ORIGINAL ARTICLE

A C G publications

Org. Commun. 6:2 (2013) 78-85

organic communications

Synthesis and antimicrobial activity of some novel fused heterocyclic moieties

Nareshvarma Seelam[1*], Satya P. Shrivastava[2] and Somarouthu Prasanthi [1]

[1]*Department of Chemistry, K. L. University, Vaddeswaram, Guntur, A.P, India-522502*

[2]*Heterocyclic Research laboratory, Department of Chemistry, Dr. H.S. Gour. Central University, Sagar, M.P, India-470003*

(Received February 4, 2013; Revised June 5, 2013; Accepted June 16, 2013)

Abstract: Here a new class of 1, 3, 4-thiadiazoles which are incorporating with isoxazolo-thiazole moieties were synthesized by the reaction of chalcone derivatives of [1, 3, 4] thiadiazol-2-yl)-thiazolidin-4-one with hydroxylamine hydrochloride. The chemical structures of these compounds were confirmed by IR, NMR (^{1}H & ^{13}C) and mass spectral studies. The new synthesized compounds were evaluated for their antimicrobial activity. The final results revealed that some of the compounds were exhibited well antimicrobial activity compared to the standard drugs.

Keywords: Thiadiazole; pyrazole; thiazolidinones; antimicrobial studies; anti-tuberculosis studies.

1. Introduction

One of the major objectives of organic and medicinal chemistry is the design, synthesis and production of molecules, which are having highly therapeutic interest. On the other hand because of the resistance of pathogenic bacteria towards available anti- biotics is rapidly becoming a worldwide undigestive problem, in the same way fungal infections continue to increase rapidly because of the increased number of immuno compromised patients. So in view of the above discussions it will be necessary to design a new class of molecules to deal with resistant bacteria and fungi has become one of the most important areas of antimicrobial research today.

Since the discovery of heterocyclic nucleus the chemistry of isoxazole and their fused derivatives continue to draw attention of organic chemists due to their various biological activities such as antithrombotic agents[1], antitumor activity[2], antinociceptive activity[3], anti-inflammatory activity[4], anti-oxidants[5], antibacterial[6], antifungal[7], nematicidal agents[8], antifungal[9], anti-viral[10], anti inflammatory and hypo glycemic agents[11].

On the other hand thiazoles are basic class of heterocyclic moieties which possess a wide range of therapeutic interest and their importance is also very much-established in medicine[12] such as antibacterial and antifungal activities[13-14], antitubercular activity[15-16], anti HIV agents[17]. Among the important heterocyclic compounds 1,3,4-thiadiazoles are one of the important structural fragments in medicinal chemistry due to their various biological activities, such as Ca^{+2} channel blockers[18], anti

* Corresponding author: Email: utd_naresh@yahoo.co.in

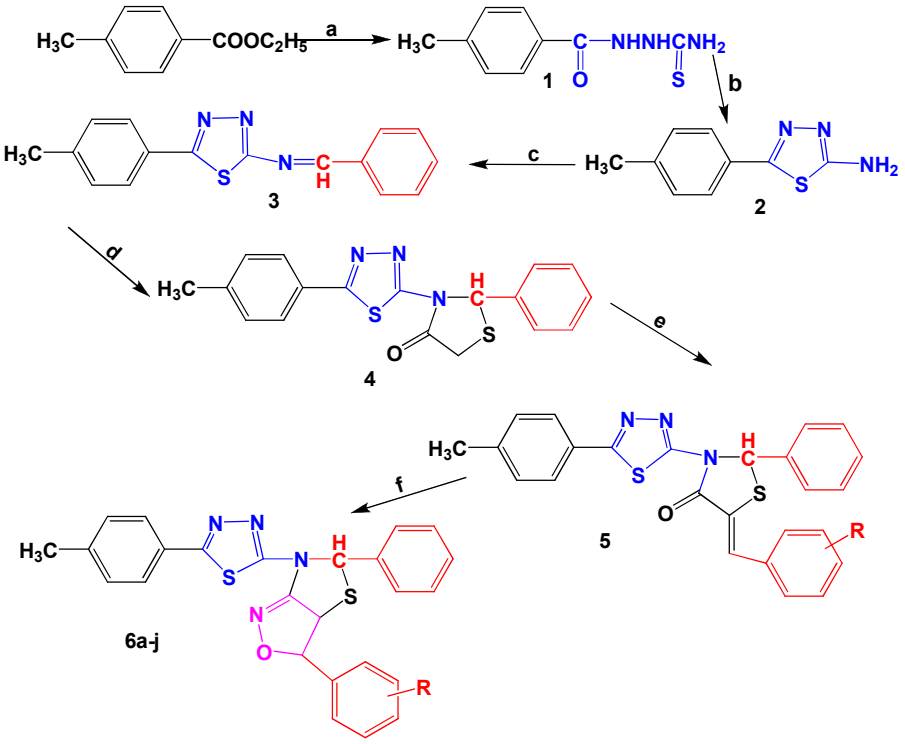
The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/OC/index.htm © Published 6/25/2013 EISSN:1307-6175

inflammatory active agents[19], antitubercular activity[20], antiinfective agents[21], antibacterial[22], antidepressants[23], anti cancer agents.[24]

