Dziewiatkowski, School of Dentistry, The University of Michigan, for furnishing us with the chondrosarcoma tumor line.

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A New Chemical Series Active against African Trypanosomes: Benzyltriphenylphosphonium Salts

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Antitrypanosomal activity for benzyltriphenylphosphonium salts is reported for the first time. Testing was conducted using *Trypanosoma rhodesiense* infected mice. Of 70 phosphorus-containing compounds tested, 21 were active. Sixteen of these active chemical species were benzyltriphenylphosphonium salts. Four were nonbenzyl triphenyl compounds. The remaining active drug was a benzyldiphenylphosphonium salt.

The effective drug control of human sleeping sickness in the last 35 years has rested principally upon suramin, tryparsamide, pentamidine, and the melaminyl arsenicals, especially melarsoprol (Mel B).[2] The toxicity and other undesirable side effects of these drugs are well documented.[3] Newer chemical agents are urgently needed. In this report, data on the antitrypanosomal activity of a new series of chemical compounds, benzyltriphenylphosphonium salts, are given. Activity of this series against the African trypanosomes has not been previously reported.

Materials and Methods

The test system used to make drug evaluations has been described in detail.[4] Briefly, ICR/HA Swiss mice of either sex, 6 weeks of age weighing 28 to 30 g, are inoculated intraperitoneally with 0.5 mL of a 1:50000 dilution of heparinized heart blood drawn from donor mice infected 3 days earlier with the Wellcome CT strain of *Trypanosoma rhodesiense*. Test compounds, after they have been ground to a fine powder with mortar and pestle, are administered within 2 h of parasite inoculation as a single dose subcutaneously or orally in peanut oil (USP peanut oil, Durkee Foods, Coral Gables, Fla.).

The test system is based on a comparison of the mean survival time of untreated control mice and animals treated with the test compound. An increase of 100% in mean survival time is taken as the minimum response for a candidate compound to be considered as active. Mice alive at the end of 30 days are scored as cured. Deaths prior to the 4th day, before deaths occur in untreated controls, are regarded as from nonparasitic causes and become the basis for an evaluation of chemical toxicity. In calculating mean survival time, toxic deaths and 30-day survivors are not included. Twenty infected, untreated (negative) controls and 20 infected, positive controls are routinely used per test. Positive controls are mice infected and treated at 26.5 mg/kg with either stilbamidine isethionate or hydroxystilbamidine isethionate. Both positive control compounds are essentially 100% curative when given subcutaneously. Stilbamidine and hydroxystilbamidine are 98 and 71% curative, respectively, when given orally.

The phosphonium salts have attracted much attention because of their use in the Wittig reaction.[5-7] A number of such compounds are available commercially and were the source of compounds 7, 10, 11, and 18–21 (Tables I and II). The other compounds were generously supplied by individuals as noted. Compound 8 was reported by Novotny et al.[8] Phosphonium salt 9 was prepared after the method of Coombs and Houghton[9] and Hirao et al.[10] Those numbered 12 through 16 were disclosed by McErven et al.[11,12] Compound 17 was made by Aguiar.[13] In the general mode for preparation of such salts (cf. ref 5 and 6) the tris-substituted phosphine and requisite halide are reacted in a suitable solvent, usually one of low polarity such as benzene, xylene, or diethyl ether. The phosphonium salt is then collected and crystallized to afford a good yield of the product.

