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Synthesis of New Benzofuran Derivatives and Evaluation of their Antimicrobial Activities.

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ABSTRACT

Starting from 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-hydrazinyl-4- (4-methoxyphenyl) nicotinonitrile **5**, novel heterocyclic derivatives of benzofuran were synthesized **6-18**. Biological evaluation in vitro revealed that compounds **15, 6, 10** and **18** exhibited a wide range of antibacterial activity against gram positive and gram negative bacteria.

Keywords: Benzofuran; Nicotinonitrile; Antibacterial; Antimicrobial.

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INTRODUCTION

Benzofurans play an important role in heterocyclic chemistry, several derivatives of which have been marked as biologically and pharmacologically active product [1]. Widespread interest in the chemistry of benzofuran in a large number of natural products has attracted due to their biological activities and their potential applications as pharmacological agents. Also benzofurans and their derivatives possess a broad range of important biological activities including anti-inflammatory [2], pesticide & insecticidal [3], antihistaminic [4], anticonvulsant [5], antiallergic [6] and In-vitro anti-HIV-1, anticancer and anti-microbial activities [7]. The antimicrobial activity of benzofuran derivative appears to be dependent on substitution at the heterocyclic furan ring than the aromatic moiety [8].

They have already attracted considerable attention amongst organic and medicinal chemists in the last few years [9, 10]. In addition benzofuran derivatives are a major group of biologically active heterocycles, which are usually important constituents of plant extracts used in medicinal chemistry for their various biological activities [11-14]. Due to their diverse activities, much attention has been paid to synthetic strategies to access these systems, and a number of methods have been developed [15-17].

RESULTS AND DISCUSSION

Chemistry

Compound 1-(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)ethanone **(1)** reacted with bromine in the presence of acetic acid via stirring to produce 1-(2,6-dibromo-4,7-di methoxybenzofuran-5-yl)ethanone **(2)**, which was allowed to react with ethyl cyano acetate, anhydrous ammonium acetate and 4-anisaldehyde to give 6-(2,6-dibromo-4,7 -dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile **(3)** (Scheme 1). Compound **(3)** was chlorinated with phosphorus penta chloride in phosphorus oxychloride to give the corresponding compound 2-chloro-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile **(4).** Compound **(4)** reacted with hydrazine hydrate to give compound 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-hydrazinyl-4-(4-methoxyphenyl)nicotinonitrile **(5)** (Scheme 1). Compound **(5)** was used as a precursor for synthesizing a number of new benzofuran derivatives. Thus, the reaction of compound **(5)** with phthalic anhydride gave the corresponding analogue 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-(1,4-dioxo-3,4-dihydrophthalazin-2(1*H*)-yl)-4-(4-methoxyphenyl)nicotinonitrile **(6)** (Scheme 1). Furthermore, compound **(5)** was allowed to react with DMF to give the compound 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **(7)** (Scheme 1). Also, compound **(5)** was condensed with acetic anhydride to give the corresponding compound 6-(2,6-dibromo-4,7-di methoxybenzofuran-5-yl)-4-(4-methoxyphenyl)-2-(2-triacetylhydrazide)nicotino nitrile **(8)** (Scheme 1). In addition, compound ethyl *N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)formohydrazonate **(9)** was synthesized by the reaction of compound **(5)** with triethyl orthoformate (Scheme 2). At the same time, the reaction of compound **(5)** with acetylacetone yielded 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-(4-methoxyphenyl) nicotinonitrile **(10)** (Scheme 2). Further treatment of compound **(5)** with ethyl aceto acetate gave the corresponding 6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)-2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)nicotinonitrile **(11)** (Scheme 2), while the reaction of compound **(5)** with formic acid gave the corresponding derivative *N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)formohydrazide **(12)** (Scheme 2). Compound 2-chloro-*N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)acetohydrazide **(13)** was prepared by the reaction of compound **(5)** with chloroacetyl chloride (Scheme 2). Also, compound **(5)** was condensed with isatoic anhydride to give the corresponding compound 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-((2,4-dioxo-1,2-dihydroquinazolin-3(4*H*)-yl)amino)-4-(4-methoxyphenyl)nicotinonitrile **(14)** (Scheme 3). Furthermore, compound **(5)** was allowed to react with tetrabromo phthalic anhydride to give the compound 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)-2-((4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)amino)nicotinonitrile **(15)** (Scheme 3). Further treatment of compound **(5)** with aromatic aldehydes namely, 2-chloro-5-nitro-benzaldehyde and/or 3-anisaldehyde gave the corresponding derivatives 2-(2-(substituted)hydrazinyl)-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile **(16a, b)** (Scheme 3), while the reaction of compound **(5)** with benzoyl chloride gave *N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)benzohydrazide **(17)** (Scheme 3). Compound 2-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxy phenyl)pyridin-2-yl)-*N*-

