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Research Article

SYNTHESIS AND PHARMACOLOGICAL EVALUATION: ANTIMICROBIAL, ANTI-INFLAMMATORY, ANALGESIC, ULCEROGENIC PROPERTIES OF SEVERAL *BIS*-HETEROCYCLIC DERIVATIVES

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ABSTRACT

In Medicinal chemistry, heterocyclic derivatives contributed the largest classical division of organic chemistry. Thiadiazole compounds have been known for many biological activities such as hypoglycemic, anticancer, anti-inflammatory, anti-asthmatic, antihypertensive etc. On this research, several 1,1-*bis* [2-{2-(5-(arylidene)imino-1,3,4-thiadiazol-2-yl) methyl amino}- 1,3,4-thiadiazo-5-yl] cyclopropane 5(a-h) have been synthesized from cyclopropane dicarboxylic acid comprising thiadiazole moieties. All the synthesized compounds have been characterized by elemental (C,H,N) and spectral (I.R., ^{1}H- NMR, Mass) analysis. Furthermore, all synthesized compounds were screened for their antifungal, antimicrobial, anti-inflammatory, analgesic, ulcerogenic and toxic activities. The Compound **5f**, 3-chlorophenyl substituted was found the most potent in antimicrobial spectrum against all the used microbes. On the other hand, compound **5g,** 4-hydroxy-3-methoxy phenyl substituted was found the most potent in anti-inflammatory, analgesic, and ulcerogenic activities which further evaluated for lesser toxicity test.

Key words: *bis*-phenylarylidinylthiadiazolidinone cyclopropane, anti-microbial, anti-inflammatory, analgesic, ulcerogenic and toxicity studies.

INTRODUCTION

Currently using non-steroidal anti-inflammatory drugs (NSAIDs) viz. ibuprofen, diclofenac, indomethacin and flurbiprofen cause gastric toxicity. Their prolong use resulted into gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity (Kimmey 1992). On the other hand, there is no chemotherapeutic agent which is found to show anti-inflammatory as well as known antibacterial activities. In medicinal chemistry, heterocyclic derivatives contributed the largest classical division of organic chemistry. Organic chemistry is providing facile and efficient methodology for the synthesis of different heterocyclic moieties due to their versatile biological spectrum. Microbial infections cause pain and inflammation. In the current study, we have focused on thiadiazoles due to its versatile biological profile such as hypoglycemic (Avetisyan *et al*., 1981), anticancer (Parkanyi *et al.* 1992), anti-inflammatory (Boschelli *et al*., 1993; Labanauskas *et al*., 2001; Schenone *et al*., 2001), anti-asthmatic (Bhattacharya *et al*., 2005), antihypertensive (Vio *et al*., 1989), antidepressant (Clerici *et al*., 2001), anxiolytic (Clerici *et al*., 2001), fungicidal (Chen and Hen, 2000; Zou *et al.,* 2002; Zou *et al*., 2002) and anti-tuberculosis (Foroumadi *et al*., 2001). On the other hand, esters of 1,1-cyclopropane dicarboxylic acid were found to possess important insecticidal activity (Ronald and Leland 1998). Furthermore bulk of literature is available to explore the biological importance of Schiff bases (Ibrahim *et al*., 2011; Sah *et al*., 2011; Sanjeeva *et al*., 2010) especially antimicrobial and thus aroused our interest in the synthesis of some novel *bis* Schiff bases. Based on the wide spectrum of biological profile of 1,1-propane dicarboxylic acid, thiadiazole, Schiff base and their importance in pharmaceutical, biological field and in continuation of our ongoing research on biologically active heterocycles (Panwar *et al*., 2006; Goel *et al*., 2004; Kumar *et al*., 2003; Panwar *et al*., 2011; Panwar *et al*., 2011), it was thought of interest to accommodate 1,1-propane dicarboxylic acid, thiadiazole and aryl

aldehydes in a single molecular frame work of designing to synthesize some novel *bis* heterocycles for enhancing biological activity with lesser toxicity.

MATERIAL AND METHODS

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. Reference drugs ampicillin trihydrate and fluconazole were procured from Ind-Swift Pharmaceutical, Panjab, India and Macleods Pharmaceutical, Mumbai, India respectively. The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogenity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus C H N rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and ^{1}H- NMR spectra on Bruker DPX 200 using TMS as internal standard.

