Chiral Resolution and Stereospecificity of 6-Phenyl-4-phenylethynyl-1,4-dihydropyridines as Selective A_3 Adenosine Receptor Antagonists

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Racemic 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivatives have been shown to be highly selective A_3 adenosine receptor antagonists (Jiang et al. *J. Med. Chem.* **1997**, *40*, 2596−2608). Methods for resolving the optical isomers at the C4 position, involving selective crystallization or chromatographic separation of diastereomeric ester derivatives, have been developed. Optically pure glycerol and threitol derivatives were used as chiral auxiliary groups for ester formation at the 3-position, resulting in diastereomeric mixtures of dihydropyridines. Esterification of a 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivative at the 3-position with a chiral, protected glycerol moiety, *(S)*-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, allowed the selective crystallization of a pure diastereomer, **9**. The ^{1}H NMR spectrum of **9** using the lanthanide shift reagent Eu(fod)$_3$ indicated optical purity, and the *(4S,2′R)*-configuration was assigned using X-ray crystallography. The noncrystalline *(4R,2′R)*-isomer **10** was also isolated and shown to be 3-fold more potent than the *(4S,2′R)*-isomer in binding to A_3 receptors. The 2,2-dimethyl-1,3-dioxolane moiety also served as a protected form of a diol, which showed selective reactivity versus a 5-ethyl ester in basic transesterification reactions. A racemic 5-carboxylic acid derivative could not be resolved through crystallization of diastereomeric salts. Enantiomers of 5-benzyl 3-ethyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**2**) were obtained via an ester derived from (*4R,5R*)-(−)-2,3-*O*-isopropylidene-D-threitol at the 3-position, which was resolved using HPLC, and each diastereomer was subsequently deprotected in acidic conditions. The resulting diols were exchanged for ethyl ester groups by base-catalyzed transesterification. The binding of pure enantiomers of **2** at A_3 adenosine receptors indicated a 35-fold stereoselectivity for the *(4S)*-isomer **21**. A receptor docking hypothesis, using a previously derived human A_3 receptor model, shows the bulkier of the two ester groups (5-Bn) of **21** oriented toward the exofacial side and the 4-position phenylethynyl group situated between transmembrane helical domain TM6 and TM7.

Introduction

Antagonists that interact selectively with A_3 adenosine receptors recently have been reported.[1−7] A_3 adenosine receptor antagonists have been proposed to have potential as antiasthmatic, antiinflammatory, and cerebroprotective agents.[8−12] Diverse structural classes of A_3 antagonists are 1,4-dihydropyridines (e.g. MRS 1191),[1,3] flavonoids (e.g. MRS 1067),[4] triazoloquinazolines (e.g. MRS 1220),[5] triazolonaphthyridines (e.g. L-249313),[6] pyridines (e.g. MRS 1523),[7] and pyridylisoquinolines (e.g. VUF 8504).[2]

We have used the 1,4-dihydropyridine[1,3] nucleus as a template for probing structure−activity relationships (SAR) at the subtypes of adenosine receptors. Dihydropyridines are privileged structures in medicinal chemistry; i.e. they display low-affinity binding at a variety of receptor sites.[13−17] Varying substituents on the 1,4-dihydropyridine template can have dramatic effects on their interactions with these receptors. We observed that at adenosine receptors, the affinity of many 1,4-dihydropyridines, even commercial L-type calcium channel blockers, is in the micromolar range, and this lead has been optimized in the design of selective A_3 adenosine receptor antagonists. The essential modifications leading to A_3 selectivity and preventing binding to L-type calcium channels and other sites are a phenyl ring at the 6-position and at the 4-position either a styryl or a phenylethynyl group (e.g. **1** and **2**, Figure 1). The trisubstituted analogue 5-benzyl 3-ethyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate, **2**, is 1300-fold selective for the human A_3 receptor versus A_1 receptors, with a K_i value of 31 nM.

While the stereospecificity of the binding of chiral ligands is a general property of receptors, the enantiomeric ratios of affinities may vary widely. At L-type calcium channels, the potency ratios between *(4R)*- and *(4S)*-1,4-dihydropyridines are generally 10−100-fold, with the *(4S)*-enantiomer being more potent.[18−20] For most of those enantiomeric pairs of calcium channel

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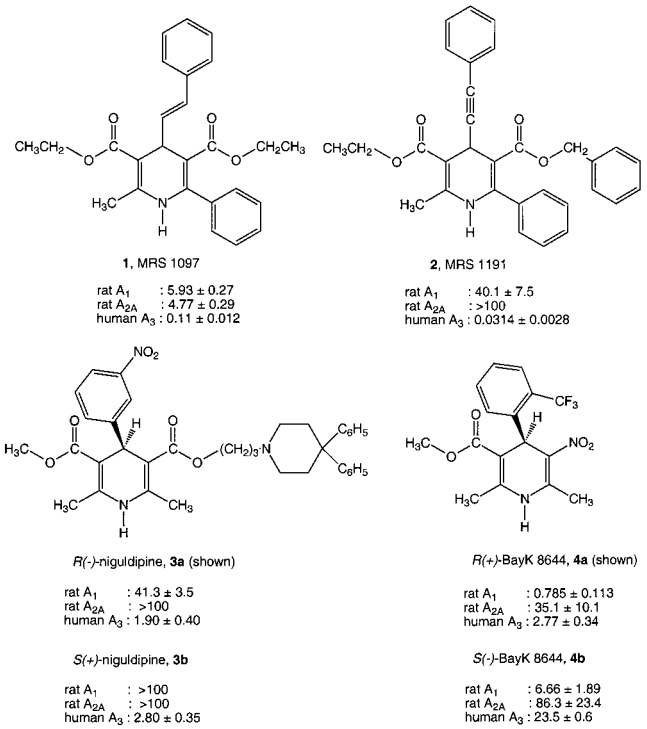
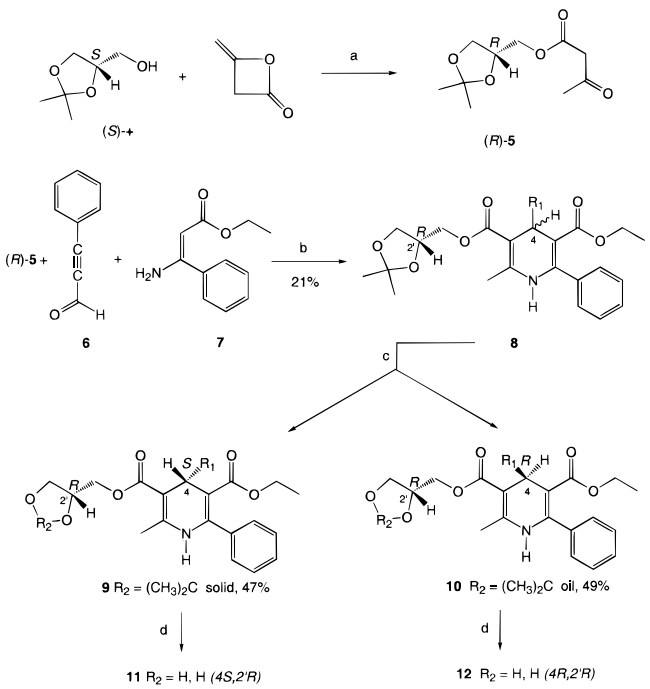


Figure 1. Structures of key racemic dihydropyridine A_3 adenosine receptor-selective antagonists **1** and **2** and optically pure L-type calcium channel ligands **3** and **4** for which the adenosine receptor affinity has been measured. K_i values in micromolar are reported in refs 1 and 3.

Scheme 1. Synthesis of Diastereomeric 5-Ethyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridines Using the Hantzsch Reaction and Resolution by Crystallization (R_1 = C≡C−Ph)[a]



[a] Reagents: (a) toluene, NaOAc, 90 °C, 4 h; (b) EtOH, 80 °C; (c) crystallization; (d) THF, 25 °C, 1 N HCl.

ligands, e.g. **3a** and **3b**, the key factor determining stereospecificity is a bulky ester substituent at the 5-position. At adenosine receptors, a previous study including several pairs of enantiomers of 1,4-dihydropyridines bearing 2,6-dimethyl groups (i.e. the antagonist niguldipine, **3**, and the antagonist/agonist pair BayK 8644, **4**) demonstrated a slight preference for the *(4R)*-enantiomers (2- and 8-fold, respectively, at human A_3 receptors).[1] The effects of the 6-phenyl substituent of 1,4-dihydropyridines on stereospecificity of binding to adenosine receptors have not previously been studied. Also, prior information on stereospecificity of dihydropyridine binding concerns mainly the 4-aryl derivatives.

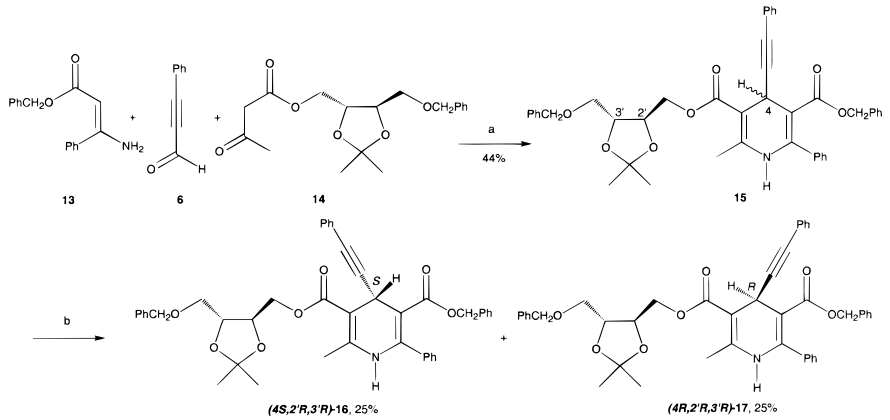
Since racemic 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivatives have displayed exceptionally high A_3 selectivity (in some cases having no measurable affinity at A_1 and A_{2A} adenosine receptors),[3] we sought a facile method to chemically resolve compounds in this series and to study the biological properties of pure stereoisomers. Such a method for resolving the enantiomers at the C4 position consisted of either the selective crystallization or the HPLC purification of diastereomeric ester derivatives. The chiral ester moieties, derivatives of 2,2-dimethyl-1,3-dioxolane-4-methanol and 2,3-*O*-isopropylidene-D-threitol, could be selectively replaced with an ethyl moiety in two steps: i.e. deprotection of a diol followed by transesterification in ethanol.

