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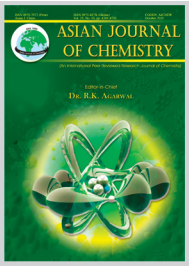

Synthesis, Characterization and Evaluation of Some 5-Substituted 1,3,4-Oxadiazole-2-thioesters as Antifungal Agents

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A series of 5-substituted 1,3,4-oxadiazole-2-thioesters was synthesized by converting several substituted organic acids successively into the corresponding esters, hydrazides and 5-substituted 1,3,4-oxadiazole-2-thiols. Finally the target compounds: 5-substituted 1,3,4-oxadiazole-2-thioesters were obtained by refluxing 5-substituted 1,3,4-oxadiazole-2-thiols in the presence of acid chloride and potassium hydroxide. The structures of the synthesized compounds were established by physicochemical and spectroscopic methods. The synthesized compounds were evaluated for their *in vitro* antifungal activity. Some of the evaluated compounds possessed significant antifungal activity as compared to a terbinafine standard.

Key Words: Oxadiazoles, Thiols, Thioesters, Synthesis, *In vitro* antifungal activity.

INTRODUCTION

Oxadiazoles and their derivatives have been normally utilized pharmacophores due to their ability to engage in hydrogen bonding. Numerous 1,3,4-oxadiazole derivatives have been reported to possess antihypertensive[1,2] as well as antibiotics properties[3]. Some other deravatives containing 1,3,4-oxadiazoles have demonstrated biological activity such as muscle relaxants[4] and antiomitotics[5], sedatives[6], platelet aggregation inhibitors[7] and have shown diuretic, analgesic, antiinflammatory, anti-convulsive and antiemetic properties[8]. The widespread use of 1,3,4-oxadiazoles as a scaffold in medicinal chemistry as demonstrated by these examples establishes this moiety as a member of the privileged structures class. During the last decades, a number of derivatives of oxadiazoles have been synthesized and tested for their biological activity[9-12].

In view of the immense biological importance of derivatives, the present work was undertaken to design, synthesize and investigate the *in vitro* antifungal activity of some 5-substituted 1,3,4-oxadiazole-2-thioesters (**Scheme-I**). The structures of the compounds were assigned on the basis of physical and spectral data.

EXPERIMENTAL

Melting points of the synthesized compounds were recorded on Gallenkamp digital melting point apparatus MFB-595-101 M in open-end capillary tubes and are uncorrected. Thin layer chromatography was carried out on pre-coated silica gel plates (0.2 mm, E. Merck, 20 × 20 cm, $60F_{254}$). FTIR spectra were recorded on a bio-red Merlin spectrophotometer using KBr discs. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.43 MHz) spectra were recorded on Bruker AM-250 spectrometer in DMSO and $CDCl_3$ solutions using TMS as internal standard. EIMS were recorded on Agilent VG: 70 SE Mass spectrometer. Elemental analysis was carried out on Leco CHNS-932 analyzer.

Synthetic method

Synthesis of 2(4-methoxyphenyl) acetate (1a): 2(4-Methoxyphenyl) acetic acid (5 g, 0.030 mol) was taken in a 250 mL round bottom flask fitted with a reflux condenser and a calcium chloride guard tube. Absolute methyl alcohol (25 mL) and few drops of concentrated sulphuric acid were added and the reaction mixture was refluxed for 4 h. The reflux time was monitored by thin layer chromatography (TLC) (silica; ethyl acetate-pet. ether, 1:2). After the completion of the reaction the excess of alcohol was distilled off on a rotary evaporator. The residue was poured into 250 mL of water contained in a separating funnel. Dichloromethane (20 mL) was added to the separating funnel and the mixture was shaken vigorously. The solution was allowed to stand and the 2(4-methoxyphenyl) acetate in the dichloromethane separated and settled at the bottom of the separating funnel. The lower layer was carefully separated and the upper aqueous layer was rejected. The 2(4-methoxyphenyl) acetate was returned to the funnel and shaken with a strong solution of sodium bicarbonate until all the free acid was removed. 2(4-Methoxyphenyl) acetate was washed

once with water and dried by pouring into a small dry conical flask containing 2 g of anhydrous magnesium sulphate. It was shaken for 5 min and allowed to stand for 1 h. The 2(4-methoxyphenyl) acetate solution was filtered through a small fluted filter paper into a distillation flask. The flask was fitted with 360º thermometer, a condenser and a receiving flask. Dichloromethane was distilled off at 40 ºC and the solid 2(4-methoxyphenyl) acetate was obtained from the flask. Ethanol was used as solvent for recrystallization of the ester.

