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Synthesis, characterization and antimicrobial activity of novel substituted aryl-1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine derivatives

Deepak Kumar Basedia[1*], Birendra Shrivastava[2], B. K. Dubey[1], Pankaj Sharma[2]

*Corresponding author:

Deepak Kumar Basedia

1. T.I.T. College of Pharmacy, Anand Nagar, BHEL, Bhopal, M.P., India.
2. School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.

Abstract

Bioactive heterocyclic rings 1,3,4-oxadiazole and 1,3,5-triazine are fused with expectation of enhanced biological activity of the newly synthesized compounds. Hence Synthesized fused heterocyclic compounds as a substituted aryl- 1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine derivatives

The structures of all the compounds were confirmed by physical and spectral analysis. The newly synthesized compounds were evaluated for antimicrobial activity against a variety of bacterial strains and fungal strains. Some of these compounds have shown significant antibacterial and antifungal activity.

Keywords: 1,3,5-triazine, 1,3,4-Oxadiazole, 1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine, Schiff's base, Antimicrobial activity

Introduction

1,3,5-triazines are amongst the oldest known organic molecules; originally they were called the symmetric triazines usually abbreviated as S- or Syn-triazines. Some of the substituted 1,3,5-s-triazine have reported to possess interested biological activities. A wide range of 1,3,5-triazines exhibit selective herbicidal properties, Simazine and atrazines are the organic compounds containing s-triazine skeleton are most important herbicides.[1] Triazines derivative Altretamine and triethylene melamine (TEM) shown activity against leukemia. Compounds contain 1,3,5-triazine as a lead moiety possessing a wide spectrum of biological activities such as anti-cancer[2-6], antiviral [7], bactericidal [8-14], fungicidal[15-17], antimalarial agents[18-20] and anti-tuberculosis[21]. In addition, the interest in 1,3,4-oxadiazole also one of important heterocyclic compound show significant biological activity such as antibacterial and fungicidal.[22-25]

In view of these observations and in continuation of our work synthesized novel compounds 1,3,4-oxadiazolo[3,2-a]-s-triazine derivatives (3a-3n and 4a-4n) by bridging of both bioactive heterocyclic rings as fused heterocycles with expectation of enhanced biological activity of the molecule. Hence we have carried out synthesis, characterization and antimicrobial activities of title compounds which are new and are not reported so far elsewhere.

Required semicarbazones were prepared by using the reported method as a reaction between aldehyde and semicarbazide (Vogel's,1996). Starting compound 2-amino-5-phenyl-1,3,4-oxadiazole were synthesized by using Semicarbazone (0.01 M) and sodium acetate (0.02 M) were dissolved in 30–40 ml of glacial acetic acid taken in a round-bottomed flask equipped with a separating funnel for the addition of bromine. Bromine (0.7 ml in 5ml glacial acetic acid) was added slowly to it, while stirring magnetically. After half an hour stirring, the solution was poured on crushed ice. The resulting solid was separated, dried and recrystallized from ethanol (Vogel's, 1996). Respective Schiff's base were synthesized reaction between 2-amino-5-phenyl-1,3,4-oxadiazole and aromatic aldehyde.[26] The present study report the synthesis of various substituted aryl-1,3,4-oxadiazole-[3,2-a]-1,3,5-triazine derivatives (Scheme-1 and Scheme-2). The compounds synthesized were characterized by using their spectral data. (IR, NMR and Mass) All synthesized molecules are evaluated to in-vitro antimicrobial activities against various microbial strains.

Experimental Section

Melting points were determined in open capillary tubes and were found uncorrected. The temperatures were expressed in ºC. TLC was performed to monitor the progress of reactions. All compounds were purified by recrystallization with suitable organic solvents. The Purity of all the compounds was checked by thin layer chromatography (TLC) on Precoated silica gel-G. Iodine chamber and UV lamp were used for the visualization of TLC spots.

All the Fourier transform infra red (FTIR) spectra were recorded in KBr pellets on a Perkin Elmer spectrometer. The ^{1}H-NMR spectra were taken on Bruker Avance-400 MHz NMR spectrometer. Chemical shifts are expressed in parts per million relative to

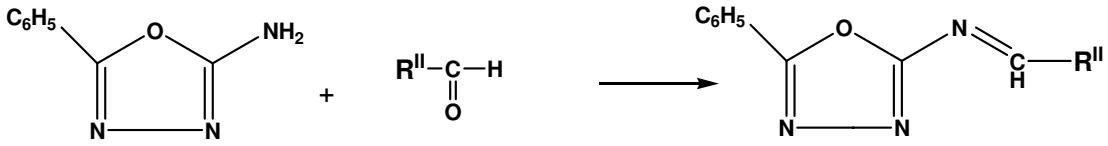
tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on LC-MSD-Tranp-SL2010A Shimadzu.

