Antibacterial and Antifungal activities of some new synthesized 1,3,4-oxadiazole containing pyrazolo[3,4-b]pyridin scaffold

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ABSTRACT
A series of novel compounds 2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(substituted) phenyl-1,3,4-oxadiazole **OP1-OP9** (Oxadiazole with Pyarazole) and derivatives N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(substituted)phenyl-1,3,4-oxadiazol-2-amine **OP10-OP18** were synthesized via intermediate 5-bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine that combined with different **1,3,4-oxadaizole-2-thiol derivatives** and **1,3,4-oxadiazol-2-amine derivatives** in presence of potassium carbonate and CuI with good yield. Intermediate 5-bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine was prepared with iodination of 5-bromo-1H-pyrazollo[3,4-b]pyridine in DMF. All novel series of **OP1-OP18** were identified via different spectral analysis and all final derivatives were tested for their antibacterial activity against gram +ve and gram -ve strains and antifungal activity. All results for scaffolds compare against the standard drug. Also studied their MIC (minimal inhibitory concentration).

Keywords 1,3,4 oxadiazole, Pyrazolo[3,4-b]pyridin, Antibacterial, Antifungal, Spectral studies.

INTRODUCTION

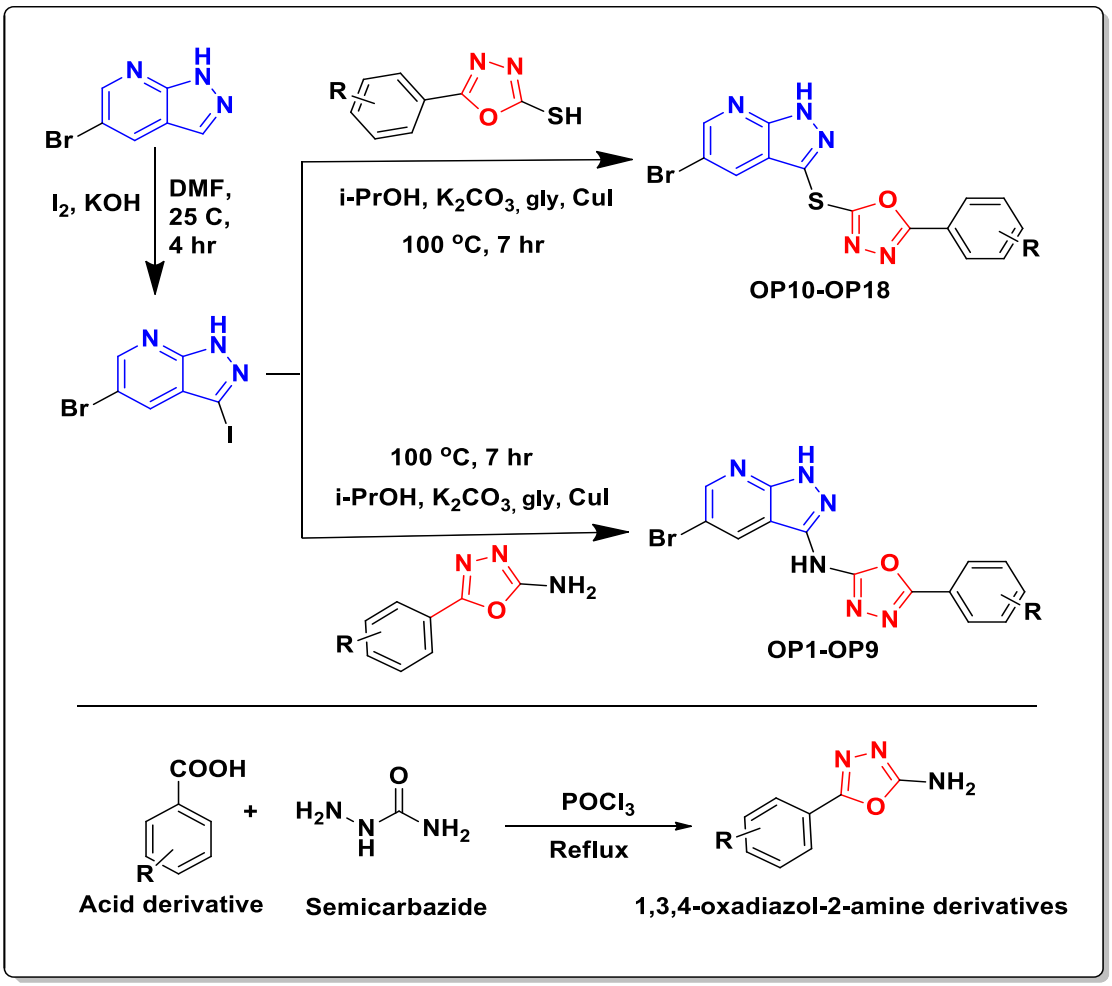
In the heterocyclic compound, most of the heterocycles contain a nitrogen atom. Nitrogen covering heterocycles are very useful important components in natural drug and pharmaceuticals. Pyrazolo[3,4-b]pyridins which belong to significant moiety of heterocyclic compound, have been widely explored for their application in the field of medicine and ring structure shows a variety of biological activities such as antimicrobial[1], hypnotic[2], inhibitors of xanthine oxidases, analgesic[3], antiviral[4], Anticancer[5][6], Antioxidant[7][8], Anti HIV[4],Alzheimer's disease, drug addiction, infertility, drug, and alcohol withdrawal symptoms[9]. Pyrazolo[3,4-b]pyridins have also been evaluated as effective and selective inhibitors of CDK(cyclin dependent kinase)[10], A1 adenosine receptors[11], phosphodiesterase-4 (PDE4) inhibitors in inflammatory cells and immune[12], GSK-3(glycogen synthase kinase-3) inhibitors[13], anti- antiplatelet and inflammatory agents[14]. That also developed as inhibitors of interleukin-6(IL-6) and tumor necrosis factor-alpha (TNF-α) which are two multifunctional pro-inflammatory cytokines complex in the pathogenesis of cardiovascular and neurodegenerative viruses and cancer[15]

