

## Synthesis, anti-fungal, anti-tumor and anti-inflammatory activities of some new 5-(N-substitutedarylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidines

Paresh Manna\* and K K Narang

Department of Applied Chemistry, Institute of Technology, Banaras Hindu University, Varanasi 221 005

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5-(N-Substitutedarylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidines (IVa-e) have been synthesized by oxidative debenzoylation and cyclization with bromine in chloroform from the corresponding 5-(4-chloroaryl)-1-(substitutedarylideneamino)-2-S-benzyliso-4-biurets (IIIa-e) or by the oxidation of corresponding 1-(substituted-arylideneamino)-5-(4-chloroaryl)-2,4-thiobiurets (Va-e), obtained from IIIa-e by reductive debenzoylation with hydrogen sulphide in a mixture of pyridine and triethylamine. 2-S-Benzyliso-4-biurets (IIIa-e), in turn, were prepared by the condensation of 2-S-benzyliso-1-substitutedarylthiosemi-carbazone (IIa-e) with 4-chlorophenylisocyanate. Thiadiazolidines (IVa-e) have been characterized by their element analyses, IR,  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ . Anti-fungal screening of these compounds against ten fungi has been done by glass slides methods. The compound (1000  $\mu\text{g/ml}$ ) containing dichlorogroup (IVe) showed maximum inhibitory effect (99.6 %) against *Fusarium udum*. The compound possessing dichloro group (IVe) have potent anti-inflammatory and anti-tumor properties as they down regulate NF-kB transcription factor.

**Keywords:** 1,2,4-Thiadiazolidines, Synthesis, Characterization, Anti-fungal, Anti-tumor, Anti-inflammatory

**IPC Code:** C10J1/20

### Introduction

Thiazolidinones and thiadiazoles show local anaesthetic<sup>1</sup>, anti-bacterial<sup>2</sup>, anti-fungal<sup>2</sup>, selective Muscarine Agonists<sup>3</sup> and anti-inflammatory<sup>4</sup> properties. 1,3,4-Thiadiazole derivatives also possess anti-microbial<sup>5</sup>, anti-convulsant<sup>6</sup>, carbonic anhydrase inhibitors<sup>7</sup> and tumor associated isozyme IX inhibition<sup>7</sup> activities. 3-Oxo-1,2,4-thiadiazolidines possess promising anti-fungal<sup>8</sup>, anti-bacterial<sup>8</sup> and plant growth regulator<sup>9</sup> activities. There have been efforts to synthesize related thiadiazolidines by oxidative debenzoylation technique<sup>10-13</sup>. This study extends this work, introduces newer groups and studies anti-fungal, anti-tumor and anti-inflammatory activities.

Accordingly, 5-(N-substitutedarylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidines (IVa-e) (Scheme 1) were synthesized from the related 5-(4-chloroaryl)-1-(substitutedarylideneamino)-2-S-benzyliso-4-biurets (IIIa-e) by oxidative debenzoylation technique or by oxidation of 1-(substitutedarylideneamino)-5-(4-chloroaryl)-2,4-thiobiurets (Va-e)<sup>14</sup>. 5-(4-Chloroaryl)-1-(substitutedarylideneamino)-2-S-benzyliso-4 biurets (IIIa-e) were synthesized by

