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Synthesis and Biological Screening of Novel 5-(5-Aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole Derivatives

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A new series of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) have been synthesized by a cyclocondensation reaction of ethyl 5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**) with aryl imidoxime (**5a-e**). The newly synthesized pyrazolyl-1,2,4-oxadiazole (**6a-o**) derivatives were characterized by spectroscopic techniques and screened for *in vitro* antibacterial activity against *Bacillus subtilis* (NCIM 2063), *Staphylococcus albus* (NCIM 2178), *Escherichia coli* (NCIM 2574), *Proteus mirabilis* (NCIM 2388) and *in vitro* antifungal activity against *Aspergillus niger* (ATCC 504) *Candida albicans* (NCIM 3100).

Keywords: Pyrazole, 1,2,4-Oxadiazoles, Antibacterial activity, Antifungal activity.

INTRODUCTION

Two or more bioactive scaffolds clubbed in one molecule plays the significant role in the development of new drugs [1]. Nitrogen containing heterocyclic scaffolds are continuously utilized in the field of drug discovery and development [2]. Several drug containing 1,2,4-oxadiazole ring are commercially available, such as oxolamine (anti-inflammatory), butalamine (vasodilator), fasiplon (anxiolytic) pleconaril (antiviral) [3]. 1,2,4-Oxadiazole scaffolds are often used as bioisoster of ester and amide functionalities in modification and development of pharmacological properties [4]. 1,2,4-Oxadiazole derivatives were widely studied against infectious and other diseases such as antibacterial [5], antifungal [6], antitubercular [7], anticancer [8], antiviral [9], neuroprotective [10], anti-inflammatory and antioxidant [11] activities. Pyrazole ring is the privileged pharmacophore for the architecture of lead molecules and have received much attention in recent years due to their promising antimicrobial activity [12-14]. The pyrazole derivatives are reported as antitubercular [15-17], antimicrobial [18,19], antifungal [20], anticancer [21,22], anti-inflammatory [23], antimalarial activity [24], *etc*.

The 1,2,4-oxadiazole as five member heterocyclic compound was firstly synthesized in 1884 by Tiemann and Krüger. The 1,2,4-oxadiazole attract its importance towards researcher/chemists after approximately 80 year after its discovery, when new techniques/method were developed for synthesis approach such as photochemical rearrangement of it with other heterocyclic systems [25,26].

According to literature survey, there are no reports on the designing of the molecules having 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole and it's derivatives in single molecular framework. Keeping in view the multifarious applications of 1,2,4-oxadiazole incorporated diaryl pyrazolyl and our interest in designing and synthesizing bioactive heterocycles, we propose to synthesize a novel 1,2,4-oxadiazole incorporated diaryl pyrazolyl showing potent moiety (**Scheme-I**) as pharmacophore to study the additive effect of these scaffolds toward the antimicrobial activity. Therefore, herein we report a synthesis and antimicrobial activity of novel 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazoles.

EXPERIMENTAL

All reagents and solvents were purchased from Merck and Spectrochem used without further purification. Melting points of all the synthesized compounds were determined in open capillary tube and are uncorrected. ^{1}H NMR spectra were recor-

ded on a Bruker DRX-500 MHz NMR spectrometer and ^{13}C NMR spectra were recorded on a Bruker DRX-126 MHz NMR in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard and chemical shifts are in δ ppm. High-resolution mass spectra (HRMS) were recorded on Agilent 6520 (QTOF) ESI-HRMS instrument and LC-MS spectrometer. The purity of each of the compound was checked by thin-layer chromatography (TLC) using silica-gel, (60 F_{254}) and visualization was accomplished by iodine/ultraviolet light.

General procedure: The synthetic route of 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a-o**) is presented in **Scheme-I**. Substituted acetophenone (**1a-c**) upon reaction with diethyl oxalate and sodium ethoxide in ethanol gave ethyl 2,4-dioxo-4-arylbutanoate (**2a-c**) (85%), which upon reaction with phenyl hydrazine in ethanol gave ethyl 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**). Substituted phenyl nitrile (**4a-e**) upon reaction with hydroxylamine in ethanol gave substituted *N*′-hydroxybenzimidamide (**5a-e**) followed by reaction with 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**) furnished target compounds 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a-o**).

