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Recent Developments in the Discovery of Novel Adenosine Kinase Inhibitors: Mechanism of Action and Therapeutic Potential

Steve McGaraughty, Marlon Cowart, and Michael F. Jarvis

Neuroscience, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbot Park, IL, USA

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ABSTRACT

Adenosine (ADO) is an endogenous inhibitory neuromodulator that limits cellular excitability in response to tissue trauma and inflammation. Adenosine kinase (AK; EC 2.7.1.20) is the primary metabolic enzyme regulating intra- and extracellular concentrations of ADO. AK inhibitors have been shown to significantly increase ADO concentrations at sites of tissue injury and to provide effective antinociceptive, antiinflammatory, and anticonvulsant activity in animal models. Structurally novel nucleoside and nonnucleoside AK inhibitors that demonstrate high specificity for the AK enzyme compared with other ADO metabolic enzymes, transporters, and receptors have recently been synthesized. These compounds have also demonstrated improved cellular and tissue penetration compared with earlier tubercidin analogs. These compounds have been shown to exert beneficial effects in animal models of pain, inflammation and epilepsy with reduced cardiovascular side effects compared with direct acting ADO receptor (P1) agonists, thus supporting the hypothesis that AK inhibitors can enhance the actions of ADO in a site- and event-specific fashion.

INTRODUCTION

The prototypic endogenous inhibitory neuromodulator adenosine (ADO) is one component of a purinergic cascade that results from the metabolic inactivation of ATP (78)

Address correspondence and reprint requests to: Michael F. Jarvis, PhD, D-4PM, AP9A/2, 100 Abbott Park Road, Abbott Park, IL 60064-6123, USA.
Tel: +1 (847) 937-3919. Fax: +1 (847) 937-9195. E-mail: michael.jarvis@abbott.com.

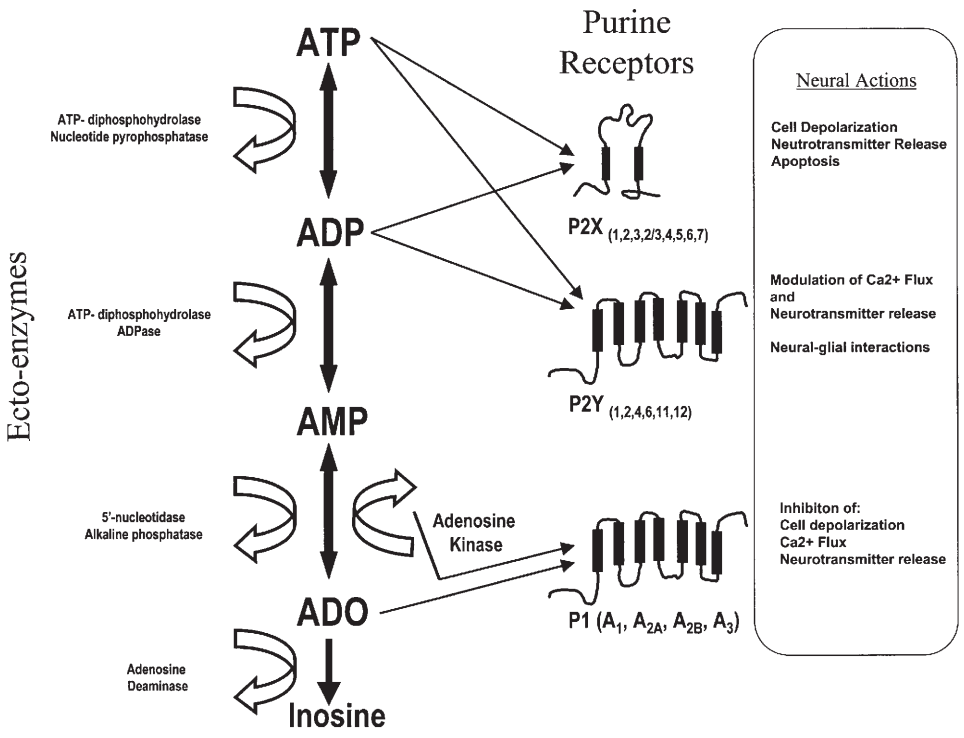

Fig. 1. The purinergic cascade. ATP, ADP, AMP, and ADO are released from nerves or cells into the extracellular space. These purines are readily metabolized by ecto-nucleotidases that are located both intra- and extracellularly. The phosphorylation of ADO to AMP is mediated primarily by the action of intracellular AK. Specific purinergic cell surface receptor families include: P1, ADO (A_1, A_{2A}, A_{2B}, A_3) receptor subtypes; P2X, ATP-sensitive ionotropic receptor subtypes; and P2Y, ATP- and ADP-sensitive metabotropic receptor subtypes. Activation of P1 and P2 receptors mediate a diverse constellation of physiological processes (see text). Specific actions related to modulation of neurotransmission are indicated in the panel.

(Fig. 1). The availability of these purines (ADO, AMP, ADP, and ATP) is under tight metabolic control in the extracellular milieu and each has distinct, receptor-mediated activities (12). ADO functions as an inhibitory brake that limits cellular energy demand and results in a variety of neuroprotective actions including decreased seizure activity, reductions in hypoxic cell death, antiinflammatory actions, and antinociception (78). Consequently, ADO has been termed a "retaliatory" or "homeostatic" modulator of cellular activity (60). ADO produces these effects by activating a family of P1 G-protein-coupled receptors (A_1, A_{2A}, A_{2B}, A_3). In contrast, activation of ATP-sensitive P2 receptor subtypes stimulates both neuronal excitability and neural-glial interactions (12,27). The extracellular actions of ATP are mediated via interactions with separate families of P2X ligand-gated ion channels and P2Y G-protein-coupled receptors (12) (Fig. 1).

