Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2013, 2 (5):1080-1101

(International Peer Reviewed Journal)

Condensed Bridge Head Nitrogen Heterocyclic Compounds: Facile Synthesis, Characterization and Bioactivity Studies of Some Substituted-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines

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Received on 25th July and finalized on 22nd August 2013.

ABSTRACT

*Two series of compounds namely, 3-(2-bromo-5-methoxyphenyl)-7-(substituted-benzylidene)-6-(substituted-phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**13a-h**) and 3-(2-bromo-5-methoxyphenyl)-6-(substituted-phenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (**14a-h/16**) were synthesized by cyclocondensation between 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3H-[1,2,4]triazole-3-thione (**4**) and 2-bromo-1,3-diaryl-prop-2-en-1-ones (**9a-h**) or 2-bromo-1-aryl-ethanones (**11a-h** and **15**) in ethanol with an aim to explore their antioxidant, analgesic, anti-inflammatory activity and effect on in vitro growth of micro-organism causing microbial infection. The antioxidant properties of synthesized compounds were evaluated by scavenging effect on DPPH radical method. In vitro antimicrobial activity was performed against four bacterial and four fungal strains. The analgesic and anti-inflammatory activities were evaluated by applying carrageenan-induced paw oedema bioassay and tail flick methods respectively. Some of the compounds were associated with moderate to good antioxidant, antimicrobial, analgesic and anti-inflammatory activity.*

Keywords: [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines, Antioxidant activity, Anti-microbial activity, Analgesic activity, Anti-inflammatory activity.

INTRODUCTION

As a result of the dramatic increase in infectious diseases and resistance of microorganisms towards currently exciting drugs, serious attention has been directed in recent years towards the discovery and development of new drugs. Fungal infections are often aggravated through the widespread use of broad-spectrum antibiotics, immunosuppressive agents, anticancer and anti-AIDS drugs [1, 2]. Despite advances in antimicrobial therapy, many problems remain to be solved for a new generation of antimicrobial agents. The main problem in the treatment of microbial infections is the increasing prevalence of drug resistance especially in patient's chronically subjected to antimycotic therapy such as persons infected with HIV [3]. Therefore, the development of new and efficacious antimicrobial drugs is a

very important goal and most of the research efforts in this field are directed towards the design of new agents. A review of the recent literature shows that many effective antimicrobial agents contain a heterocyclic moiety within their structure [4, 5] with special emphasis on those agents incorporating a triazole moiety [6-10]. In searching for new compounds with a promising antimicrobial profile and as part of our ongoing studies in the development of new chemotherapeutic agents, we embarked upon the synthesis of a series of novel condensed bicyclic derivatives in anticipation of the development of new broad spectrum antimicrobial agents which are devoid of the side effects associated with current therapeutic regimes.

The therapeutic effects of 1,2,4-triazole containing compounds have been well studied for a number of pathological conditions including inflammation, cancer, pain, tuberculosis or hypertension. Moreover, synthesis of triazoles fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as antimicrobial [11], molluscicidal agents [12], antidepressant [13], pesticides, herbicides [14], antiviral [15], antitumorial [16], analgesic [17], anti-inflammatory [18] agents, dyes, lubricant and analytical reagents. Among these, the commonly known systems are generally triazoles fused to thiadiazines, pyridines, pyridazines, pyrimidines, pyrazines and triazines. Although there are not many triazoles fused to thiadiazines, a number of them are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities such as anticancer [19], antibacterial [20], anti-fungal [21], anti-inflammatory [22], diuretic [23], analgesic [24], hypoglycaemic [25] agents.

In view of these marked activities of fused [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives [26] and continuation of our previously reported [27-30] studies on heterocyclic derivatives of methyl ester of 2-bromo-5-methoxybenzoic acid (**1**), it was contemplated to synthesize the title compounds and evaluate their biological potency.

MATERIALS AND METHODS

All solvents were of analytical grade and the reagents were used as purchased. DPPH was procured from Sigma-Aldrich. All melting points were determined by open capillary method and are uncorrected. IR spectra were obtained in KBr disc on a Shimadzu FT-IR 157 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded either on a Perkin-Elmer EM-390 or on a Bruker WH-200 (400 MHz or 300 MHz) in $CDCl_3$ or DMSO-d_6 as a solvent, using TMS as an internal standard and chemical shifts are expressed as ppm. Mass spectra were determined on a Jeol SX 102/Da-600 mass spectrometer/ Data System using Argon/Xenon (6kv, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The LCMS were recorded in MDSSCIEX, API 4000 spectrometer. The elemental analyses (CHN) were performed on CHNS-O-analyser Flash EA 1112 series. The progresses of the reactions were monitored by TLC on pre-coated silica gel G plates. All the spectral data's of newly synthesized compounds are consistent with proposed structure and microanalysis within ±0.3 of the calculated values.

Conventional synthesis of 2-bromo-5-methoxy-benzoic acid hydrazide (2): A mixture of 2-bromo-5-methoxy-benzoic acid methyl ester (**1**) (0.01 mol) in ethanol (10 mL) and hydrazine hydrate (0.01 mol) was refluxed for 4 h. The completion of reaction was monitored by TLC. After reaction completion the excess solvent was removed by distillation. The reaction mass was then cooled to 5 ^{0}C, the solid separated was filtered, dried and recrystallized from ethanol.

IR (KBr) γ/cm^{-1}: 3352.4 (-NH_2 stretch), 3230.3 (>NH stretch), 2845 (-OCH_3), 1640.65 (>C=O stretch), 764 (>C-Br); ^{1}H NMR: (400 MHz, DMSO-d_6): δ 3.77 (ss, 3H, -OCH_3), 4.45 (ss, 2H, -NH_2), 6.89-6.92 (m, 1H, ArH), 6.94-6.95 (d, 1H, J = 3.09 Hz, ArH), 7.49-7.52 (d, 1H, J = 8.67 Hz, ArH), 9.52 (ss, 1H, NH); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 55.04 (-OCH_3 carbon), 113.73, 119.42, 121.91, 129.13, 131.39, 159.45,

166.32; ^{13}C NMR-DEPT-135: (100 MHz, DMSO-d_6): δ 55.01(-OCH_3 carbon), 113.69, 119.39, 121.87; MS: *m/z* =246 (M^++1, 84 %); Anal. calcd. for $C_8H_9BrN_2O_2$: C, 39.28; H, 3.77; N, 11.48; Found: C, 39.20; H, 3.70; N, 11.43 %.

Conventional synthesis of 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-[1,2,4]triazole-3-thione (4): The acid hydrazide (**2**) (0.01 mol) was added to absolute alcohol (15 mL) containing KOH (1.6 g) at ambient temperature. Carbon disulphide (0.013 mol) was added and the mixture was stirred at ambient temperature for 10 h. The mixture was diluted with ether (10 mL) and stirred for further 1 h. The potassium salt (**3**) separated was filtered and washed with ether (5 mL). The potassium salt (**3**) was used for the next stage without further purification. Hydrazine hydrate (99 %) (0.02 mol) was gradually added to the above potassium salt (0.01 mol) dissolved in water (12 mL) with stirring and the mixture was refluxed gently for 3 h during which hydrogen sulphide evolved and the colour of the reaction mixture changed to dark green colour. It was then cooled to 5 ^{0}C and acidified with Conc. HCl to pH 1.00. A yellow solid separated out was filtered, washed with water and crystallized from ethanol to obtain pure triazole (**4**).

IR (KBr) γ/cm^{-1}: 3171.36-3300.57 (NH_2 stretch), 3071.36 (aromatic CH stretching), 2939.95 (methyl CH stretch), 1611.23 (C=N stretching), 1567.84, 1549.52 and 1428.03 (C=C ring stretching), 1472.38 (tautomeric C=S), 1337.39 (C-N stretching), 1223.61 (N-N=C), 722.21(>C-Br); ^{1}H NMR: (400 MHz, DMSO-d_6): δ 3.76 (ss, 3H, -OCH_3), 5.46 (ss, 2H, -NH_2), 7.0580-7.1788 (m, 1H, ArH), 7.34-7.35 (d, 1H, *J* = 2.96 Hz, ArH), 7.62-7.72 (dd, 1H, *J* = 8.88 and 8.84 Hz, ArH), 13.95 (ss, 1H, SH); ^{13}C NMR: (100 MHz, DMSO-d_6): δ 56.31 (-OCH_3 carbon), 116.79, 118.74, 120.20, 128.43, 133.98, 150.23, 159.03, 166.96; ^{13}C NMR-DEPT-135: (100 MHz, DMSO-d_6): δ 56.32 (-OCH_3 carbon), 116.81, 118.74, 120.20; MS: *m/z* = 303.1 (M^++2, 92 %); Anal. calcd. for $C_9H_9BrN_4OS$: C, 35.89; H, 3.01; N, 18.60; Found: C, 35.94; H, 3.06; N, 18.54 %.

General procedure for the conventional synthesis of 1,3-diaryl-prop-2-en-1-ones [7a-h]: To a mixture of substituted aromatic aldehydes (**5a-d**) (0.01 mol) and substituted acetophenones (**6a**, **b**) (0.01 mol) in ethanol, a solution of KOH (5 %, 10 mL) in water was added slowly with stirring at room temperature and it was continued for 18-24 h. The precipitated solid was filtered, washed with water, dried and recrystallized from ethanol. The products are confirmed by comparing their melting points with literature melting points. The yields varied in the range 89-94 %. The propenones prepared as per this procedure are;

a) 3-(4-Chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one (29a): yield 90 %, m.p. 130-132^{o}C (Lit[32]. 132-134 ^{o}C).
b) 3-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one (29b): yield 92%, m.p. 120-122^{o}C (Lit[33]).
c) 1-(4-Fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (29c): yield 91%, m.p. 98-100^{o}C (Lit[34]. 98 ^{o}C).
d) 1-(2,4-Dichlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (29d): yield 89%, m.p. 138-140^{o}C (Lit[35]).
e) 3-(3,4-Dimethoxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one (29e): yield 94%, m.p. 88-90^{o}C (Lit[36]. 90-92 ^{o}C).
f) 1-(2,4-Dichlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (29f): yield 90%, m.p. 132-134 ^{o}C (Lit[37]).
g) 1-(4-Fluorophenyl)-3-(2,4,5-trimethoxyphenyl)prop-2-en-1-one (29g): yield 92%, m.p. 88-90^{o}C (Lit[38]).
h) 1-(2,4-Dichlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (29h): yield 91%, m.p. 60-62 ^{o}C (Lit[39]. 62-64 ^{o}C).

General procedure for the conventional synthesis of 2,3-dibromo-1,3-diaryl-prop-2-en-1-ones [8a-h]: The compounds (**7a-h**) (0.01 mol) are dissolved in glacial acetic acid and cooled to 18-20 ^{o}C. Cautiously added a solution of liquid bromine (0.01 mol) in glacial acetic acid until the colour of bromine persists and allowed the mixture to stand for 3-4 h. The completion of reaction was monitored by TLC. After completion of reaction, the reaction mass was poured in to ice cold water, filtered off the dibromo compound, washed with little cold water. The compound was dried and recrystallized from dilute ethanol. The product obtained as such taken for dehydrobromination as discussed below.

