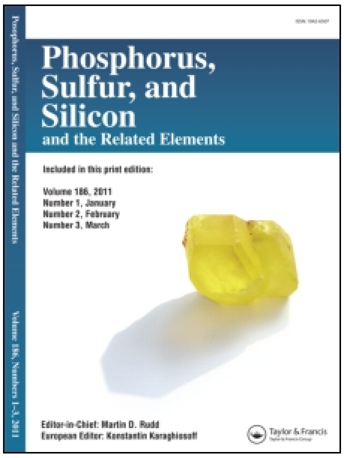

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Synthesis and Antimicrobial Activities of Some novel 1,3,4-Oxadiazoles Carrying Alkylthio and Alkylsulphonyl phenoxy Moieties

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To cite this Article Karabasanagouda, T. , Adhikari, Airody Vasudeva and Shetty, N. Suchetha(2007) 'Synthesis and Antimicrobial Activities of Some novel 1,3,4-Oxadiazoles Carrying Alkylthio and Alkylsulphonyl phenoxy Moieties', Phosphorus, Sulfur, and Silicon and the Related Elements, 182: 12, 2925 — 2941

To link to this Article: DOI: 10.1080/10426500701542981

URL: http://dx.doi.org/10.1080/10426500701542981

Phosphorus, Sulfur, and Silicon, 182:2925–2941, 2007
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ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701542981

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*A series of new 2-[(4-alkylthio / alkylsulfonyl phenoxy) methyl]-5-substituted-1,3,4-oxadiazoles (**4a–y**, **5a–h**) have been synthesized from 4-alkylthio phenol (**1a–b**) through a multi-step reaction sequence. Compounds **1a–b** reacted with ethyl chloroacetate to give ethyl [4-(alkylthio) phenoxy] acetate (**2a–b**) which on oxidation yielded ethyl [4-(alkylsulfonyl)phenoxy]acetate (**2c–d**). They were readily converted to the corresponding hydrazides **3a–d**, and then cyclized to the title compounds 2-{[4-(alkylthio / alkylsulfonyl) phenoxy] methyl}-5-aryl-1,3,4-oxadiazoles (**4a–y**) by condensing with aromatic carboxylic acids. Further, **3a–b**, on condensation with carbon disulphide afforded the title compounds 2-{[4-(alkylthio) phenoxy]methyl}-1,3,4-oxadiazole-5-thiols(**5a–b**), which on alkylation yielded the title compounds 2-[(4-alkylthio phenoxy) methyl]-1,3,4-oxadiazole-5-alkyl thiols (**5c–f**), while with chloroacetic acid gave the target compounds 2-[(4-alkylthio phenoxy) methyl]-1,3,4-oxadiazole-5-thioacetic acid (**5g–h**). The structures of new compounds **4a–5h** were established on the basis of their elemental analysis and IR, ^{1}H NMR, ^{13}C NMR & MASS spectral data. All the title compounds were subjected to in vitro antibacterial*

Received March 24, 2007; accepted June 5, 2007.

We are grateful to the Heads of Chemistry Department, National Institute of Technology Karnataka, Surathkal, and Strides Research & Specialty Chemicals Ltd., New Mangalore, for providing laboratory facilities. The authors are also thankful to Prof. A. Srikrishna, IISc Bangalore and the Head, SAIF, CDRI for providing ^{1}H NMR, ^{13}C NMR, and Mass spectral facilities.

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testing against four pathogenic strains and antifungal screening against three fungi. Preliminary results indicate some of them exhibited promising activities and they deserve more consideration as potential antimicrobials.

Keywords 2-{[4-(Alkylthio/alkylsulfonyl) phenoxy] methyl}-5-aryl-1,3,4-oxadiazoles; 2-{[4-(alkylthio) phenoxy]methyl}-1,3,4-oxadiazole-5-thiols; 5-{[4-(alkylthio) phenoxy] methyl}-1,3,4-oxadiazole-2-substituted thiols; antibacterial & antifungal screening

INTRODUCTION

Various 1,3,4-oxadiazole derivatives have been reported to possess diverse types of biological properties such as antibacterial,[1,2] antifungal,[3] anti-inflammatory,[4] antiviral,[5] anticonvulsant, antitubercular,[6] and antipyretic activities.[7] A thorough literature survey reveals that presence of 4-alkylthiophenoxy and 4-alkylsulphonyl phenoxy moieties is an important structural feature of wide variety of synthetic drugs.[8–10] It has been established that introduction of 4-methylmercapto phenyl group and 4-methylsulphonyl phenyl groups to different heterocycles have yielded many biologically active compounds endowed with wide spectrum of pharmacological and antimicrobial activities.[11–12] Encouraged by the above reports, we planned to synthesize new 5-substituted 1,3,4-oxadiazoles containing 4-alkylthio/sulphonyl phenoxy group at position 2 of it, aiming at investigation of new heterocycles of enhanced pharmacological activities. The present study describes the synthesis of hitherto unreported 2-{[4-(alkylthio or alkylsulfonyl) phenoxy] methyl}-5-aryl-1,3,4-oxadiazoles and 2-{[4-(alkylthio or alkylsulfonyl) phenoxy] methyl}-1,3,4-oxadiazole-5-substituted thiols (**4a–y** and **5a–h**) and evaluation of their antibacterial and antifungal activities.

