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Synthesis, Characterization and Biological Evaluation of Novel Halogenated 1,3,4-Oxadiazoles Derieved from Substituted Pyrazole-3-Carbohydrazide

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ABSTRACT

5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **1** underwent a series of hetero-cyclization reactions with different chemical reagents such as triethylorthoformate, acetic acid in phosphorous oxychloride, benzoic acid in phosphorous oxychloride, *N*,*N*'carbonyldiimidazole in dioxane, carbon disulphide in pyridine to afford substituted 1,3,4-oxadiazoles 2, 3, 4, 5 and 6 respectively. Extending the reaction of 6 with 4-(2-chloroethyl) morpholine hydrochloride afforded 7. The structures of the newly synthesized compounds were established on the basis of spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral data. The synthesized compounds were screened for their antimicrobial activity against two gram positive and gram negative bacteria and a fungus and found to possess good activity against selected strains.

Keywords: Pyrazole-3-carbohydrazide, 1,3,4-oxadiazole, 1,3,4-oxadiazol-one, 1,3,4-oxadiazole-thione

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INTRODUCTION

Carbohydrazides or carboxylic acid hydrazide (–$CONHNH_2$) group is very reactive group because of its electron withdrawing nature. Thus providing a very good site for attract by the electrophiles or simply for addition reaction with dehydration. Carbohydrazides have been commonly used as an important intermediate in several heterocyclic transformations. On reaction with different functionalized compounds[1,2] they give 1,3,4-oxadiazole derivatives. These are one of the most important class of compounds possessing wide range of biological activities such as antimalarial[3], antituberculosis[4], anticancer[5] etc. Background of the earlier work on 1,3,4-oxadiazole reveals that it has been synthesized by many researches[6-10] utilizing different routes. A review published recently provides an overview of the main synthetic methodologies for 1,3,4-oxadiazoles and their broad spectrum of pharmacological activities[11].

The significance of 1,3,4-oxadiazoles in heterocyclic chemistry and in continuation of previous work prompted us to utilize substituted pyrazole-3-carbohydrazide as a substrate having reactive –$CONHNH_2$ group for the synthesis of bridgehead 1,3,4-oxadiazole derivatives of better biological activities by treating it with different reagents like triethylorthoformate, acetic acid in phosphorous oxychloride, benzoic acid in phosphorous oxychloride, *N*,*N*'carbonyldiimidazole in dioxane, carbon disulphide in pyridine by adopting the reported procedures[1] and to investigate their possible antibacterial and antifungal activities.

MATERIAL AND METHODS

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, v max in cm^{-1}). ^1H NMR and ^{13}C NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 and $CDCl_3$ as solvent. Chemical Shifts are given in parts per million (ppm). Positive-ion electrospray ionisation (ESI) mass spectra were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer. Elemental analysis (CHN) was done using Elemental analyzer, Vario EL III. All the chemicals used for the synthesis were of AR grade of Merck, S.D.Fine and Aldrich. The reactions were monitored by E.Merck TLC aluminum sheet silica $gel_{60}F_{254}$ and visualizing the spot in UV Cabinet and iodine chamber. The compounds were analyzed for carbon, hydrogen, nitrogen and sulphur and the results were in good agreement with the calculated values.

Experimental Procedure

Procedure for the synthesis of 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide[12] (1)

To a mixture of methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate (10 mmol) in ethanol (100mL), hydrazine hydrate (100%, 1.7mL) was added and refluxed for 8 hrs. Then it was concentrated, cooled, filtered and washed with water, dried to give **1**, recrystallized from acetic acid as white crystalline solid.

Procedure for the synthesis of 2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazole (2)

Triethylorthoformate (20 mL) was added to **1** (4 mmol) and reaction mixture was refluxed for 12h. Then the content was cooled, solid obtained was filtered and further recrystallized from ethanol to furnish **2**.

Procedure for the synthesis of 2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (3)

A mixture of **1** (4 mmol), acetic acid (4 mmol) and phosphorous oxychloride (40 mL) were refluxed for 2h. After cooling, the resultant solution was neutralized with 20% ammonium hydroxide. The solid obtained was filtered off, washed with water, dried and further purified by recrystallization from ethanol to afford **3**.