General procedure for the conventional synthesis of 2-bromo-1,3-diaryl-prop-2-en-1-ones [9a-h]: A mixture of dibromopropanone (**8a-h**) (0.01 mol) and triethylamine (0.05 mol) in dry toluene was stirred for 24 h. The precipitated triethylammoniumhydrobromide was filtered. The filtrate was concentrated under reduced pressure. The precipitated solid was filtered, dried and recrystallized from chloroform.

2-bromo-3-(4-chlorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one [9a]: IR (KBr) γ/cm^{-1}: 3064.5 (Ar-CH-), 1650.00 (-C=O), 1594.60 (-CH=CH-), 812.50 (C-Br), 746.20 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14-7.18 (m, 2H, ArH), 7.39-7.41 (dd, 2H, ArH, J = 8.38, 1.6 Hz,), 7.58 (s, 1H, =CH), 7.77-7.86 (m, 4H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 116.00, 116.22, 122.63, 129.12, 131.72, 132.20, 132.57, 132.66, 132.75, 136.62, 140.67, 164.56, 167.10 (14C, Ar-C), 190.17 (C=O); ^{13}C NMR-DEPT-135 (100 MHz, DMSO-d_6): δ 115.73, 115.95, 128.85, 131.45, 132.40, 132.49, 140.41 (9C, $C_{nonquaternary}$); Anal. calcd. for $C_{15}H_9BrClFO$: C, 53.05; H, 2.67; Found: C, 53.06; H, 2.67 %.

2-bromo-3-(4-chlorophenyl)-1-(2,4-dichlorophenyl)prop-2-en-1-one [9b]: IR (KBr) γ/cm^{-1}: 3072.70, 3009.00 (Ar-CH-), 1663.50 (-C=O), 1585.60 (-CH=CH-), 819.70 (C-Br), 757.30 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.32-7.41 (4H, m, ArH), 7.48 (s, 1H, Ar-$H_{dichlorochloro\ phenyl}$), 7.62 (s, 1H, =CH), 7.79 (d, 2H, Ar-$H_{dichlorochloro\ phenyl}$, J = 8.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 124.19, 127.68, 129.19, 130.25, 130.43, 131.97, 132.22, 132.54, 136.11, 137.42, 144.30 (14C), 189.37 (C=O); ^{13}C NMR (100 MHz, DMSO-d_6): δ 127.41, 128.92, 129.98, 130.16, 131.95, 144.04 (8C, $C_{nonquaternary}$); Anal. calcd. for $C_{15}H_8BrCl_3O$: C, 46.14; H, 2.06; Found: C, 46.14; H, 2.07 %.

(2-bromo-1-(4-fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one [9c]: IR (KBr) γ/cm^{-1}: 2950.42, 2830.64 (Ar-CH-), 1664.18 (-C=O), 1596.92 (-CH=CH-), 1224.82, 1028.52 (CO-C), 834.09 (C-Br), 745.06 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.91 (s, 3H, OCH_3), 7.14-7.58 and 7.60- 8.00 (m, 8H, ArH), 7.59 (s, 1H, =CH); Anal. calcd. for $C_{16}H_{12}BrFO_2$: C, 57.34; H, 3.61; Found: C, 57.33; H, 3.60 %.

1-(2,4-dichlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one [9d]: IR (KBr) γ/cm^{-1}: 2952.49, 2834.70 (Ar-CH-), 1664.71 (-C=O), 1598.19 (-CH=CH-), 1226.68, 1031.48 (CO-C), 832.98 (C-Br), 745.07 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.91 (s, 3H, OCH_3), 7.11-7.59 and 7.61- 7.79 (m, 7H, ArH), 7.60 (s, 1H, =CH); Anal. calcd. for $C_{16}H_{11}BrCl_2O_2$: C, 49.78; H, 2.87; Found: C, 49.77; H, 2.86 %.

2-bromo-3-(3,4-dimethoxyphenyl)-1-(4-fluorophenyl)prop-2-en-1-one [9e]: IR (KBr) γ/cm^{-1}: 2950.92, 2833.94 (Ar-CH-), 1664.95 (-C=O), 1599.90 (-CH=CH-), 1222.67, 1032.89 (CO-C), 836.92 (C-Br), 745.08 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.91 (s, 6H, $2OCH_3$), 7.08-7.71 and 7.73- 8.03 (m, 7H, ArH), 7.72 (s, 1H, =CH); Anal. calcd. for $C_{17}H_{14}BrFO_3$: C, 55.91; H, 3.86; Found: C, 55.92; H, 3.82 %.

2-bromo-1-(2,4-dichlorophenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one [9f]: IR (KBr) γ/cm^{-1}: 2951.09, 2839.23 (Ar-CH-), 1664.44 (-C=O), 1600.95 (-CH=CH-), 1220.37, 1035.43 (CO-C), 842.02 (C-Br), 745.04 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.92 (s, 6H, $2OCH_3$), 7.08-7.60 and 7.62- 7.79 (m, 6H, ArH), 7.41 (d, 1H, =CH, J = 15.64 Hz), 7.61 (s, 1H, =CH); Anal. calcd. for $C_{17}H_{13}BrCl_2O_3$: C, 49.07; H, 3.15; Found: C, 49.10; H, 3.13 %.

2-bromo-1-(4-fluorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one [9g]: IR (KBr) γ/cm^{-1}: 2958.42, 2838.14 (Ar-CH-), 1664.94 (-C=O), 1598.38 (-CH=CH-), 1221.54, 1032.15 (CO-C), 836.62 (C-Br), 745.49 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.91 (s, 6H, 2OCH$_3$), 3.92 (s, 3H, OCH$_3$), 6.59-7.40 and 7.42- 7.80 (m, 6H, ArH), 7.41 (s, 1H, =CH); Anal. calcd. for $C_{18}H_{16}BrFO_4$: C, 54.70; H, 4.08; Found: C, 54.71; H, 4.10 %.

2-bromo-1-(2,4-dichlorophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one [9h]: IR (KBr) γ/cm^{-1}: 2965.91, 2841.44 (Ar-CH-), 1664.55 (-C=O), 1601.28 (-CH=CH-), 1223.72, 1031.05 (CO-C), 836.20 (C-Br), 745.87 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.91 (s, 6H, 2OCH$_3$), 3.92 (s, 3H, OCH$_3$), 6.89-7.53 and 7.55- 7.79 (m, 5H, ArH), 7.54 (s, 1H, =CH); Anal. calcd. for $C_{18}H_{15}BrCl_2O_4$: C, 48.46; H, 3.39; Found: C, 48.45; H, 3.40 %.

General procedure for the conventional synthesis of 2-bromo-1-(aryl) ethanone [11a-h]: To a cold solution of substituted acetophenone **10a-i** (0.01 mol) in chloroform (25 mL), bromine (0.012 mol) in chloroform (10 mL) was gradually added for about 30 minutes with continuous stirring and maintaining the temperature of the reaction mixture at 0-5 ^{o}C. After the addition was complete, the reaction mixture was slowly brought to the room temperature and stirring was continued for another 60 minutes until the evolution of hydrogen bromide gas ceased. The solvent was removed under reduced pressure and the residue obtained was recrystallized from ethanol to afford the pure phenacyl bromide. The products are confirmed by comparing their melting points with standard. The yields varied in the range 78-88 %. The phenacyl bromides prepared according to this procedure are:

a) 4-Nitro phenacyl bromide (CAS No. 99-81-0), mp 96 ^{o}C (Lit. 94-99^{o}C).
b) 4-Bromo phenacyl bromide, mp 110 ^{o}C (Lit [40]. 109-110^{o}C).
c) 4-Chloro phenacyl bromide, mp 96 ^{o}C (Lit [40]. 96-96.5^{o}C).
d) 2,4-dichloro phenacyl chloride (CAS No. 425-78-2), mp 54 ^{o}C (Lit. 50-57^{o}C).
e) Phenacyl bromide, mp 50 ^{o}C (Lit [40]. 50^{o}C).
f) 4-Methyl phenacyl bromide, mp 61 ^{o}C (Lit [41]. 61^{o}C).
g) 4-Methoxy phenacyl bromide, mp 75 ^{o}C (Lit [41]. 75-76^{o}C).
h) 5-(Bromoacetyl)-salicylamide, mp 210 ^{o}C (Lit [42]. 208-210^{o}C).

36) 3-(bromoacetyl)-5-chlorothiophene-2-sulfonamide, (CAS No. 160982-11-6), mp 144 ^{o}C (Lit. 142-144^{o}C).

General procedure for the conventional synthesis of 3-(2-bromo-5-methoxyphenyl)-7-(substituted benzylidene)-6-(substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines [13a-h]: An equimolar mixture of 4-amino-5-(2-bromo-5-methoxyphenyl)-4*H*-[1,2,4]triazole-3-thione (0.01 mol) (**4**) and 2-bromo-1,3-diaryl-prop-2-en-1-ones (0.01 mol) (**9a-h**) was taken in ethanol (20 mL). To this the solution of potassium hydroxide (10 %) in ethanol (5 mL) was added. The reaction mass was heated under reflux on a water bath for about 5 h. The completion of reaction was monitored by TLC. After reaction completion, the reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallized from ethanol or dioxane.

3-(2-bromo-5-methoxyphenyl)-7-(4-chlorobenzylidene)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine [13a]: IR (KBr) γ/cm^{-1}: 3051.8 (C–H), 2937.06, 2833.88 (methyl C-H str), 1592.91 (C=N), 1462.74 (N=C-S), 1085.73 (C–F), 840.81 (C–Cl), 759.81 (C-Br) and 684.60 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.74 (s, 3H, -OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 7.07-7.09 (d, 1H, 4-Cl-phenyl, J = 8.6 Hz), 7.20 (s, 1H, 2-Br-5-OCH$_3$ phenyl), 7.32 (m, 3H, ArH protons), 7.54 (s, 1H, =CH proton), 7.66-7.72 (m, 6H, ArH protons); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ 56.23 (-OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 113.73, 116.48, 116.70, 118.57, 119.16, 128.16, 129.11, 131.23, 132.16, 132.35, 132.44, 132.54, 134.22, 135.21, 139.54, 140.98, 151.91, 156.28, 158.87, 163.10 and 165.60 (23C); ^{13}C NMR-DEPT-135 (100 MHz, DMSO-d$_6$): δ 56.23 (-OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 116.48, 116.70, 118.56, 119.16, 129.11, 132.35, 132.45, 132.54, 134.22 and 140.99 (12C, C$_{nonquaternary}$); MS: *m/z* = 541.8 (M$^+$, 98%), 542.7 (M$^+$+1,

98%); Anal. calcd. for $C_{24}H_{15}BrClFN_4OS$: C, 53.20; H, 2.79; N, 10.34; Found: C, 53.20; H, 2.80; N, 10.35 %.

