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USE OF TRANSESTERIFIED 1,3-DIKETOESTERS IN THE SYNTHESIS OF TRISUBSTITUTED PYRAZOLES AND THEIR BIOLOGICAL SCREENING

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ABSTRACT. Starting from 2-acetylbenzofuran derivatives **1a-d**, methyl/ethyl 4-substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoate **2a-d** and **3a-d** have been synthesized by Claisen's condensation reaction with diethyloxalate. The transesterified product, 1,3-diketoester **2a-d** on condensation with phenyl hydrazine undergo cyclization to afford the corresponding methyl 5-(substituted/unsubstituted benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate **4a-d**, which upon further condensation with hydrazine hydrate yielded 5-(substituted/unsubstituted benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **5a-d**. The structures of the newly synthesized compounds **2a-d**, **3a-d**, **4a-d** and **5a-d** were characterized by their elemental analysis and spectral studies such as IR, ^{1}H NMR, ^{13}C NMR and MS. All the synthesized compounds were screened for their antimicrobial activity. Most of the synthesized compounds showed high sensitivity against the selected bacteria and fungi at various concentrations.

KEY WORDS: 2,4-Dioxobutanoate, Prazole-3-carboxylate, Pyrazole-3-carbohydrazide

INTRODUCTION

As 1,3-diketoester derivatives have been found very reactive towards organic reagent such as hydrazine hydrate, phenyl hydrazine, semicarbazide hydrochloride, hydroxyl amine hydrochloride and hence utilized for the synthesis of substituted pyrazole derivatives[1-3]. Pyrazoles are important class of nitrogen containing five membered heterocyclic compounds. Compounds with pyrazole ring are of interest due to their broad spectrum of biological activities like antibacterial [4, 5], antiameobic [6], fungicidal [7, 8], antidiuretic [9], anticancer [10], potent antidiabetic agent [11], anti-inflammatory [12], antidepressant [13], and antiviral [14]. Moreover N-phenyl pyrazole derivatives play an important role in antitumor screening [15] as well as potent antimicrobial activity [16, 17]. Some substituted pyrazoles also exhibits cyclo-oxygenes-2-(Cox2) selective inhibitors [18, 19]. Literature survey indicated that the hydrazone group plays an important role for the antimicrobial activity. A number of hydrazide-hydrazone derivatives also have been claimed to possess interesting bioactivity such as antibacterial-antifungal [20, 21], anti-inflammatory [22], antimalarial [23], anticonvulsant [24], antituberculosis [25, 26], and anticancer [27] activities. So a few pyrazole carbohydrazide hydrazone derivatives have also been reported, which have been synthesized by many methods [28-30]. Encouraged by the importance of pyrazole rings in various pharmacological lead molecules we thought of incorporating this moiety to our base material for research. As a part of our continuing interest in heterocyclic chemistry we turned our attention with the aim to synthesize and evaluate the antimicrobial activities of different trisubstituted pyrazole derivatives derived from methyl 4-(substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoate as a stating material.

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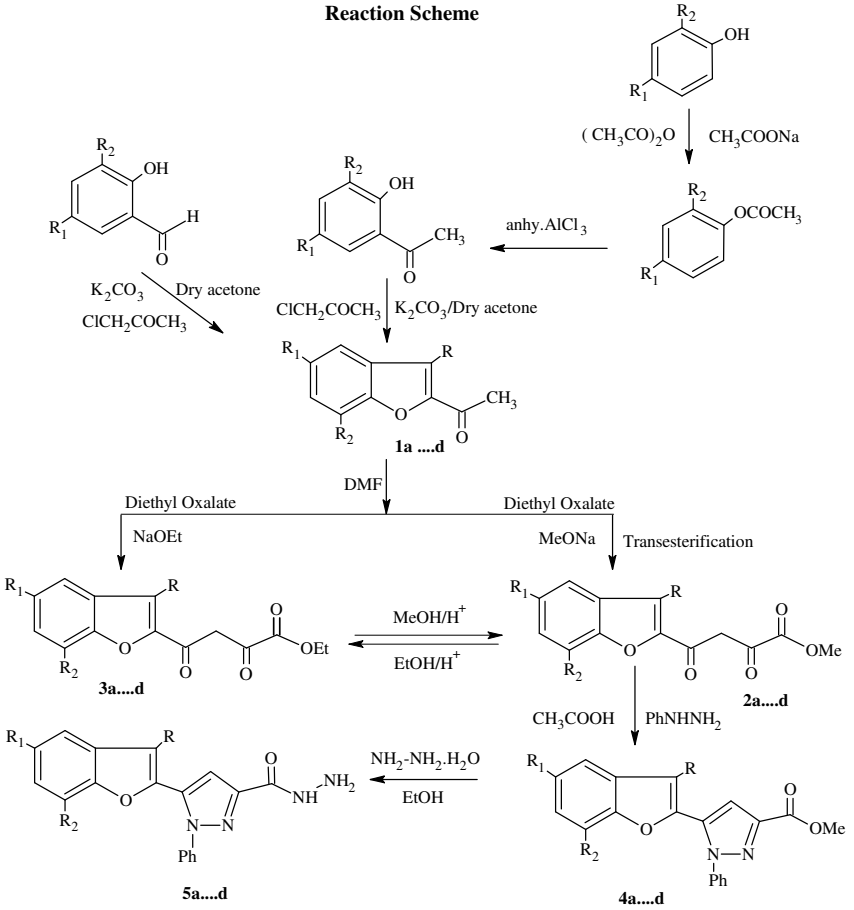
RESULTS AND DISCUSSION

