Indian Journal of Chemistry
Vol. 65, August 2026, pp. 729-735
DOI: 10.56042/ijc.v65i8.14847

Synthesis and antimicrobial screening of novel oxadiazole doped pyrazoline derivatives

Ajay Rathod*[a], Chirag Patel*[a], Akhil Hadiya[b] & Falguni Bhabhor[c]

[a] Department of Chemistry, KSKV Kachchh University, Bhuj 370 015, Gujarat, India

[b] Department of Chemistry, Saurashtra University, Rajkot 360 005, Gujarat, India

[c] Bhavan's Sheth R A College of Science,Gujarat University, Ahmedabad 380 001, Gujarat, India

E-mail: ajayrathod.chem@gmail.com, patel87chirag@gmail.com

Received 4 November 2024; accepted (revised) 1 August 2026

The numerous therapeutic uses of oxadiazole and pyrazoline make them significant pharmaceutical scaffolds. Oxadiazole clubbed derivatives have drawn the attention of numerous researchers in their hunt for lead compounds. This study has sought to synthesize a novel series of 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol)-1-(5-(phenyl)-3-phenyl-1*H*-pyrazol-1-yl)ethanones from 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol. FT-IR, ^{1}H and ^{13}C NMR, and mass spectra have been used to characterize each new chemical. The disk diffusion method has been used for antimicrobial screening. Compound **6e** bearing R= 4-N$(CH_3)_2$, R'= H displays 33, 24, 19, 18, 19 and 18 mm ZI towards *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Candida albican* and *Aspergillus niger* respectively. Compound **6l** having R= 4-NO_2, R'= 4-OCH_3 substituent has shown maximum potency against *B. subtilis* and *E. aerogenes* with ZI value of 24 and 22 mm. Compound **6o** (R= 4-N$(CH_3)_2$, R'= 4-Cl) is found most active against *C. albican* and *A. niger* with the ZI value of 22 mm.

Keywords: Oxadiazole, Pyrazoline, Antibacterial, Antifungal

These days, many commercially available medications are becoming less effective against specific cancer cells, germs, fungi, and other illnesses, which increase the rate of disability and death and prolongs the course of the illness[1,2].

It has been noted that Carbapenem, methicillin, and vancomycin develop resistance to Enterobacteriaceae, *S. aureus*, and Enterococci, respectively[3]. Anti Microbial Resistance rises as a result of an inherent characteristic of the microbes, such as DNA/RNA transfer, interaction with a different strain, or mutation[4]. Researchers are interested in clubbed heterocycles because of their possibility as new drug candidates.

In medicinal chemistry, oxadiazole and pyrazolines scaffolds are essential pharmacophores to design biological active new chemical entities[5]. They possess therapeutic applications like analgesic[6], hypoglycemic[7], antihistaminic[8], anticancer[9], tirosinase inhibitor[10], antibacterial[11], *etc*.

Pyrazoline based molecule possess remarkable medicinal properties such as non-nucleoside HIV reverse transcriptase inhibitors[12], antagonists[13], antidiabetic[14], anti-inflammatory[15], *etc*. Fig. 1 illustrates the wide availability of pyrazoline-based and oxadiazole based medications worldwide[16-19].

To identify the effective compounds, we have recently designed unique clubbed heterocycles of oxadiazole and pyrazoline and tested them against both bacterial and fungal strains.

Materials and Methods

All starting material purchased from Sigma Aldrich. FTIR spectra were recorded in a Perkin– Elmer equipment mod. RX1. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions through Brucker 500 MHz spectrometer. Elemental analysis was carried out by ElementarVario EL III elemental analyzer. Mass was carried out through ESI method by shimadzu 2010 int.

Experimental Section

General procedure

Synthesis of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol

5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-thiol was prepared as per reported method[20].

Synthesis of chloro acetyl pyrazoline

Pyrazolines were isolated as per reported method[21-23].

