6-Phenyl-1,4-dihydropyridine Derivatives as Potent and Selective A_3 Adenosine Receptor Antagonists

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An approach to designing dihydropyridines that bind to adenosine receptors without binding to L-type calcium channels has been described. 1,4-Dihydropyridine derivatives substituted with β-styryl or phenylethynyl groups at the 4-position and aryl groups at the 6-position were synthesized and found to be selective for human A_3 receptors. Combinations of methyl, ethyl, and benzyl esters were included at the 3- and 5-positions. Affinity was determined in radioligand binding assays at rat brain A_1 and A_{2A} receptors using [^{3}H]-(*R*)-PIA [[^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine] and [^{3}H]CGS 21680 [[^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine], respectively. Affinity was determined at cloned human and rat A_3 receptors using [^{125}I]AB-MECA [N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)-adenosine]. Structure−activity analysis indicated that substitution of the phenyl ring of the β-styryl group but not of the 6-phenyl substituent was tolerated in A_3 receptor selective agents. Replacement of the 6-phenyl ring with a 3-thienyl or 3-furyl group reduced the affinity at A_3 receptors by 4- and 9-fold, respectively. A 5-benzyl ester 4-*trans*-β-styryl derivative, **26**, with a K_i value of 58.3 nM at A_3 receptors, was >1700-fold selective vs either A_1 receptors or A_{2A} receptors. Shifting the benzyl ester to the 3-position lowered the affinity at A_3 receptors 3-fold. A 5-benzyl, 3-ethyl ester 4-phenylethynyl derivative, **28**, displayed a K_i value of 31.4 nM at A_3 receptors and 1300-fold selectivity vs A_1 receptors. The isomeric 3-benzyl, 5-ethyl diester was >600-fold selective for A_3 receptors. Oxidation of **28** to the corresponding pyridine derivative reduced affinity at A_3 receptors by 88-fold and slightly increased affinity at A_1 receptors.

The physiological role of A_3 adenosine receptors,[1−4] the most recently cloned receptors of the adenosine,[5,6] is under investigation, and potent and selective antagonists are needed as pharmacological probes and as potential therapeutic agents. Activation of the A_3 receptor in the rat results in hypotension[7] by promotion of release of inflammatory mediators from mast cells.[8] IB-MECA, a selective A_3 receptor agonist,[4] was shown to inhibit the release of TNF-α in macrophages.[9] A_3 adenosine receptor antagonists are being sought as potential antiasthmatic,[10] antiinflammatory,[10] and cerebroprotective agents.[11] Selective A_3 receptor agonists at high concentrations were found to induce apoptosis in a HL-60 human leukemia cell line.[12] There may also be an involvement of A_3 receptors in the cytolytic activity of antitumor killer lymphocytes.[13]

1,4-Dihydropyridine blockers of L-type calcium channels[14] are used extensively in the treatment of cardiovascular disorders as dilators of coronary arteries. We have found that, in addition to binding to calcium channels, this class of 1,4-dihydropyridines tends to bind to three subtypes of adenosine receptors, *i.e.* A_1, A_{2A}, and A_3.[15,16] For example, nifedipine (Figure 1, **1**) is bound by these three adenosine receptors with micromolar affinity, although it is much more potent at L-type channels. A newer generation calcium channel blocker, nicardipine,[17] **2**, within the family of adenosine receptors is actually selective for the A_3 subtype. The (*S*)-enantiomer of niguldipine, **3**, which is the more potent enantiomer at L-type channels, binds to human A_3 adenosine receptors with a K_i value of 2.8 μM and is totally inactive at A_1 and A_{2A} receptors. Thus, with respect to adenosine receptors alone it is highly specific for the A_3 subtype.

We have recently described approaches to designing 1,4-dihydropyridines that bind to adenosine receptors without binding to L-type calcium channels.[16] Furthermore, the moderate affinity for A_3 adenosine receptors of readily available 1,4-dihydropyridines has provided leads for novel antagonists for that subtype. Inclusion of 4-*trans*-β-styryl and 6-phenyl substituents enhanced A_3 receptor selectivity in an additive fashion and completely abolished recognition at L-type channels. Compound **4** (3,5-diethyl 2-methyl-6-phenyl-4-[2-phenyl-(*E*)-vinyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate, MRS 1097) was 55-fold selective for human A_3 receptors vs rat A_1 receptors and 44-fold selective vs rat A_{2A} receptors. In a functional assay, compound **4** attenuated the A_3 agonist-elicited inhibitory effect on adenylyl cyclase. Furthermore, whereas nicardipine, **2**, displaced radioligand ([^{3}H]-*S*-(4-nitrobenzyl)-6-thioinosine) from the Na^+-independent adenosine transporter with an apparent affinity of 5.4 μM, compound **4** displaced less than 10% of total binding at a concentration of 100 μM. The selectivity of compound **4** for adenosine receptors was further demonstrated through its inactivity in binding assays at other receptors, ion channels, and second messenger components. The present study examines combinations and compatibility of dihydropyridine substituents found previously to enhance affinity at A_3

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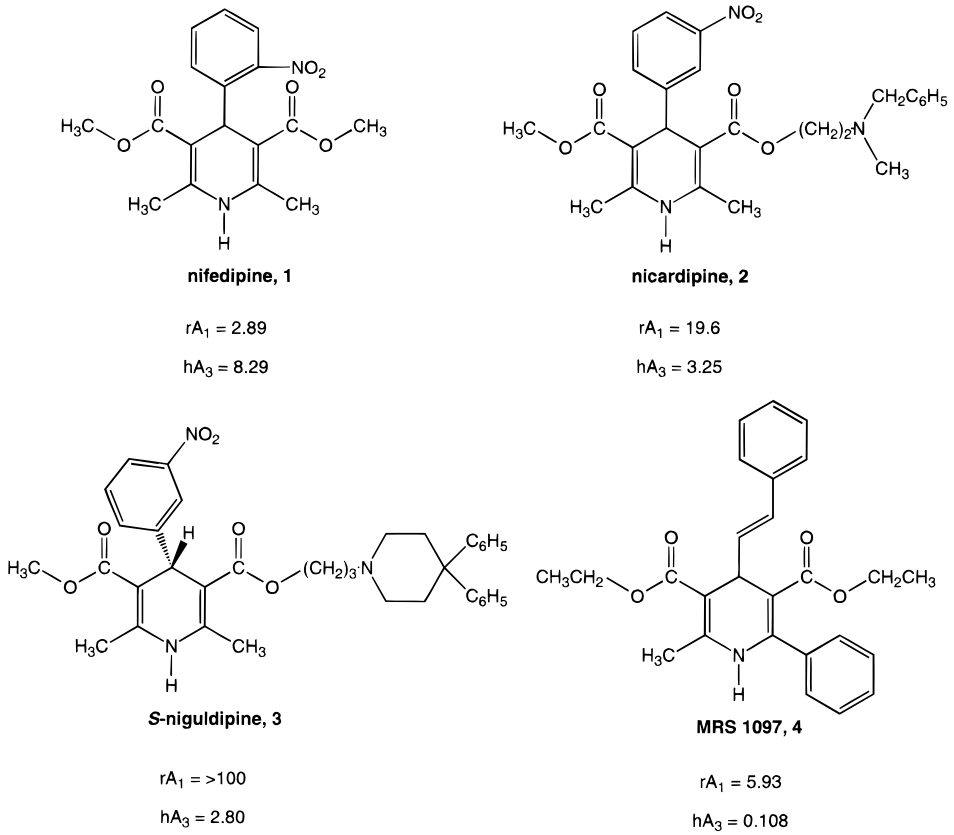


Figure 1. Structures of 1,4-dihydropyridines as potent calcium channel antagonists (**1**−**3**) and an adenosine receptor antagonist (**4**). K_i values (μM) are shown for rat brain A_1 receptors (rA_1) and for cloned human A_3 receptors (hA_3).

receptors. New derivatives of exceptionally high potency and selectivity have been discovered.

