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SYNTHESIS OF PHARMACEUTICALLY IMPORTANT 1, 3, 4-THIADIAZOLE DERIVATIVES AS ANALGESIC AND ANTIPYRETIC AGENTS

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ABSTRACT

Objective: Thiadiazole derivatives were reported to have wide range of biological activities. Hence present work was planned to synthesize thiadiazole derivatives and screen for their analgesic, antipyretic and anti-inflammatory. **Methods:** Thiosemicarbazide was made to react with aryl carboxylic acid in presence of concentrated sulphuric acid to form 5-(substituted phenyl)-2-amino -1, 3, 4-thiadiazole. To aromatic amines, chloroacetyl chloride was added drop wise in presence of glacial acetic acid and saturated solution of sodium acetate to form 2-chloro-*N*-substituted-phenyl-acetamide. This acetamide compound was reacted with 5-(substituted phenyl)-2-amino -1, 3, 4-thiadiazole in 1, 4-dioxane and triethylamine (TEA), refluxed for 3 hrs to form *N*-(substuted-phenyl)-2-[5-(3-substituted-phenyl)-1, 3, 4-thiadiazol-2-yl amino]-acetamide. Characterization of all the compounds was performed by IR, ^{1}HNMR, Mass spectroscopic and elemental analysis. **Results:** The compounds **IIIA10**, **VA17, 2b** and **3b** have showed significant analgesic, antipyretic and anti-inflammatory activity. **Conclusion:** The compounds bearing *p*-chlorophenyl and 2-nitrophenyl group at C5 position of the thiadiazole moiety have shown profound activity when compared to compounds which were lacking of these groups.

KEYWORDS: Synthesis; characterization; 1, 3, 4-Thiadiazole; Analgesic activity; Antipyretic activity.

INTRODUCTION

Five membered heterocyclic compounds show various types of biological activities, among them 2,5-disubstituted 1, 3, 4-thiadiazoles have been studies extensively because of its ready accessibility, diverse chemical reactivity and associated with potential chemotherapeutic as well as pharmacotherapeutic activities, probably by the virtue of -N=C-S- grouping.[1] The scientific literature also states that antiviral and antibacterial of the thiourea derivatives are due to the presence of - NH - C(S) - NH function in the molecule and the change in the activity depends on the nature of the substituent. Thiadiazole moiety acts as "hydrogen binding domain" and "two-electron donor system". It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole, etc. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. 1, 3, 4 Thiadiazole and its derivatives represents one of the most biological active classes of compound possessing a wide spectrum of activities. Literature survey shows that the Schiff bases of 1, 3, 4- thiadiazole derivatives were versatile moiety have been reported to exhibita wide variety of biological activities like antibacterial[2-4] antifungal[5] anticonvulsant[6] antioxidant[7] anticancer[8-14] antidepressant,[15,16] radio protective[17] and anti-leishmanial[18] activities when properly substituted in the 2-and 5-positions and also the strong aromaticity of this ring system, which leads to great in vivo stability and generally, a lack of toxicity for higher vertebrates, including humans. These findings prompted us to develop a hybrid molecules with expect biological activity.

MATERIALS AND METHODS

Synthesis

Melting points of all synthesized compounds were determined by open capillary tube method expressed in ^{0}C and were uncorrected. Purity of all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel GF plates) and iodine was used as visualizing agent. IR spectra were recorded on THERMO NICOLET iS10 FT-IR spectrometer using KBr disc method. Elemental analysis was performed using a Euro EA Elemental Analyser. Spectral and Elemental analysis was carried out at Central Analytical Instrument Facility (CAIF), spectra were recorded on 400-MHz BRUKER spectrometer in dimethylsulfoxide-d_6 as solvent and tetramethylsilane (TMS) as internal standard and chemical shift was expressed in δ or ppm. The synthesis of compounds were carried according to **Scheme-1.**

General Procedure

Synthesis of 5-(2-hydroxyphenyl)-2-amino - [1, 3, 4]-thiadiazole 1(a, b, c)[19, 20]

A mixture of thiosemicarbazide (0.1mole), aryl carboxylic acid (0.1mole) and conc. Sulphuric acid (5ml) in 50 ml of ethanol was refluxed for 2-3 h. Reaction was monitored by TLC using mobile phase Chloroform: methanol (4:1).After completion of the reaction the reaction mixture was poured on to crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give colourless crystals.

Molecular Formula: $C_8H_7N_3OS$; % yield:90% ; Melting point: 183-186 ^{0}C ; IR (KBr) *v*/(cm^{-1}): 3514.22 (O-H, st.), 663.92, 688.65 (C-S-C, st.), 3428.26 (NH_2, N-H,st.), 1425.76 (Aryl C=C, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.89-7.20 (m, 4H, ArH), 10.32 (s, 1H, OH), 2.60-2.65 (bs, 2H, NH_2); Mass (m/z): 193 (M^+).

5-(4-chlorophenyl) -2-amino - [1, 3, 4]-thiadiazole (1b)

Molecular Formula: $C_8H_6ClN_3S$; % yield: 79% ; Melting point: 165-167 ^{0}C ; IR (KBr) *v*/(cm^{-1}): 762.35 (C-Cl, st.), 3447.21 (NH_2, N-H, st.), 1647.11 (C=N, st.), 1425.79 (Aryl C=C, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.95-7.35 (m, 4H, ArH), 2.48 (bs, 2H, NH_2); Mass (m/z) : 211(M^+).

5-(2-nitrophenyl) -2-amino - [1, 3, 4]-thiadiazole (1c)

Molecular Formula: $C_8H_6N_4O_2S$; % yield: 88%; Melting point: 225-227 ^{0}C ; IR (KBr) *v*/(cm^{-1}): 1375.53 , 1545.11, (NO_2, st.), 687.73 (C-S-C, st.), 34.50.78 (NH_2, N-H,st.), 1649.95 (C=N, st.), 1416.45 (Aryl C=C, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.30-7.73(m, 4H, ArH), 2.59 (bs, 2H, NH_2); Mass (m/z): 222 (M^+).

Synthesis of *N*-(substituted-phenyl)-2-[5-(3-substituted-phenyl)-1, 3, 4-thiadiazol-2-yl amino]-acetamide (III A1-A12)

5-(2-hydroxyphenyl) -2-amino-[1, 3, 4]-thiadiazole **1**(**a, b, c**) (0.05mole) and 2-chloro-*N*-substituted-phenyl-acetamide **(II)** (0.05mole) were mixed in 15 ml of 1, 4-dioxane. To this (0.005 ml) of triethylamine (TEA) solution was added and the reaction mixture was refluxed for 3h. It was then cooled and poured into crushed ice. The solid separate out and filtered it. The filtered was washed with 10% K_2CO_3 and water.

