Structure−Activity Relationships of 4-(Phenylethynyl)-6-phenyl-1,4-dihydropyridines as Highly Selective A_3 Adenosine Receptor Antagonists

Ji-long Jiang, A. Michiel van Rhee, Louis Chang, Abraham Patchornik,† Xiao-duo Ji, Patricia Evans, Neli Melman, and Kenneth A. Jacobson*

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0810

Received February 11, 1997⊗

4-(Phenylethynyl)-6-phenyl-1,4-dihydropyridine derivatives are selective antagonists at human A_3 adenosine receptors, with K_i values in a radioligand binding assay vs [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine) in the submicromolar range. In this study, structure−activity relationships at various positions of the dihydropyridine ring (the 3- and 5-acyl substituents, the 4-aryl substituent, and 1-methyl group) were probed synthetically. Using the combined protection of the 1-ethoxymethyl and the 5-[2-(trimethylsilyl)ethyl] ester groups, a free carboxylic acid was formed at the 5-position allowing various substitutions. Selectivity of the new analogues for cloned human A_3 adenosine receptors was determined vs radioligand binding at rat brain A_1 and A_{2A} receptors. Structure−activity analysis at adenosine receptors indicated that pyridyl, furyl, benzofuryl, and thienyl groups at the 4-position resulted in, at most, only moderate selectivity for A_3 adenosine receptors. Ring substitution (*e.g.*, 4-nitro) of the 4-phenylethynyl group did not provide enhanced selectivity, as it did for the 4-styryl-substituted dihydropyridines. At the 3-position of the dihydropyridine ring, esters were much more selective for A_3 receptors than closely related thioester, amide, and ketone derivatives. A cyclic 3-keto derivative was 5-fold more potent at A_3 receptors than a related open-ring analogue. At the 5-position, a homologous series of phenylalkyl esters and a series of substituted benzyl esters were prepared and tested. (Trifluoromethyl)-, nitro-, and other benzyl esters substituted with electron-withdrawing groups were specific for A_3 receptors with nanomolar K_i values and selectivity as high as 37000-fold. A functionalized congener bearing an [(aminoethyl)amino]carbonyl group was also prepared as an intermediate in the synthesis of biologically active conjugates.

Medicinal chemists are currently developing agonists and antagonists that interact selectively with receptors for adenosine, of which A_1, A_{2A}, A_{2B}, and A_3 subtypes are known.[1] Relatively recently identified through cloning,[2,3] the A_3 adenosine receptor has provided new therapeutic opportunities in the adenosine field, due to its unique biological effects.[4] Activation of A_3 receptors would require relatively high physiological concentrations of adenosine; the K_i value of adenosine in binding to the rat A_3 receptor has been estimated to be ~1 μM vs 10 and 30 nM at rat A_1 and A_{2A} receptors, respectively.[4] Since activation may occur only under conditions of severe stress, the physiological role of A_3 receptors may be very different from that of A_1 and A_{2A} subtypes, which are likely to partially activated by adenosine under basal conditions. The A_3 receptor has a unique structure−activity relationship (SAR) profile and tissue distribution.[4] Activation of the A_3 receptor has been shown to stimulate phospholipases C[5] and D[6] and to inhibit adenylate cyclase.[1]

The varied effects of A_3 receptor agonists appear to be dual and opposite, *i.e.*, either cytoprotective or cytotoxic, depending on the level of receptor activation and the system studied. Apoptosis (programmed cell death) has been shown to occur in the HL-60 human leukemia cell line and in human blood eosinophils in response to a high concentration (≥10 μM) of A_3 selective agonists developed in our laboratory.[7,8] In cultured chick cardiac myocytes, a brief prior exposure to nanomolar concentrations of the A_3 receptor agonist 2-chloro-N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide (Cl-IB-MECA) protected cells from damage induced by subsequent hypoxia,[9] through a phenomenon termed "preconditioning." High concentrations of the same agonist induce apoptosis in rat cardiac myocytes.[10] The first cytoprotective effects of an A_3 agonist were shown following its chronic administration in gerbils in a model of stroke, in which the agonist was highly cerebroprotective and depressed nitric oxide synthase.[11,12] Acute presence of the agonist during the ischemia exacerbated damage. In astroglial cultures, A_3 agonists induced differentiation and protection at nanomolar concentrations, while promoting cell death at high concentrations.[13] There may be an involvement of A_3 receptors in cancer.[14] An A_3 agonist inhibited the release of potentially damaging TNF-α in activated macrophages; thus A_3 agonists may be protective in models of inflammation.[15]

A_3 adenosine receptor antagonists, although only recently introduced,[16−20] were previously hypothesized to act as potential anti-asthmatic,[21] anti-inflammatory,[21] or cerebroprotective agents.[11] The most promising leads for A_3 receptor antagonists have appeared recently among chemically diverse non-xanthine heterocycles (Figure 1). For example, the 1,4-dihydropyridines,

* Address correspondence to: Dr. K. A. Jacobson, Chief, Molecular Recognition Section, LBC, NIDDK, NIH, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810. Tel: (301) 496-9024. Fax: (301) 480-8422. E-mail: kajacobs@helix.nih.gov.

† Permanent address: Poly Chem PCJ, Box 271, Ness-Ziona, Israel.

⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

S0022-2623(97)00091-5

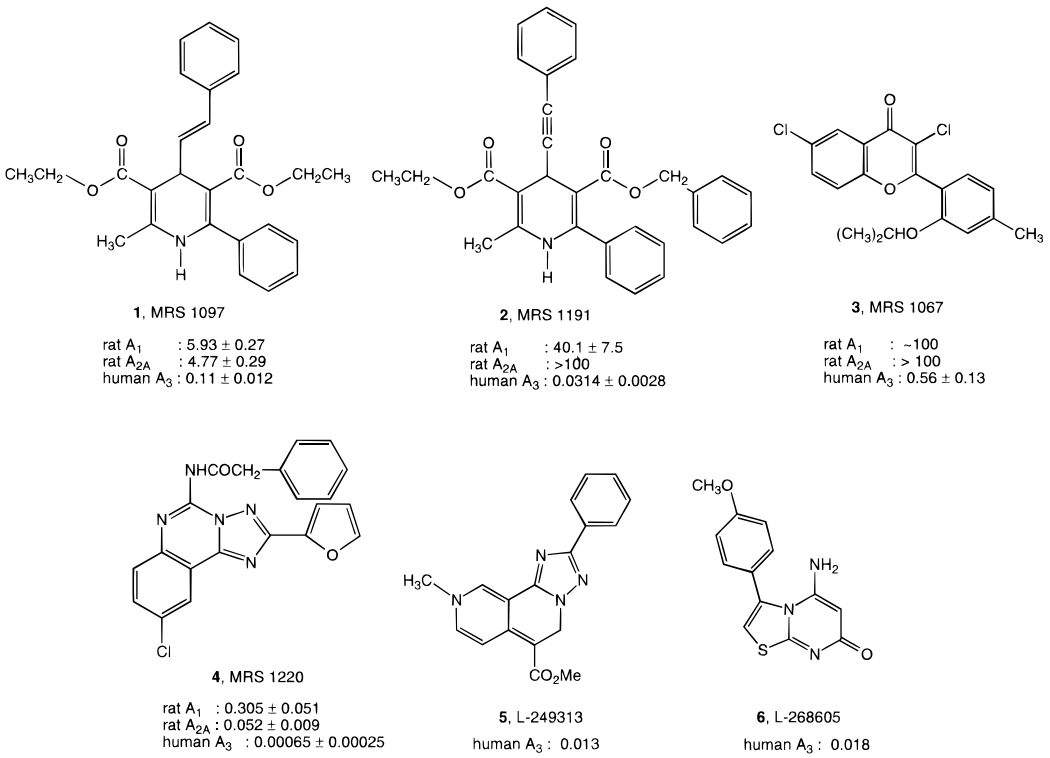


Figure 1. Structures of key A_3 adenosine receptor selective antagonists reported in 1996. K_i values (μM) are reported in refs 16−20.

known commonly as potent blockers of L-type calcium channels that are used widely in treating coronary heart disease, have provided leads for designing adenosine antagonists, particularly with selectivity for the A_3 receptor subtype.[17,18] In the present study and in two previous studies, we have used the 1,4-dihydropyridine nucleus as a template for probing structure−activity relationships at adenosine receptors. By careful structural modification, it has been possible to select for affinity at adenosine receptors and deselect for affinity at L-type Ca^{2+} channels. For example, a dihydropyridine derivative, 3,5-diethyl 2-methyl-6-phenyl-4-[2-phenyl-(*E*)-vinyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS 1097, **1**; Figure 1),[17] has been found to inhibit binding of radioligand at the human A_3 receptor with an affinity of 108 nM, while the same derivative was inactive at ion channels and other receptor sites. Furthermore, MRS 1097 antagonized the effects of IB-MECA, an A_3 receptor selective agonist, on inhibition of adenylate cyclase via the cloned rat A_3 receptor. Affinity and selectivity for the human A_3 receptor within this series was further enhanced in the trisubstituted analogue MRS 1191, 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-(phenylethynyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (**2**; Figure 1,).[18] Other A_3 selective antagonists that have been recently reported include a flavonoid derivative (MRS 1067, **3**),[16] a derivative of the triazoloquinazoline CGS 15943 (MRS 1220, **4**),[20] a triazolonaphthyridine (L-249313, **5**),[19] and a thiazolopyrimidine (L-268605, **6**).[19] Although having high affinity, L-249313 appears to bind noncompetitively to the human A_3 receptor.

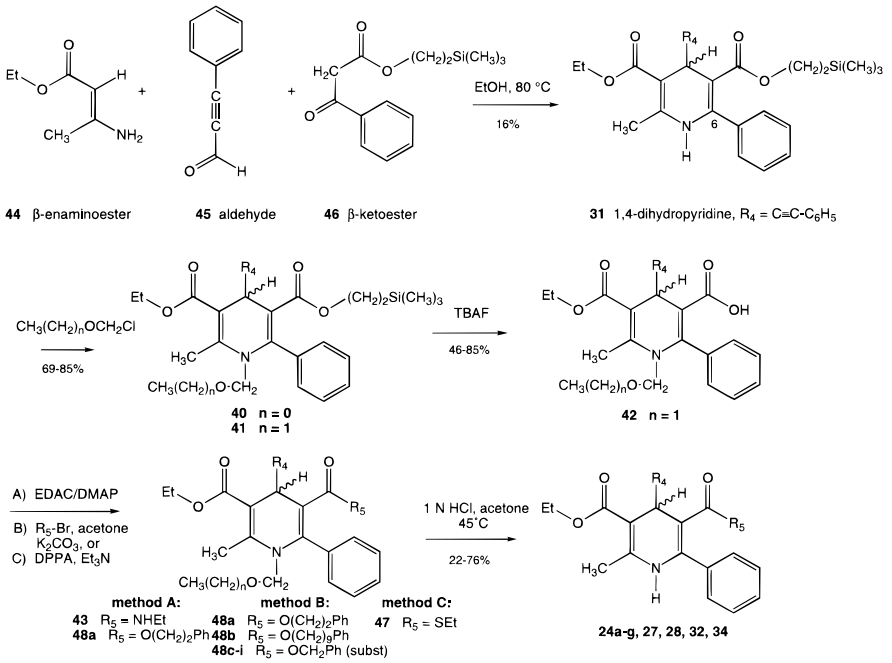
In the present study, we have explored the structure−activity relationships of known selective antagonists, MRS 1097 and MRS 1191, containing both subtle and drastic structural changes at various positions of the dihydropyridine ring (its 3- and 5-acyl substituents, the 4-aryl substituent, and 1-methyl group). We have discovered that substitutions of the 5-ester group provide the greatest versatility for improving A_3 receptor selectivity dramatically and achieving nanomolar potency. The efficient synthesis of these new analogues has been accomplished through a general approach using orthogonal protecting groups at the N1 and 5-ester positions.