Biological Results and Discussion

Use of the triphenylmethane dyes marked a point of departure from arsenicals in early work devoted to finding effective trypanocidal agents.[14] That departure ultimately led to the synthesis of suramin sodium, the present drug of choice in the treatment of the early stages of African trypanosomiasis. Several of these dyes have activity against the African trypanosomes. The structures of five such agents are shown in Figure 1. These compounds also have activity against other parasites. The salt of the triphenylmethane dye *p*-rosaniline and pamoic acid, TAC pamoate, is active against schistosomiasis,[15] schizotrypanosomiasis,[16] as well as African trypanosomiasis.[4]

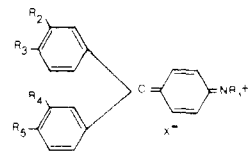
In view of evidence such as this, it follows that substitution of phosphorus, which has important and multifaceted functions in the biochemistry of host and parasite metabolism, would likely result in compounds with trypanocidal properties. A total of 70 phosphorus-containing compounds were tested. Forty-nine of these were drugs having three phenyl moieties connected directly to the phosphorus atom of the chemical species. Of the 70 tested, 21 were active (Tables I and II). Twenty of these

Table I. Benzyltriphenylphosphonium Salts Having Activity against *Trypanosoma rhodesiense* Infections of Mice

$[Ar]_3\overset{+}{P}CH_2R\ X^-$

compd[h]	Ar	R	X	WR[a]	route[b]	no. cured/no. treated, mg/kg 424	212	106	53	26.5	13.3	6.5
1	C_6H_5	C_6H_5	Br	18 868	sc	0/5 (5T)[c]		0/10 (10T)	0/5	0/10	0/5	0/5
					O		0/5 (5T)	6/10	0/5	0/10	0/5	
2	C_6H_5	$C_6H_4Cl(2)$	Cl	162 997	sc	0/10 (10T)	0/5 (5T)	2/10 (8T)	2/5 (1T)	6/10	0/5	
					O	0/10 (10T)	0/5 (5T)	0/10*	0/5	0/10	0/5	
3	C_6H_5	$C_6H_4CH_3(2)$	Cl	154 744	sc	0/10 (10T)	0/5 (5T)	2/10 (8T)	2/5 (1T)	4/10	0/5	
4	C_6H_5	$C_6H_4CH_3(4)$	Cl	132 504	sc	0/10 (10T)	2/5 (3T)	10/10	5/5	2/10	0/5	
					O	0/10 (4T)*[d]	0/5*	0/10	0/5	0/10	0/5	
5	C_6H_5	$C_6H_4CH_3(2)$	Br	17 490	sc	0/5 (5T)	0/5 (5T)	0/10 (10T)	0/5 (1T)*	0/10	0/5	
					O	0/10 (10T)	0/5*	0/10	0/5	0/10	0/5	
6	C_6H_5	$C_6H_4CH_3(4)$	Br	15 636	sc	0/5 (5T)	0/5 (5T)	0/5 (2T)*	0/5	0/5	0/5	0/5
					O	0/10 (10T)	0/10 (1T)*	0/10	0/5	0/10	0/5	
7	C_6H_5	$C_6H_4OC_2H_5(4)$	Cl	183 035	sc	0/10 (10T)	0/5 (5T)	4/10 (6T)	3/5 (1T)	0/10	0/5	
8	C_6H_5	$C_6H_4CF_3(4)$	Cl	149 136	sc	0/10 (10T)	2/5 (3T)	2/10 (2T)	1/5	0/10	0/5	
					O	0/5 (5T)	0/5 (3T)	0/5 (1T)	0/5	0/5	0/5	
9	C_6H_5	$C_6H_4CO_2CH_3(2)$	Br	116 177	sc			2/10 (8T)	2/5 (3T)	0/10	0/5	0/10
10	C_6H_5	$C_6H_3OH(4)$-*t*-$Bu_2(3,5)$	Br	179 422	sc	0/10*	0/5*	0/10*	0/5	0/10	0/5	
					O	0/10*	0/5	0/10	0/5	0/10	0/5	
11	C_6H_5	C_9H_6N[e]	Br	119 690	sc	0/10 (10T)	1/5 (3T)	6/10	0/5	0/10	0/5	
12	$C_6H_4CH_3(2-)$	C_6H_5	Cl	61 987	sc	0/5 (5T)		0/10 (10T)	3/5 (2T)	6/10	0/5*	
					O	4/10 (6T)	4/5 (1T)	0/10*	0/5	0/10	0/5	
13	$2C_6H_4CH_3(2-)$[f] C_6H_5	C_6H_5	Cl	58 915	sc	0/5 (5T)	0/5 (5T)	6/15 (9T)	8/10	12/15	0/10	0/10
					O		0/5 (5T)		0/5		0/5	
14	$C_6H_4CH_3(2-)$[f] $2C_6H_5$	C_6H_5	Cl	58 909	sc	0/5 (5T)		0/10 (10T)	0/5 (3T)*	6/10	0/5	0/5
					O	0/10 (10T)	1/5 (2T)	4/10	0/5	0/10	0/5	
15	$C_6H_4CH_3(4-)$[f] $2C_6H_5$	C_6H_5	Cl	58 912	sc	0/5 (5T)	0/5 (5T)	8/10 (2T)	3/5	0/10	0/5	0/5
					O	0/10 (10T)	0/5*	0/10*	0/5	0/10	0/5	
16	$C_6H_4CH_3(2-)$	C_6H_5	Cl	61 986	sc	0/5 (5T)		0/10 (10T)	2/5 (1T)	0/10	0/5	
					O	0/5		0/5		0/5		
	stilbamidine[g]	*trans*-4,4′-bis(guanyl)stilbene			sc	5/5	5/5	5/5	5/5	798/800	20/20	30/30
	standard				O	5/5	5/5	5/5	5/5	187/200	4/10	0/5