In view of the above observations it was a thought of interest to design and synthesize a new class of isoxazole derivatives incorporating with 1,3,4-thiadiazole moiety. So, in this present communication we report a facile synthesis of diverse 3-(4-chloro-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3, 4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene derivatives and their antimicrobial activities against various organisms.

2. Results and discussion

Chemistry: The synthesis of heterocycles **(6a-j)** including three different ring were started from intermediate **4**.The compound **4** was prepared according to the procedure outlined in **Scheme-1.** Condensation of ethyl p-methyl benzoate with thiosemicarbazide yielded the compound **1**. This compound was dehydrated with conc. H_2SO_4 to give compound **2**. Compound **2** was further reacted with benzaldehyde to give benzylidene **3**. This was then reacted with mercapto acetic acid in the presence of anhydrous $ZnCl_2$ under conventional heating conditions to give compound **4** (Scheme 1).



R= 4-Cl, 2-Cl, 4-NO_2, 4-N(Me_2), 3-NO_2, 4-OCH_3, 2-OCH_3, 4-CH_3, 3,4,5-$(OCH_3)_3$,4-Br

Scheme 1. Synthesis of compounds **6a-g**.

Compound **4** was then reacted with various aromatic aldehydes in the presence of anhydrous NaOAc in glacial AcOH at reflux temperature to give chalcone derivatives of thiazolidinones **5a-j**. Compounds **5a-j** on cyclocondensation with hydroxylamine hydrochloride in the presence of anhydrous NaOAc in glacial AcOH at reflux temperature gave titled compounds. Their IR, ^{1}H NMR, ^{13}C NMR spectral data confirmed the structures of the synthesized compounds.

The structures of the compounds were elucidated by IR, ^{1}H NMR, and ^{13}C NMR analyses. In the IR spectra of compounds **6a-j** disappearance of amide carbonyl (C=O) absorption band at ~1683cm^{-1}, which was present in compounds **5a-j**, confirmed the cyclization or involvement of α, β - unsaturated carbonyl system. On the other hand O-CH band of the pyrazole moiety were appeared at about 920 cm^{-1} respectively.

In the ^{1}H NMR spectra of compounds 6a-j recorded in DMSO-d_6, the N-CH-Ar proton of thiazole ring at 5.17- 5.70 ppm as a singlet, S-CH fused proton at 4.23-4.49 ppm as a doublet and O-CH fused proton at 4.63-4.98 ppm as a doublet. These signals demonstrate that the cyclization step had occurred. All the other aromatic protons of **6a-j** were observed at the expected regions.

The ^{13}C NMR spectra of compounds **6a-j** were recorded in DMSO-d_6. The prominent signals corresponding to the carbons of thiazolo-pyrazole ring in all compounds observed nearly at 53, 57.5 and 157.1 ppm, are proof of further evidence of their structures.

Antimicrobial activity:

The results depicted in **Table–1** revealed that most of the tested compounds displayed variable inhibitory effects on the growth of tested bacterial and fungal strains. Amongst the screened **6a**, **6b**, **6c**, **6e** and **6j** compounds have shown highly antibacterial activity against all the strains employed. In this view compound **6a**, **6b**, **6c**, **6e** and **6j** were equipotent to Streptomycin against B. subtilis and B. thuringiensis (MIC, 3.125µg/ml), while its activity were 50% or more lower than Streptomycin against remaining strains employed (MIC, 6.25 µg/ml and MIC, 12.5 µg/ml). On the other hand compounds **6f, 6g** and **6i** have shown highly antifungal activity against all the strains employed. In this view compound **6f** was equipotent to treflucan against all the strains employed (MIC, 3.125 µg/ml). As well as compound **6g** and **6i** were equipotent to treflucan against C. albicans (MIC, 3.125 µg/ml) while its activity were 50% lower than Treflucan against B.fabae, F. oxysporam (MIC, 6.25 µg/ml). The remaining compounds have also shown moderate to good antifungal activity against all the strains employed.