3-(2-bromo-5-methoxyphenyl)-7-(4-chlorobenzylidene)-6-(2,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines [13b]: IR (KBr) γ/cm^{-1}: 3094.2 (ArC–H), 2932.2, 2833.9 (methyl C-H), 1586.2 (C=N), 1478.2 (N=C-S), 858.2 (C–Cl), 757.9 (C-Br) and 670.1 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 3H, -OCH_3), 6.91-6.93 (dd, 1H, 2-Br-5-OCH_3-phenyl, J = 6.0 Hz and 2.8 Hz,), 7.13 (d, 1H, 2-Br-5-OCH_3 phenyl, J_{HH} = 2.8 Hz), 7.42 (s, 1H, =CH), 7.37-7.44(m, 3H, ArH), 7.48-7.57 (m, 4H, ArH); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.69 (-OCH_3 carbon), 114.17, 117.40, 117.65, 118.77, 127.71, 128.01, 129.15, 130.25, 130.86, 131.51, 132.57, 133.81, 134.12, 136.15, 136.36, 137.31, 138.61, 152.63, 153.45 and 158.77 (23C); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.68 (-OCH_3), 117.64, 118.78, 128.01, 129.15, 130.25, 130.87, 132.58, 133.82 and 136.37 (11C, $C_{nonquaternary}$); MS: *m/z* = 593.1 (M^++1, 74%), 594.9 (M^++2, 98%); Anal. calcd. for $C_{24}H_{14}BrCl_3N_4OS$: C, 48.63; H, 2.38; N, 9.45; Found: C, 48.64; H, 2.38; N, 9.43 %.

3-(2-bromo-5-methoxyphenyl)-7-(4-methoxybenzylidene)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines[13c]: IR (KBr) γ/cm^{-1}: 3060.78 (C–H), 1596.45 (C=N), 1462.74 (N=C-S), 1091.72 (C–F), 760.41 (C-Br) and 683.56 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.74 (s, 3H,-OCH_3 of 4-OCH_3 phenyl), 3.78 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 7.08-7.10 (dd, 1H, 2-Br-5-OCH_3-phenyl, J = 5.92 Hz and 2.97 Hz), 7.19 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.34 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 3.0 Hz,), 7.33-7.53 and 7.55-7.78 (m, 4H, p-OCH_3-phenyl protons), 7.54 (s, 1H, =CH proton); MS: *m/z* = 538.39 (M^++1, 98%); Anal. calcd. for $C_{25}H_{18}BrFN_4O_2S$: C, 55.87; H, 3.38; N, 10.43; Found: C, 55.87; H, 3.40; N, 10.45 %.

3-(2-bromo-5-methoxyphenyl)-7-(4-methoxybenzylidene)-6-(2,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [13d]: IR (KBr) γ/cm^{-1}: 3000.98 (ArC–H), 2967.21, 2883.02 (methyl C-H), 1582.87 (C=N), 1469.54 (N=C-S), 853.67 (C–Cl), 756.56 (C-Br) and 671.90 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (s, 6H, -OCH_3), 6.96-7.02 (dd, 1H, 2-Br-5-OCH_3-phenyl, J = 5.98 Hz and 2.9 Hz), 7.09 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 3.0 Hz), 7.35 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.8 Hz), 7.45 (s, 1H, =CH), 7.12-7.43 and 7.46-7.83 (m, 7H, ArH); MS: *m/z* = 588.29 (M^+, 96%), 589.3 (M^++1, 82%); Anal. calcd. for $C_{25}H_{17}BrCl_2N_4O_2S$: C, 51.04; H, 2.91; N, 9.52; Found: C, 51.06; H, 2.95; N, 9.55 %.

3-(2-bromo-5-methoxyphenyl)-7-(3,4-dimethoxybenzylidene)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [13e]: IR (KBr) γ/cm^{-1}: 2990.92, 2865.87 (Ar-CH-), 1596.76 (=CH-), 1221.32, 1031.75 (CO-C), 1582.21 (C=N), 1468.54 (N=C-S), 1082.99 (C–F), 832.87 (C-Br), 690.19 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 1H, OCH_3), 3.79 (s, 6H, 2-OCH_3), 7.08 (dd, 1H, 2-Br-5-OCH_3-phenyl, J = 5.92 Hz and 2.97 Hz), 7.17 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.32 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 2.92 Hz), 7.50 (s, 1H, =CH proton), 7.18-7.49 and 7.51- 8.04 (m, 7H, ArH); MS: *m/z* = 568.40 (M^++1, 100 %); Anal. calcd. for $_{26}H_{20}BrFN_4O_3S$: C, 55.03; H, 3.55; N, 9.87; Found: C, 55.04; H, 3.54; N, 9.90 %.

3-(2-bromo-5-methoxyphenyl)-7-(3,4-dimethoxybenzylidene)-6-(2,4-dichlorophenyl)-7*H*-1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [13f]: IR (KBr) γ/cm^{-1}: 3000.17, 2994.65, 2883.82 (Ar-CH-), 1600.01 (=CH-), 1228.91, 1045.54 (CO-C), 1589.78 (C=N), 1472.03 (N=C-S), 858.98 (C–Cl), 837.14 (C-Br), 691.23 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 1H, OCH_3), 3.79 (s, 6H, 2-OCH_3), 7.11 (d, 1H, 2-Br-5-OCH_3-phenyl, J = 5.92 Hz,), 7.25 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.32 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.47 (s, 1H, =CH proton), 7.12-7.24 and 7.48- 7.89 (m, 6H, ArH); MS: *m/z* = 619.4 (M^++1,

96%); Anal. calcd. for $C_{26}H_{19}BrCl_2N_4O_3S$: C, 50.50; H, 3.10; N, 9.06; Found: C, 50.52; H, 3.12; N, 9.06 %.

3-(2-bromo-5-methoxyphenyl)-7-(3,4,5-trimethoxybenzylidene)-6-(4-fluorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [13g]: IR (KBr) γ/cm^{-1}: 3002.92, 2998.02, 2889.78 (Ar-CH-), 1598.42 (=CH-), 1223.72, 1046.54 (CO-C), 1590.12 (C=N), 1474.42 (N=C-S), 1090.12 (C–F), 838.65 (C-Br), 688.54 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.78 (s, 6H, 2-OCH$_3$), 3.79 (s, 6H, 2-OCH$_3$), 7.52 (s, 1H, =CH proton), 6.08- 7.51 and 7.53-7.89 (m, 9H, ArH); MS: *m/z* = 597 (M$^+$, 94%); Anal. calcd. for $C_{27}H_{22}BrFN_4O_4S$: C, 54.28; H, 3.71; N, 9.38; Found: C, 54.30; H, 3.73; N, 9.39 %.

3-(2-bromo-5-methoxyphenyl)-7-(3,4,5-trimethoxybenzylidene)-6-(2,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [13h]: IR (KBr) γ/cm^{-1}: 3004.13, 2988.94, 2883.61 (Ar-CH-), 1592.54 (=CH-), 1221.41, 1039.78 (CO-C), 1591.45 (C=N), 1472.98 (N=C-S), 860.26 (C–Cl), 836.54 (C-Br), 684.98 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.79 (s, 12H, 4-OCH$_3$), 7.48 (s, 1H, =CH proton), 6.06- 7.47 and 7.49-7.82 (m, 8H, ArH); MS: *m/z* = 649 (M$^+$+1, 98%); Anal. calcd. for $C_{27}H_{21}BrCl_2N_4O_4S$: C, 50.02; H, 3.26; N, 8.64; Found: C, 50.05; H, 3.28; N, 8.64 %.

General procedure for the conventional synthesis of 3-(2-bromo-5-methoxyphenyl)-6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14a-h]: A mixture of 4-amino-5-(2-bromo-5-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (**4**) (0.01 mol), sodium acetate (2.5 mol) and 2-bromo-1-(aryl) ethanone (**11a-h**) (0.01 mol) in absolute ethanol (30 mL) was refluxed for 4 h. After cooling, the solvent was removed under vacuum and the precipitate formed was filtered, washed with water and the solid product obtained was recrystallized from ethanol.

3-(2-bromo-5-methoxyphenyl)-6-(4-nitrophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14a]: IR (KBr) γ/cm^{-1}: 3052.2 (C–H), 2965.2, 2907.5, 2839.1 (methyl C-H str), 1569.5 (C=N), 1444.2 (N=C-S), 753.0 (C-Br) and 678.0 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.78 (s, 3H, -OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 4.50 (s, 2H, -CH$_2$ proton of thiadazine ring), 7.11 (d, 1H, 2-Br-5-OCH$_3$ phenyl, *J* = 8.94 Hz), 7.22 (s, 1H, 2-Br-5-OCH$_3$ phenyl), 7.68 (d, 1H, 2-Br-5-OCH$_3$ phenyl, *J* = 8.94 Hz,), 8.07 (d, 2H, 4-nitro phenyl protons, *J* = 8.55 Hz), 8.3 (d, 2H, 4-nitro phenyl protons, *J* = 8.55 Hz); MS: *m/z* = 448.0 (M$^+$+2, 100%); Anal. calcd. for $C_{17}H_{12}BrN_5O_3S$: C, 45.75; H, 2.71; N, 15.69; Found: C, 45.75; H, 2.73; N, 15.71 %.

5-[3-(2-bromo-5-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl]-2-hydroxybenzamide [14b]: IR (KBr) γ/cm^{-1}: 3548.38-3213.79 (0H and NH$_2$), 2963.09 (C–H), 2945.73, 2924.52, 2845.45 (methyl C-H str), 1671.02 (C=O of CONH$_2$), 1592.91 (C=N), 1459.85 (N=C-S), 730.88 (C-Br) and 673.99 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.78 (s, 3H, -OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 5.09 (s, 2H, CH$_2$ 0f thiadiazine ring), 7.01-7.03 (d, 1H, 2-Br-5-OCH$_3$-phenyl, *J* = 8.7 Hz), 7.09-7.12 (dd, 1H, 2-Br-5-OCH$_3$-phenyl, *J* = 5.88 and 3.00 Hz), 7.36 (d, 1H, 2-Br-5-OCH$_3$-phenyl, *J* = 2.96 Hz), 7.71 (d, 1H, 2-hydroxy benzamide ring, *J* = 8.89 Hz), 8.06-8.08 (d, 1H, 2-hydroxy benzamide ring, J_{HH} = 8.70 Hz), 8.15 (s, 1H, 2-hydroxy benzamide ring), 8.64 (s, 1H, NH$_2$ of CONH$_2$), 13.94 (s, 1H, OH proton); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ 40.66 (CH$_2$ 0f thiadiazine ring), 56.26 (-OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 111.36, 114.56, 116.96, 118.59, 119.95, 125.37, 126.52, 130.61, 134.58, 135.66, 159.03, 164.07, 164.59, 166.30, 171.86 (15C) and 190.73 (C=O); ^{13}C NMR-DEPT-135 (100 MHz, DMSO-d$_6$): δ 40.66 (CH$_2$ of thiadiazine ring), 56.26 (-OCH$_3$ of 2-Br-5-OCH$_3$ phenyl), 116.96, 118.59, 119.96, 130.61, 134.58 and 135.66 (6C, $C_{nonquaternary}$); MS: *m/z* = 461.3 (M$^+$+1, 98%), 463.1 (M$^+$+3, 84%); Anal. calcd. for $C_{18}H_{14}BrN_5O_3S$: C, 46.97; H, 3.07; N, 15.21; Found: C, 46.98; H, 3.07; N, 15.20 %.