Procedure for the synthesis of 2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5-phenyl-1,3,4-oxadiazole (4)

A mixture of **1** (10 mmol), benzoic acid (10 mmol) and phosphorous oxychloride (15 mL) was refluxed on steam bath for 6h. The reaction mixture was cooled and poured on ice-water; ammonia was then added and solid separated was filtered off, washed with water, dried and further recrystallized from ethanol to yield **4**.

Procedure for the synthesis of 5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazol-2(3*H*)-one (5)

A mixture of **1** (10 mmol) and *N*,*N*'carbonyldiimidazole (15 mmol) in 1,4-dioxane (100 mL) was refluxed for 8h. Then the reaction mixture was cooled, residue obtained was triturated with ice-water mixture. The solid obtained was filtered off, dried and further recrystallized from ethanol to give **5**.

Procedure for the synthesis of 5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazole-2(3*H*)-thione (6)

A mixture of **1** (10 mmol), carbon disulphide (30 mL) and pyridine (100 mL) was refluxed on water bath for 6h. The reaction mixture was cooled and the residue obtained was triturated with ice-water mixture and neutralized with dilute HCl. The solid obtained was filtered and further recrystallized from ethanol to afford **6.**

Procedure for the synthesis of 4-(2-(5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazol-2-ylthio)ethyl)morpholine (7)

A mixture of **6** (10 mmol), sodium acetate (50 mmol) and 4-(2-chloroethyl) morpholine hydrochloride (10 mmol) in ethanol (100 mL) was refluxed for 6h. Then reaction mixture was cooled, residue was triturated with water, solid obtained was filtered off, dried and further recrystallized from ethanol to give **7**.

Antimicrobial Activity

All the novel synthesized compounds from **1-7** have been screened for their *in vitro* antibacterial activity against two gram positive strains i.e. *Bacillus subtilis* (NCIM 2439) and *Staphylococcus aureus* (NCIM 2079) and two gram negative strains i.e. *Escherichia coli* (NCIM 2064) and *Pseudomonas aeruginosa* (NCIB 8650) by using Mueller Hinton Agar and antifungal activity against a fungus *Aspergillus niger* (NCIM 501) using Sabouraud Dextrose agar using cup plate agar diffusion method[13-14] by measuring the inhibition zone in mm. The compounds were taken at a concentration of 1 mg/mL using Dimethyl Sulphoxide (DMSO) as solvent. Chloramphenicol (100 µg/mL) was used as standard for antibacterial and Kanamycin (100 µg/mL) for antifungal activity. 10ml of this sterilized agar media were poured into petridishes and allowed to solidify. On the surface of media microbial suspension were spread with the help of sterilized triangular loop. A stainless steel cylinder of 10mm diameter (pre-sterilized) was used to bore the cavity. Into these wells were added 0.1mL portion of the test compound in the solvent. The drug solution was allowed to diffuse for about an hour into the medium. The plates were incubated at 37°C for 24 hours for bacteria and 28°C for 72-96 hrs for fungus. Zone of inhibition observed around the cup after respective incubation was measured in four directions with the help of Vernier Calipers. Minimum Inhibitory Concentration (MIC µg/mL) was determined by broth dilution method. The stock solutions of synthesized compounds were prepared in DMSO as a solvent, starting with maximum concentration of 800µg/mL and then reducing it successively by two fold dilution methods using a calibrated micropipette to get concentrations of 400-6.25 µg/mL in nutrient broth. Similarly, serial dilution tubes for standard drug with its stock solution 100 µg/mL were also prepared. Double strength nutrient broth was used as a growth media. MIC of the sample was carried out by inoculation of these serial dilutions with test organisms. The inoculum size was

approximately 10^6 colony forming units (CFC/mL). The inoculated tubes were incubated for 24h at 37(±1) °C (bacteria) and for 72h at 28°C (fungus). After 24h and 72h the inoculated culture tubes were macroscopically examined for turbidity. Each test was performed in triplicate and MIC was considered to be the lowest concentration of the tested compound which inhibits the visible growth of bacteria or fungus after a period of incubation. The results of antifungal and antibacterial activity are given in the Table **1**.