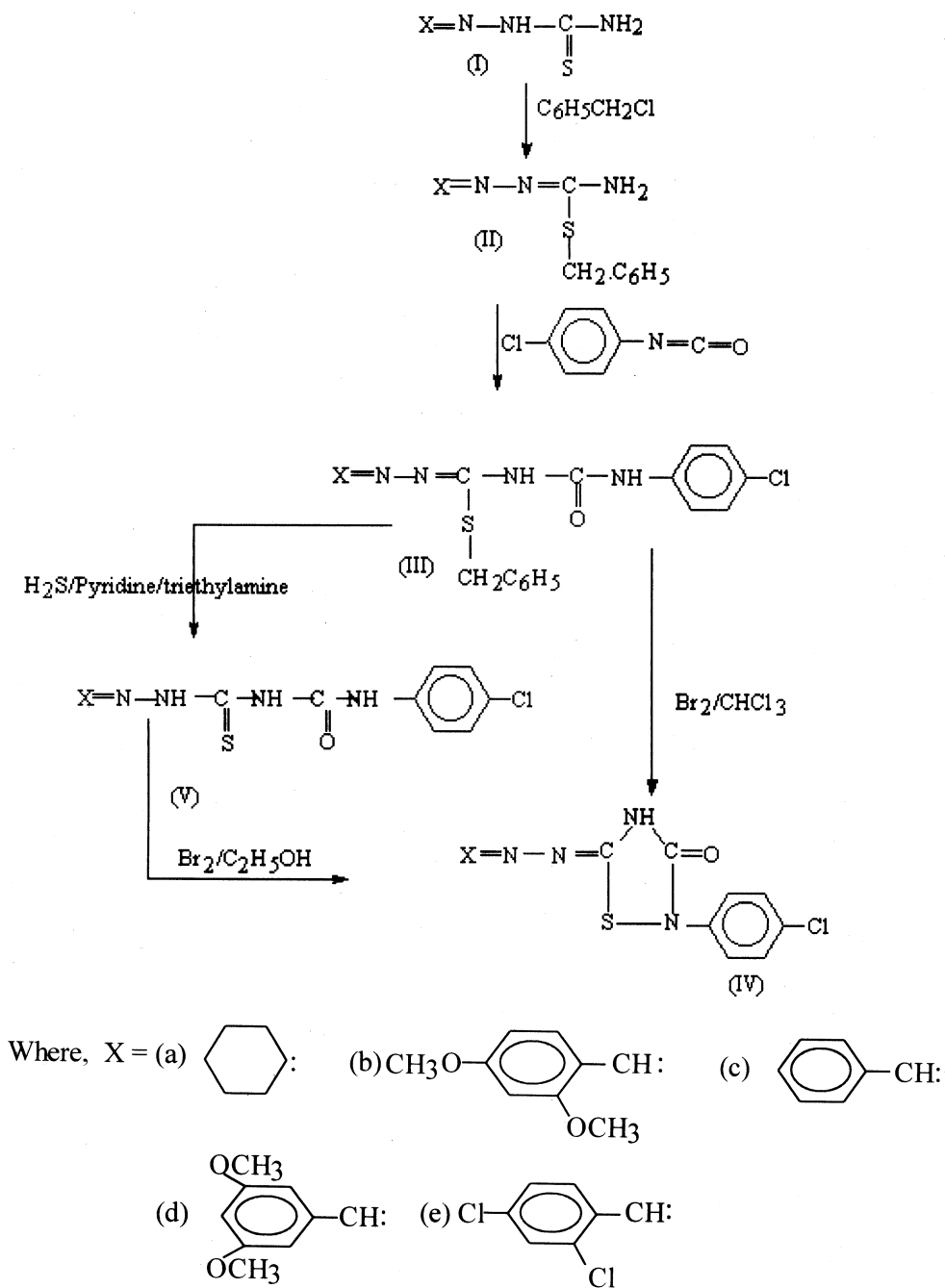
condensation of related 2-S-benzyliso-1-substituted-arylthiosemicarbazone (IIa-e) and 4-chloroaryliso-cyanate in benzene medium at its refluxing temperature for 6h. 1-(Substitutedarylideneamino)-5-(4-chloroaryl)-2,4-thiobiurets (Va-e) were prepared by reductive debenzoylation<sup>15</sup> of the corresponding 5-(4-chloroaryl)-1-(substitutedarylideneamino)-2-S-benzyliso-4 biurets (IIIa-e) with hydrogen sulphide in solution of pyridine and triethylamine. The structures of compound IVa-e were confirmed by their synthesis through other routes as well as by studying the mixed melting points of the compounds synthesized by different routes, element analyses, IR<sup>16,17</sup>,  $^1\text{H-NMR}$ <sup>17</sup> and  $^{13}\text{C-NMR}$ <sup>17</sup>.

### Anti-fungal Activity

The effect of newly synthesized compounds on spore germination method against fungi was tested at different concentrations (500, 1000 and 1500  $\mu\text{g/ml}$ ). The compounds were dissolved in methanol and then mixed in sterilized distilled water to make a stock suspension; the methanol was evaporated at room temperature. About 200 spores were picked up with a sterile needle and mixed with a drop of the solution of appropriate compound of desired concentration of grease-free glass slide. Control consisted of a similar number of spores of each fungus in sterile distilled water and sterile distilled water + methanol. All slides

\*Author for correspondence.

E-mail: mannaparesh444@rediffmail.com



Scheme 1

were incubated in moist chambers for 24 h at  $25 \pm 2^\circ\text{C}$ . Each slide was observed under the microscope for germination. All the experiments were conducted in triplicate.

Anti-fungal activity of compounds IVa-e was done by glass-slides method<sup>18</sup> against pathogenic agricultural fungi (*Alternaria alternata*, *A. tenuissima*,

*A. solani*, *Colletotrichum* sp., *Fusarium udum*, *Helminthosporium sativum*, *Aspergillus flavus*, *A. pisi*, *Erysiphe pisi* and *Curvularia* sp.), which cause serious plant diseases in India. The effect of these compounds on spore germination of plant pathogenic fungi was carried out at different concentrations (500, 1000 and 1500  $\mu\text{g/ml}$ ). Compound IVe (1000  $\mu\text{g/ml}$ )

Table 1— Fungi (1000 mg/ml) inhibition of the compounds (IVa-e)

| S<br>No | Fungi                           | Compound |          |          |          |          |
|---------|---------------------------------|----------|----------|----------|----------|----------|
|         |                                 | IVa<br>% | IVb<br>% | IVc<br>% | IVd<br>% | IVe<br>% |
| 1       | <i>Alternaria alternata</i>     | 62.0     | 92.1     | 77.7     | 88.1     | 91.5     |
| 2       | <i>A. tenuissimal</i>           | 69.3     | 87.3     | 79.1     | 86.2     | 90.8     |
| 3       | <i>A. solani</i>                | 70.8     | 88.5     | 84.5     | 89.0     | 94.3     |
| 4       | <i>Colletotrichum sp.</i>       | 60.2     | 97.3     | 87.2     | 89.8     | 97.6     |
| 5       | <i>Fusarium udum</i>            | 66.5     | 90.3     | 83.5     | 90.0     | 99.6     |
| 6       | <i>Helminthosporium sativum</i> | 69.1     | 89.3     | 80.1     | 88.8     | 91.1     |
| 7       | <i>Aspergillus flavus</i>       | 58.1     | 90.1     | 88.2     | 87.2     | 97.2     |
| 8       | <i>A. pisi</i>                  | 59.2     | 94.2     | 83.8     | 88.1     | 93.4     |
| 9       | <i>Erysiphe pisi</i>            | 60.5     | 93.5     | 89.5     | 88.7     | 95.2     |
| 10      | <i>Curvularia sp.</i>           | 61.2     | 89.5     | 86.5     | 91.2     | 94.5     |