The synthesis of the novel compounds **2a-d**, **3a-d**, **4a-d** and **5a-d** is described in reaction scheme. At every stage the purity of the compounds were monitored by TLC technique. The identities of the newly synthesized compound 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. All the obtained products were screened for their antimicrobial activities.

Reaction Scheme



	R	R_1	R_2		R	R_1	R_2
2a	H	H	H	**4a**	H	H	H
2b	H	Br	H	**4b**	H	Br	H
2c	CH_3	Cl	H	**4c**	CH_3	Cl	H
2d	CH_3	Cl	Cl	**4d**	CH_3	Cl	Cl
3a	H	H	H	**5a**	H	H	H
3b	H	Br	H	**5b**	H	Br	H
3c	CH_3	Cl	H	**5c**	CH_3	Cl	H
3d	CH_3	Cl	Cl	**5d**	CH_3	Cl	Cl

The syntheses of the key intermediates 2-acetyl substituted/unsubstituted benzofuran **1a-d** were prepared in quantitative yields according to the reference method [31]. The reaction of **1a-d** with diethyl oxalate in presence of sodium methoxide solution as a base and DMF as solvent afforded **2a-d** via transesterification, while the same reaction with sodium ethoxide furnished **3a-d**. The ^{1}H NMR and IR spectra of **2a-d** exhibited characteristic band of enolic group due to keto-enol tautomerism of reactive methylene group which was also confirmed by the test with alcoholic $FeCl_3$ which gave wine red colouration. The IR spectrum of methyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2a** showed the –OH stretch of enol form at 3475 cm^{-1} and C=O stretching in ester group at 1759 cm^{-1}. The ^{1}H NMR spectrum showed singlet signal at δ 14.24 ppm for one proton of –OH group, confirms the enolic form, singlet signal at δ 3.95 ppm due to OCH_3 confirms the methyl ester and multiplet signals at δ 7.20-7.71 ppm for five aromatic protons and singlet at δ 7.10 ppm confirms one proton due to the vinylic =CH. The chemical shift value of the methoxy carbon in ^{13}C NMR is observed at δ 53 ppm (-O$\underline{C}H_3$), the carbon atoms connected to methoxy group are observed at the δ 156-167 ppm range, signal δ 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 181 ppm, the aromatic carbons were observed in expected region. The mass spectrum [32] of this product reveals a molecular ion at *m/z* 247 $[M+H]^+$ and 269 $[M+Na]^+$ is in consistent with the molecular formula $C_{13}H_{10}O_5$.

The IR spectra of ethyl 4-[5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate **3c** showed –OH stretch at 3632 cm^{-1} and C=O stretching in ester group at 1724 cm^{-1}, respectively. The ^{1}H NMR spectrum showed singlet signal at δ 14.79 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 1.41-1.44 ppm for two proton of -CH_2 group, quartet signal at δ 4.38-4.44 ppm confirms -OCH_2CH_3 the ethyl ester and multiplet signals at δ 7.26-7.52 ppm for three aromatic protons and singlet at δ 7.11 ppm confirms one proton due to the vinylic =CH of OHC=CH-. The chemical shift values of the ethoxy carbons in ^{13}C NMR spectrum is observed at δ 14 ppm (-$OCH_2\underline{C}H_3$) and 62 ppm (-O$\underline{C}H_2CH_3$), the carbon atoms connected to ethoxy group are observed at the δ 152-167 ppm range, C_4 carbon in C=O group appears at δ 183 ppm while signal at δ 167 ppm is due to C_1 of C=O carbon of the ester group. The molecular ion peak at *m/z* 309 M^+, 331 $[(M+Na)^+, {}^{35}Cl]$, 333 $[(M+Na)^+, {}^{37}Cl]$; is in agreement with the molecular formula $C_{15}H_{13}O_5Cl$.