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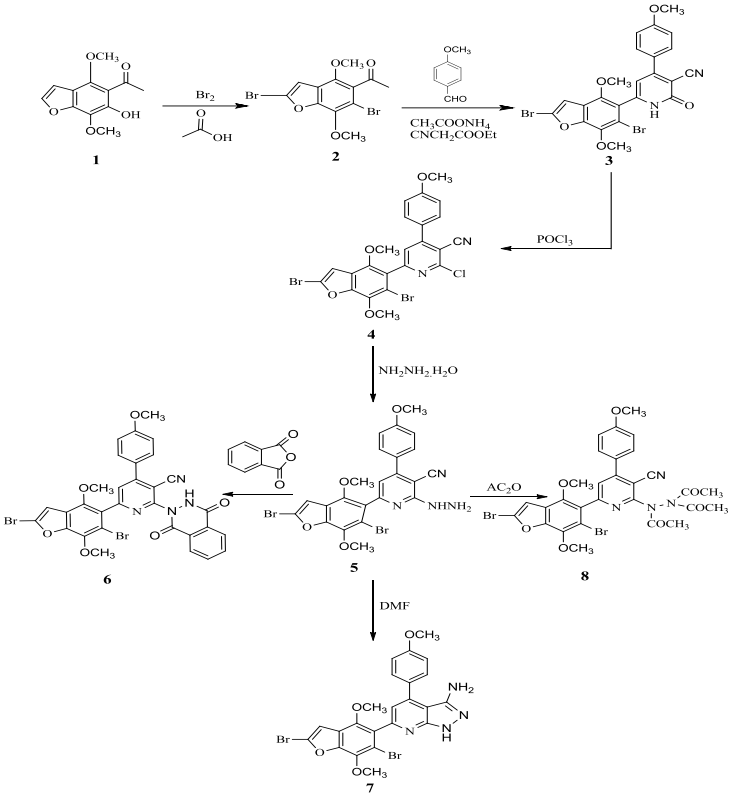
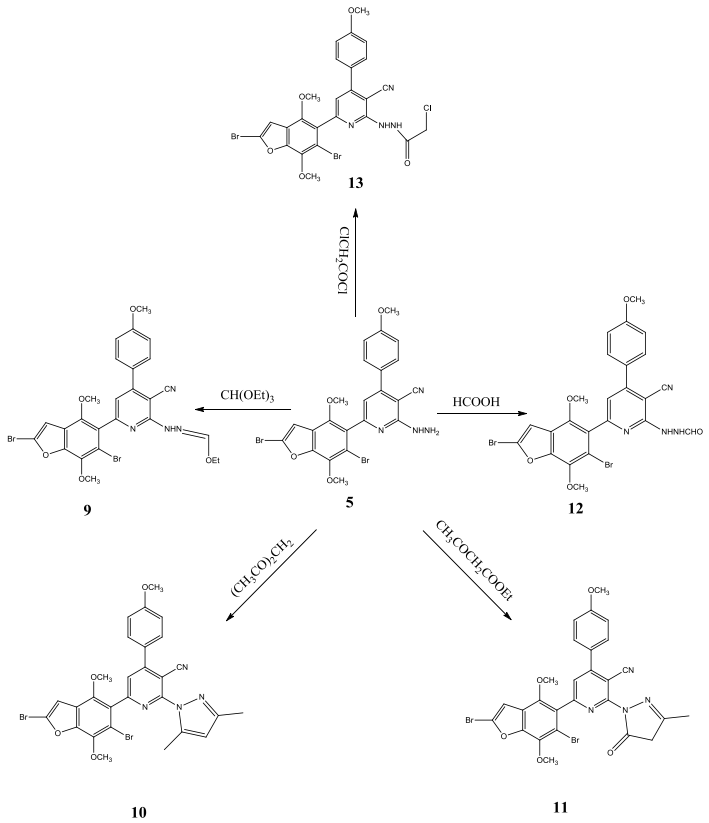
phenylhydrazinecarbothioamide **(18)** was prepared by the reaction of compound **(5)** with phenyl isothiocyanate (Scheme 3).



Scheme 1



Scheme 2:

