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Solid phase synthesis of novel 1,3,4-oxadiazole derivatives and evaluation of their antimicrobial activity

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ABSTRACT

A series of 5-(N-Aryl/Arylalkyl)-2-(3-Chclorophenyl)-1,3,4-oxadiazole have been synthesized. The constitution of the products has been delineated by elemental analysis and spectral analyses. All the synthesized compounds were screened for their antibacterial activity against Staphylococcus aureus MTCC-96, Escherichia coli MTCC-443, B. subtilis MTCC-441, P. aeruginosa MTCC-1688, and antifungal activity against Aspergillus niger MTCC-282 and Penicillium SP.at different concentration and compared with standards drugs. The minimum inhibition concentration (MIC) of the compounds was studied by micro broth dilution method.

***Keywords*:** Aminoazole, 1,3,4-oxadiazole, in vitro antimicrobial assay, s-methylthioamide

1. INTRODUCTION

The presence of two nitrogen and one oxygen heteroatoms in five membered rings Systems differentiate a unique class of compounds, the oxadiazole. Oxadiazoles belong to an imperative group of heterocyclic compounds having a toxophoric –N=C-O– linkage. 1,3,4-Oxadiazole is a thermally static aromatic molecule [1]. They have drawn very usefulness to medicinal chemists from two decades due to its spacious diversity of activity, low toxicity and

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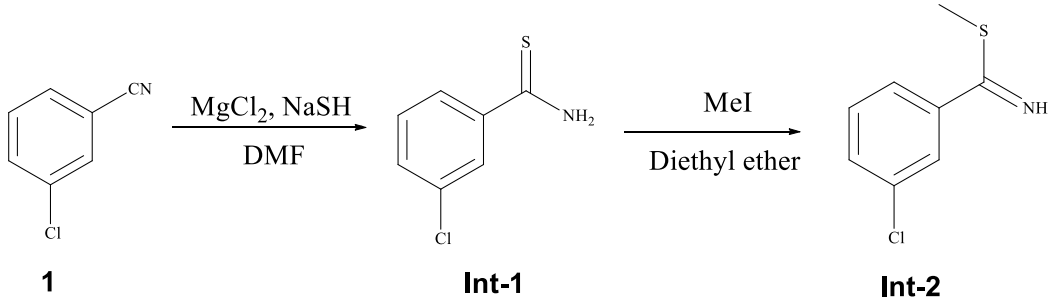
good Pharmacokinetic and Pharmacodynamics outlines [2]. This is because of massive number of applications of 1,3,4-oxadiazoles in the most distinct areas specially drug synthesis, dye stuff industry, heat resistant materials, heat resistant polymers and scintillates, change of the respective article before to 1965 are suitable [3].

Literature survey explain that the nitrogen and oxygen containing heterocyclic compounds crucial to antibacterial and antifungal drugs [4]. Among the various types of five-member heterocyclic rings, the 1,3,4-oxadiazoles are the class of heterocyclic compounds with a wide range of pharmaceutical and biological action [5, 6]. The 1,3,4-oxadiazoles possessed various biological activity like antimicrobial [7-9], antifungal [10], antiproliferative [11], anticancer [12, 13], tyrosinase inhibitor [14] and Tiodazosin possessing oxadiazole nucleus are broadly used as antihypertensive [15]. Other biological properties related with 1,3,4-oxadiazole skeletal includes anti-inflammatory as well as analgesic activity [16-18]. In context to vast medicinal significance; a number of ways have been advanced for synthesizing 1,3,4-oxadiazoles [19-21]. A number of synthetic methods under hard circumstances using various reagents viz. Cu(II) and phosphorous oxy-chloride have been established [22, 23].

The desired compounds were prepared by multi-step synthesis process. The creation of intermediates and their corresponding derivatives was proved by spectral characterization such as ^{1}H NMR, ^{13}C NMR, mass spectra, IR and elemental analysis. The compounds were reserved for their antimicrobial properties against a broad spectrum of gram-positive and gram-negative bacteria as well as fungi.