2a-d on reaction with phenyl hydrazine gave corresponding **4a-d**. The IR spectra of methyl 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate **4a** showed absorption band in the region 1734 cm^{-1} due to C=O stretching in ester group. The characteristic band at 1618 cm^{-1} shows strong C=N stretching bands. This is in evidence with the ring closure of pyrazole ring. The ^{1}H NMR spectrum showed singlet signal at δ 3.97 ppm due to three proton confirms the –$COOCH_3$ group in the ester, multiplet at δ 7.17-7.54 ppm for ten aromatic proton and one singlet signals at δ 6.22 ppm for one proton of pyrazole ring. ^{13}C NMR spectrum shows that the five membered heterocycle pyrazole is formed via cyclization and its signal is characteristically influenced by the phenyl substituent. The C_3 atom of pyrazole is found to resonate at about δ 145 ppm while C_5 atom at δ 154 ppm, signal at δ 162 ppm is due to the C=O carbon atom of ester and methoxy carbon (-O$\underline{C}H_3$) gives signal at δ 53 ppm. Similarly the mass spectra reveals a molecular ion peak at m/z 319 $[M+H]^+$ and 341 $[M+Na]^+$ is same as the molecular formula $C_{19}H_{14}O_3N_2$.

Formulation of the reaction product designed as **4a-d**, was based upon the comparative reactivity of two carbonyl groups in **2a-d**. The C_2 carbonyl group being more reactive than C_4 carbonyl group [1], it gets preferably attacked by the nucleophilic reagent such as phenyl hydrazine to give corresponding hydrazone intermediate which simultaneously undergo ring

closure with elimination of water molecule from imino proton of hydrazone residue and the –OH group of enolized C_4 carbonyl group.

The reaction of **4a-d**, with hydrazine hydrate in ethanol gave **5a-d**. Its structure was supported by IR revealing the presence of C=O group and –NH stretch. The IR spectra of 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide **5a** showed characteristic absorption band at 3429 cm^{-1} indicates the stretch due to $–NHNH_2$ and C=O stretch in $–CONHNH_2$ at 1683 cm^{-1} and C=N stretch in pyrazole ring at 1649 cm^{-1}, respectively. The ^{1}H NMR spectrum showed singlet signal at δ 3.69-3.71 ppm due to two proton of $–NH_2$ in $–CONHN\underline{H}_2$ and a broad band at δ 8.49 ppm for one proton of –NH in $CON\underline{H}NH_2$ and multiplet signals at δ 7.17-7.51 ppm for ten aromatic protons and singlet at δ 6.22 ppm is due to one proton of pyrazole. In ^{13}C NMR spectrum, the absence of signal corresponding to methoxy carbon as in **4a-d** at δ 53 ppm clearly indicates that ester group has been converted to carbohydrazide $–CONHNH_2$, C=O carbon of carbohydrazide gives signal at δ 162 ppm while the aromatic carbons, C_3 and C_5 in the pyrazole ring are at their expected regions. The elemental analysis of this product gave C, 67.50; H, 4.35; N, 16.88; and mass spectrum reveals a molecular ion at m/z 319 $[M+H]^+$ and 341 $[M+Na]^+$ is in consistent with the molecular formula $C_{18}H_{14}O_2N_4$.

The structures of the other novel synthesized compounds, **2b-d**, **3b-d**, **4b-d** and **5b-d** were also confirmed by CHN and spectral investigation such as IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. Simultaneously, the physical constant, yield, spectroscopic and analytical data are also mentioned.