ADO has a half-life on the order of seconds in physiological fluids (57), and its beneficial actions are, therefore, restricted to the tissue and cellular site where it is released. Reuptake of ADO into the cell, followed by its intracellular metabolism, is responsible for its rapid disappearance of ADO from the extracellular space (1). Since ADO has been

shown to effectively limit pathophysiologic processes resulting from tissue trauma and injury, the identification of ligands that mimic or potentiate the beneficial actions of ADO has been the subject of substantial drug discovery efforts (12,78). One approach that has received attention in recent years is the discovery of compounds that augment the concentrations and actions of endogenous ADO by inhibiting the ADO-metabolizing enzyme adenosine kinase (AK).

AK (ATP: adenosine 5′-phosphotransferase, EC 2.7.1.20) is a cytosolic enzyme that catalyzes the phosphorylation of ADO to AMP and is one of two enzymes responsible for ADO metabolism. ADO deaminase (ADA) also contributes to ADO conversion, but AK appears to predominate under physiologic conditions (1). The mammalian AK enzyme has been cloned (53,70), crystallized (51), and found to contain two ADO binding sites, a catalytic site with high affinity for ADO and a low-affinity regulatory site that may be the $MgATP^{2-}$ binding site (22,35,48). Since the predominant ADO-specific transport system operates as a nonconcentrative, bidirectional, facilitated diffusion transporter, ADO transport is largely driven by its concentration gradient across the cell membrane. Thus, AK inhibition has the net effect of decreasing cellular reuptake of ADO (19), thus potentiating the local concentration of ADO in the extracellular compartment.

Since the actions of endogenous ADO are highly localized and AK blockade may be more effective in cells undergoing accelerated ADO release (59), the effects of AK inhibitors may be more pronounced at tissue sites where pathophysiologic changes result in ADO release, thereby limiting systemic side effects (23,58). The ability of AK inhibitors to augment ADO availability has been demonstrated in hippocampal and spinal cord slices *in vitro* (32,62) and during excitotoxic insults to rat striatum *in vivo* (10). These data demonstrate that a systemically administered AK inhibitor can elicit a site- and event-specific enhancement of endogenous ADO levels *in vivo*. Furthermore, AK inhibitors can amplify the actions of ADO independent of ADO receptor subtype. This may be a potential advantage in cases where a multiplicity of ADO receptor subtypes is involved in the therapeutic effect, such as in inflammation.

Novel nucleoside and nonnucleoside inhibitors of AK have recently been reported that show a high degree of selectivity for the AK enzyme and have *in vivo* effects consistent with the augmentation of the actions of endogenous ADO in animal models of pain, inflammation, and seizure activity (38,46,52). ADO receptor antagonists block these effects, thus providing further support that increasing endogenous ADO concentrations, which in turn activates ADO (P1) receptors, is an underlying mechanism mediating the effects of AK inhibitors *in vivo*. This report provides an overview of the preclinical activity of several structurally diverse potent and selective AK inhibitors that have shown efficacy in animal disease models.

STRUCTURE ACTIVITY RELATIONSHIPS FOR NOVEL AK INHIBITORS

Early investigations of the structure-activity relationships (SAR) for nucleoside-like compounds as inhibitors and substrates of AK were reported by Miller et al. (56), and subsequent work by other groups (11,14,15,34,73,74) have replicated and expanded those

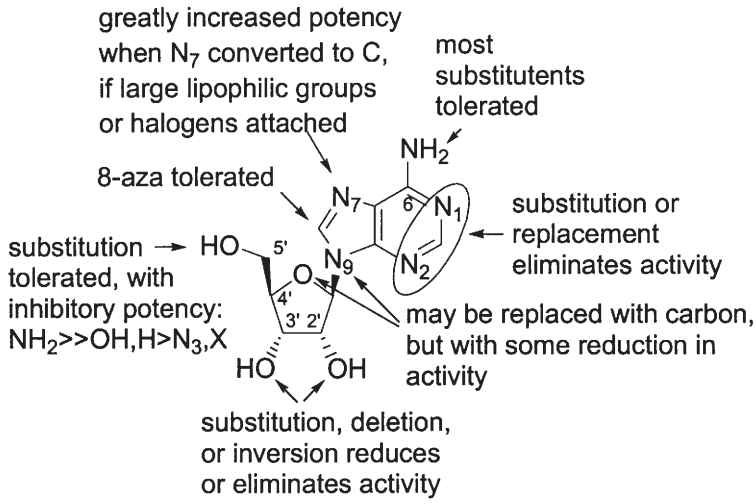

Fig. 2. General structure activity relationships for nucleoside-containing AK inhibitors.