General procedure for synthesis of ethyl 2,4-dioxo-4-arylbutanoate (2a-c): To a solution of dry ethanol, sodium (0.11 mol) was added, refluxed for 10 min and then cool preferably to 30-35 ºC and substituted acetophenone (0.1 mol) was added dropwise under stirring. Reaction mixture was refluxed for 10 min then gradually cool to 30-35 ºC. To a above solution, diethyl oxalate was added drop wise under stirring. The reaction mixture was refluxed for 4-6 h (reaction progress was monitored by TLC). After the completion of reaction distilled out the solvent under vacuum, the residue was dissolved in water and extracted in ethyl acetate (3 × 50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and distilled on rotary evaporator. The crude product was used for further reaction.

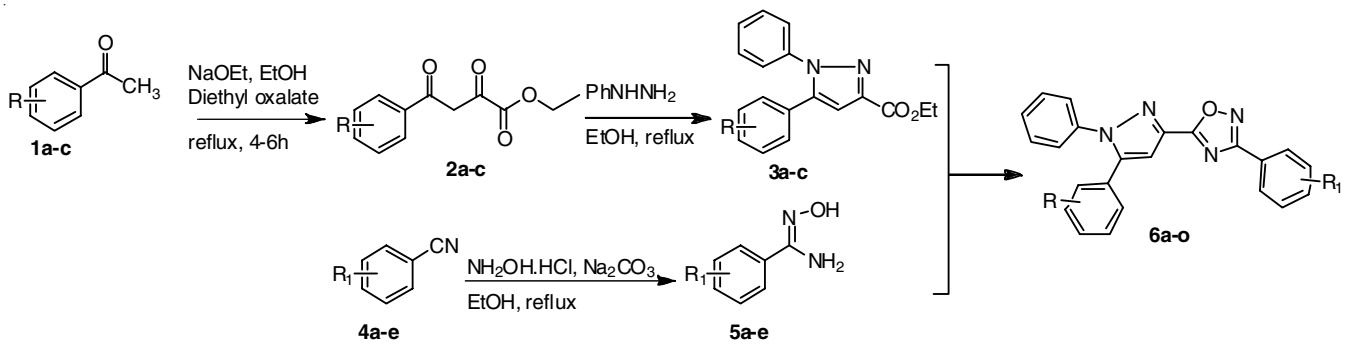
General procedure for synthesis of ethyl-5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (3a-c): To a solution of ethyl 2,4-dioxo-4-arylbutanoate (**2a-c**) (0.08 mol) and phenyl hydrazine (0.08 mol) in ethanol (50 mL) was refluxed for 3-4 h (reaction progress was monitored by TLC). After completion of the reaction solvent was distilled under vacuum, residue was dissolved in water and at acidic pH (preferably pH 1-2) extracted twice with ethyl acetate. Organic layer was distilled on rotary evaporator. The crude product was purified on column chrom-atography using ethyl acetate:hexane (2:8) as eluent (yield: 65-80%) (**3a-e**).

General procedure for synthesis of *N*-hydroxybenzamidine (5a-e): To a solution of ethanol, added Na_2CO_3 (1.5 mol) followed by charged benzonitrile (0.20 mol) and hydroxylamine hydrochloride (1.5 mol) at ambient temperature the reaction mixture was slowly heat to refluxed for 5-6 h. (reaction progress was monitored by TLC). After completion of the reaction solvent was distilled under vacuum below 45 ºC, to the residue added ice cold water and stirred to get fine crystals and filtered to get a desired product (**5a-e**).

General procedure for synthesis of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (6a-o): A solution of *N*′-hydroxy substituted benzimidamide (**5a-e**) (0.01 mol) NaOH (0.01 mol) and 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**) (0.011 mol) in DMSO (5 mL) was stirred for 2-4 h at ambient temperature. After the completion reaction (reaction progress was monitored by TLC), the reaction mixture was quenched in water and extracted with ethyl acetate (3 × 25 mL). The combined organic layer was dried over anhydrous sodium sulphate and distilled on rotary evaporator. The crude product was purified on column chromatography using ethyl acetate: hexane (3:7) as eluent furnished 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) (yield: 60-70%) (Table-1).