Results

Synthesis. The structures of the 1,4-dihydropyridines and related derivatives (**1**, **2**, and **7**−**43**) tested for affinity in radioligand binding assays at adenosine receptors are shown in Table 1. The dihydropyridine analogues were prepared by methods outlined in Schemes 1−4.

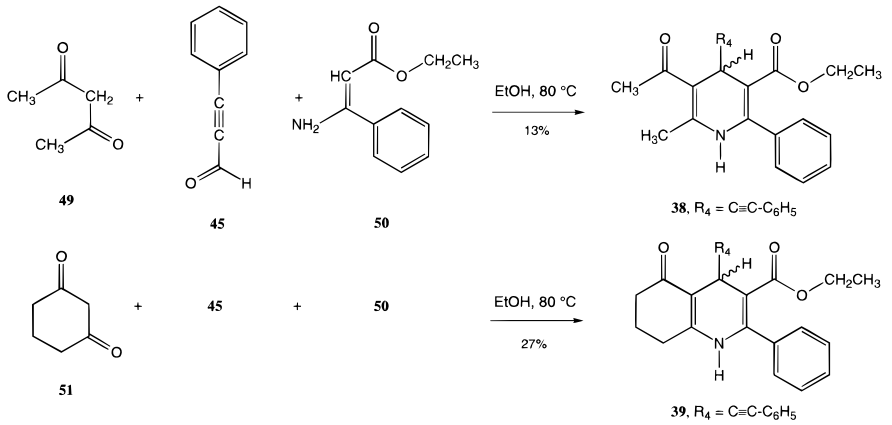
As in the previous studies,[17,18] the basic synthesis of the 1,4-dihydropyridine nucleus consisted of the Hantzsch condensation (reviewed in ref 22), an example of which is shown in Scheme 1. This method involves a three-component reaction of a 3-amino-2-propenoate ester, such as ethyl 2-aminocrotonate (**44**), an aldehyde, such as propiolaldehyde (**45**), and a benzoylacetate ester, such as **46**, that were dissolved in ethanol and refluxed. In some reactions, other β-keto esters[27] were used to synthesize analogues having groups other than phenyl in the 6-position. For example, the 6-trifluoromethyl group was introduced in compound **4** using ethyl trifluoroacetoacetate instead of **46**. Good yields of 1,4-dihydropyridines containing the 6-phenyl group were obtained using a 72 h reaction time. Alternately, the 6-phenyl group was introduced using ethyl 3-aminocinnamate, **50**, prepared as reported previously. An example of the latter approach is given in Scheme 2. This allowed the introduction of the 3-keto group in the 1,4-dihydropyridine, of which both open-ring, *e.g.*, **38**, and

Scheme 1. Synthesis of 5-Ester-Substituted 1,4-Dihydropyridines Using the Hantzsch Reaction and an Orthogonal Protecting Scheme[a]



[a] R_4 = phenylethynyl.

Scheme 2. Synthesis of Open-Chain and Ring-Constrained 3-Keto-Substituted 1,4-Dihydropyridines Using a Variation of the Hantzsch Reaction

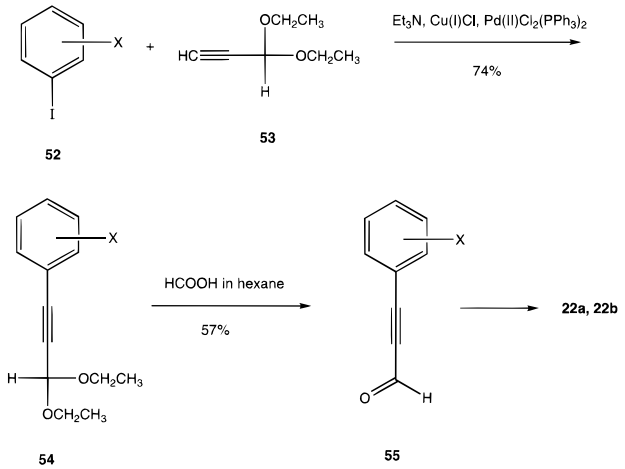


ring-constrained, *e.g.*, **39**, examples were prepared, using 2,4-pentanedione or 1,3-cyclohexanedione,[23] respectively.

The specific example shown in Scheme 1 incorporates a protecting group at the 5-ester position that allows for subsequent substitution of this ester.[28] Using the combined protection of the 1-position with the ethoxymethyl group[24] and the 5-[2-(trimethylsilyl)ethyl] ester,[25] a free carboxylic acid was formed at the 5-position allowing subsequent esterification by means of alkylation or condensation (Scheme 1). These protecting groups could then be removed separately to allow selective derivatization of the carboxylic acid. The 5-[2-(trimethylsilyl)ethyl] ester group was introduced at the stage of the Hantzsch condensation, using the appropriate 3-ketopropionate ester **46**. The silyl group was later deprotected using tetrabutylammonium fluoride in tetrahydrofuran. The carboxylic acid could then be converted to an amide, a thioester, or an ester using a carbodiimide-condensing reagent in DMF or methylene chloride, in the presence of catalytic 4-(*N*-dimethylamino)pyridine, although esters were obtained in only low yield (<20%) by this method. Alternately, the carboxylic acid could be esterified in very high yield (>90%) under alkylating conditions (alkyl bromide with potassium carbonate in acetone). Thus, esterification by means of alkylation was preferred over the condensation reaction, since the latter was sluggish, presumably due to the steric hindrance at the carboxylic acid. The 1-ethoxymethyl group was finally removed upon heating at 45 °C for 1 h with 1 N HCl in acetone.

Ring substitution of the 4-phenylethynyl group of **2** was accomplished through the preparation of the appropriate aldehyde intermediates **55**. These substituted phenylpropargyl aldehyde derivatives were prepared as shown in Scheme 3 using a modified application of the Stephans−Castro reaction.[26] A substituted iodobenzene, **52**, was coupled to propiolaldehyde diethyl acetal, **53**, using copper(I) and palladium(II) catalysis to give the protected phenylpropiolaldehyde **54**. Deprotection of the diethyl acetal was accomplished using formic acid in hexanes, since hydrolysis in aqueous medium led to decomposition.

Scheme 3. Synthesis of Phenylpropiolaldehyde Intermediates and Preparation of Ring-Substituted 4-(Phenylethynyl)dihydropyridines Using the Stephans−Castro Methodology[26] [a]



[a] X = 3-CH_3 or 4-NO_2.

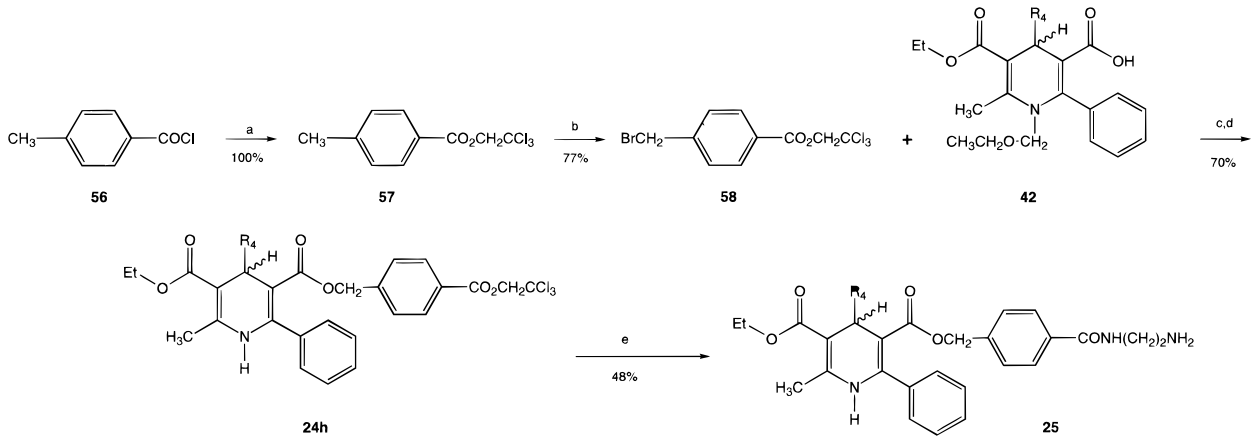
Most of the derivatives in Table 1 represent a classical medicinal chemical approach to drug design. An alternate approach, called the "functionalized congener approach",[35] in which an easily derivatized functional group is incorporated at the end of a strategically designed and attached chain substituent, was also applied in the present study. Thus, an amine-functionalized congener, **25**, was synthesized as shown in Scheme 4. The reaction involved alkylation of the 5-carboxylic acid **42** using a benzyl bromide intermediate, **58**. This intermediate was formed in two steps from *p*-toluic acid chloride, **56**, which reacted with trichloroethanol to form ester **57**. The ester was brominated using *N*-bromosuccinimide to give **58**. After acidic deprotection of the 1-ethoxymethyl protecting group of the dihydropyridine **59**, the *p*-(trichloroethyl ester) group of **24h** could be aminolyzed selectively using neat ethylenediamine at room temperature. The ester groups at the 3- and 5-positions of the dihydropyridine ring were much less susceptible to reaction with amines. This gave directly the desired primary amine congener **25**. An alternate route that proved unsuccessful used instead of **58** an intermediate benzyl bromide already containing a Boc-protected ethylenediamine moiety, previously reported for the purpose of introducing a prosthetic group in peptides and other derivatized receptor ligands for facile radiofluorination reactions.[28]

Pharmacology. van Rhee *et al.*[17] demonstrated that A_3 receptor selectivity could be achieved in 1,4-dihydropyridines through a combination of large substituents at the 4- and 6-positions. 4-Styryl or 4-phenylethynyl groups together with 6-phenyl groups are particularly suited for this pharmacological profile. In a second study by Jiang *et al.*,[18] we have explored the structure−activity relationships at the 6-position. In the present study we have introduced a variety of heterocyclic aromatic groups at the 4-position and modified other positions. Compound **7** was reported in van Rhee *et al.*[17] showing that the 4-phenyl group is tolerated in binding at adenosine receptors; although lacking the 6-phenyl group it does not provide A_3 receptor selectivity. The comparable affinity of the phenyl, **7**, and thienyl, **8**, derivatives at A_1 and A_3 receptors indicates that other aryl groups are acceptable at the 4-position. Unlike the 4-phenyl analogue **7**, which is nonselective, elongating the connection to the phenyl group with an acetylene linkage in **9** provides A_3 receptor selectivity. The 4-phenylacetylene substituent has been combined with other favorable modifications of the dihydropyridines[18] to result in analogues with as great as 1700-fold selectivity for A_3 vs A_1 receptors.

At the 6-position, substitution of the methyl group with trifluoromethyl, **10**, appeared to reduce the affinity of the dihydropyridine at A_1 and A_{2A} but not A_3 receptor subtypes, resulting in 15-fold selectivity. Since we have shown that the phenyl group is optimal at the 6-position,[18] further alkyl or haloalkyl derivatization at this position was not carried out.

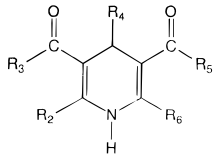
The 4-phenyl group in combination with a 6-phenyl group, in **11**, resulted in A_3 receptor selectivity (4.7-fold vs A_1 receptors). Thus, analogues **12**−**17** were synthesized to probe the effects of aromatic heterocycles directly attached at the 4-position in combination with the 6-phenyl group. The 2-, 3-, and 4-pyridyl analogues **12**−**14** were neither potent nor selective in binding. Among the positional isomers of the nitrogen in 4-pyridyl derivatives, no significant differences were observed at A_1 receptors, and the affinity at A_3 receptors varied slightly in the order of 4- > 2- > 3-position.

Scheme 4. Synthesis of an Amine-Functionalized Congener of the A_3 Adenosine Receptor Selective Antagonists Based on Dihydropyridines[a]

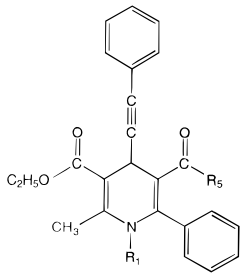


[a] Reagents: (a) 2,2,2-trichloroethanol; (b) *N*-bromosuccinimide, benzene; (c) acetone, potassium carbonate; (d) HCl; (e) ethylenediamine, rt.