Table 1. Antimicrobial activity of compounds 6a-j (MIC µg/ml)

	Bacteria[b)]				Fungi[c)]		
Comp' No.	*B. subtilis*	*B. thuringiensis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*B. fabae*	*F. oxysporam*
6a	>3.125	6.25	>6.25	6.25	25	25	12.5
6b	3.125	>3.125	6.25	6.25	25	>12.5	12.5
6c	3.125	3.125	25	3.125	>12.5	25	12.5
6d	12.5	12.5	25	>12.5	12.5	6.25	>6.25
6e	3.125	3.125	12.5	>6.25	25	12.5	>12.5
6f	12.5	25	>25	50	3.125	>3.125	3.125
6g	>12.5	25	>25	50	3.125	>3.125	6.25
6h	25	>25	>6.25	>50	>6.25	6.25	12.5
6i	>12.5	12.5	6.25	>6.25	3.125	>6.25	6.25
6j	3.125	3.125	>6.25	6.25	25	12.5	>25
Streptomycin	3.125	6.25	6.25	6.25	NA[a)]	NA[a)]	NA[a)]
Treflucan	NA[a)]	NA[a)]	NA[a)]	NA[a)]	3.125	3.125	3.125

[a)] Not Active

[b)] *B. subtilis* (MTCC No: 1133), *B. thuringiensis* (MTCC No: 4714), *E. coli* (MTCC No: 443), and *P.aeruginosa* (MTCC No: 2297).

[c)] *C. albicans* (MTCC No: 183), *B. fabae* (ATCC No: 14862) and *F. oxysporam* (MTCC No: 7392)

3. Conclusion

In the present paper our aim has been verified by the synthesis of 3-(substituted-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3,4] thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene **(6a-j)** derivatives, in which 1,3,4-thiadiazole and thiazolo-isoxazole moiety in a same molecular framework. Obtained results clearly revealed that most of the tested compounds **6a-j** showed moderate antibacterial, antifungal activities comparable with standard drugs. Finally it can be concluded that these molecules will be useful as a class of antibacterial and antifungal agents.

4. Experimental

Chemistry: All melting points were measured on open capillary method. IR spectra were recorded for KBr disc on Schimadzu-8400 FTIR spectrophotometer. ^{1}H NMR, ^{13}C NMR spectra were measured on a Bruker Avance II 400 spectrometer, operating at 400, 100.6 MHz respectively. Chemical shifts (δ) are reported in parts per million and TMS as an internal standard. Reactions were monitored by thin layer chromatography (TLC) on silica gel, plates were visualizing with ultraviolet light or iodine. Column chromatography was performed on silica gel 60(0.043-0.06mm) Merck.

General procedure for the synthesis of p-methyl benzoyl thiosemicarbazide (1): A mixture of Ethyl-p-methyl benzoate (0.01mol) and thiosemicarbazide (0.01mol) in methanol (25 ml) was refluxed for 10 h. The solvent was removed under reduced pressure and the viscous mass poured over ice water, filtered and recrystallized from methanol-water to afford compound 1. m.p. 128°-30°C. Yield 75%, IR (KBr) ν in cm^{-1}: 2942.16 (CH_3), 3060 (C-H in aromatic), 3175 (N-H), 1661.53 (C=O), 1071.6 (C=S), ^{1}H NMR (400 MHz, $CDCl_3$) & in ppm: 7.60-7.45 (m, 4H, Ar-H), 8.15 (m, 4H, NHNHCSNH_2 exchangeable with D_2O), 2.91 (s, 3H, CH_3).

General procedure for the synthesis of 5-p-Tolyl-[1, 3, 4]thiadiazol-2-yl amine (2): A mixture of compound 1(0.05 mol) and conc, H_2SO_4 (20 ml) was kept over night at room temperature, then poured in to cold water, neutralized with liquid ammonia and filtered. The product thus obtained was recrystallised from ethanol-water. m.p. 135°C. Yield 61%; IR (KBr) ν in cm^{-1} 3350 (NH_2); 3058 (C-H in aromatic), 2968.63 (CH_3); 1598 (C=N), 1221 (C-N), 1045 (N-N), 732.27 (C-S-C); ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm; 7.62-7.35 (m, 4H, Ar-H), 6.35 (bs, 2H, NH_2 exchangeable with D_2O), 3.01 (s, 3H, CH_3).

General procedure for the synthesis of Benzylidene-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-amine (3): A mixture of 2(0.01 mol) and benzaldehyde (0.01 mol) was refluxed in ethanol for 5h with a few drops of glacial acetic acid. The solid separated on cooling was filtered, dried and recrystallized from benzene as needle shaped crystals were obtained. Yield 73.2%, m.p.162°-164°C.IR (KBr) ν in cm^{-1}: 3032.2 (Ar-H), 2946.76 (C-H in CH_3), 1040.6 (N-N), 1608.61 (C=N cyclic), 1221 (C-N), 683.7 (C-S); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.79-7.21 (m, 9H, Ar-H), 7.9 (s, 1H, N=CH), 2.98 (s, 3H,CH_3).