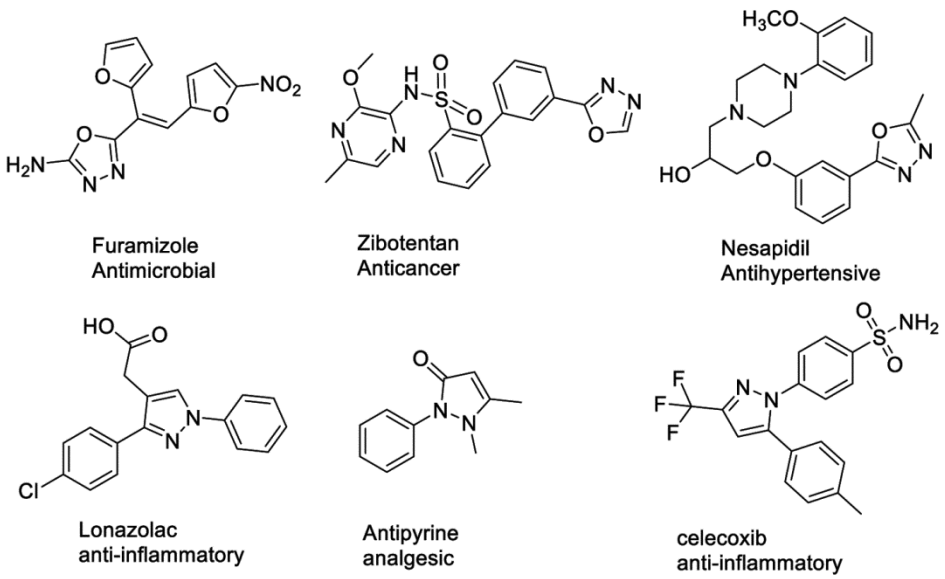
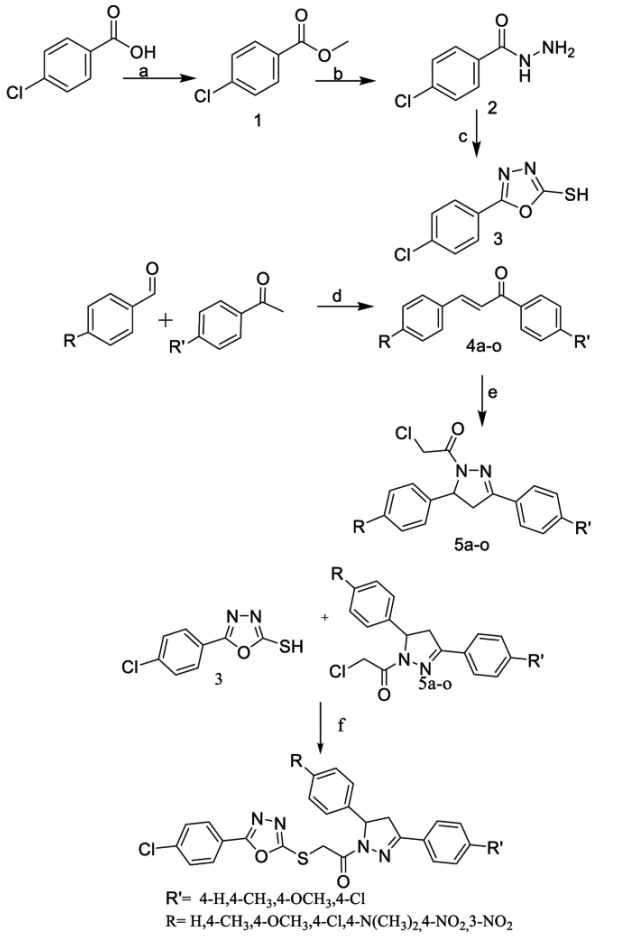


Fig. 1 — Marketed drugs of oxadiazole and pyrazoline

General procedure for synthesis of 2-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-thiol)-1-(5-(phenyl)-3-phenyl-1*H*-pyrazol-1-yl)ethanone derivatives

5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-thiol(2.12 g, 0.01 mol), 2-chloro-1-(4,5-dihydro-5-(3-aryl)-3-arylpyrazol-1-yl)ethanone (0.01mol) and (1.24 g, 0.01mol) of anhydrous K_2CO_3 were dissolved in 100 mL of DMF in a FBF. The reaction mass was agitated at 30-32°C for 8–9 hours**.** TLC (hexane: ethyl acetate; 70:30) was used to monitor the reaction. The mixture was added to cooled water once the reaction was completed. Solids were filtered off as well as recrystallized with ethyl alcohol (Scheme 1).



Scheme 1 — Synthetic pathway of compounds **6a-o**

Spectral Data

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6a: White solid. Mol. Formula: $C_{25}H_{19}ClN_4O_2S$. FT-IR (KBr): 3064, 2925, 2817 (CH), 1668 (C=O), 1610 (C=N), 1526 (C=C), 1342, 1178, 1021, 801, 689 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.3, 3.5 (dd of H_A and H_M), 4.60 (2H,CH_2), 5.57 (dd of Hx), 6.78-7.81 (14H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.41, 40.11, 60.28, 112.87, 114.15, 122.15, 126.45, 129.27, 130.48, 132.12, 155.72, 163.3,164.22, 165.42; MS: *m/z* 474 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6b: White solid. Mol. Formula: $C_{25}H_{18}Cl_2N_4O_2S$. FT-IR (KBr):