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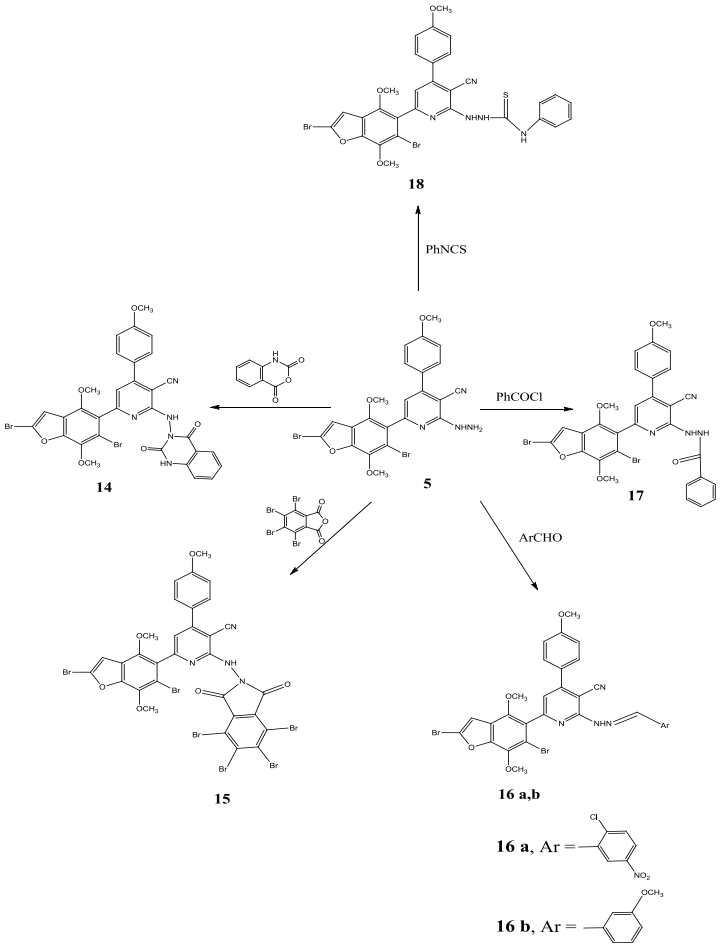

Scheme 3

Biological activity:

Antibacterial activity:

Results of antibacterial testing of the synthesized compounds **3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16a, 17** and **18** are shown in (Table 1), revealed that compounds **15, 6, 10** and **18** exhibited a wide range of antibacterial activity against gram positive and gram negative bacteria. In addition, compounds **4, 5** and **13** showed moderate inhibitory activity against gram positive bacteria only, with zones of inhibition range from 6 to 10 mm. But the compounds **16a, 9** and **12** showed moderate inhibitory activity against gram negative bacteria only, with zones of inhibition range from 7 to 9 mm, in comparison with the standard drug (Thiophenicol). Compound **15** showed good activity against *B. subtilis* and *S. aureus* with zones of inhibition 10 mm and minimum inhibitory concentrations (MIC) 25 and 50 µg, respectively (Table 2).

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Table (1): Antimicrobial activity of newly synthesized compounds (3, 4, 5, 6, 7, 9, 10, 12, 13, 15, 16a, 16b, 17 and 18) [a,b].

Entry	Gram positive bacteria		Gram negative bacteria		Fungi					
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC 25922	*P.aeruginosa* ATCC27953	*C.albicans* ATCC 10321	*A.niger* NRRL-363	*F.solani* NRC15	*F.oxysporium* NRC23	*A.alternata* NRC43	*A.tenuissima* KM651985
3	N.A.	6	N.A.	N.A.	N.A.	9	8	10	N.A.	N.A.
4	7	7	N.A.	N.A.	6	9	8	12	N.A.	N.A.
5	10	8	N.A.	N.A.	7	N.A.	N.A.	N.A.	N.A.	N.A.
6	6	8	8	6	6	N.A.	N.A.	N.A.	N.A.	N.A.
7	N.A.	N.A.	N.A.	8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
9	N.A.	N.A.	8	8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
10	7	7	8	6	6	N.A.	N.A.	N.A.	N.A.	N.A.
12	N.A.	N.A.	7	9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
13	6	6	N.A.	N.A.	6	N.A.	N.A.	N.A.	N.A.	N.A.
15	10	10	8	8	9	7	N.A.	N.A.	N.A.	N.A.
16a	N.A.	N.A.	7	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
16b	6	7	N.A.	7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
17	N.A.	N.A.	N.A.	8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
18	7	8	9	10	6	8	N.A.	N.A.	N.A.	N.A.
Thiophenicol	13	12	10	11	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Treflucan	N.A.	N.A.	N.A.	N.A.	12	9	11	13	10	11

[a] Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm) of the tested compounds against the pathological strains based on the agar diffusion technique.
[b] N.A. No activity.

Table (2): Minimal inhibitory concentration (µg/disc) against the pathological strains.

Entry	Gram positive bacteria		Gram negative bacteria		Fungi					
	B. subtilis ATCC6633	*S. aureus* ATCC29213	*E. coli* ATCC 25922	*P.aeruginosa* ATCC27953	*C.albicans* ATCC 10321	*A.niger* NRRL-363	*F.solani* NRC15	*F.oxysporium* NRC23	*A.alternata* NRC43	*A.tenuissima* KM651985
3	-	-	-	-	-	100	-	50	-	-
4	-	-	-	-	-	100	-	25	-	-
5	50	-	-	-	-	-	-	-	-	-
12	-	-	-	50	-	-	-	-	-	-
15	25	50	-	-	50	-	-	-	-	-
18	-	-	100	50	-	-	-	-	-	-
Thiophenicol	3.13	3.13	25	25	-	-	-	-	-	-
Treflucan	-	-	-	-	25	100	50	50	100	100

Antifungal activity:

All of the synthesized compounds **3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16a, 17** and **18** were tested for their antifungal activity against *C. albicans* and five pathogenic fungi. The result shown in (Table 1) displayed that compound **4** exhibited good inhibitory activity against four of the tested fungi compared to the standard antifungal drug (Treflucan), followed by compound **3**. In addition compounds **15** and **18** exhibited antifungal activity against *C. albicans* and A*. niger* (zones of inhibition range from 6 to 9 mm). Compounds **3** and **4** showed good activity against *F. oxysporium* with zones of inhibition 10 and 12 mm, respectively and minimum inhibitory concentrations (MIC) 50 and 25 µg, respectively (Table 2).