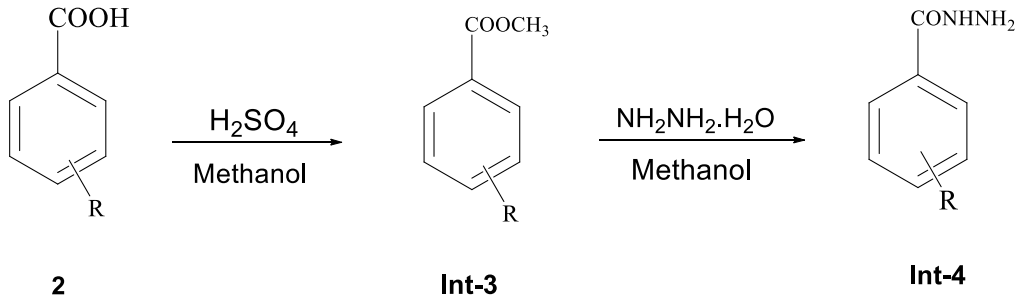
2. EXPERIMENTAL

All research chemicals were purchased from Sigma-Aldrich Chemicals and used as received. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel GF254 plates (E-Merck Co) by using appropriate solvent systems. Melting points were determined in open capillaries and are uncorrected. Infrared (IR) spectra (KBr pellets) were recorded on a Shimadzu-FTIR-8400 spectrophotometer over frequencies ranging from 4000-400 cm^{-1}. The NMR Spectra (^{1}HNMR & ^{13}C NMR) were recorded on a Bruker Avance Spectrospin 400 MHz spectrometer using $CDCl_3$ as solvents and TMS as an internal standard. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 spectrometer by using Electron Impact (EI) (0.7 kV) ionization source. The ion source temperature was 220 °C and interface temperature was 240 °C.



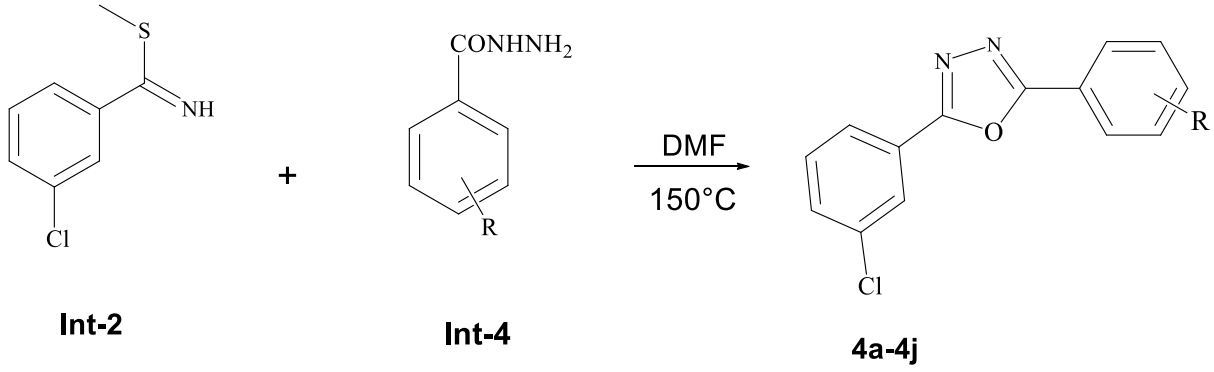
1 **Int-1** **Int-2**

Scheme-1 synthesis of methyl 3-chlorobenzimidothioate(Int-2)



2 **Int-3** **Int-4**

Scheme - 2: Synthesis of hydrazides (Int-4):



Int-2 **Int-4** **4a-4j**

Scheme - 3 : Synthesis of 5-(N-Aryl/Arylalkyl)-2-(3-Chclorophenyl)-1,3,4-oxadiazole (4a-aj)

General procedure:

A. Synthetic procedures for Intermediates

- **Synthesis of 3-chlorobenzothioamide (Int-1)**

To the stirred solution of 3-chlorobenzonitrile **1** (10 g, 0.072 mol) in DMF (100 mL), $MgCl_2 \cdot 6H_2O$ (36.89 g, 0.1817 mol) was added at 0-5 °C. The resultant solution stirred for 10 min, and then sodium hydrogensulphide (8.54 g, 0.1526 mol) was added portion wise in cooling. A mixture was stirred at room temperature for 1 h. After the completion of reaction, mixture was poured in to cold water. The separated product was filtered and washed with water and dried it in hot air oven (10.8 g, 87%).