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



1, 2, 7 - 39, R_2 = Me and R_6 = Ph, unless noted.



40 - 43

compound	R_3	R_4	R_5	K_i (μM) or % inhibition			
				rA_1[a]	rA_{2A}[b]	hA_3[c]	rA_1/hA_3
7[e] $R_6 = CH_3$	OCH_3	Ph-	OCH_2CH_3	11.0 ± 1.6	2.74 ± 0.85	12.0 ± 3.3	0.92
8 $R_6 = CH_3$	OCH_2CH_3	2-thienyl-	OCH_2CH_3	4.48 ± 0.90	25 ± 5% (10^{-4})	8.56 ± 1.22	0.52
9[e] $R_6 = CH_3$	OCH_3	Ph-C≡C-	OCH_2CH_3	5.39 ± 0.33	38.3 ± 7.9	0.940 ± 0.070	5.7
10 $R_6 = CF_3$	OCH_2CH_3	Ph-C≡C-	OCH_2CH_3	23.6 ± 2.9	25 ± 3% (10^{-4})	1.58 ± 0.56	15
11	OCH_2CH_3	Ph-	OCH_2CH_3	8.03 ± 2.05	*d* (10^{-4})	1.71 ± 0.36	4.7
12	OCH_2CH_3	2-pyridyl-	OCH_2CH_3	17.4 ± 16.0	25 ± 3% (10^{-4})	12.2 ± 0.3	1.4
13	OCH_2CH_3	3-pyridyl-	OCH_2CH_3	26.6 ± 1.2	40 ± 2% (10^{-4})	19.2 ± 2.6	1.4
14	OCH_2CH_3	4-pyridyl-	OCH_2CH_3	14.9 ± 0.2	44.7 ± 3.7	8.31 ± 1.84	1.8
15	OCH_2CH_3	2-thienyl-	OCH_2CH_3	8.59 ± 1.90	36 ± 4% (10^{-4})	1.04 ± 0.26	8.3
16	OCH_2CH_3	2-furyl-	OCH_2CH_3	15.4 ± 1.6	45 ± 2% (10^{-4})	0.507 ± 0.104	30
17	OCH_2CH_3	2-benzofuryl-	OCH_2CH_3	3.65 ± 0.45	*d* (10^{-4})	0.314 ± 0.056	12
1, MRS 1097[e]	OCH_2CH_3	Ph-CH=CH- (*trans*)	OCH_2CH_3	5.93 ± 0.27	4.77 ± 0.29	0.108 ± 0.012	55
18[e]	OCH_2CH_3	4-NO_2-Ph-CH=CH- (*trans*)	OCH_2CH_3	23 ± 9% (10^{-4})	33% (10^{-4})	0.0585 ± 0.0164	>1700
19	$OCH_2CH{=}CH_2$	4-NO_2-Ph-CH=CH- (*trans*)	OCH_2CH_3	*d* (10^{-4})	15 ± 2% (10^{-4})	0.296 ± 0.063	>300
20[e]	OCH_2CH_3	4-NH_2-Ph-CH=CH- (*trans*)	OCH_2CH_3	31 ± 3% (10^{-4})	26 ± 6% (10^{-4})	0.198 ± 0.047	>500
21[e]	OCH_2CH_3	Ph-C≡C-	OCH_2CH_3	11.0 ± 0.1	26 ± 12% (10^{-4})	0.0766 ± 0.0151	140
22a	OCH_2CH_3	4-NO_2-Ph-C≡C-	OCH_2CH_3	34.5 ± 6.8	24 ± 4% (10^{-4})	2.58 ± 0.66	13
22b	OCH_2CH_3	3-CH_3-Ph-C≡C-	OCH_2CH_3	41 ± 5% (10^{-4})	*d* (10^{-4})	0.220 ± 0.108	>400
2, MRS 1191[e]	OCH_2CH_3	Ph-C≡C-	OCH_2Ph	40.1 ± 7.5	*d* (10^{-4})	0.0314 ± 0.0028	1300
23 $R_2 = CH_2CH_3$	OCH_2CH_3	Ph-C≡C-	OCH_2Ph	25 ± 4% (10^{-4})	*d* (10^{-4})	0.0695 ± 0.0131	>1400
24a	OCH_2CH_3	Ph-C≡C-	OCH_2(2-CH_3)Ph	16 ± 1% (10^{-4})	13 ± 1% (10^{-4})	0.112 ± 0.015	>1000
24b	OCH_2CH_3	Ph-C≡C-	OCH_2(3-CH_3)Ph	*d* (10^{-4})	16 ± 2% (10^{-4})	0.0524 ± 0.017	>2000
24c	OCH_2CH_3	Ph-C≡C-	OCH_2(4-CH_3)Ph	*d* (10^{-4})	17 ± 3% (10^{-4})	0.110 ± 0.033	>1000
24d	OCH_2CH_3	Ph-C≡C-	OCH_2(4-CF_3)Ph	32 ± 3% (10^{-4})	15 ± 1% (10^{-4})	0.0177 ± 0.0015	>5000
24e	OCH_2CH_3	Ph-C≡C-	OCH_2(3-I)Ph	14 ± 1% (10^{-4})	19 ± 7% (10^{-4})	0.0937 ± 0.0333	>1000
24f	OCH_2CH_3	Ph-C≡C-	OCH_2(3-NO_2)Ph	28 ± 2% (10^{-4})	*d* (10^{-4})	0.00858 ± 0.00426	>11000
24g	OCH_2CH_3	Ph-C≡C-	OCH_2(4-NO_2)Ph	29 ± 2% (10^{-4})	*d* (10^{-4})	0.00269 ± 0.00096	>37000
24h	OCH_2CH_3	Ph-C≡C-	OCH_2-(4-$CO_2CH_2CCl_3$)Ph	33 ± 6% (10^{-4})	11% (10^{-4})	5.00	>20
24i	OCH_2CH_3	Ph-C≡C-	OCH_2-3,5-$(CF_3)_2$Ph	*d* (10^{-4})	29 ± 5% (10^{-4})	0.0374 ± 0.007	>2600
24j	OCH_2CH_3	Ph-C≡C-	OCH_2-3,5-$(NO_2)_2$Ph	20% (10^{-4})	40 ± 7% (10^{-4})	0.036	>3000
25	OCH_2CH_3	Ph-C≡C-	OCH_2Ph-4-CO-NH$(CH_2)_2NH_2$	68 ± 2% (10^{-4})	39 ± 4% (10^{-4})	*d* (10^{-7})	
26	$OCH_2CH_2CH_3$	Ph-C≡C-	OCH_2Ph	27 ± 7% (10^{-4})	16 ± 2% (10^{-4})	0.0682 ± 0.0149	>1400
27	OCH_2CH_3	Ph-C≡C-	$O(CH_2)_2Ph$	*d* (10^{-4})	*d* (10^{-4})	0.146 ± 0.012	> 1000
28	OCH_2CH_3	Ph-C≡C-	$O(CH_2)_3Ph$	*d* (10^{-4})	*d* (10^{-4})	0.0757 ± 0.0258	>1300

compound	R_3	R_4	R_5	K_i (μM) or % inhibition			
				rA_1[a]	rA_{2A}[b]	hA_3[c]	rA_1/hA_3
29	OCH_2CH_3	Ph-C≡C-	$OC(CH_3)_3$	25.3 ± 2.7	27 ± 5% (10^{-4})	3.10 ± 0.64	8.2
30	OCH_2CH_3	Ph-C≡C-	$OCH_2CH(OCH_3)Ph$ (*R*)	8.58 ± 1.34	33 ± 6% (10^{-4})	1.72 ± 0.45	5.0
31	OCH_2CH_3	Ph-C≡C-	$O(CH_2)_2Si(CH_3)_3$	18.8 ± 5.4	18 ± 5% (10^{-4})	0.0596 ± 0.0199	310
32a	OCH_2CH_3	Ph-C≡C-	SCH_2CH_3	53.0 ± 13.6	17 ± 3% (10^{-4})	0.567 ± 0.185	93
32b	SCH_2CH_3	Ph-C≡C-	OCH_2CH_3	31.5 ± 11.3	5.15 ± 1.83	0.290 ± 0.082	110
33	OCH_2CH_3	Ph-C≡C-	OH	8.20 ± 0.40	16 ± 6% (10^{-4})	*d* (10^{-5})	<1
34	OCH_2CH_3	Ph-C≡C-	$NHCH_2CH_3$	16.2 ± 5.6	47 ± 2% (10^{-4})	5.56 ± 1.69	2.9
35[e]	OCH_2Ph	Ph-C≡C-	OCH_2CH_3	24 ± 4% (10^{-4})	*d* (10^{-4})	0.169 ± 0.026	>600
36	$OC(CH_3)_3$	Ph-C≡C-	OCH_2CH_3	23.1 ± 1.6	20 ± 2% (10^{-4})	1.04 ± 0.22	22
37	$NHCH_2CH_3$	Ph-C≡C-	OCH_2CH_3	65.6 ± 15.1	19 ± 6% (10^{-4})	2.44 ± 0.13	27
38	CH_3	Ph-C≡C-	OCH_2CH_3	12.6 ± 1.9	17 ± 7% (10^{-4})	2.27 ± 0.81	5.6
39	R_2-R_3 = $(CH_2)_3$	Ph-C≡C-	OCH_2CH_3	12.5 ± 1.5	22 ± 4% (10^{-4})	0.443 ± 0.086	28
40	$R_1 = CH_2OCH_3$		$R_5 = O(CH_2)_2Si(CH_3)_3$	*d* (10^{-4})	27 ± 4% (10^{-4})	170 ± 80	>1
41	$R_1 = CH_2OCH_2CH_3$		$R_5 = O(CH_2)_2Si(CH_3)_3$	13 ± 3% (10^{-4})	19 ± 5% (10^{-4})	nd	
42	$R_1 = CH_2OCH_2CH_3$		R_5 = OH	19.1 ± 3.1	36 ± 7% (10^{-4})	>100	>1
43	$R_1 = CH_2OCH_2CH_3$		$R_5 = NHCH_2CH_3$	18.5 ± 2.3	35 ± 1% (10^{-4})	10.9 ± 0.9	1.7

[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.*[16] or Jiang *et al.*[17]

Other aromatic moieties, such as in the thienyl and furyl derivatives **15**−**17**, were substituted at the 4-position resulting in moderate A_3 selectivity.

Several analogues of 4-styryl-1,4-dihydropyridines[17] were prepared. In the case of compound **17**, the benzofuryl group may be considered a ring-constrained version of the 4-styryldihydropyridines, such as **1**, which were shown to provide A_3 selectivity. Compound **17**, with a 3-fold lower affinity, was less selective for A_3 receptors than **1**. Jiang *et al.*[18] showed that the styryl ring may be substituted with a nitro, **18**, or an amino, **20**, group with enhancement of high selectivity for A_3 vs A_1 receptors. Elongation of the 3-ester group of **18**, resulting in the allyl ester **19**, diminished the A_3 receptor affinity by 5-fold, yet A_3 receptor selectivity remained relatively high (>300-fold).

In combination with the 6-phenyl substituent, the 4-phenylethynyl group in analogue **21** resulted in slightly higher A_3 receptor potency and selectivity (140- vs 55-fold) than the 4-styryl group in analogue **1**.[18] In comparison to the corresponding 6-trifluoromethyl analogue **10**, the affinity of **21** had increased by 44-fold at A_3 receptors, while at A_1 and A_{2A} receptors the difference was not significant. Therefore, ring substitutions of the 4-phenylethynyl group (4-nitro, **22a**, and 3-methyl, **22b**) of the 3,5-diethyl ester **21** were prepared in the hope that these relatively minor changes carried out at a distance from the dihydropyridine pharmacophore would be tolerated at A_3 receptors. Yet, both of these modifications, especially **22a**, diminished A_3 receptor affinity and selectivity. The effects of ring substitution of the 4-phenylethynyl group appear to be unlike the case of the 4-styryl analogues, in which a 4-nitro group enhanced potency and selectivity for A_3 receptors. The 4-nitro substitution of the ring of the 4-phenylethynyl group of **22a** decreased the A_3 receptor affinity 48-fold while decreasing the selectivity vs A_1 receptors from 140- to 9-fold.