showed maximum inhibitory effect (99.6 %) against *F. udum* and more than 90.8 percent for others. Compound IVb showed remarkable inhibitory effect (97.3%) against *Colletotrichum sp.* and more than 88.5 percent with others. Compound IVd showed maximum inhibitory effect (92.2%) in the case of *Curvularia sp.*, whereas other fungi also exhibited good inhibition (86.2%). Compound IVc showed maximum inhibitory effect (89.5%) in the case of *E. pisi* and more than 77.7 percent with others. Compound IVa showed maximum inhibitory effect (70.8 %) against *A. solani* whereas other fungi exhibited less inhibition (58.1%). Thus, order of inhibitory effect against all tested fungi from synthesized compounds is as follows: IVe > IVb > IVd > IVc > IVa (Table 1).

#### Anti-tumor and Anti-inflammatory Activity

Nuclear transcription factor-kappa B (NF- $\kappa$ B), an ubiquitous nuclear transcription factor<sup>19</sup>, regulates the expression of various genes that play critical roles in apoptosis, viral replication, tumor genesis, various autoimmune diseases, and inflammation<sup>20,21</sup>. NF- $\kappa$ B is activated in response to various inflammatory stimuli including cytokines, nitrogen, bacterial products, viral proteins, and apoptosis-inducing agents<sup>19,20</sup>. Under normal conditions, NF- $\kappa$ B is present in the cytoplasm as an inactive heterotrimer consisting of p50, p65 and I $\kappa$ B $\alpha$  subunits. On activation, degradation of I $\kappa$ B $\alpha$

exposes nuclear localization signals on the p50-p65 heterodimer, leading to nuclear translocation and binding to a specific sequence in DNA, which in turn, results in gene transcription. Any compound, which has inhibitory activity for NF- $\kappa$ B, may have anti-inflammatory and anti-tumor property.

To detect the efficacy of different compounds (IVa-e) for anti-tumor activity, apoptotic cells were calculated by deducing the absorbance of treated cells from untreated cells. The cell viability was assayed by modified MTT assay method<sup>22</sup>. U-937 cells (10,000/well of 96-well plate in 100  $\mu$ l RPMI-1640 medium containing 100  $\mu$ g of penicillin-streptomycin and 10 % fetal bovine serum) were treated with different concentrations (0-10  $\mu$ M) of compounds (IVa-e) in triplicate samples for 72 h at the same culture condition at 37°C in a CO<sub>2</sub> incubator. MTT dye (25  $\mu$ g in 5  $\mu$ l sterile phosphate buffer saline, pH 7.4) was added in each well and after 2h, 100  $\mu$ l lysis buffer (10 % SDS in 50 % dimethyl formamide) was added and kept over night to lyse the cells and dissolve the granules. The absorbance was taken at 570 nm and the mean absorbance  $\pm$  SD for triplicate samples was indicated (Fig. 1).

Compound IVe was found more cytotoxic, whereas compounds having ethoxy substituent at *p*-position showed less potency (Fig. 1). The order of effect of tested compounds on cell apoptosis is: IVe > IVb > IVd > IVc > IVa. For compound IVe, IC<sub>50</sub> is 0.012  $\mu$ M (12 nM).

As NF- $\kappa$ B is activated in all sorts of inflammation, the agent(s) which inhibit its activation should be considered as a potential anti-inflammatory agent. Tumor necrosis factor (TNF) is known to induce inflammatory response in all sorts of cells. Hence to induce inflammation, TNF was used and NF- $\kappa$ B was assayed by electrophoresis mobility shift assay (EMSA) as a marker of inflammation. U-937 cells (2 x 10<sup>6</sup>/ml) were treated with different concentrations (0-1  $\mu$ M) of compounds IVa-e for 4 h at 37°C in a CO<sub>2</sub> incubator, and then stimulated with 1 nM TNF for 1 h at the same culture condition. Nuclear extracts were then prepared from cells and incubated with <sup>32</sup>P-end-labeled 45-mer double-stranded NF- $\kappa$ B oligonucleotide (4  $\mu$ g of protein with 16 fmol of DNA) from the human immuno deficiency virus with long terminal repeat unit, 5'-TTGTTACAAGGGACTTTCCGCTGGGGACTTTCAGGGAGGCGTGG-3' (underline indicates NF- $\kappa$ B

binding sites) for 15 min at 37°C. DNA-protein complex formed was separated from free oligonucleotide on native polyacrylamide gel (6.6%) which was dried and exposed into Phosphor-Imager screen and scanned in a Phosphor-Imager (Fuji, Japan) using Image Reader software and radioactive bands were detected.