5-(1,5-Diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (6a): IR (KBr, ν_{max}, cm^{-1}): 3122, 3050 aromatic C-H *str.*, 1958 overtone, 1491, 1434 aromatic C=C *str.* (in-ring), 749 aromatic C–H bending out-of-plane, 691 C=C bending out-of-plane ring. ^1H NMR (500 MHz, $CDCl_3$): δ 8.26-8.20 (m, 2H), 7.55-7.46 (m, 4H), 7.39 (s, 5H), 7.37-7.35 (m, 2H), 7.28 (dd, *J* = 8.0, 1.6 Hz, 2H), 7.25 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.83, 168.88, 145.25, 139.29, 138.48, 131.23, 129.09, 129.02, 128.82, 128.81, 128.71, 128.59, 127.69, 127.33, 126.73, 125.64, 108.82. HRMS *m/z* 365.1398 $(M+H)^+$.

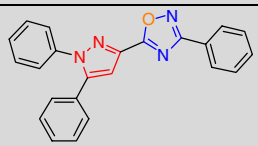
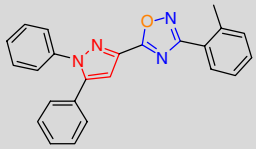
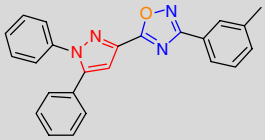
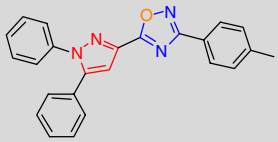
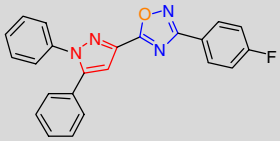
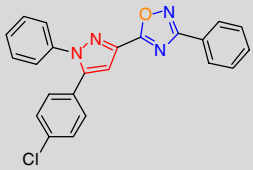
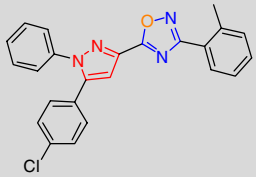
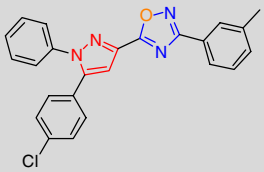
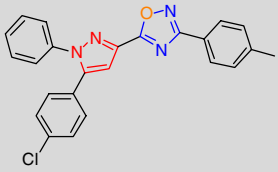
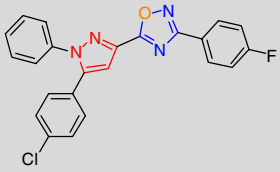
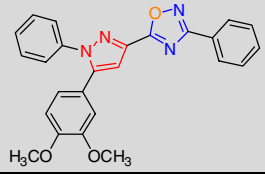
5-(1,3-Diphenyl-1*H*-pyrazol-5-yl)-3-(*o*-tolyl)-1,2,4-oxa-diazole (6b): IR (KBr, ν_{max}, cm^{-1}): 2923 alkyl C-H *str.*, 1955 overtone 1488, 1449 aromatic C=C *str.* (in-ring), 753

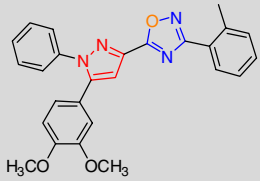
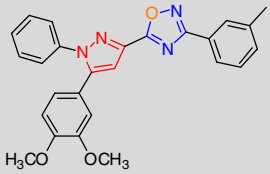
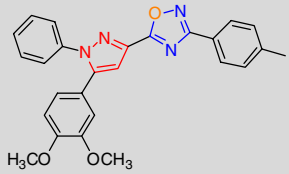
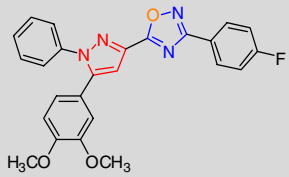


Comp	R	R_1	Comp	R	R_1	Comp	R	R_1
6a	H	H	**6f**	4-Cl	H	**6k**	3,4-$(OCH_3)_2$	H
6b	H	2-CH_3	**6g**	4-Cl	2-CH_3	**6l**	3,4-$(OCH_3)_2$	2-CH_3
6c	H	3-CH_3	**6h**	4-Cl	3-CH_3	**6m**	3,4-$(OCH_3)_2$	3-CH_3
6d	H	4-CH_3	**6i**	4-Cl	4-CH_3	**6n**	3,4-$(OCH_3)_2$	4-CH_3
6e	H	4-F	**6j**	4-Cl	4-F	**6o**	3,4-$(OCH_3)_2$	4-F