Anti-inflammatory activity

Anti-inflammatory activity was determined against carrageenan induced rat's paw edema (Winter *et al*., 1962). The rats were divided into three groups (control, drug treated, and standard, each groups contain 6 animals). A freshly prepared suspension of carrageenan (1% in 0.9% saline). 0.05mL was injected under the planter aponeurosis of the right hind paw of each rat. Test compounds and standard drug were administered orally to the animals of drug treated groups and the standard drug group, respectively, 1 h before the carrageenan injection. The paw volume of each rat was measured before 1 and after 3h of carrageenan treatment with the help of a plethymometer. The percent anti-inflammatory activity was calculated according to the formula given below:

$$\text{Percentage of inhibition of edema} : \frac{1 - V_t}{V_c} \text{ X } 100$$

Where, V_t and V_c are the mean increase in paw volume of rats of the treated and the control group, respectively. Results obtained were statistically analyzed.

Analgesic activity

Analgesic activity was performed following the method of Berkowitz *et al*., 1977. This method is based on the property of the test compound to antagonize the phenylquinone-induced pain syndrome in mice. Groups of five mice were injected intraperitonealy with 0.25mL of a 0.02% solution of phenylquinone in ethanol (5%) 1h after oral administration of the test compound. The number of writhes induced in each mouse was counted for 5min (between 5 and 10min) after injection of an irritant. The analgesic effect was expressed as percent protection in comparison to control.

% protection = (1 - mean no. of writhes in mice of test groups/ mean number of writhes in mice of control group) X 100

Ulcerogenic activity

Ulcerogenic labilities of newly synthesized compounds were checked (Verma *et al*., 1981) in albino mices. Albino rats were fasted for 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment, and their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

COX-1 and COX-2 activities

The compounds prepared were tested for cyclooxygenase-1 and cyclooxygenase-2 inhibitory activities (Copeland *et al*., 1994) followed to determine the IC_{50} values. The enzyme activity is measured using chromogenic assay based on oxidation of *N, N, N', N'*-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandin G_2 to prostaglandin H_2 by COX-1 and COX-2

enzymes. COX-1 and COX-2 enzymes used in the assay were purified from microsomal fraction. The compounds were dissolved in DMSO and stock solution was diluted to required assay concentration. The assay mixture consists of Tris-HCl. Tris-HCl buffer (pH 8.0, 100μM). hematin (15μM), EDTA (3mm), enzyme (COX-1 or COX-2, 100μM) and test compound. The mixture was pre-incubated at 25°C for 15min and then the reaction was initiated by the addition of arachidonic acid (100μM) and TMPD (120μM) in total volume of 1.0mL. The enzyme activity was measured by estimating the initial velocity of TMPD oxidation for the first 25s of the reaction following the increase in absorbance at 603nm. IC_{50} values are calculated from four parameter least squares non-linear regression analysis of the log dose vs percentage inhibition plot. However, none of the compound studied here exhibited significant inhibitory activity when compared to standard inhibitors indomethacin (for COX-1) and celecoxib (for COX-2).

Antimicrobial screening

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial and fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Bacterial inhibition diameter was measured in mm. Microorganisms employed antibacterial studies were *Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method (Cruickshank *et al*., 1975; Collins 1976) was used for determination of the preliminary antibacterial activity. Sterile 5mm Whatman no. 1 filter paper discs were used in the disc diffusion method. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140°C for 1h. The test compounds were prepared with different concentrations using DMF. One hundred milliliters of the microbial suspension was spread onto nutrient agar plates. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37°C for 24h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted (Table I). On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigatus* (plant isolate), *Candida glabrata, Candida albacans* and *Candida krusei* in DMSO by the serial plate dilution method (Khan, 1997; Varma, 1998). Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1g), D-glucose (4g), and agar (2g) in distilled water (100mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1h. Using an agar punch wells were made into each well labeled. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone in mm (Table I).

Acute toxicity test

Lethal dose (LD_{50}) (Carrol 1952) of test compounds were determined in albino mice. After 24h of drug administration, percent mortality in each group was observed from the data obtained LD_{50}. Data revealed that compound **5f** does not show any toxicity up to dose of 9.95mg/mL body weight in mice.