General procedure for the synthesis of 2-Phenyl-3-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-thiazolidin-4-one (4): A mixture of compound 3 (0.01 mol) and mercapto acetic acid (0.01 mol) in 1, 4–Dioxane (30 ml) containing a pinch of $ZnCl_2$ was refluxed for 8 h. The hot solution was filtered and cooled in an ice bath. The solid obtained was filtered, washed with 10% $NaHCO_3$ solution and was recrystallized from alcohol. Yield 70.9%, m.p. 181°-184°C; IR (KBr) ν in cm^{-1}: 2946.73 (C-H in CH_3), 2918.69 (C-H in CH_2), 1665 (C=O), 1608.61 (C=N), 1045.1 (N-N), 691.6 (C-S), ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.91-7.8 (m, 9H, Ar-H), 5.13 (s, 1H, N-CH-Ar), 4.25 (s, 2H, SCH_2C=O), 2.91 (s, 3H, CH_3).

General procedure for the synthesis of 5-(4-Chloro-benzylidene)-2-phenyl-3-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-thiazolidin-4-one (5a): A mixture of compound **4** (0.01 mol), 4-chlorobenzaldehyde (0.01 mol) and anhydrous NaOAc (0.005 mol) in anhydrous glacial AcOH (50 mL), was refluxed for 3h. The reaction mixture was concentrated and then poured in to ice cold water,

the solid thus separated was filtered, washed with water and recrystallised from glacial AcOH to afford pure brown colour solid. Yield: 59.5%; m.p.206°-208°C; IR (KBr) ν in cm^{-1}; 3032.2 (Ar-H), 2946.73 (CH in CH_3), 1608.61 (C=N), 1099.07 (C-Cl), 1042.61 (N-N), 1665 (C=O), ^{1}H NMR (400MHz, DMSO-d6) δ: 7.22-7.90 (m, 11H, Ar-H), 7.13 (d, J=9.1Hz, 2H, Ar-H near Cl), 6.89(s, 1H, =CH), 5.13 (s, 1H, N-CH-Ar), 2.93 (s, 3H, CH_3); ^{13}C NMR: δ: 157.1 (C=O in ring), 135.5 (=CH), 131.9(=CH), 130.7(=CH), 129.5(=CH), 128.0(=CH), 127.7(=CH), 118.9 ((=CH)), 154.2, 152.1(C in thiadiazole ring), 69.9 (N-CH-S), 22.5 (CH_3).
Similarly other compounds **5b-j** was also synthesized and their characteristic analytical data are given below.

General procedure for the synthesis of 3-(4-Chloro-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (**6a):** A mixture of compound **5a** (0.01mol), $NH_2OH.HCl$ (0.01mol) and anhydrous NaOAc (0.005mol) in anhydrous AcOH (50 ml), was refluxed for 8h. The reaction mixture was concentrated and then poured in to ice cold water. The solid thus separated was filtered, washed with water and recrystallized from ethanol to afford pure compound **6a**; Yield 60%, m.p. 265°-267°C; IR (KBr) ν in cm^{-1}: 3028.2 (CH in Ar-H), 2948.21 (CH in CH_3), 1551.98 (C=N), 1094.16 (C-Cl), 918.14 (O-CH); ^{1}H NMR (400MHz, DMSO-d_6) δ: 7.35-7.78 (m, 11H, Ar-H), 7.27 (d, J=9.1Hz, 2H, Ar-H near Cl), 5.08 (s, 1H, N-CH-S), 4.98 (d, J=2.3Hz, 1H, CH-O), 4.11 (d, J=2.3Hz, 1H, CH-S), 2.68 (s, 3H, CH_3). ^{13}C NMR: δ: 153.4 (C=N in fused ring), 153.5, 151.6 (C in thiadiazole ring), 141.6 (=CH), 139.4 (=CH), 135.9(=CH), 131.2(=CH), 130.8(=CH), 127.7(=CH), 118.9(=CH), 66.1(N-CH-S), 53.1(CH-O), 53.7(CH), 25.6(CH_3).

Other compounds **6b-j** was also synthesized in the similar manner using compounds **5b-j**. Characterization data are given below.