Appending the 3,5-diethyl ester **21** with an additional aromatic group in the 5-position, in the form of a benzyl ester, **2**, was found previously to greatly enhance A_3 receptor potency and selectivity. There is considerable flexibility of substitution of the 3-ester group as well as the 5-ester group in A_3 receptor selective dihydropyridines. For example, the 3-benzyl ester **35** is >600-fold selective, although not as potent as the isomer 5-benzyl ester derivative **2**. Thus, in the present study the structure−activity relationships at the 3- and 5-ester positions were systematically probed.

Substituted 5-benzyl esters (**24a**−**j**) and a homologous series of 5-phenylalkyl esters (**2**, **27**, and **28**) were prepared and tested. The parent benzyl ester **2** was 1300-fold selective for A_3 receptors with a K_i value of 31 nM. The next higher homologue, the 2-phenylethyl derivative **27**, displayed a K_i value of 146 nM. The 3-phenylpropyl ester **28** was more potent than **27**, with a K_i value of 76 nM. Since no advantage was apparent to homologation of the benzyl ester, the ring substitution of the preferred 5-benzyl ester was examined in detail. The A_3 affinity of the methylbenzyl esters **24a**−**c** varied in the order m > p, o. A *p*-(trifluoromethyl)benzyl ester, **24d**, was specific for A_3 receptors with a K_i value of 18 nM and had an estimated selectivity ratio of greater than 5000-fold vs either A_1 or A_{2A} receptors. A 3-iodo analogue, **24e**, included for its structural similarity to

Table 2. Characterization of Dihydropyridine and Pyridine Derivatives

compd	T_m (°C)	formula	MS	anal.	yield[a] (%)
8	167−168	$C_{17}H_{21}NO_4S$	335 (EI)	C,H,N	48
10	oil	$C_{21}H_{20}NF_3O_4$	407 (CI)	*b*	35
11	140−145	$C_{24}H_{25}NO_4$	391 (EI)	*c*	17
12	125−128	$C_{23}H_{24}N_2O_4$	392 (EI)	C,H,N	21
13	137−140	$C_{23}H_{24}N_2O_4$	392 (EI)	C,H,N	32
14	157−163	$C_{23}H_{24}N_2O_4$	392 (EI)	C,H,N	26
17	131−134	$C_{26}H_{25}NO_5$	431 (EI)	C,H,N	31
22b	118−123	$C_{27}H_{27}NO_4$	429 (EI)	C,H,N	55
23	oil	$C_{32}H_{29}NO_4$	491 (EI)	*d*	17
24a	95−100	$C_{32}H_{29}NO_4$	491 (EI)	*e*	76
24b	150−152	$C_{32}H_{29}NO_4$	491 (EI)	*f*	22
24c	139−144	$C_{32}H_{29}NO_4$	491 (EI)	*g*	28
24d	140−144	$C_{32}H_{26}F_3NO_4$	545 (EI)	C,H,N	53
24e	115−117	$C_{31}H_{26}INO_4$	603 (EI)	C,H,N	53
24f	146−147	$C_{31}H_{26}N_2O_6$	522 (EI)	C,H,N	34
24g	150−152	$C_{31}H_{26}N_2O_6$	522 (EI)	C,H,N	41
24h	70−74	$C_{34}H_{28}Cl_3NO_6$	652 (EI)	C,H,N	70
24i	179−180	$C_{33}H_{25}F_6NO_4$	613 (EI)	C,N; H[n]	58
25	101−105	$C_{34}H_{33}N_3O_5$	564 (FAB, M + H)	*h*	48
26	oil	$C_{32}H_{29}NO_4$	491 (EI)	*i*	15
27	oil	$C_{32}H_{29}NO_4$	491 (EI)	C,H,N	81
28	oil	$C_{33}H_{31}NO_4$	505 (EI)	C,H,N	95
29	oil	$C_{28}H_{29}NO_4$	443 (EI)	C,H,N	14
30	oil	$C_{33}H_{31}NO_5$	521 (CI)	*j*	38
31	146−147	$C_{29}H_{33}NSiO_4$	487 (EI)	C,H,N	16
32a	oil	$C_{26}H_{25}NO_3S$	(EI)	*k*	15
32b	72−74	$C_{26}H_{25}NO_3S$	432 (CI, M + H)	C,H,N	17
33	173−175	$C_{24}H_{21}NO_4 \cdot 0.50H_2O$	387 (CI)	C,H,N	6
34	oil	$C_{26}H_{26}N_2O_3 \cdot 0.85H_2O$	414 (EI)	C,H,N	61
36	oil	$C_{28}H_{29}NO_4$	443 (EI)	C,H,N	29
37	199−200	$C_{26}H_{28}N_2O_3 \cdot 0.75H_2O$	414 (EI)	C,H,N	20
38	158−159	$C_{25}H_{23}NO_3$	385 (EI)	C,H,N	13
39	oil	$C_{26}H_{23}NO_3 \cdot 0.75H_2O$	397 (EI)	C,H,N	27
40	oil	$C_{31}H_{37}NSiO_5$	531 (CI)	*l*	85
41	oil	$C_{32}H_{39}NSiO_5$	545 (CI)	*m*	85
42	182−183	$C_{27}H_{27}NO_5$	445 (EI)	C,H,N	85
43	oil	$C_{29}H_{32}N_2O_4$	472 (EI)	C,H,N	47

[a] Purification was achieved by thin layer chromatography (silica gel 60, 1000 μm layer thickness). [b] **10:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.21; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.46; EI calcd 462.1806, found 462.1791. [c] **11:** pure on analytical TLC (silica gel 60, 250 μm) $CHCl_3$:MeOH = 40:1 (v/v), R_f = 0.3; EI calcd 391.1784, found 391.1774. [d] **23** ($C_{32}H_{29}NO_4$): insufficient quantity for CHN, pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.35; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.47; EI calcd 491.2096, found 491.2096. [e] **24a:** pure on analytical TLC (silica gel 60, 250 μm) hexanes:EtOAc = 2:1 (v/v), R_f = 0.6; EI calcd 491.2097, found 491.2095. [f] **24b:** pure on analytical TLC (silica gel 60, 250 μm) hexanes:EtOAc = 2:1 (v/v), R_f = 0.6; EI calcd 491.2097, found 491.2094. [g] **24c:** pure on analytical TLC (silica gel 60, 250 μm) hexanes:EtOAc = 2:1 (v/v), R_f = 0.6; EI calcd 491.2097, found 491.2109. [h] **25:** pure on analytical TLC (silica gel 60, 250 μm) $CHCl_3$:MeOH = 5:1 (v/v), R_f = 0.1; FAB (M + H) 564.4. [i] **26:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.30; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.43; EI calcd 491.2096, found 491.2069. [j] **30:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.41; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.50; EI calcd 521.2202, found 521.2172. [k] **32a:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.29; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.41; EI calcd 431.1555, found 431.1541. [l] **40:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.50; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.58; EI calcd 531.2441, found 531.2418. [m] **41:** pure on analytical TLC (silica gel 60, 250 μm) EtOAc:petroleum ether = 20:80 (v/v), R_f = 0.54; CH_2Cl_2:MeOH = 40:1 (v/v), R_f = 0.60; EI calcd 545.2597, found 545.2606. [n] **24i**: H calcd, 4.12; found, 4.94.

the N^6-(3-iodobenzyl) adenosine derivatives,[4] which are selective agonists at A_3 receptors, was not the most potent analogue yet was >1000-fold selective. The presence of one or two electron-withdrawing groups on the benzyl ring, as in 3- and 4-nitrobenzyl esters **24f**,**g** and the disubstituted ester **24i**, resulted in exceptionally high potency and selectivity for A_3 receptors. Compound **24f** displayed an A_3 receptor selectivity ratio of >11000-fold, and compound **24g** was at least 37000-fold selective for human A_3 receptors vs either A_1 or A_{2A} receptors. The 4-[[(trichloroethyl)oxy]carbonyl] ester **24h** was much less potent at A_3 receptors.

A functionalized congener bearing an [(aminoethyl)-amino]carbonyl group, **25**, was also prepared as an intermediate for the synthesis of biologically active conjugates. Unfortunately, the affinity was much less than anticipated. At a concentration of 100 nM, no displacement of the radioligand was observed.

Other 5-ester derivatives were prepared and examined in receptor binding. Branching of the ester chain was found to have variable effects on receptor affinity. A (trimethylsilyl)ethyl ester, **31**, was highly potent and selective at A_3 receptors, while a 5-*tert*-butyl ester, **29**, was less well tolerated at A_3 receptors. Adding a methoxy group in the *R*-configuration to the α-position of the 2-phenylethyl ester, in **30** (tested as a diastereomeric mixture), was also not well tolerated in binding at A_3 receptors.

A 5-thioester derivative, **32a**, was not well tolerated in receptor binding, and the affinity at A_1 and A_3 receptors was 5- and 7-fold less, respectively, than the ester **21**. The corresponding 3-thioester derivative **32b** was more potent than the 5-thioester at A_3 receptors but was still 3.8-fold less potent than **21**. Curiously the affinity of **32b** at A_{2A} receptors was greater than at A_1

receptors. This represents a >20-fold affinity enhancement at the A_{2A} subtype by replacing oxygen with sulfur.

Other structurally simple modifications of the ethyl ester at the 5-position of the dihydropyridine ring were also examined. A free carboxylic acid at the 5-position, **33**, greatly decreased affinity at A_3 but not A_1 receptors. A 5-ethylamide, **34**, was nearly as potent as the corresponding ethyl ester **21** at A_1 receptors; however, it was 73-fold less potent at A_3 receptors. Thus, it appears that the 5-ester linkage is preferred for dihydropyridines with high A_3 receptor selectivity.

Increasing the size of the 3-position ester was also examined. Previously, compound **35** was shown to be highly selective for A_3 receptors, suggesting that added steric bulk on the side opposite the 6-phenyl ring might be beneficial for selectivity by reducing affinity at the other subtypes. Nevertheless, the homologation of the 3-ethyl ester of **2**, to the propyl ester **26**, diminished A_3 affinity 2-fold. The effect of chain branching of the 3-ester group was also probed. As for the 5-ester series, introduction of the bulky *tert*-butyl group, **36**, greatly decreased the A_3 receptor potency and selectivity. Much like the 5-ester series, the presence of a 3-amide was not well tolerated at the A_3 receptors. Thus, the 3-ethylamide **37** was 6-fold less potent than the corresponding ethyl ester **21** at A_1 receptors and ~30-fold less potent at A_3 receptors. The 3-methyl ketone **38** was also much less potent and selective at A_3 receptors than the 3-ethyl ester **21**. Thus, it appears that both the 3- and 5-ester linkages in the dihydropyridines are highly favored in A_3 receptor binding. A ring-constrained analogue, **39**, related to the 3-methyl ketone **38** was of similar affinity vs **38** at A_1 and A_{2A} receptors yet 5-fold more potent at A_3 receptors and 28-fold selective vs A_1 receptors.

During the synthesis of a number of the above analogues (Scheme 1), the N1-position was protected with methoxymethyl, **40**, or ethoxymethyl groups, **41**−**43**. Although this N-alkylation tended to decrease both water solubility and affinity (*e.g.*, **40** and **41** vs the corresponding 1-NH derivative **31**), several of these intermediates were tested in binding. Although affinity generally was decreased, one of these derivatives was nearly as potent as the N-unprotected form (**43** vs **34** at A_1 and A_3 receptors).

