30 ml of MeOH. $NaBH_4$ (1.20 g) was added over a period of 45 min; then the mixture was stirred at ambient temperature for 15 hr. The nearly clear solution was clarified by filtration, then spin-evaporated *in vacuo* to about 10 ml, and diluted with 70 ml of H_2O. The product was collected on a filter and washed with water. Two recrystallizations from aqueous EtOH gave 340 mg (38%) of light yellow crystals, mp 178–179° dec. See Table II for additional data.

6-(*p*-Chloroacetylanilinomethyl)-5-(*p*-chlorophenyl)-2,4-diaminopyrimidine (10a) Hydrochloride. Method B.—A mixture of 180 mg (0.4 mmole) of **9a** and 10 ml of 0.1 *N* HCl was refluxed with stirring for 1 hr, then cooled to 0° for several hr. The product was collected on a filter and washed with 2 ml of ice water. Recrystallization from EtOH by addition of 0.1 *N* HCl gave 146 mg (80%) of white plates, mp 180–182° dec. See Table II for additional data.

2,2′-Hydrazobis(5-nitrothiazoles) and Analogs, a New Type of Antiprotozoal Agents

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A series of 1,2-diacyl-1,2-bis(5-nitro-2-thiazolyl)hydrazines and several bis(5-nitro-2-thiazolyl) derivatives have been prepared and tested for antiprotozoal activity. Some of the compounds show a very strong *in vitro* but no *in vivo* activity.

The heterocyclic nitro compounds belong to one of the most thoroughly investigated, versatile, and useful systems in the services of chemotherapy of infectious diseases. The best examples are the nitrofurans, exhibiting pronounced trypanocidal, coccidistatic, and very strong antibacterial activity.[2] Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] is today the drug of choice in the systemic treatment of trichomoniasis.[3] This fact has led to the preparation of a great number of substituted 5-nitroimidazoles as potential chemotherapeutic agents.[4] 2-Amino-5-nitropyridine,[5,6] 2-amino-5-nitropyrimidine,[6] and a number of 4-nitropyrazoles[7] and nitropyrroles[8] show marked trichomonacidal activity. The 2-amino-5-nitrothiazole nucleus seems to possess one of the broadest profiles of antiparasitic activity, ranging from trichomonal and helminthic infections, especially schistosomiasis, to histomoniasis and amebiasis.[9] 2-Acetamido-5-nitrothiazole has also a suppressive action on infections with *Trypanosoma cruzi* in mice.[10] These results encouraged the study of a further number of 5-nitrothiazoles with different substituents at the 2-amino group.[11,12]

One common feature found in many chemotherapeutic agents is their symmetrical structure. These molecules have been described as "dumb-bell" shaped[13] or as "butterfly structures."[14] Typical examples are the aromatic diamidines used in the treatment of trypanosomiasis[15] and leishmaniasis[16] and the derivatives of 4,4′-diaminodiphenyl sulfone, used in the therapy of all forms of leprosy.[17] Bis(4,6-diaminoquinaldine) derivatives show a very marked antitrypanosomal[18] and antibacterial activity,[19] polymethylenebisquinolinium and -isoquinolinium salts possess a wide bacteriostatic[19] and fungistatic profile,[20] while diaminodiphenoxyalkanes are considered potential schistosomicides.[21]

The combination of these two important features, nitro heterocyclic compounds and symmetrical molecules, led us to consider the investigation of a new type

(1) (a) To whom inquiries should be addressed. (b) Deceased.

(2) (a) F. Hawking in "Experimental Chemotherapy," Vol. I, R. J. Schnitzer and F. Hawking, Ed., Academic Press Inc., New York, N. Y., 1963, p 197; (b) L. P. Joguer, S. F. M. Havier, and S. B. Kendal, *ibid.*, p 460; (c) H. E. Paul and M. F. Paul, *ibid.*, Vol. II, 1964, p 307.

(3) I. M. Rollo in "The Pharmacological Basis of Therapeutics," L. S. Goodman and A. Gilman, Ed., 3rd ed, The Macmillan Co., New York, N. Y., 1965, p 1135.

(4) (a) Rhone-Poulenc S.A., French Patents 1,379,787 (1964), M 3270 (1965), M 3342 (1965); (b) Netherlands Patent 6,411,717 (1965); (c) Merck and Co., Netherlands Patents 6,409,117 (1965), 6,409,120 (1965), 6,413,815 (1965); (d) Belgian Patents 660,836 (1965), 661,262 (1965); (e) May and Baker Ltd., Belgian Patents 639,372 (1964), 639,469 (1964); (f) U. S. Patent 3,236,856 (1966); (g) Carlo Erba S.p.A., Belgian Patent 667,262 (1965).

(5) N. D. Knoop and N. P. Buu-Hoï, *Compt. Rend.*, **253**, 3115 (1961).

(6) (a) R. M. Michaels and R. E. Strube, *J. Pharm. Pharmacol.*, **13**, 601 (1961); (b) R. M. Michaels, *J. Protozool.*, **9**, 478 (1962).

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(9) (a) R. J. Schnitzer, ref 2a, p 298; (b) A. C. Cuckler, A. B. Kupferberg, and N. Millman, *Antibiot. Chemotherapy*, **5**, 540 (1955); (c) ref 2b, p 341.

(10) Reference 2a, p 205.

(11) 1-(5-Nitro-2-thiazolyl)-2-imidazolidinimine is considered today the best schistosomicide: C. R. Lambert, M. Willhelm, H. Striebel, F. Kradolfer, and P. Schmidt, *Experientia*, **20**, 452 (1964); C. R. Lambert, *Ann. Trop. Med. Parasitol.*, **58**, 292 (1964). Many analogs have been prepared by CIBA Ltd., Netherlands Patents 6,410,031 (1965), 6,505,225 (1965), 6,511,486 (1966).

