reminimized, from their energies in the complexes. In addition, we have evaluated the energies of interaction between the drugs and nucleotide residues (sugars, phosphates, and bases) located close to them. These are schematically illustrated in Figure 2.

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**Registry No.** GC10, 76957-82-9.

**Supplementary Material Available:** Force field parameters for BMY-25282 (1 page). Ordering information is given on any current masthead page.

# Activity of $N^6$-Substituted 2-Chloroadenosines at $A_1$ and $A_2$ Adenosine Receptors

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Radioligand binding studies of $N^6$-substituted adenosines at the $A_1$ and $A_2$ adenosine receptors of rat brain cortex and rat brain striatum, respectively, show that a 2-chloro substituent does not consistently change the affinity or the selectivity of these analogues for the $A_1$ receptor. A 2-chloro substituent lowers the characteristic stereoselectivity of the $A_1$ receptor toward the *R* diastereomer of $N^6$-(1-phenyl-2-propyl)adenosine. A 2-chloro substituent consistently increases potency of $N^6$-substituted adenosines as agonists at an adenosine $A_2$ receptor stimulatory to adenylate cyclase in PC12 cell membranes.

The ubiquity of $A_1$ and $A_2$ adenosine receptors ($A_1$AR, $A_2$AR) and the several responses that these receptors mediate create side effects that could limit the therapeutic usefulness of this nucleoside. Accordingly, a considerable effort has gone into the synthesis of agonists and antagonists selective for one or the other type of receptor.[1,2] It is now clear that certain $N^6$-alkyl and $N^6$-cycloalkyl substituents promote selectivity for the $A_1$AR[3,4] and certain $N^6$-aralkyl substituents confer potency and selectivity for the $A_2$AR.[5,6] Attempts to improve the potency and selectivity of adenosine by combining modifications in different parts of the molecule have been only partly successful. Whereas an *N*-ethyl 5′-uronamide modification of the ribose increases the potency of adenosine,[7] such a modification of an $N^6$-cycloalkyladenosine has little effect on activity at the $A_1$AR.[8] A 2-chlorosubstituent enhances the potency and selectivity for the $A_1$AR of $N^6$-cyclopentyl-1-deazaadenosine, but not of other $N^6$-substituted 1-deazaadenosines.[9] That discovery led to the development of 2-chloro-$N^6$-cyclopentyladenosine[10] (CCPA), which is more potent at and selective for the $A_1$AR than $N^6$-cyclopentyladenosine (CPA), which until that time was the standard for selective $A_1$AR agonists.[3,11]

Here we report measurements of the affinity for $A_1$AR and $A_2$AR of $N^6$-cyclopentyladenosine, $N^6$-phenyladenosine, and $N^6$-(1-phenyl-2(*R*)-propyl)adenosine ((*R*)-PIA) and its *S* diastereomer ((*S*)-PIA) and comparison of those measurements with the affinities of the corresponding 2-chloroadenosines. In general, our observations do not support the notion that a 2-chloro substituent enhances the potency and selectivity of an $N^6$-substituted adenosine for the $A_1$AR, nor does a 2-chloro substituent appear to enhance the stereoselective recognition of the

**Scheme I**

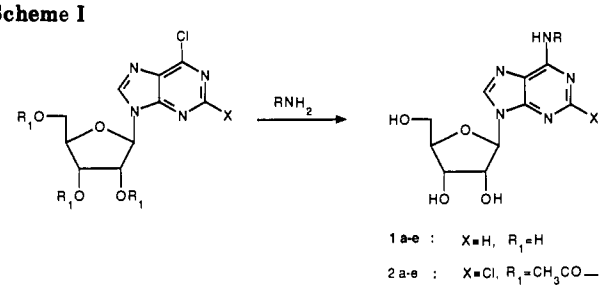


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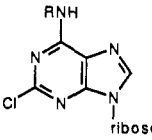
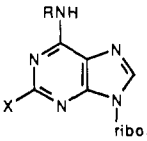
**Table I.** Properties of $N^6$-Substituted 2-Chloroadenosines **2b–e**




| no. | R | formula | anal. | purification[a] | mp, °C | UV, $\lambda_{max}$ ($\epsilon$) |
|---|---|---|---|---|---|---|
| **2b** | c-Pent | $C_{15}H_{19}ClN_5O_4$ | CHNCl | A (50–70) | 105–107 | 274 (18800) |
| **2c** | Ph | $C_{16}H_{15}ClN_5O_4 \cdot H_2O$ | CHNCl | B | 195 | 295 (29700) |
| **2d** | 1-Ph-2(*R*)-Pr | $C_{19}H_{21}ClN_5O_4$ | CHNCl | A (45–70) | 106 | 274 (18800) |
| **2e** | 1-Ph-2(*S*)-Pr | $C_{19}H_{21}ClN_5O_4$ | CHNCl | A (45–70) | 110 | 274 (18300) |

[a] A: LPLC, 40–60 μm C-18 silica gel, eluted with a linear gradient of methanol/water. Numbers in parentheses are initial and final concentrations of methanol, % v/v. B: Recrystallized from ethanol/water.

