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Activity of Antitumor Drugs Against African Trypanosomes

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Of 49 compounds known to have antitumor properties, 6 were found to have significant activity against *Trypanosoma rhodesiense* infections in mice. Activity against the African trypanosomes has not been reported previously for any of these six compounds. In order of decreasing activity these compounds were: (i) imidazole-4-carboxamide, 5-(3,3-dimethyl-1,1-triazene), (ii) inosine diglycolaldehyde, (iii) *cis*-diamminedichloro-platinum, (iv) streptozotocin, (v) coralyne sulfate, and (vi) 5-fluoro-2'-deoxyuridine. The percentage of "hits" (12.2%) from these known antitumor agents was approximately twice as great as when other means are employed for the selection of compounds for this test system.

Certain aspects of the metabolism of the predominant bloodstream form of African trypanosomes are quite similar to those of some tumor cells (1). Both undergo a high rate of aerobic glycolysis as a result of inefficient or nonfunctional mitochondrial systems (8). In each, the key "pacemaking" glycolytic enzymes are hexokinase, phosphofructokinase, and pyruvic kinase (7). It is known that this metabolic pathway may be altered by agents, including arsenical antitrypanosomal drugs, which are effective against both trypanosomes and tumors (2, 3). One might suspect that compounds with known anti-trypanosomal properties may have antitumor properties and vice versa. Actually, a number of anti-trypanosomal compounds have been found to be active against experimental tumors (5, 8); conversely, a number of antitumor agents have shown anti-trypanosomal effects (4, 8, 9).

The foregoing encouraged us to examine a selected group of compounds from diverse chemical classes with well-documented capacities to modify the growth of human and/or animal neoplasms. The compounds selected were tested by utilizing a mouse model which employs experimental infections produced by *Trypanosoma rhodesiense* (6).

MATERIALS AND METHODS

The test system has been described elsewhere (6). Briefly, ICR/Ha Swiss mice of either sex, weighing 30 to 32 g, were given an intraperitoneal injection of 0.5 ml of a 1:50,000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier with blood parasitized with the Wellcome CT strain of *T. rhodesiense*. Untreated animals died within 4 to 6 days, with a mean survival time of 4.45 ± 0.25 days.

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Drugs were administered orally or subcutaneously in a single dose in peanut oil at a concentration of 3.2% at 2 h after intraperitoneal inoculation of parasites. Stilbamidine isethionate or 2-hydroxystilbamidine isethionate were used at 26.5 mg/kg as positive control drugs. At this drug level both compounds were essentially 100% curative, if given by the subcutaneous route.

Surviving animals were observed for 30 days. An increase of 100% in mean survival time of animals given the test compound when compared with infected untreated controls was the minimum response for a candidate compound to be considered active. Animals surviving for 30 days were scored as cures; those dying before day 4 after drug administration were scored as toxic deaths. (Although delayed toxic deaths [to 14 days] are known to occur after a single dose of some oncolytic agents, the previously established criteria for routine screening were followed [6].) Cures and toxic deaths were not included in calculating the mean survival time of test animals.

Compounds were selected from two listings furnished by the Drug Development Branch, National Cancer Institute (10, 11). The criterion for selecting these agents with anticancer activity was the availability of the compounds (at least 100 mg) for testing in the *T. rhodesiense*-mouse model. A total of 49 compounds from these two lists (there was a total of 82 compounds on both lists) met this criterion and are the basis of the present report.

RESULTS

Results for the 6 active compounds of the 49 tested in this study are summarized in Table 1. The chemical formulas for these are shown in Fig. 1. Compounds which were inactive against these *T. rhodesiense* infections are listed in Table 2. The most active compound was the alkylating agent designated WR 139 007 (Table 1). The drug was curative at doses as low as 13.0 mg/kg when given subcutaneously and was cu-

TABLE 1. *Anticancer compounds having activity against T. rhodesiense infections of mice*