3032, 2919, 2826, 1668 (C=O), 1598 (C=N), 1507 (C=C), 1339, 1189, 1009, 854, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.38, 3.52 (dd of H_A and H_M), 4.65 (2H,CH_2), 5.57 (dd of Hx), 6.59-7.97(13H, m, Ar-H); ^{13}C NMR(500 MHz, $CDCl_3$): δ 36.48, 40.89, 60.28, 112.87, 122.21, 126.45, 129.56, 130.78, 132.15, 155.68, 163.41, 164.26, 165.44; MS: *m/z* 508 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-phenyl-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6c: White solid. Mol. Formula: $C_{26}H_{21}ClN_4O_2S$. FT-IR (KBr): 3051, 2923, 2821, (CH), 1671 (C=O), 1602 (C=N), 1535 (C=C), 1334, 1175, 1009, 863, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.49 (3H,s, CH_3) 3.35 , 3.50 (dd of H_A and H_M), 4.59 (2H,CH_2), 5.67 (dd of Hx), 6.23-7.82 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.82, 36.42, 40.78, 42.21, 60.12, 112.87, 122.25, 126.46, 129.48, 130.75, 132.87, 155.67, 163.36, 164.29, 165.51; MS: *m/z* 488 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl) thio)-1-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6d: White solid. Mol. Formula: $C_{26}H_{21}ClN_4O_3S$. FT-IR (KBr): 3029, 2908 (CH), 2843, 1663 (C=O), 1612 (C=N), 1531 (C=C), 1336, 1175, 1005, 851, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.31(dd of H_A), 3.56 (3H,s,OCH_3) 4.66 (2H,CH_2), 5.57 (dd of Hx), 6.59-7.93 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.60, 40.55, 55.57, 60.09, 112.69, 122.32, 126.78, 126.85, 127.94, 128.22, 129.35, 129.52, 137.79, 141.22, 155.83, 163.79, 164.30, 165.09; MS: *m/z* 504 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(4-(dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6e: White solid. Mol. Formula: $C_{27}H_{24}ClN_5O_2S$. FT-IR (KBr): 3035, 2919(CH), 1659 (C=O), 1607 (C=N), 1525 (C=C), 1346, 1181, 1013, 840, 694 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.90 6H,s, CH_3) 3.23, 3.75 (dd of H_A and H_M), 4.63 (2H,CH_2), 5.53 (dd of Hx), 6.69-7.89 (13H, m, Ar-H) ; ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.51, 40.81, 42.27, 60.31, 112.99, 122.15, 126.10, 129.45, 130.60, 132.14, 155.72, 163.3, 164.22, 165.02; MS: *m/z* 517 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-phenyl-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6f: White solid. Mol. Formula: $C_{26}H_{21}ClN_4O_3S$. FT-IR (KBr): 3042, 2911 (CH), 1669 (C=O), 1598 (C=N), 1509 (C=C), 1331, 1178, 1000, 844, 691 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.41 (3H,s, CH_3), 3.22, 3.67 (dd of H_A and H_M), 4.66 (2H,CH_2), 5.54 (dd of Hx), 6.69-7.93 (13H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.50, 36.55, 40.49, 60.18, 112.68, 122.26, 126.70, 126.81, 127.89, 128.25, 129.31, 129.47, 137.69, 141.22, 155.79, 163.85, 164.25, 165.05; MS: *m/z* 488 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(4-chlorophenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6g: White solid. Mol. Formula: $C_{26}H_{20}Cl_2N_4O_2S$. FT-IR (KBr): 3042, 2921 (CH), 1671 (C=O), 1614 (C=N), 1519 (C=C), 1382, 1154, 1007, 861, 703 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.41 (3H,s, CH_3), 3.24, 3.62 (dd of H_A&H_M), 4.63 (2H,CH_2), 5.54 (dd of Hx), 6.61-7.87 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.51, 36.53, 40.62, 60.16, 112.69, 122.08, 126.64, 126.72, 127.85, 128.17, 129.30, 129.44, 137.66, 141.09, 155.79, 163.75, 164.32, 165.08; MS: *m/z* 522 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(3,4-dimethoxyphenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6h: White solid. Mol. Formula: $C_{28}H_{25}ClN_4O_4S$. FT-IR (KBr): 3001, 2931, 2839 (OCH_3), 1666 (C=O), 1597 (C=N) 1520 (C=C), 1342, 1257, 1172, 1018, 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.40 (3H,s, CH_3) 3.20, (dd of H_A) 3.73, 3.79 (3H,s, OCH_3&dd of H_M) 4.65 (2H,CH_2), 5.54 (dd of Hx), 6.76-7.90 (11H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.55, 36.39, 42.60, 55.92, 55.98, 60.40, 109.07, 11.53, 117.86, 122.13, 126.86, 127.89, 129.39, 133.62, 137.86, 141.35, 148.73, 149.35, 155.76, 164.04, 164.11, 165.10; MS: *m/z* 549 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(4-(dimethylamino)phenyl)-3-(*p*-tolyl) -4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6i: White solid. Mol. Formula: $C_{28}H_{26}ClN_5O_2S$. FT-IR (KBr): 3078, 2931 (CH Str), 1674 (C=O), 1604 (C=N), 1527 (C=C), 1188, 1010, 771, 694 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.40 (3H,s, CH_3), 2.89 (6H,s, CH_3) 3.20, 3.69(dd of H_A and H_M), 4.65 (2H,CH_2), 5.51 (dd of Hx), 6.64-7.90 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.54, 36.60, 40.59, 42.30, 60.21, 112.79, 122.16, 126.84, 126.89, 127.99, 128.21, 129.36, 129.50, 137.76, 141.16, 155.81, 163.81, 164.28, 165.00; MS: *m/z* 533 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(3-nitrophenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6j: White solid. Mol. Formula: $C_{26}H_{20}ClN_5O_4S$. FT-IR (KBr): 3052, 2935 (CH), 1666 (C=O), 1602 (C=N), 1532 (C=C), 1339, 1175, 1006, 857, 704 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.41 (3H,s, CH_3) 3.31, 3.61(dd of H_A&H_M), 4.68 (2H,CH_2), 5.50 (dd of Hx), 6.62-7.92 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 21.49, 36.51, 40.54, 60.15, 112.75, 122.08, 126.72, 126.81, 127.93, 128.17, 129.35, 129.46, 137.71, 141.10, 155.76, 163.78, 164.30, 165.05; MS: *m/z* 533 $[M+H]^+$.

