Full Paper

Convenient Synthesis and Antimicrobial Activity of New 3-Substituted 5-(Benzofuran-2-yl)-pyrazole Derivatives

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The reaction of ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2** with two moles of hydrazine hydrate afforded 5-(benzofuran-2-yl)-1*H*-pyrazole-3-carbohydrazide **4a**, while its reaction with equimolar amount of phenylhydrazine gave ester **3b** which then converted to 5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **4b**. Various new compounds such as imides **5** and **6**, acyl hydrazones **7** and **8**, bi-pyrazoles **9-12**, and 1,3-thiazole derivatives **14** and **15** were prepared from carbohydrazide derivatives **4a**, **b**. The new compounds are tested for their antimicrobial activity. Compounds **2**, **5**, **7**, and **8** showed antifungal activities against *C. albicans*. Also, compounds **2**, **6**, **8**, and **15** showed antibacterial activities.

Keywords: 2-Acetylbenzofuran / Acid hydrazides / Antimicrobial activity / Bis-pyrazoles / Ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate / 4-Thiazolidinones

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Introduction

Several benzofurans bearing various substituents at the C-2 position are widely distributed in nature [1]. There are other well known natural products having related benzofuran ring structures, which can get isolated particularly from *Machilus glaucescens*, *Ophryosporus charua*, *Ophryosporus lorentzii*, *Krameria ramosissima*, and *Zanthoxylum ailanthoidol* [2]. The most recognized benzofurans are ailanthoidol, amiodarone, and bufuralol compounds (Fig. 1). Ailanthoidol, a neolignan with a 2-arylbenzofuran skeleton, was isolated from the Chinese herbal medicine *Zanthoxylum ailanthoides*. It has been reported that neolignans and lignans possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, antifungal, and antifeedant activities [3]. Amiodarone is a highly effective anti-arrhythmic agent with class-III activity according to the classification of Vaughan – Williams. It is used in the treatment and prophylaxis of both ventricular and supraventricular arrhythmias, in particular in patients with heart insufficiency, because it has no significant negative inotropic effect [4]. Bufuralol is a non-selective β-adrenoceptor antagonist developed by Hoffman La Roche [5]. 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 activities [6 – 14]. In view of these reports and in continuation of our previous work in the synthesis of biologically active heterocycles [15 – 20] we have herein synthesized some new benzofuran derivatives and tested them for their antimicrobial activities.

Results and discussion

Chemistry

The reaction of 2-acetylbenzofuran **1** with diethyl oxalate in sodium methoxide afforded ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2** [21] which is used as starting material in this study. Reaction of **2** with two moles hydrazine hydrate in refluxing ethanol gave directly 5-(benzofuran-2-yl)-1*H*-pyrazole-3-carbohydrazide **4a** via the non-isolable

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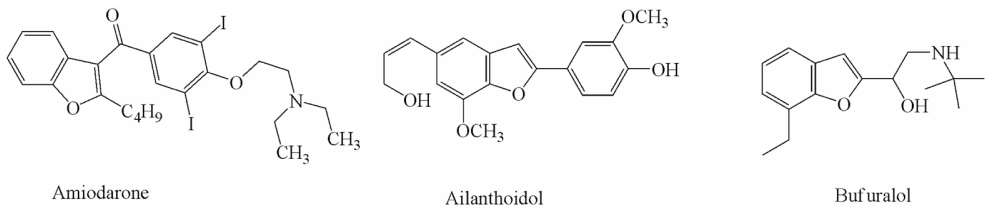
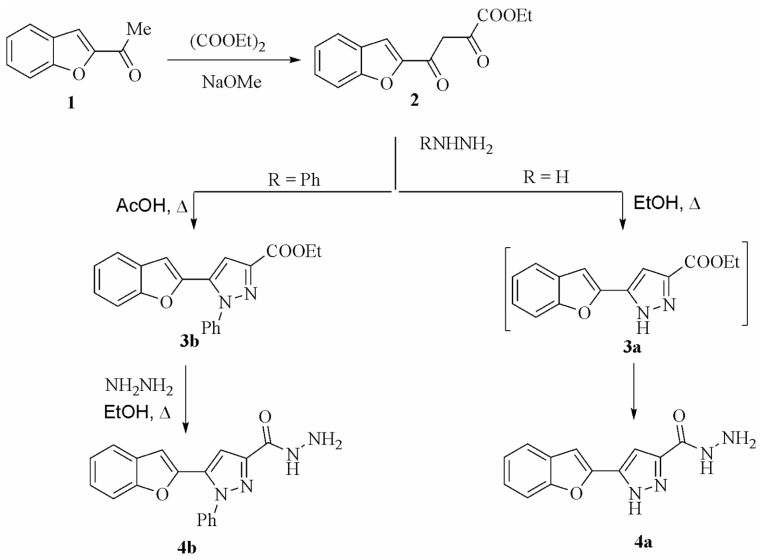