In Addition, 1,3,4-Oxadiazole emerge as a most interesting scaffold in medicinal chemistry, have been shown an interesting therapeutic activities and wide range of application such as anti-microbial [16], anti-cancer [17], antiviral [18], anti-inflammatory [19], hypoglycemic[20], anti-anxiety[21], anti-depressant[22], etc.

In correlation of extensively Biologically applications for this most efficient compounds, for the initially, we have designed a synthetic approach to produce a set of new **OP1-OP18**. derivatives by consequential procedure were characterized by elemental analysis, spectral data scrutinize by well existing different range of activity such as antibacterial activity against gram +ve and gram -ve strains with minimum inhibition concentration (MIC) and Antifungal with minimum inhibition concentration (MIC) compared to standard.

2. MATERIALS AND METHODS

TLC with 0.2 mm precoated plates of silica gel G60 F254(Merck) was Consummate and visualization under UV light. IR spectra were recorded on FTIR spectrophotometer using DRS prob in DBLS, Ahmedabad University.NMR spectra were recorded on Bruker AVANCE II spectrometer in $CDCl_3$ and DMSO. Chemical shift stated in δ *ppm* andTMSusingas an internal standard. Mass spectra were determined using GCMS-QP 2010 Mass spectrometer (Shimadzu) using inlet prob. Solvent were evaporated using BUCHI rotary evaporator. MP was measured in open capillaries and is uncorrected.



Scheme-1 Synthesis of compounds **OP 1-18**

General procedure for preparation of the 1,3,4-oxadaizole-2-thiol derivatives

Substituted Phenyl hydrazide (0.01 mol) was taken in R.B.F. containing ethanol (40 ml) and KOH (0.01 mol), CS_2 (0.02 mol) was added drop wise to the well stirred solution and reflux for 13-15 hrs. The completion of the reaction was checked by TLC with mobile phase n-hexane/EtOAc 8:2. Then ethanol was distilled off and cooled to room temp. The content was poured into ice cold water and acidified with dilute HCL till the precipitates were obtained. The separated solid was washed with cold water and dried to get desired product and crystalline in Ethanol.

General procedure for preparation of 1,3,4-oxadiazol-2-amine derivatives

Different organic acid was reacted with semicarbazide in presence of $POCl_3$ at 90˚C temp for 4-5 hrs. Then it was allowed to stir 12 hrs. The completion of the reaction was monitored using TLC plate using ethyl acetate: hexane as mobile phase.

General procedure for preparation of 5-bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine

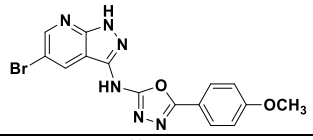
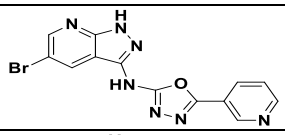
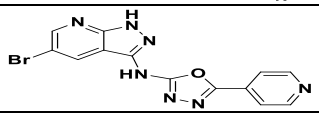
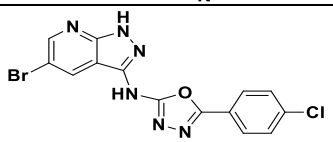
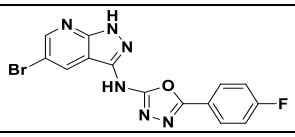
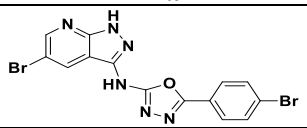
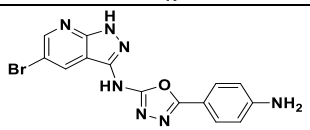
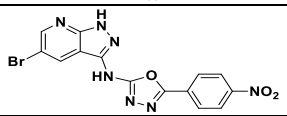
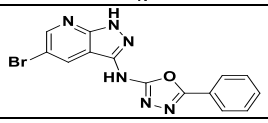
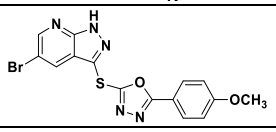
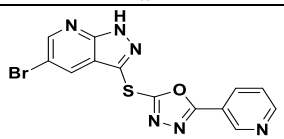
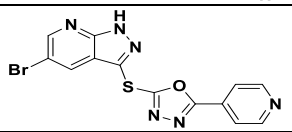
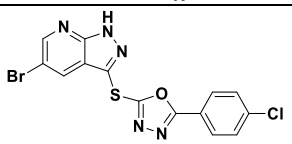
A solution of 5-bromo-1H-pyrazollo[3,4-b]pyridine (0.01 mol) in 20 ml DMF and KOH (0.02 mol) was added at 25˚C for 4 hr. the ensuing mixture was diluted with water and extracted with ethyl acetate. The organic phase was washed with saturated sodium thiosulphate solution and brine. Dry the compound and get a brown color solid.