findings. A general SAR for ribose-containing AK ligands is depicted in Fig. 2, and representative AK inhibitory potencies and *in vivo* activities are illustrated in Table 1. For ribonucleoside-like ligands, the trans hydroxy groups are necessary for substrate or inhibitor activity (2,56). Deletion, replacement, or inversion of the hydroxyl group eliminates ligand recognition by the enzyme. Replacing the ribofuranosyl ring oxygen with a methylene group (aristeromycin, Fig. 3) results in AK inhibitory activity that is approximately two orders of magnitude weaker than that for ADO (2,3). ADO is phosphorylated at the 5′ position, and removal of the 5′-hydroxyl phosphorylation site or substitution at this position would be expected to eliminate substrate activity. However, many analogs with 5′ substitution have potent activity as enzyme inhibitors, such as 5′-deoxyadenosine (Fig. 3). A particularly interesting observation is that additional substitution of an amino group at the 5′ position enhances potency still further as shown by 5′-aminoadenosine (Fig. 3, $IC_{50} = 9$ nM). The stereochemical configuration of the 5′-hydroxymethyl moiety can be inverted, and the resulting α-lyxofuranoside analogs potently inhibit AK. While acyclic adenosine analogs are generally not potent substrates or inhibitors of the enzyme, AK is responsible for the intracellular activation of many pharmacologically active nucleosides by phosphorylation (56). The heterocyclic adenine ring of adenosine may also be modified, and conversion of the C8 of adenosine to 8-azaadenine allows activity, as does the related pyrazolopyrimidine analog. The 1-aza and 3-aza nitrogens in the adenine ring are necessary, and their replacement leads to a loss of activity. A broad variety of C_6 substituents (e.g., methoxy, mercapto) retain activity, while substitution at C_2 eliminates activity.

Replacement of the N_7 nitrogen of the adenine ring with carbon leads to tubercidin (Fig. 3), which shows reduced activity compared with the parent ADO. However, some very potent pyrrolopyrimidine analogs can be created by appending lipophilic aryl or halogen moieties on the C_6 of the heterocyclic ring in tubercidin. One of the first potent AK inhibitors found to utilize this principle was created not by man, but by but by nature.

*TABLE 1. Biological activity of novel AK inhibitors**

	In vitro activity (IC_{50}),nM		*In vivo* activity (ED_{50}), i.p.	
Compound	AK	Cell AK	Epilepsy models (mg/kg, i.p.)	Pain models (μmol/kg i.p.)
5′-deoxyadenosine	410			
tubercidin	2640	25,000		
nor-aristeromycin	23,000	>100,000		
nor-tubercidin	70,000	>100,000		
aristeromycin	30,000	30,000		
NH_2dADO	9	6630	153	
5-IT	9	16	6.2	
5′d-5-IT	1	68	0.3	
GP3269	11			
GP515	4.6	88	16	
GP683	0.5	12	1.1	
GP947	0.1	0.08	0.2	
A-134974	0.06	45	5	1
ABT-702	2	50		0.6
1	0.6		7.1	
2	0.2			
3	200	100,000		
4	120	30,000		
5	13	1530		
6	400	3640		
7	5	170		30
8	0.17	33	3	3
9	3	57		10
10	17	800		2
11	0.25	30		10
12	21	304		20
13	10	371		3

* *In vitro* data represents activity to inhibit AK (rat brain cytosol and/or recombinant) and ADO phosphorylation in intact cell assays. *In vivo* data represent activity in rodent epilepsy models (e.g., PTZ, and maximal electroconvulsive shock) and pain models (e.g., carrageenan-induced thermal hyperalgesia). The data depicted were taken from references (16,19,24,33,38,42,43,55,56,73,74,77).

Davies et al. (19) isolated and elucidated the structure of a potent muscle-relaxing and hypothermia-inducing natural product in the algae *Hypnea valentiae* as 5′-deoxy-5-iodotubercidin (Fig. 2). With an IC_{50} value of 1 nM, this compound stands out as one of the more potent early AK inhibitors, and it is still used as a pharmacologic tool to study adenosine pharmacology. A related synthetic analog (5-IT, 17 nM) was prepared and reported as early as 1969 (36).

Recent research efforts have made extensive use of the aforementioned SAR to design classes of pyrrolopyrimidine and pyrazolopyrimdine lipophilic inhibitors (8,14,73,74). Ugarkar et al. (73,74) have described the evolution of the SAR for their ribonucleosides

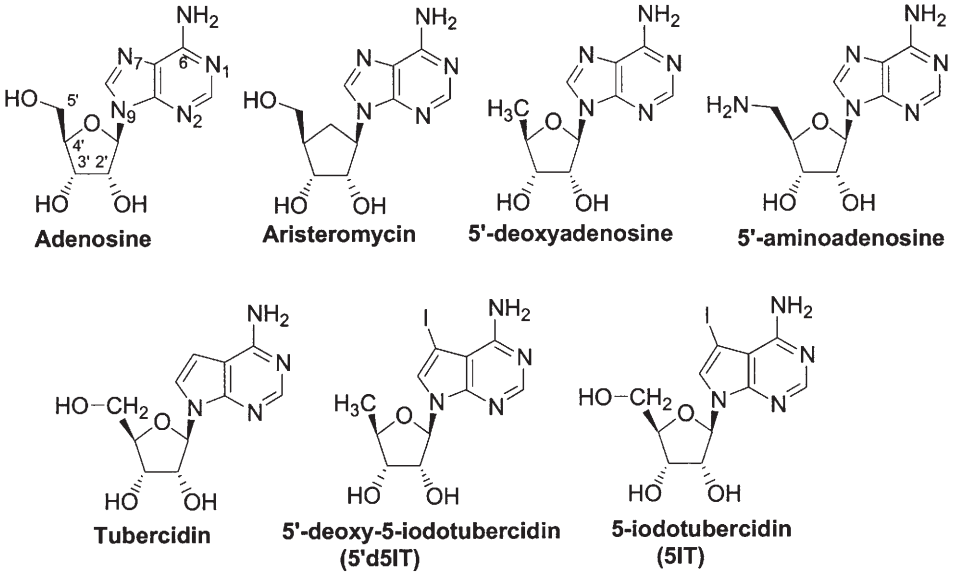

Fig. 3. Prototypic AK inhibitors derived from aristeromycin and tubercidin.