- **Synthesis of methyl 3-chlorobenzimidothioate (Int-2)**

To a solution of 3-chlorobenzothioamide (Int-1) (10 g, 0.0582 mol) in diethyl ether (100 mL), methyl iodide (10.7 g, 0.0757 mmol) was added at 0 °C. This solution was stirred for 30 min at 0 °C then warmed to room temperature for 10 h. The resulting solid was filtered and washed with diethyl ether (20 mL) to obtain pure product (Int-2) (9.5 g, 90%).

- **Synthesis of ester of various benzoic acid (Int-3)**

To the stirred solution of various substituted benzoic acid (2 g, 0.01637 mol) in methanol (4 mL), concentrated sulfuric acid was added in a catalytic amount and the mixture was refluxed for 6 hr. After the completion of reaction, methanol was distilled out on rota-evaporator and water was added to the residue and extracted with ethyl acetate. Then ethyl acetate layer was washed with 5% sodium bicarbonate solution and followed by water. Ethyl acetate layer dried over sodium sulphate and filtered through cotton plug and distilled out ethyl acetate under vacuum to get solid product (Int-3) (1.7 g, 85%).

- **Synthesis of hydrazides (Int-4):**

To the stirred solution of various substituted ester of benzoic acid (Int-3) (1 g, 0.0073 mol) in methanol (4 mL), hydrazine hydrate was added and the mixture was refluxed for 4 hr. After completion of reaction, cool the reaction mass to 0-5 °C and filter the separated solid. Solid material washed with prechilled methanol to obtained pure product (Int-4) (0.8 g, 80%).

B. General Synthetic procedure of Compounds (4a-4j)

- **General Procedure for preparation of 5-(N-Aryl/Arylalkyl)-2-(3-Chclorophenyl)-1,3,4-oxadiazole (4a-4j)**

A mixture of methyl 3-chlorobenzimidothiate (Int-2) (3.26 mmol) and hydrazides (Int-4) (3.26 mmol) in DMF (40 mL) was heated at 120 °C for 3-4 h. After completion of the reaction, the mixture was poured in to ice cold water. The solid crude of 1,3,4-oxadiazole was obtained which was filtered and dried. The crude product was purified by column chromatography using ethylacetate and hexane as mobile phase.

2-(3-chclorophenyl)-5-(p-tolyl)-1,3,4-oxadiazole (4a)

White solid; melting range 162-164 °C; (4:6 hexane-EtOAc); IR (KBr): 3092, 3039, 2950, 1787, 1635, 1554, 1486, 1368, 1255, 1072, 825, 773, 732 cm^{-1}, ^{1}H NMR (DMSO-d_6): δ = 2.51 (s, 3H, CH3), 7.28-7.30 (d, 2H, Ar-H), 7.44-7.46 (d, 1H, Ar-H) 7.6 (d, 2H, Ar-H), 8.0-8.2 (d, 3H, Ar-H) ^{13}C NMR: 21.76, 39.47, 120-142 143.02, 161.39, 162.20, 163.88, 165.49 MS: m/z 270.06 (M^{+1}) Anal. Calcd for $C_{15}H_{11}ClN_2O$: C, 66.55; H, 4.10; N,10.35; Found: C, 66.61; H, 4.13; N, 10.31.