Antimicrobial activity

The investigation of the microbial screening data revealed that all the tested compounds showed variable activities towards the fungus and bacteria used, which showed that these compounds are biologically active due to the presence of different heterocycles and functional groups. The test compound **2a**, **2b**, **2d**, **3a**, **3b**, **3c**, **3d**, **4a**, **4c**, **4d**, **5b**, **5c** were found to possess moderate to high activity, whereas **2c**, **4b**, **4c**, **5a**, **5d** were found to be poorly active at 300-500 µg/mL, but inactive at 100 or 200 µg/mL concentration against the fungus *Aspergillus niger* as given in the Table 1. Similarly the result of antibacterial activity are also tabulated which clearly indicates that the synthesized compounds **2a**, **3a**, **3b**, **4a**, **4b**, **4d**, **5a**, are highly active, compounds **2b**, **3c**, **4c**, **5b**, **5c** are moderately active while **2c**, **2d**, **3d**, **5d** are poorly active against *S. aureus*. Compounds **3b**, **3c**, **4a**, **5b**, **5c** are highly active, **2b**, **2c**, **2d**, **3a**, **3d**, **4c**, **5a** are moderately active and **2a**, **4b**, **4d** and **5d** are poorly active against *E. coli.*

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}). ^{1}H 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 antimicrobial screening of the synthesized compounds were carried out at microbiology laboratory.

Table 1. Antimicrobial activity of the synthesized compounds **2a-d**, **3a-d**, **4a-d** and **5a-d**.

No.	Compound	*Antifungal activity*					*Antibacterial activity	
		Zone of inhibition in mm						
		A. niger					*S. aureus*	*E. coli*
Concentration		µg/mL					1 mg/mL	
		100	200	300	400	500		
1	**2a**	7	9	9	11	12	26	11
2	**2b**	8	9	11	11	13	21	14
3	**2c**	-	-	7	9	11	15	12
4	**2d**	7	7	8	9	11	16	12
5	**3a**	9	11	15	16	16	25	14
6	**3b**	9	11	13	15	17	28	16
7	**3c**	7	9	10	13	16	21	15
8	**3d**	8	8	10	12	14	17	13
9	**4a**	7	9	9	10	11	28	17
10	**4b**	-	-	7	7	9	24	10
11	**4c**	-	7	9	10	14	20	13
12	**4d**	7	7	8	8	9	25	11
13	**5a**	-	6	7	8	9	26	12
14	**5b**	8	9	10	12	14	20	17
15	**5c**	-	9	10	12	15	21	18
16	**5d**	-	-	-	8	10	13	10
Kanamycin		8	9	14	18	23	-	-
Chloramphenicol		-	-	-	-	-	32	21
DMSO		-	-	-	-	-	-	-

*General procedure for the synthesis of (**1a-d**)*

2-Hydroxy acetophenone/2-hydroxy benzaldehyde derivatives (10 mmol) were taken in dry acetone (40 mL) and chloroacetone (10 mmol) was added dropwise at room temperature for 1 h. Then freshly ignited K_2CO_3 (15 mmol) was added, the reaction mixture was refluxed on steam bath for 8 h. K_2CO_3 was removed by washing with acetone. This combined acetone extract was distilled on reduced pressure then cooled and kept overnight, product obtained was filtered, washed with water, dried and recrystallized from ethanol [31].

*General procedure for the synthesis of (**2a-d**)*

To a solution of **1a-d** (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 h at room temperature; the product so obtained was acidified by 1:1 ice-cold HCl, filtered, washed with water and recrystallized from suitable solvent.

Methyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate ***(2a)*** [33]. Yellow crystals, yield: (85%); m.p.:131-133 ^{0}C (from DMF or acetone); IR (KBr, ν in cm^{-1}) 3475 (-OH), 3059, 3020 (ArH), 2968, 2879 (CH_3) 1805, 1759 (C=O, ester), 1624, 1573, 1521 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.95 (s, 3H, CH_3),7.20-7.71 (m, 5H, ArH), 7.19 (s, 1H, =CH), 14.24 (s, 1H, -OH); ^{13}C NMR δ (ppm): 53, 99, 112, 114, 123, 124, 127, 128, 150, 156, 162, 167, 181; ESI(+)-MS: *m/z* 247 $(M+H)^+$, 269 $(M+Na)^+$; anal. calcd. for $C_{13}H_{10}O_5$: C, 63.41; H, 4.06; found: C, 62.52; H, 4.13.

*Methyl 4-(5-bromobenzofuran-2-yl)-2,4-dioxobutanoate (**2b**).* Yellow crystals, yield: (79%); m.p.: 170-171 ^{0}C (from DMF or acetone); IR (KBr, ν in cm^{-1}), 3450, 3119(-OH), 3086, 3022 (ArH), 2962, 2879 (CH_3), 1898, 1732 (C=O ester), 1620, 1570 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.96 (s, 3H, CH3), 7.20-7.85 (m, 4H, ArH), 7.10 (s, 1H, =CH), 14.28 (s, 1H, -OH), ^{13}C NMR δ (ppm): 53, 99, 112, 113, 117, 125, 129, 131, 151, 154, 162,168, 180; ESI(+)-MS: *m/z* 326 $(M+H)^+$, 347 [$(M+Na)^+$, ^{79}Br], 349 [$(M+Na)^+$, ^{81}Br]; anal. calcd. for $C_{13}H_9O_5Br$: C, 48.00;H, 2.77; found: C, 47.89; H, 2.59.

