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Bioorganic & Medicinal Chemistry 13 (2005) 4796–4805
Bioorganic & Medicinal Chemistry

A new structural alternative in benzo[*b*]furans for antimicrobial activity

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Received 24 September 2003; revised 8 May 2005; accepted 9 May 2005
Available online 17 June 2005

Abstract—Two series of 2-substituted and three new diacetyl benzofurans were synthesized through palladium-catalyzed reactions, and their in vitro antimicrobial spectra were assessed. The compounds demonstrated mild to significant growth inhibition against antibiotic-susceptible standard and clinically isolated strains of Gram-positive and Gram-negative bacteria as well as human fungal pathogens. Ampicillin and kanamycin were used as references for antibacterial screening; nystatin and amphotericin B were used for antifungal screening. Varying substitution at the benzofuran moiety and subsequent antimicrobial screening identified the C-3-acetyl functionality as a new structural alternative for optimal antimicrobial property in the benzofuran class of compounds.
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1. Introduction

Human struggle against the affliction of disease, decay and death is eternal. The deterioration of human population due to an enhanced prevalence of infectious diseases is becoming a global problem.[1] The contemporary treatment of infectious diseases involves administration of a multidrug regimen over a long period of time, which has led to the rapid emergence of multidrug-resistant strains plus a high level of patient noncompliance.[1] The rising prevalence of multidrug-resistant superbugs like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) continues to provide impetus for the search and discovery of novel antimicrobial agents. A potential approach to overcome this resistance problem is to design new and innovative agents with a completely different mode of action so that no cross-resistance with the present therapeuticals can occur.

Benzofurans have drawn considerable attention over the last few years due to their profound physiological and chemotherapeutic properties as well as their widespread occurrences in nature.[2,3] Attempts were made to study the effects of different functional groups on the homocycle and/or the heterocycle for bioactivity. The antimicrobial activity of benzofuran derivatives appears to be dependent on substitution at the heterocyclic furan ring than the aromatic moiety. Most of the early SAR relationship studies on benzofuran were concentrated to variable nitro substitutions. The 3-nitrobenzofurans were always less active than the corresponding 2-nitro compounds both as antibacterials and protozoacides in vitro. This demonstrated the specificity of nitro position for bioactivity.[4] Recently, the use of benzofurans as fungal *N*-myristoyltransferase (Nmt) inhibitors against human pathogenic *Candida albicans* has led to a novel group of fungicides.[5–7] There are a few other biomolecular targets discovered for benzofurans to have in vitro antibacterial activity as well. Examples include bacterial enzymes involved in methionine cycle such as methionine aminopeptidase,[8] deformylase,[9] enzymes involved in peptidoglycan synthesis such as UDP-*N*-acetylmuramyl-L-alanine ligase,[10] and chorismate synthase, an enzyme in the shikimate pathway, essential for bacterial viability.[11] As a result, there is a growing interest in developing general and versatile methods for the synthesis of 2-benzofurans. Various classical and metal-mediated procedures have been developed over the years for elaborating the benzofuran structures.[12] For the last few decades palladium-catalysed reactions have been of

Keywords: Benzofuran; Friedel–Crafts acylation; Terminal alkynes; Antimicrobial; Cytotoxicity.
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doi:10.1016/j.bmc.2005.05.009

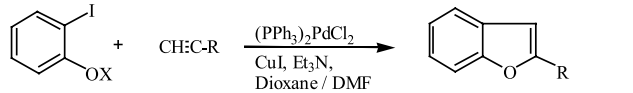
great significance in carbon–carbon[13] and carbon–heteroatom bonds formation.[14] The heteroannulations of 2-iodophenols with acetylenic compounds containing a terminal acetylenic group leading to 2-substituted[15] and 2,3-disubstituted[16] benzofurans have been reported. The synthesis of 2,3-disubstituted benzofurans by palladium-catalysed annulation of internal alkynes has also been documented.[17]