2-{[5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]amino}-N-phenylacetamide (IIIA1)

Molecular Formula: $C_{16}H_{14}N_4O_2S$; Mol.Wt. 326.37 ; % yield: 57%; Melting point: 141-143^{0}C; Rf : 0.72 (Benzene : acetone) (9:1); Composition : C (58.88%), H (4.32%), N (17.17%), Found: C (59.08%), H (4.42%), N (17.25%); IR (KBr) *v*/(cm^{-1}): 3517.52 (O-H, st.), 659.11, 697.43 (C-S-C, st.), 3439.40 (N-H, st.), 1612.58 (C=N, st.), 1654.84 (C=O, st.), 2859.23 (CH_2, C-H, st.), 3237.97 (CON-H, st.), 1442.99 (Aryl C=C, st.), 3020.93 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.88-6.94 (m, 4H, ArH), 7.57-7.89 (m, 5H, ArH), 10.13 (s, 1H, OH), 4.19 (d, 2H, CH_2), 4.69 (t, 1H, aro. C-NH), 8.95 (s, 1H, CONH); Mass (*m/z*): 326(M^+)

2-{[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl]amino}-N'-phenylacetohydrazide (IIIA2)

Molecular Formula: $C_{16}H_{15}N_5O_2S$; Mol. Wt. 341.38 ; % yield: 83%; Melting point: 154-156^{0}C; Rf : 0.79 (Benzene : acetone) (9:1); Composition : C(56.29%), H(4.43%), N(20.51%), Found: C(56.35%), H(4.40%), N(20.65%); IR (KBr) *v*/(cm^{-1}): 3517.87 (O-H, st.), 659.14, 698.01 (C-S-C, st.), 3449.27 (N-H, st.), 1641.12 (C=N, st.), 1484.69 (C-N, st.), 1669.79 (C=O, st.), 3125.50 (CH_2, C-H, st.), 3234.81 (CON-H, st.), 1443.38 (Aryl C=C, st.), 2973.65, 3010.85 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.89-7.52 (m, 4H, ArH), 7.76-7.79 (m, 5H, ArH), 10.42 (s, 1H, OH), 3.23 (d, 2H, CH_2), 4.29 (d, 1H, aro. C-NH), 8.58 (d, 1H, CONH), 3.83 (t, 1H, aro. C-NH); Mass (m/z): 341(M^+)

2-{[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl] amino}-N, N-diphenylacetamide (IIIA3)

Molecular Formula: $C_{22}H_{18}N_4O_2S$; Mol. Wt. 402.46; % yield: 65%; Melting point: 187-189^{0}C; Rf :0.81 (Benzene : acetone) (9:1); Composition : C(65.65%), H(4.51%), N(13.92%), Found: C(65.05%), H(4.59%), N(12.92%); IR (KBr) *v*/(cm^{-1}): 3525.40 (O-H, st.), 689.76 ,700 (C-S-C, st.), 3489.40 (N-H, st.), 1595.68 (C=N, st.), 1494.27 (C-N, st.), 1670.80 (C=O, st.), 2540.58 (CH_2, C-H, st.), 1457.87 (Aryl C=C, st.), 3040.77 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.76-6.82 (m, 5H, ArH), 7.05-7.26 (m, 10H, ArH), 9.98 (s, 1H, OH), 3.57 (d, 2H, CH_2), 4.50 (t, 1H, aro. C-NH); Mass (m/z): 402(M^+).

N'-(2, 4-dinitrophenyl) -2-{[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl]-amino} acetohydrazide (IIIA4)

Molecular Formula: $C_{16}H_{13}N_7O_6S$; Mol. Wt. 431.38; % yield: 91%; Melting point: 180-182^{0}C; Rf : 0.81 (Benzene : acetone) (9:1) ; Composition : C (44.55%), H (3.04%), N (22.73%), Found: C (43.95%), H (3.12%), N (22.88%); IR (KBr) *v*/(cm^{-1}): 3515.27 (O-H,st.), 636.99 (C-S-C, st.), 3311.91 (N-H, st.), 1589.16 (C=N, st.), 1708.82 (C=O, st.), 3110.01

(CH_2, C-H, st.), 3230.73 (CON-H, st.), 1423.31 (Aryl C=C, st.), 3004.61 (Aryl C-H, st.), 1307.61, 1507.92 (NO_2); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.05-7.58 (m, 4H, ArH), 7.91-8.31 (m, 3H, ArH), 10.26 (s, 1H, OH), 3.55 (d, 2H, CH_2), 4.20 (d, 1H, aro. C-NH), 9.04 (d, 1H, CONH), 4.23 (t, 1H, aro. C-NH); Mass (m/z): 431(M^+).

2-{[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-amino}-N-phenylacetamide (IIIA5)

Molecular Formula: $C_{16}H_{13}ClN_4OS$; Mol. Wt. 344.81; % yield: 54%; Melting point: 188-190^{0}C; Rf: 0.86 (Benzene : acetone) (9:1) ; Composition : C (55.73%), H (3.80%), N (16.25%), Found: C (55.87%), H (3.87%), N (16.23%); IR (KBr) *v*/(cm^{-1}): 758.11 (C-Cl, st.), 650.18, 682.98 (C-S-C, st.), 3426.28 (N-H, st.), 491.93 (C=N, st.), 1700.62 (C=O, st.), 2868.17 (CH_2, C-H, st.), 3162.19 (CON-H, st.), 1418.12 (Aryl C=C, st.), 3094.00 (Aryl C-H, st.) ; ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.90-7.48 (m, 4H, ArH), 7.49-7.52 (m, 5H, ArH), 4.23 (d, 2H, CH_2), 5.99 (t, 1H, aro. C-NH), 8.02 (s, 1H, aro. C-NH); Mass (m/z): 344(M^+).

2-{[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-amino}-N'-phenylacetohydrazide (IIIA6)

Molecular Formula: $C_{16}H_{14}ClN_5OS$; Mol. Wt. 359.83; % yield: 84%; Melting point: 197-199^{0}C; Rf = 0.76 (Benzene : acetone) (9:1) ; Composition : C (53.41%), H (3.92%), N (19.46%), Found: C (53.41%), H (3.92%), N (19.46%); IR (KBr) *v*/(cm^{-1}): 762.11 (C-Cl, st.), 681.95 (C-S-C, st.), 3455.46 (N-H, st.), 1685.89 (C=O, st.), 2918.15 (CH_2, C-H, st.), 3128.79 (CON-H, st.), 1424.75, 1491.75 (Aryl C=C, st.), 3082.11 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.53-7.56 (m, 4H, ArH), 7.91-8.16 (m, 5H, ArH), 3.64 (d, 2H, CH_2), 4. 30 (t, 1H, aro. C-NH), 9.18 (d, 1H, CONH), 4.29 (d, 1H, aro. C-NH); Mass (m/z): 359(M^+).