RESULTS AND DISCUSSION

The reaction sequences employed for the synthesis of title compounds is shown in Figures 1 and 2. The key intermediate, ethyl [4-(alkylthio) phenoxy] acetate **(2a–b)**, was prepared by treating ethyl chloroacetate with 4-(alkylthio) phenol (**1a–b**) in boiling dry acetone in presence of potassium carbonate. The compounds, ethyl [4-(alkylsulfonyl phenoxy] acetate **(2c–d)** were obtained by the oxidation of ethyl [4-(alkylthio) phenoxy] acetate (**2a–b**) with 30% hydrogen peroxide in acetic acid. These esters (**2a–d)** were conveniently converted to 2-[4-(alkylthio/alkyl sulphonyl phenoxy] acetohydrazides (**3a–d)** by refluxing with hydrazine hydrate in methanol. Further condensation of (**3a–d)** with various aromatic carboxylic acids in presence of phosphorous oxychloride afforded the title compounds 2-{[4-(alkylthio

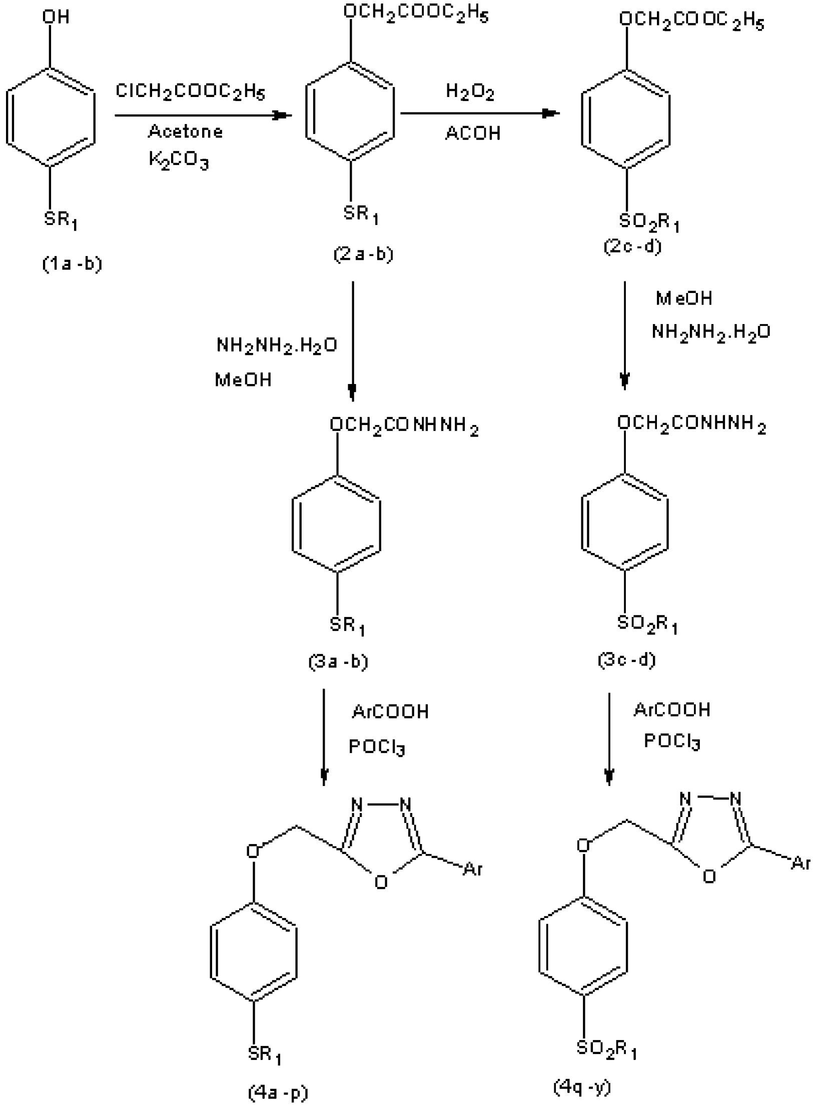

FIGURE 1 Where $R_1 = CH_3, C_2H_5$.

or alkylsulfonyl) phenoxy] methyl}-5-aryl-1,3,4-oxadiazoles (**4a–y**). Prolonged reaction leads to the formation of by-products instead of the desired **4a–y**. The acetohydrazides (**3a**,**b**), on refluxing with CS_2 in methanolic KOH afforded the 2-{[4-(alkylthio or alkylsulfonyl) phenoxy] methyl}-1,3,4-oxadiazole-5-thiols (**5a**,**b**) which on treatment with dimethylsulphate or diethylsulphate in alkaline medium gave the

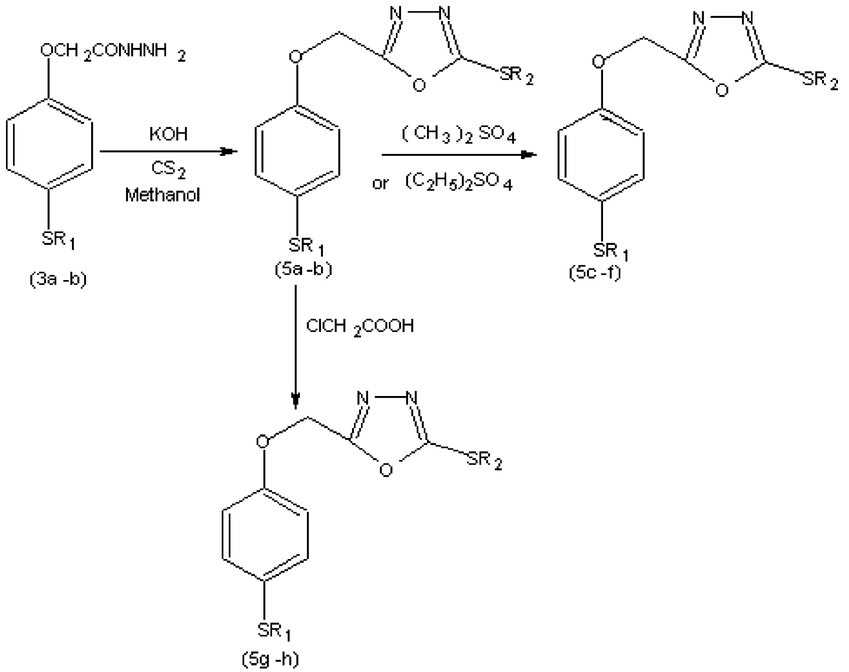

FIGURE 2 Where $R_1 = CH_3$, C_2H_5 and $R_2 = H$, CH_3, C_2H_5, CH_2COOH.

2-[(4-alkylthio phenoxy) methyl]-1,3,4-oxadiazole-5-alkyl thiols (**5c–f**). Reactions of **5a**,**b** with chloroacetic acid in presence of alkali yielded the 2-[(4-alkylthio phenoxy) methyl]-1,3,4-oxadiazole-5-thioacetic acids (**5g–h**) in good yield.

