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Original article

Convenient synthesis and antimicrobial evaluation of some novel 2-substituted-3-methylbenzofuran derivatives

Hatem A. Abdel-Aziz [a,*], Amal A.I. Mekawey [b], Kamal M. Dawood [c]

[a] Department of Applied Organic Chemistry, National Research Centre, Dokki, Cairo 12622, Egypt
[b] Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt
[c] Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt

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ABSTRACT

The reaction of 3-methylbenzofuran-2-carbohydrazide (**1**) with l-phenyl-2-bromoethanone (**2a**) or 2-chloro-1-(4-chlorophenyl)ethanone (**2b**) afforded (*Z*)-1,2-di[(3-methylbenzofuran-2-carbohydrazido]-1-arylethenes **5a** and **5b**, respectively. Single crystal X-ray analyses of compound **5a** proved that the reaction proceeds in 2:1 molar ratio and ruled out the other possible structures 1,3,4-oxadiazine derivative **6** or *E*-isomer **7**. Furthermore, both of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) and 3-methyl-2-benzofuranoyl chloride (**15**) were used as starting materials for the synthesis of several compounds, such as pyrazoles **10** and **14**, oxime **11**, hydrazones **12a**, **b** and 3,1-bezoxazine **19**. The newly synthesized compounds were tested for their antimicrobial activity against five fungal species and four bacterial species also their minimum inhibitory concentration (MIC) against most of test organisms was performed. Some of these compounds exhibited a significant antimicrobial activity.

1. Introduction

In the recent years, benzofuran derivatives have attracted much interest due to their useful biological and pharmacological properties [1–3], such as anticonvulsant [4,5], anti-inflammatory [4,5], antitumor [6,7] and antihistaminic [8] activities. They were also found to be useful as antifungal [9,10], anthelmintic [11] and antihyper-glycemic [12] agents. In addition, the benzofuran derivative *Amiodarone* is one of the most important benzofuran-based synthetic pharmaceutics, it is a highly effective antiarrhythmic agent and used in the treatment of both ventricular and supraventricular arrhythmias [13]. The recently developed *R*-(−)-1-(benzofuran-2-yl)-2-propylaminopentane, (−)-BPAP, is hundred times more potent than the well-known antidepressant agent (−)-*Deprenyl* in drug therapy of major depression with unusual safeness [14]. On the other hand, C-2-substituted benzofurans constitute a structural unit of a series of natural products [15–17] such as *Cicerfuran*, antifungal benzofuran derivative, was first obtained from the roots of wild species of chickpea, *Cicer bijugum*, reported to be a major factor in the defense system against Fusarium wilt [18]. Furthermore, *1′S*-Bufuralol is a non-selective β-adrenoceptor antagonist, it is a good substrate of cytochrome P450 (CYP) and undergoes enantioselective and regioselective oxidations in liver [19] (Fig. 1).

Encouraged by our recently reported results on the preparation of new biologically active benzofuran derivatives [4,5,20–24], we herein continue our research work on the synthesis of some new 2-substituted-3-methylbenzofuran derivatives to evaluate their antimicrobial activity.

2. Results and discussion

2.1. Chemistry

The reaction of 3-methylbenzofuran-2-carbohydrazide (**1**) with l-phenyl-2-bromoethanone (**2a**) in refluxing ethanol afforded a single product based on TLC. The elemental analysis and mass spectrum of the reaction product proved that the reaction proceeded in 2:1 molar ratio (**1**:**2a**), compatible with the molecular formula $C_{28}H_{24}N_4O_4$. Spectroscopic data (IR, ^1H and ^{13}C NMR) and X-ray single crystal analysis of the reaction product confirmed its structure as (*Z*)-1,2-di[(3-methylbenzofuran-2-carbohydrazido)]-1-phenylethene (**5a**) (Scheme 1) and ruled out the other possible structure **6** [25] (Fig. 2). Essential bond lengths of **5a** are listed in Table 1. 3-Methylbenzofuran-2-carbohydrazide (**1**) reacted similarly with 2-chloro-1-(4-chlorophenyl)ethanone (**2b**) under the

* Corresponding author. Tel.: +20 2 3371635; fax: +20 2 37601877.
E-mail address: hatem_741@yahoo.com (H.A. Abdel-Aziz).

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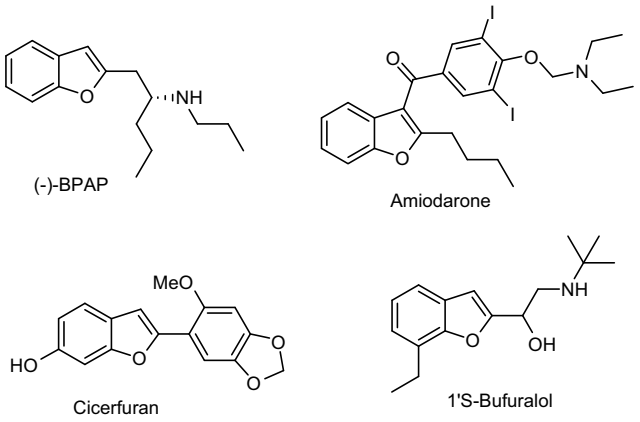