Results

Synthesis. The structures of the 1,4-dihydropyridines (**4**−**31**) and related pyridine derivatives (**32**−**34**) tested for affinity in radioligand binding assays at adenosine receptors are shown in Table 1. New dihydropyridine derivatives were synthesized using standard methodology (Scheme 1),[18−20] as reported in Table 2. The synthesis consisted of the Hantzsch condensation of a 3-amino-2-butenoate ester, **35a,b**, an aldehyde, **36a**−**h**, and a 3-ketopropionate ester derivative, **37a**−**i**, that were dissolved in ethanol and refluxed under N_2. In order to obtain substitution at the 4-position the aldehyde component, **36a**−**h**, was varied. Substitution at the 6-position was achieved by varying the 3-ketopropionate ester component, **37e**−**g**,**i** (Scheme 2), prepared according to method of Straley and Adams.[21] In the synthesis of compounds **29**−**31**, the precursor, ethyl 3-aminocinnamate was prepared from the imidate hydrochloride and Meldrum's acid (Scheme 3).[22] The yields of the 6-phenyl-1,4-dihydropyridines obtained using a ≤72 h reaction time varied between 6 and 63%. All of the dihydropyridines examined in this study were racemic mixtures at position C-4.

An aromatic nitro group on the C-4 side chain of a dihydropyridine could be reduced selectively using zinc/acetic acid (Scheme 4). This was desired as a precursor for the potential radioiodination reaction for the preparation of an A_3 receptor radioligand, as has been carried out for an agonist.[27]

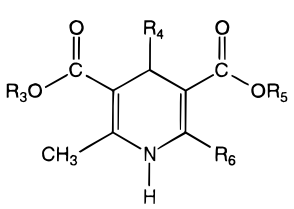
Oxidation of 1,4-dihydropyridines (**4** and **28**) to the corresponding pyridine derivatives (**33** and **34**, respectively) was carried out using tetrachloro-1,4-benzoquinone (chloranil, **45**) in tetrahydrofuran (Scheme 5).[23]

Binding at Adenosine Receptors. K_i values at A_1 and A_{2A} receptors were determined in radioligand binding assays in rat brain membranes vs [^{3}H]-(*R*)-PIA [[^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine] or [^{3}H]CGS 21680 [[^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine], respectively.[24,25] Affinity at cloned human A_3 receptors expressed in HEK-293 cells[26] was determined using [^{125}I]AB-MECA [N^6-(4-amino-3-[^{125}I]iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine].[27,28]

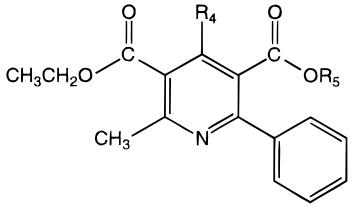
Structure−activity relationship (SAR) analysis of 1,4-dihydropyridine derivatives at adenosine receptors has indicated that increasing the size of the substituents from small alkyl either at the C-4 position or at the 5-ester position tends to increase binding affinity at A_1, A_{2A}, and A_3 subtypes (Table 1).[16] This was illustrated with the 5-benzyl ester, **6**, and the 4-ethyl analogue, **7**.[16] The benzyl ester, **6**, vs compound **5** provided a 12-fold enhancement of affinity at A_3 receptors. The 4-β-styryl substituent in **8** provided an even greater enhancement of A_3 receptor affinity (48-fold vs **5**) and selectivity. The combination of the 5-benzyl ester and 4-β-styryl substituents in **10** was compatible in its affinity enhancement at A_1 and A_3, but not at A_{2A}, subtypes.

The 6-phenyl substituent enhanced affinity particularly at A_3 receptors, thus compound **11** was slightly A_3 selective. Extending the 4-methyl group to ethyl in **12** did not result in an enhancement of affinity, as it did in the 6-methyl case (compound **7** vs **5**). The combination of the 5-benzyl ester and 6-phenyl substituents in **13** was additive in affinity enhancement at A_{2A} and A_3, but not at A_1, subtypes. The ratios of affinity

Table 1. Affinities of Dihydropyridine and Pyridine Derivatives in Radioligand Binding Assays at A_1, A_{2a}, and A_3 Receptors[a–f]



