

# ***BULLETIN***

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## CONTENTS

|                                                                                                                                                                               | PAGE |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <i>Effect of Dietary Carbohydrates (Starch/Sucrose) on Hepatic Enzyme Levels in the Albino Rat</i>                                                                            | 43   |
| U. J. DUMASWALA, R. U. DUMASWALA AND A. VENKATARAMAN                                                                                                                          |      |
| <i>Enzymatic Activities of the Venom of Enhydrina Schistosa (Common Sea Snake) from the Western Coast of India</i>                                                            | 48   |
| S. P. GAWADE AND M. B. BHIDE                                                                                                                                                  |      |
| <i>N<sup>1</sup>-Methyl, N<sup>4</sup>-Substituted Piperazine Derivatives as Antiamoebic Agents—I</i>                                                                         | 52   |
| BHARATHI SHANKER, R. A. BELLARE, ANAGHA C. DESAI AND M. B. BHIDE                                                                                                              |      |
| <i>Synthesis and Study of 2-[p-(2-Substituted-4-Thiazolyl) Arylamino]Pyrido (3, 2-d) Thiazoles as possible Anthelmintic Agents</i>                                            | 56   |
| B. G. KHADSE, D. T. CHAUDHARI, A. M. PATIL, D. M. RENAPURKAR AND VIJAYALAXMI R. KAMAT                                                                                         |      |
| <i>Synthesis and Screening of Schiff Bases as Antiinflammatory Agents—I</i>                                                                                                   | 60   |
| NIRMALA J. KHER, S. S. SABNIS AND M. B. BHIDE                                                                                                                                 |      |
| <i>Synthesis and Screening of Schiff Bases as Antiinflammatory Agents—II</i>                                                                                                  | 64   |
| NIRMALA J. KHER, P. Y. NAIK, S. S. SABNIS AND M. B. BHIDE                                                                                                                     |      |
| <i>Synthesis and Screening of Schiff Bases as Antiinflammatory Agents—III</i>                                                                                                 | 69   |
| NIRMALA J. KHER, S. S. SABNIS AND M. B. BHIDE                                                                                                                                 |      |
| <i>In vitro Antituberculosis and Antifungal Activity of p-(2-Substituted-4-Thiazolyl) Phenylacetic and p-(2-Substituted-4-Thiazolyl)Phenoxyacetic Acids</i>                   | 72   |
| MADHUBEN R. PATEL AND B. R. COLAH                                                                                                                                             |      |
| <i>In vitro Antituberculosis and Antifungal Activity of Schiff Bases from 2-Substituted-4-(4-Aminophenyl)Thiazoles and 2-Substituted-4-Formylphenoxyalkyl Acids and Ester</i> | 75   |
| PUSHPA A. KIRKE AND B. R. COLAH                                                                                                                                               |      |

## **International Cardio Pulmonary Symposium**

*Bombay, November 13 to 15, 1977*

An International Cardio Pulmonary Symposium will be held at Hotel President (India) from 13th to 15th November, 1977. The Symposium is being sponsored by the Western India Chapter—International Academy of Chest Physicians and Surgeons (affiliated with the American College of Chest Physicians) and Association of Physicians of India and its Bombay Branch and Indian Association for Chest Diseases. The Symposium wherein many international authorities are participating will deal with advances in Cardio Pulmonary Medicine and Surgery. For further information, contact Dr. M. Paul Anand, Secretary General, International Cardio Pulmonary Symposium, 4 Narendra Bhuvan, Bhulabhai Desai Road, Bombay 400 026.

## **EFFECT OF DIETARY CARBOHYDRATES (STARCH/SUCROSE) ON HEPATIC ENZYME LEVELS IN THE ALBINO RAT**

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Two groups of male weanling albino rats were fed diet containing 58 per cent starch/sucrose, 20 per cent casein and 16 per cent safflower seed oil for the period of 10 weeks. At the end of the feeding period, the specific activities of HMP shunt, isocitrate dehydrogenase and malic and citrate cleavage enzymes and pyruvate kinase were determined in the livers of the animals. Increased levels of these enzymes were observed in the liver of sucrose-fed animals. The higher liver lipid levels on sucrose diet can be correlated with the greater activities of the lipogenic enzymes in the liver.

### **Introduction**

Numerous studies have shown that the composition of the diet alters enzyme activities in liver and other tissues. The quantity (1, 2) and quality (3, 4) of dietary fat and also of protein (5, 6) have long been known to influence lipid metabolism. The effect of dietary carbohydrates on the relative participation of different pathways is a topic of current interest. Previous study from this laboratory (7) demonstrated that the dietary sucrose elevates serum lipid levels as compared to dietary starch in the presence of saturated as well as unsaturated dietary fat. The present study was aimed to investigate the effect of dietary carbohydrate (starch/sucrose) on the patterns of lipogenic enzymes in the presence of moderately high proportion (16 per cent) of a highly unsaturated dietary fat (safflower seed oil).

### **Materials and Methods**

Twelve male weanling albino rats (21-28 days old) of Haffkine strain were divided

into two groups and maintained on a synthetic diet consisting of 20 per cent vitamin-free casein, 58 per cent carbohydrate (starch/sucrose), 16 per cent fat (safflower seed oil) and adequate vitamins and salts.

The animals were fed *ad libitum* and at the end of the feeding period, they were sacrificed by decapitation, after the overnight fast. The livers were quickly dissected out, weighed and kept in ice until homogenised for enzyme assay. One portion of the liver tissue was homogenised in 0.14 M KCl solution (1:10 w/v) in a Potter-Elvehjem type homogeniser. This was centrifuged at 0–4°C for 30 min. and 27,000 × g supernatant cytosol fraction was used as the enzyme source.

### **Enzyme Methods**

The activities of malic enzyme (ME) (EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G 6 PD) (EC 1.1.1.49) and isocitrate dehydrogenase (ICD) (EC 1.1.1.42) were determined by the procedure described by Benevenga *et al.* (8). The determination of 6-phospho-gluconolactone dehydrogenase (6 PGD) (EC. 1.1.1.43) and

citrate cleavage enzyme (CCE) (EC. 4.1.3.8) was carried out according to the method of Glock and McLean (9) and Srere (10). Enzyme activities were expressed as Wroblewsky unit i.e. the amount of enzyme which changes the optical density by 0.001 at 340 m $\mu$ /minute in a 3 ml of mixture at 24 °C. The activity of pyruvate kinase (PK) (EC 2.7.1.40) was estimated by the method of Swarup *et al.* (11).

The protein concentration was determined by the method of Lowry *et al.* (12).

The second portion of the liver was processed for the lipid analysis (cholesterol and phospholipids) as described elsewhere (7).

## Results

Table I presents the effect of dietary carbohydrates (starch/sucrose) on the specific activities of various liver enzymes. It is evident from the Table that specific activities of G-6-P-D, 6-P-G-D, I-C-D, M-E, C-C-E and P-K are significantly elevated in the livers of sucrose-fed animals. The activities of HMP shunt dehydrogenases (G-6-P-D) and (6-P-G-D) are higher by about 71 per cent and 90 per cent respectively, while the levels of ICD are elevated by 12 per cent only. P K, M E and C C E activities are greater by 36 per cent, 73 per cent and 109 per cent respectively.

TABLE I

*Effect of dietary carbohydrates (starch/sucrose) on the specific activity of the liver enzymes in the albino rat*

| Enzymes                             | Units*         |                 |
|-------------------------------------|----------------|-----------------|
|                                     | Starch group   | Sucrose group   |
| Glucose-6-P dehydrogenase .. ..     | 25.2 $\pm$ 1.1 | 47.5 $\pm$ 3.5† |
| 6-P-gluconolactone dehydrogenase .. | 23.1 $\pm$ 2.1 | 39.5 $\pm$ 1.6† |
| Isocitrate dehydrogenase .. ..      | 244 $\pm$ 1.8  | 274 $\pm$ 13.6‡ |
| Pyruvate kinase .. ..               | 55.3 $\pm$ 7.6 | 75 $\pm$ 4.1‡   |
| Malic enzyme                        | 29.7 $\pm$ 4.7 | 51.8 $\pm$ 4.4§ |
| Citrate cleavage enzyme .. ..       | 21 $\pm$ 2.6   | 44 $\pm$ 2.2†   |

The figures are mean  $\pm$  S.E. of five individual estimations.

\*One unit (Wroblewsky unit) is the amount of enzyme which changes the optical density by 0.001 at 340 m $\mu$ /minute in a 3 ml of assay mixture at 24°C.

†Significantly different from starch group ( $p < 0.0025$ ).

‡Significantly different from starch group ( $P < 0.05$ ).

§ Significantly different from starch group ( $P < 0.0125$ ).

The amount of serum and liver cholesterol is significantly higher for the sucrose-fed rats as compared to starch-fed ones. The concentration of serum and liver cholesterol are higher by 45 per cent and 29 per cent respectively. It is interesting to note that the liver weight and total fat contents are about 10 per cent more in the sucrose-fed rats (Table II).

### Discussion

The enzymes investigated have been selected as representative of various metabolic pathways associated with carbohydrate and lipid metabolism and on the basis of previous reports that their activities are altered by diet composition (13, 14). In the present study significantly increased levels of M E, C-C-E, H M P-shunt dehydrogenases and P K are observed for sucrose-fed

animals than for starch-fed ones. These raised levels of enzymes may be responsible for increased lipogenesis and cholesterogenesis in sucrose group, which in turn elevates serum and liver lipids. In other words, sucrose may be stimulating lipogenic enzyme synthesis in the liver. It is very interesting to note that the sucrose diet brings about marked elevation of the lipogenic enzyme activities in the presence of moderately high proportion of unsaturated dietary fat. Several studies have pointed out that the fat synthesis is repressed in livers of animals on high fat feeding (15, 16, 17). The results of the present investigation suggest that the sucrose definitely has comparatively higher capacity to synthesize fat than starch inspite of feeding moderately high proportion of unsaturated fat.

TABLE II

*Effect of dietary carbohydrates (starch/sucrose) on serum and liver lipids in the albino rat*

|                          |    |    |    |    | Group      |             |
|--------------------------|----|----|----|----|------------|-------------|
|                          |    |    |    |    | Starch     | Sucrose     |
| SERUM (mg/100 ml)—       |    |    |    |    |            |             |
| Cholesterol              | .. |    |    |    | 110 ± 4.2  | 160 ± 5.2*  |
| Triglycerides            |    |    |    |    | 90 ± 4.4   | 99 ± 3.1†   |
| Phospholipids            | .. |    |    |    | 110 ± 6.4  | 95 ± 5.0†   |
| LIVER—                   |    |    |    |    |            |             |
| Weight (g)               |    |    |    |    | 10.8 ± 0.1 | 11.8 ± 0.1* |
| Fat (g per cent)         |    |    |    |    | 6.6 ± 0.2  | 7.2 ± 0.2‡  |
| CHOLESTEROL—             |    |    |    |    |            |             |
| Total (mg/100 g)         |    |    |    |    | 480 ± 16   | 620 ± 8.0*  |
| Free (per cent)          |    |    |    |    | 75         | 64          |
| Phospholipids (mg/100 g) | .. | .. | .. | .. | 3000 ± 60  | 3100 ± 78†  |

The figures are mean ± S.E. of five individual determinations.

\*Significantly different from starch group ( $p < 0.0025$ ).

†Not significantly different from starch group.

‡Significantly different from starch group ( $p < 0.05$ ).

Higher HMP-shunt dehydrogenases activities on sucrose diet than starch diet are reported in rats (13, 18, 19). Cohen *et al.* (20) have observed two to four-fold increase in the amount of liver P-K, G-6-P-D, M-E, acetyl CoA carboxylase and glucose-6-phosphatase in rats fed for 12 months on 72 per cent sucrose diet than 72 per cent starch diet. But many of these studies were conducted with extremely low fat diet and the type of fat was saturated.

