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Original article

Stereoselective synthesis and antimicrobial activity of benzofuran-based (1*E*)-1-(piperidin-1-yl)-N^2-arylamidrazones

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ABSTRACT

The reaction of 2-oxo-*N*-arylpropanehydrazonoyl chlorides **3a–e** with 3-methyl-2-benzofurancarboxylic acid hydrazide (**7**) furnished *N*-(aryl)propanehydrazonoyl chlorides **8a–e**. X-Ray of **8c** revealed the (1*Z*,2*E*) configuration of structure **8**. Nucleophilic substitution reaction of **8a** or **8d** with piperidine resulted in the formation of 1-(piperidin-1-yl)-N^2-arylamidrazones **9a**, **b**. The X-ray diffraction of **9b** showed its (1*E*,2*E*) configuration and it confirmed the stereoselectivity of the latter reaction. (1*E*,2*Z*,3*E*)-1-(Piperidin-1-yl)-1-(arylhydrazono)-2-[(3-methylbenzofuran-2-oyl)hydrazono]-4-arylbut-3-enes **11** were synthesized in stereoselective reaction from **8** or alternatively from **9**. X-ray analysis of **11b** showed a conversion of configuration respect to **8d** or **9b**. X-Ray analysis of **9b** and **11b** revealed the role of hydrogen interactions in the stereochemistry of their solid state structure. The *in vitro* antimicrobial activity of the newly synthesized compounds demonstrated an excellent growth inhibition of compounds **9** and **11** against clinically isolated strains of human fungal pathogens and exhibited a significant potency against Gram-positive bacteria. *Griseofulvin* and *Amoxicilline* were used as references for antifungal and antibacterial screening. The effect of most potent antifungal compound **9b** on morphological features of *Aspergillus fumigatus* and *Candida albicans* using image analyzer was studied. Furthermore, the effect of **9b** on the ultra-structures of the latter fungi was occurred by transmission electron microscope.

1. Introduction

Benzofurans with substituent(s) at C-2 and/or C-3 have attracted strong interest due to their widespread in a large number of natural products and for their useful biological and pharmacological properties [1–7]. The antifungal agent *Cicerfuran*, obtained from the roots of wild species of chickpea, *Cicer bijugum*, reported to be a major factor in the defense system against *Fusarium* wilt [8]. In addition, *Benzbromarone* is potent uricosuric agent used in the treatment of gout, especially when *Allopurinol*, a first-line treatment, fails [9]. Furthermore, the highly effective antiarrhythmic drug *Amiodarone* [10] and the unique anti-depression agent *R*-(−)-1-(benzofuran-2-yl)-2-propylaminopentane [11] are important benzofuran synthetic pharmaceutics. However, a huge number amongst the natural and synthetic benzofurans have established potency as antifungal agents [12–16] such as the fungistatic benzofuran derivative *Griseofulvin*. It is generally given for fungal infections that involve the scalp, hair, nails and skin (e.g. *Tinea corporis*, *Tinea pedis*, *Tinea cruris*, *Tinea barbae*, *Tinea capitis*, *Tinea unguium*) [16].

Although the invasive fungal diseases are now more frequent than the first half of last century, they are still difficult to diagnose clinically and present more complicate therapeutic problems with increasing incidence of chronic, often fatal, mycoses in immunocompromised patients. Fungi divided to three classes; (a) dermatophytes (superficial mycoses), (b) subcutaneous (deep mycoses) and (c) saprophytic and opportunistic fungi. Dermatophytes fungi, class a, or ringworm fungi fitted by evolution to attack the human protein keratin such as epidermis, hair and nail keratin causing fungal diseases such as *T. pedis*, *T. corporis* or *T. capidis* [17]. *Griseofulvin* used to treat ringworm infections. It binds to keratin in keratin precursor cells and makes them resistant to fungal infections [16].

Moreover, the deep mycoses fungus *Candida albicans*, class b, is endogenous inhabitant of the alimentary tract and the mucocutaneous regions of the body. Although it is not usually found in large numbers on normal human skin, it rapidly colonizes the skin after an injury [18]. *C. albicans*, have been isolated from infections in every area of the human body (skin, nails, mucous membranes, in

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addition to gasterointestinal, respiratory, vaginal, esophageal and urinary tracts) causing an enormous diversity of clinical syndromes [19]. Myristoyl-CoA:protein *N*-myristoyltransferase (Nmt) is a monomeric enzyme that catalyzes the transfer of the fatty acid myristate from myristoyl-CoA to the *N*-terminal glycine residue of a variety of eukaryotic proteins such as *C. albicans* proteins. Genetic and biochemical studies have established that Nmt is an attractive target for antifungal drugs. A series of inhibitors having a benzofuran core such as RO-09-4609 and RO-09-4879 exhibited high inhibitory activity against *C. albicans N*-myristoyltransferase (CaNmt) and, subsequently, exhibited potent *in vivo* antifungal activity [20].

However, because of the high number of therapy-resistant for fungal infections and the growing number of life-threatening invasive mycoses, there is still a great need for the development of antifungal agents with a new biological spectrum [20,21].

On the other hand, amidrazones were found to possess interesting biological activities [22–25]. Some of amidrazone-containing hetererocycles have been demonstrated potent in vitro antimicrobial activity. Amidrazone-substructural fragments were found to be the antifungal pharmacophore of the latter compounds [26–28]. Additionally, amidrazones have been reported as precursors of some effective antifungal azoles [29]. Consequently, the efficient construction of these molecules has received significant attention [30–34].

In view of the above facts and in continuation of our interest in the synthesis of bioactive benzofurans as antimicrobial agents [35–39], we hope to report herein, a convenient synthesis of a new class of benzofuran-based (1*Z*,2*E*)-*N*-arylpropanehydrazonoyl chlorides with highly synthetic potency for stereoselective synthesis of the title compounds for their antimicrobial evaluation.

2. Results and discussion

2.1. Chemistry

The reaction of α-chloroketones **1** with acid hydrazides to give the corresponding α-chlorohydrazone derivatives **2** was reported [40]. Although the hydrazone derivative of **1**, 2-oxo-*N*-arylpropanehydrazonoyl chlorides **3** have been known for more than one century [41], their reaction with acid hydrazides to afford the highly functionalized propanehydrazonoyl chlorides **4** not yet reported. On the other hand, the reactions of hydrazonoyl chlorides **5** with various secondary amines to form oxanilino-N^2-amidrazones **6** have been explored firstly by Shawali [42]. It is worthy to mention that, most structures of N^2-arylamidrazones determined by X-ray diffraction analysis were found to be *Z*-configured [43,44] (Fig. 1).

Recently, Frohberg et al. have been reported the first successfully attempts on the separation and fully characterization of *E*-isomers **6B** beside *Z*-isomers **6A** of some oxanilo-N^2-arylamidrazones **6** to correlate their exact stereochemistry with their biological activity [31]. From the latter data, it is important to combine our present study with X-ray diffraction analysis to determine the stereochemistry of the new class *N*-arylpropanehydrazonoyl chlorides **8a–e** and their reaction products 1-(piperidin-1-yl)-N^2-arylamidrazones **9a**, **b** and **11a–c**.

Thus, the reaction of 2-oxo-*N*-arylpropanehydrazonoyl chlorides **3a–e** with 3-methyl-2-benzofurancarboxylic acid hydrazide (**7**) in refluxing THF afforded, in each case, a single yellow product. IR spectra of the latter products revealed a carbonyl absorption band in the region 1683–1680 cm^{-1} in addition to the absorption bands of 2NH functions in the region 3372–3238 cm^{-1}. Their ^1H NMR spectra exhibited two D_2O exchangeable signals of 2NH groups in the regions δ 8.59–10.19 and δ 10.70–11.27 in addition to two singlet signals of two methyl groups in the region δ 2.31–2.58. The two sp^3 carbons of the latter methyl groups appeared at δ 8.8 and 13.7 in ^{13}C NMR spectra. The latter spectroscopic data of the reaction products and their satisfactory elemental analyses supported the structure 2-{[(3-methylbenzofuran-2-yl)carbonyl]hydrazono}-*N*-(aryl)propanehydrazonoyl chlorides as postulated in Scheme 1. However, X-ray diffraction of **8c** (Fig. 2) indicated without doubt that the reaction product is the stereoisomer (1*Z*,2*E*)-**8** and ruled out the other possible stereoisomers (3 isomers). Characteristic bond lengths of **8c** and the torsion angles of its two essential double bonds 1*Z* and 2*E* are listed in Table 1. There is no intra- or intermolecular hydrogen interactions were detected in **8c**.