*Methyl 4-[5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate (**2c**).* Yellow crystals, yield: (89 %) m.p.: 178-180 ^{0}C (from DMF or acetone); IR (KBr, ν in cm^{-1}) 3435, 3132 (-OH), 3016, 3086 (ArH), 2956, 2883 (CH_3), 1898, 1770, 1728 (C=O, ester), 1635, 1589 (C=C); ^{1}H NMR (DMSO) δ (ppm): 2.58-2.65 (s, 3H, CH_3), 3.76-3.94 (b, 3H, -OCH_3), 7.46-7.73 (m, 4H, ArH, -OH), 7.12 (s, 1H, =CH). ^{13}C NMR δ (ppm): 9, 53, 99, 113, 120, 125, 128, 130, 146, 152, 162, 168, 180; ESI(+)-MS: *m/z* 296 $(M+H)^+$, 317 [$(M+Na)^+$, ^{35}Cl], 319 [$(M+Na)^+$, ^{37}Cl]; anal. calcd. for $C_{14}H_{11}O_5Cl$: C, 56.95; H, 3.73; found: C, 56.84; H, 3.82.

*Methyl 4-[5,7-dichloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate (**2d**).* Yellow crystals, yield: (65%); m.p.: 200-202 ^{0}C (from DMF or acetone); IR (KBr, ν, in cm^{-1}), 3446 (-OH), 3122, 3078, 3018 (ArH), 2956 (CH_3), 1763, 1734 (C=O, ester), 1635, 1579, 1597 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.6 (s, 3H, CH_3), 3.9 (s, 3H, -OCH_3), 7.2-7.5 (m, 3H, ArH), 14.68 (b, 1H, -OH); ESI(+)-MS: *m/z* 331 $(M+2)^+$, 351[$(M+Na)^+$, ^{35}Cl], 353 [$(M +Na)^+$, ^{37}Cl]; anal. calcd. for $C_{14}H_{10}O_5Cl_2$: C, 51.06; H, 3.03; found: C, 50.73; H, 3.31.

*General procedure for the synthesis of (**3a-d**)*

To a solution of **1a-d** (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 h at room temperature; the product so obtained was acidified by 1:1 ice-cold HCl, filtered, washed with water and recrystallized from suitable solvent.

*Ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate (**3a**)* [34]. Yellow Crystalline, Yield: (55%); m.p.: 63-65 ^{0}C (from ethanol); IR (KBr, ν in cm^{-1}) 3653, 3455 (OH), 3107, 3080 (ArH), 2980, 2937, 2904, 2874 (CH_3), 1786, 1724 (C=O, ester), 1633, 1548 (C=C); anal. calcd. for $C_{14}H_{12}O_5$: C, 64.61; H, 4.61; found: C, 64.59; H, 4.60.

*Ethyl 4-(5-bromobenzofuran-2-yl)-2,4-dioxobutanoate (**3b**).* Yellow crystals, yield: (60%); m.p.: 130-131 ^{0}C (from ethanol); IR (KBr, ν in cm^{-1}) 3423, 3126 (-OH), 3072 (ArH), 2987, 2899 (CH_3), 1888, 1724 (C=O, ester), 1627, 1562 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41-1.44 (t, *J* = 7.12 Hz, 3H, -$OCH_2\underline{CH_3}$), 4.39-4.44 (q, *J* = 7.16 Hz, 2H, -$O\underline{CH_2}CH_3$), 7.26-7.85 (m, 4H, ArH), 7.09 (s,1H, =CH); ESI(+)-MS: *m/z* 339 M^+, 361 [$(M+Na)^+$,^{79}Br], 363 [$(M+Na)^+$, ^{81}Br]; anal. calcd. for $C_{14}H_{11}O_5Br$: C, 49.55; H, 3.24; found: C, 49.18; H, 3.31.

