N-Substituted Adenosines as Novel Neuroprotective A_1 Agonists with Diminished Hypotensive Effects

Lars J. S. Knutsen,*,† Jesper Lau, Hans Petersen,‡ Christian Thomsen, Jan U. Weis, Michael Shalmi, Martin E. Judge, Anker Jon Hansen, and Malcolm J. Sheardown†

Health Care Discovery and Development, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

Received March 2, 1999

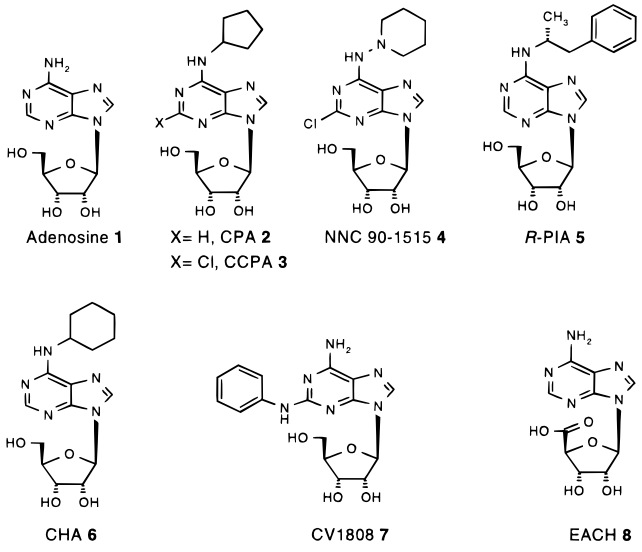
The synthesis and pharmacological profile of a series of neuroprotective adenosine agonists are described. Novel A_1 agonists with potent central nervous system effects and diminished influence on the cardiovascular system are reported and compared to selected reference adenosine agonists. The novel compounds featured are derived structurally from two key lead structures: 2-chloro-*N*-(1-phenoxy-2-propyl)adenosine (NNC 21-0041, **9**) and 2-chloro-*N*-(1-piperidinyl)adenosine (NNC 90-1515, **4**). The agonists are characterized in terms of their in vitro profiles, both binding and functional, and in vivo activity in relevant animal models. Neuroprotective properties assessed after postischemic dosing in a Mongolian gerbil severe temporary forebrain ischemia paradigm, using hippocampal CA1 damage endpoints, and the efficacy of these agonists in an A_1 functional assay show similarities to some reference adenosine agonists. However, the new compounds we describe exhibit diminished cardiovascular effects in both anesthetized and awake rats when compared to reference A_1 agonists such as (*R*)-phenylisopropyladenosine (*R*-PIA, **5**), *N*-cyclopentyladenosine (CPA, **2**), **4**, *N*-[(1*S*,*trans*)-2-hydroxycyclopentyl]adenosine (GR 79236, **26**), *N*-cyclohexyl-2′-*O*-methyladenosine (SDZ WAG 994, **27**), and *N*-[(2-methylphenyl)methyl]adenosine (Metrifudil, **28**). In mouse permanent middle cerebral artery occlusion focal ischemia, 2-chloro-*N*-[(*R*)-[(2-benzothiazolyl)thio]-2-propyl]-adenosine (NNC 21-0136, **12**) exhibited significant neuroprotection at the remarkably low total intraperitoneal dose of 0.1 mg/kg, a dose at which no cardiovascular effects are observed in conscious rats. The novel agonists described inhibit 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate-induced seizures, and in mouse locomotor activity higher doses are required to reach ED_{50} values than for reference A_1 agonists. We conclude that two of the novel adenosine derivatives revealed herein, **12** and 5′-deoxy-5′-chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine (NNC 21-0147, **13**), representatives of a new series of P_1 ligands, reinforce the fact that novel selective adenosine A_1 agonists have potential in the treatment of cerebral ischemia in humans.

Introduction

Adenosine (**1**) (Chart 1) is a naturally occurring purine nucleoside, which has a wide variety of well-documented regulatory functions and physiological effects. The central nervous system (CNS) and cardiovascular (CV) effects of this ubiquitous endogenous nucleoside have attracted particular attention in drug discovery, owing especially to the therapeutic potential of purinergic agents in CV and CNS disorders.[1−4] This therapeutic potential has resulted in a considerable recent research endeavor within this field.[5]

In the CNS adenosine modulates synaptic transmission without itself acting as a neurotransmitter.[6] It is now becoming apparent that adenosine and adenosine receptor ligands have a range of potential applications in the therapy of CNS disorders,[7] since adenosine appears to act as an endogenous neuroprotectant[8] and antiepileptic agent.[9−11] Enhanced metabolic activity, with high ATP to ADP turnover, leads in turn to elevated extracellular adenosine levels,[12] which cause hyperpolarization and reduced neuronal firing. Indeed, investigators utilizing microdialysis have measured elevated levels of adenosine postischemia,[13] giving evidence of its apparent endogenous neuroprotective

Chart 1. Structures of Reference A_1 Receptor Agonists



* To whom correspondence should be addressed. Tel: (+44) 118 977 3133. Fax: (+44) 118 989 9300. E-mail: L.Knutsen@Cerebrus.ltd.uk.

† Current address: Cerebrus Ltd., 613 Reading Road, Oakdene Court, Winnersh, Wokingham, Berkshire RG41 5UA, England.

‡ Current address: H. Lundbeck A/S, Ottiliavej 9, DK-2500 Copenhagen - Valby, Denmark.

10.1021/jm960682u CCC: $18.00
Published on Web 08/18/1999

function. These observations have been confirmed in human subjects.[14] This background data led us to investigate the concept of adenosine agonism as a mechanism for the discovery of novel drug candidates with neuroprotective properties.

Adenosine Receptor Subtypes

Adenosine receptors represent a subclass (P1) of the group of purine nucleotide and nucleoside receptors known as purinoceptors. The main pharmacologically distinct adenosine receptor subtypes are known as A_1, A_{2A}, A_{2B} (of high and low affinity), and A_3.[15] Selective ligands exist for these G-protein-coupled adenosine receptors, and the structure−activity relationships of the various reference ligands have been reviewed.[1,16] The most recently identified subtype is the A_3 receptor.[17,18]

Among the known adenosine receptor agonists most selective for the A_1 receptor over the A_{2A} receptor are the examples where the adenine nucleus is substituted with a cyclic group on the amino function, for example, the key reference agonists *N*-cyclopentyladenosine (CPA, **2**)[1,16,19] and 2-chloro-*N*-(1-piperidinyl)adenosine[20] (**4**).

Proposals have been put forward for the possible existence of subtypes of the adenosine A_1 receptor. The original suggestion for classification into A_{1A} and A_{1B} subtypes was initially put forward by Gustafsson[21] in 1989, based on the rank order of potency of a series of agonists and antagonists. Owing to a lack of subsequent studies, it appears that this postulate has yet to receive wide acceptance in the scientific community, as evidenced by lack of inclusion of these subtypes in a recent status summary of P1 receptor nomenclature.[15] However, Mahan et al. have more recently deduced the existence of a high- and low-affinity binding site at a cloned rat A_1 receptor.[22] Furthermore, Palmer and Stiles[23] have proposed the bovine brain adenosine receptor as a distinct A_1 subtype, and Meng et al.[24] have cloned an adenosine A_1 receptor from guinea pig brain with high affinity for the adenosine A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), but with uniquely low affinity for otherwise potent and selective A_1 agonists such as 2-chloro-*N*-cyclopentyladenosine (CCPA, **3**), *N*-cyclohexyladenosine (CHA, **6**), and (*R*)-phenylisopropyladenosine (*R*-PIA, **5**). One could foresee that there may be further progress toward a rational reinterpretation of the adenosine A_1 receptor in the future, possibly when radioligand binding assays featuring newer A_1 receptor ligands become available.

Of the different adenosine receptor subtypes, the A_1 receptor is apparently the most prevalent in the mammalian CNS,[25] with high density in the hippocampus, cerebral cortex, and cerebellum.

In Vivo Effects of Adenosine Agonists

Adenosine and certain adenosine receptor agonists inhibit the release of excitatory amino acids such as glycine and glutamate when acting via the adenosine A_1 receptor.[26−28] It is recognized that the unrestrained release of these neurotransmitters[29] contributes to the process causing severe neuronal damage in the period following an interruption of blood supply to the brain in humans,[30,31] for example, during stroke or cardiac arrest, as demonstrated recently by human microdialysis.[32]

However, other aspects of the pharmacological action of adenosine could also be of great utility in protection from the deleterious effects of in vivo ischemia,[33] for example, inhibition of free radical formation[34] as well as inhibition of platelet aggregation via the A_2 receptor. Adenosine also activates antioxidant enzymes during ischemia,[35] and an apparent beneficial effect on cerebral blood flow in humans has also been observed.[36] Furthermore, adenosine agonists have been shown to control levels of tumor necrosis factor-α,[37] a cytokine which has been implicated as a mediator of brain injury.[38]

The mammalian cardiovascular system is under several different forms of purinergic control. Stimulation of heart A_1 receptors results in bradycardia, whereas agonists at vascular A_2 receptors cause vasodilation. However, several articles, when taken together, reveal that the whole spectrum of adenosine's cardiovascular effects is rather complex.[39]

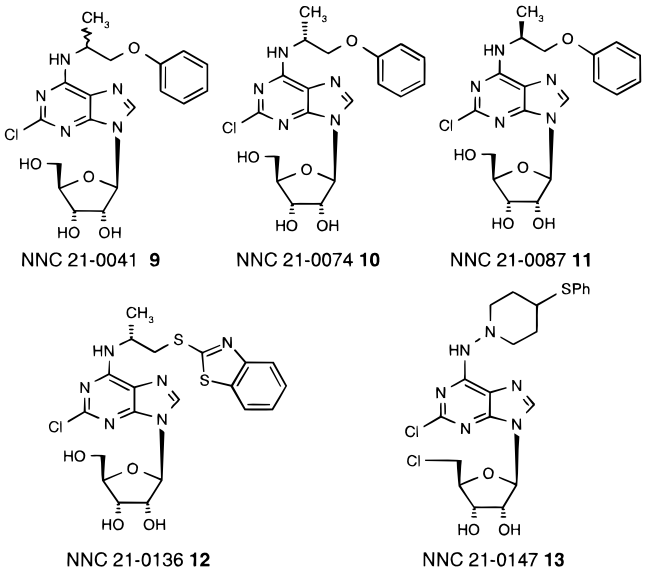
This is especially so when species differences, the traditional bugbear of pharmaceutical research, are taken into consideration. For example, the antilipolytic adenosine A_1 agonist *N*-cyclohexyl-2′-*O*-methyladenosine (SDZ WAG 994, **27**) (see Tables 1 and 2) has a potent hypotensive effect when dosed in rodents, but a milder effect is observed in primates.[40]

A similar phenomenon has been observed with other adenosine derivatives between rodent species,[41] and several different potential explanations for these species differences has been put forward.[42−44] Further complications can arise because of the potential for some central control of hypotension.[45] One particularly intriguing explanation is put forward by Cornfield et al., which proposes the existence of an A_4 adenosine receptor as an interpretation for some observations made with 2-(phenylamino)adenosine (CV 1808, **7**).[46]

The reported human clinical studies on adenosine receptor agonists, aside from the marketed drug adenosine itself, are also revealing. A range of 2-haloadenosines injected into the human forearm, reported in 1965, were found to have a stronger vasodilatory effect than adenosine.[47] *N*-[(2-Methylphenyl)methyl]adenosine (Metrifudil, **28**), an early clinical candidate,[48] later shown to be a mixed adenosine A_1 and A_2 receptor agonist[49] with some A_3 receptor affinity,[50] was tolerated in humans at 0.1 mg/kg iv or 0.5 mg/kg po without critical changes in circulatory parameters. A clinical study of *R*-PIA (**5**), which has since become a vital reference A_1 agonist, concluded that three 2-hourly 1 mg oral doses apparently caused inhibition of lipolysis, an effect associated with adenosine A_1 agonism, but no cardiovascular changes were reported.[51] The ethyl ester of adenosine-5′-carboxylic acid (EACH, **8**) has been evaluated as an antiangina agent.[52] At oral doses of 33 mg, side effects of nausea, numbness, and angina itself were noted, although normal healthy volunteers could apparently tolerate doses of 1−84 mg with minimal adverse effects.

One of the most recent adenosine agonists to be examined clinically is SDZ WAG 994 (**27**). Fifty patients with heart failure symptoms and moderate left ventricular systolic dysfunction had a balloon flotation catheter inserted. Patients received placebo or a single oral dose of either 1, 2, or 5 mg of **27**. No important

Chart 2. Structures of Selected Novo Nordisk A_1 Receptor Agonists



effects on systemic, right atrial, pulmonary artery, or pulmonary capillary wedge pressures; cardiac index; respiratory rate; or heart rate were observed. The PR interval (a reflection of A_1 receptor-mediated activity) increased significantly in a stepwise fashion. Therefore treatment with **27** resulted in no significant hemodynamic changes at rest in this subset of patients with left ventricular dysfunction.[53]

We now wish to report the synthesis and pharmacological activity of five new A_1 receptor agonists (Chart 2) featured as representatives from over 80 examples of novel nucleosides in three recently revealed series.[54−56] We have selected one structure from each of these series for detailed elucidation in this present article. The representative neuroprotectants, 2-chloro-*N*-(1-phenoxy-2-propyl)adenosine[11,54] (**9**), comprising the two diastereoisomers **10** and **11**, 2-chloro-*N*-[(*R*)-[(2-benzothiazolyl)thio]-2-propyl]adenosine[55] (NNC 21-0136, **12**), and 5′-deoxy-5′-chloro-*N*-[4-(phenylthio)-1-piperidinyl]-adenosine[56] (NNC 21-0147, **13**), all have marked CNS effects in a range of models but exhibit lowered cardiovascular effects when compared to reference A_1 agonists such as **2**, **4**, and **5**. These new agonists were selected with the goal of developing an A_1 receptor agonist as a rational treatment for acute cerebral ischemia.

Chemistry

The first adenosine agonist discovered within this project which had a mild cardiovascular profile in vivo when compared to reference A_1 agonists was **9**[11] (Chart 2). A retrosynthetic analysis provided a convenient, high-yielding synthesis of its two diastereoisomers **10** and **11**.[54] These syntheses are illustrated in Scheme 1.

The direct displacement of the 6-chlorine atom in 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine[20,57] (**14**) was accomplished using commercially available 1-phenoxy-2-propylamine which exists as a racemic mixture. The resultant diastereoisomeric mixture **15** was deblocked with methanolic ammonia to afford **9**. The diastereoisomers were not separated but rather individually synthesized from the enantiomers of alaninol. *tert*-Butyloxycarbonyl (Boc) protection of L-alaninol followed by ether formation under Mitsunobu[58] conditions provided the phenyl ether **16**, which could be converted into the amine salt **17** by treatment with hydrochloric acid in ethyl acetate. Reaction with 2,6-dichloro-9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-9*H*-purine[20] (**18**) and deblocking as previously provided the *R*-diastereoisomer **10**. The *S*-diastereoisomer **11** was obtained when D-alaninol was utilized as starting material (Scheme 1).