Next, the reactivity of **8a** or **8d** towards piperidine was examined. Heating each of the latter **8** in ethanol resulted in elimination of hydrogen chloride and the formation of a single product, in each case, as evidenced by TLC analysis of the crude product. Elemental analysis, ^1H NMR and the mass spectral data were compatible with the assigned structure **9a**, **b** in Scheme 1 (see experimental). The X-ray diffraction (Fig. 3a and 3b) showed the (1*E*,2*E*) configuration of **9b** and confirmed the sterioselectivity of the latter reaction. It is

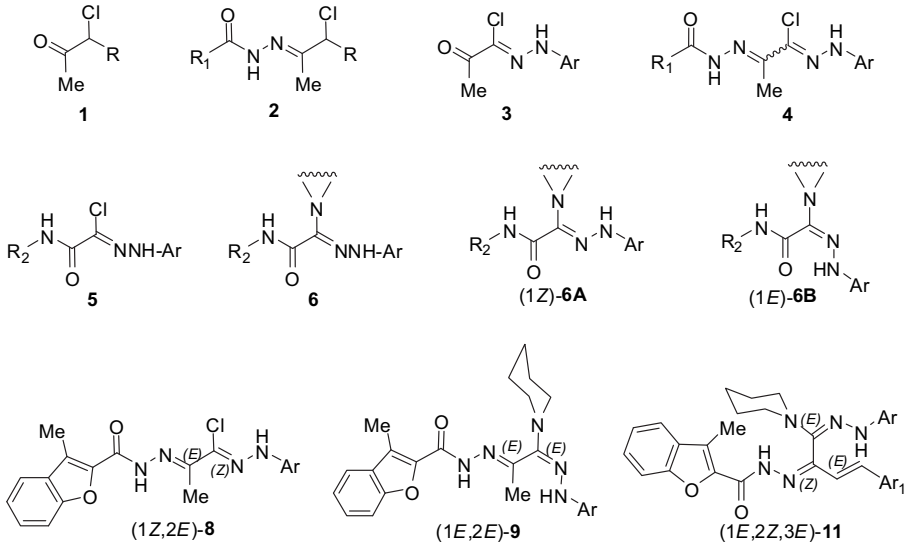
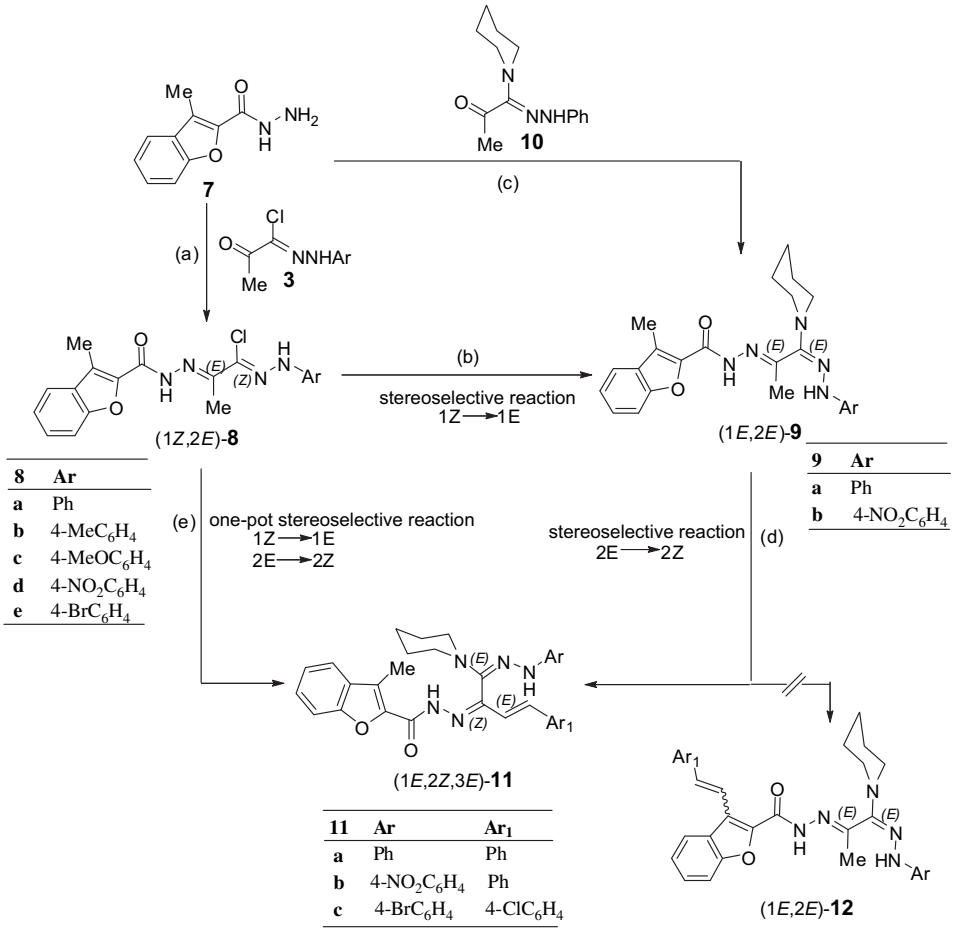


Fig. 1.



Scheme 1. Reagents and conditions: (a) THF, reflux 9-12 h, 77–83%; (b) piperidine, EtOH, heating 30 min at 100 °C, 65–72%; (c) for **9a**, THF, reflux 18 h, 23%; (d) bezaldehyde, EtOH, piperidine (2 equ.), reflux 8 h, 68–70%; (e) bezaldehyde or 4-chlorobezaldehyde, EtOH, piperidine (4 equ.), reflux 8 h, 70–76%.

noteworthy to mention here that some (1*E*)-N^2-arylamidrazones were prepared by stereochemical rearrangement in polar solvents through the preparation and/or recrystallization [31]. However, the single crystal of **9b** suitable for X-ray analysis was obtained by slow evaporation, at room temperature, for ethanol solution of **9b** and it was found to have two independent molecules in the asymmetric unit with the same stereochemistry. Essential bond distances of (1*E*,2*E*)-**9b** and the torsion angle of its double bonds 1*E* and 2*E* were given in Table 1.

An alternative synthesis of **9a** was explored. Thus, refluxing a mixture of acid hydrazide **7** with 1-(2-phenylhydrazono)-1-(piperidin-1-yl)propan-2-one **10** in THF for 18 h afforded 23% yield of **9a** (Scheme 1).

Surprisingly, upon attempts to condense benzaldehyde with **9a**, **b** to prepare compounds **12a**, **b**, where the methyl function of 3-methybenzofurans have been reported as *C*-nucleophile toward aromatic aldehydes or ketones in basic condition [45], the condensation occurred at methyl group of hydrazone chain to afford compounds **11a**, **b** not the expected **12a**, **b** (Scheme 1). Thus, the reaction of (1*Z*,2*E*)-isomer **9a**, **b** with benzaldehyde in refluxing ethanol, in the presence of piperidine, resulted in the formation of a reaction product precipitated, in each case, during reflux. The IR spectra of the latter products exhibited, in each case, a band in the region 1683–1680 cm^{-1} due to the carbonyl absorption whereas the absorption bands of 2NH functions appeared in the region 3372–3238 cm^{-1}. Their ^1H NMR spectra were characterized by the

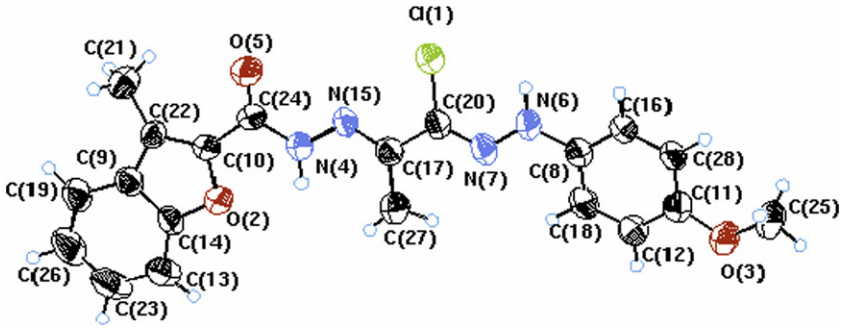
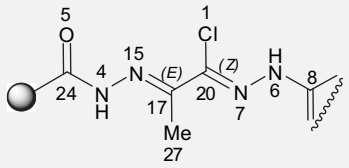
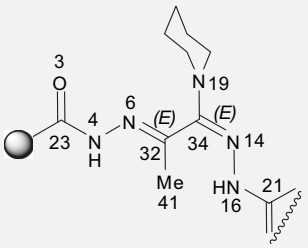
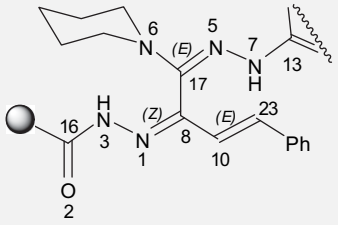


Fig. 2. X-ray structure of (1*Z*,2*E*)-**8c**.

Table 1
Characteristic bond lengths [Å] and torsion angles [°] of (1*Z*,2*E*)-**8c,** (1*E*,2*E*)-**9b (A)** and (1*E*,2*Z*,3*E*)-**11b**.

(1*Z*,2*E*)-**8c**		(1*E*,2*E*)-**9b(A)**		(1*E*,2*Z*,3*E*)-**11b**	
					
bond length [Å]					
C8–N6	1.415(3)	N16–C21	1.352(4)[a]	C13–N7	1.358(3)[a]
N6–N7	1.340(3)	N14–N16	1.402(4)[b]	N7–N5	1.385(2)[b]
N7–C20	1.284(3)	N14–C34	1.297(4)[c]	N5–C17	1.280(3)[c]
C20–Cl1	1.730(3)	N19–C34	1.376(4)[d]	C17–N6	1.382(3)[d]
C20–C17	1.471(4)	C32–C34	1.497(5)[e]	C17–C8	1.500(3)[e]
C17–N15	1.287(3)	N6–C32	1.277(4)	C8–N1	1.289(3)
N15–N4	1.372(3)	N4–N6	1.386(3)	N1–N3	1.377(2)
N4–C24	1.369(4)	N4–C23	1.366(4)	N3–C16	1.366(3)
C24–O5	1.208(3)	O3–C23	1.216(4)	C16–O2	1.217(3)
C17–C27	1.490(4)	C32–C41	1.486(5)	C8–C10	1.440(3)
				C10–C23	1.328(3)
torsion angles [°]					
(1*Z*)		(1*E*)		(1*E*)	
N6–N7–C20–Cl1	0.2(2)[f]	N16–N14–C34–N19	168.5(10)[g]	N6–C17–N5–N7	−170.0(6)[g]
(2*E*)		(2*E*)		(2*Z*)	
C20–C17–N15–N4	179.2(4)[g]	N4–N6–C32–C34	−179.8(10)[g]	N3–N1–C8–C17	−3.4(4)[f]
				(3*E*)	
				C8–C10–C23–C25	175.8(8)[g]

