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Synthesis, characterization, antimicrobial and antioxidant activity of some disubstituted [1,3,4]-oxadiazoles carrying 4-(methylsulfonyl/sulfinyl)benzyl moieties

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ABSTRACT

*Disubstituted [1,3,4]-oxadaiazoles (**6a-b**), Mannich bases (**7a-f**) and S-alkylated derivatives (**8a-f**) have been synthesized from 4-(methylsulfonyl/sulfinyl)phenylaceticacid (**3a-b**) through a multi-step reaction sequence starting from (4-methylthio)phenylacetonitrile (**1**). The structures of new compounds were established on the basis of their elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds were screened for their antioxidant, antibacterial and antifungal activity. Some of the derivatives have promising antimicrobial activity.*

Key words: [1,3,4]-Oxadaiazoles; antioxidant; antimicrobial activity; [1,3,4]-Oxadiazol-2-thiones; 4-(methyl sulfonyl/sulfinyl)benzyl moiety.

INTRODUCTION

[1,3,4]-Oxadiazoles constitute an important family of heterocyclic compounds as they have attracted significant interest in medicinal chemistry, pesticide chemistry and polymer science. Since many of [1,3,4]-oxadiazoles display a remarkable biological activity, their syntheses and transformations have been received particular interest for a long time. The [1,3,4]-oxadiazoles have been found to exhibit diverse biological activities such as antimicrobial [1-4], antitubercular [5], anti oxidant [6], antimalarial [7], analgesic [8], antiinflammatory [9,10], anticonvulsant [11], hypoglycemic [12] and other biological properties such as genotoxic [13] and lipid peroxidation inhibitory activities [14]. 5-substituted-1,3,4-oxadiazole-2-thiones possess CNS depressant [15], pesticidal [16,17] tyrosinase inhibition [18] property. We therefore are interested in exploring the biological activity of such molecules through structural modifications. In view of these observations, we hereby report syntheses, characterization and antimicrobial activity of some disubstituted oxadiazoles.

EXPERIMENTAL SECTION

Melting points were determined by the open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 4100 type A spectrophotometer. ^{1}H NMR and ^{13}C spectra were recorded on a Varian 400 MHz NMR spectrometer/Perkin-Elmer EM300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on a MDS SCIEX/API 4000 spectrophotometer. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). The progress of the reaction was monitored by thin layer chromatography (TLC) on pre-coated silica gel G plates.

Preparation of 4-(methylsulfonyl)phenylacetonitrile (2a) from (4-methylthio)phenylacetonitrile (1)
4-Methylthiophenyl acetonitrile (**1**) (0.1 mol) was dissolved in 3 volume of acetic acid and cooled to 5 °C. To the reaction mixture, 0.02 mol of sodium tungstate was added followed by 0.2 mol of 30% hydrogen peroxide which was diluted in 1.2 volume of acetic acid and water mixture (in 2:1 ratio). After the addition of hydrogen peroxide solution, the temperature of the reaction mixture was slowly brought to RT. The completion of reaction was monitored by TLC. The solid precipitated was filtered and washed with water until the pH of the fitrate was neutral. The product was dried at 65 °C for 10-12 h. The product was recrystallized from methanol.

Colourless solid, yield=92 %, mp =120-124°C. IR (KBr γ (cm^{-1}): 2255 (CN), 3075 (Ar-H), 1315 (S=O), 1145 (S=O). ^{1}H NMR (400 mHz, CD_3OD) δ = 7.88-7.90 (d, 2H, *J*= 8.0, Ar-H), 7.55-7.57 (d, 2H, *J*= 8.0, Ar-H), 3.75 (s, 2H, CH_2), 3.10 (s, 3H, CH_3); MS, m/z (%): 195(M^+).

Preparation of 4-(methylsulfinyl)phenylacetonitrile (2b) from (4-methylthio)phenylacetonitrile (1)
4-Methyl thio phenylacetonitrile (**1**) (0.1 mol) was dissolved in 3 volume of acetic acid and cooled to 5 °C. To the reaction mixture 0.02 mol of sodium tungstate was added followed by 0.2 mol of 30% hydrogen peroxide which was diluted in 1.2 volume of acetic acid and water mixture (in 2:1 ratio). After the addition of hydrogen peroxide solution, the temperature of the reaction mixture was slowly brought to RT. The completion of reaction was monitored by TLC. The solid precipitated was filtered and washed with water until the pH of the fitrate was neutral. The product was dried at 65 °C for 10-12 h. The product was recrystallized from methanol.

Colourless solid, yield = 95 %: mp =72-74 °C. IR (KBr γ (cm^{-1}), 3072 (Ar-H), 2950, 2895 (C-H), 2252 (CN), 1178 (S=O). ^{1}H NMR (400 mHz, CD_3OD) δ = 7.87-7.89 (d, 1H, *J*= 8.0, Ar-H), 7.52-7.54 (d, 1H, *J*= 8.0, Ar-H), 3.74 (s, 2H, CH_2), 3.10 (s, 3H, CH_3).

