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In Search of Anti-*Trypanosoma cruzi* Drugs: New Leads from a Mouse Model

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Nine of 25 carefully selected compounds (from a stock of more than 200 000 chemical species amassed principally as a result of testing against other parasitic diseases) were found to have significant suppressive activity against the parasites in the blood of a *Trypanosoma cruzi* mouse model. Eight of these compounds evaluated in this model had suppressive activity equal to or greater than the reference compound, nifurtimox. For the first time, suppressive activity against *T. cruzi* is reported for a 7-aminoquinoline, a phosphonium salt, and TAC pamoate. The biological model is believed to be able to serve as a means of identifying other new "leads" in seeking drugs broadly effective against *T. cruzi* infections in man.

Of the various hemoflagellate infections, Chagas' disease (or schizotrypanosomiasis) is perhaps the least responsive to presently available chemotherapeutic agents.[2-6] Newer, less toxic chemical agents are needed urgently. To that end, this paper presents results which were obtained in an attempt to identify new classes of compounds which may be effective against *Trypanosoma* (*Schizotrypanum*) *cruzi* infections. For testing, 25 carefully selected drugs were obtained from the stock of more than 200 000 compounds held by the Walter Reed Army Institute of Research. This large inventory has been amassed principally as a result of testing agents for activity against other parasitic diseases.

Experimental Section

Materials and Methods. Male albino mice (4–10 weeks old, CF_1 strain, Carworth Farms, Portage, Mich.) and a Brazil strain of *Trypanosoma cruzi*[7] were used. The organism was maintained by passage every 2 weeks in 4–6-week-old CF_1 mice. Blood was drawn into a heparinized syringe from a donor mouse by cardiac puncture. The appropriate dilution was then prepared in Hanks' balanced salt solution, pH 7.2, and 0.2 mL of this suspension containing 50 000 trypomastigotes was injected intraperitoneally into each test mouse. Twice daily intramuscular administration of the experimental compounds was begun on day 10 of the infection and continued through the afternoon of day 13, or the morning of day 14. Slight difference in treatment regimens had no apparent effect on the suppression obtained as determined by the standard reference compound, nifurtimox. Each compound was suspended in a 0.1% Tween 80 plus 0.5% hydroxyethylcellulose (HEC–Tween) and was given at three dose levels, at fourfold dilutions. Drug dosage levels ranged from 0.8125 to 104 mg/kg/day using seven animals at each dose level. Control groups consisted of mice receiving vehicle only (negative controls) and others receiving nifurtimox (positive controls). Blood smears were prepared on day 14 and numbers of parasites per milliliter of blood were determined as previously described.[8]

A comparison of the antischizotrypanosomal activity of each test compound with that of the reference compound,[9] nifurtimox [2-methyl-4-(5-nitro-2-furylidene)amino-1,4-thiazane 1,1-dioxide], WR-205 632, was made and an index (relative activity of the test compound to that of nifurtimox for each test compound) was calculated by the following formula.

$$\text{nifurtimox index } (N) = \frac{SD_{90} \text{ for nifurtimox}}{SD_{90} \text{ for test compound}}$$

In that expression, SD represents the amount of compound (mg/kg) which produced a specified degree of suppression of the infection, as, 90%. Since the compounds were tested "blind" each was administered on the basis of total compound weight, unadjusted for formula weight due to the acid component in salts. Final correction was made in salts by taking into consideration the acid combined with the base. This was done by multiplying the index obtained by the reciprocal of the fraction of base component in the salt.

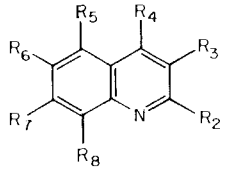
The degree of suppression (e.g., SD_{90}) was estimated graphically from plots of percent parasite suppression and dose of compound administered (mg/kg/day) on log probit paper. When the SD_{90} value could not be obtained because of low activity of the test compound, a lower SD value was used. A nifurtimox index of greater than 1 indicates that the test compound is more active than the reference compound.

Results

Nine of the 25 compounds tested significantly suppressed the parasitemia of the treated mice when compared to the untreated controls. The activity of eight of these compounds was equal to or greater than the activity found for the control compound, nifurtimox.