Table 1 – Antibacterial and Antifungal activity (diameter zones/mm) of inhibition and ([a]*MIC* µg/mL) of the compounds 1-7

	Antibacterial				*Antifungal*
Compound	***B. subtilis* (NCIM 2439)**	***S. aureus* (NCIM 2079)**	***E. coli* (NCIM 2064)**	***P. aeruginosa* (NCIB 8650)**	***A. niger* (NCIM 501)**
1	25 (6.25)	28 (6.25)	19 (6.25)	19 (6.25)	22(6.25)
2	19 (12.5)	-	12 (12.5)	-	10(25)
3	24 (6.25)	29 (6.25)	19 (6.25)	24(6.25)	18(6.25)
4	15 (12.5)	16 (12.25)	15 (12.5)	10(25)	19(6.25)
5	-	15 (12.5)	18 (6.25)	-	14(12.5)
6	28(6.25)	25 (6.25)	20 (6.25)	-	22(6.25)
7	29 (6.25)	32 (6.25)	18 (6.25)	23(6.25)	23(6.25)
Kanamycin	-	-	-	-	23(6.25)
Chloramphenicol	29 (6.25)	32 (6.25)	21 (6.25)	25 (6.25)	-
DMSO	-	-	-	-	-

[a]***MIC*:** Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period of incubation.

RESULTS AND DISCUSSION

The synthesis of the key intermediate **1** was done according to the reference method[12] as described in reaction scheme **1**.

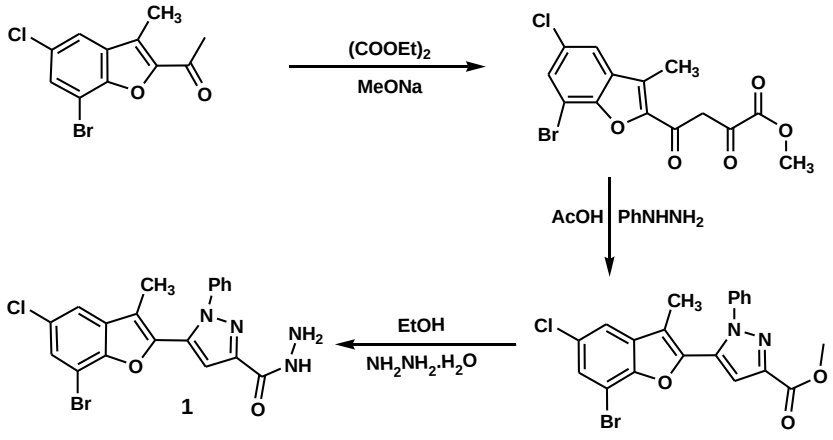

Figure1. Reaction Scheme for the Synthesis of 1

Figure 1:Reaction Scheme for Synthesis of 1

The synthesis of the novel compounds **2-7** is described in reaction scheme **2**. The reactions were monitored by TLC. The identities of the newly synthesized compound have been established on the basis of their elemental analysis and spectral data[15] such as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. The analytical data of the compounds are mentioned below. These compounds were screened for their antimicrobial activities. Reaction of **1** with triethylorthoformate yielded **2**. CH of oxadiazole ring in **2** showed a singlet at δ 9.25 ppm in ^{1}H NMR, similarly its mass spectra gave a $[M+H]^{+}$ peak at 456 which confirms formation of the 1,3,4-oxadiazole derivative having the molecular formula $C_{20}H_{12}O_2N_4ClBr$. Cyclodehydration occurred when **1** was treated with acetic acid in $POCl_3$ to give **3**. The ^{1}H NMR spectra of **3,** showed a singlet at δ 2.68 ppm due to CH_3, its mass spectra indicates $[M+H]^{+}$ peak at 470 is in agreement with its molecular formula $C_{21}H_{14}O_2N_4ClBr$. Similarly, action of benzoic acid in $POCl_3$ on **1** afforded another 1,3,4-oxadiazole derivative **4**. The structure of **4** was also confirmed from its mass spectra, where the molecular ion peak $[M+H]^{+}$ obtained at 532 is in consistent with the molecular formula $C_{26}H_{16}O_2N_4ClBr$.