The intensity of radioactive bands indicated that the unstimulated cells shown in band and all 5 compounds alone at 1  $\mu$ M concentration did not show

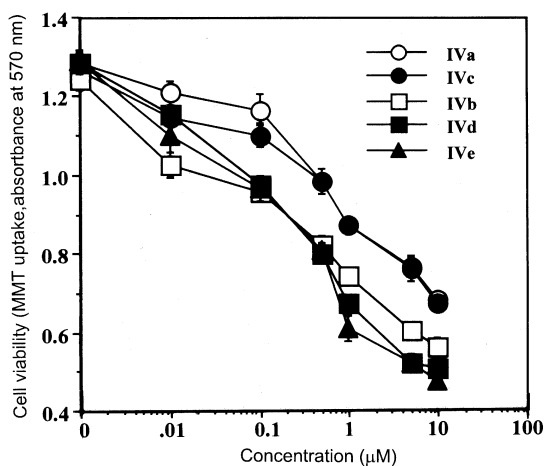


Fig. 1— Effect of compounds IVa-e on Cell Apoptosis (Cell Viability)

any activation of NF- $\kappa$ B (Fig. 2) Compounds IVb, IVd and IVe showed potent role to inhibit TNF-induced inflammatory responses, indicating potent anti-inflammatory and anti-tumor properties.

## Experimental Details

### Materials and Methods

[3-(4,5-Dimethylthiazal-2-yl) -2,5 -diphenyl -2H-tetrazo-lium bromide](MTT) and 4-chlorophenylisocyanate were obtained from Sigma-Aldrich chemicals (USA). Penicillin, streptomycin, RPM111640 medium and fetal bovine serum (FBS) were obtained from Life Technologies. TNF was obtained from Peprotech (New Jersey, USA). Benzene, ethanol, bromine, chloroform and benzylchloride were obtained from E. Merck, India. Melting points were determined using Kofler hot stage apparatus by open capillary tube method. IR Spectra were recorded in Nujol on JASCO FT/IR - 5300 spectrophotometer and  $^1$ H-NMR,  $^{13}$ C-NMR Spectra (in DMSO- $d_6$ ) on Jeol F X 90 Q spectrometer (90 MHz-instrument). The microanalyses were carried out using a Coleman carbon-hydrogen and nitrogen analyzer. All precursors were prepared in conformity with standard methods. Single spot on TLC plate and mixed melting point confirmed the purity of compounds. S-Benzylisothiosemicarbazone

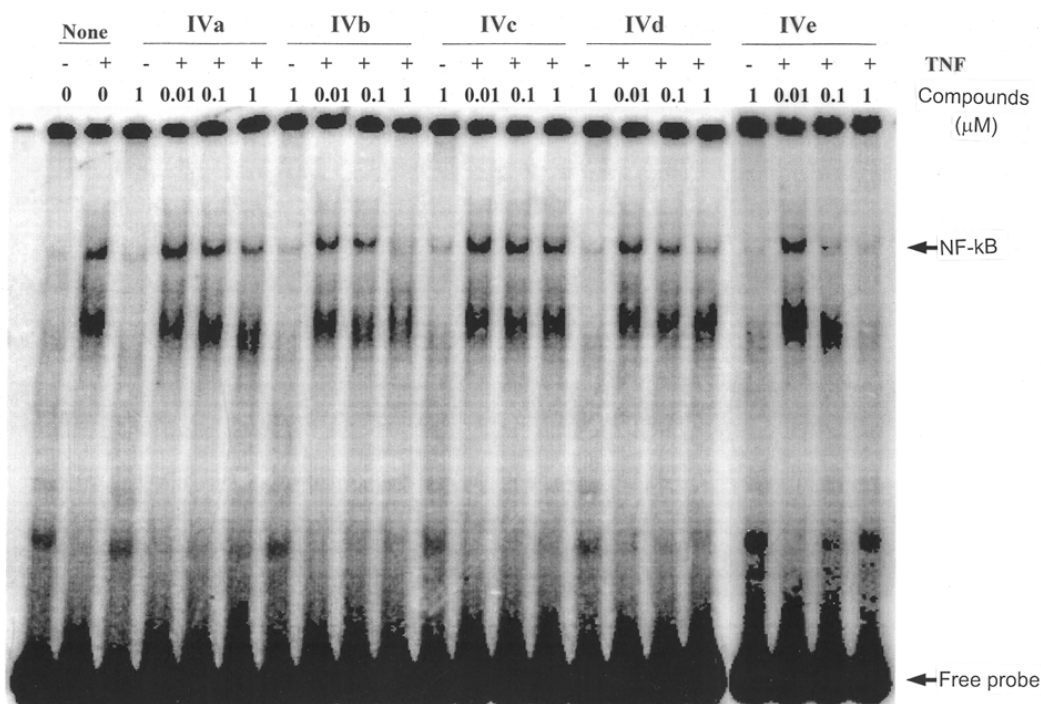


Fig. 2 — TNF-induced NF-  $\kappa$ B activation

Table 2 — Characterization data of compounds (IIIa-e)

