Anticancer Agents Suppressive for Adult Parasites of Filariasis in Mongolian Jirds

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Abstract. **Eight chemical structures not previously reported to possess antifilarial activity have been identified. A total of 79 compounds with anticancer properties were evaluated for possible macrofilaricidal activity against *Brugia pahangi* and *Acanthocheilonema viteae* transplanted into male Mongolian jirds (*Meriones unguiculatus*). All eight active compounds were suppressive for the onchocerciasis type (*Acanthocheilonema viteae*) of the disease. None was macrofilaricidal for the lymphatic form (*Brugia pahangi*). These new structures may represent a nucleus around which effective drugs can be synthesized.** [P.S.E.B.M. 2000, Vol 224:45–49]

More than one billion people (20%) of the world's population live in areas where they are at risk of infection from lymphatic filariasis (*Wucheria bancrofti, Brugia malayi, B. timori*) and "river blindness" (*Onchocerca volvulus*) (1). Drugs used most commonly to cope with these diseases are diethylcarbamazine (DEC) and ivermectin. Although both of these have marked microfilaricidal activity, neither has appreciable action against the adult worms. There is a great need for effective macrofilaricidal drugs. The discovery and development of a macrofilaricide is a mandate of the MACROFIL project of the World Health Organization (2).

A group of compounds with members known to adversely affect parasites of humans, including filaria, are anticancer agents. For example, the antineoplastic antibiotic cyanein has antinematodal activity (3). And, of more than 100 anticancer compounds tested for activity against *Trypanosoma brucei rhodesiense* infections, 18.3% were found to be positive (4, 5). Similarly, 6.7% showed activity against *Trypanosoma cruzi* infections (6). Thiosemicarbazones, a class with known antineoplastic action (7), are active against bacteria (8), viruses (9), coccidia (10), malaria (11), and filaria (12). Phosphonylmethoxyalkylpurine analogs (13), levamisole (14) and suramin (15), all structures with antitumor attributes, have been shown to have antiviral (16), general anthelminthic (17), and antifilarial (18) activity, respectively.

Although it is realized that antineoplastic agents display general cellular toxicity, it is appreciated that there is toxicity to the host with virtually all therapeutic agents. The issue is the therapeutic index. With this backdrop of information, it was of interest to us to evaluate compounds with known antineoplastic properties. We chose to evaluate 79 compounds with known anticancer activity. These were supplied to us by the National Cancer Institute.

The evaluations were carried out by assessing the macrofilaricidal activity against *Brugia pahangi* and *Acanthocheilonema viteae* in male Mongolian jirds. The animal model employed, using these two parasites, was designed to mimic, in-so-far as possible, respectively, human lymphatic

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The opinions or assertions contained herein are personal views of the authors and should not be construed as official or necessarily reflecting the views of the Walter Reed Army Institute of Research, Uniformed Services University of the Health Sciences, or the Department of Defense.

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Table I. Active Compounds in Jirds (*Meriones unguiculatus*) (Three Animals per Test Group) Transplanted with 20 Adult *Brugia pahangi* (Bp) and 10 Adult *Acanthocheilonema viteae* (Ac) and Treated Subcutaneously for 5 Consecutive Days

No.	Chemical name (WR No.[a]/NSC No.[b])	Doses tested MKD[c]	% Suppression	
			Bp	Ac
1.	Nitrogen mustard (WR1439/NSC762)	1.56	I[d]	71
2.	N,N-Bis(2-chloroethyl) phosphorodiamidic acid, compound with cyclo-hexylamine (1:1) (WR2899/NSC69945)	100	I	69
3.	Hydroxyurea (WR83799/NSC32065)	50	I	I
		25	I	84
		12.5	I	I
4.	4′,4″-Di-2-imidazolin-2-yl-isophthalanilide, dihydrochloride (WR40077/NSC53212)	50	I	98
5.	2,2′-(9,10 Anthrylenedi-methylene)bis[2-thio pseudourea], dihydrochloride dihydrate (WR138713/NSC56054)	100	I	70
6.	Tenuazonic acid (WR192150/NSC525816)	25	I	69
7.	4,4′-[Isophthaloylbis (imino-p-phenylene carbonylimino)]bis [1-methylpyridinium chloride] (WR200386/NSC73851)	100	I	80
8.	CAI 5-Amino-1-[[3,5-dichloro-4-(4-chlorobenzoyl) phenyl]methyl]-1H-1,2,3-triazole-4-carboxamide (WR25644/NSC609974)	100	I	72
Positive Control—Flubendazole (WR242630)		100	100	100
		50	100	98
		25	100	100
		12.5	100	100
		6.25	100	100
		3.13	100	100
		1.56	100	100