4 - 31

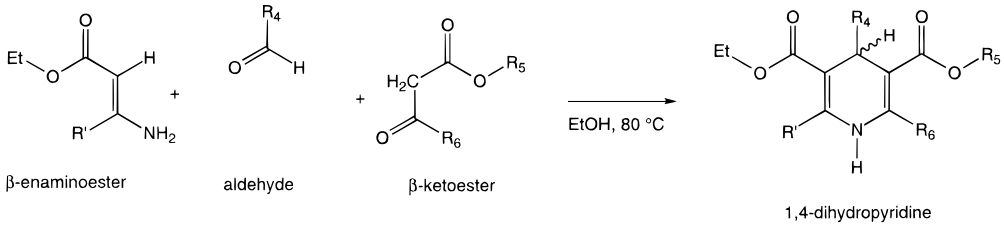


32 - 34

					K_i (μM) or % inhibition[d]			
compd	R_3	R_4	R_5	R_6	rA_1[a]	rA_{2A}[b]	hA_3[c]	rA_1/hA_3
5[e]	CH_3	CH_3	CH_2CH_3	CH_3	32.6 ± 6.3	46.1 ± 6.8	32.3 ± 5.1	1.0
6[e]	CH_3	CH_3	CH_2Ph	CH_3	6.45 ± 1.47	9.72 ± 0.63	2.78 ± 0.89	2.3
7[e]	CH_3	CH_2CH_3	CH_2CH_3	CH_3	7.52 ± 2.79	7.89 ± 2.87	13.6 ± 2.0	0.53
8[e]	CH_3	PhCH=CH-(*trans*)	CH_2CH_3	CH_3	16.1 ± 0.5	49.3 ± 12.5	0.670 ± 0.195	24
9	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	CH_3	4.65 ± 1.21	9.23 ± 3.60	0.887 ± 0.138	5.2
10	CH_2CH_3	PhCH=CH-(*trans*)	CH_2Ph	CH_3	13.7 ± 2.6	14 ± 4% (10^{-4})	3.13 ± 0.51	4.4
11[e]	CH_2CH_3	CH_3	CH_2CH_3	Ph	25.9 ± 7.3	35.9 ± 15.3	7.24 ± 2.13	3.6
12	CH_2CH_3	CH_2CH_3	CH_2CH_3	Ph	21.5 ± 2.7	14.5 ± 3.5	8.49 ± 1.74	2.5
13	CH_2CH_3	CH_3	CH_2Ph	Ph	26.0 ± 8.7	3.15 ± 0.96	1.75 ± 0.47	15
4[e]	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	Ph	5.93 ± 0.27	4.77 ± 0.29	0.108 ± 0.012	55
14	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	4-CH_3Ph	14.9 ± 4.9	37 ± 2% (10^{-4})	9.13 ± 2.43	1.6
15	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	4-OCH_3Ph	9.49 ± 1.99	*d* (10^{-4})	1.43 ± 0.37	6.6
16	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	4-ClPh	33.0 ± 7.5	12 ± 7% (10^{-4})	0.785 ± 0.272	42
17	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	4-NO_2Ph	13.1 ± 1.6	40 ± 3% (10^{-4})	4.14 ± 0.51	3.2
18	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	3-furyl	5.49 ± 0.25	35 ± 6% (10^{-4})	0.907 ± 0.307	6.1
19	CH_2CH_3	PhCH=CH-(*trans*)	CH_2CH_3	3-thienyl	7.52 ± 1.38	*d* (10^{-4})	0.407 ± 0.066	18
20	CH_2CH_3	2-OCH_3PhCH=CH-(*trans*)	CH_2CH_3	Ph	25.3 ± 2.4	17 ± 12% (10^{-4})	0.334 ± 0.059	76
21	CH_2CH_3	2-NO_2PhCH=CH-(*trans*)	CH_2CH_3	Ph	6.03 ± 1.39	*d* (10^{-4})	0.109±0.017	55
22	CH_2CH_3	4-NO_2PhCH=CH-(*trans*)	CH_2CH_3	Ph	23 ± 9% (10^{-4})	33% (10^{-4})	0.0585 ± 0.0164	>1700
23	CH_2CH_3	4-NH_2PhCH=CH-(*trans*)	CH_2CH_3	Ph	31 ± 3% (10^{-4})	26 ± 6% (10^{-4})	0.198 ± 0.047	>500
24	CH_2CH_3	$(Ph)_2C$=CH-	CH_2CH_3	Ph	1.20 ± 0.14	12 ± 6% (10^{-4})	1.42 ± 0.23	0.84
25	CH_2CH_3	PhC≡C-	CH_2CH_3	Ph	11.0 ± 0.1	26 ± 12% (10^{-4})	0.0766 ± 0.0151	140
26	CH_2CH_3	PhCH=CH-(*trans*)	CH_2Ph	Ph	35 ± 3% (10^{-4})	15 ± 3% (10^{-4})	0.0583 ± 0.0124	>1700
27	CH_2CH_3	4-NO_2PhCH=CH-(*trans*)	CH_2Ph	Ph	33 ± 1% (10^{-4})	*d* (10^{-4})	0.0724 ± 0.0377	>1300
28	CH_2CH_3	PhC≡C-	CH_2Ph	Ph	40.1 ± 7.5	*d* (10^{-4})	0.0314 ± 0.0028[f]	1300
29	CH_2Ph	PhCH=CH-(*trans*)	CH_2CH_3	Ph	*d* (10^{-4})	16 ± 11% (10^{-4})	0.142 ± 0.047	>700
30	CH_2Ph	4-NO_2PhCH=CH-(*trans*)	CH_2CH_3	Ph	*d* (10^{-4})	*d* (10^{-4})	0.286 ± 0.038	>400
31	CH_2Ph	PhC≡C-	CH_2CH_3	Ph	24 ± 4% (10^{-4})	*d* (10^{-4})	0.169 ± 0.026	>600
32[e]		CH_3	CH_2CH_3		7.41 ± 1.29	28.4 ± 9.1	4.47 ± 0.46	1.7
33		PhCH=CH-(trans)	CH_2CH_3		2.49 ± 0.47	2.40 ± 0.22	2.80 ± 1.78	0.85
34		PhC≡C-	CH_2Ph		11.6 ± 4.8	43 ± 2% (10^{-4})	2.75 ± 0.78	4.2

[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] [f] K_i value at rat A_3 receptors stably expressed in CHO cells[1] found to be 3.53 ± 0.61 μM.

Scheme 1. General Procedure for the Synthesis of 1,4-Dihydropyridine Derivatives **9**–**22** and **24**–**31**



β-enaminoester aldehyde β-ketoester 1,4-dihydropyridine

35a: R' = CH_3 **35b:** R' = Ph

36a: R_4 = CH_3
36b: R_4 = CH_2CH_3
36c: R_4 = CH=CH-Ph
36d: R_4 = CH=CH-C_6H_4-4-NO_2
36e: R_4 = CH=CH-C_6H_4-2-NO_2
36f: R_4 = CH=CH-C_6H_4-2-OCH_3
36g: R_4 = CH=$C(Ph)_2$
36h: R_4 = C=C-Ph

37a: R_5 = CH_2CH_3 R_6 = CH_3
37b: R_5 = CH_2Ph R_6 = CH_3
37c: R_5 = CH_2CH_3 R_6 = Ph
37d: R_5 = CH_2Ph R_6 = Ph
37e: R_5 = CH_2CH_3 R_6 = C_6H_4-4-OCH_3
37f: R_5 = CH_2CH_3 R_6 = C_6H_4-4-Cl
37g: R_5 = CH_2CH_3 R_6 = C_6H_4-4-NO_2
37h: R_5 = CH_2CH_3 R_6 = 3-furyl
37i: R_5 = CH_2CH_3 R_6 = 3-thienyl

of compound **13** vs the corresponding 5-ethyl ester, **11**, were 11- and 4.1-fold at A_{2A} and A_3 receptors, respectively.

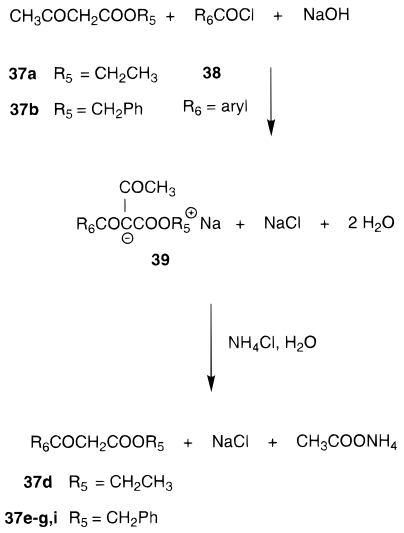
We have previously demonstrated that compound **4** is 55-fold selective for human A_3 vs rat A_1 receptors; thus the combination of the 4-β-styryl and 6-phenyl substituents is well tolerated at the A_3 receptor.[16] The compatibility of the 4-β-styryl group with other aromatic substituents at the 6-position was examined in the current study. In compounds **14**–**17**, the 6-phenyl ring

Table 2. Characterization of Dihydropyridine and Pyridine Derivatives

no.	T_m (°C)	formula	MS	analysis	yield[a] (%)
9	148−149	$C_{21}H_{25}NO_4$	355(CI)	C,H,N	16.3
10	115−117	$C_{26}H_{27}NO_4$	417 (CI)	C,H,N	69.5
12	106−108	$C_{20}H_{25}NO_4$	343 (CI)	C,H,N	25.5
13	112−114	$C_{24}H_{25}NO_4$	391 (EI)	C,H,N	62.9
14	129−131	$C_{27}H_{29}NO_4$	431 (CI)	C,H,N	32.2
15	137−139	$C_{21}H_{33}NO_4$	447 (CI)	C,H,N	6.1
16	149−151	$C_{26}H_{26}NClO_4$	451 (EI)	C,H,N	31.2
17	oil	$C_{26}H_{26}N_2O_6$	462 (EI)	*b*	15.3
18	114−116	$C_{24}H_{25}NO_5$	407 (CI)	C,H,N	25.5
19	oil	$C_{24}H_{25}NSO_4 \cdot 0.50EtOH$	423 (EI)	C,H,N	15.1
20	130−132	$C_{27}H_{29}NO_5$	447 (EI)	C,H,N	12.0
21	175−176	$C_{26}H_{26}N_2O_6$	462 (EI)	C,H,N	8.0
22	195−196	$C_{26}H_{26}N_2O_6$	462 (EI)	C,H,N	58.2
23	oil	$C_{26}H_{28}N_2O_4$	432 (EI)	*c*	24.1
24	164−165	$C_{32}H_{31}NO_4$	491 (EI)	C,H,N	8.2
25	132−134	$C_{26}H_{25}NO_4$	415 (EI)	C,H,N	36.4
26	161−163	$C_{31}H_{29}NO_4$	479 (EI)	C,H,N	27.9
27	oil	$C_{31}H_{28}N_2O_6$	524 (EI)	*d*	26.2
28	156−158	$C_{31}H_{27}NO_4$	477 (CI)	C,H,N	31.0
29	149−151	$C_{31}H_{29}NO_4$	479 (EI)	C,H,N	41.5
30	oil	$C_{31}H_{28}N_2O_6$	524 (EI)	*e*	32.9
31	124−125	$C_{31}H_{27}NO_4$	477 (CI)	C,H,N	29.3
33	oil	$C_{26}H_{25}NO_4$	415 (EI)	*f*	29.8
34	oil	$C_{31}H_{25}NO_4$	475 (EI)	*g*	74.0