Chemistry

Synthesis of Schiff's base: A solution of 2-amino-5-phenyl-1,3,4-oxadiazole (0.01 M) was prepared in 30 ml alcohol in a round-bottomed flask. Benzaldehyde (0.01 M) then added to it. The mixture was refluxed for 5–6 h. The volume of alcohol was reduced to half by distillation under reduced pressure. The resulting solution was poured on crushed ice. The precipitate which got separated was dried and recrystallized from alcohol. (compound-1)

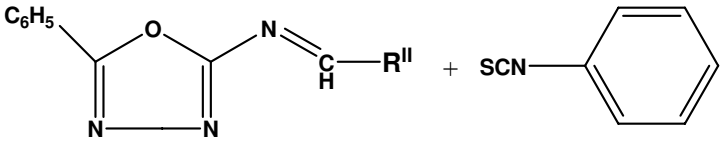
Synthesis of 7-substituted aryl-2,6-diphenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a][1,3,5]-triazine : A mixture of Schiff's base (compound-1) (0.01 mol) and phenylisothiocynate (0.01 mol) was refluxed for 7 hr in dry toluene. The solvent was distillation under reduced pressure. The residue obtained was filter, wash a small amount of ethanol followed by water and purified by recrystallized from ethanol.[27] (3a-3n) (Scheme-1)

I st STEP :



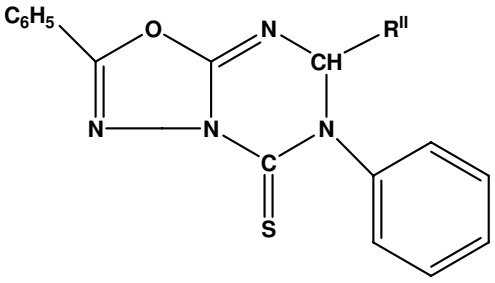
2-amino-5-phenyl-1,3,4-oxadiazole **Various aromatic aldehyde** **Schiffs base**

II nd STEP :



Schiffs base **Phenylisothiocynate**

Reflux-7 hr **Dry Toluene**



7- substituted aryl-2,6-diphenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a][1,3,5]-triazine

SCHEME -1

2,6,7-triphenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3a):

M.P.-185ºC, IR(KBr): 3124.47(=C-H str.), 2948.96(C- H str.), 1446.51(C=C str.), 1610.45(C=N str.), 1076.21(C-O-C str.), 1278.72(N-N=C str.) and 1174.57(C=S str.). ^{1}H-NMR(DMSO, ppm): 3.71(s, 1H, N-CH-N), 7.41–8.22(m, 15H, Ar-H), MS: m/z-384.3

2,6-diphenyl-7-(2-hydroxyphenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3b):

M.P.-265ºC, IR(KBr): 3467.77(O-H str.), 3103.25(=C-H str.), 2943.17(C- H str.), 1448.44 (C=C str.), 1696.95(C=N str.), 1018.34(C-O-C str.), 1222.79(N-N=C str.) and 1151.42 (C=S str.). ^{1}H-NMR(DMSO, ppm): 5.47(s, 1H, OH), 3.52(s, 1H, N-CH-N), 6.93–8.173(m, 14H, Ar-H), MS: m/z-400

2,6-diphenyl-7-(3-hydroxyphenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3c):

M.P.-254ºC, IR(KBr): 3317.34(O-H str.), 2952.81(=C-H str.), 2891.10(C- H str.), 1442.66 (C=C str.), 1647.10(C=N str.), 1107.06(C-O-C str.), 1245.93(N-N=C str.) and 1168.78 (C=S str.). MS: m/z-400.2

2,6-diphenyl-7-(4-hydroxyphenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3d):

M.P.-228ºC, IR(KBr): 3427.27(O-H str.), 2975.96(=C-H str.), 2876.74(C- H str.), 1465.80 (C=C str.), 1604.66(C=N str.), 1054.99(C-O-C str.), 1284.55(N-N=C str.) and 1207.36(C=S str.). MS: m/z-400.1