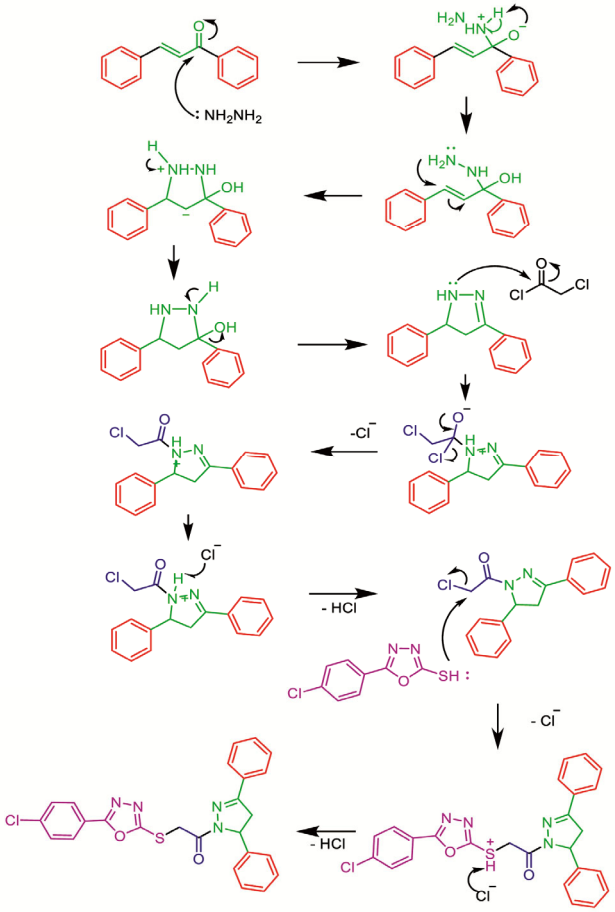
2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-(4-methoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6k: White solid. Mol. Formula: $C_{26}H_{21}ClN_4O_3S$. FT-IR (KBr): 3029, 2908 (CH), 2843, 1663 (C=O), 1612 (C=N), 1531 (C=C), 1336, 1175, 1005, 851, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.31(dd of H_A), 3.56 (3H,s,OCH_3) 4.66 (2H,CH_2), 5.57 (dd of Hx), 6.59-7.93 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.60, 40.55, 55.57, 60.09, 112.69, 122.32, 126.78, 126.85, 127.94, 128.22, 129.35, 129.52, 137.79, 141.22, 155.83, 163.79, 164.30, 165.09; MS: *m/z* 504 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-(4-methoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6l: White solid. Mol. Formula: $C_{26}H_{20}ClN_5O_5S$. FT-IR (KBr): 3042, 2925 (CH), 2843, 1658 (C=O), 1613 (C=N), 1519 (C=C), 1351, 1238, 1172, 1007, 838, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.30(dd of H_A), 3.54 (3H,s,OCH_3)3.5 (dd H_M) 4.62 (2H,CH_2), 5.53 (dd of Hx), 6.65-7.97 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.59, 40.57, 55.54, 60.09, 112.66, 122.31, 126.76, 126.87, 127.96, 128.23, 129.31, 129.50, 137.760, 141.20, 155.85, 163.83, 164.31, 165.10; MS: *m/z* 549 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6m: White solid. Mol. Formula: $C_{25}H_{18}Cl_2N_4O_2S$. FT-IR (KBr): 3034, 2917 (CH), 1653 (C=O), 1604 (C=N), 1521 (C=C), 1275, 1174, 1014, 853, 703 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.33, 3.75 (dd of H_A&H_M), 4.66 (2H,CH_2), 5.54 (dd of Hx), 6.69-7.87 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.51, 40.54, 60.05, 112.75, 122.08, 126.72, 126.81, 127.93, 128.17, 129.35, 129.46, 137.71, 141.10, 155.76, 163.78, 164.30, 165.05; MS: *m/z* 508 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-(4-chlorophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6n: White solid. Mol. Formula: $C_{27}H_{22}Cl_2N_4O_4S$. FT-IR (KBr): 3027, 2911 (CH), 2841, 1667 (C=O), 1601 (C=N), 1519 (C=C), 1246, 1176, 1018, 841, 690 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.30(dd of H_A), 3.62, 3.67 (3H,s,OCH_3) 4.68 (2H,CH_2), 5.59 (dd of Hx), 6.69-8.01 (11H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.51, 40.81, 60.31, 112.99, 122.15, 126, 129, 130,132, 155.72, 163.3, 164.22, 165.42; MS: *m/z* 568 $[M+H]^+$.