2-{[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-amino}-N, N-diphenylacetamide (IIIA7)

Molecular Formula: $C_{22}H_{17}ClN_4OS$; Mol. Wt. 420.91; % yield: 71%; Melting point: 207-209^{0}C; Rf: 0.79 (Benzene : acetone) (9:1) ; Composition : C (62.78%), H (4.07%), N (13.31%), Found: C (62.67%), H (4.17%), N (13.13%); IR (KBr) *v*/(cm^{-1}): 761.98, 783.18 (C-Cl, st.), 682.69, 700.73 (C-S-C, st.), 3350.55 (N-H, st.), 1491.65(C-N, st.), 1681.86 (C=O, st.), 2945.49 (CH_2, C-H, st.), 1422.96 (Aryl C=C, st.) ; ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.05-7.34 (m, 4H, ArH), 7.36-7.95 (m, 10H, ArH), 4.18 (d, 2H, CH_2), 5.57 (t, 1H, aro.C-NH) ; Mass (m/z): 420(M^+).

2-{[5- (4-chlorophenyl) -1, 3, 4-thiadiazol-2-yl]-amino}-N'- (2, 4-dinitrophenyl) -acetohydrazide (IIIA8)

Molecular Formula: $C_{16}H_{12}ClN_7O_5S$; Mol. Wt. 449.82; % yield: 49%; Melting point: 221-224^{0}C; Rf: 0.78 (Benzene : acetone) (9:1) ; Composition : C (42.72%), H (2.69%), N (21.80%), Found: C (42.70%), H (2.81%), N (21.98%); IR (KBr) *v*/(cm^{-1}): 762.77 (C-Cl, st.), 628.90 , 682.20 (C-S-C, st.), 3321.12 (N-H, st.), 1492.15 (C-N, st.), 1696.96 (C=O, st.), 2995.25 (CH_2, C-H, st.), 1424.61 (Aryl C=C, st.), 3094.06 (Aryl C-H, st.), 1519.45(NO_2); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.53-7.93 (m, 4H, ArH), 8.30-8.33 (m, 3H, ArH), 3.39 (d, 2H, CH_2), 4.31 (d, 1H, aro.C-NH), 8.84 (d, 1H, CONH), 4.27(s, 1H, aro.C-NH) ; Mass (m/z): 449(M^+).

2-{[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]-amino}-N-phenylacetamide (IIIA9)

Molecular Formula: $C_{16}H_{13}N_5O_3S$; Mol. Wt. 355.37; % yield: 66%; Melting point: 201-203^{0}C; Rf : 0.82 (Benzene : acetone) (9:1) ; Composition : C (54.08%), H (3.69%), N (19.71%), Found: C (53.78%), H (3.79%), N (19.43%); IR (KBr) *v*/(cm^{-1}): 1392.76, 1527.53 (NO_2), 697.90 (C-S-C, st.), 3440.22 (N-H, st.), 1631.34 (C=N, st.), 1492.84 (C-N, st.), 1700.31 (C=O, st.), 3004.19 (CH_2, C-H, st.), 3200.12 (CO N-H), 1438.88 (Aryl C=C, st.), 2939.27 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.67-7.82 (m, 5H, ArH), 7.98-8.05 (m, 4H, ArH), 3.91 (d, 2H, CH_2), 4.81 (t, 1H, aro. C-NH), 9.13 (s, 1H, CONH); Mass (m/z): 354(M^+-1)$^+$.

2-{[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl] amino}-N'-phenylacetohydrazide (IIIA10)

Molecular Formula: $C_{16}H_{14}N_6O_3S$; Mol. Wt. 370.38; % yield: 70% ; Melting point: 210-212^{0}C; Rf : 0.87 (Benzene : acetone) (9:1) ; Composition : C (51.88%), H (3.81%), N (22.69%), Found: C (51.18%), H(3.76%), N(22.54%); IR (KBr) *v*/(cm^{-1}): 1367.23, 1516.18 (NO_2), 680.81 (C-S-C, st.), 3350.42(N-H,st.), 1603.09 (C=N, st.), 1649.03 (C=O, st.), 2925.46 (CH_2, C-H, st.), 3162.68 (CO N-H), 1403.38 (Aryl C=C, st.), 3022.23 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.90-7.43 (m, 5H, ArH) , 7.54-8.38 (m, 4H, ArH), 3.76 (d, 2H, CH_2), 4.93 (t, 1H, aro.C-NH), 8.44 (d, 1H, CONH), 4.95 (d, 1H, aro.C-NH); Mass (m/z): 370(M^+).

2-{[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl] amino}-N, N-diphenylacetamide (IIIA11)

Molecular Formula: $C_{22}H_{17}N_5O_3S$; Mol. Wt. 431.46; % yield: 70%; Melting point: 225-227^{0}C; Rf : 0.82 (Benzene : acetone) (9:1) ; Composition : C (51.88%), H (3.81%), N (22.69%), Found: C (51.88%), H (4.00%), N (22.57%); IR (KBr) *v*/(cm^{-1}): 1310.04, 1338.05,

1365.98 (NO_2), 689.93 (C-S-C, st.), 3383.38, 3423.43(N-H,st.), 1602.18 (C=N, st.),1472.61 (C-N, st.), 1633.25, 1731.60 (C=O, st.), 2975.03 (CH_2, C-H, st.), 3162.68 (CO N-H), 1438.84 (Aryl C=C, st.), 3026.22 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.81-7.74 (m, 10H, ArH), 7.98-8.64 (m, 4H, ArH), 3.94 (d, 2H, CH_2), 4.92 (t, 1H, aro.C-NH) ; Mass (m/z): 431(M^+)

N'-(2, 4-dinitrophenyl)-2-{[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]-amino}acetohydrazide (IIIA12)

Rf : 0.81 (Benzene : acetone) (9:1) ; Composition : C (41.74%), H (2.63%), N (24.34%), Found: C (41.65%), H (2.60%), N (24.24%); IR (KBr) *v*/(cm^{-1}): 1392.96,1523.65 (NO_2), 670.05,695.61 (C-S-C, st.), 3424.31(N-H, st.), 1624.38 (C=N, st.),1731.62 (C=O, st.), 2979.91 (CH_2, C-H, st.), 3286.61 (CO N-H), 1427.22 (Aryl C=C, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.59-8.09 (m, 5H, ArH), 8.17-9.06 (m, 4H, ArH), 3.55 (d, 2H, CH_2), 3.91 (s, 1H, aro.C-NH), 9.29 (d, 1H, CONH), 4.34 (d, 1H, aro.C-NH) ; Mass (m/z): 460(M^+)

Synthesis of 2-Chloro-N-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide IV (d, e, f) [21, 22]

To the mixture of appropriately substituted compound **I (a, b, c)** (10 mmole) in dry benzene (15ml) and 2 ml of dry pyridine, was cooled to 0-5^{0}C.Chloro-acetyl chloride (20 mmole) dissolved in dry benzene (10 ml) was added drop wise to the solution with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. Benzene was removed *in vacuo*. The residue was poured over crushed ice. The precipitate was filtered, washed with water. The crude product was dried and crystallized from 1, 4-dioxane to yield compound **IV (d, e and f)**; the purity of compounds was analyzed by TLC using benzene: acetone (9:1) as mobile phase. % yield: 64.4%, melting point: 210 – 212 ^{0}C.