Procedure for the synthesis of 4-(methylsulfonyl/sulfinyl)phenylaceticacid, (3a-b)
4-(Methylsulphonyl/sulfinyl)phenyl acetonitrile (**2a-b**) (0.1mol) was taken in 5 volume of water. To this potassium hydroxide (0.25mol) was added and heated to 98 °C. The reaction mass was refluxed for 2 h. The completion of reaction was monitored by TLC. The reaction was cooled to 25-28 °C. The impurities were removed by Toluene wash. The aq layer was acidified to pH=2 using Conc HCl. The solid thus precipitated was filtered and washed with water until the filtrate was neutral. The product was dried at 65 °C for 10-12 h. The product was re-crystallized from methanol.

4-(Methylsulfonyl)phenylaceticacid, (3a)
Colourless solid (95 %). mp =142-144 °C. IR (KBr γ (cm^{-1}): 3369 (OH), 2960 (Ar-H), 2860 (C-H), 1693 (C=O), 1588 (C=C), 1407 (S=O), 1140 (S=O); ^{1}H NMR (400 MHz, CD_3OD): δ = 3.11 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 7.54 (d, 2H, *J* = 8.0, Ar-H*)*, 7.89 (d, 2H, *J* = 8.0, Ar-H*)*. ^{13}C NMR (100 MHz, CD_3OD): δ = 40.164, 43.06, 127.139, 130.33, 139.176, 141.29, 172.91. DEPT (100 MHz, CD_3OD): δ = CH_3: 43.06, CH_2: -40.16, 127.14, 130.32. MS, m/z (%): 214 (M^+). Anal. Calcd. (%) for $C_9H_{10}O_4S$: C, 50.46, H, 4.7; Found C, 50.44, H, 4.5.

4-(Methylsulfinyl)phenylaceticacid, (3b)
Colourless solid (90 %). mp =118-120 °C. IR (KBr γ (cm^{-1}): 3399 (O-H), 2986 (Ar-H), 2858 (C-H), 1698 (C=O), 1537 (C=C), 1186 (S=O). ^{1}H NMR (400 MHz, CD_3OD): δ = 2.77 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 7.49 (d, 2H, *J* = 8.0, Ar-H), 7.64 (d, 2H, *J* = 8.0, Ar-H). ^{13}C NMR(100 MHz, CD_3OD): δ = 40.16, 42.14, 123.65, 130.46, 138.82, 143.01, 173.29, DEPT (100 MHz, CD_3OD): δ = CH_3: 42.16, CH_2: -40.19, 123.66, 130.45; MS, m/z (%): 198 (M+1). Anal. Calcd. (%) for $C_9H_{10}O_3S$: C, 54.53, H, 5.08; Found C, 54.50, H, 5.06.

Procedure for the synthesis of ethyl-4-(methylsulfonyl/sulfinyl)phenylacetate, (4a-b)
The above esters were prepared by refluxing 4-(methylsulfonyl/sulfinyl)phenylaceticacid (**3a-b**) in excess absolute ethanol in the presence of few drops of conc. sulphuric acid and excess ethanol was distilled off and quenched to water. Aq layer was basified to neutral pH using 5% sodium bicarbonate solution. The product was extracted from aqueous layer using ethyl acetate. The solvent was distilled off. The compounds were obtained as low melting white solids.

4-(Methylsulfonyl)phenylacetate, 4a
IR (KBr γ (cm^{-1}): 3014 (Ar-H), 1691(C=O), 1407 (S=O), 1138 (S=O). Anal. Calcd. (%) for $C_{11}H_{14}O_4S$: C, 54.53, H, 5.82; Found C, 54.50, H, 5.80.

Procedure for the synthesis of 4-(methylsulfonyl/sulfinyl) acetohydrazide (5a-b)
A mixture of ethyl-4-(methylsulfonyl/sulfinil)phenylacetate (**4a-b**) (0.1mol), hydrazine hydrate (0.15mol) and 20ml of ethanol was refluxed on an oil bath for 10 h. The excess solvent was then distilled off under reduced

pressure and the concentrated solution was quenched to ice cold water. The solid separated was filtered, washed and dried. The crude product was purified by re-crystallization from ethanol.

4-(Methylsulfonyl)acetohydrazide (5a)
Colourless solid (89 %); mp =118-120 °C; IR (KBr γ (cm^{-1}): 3340, 3240 (NH), 3035 (Ar-H), 2921 (C-H), 1654 (C=O), 1598 (C=C), 1409 (S=O), 1145 (S=O).

Synthesis of 5-[4-(methylsulfonyl/sulfinyl)benzyl-[1,3,4]-oxadiazole-2(3H)-thione, (6a-b)
A mixture of 4-(methylsulfonyl/sulfinyl)acetohydrazides (**5a-b**) (0.1mol), 5 volume of methanol, carbon disulphide (0.2mol) and potassium hydroxide solution (30%, 5mL) was refluxed on a water bath for 2 h. The reaction mixture was cooled, quenched to ice cold water and acidified with acetic acid. The product separated was filtered, washed with water and dried. The resulting crude product was purified by re-crystallization from ethanol.