3-(2-Chloro-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3,4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (6b): Yield 61.3%, m.p. 261°-263°C; IR (KBr) ν in cm^{-1}: 3028.2 (CH in Ar-H), 2948.21 (CH in CH_3), 1551.98 (C=N), 1089.71 (C-Cl), 918.14 (O-CH); ^{1}H NMR (400MHz, DMSO-d_6) δ: 7.35-7.82 (m, 11H, Ar-H), 7.18(d, J=9.1Hz, 2H, Ar-H near Cl), 5.17 (s, 1H, N-CH-S), 4.98 (d, J=2.3Hz, 1H, CH-O), 4.11 (d, J=2.3Hz, 1H, CH-S), 2.68 (s, 3H, CH_3). ^{13}C NMR: δ: 153.4 (C=N in fused ring), 153.2, 151.1 (C in thiadiazole ring), 140.8(=CH), 139.4(=CH), 135.9(=CH), 131.2(=CH), 130.8(=CH), 127.7(=CH), 118.9(=CH), 66.1(N-CH-S), 53.1(CH-O), 53.7(CH), 25.6(CH_3).

3-(4-Nitro-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3,4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro -2-oxa-4-thia-1,6-diaza-pentalene (**6c):** Yield 62 %, m.p.248°-249°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2955.21 (CH in CH_3), 1564.51 (C=N), 1541.18 (N-O), 921.27 (O-CH), ^{1}H NMR (400MHz, DMSO-d_6) δ: 8.10 (s, 1H, N-H), 7.68 (d, J=8.6Hz, 2H, Ar-H near NO_2), 6.90-7.46 (m, 11H, Ar-H), 5.19 (s, 1H, N-CH-S), 4.81 (d, J=2.2Hz, 1H, CH-O), 4.38(d, J=2.2Hz, 1H, CH-S), 2.78 (s, 3H, CH_3); ^{13}C NMR: δ: 158.3 (C=N in fused ring), 155.3, 154.2 (C in thiadiazole ring), 147.12(=CNO_2), 141.4(=CH), 138.7(=CH), 133.5(=CH), 132.5 (=CH), 129.1(=CH), 123.7(=CH), 119.1(=CH), 69.9 (N-CH-Ar), 52.26(CH), 51.8(CH-S), 25.6 (CH_3).

3-(4-Dimethylamino-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3,4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2H-pyrazolo-[3, 4-d]thiazole (6d): Yield 60.4%, m.p.271°-73°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2968.55, 2935.81 (CH in CH_3), 1558.54 (C=N), 1265.77 (C-N), 927.6 (CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.95-7.6 (m, 11H, Ar-H), 6.88 (d, J=9.1Hz, 2H, Ar-H near NMe_2), 5.47 (s, 1H, N-CH-S), 4.87 (d, J=2.3Hz, 1H, CH-O), 4.11(d, J=2.3Hz, 1H, CH-S), 2.95 (s, 6H, $N(CH_3)_2$), 2.73 (s, 3H, CH_3); ^{13}C NMR: δ: 156.7 (C=N in fused ring), 148.9 (=C-NMe_2), 150.2(=CH), 134.6(=CH), 132.2(=CH), 129.7(=CH), 128.6(=CH), 116.2(=CH), 115.9 (=CH), 155.9, 154.7 (C in thiadiazole ring), 68.1 (N-CH-S), 54.1(CH), 56.3(CH-O), 42.8(C in $N(CH_3)_2$), 29.8 (CH_3).

3-(3-Nitro-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (**6e):** Yield 62%, m.p.245°-48°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar),

2957.21 (CH in CH_3), 1550.7 (N-O), 1558.54 (C=N), 911.11 (CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ: 7.81 (d, J=8.3MHz, 2H, Ar-H near NO_2), 6.99-7.67 (m, 11H, Ar-H), 5.29 (s, 1H, N-CH-S), 4.98 (d, J=2.2MHz, 1H, CH-O), 4.49(d, J=2.2MHz, 1H, CH-S), 2.78 (s, 3H, CH_3); ^{13}C NMR: δ: 158.1 (C=N in fused ring), 154.5, 152.8 (C in thiadiazole ring), 146.4(=CH), 138.7(=CH), 133.5(=CH), 132.5 (=CH), 129.1(=CH), 123.7(=CH), 119.1(=CH), 68.9 (N-CH-Ar), 52.3(CH), 51.6(CH-N), 28.5 (CH_3).

3-(4-Methoxy-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (6f): Yield 61%, m.p.254°-56°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2957.21 (CH in CH_3), 1248.7 (C-O-C), 923.18 (CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.9-7.71 (m, 13H, Ar-H), 5.41 (s, 1H, N-CH-S), 4.91 (d, J=2.2Hz, 1H, CH-O), 4.49 (d, J=2.2Hz, 1H, CH-S), 3.81 (s, 3H, OCH_3), 2.78 (s, 3H, CH_3); ^{13}C NMR: δ: 157.9 (C=N in fused ring), 160.3 (=C-OMe), 158.7(=CH), 136.1 (=CH), 129.8 (=CH), 127.6 (=CH), 121.5 (=CH), 115.9 (=CH), 155.1, 153.6(C in thiadiazole ring), 68.9 (N-CH-Ar), 55.2 (OCH_3), 53.2(CH), 53.9(CH-O), 28.6 (CH_3).