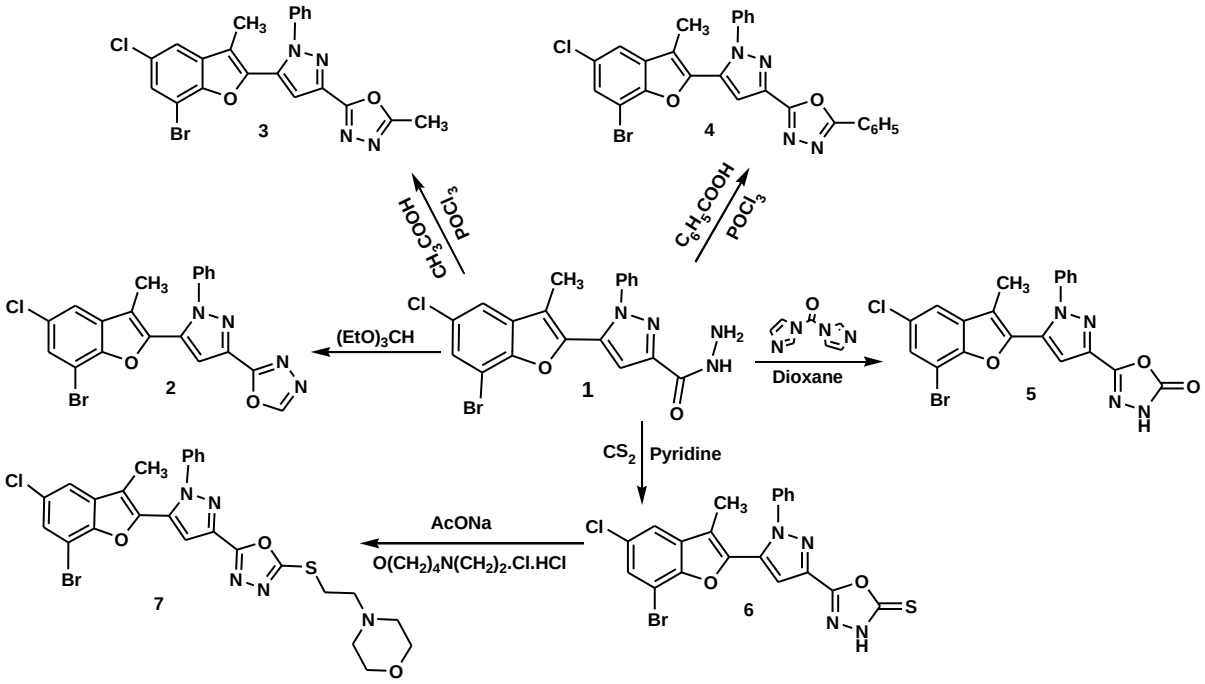

Figure 2: Reaction Scheme for the Synthesis of 2-7

Treatment of **1** with *N, N'* carbonyldiimidazole (CDI) in dioxane afforded **5**. The IR spectra of **5** showed absorption band at 1760 cm^{-1} for C=O, ^{1}H NMR spectrum showed a singlet at δ 12.75 ppm is due to one proton of NH of oxadiazole. Similarly, its mass spectrum showed M^{+} peak at 471 is consistent with the molecular formula $C_{20}H_{12}O_3N_4ClBr$. The reaction of **1** with carbon disulfide in pyridine afforded **6**. Absence of SH signal in ^{1}H NMR of **6** indicates its existence as the thione

tautomer and appearance of singlet at δ 14.81 ppm revealed that their exist an exchangeable imino proton in oxadiazole ring. A bioactive alkylating agent 4(2-chloroethyl)morpholine hydrochloride was used to alkylate the thione function of **6** in ethanol in presence of fused sodium acetate to furnish **7**. The mass spectrum of **7** displayed the $[M+H]^+$ at 601, which is good agreement with the molecular formula $C_{26}H_{23}O_3N_5SClBr$.

