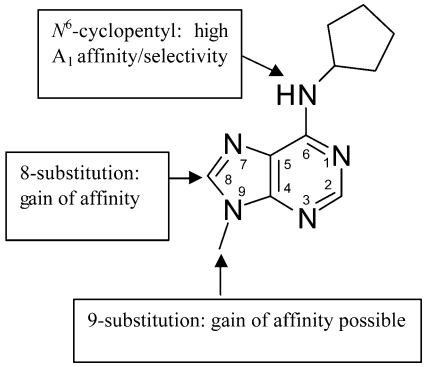

Figure 2. Features to enhance affinity and selectivity at the A_1 receptor and the numbering around the purine ring (adapted from ref 7).

* To whom correspondence should be addressed. Tel: +31 (0)71 527 4651. Fax: +31 (0)71 527 4565. E-mail: ijzerman@lacdr.leidenuniv.nl.

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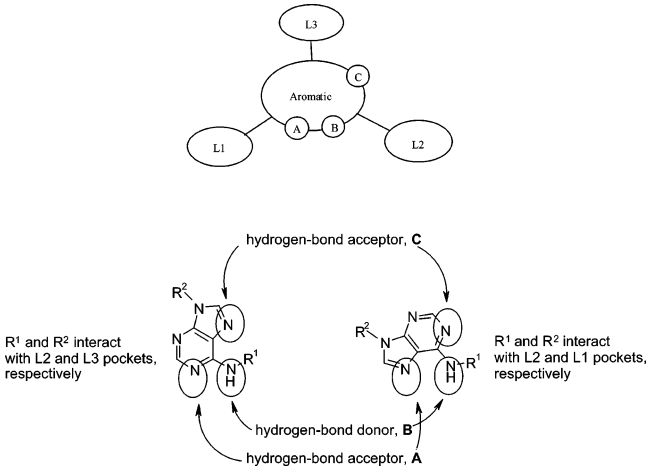


Figure 3. Model detailed in an earlier publication (upper part, see ref 2), and the two possible orientations of the traditionally substituted adenines to fit this model (see text).

with the proposed model[2] in the manner shown in Figure 3. The hydrogen-bond-donating group (denoted B) is represented by the available hydrogen on the N^6, and the hydrogen-bond-acceptor groups A or C may be one of the two ring nitrogens (N1 or N7) depending on the orientation and the size of possible substituents on the other positions of the purine ring. The lipophilic pocket denoted L2 may be filled with the substituent labeled R^1, and either L1 or L3 are satisfied by the R^2 substituent. Optimal receptor interaction may be provided by substitution in the C2 position, as found by Bianucci et al. in their investigations.[7]

In this article, a new perspective of the purines is realized according to the model proposed in our earlier publication,[2] where excellent affinity for the A_1 adenosine receptor is achieved, despite the lack of the seemingly essential N^6 and N9 groups/substituent proposed in earlier articles. Direct aromatic substitution at the C2 and C6 positions (labeled R^1 and R^2 in the purine scaffold in Figure 1) provides an analogy of the 2,6-aromatic substituents on the 4-amino-pyrimidines.[2] C8 substitution (R^3) explores the L2 pocket, and R^4 should be, according to the model, left unsubstituted to achieve high affinity at the A_1 adenosine receptor.

Chemistry

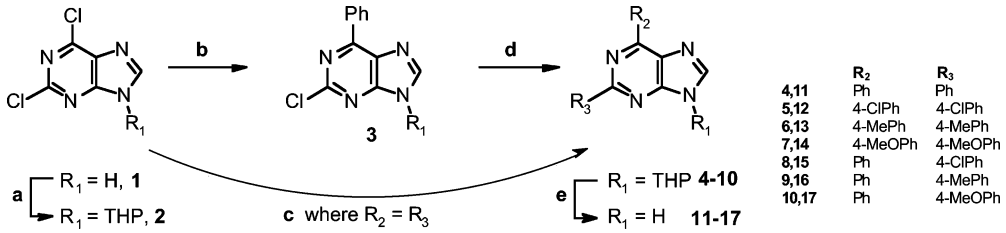
The purines were made via two routes. The 2,6-disubstituted purines were synthesized from commercially available 2,6-dichloropurine as described in Scheme 1. Substitution of the chlorines was possible through metal-mediated cross-coupling reactions. Both Stille and Suzuki−Miyaura couplings of purines have been reported in the literature.[10−17] To facilitate the Suzuki cross-coupling, protection of the nitrogen at N9 was necessary. This was successfully achieved with tetrahydropyran (THP). Initial attempts with a benzylic group rendered the product far too stable, and the removal of the protecting group was impossible under usual hydrogenation or transfer hydrogenation conditions. Substitution of both chlorines with the same phenyl derivative occurred under standard Suzuki conditions as detailed by Hocek et al. (compounds **11**−**14**).[13] However, microwave heating to a temperature of 150 °C instead of conventional heating methods reduced the reaction time from 8 h to approximately 20 min. An excess of the boronic acid (3 equivalents) encouraged the reactions to completion. Where the 2 and 6 substituents differed, one equivalent of the boronic acid provided a single substitution at the 6 position (compounds **15**−**17**). This was confirmed by X-ray crystallography. Further reaction with an excess of the second boronic acid gave the desired 2,6-diphenyl derivatives.