An overall similar synthetic strategy was used for the novel benzothiazolyl analogue **12**, but in this case 2-mercaptobenzothiazole was used as the nucleophile in place of phenol in the Mitsunobu reaction (Scheme 2).

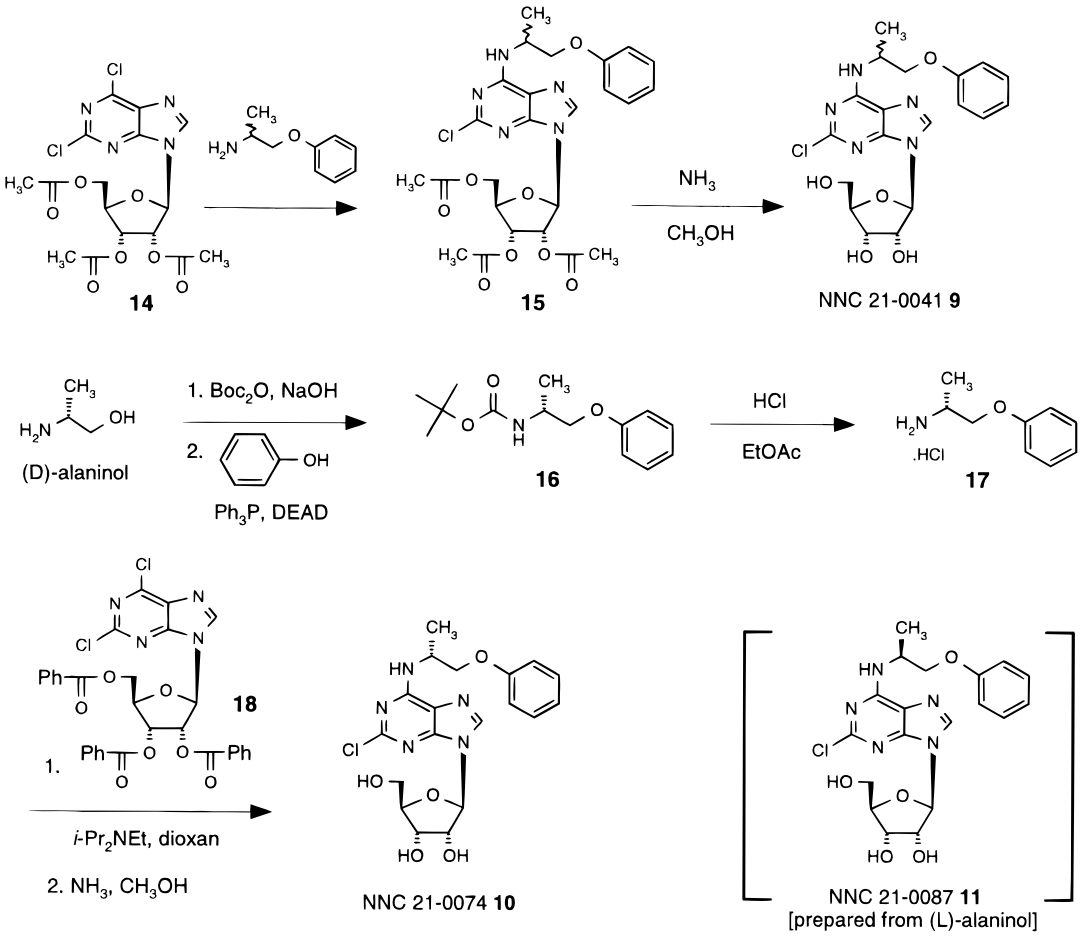
Compound **13** is a 5′-ribose-modified extension of a previously published series of novel, highly potent A_1 receptor agonists[11,20,59] and was prepared from 2-chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine (NNC 21-0129,[11,20] **24)** by a convenient 5′-chlorination which does not involve separate protection of the 2′- and 3′-ribosyl hydroxy groups[60] (Scheme 3). The parent β-D-ribofuranosyl nucleoside **24** was synthesized[20] via the Boc protection/phenyl thioether formation procedure on 4-hydroxypiperidine, followed by a classical *N*-amination procedure involving reduction of the *N*-nitroso intermediate, to afford the phenylthioether **23** (Scheme 3).

The noncommercially available reference A_1 agonists and antilipolytic agents, Glaxo Wellcome's [(1*S*,*trans*)-2-hydroxycyclopentyl]adenosine (GR 79236,[61] **26**), Novartis' SDZ WAG 994[40] (**27**) and the mixed agonist *N*-[(2-methylphenyl)methyl]adenosine[48,49] (**28),** were prepared, the two former agents via new, brief syntheses (Scheme 4). For the synthesis of **26**, we found that rather than separating the enantiomers[62] of *trans*-2-aminocyclopentanol (**29**) before the displacement of chlorine from 6-chloropurine riboside, it was preferable to react the amino alcohol **29** directly. The resultant mixture of diastereoisomers was separable by crystallization and chromatography to provide GR 79236 (**26**). Previous syntheses of the recently described adenosine A_1 agonist SDZ WAG 994 **(27**) have been based on preparing the 2′-*O*-methylated ribose moiety before reacting it with a purine derivative.[63] We evaluated strategies for 3,5-*O*-protection of *N*-cyclohexyladenosine (**6**) before attempting the simplest synthesis method, a direct 2′-*O*-methylation of **6** using methyl iodide, affording **27** in 25% isolated yield (Scheme 4). This method is now the subject of a Sandoz patent application.[64] The minor product from this direct alkylation, 3′-*O*-methyl-*N*-cyclohexyladenosine, could be removed from the reaction mixture by column chromatography. Other key commercially available reference compounds are also featured in Tables 1 and 2.

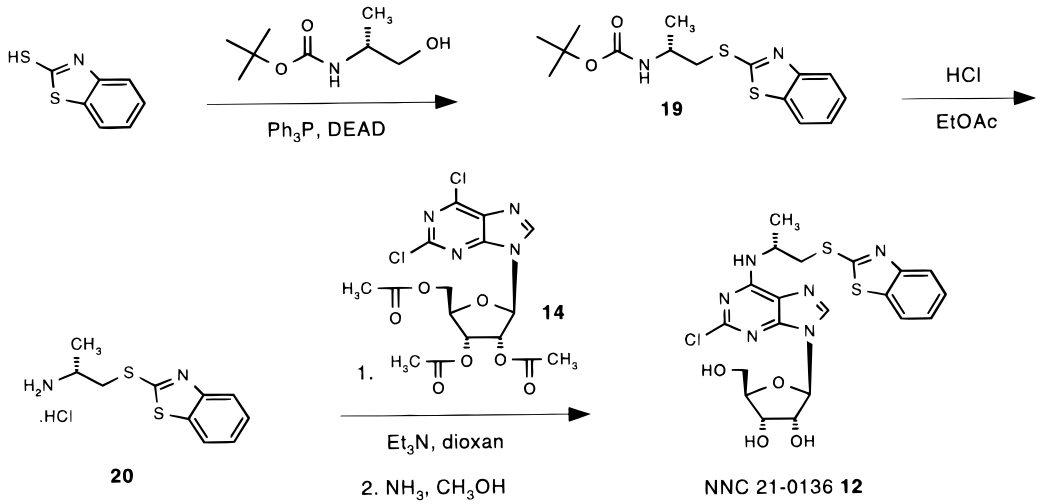
Results and Discussion

In Vitro Profiles. The in vitro biochemical profiles of the five novel adenosine A_1 agonists and eight key reference compounds are shown in Table 1. Affinity for the adenosine A_1 receptor for each compound is quantified by displacement of the binding of [^{3}H]-*R*-PIA from Wistar rat forebrain and for the A_{2A} receptor by displacement of [^{3}H]-2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-(*N*-ethylcarboxamido)adenosine (CGS 21680, **33**) from rat striatal tissue.[49] The A_1 functional assay measuring inhibition of isoprenaline-stimulated cAMP accumula-

Scheme 1. Synthesis of 2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (**9**) and the Two Corresponding 6-Amino Substituent Diastereoisomers **10** and **11**



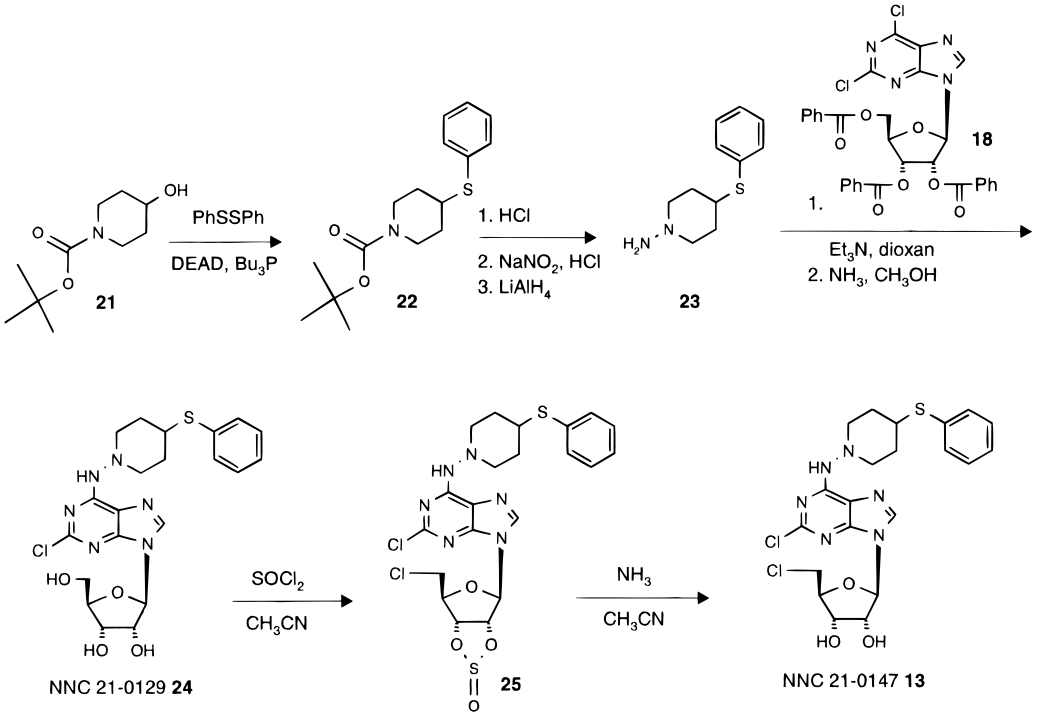
Scheme 2. Synthesis of 2-Chloro-*N*-[*R*-(2-benzothiazolyl)thio-2-propyl]adenosine (**12**)



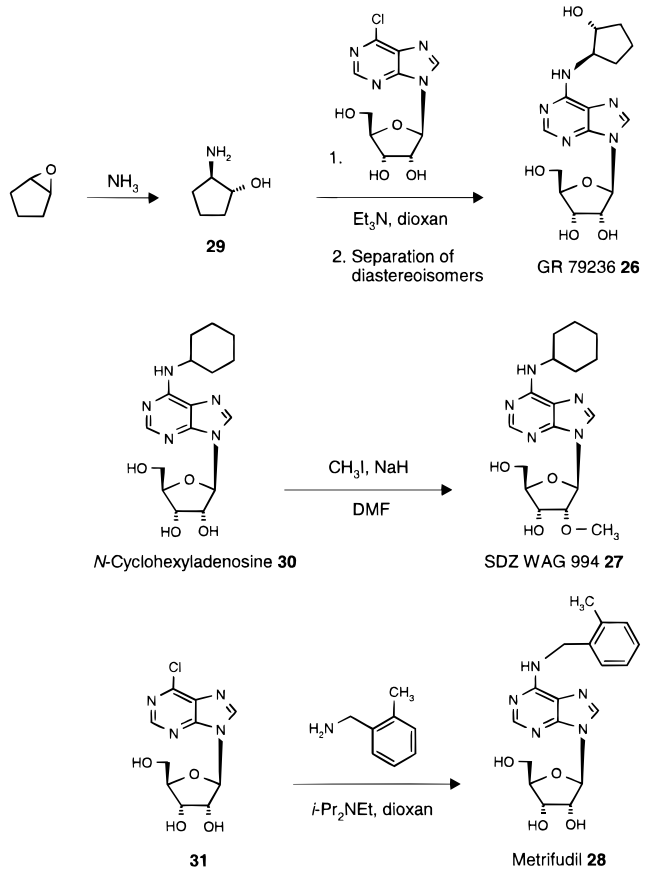
tion in DDT_1-MF2 cells[65] was used to examine whether the compounds investigated exhibited full agonist effect. In addition, the isolated guinea pig atria model was utilized to provide data for assessing bradycardic effects of the compounds studied without the influence of complicating factors which affect in vivo data, such as differences in metabolism, bioavailability, and distribution.

Reference agonists **2, 4**, **5**, and **26** exhibited the expected in vitro characteristics of potent A_1 agonists, with rat brain A_1 receptor binding in the low nanomolar range, selectivity for A_1 receptor binding over A_{2A}, and pronounced effects (IC_{50} < 5 nM) as full agonists in the A_1 functional assay. The novel adenosine agonists **10** and **12** are similar in effect and are slightly less potent than the reference agonists **2, 4**, **5**, and **26**. However, the novel compound **13**, a close structural analogue of **4**, maintains in vitro potency compared to the reference agonists, being essentially equipotent in the binding and functional assays. All the novel compounds were shown to be full agonists in the functional assay. Given that **9** is a 1:1 mixture of **10** and **11**, the functional data is not

Scheme 3. Synthesis of 2,5′-Dichloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine (**13**)



Scheme 4. Synthesis of the Key Reference Compounds GR 79236 (**26**), SDZ WAG 994 (**27**), and Metrifudil (**28**)



surprising, confirming that **10** is the more potent A_1 agonist of this diastereomeric pair.

SDZ WAG 994[40] (**27**), **11,** and *N*-(sulfophenyl)adenosine[66] (**32**) were weaker in terms of A_1 receptor binding than the potent reference A_1 agonists. Our data for **32** (A_1 K_i 126 nM) was close to the published figure (K_i 74 nM). However, our data for SDZ WAG 994 (**27**) (A_1 K_i 76 nM) indicates that it may be weaker than expected as an A_1 agonist in our hands, although there was a species difference (rat forebrain vs pig striatum) between our binding figures and those previously published.[40]

We noted in an earlier study of anticonvulsant effects that *N*-[(2-methylphenyl)methyl]adenosine (**28**) is a slightly A_{2A}-selective agonist,[49] and it has since been shown to have some A_3 receptor affinity (IC_{50} 360 nM).[50] Its property as a low-affinity A_1 receptor agonist is also reflected in the weak effect observed in the corresponding functional assay.

