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ANTIPROTOZOAL ACTIVITY OF 3′-DEOXYINOSINE

INVERSE CORRELATION TO CLEAVAGE OF THE GLYCOSIDIC BOND

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Abstract—Two nucleosides related to the known antiprotozoal agent 1-(β-D-ribofuranosyl)-1,5-dihydro-4*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (allopurinol riboside, **1**) were prepared and evaluated against *Leishmania donovani*, *Trypanosoma cruzi*, and *Trypanosoma gambiense*. 3′-Deoxyinosine (**2**) exhibited potent antiprotozoal activity against the three protozoal pathogens with minimal toxicity for host cells. It was found to be especially effective against the Columbia strain of *T. cruzi* reported to be resistant to **1**. The antiprotozoal activity of **2** appeared to be inversely related to the rate of cleavage of the glycosidic bond, as shown by metabolic profiles of **2** in the various pathogenic hemoflagellates and host cells. Combining the key structural elements of **1** and **2** led to the synthesis of 1-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (3′-deoxy-allopurinol riboside, **3**), which was found to be inactive as an antiprotozoal agent.

The rational design of chemotherapeutic agents has employed a number of approaches with varying degrees of success. One approach for the pathogenic hemoflagellates takes advantage of qualitative differences between host and parasite in the purine salvage and interconversion pathways [1–5]. As an example of this approach, 1-(β-D-ribofuranosyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (allopurinol riboside, **1**) has been shown to be a potent growth inhibitor of *Leishmania donovani* and *Trypanosoma cruzi* with significantly lower toxicity for mammalian cells [6]. This selective toxicity toward *L. donovani* and *T. cruzi* has been attributed to the metabolic conversion of **1** to its corresponding 5′-monophosphate and ultimately to 4-amino-1-(β-D-ribofuranosyl)-pyrazolo[3,4-*d*]-pyrimidine-5′-triphosphate by the protozoa, though not by mammalian cells [6–8].

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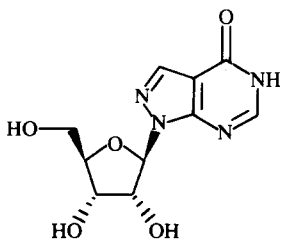
Previous studies in these laboratories had suggested that an altered purine ring was a requirement for selective antiprotozoal activity [7, 8]. In contrast to these findings, Wataya and Hiraoka [9] have reported that 3′-deoxyinosine (**2**), a compound with a modified sugar and intact purine, is a potent growth inhibitor of *L. tropica* with minimal toxicity toward a model host cell line (mouse mammary tumor FM3A). Although Wataya and Hiraoka examined the metabolism of **2** by *L. tropica*, they did not describe the metabolism of **2** by the host cell line or the sensitivity of other pathogenic hemoflagellates to the effects of 3′-deoxyinosine.

To further evaluate the selectivity of this antiprotozoal activity, we have examined the cytotoxic effects and metabolism of 3′-deoxyinosine with three other parasitic protozoa, including a strain of *T. cruzi* known to be resistant to **1**, and with a mammalian host cell line. With the thought that a compound possessing both an altered sugar and an



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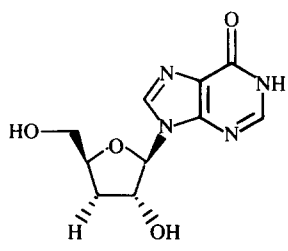

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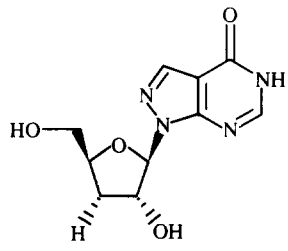

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altered purine ring would lead to enhanced antiprotozoal activity with minimal toxicity for the host cell, we have also prepared and evaluated 1-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (3′-deoxy-allopurinol riboside, **3**), a novel compound with structural features of both **1** and **2**.