2-((5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(3-(4-chlorophenyl)-5-(4-(dimethylamino) phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one, 6o: White solid. Mol. Formula: $C_{27}H_{23}Cl_2N_5O_2S$. FT-IR (KBr): 3027, 2914 (CH), 1664 (C=O), 1597 (C=N), 1517 (C=C), 1147, 1011, 851, 708 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.95 (6H,s,CH_3) 3.31, 3.62 (dd of H_A and H_M), 4.61 (2H,CH_2), 5.56 (dd of Hx), 6.61-7.82 (12H, m, Ar-H); ^{13}C NMR (500 MHz, $CDCl_3$): δ 36.60, 40.55, 55.57, 60.09, 112.69, 122.32,



Scheme 2 — Plausible mechanism

Table 1 — Elemental analysis of compounds **6a-o**

Compd	R'	R	Mol. Formula	m.p. (°C)	Yield (%)	Elemental analysis Calcd (%) C	H	N	S	Found (%) C	H	N	S
6a	H	H	$C_{25}H_{19}ClN_4O_2S$	140	63	63.22	4.03	11.80	6.75	63.15	4.0	11.73	6.70
6b	H	4-Cl	$C_{25}H_{18}Cl_2N_4O_2S$	146	67	58.95	3.56	11.00	6.29	58.82	3.50	10.90	6.22
6c	H	4-CH_3	$C_{26}H_{21}ClN_4O_2S$	118	69	63.84	4.33	11.46	6.56	63.81	4.24	11.41	6.51
6d	H	4-OCH_3	$C_{26}H_{21}ClN_4O_3S$	144	71	61.84	4.19	11.09	6.35	61.69	4.04	11.00	6.20
6e	H	4-$N(CH_3)_2$	$C_{27}H_{24}ClN_5O_2S$	150	60	62.60	4.67	13.52	6.19	62.49	4.55	13.42	6.17
6f	4-CH_3	H	$C_{26}H_{21}ClN_4O_2S$	112	65	63.84	4.33	11.46	6.56	63.75	4.25	11.41	6.50
6g	4-CH_3	4-Cl	$C_{26}H_{20}Cl_2N_4O_2S$	126	66	59.66	3.85	10.70	6.12	59.60	3.80	10.66	6.12
6h	4-CH_3	3,4-di OCH_3	$C_{28}H_{25}ClN_4O_4S$	138	71	61.25	4.59	10.20	5.84	61.2	4.52	10.15	5.79
6i	4-CH_3	4-$N(CH_3)_2$	$C_{28}H_{26}ClN_5O_2S$	82	70	63.21	4.93	13.16	6.03	63.14	4.88	13.12	6.00
6j	4-CH_3	3-NO_2	$C_{26}H_{20}ClN_5O_4S$	148	67	58.48	3.78	13.12	6.00	58.41	3.77	13.07	5.94
6k	4-OCH_3	H	$C_{26}H_{21}ClN_4O_3S$	110	66	61.84	4.19	11.09	6.35	61.75	4.13	11.03	6.30
6l	4-OCH_3	4-NO_2	$C_{26}H_{20}ClN_5O_5S$	136	64	56.78	3.67	12.73	5.83	56.72	3.62	12.66	5.79
6m	4-Cl	H	$C_{25}H_{18}Cl_2N_4O_2S$	140	69	58.95	3.56	11.00	6.29	58.9	3.51	10.94	6.22
6n	4-Cl	3,4-di OCH_3	$C_{27}H_{22}Cl_2N_4O_4S$	126	65	56.95	3.89	9.84	5.63	56.9	3.84	9.78	5.58
6o	4-Cl	4-$N(CH_3)_2$	$C_{27}H_{23}Cl_2N_5O_2S$	152	63	58.70	4.20	12.68	5.80	58.6	4.14	12.63	5.75

126.78, 126.85, 127.94, 128.22, 129.35, 129.49, 137.69, 141.25, 155.91, 163.84, 164.28, 165.11; MS: *m/z* 551 $[M+H]^+$.

Elemental Analysis

Reaction Scheme

Plausible Reaction Mechanism

The plausible mechanism for the formation of compounds **6a-o** is shown in Scheme 2.