[a] The bond lengths of (1*E*,2*E*)-**9b(A)** and (1*E*,2*Z*,3*E*)-**11b** are well within the range that typically occurred in other amidrazone derivatives: 1.391(2)–1.396(3) Å.
[b] 1.342(4)–1.351(2) Å.
[c] 1.286(3)–1.294(2) Å.
[d] 1.373(3)–1.405(4) Å.
[e] 1.491(4)–1.511(3) Å[31].
[f] The torsion angles of (1*Z*,2*E*)-**8c**, (1*E*,2*E*)- **9b(A)** and (1*E*,2*Z*,3*E*)-**11b** are well within the range that typically reported for $Z = 0° \rightarrow \pm 30°$.
[g] $E = \pm 150° \rightarrow 180$.

presence of two signals in the regions δ 8.37–8.56 and 10.60–10.88, assigned to the 2 NH groups in addition to singlet signal characteristic to benzofuran C-3 methyl group in the region δ 2.57–2.58. The ^{13}C NMR spectra showed a signal of sp^3 carbon at δ 8.8 of benzofuran C-3 methyl carbon in addition to the other sp^3 signals of piperidine carbons in the region δ 23.8–48.6. Moreover, the ^{1}H NMR spectrum of **9a** revealed a two douplets at δ 6.91 and 7.23 due to the protons of olefinic bond with $J = 16.5$ Hz consistent with the *trans* olefinic vicinal coupling constant. The above mentioned spectroscopic data provided support for **11a–c** (Scheme 1). X-Ray analysis of amidrazone **11b** (Fig. 4a and 4b) showed that, the stereochemical structure of **11** is (1*E*,2*Z*,3*E*) geometrical structure and excluded the other possible stereoselective isomers (7 isomers) or the site selective product **12** as postulated in Scheme 1. Selected bond distances in (1*E*,2*Z*,3*E*)-**11b** and the torsion angle of its essential double bonds 1*E*, 2*Z* and 3*E* were given in Table 1.

Interestingly, treatment of **8a**, **8d** with benzaldehyde refluxing in ethanol, in the presence of four molar ratio of piperidine, afforded a reaction product identical to compound **11a**, **b** that were obtained above. Similar reaction of **8e** with 4-chloro benzaldehyde furnished amidrazone **11c**.

X-Ray diffraction of **9b** showed a network of three intermolecular hydrogen contacts between the independent molecules **A** and **B** (Fig. 5). The latter three H-bonds tided the hydrazide part with amidrazone skeleton and linked the two molecules (**A** and **B**) into a dimer, these findings suggested that the hydrazide part played a unique role in the stereochemistry of amidrazone skeleton.

Beside to the intramolecular hydrogen bond N3–H3···N6, three intermolecular hydrogen contacts N7–H7···N1, N7–H7···O2 and C9–H9···O2 stabilized the structure (1*E*,2*Z*,3*E*)-**11b**. The importance of hydrogen bonds as an auxiliary binding and, in some cases, as the dominant factor in determining crystal packing [46] or molecular conformation [47] has received wide attention in biological systems [48]. However, hydrogen bonds played a crucial role in directing the molecular assembly of **11** in the solid state. The hydrogen interactions in **9b** and **11b** were outlined in Fig. 5. The hydrogen interaction between *ortho*-proton of benzene moiety and H–bond acceptors (N or O) was already known [49] (C33–H33···O2 and C28–H28···O3 in case of **9b**, and C9–H9—O2 in case of **11b**). The hydrogen bond geometry of (1*E*,2*E*)-**9b** and (1*E*,2*Z*,3*E*)-**11b** represented in Table 2.

The preliminary antimicrobial test showed an excellent activity for **9b** as promising antifungal agent. The latter findings stimulated our interest to investigate the reactivity of **8d** towards sodium benzenesulfinate and hydroxylamine with the following objective in mind. We thought it interesting to explore the utility of the **8d** as a synthon for new potent antimicrobial agents. Firstly, **8d** reacted with sodium benzenesulfinate in refluxing ethanol, yielding 3-methyl-*N*'-1-methyl-2-[(4-nitrophenyl)hydrazono-2-(phenylsulfonyl)ethylidene]-1-benzofuran-2-carbohydrazide (**13**) as shown in Scheme 2. Secondly, the reactions of **8d** with hydroxylamine hydrochloride, in refluxing ethanol, resulted in the formation of *N*-hydroxy-2-{[(3-methyl-1-benzofuran-2-yl)carbonyl]hydrazono}-*N*'-(4-nitrophenyl)propanehydrazonamide (**14**) (Scheme 2). Each of the latter

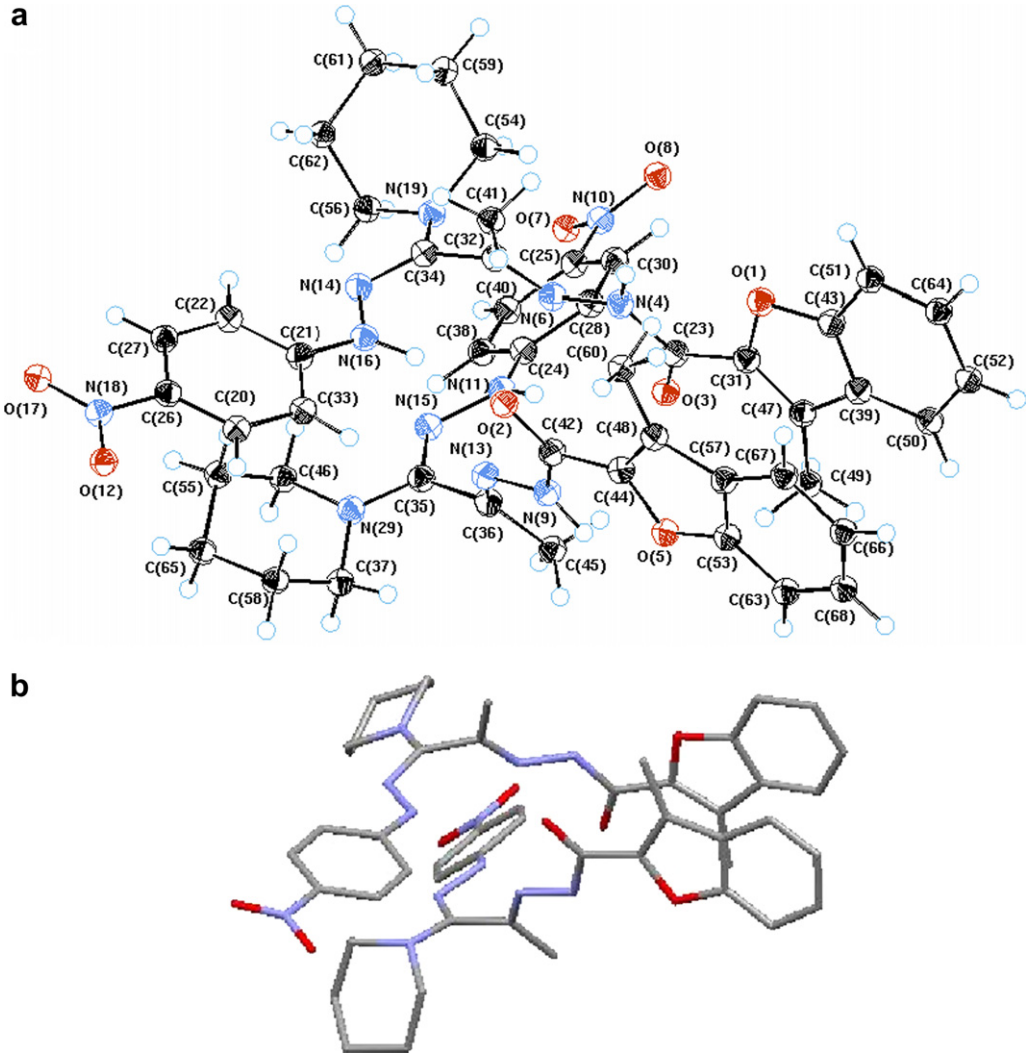


Fig. 3. (a) X-ray structure of (1*E*,2*E*)-**9b**. (b) View of (1*E*,2*E*)-**9b**, Hydrogen atoms are omitted for clarity.

reactions was found to give a single product as evidenced by TLC analysis. The structure of compound **13** or **14** was established on the basis of their elemental analysis and spectral data (see experimental). It would be of great support to get an evidence for one of their possible isomeric structures by carrying out a single crystal X-ray analysis of the reaction product, however all attempts to get such crystals for X-ray diffraction were failed Scheme 3

In an attempt to understanding the potentiality of **9a**, **b** and **11a–c** in antimicrobial test and to shed some light on their preliminary **SAR**, we synthesized hydrazones **15a**, **b**. Thus, the reaction of acid hydrazide **7** with acetaldehyde or *E*-cinnamaldehyde, in refluxing ethanol, afforded the corresponding (*E*)-hydrazones **15a** and **15b**, respectively. Their ^{1}H NMR spectra revealed, in each case, a singlet signal in the region δ 8.50–8.54 characteristic for the azomethyne proton and showed D_2O-exchangeable singlet signal integrating for one proton of ═NNH– function.

The compounds containing arylidene–hydrazide structure may exist as *E*/*Z* geometrical isomers about –C═N– double bond and *cis–trans* amide conformers. These compounds were present in higher percentage in DMSO-d_6 in the form of geometrical *E* isomer about –C═N– bond [50]. In the present study, the spectral data were obtained in DMSO-d_6 and neither the signal of *Z* isomer nor these of *cis–trans* conformers of *E* isomer were observed.

2.2. Biological activity

All synthesized compounds were screened for their antifungal and antibacterial activities at 100 μg concentration against two filamentous fungi (*Aspergillus fumigatus* and *Syncephalastrum racemosum*), two yeast (*C. albicans* and *Geotrihum candidium*), two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. *Griseofulvin* and *Amoxicilline* were used as a standard antifungal and antibacterial agent, respectively. Some of the newly synthesized compounds showed excellent antimicrobial activities with respect to the control drugs. Most of active compounds have a significant antifungal potency more than antibacterial potency. The results of the antifungal and antibacterial activities were shown in Tables 3 and 4, respectively. Susceptibilities of the fungal and bacterial isolates to our synthesized benzofurans were investigated by measuring their inhibitory effect on the growth of microorganisms compared to the solvent used.