Figure 1. Some benzofurans containing biologically active compounds.



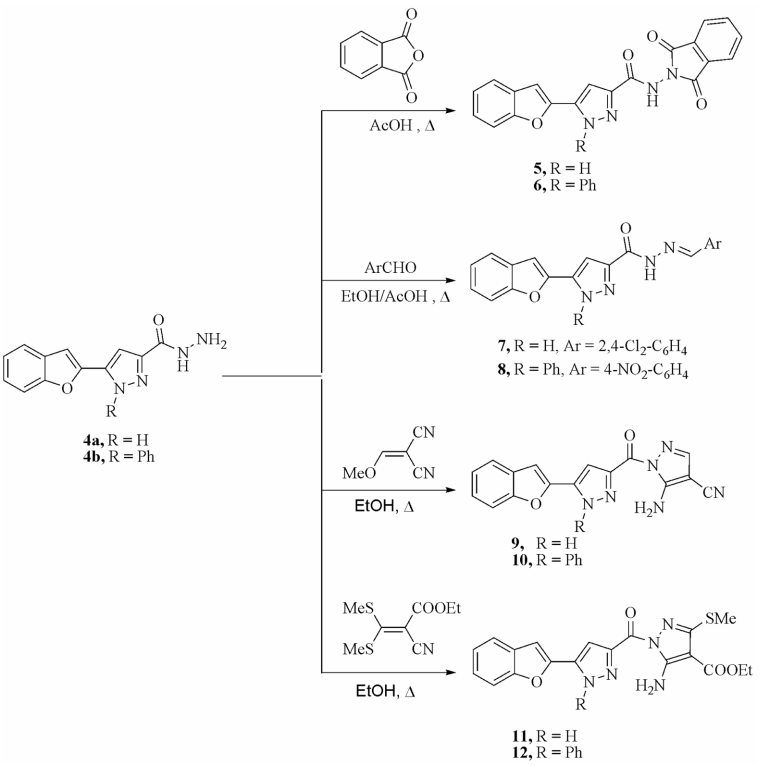
Scheme 1. Schematic diagram showing the synthesis of compounds **3** and **4**.

intermediate **3a**, while the reaction of **2** with phenylhydrazine in refluxing glacial acetic acid furnished the isolable ester **3b** which then reacted with hydrazine hydrate in absolute ethanol to afford 5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-carbohydrazide **4b** (Scheme 1). The structure of compounds **3b**, **4a**, and **4b** were established under the basis of their elemental analysis and spectral data. For example, the mass spectra of compounds **3b**, **4a**, and **4b** showed the molecular ion peaks at *m/z* 332, 242, and 318, respectively; these fit exactly with the calculated masses.