MATERIALS AND METHODS

Cordycepin (3′-deoxyadenosine), cordycepin-5′-monophosphate, 3′-dATP, and 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine were obtained from the Sigma Chemical Co., St. Louis, MO. [^{3}H(G)]Cordycepin (25 mCi/mmol) was purchased from Moravek Biochemicals, Inc., Brea, CA. 3′-Deoxyuridine was prepared by Dr. J. Wilson of the Chemical Development Laboratories, Burroughs Wellcome Co., Research Triangle Park, NC, according to literature methods [10]. All other chemicals were of the highest purity commercially available and were used without further purification.

Adenosine deaminase (EC 3.5.4.4, calf intestine) was obtained as a glycerol solution from Boehringer Mannheim, Indianapolis, IN. Uridine phosphorylase (EC 2.4.2.3) and purine nucleoside phosphorylase (EC 2.4.2.10) were purified from *Escherichia coli* as previously described [11]. One unit of enzyme activity is defined as the amount of enzyme that will catalyze the formation of 1 μmol of product/min under the defined assay conditions [11].

Melting points were determined on a Thomas Hoover UniMelt® apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian XL-200 (200.058 MHz) or a Varian XL-300 (299.945 MHz) and are reported relative to tetramethylsilane. Assignments of the two resonances of the purine or pyrazolo[3,4-*d*]pyrimidine nucleus are not unequivocal and may be reversed. Mass spectra were recorded at 70 eV (EI) by Oneida Research Services, Inc., Whitesboro, NY. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA.

3′-Deoxyinosine (**2**). Cordycepin (100 mg) was dissolved in 30 mL of 50 mM potassium phosphate, pH 7.0, and 5.0 units of adenosine deaminase was added. The mixture was allowed to proceed at 37° for 48 hr, lyophilized, and the residue applied to a column of Bio-Gel P-2 (2.5 × 45 cm) in 30% 1-propanol/water and eluted with the same solvent to afford 94 mg (87%) of the desired compound: m.p. 192–193° (lit., 197–199° [12]); ^{1}H-NMR (Me_2SO-d_6) δ 12.30 (br s, 1H, NH), 8.32 (s, 1H, H-8), 8.05 (s, 1H, H-2), 5.85 (d, 1H, H-1′, J = 1.8 Hz), 5.00 (br s, 2H, 2′-OH, 5′-OH), 4.48 (m, 1H, H-2′), 4.34 (m, 1H, H-4′), 3.35 (m, 2H, H-5′), 2.20 (ddd, 1H, H-3′$_\alpha$, J_{gem} = 13.2 Hz, $J_{2',3'\alpha}$ = 9.0 Hz, $J_{3'\alpha,4'}$ = 5.4 Hz), 1.87 (ddd, 1H, H-3′$_\beta$, J_{gem} = 13.2 Hz, $J_{3'\beta,4'}$ = 6.1 Hz, $J_{2',3'\beta}$ = 2.4 Hz); MS (EI): *m/z* 233 (M − H_2O − H), 201 (233 − CH_3OH), 165 ($C_5H_3N_4O$ + CHOH), 149 ($C_5H_3N_4O$ + CH_2), 136 ($C_5H_4N_4O$), 117 (M + H − $C_5H_3N_4O$). Analysis calculated for $C_{10}H_{12}N_4O_4 \cdot H_2O$: C, 44.44; H, 5.22; N, 20.73. Found: C, 44.43; H, 5.20; N, 20.72.