2.2.1. Antifungal activity

Data in Table 3 revealed a remarkable activity of compounds **9a, b** and **11a–c** as antifungal agents. Compound **9b** exhibited the highest potency against all tested fungal organisms with respect to reference drug *Griseofulvin*, it is an antibiotic fungistatic drug

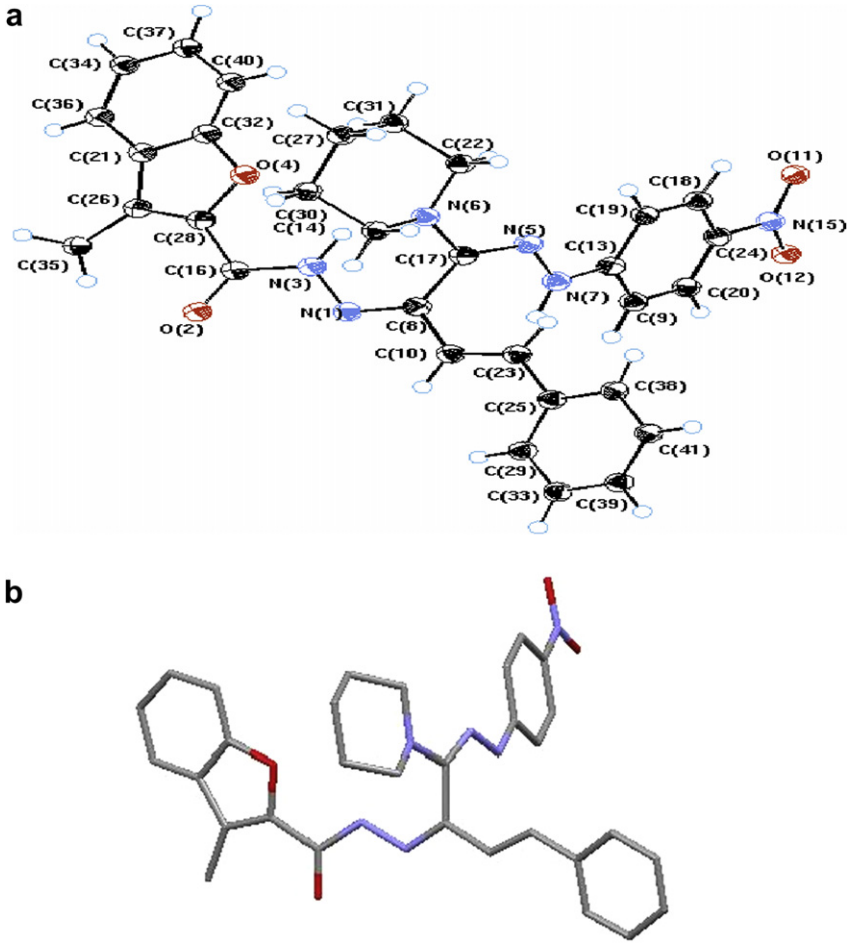


Fig. 4. (a)X-ray structure of (1*E*,2*Z*,3*E*)-**11b**. (b) View of (1*E*,2*Z*,3*E*)-**11b**, Hydrogen atoms were omitted for clarity.

administered orally in the treatment of dermatophyte and ringworm infections [16].

C. albicans was the most sensitive fungus with potency 1.8 of *Griseofulvin* followed by *A. fumigatus*, *G. candidum* and *S. racemosum* with potencies 1.28, 1.2 and 1.16, respectively. The order of activity of compounds **9a**, **b** and **11a–c** against tested fungal strains from higher to lower was **9b**, **11a**, **11b**, **9a** then **11c**. Compounds **8a–e** as well as hydrazones **15a**, **b** showed varied antifungal activity against *C. albicans* with potencies ranged from 0.5 to 1.2 and revealed no ability against most of other fungal species. On the other hand, sulfone **13** and hydroxymoyl **14** revealed potencies 1.5 and 1.05 against *C. albicans* and *S. racemosum*, respectively.

G. candidum have been isolated from infections in debilitated, from human lung, and respiratory tract, gut, and skin tissue and

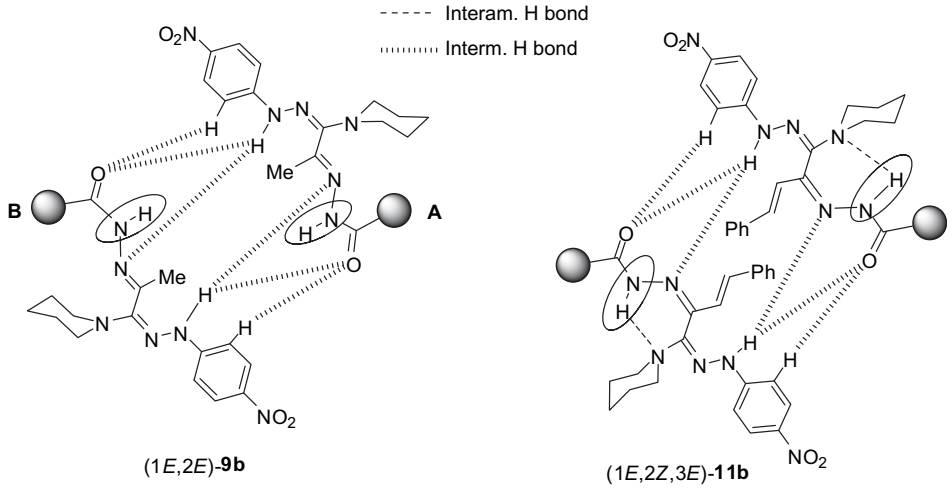


Fig. 5. Intera- and intermolecular hydrogen bonds (dashed lines) of (1*E*,2*E*)-**9b** and (1*E*,2*Z*,3*E*)-**11b**.

Table 2
Intra- and intermolecular hydrogen bonds of (1*E*,2*E*)-**9b** and (1*E*,2*Z*,3*E*)- **11**.

(1*E*,2*E*)-9b				
D–H (**A**)···A (**B**)	D–H	H···A	D···A	<(DHA)
Intermolecular				
N16—H16···N13	0.960 (3)	2.550	3.227	127.70
N16—H16···O2	0.960 (3)	2.253	3.149	154.96
C33—H33···O2	0.960 (3)	2.740	3.480	134.39
D–H (**B**)···A (**A**)	D–H	H···A	D···A	<(DHA)
Intermolecular				
N11–H11···N6	0.960 (3)	2.509	3.196	128.47
N11–H11···O3	0.960 (3)	2.503	3.370	150.06
C28–H28···O3	0.960 (3)	2.645	3.467	143.95
(1*E*,2*Z*,3*E*)-11b				
D–H···A	D–H	H···A	D···A	<(DHA)
Intramolecular				
N3–H3···-N6	0.960(2)	2.410	2.986	118.2
Intermolecular				
N7–H7···-N1	0.960(2)	2.522(2)	3.415	149.9
N7–H7···-O2	0.960(2)	2.520(2)	3.202	125.4
C9–H9···-O2	0.960(2)	2.643(2)	3.271	123.4

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

immunocompromised patients in cases of vaginitis, thrush, bronchitis, diabetic and cutaneous infections. The general term for these infections is geotrichosis [51]. Although, *G. candidum* was recorded the highest resistance for tested compounds, **9b** showed a potency of 1.2 against this fungus.

It has been estimated that over one-half of all therapeutic agents consist of heterocyclic compounds. The heterocyclic ring system in many cases comprised the very core of the active moiety or pharmacophore. Nevertheless, the bicyclic array in the therapeutic agents based on the benzofuran ring played the role of a rigid support for functional groups. In our present study, varying substitution at C-2 of benzofuran moiety in the newly synthesized compounds especially **9b**, **11b**, **13**, **14**, **15a** and **15b** (Fig. 6), and subsequent their antimicrobial results identified the functionality for optimal antimicrobial property in this class of compounds. According to structure–activity relationships (SAR), it can be concluded that both of piperidine moiety and 4-nitrophenylhydazone functions were essential for the antimicrobial activity. The piperidines, which are so frequently found in the side chains of therapeutic agents, are not usually associated with pharmacophores; they simply serve as surrogates for open-chain tertiary amines.

2.2.2. Antibacterial activity

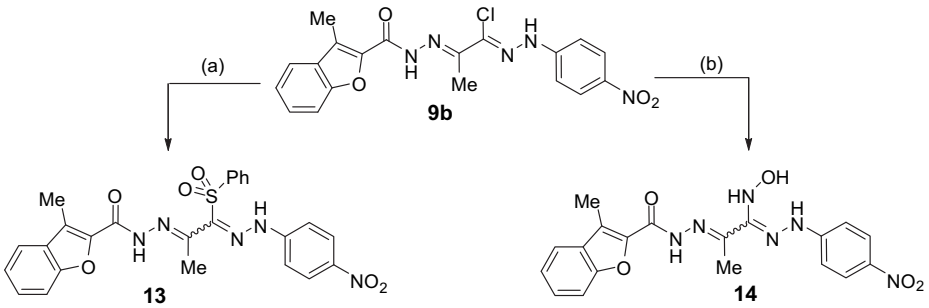
Although, the synthesized compounds showed a variable potencies against tested bacteria, their activity were lower than that of standard drug *Amoxicilline*. From the results in Table 4 Gram-positive bacteria were highly susceptible where *Bacillus subtilis* was the most affected strain against tested compounds followed by *S. aureus*. Alternatively, the tested compounds exhibited weak inhibitory effects against the Gram-negative bacterium *E. coli* **9b** whereas they revealed no effect, or very weak, against *P. aerogenosa* which emerged as one of the most problematic Gram-negative pathogens, with the alarmingly high antibiotics resistance rates [52].