Biological Evalution

Antimicrobial Screening

All novel compounds underwent antimicrobial testing utilizing the disk diffusion method against strains of Gram-negative bacteria, such as E. coli and E. aerogenes, and gram-positive bacteria, such as *S. aureus* and *B. subtilis*[24]. Screenings of **6a-o** were also carried out against *C. albican* and *A. niger*. 5 mm Disc were saturated with 200 µg/mL solution in DMSO and placed on media inoculated with bacteria. It was incubated at 35°C for 24 hours. Then Zone inhibition diameters were measured.

Results and Discussion

Chemistry

Synthetic route of 2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(sub-phenyl)-3-subphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one derivatives (**5a-o**) are illustrated in scheme 1. Oxadiazole **3** was prepared from 4-chloro benzoic acid *via* esterification followed by reaction with hydrazine hydrate.

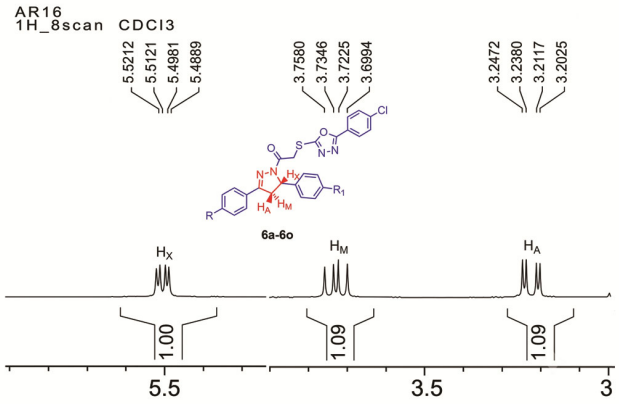


Fig. 2 — AMX pattern of pyrazoline ring shown by **6a-o**

Hydrazones were treated with carbon disulfide in the presence of KOH to achieve **3**.

Chalcones (**4a-o**) were prepared through Claisen Schmidt condensation reactions, which were further treated with chloroacetyl chloride and hydrazine to isolate pyrazoline (**5a-o**). **6a-o** were synthesized through stirring of **5a-o** and **3** in the presence of anhydrous K_2CO_3. Derivatives were characterized using elemental analysis, LCMS, FT-IR, and NMR spectroscopic techniques (Table 1, Fig. 2).

Compound **6d** have displayed IR stretching band at 3035, 2919 (Ar and aliphatic C-H), 1659 (C=O), 1607 (C=N), 1525 (C=C), 1346, 1181 (C-S), 1013, 840, 694; Similarly Compound **6h** have shown band at 2839 (OCH_3), 1666 (C=O), 1597 (C=N) 1520 (C=C), 1342, 1257, 1172 (C-S), 1018, 702 cm^{-1}.

^{13}C NMR of compound **6d** displayed pyrazoline skeleton by 36.51, 42.27, 60.31 as well as 165.02

(C=O). Compound **6h** have shown singlet peak at 21. 55 (CH_3) and 55.92, 55.98 (OCH_3), 165.10 (C=O). Compound **6d** displayed peak at 2.90 (6H,s, CH_3) 3.23, 3.75 (dd of H_A and H_M), 4.63 (q,CH_2), 5.53 (dd,Hx), 6.69-7.89 (13H, m, Ar-H). Compound **6h** have shown proton peak at 2.40 (3H,s, CH_3) 3.20 (dd of Ha) 3.73, 3.79 (3H,s, OCH_3) 4.65 (2H,q), 5.54 (dd,CH), 6.76-7.90 (11H, m, Ar-H).

The appearance of three double-doubles in the ^{1}H NMR spectrum that displayed an AMX pattern for three protons (HA, HM, and HX) of the ring, which verified the presence of the pyrazoline ring. ^{1}H NMR spectra of **6h** displayed AMX pattern through δ 3.20 (dd, 1H, *J*= 17.755 Hz for H_A), δ 3.73 (dd, 1H, *J* = 11.6 Hz for H_M), δ 5.54 (dd, 1H, *J* = 4.8 Hz for H_X). AMX pattern at δ 3.20 (dd, 1H, *J*= 17.75 Hz, H_A), δ 3.69 (dd, 1H, *J* = 11.6 Hz H_M), δ 5.51 (dd, 1H, *J* = 4.55 H_X) proves the presence of pyrazoline ring in comp. **6i**.