The main thrust of the present article is that the novel adenosine agonists we describe have diminished cardiovascular effects when compared to reference A_1 agonists. A tendency in this direction is apparent in Table 1, within the data for negative inotropy in isolated guinea pig atria; bradycardia is apparently A_1-mediated.[39] Compounds **2, 4,** and **5** exhibited submicromolar inhibition of beating rate, whereas all the novel compounds evaluated were significantly weaker. Agonist **13** is a particularly dramatic example, having similar receptor affinity and A_1 functional effect to the potent reference compounds, but it gave a 50-fold weaker bradycardic effect in this paradigm. Reference agonists **27** and **28** also show a weak effect on isolated guinea pig atria, but this is presumably a reflection of their relatively weak in vitro adenosine A_1 receptor agonism.

In Vivo Effects. Table 2 summarizes some important in vivo effects of the 13 adenosine agonists highlighted in this present study. Intraperitoneal (ip) dosing was utilized in all except the CV studies, where iv dosing was performed. These models are described in detail in the Pharmacological Methods section. Figure 1 shows results from **5** and **12** in a mouse focal ischemia model with comparison to the reference neuroprotectant, the

Table 1. In Vitro Characterization of Adenosine Agonists

compd, no.	adenosine A_1 receptor binding, inhibition of rat brain [^{3}H]-*R*-PIA binding, K_i (nM)	adenosine A_{2A} receptor binding, inhibition of rat brain [^{3}H]CGS 21680 binding, K_i (nM)	adenosine A_1 functional assay, inhibition of cAMP accumulation in DDT-MF2 cells, IC_{50} (nM)	isolated guinea pig atria, negative inotropy, EC_{50} (nM)
R-PIA, **5**	2.6 ± 0.6	117 ± 8	0.4 ± 0.1	440
CPA, **2**	1.2 ± 0.1	321 ± 36	0.5 ± 0.1	420
NNC 90-1515, **4**	4.7 ± 0.5	1485 ± 40	2.1 ± 0.3	580
GR 79236, **26**	3.1 ± 0.4	1300 ± 65	2.6 ± 0.2	1500
SDZ WAG 994, **27**	76 ± 9	5700 ± 2000	40 ± 16	18000
N-(4-sulfophenyl)adenosine, **32**	126 ± 15	4200 ± 140	35 ± 8	
CGS 21680, **33**	9900 ± 1200	11 ± 2	1260 ± 120	
Metrifudil, **28**	98 ± 6	25 ± 5	55 ± 10	37000
NNC 21-0041, **9**	43 ± 9	1086 ± 180	4.8 ± 0.3	8400
NNC 21-0074, **10**	15 ± 2	245 ± 25	3.9 ± 0.1	>22900
NNC 21-0087, **11**	100 ± 11	7420 ± 240	17 ± 3	
NNC 21-0136, **12**	10 ± 1	630 ± 120	3.1 ± 0.8	8200
NNC 21-0147, **13**	3.6 ± 1.3	1150 ± 375	1.4 ± 0.3	25500

Table 2. In Vivo Effects of Adenosine Agonists

compd, no.	reduction of mouse locomotor activity, ip 30 min, ED_{50} (mg/kg)	inhibition of mouse DMCM-induced seizures ip 30 min, ED_{50} (mg/kg)	gerbil 2 vessel occlusion ischemia, reduction in hippocampal cell death (*p*-value), dose (mg/kg)	blood pressure reduction (%) anesthetized rat, 0.1 mg/kg iv bolus, 5 min
R-PIA, **5**	0.05	0.50	24 (0.08), 0.3	78
CPA, **2**	0.07	0.17	75 (0.004), 3.0	62
NNC 90-1515, **4**	0.21	2.0	65 (0.0002), 3.0	78
GR 79236, **26**	0.13	0.30	47 (0.001), 3.0[a]	74
SDZ WAG 994, **27**	0.1	0.23	38 (0.03), 0.3[a]	77
N-(4-sulfophenyl)adenosine, **32**	0.24	3.0		69
CGS 21680, **33**	0.19	4.9	46 (0.0007), 10.0[a]	69
Metrifudil, **28**	0.9	0.54	25 (0.04), 10.0[a]	45
NNC 21-0041, **9**	3.6	3.4	49 (0.003), 1.0	0
NNC 21-0074, **10**	4.3	3.9	18 (0.037), 3.0	8
NNC 21-0087, **11**	14.0	16	6 (0.46), 3.0	15
NNC 21-0136, **12**	2.3	7.8	66 (0.0006), 10.0	6
NNC 21-0147, **13**	8.2	25	45 (0.01), 3.0[a]	10

[a] Dosed 30 and 120 min postischemia.

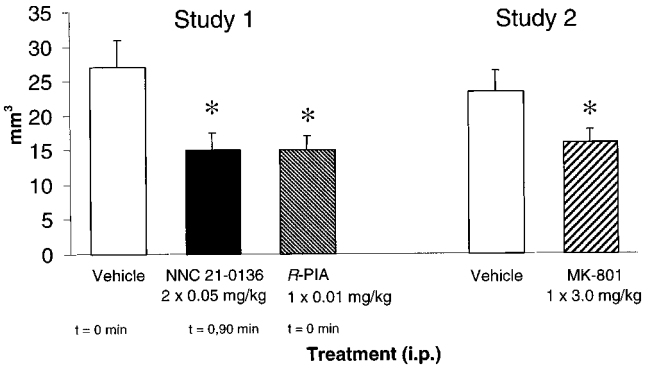


Figure 1. Results in the mouse middle cerebral artery occlusion stroke model. Results are expressed in terms of infarct size in mm^3.

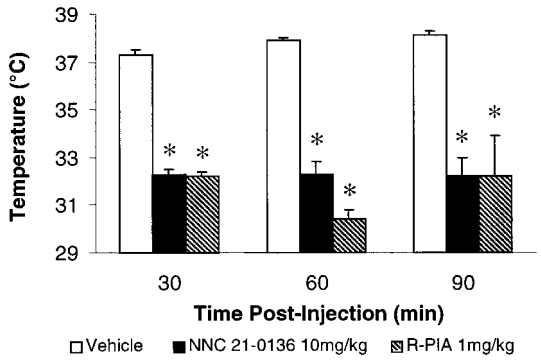


Figure 2. Rectal temperature in mice. Effects of NNC 21-0136 (**12**) and *R*-PIA (**5**).

NMDA antagonist MK-801. Figure 2 reveals mouse rectal temperature data for these compounds. At doses of <10 mg/kg, **12** had no effect on rectal temperature.

Mouse spontaneous locomotor activity is inhibited very potently by all of the eight reference compounds featured in Table 2, virtually regardless of their affinity profile for adenosine A_1 and A_{2A} receptors. It has been shown previously that adenosine receptor agonists are potent locomotor depressants,[11,44,67,68] and although most studies have been carried out on A_1 agonists, the A_{2A} agonist CGS 21680 (**33**) has also been shown previously to possess this property.[69] The data in Table 2 indicate that the five novel adenosine agonists with diminished cardiovascular effects also have markedly less inhibitory effect on spontaneous locomotor activity than the eight reference compounds.

A highly potent anticonvulsant effect is observed with several of the adenosine receptor agonists against seizures induced by the inverse agonist at benzodiazepine receptors, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM).[49,70] We have previously reviewed the background to this observed anticonvulsant effect[10] and have revealed in communication form the ED_{50} values for **2**, **4**, **5**, **9**, **24**, **26**, and **28** in protection against DMCM-induced seizures.[11] The agonist **9** and its *R*-diastereoisomer **10** are the most potent anticonvulsants in the DMCM seizure model among the five novel compounds featured in Table 2.

Table 3. Cardiovascular Effects in Rats as Determined Using Telemetry[a]

	infused iv dose of agonist tested (mg/kg/h)			
	0.0	0.005	0.01	0.05
R-PIA (**5**) MBP (*n*)	102 ± 6 (6)	98 ± 3 (4)	91 ± 7 (4)	80 ± 6 (6)
{HR}	{374 ± 10}	{361 ± 31}	{303 ± 36}	{224 ± 23}

	dose of agonist tested (mg/kg/h)				
	0.0	0.04	0.08	0.12	0.40
Metrifudil (**28**) MBP ($n = 6$ for all)	102 ± 6	101 ± 2	104 ± 3	93 ± 2	
{HR}	{383 ± 20}	{378 ± 20}	{438 ± 15}	{451 ± 13}	
NNC 21-0136 (**12**) MBP ($n = 6$)	101 ± 2	104 ± 2	97 ± 2	103 ± 3	86 ± 3
{HR}	{361 ± 12}	{359 ± 14}	{308 ± 13}	{305 ± 18}	{217 ± 10}

[a] Figures quoted are for mean blood pressure (MBP) in mmHg with number of animals (*n*) and heart rate {HR}.

Special mention of the potent effect of SDZ WAG 994 (**27**) in these two behavioral models is warranted because of its relatively weak in vitro profile. This unusual agonist is around 2 orders of magnitude weaker than, for example, reference compounds **2** and **5** in the in vitro assays in Table 1, but its effect in locomotor activity and DMCM seizure protection is equivalent to that of these two reference compounds. For this reason we conclude that **27** appears to be an atypical A_1 receptor agonist[71] compared to the other reference compounds studied and warrants further study in this respect.

Neuroprotection and Cardiovascular Studies. We demonstrate herein that a range of both novel and reference A_1 receptor-selective adenosine agonists show neuroprotectant properties when administered 30 min after a period of severe forebrain ischemia in the Mongolian gerbil (Table 2). Furthermore, the data suggest that hypothermia is not a prerequisite for a neuroprotective effect (Figure 2).

The distinguishing characteristics of the novel A_1 agonists when compared to the reference agonists reported herein are the pronounced neuroprotective effects, combined with diminished hypotensive and behavioral effects, compared to reference A_1 receptor agonists. One of the novel compounds, **12** has in addition been found to exhibit a marked reduction in infarct size in a mouse model of focal ischemia (Figure 1). Precise details of these ischemia paradigms are given in the Pharmacological Methods section.

Only a relatively small range of adenosine A_1 agonists have previously shown neuroprotectant activity against global or focal cerebral ischemia in rodents.[8] These are *R*-PIA (**5**),[72−75] CPA (**2**),[76] CHA (**6**),[77] 2-chloroadenosine,[78] adenosine amine congener (ADAC),[79] and NNC 21-0113.[37]

Cardiovascular Studies. A feature of the in vivo effects of adenosine receptor agonists, illustrated by the eight reference agonists in Table 2, is the profound dose-dependent hypotension induced in rats. This hypotensive effect is observed regardless of the in vitro binding profile of these reference agonists, because agonists at heart adenosine A_1 receptors result in bradycardia and A_2 receptor agonism leads to vasodilation.[39]

The ultimate target of our investigations is the application of new adenosine agonists to the treatment of stroke and cerebral ischemia in humans. Consequently, ideal drug candidates should be devoid of hypotensive side effects at neuroprotectant doses, as this target group of patients is clearly already in a situation of compromised cerebral blood supply. Our strategy within this research program has therefore been only to select novel agonists for evaluation in gerbil neuroprotection if they have markedly lower blood pressure (BP) effects than reference A_1 agonists.

This criterion is illustrated in Table 2, from which it is clear that all the eight reference adenosine agonists evaluated here have profound hypotensive effects. The simple blood pressure model utilized was the barbiturate-anesthetized rat ($n = 3$), with arterial BP measured 5 min after an intravenous (iv) bolus dose of 0.1 mg/kg. This model is not intended to give an absolute measure of the hypotensive effect, as the reflexes required for maintenance of blood pressure are apparently compromised by the use of an anesthetic. The evaluation of blood pressure in conscious rats by telemetry (see Table 3) provides a more objective and accurate assessment of CV effects, but the anesthetized rat is very useful for rapid data providing a comparison within a series of compounds.

Given the need for new adenosine receptor ligands which combine neuroprotection with an improved cardiovascular profile, we have synthesized and evaluated a wide range of novel adenosine derivatives.[20,54−56] This drug design process led to the discovery of **9**,[11,54] which has a significantly diminished hypotensive effect. This remarkable CV profile set agonist **9** apart from the reference A_1 agonists in Table 2, as we could observe no alteration in BP when assessed under the conditions described.

Compound **9** was therefore our first lead structure in this series with diminished cardiovascular effects compared to reference A_1 agonists and was found to have a potent effect in the cAMP A_1 functional assay. This agent also exhibited potent neuroprotection in the gerbil ischemia model and inhibited DMCM-induced seizures, as noted above. When the two components making up this agonist, the *S*-diastereoisomer **11** and the *R*-isomer **10**, were synthesized and examined separately, **11** was markedly weaker in the assays used, aside from the BP study, than **10**. This parallels observations made with the isomers of *N*-(phenylisopropyl)adenosine; *R*-PIA (**5**) is more potent as an A_1 agonist than *S*-PIA.[1]

We have noted above that compound **13** was around 50-fold weaker than expected in negative inotropy in the guinea pig atria when compared to reference agonists such as **4** or *R*-PIA (**5**), despite having a very similar binding and functional profile. This is reflected in its anesthetized rat BP-lowering value of 10% at the standard dose, which is a markedly lower value than that of the reference agonists.

Cardiovascular Effects Assessed by Telemetry. To assess the absolute CV effects of adenosine receptor agonists in conscious, nonanesthetized animals, we selected **5**, **12**, and **28** for evaluation in a model where the CV effects were assessed by telemetry (Table 3). In this model, BP and heart rate were assessed remotely from an implanted sensor in rats. This paradigm provides a more objective and accurate assessment of CV effects owing to the absence of any anesthetic agent and the fact that the baroreflex appears to be more intact in these animals. Agonist **12** is approximately 20 times weaker than *R*-PIA (**5**) in terms of producing hypotension in conscious rats, despite only a ca. 5-fold difference in their biochemical profile as A_1 receptor agonists. *N*-[(2-Methylphenyl)methyl]adenosine (**28**)[49] is included with **5** because both of these drugs have been evaluated in humans.[48,51]

Global Ischemia. As stated above, drug candidates for cerebral ischemia should ideally be devoid of hypotensive side effects at neuroprotective doses, but curiously we find that in the gerbil ischemia model, neuroprotection is observed virtually regardless of the CV profile of the adenosine agonists evaluated in this study (Table 2). This is surprising, given that the gerbil model involves reperfusion, but one explanation may be that cerebral blood flow is maintained or improved during ischemia despite peripheral hypotension.[80]

Compound **12** is derived from and structurally related to **10** but contains a 2-benzothiazolylthio moiety in place of the phenoxy function. This change confers greater lipophilicity to the molecule, to assist it in crossing the blood−brain barrier. Nucleoside **12** is a member of a later compound series[81] compared to **10**, and its pronounced neuroprotective effects have been reported in brief previously.[82,83]

The compounds showing >50% protection from hippocampal cell death in this paradigm were **2**, **4**, and **12**. Several other compounds exhibited significant neuroprotection ($p < 0.05$). The only adenosine agonists in this current series that did not prove effective in this global ischemia model were **5** and compound **11**. The surprising data we show for the A_{2A} agonist CGS 21680 (**33**), which we unexpectedly found to have neuroprotectant properties, has been discussed in greater depth elsewhere.[84]

The lack of a significant neuroprotective effect when dosing *R*-PIA (**5**) in this gerbil global ischemia model was particularly intriguing.[85] This key reference A_1 agonist is not effective despite producing a profound dose-related reduction in body temperature. The dose range from 0.01 to 1.0 mg/kg was utilized, without significant neuroprotectant effect being achieved, and hypothermia would certainly be observed at the higher doses (see Figure 2). Figure 2 illustrates rectal temperature in NMRI mice following doses of *R*-PIA (**5**) and **12**, with the conclusion that compound **12** has a much less profound hypothermic effect than reference agonist **5**. The lack of neuroprotectant effect with **5**, even though hypothermia is likely at the dose administered, indicates agreement with the finding of Welsh and Harris that postischemic hypothermia alone does not afford neuroprotection in the gerbil.[86]

The conclusion from this gerbil severe forebrain ischemia study is that adenosine A_1 receptor agonists exhibit a potent neuroprotectant effect and that a hypothermic action alone does not produce a neuroprotectant effect. The novel agonists such as **9**, **12**, and **13** with diminished hypotensive effects provide a comparable degree of neuroprotection to the reference adenosine agonists featured in Table 2.

