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UTILITY OF HALOGENATED 2,4-DIOXOBUTANOATE DERIVATIVE IN THE SYNTHESIS OF NITROGENEOUS HETEROCYCLES AND THEIR BIOLOGICAL EVALUATION

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ABSTRACT

The reaction of 7-bromo-5-chloro-2-acetyl-3-methylbenzofuran **(1)** with diethyloxalate in presence of NaOMe and NaOEt afforded **(2)** and **(3)**, respectively by Claisen's condensation reaction. Treatment of the transesterified halogenated 2,4-dioxobutanoate **(2)** with different nucleophilic reagents undergo cyclocondensation to afford the corresponding **(4)**, **(5)**, **(6)**, **(7)** and **(8)**. The structures of all the newly synthesized compounds **(2-8)** have been characterized by their elemental and spectral studies such as IR, ^{1}H NMR, ^{13}C NMR and MS. The newly synthesized compounds were tested for their antimicrobial activities against two gram positive; two gram negative bacteria and one fungus.

Key words: 2-Substituted benzofuran,2,4-dioxobutanoate, Pyrazole-3-carboxylate, Isoxazole, Pyrazole-3-carbohydrazide.

INTRODUCTION

Benzofuran derivatives are an important class of heterocyclic compounds known to possess important biological and pharmacological properties[1-2]. Several benzofurans bearing various substituents at the C-2 and C-3 position are widely distributed in nature[3]. There are other well-known natural products having related benzofuran ring structures, which can be isolated particularly from *Machilusglaucescens*, *Ophryosporuscharua, Ophryosporuslorentzii*, *Krameriaramosissima*, and *Zanthoxylumailanthoidol*[4]. The most recognized benzofurans are ailanthoidol, amiodarone, and bufuralol compounds. Furthermore, most of the compounds prepared from 2-acetylbenzofurans have antimicrobial, antitumor, antiflammatory, fungicidal weed killing activity and used for the treatment of cardiac arrhythmias[5-11]. On the other hand, compounds including pyrazole nucleus are known to possess analgesic, anti-inflammatory, antipyretic, antiarrhythmic, tranquillizing, muscle relaxant, psychoanaleptic, anticonvulsant, hypotensive, monoamine oxidase inhibitor, antidiabetic, and antibacterial

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activities[12-19]. Recently, we reported that, some of 5-(3-substituted/unsubstituted benzofuran-2-yl)-pyrazole derivatives showed significant antimicrobial activities towards various microorganisms[20]. Encouraged by the importance of benzofuran and pyrazole rings in various pharmacological lead molecules and in continuation of our previous work in the synthesis of biologically active heterocycles, we thought of incorporating these moieties to synthesize some more pyrazole derivatives from 7-bromo-5-chloro-2-acetyl-3-methylbenzofuran as the starting material and carry out the biological screening of these newly synthesized compounds.

EXPERIMENTAL

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, ν_{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. All the compounds were analyzed for carbon, hydrogen and nitrogen and the results were within ± 0.04% of the calculated values.

Synthesis of 7-bromo-5-chloro-2-acetyl-3-methylbenzofuran (1)

3-Bromo-5-chloro-2-hydroxy acetophenone (10 mmol) was taken in dry acetone (40 mL) and chloroacetone (10 mmol) was added dropwise at room temperature for about 1 hour. Then freshly ignited K_2CO_3 (15 mmol) was added and the reaction mixture was refluxed on steam bath for 8 hrs. K_2CO_3 was removed by washing with acetone. This combined acetone extract was distilled on reduced pressure, then cooled and kept overnight. The product obtained was filtered, washed with water, dried and recrystallized from ethanol[21].