that ultimately led to the selection of GP-3269 (Fig. 4) as a preclinical candidate with antiseizure activity. The potency-enhancing effects of substituting lipophilic moieties at the 4 and 5 positions of tubercidin were combined with the aforementioned favorable 5′-amino group to give a family of AK inhibitors with potent *in vitro* and *in vivo* activity, most prominently GP-515 and GP-683 (Fig. 4), along with the related α-lyxofuranosyl analog GP-947. These compounds are reported to possess potent antiseizure and antiinflammatory activity. Two of the most potent *in vitro* inhibitors are compounds 1 and 2, illustrating the beneficial effect of combining the potency-enhancing 5′-amino moiety and the iodotubercidin structure.

A potent series of nucleoside analogs that introduced the critical structural modification of a 5′-truncated carbocyclic structure have recently been described (14,15,17). In this series, the ribose ring was replaced with a cyclopentane carbocyclic ring, thereby rendering the analogs impervious to acid- and enzyme-induced hydrolytic degradation. This change also permitted the possibility of additional modification and truncation of the 5′-hydroxymethyl group, thereby giving 4′-heteroatom substituted carbocycles. Some of these extensively modified adenosine/tubercidin analogs have been shown to have potent *in vitro* and *in vivo* activity in animal models of cerebral ischemia and chronic pain (15,17), with oral bioavailability in animals approaching 100%. The starting point for this series was the weakly active natural product aristeromycin (Fig. 4). Truncation of the 5′-methylene gave nor-aristeromycin, which was virtually devoid of inhibitory potency (IC_{50} = 23,000 nM [63]). Further modification by replacement of the 4′-hydroxyl group with a 4′-amino group led to compound 3, which was comparable in potency to adenosine, as was the 8-aza analog compound 4. Replacement of the adenine ring of noraristeromycin with a 5-iodopyrrolidine gave the even more potent compound 5 (Fig. 5, IC_{50}

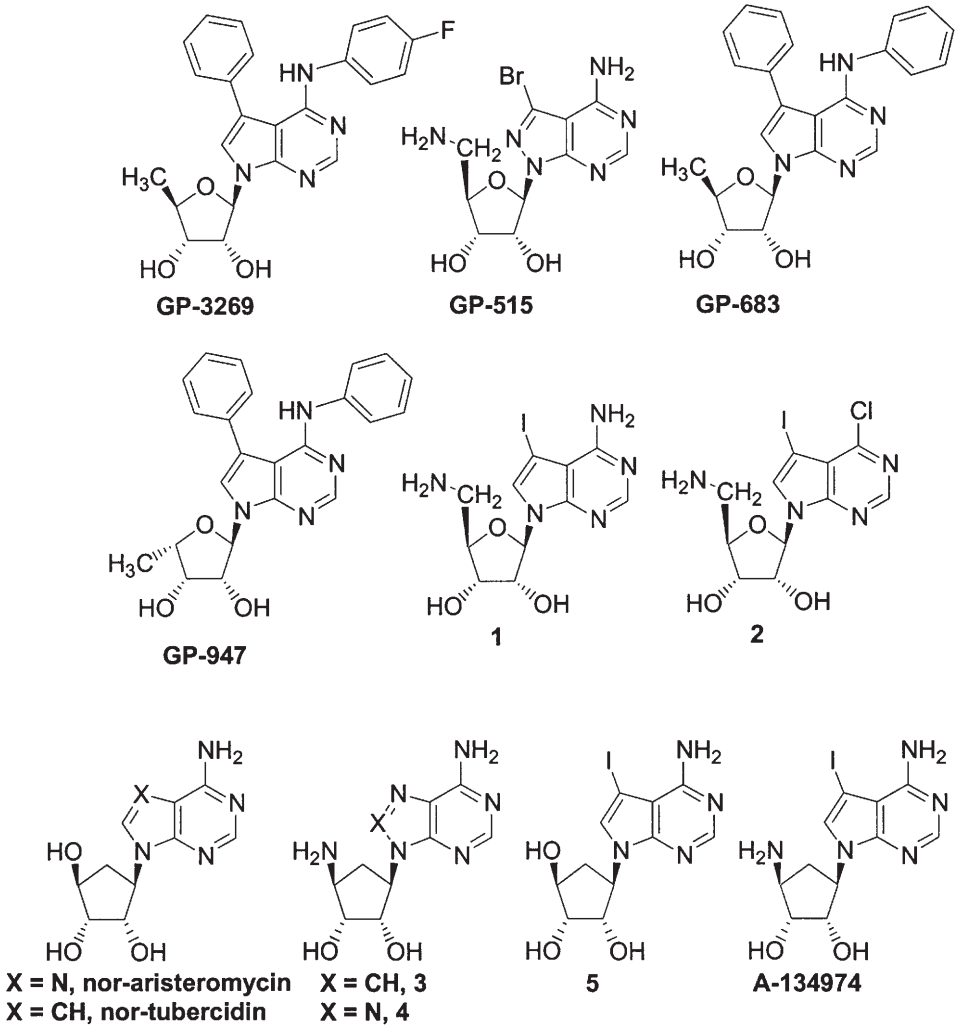

Fig. 4. Structurally novel nucleoside AK inhibitors.

