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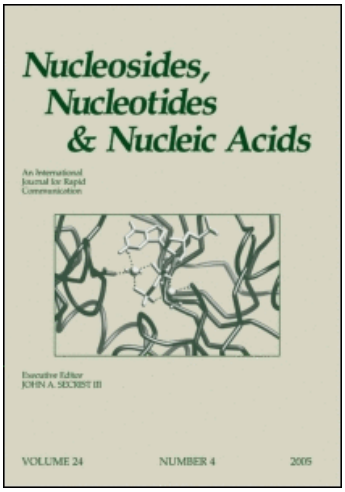

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Mark D. Erion[a]; Bheemarao G. Ugarkar[a]; Jay DaRe[a]; Angelo J. Castellino[a]; James M. Fujitaki[a]; Ross Dixon[a]; James R. Appleman[a]; James B. Wiesner[a]
[a] Gensia, Inc., San Diego, CA

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DESIGN, SYNTHESIS AND ANTICONVULSANT ACTIVITY OF THE POTENT ADENOSINE KINASE INHIBITOR GP3269

Mark D. Erion*, Bheemarao G. Ugarkar, Jay DaRe, Angelo J. Castellino, James M. Fujitaki, Ross Dixon, James R. Appleman and James B. Wiesner

Gensia, Inc., 9390 Towne Centre Drive, San Diego, CA 92121

ABSTRACT: The pyrrolopyrimidine nucleoside GP3269 (**12**) was shown to be a potent and selective inhibitor of human adenosine kinase (IC_{50} = 11 nM) and to exhibit anticonvulsant activity in rats after oral administration. Synthesis of GP3269 was accomplished in 4 steps from 4-chloro-5-iodopyrrolopyrimidine (**9**) and the protected 5-deoxy-1-α-chlororibose (**8**) using a base-catalyzed nucleoside coupling reaction and the Suzuki reaction to replace the 5-iodo substituent with phenyl.

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Adenosine is a naturally-occurring neuromodulator[1] that is purported to act as an endogenous anticonvulsant.[2] Considerable evidence exists indicating that adenosine inhibits seizure initiation and seizure propagation[3] and that seizures are controlled under normal settings because neuronal overexcitation leads to rapid ATP breakdown and concomitant adenosine production.[4] An elevation in local adenosine concentration inhibits seizure activity by attenuating excitatory neurotransmission through activation of A1 adenosine receptors. Activation of A1 receptors on the presynaptic membrane inhibits release of the excitatory amino acid glutamate whereas activation of A1 receptors on the postsynaptic membrane reduces neuronal firing by stabilizing the membrane potential.[5] Experimental support for the role of adenosine as a natural anticonvulsant is derived in large part from studies which indicate that overactivation of the glutamate neurotransmitter system is the primary mechanism of epilepsy,[6] that A1 receptor distribution in the brain corresponds to the distribution of the principle glutamate receptor subtype associated with epilepsy (i.e. N-methyl-D-aspartate receptor)[7] and that local adenosine concentration dramatically increases at sites undergoing epileptiform activity.[4] Other studies have reported that adenosine receptor antagonists are proconvulsant and therefore that basal levels of adenosine are important for controlling seizure activity.[8] Furthermore, A1

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agonists have been reported to inhibit electrically- and chemically-induced seizures in rats[9] and to cause a decrease in glutamate release from brain tissue slices.[10]

Efforts to develop A1 agonists as anticonvulsants have been hampered by A1 receptor-mediated cardiovascular (hypotension, bradycardia) and CNS (sedation and ataxia) side effects.[11] Consequently, alternative strategies have been explored that focus on finding agents that selectively activate A1 receptors at sites undergoing seizure activity and not at sites in the periphery. One potential target is adenosine kinase (AK), since AK is the major enzyme controlling adenosine metabolism and therefore its inhibition should lead to an elevation in endogenous adenosine concentration at seizure foci and to inhibition of seizure initiation, spread and propagation.[12,13] Furthermore, AK inhibitors were expected to produce greater elevations of adenosine at seizure foci relative to systemic sites based on the enhanced rate of adenosine production and, therefore, adenosine metabolism at these sites.