The more marked increase in the activity of lipogenic enzyme in the liver of sucrose as compared to starch-fed rats should be traced to the greater capacity of the liver to metabolise fructose. It may be also interesting to note at this point that the fructose metabolism is restricted to hepatic tissue only, as against glucose metabolism (20). The glucose absorbed upon the hydrolysis of starch is available

to all body tissues. As a result, the amount of monosaccharide reaching the liver may be lower after starch ingestion. This might in turn compel a greater induction of the enzymes controlling hexose disposal in the liver tissue.

#### Acknowledgements

Authors are thankful to Dr. B. B. Gaitonde Director, Haffkine Institute, for the keen interest shown during the course of this study. We are extremely thankful to Prof. Mario U. Dianzani, Professor of General Pathology and Dean of the Faculty of Medicine, University of Torino, Italy; Professor Walton W. Shreeve, Chief, Nuclear Medicine Service, Veterans Administration Hospital, North Port, New York and Professor of Medicine (Nuclear Medicine), State University of New York, at Stony Brook, for their useful suggestions.

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## ENZYMATIC ACTIVITIES OF THE VENOM OF *ENHYDRINA SCHISTOSA*\* (Common Sea Snake) FROM THE WESTERN COAST OF INDIA

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The enzymatic activities of phospholipase A, phosphodiesterase (exonuclease), alkaline phosphomonoesterase, 5' Nucleotidase, protease, cholinesterase, L-amino acid oxidase and amino acid esterase were studied in the sea snake *E. schistosa* venom. It was shown that it is very rich in phospholipase A, while L-amino acid oxidase and amino acid esterase were absent.

### Introduction

All the snakes belonging to the family Hydrophiidae are extremely venomous and are more numerous than terrestrial venomous snakes. Their venom is known to contain potent neurotoxins which are some of the most toxic substances in the world. Besides neurotoxins, sea snake venoms also contain several enzymes which makes the study of this venom very interesting. Enzymes also have important application in research. Sea snake *E. schistosa* is the most commonly available and the poisonous sea snake found along the western coast of India. An attempt has been made to study the enzymatic activities present in *E. schistosa* venom.

### Materials and Methods

**Sea Snake *E. schistosa***—Snakes were collected from the Bombay sea coast from local fishermen in the month of March/April 1975. Species were identified using keys for the identification (1, 2).

(Received for publication August 4, 1976)

\*This work is based on a thesis for the degree of M. Pharm. of the University of Bombay.

The paper presented in First Maharashtra State Pharmacological Conference held at Solapur on 27 and 28 July 1975.

**Crude Dried Venom**—Snakes were anaesthetized with pentobarbitone sodium 15 mg/kg (I.P.). Venom glands were dissected out, homogenised with a hand homogeniser at 4°C in distilled water. The suspension was centrifuged at 4,000 r.p.m. for 15 min. and the supernate was lyophilised. The lyophilised powder was used as crude dried venom. The venom preparation was kept in the dessicator over calcium chloride until use. About 2 g of venom was obtained from about 170 sea snakes.

### Enzyme Assays

For enzyme assays following substrates were used. Casein (BDH), calcium di-p-nitrophenyl phosphate (Fluka), sodium di-p-nitrophenyl phosphate (E. Merck), adenosine monophosphate (E. Merck), acetyl choline chloride (Koch light) 2 : 6-dichlorophenol indophenol (DCIP, E. Merck), L-leucine (E. Merck), Benzoyl arginine ethyl ester (BAEE, Koch light). All other chemicals and reagents used were of analytical and reagent grade. All solutions were made in distilled water.

### Assay of Phospholipase A Activity

Phospholipase A activity was determined quantitatively by turbidimetric method (3)

using egg yolk as a substrate. A fresh egg yolk suspension was prepared in 0.9 per cent sodium chloride. Working egg yolk suspension was prepared from this suspension by diluting with 0.9 per cent sodium chloride in such a way that the absorbance of this working suspension at 925 nm was around 1.2. Different concentrations of the venom (0.1 ml) were incubated with 2.0 ml of the working solution at 37°C for 20 min. The reaction was stopped by adding 3.0 ml of ice-cold 0.9 per cent sodium chloride and optical density was measured at the same wave length i.e. 925 nm on Bausch and Lomb Spectronic 20 against 0.9 per cent sodium chloride. One unit of the activity was defined as the amount of enzyme which produced a decrease of 1 nm unit of absorbance at 925 nm per minute.

#### Assay of Proteolytic Activity

The proteolytic activity was determined using casein (BDH) and denatured haemoglobin as substrates (4).

**Casein Digestion**—The casein solution was prepared by suspending 2 g of casein in 100 ml of 0.4 M Tris-HCl buffer at pH 8.5 and heating for 15 min. in a boiling water-bath to get a complete solution. A 0.5 ml of the venom solution of varying concentrations and 1.0 ml of the casein solution were incubated at 37°C for 15 min. The reaction was stopped by adding 1.0 ml of 0.44 M Trichloroacetic acid (TCA) and after standing for 30 min. the mixture was filtered. To 0.5 ml of the filtrate, 2.5 ml of 0.4 M  $\text{Na}_2\text{CO}_3$  and 0.5 ml of Folin-Ciocalteu reagent (Centron) was added and the optical density was measured at 660 nm. One unit of protease was defined as the amount of enzyme which yielded a colour equivalent to 1  $\mu\text{g}$  of tyrosine per minute under the above conditions.

**Denatured Haemoglobin Digestion**—Haemoglobin solution was prepared by dissolving 1.0 g of Hb in 40 ml of water and was denatured by heating for 30 min. at 80°C. The pH of the solution was adjusted to 8.5 with 0.1 N NaOH. The same assay procedure was followed as described above in casein digestion replacing casein with haemoglobin.

#### Assay of Phosphodiesterase Activity

The phosphodiesterase activity was determined using calcium-di-p-nitrophenyl phosphate as a substrate (5).

The reaction mixture contained 1.0 ml of 0.1 M Tris HCl buffer at pH 8.9, 1.2 ml of 0.001 M calcium di-p-nitrophenyl phosphate 0.2 ml of venom solution (of varying concentration) and distilled water to make 3.0 ml. The reaction mixture was incubated at 37°C for 30 min. and the reaction was stopped by adding 3.0 ml of 0.05 N NaOH. The absorbance was measured at 400 nm against the blank that contained all the reagents but the venom solution. One unit of phosphodiesterase activity was defined as the amount of enzyme which liberated 1  $\mu\text{mole}$  of p-nitrophenol per minute under the above conditions.

#### Assay of Alkaline Phosphomonoesterase Activity

The alkaline phosphatase activity was estimated using sodium-di-p-nitrophenyl phosphate as a substrate (5). The reaction mixture contained 1.0 ml of 0.1 M glycine NaOH buffer pH 9.5, 1.2 ml of 0.1 M  $\text{MgCl}_2$ , 0.1 ml of venom solution (of varying concentration) and water to make 3.0 ml. The reaction was stopped after 15 min. at 37°C by adding 3.0 ml of 0.05 N NaOH. The absorbance was read at 400 nm against the blank that contained all the reagents except venom solution. One unit of the activity was defined as the amount of enzyme which liberated 1  $\mu\text{mole}$  of

*p*-nitrophenol per minute under the above conditions.

#### Assay of 5' Nucleotidase Activity

The 5' Nucleotidase activity was determined (6) with a modification (5) using Adenosine monophosphate as substrate. The liberated phosphate was determined by a known method (7). The reaction mixture contained 0.1 ml of 1.0 M glycine buffer at pH 9.0, 1.0 ml of 0.1 M  $MgCl_2$ , 0.3 ml of 0.01 M Adenosine monophosphate (AMP), 0.1 ml of venom solution (of varying concentration). The reaction mixture was incubated for 15 min. at 37°C. One unit of 5'-Nucleotidase activity was defined as the amount of enzyme liberated 1  $\mu$ mole of inorganic phosphate per minute under the above conditions.

#### Assay of Cholinesterase Activity

Cholinesterase activity was determined by colorimetric method (8) using acetyl cholinechloride as a substrate. The unhydrolysed acetylcholine is converted with hydroxylamine to acetylhydroxamic acid, which can be determined as a brown ferric complex. The reaction mixture contained 2.0 ml of acetylcholine buffer salt mixture (containing 8 vol. of Veronal buffer, 0.1 M, pH 8.2; 1 vol. of 0.5 M acetylcholine chloride; 1 vol. of mixture of 0.44 M- $MgCl_2$  and 0.03 M KCl) and 0.2 ml of venom solution (of varying concentration). After incubation 2.0 ml of alkaline hydroxylamine solution (prepared just before use by mixing equal volumes of 14 per cent w/v hydroxylamine (BDH) and 14 per cent w/v NaOH) was added. After one minute, 6.0 ml of 0.5 N HCl was added with thorough shaking. From the sample and control tubes 0.5 ml hydroxamic acid formed was pipetted into centrifuge tubes containing 10 ml of ferric chloride (1 per cent w/v) solution and was centrifuged for 5 min. at 2,000 r.p.m. and the colour of the supernatant measured at 540 nm.

#### Assay of L-Amino Acid Oxidase Activity

The L-amino acid oxidase activity was determined by measuring the rate of reduction of 2:6-dichlorophenol indophenol (DCIP) at 600 nm (9). The reaction mixture contained 7.5 nm L-leucine, 0.1 nm DCIP, 0.5 M Tris-HCl buffer pH 7.4 and 0.5 M KCl in a final volume of 1.0 ml. The reaction mixture was preincubated for 2 to 3 min. before the addition of leucine in order to check endogeneous activity. One unit of the activity was defined as the amount of enzyme required to produce a decrease of one absorbance unit per minute at 37°C.

#### Assay of Amino Acid Esterase Activity

Amino acid esterase activity was determined (10) with slight modifications to comply with our laboratory conditions. The enzyme esterase hydrolyses synthetic amino acid esters to liberate free amino acids, which can be titrated with 0.05 N NaOH using phenol red as an indicator. 15  $\mu$ mole of benzoyl arginine ethyl ester (BAEE), in 4 ml of distilled water was incubated with 1.0 ml of venom solution (1 mg/ml) at 37°C in a constant temperature bath. The acid liberated was titrated using microburette with 0.05 N NaOH at pH 8.5.

#### Results

Results are listed in Table I. Specific activity was determined in units/mg of lyophilised venom.

TABLE I

| Enzyme                                         | Specific activity |
|------------------------------------------------|-------------------|
| 1 Phospholipase A (E.C. 3-1-1-4) ..            | 78.5              |
| 2 5'-Nucleotidase (E.C.3-1-3-5) ..             | 0.15              |
| 3 Phosphodiesterase (E.C. 3-1-4-5) ..          | 0.05              |
| 4 Alkaline phosphomonoesterase (E.C. 3-1-3-2). | 0.15              |
| 5 Cholinesterase (E.C.3-1-1-7) ..              | 7.6               |
| 6 L-amino acid oxidase (E.C.1-4-3-2) ..        | ....              |
| 7 Protease (Caseinolytic) ..                   | 12.0              |
| 8 Amino acid esterase ..                       | ....              |

### Discussion

It was generally believed that the ability to hydrolyse synthetic substrates, BAEE and ATEE was associated with protease activity. But it was shown that the enzyme responsible for esterolytic activity is different from the proteinase enzyme (4, 11).

*Enhydrina schistosa* venom was studied for the presence of eight enzymes. It was observed that enzymes phospholipase A, 5'-Nucleotidase, phosphodiesterase, alkaline-phosphatase, protease and cholinesterase were present, while L-amino acid oxidase and amino acid esterase enzymes were absent. Toom *et al.* (12) from isoelectric focusing showed that proteolytic activity and arginine ester hydrolysing

activity were absent in *E. schistosa* venom collected from the strait of Malacca region. However, the venom of the same species collected from the western coast of India showed proteolytic activity using casein and denatured haemoglobin as substrates. Differences are reported in the venom compositions of the species collected from different geographical origin (13, 14).

The present work showed that the venom of sea snakes from the western coast of India may be different in composition from those obtained from the strait of Malacca.

### Acknowledgements

The authors are thankful to Dr. B. B. Gaitonde, Director, Haffkine Institute for providing necessary facilities.

