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Antitrypanosomal Activity of Purine Nucleosides Can Be Enhanced by Their Conversion to O-Acetylated Derivatives

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Fifteen purine nucleosides and their O-acetylated ester derivatives were examined for in vitro antitrypanosomal activity against the LAB 110 EATRO isolate of *Trypanosoma brucei brucei* and two clinical isolates of *Trypanosoma brucei rhodesiense*. Initial comparisons of activity were made for the LAB 110 EATRO isolate. Three nucleoside analogs exhibited no significant activity (50% inhibitory concentrations [IC_{50}s] of >100 μM), whether they were O acetylated or unacetylated; three nucleosides showed almost equal activity (IC_{50}s of <5 μM) for the parent compound and the O-acetylated derivative; nine nucleosides showed significantly improved activity (≥3-fold) upon O acetylation; of these nine analogs, six displayed activity at least 10-fold greater than that of their parent nucleosides. The most significant results were those for four apparently inactive compounds which, upon O acetylation, displayed IC_{50}s of ≤25 μM. When the series of compounds was tested against *T. brucei rhodesiense* isolates (KETRI 243 and KETRI 269), their antitrypanosomal effects were comparable to those observed for the EATRO 110 strain. Thus, our studies of purine nucleosides have determined that O acetylation consistently improved their in vitro antitrypanosomal activity. This observed phenomenon was independent of their cellular enzyme targets (i.e., *S*-adenosylmethionine, polyamine, or purine salvage pathways). On the basis of our results, the routine preparation of O-acetylated purine nucleosides for in vitro screening of antitrypanosomal activity is recommended, since O acetylation transformed several inactive nucleosides into compounds with significant activity, presumably by improving uptake characteristics. O-acetylated purine nucleosides may offer in vivo therapeutic advantages compared with their parent nucleosides, and this possibility should be considered in future evaluations of this structural class of trypanocides.

Human disease caused by *Trypanosoma brucei gambiense* (West Africa) and *T. brucei rhodesiense* (East Africa) is a prominent health concern in subequatorial Africa. More than 50 million people are at risk for this disease, which is uniformly fatal if left untreated. Agents currently in use to treat these infections are either (i) highly toxic, (ii) often rendered ineffective by resistant strains, or (iii) costly and inconvenient to administer to local populations. There is a continuing and urgent need for innovative and practical approaches to chemotherapy of human African trypanosomiasis (18, 29).

New directions for the design of trypanocides were opened in the late 1970s with the discovery that African trypanosomes are particularly susceptible to treatment by the polyamine biosynthesis inhibitor, DL-α-difluoromethylornithine (DFMO; eflornithine) (2). Subsequent findings ultimately led to the clinical use of DFMO for the treatment of African sleeping sickness (24). Although DFMO treatment has proved highly effective, DFMO is a costly drug to synthesize and administer. Furthermore, DFMO is not adequately active against the East African form of the disease (28, 29).

DFMO is an enzyme-activated inhibitor of ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthetic pathways. ODC converts ornithine to the diamine putrescine, which is in turn converted to spermidine and then spermine via sequential aminopropyl group transfers from decarboxylated *S*-adenosylmethionine (AdoMet). DFMO was originally developed at Marion Merrell Dow as a potential anticancer agent but did not prove clinically efficacious for treating human malignancies. As an inhibitor of ODC, the antiproliferative activity of DFMO in tumor cells is attributable to its cytostatic effects in lowering cellular levels of putrescine and spermidine (20). In contrast, the antiproliferative effects of DFMO in trypanosomes appear to be primarily related to perturbations of AdoMet metabolism rather than polyamine biosynthesis (1).

The pathways of methionine/AdoMet and polyamine metabolism are interrelated (Fig. 1). AdoMet is synthesized from methionine and ATP by AdoMet synthetase. AdoMet is the principal biological methyl donor and participates in many critical cellular processes that require methylation for execution. Additionally, AdoMet can be decarboxylated by AdoMet decarboxylase, to produce the propylamine donor for synthesis of the polyamines, spermidine and spermine. The by-product of these transfers is 5′-deoxy-5′-(methylthio)adenosine (MTA), which is cleaved by MTA phosphorylase to yield 5-deoxy-5-methylthioribose-1-phosphate (MTRP) and adenine. MTRP is further metabolized and ultimately recycled to methionine whereas adenine is salvaged for purine synthesis. African trypanosomes, in contrast to mammalian cells, synthesize putrescine and spermidine but not spermine. African trypanosomes further utilize spermidine to form trypanothione, a bis(glutathionyl)spermidine conjugate that behaves similarly to glutathione and is uniquely found in trypanosomatids.

