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Synthesis and Biological Evaluation of Novel Series of 3-Substituted 2-Methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine Derivatives as Antimicrobial Agents

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A series of novel 2-methoxy-3-phenyl-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine (**6a-h**) have been synthesized from bromo methoxy picoline as starting material. Finally, oxadiazoles were coupled with various boronic acids using Suzuki coupling reactions to give the target compounds. The newly synthesized compounds were evaluated for their efficacy as antimicrobial agents against various Gram-positive and Gram-negative strains of bacteria and fungal strains. Compounds **6g** and **6h** possessing fluoro groups at second and fourth positions of the aniline ring exhibited broad spectrum inhibition against all the strains tested. Moreover, other compounds also found to be potential fungal agents in comparison to the standard drugs.

Keywords: 1,3,4-Oxadiazoles, Antibacterial activity, Antifungal activity, Nematicidal activity.

INTRODUCTION

In the past decades, multi-drug resistant microorganisms caused problems to the alarming level in many countries in the world. Several reports describe infections caused by these microorganisms pose serious challenges and thus require an effective therapy for antimicrobial treatment. In addition, the risk of opportunistic fungal infections increases rapidly accompanied with other disease and led to invasive infections and cause of mortality for these patients.

Heterocyclic is the largest classical division of medicinal chemistry and is display a broad range of industrial and pharmaceutical applications[1]. Oxadiazole ring system has long been known to possess interesting biological profile[2-5]. A variety of substituted 1,3,4-oxadiazoles embedded with different biological agents and a significant amount of research activity has been directed towards this class. A large number of heterocylic compounds containing the oxadiazole scaffold associated with a broad spectrum of pharmacological activities such as antimalarial[6], antiinflammatory[7], anticonvulsant[8], analgesic[9], antimicrobial[10], antimycobacterial[11], antitumor[12], herbicidal[13], vasodialatory[14], cytotoxic[15], hypolipidemic[5] and antiedema[16].

In the present investigation, some substituted 1,3,4-oxadiazole derivatives were synthesized and evaluated for their potential antibacterial and antifungal activity.

EXPERIMENTAL

All reagents and solvents were used as purchased without further purification. Crude products were purified by column chromatography on silica gel of 60-120 mesh. NMR spectra were recorded on a Varian 400 MHz and 500MHz spectrometer for ^{1}H NMR. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The chemical shifts were reported as ppm down field using TMS as an internal standard. A mass spectrum was recorded on a VG-Micro mass 7070H spectrometer operating at 70 eV.

Synthesis of 5-bromo-6-methoxy-pyridine-2-carboxylic acid methyl ester (2): To a solution of 5-bromo-6-methoxy picolinic acid (**1**) (1.0 g, 0.00431 mol) in DMF (10 mL, 10 vol), cooled to 0 °C was added Na_2CO_3 (456 mg, 0.00431 mol) and MeI (0.536 mL, 0.0086 mol) the mixture was stirred for 16 h at room temperature. Water was added, extracted with ethyl acetate, dried and purified by column chromatography using a gradient of hexane-EtOAc (9:1-8:2) to give pure compound (**2**): Off white solid; yield: 94 %, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.98 (s, 3H), 4.08 (s, 3H), 7.58 (d, 1H, Ar-H), 7.98 (d, 1H, Ar-H): MS: *m/z* (%) 247.6 [M^{+2}], HPLC purity (99.52 %).

Synthesis of 5-bromo-6-methoxy-pyridine-2-carboxylic acid hydrazide (3): To a solution of 5-bromo-6-methoxy-pyridine-2-carboxylic acid methyl ester (**2**) (1.0 g, 0.00406

mol) in EtOH (10 mL), was added $NH_2{\cdot}NH_2{\cdot}H_2O$ (813 mg, 0.0162 mol) and the mixture was stirred for 3 h at 70 °C, cooled to room temperature, solvents were evaporated, water was added, filtered the reaction mass, to give pure compound (**3**)**:** Off-white solid; yield: 85 %, m.p.: 188-190 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.02 (s, 3H, OCH_3), 4.08 (d, 2H, NH_2), 7.65 (d, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.68 (broad s, 1H, NH); MS: *m/z* (%) 247.7 [M^{+2}], HPLC purity (99.61 %).