For substitution at the 8-position, the bromination of a protected 2,6-dichloropurine to provide a more versatile intermediate was attempted following the procedure described by De Ligt et al.[9] in their evaluation of N-0840 analogues. Unfortunately, this was unsuccessful, and the 8-substituent was introduced into the purine frame at an earlier stage, as described in Scheme 2. To make vital intermediate **18**, a four step procedure described in a number of articles by Biagi et al. was broadly exploited.[18−20] The reaction of commercially available benzamidine with diethyl malonate created a pyrimidine ring, after which various substitution steps resulted in intermediate **18**. Ring closure was attempted by several different methods that are detailed in the literature, namely, using trimethylorthoformate, triethylorthoformate, or PPA as the reaction medium.[21,22] These methods were either poor in yields or were difficult to work up or purify. Finally, a two-step reaction incorporating the addition of the appropriate acid chloride followed by cyclization under basic conditions provided a more ideal synthetic pathway.[23] The 8-substituted-2,6-dichloropurine was then subjected to metal-catalyzed cross-coupling reactions as described above. It was discovered that protection at N9, although readily applicable (**24**−**27**) was no longer necessary, probably, because of the increased steric bulk around this position preventing the complexation of the catalyst and/or the boronic acid. Methylation of compound **32** following standard procedures with methyl iodide in basic conditions gave compound **37**.

Structure−Activity Relationships. The results of the radioligand-binding assays performed on these purines are shown in Table 1. Compounds **11**−**17** are unsubstituted in the C8 position and are varied at C2 and C6. 2,6-Diphenyl-9*H*-purine (**11**) has an affinity of 4 nM for the hA_1 receptor. The selectivity is approximately 10-fold better for this receptor than that for the A_{2A} and the A_3 receptors at 53 and 38 nM, respectively. An identical substitution at the 4-positions of both of the phenyl groups (**12**−**14**) is to the great detriment of the affinity for the A_1 and A_{2A} receptors, in contrast to the improved affinity (9 nM) at the A_3 receptor by the bis-4-methyl substitution (**13**). The single substitution of just the C2 phenyl group shows no enhancement for the A_1 receptor, with both the 4-Cl (**15**) and the 4-MeO (**17**) moieties reducing the affinity of the ligand for this receptor, whereas compound **16** possessing the 4-Me group retains the affinity of the unsubstituted diphenylpurine at 4 nM. The great beneficiary of this particular substitution pattern is the A_3 receptor, where both the 4-Me (**16**) and the 4-MeO (**17**) compounds display significantly enhanced affinities (K_i values of 9 and 4 nM, respectively) when compared to that of compound **11**. 4-Methoxy-substitution of phenyl groups have usually been shown to enhance the affinity for the A_3 receptor in a number of different series of adenosine antagonists;[24,25] thus, it may seem surprising that the bis-4-Me substituted variety (**13**) displayed significantly higher affinity for the A_3 receptor than the analogous bis-4-MeO ligand (**14**). However, the results of the monosubstituted compounds (**16**, 9 nM and **17**, 4 nM) confirm that the 4-MeO group is still preferred by the A_3 receptor. Bis-4-MeO substitution is probably just too large for optimal binding in the A_3 receptor pocket, accounting for its poorer affinity compared to that of the slightly smaller bis-4-Me variation. It seems that for the A_1 receptor, unsubstituted phenyl groups may be the optimal R^1 and R^2 groups for the affinity of 9*H*-purines.