Results

Chemistry. The chemical synthesis and resolution of diastereomeric mixtures of 1,4-dihydropyridine derivatives are outlined in Schemes 1−5. The strategy utilized for the successful resolution of the enantiomers of **2** (Figure 1), i.e. 5-benzyl ester derivatives, involved use of a chiral auxiliary group at the 3-ester position, which could later be exchanged for an ethyl ester. Analogues of **2** and other 1,4-dihydropyridines substituted with a variety of chiral auxiliaries at the 3-position (groups **A**−**F**, Figure 2) were prepared and compared for ease of separation of diastereomers, either by HPLC or by crystallization. Group **A** (2,2-dimethyl-1,3-dioxolan-4-ylmethyl), a protected glycerol moiety having a single chiral center, proved useful for the fractional crystallization of diastereomers. The synthesis and resolution of 1,4-dihydropyridine derivatives containing group **A** are shown in Scheme 1. While a group **A** derivative containing a 5-ethyl ester was separable by crystallization and led to the determination of the absolute configuration using X-ray crystallography (below), the corresponding 5-benzyl ester was unseparable.

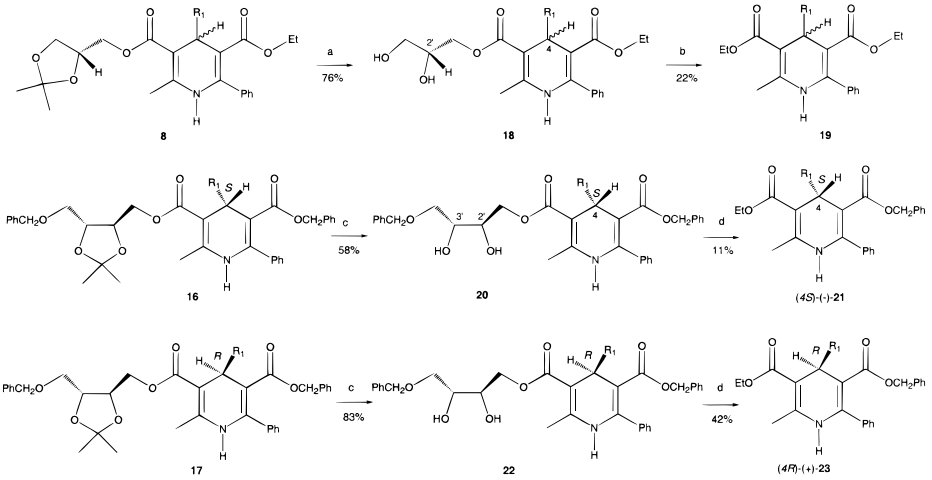
To synthesize a diastereomeric pair for resolution by crystallization, the Hantzsch reaction was carried out using a chiral *β*-ketoester derived from a protected glycerol moiety (**5**, Scheme 1). This intermediate consisted of an acetoacetate ester of *(S)*-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, which corresponds to the *(R)*-enantiomer **5** following esterification. This *β*-ketoester **5** and the other components of the reaction to form the 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivative, e.g. phenylpropargyl aldehyde, **6**, and *β*-enaminoester **7**, were dissolved in ethanol and refluxed overnight. Proton NMR in deuterated chloroform showed the isolated, gummy product **8** to be a 1:1 mixture of *(4S,2′R)*- and *(4R,2′R)*-diastereomers. The signals from each of the two ester α-methylene groups were well-resolved in the spectrum and separated by 0.1 ppm. The signals from the 4-H of the diastereomers were sepa-

Scheme 2. Synthesis of Diastereomeric 5-Benzyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridines Using the Hantzsch Reaction and Resolution by HPLC[a]



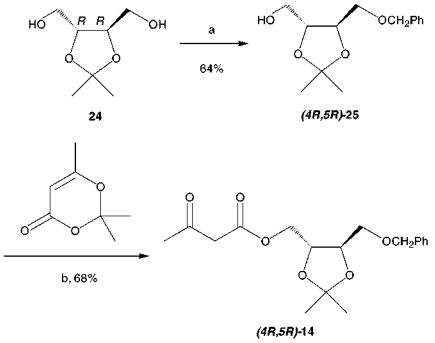
[a] Reagents: (a) EtOH, 90 °C, overnight; (b) HPLC separation.

Scheme 3. Deprotection of Isopropylidene Group from 3-Ester Chiral Auxiliary Esters and Transesterification in Ethanol (R_1 = C≡C−Ph)[a]



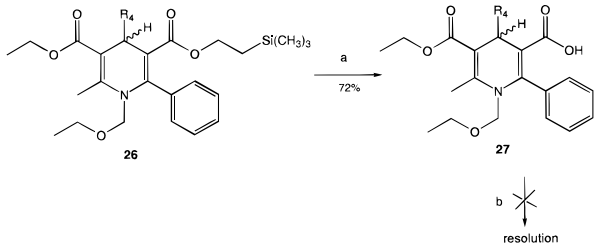
[a] Reagents: (a) THF, 25 °C, 1 N HCl; (b) 95% EtOH, 25 °C, 1.5 equiv NaOH; (c) 1 N HCl/THF (1:1), rt, overnight; (d) NaOH, 95% EtOH, rt, 3 days.

Scheme 4. Synthesis of Chiral β-Ketoester as a Starting Material for the Synthesis of Compounds **21** and **23**[a]



[a] Reagents: (a) $PhCH_2Cl$, NaOH, DMSO/H_2O (6:1); (b) toluene, 90 °C, overnight.

Scheme 5. Attempted Resolution by Crystallization of Diastereomeric Salts of a 1-Protected 6-Phenyl-4-phenylethynyl-1,4-dihydropyridine (R_4 = C≡C−Ph)[a]



[a] Reagents: (a) TBAF; (b) cinchonidine or cinchonine.

rated by 0.03 ppm. Subsequent resolution of the enantiomers (below) and addition of a chiral shift reagent verified that these peaks corresponded to pure diastereomers.

Fractional crystallization of **8** from methanol provided a pure diastereomer of this 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivative. This isomer was shown by X-ray crystallography (Figure 3, see below) to be of the *(4S,2′R)*-configuration, **9**. The remaining isomer, evidently having the *(4R,2′R)*-configuration, **10**, was obtained from the mother liquor as an oil and was shown by ^{1}H NMR to be pure. The ^{1}H NMR resonances of the two separate isomers with and without the lanthanide shift reagent Eu(fod)$_3$[21] were catalogued and are shown in Table 1. Each of the isolated isomers was shown to be a pure diastereomer and with the peaks of the other isomer not visible in the spectrum. Without Eu(fod)$_3$ the 4-H and α-methylene resonances were downfield in the *(4R,2′R)*-isomer **10** versus the *(4S,2′R)*-

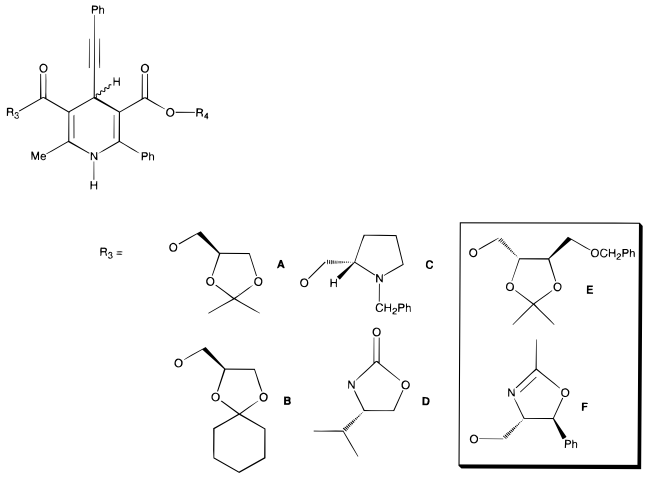


Figure 2. Chiral auxiliary ester groups (3-position) used in the resolution of 6-phenyl-4-phenylethynyl-1,4-dihydropyridines; R_4 = benzyl or ethyl.