| Compounds | Mol formulae<br>(Formula wt)                                                 | m p<br>°C | Yield<br>% | Analysis<br>% |        | IR band in Nujal |                    | <sup>1</sup> H-NMR in DMSO-d <sub>6</sub><br>TMS int. |                       |
|-----------|------------------------------------------------------------------------------|-----------|------------|---------------|--------|------------------|--------------------|-------------------------------------------------------|-----------------------|
|           |                                                                              |           |            | Found         | Calcd. | cm <sup>-1</sup> | Assignment         | δ ppm                                                 | Assignment            |
| IIIa      | C <sub>21</sub> H <sub>23</sub> N <sub>4</sub> O SCl<br>(414.5)              | 160       | 61.8       | C: 60.88      | 60.79  | 3290             | NH                 | 1.42 (10 H)                                           | Alicy-CH <sub>2</sub> |
|           |                                                                              |           |            | H: 5.50       | 5.54   | 1695             | C=O                | 4.36 (2 H)                                            | S-CH <sub>2</sub>     |
|           |                                                                              |           |            | N: 13.61      | 13.51  | 1598             | C=N                | 6.66 (2 H)                                            | NH                    |
|           |                                                                              |           |            |               |        | 1416             | CH <sub>2</sub> -S | 7.18 (9 H)                                            | Ar-H                  |
|           |                                                                              |           |            |               |        | 675              | C-Cl               |                                                       |                       |
| IIIb      | C <sub>24</sub> H <sub>23</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(482.5) | 184       | 58         | C: 59.50      | 59.68  | 3298             | NH                 | 3.30 (6 H)                                            | O CH <sub>3</sub>     |
|           |                                                                              |           |            | H: 4.66       | 4.76   | 1690             | C=O                | 4.20 (2 H)                                            | S-CH <sub>2</sub>     |
|           |                                                                              |           |            | N: 11.68      | 11.60  | 1586             | C=N                | 7.40 (2 H)                                            | NH                    |
|           |                                                                              |           |            | S: 6.60       | 6.63   | 1418             | CH <sub>2</sub> -S | 7.78 (12 H)                                           | Ar-H                  |
|           |                                                                              |           |            |               |        | 670              | C-Cl               | 8.18 (1 H)                                            | CH=N                  |
| IIIc      | C <sub>22</sub> H <sub>19</sub> N <sub>4</sub> O SCl<br>(422.5)              | 175       | 65.9       | C: 62.41      | 62.48  | 3292             | NH                 | 4.12 (2 H)                                            | S-CH <sub>2</sub>     |
|           |                                                                              |           |            | H: 4.59       | 4.49   | 1688             | C=O                | 7.18 (2 H)                                            | NH                    |
|           |                                                                              |           |            | N: 13.40      | 13.25  | 1590             | C=N                | 7.60 (14 H)                                           | Ar-H                  |
|           |                                                                              |           |            |               |        | 1426             | CH <sub>2</sub> =S | 8.24 (1 H)                                            | CH=N                  |
|           |                                                                              |           |            |               |        | 690              | C-Cl               |                                                       |                       |
| IIId      | C <sub>24</sub> H <sub>23</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(482.5) | 182       | 64.9       | C: 59.77      | 59.68  | 3290             | NH                 |                                                       |                       |
|           |                                                                              |           |            | H: 4.89       | 4.76   | 1686             | C=O                |                                                       |                       |
|           |                                                                              |           |            | N: 11.56      | 11.60  | 1588             | C=N                | -                                                     | -                     |
|           |                                                                              |           |            | S: 6.70       | 6.65   | 1420             | CH <sub>2</sub> -S |                                                       |                       |
|           |                                                                              |           |            |               |        | 666              | C-Cl               |                                                       |                       |
| IIIe      | C <sub>22</sub> H <sub>17</sub> N <sub>4</sub> O SCl<br>(491.5)              | 190       | 60.7       | C: 53.63      | 53.71  | 3310             | NH                 |                                                       |                       |
|           |                                                                              |           |            | H: 3.52       | 3.45   | 1699             | C=O                |                                                       |                       |
|           |                                                                              |           |            | N: 11.47      | 11.39  | 1578             | C=N                | -                                                     | -                     |
|           |                                                                              |           |            | S: 6.60       | 6.51   | 1430             | CH <sub>2</sub> -S |                                                       |                       |
|           |                                                                              |           |            |               |        | 682              | C-Cl               |                                                       |                       |

(II) was prepared by benzylation of substitutedarythiosemicarbazides<sup>23</sup> with benzyl chloride in sodium methoxide and methanol.

**5-(4-Chloroaryl)-1-(cyclohexylideneamino)-2-S-benzyliso-4-biuret (IIIa)**

A mixture of 2-S-benzyliso-1-cyclohexylthiosemicarbazone (II<sub>a</sub>) (5.22 g, 0.02 mol) was refluxed for 6 h on a water bath. The excess solvent was evaporated under vacuum over a rotary evaporator and semisolid was repeatedly washed with petroleum ether (40-60°C, 100 ml) followed by addition of ethanol (20 ml). The crude 5-(4-chloroaryl)-1-(cyclohexylideneamino)-2-S-benzyliso-4-biuret (IIIa), thus obtained was recrystallized from ethanol. Purity of the compound was checked by TLC, IIIa, [yield, 5.13 g (61.8 %); m p, 160°C]. Compounds, 5-(4-chloroaryl)-1-(2,4-dimethoxyarylideneamino), 5-(4-chloroaryl)-1-(arylideneamino), 5-(4-chloroaryl)-1-(3,5-dimethoxyarylideneamino), 5-(4-chloroaryl)-1-

(2,4-dichloroarylideneamino)-2-S-benzyliso-4-biurets (III b-c) were prepared and characterized (Table 2).

