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Chemotherapy of Experimental Tuberculosis with Benzothiazole Derivatives.*

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In the following experiments a new class of tuberculostatic agents is described. A systematic evaluation of the bacteriostatic action of various benzothiazole derivatives on the growth of tubercle bacilli *in vitro* was attempted. Many of the compounds were also investigated *in vivo* for their therapeutic action on tuberculous animals. A few benzothiazole derivatives have previously been evaluated by Everitt and Sullivan[1] for their fungicidal action and as antivirus agents by Kramer and associates.[2] The authors[3] have reported the effect of several benzothiazole sulfones in experimental tuberculosis.

Methods. The bacteriostatic studies were initially performed on the avirulent strain of human tubercle bacilli, No. 607 of the American Type Culture Collection, using Lowenstein's liquid asparagin media, at a pH of 7.5. Many of the compounds were also tested on the virulent human strain, H37Rv, in Proskauer and Beck media. The chemicals were dissolved in propylene glycol to make 0.5% solutions. Subsequent dilutions were made directly into the aqueous media, without further heating. Each tube was inoculated with one loopful of fresh culture of tubercle bacilli. The avirulent cultures were read at the end of 3 days; the virulent cultures at 18 days. The highest bacteriostatic dilution was taken as that dilution in which there was less than 50% of control growth.

Animal Experiments. Nine to 12 guinea pigs, averaging 400 g in weight, were used to evaluate each compound. The animals were inoculated in the left groin with 0.03 mg of H37Rv tubercle bacilli. The drugs were administered orally, suspended in water, by dropper once daily. Therapy was started on the day of infection. The dosage of the drugs was increased gradually for the first 2 weeks of the experiment. The dosage of all drugs approached the chronic maximum tolerated dose. The experiments were terminated at the end of 6 weeks. Pathology was evaluated numerically, based on a maximum of 4 for each organ (glands, spleen, liver and lungs); with a maximum of 16 for all organs. Hemoglobin and weight determinations were made at the end of the experiments.

Results. The *in vitro* bacteriostatic results are recorded in Table I. The benzothiazole ethers (Nos. 12 to 19 incl.) were the most extensively investigated group. The compound 6 - amino - 2 - (n)butoxybenzothiazole (No. 16) was bacteriostatic in extremely high dilution (1:40,000,000) on H37Rv. This activity was a thousand times as great as its bacteriostatic effect on the avirulent strain 607. All compounds tested were more bacteriostatic against the virulent culture. Five other types of benzothiazole derivatives were tuberculostatic in low concentrations: 6,2-diaminobenzothiazole (No. 20); 6-amino-2-chlorobenzothiazole (No. 24); 6-aminobenzothiazole (No. 3); 6-amino-2-mercaptobenzothiazole (No. 6) and 6-amino-2-methylbenzothiazole (No. 8). In general, the compounds were bacteriostatic rather than bactericidal. Compounds Nos. 16, 20 and 24 were tested in the presence of 20% defibrinated horse blood. They retained most of

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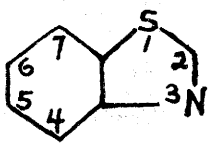
1 Everitt, E. L., and Sullivan, M. X., *J. Wash. Acad. of Sci.*, 1940, **30**, 125.

2 Kramer, S. D., Greer, H. A., and Szobel, D. A., *J. Immunol.*, 1944, **49**, 273.

3 Freedlander, B. L., and French, F. A., *Am. Rev. Tuberc.*, in press.

TABLE I.
Tuberculostatic Action on Strains 607 and H37Rv.