Synthesis of methyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2, 4-dioxobutanoate (2)

To a solution of **(1)** (10 mmol) and sodium methoxide (10 mmol) in DMF (100 mL), diethyloxalate (10 mmol) was gradually added with shaking. The reaction mixture was then stirred for 12 hrs at room temperature; the product so obtained was acidified by 1 : 1 ice-cold HCl, filtered, washed with water and recrystallized from DMF or acetone. Yellow crystals, Yield: (63%); m.p: 207-208^{0}C; IR (KBr v, in cm^{-1}), 3446 (-OH), 3126, 3075, 3014 (ArH),

2955 (CH_3), 1760, 1735 (C=O, ester), 1633, 1580, 1597 (C=C); ^{1}H NMR ($CDCl_3$): δ (ppm) 2.65 (s, 3H, CH_3), 3.97 (s, 3H, -OCH_3), 7.28-7.54 (m, 3H, ArH + =CH), 14.72 (b, 1H, -OH); ^{13}C NMR δ (ppm) 8, 52, 99, 111, 120, 125, 128, 129, 131, 146, 153, 161, 167, 180; ESI (+)-MS**:** *m/z* 374$(M+H)^+$, 396[$(M+Na)^+$, ^{79}Br], 398[$(M+Na)^+$, ^{81}Br]; Anal. calcd. for $C_{14}H_{10}O_5ClBr$: C, 45.04; H, 2.68. Found: C, 45.41; H, 2.79.

Synthesis of ethyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate (3)

To a solution of **(1)** (10 mmol) and sodium ethoxide (10 mmol) in DMF (100 mL), diethyloxalate (10 mmol) was gradually added with shaking. The reaction mixture was then stirred for 12 hrs at room temperature. The product so obtained was acidified by 1 : 1 ice-cold HCl, filtered, washed with water and recrystallized from acetone. Yellow crystals, Yield: (47%); m.p.: 154-156^{0}C (from); IR (KBr, v in cm^{-1}), 3445, 3121 (-OH), 3080, 3010 (ArH), 2985, 2949 (CH_3), 1860, 1788, 1732 (C=O, ester), 1689, 1643, 1600, 1577 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.39-1.43 (t, *J =7.12* Hz, 3H, OCH_2CH_3), 4.41-4.46 (q, *J = 7.16* Hz, 2H, -OCH_2CH_3), 2.64 (s, 3H, CH_3), 7.20-7.49 (m, 3H, ArH + =CH), 14.88(s, 1H, -OH); Anal. calcd. for $C_{15}H_{12}O_5ClBr$, C, 46.51; H,3.10. Found: C, 46.62; H, 3.44.

Synthesis of methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-carbamoyl-1H-pyrazole-3-carboxylate (4)

To a mixture of methyl 4-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** (5 mmol) in ethanol 10 mL, semicarbazide hydrochloride (10 mmol) and sodium acetate (10 mmol) was added and the reaction mixture was refluxed for 4 hrs. After that it was concentrated, cooled and poured in ice cold water. Solid separated out was filtered, washed, dried and recrystallized from ethanol to get 66% **(4)** as white crystalline solid; m.p.:215-218^{0}C; IR (KBr, v in cm^{-1}), 3457, 3415, 3255 (NH_2), 3165, 3060, 3028 (ArH), 2960, 2925, 2859 (CH_3), 1760, 1740 (C=O, ester), 1690 (C=N), 1395(C-N, amide), 1500, 1434, 1410 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.93 (s, 3H, -$COOCH_3$), 2.62 (s, 3H, CH_3), 7.26-7.96 (m, 5H, ArH + NH_2) ; ^{13}C NMR δ (ppm) 8, 51, 99, 111, 113, 119, 120, 127, 128, 129, 135, 139, 149, 152, 163,; ESI(+)-MS**:** *m/z* (%) 413 $(M+H)^+$, 435 [$(M+Na)^+$, ^{79}Br], 437[$(M+Na)^+$, ^{81}Br]; Anal. calcd. for $C_{15}H_{11}O_4N_3ClBr$: C, 43.69; H, 2.67; N, 10.19. Found: C, 43.58; H, 2.84; N, 10.00.