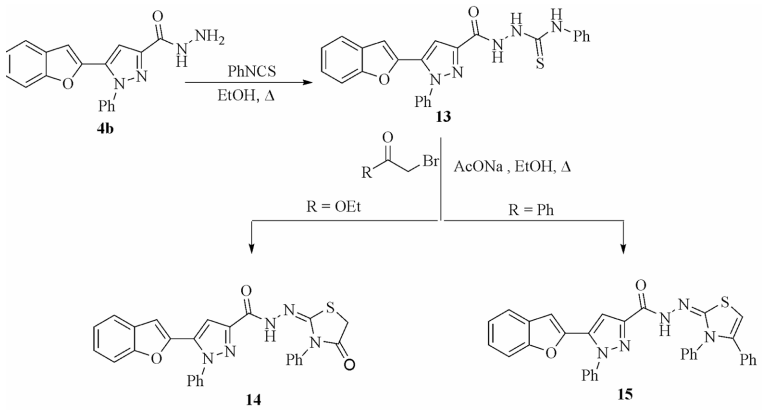
Treatment of acid hydrazides **4a**,**b** with phthalic anhydride in refluxing glacial acetic acid for 5 h afforded the imides **5** and **6**, respectively. The IR spectra of compounds **5** and **6** showed three absorption bands in the region 1666 – 1747 cm^{-1} characteristic for three carbonyl groups. In addition, the reaction of **4a** with 2,4-dichlorobenzaldehyde afforded hydrazone **7** while hydrazone **8** was formed by the reaction of 4-nitrobenzaldehyde with hydrazide **4b**. The ^{1}H-NMR of **7a** showed a singlet proton at 9.89 ppm attributed to -C*H*=N-. On the other hand, reaction of hydrazides **4a**,**b** with alkenes derivatives, namely, 2-(methoxymethylene)malononitrile or ethyl 2-cyano-3,3-bis(methylthio)acrylate in boiling ethanol afforded the bi-pyrazole derivatives **9** – **12** respectively (Scheme 2). The IR spectra of compounds **9** and **10** showed characteristic absorption bands in the range 2219 – 2221 cm^{-1} due to the presence of the carbonitrile group. The IR spectra of compounds **9** – **12** showed absorption bands in the range 3288 – 3450 cm^{-1} indicating the presence of amino groups.

Acid hydrazides have long been known to react with isothiocyanates and α-haloketones to afford a variety of heterocyclic derivatives [22 – 27]. Thus, reaction of hydrazide **4b** with phenyl isothiocyanate in absolute ethanol afforded thiosemicarbazide **13**, which was then treated with ethyl bromoacetate or phenacyl bromide in ethanol containing anhydrous sodium acetate to yield thiazolidin-4-one **14** or 1,3-thiazole derivative **15**, respectively (Scheme 3).

The mass spectrum of **13** showed a peak corresponding to its molecular ion peak at *m/z* 453. The IR spectrum of **14** showed an absorption band at 1750 cm^{-1} due to the carbonyl function of thiazolidinone moiety. The ^{1}H-NMR of compound **14** showed a singlet signal at 4.10 ppm corresponding to methylene protons of thiazolidene ring



Scheme 2. Schematic diagram showing the synthesis of compounds **5**–**12**.



Scheme 3. Schematic diagram showing the synthesis of compounds **13**–**15**.

while ^{1}H-NMR of compound **15** displayed one singlet at 5.78 ppm corresponding to thiazole ring proton.

Biology

Most of the new compounds were evaluated for their antimicrobial properties. Compounds **2**, **5**, **7**, and **8** showed inhibition zones and, therefore, antifungal activities against *C. albicans* more than the reference compound Flucanazol, while compounds **9**, **10**, and **14** showed similar antifungal activity against *C. albicans,* when compared with Flucanazol. All the tested compounds showed no antifungal activities against *Aspergillus niger*, *Aspergillus fumigatus*, *Trycophyton mentgrophytes*, or *Saccharomyas cerevisiae* (Table 1).

From the results in Table 2 we can conclude that some of our products have noticeable antibacterial activities. The inhibition zones of compounds **2**, **4a**, **6**, **7**, **8**, **9**, and **14** against *Bacillus subitilis* are larger than that of the control Amoxicillin. While some of our compounds, such as **2**, **6**, **8**, and **15** showed antibacterial activities more than the control against *E. coli*, some of the new compounds may be therefore considered as highly potential antibacterial agents. On the other hand, all of the tested compounds showed no antibacterial activities against *Pseudo-*

Table 1. Antifungal activities.