Synthesis of 2(4-methoxyphenyl) acetic acid hydrazide (2a): 2(4-Methoxyphenyl) acetate (7 g, 0.038 mol) was dissolved in absolute ethanol (40 mL) and taken in a flask fitted with a reflux condenser and a calcium chloride guard tube. Hydrazine hydrate (80 %, 13 mL) was added and the reaction mixture was refluxed for 8 h. The reflux time was monitored by TLC as before. After the completion of the reaction, the excess hydrazine was distilled off. The crude solid was collected, washed with water and recrystallized from 30 % aqueous ethanol.

Synthesis of 5-(4-methoxybenzyl)-1,3,4-oxadiazole-2-thiol (3a): 2(4-Methoxyphenyl) acetic acid hydrazide (7 g, 0.038 mol) was dissolved in absolute ethanol (40 mL) in a 250 mL flask. Carbon disulfide (2 mL, 0.034 mol) was then added to the solution followed by the addition of a solution of potassium hydroxide (1.2 g, 0.019 mol) in water (20 mL). The reaction mixture was thoroughly stirred and refluxed. It was initially yellow, which turned to green and then light yellow with the progress of the reaction. Evolution of hydrogen sulfide gas was observed during each reaction. After completion of the reaction, excess of ethanol was removed under reduced pressure. The mixture was diluted with distilled water (200 mL) and acidified with 4 N hydrochloric acid to pH 2-3. It was then filtered, washed with diethyl ether and recrystallized from ethanol.

Synthesis of 5-(4-methoxyphenyl)-4-amino-1,2,4-triazole-3-thiol (4a): 5-(4-Methoxybenzyl)-1,3,4-oxadiazole-2-thiol (4 g, 0.018 mol) and 80 % hydrazine hydrate (6 mL, 0.124 mol) in absolute ethanol (25) were refluxed in a 250 mL flask fitted with a condenser and a guard tube. After the completion of the reaction the solvent and excess of hydrazine hydrate were removed under reduced pressure using rotary evaporator. The residue was washed with diethyl ether and recrystallized from ethanol.

Synthesis of 5-(4-methoxybenzyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (5a): 5-(4-Methoxybenzyl)-1,3,4-oxadiazole-2-thiol (5 g, 0.022 mol) was dissolved in absolute ethanol (30 mL) in a 250 mL flask. Potassium hydroxide (1 g, 0.178 mol) solution in water (20 mL) was added to the solution. After 1 h reflux 1-naphthoyl chloride (4 g, 0.021 mol) was added to the reaction mixture. The reaction mixture was thoroughly stirred and refluxed for 6 h. After completion of the reaction, the excess of ethanol in the reaction mixture was under reduced pressure. The mixture left behind was diluted with distilled water (200 mL) and then filtered. The crude product was dried in an oven and recrystallized from ethanol.

Purification of all the synthesized compounds was achieved by recrystallization and purity of each compound was monitored by thin layer (TLC).

5-(4-Methoxybenzyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (5a): 5-(4-Methoxybenzyl)-1,3,4-oxadiazole-2-thiol (5.0 g; 0.024 mol), KOH (1g; 0.0178 mol) and 1-naphthoyl chloride (4 g; 0.021 mol) were reacted according to the general procedure. Colour brown; yield: 77 % (3.8 g); recrystallization from EtOH; m.p. 179-181 ºC; m.f. $C_{21}H_{16}O_3N_2S$; m.w. 376. FTIR (KBr, ν_{max}, cm^{-1}): 1660 (C=O), 1580 (C=C)Ar, 1517 (C=N), 1133 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.50 (m, 1H, ArH-1), 6.87 (m, 1H, Ar H-2), 6.50 (m, 1H, Ar H-3), 6.87 (m, 1H, Ar H-4), 7.76 (dd, 1H, *J* = 2.4, 6.2Hz, Ar H-1´), 7.42 (dd, 1H, *J* = 3.2, 6.5 Hz, Ar H-2´), 7.73 (m, 1H, Ar H-3´), 7.46 (m, 1H, Ar H-4´), 7.55 (m, 1H, Ar H-5´), 7.76 (dd, 1H, *J* = 2.4, 6.2 Hz Ar H-6´), 2.50, (s, 2H, methylene protons) 3.56 (s, 3H, methoxy protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 30.06 (C-1), 55.90 (C-2), 128.63 (C-3), 130.10 (C-4), 114.20 (C-5), 157.73 (C-6), 166.44 (C-7), 175.13 (C-8), 179.53 (C-9), 130.10 (C-10), 114.21 (C-11),134.35 (C-12), 127.42 (C-13), 129.44 (C-14), 125.61 (C-15), 127.21 (C-16), 118.44 (C-17), 126.70 (C-18), 130.41 (C-19), 127.21 (C-20), 133.27 (C-21); MS (m/z) 376 (40 % F) (M^+), 155 (100 % F) 147 (75 % F), 229 (50 % F), 127 (60 % F) and 121 (65% F); Anal. calcd. (%) for $C_{21}H_{16}N_2O_3S$: C, 67.02; H, 4.25; N, 7.45; S, 8.51 found (%) C, 66.51; H, 4.02; N, 7.40.; S, 8.23.