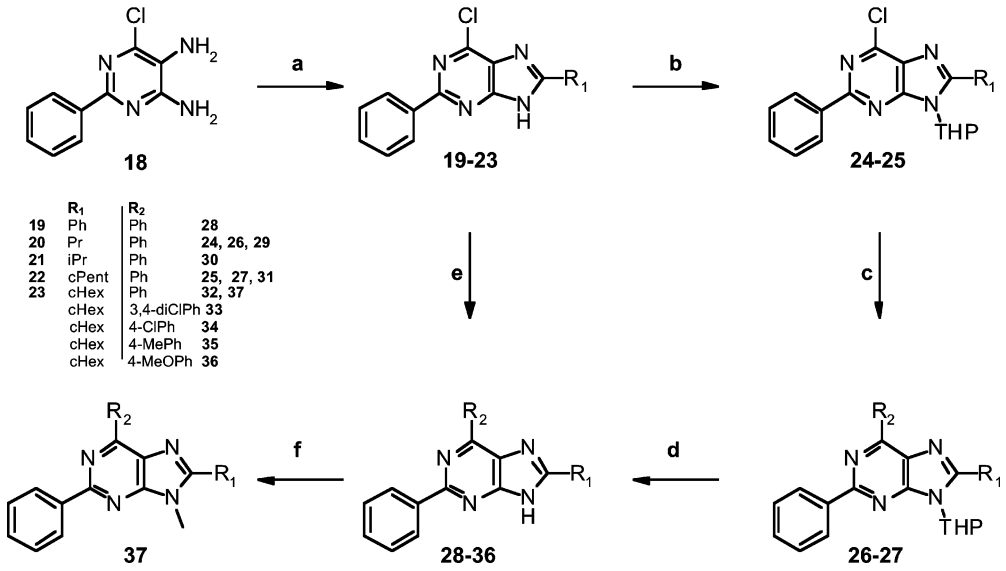
Exploration of the C8 position of the purine led, in general, to significant improvements in the affinity for the A_1 receptor.

Scheme 1[a]



[a] (a) dihydropyran, pTSA, THF; (b) 1 equiv $Ph_2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; (c) 3 equiv $R_2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; (d) 1.2 eq $R_3B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; (e) dowex, EtOH.

Scheme 2[a]



[a] (a) (i) R_1COCl, pyridine, DCM; (ii) 2M NaOH; (b) dihydropyran, pTSA, THF; (c) 1.5 equiv $R_2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; (d) dowex, EtOH; (e) 1.5 equiv $R_2B(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, PhMe; (f) NaH, MeI, DMF.

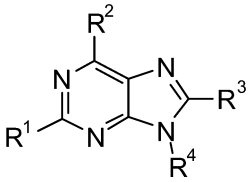
A phenyl group in the 8-position (**28**) is evidently less appreciated in the A_1 receptor binding pocket with a drop in affinity to 21 nM. Both the A_{2A} and A_3 receptors seem to tolerate this much larger substituent well, on the whole retaining the affinity achieved by the comparable unsubstituted compound **11**. This indicates, perhaps, more space in this part of the respective A_{2A} and A_3 receptor pockets than in the A_1 receptor site. The single straight-chained alkyl group (nPr, **29**) showed a K_i value of 4 nM, improving on the selectivity over the A_{2A} receptor when compared to that of the unsubstituted form (**11**) by a factor of 3. However, the affinity at the A_3 receptor also showed an improvement to 17 nM. The most active compounds at the adenosine A_1 receptor registered subnanomolar affinity. These were the C8-isopropyl (**30**), cyclopentyl (**31**), and cyclohexyl (**32**) derivatives at 0.82, 0.29, and 0.73 nM, respectively. In particular, the cyclopentyl moiety (**31**) displayed an impressive gain in affinity at the A_1 receptor, while retaining the same degree of affinity at the A_{2A} and A_3 receptors in comparison to that of ligand **11**. In comparison to the cyclopentyl derivative, the cyclohexyl compound (**32**) was only slightly lower in affinity at the A_1 receptor, yet showed a significant drop in affinity at the A_{2A} and A_3 receptors. Thus, this compound had, on the whole, better A_{2A}/A_1 and A_3/A_1 selectivity ratios at 160 and 274, respectively (for **31** $A_{2A}/A_1 = 190$ and $A_3/A_1 = 121$). Its cyclohexyl substituent is also more flexible than the similarly sized phenyl group in **28**, which is apparently capable of accommodating the binding pocket much better.

To see whether both C8 substitution and the substitution at one of the phenyl groups would be well tolerated, compounds **33**−**36** were synthesized and tested. Again, a significant loss in affinity was noted across the three receptors, although the 4-MeO moiety seemed to interfere the least, retaining an affinity of 4 nM for the A_1 receptor. Compound **37** highlights the importance of the free N9 proton to act as a hydrogen-bond donor because the methyl substitution results in a complete loss of affinity at the adenosine receptors.

