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HALOGENATED PYRROLOPYRIMIDINE ANALOGUES OF ADENOSINE FROM MARINE ORGANISMS: PHARMACOLOGICAL ACTIVITIES AND POTENT INHIBITION OF ADENOSINE KINASE

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Abstract—Two novel halogenated pyrrolopyrimidine analogues of adenosine, isolated from marine sources, have been examined for pharmacological and biochemical activities. 4-Amino-5-bromopyrrolo[2,3-d]pyrimidine, from a sponge of the genus *Echinodictyum*, had bronchodilator activity at least as potent as theophylline but with a different biochemical profile; unlike theophylline it had no antagonist activity at CNS adenosine receptors and it was quite a potent inhibitor of adenosine uptake and adenosine kinase in brain tissue. 5′-Deoxy-5-iodotubercidin, isolated from the red alga *Hypnea valentiae*, caused potent muscle relaxation and hypothermia when injected into mice. This compound was a very potent inhibitor of adenosine uptake into rat and guinea-pig brain slices and an extremely potent inhibitor of adenosine kinase from guinea-pig brain and rat brain and liver. Neither of these two pyrrolopyrimidine analogues was a substrate for, or an inhibitor of, adenosine deaminase. Neither compound appeared to have any direct agonist activity on guinea-pig brain adenosine-stimulated adenylate cyclase (A2 adenosine receptors). 5′-Deoxy-5-iodotubercidin is unique in two respects: it appears to be the first naturally-occurring example of a 5′-deoxyribosyl nucleoside and is the first example of a specifically iodinated nucleoside from natural sources. It may be the most potent adenosine kinase inhibitor yet described and, by virtue of its structure, may prove to be the most specific.

Marine organisms, particularly the sponges, have yielded a number of novel nucleosides. Some of these, such as spongothymidine [1] and 1-methylisoguanosine [2], exhibit potent pharmacological activity when administered to animals (e.g. [3]). Compared to the variety of other natural product classes such as the terpenes, nitrogen heterocycles form only a small percentage of the vast number of new metabolites isolated from marine sources in recent years [4], possibly a reflection of the fact that they are relatively minor constituents and present greater separation problems than the other more prevalent classes.

We now report some of the biological activities of two new compounds structurally related to adenosine which have been isolated from biologically-active crude extracts of marine organisms, using bioassay as a dominant criterion to aid purification. Details of the isolation and structural elucidation of these compunds, both pyrrolo[2,3-d]pyrimidines, have been recently reported [5].

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MATERIALS AND METHODS

In vivo testing in mice. Male Füllinsdorf mice (20–25 g) were used throughout and housed at room temperature with food and water available *ad lib.* For each experiment, groups of five mice were acclimatized in white plastic boxes with sawdust bedding for approximately 1 hr prior to intraperitoneal administration of the extract or compound. Where limited solubility presented a problem, the compounds were given as a fine suspension with Tween 80 (2% v/v) in sterile saline. Volumes did not exceed 0.2 ml per 10 g body weight. Rectal temperature was measured using a YSI thermistor probe (Model No. 423) and Tele-thermometer (Yellow Springs Instrument Co., Ohio). All measurements were compared with a vehicle-treated control group.

Bronchodilator activity. An *in vitro* method using isolated intact trachea was used [6]. Adult guinea-pigs (Himalayan white, 600–800 g) were stunned and the whole of the trachea removed and dissected free of extraneous tissues. The trachea was washed in Krebs bicarbonate buffer, stored overnight at 2–4°, then cannulated with a U-tube connected to a syringe at one end and connected to a Statham pressure transducer at the other end. Experiments were per-

formed at 37°. An *in vivo* method using a modified Konzett–Rössler technique [7] was also employed. Guinea-pigs (400–600 g) were anaesthetized with urethane (approximately 1.5 g/kg i.p.). Compounds were administered in the left jugular vein, blood pressure was recorded from a cannula in the right carotid artery and the intrapulmonary pressure recorded by a pressure transducer connected via a 3-way cannula to the trachea and an animal respirator. Respiration rate and stroke volume were set to keep blood pressure at or near normal [6]. Bronchodilation was assessed by inhibition of pressure increases caused by i.v. administration of histamine (1–10 μg/kg). A second *in vivo* method for testing bronchodilation used the conscious guinea-pig with an aerosol histamine challenge [8]. Animals were placed in a Perspex chamber (10 × 40 × 18 cm) and a 5 mM solution of histamine (10% glycerol) was sprayed in using a Johnson & Johnson 'Maximyst' nebulizer. Prior administration of bronchodilators prolonged the time taken for the guinea-pig to collapse.

Cardiovascular effects. Blood pressure and heart rate effects were determined in Füllinsdorf rats implanted with chronic indwelling carotid cannulae. Details have been previously described [3]. In a number of studies hypertensive rats were used. Rats were made hypertensive by intramuscular injection of 100 mg/kg deoxycorticosterone acetate (DOCA) when 4 weeks old and then maintained on 1% saline until the time of cannulation at approximately 3 months (220–270 g body weight).