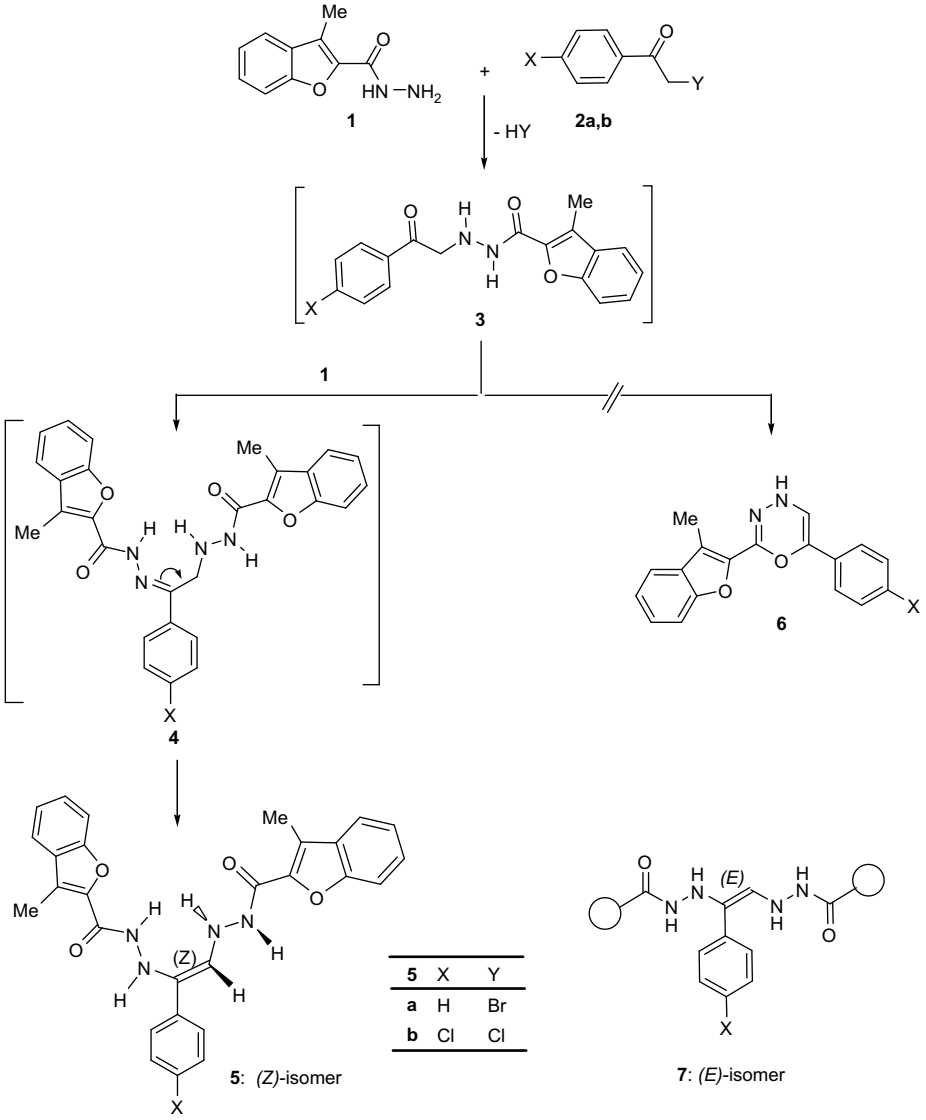
Fig. 1. Structures of Amiodarone, (−)-BPAP, Cicerfuran and Bufuralol.

same reaction condition to give (*Z*)-1,2-di[(3-methylbenzofuran-2-oyl)hydrazino]-1-(4-chlorophenyl)ethene (**5b**) (Scheme 1).

Single crystal X-ray analysis of compound **5a** showed that its structure exists predominantly in the *Z*-configuration and not the *E*-one **7**, which may be attributed to the hindrance factor of aryl groups. Also, intra- and intermolecular hydrogen bonding may play an important role in directing the molecular assembly of structure **5** in its solid state [26,27] as outlined in Fig. 3. Distances and angles of hydrogen bonds are listed in Table 2.

The mechanistic pathway of latter reaction is assumed to proceed via a preliminary formation of the non-isolable intermediates **3a**, **b** followed by its reaction with another molecule of hydrazide **1** with elimination of water molecule to form the intermediate **4** which was consequently tautomerized into compounds **5a**, **b** as final products. The ^{1}H NMR spectrum of **5a** revealed two doublets at δ 7.63 and 8.01 due to H24 and H6, respectively, with $J = 8.1$ Hz. When D_2O was added to the DMSO-d_6 in the NMR tube of compound **5a**, the doublet signal of H24 at δ 7.63 was changed to a singlet one and the doublet one at δ 8.01 of H6 was disappeared. Single crystal X-ray analysis of compound **5a** showed unequivocally that H24 and H6 are *trans* to each other.

3-(3-Methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) was prepared from the reaction of 2-bromo-1-(3-methylbenzofuran-2-yl)ethanone (**8**) with potassium cyanide in ethanol at ambient temperature. The mp, IR, mass, and ^{1}H NMR spectra of compound **9** were found to be identical with that reported by us [23]. The



Scheme 1.

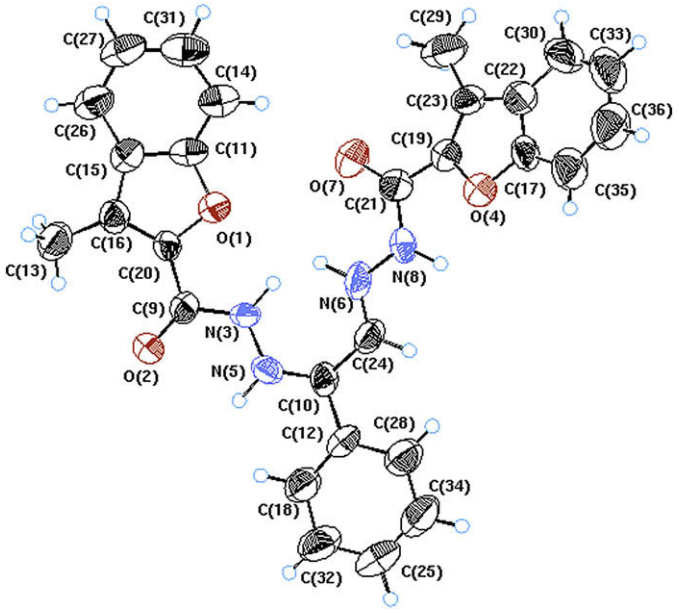


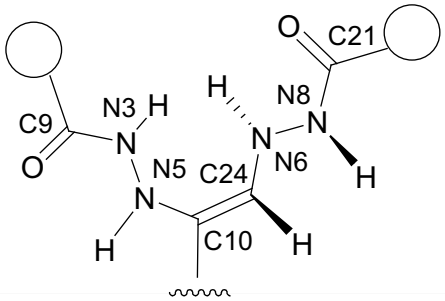
Fig. 2. X-Ray structure of compound **5a**.

reaction of compound **9** with phenylhydrazine in refluxing ethanol afforded the corresponding 5-amino-3-(3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole (**10**) (Scheme 2). The IR spectrum of **10** showed absorption bands at 3449 and 3233 cm^{-1} due to NH_2 group whereas its ^1H NMR appeared D_2O-exchangeable broad signal of NH_2 group at δ 3.45. The mass spectrum of **10** revealed a peak at *m*/*z* 289 corresponding to its molecular ion.

