and investigate them for biological and pharmacological activities. The key starting materials, 1-H-indole 2-3 dione and 2-aminothiazole, were refluxed in the presence of alcohol and glacial acetic acid to get Schiff base 3 (1’-3’tiazole 2-yl imino) 1-3 dihydro 2-H-indole-2-one (A); mannich bases are prepared with different secondary amines in the presence of formaldehyde (A1-A6). Meanwhile, Spiro isatin derivatives are prepared with Azetidine (Aa) and thiazolidone (Ab) in the presence of chloroacetyl chloride (Cl-CH2-CO-Cl) and thioglycolic acid (SH-CH2-COOH).

**MATERIALS AND METHODS**

Melting points are recorded in open capillary tubes and are uncorrected. The IR spectra (KBr) were recorded on a...
SHIMADZU FTIR-8300, spectrophotometer, Japan. The H-NMR spectra were recorded on a Bruker Advance-400 MHz spectrometer, USA. Purity of the compounds was checked by Thin layer chromatography. For analgesic, anti-inflammatory activity studies, adult healthy albino rats (Swiss strain) of either sex weighing 80–120 g were used. All the animals were maintained under standard conditions and had access to pelleted animal feed and water. The study protocol was approved by the Institutional Animal Ethics Committee.

**Synthesis**[7] of 3(1’-3’Thiazole-2-yl Imino) 1-3 Dihydro-2H Indole-2-One (A)

Equimolar quantity of isatin and 2-amino thiazole was taken in a round bottom flask, dissolving the content with a sufficient amount of ethanol and two to three drops of glacial acetic acid was added. The content was refluxed for 4–5 h and then poured into ice cold water. The solid obtained was filtered, dried and recrystallized from ethanol.

**Synthesis**[7] of 1[(Diphenyl Amino) Methyl]-3-(1’-3’ Thiazole-2-yl Imino) 1-3 Dihydro-2H Indole-2-One (A)

To a slurry containing A (0.003 mol), ethanol (5 ml) and 37% formalin (1 ml), diphenylamine (0.003 mol) was added slowly with good stirring. The reaction mixture was cooled and allowed to stand at room temperature for 1 h, with occasional shaking. Then, it was warmed on a steam bath for 15 min, cooled and the product was recovered. The product was recrystallized from chloroform–methanol (1:1) mixture.

The compounds 1(piperazin-1-yl) methyl-3-(1’-3’ thiazole-2-yl imino) 1-3 dihydro-2H indole-2-one (A), 1[(dicyclohex-y1) methyl]-3-(1’-3’ thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A), 1[(dimethylamino) methyl]-3-(1’-3’ thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A), 1-[(diethy lamino) methyl]-3-(1’-3’ thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A) and 1-[(morpholin-4”yl) methyl]-3-(1’-3’ thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A) were prepared by following a similar procedure.

**Synthesis of 3’Chloro-1-(1”-3”Thiazo-2-yl) 4’-H-Spiro [Azetidine-2, 3’Indole] 2’4-(1-H) Di One (A)***

Equimolar quantity of compound A and thioglycollic acid was dissolved in a round bottom flask. Then, 1,4-dioxane and a pinch of zinc chloride was added to it. The contents of the mixture were refluxed for 12–14 h, concentrating the content of the flask, and poured into ice cold water. The solid mass was collected and dried. The product was recrystallized from chloroform–methanol mixture (1:1).

**Antimicrobial activity**

The *in vitro* antimicrobial activity[9] was carried out against 24-h-old cultures of microorganisms by the cup–plate method. Compound A, A, A, A, A were tested against four pathogenic bacteria, *Pseduomonas miribelis, Pseduomonas aeroginosa, Escherichia coli* and *Staphylococcus aureus*. The antifungal activity was tested against *Aspergillus niger* and *Candida albicans*. Ampicillin and cotrimoxazole was taken as an internal standard for antibacterial and antifungal activity, respectively. The compound was tested at a concentration of 100 µg/ml in DMF against all organisms. The zone of inhibition was compared with the standard drug after 24-h incubation at 37°C for antibacterial and 48 h at 25°C for antifungal activity.