**Table II.** Agonist Potencies of $N^6$-Substituted Adenosine and 2-Chloroadenosines at $A_1$ and $A_2$ Adenosine Receptors




| | | | $K_i$ or $EC_{50}$, μM[a] | | | |
|---|---|---|---|---|---|---|
| no. | R | X | binding vs [$^3$H](*R*)-PIA at rat brain $A_1$AR | binding vs [$^3$H]NECA at rat striatum $A_2$AR | $A_2$AR-mediated stimulation of PC12 adenylate cyclase[b] | $A_2$AR/$A_1$AR activity ratio[c] |
| **1a** | H | H | *d* | *d* | 0.15 ± 0.01 | |
| **2a** | H | Cl | 0.0067 ± 0.001 | 0.076 ± 0.012 | 0.46 ± 0.05 | 1.1 |
| **1b** | c-Pent | H | 0.00045 ± 0.00004 | 0.51 ± 0.12 | 3.2 ± 0.3 | 1130 |
| **2b** | c-Pent | Cl | 0.0006 ± 0.0001 | 0.95 ± 0.09 | 0.73 ± 0.11 | 1500 |
| **1c** | Ph | H | 0.016 (0.011–0.028) | 1.2 ± 0.37 | 3.1 ± 0.1 | 75 |
| **2c** | Ph | Cl | 0.0076 ± 0.0003 | 1.7 ± 0.2 | 1.6 ± 0.4 | 220 |
| **1d** | 1-Ph-2(*R*)-Pr | H | 0.0012 (0.0009–0.0015) | 0.22 ± 0.06 | 0.98 ± 0.12 | 180 |
| **2d** | 1-Ph-2(*R*)-Pr | Cl | 0.0014 ± 0.0001 | 0.22 ± 0.04 | 0.32 ± 0.06 | 160 |
| **1e** | 1-Ph-2(*S*)-Pr | H | 0.050 (0.044–0.057) | 3.0 ± 1.0 | 4.2 ± 1.0 | 60 |
| **2e** | 1-Ph-2(*S*)-Pr | Cl | 0.020 ± 0.001 | 3.0 ± 0.3 | 3.0 ± 0.6 | 150 |

[a] Values are means ± SEM ($n$ = 3), or in three instances of data from prior studies,[16,18] values are means with 95% confidence limits in parentheses. [b] The maximum stimulation of PC12 adenylate cyclase by adenosine was 70% that of NECA, and stimulation by the $N^6$-substituted adenosines was about 80% that of NECA. [c] $K_i$ of binding at the $A_2$AR divided by the $K_i$ of binding at the $A_1$AR. [d] Adenosine cannot be assayed because of the presence of adenosine deaminase added to the assay mixture.

diastereomers of PIA that is characteristic of the $A_1$AR.[4]

## Results and Discussion

**Chemistry.** The reaction of an amine with 6-chloropurine riboside[12] affords $N^6$-substituted adenosines **1a–e**. Similarly, the reaction of an amine with 2,6-dichloro-9-(2′,3′,5′-*O*-triacetyl-β-D-ribofuranosyl) purine[13] is a known route to $N^6$-substituted 2-chloroadenosines **2a–e**. Table I lists the properties of **2b–e**.

**Agonist Activity.** Table II summarizes assays of the affinities of **1b–d** and **2a–d** for the $A_1$AR of rat brain cortex and for the $A_2$AR in rat brain striatum as well as stimulation of cyclic AMP production by the $A_2$AR in PC12 cell membranes. As expected, 2-chloroadenosine (**2a**) was an unselective agonist, the $A_2/A_1$ potency ratio being 11. At the $A_1$AR, 2-chloro-$N^6$-cyclopentyladenosine (**2b**) was only 75% as potent as CPA (**1b**). However, **2b** appeared to be more selective for the $A_1$AR; the $A_2/A_1$ potency ratio of **1b** was 1100 while that of **2b** was almost 1600. A 2-chloro substituent improved the potency of $N^6$-phenyladenosine at the $A_1$AR (**1c** vs **2c**) and also increased selectivity for the $A_1$AR by 3-fold. Stereoselective binding of (*R*)-PIA (**1d**), in preference to its *S* diastereomer **1e**, is characteristic of the $A_1$AR; in this instance the potency ratio of diastereomers, **1d**/**1e**, was 42. The 2-chloro derivative of (*R*)-PIA (**2d**) was no more potent that **1d** at the $A_1$AR, and the stereoselectivity ratio, **2d**/**2e**, was 14, lower by 3-fold. Although **1d** and **2d** are less potent at the $A_2$AR, their potencies were equal, so the 2-chloro substituent did not improve selectivity for the $A_1$AR.

As stimulants of the adenylate cyclase in PC12 cell membranes, neither **2a** nor any of the $N^6$-substituted adenosines was as active as adenosine. Among the $N^6$-substituted analogues, however, a 2-chloro substituent consistently lowered the $EC_{50}$ of adenylate cyclase stimulation, but at most by only 4-fold.