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■ ■

## ***N*<sup>1</sup>-METHYL, *N*<sup>4</sup>-SUBSTITUTED PIPERAZINE DERIVATIVES AS ANTIAMOEBCIC AGENTS—I**

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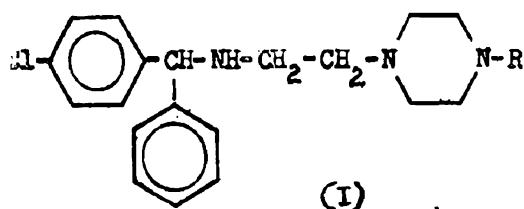
*N*<sup>1</sup>-Methyl, *N*<sup>4</sup>-substituted piperazines were synthesised and tested *in vitro* for antiamoebic activity against *E. histolytica* and structure activity is discussed. Compound with six carbon atom chain is found active *in vitro* against *E. histolytica* (5 µg/ml).

### **Introduction**

Though a number of drugs are used in amoebiasis, chronic amoebiasis has still remained a problem. Today metronidazole and emetine are preferred but these two are also not ideal as they failed to cure number of cases (1-6), and also they have shown some serious toxic effects like cardiotoxicity (7) or tendency to produce malignancy in experimental rodents (8-12). All these necessitated a search for new better drug.

A series of *N*<sup>4</sup>-[β-(*p*-chlorobenzhydryl) aminoethyl]-*N*<sup>1</sup>-substituted piperazines(I), synthesized primarily to study their CNS depressant activity (13), were subjected to 'mass screening programme'.

(Received for publication July 12, 1976)



One out of all the compounds synthesized (Formula I, R=CH<sub>3</sub>), showed anti-amoebic activity against *E. histolytica*, *in vitro* as good as emetine.

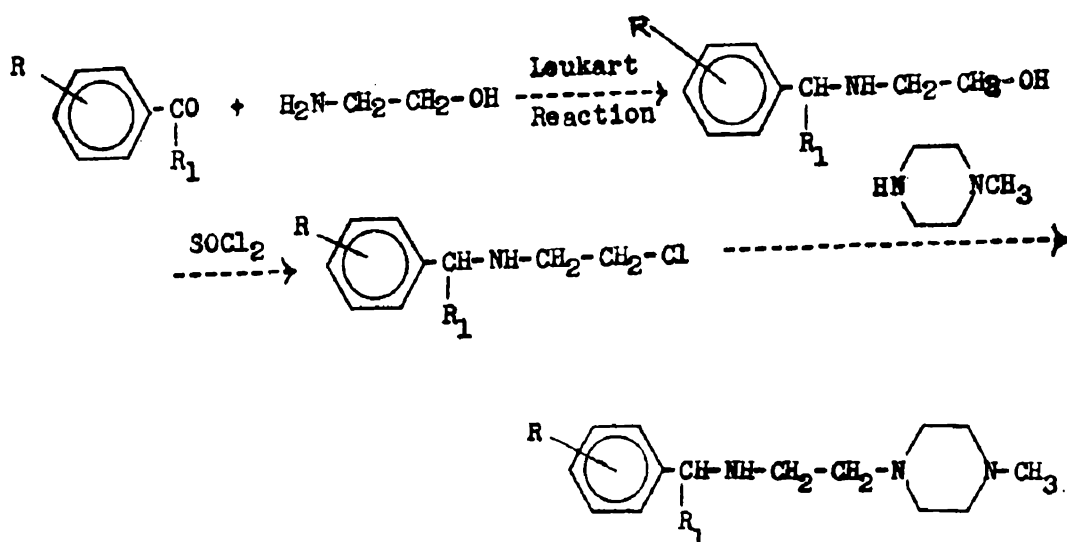
This was really a novel moiety for antiamoebic activity against *E. histolytica*. Having obtained the lead, a systematic study of the molecule was taken up, and

as so doing the following two points have been investigated :

- (i) importance of the Cl atom in the para position and
- (ii) effect of replacement of one phenyl ring with small alkyl chains.

### Chemistry

Compounds were synthesised through the following series of reactions :



When  $R_1$  was phenyl i.e. for benzophenone the yield of Leukart reaction was low. Hence the ketone was reduced to benzhydrol, then converted into benzhydryl chloride and then condensed with 2-amino ethanol to give desired intermediate.

### Experimental

Following is the typical synthetic method for the preparation of piperazine derivatives discussed in this paper and all the compounds are listed in Table I.

*Synthesis of N<sup>1</sup>-[β-(o-chloro-α-phenylbenzylamino)-ethyl]-N<sup>4</sup>-methylpiperazine—* A mixture of N-methylpiperazine (10 g, 0.1 mole), triethylamine (5.0 g, 0.05

mole) and β-(o-chloro-α-phenylbenzylamino) ethylchloride hydrochloride (6.33g, 0.02 mole) in EtOH (35 ml) was refluxed for 24 hrs. The residue obtained after removal of solvent was basified with cold aqueous 40 per cent NaOH and was extracted with  $\text{CHCl}_3$ . The combined  $\text{CHCl}_3$  extracts were dried ( $\text{Na}_2\text{SO}_4$ ), concentrated and the residual viscous oil was subjected to fractional distillation *in vacuo*.

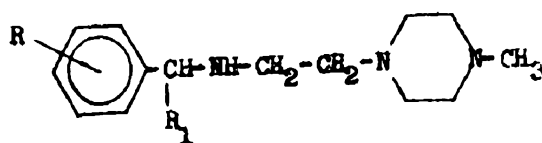
The fraction distilling between 210–220°C at 3 to 5 mm was collected.

The maleate salt of the above base was prepared by adding the ethereal solution of the base to a solution of maleic acid in alcohol. The solid thus obtained was recrystallized from alcohol, m.p. 160–162°C, yield : 60 per cent.

Anal. calcd. for  $\text{C}_{32}\text{H}_{38}\text{ClN}_3\text{O}_{12}$  : C, 55.51; H, 5.49; N, 6.07 per cent

Found : C, 55.72; H, 5.65; N, 6.13 per cent.

TABLE I

*N*<sup>1</sup>-[β-(substituted benzylamino)ethyl]-*N*<sup>4</sup>-methyl piperazines

| No. | R                    | R <sub>1</sub>                              | Yield*†<br>(per cent) | Salt<br>prepared                               | M.p.°C<br>(uncorr.) | Minimum inhibitory<br>concentration‡<br>(μg/ml) |
|-----|----------------------|---------------------------------------------|-----------------------|------------------------------------------------|---------------------|-------------------------------------------------|
| 1   | H                    | Ph                                          | 50                    | 3-HCl                                          | 250–252             | 100                                             |
| 2   | 2-Cl                 | Ph                                          | 40                    | 3-HCl                                          | 262–263             | 100                                             |
|     |                      |                                             |                       | 3-C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> | 160–162             | 100                                             |
| 3   | 3-Cl                 | Ph                                          | 42                    | 3-C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> | 158–159             | 100                                             |
| 4   | 2, 4-Cl <sub>2</sub> | Ph                                          | 45                    | 3-HCl                                          | 260–261             | 100                                             |
| 5   | 4-Cl                 | H                                           | 62                    | 3-HCl                                          | 240–242             | NS                                              |
| 6   | 4-Cl                 | CH <sub>3</sub>                             | 42                    | 3-HCl                                          | 248–250             | NS                                              |
| 7   | 4-Cl                 | C <sub>2</sub> H <sub>5</sub>               | 47                    | 3-HCl                                          | 240–242             | NS                                              |
|     |                      |                                             |                       | 3-C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> | 188–190             | NS                                              |
| 8   | 4-Cl                 | C <sub>3</sub> H <sub>7</sub> ( <i>n</i> )  | 45                    | 3-HCl                                          | 242–244             | NS                                              |
| 9   | 4-Cl                 | C <sub>4</sub> H <sub>9</sub> ( <i>n</i> )  | 42                    | 3-HCl                                          | 239–242             | 100                                             |
| 10  | 4-Cl                 | C <sub>5</sub> H <sub>11</sub> ( <i>n</i> ) | 47                    | 3-C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> | 162–163             | 50                                              |
| 11  | 4-Cl                 | C <sub>6</sub> H <sub>13</sub> ( <i>n</i> ) | 45                    | 3-C <sub>4</sub> H <sub>4</sub> O <sub>4</sub> | 164–166             | 5                                               |

\* Yields reported are the results of single experiment and are calculated after the material melting not less than 3–4° below the highest melting point obtained.

† Analysed correctly for C, H, N.

‡ NS = Not significant (> 100 μg/ml).

## IN VITRO SCREENING

### Materials and Methods

Culture of *E. histolytica* used for this purpose was obtained from Ciba Research Centre. It was maintained in Dobell's medium (14) and L.M.S. medium (15).

Few standard drugs and synthetic compounds were screened *in vitro* by using 'Woelfes capillary method' (1957).

Solutions of the drugs of required concentration were prepared in the medium itself. If the drugs were not soluble very fine suspensions were used, prepared either by titration in the presence of a wetting agent tween-80 or by adding gum acacia. These two were found to exhibit no effect on amoebiasis at the concentration used. The capillaries were

incubated at 37°C for 48 hrs. The amoebicidal end point was taken as the minimum concentration of the drug in the tube in which no amoeba were seen. All the compounds were tested at the concentration ranging from 5 μg/ml to 100 μg/ml.

### Results

Emetine hydrochloride and metronidazole were found active *in vitro* at the concentration of 5 μg/ml and 10 μg/ml respectively. The reported values in the literature are emetine 0.2–60 μg/ml (16) and metronidazole 0.25 μg/ml (17).

In case of synthetic compounds eight were found active *in vitro* against *E. histolytica* at the concentration ranging from 5–100 μg/ml (Table I).

### Conclusions

The position of chloro from *para* to *ortho* or to *meta* or the complete removal of the same did not make appreciable change in activity. Also addition of one more Cl atom in the molecule (2, 4-dichloro compound) did not alter the activity indicating that chlorine atom does not play important role for activity.

When the phenyl ring was removed, no significant activity was observed for compounds having H or small alkyl chain.

When alkyl chain was increased to six carbon atom (Compound No. 11) it showed activity of 5  $\mu$ g/ml against *E. histolytica* *in vitro*.

### Acknowledgements

The authors thank Mr. M. T. Jaokar and his associates for microanalyses, Dr. H. K. Sen from CIBA Research Centre for providing the amoebic culture, and Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay, for providing facilities to carry out the present work.

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# SYNTHESIS AND STUDY OF 2-[p-(2-SUBSTITUTED-4-THIAZOLYL) ARYLAMINO]PYRIDO (3, 2-d) THIAZOLES AS POSSIBLE ANTHELMINTIC AGENTS

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Pyridothiazole derivatives reported from this laboratory have been found to possess good anthelmintic activity against *N. dubius* in mice. In view of the above findings, it was proposed to synthesise more compounds and test them for the anthelmintic activity.

## Introduction

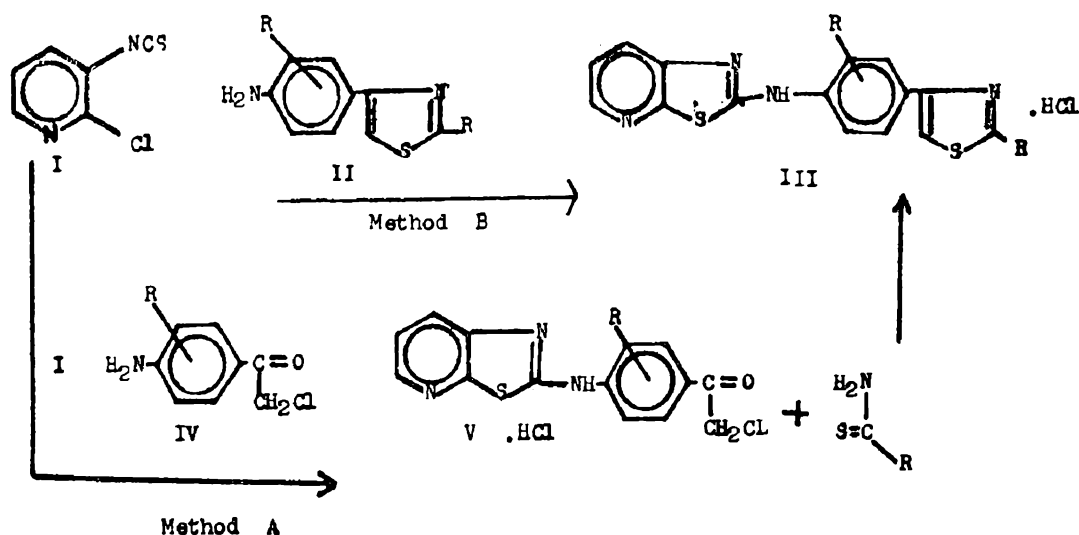
Pyrido thiazole derivatives reported from this laboratory have been found to possess anthelmintic activity against *N. dubius* in mice and one of these compounds, showed 92 per cent mortality rate of the adult worm at the dose level of 0.2 mg. It was therefore decided to synthesize a series of 2-[p-(substituted-4-thiazolyl) arylamino] pyrido (3, 2-d) thiazoles and test them against *N. dubius*.