Comparing the most potent compound at the human A_1 receptor of this series, **31** (0.29 nM), to the most potent purine published to date, N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine (7.7 nM),[9] it may be speculated that the affinity to the A_1 receptor is much improved (almost 30-fold) because both the L1 and the L3 pockets are now subject to interaction with the ligand. In Figure 4, the two possible ways of superimposing these compounds is depicted, following the proposed model as mentioned in Figure 3. The superimposition shown in Figure 4a suggests that there are no interactions of the L1 pocket with N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine, as provided by the C2-phenyl group of **31**, whereas the one shown in 4b suggests that there likewise is little interaction of the L3 pocket with N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine, as provided by the C6-phenyl of **31**. In the lowest energy state of the compound, the 8-substituent of N^6-cyclopentyl-8-(*N*-methyl-isopropylamino)-9-methyladenine is also, most probably, orientated out of the plane of the core heterocycle, although this may not be a significant factor upon the final ligand−receptor complex.

By immobilizing the H-bond acceptor at the top of the molecule to form the fused heterocyclic ring, the position and, thus, the orientation toward the receptor of the L2 group is shifted slightly higher than that predicted in the model detailed

Table 1. Affinities of the Di- and Trisubstituted Purines **11**−**35** in Radioligand-Binding Assays of Human Adenosine Receptors



					K_i (nM) or % disp.[a]		
	R^1	R^2	R^3	R^4	hA_1[b]	hA_{2A}[c]	hA_3[d]
11	Ph	Ph	H	H	4.1 ± 0.5	53 ± 14	38 ± 12
12	4-ClPh	4-ClPh	H	H	21%	11%	68 ± 11
13	4-MePh	4-MePh	H	H	235 ± 25	4%	9.3 ± 3
14	4-MeOPh	4-MeOPh	H	H	40%	37%	165 ± 38
15	4-ClPh	Ph	H	H	27 ± 6	315 ± 96	94 ± 10
16	4-MePh	Ph	H	H	4.0 ± 0.6	289 ± 19	9.4 ± 4
17	4-MeOPh	Ph	H	H	32 ± 6	47%	4.0 ± 2
28	Ph	Ph	Ph	H	21 ± 12	66 ± 4	49 ± 19
29	Ph	Ph	Pr	H	4.4 ± 0.7	167 ± 25	17 ± 9
30 (LUF 5956)	Ph	Ph	^{i}Pr	H	0.82 ± 0.08	148 ± 27	9.5 ± 0.9
31 (LUF 5962)	Ph	Ph	cPent	H	0.29 ± 0.07	55 ± 3	35 ± 13
32 (LUF 5957)	Ph	Ph	cHex	H	0.73 ± 0.07	118 ± 17	200 ± 46
33	Ph	4-ClPh	cHex	H	25 ± 5	24%	243 ± 89
34	Ph	3,4-diClPh	cHex	H	37 ± 10	20.3%	37.2%
35	Ph	4-MePh	cHex	H	36 ± 4	9%	41%
36	Ph	4-MeOPh	cHex	H	3.7 ± 0.5	27%	138 ± 42
37	Ph	Ph	cHex	Me	24%	0%	25%

[a] K_i ± SEM ($n = 3$), % displacement ($n = 2$). [b] Displacement of specific [^{3}H]DPCPX binding in CHO cells expressing human adenosine A_1 receptors or % displacement of specific binding at 1 μM concentrations. [c] Displacement of specific [^{3}H]ZM 241385 binding in HEK 293 cells expressing human adenosine A_{2A} receptors or % displacement of specific binding at 1 μM concentrations. [d] Displacement of specific [^{125}I]AB-MECA binding in HEK 293 cells expressing human adenosine A_3 receptors or % displacement of specific binding at 1 μM concentrations.

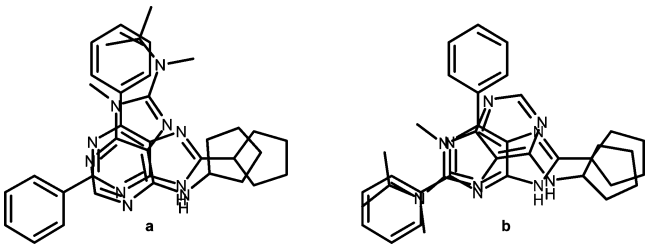


Figure 4. Two possible ways of superimposing compounds **31** and N^6-cyclopentyl-8-(*N*-methylisopropylamino)-9-methyladenine (ref 9).

