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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF IMINOSULFURANE ANALOGS OF PHENOTHIAZINE DERIVATIVES

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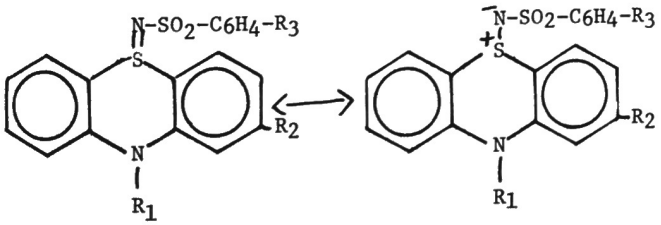
ABSTRACT

A new series of iminosulfurane analogs of phenothiazine derivatives has been prepared. These compounds were tested against *Sarcina lutea* and *Staphlococcus epidermis* in the presence of antibiotic media I and IV. Also studied was the toxicity in mice.

INTRODUCTION

The amino acids L-methionine and L-cysteine, thioxanthone, diphenylene methanesulfide and phenarsazine chloride were made to react with chloramines to obtain the corresponding iminosulfuranes (Shah, 1975). We have found phenothiazine and its derivatives to react with Chloramine-B, Chloramine-T and a new reagent, N-chloro-N-sodio-p-ethyl benzene sulfonamide, to obtain the corresponding phenothiazine iminosulfuranes (Shah, 1972; Claypool and Shah, 1971). We have examined infrared and ultraviolet spectra of various iminosulfuranes and P, As and Sb analogs.

The structures of these compounds are as follows:



R_1 & R_2= Depend upon the phenothiazine derivatives

R_3= -H, $-CH_3$ or $-C_2H_5$

These compounds were tested against *Sarcina lutea* and *Staphylococcus epidermis* in the presence of Bacto-Antibiotic Medium I (Code 0263, Bacto-Penassay seed agar) and Bacto-Antibiotic Medium IV (Code 0244, Bacto yeast beef agar), pH 6 phosphate buffer with 16-20 hours incubates at 30-31°C. This method was first described by Abraham (1941) for the assay of penicillin. It was later modified by Foster and Woodruff (1943) and by Schmidt and Moyer (1944). These compounds were shown to be inactive against *Plasmodium berghei* in birds. The acute oral LD_{50} of compounds 2, 5, 6, 7 and 9 for male albino mice is greater than or in the range of 5.0 gm/kg of body weight.

EXPERIMENTAL

Melting points (capillary tube) are uncorrected. Infrared spectra and ultraviolet spectra were recorded on a Beckman Infrared Spectrophotometer 620 and Beckman Spectrophotometer Acta CIII. Elemental analyses were determined at M-H-W Laboratories, Garden City, Michigan 48135.

GENERAL METHOD FOR PREPARATION OF IMINOSULFURANES [1]

The mixed solution of 0.02 mole of sulfide (phenothiazine and its derivatives) and of 0.03 mole of chloramine (in 50 ml of 50% ethyl alcohol-water solution) was heated in a water-bath for 30 minutes. It was then covered and allowed to stand overnight at room temperature. The product formed on standing was filtered, washed thoroughly with water, dried and recrystallized from ethyl alcohol or methyl alcohol.

(2) *Phenothiazine-Benzene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 168°C; ir($CHCl_3$): 923 (S=N), 1132 (Sym SO_2), 1035, 1200 (Asym SO_2) cm^{-1}; UV (CH_3OH): 244, 302.5 nm (λ max).

Anal. calcd for $C_{18}H_{14}N_2S_2O_2$: C, 61.01; H,3.95; N, 7.90; S, 18.07

Found: C, 60.90; H,4.02; N,7.81; S, 18.15

(3) *Promethazine-Benzene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 78-79°C; ir($CHCl_3$): 926 (S=N), 1138 (Sym SO_2), 1045 and 1199 (Asym SO_2) cm^{-1}. UV (CH_3OH): 221 nm (λ max).

Anal. calcd for $C_{23}H_{25}N_3S_2O_2$:*

(4) *Mepazine-Benzene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 73-74°C; ir($CHCl_3$): 928 (S=N), 1137 (Sym SO_2), 1042, 1200 (Asym SO_2) cm^{-1}. UV (CH_3OH): 221.5 nm (λ max).