The success of DFMO against trypanosomiasis has raised

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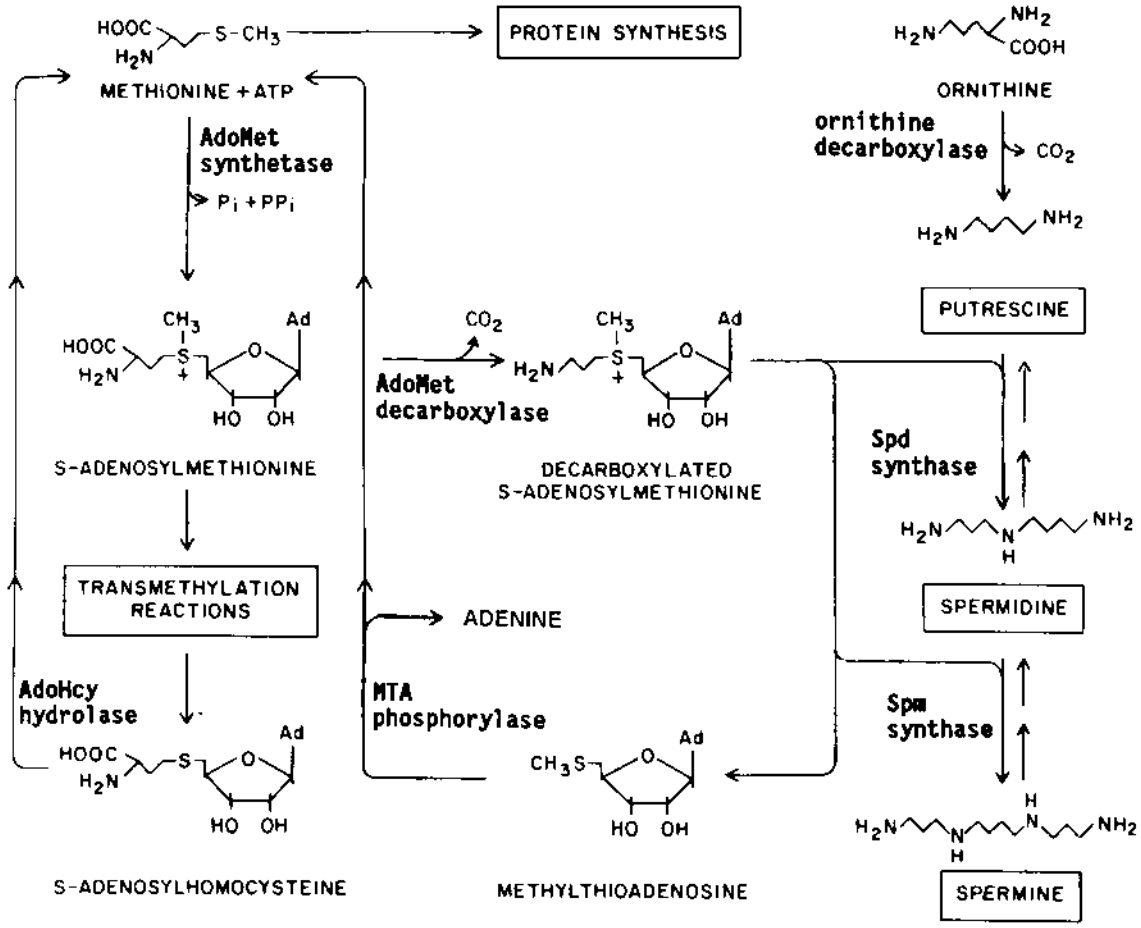