Focal Ischemia. Two of the compounds from Table 2 with a neuroprotective effect in the gerbil 2VO model, *R*-PIA (**5**) and **12**, were selected for investigation in a focal stroke model, in which permanent middle cerebral artery occlusion (MCAO) was performed in NMRI mice (Figure 1). The test drug was administered ip rapidly post-ischemia, and after 24 h the infarct volume was estimated by histology of coronal sections as described in the Experimental Section.

We found that significant and reproducible neuroprotection could be obtained with remarkably low doses of these compounds in this model, which was further validated by comparison with the NMDA antagonist MK-801 administered at 3 mg/kg (Figure 1). The dose of **5** administered was 0.01 mg/kg ip immediately following ischemia, and a similar highly significant neuroprotection was obtained when **12** (0.05 mg/kg ip) was administered 0 and 90 min postischemia.

One of the problems involved in interpreting the investigations described herein is the fact that different rodent species were utilized in the in vivo paradigms. In an ideal world this would not be the case, but the recognized paradigms we chose to utilize have been established in different species. However, it is documented from cloning of A_1 adenosine receptors from a range of species that the overall structures of these clones and the expressed protein are remarkably similar in that each encodes a protein of ~326 amino acids. The bovine brain receptor does exhibit pharmacological differences, but we have avoided that species.[87] Furthermore, there may be differences in metabolism, bioavailability, and distribution of the agonists between species which could lead to apparent interspecies differences, but we have set out to provide a full set of data for the agonists in each paradigm which allows comparison across the whole series of compounds.

Further mechanistic studies are in progress which will assist in elucidating the nature of this apparent selectivity, and we have recently published a profile of the radioligand of **12**.[91] This radioligand did show some differences in ligand binding compared to *R*-PIA (**5**) but overall did possess the characteristics of an adenosine A_1 receptor ligand. However, a potential explanation for the findings revealed above may lie in tissue differences between adenosine A_1 receptors or their coupling, where observable differences have been brought about by these novel agonists.

Conclusion

We have described the discovery of a series of novel adenosine receptor agonists. A potential drawback to the development of adenosine agonists for stroke therapy has formerly been their receptor-mediated cardiovascular effects, resulting in dose-dependent hypotension.

We have found that in the gerbil model of severe temporary forebrain ischemia with postischemic dosing, the novel adenosine agonists such as **9**, **12,** and **13** provide a comparable degree of neuroprotection to

several reference adenosine agonists. However, they show lowered CV effects evidenced by diminished hypotension when compared to reference adenosine agonists in two models. The observed neuroprotection is not explained by hypothermic effects.[86] Furthermore, in mouse MCAO focal ischemia, **12** exhibited significant neuroprotection at a total dose of 0.1 mg/kg, a dose at which no cardiovascular effects are observed in conscious rats. CNS effects are also evidenced by inhibition of DMCM-induced seizures, and the compounds show lowered behavioral effects when compared to reference A_1 agonists in mouse locomotor activity.

Given the promising profile described above, we conclude that neuroprotective adenosine agonists with diminished hypotensive effects show considerable potential for the treatment of cerebral ischemia in humans. The full SAR of compounds from these three new series of adenosine agonists[54−56] will be the subject of future publications.

Experimetal Section

Chemistry. TLC is thin-layer chromatography, THF is tetrahydrofuran, TFA is trifluoroacetic acid, and mp is melting point. Where melting points are given, these are uncorrected. The structures of the compounds are confirmed by assignment of 400-MHz NMR spectra (from which representative peaks are quoted) and by microanalysis where appropriate. Compounds used as starting materials are either known compounds or compounds which can be prepared by methods known per se. Flash chromatography was carried out using the technique described by Still et al.[88] on Merck silica gel 60 (Art 9385). HPLC was carried out on a Merck Hitachi model L6200A Intelligent chromatograph interfaced to a Merck Hitachi L4000A UV detector to a LiChrospher 100 reversed-phase C_{18} column (5 μm, 250 × 4 mm, 5 mm, 100 Å; eluent flow rate 1 mL/min). Retention times are given in min.

2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (9). 1-Phenoxy-2-propylamine (16.62 g, 110 mmol) and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloro-9*H*-purine (**14**)[20,89] (24.6 g, 55 mmol) were stirred in dioxane (250 mL) in the presence of Et_3N (7.23 g, 71.5 mmol) for 18 h. The reaction mixture was filtered and evaporated. The residue was dissolved in a mixture of H_2O (200 mL) and CH_2Cl_2 (200 mL). The CH_2Cl_2 phase was separated, washed with H_2O (2 × 100 mL), and dried ($MgSO_4$). Evaporation provided **15** as a residue which was deprotected with a solution of Na (0.15 g, 6.5 mmol) in CH_3OH (250 mL) at room temperature. After 2 h the reaction mixture was neutralized with citric acid (2.0 g, 10.4 mmol) and treated with a mixture of EtOAc (300 mL) and H_2O (200 mL). The EtOAc phase was separated, dried ($MgSO_4$), and evaporated before being purified by flash chromatography (initially CH_2Cl_2, then with CH_2Cl_2/EtOH, 90/10) to provide **9** (18.2 g, 76%) as an amorphous foam (mixture of diastereoisomers): ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, d, $-CH_3$), 3.53−3.59 (1H, m, H-5′$_a$), 3.64−3.71 (1H, m, H-5′$_b$), 3.95 (1H, q, H-4′), 4.06−4.20 (3H, 2 m, H-3′ and $-CH_2-$), 4.54 (1H, m, H-2′), 4.65 (1H, m, $-CHCH_3$), 5.07 (1H, t, 5′-OH), 5.21, 5.50 (2H, 2d, 2′- and 3′-OH), 5.84 (1H, d, H-1′), 6.87−7.00 (3H, m, Ar−H), 7.23−7.32 (2H, t, Ar−H), 8.31−8.45 (2H, m, H-8 and N−H); HPLC purity 97.8%.

The maleate salt was prepared by dissolving **9** (1.7 g, 3.9 mmol) in THF (10 mL) and adding diethyl ether (60 mL) followed by maleic acid (0.45 g, 3.9 mmol). The residue on evaporation was treated with Et_2O (50 mL), and the maleate salt precipitated and was collected by filtration (1.15 g): mp 102−104 °C. Anal. ($C_{23}H_{26}ClN_5O_9$) C, H, N.

(*R*)-2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (10). 1. 2-[(*R*)-*N*-(*tert*-Butyloxycarbonyl)amino]-1-phenoxypropane (16). (*R*)-2-Amino-1-propanol (D-alaninol) (15.0 g, 200 mmol) was dissolved in 1 N sodium hydroxide (200 mL), and THF (300 mL) was introduced. The reaction mixture was cooled to 0 °C, and di-*tert*-butyl dicarbonate (52.4 g, 240 mmol) in THF (100 mL) was added dropwise over 0.5 h. The reaction mixture was stirred at ambient temperature for 24 h before the phases were separated. The aqueous phase was washed with EtOAc (2 × 200 mL). The organic phases were combined, washed with saturated brine, dried ($MgSO_4$), and evaporated to a residue. Crystallization from *n*-hexane provided 2-[(*R*)-*N*-(*tert*-butyloxycarbonyl)amino]-1-propanol (24.05 g, 69%): mp 59−61 °C; ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, d, $-CHCH_3$), 1.45 (9H, s, butyl-CH_3), 3.50 (1H, dd, $-CH_2$a−), 3.65 (1H, dd, $-CH_2$b−), 3.70−3.80 (1H, m, CH).

This 2-[(*R*)-*N*-(*tert*-butyloxycarbonyl)amino]-1-propanol (10.0 g, 57 mmol), Ph_3P (22.5 g, 86 mmol), and PhOH (5.4 g, 57 mmol) were dissolved in $PhCH_3$ (200 mL). Diethyl azodicarboxylate (14.9 g, 86 mmol) in $PhCH_3$ (100 mL) was slowly added while keeping the temperature below 35 °C.[58] The resultant yellow solution was stirred for 16 h at room temperature before being washed with 1 N aqueous HCl (3 × 100 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo, and the residual oil was purified by flash chromatography (*n*-heptane/EtOAc, 4/1) to provide **16** (8.0 g, 59%): ^{1}H NMR (DMSO-d_6) δ 1.10 (3H, d, $-CH_3$), 1.38 (9H, s, butyl-CH_3), 3.70−3.90 (3H, m, $-CH-CH_2-$), 6.85−6.95 (3H, m, Ar−H), 7.25 (2H, t, Ar−H).

2. (*R*)-*N*-1-Phenoxy-2-propylamine (17). 16 (8.0 g, 33 mmol) was dissolved in EtOAc (100 mL), and a solution of HCl-(g) in dry EtOAc (saturated at room temperature, ca. 5.5 N) (100 mL) was added. The reaction mixture was stirred at room temperature for 18 h during which time a heavy precipitate was formed. The reaction mixture was concentrated to one-half the original volume before the product was collected by filtration and dried in vacuo to provide **17** as a white solid hydrochloride (4.3 g, 69%): mp 186−189 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, d, $-CH_3$), 3.51−3.60 (1H, m, −CH−), 4.05 (1H, dd, $-CH_2$a−), 4.12 (1H, dd, $-CH_2$b−), 6.95−7.00 (3H, m, Ar−H), 7.32 (2H, t, Ar−H).

3. (*R*)-2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (10). **17** (4.3 g, 23 mmol) and 9-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloro-9*H*-purine (**18**)[89] (11.2 g, 18 mmol) were added to a solution of diisopropylethylamine (5.3 g, 41 mmol) in dioxane (150 mL). The reaction mixture was stirred at room temperature for 18 h, heated at 50 °C for 4 h, and stirred at room temperature for 60 h before being filtered and evaporated. The residue was purified by flash chromatography (*n*-heptane/EtOAc, 60/40) yielding **15** before being treated with methanolic ammonia (150 mL). The yellowish solution was evaporated to a residue and purified by flash chromatography (CH_2Cl_2/EtOH/aq NH_3, 90/10/1) to provide **10** as a foam (4.2 g, 64%): ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, d, $-CH_3$), 3.52−3.59 (1H, m, H-5′$_a$), 3.63−3.72 (1H, m, H-5′$_b$), 3.92−3.99 and 4.10−4.21 (4H, 2 m, H-3′, H-4′ and $-CH_2-$), 4.52 (1H, dd, H-2′), 4.65 (1H, m, $-CH_3CH-$), 5.07 (1H, t, 5′-OH), 5.22, 5.49 (2H, 2d, 2′ and 3′-OH), 5.84 (1H, d, H-1′), 6.88−7.02 (3H, m, Ar−H), 7.24−7.33 (2H, dd, Ar−H), 8.32−8.45 (2H, s & m, H-8 and N−H). Anal. ($C_{19}H_{22}ClN_5O_5$) C, H, N.

(*S*)-2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (11). (*S*)-2-Chloro-*N*-(1-phenoxy-2-propyl)adenosine (**11**) was prepared by the procedure described for **10**, except that (*S*)-2-amino-1-propanol (L-alaninol) was used in the first step, thereby providing the opposite diastereoisomer to **10**. Anal. ($C_{19}H_{22}ClN_5O_5$·0.25(H_2O) C, H, N.

***N*-[(*R*)-1-[(2-Benzothiazolyl)thio]-2-propyl]-2-chloroadenosine (12). 1. 2-[(*R*)-[(*N*-(*tert*-Butyloxycarbonyl)amino]-1-propylthio]benzothiazole (19).** To a solution of 2-[(*R*)-*N*-(*tert*-butyloxycarbonyl)amino]-1-propanol (see precursor of **16**) (15.0 g, 85.6 mmol) in THF (200 mL) 2-mercaptobenzothiazole (14.3 g, 85.6 mmol) and Ph_3P (24.7 g, 94.2 mmol). After stirring for 0.25 h a solution of diethyl azodicarboxylate (14.8 mL, 94.2 mmol) in dry THF (100 mL) was added dropwise over 0.5 h. The reaction mixture was stirred for 1 h at 20 °C and filtered. The filtrate was evaporated and stirred with EtOAc (100 mL), and the product was collected by filtration to provide **19** (20.66 g, 74%) as a solid: mp 120−122 °C (EtOAc/*n*-heptane). The filtrate was evaporated and purified by flash chromatography

(initially with *n*-heptane, then *n*-heptane/EtOAc, 70/30) to give a further batch of **19** (5.9 g, 21%).

2. 2-[(*R*)-2-Amino-1-propylthio]benzothiazole Hydrochloride (20). 19 (26.5 g, 81.6 mmol) was dissolved in EtOAc (100 mL), and a solution of HCl(g) in EtOAc (100 mL) (see **17**) was added. The reaction mixture was stirred at room temperature for 18 h, and the precipitated solid was collected by filtration to provide a first batch of **20** (5.3 g, 32%): mp 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, d, −CHCH_3), 3.56−3.77 (3H, m, −CHCH_2−), 7.39, 7.50 (2H, 2t, Ar−H), 7.89, 8.05 (2H, 2d, Ar−H). Anal. ($C_{10}H_{13}N_2S_2Cl$) C, H, N. Evaporation of the filtrate to ca. 50% of its original volume gave a further batch of **20** (14.0 g, 57%).