At the start of our work, several AK inhibitors were known (**1-5**). These compounds, however, were of limited use due to their short half-life in vivo (e.g. **1**) or their toxicity (e.g. **2**, iodotubercidin, $LD_{50} \approx 13$ mg/kg in rats, i.p.). Some of the toxicity of **2** was suspected to arise from intracellular accumulation of the corresponding 5'-triphosphate metabolite, which is generated by a kinase-mediated phosphorylation of the 5'-hydroxyl.

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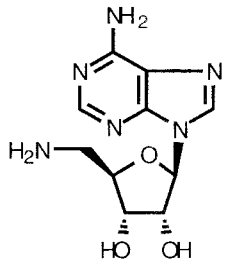

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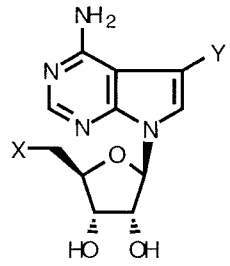

	X	Y
2	OH	I
3	H	I
4	OH	CN
5	OH	$CONH_2$
6	H	CH_3

Furthermore, the structural similarity of **2** with adenosine suggested that some of the side effects might result from non-selective binding to other adenosine-binding proteins. Consequently, our efforts focused on the design of AK inhibitors that lacked the 5'-OH and contained structural features that could enhance selectivity for AK.

RESULTS AND DISCUSSION

To block phosphorylation of the 5'-hydroxyl, the 5'-deoxy analog of iodotubercidin (**3**)[14] was prepared and shown to retain AK inhibitory potency. Study of **3** in vivo, however, revealed a variety of severe side effects and apparent toxicities. Further efforts to design potent and selective AK inhibitors focused on modifications of the heterocyclic base, since the heteroatoms located at e.g. the 1, 6 and 7 positions of the purine base are frequently important for purine nucleoside binding affinity and specificity and often are in contact with residues within the purine-binding site. One site extensively explored during the course of our work was the 5-position of the pyrrolopyrimidine base. Replacement of the iodo group with hydrogen or alkyl groups (e.g. methyl, **6**) led to complete loss in inhibitory activity. Surprisingly, however, replacement of the iodo substituent with phenyl led to potent AK inhibition. Incorporation of a second aryl group attached to the N4 amino group led to compounds with IC_{50}s between 0.5 - 40 nM (e.g. diphenyl analog, **11**, Scheme 1, Table 1).

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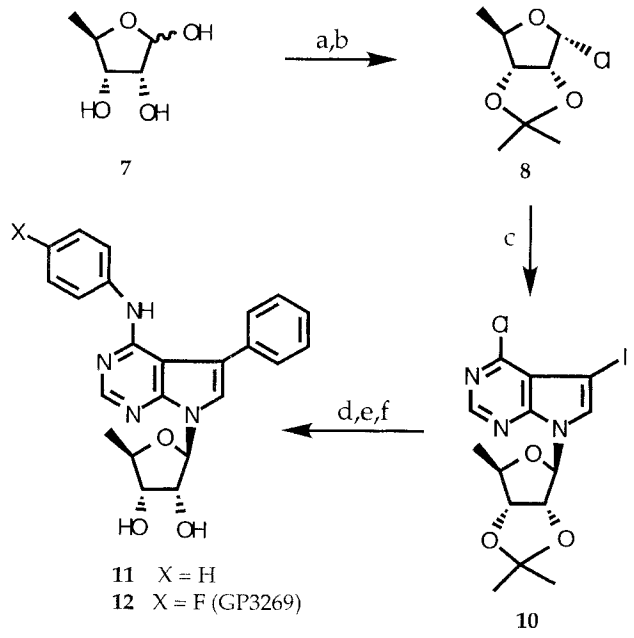
Synthesis of **11** was readily accomplished following the route detailed in Scheme 1. Preparation of the nucleoside **10** from the known base[15] and IP-protected sugar was achieved using the sodium salt coupling procedure.[16] The α-chlorosugar was prepared from the corresponding ribose analog using HMPT and CCl_4.[17] The α-chlorosugar was then reacted immediately with the sodium salt of **9** due to the propensity of the sugar to irreversibly isomerize to the β-anomer over the course of a few hours. Aniline was used to displace the 4-chloro group whereas replacement of the 5-iodo group with phenyl was readily accomplished using the Suzuki reaction.[18] Treatment of the 5-iodo pyrrolopyrimidine nucleoside with phenylboronic acid and tetrakistriphenylphosphine palladium gave **11** after deprotection in 70% yield from **10**. Other aryl groups were subsequently incorporated at this position using the same reaction.