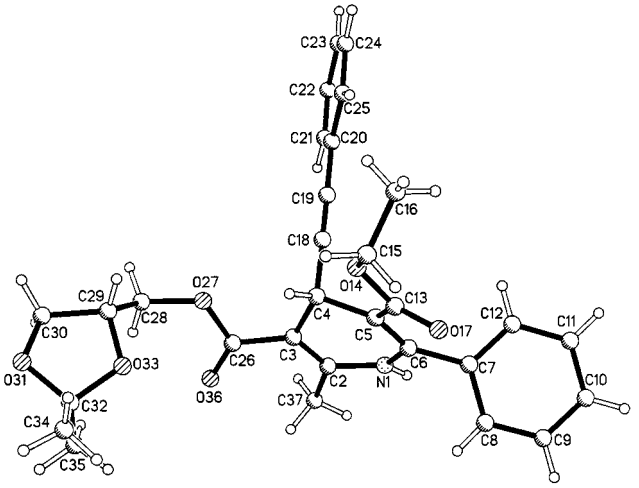
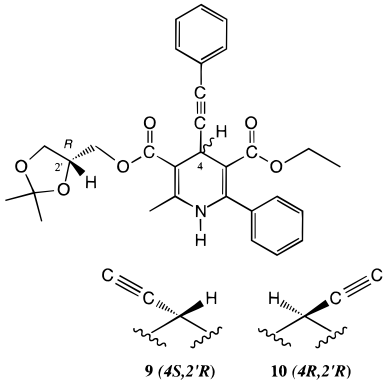


Figure 3. X-ray crystallographic structure determined for **9**, 3-*(2′R)*-(2,2-dimethyl-1,3-dioxolan-4-ylmethyl) 5-ethyl 4-*(S)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate.

isomer **9**. Eu(fod)$_3$ (4 mg/mL) caused a downfield shift of all of the resonances. In each case the resonances of the *(4S,2′R)*-isomer were shifted downfield to a greater degree in the presence of the NMR shift reagent. For example, the signals from the 4-H were shifted by Eu(fod)$_3$ by >0.7 ppm in the *(4S,2′R)*-isomer and by only 0.3 ppm in the *(4R,2′R)*-isomer. Thus, in the presence of Eu(fod)$_3$ this important resonance was separated by 0.4 ppm between the two diastereomers. The resonances of the two ester α-methylene groups, which were the best-resolved in the spectrum measured in the absence of Eu(fod)$_3$, were separated by ~0.3 ppm in the presence of Eu(fod)$_3$.

To establish the absolute configuration at the C4 position of compound **9**, an X-ray crystallographic structure was determined. Small needles of **9** were grown by vapor diffusion from methanol/water solution. X-ray diffraction studies[22,23] were performed with a Siemens P4 diffractometer (Mo Kα radiation). Crystals of **9** were found to be monoclinic ($P2_1$) with lattice parameters a = 11.059(2) Å, b = 8.212(2) Å, c = 15.629(3) Å, β = 104.46(1)°, Z = 2. The spatial coordinates of the X-ray structure are provided as Supporting Information. The carbonyl groups were in the cis/cis orientation (Figure 3), which is consistent with crystal structures determined for other 1,4-dihydropyridines in which the 4-position substituent is not highly hindered.[18]

Table 1. Proton NMR Chemical Shifts of Diastereomeric 1,4-Dihydropyridine Derivatives Resolved by Crystallization



	chemical shift (ppm from TMS)[a]			
resonance	**9**	**9** + Eu(fod)$_3$	**10**	**10** + Eu(fod)$_3$
2-CH_3	2.37	2.59	2.37	2.49
4-H	5.10	5.83	5.13	5.43
3-OCH_2	4.26	4.79	4.36	4.46
5-OCH_2	3.99	4.47	4.09	4.19
N-H	5.90	6.03	5.88	5.94

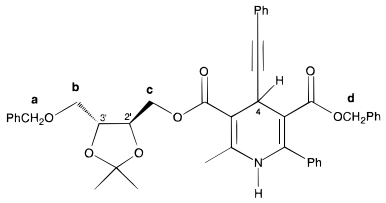
[a] In $CDCl_3$, in the presence or absence of 4 mg/mL Eu(fod)$_3$ (europium tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate)). The dihydropyridines were dissolved at a concentration of 5 mg/mL.

The 2,2-dimethyl-1,3-dioxolane moiety also served as a protected form of a diol, **18**, obtained following deprotection of **8** in HCl/THF (Scheme 3). This diol showed a selective reactivity versus the 5-ester in basic transesterification reactions. The 3-ethyl ester **19** was obtained in this manner using sodium hydroxide in 95% ethanol. An attempt to synthesize the corresponding 3-thioester via transesterification in ethanethiol was unsuccessful. During the basic transesterification reaction, a simple 5-alkyl ester group was expected to be unreactive, as found previously with similar derivatives. The resolved diastereomers **9** and **10** were also deprotected separately, to give **11** and **12**. These diols were then tested for biological activity.

The analogue of **2**, corresponding to compound **8** except containing a 5-benzyl ester instead of ethyl, was prepared (scheme not shown); however, it could not be resolved by crystallization. Since the more biologically interesting dihydropyridine derivatives contained a bulkier ester, e.g. benzyl ester, at the 5-position, alternate methods (Schemes 2−4, see below) were required for the resolution. Groups **B**−**D** (Figure 2: **B**, 1,4-dioxaspiro[4.5]decan-2-ylmethyl; **C**, 1-benzyl-2-pyrrolidinylmethyl; **D**, 4-isopropyl-2-oxazolidinon-3-yl), also containing a single chiral center, were likewise incorporated into analogues of **2** as the 3-position substituent (schemes not shown); however, none of these analogues were amenable to resolution by fractional crystallization.

Another approach to the resolution of these enantiomers is the crystallization of diastereomeric salts of the 5-carboxylic acid derivative **27** using a chiral base (Scheme 5). Methods for synthesizing 5-carboxylic acid

Table 2. Proton NMR Chemical Shifts of Diastereomeric 1,4-Dihydropyridine Derivatives Resolved by HPLC[a]



number or position	racemic mixture		**16** (t_R = 32 min) *(4S,2′R,3′R)*		**17** (t_R = 35 min) *(4R,2′R,3′R)*	
	chemical shift (ppm)	+Eu(fod)$_3$ (ppm)	chemical shift (ppm)	+Eu(fod)$_3$ (ppm)	chemical shift (ppm)	+Eu(fod)$_3$ (ppm)
$(CH_3)_2$	s, 1.41		s. 1.42		s, 1.42	
2-CH_3	s, 2.34	2.55, −2.61	s, 2.37	2.53	s, 2.37	2.56
a	m, 3.60−3.70		s, 3.67		s, 3.62	
b	bs, 4.16		m, 4.17−4.23		m, 4.17−4.22	
c	bs, 4.32		m, 4.32−4.40		bs, 4.35	
d	d, 4.51−4.54		s, 4.54		bs, 4.51	
4-H	d, 5.16−5.18	5.78, 5.96	s, 5.17	5.89	s, 5.20	5.64
2CH	m, 4.89−5.13		m, 4.93−5.12		m, 4.91−5.15	
NH	d, 6.05−6.08	6.02, 6.07	s, 5.93	6.06	s, 5.90	5.99
C_6H_5	m, 7.19−7.35		m, 7.20−7.39		m, 7.20−7.37	

[a] In $CDCl_3$, in the presence or absence of 4 mg/mL Eu(fod)$_3$ (europium tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate)). The dihydropyridines were dissolved at a concentration of 5 mg/mL.

derivatives of 1,4-dihydropyridines have already been explored,[18] and we have reported a method specifically with 6-phenyl-4-phenylethynyl-1,4-dihydropyridine derivatives.[39] This method utilizes the 2-trimethylsilylethyl ester group at the 5-position, as in **26**, combined with the acid-sensitive 1-ethoxymethyl group. However, the attempted crystallization of the carboxylic acid intermediate **27** with cinconidine failed. From a 1:1 mixture in ethyl ether, only cinconidine itself precipitated. Thus, it appears that the cinconidine salt does not form, presumably due to steric hindrance. In previous examples of resolution of 4-aryldihydropyridine salts,[18] generally there was a methyl group rather than a phenyl group at the 6-position.

None of the diastereomeric mixtures of either 5-ethyl or 5-benzyl esters containing chiral auxiliaries **A**−**D** (including compound **8** and the four corresponding analogues of **2**) were separable by normal-phase HPLC. The separation of diastereomers by HPLC required introduction of group **E** or **F** (Figure 2), each containing two chiral centers. Group **E** esters ((*4R,5R*)-5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-ylmethyl)[24] were chemically stable, while group **F** derivatives ((*4S,5S*)-2-methyl-5-phenyl-2-oxazolin-4-ylmethyl) decomposed rapidly upon storage; thus, group **E** was selected for resolution of enantiomers of **2**. The incorporation of group **E** as a 3-position ester leading to the successful synthesis of the pure enantiomers is shown in Schemes 2−4. Thus, the subsequent transesterification reaction at the 3-position completed the synthesis of pure enantiomers of the highly potent A_3 adenosine receptor antagonist.

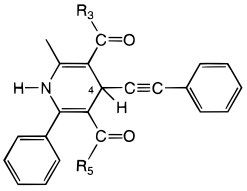
The assignment of absolute configuration at the 4-position in the two diastereomers separated by HPLC, **16** and **17**, and consequently by analogy the two enantiomers of **2**, i.e. **21** and **23**, was based on NMR analysis. In the absence of Eu(fod)$_3$, the chemical shifts of the 4-hydrogen in (*4S*)-**9** and (*4R*)-**10** were 5.10 and 5.13 ppm, respectively. The chemical shift of the *(4S)*-form was more upfield than that of the *(4R)*-form. After the chemical shift reagent Eu(fod)$_3$ was added, the chemical shifts of the 4-hydrogen in *(4S)*-**9** and *(4R)*-**10** (Table 1) were 5.83 and 5.43 ppm, respectively. The chemical shift of the *(4S)*-form was downfield from that of *(4R)*-form. The differences in chemical shift caused by Eu(fod)$_3$ in *(4S)*-**9** and *(4R)*-**10** were 0.73 and 0.30 ppm, respectively. Thus, Eu(fod)$_3$ caused larger chemical shift differences in the *(4S)*-form than in the *(4R)*-form. For diastereomers **16** and **17** (Table 2), which were structurally similar to *(4S)*-**9** and *(4R)*-**10**, without Eu(fod)$_3$, the chemical shift of the 4-hydrogen in **16** (5.17 ppm) was upfield from that of **17** (5.20 ppm). After addition of Eu(fod)$_3$, the chemical shift of the 4-hydrogen in **16** (5.89 ppm) shifted downfield from that of **17** (5.64 ppm). The difference of chemical shift caused by Eu(fod)$_3$ in **16** (0.72 ppm) was also larger than in **17** (0.44 ppm). Thus, the NMR analysis was consistent with the absolute configuration of the 4-position in **16** being *(S)* and in **17** being *(R)*, and by direct analogy, the absolute configurations of enantiomers **21** and **23** were assigned as *(S)* and *(R)*, respectively.