Next, the reaction of propanenitrile **9** with nitrous acid afforded 3-(3-methylbenzofuran-2-yl)-2-hydroximoyl-3-oxopropanenitrile (**11**) as shown in Scheme 2. Its IR spectrum revealed absorption band at 1643 cm^{-1} assignable to carbonyl group in addition to broad band of OH function at 3263 cm^{-1}. Interestingly, the IR spectrum of **11** was free of nitrile absorption band due to the presence of both nitrile function and hydroximoyl group attached to the same carbon, in fact such cases are already known [28]. ^{13}C NMR spectrum of compound **11** exhibited signal at δ 109.0 due to the carbon of nitrile function.

Propanenitrile derivative **9** reacted also with diazonium chlorides of 4-toluidine or 4-chloroaniline in cold ethanol in the presence of sodium acetate and afforded high yields of the

Table 1
Characteristic bond length [Å] of **5a**.



N3–N5	1.351 (3)
N3–C9	1.377 (4)
N5–C10	1.293 (3)
N6–N8	1.364 (3)
N6–C24	1.275 (4)
N8–C21	1.358 (4)
C10–C24	1.447 (4)

corresponding hydrazones **12a**, **b**, respectively. The IR spectra of the latter products showed, in each case, absorption bands around 3210, 2220 and 1620 cm^{-1} corresponding to hydrazone NH, nitrile and carbonyl groups, respectively.

Moreover, 3-ethoxy-2-[(3-methylbenzofuran-2-yl)carbonyl]acrylonitrile (**13**) was synthesized by neat refluxing of equimolar quantities of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) and triethyl orthoformate. The ^1H NMR spectrum of compound **13** revealed a triplet and quartet signals at δ 0.86 and 3.7 due to the ethoxy protons in addition to two singlets at δ 2.59 and 8.1 due to 3-methyl and C═CH– protons, respectively. Reaction of compound **13** with hydrazine hydrate in absolute ethanol afforded a compound identified as 3-(3-methylbenzofuran-2-yl)-1*H*-pyrazole-4-carbonitrile (**14**) (Scheme 2). The IR spectrum of the latter compound revealed two absorption bands at 3125 and 2230 cm^{-1} assignable to NH function and nitrile group, respectively and its ^1H NMR spectrum displayed a D_2O-exchangeable singlet at δ 13.9 due to pyrazole NH and characteristic singlet at δ 8.7 due to H-4 of pyrazole moiety in addition to a multiplet in the region δ 7.3–7.72 of four aromatic protons.

Furthermore, treatment of the hydrazide **1** with 3-methyl-2-benzofuranoyl chloride (**15**) in pyridine afforded the bis-benzofuranoylhydrazide derivative **16** as shown in Scheme 3. The structure of the latter product was assigned on the basis of its

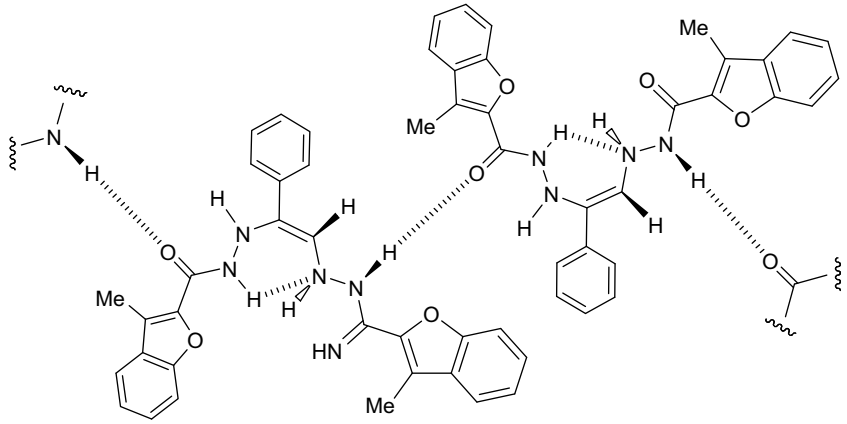


Fig. 3. Part of the hydrogen bonding scheme in the structure of **5a**.

Table 2
Intra- and intermolecular hydrogen bonds of **5a**.

Type, D–H···A	D–H	H···A	D···A	<(DHA)
Intramolecular				
N3–H3···N6	0.960(2)	1.910	2.658	132.9
Intermolecular				
N8–H8···O2	0.960(2)	2.145(2)	3.066	157.9

Distances (D–H, H···A, D···A) are given in Å, angles in °, D: donor, A: acceptor.

elemental analysis and spectral data (IR, ^{1}H and ^{13}C NMR). Similarly, the reaction of anthranilic acid with 3-methyl-2-benzofuranoyl chloride (**15**) in refluxing pyridine afforded 2-(3-methylbenzofuran-2-carboxamido)benzoic acid (**18**). Our attempts to synthesize compound **18** by reaction of the ester **20** with anthranilic acid under different reaction conditions were failed. The IR spectrum of compound **18** showed absorption bands at 3171 and at 1697, 1659 cm^{-1} due to one NH and two carbonyl functions, respectively, whereas its mass spectrum showed a peak at *m*/*z* 295 corresponding to its molecular ion.

Compound **18** was cyclized by anhydrous sodium acetate in acetic anhydride with heating at 140 °C to afford 2-(3-methylbenzofuran-2-yl)-4*H*-3,1-benzoxazin-4-one (**19**) (Scheme 3). The ^{1}H NMR spectrum of compound **23** showed the disappearance of signals due to protons of amide and acid functions and its mass spectrum revealed a peak at *m*/*z* 277 corresponding to its molecular ion.