(12) (a) E. Piscaro, H. Tefas, A. Berechi, and C. Chirita, *Farmacia (Bucharest)*, **7**, 513 (1959); *Chem. Abstr.*, **54**, 14163 (1960); (b) Wallace and Tiernan Inc., British Patent 867,273 (1961); (c) J. M. Garcia-Maroquina Rodrigo and R. F. Graner, *Farm. Nueva* (Madrid), **27**, 503 (1962); (d) S. Hillers, N. Saldabols, and A. Medne, *Zh. Obshch. Khim.*, **33**, 317 (1963); (e) NOPCO Chemical Co., French Patent M 1864 (1963); (f) CIBA Ltd., French Patent 1,345,059 (1963); (g) Belgian Patent 636,256 (1964); (h) Chemie Gruenenthal G.m.b.H., German Patent 1,189,096 (1965); (i) N. Steiger, U. S. Patent 3,188,316 (1965); (j) CIBA Ltd., Netherlands Patent 6,505,226 (1965); (k) D. B. Reisner, S. Gister, and H. C. Klein, U. S. Patent 3,262,848 (1966); (l) G. L. Dunn, P. Actor, and V. J. DiPasquo, *J. Med. Chem.*, **9**, 751 (1966).

(13) L. P. Walls, *Chem. Ind.* (London), 606 (1951).

(14) F. C. Goble, *J. Pharmacol. Exptl. Therap.*, **98**, 49 (1950).

(15) References 2a, p 170.

(16) E. Beveridge, ref 2a, p 275.

(17) L. Weinstein, ref 3, p 1309.

(18) Reference 2a, p 191.

(19) R. J. Schnitzer, ref 2c, p 376.

(20) (a) Reference 2a, p 907; (b) W. C. Austin, M. D. Porter, and E. P. Taylor, *J. Chem. Soc.*, 1480 (1958); (c) R. Strauss, A. McBurney, S. Rhonisher, and J. M. Beiler, *Antimicrobial Agents Chemotherapy 1963*, 578 (1964).

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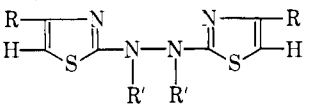
TABLE I

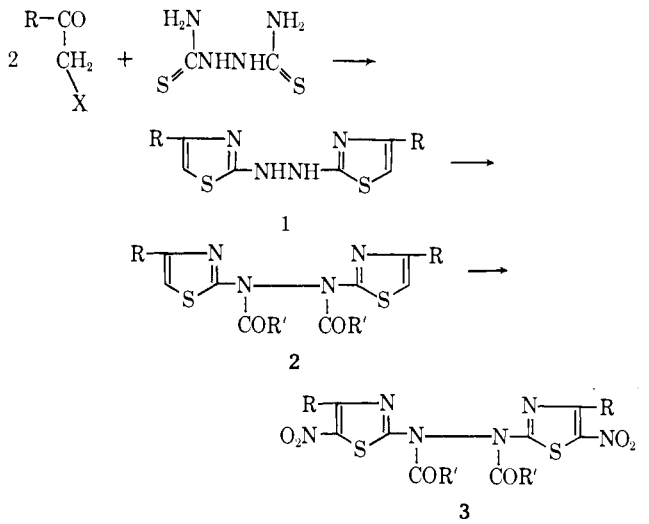
2,2′-HYDRAZOBISTHIAZOLES



Compd			Yield,[a]		Crystn		Calcd, %			Found, %[d]		
1	R	R′	%	Mp, °C	solvent	Formula	C	H	N	C	H	N
a	H	H[b,c]										
b	CH_3	H[c]										
c	C_2H_5	H	61	174	AcOEt	$C_{10}H_{14}N_4S_2$	47.24	5.55	22.04	46.89	5.67	21.51
d	n-C_4H_9	H	52	181	EtOH	$C_{14}H_{22}N_4S_2$	54.18	7.15	18.05	54.71	7.57	17.56
e	t-C_4H_9	H	68	199	MeOH	$C_{14}H_{22}N_4S_2$	54.18	7.15		54.92	7.40	
g	$ClCH_2$	H[b]	87									
l	Et_2NCH_2	H[b]	77									
n	m-H_2NSO_2-C_6H_4	H	93	225-228	None	$C_{18}H_{16}N_6O_4S_4$	42.50	3.17	16.52	42.38	3.29	16.68
o	CH_3	CH_3	65	296-299	n-PrOH	$C_{10}H_{14}N_4S_2$	47.25	5.55	22.04	47.14	5.72	21.61

[a] Crude product. [b] Obtained only in the form of crude hydrohalide. [c] Reference 22. [d] The unsatisfactory analytical data of compounds **1c-e** are attributed to the instability of these hydrazines. The final evidence for these structures is shown by the data of the stable acetylated derivatives **2c-e** in Table II.

of potential chemotherapeutic agents, symmetrical heterocyclic nitro compounds. The first system to be investigated was the dimer of the versatile 2-amino-5-nitrothiazole and derivatives of it. A series of 2,2′-hydrazobis(5-nitrothiazoles) (**3**) was therefore prepared, carrying different substituents in the free positions of the thiazole rings and different acyl groups on the hydrazine bridge. Several 2,2′-hydrazobisthiazoles have been prepared by Beyer using the Hantzsch thiazole synthesis, namely, the condensation of dithiobiurea with different α-halogeno ketones.[22] Since these hydrazines were very sensitive to oxygen, some of them were isolated only in the form of their salts. Acetylation of the hydrochlorides led to the more stable diacetyl derivatives. In the same way we were able to obtain a series of 2,2′-hydrazobis(4-alkylthiazoles) (**1**) by condensing the corresponding halogenomethyl ketones with dithiobiurea in boiling ethanol (Table I). The bischloromethyl derivative (**1g**) was obtained by carrying out the condensation with 1,3-dichloropropanone in methanol at room temperature in order to avoid the formation of polymers by reaction of the bifunctional halogeno ketone with the bifunctional urea. The condensation of chloroacetone with 3,4-dimethyldithiobiurea, prepared from *sym*-dimethylhydrazine dihydrochloride and ammonium thiocyanate, afforded 1,2-dimethyl-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**1o**). The introduction of functional groups at the 4 position of the thiazole nucleus presented many difficulties. All attempts to exchange the allylic chlorine atom of the relatively stable acetylated 4-chloromethyl derivative (**2g**) by a dialkylamino group or to quaternize it with a tertiary amine led solely to colored oxidation products. The same reactions have been performed successfully with simple 4-chloromethylthiazoles.[23] The desired 2,2′-hydrazobis(4-diethylaminomethylthiazole) (**1l**) was prepared by the condensation of dithiobiurea with 1-bromo-3-diethylaminopropanone hydrobromide. Similarly, 2,2′-hydrazobis[4-(*m*-sulfamoylphenyl)thiazole] (**1n**) was prepared from α-bromo-*m*-sulfamoylacetophenone. The hydrazobisthiazoles in the form of the hydrohalides or the free bases were treated with acetic anhydride under reflux to yield the corresponding diacetyl derivatives (**2**) (Table II). In the case of the *m*-sulfamoylphenyl compound (**1n**), the sulfonamido nitrogen atoms were also attacked and the tetraacetyl derivative (**2n**) was obtained.



