UR 10 Carl Zeiss Jena spectrophotometer in KBr pellets. The nmr spectra were run on a JEOL 100-MHz spectrometer in DMSO-d_6. Paper chromatograms were run on Whatman No. 1 paper and the spots were detected under uv light.

***N*-[4-[[(2,4-Diamino-7-pteridyl)methyl]-N^{10}-methylamino]benzoyl]glutamic Acid (II).** A solution of tetraaminopyrimidine dihydrochloride (2.1 g, 0.01 mol) (prepared from tetraaminopyrimidine bisulfite[10] and HCl) in H_2O (50 ml) was added dropwise, during 6 hr and at room temperature, to a stirred solution of 1,1,3-trichloroacetone[5,19,20] (IV, 4.8 g, 0.03 mol) in 40 ml of H_2O and 8.2 g of AcONa at pH 6.0 (adjusted with concentrated HCl). NaOH solution (0.5 *M*) was occasionally added to keep the pH of the solution at 5.5-6.0. The reaction mixture was stirred overnight at room temperature and then cooled at 0°. A brown solid was collected by filtration and washed (H_2O, EtOH, EtOH-Et_2O (1:1), Et_2O) to afford 1.7 g of 2,4-diamino-7-chloromethylpteridine (V, 80%).

Paper chromatography in BuOH-EtOH-H_2O (100:35:72, ascending) showed a major spot at R_f 0.57 and a minor impurity at R_f 0.49.

To a warm (45°) solution of *N*-[4-(*N*-methylamino)benzoyl]glutamic acid[21,22] (VIII, 2.8 g, 0.01 mol) in 300 ml of 4 *N* AcONa-AcOH buffer (pH 4) was added dropwise over a period of 1 hr a solution of V (2.1 g, 0.01 mol). The mixture was maintained at 45° for 40 hr and then gradually warmed at 55° for 2 hr, at 65° for 2 hr, and finally at 75° for 1 hr, the pH value being kept at 4 by occasional addition of 1 *N* NaOH. The mixture was cooled and left overnight at 4°. The solid was filtered off, washed (cold H_2O, EtOH, EtOH-Et_2O (1:1), Et_2O; 40 ml, respectively), and dried *in vacuo* at 50° for 24 hr, giving about 3 g of crude II.

The above crude II was suspended in H_2O (150 ml), basified (Na_2CO_3) to pH 11-12, heated at 50°, and the insoluble residue was removed by filtration. The filtrate was neutralized (1 *N* HCl) to pH 7, left overnight at 4°, treated with active charcoal, and filtered. The filtrate was brought to pH 3.5-4.0 (1 *N* HCl), left overnight at 4°. The precipitate was filtered, washed (cold H_2O, EtOH, EtOH-Et_2O (1:1), Et_2O; 20 ml, respectively), and dried *in vacuo* at 50° for 24 hr giving 1.8 g of partially purified II.

The product thus obtained was suspended in 20 ml of distilled water and the pH adjusted to 7.0 with 0.5 *N* NaOH. The insoluble impurities were removed by filtration. The solution was passed over a column (ϕ 22 mm) of 100 g of cellulose (Schuchardt) suspended in an aqueous solution of 0.1 *M* Na_2HPO_4 (pH adjusted to 7.0 with concentrated HCl). Elution with the same phosphate buffer was started 1 hr after introducing the sample. The fractions containing II (uv monitoring) were combined, the pH was adjusted to 3.5-4.0 (glacial AcOH) and cooled overnight at 4°, and the precipitate was worked up as above giving 1.2 g of a still impure II with R_f 0.60 (paper chromatography, descending, eluent 0.1 *M* phosphate buffer pH 7.0); additional spots R_f 0.3 and in the start.