Synthesis of 3-bromo-2-methoxy-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)-pyridine (5): To a solution of 5-bromo-6-methoxy-pyridine-2-carboxylic acid hydrazide (**3**) (800 mg, 0.00325 mol) and benzoic acid **4** (460 mg, 0.00325 mol) in $POCl_3$ (16 mL) and the mixture was stirred for 12 h at 115 °C, cooled to room temperature, ethyl acetate was added, washed with sat $NaHCO_3$ sol, dried with Na_2SO_4, filtered and concentrated under reduced pressure, purified by column chromatography EtOAc-Hexane (5:95-20:80) to give pure compound (**5**): Off-white solid; yield: 66 %, m.p.: 198-200 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.18 (s, 3H, OCH_3), 7.58 (m, 3H, Ar-H), 7.78 (d, 2H, Ar-H), 7.98 (d, 1H, Ar-H), 8.20 (s, 2H, Ar-H); MS: *m/z* (%) 333.7 [M^{+2}], HPLC purity (95.83 %).

Synthesis of 3-substituted 2-methoxy-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)pyridine (7a-h): To a solution of 3-bromo-2-methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine (**4**) (50 mg, 0.00015 mol), compound **6** (27.5 mg, 0.000225 mol), $Pd(dppf)_2Cl_2$ (11 mg, 0.000015 mol), LiOH (12.6 mg, 0.00030 mol), dioxane (0.8 mL), water (0.2 mL), and the mixture was stirred for 6 h at 120 °C, cooled to room temperature, water was added, extracted with EtOAc (2 × 30 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure, purified by column chromatography EtOAc:Hexane (1-9:2-8) to give pure compound (**7a-h**).

2-Methoxy-3-phenyl-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine (7a): Off-white solid, yield: 66 %, m.p.: 196-198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.16 (s, 3H, OCH_3), 7.42 (m, 1H, Ar-H), 7.48 (m, 2H, Ar-H), 7.58 (m, 3H, Ar-H), 7.62 (m, 2H, Ar-H), 7.80 (d, 1H, Ar-H), 7.98 (d, 1H, Ar-H) 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 329.9 [M^+], HPLC purity (96.85 %).

2-Methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)-3-*o*-tolyl-pyridine (7b): Off-white solid; yield: 64 %, m.p.: 187-190 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 2.32 (s, 3H, CH_3), 4.16 (s, 3H, OCH_3), 7.44 (m, 1H, Ar-H), 7.54 (m, 2H, Ar-H), 7.62 (m, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 7.86 (d, 1H, Ar-H), 7.98 (d, 1H, Ar-H) 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 343.9 [M^+], HPLC purity (97.82 %).

2-Methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)-3-*p*-tolyl-pyridine (7c): Off-whitesolid; yield: 69 %, m.p.: 183-185 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 2.32 (s, 3H, CH_3), 4.16 (s, 3H, OCH_3), 7.42 (m, 1H, Ar-H), 7.50 (m, 2H, Ar-H), 7.61 (m, 2H, Ar-H), 7.65 (m, 2H, Ar-H), 7.84 (d, 1H, Ar-H), 7.95 (d, 1H, Ar-H) 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 343.9 [M^+], HPLC purity (98.12 %).

3-Cyclopropyl-2-methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine (7d): Off-white solid; yield: 66 %, m.p.: 178-180 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.78 (m, 2H), 1.04 (m, 2H), 2.18 (m, 1H), 4.18 (s, 3H, OCH_3)7.22 (d, 1H, Ar-H), 7.46-7.50 (m, 3H, Ar-H), 7.78 (d, 1H, Ar-H), 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 293.9 [M^+], HPLC purity (95.67 %).

2-Methoxy-3-(2-methoxy-phenyl)-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)pyridine (7e): Off-white solid; yield: 68 %, m.p.: 168-170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 7.02 (m, 2H, Ar-H), 7.32 (d, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.56 (m, 3H, Ar-H), 7.70 (d, 1H, Ar-H), 7.92 (d, 1H, Ar-H) 8.21 (m, 2H, Ar-H); MS: *m/z* (%) 359.9 [M^+], HPLC purity (98.81 %).

2-Methoxy-3-(4-methoxy-phenyl)-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)pyridine (7f): Off-white solid; yield: 62 %, m.p.: 153-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.80 (s, 3H, OCH_3), 4.08 (s, 3H, OCH_3), 7.00 (m, 2H, Ar-H), 7.32 (d, 1H, Ar-H), 7.38 (m, 1H, Ar-H), 7.52 (m, 3H, Ar-H), 7.68 (d, 1H, Ar-H), 7.92 (d, 1H, Ar-H) 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 359.9 [M^+], HPLC purity (96.85 %).