In order to study the influence of the different acyl groups attached to the hydrazine moiety, 2,2′-hydrazobis(4-methylthiazole), which could be isolated as the free base, was acylated with butyric, octanoic, and lauric anhydrides to give **2h**, **2i**, and **2j**, respectively. The reaction with glutaric anhydride afforded the diacid **2m**. Attempts to prepare the bis(dichloroacetyl) derivative by the same method, treatment of the hydrochloride or the free hydrazobisthiazole with dichloracetic anhydride, yielded a product with the correct elemental analysis. The failure of this compound to undergo nitration led us to reconsider its structure. The absence of an aromatic hydrogen signal in the nmr spectrum indicated the possibility that a molecular rearrangement had taken place. Beyer and Kreutzberger observed the benzidine rearrangement of 2,2′-hydrozobisthiazoles when they treated them with phthalic anhydride at elevated temperature.[24] In our case, the use of dichloroacetic anhydride [the acid being even stronger (pK_a =

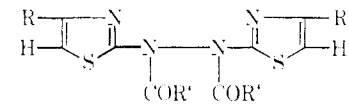
(22) H. Beyer, *Chem. Ber.*, **82**, 143 (1949).

(23) (a) J. M. Sprague, A. H. Land, and C. Ziegler, *J. Am. Chem. Soc.*, **68**, 2155 (1946); (b) A. Silberg, Z. Frenkel, and L. Cormos, *Chem. Ber.*, **96**, 2992 (1963).

(24) H. Beyer and A. Kreutzberger, *ibid.*, **84**, 518 (1951).

TABLE II

1,2-DIACYL-1,2-BIS(2-THIAZOLYL)HYDRAZINES

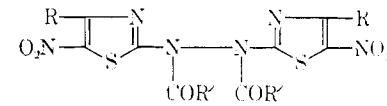


Compd 2	R	R'	Yield,[a] %	Mp, °C	Cryst solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
a	H	CH_3[b]										
b	CH_3	CH_3[b]										
c	C_2H_5	CH_3	80	128-129	EtOH	$C_{14}H_{18}N_4O_2S_2$	49.70	5.36	16.56	49.72	5.34	16.76
d	n-C_4H_9	CH_3	53	106-108	EtOH	$C_{18}H_{26}N_4O_2S_2$	54.81	6.64	14.21	55.06	6.65	14.49
e	t-C_4H_9	CH_3[c]	31	123	EtOH	$C_{18}H_{26}N_4O_2S_2$	54.81	6.64		55.02	6.73	
f	C_6H_5	CH_3	87	209 dec	AcOH	$C_{22}H_{18}N_4O_2S_2$	60.82	4.18	12.90	60.58	4.49	13.06
g	$ClCH_2$	CH_3	17	157-159	Cyclohexane	$C_{12}H_{12}Cl_2N_4O_2S_2$	38.00	3.19	14.77	38.47	3.29	14.91
h	CH_3	n-C_3H_7	83	168	n-PrOH	$C_{16}H_{22}N_4O_2S_2$	52.43	6.05		52.50	6.22	
i	CH_3	n-C_7H_{15}	78	149-150	i-PrOH	$C_{24}H_{38}N_4O_2S_2$	60.21	8.00		60.43	7.98	
j	CH_3	n-$C_{11}H_{23}$	79	121	EtOH	$C_{32}H_{54}N_4O_2S_2$	65.04	9.21	9.48	65.23	9.18	9.47
k	CH_3	Cl_2CH	53	133-134	i-PrOH	$C_{12}H_{14}Cl_4N_4O_2S_2$[d]	32.16	2.25		32.64	2.17	
l	Et_2NCH_2	CH_3	77[e]	73	Petr ether	$C_{20}H_{32}N_6O_2S_2$	53.08	7.13	18.56	53.19	7.04	18.57
m	CH_3	$H_2OCC_2H_4$	61	195-196	EtOH	$C_{18}H_{22}N_4O_6S_2$[f]	47.55	4.88	12.32	47.63	4.91	12.21
n	m-CH_3CONH-$C_6H_4SO_2$-	CH_3	51	180-183	MeOH	$C_{26}H_{24}N_6O_8S_4$	46.14	3.57	12.42	46.11	3.52	12.69

[a] Yields are based on the crude bases or dihydrohalides used in the preparations. [b] Reference 22. [c] The Ac_2O solution was decomposed and diluted only with an equal volume of water. [d] *Anal.* Calcd: Cl, 31.64. Found: Cl, 31.61. [e] Obtained as dihydrobromide. [f] *Anal.* Calcd: mol wt, 454. Found: acid equiv, 220.