3-(2-Methoxy-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3, 3a, 5, 6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (6g): Yield 59%, m.p. 238°-40°C; IR (KBr) ν in cm^{-1}; 3032.2 (CH in Ar), 2952.21 (CH in CH_3), 1248.7 (C-O-C), 923.71(CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ:6.97-7.84 (m, 13H, Ar-H), 5.43(s, 1H, N-CH-S), 4.98 (d, J=2.2Hz, 1H, CH-O), 4.49(d, J=2.2Hz, 1H, CH-S), 3.81 (s, 3H, OCH_3), 2.78 (s, 3H, CH_3); ^{13}C NMR: δ: 158.1 (C=N in fused ring), 160.3 (=COMe), 158.7(=CH), 136.1 (=CH), 129.8 (=CH), 127.6 (=CH), 121.5 (=CH), 115.9 (=CH), 155.1, 153.6(C in thiadiazole ring), 68.9 (N-CH-S), 55.2 (OCH_3), 53.2(CH), 53.9(CH-O), 28.6 (CH_3).

3-(4-Methyl-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3, 3a, 5, 6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (6h): Yield 62.8%, m.p.267°-68°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2971.55, 2935.81 (CH in CH_3), 1558.54 (C=N), 1265.77 (C-N), 927.6 (CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.91-7.53 (m, 11H, Ar-H), 6.91 (d, J=9.1Hz, 2H, Ar-H near NMe_2), 5.47 (s, 1H, N-CH-S), 4.87 (d, J=2.3Hz, 1H, CH-O), 4.14 (d, J=2.3Hz, 1H, CH-S), 2.82 (s, 3H, CH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR: δ: 156.7 (C=N in fused ring), 155.9, 154.7 (C in thiadiazole ring), 148.93 (=CH), 134.6 (=CH), 132.2 (=CH), 128.6 (=CH), 116.2(=CH), 115.9 (=CH), 68.1 (N-CH-Ar), 54.1(CH), 56.3(CH-O), 29.8 (CH_3).

3-(3,4,5-Tri-Methoxy-phenyl)-5-phenyl-6-(5-p-tolyl-[1,3,4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene (6i): Yield 59%, m.p. 246°-248°C; IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2957.21 (CH in CH_3), 1251.84 (C-O-C), 913.78 (CH-O), ^{1}H NMR (400MHz, DMSO-d_6) δ: 7.01-7.89 (m, 11H, Ar-H), 5.70 (s, 1H, N-CH-S), 4.86 (d, J=2.2Hz, 1H, CH-O), 4.32(d, J=2.2Hz, 1H, CH-S), 3.95 (s, 6H, OCH_3), 3.71 (s, 3H, OCH_3), 2.31(s, 3H, CH_3); ^{13}C NMR: δ: 158.1 (C=N in fused ring), 156.75 (=COMe), 158.7(=CH), 154.2, 152.9 (C in thiadiazole ring), 136.1 (=CH), 129.8 (=CH), 127.6 (=CH), 121.5 (=CH), 115.9 (=CH), 65.1 (N-CH-Ar), 54.88 (OCH_3), 53.2 (CH), 56.5 (CH-O), 23.9 (CH_3).

3-(4-Bromo-phenyl)-5-phenyl-6-(5-p-tolyl-[1, 3, 4]thiadiazol-2-yl)-3,3a,5,6-tetrahydro-2-oxa-4-thia-1,6-diaza-pentalene 6j: Yield 64%, m.p. 252°-54°C, IR (KBr) ν in cm^{-1}: 3032.2 (CH in Ar), 2957.21 (CH in CH_3), 917.8 (O-CH), ^{1}H NMR (400MHz, DMSO-d_6) δ: 6.87-7.68 (m, 13H, Ar-H), 5.68 (s, 1H, N-CH-S), 4.65 (d, J=2.2Hz, 1H, CH-O), 4.45(d, J=2.2Hz, 1H, CH-S), 2.59 (s, 3H, CH_3): ^{13}C NMR: δ: 158.3(C=N in fused ring), 155.3, 152.9 (C in thiadiazole ring), 141.3(=CH), 139.4(=CH), 135.9(=CH), 131.2(=CH), 130.8(=CH), 127.7(=CH), 118.9(=CH), 67.3 (N-CH-Ar), 52.8(CH), 53.7(CH-O), 25.7 (CH_3).