Adenosine uptake. Uptake of [2-^{3}H]adenosine was measured in cerebral cortex slices (0.1 × 0.1 × approximately 1 mm) prepared from rat (Füllinsdorf strain) or guinea-pig. Slices were washed 4–5 times by decantation in Krebs bicarbonate buffer before use, to remove adenosine and adenosine deaminase released by tissue damage. Other details have been published [9].

Adenosine kinase. Brains were homogenized in a glass–glass homogenizer in 50 mM sodium acetate buffer, pH 5.6, and the homogenate centrifuged at 20,000 *g* for 1 hr. Enzyme activity was assayed in the supernatant. Adenosine kinase (AK) activity was assayed essentially according to the procedure of De Jong [10], as detailed in ref. [11], using small discs of DEAE ion-exchange paper. A simple apparatus for washing the discs has been described [12].

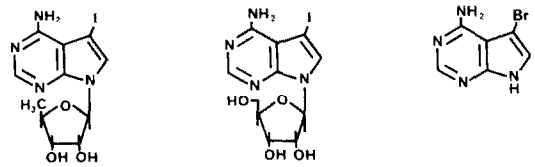

Fig. 1. Structures of (a) the natural product 5′-deoxy-5-iodotubercidin, isolated from a methanol extract of the red alga *Hypnea valentiae*; (b) the related synthetic compound 5-iodotubercidin; and (c) the natural product 4-amino-5-bromopyrrolo[2,3,d]pyrimidine.

Adenosine-stimulated adenylate cyclase. Cerebral cortex slices from guinea-pigs (Himalayan white, 300–500 g) were used. The method was the same as previously described [9]. After exposure of the slices to stimulating agents, cAMP was extracted and determined using kits supplied by Amersham International (Bucks., U.K.).

cAMP phosphodiesterase activity. The assay method used was essentially as outlined by Rangel-Aldao *et al.* [13], using mini-TLC plates of PEI-cellulose to separate [8-^{3}H]cyclic AMP from the hydrolysis product, 5-AMP. After development in 40 mM KCl for 8 min, the plates were dried, cut into strips and the strips eluted directly into scintillation vials with concentrated KCl (in order to ensure that the tritium was not quenched by being tightly bound to the PEI-cellulose). A crude preparation of the soluble enzyme from rat brain was used.

Adenosine deaminase activity. The enzyme used was purified intestinal mucosa adenosine deaminase (ADA; Calbiochem, La Jolla, CA) or dialysed high speed supernatant from rat brain homogenates. Activity was assayed colorimetrically as previously described [14].

Materials. [2-^{3}H]Adenosine, [8-^{3}H]cyclic AMP and cAMP assay kits were supplied by Amersham International (Bucks, U.K.). 5-Iodotubercidin was a kind gift from Prof. L.B. Townsend (University of Michigan) and dilazep, from Prof. B. B. Fredholm (Karolinska Institute). Tubercidin, dipyridamole and hexobendine were supplied by Hoffmann-La Roche (Basel, Switzerland). Other fine chemicals were from Sigma Chemical Co. (St. Louis, MO).

Table 1. *In vivo* and *in vitro* bronchodilator actions of 4-amino-5-bromopyrrolo[2,3-d] pyrimidine

Compound	Approx. ED_{50} concentration: Isolated trachea (g/ml)	Konzett–Rössler technique (mg/kg)
4-Amino-5-bromopyrrolo-[2,3-d]pyrimidine	10^{-5} (12)	0.8 i.v. (4) 50 p.o. (2)
4-Amino--pyrrolo-[2,3-d]pyrimidine	3×10^{-5} (3)	1.0 i.v. (2) 50 p.o. (2)
Theophylline	2×10^{-5} (3)	1.5 i.v. (5)

Compounds were tested *in vitro* in the isolated guinea-pig trachea preparation and *in vivo*, after intravenous or oral dosing, using the ventilated, anaesthetized guinea-pig (see Materials and Methods). The number of experiments is given in parentheses. All compounds were dissolved in DMSO for *in vitro* experiments or a Tween/saline suspension for *in vivo* dosing.

Table 2. Effect of compounds on histamine-induced bronchoconstriction in the anaesthetized guinea-pig

Compound	Intravenous dose (mg/kg)	% Inhib. of* bronchoconstriction
4-Amino-5-bromopyrrolo-	0.5	40 ± 7 (4)
[2,3-d]pyrimidine	1.0	64 ± 8 (7)
	2.0	85 ± 10 (4)
4-Amino-pyrrolo-	1.0	50 ± 1 (2)
[2,3-d]pyrimidine	2.0	97 ± 3 (2)
Theophylline	2.0	75 ± 14 (5)
	5.0	100 ± 1 (3)

* Histamine challenge was given 1 min after injection of the test compound. The compounds were administered as a Tween/saline suspension. Data are means ± S.E.M. (or range) for the number of experiments in parentheses.