Compounds	Inhibition zone [mm]				
	Aspergillus niger	*Aspergillus fumigatus*	*Trycophyton mentgrophytes*	*Candida albicans*	*Saccharomyas cerevisiae*
2	–	–	–	30	–
4a	–	–	–	–	–
5	–	–	–	30	–
6	–	–	–	–	–
7	–	–	–	30	–
8	–	–	–	30	–
9	–	–	–	20	–
10	–	–	–	20	–
11	–	–	–	–	–
12	–	–	–	–	–
13	–	–	–	–	–
14	–	–	–	20	–
15	–	–	–	–	–
Fluconazole	20	20	20	20	20

Table 2. Antibacterial activities.

Compounds	Inhibition zone [mm]				
	Escherichia coli	*Bacillus subitilis*	*Pseudomonas aeroginosa*	*Staphylo-coccus aureus*	*Strepto-coccus sp.*
2	40	40	–	–	–
4a	–	40	–	–	–
5	–	–	–	–	–
6	35	40	–	–	–
7	–	40	–	–	–
8	40	40	–	–	–
9	–	40	–	–	–
10	–	–	–	–	–
11	–	35	–	–	–
13	–	20	–	–	–
14	–	40	–	–	–
15	40	–	–	–	–
Amoxicilline	25	25	25	25	25

monas aeroginosa, Staphylococcus aurous and *Streptococcus sp*.

Structure-activity relationship of antimicrobial activities (SAR)

(i) The benzofuran, pyrazole, thiazolidinone, and thiazole moieties are essential for antimicrobial activity. (ii) Increasing the number of nitrogen atoms sharply increases the antimicrobial activity. (iii) The phthalimide moieties in compounds **5** and **6** are essential for the antimicrobial activities of these compounds.

The authors have declared no conflict of interest.

Experimental

Chemistry

All melting points were taken on Electrothermal IA 9000 series digital melting point apparatus (Electrothermal, Essex, U.K.). Elemental analytical data (in accord with the calculated values) were obtained from the microanalytical unit, Cairo University, Giza, Egypt. The IR spectra (KBr) were recorded on a Shimadzu CVT-04 spectrophotometer (Shimadzu, Tokyo, Japan). The ^{1}H-NMR spectra were recorded at 270 MHz on a Varian EM-360 spectrometer (Varian Inc., Palo Alto, CA, USA) using TMS as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift (δ) values are given in parts per million. The mass spectra were performed using a Varian MAT CH-5 spectrometer (Varian) (70 eV). 2-Acetylbenzofuran **1** [28] and ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2** [21] were prepared according to the procedures reported in literature.

*Ethyl 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carboxylate **3b***

To a solution of ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2** (2.6 g, 10 mmol) in glacial acetic acid (30 mL), phenylhydrazine (1.1 g, 10 mmol) was added and the resulting mixture was boiled under reflux for 30 min; it was then cooled and poured into 200 mL water. The precipitated was filtered off and recrystallized from dilute ethanol, giving 82% of **3b** with m.p.: 165 – 166°C; IR (KBr) ν_{max}/cm^{-1}: 1617 (C=N), 1754 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 1.51 (t, 3H,CH_3), 4.22 (q, 2H, CH_2), 6.63 (s, 1H, benzofuran-H), 7.09 (s, 1H, pyrazole-H), 7.13 – 7.63 (m, 9H, Ar-H); Ms *m/z* (%): 332 [M$^+$] (0.6), 318 (100). Anal. Calcd. for $C_{20}H_{16}N_2O_3$: C, 72.28; H, 4.85; N, 8.43. Found: C, 72.41; H, 4.98; N, 8.61.