[a] WR No. = Number assigned by the Walter Reed Army Institute of Research
[b] NSC No. = Number assigned by the Drug Synthesis and Chemistry Branch, Developmental Therapeutic Program, National Cancer Institute
[c] MKD = Highest dose level tested, in mg/kg/day for 5 days
[d] I = Inactive

filariasis and "river blindness" (onchocerciasis). The model, actually a double-model, was based upon experience gained during studies following interaction with those of the World Health Organization (19). The creation of these "lymphatic type" (*Brugia pahangi*) and "onchocerciasis type" (*Acanthocheilonema viteae*) models is discussed more fully elsewhere (20).

Materials and Methods

Animals used were male Mongolian jirds (*Meriones unguiculatus*) weighing 50–60 g. The experiments reported herein were conducted according to principles set forth in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council, NIH Pub. no. 85–23. *Brugia pahangi* was maintained by alternate passage through beagle dogs, and *Aedes aegypti* mosquitoes (selected Liverpool strain) and *A. viteae* were cyclically maintained in jirds or outbred Syrian hamsters, and the soft tick, *Ornithodoros tartakovski*, as described elsewhere (21). The dosage level of the compound selected for testing depended upon the available sample size and toxicity of the drug. Compounds were given once daily, at the mg/kg/day (MKD) dosage shown, for 5 days by subcutaneous injection. All compounds were suspended in hydroxyethylcellulose (0.5%) and Tween 80 (0.1%) by sonication at 20 kilocycles for 10 min. During dosing, suspensions were agitated as needed using a vortex mixer.

The test regimen employed 8-week-old *A. viteae* collected from donor hamsters and given to jirds (21). Each of these jirds was given 10 (5 male and 5 female) of these adult worms by subcutaneous (SC) transplantation under sodium pentobarbital anesthesia at a level of 48 mg/kg (22). Two weeks later, 20 (10 male and 10 female) adult *B. pahangi*, taken from jirds infected intraperitoneally (IP) 8 weeks earlier (4) were transplanted into the peritoneal cavity of each of these jirds, under sodium pentobarbital anesthesia. The following week, they were randomly allocated to form a "run," and dosing was initiated (Day 0). Each run consisted of 18 test groups of 3 jirds each, a negative control group of 3–6 jirds given the hydroxyethylcellulose-Tween 80 carrier, and a positive control group of 3 jirds treated subcutaneously with 2 MKD for 5 days of flubendazole. Body weights were recorded daily for 5 days to determine the doses needed and to look for signs of toxicity. A drug was considered to be toxic if jirds were moribund or dead or if there was a 15% (group mean) weight loss. Dosing of a group of jirds was discontinued if toxicity was noted.

All jirds were sacrificed on Day 56 after dosing was initiated to determine the effects of the drugs on the adults of both *A. viteae* in subcutaneous tissue and between facial planes of muscle and *B. pahangi* in the peritoneal cavity.

Table II. Inactive Compounds in Jirds (*Meriones unguiculatus*) (Three Animals per Test Group) Transplanted with 20 Adult *Brugia pahangi* and 10 Adult *Acanthocheilonema viteae* and Treated Subcutaneously for 5 Consecutive Days