EXPERIMENTAL

Chemistry

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer

(Japan) at cm^{-1} scale using KBr disc technique at the Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt. ^1H NMR spectra were determined by using a JEOl EX-270 NMR spectrometer (Japan) at the Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at the Central Services Laboratory, Cairo University, Giza, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV analysis lamp at λ 254/366 nm for few seconds.

Synthesis of 1-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)ethanone (2):

Bromine (0.001 mol) was added drop-wise to a solution of compound **1** (0.001 mol) in acetic acid (40 mL). The solution was stirred for 2 h at room temperature. The reaction mixture was poured onto ice water and treated with 5% sodium bisulfate solution to remove excess bromine. The resultant solid residue was filtered, washed with water and recrystallized from ethanol to obtain compound **2**.

Yield: 65%; Brown crystalline powder; mp: 123-125 °C, IR (KBr): υ(cm^{-1}) 1,700 (C=O). LC-MS (ESI) m/z ≈ 378 (M^+, 8.28 %). ^1H NMR (DMSO-d_6, δ ppm): 2.18 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 6.60 (s, 1H, CH of benzofuran). Anal. Calcd. for $C_{12}H_{10}Br_2O_4$ (378.01): C, 38.13; H, 2.67; Found: C, 38.01; H, 2.59.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl) -2-oxo-1,2-dihydropyridine-3-carbonitrile (3):

A mixture of compound **2** (0.002 mol), ethyl cyanoacetate (0.002 mol), anhydrous ammonium acetate (0.016 mol) and 4-anisaldehyde (0.002 mol) in n-butanol (10 mL) was refluxed for 5 h. The reaction mixture was then concentrated to a third of its volume under reduced pressure. After cooling, the formed precipitated solid was filtered, air dried and recrystallized from isopropanol to obtain compound **3**.

Yield: 80%; Yellow crystalline powder; mp: 110-112 °C, IR (KBr): υ(cm^{-1}) 1,716 (C=O), 2,213 (C≡N) and 3,142 (NH). LC-MS (ESI) m/z ≈ 560 (M^+, 15 %). ^1H NMR (DMSO-d_6, δ ppm): 3.60 (s, 3H, OCH_3 of benzofuran), 3.75 (s, 3H, OCH_3 of benzofuran), 3.80 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.81 (s, 1H, CH of benzofuran), 6.12 (s, 1H, CH of pyridine), 6.53-7.46 (m, 4H, Ar-H), 8.81 (exch br s, 1H, NH). Anal. Calcd. for $C_{23}H_{16}Br_2N_2O_5$ (560.19): C, 49.31; H, 2.88; N, 5.00; Found: C, 49.12; H, 2.59; N, 4.75.

Synthesis of 2-chloro-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile (4):

A mixture of compound **3** (0.01 mol) and phosphorus pentachloride (0.01 mol) in phosphorus oxychloride (20 mL) was heated on a steam bath for 3 h. The mixture was poured on crushed ice. The isolated solid was filtered and recrystallized from methanol to afford compound **4**.
Yield: 75%; Brown crystalline powder; mp: 88-90 °C, IR (KBr): υ(cm^{-1}) 2,209 (C≡N). LC-MS (ESI) m/z ≈ 578 (M^+, 86.44 %), 580 (M+2, 61.86 %). ^1H NMR (DMSO-d_6, δ ppm): 3.55 (s, 3H, OCH_3 of benzofuran), 3.60 (s, 3H, OCH_3 of benzofuran), 3.75 (s, 3H, OCH_3 of 4-methoxyphenyl), 6.43 (s, 1H, CH of benzofuran), 9.11 (s, 1H, CH of pyridine), 7.13-7.81 (m, 4H, Ar-H). Anal. Calcd. for $C_{23}H_{15}Br_2ClN_2O_4$ (578.64): C, 47.74; H, 2.61; N, 4.84; Found: C, 47.65; H, 2.52; N, 4.75.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-hydrazinyl-4-(4-methoxyphenyl)nicotinonitrile (5):

A mixture of compound **4** (0.01 mol) and hydrazine hydrate (98%, 0.01 mol) in ethanol (20 mL) was heated under reflux for 6 h. The excess of solvent was removed under reduced pressure, and the resulting precipitate was filtered off, washed with ethanol and recrystallized from methanol to give compound **5**.