We have previously reported the synthesis of isoindolinones[18] and isobenzofurans[19] by the palladium-catalysed annulation of terminal alkynes with 2-iodobenzamides and 2-iodobenzyl alcohol, respectively. As part of our ongoing studies in developing new broad spectrum antimicrobial agents, we herein, report a novel approach for the synthesis of 2-acyl(aroyl)benzofurans by the palladium-catalyzed annulation of (trimethylsilyl)acetylene with 2-iodophenol (**1**) or 2-acetoxyphenyl iodide (**2**), followed by Friedel–Crafts acylation with acyl(aroyl) chloride or acetic anhydride (Schemes 1, 2) and also a new strategy for a one-pot synthesis of 2-alkyl(aryl)benzofurans from the same starting materials (**1**, **2**) with terminal alkynes (Scheme 3).

In the present investigation, eight newly synthesized 2-benzofuran derivatives (**9**–**11**, **19**–**23**), three diacetylated benzofurans (**12a**–**c**) together with the starting materials (**1**, **2**, **4** and **5**) were screened for in vitro antimicrobial activity.[20] The most potent compounds, **9** and the isomeric mixture (**12abc**) of **12a**–**c**, were further evaluated for their minimum inhibitory concentrations (MIC) against Gram-positive, *Bacillus subtilis* QL 40 and *S. aureus* ATCC 25923 and Gram-negative *Escherichia coli* ATCC 25922, *Salmonella typhi* AM 16406, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio parahemolyticus* AM 16362, plus a pathogenic fungus, *C. albicans* ATCC 10231, and for their cytotoxicity against brine shrimp nauplii.[21]

2. Syntheses

The syntheses of the title compounds have been carried out as depicted in Schemes 1–3. 2-Iodophenol (**1**) and 2-acetoxyphenyl iodide (**2**) were used as starting materials because of their easy availability from 2-aminophenol. It was found that 2-iodophenol (**1**) and 2-acetoxyphenyl iodide (**2**) underwent facile reaction with (trimethylsilyl)acetylene (**3**) in the presence of bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide at 45 °C to yield 2-(trimethylsilyl)ethynyl phenol (**4**) and 2-((trimethylsilyl)ethynyl)phenyl acetate (**5**), respectively, in excellent yields (95–91%).[22] The open chain compounds **4** and **5** were subjected to Friedel–Crafts acylation with acid chloride (**6**–**8**) to afford the 2-aroylbenzofurans (**9**–**11**) in 83–81% yields as shown in Scheme 1. Similar reaction of compound **5** with acetyl chloride or acetic anhydride afforded diacetylbenzofurans **12a** in 26%, **12b** in 19% and **12c** in 33% yields as a result of secondary reactions induced by an excess $AlCl_3$ (Scheme 2). It has been reported that alkylbenzofurans are readily acetylated in the furan ring and in the benzene ring when the furan ring is fully substituted. These acetylation patterns of alkylbenzofurans seem to be due to the contributions of extreme resonance structures in Figure 1.[23]

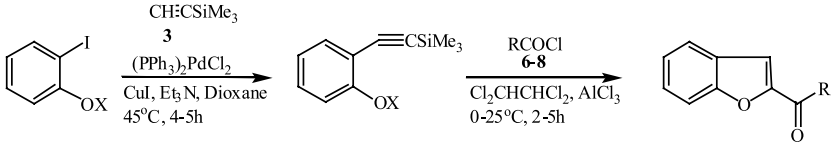


1: X = H
2: X = Ac

13: R = C_6H_5
14 R = C_4H_9
15: R = C_5H_{11}
16: R = $CO_2CH_2CH_3$
17: R = $CH_2CH(CO_2CH_3)_2$
18: R = CH_2OH

19: R = C_6H_5
20: R = C_4H_9
21: R = C_5H_{11}
22: R = $CO_2CH_2CH_3$
23: R = $CH_2CH(CO_2CH_3)_2$
24: R = CH_2OH

Scheme 3.