Synthesis of 2-(Substituted-amino)-N-[5-(Substituted-phenyl)-1, 3, 4-thiadiazol-2-yl]-acetamide, V (A13-A21)

The compound **IV (d, e, f)** 2-Chloro-*N*-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide (0.01mole) was taken in about 25 ml of dry alcohol and 0.01 mole of thiourea /hydrazine hydrate / piperidine was added to it and the mixture was heated on water bath for 9 h. The content was cooled under tap water, filter, dried and recrystalized from alcohol. Purity of the compounds was analyzed by petroleum ether: acetone (9:1) as mobile phase. The structures of synthesized compounds under investigation were supported by the ^{1}H-NMR, FTIR and MASS spectral measurement.

2-(carbamothioylamino)-N-[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl]acetamide (VA13)

Molecular Formula: $C_{11}H_{11}N_5O_2S_2$; Mol. Wt. 309.36; % yield: 45%; Melting point: 169-172 ^{0}C; Rf : 0.76 (Petroleum ether : acetone) (9:1) ; Composition : C (42.71%), H (3.58%), N (22.64%), Found: C (42.45%), H (3.50%), N (22.81%); IR (KBr) *v*/(cm^{-1}): 3559.56 (O-H), 688.51 (C-S-C, st.), 3411.10 (N-H, st.), 1689.70 (C=O, st.), 3119.59 (CH_2, C-H, st.), 1122.86 (C=S), 1426.53 (Aryl C=C, st.), 3080.72 (Aryl C-H, st.); 3490.53 (NH_2, st.); ^{1}H NMR (400MHz, DMSO-*d6*) δ 6.90-7.52 (m, 4H, ArH), 10.12 (s, 1H, OH), 3.45 (d, 2H, CH_2), 6.16 (t, 1H, CH_2-NH), 9.22 (s, 1H, CONH), 2.87, 2.90 (d, 2H, NH_2); Mass (m/z): 309(M^+)

N-[5-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl]-2-(piperidin-1-yl)acetamide (VA14)

Molecular Formula: $C_{15}H_{18}N_4O_2S$; Mol. Wt. 318.39; % yield: 52%; Melting point: 173-175^{0}C; Rf : 0.75 (Petroleum ether : acetone) (9:1); Composition : C (56.58%), H (5.70%), N (17.60%), Found: C (57.08%), H (5.60%), N (17.76%); IR (KBr) *v*/(cm^{-1}): 3518.34 (O-H), 697.28, 659.15 (C-S-C, st.), 3237.91 (N-H, st.), 1655.19 (C=O, st.), 3040.95 (CH_2, C-H, st.), 1612.63 (C=N), 1324.90 (C-N, st. piperidine), 2860.33 (CH_2, C-H, st. piperidine), 1445.93 (Aryl C=C, st.); 2998.01 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d6*) δ6.88-7.51 (m, 4H, ArH), 10.15 (s, 1H, OH), 3.12 (s, 2H, CH_2), 8.69 (s, 1H, CO NH), 1.51-1.69 (m, 6H, piperidine), 2.60, 2.71 (t, 4H, piperidine); Mass (m/z): 318(M^+)

2-hydrazinyl-N-[5-(2-hydroxyphenyl)-1, 3,4-thiadiazol-2-yl]acetamide (VA15)

Molecular Formula: $C_{10}H_{11}N_5O_2S$; Mol. Wt. 265.29; % yield: 47%; Melting point: 145-147^{0}C; Rf : 0.79 (Petroleum ether : acetone) (9:1); Composition : C (45.27%), H (4.18%), N (26.40%), Found: C (45.20%), H (4.10%), N (25.89%); IR (KBr) *v*/(cm^{-1}): 3521.96 (O-H), 680.47 (C-S-C, st.), 3420.28 (N-H, st.), 1640.57 (C=O, st.), 3088.23 (CH_2, C-H, st.), 1621.31 (C=N), 1464.43 (C-N), 1449.63 (Aryl C=C, st.), 3014.04 (Aryl C-H, st.), 3453.36(NH_2, st.); ^{1}H NMR (400MHz, DMSO-*d6*) δ 6.28-7.94 (m, 4H, ArH), 9.64 (s, 1H, OH), 3.91 (d, 2H, CH_2), 2.54 (m, 1H, CH_2-NH), 8.85 (s, 1H, CONH), 2.45 (d, 2H, NH_2); Mass (m/z): 265(M^+)

2-(carbamothioylamino)-N-[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]acetamide (VA16)

Molecular Formula: $C_{11}H_{10}ClN_5OS_2$; Mol. Wt. 327.81; % yield: 40%; Melting point: 198-200^{0}C; Rf :0.78 (Petroleum ether : acetone) (9:1); Composition : C (40.30%), H (3.07%), N (21.36%), Found: C (40.30%), H (3.07%), N (21.36%); IR (KBr) *v*/(cm^{-1}): 762.38 (C-Cl), 682.01 (C-S-C, st.), 3410.65 (N-H, st.), 1681.41 (C=O, st.), 2991.53 (CH_2, C-H, st.) ,1591.77 (C=N), 1491.66 (C-N), 1128.66, 1175.69 (C=S), 1424.68 (Aryl C=C, st.), 2836. 65 (Aryl C-

H, st.), 3289.64 (NH_2, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.45-7.94(m, 4H, ArH), 3.55 (d, 2H, CH_2), 2.81 (m, 1H, CH_2-NH), 8.83 (s, 1H, CONH), 2. 60 (s, 2H, NH_2); Mass (m/z): 328 $(M^+ +1)^+$

N-[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-2-(piperidin-1-yl)acetamide (VA17)

Molecular Formula: $C_{15}H_{17}ClN_4OS$; Mol. Wt. 336.83; % yield: 65%; Melting point: 135-138^{0}C; Rf : 0.81 (Petroleum ether : acetone) (9:1); Composition : C (53.49%), H (5.09%), N (16.63%), Found: C (53.59%), H (5.20%), N (15.93%); IR (KBr) *v*/(cm^{-1}): 762.40 (C-Cl), 650.06, 681.85 (C-S-C, st.), 3410.63 (N-H, st.), 1655.19, 1700.08 (C=O, st.), 3083.19 (CH_2, C-H, st.), 1593.13 (C=N), 1491.75(C-N), 1423.33 (Aryl C=C, st.), 2991.53 (Aryl C-H, st.); ^{1}HNMR (400MHz, DMSO-*d*6) δ 7.33-7.43 (m, 4H, ArH), 3.72 (s, 2H, CH_2), 9.31 (s, 1H, CONH), 1.78-1.98 (m, 6H, piperidine), 2.57-2.69 (t, 4H, piperidine); Mass (m/z): 336(M^+)

N-[5-(4-chlorophenyl)-1, 3, 4-thiadiazol-2-yl]-2-hydrazinylacetamide (VA18)