The structural assignments of the compounds were based on their elemental analysis and spectral (IR, ^{1}H NMR ^{13}C NMR and MASS) data. The characterization data of all the new compounds were summarized in Tables I and II.

The formation of 2-[4-(methylthio) phenoxy] acetohydrazide (**3a**) from ethyl [4-(methylthio) phenoxy] acetate (**2a**) was confirmed by its IR, ^{1}H NMR spectral and elemental analysis. IR spectrum of it showed absorption band at 3310, 3224, 3045, 1680, 1544 cm^{-1} due to NH_2, NH, SCH_3, C=O, and C=C groups respectively, while ^{1}H NMR showed sharp singlets at δ 2.40 and 4.46, which correspond to SCH_3 and CH_2 protons respectively. It appeared two doublets at δ 6.94 and 7.24 indicating the presence of four aromatic protons. Further cyclization of **3a** to 2-{[4-(methylthio) phenoxy] methyl}-5-phenyl-1,3,4-oxadiazole (**4a**) was confirmed by IR spectrum which showed peaks at 3009, 1697, 1494, and 1242 cm^{-1} indicating the presence of SCH_3, C=N, phenyl

TABLE I Characterization Data of Compounds 4a–y

							Elemental analysis % found (cal)		
Compd.	Ar	R_1	Mol. formula	Mol. mass	M.P. (°C)	Yield (%)	C	H	N
4a	C_6H_5	CH_3	$C_{16}H_{14}N_2O_2S$	298.36	170	65	64.43 (64.41)	4.75 (4.73)	9.36 (9.39)
4b	4-$CH_3C_6H_4$	CH_3	$C_{17}H_{16}N_2O_2S$	312.38	150	60	65.33 (65.36)	5.17 (5.16)	8.99 (8.97)
4c	2-ClC_6H_4	CH_3	$C_{17}H_{13}ClN_4S_2$	332.80	110	63	57.75 (57.74)	3.92 (3.94)	8.43 (8.42)
4d	2-$CH_3C_6H_4$	CH_3	$C_{17}H_{16}N_2O_2S$	312.38	155	60	65.33 (65.36)	5.17 (5.16)	8.99 (8.97)
4e	3-$CH_3C_6H_4$	CH_3	$C_{17}H_{16}N_2O_2S$	312.38	135	60	65.33 (65.36)	5.17 (5.16)	8.99 (8.97)
4f	2-$OCH_3C_6H_4$	CH_3	$C_{17}H_{16}N_2O_3S$	328.38	130	64	62.15 (62.18)	4.93 (4.91)	8.55 (8.53)
4g	2-OHC_6H_5	CH_3	$C_{16}H_{14}N_2O_3S$	314.36	160	66	61.15 (61.13)	4.45 (4.49)	8.55 (8.53)
4h	4-CH_3-2-OHC_6H_3	CH_3	$C_{17}H_{16}N_2O_3S$	328.38	210	63	62.15 (62.18)	4.93 (4.91)	15.16 (15.15)
4i	3,5-$(CH_3)_2C_6H_3$	CH_3	$C_{18}H_{18}N_2O_2S$	326.41	135	68	66.25 (66.23)	5.58 (5.56)	8.55 (8.58)
4j	C_6H_5	C_2H_5	$C_{17}H_{16}N_2O_2S$	312.38	152	66	65.36 (65.36)	5.18 (5.16)	8.98 (8.97)
4k	2-ClC_6H_4	C_2H_5	$C_{17}H_{15}ClN_2O_2S$	346.83	130	67	58.89 (58.87)	4.38 (4.36)	8.05 (8.08)
4l	4-$CH_3C_6H_4$	C_2H_5	$C_{18}H_{18}N_2O_2S$	326.41	200	63	66.26 (66.23)	5.58 (5.56)	8.59 (8.58)
4m	3-$CH_3C_6H_4$	C_2H_5	$C_{18}H_{18}N_2O_2S$	326.41	130	63	66.26 (66.23)	5.58 (5.56)	8.59 (8.58)
4n	2-$CH_3C_6H_4$	C_2H_5	$C_{18}H_{18}N_2O_2S$	326.41	100	63	66.26 (66.23)	5.58 (5.56)	8.59 (8.58)

(Continued on next page)

TABLE I Characterization Data of Compounds 4a–y *(Continued)*

Compd.	Ar	R_1	Mol. formula	Mol. mass	M.P. (°C)	Yield (%)	Elemental analysis % found (cal) C	H	N
4o	2-$OCH_3C_6H_4$	C_2H_5	$C_{18}H_{18}N_2O_3S$	342.41	98	64	63.15 (63.14)	5.35 (5.3)	8.15 (8.18)
4p	3,5-(CH_3)-C_6H_3	C_2H_5	$C_{19}H_{20}N_2O_2S$	340.44	160	64	67.05 (67.03)	5.95 (5.92)	8.25 (8..23)
4q	C_6H_5	CH_3	$C_{16}H_{14}N_2O_4S$	330.35	184	65	58.18 (58.17)	4.25 (4.27)	8.49 (8.48)
4r	2-ClC_6H_4	CH_3	$C_{16}H_{13}ClN_2O_4S$	364.80	118	70	52.69 (52.68)	3.57 (3.59)	7.67 (7.68)
4s	4-$CH_3C_6H_5$	CH_3	$C_{17}H_{16}N_2O_4S$	344.38	200	65	59.25 (59.29)	4.69 (4.68)	8.15 (8.13)
4t	3-$CH_3C_6H_5$	CH_3	$C_{17}H_{16}N_2O_4S$	344.38	125	65	59.25 (59.29)	4.69 (4.68)	8.15 (8.13)
4u	2-$CH_3C_6H_5$	CH_3	$C_{17}H_{16}N_2O_4S$	344.38	145	65	59.25 (59.29)	4.69 (4.68)	8.15 (8.13)
4v	2-$OCH_3C_6H_5$	CH_3	$C_{17}H_{16}N_2O_5S$	360.38	150	65	56.67 (56.66)	4.49 (4.47)	7.79 (7.77)
4w	C_6H_5	C_2H_5	$C_{17}H_{16}N_2O_4S$	344.38	160	65	59.23 (59.20)	4.65 (4.68)	8.16 (8.15)
4x	4-$CH_3C_6H_5$	C_2H_5	$C_{18}H_{18}N_2O_4S$	358.41	210	65	60.35 (60.32)	5.03 (5.06)	7.8 (7.82)
4y	2-$OCH_3C_6H_5$	C_2H_5	$C_{18}H_{18}N_2O_5S$	374.41	170	65	57.70 (57.74)	4.87 (4.85)	7.49 (7.48)