Scheme-I: Synthesis of 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a-o**)

TABLE-1
YIELD, MELTING POINT AND PHYSICAL NATURE OF COMPOUNDS **6a-o**

Compd.	Structure	R	R_1	Yield (%)	m.p. (°C)	Physical nature
6a		H	H	60	146	White solid
6b		H	2-CH_3	65	136	White solid
6c		H	3-CH_3	65	148	White solid
6d		H	4-CH_3	70	140	White solid
6e		H	4-F	55	140	White solid
6f		4-Cl	H	65	148	White solid
6g		4-Cl	2-CH_3	62	158	White solid
6h		4-Cl	3-CH_3	65	160	White solid
6i		4-Cl	4-CH_3	70	134	White solid
6j		4-Cl	4-F	60	142	White solid
6k		3,4-$(OCH_3)_2$	H	65	152	White solid

6l		3,4-$(OCH_3)_2$	2-CH_3	60	156	White solid
6m		3,4-$(OCH_3)_2$	3-CH_3	62	142	White solid
6n		3,4-$(OCH_3)_2$	4-CH_3	65	158	White solid
6o		3,4-$(OCH_3)_2$	4-F	52	150	White solid

aromatic C–H bending out-of-plane, 694 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.8 Hz, 1H), 7.42-7.32 (m, 11H), 7.28 (dd, *J* = 7.9, 1.6 Hz, 2H), 7.24 (s, 1H), 2.70 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.89, 169.45, 145.23, 139.35, 138.58, 138.34, 131.31, 130.60, 130.40, 129.21, 129.11, 129.03, 128.85, 128.73, 128.57, 125.95, 125.64, 108.88, 22.22; HRMS *m/z* 379.1565 $(M+H)^+$.

5-(1,3-Diphenyl-1*H*-pyrazol-5-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (6c): IR (KBr, ν_{max}, cm^{-1}): 2921 alky C-H *str.*, 1937 overtone, 1604, 1453 aromatic C=C *str.* (in-ring), 757 aromatic C–H bending out of plane, 695 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (d, *J* = 0.6 Hz, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.41-7.33 (m, 10H), 7.30-7.27 (m, 2H), 7.25 (s, 1H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.76, 168.99, 145.25, 139.33, 138.61, 138.53, 132.01, 129.17, 129.09, 129.02, 128.83, 128.71, 128.58, 128.29, 126.58, 125.67, 124.79, 108.80, 21.33; HRMS *m/z* 379.1565 $(M+H)^+$.

5-(1,3-Diphenyl-1*H*-pyrazol-5-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole (6d): IR (KBr, ν_{max}, cm^{-1}): 3117 aromatic C-H *str.*, 2915 alkyl C-H *str.*, 1613, 1493, 1428, 1347 aromatic C=C *str.* (in-ring), 828, 764 aromatic C–H bending out-of-plane, 695 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (d, *J* = 8.1 Hz, 2H), 7.41-7.26 (m, 12H), 7.24 (s, 1H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.69, 168.91, 145.24, 141.56, 139.35, 138.58, 129.54, 129.19, 129.10, 129.02, 128.84, 128.72, 128.58, 127.64, 125.68, 123.94, 108.82, 21.62; HRMS *m/z* 379.1565 $(M+H)^+$.

5-(1,5-Diphenyl-1*H*-pyrazol-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (6e): IR (KBr, ν_{max}, cm^{-1}): 2920, 2851, alkyl C-H *str.*, 2002 overtone, 1595, 1488, 1410 aromatic C=C *str.* (in-ring), 1339, 1225 C-F *str.*, 844, 762 aromatic C–H bending out-of-plane, 692 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.22 (dd, *J* = 9.0, 5.4 Hz, 2H), 7.45-7.38 (m, 5H), 7.34-7.20 (m, 5H), 7.14 (s, 1H), 7.05 (t, *J* = 8.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.62, 168.00, 165.65, 163.65, 144.13, 139.04, 138.51, 130.28, 129.92, 129.68, 129.54, 129.29, 129.09, 128.89, 127.54, 125.66, 122.95, 122.93, 116.14, 115.97, 108.90.