2,6-diphenyl-7-(2-nitrophenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3e):

M.P.-220ºC, IR(KBr): 3132.18(=C-H str.), 2950.89(C-H str.), 1446.51(C=C str.), 1610.45(C=N str.), 1074.57(C-O-C str.), 1278.72(N-N=C str.), 1571.88(C-NO_2 str.) and 1188.07(C=S str.). ^{1}H-NMR (DMSO, ppm): 3.9(s, 1H, N-CH-N), 7.19-7.83(m, 14H, Ar-H), MS: m/z-429.4

2,6-diphenyl-7-(3-nitrophenyl)-[1,3,4]oxadiazolo[3,2-*a*][1,3,5]triazine-5-thione(3f):

M.P.- 195ºC, IR(KBr): 2954.74(=C-H str.), 2912.31(C- H str.), 1452.30(C=C str.), 1618.17 (C=N str.), 1058.92(C-O-C str.), 1255.57(N-N=C str.), 1348.05(C-NO_2 str.) and 1207.36 (C=S str.). MS: m/z-429.2

2,6-diphenyl-7-(4-nitrophenyl)-[1,3,4]oxadiazolo[3,2-*a*][1,3,5]triazine-5-thione(3g):

M.P.- 234ºC, IR(KBr): 2948.42(C- H str.), 1479.30(C=C str.), 1639.38(C=N str.), 1051.31 (C-O-C str.), 1253.64(N-N=C str.), 1566.09(C-NO_2 str.) and 1163.00(C=S str.). MS: m/z-429.2

2,6-diphenyl-7-(2-chlorophenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3h):

M.P.-247ºC, IR(KBr): 3052.11(=C-H str.), 2941.24(C- H str.), 1458.08(C=C str.), 1664.45 (C=N str.), 1070.42(C-O-C str.), 1232.43(N-N=C str.), 1151.42(C=S str.) and 775.33(C-Cl str.). ^{1}H-NMR(DMSO, ppm): 4.13(s, 1H, N-CH-N), 7.15 – 7.97(m, 14H, Ar-H), MS: m/z-418.1

2,6-diphenyl-7-(4-chlorophenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3i):

M.P.- 174ºC, IR(KBr): 3015.70(=C-H str.), 2955.34 (C- H str.), 1454.23 (C=C str.), 1674.10 (C=N str.), 1151.42(C-O-C str.), 1271.00(N-N=C str.), 1191.93(C=S str.) and 742.54(C-Cl str.). MS: m/z-418.2

2,6-diphenyl-7-(4-dimethylaminophenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3j):

M.P.- 217ºC, IR(KBr):2991.39(=C-H str.), 2839.02(C- H str.), 1433.01(C=C str.), 1654.81(C=N str.), 1074.28(C-O-C str.), 1232.43(N-N=C str.) and 1201.57(C=S str.). ^{1}H-NMR(DMSO, ppm): 3.96(s, 1H, N-CH-N), 2.88(s, 6H, $2CH_3$), 7.24 – 7.98(m, 14H, Ar-H), MS: m/z-427.2

2,6-diphenyl-7-(3,4,5-trimethoxyphenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3k):

M.P.- 284ºC, IR(KBr): 3001.03(=C-H str.), 2839.02(C- H str.), 1442.66(C=C str.), 1622.02 (C=N str.), 1016.42(C-O-C str.), 1238.21(N-N=C str.) and 1188.07(C=S str.). MS: m/z-474.5

2,6-diphenyl-7-(4-methoxy phenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3l):

M.P.- 158ºC, (KBr): 2985.67(=C-H str.), 2870.95(C-H str.), 1471.59(C=C str.), 1596.95(C=N str.), 1105.14(C-O-C str.), 1271.00(N-N=C str.) and 1213.14(C=S str.). MS: m/z-414.2

2,6-diphenyl-7-(4-hydroxy-3-methoxyphenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3m):

M.P.- 205ºC, IR(KBr): 3355.91(O-H str.), 2977.89(=C-H str.), 2837.09(C-H str.), 1448.94(C=C str.), 1693.38(C=N str.), 1054.99(C-O-C str.), 1276.79(N-N=C str.) and 1203.50(C=S str.). MS: m/z-430.2

2,6-diphenyl-7-(2,5-dichlorophenyl)-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(3n):