[a] WR denotes the compound number assigned by the Walter Reed Army Institute of Research. [b] sc, subcutaneous; O, oral. [c] T, death attributed to drug toxicity. [d] An asterisk denotes activity, i.e., at least 100% increase in mean survival time when compared to infected untreated controls. [e] Denotes 8-quinolyl grouping. [f] Differing aryl groups indicated. [g] Activity of nonphosphorus containing standard 4. [h] Source of compounds: 1 and 12–16, Carpino, University of Massachussets, Amherst, MA 01003; 2, 5–7, and 10, Aldrich Chemical Co., Inc., Milwaukee, WI 53210; 3, Eastman Organic Chemicals, Division of Eastman Kodak Co., Rochester, NY 14600; 4 and 11, Maybridge Chemical Co. Ltd., Tintagel, North Cornwall, England; 8, F. W. Starks, Starks Associates, Inc., Buffalo, NY 14213; 9, J. C. Martin, Department of Chemistry, University of Illinois, Urbana, Ill. 61801; standard, J. H. Hill, May and Baker, Ltd., Dagenham, Essex, England.



	R_1	R_2	R_3	R_4	R_5	X^-
1. Malachite Green	$(CH_3)_2$	H	$N(CH_3)_2$	H	H	Cl^-
2. Brilliant Green	$(C_2H_5)_2$	H	$NC_2H_3I_2$	H	H	HSO_4^-
3. Rosaniline (Fuchsin)	H_2	NH_2	CH_3	H	NH_2	Cl^-
4. *p*-Rosaniline (*p*-Fuchsin)	H_2	H	NH_2	H	NH_2	Cl^-
5. Tryparsen	H_2	Cl^-	NH_2	Cl^-	NH_2	Cl^-

Figure 1. Structures of five triphenylmethane dyes that have activity against the African trypanosomes.

active drugs were compounds having three phenyl rings attached directly to the phosphorus; the one remaining agent was 1,2-diphenylethylethyldiphenylphosphonium iodide (compound **17**). Sixteen of these 21 active agents were benzyltriphenylphosphonium salts.

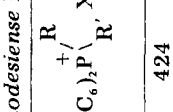
The unsubstituted benzyltriphenylphosphonium salt (compound **1**) had modest activity. The dibenzyldiphenyl-, the tribenzylphenyl-, and the phenacyltriphenylphosphonium salts were inactive, while the *p*-chlorophenoxymethyltriphenylphosphonium salt (compound **18**) was modestly active.