Analytical data

5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide (1)

White crystalline solid; Yield: 72%; m.p: 210-212°C; IR(KBr, v in cm^{-1}): 3321, 3145(-$NHNH_2$), 3060 (ArH), 2929(CH_3), 1710(C=O), 1668 (C=N), 1620, 1580, 1500(C=C); ^{1}HNMR ($CDCl_3$) δ (ppm): 2.15 (s, 3H, CH_3), 3.64(b, 2H, -CONH$\underline{NH_2}$), 8.20(b, 1H,-CO$\underline{NH}NH_2$), 7.23-7.51(m, 8H, ArH + pyrazole CH); ^{13}C NMR δ (ppm), 8, 105, 110, 111, 120, 121, 125, 127, 128, 129, 130, 133, 141, 143, 153, 156, 162; ESI(+)-MS**:** *m/z* 445 M^+, 446 $(M+H)^+$ 468$(M+Na)^+$; Anal. calcd. for $C_{19}H_{14}O_2N_4ClBr$: C, 51.24; H, 3.15; N,12.58. Found: C, 51.30; H, 3.28; N, 12.66

2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazole (2)

White crystalline solid; Yield: 68% ; m.p: 182-184°C; IR(KBr, v in cm^{-1}): 3058 (ArH), 2923(CH_3), 1607, 1593 (C=N-N=C), 1499, 1455(C=C), 1260 (C-O-C); ^{1}H NMR (DMSO-d_6) δ (ppm): 2.13 (s, 3H, CH_3), 6.52 (s, 1H, Pyrazole CH), 7.64-7.77 (m, 7H, Ph), 9.25 (s, 1H, CH of oxadiazole); ^{13}C NMR δ (ppm): 8, 105, 109, 111, 119, 122, 125, 128, 129, 135, 137, 138, 145, 152, 154, 158; ESI(+)-MS: *m/z* 456$(M+H)^+$, 478$(M+Na)^+$; Anal. calcd. for $C_{20}H_{12}O_2N_4ClBr$: C, 52.75; H, 2.64; N, 12.31 Found: C, 52.69; H, 2.52; N, 12.12

2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5-methyl-1,3,4-oxadiazole (3)

White crystalline solid; Yield 65%; m.p.:227-229°C; IR (KBr, ν_{max}): 3062 (ArH), 2970, 2926(CH_3), 1612, 1594(C=N-N=C), 1441, 1459 (C=C), 1256(C-O-C); ^{1}H NMR(DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.68(s, 3H, -CH_3), 6.54(s, 1H, pyrazole –CH), 7.29-7.81(m, 7H, ArH) ppm; ^{13}C NMR(DMSO-d_6): δ 8, 10, 105, 106, 110, 121, 123, 125, 127, 130, 134, 139, 138, 144, 152, 158, 162 ppm; ESI(+)-MS**:** *m/ z* 470$(M+H)^+$, 492 $(M+Na)^+$; Anal. calcd. for $C_{21}H_{14}O_2N_4ClBr$**:** C,53.73; H, 2.98; N, 11.94 Found**:** C, 53.70; H, 3.00; N, 11.93

2-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5-phenyl-1,3,4-oxadiazole (4)

White crystalline solid; Yield 81%; m.p.:243-245°C; IR (KBr, ν_{max}): 3068(ArH), 1694, 1591(C=N-N=C), 1461, 1493, 1445 (C=C), 1258(C-O-C); ^{1}H NMR (DMSO-d_6): δ 2.68 (s, 3H, CH_3), 6.53 (s,

1H, pyrazole CH), 7.54-7.88(m, 12H, ArH) ppm; ESI(+)-MS**:** *m/z* 532$(M+H)^+$, 554$(M+ Na)^+$; Anal. calcd. for $C_{26}H_{16}O_2N_4ClBr$: C, 58.75; H, 3.01; N, 10.54 Found: C, 58.78; H, 3.07; N, 10.55

5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazol-2(3*H*)-one (5)