*5-(Benzofuran-2-yl)-1H-pyrazole-3-carbohydrazide **4a***

Hydrazine hydrate (1.0 g, 20 mmol) was given to ethyl 4-(benzofuran-2-yl)-2,4-dioxobutanoate **2** (2.6 g, 10 mmol) in absolute ethanol (40 mL). The reaction mixture was boiled under reflux for 4 h and was then cooled and poured into 200 mL ice-water. The precipitate was filtered off and recrystallized from dilute ethanol, giving 76% of **4a** with m.p.: 291 – 292°C; IR(KBr) ν_{max}/cm^{-1}: 1612 (C=N), 1666 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 4.47 (s, 2H, NH_2), 7.09 (s, 1H, pyrazole-H), 7.19 (s, 1H, benzofuran-H), 7.25 – 7.69 (m, 4H, Ar-H), 9.90 (s, 1H, NH), 13.81 (s, 1H, NH); Ms *m/z* (%): 242 [M$^+$] (100). Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 13.21. Found: C, 59.71; H, 4.33; N, 13.41.

*5-(Benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide **4b***

To a solution of **3b** (1.66 g, 5 mmol) in absolute ethanol (25 mL) hydrazine hydrate (~1 mL, 15 mmol) was added and the mixture was boiled for 4 h. The ethanol was distilled off and the residue was poured into 200 mL ice-water, the precipitate was filtered off and recrystallized from dilute ethanol, giving 83% of **4b** with m.p.: 140 – 142°C; IR(KBr) ν_{max}/cm^{-1}: 1614 (C=N), 1667 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 4.21 (s, 2H, NH_2), 6.74 (s, 1H, pyrazole-H), 6.83 (s, 1H, benzofuran-H), 7.15 – 7.60 (m, 9H, Ar-H), 9.21 (s, 1H, NH); Ms *m/z* (%): 318 [M$^+$] (100). Anal. Calcd. for $C_{18}H_{14}N_4O_2$: C, 67.91; H, 4.43; N, 17.60. Found: C, 68.08; H, 4.61; N, 17.79.

*Synthesis of imides **5** and **6***

A mixture of **4a**,**b** (2 mmol) and phthalic anhydride (0.3 g, 2 mmol) in glacial acetic acid (25 mL) was refluxed for 5 h. The reaction mixture was evaporated and the obtained residue was solidified with ether, filtered off, and crystallized from AcOH-H_2O to give **5** and **6**.

5-(Benzofuran-2-yl)-N-(1,3-dioxoisoindolin-2-yl)-1H-pyrazole-3-carboxamide ***5***

Yield (82%); m.p.: 292 – 294°C; IR(KBr) ν_{max}/cm^{-1}: 1612 (C=N), 1666, 1712, 1747 (3C=O); ^{1}H-NMR (DMSO-d_6) δ: 6.52 (s, 1H, pyrazole-H), 6.77 (s, 1H, benzofuran-H), 7.28 – 7.99 (m, 8H, Ar-H), 9.09 (s, 1H, NH), 13.76 (s, 1H, NH); Ms *m/z* (%): 372 [M$^+$] (43), 287 (100). Anal. Calcd. for $C_{20}H_{12}N_4O_4$: C, 64.52; H, 3.25; N, 17.19. Found: C, 64.71; H, 3.07; N, 17.38.

5-(Benzofuran-2-yl)-N-(1,3-dioxoisoindolin-2-yl)-1-phenyl-1H-pyrazole-3-carboxamide ***6***

Yield (86%); m.p.: >300°C; IR(KBr) νmax/cm^{-1}: 1610 (C=N), 1667, 1712, 1747 (3C=O); ^{1}H-NMR (DMSO-d_6) δ: 6.55 (s, 1H, pyrazole-H), 6.76 (s, 1H, benzofuran-H), 7.28 – 7.99 (m, 13H, Ar-H), 9.09 (s, 1H, NH); Ms *m/z* (%): 448 [M$^+$] (48), 287 (100). Anal. Calcd. for $C_{26}H_{16}N_4O_4$: C, 69.64; H, 3.60; N, 12.49. Found: C, 69.71; H, 3.69; N, 12.53.