5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-phenyl-1,2,4-oxadiazole (6f): IR (KBr, ν_{max}, cm^{-1}): 3121 aromatic C-H *str.*, 1618, 1525 aromatic C=C *str.* (in-ring), 1349, 1094 aromatic C–H bending in-plane, 811, 746, aromatic C–H bending out-of-plane, 692, 487 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.22 (dd, *J* = 7.7, 1.9 Hz, 2H), 7.54-7.50 (m, 3H), 7.43-7.37 (m, 5H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.24 (s, 1H), 7.21 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.68, 168.92, 144.08, 139.07, 138.63, 135.28, 131.29, 130.06, 129.27, 129.08, 128.96, 128.84, 127.70, 127.59, 126.70, 125.67, 108.92; HRMS *m/z*: 399.1011 $(M+H)^+$.

5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*o*-tolyl)-1,2,4-oxadiazole (6g): IR (KBr, ν_{max}, cm^{-1}): 2957, 2925 alkyl C-H *str.*, 1727 overtone, 1615, 1486 aromatic C=C *str.* (in-ring), 1327, 1070 aromatic C-H bending in-plane, 806, 758, aromatic C–H bending out-of-plane, 694, 455 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (d, *J* = 8.0 Hz, 1H), 7.35-7.29 (m, 6H), 7.25 (dd, *J* = 8.7, 6.9 Hz, 4H), 7.15 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 2.62 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.71, 169.46, 144.02, 139.10, 138.70, 138.34, 135.25, 131.33, 130.64, 130.38, 130.07, 129.26, 129.07, 128.81, 127.64, 126.00, 125.96, 125.64, 108.95, 22.22; HRMS *m/z*: 413.1166 $(M+H)^+$.

5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (6h): IR (KBr, ν_{max}, cm^{-1}): 3118, 3058 aromatic C-H *str.*, 2919 alkyl C-H *str.*, 1724 overtone, 1617, 1458 aromatic C=C *str.* (in-ring), 1333, 1090 aromatic C-H bending in-plane, 803, 761, aromatic C–H bending out-

of-plane, 690, 438 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.43-7.36 (m, 6H), 7.35-7.30 (m, 3H), 7.24 (s, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 2.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.54, 168.97, 144.02, 139.03, 138.60, 135.23, 132.02, 130.01, 129.21, 129.02, 128.79, 128.70, 128.24, 127.55, 126.49, 125.65, 125.76, 124.74, 108.84, 21.29; HRMS *m/z*: 413.1174 $(M+H)^+$.

5-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole (6i): IR (KBr, ν_{max}, cm^{-1}): 3123, 3052 aromatic C-H *str.*, 2957 alkyl C-H *str.*, 1726 overtone, 1616, 1490 aromatic C=C *str*. (in-ring), 1343, 1092 aromatic C-H bending in-plane, 817, 761, aromatic C–H bending out-of-plane, 692, 496 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (d, *J* = 8.2 Hz, 2H), 7.40 (t, *J* = 3.1 Hz, 2H), 7.32 (d, *J* = 2.0 Hz, 2H), 7.23 (s, 1H), 7.21 (d, *J* = 8.6 Hz, 2H), 7.15 (dd, *J* = 8.4, 7.4 Hz, 2H), 6.78-6.73 (m, 1H), 6.69 (dd, *J* = 8.5, 1.0 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.51, 168.93, 144.04, 141.62, 139.09, 138.70, 135.26, 130.06, 129.56, 129.30, 129.26, 129.07, 128.83, 127.63, 125.68, 123.87, 118.58, 115.12, 108.89, 21.62; HRMS *m/z*: 413.1168 $(M+H)^+$.

5-(5-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (6j): IR (KBr, ν_{max}, cm^{-1}): 3120 aromatic C-H *str.*, 2921 alkyl C-H *str.*, 1999 overtone, 1615 aromatic C=C *str.* (in-ring), 1334 aromatic C-F *str.*, 1090 aromatic C-H bending in-plane, 808, 762, aromatic C–H bending out-of-plane, 688, 489, 434 C-C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.22 (dd, *J* = 8.9, 5.4 Hz, 2H), 7.43-7.37 (m, 5H), 7.33 (d, *J* = 8.6 Hz, 2H), 7.24-7.17 (m, 5H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.77, 168.12, 165.67, 163.67, 144.13, 139.04, 138.51, 135.32, 130.06, 129.91, 129.84, 129.29, 129.09, 128.89, 127.54, 125.66, 122.95, 122.93, 116.13, 115.96, 108.90; HRMS *m/z*: 417.0918 $(M+H)^+$.