3-(2-bromo-5-methoxyphenyl)-6-(4-chlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14c]: IR (KBr) γ/cm^{-1}: 3049.87 (C–H), 2964.05, 2938.98, 2920.66 (methyl C-H str), 1574.59 (C=N), 1434.78

(N=C-S), 858.16 (C–Cl), 727.99 (C-Br) and 672.07 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 4.74 (s, 2H, -CH_2 proton of thiadazine ring), 7.21 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.94 Hz,), 7.23 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.54 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.94 Hz), 8.05 (d, 2H, 4-chloro-phenyl, J = 9.0 Hz), 8.23 (d, 2H, 4-chloro-phenyl J = 9.0 Hz); MS; *m/z* = 436.80 (M^++1, 96%); Anal. calcd. for $C_{17}H_{12}BrClN_4OS$: C, 46.86; H, 2.78; N, 12.86; Found: C, 46.85; H, 2.76; N, 12.83 %.

3-(2-bromo-5-methoxyphenyl)-6-(2,4-dichlorophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14d]: IR (KBr) γ/cm^{-1}: 3052.32 (C–H), 2960.55, 2940.04 (methyl C-H str), 1570.98 (C=N), 1442.58 (N=C-S), 860.34 (C–Cl), 731.56 (C-Br) and 668.97 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 4.92 (s, 2H, -CH_2 proton of thiadazine ring), 7.18 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.94 Hz), 7.20 (s, 1H, 2-Br-5-OCH_3 phenyl), 7.48 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.94 Hz,), 8.02 (d, 1H, , 2,4-dichloro-phenyl, J = 9.0 Hz), 8.18 (d, 1H, 2,4-dichloro-phenyl, J = 8.88 Hz,), 8.22 (d, 1H, 2,4-dichloro-phenyl, J = 8.85 Hz); MS: *m/z* = 471 (M^++1, 100%); Anal. calcd. for $C_{17}H_{11}BrCl_2N_4OS$: C, 43.43; H, 2.36; N, 11.92; Found: C, 43.41; H, 2.32; N, 11.95 %.

3-(2-bromo-5-methoxyphenyl)-6-(phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14e]: IR (KBr) γ/cm^{-1}: 3099.88, 2962.75, 2950.67 (C-H str), 1569.91 (C=N), 1441.98 (N=C-S), 744.79 (C-Br) and 672.43 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 5.00 (s, 2H, -CH_2 proton of thiadazine ring), 6.94 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 8.8 Hz and 2.9 Hz), 7.23-7.30 (m, 3H, phenyl ring), 7.37 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 3.0 Hz,), 7.70 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.88 Hz,), 7.74 (m, 2H, phenyl); MS: *m/z* = 402.3 (M^++1, 99%); Anal. calcd. for $C_{17}H_{13}BrN_4OS$: C, 50.88; H, 3.27; N, 13.96; Found: C, 50.89; H, 3.27; N, 13.97 %.

3-(2-bromo-5-methoxyphenyl)-6-(4-methylphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines [14f]: IR (KBr) γ/cm^{-1}: 3094.18 (C–H), 2958.28, 2946.42 (methyl C-H str), 1568.53 (C=N), 1440.14 (N=C-S), 740.42 (C-Br) and 670.44 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42 (s, 3H, -CH_3 of 4-CH_3 phenyl), 3.79 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 5.02 (s, 2H, -CH_2 proton of thiadazine ring), 6.96 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 8.8 Hz and 2.9 Hz), 7.23-7.30 (m, 3H, ArH), 7.58 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.88 Hz), 7.74 (d, 2H, 4-CH_3 phenyl, J_{HH} = 8.01 Hz); MS: *m/z* = 416.3 (M^++1, 98%); Anal. calcd. for $C_{18}H_{15}BrN_4OS$: C, 52.06; H, 3.64; N, 13.49; Found: C, 52.08; H, 3.67; N, 13.51 %.

3-(2-bromo-5-methoxyphenyl)-6-(4-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14g]: IR (KBr) γ/cm^{-1}: 3089.23 (C–H), 2956.81, 2945.12 (methyl C-H str), 1571.90 (C=N), 1441.16 (N=C-S), 741.92 (C-Br) and 675.97 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3 of 4-OCH_3 phenyl), 3.79 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 4.99 (s, 2H, -CH_2 proton of thiadazine ring), 6.98 (dd, 1H, 2-Br-5-OCH_3 phenyl, J = 8.8 Hz and 2.9 Hz), 7.21-7.32 (m, 2H, p-OCH_3 phenyl ring), 7.34 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 2.99 Hz), 7.69 (d, 1H, 2-Br-5-OCH_3 phenyl, J = 8.88 Hz), 7.72 (m, 2H, p-OCH_3 phenyl ring); MS: *m/z* = 431.29 (M^+, 100%); Anal. calcd. for $C_{18}H_{15}BrN_4O_2S$: C, 50.13; H, 3.51; N, 12.99; Found: C, 50.14; H, 3.52; N, 13.00 %.

3-(2-bromo-5-methoxyphenyl)-6-(4-bromophenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine [14h]: IR (KBr) γ/cm^{-1}: 3095.19 (C–H), 3002.62, 2965.02, 2920.66 (methyl C-H str), 1570.74 (C=N), 1434.78 (N=C-S), 728.96 (C-Br) and 673.99 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 5.1 (s, 2H, CH_2 of thiadiazine ring), 7.10-7.13 (dd, 1H, 2-Br-5-OCH_3-phenyl, J = 5.93 Hz and 2.96 Hz), 7.38 (d, 1H, 2-Br-5-OCH_3-phenyl, J = 2.96 Hz), 7.71 (d, 1H, 2-Br-5-OCH_3-phenyl, J = 8.88 Hz), 7.79 (d, 2H, 4-Br-phenyl, J = 8.34 Hz), 7.99 (d, 2H, 4-Br-phenyl, J = 8.39 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.83 (CH_2 of thiadiazine ring), 56.27 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 111.35, 116.98, 119.99, 125.35, 128.69, 130.92, 132.47, 134.52, 135.67, 159.04, 164.10, 164.42, 192.23 (15C); ^{13}C NMR-DEPT-135 (100 MHz, DMSO-d_6): δ 40.83 (CH_2 0f thiadiazine ring), 56.27 (-OCH_3 of 2-Br-5-OCH_3

phenyl), 116.98, 119.99, 130.92, 132.47, 135.67 ($7C_{nonquaternary}$); MS: *m/z* = 481 (M^++1, 96%); Anal. calcd. for $C_{17}H_{12}Br_2N_4OS$: C, 42.52; H, 2.52; N, 11.67; Found: C, 42.52; H, 2.53; N, 11.66 %.

3-[3-(2-bromo-5-methoxyphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-6-yl]-5-chlorothiophene-2-sulfonamide [16]: IR (KBr) γ/cm^{-1}: 3420.42 (NH_2 stretching of SO_2NH_2), 3092.19, 3002.62, 2965.02, 2920.66 (C-H str), 1644.12 (C=N), 1578.26 (C=C), 1434.78 (N=C-S), 748.09 (C-Cl), 726.94 (C-Br) and 671.89 (C-S-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, -OCH_3 of 2-Br-5-OCH_3 phenyl), 4.27 (s, 2H, CH_2 of thiadiazine ring), 7.07-7.09 (dd, 1H, 2-Br-5-OCH_3-phenyl, *J* = 6.80 Hz and 1.78 Hz), 7.19 (s, 1H, =CH of thienyl ring), 7.43 (s, 1H, 2-Br-5-OCH_3-phenyl), 7.65 (s, 3H, NH_2 and 1H of 2-Br-5-OCH_3-phenyl); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.42 (CH_2 of thiadiazine ring), 56.21 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 113.68, 118.46, 119.12, 128.28, 130.03, 133.05, 134.30, 135.16, 141.84, 143.74, 151.55, 151.98, 158.88 (13C); ^{13}C NMR-DEPT-135 (100 MHz, DMSO-d_6): δ 26.42 (CH_2 0f thiadiazine ring), 56.21 (-OCH_3 of 2-Br-5-OCH_3 phenyl), 118.46, 119.11, 130.03, 134.30 ($4C_{nonquaternary}$); MS: *m/z* = 520.80 (M^+, 96%), 521.81 (M^++1, 76%), 522.78 (M^++2, 84%); Anal. calcd. for $C_{15}H_{11}BrClN_5O_3S_3$: C, 34.59; H, 2.13; N, 13.45; Found: C, 34.60; H, 2.13; N, 13.45 %.

Anti-oxidant activity

DPPH radical scavenging activity: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which has maximum optical absorbance at 517 nm. The reaction of DPPH with free radical scavenger causes decline in the absorbance value at 517 nm [43]. The MDC solutions of newly synthesized compounds at 200, 400 and 800 ug/mL concentrations were prepared and 4 mL of 0.1 mM methanolic solution of DPPH was added. The test tubes were kept at an ambient temperature for 20 mins and the absorbances were measured at 517 nm against control. Ascorbic acid was used as a positive control. These measurements were run in triplicate. The percentage of scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [(A_{DPPH}-A_{TEST})/A_{DPPH}] \times 100$$

Where A_{DPPH} is the absorbance of DPPH without test sample (control) and A_{TEST} is the absorbance of DPPH in the presence of test sample.

Antibacterial activity: All the newly synthesized compounds were screened for their antibacterial activity against four bacterial strains, *viz*., *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* (recultured) by serial plate dilution method [44]. Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using a phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16-18 h at 37 ^oC. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no conspicuous growth.

A number of antibacterial discs were placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. The excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^oC for an h. Using a punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethyl sulfoxide (DMSO) was added into each labelled well. A control was also prepared for the plates in the same way using DMSO as a solvent. The Petri dishes were prepared in triplicate and maintained at 37 ^oC for 3-4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with taking ampicillin as standard.

Antifungal Activity: All those compounds screened for antibacterial activity were also tested for their antifungal activity against *Penicillium marneffei* (recultred*), Trichophyton mentagrophytes* (recultured), *Aspergillus flavus* (NICM No.524) and *Aspergillus fumigatus* (NCIM No.902) by serial plate dilution method [45]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of sore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to

get a suspension of the corresponding species. Twenty mL of agar media was poured in to each petri dish. The excess of suspension was decanted and the plates were dried by placing in an incubator at 37 ^{o}C for 1h. Using a punch, wells were made on these seeded agar plates. Minimum inhibitory concentrations of the test compounds in DMSO were added into each labelled well. A control was also prepared in triplicate and maintained at 37 ^{o}C for 3-4 days. Activity of each compound was compared with itraconazole as standard. Itraconazole has a MIC value of 0.03-16 µg/mL. The minimum inhibitory concentrations of each compound were determined.