[a] Purification was achieved by thin layer chromatography, silica 60, 1000 μm layer thickness, using EtOAc−petroleum ether (pe) 35−60, 20:80 (v/v) as eluent. [b] **17** pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 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] **23** ($C_{26}H_{28}N_2O_4$) insufficient quantity for CHN, pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 20:80 (v/v), R_f = 0.06; $CHCl_3$−MeOH−CH_3COOH = 94:6:1 (v/v/v), R_f = 0.47; EI calcd 432.2049, found 432.2042. [d] **27** pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 20:80 (v/v), R_f = 0.23; CH_2Cl_2−MeOH = 40:1 (v/v), R_f = 0.68; EI calcd 524.1938, found 524.1947. [e] **30** pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 20:80 (v/v), R_f = 0.21; CH_2Cl_2−MeOH = 40:1 (v/v), R_f = 0.71; EI calcd 524.1938, found 524.1928. [f] **33** pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 20:80 (v/v), R_f = 0.62; CH_2Cl_2−MeOH = 40:1 (v/v), R_f = 0.69; EI calcd 415.1772, found 415.1783. [g] **34** pure on analytical TLC (silica 60, 250 μm) EtOAc−pe = 20:80 (v/v), R_f = 0.52; CH_2Cl_2−MeOH = 40:1 (v/v), R_f = 0.75; EI calcd 475.1776, found 475.1783.

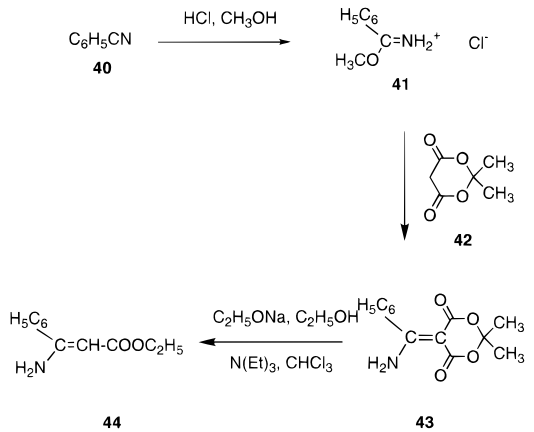
Scheme 2. General Procedure for the Synthesis of β-Keto Esters



was substituted at the para position with methyl, methoxy, chloro, and nitro groups. All substitutions were less potent than 6-phenyl at A_3 receptors, although the 4-chloro derivative, **16**, retained 42-fold selectivity vs A_1 receptors. The 4-methoxy derivative, **15**, was 85-fold less potent than **4** at A_3 receptors. Affinity at A_{2A} receptors was particularly sensitive to substitution of the 6-phenyl ring. Substitution of the 6-phenyl ring with 3-furyl, **18**, or 3-thienyl, **19**, groups reduced the affinity at A_3 receptors by 9- and 4-fold, respectively.

Substitutions of the phenyl ring of the β-styryl group, **20**−**23**, suggested much more freedom in this region in binding at A_1 and A_3, but not A_{2A}, subtypes. The 4-nitro analogue, **22** (MRS 1222), was slightly more potent at

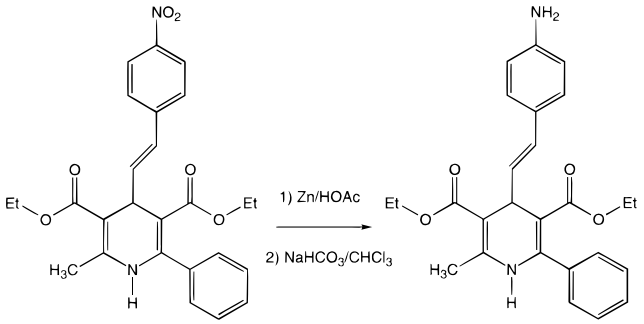
Scheme 3. Procedure for the Synthesis of Ethyl 3-Aminocinnamate



A_3 receptors than **4**. Phenyl substitution at the β-olefinic carbon in **24** resulted in relatively weak affinity at adenosine receptors (A_1, A_3 > A_{2A}). Compound **24** was 5-fold more potent than **4** at A_1 receptors and 13-fold less potent at A_3 receptors. The affinity of the dehydro equivalent (4-phenylethynyl substituent) of **4**, *i.e.* compound **25**, was very similar to its affinity at A_1 and A_3 receptors.

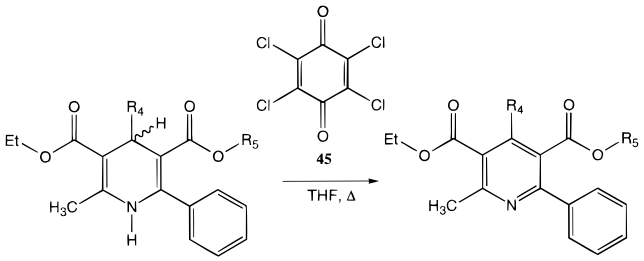
Triple substitutions of the simple dihydropyridines were also probed in compounds **26**−**31**. All contained the 6-phenyl substituent and either a 4-β-styryl or a 4-phenylethynyl group. Benzyl esters at the 3- (**29**−**31**) or 5-positions (**26**−**28**) were included. A 5-benzyl ester 4-*trans*-β-styryl derivative, **26**, with a K_i value of 58.3 nM at A_3 receptors, was >1700-fold selective vs either A_1 receptors or A_{2A} receptors. The enhanced selectivity was mainly the result of loss of affinity at A_1 receptors. Shifting the benzyl ester from the 5- to

Scheme 4. Method for Reduction of the Nitro Group on 1,4-Dihydropyridines



22 **23**

Scheme 5. General Procedure for the Oxidation of 1,4-Dihydropyridine Derivatives Using Tetrachlorobenzoquinone, **45**



4 R_4 = CH=CH-Ph, R_5 = C_2H_5 **33**

28 R_4 = C≡C-Ph, R_5 = CH_2Ph **34**

the 3-position as in **29** lowered the affinity at A_3 receptors 3-fold. The presence of a 4-nitro group on the β-styryl substituent, as in **27** and **30**, had a negligible effect on affinity at any of the receptors studied. 3-Ethyl 5-benzyl 2-methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate, **28** (MRS 1191), displayed a K_i value of 31.4 nM at A_3 receptors and 1300-fold selectivity vs A_1 receptors. Relative to the corresponding 5-ethyl ester derivative, **25**, compound **28** was 2.4-fold more potent at A_3 receptors and less potent at A_1 receptors. A derivative isomeric to compound **28**, the corresponding 3-benzyl 5-ethyl diester, **31**, was >600-fold selective for A_3 receptors. Thus, bulky groups at combinations of either 3-, 4-, and 6-positions or 4-, 5-, and 6-positions still resulted in high A_3 receptor selectivity as a result of very low affinity at A_1 and A_{2A} receptors.

Oxidation of a simple 6-phenyl-1,4-dihydropyridine, **11**, to the corresponding pyridine derivative increased affinity at A_1 but not A_3 receptors. The presence of additional bulky substituents, *e.g.* at the 4- and 5-positions, caused the effects of oxidation to be less tolerated in binding, at A_3 receptors in particular. Oxidation of **28** to the corresponding pyridine derivative, **34**, reduced affinity at A_3 receptors by 88-fold, while affinity at A_1 receptors increased 4-fold.