Compound designations[a]	Route[b]	No. cured/no. treated with the following dose (mg/kg):[c]					
		424	212	106	53	26.5	13.3
WR 139 007[c]	SC	15/15	5/5	15/15	5/5	19/20	4/10
(NSC 45388)	O	5/5	5/5	5/5		5/5	0/5
WR 220 078	SC	15/15	5/5	14/15	2/5	0/10	0/5
(NSC 118994)	O	3/5	0/5	0/5		0/5	0/5
WR 177 529	SC	0/15(15 T)[d]	0/5(5 T)	0/15(15 T)	0/5[e]	0/15	0/5
(NSC 119875)	O		0/5(2 T)	0/5	0/5		
WR 139 502	SC	9/15	2/5	0/15	0/5	0/15	0/5
(NSC 85998)	O	0/5	0/5	0/5			
WR 158 221	SC	10/20	0/10[e]	0/20	0/10	0/20	0/10
(NSC 154890)							
WR 138 720	SC	0/15[e]		0/10		0/10	
(NSC 27640)	O	0/5	0/5	0/5			

[a] WR numbers are those assigned by the Walter Reed Army Institute of Research; NSC numbers are those assigned by the National Cancer Institute.

[b] SC, Subcutaneous; O, oral.

[c] Five animals per group were also treated subcutaneously or orally with doses of 6.65, 3.33, 1.66, and 0.83 mg/kg. No activity was noted for any of these eight treatment groups.

[d] T, Death attributed to drug toxicity.

[e] These treatments showed activity, i.e., at least a 100% increase in mean survival time when compared with infected untreated controls.

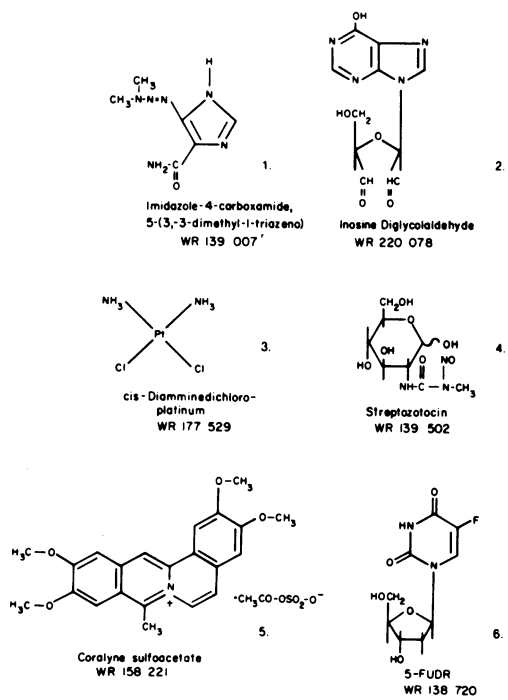

FIG. 1. *Structures of anticancer compounds having activity against T. rhodesiense infections in mice. 5-FUDR, 5-Fluoro-2'-deoxyuridine.*

rative at doses as low as 26.5 mg/kg when given orally. The antimetabolite inosine diglycolaldehyde, designated WR 220 078, was curative at doses of 53 mg/kg by the subcutaneous route but curative only at the highest dose (424 mg/kg) by the oral route. Streptozotocin (WR 139 502) and coralyne sulfoacetate (WR 158 221) were curative only at the higher doses.

The platinum complex cisplatin, designated WR 177 529, and the nucleoside floxuridine (WR 138 720) failed to show curative effects at any level tested but did produce at least a 100% increase in survival time of infected mice at the higher dose levels when the drugs were given subcutaneously. Neither was active when given orally. It should be noted that the platinum complex was very toxic, producing lethality at doses as low as 106 mg/kg.

DISCUSSION

The present screening of 49 compounds selected from known antineoplastic drugs has shown that 6 possess significant activity against *T. rhodesiense*. To our knowledge, activity against the African trypanosomes has not been reported previously for any of these six compounds.

It is realized that these data are preliminary. Certainly more extensive definitive testing to determine the efficacy of one or more of these compounds must be accomplished. Also, if secondary efficacy evaluations appear promising, it is imperative that the degree of toxicity be carefully ascertained before any human trials are attempted. It should be noted that no evidence of toxicity was observed in this preliminary testing for five of these six active chemical species.