## Chemistry

These compounds were synthesised by two routes :

(1) 2-Chloro-3-isothiocyanatopyridine was condensed with 2-substituted-4'-(p-aminophenyl)thiazole. The various 4-(p-aminophenyl) thiazoles were synthesised by reported methods (2).

(2) 2-Chloro-3-isothiocyanatopyridine was condensed with 4'-amino-2-chloroacetophenone (Table I) and



(Received for publication November 8, 1976)

3-chloro-4-amino-2-chloroacetophenone the resultant compound was condensed with different thioamides to give 2-[*p*-(2-substituted-4-thiazolyl) arylamino]pyrido (3,2-*d*)thiazoles and 2-[2-chloro-4-(2-substituted-4-thiazolyl)phenyl] amino]pyrido (3, 2-*d*) thiazoles (Table II) respectively.

### Experimental

**2-(4-Chloroacetylphenyl)amino pyrido (3, 2-*d*)thiazole hydrochloride**—To a solution of 2-chloro-3-isothiocyanatopyridine (1.7 g, 0.01 mole) in absolute ethanol (20 ml) was added 4'-amino-2-chloroacetophenone (1.69 g, 0.01 mole) and the reaction mixture refluxed on water-bath for 6-7 hrs. The crystalline compound that separated was filtered hot and washed with warm alcohol.

Yield : 60 per cent, m.p. 272–274°C.

Similarly 2-(2'-chloro-4-chloroacetylphenyl) aminopyrido (3, 2-*d*)thiazole was prepared in 52 per cent yield m.p. 279–283°C.  $C_{14}H_9Cl_2N_3O.S.HCl$ .

**2-[4-(2-(3-Ethoxy-4-propoxyphenyl)-4-thiazolylphenyl)-amino]pyrido (3, 2-*d*)thiazole (5): Method A**—A mixture of 2-(4'-chloroacetylphenyl) amino pyrido (3, 2-*d*)thiazole hydrochloride (1.7 g, 0.005 mole) and 3-ethoxy-4-propoxythiobenzamide (1.32 g, 0.005 mole) in dry alcohol (100 ml) was refluxed for 1 hr, cooled, filtered, washed with alcohol and then with ether.

Yield : 2.4 g, 84 per cent, m.p. 231–235°C.

The above hydrochloride was basified to give the free base m.p. 171–173°C.

**2-[4-(2-(3-Ethoxy-4-propoxyphenyl)-4-thiazolylphenyl)-amino]pyrido (3, 2-*d*)thiazole (5): Method B**—2-Chloro-3-isothiocyanatopyridine 1.7 g, 0.01 mole (20 ml) and 2-(3-ethoxy-4-propoxy)-4-(*p*-aminophenyl)thiazole (3.54 g, 0.01 mole) in absolute alcohol (20 ml) were refluxed on water-bath for 6-8 hrs. The crystalline

solid that separated was filtered while hot and washed with warm alcohol.

Yield : 3.69 g, 70 per cent, m.p. 192–194°C, free base, m.p. 171–173°C.

Basification with aq.  $NH_4OH$  gave the free base, m.p. 171–173°C, which showed no depression in mixed m.p. sample obtained from Method A.

**2-[2'-Chloro-4[2-(3-Methoxy-4-benzoyloxyphenyl)-4-thiazolyl]-phenyl]amino]pyrido (3, 2-*d*)thiazole dihydrochloride (13)**—A mixture of 2-(2'-chloro-4-chloroacetylphenyl)pyrido (3, 2-*d*)thiazole hydrochloride (1.87 g, 0.005 mole) and 3-methoxy-4-benzoyloxythiobenzamide (1.35 g, 0.005 mole) were taken in 100 ml of dry alcohol and refluxed for one hr. cooled and the product filtered, washed with alcohol and then with ether.

Yield : 2.52 g, 80%, m.p. 207–210°C.  $C_{29}H_{21}ClN_4O_2S_2 \cdot 2HCl$

### Biological Screening Results

The compounds reported herein were tested against *N. dubius* in mice by the method described in literature (3). Ten mice were used for infection for each compound. L-Tetramisole was taken as the standard drug, which shows 100 per cent mortality rate at the dose level of 0.1 mg. Compound No. 1 (Table I) showed mortality of the adult worms upto 90 per cent at 1 mg dose level and 94 per cent at 5 mg dose. Compound No. 2 showed 86 per cent mortality rate at 5 mg dose level; and compound No. 5 showed 74 per cent and 88 per cent mortality at 1 mg and 5 mg dose level respectively. The other compounds did not show significant activity.

### Acknowledgements

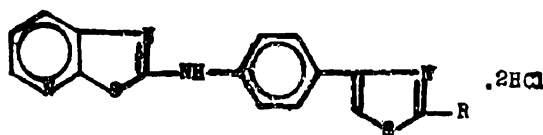
The authors wish to thank Dr. M. H. Shah, Assistant Director, Department of Chemotherapy, Haffkine Institute, Bombay for his keen interest in present work. They are

indebted to Mr. M. T. Jaokar and his colleagues for elemental microanalyses. They are also grateful to Dr. B.B. Gaitonde

Director, Haffkine Institute, Bombay for providing facilities to carry out the present work.

TABLE I

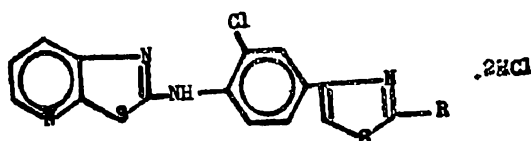
*2-4-[(2-substituted-4-thiazolyl)phenyl]amino]pyrido (3, 2-d) thiazoles*



| Serial No. | R                                 | M.p. °C* | Molecular formula†                                                                 |
|------------|-----------------------------------|----------|------------------------------------------------------------------------------------|
| 1          | Methyl .. ..                      | 249-252  | C <sub>16</sub> H <sub>12</sub> N <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 2          | 3, 4-Dimethoxyphenyl .. ..        | 246-249  | C <sub>23</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 3          | 3, 4-Diethoxyphenyl .. ..         | 242-246  | C <sub>26</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 4          | 3-Methoxy-4-propoxyphenyl .. ..   | 230-233  | C <sub>26</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 5          | 3-Ethoxy-4-propoxyphenyl .. ..    | 231-235  | C <sub>26</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 6          | 3-Methoxy-4-benzyloxyphenyl .. .. | 196-199  | C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 7          | 3-Ethoxy-4-benzyloxyphenyl .. ..  | 213-216  | C <sub>30</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 8          | <i>p</i> -Tolyl .. ..             | 256-259  | C <sub>22</sub> H <sub>16</sub> N <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 9          | 3-Nicotinyl .. ..                 | 213-216  | C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> S <sub>2</sub> ·2HCl                |

TABLE II

*2-[2-Chloro-4-[(2-substituted-4-thiazolyl)phenyl]amino] pyrido (3, 2-d) thiazoles dihydrochloride*



| Serial No. | R                                 | M.p. °C* | Molecular formula†                                                                   |
|------------|-----------------------------------|----------|--------------------------------------------------------------------------------------|
| 10         | 3, 4-Diethoxyphenyl .. ..         | 213-216  | C <sub>26</sub> H <sub>21</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 11         | 3-Methoxy-4-propoxyphenyl .. ..   | 210-213  | C <sub>26</sub> H <sub>21</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 12         | 3-Ethoxy-4-propoxyphenyl .. ..    | 205-209  | C <sub>26</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 13         | Methyl .. ..                      | 253-256  | C <sub>16</sub> H <sub>11</sub> ClN <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 14         | Benzyl .. ..                      | 219-222  | C <sub>22</sub> H <sub>15</sub> ClN <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 15         | 3, 4-Dimethoxyphenyl .. ..        | 248-251  | C <sub>23</sub> H <sub>17</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 16         | 3-Ethoxy-4-methoxyphenyl .. ..    | 228-231  | C <sub>24</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 17         | 3-Ethoxy-4-benzyloxyphenyl .. ..  | 207-210  | C <sub>30</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 18         | 3-Methoxy-4-benzyloxyphenyl .. .. | 207-210  | C <sub>28</sub> H <sub>21</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 19         | <i>p</i> -methoxyphenyl .. ..     | 238-241  | C <sub>22</sub> H <sub>15</sub> ClN <sub>4</sub> O <sub>2</sub> S <sub>2</sub> ·2HCl |
| 20         | Phenyl .. ..                      | 245-248  | C <sub>21</sub> H <sub>13</sub> ClN <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 21         | <i>p</i> -Tolyl .. ..             | 245-248  | C <sub>22</sub> H <sub>15</sub> ClN <sub>4</sub> S <sub>2</sub> ·2HCl                |
| 22         | 3-Nicotinyl .. ..                 | 215-219  | C <sub>20</sub> H <sub>13</sub> ClN <sub>5</sub> S <sub>2</sub> ·2HCl                |

\*Melting points are capillary melting points and are uncorrected.

†C, H, and N are analysed correctly within  $\pm 0.4$  per cent.

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## SYNTHESIS AND SCREENING OF SCHIFF BASES AS ANTIINFLAMMATORY AGENTS—I

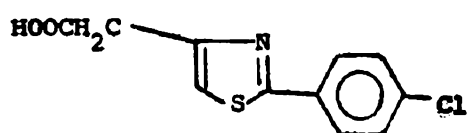
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A series of Schiff bases from 2-(*p*-aminophenyl) thiazoles and indole-3-aldehydes have been synthesized and screened for antiinflammatory activity. One compound showed 40 per cent inhibition of edema in the carrageenin induced rat paw edema test.

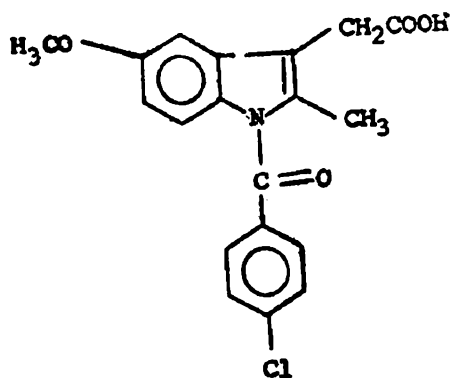
### Introduction

Fenclozic acid (I), 2-(4-chlorophenyl) thiazol-4-ylacetic acid, a thiazole derivative has significant antiinflammatory activity (1).



(I)

Indomethacin (II), an indole derivative (2) is a well-known antiinflammatory drug in clinical use today.

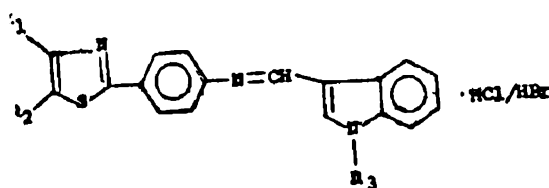


(II)

It was therefore thought worthwhile to prepare compounds having these two moieties which would show good antiinflammatory activity.

