ANTHELMINTIC SCREENING OF SUBSTITUTED BIS - BENZOTHIAZOLES

By

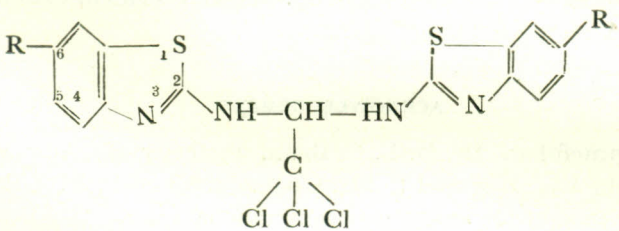
R. C. TRIPATHI*, MAN MOHAN SINGH** AND J. D. KOHLI

Central Drug Research Institute, Lucknow.

(*Received March 28, 1958*)

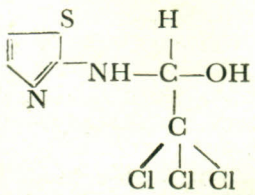
Derivatives of rhodanine and 2:3-dihydro-3-ketobenzol-1:4-thiazine have been tested against liver flukes and Ascaris lumbricoides by the group of workers led by Mackie[1,2,3], as all these compounds contain certain groups which appear among well known anthelmintics phenothiazine and filicic acid. Following this work, Mackie and Misra[4] further synthesized several derivatives of thiazole and benzothiazoles among which 2-mercapto-6-nitrobenzo-thiazole was found to be paralysant at 1:80,000 towards liver fluke *in vitro*. This work has now been extended by Misra and Shah[5] to a new series of benzothiazoles which has been tested for anthelmintic activity against intestinal helminths.

The following compounds‡ have been screened:

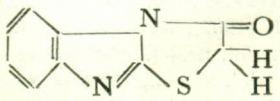


Code No.	R
B. T. 1	H
B. T. 2	CH_3
B. T. 3	OCH_3
B. T. 4	Cl
B. T. 5	OC_2H_5
B. T. 6	$OOC(CH_2)_2 N(C_2H_5)_2 . 2HCl$

B. T. 7.



A. 1



*On deputation from Orissa Government Veterinary Service.

**On deputation from Bihar Government Veterinary Service.

‡Kindly supplied by Dr. R. C. Shah, National Chemical Laboratory, Poona.

METHODS AND RESULTS

In vitro screening for vermicidal activity was carried out on *Pheretima posthuma* (earthworm), *Hirudinaria granulosa* (Leeches), *Ankylostoma caninum* (Dog hook worm) and *Moniezia expansa* (Goat-tape-worm). The method followed was essentially the same as described by Singh *et al*[6]. All the compounds tested were insoluble in water and were, therefore, suspended with gum tragacanth and then diluted, as required, with water.

Experiments were designed to record (*i*) the time required to paralyse all the worms and (*ii*) the time taken for all the worms to recover to normalcy from paralysed condition when washed and kept in tap water after a certain period of exposure to the drug. Control experiments were run side by side against each drug.

Paralysing activity of the compounds is shown in Table I. The worms were observed for 4 hours and then again next morning. A dash (—) means no effect noticed during the period of observation.

TABLE I

Time in minutes taken to paralyse the worms.

Sl. No.	Test Object	Concentration of solution	Code No. of synthetic compounds							
			BT. 1	BT. 2	BT. 3	BT. 4	BT. 5	BT. 6	BT. 7	A. 1
1.	Earthworms	1 in 1000	—	—	4	5	7	8	—	—
	(*P. posthuma*)	1 in 2000	—	—	8	7	17	13	—	—
		1 in 5000	—	—	12	11	—	20	—	—
2.	Leeches	1 in 1000	—	—	34	6	—	37	—	—
	(*H. granulosa*)	1 in 2000	—	—	60	11	—	59	—	—
		1 in 5000	—	—	—	18	—	145	—	—
3.	Hookworms	1 in 1000	—	—	—	42	—	—	—	—
	(*A. caninum*)	1 in 2000	—	—	—	70	—	—	—	—
		1 in 5000	—	—	—	90	—	—	—	—
4.	Tapeworms	1 in 1000	—	—	—	30	—	—	—	—
	(*M. expansa*)	1 in 2000	—	—	—	40	—	—	—	—
		1 in 5000	—	—	—	60	—	—	—	—

N. B.—Each figure is an average of 3 experiments consisting of 5 worms each.

All the worms employed in this study showed irritability on exposure to these compounds as evidenced by increased motility. In case of the earthworm, mucoid slimy discharge appeared when the worms were brought in contact with the solutions and in case of leeches bloodly discharge was noticed.

Among these compounds BT. 4, 3, 6 and 5 showed paralysing activity in that order against earthworm. Against leeches same order of activity was retained except that compound 5 was practically inactive. Against hook-worm and tapeworm segments BT. 4 was alone active.

The capability of worms to recover from paralytic stage could not be studied in case of tape-worm segments. The results in respect of other test objects are given in Table II.

TABLE II.

Time taken to regain normal motion (in minutes).

Synthetic compound	Earthworms (after 15 ′ exposure)			Leeches (after 30 ′ exposure)			Hookworms (after 30 ′ exposure)		
	1 in 1000	1 in 2000	1 in 5000	1 in 1000	1 in 2000	1 in 5000	1 in 1000	1 in 2000	1 in 5000
BT. 3	126	87	26	30	20	–	–	–	–
BT. 4	83	61	47	*	*	*	5	4	2
BT. 5	63	20	–	–	–	–	–	–	–
BT. 6	34	30	17	143	95	2	–	–	–

* = No recovery upto 24 hours.