Molecular Formula: $C_{10}H_{10}ClN_5OS$; Mol. Wt. 283.73; % yield: 79%; Melting point: 149-152^{0}C; Rf : 0.72 (Petroleum ether : acetone) (9:1); Composition : C (42.33%), H (3.78%), N (24.99%), Found: C (42.63%), H (5.20%), N (15.93%); IR (KBr) *v*/(cm^{-1}): 703.34 (C-Cl), 653.01 (C-S-C, st.) , 3310.44 (N-H, st.) , 1688.21 (C=O, st.), 3104.36 (CH_2, C-H, st.), 1624.25 (C=N), 1443.31 (Aryl C=C, st.), 3091.58 (Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.33-8.33(m, 4H, ArH), 4.27 (d, 2H, CH_2) 2.49 (m, 1H, CH_2-NH), 8.85 (s, 1H, CONH), 1.99 (d, 2H, NH_2); Mass (m/z): 282$(M^+ -1)^+$

2-(carbamothioylamino)-N-[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]acetamide (VA19)

Molecular Formula: $C_{11}H_{10}N_6O_3S_2$; Mol. Wt. 338.36; % yield: 48%; Melting point: 215-217^{0}C; Rf : 0.73 (Petroleum ether : acetone) (9:1); Composition : C (39.05%), H (2.98%), N (24.84%), Found: C (39.05%), H (2.98%), N (24.84%); IR (KBr) *v*/(cm^{-1}): 1345.95, 1392.86 (NO_2), 670.11 (C-S-C, st.), 3340.70 (N-H, st.), 1632.11, 1731.44 (C=O, st.), 2770.99 (CH_2, C-H, st.), 1650.21 (C=N), 1345.95, 1392.86 (C-N), 1202.56, 1077.95, 1054.39 (C=S), 1441.47 (Aryl C=C, st.), 3039.76 (Aryl C-H, st.), 3400.35 (NH_2, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.01-8.45 (m, 4H, ArH), 3.55 (d, 2H, CH_2), 2.49 (t, 1H, CH_2-NH), 8.81 (s, 1H, CONH), 1. 98 (d, 2H, NH_2); Mass (m/z): 338(M^+).

N-[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]-2-(piperidin-1-yl)acetamide (VA20)

Molecular Formula: $C_{15}H_{17}N_5O_3S$; Mol. Wt. 347.39; % yield: 57%; Melting point: 220-222^{0}C; Rf : 0.77 (Petroleum ether : acetone) (9:1); Composition : C (51.86%), H (4.93%), N

(20.16%), Found: C (51.96%), H (4.63%), N (20.06%); IR (KBr) *v*/(cm^{-1}): 1398.11, 1515.18 (NO_2), 681.85 (C-S-C, st.), 1725.98 (C=O, st.), 3116.52 (CH_2, C-H, st.), 1653.50 (C=N), 1490.83 (Aryl C=C, st.), 3055.76 (Aryl C-H, st.); ^{1}HNMR (400MHz, DMSO-*d*6) δ 7.76-8.15 (m, 4H, ArH); 3.31 (s, 2H, CH_2); 8.95 (s, 1H, CONH); 1.59-1.63 (m, 6H, piperidine), 2.51-2.62 (t, 4H, piperidine); Mass (m/z): 347(M^+).

2-hydrazinyl-N-[5-(2-nitrophenyl)-1, 3, 4-thiadiazol-2-yl]acetamide (VA21)

Mol. formula: $C_{10}H_{10}N_6O_3S$; Mol. Wt. 294.28; % yield: 43%; Melting point: 228-230; Rf = 0.72 (Petroleum ether : acetone) (9:1); Composition : C (40.81%), H (3.42%), N (28.56%), Found: C (40.81%), H (3.42%), N (28.56%); IR (KBr) *v*/(cm^{-1}): 1521.33 (NO_2), 650.50 (C-S-C, st.), 3450.05 (N-H, st.), 1626.76 (C=O, st.), 1570.14, 1606.78 (C=N), 1443.31 (Aryl C=C, st.), 3096.47 (Aryl C-H, st.), 3190.43 (CH_2 N-H), 3434.03 (NH_2, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.43-7.91 (m, 4H, ArH), 3.68 (d, 2H, CH_2), 2.90 (m, 1H, CH_2-N<u>H</u>), 9.10 (s, 1H, CON<u>H</u>), 2.89 (d, 2H, NH_2); Mass (m/z): 294(M^+)

Biological Evaluation

Experimental Animals

Adult Swiss albino mice (20–25 g) and albino rats weighing (150–200 g) of either sex were used as experimental animals. All the animals were housed in groups of 4–8 per cage at a temperature of 25 ±1^{0}C and a relative humidity of 45–55%. A 12 h dark and 12 h light cycle was followed during the experiments. Animals were allowed free access to food and water *ad libitum*. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals.

Acute toxicity studies

The acute toxicity studies were carried out in groups of six Swiss albino mice, weighing 20–25 g which was fasted overnight and treated intraperitonially with the test compounds. The dosage was 175-1750 mg/kg body weight intraperitonially. All the animal experiments were performed with the approval of Institutional Animal Ethics Committee.

Analgesic activity using acetic acid-induced writhing method[23-28]

Weigh and number the animals. Divide the animals into 23 groups, each containing of six animals of either sex (25-30 gm). Among these 23 groups one was kept as control, one as standard and rest 21 as test group for different synthesized compounds. Administer

intraperitonealy 1% v/v [100 mg/kg body weight] volume of acetic acid solution to the first group (which serve as control), place them individually under glass jar for observation. Note the onset on writhes. Record the number of abdominal contractions, trunk twist response and extension of hind limbs as well as the number of animals showing each response during a period of 20 min. The test solutions were administered intraperitonialy to test group animals (50-100 mg/kg body weight). The group 2 of animals received the standard drug, aspirin (100 mg/kg body weight) orally. Fifteen minutes later, administer acetic acid solution to these animals and observed by placing the animal in transparent glass jar for 20 min, note the onset and severity of writhing response as done above.

Calculate the mean writhing scores in control and aspirin treated groups. Record the inhibition of pain response by aspirin.

Analgesic activity of compounds was calculated using following formula,

% Analgesic activity = [1-At / Ac] x 100

Where, At = No. of writhings for control group

Ac =No. of writhings for test group

The results are shown in Table 1.

Antipyretic activity by Brewer's Yeast method[29, 30]

Inductions of Brewer's Yeast-Induced Pyrexia, the rats were divided into 23 groups of six each. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. The rats were trained to remain quiet in a restraint cage. A thermometer probe was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. Temperature was measured on a digital thermometer. After measuring the basal rectal temperature, the animals were injected subcutaneously with 10 ml/kg body weight of 15% w/v suspension of brewer's yeast, suspended in 0.9% w/v NaCl solution. The rats were then returned to their housing cages. Nineteen hours after the yeast injection, the animals were again restrained in individual cages for rectal temperature recording. Nineteen hours after yeast injection, the test solutions were administered i.p. to test group animals (50-100 mg/kg body weight). A similar volume (5 ml/kg body weight) of 0.9 % NaCl solutions was administered i.p. to the control group. The second group of animals received the standard drug, aspirin (100 mg/kg body weight) p.o. The rats were restrained for rectal temperature recording at the nineteenth hour, immediately before test compounds or 0.9 % NaCl solution or aspirin administration, and again at one hour intervals up to the five hours after yeast

injection [31]. Anti pyretic activity was evaluated by comparing initial rectal temperature (^{0}C) before yeast injection, with rectal temperature (^{0}C) after 18 h of yeast injection at different time intervals.[32-34]

The results are summarized in Table 2.