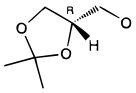
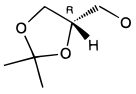
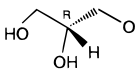
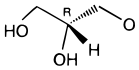
Pharmacology. The structures of the 1,4-dihydropyridines derivatives (**2**, **9**−**12**, **21**, **23**, and **28**) tested for affinity in standard radioligand binding assays[25−28] at adenosine receptors are shown in Table 3. Ordinarily, a comparison of A_1, A_{2A}, and A_3 affinities within one species would be preferred. We chose the human A_3 receptor for the present study instead of the rat A_3 receptor since the affinity of dihydropyridine antagonists is only marginal at rat A_3 receptors.[3] In contrast, at non-A_3 adenosine receptors, the affinity of dihydropyridines does not show as marked a species dependence.

The *(4S,2′R)*-isomer of the 5-ethyl derivative **9** was >70-fold selective for human A_3 receptors versus rat A_1 receptors. The corresponding *(4R,2′R)*-isomer **10**, although less selective, was approximately 3-fold more potent at human A_3 receptors, with a K_i value of 0.426 μM. Following removal of the isopropylidene groups, there was no stereoselectivity of binding. Thus, compounds **11** and **12** each bound to human A_3 receptors, with a K_i value of 0.5−0.6 μM.

Introduction of 3-ethyl and 5-benzyl esters in the enantiomers of **2**, compounds **21** and **23**, selectively

Table 3. Affinities of 1,4-Dihydropyridine Derivatives in Radioligand Binding Assays at A_1, A_{2A}, and A_3 Receptors



compd	R_3	chirality at 4-position	R_5	K_i (μM) or % inhibition[d] rA_1[a]	rA_{2A}[b]	hA_3[c]	rA_1/hA_3
2	OCH_2CH_3	racemic	OCH_2Ph	40.1 ± 7.5[e]	*d* (10^{-4})	0.0222 ± 0.0038 (0.0314 ± 0.0028)[e]	1800
9		*S*	OCH_2CH_3	32 ± 4% (10^{-4})	*d* (10^{-4})	1.31 ± 0.57	>70
10		*R*	OCH_2CH_3	16.4 ± 3.1	*d* (10^{-4})	0.426 ± 0.133	38
11		*S*	OCH_2CH_3	14.8 ± 1.0	39 ± 8% (10^{-4})	0.52	28
12		*R*	OCH_2CH_3	6.83 ± 0.20	*d* (10^{-4})	0.65	11
21	OCH_2CH_3	*S*	OCH_2Ph	32 ± 12% (10^{-4})	14 ± 6% (10^{-4})	0.0213 ± 0.0065	>400
23	OCH_2CH_3	*R*	OCH_2Ph	25 ± 11% (10^{-4})	11 ± 4% (10^{-4})	0.750 ± 0.151	>15
28[e]	OCH_2CH_3	racemic	OCH_2CH_3	11.0 ± 0.1	26 ± 12% (10^{-4})	0.0766 ± 0.0151	140

[a] Displacement of specific [^{3}H]*R*-PIA binding in rat brain membranes, expressed as K_i ± SEM in μM (n = 3−5), or as a percentage of specific binding displaced at the indicated concentration (M). [b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as K_i ± SEM in μM (n = 3−6), or as a percentage of specific binding displaced at the indicated concentration (M). [c] Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK cells, in membranes, expressed as K_i ± SEM in μM (n = 3−4). [d] Displacement of 10% of specific binding at the indicated concentration (M). [e] Values taken from van Rhee et al.[1] or from Jiang et al.[3]

enhanced affinity at human A_3 receptors for the *(4S)*-derivative **21**. The affinity of **21** was similar to that of the racemic mixture **2**, while the affinity of the opposite enantiomer **23** at human A_3 receptors was 35-fold less than that of **21**. The effect of the benzyl ester alone was determined by comparison to the corresponding racemic 3,5-diethyl ester **28**, which was 4-fold less potent than **2**. The stereoselectivity of the weak binding of **21** and **23** at A_1 and A_{2A} receptors could not be analyzed due to limited solubility.

Molecular Modeling. A symmetric 1,4-dihydropyridine[29] and related pyridine[7] antagonists have been docked in the putative ligand binding site of a rhodopsin-based molecular model of the human A_3 receptor.[29,31] To illustrate a possible conformational explanation of the results of the present study, the enantiomers of **2** were similarly docked in this site. According to our model, it is possible to dock both *(4R)*- and *(4S)*-enantiomers in energetically favorable conformations, simply by rotating the 1,4-dihydropyridine ring by 180° about the NH−C4 axis. In this way, the bulky 4-phenylethynyl substituent of both enantiomers may still be arranged between TM6 and TM7 (see Figure 4 and Discussion). Thus, for the *(4S)*-enantiomer **21**, the bulky 5-benzyl ester is facing the exofacial side of the helical bundle, while the 3-ethyl ester of the opposite enantiomer **23** points in the same direction.

Discussion

Previously, analysis of the structure−activity relationships and receptor docking of the series of mainly racemic 1,4-dihydropyridines has been limited by lack of pharmacological information about the two pure enantiomers. In the present study, we have explored the stereoselectivity of A_3 adenosine receptor-selective 1,4-dihydropyridines related to the 4-phenylethynyl derivative **2**.

It was necessary to resolve the analogues by crystallization or by HPLC of diastereomeric derivatives. In one synthetic approach, a free carboxylic acid was created at the 5-position; however, attempted crystallization with chiral alkaloids, such as cinconidine, as counterion was unsuccessful. Alternately, a chiral ester group such as a glycerol acetonide (group **A**) was introduced at the 3-position, thus allowing the crystallization of a pure *(4S,2′R)*-isomer, **9**, as determined by X-ray crystallography. The effect of the glycerol group on receptor affinity was also of interest, since the hydroxyl groups would be expected to enhance water solubility; however, only moderate affinity was achieved. After removal of the acetonide, the glycerol ester was removable with base and could be exchanged through basic transesterification. This allowed for greater flexibility of substitution. The previous methods for resolution of 1,4-dihydropyridines via separation of diastereomeric esters[18] relied on subsequent removal of the chiral esterifying group by one of three methods: (1) activation of the ester for selective reaction, (2) removal via β-elimination, and (3) removal through hydrogenolysis. Hydrogenolysis may not be used for the present target compounds due to the unsaturated 4-substituent which would also be reduced. A scheme not involving β-elimination was also desired, since this method may be needed for optional substitution at the 5-position.[39]

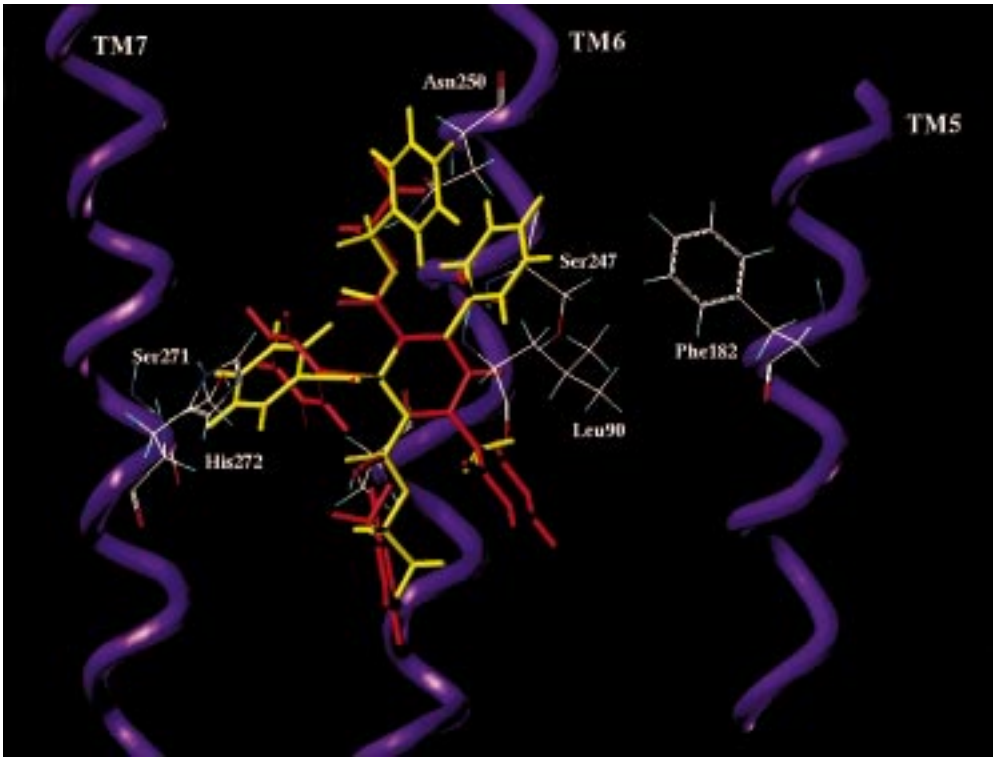


Figure 4. Overlapped compounds **21** ((*4S*), yellow structure) and **23 (**(*4R*), red structure) computer-docked inside the putative binding pocket of the human A_3 receptor.[29,31] Three of the seven transmembrane helices are shown as viewed from the plane of the plasma membrane. The side chains of the important residues in proximity (≤5 Å) to the docked dihydropyridine molecules are highlighted and labeled: Leu90 (TM3), Phe182 (TM5), Ser242 (TM6), Ser247 (TM6), Asn250 (TM6), Ser271 (TM7), and His272 (TM7).