Affinity at rat A_3 receptors of selected compounds is shown in Table 3. As expected from previous studies of A_3 antagonists, the affinity of the dihydropyridine derivatives in the rat was considerably less than that at human A_3 receptors. Nevertheless, certain analogues (**24b**,**c** and **26**−**28**) were still moderately selective (>50-fold) for rat A_3 vs rat A_1/A_{2A} receptors. The highest potency at rat A_3 receptors was observed for compound **26**, with a K_i of 1.12 μM.

Table 3. Affinities of 4-(Phenylethynyl)-6-phenyl-1,4-dihydropyridine Derivatives in Radioligand Binding Assays at Rat A_3 receptors

	K_i (μM)	
compd	rA_3[a]	rA_1/rA_3
2, MRS 1191	1.42 ± 0.19	28
24b, MRS 1328	1.52 ± 0.65	>60
24c, MRS 1326	1.69 ± 0.42	>50
24d, MRS 1329	3.00 ± 0.58	>30
24e, MRS 1330	4.02 ± 1.26	>20
24f, MRS 1333	5.07 ± 0.69	>10
24g, MRS 1334	3.85 ± 0.92	>20
24h, MRS 1353	13.0 ± 2.3	>7
24j, MRS 1355	5.07 ± 2.00	>10
26, MRS 1323	1.12 ± 0.05	>80
27, MRS 1321	1.32 ± 0.11	>70
28, MRS 1322	1.38 ± 0.27	>70

[a] Displacement of specific [^{125}I]AB-MECA binding at rat A_3 receptors stably expressed in CHO cells[2,32] (n = 3−5).

Discussion

Although highly selective antagonists have been reported for both A_1 and A_{2A} subtypes of adenosine receptors,[1] the development of such ligands for A_3 receptors has lagged behind the other subtypes.[4] In the present study, we have probed the SAR of A_3 adenosine receptor selective 1,4-dihydropyridines based on the 4-styryl derivative MRS 1097, [17] **1**, and the 4-phenylethynyl derivative MRS 1191, **2**,[18] and have designed ligands of exceptionally high selectivity. In addition to the 4-styryl and the 4-phenylethynyl substituents, various other aromatic groups were placed at the 4-position. Some differences in affinity were observed for the heterocyclic derivatives **12**−**17** in comparison to the 4-phenyl derivative **11**. At A_3 receptors, the furyl and benzofuryl derivatives **16** and **17** were more potent than the phenyl derivative, although the selectivity was only moderate. Ring substitution of the 4-styryl group maintained high A_3 receptor selectivity, but similar substitution of the 4-phenylethynyl substituent in compounds **22a**,**b** reduced affinity.

At the 2-position, only one homologue, the 2-ethyl derivative **23**, was prepared and found not to enhance the affinity at A_3 receptors over the 2-methyl derivative. An effort to produce a ring-constrained analogue, **39**, in which 2- and 3-substituents are joined and which may be useful for purposes of eventual QSAR molecular modeling of the dihydropyridines in binding to adenosine receptors, displayed only moderate potency, but this was likely due to the presence of a 3-keto group rather than an ester group. Nevertheless, the cyclic 3-keto derivative **39** was 5-fold more potent at A_3 receptors than the related open-ring analogue, **38**.

Functional group replacement at the 3- and 5-positions produced analogues that were less potent and selective than the parent 1,4-dihydropyridine 3,5-diesters, indicating the requirement for ester groups at these positions for binding with high affinity to A_3 adenosine receptors. At A_1 receptors, the ester groups appeared to be less critical, since their replacement with 3- or 5-ethylamide groups (**37** and **34**) resulted in only 6- and 3-fold loss of affinity vs **21**, respectively. Thioester derivatives **32a**,**b**, although similar in electronic structure to the corresponding ester **21**, were less potent in binding at A_3 adenosine receptors.

A homologous series of arylalkyl esters at the 5-position, **2**, **27**, and **28**, indicated the affinity at A_3 receptors varied in the order benzyl > 3-phenylpropyl > 2-phenylethyl. Thus, for achieving selectivity there appeared to be no advantage to homologation of the benzyl ester at this position, since the affinity of all three analogues was very weak at both A_1 and A_{2A} receptors. Thus, the benzyl ester group of **2** remained the most promising structural lead in the present study, and consequently the most selective pharmacological probes arose from ring substitution of the benzyl group. It was possible

to vary considerably the electronic and steric properties surrounding this ester group. Electron-withdrawing groups, particularly at the para- and meta positions, provided A_3 receptor selectivity of many thousand-fold, *i.e.*, the affinity at A_1 and A_{2A} receptors was essentially negligible, and the affinity at A_3 receptors vs **2** was either maintained or enhanced. The most selective compounds (human) in this study were 4-nitrobenzyl > 3-nitrobenzyl > 4-(trifluoromethyl)benzyl > 3,5-dinitrobenzyl esters. It will be interesting to probe which region of the human A_3 receptor contains an electron-rich binding site for this benzyl moiety. At rat A_3 receptors, the most potent and selective was the 3-propyl ester **26**; however, this compound was not significantly more potent than **2**.

The indication that the 5-ester position is very flexible for substitution in relation to A_3 receptor affinity has suggested the design of an amine-functionalized congener, **25**. As in our previous studies of purines derivatized with long chains for the purpose of conjugating with other molecules while retaining the biological potency, this derivative may prove to be a key intermediate in the design of much higher molecular weight derivatives bearing "carrier" moieties, reporter groups, prosthetic groups, etc. The presence of the amino group also increased water solubility; unfortunately it was not potent in binding at A_3 receptors.

A persistent problem during the development of selective A_3 receptor antagonists has been species differences.[4] Which of the novel antagonists[16−20] (screened for affinity principally at the human A_3 receptor) may be useful for pharmacological studies in rat and other species of interest? This study has demonstrated that considerable selectivity for rat A_3 receptors is still present (Table 3). Compound **2**, which has a ratio of selectivity in binding of only 28-fold, has been demonstrated to antagonize agonist action at the A_3 receptor in rat hippocampal slices.[33] In the latter study A_3 receptor activation antagonized the effects of presynaptic A_1 receptor activation to depress EPSPs. Compound **2** at a concentration of 10 μM selectively blocked the A_3 receptor activity without any measurable effect on A_1 receptors. Thus, certain compounds in this series having exceptionally high selectivity, such as **24b** and **26**−**28**, are likely of broad utility as pharmacological probes across species.

In conclusion, the dihydropyridines have served as a structural scaffold on which to add substituents to enhance the potency at the desired (A_3) receptor.[17] Nearly complete specificity for this subtype has now been achieved. Although these selective A_3 antagonists have not been evaluated at the A_{2B} receptor, it is likely that the affinity at that subtype is also low. A lead dihydropyridine derivative, nimodipine,[17] was found to be inactive at 100 μM in antagonizing the adenosine agonist effects on intracellular calcium via the cloned human A_{2B} receptor expressed in CHO cells (IJzerman *et al.*, unpublished results). Remaining challenges are to enhance potency to the subnanomolar level and to prepare radioligands. In the future, methods for resolving C-4 enantiomers of the present racemic compounds will be reported. In the previous and present studies we have probed systematically all of the positions of the 6-phenyl-1,4-dihydropyridines for the flexibility of substitution and have found that substitutions at the 4- and 5-positions, especially electron-poor benzyl esters, are most likely to result in high selectivity as A_3 receptor antagonists.

Experimental Section

Synthesis. 1. Materials. Ethyl 3-aminocrotonate (**44**), phenyl propargylaldehyde (**45**), 2,4-pentanedione (**49**), and 1,3-cyclohexanedione (**51**) were from Aldrich (St. Louis, MO). Compounds **1, 2, 7, 9, 18, 20, 21,** and **35** were prepared as described in van Rhee *et al.* or Jiang *et al.*[17,18] (*R*)-PIA and 2-chloroadenosine were purchased from Research Biochemicals International (Natick, MA). All other materials were obtained from commercial sources.

2. Synthesis. Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer, and spectra were taken in DMSO-d_6, CH_3OH-d_4, or $CHCl_3$-*d*. 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). All melting points were determined with a Unimelt capillary melting point apparatus (Arthur H. Thomas Co., PA) and are uncorrected.

General Procedure for Preparation of 1,4-Dihydropyridine-3,5-dicarboxylate Esters 8, 10−17, 22, 26, 29−31, 32b, and 36−39. Equimolar amounts (0.5 mmol) of the appropriate 3-amino-2-propenoate ester, aldehyde, and 3-ketopropionate ester derivative were dissolved in 2−5 mL of absolute ethanol. The solution was sealed in a glass tube and heated to 100 °C (for volatile aldehydes) or was refluxed under N_2 for at least 24 h and, at most, 72 h. The solvent was then evaporated, and products were purified either by crystallization, column chromatography (silica gel 60, 220−440 mesh; Fluka, Buchs, CH; 20% ethyl acetate−80% petroleum ether, 35−60), or preparative TLC (silica gel 60, 1000 μm; Analtech, Newark, DE; 2.5% methanol−97.5% dichloromethane). All procedures were performed under nitrogen and low-light conditions to prevent oxidation of the products. The products were shown to be homogeneous by analytical TLC and were stored at −20 °C.

3,5-Diethyl 2-Methyl-6-phenyl-4-(2-phenylethynyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (2). Equimolar amounts (0.5 mmol) of ethyl 3-amino-2-propenoate (65 mg), phenylpropargylaldehyde (65 mg) and benzyl benzoylacetate[18] (127 mg) were dissolved in 2 mL of absolute ethanol. The solution was sealed in a glass tube and refluxed under N_2 for 24 h. The solvent was then evaporated, and products were purified by preparative TLC (silica gel 60, 1000 μm; ethyl acetate:petroleum ether, 2:8). The product (R_f 0.36, same solvent) was isolated (73 mg) as white crystals, which were recrystallized from methanol:water, 7:3. ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, 5-methyl, J = 7.1 Hz), 2.36 (s, 6H, 2-, 6-CH_3), 3.80 (s, 3H, 3-methyl), 4.23−4.31 (m, 2H, 5-methylene), 4.99 (s, 1H, H-4), 5.71 (br, 1H, H-1), 7.24 (t, 3H, H-3′, H-4′, H-5′, J = 3.2 Hz), 7.36 (d, 2H, H-2′, H-6′, J = 3.6 Hz). MS (CI/NH_3): m/z 478 MH^+, 376 (M − Ph-CC)$^+$, 242 (376 − CO_2CH_2Ph)$^+$, base. UV absorbance peaks (MeOH) at 242 (λ_{max}, ϵ = 20 200), 351 nm.

3,5-Diethyl 2,6-Dimethyl-4-(2-thienyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (8). ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 6H, J = 7.75 Hz, 3, 5-CH_2CH_3), 2.3 (s, 6H, 2-, 6-CH_3), 4.17 (m, 4H, 3-, 5-OCH_2), 5.35 (s, 1H, 4-H), 5.92 (br, 1H, NH), 6.8 (d, 1H, J = 3.9 Hz, 3′-H), 6.85 (m, 1H, 4′-H), 7.06 (d, 1H, J = 4.89 Hz, 5′-H).

3,5-Diethyl 2-Methyl-4-(phenylethynyl)-6-(trifluoromethyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (10). ^{1}H NMR ($CDCl_3$): δ 1.32 (m, 6H, 3-, 5-CH_2CH_3); 2.35 (s, 3H, 7-CH_3), 4.20−4.38 (m, 4H, 3-, 5-OCH_2), 4.84 (s, 1H, 4-H), 6.29 (br, 1H, NH), 7.25−7.35 (m, 5H, C_6H_5). MS (EI): m/z 415 $(M)^+$; 386 (M − C_2H_5)$^+$, 342 (M − $CO_2C_2H_5$)$^+$, base.

3,5-Diethyl 2-Methyl-4,6-diphenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (11). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H, J = 6.83 Hz, 5-CH_2CH_3), 1.24 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.36 (s, 3H, 2-CH_3), 3.84 (m, 2H, 5-OCH_2), 4.12

(q, 2H, J = 6.83 Hz, 3-OCH_2), 5.12 (s, 1H, 4-H), 5.76 (br, 1H, NH), 7.18−7.44 (m, 10H, 4-, and 6-C_6H_5).