RESULTS AND DISCUSSION

Synthesis

Various substituted benzoic acids were initially treated with thiosemicarbazide in presence of cyclizing agent H_2SO_4 to give compounds **1(a-c)**. The formation of the intermediate was confirmed on the basis of their IR and 1H NMR data. Treatment of aryl carboxylic acid in absolute ethanol with thiosemicarbazide afforded the corresponding 2-amino-5 (substituted phenyl)-1, 3, 4-thiadiazole **I** (a, b and c). Molecular formula of the compounds derived from elemental analyses data are supported by their molecular weight also %yield, Melting point, Rf value and all the synthesized compounds were further confirmed by FTIR, ^{1}H NMR, Mass data. The IR spectrum of **1a** showed characteristic absorption bands at 3428 cm^{-1} characteristic due to NH_2 functions in addition to the -OH absorption band at 3514 cm^{-1}, C-S-C absorption band at 688 cm^{-1}. Its ^{1}H NMR spectrum revealed the characteristic signal at δ 10.32 assigned to OH protons, two characteristic signals at δ 2.60 and 2.65 assigned to NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. Also, its mass spectrum showed the molecular ion peak at *m/z* 193 [M$^+$] and the base peak at *m/z* 94.

The IR spectrum of **1b** showed characteristic absorption bands at 3447 cm^{-1} characteristic which is due to NH_2 functions in addition to the C-Cl absorption band at 762 cm^{-1}, C-S-C absorption band at 682 cm^{-1} and C=C (aromatic)absorption band at 1425 cm^{-1}. Its ^{1}H NMR spectrum revealed the characteristic signal at δ 2.48 assigned to NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. The mass spectrum showed the molecular ion peak at *m/z* 211 [M$^+$] and the base peak at *m/z* 42.

The IR spectrum of **1c** has exhibited characteristic absorption bands at 3450 , 682 and 1416 cm^{-1} due to NH_2 , C-S-C and C=C (aromatic) functions respectively .Two characteristic absorption band at 1375, 1545 cm^{-1} which are due to NO_2 function. It was also showed proton signals at: δ 2.59 (NH_2) and δ 7.30-7.73 (Ar-H), respectively. Mass spectrum **(1c)** of the compound exhibited its molecular ion (M+) at m/z 222 and the base peak at *m/z* 206.

For yielding the compound **II** (2-substituted-N-substituted-phenyl-acetamide) by stirring the aromatic amines with chloroacetyl chloride in the solution of glacial acetic acid and saturated solution of sodium acetate.

Compound **1 (a, b, c)** was refluxed for 3h with **II** in TEA and 1, 4-dioxan, yielding **III (A1-A12).** Structures of the synthesized compounds were identified by FTIR and ^{1}H NMR. The structures of the **IIIA1** was confirmed by the appearance of -OH, C=O, NH (aromatic), C-H (CH_2), C=C (aromatic) and C-S-C absorption bands at 3517, 1654, 3439, 2859, 1442 and 659 respectively. ^{1}HNMR spectrum of its showed proton signals at: δ 10.13 (OH), 4.69 (NH), 4.19 (CH_2), 6.88-7.89 (Ar-H), respectively. Mass spectrum of the compound exhibited its molecular ion (M+) at m/z 326 and the base peak at *m/z* 121.

The IR spectrum of **IIIA2** exhibited characteristic absorption bands at 3517 cm^{-1} which is due to OH functions in addition to the C=O absorption band at 1669 cm^{-1}and C-H (CH_2) absorption band at 3125 cm^{-1}.Its ^{1}H NMR spectrum revealed the characteristic signal at δ 10.42 assigned to OH protons, δ 3.23 for CH_2 protons and δ 6.89-7.79 assigned to aromatic protons respectively. The results of its mass spectrum showed the molecular ion peak at *m/z* 341 [M^{+}] and the base peak at *m/z* 142.

The structures of the products **IIIA3** and **III4** were confirmed by the appearance of -OH, C=O and C-H (CH_2) bands at 3525, 1670, 2540 cm^{-1} and 3515, 1708, 3110 cm^{-1} stretching vibrations, respectively. Compounds **IIIA5**, **IIIA6**, **IIIA7** and **IIIA8** were showed the characteristic absorption band at 758, 762, 761 and 762 cm^{-1} respectively for C-Cl function, 3426, 3455, 3350 and 3321 stretching vibrations, respectively for NH group and at 2868, 2978, 2945 and 2995 cm^{-1} due to C-H (CH_2) stretching vibrations, respectively. Also ^{1}HNMR spectrum exhibited signal at δ 5.99, 4.30, 5.57 and 4.31, respectively, for NH proton. The signals were appears at δ 4.23, 3.64, 4.18 and 3.39 assigned to CH_2 protons. While the mass spectrum were showed the molecular ion peak at *m/z* 344 [M^{+}], 431 [M^{+}], 359 [M^{+}] , 420 [M^{+}] and 449 [M^{+}] respectively and the base peak at *m/z* 134, 93, 197 and 255 respectively. The ^{1}H NMR spectrum of the compounds **IIIA9**, **IIIA10**, **IIIA11** and **IIIA12** revealed the characteristic signal at δ 4.81, 4.93, 4.92 and 4.34, respectively, for NH proton. Also the compounds were showed the presence of methylene (CH_2) group protons appeared at δ 3.91, 3.76, 3.94 and 3.55, respectively. The mass spectrum of all the above compounds exhibited the molecular ion peak at *m/z* 354[M -1] $^{+}$, 370[M^{+}], 431[M^{+}] and 460[M^{+}], respectively, and

the base peak at *m/z* 142, 136, 221 and 183, respectively, corresponding to the molecular formula $C_{16}H_{13}N_5O_3S$, $C_{16}H_{14}N_6O_3S$, $C_{22}H_{17}N_5O_3S$ and $C_{16}H_{12}N_8O_7S$.

To the mixture of compounds I**(a, b, c)**, added solution of chloro-acetyl chloride with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. The precipitate was filtered, washed with water to yield compound **(IV d, e, f)**; %yiels: 64.4%, Melting point: 210 – 212 ^{0}C.