3. *N*-[(*R*)-1-[(2-Benzothiazolyl)thio]-2-propyl]-2-chloroadenosine (12). To a solution of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloro-9*H*-purine[89] (**14**) (6.27 g, 14.0 mmol) in dry dioxane (100 mL) were introduced **20** (5.0 g, 19.1 mmol) and Et_3N (4.96 g, 6.8 mL, 49.1 mmol) at 20 °C. After stirring at 20 °C for 40 h the reaction mixture was filtered and evaporated to a residue, which was purified by flash chromatography (heptane/EtOAc, 1/1) to afford 2′,3′,5′-tri-*O*-acetyl-2-chloro-*N*-[(*R*)-1-[(2-benzothiazolyl)thio]-2-propyl]adenosine (8.5 g, 86%) as a foam: ^{1}H NMR ($CDCl_3$) δ 1.48 (3H, d, −CHCH_3), 2.09, 2.14, 2.17 (3H, 3s, 3 x −COCH_3), 5.58 (1H, dd, H-3′), 5.74 (1H, dd, H-2′), 6.13 (1H, d, H-1′), 7.78 (1H, s, H-8), 8.49 (1H, d, N−H); TLC R_f 0.19 (SiO_2; heptane/EtOAc, 1/1).

This 2′,3′,5′-tri-*O*-acetyl-2-chloro-*N*-[(*R*)-1-[(2-benzothiazolyl)-thio]-2-propyl]adenosine (8.5 g, 13.4 mmol) was dissolved in CH_3OH (200 mL), and Na metal (0.031 g, 1.34 mmol) was introduced. The solution was stirred at room temperature for 2 h, treated with citric acid (0.1 g), and evaporated to a residue. Purification by flash chromatography (initially with CH_2Cl_2, then CH_2Cl_2/EtOH, 5/1) provided **12** (6.11 g, 89%): ^{1}H NMR (DMSO-d_6) δ 1.38 (3H, d, −CHCH_3), 3.50−3.68 (4H, m, H-5′$_a$ and H-5′$_b$ and −CH_2−), 3.95 (1H, d, H-4′), 4.12 (1H, d, H-3′), 4.51 (1H, q, H-2′), 5.07 (1H, t, 5′-OH), 5.22, 5.50 (2H, 2d, 2′- and 3′-OH), 5.83 (1H, d, H-1′), 7.34, 7.45 (2H, 2t, Ar−H), 7.85, 7.98 (2H, 2d, Ar−H), 8.40 (1H, s, H-8), 8.53 (1H, d, N−H); HPLC retention time 16.6 min [gradient elution, 20−80% acetonitrile/H_2O (containing 0.1% TFA)]. An analytical sample was obtained by crystallization from EtOH: mp 194−195 °C. Anal. ($C_{20}H_{21}ClN_6O_4S_2$·0.5EtOH) C, H, N.

5′-Deoxy-2,5′-dichloro-*N*-[4-(phenylthio)-1-piperidinyl]-adenosine (13). 1. 1-Amino-4-(phenylthio)piperidine (23). 1-(*tert*-Butyloxycarbonyl)-4-hydroxypiperidine (**21**) (prepared from 4-hydroxypiperidine using the method outlined in the synthesis of **16**) (10.06 g, 50 mmol) was dissolved in THF (50 mL), and PhSSPh (16.38 g, 75 mmol) was introduced, followed by *n*-Bu_3P (20.2 g, 24.9 mL, 100 mmol).[86] The reaction mixture was heated at 80 °C for 18 h and cooled. Purification by flash chromatography (initially heptane, later heptane/EtOAc, 90/10) afforded 1-(*tert*-butyloxycarbonyl)-4-(phenylthio)piperidine (**22**) (8.42 g, 57%) as an oil. This phenyl thioether was dissolved in EtOAc (200 mL), and a solution of HCl(g) in dry EtOAc (see procedure for **17**) (40 mL) was added. The solution was stirred at room temperature for 72 h, and the precipitated crystals were collected by filtration and washed with EtOAc to provide 4-(phenylthio)piperidine hydrochloride (5.46 g, 83%): mp 162−163 °C; ^{1}H NMR (DMSO-d_6) δ 1.71 (2H, dq, CH_2), 2.94 (2H, br t, CH_2), 3.47−3.56 (1H, m, −CHS−), 3.95 (1H, d, H-4′), 7.25−7.44 (5H, m, Ar−H).

This hydrochloride (4.47 g, 19.4 mmol) was dissolved in a 1:1 mixture of H_2O and EtOH (50 mL), and a solution of $NaNO_2$ (8.3 g, 120 mmol) in H_2O (85 mL) was gradually introduced. The pH was reduced to 2 with 2 N HCl solution, and the solution was heated at 70 °C for 0.5 h. The reaction mixture was cooled and extracted with heptane (150 mL) and EtOAc (2 × 150 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to afford 1-nitroso-4-(phenylthio)-piperidine (4.24 g, 63%) as an oil which was dissolved in dry THF (150 mL). A 1 M solution of $LiAlH_4$ in Et_2O (19 mL) was added, and the reaction mixture was heated gradually to 70 °C and maintained at that temperature for 1.5 h. Aqueous 1 N NaOH (3.6 mL) was added slowly, followed by H_2O (22 mL). The mixture was stirred vigorously for 5 min and filtered. Further H_2O (100 mL) was introduced, the THF was removed in vacuo, and the aqueous mixture was extracted with CH_2Cl_2 (2 × 100 mL). The combined extracts were dried ($MgSO_4$) and evaporated to afford **23** (4.07 g, 39% from **21**) as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.51 (2H, dq, CH_2), 1.85 (2H, br d, CH_2), 2.18 (2H, br t, CH_2), 7.20−7.41 (5H, m, Ar−H).

2. 2-Chloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine (24). 23 (1.10 g, 6.7 mmol) was reacted with 9-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-2,6-dichloro-9*H*-purine (**18**) (2.5 g, 4 mmol) utilizing the procedure described for **10** (except triethylamine was used as base) followed by debenzoylation of the purified tribenzoyl product using methanolic ammonia. This provided **24**[11,20,59] (1.25 g, 65%) as a foam: ^{1}H NMR (DMSO-d_6) δ 3.51−3.60 (1H, m, H-5′$_a$), 3.62−3.68 (1H, m, H-5′$_b$), 3.95 (1H, q, H-4′), 4.14 (1H, q, H-3′), 4.50 (1H, q, H-2′), 5.08 (1H, t, 5′-OH), 5.21, 5.50 (2H, 2d, 2′-and 3′-OH), 5.83 (1H, d, H-1′), 7.24−7.45 (5H, 2m, Ar−H), 8.41 (1H, s, H-8), 9.44 (1H, s, N−H). Anal. ($C_{21}H_{25}ClN_6O_4S$·0.75H_2O) C, H, N.

3. 5′-Deoxy-2,5′-dichloro-*N*-[4-(phenylthio)-1-piperidinyl]adenosine (13). **24** (5.49 g, 11.1 mmol) was suspended in CH_3CN (55 mL) and cooled on an ice bath, and a solution of $SOCl_2$ (2.7 mL, 33.4 mmol) in CH_3CN (25 mL) was introduced dropwise.[57] The reaction mixture mixture was stirred for 1 h, after which time it was homogeneous. Pyridine (1.79 mL) was added, and the reaction mixture was stirred at ambient temperature for 18 h before being poured onto ice (600 mL). The mixture was stirred until the ice had melted and was basified with saturated aqueous $NaHCO_3$ (150 mL). The solid 2,3-*O*-sulfonyl intermediate **25** were collected by filtration and dissolved in CH_3OH (200 mL), whereupon 25% aqueous NH_3 solution (2.7 mL) was added. The product precipitated from the aqueous methanolic solution, and recrystallization from CH_3OH/H_2O provided **13** as a solid (3.93 g, 69% from **24**): mp 154−157 °C; ^{1}H NMR (DMSO-d_6) δ 1.74−1.84 (2H, br, piperidine C−H), 1.95−2.05 (2H, br, piperidine C−H), 2.80−2.90 (1H, br, piperidine C−H), 3.04−3.12 (2H, br, piperidine C−H), 3.84, 3.93 (2H, ABX, H-5′$_a$ and H-5′$_b$), 4.10 (1H, q, H-4′), 4.17 (1H, q, H-3′), 4.64 (1H, dd, H-2′), 5.48, 5.62 (2H, 2d, 2′- and 3′-OH), 5.87 (1H, d, H-1′), 7.26 (1H, t, Ar−H), 7.35 (2H, t, Ar−H), 7.42 (2H, d, Ar−H), 8.38 (1H, s, H-8), 9.49 (1H, s, N−H); HPLC retention time 22.19 min (gradient elution over 30 min; 20−80% acetonitrile/0.1% TFA in H_2O, 100% purity at 250 nm). Anal. ($C_{21}H_{24}Cl_2N_6O_3S$·0.75H_2O) C, H, N.

***N*-[(1*S*,*trans*)-2-Hydroxycyclopentyl]adenosine (GR 79236) (26).** *trans*-2-Hydroxycyclopentylamine **(29)** was prepared as a mixture of enantiomers by reaction of cyclopentene epoxide (8.0 g, 95.1 mmol) with a 25% aqueous ammonia solution (35 mL) in a sealed glass vessel at 110 °C for 1.5 h. The reaction mixture was cooled and evaporated to an oil (9.14 g). A sample of **29** (0.35 g, 3.46 mmol) was then reacted with 6-chloropurine riboside (0.5 g, 1.7 mmol) in dioxane (30 mL) in the presence of triethylamine (0.93 g, 9 mmol). The reaction mixture was heated at 100 °C for 70 h, cooled, and evaporated. The resultant residue was purified by flash chromatography eluting with a mixture of ethyl acetate and methanol (19:1). The fractions found to contain the highest amounts of *N*-[(1*R*,*trans*)-2-hydroxycyclopentyl]adenosine following HPLC examination were combined and evaporated to a solid (0.17 g). Recrystallization from methanol provided the opposite diastereoisomer *N*-[(1*R*,*trans*)-2-hydroxycyclopentyl]adenosine (0.11 g, 18%): mp 233−235 °C; ^{1}H NMR (DMSO-d_6) δ 1.43−2.12 (6H, 4m, −CH_2CH_2CH_2−), 3.52−3.59 (1H, m, H-5′$_a$), 3.54−3.71 (1H, m, H-5′$_b$), 3.97 (1H, q, H-4′), 4.15 (1H, q, H-3′), 4.61 (1H, q, H-2′), 5.21, 5.41−5.47 (3H, d & m, 2′, 3′ and 5′-OH), 5.89 (1H, d, H-1′), 7.75 (1H, br d, −NH), 8.21 and 8.37 (H-2 and H-8).

The mother liquors from the above recrystallization were evaporated and purified by short path chromatography on silica gel (Art. 7729), and the product recrystallized to provide **26**[62] (0.05 g, 4%): ^{1}H NMR (DMSO-d_6) δ 1.44−2.13 (6H, 4m, −CH_2CH_2CH_2−), 3.52−3.59 (1H, m, H-5′$_a$), 3.54−3.71 (1H, m, H-5′$_b$), 3.96 (1H, q, H-4′), 4.15 (1H, q, H-3′), 4.60 (1H, q, H-2′),

5.20, 5.41−5.47 (3H, d & m, 2′, 3′ and 5′-OH), 5.88 (1H, d, H-1′), 7.75 (1H, br d, −NH), 8.19 and 8.36 (H-2 and H-8); HPLC retention time 6.05 min (gradient elution over 30 min; 20−80% acetonitrile/0.1% TFA in H_2O, 97.3% purity at 250 nm).

2′-*O*-Methyl-*N*-cyclohexyladenosine (27). *N*-Cyclohexyladenosine[19] **(30)** (0.35 g, 1.0 mmol) was dissolved in DMF (5 mL), and the solution was cooled to 0 °C. NaH (0.046 g, 1.15 mmol) (60% oil dispersion) was added. The reaction mixture was stirred for 0.75 h at 0 °C, CH_3I (0.06 mL, 1.0 mmol) was introduced, and stirring was continued for 4 h at 0 °C, followed by 0.5 h at ambient temperature. The reaction mixture was filtered and evaporated, and to the residue were added H_2O (100 mL) and CH_2Cl_2 (100 mL). The phases were separated, and the organic phase was washed with H_2O (2 × 50 mL) before being dried ($MgSO_4$). The residue on evaporation was purified by flash chromatography (initially with CH_2Cl_2, then CH_2Cl_2/EtOH, 19/1) provided **27** (0.09 g, 25%) as a foam: ^{1}H NMR (DMSO-d_6) δ 1.1−1.4 (10H, m, cyclohexyl), 3.31 (3H, s, $-OCH_3$), 3.52−3.60 and 3.64−3.72 (2H, 2m, H-5′$_a$ and H-5′$_b$), 3.98 (1H, d, H-4′), 5.28 (1H, d, 2′-OH), 5.45 (1H, t, 5′-OH), 6.00 (1H, d, H-1′), 7.67 (1H, d, N−H), 7.85, 7.98 (2H, 2d, Ar−H), 8.20 (1H, s, H-2), 8.39 (1H, s, H-8).

***N*-[(2-Methylphenyl)methyl]adenosine (28).** A mixture of 6-chloropurine riboside (0.57 g, 2.0 mmol), diisopropylethylamine (0.52 mL, 3.0 mmol), and 2-methylbenzylamine (0.30 g, 2.5 mmol) in dioxane (30 mL) was heated at 80 °C for 4 h. After cooling, the residue on evaporation was purified by flash chromatography initially with CH_2Cl_2, then with CH_2Cl_2/EtOH/aq NH_3 (90/10/1) and provided *N*-[(2-methylphenyl)-methyl]adenosine[49] (0.58 g, 81%). Recrystallization from CH_3-OH provided **28** as a white solid (0.34 g, 46%): mp 161.5−163.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.35 (3H, s, $-CH_3$), 3.50−3.58 and 3.54−3.70 (2H, 2m, H-5′$_a$ and H-5′$_b$), 3.97 (1H, q, H-4′), 4.15 (1H, d, H-3′), 4.60−4.71 (3H, m, H-2′ and $-CH_2-$), 5.40 (1H, t, 5′-OH), 5.20, 5.46 (2H, 2d, 2′-and 3′-OH), 5.90 (1H, d, H-1′), 7.05−7.22 (4H, m, Ar−H), 8.20 (1H, s, H-2), 8.40 (2H, br s, H-8 and N−H), 8.53 (1H, d, N−H). Anal. ($C_{18}H_{21}N_5O_4\cdot 0.25H_2O$) C, H, N.

Pharmacological Methods. 1. Drugs. The reference adenosine agonists *R*-PIA, CPA, CGS 21680, and (5*R*,10*S*)−(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) were obtained from Sigma (St. Louis, MO) or Research Biochemicals Inc. (Natick, MA). The adenosine A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was purchased from Research Biochemicals Inc. DMCM was a generous gift of Dr. L. Turski, Schering AG, Berlin, Germany. *N*-(Sulfophenyl)adenosine (**32**)[64] was provided by Research Biochemicals International as part of the Chemical Synthesis Program of the National lnstitute of Mental Health, Contract NIMH30003. The novel adenosine receptor agonists featured in Tables 1 and 2 as well as CPA, *R*-PIA, and *N*-[(2-methylphenyl)methyl]adenosine were generally dissolved in 5% aqueous cremophor (BASF) prior to treatment. For the anesthetized rat studies agonists were first dissolved at 10 mg/mL in DMSO before being diluted further with 5% aqueous cremophor. DMCM was dissolved in 0.02 N HCl; MK-801 was dissolved in water.

