REPORT

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME 1, 3, 4-THIADIAZOL DERIVATIVES

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ABSTRACT

Thiadiazoles are their derivatives exhibit a wide variety of pharmacological activities such as Antibacterial and anti-inflammatory. In the present study we have synthesized derivatives some 2,5 substituted 1,3,4-thiadiazoles. The structures of these synthesized compounds were confirmed by IR, NMR, and MASS spectra data. These compounds were evaluated for varies biological activities such as antibacterial and anti-inflammatory activity.

Keywords: 1, 3, 4-thiadiazoles, anti-inflammatory and, antibacterial.

INTRODUCTION

Mostly five membered aromatic system having three heteroatom's at symmetrical position have been studied because of their physiological properties (Saad, 1996, Nanjunda *et al*., 2006), it is also well established that various derivatives of 1,3,4-thiadiazole exhibit broad spectrum of pharmacological properties such as antibacterial and antifungal activities (Saad, 1996 and Hui *et al*., 2002). 1,3,4- thiadiazole showed antibacterial properties similar to those of well known sulfonamide drugs (Golgolab *et al*., 1973) prompted by their finding and in continuation of our efforts in synthesizing various condensed bridge pharmaceutically active groups (Sandstorm 1968).

MATERIAL AND METHODS

General procedure

1:1 (molar ratio) of aromatic acid and phosphorous penta chloride were taken in a r.b. fitted with air condenser and calcium chloride guard tube. This mixture was heated gently to melt with vigorous shaking at around 50 C. After 30 min excess $POCl_3$ was distilled out. The residue was dried well and used for next step of the reaction.Then the Thiosemicarbazide added to the respected acid chloride and refluxed for 4-5 hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 x 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure.

Purification of the compounds

The compounds were purified by column chromatography using silica gel (60-120 mess) and 5% EtOAc in pet petroleum ether. The actual fraction was collected by regular checking of TLC. Finally the compounds were further purified by preparative TLC.

Compounds characterization (C-1 to C-7) data

Compound 1: [*N*-(5-Phenyl-[1, 3, 4] Thiadiazol-2-yl)-benzamide]

Thiosemicarbazide added to the benzoyl choride and refluxed for 4-5hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 62%, mp: 82^{o}C, Rf: 0.715: IR (KBr) 1601, 1446, 1312, 1172, 690 (thiadiazole), 3213 (NH), 1662 (C=O). ^{1}HNMR ($CDCl_3$, 300MHz): δ8.76 (Bs, 1H), 7.77 (d,3H, J=7.9Hz), 7.81 (d, 1H, J=7.9Hz), 7.62 (d,1H, J=7.5Hz), 7.567.42 (m, H) Mass(ESI): m/z (%): $C_{15}H_{11}N_3SO$: 281(23), 269(14), 264(18), 249(17), 248(100)

Compound 2: [2-Chloro-*N*-[5-(2-chloro-phenyl)-[1,3,4]Thiadiazol-2-yl]benzamide]

Thiosemicarbazide added to the 2-chloro-benzoyl choride and refluxed for 4-5hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. NaHCO3 and the compound was extracted with $CHCl_3$ (25 x 4 ml). The crude was obtained by distillation of CHCl3 under reduced pressure. Yield: 58% mp: 850C, Rf: 0.691: IR (KBr) 1602, 1445, 1315, 1171, 691 (thiadiazole), 3212 (NH), 1663 (C=O). 1HNMR (DMSO ds, 400MHz): 7.96 (d, 1H, J=7.5), 7.8 (d, 1H, J=7.4), 7.7 (d, 2H, J=8.8), 7.67.5 (m,3H), 7.4 (m.2H) Mass (CPS): m/z (%) $C_{15}H_9N_3SOC_l$, calculated: C=52.61, H=2.31, N=13.35%, found: C=52.62, H=2.2.97, N=13.23%).

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Compound 3: [4-Chloro-*N*-[5-(4-chloro-phenyl)-[1,3,4] Thiadiazol-2-yl]-benzamide]

Thiosemicarbazide added to the 4-chloro benzoyl choride and refluxed for 4-5 hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 59%, mp: 86°C, Rf: 0.672, IR (KBr) 1602, 1445, 1315, 1171, 691 (thiadiazole), 3212 (NH), 1663 (C=O). ^{1}HNMR (DMSO) ds, 400MHz): 6.56 (S,1H), 7.74 (d, 1H, J=7.3Hz), 7.5 (d, 1H, J=8.24 Hz), 7.63 (m, 3H), 7.7 (d, 1H, J=4.4 Hz), 7.9(d, 1H, J=8.6Hz), 8.1 (d, 1H, J=4.8Hz) Mass (LCMS2): Intensity (CPS): $C_{15}H_9N_3SOCl$, calculated: C=52.64, H=2.30, N=13.33, found: C=52.61, H=2.2.95, N=13.27%).