Synthesis of 1,1-*bis* (2-amino-1,3,4-thiadiazol-5-yl) cyclopropane (1)

A mixture of 1,1-cyclopropane dicarboxylic acid (0.01mol), thiosemicarbazide (0.02mol) and phosphorous oxychloride (5mL) was gently refluxed for 30min. After cooling, water (10mL) was added and the reaction mixture was refluxed for four hours and filtered. The completion of reaction of reaction was checked by TLC. The cooled, refluxed residue was poured into ice-water slowly with continuous stirring, the solution was neutralized with aqueous 3% KOH solution and the precipitate was filtered and recrystallized by methanol: Yield: 67%; m.p.: 235-238°C; R_f: 0.50; IR (KBr, cm^{-1}): 3280 (NH_2), 1605 (C=N), 665 (C-S-C); ^{1}H-NMR ($CDCl_3$, δ/ ppm): 6.40 (s, 4 H, 2 X 2H, NH_2), 1.18-1.00 (m, 4H, 2 X

2H, CH_2); MS (*m/z*, %): 240.31. Anal. calcd. for $C_7H_8N_6S_2$: C, 34.99; H, 3.36; N, 34.97%. Found: C, 35.00; H, 3.35; N, 34.98%.

Synthesis of 1,1-*bis* (2-Carboethoxy-methylamino-1,3,4-thiadiazol-5-yl) cyclopropane (2)

A mixture of compound **1** and chloroethylacetate (0.01mol) in dry dioxane (50 ml) was refluxed for 8h. The reaction mixture was further stirred for 1h, and poured in water. The resulting mixture was filtered and recrystallized from ethanol to yield compound **2**: Yield: 54%; m.p.: 209°C; R_f : 0.61; IR (KBr, cm^{-1}): 3185(N-H), 2950 (C-H aliphatic), 1695(C=O), 1603 (C=N), 1295(N-N), 1242(C-N), 670 (C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 6.02 (t, 2H, 2X1H, NH, exchangeable with D_2O), 4.55 (d, 4H, 2X 2H, NH-CH_2), 4.12(q, 4H, 2 X 2H, 2H-$COOCH_2$-CH_3), 1.45 (t, 6H, 2X3H, $COOCH_2$-CH_3), 1.20-1.05 (m, 4H, 2 X 2H, CH_2); MS (*m/z*, %): 412.49. Anal. calcd. for $C_{15}H_{20}N_6S_2O_4$: C, 43.68; H, 4.89; N, 20.37 %. Found: C, 43.66; H, 4.87; N, 20.43 %.

Synthesis of 1, 1-*bis* [2-{(2-Thiosemi-carbazido)carbonylmethylamino}- 1,3,4 -thiadiazo-5-yl] cyclopropane (3)

The equimolar mixture (0.02mol) of compound **2** and thiosemicarbazide (0.02mol) in methanol (50mL) was refluxed for 10h. The excess of solvent was distilled off and viscous mass was poured into water, dried and recrystallized from methanol to yield compound **3**.

Yield: 56 %; m.p.: 181 °C; R_f : 0.63; IR (KBr, cm^{-1}): 3189(N-H), 2956 (C-H aliphatic), 1692 (C=O), 1606 (C=N), 1298(N-N), 1240(C-N), 677 (C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.12-7.48 (m, 8H, 2 X 4H, $NHNHCSNH_2$, exchangeable with D_2O), 6.12(t, 2H, 2X 1H, NH, exchangeable with D_2O), 4.47(d, 4H, 2 X 2H, NH-CH_2), 1.08-0.96(m, 4H, 2 X 2H,CH_2); MS (*m/z*, %): 502.62. Anal. calcd. for $C_{13}H_{18}N_{12}O_2S_4$: C, 31.06; H, 3.61; N,33.44 %. Found: C, 31.12; H, 3.65; N, 33.50 %.

Synthesis of 1, 1-*bis* [2-{2-(5-amino -1,3,4-thiadiazol-2-yl) methylamino}-1,3,4-thiadiazo-5-yl] cyclopropane (4)

A mixture of compound **3** (0.01mol) and conc. H_2SO_4 (20mL) was kept overnight at room temperature, poured into ice-cold water, neutralized with liquid ammonia and filtered. The product thus obtained was recrystallized from ethanol water to furnish compound **4**. Yield: 56%; m.p.: 279°C; R_f :0.67; IR (KBr, cm^{-1}): 3190(N-H), 2952 (C-H aliphatic), 1689 (C=O), 1609 (C=N), 1288 (N-N), 1238(C-N), 671 (C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 6.26(bs, 4H, 2 X 2H, NH_2), 5.75(s, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.47(d, 4H, 2 X 2H, NH-CH_2), 1.08-0.96(m, 4H, 2 X 2H,CH_2); MS (*m/z*, %): 466.59. Anal. calcd. for $C_{13}H_{14}N_{12}S_4$: C, 33.46; H, 3.02; N,36.02 %. Found: C, 33.41; H, 3.02; N, 36.30 %.