Compound **IV (d, e, f)** was refluxed for 9h with thiourea /hydrazine hydrate / piperidine in alcohol, to yield **V (A13-A21).** The FTIR spectrum of compound **VA13, VA14** and **VA15** showed a medium intensity band at 1622, 1612 and 1624 cm^{-1} that could correspond with (C=N) stretching in the vicinity of 1, 3, 4-thiadiazole ring [35]. In this spectrum there are two other characteristic bands at 3559, 3518, 3521 and 1689, 1655, 1640 cm^{-1} due to (O-H) and (C=O) stretching vibrations, respectively. Whereas the compound **VA13**, showed two absorption band at 1122 and 3490 cm^{-1} for (C=S) and (NH_2) stretching vibrations, respectively and in the compound **VA14** two absorption band was appeared at 1324 and 2860 cm^{-1} for (C-N, st. piperidine) and (CH_2, st. piperidine) stretching vibrations, respectively. Two characteristic band was found to be at 3453 and 3088 cm^{-1} stretching vibrations, respectively, indicated the presence of (N-H, NH_2, st.) and (C-H, CH_2, st.) functions in compound **VA15.** The structures of compounds **VA16, VA17 and VA18** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures. IR spectra of compound **VA16, VA17** and **VA18** showed characteristic bands for NH, CH–aliphatic, C-Cl and C=O groups. Mass spectrum of compounds **VA16**, **VA17** and **VA18** were showed the molecular ion peak at *m/z*: 328 $[M+1]^+$, 336 and 282 $[M-1]^+$, with a base peak at *m/z*: 100, 126 and 157 respectively.

The structures of compounds **VA19**, **VA20** and **VA21** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures. The primary amino group in compounds **VA19** and **VA21** was depicted by the presence of NH asymmetric stretch at 3400 and 3434 cm^{-1}. The IR bending vibration corresponding to C=S of compound **VA19** appeared at 1202 cm^{-1}. The presence of heterocyclic pyrrolidine moiety in compound **VA20** was demonstrated by the presence of C-N at 1345 cm^{-1}. The appearance of C=O stretch in the range of 1626 -1725 cm^{-1} indicated the formation of secondary amides (**VA19 -VA21**) by the reaction of hydrazine/ thiourea/ piperidine with the 2-chloro-*N*-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl] acetamide. The appearance of singlet at δ 8.81, 8.95 and

9.10, respectively, corresponds to the proton of CONH in the NMR of all the compounds indicated the presence of secondary amide to the 2nd position of synthesized 1, 3, 4-thiadiazoles moiety (**VA19- VA21**).

Acute Toxicity Studies

Acute toxicity and gross behavior studies revealed that the tested compounds in the present investigation were found to be nontoxic up to 1750 mg/kg i. p.

Analgesic Activity

Table 1, revealed that almost all the compounds showed very potent analgesic activity when compared with standard aspirin. Among the all tested compounds IIIA6, IIIA10 , VA17 and VA20 at different doses (75mg/kg) showed profound analgesic activity($P<0.01$) by increasing the threshold potential of pain (increase reaction time) in comparison to normal control (saline) and aspirin. The dose at 75 mg/kg body weight was found more effective when compared with positive control (aspirin).

Antipyratic activity

The antipyretic activity of all tested compounds were performed using Brewer's yeast induced pyrexia rats and exhibited significant ($p<0.05$) results by decreasing in the rectal temperature at 5th h after tested compounds administration (Table **2**). The dose of 75 mg/kg, compounds showed remarkable antipyretic activity when compared with positive control (aspirin). It is a well known fact that hypothalamus gland is responsible for rising or decreasing the normal body temperature (37 ^{0}C) of an individual which ensures a balance between heat production and heat loss. The disturbance of hypothalamic thermostat leads to rising of body temperature which results in a complaint of fever. The IIIA6, IIIA10, VA17 and VA20 at 75 mg/kg body weight doses decreased the rectal temperature of Brewer's yeast induced pyrexia rats up to 1^{0}C, suggesting that the synthesized compounds can act as a potential antipyretic drug. Aspirin ensures a balance between heat production and heat loss of the body by acting on the hypothalamus gland and thus used as an effective antipyretic agent.

Table 1: Acetic acid (1% v/v) induced writhing response in mice

Group	Compd. No.	Body wt. (gm)	Dose (mg/kg)	No. of Writhings in 20 minute	Analgesic activity (%)
1	Acetic acid	25	100	53.4±1.150	0.00
2	Aspirin	25	100	8.2 ±0.812***	84.64
3	IIIA1	26	100	50.0±0.234	6.36

4	IIIA2	25	100	48.4±0.413	9.36
5	IIIA3	28	100	50.4±0.584	5.61
6	IIIA4	27	100	50.2±0.782	5.99
7	IIIA5	29	100	42.6±0.821*	20.22
8	IIIA6	27	85	17.8±0.033**	66.66
9	IIIA7	25	100	51.4±0.312	3.74
10	IIIA8	25	100	47.6±0.752	10.86
11	IIIA9	30	100	25.8± 0.902**	51.68
12	IIIA10	25	85	14.8±0.043***	72.28
13	IIIA11	30	100	30.4±0.495**	43.07
14	IIIA12	28	100	28.6 ±0.275**	46.44
15	VA13	25	100	51.0 ±0.842	4.49
16	VA14	27	100	49.8±0.231	6.74
17	VA15	29	100	52.8±0.345	1.12
18	VA16	25	100	47.8±0.652	10.48
19	VA17	26	75	16.4±0.042***	69.28
20	VA18	25	100	45.0±±0.671	15.73
21	VA19	29	125	44.2±0.425*	17.22
22	VA20	30	75	16.6 ±0.033**	68.91
23	VA21	25	125	46.8±0.422	12.35

*Mean ± S.E.M; n=6; Standard = Aspirin 100 mg/kg (body weight) p.o; Control-vehicle (Acetic acid 1% v/v); *P<0.05; **P<0.01; ***P<0.001 when compared with standard.*

Table II: Effect of test compounds on brewer's yeast-induced pyrexia rats

Group No.	Compd. No.	Dose mg/kg	Rectal Temperature in ^{0}C at Time (h)						
			-18[a]	0[b] hr	19hr	20hr	21hr	22hr	23hr
1	Control.	5ml	36.30±0.1451	37.26 ±0.102 (+0.96)[c]	39.80±0.098	39.60±0.094	39.30±0.0371	39.10±0.023	39.10±0.435
2	Std.	100	36.20±0.027	37.13±0.099 (+0.93)[c]	39.50±0.034	39.10±0.157	38.00±0.021	37.40±0.062*	37.60±0.016*
3	IIIA1	100	36.73±0.092	37.39±0.075 (+0.66)[c]	39.83±0.061	39.74±0.082	39.08±0.006	38.52±0.034	38.19±0.147
4	IIIA2	120	36.54±0.024	37.27±0.094 (+0.73)[c]	39.64±0.025	39.76±0.165	39.17±0.325	38.51±0.041	38.02±0.275
5	IIIA3	120	36.70±0.012	37.30±0.131 (+0.60)[c]	39.71±0.122	39.35±0.151	39.36±0.225	38.23±0.176	38.08±0.462
6	IIIA4	100	36.61±0.101	37.10±0.152 (+0.49)[c]	39.38±0.146	39.58±0.116	39.45±0.366	38.33±0.018	38.03±0.283
7	IIIA5	100	36.08±0.452	37.81±0.123 (+0.73)[c]	39.60±0.081	39.50±0.158	39.50±0.472	38.15±0.116	38.07±0.340
8	IIIA6	85	36.10±0.473	37.01±0.012 (+0.91)[c]	39.77±0.123	39.30±0.257	39.40±0.059	37.95±0.163*	37.91±0.224*
9	IIIA7	100	36.59±0.142	37.44±0.017 (+0.81)[c]	39.89±0.158	39.75±0.167	39.64±0.266	38.17±0.172	38.10±0.339
10	IIIA8	100	36.33±0.421	37.17±0.034 (+0.84)[c]	39.67±0.091	39.59±0.133	39.86±0.201	38.67±0.311	38.45±0.418
11	IIIA9	100	36.93±0.105	37.69±0.052 (+0.76)[c]	39.80±0.052	39.96±0.092	39.82±0.346	38.18±0.155	38.20±0.537
12	IIIA10	85	36.32±0.093	37.11±0.031 (+0.79)[c]	39.30±0.076	39.20±0.157	38.30±0.291	37.80±0.137*	37.79±0.024
13	IIIA11	100	36.35±0.211	37.06±0.037 (+0.71)[c]	39.20±0.034	39.49±0.093	39.61±0.424	38.28±0.184	38.03±0.455
14	IIIA12	100	36.87±0.166	37.03±0.074 (+0.16)[c]	39.79±0.158	39.70±0.101	39.63±0.132	38.45±0.312	38.30±0.321
15	VA13	100	36.30±0.365	37.29±0.043	39.40±0.102	39.61±0.084	39.80±0.271	38.11±0.271	38.05±0.334