5-(Benzofuran-2-yl)-N'-arylidene-pyrazole-3-carbohydrazides ***7*** and ***8***

A mixture of **4a**,**b** (1 mmol) and appropriate aromatic aldehydes (1 mmol) in 30 mL absolute ethanol in the presence of few drops of glacial acetic acid was refluxed for 2 h. The formed solid was filtered off, dried, and crystallized from EtOH / DMF (10 : 1 v/v) to give **7** and **8**.

5-(Benzofuran-2-yl)-N'-(2,4-dichlorobenzylidene)-1H-pyrazole-3-carbohydrazide ***7***

Yield (81%); m.p.: >300°C; IR(KBr) ν_{max}/cm^{-1}: 1608 (C=N), 1671 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 6.89 (s, 1H, pyrazole-H), 7.21 (s, 1H, benzofuran-H), 7.30 – 8.5 (m, 7H, Ar-H), 9.89 (s, 1H, CH), 12.3 (s, 1H, NH), 13.89 (s, 1H, NH); Ms *m/z* (%): 399 [M$^+$] (100). Anal. Calcd. for $C_{19}H_{12}Cl_2N_4O_2$: C, 57.16; H, 3.03; N, 17.76. Found: C, 56.98; H, 3.12; N, 17.88.

5-(Benzofuran-2-yl)-N'-(4-nitrobenzylidene)-1-phenyl-1H-pyrazole-3-carbohydrazide ***8***

Yield (84%); m.p.: 238 – 340°C; IR(KBr) ν_{max}/cm^{-1}: 1611 (C=N), 1664 (C=O); ^{1}H-NMR (DMSO-d_6) δ: 6.71 (s, 1H, pyrazole-H), 7.03 (s, 1H, benzofuran-H), 7.19 – 8.12 (m, 12H, Ar-H), 9.88 (s, 1H, CH), 12.42 (s, 1H, NH); Ms *m/z* (%): 451 [M$^+$] (100). Anal. Calcd. for $C_{25}H_{17}N_5O_4$: C, 66.51; H, 3.80; N, 15.51. Found: C, 66.53; H, 3.69; N, 15.43.

Synthesis of bi-pyrazoles ***9 – 12***

To a solution of **4a**,**b** (1 mmol) in anhydrous ethanol (20 mL), 2-[bis(methylthio)methylene]malononitrile or ethyl 2-cyano-3,3-bis(methylthio)acrylate (1 mmol) was added and the reaction mixtures were refluxed for 2 h and 4 h, respectively. The products, which separated on cooling, were collected by filtration and recrystallized from ethanol to give compounds **9** – **12**.

5-Amino-1-(5-(benzofuran-2-yl)-1H-pyrazole-3-carbonyl)-1H-pyrazole-4-carbonitrile ***9***

Yield (81%); m.p.: >300°C; IR(KBr) ν_{max}/cm^{-1}: 1624 (C=N), 1655 (C=O), 2219 (CN), 3288, 3200 (NH_2); ^{1}H-NMR (DMSO-d_6) δ: 6.06 (s, 1H, pyrazole-H), 7.19 (s, 1H, benzofuran-H), 7.28 – 7.70 (m, 4H, Ar-H), 7.72 (s, 1H, CH), 9.92 (s, 2H, NH_2), 13.53 (s, 1H, NH); Ms *m/z* (%): 318 [M$^+$] (32), 287 (100). Anal. Calcd. for $C_{16}H_{10}N_6O_2$: C, 60.38; H, 3.17; N, 26.40. Found: C, 60.53; H, 3.10; N, 26.51.