2-(3-chclorophenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (4b)

Light yellow solid; melting range 146-148 °C; (4:6 hexane-EtOAc); IR (KBr): 3088, 3014, 2854, 1796, 1604, 1518, 1342, 1261, 1070, 1006, 820, 725, 677 cm^{-1}, ^{1}H NMR (DMSO-d_6): δ = 2.51 (s, 3H, CH3), 3.35 (s, 3H, OCH_3), 7.28-7.30 (d, 2H, Ar-H), 8.09-8.11 (d, 1H, Ar-H), 8.35 (s, 1H, Ar-H), 8.38-8.40 (d, 1H, Ar-H), 8.46-8.51 (m, 1H, Ar-H), ^{13}C NMR: 21.76, 39.47, 124-134 146.02, 163, 162.20, 167.88, 169.49 MS: m/z 301.03 (M^{+1}) Anal. Calcd for $C_{14}H_8ClN_3O_3$: C, 55.74; H, 2.67; N, 13.93; Found: C, 55.72; H, 2.64; N, 13.96.

2-(4-bromophenyl)-5-(3-chclorophenyl)-1,3,4-oxadiazole (4c)

White solid; melting range 142-144 °C; (4:6 hexane-EtOAc); IR (KBr): 3045, 2952, 2844, 1763, 1664, 1512, 1452, 1086, 822, 757, 697, 628 cm^{-1} MS: m/z 333.99 (M^{+1}) Anal. Calcd for $C_{14}H_8ClBrN_2O$: C, 50.11; H, 2.40; N, 8.35; Found: C, 50.15; H, 2.36; N, 8.38.

2-(2-chclorobenzyl)-5-(3-chclorophenyl)-1,3,4-oxadiazole (4d)

White solid; melting range 174-176 °C; (4:6 hexane-EtOAc); IR (KBr): 3082, 2991, 1778, 1604, 1546, 1479, 1373, 1330, 1259, 1105, 1095, 922, 837, 762, 692, 589 cm^{-1}, ^1H NMR (DMSO-d_6): δ = 7.46-7.49 (m, 3H, Ar-H), 7.97-7.99 (d, 2H, Ar-H), 8.02-8.04 (d, 2H, Ar-H) MS: m/z 304.02 (M^{+1}) Anal. Calcd for $C_{15}H_{10}Cl_2N_2O$: C, 59.04; H, 3.30; N, 9.18; Found: C, 59.07; H, 3.26; N, 9.21.

2-(3-chclorobenzyl)-5-(3-chclorophenyl)-1,3,4-oxadiazole (4e)

White solid; melting range 170-172 °C; (4:6 hexane-EtOAc); IR (KBr): 2945, 2850, 1901, 1784, 1608, 1550, 1487, 1448, 1373, 1321, 1261, 1110, 1024, 916, 831, 764, 698, 610 cm^{-1}, ^1H NMR (DMSO-d_6): δ = 3.87 (s, 3H, OCH3), 7.15-7.19 (d, 2H, Ar-H), 8.025-8.047 (d, 1H, Ar-H), 8.12-8.16 (d, 2H, Ar-H), 8.28-8.30 (d, 1H, Ar-H MS: m/z 304.02 (M^{+1}) Anal. Calcd for $C_{15}H_{10}Cl_2N_2O$: C, 59.04; H, 3.30; N, 9.18; Found: C, 58.99; H, 3.33; N, 9.17.

2-(3-chclorophenyl)-5-(4-chclorophenyl)-1,3,4-oxadiazole (4f)

White solid; melting range 138-140 °C; (4:6 hexane-EtOAc); IR (KBr): 3132, 3045, 2950, 2867, 1630, 1545, 1465, 1320, 1213, 1164, 1098, 950, 864, 787, 699, 628 cm^{-1} MS: m/z 290.00 (M^{+1}) Anal. Calcd for $C_{14}H_8Cl_2N_2O$: C, 57.76; H, 2.77; N, 9.62; Found: C, 57.73; H, 2.80; N, 9.59.

2-(3-chclorophenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (4g)

White solid; melting range 126-128 °C; (4:6 hexane-EtOAc); IR (KBr): 3073, 1632, 1539, 1454, 1335, 1264, 1139, 1109, 865, 771, 696, 629, 552 cm^{-1} MS: m/z 286.05 (M^{+1}) Anal. Calcd for $C_{15}H_{11}ClN_2O_2$: C, 62.84; H, 3.87; N, 9.77; Found: C, 62.81; H, 3.90; N, 9.74.