The anticonvulsant activity of AK inhibitors was evident from their ability to suppress maximal electrical shock (MES)-induced seizures in rats. Analysis of **11** in the MES assay gave an ED_{50} of 0.8 and 5.5 mg/kg after i.p. and p.o. administration, respectively. Further studies of **11**, however, indicated that its oral bioavailability was < 20% in rats and < 10% in dogs and monkeys. In addition, PK studies indicated that **11** had a relatively short half-life (≈1.1 hours) in dogs and a high hepatic clearance in all three species. Analysis of the plasma from drug-treated animals by HPLC showed a new peak in the chromatogram that was related in structure to **11** based on spectral analysis. MS analysis of the peak showed a molecular ion consistent with the addition of one hydroxyl group. The identical metabolite was also produced when **11** was incubated with rat, dog and



a: 2,2-Dimethoxypropane, DMF, PTSA; b: THF, CCl_4, HMPT, -78°C; c: 4-Chloro-5-iodopyrrolo[2,3-d]pyrimidine (**9**), NaH, CH_3CN; d: Aniline or *p*-Fluoroaniline, EtOH, CH_3COONa; e: $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3; f: 70% TFA.

SCHEME 1

TABLE 1. Adenosine Kinase Inhibition

Compound	IC_{50} (nM)	Compound	IC_{50} (nM)
1	120	**6**	> 10,000
2	63	**11**	2
3	17	**12**	11
4	310	**13**	32
5	470		

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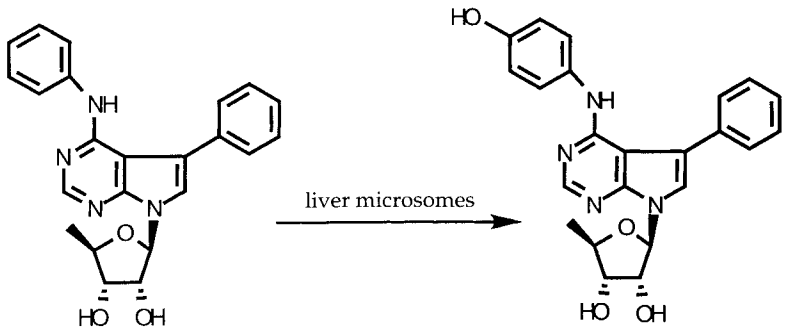

11 13

SCHEME 2

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human S9 microsomes. Since *p*-hydroxylation of phenyl rings is a common metabolic pathway, the corresponding analog in which the 4-phenyl group was substituted by a hydroxyl group, **13**, was prepared and shown to comigrate by HPLC and to have spectral features identical to the isolated metabolite. In addition to **13**, the glucuronidide of **13** was also evident in dogs treated with **11** based on the increased levels of **13** found after treatment of plasma samples with β-glucuronidase.

To extend the half-life of **11** and prevent first pass metabolism, a number of analogs were prepared with substituents at the *para*-position of the 4-anilino group. Analysis of the analog with a fluoro substituent, GP3269 (**12**), showed a large enhancement in oral bioavailability (60%) and the terminal plasma half-life (4.2 hours) in dogs. The compound was also shown to be a potent AK inhibitor (IC_{50} = 11 nM) and to inhibit seizures in the MES rat model with an ED_{50} of 5.1 mg/kg after oral administration. In contrast to A1 agonists, **12** produced no hemodynamic effects (blood pressure, heart rate) in rats.[19]

In summary, GP3269 (**12**) is a potent, orally-bioavailable, adenosine kinase inhibitor that inhibits electrically-induced seizures in rats. The anticonvulsant activity of GP3269 is postulated to result from elevation of adenosine levels at seizure foci and concomitant activation of A1 receptors on excitatory neurons. The ability of GP3269 to inhibit seizure activity without eliciting cardiovascular side effects suggests that AK inhibitors will have significant advantages over adenosine receptor agonists based on their ability to activate A1 receptors on excitatory neurons without simultaneously activating A1 receptors in the periphery.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined at 200 MHz with a Varian Gemini-200 spectrometer.