No.	Compound	Formula	Mg % 607	Mg % H37Rv
1	Benzothiazole		10.	5.
2	2-Aminobenzothiazole	$C_7H_4NSNH_2$	>10.	
3	6- "	$C_7H_4NSNH_2$	0.25	0.1
4	6-Nitrobenzothiazole	$C_7H_4NSNO_2$	10.	
5	2-Mercaptobenzothiazole	C_7H_4NSSH	5.	
6	6-Amino,2-mercaptobenzothiazole	$H_2NC_7H_3NSSH$	5.	0.1
7	2-Methylbenzothiazole	$C_7H_4NSCH_3$	10.	
8	6-Amino,2-methylbenzothiazole	$H_2NC_7H_3NSCH_3$	0.1	0.01
9	6 Nitro,2- "	$O_2NC_7H_3NSCH_3$	10.	
10	2-Phenylbenzothiazole	$C_7H_4NSC_6H_5$	10.	
11	2-Chlorobenzothiazole	C_7H_4NSCl	5.	
12	6-Amino,2-ethoxybenzothiazole	$H_2NC_7H_3NSOC_2H_5$	10.	0.7
13	6-Nitro,2 "	$O_2NC_7H_3NSOC_2H_5$	>10.	
14	6-Amino,2-isopropoxybenzothiazole	$H_2NC_7H_3NSOCH(CH_3)_2$	2.5	0.35
15	6-Nitro,2- "	$O_2NC_7H_3NSOCH(CH_3)_2$	10.	
16	6-Amino,2-(n)butoxybenzothiazole	$H_2NC_7H_3NSOC_4H_9$	2.5	0.0025
17	6-Nitro,2- "	$O_2NC_7H_3NSOC_4H_9$	1.	
18	6-Amino,2-butoxyethoxybenzothiazole	$H_2NC_7H_3NSOC_2H_4OC_4H_9$	>10.	
19	6-Nitro,2- "	$O_2NC_7H_3NSOC_2H_4OC_4H_9$	5.	
20	6,2-Diaminobenzothiazole	$H_2NC_7H_3NSNH_2$	0.25	0.01
21	6-Nitro,2-aminobenzothiazole	$O_2NC_7H_3NSNH_2$	2.5	
22	6-Amino,2-hydroxybenzothiazole	$H_2NC_7H_3NSOH$	10.	
23	6-Nitro,2- "	$O_2NC_7H_3NSOH$	2.5	
24	6-Amino,2-chlorobenzothiazole	$H_2NC_7H_3NSCl$	0.025	0.0025
25	6-Nitro,2- "	$O_2NC_7H_3NSCl$	1.	
26	6'-Aminobenzothiazolyl,2'-(N)-morpholine	$H_2NC_7H_3NSNC_4H_8O$	>10.	
27	6'-Nitrobenzothiazolyl,2'-(N)-morpholine	$O_2NC_7H_3NSNC_4H_8O$	>10.	
30	5-Amino-2-isopropoxypyridine	$H_2NC_5H_3NOCH(CH_3)_2$	1.	0.1

The above compounds were prepared in this laboratory under the direction of Dr. F. A. French with the following exceptions: Nos. 1, 2, 5, 6, 7, 10 and 11 were obtained from the Eastman Kodak Co. (highest purity).

Control experiments were performed with propylene glycol alone in 1:50 dilution. No bacteriostatic effect was noted.

their bacteriostatic activity. Compounds Nos. 16 and 20 were tested against the following organisms: *S. aureus, E. coli, Proteus vulgaris, Streptococcus pyogenes* and *Ps. pyocyaneus*. The results were essentially negative.

The *in vivo* results are summarized in Table II. 6-amino-2-(n)butoxybenzothiazole and 6,2-diaminobenzothiazole were highly effective. These compounds were approximately twice as active as 4,4'diaminodiphenylsulfone (No. 28) and about ¾ as effective as streptomycin (No. 29). 6-Amino-2-isopropoxy and 6-amino-2-ethoxybenzothiazole (Nos. 14 and 12) were also significantly active therapeutically. Animals treated with 6,2-diaminobenzothiazole showed no enlargement of the thyroid gland. This is of interest because this compound contains a thioureoid structure similar to that found in 2-aminothiazole which has been reported to inhibit thyroid activity.[4]

Discussion. 6-Aminobenzothiazoles with 5 types of substituents in the 2- position have been found to be highly active against tubercle bacilli. The 2- substituents included alkoxy, alkyl, amino, chloro and mercapto. The invariant characteristic of the group is the possession of an aromatic amino group

[4] Williams, R. H., Kay, G. A., and Solomon, B., *Am. J. Med. Sci.*, 1947, **213**, 198.

TABLE II.
Summary of *in vivo* Experimental Data.

No.		Avg daily dosage mg per animal	*Path. ratio, Controls / Treated	Relative wt gain, g deviation from controls	Hglb. % deviation from controls
12	6-Amino-2-ethoxybenzothiazole	50-150	2.40	—87.	+14.
13	6-Nitro-2- "	50-150	1.60	—72.	0.
14	6-Amino-2-isopropoxybenzothiazole	50-150	2.28	—58.	+14.
16	6-Amino-2-(n)butoxybenzothiazole	50-100	3.05	—67.	— 3.
18	6-Amino-2-butoxyethoxybenzothiazole	75-125	1.75	—80.	0.
19	6-Nitro-2- "	50-130	1.04	+25.	0.
20	6,2-Diaminobenzothiazole	50-150	3.03	—68.	0.
24	6-Amino-2-chlorobenzothiazole	50-100	1.59	—50.	— 8.
28	4,4'-Diaminodiphenylsulfone†	50-100	1.66	—32.	—20.
29	Streptomycin†	20 (hypo)	3.9	— 5.	— 5.
30	5-Amino-2-isopropoxypyridine	30	1.14	—137	— 9.

* "Pathological Ratio" is the result of the average numerical pathological rating of the control animals divided by that of the treated animals.
† Diaminodiphenylsulfone and streptomycin are included for comparison.

in the 6- position and of an activating substituent in the 2- position. Kumler and Daniels[5,6] proposed a theory of activity of sulfonamides and aminoacridines in which the reactive character of the aromatic amine group is correlated with bacteriostatic activity. The aromatic amino group must be present, of course, in a molecule of suitable overall structure. This theory may be applied to these new tuberculostatic agents. The 6-amino group is an aromatic amino group and the 2- substituents may be regarded as activating groups. Using 6-aminobenzothiazole, where the 2- position is occupied by hydrogen, as a base level, one may calculate activity ratios with strain H37Rv as the test organism. With the following 2-substituents the activity ratios are: CH_3 -10, SH -1, OC_2H_5 -0.14, NH_2 -10, OC_4H_9(n) -40. In a previous paper[3] it was shown that a 6-aminobenzothiazole substituted with 2-arylsulfonyl groups, which are electroacceptors, had only a low order of activity. The present compounds with electrodonor groups in the 2- position are as much as 1,000 times as active. Unfortunately, as yet, no adequate quantitative measure of the reactive character of the aromatic amine group is available. Qualitative comparisons can be made if adequate allowance is made for other important factors affecting drug activity.