= 13 nM). There was a considerable synergistic effect when the 4′-amino truncated carbocyclic moiety of compound 3 was combined with the heterocyclic ring of compound 5. In this case, A-134974 was produced with low picomolar potency (IC_{50} = 0.06 nM).

A unique family of nonnucleoside AK inhibitors (Fig. 5) that were obtained through modification of a compound (compound 6; IC_{50} = 400 nM), that was discovered from high throughput screening, has also been reported (47). A number of analogs of the lead structure were examined, and it was found that replacement of the 5-aza atom with a carbon, when coupled with concurrent C_5-lipophilic substitution at the 5-position, gave compounds with potent *in vitro* AK activity. For example, the 3-bromophenyl analog compound 7 showed an IC_{50} of 5 nM. Unfortunately, this class of 7-dimethylaminophenyl-

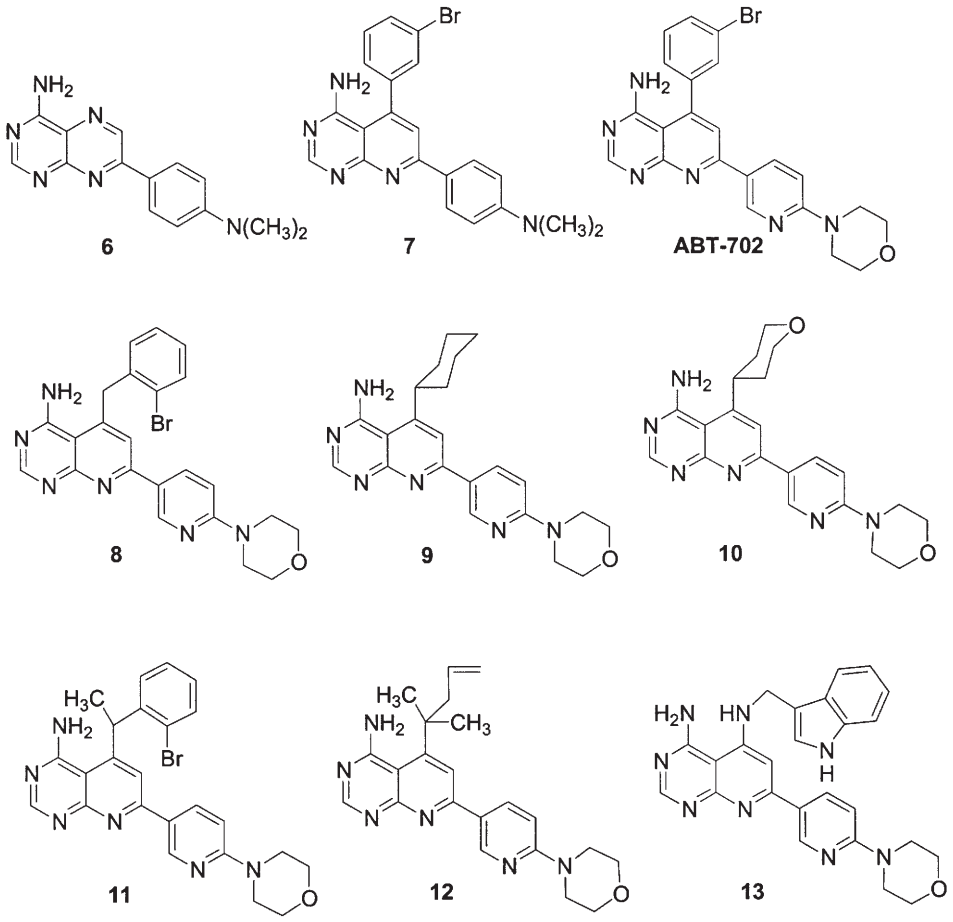

Fig. 5. Structurally novel nonnucleoside AK inhibitors.

substituted compounds often suffered from especially low water solubility. Aqueous solubility, as well as *in vivo* activity, was considerably improved through further modification of this structural class by replacement of the 7-phenyl moiety of compound 7 with a 7-morpholino(2-pyridyl) moiety as seen in ABT-702, which was shown to have potent antinociceptive actions *in vivo* (38,46). An examination of the SAR of many analogs showed that compounds bearing a wide variety of lipophilic C_5-substituents displayed potent *in vitro* inhibitory activity; for example, compounds 8 and 11 each have subnanomolar affinity to inhibit AK. Many analogs also display efficacy in animal pain models when administered by systemic (i. p.) administration. These compounds also showed potent activity following oral administration; for example, ABT-702 and compound 9 were active in a carageenan-induced thermal hyperalgesia assay with ED_{50} values of 5–10 μmol/kg, p.o. ([46] and unpublished observations). The structural variety of C_5 substituents that allowed potent activity ranged from aryl to quaternary alkyl through to even aminoalkyl indole (13,32).

Potent nucleoside (77) and nonnucleoside AK (38) inhibitors have demonstrated a high degree of selectivity for inhibiting AK compared with their respective activities at other sites of ADO action (e.g., P1 receptors, ADO transporters, ADO deaminase) and a range of other cell surface receptors, ion channels, and kinases. In the case of ABT-702, kinetic studies have shown that this compound is also a competitive AK inhibitor with respect to ADO, noncompetitive with respect to $MgATP^{2-}$, and reversible following washout (38). As illustrated by the compounds described above, there are now a number of structurally diverse and pharmacologically selective AK inhibitors that are useful for elucidating the physiological significance of ADO in various disease models.