Anti-inflammatory activity: The anti-inflammatory activity of newly synthesized 3-(2-bromo-5-methoxyphenyl)-7-(substituted benzylidene)-6-(substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**13a-h**) and 3-(2-bromo-5-methoxyphenyl)-6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**14a-h**) were evaluated by applying carrageenan-induced paw oedema bioassay in rats of either sex (100-140 g) by following the method of Winter *et al*. [46] using Diclofenac sodium as a reference standard. Rats were selected by random sampling technique. The test compounds were administrated at dose level of 100 mg/kg orally 30 min. prior to the administration of carrageenan in the right hind paw of the rats. The paw thickness was measured using vernier callipers at regular intervals of 60, 120 and 180 mins. after carrageenan administration.

Analgesic activity: The analgesic activity of the above mentioned derivatives were also evaluated by applying tail flick method [47] using pentazocin as a standard reference. Wistar albino mice of either sex (20-30 g) in the groups of six animals each one was selected by random sampling technique. The test compounds at dose level of 100 mg/kg were administered orally by intragastric tube. The animals were held in position by a suitable restrained the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 50- 55 ^{o}C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at regular intervals of 60, 80 and 120 mins. after administration of compounds. A cut off point of 10 sec. was observed to prevent the tail damage.

RESULTS AND DISCUSSION

Syntheses and Chemistry: The acid hydrazide (**2**) was prepared by the esterification of 2-bromo-5-methoxybenzoic acid (**1**) followed by treatment with hydrazine hydrate in absolute ethanol. The acid hydrazide (**2**) was allowed to react with carbon disulphide in the presence of potassium hydroxide in ethanol to afford the corresponding intermediate potassium dithiocarbazinate (**3**). This salt underwent ring closure with an excess of 99 % hydrazine hydrate to give 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-[1,2,4]triazole-3-thione (**4**). The reaction sequences employed for the synthesis of the compound (**4**) is shown in figure 1. Compound (**4**) can exist in two tautomeric forms [31], 4-amino-5-(2-bromo-5-methoxyphenyl)-4*H*-[1,2,4]triazole-3-thiol and 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-[1,2,4]triazole-3-thione. The spectral analysis showed that it exists in the latter form, *e.g.*, in the ^{1}H NMR spectrum, a broad singlet at δ 13.96 can be only attributed to NH-C=S rather than to S–H and in the IR spectrum, the S–H vibration band (2500 cm^{-1}) is absent.

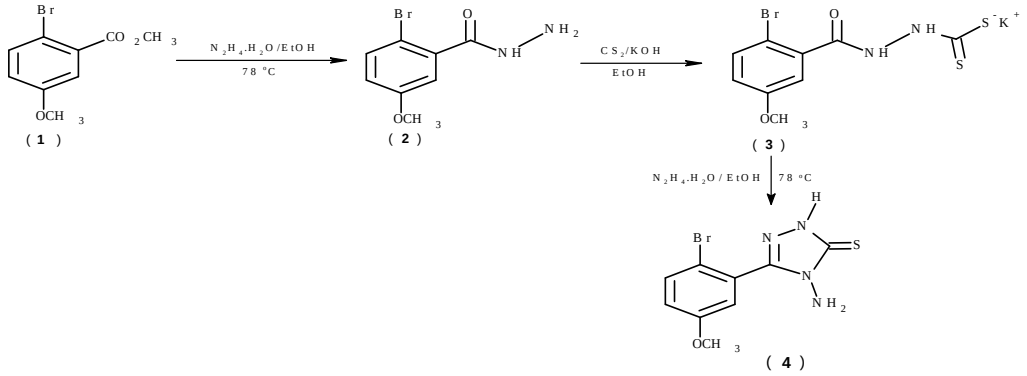
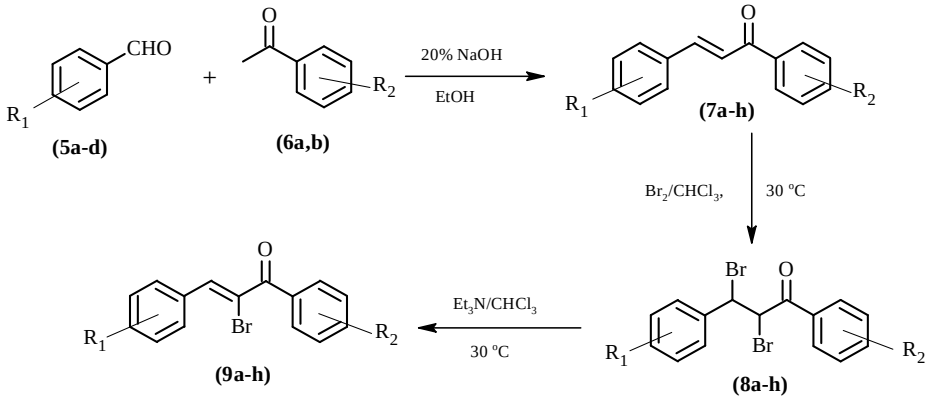


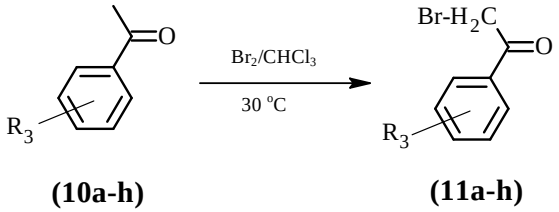
Figure 1: Synthetic route for the compound **4**.

2-Bromo-1,3-diaryl-prop-2-en-1-ones (**9a-h**) were synthesized by the Claisen-Schmidt condensation of substituted benzaldehydes with substituted acetophenones followed by bromination and dehydrobromination. The synthetic route is depicted in figure 2. The reaction of substituted acetophenones with bromine afforded required 2-bromo-1-aryl-ethanones (**11a-h**). The synthetic route is outlined in figure 3.



R_1 = 4-Cl, 4-OCH_3, 3,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$; R_2 = 4-F, 2,4-Cl_2

Figure 2: Synthetic route for the compounds **9a-h**.

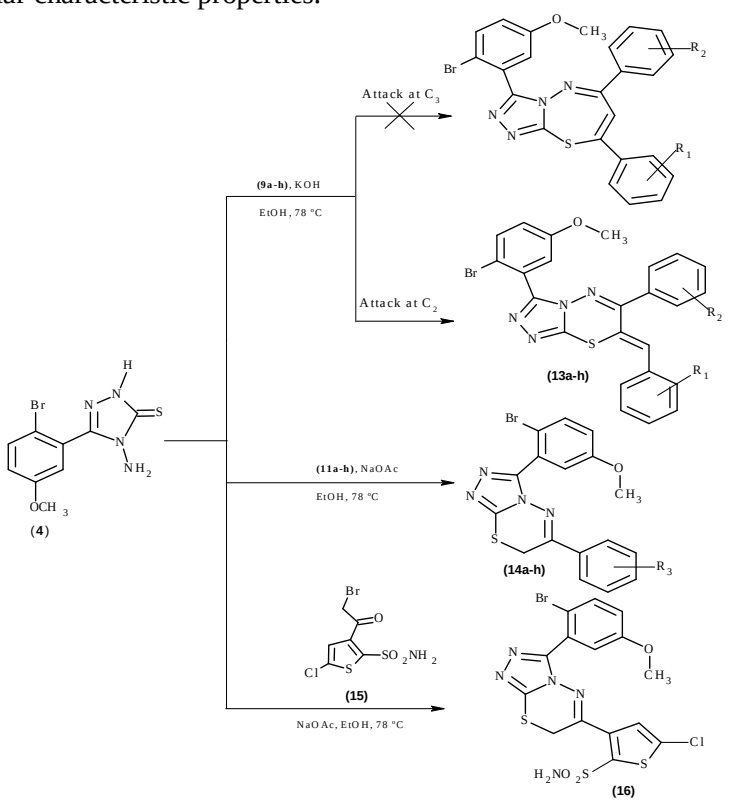


R_3 = 4-NO_2, 3-$CONH_2$-4-OH, 4-Cl, 2,4-Cl_2, H, 4-CH_3, 4-OCH_3, 4-Br

Figure 3: Synthetic route for the compounds **11a-h**.

The condensation of [1,2,4]triazole (**4**) with 2-bromo-1,3-diaryl-prop-2-en-1-ones (**9a-h**) afforded a series of 3-(2-bromo-5-methoxyphenyl)-7-(substituted benzylidene)-6-(substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**13a-h**). The second series of 3-(2-bromo-5-methoxyphenyl)-6-(substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines (**14a-h, 16**) were obtained by the cyclocondensation of (**4**) with 2-bromo-1-aryl-ethanones (**11a-h, 15**). The reaction sequences employed is shown in figure 4.

All structures of the title compounds were confirmed by recording their IR, ^{1}H NMR, ^{13}C NMR and mass spectra. IR spectrum of (**13a**) showed absorption bands at 3051.8 (ArC–H), 2937.06, 2833.88 (methyl C-H), 1592.91 (C=N), 1462.74 (N=C-S), 1085.73 (C–F), 840.81 (C–Cl), 759.81 (C-Br) and 684.60 (C-S-C) cm^{-1} respectively. The absence of the absorption bands corresponding to –NH_2, C=O and -N=C-SH (-NH-C=S of the tautomer) stretching frequencies of the reactants clearly revealed the formation of triazolothiadiazines (**13a-h**). The ^{1}H NMR spectrum of (**13a**) showed a singlet at δ 7.20 corresponding to exocyclic vinylic proton along with other characteristic signals. The signals due to NH_2 and NH-C=S protons of the parent [1,2,4]triazole were disappeared. The IR and ^{1}H NMR spectra of other compounds of the series showed similar characteristic properties.



R_1 = 4-Cl, 4-OCH_3, 3,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$; R_2 = 4-F, 2,4-Cl_2: R_3 = 4-NO_2, 3-$CONH_2$-4-OH, 4-Cl, 2,4-Cl_2, H, 4-Me, 4-OMe, 4-Br

Figure 4: Synthetic route for the title compounds **13a-h**, **14a-h** and **16**.

Formation of the products (**13a-h**) may be rationalized by the mechanism shown in figure 5.