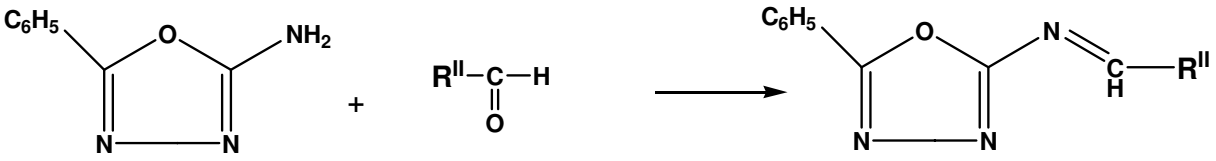
M.P.-189ºC, IR(KBr): 2998.25(=C-H str.), 2888.67(C-H str.), 1433.01(C=C str.), 1625.88(C=N str.), 1008.70(C-O-C str.),

1274.86(N-N=C str.), 1147.57(C=S str.) and 746.40(C-Cl str.). MS: m/z-452.6

Synthesis of 7-substituted aryl-2-phenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a][1,3,5]-triazine:

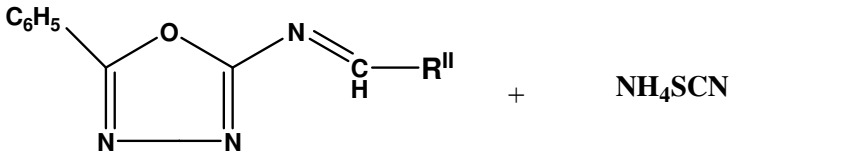
A mixture of Schiff's base (compound-1) (0.01 mol) and ammonium thiocyanate (0.01 mol) were dissolved in dioxin (20 ml) by slow warming and shaking. The reaction mixture was stirred for 30 min at room temperature and subsequently refluxed for 5 hr. The solvent was distillation under reduced pressure. The residue obtained was wash with water, dried and recrystallized from ethanol.[28] (4a-4n) (Scheme-2)

I st STEP :



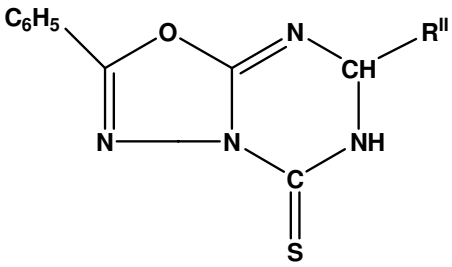
2-amino-5-phenyl-1,3,4-oxadiazole **Various aromatic aldehyde** **Schiffs base**

II nd STEP :



Schiffs base **Ammonium thiocyanate**

Refluxed 5 hr. **Dioxin**



7-substituted aryl-2-phenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a][1,3,5]-triazine

SCHEME -2:

2,7-diphenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4a):

M.P.-135ºC, IR(KBr): 3211.72(N-H str.), 3110.95(=C-H str.), 2938.47(C- H str.), 1452.39 (C=C str.), 1597.85(C=N str.), 1069.19(C-O-C str.), 1243.75(N-N=C str.) and 1189.86(C=S str). ^{1}H-NMR(DMSO, ppm): 2.14(s, 1H, NH), 3.81(s, 1H, CH), 7.14–7.81(m, 10H, Ar-H), MS: m/z-308.2

7-(2-hydroxyphenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4b):

M.P.-152ºC, IR(KBr): 3410.22(O-H str.), 3038.05(N-H str.), 2903.59(C- H str.), 1445.26(C=C str.), 1659.13(C=N str.), 1075.56(C-O-C str.), 1259.94(N-N=C str.) and 1202.31(C=S str). ^{1}H-NMR(DMSO, ppm): 2.57(s, 1H, NH), 5.37(s, 1H, OH), 3.77(s, 1H, CH), 6.96-7.79(m, 9H, Ar-H), MS: m/z-324.4

7-(3-hydroxyphenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4c):

M.P.-169ºC, IR(KBr): 3423.00(O-H str.), 3158.93(=C-H str.), 2933.34(C- H str.), 1477.37 (C=C str.), 1670.41(C=N str.), 1133.34(C-O-C str.), 1241.42(N-N=C str.) and 1153.82(C=S str). MS: m/z-324.1

7-(4-hydroxyphenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4d):