The first compound of interest was isolated from a sponge of the genus *Echinodictyum* (new species) collected by trawler from deep water off the coast of South Australia. The activity was detected in the dichloromethane extract of the freeze-dried sponge. Fractionation followed by biossay (guinea-pig isolated trachea) led to the isolation of a pure compound which was identified as 4-amino-5-bromopyrrolo[2,3-d]pyrimidine (Fig. 1c) using high resolution mass spectrometry, ^{1}H- and ^{13}C-nuclear magnetic resonance [5]. This compound, although not previously reported as a natural product, has been synthesized as an analogue of tubercidin [15]. The compound and an analogue lacking the 5-Br were also synthesized by one of us (R.K.) and by Dr. A. F. Cook (Hoffmann-La Roche, Nutley).

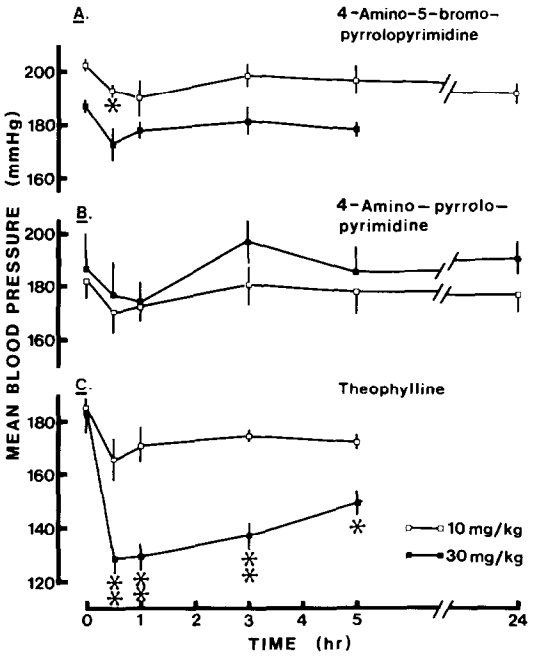

Fig. 2. The effect on blood pressure in DOCA/salt hypertensive rats of compounds administered orally at 10 and 30 mg/kg. Blood pressures were recorded in conscious unrestrained animals through chronic, indwelling carotid cannulae. (A) The natural product 4-amino-5-bromopyrrolopyrimidine. (B) A synthetic analogue of the natural product lacking the 5-Br. (C) Theophylline. Data given are means ± S.E.M. of results from four rats at each dose for theophylline (C) and from five rats at each dose for the two pyrrolopyrimidine compounds (A and B). *Significantly different from predosing blood pressure at $P < 0.01$ and **$P < 0.001$.

The second compound, isolated from a methanol extract of the red alga *Hypnea valentiae* (collected at Quobba Lagoon, Western Australia), was initially detected as a potent muscle-relaxant and hypothermic agent in mice. Fractionation, purification and structural elucidation [5] determined that the active component was 5′-deoxy-5-iodotubercidin[7(5′-deoxyribos-1′-β-yl)-4-amino-5-iodopyrrolo-[2,3-*d*]pyrimidine] (Fig. 1a).

RESULTS

Pharmacological activities in vivo

4-Amino-5-bromopyrrolo[2,3-d]pyrimidine. *Bronchodilator activity:* The pure natural product was marginally more potent than theophylline both *in vivo* and *in vitro* (Tables 1 and 2). When tested against histamine-induced bronchoconstriction in the anaesthetized guinea-pig, the onset of effect of the natural compound (and the synthetic analogue lacking the 5-Br) was faster than for theophylline but the duration of action was somewhat less, i.e. the recovery of the control constrictor response to histamine after oral dosing (50–100 mg/kg) was slower for theophylline (approximately 4 hr) than for the natural product (2.5–3 hr). In preliminary trials in the conscious guinea-pig, oral administration of 25, 50 and 100 mg/kg of the synthetic natural product, 1 hr prior to a histamine–aerosol challenge, provided very significant protection with a potency similar to that of theophylline.

Toxicity and CNS effects: Because of insufficient compound we were not able to determine LD_{50}s accurately and thus minimum lethal doses were determined. The MLD for the brominated natural product in mice was 400 mg/kg i.p., compared with an MLD for the synthetic analogue 4-amino-pyrrolo[2,3-d]pyrimidine of 100 mg/kg i.p. and 200 mg/kg i.p. for theophylline. With the natural product, slight sedation at 100 mg/kg i.p. was observed. This was quite pronounced at 200 mg/kg and above. This compound was also observed to potentiate barbiturate-induced sleeping times and to have anti-amphetamine activity. This is in contrast to theo-

phylline and to the synthetic analogue lacking the 5-Br; this latter compound produced hyperactivity at 100 mg/kg i.p., preconvulsive symptoms at 200 mg/kg and clonic, progressing to tonic-extensor convulsions at 500 mg/kg. Our data for theophylline toxicity agree closely with literature values [16].