2.2.3. Minimum inhibitory concentration (MIC)

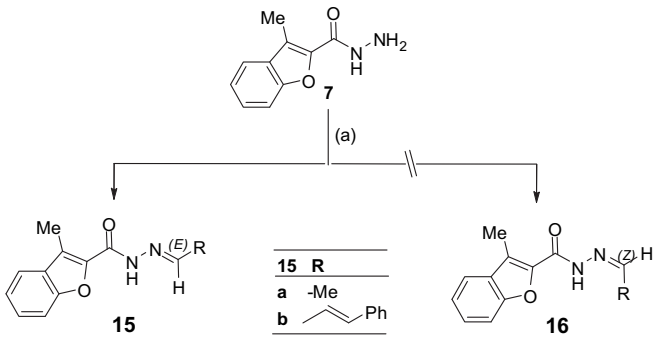
The minimum inhibitory concentration (MIC) of **9b** was measured against the most sensitive fungi *A. fumigatus* and *C. albicans*, and against the most effective bacterial strain *S. aureus*. Compound **9b** showed low MIC (25 μg/ml) against *A. fumigatus* and revealed MIC of 75 μg/ml against *C. albicans* respect to that of *Griseofulvin* which showed 50 and 75 μg/ml against *A. fumigatus* and *C. albicans*, respectively. On the other hand, **9b** exhibited high MIC 75 μg/ml against *S. aureus* respect to *Amoxicilline* (50 μg/ml) as reference drugs. The minimum inhibitory concentration (MIC) of **9b** against highly inhibited organisms reported in Table 5.

2.2.4. Effect of *9b* on the morphological features of A. fumigatus and C. albicans

Prompted by the antifungual potentialities of compound **9b**, it was selected for further assessment. The effect of **9b** on *A. fumigatus* and *C. albicans* was carried out using image analyzer in the RCMB center, Al-Azhar University. Image analysis studies showed that, the size of all morphological features of *A. fumigatus* was reduced by *Griseofulvin* and **9b** respect to their original measurements. Furthermore, many of chlamydospores were obtained after incubation of *A. fumigatus* with 100 μL of **9b**. On the other hand, *Griseofulvin* caused aggregation of *C. albicans* cells while **9b** caused elongation and swollen for the same cells with great reduction in their number.



Scheme 2. Reagents and conditions: (a) $PhSO_2Na.2H_2O$, reflux 10 h, 53%; (b) $NH_2OH.HCl$, K_2CO_3, EtOH, reflux 45 min, 47%.



Scheme 3. Reagents and conditions: (a) R–CHO, EtOH, reflux 4 h, 62–78%.

*2.2.5. Effect of **9b** on the ultra-structures of A. fumigatus and C. albicans*

Effect of DMSO, *Griseofulvin* and **9b** on the ultra-structures of *A. fumigatus* and *C. albicans* was occurred by transmission electron microscope at 25,000 magnifications. The results revealed a great effect of **9b** against both of *A. fumigatus* and *C. albicans* such as shrinking of cell membrane, distortion of most of sub-organs, aggregation of lysised mitochondria in groups and most cells became semi-empty. In hyphae cell, cell wall and cell membrane increased in thickness in both of *A. fumigatus* and *C. albicans*.

3. Conclusion

In conclusion, we described an efficient synthesis of functionally (1*Z*,2*E*)-*N*-(aryl)propanehydrazonoyl chlorides bearing active methyl group used as *C*-nucleophiles. These derivatives were used in economical and versatile synthetic approach for stereoselective synthesis of the title derivatives. The results of the present study showed that, some of the newly synthesized benzofuran-based (1*E*)-1-(piperidin-1-yl)-N^2-arylamidrazones have significant antifungal potencies and can be consider very promising in the perspective of new drugs discovery with respect to the medical importance of the tested fungal strains. Accordingly, generalization of the latter established method can be widely used in synthesis of library of (1*E*)-N^2-arylamidrazones with expected biological activity. In this regard as well as in the light of interesting structural and biological results the situation definitely calls for some additional research towards understanding the relation between biological activity and structure of such class of compounds. However, to confirm this suggestion, further study for similar compounds is in progress and their biological evaluations are currently underway.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were measured with a Gallenkamp apparatus. IR spectra were recorded on Shimadzu FT-IR 8101 PC infrared spectrophotometer. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^{1}H spectra were run at 300 MHz and ^{13}C spectra of compounds **8a**, **8c**, **8d**, **9a, b** and **11a, b** were run at 75.46 MHz in deuterated dimethylsulphoxide (DMSO-d_6). Chemical shifts (δ_H) are reported relative to TMS as internal standard. All coupling constant (J) values are given in hertz. Chemical shifts (δ_C) are reported relative to DMSO-d_6 as internal standards. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet; D_2O exch., exchanged with D_2O; all the NH exchanged with D_2O. Mass spectra were measured on a GCMS-QP1000 EX spectrometer at 70 e V. Elemental analyses were carried out at the Microanalytical center of Cairo University. X-Ray crystallography was carried out on a Kappa CCD Enraf Nonius FR 590 diffractometer, National Research Center, Dokki, Cairo, Egypt. The X-ray diffraction measurements of compounds **8c, 9b** and **11b** were made using maXus (Bruker Nonius, Delft & Mac Science, Japan) [53], at wavelength $\lambda = 0.71073$ Å and a graphite monochromator was used for data collection. 3-Methylbenzofuran-2-carboxylic acid hydrazide (**7**) [54], 2-oxo-*N*-arylpropanehydrazonoyl chlorides **3a–e** [41], and 1-(2-phenylhydrazono)-1-(piperidin-1-yl)propan-2-one (**10**) [22] were prepared according to the literature procedures.

4.1.2. Synthesis of (1Z,2E)-2-{[(3-methylbenzofuran-2-yl)carbonyl] hydrazono}-N-(aryl)propanehydrazonoyl chlorides 8a–e

A mixture of 3-methyl-2-benzofurancarboxylic acid hydrazide (**7**) (1.9 g, 10 mmol) and the appropriate 2-oxo-*N*-arylpropanehydrazonoyl chloride **3a-e** (10 mmol) in dry THF (50 mL) was heated under refluxing temperature. The reaction was controlled by TLC and continued until the starting substrates were completely consumed (9–12 h), then left to cool to room temperature. The solvent was removed, in each case, under reduced pressure and

Table 3
In vitro antifungal activity of the synthesized compounds.

Compound	*Aspergillus fumigatus*		*Syncephalastrum racemosum*		*Candida albicans*		*Geotrichum candidum*	
	I.Z.[a]	P.[b]	I.Z.[a]	P.[b]	I.Z.[a]	P.[b]	I.Z.[a]	P.[b]
8a	–	0	–	0	6 ± 0.5	0.6	–	0
8b	–	0	–	0	6 ± 0.5	0.6	–	0
8c	–	0	–	0	10 ± 0.5	1	–	0
8d	14 ± 1.3	0.56	–	0	12 ± 0.8	1.2	5 ± 0.5	0.5
8e	–	0	5 ± 0.5	0.26	10 ± 1	1	–	0
9a	28 ± 2.2	1.12	16 ± 1.2	0.84	14 ± 0.9	1.4	–	0
9b	32 ± 2.5	1.28	22 ± 1.2	1.16	18 ± 1.3	1.8	12 ± 0.8	1.2
11a	21 ± 2	0.84	19 ± 1.4	1	14 ± 1	1.5	–	0
11b	–	0	21 ± 1.7	1.1	17 ± 1.4	1.7	–	0
11c	–	0	20 ± 1.8	1.05	14 ± 1.1	1.4	–	0
13	–	0	–	0	15 ± 1	1.5	–	0
14	–	0	20 ± 1.5	1.05	9 ± 0.8	0.9	–	0
15a	–	0	–	0	8 ± 0.5	0.8	–	0
15b	–	0	–	0	5 ± 0.5	0.5	–	0
Grisofulvin	25 ± 2.3	1	19 ± 1.6	1	10 ± 0.7	1	10 ± 0.8	1

[a] Inhibition zone in mm.; –: not detected.
[b] potency = I.Z. of compound / I.Z. of *Grisofulvin*.

Table 4
In vitro antibacterial activity of the synthesized compounds.

Compound	*Staphylococcus aureus*		*Bacillus subtilis*		*Escherichia coli*		*Pseudomonas aeruginosa*	
	I.Z.[a]	P[b]	I.Z.[a]	P[b]	I.Z.[a]	P[b]	I.Z.[a]	P[b]
8a	–	0	–	0	–	0	–	0
8b	–	0	7 ± 0.5	0.29	–	0	–	0
8c	–	0	–	0	–	0	–	0
8d	12 ± 1	0.4	6 ± 0.5	0.25	10 ± 0.8	0.36	5 ± 0.5	0.25
8e	–	0	9 ± 0.5	0.38	–	0	–	0
9a	15 ± 1.2	0.5	–	0	17 ± 1.1	0.61	–	0
9b	26 ± 1.7	0.87	20 ± 1.5	0.83	20 ± 1	0.71	7 ± 0.5	0.35
11a	16 ± 1	0.53	15 ± 1.6	0.63	14 ± 1	0.5	–	0
11b	–	0	–	0	8 ± 0.7	0.29	–	0
11c	10 ± 1	0.33	16 ± 1.5	0.67	10 ± 0.8	0.36	–	0
13	8 ± 1	0.27	10 ± 1.3	0.42	6 ± 0.5	0.21	–	0
14	–	0	–	0	–	0	–	0
15a	16 ± 1.5	0.53	–	0	20 ± 1.6	0.71	–	0
15b	–	0	8 ± 0.6	0.33	–	0	–	0
Amoxicilline	30 ± 2.5	1	24 ± 2	1	28 ± 2	1	20 ± 2.2	1

[a] Inhibition zone in mm.; –: not detected.
[b] potency = I.Z. of compound / I.Z. of *Amoxicilline*.

the residue was triturated with ethanol to give yellow colored product. The solid products that formed were filtered off, washed with ethanol and recrystallized from EtOH/DMF to afford the corresponding (1*Z*,2*E*)-*N*-arylpropanehydrazonoyl chlorides **8a–e** in 77–83% yields.