Synthesis of methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1H-pyrazole-3-carboxylate (5)

To a mixture of methyl 4-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** (10 mmol) in CH_3COOH (10 mL), hydrazine hydrate (30 mmol) was

added gradually with constant stirring and refluxed for 2 hrs. After that it was poured in ice cold water, filtered, washed, dried and recrystallized from ethanol to get 85% **(5)** as white crystalline solid; m.p.:231-232^{0}C; IR (KBr, v in cm^{-1}), 3258, 3168 (NH), 3060, 3015 (ArH), 2965 (CH_3), 1738 (C=O), 1690 (C=N), 1494, 1462, 1442, (C=C) ; ^{1}H NMR (DMSO-d_6) δ (ppm) 2.61 (s, 3H, CH_3), 3.94 (s, 3H, -$COOCH_3$), .6.20 (s, 1H, pyrazole CH) 7.20-7.60 (m, 3H, Ar H+NH). ^{13}C NMR δ (ppm) 9, 51, 103, 105, 111, 120, 128, 129, 131, 136, 142, 153, 156, 162; ESI(+)-MS**:** *m/z* 370(M +H)$^+$, 392 [(M+Na)$^+$, ^{79}Br],394 [(M +Na)$^+$, ^{81}Br];; Anal. calcd. for $C_{14}H_{10}O_3N_2ClBr$: C, 45.53; H, 2.71; N, 7.59. Found: C, 45.50; H, 2.70; N, 7.60.

Synthesis of methyl 5-(7-bromo-5-chloro -3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate (6)

To a mixture of methyl 4-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** (10 mmol) in CH_3COOH (10 mL), phenyl hydrazine (15 mmol) was added and the reaction mixture was refluxed for 4 hrs. After that it was concentrated, cooled and poured in crushed ice, filtered, dried to get 85% of **(6)** and recrystallized from ethanol as white crystalline solid; m.p: 155-158^{0}C; IR (KBr, v in cm^{-1}), 3060 (ArH), 2960 (CH_3), 1620 (C=N), 1740 (C=O, ester), 1594, 1500, 1438, 1410 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.60 (s, 3H, CH_3) 3.99 (s, 3H,-$COOCH_3$), 6.18 (s, 1H, pyrazole CH), 7.19-7.61 (m, 7H, ArH), ^{13}C NMR δ (ppm) 9, 52, 105, 111, 113, 120, 121(2C), 125, 127, 128, 129, 130 (2C), 133, 138, 147, 152, 156, 160; ESI (+)-MS**:** *m/z* 446 (M+H)$^+$, 468 [(M+Na)$^+$, ^{79}Br], 470[(M+Na)$^+$, ^{81}Br]; Anal. calcd. for $C_{20}H_{14}O_3N_2ClBr$: C, 53.93; H, 3.15; N, 6.29. Found: C, 53.88; H, 3.20; N, 6.48.

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

To a mixture of methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate **(6)** (10 mmol) in ethanol (100 mL), hydrazine hydrate (100%, 1.7 mL) was added and refluxed for 8 hrs. Then it was concentrated, cooled, filtered and washed with water, dried to get **(7)**, recrystallized from acetic acid as white crystalline solid; Yield: (72%); m.p: 210-212^{0}C; IR (KBr, v in cm^{-1}), 3321, 3145 (-$NHNH_2$), 3060, 3011 (ArH), 2929 (CH_3), 1710 (C=O), 1668 (C=N), 1620, 1580, 1500 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.15 (s, 3H, CH_3), 3.64 (b, 2H, -$CONHNH_2$), 8.20(b, 1H, -$CONHNH_2$), 7.23-7.51 (m, 8H, ArH+ pyrazole CH); ^{13}C NMR δ (ppm), 8, 105, 110, 111, 120, 121 (2C), 125, 127, 128, 129, 130 (2C), 133, 141, 143, 153, 156, 162; ESI(+)-MS**:** *m/z* 445 M$^+$, 446 (M+H)$^+$ 468[(M+Na)$^+$, ^{79}Br], 470 [(M +Na)$^+$, ^{81}Br]; 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.