**5-(N-Cyclohexylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidine (IVa)**

*Method A: Oxidative debenzylation of IIIa*

5-(4-Chloroaryl)-1-(cyclohexylideneamino)-2-S-benzyliso-4-biuret (IIIa) (4.145g; 0.01 mol) was made into a paste with chloroform (1 ml) and treated with molecular bromine (2 ml) with vigorous and continuous stirring, till the colour of bromine persisted. Reaction mixture warmed up considerably, evolving lachrymatory fumes of benzylbromide. The mixture was allowed to stand for ½ h and then repeatedly washed with ether (125 ml) on addition of ethanol (25 ml). The hydrobromide of 5-(N-cyclohexylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidine (IVa) separated out as a solid mass, which was treated with ammonia solution (30

Table 3 — Characterization data of compounds (IVa-e)

| Com-<br>pounds | Mol formulae<br>(Formula wt)                                                 | m p<br>°C | Yield <sup>a</sup><br>% | Analysis<br>% |        | IR band in Nujal |                    | <sup>1</sup> H-NMR in DMSO-d <sub>6</sub><br>TMS int. |                       | <sup>13</sup> C-NMR in<br>DMSO-d <sub>6</sub> |                 |
|----------------|------------------------------------------------------------------------------|-----------|-------------------------|---------------|--------|------------------|--------------------|-------------------------------------------------------|-----------------------|-----------------------------------------------|-----------------|
|                |                                                                              |           |                         | Found         | Calcd. | cm <sup>-1</sup> | Assignment         | δ ppm                                                 | Assignment            | δ ppm                                         | Assign-<br>ment |
| IVa            | C <sub>14</sub> H <sub>15</sub> N <sub>4</sub> O SCl<br>(322.5)              | 141       | 78.9<br>(47.8)          | C: 52.16      | 52.09  | 3315             | NH                 | 1.24 (10 H)                                           | Alicy-CH <sub>2</sub> |                                               |                 |
|                |                                                                              |           |                         | H: 4.74       | 4.65   | 1705             | C=O                | 6.54 (1H)                                             | NH                    |                                               |                 |
|                |                                                                              |           |                         | N: 17.14      | 17.36  | 1594             | C=N                | 7.30 (4 H)                                            | Ar-H                  | -                                             | -               |
|                |                                                                              |           |                         | S: 9.80       | 9.92   | 1245             | ring C-N           |                                                       |                       |                                               |                 |
|                |                                                                              |           |                         |               |        | 705              | ring C-N           |                                                       |                       |                                               |                 |
| IVb            | C <sub>17</sub> H <sub>15</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(390.5) | 85        | 76.6<br>(48.9)          | C: 52.20      | 52.24  | 3320             | NH                 | 3.84 (6 H)                                            | O CH <sub>3</sub>     | 166.33                                        | C=N             |
|                |                                                                              |           |                         | H: 3.78       | 3.84   | 1703             | C=O                | 6.78 (1 H)                                            | NH                    | 160.52                                        | C=O             |
|                |                                                                              |           |                         | N: 14.28      | 14.34  | 1576             | C=N                | 7.42 (7 H)                                            | Ar-H                  | 113.34-                                       | Aromatic        |
|                |                                                                              |           |                         | S: 8.13       | 8.19   | 1250             | ring C-N           | 8.12 (1 H)                                            | CH=N                  | 133.12                                        | Carbon          |
|                |                                                                              |           |                         |               |        | 710              | ring C-S           |                                                       |                       | 55.25                                         | CH <sub>3</sub> |
| IVc            | C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O SCl<br>(330.5)              | 169       | 76.8<br>(53.7)          | C: 54.39      | 54.46  | 3312             | NH                 | 6.66 (1 H)                                            | NH                    | 166.25                                        | C=N             |
|                |                                                                              |           |                         | H: 3.28       | 3.32   | 1675             | C=O                | 7.60 (9 H)                                            | Ar-H                  | 159.30                                        | C=O             |
|                |                                                                              |           |                         | N: 16.88      | 16.94  | 1586             | C=N                | 8.42 (1 H)                                            | CH=N                  | 112.52                                        | Aromatic        |
|                |                                                                              |           |                         | S: 9.60       | 9.68   | 1268             | ring C-N           |                                                       |                       | 132.35                                        | Carbon          |
|                |                                                                              |           |                         |               |        | 704              | ring C-S           |                                                       |                       |                                               |                 |
| IVd            | C <sub>17</sub> H <sub>15</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(390.5) | 148       | 78.9<br>(49.2)          | C: 52.18      | 52.24  | 3940             | NH                 | 3.36 (6 H)                                            | O-CH <sub>3</sub>     |                                               |                 |
|                |                                                                              |           |                         | H: 3.77       | 3.84   | 1696             | C=O                | 7.24 (1 H)                                            | NH                    |                                               |                 |
|                |                                                                              |           |                         | N: 14.28      | 14.34  | 1595             | C=N                | 7.54 (7 H)                                            | Ar-H                  | -                                             | -               |
|                |                                                                              |           |                         | S: 8.24       | 8.19   | 1260             | ring C-N           | 8.48 (1 H)                                            | CH=N                  |                                               |                 |
|                |                                                                              |           |                         |               |        | 700              | ring C-S           |                                                       |                       |                                               |                 |
| IVe            | C <sub>15</sub> H <sub>9</sub> N <sub>4</sub> O SCl<br>(399.5)               | 163       | 75.4<br>(50.6)          | C: 45.11      | 45.05  | 3345             | NH                 | 6.48 (1 H)                                            | NH                    | 168.32                                        | C=N             |
|                |                                                                              |           |                         | H: 2.18       | 2.25   | 1698             | C=O                | 7.78 (7 H)                                            | Ar-H                  | 161.12                                        | C=O             |
|                |                                                                              |           |                         | N: 13.98      | 14.01  | 1584             | C=N                | 7.42 (7 H)                                            | CH= N                 | 116.21-                                       | Aromatic        |
|                |                                                                              |           |                         | S: 8.12       | 8.01   | 1236             | CH <sub>2</sub> -S | 8.36 (1 H)                                            |                       | 138.25                                        | Carbon          |
|                |                                                                              |           |                         |               |        | 707              | C-Cl               |                                                       |                       |                                               |                 |
|                |                                                                              |           |                         |               |        | 688              |                    |                                                       |                       |                                               |                 |