5-(3-(3,4-Dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-phenyl-1,2,4-oxadiazole (6k): IR (KBr, ν_{max}, cm^{-1}): 3123 aromatic C-H *str.*, 2922 alkyl C-H *str.*, 1962 overtone, 1616, 1490 aromatic C=C *str*. (in-ring), 1342, 1092 aromatic C-H bending in-plane, 816, 761, aromatic C–H bending out-of-plane, 692, 496 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.22 (dd, *J* = 7.7, 1.9 Hz, 2H), 7.54-7.49 (m, 3H), 7.45-7.38 (m, 5H), 7.22 (s, 1H), 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.91, 168.89, 149.62, 148.77, 145.23, 139.49, 138.42, 131.25, 129.12, 128.83, 128.60, 127.71, 126.77, 125.85, 121.67, 121.53, 111.78, 111.12, 108.16, 55.92, 55.71, HRMS *m/z* 425.1614 $(M+H)^+$.

5-(3-(3,4-Dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*o*-tolyl)-1,2,4-oxadiazole (6l): IR (KBr, ν_{max}, cm^{-1}): 3058 aromatic C-H *str.*, 2922 alkyl C-H *str.*, 1609, 1499, 1455 aromatic C=C *str*. (in-ring), 1329, 1258 asymmetric C-O-C *str.*, 1140 symmetric C-O-C *str.*, 1022 aromatic C-H bending in-plane, 796, 756 aromatic C–H bending out-of-plane, 697, 452 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.9 Hz, 1H), 7.43-7.39 (m, 5H), 7.34 (d, *J* = 7.9 Hz, 2H), 7.21 (s, 1H), 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.65 (s, 3H), 2.70 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.90, 168.39, 148.58, 147.75, 144.13, 138.49, 137.45, 137.29, 130.27, 129.55, 129.34, 128.07, 127.51, 125.04, 124.89, 124.77, 120.64, 120.55, 110.78, 110.10, 107.16, 54.87, 54.67, 21.16. HRMS *m/z* 439.1772 $(M+H)^+$.

5-(3-(3,4-Dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (6m): IR (KBr, ν_{max}, cm^{-1}): 2915 alkyl C-H *str.*, 1615, 1503, 1460, 1429 aromatic C=C *str*. (in-ring), 1252 asymmetric C-O-C *str.*, 1139, 1023 symmetric C-O-C *str.* and aromatic C-H bending in-plane, 803, 759 aromatic C–H bending out-of-plane, 697 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (s, 1H), 8.02 (d, *J* = 7.6 Hz, 1H), 7.46-7.37 (m, 6H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.22 (s, 1H), 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.65 (s, 3H), 2.44 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.79, 167.94, 148.58, 147.75, 144.19, 138.47, 137.59, 137.40, 130.99, 128.08, 127.71, 127.55, 127.25, 125.56, 124.81, 123.75, 120.64, 120.50, 110.77, 110.11, 107.10, 54.87, 54.67, 20.30. HRMS *m/z* 439.1772 $(M+H)^+$.

5-(3-(3,4-Dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-5-yl)-3-(*p*-tolyl)-1,2,4-oxadiazole (6n): IR (KBr, ν_{max}, cm^{-1}): 2920, 2849 alkyl C-H *str.*, 1614, 1538, 1458 aromatic C=C *str.* (in-ring), 1257 asymmetric C-O-C *str.*, 1023 symmetric C-O-C *str.* and aromatic C-H bending in-plane, 810, 760 aromatic C–H bending out-of-plane, 697, 502 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11 (d, *J* = 8.2 Hz, 2H), 7.45-7.38 (m, 5H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.21 (s, 1H), 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.65 (s, 3H), 2.43 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.71, 167.85, 148.57, 147.74, 144.15, 140.52, 138.48, 137.45, 128.50, 128.06, 127.53, 126.59, 124.81, 122.91, 120.63, 120.52, 110.77, 110.10, 107.10, 54.87, 54.67, 28.67, 20.57. HRMS *m/z* 439.1772 $(M+H)^+$.