3,5-Diethyl 2-Methyl-4-(2-pyridyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (12). ^{1}H NMR ($CDCl_3$): δ 0.81 (t, 3H, J = 6.84 Hz, 5-CH_2CH_3), 1.22 (t, 3H, J = 6.83 Hz, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.82 (m, 2H, 5-OCH_2), 4.11 (q, 2H, J = 6.84 Hz, 3-OCH_2), 5.25 (s, 1H, 4-H), 5.88 (br, 1H, NH), 7.08−7.56 (m, 3H, pyridyl 3′-, 4′-, 5′-H), 7.37 (m, 5H, 6-C_6H_5), 8.55 (d, 1H, J = 4.89, pyridyl 6′-H).

3,5-Diethyl 2-Methyl-4-(3-pyridyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (13). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H, J = 6.83 Hz, 5-CH_2CH_3), 1.24 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 3.83 (m, 2H, 5-OCH_2), 4.11 (q, 2H, J = 6.84 Hz, 3-OCH_2), 5.09 (s, 1H, 4-H), 6.43 (br, 1H, NH), 7.2−7.7 (m, 2H, pyridyl 4′, 5′-H), 7.3−7.4 (m, 5H, 6-C_6H_5), 8.33 (d, 1H, pyridyl 6′-H), 8.64 (s, 1H, pyridyl 2′-H).

3,5-Diethyl 2-Methyl-4-(4-pyridyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (14). ^{1}H NMR ($CDCl_3$): δ 0.83 (t, 3H, J = 6.84 Hz, 5-CH_2CH_3), 1.24 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.4 (s, 3H, 2-CH_3), 3.84 (m, 2H, 5-OCH_2), 4.12 (q, 2H, J = 7.81 Hz, 3-OCH_2), 5.12 (s, 1H, 4-H), 6.35 (br, 1H, NH), 7.3−7.4 (m, 7H, 6-C_6H_5, pyridyl 3′-, 5′-H), 8.42 (d, 2H, J = 5.86 Hz, pyridyl 2′-, 6′-H).

3,5-Diethyl 2-Methyl-4-(2-benzofuryl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (17). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, J = 6.84 Hz, 5-CH_2CH_3), 1.31 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.39 (s, 3H, 2-CH_3), 3.92 (m, 2H, 5-OCH_2), 4.21 (m, 2H, 3-OCH_2), 5.48 (s, 1H, 4-H), 5.96 (br, 1H, NH), 6.48 (s, 1H, benzofuryl 3′-H), 7.1−7.5 (m, 9H, 6-C_6H_5, benzofuryl 4′-, 5′-, 6′-, 7′-H).

3,5-Diethyl 2-Methyl-4-[2-(4-nitrophenyl)ethynyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate (22a). ^{1}H NMR ($CHCl_3$-d): δ 1.25−1.34 (m, 6H, 3-, 5-CH_3), 2.49 (s, 3H, 2-CH_3), 3.47−3.59 (m, 2H, 3-CH_2), 4.23 (q, 2H, 5-CH_2, J = 7.8 Hz), 5.42 (s, 1H, H-4), 6.29 (d, 1H, H-1, J = 2.9 Hz), 7.55 (d, 2H, H-2′, H-6′, J = 8.8 Hz), 8.21 (d, 2H, H-3′, H-5′, J = 8.8 Hz), 8.26 (wide, 1H, H-6).

3,5-Diethyl 2-Methyl-4-[(3-methylphenyl)ethynyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (22b). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, J = 6.84 Hz, 5-CH_2CH_3), 1.35 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.29 (s, 3H, 3′-CH_3), 2.36 (s, 3H, 2-CH_3), 4.0 (m, 2H, 5-OCH_2), 4.3 (m, 2H, 3-OCH_2), 5.11 (s, 1H, 4-H), 5.92 (br, 1H, NH), 7.0−7.43 (m, 9H, 4-C_6H_4, 6-C_6H_5).

3-Ethyl 5-Benzyl 2-Ethyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (23). ^{1}H NMR ($CDCl_3$): δ 1.22 (t, J = 6.9 Hz, 3H, 2-CH_2CH_3), 1.34 (t, J = 6.9 Hz, 3H, 3-CH_3 CH_2), 2.55, 3.01 (2m, 2H, 2-CH_2CH_3), 4.25 (m, 2H, 3-OCH_2), 5.06 (AB, J = 12.7 Hz, 2H, 5-OCH_2), 5.18 (s, 1H, 4-H), 5.95 (br, 1H, NH), 7.05−7.39 (m, 15H, 3 × C_6H_5). MS (EI): m/z 491 $(M)^+$, 462 $(M - C_2H_5)^+$, 418 $(M - CO_2C_2H_5)^+$, 356 $(M - CO_2CH_2C_6H_5)^+$, base.

General Procedure for the Preparation of Compounds 24a−j, 27, and 28. Compound **42** (0.2 mmol) was dissolved in dry acetone (10 mL), anhydrous potassium carbonate (0.5 g) and the appropriate phenylethyl bromide or phenylpropyl bromide (5 equiv) were added, and the mixture was refluxed for 2−6 h. After the mixture was filtered, the solvent was evaporated in vacuo. The residue was separated by preparative TLC (40:1 $CHCl_3$:MeOH), and the desired N-protected 5-benzyl ester DHP was isolated. Deprotection was achieved by dissolving the DHP in acetone (1 mL), adding excess 1 N HCl (200 μL), and heating at 45 °C for 2−3 h. An equivolume amount of water was added to the solution followed by extraction with CH_2Cl_2 (2 mL × 2). The extracts were combined, washed with water and brine, and dried over sodium sulfate. The solvent was removed in vacuo, and the residue was purified by preparative TLC (2:1 hexanes:EtOAc) to afford the desired 5-benzyl ester dihydropyridine.

3-Ethyl 5-(2-Methylbenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24a). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.16 (s, 3H, 2′-CH_3), 2.37 (s, 3H, 2-CH_3), 4.26 (m, 2H, 3-OCH_2), 5.04 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.17 (s, 1H, 4-H), 5.92 (br, 1H, NH), 7.06−7.36 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-(3-Methylbenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24b). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.25 (s, 3H, 2′-CH_3), 2.36 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 4.99 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.18 (s, 1H, 4-H), 5.91 (br, 1H, NH), 6.88−7.39 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-(4-Methylbenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24c). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.3 (s, 3H, 2′-CH_3), 2.37 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 4.99 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.17 (s, 1H, 4-H), 5.86 (br, 1H, NH), 7.0−7.4 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-[4-(Trifluoromethyl)benzyl] 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24d). ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.15 (AB, 2H, J = 13.7 Hz, 5-OCH_2), 5.18 (s, 1H, 4-H), 5.94 (br, 1H, NH), 7.13−7.45 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-(3-Iodobenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24e). ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 4.93 (AB, 2H, J = 13.7 Hz, 5-OCH_2), 5.16 (s, 1H, 4-H), 5.92 (br, 1H, NH), 6.9−7.4 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-(3-Nitrobenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24f). ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.15 (AB, 2H, J = 13.7 Hz, 5-OCH_2), 5.17 (s, 1H, 4-H), 5.94 (br, 1H, NH), 7.3−8.1 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-(4-Nitrobenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24g). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.12 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.20 (s, 1H, 4-H), 5.94 (br, 1H, NH), 7.1−8.0 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-[4-[(2,2,2-Trichloroethoxy)carbonyl]benzyl] 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24h). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.12 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.20 (s, 1H, 4-H), 5.94 (br, 1H, NH), 7.1−8.0 (m, 14H, 4-C_6H_5, 5-C_6H_4, 6-C_6H_5).

3-Ethyl 5-[3,5-Bis(trifluoromethyl)benzyl] 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24i). ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, J = 6.83 Hz, 3-CH_2CH_3), 2.39 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.08 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.15 (s, 1H, 4-H), 5.94 (br, 1H, NH), 7.34 (s, 10H, 4-C_6H_5, 6-C_6H_5), 7.52 (s, 2H, 5-Ar), 7.75 (s, 1H, 5-Ar).

3-Ethyl 5-(3,5-Dinitrobenzyl) 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24j). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.39 (s, 3H, 2-CH_3), 4.3 (m, 2H, 3-OCH_2), 5.17 (AB, 2H, J = 13.7 Hz, 5-OCH_2), 5.16 (s, 1H, 4-H), 5.97 (br, 1H, NH), 7.37 (s, 10H, 4-C_6H_5, 6-C_6H_5), 8.24 (s, 2H, 5-Ar), 8.9 (s, 1H, 5-Ar).

3-Ethyl 5-[4-[[(2-Aminoethyl)amino]carbonyl]benzyl] 4-(Phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (25). Compound **24h** (16.2 mg, 0.03 mmol) was mixed with ethylenediamine (12.4 mg, 0.21 mmol). After 30 min at room temperature, the mixture was quenched with H_2O (1 mL) and extracted with CH_2Cl_2 (3 mL × 3). The product was purified with preparative TLC (5:1 $CHCl_3$:MeOH) to yield 7.0 mg of a white solid (47.9%). ^{1}H NMR (CD_3OD): δ 1.39 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 2.40 (s, 3H, 2-CH_3), 2.91 (br, 2H, -CH_2NH_2), 3.51 (t, 2H, J = 5.86 Hz, -$NHCH_2$), 4.3 (m, 2H, 3-OCH_2), 5.12 (AB, 2H, J = 12.7, Hz, 5-OCH_2), 5.14 (s, 1H, 4-H), 7.14 (d, 2H, J = 7.81 Hz, 5-Ar), 7.3−7.4 (m, 10H, 4-C_6H_5, 6-C_6H_5), 7.74 (d, 2H, J = 7.81 Hz, 5-Ar), 7.96 (s, 1H, NH).

3-Propyl 5-Benzyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (26). ^{1}H NMR ($CDCl_3$): δ 1.01 (t, J = 6.9 Hz, 3H, 3-CH_2CH_3), 1.73 (m, 2H, 3-CH_3 CH_2), 2.37 (s, 3H, 2-CH_3), 4.15 (m, 2H, 3-OCH_2), 5.06 (AB, J = 12.7 Hz, 2H, 5-OCH_2), 5.20 (s, 1H, 4-H), 5.88 (br, 1H, NH), 7.07−7.37 (m, 15H, 3 × C_6H_5). MS (EI): m/z 491

$(M)^+$, 448 $(M - C_3H_7)^+$, 404 $(M - CO_2C_3H_7)^+$, 356 $(M - CO_2CH_2C_6H_5)^+$, base.

3-Ethyl 5-Phenylethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (27). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 2.69 (t, J = 6.8 Hz, 2H, 5-$CH_2C_6H_5$), 4.15 (m, 2H, 3-OCH_2), 4.30 (m, 2H, 5-OCH_2), 5.11 (s, 1H, 4-H), 5.87 (br, 1H, NH); 7.10−7.41 (m, 15H, 3 × C_6H_5). MS (EI): m/z 491 $(M)^+$, 462 $(M - C_2H_5)^+$, 418 $(M - CO_2C_2H_5)^+$, 342 $(M - CO_2(CH_2)_2C_6H_5)^+$.

3-Ethyl 5-Phenylpropyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (28). ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 1.65 (m, 2H, 5-$CH_2CH_2CH_2$), 2.35 (s, 3H, 2-CH_3), 2.40 (m, 2H, 5-$CH_2C_6H_5$), 3.98 (m, 2H, 3-OCH_2), 4.26 (m, 2H, 5-OCH_2); 5.14 (s, 1H, 4-H), 5.85 (br, 1H, NH), 7.20−7.41 (m, 15H, 3 × C_6H_5). MS (EI): m/z 505 $(M)^+$, 476 $(M - C_2H_5)^+$, 432 $(M - CO_2C_2H_5)^+$, 342 $(M - CO_2(CH_2)_3C_6H_5)^+$.