<sup>a</sup> Numbers in parenthesis indicate yields obtained by Method B.

ml) to obtain the free base, (IVa), which was filtered and purified by recrystallization from ethanol [m p, 141°C; yield, 2.55 g (78.9 %)]. Similarly, 5-(N-2,4-dimethoxyarylidenehydrazono), 5-(N-arylidenehydrazono), 5-(N-3,5-dimethoxyarylidenehydrazono), 5-(N-2,4-dichloroarylidenehydrazono)-2-(4-chloroaryl)-

3-oxo-1,2,4-thiadiazolidines (IVb-e) were prepared and characterized (Table 3).

#### Method B: Oxidation of Va

1-(Cyclohexylideneamino)-5-(4-chloroaryl)-2,4-thiobiuret (Va) (1.30 g, 0.004 mol) was oxidized by bromine (1 ml) in dilute ethanol (20 ml). Addition of excess ether afforded the hydrobromide of

Table 4 — Characterization data of compounds (Va-e)

| Compounds | Mol formulae<br>(Formula wt)                                                 | m.p<br>°C | Yield<br>% | Analysis<br>% |        | IR band in Nujal |            | ‘H-NMR in DMSO-d <sub>6</sub><br>TMS int |                       |
|-----------|------------------------------------------------------------------------------|-----------|------------|---------------|--------|------------------|------------|------------------------------------------|-----------------------|
|           |                                                                              |           |            | Found         | Calcd. | cm <sup>-1</sup> | Assignment | δ ppm                                    | Assignment            |
| Va        | C <sub>14</sub> H <sub>17</sub> N <sub>4</sub> O SCl<br>(324.5)              | 155       | 55.08      | C: 51.76      | 51.77  | 3294             | NH         | 1.40 (10 H)                              | Alicy-CH <sub>2</sub> |
|           |                                                                              |           |            | H: 5.18       | 5.23   | 1690             | C=O        | 6.66 (3H)                                | NH                    |
|           |                                                                              |           |            | N: 17.19      | 17.25  | 1140             | C=S        | 7.12 (4 H)                               | Ar-H                  |
|           |                                                                              |           |            |               |        | 674              | C-Cl       |                                          |                       |
| Vb        | C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(392.5) | 178       | 51.06      | C: 51.87      | 51.97  | 3300             | NH         |                                          |                       |
|           |                                                                              |           |            | H: 4.36       | 4.33   | 1692             | C=O        |                                          |                       |
|           |                                                                              |           |            | N: 14.32      | 14.26  | 1160             | C=S        | -                                        | -                     |
|           |                                                                              |           |            |               |        | 675              | C-Cl       |                                          |                       |
| Vc        | C <sub>15</sub> H <sub>13</sub> N <sub>4</sub> O SCl<br>(332.5)              | 172       | 57.9       | C: 54.17      | 54.13  | 3290             | NH         | 7.12 (3 H)                               | NH                    |
|           |                                                                              |           |            | H: 3.96       | 3.90   | 1694             | C=O        | 7.60 (9 H)                               | Ar-H                  |
|           |                                                                              |           |            | N: 16.92      | 16.84  | 1152             | C=S        | 8.18 (1 H)                               | CH=N                  |
|           |                                                                              |           |            |               |        | 692              | C-Cl       |                                          |                       |
| Vd        | C <sub>17</sub> H <sub>17</sub> N <sub>4</sub> O <sub>3</sub> SCl<br>(392.5) | 165       | 54.0       | C: 51.85      | 51.97  | 3290             | NH         |                                          |                       |
|           |                                                                              |           |            | H: 4.21       | 4.33   | 1684             | C=O        |                                          |                       |
|           |                                                                              |           |            | N: 14.18      | 14.26  | 1145             | C=S        | -                                        | -                     |
|           |                                                                              |           |            |               |        | 660              | C-Cl       |                                          |                       |
| Ve        | C <sub>15</sub> H <sub>11</sub> N <sub>4</sub> O SCl<br>(401.5)              | 180       | 75.4       | C: 44.78      | 44.83  | 3300             | NH         | 7.36 (3 H)                               | NH                    |
|           |                                                                              |           |            | H: 2.80       | 2.73   | 1700             | C=O        | 7.66 (7 H)                               | Ar-H                  |
|           |                                                                              |           |            | N: 13.86      | 13.94  | 1155             | C=S        | 8.24 (1 H)                               | CH=N                  |
|           |                                                                              |           |            |               |        | 680              | C-Cl       |                                          |                       |

corresponding 5-(N-cyclohexylidene hydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidine (IVa) on treatment with ammonia (25 ml), the free base IVa was obtained, which was filtered and purified by recrystallization from ethanol, [mp, 141°C, yield; 6.2 g (47.8 %)]. Compounds IVb-c were similarly prepared and characterized (Table 3).