No.	Chemical name	Highest dose tested MKD[a]	WR No.[b]	NSC No.[c]
9.	4-(p-Dimethylamino styryl)quinoline methiodide	6.25	66,306	4239
10.	Cytembena	100	149,912	104801
11.	Prednisone	100	6,501	10023
12.	Streptozotocin & cofactor	100	192,720	37917
13.	5-Bromo-2'-deoxyuridine	100	262	38297
14.	2'-Deoxy-5-iodouridine	100	2,620	39661
15.	6-Mercaptopurine	100	2,785	755
16.	2-Acetamido-1,3,4-thiadiazole	100	2,792	4729
17.	Hydrocortisone	100	6,208	10483
18.	Diethylstilbestrol	100	6,277	3070
19.	Hadacidin	100	6,433	521778
20.	Adrenocorticotropin	100	6,975	25933
21.	Prednisolone	100	8,599	9900
22.	Progesterone	100	8,603	9704
23.	as-Triazine-3,5(2H,4H)dione	100	10,492	3425
24.	1,1-Dicholor-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane (Mitotane)	100	13,045	38721
25.	Trimethylpurin-6-yl-ammonium chloride	100	17,593	51095
26.	6-Azauridine	100	18,686	32074
27.	Methotrexate	100	19,039	740
28.	Busulfan	100	19,508	750
29.	Cortisone	75	20,068	9703
30.	4',4"-Di-2-imidazolin-2-yl-2-nitroterephthalanilide, dihydrochloride	12.5	40,076	35847
31.	4',4"-Bis(4-methyl-2-imidazolin-2-yl)terephthalanilide, dihydrochloride	100	40,080	57148
32.	4',4"-Di-2-imidazolin-2-yl-terephthalanilide	100	40,083	59503
33.	4',4"-Bis[N-(3-methoxypropyl)N'-methylamidino]terephthalanilide	6.25	40,085	61614
34.	4-[p-(Dimethylamino)styryl]quinoline	100	67,346	4236
35.	4-[p-Diethylamino)styryl]quinoline, monohydrochloride	100	67,376	4241
36.	5-Fluorouracil	100	69,596	19893
37.	Ethanesulfonic acid compound with N-[m-(4,6-diamino-2,2-dimethyl-S-triazin-1(2H)hydrocinnamoyl]sulfanilyl fluoride (1:1)	100	85,944	113907
38.	Hexamethylmelamine	100	95,704	13875
39.	Patulin	6.25	108,308	32951
40.	Fluoxymesterone	100	120,935	12165
41.	Gallium nitrate	100	135,675	15200
42.	4-(o-Methoxystyryl)quinoline	100	137,895	15783
43.	Dromostanolone propionate	100	138,718	12198
44.	2'-Deoxy-5-flurouridine	100	138,720	27640
45.	11B-Hydroxy-12alpha-methyltestosterone	100	138,722	72256
46.	Dromostanolone	100	138,728	26198
47.	3-Ethyl-4-(p-methoxyphenyl)-2-methyl-3-cyclohexane-1-carboxylic acid	100	138,732	19962
48.	Fluorometholone	100	138,735	33001
49.	Dibromodulcitol	100	138,743	104800
50.	Imidazole-4-carboxamide, 5-(3,-3-dimethyl-1-triazeno)	100	139,007	45388
51.	Chlorambucil	12.5	139,013	3088
52.	1-(2-Chloroethyl)-3-cyclohexyl-nitrosourea	100	139,017	79037
53.	5-[3,3-Bis(2-chloroethyl)-1-triazino]imidazole-4-carboxamide	100	139,019	82196
54.	Streptozotocin	100	139,502	85998
55.	Actinbolin	100	144,524	31083
56.	cis-Diamminedechloroplatinum	100	177,529	119875
57.	4'-(9-Acridinylamino)methane-sulfonyl-m-anisidine	100	177,550	141549
58.	14-Methyl-14H-dibenzo[a,h]phenothiazine	100	192,163	60902
59.	3-Deazauridine	100	199,830	126849
60.	4',4"-Bis(N'-methyl-N-propyl-amidino)terephthalanilide, dihydrochloride	100	200,367	57136
61.	5-Methyl-tetrahydrohomofolic acid	100	211,454	139490
62.	Ellipticine	100	215,789	71795
63.	Fluorodopan	25	218,929	73754
64.	Baker's Antifol	100	219,427	139105
65.	Ftorafur	100	220,066	148958
66.	Inosine diglycolaldehyde	100	220,078	118994
67.	N-Isopropyl-alpha-(2-methylghdrazino)-p-toluamide, hydrochloride (procarbazine hydrochloride)	100	220,086	77213
68.	Nitidine chloride	100	220,104	146397
69.	Nafoxidine hydrochloride	100	220,110	70735
70.	6-Amino-1-methyl-4-[[[[[[(1-methylpyridinium-4-yl)amino]phenyl]amino]carbonyl]phenyl]amino]quinolinium, dibromide	100	221,122	176319
71.	11-Demethylellipticine	100	222,624	87206
72.	4-[p-[Bis(2-chloroethyl)amino]styryl]quinoline	100	222,668	59634
73.	9-Methoxellipticine	100	222,840	69187
74.	N-Oxide indicine (from *Heliotropium indicum*)	100	223,060	132319
75.	Thalicarpine	100	225,567	68075
76.	N1,N6-bis(7-dimethylamino quinoline-4-yl)-1,6-hexamidiamine	100	249,790	313159
77.	Cycloheximide	25	13,255	185
78.	Imidazole-pyrazole (IMPY)	100	215,036	51143
79.	5-Azacytidine	6.25	183,027	102816