5-(5-(3,4-Dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)-3-(4-fluorophenyl)-1,2,4-oxadiazole (6o): IR (KBr, ν_{max}, cm^{-1}): 3123 aromatic C-H *str.*, 2928, 2832 alkyl C-H *str.*, 1985, 1767 overtone, 1612, 1504, 1430 aromatic C=C *str.* (in-ring), 1249 asymmetric C-O-C *str.*, 1139, 1023 symmetric C-O-C *str*. and aromatic C-H bending in-plane, 811, 755 aromatic C–H bending out-of-plane, 694 C=C bending out-of-plane ring. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (dd, *J* = 8.9, 5.4 Hz, 2H), 7.44-7.37 (m, 5H), 7.22-7.17 (m, 3H), 6.91 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.68 (d, *J* = 2.0 Hz, 1H), 3.90 (s, 3H), 3.65 (s, 3H). HRMS *m/z* 443.1522 $(M+H)^+$.

RESULTS AND DISCUSSION

A synthetic route for the synthesis of a series of novel 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) as potential antimicrobial agents from commercially available starting materials is shown in **Scheme-I**. The synthesis of substituted acetophenone (**1a-c**) on reaction with diethyl-oxalate and sodium ethoxide in ethanol gave ethyl 2,4-dioxo-4-arylbutanoate (**2a-c**) (85%) which upon reaction with phenyl hydrazine in ethanol gave ethyl 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**). Substituted phenyl nitrile, **4a-e** upon reaction with hydroxyl amine in ethanol gave substituted *N*′-

hydroxybenzimidamide (**5a-e**) followed by reaction with 5-aryl-1-phenyl-1*H*-pyrazole-3-carboxylate (**3a-c**) furnished target compounds 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a-o**).

The synthesis of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) has been confirmed by physical and spectroscopic ^{1}H NMR, ^{13}C NMR and HRMS data. As a representative, ^{1}H NMR spectrum of 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a**) revealed a singlet at δ 7.25 ppm integrated for one proton was assigned to the C-4 pyrazole proton. The fifteen aromatic protons are resonated between at δ 8.26-7.28 ppm. The ^{13}C NMR spectrum of compound **6a** showed a signal at δ 108.82 ppm was assigned to C-4-pyrazole carbon. The other aromatic, pyrazole and 1,2,4-oxadiazole carbons are appeared from δ 170.83 to 125.64 ppm. The structure of compound **6a** was confirmed by HRMS at molecular ion peaks *m/z* 365.1398 $(M+H)^{+}$. Structure of other synthesized compounds was also confirmed accordingly.

Biological evaluation

Antimicrobial activity: The antimicrobial susceptibility of all newly synthesized derivatives of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) were evaluated *in vitro*. The antibacterial activity was carried against Gram-positive bacteria *S. albus* and *B. subtilis* and Gram-negative bacteria *E. coli* and *P. mirabilis* and using the well diffusion method [27,28]. Standard drug streptomycin and DMSO were used as positive and negative control, respectively. The *in vitro* antifungal activity was performed against *C. albicans* and *A. niger* using well diffusion method [25,26]. The antifungal drugs fluconazole and ravuconazole were used as reference. All the test solutions were prepared in DMSO at 1000 µg/mL concentrations and the wells were filled with 80 µL of the samples. The result of antimicrobial activity in zone of inhibition (mm) and minimum inhibition concentration (µg/mL) are presented in Tables 2 and 3, respectively.

The antimicrobial activity data of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) revealed that among the synthesized derivatives, compounds **6a** (R = H, R^1 = H) and **6c** (R = H, R^1 = 3-CH_3) showed moderate activity against *P. mirabilis*. Also compounds **6a** (R = H, R^1 = H), **6b** (R = H, R^1 = 2-CH_3) and **6c** (R = H, R^1 = 3-CH_3) showed good activity against *A. niger* with MIC 62.5 µg/mL, which was only two fold-less than the standard drug ravuconazole. Compound **6e** (R = H, R^1 = 4-F) showed moderate activity against *B. subtilis*. Among the compounds 5-(5-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-3-yl)-3-(substituted phenyl)-1,2,4-oxadiazole (**6f-j**) compound **6j** (R = Cl, R^1 = 4-F) showed moderate activity against *A. niger*, whereas other derivatives found less active against all tested strains. Moreover compound 5-(5-(3,4-dimethoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)-3-(substituted phenyl)-1,2,4-oxadiazole, compound **6o** (R = 3,4-$(OMe)_2$, R^1 = 4-F) showed moderate activity against *A. niger.*