Cardiovascular effects: Thirty minutes after oral dosing with 10 or 30 mg/kg of the natural product, there was only a small (approximately 5%) fall in the blood pressure of DOCA/salt hypertensive rats (Fig. 2A). Theophylline at these same doses had a much greater and more prolonged effect (Fig. 2C). The analogue lacking the 5-Br had non-significant effects (Fig. 2B). None of the compounds at 10 mg/kg p.o. affected heart rate, while at 30 mg/kg only theophylline produced an effect, a significant and prolonged heart rate increase (26.5% increase 0.5 hr after dosing, gradually falling over the next 5 hr). At 10 or 30 mg/kg p.o. in normotensive rats the natural product had no significant effect on heart rate or blood pressure. Given intravenously at 10 mg/kg, the natural product did lower blood pressure in DOCA/salt hypertensive rats.

Similar results were noted in the anaesthetized guinea-pig. Whereas theophylline at doses from 2 mg/kg i.v. lowered blood pressure, with falls greater than 50% at 5 mg/kg i.v., neither the natural product nor the synthetic analogue lacking the 5-Br had any effect at 2 mg/kg i.v., while at 5 and 10 mg/kg i.v., only transient falls were recorded (no greater than 50% at 10 mg/kg).

5′-Deoxy-5-iodotubercidin.

Muscle-relaxation/hypothermia: The initial crude methanol extract of *Hypnea valentiae* and the purified active compound produced pronounced muscle relaxation and hypothermia when administered i.p. in mice (Fig. 3). Doses of 10 mg/kg led to prolonged hypothermia and death unless the animals were warmed.

A paucity of the isolated pure compound limited further *in vivo* studies, although in one other experiment it blocked mono- and polysynaptic spinal reflexes in anaesthetized Füllinsdorf mice [17]. To date, this compound has proved difficult to synthesize.

Biochemical studies

Adenosine-stimulated adenylate cyclase. In view of its structural and pharmacological similarity to theophylline, 4-amino-5-bromopyrrolopyrimidine was tested for its ability to block adenosine receptors, using the adenosine-stimulated adenylated cyclase of guinea-pig brain slices as the test system. Theophylline antagonizes the stimulatory action of adenosine on cAMP levels [18]. Adenosine in the range 15–100 μM caused dramatic increases in the cAMP levels of the brain slices (up to 15-fold) (Fig. 4). This response was not blocked by 4-amino-5-bromopyrrolopyrimidine but in fact very significantly potentiated by it to produce more than additive effects. When tested alone, the compound caused only minimal increases (2- to 3-fold) in the basal level of cAMP and there was no dose-related response (in the range 15–500 μM). Theophylline (250 μM) blocked this small stimulatory effect. These results suggested that

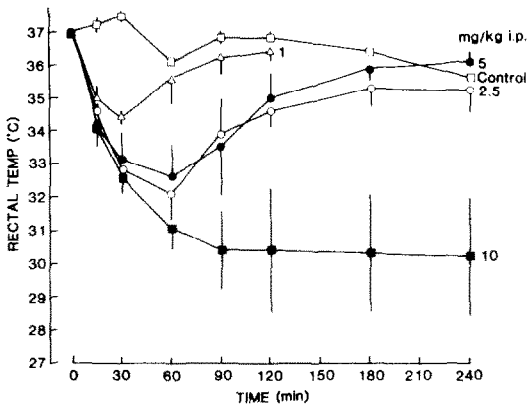

Fig. 3. The rectal temperature was recorded in male Füllinsdorf mice (five per group) at various times after administration of varying doses of 5′-deoxy-5-iodotubercidin by the intraperitoneal route. (△) 1 mg/kg; (○) 2.5 mg/kg; (●) 5 mg/kg; (■) 10 mg/kg; (□) control. The ED_{50} for muscle relaxation was scored (using a subjective rating scale) and an ED_{50} of 1.7 mg/kg (1.0–2.8, 95% confidence limits) calculated. The potency of this compound in producing muscle relaxation was similar to that for 1-methylisoguanosine with an ED_{50} of 3.0 mg/kg i.p. (1.9–4.6, 95% confidence limits) [3].

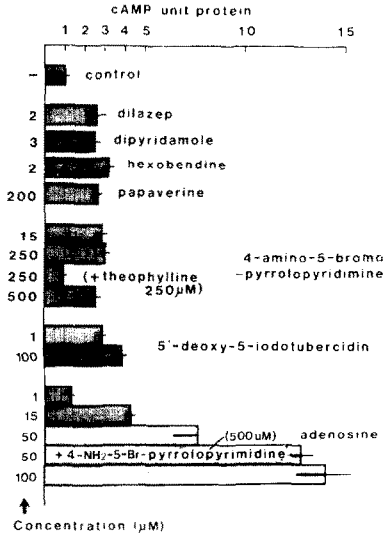

Fig. 4. Cyclic AMP accumulation in 250 μm guinea-pig cerebral cortex slices in the presence of four standard adenosine-uptake blockers (dilazep, dipyridamole, hexobendine and papaverine), 4-amino-5-bromopyrrolopyrimidine, 5′-deoxy-5-iodotubercidin and adenosine. Results from each experiment (pmole cAMP/mg protein) were normalized to enable comparison of results from separate experiments in which the basal cAMP level varied (the mean ± S.E.M. of the absolute control cAMP concentration was 16.6 ± 2.0 pmole/mg; 18 determinations). Data are means ± S.E.M. The number of determinations for each bar was (top to bottom): 18, 3, 2, 3, 3, 6, 4, 4, 4, 7, 4, 3, 3, 3, 3 and 3. The four standard adenosine-uptake blockers were tested at concentrations chosen to be in excess of 20 times their respective IC_{50}'s for inhibition of [2-^{3}H]adenosine uptake.