Yield: 75%; Orange crystalline powder; mp: 165-167 °C, IR (KBr): υ(cm^{-1}) 2,220 (C≡N), 3,147 (NH) and 3,243, 3,330 (NH_2). LC-MS (ESI) m/z ≈ 574 (86.44%). ^1H NMR (DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of benzofuran), 3.88 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.30 (s, 1H, CH of benzofuran), 6.91-7.40 (m, 4H, Ar-H), 8.31 (s, 1H, CH of pyridine), 8.52 (s, 1H, NH, exch br s), 8.79 (s, 2H, NH_2, exch br s). Anal. Calcd. for $C_{23}H_{18}Br_2N_4O_4$ (574.22): C, 48.11; H, 3.16; N, 9.76; Found: C, 47.95; H, 2.98; N, 9.68.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-(1,4-dioxo-3,4-di hydrophthalazin-2(1*H*)-yl)-4-(4-methoxyphenyl)nicotinonitrile (6):

A solution of compound **5** (0.01 mol) and phthalic anhydride (0.01 mol) in acetic acid (20 mL) was refluxed for 6 h. The reaction mixture was concentrated, allowed to cool and poured on cold water and obtained solid was filtered off and recrystallized from methanol to give compound **6**.
Yield: 65%; Yellow crystalline powder; mp: 200-202 °C, IR (KBr): υ(cm^{-1}) 1,689, 1,710 (2C=O), 2,220 (C≡N) and 3,079 (NH). LC-MS (ESI) m/z ≈ 704 (3.21%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.84 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.87 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.80 (s, 1H, CH of benzofuran), 6.91-7.88 (m, 8H, Ar-H), 8.34 (s, 1H, CH of pyridine), 8.55 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{31}H_{20}Br_2N_4O_6$ (704.32): C, 52.86; H, 2.86; N, 7.95; Found: C, 52.65; H, 2.79; N, 7.75.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl) -1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (7):

Compound **5** (0.005 mol) in DMF (20 mL) was refluxed for 12 h, and then allowed to cool. The solid product that precipitated on cooling was filtered off, dried and recrystallized from isopropanol to give compound **7**.

Yield: 80%; Yellow crystalline powder; mp: 170-172 °C, IR (KBr): υ(cm^{-1}) 3,155 (NH) and 3,205, 3,429 (NH_2). LC-MS (ESI) $[M-1]^+$ m/z ≈ 573 (20.37%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.62 (s, 1H, CH of benzofuran), 6.74-7.97 (m, 4H, Ar-H), 8.54 (s, 1H, CH of pyridine), 8.78 (s, 2H, NH_2, exch br s), 12.80 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{23}H_{18}Br_2N_4O_4$ (574.22): C, 48.11; H, 3.16; N, 9.76; Found: C, 47.92; H, 2.86; N, 9.65.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl) -2-(2-triacetylhydrazide)nicotinonitrile (8):

Compound **5** (0.002 mol) in acetic anhydride (10 mL) was heated under reflux for 1 h. After cooling, the solvent was concentrated under reduced pressure, and then the reaction mixture was poured into ice-water (20 mL) to give a solid precipitate which was filtered off and recrystallized from benzene to give compound **8**.

Yield: 60%; Yellow crystalline powder; mp: 80-82 °C, IR (KBr): υ(cm^{-1}) 1,685, 1,701, 1,720 (3C=O) and 2,226 (C≡N). LC-MS (ESI) m/z ≈ 700 (15.21%). ^{1}H NMR (DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.88 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.97 (s, 1H, CH of benzofuran), 6.54-7.87 (m, 4H, Ar-H), 8.21 (s, 1H, CH of pyridine). Anal. Calcd. for $C_{29}H_{24}Br_2N_4O_7$ (700.33): C, 49.74; H, 3.45; N, 8.00; Found: C, 49.65; H, 3.23; N, 7.85.

Synthesis of ethyl *N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)formohydrazonate (9):

A mixture of **5** (0.001 mol), and triethyl orthoformate (10 mL) was heated under reflux for 30 min. The reaction mixture was cooled then the solid was filtered off, dried, and recrystallized from ethanol to give compound **9**.

Yield: 90%; Brown crystalline powder; mp: 175-177 °C, IR (KBr): υ(cm^{-1}) 2,225 (C≡N), 3,182 (NH). LC-MS (ESI) m/z ≈ 630 (16.44%). ^{1}H NMR (DMSO-d_6, δ ppm): 1.40 (t, 3H, CH_3), 3.83 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.88 (s, 3H, OCH_3 of 4-methoxyphenyl), 3.91 (q, 2H, CH_2), 5.91 (s, 1H, CH of benzofuran), 6.77-7.81 (m, 4H, Ar), 8.58 (s, 1H, CH of pyridine), 8.62 (s, 1H, NH, exch br s), 8.84 (s, 1H, CH=N). Anal. Calcd. for $C_{26}H_{22}Br_2N_4O_5$ (630.28): C, 49.55; H, 3.52; N, 8.89; Found: C, 49.49; H, 3.41; N, 8.78.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-4-(4-methoxyphenyl)nicotinonitrile (10):

Acetylacetone (0.015 mol) was added to a solution of compound **5** (0.01 mol) in ethanol (30 mL). The reaction mixture was heated under reflux for 6 h. Solvent was then removed under reduced pressure and the residue was recrystallized from methanol to give compound **10**.