3-Ethyl 5-*tert*-Butyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (29). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 3H, 3-CH_2CH_3), 1.31 (s, 9H, $C(CH_3)_3$), 2.31 (s, 3H, 2-CH_3), 4.22 (m, 2H, 3-OCH_2); 4.94 (s, 1H, 4-H), 5.56 (br, 1H, NH), 7.21−7.34 (m, 10H, 2 × C_6H_5). MS (EI): m/z 505 $(M)^+$, 476 $(M - C_2H_5)^+$, 432 $(M - CO_2C_2H_5)^+$, 342 $(M - CO_2(CH_2)_3C_6H_5)^+$.

3-(2-Methoxy-2-phenylethyl) 5-Ethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (30). ^{1}H NMR ($CDCl_3$): δ 0.91 and 1.00 (2t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 4.00 (m, 2H, 5-OCH_2), 4.35 (m, 1H, 3-CH), 4.50 (m, 2H, 3-OCH_2), 5.05 (s, 1H, 4-H), 5.82 (br, 1H, NH), 7.21−7.41 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 539 $(M = NH_4)^+$.

3-Ethyl 5-[2-(Trimethylsilyl)ethyl] 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (31). ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 9H, $Si(CH_3)_3$), 0.79 (t, J = 8.8 Hz, 2H, CH_2Si), 1.41 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.40 (s, 3H, 2-CH_3), 4.10 (t, J = 8.8 Hz, 2H, 5-OCH_2), 4.31 (m, 2H, 3-OCH_2), 5.15 (s, 1H, 4-H), 5.87 (br, 1H, NH), 7.28−7.46 (m, 10H, 2 × C_6H_5).

3-Ethyl 5-Thioethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (32a). Triethylamine (20 mg) was added to a mixture of compound **42** (90 mg, 0.2 mmol), diphenyl phosphorazate (56 mg, 0.2 mmol), and ethanethiol (20 mg, 0.3 mmol) in DMF (1 mL) with stirring while cooled in an ice−water bath. The bath was removed, and the mixture was stirred at room temperature for 3 h, then diluted with dichloromethane (20 mL), washed with water (10 mL × 2), and dried with sodium sulfate. The solvent was evaporated, and the residue was carried out for deprotection with 1 N HCl to give 15 mg of product. ^{1}H NMR ($CDCl_3$): δ 1.15 (t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 1.28 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 2.82 (m, 2H, 5-SCH_2), 4.31 (m, 2H, 3-OCH_2), 5.23 (s, 1H, 4-H), 5.95 (br, 1H, NH), 7.24−7.46 (m, 10H, 2 × C_6H_5).

3-Thioethyl 5-Ethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (32b). The 3-ketopropionate ester for this reaction was synthesized by adding 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (4.6 mmol) dropwise to a solution of ethanethiol (4.6 mmol) in 15 mL of toluene at 100 °C. Heating at 100 °C was continued for 5 h. The solvent was evaporated and the residue chromatographed by preparative TLC (3:1 hexanes:EtOAc) to yield 49.1 mg of a yellowish oil (7.3%). **32b** was then synthesized via the Hantszch condensation method described in the general procedure. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, J = 6.84 Hz, 3-CH_2CH_3), 1.32 (t, 3H, J = 6.84 Hz, 5-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.0 (m, 2H, 3-SCH_2), 4.02 (q, 2H, J = 6.83 Hz, 5-OCH_2), 5.27 (s, 1H, 4-H), 6.06 (br, 1H, NH), 7.25−7.44 (m, 10H, 4-C_6H_5, 6-C_6H_5).

3-(Ethoxycarbonyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-5-carboxylic Acid (33). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.29 (m, 2H, 3-OCH_2), 5.08 (s, 1H, 4-H), 5.95 (br, 1H, NH), 7.24−7.44 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 405 $(M + NH_4)$, 388 $(MH)^+$.

3-(Ethoxycarbonyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-5-carboxylic Acid Ethylamide (34). A mixture of compound **42** (75 mg, 0.17 mmol), *N*-hydroxysuccinimide (22 mg, 0.17 mmol), and EDAC (34 mg, 0.17 mmol) in DMF (1 mL) was stirred at room temperature for 4 h. Ethylamine (2.0 M in THF, 0.4 mL) was added, and the reaction mixture was stirred overnight. The solvent was removed, and the residue was diluted with dichloromethane (10 mL), washed with water (5 mL × 2) and brine (5 mL × 2), and dried with sodium sulfate. The solvent was evaporated, and the residue was purified with a preparative TLC plate to give 38 mg of compound **43**, which was carried out for deprotection with 1 N HCl to give 18 mg of product **34**.

43. ^{1}H NMR ($CDCl_3$): δ 0.72 (t, J = 6.8 Hz, 3H, 1-CH_2CH_3), 0.96 (t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 1.33 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.60 (s, 3H, 2-CH_3), 3.08 (m, 2H, 5-NCH_2), 3.62 (m, 2H, 1-OCH_2), 4.23 (m, 2H, 3-OCH_2), 4.45, 4.85 (AB, J = 11.7 Hz, N-CH_2-O), 4.92 (s, 1H, 4-H), 4.97 (br, 1H, CONH), 7.22−7.44 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 473 $(MH)^+$.

34. ^{1}H NMR ($CDCl_3$): δ 0.85 (t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 1.31 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.16 (m, 2H, 5-NCH_2), 4.25 (m, 2H, 3-OCH_2), 5.09 (s, 1H, 4-H), 5.37 (br, 1H, CONH), 5.64 (br, 1H, NH), 7.25−7.44 (m, 10H, 2 × C_6H_5).

3-*tert*-Butyl 5-Ethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (36). ^{1}H NMR ($CDCl_3$): δ 0.91 (t, J = 6.9 Hz, 3H, 5-CH_2CH_3), 1.52 (s, 9H, $C(CH_3)_3$), 2.29 (s, 3H, 2-CH_3), 3.98 (m, 2H, 5-OCH_2), 5.05 (s, 1H, 4-H), 5.71 (br, 1H, NH), 7.21−7.40 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 461 $(M + NH_4)^+$, 443 $(MH)^+$.

5-(Ethoxycarbonyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3-carboxylic Acid Ethylamide (37). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, J = 6.8 Hz, 3H, 3-CH_2CH_3), 1.18 (t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 2.24 (s, 3H, 2-CH_3), 3.38 (m, 2H, 3-NCH_2), 3.94 (m, 2H, 5-OCH_2), 4.80 (s, 1H, 4-H), 5.65 (br, 1H, NH), 6.32 (br, 1H, CONH), 7.23−7.38 (m, 10H, 2 × C_6H_5). MS (EI): m/z 414 $(M)^+$, 385 $(M - C_2H_5)^+$, base, 342 $(M - CONHC_2H_5)^+$.

3-(Ethylcarbonyl)-5-(ethoxycarbonyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine (38). ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.0 Hz, 3H, 5-CH_2CH_3), 2.33 (s, 3H, 2-CH_3), 2.48 (s, 3H, 3-$COCH_3$), 4.00 (q, 2H, J = 7.0 Hz, 5-OCH_2), 5.04 (s, 1H, 4-H), 5.88 (br, 1H, NH), 7.26−7.43 (m, 10H, 2 × C_6H_5). MS (EI): m/z 385 $(M)^+$, 443 $(M - CH_3CO)^+$, base.

Ethyl 5-Oxo-4-(phenylethynyl)-2-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (39). ^{1}H NMR ($CDCl_3$): δ 1.02 (t, J = 6.8 Hz, 3H, 5-CH_2CH_3), 2.04 (t, J = 4.9 Hz, 2H, 7-CH_2), 2.46 (m, 4H, 8-, 9-CH_2), 4.00 (m, 2H, 5-OCH_2), 5.13 (s, 1H, 4-H), 6.14 (br, 1H, NH), 7.20−7.41 (m, 10H, 2 × C_6H_5). MS (EI): m/z 397 $(M)^+$, 368 $(M - C_2H_5)^+$, base.

General Procedure of N-H Group Protection of Compound 31. Sodium hydride (60% in mineral oil, 1.5 equiv) was added to compound **31** in a solution of DMF (1.5 mL). The mixture was stirred for 5 min, chloromethyl methyl (or chloromethyl ethyl) ether (1.5 equiv) was added slowly to the solution under argon at room temperature and stirred for 2 h. The reaction was quenched by adding cold water (10 mL), the mixture was extracted with ethyl acetate (10 mL × 2), and the organic layer was washed with water (10 mL × 2) and brine (10 mL × 2) and dried with sodium sulfate. The solvent was evaporated, and the residue was purified with preparative TLC plates to give corresponding N-protected products **40** and **41**.

3-Ethyl 5-[2-(Trimethylsilyl)ethyl] 1-(Methoxymethyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (40). ^{1}H NMR ($CDCl_3$): δ −0.04 (s, 9H, $Si(CH_3)_3$), 0.63 (m, 2H, CH_2Si), 1.33 (t, J = 7.0 Hz, 3H, 3-CH_2CH_3), 2.54 (s, 3H, 2-CH_3), 3.18 (s, 3H, OCH_3), 3.97 (t, J = 7.8 Hz, 2H, 5-OCH_2), 4.27 (m, 2H, 3-OCH_2), 4.37, 4.85 (AB, J = 11.7 Hz, N-CH_2-O), 5.07 (s, 1H, 4-H), 7.21−7.40 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 549 $(M + NH_4)^+$, 532 $(MH)^+$.

3-Ethyl 5-[2-(Trimethylsilyl)ethyl] 1-(Ethoxymethyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (41). ^{1}H NMR ($CDCl_3$): δ −0.08 (s, 9H, $Si(CH_3)_3$), 0.61 (m, 2H, CH_2Si), 0.91 (t, J = 6.9 Hz, 3H,

OCH_2CH_3), 1.41 (t, J = 6.9 Hz, 3H, 3-CH_2CH_3), 2.57 (s, 3H, 2-CH_3), 3.13 (m, 2H, OCH_2CH_3), 3.95 (t, J = 7.9 Hz, 2H, 5-OCH_2), 4.12 (m, 2H, 3-OCH_2), 4.41, 4.81 (AB, J = 10.8 Hz, 2H, N-CH_2-O), 5.02 (s, 1H, 4-H), 7.20−7.39 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 563 $(M + NH_4)^+$, 546 $(MH)^+$.

1-(Ethoxymethyl)-3-(ethoxycarbonyl)-2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-5-carboxylic Acid (42). TBAF (hydrate, 208 mg, 0.8 mmol) was added to a solution of **41** (115 mg, 0.21 mmol) in DMF (1 mL). The mixture was stirred under argon at room temperature for 2 h, diluted with ethyl acetate (20 mL), washed with 1 N HCl (5 mL), H_2O (20 mL × 2), and brine (20 mL × 2), and dried with magnesium sulfate. The solvent was evaporated, and the residue was separated with preparative TLC plates to give 80 mg of product. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 6.8 Hz, 3H, OCH_2CH_3), 1.31 (t, J = 6.9 Hz, 3H, 3-CH_2CH_3), 2.59 (s, 3H, 2-CH_3), 3.09, 3.65 (2m, 2H, OCH_2CH_3), 4.27 (m, J = 7.9 Hz, 2H, 3-OCH_2), 4.39, 4.85 (2d, J = 10.7 Hz, 2H, 3-OCH_2), 4.41, 4.81 (AB, J = 10.8 Hz, 2H, N-CH_2-O), 5.02 (s, 1H, 4-H), 7.12 (br, 1H, COOH), 7.20−7.39 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 463 $(M + NH_4)^+$, 446 $(MH)^+$.

1-(4-Nitrophenyl)-2-propyn-1-al (55b). 4-Nitroiodobenzene (1.25 g, 5 mmol) was added to 20 mL of diethylamine and stirred vigorously. To the mixture were added 0.1 mmol (5%) of copper(I) iodide (20 mg) and 0.1 mmol (5%) of $Pd^{II}Cl_2(PPh_3)_2$ (70 mg). 3,3-Diethoxy-1-propyne (**53**; 790 μL, 5.5 mmol) was then slowly added to the reaction mixture. The reaction was monitored by TLC (silica gel, petroleum ether/EtOAc = 95/5) every 5 min until virtually all starting material had reacted (~25 min). The reaction was then quenched by the addition of 50 mL of cold water. The product was extracted twice with 50 mL of toluene and washed once with 50 mL of water. The combined organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated in vacuo. The acetal intermediate **54** was purified by flash column chromatography (silica gel 60, petroleum ether/EtOAc = 95/5), and 0.89 g (72% yield) of a yellowish oil was obtained.