General procedure for the Synthesis of 1, 1-*bis* [2-{2-(5-(arylidene)imino-1,3, 4-thiadiazol-2-yl) methyl amino}-1,3, 4-thiadiazo-5-yl] cyclopropane 5(a-h)

A mixture of compound **4** (0.01mol) and proper aromatic aldehydes (0.01mol) in methanol (50mL) was refluxed for 5-7h, in the presence of few drops of glacial acetic acid. The progress and completion of reaction was checked by TLC. The reaction mixtures were distilled off cooled and then poured into ice water, filtered, washed with water and dried to get compounds **5(a-h)**. The crude solids thus obtained were recrystallized from appropriate solvents.

5a. Yield: 51%; m.p.: 228 °C; R_f : 0.59; IR (KBr, cm^{-1}): 3020 (C-H-Ar), 2942 (C-H aliphatic), 1690(C=O), 1598 (C=N), 1292(N-N), 1246(C-N), 680 (C-S-C);^{1}H-NMR ($CDCl_3$, δ / ppm): 8.38 (s, 2H, 2X 1H, N=CH-Ph),7.60-6.66 (m, 10H, 2 X Ph), 6.38(brs, 2H, 2 X 1H, NH), 5.62 (s, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.63(d, 2H,NH-CH_2),1.01-0.90(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 642.80. Anal. calcd. for $C_{27}H_{22}N_{12}S_4$: C,50.45; H,3.45; 26.15%. Found: C, 50.50; H, 3.57; N, 26.21%. **5b.** Yield: 49%; m.p.: 290 °C; R_f : 0.68; IR (KBr, cm^{-1}): 3016 (C-H-Ar), 2946 (C-H), 1700(C=O), 1604 (C=N), 791(C-Cl), 676(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.42 (s, 2H, 2X 1H, N=CH-Ph),7.76-6.67 (m, 8H, 2 X Ph), 6.31(brs, 2H, 2 X 1H, NH), 5.60 (s, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.60(d, 2H,NH-CH_2),1.11-0.97(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 711.69. Anal. calcd. for $C_{27}H_{20}N_{12}S_4Cl_2$: C,45.57; H,2.83; 23.62%. Found: C, 45.54; H, 2.85; N, 23.64%. **5c.** Yield: 52%; m.p.: 166 °C; R_f : 0.62; IR (KBr, cm^{-1}):