FIG. 1. *S*-Adenosylmethionine and polyamine pathways as they occur in mammalian cells. Mammalian cells synthesize spermidine and spermine whereas African trypanosomes produce spermidine only. African trypanosomes further utilize spermidine to form a bis(glutathionyl)spermidine conjugate, trypanothione (not shown in figure).

interest in other enzymes of AdoMet/polyamine pathways as potential targets for the design of trypanocides. Two of these enzyme targets, AdoMet decarboxylase and MTA phosphorylase, have yielded new agents which are curative for various strains of African trypanosomiasis: 5′-{[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine (AbeAdo; MDL73811), an inhibitor of AdoMet decarboxylase (7, 12), and 5′-deoxy-5′-(hydroxyethylthio)adenosine (HETA), a selective substrate of trypanosome MTA phosphorylase (4). HETA was found to be curative to mice infected with *T. brucei brucei* when it was administered by continuous infusion with the use of Alza osmotic pumps (implanted subcutaneously).

Our initial discovery of the in vivo trypanocidal activity of HETA has been followed by efforts to improve its bioavailability within the infected host. Strategies to modify the solubility characteristics of HETA have included consideration of lipophilic prodrug forms that would release HETA slowly and thus partially mimic conditions of continuous infusion. 2′,3′,5′-Tri-*O*-acetyl-HETA was synthesized as a potential prodrug. Like HETA, it is soluble in dimethyl sulfoxide and poorly soluble in water; unlike HETA, it is freely soluble in alcohol and chloroform. Surprisingly, tri-*O*-acetyl-HETA was slightly more active than HETA against *T. brucei brucei* in culture. When administered to *T. brucei brucei*-infected mice, it was consistently more effective than HETA in curing the infections (3). These observations led to preparation of the O-acetylated derivatives of other purine nucleosides for comparison of their in vitro antitrypanosomal effects with those of the parent nucleosides.

MATERIALS AND METHODS

Trypanosome strains. The *T. brucei brucei* LAB 110 EATRO was obtained from W. Trager of the Rockefeller University (27) and clinical isolates of *T. brucei rhodesiense* (KETRI 243 and KETRI 269) were obtained from A. R. Njogu of the Kenya Trypanosomiasis Research Institute (KETRI). KETRI 243 is resistant to pentamidine and melarsoprol, and KETRI 269 is resistant to pentamidine (1). These strains were adapted to grow under axenic conditions in the bloodstream form in HMI-18 medium with hypoxanthine at 1 μM and 20% horse serum instead of synthetic serum (17).

Determination of in vitro antitrypanosomal activity. Drug studies were done in duplicate in 24-well plates (1 ml per well), with final inhibitor concentrations of 0.1, 1, 10, 25, and 100 μM. After 48 h, the number of parasites was determined in a Z1 Coulter counter, and the approximate range of activity was determined. The 50% inhibitory concentrations (IC_{50}s) were then determined from additional studies with closely spaced inhibitor concentrations. Inhibitors with <50% inhibition at 100 μM were not studied further. Most analogs were dissolved in water, several were dissolved in 100% dimethyl sulfoxide. Dilutions were made with HMI-18 medium, so that the dimethyl sulfoxide concentration never exceeded 0.3%, a noninhibitory concentration.

Nucleoside analogs. Compounds 1, 3, 3A, 4, 4A, 5, 6, 6A, 7, and 7A were synthesized according to our published procedures (25, 26). Compounds 1A, 2A, 5A, 8A, 9A, 11A, 12A, 13A, 14A, and 15A were obtained by O acetylation of the respective parent nucleosides under conditions described by us previously (26). Compound 1B was obtained from HETA (25) in the following manner: the 5′-hydroxy substituent of HETA was silylated with tert-butyldimethylsilyl chloride according to the general procedure of Corey et al. (13). O acetylation of the