5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (5b): 5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (4.0 g; 0.020 mol), KOH (1 g; 0.0178 mol) and 1-naphthoyl chloride (3.4 g; 0.018 mol) were reacted according to the general procedure. Colour yellow; yield: 77% (3.1 g); recrystallization from EtOH; m.p. 160-162 ºC; m.f. $C_{19}H_{11}N_3O_4S$; m.w. 377. FTIR (KBr, cm^{-1}): 1670 (C=O), 1575 (C=C)Ar, 1523 (C=N), 1052 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.74 (dd, 1H, *J* = 1.2, 4.5 Hz, ArH-1), 8.01 (dd, 1H, *J* = 2.4, 7.1 Hz, Ar H-2) 8.01 (dd, 1H, *J* = 2.4, 7.1 Hz, A H-3), 7.74 (dd, 1H, *J* = 2.2, 4.5 Hz, Ar H-4), 7.76 (m, 1H, Ar H-1´), 7.42 (m, 1H, Ar H-2´), 7.88 (m, 1H, Ar H-3´), 7.73 (m, 1H, Ar H-4´), 7.46 (m, 1H, Ar H-5´) 7.55 (dd, 1H, *J* = 2.4, 6.2 Hz Ar H-6´); ^{13}C NMR (DMSO-d_6, δ, ppm): 129.41 (C-1), 128.40 (C-2), 116.52 (C-3), 115.52 (C-4), 116.31 (C-5), 130.43 (C-6), 160.42 (C-7), 171.40 (C-8), 182.31 (C-9), 135.61 (C-10), 127.12 (C-11), 120.43 (C-12), 122.41 (C-13), 125.32 (C-14), 126.32 (C-15), 128.43 (C-16), 129.42 (C-17), 130.21 (C-18), 129.66 (C-19); MS (m/z) 377 (42 % F) (M^+), 155 (100 % F) 148 (75 % F), 229 (50 % F), 127 (60 % F) and 122 (65 % F); Anal. calcd. (%) for $C_{19}H_{11}N_3O_4S$: C, 60.48; H, 2.91; N, 11.14; S, 8.48 found (%) C, 60.22; H, 2.12; N, 10.91.; S, 8.23.

5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (5c): 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (5.0 g; 0.025 mol), KOH (1.3 g; 0.023 mol) and 1-naphthoyl chloride (3.4 g; 0.018 mol) were reacted according to the general procedure. Colour brown; yield: 80 % (4.0 g); recrystallization from EtOH; m.p. 160-162 ºC; m.f. $C_{19}H_{11}N_3O_4S$; m.w. 377. FTIR (KBr, ν_{max}, cm^{-1}): 1685 (C=O), 1568 (C=C) Ar, 1520 (C=N), 1240 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.63 (dd, 1H, *J* = 1.2, 4.5 Hz, ArH-1), 7.77 (dd, 1H, *J* = 2.4, 7.1Hz, Ar H-2) 7.02 (dd, 1H, *J* = 2.4, 7.1Hz, A H-3), 7.46 (dd, 1H, *J* = 2.2, 4.5Hz, Ar H-4), 7.88 (m, 1H, Ar H-1´), 7.77 (m, 1H, Ar H-2´), 7.62 (m, 1H, Ar H-3´), 8.85 (m, 1H, Ar H-4´), 7.21 (m, 1H, Ar H-5´) 7.50 (dd, 1H, *J* = 2.4, 6.2 Hz Ar H-6´); ^{13}C NMR (DMSO-d_6, δ, ppm): 128.40 (C-1), 127.41 (C-2), 154.50

(C-3), 126.46 (C-4), 116.61 (C-5), 127.41 (C-6), 162.61 (C-7), 174.62 (C-8), 180.37 (C-9), 136.21 (C-10), 129.11 (C-11), 122.41 (C-12), 125.61 (C-13), 128.81 (C-14), 127.48 (C-15), 130.61 (C-16), 132.81 (C-17), 126.21 (C-18), 129.44 (C-19); MS (m/z) 377 (42% F) (M^+), 155 (100 % F) 148 (72 % F), 229 (50 % F), 127 (60 % F) and 122 (65 % F); Anal. calcd. (%) for $C_{19}H_{11}N_3O_4S$: C, 60.48; H, 2.91; N, 11.14; S, 8.48 found (%) C, 60.22; H, 2.12; N, 10.91.; S, 8.23.