Synthesis of methyl 4-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2-(hydroxyimino)-4-oxobutanoate (8a)

To a mixture of methyl 4-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** (10 mmol) in 200 mL ethanol, hydroxylamine hydrochloride (20 mmol) and sodium acetate (20 mmol) was added and the reaction mixture was refluxed for 4 hrs. After that it was concentrated, cooled and poured in ice cold water and kept overnight. The solid separated out was filtered, dried and recrystallized from diluted ethanol to get 80% of **(8a)** as white crystalline solid; m.p.: 160-163^{0}C; ^{1}H NMR (DMSO-d_6 δ (ppm), 2.59 (s, 3H, CH_3), 3.83 (s, 3H, -OCH_3), 4.30 (s, 2H, CH_2), 12.39 (s, 1H, -OH), 7.20-7.75 (m, 2H, ArH); Anal. calcd. for $C_{14}H_{11}O_5NClBr$: C, 43.29; H, 2.84; N, 3.61. Found: C, 43.45; H, 2.94; N, 3.55.

Synthesis of ethyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl) isoxazole-3-carboxylate (8)

(8a) synthesized above was refluxed for 2 hrs in ethanol by adding 1 mL of conc. HCl. The solvent was evaporated under reduced pressure, cooled, filtered, and washed with water to get 75% of **(8)**. It was recrystallized from ethanol to get **(8)** as white crystalline solid; m.p.:145-148^{0}C; IR (KBr, v in cm^{-1}), 3080, 3145, 3110 (ArH), 2995, 2945 (CH_3), 1734 (C=O, ester), 1622 (C=N), 1544, 1477, 1458, 1440 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm), 2.61(s, 3H, CH_3), 1.40-1.43 (t, *J* = 8 Hz**,** 3H, -OCH_2CH_3), 4.41-4.46 (q, *J* = 8 Hz, 2H, -OCH_2 CH_3), 7.30-7.78 (m, 3H, ArH); ^{13}C NMR δ (ppm) 9, 14, 61, 99, 107, 111, 121, 126, 127, 132, 150, 150, 154, 158, 168; ESI(+)-MS**:** *m/z* 385 $(M+H)^+$, 407 $[(M+Na)^+, {}^{79}Br]$, 409 $[(M+Na)^+, {}^{81}Br]$; Anal. calcd. for$C_{15}H_{11}O_4NClBr$: C, 46.88; H, 2.86; N, 3.65. Found: C, 46.80; H, 2.90; N, 3.73.

Antimicrobial activity

All the novel synthesized compounds **(2-8)** were screened for their *in vitro* antimicrobial activity against two gram positive strains, *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative strains, *Escherichia coli* and *Pseudomonas aeruginosa* in addition to a fungus *Aspergillus niger*. Antimicrobial activity was assessed by serial two fold (broth) dilution technique, using Muller-Hinton broth for bacteria and Sabouraud dextrose agar for fungus. Ampicillin was used as a standard drug for bacteria and clotrimazole for fungus. Similarly, serial dilution tubes for standard drug with its stock solution 100 μg/cm^3 were also prepared so that the concentrations of standard drug in five tubes were 50, 25, 12.5, 6.25, 3 μg/cm^3, respectively. All the compounds were dissolved in DMSO to give a concentration of 1 mg/cm^3. Antimicrobial activity of DMSO against the test organisms were investigated, and found to be nil. Double strength nutrient broth was used as

a growth media. The stock solution was serially diluted to give concentration of 400-3 μg/cm^3 in nutrient broth. The inoculums size was approximately 10^6 colony forming units (CFC/mL). The inoculated tubes were incubated for 24 h at 37(± 1)^{0}C (bacteria) and for 72 h at 28^{0}C (fungi). After 24 h and 72 h, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The antimicrobial activities of all the screened compounds as well as standard drug ampicillin and clotrimazole, determined in terms of minimum inhibitory concentration (MIC μg/cm^3) are given in the Table 1.