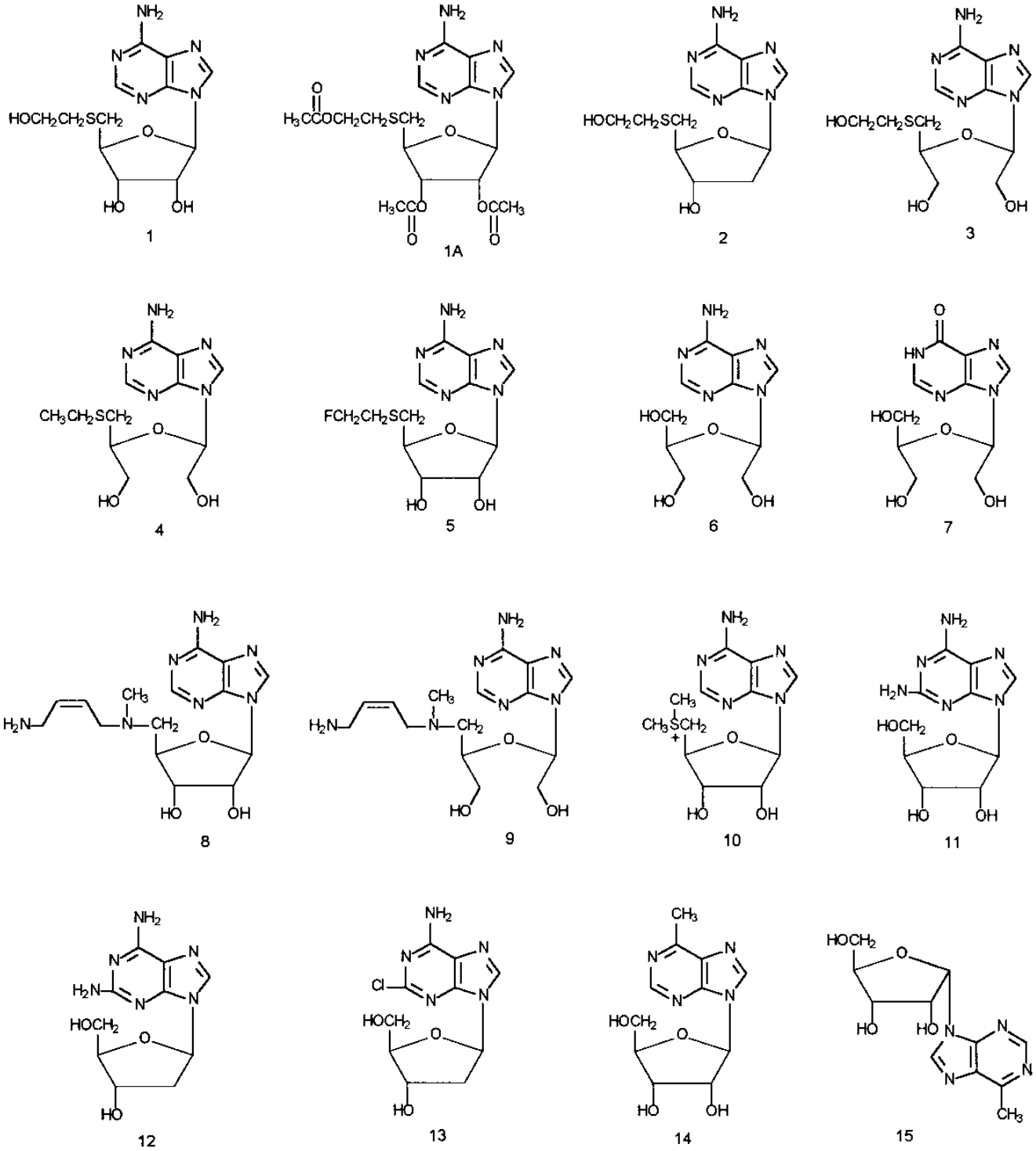

FIG. 2. Structures of purine nucleosides.

5′-protected intermediate was followed by treatment with *p*-toluenesulfonic acid (1%) in methanol to give 1B. Compound 2 was prepared by subjecting 2′-deoxyadenosine to the general synthetic conversions used to prepare HETA from adenosine (25). Compound 8 was prepared according to Casara et al. (12). Compound 9 was prepared from 8 according to our method for preparing purine 2′,3′-acyclonucleosides (26). Compound 10 was prepared according to Borchardt (8). Compound 10A was synthesized by treating 2′,3′-di-*O*-acetyl-5′-deoxy-5′-(methylthio)adenosine with methyl iodide according to the general procedure of Borchardt (8). Compound 11 was obtained from Raylo Chemicals (Edmonton, Alberta, Canada). Compounds 12 and 13 were obtained from Sigma Chemical Company (St. Louis, Mo.). Compounds 14 and 15 were synthesized according to Montgomery and Hewson (21). All compounds synthesized by us were purified (silica gel column chromatography or recrystallization) and were analyzed by thin-layer chromatography on a silica gel and by ^{1}H nuclear magnetic resonance spectrometry.