5-[4-(Methylsulfonyl)benzyl-[1,3,4]-oxadiazole-2(3H)-thione, (6a)
Colourless solid (85%). mp =162-167 °C. IR (KBr γ (cm^{-1}): 3194 (NH), 2978 (Ar-H), 2907 (Ar-H), 1669 (C=N), 1612 (C=C), 1410 (S=O), 1284 (C=S), 1138 (S=O). ^{1}H NMR (400 MHz, CDCl3): δ = 3.12 (s, 3H, CH_3), 4.87 (s, 2H, CH_2), 7.60 (d, 2H, *J* = 8.0Hz, Ar-H), 7.94 (d, 2H, *J* = 8.0 Hz, Ar-H), 11.30 (s, 1H, NH). ^{13}C (100 MHz, CDCl3): δ = 31.0, 42.95, 127.62, 129.9, 139.68, 140.06, 162.07, 179.23. DEPT (100 MHz, CDCl3): δ = CH_3, CH: 42.95, 127.62, 129.90; CH_2: -31.0; MS, m/z (%): 271 (M^+). Anal. Calcd. (%) for $C_{10}H_{10}N_2O_3S_2$: C, 44.43, H, 3.73, N, 10.36; Found C, 44.42, H, 3.71, N, 10.33.

5-[4-(Methylsulfinyl)benzyl-[1,3,4]-oxadiazole-2(3H)-thione, (6b)
Colourless solid (82%). mp =163.165 °C. IR (KBr γ (cm^{-1}): 3190 (NH), 2970 (Ar-H), 2900 (C-H), 1680 (C=N), 1609 (C=C), 1307 (C=S), 1154 (S=O). ^{1}H NMR (400 MHz, CDCl3): δ = 2.78 (s, 3H, SO-CH_3), 4.16 (s, 2H, CH_2), 7.54 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.68 (d, 2H, *J* = 8.0 Hz, Ar-H) 11.28 (s, 1H, NH); ^{13}C NMR (100 MHz, CDCl3): δ = 30.96, 42.17, 124.14, 130.03, 137.18, 144.10, 162.41, 179.25; DEPT (100 MHz, CDCl3): δ = CH_3, CH: 42.18, 124.15, 130.05; CH_2: -30.97; MS, m/z (%): 254 (M^+). Anal. Calcd. (%) for $C_{10}H_{10}N_2O_2S_2$: C, 47.23, H, 3.96, N, 11.01; Found C, 47.20, H, 3.94, N, 11.00.

Procedure for the synthesis of Mannich bases, (7a-f)
5-[4-(methylsulfonyl/sulfinyl)benzyl-[1,3,4]-oxadiazole-2(*3H*)-thione (**6a-b**) (10mmol) was dissolved in methanol, then 40% formaldehyde (1.5mL) and secondary amine (10mmol) in methanol were added under stirring. The resulting mixture was stirred overnight at room temperature. The precipitated solid was filtered, washed with water and re-crystallized from methanol to yield the title compounds (**7a-f**).

3-[(4-Methylpiperazin-1-yl)methyl]-5-[4-(methylsulfonyl)benzyl]-[1,3,4]-oxadiazole-2(3H)-thione, (7a)
IR (KBr γ (cm^{-1}): 2988 (Ar-H), 2935 (C-H), 1622 (C=N), 1579 (C=C), 1458 (S=O), 1325 (C=S), 1153 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.63 (s, 3H, N-CH3), 2.77-2.79 (t, 4H, *J* = 4.0, piperazino), 3.10 (s, 3H, SO_2-CH_3), 3.88-3.90 (t, 4H, *J* = 4.0, piperazino), 4.15 (s, 2H, CH_2), 5.12 (s, 2H, N-CH_2-N), 7.54-7.56 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.97-7.99 (d, 2H, *J* = 8.0 Hz, Ar-H). Anal. Calcd. (%) for $C_{16}H_{22}N_4O_3S_2$: C, 50.24, H, 5.80, N, 14.65; Found C, 50.22, H, 5.78, N, 14.63.

5-[4-(Methylsulfonyl)benzyl]-3-(morpholin-4-ylmethyl)-[1,3,4]-oxadiazole-2(3H)-thione, (7c)
IR IR (KBr γ (cm^{-1}): 2971 (Ar-H), 2938 (C-H), 1620 (C=N), 1580 (C=C), 1453 (S=O), 1324 (C=S), 1149 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.78-2.80 (t, 4H, *J* = 4.0, morpholino), 3.09 (s, 3H, CH_3), 3.70-3.72 (t, 4H, *J* = 4.0, morpholino), 4.13 (s, 2H, CH_2), 4.96 (s, 2H, N-CH_2-N), 7.53 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.96 (d, 2H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.97, 44.53, 50.50, 66.74, 70.34, 128.3, 130.05, 138.36, 140.43, 159.31, 178.84. DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 44.53, 128.31, 130.05, CH_2: -31.96, -50.50, -66.73, -70.33; MS, m/z (%): 3 7 0 . 8 (M^+). Anal. Calcd. (%) for $C_{15}H_{19}N_3O_4S_2$: C, 48.72, H, 5.15, N, 11.36; Found C, 48.76, H, 5.18, N, 11.37.