M.P.-174ºC , IR(KBr): 3313.59(O-H str.), 3062.14(=C-H str.), 2916.06(C- H str.), 1433.91 (C=C str.), 1676.12(C=N str.), 1026.16(C-O-C str.), 1242.34(N-N=C str.) and 1237.82(C=S str). MS: m/z-324.2

7-(2-nitrophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4e):

M.P.-187ºC, IR(KBr): 3332.47(N-H str.), 3092.27(=C-H str.), 2849.06(C- H str.), 1345.43(C=Cstr.), 1667.22(C=N str.), 1097.62(C-O-C str.), 1265.91(N-N=Cstr.), 1533.45(C-NO_2 str.) and 1103.36(C=S str).^{1}H-NMR(DMSO, ppm): 2.24(s, 1H, NH), 3.48 (s, 1H, CH), 6.81 – 7.94(m, 9H, Ar-H), MS: m/z-353.4

7-(3-nitrophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4f):

M.P.-195ºC, IR(KBr): 3407.07(N-H str.), 3108.09(=C-H str.), 2905.12(C- H str.), 1458.52 (C=C str.), 1596.15(C=N str.), 1093.60(C-O-C str.), 1250.04(N-N=C str.), 1546.13(C-NO_2 str.) and 1208.08(C=S str).MS: m/z-353.2

7-(4-nitrophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4g):

M.P.-207ºC, IR(KBr): 3401.74(N-H str.), 3030.48(C- H str.), 1490.09(C=C str.), 1643.26 (C=N str.), 1071.10(C-O-C str.), 1238.36(N-N=C str.), 1596.02(C-NO_2 str.) and 1177.93 (C=S str). MS: m/z-353.1

7-(2-chlorophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4h):

M.P.-163ºC, IR(KBr): 3311.64(N-H str.), 2917.71(C- H str.), 1480.82(C=C str.), 1688.48(C=N str.), 1109.57(C-O-C str.), 1258.80(N-N=C str.), 769.42(C-Cl str.) and 1167.77(C=S str). ^{1}H-NMR(DMSO, ppm): 2.09(s, 1H, NH), 3.53(s, 1H, CH), 7.10 – 7.79(m, 9H, Ar-H), MS: m/z-342.5

7-(4-chlorophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4i):

M.P.-186ºC, IR(KBr): 3416.90(N-H str.), 3136.90(C- H str.), 1445.38(C=C str.), 1661.74(C=N str.), 1064.01(C-O-C str.), 1265.91(N-N=C str.), 727.22(C-Cl str.) and 1233.28(C=S str). MS: m/z-342.3

7-(4-dimethylaminophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4j):

M.P.-180ºC, IR(KBr): 3456.23(N-H str.), 2921.10(=C-H str.), 2850.09(C- H str.), 1466.95 (C=C str.), 1672.31(C=N str.), 1104.83(C-O-C str.), 1381.41(N-N=C str.) and 1172.13(C=S str). ^{1}H-NMR(DMSO, ppm): 2.25(s, 1H, NH), 2.80(s , 6H, $2CH_3$), 6.49-7.43(m, 9H, Ar-H), MS: m/z-351.1

7-(3,4,5-trimethoxyphenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4k):

M.P.-225ºC, IR(KBr): 3303.83(N-H str.), 2917.74(C- H str.), 1454.73(C=C str.), 1697.98(C=N str.), 1093.52(C-O-C str.), 1242.16(N-N=C str.) and 1172.00(C=S str). MS: m/z-398.2

7-(4-methoxyphenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4l):

M.P.-160ºC, IR(KBr): 3250.67(N-H str.), 2917.00(C- H str.), 1452.35(C=C str.), 1692.50 (C=N str.), 1043.23(C-O-C str.), 1291.18(N-N=C str.) and 1243.33(C=S str) MS: m/z-338.1

7-(4-hydroxy-3-methoxyphenyl)-2-diphenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4m):

M.P.-214ºC, IR(KBr): 3371.72(O-H str.), 3312.73(N-H str.), 3078.85(C- H str.), 1505.37(C=C str.), 1689.84(C=N str.), 1038.12(C-O-C str.), 1270.29(N-N=C str.) and 1158.14 (C=S str). MS: m/z-354.2

7-(2,5-dichlorophenyl)-2-phenyl-[1,3,4]oxadiazolo[3,2-a][1,3,5]triazine-5-thione(4n):