Structures of nucleoside analogs 1 to 15 appear in Fig. 2. Compounds 3, 4, 6,

TABLE 1. In vitro activities of compounds against *T. brucei* EATRO 110, KETRI 243, and KETRI 269

Compound no.	Compound	IC_{50} (μM)			% Inhibition at 100 μM			Group[a]
		EATRO 110	KETRI 243	KETRI 269	EATRO 110	KETRI 243	KETRI 269	
1	HETA	0.54	0.44	0.45				II
1A	2′,3′,5′-Tri-*O*-acetyl-HETA	0.32	0.31	0.26				II
1B	2′,3′-Di-*O*-acetyl-HETA	1.8	1.1	0.74				
2	2′-Deoxy-HETA				26	28	45	III
2A	3′,5′-Di-*O*-acetyl-2′-deoxy-HETA (*S*5-163)	22	30.6	14.9				III
3	2′,3′-Acyclo-HETA	14	15	25				III
3A	2′,3′,5′-Tri-*O*-acetyl-2′,3′-acyclo-HETA	5.3	5.2				22	III
4	2′,3′-Acyclo-ETA				47	33		I
4A	2′,3′-Di-*O*-acetyl-2′,3′-acyclo-ETA				41	39		I
5	MFETA	0.20	0.32	0.28				II
5A	2′,3′-Di-*O*-acetyl-MFETA	0.83	0.62	0.60				II
6	2′,3′-Acycloadenosine				45	29	12	I
6A	2′,3′,5′-Tri-*O*-acetyl-2′,3′-acycloadenosine				28	16	32	I
7	2′,3′-Acycloinosine				28	29	20	III
7A	2′,3′,5′-Tri-*O*-acetyl-2′,3′-acycloinosine	5.4				21	41	III
8	AbeAdo (MDL73811)	0.099	0.045	0.22				III
8A	2′,3′-Di-*O*-acetyl-AbeAdo	0.014	0.014	0.020				III
9	2′,3′-Acyclo-AbeAdo	16	15	ND[b]				III
9A	2′,3′-Di-*O*-acetyl-2′,3′-acyclo-AbeAdo	1.8	4.6	3.8				III
10	DMTA				43	26	ND	III
10A	2′,3′-Di-*O*-acetyl-DMTA	10	28	60				III
11	2-Aminoadenosine				42	34	35	I
11A	2′,3′,5′-Tri-*O*-acetyl-2-aminoadenosine				27	25	34	I
12	2-Amino-2′-deoxyadenosine				26	28	8	III
12A	3′,5′-Di-*O*-acetyl-2′-deoxy-2-aminoadenosine	55	88	76				III
13	2-Chloro-2′-deoxyadenosine	28				12	3	III
13A	3′,5′-Di-*O*-acetyl-2-chloro-2′-deoxyadenosine	5.9	8.4	11.9				III
14	β-D-6-Methylpurine riboside	2.11	1.55	0.22				II
14A	2′,3′,5′-Tri-*O*-acetyl-β-D-6-methylpurine riboside	1.35	1.02	0.78				II
15	α-D-6-Methylpurine riboside				38	29	ND	I
15A	2′,3′,5′-Tri-*O*-acetyl-α-D-6-methylpurine riboside				31	33	ND	I

[a] Group designations are elaborated in Results.
[b] ND, not determined.

7, and 9 and their respective O-acetylated derivatives are commonly called 2′,3′-acyclo- or 2′,3′-seco-nucleosides to denote the ribonucleoside structures from which they are formally derived by bond scission. Abbreviations of compounds that appear in Table 1 are AbeAdo, MDL73811, 5′{[(*Z*)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine; DMTA, 5′-deoxy-5′-(dimethylthio)adenosine; ETA, 5′-deoxy-5′-(ethylthio)adenosine; HETA, 5′-deoxy-5′-(hydroxyethylthio)adenosine; and MFETA, 5′-deoxy-5′-(monofluoroethylthio)adenosine.