3-[(4-Methylpiperazin-1-yl)methyl]-5-[4-(methylsulfinyl)benzyl]-[1,3,4]-oxadiazole-2(3H)-thione, (7d)
IR (KBr γ (cm^{-1}): 3050 (Ar-H), 2908 (C-H), 1620 (C=N), 1568 (C=C), 1300 (C=S), 1150 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.62 (s, 3H, N-CH_3), 2.74-2.76 (t, 4H, *J* = 4.0, piperazino), 2.78 (s, 3H, SO-CH_3), 3.85-3.87 (t, 4H, *J* = 4.0, piperazine), 4.11(s, 2H, CH_2), 5.10 (s, 2H, N-CH_2-N), 7.50 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.95 (d, 2H, *J* = 8.0 Hz, Ar-H); Anal. Calcd. (%) for $C_{16}H_{22}N_4O_2S_2$: C, 52.43, H, 6.05, N, 15.29; Found C, 52.40, H, 6.03, N, 15.25.

5-[4-(Methylsulfinyl)benzyl]-3-(morpholin-4-ylmethyl)-[1,3,4]-oxadiazole-2(3H)-thione, (7f)
IR (KBr γ (cm^{-1}): 2937 (Ar-H), 2909 (C-H), 1627 (C=N), 1560 (C=C), 1325 (C=S), 1157 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.75 (s, 3H, CH_3), 2.78-2.80 (t, 4H, *J* = 4.0 Hz, morpholino), 3.70-3.72 (t, 4H, *J* = 4.0 Hz, morpholino), 4.09 (s, 2H, CH_2), 4.96 (s, 2H, N-CH_2-N), 7.48 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.66 (d, 2H, *J* = 8.0 Hz, Ar-H,). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.88, 43.99, 50.50, 66.74, 70.28, 124.41, 129.99, 135.39, 145.71, 159.82, 178.85. DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 43.99, 124.41, 129.99, CH_2: -31.88, -50.50, -66.74, -70.27; MS, m/z (%): 3 5 4 . 3 (M^+). Anal. Calcd. (%) for $C_{15}H_{19}N_3O_3S_2$: C, 50.95, H, 5.42, N, 11.89; Found C, 50.94, H, 5.40, N, 11.85.

Procedure for the synthesis of 2-thioalkyl-5-[4-(methylsulfonyl/sulfinyl)benzyl]-[1,3,4]-oxadiazoles, (8a-f)
5-[4-(methylsulfonyl/sulfinyl)benzyl-[1,3,4]-oxadiazole-2(*3H*)-thione (**6a-b**) (1mmol), alkyl chloride (1mmol) and potassium carbonate (5mmol) in acetone were stirred for 10 h at 58 °C. The completion of reaction was monitored by thin layer chromatography. After completion of the reaction, the reaction mixture was concentrated under reduced pressure and quenched to water. The precipitated solid was filtered and dried to afford the title compound. The crude product was purified by crystallization using ethanol or methanol as solvents.

2-(Ethylthio)-5-[4-(methylsulfonyl)benzyl]-[1,3,4]-oxadiazole, (8b)
IR (KBr γ (cm^{-1}): 2966 (Ar-H), 2823 (C-H), 1676 (C=N), 1572 (C=C), 1479 (S=O), 1292 (C-O-C), 1143 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43-1.46 (t, 3H, CH_3), 3.043 (s, 3H, SO_2-CH_3), 3.20 (q, 2H, S-CH_2), 4.26 (s, 2H, Ar-CH_2), 7.50 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.91 (d, 2H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.65, 26.95, 31.72, 44.54, 128.11, 129.96, 139.9, 140.01, 164.94. DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 14.65, 44.54, 128.11, 129.96, CH_2: -26.96, -31.73; MS, m/z (%): 3 0 0 . 1 (M^+). Anal. Calcd. (%) for $C_{12}H_{14}N_2O_3S_2$: C, 48.30, H, 4.73, N, 9.39; Found C, 48.29, H, 4.70, N, 9.35.