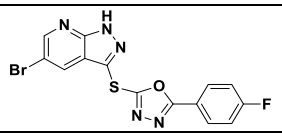
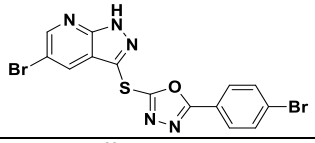
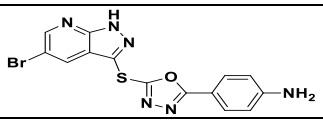
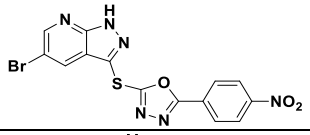
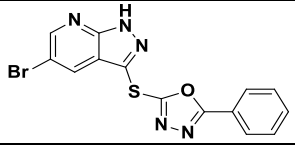
^{1}H NMR (400-MHz, DMSO-d6) δ 14.31 (s, 1H, amine), 8.66 (s, 1H, Aryl), 8.20 (s, 1H, aryl).

General procedure for preparation of 2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(Substituted)phenyl-1,3,4-oxadiazole/N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5--(Substituted)phenyl-1,3,4-oxadiazol-2-amine

The appropriate 1,3,4-oxadiazole-2-thiol or 1,3,4-oxadiazol-2-amine derivatives were reacted with 5-bromo-3-iodo-1H-pyrazolo[3,4-b]pyridine in refluxing isopropyl alcohol in the presence of potassium carbonate and CuI was added to the above mixture. Then it was allowed to stir 7hrs at temp the completion of the reaction was monitored using TLC plate using ethyl acetate:hexane as mobile phase

Table 1 Physical data and substitutions of compounds **OP1-OP18.**

Entry	Compounds	M.P(°C)	Molecular Weight	Molecular Formula	Yield %
OP1		~230	387.20	$C_{15}H_{11}BrN_6O_2$	67
OP2		~223	358.16	$C_{13}H_8BrN_7O$	76
OP3		~234	358.16	$C_{13}H_8BrN_7O$	78
OP4		~230	391.61	$C_{14}H_8BrClN_6O$	67
OP5		~240	375.16	$C_{14}H_8BrFN_6O$	68
OP6		~233	436.07	$C_{14}H_8Br_2N_6O$	67
OP7		~231	372.19	$C_{14}H_{10}BrN_7O$	61
OP8		~235	402.17	$C_{14}H_8BrN_7O_3$	70
OP9		~230	357.16	$C_{14}H_9BrN_6O$	64
OP10		~239	404.24	$C_{15}H_{10}BrN_5O_2S$	65
OP11		~229	375.20	$C_{13}H_7BrN_6OS$	71
OP12		~220	375.20	$C_{13}H_7BrN_6OS$	68
OP13		~234	408.66	$C_{14}H_7BrClN_5OS$	68

OP14		~240	392.21	$C_{14}H_7BrFN_5OS$	71
OP15		~234	453.11	$C_{14}H_7Br_2N_5OS$	75
OP16		~220	389.23	$C_{14}H_9BrN_6OS$	58
OP17		~224	419.21	$C_{14}H_7BrN_6O_3S$	66
OP18		~231	374.22	$C_{14}H_8BrN_5OS$	64

3. EXPERIMENTAL DATA

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-amine OP1.

IR(KBr) cm^{-1}:3278, 3149 , 3061,2971,2841, 1593, 1344, 1278, 1170, 1133, 1014, 651. ^1H NMR (400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, amine), 8.65 (s, 1H, aryl), 8.21 (s, 1H, Ar-H), 3.82(s, 3H, Ar-OCH_3), 7.82-8.88(m,4H, Ar), 9.75(s,1H,NH). ^{13}C NMR: 108,130,145,149(pyrazollo ring), 154,156(oxa ring), 113,127,131,162(aryl), 56(OCH_3). MS: m/z = 388.05 (M^+) Elemental Analysis: Calc. C, 46.53; H, 2.86; N, 21.71; O, 8.26Found. C, 46.65; H, 2.75; N, 21.77; O, 8.15

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine OP2.