The glycerol acetonide served the purpose as a chiral moiety which, following removal of the ketal, provided an ester that was activated for selective reaction.

The resolution of the more potent antagonist **2** was carried out for comparison using a similar approach, requiring a more complex chiral auxiliary ester (group **E**) and chromatographic resolution. The absolute configuration of the resulting stereoisomers was determined based on the effects of an NMR shift reagent in comparison with the corresponding shifts of the glycerol acetonides.

From the structural information obtained for the *(4S,2′R)*-isomer **9** and from the receptor binding of both *(4S,2′R)*- and *(4R,2′R)*-isomers, we have concluded that the more potent isomer of the group **A** esters is **10**, of the *(4R)*-configuration at the 4-position. However, the binding of pure enantiomers **21** and **23** at A_3 adenosine receptors indicates a 35-fold selectivity for the 4-position *(4S)*-isomer. Therefore, the stereoselectivity of binding at A_3 receptors appears to be a function of the orientation of the 4-position phenylethynyl group relative to the bulkier of the two ester groups, rather than relative to the 6-phenyl group, which determines its absolute *(4R)*- or *(4S)*-configuration. The substantial stereoselectivity of enantiomers of **2** in binding to A_3 receptors is noteworthy since stereoselectivity has not generally been a prominent feature of antagonists at any of the adenosine receptors.

These results are consistent with the conformational features of our previously published human A_3 receptor model, in which a symmetric dihydropyridine[29] and structurally related pyridine derivatives[7,31] have been docked in a common mode of binding. This putative recognition site is located in the upper region of the helical bundle of the receptor. According to this docking model, the pyridine or 1,4-dihydropyridine rings are most favorably oriented perpendicular to the plane of the lipid bilayer with the 3-ester position in the exofacial direction. The carbonyl of the 5-ester group is in proximity to Ser275 (TM7), and the carbonyl of the 3-ester group is close to Asn250 (TM6). Two important hydrophilic interactions, probably hydrogen-bonding interactions, seem to be involved between the two ester groups and these two polar amino acids. Two hydrophobic pockets are likely present around the 2-position, where an alkyl side chain would bind (Leu90, TM3; and Phe182, TM5), and around the 6-position, where the phenyl ring would bind (Leu90, TM3; and Ile186, TM5). The TM7 backbone is close to the 4-position of the pyridine ring. Consequently, a strong steric control is suggested around the 4-position of both 1,4-dihydropyridine and pyridine structures. In fact, bulky 4-position substituents, such as the 4-phenylethynyl group, which is affinity-enhancing in the 1,4-dihydropyridines, are not well-tolerated in the pyridine series. From a structural point of view, changing the C4 hybridization from sp^3 to sp^2, i.e. transformation of 1,4-dihydropyridine to the corresponding pyridine, would change the C5−C4−R4 angle from 68.1° to 0.2°. As already reported, in the case of 1,4-dihydropyridine derivatives, the bulky and rigid 4-phenylethynyl substituent could be situated between TM6 and TM7; thus, the nature of the 4-substituent is critical for recognition of this series inside the receptor. To maintain the critical orientation of the 4-phenylethynyl group, energetic optimization in the docking of the enantiomers of **2** required inverting

the 3- and 5-ester groups, such that the benzyl ester pointed in the exofacial direction for the more potent enantiomer **21**.

As reported in Table 3, **11** and **12**, which have 3- and 5-substitutents of similar size, show the same affinity at the human A_3 receptor. However, the presence of bulky substituents at the carbonyl of the 3- and/or 5-ester groups can change the enantioselectivity of receptor binding. In fact, secondary to the arrangement of the substituent at the 4-position, the bulkier of the two ester groups, independent of its position, seems to prefer to be located at the extracellular region of the TM domain. The *(4S)*-enantiomer **21** is 35-fold more active than the *(4R)*-isomer **23** and is more favorably docked with the 5-benzyl ester pointing toward the extracellular environment. Accordingly, the 3-(1,3-dioxolane) substituent of the *(4R)*-enantiomer **10** would be located in the upper region of the TM bundle, and its affinity is ca. 3-fold higher than that of the *(4S)*-enantiomer. Apparently, the orientation of the 6-phenyl substituent does not seem to direct stereocontrol of binding, which, rather, appears to be dominated by the presence, or not, of bulky ester groups. This is a clear example of the fact that receptor stereoselectivity is not only due to chiral properties of the ligand but also to the steric and electronic requirements of the receptor cleft. Other studies are in progress to test this hypothesis.

Materials and Methods

Synthesis. Materials and Instrumentation. Phenylpropargyl aldehyde (**6**), benzyl chloride, 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one, *(4R,5R)*-(−)-2,3-*O*-isopropylidene-D-threitol (**24**), *(S)*-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, diketene, and all other chiral auxiliaries were purchased from Aldrich (St. Louis, MO). Benzyl 3-amino-3-phenyl-2-propenoate (**13**) and ethyl 3-amino-3-phenyl-2-propenoate (**7**) were prepared by our previous method.[7] All other materials were obtained from commercial sources.

Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer, and all spectra were obtained in $CDCl_3$. Chemical shifts (δ) relative to tetramethylsilane are given. Chemical ionization (CI) mass spectrometry was performed with a Finnigan 4600 mass spectrometer and electron impact (EI) mass spectrometry with a VG7070F mass spectrometer at 6 kV. Elemental analysis was performed by Atlantic Microlab Inc. (Norcross, GA).

Preparation of 3-*(2′R)*-(2,2-Dimethyl-1,3-dioxolan-4-ylmethyl) 5-Ethyl 4-*(R* and *S)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (8) (Scheme 1). Phenylpropargyl aldehyde (**6**; 130 mg, 1.0 mmol), ethyl 3-amino-3-phenyl-2-propenoate (**7**; 191 mg, 1.0 mmol), and chiral ketoester *(R)*-**5** (174 mg, 1.0 mmol) were dissolved in 3 mL of 95% ethanol. The mixture was refluxed, with stirring, at 80 °C overnight. After cooling to room temperature, the solvent was evaporated, and the residue was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (3:1)) to afford 105 mg of compound **8** as a mixture of two diastereomers (yield: 21%).

Separation of Diastereomeric Mixture of 8 by Crystallization. Compound **8** (45 mg) was dissolved in hot methanol (2 mL), and the solution was left at room temperature for crystallization. The crystals **9** (21 mg, 47%), which were optically pure (by NMR and HPLC) and identified as the *(4S,2′R)*-isomer by X-ray crystallography, were collected by filtration, and the mother liquor was evaporated to give 22 mg (49%) of the other isomer which was also optically pure (by NMR and HPLC), i.e. *(4R,2′R)*-**10**.

3-*(2′R)*-(2,2-Dimethyl-1,3-dioxolan-4-ylmethyl) 5-ethyl 4-*(S)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (9): ^{1}H NMR: δ 0.94 (t, J = 6.9 Hz, 3 H), 1.35 (s, 3 H), 1.44 (s, 3 H), 2.37 (s, 3 H), 3.85 (m, 1), 4.10 (m, 1 H), 4.00 (q, J = 6.9 Hz, 2 H), 4.25 (d, J = 4.9 Hz, 2 H), 4.40 (m, 1 H), 5.10 (s, 1 H), 5.88 (s, br, 1 H), 7.25−7.43 (m, 10 H). MS (EI): m/z 501 (M^+), 428 ($M^+ - CO_2Et$), 386 (M^+ − (2,2-dimethyl-1,3-dioxolan-4-yl)methyl), 342 (M^+ − (2,2-dimethyl-1,3-dioxolan-4-yl)methoxycarbonyl, base). HRMS: calcd for $C_{30}H_{31}NO_6$ 501.2151, found 501.2141.

3-*(2′R)*-(2,2-Dimethyl-1,3-dioxolan-4-ylmethyl) 5-ethyl 4-*(R)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (10): ^{1}H NMR: δ 0.98 (t, J = 6.8 Hz, 3 H), 1.38 (s, 3 H), 1.49 (s, 3 H), 2.37 (s, 3 H), 3.98 (m, 2 H), 4.09 (m, 2 H), 4.22 (m, 2 H), 4.36 (m, 1 H), 5.13 (s, 1 H), 5.88 (s, br, 1 H), 7.25−7.44 (m, 10 H). MS (EI): m/z 501 (M^+), 428 ($M^+ - CO_2Et$), 386 (M^+ − (2,2-dimethyl-1,3-dioxolan-4-yl)methyl), 342 (M^+ − (2,2-dimethyl-1,3-dioxolan-4-yl)methoxycarbonyl, base). HRMS: calcd for $C_{30}H_{31}NO_6$ 501.2151, found 501.2151.

Preparation of 3-*(2′R)*-(2,3-Dihydroxy-1-propyl) 5-Ethyl 4-*(S)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (11) and 3-*(R)*-(2,3-Dihydroxy-1-propyl) 5-Ethyl 4-*(R)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (12) by Acidic Hydrolysis. Optically pure diastereomer **9** (15 mg) or **10** (9 mg) was stirred in a mixture of 1 N HCl and THF (1:1) (1.0 mL) at room temperature for 4 h. The solvents were removed, and the residue was diluted with ethyl acetate (10 mL). The organic layer was washed with water (5 mL × 2), dried over $MgSO_4$, and evaporated to give a residue, which was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (1:1)) to give 8.0 mg (yield: 58%) of compound **11** or 5.0 mg (60%) of compound **12**. ^{1}H NMR: δ 0.95 (t, J = 6.8 Hz, 3 H), 2.38 (s, 3 H), 3.71 (m, 2 H), 4.00 (m, 2 H), 4.18 (m, 2 H), 4.54 (m, 1 H), 5.11 (s, 1 H), 5.94 (s, br, 1 H), 7.25−7.45 (m, 10 H). MS (EI): m/z 461 (M^+), 432 (M^+ − Et), 388 ($M^+ - CO_2Et$), 342 ($M^+ - CO_2CH_2CH(OH)CH_2OH$, base). HRMS for **11**: calcd for $C_{27}H_{27}NO_6$ 461.1838, found 461.1861. HRMS for **12**: calcd for $C_{27}H_{27}NO_6$ 461.1838, found 461.1845.