4-Amino-1-(3-deoxy-β-D-erythro-pentofuranosyl)-1H-pyrazolo[3,4-d]pyrimidine (**4**). 4-Amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (0.405 g, 3.0 mmol) and 3′-deoxyuridine (0.684 g, 3.0 mmol) were dissolved in 200 mL of 10 mM potassium phosphate buffer, pH 7.4. To this was added 300,000 units of purine nucleoside phosphorylase and 30,000 units of uridine phosphorylase. The mixture was incubated for 76 hr at 37°, at which time HPLC indicated that the reaction had reached equilibrium (C-18, 10% MeOH in 50 mM ammonium phosphate, pH 7.3, 1.0 mL/min). After filtering, the reaction mixture was lyophilized, purified by column chromatography on Bio-Gel P-2 with 30% 1-propanol/H_2O, and the product recrystallized from water to provide 282 mg (36%) of the desired compound: ^{1}H-NMR (Me_2SO-d_6): δ 8.14 (s, 1H, H-3), 8.13 (s, 1H, H-6), 7.75 (br s, 2H, NH_2), 6.11 (d, 1H, H-1′, J = 1.0 Hz), 5.60 (br s, 1H, 2′-OH), 4.73 (br m, 1H, 5′-OH), 4.53 (m, 1H, H-2′), 4.32 (m, 1H, H-4′), 3.43 (m, 2H, H-5′), 2.30 (ddd, 1H, H-3′$_\alpha$, J_{gem} = 12.8 Hz, $J_{3'\beta,4'}$ = 5.6 Hz, $J_{2',3'\alpha}$ = 9.4 Hz), 1.98 (ddd, 1H, H-3′$_\beta$, J_{gem} = 12.8 Hz, $J_{3'\beta,4'}$ = 6.4 Hz, $J_{2',3'\beta}$ = 1.8 Hz); MS (EI): *m/z* 252 (M + H), 221 (M − CH_2O), 203 (221 − H_2O), 178 ($C_5H_4N_5$ + CH_2CHOH), 164 ($C_5H_4N_5$ + CHOH), 149 ($C_5H_4N_5$ + CH_2 + H), 135 ($C_5H_5N_5$). Analysis calculated for $C_{10}H_{13}N_5O_3 \cdot 0.5\ H_2O$: C, 45.97; H, 5.79; N, 26.81. Found: C, 45.98; H, 5.39; N, 26.87.

1-(3-Deoxy-β-D-erythro-pentofuranosyl)-1.5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (**3**). Compound **4** (100 mg) was dissolved in 30 mL of 50 mM potassium phosphate, pH 7.0, to which was added 10.0 units of adenosine deaminase. The mixture was allowed to proceed at 37° for 72 hr and then lyophilized. The residue was applied to a column of Bio-Gel P-2 (2.5 × 45 cm) in 30% 1-propanol/water; it was eluted with the same solvent. UV-absorbing material was collected and concentrated to afford 78 mg (81%) of the desired compound. ^{1}H-NMR (Me_2SO-d_6) δ 8.18 (s, 1H, H-6), 8.13 (s, 1H, H-3), 7.50 (br. s, 1H, NH), 6.13 (d, 1H, H-1′, J = 1.0 Hz), 5.53 (d, 1H, 2′-OH, J = 4.0 Hz), 4.75 (t, 1H, 5′-OH, J = 5.6 Hz), 4.56 (m, 1H, H-2′), 4.30 (m, 1H, H-4′), 3.42 (m, 2H, H-5′), 2.32 (ddd, 1H, H-3′$_\alpha$, J_{gem} = 13.2 Hz, $J_{2',3'\alpha}$ = 9.0, $J_{3'\alpha,4'}$ = 6.2 Hz), 1.97 (ddd, 1H, H-3′$_\beta$, J_{gem} = 13.2 Hz, $J_{3'\beta,4'}$ = 6.2, $J_{2',3'\beta}$ = 2.0 Hz); MS (EI): *m/z* 253 (M + H), 222 (M − CH_2O), 203 (M − CH_2OH − H_2O), 179 ($C_5H_3N_4O$ + CH_2CHOH), 165 ($C_5H_3N_4O$ + CHOH), 149 ($C_5H_3N_4O$ + CH_2), 137 ($C_5H_4N_4O$ + H). Analysis calculated for $C_{10}H_{12}N_4O_4 \cdot H_2O$: C, 44.44; H, 5.22; N, 20.73. Found: C, 44.38; H, 5.31; N, 20.79.

Growth of protozoa and host cell culture. The protozoa and host macrophages were grown, quantitated using a Coulter counter, and harvested according to previously described methods, as follows: *L. donovani* (promastigote) [13], *T. cruzi* (epimastigote) [4], *T. brucei gambiense* (procyclic form) [14], and U-937 cells [15].