IR(KBr) cm^{-1}:3280, 3229, 3051, 2956, 2885, 1591, 1349, 1247, 1271, 1170, 1016, 650. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.32 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H).7.80-8.84(m,4H, py), 9.75(s,1H,NH). ^{13}C NMR: 108,130,145,149(pyrazollo ring), 154,156(oxa ring), 123,148(py). MS: m/z = 359.04 (M^+). Elemental Analysis: Calc. C, 43.60; H, 2.25; N, 27.38; O, 4.47Found.C, 43.66; H, 2.31; N, 27.36; O, 4.42.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine OP3.

IR(KBr) cm^{-1}:3278, 3061, 2971, 2901, 2841, 1595, 1341, 1275, 1257, 1154, 1031, 762, 684. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.33 (s, 1H, NH), 8.62 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 7.82-8.83(m,4H, py), 9.82(s,1H,NH).^{13}C NMR:108,130,145, 149 (pyrazollo ring), 154,156(oxa ring), 123-150(aryl). MS: m/z = 358.1(M^+). Elemental Analysis: Calc. C, 43.60; H, 2.25; N, 27.38; O, 4.47. Found. C, 43.64; H, 2.26; N, 27.43; O, 4.41.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(4-chlorophenyl)-1,3,4-oxadiazol-2-amine OP4.

IR(KBr) cm^{-1}: 3294, 2954, 2881, 1586, 1354, 1276, 1167, 1024, 807, 698. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.29 (s, 1H, NH), 8.66 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.85-8.86(m,4H, Ar), 9.75(s,1H,NH). ^{13}C NMR:108,130,145,149 (pyrazollo ring), 154,156(oxa ring), 128-138(aryl). MS: m/z = 392.55 (M^+). Elemental Analysis: Calc. C, 42.94; H, 2.06; N, 21.46; O, 4.09. Found. C, 42.89; H, 2.01; N, 21.41; O, 4.05.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine OP5.

IR(KBr) cm^{-1}: 3279, 3065, 2959, 2882, 1599, 1347, 1298, 1164, 1126, 1026, 645. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.28 (s, 1H, NH), 8.58 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 7.88-8.90(m,4H, Ar), 9.75(s,1H,NH). ^{13}C

NMR:108,130,145,149(pyrazollo ring), 154,156(oxa ring), 116-166(aryl). MS: m/z = 476.05 (M^+). Elemental Analysis: Calc. C, 44.82; H, 2.15; N, 22.40; O, 4.26. Found. C, 44.76; H, 2.11; N, 22.37; O, 4.18.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(4-bromophenyl)-1,3,4-oxadiazol-2-amine OP6.

IR(KBr) cm^{-1}:3269, 3049, 2957, 2878, 1573, 1354, 1267, 1170, 1014, 664, 655. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.70 (s, 1H, NH), 8.55 (s, 1H, Ar-H), 8.25 (s, 1H, Ar-H), 7.86-8.85(m,4H, Ar), 9.75(s,1H,NH). ^{13}C NMR:108,130,145,149(pyrazollo ring), 154,156(oxa ring), 125-132(aryl). MS: m/z = 436.99 (M^+). Elemental Analysis: Calc. C, 38.56; H, 1.85; N, 19.27; O, 3.67. Found. C, 38.49; H, 1.81; N, 19.22; O, 3.69.

5-(4-aminophenyl)-N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-1,3,4-oxadiazol-2-amine OP7.

IR(KBr) cm^{-1}: 3351, 3289, 3055, 2958, 2889, 1593, 1344, 1278, 1170, 1014, 651. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.84-8.91(m,4H, Ar), 9.75(s,1H,NH), 5.67(s,2H,NH_2).^{13}C NMR:108,130,145,149(pyrazollo ring), 154,156(oxa ring), 114-156(aryl). MS: m/z = 372.01 (M^+). Elemental Analysis: Calc. C, 45.18; H, 2.71; N, 26.34; O, 4.30. Found. C, 45.22; H, 2.65; N, 26.31; O, 4.37.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine OP8.

IR(KBr) cm^{-1}: 3289, 3055, 2948, 2889, 1573, 1544, 1334, 1268, 1177, 1019, 659. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.23 (s, 1H, Ar-H), 7.82-8.89(m,4H, Ar), 9.75(s,1H,NH). ^{13}C NMR:108,130,145,149(pyrazollo ring), 154,156(oxa ring), 124-147(aryl). MS: m/z = 403.02 (M^+). Elemental Analysis: Calc. C, 41.81; H, 2.01; N, 24.38; O, 11.93. Found. C, 41.75; H, 2.10; N, 24.32; O, 11.86.

N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-phenyl-1,3,4-oxadiazol-2-amine OP9.

IR(KBr) cm^{-1}:3279, 3057, 2955, 2881, 1583, 1335, 1278, 1170, 1024, 651. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.29 (s, 1H, NH), 8.68 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.75-8.85(m,5H, Ar), 9.75(s,1H,NH). ^{13}C NMR:108,130,145,149(pyrazollo ring), 154,156(oxa ring), 126-131(aryl). MS: m/z = 458.08 (M^+). Elemental Analysis: Calc. C, 47.08; H, 2.54; N, 23.53; O, 4.48. Found. C, 47.15; H, 2.50; N, 23.48; O, 4.42.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(4-methoxyphenyl)-1,3,4-oxadiazole OP10.