Some compounds with substitutions on the benzyl function of the basic benzyltriphenylphosphonium molecule (compound **1**) were more active than the unsubstituted parent agent. The most effective was the substitution of Cl at the 2 position (compound **2**). This was curative at 26.5 mg/kg. Methyl substitution at this same 2 position (compound **3**) or at the 4 position (compound **4**) yielded drugs that were 40 and 20% curative, respectively, at this same dosage level. It should be noted, however, that the results for these latter two agents were obtained with the chloride salts. The bromide salts of these two (compounds **5** and **6**), although active, were less active than their chloride counterparts. Substitution of either an ethoxy (compound **7**) or trifluoromethyl (compound **8**) at the 4 position or a methyl ester at the 2 position (compound **9**) yielded agents that were curative to 53 mg/kg. Substitution of 3,5-di-*tert*-butyl-4-hydroxyphenyl (compound **10**) yielded a compound that was active as low as 106 mg/kg. However, substitution to render the benzyl function a quinolyl (compound **11**) provided a chemical species that was curative as low as 106 mg/kg. Benzyl ring substitutions by the following groupings yielded compounds that were inactive: 2-CH_2Br (Br) (abbreviations with parentheses designate the salt(s) tested), 3-Cl (Cl and Br), 3-CH_3 (Br), 4-NO_2 (Cl and Br), 4-CH_2Br (Br), 4-OCH_3 (Cl), 4-Br (Br), 4-C≡N (Br).

As with substitutions on the benzyl function, some compounds with substitutions on the triphenyl function of the basic benzyltriphenylphosphonium species (compound **1**) were more active than the parent compound. The chemical species with the methyl grouping in the meta position of all three phenyl rings (compound **12**) was active at doses as low as 13.3 mg/kg. This same methyl substitution in the ortho position of the three rings (compound **16**) was only curative to 53 mg/kg. Compounds with *o*-methyl substitution on one (compound **14**) or two (compound **13**) of the two phenyl rings were curative to 26.5 mg/kg. Compound **15** with a *p*-methyl group on one of the three phenyl rings was curative to 53 mg/kg.

Of the five nonbenzyl triphenylphosphonium compounds shown in Table II, four are triphenyl salts (compounds **18**–**21**); the remaining (compound **17**) is a diphenyl salt. The most active compound in this group

Table II. Diphenyl(disubstituted)phosphonium Salts Active against *Trypanosoma rhodesiense* Infections in Mice



compd[h]	R	R′	X	WR[a]	route[b]	no. cured/no. treated, mg/kg					
						424	212	106	53	26.5	13.3
17	C_2H_5	$CH(C_6H_5)CH_2C_6H_5$	I	104 005	sc	0/5 (5T)[c]		0/10 (8T)*[d]	0/5*	0/10	0/5
18	C_6H_5	$CH_2OC_6H_4Cl(4-)$	Cl	179 271	sc	0/5 (5T)		1/10 (4T)	0/5	0/10	0/5
19	C_6H_5	C_6H_5	Br	122 536	sc	0/10 (10T)	0/5 (5T)	0/10 (8T)	0/5 (5T)	5/5	
20	C_6H_5	$CH_2CH{=}CH_2$	Br	14 934	sc	0/5 (5T)		1/5 (1T)	0/5*	0/10	0/5
21	C_6H_5	$C_{12}H_{25}$	Br	145 048	sc	0/10*	0/5*	0/10*	0/5*	0/5	
	stilbamidine,[g] *trans*-4,4′-bis(guanyl)stilbene				sc	5/5	5/5	5/5	5/5	798/800	20/20
	standard				O	5/5	5/5	5/5	5/5	187/200	4/10