Discussion

Among the most selective ligands in the present study were compounds **26**−**28**, *i.e.* 5-benzyl esters having ≥1300-fold selectivity for A_3 vs A_1 adenosine receptors, and compounds **29**−**31**, *i.e.* 3-benzyl esters having ≥ 400-fold selectivity. The para-substituted 4-styryl-6-phenyl derivatives **22** and **23** were also highly selective. Structure−activity analysis at A_3 adenosine receptors indicated that sterically bulky groups in 1,4-dihydropyridines are tolerated at the 3-, 4-, 5-, and 6-positions. In the present study simultaneous substitution at two or three, but not all four, of these positions was carried out. In this study large substituents at the 4-(β-styryl or phenylethynyl) and 6-positions (phenyl) were combined with bulky esters at either the 3- or 5-position, and a beneficial effect on selectivity for human A_3 receptors was observed. In combination with the 4- and 6-position substitutions mentioned above, a benzyl ester group preserved affinity (vs **4**) at the A_3 receptors to a greater extent when located at the 5-position (*e.g.* **26**) than at the 3-position (*e.g.* **29**). Nevertheless, it is surprising that combinations of bulky substituents at the 3-, 4-, and 6-positions result in receptor affinity profiles not radically different from those combinations at the 4-, 5-, and 6-positions. This suggests that the A_3 receptor binding site is not highly sterically constrained. Also, similar enhancements of affinity at A_3 receptors by either a 4-*trans*-β-styryl or a 4-phenylethynyl group point to the same conclusion. Both of these groups are highly rigid, yet occupy nonidentical spatial regions.

A series of 8-styrylxanthines[30,31] were found to be subject to photoisomerization about the olefinic bond, which reduces their utility as pharmacological probes. One might expect a similar complication with the 4-styryldihydropyridine derivatives. Incorporation of a linear phenylethynyl group instead of the styryl group avoids this potential problem of isomerization.

Pyridine derivatives, which have a distinctly more planar geometry than that of the corresponding dihydropyridines, lost affinity for A_3 adenosine receptors but, remarkably, gained affinity for A_1 receptors. Numerous classes of adenosine receptor non-xanthine antagonists have been found for the A_1 receptor, and all tend to have a planar geometry.[29] It is possible that the A_3 receptor does not share this preference for planar antagonists.

There appears to be more flexibility of substitution of the phenyl ring of 4-β-styryl derivatives (*e.g.* compounds **20**−**23**) than of the 6-phenyl substituent in A_3 receptor selective agents. Substitution of the 6-phenyl ring in the para position with a variety of electron-donating (methyl, methoxy) or -withdrawing (nitro, chloro) groups or its replacement with 3-thienyl or 3-furyl groups (*e.g.* compounds **14**−**19**) all reduced the affinity at A_3 receptors.

It will now be necessary to investigate the regio- (and stereo-) selectivity of the ligand receptor interaction more fully. Many of the dihydropyridines examined in our previous study[16] are asymmetric only on account of nonequivalent ester substitutions at the 3- and 5-positions. One such pair of enantiomers, (*R*)- and (*S*)-niguldipine, was studied at adenosine receptors. The difference in affinity at human A_3 receptors between (*R*)- and (*S*)-niguldipine, **3**, was insignificant (K_i values of 1.9 and 2.8 μM, respectively). Thus, by analogy to the present set of mixed ethyl and benzyl esters, it is likely that there is no dramatic difference in A_3 receptor affinity between (*R*)- and (*S*)-enantiomers of 3,4- or 4,5-disubstituted dihydropyridines (*i.e.* in which methyl groups occur at both the 2- and 6-positions). The effects of 6-aryl substitution on stereoselectivity of binding at adenosine receptors have yet to be explored.

At the rat A_3 receptor, the affinity of 1,4-dihydropyridines is considerably lower than at human A_3 recep-

tors.[16] For example the K_i value of **28** at the rat A_3 receptor was found to be 3.53 μM, while at the human A_3 receptor the K_i value is only 31 nM. Although compound **28** is still somewhat selective for A_3 receptors in the rat (11-fold), it is 110-fold less potent than at human A_3 receptors. This species dependence of A_3 receptor affinity has also been demonstrated for xanthines[2,4] and other classes of adenosine antagonists, such as flavonoids,[28] which tend to bind with lower K_i values at human vs rat A_3 receptors.

It is expected that all of the 4-(arylalkyl)-6-phenyl-1,4-dihydropyridines prepared in the present study are selective for adenosine receptors vs L-type Ca^{2+} channels, since this principle was demonstrated in a previous study for compound **4**.[16] Moreover, compound **4** was also shown to have negligible affinity for the adenosine transporter.[16] Furthermore, it was demonstrated that dihydropyridines such as compound **4** effectively attenuate the IB-MECA elicited inhibition of adenylyl cyclase in CHO cells expressing the cloned rat A_3 adenosine receptor.[16] Thus, the selective ligands introduced here should be useful as antagonists in probing the role of A_3 receptors, especially for the human homologue of the receptor. These selective agents are now suitable for study in functional assays and in investigations of the physiological role of A_3 receptors. With slight additional improvement of affinity, dihydropyridines may also provide affinity probes such as radioligands for human A_3 receptors, although it has not been determined if the binding is competitive.

Experimental Section

Materials. Ethyl acetoacetate (**37a**), acetaldehyde (**36a**), propionaldehyde (**36b**), ethyl 3-aminocrotonate (**35a**), and *trans*-cinnamaldehyde (**36c**) were obtained from Fluka (Ronkonoma, NY). Ethyl 3-aminocrotonate (**35a**), 4-nitro-*trans*-cinnamaldehyde (**36d**), 2-nitro-*trans*-cinnamaldehyde (**36e**), 2-methoxy-*trans*-cinnamaldehyde (**36f**), β-phenylcinnamaldehyde (**36g**), phenyl propargylaldehyde (**36h**), benzyl acetoacetate (**37b**), ethyl benzoylacetate (**37c**), and tetrachloro-1,4-benzoquinone (**45**) were from Aldrich (St. Louis, MO). Compounds **4**−**8** and **32** were prepared as described in van Rhee et al.[16] (*R*)-PIA and 2-chloroadenosine were purchased from Research Biochemicals International (Natick, MA). All other materials were obtained from commercial sources.

Synthesis. Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer, and spectra were taken in DMSO-d_6 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 were uncorrected.

General Procedure for the Preparation of 1,4-Dihydropyridine-3,5-dicarboxylate Esters (9−22, 24−31). Equimolar amounts (0.5 mmol) of the appropriate 3-amino-2-propenoate ester (**35a,b**), aldehyde (**36a**−**h**), and 3-ketopropionate ester (**37a**−**i**) derivative were dissolved in 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 72h. The solvent was then evaporated, and products were purified either by crystallization, column chromatography (silica 60; 220−440 mesh; Fluka, Buchs, CH; 20% ethyl acetate−80% petroleum ether 35−60), or preparative TLC (silica 60; 1000 μm; Analtech, Newark, DE; 20% ethyl acetate−80% petroleum ether 35−60). 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.

3,5-Diethyl 2,6-Dimethyl-4-[2-phenyl-(*E*)-vinyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate (9). ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 6H, J = 6.8 Hz, 3 and 5-CH_2CH_3), 2.33 (s, 6H, 2 and 6-CH_3), 4.16 (m, 4H, 3 and 5-OCH_2), 4.61 (d, 1H, J = 5.8 Hz, 4-H), 5.60 (br, 1H, NH), 6.17 (dd, 1H, J = 5.9, 16.6 Hz, C_6H_5C=CH), 6.25 (d, 1H, J = 16.6 Hz, C_6H_5CH=C), 7.16−7.33 (m, 5H, C_6H_5). MS (CI/NH_3): m/z 356 $(MH)^+$, 252 $(M - C_6H_5CH{=}CH)^+$, base.