*Ethyl 4-[5-chloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate (**3c**).* Yellow crystals, yield: (60%); m.p.: 145-147 ^{0}C(from acetone); IR (KBr, ν in cm^{-1}), 3632, 3132 (-OH), 3095 (ArH), 2999 (CH_3), 1871, 1724 (C=O, ester), 1678, 1639, 1599, 1575 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41-1.44 (t, *J* = 7.12 Hz, 3H, -$OCH_2\underline{CH_3}$), 4.38-4.44 (q, *J* = 7.16 Hz, 2H, -$O\underline{CH_2}CH_3$), 2.59 (s, 3H, CH_3), 7.11(s, 1H, =CH), 7.26-7.52(m, 3H, ArH), 14.79(b, 1H, -OH); ^{13}C NMR δ (ppm): 9, 14, 62, 99, 113, 120, 126, 128, 129, 130, 146, 152, 161,167,183; ESI(+)-MS: *m/z* 309 M^+, 331 [$(M + Na)$,$^+$ ^{35}Cl], 333 [$(M+Na)^+$, ^{37}Cl]; anal. calcd. for $C_{15}H_{13}O_5Cl$: C, 58.25; H, 4.21; found: C, 58.00; H, 4.32.

*Ethyl 4-[5,7-dichloro-3-methylbenzofuran-2-yl)-2,4-dioxobutanoate (**3d**).* Yellow crystals, yield: (45%); m.p.: 125-127 ^{0}C (from acetone); IR (KBr, ν in cm^{-1}), 3435, 3117 (-OH), 3080, 3007 (ArH), 2987, 2945 (CH_3), 1855, 1782, 1728 (C=O, ester), 1683, 1639, 1604, 1577 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41-1.45 (t, *J* = 7.12 Hz, 3H, $OCH_2\underline{CH_3}$), 4.40-4.45 (q, *J* = 7.16 Hz, 2H, -$O\underline{CH_2}CH_3$), 2.62 (s, 3H, CH_3), 7.17 (s, 1H, =CH) ,7.47 (d, 2H, ArH), 7.5 (d, 1H, ArH), 14.83 (s, 1H, -OH); anal. calcd. for $C_{15}H_{12}O_5Cl_2$,: C, 54.71; H, 3.65; found: C, 54.68; H, 3.68.

*General procedure for the synthesis of (**4a-d**)*

To a mixture of **2a-d** (10 mmol) in CH_3COOH (10 mL), phenyl hydrazine (15 mmol) was added and the reaction mixture was refluxed for 4 h. After that it was concentrated, cooled and poured in crushed ice, filtered, dried and recrystallized from suitable solvent.

*Methyl 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate (**4a**).* White crystals, yield: (85%); m.p.: 161-163 ^{0}C (from acetic acid); IR (KBr, ν in cm^{-1}), 3061 (ArH), 2955 (CH_3), 1618 (C=N), 1734 (C=O, ester), 1593, 1500, 1436, 1408 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H, -$COOCH_3$), 7.17-7.54 (m, 10H, ArH), 6.22 (s, 1H, pyrazole CH); ^{13}C NMR δ (ppm): 52, 105, 109, 111, 121, 123, 125, 126, 127, 129, 136, 139, 144, 145, 154, 162; ESI(+)-MS: *m/z* 319 $(M+H)^+$, 341 $(M+Na)^+$; anal. calcd. for $C_{19}H_{14}O_3N_2$: C, 71.69; H, 4.40; N, 8.81; found: C, 71.05; H, 4.42; N, 8.42.

*Methyl 5-(5-bromobenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate (**4b**).* White crystals, yield: (90%); m.p.: 188-190 ^{0}C (from acetic acid); IR (KBr, ν in cm^{-1}), 3146, 3001 (ArH), 2953 (CH_3), 1734 (C=O, ester), 1589 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.97 (s, 3H, -$COOCH_3$), 7.27-7.54 (m, 9H, ArH), 6.12 (s, 1H, pyrazole CH); ^{13}C NMR δ (ppm): 52, 104, 109, 112, 116, 124, 126, 128, 129, 135, 139, 144 , 146, 153, 162; ESI(+)-MS: *m/z* 397 M^+, 419 [$(M+Na)^+$, ^{79}Br], 421 [$(M+Na)^+$, ^{81}Br]; anal. calcd. for $C_{19}H_{13}O_3N_2Br$: C, 57.43; H, 3.27; N, 7.05; found: C, 56.98; H, 3.22; N, 6.62.