2.2. Biological activity

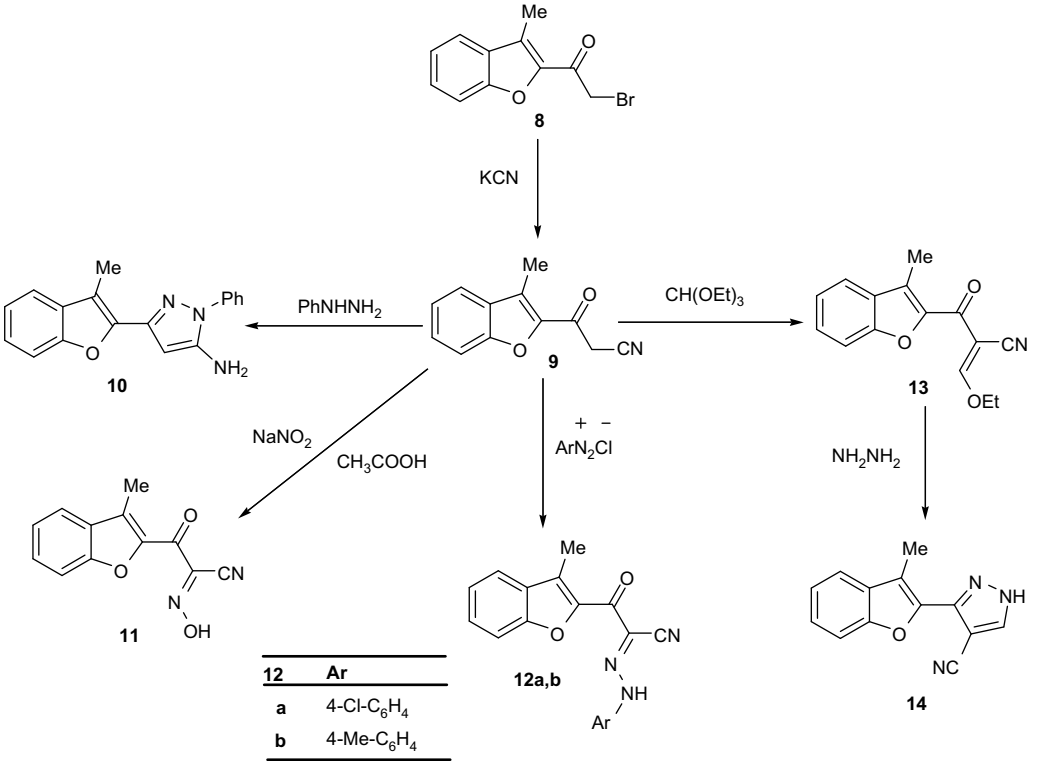
All synthesized compounds were screened for their antibacterial and antifungal activities at 100 μg concentration. Some of our compounds showed excellent antimicrobial activities with respect to the control drugs. The results of the antifungal and antibacterial activities are shown in Tables 3 and 4, respectively. The results revealed that most of the synthesized compounds showed variable degrees of inhibition against the tested microorganisms. Susceptibilities of the fungal and bacterial isolates to our synthesized benzofuran derivatives were investigated by measuring their inhibitory effect on the growth of microorganisms compared to the solvent used.

2.2.1. Antifungal activity

The results obtained from the present study recorded a remarkable difference in the antifungal effect of compounds **13**, **14**, **16** and **19**. The inhibition zone of the latter compounds ranged from 5 mm against *Aspergillus niger* to 30 mm against *Syncephalastrum racemosum*. Compounds **12b** and **18** showed weak antifungal activities against tested organisms where *S. racemosum* was the most susceptible strain (22 mm) followed by *Candida albicans* (20 mm), *Penicillium italicum* (13 mm) and *Aspergillus fumigatus* (12 mm). In addition, compound **12a** showed 16 mm inhibition against *C. albicans* (Table 3). Alternatively, compounds **5b**, **10** and **11** recorded different effects where their inhibition of fungal growth ranged from 5 mm against *A. niger* to 18 mm against *C. albicans*. *P. italicum* and *S. racemosum* showed high ability to resist the latter compounds. Compound **5a** exhibited no ability to inhibit the growth of any fungal species (Table 3).

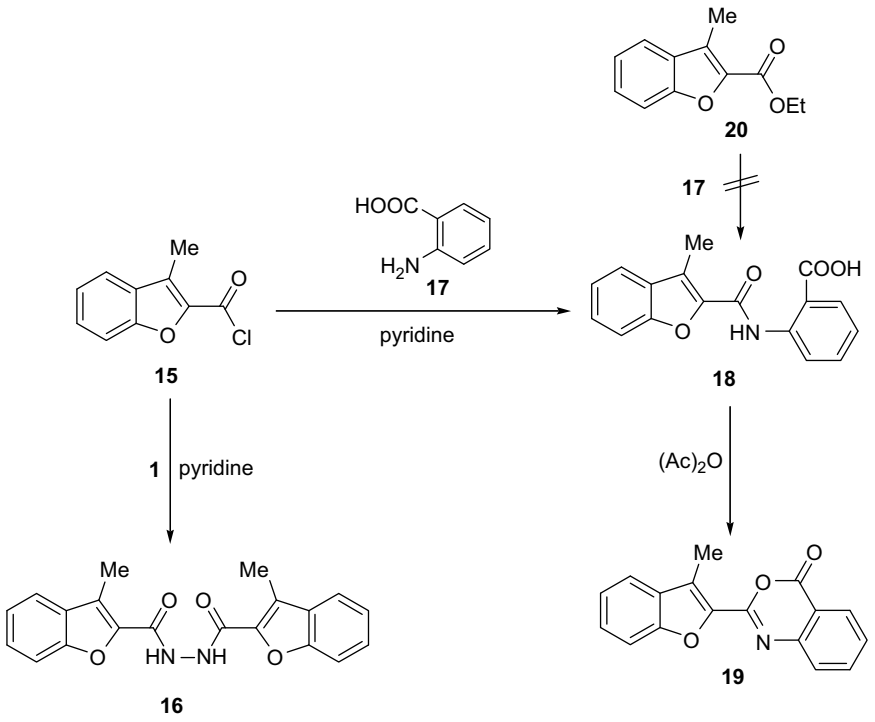
2.2.2. Antibacterial activity

Compounds **12b** and **18** showed moderate effects against *Staphylococcus aureus* and *Bacillus subtilis* whereas they revealed no effect against *Pseudomonas aeruginosa* and *Escherichia coli* (Table 4). Compound **5a** showed moderate activity against all bacterial species (inhibition zone varied from 5 mm against *E. coli* to 15 mm against *S. aureus*). On the other hand, compound **12a** showed weak activity against *S. aureus* (5 mm). In addition, data in Table 4 revealed that Gram-positive bacteria were highly susceptible



12	Ar
a	4-Cl-C_6H_4
b	4-Me-C_6H_4

Scheme 2.