TABLE III

1,2-DIACYL-1,2-BIS(5-NITRO-2-THIAZOLYL)HYDRAZINES



Compd 3	R	R'	Reaction Temp, °C	Time, hr	Yield, %	Mp, °C	Cryst solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
a	H	CH_3	26	2	70	226 dec	n-PrOH	$C_{10}H_8N_6O_6S_2$	32.27	2.17	22.58	32.28	2.50	22.31
b	CH_3	CH_3	0	1	54	241 dec	AcOH	$C_{12}H_{12}N_6O_6S_2$	36.07	3.02	21.00	35.84	3.29	21.45
c	C_2H_5	CH_3	0	1	40	215 dec	AcOH	$C_{14}H_{16}N_6O_6S_2$	39.26	3.77	19.83	39.50	4.13	18.97
d	n-C_4H_9	CH_3	0	1	73	179-180	AcOH	$C_{18}H_{24}N_6O_6S_2$	44.61	4.99		44.93	5.34	
e	t-C_4H_9	CH_3	22	1	39	247 dec	n-PrOH	$C_{18}H_{24}N_6O_6S_2$	44.61	4.99	17.35	44.80	5.06	17.41
f	C_6H_5	CH_3	0	1	47[a]	216-218	Benzene or AcOH	$C_{22}H_{16}N_6O_6S_2$	50.39	3.08	16.03	50.94	2.99	16.29
g	$ClCH_2$	CH_3	22	2	61	200 dec	Toluene or AcOH	$C_{12}H_{10}Cl_2N_6O_6S_2$	30.71	2.15	17.91	31.05	2.07	17.23
h	CH_3	n-C_3H_7	22	2	76	201-203	n-PrOH	$C_{16}H_{20}N_6O_6S_2$	42.09	4.42		42.40	4.53	
i	CH_3	n-C_7H_{15}	50	0.5	87	125	i-PrOH	$C_{24}H_{36}N_6O_6S_2$	50.69	6.38	14.78	50.37	6.16	14.95
j	CH_3	n-$C_{11}H_{23}$	22	1	46	103	MeOH	$C_{32}H_{52}N_6O_6S_2$	56.44	7.70	12.34	56.53	7.68	12.48
k	CH_3	Cl_2CH	22	1	44	215 dec	AcOEt–petr ether	$C_{14}H_8Cl_4N_6O_6S_2$[b]	26.78	1.50	15.62	26.97	1.45	15.88

[a] The crude product was washed with hot 2-propanol before recrystallization. [b] *Anal.* Calcd: Cl, 26.35. Found: 26.52.

1,3) than phthalic acid (pK_a = 2.9)] had also led to the benzidine rearrangement. The resulting 2,2′-diamino-4,4′-dimethyl-5,5′-bithiazole was further acylated to the corresponding 2,2′-bis(dichloroacetamide) **4**. This structure was established by the nmr spectrum and by hydrolysis of **4** to the free diamine, described by Erlenmeyer and Menzi.[25] In order to obtain the required dichloroacetyl compound, we had to apply a different method, avoiding the use of the anhydride or the free acid. Ronwin acylated a number of amino acids by treatment with acyl chlorides in boiling ethyl acetate, the resulting mixed anhydride being the active moiety.[26] This method enabled us to prepare the desired 1,2-bis(dichloroacetyl)-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**2r**); no benzidine rearrangement occurred despite markedly acidic conditions.

The nitration of the acylated hydrazobisthiazoles to the 1,2-bis(5-nitro-2-thiazolyl)hydrazines (**3**) was performed by nitric acid in acetic anhydride (Table III).[27]

The nitrate of the diethylaminomethyl compound **2l** was not attacked by this nitration mixture, probably due to the interference of the basic side chain. Treatment of the nitrate with sulfuric acid at room temperature yielded a product which was identified as 2,2′-azobis(4-diethylaminomethylthiazole) (**5c**). Here the hydrolysis and the oxidation of the hydrazine moiety had taken place before the possible nitration of the thiazole nucleus. In the cases of 1,2-dimethyl-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**1o**), the glutaric acid derivative **2m**, and the sulfamoylphenyl compound **2n**, we were unable to isolate any identifiable products even when various nitration conditions were tried. Attempts to remove the acetyl groups in **3b** by means of concentrated hydrochloric acid at room temperature afforded a yellow substance which darkened in the absence of concentrated acid and was not further investigated.

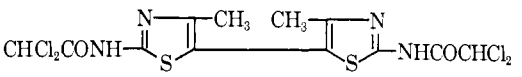
In order to ascertain the importance of the hydrazine bridge connecting the two nitrothiazole nuclei, several bis(5-nitro-2-thiazolyl) derivatives were prepared. Considering the instability of the hydrazobisthiazoles, we assumed an oxidative degradation product, an azobisthiazole, to be the active chemotherapeutic agent. Azobisthiazoles have been prepared by the oxidation

(25) H. Erlenmeyer and K. Menzi, *Helv. Chim. Acta*, **31**, 2065 (1948).

(26) E. Ronwin, *J. Org. Chem.*, **18**, 127 (1953).

(27) Structure **3f** is assigned to the nitration product of the 4-phenylthiazole derivative **2f**, in analogy with the structure of the nitration product of 2-acetamido-4-phenylthiazole [J. B. Dickey and E. B. Towne, U. S. Patent 2,659,719 (1953)].

of the hydrazines with nitrous or nitric acid.[22,28] We prepared 2,2′-azobis(5-nitrothiazole) (**5a**) and the 4-methyl derivative **5b** by oxidation of the corresponding diacetylhydrazines **3a** and **3b** with nitric acid. The hydrazine bridge was further replaced by the piperazine molecule. The desired 1,4-bis(5-nitro-2-thiazolyl)piperazine (**6**) was obtained from piperazine hydrate and 2-bromo-5-nitrothiazole. This method has been described[12b] for the preparation of 1-acyl-4-(5-nitro-2-thiazolyl)piperazines. Attempts to replace piperazine by ethylenediamine in this reaction or, conversely, to alkylate 2-aminothiazole with ethylene dibromide did not lead to any positive result.[29] 2,2′-Ethylenediiminobis(4-methylthiazole) (**7a**), prepared by the Hantzsch synthesis as described by Tanaka, *et al.*,[30] and its diacetyl derivative **7b** were nitrated to the corresponding 5-nitrothiazole derivatives **7c** and **7d**. 2,2′-Iminobis(4-methyl-5-nitrothiazole)[31] (**8**) was chosen as an example of a compound in which the hydrazine bridge has contracted into a secondary amine.