5-Amino-1-(5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbonyl)-1H-pyrazole-4-carbonitrile ***10***

Yield (81%); m.p.: 210°C; IR(KBr) ν_{max}/cm^{-1}: 1629 (C=N), 1665 (C=O), 2221 (CN), 3288, 3224 (NH_2); ^{1}H-NMR (DMSO-d_6) δ: 6.18 (s, 1H, pyrazole-H), 7.10 (s, 1H, benzofuran-H), 7.28 – 7.70 (m, 9H, Ar-H), 7.78 (s, 1H, CH), 9.96 (s, 2H, NH_2); Ms *m/z* (%): 394 [M$^+$] (26), 287 (100). Anal. Calcd. for $C_{22}H_{14}N_6O_2$: C, 67.00; H, 3.58; N, 21.31. Found: C, 67.21; H, 3.62; N, 21.49.

Ethyl 5-amino-1-(5-(benzofuran-2-yl)-1H-pyrazole-3-carbonyl)-3-(methylthio)-1H-pyrazole-4-carboxylate ***11***

Yield (76%); m.p.: 258 – 260°C; IR(KBr) ν_{max}/cm^{-1}: 1629 (C=N), 1659 (C=O), 1694 (C=O, ester), 3292, 3202 (NH_2); ^{1}H-NMR (DMSO-d_6) δ: 1.24 (t, 3H, CH_3), 2.49 (s, 3H, SCH_3), 4.39 (q, 2H, CH_2), 7.19 (s, 1H, pyrazole-H), 7.25 (s, 1H, benzofuran-H), 7.29 – 7.76 (m, 4H, Ar-H), 9.89 (s, 2H, NH_2), 13.89 (s, 1H, NH); Ms *m/z* (%): 411 [M$^+$] (43), 287 (100). Anal. Calcd. for $C_{19}H_{17}N_5O_4S$: C, 55.47; H, 4.16; N, 17.02. Found: C, 55.31; H, 4.23; N, 17.22.

Ethyl 5-amino-1-(5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbonyl)-3-(methylthio)-1H-pyrazole-4-carboxylate ***12***

Yield (78%); m.p.: 196 – 198°C; IR(KBr) ν_{max}/cm^{-1}: 1623 (C=N), 1656 (C=O), 1697 (C=O, ester), 3292, 3228 (NH_2); ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, 3H, CH_3), 2.51 (s, 3H, SCH_3), 4.37 (q, 2H, CH_2), 7.12 (s, 1H, pyrazole-H), 7.22 (s, 1H, benzofuran-H), 7.30 – 7.78 (m, 9H, Ar-H), 9.98 (s, 2H, NH_2); Ms *m/z* (%): 487 [M$^+$] (36), 287 (100). Anal. Calcd. for $C_{25}H_{21}N_5O_4S$: C, 61.59; H, 4.34; N, 14.36. Found: C, 61.37; H, 4.51; N, 14.48.

2-(5-(Benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbonyl)-N-phenylhydrazinecarbo-thioamide ***13***

A mixture of **4b** (1.6 g, 5 mmol) and phenylisothiocyante (0.67 g, 5 mmol) in absolute ethanol (30 mL) was heated under reflux for 3 h. The formed solid was filtered off and recrystallized from EtOH / DMF to give **13** in 83% yield; m.p.: 209 – 210°C; IR(KBr) ν_{max}/cm^{-1}: 1259 (C=S), 1614 (C=N), 1656 (C=O), 3315-3154 (NH); ^{1}H-NMR (DMSO-d_6) δ: 6.61 (s, 1H, pyrazole-H), 7.15 (s, 1H, benzofuran-H), 7.25 – 7.64 (m, 14H, Ar-H), 9.47, 9.49, 10.48 (3s, 3H, 3NH); Ms *m/z* (%): 453 [M$^+$] (2), 287 (100). Anal. Calcd. for $C_{25}H_{19}N_5O_2S$: C, 66.21; H, 4.22; N, 15.44. Found: C, 66.42; H, 4.41; N, 15.58.