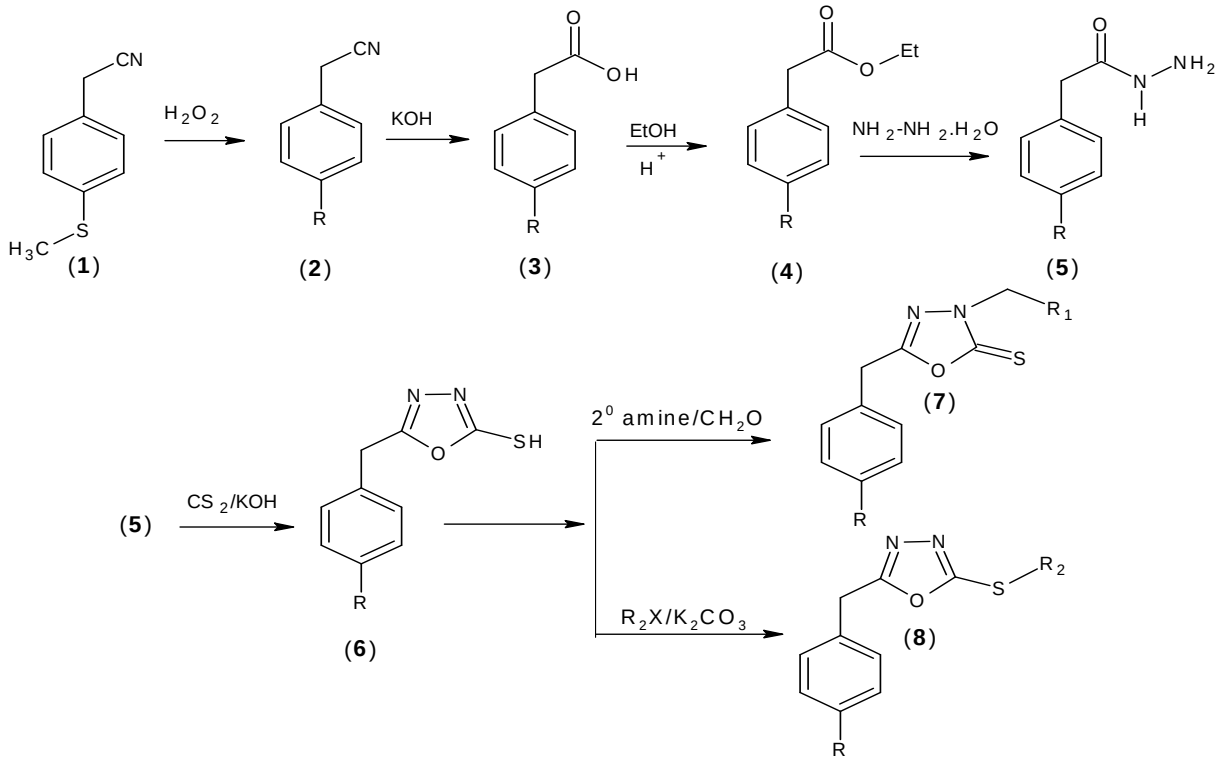
2-[4-(Methylsulfonyl)benzyl]-5-(propylthio)-[1,3,4]-oxadiazole, (8c)
IR (KBr γ (cm^{-1}): 2966 (Ar-H), 2923 (C-H), 1625 (C=N), 1568 (C=C), 1479 (S=O), 1292 (C-O), 1143 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.02-1.06 (t, 3H, *J* = 8.0 Hz, CH_3), 1.79 (m, 2H, CH_2), 3.052 (s, 3H, SO_2-CH_3), 3.18-3.22 (t, 2H, S-CH_2), 4.26 (s, 2H, Ar-CH_2), 7.51 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.92 (d, 2H, *J* = 8.0 Hz, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.18, 22.63, 31.74, 34.43, 44.55, 128.13, 129.96, 139.91, 140.02, 164.89. DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 13.18, 44.55, 128.13, 129.96, CH_2: -22.63, -31.74, -34.43; MS, m/z (%): 3 1 3 . 2 (M^+). Anal. Calcd. (%) for $C_{13}H_{16}N_2O_3S_2$: C, 49.98, H, 5.16, N, 8.97; Found C, 49.93, H, 5.14, N, 8.99.

2-(Ethylthio)-5-[4-(methylsulfinyl)benzyl]-[1,3,4]-oxadiazole, (8e)
IR (KBr γ (cm^{-1}): 2970 (Ar-H), 2933 (C-H), 1676 (C=N), 1578 (C=C), 1263 (C-O-C), 1145 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40-1.44 (t, 3H, CH_3), 2.69 (s, 3H, SO-CH_3), 3.17 (q, 2H, *J* = 8.0 Hz, S-CH_2), 4.20 (s, 2H, Ar-CH_2), 7.44 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.59 (d, 2H, *J* = 8.0, Ar-HHz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.66, 26.92, 31.62, 43.99, 124.2, 129.91, 136.91, 145.22, 165.15, 165.40. DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 14.67, 44.0, 124.21, 129.92, CH_2: -26.93, -31.63. MS, m/z (%): 2 8 3 . 1 (M^+). Anal. Calcd. (%) for $C_{12}H_{14}N_2O_2S_2$: C, 51.04, H, 5.00, N, 9.92; Found C, 51.02, H, 5.00, N, 9.93.

2-[4-(Methylsulfinyl)benzyl]-5-(propylthio)-[1,3,4]-oxadiazole, (8f)
IR (KBr γ (cm^{-1}): 2930 (Ar-H), 2935 (C-H), 1626 (C=N), 1575 (C=C), 1130 (S=O). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.00-1.04 (t, 3H, *J* = 8.0 Hz, CH_3), 1.77 (m, 2H, CH_2), 2.70 (s, 3H, SO-CH_3), 3.16-3.20 (t, 2H, *J*= 8.0 Hz, *S*-CH_2), 4.21 (s, 2H, Ar-CH_2), 7.45 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.60 (d, 2H, *J* = 8.0 Hz, Ar-H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.17, 22.64, 31.64, 34.4, 43.99, 124.21, 129.92, 136.94, 145.21, 165.39, DEPT (100 MHz, $CDCl_3$): δ = CH and CH_3: 13.18, 44.00, 124.22, 129.93, CH_2: -22.65, -31.65, -34.41; MS, m/z (%): 2 9 7 . 3 (M^+); Anal. Calcd. (%) for $C_{13}H_{16}N_2O_2S_2$: C, 52.68, H, 5.44, N, 9.45; Found C, 52.66, H, 5.40, N, 9.46.