The compounds possessing the azomethine linkage have biological activities such as antitubercular, antibacterial, antiviral, antimalarial, antileprotic and anticancer. Further a series of naphthalene Schiff bases has been shown to possess antiinflammatory activity (3).

In the present work, both the moieties e.g. thiazole and indole have been linked through a Schiff base linkage to form compounds with antiinflammatory activity. A series of Schiff bases (III) has been synthesized and screened for its antiinflammatory activity.



(III)

### Chemistry

The general method adopted for the preparation of Schiff bases (III) is the heating of a mixture of the amine hydrochloride and aldehyde in alcohol.

Indole-3-aldehyde was prepared from the indole and dimethylformamide and phosphorus oxychloride. Substituted indole-3-aldehydes were prepared from indole-3-aldehyde by methylation, acetylation, benzylation and benzoylation.

The required 2-(*p*-aminophenyl)thiazoles were prepared as their hydrochlorides by condensing equimolecular quantities of appropriate  $\alpha$  haloketones and *p*-aminothiobenzamide in boiling ethanol. The thiazole hydrochlorides were obtained in a sufficiently pure state for further reactions.

### Experimental

*1-(p-chlorobenzoyl) indole-3-aldehyde*—To a constantly stirred mixture of indole-3-aldehyde (2.9 g, 0.02 mole) in dry pyridine (30 ml) was added *p*-chlorobenzoyl chloride (4.85 g, 0.03 mole) dropwise. After 5 hrs. of heating on a steam bath, the mixture was treated with dilute hydrochloric acid. Stirring was continued for 30 min. and the resultant brown coloured solid was filtered, dried and crystallized from ethyl alcohol to give pure 1-(*p*-chlorobenzoyl indole-3-aldehyde.

Yield : 3.69 g, 65 per cent, m.p. 125–127 °C. Anal. calcd. for  $C_{16}H_{10}ClNO_2$  : C, 67.63 per cent; H, 3.52 per cent; N, 4.93 per cent. Found : C, 67.86 per cent; H, 3.52 per cent; N, 5.24 per cent.

*5-Carbethoxy-4-methyl-2-(p-aminophenyl) thiazole hydrochloride*—*p*-Aminothiobenzamide (0.76 g, 0.005 mole) was dissolved in boiling acetone (2 ml). When this cooled down  $\alpha$ -chloroethylacetoacetate (0.82 g, 0.005 mole) was added to it with shaking.

This mixture was kept aside for about 3 to 4 hrs. Then a solid was formed which was filtered, washed with ether and dried in air.

Yield : 0.98 g, 65.8 per cent, m.p. 214–218 °C. Anal. calcd. for  $C_{13}H_{15}ClN_2O_3S$  : C, 52.3 per cent; H, 5.0 per cent; N, 9.38 per cent. Found : C, 52.5 per cent; H, 5.2 per cent; N, 9.44 per cent.

*p-[2-(Aminophenyl)-4-thiazolyl] phenoxyacetic acid hydrochloride*—A mixture of 4-chloroacetylphenoxyacetic acid (1.14 g, 0.005 mole), *p*-aminothiobenzamide (0.76 g, 0.005 mole) and ethyl alcohol (10 ml) was heated gently at reflux temperature for about 3 hrs. A compound was thrown out while refluxing. The mixture was cooled and the solid filtered, washed with ether and dried in air.

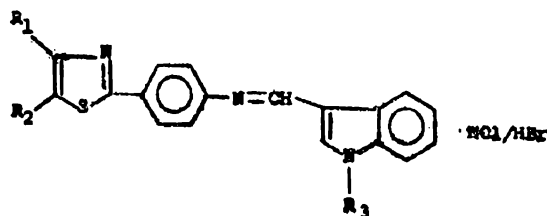
Yield : 0.92 g, 48.4 per cent, m.p. 303–305 °C. Anal. calcd. for  $C_{17}H_{15}ClN_2O_3S$  : C, 56.30 per cent; H, 4.14 per cent; N, 7.73 per cent. Found : C, 56.06 per cent; H, 3.93 per cent; N, 7.38 per cent.

*4-Phenyl-2-[p-(3-indolydine)amino]phenylthiazole hydrobromide*—To a solution of 4-phenyl-2-(*p*-aminophenyl)thiazole hydrobromide (3.33 g, 0.01 mole) in ethyl alcohol was added a hot solution of indole-3-aldehyde (1.45 g, 0.01 mole) in ethyl alcohol. The resulting yellowish-orange solution was boiled for 2–3 min. On cooling to room temperature, it deposited a solid. This was collected by filtration, washed with dry ethyl alcohol and then with a little ether to give the desired Schiff base as its hydrobromide salt.

Yield : 3.7 g, 80.0 per cent, m.p. 284–285 °C.

All the other Schiff bases were similarly prepared and are listed in Table I.

TABLE I

*Schiff bases from 4, 5 disubstituted 2-(p-aminophenyl) thiazoles and indole-3-aldehydes*

| Serial No. | R <sub>1</sub>                                                  | R <sub>2</sub>                   | R <sub>3</sub>                                      | M.p. C.*<br>(Uncorr.) | Molecular formula†                                                    | Per cent of edema‡ |
|------------|-----------------------------------------------------------------|----------------------------------|-----------------------------------------------------|-----------------------|-----------------------------------------------------------------------|--------------------|
| 1          | C <sub>6</sub> H <sub>5</sub>                                   | H                                | H                                                   | 284–285               | C <sub>24</sub> H <sub>17</sub> N <sub>3</sub> S·HBr.                 | 6.2                |
| 2          | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | H                                | H                                                   | 238–240               | C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> OS·HCl                 | 8.1                |
| 3          | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | H                                                   | 209–212               | C <sub>22</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub> S·HCl   | 13.9               |
| 4          | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | H                                                   | 180–182               | C <sub>26</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub> S·HCl   | 20.0               |
| 5          | C <sub>6</sub> H <sub>5</sub>                                   | H                                | CH <sub>3</sub>                                     | 274–276               | C <sub>25</sub> H <sub>19</sub> N <sub>3</sub> S·HBr.                 | 24.5               |
| 6          | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | H                                | CH <sub>3</sub>                                     | 245–248               | C <sub>27</sub> H <sub>22</sub> N <sub>4</sub> OS·HCl                 | Nil                |
| 7          | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub>                                     | 246–248               | C <sub>28</sub> H <sub>21</sub> N <sub>3</sub> O <sub>2</sub> S·HCl   | 21.0               |
| 8          | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | CH <sub>3</sub>                                     | 205–208               | C <sub>27</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S·HCl   | 9.3                |
| 9          | C <sub>6</sub> H <sub>5</sub>                                   | H                                | COCH <sub>3</sub>                                   | 220–225               | C <sub>26</sub> H <sub>19</sub> N <sub>3</sub> OS·HBr.                | 17.1               |
| 10         | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | H                                | COCH <sub>3</sub>                                   | 213–215               | C <sub>28</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> S·HCl   | 9.0                |
| 11         | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | COCH <sub>3</sub>                                   | 225–228               | C <sub>24</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S·HCl   | 6.4                |
| 12         | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | COCH <sub>3</sub>                                   | 204–205               | C <sub>28</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub> S·HCl   | 1.6                |
| 13         | C <sub>6</sub> H <sub>5</sub>                                   | H                                | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>       | 270–272               | C <sub>31</sub> H <sub>23</sub> N <sub>3</sub> S·HBr.                 | 40.2               |
| 14         | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | H                                | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>       | 250–252               | C <sub>33</sub> H <sub>26</sub> N <sub>4</sub> OS·HCl                 | 36.9               |
| 15         | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>       | 238–240               | C <sub>29</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> S·HCl   | 4.2                |
| 16         | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>       | 208–211               | C <sub>33</sub> H <sub>25</sub> N <sub>3</sub> O <sub>3</sub> S·HCl   | Nil                |
| 17         | C <sub>6</sub> H <sub>5</sub>                                   | H                                | CO·C <sub>6</sub> H <sub>5</sub>                    | 229–232               | C <sub>31</sub> H <sub>21</sub> N <sub>3</sub> OS·HBr.                | 4.5                |
| 18         | C <sub>6</sub> H <sub>5</sub>                                   | H                                | CO( <i>p</i> -Cl<br>C <sub>6</sub> H <sub>4</sub> ) | 158–160               | C <sub>31</sub> H <sub>20</sub> ClN <sub>3</sub> OS·HBr.              | 6.0                |
| 19         | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | COC <sub>6</sub> H <sub>5</sub>                     | 168–170               | C <sub>29</sub> H <sub>23</sub> N <sub>3</sub> O <sub>3</sub> S·HCl   | 17.4               |
| 20         | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | CO( <i>p</i> -Cl<br>C <sub>6</sub> H <sub>4</sub> ) | 159–161               | C <sub>29</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>3</sub> S·HCl | 4.0                |

\* All melting points are capillary melting points and are uncorrected.

† Analysed correctly for C, H, N.

‡ Reference : Phenylbutazone (50.5 per cent inhibition of edema). Dose = 100mg/kg.

Test compound, dose = 100 mg/kg each; Number of animals used = 6 rats per group.

### Results and Discussion

The compounds synthesized in the present work were screened for their antiinflammatory activity by the method of carrageenin induced edema in rat hind paw (4).

As seen in Table I compounds 13 and 14 displayed good activity. Compound 13 was the most active of the series, showing 40 per cent inhibition of edema. This compound had benzyl substitution at the N position of indole.

### Acknowledgements

The authors are indebted to Dr. M. H. Shah, Assistant Director, Department of Chemotherapy, for his helpful suggestions and also to Dr. D. T. Chaudhari, S.S.O.I. for the encouragement and to Mr. M. T. Jaokar and his associates for the elemental microanalyses. They are thankful to Mr. P. Y. Naik, Assistant Research Officer, C.C.R.I.M.H. Unit No. 2 for his co-operation. They are grateful to Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay 400 012, for providing facilities to carry out the present work.

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## SYNTHESIS AND SCREENING OF SCHIFF BASES AS ANTIINFLAMMATORY AGENT—II

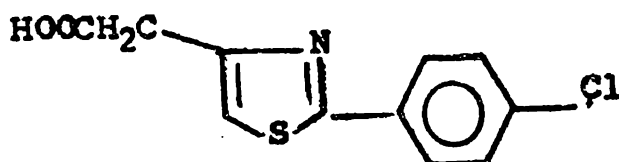
NIRMALA J. KHER, P. Y. NAIK, S. S. SABNIS AND M. B. BHIDE

*Haffkine Institute, Parel, Bombay 400 012*

A series of Schiff bases from 4, 5 di-substituted 2-(*p*-aminophenyl) thiazoles and 4-formylphenoxyacetic acids was synthesised and screened for their antiinflammatory activity. 4-(*p*-Acetamidophenyl)-2-[4-(*p*-carboxy methoxy) benzylideneaminophenyl] thiazole hydrochloride was found as active as phenylbutazone in carrageenin induced edema in rat hind paw. The same compound showed significant analgesic and antipyretic activity.

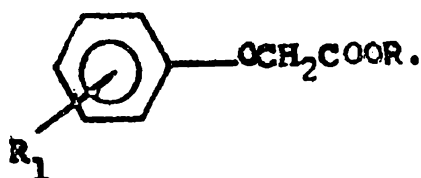
### Introduction

Fenclozic acid (I) 2-(4-chlorophenyl) thiazole 4-ylacetic acid is reported to have antiinflammatory activity (1).



( I )

Northover and others have stated that certain derivatives of phenoxyacetic acids (II) are effective against inflammation (2).

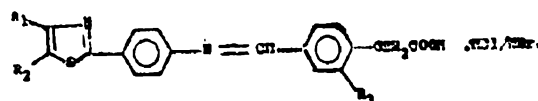


( II )

In the present work it was therefore decided to introduce these two moieties in the new compounds to be prepared.

(Received for publication November 8, 1976)

Further, certain naphthalene Schiff bases are known to be antiinflammatory (3). In view of the important, once of the azomethine linkage in medicinal chemistry, a series of Schiff bases (III) from 4, 5 di-substituted 2-(*p*-aminophenyl)thiazoles and 4 formyl phenoxyacetic acids was synthesized and screened for antiinflammatory activity.