In the case of the 6-amino-2-alkoxybenzothiazoles it is apparent that the distribution coefficient is of considerable significance and this effect is much more noticeable with H37Rv than with strain No. 607. 6-Amino-2-ethoxy, isopropoxy, and (n)butoxybenzothiazoles all have about the same activity against strain No. 607 but the butyl ether is 300 times as active as the ethyl ether against strain H37Rv. The generic similarity of these benzothiazole ethers to the pyridine and other ether-amines studied by Feinstone, Friedman and co-workers[7-11] suggests that certain generalizations of some validity concerning both groups of compounds can be made. They found an optimum activity with ether chains of 4 to 6 carbon atoms. In addition it is noticeable from their data that the

[5] Kumler, W. D., and Daniels, T. C., *J. Am. Chem. Soc.*, 1943, **65**, 2190.

[6] Daniels, T. C., personal communications.

[7] Friedman, H. L., Braitberg, L. D., Tolstoouhov, A. V., and Tisza, E. T., *J. Am. Chem. Soc.*, 1947, **69**, 1795.

[8] Braitberg, L. D., Robin, M., Friedman, H. L., and Tisza, E. T., *J. Am. Chem. Soc.*, 1947, **69**, 2005.

[9] Friedman, H. L., Braitberg, L. D., Tolstoouhov, A. V., and Tisza, E. T., *J. Am. Chem. Soc.*, 1947, **69**, 1204.

[10] Feinstone, W. H., Friedman, H. L., Rothlauf, M. V., Kelly, A. M., and Williams, R. D., *J. Pharm. and Exp. Therap.*, 1947, **89**, 153.

[11] Feinstone, W. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 153.

symmetry of the ether group is very important. The chain length and symmetry of the ether group may very well maximize a tendency for proper molecular orientation and binding at some very important intracellular interface or enzyme locus. As evidence of the importance of symmetry the data of Friedman *et al.*[9] may be cited. With the 2-alkoxy-5-aminopyridines, using the n-amyl ether as unity the activities against strain No. 607 are: i-amyl -0.03, 3-n-amyl -0.5, n-butyl -1, i-butyl -.07, sec-butyl -0.016. These orientation factors may be much more important than the commonly known one of an optimal distribution coefficient, dependent on chain length, producing a maximal drug concentration in some lipoid phase.

The high order of specificity of some of the benzothiazole derivatives for strain H37Rv relative to strain No. 607 is encouraging. In the case of 6-amino-2-(n)-butoxybenzothiazole the activity ratio for the two strains is 1000. With other benzothiazole derivatives the ratio varies from 2 to 50. With the arylsulfones (extensively studied) the activity ratios were close to unity.

Those benzothiazole derivatives which were tested *in vivo* showed a low or moderate chronic toxicity. In the case of 6-amino-2-(n) butoxybenzothiazole a definite hypnotic effect appeared at the maximum tolerated dose. This is not unexpected for an ether-amine nor for a compound which may be regarded as a cyclic thiocarbamic ester. Hemotoxic effects which are so prominent with derivatives of diaminodiphenylsulfone were absent with the benzothiazole compounds. For comparison of toxicity and activity, 2-isopropoxy-5-aminopyridine (No. 30) (somewhat comparable to 6-amino-2-isopropoxybenzothiazole of this series) was evaluated *in vivo*. While this compound is not at the pyridine series optimum the comparison to equivalent benzothiazole compounds is significant. It may be noted that much smaller dosages of the pyridine ether were tolerated, marked liver damage occurred, and no significant effect on the infection was noticed.

Many types of compounds possessing active aromatic amino groups are tuberculostatic. Feinstone, Friedman and co-workers found many amino pyridines, pyrimidines and benzenes to be active. The authors[12] have noted fair activities with some amino acridines and phenothiazines. It may be suspected that some tuberculostatic activity is possessed by a great variety of amino-cyclic compounds in which the amino group is aromatic in character and is suitably activated.

Further studies of benzothiazole derivatives, including solublized derivatives of 6-amino-2-(n)butoxybenzothiazole, are being undertaken.

Summary. 1. A new class of highly tuberculostatic agents of low toxicity is described. This group comprises many 2,6- substituted derivatives of benzothiazole. 2. Several of these derivatives are active therapeutic agents in guinea pig tuberculosis. 3. Structure activity relations and possible modes of action are discussed.

[12] French, F. A., and Freedlander, B. L., unpublished data.