In summary, a 2-chloro substituent does not consistently increase the potency of an $N^6$-substituted adenosine at the $A_1$AR of rat brain cortex, the potency of such analogues for the $A_2$AR of rat brain striatum, or reduce the stereo-

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selective recognition of (*R*)-PIA at the $A_1$AR. The $N^6$-substituted 2-chloroadenosines tend to be better stimulants of the adenylate cyclase of PC12 cell membranes than the corresponding deschloro adenosines.

## Experimental Section

Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. $^1$H NMR spectra of solutions of nucleosides in DMSO-$d_6$ obtained on a Varian EM 360L spectrograph were consistent with the assigned structures. M-H-W Laboratories, Tucson, AZ, performed the elemental analyses, which agreed to within ±0.4% of theoretical composition. Assays of purity by reverse-phase HPLC revealed that product accounted for >99% of the UV-absorbing material in samples submitted for assay.

**2-Chloro-$N^6$-cyclopentyladenosine (2b).** A mixture of 2,6-dichloro-9-(2,3,5-*O*-triacetyl-β-D-ribofuranosyl)purine (2.0 g, 4.5 mmol), cyclopentylamine (0.77 g, 9.0 mmol), *N*,*N*-diisopropylethylamine (1.6 mL, 9.2 mmol), and 70 mL of 100% ethanol was refluxed for 24 h. The resulting solution was cooled to 5–10 °C in an ice bath and saturated with dry ammonia. The solution was stirred at room temperature for 5 days. Evaporating the solvents in vacuo yielded a syrup, which was purified according to Table I.

**Assays of Receptor Binding and Adenylate Cyclase.** Inhibition of the binding of [$^3$H]-$N^6$-(1-phenyl-2(*R*)-propyl)adenosine ((*R*)-PIA) to the $A_1$AR in rat cerebral cortex membranes and of [$^3$H]-*N*-ethyladenosine-5′-uronamide (NECA) to rat striatal membranes were assayed as described.[11,14] Both assays employed binding in the presence of 5 mM theophylline to define unspecific binding, and in the assays of binding to the $A_2$AR, 50 nM CPA was present to block binding to the $A_1$AR. Calculations of $K_i$ from measurements of $IC_{50}$ employed the Cheng–Prusoff equation.[15] Previously described assays[16,17] measured $A_2$AR-mediated stimulation of the adenylate cyclase in membranes from PC12 rat pheochromocytoma cells.

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**Registry No.** **1b**, 41552-82-3; **1c**, 23589-16-4; **1d**, 38594-96-6; **1e**, 38594-97-7; **2a**, 146-77-0; **2b**, 37739-05-2; **2c**, 29204-70-4; **2d**, 23558-58-9; **2e**, 23559-45-7; 6-chloropurine riboside, 5399-87-1; 9-(2′,3′,5′-*O*-triacetyl-β-D-ribofuranosyl)purine, 3056-18-6; cyclopentylamine, 1003-03-8; (*R*)-1-phenyl-2-propylamine, 156-34-3; (*S*)-1-phenyl-2-propylamine, 51-64-9; adenylate cyclase, 9012-42-4.

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# Synthesis and Antibacterial Activities of C-21 Functionalized Derivatives of (9*R*)-9-Amino-9-deoxoerythromycins A and B

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Selective protection of (9*R*)-9-amino-9-deoxoerythromycin A allowed for elimination of the 12-hydroxyl group to afford a versatile 12,21-olefin intermediate. Further modifications of the intermediate led to the syntheses of (9*R*)-9-deoxo-9-(*N*,*N*-dimethylamino)-12,21-epoxyerythromycin B, (9*R*)-9-deoxo-9-(*N*,*N*-dimethylamino)-21-hydroxyerythromycin A, and (9*R*)-9-deoxo-9-(*N*,*N*-dimethylamino)-21-hydroxyerythromycin B. All three compounds retained antibacterial activity against several organisms normally susceptible to (9*R*)-9-deoxo-9-(*N*,*N*-dimethylamino)erythromycin A. However, the 21-hydroxylated erythromycin A analogue was weaker in potency than the corresponding erythromycin B congener and much weaker than the epoxy derivative. This suggests that while substitution of a polar functionality at C-21 does not abolish antibacterial activity, introduction of vicinal polar groups at both C-12 and C-21 may lead to reduction in potency. Nevertheless, these 21-functionalized derivatives of (9*R*)-erythromycylamine provide an entry into novel analogues of the important macrolide antibiotic erythromycin.

## Introduction

The macrolide antibiotic erythromycin A (1) has enjoyed successful clinical use for over 35 years. This longevity is due to its proven efficacy in Gram-positive infections and infections caused by organisms of emerging importance, such as *Legionella* and *Chlamydia*,[1] while showing a relative lack of toxicity. The success of 1 has led to several synthetic modifications aimed at improving its activity, antibacterial spectrum, and pharmacokinetics or at exploring its structure–activity relationships.[2] One such

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