*4.1.2.1. (1Z,2E)-2-{[(3-Methylbenzofuran-2-yl)carbonyl]hydrazono}-N-phenylpropanehydrazonoyl chloride (**8a**).* Reaction time 11 h, pale yellow crystals (2.84 g, 79%); mp 200–202 °C; IR (KBr) ν 3371, 3248 (2NH), 1682 (C=O), 1605 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.43 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 6.90–7.78 (m, 9H, ArH), 10.19 (s, D_2O exch., 1H,=NNH–), 10.75 (s, D_2O exch., 1H, –CONH–); ^{13}C NMR δ 8.8 (1C of benzofuran $\underline{C}H_3$), 13.7 (1C of propane $\underline{C}H_3$), 111.7, 113.8, 114.8, 121.0, 121.2, 122.6, 123.4, 127.5, 128.8, 129.1, 129.2, 142.3, 143.4, 152.9; MS *m*/*z* (%) 370 ($M^+ + 2$, 3.0), 369 ($M^+ + 1$, 2.5), 368 (M^+, 8.6), 159 (100), 77 (12.4). Anal. Calcd for $C_{19}H_{17}ClN_4O_2$: C, 61.87; H, 4.65; N, 15.19%. Found: C, 62.03; H, 4.51; N, 15.27%.

*4.1.2.2. (1Z,2E)-2-{[(3-Methylbenzofuran-2-yl)carbonyl]hydrazono}-N-(4-tolyl)propanehydrazonoyl chloride (**8b**).* Reaction time 9.5 h, yellow crystals (3.10 g, 77%); mp 208–210 °C; IR (KBr) ν 3302, 3256 (2NH), 1682 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.31 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.28–7.85 (m, 8H, ArH), 8.59 (s, D_2O exch., 1H,=NNH–), 10.77 (s, D_2O exch., 1H, –CONH–); MS *m*/*z* (%) 384 ($M^+ + 2$, 8.3), 383 ($M^+ + 1$, 4.8), 382 (M^+, 25.9), 159 (100), 77 (17.4). Anal. Calcd for $C_{20}H_{19}ClN_4O_2$: C, 62.74; H, 5.00; N, 14.63%. Found: C, 62.60; H, 5.12; N, 14.55%.

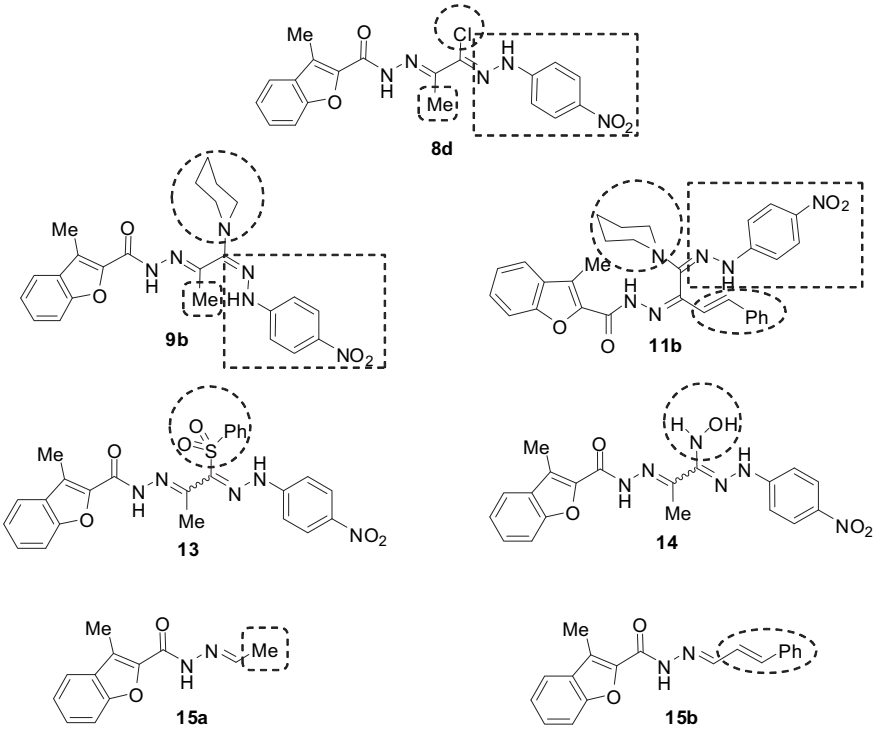


Fig. 6. Structure features of compounds **8d**, **9b**, **11b**, **13**, **14**, **15a** and **15b**.

Table 5
Minimum inhibitory concentration (MIC) of **9b**.

Compound	MIC (μg/ml)		
	Aspergillus fumigatus	*Candida albicans*	*Staphylococcus aureus*
9b	25	75	75
Standard	50 (*Grisofulvin*)	75 (*Grisofulvin*)	50 (*Amoxicilline*)

*4.1.2.3. (1Z,2E)-2-{[(3-Methylbenzofuran-2-yl)carbonyl]hydrazono}-N-(4-methoxyphenyl)propanehydrazonoyl chloride (**8c**).* Reaction time 9 h, yellow crystals (3.27 g, 82%); mp 217–219 °C; IR (KBr) ν 3346, 3238 (2NH), 1680 (C=O), 1604 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.67 (s, 3H, –OCH_3), 7.28–7.87 (m, 8H, ArH), 8.89 (s, D_2O exch., 1H,=NNH–), 10.70 (s, D_2O exch., 1H, –CONH–); 54.8, ^{13}C NMR δ 8.8 (1C of benzofuran $\underline{C}H_3$), 13.7 (1C of propane $\underline{C}H_3$), 56.1 (1C of –O$\underline{C}H_3$), 111.5, 114.1, 114.4, 121.0, 121.4, 122.6, 123.8, 127.8, 128.4, 129.2, 129.4, 129.8, 141.8, 143.7, 153.1; MS *m/z* (%) 400 ($M^+ + 2$, 12.3), 399 ($M^+ + 1$, 7.9), 398 (M^+, 39.6), 159 (100). Anal. Calcd for $C_{20}H_{19}ClN_4O_3$: C, 60.23; H, 4.80; N, 14.05%. Found: C, 60.44; H, 4.67; N, 14.13%. The purified product **8c** was dissolved in ethanol/DMF (v/v = 3/1) and yellow single crystals were separated after 3 days. Crystal data for compound **8c**: $C_{20}H_{19}ClN_4O_3$, M_r, 398.850; system, monoclinic; Space group, $P2_1/c$; unit cell dimensions, *a* 12.9456 (5) Å, *b* 11.2257 (6) Å, *c* 13.5705 (6) Å, α 90.00°, β 95.137 (4)°; *V*, 1964.2 (2) $Å^3$; *Z*, 4; D_x,1.349 $mg.m^{-3}$; θ range for data collection, 2.910–19.980°; μ(Mo-K_α), 0.22 mm^{-1}; T, 298 K; measured reflections, 2999; independent reflections, 1807; observed reflections, 1453; R_{int}, 0.026; *R*(*all*), 0.048; *wR*(*ref*), 0.077; *wR*(*all*), 0.079; *S*(*ref*), 1.359; *S*(*all*), 1.577; Δ/σ_{max}, 0.046; $\Delta\rho_{max}$, 0.17 $eÅ^3$, $\Delta\rho_{min}$, –0.16 $eÅ^3$.

*4.1.2.4. (1Z,2E)-2-{[(3-Methylbenzofuran-2-yl)carbonyl]hydrazono}-N-(4-nitrophenyl)propanehydrazonoyl chloride (**8d**).* Reaction time 12 h, yellow powder (3.43 g, 83%); mp 238–240 °C; IR (KBr) ν 3367, 3250 (2NH), 1683 (C=O), 1600 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.34 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.11–7.89 (m, 8H, ArH), 9.12 (s, D_2O exch., 1H,=NNH–), 11.27 (s, D_2O exch., 1H, –CONH–); ^{13}C NMR δ 8.8 (1C of benzofuran $\underline{C}H_3$), 13.7 (1C of propane $\underline{C}H_3$), 111.7, 112.6, 114.7, 120.9, 121.12, 122.56, 123.4, 127.4, 128.8, 129.1, 129.3, 129.4, 142.3, 142.5, 143.4, 152.9; MS *m/z* (%) 415 ($M^+ + 2$, 23.2), 414 ($M^+ + 1$, 13.9), 413 (M^+, 68.8), 287 (15.7), 159 (100). Anal. Calcd for $C_{19}H_{16}ClN_5O_4$: C, 55.15; H, 3.90; N, 16.92%. Found: C, 55.03; H, 4.02; N, 16.83%.

*4.1.2.5. (1Z,2E)-2-{[(3-Methylbenzofuran-2-yl)carbonyl]hydrazono}-N-(4-bromophenyl)propanehydrazonoyl chloride (**8e**).* Reaction time 11 h, Yellow needles (3.58 g, 80%); mp 235–237 °C; IR (KBr) ν 3372, 3257 (2NH), 1680 (C=O), 1600 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.44 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.24–7.62 (m, 8H, ArH), 8.89 (s, D_2O exch., 1H,=NNH–), 10.58 (s, D_2O exch., 1H, –CONH–); MS *m/z* (%) 449 ($M^+ + 2$, 9.6), 448 ($M^+ + 1$, 9.5), 447 (M^+, 10.1), 446 (10.3), 159 (100). Anal. Calcd for $C_{19}H_{16}Cl_2N_4O_2$: C, 50.97; H, 3.60; N, 12.51%. Found: C, 50.76; H, 3.62; N, 12.34%.