2. In Vitro Experiments. Adenosine A_1 and A_{2A} Receptor Binding.[92] [^{3}H]-(*R*)-(Phenylisopropyl)adenosine binding to cortical membranes: These determinations were carried out using experimental procedures we have described previously.[11,20,49,91] [^{3}H]-*R*-PIA receptor binding assays were performed in crude membrane preparations from male Wistar rat whole forebrain. Washed tissue homogenates were incubated for 60 min in 50 mM Tris-HCl (pH 7.7) + 0.1 U/L adenosine deaminase (Sigma) at 25 °C with 1 nM [^{3}H]-*R*-PIA (50 Ci/mmol; Amersham U.K.). Free and bound ligands were separated as previously described.

[^{3}H]CGS 21680 binding to striatal membranes: Ligand binding with [^{3}H]CGS 21680[93] (48.1 Ci/mmol; Amersham) was measured as previously described, except that the concentration of radioligand was 2 nM in displacement studies. IC_{50} values were calculated by a nonlinear regression analysis using the GRAPHPAD program (I.S.I., Philadelphia, PA). Inhibitory constants (K_i) were calculated from the equation: $K_i = IC_{50}/(1 + [Ld]/K_d)$, where [Ld] is the concentration of radioligand and K_d is the equilibrium dissociation constant for the ligand.

Inhibition of isoprenaline-stimulated cAMP production in DDT_1-MF2 cells: The smooth muscle cell line DDT_1-MF2[94] contains both A_1 and A_2 adenosine receptors which appears to be functionally coupled to inhibition and stimulation of cAMP production, respectively. The assay protocol was a modification of a previously described method, which we have referred to previously.[10,20]

Cells were resuspended in DMEM + 20 mM HEPES, pH 7.4, and preincubated with test compounds 5 min prior to the addition of 10 mM isoprenaline in a final volume of 500 mL. Following a 10-min incubation at 37 °C, the tubes were placed on ice and 1 mL of ice-cold ethanol was added. The protein was removed by centrifugation, and the ethanol was evaporated under a stream of nitrogen at 70 °C. The cAMP content was determined using a radioimmunoassay. It was found that isoprenaline stimulated cAMP production to a multiple of 50−80-fold the basal level. No stimulation of cAMP production was observed with the A_{2A} agonist CGS 21680, which may be due to the inhibitory effects of the A_1 receptors. The A_1 antagonists 8-cyclopentyltheophylline (CPT) and DPCPX did not reduce cAMP production at concentrations up to 100 mM.

Spontaneously Beating Isolated Guinea Pig Atria. Male guinea pigs were killed by cervical dislocation and the hearts rapidly removed and placed in oxygenated Krebs Henselit solution of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$, 1.64; $NaHCO_3$, 24.88; $KHPO_4$, 1.18; glucose, 5.55, bubbled with 5% CO_2 and 95% O_2. The test compounds were added to the bath, and the amplitude of the contractions were measured after 0.5 min of incubation, after which the compounds were washed off by overflow. In this way dose-response curves were constructed for each compound with three to five preparations at each concentration. From these curves EC_{50} values were obtained by extrapolation, and the percentage maximum response obtained was noted (acetylcholine and carbachol give a maximum 100% inhibition of the contractions).[95]

3. In Vivo Methods. Mouse Locomotor Activity.[44,67,69] Male NMRI mice (Moellegaard's Breeding Labs, Ll. Skensved, Denmark) weighing an average of 25 g were used. The mice were kept on a 12/12-h light/dark cycle (light from 6.00 to 18.00 h) and were housed at a temperature of 20 ± 1 °C and at a humidity of 55 ± 5%. The animals had free access to standard pellet food and water before testing. All testing was performed between 9.00 and 15.00 h.

Locomotor activity testing: Spontaneous locomotor activity was evaluated in standard photocell activity test chambers. Six-week-old male NMRI mice, naive to the apparatus, were used for testing; 30 min after an ip injection of the test compound, dissolved in 5% aqueous cremophor, individual mice were placed in a test chamber and their spontaneous locomotor activity was recorded for a 10-min period. Four to six doses in half-log steps were used to determine an ED_{50} value (mg/kg) for each compound.

DMCM-Induced Seizure Test. Male NMRI mice (Moellegaard's Breeding Labs, Ll. Skensved, Denmark) weighing 18−27 g were used. The mice were kept on a 12/12-h light/dark cycle (light from 6.00 to 18.00 h) and were housed at a temperature of 20 ± 1 °C and at a humidity of 55 ± 5%. The mice had free access to standard pellet food and water before testing. All testing was performed between 9.00 and 15.00 h.

Seizures involving the GABA/benzodiazepine receptor complex were induced by DMCM[11,49,70] (18 mg/kg dosed ip). The animals were placed individually in Perspex cages and observed for the presence of clonic convulsions during the following 15 min. Animals were pretreated with the test compound, dissolved in 5% aqueous cremophor, by ip administration 30 min before testing. All values are means for groups of 6−8 animals. An anticonvulsive effective dose protecting 50% of the animals against clonic convulsions (ED_{50}) and their associated 95% confidence intervals were calculated using a

log−probit program. These values were calculated from the effect of 3−4 different doses of each compound.

Blood Pressure in Barbiturate-Anesthetized Rats.[96] Female Sprague−Dawley rats (n = 2 or 3) were anesthetized with pentobarbitol (625 mg/kg ip). Catheters (PE 50) were implanted in one jugular vein for injection and in one carotid artery for BP measurement. BP was registered by means of a pressure transducer connected to a pressure coupler, and the heart rate was derived from the pressure signal by an EEG/rate coupler linked to a Watanabe Linearcorder (Hugo Sachs Electronik, Germany). Vehicle and test agonist were injected over 1 min in a fixed volume of 1 mL/kg. The doses 0 (vehicle), 0.01, 0.1, and 1 mg/kg were administered within an interval of 15−20 min. Mean blood pressure (MBP) and heart rate were measured 5 min after the injection, and the percentage changes, related to the values immediately before the 0.01 mg/kg dose, were calculated.

Blood Pressure and Heart Rate Measurement by Telemetry. Female Sprague−Dawley rats (body weight 252−328 g) (Moellegaard's Breeding Labs, Ll. Skensved, Denmark)-were housed individually in Macrolon cages and kept on a 12/12-h light/dark cycle (light from 6.00 to 18.00 h) at a temperature of 20 ± 1 °C and at a humidity of 40−70%. The animals had free access to standard pellet food and water before testing.

At least 1 week prior to any experimentation rats were surgically prepared for the study by implantation of a Data Sciences telemetry transmitter (model TL11M2-C50-PXT or TA11PA-C40) in the abdominal aorta and a chronic sterile venous Tygon catheter into the femoral vein, according to standard operating procedures. All surgical procedures were performed during halothane/N_2O anesthesia using aseptic techniques. In the 2−4 days following surgery the rats were allowed free access to an isotonic NaCl drinking solution, in addition to their normal drinking water, to facilitate recovery. Some of the rats used in the present study had previously been used in other cardiovascular pharmacology studies. However, the rats were always allowed a minimum recovery period of 7 days between different experimental series.

The experimental procedures were not commenced until the rats had recovered from the surgical procedures−typically at least 7 days after the initial operation. In the experiments drug solutions or the vehicle was administered by iv infusion together with the baseline infusion solution by separate computer-controlled infusion pumps using a purpose constructed Labview virtual instrument driver. This system allows the infusion rate of these solutions to be changed remotely without disturbing the animals. In addition it is designed to reduce the baseline infusion rate equivalent to the flow of the test substance infusion rate so the animals receive a constant volume of fluid per unit time even though the dosage is altered.

On each day of experimentation the chronic venous catheter was opened and flushed (<250 μL of 0.9% NaCl), and the line from the infusion pumps was connected to the catheter. The rats were thereafter positioned on the telemetry receivers, and the telemetry transmitter was turned on, following which the infusion of the baseline solution (8 or 6 mL/h) was started. After 30 min of equilibration the data acquisition was commenced. Systemic arterial BP variables (systolic, diastolic, and mean arterial BP as well as heart rate) were recorded continuously via a modified Data Sciences telemetry system, using a Data Sciences UA-10 digital to analog converter and a PONEMAH data acquisition system. The sampling frequency of the analog signal was set to 250 Hz. In addition to the digitized signal, direct BP traces were recorded in parallel on a four-channel and a two-channel Linearcorder thermal recorder. The data acquisitions system used a logging rate of 1 value per 15 s and a line average of 4, thereby revealing 1 value/animal/min of all variables recorded.

The compounds were dissolved at 0.05 mg/mL in 150 mM glucose solution with 1% ethanol added. The baseline infusion rate was 8 mL/h. Each infusion rate, quoted in mg/kg/h in Figure 1, was maintained for 30 min. Increasing doses of either vehicle or drug were administered iv to the rats (n = 4 or 6) on separate experimental days during determination of the biological variables specified above. After 30 min of equilibration, a test dose of acetylcholine was given over 60 s (manually into the connector), and after an additional 15 min the infusion of **5**, **12,** or **28** at the doses indicated was commenced. If the systolic BP decreased to below 70 mmHg the infusion of drug was stopped. Once the constant infusion at the highest dose of these adenosine agonists was completed, another test dose of acetylcholine was administered. A baseline infusion of 150 mmol/L glucose with 1% ethanol added at a rate of 8 or 6 mL/h was started at the beginning of the equilibration period. The rate of this infusion was varied in parallel with administration of either of the test substance solution, so the animals received the same fluid volume per unit time throughout the experiment.

Some animals were used in more than one of the experimental series; however, their participation in the other series was always separated by at least 1 week. Effects of test substance administration on systemic arterial BP and HR variables are presented as average mean values of the final 5 min 25 min after start of the dosing period, i.e., at a time point were the entire dose had been administered.

Severe Temporary Forebrain Ischemia in Mongolian Gerbils. Transient forebrain ischemia[97] was produced in Mongolian gerbils (male 60−70 g; Charles River, Germany) anesthetized with 2% halothane in 70% nitrous oxide and 30% carbogen (95% oxygen, 5% carbon dioxide). The common carotid arteries were occluded for 5 min using microaneurism clips, and the animals were placed in recovery cages and allowed to survive for 4 days. The animals were then reanesthetized with Sombrevin (50 mg/kg ip) and decapitated and the brains rapidly frozen in powdered dry ice and prepared for histology. Coronal sections were taken through the brain at the level of the hippocampus and stained with cresyl violet and hematoxylin and eosin.

The resultant sections were rated for pyramidal cell loss in the CA1 region of the hippocampus using a semiquantitative scale from 0 (no cell loss) to 3 (almost total cell loss). The score for each hippocampus was summed to give the total score for each animal, thus giving a maximal cell loss score of 6 and a score of 0 for an animal with no ischemic damage. This evaluation was carried out by two independent operatives each without knowledge of the treatment given to the respective animals. The body temperature of all animals was maintained at 37 °C throughout surgery, and the animals were placed in warm boxes during the recovery period. Each experiment consisted of a drug-treated group and a vehicle control (n = 10−15), and statistical analysis of the cell loss data was carried out using the Mann−Whitney U-test.

Rectal Temperature. Male NMRI mice, housed as described above and weighing 25−30 g, were examined for effects on body temperature[98] at the postdosing time points shown in Figure 2.

Mouse Focal Ischemia. Male NMRI mice, housed as described above and weighing 25−30 g, were anesthetized using Avertin (tribromethanol dissolved in amylene hydrate) (400 mg/kg in a volume of 10 mL/kg ip). When positioned on the left side, the head was disinfected by 96% ethanol and secured to the underlayer by pushpins. A skin incision was made between the right ear and the right orbit. The underlying temporalis muscle was loosened and displaced downward, and a craniotomy was performed using a dental drill to expose the middle cerebral artery. Using bipolar electrocoagulation (Erbotom T71) the artery was severed at the point where it crosses the upper level of the zygomatic arc. Hereafter, the muscle was repositioned, the skin incision closed by sutures, and the mouse placed on a heating blanket at 30−32 °C for 45−60 min until it regained consciousness. The test substance was dosed ip, and the mouse was then placed in a box at room temperature with free access to water and food (5 mice/box).

After 24 h the mice were reanesthetized using Avertin, the chest was opened, and the brain was perfused with 1 mL of a 10% solution of 2,3,5-triphenyltetrazolium chloride (TTC) in

saline administered through the aorta after opening of the right atrium. After 2 min the animal was decapitated, and the brain was removed and placed in buffered formalin solution. The TTC is converted by mitochondria in viable cells to a red dye (formazan) which is bound to the cell constituents;[99] the infarcted regions lack certain enzymes and remain white. After the brains had been fixated (2−4 days) they were embedded in 5% agar in saline to support the division of the brain in 1-mm coronal sections (9−10 slices). The section images were transferred to a Macintosh computer via a TV camera and analyzed using the Image program. Areas of both hemispheres, as well as the infarct on the left side, were measured in each slice in order to calculate the volume of the infarct and the volumes of the hemispheres. We have shown that there is a high concordance between the infarct size determined by the TTC method and with conventional histological techniques.

Statistical Analysis. When the study comprises one control and one drug group, they are compared by an unpaired Student's *t*-test. In case of several drug groups (and one control), comparisons were made by an ANOVA with Dunnet's multiple comparison test.

Acknowledgment. We are grateful to Paw Bloch, Claus Bruun Jensen, Peter Ellegård, and Henrik Stephensen for their skilled syntheses of target compounds. In vitro and in vivo examinations of adenosine agonists were carried out by Iben Bredmose, Mie Bøgelund, Lisbeth Hansen, Lone Igel, Vibeke Johansson, Dorothe Larsen, Anne Lund, Anna Meincke, Kira Meyhoff, Camilla Nielsen, Mette Okkels, Helle Petersen Jette Platou, and Anne Vinter. We also wish to thank Scott Weiss of Cerebrus Ltd. for assistance with data presentation.