Preparation of *(R)*-(+)-2,2-Dimethyl-1,3-dioxolan-4-ylmethyl Acetoacetate (5) (Scheme 1). *(S)*-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol (1.32 g, 10 mmol) and diketene (912 mg, 11 mmol) were dissolved in toluene (10 mL). Sodium acetate (0.2 g) was added, and the mixture was heated at 90 °C for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica 60; CH_2Cl_2) to give 0.932 g of compound **5**, yield: 54%. ^{1}H NMR: δ 1.37 (s, 3 H), 1.44 (s, 3 H), 2.29 (s, 3 H), 3.77 (m, 1 H), 4.07 (m, 1 H), 3.52 (s, 2 H), 4.22 (m, 2 H), 4.35 (m, 1 H).

Preparation of 5-Benzyl 3-*(4′R,5′R)*-(5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-ylmethyl) 4-*(R* and *S)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (15) (Scheme 2). Phenylpropargyl aldehyde (**6**; 65 mg, 0.5 mmol), benzyl 3-amino-3-phenyl-2-propenoate (**13**; 127 mg, 0.5 mmol), and chiral ketoester **14** (168 mg, 0.5 mmol) were dissolved in 3 mL of 95% ethanol. The mixture was sealed in a Pyrex tube and heated, with stirring, to 90 °C overnight. After cooling to room temperature, the solvent was evaporated and the residue was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (2:1)) to afford 150 mg of compound **15** as a mixture of two diastereomers (yield: 44%).

Chromatographic Separation of Diastereomers. Samples of the mixture of diastereomers were separated by HPLC (Hewlett-Packard 1090 liquid chromatography system) using a silica column (300 × 10 mm) with the mobile phase consisting of a linear gradient of hexane/10% 2-propanol in ethyl acetate from 90:10 to 85:15 in 50 min. Samples dissolved in the mobile phase were injected at a flow rate of 2.5 mL/min and with a column temperature of 50° C. Peaks were detected by UV absorption using a diode array detector. From 40 mg of a 50:50 mixture of diastereomers, 10 mg (25%) of 5-benzyl 3-*(4′R,5′R)*-(5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl-

methyl) 4-*(S)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**16**) and 10.1 mg (25%) of 5-benzyl 3-*(4′R,5′R)*-(5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-ylmethyl) 4-*(R)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**17**) were obtained.

The diastereomeric excess of each fraction was determined by analytical HPLC using a silica column (250 × 4.6 mm) and a flow rate of 1.0 mL/min with the same mobile phase gradient system and column temperature as above. The diastereomeric purities of compounds **16** and **17** were 99.0% and 95.0%. The specific rotations for **16** and **17**, $[\alpha]_D$ (c = 0.5, $CHCl_3$), were +0.05 and +0.26, respectively.

***(4S)*-Diastereomer 16:** ^{1}H NMR: δ 1.42 (s, 6 H), 2.37 (s, 3 H), 3.67 (s, 2 H), 4.17−4.23 (m, 2 H), 4.32−4.40 (m, 2 H), 4.54 (s, 2 H), 5.17 (s, 1 H), 4.93−5.12 (m, 2 H), 5.93 (s, br, 1 H), 7.20−7.39 (m, 20 H). MS (EI): m/z 683 (M^+), 668 ($M^+ - Me$), 592 ($M^+ - CH_2Ph$), 91 ($^+CH_2Ph$, base).

***(4R)*-Diastereomer 17:** ^{1}H NMR: δ 1.42 (s, 6 H), 2.37 (s, 3 H), 3.62 (s, 2 H), 4.17−4.22 (m, 2 H), 4.35 (m, 2 H), 4.51 (m, 2 H), 5.20 (s, 1 H), 4.91−5.15 (m, 2 H), 5.90 (s, br, 1 H), 7.20−7.37 (m, 20 H). MS (CI/NH_3): m/z 701 ($M^+ + NH_4$, base).

^{1}H NMR Resonances of Diastereomeric 1,4-Dihydropyridine Derivatives Resolved by HPLC with the Lanthanide Shift Reagent Eu(fod)$_3$. Proton NMR chemical shifts of compounds **16** and **17** were measured in the presence of 4 mg/mL Eu(fod)$_3$ (europium tris(6,6,7,7,8,8,8,-heptafluoro-2,2-dimethyl-3,5-octanedionate)). Samples were dissolved at a concentration of 5 mg/mL $CDCl_3$.

Hydrolytic Deprotection of Chiral Compounds 16 and 17. Optically pure diastereomer **16** (10 mg) or **17** (10.1 mg) was stirred in a mixture of 1 N HCl and THF (1:1) (2 mL) at room temperature overnight. The solvents were removed under reduced pressure, and the residue was subjected to preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (1:1)) to give 5.5 mg of 5-benzyl 3-*(2′R,3′R)*-(4-benzyloxy-2,3-dihydroxy-1-butyl) 4-*(S)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**20**) (yield: 58%) or 7.8 mg of 5-benzyl 3-*(2′R,3′R)*-(4-benzyloxy-2,3-dihydroxy-1-butyl) 4-*(R)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**22**) (yield: 83%).

Compound 20: ^{1}H NMR: δ 2.37 (s, 3 H), 3.60 (d, J = 5.7 Hz, 2 H), 3.92 (m, 1 H), 3.99 (m, 1 H), 4.17 (m, 1 H), 4.50 (m, 1 H), 4.51 (s, 2 H), 5.02 (AB, J = 12.6 Hz, 2 H), 5.15 (s, 1 H), 5.89 (s, br, 1 H), 7.05 (m, 1 H), 7.20−7.39 (m, 19 H). MS (CI/NH_3): m/z 661 ($M^+ + NH_4$, base), 644 ($M^+ + 1$).

Compound 22: ^{1}H NMR: δ 2.37 (s, 3 H), 3.59 (d, J = 4.8 Hz, 2 H), 3.91 (m, 1 H), 4.02 (m, 1 H), 4.23 (m, 1 H), 4.40 (m, 1 H), 4.51 (s, 2 H), 5.02 (AB, J = 12.6 Hz, 2 H), 5.16 (s, 1 H), 5.90 (s, br, 1 H), 7.07 (m, 1 H), 7.19−7.39 (m, 19 H). MS (CI/NH_3): m/z 404 ($M^+ - CO_2CH_2CH(OH)CH(OH)CH_2OCH_2Ph$).

Preparation of 3-*(2′R)*-(2,3-Dihydroxy-1-propyl) 5-Ethyl 4-*(R and S)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (18) by Acidic Hydrolysis (Scheme 3). A mixture of diastereomers **8** (50 mg, 0.1 mmol) was stirred in a mixture of 1 N HCl and THF (1:1) (4.0 mL) at room temperature for 4 h. The solvents were removed, and the residue was diluted with ethyl acetate (10 mL). The organic layer was washed with water (5 mL × 2), dried over $MgSO_4$, and evaporated to give a residue, which was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (1:1)) to give 35 mg (yield: 76%) of compound **18**. The analytical data are identical with its pure diastereomers **11** and **12**.

Preparation of 3,5-Diethyl 4-*(R and S)*-2-Methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (19) by a Transesterification Reaction (Scheme 3). Compound **18** (30 mg, 0.065 mmol) and sodium hydroxide (4 mg, 1.5 equiv) were stirred in 95% ethanol (1 mL) at room temperature for overnight. The solvents were removed under reduced pressure, and the residue was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (4:1)) to give 6 mg (yield: 22%) of **19**, which has the same analytical data with **28** (Table 3).[3]

Preparation of Optically Pure 1,4-Dihydropyridines (−)-21 and (+)-23 by a Transesterification Reaction. Optically pure compound **7** (5.0 mg) or **8** (7.3 mg) and sodium hydroxide (3 equiv) were stirred in 95% ethanol at room temperature for 3 days. The solvents were removed under reduced pressure, and the residue was purified by preparative TLC (silica 60, 1000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (4:1)) to give 0.4 mg of *(S)*-(−)-**21** (yield: 11%) or 2.28 mg of *(R)*-(+)-**23** (yield: 42%).

5-Benzyl 3-ethyl 4-*(S)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (21): ^{1}H NMR: δ 1.35 (t, J = 6.9 Hz, 3 H), 2.37 (s, 3 H), 4.28 (m, 2 H), 5.04 (AB, J = 12.6 Hz, 2 H), 5.19 (s, 1 H), 5.86 (s, br, 1 H), 7.07 (m, 2 H), 7.20−7.27 (m, 7 H), 7.37 (m, 6 H). MS (EI): m/z 477 (M^+), 448 ($M^+ - Et$), 404 ($M^+ - CO_2Et$), 384 ($M^+ - 1 - Me$-Ph), 356 ($MH^+ - Me - OCH_2Ph$), 342 ($M^+ - CO_2CH_2Ph$), 91 ($^+CH_2Ph$, base). HRMS: calcd for $C_{31}H_{27}NO_4$ 477.1940, found 477.1937. $[\alpha]^{20}_D$ = −70 ° (c = 0.3, MeOH).