TABLE II Characterization Data of Compounds 5a–h

							Elemental analysis % found (cal)		
Compd.	Ar	R_1	Mol. formula	Mol. mass	M.P. (°C)	Yield (%)	C	H	N
5a	SH	CH_3	$C_{10}H_{10}N_2O_2S_2$	254.33	102	69	47.20 (47.22)	3.98 (3.96)	11.04 (11.07)
5b	SH	C_2H_5	$C_{11}H_{12}N_2O_2S_2$	268.30	99	69	49.26 (49.23)	4.54 (4.51)	10.46 (10.44)
5c	SCH_3	CH_3	$C_{11}H_{12}N_2O_2S_2$	268.35	70	73	49.20 (49.23)	4.50 (4.51)	10.46 (10.44)
5d	SCH_3	C_2H_5	$C_{12}H_{14}N_2O_2S_2$	282.38	50	73	51.06 (51.04)	5.03 (5.00)	9.94 (9.92)
5e	SC_2H_5	CH_3	$C_{12}H_{14}N_2O_2S_2$	282.38	46	70	51.03 (51.04)	5.01 (5.00)	9.93 (9.92)
5f	SC_2H_5	C_2H_5	$C_{13}H_{16}N_2O_2S_2$	296.41	35	65	52.65 (52.68)	5.43 (5.44)	9.43 (9.45)
5g	SCH_2COOH	CH_3	$C_{12}H_{12}N_2O_4S_2$	312.36	72	75	47.15 (46.14)	4.89 (3.87)	8.95 (8.97)
5h	SCH_2COOH	C_2H_5	$C_{13}H_{14}N_2O_4S_2$	326.39	60	70	47.87 (47.84)	4.34 (4.32)	8.56 (8.58)

and C—O—C groups respectively. In its ^{1}H NMR spectrum, peaks due to SCH_3 and CH_2 protons appeared at δ 2.45 and 5.30, respectively. Peaks due to two phenyl groups appeared at δ 6.80, 7.28, and 8.00 as doublets and at 7.50 as multiplet. Further, FAB Mass Spectrum showed molecular ion peak at m/z 317 (100%) and 316 which is in agreement with the molecular formula [$C_{16}H_{14}N_2O_2S$ NH_4]+1 and $C_{16}H_{14}N_2O_2S$ NH_4 ^{13}C NMR spectrum showed signals at δ 17.32 due to SCH_3 while peaks at δ 60.14, 115.41, 129.08, 129.52, 129.61, 131.02, 132.06, 132.50, 155.89, 162.19, and 164.97 are due to, OCH_2, C_2 & C_6 of phenoxy group, C_2 & C_6 of 6-phenyl moiety, C_3 & C_5 of phenoxy moiety, C_4 of phenoxy moiety, C_4 of 6-phenyl moiety, C_3 & C_5 of 6-phenyl moiety, C_1 of 6-phenyl moiety, C_1 of phenoxy moiety, C_5 of oxadiazole and C_2 of oxadiazole moiety respectively, The peaks due to quaternary carbon atoms of the structure disappeared on DEPT experimentation

The buildup of **5b** from **3b** is evidenced by its IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. IR spectrum of **5b** indicates the presence of SC_2H_5, C=N, phenyl and C—O—C groups due to peaks at 3089, 1641, 1599, and 1253 cm^{-1}, respectively. ^{1}H NMR spectrum showed the presence of SCH_2CH_3 at δ 1.26 as triplet δ 2.85 as quartet. The singlet at δ 5.00 shows presence of OCH_2 and also two doublets at δ 6.88 and δ 7.32 are due to four aromatic protons. ^{13}C-NMR spectrum of **5b** showed signals at δ 29.16 and 14.49 due to SCH_2CH_3, while peaks at δ 59.91,

TABLE III Antibacterial Activity of the Prepared Compounds

	MIC in μg/ml and zone of inhibition (mm)			
Compd.	*E. coli*	*K .pneumoniae*	*P.aeruginosa*	*S. aureus*
4a	12.5 (11–15)	12.5 (11–15)	6.25 (16–20)	25 (<10)
4b	12.5 (11–15)	12.5 (11–15)	25 (<10)	12.5 (11–15)
4c	6.25 (16–20)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
4d	12.5 (11–15)	12.5 (11–15)	25 (<10)	25 (<10)
4e	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	25 (<10)
4f	25 (<10)	25 (<10)	25 (<10)	25 (<10)
4g	12.5 (11–15)	25 (<10)	12.5 (11–15)	6.25 (16–20)
4h	25 (<10)	25 (<10)	25 (<10)	25 (<10)
4i	12.5 (11–15)	12.5 (11–15)	25 (<10)	6.25 (16–20)
4j	12.5 (11–15)	25 (<10)	25 (<10)	25 (<10)
4k	25 (<10)	25 (<10)	25 (<10)	25 (<10)
4l	25 (<10)	25 (<10)	12.5 (11–15)	25 (<10)
4m	25 (<10)	25 (<10)	25 (<10)	12.5 (11–15)
4n	25 (<10)	25 (<10)	25 (<10)	25 (<10)
4o	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	25 (<10)
4p	25 (<10)	25 (<10)	25 (<10)	25 (<10)
4q	12.5 (11–15)	12.5 (11–15)	25 (<10)	25 (<10)
4r	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4s	6.25 (16—20)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
4t	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
4u	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4v	12.5 (11–15)	25 (<10)	12.5 (11–15)	12.5(11–15)
4w	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4x	6.25 (16–20)	12.5(11–15)	6.25 (16–20)	6.25 (16–20)
4y	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)
5a	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5b	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
5c	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	6.25 (16–20)
5d	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5e	25 (<10)	12.5 (11–15)	12.5 (11–15)	25 (<10)
5f	12.5 (11–15)	12.5 (11–15)	25 (<10)	12.5 (11–15)
5g	12.5 (11–15)	25 (<10)	25 (<10)	25 (<10)
5h	25 (<10)	25 (<10)	25 (<10)	12.5 (11–15)
Standard (ciprofloxacin)	6.25 (30–40)	6.25 (23–27)	6.25 (25–33)	1.56 (22–30)