White crystalline solid; Yield 74%; m.p.:218-220°C; IR (KBr, ν_{max}): 3221, 3150(-NH), 3055(ArH), 1760(C=O), 1627(C=N), 1499, 1440(C=C), 1256 (C-O-C); ^{1}H NMR (DMSO-d_6): δ 2.19 (s, 3H, CH_3), 6.48 (s, 1H, pyrazole, CH), 7.15-7.49(m, 7H, ArH), 12.75 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6): δ 8, 105 111, 120, 121, 125, 128, 129, 135, 138, 144, 149, 153, 154 ppm; ESI(+)-MS**:** *m/z* 471 M^+, 494 $(M +Na)^+$; Anal. calcd. for $C_{20}H_{12}O_3N_4ClBr$: C, 50.95; H, 2.54; N, 11.88 Found: C, 50.93; H, 2.56; N, 11.93

5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazole-2(3*H*)-thione (6)

White crystalline solid; Yield 74%; m.p.:212-214°C; IR(KBr, ν_{max}): 3410 (-NH), 3067 (ArH), 1636, 1594, (C=N-N=C), 1497, 1472 (C=C), 1255 (C-O-C), 1234 (C=S) cm^{-1}; ^{1}H NMR(DMSO-d_6): δ 2.71 (s, 3H, CH_3), 6.58 (s, 1H pyrazole CH), 7.47-7.68 (m, 7H, ArH), 14.81 (s, 1H, -NH) ppm; ^{13}C NMR (DMSO-d_6): δ 8, 105, 106, 111, 122, 124, 125, 127, 129, 134, 137, 138, 145, 152, 155, 176 ppm; ESI(+)-MS**:** *m/z* 488$(M+H)^+$, 510$(M +Na)^+$; Anal. calcd. for $C_{20}H_{12}O_2N_4SClBr$: C, 49.28; H, 2.46; N, 11.49; S, 6.57 Found: C, 49.33; H, 2.48; N, 11.50; S, 6.55

4-(2-(5-(5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-1,3,4-oxadiazol-2-ylthio)ethyl)morpholine (7)

White crystalline solid; Yield 65 %; m.p.:201-203°C; IR (KBr, ν_{max}): 3054 (ArH), 1622, 1594 (C=N), 1499, 1440(C=C), 1257(C-O-C), 694 (C-S); ^{1}H NMR(DMSO-d_6): δ 2.15 (s, 3H, CH_3), 2.46 (s, 4H, CH_2NCH_2), 2.87-2.90 (t, *J*=6.00 Hz, 2H, SCH_2), 3.50-3.53 (t, *J*=6.00 Hz, 2H, -CH_2N), 3.72-3.74 (t, 4H, -CH_2OCH_2), 6.39 (s, 1H, pyrazole CH), 7.24-7.59 (m, 7H, ArH) ppm; ^{13}C NMR(DMSO-d_6): δ 8, 29, 52, 56, 66, 105, 106, 110, 121, 123, 125, 127, 129, 135, 137, 138, 144, 153, 159, 164 ppm; ESI(+)-MS**:** *m/z* 601$(M+H)^+$, 623 $(M+Na)^+$; Anal. calcd. for $C_{26}H_{23}O_3N_5SClBr$: C, 52.00; H, 3.83; N, 11.66; S, 5.33 Found: C, 51.97; H, 3.87; N, 11.65; S, 5.34

Antimicrobial Activity

The diameter of the zone of inhibition and minimum inhibitory concentration values are given in the Table **1.** The investigation of the microbial screening data revealed that the tested compounds **1, 3**, **4, 6** and **7** showed good activity against the fungus *Aspergillus niger* while the result of antibacterial activity indicates that the synthesized compounds **1**, **3**, **6** and **7** were active against *B.*

subtilis and *S. aureus*. Compounds **1, 3, 5, 6** and **7** are active against *E. coli* whereas compounds **1**, **3** and **7** are active against *P. aeruginosa*.

CONCLUSIONS

5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide was utilized for the synthesis of newer substituted 1,3,4-oxadiazole derivatives which were characterized by spectral analysis for their structure elucidation. Spectral data and chemical reactions supported the structures. The newly synthesized 1,3,4-oxadiazole derivatives especially, **1, 3, 6** and **7** exhibited good antibacterial and antifungal activity against all tested bacterial and fungal strains almost equivalent to standard drugs.

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