The above II (1.2 g) was suspended in 25 ml of distilled H_2O, dissolved by neutralization (1 *N* NaOH) to pH 7.0, and passed over a three-layer column (1 g of cellulose; 12 g of cellulose and 2.5 g of charcoal, and finally 12 g of cellulose, suspended in water) with distilled H_2O as eluent. From the uv, selected fraction II was precipitated and worked up as above giving 0.55-0.75 g of pure II (12-16% based on VII): mp 203°; uv spectra λ_{max} (nm) ($\epsilon \times 10^{-3}$) 0.1 *N* HCl for II (7-MTX) 243.6 (sh), 311.6 (24.1); for I (6-MTX) 240, 306 (21.7); ir (KBr) ν_{max} (cm^{-1}) for II (7-MTX) 767, 811, 832, 924, 946, 1007, 1100, 1208, 1248, 1309, 1368, 1404 (sh), 1451, 1507, 1515, 1557, 1595 (sh), 1609, 1635, 1643; for I (6-MTX) 766, 803, 828, 938, 961, 1018, 1099, 1113, 1206, 1252, 1291, 1385 (sh), 1395, 1452, 1506, 1514, 1551, 1595 (sh), 1608, 1635, 1643; nmr (DMSO-d_6) δ for II (7-MTX) 8.51 (1 H, s), 8.08 (2 H, m), 7.14 (2 H, m) (arom region); for I (6-MTX) 9.01 (1 H, s), 8.18 (2 H, m), 7.14 (2 H, m). *Anal.* ($C_{20}H_{28}N_8O_5 \cdot 2H_2O$) C, H, N; H_2O (K. Fischer).

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In Vitro and *in Vivo* Activity of Certain Thiosemicarbazones against *Trypanosoma cruzi*

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From the time thiosemicarbazones were first prepared as derivatives for the identification of aldehydes, ketones, and quinones,[1] biological activity in many areas has been noted including antibacterial activity (particularly antitubercular[2]), fungistatic activity,[3] antiinflammatory activity,[4] antiparasitic activity (*Trichomonas vaginalis*[5]), antiviral activity (vaccinia,[6-8] herpes, and cytomegalo),[9,10] and antitumor activity.[11] Recent investigations in our laboratory have revealed that certain members of this chemical group possess significant *in vitro* and *in vivo* activity against *Trypanosoma cruzi* infections (Chagas' disease).

Biological Results and Discussion. Of the compounds found active *in vitro* (Table I) against *T. cruzi*, **4**, **5**, **6**, and **10** were examined for *in vivo* activity. All produced significant increases in the mean survival times of infected mice (Table II) although no radical cures were effected at near toxic doses.

General conclusions regarding the structure-activity relationship of thiosemicarbazone structure and *in vitro T. cruzi* activity parallel the conclusions drawn by Bernstein, *et al.*,[12] and Young, *et al.*,[13] with respect to antitubercular structure-activity relationships, namely, (a) the sulfur cannot be replaced by oxygen, (b) substitution at 3 or 4 of the thiosemicarbazone eliminates activity, and (c) thiosemicarbazides and aliphatic thiosemicarbazones are inactive.

Since it has recently been shown[2,9,10] that there are certain parallels between the structure-activity relationships of thiosemicarbazones and antitubercular and antiviral activities, it may be that the antitrypanosomal ac-

Table I. Physical Data and *in Vitro* Activity *vs. Trypanosoma cruzi* of Certain Aryl Aldehyde Thiosemicarbazones

Compd no.	$R_1CH{=}NN(H){-}C(=X)R_2$ R_1	R_2	X	Mp, °C	Concn, μg/ml[a] MIC[b]	MLC[c]
1[d]	Benzimidazol-2-yl	NH_2	S	258	>100	
2[e]	Pyridin-3-yl	NH_2	S	213–216	>100	
3[f]	Pyridin-4-yl	NH_2	S	216	>100	
4	3-Methylthien-2-yl	NH_2	S	199–202	10	10
5[g]	5-Methylthien-2-yl	NH_2	S	158–160	1.0	100
6[h]	*p*-Dimethylaminophenyl	NH_2	S	210–211	1.0	10
7	*p*-Dimethylaminophenyl	$NHCH_3$	S	226–227	>100	
8[i]	*p*-Dimethylaminophenyl	NH_2	O	222	>100	
9	*p*-Dimethylaminophenyl	OCH_2Ph	O	161–163	>100	
10	2-Chloro-4-dimethylaminophenyl	NH_2	S	205–208	3.2	3.2
11[h]	*p*-Acetamidophenyl	NH_2	S	246–247	>100	
12[j]	*p*-[*N*-Bis(2-chloroethyl)]aminophenyl	NH_2	S	196	5.0	10
13[k]	*p*-[β-(Diethylamino)ethoxy]phenyl	NH_2	S	144–145	100	