Table 3. Inhibition of soluble cAMP phosphodiesterase from rat brain

Compound	Concentration (μM)	% Inhibition
4-Amino-5-bromopyrrolo-[2,3-*d*]pyrimidine	100	27.5
IBMX	100	68.6
Papaverine	100	75.6
1-Methylisoguanosine	100	2.5
Adenosine	100	2.8
2-Chloroadenosine	100	17.3

Inhibition of a crude preparation of soluble cAMP phosphodiesterase activity prepared from rat brain using 1 μM [8-^{3}H]cAMP as substrate. The rate of hydrolysis of cAMP in the presence or absence of the compounds was determined from initial slopes of time–course curves, measured at five time points over 60 min, with a dilute preparation of the enzyme. Data are per cent inhibition of the hydrolysis rate relative to controls (no added compound).

the compound might be acting to inhibit re-uptake of endogenously-released adenosine which could then act on the cyclase receptors. In support of this, the compound gave maximal cAMP stimulations equal to the cAMP stimulations given by high concentrations of four standard adenosine-uptake inhibitors—dilazep, dipyridamole, hexobendine and papaverine (tested at concentrations approximately 20 times their IC_{50}s for inhibition of [^{3}H]adenosine uptake) (Fig. 4).

When 5′-deoxy-5-iodotubercidin (1–100 μM) was tested in washed guinea-pig brain slices, a small (2- to 3-fold) increase in cAMP levels was observed, but as with 4-amino-5-bromopyrrolopyrimidine, there was no dose-related response.

Adenosine deaminase. Neither of the two marine natural products were deaminated by purified intestinal mucosa ADA or by a crude rat brain homogenate (containing ADA and guanine deaminase [14]). Furthermore, neither compound inhibited the deamination of adenosine by ADA from either source.

cAMP phosphodiesterase. A possible mechanism causing potentiation of adenosine in the cAMP assay is by an inhibition of cAMP phosphodiesterase. 4-Amino-5-bromopyrrolopyrimidine did have some activity against soluble cAMP PDE from rat brain, but much less than isobutylmethylxanthine (IBMX) or papaverine (Table 3). 5′-Deoxy-5-iodotubercidin was not tested against cAMP PDE.

Adenosine uptake. Evidence from the cAMP-stimulation assay suggested that both compounds might be acting to inhibit adenosine uptake. When tested against uptake of [2-^{3}H]adenosine into well-washed rat or guinea-pig brain slices, both com-

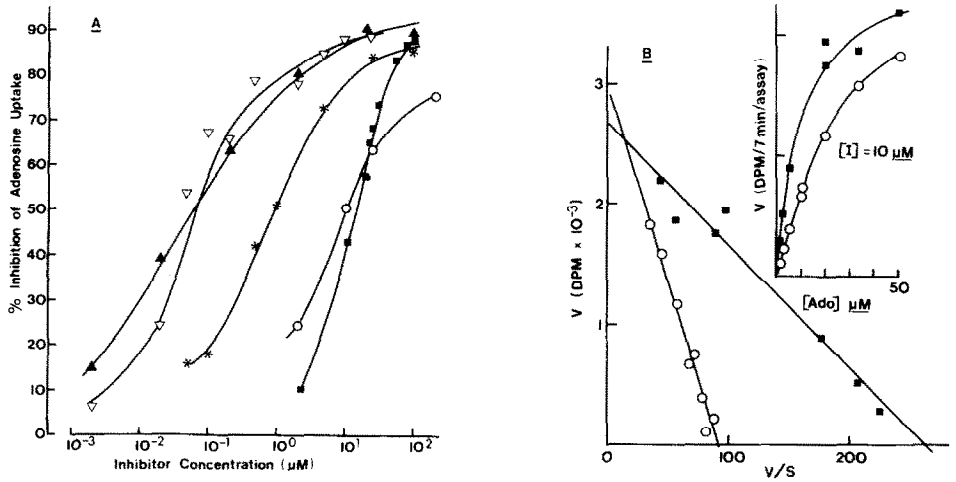

Fig. 5. (A) Percentage inhibition of 5 μM [2-^{3}H]adenosine uptake into guinea-pig cerebral cortex slices (100 μm mini-slices) by: (▲) dipyridamole; (○) papaverine; (■) adenosine; (*) 4-amino-5-bromopyrrolopyrimidine and (▽) 5′-deoxy-5-iodotubercidin. Each point represents the mean of one to three experiments, each performed in triplicate. Uptake was measured as the total dpm in the slices minus blank dpm (accumulation measured at 0°, in the presence of a 1000-fold excess of unlabelled adenosine). The inhibition curve for adenosine was not shifted in the presence of 50 nM 2′-deoxycoformycin, a very potent adenosine deaminase inhibitor, demonstrating that in these well-washed slices ADA was not degrading the substrate in the extracellular environment and influencing the amount of adenosine uptake observed. Calculated IC_{50} values are given in Table 4. (B) Eadie–Hofstee plot illustrating the competitive nature of the inhibition of high-affinity adenosine uptake in guinea-pig brain slices (measured at seven concentrations over the range 1.25–50 μM) by 10 μM papaverine.