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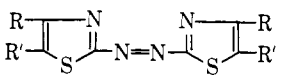
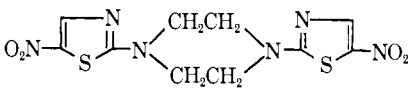


5a, R = H; R′ = NO_2
b, R = CH_3; R′ = NO_2
c, R = Et_2NCH_2; R′ = H



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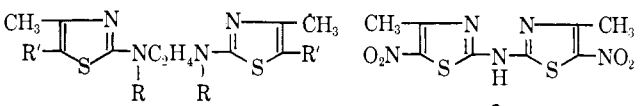

7a, R = R′ = H
b, R = CH_3CO; R′ = H
c, R = H; R′ = NO_2
d, R = CH_3CO; R′ = NO_2

8

Biological Results.—The nitroheterocyclic compounds described above were screened against *Trypanozoma cruzi* and *Leishmania brasiliensis in vitro* (Table IV). Two compounds, 1,2-diacetyl-1,2-bis(5-nitro-2-thiazolyl)hydrazine (**3a**) and the 4-methyl derivative (**3b**) showed very significant activity (MIC between 0.02 and 0.05 μg/ml) against *Tryp. cruzi*. Replacement of the acetyl groups in **3b** by butyl groups (**3h**) decreased the activity markedly, while the introduction of higher aliphatic acyl or dichloracetyl groups caused inactivation. The same results were obtained by replacement of the methyl groups in **3b** by phenyl or by various alkyl radicals. Replacement of the hydrazine bridge in the molecule by other symmetrical nitrogen-bearing radicals or by a secondary amine group also caused inactivation. No appreciable activity was shown by the parent compound **2b**, which did not possess the nitro groups. A number of compounds tested against *L. brasiliensis* showed comparable rssults. While **3a** and **3b** were the most active ones and **3h** occupied an intermediate position, all other compounds were only slightly active (MIC 10 μg/ml).

TABLE IV

In Vitro ACTIVITY OF NITROHETEROCYLIC COMPOUNDS

Compd	MIC, μg/ml[a] against *Tryp. cruzi*	MIC, μg/ml[a] against *L. brasiliensis*
3a	0.02	0.1
3b	0.05	0.1
3c	50	5
3d	50	10
3e	100	10
3f	50	
3g	>2[b]	
3h	10	1
3i	100	10
3j	100	10
3k[c]	>100	>100
5a	>20	
5b	20	
6	>10	
7c	>20	
7d[d]	>20	
2b	>20	
8	100	10

[a] See Biological Methods (p 1143) for a definition of MIC. [b] Maximum concentration achieved because of low solubility of the compound. [c] In olive oil solution. [d] In DMSO solution.

TABLE V

In Vitro ANTIPROTOZAL ACTIVITY OF **3a** AND **3b**

Strain	MIC, μg/ml[a] of **3a**	MIC, μg/ml[a] of **3b**
Tryp. cruzi	0.02	0.05
L. brasiliensis	0.1	0.1
L. arvicanthis	0.1	0.05
T. vaginalis[b]	0.1	0.1
E. histolytica[c]	0.1	

[a] See Biological Methods (p 1143) for a definition of MIC. [b] Metronidazole, used as reference compound, had a MIC of 0.5 μg/ml. [c] Emetine hydrochloride, used as reference compound, had a MIC of 5 μg/ml.

The high activity of **3a** and **3b** was also demonstrated when tested against *Leishmania arvicanthis*, *Trichomonas vaginalis*, and *Entamoeba histolytica* (Table V), as compared to the trichomonacide, metronidazole, and especially to the amebacide, emetine.

In vivo experiments met at first with difficulties owing to the fact that the compounds to be tested were soluble only in dimethylacetamide or in dimethyl sulfoxide. A high concentration of these solvents was found to be toxic to mice by all routes of administration. Therefore an attempt was made to administer the drugs orally in the form of a suspension in mucilage of tragacanth.

Experiments on *Tryp. cruzi* were performed on laboratory mice (approximate weight 20 g) infected intraperitoneally. Medication was started 5 days after inoculation. Out of 15 mice, five received **3a**, five received **3b**, and five served as controls. Medication was given by the intramuscular route, 20 mg/kg of the drug in dimethylacetamide solution being administered twice daily for 4 days. Oral medication was given to a similar group of mice, 100 mg/kg of the drug being given twice daily by gavage in form of a suspension (20 mg/ml) in 1% mucilage of tragacanth,

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(29) This is in accordance with similar negative results described by I. A. Kaye and C. L. Parris, *J. Am. Chem. Soc.*, **74**, 2921 (1952).
(30) H. Tanaka, M. Yano, and M. Machida, *Chem. Pharm. Bull.* (Tokyo), **7** 252 (1959).
(31) H. Beyer and G. Berg, *Chem. Ber.*, **89**, 1602 (1956).

for 12 days. In both methods of treatment no differences were observed between treated and control mice with respect to number of parasites in the blood or to length of survival of the animals. The drugs proved to be nontoxic since control mice, given the same oral dosage, remained unaffected.

The oral administration of **3a** or **3b**, 200 mg/kg/day for 14 days, on mice showed no effect whatever on the *Trichomonas muris* population. The administration of metronidazole, on the other hand, showed a complete disappearance of the flagellates.

Experiments were performed on hamsters with four different strains of *E. histolytica*, inoculated intraperitoneally. These strains frequently produce liver abcess. The administration of **3a** or **3b** either intraperitoneally or orally (six daily doses of 100 mg/kg, starting 2 days before inoculation) gave the same results. An inconstant number of treated hamsters and control hamsters developed liver abcess. The administration of emetine, on the other hand, prevented, in the majority of cases, the formation of liver abscess.

These biological findings demostrate the vast difference between *in vitro* and *in vivo* effects of the same drugs. This difference may be attributed to the insolubility of the compounds.