IR(KBr) cm^{-1}:3259, 3075, 2957, 2889, 1593, 1344, 1279, 1180, 1132, 1014, 697, 659. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.31 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.17 (s, 1H, Ar-H), 7.86-8.93 (m,4H, Ar), 3.75(s,3H,OCH_3).^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 113-162(aryl), 56(OCH_3). MS: m/z = 405.12 (M^+). Elemental Analysis: Calc. C, 44.57; H, 2.49; N, 17.32; O, 7.92. Found. C, 44.52; H, 2.44; N, 17.37; O, 7.95.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(pyridin-3-yl)-1,3,4-oxadiazole OP11.

IR(KBr) cm^{-1}: 3278, 3055, 2958, 2889, 1593, 1576, 1344, 1278, 1170, 1014, 699, 652. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, NH), 8.62 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.81-8.82(m,4H, Ar). ^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 128-149(aryl). MS: m/z = 375.0 (M^+). Elemental Analysis: Calc. C, 41.61; H, 1.88; N, 22.40; O, 4.26. Found. C, 41.67; H, 1.82; N, 22.35; O, 4.31.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(pyridin-4-yl)-1,3,4-oxadiazole OP12.

IR(KBr) cm^{-1}: 3279, 3057, 2955, 2881, 1583, 1409, 1335, 1278, 1170, 1076, 704, 594. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.33 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.82-8.33(m,4H, Py). ^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 123-150(aryl). MS: m/z = 375.0 (M^+). Elemental Analysis: Calc. C, 41.61; H, 1.88; N, 22.40; O, 4.26. Found. C, 41.57; H, 1.83; N, 22.45; O, 4.16

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(4-chlorophenyl)-1,3,4-oxadiazole OP13.

IR(KBr) cm^{-1}: 3281, 3059, 2958, 2889, 1593, 1346, 1273, 1170, 1014, 762, 689, 654. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.34 (s, 1H, NH), 8.64 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 8.18-9.16(m,4H, Py).^{13}C

NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 128-137(aryl). MS: m/z = 409.54 (M^+). Elemental Analysis: Calc. C, 41.15; H, 1.73; N, 17.14; O, 3.92. Found. C, 41.19; H, 1.63; N, 17.05; O, 3.84.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(4-fluorophenyl)-1,3,4-oxadiazole OP14.

IR(KBr) cm^{-1}: 3286, 3179, 3059, 2958, 2881, 1599, 1356, 1298, 1178, 1143, 1014, 702,664. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, NH), 8.65 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.78-8.86(m,4H, Ar). ^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 116-166(aryl).MS: m/z = 393.02 (M^+). Elemental Analysis: Calc. C, 42.87; H, 1.80; N, 17.86; O, 4.08. Found. C, 42.87; H, 1.80; N, 17.86; O, 4.08

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(4-bromophenyl)-1,3,4-oxadiazole OP15.

IR(KBr) cm^{-1}: 3289, 3055, 2958, 2889, 1593, 1344, 1278, 1170, 1014, 687, 642, 661. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.34 (s, 1H, NH), 8.58 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.79-8.84(m,4H, Ar). ^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 125-132(aryl). MS: m/z = 454.04 (M^+). Elemental Analysis: Calc. C, 37.11; H, 1.56; N, 15.46; O, 3.53. Found. C, 32.11; H, 1.51; N, 15.52; O, 3.56.

4-(5-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-1,3,4-oxadiazol-2-yl)aniline OP16.

IR(KBr) cm^{-1}:3310, 3299, 3015, 2928, 2839, 1573, 1324, 1268, 1174, 1014, 697, 651.^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.31 (s, 1H, NH), 8.57 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.82-8.87(m,4H, Ar). 5.78(s,2H,NH_2).^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 114,118,127,156(aryl). MS: m/z = 399.08 (M^+). Elemental Analysis: Calc. C, 43.20; H, 2.33; N, 21.59; O, 4.11. Found. C, 43.18; H, 2.23; N, 21.63; O, 4.15.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(4-nitrophenyl)-1,3,4-oxadiazole OP17.

IR(KBr) cm^{-1}:3212,3145, 2858, 2759, 1653, 1466, 1324, 1268, 1170, 1014, 699, 655. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.30 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 8.20 (s, 1H, Ar-H), 7.76-8.88(m,4H, Ar). ^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 124,127,132,147(aryl). MS: m/z = 420.08 (M^+). Elemental Analysis: Calc. C, 40.11; H, 1.68; N, 20.05; O, 11.45. Found. C, 40.15; H, 1.67; N, 20.15; O, 11.40.

2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-phenyl-1,3,4-oxadiazole OP18.