3021 (C-H-Ar), 2944 (C-H), 1706(C=O), 1627 (C-C of aromatic ring),1607 (C=N), 672(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.46 (s, 2H, 2X 1H, N=CH-Ph), 7.85-6.64 (m, 8H, 2 X Ph), 6.22 (brs, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.56(d, 4H, 2 X 2H, NH-CH_2), 2.41 (s, 6H, 2X 3H,H_3C-Ph), 1.16-1.04(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 670.86. Anal. calcd. for $C_{29}H_{26}N_{12}S_4$: C,51.92; H,3.91; 25.05%. Found: C, 51.89; H, 3.92; N, 25.09%. **5d.** Yield: 46%; m.p.: 237 °C; R_f : 0.68; IR (KBr, cm^{-1}): 3025 (C-H-Ar), 2947 (C-H), 1704 (C=O), 1628 (C-C of aromatic ring), 1604 (C=N), 790(C-Cl), 677(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.40(s, 2H, 2X 1H, N=CH-Ph), 7.83-6.69 (m, 8H, 2 X Ph), 6.25 (brs, 2H, 2 X 1H, NH exchangeable with D_2O), 4.65(d, 2H, NH-CH_2), 2.44 (s, 6H, 2X 3H,H_3C-Ph),1.14-1.00(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 711.69. Anal. calcd. for $C_{27}H_{20}N_{12}S_4Cl_2$: C,45.57; H,2.83; 23.62%. Found: C, 45.53; H, 2.80; N, 23.59%. **5e.** Yield: 52%; m.p.: 249°C; R_f : 0.62; IR (KBr, cm^{-1}): 3018 (C-H-Ar), 2939 (C-H), 1698(C=O), 1618 (C-C of aromatic ring), 1610 (C=N), 668(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.35 (s, 2H, 2X 1H, N=CH-Ph), 7.83-6.69 (m, 8H, 2 X Ph), 6.25 (brs, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.65(d, 4H, 2 X 2H, NH-CH_2), 2.39 (s, 6H, 2X 3H,H_3C-Ph), 1.18-1.05(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 670.86. Anal. calcd. for $C_{29}H_{26}N_{12}S_4$: C,51.92; H,3.91; 25.05%. Found: C, 51.90; H, 3.90; N, 25.15%. **5f.** Yield: 44%; m.p.: 265°C; R_f : 0.63; IR (KBr, cm^{-1}): 3020 (C-H-Ar), 2942 (C-H), 1697(C=O), 1622 (C-C of aromatic ring), 1600 (C=N), 787(C-Cl), 671(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 8.37 (s, 2H, 2X 1H, N=CH-Ph),7.78-6.72 (m, 8H, 2 X Ph), 6.31(brs, 2H, 2 X 1H, NH), 5.60 (s, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.60(d, 2H,NH-CH_2),1.11-0.97(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 711.69. Anal. calcd. for $C_{27}H_{20}N_{12}S_4Cl_2$: C,45.57; H,2.83; 23.62%. Found: C, 45.52; H, 2.81; N, 23.60%. **5g.** Yield: 47%; m.p.: 133 ^{0}C; R_f : 0.66; IR (KBr, cm^{-1}): 3016 (C-H-Ar), 2947 (C-H), 1701(C=O), 1620 (C-C of aromatic ring), 1605 (C=N), 670(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 10.45 (s, 2H, 2X1H, HO-Ph), 8.30 (s, 2H, 2X 1H, N=CH-Ph), 7.80-6.69 (m, 8H, 2 X Ph), 6.26 (brs, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.56(d, 4H, 2 X 2H, NH-CH_2), 2.39 (s, 6H, 2X 3H,H_3C-Ph), 1.18-1.06(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 674.80. Anal. calcd. for $C_{27}H_{22}N_{12}S_4O_2$: C,48.06; H, 3.29; 24.91%. Found: C, 51.90; H, 3.90; N, 25.15%. **5h.** Yield: 50%; m.p.: 216°C; R_f : 0.63; IR (KBr, cm^{-1}): 3022 (C-H-Ar), 2945 (C-H), 1710(C=O), 1625 (C-C of aromatic ring), 1610 (C=N), 1165(C-O-C), 679(C-S-C); ^{1}H-NMR ($CDCl_3$, δ / ppm): 10.45 (s, 2H, 2X1H, HO-Ph), 8.30 (s, 2H, 2X 1H, N=CH-Ph), 7.80-6.69 (m, 8H, 2 X Ph), 6.26 (brs, 2H, 2 X 1H, NH, exchangeable with D_2O), 4.56(d, 4H, 2 X 2H, NH-CH_2), 2.39 (s, 6H, 2X 3H,H_3C-Ph), 1.18-1.06(m, 4H, 2X 2H, CH_2); MS (*m/z*, %): 734.85. Anal. calcd. for $C_{29}H_{26}N_{12}S_4O_2$: C,47.40; H, 3.57; 22.87%. Found: C, 47.45; H, 3.60; N, 22.85%.