3-Ethyl 5-Benzyl 2,6-Dimethyl-4-[2-phenyl-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (10). ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 2.34 (s, 3H, 2-CH_3), 2.38 (s, 3H, 6-CH_3), 4.22 (m, 2H, 3-OCH_2), 4.70 (d, 1H, J = 5.8 Hz, 4-H), 5.20 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 6.16 (dd, 1H, J = 5.9, 15.8 Hz, C_6H_5C=CH), 6.21 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 7.20−7.44 (m, 10H, 2 × C_6H_5). MS (CI/NH_3): m/z 418 (MH^+), base.

3,5-Diethyl 2-Methyl-4-ethyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (12). ^{1}H NMR ($CDCl_3$): δ 0.88 (2t, 6H, J = 6.8 Hz, 3 and 5-CH_2CH_3), 1.32 (t, 3H, J = 7.0 Hz, 4-CH_2CH_3), 1.55 (m, 2H, 4-CH_2CH_3), 2.33 (s, 3H, 2-CH_3), 3.90 (m, 2H, 3-OCH_2), 4.06 (t, 1H, J = 6.5 Hz, 4-H), 4.22 (m, 2H, 5-OCH_2), 5.63 (br, 1H, NH), 7.29−7.41 (m, 5H, 6-C_6H_5). MS (CI/NH_3): m/z 361 $(M + NH_4^+)$, base, 344 (MH^+).

3-Ethyl 5-Benzyl 2,4-Dimethyl,6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (13). ^{1}H NMR ($CDCl_3$): δ 1.15 (d, 3H, J = 6.6 Hz, 4-CH_3), 1.32 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 2.31 (s, 3H, 2-CH_3), 4.02 (q, 1H, J = 6.7 Hz, 4-H), 4.21 (m, 2H, 3-OCH_2), 4.95 (AB, 2H, J = 12.7 Hz, 5-OCH_2), 5.70 (br, 1H, NH), 6.95−7.36 (m, 10H, 2 × C_6H_5). MS (EI): m/z 376 $(M - CH_3)^+$, 91 $(C_6H_5CH_2^+)$, base.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(4-toluyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (14). ^{1}H NMR (DMSO-d_6): δ 0.78 (t, 3H, J = 7.0 Hz, $3CH_2CH_3$), 1.20 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.28 (s, 3H, toluyl-CH_3), 2.34 (s, 3H, 2-CH_3), 3.78 (q, 2H, J = 6.7 Hz, 3-OCH_2), 4.10 (m, 2H, 5-OCH_2), 4.49 (d, 1H, J = 7.3 Hz, 4-H), 6.20 (m, 2H, CH=CH), 7.18−7.36 (m, 9H, C_6H_4 and C_6H_5), 9.01 (br, 1H, NH). MS (CI/NH_3): m/e 432 (MH^+), 328 $(M - C_6H_5CH{=}CH^+)$, base.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(4-methoxyphenyl)-1,4-(*R,S*)-dihydropyridine-3,5-dicarboxylate (15). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 1.33 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 3.85 (s, 3H, 4′-OCH_3), 4.00 (q, 2H, J = 6.7 Hz, 3-OCH_2), 4.22 (m, 2H, 5-OCH_2), 4.74 (d, 1H, J = 6.1 Hz, 4-H), 5.76 (br, 1H, NH), 6.32 (dd, 1H, J = 6.6, 15.8 Hz, C_6H_5C=CH), 6.41 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 6.94 (d, 2H, J = 8.7 Hz, 2′- and 6′-H), 7.19−7.31 (m, 5H, C_6H_5), 7.37 (d, 2 H, J = 7.3 Hz, 3′- and 6′-H). MS (CI/NH_3): m/z 448 $(MH)^+$, 344 $(M - C_6H_5$-$CH{=}CH)^+$, base.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(4-chlorophenyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (16). ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, J = 7.2 Hz, 3-CH_2CH_3), 1.29 (t, 3H, J = 7.2 Hz, 5-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.97 (q, 2H, J = 7.0 Hz, 3-OCH_2), 4.22 (m, 2H, 5-OCH_2), 4.75 (d, 1H, J = 6.3 Hz, 4-H), 5.71 (br, 1H, NH), 6.28 (dd, 1H, J = 6.6, 15.8 Hz, C_6H_5C=CH), 6.40 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 7.20−7.41 (m, 9H, C_6H_4 and 6-C_6H_5). MS (EI): m/z 451 $(M)^+$, 422 $(M - C_2H_5)^+$, 378 $(M - CO_2C_2H_5)^+$, base, 348 $(M - C_6H_5$-$CH{=}CH)^+$.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(4-nitrophenyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (17). ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 1.28 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.13 (q, 2H, J = 7.0 Hz, 3-OCH_2), 4.25 (m, 2H, 5-OCH_2), 4.77 (d, 1H, J = 5.9 Hz, 4-H), 5.67 (br, 1H, NH), 6.26 (dd, 1H, J = 6.6, 15.8 Hz, C_6H_5C=CH), 6.37 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 7.21−7.54 (m, 7H, 3′,5′-H and 6-C_6H_5), 8.28 (m, 2H, 2′ and 6′-H). MS (EI): m/z 462 $(M)^+$, 433 $(M - C_2H_5)^+$, 389 $(M - CO_2C_2H_5)^+$, base, 359 $(M - C_6H_5CH{=}CH)^+$.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(3-furyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (18). ^{1}H NMR δ 1.13 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 1.31 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.85 (s, 3H, 4′-OCH_3), 4.08 (m, 2H, 3-OCH_2), 4.21 (q, J = 7.1 Hz, 2H, 5-OCH_2), 4.73 (d, 1H, J = 6.3 Hz, 4-H), 5.73 (br, 1H, NH), 6.24 (dd, 1H, J = 6.7, 15.8 Hz, C_6H_5C=CH), 6.35 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 6.52 (m, 1H, 3′-H), 7.19−7.37 (m, 5H, C_6H_5), 7.45 (m, 1 H, 2′-H), 7.65 (s, 1H, 5′-H). MS (CI/NH_3): m/z 408 $(MH)^+$, 304 $(M - C_6H_5CH{=}CH)^+$, base.

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-(3-thienyl)-1,4-(±)-dihydropyridine-3,5-dicarboxylate (19). ^{1}H NMR ($CDCl_3$): δ 1.05 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 1.27 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.05 (m, 2H, 3-OCH_2), 4.22 (m, 2H, 5-OCH_2), 4.73 (d, 1H, J = 6.2 Hz, 4-H), 5.88 (br, 1H, NH), 6.30 (dd, 1H, J = 6.5, 15.8 Hz, C_6H_5C=CH), 6.38 (d, 1H, J = 15.8 Hz, C_6H_5CH=C), 7.06 (m, 1H, 4′-H), 7.18−7.31 (m, 5H, C_6H_5), 7.35 (d, 1H, J = 5.2 Hz, 3′-H), 7.40 (d, 1H, J = 4.8 Hz, 5′-H). MS (EI): m/z 423 $(M)^+$, 350 $(M - CO_2CH_2CH_3)$, base, 320 $(M - C_6H_5CH{=}CH)^+$.