5-Phenyl-1,3,4-oxadiazole-2-ylbenzothioate (5d): 5-Phenyl-1,3,4-oxadiazole-2-thiol (3.0 g; 0.016 mol), KOH (0.8 g; 0.014 mol) and benzoyl chloride (2.0 g; 0.012 mol) were reacted according to the general procedure. Colour reddish brown; yield: 79 % (2.4 g); recrystallization from EtOH; m.p. 214-215 ºC; m.f. $C_{15}H_{10}N_2O_2S$; m.w. 282. FTIR (KBr, ν_{max}, cm^{-1}): 1666 (C=O), 1572 (C=C)Ar, 1526 (C=N), 1190 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.48 (m, 1H, ArH-1), 7.32 (m, 1H, Ar H-2) 7.22 (m, 1H, A H-3), 7.32 (m, 1H, Ar H-4), 7.97 (dd, 1H, *J* = 3.1, 6.7Hz, Ar H-1´), 7.45 (dd, 1H, *J* = 2.5, 4.7Hz, Ar H-2´), 7.58 (m, 1H, Ar H-3´), 8.85 (dd, 1H, *J* = 2.5, 4.7Hz, Ar H-4´), 7.97 (dd, 1H, *J* = 3.1, 6.7 Hz, Ar H-5´) ^{13}C NMR (DMSO-d_6, δ, ppm): 130.01 (C-1), 126.21 (C-2), 115.24 (C-3), 120.32 (C-4), 115.24 (C-5), 126.21 (C-6), 166.04 (C-7), 176.23 (C-8), 184.33 (C-9), 134.03 (C-10), 130.71 (C-11), 126.23 (C-12), 125.42 (C-13), 126.23 (C-14), 130.71 (C-15); MS (m/z) 282 (40 % F) (M^+), 105 (100 % F) 103 (74 % F), 179(53 % F) and 77 (58 % F); Anal. calcd. (%) for $C_{15}H_{10}N_2O_2S$: C, 63.82; H, 3.54; N, 9.92; S, 11.34 found (%) C, 63.22; H, 3.12; N, 9.11.; S, 11.23.

5-Phenethyl-1,3,4-oxadiazole-2-yloctanethioate (5e): 5-Phenethyl-1,3,4-oxadiazole-2-thiol (4.0 g; 0.019 mol), KOH (1.1 g; 0.02 mol) and heptoyl chloride (2.8 g; 0.018 mol) were reacted according to the general procedure. Colour orange; yield: 82 % (3.3 g); recrystallization from EtOH; m.p. 181-183 ºC; m.f. $C_{18}H_{24}N_2O_2S$; m.w. 322. FTIR (KBr, ν_{max}, cm^{-1}): 1677 (C=O), 1550 (C=C)Ar, 1519 (C=N), 1265 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.12 (m, 1H, ArH-1), 7.21 (dd, 1H, J=2.4, 6.7Hz, Ar H-2) 7.08 (m, 1H, A H-3), 7.21 (dd, 1H, *J* = 2.4, 6.7Hz, Ar H-4), 2.88 t, 4H, *J* = 2.4 Hz, t, 2H, *J* = 2.1, 1.5 Hz, m, 10 H, other protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 30.06 (C-1), 30.18 (C-2), 127.21 (C-3), 125.41 (C-4), 126.33 (C-5), 116.24 (C-6), 118.44 (C-7), 116.24 (C-8), 166.33 (C-9), 175.88 (C-10), 182.63 (C-11), 45.43 (C-12), 33.66 (C-13), 26.21 (C-14), 25.80 (C-15), 20.21 (C-16), 18.44 (C-17), 22.37 (C-18); MS (m/z) 332 (48 % F) (M^+), 127 (100 % F) 131 (70 % F), 201(50 % F), 99 (60 % F) and 105 (62 % F); Anal. calcd. (%) for $C_{18}H_{24}N_2O_2S$: C, 65.06; H, 7.22; N, 8.43; S, 9.63 found (%) C, 64.88; H, 7.02; N, 7.91.; S, 8.89.