The MIC values were evaluated at concentration range, 1.56–25 μg/ml. The figures in the table show the MIC values and the corresponding zone of inhibition (in mm).

115.49, 129.38, 132.39, 156.04, 159.41, and 178.76 are due to OCH_2, C_3 & C_5 of phenoxy group, C_4 of phenoxy moiety, C_2 & C_6 of phenoxy moiety, C_1 of phenoxy moiety, C_3 of oxadiazole and C_5 of oxadiazole, respectively. The peaks due to quaternary carbon atoms disappeared on DEPT experimentation. Further, FAB MS of **5b** showed the molecular ion peak at m/z 269 $[M+H]^+$ and 268 $[M]^+$ which is in agreement with their molecular formula, $C_{11}H_{12}N_2O_2S_2$.

EXPERIMENTAL

Melting points were determined in open capillaries and uncorrected [melting point apparatus: SERWELL Instruments INC, India]. Purity of the compounds was checked by thin-layer chromatography (TLC) on a silica coated aluminum sheet (silica gel $60F_{254}$) using chloroform and methanol (9:1, v/v). IR spectra were recorded on NICOLET AVATAR 330-FTIR Spectrometer. ^1H and ^{13}C NMR spectra recorded on a Varian 300 MHz NMR spectrometer using TMS as an internal standard. Chemical shifts are reported in ppm (δ) and signals are described as singlet (s), doublet (d), triplet (t), quartet (q), broad (br,), and multiplet (m). The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 spectrophotometer/Data system using Argon/Xenon (6KV, 10 mA) FAB gas, at 70 eV. Elemental analysis was carried out using Flash EA 1112 Series, CHNSO Analyzer (Thermo). Solvents and reagents were purchased from the commercial venders in the appropriate grade and were used without purification.

General Procedure for the Preparation of Ethyl [4-(Thioalkyl) phenoxy]acetate (2a–b)

4-Alkylthio phenol, **1a–b**, (**1a**: 1.4 g, **1b**: 1.54 g, 10 mmol) was dissolved in 50 mL of dry acetone and mixed with (1.6 g, 12 mmol) of anhydrous potassium carbonate. This was treated with (1.47 g, 12 mmol) of ethyl chloroacetate slowly with shaking over 10 min, and the mixture was heated under reflux for 3 h. When the reaction was complete (monitored by TLC), the reaction mixture was filtered and the filtrate was distilled under 10-mm pressure. The fraction collected at appropriate boiling point (140–144°C for **2a** and 130–135°C for **2b**) was directly used for the next step without further purification.

Procedure for the Preparation of Ethyl [4-Alkyl sulphonyl phenoxy] acetate (2c–d)

The compound **2a–b** (**2a**: 2.26 g, **2b**: 2.4 g 10 mmol), dissolved in 50 ml of glacial acetic acid was added slowly with (2.2 g, 20 mmol) of 30–33% hydrogen peroxide over the period of 1 h, below 70°C. It was further kept at 70–75°C with stirring for 3 h, cooled to ambient temperature, and quenched to 100 ml ice-cold water. The compound **2c–d** was extracted with ethyl acetate and after giving water wash, the solvent was removed by distillation; the liquid left over was further purified by distillation under reduced pressure. The fraction collected at appropriate boiling point (160–164°C for **2c** and 155–159°C for **2d**) at 10-mm pressure was used directly for the next step.

General Procedure for the Preparation of 2-[4-(Alkylthio) phenoxy] acetohydrazide (3a–b) and 2-[4-Alkyl sulphonyl phenoxy] acetohydrazide (3c–d)

A mixture of [4-(alkylthio)/alkyl sulphonyl phenoxy] acetate (**2a**: 2.26 g, **2b**: 2.4 g, **2c**: 2.58, **2d**: 2.72 g, 10 mmol), hydrazine hydrate (1.0 g, 20 mmol) in 50 ml of ethanol was heated under reflux for 5–6 h. The reaction mixture was left overnight at room temperature, and the solid separated was collected by filtration. It was washed with ethanol, dried and recrystallized from ethanol.

3a. 2-[4-(Methylthio) phenoxy] acetohydrazide

This compound was obtained as white solid in a yield of 90%, m.p. 128–129°C, IR: 3310 cm^{-1} (NH_2), 3224 cm^{-1} (NH), 3045 cm^{-1} (SCH_3), 1680 cm^{-1} (>C=O), 1544 cm^{-1} (C=C), 814 cm^{-1} (SCH_3); ^{1}H NMR (DMSO-d$_6$): δ2.4 (s, 3H, SCH_3), δ4.46 (s, 2H, CH_2), δ6.94 (d, 2H, C_2, C_6-H of 4-methylthio phenoxy moiety J = 8.5 Hz), δ 7.24 (d, 2H, C_3, C_5-H of 4-ethylthio phenoxy moiety J = 8.5 Hz); *Anal.* Calculated for $C_9H_{12}N_2O_2S$: C, 50.92; H, 5.70; N, 13.20; found: C, 50.8; H, 5.9; N, 13.1.