(III)

$R_1, R_2 = \text{alkyl or aryl}$

$R_3 = \text{H or OCH}_3$

### Chemistry

The required 4, 5 di-substituted 2-(*p*-aminophenyl)thiazoles were prepared by condensing equimolecular quantities of respective haloketones with *p*-aminothiobenzamide.

The 4-formylphenoxyacetic acids were synthesized by treating respective hydroxybenzaldehydes with chloroacetic acid.

The Schiff bases were prepared by the general method of heating the appropriate

amine hydrochloride or hydrobromide with aldehyde in alcohol.

All the Schiff bases thus synthesized are listed in Table I.

### Experimental

4-(*p*-Acetamidophenyl)-2-[4(*p*-carboxymethoxy)benzylideneaminophenyl]thiazole hydrochloride—4-(*p*-Acetamidophenyl)-2-(*p*-aminophenyl)thiazole hydrochloride (3.45 g, 0.01 mole) was dissolved in minimum quantity of hot ethyl alcohol.

A solution of 4-formylphenoxyacetic acid (1.80 g, 0.01 mole) in hot ethyl alcohol was added to the thiazole solution. The resulting yellowish-orange coloured solution was boiled on a steam bath and then cooled. This resulted in deposition of a solid which was filtered and washed with ether and dried.

Yield: 3.9 g, 73.5 per cent, m.p. 234–235°C. Anal. Calcd. for  $C_{26}H_{22}ClN_3O_4S$ : C, 61.4 per cent; H, 4.33 per cent; N, 8.27 per cent; Found: C, 61.03 per cent; H, 4.32 per cent; N, 8.02 per cent.

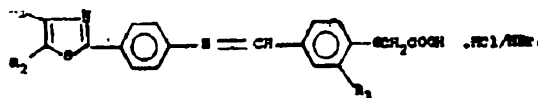
### Biological Activity

The compounds synthesized were screened for their probable antiinflammatory activity by the method of carrageenin induced hind paw edema in rats (4). The compound under study were administered orally one hour prior to the injection of carrageenin.

The compound 2 showing promising activity as compared to phenylbutazone in carrageenin induced hind paw edema was screened further following the method of cotton pellet granuloma (5), and heat induced hemolysis method (6).

TABLE I

*Schiff bases from 2-(p-aminophenyl) thiazole and 4-formylphenoxyacetic acid*



| Serial No. | R <sub>1</sub>                                                         | R <sub>2</sub>                   | R <sub>3</sub>   | M.p. °C* (uncorr) | Molecular Formula†                                                     | Per cent inhibition of edema‡ |
|------------|------------------------------------------------------------------------|----------------------------------|------------------|-------------------|------------------------------------------------------------------------|-------------------------------|
| 1          | C <sub>6</sub> H <sub>5</sub>                                          | H                                | H                | 232–235           | C <sub>24</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub> S · HBr. | 18.3                          |
| 2          | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>            | H                                | H                | 234–235           | C <sub>26</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub> S · HCl  | 45.8                          |
| 3          | CH <sub>3</sub>                                                        | COOC <sub>2</sub> H <sub>5</sub> | H                | 224–225           | C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S · HCl  | 12.8                          |
| 4          | <i>p</i> -HOOC · H <sub>2</sub> C · O · C <sub>6</sub> H <sub>5</sub>  | H                                | H                | 240–242           | C <sub>26</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub> S · HCl  | Nil                           |
| 5          | C <sub>6</sub> H <sub>5</sub>                                          | H                                | OCH <sub>3</sub> | 284–288           | C <sub>25</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> S · HBr. | 2.7                           |
| 6          | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>            | H                                | OCH <sub>3</sub> | 235–238           | C <sub>27</sub> H <sub>23</sub> N <sub>3</sub> O <sub>5</sub> S · HCl  | 1.8                           |
| 7          | CH <sub>3</sub>                                                        | COOC <sub>2</sub> H <sub>5</sub> | OCH <sub>3</sub> | 200–202           | C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub> S · HCl  | 13.6                          |
| 8          | <i>p</i> -HOOC · H <sub>2</sub> C · O · OC <sub>6</sub> H <sub>4</sub> | H                                | OCH <sub>3</sub> | 208–210           | C <sub>27</sub> H <sub>22</sub> N <sub>2</sub> O <sub>7</sub> S · HCl  | 10.3                          |

\* All melting points are capillary melting points and are uncorrected.

† Analysed correctly for C, H, N.

‡ Reference : Phenylbutazone (50.5 per cent inhibition of edema). Dose = 100 mg/kg.

Test compound, dose = 100 mg/kg each.

Number of animals used = 6 rats per group.

**Cotton Pellet Granuloma Test**—Cotton pellets implanted subcutaneously produce granuloma which can be removed and weighed. The antiinflammatory drugs inhibit the growth of granuloma. The compound 2 was screened to study its effects on chronic types of inflammation by the method described elsewhere (5).

Phenylbutazone (100 mg/kg) and compound 2 (100 mg/kg) caused 52.1 and 34.4 per cent inhibition of growth of granuloma respectively (Table II).

TABLE II

*Cotton-pellet granuloma method*

| Drug           | Dose<br>mg/kg<br>daily | Dry weight<br>mg $\pm$ S.E. | Inhibition of<br>granuloma<br>per cent |
|----------------|------------------------|-----------------------------|----------------------------------------|
| Control        |                        | 93.5 $\pm$ 1.81             |                                        |
| Phenylbutazone | 100                    | 44.8 $\pm$ 2.3              | 52.1                                   |
| Compound (2)   | 100                    | 61.0 $\pm$ 1.0              | 34.4                                   |

### Heat Induced Hemolysis Test

Lysosomal rupture results in cell death or injury to surrounding tissues and is also documented to mediate acute inflammation. A number of non-steroidal anti-inflammatory agents are known to inhibit heat induced lysis of erythrocytes. The membrane of the erythrocytes may be considered as a model of the lysosomal membrane. Therefore inhibition of heat induced lysis of erythrocytes by compound 2 was determined by the method described elsewhere (6).

The inhibition of heat induced hemolysis was 59.9 per cent and 42.1 per cent by phenylbutazone and compound 2 respectively, at the concentration of  $5 \times 10^{-5}$ M. Whereas at the concentration of  $5 \times 10^{-5}$ M

the inhibition was 74.5 per cent and 69.5 per cent respectively (Table III).

TABLE III

*Heat-induced hemolysis method*

| Drug           | Concentration     | Inhibition<br>per cent |
|----------------|-------------------|------------------------|
| Phenylbutazone | $5 \pm 10^{-4}$ M | 74.5                   |
|                | $5 \pm 10^{-5}$ M | 59.9                   |
| Compound 2     | $5 \pm 10^{-4}$ M | 69.5                   |
|                | $5 \pm 10^{-5}$ M | 42.1                   |

The compound 2 exhibiting promising antiinflammatory activity was screened to probe into its potential analgesic and antipyretic activities as these two activities are associated with most of the antiinflammatory agents.

### Analgesic Activity

Analgesic activity was evaluated in mice by tail clip method (7) and by writhing method (8). The compound 2 and aspirin were administered orally in the dose of 200 mg/kg.

Table IV and Table V illustrate the analgesic activity of compound 2 as compared to aspirin.

### Antipyretic Activity

The antipyretic activity was determined (9) by injecting yeast suspension subcutaneously. Rectal temperature was measured before administration of the compound and 1, 3 and 5 hrs. after the administration of compound. Compound 2 reduced the rectal temperature of hyperthermic rats in the dose of 100 mg/kg (Table VI).

TABLE IV

*Tail Clip Method*

| Drug               | Dose mg/kg orally | Time in minutes | No. of animals prolonged reaction time | Analgesic activity per cent |
|--------------------|-------------------|-----------------|----------------------------------------|-----------------------------|
| Control            | ....              | 15              | 0                                      |                             |
|                    |                   | 30              | 0                                      |                             |
|                    |                   | 45              | 0                                      |                             |
|                    |                   | 60              | 0                                      |                             |
| Aspirin .. .. .    | 200               | 15              | 4                                      | 40                          |
|                    |                   | 30              | 2                                      | 20                          |
|                    |                   | 45              | 2                                      | 20                          |
|                    |                   | 60              | 0                                      | ....                        |
| Compound 2 .. .. . | 200               | 15              | 3                                      | 30                          |
|                    |                   | 30              | 2                                      | 20                          |
|                    |                   | 45              | 2                                      | 20                          |
|                    |                   | 60              | 0                                      | 0                           |

TABLE V

*Writhing Method*

| Drug               | Dose mg/kg | No. of writhing episodes $\pm$ S.E. | Analgesic activity per cent |
|--------------------|------------|-------------------------------------|-----------------------------|
| Control            |            | 40.6 $\pm$ 4.08                     |                             |
| Aspirin .. .. .    | 200        | 19.66 $\pm$ 2.43                    | 51.5                        |
| Compound 2 .. .. . | 200        | 20.3 $\pm$ 3.53                     | 50.0                        |

TABLE VI

*Action of (2) on Body Temperature of Pyretic Rats*

| Drug               | Dose mg/kg I.P. | Temperature fall in degrees (F) |                   |              |
|--------------------|-----------------|---------------------------------|-------------------|--------------|
|                    |                 | After 1 hr.                     | After 3 hrs.      | After 5 hrs. |
| Control            |                 | 0.25 <sup>R</sup>               | 0.20 <sup>R</sup> | 0.70         |
| Aspirin            | 100             | 1.8                             | 3.2               | 2.75         |
| Compound 2 .. .. . | 100             | 1.25                            | 1.95              | 2.1          |

R = Rise in temperature compared to febrile temperature.

### Discussion

A large number of thiazolyl derivatives were screened with special reference to their antiinflammatory activity. Suppression of carrageenin induced edema as advocated by Winter *et al.* was chosen as the screening method because of its specificity in evaluation of antiinflammatory compounds. Compound 2 is found to be as active as phenylbutazone in inhibiting carrageenin induced edema in rats. It inhibited the growth of granuloma indicating that it is active in chronic type of inflammation. Like other known antiinflammatory drugs, compound 2 also exhibited analgesic and antipyretic activity. Thus our study

reveals that further manipulation in the structure of this moiety may lead to a better antiinflammatory agent.

### Acknowledgements

The authors are indebted to Dr. M. H. Shah, Assistant Director, Department of Chemotherapy, for his helpful suggestions and also to Dr. D. T. Chaudhary, S.S.O.I., for their encouragement and to Mr. M. T. Jaokar and his associates for the elemental microanalyses. They are grateful to Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay 400 012, for providing facilities to carry out the present work.

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□ □

## SYNTHESIS AND SCREENING OF SCHIFF BASES AS ANTIINFLAMMATORY AGENTS—III

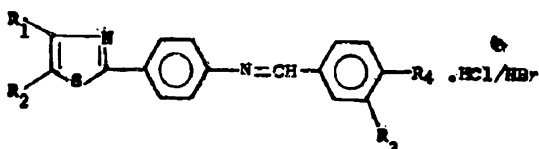
NIRMALA J. KHER, S. S. SABNIS AND M. B. BHIDE

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A series of Schiff bases from 4, 5-disubstituted 2-(*p*-aminophenyl) thiazoles and substituted benzaldehydes was synthesized and screened for their antiinflammatory activity by the method of carrageenin induced edema in hind paw of rats. A Schiff base with methoxy groups substituted at 3, 4-positions of benzaldehyde ring is the most active in the series.

### Introduction

In continuation of our previous work (1) we have synthesized another series of Schiff bases (I) and screened them for anti-inflammatory activity.



$R_1, R_2, R_3, R_4 = H, \text{ alkyl, Aryl.}$

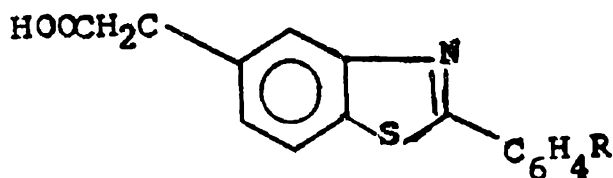
(I)

We have synthesized substituted 2-(*p*-aminophenyl) thiazoles and condensed them with various aromatic aldehydes.