**Analgesic activity**

The analgesic activity[10] was evaluated by the acetate acid-induced writhing test[5] using adult Swiss albino mice of either sex. In this method, mice are made to writhe by a simple intraperitoneal injection of 0.6% v/v aqueous acetic acid (0.1 ml/kg). Test substances were administered 30 min before the injection of acetic acid. The number of writhes (full extension of hind paws) was recorded.

**Anti-inflammatory activity**

The anti-inflammatory activity[10] was evaluated by rat paw edema. Edema represents the early phase of inflammation and carragenin-induced paw edema is the simplest and most widely used method[8] for studying the anti-inflammatory activity of the chemical compounds. This method is based on the plethysmographic measurement.
of carragenin-induced acute rat paw edema. For this study, Wister rats of either sex, weighing between 80 and 100 g were divided into 10 groups of five animals each. Group 1 serves as a solvent control, and received 0.5% Carboxy Methyl Cellulose in normal saline orally. Group 2 received indomethacin (10 mg/kg) in solvent as a standard. Groups 3–10 received test drugs at a dose of 100 mg/kg orally. These drugs were administered 1 h before the injection of an irritant, carragenin. After 1 h, all the animals were injected subcutaneously with a suspension of carragenin in CMC solution (0.1 ml) to the left hind paw in the subplantar region and the paw volume was measured immediately. After 3 h, the paw volume was measured for the control, standard and test groups. Percentage inhibition of paw volume was calculated.

RESULTS AND DISCUSSION

The structures of newly synthesized compounds were elucidated by IR, 1H-NMR and elemental analysis and are reported as follows:

The IR spectrum of A in KBr (in cm⁻¹): 3446 (NH str), 1728 (C=O), 1610.95 (C=N), 1469.01 (CH=CH of Ar), 1096.66 (C-S-C).

1H-NMR of A (δ values): 11.04 (S, 1H, NH), 6.89–7.61 (m, Ar-H), 3.39 (1H,CH), 3.16 (1H, CH).

### Table 1: Characterization data of the synthesized compounds

| Compounds | M.P (°C) | Yield% | Molecular formula | Found (calculated) % |
|-----------|----------|--------|-------------------|----------------------|
| A₁        | 108–110  | 59     | C₆H₁₂N₄O₅S       | C₆H₁₂N₄O₅S          |
| A₂        | 238–240  | 68     | C₆H₁₂N₄O₅S       | 69.90 (70.22)        |
| A₃        | 110–114  | 72     | C₆H₁₂N₄O₅S       | 67.01 (68.21)        |
| A₄        | 220–223  | 72     | C₆H₁₂N₄O₅S       | 58.60 (58.72)        |
| A₅        | 252–254  | 61     | C₆H₁₂N₄O₅S       | 61.01 (61.12)        |
| A₆        | 248–250  | 65     | C₆H₁₂N₄O₅S       | 58.71 (58.82)        |
| A₇        | 340–342  | 65     | C₆H₁₂N₄O₅S       | 50.88 (51.47)        |
| A₈        | 343–344  | 62     | C₆H₁₂N₄O₅S       | 51.12 (51.47)        |

### Table 2: Results of antimicrobial activity

| Compounds | P. mirebels | P. auriginosa | E. coli | S. aureus | A. niger | C. albicans |
|-----------|-------------|---------------|---------|-----------|----------|-------------|
| A₁        | 12          | 14.2          | 10      | 9.5       | 22.2     | 17.3        |
| A₂        | 13          | 12.9          | 10      | 8.3       | 21       | 16.5        |
| A₃        | 10          | 10.3          | 11.2    | 9.8       | 23       | 20          |
| A₄        | 11.1        | 11            | 10      | 8.8       | 17       | 17.8        |
| A₅        | 11          | 11.2          | 13.6    | 9.3       | 21       | 15.3        |
| A₆        | 12.2        | 12.5          | 14      | 9.1       | 22.9     | 19          |
| A₇        | 15.2        | 17            | 20      | 10        | 5        | 21.2        |
| A₈        | 13.5        | 16.1          | 17.5    | 10        | 24.3     | 20          |
| Ampicillin| 23          | 22.5          | 24      | 26        | 27.6     | 28          |
| Cotrimoxazole| DMF    | -             | -       | -         | -        | -           |

*average of three readings.