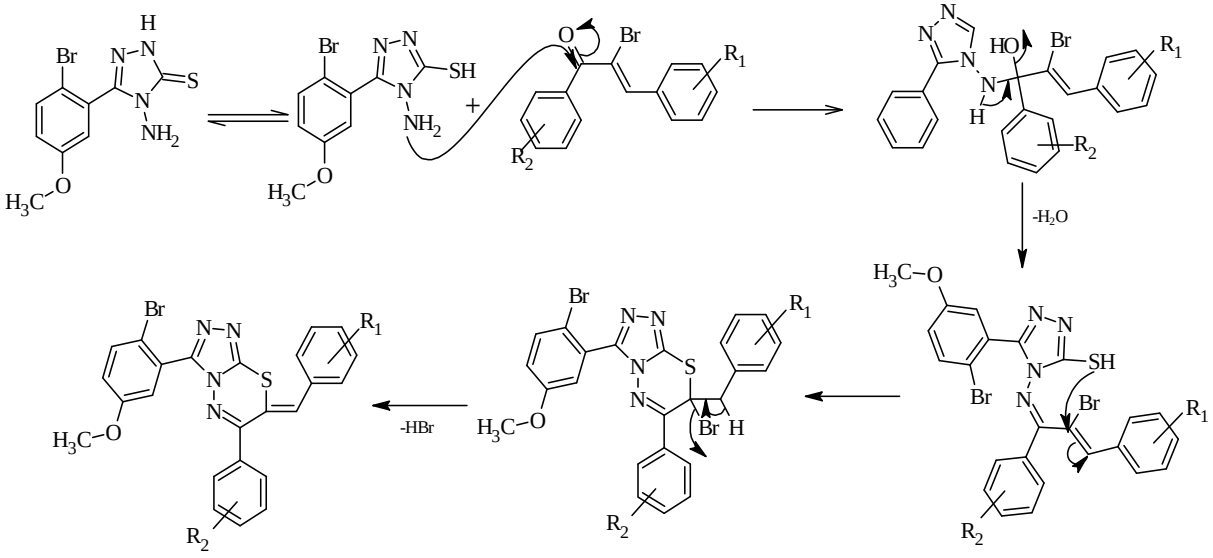


Figure 5: Reaction mechanism of formation of compounds **13a-h**.

In the ^{1}H NMR spectrum of (**13a**), the signals due to three protons of methoxy group appeared as a sharp singlet at δ 3.79. The one aromatic proton of 4-chlorophenyl ring resonated as a doublet of doublet in the region δ 6.91–6.93 with a coupling constant of J = 8.8 and 2.8 Hz. The one aromatic proton of 2,4-chlorophenyl ring resonated as a doublet at δ 7.1 with a coupling constant of J = 2.8. The remaining two aromatic protons of the 2,4-dichlorophenyl ring, three aromatic protons of 4-chlorophenyl ring and three aromatic protons of 2-bromo-5-methoxy phenyl ring resonated as multiplets in the region δ 7.26-7.57. This data confirmed the formation of compound (**13a**). In the ^{13}C NMR spectrum of the compound (**13a**), the chemical shift values of carbon-nitrogen double bonds are appeared at δ 158.77, 153.45 and 152.63. The peaks of remaining cabon atoms of 2,4-dichlorophenyl, 4-chlorophenyl and 2-bromo-5-methoxyphenyl rings are appeared at δ 138.61, 137.31, 136.36, 136.15, 134.12, 133.81, 132.57, 131.51, 130.86,130.25, 129.15, 128.01, 127.71,118.77,117.65,117.40 and 114.17. The carbon atom of methoxy group was resonated at δ 55.69. The ^{13}C NMR-DEPT-135 spectrum of the compound (**13a**) gives the strong evidence for the presence of nine non-quaternary aromatic carbon atoms, giving the peaks at δ 136.37, 133.82, 132.58, 130.87, 130.25, 129.15, 128.01, 118.78 and 117.64 (two pairs of carbon atoms have similar chemical environment) and at δ 55.68 for methoxy cabon atom. The compound (**13a**) showed molecular ion peak at 541.8 (M^+) and 542.7 (M^++1) in conformity with its molecular formula $C_{24}H_{15}BrClFN_4OS$, revealing the stability of the compound.

In the IR spectrum of the compound (**14b**), the absence of $–NH_2$ and -NH-C=S stretching bands (3300, 3260 $cm^{–1}$) indicated the cyclocondensation. The other characteristic stretching vibrations of the product is at 3213.79-3548.38 cm^{-1} (O-H, strong), 1592.91-1671.02 cm^{-1} (C=N), 1636, 1600, 1556.27, 1503.24, 1484.92, 1459.85 cm^{-1} (aromatic ring skeleton vibrations). The C-S-C bending vibrations are in the region 623.85 cm^{-1}. The stretching vibration peaks of CH_2 are observed at about 2924.52 cm^{-1}.
The proposed reaction mechanism is summarized in figure 6.

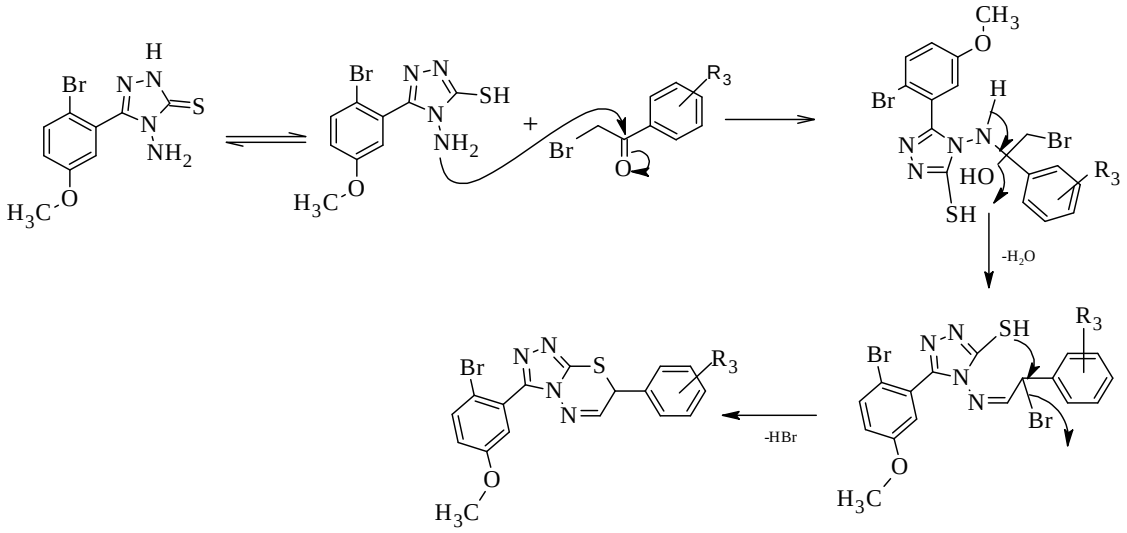


Figure 6: Reaction mechanism of formation of compounds **14a-h** and **16**.

In the ^{1}H NMR spectrum of (**14b**), the disappearance of NH_2 (δ 5.47) and -N=C-SH (-NH-C=S of the tautomer) (δ 13.96) confirm the ring-closure. As a result of the overall action of inductive effect and magnetic anisotropic effect of the aromatic rings, the chemical shift of the SCH_2 group in the thiadiazine moiety appears downfield at δ 5.09. The phenolic OH proton resonated at δ 13.94. The amide NH_2 proton appeared as a broad singlet in the downfield region at δ 8.64. The one aromatic proton of 3-hydroxy-4-benzamide ring resonated as a singlet at δ 8.15. The one more aromatic proton of 3-hydroxy-4-benzamide ring resonated as a doublet at δ 8.06 with a coupling constant of J = 8.7 Hz. The one aromatic protons of 4-methoxyphenyl ring appeared as a doublet in the region δ 7.68 with a coupling constant of J = 8.89 Hz. The one aromatic proton of 2-bromo-5-methoxyphenyl ring showed doublet at δ 7.36 with a coupling constant of J = 8.78 Hz. The one more aromatic proton of 2-bromo-5-methoxyphenyl ring undergoes meta coupling and appeared as a doublet at δ 7.36 with a coupling constant of J = 2.96 Hz. The remaining one aromatic proton of 3-hydroxy-4-benzamide ring resonated as a doublet of doublet in region δ 7.09-7.12 with a coupling constant of J = 8.9 and 3.0 Hz. The aromatic proton of 2-bromo-5-methoxyphenyl ring appeared as a doublet at δ 7.01 with a coupling constant of J = 8.7 Hz. The ^{13}C NMR spectrum of the compound (**14b**), for instance, exhibits the expected absorption peak at δ 190.73, which is assigned to CO-NH_2, one at δ 56.26, which is attributed to the O-CH_3 and another at δ 40.66, which is assigned to the S-CH_2 in the thiadiazine ring. Because of the existence of carbon-nitrogen double bonds, the chemical shifts of the three carbon atoms in the triazole ring appear at δ 159.03, 166.30 and 171.86. When the triazole ring is transformed into a triazolothiadiazine ring, the difference between chemical shifts of the two triazole carbons (δ 177.9, 150.23) is reduced (δ 171.86, 159.0). All the benzene carbons of the title compounds exhibit chemical shifts at δ 111.36, 114.56, 116.96, 118.59, 119.95, 125.37, 126.52, 130.61, 134.58 and 135.66 respectively. The ^{13}C NMR-DEPT-135 spectrum of the compound (**14b**) identifies and confirms the presence of six –CH aromatic protons in region δ 116.96, 118.59, 119.96, 130.61, 134.58 and 135.66. It also gives the strong support for the presence of CH_3 carbon at δ 56.26 and CH_2 carbon of triazolothiadiazine ring at δ 40.66 in downward pattern. In the LC mass spectrum of the compound (**14b**), the (M^++1) peak observed at 461.3 and (M^++3) observed at 463.1 is in good agreement with the molecular formula of the compound $C_{18}H_{14}BrN_5O_3S$ (mol. wt = 460.3). The physicochemical properties of synthesized compounds were presented in table 1, table 2 and table 3.

Table 1. Physical Constants and Characterization Data of Compounds **9a-h**.

Comp. No.	R_1	R_2	Recryst. Solvent	Mol. Formula (Mol. wt)	M.p. (^oC)	Yield (%)
9a	4-Cl	4-F	Chloroform	$C_{15}H_9BrClFO$ (339.58)	90-92	82
9b	4-Cl	2,4-Cl_2	Chloroform	$C_{15}H_8BrCl_3O$ (390.48)	84-86	81
9c	4-OCH_3	4-F	Chloroform	$C_{16}H_{12}BrFO_2$ (335.16)	126-128	81
9d	4-OCH_3	2,4-Cl_2	Chloroform	$C_{16}H_{11}BrCl_2O_2$ (386.06)	158-160	78
9e	3,4-$(OCH_3)_2$	4-F	Chloroform	$C_{17}H_{14}BrFO_3$ (365.19)	98-100	84
9f	3,4-$(OCH_3)_2$	2,4-Cl_2	Chloroform	$C_{17}H_{13}BrCl_2O_3$ (416.09)	130-132	80
9g	3,4,5-$(OCH_3)_3$	4-F	Chloroform	$C_{18}H_{16}BrFO_4$ (395.21)	108-110	85
9h	3,4,5-$(OCH_3)_3$	2,4-Cl_2	Chloroform	$C_{18}H_{15}BrCl_2O_4$ (446.12)	122-124	80

Table 2. Physical Constants and Characterization Data of compounds **13a-h**.