RESULTS

A group of 15 purine nucleosides and their respective O-acetylated derivatives were tested for in vitro antitrypanosomal activity against the LAB 110 EATRO isolate of *T. brucei brucei* and two clinical isolates of *T. brucei rhodesiense*, KETRI 243 (melarsoprol and pentamidine resistant) and KETRI 269 (pentamidine resistant) (Table 1). Initial comparison of the activity of each parent nucleoside with that of its O-acetylated derivative was made in the EATRO 110 isolate, which serves as the primary screen for evaluating antitrypanosomal activity in our laboratory. On the basis of the results obtained for EATRO 110, the purine nucleosides were placed into three groups: group I, nucleoside analogs which exhibited no significant activity (IC_{50}s of >100 μM), whether they were O acetylated or unacetylated (compound pairs 4/4A, 6/6A, 11/11A, and 15/15A); group II, nucleoside analogs which showed almost equal activity (IC_{50}s of <5 μM) for the parent compound and the O-acetylated derivative (compound pairs 1/1A, 5/5A, and 14/14A); and group III, nucleoside analogs whose activity was significantly improved (≥threefold) by O acetylation (compound pairs 2/2A, 3/3A, 7/7A, 8/8A, 9/9A, 10/10A, 12/12A, and 13/13A). In fact, six of the nine O-acetylated nucleosides in group III, i.e. 2A, 7A, 8A, 9A, 10A, and 12A, displayed activity at least 10-fold greater than that of their parent nucleosides.

The drug-resistant KETRI isolates were included in these studies because these are clinical isolates containing uncloned, heterogeneous populations, thus providing a screening system that has direct clinical relevance. When the series of compounds was tested against the KETRI 243 and KETRI 269 isolates, their antitrypanosomal effects were consistently comparable to those observed for the EATRO 110 strain. The only exceptions were the greater activity (10-fold) observed for compound 14 in KETRI 269 versus EATRO 110 and the inactivity of compound 3A versus KETRI 269 as opposed to IC_{50}s of ~5 μM for EATRO 110 and KETRI 243.

The most significant results of our studies were observed in EATRO 110 for four compounds in group III, namely, 2, 7, 10, and 12. These inactive compounds, on O acetylation, were transformed to compounds with IC_{50}s of ≤25 μM. Also noteworthy was the enhanced activity of compound 8A compared with that of 8, a highly effective in vivo trypanocide (7).

DISCUSSION

Protozoan parasites cannot synthesize purines and must salvage them from their host (5). In contrast, mammalian cells have elaborate biochemical pathways for de novo purine synthesis. This striking difference provides an exploitable biochemical feature that has received considerable attention in

the rational design of antiparasitic agents (5, 19). Uptake mechanisms for purines and purine nucleosides assume primary importance in these parasites and, in theory, can be used to concentrate a broad spectrum of therapeutically active nucleosides inside the microorganism. African trypanosomes efficiently salvage purines from purine nucleosides (19) whose transport is mediated through two high-affinity adenosine transport systems, P1, which also transports inosine, and P2, which also transports adenine and pentamidine, and the arsenicals, melarsen and melarsen oxide. P2 is absent or modified in drug-resistant trypanosomes (6, 10, 11). Antitrypanosomal purine nucleosides, such as sinefungin (23), MDL73811 (9), and HETA (4), which target enzymes of AdoMet metabolism, presumably use these two purine nucleoside transporters to gain entry into the parasites, although our recent studies indicate that trypanosomes have a dedicated AdoMet transporter which also takes up several of these agents (15).