References

(1) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine receptors: Pharmacology, structure−activity relationships and therapeutic potential. *J. Med. Chem.* **1992**, *35*, 407−422.
(2) DeNinno, M. P. Adenosine. *Annu. Rep. Med. Chem.* **1998**, *33*, 111−120.
(3) Poulsen, S.-A.; Quinn, R. J. Adenosine receptors: new opportunities for future drugs. *Bioorg. Med. Chem.* **1998**, *6*, 619−641.
(4) Appleman, J. R.; Erion, M. D. The therapeutic potential of agents acting via purine receptors. *Exp. Opin. Invest. Drugs* **1998**, *7*, 225−243.
(5) Bhagwhat, S. S.; Williams, M. Recent progress in modulators of purinergic activity. *Exp. Opin. Ther. Patents* **1995**, *5*, 547−558.
(6) Williams, M. Adenosine − a selective neuromodulator in the mammalian CNS? *TINS* **1984**, 164−168.
(7) For reviews see: Daval, J.-L.; Nehlig, A.; Nicolas, F. Physiological and pharmacological properties of adenosine: therapeutic implications. *Life Sci.* **1991**, *49*, 1435−1453. Marangos, P. J. Potential therapeutic roles for adenosine in neurologic disease. In *Adenosine in the Nervous System*; Stone, T. W., Ed.; Academic Press Ltd.: London, 1991; pp 217−226.
(8) Rudolphi, K. A.; Schubert, P.; Parkinson, F. E.; Fredholm, B. B. Neuroprotective role of adenosine in cerebral ischaemia. *TIPS* **1992**, 439−445. Miller, L. P.; Hsu, C. Therapeutic potential for adenosine receptor activation in ischemic brain injury. *J. Neurotrauma* **1992**, *9*, S563−S577. von Lubitz, D. K. J. E.; Carter, M. F.; Beenhakker, M.; Lin, R. C.-S.; Jacobson, K. A. Adenosine, a prototherapeutic concept in neurodegeneration. *Ann. N. Y. Acad. Sci.* **1995**, *765*, 163−178. Sweeney, M. I. Neuroprotective effects of adenosine in cerebral ischemia: window of opportunity. *Neurosci. Biobehav. Rev.* **1997**, *21*, 207−217. Fredholm, B. B. Adenosine and neuroprotection. *Int. Rev. Neurobiol.* **1997**, *40*, 259−280. Rudolphi, K. A.; Keil, M.; Grome, J. J. Adenosine − a pharmacological concept for the treatment of cerebral ischemia? In *Pharmacology of Cerebral Ischemia*; Kriegelstein, J., Oberpichler, H., Eds.; Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, 1990; pp 439−448. Bischofberger, N.; Jacobson, K. A.; von Lubitz, D. K. J. E. Adenosine A_1 receptor agonists as clinically viable agents for treatment of ischemic brain disorders. *Ann. N. Y. Acad. Sci.* **1997**, *825*, 23−29. von Lubitz, D. K. J. E. Adenosine and cerebral ischemia: therapeutic future or death of a brave concept? *Eur. J. Pharmacol.* **1999**, *365*, 9−25.
(9) Young, D.; Dragunow, M. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. *Neuroscience* **1994**, *58*, 245−261.
(10) Knutsen, L. J. S.; Murray, T. F. Adenosine and ATP in Epilepsy. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997; pp 423−447.
(11) Knutsen, L. J. S.; Lau, J.; Eskesen, K.; Sheardown, M. J.; Thomsen, C.; Weis, J. U.; Judge, M. E.; Klitgaard, H. Anticonvulsant actions of novel and reference adenosine agonists. In *Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology*; Belardinelli, L., Pelleg, A., Eds.; Kluwer: Boston, 1995; pp 479−487. See also NNC-21-0041. *Annu. Drug Data Rep.* **1995**, *17*, 981.
(12) Deckert, J.; Gleiter, C. H. Adenosine − an endogenous neuroprotective metabolite and neuromodulator. *J. Neural Transm.* **1994**, *43* (Suppl.), 23−31.
(13) Dux, E.; Fastbom, J.; Ungerstedt, U.; Rudolphi, K.; Fredholm, B. B. Protective effect of adenosine and a novel xanthine derivative propentofylline on the cell damage after bilateral carotid occlusion in the gerbil hippocampus. *Brain Res.* **1990**, *516*, 248−256.
(14) During, M. J.; Spencer, D. D. Adenosine: A potential mediator of seizure arrest and postictal refractoriness. *Ann. Neurol.* **1992**, *32*, 618−624. Sollevi, A. Cardiovascular effects of adenosine in man. *Prog. Neurobiol.* **1986**, *27*, 319−349.
(15) Fredholm, B. B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Williams, M. Nomenclature and classification of purinoceptors. *Pharmacol. Rev.* **1994**, *46*, 143−156.
(16) Van Galen, P. J. M.; Stiles, G. L.; Michaels, G.; Jacobson, K. A. Adenosine A_1 and A_2 receptors: structure activity relationships. *Med. Chem. Rev.* **1992**, *12*, 423−471.
(17) Linden, J. Cloned adenosine A_3 receptors: pharmacological properties, species differences and receptor functions. *TIPS* **1994**, *15*, 298−306.
(18) Jacobson, K. A.; Kim, H. A.; Siddiqi, S. M.; Olah, M. E.; Stiles, G. L.; von Lubitz, D. K. J. E. A_3 adenosine receptors: design of selective ligands and therapeutic prospects. *Drugs Future* **1995**, *20*, 689−699. Collis, M. G.; Hourani, S. M. O. Adenosine receptor subtypes. *TIPS* **1993**, *14*, 360−366.
(19) Moos, W. H.; Szotek, D. S.; Bruns, R. F. N^6-Cycloalkyladenosines. Potent A_1-selective adenosine agonists. *J. Med. Chem.* **1985**, *28*, 1383−1384.
(20) Knutsen, L. J. S.; Lau, J.; Sheardown, M. J.; Thomsen, C. The synthesis and biochemical evaluation of new A_1 selective adenosine receptor agonists containing a 6-hydrazinopurine moiety. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2661−2666.
(21) Gustafsson, L. E.; Wiklund, C. U.; Wiklund, N. O.; Stelius, L. Subclassification of neuronal adenosine receptors. In *Purines in Cellular Signalling*; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer-Verlag: New York, 1990; pp 200−205.
(22) Mahan, L. C.; McVittie, L. D.; Smyk-Randall, E. M.; Nakata, H.; Monsma, F. J., Jr.; Gerfen, C. R.; Sibley, D. R. Cloning and expression of an A_1 adenosine receptor from rat brain. *Mol. Pharmacol.* **1991**, *40*, 1−7.
(23) Palmer, T. M.; Stiles, G. L. The new biology of adenosine receptors. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1994**, *69*, 83−120.
(24) Meng, F.; Xie, G.; Chalmers, D.; Morgan, C.; Watson, S. J.; Akil, H. Cloning and characterization of a pharmacologically distinct A_1 adenosine receptor from guinea pig brain. *Mol. Brain Res.* **1994**, *26*, 143−155.
(25) Fredholm, B. B. Adenosine receptors in the central nervous system. *News Physiol. Sci.* **1995**, *10*, 122−128.
(26) Dolphin, A. C.; Prestwich, S. A. Pertussis toxin reverses adenosine inhibition of neural glutamate release. *Nature* **1985**, *316*, 148−150.
(27) Simpson, R. E.; O'Regan, M. H.; Perkins, L. M.; Phillis, J. W. Excitatory transmitter release from the ischemic rat cerbral cortex: effects of adenosine receptor agonists and antagonists. *J. Neurochem.* **1992**, *58*, 1683−1690.
(28) Abbracchio, M. P.; Brambilla, R.; Camisa, M.; Rovati, G. E.; Ferrari, R.; Canevari, L.; Dagani, F.; Catabeni, F. Adenosine A_1 receptors in rat brain synaptosomes: transductional mechanisms, effects on glutamate release, and preservation after metabolic inhibition. *Drug Dev. Res.* **1995**, *35*, 119−129.
(29) Collingridge, G. L.; Lester, R. A. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.* **1989**, *40*, 143−210.
(30) Benveniste, H.; Drejer, J.; Schousboe, A.; Diemer, N. H. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischaemia monitored by intracerebral microdialysis. *J. Neurochem.* **1984**, *43*, 1369−1374.
(31) Simon, R. P.; Swan, J. H.; Griffiths, T.; Meldrum, B. S. Blockade of *N*-Methyl-D-aspartate receptors may protect against ischemic damage in the brain. *Science* **1984**, *266*, 850−852.
(32) Bullock, R.; Zauner, A.; Woodward, J.; Young, H. F. Massive persistent increase of excitatory amino acids following human occlusive stroke. *Stroke* **1995**, *26*, 2187−2189.

(33) Schubert, P.; Rudolphi, K. A.; Fredholm, B. B.; Nakamura, Y. Modulation of nerve and glial function by adenosine − role in the development of ischemic damage. *Int. J. Biochem*. **1994**, *26*, 1227−1236.
(34) Cronstein, B. N. Adenosine, an endogenous antiinflammatory agent. *J. Appl. Physiol*. **1994**, *76*, 5−13.
(35) Ramkumar, V.; Nie, Z.; Rybak, L. P.; Maggirwar, S. B. Adenosine, antioxidant enzymes and cytoprotection. *TIPS* **1995**, *16*, 283−285.
(36) Soricelli, A.; Postiglione, A.; Cuocolo, A.; De Chiara, S.; Ruocco, A.; Brunetti, A.; Salvatore, M.; Ell, P. J. Effect of adenosine on cerebral blood flow as evaluated by single-photon emission computed tomography in normal subjects and in patients with occlusive carotid disease. *Stroke* **1995**, *26*, 1572−1576.
(37) Knutsen, L. J. S.; Sheardown, M. J.; Roberts, S. M.; Mogensen, J. P.; Olsen, U. B.; Thomsen, C.; Bowler, A. N. Adenosine A_1 and A_3 selective *N*-alkoxypurines as novel cytokine modulators and neuroprotectants. *Drug Dev. Res*. **1999**, *45*, 214−221.
(38) Feuerstein, G. Z.; Wang, X.; Barone, F. C. Inflammatory gene expression in cerebral ischemia and trauma: potential new therapeutic targets. *Ann. N. Y. Acad. Sci*. **1997**, *825*, 179−193.
(39) Mullane, K. M.; Williams, M. Adenosine and cardiovascular function. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana Press Inc.: Clifton, NJ, 1990; pp 289−333. Shryock, J. C.; Belardinelli, L. Adenosine and adenosine receptors in the cardiovascular system: biochemistry, physiology, and pharmacology. *Am. J. Cardiol*. **1997**, *79*, 2−10. Olsson, R. A. Adenosine receptors in the cardiovascular system. *Drug Dev. Res*. **1997**, *39*, 301−307. Collis, M. G. Effect of adenosine on the coronary circulation. In *Adenosine and Adenosine Nucleotides as Regulators of Cellular Function*; Phillis, J. W., Ed.; CRC Press: Boca Raton, FL, 1991; pp 171−180.
(40) Fozard, J. R.; Menninger, K.; Tapparelli, C. Cardiovascular and metabolic effects of SDZ WAG-994, a selective adenosine A_1 receptor agonist, after acute and chronic dosing in the rhesus monkey. *Br. J. Pharmacol*. **1994**, *112*, U69. Wagner, H.; Milavec-Krizman, M.; Gadient, F.; Menninger, C.; Schoeffter, P.; Tapparelli, C.; Pfannkuche, H.-J.; Fozard, J. R. General pharmacology of SDZ WAG 944, a potent selective and orally active adenosine A_1 agonist. *Drug Dev. Res*. **1995**, *34*, 276−288. Belardinelli, L.; Lu, J.; Dennis, D.; Martens, J.; Shryock, J. C. The cardiac effects of a novel A_1-adenosine receptor agonist in guinea pig isolated heart. *J. Pharmacol. Exp. Ther*. **1994**, *271*, 1371−1382. Cox, B. F.; Clark, K. L.; Perrone, M. H.; Welzel, G. E.; Greenland, B. D.; Colussi, D. J.; Merkel, L. A. Cardiovascular and metabolic effects of adenosine A_1-receptor agonists in streptozotocin-treated rats. *J. Cardiovasc. Pharmacol*. **1997**, *29*, 417−426. Ishikawa, J.; Mitani, H.; Bandoh, T.; Kimura, M.; Totsuka, T.; Hayashi, S. Hypoglycemic and hypotensive effects of 6-cyclohexyl-2′-O-methyl-adenosine, an adenosine A_1 receptor agonist, in spontaneously hypertensive rat complicated with hyperglycemia. *Diabetes Res. Clin. Pract*. **1998**, *39*, 3−9.
(41) Ueeda, M.; Thompson, R. D.; Padgett, W. L.; Secunda, S.; Daly, J. W.; Olsson, R. A. Cardiovascular actions of adenosines, but not adenosine receptors, differ in rat and guinea pig. *Life Sci*. **1991**, *49*, 1351−1358.
(42) Martin, P. Evidence that adenosine receptors in the dog left atrium are not of the typical A_1 or A_2 adenosine receptor subtypes. *Eur. J. Pharmacol*. **1992**, *214*, 199−205.
(43) Barraco, R. A.; Phillis, J. W. Subtypes of adenosine receptors in the brainstem mediate opposite blood pressure changes. *Neuropharmacology* **1991**, *30*, 403−407.
(44) Barraco, R. A.; Aggarwal, A. K.; Phillis, J. W.; Moron, M. A.; Wu, P. H. Dissociation of the locomotor and hypotensive effects of adenosine analogues in the rat. *Neurosci. Lett*. **1984**, *48*, 139−144.
(45) White, P. J.; Rosé Meyer, R. B.; Hope, W. Functional characterization of adenosine receptors in the nucleus tractus solaritus mediating hypotensive responses in the rat. *Br. J. Pharmacol*. **1996**, *117*, 305−308.
(46) Cornfield, L. J.; Hu, S.; Hurt, S. D.; Sillis M. A. ^{3}H-2-Phenylaminoadenosine (^{3}H-CV 1808) labels a novel adenosine receptor in rat brain. *J. Pharmacol. Exp. Ther*. **1992**, *263*, 552−561.
(47) Born, G. V. R.; Haslam, R. J.; Goldman, M.; Lowe, R. D. Comparative effectiveness of adenosine analogues as inhibitors of blood-platelet aggregation and as vasodilators in man. *Nature* **1965**, *205*, 678−680.
(48) Schaumann, E.; Kutscha, W. Clinical-pharmacological studies with a new orally active adenosine derivative. *Drug Res*. **1972**, *22*, 783−790.
(49) Klitgaard, H.; Knutsen, L. J. S.; Thomsen, C. Contrasting effects of adenosine A_1 and A_2 receptor ligands in different chemoconvulsive models. *Eur. J. Pharmacol*. **1993**, *224*, 221−228.
(50) Siddiqi, S. M.; Jacobson, K. A.; Esker, J. L.; Olah, M. E.; Li, X.-d.; Melman, N.; Tiwari, K. N.; Secrist, J. A., III; Schneller, S.; Cristalli, G.; Stiles, G. L.; Johnson, C. R.; IJzerman, A. P. Search for new purine- and ribose-modified adenosine analogues as selective agonists and antagonists at adenosine receptors. *J. Med. Chem*. **1995**, *38*, 1174−1178.
(51) Schaumann, E.; Sclierf, G.; Pfleiderer, T.; Weber, E. The effect of administering repeated doses of phenyl-isopropyl-adenosine on fat and carbohydrate metabolism in healthy, fasted human volunteers. *Drug Res*. **1972**, *22*, 593−596.
(52) Irshad, F.; Dagenais, G. R.; Fedor, E. J.; Cavanaugh, J. H. Trials with an adenosine analogue as antianginal medication. *Clin. Pharmacol. Ther*. **1977**, *22*, 470−474.
(53) Bertolet, B. D.; Anand, I. S.; Bryg, R. J.; Mohanty, P. K.; Chatterjee, K.; Cohn, J. N.; Khurmi, N. S.; Pepine, C. J. Effects of A_1 adenosine receptor agonism using N^6-cyclohexyl-2′-O-methyladenosine in patients with left ventricular dysfunction. *Circulation* **1996**, *94*, 1212−1215.
(54) Knutsen, L. J. S.; Lau, J. Purine derivatives. Novo Nordisk A/S, WO 93/23418, Nov 25, 1993.
(55) Lau, J.; Knutsen, L. J. S.; Sheardown, M. J.; Hansen, A. J. Purine derivatives. Novo Nordisk A/S, WO 94/14832, July 7, 1994.
(56) Lau, J.; Knutsen, L. J. S. Purine derivatives. Novo Nordisk A/S, WO 95/07921, Mar 23, 1995.
(57) Robins, M. J.; Uznanski, B. Improved acetylation and deoxygenative chlorination of guanosine; nonaqueous diazotisation-dediazotisation of a 2-aminopurine nucleoside. In *Nucleic Acid Chemistry;* Townsend, L. B., II., Tipson, R. S., Eds.; John Wiley and Sons: New York 1986; Vol. 3, pp 144−148.
(58) Mitsunobu, O. The use of diethyl azocarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1−28.
(59) Knutsen, L. J. S.; Lau, J. C2,N^6-Disubstituted adenosine derivatives. U.S. 5,432,164, July 11, 1995. Knutsen, L. J. S.; Lau, J. 2-Chloro-N^6-substituted adenosines, their pharmaceutical compositions and activity in treating ischemias. U.S. 5,430,027, July 4, 1995.
(60) Borchardt, R. T.; Huber, J. A.; Wu, Y. S. Convenient preparation of S-adenosylhomocysteine and related compounds. *J. Org. Chem*. **1976**, *41*, 565−567. See also: Taylor, M. D.; Moos, W. M.; Hamilton, H. M.; Szotek, D. S.; Patt, W. C.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Heffner, T. G.; Mertz, T. G. Ribose-modified adenosine analogues as adenosine receptor agonists. *J. Med. Chem*. **1986**, *29*, 346−353.
(61) Gurden, M. F.; Coates, J.; Ellis, F.; Evans, B.; Foster, M.; Hornby, E.; Kennedy, I.; Martin, D. P.; Strong, P.; Vardey, C. J.; Wheeldon, A. Functional characterization of three adenosine receptor types. *Br. J. Pharmacol*. **1993**, *109*, 693−698. Merkel, L. A.; Hawkins, E. D.; Colussi, D. J.; Greenland, B. D.; Smits, G. J.; Perrone, M. H.; Cox, B. F. Cardiovascular and antilipolytic effects of the adenosine agonist GR 79236. *Pharmacology* **1995**, *51*, 224−236. Thompson, C. S.; Strong, P.; Mikhailidis, D. P. Triacylglycerol lowering properties of the adenosine analogue GR 79236 during 5 days of dosing large, old rats: a pilot study. *Nutr. Res*. **1996**, *16*, 1925−1932.
(62) Evans, B. 2,N-6-Disubstituted adenosines and their antihypertensive methods of use. U.S. 5,032,583, July 16, 1991.
(63) Gadient, F.; Milavec, M.; Muller, E. New use of organic compounds. Sandoz Ltd., European Patent Application 0 490 818 A1, June 17, 1992.
(64) Gadient, F.; Mangold, B. M.; Fozard, J. R.; Prashad, M.; Kapa, P. K. Adenosine derivatives. Sandoz Ltd., WO 95/30683, Nov 16, 1995.
(65) Gerwins, P.; Norstedt, C.; Fredholm, B. B. Characterization of adenosine A_1 receptors in intact DDT_1 MF2 smooth muscle cells. *Mol. Pharmacol*. **1990**, *38*, 660−666.
(66) Jacobson, K. A.; Nikodijevic, O.; Ji, X.-d.; Berkich, D. A.; Eveleth, D.; Dean, R. L.; Hiramatsu, K.-I.; Kassel, N. F.; Van Galen, P. J. M.; Lee, K. S.; Bartus, R. T.; Daly, J. W.; LaNoue, K. F.; Maillard, M. Synthesis and biological activity of N^6-(*p*-sulfophenyl)alkyl and N^6-sulfoalkyl derivatives of adenosine: water-soluble and peripherally selective adenosine agonists. *J. Med. Chem*. **1992**, *35*, 4143−4149. Van Schaick, E. A.; De Greef, H. J. M. M.; Langemeijer, M. W. E.; Sheehan, M. J.; Ijzerman, A. P.; Danhof, M. Pharmacokinetic-pharmacodynamic modeling of the anti-lipolytic and anti-ketotic effects of the adenosine A_1-receptor agonist N^6-(*p*-sulfophenyl)adenosine in rats. *Br. J. Pharmacol*. **1997**, *122*, 525−533. Van Schaick, E. A.; Zuideveld, K. P.; Tukker, H. E.; Langemeijer, M. W. E.; Ijzerman, A. P.; Danhof, M. Metabolic and cardiovascular effects of the adenosine A_1 receptor agonist N^6-(*p*-sulfophenyl)adenosine in diabetic Zucker rats: influence of the disease on the selectivity of action. *J. Pharmacol. Exp. Ther*. **1998**, *287*, 21−30.
(67) Nikodijevic, O.; Sarges, R.; Daly, J. W.; Jacobson, K. A. Behavioral effects of A_1 and A_2-selective adenosine agonists and antagonists: evidence for synergism and antagonism. *J. Pharmacol. Exp. Ther*. **1991**, *259*, 286−294.