[a] MKD = Highest dose level tested in mg/kg/day for 5 days
[b] WR No. = Number assigned by the Walter Reed Army Institute of Research
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Live worms were identified to species, noted as to sex, and counted. The number of dead and/or encapsulated worms was also recorded. If a compound caused a 60% or greater reduction in the adult worm burden of one or both species, it was considered active. Arcsin conversions were done before percentage computations were undertaken.

Results

Results for the eight anticancer agents exhibiting antifilarial activity are shown in Table I. The 71 compounds that were inactive are listed in Table II. All eight actives were suppressive for the parasite that causes the onchocerciasis form (*A. viteae*) of the disease. None of the compounds was found to be effective against the lymphatic filariasis form (*B. pahangi*). Although Table II enumerates compounds found to be inactive, it is believed that the information is valuable. Among drugs that are listed in Table II are many standard anticancer agents.

Why hydroxyurea (Compound 3, Table I) was more active at 25 MKD than at 50 MKD is not known. The worm location within the test animals at the higher dose level may have been more inaccessible to the subcutaneously administered compound than those at the lower dose level (i.e., 25 MKD). Please note that a similar result was noted for the positive control drug (flubendazole) at 50 MKD and 25 MKD.

Discussion

None of the eight compounds found to be suppressive in the present study have been reported previously to possess activity against parasites that produce either the lymphatic form or the onchocerciasis form of filariasis. Why certain anticancer agents are effective against certain parasites is not understood. Some metabolic similarities between neoplastic cells and the African trypanasomes have been offered as a reason for the effectiveness in those organisms (23–25). However, unlike the protozoan diseases in which the parasite undergoes replication within the host, once the infective larvae of helminths have reached the final host, the only cell division that takes place is within the reproductive organs (26).

The antitumor mechanism of action of suramin, the only WHO-recommended macrofilaricidal agent, has been examined. Inhibition of various growth factors favoring cell transformation of tumor progression represents a major mechanism of action by this drug as an anticancer agent (15, 27). As an antifilarial, suramin is known to destabilize filarial DNA (28) and adversely affect protein kinases and enzymes associated with glucose catabolism (i.e., glyceraldehyde-3-phosphate dehydrogenase, lactic dehydrogenase, malic dehydrogenase) (29–31). Its limited efficacy against adult worms in onchocerciasis is not because of the poor penetration of the nodules by the drug (32, 33). How these various attributes might be linked is not presently understood.

Other metabolic similarities of cancerous tissues and parasites worthy of mention are seen in certain polyamine relationships. The interconversion of spermine to spermidine to putrescine is similar in mammalian cells and filarial worms (34, 35). However, it is important to note that this functional reverse pathway, is much more important in filaria than mammals. In the parasite, it is the mechanism by which the worms control and reduce the level of higher polyamines and supply putrescine for recycling *via* the synthetic pathway. This difference may represent a rewarding avenue by which filaricides may be designed.

The "hits" reported in the present study may represent a nucleus around which chemical analogs can be synthesized. The approach envisioned is not dissimilar to one employed in the search for anticancer agents where the aim is to take advantage of the numerous molecular alterations identified in tumor cells (36). But rather than the focus being on the deviation of the tumor cells from those that are normal in the anticancer drug search, concentration is on the metabolic differences between the host's normal cells and those of the parasitic worm. One can be optimistic that the eight structures identified in the present work represent a harbinger of drugs with therapeutic advantage that can interfere with a critical metabolic step and thus use an effective killing mechanism for the nematode worms without adversely affecting the host.

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