in our previous publication.[2] This slight relocation of the L2 group has brought about substantial improvements in the affinity for the A_1 receptor. This is best illustrated by comparing the analogous compounds, cyclopentanecarboxylic acid, (2,6-diphenyl-pyrimidin-4-yl)-amide,[2] and 8-cyclopentyl-2,6-diphenyl-9*H*-purine (**31**). In both series, these two compounds displayed the highest affinity for the A_1 receptor, yet **31** was, at 0.29 nM, significantly more active than cyclopentanecarboxylic acid (2,6-diphenyl-pyrimidin-4-yl)-amide (2.14 nM). The selectivity for the A_1 receptor over the A_{2A} and A_3 receptors was also somewhat better for the purine compound (with a A_{2A}/A_1 ratio of 190 and A_3/A_1 ratio of 121) than that for the pyrimidine (92 and 79, respectively). It is therefore appropriate to take into consideration these results and refine the pharmacophoric model. Figure 5 schematically displays the new refinements. It has previously been discovered and discussed in both the pyrimidines series and in this current article that the optimal L2 group seems to be somewhat smaller than the L1 and L3 groups, and the lipophilic pocket that this group fills lacks the ability to interact with π-electrons. The ideal L2 group is, therefore, an alkyl group that consists of an alkyl chain of 2 to 3 carbons in length (i.e., ethyl or propyl) and a secondary branched moiety (i.e., isopropyl, cyclopentyl, etc). Although this article details the development of a fused bicyclic ring, the central aromatic region was sufficiently covered by a single ring, as detailed in the previous publication.[2] Thus, it is appropriate to assume that it is not the size of the aromatic ring that is particularly important but the relative positioning to the aromatic center of the hydrogen-bond donor B and the acceptor C.

We next performed functional assays with these compounds to determine their effect at the adenosine A_1 receptor. First, the three most potent compounds at the human A_1 receptor (**30**, **31**, and **32**, $K_i = 0.82$, 0.29, and 0.73 nM, respectively) were selected and tested in cAMP assays to assess their ability to agonize or antagonize the A_1 receptor. Figure 6 depicts the results of these experiments. Because of the adenosine A_1 receptor's coupling to an inhibitory G protein, which leads to a decrease in the levels of cAMP upon stimulation, forskolin was added to generate a measurable amount of cAMP in the system. The amount of cAMP generated by the sole addition of forskolin was thus set at 100%. CPA (1 μM), as the reference full agonist of the A_1 receptor, reduced the levels of cAMP present in the system, and this was thus set at 0% for the calculations. N0840 (reported in [^{35}S]GTPγS assays to be a neutral antagonist)[9,26] was shown in this whole cell cAMP assay to have properties consistent with an inverse agonist at a concentration of 100 μM, although on lower levels than DPCPX (the reported full inverse agonist, at a concentration of 1 μM). This discrepancy between the two assays is not necessarily conflicting because intrinsic activity is also dependent on the assay system examined. At a concentration of 100 nM (>100 × K_i values, to ensure full receptor occupancy), compounds **30**, **31**, and **32**, all increased the measured-cAMP levels above the levels measured with forskolin alone. Compounds **30** and **32** were less efficacious than N-0840, however, suggesting that the behavior is that of neutral antagonists. We next recorded a full concentration−response curve for compound **32** (Figure 7), antagonizing the effect of 100 nM CPA on forskolin-induced (10 μM) cAMP production. A similar experiment was performed for compound **30**. The IC_{50} values for the two compounds ($n = 3$) were 460 ± 100 nM (**30**) and 477 ± 137 nM (**32**), yielding K_B values of

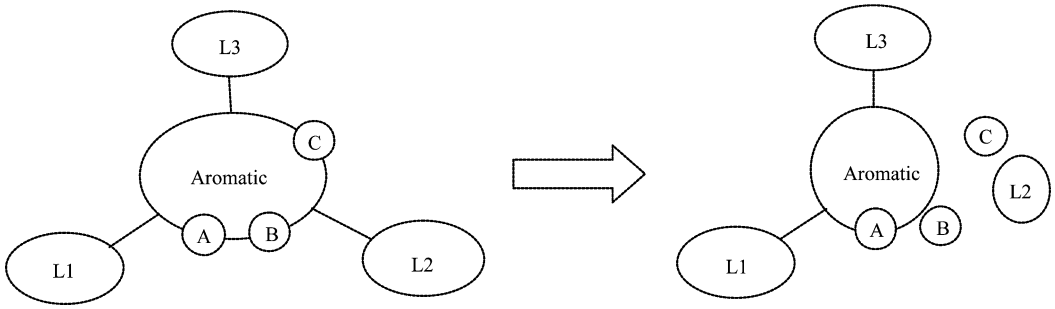


Figure 5. Refinement of the model proposed in an earlier publication (ref 2), showing, among others, the smaller size of the L2 group (see text).