RESULTS AND DISCUSSION

The target compounds (**6a-b**), (**7a-f**), (**8a-f**) were synthesized as illustrated in **Scheme 1**. The 4-methylthio phenylacetonitrile (**1**) was oxidized to 4-(methylsulfonyl/sulfinyl)phenylacetonitrile (**2a-b**). This on hydrolysis (**3a-b**) followed by esterification (**4a-b**) and treatment with hydrazinehydrate gave acid hydrazides (**5a-b**). The reaction of the acid hydrazides with carbon disulphide under basic conditions yielded [1,3,4]-oxadiazol-2-thiones (**6a-b**), which underwent N-aminomethylation reaction on treating with formaldehyde and secondary amines to afford the compounds (**7a-f**). Further the alkylation of (**6a-b**) yielded s-alkylated products (**8a-f**). All the compounds were characterized by analytical and spectroscopic (IR, ^{1}H NMR, ^{13}C NMR and Mass) data. The characterization data of the newly synthesized compounds are given in **Table 1**.



R= SO_2CH_3, $SOCH_3$

R_1= N-Methylpiperazino,Piperidino,Morpholino R_2= CH_3, -CH_2-CH_3, CH_2-CH_2-CH_3

Scheme 1 Formation of 1,3,4-oxadiazole derivatives

IR spectrum of 5-[4-(methylsulfonyl)benzyl-[1,3,4]-oxadiazole-2(3H)-thione, **6a** showed absorption bands at 3194 cm^{-1} for (NH), 1612 cm^{-1} for (C=N), 1410 cm^{-1} for (S=O), 1284 cm^{-1} for (C=S), 1138 cm^{-1} for (S=O). The 400 MHz ^{1}H NMR spectrum of **6a** showed the down-field broad singlets at δ11.30 for the NH proton indicating the formation by simple cyclo condensation process. The compound **6a** also showed prominent singlets at δ3.12 and δ4.87 for its CH_3 and CH_2 protons respectively. The four protons of 4-methylsulfonylphenyl moiety appeared as two doublets at δ7.60 and 7.94. The 100 MHz ^{13}C NMR spectrum of **6a** showed characteristic signals at δ31.00, 42.95, 127.62, 129.90, 139.68, 140.06, 162.07 and 179.23 thereby accounting for the presence of ten different carbon atoms in the molecule. Further, MS spectrum of **6a** showed a (M+1) peak at *m/z* 271 corresponding to its molecular formula, $C_{10}H_{10}N_2O_3S_2$.

Formations of the Mannich bases, 3-substituted-5-(4-(methylsulfonyl/sulfinyl)benzyl)-[1,3,4]-oxadiazole-2(3*H*)-thiones (**7**) were also confirmed by their IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data.

The IR spectrum of Mannich base, 5-[4-(methylsulfonyl)benzyl]-3-(morpholin-4-ylmethyl)-[1,3,4]-oxadiazole-2(3*H*)-thione, **7c** showed the absence of absorption bands corresponding to the (NH) group of the parent oxadiazole. It showed absorption bands at 1620 cm^{-1} for (C=N), 1453 cm^{-1} for (S=O), 1324 cm^{-1} for (C=S) and 1149 cm^{-1} for (S=O) stretching vibrations. The 400 MHz ^{1}H NMR spectrum **7c** showed the signals corresponding to the NH proton was absent and a new singlet for N-CH_2-N was observed at δ4.96, thus confirming the aminomethylation. It also showed prominent singlets at δ3.09 and δ4.13 for its SCH_3 and CH_2 protons respectively. Two characteristic triplets at δ2.78 and δ 3.70 each integrating for four protons were due to the methylene protons of the morpholine ring. The four protons of 4-methylsulfonylphenyl moiety appeared as two doublets at δ 7.53 and 7.96. The 100 MHz ^{13}C NMR spectrum of compound **7c** showed characteristic signals at δ 31.97, 44.53, 50.50, 66.74, 70.34, 128.3, 130.05, 138.36, 140.43, 159.31 and 178.84. The mass spectrum of **7c** showed a molecular ion (M+1) peak at *m/z* 370.8, consistent with its molecular formula $C_{15}H_{19}N_3O_4S_2$.

IR spectrum of compound, 2-(ethylthio)-5-[4-(methylsulfonyl)benzyl]-[1,3,4]-oxadiazole, **8b** showed absorption bands at 2966 and 2823 cm^{-1} for its aromatic and aliphatic (C-H) stretching vibrations. The (C=N) and (C-O) have showed their characteristic absorption bands at 1676 and 1292 cm^{-1} respectively. The 400 MHz ^{1}H NMR spectrum **8b** showed a sharp singlet at δ3.04 in the ^{1}H NMR spectrum was assigned for ring SCH_3 protons. Another triplet observed at δ1.43 integrating for CH_3 of ethyl group. The CH_2 protons of ethyl group resonated as a quartet at δ