Seven 8-aminoquinolines were evaluated. Results are shown in Table I. Primaquine (compound **2**) and its 2-methyl analogue (compound **3**) were almost four times as effective as the control drug. Methyl substitution at position 3 (compound **4**) or 4 (compound **5**) in primaquine made the compounds nine times as effective and ineffective, respectively, as their corresponding positive controls. Random substitution (i.e., unspecified as to position) of the *p*-carboxyphenylazo moiety upon the

Table I. Aminoquinolines Active against *T. cruzi* Infections in Mice



Compd no.[a]	Aminoquinoline derivative	WR no.[b]	% suppression at dose in mg/kg/day[c] 104	52	26	13	3.25	SD value[d] in mg/kg/day[c]	*N* index[e]	Remarks
1	Nifurtimox	205 632	99	92		57		41.5–54.0 (SD_{90})	1	Positive control[f]
2	$R_2 = R_3 = R_4 = R_5 = R_7 = H$; $R_6 = OCH_3$; $R_8 = P^g$ (as dihydroiodide)	2 975	100	97		80		21.8 (SD_{90})	3.8	Primaquine dihydroiodide
3	$R_3 = R_4 = R_5 = R_7 = H$; $R_2 = CH_3$; $R_6 = OCH_3$; $R_8 = P^g$ (as dihydrochloride monohydrate)	182 234			82	86	38	10.5 (SD_{80})	3.9	2-Methylprimaquine dihydrochloride monohydrate
4	$R_2 = R_4 = R_5 = R_7 = H$; $R_3 = CH_3$; $R_6 = OCH_3$; $R_8 = P^g$ (as diphosphate)	211 814			99	98	92	<3.25 (SD_{90})	>9.2	3-Methylprimaquine diphosphate
5	$R_2 = R_3 = R_5 = R_7 = H$; $R_4 = CH_3$; $R_6 = OCH_3$ $R_8 = P^g$ (as diphosphate)	181 023	*h*	*h*		*h*			*h*	4-Methylprimaquine diphosphate
6	$R_2 = R_3 = R_4 = R_7 = H$; $R_5 = -N{=}N\text{-}C_6H_4\text{-}CO_2H$; $R_6 = OCH_3$; $R_8 = P^g$	157 835	100	99		98		<3 (SD_{90})	>4	
7	$R_2 = R_3 = R_4 = R_5 = R_7 = H$; $R_6 = OCH_3$; $R_8 = Pn^g$ (as monophosphate)	214 420	99	93		54		43.5 (SD_{90})	1.2	
8	$R_2 = R_3 = R_5 = R_7 = H$; $R_4 = CH_3$; $R_6 = OCH_3$; $R_8 = He^g$ (as dihydrochloride)	6 026			*h*	*h*	*h*		*h*	
9	$R_2 = R_3 = R_4 = R_6 = H$; $R_5 = R_8 = OCH_3$; $R_7 = Pa^g$ (as dinitrate monohydrate)	213 640	100	99		93		<13 (SD_{90})	>3	
10	$R_3 = R_6 = H$; $R_2 = R_4 = CH_3$; $R_5 = R_8 = OCH_3$; $R_7 = P^g$ (as triphosphate)	217 270	*h*	*h*		*h*			*h*	

[a] Sources of compounds: 1, Sparta Pharma Produkt-Koordinierung, Werk Elberfeld, Bayer A.G., 56 Wuppertal 1, German Federal Republic; 2, D. L. Klayman and T. S. Woods, Walter Reed Army Institute of Research, Washington, D.C. 20012; 3, F. Starks, Starks Associates, Inc., Buffalo, N.Y. 14213; 4, 9, and 10, A. L. Ash, Ash Stevens, Inc., Detroit, Mich. 48202; 5, L. H. Schmidt, Southern Research Institute, Birmingham, Ala. 35205; 6, E. A. H. Friedheim, Geneva, Switzerland; 7, F. C. Nachod, Sterling-Winthrop Research Institute, Rensselaer, N.Y. 12144; 8, R. D. Powell, Department of Medicine, University of Chicago, Chicago, Ill. 60637. [b] Designation of compounds in files of Walter Reed Army Institute of Research. [c] Mg unadjusted for formula weight due to the acid component in salts. [d] Graphically determined suppressive dose (SD) at which experimental drug was compared to dose of nifurtimox giving the same percent suppression. [e] Nifurtimox used as a reference drug for *N* index. *N* index = SD for nifurtimox/SD for test compound × 1/[(1 − formula wt due to salt)/(total formula wt)]. [f] Nifurtimox included for comparison purposes; not an aminoquinoline derivative. See ref 25. [g] Legend: He = $-NH(CH_2)_6N(C_2H_5)_2$; P = $-NHCH(CH_3)CH_2CH_2NH_2$; Pa = $-NHCH(CH_3)CH_2CH_2CH_2N(C_2H_5)_2$; Pn = $-NH(CH_2)_5NH\text{-}n\text{-}C_3H_7$. [h] Not active.