5-Benzyl 3-ethyl 4-*(R)*-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (23): ^{1}H NMR: δ 1.35 (t, J = 6.9 Hz, 3 H), 2.37 (s, 3 H), 4.28 (m, 2 H), 5.04 (AB, J = 12.6 Hz, 2 H), 5.19 (s, 1 H), 5.87 (s, br, 1 H), 7.06 (m, 2 H), 7.20−7.25 (m, 7 H), 7.36−7.40 (m, 6 H). MS (EI): m/z 477 (M^+), 448 ($M^+ - Et$), 432 ($M^+ - OEt$), 404 ($M^+ - CO_2Et$), 386 ($M^+ - CH_2Ph$), 342 ($M^+ - CO_2CH_2Ph$), 91 ($^+CH_2Ph$, base). HRMS: calcd for $C_{31}H_{27}NO_4$ 477.1940, found 477.1949. $[\alpha]^{20}_D$ = +70.2 ° (c = 1.14, MeOH).

Chromatographic Purity of 9−12, 21, and 23. Purity of diastereomers **9**−**12** and enantiomers of MRS 1191, compounds **21** *(4S)* and **23** *(4R)*, was checked by HPLC using SMT OD-5-60 RP C18 (250 × 4.6 mm) as an analytical column in two solvent systems. System A consisted of a linear gradient solvent system of 0.1 M TEAA/CH_3CN from 80:20 to 20:80 in 40 min, and flow rate was 1 mL/min. System B consisted of a linear gradient solvent system of CH_3CN/H_2O from 60:40 to 80:20 in 30 min, and flow rate was 1 mL/min. Peaks were detected by UV absorption in the range of 200−400 nm using a diode array detector. Peaks showed >95% purity for all compounds in these systems. The retention times of **9**, **10**, **11**, **12**, **21**, and **23** were 23.83, 23.73, 19.12, 19.14, 20.86, and 20.82 min, respectively, in system A; and 8.92, 8.86, 3.00, 3.00, 10.03, and 9.96 min, respectively, in system B.

Preparation of *(4R,5R)*-(5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (25) (Scheme 4).[24] *(4R,5R)*-(−)-2,3-*O*-Isopropylidene-D-threitol (**24**; 1.00 g, 6.16 mmol) and NaOH (0.444 g, 11 mmol) were stirred in a mixture of DMSO (6 mL) and water (1 mL) at room temperature for 1 h. Benzyl chloride (0.937 g, 7.4 mmol) was then added, and stirring was continued for 12 h. The reaction mixture was diluted with brine (20 mL) and extracted with ether (3 × 60 mL). The ether extract was washed with water and dried over $MgSO_4$. The solvent was removed, and the residue was purified by preparative TLC (silica 60, 2000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (3:1)) to give 1.00 g of product **25**, yield: 64%. ^{1}H NMR: δ 1.42 (s, 6 H), 3.56 (dd, J = 6.0, 3.9 Hz, 1 H), 3.66−3.72 (m, 2 H), 3.78 (dd, J = 7.8, 3.9 Hz, 1 H), 3.95 (m, 1 H), 4.06 (m, 1 H), 4.59 (s, 2 H), 7.29−7.39 (m, 5 H). MS (CI/NH_3): m/z 270 ($M^+ + NH_4$, base).

Preparation of *(4R,5R)*-(5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)methyl Acetoacetate (14) (Scheme 4). *(4R,5R)*-(5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (**25**; 1.00 g, 3.96 mmol) and 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (0.677 g, 4.76 mmol) in toluene (3 mL) were heated at 90 °C in a sealed tube overnight. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by preparative TLC (silica 60, 2000 μm; Analtech, Newark, DE; petroleum ether−ethyl acetate (3:1)) to afford 0.911 g of compound **14**, yield: 68%. ^{1}H NMR: δ 1.42 (s, 6 H), 2.26 (s, 3 H), 3.47 (s, 2 H), 3.61 (m, 2 H), 4.00−4.12 (m, 2 H), 4.18 (dd, J = 6.0, 5.7 Hz, 1 H), 4.40 (dd, J = 7.0, 9.2 Hz, 1 H), 4.59 (s, 2 H), 7.30−7.36 (m, 5 H). MS (CI/NH_3): m/z 354 ($M^+ + NH_4$, base), 337 ($M^+ + 1$).

X-ray Structural Determination of Compound 9. Small needle crystals of compound **9** were grown by vapor diffusion from methanol/water solution. X-ray diffraction studies were

performed with Siemens P4 diffractometer (Mo Kα radiation). Crystals of $C_{30}H_{31}NO_6$ are monoclinic ($P2_1$) with lattice parameters $a = 11.059(2)$ Å, $b = 8.212(2)$ Å, $c = 15.629(3)$ Å, $\beta = 104.46(1)°$, $Z = 2$. Intensities were measured with variable speed $\omega/20$ scans from a crystal size of $0.80 \times 0.12 \times 0.06$ mm. No significant variation was observed in the intensities of three standard reflections monitored at regular intervals; no corrections were necessary for absorption.

The structure was determined by direct methods [1] and refined on F^2 by full-matrix least-squares method [2] from 1895 unique reflections ($2\theta_{max} = 45°$, $R_{int} = 0.029$). Isotropic hydrogen atoms were refined with a riding model; all other atoms were assigned anisotropic displacement parameters. The final conventional $R = 5.50\%$ on F values of 1345 reflections having $F_0 > 4\sigma(F_0)$.

Pharmacological Methods. Binding at Adenosine Receptors. Binding of [^{3}H](*R*)-N^6-phenylisopropyladenosine ([^{3}H]-*R*-PIA; Amersham, Chicago, IL) to A_1 receptors from rat cerebral cortex membranes,[25] binding of [^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-ethylcarbamoyladenosine ([^{3}H]-CGS 21680; DuPont NEN, Boston MA) to A_{2A} receptors from rat striatal membranes,[26] and binding of [^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine ([^{125}I]AB-MECA; Amersham, Chicago, IL) to membranes prepared from HEK-293 cells stably expressing the human A_3 receptor[27,28] (Receptor Biology, Inc., Baltimore MD) were performed as described.

All nonradioactive compounds were initially dissolved in DMSO and diluted with buffer to the final concentration, where the amount of DMSO never exceeded 2%.

Incubations were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). The tubes were rinsed three times with 3 mL of buffer each.

At least five different concentrations of competitor, spanning 3 orders of magnitude adjusted appropriately for the IC_{50} of each compound, were used. IC_{50} values, calculated with the nonlinear regression method implemented in the InPlot program (Graph-PAD, San Diego, CA), were converted to apparent K_i values using the Cheng−Prusoff equation[30] and K_d values of 1.0 and 14 nM for [^{3}H]*R*-PIA and [^{3}H]CGS 21680, respectively, and 0.59 nM for binding of [^{125}I]AB-MECA at human A_3 receptors.

Molecular Modeling. All calculations were performed on a Silicon Graphics Indigo 2 R8000 workstation. All *(4R)*- and *(4S)*-enantiomer models of 1,4-dihydropyridine derivatives were constructed using the "Sketch Molecule" of SYBYL 6.4.2.[32] Semiempirical molecular orbital calculations were done using the AM1 Hamiltonian[33] as implemented in MOPAC 6.0[34] (keywords: PREC, GNORM = 0.1, EF, MMOK if necessary).

The three-dimensional human A_3 receptor model was built and optimized using SYBYL 6.4.2 and Macromodel 5.0,[35] respectively, based on the approach described by Moro et al.[29,31] Briefly, the seven transmembrane helical domains were identified with the aid of Kyte−Doolittle hydrophobicity[36] and E_{mini}[36] surface probability parameters. The helices were built and energy-minimized for each transmembrane sequence. The minimized helices were then grouped together to form a helical bundle matching the overall characteristics of the electron density map of rhodopsin. The helical bundle was energy-minimized using the AMBER[37] all-atom force field, until the rms value of the conjugate gradient (CG) was <0.1 kcal/mol/Å. A fixed dielectric constant = 4.0 was used throughout these calculations.

Compounds **21** and **23** were each rigidly docked into the helical bundle using graphical manipulation with continuous energy monitoring (Dock module of SYBYL). The manually docked local energy-minimized receptor−ligand complexes were subjected to an additional CG minimization run of 300 steps. Partial atomic charges for the ligands were taken from the MOPAC output files. A fixed dielectric constant = 4.0 was used throughout the docking calculations. We have recently introduced the *cross-docking* procedure to obtain energetically refined structures of $P2Y_1$ receptor−ligand complexes.[38] We applied this technique to predict the structure of both **21**− and **23**−A_3 receptor complex. Cross-docking allowed possible ligand-induced rearrangements of the 7TM bundle to be explored by sampling 7TM conformations in the presence of the docked ligands. Small translations and rotations were applied to each helix relative to its original position until a new lower-energy geometry was obtained. These manual adjustments were followed by 25 ps of molecular dynamics (MD module of Macromodel) performed at a constant temperature of 300 K using a time step of 0.001 ps and a dielectric constant = 4.0. This procedure was followed by another sequence of CG energy minimization to a gradient threshold of <0.1 kcal/mol/Å. Energy minimization of the complexes was performed using the AMBER all-atom force field in MacroModel.