Yield: 70%; Black crystalline powder; mp: > 300 °C, IR (KBr): υ(cm^{-1}) 2,219 (C≡N). LC-MS (ESI) m/z ≈ 638 (10.21%). ^{1}H NMR (DMSO-d_6, δ ppm): 2.30 (s, 3H, CH_3), 2.91 (s, 3H, CH_3), 3.81 (s, 3H, OCH_3 of benzofuran), 3.83 (s, 3H, OCH_3 of benzofuran), 3.87 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.50 (s, 1H, CH of benzofuran), 6.50 (s, 1H, CH of pyrazole), 6.71-7.62 (m, 4H, Ar-H), 8.45 (s, 1H, CH of pyridine). Anal. Calcd. for $C_{28}H_{22}Br_2N_4O_4$ (638.31): C, 52.69; H, 3.47; N, 8.78; Found: C, 52.47; H, 3.28; N, 8.69.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl) -2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)nicotinonitrile (11):

Ethyl acetoacetate (0.015 mol) was added to a solution of compound **5** (0.01 mol) in ethanol (30 mL). The reaction mixture was heated under reflux for 6 h. Solvent was then removed under reduced pressure and the residue was recrystallized from benzene to give compound **11**.

Yield: 55%; Brown crystalline powder; mp: > 300 °C, IR (KBr): υ(cm^{-1}) 1,729 (C=O) and 2,228 (C≡N). LC-MS (ESI) m/z ≈ 640 (21.52%). ^{1}H NMR (DMSO-d_6, δ ppm): 1.29 (s, 3H, CH_3), 2.21 (s, 2H, CH_2 of pyrazole), 3.82 (s, 3H, OCH_3 of benzofuran), 3.84 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.84 (s, 1H, CH of benzofuran), 6.65-7.81 (m, 4H, Ar-H), 8.61 (s, 1H, CH of pyridine). Anal. Calcd. for $C_{27}H_{20}Br_2N_4O_5$ (640.28): C, 50.65; H, 3.15; N, 8.75; Found: C, 50.57; H, 2.89; N, 8.64.

Synthesis of *N*'-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)formohydrazide (12):

A solution of compound **5** (0.001 mol) in formic acid (25 mL) was heated under reflux for 30 min. The solid separated upon cooling was filtered off, washed with petroleum ether, dried, and recrystallized from ethanol to give compound **12**.

Yield: 65%; Brown crystalline powder; mp: > 300 °C, IR (KBr): υ(cm^{-1}) 1,689 (C=O) ,2,222 (C≡N) and 3,178 (NH). LC-MS (ESI) $[M+H_2O]^+$ m/z ≈ 620 (1.49%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.81 (s, 3H, OCH_3 of benzofuran), 3.82 (s, 3H, OCH_3 of benzofuran), 3.84 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.59 (s, 1H, CH of benzofuran), 6.81-7.88 (m, 4H, Ar-H), 8.23 (s, 1H, CH of CHO), 8.62 (s, 1H, CH of pyridine), 8.97 (s, 1H, NH, exch br s), 9.40 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{24}H_{18}Br_2N_4O_5$ (602.23): C, 47.86; H, 3.01; N, 9.30; Found: C, 47.75; H, 2.87; N, 9.15.

Synthesis of 2-chloro-*N*'- (3-cyano-6-(2,6-dibromo-4,7- dimethoxybenzofuran-5-yl)- 4-(4-methoxyphenyl)pyridin-2-yl) acetohydrazide (13):

Chloroacetyl chloride (0.001 mol) was added drop wise to a solution of compound **5** (0.001 mol) in dimethylformamide (20 mL) and stirred at room temperature for 1 h, then poured into ice-water. The solid was filtered off, dried, and recrystallized from methanol to give compound **13**.

Yield: 65%; Brown crystalline powder; mp: 195-197 °C, IR (KBr): υ(cm^{-1}) 1,729 (C=O), 2,226 (C≡N) and 3,006 (NH). LC-MS (ESI) m/z ≈ 650 (12.40%), $[M+2]^+$ m/z ≈ 652 (9%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3 of benzofuran), 3.84 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of 4-methoxyphenyl), 4.52 (s, 2H, CH_2 of methylene), 5.61 (s, 1H, CH of benzofuran), 6.72-7.91 (m, 4H, Ar-H), 8.71 (s, 1H, CH of pyridine), 8.81 (s, 1H, NH, exch br s), 9.32 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{25}H_{19}Br_2ClN_4O_5$ (650.70): C, 46.15; H, 2.94; N, 8.61; Found: C, 45.95; H, 2.82; N, 8.48.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-((2,4-dioxo-1,2-di hydroquinazolin-3(4*H*)-yl)amino)-4-(4-methoxyphenyl)nicotinonitrile (14):

A solution of compound **5** (0.01 mol) and isatoic anhydride (0.01 mol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool and the obtained solid was filtered off and recrystallized from ethanol to give compound **14**.