Biological Studies

All novel compounds **6a-o** were investigated for antimicrobial potential against gram- positive bacterial culture *S. aureus* (ATCC-25923) and *B. subtilis* (ATCC 6633) and Gram-negative bacteria strains *E. coli* (ATCC-25922) and *E. aerogenes* (ATCC-27853) diffusion method[25]. Screenings of **6a-o** were carried out against *C. albican* and *A. niger*. Amoxicillin, ciprofloxacin and fluconazole were used as the reference standard. The results show that all synthesized compounds are potent against gram-negative bacteria strain as well as gram-positive bacterial strains (Table 2). Among the series, comp. **6e** (R= 4-$N(CH_3)_2$, R'= H) was found most active against *S. aureus*, *B. subtilis* and *E. coli* with the zone of inhibition value 33, 24, 19 mm respectively. Comp. **6l** (R= 4-NO_2, R'= 4-OCH_3) was found most active against *E.aerogenes* (22 mm).Comp. **6d** (R= -4-OCH_3, R'= -H) have shown good to moderate activities towards bacterial stain. Comp. **6j** (R= 3-NO_2, R'= 4-CH_3) displayed 30, 22, 20 and 19 mm inhibition against *S. aureus*, *B. subtilis*, *C. albican* and *A. niger* respectively. comp. **6b**(R= 4-Cl, R'=H) found less to moderate active against bacterial stains. Comp. **6i** (R=4-$N(CH_3)_2$, R'= 4-CH_3) have shown highest antifungal activities against *C. albican* and *A. niger* with MIZ of 24 and 22 mm. Compound **6o** (R= 4-$N(CH_3)_2$, R'= 4-Cl) have shown excellent inhibition of *C. albican* and *A. niger* with MIZ of 22 and 22 mm while **6b** (R= 4-Cl, R'= -H), **6f** (R= -H, R'= 4-CH_3), **6g** (R= -4-Cl, R'= -4-CH_3) found less active towards fungal strains.

Table 2 — Antimicrobial screening result of 6a-o

Compd	Zone of Inhibition (mm)					
	Gram Positive		Gram negative		Fungal strains	
	S. aureus	*B. subtilis*	*E. coli*	*E. aerogenes*	*C. albican*	*A. niger*
6a	19	16	15	14	11	13
6b	24	19	17	16	18	17
6c	19	17	14	13	18	17
6d	30	20	16	17	18	18
6e	33	24	19	18	19	18
6f	28	18	15	16	15	14
6g	27	21	16	14	14	15
6h	28	19	18	19	22	21
6i	27	21	17	17	24	22
6j	30	22	18	17	20	19
6k	29	20	18	20	19	17
6l	30	24	19	22	18	19
6m	28	20	17	19	20	19
6n	27	20	19	18	19	18
6o	29	22	18	18	22	22
Amoxicillin	40	28	22	24	–	–
Ciprofloxacin	40	36	28	30	–	–
Fluconazole	–	–	–	–	28	26

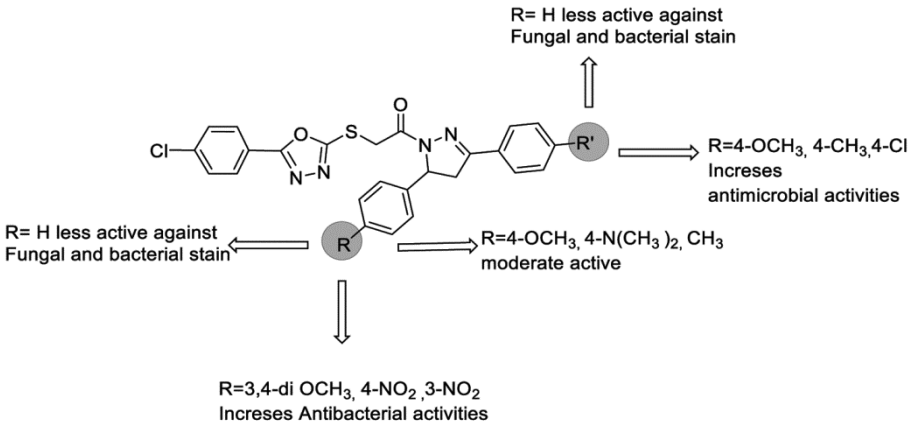


Fig. 3 — Structure Activity Relationship of 6a-o

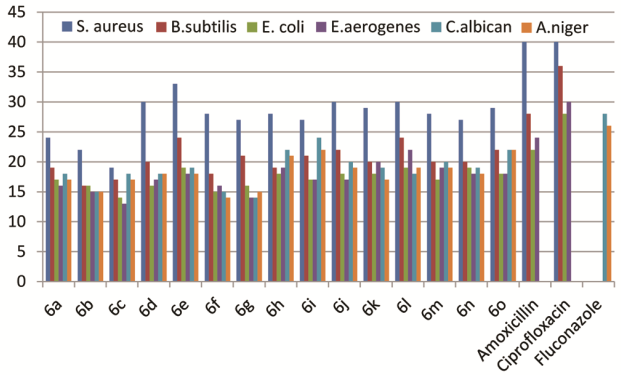


Fig. 4 — Zone of Inhibition (mm) of compounds 6a-o

A brief investigation of the structure-activity relationship (SAR) revealed that *para* and *meta* position of benzene ring was substituted with an electron withdrawing group like NO_2, $N(CH_3)_2$ and *para* position of second benzene ring was substituted with an electron-donating group like $–OCH_3$ and $-CH_3$ substitution at the benzene ring contributed to better antibacterial activity shown in Fig. 3 (such as Compounds **6e**, **6l** and **6j**). Fig. 4 shows the zone of Inhibition (mm) of compounds **6a-o** for all the tested microbes.