From the antimicrobial activity analysis, it was noticed that compounds **6a, 6b** and **6c** which contains R = H and R^1 = H/2- CH_3 or 3-CH_3 showed good activity against *A. niger* with MIC 62.5 µg/mL.

Conclusion

A new series of 5-(5-aryl-1-phenyl-1*H*-pyrazol-3-yl)-3-aryl-1,2,4-oxadiazole (**6a-o**) have been synthesized and characterized successfully. Biological evaluation study of 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-phenyl-1,2,4-oxadiazole (**6a**), 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-(*o*-tolyl)-1,2,4-oxadiazole (**6b**) and 5-(1,5-diphenyl-1*H*-pyrazol-3-yl)-3-(*m*-tolyl)-1,2,4-oxadiazole (**6c**) reported good activity against *A. niger*, whereas compounds **6a** and **6c** also exhibited moderate antibacterial activity against *P. mirabilis*.

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TABLE-2
ANTIMICROBIALACTIVITY IN ZONE OF INHIBITION (mm) OF SYNTHESIZED COMPOUNDS

Compd.	*E. coli*	*P. mirabilis*	*B. subtilis*	*S. albus*	*C. albicans*	*A. niger*
6a	12.2	15.6	9	12.6	11.5	15.6
6b	11.3	11.8	10	12	11.4	16.4
6c	9.8	13.75	10	11.6	10.6	15
6d	12.2	Inactive	10.25	12	12.6	10.2
6e	12.6	10.25	15.6	10	10.4	11.2
6f	9.75	11	13.4	10	11.6	13.4
6g	12	Inactive	13	10.8	10.25	12.25
6h	10.5	11.4	12	9.8	10.75	11
6i	Inactive	10.2	Inactive	11	11.2	11.4
6j	13	10.75	10.75	10.8	10.4	14
6k	11	11	Inactive	10	12.4	12.6
6l	12	Inactive	Inactive	10.4	10.4	12.2
6m	12.4	9.3	13	12	10.2	11.4
6n	11.2	9	11	9.8	10.4	12.6
6o	10.4	12.75	Inactive	10.6	10.4	13.75
Streptomycin	25.0	18.5	21.6	21.6	NA	NA
Fluconazole	NA	NA	NA	NA	20.3	18.4
Ravuconazole	NA	NA	NA	NA	28.5	20.2

TABLE-3
ANTIBACTERIAL ACTIVITY IN MINIMUM INHIBITORY CONCENTRATION (µg/mL) OF SYNTHESIZED COMPOUNDS

Compd.	*E. coli*	*P. mirabilis*	*B. subtilis*	*S. albus*	*C. albicans*	*A. niger*
6a	> 250	125	> 250	> 250	> 250	62.5
6b	> 250	> 250	> 250	> 250	> 250	62.5
6c	> 250	125	> 250	> 250	> 250	62.5
6d	> 250	–	> 250	> 250	> 250	> 250
6e	> 250	> 250	125	> 250	> 250	> 250
6f	> 250	> 250	250	> 250	> 250	250
6g	> 250	–	250	> 250	> 250	> 250
6h	> 250	> 250	250	> 250	> 250	> 250
6i	–	> 250	–	> 250	> 250	> 250
6j	250	> 250	> 250	> 250	> 250	125
6k	> 250	> 250	–	> 250	> 250	> 250
6l	> 250	–	–	> 250	> 250	> 250
6m	> 250	> 250	250	> 250	> 250	> 250
6n	> 250	> 250	> 250	> 250	> 250	> 250
6o	> 250	> 250	–	> 250	> 250	125
Streptomycin	7.81	15.62	7.81	7.81	NA	NA
Fluconazole	NA	NA	NA	NA	7.81	7.81
Ravuconazole	NA	NA	NA	NA	7.81	31.25

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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