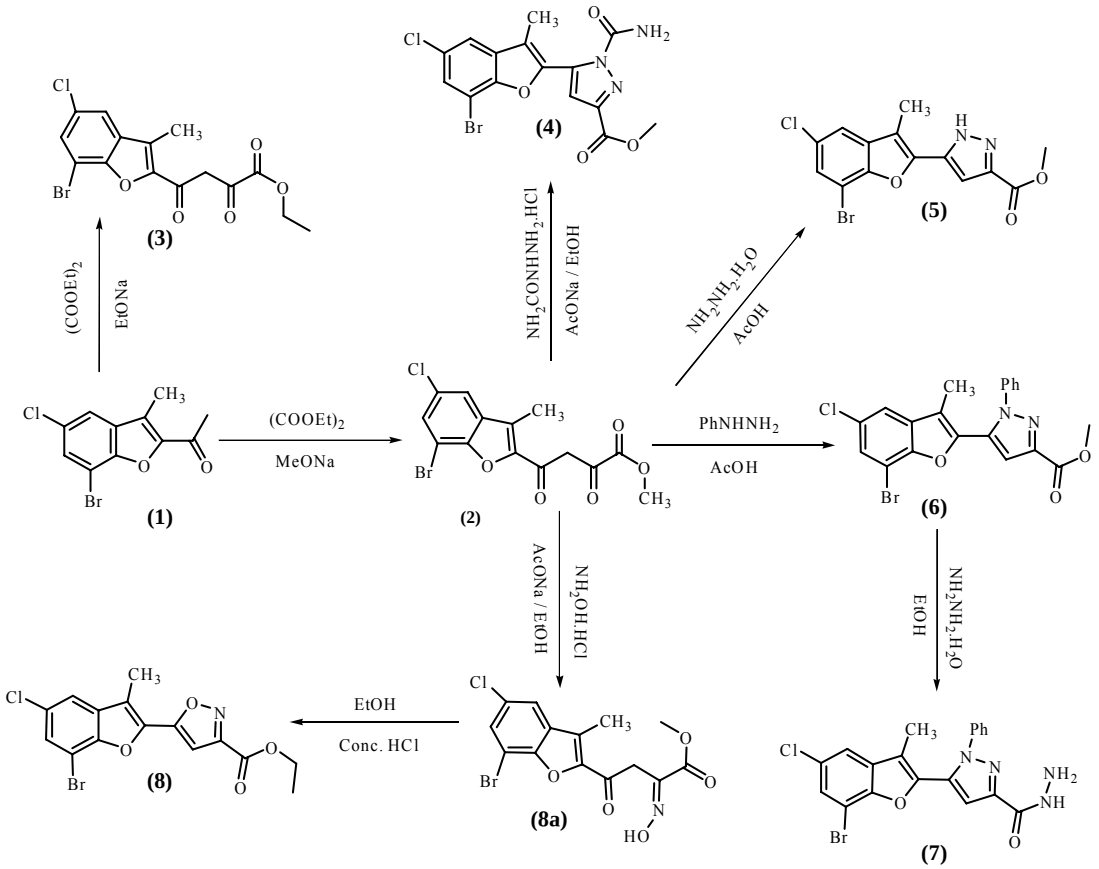
RESULTS AND DISCUSSION

The synthesis of the novel compounds **(2-8)** is described in **Scheme**. At every stage, the purity of the compounds were monitored by TLC technique. The identities of the newly synthesized compounds have been established on the basis of their elemental analysis and spectral data such as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. These compounds were screened for their antimicrobial activities.