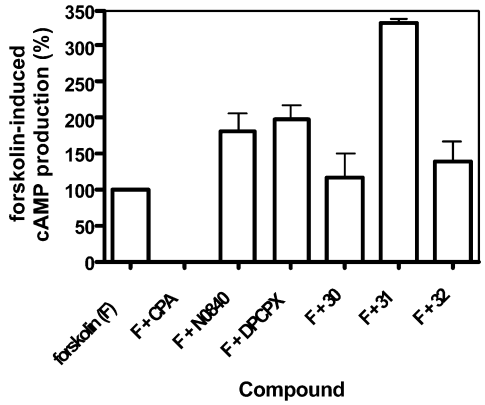


Figure 6. Functional assays performed with reference ligands and compounds **30**, **31**, and **32** on the adenosine A_1 receptor. 100% cAMP amounts to approximately 15 pmol/well. 0% corresponds to approximately 1.3 pmol/well.

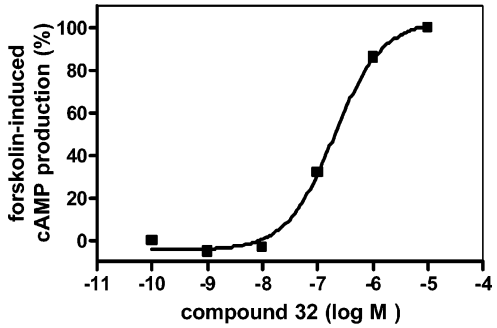


Figure 7. Concentration-dependent antagonism of the effect of CPA (10^{-7} M) on forskolin (10^{-5} M)-induced cAMP production by compound **32**.

approximately 4 nM for both compounds in this functional assay. These K_B values are somewhat higher but not inconsistent with the affinity values determined in the radioligand-binding assays. As a control experiment, we examined the effects of N-0840, **30**, **31**, and **32** when given alone (10 μM), that is, in the absence of both CPA and forskolin. As expected, no measurable changes from control levels were recorded. Thus, the purines presented in the present article display antagonistic-inverse agonistic behavior, typical of most nonribose ligands for adenosine receptors.

Conclusions

This article describes a series of 2,6-di- and 2,6,8-trisubstituted purines as antagonists for the human adenosine A_1 receptor synthesized as a consequence of the refinement of the model proposed in an earlier publication.[2] The fixation of the H-bond acceptor at the top of the molecule repositions the relative location of the L2 group. Exchanging the heteroatom for nitrogen creates the imidazole ring. The benefits of these modifications are highlighted by compound **31**, LUF 5962, 8-cyclopentyl-2,6-diphenyl-9*H*-purine, which has an affinity of 0.29 nM at the human adenosine A_1 receptor.

Experimental Section

Chemistry. Materials and Methods. All reagents used were obtained from commercial sources and all solvents were of an analytical grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 (^{1}H NMR, 200 MHz; ^{13}C NMR, 50.29 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in δ (ppm), and the following abbreviations are used: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, and ar = aromatic protons. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Leiden Institute of Chemistry and are within 0.4% of the theoretical values unless otherwise stated. The reactions were routinely monitored by TLC using Merck silica gel F_{254} plates. Microwave reactions were performed on an Emrys Optimizer (Biotage AB, formerly Personal Chemistry). The wattage was automatically adjusted so as to maintain the desired temperature.

A procedure to protect the purines has been reported in the literature by Cassidy et al.[27] Compounds **2**[27] and **18**[20] have been reported previously.

General Preparation for the Suzuki−Miyaura Cross-Coupling Under Microwave Conditions. THP-protected 2,6-dichloropurine **2** (1 equiv) was dissolved in dry toluene (5 mL). To this was added the appropriate boronic acid (3 equiv if double substitution was required or 1 equiv if single substitution was desired), K_2CO_3 (1.5 equiv or 1 equiv, respectively), and $Pd(PPh_3)_4$ (0.052 equiv). The reaction vial was then sealed and heated at 150 °C for 20 min. Upon completion of the reaction (monitored by TLC), the solvents were evaporated and the crude product preabsorbed on silica. Purification with column chromatography gave the desired product.

2-Chloro-6-phenyl-9-(tetrahydro-pyran-2-yl)-9*H*-purine (3). White solid, 71%.

2,6-Diphenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (4). White solid, 80%.

2,6-Bis(4-chlorophenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (5). White solid, 91%.

2,6-Bis(4-tolyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (6). White solid, 77%.

2,6-Bis(4-methoxyphenyl)-9-(tetrahydropyran-2-yl)-9*H*-purine (7). White solid, 82%.

2-(4-Chlorophenyl)-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (8). White solid, 87%.

2-Tolyl-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (9). White solid, 90%.

2-(4-Methoxyphenyl)-6-phenyl-9-(tetrahydropyran-2-yl)-9*H*-purine (10). Oil, 97%.

Deprotection of the purines was performed following a procedure described by Hocek et al.[28]

2,6-Diphenyl-9*H*-purine (11). Recrystallized from EtOAc/PE. White solid, 53%; mp: >252 °C dec MS (ESI): 273.0. Anal. ($C_{17}H_{12}N_4$·0.4EtOAc) C, H, N.