### Table 3: Analgesic activity of the compounds

| Compound code | Dose (mg/kg) | Number of writhing movements | % of protection |
|---------------|-------------|------------------------------|----------------|
| 0.5% CMC      | -           | 58.8 ± 0.95                  | -79.5632.1128.46|
| Indomethacin  | 10          | 11.2 ± 4.32**                | 43.7933.2127.91|
| A₁            | 100         | 37.2 ± 3.21*                 | 49.8167.5164.78|
| A₂            | 100         | 39.2 ± 3.48                  |                |
| A₃            | 100         | 30.8 ± 2.23*                 |                |
| A₄            | 100         | 36.6 ± 2.97*                 |                |
| A₅            | 100         | 39.5 ± 3.27                  |                |
| A₆            | 100         | 27.5±2.85**                  |                |
| A₇            | 100         | 17.8 ± 2.19**                |                |
| A₈            | 100         | 19.3 ± 2.98**                |                |

Values are expressed as mean ± SE (n = 6). **P<0.05, ***P<0.01 and ****P<0.001 compared with vehicle control (ANOVA followed by Dunnet’s t-test).

IR of A₁ (in cm⁻¹): 2890 (CH₂), 1730 (C=O), 1670 (C=N), 1459.37 (CH=CH), 1200 (C-N), 1090 (C=S-C), 766.88 (C=C aromatic hydrocarbon).

1H-NMR of A₁ (δ values): 6.89–7.09 (m, Ar-H), 2.50 (S, 2H, N=CH₂), 3.38 (S, 2H, CH of thiazole).

IR of A₆ (in cm⁻¹): 2832 (CH₂), 1720 (C=O), 1609 (C=N), 1480 (CH=CH), 1363 (C-N), 1164.30 (C-S-C str), 3305 (N-H).

1H-NMR of A₆ (δ values): 6.89–7.92 (m, Ar-H), 4.38 (S, 1H, N-H), 3.06–3.38 (8, 1H, CH), 1.23 (t, 2H, CH₂ piperazine).

IR of A₇ (in cm⁻¹): 2946 (CH), 2854 (C-H), 1718.84 (C=O),
1647.58 (C-N), 1517 (C=N), 1458 (CH = CH of Ar), 1105 (C=S-C).

IR of A₄ (in cm⁻¹): 2942 (CH₃), 1735 (C=O), 1676 (C=N), 1654 (C-N), 1542 (CH = CH of Ar), 1042 (C-S-C str), 758 (C-Cl).

IR of A₅ (in cm⁻¹): 1718 (C=O), 1560 (C-N str), 1458 (CH = CH of Ar), 1105 (C-S-C str), 717 (C=C Ar).

IR of A₆ (in cm⁻¹): 3445 (NH str), 1720 (C=O), 1640 (C=N), 649 (C-Cl).

1H-NMR A₄: 11.08 (s, 1H, NH), 6.93–7.79 (m-Ar-H), 3.39 (δ, 1H, CH of thiazole), 2.27 (s, 1H, CH₂Cl).

IR of A₆ (in cm⁻¹): 3442 (NH str), 1724 (C=O), 1708 (C=O), 1645 (C-N), 1090 (C-S-C).

1H-NMR of A₄ (δ values): 11.08 (s, 1H, NH), 6.71–7.78 (m-Ar-H), 3.39 (δ, 1H, CH of thiazole), 2.50 (s, 2H, CH₂Cl).

The characterization of synthesized compounds is given in Table 1. Analytical data of the compounds supports the proposed structures.

Results of antimicrobial activity, analgesic activity and anti-inflammatory activity are shown in Tables 2–4, respectively. The results of the antimicrobial activity revealed that the compounds A₁, A₂, A₃, A₄, A₅ and A₆ were found to be active against bacteria and fungi. The compounds A₃, A₄, A₅ and A₆ showed significant analgesic activity, with percentage of inhibition of 43.69, 49.81, 67.51, 64.78, respectively, as compared to indomethacin, with percentage inhibition of 79.56. The compounds A₃, A₅, A₆ showed considerable anti-inflammatory activity, having percentage protection of 38.77, 32.65 and 36.73 as compared with indomethacin, having percentage protection value of 46.93.

Thus, it is observed that the synthesized compounds showed promising antimicrobial, analgesic and anti-inflammatory activities. Further studies are needed to discover new novel compounds of this class with profound activities.

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