(68) Dunwiddie, T. V.; Worth, T. Sedative and anticonvulsant effects of adenosine analogues in mouse and rat. *J. Pharmacol. Exp. Ther*. **1982**, *220*, 70−76.
(69) Janusz, C. A.; Berman, R. F. Adenosinergic modulation of the EEG and locomotor effects of the A_2 agonist CGS 21680. *Pharmacol. Biochem. Behav*. **1993**, *45*, 913−919.
(70) Petersen, E. N. Selective protection by adenosine receptor agonists against DMCM-induced seizures. *Eur. J. Pharmacol*. **1991**, *195*, 261−265.
(71) Sheardown, M. J.; Judge, M. E.; Eskesen, K.; Thomsen, C.; Lau, J.; Knutsen, L. J. S. Neuroprotective actions of novel and reference adenosine receptor agonists. *Drug Dev. Res.* **1994**, *31*, 320 (abstract of poster presented at 5th International Symposium on Adenosine and Adenine Nucleotides, Philadelphia, PA, May 13−16, 1994).
(72) Bloch, G. A.; Pulsinelli, W. A. The adenosine receptor agonist, R-phenylisopropyladenosine, attenuates ischemic neuronal damage. *J. Cereb. Blood Flow Metab*. **1987**, *7* (Suppl. 1), S258.
(73) Roucher, P.; Meric, P.; Correze, J. L.; Mispelter, J.; Tiffon, B.; Lhoste, J. M.; Seylaz, J. Metabolic effects of *R*-phenylisopropyladenosine during reversible forebrain ischemia studied by in vivo ^{13}P nuclear magnetic resonance spectroscopy. *J. Cereb. Blood Flow Metab*. **1991**, *11*, 453−458.
(74) Bielenberg, G. W. *R*-PIA Protects Cortical Tissue in Permanent MCA occlusion in the rat. *J. Cereb. Blood Flow Metab*. **1989**, *9* (Suppl. 1), S645.
(75) Roussel, S.; Pinard, E.; Seylaz, J. Focal cerebral ischemia in chronic hypertension: no protection by (*R*)-phenylisopropyladenosine. *Brain Res*. **1991**, *545*, 171−174.
(76) Von Lubitz, D. K. J. E.; Lin, R. C.-S.; Melman, N.; Ji, X.-d.; Carter, M. F.; Jacobson, K. A. Chronic administration of selective adenosine A1 receptor agonist or antagonist in cerebral ischemia. *Eur. J. Pharmacol*. **1994**, *256*, 161−167.
(77) von Lubitz, D. K. J. E.; Dambrosia, J. M.; Kempski, O.; Redmond, D. Cyclohexyl adenosine protects against neuronal death following ischemia in the CA1 region of the gerbil hippocampus. *Stroke* **1987**, *19*, 1133−1139. Daval, J.−L.; von Lubitz, D. K. J. E.; Deckert, J.; Redmond, D. J.; Marangos, P. *Brain Res*. **1989**, *491*, 212−226.
(78) Evans, M. C.; Swan, J. H.; Meldrum, B. S. An adenosine analogue, 2-chloroadenosine protects against long-term development of ischaemic cell loss in the rat hippocampus. *Neurosci. Lett*. **1987**, *83*, 287−292.
(79) Von Lubitz, Dag K. J. E.; Lin, Rick C.-S.; Paul, I. A.; Beenhakker, M.; Boyd, M.; Bischofberger, N.; Jacobson, K. A. Postischemic administration of adenosine amine congener (ADAC): analysis of recovery in gerbils. *Eur. J. Pharmacol*. **1996**, *316*, 171−179. Von Lubitz, D. K. J. E.; Beenhakker, M.; Lin, R. C.-S.; Carter, M. F.; Paul, I. A.; Bischofberger, N.; Jacobson, K. A. Reduction of postischemic brain damage and memory deficits following treatment with the selective adenosine A_1 receptor agonist. *Eur. J. Pharmacol*. **1996**, *302*, 43−48.
(80) Kulinskii, V. I.; Sufianova, G. Z.; Usov, L. A.; Ekimov, E. N. Effect of the adenosine receptor agonists on the cerebral blood flow in normal conditions and in brain ischemia. *Eksp. Klin. Farmakol*. **1996**, *59*, 37−39.
(81) Baudy, R. B. Patent update: central and peripheral nervous systems. Agents for the treatment of neurodegenerative diseases: Part 4. *Exp. Opin. Ther. Patents* **1995**, *5*, 299−326.
(82) Knutsen, L. J. S.; Lau, J.; Thomsen, C.; Eskesen, K.; Judge, M. E.; Weis, J. U.; Klitgaard, H.; Sheardown, M. J. Promising anticonvulsant and antiischemic effects of new, selective adenosine agonists in animal models. *33rd Annu. Meeting Am. Coll. Neuropsychopharmacol*. **1994**, 49 (abstract of lecture presented at ACNP Meeting, Puerto Rico, December 1994).
(83) Sheardown, M. J.; Hansen, A. J.; Thomsen, C.; Judge, M. E.; Knutsen, L. J. S. Novel adenosine agonists: a strategy for stroke therapy. In *Ischemic Stroke: Recent Advances in Understanding and Therapy:* Grotta, J., Miller, L., Buchan, A. M., Eds.; International Business Communications, 1995; pp 187−214.
(84) Sheardown, M. J.; Knutsen, L. J. S. Unexpected neuroprotection observed with the adenosine A_{2A} receptor agonist CGS 21680. *Drug Dev. Res*. **1996**, 39, 108−114.
(85) Heron, A.; Lekieffre, D.; Le Peillet, E.; Lasbennes, F.; Seylaz, J.; Plotkine, M.; Boulu, R. G. Effects of an A_1-adenosine receptor agonist on the neurochemical, behavioral and histological consequences of ischemia. *Brain Res*. **1994**, *641*, 217−224.
(86) Welsh, F. A.; Harris, V. A. Postischemic hypothermia fails to reduce ischemic injury in gerbil hippocampus. *J. Cereb. Blood Flow Metab*. **1991,** *11*, 617−620.
(87) Stiles, G. L. Adenosine Receptor Subtypes: New Insights From Cloning and Functional Studies. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997; pp 29−37.
(88) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem*. **1978**, *43*, 2923−2925.
(89) Imai, K.; Nohara, A.; Honjo, M. Synthesis of purine nucleosides using iodine as catalyst. *Chem. Pharm. Bull*. **1966**, *14*, 1377−1381.
(90) Kotsuki, H.; Matsumoto, K.; Nishizawa, H. High pressure-promoted transformation of alcohols into the corresponding phenylsulphides with Bu_3P−PhSSPh. *Tetrahedron Lett*. **1991**, *32*, 4155−4158.
(91) Thomsen, C.; Valsborg, J. S.; Foged, C.; Knutsen, L. J. S. Characterization of [^{3}H]-*N*-[R-(2-Benzothiazolyl)thio-2-propyl]-2-chloroadenosine ([^{3}H]-NNC 21-0136) binding to rat brain: profile of a novel selective adenosine receptors for adenosine A_1 receptors. *Drug Dev. Res*. **1997**, *42*, 86−97.
(92) Williams, M.; Jacobson, K. A. Radioligand Binding Assays for Adenosine Receptors. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; Humana Press Inc.: Clifton, NJ, 1990; pp 17−55.
(93) Jarvis, M. F.; Schultz, R.; Hutchison, A. J.; Do, U. H.; Sills, M. A.; Williams, M. [^{3}H]-CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J. Pharmacol. Exp. Ther*. **1989**, *251*, 888−893.
(94) Gerwins, P.; Norstedt, C.; Fredholm, B. B. Characterization of adenosine A_1 receptors in intact DDT_1 MF2 smooth muscle cells. *Mol. Pharmacol*. **1990**, *38*, 660−666. Ramkumar, V.; Barrington, W. W.; Jacobson, K. A.; Stiles, G. L. Demonstration of both A_1 and A_2 receptors in DDT_1 MF2 smooth muscle cells. *Mol. Pharmacol*. **1990**, *37*, 149−156.
(95) Sauerberg, P.; Olesen, P. H.; Nielsen, S.; Treppendahl, S.; Sheardown, M. J.; Honore, T.; Mitch, C. H.; Ward, J. S.; Pike, A. J.; Bymaster, F.; Sawyer, B. D.; Shannon, H. E. Novel functional M_1 selective muscarinic agonists. Synthesis and structure−activity relationships of 3-(1,2,5-thiadiazolyl)-1,2,5,6-tetrahydro-1-methylpyridines. *J. Med. Chem*. **1992**, *35*, 2274−2283.
(96) Olsen, U. B.; Bille-Hansen, V. Exaggerated hypotension by N-formylmethionylleucylphenylalanine in indomethacin-pretreated rats. Role of toxic oxygen. *Agents Actions* **1985**, *17*, 489−494.
(97) Sheardown, M. J.; Suzdak, P. D.; Nordholm, L. AMPA, but not NMDA, receptor antagonism is neuroprotective in gerbil global ischaemia, even when delayed for 24 h. *Eur. J. Pharmacol*. **1993**, *236*, 347−353.
(98) Mehta, A. K.; Kulkarni, S. K. Effect of purinergic substances on rectal temperature in mice: involvement of P_1-purinoceptors. *Arch. Int. Pharmacodyn. Ther*. **1983**, *264*, 180−186.
(99) Bederson, J. B.; Pitts, L. H.; Tsuji, M.; Nishimura, M. C.; Davis, R. L. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. *Stroke* **1986**, *17*, 472−476.

JM960682U