TABLE 2. *Anticancer compounds inactive in the T. rhodesiense-mouse model*[a]

Group and name	Highest dose at which no toxic deaths were observed[b] (mg/kg)	WR no.[c]	NSC no.[d]
Alkylating agent			
Busulfan	26.5	19508	750
Melphalan	424	19813	8806
Thiotepa	26.5	45312	6396
Hydroxyurea	424	83799	32065
Hexamethylmelamine	424	95704	13875
Cyclophosphamide	106(106)	138719	26271
Dibromodulcitol	212(212)	138743	104800
Chlorambucil	53(212)	139013	3088
1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea	424	139017	79037
1,3-Bis(2-chloroethyl)-1-nitrosourea	53(212)	139021	409962
Nitrogen mustard	26.5	147650	762
Cytembena	212	149912	104801
Fluorodopan	26.5	218929	73754
Dibromomannitol	424	220057	94100
Methyl-1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea	212	220076	95441
Steroid (probable effects on protein synthesis)			
Hydrocortisone	424	6208	10483
Prednisone	424	6501	10023
Testosterone propionate	424	6518	9166
Adrenocorticotropin	424	6975	25933
Prednisolone	424	8599	9900
Progesterone	424	8603	9704
Cortisone	424	20068	9703
Fluoxymesterone	424	120935	12165
Calusterone	424	138742	88536
Steroid-like effects			
Diethylstilbestrol	424	6277	3070
Nafoxidine hydrochloride	424	220110	70735
Inhibits steroid formation			
1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (Mitotane)	424	13045	38721
Interferes with purine biosynthesis and/or interconversions			
Thioguanine	424	1141	752
6-Mercaptopurine	424	2785	755
DNA synthesis inhibitor			
2,3-Dihydro-1H-pyrazolo-(2,3-a)imidazole	424	215038	51143
Binds with deoxyribonucleic acid			
Actinomycin D	1.66(106)	2878	3053
4′-(9-Acridinylamino) methanesulfonyl-*m*-anisidide	424	177550	141549
Ellipticine	424	215789	71795
Nitidine chloride	424	220104	146397
Nucleoside analog			
Cytosine arabinoside	424	28453	63878
5-Azacytidine	212	183027	102816
3-Deazauridine	424	199830	126849
Ftorafur	424	220066	148958
Antimetabolite			
Methotrexate	53	19039	740
5-Fluorouracil	424	69596	19893
5-Methyl-tetrahydrohomofolic acid	424	211454	139490
Baker's Antifol	424	219427	139105
Uncertain mode of action			
Gallium nitrate	424	135675	15200

[a] A compound was considered inactive if, in ICR/Ha Swiss mice infected with blood parasitized with the Wellcome CT strain of *T. rhodesiense*, it does not (after a single subcutaneous or oral injection) effect at least an increase of 100% in mean survival time of animals given the test compound when compared with infected untreated controls.

[b] Animals dying before day 4 after drug administration were scored as toxic deaths. The highest dose tested was 424 mg/kg in all cases, except where the highest level is given in parentheses.

[c] WR numbers are those assigned by the Walter Reed Army Institute of Research.

[d] NSC numbers are those assigned by the National Cancer Institute.

Furthermore, the most active agent (WR 139 007), although tested at concentrations as high as 424 mg/kg without signs of toxicity, was curative at 13.3 mg/kg. The compound WR 177 529, which was found to exhibit toxicity, was lethal at all doses of 106 mg/kg and above. The fact that it was not curative at the next lower dose tested (although it did meet the defined criteria for being classified as an active compound) renders it an unlikely candidate for a clinically usable drug.

Perhaps most important is the finding that anti-trypanosomal activity for new classes of compounds has been found. These "hits" represent a nucleus around which chemical analogs may be synthesized. One or more of these newly synthesized compounds may demonstrate a substantial increase in anti-trypanosomal activity with no concomitant increase in toxicity to the host.

The positive relationship which apparently exists between the antitumor properties and anti-trypanosomal properties of certain compounds is poorly understood. Of 49 compounds tested, however, 6 were active, and this represents 12.2% hits. This is approximately two times as great as when other methods are employed to select compounds for testing with this same standard test (6). In view of this, consideration of further screening of known antitumor agents from classes with previously unknown anti-trypanosomal activity seems warranted.

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