Abbreviations: [^{125}I]AB-MECA, [^{125}I]N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine; CGS 21680, 2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-ethylcarbamoyladenosine; DMAP, *N*,*N*-(dimethylamino)pyridine; DMSO, dimethyl sulfoxide; Eu(fod)$_3$, europium tris(6,6,7,7,8,8,8,-heptafluoro-2,2-dimethyl-3,5-octanedionate); HEK cells, human embryonic kidney cells; IB-MECA, N^6-(3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine; K_i, equilibrium inhibition constant; MRS 1097, 3,5-diethyl 2-methyl-6-phenyl-4-[2-phenyl-(*E*)-vinyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate; MRS 1191, 5-benzyl 3-ethyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate; *R*-PIA, (*R*)-N^6-phenylisopropyladenosine; SAR, structure−activity relationship; TBAF, tetrabutylammonium fluoride; Tris, tris(hydroxymethyl)aminomethane; TEAA, triethylamine−acetic acid buffer.

Supporting Information Available: Spatial coordinates of the X-ray structure of **9**. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) van Rhee, A. M.; Jiang, J. L.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Interaction of 1,4-dihydropyridine and pyridine-derivatives with adenosine receptors − selectivity for A_3 receptors. *J. Med. Chem.* **1996**, *39*, 2980−2989.

(2) Van Muijlwijk-Koezen, J. E.; Timmerman, H.; Link, R.; Van der Goot, H.; IJzerman, A. P. A novel class of adenosine A_3 receptor ligands. II. Structure affinity profile of a series of isoquinoline and quinazolinecompounds. *J. Med. Chem.* **1998**, *41*, 3994−4000.

(3) Jiang, J.-l.; van Rhee, A. M.; Chang, L.; Patchornik, A.; Evans, P.; Melman, N.; Jacobson, K. A. Structure activity relationships of 4-phenylethynyl-6-phenyl-1,4-dihydropyridines as highly selective A_3 adenosine receptor antagonists. *J. Med. Chem.* **1997**, *40*, 2596−2608.

(4) Karton, Y.; Jiang, J. L.; Ji, X. D.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Synthesis and biological-activities of flavonoid derivatives as as adenosine receptor antagonists. *J. Med. Chem.* **1996**, *39*, 2293−2301.

(5) Kim, Y.-C.; de Zwart, M.; Chang, L.; Moro, S.; von Frijtag Drabbe Künzel, J. K.; Melman, N.; IJzerman, A. P.; Jacobson, K. A. Derivatives of the triazoloquinazoline adenosine antagonist (CGS15943) having high potency at the human A_{2B} and A_3 receptor subtypes. *J. Med. Chem.* **1998**, *41*, 2835−2841.

(6) Jacobson, M. A.; Chakravarty, P. K.; Johnson, R. G.; Norton, R. Novel selective nonxanthine selective A_3 adenosine receptor antagonists. *Drug Devel. Res.* **1996**, *37*, 131.

(7) Li, A.-H.; Moro, S.; Melman, N.; Ji, X.-d.; Jacobson, K. A. Structure activity relationships and molecular modeling of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A_3 adenosine receptor antagonists. *J. Med. Chem.* **1998**, *41*, 3186−3201.

(8) Beaven, M. A.; Ramkumar, V.; Ali, H. Adenosine-A_3 receptors in mast-cells. *Trends Pharmacol. Sci.* **1994**, *15*, 13−14.

(9) Knight, D.; Zheng, X.; Rocchini, C.; Jacobson, M. A.; Bai, T.; Walker, B. Adenosine A_3 receptor stimulation inhibits migration of human eosinophils. *J. Leukocyte Biol.* **1997**, *62*, 465−468.

(10) von Lubitz, D. K. J. E.; Lin, R. C. S.; Popik, P.; Carter, M. F.; Jacobson, K. A. Adenosine A_3 receptor stimulation and cerebral ischemia. *Eur. J. Pharmacol.* **1994**, *263*, 59−67.

(11) Jacobson, K. A. A_3 adenosine receptors: Novel ligands and paradoxical effects. *Trends Pharmacol. Sci.* **1998**, *19*, 184−191.

(12) Triggle, D. J. Drugs acting on ion channels and membranes. In *Comprehensive Medicinal Chemistry*; Emmett, J. C., Ed.; Pergamon Press: London, 1985; Vol. 3, pp 1047−1099.

(13) Coburn, R. A.; Wierzba, M.; Suto, M. J.; Solo, A. J.; Triggle, A. M.; Triggle, D. J. 1,4-Dihydropyridine antagonist activities at the calcium channel: a quantitative structure−activity relationship approach. *J. Med. Chem.* **1988**, *31*, 2103−2107.

(14) Wong, W. C.; Chiu, G.; Wetzel, J. M.; Marzabadi, M. R.; Nagarathnam, D.; Wang, D.; Fang, J.; Miao, S. W.; Hong, X.; Forray, C.; Vaysse, P. J.; Branchek, T. A.; Gluchowski, C.; Tang, R.; Lepor, H. Identification of a dihydropyridine as a potent α1a adrenoceptor-selective antagonist that inhibits phenylephrine-induced contraction of the human prostate. *J. Med. Chem.* **1998**, *41*, 2643−2650.
(15) Sunkel, C. E.; de Casa-Juana, M. F.; Santos, L.; Gomez, M. M.; Villarroya, M.; Gonzalez-Morales, M. A.; Priego, J. G.; Ortega, M. P. 4-Alkyl-1,4-dihydropyridines derivatives as specific PAF-acether antagonists. *J. Med. Chem.* **1990**, *33*, 3205−3210.
(16) Lopez, M. G.; Fonteriz, R. I.; Gandia, L.; de la Fuente, M.; Villarroya, M.; Garcia-Sancho, J.; Garcia, A. G. The nicotinic acetylcholine receptor of the bovine chromaffin cell, a new target for dihydropyridines. *Eur. J. Pharmacol.* **1993**, *247*, 199−207.
(17) Goldmann, S.; Stoltefuss, J. 1,4-Dihydropyridines: Effects of Chirality and Conformation on the Calcium Antagonist and Calcium Agonist Activities. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1559−1578.
(18) Höllt, V.; Kouba, M.; Dietel, M.; Vogt, G. Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. *Biochem. Pharmacol.* **1992**, *43*, 2601−2608.
(19) Rovnyak, G. C.; Kimball, S. D.; Beyer, B.; Cucinotta, G.; DiMarco, J. D.; Gougoutas, J.; Hedberg, A.; Malley, M.; McCarthy, J. P.; Zhang, R.; Moreland, S. Calcium entry blockers and activators: conformational and structural determinants of dihydropyrimidine calcium channel modulators. *J. Med. Chem.* **1995**, *38*, 119−129.
(20) Wenzel, T. J.; Bettes, T. C.; Sadlowski, J. E.; Sievers, R. E. New Binuclear Lanthanide NMR Shift Reagents Effective for Aromatic Compounds. *J. Am. Chem. Soc.* **1980**, *102*, 5903−5904.
(21) Sheldrick, G. M. *SHELX-86. A Computer Program for Crystal Structure Determination*; University of Gottingen: Gottingen, 1986.
(22) Sheldrick, G. M. *SHELX-93. Program for the Refinement of Crystal Structures*; University of Gottingen: Gottingen, 1993.
(23) Suemune, H.; Harabe, T.; Sakai, K. Syntheses of Unsaturated Trihydroxy C-18 Fatty Acids Isolated from Rice Plants Suffering from Rice Blast Disease. *Chem. Pharm. Bull.* **1988**, *36*, 3632−3637.
(24) Schwabe, U.; Trost, T. Characterization of adenosine receptors in rat brain by (−) [^{3}H]N^6-phenylisopropyladenosine. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980**, *313*, 179−187.
(25) Jarvis, M. F.; Schutz, R.; Hutchison, A. J.; Do, E.; Sills, M. A.; Williams, M. [^{3}H]CGS 21680, an A_2 selective adenosine receptor agonist directly labels A_2 receptors in rat brain tissue. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888−893.
(26) Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L. [^{125}I]AB-MECA, a high affinity radioligand for the rat A_3 adenosine receptor. *Mol. Pharmacol.* **1994**, *45*, 978−982.
(27) Ji, X.-d.; von Lubitz, D.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Species differences in ligand affinity at central A_3-adenosine receptors. *Drug Devel. Res.* **1994**, *33*, 51−59.
(28) Moro, S.; Li, A. H.; Jacobson, K. A. Molecular modeling studies of human A_3 adenosine receptor antagonists: structural homology and receptor docking. *J. Chem. Inf. Comput. Sci.* **1998**, in press.
(29) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(30) Li, A.-H.; Moro, S.; Forsyth, N.; Melman, N.; Ji, X.-d.; Jacobson, K. A. Synthesis, CoMFA analysis, and receptor docking of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A_3 adenosine receptor antagonists. *J. Med. Chem.* **1999**, *42*, 706−721.
(31) The program SYBYL 6.3 is available from TRIPOS Associates, St. Louis, MO; 1993.
(32) Dewar, M. J. S. E.; Zoebisch, G.; Healy, E. F. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.
(33) MOPAC 6.0 is available from Quantum Chemistry Program Exchange.
(34) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel-An Integrated Software System for Modeling Organic and Bioorganic Molecules using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−450.
(35) Kyte, J.; Doolittle, R. F. A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.* **1982**, *157*, 105−132.
(36) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. An all-atom force field for simulation of protein and nucleic acids. *J. Comput. Chem.* **1986**, *7*, 230−252.
(37) Moro, S.; Guo, D.; Camaioni, E.; Boyer, J. L.; Harden, K. T.; Jacobson, K. A. Human $P2Y_1$ receptor: molecular modeling and site-directed mutagenesis as tools to identify agonist and antagonist recognition sites. *J. Med. Chem.* **1998**, *41*, 1456−1466.
(38) Li, A.-H.; Chang, L.; Ji, X.-d.; Melman, N.; Olah, M.; Stiles, G.; Jacobson, K. A. Functionalized Congeners of 1,4-Dihydropyridines as Molecular Probes for A_3 Adenosine Receptors. *Bioconjugate Chem.* **1999**, *10*, 667−677.

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