In vitro studies indicated that compounds BT. 3, 4, 5 and 6 possessed certain paralysing activity. Among these, BT4 was the most active. These compounds were, therefore, taken up for *in vivo* study.

In vivo Tests.

In vivo anthelmintic activity was studied against nematodes. The selection of the test material was based on the availability of a natural infection and cost of the material. As such, testing was limited to dogs who are natually infected with *A. caninum* besides several other infestations, and fowls who harbour naturally *Ascaridia galli.*

Dogs – Mongrel dogs were obtained and kept under observation for a few days during which the stool was examined for the evidence of *A. caninum* infection. Extent of infestation was estimated by ova count on two successive days following the method of Monnig[7]. Following a 24 hours fasting period, the drug was administered in gelatin capsules and one hour later a purgative dose of magnesium sulphate (3 g./kg.) in 10% solution was administered by stomach tube. The stool was thereafter observed for the presence of adult worms passed on 3 successive days. On the fourth morn-

ing quantitative ova estimation was carried out. The animal was then sacrificed and the gut was examined for living worms, which were picked up and counted.

Two animals were used for each compound, one received 50 mg./kg. and the other 100 mg./kg. of the drug. A positive control was treated with tetrachloroethylene and another control group was fed magnesium sulphate only. A comparison of number of ova in the stools before and after treatment as also a comparison between number of worms passed after treatment and number of worms still left in gut after treatment gave an idea of the anthelmintic activity of the compound.

The results are tabulated in Table III.

TABLE III.

Drug.	Dosage.	Weight in Kg.	Pre-treatment ova count per gm. of stool × 100 (average of two readings on successive days).	No. of worms passed out in stool.	Post-treatment ova count per gm. of stool × 100.	No. of worms found in gut at autopsy.
Tetrachloroethylene	.02 cc./kg.	8.5	584	32	7	6
Magnesium Sulphate	3 g./kg.	8.5	316	0	120	31
		7.5	691	0	320	545
BT. 3	100 mg./kg.	6.5	70	0	64	83
	50 mg./kg.	7.5	85	0	90	105
BT. 4	100 mg./kg.	7.0	28	0	96	48
	50 mg./kg.	7.0	50	0	22	71
BT. 5	100 mg./kg.	8.0	222	0	200	212
	50 mg./kg.	8.5	23	0	30	35
BT. 6	100 mg./kg.	6.0	375	0	105	238
	50 mg./kg.	12.0	40	0	60	76

Chickens—The mothod followed was essentially the same as in dogs. Fowls weighing 600-900 g. were obtained and an estimation of the infection with *Ascaridia galli* determined.

Here again positive control was carried out with 'Antepar' brand of piperazine (Burroughs Wellcome) and tetrachloroethylene. Effect of magnesium sulphate alone was also noted. 1 g. of each compound in capsules was fed to

each bird after 24 hours fasting period and 10% magnesium sulphate solution was substituted for drinking water an hour after feeding. As in dogs the birds were sacrificed on the 4th day and post mortem count of living worms in the gut carried out. The results are given in Table IV.

TABLE IV

Drug	Dosage	Pre-treatment ova count per gm. of stool × 50 (average of two readings on successive days)	No. of worms passed out in stool	Post-treatment ova count per gm. of stool × 50	No. of worms found in gut at autopsy
Terachloroethylene	0.85 cc. each	10	2	1	3
		5	9	0	0
		13	4	0	0
'Antepar' brand of piperazine	1gm. each.	326	45	0	0
		138	13	0	0
		38	4	0	0
Magnesium Sulphate	10% solution as drinking water.	25	0	24	8
		5	0	4	2
BT. 3	1 gm. each.	6	0	6	3
		130	0	160	18
		3	0	3	2
BT. 4	1 gm. each.	19	0	24	6
		3	0	2	1
		89	0	81	20
BT. 5	1 gm. each.	11	0	12	4
		37	0	30	12
BT. 6	1 gm. each.	110	0	96	12
		68	0	80	9
		184	0	30	74

DISCUSSION

The value of *in vitro* screening in general and the use of annelids in particular for the purpose of screening anthelmintics has often been questioned. However, the value of this procedure for the purpose of determining comparative effectiveness from amongst a chemical series as a guide to select potent analogues for *in vivo* screening has been recognised[8]. As such the preliminary screening was carried out using annelids, cestodes and nematodes and compounds BT. 3, 4, 5 and 6 were selected for *in vivo* screening.

In vivo screening has been carried out in dogs and chickens. Both the animals have been employed previously for the purpose by several workers. However, in either case positive controls were kept by using known anthelmintic drugs. In case of chickens the method was checked by using tetrachloroethylene and 'Antepar' brand of piperazine (Burroughs Wellcome). It is worthwhile recording that both these compounds which are effective ascaricides in clinical work gave clear cut positive results against *A. galli.* It may therefore be mentioned that *A. galli* may prove a sensitive test object for screening and standardising ascaricidal drugs.

SUMMARY

In vitro and *in vivo* screening of substituted-bis-benzothiazoles has been carried out. By *in vitro* tests the compounds that showed paralysing activity could be arranged in the following order BT. 4, 3, 6 and 5. None of the compounds in the dosage employed showed any anthelmintic activity *in vivo.*

ACKNOWLEDGEMENT

Our thanks are due to Dr. B. Mukerji and Dr. N. N. De for their interest in the work.

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