2-(3-chclorophenyl)-5-(2,4-dimethoxyphenyl)-1,3,4-oxadiazole (4h)

White solid; melting range 148-150 °C; (4:6 hexane-EtOAc); IR (KBr): 3065, 1653, 1545, 1487, 1365, 1245, 1158, 1112, 898, 845, 764, 699, 658, 563 cm^{-1} MS: m/z 316.06 (M^{+1}) Anal. Calcd for $C_{16}H_{13}ClN_2O_3$: C, 60.67; H, 4.14; N, 8.84; Found: C, 60.69; H, 4.11; N, 8.87.

2-(3-chclorophenyl)-5-(2,6-dichlorophenyl)-1,3,4-oxadiazole (4i)

White solid; melting range 144-146 °C; (4:6 hexane-EtOAc); IR (KBr): 3165, 3039, 2950, 2856, 1664, 1554, 1476, 1364, 1282, 1178, 1007, 777, 741, 696, 658 cm^{-1} MS: m/z 323.96 (M^{+1}) Anal. Calcd for $C_{14}H_7Cl_3N_2O$: C, 51.65; H, 2.17; N, 8.60; Found: C, 51.68; H, 2.15; N, 8.58.

2-(3-chclorophenyl)-5-(2,4-dichlorophenyl)-1,3,4-oxadiazole (4j)

White solid; melting range 132-134 °C; (4:6 hexane-EtOAc); IR (KBr): 3062, 2878, 1632, 1564, 1422, 1368, 1254, 1134, 896, 787, 737, 698, 666 cm^{-1} MS: m/z 323.96 (M^{+1}) Anal. Calcd for $C_{14}H_7Cl_3N_2O$: C, 51.65; H, 2.17; N, 8.60; Found: C, 51.67; H, 2.19; N, 8.63.

3. RESULT AND DISCUSSION

3. 1. Chemistry

The present work reports solid phase synthesis approach for the synthesis of 1,3,4-oxadiazole derivatives **4a-4j**. 3-chclorobenzonitrile **1** was reacted in presence of DMF in

magnesium chcloride and sodium hydrogensulphide at 0-5 °C temperature to give 3-chclorobenzothioamide **(Int.1)** which was then converted into methyl 3-chclorobenzimido thioate **(Int.2)** by reaction with diethyl ether and methyl iodide. The product was then reacted with substituted benzoic acid **2** in methanol in the presence of catalytic amount of concentrated sulfuric acid at reflux temperature to give ester of various benzoic acids **(Int.3).** Which was then reacted with hydrazine hydrate at reflux temperature in the presence of methanol to give hydrazides **(Int.4).**

The mixture of methyl 3-chclorobenzimidothioate **(Int.2)** and hydrazides **(Int.4)** carried out in the presence of DMF at 150 °C for appropriate time to afford targeted compound 4a-4j in moderate to excellent yield (75-80%).

The physical characterization data are listed in Table 1.

Table 1. Physical data of N-substituted Oxadiazole (4a-j).

Sr. No.	Compound Code	R	Time (min)	Yield (%)	M.P. (°C)
1	4a	4-CH_3 C_6H_4	15	74	162-164
2	4b	4-NO_2 C_6H_4	21	63	146-148
3	4c	4-Br C_6H_4	17	84	142-144
4	4d	CH_2-(2-Cl C_6H_4)	14	66	174-176
5	4e	CH_2-(3-Cl C_6H_4)	22	68	170-172
6	4f	4-Cl C_6H_4	19	74	138-140
7	4g	4-OMeC_6H_4	15	72	126-128
8	4h	2,4-di-OMe C_6H_3	18	80	148-150
9	4i	2,6-di-Cl C_6H_3	21	81	144-146
10	4j	2,4-di-Cl C_6H_3	16	78	132-134