Experimental Section[32]

Dithiobiurea, 1,2-diacetyl-1,2-bis(2-thiazolyl)hydrazine (**2a**), 2,2′-hydrazobis(4-methylthiazole) (**1b**), its diacetyl derivative **2b**, and 2,2′-hydrazobis(4-phenylthiazole) (**1f**) were prepared as described by Beyer.[22] 2,2′-Iminobis(4-methyl-5-nitrothiazole) has been described by Beyer and Berg.[31]

2,2′-Hydrazobis(4-alkylthiazoles) (**1c–e**) were obtained from dithiobiurea and the corresponding halomethyl ketones. 1-Chloro-2-butanone[33] and 1-chloro-2-hexanone[34] were prepared from diazomethane and propionyl chloride or valeroyl chloride, respectively. 1-Bromo-3,3-dimethyl-2-butanone was obtained by the bromination of pinacolone.[35]

The dihydrohalides were prepared as follows. A mixture of 0.1 mole of dithiobiurea and 0.2 mole of the corresponding halomethyl ketone in 200 ml of EtOH was refluxed with stirring for 0.5 hr. The cooled thick paste was filtered, washed with EtOH, and dried to yield the crude dihydrohalide, which was acetylated without further purification. The free bases (**1c–e**) were precipitated from the aqueous solutions of the hydrohalides by NaOAc solution. They are unstable and turn colored on standing. Their physical data and analyses together with the data of the following hydrazothiazoles are given in Table I.

2,2′-Hydrazobis(4-chloromethylthiazole) (**1g**).—A mixture of 10.5 g (0.07 mole) of dithiobiurea and 17.8 g (0.14 mole) of 1,3-dichloropropanone in 150 ml of MeOH was stirred for 24 hr at room temperature to give a clear solution. An excess of acetone precipitated part of the crude product and evaporation of the organic solvents afforded a second crop, to give together 24.0 g of crude **1g·2HCl**. The free base, liberated by NaOAc solution, turned immediately into a dark gummy substance.

2,2′-Hydrazobis(4-diethylaminomethylthiazole) (**1l**).—A mixture of 1.5 g (0.01 mole) of dithiobiurea and 5.8 g (0.02 mole) of 1-bromo-3-diethylamino-2-propanone hydrobromide[23a] (mp 121–123° from 2-PrOH) in 100 ml of MeOH was refluxed for 1 hr. The clear solution was evaporated to dryness; the residue was boiled with 25 ml of absolute EtOH, cooled, filtered, and washed with absolute EtOH to give 5.3 g (77%) of crude **1l·4HBr**, mp 201–205°. The free base, liberated by $NaHCO_3$ solution, turned immediately into a dark mass.

α-Bromo-*m*-sulfamoylacetophenone.[36]—To a suspension of 6.61 g (0.03 mole) of *m*-sulfamoylacetophenone[37] (mp 147–148°) in 70 ml of EtOAc was added with stirring during 20 min a solution of 4.8 g (0.03 mole) of Br_2 in 10 ml of EtOAc. The reaction was initiated by gentle heating. The clear solution was left for 1 hr and then evaporated to dryness. The oily residue was washed with hot $CHCl_3$ to obtain a solid product, which, upon recrystallization from 1-PrOH, gave 5.9 g (71%), mp 129–131°.

Anal. Calcd for $C_8H_8BrNO_3S$: Br, 28.73. Found: Br, 28.15.

2,2′-Hydrazobis[4-(*m*-sulfamoylphenyl)thiazole] (**1n**).—A mixture of 1.5 g (0.01 mole) of dithiobiurea and 5.6 g (0.02 mole) of α-bromo-*m*-sulfamoylacetophenone in 50 ml of EtOH was refluxed for 0.5 hr, cooled, filtered, and washed (EtOH, hot H_2O, Me_2CO) to give 4.7 g, soluble in DMF only.

3,4-Dimethyldithiobiurea.—A solution of 13.3 g (0.1 mole) of *sym*-dimethylhydrazine dihydrochloride and 15.0 g (0.2 mole) of ammonium thiocyanate in 50 ml of water was refluxed for 4 hr. The precipitate formed in the cold solution was filtered and recrystallized from H_2O to yield 3.60 g (19%), mp 234°.

Anal. Calcd for $C_4H_{10}N_4S_2$: S, 35.92. Found: S, 35.88.

1,2-Dimethyl-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**1o**).—3,4-Dimethyldithiobiurea (3.6 g, 20 mmoles) and chloroacetone (4.0 g, 44 mmoles) in H_2O (15 ml) were refluxed for 1 hr. The free hydrazobisthiazole was precipitated by NaOAc solution.

1,2-Diacetyl-1,2-bis(2-thiazolyl)hydrazines (**2c–g**) were prepared by refluxing the corresponding crude hydrazobisthiazoles dihydrohalides or the free bases with ten parts of Ac_2O for 1 hr. Addition of H_2O decomposed the excess anhydride and precipitated the diacetyl compounds. Their physical properties and analyses are given in Table II.

1,2-Dibutyryl-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**2h**).—A mixture of **1b·2HCl** in ten parts of butyric anhydride was heated with stirring for 1.5 hr at 100°. Addition of H_2O decomposed the excess anhydride and precipitated an oil. The acid so obtained was neutralized with $NaHCO_3$ and the product was extracted with ether. The organic solution was washed with $NaHCO_3$ solution, dried, and evaporated to yield the required compound.

1,2-Dioctanoyl-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**2i**) **and 1,2-dilauroyl-1,2-bis(4-methyl-2-thiazolyl)hydrazine** (**2j**) were obtained by the same procedure, using **1b** base and octanoic anhydride[38] [bp 125° (0.2 mm)] or lauric anhydride,[39] respectively, benzene being the extracting solvent.

1,2-Bis(β-carboxypropionyl)-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**2m**).—A mixture of **1b** and eight parts of glutaric anhydride[40] was heated for 1 hr at 100° and then poured into water. The precipitate was filtered and washed with hot water by trituration.

The **acetylation of 1n** was performed by refluxing a mixture of equal parts of **1n** and of KOAc in ten parts of Ac_2O for 1.5 hr to give **2n**.