#### 1-(Cyclohexylideneamino) -5-(4-chloroaryl)-2,4-thiobiuret (Va)

5-(4-Chloroaryl)-1-(cyclohexylideneamino)-2-S-benzyliso-4 biuret (IIIa) (1.30 g; 0.004 mol) were dissolved in a mixture of pyridine (5 ml) – triethylamine (30 ml) (1:6) and subjected to reduction in a stream of hydrogen sulphide for 4 h. The resulting mixture was then filtered, poured over crushed ice and acidified with dilute HCl (10 ml), when the expected 1-(cyclohexylideneamino)-5-(4-chloroaryl)-2,4-thiobiuret (Va) was precipitated. It was filtered and purified by recrystallization from

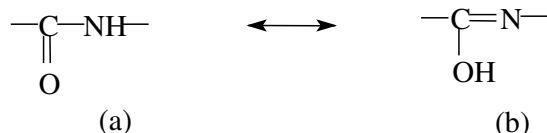
ethanol, [m p, 155°C; yield, 7150g (55.08 %). 1-(2,4-Dimethoxyarylideneamino), 1-(arylideneamino), 1-(3,5-dimethoxyarylidene-amino), 1-(2,4-dichloroarylideneamino)-5-(4-chloroaryl)-2,4-thiobiurets (Vb-c) were similarly prepared and characterized (Table 4).

#### Results and Discussion

Oxidation of 5-(4-chloroaryl)-1-(cyclohexylideneamino)-2-S-benzyliso-4-biurets (IIIa-e) with bromine in chloroform afforded the corresponding 1,2,4-thiadiazolidines. The position of the substituents at 1 and 5 did not appear to have any marked effect on this mode of reaction. The benzyl group could only be eliminated when bromine was the oxidant. However, iodine failed to bring about the required reaction. The oxidative debenzylation and cyclisation of related 2-S-benzyliso-4-biurets to the corresponding 1,2,4-thiadiazolidines using chloroform or benzene as a solvent were successfully observed. Such oxidation in ethanol or methanol could not eliminate benzyl

group and did not form the expected compounds. It may, therefore, be concluded that the polarity of the solvent has some relevant effect on the oxidative debenzoylation and cyclisation reaction that with decrease in polarity of the solvent, the oxidative debenzoylation is favoured and vice-versa. The reductive debenzoylation of related 2-S-benzyliso-4-biurets to the corresponding 2-(substituted-arylideneamino)-5-(4-chloroaryl)-2,4-thiobiurets (Va-e) with hydrogen sulphide could be effected either in ethanol sodium sulphide or pyridine-triethylamine medium.

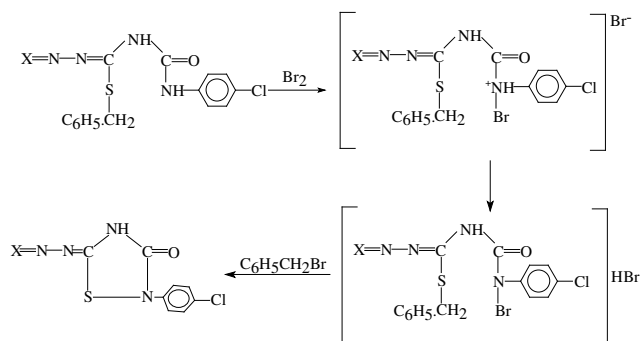
The oxidative debenzoylation and cyclisation could be easily affected at 30-40°C, but below 15°C, the expected reaction leading to the formation of the corresponding 1,2,4-thiadiazolidines did not take place. Thus, the nature of the solvent, temperature and oxidant appear to be important factors in the reaction for synthesis of compound from II-V (Scheme). 5-(N-substitutedarylidenehydrazono)-2-(4-chloroaryl)-3-oxo-1,2,4-thiadiazolidines (IVa-e) have been studied for their structure with the help of IR, <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra (Table 3). -CO.NH group, may exists in two tautomeric forms:



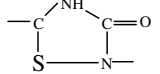
But, from the IR spectra<sup>16,17</sup>, presence of  $\nu$  C=O (in  $\text{cm}^{-1}$ ) at 1705 (IVa), 1703 (IVb), 1675 (IVc), 1696 (IVd), 1698 (IVe) firmly rule out the existence of tautomeric forms.

Similarly, from <sup>1</sup>H-NMR<sup>17</sup>, the signals of -NH at 6.54 (IVa), 6.78 (IVb), 6.60 (IVc), 7.24 (IVd), 6.48 (IVe) (ppm) and from <sup>13</sup>C-NMR<sup>17</sup>, the signals of C=O at 160.52 (IVb), 159.30 (IVc), 161.12 (IVe) (ppm), rule out the existence of tautomerism in the compound.

The expected mechanism of the oxidative debenzoylation and cyclization may be given as follows.



## Conclusion

1,2,4-thiadiazolidine, , is a biologi-

cally active moiety, the activity of which can be varied by substituents. This point has been studied and to some extent established in this paper by preparing newer compounds using different substituents and a number of fungi and cancer affecting cells. The activity for a particular fungi varies as: IVe > IVb > IVd > IVc > IVa, the most active being 2,4-dichloro arylidene substituted 1,2,4 thiadiazolidine. Further, the same compound is a potential anti-inflammatory and anti-tumor agent, which significantly down regulates NF- $\kappa$ B transcription factor.

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