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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: PART II. SULPHONAMIDES

BY

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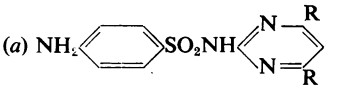
Rich and Follis (1938) showed that sulphanilamide had a retarding effect upon tuberculosis in guinea-pigs and later (1939) they were also able to demonstrate a slightly favourable response against " bovine " tuberculosis in rabbits. Sulphapyridine had no significant effect in the latter experiments, though other workers—for example, Feldman and Hinshaw (1940)—have shown that this drug has some slight action on tuberculosis in guinea-pigs. A more favourable effect in guinea-pigs was obtained with sulphadiazine (though not with sulphapyridine or sulphathiazole) by Smith, Emmart, and Westfall (1942). In all experiments the observed therapeutic benefit has been slight and could be demonstrated only when the drugs were given at maximum tolerated doses. Examination of a number of the commoner sulphonamide drugs in mice by the method previously described by one of us (Martin, 1946) is in agreement with these earlier results. We obtained a significant increase of survival time with the higher doses of sulphadiazine (No. 2052) and with the highest dose of No. 3536 (which corresponds to sulphamerazine) but not with the other sulphonamide drugs used (Table II).

Though none of these results is of practical importance, we were led to return to this group of compounds by the remarkable pharmacological properties of 2-sulphanilamido-4 : 6-dimethoxypyrimidine (No. 3445). The preparation of this compound and some homologues has been described by Rose and Tuey (1946); it was developed in these laboratories in the course of a search for improved sulphonamides for the treatment of non-tuberculous bacterial infections. It has been shown to be well absorbed, extremely persistent in the body and very efficient in the treatment of streptococcal infections in mice (Gage, Martin, Rose, Spinks, and Tuey, 1947). *In vitro* the drug No. 3445 had only a very feeble activity against *Mycobacterium tuberculosis*, as indicated by its *in vitro* index $< 1/6$ (for description of our method of measuring *in vitro* activity, see Hoggarth and Martin, 1948), and failed to show any activity in mice. Our previous experience with sulphones led us to believe that the *in vitro* activity of drug No. 3445 might be raised by suitable modification of its structure. Provided that the favourable pharmacological properties were retained, it was reasonable to expect activity *in vivo*.

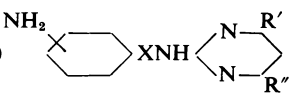
TABLE I

(*a*)



Number	R	*In vitro* index
3445	methoxy	$<1/6$
3706	ethoxy	$<1/3$
4583	*n*-propoxy	5/7
4584	*iso*propoxy	4/8
5105	*n*-butoxy	5/7
5239	*iso*butoxy	6/8
5033	1-methylpropoxy	4/7
5066	methylthio	$<1/7$

(*b*)

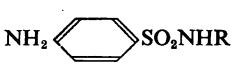


Number	Position of amino group	X	R′	R″	*In vitro* index
3052	*p*	SO_2	methyl	methoxy	$<1/1$
5162	*p*	S	methoxy	methoxy	$<1/4$
4594	*m*	SO_2	methoxy	methoxy	2/6

Examination of the *in vitro* indices for a series of 2-sulphanilamido-4 : 6-dialkoxypyrimidines (Table I) shows that, as expected, the *in vitro*

activity increases with increasing size of the alkyl groups, at least as far as 4 carbon atoms. Prior to examination of these compounds *in vivo*, the blood levels attained in mice dosed orally with these new compounds were examined by our colleague Dr. Spinks (Spinks, 1947), who found that whilst the property of persisting in the blood stream was retained, the maximum concentrations which could be attained fell off with increasing molecular weight. With the butoxy compounds, the maximum blood level was so small that it seemed unnecessary to carry the synthetical work any further. The *in vitro* activity of 2-sulphanilamido-4:6-dimethoxypyrimidine (No. 3445) was not increased by replacing the methoxy groups by methylthio groups (No. 5066), by replacing one methoxy group by methyl (No. 3052), or by replacing the sulphonamide linkage (-SO_2-NH-) by sulphenamide (-S-NH-) (No. 5162), though by changing the position of the amino group a slight increase was noted (No. 4594). As this last change led to loss of power to persist in the blood (Spinks, 1947), it was not considered that any of these variations applied to compounds with larger alkoxy groups would result in increased activity *in vivo*.