THERAPEUTIC UTILITY

Analgesia

Significant progress during the last decade has been made in the study of pain, and it is now appreciated that distinct mechanisms contribute to acute nociceptive states, to pain arising from tissue damage (inflammatory pain), and to pain arising from injury to the nervous system (neuropathic pain) (80). The psychophysical parameters used to describe nociceptive processing have been refined to differentiate acute withdrawal behaviors in response to acute noxious stimuli (acute nociception) from increased sensitivity to mildly painful stimuli (hyperalgesia) or to innocuous stimuli (allodynia). Tissue trauma associated with nerve injury leads to the release of a variety of pronociceptive neuropeptides and neuromodulators that sensitize peripheral nerve terminals (peripheral sensitization) and contribute to phenotypic alterations of sensory neurons and increased excitability of spinal cord dorsal horn neurons (central sensitization) (80). Thus, pain is not a unimodal sensory phenomenon, but rather a series of complex neurophysiologic responses to distinct acute and persistent noxious stimulation.

Inhibitors of AK have emerged as potent antinociceptive agents with a potentially improved therapeutic window over direct acting ADO receptor agonists. Systemic delivery of prototypic nucleoside (5′d-5IT, NH_2dADO, and 5-IT; Fig. 3), as well as structurally novel nonnucleoside (ABT-702) and carbocyclic (A-134974) AK inhibitors have demonstrated antinociceptive activity in a diverse array of nociceptive models (38,43–46,52). In animals, systemic administration of AK inhibitors alleviates acute nociception (thermal stimulation and chemically-induced abdominal constriction), neuropathic allodynia (diabetic and nerve ligation), persistent pain (formalin), chronic adjuvant arthritis, and inflammatory thermal hyperalgesia (carrageenan and complete Freund's adjuvant). Table 2 summarizes the antinociceptive profile of two well-characterized and chemically distinct AK inhibitors, ABT-702 and A-134974. Both compounds have oral activity and were most effective in the carrageenan model of thermal hyperalgesia with slightly improved efficacy over morphine (46,52). These AK inhibitors were more potent to attenuate carrageenan-induced hyperalgesia than the accompanying paw edema, suggesting that pain relief was not secondary to a significant reduction of inflammation. The antihyperalgesic effects of ABT-702 and A-134974 were selective for the inflamed but not the noninflamed hind paw, a result consistent with the proposed beneficial effects of increased ADO concentrations at sites of injury or trauma (23,58). The systemic antihyperalgesic actions of these

compounds were also separable from their respective effects on motor function. A 6- to 16-fold separation between ED_{50} values for hypomotive and antihyperalgesic actions was observed while an even greater separation was observed in coordinative motor function, as measured in the rotorod test. Furthermore, in the carrageenan hyperalgesia experiments withdrawal latencies of the noninflamed hind paws did not differ between groups receiving either an AK inhibitor or vehicle, which demonstrated that administration of therapeutic doses of AK inhibitors does not impair withdrawal reflexes.

Local administration of AK inhibitors into relevant sites of action also has significant antinociceptive activity. For example, intrathecal infusion of NH_2dADO to animals has been shown to alleviate acute thermal nociception (40) and tactile allodynia after nerve injury (42). In the formalin model of persistent pain, intrathecal or intraplantar injection of NH_2dADO produced antinociception (65,68). Furthermore, in the carrageenan model of inflammatory pain, intrathecally administered 5-IT and NH_2dADO were antihyperalgesic (66). Unlike direct-acting A_1 and A_{2A} receptor agonists (N^6-cyclohexyladenosine and CGS 21680), the intrathecal administration of 5-IT and NH_2dADO did not produce noticeable motor impairment (66). In contrast to the local antinociceptive actions of AK inhibitors, intrathecal or intraplantar administration of ADA inhibitors such as deoxycoformycin, was ineffective in animal models of acute thermal nociception, persistent pain, and thermal hyperalgesia (66,68). These data suggest a more prominent role for AK than ADA inhibitors in regulating endogenous ADO in the spinal cord and at peripheral sites of tissue injury.

Recent studies demonstrate that spinal sites of action are the primary contributors to the antinociceptive actions of AK inhibition (52). Antihyperalgesia following intracerebroventricular (ED_{50} = 100 nmol) or intraplantar (ED_{50} > 300 nmol) injection of A-134974 was relatively weak compared with the effect following intrathecal administration (ED_{50} = 6 nmol; Table 3). Furthermore, site-specific antagonism of the systemic effects of A-134974 was observed after theophylline administration into the spinal cord or inflamed hind paw but not the brain. Theophylline administered into the noninflamed hind paw also

TABLE 2. Antinociceptive profile of two structurally diverse orally active AK inhibitors.

	ABT 702 ED_{50} (μmol/kg)	A-134974 ED_{50} (μmol/kg)
Acute pain		
Mouse hot plate	65	50
Rat acute thermal nociception	40	–
Inflammatory pain		
Carrageenan-induced thermal hyperalgesia	5	3
Carrageenan-induced paw edema	70	20
Formalin test (second phase)	60	15
Neuropathic pain		
L5/L6 nerve ligation	50	6
Motor effects		
Exploratory locomotor activity	30	20
Rotorod performance	>300	>30

antagonized the antihyperalgesic effects of systemic A-134974, suggesting that the actions of theophylline were systemic in nature. Contributions from mechanisms local to the inflamed hind paw cannot be dismissed since Sawynok et al. (68) demonstrated that coadministration of NH_2dADO with low concentrations of formalin into a hind paw site-specifically reduced formalin-induced nociceptive responses. The actions of endogenously released ADO to inhibit peripheral neurotransmitter release (30) and to attenuate inflammatory processes may contribute to ADO modulation of nociception in the periphery.