Scheme 3.

where *B. subtilis* was the most susceptible strain against compound **18** (12 mm) while *S. aureus* showed weak effect against compound **16** (3 mm). Furthermore, compounds **5b**, **10** and **11** showed ability to inhibit the growth of all bacterial species except *P. aeruginosa* (no inhibition) (Table 4).

2.2.3. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized compounds against highly inhibited organisms is reported in Table 5. Compounds **5a** and **5b** revealed high MIC (500 μg/ml) against *S. aureus* and *A. fumigatus*, respectively. On the other hand, compounds **12b**, **14** and **16** exhibited low MIC (50 μg/ml) against *C. albicans*, *A. fumigatus*, *P. italicum* and *S. racemosum*. Compound **11** showed MIC 125 μg/ml against *B. subtilis*. Additionally, compounds **10**, **12a**, **13**, **18** and **19** exhibited MIC 250 μg/ml against *B. subtilis*, *C. albicans*, *A. niger* and *S. racemosum* (Table 5).

In conclusion, eleven benzofuran-based compounds were synthesized and screened for their antimicrobial activity as well as their MIC against all test organisms. The highest antifungal and antibacterial activities were showed by ethoxymethylene derivative **13** and ethylene **5a**, respectively. The presence of pyrazole moiety beside benzofuran ring in compounds **10** and **14** was found to be essential for their high antifungal and antibacterial activities. The significant antimicrobial activity of **5a** and **16** may be due to the presence of two benzofuran moieties in addition to hydrazide function in both of them. The MIC of compounds **5a**, **10**, **13**, **14** and **16** are 500, 250, 250, 50, 50 μg/ml, respectively.

3. Experimental

3.1. Chemistry

3.1.1. General

Melting points were measured on a Gallenkamp apparatus. IR spectra were recorded on Shimadzu FT-IR 8101PC infrared spectrophotometer. NMR spectra were determined in DMSO-d_6 at

Table 3
In vitro antifungal activity of the synthesized compounds.

Compound	Inhibition zone [mm]				
	Aspergillus fumigatus	*Aspergillus niger*	*Penicillium italicum*	*Syncephalastrum racemosum*	*Candida albicans*
5a	–	–	–	–	–
5b	10	5	–	6	–
10	10	7	–	–	9
11	8	12	–	–	18
12a	–	–	–	–	16
12b	12	–	–	–	20
13	19	18	10	22	10
14	20	6	–	30	12
16	4	13	12	18	6
18	–	–	13	22	–
19	8	5	6	18	7
Terbinafin	30	25	20	35	30

Table 4
In vitro antibacterial activity of the synthesized compounds.

Compound	Inhibition zone [mm]			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Escherichia coli*
5a	15	6	14	5
5b	4	–	4	3
10	4	–	25	5
11	15	–	20	8
12a	5	–	–	–
12b	4	–	6	–
13	13	–	7	–
14	10	–	18	–
16	3	–	12	–
18	3	–	12	–
19	4	8	8	–
Amoxicilline	30	20	25	30

Table 5
Minimum inhibitory concentration (MIC).

Compound[a]	Microorganism	MIC [μg/ml]
5a	*Staphylococcus aureus*	500
5b	*Aspergillus fumigatus*	500
10	*Bacillus subtilis*	250
11	*Bacillus subtilis*	125
12a	*Candida albicans*	250
12b	*Candida albicans*	50
13	*Aspergillus niger*	250
14	*Aspergillus fumigatus*	50
	Syncephalastrum racemosum	50
16	*Penicillium italicum*	50
	Syncephalastrum racemosum	50
18	*Syncephalastrum racemosum*	250
19	*Syncephalastrum racemosum*	250

[a] Terbinafin and Amoxicilline exhibited 50 μg/ml against all MIC measured organisms.

300 MHz (^{1}H NMR) and at 75 MHz (^{13}C NMR) on a Varian Mercury VX 300 NMR spectrometer using TMS as an internal standard. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 eV. Elemental analysis was carried out at the Microanalytical Center of Cairo University. 3-Methyl-2-benzofurancarbohydrazide (**1**) [29], 2-bromo-1-(3-methylbenzofuran-2-yl)ethanone (**8**) [30], 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) [23], 3-methyl-2-benzofuranoyl chloride (**13**) [31] and ethyl 3-methyl-2-benzofurancarboxylate (**20**) [32] were prepared by the reported methods. 2-Bromo-1-phenylethanone **2a** and 2-chloro-1-(4-chlorophenyl)ethanone **2b** were used as commercially received.

*3.1.2. General procedure for the synthesis of (Z)-1,2-di[(3-methylbenzofuran-2-carbohydrazido)]-1-arylethene **5a**, **b***

To a solution of 3-methyl-2-benzofurancarbohydrazide (**1**) (1.9 g, 10 mmol) in ethanol (50 ml), 2-bromo-1-phenylethanone **2a** or 2-chloro-1-(4-chlorophenyl)ethanone **2b** (5 mmol) was added. The reaction was refluxed for 7 h, then to cool to room temperature. The formed solid was filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford compounds **5a** and **5b**, respectively.

*3.1.2.1. (Z)-1,2-Di[(3-methylbenzofuran-2-carbohydrazido)]-1-phenylethene (**5a**).* Pale yellow crystals, yield (67%); mp 292–294 °C; IR (KBr) ν_{max}/cm^{-1}: 3450–3139 (4NH), 1688, 1658 (2C=O), 1602 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.61 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.31–7.55 (m, 9H, ArH), 7.63 (d, 1H, J = 8.1 Hz, C=CH–), 7.71–7.81 (m, 4H, ArH), 8.01 (d, 1H, D_2O exchangeable, J = 8.1 Hz, NH), 8.86 (s, 1H, D_2O exchangeable, NH), 12.39 (s, 1H, D_2O exchangeable, NH), 14.33 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6) δ: 8.7, 8.8, 111.6, 112.6, 120.9, 121.3, 123.2, 123.5, 123.8, 124.4, 126.5, 127.5, 127.9, 128.6, 128.9, 129, 129.2, 136.7, 140.9, 141.5, 142.8, 153, 153.4, 155.7, 156.7; MS m/z (%): 481 (M^+ + 1, 30.7), 480 (M^+, 33.0), 291 (6.8), 159 (100), 116 (22.5), 77 (28.7). Anal. Calcd for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.86; H, 4.92; N, 11.79%.