Yield: 85%; Brown crystalline powder; mp: 125-127 °C, IR (KBr): υ(cm^{-1}) 1,671, 1,700 (2C=O), 2,209 (C≡N) and 3,168 (NH). LC-MS (ESI) m/z ≈ 719 (14.25%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.78 (s, 1H, CH of benzofuran), 6.72-8.21 (m, 8H, Ar-H), 8.65 (s, 1H, CH of pyridine), 8.83 (s, 1H, NH, exch br s), 9.15 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{31}H_{21}Br_2N_5O_6$ (719.34): C, 51.76; H, 2.94; N, 9.74; Found: C, 51.69; H, 2.87; N, 9.67.

Synthesis of 6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl) -2-((4,5,6,7-tetrabromo-1,3-dioxoisoindolin-2-yl)amino)nicotinonitrile (15):

A solution of compound **5** (0.01 mol) and tetrabromo phthalic anhydride (0.01 mol) in ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool and the obtained solid was filtered off and recrystallized from methanol to give compound **15**.

Yield: 65%; Brown crystalline powder; mp: > 300 ^{0}C, IR (KBr): υ(cm^{-1}) 1,722 (2C=O), 2,222 (C≡N) and 3,164 (NH). LC-MS (ESI) $[M+H_2O]^+$ m/z ≈ 1037 (6.49%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3 of benzofuran), 3.83 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.84 (s, 1H, CH of benzofuran), 6.72-7.22 (m, 4H, Ar-H), 8.71 (s, 1H, CH of pyridine), 8.83 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{31}H_{16}Br_6N_4O_6$ (1019.91): C, 36.51; H, 1.58; N, 5.49; Found: C, 36.47; H, 1.48; N, 4.95.

Synthesis of 2-(2-(substituted)hydrazinyl)-6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile (16a, b):

A mixture of **5** (0.001 mol) and 2-chloro-5-nitro-benzaldehyde and/or 3-anisaldehyde (0.0015 mol) in glacial AcOH (30 mL) was heated under reflux for 6 h. The reaction mixture was cooled, and the solid was filtered off, washed with petroleum ether, dried, and recrystallized from methanol to give compounds **16a, b** respectively.

2-(2-(2-chloro-5-nitrobenzylidene)hydrazinyl)-6-(2,6-dibromo-4,7-dimethoxy benzofuran-5-yl)-4-(4-methoxyphenyl)nicotinonitrile (16a):

Yield: 65%; White crystalline powder; mp: 135-137 °C, IR (KBr): υ(cm^{-1}) 2,225 (C≡N) and 3,148 (NH). LC-MS (ESI) m/z ≈ 741 (10.52%), $[M+2]^+$ m/z ≈ 743 (7%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.81 (s, 3H, OCH_3 of benzofuran), 3.83 (s, 3H, OCH_3 of benzofuran), 3.84 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.74 (s, 1H, CH of benzofuran), 6.68-7.87 (m, 7H, Ar-H), 8.75 (s, 1H, CH of pyridine), 8.82 (s, 1H, CH=N), 8.91 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{30}H_{20}Br_2ClN_5O_6$ (741.77): C, 48.58; H, 2.72; N, 9.44; Found: C, 47.95; H, 2.52; N, 9.25.

6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-2-(2-(3-methoxybenzylidene) hydrazinyl)-4-(4-methoxyphenyl)nicotinonitrile (16b):

Yield: 68%; Brown crystalline powder; mp: 155-157 °C, IR (KBr): υ(cm^{-1}) 2,222 (C≡N) and 3,058 (NH). LC-MS (ESI) m/z ≈ 692 (14.21%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.82 (s, 3H, OCH_3 of benzofuran), 3.83 (s, 3H, OCH_3 of benzofuran), 3.85 (s, 3H, OCH_3 of 4-methoxyphenyl), 3.86 (s, 3H, OCH_3 of anisaldehyde), 5.84 (s, 1H, CH of benzofuran), 6.71-7.95 (m, 8H, Ar-H), 8.74 (s, 1H, CH of pyridine), 8.83 (s, 1H, CH=N), 8.95 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{31}H_{24}Br_2N_4O_5$ (692.35): C, 53.78; H, 3.49; N, 8.09; Found: C, 53.75; H, 3.36; N, 7.95.

Synthesis of *N'*-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)benzohydrazide (17):

A mixture of compound **5** (0.01 mol) and benzoyl chloride (0.015 mol) in dimethylformamide (25 mL) was heated under reflux for 5 h. The reaction mixture was cooled, poured onto ice. The separated solid was filtered off, washed with water, dried, and recrystallized from DMF to give compound **17**.