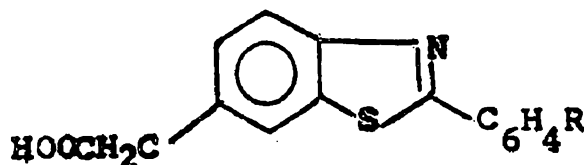
Wada *et al.* ((2) have reported 2-substituted 5 or 6 benzothiazole acetic acids (II), (III) as antiinflammatory. They have reported the compound with hydroxyl substituent to have enhanced the activity of parent compound.

Therefore we have selected benzaldehydes with substituents such as hydroxyl, acetamide, methoxy for condensation with thiazoles.

(Received for publication November 19, 1976)



( II )



( III )

### Chemistry

The Schiff bases were prepared by heating a mixture of 2-(*p*-aminophenyl)thiazoles and appropriate benzaldehydes in alcohol.

### Experimental

4-Phenyl-2-[(3, 4-dimethoxybenzylidene) aminophenyl]thiazole hydrobromide—1.66 grams (0.05 mole) of 4-phenyl-2-(*p*-aminophenyl)thiazole hydrobromide was dissolved in a minimum quantity of ethyl alcohol. 3, 4-dimethoxybenzaldehyde, 0.84 grams (0.05 mole) was dissolved in hot ethyl alcohol. Then these two solutions were mixed and refluxed for a few minutes. The resulting solution was concentrated and then cooled and the deposited solid was collected by filtration and washed with ether and air dried.

### Results and Discussion

The Schiff bases were screened for their antiinflammatory activity by the carrageenin induced adema in rat hind paw as described by Winter *et al.* (3). The results are shown in Table I. It can be observed from the results that in compound 4 the presence

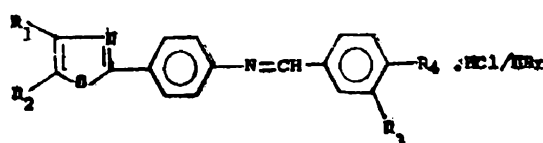
of hydroxyl group at para position and also the presence of phenoxyacetic acid grouping enhances the activity of the compound. Also the presence of dimethoxy group contributes well towards the activity of compound 6.

### Acknowledgements

The authors are indebted to Dr. M. H. Shah, Assistant Director, Department of Chemotherapy, for his helpful suggestions and also to Dr. D. T. Chaudhary, S.S.O.I. for the encouragement and to Mr. M. T. Jaokar and his associates for the elemental microanalyses. They are thankful to Mr. P. Y. Naik, Assistant Research Officer, C.C.R.I.M.H. Unit No. 2 for his co-operation. They are grateful to Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay 400 012, for providing facilities to carry out the present work.

TABLE I

*Schiff bases from 2-(p-aminophenyl)thiazoles and substituted benzaldehydes*



| Sr. No. | R <sub>1</sub>                                                  | R <sub>2</sub>                      | R <sub>3</sub>   | R <sub>4</sub>   | M.p.C°  | Molecular Formula†                                                   | Per cent inhibitions of edema‡ |
|---------|-----------------------------------------------------------------|-------------------------------------|------------------|------------------|---------|----------------------------------------------------------------------|--------------------------------|
| 1       | C <sub>6</sub> H <sub>5</sub>                                   | .. H                                | OCH <sub>3</sub> | OH               | 246–248 | C <sub>23</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub> S·HBr. | 7.6                            |
| 2       | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | .. H                                | OCH <sub>3</sub> | OH               | 238–240 | C <sub>25</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S·HCl  | 13.3                           |
| 3       | CH <sub>3</sub>                                                 | .. COOC <sub>2</sub> H <sub>5</sub> | OCH <sub>3</sub> | OH               | 242–245 | C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> S·HCl  | 15.8                           |
| 4       | <i>p</i> -HOOC-H <sub>2</sub> C.O.C <sub>6</sub> H <sub>4</sub> | H                                   | OCH <sub>3</sub> | OH               | 213–215 | C <sub>25</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S·HCl  | 36.2                           |
| 5       | C <sub>6</sub> H <sub>5</sub>                                   | .. H                                | OCH <sub>3</sub> | OCH <sub>3</sub> | 224–225 | C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S·HBr. | 24.7                           |
| 6       | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>     | .. H                                | OCH <sub>3</sub> | OCH <sub>3</sub> | 215–218 | C <sub>26</sub> H <sub>23</sub> N <sub>3</sub> O <sub>3</sub> S·HCl  | 33.0                           |
| 7       | CH <sub>3</sub>                                                 | .. COOC <sub>2</sub> H <sub>5</sub> | OCH <sub>3</sub> | OCH <sub>3</sub> | 235–238 | C <sub>22</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub> S·HCl  | 15.3                           |

TABLE I—*contd.*

| Sr. No. | R <sub>1</sub>                                                  | R <sub>2</sub>                   | R <sub>3</sub>   | R <sub>4</sub>      | M.p. °C* | Molecular Formula†                                                  | Per cent inhibitions of edema‡ |
|---------|-----------------------------------------------------------------|----------------------------------|------------------|---------------------|----------|---------------------------------------------------------------------|--------------------------------|
| 8       | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | OCH <sub>3</sub> | OCH <sub>3</sub>    | 203–205  | C <sub>26</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub> S·HCl | 18·6                           |
| 9       | C <sub>6</sub> H <sub>5</sub> ..                                | .. H                             | H                | NHCOCH <sub>3</sub> | 255–258  | C <sub>24</sub> H <sub>18</sub> N <sub>3</sub> O S·HBr.             | 13·5                           |
| 10      | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>5</sub> ..  | H                                | H                | NHCOCH <sub>3</sub> | 228–230  | C <sub>26</sub> H <sub>22</sub> N <sub>4</sub> O <sub>2</sub> S·HCl | Nil.                           |
| 11      | CH <sub>3</sub>                                                 | COOC <sub>2</sub> H <sub>5</sub> | H                | NHCOCH <sub>3</sub> | 235–238  | C <sub>22</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> S·HCl | 19·3                           |
| 12      | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | H                | NHCOCH <sub>3</sub> | 245–248  | C <sub>26</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub> S·HCl | Nil.                           |
| 13      | C <sub>6</sub> H <sub>5</sub>                                   | H                                | H                | OCH <sub>3</sub>    | 230–232  | C <sub>23</sub> H <sub>18</sub> N <sub>2</sub> OS·HBr.              | 3·8                            |
| 14      | <i>p</i> -CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub> ..  | H                                | H                | OCH <sub>3</sub>    | 250–253  | C <sub>25</sub> H <sub>21</sub> N <sub>3</sub> O <sub>2</sub> S·HCl | 20·8                           |
| 15      | CH <sub>3</sub> ..                                              | COOC <sub>6</sub> H <sub>5</sub> | H                | OCH <sub>3</sub>    | 235–240  | C <sub>21</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> S·HCl | 3·7                            |
| 16      | <i>p</i> -HOOC·H <sub>2</sub> C·O·C <sub>6</sub> H <sub>4</sub> | H                                | H                | OCH <sub>3</sub>    | 230–235  | C <sub>25</sub> H <sub>20</sub> N <sub>3</sub> O <sub>4</sub> S·HCl | 14·8                           |

\* All melting points are capillary melting points and are uncorrected.

† Analysed correctly for C, H, N.

‡ Reference Drug = Phenylbutazone (50·5 per cent inhibition of edema).

Dose = 100 mg/kg.

Test compound, dose = 100 mg/kg each.

Number of animals used = 6 rats per group.

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## IN VITRO ANTITUBERCULOSIS AND ANTIFUNGAL ACTIVITY OF *p*-(2-SUBSTITUTED-4-THIAZOLYL)PHENYLACETIC AND *p*-(2-SUBSTITUTED-4-THIAZOLYL)PHENOXYACETIC ACIDS

MADHUBEN R. PATEL AND B. R. COLAH

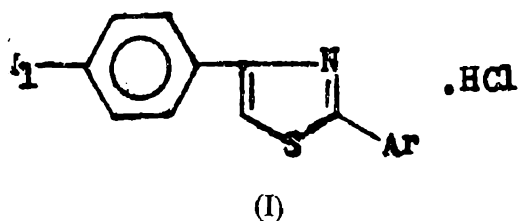
Department of Chemotherapy, Haffkine Institute, Parel, Bombay 400 012

A series of *p*-(2-substituted-4-thiazolyl)phenylacetic and *p*-(2-substituted-4-thiazolyl)phenoxyacetic acids has shown activity against *M. tuberculosis in vitro*. A few of the compounds showed *in vitro* activity against *T. rubrum* and *T. mentagrophytes*.

### Introduction

Sulphathiazole and promizole, both thiazole derivatives, were used in clinical practice as antimicrobial agents. Several thiazoles reported earlier from our laboratory also possessed good antituberculosis activity (1, 2).

Thiazoles of the general formula (I) were synthesized as potential antiinflammatory agents in this laboratory and studied for their pharmacological action (3).



$R_1 = \text{CH}_2 \text{COOH}, \text{OCH}_2 \text{COOH}.$

Compounds containing the phenoxyacetic and phenoxypropionic acid groups have shown good activity against fungi pathogenic to man (4). PAS which is one of the most widely used drugs against

tuberculosis also contains the carboxylic acid group.

Taking the above facts into account, the thiazoles I containing the carboxylic acid group were subjected to *in vitro* antituberculosis and antifungal screening.

### Materials and Methods

Table I shows the results of the antituberculosis and antifungal screening which was carried out by using the serial dilution method. Youmans basal medium containing 10 per cent horse serum was used for testing the compounds against *M. tuberculosis var hominis* (H<sub>37</sub>Rv strain) by a modified method reported earlier from this laboratory (5). The medium of Shraufstätter *et al.* (6) was used for the antifungal testing.

### Results

The compounds showed good *in vitro* tuberculostatic activity. Compound 2 showed activity against *M. tuberculosis in vitro* at 0.5 µg/ml concentration and compound 9 showed activity at 1 µg/ml concentration. *In vitro* antifungal activity

was shown by compound 2 at 20  $\mu\text{g/ml}$  concentration against *T. rubrum* and *T. mentagrophytes*.

#### Acknowledgements

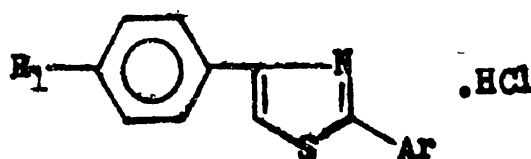
The authors are grateful to Dr. M. H. Shah, Assistant Director, Department of

Chemotherapy, for his keen interest in the present work.

The authors are also indebted to Dr. B. B. Gaitonde, Director, Haffkine Institute for providing facilities to carry out the present work.