*3.1.2.2. (Z)-1,2-Di[(3-methylbenzofuran-2-carbohydrazido)]-1-(4-chlorophenyl)ethene (**5b**).* Pale yellow crystals, yield (63%); mp >300 °C; IR (KBr) ν_{max}/cm^{-1}: 3157 (4NH), 1680, 1658 (2C=O), 1600 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.62 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 7.36–7.65 (m, 5H, ArH), 7.66 (d, 1H, D_2O exchangeable, J = 8.4 Hz, C=CH–), 7.77–7.83 (m, 4H, ArH), 8.02 (d, 1H, D_2O exchangeable, J = 8.4 Hz, NH), 8.90 (s, 1H, D_2O exchangeable, NH), 12.51 (s, 1H, D_2O exchangeable, NH), 14.33 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 516 (M^+ + 2, 29.8), 515 (M^+ + 1, 31.2), 514 (M^+, 97.9), 291 (9.5), 159 (100), 116 (26.3). Anal. Calcd for $C_{28}H_{23}ClN_4O_4$: C, 65.31; H, 4.50; N, 10.88. Found: C, 65.18; H, 4.68; N, 10.81%.

*3.1.2.3. X-ray crystallography of compound **5a**.* The single crystal X-ray measurement was made using maXus (Bruker Nonius, Delft & MacScience, Japan) [33]. Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator were used for data collection. Crystal data for compound **5a**: $C_{28}H_{24}N_4O_4$, M_r, 480.524; system, monoclinic; Space group, $P2_1/c$; unit cell dimensions, a = 7.6399 (3) Å, b = 22.4018 (10) Å, c = 14.0064 (7) Å, α = 90.00°, β = 99.357 (3)°; V = 2365.3 (2) Å^3; Z = 4; D_x = 1.349 mg m^{-3}; θ range for data collection, 2.910–19.980°; μ(Mo Kα), 0.09 mm^{-1}; T, 298 K; measured reflections, 3908; independent reflections, 2397; observed reflections, 1325; R_{int}, 0.030; R (all), 0.093; wR (ref), 0.094; wR (all), 0.105; S (ref), 1.778; S (all), 1.795; Δ/σ_{max}, 0.012; $\Delta\rho_{max}$, 0.31 e Å^3, $\Delta\rho_{min}$, −0.43 e Å^3. Crystallographic data for the structure **5a** has been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 700682. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk].

*3.1.3. 3-(3-Metylbenzofuran-2-yl)-3-oxopropanenitrile (**9**)*

To a solution of 2-bromo-1-(3-methylbenzofuran-2-yl)ethanone (**8**) (5.06 g, 50 mmol) in absolute ethanol (30 ml) was added a solution of potassium cyanide (1.3 g, 50 mmol in 5 ml water) with stirring. The reaction mixture was stirred at room temperature for further 4 h, then diluted with water. The solid that precipitated was filtered off, washed with water, dried and finally recrystallized from ethanol to afford a product identical in all respects (mp, mixed mp, IR, mass and ^{1}H NMR) with that we reported in previous study [23], mp. 115–117 °C.

*3.1.4. 5-Amino-3-(3-methylbenzofuran-2-yl)-1-phenyl-1H-pyrazole (**10**)*

A mixture of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) (1.0 g, 5 mmol) and phenylhydrazine (0.54 g, 5 mmol) in ethanol (20 ml) was refluxed for 5 h, then left to cool to room temperature. The precipitated product was collected by filtration, washed with ethanol and dried. Recrystallization from ethanol afforded the pyrazole derivative **10**. Yield (68%); mp 150–152 °C; IR (KBr) ν_{max}/cm^{-1}: 3449, 3233 (NH_2), 1620 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.58 (s, 3H, CH_3), 3.45 (s, 1H, D_2O exchangeable), 6.42–7.65 (m, 10H, ArH); ^{13}C NMR (DMSO-d_6) δ: 9.1, 111.8, 121.4, 123.2, 124.2, 127.7, 128.3, 128.7, 129.0, 129.5, 142.0, 153.5, 161.1; MS m/z (%): 289 (M^+, 12.8), 253 (11.3), 176 (100), 159 (22.9), 131 (86.7), 102 (44.9), 77 (51.3), 51 (80.9). Anal. Calcd for $C_{18}H_{15}N_3O$: C, 74.72; H, 5.23; N, 14.52. Found: C, 74.96; H, 5.13; N, 14.30%.

*3.1.5. 3-(3-Methylbenzofuran-2-yl)-2-hydroximoyl-3-oxopropanenitrile (**11**)*

To a stirred cold solution of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) (1.99 g, l0 mmol) in glacial acetic acid (30 ml), cold solution of sodium nitrite (0.7 g, 10 mmol) in water (10 ml) is added drop-wise with stirring at such a rate that the temperature remains in the range 0–5 °C over a period of 30 min The mixture is stirred for extra 30 min and then allowed to stand for 4 h, during which time it warms up to room temperature. The solid that precipitated was collected, washed with water and dried. Recrystallization from EtOH/DMF afforded 89% yield of compound **11**; mp 205–207 °C; IR (KBr) ν_{max}/cm^{-1}: 3263 (OH), 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.57 (s, 3H, CH_3), 3.50 (br s, D_2O exchangeable, 1H, OH), 7.39–7.44 (m, 1H, ArH), 7.57–7.67 (m, 2H, ArH), 7.89 (d, 1H, ArH, J = 7.83 Hz); ^{13}C NMR (DMSO-d_6) δ: 9.8, 109, 112.1, 122.2, 123.8, 128.3, 128.4, 129.4, 132.9, 145.8, 154.1, 175.2; MS m/z (%): 228 (M^+, 16.7), 211 (31.1), 159 (100), 103 (39.1), 77 (54.9), 51 (57.4). Anal. Calcd for $C_{12}H_8N_2O_3$: C, 63.16; H, 3.53; N, 12.28. Found: C, 62.95; H, 3.45; N, 12.43%.