2,6-Bis(4-chlorophenyl)-9*H*-purine (12). Recrystallized from DCM. White solid, 43%; mp: >290 °C dec. MS (ESI): 340.7. Anal. ($C_{17}H_{10}N_4Cl_2 \cdot 0.1H_2O.0.05CH_2Cl_2$) C, H, N.

2,6-Bis(4-tolyl)-9*H*-purine (13). Recrystallized with DCM. White solid, 52%; mp: >288 °C dec MS (ESI): 300.9. Anal. ($C_{19}H_{16}N_4 \cdot 0.06CH_2Cl_2$) C, H, N.

2,6-Bis(4-methoxyphenyl)-9*H*-purine (14). Recrystallized several times from various solvents, including CH2Cl2, EtOH, MeOH, and EtOAc/PE mixtures. White solid; mp: 282 °C. MS (ESI): 332.0.

2-(4-Chlorophenyl)-6-phenyl-9*H*-purine (15). Recrystallized from MeOH. White solid, 46%; mp: 262 °C. MS (ESI): 306.8. Anal. ($C_{17}H_{11}ClN_4$) C, H, N.

2-Tolyl-6-phenyl-9*H*-purine (16). Recrystallized from MeOH. White solid, 43%; mp: 251 °C. MS (ESI): 286.8. Anal. ($C_{18}H_{14}N_4 \cdot$ 0.16MeOH) C, H, N.

2-(4-Methoxyphenyl)-6-phenyl-9*H*-purine (17). Recrystallized from MeOH. White solid, 55%; mp: 269 °C. MS (ESI): 302.8. Anal. ($C_{18}H_{14}N_4$) C, H, N. Ring closure was performed according to a procedure described by Scammells et al.[23]

6-Chloro-2,8-diphenyl-9*H*-purine (19). White solid, 66%.

2-Phenyl-6-chloro-8-propyl-9*H*-purine (20). White solid, 51%.

2-Phenyl-6-chloro-8-isopropyl-9*H*-purine (21). White solid, 78%.

2-Phenyl-6-chloro-8-cyclopentyl-9*H*-purine (22). White solid, 44%.

2-Phenyl-6-chloro-8-cyclohexyl-9*H*-purine (23). White solid, 99%.

2-Phenyl-6-chloro-8-propyl-9-(tetrahydropyran-2-yl)-9*H*-purine (24). White solid, 67%.

2-Phenyl-6-chloro-8-cyclopentyl-9-(tetrahydropyran-2-yl)-9*H*-purine (25). White solid, 75%.

2,6-Diphenyl-8-propyl-9-(tetrahydropyran-2-yl)-9*H*-purine (26). White solid, 53%.

2,6-Diphenyl-8-cyclopentyl-9-(tetrahydropyran-2-yl)-9*H*-purine (27). White solid, 57%.

2,6,8-Triphenyl-9*H*-purine (28). Recrystallized with MeOH. White solid, 87%; mp: 233 °C. MS (ESI): 348.7. Anal. ($C_{23}H_{16}N_4 \cdot$ 0.12 MeOH) C, H, N.

8-Propyl-2,6-diphenyl-9*H*-purine (29). Recrystallized from MeOH. White solid, 52%; mp: 149 °C. MS (ESI): 314.8. Anal. ($C_{20}H_{18}N_4$) C, H, N.

8-Isopropyl-2,6-diphenyl-9*H*-purine (30). Recrystallized from MeOH. White solid, 36%; mp: 214 °C. MS (ESI): 314.8. Anal. ($C_{20}H_{18}N_4 \cdot 0.1$EtOH) C, H, N.

8-Cyclopentyl-2,6-diphenyl-9*H*-purine (31). Recrystallized from DCM. White solid, 45%; mp: 224 °C. MS (ESI): 341.0. Anal. ($C_{22}H_{20}N_4 \cdot 0.04CH_2Cl_2$) C, H, N.

8-Cyclohexyl-2,6-diphenyl-9*H*-purine (32). Recrystallized from EtOH. White solid, 49%; mp: 209 °C. MS (ESI): 354.8. Anal. ($C_{23}H_{22}N_4 \cdot 0.1H_2O$) C, H, N.

8-Cyclohexyl-6-(4-chlorophenyl)-2-phenyl-9*H*-purine (33). Recrystallized from MeOH. White solid, 86%; mp: 156 °C. MS (ESI): 388.9, 390.2. Anal. ($C_{23}H_{21}ClN_4 \cdot 0.7H_2O \cdot 0.5$MeOH) C, H, N.