M.P.-220ºC, IR(KBr): 3366.22(N-H str.), 3113.59(=C-H str.), 2936.90(C-H str.), 1550.28(C=C str.), 1647.93(C=N str.),

1047.94(C-O-C str.), 1237.90(N-N=C str.), 1127.64(C=S str) and 799.34(C-Cl str.), MS: m/z-377.3

Antimicrobial activity

All the newly synthesized compounds (3a-3n) and (4a-4n) were evaluated for *in vitro* Antimicrobial activity against gram positive and gram negative bacterial strains such *Escherichia coli* (Gram -ve), *Klebsiella pneumonia* (Gram – ve), *Staphylococcus aureus* (Gram +ve), *Bacillus subtilis* (Gram +ve) and fungal strains *Aspergillus niger* at three different concentration 100 μg/ml, 200 μg/ml and 300 μg/ml by Cup-Plate method and nutrient agar was employed as culture media(beef extract 3 gm, Agar 15 g, Peptic Digest of Animal Tissue 5 g, sodium chloride 5 g and distilled water-q.s. to 1,000 ml) was employed as culture media for antimicrobial activity. The sterilization of the nutrient broth, culture tubes, pipette and other glassware was done by autoclaving. For antibacterial studies, incubation was carried out at 37 C for 24 hr and for antifungal studies, incubation was carried out at 25±2ºc for 72 hr. The zone of inhibition was measured in mm.[29-30] The activity was compared with known antibiotic Ciprofloxacin and Ketoconazole as standards for antibacterial and antifungal studies respectively and zone of inhibition were measured for all synthesized compounds.

Results and discussion

The present study report the synthesis of novel substituted aryl-1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine derivatives. The first step involve synthesized the Schiff's base as reaction between 2-amino-5-phenyl-1,3,4-oxadiazole and aromatic aldehyde.

In Step -2: synthesized 7-substituted aryl -2,6-diphenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a] [1,3,5]-triazine (Scheme-1) and 7-substituted aryl-2-phenyl-5-thioxo-[1,3,4]-oxadiazolo-[3,2-a][1,3,5]-triazine (Scheme-2). Series of compounds 3a-3n and 4a-4n synthesized and reported.

The synthesized compounds were recrystallized and identified by TLC. The melting point were found uncorrected. The difference in the R_f value and melting point show the change in the structure between the molecules. All Physical data of the compounds are recorded in table-01 and table-02

IR Spectra were recorded in KBr pellets on Perkin Elmer FT-IR instrument. All the compounds show aromatic C-H stretching between 3124.2 to 2952.8 cm^{-1}, aliphatic C-H stretching between 2948.9 to 2876.7 cm^{-1}, C=N in ring between 1647.1 -1597.8 cm^{-1} and C-O-C stretching between 1075.5- 1018.3 cm^{-1} presence of cyclic ring system in [1,3,4] oxadiazoline. All the compounds show C=C stretching vibration at 1479.3-1452.3 cm^{-1} and C=S stretching vibration at 1189.11-1151.4 cm^{-1} . Compound 3a-3n show stretching vibration between 3467.7 to 3317.3 the presence of 0-H group. Compounds 4a-4n show the presence of N-H stretching vibration in amine between 3432.1 to 3410.2 cm^{-1},

In compound 3j and 4j show C-H str. due to CH_3 at 2839 and 2917cm^{-1}. Compounds 3e, 3f, 3g and Compounds 4e, 4f, 4g show characteristic C-NO_2 stretching vibration between 1571.8 – 1560.3 cm^{-1} and 1596 – 1533.4 cm^{-1} respectively may be due to nitro group in compounds. Compound 3h, 3i, 3n and 4h, 4i, 4n show C-Cl stretching vibration at 775.3 – 742.5 cm^{-1} and 769.4 - 727 cm^{-1} presence of chlorine substitution in respective compounds .

^{1}NMR was recorded on Bruker Avance-400 MHz NMR spectrometer chemical shift was measured at part per million downfield from tetra methyl silane. Compounds 4a-4n show sharp singlet near 2.09 to 2.57 might be due to NH proton. All compounds show multiple between 7.15 to 7.94 showed the presence of aromatic proton (Ar-H). The singlet formed between 5.37 to 5.37 might be due to presence of OH in compounds 3b and 4b respectively. Compound 3j and 4j show sharp singlet at 2.88 and 2.80 respectively due to presence of CH_3 in dimethylamine. All compounds show sharp singlet near 3.48 to 3.93 might be due to CH proton in C-7 position in ring.