RESULTS AND DISCUSSION

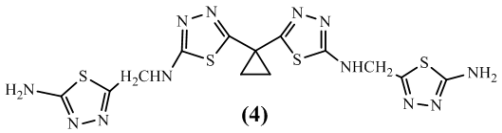
Chemistry

1,1-Cyclopropane dicarboxylic acid prepared according to the method in the literature (Singh and Danishefsky, 1990). 1,1-Cyclopropane dicarboxylic acid was refluxed with thiosemicarbazide and phosphorous oxychloride to furnish1,1-*bis*(2-amino-1,3,4-thiadiazol-5-yl) cyclopropane **(1)** in good yield. Reaction of compound **(1)** with chloroethyl-acetate in dry dioxane afforded 1,1-*bis* (2-carboethoxymethylamino-1,3,4-thiadiazol-5-yl) cyclopropane **(2)** which on further reaction with thosemicarbazide yielded 1,1-*bis* [2-{(2-thiosemicarbazido) carbonylmethylamino}- 1,3, 4-thiadiazo-5-yl] cyclopropane **(3)**. Compound **(3)** in presence of concentrate sulphuric acid, cyclised to give 1,1-*bis* [2-{2-(5-amino -1, 3, 4-thiadiazol-2-yl) methylamino}- 1,3,4-thiadiazo-5-yl]cyclo propane **(4)**. The synthesis of target derivatives, schiff bases i.e. 1, 1-*bis* [2-{2-(5-(arylidene)imino- 1,3,4-thiadiazol-2-yl) methyl amino}- 1,3,4-thiadiazo-5-yl] cyclopropane **5(a-h)** were afforded by the reaction of different aromatic aldehydes with compound **(4)** in presence of glacial acetic acid (Figure 1).

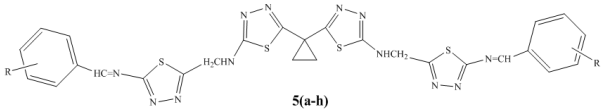
Pharmacology

Compounds 4, 5a-f wrere evaluated for antimicrobial, anti-inflammatory, analgesic and ulcerogenic activities. The biological evaluation data were summarized in table I and II.

Table I. Antimicrobial data for the synthesized compounds 4 and 5(a-h).



(4)



5(a-h)

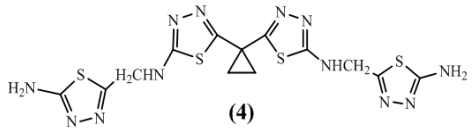
Comp. no.	Antibacterial inhibition (mm)				Antifungal inhibition (mm)			
	S. *aureus*	**E. *Coli***	**K. *pneumoniae***	**P. *vulgaris***	**A. *fumigatus***	**C. *glabrata***	**C. *albacans***	**C. *krusei***
4.	-	-	5	8	-	-	5	-
5a.	-	5	5	8	5	-	-	-
5b.	12	8	6	12	10	8	10	10
5c.	8	7	8	15	14	5	5	-
5d.	14	15	12	15	6	10	8	12
5e.	6	-	10	8	10	-	-	6
5f.	15	20	26	22	15	10	12	13
5g.	6	-	8	8	-	6	-	5
5h.	6	10	10	6	-	8	-	-
Ampicillin trihydrate (std.)	16	20	20	20	-	-	-	-
Fluconazole (std.)	-	-	-	-	20	15	16	15
DMF (control)	-	-	-	-	-	-	-	-

-: showing no activity

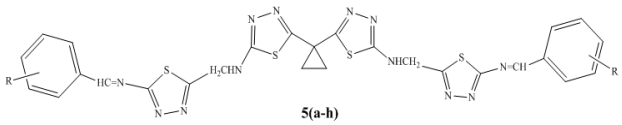
Synthetically derived molecules 4, 5a-f were assayed by using the disk diffusion method and serial plate method for antimicrobial activity against selected pathogenic clinical strains. The screening results were compared with standard ampicillin trihydrate and fluconazole respectively for antibacterial and antifungal testing respectively. From the antimicrobial screening data of compounds 4 and 5a-h, it was found that conversion of thiadiazole derivative i.e. 4 into Schiff bases i.e. 5a-h, brought enhancing antibacterial and antifungal activity. Compound 5f claimed the most remarkable antimicrobial spectrum against all the used microbes. It showed more potency against K. *pneumoniae*, P. *vulgaris* and comparable against S. *aureus* with standard drug ampicillin trihydrate while on the other hand, remaining tested compounds exhibited mild to moderate activity. Among the derivatives 5(a-h), derivative 5b, 5c and 5d showed moderate potency while compounds 5a, 5e, 5g and 5h showed poor antimicrobial activity against the used pathogenic strains.