Growth inhibition assay. The compounds were evaluated for their ability to inhibit the growth of the various organisms during 5 days and U-937 cells during 7 days as previously described [16]. Control

Table 1. Approximate EC_{50} values for growth inhibition of protozoa and host macrophages by selected nucleosides

Protozoan/Host cell line	EC_{50} (μM)		
	Allopurinol riboside (**1**)	3′-Deoxyinosine (**2**)	3′-Deoxy-allopurinol riboside (**3**)
Leishmania donovani	7.0	2.5	>100
Trypanosoma cruzi (Peru)	2.0–10	5	>100
Trypanosoma cruzi (Colombia)	>50	1.5	>100
Trypanosoma gambiense	20	40	>100
U-937 macrophages	>1000	150	>100

growth assays showed a 2-log increase in the number of cells during these time periods. The EC_{50} values were estimated from semi-logarithmic plots of the percent growth inhibition versus compound concentration.

Metabolism of 3′-deoxyinosine. [^{3}H(G)]-3′-Deoxyinosine (sp. act. 25.0 mCi/mmol), prepared as described above for unlabeled material, was incubated with the hemoflagellated protozoa at the concentrations given in Table 2 for 24 hr; the cells were quantitated and harvested, and then extracted with perchloric acid as previously described [17]. Analysis of the extracts for endogenous nucleotides and nucleotide metabolites was carried out by HPLC as previously described [17].

RESULTS AND DISCUSSION

The estimated EC_{50} values for **2** with the four protozoa and host U-937 macrophages are shown in Table 1. Also given are the corresponding values for allopurinol riboside (**1**) and 3′-deoxy-allopurinol riboside (**3**). These data show that 3′-deoxyinosine was active against a range of protozoa other than *L. tropica*.

The concentration–response curves for the four protozoa (Fig. 1) were all similar, showing an exponential decline in protozoal growth with increasing concentrations of **2**. In contrast, the concentration–response curve for the host U-937 cell line (Fig. 1) showed little evidence of cellular toxicity at concentrations up to 100 μM, but marked toxicity at higher concentrations.

A comparison of the data in Table 1 to a previous report [16] reveals several interesting points. First, 3′-deoxyinosine was approximately equipotent with allopurinol riboside against *L. donovani*, *T. b. gambiense*, and the Peru strain of *T. cruzi*. Second, the Colombia strain of *T. cruzi*, although resistant to the inhibitory activity of **1**, was quite sensitive to **2**. Third, 3′-deoxyinosine was more toxic to host cells than was allopurinol riboside. This may reflect the ability of host cells to convert **2** to the corresponding 3′-dATP, a known cytotoxic agent capable of inhibiting RNA synthesis [18]. In contrast, it has been shown that host cells lack the ability to effect a similar transformation of allopurinol riboside monophosphate to the corresponding 4-amino nucleotide analogue and ultimately the triphosphate [19].

As a test of this hypothesis, we examined the metabolism of [^{3}H-(G)]-**2** in the four hemoflagellates and the U-937 host cells. The results and experimental conditions are given in Table 2. All of the cells examined metabolized **2** to the corresponding 3′-deoxyadenosine nucleotides (3′-dAMP, 3′-dADP, and 3′-dATP). This probably involved direct phosphorylation of **2**, as suggested by the presence of 3′-deoxyinosine monophosphate in the extracts from the Colombia strain of *T. cruzi*. This was then putatively converted to 3′-dAMP by succino-AMP synthetase (EC 6.3.4.4) and succino-AMP lyase (EC 4.3.2.2). This proposed pathway is consistent with previous metabolic studies of inosine analogues in these cells [13, 16, 17, 20–22] and with the proposed pathway described for **2** in *L. tropica* [9].

In addition to being incorporated into 3′-deoxyadenosine nucleotides, **2** was found to be incorporated into a variety of endogenous ribonucleotides, presumably by cleavage of the glycosidic bond and incorporation of the released hypoxanthine. This pathway was not examined previously with *L. tropica* [9] and may explain some of the unidentified peaks observed by Wataya and Hiraoka. The partitioning of metabolites of **2** between these two pathways is apparently significant to the biological activity of **2**, as seen in the close correlation observed when the ratio of the level of radioactivity in ATP versus 3′-dATP was plotted against the approximate EC_{50} values seen with these organisms (Fig. 2). The lower toxicity of **2** toward the U-937 cells may therefore simply be an indication of the ability of these cells to rapidly degrade the compound to hypoxanthine and a relatively innocuous sugar analogue. Although greater quantities of 3′-dATP were seen in the U-937 cells, the higher ribonucleotide pools and a greater volume of the mammalian cell would be expected to produce a significantly lower effective intracellular concentration of 3′-dATP.