*4.1.3. Synthesis of (1E,2E)-1-(Piperidin-1-yl)-1-(arylhydrazono)-2-[(3-methylbenzofuran-2-oyl)hydrazono]propanes **9a, b***

Method A. To a solution of propanehydrazonoyl chloride **8a** or **8d** (2 mmol) in ethanol (50 mL), piperidine (0.32 g, 4 mmol) was added. The reaction mixture was heated 30 min at 100 °C then left to cool at room temperature overnight. The precipitated product was filtered off, washed with ethanol and dried. Recrystallization from EtOH afforded compounds **9a** and **9b,** respectively.

Method B (for **9a**). To a solution of 3-methyl-2-benzofurancarboxylic acid hydrazide (**7**) (0.19 g, 1 mmol) in THF (50 mL), 1-(2-phenylhydrazono)-1-(piperidin-1-yl)propan-2-one (**10**) (0.25 g, 1 mmol) was added. The reaction mixture was refluxed for 18 h then left to cool. The precipitated product was filtered off, washed with ethanol and dried. Recrystallization from EtOH afforded 0.1 g (23% yield) of compound **9a**.

*4.1.3.1. (1E,2E)-1-(Piperidin-1-yl)-1-phenylhydrazono-2-[(3-methylbenzofuran-2-oyl)hydrazono]propane (**9a**).* Orange needles in yield (0.54 g, 65%) ; mp 206–208 °C; IR (KBr) ν 3362, 3215 (2NH), 1682 (C=O), 1599 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.61 (m, 6H, $3CH_2$ of piperidine), 2.22 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 3.16 (m, 4H, $2CH_2$ of piperidine), 7.02–8.04 (m, 9H, ArH), 9.74 (s, D_2O exch., 1H,=NNH–), 10.42 (s, D_2O exch., 1H, –CONH–) ppm; ^{13}C NMR δ 8.7 (1C of benzofuran $\underline{C}H_3$), 13.6 (1C of propane $\underline{C}H_3$), 23.7 (1C of piperidine), 25.3 (2$\underline{C}$ of piperidine), 48.7 (2C of piperidine), 111.6, 113.7, 115.0, 121.0, 121.2, 122.6, 123.4, 127.2, 128.7, 130.1, 143.3, 147.5, 152.5; MS *m/z* (%), 418 ($M^+ + 1$, 9.1), 417 (M^+, 34.7), 159 (100). Anal. Calcd for $C_{24}H_{26}N_6O_4$: C, 69.04; H, 6.52; N, 16.77%. Found: C, 68.88; H, 6.64; N, 16.70%.

*4.1.3.2. (1E,2E)-1-(Piperidin-1-yl)-1-[(4-nitrophenyl)hydrazono]-2-[(3-methylbenzofuran-2-oyl)hydrazono]propane (**9b**).* Red crystals in yield (0.67 g, 72%) ; mp 232–234 °C; IR (KBr) ν 3370, 3219 (2NH), 1680 (C=O), 1597 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62 (m, 6H, $3CH_2$ of piperidine), 2.22 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 3.18 (m, 4H, $2CH_2$ of piperidine), 6.98–8.07 (m, 8H, ArH), 9.70 (s, D_2O exch., 1H,=NNH–), 10.55 (s, D_2O exch., 1H, –CONH–) ppm; ^{13}C NMR δ 8.7 (1C of benzofuran $\underline{C}H_3$), 13.7 (1C of propane $\underline{C}H_3$), 23.7 (1C of piperidine), 25.3 (2$\underline{C}$ of piperidine), 48.8 (2C of piperidine), 111.6, 113.7, 114.8, 120.9, 121.1, 122.6, 123.4, 127.3, 128.7, 130.0, 143.1, 147.2, 152.9; MS *m/z* (%) 464 ($M^+ + 2$, 22.3), 463 ($M^+ + 1$, 52.1), 462 (M^+, 71.3), 288 (21.7), 159 (58.1), 84 (100). Anal. Calcd for $C_{24}H_{26}N_6O_4$: C, 62.33; H, 5.67; N, 18.17%. Found: C, 62.26; H, 5.48; N, 18.15%. The red single crystal of **9b** was cultured from EtOH by slow evaporation at room temperature. Crystal data for compound **9b**: $C_{48}H_{52}N_{12}O_8$, M_r, 925.020; system, triclinic; Space group, $P\bar{1}$; unit cell dimensions, *a* 9.6172 (4) Å, *b* 14.1318 (5) Å, *c* 18.7384 (9) Å, α 72.960 (3)°, β 84.802 (2)°; *V*, 2407.5 (2) $Å^3$; *Z*, 2; D_x, 1.276 $mg.m^{-3}$; θ range for data collection, 2.910–19.980°; μ(Mo-K_α), 0.09 mm^{-1}; T, 298 K; measured reflections, 7451; independent reflections, 5088; observed reflections, 2130; R_{int}, 0.042; *R*(*all*), 0.127; *wR*(*ref*), 0.081; *wR*(*all*), 0.104; *S*(*ref*), 1.864; *S*(*all*), 2.002; Δ/σ_{max}, 0.048; $\Delta\rho_{max}$, 0.41 $eÅ^3$, $\Delta\rho_{min}$, −0.37 $eÅ^3$.

*4.1.4. Synthesis of (1E,2Z,3E)-1-(piperidin-1-yl)-1-(arylhydrazono)-2-[(3-methylbenzofuran-2-oyl)hydrazono]-4-(aryl[1]) but-3-enes **11a–c***

Method A. To a solution of the appropriate amidrazone **9a** or **9b** (1 mmol) in ethanol (50 mL), piperidine (0.17 g, 2 mmol) and benzaldehyde (0.1 g, 1 mmol) were added. The reaction mixture was refluxed for 8 h. The precipitated product was filtered off, washed with ethanol and dried. Recrystallization from EtOH/DMF afforded compounds **11a** and **11b**, respectively.

Method B. To a solution of propanehydrazonoyl chloride **8a**, **8d** or **8e** (1 mmol) in ethanol (50 mL), piperidine (0.34 g, 4 mmol) and the appropriate aldehyde (1 mmol) were added. The reaction mixture was refluxed for 8 h. The precipitated product was filtered off, washed with ethanol and dried. Recrystallization from EtOH /DMF afforded compounds **11a**–**c**, respectively.

*4.1.4.1. (1E,2Z,3E)-1-(Piperidin-1-yl)-1-(phenylhydrazono)-2-[(3-methylbenzofuran-2-oyl)hydrazono]-4-phenylbut-3-ene (**11a**).* Orange needles (0.35 g, 70% method A; 0.38 g, 76%, method B); mp 208–210 °C; IR (KBr) ν 3375, 3254 (2NH), 1681 (C=O), 1603 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.63 (m, 6H, $3CH_2$ of piperidine), 2.58 (s, 3H, CH_3), 3.28 (m, 4H, $2CH_2$ of piperidine), 6.61(m, 1H, ArH), 6.91

(d, $J = 16.5$ Hz, 1H, olefinic=CH–), 6.98–7.11 (m, 4H, ArH), 7.23 (d, $J = 16.5$ Hz, 1H, olefinic=CH–), 7.33–7.52 (m, 7H, ArH), 7.62 (d, $J = 8.1$ Hz, 1H, ArH), 7.79 (d, $J = 8.1$ Hz, 1H, ArH), 8.37 (s, D_2O exch., 1H,=NNH–), 10.70 (s, D_2O exch., 1H, –CONH–); ^{13}C NMR δ 8.8 (1C of benzofuran $\underline{C}H_3$), 23.83 (1C of piperidine), 25.2 (2C of piperidine), 48.6 (2C of piperidine), 111.8, 113.7, 114.9, 121.4, 121.9, 122.6, 123.3, 123.6, 124.5, 127.6, 128.3, 128.9, 129.3, 130.1, 138.6, 139.0, 142.4, 143.4, 148.8, 154.7; MS *m/z* (%) 507 ($M^+ + 2$, 6.3), 506 ($M^+ + 1$, 31.5), 505 (M^+, 23.7), 159 (100), 77 (10.7). Anal. Calcd for $C_{31}H_{31}N_5O_2$: C, 73.64; H, 6.18; N, 13.85%. Found: C, 73.52; H, 6.16; N, 13.62%.

*4.1.4.2. (1E,2Z,3E)-1-(Piperidin-1-yl)-1-[(4-nitrophenyl)hydrazono]-2-[(3-methylbenzofuran-2-oyl)hydrazono]-4-phenylbut-3-ene (**11b**).* Red crystals (0.37 g, 68%, method A; 0.4 g, 72%, method B); mp 261–263 °C; IR (KBr) ν 3367, 3252 (2NH), 1684 (C=O), 1604 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.61 (m, 6H, $3CH_2$ of piperidine), 2.58 (s, 3H, CH_3), 3.18 (m, 4H, $2CH_2$ of piperidine), 6.99–7.53 (m, 6H, ArH), 7.56–7.66 (m, 5H, ArH), 7.68 (d, $J = 8.1$ Hz, 1H, ArH), 8.00 (d, $J = 8.1$ Hz, 1H, ArH), 9.34 (s, D_2O exch., 1H,=NNH–), 10.88 (s, D_2O exch., 1H, –CONH–); ^{13}C NMR δ 8.8 (1C of benzofuran $\underline{C}H_3$), 23.7 (1C of piperidine), 25.2 (2C of piperidine), 48.6 (2C of piperidine), 111.7, 113.7, 114.8, 121.2, 121.3, 122.6, 123.0, 123.4, 124.5, 127.6, 128.3, 129.1, 129.3, 138.3, 138.7, 142.5, 143.4, 148.8, 154.1; MS *m/z* (%) 552 ($M^+ + 2$, 7.2), 551 ($M^+ + 1$, 33.0), 550 (M^+, 28.3), 159 (100). Anal. Calcd for $C_{31}H_{30}N_6O_4$: C, 67.62; H, 5.49; N, 15.26%. Found: C, 67.56; H, 5.42; N, 15.20%. The red single crystal of **11b** was cultured from a mixture solution of CH_3CN/DMF (v/v = 5/1) by slow evaporation at room temperature. Crystal data for compound **11b**: $C_{31}H_{30}ClN_6O_4$, M_r, 480.524; system, monoclinic; Space group, $P2_1/c$; unit cell dimensions, *a* 13.8406 (7) Å, *b* 12.1451 (6) Å, *c* 20.5992 (13) Å, α 90.00°, β 124.282 (18)°; *V*, 2861.1 (3) Å^3; *Z*, 4; D_x, 1.278 mg.m^{-3}; θ range for data collection, 2.910–25.350°; μ(Mo-K_α), 0.09 mm^{-1}; T, 298 K; measured reflections, 9015; independent reflections, 6216; observed reflections, 1700; R_{int}, 0.044; *R*(*all*), 0.291; *wR*(*ref*), 0.087; *wR*(*all*), 0.133; *S*(*ref*), 1.894; *S*(*all*), 2.125; Δ/σ_{max}, 0.025; $\Delta\rho_{max}$, 0.55 eÅ^3, $\Delta\rho_{min}$, –0.67 eÅ^3.