TABLE II



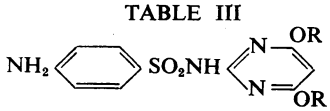
Number	R	Dose (mg. per 20 g. mouse)	Increased mean survival time	Increase required for significance
2052	2-pyrimidyl ..	10.0	+3.9	1.9
		8.0	+2.9	1.7
		5.0	+1.3	1.7
		2.0	0	1.7
3536	2-(4-methyl-pyrimidyl)	8.0	+1.7	1.7
		5.0	+0.5	1.7
		2.0	+1.2	1.7
1968	2-(4:6-dimethyl-pyrimidyl)	10.0	−1.0	1.8
		2.5	−1.1	1.8
2347	2-thiazyl ..	10.0	−1.1	1.5
6131	-CS.NH_2 ..	10.0	+0.5	1.1

The tests summarized in the following tables were carried out as described in the preceding paper by Hoggarth and Martin (1948). Drugs were given orally, usually twice daily for five days and once on Saturdays, but the remarkable persistence of the 2-sulphanilamido-4:6-dialkoxypyrimidines in the blood made it unnecessary to dose oftener than once daily with some of these compounds. Infection was carried out intravenously, using 1 mg. of moist growth of "human" tubercle bacillus from a culture on Lowenstein's medium suspended in water.

Discussion of Results

The therapeutic results show (Table III) that *in vivo* activity against *Mycobacterium tuberculosis* can be attained by compounds of the 2-sulphanilamido-4:6-dialkoxypyrimidine class. Activity in this group seems to be limited by the same considerations as were found to be operative with the sulphones (Hoggarth and Martin, 1948)—namely, that substituents sufficiently large to confer high activity *in vitro* result in such poor absorption that activity *in vivo* cannot be expected. This is certainly the explanation, at least in part, of the activity of the *n*-propoxy and *iso*propoxy compounds (Nos. 4583 and 4584) on the one hand, and the absence of activity in all the butoxy compounds (Nos. 5105, 5239, and 5033) on the other, although all show about the same high activity *in vitro* (Table I).

TABLE III



Number	R	Dose (mg. per 20 g. mouse)	Increased mean survival time	Increase required for significance
3445	methyl ..	5.0*	+1.1	1.4
4583	*n*-propyl ..	5.0*	+1.1	1.4
		2.5*	−0.5	1.5
		2.5	+2.0	1.4
4584	*iso*propyl ..	7.5*	+1.1	1.4
		5.0*	+6.4	1.4
		4.0	+4.0	2.2
		2.5*	−1.5	1.5
5105	*n*-butyl ..	5.0	+0.1	1.3
5033	l-methylpropyl	5.0	+1.2	1.4
5239	*iso*butyl ..	3.0	+1.0	1.3

*Dosed once daily only.

Our interest in 2-sulphanilamido-4:6-di-*iso*-propoxypyrimidine (No. 4584), which produced a very striking increase in mean survival time at the optimal dose, was lessened by the observation that this beneficial effect is almost lost when dosing (which normally begins just before infection) was delayed by so little as 24 hours. With other un-

related compounds (to be reported) we have shown a definite prolongation of life of treated animals even when dosing was delayed for as long as 7 days. The anti-tuberculous activity of No. 4584 is therefore of no practical significance.

SUMMARY

1. The activity of a series of 2-sulphanilamido-4:6-dialkoxyprimidines and some closely related compounds against *Mycobacterium tuberculosis in vitro* has been studied. In the former group activity *in vitro* increases with increasing size of the alkoxy groups.

2. The therapeutic action of certain of these compounds in mice infected with *Mycobacterium tuberculosis* was examined. The di-*n*-propoxy and di-*iso*propoxy compounds produced a significant increase in the mean survival time of groups of mice treated with them when the drug was given both before and after the mice were infected. When drug treatment was delayed for 24 hours, no therapeutic effect was demonstrated.

3. The higher members of the series were very poorly absorbed and failed to show any therapeutic action.

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