Although **2** shows good activity with a variety of protozoa, especially with a strain of *T. cruzi*

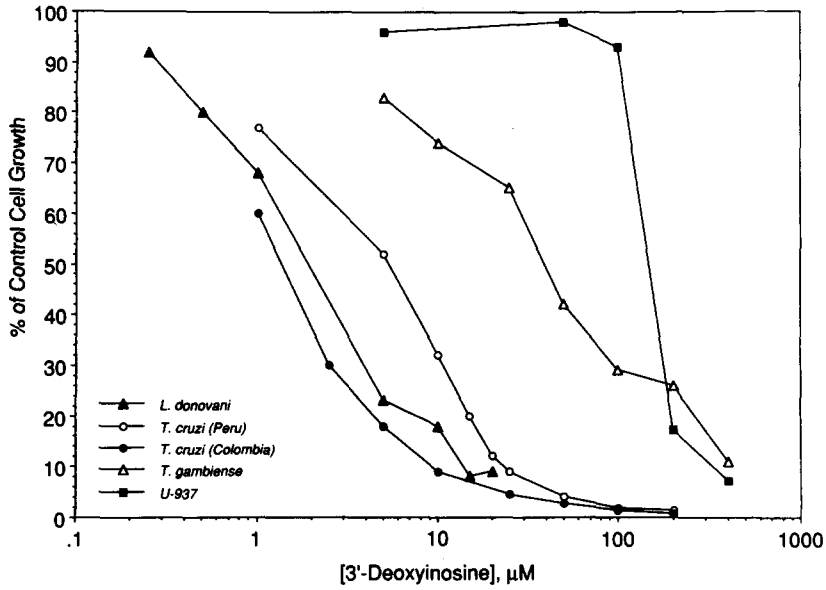

Fig. 1. Growth inhibitory effect of 3′-deoxyinosine on *L. donovani*, *T. cruzi* (Peru), *T. cruzi* (Colombia), *T. gambiense*, and U-937 macrophages.

Table 2. Concentrations of endogenous nucleotides and metabolites of [^{3}H]-3′-deoxyinosine

	Concentration (pmol/10^6 cells)				
		T. cruzi			
	L. donovani	Peru	Colombia	*T. gambiensis*	U-937
Endogenous nucleotides					
NAD + AMP	23 (1.500)*	78 (0.070)	82 (1.170)	49 (1.800)	916 (26.000)
NADP	1.8 (0.070)	3.4 (0.000)	29 (0.004)	2.9 (0.050)	52 (0.760)
ADP	22 (2.500)	44 (0.040)	74 (0.160)	33 (1.400)	1400 (33.000)
GDP	14 (0.220)	21 (0.010)	16 (0.000)	16 (0.120)	516 (4.800)
CTP	1.5 (0.000)	12 (0.000)	3.5 (0.000)	1.7 (0.000)	365 (0.000)
UTP	45 (0.000)	214 (0.000)	99 (0.000)	85 (0.000)	1312 (0.000)
ATP	62 (3.300)	191 (0.170)	85 (0.110)	101 (3.900)	3802 (75.000)
GTP	20 (0.150)	44 (0.020)	15 (0.000)	34 (0.170)	1213 (6.400)
Nucleotide metabolites					
3′-dIMP	<0.005	<0.005	0.073	<0.005	<0.005
3′-dAMP	<0.005	0.008	0.036	<0.005	<0.005
3′-dADP	0.560	0.076	0.210	<0.005	<0.005
3′-dATP	0.560	0.310	0.150	0.120	0.740
[^{3}H]-ATP:[^{3}H]-3′-dATP	5.89	0.55	0.73	32.50	101.35
Experimental parameters					
Cell density (10^6/mL)	13	13	22	7.8	0.97
Incubation time (hr)	24	24	24	24	24
Concentration of **2** (μM)	8.8	8.9	10	7.6	6.8