3.20. The two hydrogen atoms of the aryl methylene group were appeared as a singlet at δ 4.26. The aromatic protons of 4-(methylsulfonyl) phenyl appeared as two distinct doublets at δ 7.50 (J = 8.0 Hz) and δ 7.91 (J = 8.0 Hz). Further, ^{13}C NMR spectrum of **8b** manifested signals at δ 14.66 (CH_3 of ethyl), 26.96 (CH_2 of ethyl), 31.73 (CH_2 of benzyl), 44.55 (SCH_3), C-S carbon appeared at δ 165.38 and δ 164.94 was due to (C-O). The peaks at δ 128.11, 129.96, 139.9 and 140.01 were due to the aromatic carbon atoms. Further, Mass spectrum of **8b** showed the molecular ion peak at m/z 300 (M+1), which corresponds to its molecular formula $C_{12}H_{14}N_2O_3S_2$.

Table 1 Characterization data of [1,3,4]-oxadiazoles (7a-f) and (8a-f)

Sample	$R/R_1/R_2$	Mol Formula	Mol wt	M.P. (°C)	Yield (%)	Analysis (%) calculated (Found)		
						C	H	N
2a	R= SO_2-CH_3	$C_9H_9NO_2$ S	195.23	120-124	92	55.37 (55.33)	4.65 (4.62)	7.17 (7.12)
2b	R= SO-CH_3	C_9H_9NO S	179.23	72-74	95	60.31 (60.30)	5.06 (5.04)	7.81 (7.78)
3a	R= SO_2-CH_3	$C_9H_{10}NO_4$ S	214.23	142-144	95	50.46 (50.44)	4.7 (4.65)	- -
3b	R= SO-CH_3	$C_9H_{10}NO_3$ S	198.23	118-120	90	54.53 (54.50)	5.08 (5.02)	- -
5a	R= SO_2-CH_3	$C_9H_{12}N_2O_3$ S	228.26	118-120	88	47.35 (47.33)	5.3 (5.2)	12.27 (12.2)
5b	R= SO-CH_3	$C_9H_{12}N_2O_2$ S	212.26	98-100	89	50.92 (50.90)	5.7 (5.66)	13.2 (13.0)
6a	R= SO_2-CH_3	$C_{10}H_{10}N_2O_3$ S_2	270.32	162-167	85	44.40 (44.43)	3.70 (3.73)	10.33 (10.36)
6b	R= SO-CH_3	$C_{10}H_{10}N_2O_2$ S_2	254.32	163-165	82	47.25 (47.28)	3.95 (3.96)	11.00 (11.01)
7a	R_1= N-Methylpiperazino	$C_{16}H_{22}N_4O_3$ S_2	382.5	135-137	92	50.25 (50.24)	5.76 (5.80)	14.62 (14.65)
7b	R_1= piperidino	$C_{16}H_{21}N_3O_3$ S_2	367.48	130-136	90	52.25 (50.29)	5.76 (5.76)	11.42 (11.43)
7c	R_1= N-Methylmorpholino	$C_{15}H_{19}N_3O_4$ S_2	369.45	128-130	90	48.72 (48.76)	5.15 (5.18)	11.36 (11.37)
7d	R_1= N-Methyl piperazino	$C_{16}H_{22}N_4O_2$ S_2	366.50	145-147	93	52.40 (52.43)	6.03 (6.05)	15.25 (15.29)
7e	R_1= piperidino	$C_{16}H_{21}N_3O_2$ S_2	351.48	122-124	92	54.65 (54.67)	6.01 (6.02)	11.94 (11.95)
7f	R_1=N-Methyl morpholino	$C_{15}H_{19}N_3O_3$ S_2	353.46	138-140	95	50.95 (50.97)	5.40 (5.42)	11.85 (11.89)
8a	R_2= CH_3	$C_{11}H_{12}N_2O_3S_2$	284.35	90-94	88	46.42 (46.46)	4.25 (4.25)	9.84 (9.85)
8b	R_2= CH_2-CH_3	$C_{12}H_{14}N_2O_3S_2$	298.38	60-62	87	47.28 (48.3)	4.73 (4.73)	9.38 (9.39)
8c	R_2= CH_2-CH_2-CH_3	$C_{13}H_{16}N_2O_3S_2$	312.4	60-64	83	49.95 (49.98)	5.16 (5.16)	8.98 (8.97)
8d	R_2= CH_3	$C_{11}H_{12}N_2O_2S_2$	268.35	70-74	86	49.20 (49.23)	4.50 (4.51)	10.43 (10.44)
8e	R_2= CH_2-CH_3	$C_{12}H_{14}N_2O_2S_2$	282.38	74-76	87	51.0 (51.04)	5.00 (5.00)	9.90 (9.92)
8f	R_2= CH_2-CH_2-CH_3	$C_{13}H_{16}N_2O_2S_2$	296.40	78-80	86.5	52.65 (52.68)	5.44 (5.44)	9.43 (9.45)