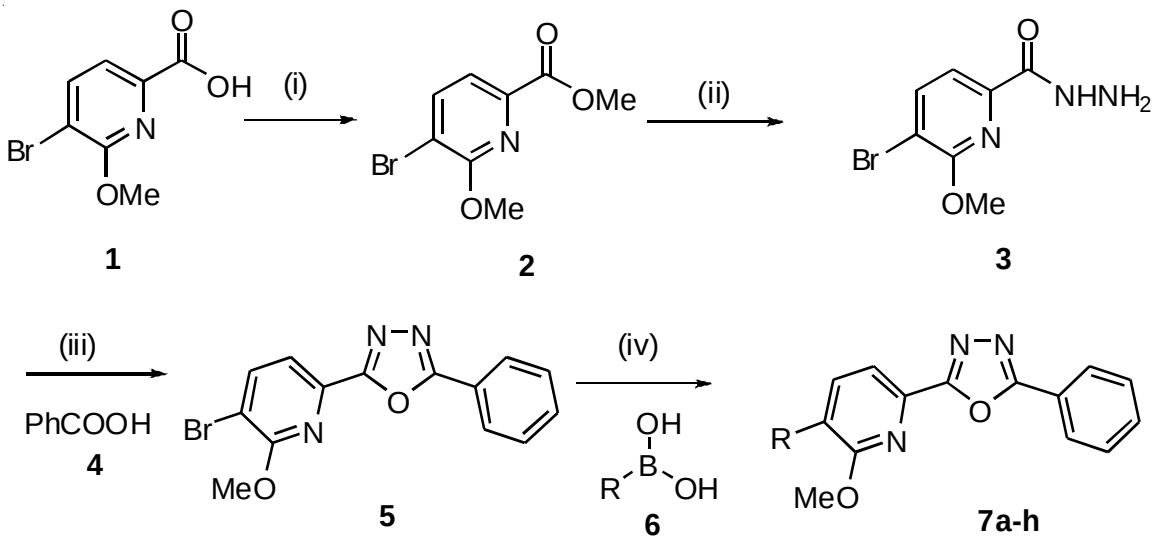
3-(2-Fluoro-phenyl)-2-methoxy-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)pyridine (7g): Off-white solid; yield: 60 %, m.p.: 167-170 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.08 (s, 3H, OCH_3), 7.04 (m, 2H, Ar-H), 7.36 (d, 1H, Ar-H), 7.44 (m, 1H, Ar-H), 7.56 (m, 3H, Ar-H), 7.72 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H) 8.22 (m, 2H, Ar-H); MS: *m/z* (%) 347.9 [M^+], HPLC purity (95.81 %).

3-(4-Fluoro-phenyl)-2-methoxy-6-(5-phenyl-[1,3,4]-oxadiazol-2-yl)pyridine (7h): Off-white solid; yield: 60 %, m.p.: 176-178 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.08 (s, 3H, OCH_3), 7.02 (m, 2H, Ar-H), 7.36 (d, 1H, Ar-H), 7.42 (m, 1H, Ar-H), 7.54 (m, 3H, Ar-H), 7.72 (d, 1H, Ar-H), 7.94 (d, 1H, Ar-H) 8.20 (m, 2H, Ar-H); MS: *m/z* (%) 347.9 [M^+], HPLC purity (95.17 %).

RESULTS AND DISCUSSION

Synthesis of a novel series of 3-substituted 2-methoxy-6-(5-phenyl-[1,3,4]oxadiazol-2-yl)pyridine derivatives is shown in **Scheme-I**. Esterification of **1** followed by treating with hydrazine hydrate afforded the compound **3**, which upon reaction with benzoic acid in the presence of $POCl_3$ furnished oxadiazloe derivatives **5**[17]. Finally, the key intermediates 1,3,4-oxadiazoles (**5**) were coupled with various boronic acids (**6a-h**) using Suzuki couling reaction gave the final compounds (**7a-h**). The chemical structures of all the newly synthesized compounds were confirmed by their ^{1}H NMR, ^{13}C NMR, mass spectral data and elemental analysis. Further these compounds have been screened for their antibacterial, antifungal and nematicidal activities.

Antibacterial activity: The *in vitro* antibacterial activity of the synthesized compounds **6a-h** was evaluated against three Gram-positive bacteria *Bacillus subtilis* MTCC 2415, *Staphylococcus aureu*s MTCC 9886 and *Micrococcus luteus* MTCC 1538 and Gram-negative bacteria *Escherichia coli* MTCC 448. Ampicillin was used as a standard in the assay. The minimum inhibitory concentrations (MIC) of the synthesized compounds were determined using broth dilution method according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS)[18]. The observed minimum inhibitory concentrations (MICs) are given in Table-1. In all determinations, tests were performed in duplicate and results were reported as mean of at least three determinations. According to the results displayed in Table-1, all the tested compounds exhibited moderated to significant antibacterial activity. The investigational data of antibacterial screen revealed that compounds