Conclusion

In present study, we have designed, prepared and characterized new derivatives of 2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)-1-(5-(sub-phenyl)-3-subphenyl-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one derivatives (**6a-o**) where 2-pyrazolines were combined with oxadiazole. All novel derivatives were investigated for antibacterial against gram +ve and gram -ve strains where compounds **6e**, **6l**, **6j** and **6o** bearing nitro, methoxy and chloro substitution found most active against *S. aureus*, *B. subtilis*, *E. coli* and *E. aerogenes*. Compound **6d**, **6m**, **6n** and **6b** were found less to moderately active against bacterial stains. Compound **6i**, **6o** and **6h** have shown excellent antifungal activities against *C. albican* and *A. niger* with more than 20 mm inhibition. Compound **6b**, **6f** and **6g** were found less active toward fungal strains.

Supplementary Information

Supplementary information is available in the website https://nopr.niscpr.res.in/handle/123456789/58776.

Funding

None.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

Authors are thankful to Department of Chemistry, KSKV Kachchh University,Bhuj for providing laboratory facilities.The authors are also thankful to SAIF, Punjab University, Chandigadh for providing Analysis facilities

References

1 Chen L, Cheng M, Wang S, Zheng Q, & Liu Q, *RSC Adv*, 8 (2018) 41192.
2 Bossche H V, Marichal P & Odds F. C, *Trends Microbio*, 2 (1994) 393.
3 Bouchillon S K, Johnson B M, Hoban D J, Johnson J L, Dowzicky M J, Wu D H & Bradford P A, *Int J Antimicro Agents*,24 (2004) 119.
4 Coates A, Hu Y, Bax R & Page C, *Nature Rev Drug Disc*, 1 (2002) 895.
5 Shukla C & Srivastava S, *J Drug Del Therap*, 5 (2015) 8.
6 Dewangan D, Pandey A, Sivakumar T, Rajavel R, & Dubey R D, *Int J Chem Tech Res,* 2 (2010) 1397.
7 O'Neal J B, Rosen H, Russell P B, Adams A C, & Blumenthal A, *J Med Chem*, 5 (1962) 617.
8 Rao A R, & Reddy V M, *J Pharm Sci*, 83 (1994) 953.
9 Glomb T, Szymankiewicz K & Świątek P, *Molecules*, 23 (2018) 3361.
10 Sadek M M E, Hassan S Y, Abdelwahab H E & Yacout G A, *Molecules*, 18 (2013) 8550.
11 Hannoun M H, Hagras M, Kotb A, El-Attar A A M & Abulkhair H S, *Bioorg Chem*, 94 (2020) 103364.
12 Mowbray C E, Burt C, Corbau R, Perros M, Tran I, Stupple P A & Wood A, *BMC Lett*, 19 (2009) 5599.
13 Acker T M, Khatri A, Vance K M, Slabber C, Bacsa J, Snyder J P & Liotta D C, *J Med Chem*, 56 (2013) 6434.
14 Kumar A, Kumar P, Shetty C R, James J P & Shetty H C, *Indian J Pharm Edu Res*, 55 (2021) 574.
15 Sahu S K, Banerjee M, Samantray A, Behera C & Azam M A, *Tropical J Pharm Res*, 7 (2008) 961.
16 Desai N C, Bhatt K,Jadeja D J, Mehta H K, Khedkar V M & Sarkar D, *Drug Dev Res*, 83 (2022) 416.
17 Nehra B, Rulhania S, Jaswal S, Kumar B, Singh G & Monga V, *European J Med Chem*, 205 (2020) 112666.
18 Tripathi G, Kumar A, Rajkhowa S & Tiwari V K, *Elsevier*, (2021) 421.
19 A Siwach & Verma P K, *BMC Chem*, 14 (2020) 1-40.
20 Gul S, Abbasi M A, Nafeesa K, Siddiqa A, Khan K M, Shahid M & Subhani Z, *J Chem Soc Pak*, 36 (2014) 503.
21 Hadiyal A, Rathod A & Dhakhda A, *WJPPS*, 13 (2024) 1391.
22 Dhakhda S, Baxi G, Gusai R, Hadiyal A, Rathod A, *Heterocyclic Lett*, 13 (2023) 811.
23 Baxi G, Rathod A, Bhatt J & Hadiyal A, *Rasayan J Chem*, 16 (2023) 1805.
24 Biemer J J, *Annals Clin Lab Sci*, 3 (1973) 135.
25 Bhatt J J, Hadiyal A, Baxi G, & Rathod A, *IJSRSET*, 10 (2023) 244.