Compound 4:[3-Chloro-N-[5-(3-Chloro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide

Thiosemicarbazide added to the 3- chloro benzoyl choride and refluxed for 3-5hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 61%, mp: 85°C, Rf: 0.712, IR (KBr) 1602, 1445, 1315, 1171, 691 (thiadiazole), 3212(NH), 1663 (C=O). ^{1}HNMR (DMSO ds, 400Hz): 7.86 (d, 1H, J=7.2), 7.62 (d,1H, J=7.2), 7.52 (d, 2H, J=8.2), 7.57.6 (m, 3H), 7.41 (m, 2H), 7.39 (d, 1H, J=7.2). Mass (LCMS2): Intensity (CPS): m/z, $C_{15}H_9N_3SOCl$, calculated: C=52.63, H=2.29, N=13.31, found: C=52.62, H=2.2.92, N=13.25%).

Compound 5: [2-Nitro-N-[5-(2-Nitro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide

Thiosemicarbazide added to the 2- Nitro benzoyl-choride and refluxed for 3-6hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 57%, mp: 92°C, Rf: 0.693, IR (KBr) 1603, 1447, 1312, 1170, 691 (thiadiazole), 3213 (NH), 1662(C=). ^{1}HNMR (DMSO-ds, 400MHz): 7.95 (d,1H, J=7.5), 7.7 (d, 1H, J=7.3), 7.8 (d, 2H, J=8.9), 7.6-7.5 (m,3H), 7.3 (m.2H) Mass (CPS): m/z (%), $C_{15}H_9N_5SO_5$: calculated :C=52.59, H=2.29, N=13.31%). found: C=52.57, H=2.28, N=13.25%).

Compound 6: [3-Nitro-N-[5-(3-Nitro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide

Thiosemicarbazide added to the 3-Nitro benzoyl choride and refluxed for 3-6 hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 62%, mp: 88°C, Rf: 0.692**,** IR (KBr) 1605, 1448, 1315, 1172, 691 (thiadiazole), 3213 (NH), 1663(C=O). ^{1}HNMR (DMSO, ds, 400MHz): 7.91 (d, 1H, J=7.5), 7.9 (d, 1H, J=7.3), 7.8 (d, 2H, J=8.9), 7.6 (m,3H), 7.3 (m.2H) Mass (CPS): m/z (%), $C_{15}H_9N_5SO_5$: calculated: C=52.59, H=2.29, N=13.31%), found: C=52.52, H=2.29, N=13.27%).

Compound 7: [4-Nitro-N-[5-(4-Nitro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide

Thiosemicarbazide added to the 4-Nitro benzoyl-choride and refluxed for 3-6 hrs. The progress of the reaction was monitored by checking the TLC. Then excess benzene was distilled out, neutralized with aq. $NaHCO_3$ and the compound was extracted with $CHCl_3$ (25 X 4 ml). The crude was obtained by distillation of $CHCl_3$ under reduced pressure. Yield: 57%, mp: 87°C, Rf: 0.681, IR (KBr) 1602, 1444, 1316, 1172, 692 (thiadiazole), 3211 (NH), 1663(C=O). ^{1}HNMR (DMSO-ds, 400MHz): 7.95 (d, 1H, J=7.5), 7.8 (d, 1H, J=7.3), 7.9 (d, 2H, J=8.9), 7.7 (m, 3H), 7.3 (m.2H) Mass (CPS):m/z(%), $C_{15}H_9N_5SO_5$: calculated: C=52.57, H=2.29, N=13.31%), found: C=52.54, H=2.31, N=13.28%).

Anti-inflammatory activity (Winter *et al*., 1962)

Anti-inflammatory activities of all synthesized derivatives were determined by the carrageenean induced rat paw oedema model. Both (male and female) sex of Albino rat (100-200 gm) in different groups divided as control, test and standard and six animals were in each group. Overnight fasted animals were used and during that period only tap water was given. Generally, Indomethacine was used as standard drug, both test and standard drugs was suspended in 1% carboxy-methyl cellulose (CMC) and administered orally through gastric gavage needle and in control group 1% CMC is administered. After one hour of the compound administration we induced the carrageenean (1%) by the sub-planner surface of the right hind paws of animals. Initial paw volume and after paw volume was measure 3 hour and 6 hour after the administration of carrageenan. Percent paw oedema inhibition was calculated (table 1).