Table 4. Inhibition of [2-^{3}H]adenosine uptake into rat and guinea-pig brain slices

Compound	IC_{50} for adenosine uptake (μM)	
	Rat	Guinea-pig
4-Amino-5-bromopyrrolo-[2,3-*d*]pyrimidine	7.32 ± 1.72	0.986 ± 0.137
5′-Deoxy-5-iodotubercidin	0.040 ± 0.009	0.133 ± 0.023
5-Iodotubercidin	0.022 ± 0.004	0.071 ± 0.021
Tubercidin	n.t.	65.8 ± 9.96
2′-Deoxyadenosine	70.0 ± 15.7	n.t.
Adenosine	13.2 ± 1.42	14.1 ± 1.11
1-Methylisoguanosine	n.t.	⩾250
Dilazep	1.38 ± 0.29	0.063 ± 0.016
Dipyridamole	1.31 ± 0.23	0.084 ± 0.020
Hexobendine	1.39 ± 0.39	0.019 ± 0.006
Lidoflazine	51.7 ± 7.4	n.t.
Papaverine	51.3 ± 17.1	9.78 ± 1.12

IC_{50} values for inhibition of 5 μM [2-^{3}H]adenosine uptake into 100 μm mini-slices of cerebral cortex are given as means ± S.E.M. for 2–4 separate experiments, each using 3–5 separate inhibitor concentrations. IC_{50} values were calculated by computer-assisted long-probit analysis. n.t. = Not tested.

pounds were found to be quite potent inhibitors (Table 4 and Fig. 5A). In fact, in rat brain slices at least, 5′-deoxy-5-iodotubercidin was significantly more potent than dipyridamole, hexobendine or dilazep. Kinetic analyses of the inhibition (Fig. 6) demonstrated that both compounds were non-competitive inhibitors of adenosine uptake. This is in contrast to papaverine which inhibits uptake competitively (Fig. 5B), in agreement with previous observations [19].

Adenosine kinase. Both compounds were potent inhibitors of rat and guinea-pig brain AK activity (Table 5). In fact, 5′-deoxy-5-iodotubercidin was significantly more potent (at least an order of magnitude) than 5-iodotubercidin when measured against guinea-pig brain and rat brain and liver AK activity (Fig. 7).

Kinetic analysis using guinea-pig brain cortex AK activity suggested that the inhibition by 5′-deoxy-5-iodotubercidin was competitive whereas that by 4-amino-5-bromopyrrolopyrimidine was non-competitive (Fig. 8).

Table 5. Inhibition of rat and guinea-pig brain adenosine kinase activity

Compound	Concentration (μM)	% Inhibition	
		Rat	Guinea-pig
4-Amino-5-bromopyrrolo-[2,3-d]pyrimidine	100	80 ± 1 (4)	79 ± 2 (3)
5′-Deoxy-5-iodotubericidin	10	98 ± 1 (4)	91 ± 3 (2)
5-Iodotubercidin	10	67 ± 4 (2)	83 ± 1 (2)
2′-Deoxyadenosine	100	6 ± 1 (3)	n.t.
Dilazep	100	−10 ± 1 (2)	−6 ± 3 (2)
Dipyridamole	10	3 ± 2 (4)	−6
Hexobendine	100	1 ± 8 (2)	7
Lidoflazine	100	1 ± 3 (4)	n.t.
Papaverine	100	−1 ± 15 (2)	n.t.

Inhibition of adenosine kinase was determined at a single concentration of inhibitor given in the left-hand column. Per cent inhibitions are expressed as means ± S.E.M. (or range) for the number of separate experiments in parentheses; in each experiment determinations were performed in triplicate.

K_ms for adenosine of 26.0 ± 4.7 μM (n = 8) and 12.9 ± 2.1 μM (n = 6) were measured using crude preparations of rat and guinea-pig brain adenosine kinase, respectively. The respective K_i values for inhibition of the rat and guinea-pig brain enzyme by 5′-deoxy-5-iodotubercidin were 0.46 ± 0.14 μM (n = 4) and 0.11 ± 0.03 μM (n = 6), while for 4-amino-5-bromopyrrolopyrimidine the respective K_i values were 47.5 ± 6.8 μM (n = 4) and 31.3 ± 5.9 μM (n = 3).