Yield: 75%; White crystalline powder; mp: 160-162 °C, IR (KBr): υ(cm^{-1}) 1,698 (C=O), 2,285 (C≡N) and 3,155 (NH). LC-MS (ESI) m/z ≈ 678 (23.85%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.83 (s, 3H, OCH_3 of benzofuran), 3.84 (s, 3H, OCH_3 of benzofuran), 3.86 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.52 (s, 1H, CH of benzofuran), 6.68-8.15 (m, 9H, Ar-H), 8.64 (s, 1H, CH of pyridine), 8.93 (s, 1H, NH, exch br s), 9.89 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{30}H_{22}Br_2N_4O_5$ (678.33): C, 53.12; H, 3.27; N, 8.26; Found: C, 52.98; H, 3.18; N, 8.11.

Synthesis of 2-(3-cyano-6-(2,6-dibromo-4,7-dimethoxybenzofuran-5-yl)-4-(4-methoxyphenyl)pyridin-2-yl)-*N*-phenylhydrazinecarbothioamide (18):

A mixture of compound **5** (0.01 mol) and the phenyl isothiocyanate (0.012 mol) in dioxane (20 mL) was heated under reflux for 4 h. The solid was filtered, dried, and recrystallized from ethanol to give compound **18**.

Yield: 70%; Black crystalline powder; mp: > 300 °C, IR (KBr): υ(cm^{-1}) 2,097 (C≡N) and 3,056 (NH). LC-MS (ESI) m/z ≈ 709 (11.25%). ^{1}H NMR (DMSO-d_6, δ ppm): 3.69 (s, 3H, OCH_3 of benzofuran), 3.75 (s, 3H, OCH_3 of benzofuran), 3.80 (s, 3H, OCH_3 of 4-methoxyphenyl), 5.71 (s, 1H, CH of benzofuran), 6.85-7.89 (m, 9H, Ar-H), 8.56 (s, 1H, CH of pyridine), 9.81 (s, 1H, NH, exch br s), 9.87 (s, 1H, NH, exch br s), 10.17 (s, 1H, NH, exch br s). Anal. Calcd. for $C_{30}H_{23}Br_2N_5O_4S$ (709.41): C, 50.79; H, 3.27; N, 9.87; Found: C, 50.68; H, 2.97; N, 9.75.

Biological activity

In-vitro antibacterial and antifungal activities:

Antimicrobial activity of the synthesized compounds **3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16a, 17** and **18** were screened in vitro against a panel of gram positive and gram negative bacterial pathogens, and fungi, in comparison with control drugs Thiophenicol (Thiamphenicol, Sanofiaventis, France) as an antibacterial agent, and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company. EIPICO) as an antifungal agent, by the agar diffusion technique [18, 19].

Compounds **3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15, 16a, 17** and **18** were individually tested against gram positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213), gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27953), and fungi (*Candida albicans* ATCC 10321, *Aspergillus niger* NRRL-363, *Fusarium solani* NRC15, *Fusarium oxysporium* NRC23, *Alternaria alternata* NRC43 and *Alternaria tenuissima* KM651985). All microorganisms used were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Centre, Giza, Egypt. The microorganisms were passaged at least twice to ensure purity and viability. The compounds were mounted on a paper disc prepared from blotting paper (5 mm diameter) on a concentration of 100µg/5µL DMSO/disc.

Thiophenicol and Treflucan were used as positive controls for antibacterial and antifungal activity in a concentration of 50 µg/disc. DMSO showed no inhibition zone used as a negative control.

Preparation of agar plates

Agar plates were prepared by using 100 mL of suspension containing 1 x10^8 CFU/mL of pathological tested bacteria and 1 x10^6 CFU/mL of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA), respectively. After the media had cooled and solidified, the discs were applied on the inoculated agar plates and incubated for 24 h at 30 ºC for bacteria and 72 h at 28 ºC for fungi.

After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition around the disc in millimeters (mm) and compared with that of the controls. The observed zones of inhibition against the test microorganisms are presented in (Table 1).

Minimal inhibitory concentration (MIC) measurement

The active compounds (having inhibition zones (IZ) ≥ 9 mm) was then evaluated for its minimal inhibitory concentration MIC. The final concentrations tested were; 50, 25, 12.5, 6.25, 3.13 µg. The lowest concentration showing inhibition zone around the disc was taken as the minimum inhibitory concentration (MIC).

Structural-Activity Relationship

The activity of the tested compounds could be correlated to structure variation and modifications. By investigating the variation in the selectivity of the tested compounds it was revealed that: (1) Cyclization of some compounds such as **15, 6** and **10** increase their activities against gram positive and gram negative bacteria. (2) The presence of Cl in some compounds such as **4** and **13** results in moderate activities against gram positive bacteria. (3) On the other hand the presence of Cl in compound **4** results in good inhibitory activity against four of the tested fungi compared to the standard antifungal drug (Treflucan).

CONCLUSION

A novel series of some new benzofuran derivatives were synthesized and evaluated as antibacterial and antifungal agents. Antibacterial activity results exhibited that compounds **15, 6, 10** and **18** exhibited a wide range of antibacterial activity against gram positive and gram negative bacteria. On the other hand, compound **4** exhibited good inhibitory activities against four of the tested fungi compared to the standard antifungal drug (Treflucan), followed by compound **3**.

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CONFLICT OF INTEREST

No conflict of interest is there to declare.

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