IR(KBr) cm^{-1}:3238, 3110, 3055, 2958, 2889, 1593, 1394, 1310, 1178, 1011, 706, 659. ^1H NMR(400-MHz, DMSO-d_6) δ (ppm):δ 14.33 (s, 1H, NH), 8.57 (s, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 7.75-8.80(m,5H, Ar).^{13}C NMR:110,114,132,142,147,149 (pyrazollo ring), 152,159(oxa ring), 126,127,129,131(aryl). MS: m/z = 375.15 (M^+). Elemental Analysis: Calc. C, 44.93; H, 2.15; N, 18.71; O, 4.28. Found. C, 44.95; H, 2.17; N, 18.77; O, 4.21.

4. RESULTS AND DISCUSSION

4.1 Antibacterial activity

All the synthesized compounds were carried out for their antibacterial activity on nutrient-agar plates by well-diffusion assay method compared to test culture. Cultures were produced in Nutrient broth. Isolates inhibits the above mentioned organisms or not were studied and zone of inhibition was measured in terms of Zone diameter and with the help of that zone index was calculated where streptomycin was used as standard drug.

$$\boldsymbol{Activityindex\ (A.I.)} = \frac{\boldsymbol{MeanofZoneofinhibitionofderivative}}{\boldsymbol{Zoneofinhibitionobtainedforstandardantibioticdrug}}$$

Note: Standard drug used streptomycin with 1000 µg/ml concentration

4.2 Determination of antibacterial activity (zone inhibition and MIC)

The newly synthesized compounds were screened in vitro for antibacterial activity against Gram positive *Micrococcus luteus* (MTCC No. 11948), *Bacillus Cereus* (MTCC No. 8558) and Gram negative

Enterobacteraerogens (MTCC No. 8558), *Escherichia coli* (MTCC No. 1610) by determining the zone of inhibition in mm. These four bacterial strains were tested on 24 h old cultures. Bacterial strains were maintained under ±37 ºC for 24 h by using nutrient agar medium. Activated culture was centrifuged for 15 min at 3000 rpm and after centrifuged all the supernatant was collected and used to study for anti-bacterial activity. Prepare a 1000µg/ml solution of all compounds using 95% DMF and directly pour into agar plates. The plates were inoculated for 37 ºC for 24 hour. The inhibitions zone was measured were the microorganism inhibited after the incubation was done.All the compounds were compared to standard streptomycin drug. Shown in **Table-2**

Broth micro dilution technique was used to evaluate the minimum Inhibitory concentration (MIC) of all synthesized compounds. The serially two fold dilutions of tested compounds and control inoculated with actively bacterial cell which were the nutrient broth and monitoring by spectrophotometer. The maximum dilution or minimum concentration which was required for kill bacterial growth regard as minimum Inhibitory concentration (MIC). MIC values shown in **Table-3**

This activity was done by in vitro agar well diffusion method. The percentage of zone inhibition was calculated in term of active zone index in which the streptomycin was used as standard drug.

Table 2 Antibacterial activity of **OP1-OP18** compounds

Derivatives	***Enterobacter aerogens* MTCC No. 8558**		***Escherichia coli* MTCC No. 1610**		***Micrococcus luteus* MTCC No. 11948**		***Bacillus cereus* MTCC No. 8558**	
	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)
OP1	18	0.750	17	0.708	15	0.625	15	0.625
OP2	17	0.708	19	0.792	19	0.79167	21	0.875
OP3	30	1.250	27	1.125	25	1.04167	26	1.083
OP4	30	1.250	32	1.333	29	1.20833	28	1.167
OP5	24	1.000	23	0.958	26	1.08333	24	1.000
OP6	15	0.625	23	0.958	19	0.79167	15	0.625
OP7	15	0.625	14	0.583	15	0.625	17	0.708
OP8	30	1.250	36	1.500	30	1.25	36	1.500
OP9	20	0.833	19	0.792	16	0.66667	21	0.875
OP10	19	0.792	16	0.667	17	0.70833	17	0.708
OP11	15	0.625	14	0.583	19	0.79167	20	0.833
OP12	22	0.917	26	1.083	29	1.20833	28	1.167
OP13	28	1.167	28	1.167	30	1.25	29	1.208
OP14	21	0.875	27	1.125	22	0.91667	24	1.000
OP15	17	0.708	19	0.792	22	0.91667	20	0.833
OP16	30	1.250	19	0.792	19	0.79167	19	0.792
OP17	32	1.333	30	1.250	30	1.25	29	1.208
OP18	17	0.708	15	0.625	15	0.625	17	0.708
Std	24	-	24	-	24	-	24	-