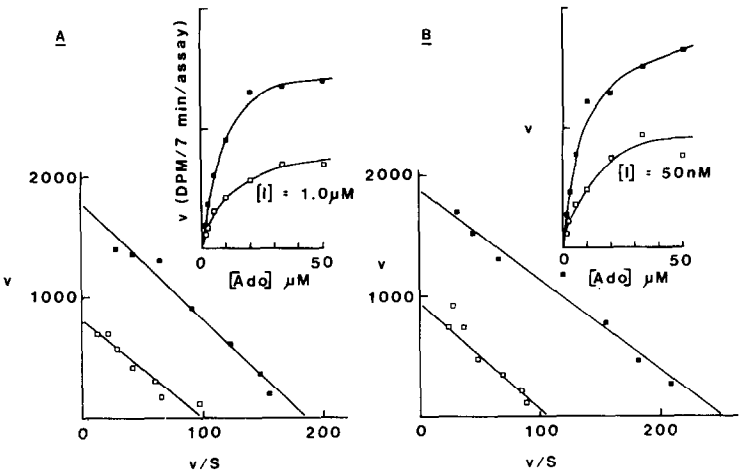

Fig. 6. [2-^{3}H]Adenosine uptake into guinea-pig cerebral cortex mini-slices measured at seven concentrations over the range 1.25–50 μM, in the absence (■) or presence (□) of inhibitor. The K_m for high-affinity uptake of adenosine was determined to be 8.5 μM (S.E.M. = 0.5 for four separate experiments). (A) Eadie–Hofstee plot showing non-competitive inhibition by 1.0 μM 4-amino-5-bromopyrrolopyrimidine. (B) Non-competitive inhibition by 50 nM 5′-deoxy-5-iodotubercidin. Data illustrated represent triplicate determinatioins from single experiments.

DISCUSSION

Two novel halogenated pyrrolopyrimidine analogues of adenosine have been isolated from marine organisms. One of these, 4-amino-5-bromopyrrolo[2,3-d]pyrimidine from a sponge (genus *Echinodictyum*) had bronchodilator activity at least as potent as theophylline, if not marginally more so, when tested in several animal models. However, the compound had a markedly different biochemical profile. In particular, the marine natural product had no antagonist activity at adenosine receptors mediating cAMP increases in guinea-pig brain slices. This result supports recent observations by Persson *et al.* [20] that among xanthine derivatives there is a clear differentiation between bronchodilation and adenosine antagonism; in fact adenosine antagonism seems to be neither necessary nor desirable with xanthine anti-asthmatics. Another difference is that the compound was quite a potent inhibi-

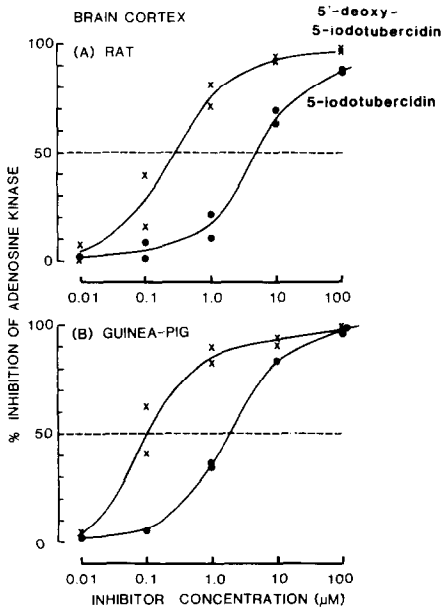

Fig. 7. Per cent inbihition of adenoside kinase from (A) rat brain and (B) guinea-pig brain by (×) 5′-deoxy-5-iodotubercidin and by (●) 5-iodotubercidin. The adenosine substrate concentration was 50 μM.

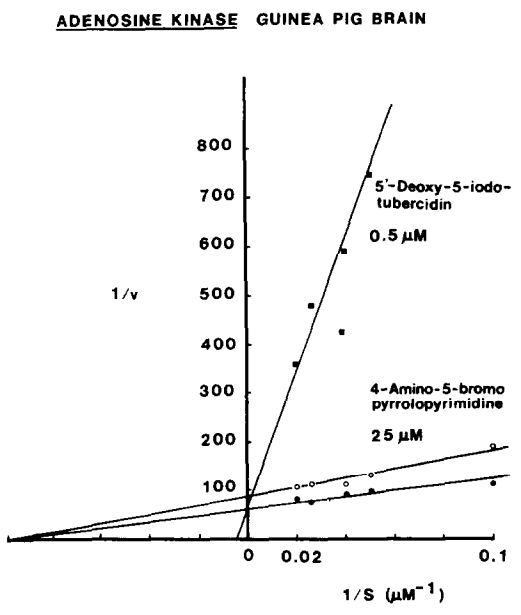

Fig. 8. Lineweaver–Burk plots of the inhibition of guinea-pig brain cortex adenosine kinase activity (●) by 0.5 μM 5-deoxy-5-iodotubercidin (■) and by 25 μM 4-amino-5-bromopyrrolopyrimidine (○) at five concentrations of [2-^{3}H]adenosine in the range 10–50 μM. The plotted data are from a single experiment in triplicate. Averaged inhibition constants from a number of experiments are given in Table 5.

tor of adenosine uptake into CNS tissue slices, unlike theophylline which is extremely weak.