3,5-Diethyl 2-Methyl-4-[2-(2-methoxyphenyl)-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (20). ^{1}H NMR ($CDCl_3$): δ 0.93 (t, 3H, J = 7.3 Hz, 3-CH_2CH_3), 1.33 (t, 3H, J = 6.8 Hz, 5-CH_2CH_3), 2.35 (s, 3H, 2-CH_3), 3.82 (s, 3H, 2′-OCH_3), 3.92 (m, 2H, 3-OCH_2), 4.20 (m, 2H, 5-OCH_2), 4.75 (d, 1H, J = 5.9 Hz, 4-H), 5.76 (br, 1H, NH), 6.26 (dd, 1H, J = 5.9, 15.6 Hz, C_6H_5C=CH), 6.84 (d, 1H, J = 16.6 Hz, C_6H_5-CH=C), 7.15−7.48 (m, 9H, 4-C_6H_4 and 6-C_6H_5). MS (EI): m/z 447 $(M)^+$, 402 $(M - OC_2H_5)^+$, 374 $(M - CO_2C_2H_5)^+$.

3,5-Diethyl 2-Methyl-4-[2-(2-nitrophenyl)-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (21). ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, J = 7.3 Hz, 3-CH_2CH_3), 1.33 (t, 3H, J = 7.3 Hz, 5-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.96 (q, 2H, J = 7.2 Hz, 3-OCH_2), 4.27 (q, 2H, J = 7.2 Hz, 5-OCH_2), 4.78 (d, 1H, J = 5.9 Hz, 4-H), 5.93 (br, 1H, NH), 6.34 (dd, 1H, J = 5.9, 15.6 Hz, C_6H_5C=CH), 6.90 (d, 1H, J = 16.6 Hz, C_6H_5-CH=C), 7.30−7.48 (m, 8H, 4-C_6H_3 and 6-C_6H_5), 7.90 (d, 1H, J = 9.8 Hz, 3′-H). MS (EI): m/z 462 $(M)^+$, 417 $(M - OC_2H_5)^+$, 389 $(M - CO_2C_2H_5)^+$.

3,5-Diethyl 2-Methyl-4-[2-(4-nitrophenyl)-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (22). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, J = 7.3 Hz, 3-CH_2CH_3), 1.32 (t, 3H, J = 7.3 Hz, 5-CH_2CH_3), 2.39 (s, 3H, 2-CH_3), 3.94 (q, 2H, J = 6.8 Hz, 3-OCH_2), 4.24 (q, 2H, J = 6.8 Hz, 5-OCH_2), 4.80 (d, 1H, J = 4.9 Hz, 4-H), 5.82 (br, 1H, NH), 6.47 (dd, 1H, J = 3.9, 16.1 Hz, C_6H_5C=CH), 7.32−7.51 (m, 8H, C_6H_5CH=C and 4-C_6H_2 and 6-C_6H_5), 8.15 (d, 2H, J = 8.8 Hz, 3′- and 5′-H). MS (EI): m/z 462 $(M)^+$, 417 $(M - OC_2H_5)^+$, 389 $(M - CO_2C_2H_5)^+$.

3,5-Diethyl 2-Methyl-4-[2-(4-aminophenyl)-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (23). Compound **23** was prepared by the catalytic reduction of compound **22** with zinc and acetic acid as described previously.[30] Compound **22** (23 mg, 0.05 mmol) was dissolved in 1.5 mL of glacial acetic acid. Zn powder (0.15 mmol, 10 mg) was added to the solution, and the reaction mixture was stirred with a magnetic stirring bar at room temperature. Six hours after the start of the reaction, another batch of zinc powder was added. At 9 h reaction time, TLC (silica 60; petroleum ether 35−60−EtOAc = 80:20) analysis of the reaction mixture indicated that all starting material had been converted. The reaction mixture was diluted with 30 mL of water and neutralized with saturated $NaHCO_3$ solution. The aqueous solution was extracted three times with 15 mL of chloroform, and the organic phase was washed once with 20 mL of water. The organic phase was separated and dried over anhydrous $MgSO_4$. The product was purified by preparative TLC (1000 μm of silica 60; petroleum ether 35−60−EtOAc, 90:10) to yield 5.2 mg (24%) of a slightly yellow oil, which was shown to be pure by HPLC (OD-5-60, C-18 column, Separation Methods Technologies, Inc, Newark, DE; 0.1 M triethylammonium acetate buffer−CH_3CN, 40:60 gradient to 10:90 in 20 min; 1 mL/min flow; retention time = 4.45 min), and analytical TLC (silica 60; petroleum ether 35−60−EtOAc, 80:20, R_f = 0.06; $CHCl_3$−MEOH−HOAc, 94:6:1, R_f = 0.47). ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 1.31 (t, 3H, J = 6.8 Hz, 5-CH_2CH_3), 2.34 (s, 3H, 2-CH_3), 3.67 (br, 2H, 4′-NH_2), 3.92 (m, 2H, 3-OCH_2), 4.17 (m, 2H, 5-OCH_2), 4.70 (d, 1H, J = 6.8 Hz, 4-H), 5.78 (br, 1H, NH), 6.08 (dd, 1H, J = 6.8, 15.6 Hz, C_6H_5C=CH), 6.30 (d, 1H, J = 15.6 Hz, C_6H_5CH=C), 6.60 (d, 2H, J = 8.8 Hz, 3′- and 5′-H), 7.18 (d, 2H, J = 8.8 Hz, 2′- and 6′-H), 7.32−7.42 (m, 5H, 6-C_6H_5). MS (EI): m/z 432 $(M)^+$, 387 $(M - OC_2H_5)^+$, 359 $(M - CO_2C_2H_5)^+$.

3,5-Diethyl 2-Methyl-4-(2,2-diphenylvinyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (24). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 1.10 (t, 3H, J = 7.2 Hz, 5-CH_2CH_3), 2.32 (s, 3H, 2-CH_3), 3.70−4.18 (m, 4H, 3-OCH_2 and 5-OCH_2), 4.95 (d, 1H, J = 9.9 Hz, 4-H), 5.82 (br, 1H, NH), 6.02 (d, 1H, J = 10.3 Hz, C_6H_5C=CH), 7.17−7.46 (m, 15H, 4-C_6H_5 and 4-C_6H_5 and 6-C_6H_5). MS (EI): m/z 491 $(M)^+$, 446 $(M - OC_2H_5)^+$, 418 $(M - CO_2C_2H_5)^+$.

3,5-Diethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (25). ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, J = 7.0 Hz, 3-CH_2CH_3), 1.32 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 3.98 (m, 2H, 3-OCH_2), 4.27 (m, 2H, 3-OCH_2), 5.12 (s, 1H, 4-H), 5.92 (br, 1H, NH), 7.23−7.43 (m, 10H, 2 × C_6H_5). MS (EI): m/z 415 $(M)^+$, 386 $(M - C_2H_5)^+$, 342 $(M - CO_2C_2H_5)^+$, base.

3-Ethyl 5-Benzyl 2-methyl-4-[2-phenyl-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (26). ^{1}H NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.22 (m, 2H, 3-OCH_2), 4.78 (d, 1H, J = 6.2 Hz, 4-H), 4.99 (AB, J = 12.7 Hz, 5-OCH_2), 5.76 (br, 1H, NH), 6.30 (dd, 1H, J = 6.5, 16.2 Hz, C_6H_5C=CH), 6.38 (d, 1H, J = 16.2 Hz, C_6H_5CH=C), 6.98 (m, 2H, 2′- and 6′-H), 7.21−7.38 (m, 13H, 2 × C_6H_5, 3′-, 4′-, and 5′-H). MS (EI): m/z 479 $(M)^+$, 388 $(M - C_6H_5CH_2)^+$, 376 $(M - C_6H_5CH{=}CH)^+$, 344 $(M - CO_2CH_2$-$CH_3)$, 91 $(C_6H_5CH_2)^+$, base.