Mass spectra were recorded on LC-MSD-Trap-SL which show characteristic molecular ion and base peak and further confirmed the compounds.

Antimicrobial activity of synthesized compounds was evaluated by cup-plate method. All the synthesized compounds show a moderate biological activity. Compound 3c, 3h, 3m and 4c, 4k, 4m showed very good activities against the entire test microorganism. Increase the antibacterial activity of compounds due to presence of hydroxyl substitution in the *m*-position (3c and 4c) and compounds 3m, 4m show higher antibacterial activity against all the test microorganisms due to 4-hydroxy-3-methoxy substitution. 2-Cholro and 3,4,5-trimethoxy substituted group also show good antibacterial agent (3h and 4k). All synthesized compounds are active against gram positive and negative microorganism. Compound 3c, 3h, 3m and 4c, 4k, 4m has highest antibacterial activity against all the test microorganisms.

All synthesized compounds show antifungal against fungal strains *Aspergillus niger.* The compound 3c, 3k, 3f, 4a and 4c are most active and show better significant antifungal activity. Antimicrobial activity of synthesized compounds are recorded in table-03, table-04 and table-05

Table-01: Physical data compounds (3a-3n)

S. No.	Compound code	Molecular formula	Molecular weight	Melting point ^{0}C	R_f value	% yield	Appearance
1.	3a	$C_{22}H_{16}N_4OS$	384.45	185^{o}C	0.71	77	Light Yellow
2.	3b	$C_{22}H_{16}N_4O_2S$	400.45	265^{o}C	0.80	69	Brown
3.	3c	$C_{22}H_{16}N_4O_2S$	400.45	254^{o}C	0.69	81	Yellow
4.	3d	$C_{22}H_{16}N_4O_2S$	400.45	228^{o}C	0.78	78	Yellow
5.	3e	$C_{22}H_{15}N_5O_3S$	429.45	220^{o}C	0.73	65	Brown
6.	3f	$C_{22}H_{15}N_5O_3S$	429.45	195^{o}C	0.77	80	Yellow
7.	3g	$C_{22}H_{15}N_5O_3S$	429.45	234^{o}C	0.83	82	Light brown
8.	3h	$C_{22}H_{15}ClN_4OS$	418.89	247^{o}C	0.66	60	White
9.	3i	$C_{22}H_{15}ClN_4OS$	418.89	174^{o}C	0.74	80	Yellow
10.	3j	$C_{24}H_{21}N_5OS$	427.52	217^{o}C	0.82	73	Dark brown
11.	3k	$C_{25}H_{22}N_4O_4S$	474.53	284^{o}C	0.63	78	Yellow
12.	3l	$C_{23}H_{18}N_4O_2S$	414.47	158^{o}C	0.75	70	Light green
13.	3m	$C_{23}H_{18}N_4O_3S$	430.47	205^{o}C	0.60	75	White
14.	3n	$C_{22}H_{14}Cl_2N_4OS$	452.34	189^{o}C	0.64	83	Yellow

Table-02: Physical data compounds (4a-4n)

S. No.	Compound code	Molecular formula	Molecular weight	Melting point ^{0}C	R_f value	% yield	Appearance
1.	4a	$C_{16}H_{12}N_4OS$	308.36	135^{o}C	0.76	70	Yellow
2.	4b	$C_{16}H_{12}N_4O_2S$	324.36	152^{o}C	0.72	82	Light brown
3.	4c	$C_{16}H_{12}N_4O_2S$	324.36	169^{o}C	0.59	86	White
4.	4d	$C_{16}H_{12}N_4O_2S$	324.36	174^{o}C	0.81	63	Yellow
5.	4e	$C_{16}H_{11}N_5O_3S$	353.36	187^{o}C	0.66	74	Dark brown
6.	4f	$C_{16}H_{11}N_5O_3S$	353.36	195^{o}C	0.62	85	Light Yellow
7.	4g	$C_{16}H_{11}N_5O_3S$	353.36	207^{o}C	0.73	65	White
8.	4h	$C_{16}H_{11}ClN_4OS$	342.80	163^{o}C	0.76	68	Light Yellow
9.	4i	$C_{16}H_{11}ClN_4OS$	342.80	186^{o}C	0.66	72	Light brown
10.	4j	$C_{18}H_{17}N_5OS$	351.43	180^{o}C	0.74	69	White
11.	4k	$C_{19}H_{18}N_4O_4S$	398.44	225^{o}C	0.71	78	Yellow
12.	4l	$C_{17}H_{14}N_4O_2S$	338.38	160^{o}C	0.65	73	Yellow
13.	4m	$C_{17}H_{14}N_4O_3S$	354.38	214^{o}C	0.77	80	Light brown
14.	4n	$C_{16}H_{10}C_{l2}N_4OS$	377.25	220^{o}C	0.64	69	White