5-Phenethyl-1,3,4-oxadiazole-2-ylbenzothioate (5f): 5-Phenethyl-1,3,4-oxadiazole-2-thiol (3.0 g; 0.015 mol), KOH (0.9 g; 0.016 mol) and benzoyl chloride (2.0 g; 0.014 mol) were reacted according to the general procedure. Colour light brown; yield: 80 % (2.4 g); recrystallization from EtOH; m.p. 168-169 ºC; m.f. $C_{17}H_{14}N_2O_2S$; m.w. 310. FTIR (KBr, ν_{max}, cm^{-1}): 1681 (C=O), 1574 (C=C)Ar, 1530 (C=N), 1085 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.14 (m, 1H, ArH-1), 7.47 (m, 1H, Ar H-2) 7.12 (m, 1H, A H-3), 7.67(m, 1H, Ar H-4), 7.97 (dd, 1H, *J* = 2.4, 6.5 Hz, ArH-1´) 7.58 (dd, 1H, *J* = 3.1, 4.5 Hz, ArH-2´), 7.77 (m, 1H, AH-3´), 7.67 (m, 1H, ArH-4´), 2.90 (t, 4H, *J* = 2.8 Hz, other protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 28.33 (C-1), 32.71 (C-2), 130.43 (C-3), 125.61 (C-4), 118.33 (C-5), 115.47 (C-6), 118.23 (C-7), 125.61 (C-8), 159.41 (C-9), 169.68 (C-10), 183.47 (C-11), 134.22 (C-12), 127.43 (C-13), 130.53 (C-14), 126.31 (C-15), 130.53 (C-16), 127.43 (C-17); MS (m/z) 310 (42 % F) (M^+), 105 (100 % F) 131 (70 % F), 179 (50 % F), 77 (55 % F) and 105 (60 % F); Anal. calcd. (%) for $C_{17}H_{14}N_2O_2S$: C, 65.80; H, 4.51; N, 9.03; S, 10.32 found (%) C, 65.14; H, 4.01; N, 10.91.; S, 11.23.

5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-ylheptanethioate (5g): 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (5.0 g; 0.022 mol), KOH (1.3 g; 0.023 mol) and hexoyl chloride (3.8 g; 0.025 mol) were reacted according to the general procedure. Colour golden; yield: 78 % (3.9 g); recrystallization from EtOH; m.p. 127-129 ºC; m.f. $C_{15}H_{17}N_3O_4S$; m.w. 335. FTIR (KBr, ν_{max}, cm^{-1}): 1674 (C=O), 1564 (C=C)Ar, 1525 (C=N), 1008 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.47 (dd, 1H, *J* = 3.2, 6.4 Hz, ArH-1), 7.27 (dd, 1H, *J* = 2.1, 7.2Hz, Ar H-2) 6.97 (dd, 1H, *J* = 3.2, 7.3Hz, A H-3), 7.47 (dd, 1H, *J* = 3.2, 6.4 Hz, Ar H-4), 2.50 (t, 2H, *J* = 3.2, 1.5 Hz, m, 10 H, other protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 130.33 (C-1), 127.25 (C-2), 152.44 (C-3), 126.69 (C-4), 127.25 (C-5), 116.21 (C-6), 161.37 (C-7), 170.66 (C-8), 182.03 (C-9), 54.33 (C-10), 44.21 (C-11), 33.33 (C-12), 24.61 (C-13), 25.62 (C-14), 20.22 (C-15); MS (m/z) 335(40 % F) (M^+), 113 (100 % F) 148 (72 % F), 187(50 % F), 85 (58 % F) and 122 (62 % F); Anal. calcd. (%) for $C_{15}H_{17}N_3O_4S$: C, 53.73; H, 5.07; N, 12.53; S, 9.55 found (%) C, 53.70; H, 4.93; N, 12.03.; S, 9.54.

5-Phenyl-1,3,4-oxadiazole-2-ylnonanethioate (5h): 5-Phenyl-1,3,4-oxadiazole-2-thiol (3 g; 0.016 mol), KOH (0.8 g; 0.014 mol) and nonanoyl chloride (2.0 g; 0.011 mol) were reacted according to the general procedure. Colour reddish yellow; yield: 82 % (2.4 g); recrystallization from EtOH; m.p. 194-196 ºC; m.f $C_{17}H_{22}N_2O_2S$; m.w. 318. FTIR (KBr, ν_{max}, cm^{-1}): 1690 (C=O), 1582 (C=C)Ar, 1518 (C=N), 1245 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 6.52 (m, 1H, ArH-1), 6.77 (m, 1H, Ar H-2) 7.14 (m, 1H, A H-3), 7.02 (m, 1H, Ar H-4), 2.50 (t, 2H, *J* = 3.2, 1.6 Hz, m, 12H, other protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 130.46 (C-1), 125.33 (C-2), 116.73 (C-3), 118.62 (C-4), 116.73 (C-5), 125.33 (C-6), 159.68 (C-7), 168.73 (C-8), 184.66 (C-9), 53.62.03 (C-10), 44.65 (C-11), 33.72 (C-12), 28.69 (C-13), 24.71 (C-14), 25.69 (C-15), 20.01 (C-16), 18.33 (C-17); MS (m/z) 318 (43 % F) (M^+), 141 (100 % F) 103 (73 % F), 215 (49 % F), 125 (56 % F) and 77 (60 % F); Anal. calcd. (%) for $C_{17}H_{22}N_2O_2S$: C, 65.80; H, 7.10; N, 9.03; S, 10.32 found (%) C, 65.22; H, 6.98; N, 8.91; S, 10.02.