Spinal sites, but not the supraspinal or peripheral hind paw sites, were also responsible for the antiallodynic action of A-134974 (82). Direct administration of this compound into the spinal lumbar enlargement potently relieved tactile allodynia (ED_{50} = 10 nmol) in rats with ligated L5/L6 nerves. Supraspinal or intraplantar administrations were ineffective. Additionally, intrathecal administration of theophylline antagonized the antiallodynic effects of systemic A-134974. Like A-134974, direct-acting ADO receptor agonists have also shown improved antinociceptive efficacy after injection into the spinal cord compared with delivery into the brain (38). Supraspinal mechanisms are more likely to contribute to the hypomotive actions of AK inhibitors. Administration of A-134974 via three different routes of injection (i.c.v., i.t., and i.p.) depressed locomotor activity and each of these effects was selectively antagonized by supraspinal administration of theophylline (Table 3). The mechanisms underlying the antinociceptive and the hypomotive actions of AK inhibitors are clearly distinct.

Additional studies with selective ADO receptor antagonists suggest there are different control mechanisms for the relief of inflammatory hyperalgesia compared with neuropathic and acute pain. The antiallodynic effects of ABT-702 in a nerve injury model of neu-

TABLE 3. AK inhibitor sites of action*

Route	Antagonist	Antinociception	Motor Activity
Systemic			
i.p. ED_{50}		10 μmol/kg	16 μmol/kg
	i.t. Theophylline	yes	no
	i.c.v. Theophylline	no	yes
Spinal			
i.t. ED_{50}		6 nmol	>100 nmol
	i.t. Theophylline	yes	no
	i.c.v. Theophylline	no	yes
Supraspinal			
i.c.v. ED_{50}		100 nmol	1 nmol
	i.t. Theophylline	—	no
	i.c.v. Theophylline	no	yes

* The effects of A-134974, a potent and water soluble AK inhibitor were evaluated following systemic, spinal, and supraspinal administration (52). The ability of the nonselective ADO receptor antagonist theophylline to block the actions of A-134974 was evaluated following spinal and supraspinal administration. Antinociception was assessed using carrageenan-induced thermal hyperalgesia and motor activity was defined as exploratory locomotor activity (30 min) in a novel environment.

ropathy were attenuated by the selective A_1 receptor antagonist CPT but not by the A_{2A} receptor antagonist DMPX (46). The A_1 receptor also selectively mediated AK inhibitor-induced antinociception in models of acute thermal pain (38,40,43). In contrast, in the carrageenan model of inflammatory thermal hyperalgesia both A_1 and A_{2A} receptor subtypes contributed to the antinociceptive action of systemically administered ABT-702 and NH_2dADO (42,46). The spinal component of this response, however, is most likely mediated by the A_1 receptor (66). Nonetheless, in both carrageenan and nerve-injured animals, systemic administration of ABT-702 reduced noxious evoked (thermal and mechanical) firing of wide dynamic range neurons in the spinal cord (71), suggesting that ABT-702 modulates the activity of these spinal neurons under both pathological conditions.

AK inhibitors may also have utility as analgesic- and anesthetic-sparing agents during the perioperative period, as previously demonstrated clinically with ADO itself (69). Thus, the AK inhibitor, GP683 decreased the desflurane anesthetic requirement in dogs without producing adverse effects on the cardiovascular system (76). This compound is a potent AK inhibitor that is reported to have good oral bioavailability and efficacy in preclinical seizure models (24,77).

Inflammation

As discussed earlier, AK inhibitors clearly modulate nociceptive signals associated with states of tissue trauma that are accompanied by inflammation. Administration of ADO or ADO receptor agonists have shown antiinflammatory actions in a variety of animal models (9,18,29,42) and endogenous levels of ADO are increased at sites of inflammation (18). ADO acts at various ADO receptor subtypes to modulate neutrophil function when administered either peripherally or centrally (A_{2A} and A_1, respectively), endothelial cell permeability (A_1 and A_{2A}), TNF-α production *in vitro* and *in vivo* (A_3), and collagenase (MMP-1) production and gene expression on synoviocytes *in vitro* (A_{2B}) (7,28,29). Thus, enhancing levels of endogenous ADO by inhibiting AK may advantageously attenuate both nociception and inflammation.

AK inhibitors are effective in various animal models of inflammation. ABT-702, GP515, and other AK inhibitors reduced edema, pleurisy, and neutrophil accumulation at the site of inflammation following local administration of carrageenan or other proinflammatory agents (13,18,29,46,67). The antiinflammatory actions of systemic ABT-702 were attenuated by selective A_1 and A_{2A} receptor antagonists (46), and the effects of other systemically administered AK inhibitors were blocked by the peripherally selective ADO receptor antagonist 8-(*p*-sulfophenyl)theophylline (8-SPT) and by local application of exogenous ADA (to degrade endogenously released ADO) at sites of inflammation (18). The latter outcome suggests that the action of the AK inhibitors might be at least partially mediated at the peripheral site of inflammation. In adjuvant arthritis, ABT-702 and another AK inhibitor with an undisclosed structure (AKI-2, Metabasis Therapeutics) reduced edema, as well as bone and cartilage degeneration (8,28,29). The effects of the AK inhibitor on bone protection observed in the rat adjuvant arthritis model may reflect actions of ADO on MMP regulation, and on modulation of TNF-α production (8,28). These antiinflammatory actions of ABT-702 were reversed by the nonselective antagonist theophylline. GP515 also significantly improved survival in animal models of septic shock (murine endotoxic shock and a rat model of bacterial peritonitis, [28]). Since sepsis in the latter

model results from an infectious process, improved survival by the AK inhibitor suggests that the antiinflammatory actions of ADO do not suppress the normal immune response to infection (28,29).