Anal. calcd for $C_{25}H_{27}N_3S_2O_2$:*

(5) *Prochlorperazine-Benzene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. —°C; ir

[1] M. A. McCall, D. S. Tarbey and M. A. Havill, 1951.

($CHCl_3$), not enough sample. UV (CH_3OH): 255, 267.5, 272.5 nm (λ max).

Anal. calcd for $C_{26}H_{26}N_4S_2O_2$:*

(6) *Phenothiazine-p-Toluene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 122°C; ir ($CHCl_3$): 923 (S=N), 1132 (Sym SO_2), 1035 and 1200 (Asym SO_2) cm^{-1}. UV (CH_3OH): 254, 306 nm (λ max).

Anal. calcd for $C_{19}H_{16}N_2S_2O_2$: C, 61.95; H, 4.34; N, 7.60; S, 17.39

Found: C, 61.86; H, 4.30; N, 7.51; S, 17.48

(7) *Promethazine-p-Toluene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. —°C; ir ($CHCl_3$): 923 (S=N), 1136 (Sym SO_2), 1040, 1196 (Asym SO_2) cm^{-1}. UV (CH_3OH): 222 nm (λ max).

Anal. calcd for $C_{24}H_{27}N_3S_2O_2$:*

(8) *Mepazine-p-Toluene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 120°C; ir ($CHCl_3$): 923 (S=N), 1135 (Sym SO_2), 1035, 1205 (Asym SO_2) cm^{-1}. UV (CH_3OH): 221 nm (λ max).

Anal. calcd for $C_{26}H_{29}N_3S_2O_2$:*

(9) *Phenothiazine-p-Ethyl Benzene-Sulfonylimine:*

The recrystallized product was obtained by the general procedure outlined above: m.p. 120°C; ir($CHCl_3$): 925 (S=N), 1135 (Sym SO_2), 1035, 1205 (Asym SO_2) cm^{-1}. UV (CH_3OH): 256.5, 306 nm (λ max).

Anal. calcd for $C_{20}H_{18}N_2S_2O_2$: C, 62.82; H, 4.71; N, 7.33; S, 16.75

Found: C, 62.71; H, 4.63; N, 7.24; S, 16.84

*(Not enough sample for analyses)

Microbiological Test [2]

Weigh approximately 10 mg ($\pm$0.1 mg) of each compound in separate 100 ml volumetric flask and dissolve in 10 ml HCl and enough pH 6 phosphate buffer to give an exact concentration of 100 μg/ml (solution a).

Dilute appropriate aliquots of stock solution (a) with enough pH 6 buffer to obtain concentrations of 5, 4, 3, 2 and 1 μg/ml. Add 10 ml melted Bacto-Antibiotic Medium I to sterile petri dishes, distribute evenly and let harden on level surface. For actual assay approximate amounts of organism—suspensions are added to (1 ml) Bacto-Antibiotic Medium IV previously melted and cooled to 48°C. Mix thoroughly and add 4.0 ml to each plate containing base layer of Bacto-Antibiotic Medium I. Distribute media evenly by tilting plates from side to side with circular motion and let harden.

Place three cylinders on each plate at approximately 60° intervals on a 2.8 cm radius. Fill all 3 cylinders with the test solution. Incubate plates overnight at 30-31°C and measure the diameters of zones of inhibition by means of mm ruler. Three plates were used for each assay solution and three plates for the standard. Plotting values of x^2 (square of the zone site) against 1n m_o (logarithm of the concentration in the reservoir) gives a straight line intercepting the concentration axis at 1n m' (critical concentration).

Toxicity Tests (Mice) [3]

Each test sample was administered orally by stomach tube to a group of 7, 8, 9 or 10 male albino mice (Laboratory Supply Company, weight range 15.0 to 28.4 grams). Each sample was administered as a 25 or 50% weight per volume suspension in distilled water at a dosage level of 5.0 grams per kilogram of body weight.

Food was withheld from the mice for approximately 18 hours prior to dosage. Following dosage, food consisting of commercial pellets and water was available ad libitum. The mice were housed in groups in plastic cages with metal lids. All animals were observed closely for gross signs of systemic toxicity and mortality at frequent intervals during the day of dosage, and at least once daily thereafter for a total of 14 days. At the end of the 14 day observation period the surviving mice were weighed, sacrificed by cervical dislocation and gross necropsies performed.

Biological Activity

The compounds were tested for their antimalarial activity against *Plasmodium berghei* in birds according to a procedure already published (Osdene, 1967). Compounds 2, 6 and 9 are inactive against *Plasmodium berghei* in birds. The test results are given in Table III.