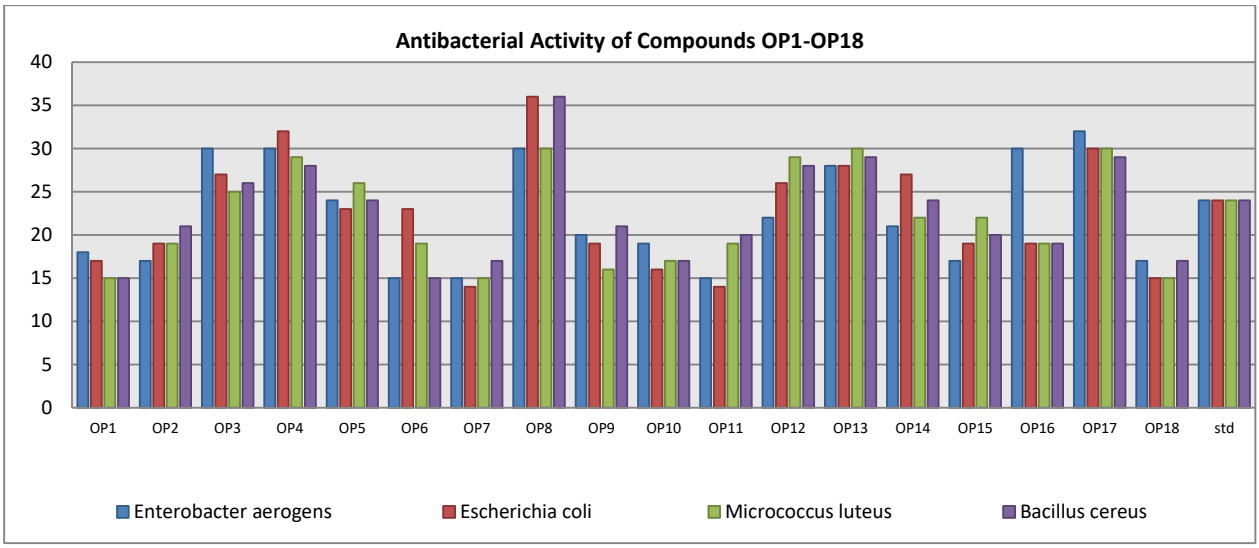


Figure-1 Antibacterial activity for compounds **OP1 to OP18.**

For all synthesized compounds **OP3, OP4, OP8, OP12** and **OP13** scaffold showed very good MIC values near to streptomycin shown in table-6 and other compound shown good, moderate and leaser MIC values. However, the compound **OP14 and OP17**showed very good zone inhibition activity as well as in MIC for all bacterial strains.

Table 3 MIC results of **OP1-OP18**compounds

Derivatives	***Enterobacter aerogens*** **MTCC No. 8558**	***Escherichia coli*** **MTCC No. 1610**	***Micrococcus luteus*** **MTCC No. 11948**	***Bacillus cereus*** **MTCC No. 8558**
	MIC(µg/ml)	**MIC(µg/ml)**	**MIC(µg/ml)**	**MIC(µg/ml)**
OP1	200	100	200	100
OP2	50	100	100	100
OP3	12.5	25	50	50
OP4	25	25	50	25
OP5	25	12.5	50	25
OP6	100	50	100	200
OP7	100	200	200	200
OP8	12.5	25	12.5	25
OP9	100	100	200	100
OP10	100	200	200	100
OP11	100	200	100	50
OP12	50	100	100	100
OP13	100	25	25	50
OP14	25	12.5	50	25
OP15	100	100	100	200
OP16	200	200	200	200
OP17	25	25	12.5	25
OP18	200	100	50	200
std	6.25	6.25	3.125	6.25

4.2 Antifungal activity

The antifungal activity was done by using Sabourauds agar media well diffusion method. The percentage of zone inhibition was calculated in term of active zone index in which the voriconazole was used as standard drug.

Determination of activity index

$$Activityindex(A.I) = \frac{meanofzoneofinhibitionofderivatives}{zoneofinhibitionobtainedforstandardantibioticdrug}$$

Determination of antifungal activity (zone inhibition and MIC)

All the synthesized Compounds were screened for their antifungal activity against *Candida albicans* and *Aspergillus niger* in DMF. vitro agar well diffusion method was used to evaluated antifungal activity. The materials require for the antifugal activity was given below. Pepton (1g), D-glucose (4g), Distilled water (100 ml), Agar (2g), Prepare a mixture of all material to form a Saubourauds agar media. Use a Distilled water to maintain 5.7 pH. After preparation make a suspension for fungal strain. However the making suspension of corresponding species, the fungal transferred into 3ml salin and make a disc by adding 20 ml of fungal media for each Petri dish and put into the Incubator at 37 ºC for 1 day. After dry a prepared control was allowed for 3-4 day at 37 ºC and the fungal inhibitions zone was measured were the microorganism inhibited after the incubation was done. All the compounds were compared to standard voriconazole drug.