*3.1.6. General procedure for the synthesis of 3-(3-methylbenzofuran-2-yl)-2-(4-arylhydrazono)-3-oxopropanenitrile **12a**, **b***

To a stirred cold solution of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) (1.99 g, l0 mmol) in ethanol (30 ml) and sodium acetate trihydrate (2 g), was added the appropriate diazonium chloride solution (20 mmol) portion-wise over a period of 30 min at 0–5 °C. After complete addition, the reaction mixture was stirred for further 3 h at 0–5 °C. The solid that precipitated was collected, washed with water and dried. Recrystallization from EtOH/DMF afforded the corresponding hydrazone **12a**, **b**, respectively.

*3.1.6.1. 3-(3-Methylbenzofuran-2-yl)-2-(4-chlorophenylhydrazono)-3-oxopropanenitrile (**12a**).* Yield (85%); mp 203–205 °C (DMF/H_2O); IR (KBr) ν_{max}/cm^{-1}: 3217 (NH), 2222 (C≡N), 1651 (C═O), 1543 (C═N); ^1H NMR (DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 7.19–7.54 (m, 8H, ArH), 15.11 (s, 1H, D_2O exchangeable, NH); MS *m*/*z* (%): 339 ($M^+ + 2$, 20.1), 338 ($M^+ + 1$, 31.0), 337 (M^+, 89.8), 159 (100). Anal. Calcd for $C_{18}H_{12}ClN_3O_2$: C, 64.01; H, 3.58; N, 12.44. Found: C, 64.19; H, 3.35; N, 12.27%.

*3.1.7. 3-(3-Methylbenzofuran-2-yl)-2-(4-tolylhydrazono)-3-oxopropanenitrile (**12b**)*

Yield (83%); mp 177–179 °C; IR (KBr) ν_{max}/cm^{-1}: 3209 (NH), 2214 (C≡N), 1620 (C═O), 1542 (C═N); ^1H NMR (DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 7.24–7.87 (m, 8H, ArH), 12.48 (br s, 1H, NH); MS *m*/*z* (%): 317 (M^+, 46.2), 159 (100). Anal. Calcd for $C_{19}H_{15}N_3O_2$: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.14; H, 4.49; N, 13.07%.

*3.1.8. 3-Ethoxy-2-[(3-methylbenzofuran-2-yl) carbonyl]acrylonitrile (**13**)*

A mixture of 3-(3-methylbenzofuran-2-yl)-3-oxopropanenitrile (**9**) (1.99 g, l0 mmol) and triethyl orthoformate (1.5 g, 10 mmol) was refluxed for 2 h, then left to cool, the resulting brown precipitate was collected by filtration, washed with ethanol, dried and finally recrystallized from ethanol to afford compound **13** in 65% yield; mp 158–160 °C; IR (KBr) ν_{max}/cm^{-1}: 3402 (NH_2), 2214 (C≡N), 1682 (C═O), 1589 (C═N); ^1H NMR (DMSO-d_6) δ: 0.86 (t, 3H, CH_3, J = 7.2 Hz), 2.59 (s, 3H, CH_3), 3.7 (q, 2H, CH_2, J = 7.2 Hz), 7.26–7.68 (m, 4H, ArH), 8.1 (s, 1H, CH); MS *m*/*z* (%): 254 (M^+, 5.8), 227 (45.0), 210 (21.2), 199 (22.0), 159 (100), 131 (74.8), 105 (52.1), 77 (98.2), 51 (78.8). Anal. Calcd for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.36; H, 5.30; N, 5.74%.

*3.1.9. 3-(3-Methylbenzofuran-2-yl)-1H-pyrazole-4-carbonitrile (**14**)*

A mixture of 3-ethoxy-2-[(3-methylbenzofuran-2-yl)carbonyl]acrylonitrile (**13**) (1.27 g, 5 mmol) and hydrazine hydrate (1 ml, 80%) in ethanol (20 ml) was refluxed for 6 h, then cooled. The precipitated product was collected by filtration, washed with ethanol and dried. Recrystallization from ethanol afforded the pyrazole **14** in 62% yield; mp 168–170 °C; IR (KBr) ν_{max}/cm^{-1} 3125 (NH), 2230 (C≡N), 1628 (C═O); ^1H NMR (DMSO-d_6) δ: 2.52 (s, 3H, CH_3), 7.31–7.43 (m, 2H, ArH), 7.61 (d, 1H, ArH, J = 7.96 Hz), 7.72 (d, 1H, ArH, J = 7.35 Hz), 8.74 (s, 1H, pyrazole), 13.9 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6) δ: 8.7, 88.9, 111.2, 114.1, 120.2, 121.0, 123.1, 125.6, 126.3, 129.5, 153.4; MS *m*/*z* (%): 241 (M^+, 18.5), 210 (22.9), 159 (100). Anal. Calcd for $C_{13}H_9N_3O$: C, 69.95; H, 4.06; N, 18.82. Found: C, 70.17; H, 4.13; N, 18.74%.

*3.1.10. 1,2-Di(3-methylbenzofuran-2-ylcarbonyl)hydrazine (**16**)*

To a cold solution of 3-methyl-2-benzofurancarbohydrazide (**1**) (1.90 g, 10 mmol) in pyridine (20 ml), 3-methyl-2-benzofuranoyl chloride (**15**) (1.94 g, 10 mmol) was added portion-wise while stirring over a period of 30 min. After complete addition, the reaction mixture was stirred for further 1 h at room temperature and then the reaction mixture was poured onto an ice–water mixture with stirring. The precipitated solid was collected by filtration, washed with dilute hydrochloric acid followed by cold water, then finally dried and recrystallized from EtOH/DMF to afford 1,2-di(3-methylbenzofuran-2-ylcarbonyl)hydrazine (**16**) as white powder in 75% yield; mp 224–226 °C; IR (KBr) ν_{max}/cm^{-1}: 3222 (NH), 1658 (C═O); ^1H NMR (DMSO-d_6) δ: 2.57 (s, 6H, $2CH_3$), 7.37–7.42 (m, 2H, ArH), 7.51–7.56 (m, 2H, ArH), 7.65 (d, 2H, ArH, J = 8.28 Hz), 7.80 (d, 2H, ArH, J = 7.59 Hz), 10.58 (s, 2H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6) δ 8.7, 111.7, 121.2, 122.5, 123.4, 127.6, 128.8, 141.8, 152.9, 158.8; MS *m*/*z* (%): 349 ($M^+ + 1$, 2.8), 348 (M^+, 11.1), 159 (100), 103 (18.2). Anal. Calcd for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 8.04. Found: C, 69.18; H, 4.46; N, 8.22%.