5-Deoxy-2,3-O-isopropylidene-ß-D-ribofuranose: A mixture of 5-deoxy-D-ribose[20] (**7**, 8.1 g, 60.4 mmol), 2,2-dimethoxypropane (18 mL, 150 mmol) and PTSA (150 mg) in dry DMF (25 mL) was stirred at room temperature for 8 hours. The reaction mixture was concentrated under vacuum and the residue was chromatographed on silica gel (5% methanol in CH_2Cl_2) to obtain an 85:15 anomeric mixture of the protected **7** as an oily product (7.8 g, 74%). ^{1}H-NMR (major isomer, $CDCl_3$) 1.1- 1.7 (m, 9H, 5-CH and isopropylidene), 4.35 (m, 1H, 4-CH), 4.52 and 4.65 (2d, 2H, 2-CH and 3-CH), 5.4 (d, $J_{1'-2'}$ = 2.6 Hz, 1-CH) and 5.79 (bs, 1H, OH).

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4-Chloro-5-iodo-7-(5-deoxy-2,3-O-isopropylidene-ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (10): A solution of 5-deoxy-2,3-O-isopropylidene-D-ribose (4.1 g, 23.56 mmol), CCl_4 (3.15 mL, 30 mmol) in dry THF (35 mL) was cooled to -78°C. HMPT (5.1 mL, 26.5 mmol) was added over a period of 15 minutes maintaining the reaction temperature below -68°C. After stirring for an additional 45 minutes, the reaction mixture was allowed to warm to -15°C. The pale yellow solution was canulated into a stirred mixture of 4-chloro-5-iodopyrrolo[2,3-d]pyrimidine (**4**, 7.0 g, 25 mmol), NaH (80% dispersion in oil, 0.75 g, 25 mmol) in dry CH_3CN (50 mL). After stirring at room temperature for 16 hours, the reaction mixture was concentrated under high vacuum and the residue redissolved in ethyl acetate (50 mL) and filtered. The filtrate was evaporated and the resulting oil chromatographed on silica gel (25% ethyl acetate in hexane). Fractions containing the product were pooled and evaporated to obtain **10** as a glassy product (4.7 g, 43%). ^{1}H-NMR (DMSO-d_6) 1.26-1.29 (d, 3H, 5'-CH_3), 1.31 and 1.54 (2s, 6H, isopropylidene), 4.2 (m, 1H, 4'-CH), 4.77 and 5.32 (2m, 2H, 3'-CH and 2'-CH), 6.28 (d, $J_{1'-2'}$ =2.8 Hz, 1'-CH), 8.22 (s, 1H, 6-CH) and 8.72 (s, 1H, 2-CH). Anal. calcd. for $C_{14}H_{15}N_3O_3Cl$ I: C, 38.6; H, 3.47; N, 9.65; Cl, 8.14; I, 29.13. Found: C, 38.89; H, 3.2; N, 9.56; Cl, 8.48; I, 28.89.