1,2-Bis(dichloroacetyl)-1,2-bis(4-methyl-2-thiazolyl)hydrazine (**2k**).—A suspension of **1b·2HCl** (0.60 g) in EtOAc (20 ml) and dichloroacetyl chloride (1.6 ml) was refluxed with stirring for 12 hr. The clear solution was washed with dilute HCl and $NaHCO_3$ solution and dried (Na_2SO_4). The residue, left after the evaporation of the solvent, was treated with several milliliters of boiling 2-PrOH, cooled, filtered, and washed with the same solvent to give 0.47 g, mp 131–132°. An analytical sample, recrystallized from 2-PrOH, melted at 133–134°; nmr (deuterated DMSO), singlets at τ 7.12 and 7.28 (1 H at aromatic C-5 and 1 H of $CHCl_2CO$), singlet at 2.15 (3 H of benzylic CH_3 at C-4).

Hydrolysis of **2k** was performed with 30 parts of concentrated HCl for 8 days at room temperature. Partial neutralization of the diluted solution with $NaHCO_3$ and then precipitation with NaOAc solution yielded 2,2′-hydrazobis(4-methylthiazole), mp 171°, mmp 172–174°.

(32) Melting points were determined in open capillary tubes and are corrected. Elemental analyses were performed by the Microanalytical Laboratory of the Weizmann Institute. Nmr spectra, recorded on a Varian A-60 spectrometer with tetramethylsilane as an internal standard, are reported in parts per million.

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(38) Prepared by the general procedure for acid anhydrides, described by C. F. H. Allen, C. J. Kibler, D. M. McLachlin, and C. V. Wilson, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p 28.

(39) J. M. Wallace, Jr., and J. E. Copenhaver, *J. Am. Chem. Soc.*, **63**, 199 (1941).

(40) H. D. Zook and J. A. Knight, *ibid.*, **76**, 2302 (1954).

1,2-Diacetyl-1,2-bis(4-diethylaminomethyl-2-thiazolyl)hydrazine (2l).—A mixture of the crude 1l·4HBr (4.0 g) and Ac_2O (20 ml) was refluxed with stirring for 0.5 hr. The precipitation was completed by addition of ether to the cold reaction mixture. The product was filtered, washed with ether, and recrystallized from 2-PrOH to yield 2.7 g (77%) of **2l·2HBr**, mp 240° dec.

Anal. Calcd for $C_{20}H_{34}Br_2N_6O_2S_2$: C, 39.09; H, 5.58; Br, 26.01. Found: C, 39.52; H, 5.46; Br, 26.42.

The base was liberated by $NaHCO_3$ solution. Its nmr spectrum (in $CDCl_3$) showed the following absorptions: τ 6.85 (1 H at C-5), 3.65 (2 H of the benzylic CH_2 at C-4), 2.1-2.5 quartet (CH_2 of Et_2N) overlapping 2.33 singlet (CH_3CO) (total 7 H), 0.9-1.1 triplet (CH_3 of Et_2N, 6 H).

The **dinitrate of 2l** was prepared by addition of concentrated HNO_3 to the solution of the base in AcOH and was precipitated with ether, mp 207-209° dec (from PrOH).

Anal. Calcd for $C_{20}H_{34}N_8O_8S_2$: N, 19.37; S, 11.08. Found: N, 19.41; S, 11.31.

1,2-Diacyl-1,2-bis(5-nitro-2-thiazolyl)hydrazines (3a-j) were prepared by the general procedure illustrated below. The reaction period and temperature in each case, as well as the physical data and analyses of the new compounds, are listed in Table III.

The 1,2-diacyl-1,2-bis(2-thiazolyl)hydrazine (**2**) (1.00 g) was added to a stirred solution of 98% HNO_3 (0.50 ml) in Ac_2O (3 ml) cooled to 0°. The reaction mixture was left for the specified period of time at the given temperature and then poured into ice. The product was filtered, washed (H_2O), and recrystallized.

1,2-Bis(dichloroacetyl)-1,2-bis(4-methyl-5-nitro-2-thiazolyl)hydrazine (3k).—Compound **2k** (300 mg) was added at 0° to a solution of 98% HNO_3 (0.30 ml) in Ac_2O (3 ml), and the mixture was stirred for 1 hr at room temperature. The precipitate was filtered, washed (AcOH), and dried *in vacuo* (NaOH) to give 205 mg, mp 207° dec. The product was dissolved in EtOAc, precipitated by excess petroleum ether (bp 60-90°), and left overnight at 0° to give 155 mg, mp 215° dec. This compound is very unstable, in the solid state or in solution of dimethylacetamide, DMF, or DMSO; it turns dark after a very short while. Only its solution in olive oil, prepared by gentle heating, stays colorless at room temperature for several days.

2,2′-Azobis(5-nitrothiazole) (5a).—A mixture of **3a** (300 mg) in 70% HNO_3 (3 ml) was heated in a boiling-water bath for several minutes, until the evolution of nitrous oxides had ceased, and then poured into ice. The red precipitate was filtered, washed (H_2O), dried, and recrystallized from EtOAc to yield 62 mg (27%), mp 207° dec.

Anal. Calcd for $C_6H_2N_6O_4S_2$: C, 25.18; H, 0.70; N, 29.36. Found: C, 25.44; H, 1.00; N, 29.14.

2,2′-Azobis(4-methyl-5-nitrothiazole) (5b) was prepared from **3b** by the above procedure in 41% yield, mp 220° dec (from AcOH).

Anal. Calcd for $C_8H_6N_6O_4S_2$: C, 30.58; H, 1.93; N, 26.75. Found: C, 31.03; H, 1.85; N, 27.48.

1,4-Bis(5-nitro-2-thiazoyl)piperazine (6).—A mixture of 1.04 g (5 mmoles) of 2-bromo-5-nitrothiazole,[41] 0.49 g (2.5 mmoles) of piperazine hydrate, and 0.46 g (5.5 mmoles) of $NaHCO_3$ in 10 ml of EtOH was refluxed with stirring on a water bath for 0.5 hr. The heavy, yellow precipitate was filtered, washed (H_2O, MeOH), dried, and recrystallized from DMF to give 0.45 g (53%), mp 286° dec.

Anal. Calcd for $C_{10}H_{10}N_6O_4S_2$: C, 35.09; H, 2.94; N, 24.56; S, 18.70. Found: C, 35.39; H, 3.27; N, 24.25; S, 18.35.