* Numbers in parentheses are the concentration of radiolabeled endogenous nucleotide formed during the incubation period.

previously shown to be resistant to the activity of allopurinol riboside, we feel that there is reason for concern with this compound. Compound **2** can be metabolized by mammalian cells to a known toxic agent, 3′-dATP. Further, the extensive metabolism of **2** by mammalian cells would serve to limit the delivery of effective concentrations of **2** to the intracellular amastigote forms of *L. donovani* and *T. cruzi*. Combined, these factors make **2** an unlikely candidate for use as a chemotherapeutic agent in diseases caused by these pathogenic hemoflagellates.

Since allopurinol riboside (**1**) lacks significant

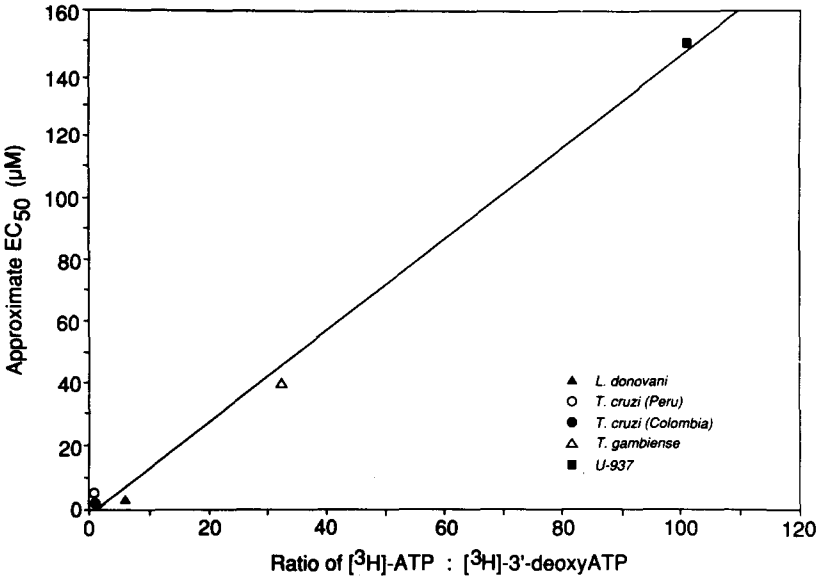

Fig. 2. Plot of the ratio of [^{3}H]-ATP to [^{3}H]-3′-dATP derived from [^{3}H]-3′-deoxyinosine versus approximate EC_{50} (μM) for the four protozoal and U937 cell lines.

toxicity for mammalian cells due to the specificity of succino-AMP synthetase and succino-AMP lyase, we felt that the corresponding 3′-deoxy-allopurinol riboside (**3**) may offer the advantage of good antiprotozoal activity, especially against the Colombia strain of *T. cruzi*, with diminished toxicity for the host. As seen in Table 1, compound **3** was devoid of any significant antiprotozoal activity at concentrations up to 100 μM. The reasons for this are unknown at this time, but may be indicative of a failure by the protozoa to phosphorylate this analog.