Biological activity

Antioxidant activity

DPPH radical-scavenging assay

The antioxidant activity of the compounds (**7a-f**), (**8a-f**) and the standard (Rutin) were assessed on the basis of radical scavenging effect of the stable DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical, with minor modification of the method of Germano *et. al.*,[19]. The test samples and the Rutin (Std) were dissolved in methanol, aliquots (0.5 mL) were mixed with 3 mL of a 39.4 µg/mL methanolic solution of DPPH. The mixture was shaken vigorously and then kept in the dark for 30 min at room temperature. The decrease in absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601). All tests were performed in triplicate and the results were expressed as mean ±S.D. The inhibition percentage (% I) of the DPPH radical was calculated according to the following formula

$$I\% = \frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}} \times 100$$

where $A_{C(0)}$ is the absorbance of the control DPPH solution at ***t***=0 min and $A_{A(t)}$ is the absorbance after addition of test samples at t=30 min. The concentration of the compound required to reduce the absorbance of DPPH control solution by 50% (IC_{50}) was calculated. The **Table 2** contains the anti oxidant activity data of the compounds (**7a-f**), (**8a-f**). It was found that the compounds have moderate antioxidant activity.

Table 2 Anti oxidant (DPPH Scavenging) activity data of [1,3,4]-oxadiazoles (7a-f) and (8a-f)

Compounds	IC_{50} (µg/mL)
Rutin(std)	5.80 ±2.08
7a	183.08±0.05
7b	173.08±0.08
7c	165.08±0.01
7d	>200
7e	>200
7f	>200
8a	195±0.06
8b	182±0.05
8c	173±0.06
8d	>200
8e	>200
8f	>200

Antimicrobial studies

All the newly synthesized oxadiazoles were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *Staphylococcus aureus*, *E.coli*, *P. aeruginosa* and *B. cereu*s. For antifungal screening, *C. albicans* strain was used. Both microbial studies were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [20]. The compound whose MIC has to be determined was dissolved in serially diluted DMF. Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 h at 37 °C. MIC is the highest dilution of the compound which shows clear fluid with no development of turbidity. The anti-microbial activity data are presented in **Table 3** and **Table 4**. The MIC values were evaluated at concentration range, 0.0240-50 µg/mL. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. The figures in the tables show the MIC values in µg/mL.

Table 3 Antibacterial activity data of [1,3,4]-oxadiazoles (7a-f) and (8a-f)

Compound	MIC in µg /mL			
	S. aureus	***E.Coli***	***P. aeruginosa***	***Bacillous cereus***
7a	6.25	6.25	6.25	6.25
7b	6.25	6.25	3.125	6.25
7c	6.25	6.25	6.25	6.25
7d	12.5	6.25	6.25	6.25
7e	6.25	3.125	3.125	3.125
7f	6.25	6.25	6.25	6.25
8a	12.5	6.25	6.25	12.5
8b	12.5	3.125	3.125	6.25
8c	6.25	3.125	6.25	6.25
8d	12.5	6.25	6.25	12.5
8e	3.125	3.125	0.781	3.125
8f	12.5	6.25	6.25	12.5
Penicillin	0.12	0.12	0.12	0.12

Among the screened samples, compounds **7b** showed good antibacterial activity against *E.Coli* where as **7e** showed better antibatcterial activity against *E.Clil, P. aeruginosa* and *B. cereus* even at low concentration of 3.125µg/mL. The enhanced activity may be due to the presence of pipyridyl group at position 3 of the oxadiazole ring. In **7e** the benzyl sulfinyl group at C-5 position of the oxadiazole ring is responsible for better activity than the **7b** where at C-5 position of oxadiazole ring benzyl sulfonyl group is present. Similarly incase of S-alkylated derivatives **8e** showed excellent antibacterial activity againt *P. aeruginosa* even at low concentration of 0.781 µg/mL due to the presence of ethyl group at C-2 position of oxadaizole ring. The derivatives **8d** and **8e** showed better activities than **8a**, **8b** and **8c** due to the presence of benzyl sulfinyl group at C-5 position of the oxadiazole ring.

The antifungal activity of the derivatives had much better results for sulfinyl moiety than for sulfonyl moiety. The screening studies have demonstrated that the newly synthesized compounds have promising antibacterial and antifungal properties. Therefore, it was concluded that there exists better scope for further study on this class of compounds.

Table 4 Antifungal activity data of [1,3,4]-oxadiazoles 7(a-f) and 8(a-f)

Compound	MIC in µg /mL
	Candida albicans
7a	6.25
7b	3.125
7c	6.25
7d	3.125
7e	3.125
7f	3.125
8a	12.5
8b	25.0
8c	6.25
8d	6.25
8e	3.125
8f	6.25

CONCLUSION

1,3,4-oxadaiazoles, 1,3,4-oxadaiazole Mannich bases and S-alkylated derivatives derived from 4-(methyl sulfonyl/sulfinyl)phenyl acetic acid were successfully synthesized in good yields. The structures of all the compounds were confirmed by recording their ^{1}H NMR, ^{13}C NMR, Mass and IR spectra. All the newly synthesized compounds were screened for their antioxidant, antibacterial and antifungal properties. It is found that the sulfinyl derivative is showing more activity than the sulphonyl derivatives. The results of our study indicate that the compounds have the potential to generate novel antimicrobial properties by displaying moderate to high affinities for most of the receptors.Therefore it was concluded that there exists better scope for further study on this class of compounds.

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