2,2′-Ethylenediiminobis(4-methylthiazole) (7a).—Ethylenebis(thiourea) was prepared from silicon isothiocyanate and ethylenediamine, according to the procedure of Neville and McGee,[42] mp 148-150° (from 2-PrOH, lit.[42] 202°).

Anal. Calcd for $C_4H_{10}N_4S_2$: C, 26.96; H, 5.66; S, 35.92. Found: C, 27.21; H, 6.13; S, 35.87.

Compound **7a** was prepared from choloroacetone and ethylenebis(thiourea), as described by Tanaka, *et al.*,[30] mp 218-220° (lit.[30] 214°).

Anal. Calcd for $C_{10}H_{14}N_4S_2$: C, 47.21; H, 5.55; S, 25.21. Found: C, 47.24; H, 5.33; S, 24.92.

2,2′-Ethylenediiminobis(4-methyl-5-nitrothiazole) (7c).—To a stirred solution of **7a** (1.0 g) in concentrated H_2SO_4 (5 ml) at 0° was added 95% HNO_3 (0.35 ml). The mixture was stirred for 2 hr at 0°, then for 2 hr at room temperature, and finally poured into ice. The precipitate was filtered, washed (H_2O), and recrystallized from AcOH to yield 0.42 g (31%), mp 239°.

Anal. Calcd for $C_{10}H_{12}N_6O_4S_2$: C, 34.89; H, 3.51. Found: C, 34.88; H, 4.11.

N,N′-Diacetyl-2,2′-ethylenediiminobis(4-methylthiazole) (7b) was prepared in 64% yield by refluxing **7a** with seven parts of Ac_2O for 2 hr, mp 284-286°. An analytical sample, from DMF, melted at 285-286°.

Anal. Calcd for $C_{14}H_{18}N_4O_2S_2$: C, 49.70; H, 5.36. Found: C, 50.17; H, 5.72.

N,N′-Diacetyl-2,2′-ethylenediiminobis(4-methyl-5-nitrothiazole) (7d).—To a stirred solution of **7b** (1.0 g) in concentrated H_2SO_4 (5 ml) at 0° was added 70% HNO_3 (0.5 ml). The mixture was stirred for 2 hr at 0° and then poured into ice. The precipitate was filtered, washed with water, and recrystallized from DMF to yield 1.1 g (87%), mp >300°.

Anal. Calcd for $C_{14}H_{16}N_6O_6S_2$: C, 39.26; H, 3.77; N, 19.62. Found: C, 39.55; H, 4.05; N, 19.16.

2,2′-Azobis(4-diethylaminomethylthiazole) (5c).—A solution of **2l·2HNO₃** (0.30 g) in H_2SO_4 (3 ml) was left for 2 hr at room temperature, then poured into ice, treated with excess Na_2CO_3, and finally extracted with $CHCl_3$. The residue, obtained by evaporation of the dried organic solvent, was dissolved in AcOH (2 ml), treated with 70% HNO_3 (0.1 ml), and precipitated with ether, to give after recrystallization from EtOH, 0.11 g (43%) of **5c·2HNO₃**, mp 190° dec.

Anal. Calcd for $C_{16}H_{28}N_8O_6S_2$: C, 39.02; H, 5.73; N, 22.76. Found: C, 39.81; H, 5.86; N, 22.28.

The free base **5c**, liberated by $NaHCO_3$ solution and recrystallized from cyclohexane, melted at 103-104°; nmr ($CDCl_3$), τ 7.4 (1 H at C-5), 3.85 (2 H of the benzylic CH_2 at C-4), 2.45-2.8 quartet (CH_2 of Et_2N, 4 H), 0.95-1.2 triplet (CH_3 of Et_2 of Et_2H, 6 H).

Anal. Calcd for $C_{16}H_{26}N_6S_2$: C, 52.44; H, 7.15; N, 22.94. Found: C, 53.12; H, 7.70; N, 22.60.

2,2′-Bis(dichloroacetamido)-4,4′-dimethyl-5,5′-bithiazole (4).—A suspension of **1b·2HCl** (0.54 g) in dichloroacetic anhydride[43] (8 ml) was heated for 3 hr at 130° and then poured into water. The oily residue was washed by decantation several times with H_2O and then recrystallized twice from MeOH to give 0.27 g (33%), mp 250-251°; nmr (in *d*-DMSO), singlets at τ 6.68 (1 H of $CHCl_2CO$) and 2.23 (3 H of benzylic CH_3 at C-4).

Anal. Calcd $C_{12}H_{10}Cl_4N_4O_2S_2$: C, 32.16; H, 2.25; Cl, 31.64; N, 12.50. Found: C, 32.58; H, 2.35; Cl, 31.75; N, 12.62.

Hydrolysis of **4** with 30 parts of concentrated HCl for 8 days at room temperature afforded 2,2′-diamino-4,4′-dimethyl-5,5′-bithiazole, mp 276° (lit.[25] 275-276°).

Biological Methods.—Strains of *T. cruzi* Sonia, *T. cruzi* 125, *L. brasiliensis* muco-cutaneous 6, and *L. arricanthis* from Sudan were used. The strains were cultivated on Locke agar to which 10% defibrinated rabbit blood was added, sterile conditions being stringently observed throughout. The compounds tested were dissolved in dimethylacetamide (1 mg/ml). Further dilutions were made with saline.

Screening was carried out in Kahn tubes. Solutions of the test material were added to the culture medium to make a total volume of 2 ml and concentrations of the drug ranging from 10 to 0.02 μg/ml. A culture of the protozoa (0.1 ml) containing 800,000 to 2 million flagellates) was added to each tube, as well as to control tubes, and incubated at 28°. In some cases a parallel batch of tubes was incubated at 37° for 48 hr and then transferred to 28°.

Compound activity was described as the minimum inhibitory concentration (MIC), micrograms per milliliter, that completely inhibited growth of the organism after 4 days of incubation.

Acknowledgment.—The authors are indebted to Miss Blanca Montilio for her help in the *in vivo* experiments.

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