3. 2. Biological activities

Antibacterial and antifungal activities: Oxadiazole derivatives were screened for their in-vitro antibacterial and antifungal activities following micro broth dilution method [24-26]. Antibacterial activity was screened aginst gram-positive (Bacillus subtillis (MTCC 441), Staphylococcus aureus (MTCC 96)) and gram-negative (Escherichia coli (MTCC 443), P.

aeruginosa (MTCC-1688) microorganisms. Antifungal activity was screened against Aspergillus niger (MTCC 282), and Penicillium SP.microorganisms. The standard drugs used for this study were Penicillin and Streptomycin was used for antibacterial screening. Griseofulvin was used for antifungal screening. The standard strains used for screening of antibacterial and antifungal activities were procured from the Culture collection and gene bank (MTCC), Chandigarh, India. Mueller Hinton Broth was used as a nutrient medium for bacteria and Sabouraud dextrose Broth for fungal growth. Inoculums size for test strain was adjusted to 108 CFU/mL by comparing the turbidity.

The results were recorded in the form of primary and secondary screening. The stock solution (2000 µg/mL) of the compounds under investigation and standard drugs were prepared by successive twofold dilution. In primary screening, 1000, 500 and 250 µg/mL concentrations of the compounds were used. The compounds found to be active in this primary screening were further screened. In secondary screening, 200, 100, 50, 25, 12.5 and 6.25 µg/mL concentrations were used. The inoculated wells were incubated overnight at 37 °C in a humid atmosphere. The highest dilution showing complete inhibition was considered as a minimum inhibition concentration (MIC).

The MIC values revealed that the synthesized compounds showed moderate to good inhibition. Compounds 6b, 6d, 6e, 6h and 6i exhibited good activities against bacterial strains. The MIC values of antifungal activity shown that compound 6b and 6h exhibited good activity against all fungal strain. Antimicrobial activity of compounds (4a-j) is listed in Table 2.

Table 2. Antimicrobial activity of compounds (4a-j)

	Antibacterial MIC (µg/mL)				**Antifungal MIC (µg/mL)**	
Compound	***B. subtilis***	***Staphyaloccoc us aureus***	***P. aeruginosa***	***E. coli***	***Penicillium* sp.**	***Aspergillus niger***
Penicillin	50	50	-	-	-	-
Streptomycin	-	-	100	100	-	-
Griseofulvin	-	-	-	-	100	100
4a	250	500	500	500	500	250
4b	500	250	500	1000	500	1000
4c	250	125	500	250	250	500
4d	500	500	1000	500	1000	500
4e	1000	1000	500	500	1000	1000

4f	500	250	125	250	500	250
4g	1000	500	1000	500	1000	1000
4h	1000	500	500	1000	500	1000
4i	500	1000	1000	500	1000	1000
4j	250	500	500	250	250	500

4. CONCLUSION

In summary, synthesis of novel 1,3,4-oxadiazol derivatives **4a-j** using solid phase synthesis reaction. The entire synthesized compound characterized and confirmed by spectroscopic data and elemental analysis. Additionally, all the synthesized compounds were screened for their antimicrobial activity against the selected pathogens and compared with standard drugs. Compound **4c** and **4f** substituent were observed as most active against tested antibacterial and antifungal strains.

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References:

[1] *Ainswarth, J. Am. Chem. Soc.*, 87(24), 5800-5801 (1965). https://doi.org/10.1021/ja00952a056

[2] Kumar, A., D'Souza, S. S., Nagaraj, S. R. M., Gaonkar, S. L., Salimath, B. P., & Rai, K. L. (2009). Antiangiogenic and antiproliferative effects of substituted-1, 3, 4-oxadiazole derivatives is mediated by down regulation of VEGF and inhibition of translocation of HIF-1α in Ehrlich ascites tumor cells. *Cancer Chemotherapy and Pharmacology*, 64(6), 1221-1233. https://doi.org/10.1007/s00280-009-0992-y

[3] A. Hetzheim, K. Moeckel, *Adv. Heterocyclic Chem.* 7, 183-224 (1966). https://doi.org/10.1016/s0065-2725(08)60591-7