The synthesis of the key intermediate **(1)**, 7-bromo-5-chloro-2-acetyl-3-methylbenzofuran was prepared in quantitative yields according to the reference method[21]. The reaction of **(1)** with diethyl oxalate in presence of sodium methoxide or sodium ethoxide solution as a base and DMF as solvent medium gave **(2)** and **(3)**, respectively. The ^{1}H NMR and IR spectrum of **(2)** exhibited characteristic band of enolic group due to keto-enol tautomerism of reactive methylene group, which was confirmed by the test with alcoholic $FeCl_3$ giving wine red colouration. The IR spectrum of methyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** showed the – OH stretch of enol form at 3446 cm^{-1} and C=O stretching in ester group at 1760 cm^{-1}. The ^{1}H NMR spectrum showed singlet signal at δ 14.72 ppm for one proton of –OH group, confirms the enolic form, singlet signal at δ 3.97ppm due to OCH_3 confirms the methyl ester and multiplet signals at δ 7.28-7.54 ppm was obtained for three aromatic protons. The chemical shift value of the methoxy carbon in ^{13}C NMR is observed at δ 52 ppm (-OCH_3), the carbon atoms connected to methoxy group are observed at the δ 153-167 ppm range, signal 167 ppm is due to C-1 carbon in C=O of the ester group whereas C-4 carbon in C=O group under the influence of strong electronegative environment appears downfield at δ 180 ppm, the aromatic carbons were observed in expected region. The mass spectrum[22] of this product reveals amolecular ion at *m/z* 374 $[M+H]^+$ and 396 $[(M+Na)^+, {}^{79}Br]$, 398$[(M+Na)^+, {}^{81}Br]$ is in consistent with the molecular formula $C_{14}H_{10}O_5ClBr$.

The IR spectra of ethyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(3)** showed –OH stretch at 3445 cm^{-1} and C=O stretching in ester group at 1732 cm^{-1}, respectively. The ^1H NMR spectrum showed singlet signal at δ 14.88 ppm due to one proton confirms the –OH group in the enolic form of -OHC=CH- due to keto-enol tautomerism, triplet signal at δ 1.39-1.43 ppm for three proton of $–CH_3$ group, quartet signal at δ 4.41-4.46 ppm confirms $-OCH_2CH_3$ the ethyl ester and multiplet signals at δ 7.20-7.49 ppm for two aromatic and one proton due to the vinylic =CH of -OHC=CH.

Methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-carboxamido-1*H*-pyrazole-3-carboxylate **(4)**, methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1*H*-pyrazole-3-carboxylate **(5)**, methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate **(6)**, were obtained by the treatment of **(2)** with semicarbazide hydrochloride and sodium acetate in ethanol, hydrazine hydrate in acetic acid and phenyl hydrazine in acetic acid, respectively.



Scheme 1

Formulation of the reaction product designed as **(4)**, **(5)** and **(6)** was based upon the comparative reactivity of two carbonyl groups in **(2)**[23]. The C-2 carbonyl group being more reactive than C-4 carbonyl group, it gets preferably attacked by the nucleophilic reagent such as hydroxylamine hydrochloride, semicarbazide hydrochloride, hydrazine hydrate and phenyl hydrazine to give corresponding intermediate, which simultaneously undergo ring closure with elimination of water molecule from imino proton and the –OH group of enolized C-4 carbonyl group forming **(4)**, **(5)** and **(6)**. The ^{1}H NMR spectrum of **(4)** showed multiplet at δ 7.26-7.96 ppm for three aromatic and two protons of NH_2. The characteristic strong stretching band at 1690 cm^{-1} in IR spectrum due to C=N is in evidence with the closure of pyrazole ring. The mass spectra reveals a molecular ion peak at m/z 413 $[M+H]^+$ and is in agreement with the molecular formula $C_{15}H_{11}O_4N_3ClBr$. The ^{1}H NMR spectrum of **(5)** showed multiplet at δ 7.20-7.60 ppm for two aromatic protons and one NH proton and a singlet signal at δ 6.20 ppm for one proton of pyrazole ring. Similarly, the IR spectrum of **(6)** showed the characteristic band at 1620 cm^{-1} shows strong C=N stretching band. The ^{1}H NMR spectrum showed multiplet at δ 7.19-7.61 ppm for seven aromatic protons and one singlet signal at δ 6.18 ppm for one proton of pyrazole ring. The mass spectra also confirmed the molecular formula $C_{20}H_{14}O_3N_2ClBr$, as a molecular ion peak is obtained at m/z 446 $[M+H]^+$.