Anti-inflammatory, analgesic and ulcerogenic activities of all the compounds 4, 5(a-h) showed significant results. The Compound 4 displayed poor activity data than the compounds 5a-h. All the compounds 5a-h has shown promising degree (32.60-42.00%) of anti-inflammatory activity in comparison to phenylbutazone. Derivatives 5b, 5d and 5f bearing 2-chlorophenyl, 4-chlorophenyl and 3-chlorophenyl substitution showed similar inhibition of edema. Results revealed that compounds 5a, 5b, 5c, 5d, 5e, 5f and 5h showed 32.78%, 36.00%, 35.70%, 35.97%, 32.60%, 36.25% and 34.10% inhibition of edema. Compound 5g that was 4-hydroxy-3-methoxy phenyl substituted, has shown the maximum percentage of anti-inflammatory activity, i.e. 42.00% at a dose of 50 mg/kg/ p.o. Considering, the potentiality of compounds 5g, it was studied in detail at three graded doses 25, 50, 100 mg/kg/ p.o. and exhibited better anti-inflammatory activity as compared to phenylbutazone. The ulcerogenic liability of compound 5g (132.10mg/kg/i.p.) is much

Table IIa. Antimicrobial data for the synthesized compounds 4 and 5(a-h).



(4)



5(a-h)

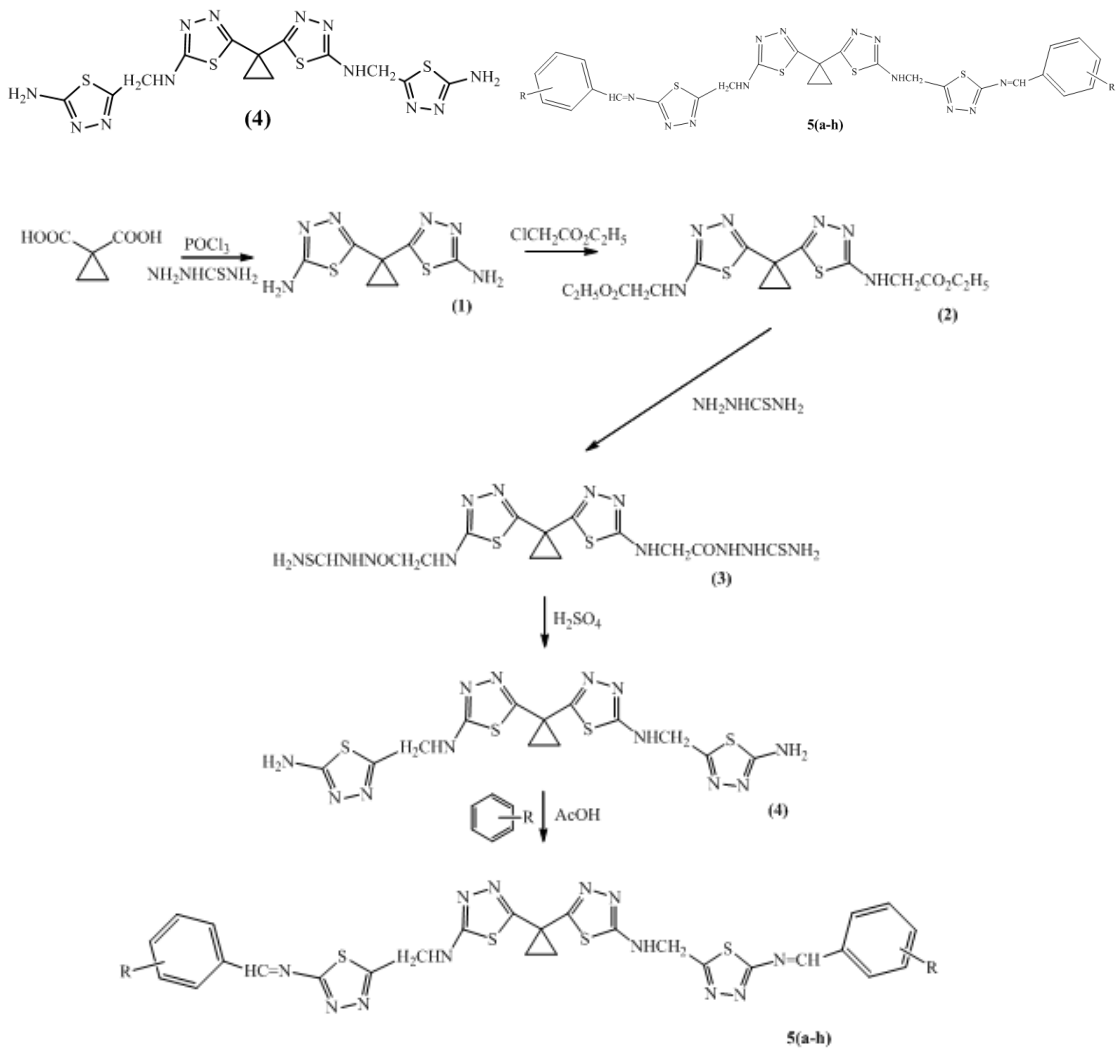
Comp. No.	R	Anti inflammatory activity		Analgesic activity	
		Dose (mg/kg /p.o.)	% inhibition of edema	Dose (mg/kg/p.o.)	% Protection
Phenylbutazone		25	26.60***	25	14.25***
		50	37.20***	50	32.55***
		100	64.50**	100	54.58***
Indomethacin		5.0	52.50***	5.0	42.30***
		7.5	63.00***	7.5	59.25***
		10.0	92.40***	10.0	65.30***
4.		50	31.15***	50	21.10**
5a.	H	50	32.78***	50	20.90**
5b.	2-Cl	50	36.00***	50	35.10***
5c.	4-CH_3	50	35.70***	50	21.20**
5d.	4-Cl	50	35.97***	50	19.90**
5e.	2-CH_3	50	32.60***	50	22.60***
5f.	3-Cl	50	36.25***	50	18.75**
5g.	4-OH	25	28.70***	25	19.00***
		50	42.00***	50	38.00***
		100	66.75***	100	58.00***
5h.	4-OH, 3-OCH_3		34.10***	50	19.95**