*4.1.4.3. (1E,2Z,3E)-1-(Piperidin-1-yl)-1-[(4-bromophenyl)hydrazono]-2-[(3-methylbenzofuran-2-oyl)hydrazono]-4-(4-chlorophenyl)but-3-ene (**11c**).* Orange needles (0.43 g, 70%, method B); mp 256–258 °C; IR (KBr) ν 3364, 3245 (2NH), 1684 (C=O), 1558 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.61 (m, 6H, $3CH_2$ of piperidine), 2.57 (s, 3H, CH_3), 3.18 (m, 4H, $2CH_2$ of piperidine), 6.85–6.96 (m, 2H, ArH), 7.09–7.23 (m, 2H, ArH), 7.32–7.56 (m, 6H, ArH), 7.64 (d, $J = 8.1$ Hz, 1H, ArH), 7.77 (d, $J = 8.1$ Hz, 1H, ArH), 8.56 (s, D_2O exch., 1H,=NNH–), 10.60 (s, D_2O exch., 1H, –CONH–); MS *m/z* (%) 620 ($M^+ + 2$, 17.4), 619 ($M^+ + 1$, 18.5), 618 (M^+, 13.1), 617 (13.4), 444 (34.9), 175 (36.8), 159 (100). Anal. Calcd for $C_{31}H_{29}BrClN_5O_2$: C, 60.16; H, 4.72; N, 11.31%. Found: C, 59.97; H, 4.78; N, 11.36%.

*4.1.5. 3-Methyl-N′-1-methyl-2-[(4-nitrophenyl)hydrazono-2-(phenylsulfonyl)ethylidene]-1-benzofuran-2-carbohydrazide (**13**)*

To a solution of **8d** (0.41 g, 1 mmol) in absolute ethanol (50 mL), sodium benzenesulfinate dihydrate (0.4 g, 2 mmol) was added. The mixture was refluxed for 10 h, then left to cool. The reaction mixture was poured into cold water and the solid product filtered off, washed with water, dried and finally recrystallized from EtOH/DMF to afford sulfone **13** as yellow fibers in 0.28 g (53%) yield; mp >300 °C; IR (KBr) ν 3345, 3256 (2NH), 1674 (C=O), 1588 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 6.78–7.01 (m, 4H, ArH), 7.09–7.89 (m, 9H, ArH), 8.48 (s, D_2O exch., 1H,=NNH–), 9.98 (s, D_2O exch., 1H, –CONH–); MS *m/z* (%) 520 ($M^+ + 1$, 11.3), 519 (M^+, 35.0), 159 (100). Anal. Calcd for $C_{25}H_{21}N_5O_6S$: C, 57.80; H, 4.07; N, 13.48; S, 6.17%. Found: C, 57.68; H, 4.11; N, 13.60; S, 6.13%.

*4.1.6. N-Hydroxy-2-{[(3-methyl-1-benzofuran-2-yl) carbonyl]hydrazono}-N′-(4-nitrophenyl) propanehydrazonamide (**14**)*

A mixture of **8d** (0.41 g, 1 mmol), of hydroxylamine hydrochloride (0.11 g, 1.5 mmol) and anhydrous potassium carbonate (0.21 g, 1.5 mmol) in ethanol (50 mL) was refluxed for 45 min, then left to cool. The reaction mixture was poured into cold water and the solid product was filtered off, washed with water, dried and finally recrystallized from EtOH/DMF to afford **14** as yellow fibers in 0.19 g (47 %) yield; mp 286–288 °C; IR (KBr) ν 3390–3150 (3NH + OH), 1686 (C=O), 1588 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 5.72 (s, D_2O exch., 1H, –OH), 7.02–7.05 (m, 1H, ArH), 7.08 (s, D_2O exch., 1H, –N$\underline{H}$–OH), 7.2–7.81 (m, 7H, ArH), 9.70 (s, D_2O exch., 1H,=NNH–), 10.6$\underline{0}$ (s, D_2O exch., 1H, –CONH–); MS *m/z* (%) 411 ($M^+ + 1$, 15.5), 410 (M^+, 68.0), 159 (100). Anal. Calcd for $C_{19}H_{18}N_6O_5$: C, 55.61; H, 4.42; N, 20.48%. Found: C, 55.49; H, 4.48; N, 20.36%.

*4.1.7. Synthesis of hydrazones **15a**, **b***

A mixture of 3-methyl-2-benzofurancarboxylic acid hydrazide (**7**) (0.19 g, 1 mmol) and acetaldehyde or *E*-cinnamaldehyde (1 mmol) in absolute ethanol (30 mL) was refluxed for 4 h then left to cool. The solid product so formed was collected by filtration, washed with ethanol and dried. Recrystallization from the proper solvent afforded the corresponding hydrazones **15a**, **b**.

*4.1.7.1. N′-[(1E)-Ethylidene]-3-methyl-1-benzofuran-2-carbohydrazide (**15a**).* White powder (0.13 g, 62%); mp. 170–172 °C (EtOH); IR (KBr) ν 3217 (NH), 1659 (C=O), 1605 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.14 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 7.30–7.82 (m, 4H, ArH), 8.50 (s, 1H, –CH=N–), 11.95 (s, D_2O exch., 1H,=NNH–); MS *m/z* (%) 217 ($M^+ + 1$, 7.0), 216 (M^+, 58.8), 159 (100). Anal. Calcd for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96%. Found: C, 66.73; H, 6.06; N, 13.08%.

*4.1.7.2. 3-Methyl-N′-[(1E,2E)-3-phenylprop-2-en-1-ylidene]-1-benzofuran-2-carbohydrazide (**15b**).* White fibers (0.24 g, 78%); mp 195–197 °C (EtOH/DMF); IR (KBr) ν 3225 (NH), 1664 (C=O), 1603 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.57 (s, 3H, CH_3), 6.88 (d, $J = 16.4$ Hz, 1H, olefinic=CH–), 6.96–7.15 (m, 4H, ArH), 7.25 (d, $J = 16.5$ Hz, 1H, olefinic=CH–), 7.29–7.78 (m, 5H, ArH), 8.54 (s, 1H, –CH=N–), 12.20 (s, D_2O exch., 1H,=NNH–); MS *m/z* (%) 305 ($M^+ + 1$, 8.2), 304 (M^+, 38.9), 159 (100). Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20%. Found: C, 74.82; H, 5.46; N, 9.33%.

4.2. Antimicrobial activity

4.2.1. 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 [55].

4.2.2. Microorganisms

Eight clinical strains employed for this investigation include two filamentous fungi (*A. fumigatus*, *S. racemosum*), two yeast (*C. albicans* and *G. candidum*), two Gram-positive (*S. aureus* and *Bacillus subtilis*) and 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.

4.2.3. Antimicrobial assays

By diffusion agar technique, the antifungal and antibacterial potentialities against the tested 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 ($\pm$ 2) °C for 1 h and then incubated at 37 ($\pm$ 2) °C for bacterial isolates and incubated at 28 ($\pm$ 2) °C for fungal isolates used. Inhibition zones developed due to active ingredients were measured after 24–48 h of incubation time. *Griseofulvin* was used as a standard antifungal agent while *Amoxicilline* was used as a standard antibacterial agent.

4.2.4. Minimum inhibitory concentration (MIC) assays

Determination of MIC was performed by a serial dilution technique described by Irobi et al. [56]. Appling DMSO solvent of the synthesized compounds started with a maximum concentration of 500 mg/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 organism's growth [57].

4.2.5. Microscopic examination by image analyzer

The action of most active antifungal compound **9b** on the morphological structure of *A. fumigatus* and *C. albicans* fungi was investigated by subjecting the agar cultures of them to the direct microscopic examination. An image analysis system, soft imaging system GmbH software (analySIS® pro ver. 3.0), in the Regional Center for Mycology and Biotechnology, AL-Azhar University was used for examination of the alteration of the tested fungal morphological features at magnification of ×400 for multicellular fungi and ×1000 for unicellular fungi using either phase-contrast or bright field optics under video camera. For each isolate 3–5 plates were prepared and a minimum of 20 microscopic fields were examined.

4.2.6. Transmission electron microscopy

The effect of compound **9b** to on the ultra-structures of *A. fumigatus* and *C. albicans* was examined at the transmission electron microscopy level. The preparation of samples, fixation, dehydration and staining was occurred and examined with a JEOL 1010 transmission electron microscope at 80 KV in The Regional Center for Mycology and Biotechnology, AL- Azhar University.

4.2.7. Statistical analysis

Data were expressed as mean ± SE. The one-way analysis of variance (ANOVA) test was used to detect the statistical significance followed by Duncan's new multiple range test. *P* value more than 0.05 was considered statistically insignificant.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.09.002.

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