[4] Maslat, A. O., Abussaud, M., Tashtoush, H., & AL-Talib, M. (2002). Synthesis, antibacterial, antifungal and genotoxic activity of bis-1,3,4-oxadiazole derivatives. *Polish Journal of Pharmacology*, 54(1), 55-60. https://doi.org/10.1080/02772240410001665490

[5] Küçükgüzel, Ş. G., Oruç, E. E., Rollas, S., Şahin, F., & Özbek, A. (2002). Synthesis, characterisation and biological activity of novel 4-thiazolidinones, 1, 3, 4-oxadiazoles and some related compounds. *European Journal of Medicinal Chemistry*, 37(3), 197-206. https://doi.org/10.1016/s0223-5234(01)01326-5

[6] Karad, S. C., Purohit, V. B., Avalani, J. R., Sapariya, N. H., & Raval, D. K. (2016). Design, synthesis, and characterization of a fluoro substituted novel pyrazole nucleus clubbed with 1, 3, 4-oxadiazole scaffolds and their biological applications. *RSC Advances*, 6(47), 41532-41541. https://doi.org/10.1039/c6ra01349j

[7] Rane, R. A., Bangalore, P., Borhade, S. D., & Khandare, P. K. (2013). Synthesis and evaluation of novel 4-nitropyrrole-based 1,3,4-oxadiazole derivatives as antimicrobial and anti-tubercular agents. *European Journal of Medicinal Chemistry*, 70, 49-58. https://doi.org/10.1016/j.ejmech.2013.09.039

[8] Farghaly, A. H. (2004). Synthesis, Reactions and Antimicrobial Activity of Some New Indolyl-1, 3, 4-Oxadiazole, Thiazole and Pyrazole Derivatives. *Journal of the Chinese Chemical Society*, 51(1), 147-156. https://doi.org/10.1002/jccs.200400023

[9] Desai, N. C., Bhatt, N. B., Somani, H. C., & Bhatt, K. A. (2016). Synthesis and antimicrobial activity of some thiazole and 1, 3, 4-oxadiazole hybrid heterocycles. *Indian Journal of Chemistry*, 55, 94-101. https://doi.org/10.1007/s00044-014-1108-8

[10] Sangshetti, J. N., Chabukswar, A. R., & Shinde, D. B. (2011). Microwave assisted one pot synthesis of some novel 2, 5-disubstituted 1, 3, 4-oxadiazoles as antifungal agents. *Bioorganic & medicinal chemistry letters*, 21(1), 444- 448. https://doi.org/10.1016/j.bmcl.2010.10.120

[11] Terenzi, A., Fanelli, M., Ambrosi, G., Amatori, S., Fusi, V., Giorgi, L & Barone, G. (2012). DNA binding and antiproliferative activity toward human carcinoma cells of copper (II) and zinc (II) complexes of a 2, 5-diphenyl [1, 3, 4] oxadiazole derivative. *Dalton Transactions*, 41(15), 4389-4395. https://doi.org/10.1039/c2dt11759b

[12] Bhat, K. S., Karthikeyan, M. S., Holla, B. S., & Shetty, N. S. (2004). Synthesis of some new fluorine containing 1, 3, 4-oxadiazole derivatives as potential antibacterial and anticancer agents. *Indian J Chem B*, 43, 1765-1769. https://doi.org/10.1002/chin.200450090

[13] Abdel-Aziz, M., A Metwally, K., Gamal-Eldeen, A. M., & M Aly, O. (2016). 1, 3, 4-oxadiazole-2-thione Derivatives; Novel Approach for Anticancer and Tubulin Polymerization Inhibitory Activities. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*, 16(2), 269-277. https://doi.org/10.2174/1871520615666150907093855

[14] Khan, M. T. H., Choudhary, M. I., Khan, K. M., & Rani, M. (2005). Structure–activity relationships of tyrosinase inhibitory combinatorial library of 2, 5-disubstituted-1, 3, 4-oxadiazole analogues. *Bioorganic & Medicinal Chemistry*, 13(10), 3385-3395.https://doi.org/10.1016/j.bmc.2005.03.012