*Methyl 5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate (**4c**).* White crystals, yield: (90%); m.p.: 148-150 ^{0}C (from acetic acid); IR (KBr, ν in cm^{-1}) 3151, 3072 (ArH), 2995, 2951 (CH_3), 1720 (C=O, ester), 1689 (C=N), 1595, 1527 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (s, 3H, CH_3), 3.99 (s, 3H, -$COOCH_3$), 7.22-7.4 (m, 8H, ArH), 6.22 (s, 1H, pyrazole CH); ^{13}C NMR δ (ppm): 8, 52, 112, 116, 119, 124, 125, 126, 128, 129, 130, 133, 139, 141, 144, 152, 162; ESI(+)-MS: *m/z* 389 [$(M+Na)^+$, ^{35}Cl], 391 [$(M+Na)^+$, ^{37}Cl]; anal. calcd. for $C_{20}H_{15}O_3N_2Cl$: C, 65.39; H, 4.08; N, 7.63; found: C, 65.00; H, 4.13; N, 7.34.

*Methyl 5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate (**4d**).* White crystals, yield: (80%); m.p.: 149-151 ^{0}C (from acetic acid); IR (KBr, ν in cm^{-1}), 3082 (ArH), 2993, 2953 (CH_3), 1724, 1749 (C=O, ester), 1600 (C=N), 1579, 1552, 1523 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.06 (s, 3H, CH_3), 3.99 (s, 3H, -$COOCH_3$), 7.26-7.46 (m, 8H, ArH + pyrazole CH); ^{13}C NMR δ (ppm): 8, 52, 111, 112, 113, 116, 117, 118, 120, 123, 124, 125, 128, 129, 131, 133, 135, 139, 142, 144, 148, 162; ESI(+)-MS: *m/z* 401 M^+, 423 [$(M+Na)^+$, ^{35}Cl], 425 [$(M+Na)^+$, ^{37}Cl]; anal. calcd. for $C_{20}H_{14}O_3N_2Cl_2$: C, 59.85; H, 3.49; N, 6.98; found: C, 59.80; H, 3.50; N, 6.45.

*General procedure for the synthesis of (**5a-d**)*

To a mixture of **4a-d** (10 mmol) in ethanol (100 mL), hydrazine hydrate (100%, 1.7 mL) was added and refluxed for 8 h. Then it was concentrated, cooled, filtered, washed and recrystallized from suitable solvent.

*5-(Benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (**5a**)* [35]. White crystals, yield: (88%); m.p.: 145-146 ^{0}C (from ethanol). IR(KBr, ν in cm^{-1}) 3429, 3317, 3225, 3159 (-NH-NH_2), 3066 (ArH), 1683 (C=O), 1649 (C=N), 1531, 1597 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.69-3.71 (s, 2H, -CONH$\underline{NH_2}$), 8.49 (b, 1H, -CO$\underline{NH}NH_2$), 7.17-7.54 (m, 10H, ArH), 6.22 (s, 1H, pyrazole CH); ^{13}C NMR δ (ppm): 105, 107, 111, 121,123, 125, 126, 127, 129, 136, 139, 145, 145, 154, 162; ESI(+)-MS: *m/z* 319 $(M+H)^+$, 341 $(M+Na)^+$; anal. calcd. for $C_{18}H_{14}O_2N_4$,: C, 67.92; H, 4.40; N, 17.61; found: C, 67.50; H, 4.35; N, 16.88.

*5-(5-Bromobenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (**5b**).* White crystals, yield: (90%); m.p.: 174-176 ^{0}C (from ethanol); IR (KBr, ν in cm^{-1}), 3323 (-$NHNH_2$), 3068 (ArH), 1683 (C=O, ester), 1664 (C=N), 1620, 1541, 1599 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 3.70-3.72 (b, 2H, -CONH$\underline{NH_2}$), 8.29 (b, 1H, -CO$\underline{NH}NH_2$), 7.27-7.56 (m, 9H, ArH), 6.16 (s, 1H, pyrazole CH); ^{13}C NMR δ (ppm): 104, 108, 112, 116, 123, 128, 129, 135, 139, 145, 146, 153, 162; ESI(+)-MS: *m/z* 397 M^+, 399 $(M+2)^+$, 421 [$(M+Na)^+$, ^{79}Br], 422 [$(M+Na)^+$, ^{81}Br]; anal. calcd. for $C_{18}H_{13}O_2N_4Br$: C, 54.40; H, 3.27; N, 14.11; found: C, 53.51; H, 3.48; N, 13.54.