Reaction and condition: 1 (i) Na_2CO_3, MeI, DMF, (ii) $NH_2NH_2.H_2O$, EtOH, 3 h, reflux, (iii) $POCl_3$, 16h, 110 °C, (iv) $Pd(dppf)_2Cl_2$, LiOH, Dioxane, water, 6 h, 120 °C, R = (a) Ph, (b) 2-Me-Ph, (c) 4-Me-Ph, (d) cyclopropyl, (e) 2-OMe-Ph, (f) 4-OMe-Ph, (g) 2-F-Ph. (h) 4-F-Ph

Scheme-I

TABLE-1
ANTIBACTERIAL ACTIVITY (MIC IN µg/mL) OF 3-SUBSTITUTED 2-METHOXY-6-(5-PHENYL-[1,3,4]OXADIAZOL-2-YL)-PYRIDINE DERIVATIVES (**6a-h**)

Compd.	Antibacterial strains			
	S. aureus[a]	*M. luteus*[a]	*B. subtilis*[a]	*E. coli*[b]
6a	100	-	100	-
6b	50	50	50	-
6c	200	100	-	-
6d	-	100	200	-
6e	200	200	100	200
6f	100	100	50	100
6g	25	100	50	50
6h	50	50	25	100
Ampicillin	12.5	50	25	100

[a]Gram-positive bacteria: *Staphylococcus aureus* MTCC 9886, *Micrococcus luteus* MTCC 1538, *Bacillus subtilis* MTCC 2415 and [b]Gram negative bacteria: *Escherichia coli* MTCC 448. - Means inactive.

TABLE-2
ANTIFUNGAL ACTIVITY (ZONE OF INHIBITION IN mm AT 100 µg/mL) OF 3-SUBSTITUTED 2-METHOXY-6-(5-PHENYL-[1,3,4]OXADIAZOL-2-YL)-PYRIDINE DERIVATIVES (**6a-h**)

Compd.	*C. albicans*	*T. rubrum*	*A. fumigatus*	*T. mentagropytes*
6a	-	3	5	-
6b	11	10	12	15
6c	12	7	8	10
6d	10	-	5	4
6e	8	4	10	13
6f	4	8	6	7
6g	5	-	-	-
6h	-	5	4	6
Amphotericin-B	10	8	12	15

- Means inactive.

with fluoro substituents at 2- and 4-positions of the aniline ring in compounds **6g** and **6h** exhibited potent antibacaterial inhibition with MIC value in the range of 25-100 µg/mL. However, the other compounds displayed mild to moderate antibacterial activity against other organisms tested. Overall, these compounds showed mild or no inhibition against *E. coli.*

Antifungal activity: All the synthesized compounds **6a-h** were also assayed for their antifungal activity against four fungal strains. The antifungal screening data from Table-2 showed that all he tested compound showed moderate to comparable fungal inhibition. Compounds **6b,c** & **e** possessing methyl and methoxy positions at the *ortho* and *para* positions of the aniline ring exhibited significant antifungal inhibition. 4-Methoxy substituent in compound **6c** exhibited moderate broad rang of antifungal activity against, whereas the remaining compounds exhibited mild antifungal inhibition.

Nematicidal activity: The synthesized compounds **6a-h** were also evaluated for their nematicidal activity against *Ditylenchus myceliophagus* and *Caenorhabditis elegans* by aqueous *in vitro* screening technique at various concentrations. Levamisole was used as a standard for nematicidal activity. The results are shown in terms of LD_{50} *i.e.* median lethal dose

at which 50 % of nematodes became immobile (dead). The nematicidal screening data (Table-3) displayed that the compound **6g** is the most effective against *D. myceliophagus* and *C. elegans* with LD_{50} of 210 and 260 ppm, respectively and is almost equally active as the standard. Compounds **6h** also showed significant activity against *C. elegans* with LD_{50} of 220 ppm and 270 ppm, respectively. The other tested compounds showed moderate activity.

TABLE-3
MEDIAN LETHAL DOSE (LD_{50}, ppm) OF 3-SUBSTITUTED 2-METHOXY-6-(5-PHENYL-[1,3,4]OXADIAZOL-2-YL)-PYRIDINE DERIVATIVES (**6a-h**)

Compd.	*D. myceliophagus*	*C. elegans*
6a	390	510
6b	540	510
6c	650	480
6d	410	740
6e	450	390
6f	840	420
6g	210	260
6h	220	270
Levmisole	180	210

Conclusion

In conclusion, synthesis and evaluation of antibacterial and antifungal activities for a novel series of 1,3,4-oxadiazoles conjugates have been investigated. All the compounds have shown mild to potent inhibitory activity against all the antibacterial starins tested. Compounds **6g** and **6h** were found to be the most active against most of the tested organisms. Some of the compounds have shown significant antifungal activity in comparison to the controls.

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