This compound has a further pharmacological difference in that unlike theophylline, a CNS stimulant, it had a general CNS depressant activity. Recently other workers noted that several synthetic pyrrolo[2, 3-d]pyrimidines (bearing methyl groups in the 5-position) had some anticonvulsant activity against PTZ-induced seizures [21]. The observation that this bronchodilator, structurally related to theophylline and caffeine, is neither an adenosine antagonist nor a CNS stimulant indirectly lends support to the hypothesis that the CNS excitatory properties of xanthines may result from an antagonism of the depressant effects of endogenously-released adenosine [22].

5'-Deoxy-5-iodotubercidin is unique in two respects. Firstly, it appears to be the first naturally-occurring example of a 5'-deoxyribosyl nucleotide and secondly, it is the first example (with the exception of amino acid derivatives) of a specifically iodinated metabolite from natural sources [5].

The compound produced intense muscle relaxation and hypothermia when administered i.p. to mice. In this respect it is similar to 1-methylisoguanosine, another adenosine analogue isolated from marine sources [2, 23]. In view of its structural similarity to adenosine, it was examined for activity as an adenosine agonist in several biochemical and pharmacological assays, in an attempt to find a basis for its *in vivo* activity. Like 1-methylisoguanosine it was completely resistant to ADA (prepared from rat brain or calf intestinal mucosa). It was without activity as an inhibitor of ADA, compared with 1-methylisoguanosine which was a weak inhibitor [18]. When tested in the range 1–100 μM, 5'-deoxy-5-iodotubercidin caused a small but dose-independent increase in cAMP levels in well-washed, preincubated guinea-pig brain slices. Similar small increases were caused by standard adenosine uptake blockers such as dipyridamole, hexobendine and papaverine. Therefore this action is likely to be due to an inhibition of the re-uptake of endogenous adenosine, released from the brain slices during the course of the incubation. This lack of a dose-dependent response is in contrast to 1-methylisoguanosine which has direct agonist activity at the A2 adenosine receptors mediating increases in cAMP.

When tested against [^{3}H]adenosine uptake into guinea-pig and rat brain slices, 5'-deoxy-5-iodotubercidin was an extremely potent inhibitor, with IC_{50}s in guinea-pig and rat brain slices (using 5 μM [^{3}H] adenosine substrate concentration) of 40 and 133 nM, respectively [the respective K_ms for adenosine are 9.6 ± 0.5 μM ($n = 6$) and 21.1 ± 7.3 μM ($n = 4$)].

Kinetic studies in mammalian brain slices have demonstrated that adenosine reaches the cells by carrier-mediated diffusion, facilitated or driven by rapid intracellular phosphorylation to AMP by cytosolic AK [24]. Since the adenosine uptake measured in our assay is a result of both membrane transport and subsequent intracellular metabolism, it is possible that the inhibition of uptake by both 4-amino-5-bromopyrrolopyrimidine and by 5'-deoxy-5-iodotubercidin is not at the level of the membrane nucleoside transporter but by an inhibition of intracellular AK. Both natural products strongly inhibited rat and guinea-pig brain and rat liver adenosine kinase. In fact, 5'-deoxy-5-iodotubercidin was at least an order of magnitude more potent than 5-iodotubercidin, one of the most potent AK inhibitors previously described [25]. When measured against adenosine uptake, however, 5-iodotubercidin was marginally more potent than 5'-deoxy-5-iodotubercidin. This relative difference in potency in the two assays may possibly be explained by the accessibility of the two nucleoside analogues to the intracellular enzyme. Whereas the AK assay uses a relatively crude tissue homogenate (no permeability barriers to consider), the uptake assays are carried out with intact tissue slices. The degree of inhibition of [^{3}H]adenosine uptake by a 'pure' AK inhibitor will be determined by its ability to cross the membrane (presumably as a substrate for the nucleoside transporter). It remains to be determined whether 5'-deoxy-5-iodotubercidin also inhibits the neucleoside transporter; these studies will be performed when compound becomes available.

5'-Deoxy-5-iodotubercidin, as a possible specific AK inhibitor, has exciting potential as a research tool. 5-Iodotubercidin, a previously known compound, is a potent AK inhibitor but it is also a substrate for the enzyme and the resulting phosphorylated form subsequently inhibits other enzymes of nucleotide metabolism. By virtue of the fact that the marine natural product lacks the 5'-OH and therefore will not be phosphorylated, it has the potential of being far more selective as an inhibitor of AK. A specific inhibitor of AK would be a valuable tool, not only in pharmacological studies on the role adenosine as a possible neuromodulator or neurotransmitter within the CNS but also in various areas of cancer research or in studies on those severe combined immunodeficiency diseases (SCID) which result from an inherited deficiency of adenosine deaminase. Certain lymphoblastic leukaemias respond to inhibition of adenosine deaminase by 2'-deoxycoformycin, apparently due to the lymphotoxicity of the raised levels of adenosine (or deoxyadenosine) which result. In basic research into these cancers or into SCID, it is not yet clear whether the adenosine (or deoxyadenosine) must be phosphorylated first (by AK) in order for toxicity to be expressed. It is clear that a specific AK inhibitor might help solve these problems.

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