5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-ylbenzothioate (5i): 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (6.0 g; 0.026 mol), KOH (1.5 g; 0.026 mol) and benzoyl chloride (4.5 g; 0.032 mol) were reacted according to the general procedure. Colour dull yellow; yield: 80 % (4.8 g); recrystallization from EtOH; m.p. 170-171 ºC; m.f. $C_{15}H_9N_3O_4S$; m.w. 327. FTIR (KBr, ν_{max}, cm^{-1}): 1688 (C=O), 1590 (C=C)Ar, 1514 (C=N), 1132 (C-O); ^{1}H NMR (DMSO-d_6, δ, ppm): 7.12 (dd, 1H, *J* = 2.3, 6.4 Hz, ArH-1), 7.27 (dd, 1H, *J* = 3.2, 7.4 Hz, Ar H-2) 6.98 (dd, 1H, *J* = 2.1, 4.5 Hz, A H-3), 7.12 (dd, 1H, *J* = 2.3, 6.4 Hz, Ar H-4), 7.77 (m, 1H, Ar H-1´),7.67 (m, 1H, Ar H-2´), 7.58 (m, 1H, Ar H-3´), 7.67 (m, 1H, Ar H-4´), 7.77; ^{13}C NMR (DMSO-d_6, δ, ppm): 133.06 (C-1), 127.43 (C-2), 153.44 (C-3), 125.21 (C-4), 127.43 (C-5), 116.66 (C-6), 163.23 (C-7),

177.45 (C-8), 181.08 (C-9), 136.38 (C-10), 127.21 (C-11), 116.31 (C-12), 125.64 (C-13), 127.21 (C-14), 125.64 (C-15); MS (m/z) 333 (40 % F) (M^+), 105 (100 % F) 148 (70 % F), 179 (50 % F), 77 (58 % F) and 122 (60 % F); Anal. calcd. (%) for $C_{15}H_9N_3O_4S$: C, 55.05; H, 2.75; N, 12.84; S, 9.79 found (%) C, 54.92; H, 2.12; N, 11.91.; S, 9.23.

Antifungal evaluation: The synthesized compounds were tested by agar tube dilution method[13] for their *in vitro* fungicidal activity. Terbinafine (200 mg/mL) was used as positive control. All experiments were done in three replicates. Four fungal strains: *Aspergillus flavus, Mucor species, Aspergillus niger* and *Aspergillus fumigatus* were used. All fungal strains were grown on 6.5 % SDA (Sabouraud dextrose agar, pH 5.7) at 28 ºC and preserved at 4 ºC in a refrigerator. 100 mm slants with sterilized SDA were prepared by adding each compound in 200 µg/mL concentration. Terbinafine (200 µg/mL) was used as standard drug while DMSO was used as negative control. Each slant was inoculated with 4 mm piece of respective fungal strain and incubated at 28 ºC for 7-10 days. Fungal growth was compared with negative control to get the % age inhibition.

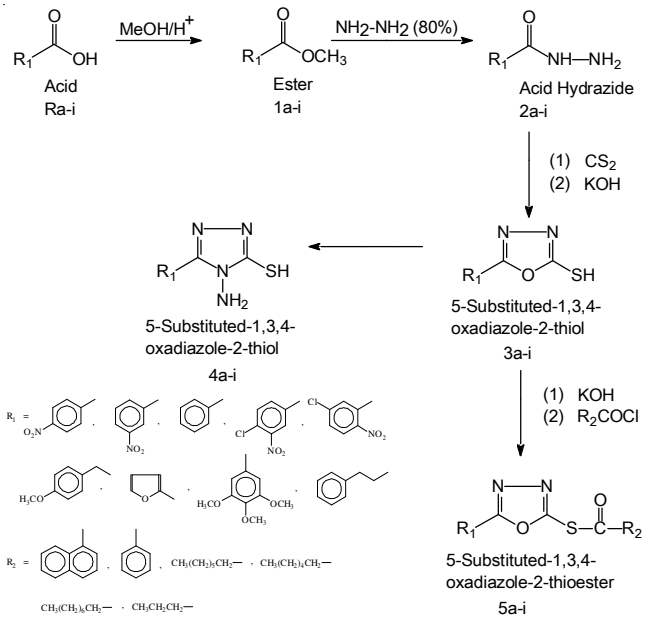
In the *in vitro* antifungal bioassay, the compounds **5a, 5b, 5c** and **5h** were found to have significant antifungal activities against the four tested fungal strains (Table-1). In particular **5a** and **5h** had high inhibitory effects on the growth of *Aspergillus. flavus, Mucor species, Aspergillus niger* and *Aspergillus fumigatus*. A structure activity relationship comparison shows that the presence of naphthyl group at the 3-position of the oxadiazole-2-thioester enhances the activity of the compounds. It is interesting to note that a similar group is also present in the structure of the reference compound terbinafine.