*3.1.11. 2-(3-Methylbenzofuran-2-carboxamido)benzoic acid (**18**)*

This compound was synthesized by the same method mentioned above for compound **16** by using 3-methyl-2-benzofuranoyl chloride (**15**) and 2-aminobenzoic acid (**17**) instead of hydrazide **1**. Yield (87%); mp 275–277 °C (EtOH/DMF); IR (KBr) ν_{max}/cm^{-1}: 3171 (NH), 1697, 1659 (2C═O); ^1H NMR (DMSO-d_6) δ: 2.58 (s, 3H, CH_3), 4.34 (s, 2H, D_2O exchangeable), 6.36–7.86 (m, 8H, ArH), 9.28 (s, 1H, D_2O exchangeable); MS *m*/*z* (%): 295 (M^+, 21.9), 159 (100), 120 (40.2), 77 (37.8). Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.33; H, 4.68; N, 4.50%.

*3.1.12. 2-(3-Methylbenzofuran-2-yl)-4H-3,1-benzoxazin-4-one (**19**)*

A solution of compound **18** (0.59 g, 2 mmol) and anhydrous sodium acetate (0.16 g, mmole) in acetic anhydride (10 ml) was heated at 140 °C while stirring for 2 h, then left to cool to room temperature. The reaction mixture was poured onto crushed ice and the solid that formed was filtered off, washed with water and dried. Recrystallization from EtOH/DMF afforded compound **23** in 72% yield; mp 190–192 °C; IR (KBr) ν_{max}/cm^{-1}: 1762 (C═O), 1593 (C═N); ^1H NMR (DMSO-d_6) δ: 2.73 (s, 3H, CH_3), 7.37–7.42 (m, 1H, ArH), 7.52–7.57 (m, 1H, ArH), 7.60–7.66 (m, 1H, ArH), 7.72–7.76 (m, 2H, ArH), 7.84 (d, 1H, ArH, J = 7.80 Hz), 7.93–7.99 (m, 1H, ArH), 8.16 (d, 1H, ArH, J = 7.87 Hz); ^{13}C NMR (DMSO-d_6) δ: 9.6, 111.8, 116.9, 121.3, 123.6, 124, 126.8, 128, 128.1, 128.6, 129.1, 136.9, 140.5, 146.3, 150.7, 153.9, 158.2; MS *m*/*z* (%): 277 (M^+, 100), 249 (20.2), 220 (39.0), 146 (17.0), 125 (10.4), 102 (26.9), 77 (33.4), 51 (21.0). Anal. Calcd for $C_{17}H_{11}NO_3$: C, 73.64; H, 4.00; N, 5.05. Found: C, 73.86; H, 3.82; N, 4.80%.

3.2. Antimicrobial activity

3.2.1. Culture media

Two specific media were used for detecting the antimicrobial activity, malt extract agar (MEA) for fungal isolates [malt extract, 20 g; bacteriological peptone, 5 g; agar, 20 g, the pH was adjusted to 5.4 ± 0.2 at 25 (±2) °C] while nutrient agar medium was used for bacterial growth [beef extract, 3 g; bacteriological peptone, 5 g; agar, 20 g, the pH was adjusted to 6.2 ± 0.2 at 25 (±2) °C]. Each medium was prepared by dissolving the solid ingredients in 1 l of cold distilled water and then heated to 60–70 °C with stirring. Media were sterilized by autoclaving at 121 °C (1.5 atm) for 15–20 min [34].

3.2.2. Microorganisms

Nine clinical fungal strains employed for this investigation include four filamentous fungi (*A. fumigatus*, *A. niger*, *P. italicum* and *S. racemosum*) and one unicellular fungi (*C. albicans*) and two Gram

positive (*S. aureus*, *B. subtilis*), two Gram negative (*E. coli* and *P. aeruginosa*) bacteria. All strains were kindly provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

3.2.3. Antimicrobial assays

By diffusion agar technique, the antifungal and antibacterial potentialities against several species were expressed as the measurement of diameter of their inhibition zone. Hole-plate diffusion method was used; six equidistant (1 cm diameter) holes were made using sterile cork borer in Malt extract agar and Nutrient agar sterile plates (10×10 cm), which had previously been seeded with tested fungal and bacterial isolates. Holes were filled with 100 μL of 5 mg/ml concentration of each of the synthesized compounds after completely dissolving in DMSO. Control holes were filled with DMSO solvent. Plates were left in a cooled incubator at 4 (±2) °C for 1 h and then incubated at 37 (±2) °C for bacterial isolates and incubated at 28 (±2) °C for fungal isolates used. Inhibition zones developed due to active ingredients were measured after 24–48 h of incubation time. Terbinafin was used as a standard antifungal agent while Amoxicilline was used as a standard antibacterial agent.

3.2.4. Minimum inhibitory concentration (MIC) assays

Determination of MIC was performed by a serial dilution technique described by Irobi et al. [35] Appling DMSO solvent of the synthesized compounds started with a maximum concentration of 500 μg/ml and then reduced it by successive twofold dilutions of that stock solution using a calibrated micropipette. MIC of the sample determination was carried out by inoculation of their serial dilutions with test organisms and measurement of inhibition zones using diffusion agar technique. MIC was expressed as the lowest concentration inhibiting test organisms' growth. Samples that showed no antimicrobial activity at concentrations of 5 mg/ml were considered inactive [36].

Appendix. Supplementary material

Supplementary material associated with this article can be found in the online version, at doi: 10.1016/j.ejmech.2009.02.020.

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