Pharmacology:

Antimicrobial activity: The newly prepared compounds **6a-j** were screened for their antimicrobial activity. The preliminary activity was determined by disc diffusion method. Because of the limitation in disc diffusion method the activity of the compounds at lower concentrations have been tested using broth dilution technique with the help of spectrophotometer. In this work *Bacillus subtilis*, *Bacillus*

thuringiensis, Escherichia coli and *Pseudomonas aeruginosa* bacterial strains were used, and *Candida albicans, Botrytis fabae and Fusarium oxysporam* fungal strains were used. The compounds were dissolved in DMSO at concentration of $1mgmL^{-1}$. The antibacterial activity of each compound was compared with the standard drug Streptomycin and antifungal activity with Treflucan.

Acknowledgments

The authors are grateful to The Head, Department of Chemistry Dr. H. S. Gour University Sagar for providing necessary facilities to carry out this work. We sincerely express our gratitude to Head, Biotechnology laboratory, Department of Botany, Dr. H. S. Gour University Sagar for providing antimicrobial data. We are grateful to The Director, SAIF, Panjab University for providing spectral data and The Director Micro care laboratory Surat, for providing antitubercular data.

References

[1] Batra, S.; Roy, A. K.; Patra, A.; Bhaduri, A. P.; Surin, W. R.; Raghavan, S. A. V.; Sharma, P.; Kapoor, K.; Dikshit, M. Baylis–Hillman reaction assisted parallel synthesis of 3,5-disubstitu- ted isoxazoles and their in-vivo bioevaluation as antithrombotic agents. *Bioorg. Med. Chem.* **2004,** *12*, 2059-2077.

[2] Diana, P.; Carbone, A.; Barraja, P.; Kelter, G.; Fiebig, H. H.; Cirrincione, G. Synthesis and antitumor activity of 2,5-bis(30-indolyl)-furans and 3,5- bis(30-indolyl)-isoxazoles nortopsentin analogues, *Bioorg. Med. Chem*. **2010,** *18*, 4524–4529.

[3] Karthikeyan, K.; Seelam, T. V.; Lalitha, K. G.; Perumal, P. T. Synthesis and antinociceptive activity of pyrazolyl isoxazolines and pyrazolyl Isoxazoles, *Bioorg. Med. Chem. Lett.* **2009,** *19*, 3370–3373.

[4] Mazzei, M.; Sottofattori, E.; Dondero, R.; Ibrahim, M.; Melloni, E.; Michetti, M. *N*,*N*-Dialkyl amino substituted chromones and isoxazoles as potential anti-inflammatory agents. *Il Farmaco*. **1999**, *54*, 452-460.

[5] Padmaja, A.; Payani, T.; Dinneswara Reddy, G.; Padmavathi, V. Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives. *Eur. J. Med. Chem.* **2009**, *44*, 4557–4566.

[6] Pae, A. N.; Kim, H. Y.; Joo, H. J.; Kim, B. H.; Cho, Y. S.; Choi, K.; Choi, J. H.; Koh, H. Y. Synthesis and in vitro activity of new oxazolidinone antibacterial agents having substituted isoxazoles. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2679-2684.

[7] Santos, M. M. M.; Faria, N.; Iley, J.; Coles, S. J.; Hursthouse, M. B.; Martins, M. L.; Moreira, R. Reaction of naphthoquinones with substituted nitromethanes. Facile synthesis and antifungal activity of naphtho[2,3-d]isoxazole-4,9-diones. *Bioorg. Med.Chem. Lett.* **2010**, *20*, 193-195.

[8] Avula, S.; Adki, N.; Cherkupally, S. R. Synthesis and in-vitro study of methylene-bis-tetra hydro [1,3] thiazolo [4,5-c] isoxazoles as potential nematicidal agents. *Eur. J. Med. Chemistry*. **2010**, *45*, 2353-2358.

[9] Desai, J. T.; Desai, C. K.; Desai, K. R. A Convenient, Rapid and Eco-Friendly Synthesis of Isoxazoline heterocyclic Moiety Containing Bridge at 2°-Amine as Potential Pharmacological Agent. *J. Iran. Chem. Soc.* **2008**, *5*, 67-73.

[10] Lee, Y. S.; Park, S. M.; Kim, B. H. Synthesis of 5-isoxazol-5-yl-2′-deoxyuridines exhibiting antiviral activity against HSV and several RNA viruses. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 1126-1128.

[11] Conti, P.; Dollanoce, C.; Amici, M. De.; Micheli, C. De.; DK.-N. Klotz, D. K. N Synthesis of new Δ^2-isoxazoline derivatives and their pharmacological characterization as β-adrenergic receptor antagonists. *Bioorg. Med. Chem.* **1998**, *6*, 401-408.