Further the reaction of **(6)** with hydrazine hydrate in ethanol gives 5-(7-bromo-5-chloro-3-methylbenzofuran-2-ylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **(7)**. The IR spectrum of **(7)** showed absorption band at 3321 cm^{-1}, which indicates the stretch due to $–NHNH_2$ and C=O stretch in $–CONHNH_2$ at 1710 cm^{-1} and C=N stretch in pyrazole ring at 1668 cm^{-1}, respectively. The ^{1}H NMR spectrum showed singlet signal at δ 3.64 ppm due to two protons of $–NH_2$ in $-CONHNH_2$ and a broad band at δ 8.20 ppm for one proton of –NH in $CONHNH_2$. Mass spectrum reveals a molecular ion M^+ at m/z 445, in consistent with the molecular formula $C_{19}H_{14}O_2N_4ClBr$.

Methyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** was then reacted with hydroxyl amine hydrochloride and sodium acetate in ethanol to yield **(8a)**, which was confirmed from ^{1}H NMR spectrum. Singlet signal at δ 12.39 ppm due to –OH group, multiplet signal at δ 7.20-7.75 for two aromatic protons confirms that cyclization didn't occurred to form isoxazole ring. Hence **(8a)** was heated in ethanol in presence of conc. HCl for 2 hrs to get ethyl 5 (7-bromo-5-chloro-3-methylbenzofuran-2-yl)-isoxazole-3-carboxylate **(8)**, where a multiplet signal at δ 7.30-7.78 ppm due to three protons and quartet at δ 4.41-4.46 and triplet at 1.40-1.43 ppm was obtained due to the presence of $–COOCH_2CH_3$ group confirming that trans esterification has also occurred simultaneously.

The structures of all the novel synthesized compounds, **(2-8)** were also confirmed by CHN and spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR, and Mass spectra (See Experimental).

Antimicrobial activity

Biological screening results of the novel synthesized compounds revealed that compounds **(6)** and **(7)** have shown better activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa* using ampicillin as a standard drug. While rest of all the derivatives possesses good activity in the range of 25-50 µg/cm^{3}and moderate in 50-100 µg/cm^3 range against all the bacteria. Compound **(2)**, **(5)**, **6** and **(7)** were found to have significant antifungal activity against *A. niger,* while rest of all the derivatives were found to be moderate to poorly active using clotrimazole as a standard drug (MIC 12.5 µg/cm^3).

Table 1: Antimicrobial activity of compounds (2-8)

Comp. No.	Minimum inhibitory concentration (MIC, µg/cm^3)				
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***A. niger***
2	50	50	200	200	12.5
3	50	100	100	50	50
4	100	50	100	50	100
5	> 200	100	25	100	12.5
6	12.5	12.5	50	25	6
7	25	200	12.5	12.5	12.5
8	100	> 200	50	100	50
Ampicillin	25	12.5	25	25	-
Clotrimazole	-	-	-	-	12.5
DMSO	-	-	-	-	-

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

CONCLUSION

Methyl 4-[7-bromo-5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **(2)** was reacted with various nucleophilic reagents to synthesize novel pyrazole-3-carboxylates derivatives **(4)**, **(5)**, **(6)** and isoxazole derivative **(8)**. Methyl 5-(7-bromo-5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate **(6)** was further utilized for the

synthesis of 5-(7-bromo-5-chloro-3-methylbenzofuran-2-ylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **(7)**. The compounds exhibited good antibacterial activity *in vitro* against both gram positive and gram negative strains of bacteria while significant activity for the fungus. It can be concluded that the structural and electronic diversities of these synthesized compounds influenced their activity.

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