Table II. Miscellaneous Compounds Active against *T. cruzi* Infections in Mice

Compd no.[g]	Chemical name	WR no.[a]	% suppression at dose in mg/kg/day[b] 104	52	26	13	3.25	SD value[c] in mg/kg/day[b]	*N* index[d]	Remarks
11	Tris(4-aminophenyl-carbonium) pamoate	3 709	52[e]	39	43			96.0 (SD_{50})	0.3	Antischistosomal agent; TAC pamoate
12	*trans*-5-Amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole	169 916	90	91	49			48.5 (SD_{90})	1.13	Antischistosomal agent; SQ-18506
13	*p*-Methylbenzyltriphenyl-phosphonium chloride	132 504	*f*	85	80			13.0 (SD_{80})	3.6	

[a] See Table I, footnote *b*. [b] See Table I, footnote *c*. [c] See Table I, footnote *d*. [d] See Table I, footnote *e*. [e] 2/7 deaths attributed to drug toxicity. [f] 7/7 deaths attributed to drug toxicity. [g] Sources of compounds: 11, E. F. Elslager, Parke, Davis & Co., Ann Arbor, Mich. 48106; 12 and 13, Maybridge Chemical Co., Tinagel, Cornwall, United Kingdom.

Table III. Compounds Inactive against *T. cruzi* Infections in Mice[a]

Compd no.[i]	Chemical name	WR no.	Remarks
14	1,4-Bis[3-(6-methoxy-8-quinolylamino)propyl]piperazine	187 428	Active against *L. donovani*[b]
15	1,6-Bis(6-amino-2-methyl-4-quinolylamino)hexane	167 577	
16	5-Fluoroortic acid	152 520	
17	2,4-Diamino-6-(2-naphthylsulfonyl)quinazoline	158 122	Class active against malaria[c]
18	2,4-Diamino-6-(2-naphthylsulfonyl)-5,6,7,8-tetrahydroquinazoline	180 827	Class active against malaria[c]
19	6-Hydroxymethyl-2-isopropylamino-methyl-7-nitro-1,2,3,4-tetrahydroquinoline	188 439	Oxamniquine[d]
20	(−)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole hydrochloride	194 184	Levamisole[e]
21	2-Benzimidazolinone 5-arsinoxide hemihydrate	178 340	Active against *Plasmodium bergheiⁱ*
22	2,5-Bis(4-guanylphenyl)-furan dihydrochloride	199 385	
23	1-Aminocyclopentane-1-carboxylic acid	14 997	Active against malaria[c] and *T. rhodesiense*[g]
24	6-(3-Diethylaminopropylamino)-5,8-dimethoxy-2-methylquinoline bis(2,4-dihydroxybenzoate)	27 799	
25	6-(4-Amino-1-methylbutylamino)-5,8-dimethoxy-2,4-dimethylquinoline dihydrochloride monohydrate	182 146	Class has activity against *L. donovani*[b] and malaria[h]
26	6-(4-Amino-1-methylbutylamino)-5,8-dimethoxy-4-methylquinoline bis(2,4-dihydroxybenzoate)	203 766	

[a] Doses: 13, 52, and 104 mg/kg/day. [b] See ref 11. [c] See ref 26. [d] See ref 27-30. [e] See ref 31-34. [f] Unpublished data. [g] See ref 14. [h] See ref 35 and 36. [i] Sources of compounds: 14, C. Blanton, School of Pharmacy, University of Georgia, Athens, Ga. 30601; 15, A. Geiszler, Abbott Laboratories, North Chicago, Ill. 60604; 16, L. Garson, School of Pharmacy, University of Tennessee Medical Units, Memphis, Tenn. 38103; 17, F. Starks, Starks Associates, Inc., Buffalo, N.Y. 14213; 18, 21, and 24, H. Koppel, Aldrich Chemical Co., Inc., Milwaukee, Wis. 53210; 19, Pfizer, Ltd., Sandwich, Kent, England, United Kingdom; 20, H. Wood, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. 20014; 22, R. Olsen, Cordova Chemical Co., Sacramento, Calif. 95813; 23, C. Champlain, Dow Chemical Co., Midland, Mich. 48640; 25 and 26, Tara, Aldrich Chemical Co., Milwaukee, Wis. 53233.

primaquine moiety (i.e., compound 6) resulted in activity approximately fourfold greater than the control drug. The 4-methyl-8-aminoquinoline (compound 8) was ineffective. However, in an 8-aminoquinoline (compound 9) with a five-carbon chain and lacking a 4-methyl function, activity was about the same as for nifurtimox.