4-N-(4-Fluorophenylamino)-5-phenyl-7-(5-deoxy-ß-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (12): A mixture of **10** (4.35 g, 10 mmol), 4-fluoroaniline (3.0 g, 27 mmol), sodium acetate trihydrate (3.4 g, 25 mmol) and

ethanol (30 mL) was refluxed for 72 hours. After solvent evaporation, the residue was dissolved in ethyl acetate (50 mL) and washed with 10% HCl solution (25 mL) followed by water (25 mL). The organic layer was dried ($MgSO_4$), evaporated and the residue triturated with cold ethanol. The solid was collected by filtration, washed with ethanol (5 mL) and dried under vacuum (3.9 g, 76%), mp 110-111°C. ^{1}H-NMR (DMSO-d_6) 1.18-1.28 (d, 3H, 5'-CH_3), 1.32 and 1.52 (2s, 6H, isopropylidene), 4.18 (m, 1H, 4'-CH), 4.75 and 5.35 (2m, 2H, 3'-CH and 2'-CH), 6.23 (d, $J_{1',2'}$=2.9 Hz, 1'-CH), 7.2-7.8 (2 m, 4H, phenyl), 7.86 (s, 1H, 6-CH), 8.3 (bs, 1H, NH), and 8.4 (s, 1H, 2-CH). The 5-iodo nucleoside (3.5 g, 6.8 mmol) was then treated with phenylboronic acid (2.5 g, 20.5 mmol), tetrakistriphenylphosphine palladium (314 mg, 0.27 mmol), saturated Na_2CO_3 solution (20 mL) and ethanol (4 mL) in diglyme (130 mL) and the reaction mixture heated at 100-105°C. After four hours, the reaction mixture was filtered through a celite pad and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in water and extracted with ethyl acetate (2x100 mL). The organic layers were combined, dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (25% ethyl acetate in hexane). Fractions containing the product were collected and evaporated to provide a colorless glassy product (2.6 g, 83%). ^{1}H-NMR (DMSO-d_6) 1.28-1.33 (d, 3H, 5'-CH_3), 1.34 and 1.54 (2s, 6H, isopropylidene), 4.2 (m, 1H, 4'-CH), 4.78 and 5.36 (2m, 2H, 3'-CH and 2'-CH), 6.23 (d, $J_{1',2'}$=2.92 Hz, 1'-CH), 7.2-7.8 (m, 11H, aromatic and NH), and 8.38 (s, 1H, 2-CH). Removal of the isopropylidene group was accomplished by stirring the arylated product in 120 mL of 70% TFA at room temperature. After 45 min., the solution was evaporated under high vacuum and the residue coevaporated with water (3x50 mL) followed by ethanol (25 mL). The resulting semi-solid was triturated with a $NaHCO_3$ solution and the solid collected by filtration, washed with water and dried under vacuum. This product was crystallized from boiling ethanol to provide **12** as a white crystalline solid (1.86 g, 65% for the final two steps), mp 187-189°C. ^{1}H-NMR (DMSO-d_6) 1.29-1.32 (d, 3H, 5'-CH_3), 3.95 (m, 2H, 3'-CH and 4'-CH), 4.52 (m, 1H, 2'-CH), 5.13 and 5.37 (2d, 2H, 2'-OH and 3'-OH), 6.16 (d, $J_{1',2'}$ = 5.16 Hz, 1'-CH), 7.1-7.65 (m, 11H, aromatic and NH), and 8.38 (s, 1H, 2-CH). Anal. calcd. for $C_{23}H_{21}N_4O_3F$: C, 65.71; H, 5.03; N, 13.33; F, 4.52. Found: C, 65.61; H, 5.15; N, 13.33; F, 4.42.

Biological Assays: Inhibition of enzyme activity was determined using a 0.1 mL assay mixture containing 50 mM Tris-maleate pH 7.0, 0.1% (w/v) BSA, 1 mM ATP, 1 mM $MgCl_2$, 0.5 µM (U-^{14}C] adenosine (500 mCi/mmol) and 0.1 µg of purified pig heart adenosine kinase. Different concentrations of the inhibitors were incubated in the assay mixture for 20 minutes at 37 °C. ^{14}C-AMP was separated from unreacted ^{14}C-adenosine

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by absorption to anion exchange paper (Whatman) and quantified by scintillation counting.[12,21] Seizure activity was determined by administration of the AK inhibitor to rats prior to electroshock treatment. After 1 hour, corneal electrodes were applied to the eyes of each animal and an electrical stimulus of 160 mA delivered for 0.2 seconds. The reported ED_{50} values were derived from a dose-response curve and represent the dose needed to protect 50% of the animals against the hind-limb extensor component.[12]

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