Anticonvulsant and Neuroprotective Activity

Extracellular ADO levels, as measured by intra-hippocampal microdialysis, increased up to 30-fold in patients with intractable complex partial epilepsy during spontaneously occurring seizures, supporting a role of endogenous ADO in mediating seizure arrest and postictal refractoriness (20,21). AK inhibitors have also been shown to effectively block seizure activity in a number of different animal models (4,5,44,77,81). The direct administration of AK inhibitors into the central nervous system has been shown to effectively reduce bicuculline methiodide-induced seizures in rats (80). Furthermore, as noted above, AK inhibitors (NH_2dADO and 5-IT) were found to be significantly more effective than the ADA inhibitor, 2′-deoxycoformycin, and more potent than the nucleoside transport inhibitor, dilazep, in reducing chemically induced seizures (81). Following intraperitoneal administration, 5′d-5-IT was more potent and effective in reducing PTZ-induced seizures in mice than NH_2dADO and 5-IT (4). The rank order of potency for these AK inhibitors is consistent with their ability to inhibit ADO phosphorylation in intact cells and may also reflect the relative degree to which they penetrate into the CNS following systemic administration (44). These effects appear to be centrally mediated since the peripherally acting ADO receptor antagonist 8-PST was ineffective in blocking the anticonvulsant actions of 5′d-5-IT (44). The anticonvulsant actions of AK inhibitors appear to result from the enhanced ability of extracellular ADO to activate A_1 receptors since the highly A_1-selective antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT) was found to completely block 5′d-5-IT-mediated inhibition of PTZ-induced seizures in mice (44).

Several novel nucleoside-like AK inhibitors that have been designed to improve central nervous system penetration, including GP515, GP683, and GP3269, demonstrate improved *in vivo* potency in reducing PTZ-induced and maximal electroshock seizures in rats relative to NH_2dADO (77) and to effectively reduce seizure severity and duration (4). The *in vivo* potency of these novel AK inhibitors was positively correlated with their ability to inhibit AK, to block adenosine phosphorylation in intact cells, and to inhibit epileptigenic activity in a rat *in vitro* neocortical slice preparation (77). The anticonvulsant effects associated with AK inhibition appear to be separable from cardiovascular and other side effects. For example, GP683 has been shown to effectively block rat maximal electroshock seizures (ED_{50} = 1 mg/kg, i.p.) and to not significantly alter hemodynamic function (heart rate and blood pressure) at doses as high as 40 mg/kg, i.p. (77). Similar to these actions, other AK inhibitors including A-134974 and ABT-702, have been shown to effectively block PTZ-induced seizure activity in mice at doses that do not affect motor function (44 and unpublished observations).

Consistent with its anticonvulsant actions, ADO concentrations increase in response to hypoxic stress (31,49,54,55,79) and serve as an endogenous defense mechanism to limit ischemic brain damage (26,61). In experimental models, ADO receptor agonists reduce cerebral ischemic damage while ADO receptor antagonists exacerbate ischemic trauma (54,75). An initial evaluation of 5-IT in a gerbil global ischemia model indicated that AK inhibition did not provide effective neuroprotection (64). However, subsequent studies using AK inhibitors (5′d-5-IT, A-134974, and GP683) with increased central nervous

system penetrability demonstrated significant neuroprotection in terms of both brain infarct size (39,45,55,72) and neurological deficits (39) in the middle cerebral artery occlusion model. These neuroprotective effects of 5′d-5-IT were not accompanied by drug-induced hemodynamic depression (60) or sufficient hypothermia to account for the extent of neuroprotection observed (39).

CONCLUSION

The realization that ADO functions as an endogenous homeostatic modulator of pathophysiologic responses has resulted in significant efforts to identify pharmaceutical agents that mimic or modulate the actions of ADO. As shown above, the recent development of structurally novel AK inhibitors has emerged as one approach to exploit the effects of beneficial effects of enhancing endogenous ADO concentrations at sites of tissue trauma. AK inhibitors can selectively enhance the protective actions of ADO released locally at the site of cellular stress, potentially minimizing the undesirable side effects associated with systemic administration of direct acting ADO receptor agonists. Recent medicinal chemistry efforts have yielded a variety of novel, potent and selective AK inhibitors based on the purine nucleoside pharmacophore and, more recently, on a nonnucleoside pharmacophore. The preclinical profile of AK inhibitors indicates that these agents may have therapeutic potential in the management of pain, inflammation, and seizure disorders. To date, only the nucleoside AK inhibitor GP3269 has been reported to have been studied in man (phase I), but data from these studies has not been disclosed. Additionally, the clinical development of another nucleoside AK inhibitor that shows antinociceptive effects in the absence of significant alterations of hemodynamic function in animal models, GP3966, was halted due to a preliminary report of central nervous system hemorrhage in rats and dogs (25). Thus, additional data are clearly needed to properly evaluate the therapeutic window for both nucleoside and nonnucleoside AK inhibitors. Whether the potential therapeutic benefits that AK inhibitors offer in preclinical models can be realized for clinical practice remains to be established.

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