Broth micro dilution method was used to evaluate a minimum Inhibitory concentration (MIC) of all synthesized compounds. *Aspergillus niger* and *Candida albicans* was used as standard fungal strains to evaluate a antifungal activity. The serially two fold dilutions of tested compounds and control inoculated with actively fungal cell which were the nutrient broth and monitoring by spectrophotometer. The maximum dilution or minimum concentration which was required for kill bacterial growth regard as minimum Inhibitory concentration (MIC). MIC values shown in **table-5**

Table 4 Antifungal activity of **OP1-OP18** compounds

Derivatives	***Aspergillus niger***		***Candida albicans***	
	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)	Mean value for Zone of Inhibition (mm)	Activity Index (A.I.)
OP1	24	0.857	21	0.875
OP2	28	1.000	23	0.958
OP3	18	0.643	16	0.667
OP4	22	0.786	21	0.875
OP5	24	0.857	23	0.958
OP6	28	1.000	22	0.917
OP7	22	0.786	20	0.833
OP8	25	0.893	26	1.083
OP9	20	0.714	21	0.875
OP10	15	0.536	17	0.708
OP11	27	0.964	17	0.708
OP12	28	1.000	25	1.042
OP13	28	1.000	21	0.875
OP14	25	0.893	21	0.875
OP15	27	0.964	22	0.917
OP16	15	0.536	17	0.708
OP17	30	1.071	20	0.833
OP18	25	0.893	21	0.875
std	28	-	24	-

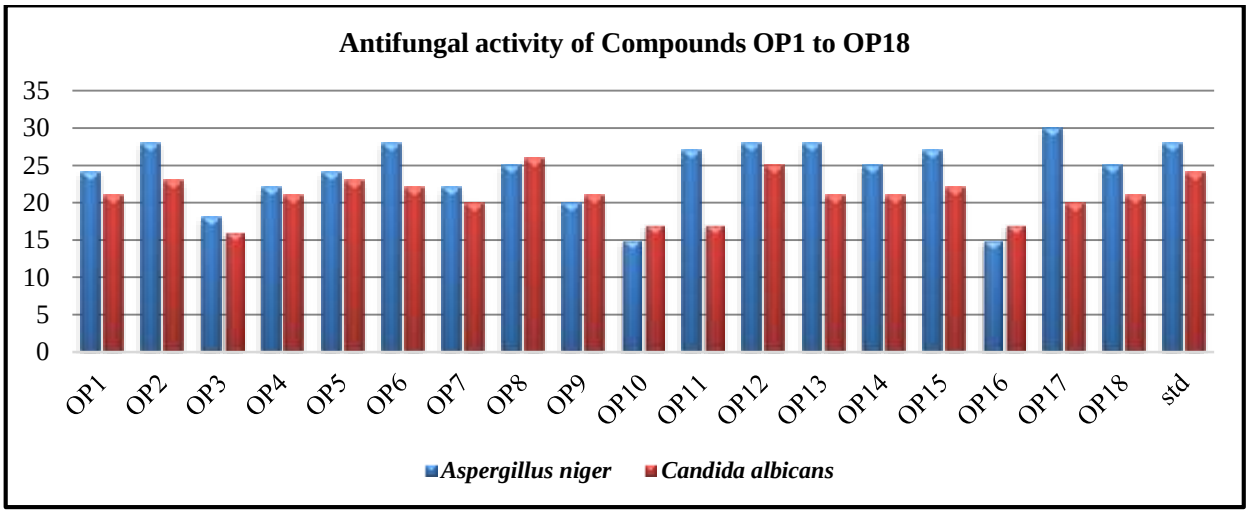


Figure -2 Antifungal activity for Compounds **OP1 to OP18.**

Minimum inhibition concentration (MIC) of antifungal For all synthesized compounds the **OP3, OP11** and **OP14**scaffold showed moderate MIC values compared to standard drug voriconazole shown in table-**14** and other compound shown average MIC values. However, the compound **OP2, OP12** and **OP8** showed very good zone inhibition activity as well as in MIC for all fungal strains.

Table 5 **MIC results of** OP1-OP18 **compounds**

Derivatives	***Aspergillus niger*** **MIC (µg/ml)**	***Candida albicans*** **MIC (µg/ml)**
OP1	200	200
OP2	100	400
OP3	12.5	12.5
OP4	200	200
OP5	400	200
OP6	100	50
OP7	100	400
OP8	200	400
OP9	50	100
OP10	100	200
OP11	6.25	12.5
OP12	50	200
OP13	12.5	100
OP14	6.25	6.25
OP15	100	50
OP16	200	400
OP17	400	200
OP18	100	50
Std	6.25	3.125

CONCLUSIONS

In this current work we describe the synthesis and biological properties of 2-((5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)thio)-5-(substituted)phenyl-1,3,4-oxadiazole **OP1-OP9** (Oxadiazole with Pyarazole) and derivatives N-(5-bromo-1H-pyrazolo[3,4-b]pyridin-3-yl)-5-(substituted)phenyl-1,3,4-oxadiazol-2-amine **OP10-OP18** and evaluated for their in vitro antibacterial against two Gram-positive and two gram-negative strains, the examined results **OP3, OP4, OP8, OP12** and **OP13** scaffold showed very good inhibitions and MIC values near to streptomycin. Moreover, compound **OP2, OP12** and **OP8** showed very good zone inhibition activity as well as in MIC for all fungal strains. All compounds accepted by spectral analysis.

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