The antifungal assay results also show that the activity of the compounds **5d, 5e, 5f, 5g** and **5i** is relatively low as compared to other members of the series due to the absence of this group in these structures. It is therefore concluded that the potential pharmacophore in the oxadiazole -2-thioesters is probably naphthalene-1-carbothioate.

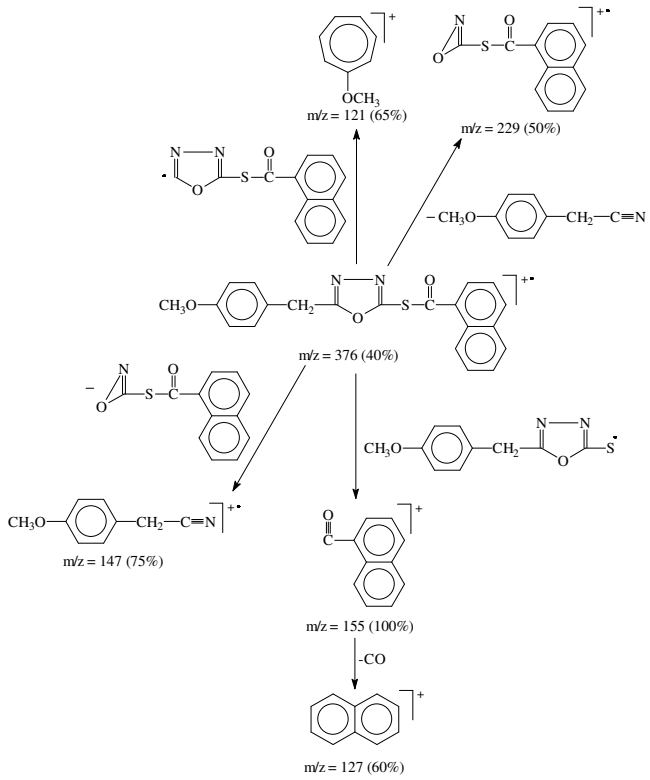
RESULTS AND DISCUSSION

Nine 5-substituted 1,3,4-oxadiazole-2-thioesters were obtained in 77-82 % yield by converting variously substituted organic acids (**Ra-i**) to the corresponding esters (**1a-i**) and acid hydrazides (**2a-i**) by reaction with methanol and hydrazine hydrate, respectively. The hydrazides were converted to 5-substituted 1,3,4-oxadiazole-2-thiols (**3a-i**) and 5-substituted 1,2,4-triazole-3-thiols (**4a-i**) as described in previous work[14-16]. 5-Substituted 1,3,4-oxadiazole-2-thiols were next converted to 5-substituted 1,3,4-oxadiazole-2-thioesters (**5a-i**) by refluxing with potassium hydroxide and acid chloride as outlined in **Scheme-I**.

Mass spectral data support the proposed structures. **Scheme-II** shows the fragmentation pattern for 5-(4-methoxybenzyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (**5a**), which serves as an example for all the compounds mutatis mutandis. The mass spectrum showed various characteristic peaks. A peak at m/z 376 was attributed to molecular ion. A peak at m/z 155 was assigned as the base peak produced due to the naphthoyl fragment. A characteristic peak at m/z 121 was assigned to the tropylium ion due to the loss of oxadiazole-thioester moiety.