Table IIb. Antimicrobial data for the synthesized compounds 4 and 5(a-h).

UD50 (mg/kg/i.p.)	IC50 % of inhibition		ALD50 (mg/kg/i.p.)
	COX-1	COX-2	>800
66.21	-	-	>800
55.56	-	-	>800
-	37.45***	76.56***	>800
-	38.15***	72.42***	>800
-	38.70***	64.33***	>800
-	39.55***	66.17***	>800
-	39.25**	71.23***	>800
-	37.10**	65.80***	>800
-	37.45**	63.56***	>800
132.10	68.40**	92.20***	>800
-	39.25**	70.40***	>800

*P< 0.05, **P<0.01, ***P<0.001, - denotes not tested; Propylene glycol standard for control group.

less than that of indomethacin (55.56mg/kg/ i.p.) and phenylbutazone (66.21mg/kg/i.p.). The most active compound of this series was 5g which has shown potent analgesic activity, i.e. 42.00% at a dose of 50mg/kg/ p.o. Moreover, at three (25, 50 and 100) graded doses, compound 5g displayed better analgesic activity than phenylbutazone, while it was less active then indomethacin. All these compounds were also evaluated for COX-1 and COX-2 inhibitory activities. Compound 5g exhibited 68.40% and 92.20%



R = C_6H_5, 2-ClC_6H_4, 4-$CH_3C_6H_4$, 4-ClC_6H_4, 2-$CH_3C_6H_4$, 3-ClC_6H_4, 4-OHC_6H_4, 3-OCH_3(4-OH)C_6H_3

Figure 1. The synthesis of target derivatives, schiff bases i.e. 1, 1-*Bis* [2-{2-(5-(arylidene)imino-1,3,4-thiadiazol-2-yl) methyl amino}- 1,3,4-thiadiazo-5-yl] cyclopropane **5(a-h)** were afforded by the reaction of different aromatic aldehydes with compound **(4)** in presence of glacial acetic acid.

inhibition of action. Moreover, rest of the compounds showed moderate degree of COX-1 and COX-2 inhibitory actions suggesting that these compounds showed anti-inhibitory activity by inhibition of both COX-1 and COX-2 enzymes. ALD_{50} of all these compounds were >800 mg/kg/ i.o. (Table-II).

CONCLUSION

Antimicrobial evaluation of compound **4** and 1,1-*bis* (2-phenyl-5-arylidine-1,3-thiadiazolidin-4-one) cyclopropanes (**5a-h**) revealed that conversion of compound 4 into Schiff bases (**5a-h**) enhances biological potency viz. antimicrobial, anti-inflammatory, analgesic and ulcerogenic activities. Compound **5f** possessing 3-chlorophenyl substitution; displayed the most potent antimicrobial spectrum as well as anti-inflammatory and analgesic activity. On the other hand, compound **5g,** 4-hydroxy-3-methoxy phenyl substituted was found the most potent in anti-inflammatory, analgesic, and ulcerogenic activities and deserves further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed.

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