3-Ethyl 5-Benzyl 2-Methyl-4-[2-(4-nitrophenyl)-*E*)-vinyl]-6-phenyl-1,4-(*R*,*S*)-dihydropyridine-3,5-dicarboxylate (27). ^{1}H NMR ($CDCl_3$): δ 1.31 (t, 3H, J = 6.9 Hz, 3-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.24 (m, 2H, 3-OCH_2), 4.84 (d, 1H, J = 5.8 Hz, 4-H), 5.02 (AB, J = 12.7 Hz, 5-OCH_2), 5.82 (br, 1H, NH), 6.43 (dd, 1H, J = 6.5, 16.0 Hz, $NO_2C_6H_4C$=CH), 6.45 (d, 1H, J = 16.0 Hz, $NO_2C_6H_4CH$=C), 6.96 (dd, 2H, J = 2.0, 7.7 Hz, 3′- and 5′-H), 7.19−7.39 (m, 5H, C_6H_5), 8.13 (d, 2H, J = 8.8 Hz, 2′- and 6′-H). MS (EI): m/z 524 $(M)^+$, 389 $(M - CO_2C_6H_5$-$CH_2)^+$, 376 $(M - NO_2C_6H_5CH{=}CH)^+$, 91 $(C_6H_5CH_2)^+$, base.

3-Ethyl 5-Benzyl 2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (28). ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 2.37 (s, 3H, 2-CH_3), 4.27 (m, 2H, 3-OCH_2), 5.10 (AB, J = 12.7 Hz, 5-OCH_2), 5.19 (s, 1H, 4-H), 5.87 (br, 1H, NH), 7.08−7.39 (m, 15H, 3 × C_6H_5). MS (CI/NH_3): m/z 478 $(MH)^+$, 376 $(M - C_6H_5C{=}C)^+$, 242 $(M - CO_2C_6H_5CH_2)^+$, base.

3-Benzyl 5-Ethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (29). ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, J = 7.0 Hz, 5-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 3.92 (q, 2H, J = 6.7 Hz, 5-OCH_2), 4.81 (d, 1H, J = 5.9 Hz, 4-H), 5.18 (AB, J = 12.7 Hz, 2H, 3-OCH_2), 5.78 (br, 1H, NH), 6.31 (dd, 1H, J = 5.9, 16.0 Hz, C_6H_5C=CH), 6.36 (d, 1H, J = 16.0 Hz, C_6H_5CH=C), 7.20−7.42 (m, 10H, 2 × C_6H_5). MS (EI): m/z 406 $(M - CO_2CH_2)^+$, 376 $(M - C_6H_5$-$CH{=}CH)^+$, 91 $(C_6H_5CH_2)^+$, base.

3-Benzyl 5-Ethyl 2-Methyl-4-[2-(4-nitrophenyl)-(*E*)-vinyl]-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (30). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J = 6.8 Hz, 5-CH_2CH_3), 2.39 (s, 3H, 2-CH_3), 4.09 (q, 2H, J = 6.8 Hz, 3-OCH_2), 4.84 (d, 1H, J = 5.8 Hz, 4-H), 5.30 (AB, J = 12.2 Hz, 3-OCH_2), 5.87 (br, 1H, NH), 6.40 (d, 1H, J = 16.0 Hz, $NO_2C_6H_4$-CH=C), 6.50 (dd, 1H, J = 5.9, 16.6 Hz, $NO_2C_6H_4C$=CH), 6.96 (dd, 2H, J = 2.0, 7.7 Hz, 3′- and 5′-H), 7.19−7.39 (m, 5H, C_6H_5), 8.14 (d, 2H, J = 7.8 Hz, 2′- and 6′-H). MS (EI): m/z 524 $(M)^+$, 389 $(M - CO_2C_6H_5CH_2)^+$, 376 $(M - NO_2C_6H_5CH{=}CH)^+$, 91-$(C_6H_5CH_2)^+$, base.

3-Benzyl 5-Ethyl 2-Methyl-4-(phenylethynyl)-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (31). ^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, J = 6.8 Hz, 5-CH_2CH_3), 2.38 (s, 3H, 2-CH_3), 4.00 (q, 2H, J = 6.8 Hz, 5-OCH_2), 5.21 (s, 1H, 4-H),

5.30 (AB, J = 13.6 Hz, 3-OCH_2), 5.87 (br, 1H, NH), 7.30−7.49 (m, 15H, 3 × C_6H_5). MS (CI/NH_3): m/z 478 $(MH)^+$, 376 $(M - C_6H_5C{=}C)^+$, base.

Procedure for Oxidation of 1,4-Dihydropyridine-3,5-dicarboxylate Esters (33−34). Equimolar amounts (0.25 mmol) of the 1,4-dihydropyridine-3,5-dicarboxylate ester (**4**, **28**) and tetrachloro-1,4-benzoquinone (**45**) in tetrahydrofuran (2 mL) were mixed and refluxed for up to 4 h. The solvent was then evaporated, and products were purified by preparative TLC (silica 60; 1000 μm; Analtech, Newark, DE; 20% ethyl acetate−80% petroleum ether 35−60).

3,5-Diethyl 2-Methyl-4-[2-phenyl-(*E*)-vinyl]-6-phenylpyridine-3,5-dicarboxylate (33). ^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, J = 6.9 Hz, 3-CH_2CH_3), 1.26 (t, 3H, J = 6.8 Hz, 5-CH_2CH_3), 2.67 (s, 3H, 2-CH_3), 4.07 (q, 2H, J = 6.8 Hz, 3-OCH_2), 4.35 (q, 2H, J = 6.8 Hz, 5-OCH_2), 6.88 (d, 1H, J = 16.6 Hz, $C_6H_5C{=}CH$), 6.88 (d, 1H, J = 16.6 Hz, $C_6H_5CH{=}C$), 7.31−7.61 (2m, 10H, 2 × C_6H_5). MS (EI): m/e 415 $(M)^+$, base, 370 $(M - OC_2H_5)^+$, 358 $(386 - CH_2{=}CH_2)^+$.

3-Ethyl 5-Benzyl 2-Methyl-4-(phenylethynyl)-6-phenylpyridine-3,5-dicarboxylate (34). ^{1}H NMR ($CDCl_3$): δ 1.42 (t, 3H, J = 6.8 Hz, 3-CH_2CH_3), 2.68 (s, 3H, 2-CH_3), 4.48 (q, 2H, J = 6.8 Hz, 3-OCH_2), 5.17 (s, 2H, 5- OCH_2), 7.10−7.60 (4m, 15H, 3 × C_6H_5). MS (EI): m/e 475 $(M)^+$, 438 $(M - OC_2H_5)^+$, 384 $(M - C_6H_5CH_2)^+$, 356 $(M - CH_2{=}CH_2)^+$, base.

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]ethylamino]-5′-(*N*-ethylcarbamoyl)-adenosine ([^{3}H]CGS 21680) to A_{2A} receptors from rat striatal membranes was performed as described previously.[24,25] 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 (Receptor Biology, Inc., Baltimore, MD) or to membranes prepared from CHO cells stably expressing the rat A_3 receptor was performed as described.[27,28] The assay medium consisted of a buffer containing 50 mM Tris, 10 mM Mg^{2+}, 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 200 μM NECA.

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[32] 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, 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]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine; CHO cells, Chinese hamster ovary cells; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; HEK cells, human embryonic kidney cells; IB-MECA, N^6-(3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine; K_i, equilibrium inhibition constant; NECA, 5′-(*N*-ethylcarbamoyl)adenosine; (*R*)-PIA, (*R*)-N^6-(phenylisopropyl)adenosine; SAR, structure−activity relationship; Tris, tris(hydroxymethyl)aminomethane.

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