5-(Benzofuran-2-yl)-N'-(4-oxo-3-phenylthiazolidin-2-ylidene)-1-phenyl-1H-pyrazole-3-carbohydrazide ***14***

A mixture of **13** (0.45 g, 1 mmol) and ethyl bromoacetate (0.17 g, 1 mmol) in absolute ethanol (30 mL) containing anhydrous sodium acetate (0.33 g, 4 mmol) was heated under reflux for 6 h. The reaction mixture was cooled, diluted with water, and allowed to stand overnight. The precipitate solid was filtered off and recrystallized from ethanol to give **14** in 63% yield; m.p.: 159 – 160°C; IR(KBr) ν_{max}/cm^{-1}: 1614 (C=N), 1643 (C=O), 1739 (C=O), 3423 (NH); ^{1}H-NMR (DMSO-d_6) δ: 4.10 (s, 2H, CH_2), 4.17 (s, 1H, NH), 6.66 (s, 1H, pyrazole-H), 7.07 (s, 1H, benzofuran-H), 7.22-7.62 (m, 14H, Ar-H); Ms *m/z* (%): 493 [M$^+$] (11), 77 (100). Anal. Calcd. for $C_{27}H_{19}N_5O_3S$: C, 65.71; H, 3.88; N, 14.19. Found: C, 65.89; H, 3.96; N, 14.07.

5-(Benzofuran-2-yl)-N′-(3,4-diphenylthiazol-2-(3H)-ylidene)-1-phenyl-1H-pyrazole-3-carbohydrazide ***15***

A mixture of **13** (0.45 g, 1 mmol) and phenacylbromide (0.2 g, 1 mmol) in absolute ethanol (30 mL) containing anhydrous sodium acetate (0.33 g, 4 mmol) was heated under reflux for 6 h. The reaction mixture was cooled; the formed solid was filtered off, washed with water, and recrystallized from EtOH / DMF to give **15** in 64% yield; m.p.: 279 – 80°C; IR (KBr) ν_{max}/cm^{-1}:1608 (C=N), 1641 (C=O), 3402 (NH); ^{1}H-NMR (DMSO-d_6) δ: 5.78 (s, 1H, thiazole-H), 6.63 (s, 1H, pyrazole-H), 7.03 (s, 1H, benzofuran-H), 7.22 – 7.62 (m, 19H, Ar-H), 10.32 (s, 1H, NH); Ms *m/z* (%): 553 [M^+] (19), 77 (100). Anal. Calcd. for $C_{33}H_{23}N_5O_2S$: C, 71.59; H, 4.19; N, 12.65. Found: C, 71.73; H, 4.24; N, 12.81.

Biology

Antifungal activities

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NCIM no. 524), *Aspergillus fumigatus* (NCIM no. 902), *Candida albicans* (NCIM no. 300), *Penicillium marneffei*, and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method [29, 30]. Sabourand's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting the pH to 5.7. Normal saline was used to make a suspension of spores of the fungal strain for lawning. A loopful of the articular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Agar media (20 mL) were poured into each Petri dish. The excess suspension was decanted and the plates were dried by placing them in an incubator at 37°C for 1 h. Using an agar punch, wells were made and each well were labeled. A control was also prepared in triplicate and maintained at 37°C for 3 – 4 days. The zone of inhibition was noted. The activity of each compound was compared with Fluconazole as the standard drug. The results of antifungal studies are given in Table 1.

Antibacterial activities

The new compounds were tested for their antibacterial activities by the disc-diffusion method [31] using nutrient broth medium (contained (g/L): beef extract 3 g; peptone 5 g; pH = 7.0). The Gram-positive bacteria utilized in this study consisted of *Bacillus subitilis*, *Staphylococcus aureus*, and *Streptococcus sp*. The Gram-negative bacteria included *Escherichia coli* and *Pseudomonas aeruginos.* In the disc-diffusion method, sterile paper discs (0.5 mm) impregnated with compounds dissolved in dimethylsulfoxide (DMSO) at a concentration of 200 μg/mL were used. Then, the paper discs impregnated with the solution of the compound tested were placed on the surface of the media inoculated with microorganism. The plates were incubated at 35°C for 24 h. The growth inhibition zones measured after incubation, are documented in Table 2.

Generally, the results were taken in duplicate. Results with difference higher than 5% were neglected and repeated.

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