3b. 2-[4-(Ethylthio) phenoxy] acetohydrazide

This compound was obtained as white solid in a yield 92%, m.p. 126°C (methanol), IR:3310 cm^{-1} (NH_2), 3224 cm^{-1} (NH), 3041 cm^{-1} (SCH_3), 1680 cm^{-1} (C=O), 1547cm^{-1} (C=C), 813cm^{-1} (SCH_3); *Anal.* Calculated for $C_{10}H_{14}N_2O_2S$: C, 53.08; H, 6.24; N, 12.38; found: C, 53.12; H, 6.30; N, 12.45.

3c. 2-[4-Methyl sulphonyl phenoxy] acetohydrazide

This compound was obtained as white solid in a yield 85%, m.p. 190°C, IR: 3315 cm^{-1} (NH_2), 3276 cm^{-1} (NH), 3101 cm^{-1} (SCH_3), 1689 cm^{-1} (C=O), 1615 cm^{-1} (C=C), 1496 cm^{-1} (C=C); *Anal.* Calculated for $C_9H_{12}N_2O_4S$: C, 44.25, H, 4.95; N, 11.47; found: C, 44.28; H, 4.99; N, 11.45.

3d. 2-[4-Ethyl sulphonyl phenoxy] acetohydrazide

This compound was obtained as white solid in a yield 85%, mp 176°C IR: 3317 cm^{-1} (NH_2), 3276 cm^{-1} (NH), 3104 cm^{-1} (SCH_3), 1688 cm^{-1} (C=O), 1614 cm^{-1} (C=C), 1496 cm^{-1} (C=C).

General Procedure for the Preparation of 2-{[4-Alkyl thio/alkyl sulphonyl) phenoxy] methyl}-5-phenyl-1,3,4-oxadiazole (4a–y)

A mixture of 2-[4-(alkyl thio/alkyl sulphonyl) phenoxy] acetohydrazide **(3a–d)** (1mmol), aromatic acid (1 mmol), and phosphorous oxy chloride (5 ml) was refluxed for 5 to 10 min. The reaction mixture was gradually poured onto crushed ice with stirring and neutralized with solid sodium bicarbonate. The aqueous layer was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and finally distilled under reduced pressure to remove the solvent. The resulting white solid was recrystallized using ethyl acetate.

2-{[4-(Methylthio)phenoxy]methyl}-5-phenyl-1,3,4- oxadiazole (4a)

IR: 3009 cm^{-1} (SCH_3), 1696 cm^{-1} (C=N), 1494 cm^{-1} (C=C), 1242 (C—O—C), ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3), δ 5.30 (s, 2H, OCH_2), δ 6.89 (d, 2H, C_2, C_6-H of 4-methyl thio phenoxy moiety J = 8.4), δ 7.28 (d, 2H, C_3, C_5-H of 4-methylthio phenoxy moiety J = 7.2), δ 7.50 (m, C_3, C_4,C_5-H 5-phenyl moiety J = 8.1), δ 8.00 (d 2H, C_2, C_6-H of 5-phenyl moiety J = 7.1), ^{13}C-NMR δ: 17.32, 60.14, 115.41, 129.08, 129.52, 129.6, 131.02, 131.22, 132.5, 155.89, 162.19, 164.97; DEPT: CH and $CH_3\delta$: 17.32, 115.41, 129.08, 129.5 131.02, 131.22, $CH_2\delta$: 60.14; MS FAB$^+$: m/z (%) = 317 (100) $[M+NH_4+H]^+$, 316(80) $[M+NH_4]^+$.

2-(4-Methylphenyl)-5-{[4-(methylthio) phenoxy] methyl}-1,3,4-oxadiazole (4b)

IR: 3043 cm^{-1} (SCH_3), 1736 cm^{-1} (C=N), 1496 cm^{-1} (C=C), 1239 (C—O—C) , ^1H NMR (DMSO-d_6): δ 2.50 (s, 3H, SCH_3), δ 3.33 (s, 3H, CH_3 of 5-phenyl moiety), δ 5.45 (s, 2H, OCH_2), δ 7.00 (d, 2H, C_2, C_6-H of 4-methyl thio phenoxy moiety J = 7.2), δ 7.28 (d, 2H, C_3, C_5-H of 4-methylthio phenoxy moiety J = 8.1), δ 7.42 (d 2H , C_3, C_5-H of 5-phenyl moiety J = 6.9), δ 7.90 (d 2H, C_2, C_6-H of 5-phenyl moiety J = 8.1); MS FAB$^+$: m/z (%) = 313 (100%) $[M+ 1]^+$, 312 (60%) M^+, 173(30%), 119 (30%), 91 (10%).

2-(2-Methoxyphenyl)-5-{[4-(methylthio) phenoxy] methyl}-1,3,4-oxadiazole (4f)

IR: 3045 cm^{-1} (SCH_3), 1612 cm^{-1} (C=N), 1493 cm^{-1} (C=C),1243 (C—O—C); ^1H NMR (DMSO-d_6): δ 2.50 (s, 3H, SCH_3), δ 3.80 (s, 3H, OCH_3 of 5-phenyl moiety), δ 4.65 (s, 2H, OCH_2), δ 7.00 (d, 2H, C_2, C_6-H of 4-methyl thio phenoxy moiety J = 8.7), δ 7.28 (d 2H , C_3, C_5-H of 4-methylthio phenoxy moiety J = 8.5), δ 7.49 (m, 4H, C_2, C_4, C_5, C_6-H of

5-phenyl moiety J = 7.8); MS FAB$^+$: m/z (%) = 347 (100%) [M+NH$_4$+H]$^+$, 346 (60%) [M+NH$_4$]$^+$, 91 (10%).

2-{[4-(Ethyl thio) phenoxy] methyl}-5-phenyl-1,3,4-oxadiazole (4j)

IR: 3007 cm^{-1} (SCH$_3$), 1703 cm^{-1} (C=N), 1494 cm^{-1} (C=C),1245 (C—O—C); ^{1}H NMR (DMSO-d$_6$): δ 1.20 (t, 3H, CH$_3$ of SCH$_2$CH$_3$), δ 2.86 (q, 2H, CH$_2$ of SCH$_2$ CH$_3$), δ 4.66 (s, 2H, OCH$_2$), δ 6.90 (d, 2H, C$_2$, C$_6$-H of 4-methyl thio phenoxy moiety J = 8.9), δ 7.32 (d 2H, C$_3$, C$_5$-H of 4-ethylthio phenoxy moiety J = 8.7), δ 7.50 (m, C$_3$, C$_4$, C$_5$-H of 5-phenyl moiety J = 7.5), δ 7.90 (d 2H, C$_2$, C$_6$-H of 5-phenyl moiety J = 8.4); LCMS: m/z (%) = 313 (100%) M$^+$, 154 (10%).