				(+0.93)[c]					
16	VA14	100	36.10±0.307	37.09±0.012 (+0.99)[c]	39.68±0.164	39.82±0.141	39.43±0.172	38.19±0.022	38.06±0.236
17	VA15	100	36.15±0.211	37.13± 0.031 (+0.98)[c]	39.67±0.205	39.83±0.083	39.83±0.245	38.41±0.153	38.20±0.372
18	VA16	100	36.65±0.365	37.32±0.088 (+0.67)[c]	39.69±0.126	39.77±0.241	39.86±0.284	38.26±0.056	38.09±0.28
19	VA17	75	36.40±0.333	37.32±0.013 (+0.92)[c]	39.40±0.087	38.90±0.283	39.10±0.273	37.90±0.085[*]	37.71±0.035
20	VA18	100	36.25±0.223	37.07±0.015 (+0.80)[c]	39.83±0.158	39.78±0.211	39.85±0.451	38.17±0.312	38.07±0.155
21	VA19	120	36.84±0.258	37.53±0.052 (+0.69)[c]	39.68±0.165	39.51±0.267	39.79±0.154	38.21±0.357	38.10±0.512
22	VA20	75	36.50±0.183	37.05±0.022 (+0.55)[c]	39.88±0.177	39.20±0.136	38.59±0178	37.87±0.214[*]	37.83±0.013
23	VA21	120	36.26±0.211	37.16±0.026 (+0.90)[c]	39.82±0.174	39.86±0.182	38.85±0.071	38.10±0.212	38.06±0.082

The data were analyzed by one way ANOVA followed by Dunnett's t test using Graph Pad Instat software.

Mean ± SEM; n=6;

Standard (Std.): Aspirin 10 mg/kg (body weight) p. o;

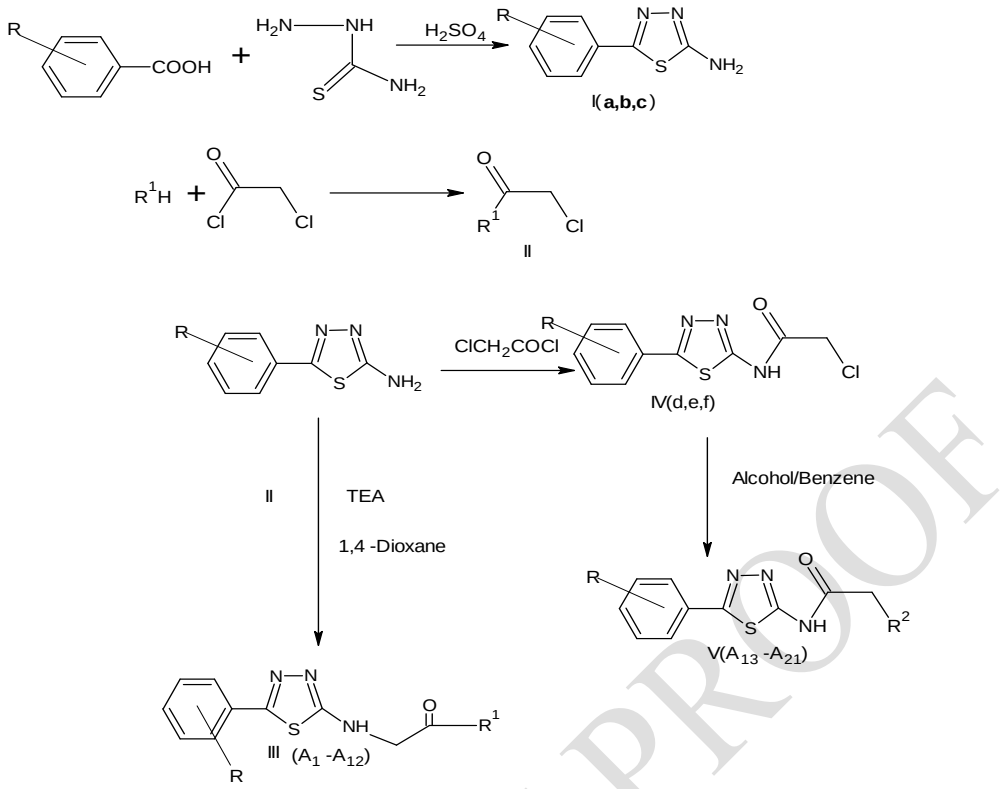
Control: normal saline (0.9%w/v NaCl);

a: temperature just before yeast injection;

b: temperature just before drug administration;

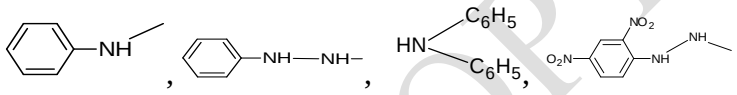
c: change in temperature following yeast injection;

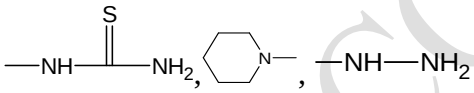
[*]*p <0.05 (p value as compared to control group).*



Scheme 1

R= 2OH, 4Cl, $2NO_2$

R^1 = 

R^2 = 

CONCLUSION

A series of novel 1, 3, 4-Thiadiazole derivatives were synthesized and the structure of the entire compounds were confirmed by recording by their ^{1}H NMR, and IR spectra. In conclusion, we feel that the preliminary *in vivo* activity results of this class of compounds may possess potential for design of future molecules with modifications on the aryl substituent's as well as NH_2 side chain. All the synthesized compounds showed significant activity.

The screening studies have demonstrated that the newly synthesized compounds exhibit promising antipyretic and anti-inflammatory properties. Therefore, it is concluded that there exists ample scope for further study in this class of compounds.

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