Table-03 : Antimicrobial Activities of Compounds (3a-3n)

Compound Code	Diameter of Zone of Inhibition in mm											
	E.coli			*K.pneumoniae*			*S.aureus*			*B.subtilis*		
	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml
3a	12	13	15	12	13	15	12	13	15	11	13	15
3b	13	15	17	13	15	17	13	15	17	12	14	15
3c	14	16	19	16	18	20	15	17	19	13	15	17
3d	13	14	15	12	13	15	12	14	15	12	14	15
3e	10	12	13	13	15	17	10	12	14	10	12	14
3f	11	13	15	13	15	17	13	14	15	11	12	13
3g	13	15	17	11	13	15	11	13	15	11	13	15
3h	14	15	17	14	16	18	14	16	18	13	15	18
3i	11	13	15	13	15	17	13	15	17	13	14	15
3j	12	13	15	13	14	16	10	13	15	12	13	15
3k	13	15	17	12	13	15	11	13	15	10	11	13
3l	13	14	16	13	15	17	13	15	17	12	13	14
3m	15	17	19	15	17	20	14	17	19	13	15	17
3n	13	15	17	13	15	17	13	14	16	12	13	15
Std (10µg/ml)	22	22	22	23	23	23	21	21	21	20	20	20

Table-04 : Antimicrobial Activities of Compounds (4a-4n)

Compound Code	Diameter of Zone of Inhibition in mm											
	E.coli			*K.pneumoniae*			*S.aureus*			*B.subtilis*		
	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml
4a	12	14	16	12	14	16	12	14	16	11	13	15
4b	12	14	17	13	15	17	12	13	14	12	14	16
4c	14	16	18	16	17	19	14	16	18	14	16	18
4d	12	14	16	12	14	16	13	14	15	13	14	16
4e	13	15	17	12	13	15	12	13	14	12	13	14
4f	12	14	16	11	13	15	11	13	15	12	14	16
4g	13	14	16	12	14	16	12	14	16	11	13	15
4h	13	15	17	14	15	17	13	14	15	13	15	16
4i	12	13	15	14	15	17	12	14	16	12	13	14
4j	13	15	17	11	13	15	13	15	17	12	14	16
4k	15	17	19	15	17	19	15	17	19	14	15	17
4l	12	13	15	12	14	16	13	14	15	12	13	14
4m	14	16	18	16	17	20	14	16	18	14	16	18
4n	12	14	16	13	14	15	13	15	17	11	13	15
Std (10µg/ml)	22	22	22	23	23	23	21	21	21	20	20	20

Table-05 : Antifungal Activity of the Compounds Against *Aspergillus niger*

Concentration in µg/ml	Diameter of the Inhibition Zone (mm)														
	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	3m	3n	Std 10µg/ml
100	12	14	15	12	13	16	11	10	12	14	16	12	14	12	24
200	14	16	18	13	15	18	13	12	14	15	18	14	15	15	24
300	15	18	20	15	17	21	15	14	16	17	20	16	17	18	24
Concentration in µg/ml	Diameter of the Inhibition Zone (mm)														
	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	Std 10µg/ml
100	16	12	16	13	12	13	12	13	12	13	12	14	12	13	24
200	18	14	19	15	13	15	14	14	14	15	14	16	13	14	24
300	20	16	21	16	16	17	15	15	16	18	15	18	16	15	24

Conclusion

A series of novel fused heterocyclic compounds 1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine derivatives has been successfully synthesized by bridging between 1,3,5-triazine nucleus which is one of the active lead present in many standard drugs and bioactive heterocyclic rings 1,3,4-oxadiazole. Hence, it is concluded that 1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine derivatives more active against all representative panel of bacterial and fungal strains and thus, there is enough scope for further study in developing such compounds as a good bioactive molecules.

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