Compd. No.	R_1	R_2	Recryst. Solvent	Mol. Formula (Mol. wt)	M.p. (^oC)	Yield (%)
13a	4-Cl	4-F	Ethanol	$C_{24}H_{15}BrClFN_4OS$ (541.82)	210-212	74
13b	4-Cl	2,4-Cl_2	Ethanol	$C_{24}H_{14}BrCl_3N_4OS$ (592.72)	208-210	76
13c	4-OCH_3	4-F	Ethanol	$C_{25}H_{18}BrFN_4O_2S$ (537.40)	214-216	72
13d	4-OCH_3	2,4-Cl_2	Ethanol	$C_{25}H_{17}BrCl_2N_4O_2S$ (588.30)	202-204	70
13e	3,4-$(OCH_3)_2$	4-F	Dioxane	$C_{26}H_{20}BrFN_4O_3S$ (567.42)	214-216	68
13f	3,4-$(OCH_3)_2$	2,4-Cl_2	Dioxane	$C_{26}H_{19}BrCl_2N_4O_3S$ (618.33)	226-228	74
13g	3,4,5-$(OCH_3)_3$	4-F	Dioxane	$C_{27}H_{22}BrFN_4O_4S$ (597.45)	232-234	72
13h	3,4,5-$(OCH_3)_3$	2,4-Cl_2	Dioxane	$C_{27}H_{21}BrCl_2N_4O_4S$ (648.35)	240-242	76

Table 3. Physical Constants and Characterization Data of Compounds **14a-h** and **16**.

Compd. No.	R_3	Recryst. Solvent	Mol. Formula (Mol. wt.)	M.p. (^oC)	Yield (%)
14a	4-NO_2	Ethanol	$C_{17}H_{12}BrN_5O_3S$ (446.27)	146-148	70
14b	3-$CONH_2$-4-OH	Ethanol	$C_{18}H_{14}BrN_5O_3S$ (460.30)	136-138	72
14c	4-Cl,	Ethanol	$C_{17}H_{12}BrClN_4OS$ (435.72)	156-158	70
14d	2,4-Cl_2	Ethanol	$C_{17}H_{11}BrCl_2N_4OS$ (470.17)	158-160	71
14e	H	Ethanol	$C_{17}H_{13}BrN_4OS$ (401.28)	156-158	70
14f	4-Me	Ethanol	$C_{18}H_{15}BrN_4OS$ (415.30)	188-190	74
14g	4-OMe	Ethanol	$C_{18}H_{15}BrN_4O_2S$ (431.30)	136-138	69
14h	4-Br	Ethanol	$C_{17}H_{12}Br_2N_4OS$ (480.17)	172-174	75
16	-	Ethanol	$C_{15}H_{11}BrClN_5O_3S_3$ (520.83)	224-226	74

Pharmacological screening

Pharmacology: All newly synthesized compounds were screened for their *in vitro* antioxidant activity by DPPH scavenging method. Ascorbic acid was used as a positive control and measurements was run in triplicate. The percentage of scavenging activity was calculated. We investigated all the newly synthesized compounds for their antibacterial activity against four bacterial strains, *viz*., *Staphylococcus aureus*

(ATTC-25923), *Escherichia coli* (ATTC-25922), *Psuedomonus aeruginosa* (ATTC-27853) and *Klebsiella pneumonia* (recultured). Newly synthesized compounds were also tested for antifungal activity against *Penicillium marneffei* (recultred), *Trichophyton mentagrophytes* (recultured), *Aspergilus flavus* (NICM No.524) and *Aspergilus fumigatus* (NCIM No.902) fungal strains.

The newly synthesized compounds were also subjected for their anti-inflammatory and analgesic activity studies. The tested compounds were administered in the form of a suspension (1 % carboxy methyl cellulose as vehicle). Anti-inflammatory and analgesic activities of the tested compounds were measured with respect to the control and compared with the standard drugs Diclofenac sodium and pentazocine respectively. All the pharmacological data are expressed as mean±SEM; statistical analysis was applied to determine the significance of the difference between the control group and group of animals tested with the tested compounds.

Anti-oxidant activity

DPPH radical scavenging activity: The newly synthesized compounds were tested for their antioxidant activity. The compounds **13d**, **13h, 14b, 14d, 14f, 14g** and **16** showed comparatively significant antioxidant activities. These compounds contain para methoxy, 3,4,5-trimethoxy, 3-amido-4-hydroxy, 2,4-dichloro or para methyl groups in their structure. The compound **16** contain biologically active 5-chloro-thienyl-2-sulphonamide moiety in their structure. The compounds **13b**, **13c**, **13f**, **14a**, **14c**, **14e** and **14h** showed good activity compared with ascorbic acid as a positive control. The good activity attributed due to the presence of fluoro, chloro, nitro or bromo groups in their structure. The remaining compounds **4, 13a, 13b, 13e, 13g** and **14e** showed moderate activity compared to the positive control. From the study it was observed that, the formation of thiadiazine ring fused to [1,2,4]triazole increased the antioxidant activity of 1,2,4-triazole alone. The results of antioxidant activity study were tabulated in table 4. Figure 7 depicts the percentage of free radical scavenging activity of tested compounds by using DPPH.

Table 4. DPPH radical scavenging assay for **4**, **13a-h**, **14a-h** and **16**.

Compounds	Concentration (ug/mL)		
	200	400	800
	% inhibition	% inhibition	% inhibition
4	52.67	56.84	67.45
13a	53.24	57.86	69.24
13b	62.15	70.32	78.89
13c	64.05	69.98	75.87
13d	58.58	69.81	81.67
13e	53.98	57.21	64.98
13f	58.45	66.97	74.85
13g	55.32	59.89	69.67
13h	60.12	76.65	84.09
14a	53.91	65.77	76.99
14b	64.21	75.97	89.02
14c	57.45	63.98	75.45
14d	60.43	76.90	82.89
14e	59.76	63.31	68.43
14f	66.41	74.82	84.47

14g	61.90	75.42	83.42
14h	54.19	67.71	76.98
16	69.17	78.08	89.04
Ascorbic acid	87.57	94.97	96

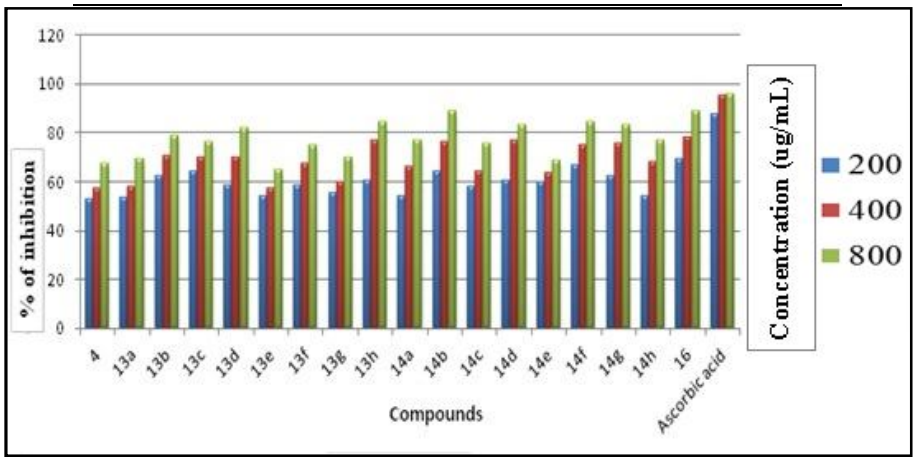


Figure 7: Radical scavenging assay for compounds **4**, **13a-h**, **14a-h** and **16**.

Antibacterial activity: The investigation of antibacterial screening data revealed that all the tested compounds, **4**, **13a-h**, **14a-h** and **16** showed only moderate to good activities. The compound 4-amino-5-(2-bromo-5-methoxyphenyl)-2,4-dihydro-3*H*-[1,2,4]triazole-3-thione (**4**) alone showed moderate activity against *Escherichia coli* (ATTC-25922) and *Klebsiella pneumoniae* (recultured) bacterial strains. The compound **13b**, **13d**, **13e** and **16** showed good activity towards all the four bacterial strains. This may be attributed because of the presence of dichloro, para methoxy, 3,4-dimethoxy, fluoro or 5-chloro-thienyl-2-sulphonamide group in their structure. The compound **14c** showed very less activity towards all the four bacterial strains. The remaining compounds showed moderate activity compared to a standard drug Ampicillin. On after going through structure activity relationship, it is observed that the construction of thiadiazine ring fused to triazole moiety widen the activity of the compound compared to 4-amino-3-(2-bromo-5-methoxyphenyl)-5-mercapto-1,2,4-triazole **4** alone. The results were summarized in table 5.

Table 5. Antibacterial activity of the compounds 4, 13a-h, 14a-h and 16.

Products	MIC (in µM) and zone of inhibition (mm) in parentheses			
	S. aureus **(ATTC-25923)**	***E. coli*** **(ATTC-25922)**	***P. aeruginosa*** **(ATTC-27853)**	***K. pneumoniae*** **(recultured)**
4	41.51 (11-15)	20.75 (16-20)	41.51 (11-15)	20.75 (16-20)
13a	23.07 (11-15)	23.07 (11-15)	11.53 (16-20)	23.07 (11-15)
13b	10.54(16-20)	10.54 (16-20)	10.54 (16-20)	10.54 (16-20)
13c	11.63 (16-20)	23.26(11-15)	23.26 (11-15)	11.63 (16-20)
13d	10.62 (16-20)	10.62 (16-20)	10.62 (16-20)	10.62 (16-20)
13e	11.01 (16-20)	11.01 (16-20)	11.01 (16-20)	11.01 (16-20)
13f	20.21 (11-15)	20.21 (11-15)	10.10 (16-20)	10.10 (16-20)
13g	12.5 (11-15)	10.46 (16-20)	10.46 (16-20)	20.92 (11-15)
13h	9.64 (16-20)	19.28 (11-15)	9.64 (16-20)	19.28 (11-15)
14a	28.01 (11-15)	28.01 (11-15)	14.00 (16-20)	14.00 (16-20)
14b	13.57 (16-20)	-	27.16 (11-15)	27.16 (11-15)

14c	28.68 (11-15)	28.68 (11-15)	28.68 (11-15)	28.68 (11-15)
14d	13.30 (16-20)	26.58 (11-15)	-	13.30 (16-20)
14e	15.57 (16-20)	31.15 (11-15)	15.57 (16-20)	31.15 (11-15)
14f	30.10 (11-15)	-	15.05 (16-20)	30.10 (11-15)
14g	14.50 (16-20)	14.50 (16-20)	28.10 (11-15)	14.50 (16-20)
14h	26.03 (11-15)	13.01 (16-20)	13.01 (16-20)	26.03 (11-15)
16	12.00 (16-20)	12.00 (16-20)	12.00 (16-20)	12.00 (16-20)
Ampicillin	4.46 (16-22)	17.88 (16-22)	17.88 (16-22)	17.88 (16-22)

(-) indicates inactive.