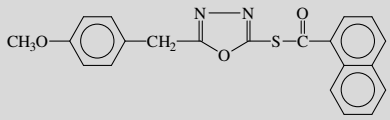
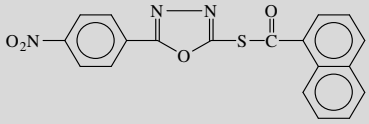
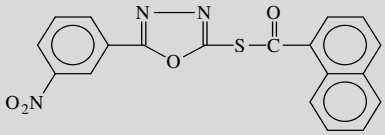
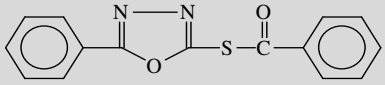
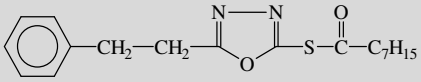
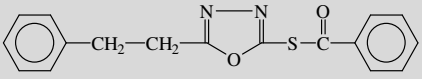
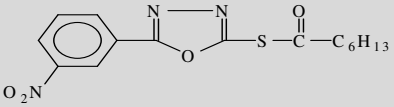
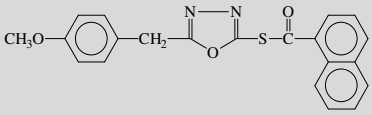
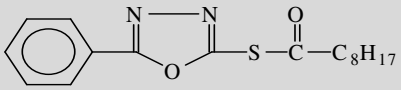
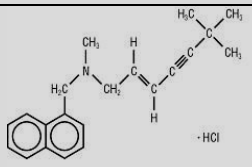


Scheme-I Synthesis of 5-substitued-1,3,4-oxadiazole-2-thioesters



Scheme-II Mass fragmentation of **5a**

TABLE-1
ANTIFUNGAL ACTIVITY OF 5-SUBSTITUTED-1,3,4-OXADIAZOLE-2-THIOESTERS

Sample No.	Structure of 5-substituted-1,3,4-oxadiazole-2-thioesters	Fungal strains/inhibition (%)			
		Aspergillus flavus	*Mucor species*	*Aspergillus niger*	*Aspergillus fumigatus*
5a		99.22	92.66	84.79	97.20
5b		84.12	100.00	81.20	100.00
5c		83.00	80.90	75.20	93.60
5d		61.00	78.36	59.19	87.00
5e		71.00	45.89	69.99	73.00
5f		27.00	15.58	24.39	75.00
5g		54.00	21.64	28.00	52.00
5h		100.00	88.22	96.20	98.10
5i		50.00	19.48	51.99	58.00
Terbinafine standard drug		100.00	90.00	110.80	98.40

The FTIR spectrum of (**5a**) showed absorption bands at 1517, 1660, 1133 and 1580 cm^{-1} which indicated the presence of C=N, C=O, C-O and C=C (aromatic) respectively. The ^{1}H NMR spectrum showed characteristic signals at 6.86 to 7.83 ppm, which were assigned to the aromatic protons. A signal at 2.50 ppm was assigned to the methylene proton. Similarly, a singlet at 3.87 ppm was attributed to the methoxy protons.

The ^{13}C NMR spectrum showed characteristic peaks at 120.5-134.5 ppm, which were assigned to the aromatic carbon atoms. The peak at 179.53 ppm was attributed to the carbonyl carbon atom. The peaks at 166.4 ppm and 175.13 ppm were attributed to the carbon atoms of oxadiazole moiety. The peaks at 30.6 ppm and 55.9 ppm were assigned to methylene and methoxy carbon atoms. On the basis of the combined physical

and spectral data compound **5a** was assigned the following structure, corresponding to 5-(4-methoxybenzyl)-1,3,4-oxadiazole-2-ylnaphthalene-1-carbothioate (Fig. 1).

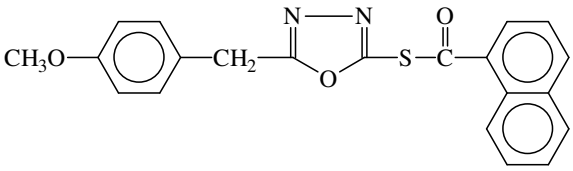


Fig. 1. Structure of compound **5a**

Similarly, other members of the series of 5-substituted 1,3,4-oxadiazole-2-thioester (**5b-i**) were synthesized from 5-substituted 1,3,4-oxadiazole-2-thiols using potassium hydroxide and acid chloride respectively

Conclusion

Nine 5-substituted 1,3,4-oxadiazole-2-thiols derivatives (**3a-i**) containing different groups were obtained *via* the cyclization reaction of acid hydrazides in the presence of carbon disulfide and potassium hydroxide. The resulting 5-substituted 1,3,4-oxadiazole-2-thiols were subjected to reactions with potassium hydroxide and acid chloride to furnish nine new 5-substituted 1,3,4-oxadiazole-2-thioesters (**5a-i**). Their structures were confirmed by infrared, ^{1}H- and ^{13}C -NMR, mass spectrometric and elemental analysis. In the *in vitro* antifungal bioassay, compounds **5a, 6b, 6c** and **6h** were found to have significant antifungal activities against the four tested fungal strains: *Aspergillus flavus, Mucor species, Aspergillu niger* and *Aspergillu. fumigatus* structure activity relationship comparison shows that the presence of naphthyl group at 3-position of the oxadiazole-2-thioester enhances the activity of the compounds, probably because a similar group is also present in the structure of the reference compound terbinafine.

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