TABLE I

*In vitro* antituberculosis and antifungal activity of *p*-(2-substituted-4-thiazolyl)phenylacetic and *p*-(2-substituted-4-thiazolyl)phenoxyacetic acids



| Serial No. | $R_1$                     | Ar                                                               | Minimum inhibitory concentration ( $\mu\text{g/ml}$ ) |                  |                          |
|------------|---------------------------|------------------------------------------------------------------|-------------------------------------------------------|------------------|--------------------------|
|            |                           |                                                                  | <i>M. tuberculosis</i>                                | <i>T. rubrum</i> | <i>T. mentagrophytes</i> |
| 1          | $\text{CH}_2\text{COOH}$  | $\text{C}_6\text{H}_5$                                           | 5                                                     | 40               | 40                       |
| 2          | $\text{CH}_2\text{COOH}$  | 4- $\text{ClC}_6\text{H}_4$                                      | 0.5                                                   | 20               | 20                       |
| 3          | $\text{CH}_2\text{COOH}$  | $\text{C}_6\text{H}_5\text{CH}_2$                                | 2                                                     |                  |                          |
| 4          | $\text{CH}_2\text{COOH}$  | 3, 4-( $\text{CH}_3\text{O}$ ) $_2\text{C}_6\text{H}_3$          | 10                                                    |                  | ....                     |
| 5          | $\text{CH}_2\text{COOH}$  | 3, 4-( $\text{C}_2\text{H}_5\text{O}$ ) $_2\text{C}_6\text{H}_3$ | 5                                                     |                  |                          |
| 6          | $\text{CH}_2\text{COOH}$  | 3, 4, 5-( $\text{CH}_3\text{O}$ ) $_3\text{C}_6\text{H}_2$       | 10                                                    | ....             |                          |
| 7          | $\text{CH}_2\text{COOH}$  | 4- $\text{CH}_3\text{CONHC}_6\text{H}_4$                         | 20                                                    | ....             |                          |
| 8          | $\text{OCH}_2\text{COOH}$ | $\text{C}_6\text{H}_5$                                           | 10                                                    | 40               | 40                       |
| 9          | $\text{OCH}_2\text{COOH}$ | 4- $\text{ClC}_6\text{H}_4$                                      | 1                                                     | 100              | 100                      |
| 10         | $\text{OCH}_2\text{COOH}$ | $\text{C}_6\text{H}_5\text{CH}_2$                                | 40                                                    | 40               | 40                       |
| 11         | $\text{OCH}_2\text{COOH}$ | 3, 4-( $\text{CH}_3\text{O}$ ) $_2\text{C}_6\text{H}_3$          | 10                                                    | 100              | 100                      |
| 12         | $\text{OCH}_2\text{COOH}$ | 3, 4-( $\text{C}_2\text{H}_5\text{O}$ ) $_2\text{C}_6\text{H}_3$ | 10                                                    | ....             | ....                     |
| 13         | $\text{OCH}_2\text{COOH}$ | 3, 4, 5-( $\text{CH}_3\text{O}$ ) $_3\text{C}_6\text{H}_2$       | 10                                                    | ....             |                          |
| 14         | $\text{OCH}_2\text{COOH}$ | 4- $\text{CH}_3\text{CONHC}_6\text{H}_4$                         | 20                                                    |                  |                          |

INH is active at 0.04  $\mu\text{g/ml}$  concentration against *M. tuberculosis*.

PAS is active at 0.4  $\mu\text{g/ml}$  concentration against *M. tuberculosis*.

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# IN VITRO ANTITUBERCULOSIS AND ANTIFUNGAL ACTIVITY OF SCHIFF BASES FROM 2-SUBSTITUTED-4-(4-AMINOPHENYL)THIAZOLES AND 2-SUBSTITUTED-4-FORMYLPHENOXYALKYL ACIDS AND ESTER

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*In vitro* antituberculosis and antifungal screening has been carried out on a series of Schiff bases from 2-substituted-4-(4-aminophenyl)thiazoles and 2-substituted-4-formylphenoxyalkyl acids and ester. Most of the compounds tested showed *in vitro* tuberculostatic activity.

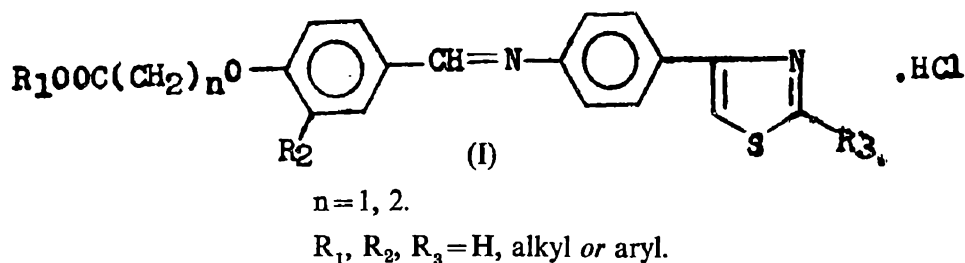
## Introduction

In continuation of the work reported earlier from this laboratory (1), Schiff bases of the general formula (I) containing the thiazole moiety have been subjected to *in vitro* antituberculosis and antifungal screening. The synthesis and pharmacological action of these compounds has been reported earlier (2). The above compounds were subjected to *in vitro* antituberculosis and antifungal screening on the basis that a number of phenoxyacetic acids and phenoxypropionic acids were earlier shown to be active against human pathogenic fungi (3).

(H<sub>37</sub>Rv strain) cultivated in Youmans medium containing 10 per cent horse serum, by a modified method reported from this laboratory (4). The medium of Schraufstatter *et al.* (5) was used for the antifungal testing.

## Results

Most of the compounds had *in vitro* tuberculostatic activity, the results of which are shown in Table I. Compounds 10 and 12 showed activity against *M. tuberculosis* *in vitro* at 1 µg/ml concentration. Compound 22 showed *in vitro* antifungal activity at 20 µg/ml concentration against *T. rubrum* and *T. mentagrophytes*.



## Materials and Methods

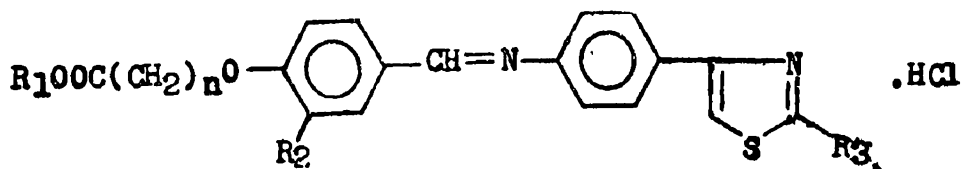
All compounds were tested for antituberculosis and antifungal activity by the serial dilution method and the results are given in Table I. The *in vitro* tuberculostatic activity was determined against the culture of *M. tuberculosis* *var hominis* (Received for publication August 20, 1976)

## Acknowledgements

The authors are grateful to Dr. M. H. Shah, Assistant Director, Department of Chemotherapy for his continuous advice. They are indebted to Dr. B. B. Gaitonde, Director, Haffkine Institute for providing facilities to carry out the present work.

TABLE I

*In Vitro* Antituberculosis and Antifungal activity of Schiff bases from 2-substituted-4-(4-aminophenyl) thiazoles and 2-substituted-4-formylphenoxyalkyl acids and ester



| Serial No. | n | R <sub>1</sub>                | R <sub>2</sub>    | R <sub>3</sub>                                                                     | Minimum inhibitory concentration<br>μg/ml |                  |                          |
|------------|---|-------------------------------|-------------------|------------------------------------------------------------------------------------|-------------------------------------------|------------------|--------------------------|
|            |   |                               |                   |                                                                                    | <i>M. tuberculosis</i>                    | <i>T. rubrum</i> | <i>T. mentagrophytes</i> |
| 1          | 1 | H                             | H                 | C <sub>6</sub> H <sub>5</sub>                                                      | 10                                        | 200              | 200                      |
| 2          | 1 | H                             | H                 | 4-ClC <sub>6</sub> H <sub>4</sub>                                                  | 5                                         | ....             | ....                     |
| 3          | 1 | H                             | H                 | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                      | 5                                         | ....             | ....                     |
| 4          | 1 | H                             | H                 | 3, 4-(CH <sub>2</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>                | 5                                         | ....             | ....                     |
| 5          | 1 | H                             | H                 | 3, 4-(C <sub>2</sub> H <sub>5</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>  | 2                                         | ....             | ....                     |
| 6          | 1 | H                             | H                 | 3, 4, 5-(CH <sub>3</sub> O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>             | 5                                         | ....             | ....                     |
| 7          | 1 | H                             | H                 | 4-CH <sub>3</sub> CONH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>                  | 5                                         | ....             | ....                     |
| 8          | 1 | H                             | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub>                                                      | 5                                         | ....             | ....                     |
| 9          | 1 | H                             | CH <sub>3</sub> O | 4-ClC <sub>6</sub> H <sub>4</sub>                                                  | 2                                         | ....             | ....                     |
| 10         | 1 | H                             | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                      | 1                                         | ....             | ....                     |
| 11         | 1 | H                             | CH <sub>3</sub> O | 3, 4-(CH <sub>3</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>                | 2                                         | ....             | ....                     |
| 12         | 1 | H                             | CH <sub>3</sub> O | 3, 4-(C <sub>2</sub> H <sub>5</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>  | 1                                         | ....             | ....                     |
| 13         | 1 | H                             | CH <sub>3</sub> O | 3, 4, 5-(CH <sub>3</sub> O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>             | 5                                         | ....             | ....                     |
| 14         | 1 | H                             | CH <sub>3</sub> O | 4-CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>                                | 2                                         | ....             | ....                     |
| 15         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub>                                                      | 10                                        | 200              | 200                      |
| 16         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | 4-ClC <sub>6</sub> H <sub>4</sub>                                                  | 5                                         | 200              | 200                      |
| 17         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                      | 5                                         | 100              | 100                      |
| 18         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | 3, 4-(CH <sub>3</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>                | 5                                         | ....             | ....                     |
| 19         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | 3, 4-(C <sub>2</sub> H <sub>5</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>  | 2                                         | ....             | ....                     |
| 20         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | 3, 4, 5-(CH <sub>3</sub> O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>             | 2                                         | ....             | ....                     |
| 21         | 1 | C <sub>2</sub> H <sub>5</sub> | CH <sub>3</sub> O | 4-CH <sub>3</sub> CONH <sub>2</sub> C <sub>6</sub> H <sub>4</sub>                  | 5                                         | 200              | 200                      |
| 22         | 2 | H                             | H                 | C <sub>6</sub> H <sub>5</sub>                                                      | 5                                         | 20               | 20                       |
| 23         | 2 | H                             | H                 | 4-ClC <sub>6</sub> H <sub>4</sub>                                                  | 5                                         | ....             | ....                     |
| 24         | 2 | H                             | H                 | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                      | 5                                         | 100              | 100                      |
| 25         | 2 | H                             | H                 | 3, 4-(CH <sub>3</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>                | 5                                         | ....             | ....                     |
| 26         | 2 | H                             | H                 | 3, 4-(C <sub>2</sub> H <sub>5</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>  | 10                                        | ....             | ....                     |
| 27         | 2 | H                             | H                 | 3, 4, 5-(CH <sub>3</sub> O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>             | 5                                         | ....             | ....                     |
| 28         | 2 | H                             | H                 | 4-CH <sub>3</sub> CONC <sub>6</sub> H <sub>4</sub>                                 | 10                                        | ....             | ....                     |
| 29         | 2 | H                             | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub>                                                      | 10                                        | ....             | ....                     |
| 30         | 2 | H                             | CH <sub>3</sub> O | 4-ClC <sub>6</sub> H <sub>4</sub>                                                  | 10                                        | ....             | ....                     |
| 31         | 2 | H                             | CH <sub>3</sub> O | C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>                                      | 2                                         | 200              | 200                      |
| 32         | 2 | H                             | CH <sub>3</sub> O | 3, 4-(CH <sub>3</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub>                | 5                                         | ....             | ....                     |
| 33         | 2 | H                             | CH <sub>3</sub> O | 3, 4, (C <sub>2</sub> H <sub>5</sub> O) <sub>2</sub> C <sub>6</sub> H <sub>3</sub> | 5                                         | ....             | ....                     |
| 34         | 2 | H                             | CH <sub>3</sub> O | 3, 4, 5-(CH <sub>3</sub> O) <sub>3</sub> C <sub>6</sub> H <sub>2</sub>             | 10                                        | ....             | ....                     |
| 35         | 2 | H                             | CH <sub>3</sub> O | 4-CH <sub>3</sub> CONHC <sub>6</sub> H <sub>4</sub>                                | 10                                        | ....             | ....                     |

INH is active at 0.04 μg/ml concentration against *M. tuberculosis*.

PAS is active at 0.4 μg/ml concentration against *M. tuberculosis*.

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Edited by Dr. M. V. N. Shirodkar, Head, Department of Virology, Haffkine Institute for Training, Research and Testing. Published by the Director, Haffkine Institute for Training, Research and Testing, Bombay 400 012 and printed at the Government Central Press, Bombay 400 004