*5-(5-Chloro-3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole-3–carbohydrazide (**5c**).* White crystals, yield: (90%); m.p.: 159-160 ^{0}C (from ethanol); IR (KBr, ν in cm^{-1}) 3313, 3279 (-$NHNH_2$), 3068 (ArH), 1678 (C=O, ester), 1618 (C=N), 1541, 1518 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.10 (s, 3H, CH_3), 3.86 (b, 2H, -CONH$\underline{NH_2}$), 8.33 (b, 1H, -CO$\underline{NH}NH_2$), 7.17-7.46 (m, 9H, ArH); ^{13}C NMR δ (ppm): 8, 110, 112, 116, 119, 124, 125, 128, 129, 130, 133, 139, 141, 145, 152, 162; ESI(+)-MS: *m/z* 367 M^+, 389 [$(M +Na)^+$, ^{35}Cl], 391 [$(M+Na)^+$, ^{37}Cl]; anal. calcd. for $C_{19}H_{15}O_2N_4Cl$: C, 62.12; H, 4.08; N, 15.25; found: C, 62.28; H, 4.00; N, 14.84.

*5-(5,7-Dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (**5d**).* White crystals, yield: (85%); m.p.: 199-200 ^{0}C (from acetic acid); IR (KBr, ν in cm^{-1}), 3313, 3140 (-$NHNH_2$), 3006 (ArH), 2924 (CH_3), 1714 (C=O, ester), 1660 (C=N), 1604, 1575, 1500 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm): 2.10 (s, 3H, CH_3), 3.24 (b, 2H, -CONH$\underline{NH_2}$), 8.17 (b, 1H, -CO$\underline{NH}NH_2$), 7.2-7.42 (m, 8H, ArH); ^{13}C NMR δ (ppm): 8, 109, 116, 117, 124, 128, 131, 132, 139, 142, 145,148, 159, 162; ESI(+)-MS: *m/z* 401 M^+, 403 $(M+2)^+$; anal. calcd. for $C_{19}H_{14}O_2N_4Cl_2$: C, 56.85; H, 3.49; N,13.96; found: C, 55.65; H, 3.45; N, 12.74.

Antifungal activity

Test solution was prepared by dissolving known weight of each compound **2a-d**, **3a-d**, **4a-d** and **5a-d** in dimethyl sulphoxide (DMSO) as solvent and diluted suitably to give the resultant concentration of 100, 200, 300, 400, 500 µg/mL. Whatmann No. 1 sterile paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. In vitro antifungal activity was determined by using Sabouraud Dextrose Agar obtained from Himedia Ltd/Mumbai. Twenty four hours old culture of selected fungi, *Aspergillus niger* was mixed with physiological saline and the turbidity was corrected by adding sterile physiological saline and sub cultured on Sabouraud Dextrose and suspended in sterile distilled water to an absorbance of 0.6 at 450 nm. Petri plates were prepared by pouring 10 mL Sabouraud Dextrose Agar for fungi containing microbial culture and was allowed to solidify. The discs were then applied and the

plates were incubated at 28 ^{0}C for 72-96 h (fungi) and the inhibition zone was measured in four directions and expressed as mean and the results were compared by using Kanamycin as antifungal standard.

Antibacterial study

As the sensitivity was not observed at conc. < 500 µg/mL, the antibacterial activity of the test compounds has been screened at concentration of 1 mg/mL using dimethyl sulphoxide (DMSO) as solvent and chloramphenicol (100 µg/mL) as standard for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in Mueller Hinton Agar by using cup plate agar diffusion method [36, 37]. 10 mL 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 10 mm diameter (pre-sterilized) was used to bore the cavity. Into these wells were added 0.1 mL 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 ^{0}C for 24 h. Zone of inhibition observed around the cup after respective incubation was measured with the help of Vernier Calipers. The results of antifungal and antibacterial activity are given in the Table 1.

CONCLUSION

In summary, we have demonstrated in the present study that transesterification reaction was succeeded for the conversion of **1a-d** to methyl 4-substituted/unsubstituted benzofuran-2-yl)-2,4-dioxobutanoate **2a-d**; which upon condensation with phenyl hydrazine yielded methyl 5-(substituted/unsubstituted benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carboxylate **4a-d**. This method provides an easy and versatile access to cyclize 1,3-diketoester for synthesizing pyrazoles of significant biological interest. **4a-d** were easily converted to 5-(substituted/ unsubstituted benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **5a-d** by reaction with hydrazine hydrate. The structures of synthesized compounds were confirmed by spectroscopic investigation and elemental analysis. The biological screenings of the newly synthesized derivatives were carried out against two bacteria and one fungus and it was concluded that the test compounds revealed the antifungal activity much better than their antibacterial activities at lower concentrations.

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