The intermediate diethyl acetal was then dissolved in hexane (mixed isomers), and 2.2 mol equiv of formic acid was added to the solution. The product precipitated from the solution, was recovered by decantation, and was purified by crystallization from chloroform; 0.30 g (57% yield) of pure red-brown crystals (needles) of **55b** was thus obtained.

1-(3-Toluyl)-2-propyn-1-al (55c). **55c** was prepared in a similar fashion as described for compound **55b** from 3-iodotoluene (1.09 g, 5 mmol) and 3,3-diethoxy-1-propyne (790 μL, 5.5 mmol) in 15 mL of anhydrous triethylamine. Deprotection of the aldehyde required heating to 40 °C.

2,2,2-Trichloroethyl *p*-Methylbenzoate (57). *p*-Toluyl chloride (**56**) (Aldrich; 1.07 g, 6.92 mmol) and 2,2,2-trichloroethanol (2 mL) were dissolved in 3 mL of chloroform. Triethylamine (1 mL, 7.19 mmol) was added dropwise with stirring at room temperature. The reaction mixture was washed with water (2×), saturated sodium bicarbonate, 0.1 N HCl, and water. The organic layer was separated and dried leaving 1.45 g of a clear oil (100% yield). ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 3H, -CH_3), 5.0 (s, 2H, -CH_2CCl_3), 7.3 (d, 2H, J = 7.82 Hz, Ar), 8.1 (d, 2H, J = 7.81 Hz, Ar).

2,2,2-Trichloroethyl 4-(Bromomethyl)benzoate (58). A solution of **57** (0.96 g, 3.6 mmol), *N*-bromosuccinimide (0.71 g, 4.0 mmol), and a catalytic amount of benzoyl peroxide dissolved in 4 mL of benzene was refluxed for 1 h. After cooling, the succinimide was filtered off and the filtrate chromatographed with preparative TLC (95% petroleum ether, 5% ethyl acetate) to yield 0.98 g of a yellow oil (76.8%). ^{1}H NMR ($CDCl_3$): δ 4.52 (s, 2H, -CH_2Br), 4.98 (s, 2H, -CH_2CCl_3), 7.52 (d, 2H, J = 7.82 Hz, Ar), 8.12 (d, 2H, J = 7.81 Hz, Ar).

Pharmacology: Radioligand Binding Studies. Binding of [^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine ([^{3}H]-(*R*)-PIA) to A_1 receptors from rat cerebral cortex membranes and of [^{3}H]-2-[[[4-(2-carboxyethyl)phenyl]ethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680) to A_{2A} receptors from rat striatal membranes was performed as described previously.[30,31] Adenosine deaminase (3 units/mL) was present during the preparation of the brain membranes, in a preincubation of 30 min at 30 °C, and during the incubation with the radioligands.

Binding of [^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-*N*-methylcarbamoyl)adenosine ([^{125}I]AB-MECA) to membranes prepared from HEK-293 cells stably expressing the human A_3 receptor,[3] clone HS-21a (Receptor Biology, Inc., Baltimore, MD), or to membranes prepared from CHO cells stably expressing the rat A_3 receptor was performed as described.[13,32] The assay medium consisted of a buffer containing 10 mM Mg^{2+}, 50 mM Tris, and 1 mM EDTA, at pH 8.0. The glass incubation tubes contained 100 μL of the membrane suspension (0.3 mg of protein/mL, stored at −80 °C in the same buffer), 50 μL of [^{125}I]-AB-MECA (final concentration 0.3 nM), and 50 μL of a solution of the proposed antagonist. Nonspecific binding was determined in the presence of 100 μM N^6-(phenylisopropyl)adenosine ((*R*)-PIA).

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[41] and K_d values of 1.0M 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, respectively.

Abbreviations: [^{125}I]AB-MECA, [^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine; CGS 21680, 2-[[[4-(2-carboxyethyl)phenyl]ethyl]amino]-5′-(*N*-ethylcarbamoyl)-adenosine; CHO cells, Chinese hamster ovary cells; DMAP, (*N*,*N*-dimethylamino)pyridine; DMSO, dimethyl sulfoxide; DPPA, diphenyl phosphorazidate; EDAC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; HEK cells, human embryonic kidney cells; IB-MECA, N^6-(3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine; 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, 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-(phenylethynyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate; (*R*)-PIA, (*R*)-N^6-(phenylisopropyl)adenosine; SAR, structure−activity relationship; TBAF, tetrabutylammonium fluoride; TNF, tumor necrosis factor; Tris, tris-(hydroxymethyl)aminomethane.

Acknowledgment. We thank Gilead Sciences (Foster City, CA) and the Cystic Fibrosis Foundation (Silver Spring, MD) for financial support. We thank Dr. Gary L. Stiles and Dr. Mark E. Olah (Duke University, Durham, NC) for providing samples of [^{125}I]AB-MECA and Reuven Muller for technical assistance.

References

(1) Jacobson, K. A.; Suzuki, F. Recent developments in selective agonists and antagonists acting at purine and pyrimidine receptors. *Drug Dev. Res.* **1997**, *39*, 289−300.
(2) Zhou, Q. Y.; Li, C. Y.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. Molecular cloning and characterization of an adenosine receptor - The A_3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7432−7436.
(3) Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. Molecular cloning and characterization of the human A_3 adenosine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10365−10369.
(4) Jacobson, K. A.; Kim, H. O.; Siddiqi, S. M.; Olah, M. E.; Stiles, G.; von Lubitz, D. K. J. E. A_3 adenosine receptors: design of selective ligands and therapeutic prospects. *Drugs Future* **1995**, *20*, 689−699.
(5) Ramkumar, V.; Stiles, G. L.; Beaven, M. A.; Ali, H. The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J. Biol. Chem.* **1993**, *268*, 16887−16890.

(6) Ali, H.; Choi, O. H.; Fraundorfer, P. F.; Yamada, K.; Gonzaga, H. M. S.; Beaven, M. A. Sustained activation of phospholipase-D via adenosine A_3 receptors is associated with enhancement of antigen-ionophore-induced and Ca^{2+}- ionophore-induced secretion in a rat mast-cell line. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 837−845.
(7) Kohno, Y.; Sei, Y.; Koshiba, M.; Kim, H. O.; Jacobson, K. A. Induction of apoptosis in HL-60 human promyelocytic leukemia cells by selective adenosine A_3 receptor agonists. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 904−910.
(8) Kohno, Y.; Ji, X.-d.; Mawhorter, S. D.; Bochner, B.; Sei, Y.; Koshiba, M.; Jacobson, K. A. Activation of adenosine A_3 receptor on human eosinophils raises intracellular Ca^{2+} and induces apoptosis. *Drug Dev. Res.* **1996**, *37*, 182.
(9) Strickler, J.; Jacobson, K. A.; Liang, B. T. Direct preconditioning of cultured chick ventricular myocytes: novel functions of cardiac adenosine A_{2A} and A_3 receptors. *J. Clin. Invest.* **1996**, *98*, 1773−1779.
(10) Shneyvais, V.; Jacobson, K. A.; Shainberg, A. Induction of apoptosis in cultured cardiac myocytes by adenosine A_3 receptor agonist. *J. Basic Clin. Physiol. Pharmacol.* **1997**, in press.
(11) 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.
(12) von Lubitz, D. K. J. E.; Lin, R. C. S.; Sei, Y.; Boyd, M.; Abbracchio, M.; Bischofberger, N.; Jacobson, K. A. A_3 adenosine receptors and ischemic brain injury: a hope or a disaster? *Drug Dev. Res.* **1996**, *37*, 140.
(13) Abbracchio, M. P.; Malorni, W.; Ceruti, S.; Brambilla, R.; Barbieri, D.; Franceschi, C.; Giammarioli, A. M.; Rainaldi, G.; von Lubitz, D. K. J. E.; Jacobson, K. A.; Cattabeni, F. *Eur. J. Neurosci.* **1997**, submitted for publication.
(14) MacKenzie, W. M.; Hoskin, D. W.; Blay, J. Adenosine inhibits the adhesion of anti-CD3-activated killer lymphocytes to adenocarcinoma cells through an A_3 receptor. *Cancer Res.* **1994**, *54*, 3521−3526.
(15) Sajjadi, F. G.; Takabayashi, K.; Foster, A. C.; Domingo, R. C.; Firestein, G. S. Inhibition of TNF-alpha expression by adenosine - role of A_3 adenosine receptors. *J. Immunol.* **1996**, *156*, 3435−3442.
(16) 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.
(17) 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.
(18) Jiang, J.-l.; van Rhee, A. M.; Melman, N.; Ji, X.-d.; Jacobson, K. A. 6-Phenyl-1,4-dihydropyridine derivatives as potent and selective A_3 adenosine receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4667−4675.
(19) Jacobson, M. A.; Chakravarty, P. K.; Johnson, R. G.; Norton, R. Novel selective non-xanthine selective A_3 adenosine receptor antagonists. *Drug Dev. Res.* **1996**, *37*, 131.
(20) Kim, Y.-C.; Ji, X.-d.; Jacobson, K. A. Derivatives of the triazoloquinazoline adenosine antagonist (CGS15943) are selective for the human A_3 receptor subtype. *J. Med. Chem.* **1996**, *39*, 4142−4148.
(21) Beaven, M. A.; Ramkumar, V.; Ali, H. Adenosine-A_3 receptors in mast-cells. *Trends Pharmacol. Sci.* **1994**, *15*, 13−14.
(22) Stout, D. M.; Myers, A. I. Recent advances in the chemistry of dihydropyridines. *Chem. Rev.* **1982**, *82*, 223−243.
(23) Goldmann, S.; Stoltefuss, J. 1,4-Dihydropyridines: Effect of chirality and conformation on the calcium antagonist and calcium agonist activities. *Angew. Chem., Int. Ed. Engl.* **1991**, 1559−1578.
(24) Kajino, M.; Wada, Y.; Nagai, Y.; Nagaoka, A.; Meguro, K. Synthesis and biological activities of optical isomers of 2-(4-diphenylmethyl-1-piperazinyl)ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (manidipine) dihydrochloride. *Chem. Pharm. Bull.* (Tokyo) **1989**, *37*, 2225−2228.
(25) 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.
(26) Sonogashira, K.; Tohda, Y.; Hagihara, N. A convenient synthesis of acetylenes: catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, *50*, 4467−4470.
(27) Rathke, M. W.; Deitch, J. The reaction of lithium ester enolates with acid chlorides. A convenient procedure for the preparation of β-keto esters. *Tetrahedron Lett.* **1971**, *31*, 2953−2956.
(28) Meyer, H.; Bossert, F.; Wehinger, E.; Stoepel, K.; Vater, W. Synthesis and comparative pharmacological studies of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylates with non-identical ester functions. *Arzneim.-Forsch.* **1981**, *31*, 407−409.
(29) Ji, X. D.; von Lubitz, D. K. J. E.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Species differences in ligand affinity at central A_3-adenosine receptors. *Drug Dev. Res.* **1994**, *33*, 51−59.
(30) 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.
(31) 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.
(32) 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.
(33) Dunwiddie, T. V.; Diao, L.; Kim, H. O.; Jiang, J.-l.; Jacobson, K. A. Activation of hippocampal adenosine A_3 receptors produces a heterologous desensitization of A_1 receptor mediated responses in rat hippocampus. *J. Neurosci.* **1997**, *17*, 607−614.
(34) Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(35) Jacobson, K. A. Molecular probes for adenosine receptors. In *Purines in Cellular Signalling: Targets for New Drugs*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer: New York, **1990**; pp 54−64.

JM970091J