Results and Discussion

Analytical and spectra data (infrared, ultraviolet) support the structure of these compounds. Penicillin G Potassium readily inhibits *Sarcina lutea* at a very low concentration (0.025 μg/ml), but at a high concentration (0.1 μg/ml) inhibits *Staphlococcus epidermis.* These compounds inhibit *Sarcina lutea* at a low concentration (1 μg/ml), but at a high concentration (3 μg/ml) inhibit *Staphylococcus pedermis.*

These compounds are 40 times less active than Penicillin G Potassium against *Sarcina lutea* and 30 times less active than Penicillin G Potassium against *Staphylococcus epidermis.* Tables I and II show the iminosulfurane analogs of phenothiazine derivatives and their antibacterial and toxic properties.

Acknowledgment

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[2] W. Horwitz, 1970, p. 761.

[3] Hill-Top Testing Laboratories, Miamiville, Ohio 45147.

TABLE 1: *Microbiological Data of Iminosulfurane Analogs of Phenothiazine.*

Number	Concentration m_o(μg/ml)	*Sarcina lutea* Diameter of Zones x (mm)	Critical Concentration m' (μg/ml)	Concentration m_o(μg/ml)	*Staphy. epidermis* Diameter of Zones x (mm)	Critical Concentration m' (μg/ml)
1	3	28.6	0.50	5	29.2	1.92
	2	20.5		4	25.6	
	1	12.2		3	23.3	
2	3	29.0	0.53	5	28.4	1.38
	2	21.0		4	27.2	
	1	12.0		3	23.7	
3	3	29.3	0.35	5	30.4	1.1
	2	24.8		4	27.1	
	1	17.06		3	25.0	
4	3	27.9	0.69	5	29.9	1.83
	2	23.4		4	25.5	
	1	14.93		3	23.9	
5	3	28.6	0.55	5	28.9	1.68
	2	22.6		4	26.9	
	1	15.9		3	23.6	
6	3	26.7	0.60	5	30.7	1.7
	2	19.0		4	26.9	
	1	11.00		3	24.1	
7	3	27.9	0.65	5	30.5	1.75
	2	25.1		4	27.7	
	1	15.2		3	24.0	
8	3	28.6	0.46	5	30.2	1.4
	2	23.7		4	27.4	
	1	16.3		3	24.5	
9	3	27.7	0.67	5	28.3	2.15
	2	19.30		4	26.6	
	1	11.00		3	22.5	

TABLE 2: *Antimalarial Activity of Iminosulfurane Analogs of Phenothiazine*

COMPOUND	ANIMAL	DOSE MG/KG	CURES	MSTT*	MSTC*	T-C*	TOX.	MSTX
2	Bird	30	—	8.2	8.5	−0.3	—	—
		120	—	8.2	8.5	−0.3	—	—
		480	—	8.2	8.5	−0.3	—	—
6	Bird	30	—	7.6	7.7	−0.1	—	—
		120	—	7.6	7.7	−0.1	—	—
		480	—	7.8	7.7	0.1	—	—
9	Bird	30	—	7.4	7.7	−0.3	—	—
		120	—	7.6	7.7	−0.1	—	—
		480	—	7.6	7.7	−0.1	—	—

MSTT* — Means Survival Time of Treated Animals
MSTC* — Means Survival Time of Controls
T-C* — Changes in Survival Time (MSTT-MSTC)
Tox. — Toxic Deaths. Those deaths occurring on days 2, 3, 4 and 5 after injection. Control animals do not die before day 6.

TABLE 3: *Acute Oral Toxicity Test of Iminosulfurane Analogs of Phenothiazine Derivatives.*

Sample No.	Result	Classification
2	LD_{50} in the range of or slightly greater than 5.0 gm/kg of body weight for male albino mice	Non-Toxic
5	LD_{50} in the range of or slightly greater than 5.0 gm/kg of body weight for male albino mice	Non-Toxic
6	LD_{50} in the range of or slightly greater than 5.0 gm/kg of body weight for male albino mice	Non-Toxic
7	LD_{50} in the range of or slightly greater than 5.0 gm/kg of body weight for male albino mice	Non-Toxic
9	LD_{50} in the range of or slightly greater than 5.0 gm/kg of body weight for male albino mice	Non-Toxic

ACKNOWLEDGMENT

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