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REFERENCES

1. Marr JJ, Nelson DJ and Berens RL, Purine metabolism in *Leishmania donovani* and *Leishmania braziliensis*. *Biochim Biophys Acta* **544**: 360–371, 1978.
2. Ceron CR, Caldas RA, Feliz CF, Mundim MH and Roitman I, Purine metabolism in trypanosomatids. *J Protozool* **26**: 479–483, 1979.
3. Gutteridge WE and Gaborek M, A re-examination of purine and pyrimidine synthesis in the three main forms of *Trypanosoma cruzi*. *Int J Biochem* **10**: 415–422, 1979.
4. Berens RL, Marr JJ, LaFon SW and Nelson DJ, Purine metabolism in *Trypanosoma cruzi*. *Mol Biochem Parasitol* **3**: 187–196, 1981.
5. Hammond DJ and Gutteridge WE, Purine and pyrimidine metabolism in the trypanosomatidae. *Mol Biochem Parasitol* **13**: 243–261, 1984.
6. Nelson DJ, LaFon SW, Tuttle JV, Miller WH, Miller RL, Krenitsky TA, Elion GB, Berens RL and Marr JJ, Allopurinol ribonucleoside as an antileishmanial agent. *J Biol Chem* **254**: 11544–11549, 1979.
7. Spector T, Berens RL and Marr JJ, Adenylosuccinate synthetase and adenylosuccinate lyase from *Trypanosoma cruzi*. *Biochem Pharmacol* **31**: 225–229, 1982.
8. Spector T, Jones TE, LaFon SW, Nelson DJ, Berens RL and Marr JJ, Monophosphates of formycin B and allopurinol riboside. *Biochem Pharmacol* **33**: 1611–1617, 1984.
9. Wataya Y and Hiraoka O, 3′-Deoxyinosine as an antileishmanial agent: The metabolism and cytotoxic effects of 3′-deoxyinosine in *Leishmania tropica* promastigotes. *Biochem Biophys Res Commun* **123**: 677–683, 1984.
10. Uemtsu T and Suhadolnik RJ, Synthesis of 3′-deoxyuridine. In: *Nucleic Acid Chemistry* (Eds. Townsend LB and Tipson RS), Vol. 1, pp. 405–411. Wiley, New York, 1978.
11. Krenitsky TA, Koszalka GW and Tuttle JV, Purine nucleoside synthesis, an efficient method employing nucleoside phosphorylases. *Biochemistry* **20**: 3615–3621, 1981.
12. Yamazaki A, Akiyama M, Kumashiro I and Ikehara M, Synthesis of 2′- and 3′-deoxyinosines. *Chem Pharm Bull (Tokyo)* **21**: 1143–1146, 1973.
13. Marr JJ, Berens RL, Nelson DJ, Krenitsky TA, Spector T, LaFon SW and Elion GB, Antileishmanial action of 4-thiopyrazolo(3,4-*d*)pyrimidine and its ribonucleoside. Biological effects and metabolism. *Biochem Pharmacol* **31**: 143–148, 1982.
14. Fish WR, Marr JJ and Berens RL, Purine metabolism in *Trypanosoma brucei gambiense*. *Biochim Biophys Acta* **714**: 422–428, 1982.
15. Looker DL, Martinez S, Horton JM and Marr JJ, Growth of *Leishmania donovani* amastigotes in the continuous human macrophage cell line U937: Studies of drug efficacy and metabolism. *J Infect Dis* **154**: 323–327, 1986.
16. Marr JJ, Berens RL, Cohn NK, Nelson DJ and Klein RS, Biological action of inosine analogs in *Leishmania* and *Trypanosoma* spp. *Antimicrob Agents Chemother* **25**: 292–295, 1984.
17. LaFon SW, Nelson DJ, Berens RL and Marr JJ,

Inosine analogs: Their metabolism in mouse L cells and in *Leishmania donovani*. *J Biol Chem* **260**: 9660–9665, 1985.

18. Klenow H and Overgaard-Hansen K, Effect of cordycepin triphosphate on the incorporation of [8-^{14}C]adenine and [^{32}P]orthophosphate into the acid-soluble ribotides of Ehrlich ascites tumor cells *in vitro*. *Biochim Biophys Acta* **80**: 500–504, 1964.
19. Spector T and Miller RL, Mammalian adenylosuccinate synthetase nucleotide monophosphate substrates and inhibitors. *Biochim Biophys Acta* **445**: 509–517, 1976.
20. Fish WR, Marr JJ, Berens RL, Looker DL, Nelson DJ, LaFon SW and Balber AE, Inosine analogs as chemotherapeutic agents for African trypanosomes: Metabolism in trypanosomes and efficacy in tissue culture. *Antimicrob Agents Chemother* **27**: 33–36, 1985.
21. Marr JJ and DoCampo R, Chemotherapy for Chagas' disease: A perspective of current therapy and considerations for future research. *Rev Infect Dis* **8**: 884–903, 1986.
22. Rainey P, Nolan PA, Townsend LB, Robins RK, Fox JJ, Secrist JA III and Santi DV, Inosine analogs as anti-leishmanial agents. *Pharmaceut Res* 217–220, 1985.