Two 7-aminoquinolines were tested (Table I). The compound with a terminal tertiary amine grouping (compound 9) was greater than three times as effective as the control compound. On the other hand, the compound having a terminal primary amine function and methyl groups at positions 2 and 4 (compound 10) was ineffective.

Conclusions related to toxicity for the 7- and 8-aminoquinoline types are limited, especially since some of them were not tested at higher dose levels. It should be noted, however, that the 7-aminoquinolines (compounds 9 and 10) and the "randomly substituted primaquine" (compound 6) were apparently not toxic at the higher levels. Other compounds in these classes, including primaquine (compound 2), produced death at the higher drug levels.

TAC pamoate (compound 11) was less effective (nifurtimox index, 0.2), the nitrofuran compound 12 was approximately as effective (index = 1.13), and the phosphonium salt compound 13 was 3.6 times as effective as nifurtimox, the standard drug. Results are shown in Table II. Deaths attributed to toxicity were observed at the highest treatment level with TAC pamoate (compound 11) and the phosphonium salt (compound 13).

The remaining 13 compounds of the 25 tested were found to be inactive. The compounds and some descriptive information are given in Table III.

Discussion

Eight of 25 compounds tested were noted to have suppressive activity equal to or greater than the control

compound, nifurtimox, in reducing parasitemia of *T. cruzi* infected mice. Five of these were 8-aminoquinoline derivatives, a class known to be effective in reducing the parasitemia.[2,3,6] It was of considerable interest to note that methyl substitution in the 3 position (but not the 2 or 4 position) or random *p*-carboxyphenylazo substitution on the quinoline moiety increases apparent activity over primaquine (compound **2**) by a factor of 2 or more.

The 7-aminoquinoline designated compound **9**, which was more than three times as active as the control compound, is known to have antimalarial[10] and antileishmanial activity.[11] To our knowledge, antischizotrypanosomal activity for this class of compounds has not been reported previously. The same is true regarding antischizotrypanosomal activity of the phosphonium salts; activity in other antiprotozoal test systems[11,12] has been noted for phosphonium salts, but activity against *T.* (*Schizotrypanum*) *cruzi* has not been reported. The phosphonium salt, compound **13**, was approximately 3.5 times as effective as nifurtimox. The triphenylmethane derivative, TAC pamoate, is known to have antischistosomal effects[13] and activity against African trypanosomes.[14,15] Here, it was found to be active in the *T. cruzi* mouse model, but it was less effective than nifurtimox. The activity observed for the nitrofuran compound **12** was not unexpected since the nitrofurans have activity against *T. cruzi*[2,3,6] as well as other microorganisms (e.g., see ref 16–20). Since completion of our testing the *trans*-5-amino-3-[2-(5-nitro-2-furyl)-vinyl]-1,2,4-oxadiazole, there have been preliminary reports[21,22] that this compound has been effective in therapy of acute experimental *T. cruzi* infections at levels comparable with nifurtimox (compound **1**).

It is recognized that several classes of compounds such as the 8-aminoquinolines and the nitrofurans can reduce numbers of the circulating blood forms of *T. cruzi* without eradicating the intracellular forms.[2,3,6] Obviously, the aim is to find chemotherapeutic agents which will eliminate all stages of the trypanosomes. Whether the newer compounds reported here may have such effect remains to be seen. However, it is believed that the biological model reported here may serve as a means of identifying new "leads" in seeking drugs broadly effective against *T. cruzi* infections in man. Chagas' disease is recognized as a serious public health problem in Ibero-American areas. It occurs in some 7 million persons among more than 35 million at potential risk and results in an estimated one death in ten persons acutely infected with *T. cruzi* plus markedly increased rate in morbidity and mortality among those chronically infected.[5,6,23,24]

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