[15] Dolman, S. J., Gosselin, F., O'Shea, P. D., & Davies, I. W. (2006). Superior reactivity of thiosemicarbazides in the synthesis of 2-amino-1, 3, 4-oxadiazoles. *The Journal of Organic Chemistry*, 71(25), 9548-9551. https://doi.org/10.1021/jo0618730

[16] El-Samii, Z. K. (1992). Synthesis and anti-inflammatory activity of some novel 1,3,4-oxadiazole derivatives. *Journal of Chemical Technology and Biotechnology*, 53(2), 143-146. https://doi.org/10.1002/jctb.280530206

[17] Ilango, K., Valentina, P., Kumar, G., Dixit, D., Nilewar, S., & K Kathiravan, M. (2015). Design, Synthesis and QSAR Studies on a Series of 2, 5- Disubstituted-1, 3, 4-oxadiazole Derivatives of Diclofenac and Naproxen for Analgesic and Anti-inflammatory Activity. *Medicinal Chemistry*, 11(8), 753-763. https://doi.org/10.2174/1573406411666150519112037

[18] Akhter, M., Husain, A., Azad, B., & Ajmal, M. (2009). Aroylpropionic acid based 2, 5-disubstituted-1, 3, 4-oxadiazoles: Synthesis and their anti-inflammatory and analgesic activities. *European Journal of Medicinal Chemistry*, 44(6), 2372-2378. https://doi.org/10.1016/j.ejmech.2008.09.005

[19] Johns, B. A., Weatherhead, J. G., Allen, S. H., Thompson, J. B., Garvey, E. P., Foster, S. A., ... & Miller, W. H. (2009). 1, 3, 4-Oxadiazole substituted naphthyridines as HIV-1 integrase inhibitors. Part 2: SAR of the C5 position. *Bioorganic & Medicinal Chemistry Letters*, 19(6), 1807-1810. https://doi.org/10.1016/j.bmcl.2009.01.089

[20] Khatale, P. N., & Sivakumar, T. (2015). Methods of Synthesis of 1,3,4-Oxadiazole-2-thiones: A Review. *ChemInform*, 46(13). https://doi.org/10.1002/chin.201513324

[21] Khalilullah, H., J Ahsan, M., Hedaitullah, M., Khan, S., & Ahmed, B. (2012). 1,3,4-Oxadiazole: A biologically active scaffold. *Mini reviews in medicinal chemistry*, 12(8), 789-801. https://doi.org/10.2174/138955712801264800

[22] Parikh, K., & Joshi, D. (2014). Synthesis and evaluation of 2-(5-(aryl)-1,3,4-oxadiazol-2-ylthio)-N-(3-(trifluoromethyl) phenyl) acetamides and N-(4- chloro-3-fluorophenyl)-2-(5-(aryl)-1, 3, 4-oxadiazol-2-ylthio) acetamides as antimicrobial agents. *Journal of Chemical Sciences*, 126(3), 827-835. https://doi.org/10.1007/s12039-014-0625-9

[23] Hajdok, I., Bona, A., Werner, H. J., & Kerres, J. (2014). Synthesis and characterization of fluorinated and sulfonated poly (arylene ether-1, 3, 4- oxadiazole) derivatives and their blend membranes. *European Polymer Journal*, 52, 76-87. https://doi.org/10.1016/j.eurpolymj.2013.12.003

[24] National committee for clinical laboratory standards. (M7A5), 5th ed.; NCCLS: Wayne, PA, (2000). https://doi.org/10.1093/clinchem/34.8.1665

[25] Patel, N.B.; Shaikh, A.R. *Indian J. Chem.* 49B, 929-936 (2010). https://doi.org/10.4103/0970-0218.62571

[26] Rattan, A. Antimicrobial in laboratory medicine; BI Churchill Livingstone: India, p. 85, (2000). https://doi.org/10.1111/j.1365-2257.1985.tb00041.x