2-{[4-(Methylsulphonyl) phenoxy]methyl}-5-(4-methylphenyl)-1,3,4-oxadiazole (4q)

IR: 3021 cm^{-1} (SO$_2$CH$_3$), 1694 cm^{-1} (C=N), 1514 cm^{-1} (C=C), 1253 (C—O—C); ^{1}H NMR (DMSO-d$_6$): δ 3.10 (s, 3H, SO$_2$CH$_3$), δ 4.90 (s, 2H, OCH$_2$), δ 7.57 (d, 2H, C$_2$, C$_6$-H of 4-methylsulphonyl phenoxy moiety J = 6.9). δ 7.66 (m, C$_{2,}$ C$_3$, C$_4$, C$_{5,}$ C$_6$-H of 5-phenyl moiety J=6.0), δ 8.00 (d, 2H, C$_3$, C$_5$-H of 4-methyl sulphonyl phenoxy moiety J = 8.6); MS FAB$^+$ m/z (%) = 349 (80%) [M+NH$_4$+1]$^+$, 348 (60%) [M+NH$_4$]$^+$, 165 (40%), 115 (40%).

2-{[4-(Ethylsulphonyl) phenoxy] methyl}-5-phenyl-1,3,4-oxadiazole (4w)

IR: 3006 cm^{-1} (SO$_2$C$_2$H$_5$), 1703 cm^{-1} (C=N), 1513 cm^{-1} (C=C),1260 (C—O—C); ^{1}H NMR (DMSO-d$_6$): δ 1.12 (t, 3H, CH$_3$ of SO$_2$C$_2$H$_5$), δ 3.26 (q, 2H, CH$_2$ of SO$_2$C$_2$H$_5$), δ 4.82 (s, 2H, OCH$_2$), δ 7.23 (d, 2H, C$_2$, C$_6$-H of 4-methylthio phenoxy moiety J = 8.7). δ 7.53 (d, 2H, C$_3$, C$_5$-H of 4-methyl thio phenoxy moiety J = 7.5), δ 7.87 (m, C$_{2,}$ C$_3$, C$_4$, C$_5$, C$_6$-H of 5-phenyl moiety J = 7.2),

General Procedure for the Preparation of 5-{[4-(Alkylthio) phenoxy] methyl}-1,3,4-oxadiazole-2-thiol (5a,b)

The mixture of 2-[4-(alkyl thio) phenoxy] acetohydrazide **3a,b** (**3a**: 3.18 g, **3b**: 3.39, 15 mmol) in ethanol (25ml) was added to the solution of potassium hydroxide (1.68 g, 30 mmol) in water (20 ml) at 5–10°C and stirred for 10 min.; then CS$_2$ (3.42 g, 45 mmol) was added slowly below 10°C. Heated the reaction mass to reflux until evolution of H$_2$S ceased (5–6 h), and the reaction mixture was cooled to room temperature and poured into ice-cold water. It was then neutralized with diluted hydrochloric acid. The precipitated solid was filtered, washed with water, and dried. The product was recrystallized from ethanol.

5-{[4-(Ethylthio) phenoxy] methyl}-1,3,4-oxadiazole-2-thiol (5b)

IR: 3007 cm^{-1} (SCH_3), 1703cm^{-1} (C=N), 1492 cm^{-1} (C=C), 1238 (C—O—C); ^{1}H NMR ($CDCl_3$): δ 1.26 (t, 3H, SCH_2CH_3), δ 2.85 (q, 2H, SCH_2CH_3), δ 5.00 (s, 2H,OCH_2), δ 6.87 (d, 2H, C_2, C_6-H of 4-ethyl thio phenoxy moiety J = 8.7), δ 7.35 (d, 2H, C_3, C_5-H of 4-ethylthio phenoxy moiety J = 8.4); ^{13}C-NMR δ: 14.49, 29.16, 59.91, 115.49, 129.38, 132.39, 156.04, 159.41, 178.76; DEPT: CH and $CH_3\delta$: 14.49, 115.49, 132.39 $CH_2\delta$: 29.16, 59.91; MS FAB$^+$: m/z (%) = 269(60), [M +1]$^+$, 268[M]$^+$, 107 (30%).

General Procedure for the Preparation of 2-(Alkylthio)-5-{[4-(alkylthio) phenoxy] methyl}-1,3,4-oxadiazole (5c–f)

A solution of sodium hydroxide (0.8 g, 20 mmol) in water (25 ml) was cooled to 5–10°C and slowly added 2-{[4-(alkylthio) phenoxy] methyl}-1,3,4-oxadiazole-5-thiol **5a,b** (**5a**: 2.54 g, **5b**: 2.68 g, 10 mmol) and stirred the reaction mixture to dissolve completely. To the clear reaction mixture, 12 mmol of dimethyl sulfate (1.512 g) or diethylsulphate (1.848 g) or chloroacetic acid (1.134 g) was added slowly with stirring; and, the stirring was continued for 2 h at 50–55°C. The product was cooled to room temperature and poured into ice-cold water (100 ml). It was then neutralized with diluted hydrochloric acid. The precipitated solid was filtered, washed with water, and dried. It was recrystallized from ethanol.