[a] WR denotes the compound number assigned by the Walter Reed Army Institute of Research. [b] sc, subcutaneous; O, oral. [c] T, death attributed to drug toxicity. [d] An asterisk denotes activity, i.e., at least 100% increase in mean survival time when compared to infected untreated controls. [g] Activity of nonphosphorus containing standard 4. [h] Source of compounds: 17, A. M. Aguiar, Department of Chemistry, Fairleigh Dickinson University, Madison, NJ 07940; 18 and 20, Aldrich Chemical Co., Inc., Milwaukee, WI 53210; 19, Alfa Inorganics Inc., Beverely, MA 01915; 21, Distillation Products Industries, Rochester, NY 14603; standard, J. H. Hill, May and Baker, Ltd., Dagenham, Essex, England.

was compound **19**, tetraphenylphosphonium bromide.

Whether this new series of compounds, active also against American trypanosomiasis,[16] visceral leishmaniasis,[17] and malaria,[18] will provide the "lead" which will culminate in new drugs effective against the African trypanosomes remains to be seen. Relatively little is known of the biological activity of phosphorus as the phosphonium salt. It is known that phosphorus as phosphates is required by virtually all forms of life, have more known functions than any other mineral element within the human body, and is perhaps the single most important mineral constituent required for cellular activity. On the other hand, phosphorus in the white or yellow elemental form is very toxic, with injury occurring to the gastrointestinal tract, liver, muscles, myocardium, kidney, and central nervous system. Elemental phosphorus in the red, granular, nonabsorbable form is essentially inert to the mammalian system. In the present studies, toxicity was observed at the upper dose levels with all compounds except compound **10** (Table I). It should be noted, however, that curative activity was seen at lower doses without observable toxic side effects. For example, compound **4** was curative at a dose level of 26.5 mg/kg. Toxicity was not seen until the drug level was increased eightfold, i.e., to 212 mg/kg. Further studies seem warranted in an attempt to find new and better chemotherapeutic agents for inclusion in the armamentarium required for the control of African trypanosomiases.

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(19) The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals", Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education, and Welfare publication no. 74–23.

Synthesis and Pharmacological Activity of 2,3,4,5-Tetrahydro-1,5-methano-1*H*-3-benzazepines

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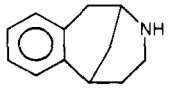
2,3,4,5-Tetrahydro-1,5-methano-1*H*-3-benzazepine (**4**) has been synthesized from 2,3-dioxobenzonorbornene. Oxidative cleavage of the diketone to *cis*-1,3-indandicarboxylic acid, followed by closure to the corresponding anhydride, conversion to the imide, and lithium aluminum hydride reduction, gave **4**. Compound **4** and its N-derivatives show no analgesic activity in the mouse hot-plate assay and little antagonist activity in a tail-flick assay.

Recently there has been much interest in the synthesis and properties of potential analgesics having a "simplified" morphine ring system. The morphine-type analgesic activities of the 6,7-benzomorphan **1**[1] (ED_{50} = 10.2 mg/kg),



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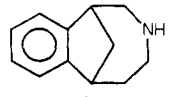

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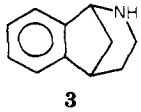

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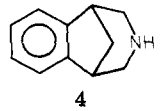

4

benzazocine **2**[2] (ED_{50} = 4.9 mg/kg), and *B*-norbenzomorphan **3**[3] (reported as one-third the activity of codeine) and their respective derivatives prompted us to investigate the structurally related compound 2,3,4,5-tetrahydro-1,5-methano-1*H*-3-benzazepine (**4**) to see if the change in the N position would dramatically affect its pharmacologic activity. We report the synthesis and analgesic testing results of several members of ring system **4**.

Chemistry. The key to the synthesis of **4** was the preparation of the heretofore unknown *cis*-1,3-indandicarboxylic acid (**8**). Our initial approach, which involved oxidative cleavage of benzonorbornadiene to **8**, was unsuccessful. Thus, ozonolysis of benzonorbornadiene[4] using a variety of solvents and workup procedures resulted mainly in the isolation of intractable material. Other traditional oxidizing methods including neutral per-

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