[12] Andrew, S.; Susan, M.; Michael, F.; Mathew, W. Penny.; Paul, L.; Anne, G..; Chris, B.; John, M.; Donald, T.; Dennis, S.; Tesfaye, B. Synthesis and biological activity of anticoccidial agents: 5,6-Diarylimidazo[2,1-*b*][1,3]thiazoles. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5263-5267.

[13] Kumar, Y.; Green, R.; Boryska, K. Z.; Wise, D. D.; Worting, L. L.; Townsend, L. B. Synthesis of 2,4-disubstituted thiazoles and selenazoles as potential antitumor and antifilarial agents.1.Methyl 4-isothiocyanatomethyl)thiazole-2-carbamates-selenazole-2-carbamates and related derivatives. *J. Med. Chem.* **1993**, *36*, 3843-3848.

[14] Jung, K. Y.; Kim, S. K.; Gao, Z. G.; Ariel, S. G.; Neil, M.; Denneth, A. J.; Kim, Y. C. Structure activity relationships of thiazole and thiadiazole derivatives as potent and selective human adenosine A_3 receptor antagonists. *Bioorg. Med. Chem.* **2004**, *12*, 613-623.

[15] Andrean, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M. Synthesis and antitubercular activity of imidazo [2, 1-b] thiazoles. *Eur. J. Med. Chem.* **2001**, *36*, 743- 746.

[16] Aridoss, G.; Amirthaganesan, S.; Kim, M. S.; Kim, J. T.; Jeong, Y. T. Synthesis, spectral and biological evaluation of some new thiazolidinones and thiazoles based on t-3-alkyl-r- 2, C-6-diaryl piperidin-4-ones. *Eur. J. Med. Chem.* **2009**, *44*, 4199-4210.

[17] Bell, F. M.; Cantrell, A. S.; Hoegberg, M.; Jaskunas, S. R.; Johansson, N. G.; Jordan, C. L.; Kinnick, M. D.; Lind, P.; Mortin Jr, J. M. Phenethyl thiazolethiourea (PETT) Compounds, a New Class of HIV-1 Reverse Transcriptase Inhibitors. 1. Synthesis and Basic Structure-Activity Relationship Studies of PETT Analogs. *J. Med. Chem.* **1995**, *38*, 4929-4936.

[18] Bankar, G. R.; Nandakumar, K.; Nayak, P. G.; Thakur, A.; Mallikarjuna, R. C.; Nampurath, G. K. Vasorelaxant effect in rat aortic rings through calcium channel blockage: A preliminary invitroassessment of a 1,3,4-oxadiazole derivative. *Chem. Biol. Interactions.* **2009,** *181*, 377-382.

[19] Bhati, S. K.; Ashok K. Synthesis of new substituted azetidinoyl and thiazolidinoyl-1,3,4-thiadiazino (6,5-b) indoles as promising anti-inflammatory agents. *Eur. J. Med. Chem.* **2008**, *43*, 2323-2330.

[20] Kolavi, G.; Hegde, V.; Khazi, I. A.; Gadad, P. Synthesis and evaluation of antitubercular activity of imidazo [2,1-b][1,3,4]thiadiazole derivatives. *Bioorg. Med. Chem.* **2006,** *14*, 3069-3080.

[21] Kucukguzel, S. G.; Kucukguzel, I.; Tatar, E.; Rollas, S.; Sxahin, F.; Gulluce, M.; Clercq, E. De.; Kabasakal, L. Synthesis of some novel heterocyclic compounds derived from diflunisal hydrazide as potential anti-infective and anti inflammatory agents. *Eur. J. Med. Chem.* **2007,** *42*, 893-901.

[22] Palekar, V. S.; Damle, A. J.; Shukla, S. R. Synthesis and antibacterial activity of some novel bis-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazoles and bis-4-thiazolidinone derivatives from terephthalic dihydrazide. *Eur. J. Med. Chem.* **2009,** *44*, 5112-5116.

[23] Varvaresou, A.; Siatra-Papastaikoudi, T.; Tsotinis, A.; Tsantili-kakoulidou, A.; Vamvakides, A. Synthesis, lipophilicity and biological evaluation of indole-containing derivatives of 1,3,4-thiadiazole and 1,2,4-triazole. *IL. Farmaco.* **1998,** *53*, 320-326.

[24] Matysiak, J. Evaluation of electronic, lipophilic and membrane affinity effects on antiproliferative activity of 5-substituted-2-(2, 4-dihydroxyphenyl)-1,3,4-thiadiazoles against various human cancer cells. *Eur. J. Med. Chem.* **2007**, *42*, 940-947.