2-(Methylthio)-5-{[4-(methylthio) phenoxy] methyl}-1,3,4-oxadiazole (5c)

IR: 2929 cm^{-1} (CH_3), 1594 cm^{-1} (C=N), 1494 cm^{-1} (C=C), 1231 (C—O—C), ^{1}H NMR (DMSO-d_6); δ 2.45 (s, 6H, SCH_3), δ 5.00 (s, 2H,OCH_2), δ 6.85 (d, 2H, C_2, C_6-H of 4-methyl thio phenoxy moiety J = 8.7), δ 7.35 (d, 2H, C_3, C_5-H of 4-methylthio phenoxy moiety J = 8.4); LC mass: m/z (%) = 269 (100%) M$^+$, 128.9 (20%),

BIOLOGICAL ACTIVITY

Antibacterial Studies

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus* *aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853), and *Klebsiella pneumoniae* (recultured) bacterial stains by serial plate dilution method.[13,14] Serial dilutions of the drug in Muller-Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37°C. The Minimum Inhibitory Concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

Twenty milliliters of above prepared agar media was poured in to each petri dishes. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for 1 h. Using a punch, wells were made on these seeded agar plates and Minimum Inhibitory Concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3–4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Ciprofloxacin as standard.[15] Zones of inhibition were determined for **4a–5h** and the results are summarized in Table III.

The investigation of antibacterial screening data revealed that all the tested compounds **4a–y** and **5a–h** showed moderate to good inhibition at 1.56–25 μg/ml in DMSO. The compounds **4r**, **4u**, **4w**, **4x**, **4y**, **5a**, **5b**, and **5d** showed comparatively good activity against all the bacterial strains. The good activity is attributed to presence of pharmacologically active Cl, CH_3, OCH_3, groups attached to phenyl group in position 5 of the oxadiazole ring and SH, SCH_3 groups attached position 2 of oxadiazole ring. The presence of SO_2CH_3, $SO_2C_2H_5$, SCH_3, and SC_2H_5 groups at position 4 of phenoxy group caused good activity against most of the strains. The compounds **4a**, **4b**, **4c**, **4d**, **4e**, **4g**, **4i**, **4o**, **4q**, **4s**, **4t**, **4v**, **4c**, **4e**, and **4f** exhibited moderate activity compared to that of standard against all the bacterial strains.

Antifungal Studies

Newly prepared compounds were screened for their antifungal activity against *Aspergilus flavus* [NCIM No.524], *Aspergilus fumigatus* [NCIM No. 902], *Penicillium marneffei* [recultured], and *Trichophyton mentagrophytes* [recultured] in DMSO by serial plate dilution method.[16,17] Sabouraud's agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was

TABLE IV Antifungal Activity of the Prepared Compounds

Compd.	MIC in μg/ml and zone of inhibition (in mm)			
	P.marneffei	*T.mentagrophytes*	*A.flavus*	*A.fumigatus*
4a	12.5 (11–15)	25 (<10)	25 (<10)	12.5 (11–15)
4b	25 (<10)	12.5 (11–15)	25 (<10)	12.5 (11–15)
4c	12.5 (11–15)	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)
4d	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	12.5 (11–15))
4e	12.5 (11–15)	12.5 (11–15)	25 (<10)	6.25 (16–20)
4f	25 (<10)	12.5 (11–15)	25 (<10)	12.5 (11–15)
4g	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)	6.25 (16–20)
4h	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)
4i	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4j	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4k	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4l	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)
4m	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4n	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4o	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
4p	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)
4q	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4r	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4s	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
4t	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
4u	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)
4v	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
4w	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)
4x	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
4y	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5a	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)	6.25 (16–20)
5b	12.5 (11–15)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
5c	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)	6.25 (16–20)
5d	6.25 (16–20)	6.25 (16–20)	12.5 (11–15)	6.25 (16–20)
5e	25 (<10)	12.5 (11–15)	12.5 (11–15)	12.5 (11–15)
5f	12.5 (11–15)	12.5 (11–15)	25 (<10)	12.5 (11–15)
5g	25 (<10)	25 (<10)	12.5 (11–15)	25 (<10)
5h	25 (<10)	25 (<10)	25 (<10)	12.5 (11–15)
(Ciclo-piroxolamine)	6.25 (20–27)	3.125 (27–33)	3.125 (25–30)	6.25 (25–30)

The MIC values were evaluated at concentration range, 1.56–25 μg/ml. The figures in the table show the MIC values and the corresponding zone of inhibition (in mm).

transferred to 3 ml saline to get a suspension of corresponding species. Twenty mL of agar media was poured in to each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for 1 h. Using a punch, wells were made on these seeded agar plates Minimum Inhibitory Concentrations of the test compounds in DMSO were added into each well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37°C for 3–4 days. Antifungal activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with Ciclopiroxolamine as standard. Zones of inhibition were determined for **4a–5h** and the results are summarized in Table IV.

The results of antifungal screening of tested compounds indicate that the compounds **4c**, **4d**, **4h**, **4i**, **4j**, **4k**, **4l**, **4m**, **4n**, **4o**, **4p**, **4q**, **4r**, **4s**, **4t**, **4u**, **4v**, **4w**, **4x**, **4y**, **5a**, **5b**, **5c**, and **5d** showed comparatively good activity against all the fungal strains. The structure of these compounds contain biologically active CH_3, Cl, OCH_3, SH, SCH_3 and SCH_2COOH groups attached to phenyl group in position 2 of the oxadiazole. The compounds **4a**, **4b**, **4e**, **4f**, and **4g** exhibited moderate activity compared to that of standard. Results of antifungal screening showed that presence of SCH_3, SC_2H_5, SO_2CH_3 and $SO_2C_2H_5$ groups at position 4 of phenoxy group caused increased activity.

CONCLUSION

The research study reports the successful synthesis and antimicrobial activity of new 1,3,4-oxadiazoles carrying 4-alkylthio and alkylsulfonyl phenoxy moieties at position 2. The antimicrobial activity study revealed that all the compounds tested showed moderate to good antibacterial and antifungal activities against pathogenic strains. Structure and biological activity relationship of the title compounds showed that presence of 4-alkylthio and 4-alkylsulfonyl phenoxy groups at position-2 and biologically active groups like CH_3, OCH_3, Cl, $(CH_3)_2$, SH, SCH_3, SCH_2COOH groups at aryl moiety attached to position-5 of title compounds are responsible for increased antimicrobial activity in newly synthesized title compounds.

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