8-Cyclohexyl-6-(3,4-dichlorophenyl)-2-phenyl-9*H*-purine (34). Recrystallized from EtOH. White solid, 64%; mp: 201 °C. MS (ESI): 422.7, 425.2. Anal. ($C_{23}H_{20}Cl_2N_4 \cdot 0.7$EtOH) C, H, N.

8-Cyclohexyl-6-(4-tolyl)-2-phenyl-9*H*-purine (35). Recrystallized from MeOH. White solid, 98%; mp: 149 °C. MS (ESI): 368.5. Anal. ($C_{24}H_{24}N_4 \cdot 0.9H_2O \cdot 0.35$MeOH) C, H, N.

8-Cyclohexyl-6-(4-methoxyphenyl)-2-phenyl-9*H*-purine (36). Recrystallized from MeOH. White solid, 29%; mp: 141 °C. MS (ESI): 384.9. Anal. ($C_{24}H_{24}N_4O \cdot 0.95H_2O \cdot 0.3$MeOH) C, H, N.

8-Cyclohexyl-9-methyl-2,6-diphenyl-9*H*-purine (37). Recrystallized from $CHCl_3$. White solid, 30%; mp: 200−202 °C. MS (ESI): 369.1. Anal. ($C_{24}H_{24}N_4 \cdot 0.09CHCl_3$) C, H, N.

Biology. Materials and Methods. [^{3}H]DPCPX and [^{125}I]AB-MECA were purchased from Amersham Biosciences (NL). [^{3}H]-ZM 241385 was obtained from Tocris Cookson, Ltd. (U.K.). CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College. London, U.K. HEK 293 cells stably expressing the human adenosine A_{2A} and A_3 receptors were gifts from Dr. Wang (Biogen, U.S.A.) and Dr. K. -N. Klotz (University of Würzburg, Germany), respectively.

All compounds were tested in radioligand-binding assays to determine their affinities at the human adenosine A_1, A_{2A}, and the A_3 receptors as described previously.[2] The human A_1 receptors were expressed in CHO cells, and [^{3}H]DPCPX was used as the radioligand. The A_{2A} and A_3 receptors were expressed in HEK 293 cells, and [^{3}H]ZM 241385 and [^{125}I]AB-MECA were used as the respective radioligands.

The compounds specified in the text were tested in functional assays for their ability to influence the levels of cAMP in the test system. The compounds were tested at concentrations of, at least, $100 \times K_i$, where the receptor sites should be fully occupied. The behavior of the compounds was observed with reference to known adenosine receptor ligands: CPA (a full agonist), DPCPX (an inverse agonist), and N0840 (reported as a neutral antagonist).

CHO cells expressing the human adenosine A_1 receptor were grown overnight as a monolayer in 24-well tissue culture plates (400 μL/well; 2×10^5 cells/well). cAMP generation was performed in a Dulbecco's Modified Eagles Medium (DMEM)/N-2-hydroxyethylpiperazin-N′-2-ethansulfonic acid (HEPES) buffer (0.60 g HEPES/50 mL DMEM pH 7.4). Each well was washed twice with the HEPES/DMEM buffer (250 μL) and the following added: adenosine deaminase (0.8 IU/mL), rolipram (50 μM), and cilostamide (50 μM). This was then incubated for 30 min at 37 °C followed by the introduction of the compound of interest. After a further 10 min of incubation, forskolin was added (10 μM). After a subsequent 15 min, incubation was stopped by aspirating the assay medium and by adding 200 μL of ice-cold 0.1 M HCl. The amount of cAMP was determined by competition with [^{3}H]cAMP for protein kinase A (PKA). Briefly, the sample, approximately 1.8 nM [^{3}H]cAMP, and 100 μL of PKA solution were incubated on ice for 2.5 h. The incubations were stopped by rapid dilution with 2 mL of ice-cold Tris HCl buffer (pH 7.4), and the bound radioactive material was then recovered by filtration through Whatman GF/C filters. The filters were additionally rinsed with 2 $\times$ 2 mL of Tris HCl buffer and then the radioactivity counted in a Packard Emulsifier Safe scintillation fluid (3.5 mL). All data reflect at least three independent experiments performed in duplicate.

Data Analysis. K_i values were calculated using a nonlinear regression curve-fitting program (GraphPad Prism, GraphPad Software Inc., San Diego, CA). K_D values of the radioligands were 1.6 nM, 1.0 nM, and 5.0 nM for [^{3}H]DPCPX, [^{3}H]ZM241385, and [^{125}I]AB-MECA, respectively. The data from the functional assays were also analyzed with GraphPad Prism, and Figures 6 and 7 were generated by evaluating the data to relate to the known ligand CPA (set at 0%) and forskolin (set at 100%).

Supporting Information Available: Elemental analyses and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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