Drug resistance in parasites often involves impairment of their purine nucleoside transport systems. Thus new ways to deliver antiparasitic purine nucleosides are potentially useful, especially if they circumvent the purine nucleoside transporters. Prodrug formulations are attractive from two viewpoints. They alter and hopefully improve the solubility characteristics of the parent compound, and they also can be designed to slowly release active drug, thereby approximating conditions of continuous infusion. The preparation of tri-*O*-acetyl-HETA, an ester conjugate of HETA, was carried out with these two objectives in mind. Comparative in vivo studies of HETA and tri-*O*-acetyl-HETA in mice infected with *T. brucei brucei* were carried out (3), and although both compounds were curative at similar dose levels, the tri-*O*-acetyl derivative was more consistent in its ability to elicit a greater percentage of cures within a given group of drug-treated mice. Accordingly, the rationale for comparing the activities of other O-acetylated purine nucleosides with their parent compounds was established and further investigated with a variety of purine nucleoside structures. The results of these comparisons, presented in Table 1, led to the grouping of these analogs into three different categories, as described in Results. Briefly, nucleoside analogs in group I exhibited no significant activity (IC_{50}s of >100 μM), whether they were O acetylated or unacetylated; nucleoside analogs in group II showed virtually equal activity (IC_{50}s of <5 μM) for the parent compound and the O-acetylated derivative; nucleoside analogs in group III showed significant improvement in activity following O acetylation. In fact, O acetylation improved the activity of six compounds in group III by at least 10-fold. Most importantly, four of these six unacetylated compounds (compounds 2, 7, 10, and 12) were regarded as inactive when tested against EATRO 110.

The lack of meaningful in vitro activity against EATRO 110 for the nucleoside analog pairs in group I indicates that the parent nucleosides 4, 6, 11, and 15 do not effectively interfere with biological targets that are essential for trypanosome viability. In contrast, the similar activity observed for each of the group II nucleoside pairs, 1/1A, 5/5A, and 15/15A, suggests that treatment with each compound leads to effective interactions of the parent nucleosides 4, 5, and 15 with strategic biological targets. The mode of action of the O-acetylated derivatives 1A, 5A, and 15A is unclear; they may be interacting directly with critical cellular targets, or alternatively, they may be acting as prodrugs that effectively release the parent nucleosides within the trypanosomes.

A common feature of compounds 1 to 15 is their purine nucleoside structure. However, these compounds were not explicitly designed or chosen for these studies because they share the same biological target(s). In fact, the observed activity profiles (i.e., group I, II, or III) are not specifically associated with interactions at a particular target. Compounds 1 to 5 are analogs of MTA, the nucleoside by-product of polyamine biosynthesis. Known cellular targets of MTA and its analogs include MTA phosphorylase (25), *S*-adenosylhomocysteine (AdoHcy) hydrolase (14), spermidine synthase (16), and, in mammalian cells, spermine synthase (22). Compounds 6, 7, 11, 12, and 14 are purine analogs whose antitrypanosomal effects are most likely linked to their ability to interfere with purine salvage. Compounds 8, 9, and 10 are analogs of the key biological intermediate, AdoMet, which participates in polyamine biosynthesis and numerous methylation reactions. More specifically, 8 is a rationally designed inhibitor of AdoMet decarboxylase (12). Clearly, O acetylation has significantly improved the in vitro antitrypanosomal activity of MTA analogs 2 and 3; AdoMet analogs 8, 9, and 10; and miscellaneous purine nucleoside analogs 7, 12, and 13.

Among the 15 purine nucleoside structures tested, three compounds, 2, 12 and 13, were 2′-deoxynucleoside analogs. Whereas all three of these compounds were inactive (IC_{50}s of ≥ 100 μM) when tested in vitro against EATRO 110, their activity markedly improved with O acetylation. Although this sampling of 2′-deoxypurine nucleosides is limited, this result suggests that purine analogs which are potentially toxic to the African trypanosome might be effectively delivered via their *O*-acetyl-2′-deoxy derivatives.

Our studies of a group of 15 purine nucleosides have determined that O acetylation of the parent nucleosides consistently improved their in vitro antitrypanosomal activity. This observed phenomenon was independent of their cellular enzyme targets. In several instances, O acetylation transformed apparently inactive nucleosides into compounds with significant antitrypanosomal activity. For this reason alone, when screening purine nucleoside analogs for in vitro antitrypanosomal activity, the routine preparation of their O-acetylated derivatives appears advantageous. O-acetylated purine nucleosides may offer in vivo therapeutic advantages compared with their parent nucleosides, and this possibility should be considered in future evaluations of this structural class of trypanocides.

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