Antimicrobial activity

After synthesis of the compounds were evaluated for their antimicrobial activity against bacterial strain *Staphylococcus aureus* (gram +ve), *E. coli* (gram -ve) and *A. niger* by cup-plate method (Barry, 1976 and Bauer, 1966). Ofloxacin was used as standard drug in the concentration of 25 µg/ml. Nutrient Agar (Beef extract 10 gm, Peptone 10 gm, Sodium Chloride 5 gm, Agar 20 gm, unfired water 1000ml) was used as culture media. The ingredients were dissolve in water, and adjust the PH to 7.2 to 7.4 by using dilute alkali/dilute acid and autoclave at 12°C for 20 min. 30-35 ml of nutrient agar was taken in Petri dish in the thickness of 5-6mm. 1000 µg/disc,

500µg/disc, 250 µg/dish concentration of the test compounds were prepared and Dimethylforamide (DMF) was used as vehicle. The plates were allowed to solidify and invert to prevent condensed falling on the agar surface. The standard inoculum was inoculated in the plates prepared earlier aseptically by dipping a sterile swab in the inoculums. The sterilized discs for the test drugs were placed in the Petri- dishes aseptically. Incubate the Petri dish at 37ºC ± 0.2ºC for about 18-24 hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion. The average zone diameter of the plates was measured and recorded (table 2).

Table-1:Anti-inflammatory activity of synthesized compounds (C_{-1} to C_{-7})

Compound No.	Inhibition of paw oedema after 3 hrs (%)[1]	Inhibition of paw oedema after 6 hrs (%)[2]
C_{-1}	14.2±0.01	10.3±0.01
C_{-2}	46.4±0.02	33.3±0.01
C_{-3}	22.3±0.01	16.6±0.02
C_{-4}	38.4±0.02	31.3±0.01
C_{-5}	60.6±0.02	47.9±0.02
C_{-6}	41.8±0.01	34.4±0.02
C_{-7}	0.00	-4.2±0.01
Control	-	-
Indomethacie	76.6±0.01	57.3±0.02

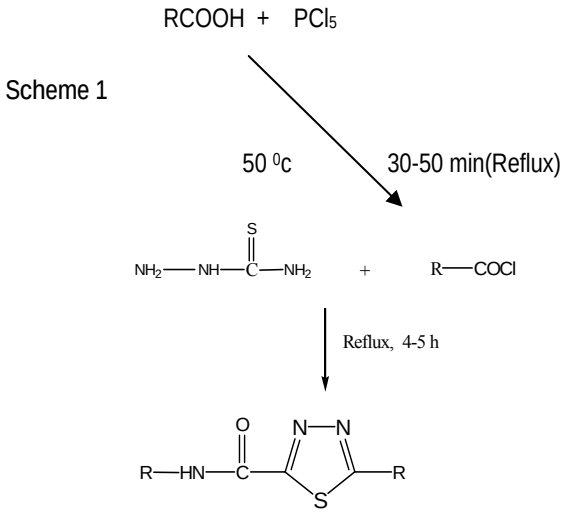
1. Dose for 1-7: 56mg/kg b.wt
2. Dose for indomethacine 10mg/kg b.wt mean ± SEM, n+6

Table 2: Antimicrobial activities of the compounds

Compounds	*S. aureus* (MIC µg/ml)	*E. coli* (MIC µg/ml)	*A. niger* (MIC µg/ml)
C_{-1}	50	50	50
C_{-2}	100	50	100
C_{-3}	100	50	25
C_{-4}	50	100	25
C_{-5}	100	100	50
C_{-6}	50	50	50
C_{-7}	50	50	50
Ofloxacin	10	12.4	--

RESULT

After the experiment it have concluded that the compounds which synthesized in the project having good yield value. The synthesize Thiadiazole compounds identified and characterize by IR, ^{1}H NMR and MASS spectra. After it the Pharmacological activity was done. The entire compound gives good response for Anti-



(C_{-1} to C_{-7})

R=C_6H_5, 2-C_6H_4Cl, 3- C_6H_4Cl, 4-C_6H_4Cl,2-

$C_6H_4NO_2$,3-$C_6H_4NO_2$,4- $C_6H_4NO_2$

inflammatory activity [2-Nitro-N-[5-(2-Nitro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide (C_{-5}), 3-Nitro-N-[5-(3-Nitro-phenyl)-[1,3,4] Thiadiazole-2yl]benzamide (C_{-6}). The MIC of all the compounds were determined by observing the zone of inhibition formed around cup after 24th of incubation for antibacterial activity compounds were found to have moderate antimicrobial activity.

DISCUSSION

All the synthetic compounds were obtained and further purified than characterization were carried out of all the compounds (C_{-1} to C_{-7}) using IR, H^1NMR, and Mass spectra. Antiinflammatory activity was carried out by using carrageenean induced paw oedema method. Coumpound-2 and compound-5 have shown the anti-inflammatory activity against Indomethacine as standard drug. Antimicrobial activity was carried out using bacterial strain *Staphylococcus aureus* (gram +ve), *E. coli* (gram -ve) and *A. niger*. All compounds have shown moderate antimicrobial activity against all the organism.

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