Antifungal Activity: The antifungal screening data also revealed that all the newly synthesized compounds were moderate to good anti-fungal agents. The compounds **13b, 13d**, **13e** and **16** showed marked activity against all the four fungal strains. Like good antibacterial agents, these compounds also contain dichloro, para methoxy, 3,4-dimethoxy, fluoro or 5-chloro-thienyl-2-sulphonamide groups in their structure. The compound **14c** is less active towards all the four fungal strains. Moreover, the antifungal activities of the other compounds against the tested organisms are weak compared to a standard drug itraconazole. The anti-fungal study revealed that, the activity of 1,2,4-triazole is enhanced by the formation of fused thiadiazine ring. Results of antifungal studies are presented in table 6.

Table 6. Antifungal activity of the compounds **4**, **13a-h**, **14a-h** and **16**.

Products	**MIC (in µM) and zone of inhibition (mm) in parentheses**			
	P. marneffei **(recultured)**	***T. mentagrophytes*** **(recultured)**	***A. flavus*** **(NCIM No.524)**	***A. fumigatus*** **(NCIM No.902)**
4	20.75 (16-20)	20.75 (16-20)	20.75 (16-20)	20.75 (16-20)
13a	11.53 (16-20)	11.53 (16-20)	23.07 (11-15)	11.53 (16-20)
13b	10.54 (16-20)	10.54 (16-20)	10.54 (16-20)	10.54 (16-20)
13c	23.26 (11-15)	-	11.63 (16-20)	11.63 (16-20)
13d	10.62 (16-20)	10.62 (16-20)	10.62 (16-20)	10.62 (16-20)
13e	11.01 (16-20)	11.01 (16-20)	11.01 (16-20)	11.01 (16-20)
13f	20.21 (11-15)	20.21 (11-15)	10.10 (16-20)	10.10 (16-20)
13g	20.92 (11-15)	10.46 (16-20)	-	10.46 (16-20)
13h	9.64 (16-20)	19.28 (11-15)	-	9.64 (16-20)
14a	28.01 (11-15)	28.01 (11-15)	14.00 (16-20)	14.00 (16-20)
14b	13.57 (16-20)	27.16 (11-15)	13.57 (16-20)	13.57 (16-20)
14c	28.68 (11-15)	28.68 (11-15)	28.68 (11-15)	28.68 (11-15)
14d	26.58 (11-15)	13.30 (16-20)	26.58 (11-15)	26.58 (11-15)
14e	-	31.15 (11-15)	15.57 (16-20)	-
14f	30.10 (11-15)	-	30.10 (11-15)	30.10 (11-15)
14g	14.50 (16-20)	28.10 (11-15)	28.10 (11-15)	28.10 (11-15)
14h	13.01 (16-20)	13.01(16-20)	26.03 (11-15)	13.01 (16-20)

16	12.00 (16-20)	12.00 (16-20)	12.00 (16-20)	12.00 (16-20)
Itraconazole	2.21 (0.03-16)	8.86 (0.03-16)	8.86 (0.03-16)	8.86 (0.03-16)

(-) indicates inactive.

Anti-inflammatory activity: The 7-(substitutedbenzylidene)-6-(substitutedphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **13a**, **13b**, **13c**, **13d**, **13e**, **13f**, **13g** and **13h** containing 4-chloro/4-fluoro, 4-chloro/2,4-dichloro, 4-methoxy/4-fluoro, 4-methoxy/2,4-dichloro, 3,4-dimethoxy/4-fluoro, 3,4-dimethoxy/2,4-dichloro, 3,4,5-trimethoxy/4-fluoro and 3,4,5-trimethoxy/2,4-dichloro groups and 6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **14c**, **14d** and **14h** containing 4-chlorophenyl, 2,4-dichlorophenyl and 4-bromophenyl respectively are the most potent agents of this series against rat-foot inflammation. According to the study, 1,2,4-triazole **4** itself showed good activity, but it is observed that activity is further increased by the formation of thiadiazine ring. Therefore according to structure activity relationship, the electron diminishing functional groups like chloro and fluoro at para position of the phenyl ring are responsible for the excellent activity against rat-foot inflammation. The remaining compounds showed moderate anti-inflammatory activity. The results were presented in table-7.

Table 7. Anti-inflammatory activity of the compounds **13a-h** and **14a-h**.

Compound	Dose (mg/kg)	60 min	120 min	180 min
Control	100	2.175±0.1797	2.375±0.1436	2.275±0.1181
Diclofenac sodium	100	0.6±0.04082***	0.625±0.04787***	0.625±0.04787***
13a	100	1.58± 0.1434*	1.63±0.1843**	1.68±0.0909**
13b	100	1.59± 0.1933*	1.68±0.2136**	1.79±0.1867
13c	100	1.48± 0.1455**	1.78±0.1089*	1.99±0.1207
13d	100	1.58± 0.1808*	1.70±0.1393**	1.76±0.1654*
13e	100	1.48± 0.1757**	1.88±0.1642	2.11±0.1735
13f	100	1.628± 0.1456**	1.86±0.114	2.04±0.1163
13g	100	1.425± 0.1651**	1.90±0.182	1.99±0.1643
13h	100	1.546± 0.1452**	1.75±0.162	1.80±0.0283
14a	100	2.18± 0.1574	2.23±0.0765	2.25±0.0795
14b	100	1.89± 0.1753	1.99±0.1402	2.085±0.1218
14c	100	1.50± 0.0345**	1.86±0.1357**	1.92±0.1382
14d	100	1.60± 0.1648**	1.86±0.1142	1.96±0.1345
14e	100	1.725± 0.1539	1.99±0.1043	2.07±0.1081
14f	100	2.04± 0.04126	2.08±0.0763	2.29±0.1265
14g	100	1.86± 0.1856	1.96±0.1651	2.09±0.1473
14h	100	1.725± 0.0952**	1.85±0.1242	1.98±0.1523

*Results were expressed in mean ± SE M. (n=6) significance levels *P<0.05, **P<0.01, ***P<0.001 as compared with the respective control*

Analgesic activity: The 4-amino-3-(2-bromo-5-methoxyphenyl)-5-mercapto-1,2,4-triazole (**4**) itself showed potent analgesic activity compared to a standard drug pentazocin. From the analgesic study it was observed that the formation of thiadiazine ring further enhanced the activity of synthesized compounds. Like 4-amino-3-(2-bromo-5-methoxyphenyl)-5-mercapto-1,2,4-triazole (**4**), the compounds 7-

(substitutedbenzylidene)-6-(substitutedphenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **13a**, **13b**, **13c**, **13d**, **13e**, **13f**, **13g** and **13h** showed potent activity compared to a standard drug pentazocin. The good activity of these compounds are attributed due to the presence of 4-chloro/4-fluoro, 4-chloro/2,4-dichloro, 4-methoxy/4-fluoro, 4-methoxy/2,4-dichloro, 3,4-dimethoxy/4-fluoro, 3,4-dimethoxy/2,4-dichloro, 3,4,5-trimethoxy/4-fluoro, and 3,4,5-trimethoxy/2,4-dichloro functional groups at para position of phenyl ring. Similarly 6-(4-substituted phenyl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **14c**, **14d** and **14h** containing 4-chlorophenyl, 2,4-dichlorophenyl and 4-bromophenyl groups respectively showed good analgesic activity. The remaining compounds showed moderate analgesic activity. The results were illustrated in table 8.

Table 8. Analgesic activity of the newly synthesized compounds **13a-h** and **14a-h**.

Compound	Dose (mg/kg)	60 min	120 min	180 min
Control	100	1.363±0.1297	1.363±0.1297	1.363±0.1297
pentazocin	100	7.075±0.1164***	6.863±0.1919***	7.025±0.2504***
13a	100	3.445±0.2986**	3.392±0.3062**	2.329±0.1431**
13b	100	3.385±0.4884**	3.274±0.3423**	2.652±0.2901**
13c	100	3.046±0.1574	3.324±0.1675**	3.216±0.1282
13d	100	3.262±0.1641*	3.601±0.1352**	2.946±0.1644*
13e	100	3.686±0.8205**	3.576±0.8011**	2.436±0.2425**
13f	100	2.906±0.0903	3.440±0.2593**	3.221±0.2831
13g	100	3.566±0.6915**	3.692±0.7791**	2.246±0.0585*
13h	100	3.478±0.5619**	3.345±0.4052**	2.845±0.4715*
14a	100	2.863±0.6135	2.878±0.6462	2.439±0.0724
14b	100	2.613±0.5113	2.788±0.4255	2.463±0.357
14c	100	3.398±0.4684**	3.287±0.3541**	2.629±0.2734**
14d	100	3.336±0.4884**	3.248±0.3446**	2.658±0.2632**
14e	100	1.942±0.2105	2.161±0.09412	2.565±0.1409
14f	100	3.185±0.6435*	3.088±0.5526*	2.368±0.09085*
14g	100	2.394±0.2452	2.582±0.2192	2.541±0.0422
14h	100	3.345±0.4682**	3.233±0.3548**	2.633±0.2732**

*Results were expressed in mean ± SEM. (n=6) significance levels *P<0.05, **P<0.01, ***P<0.001 as compared with the respective control*

APPLICATIONS

From the overall study, it is hoped that [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines may emerge as potential compounds with varied biological activities in future.

CONCLUSIONS

The research study reports the successful synthesis of 7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives in good yield. All the synthesized compounds have been characterized by analytical and spectral data. Some of the compounds showed significant antioxidant activity. The antimicrobial study revealed that synthesized compounds are moderate antimicrobial agents. The majority of the newly synthesized compounds showed good anti-inflammatory and analgesic activities. As the compounds are subjected to preliminary bioactivity study, hence we are unable to comment on SAR accurately.

ACKNOWLEDGEMENTS

The author N. Chidananda is grateful to the Managing Director, SeQuent Scientific Ltd., New Mangalore, India for encouraging the research work. N. Chidananda remember with great sense of gratitude Prof. (Late) A. Srikrishna, Department of Organic Chemistry, IISc, Bangalore for their invaluable suggestions and technical support. The authors are also thankful to Dr. N. Suchetha Kumari (Professor, Department of Biochemistry, K.S. Hegde Medical Academy, Deralakatte, Karnataka, 574 162, India) and Dr. D. Prashanth Shetty (Professor, NGSM Institute of pharmaceutical sciences, Deralakatte, Mangalore, Karnataka, 574 160, India) for providing biological activity data. We are also thankful to Smt. Ashwini Srinivas, Department of Bioscience, Mangalore University for her sincere help during the antioxidant study. Thanks are also due to CDRI-Lucknow and IISc. Bangalore for providing ^{1}H and ^{13}C NMR spectral facilities.

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