[a]Determined by serial dilution *in vitro* in liquid culture in the medium of L. G. Warren, *J. Parasitol.*, **46,** 529 (1960). [b]MIC = minimum inhibitory concentration. [c]MLC = minimum lethal concentration. [d]See ref 7. [e]C. Levaditi, A. Girard, A. Vaisman, and A. Ray, *C. R. Acad. Sci., Paris, Ser. C,* **231,** 1174 (1950). [f]E. Grunberg and B. Leiwant, *Proc. Soc. Exp. Biol. Med.*, **77,** 47 (1951). [g]U. S. Patent 2,746,972 (1956); *Chem. Abstr.*, **51,** 2871*a* (1957). [h]See ref 1. [i]N. D. Cheronis and J. B. Entrikin, "Identification of Organic Compounds," Wiley, New York–London, 1963, p 369. [j]H. C. Koppel, R. H. Springer, G. D. Daves, Jr., and C. C. Cheng, *J. Pharm. Sci.*, **52,** 81 (1963). [k]J. Bernstein, H. L. Yale, K. Losee, M. Holsing, J. Martins, and W. A. Lott, *J. Amer. Chem. Soc.*, **73,** 906 (1951).

Table II. *In Vivo* Activity of Selected Aryl Aldehyde Thiosemicarbazones against *Trypanosoma cruzi* Infections in Mice

Compd no.	$RCH{=}NN(H){-}C(=S)NH_2$ R	Effects on *T. cruzi* infected mice: Dose, mg/kg/day	Route[a]	Surv/ total	MST,[b] days	MST, inc., *P*[c]
4	3-Methylthien-2-yl	50	sc	0/7	35	<0.001
5	5-Methylthien-2-yl	50	sc	0/7	33	<0.01
6	*p*-Dimethylaminophenyl	50	sc	0/7	34	<0.01
10	2-Chloro-4-dimethylaminophenyl	25	sc	0/7	32	<0.02
	Vehicle control	0		0/7	26	

[a]sc, subcutaneous. [b]MST, mean survival time. [c]*P*, probability (Student's t test).

tivity of thiosemicarbazones is due to the inhibition of protein synthesis[14] as has been demonstrated for antiviral thiosemicarbazones (Methisazone).

Experimental Section

General Synthesis. A solution of the desired aldehyde in the minimum volume of hot ethanol was added to an equimolar amount of a thiosemicarbazide derivative dissolved likewise in the minimum amount of ethanol. One drop of hydrochloric acid was added as a catalyst, and the mixture was heated to boiling for 30 min. In most cases the thiosemicarbazone derivatives crystallized upon cooling; exceptional cases were heated to the boiling point again and water was added dropwise until incipient turbidity and the solution was allowed to cool. The crystals were filtered and recrystallized from ethanol or aqueous ethanol.

In Vivo **Antitrypanosomal Evaluation.** C_3H strain mice were infected by intraperitoneal injection of 2×10^3 bloodstream forms of a Brazilian strain of *T. cruzi*. Typically, all untreated infected mice of this strain demonstrate patent parasitemias in 10–14 days and die of a fulminating *T. cruzi* infection 19–26 days after inoculation. Activity was determined from mortality records and mean survival times of treated and untreated groups of mice.

Compounds to be evaluated were micronized to a particle size of 0.5–2.0 μ and suspended in a vehicle containing 0.9% NaCl, 1.0% sodium carboxymethylcellulose, 0.5% Tween 80, and 0.05% antifoam B. They were subsequently administered in this vehicle at a concentration where 0.01 ml/2 g of body weight provided the desired dose level. Animals were treated for 5 successive days beginning 3 days postinfection.

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Supplementary Material Available. The ultraviolet spectral data for compounds 1–13 at pH 1 and 11 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy of $2.00 for microfiche, referring to code number JMED-74-760.

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