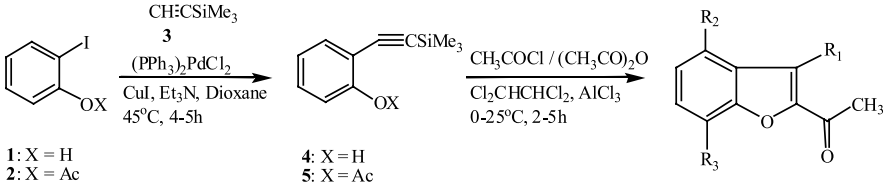
1: X = H
2: X = Ac

4: X = H
5: X = Ac

6: R = C_6H_5
7: R = C_6H_4OMe-*p*
8: R = C_6H_4Me-*p*

9: R = C_6H_5
10: R = C_6H_4OMe-*p*
11: R = C_6H_4Me-*p*

Scheme 1.



1: X = H
2: X = Ac

4: X = H
5: X = Ac

12a: R_1 = H; R_2 = Ac; R_3 = H
12b: R_1 = H; R_2 = H; R_3 = Ac
12c: R_1 = Ac; R_2 = H; R_3 = H

Scheme 2.

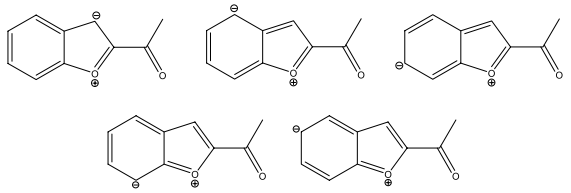


Figure 1. Resonance structures of 2-acetylbenzofuran representing the loci for further electrophilic substitution.

The palladium-catalysed reaction was carried out by stirring a mixture of 2-iodophenol **1** (4.0 g, 18.18 mmol), $Pd(Ph_3P)_2Cl_2$ (0.128 g, 0.18 mmol), CuI (0.07 g, 0.36 mmol), Et_3N (10 mL) and $HC{\equiv}CSiMe_3$ (5 mL, 36.21 mmol) in dioxane (10 mL) at 45 °C for 5 h, under nitrogen atmosphere to yield the 2-(trimethylsilyl) ethynylphenol (**4**) which was purified by column chromatography over silica gel (60–120 mesh). 2-(Trimethylsilyl)ethynylphenyl acetate (**5**) was prepared from 2-acetoxyphenyl iodide (**2**) by applying the same procedure. The Friedel–Crafts acylation reactions were carried out by stirring an ice cold solution of the acyclic product **4** or **5** (1 mmol), anhydrous $AlCl_3$ (3 mmol) and acid chloride or acetic anhydride (1.2 mmol) in tetrachloroethane (10 mL) at 0–25 °C for 2–5 h, where the 2-aroylbenzofurans (**9**–**11**) were obtained in good yields after purification by column chromatography over silica gel (60–120 mesh).

2-Alkyl(aryl)benzofurans (**19**–**24**) were synthesized from 2-iodophenol (**1**) or 2-acetoxyphenyl iodide (**2**) after reactions with terminal alkynes (**13**–**18**). The reactions were carried out by heating a mixture of 2-iodophenol (**1**) or 2-acetoxyphenyl iodide (**2**) and alkynes (**13**–**18**) in dioxane or dimethylformamide at 70 °C for 20 h in the presence of $Pd(Ph_3P)_2Cl_2$ (2.5 mol %), CuI (3–5 mol %) and Et_3N to afford the 2-substituted benzofurans (**19**–**24**) in good yields (Scheme 3). $Pd(Ph_3P)_2Cl_2$ (2–3.5 mol %) was found to be the catalyst of choice with CuI (3–5 mol %) needed as a co-catalyst.[22] Dioxane was found to be a better solvent than DMF in regard to product yield. Uses of Et_3N as a base and dioxane as a solvent were found to be the best due to easier workup and yield of cleaner products. In case of 2-acetoxyphenyl iodide (**2**), we obtained better yields (81–85%) than unprotected 2-iodophenol (**1**). The formation of Pd(0) from the interaction of $Pd(Ph_3P)_2Cl_2$ and CuI was proposed by Sonogashira et al.[22]

All cyclic and acyclic synthesized products obtained were stable at room temperature and well characterized by spectroscopic data (UV, IR, ^1H NMR and ^{13}C NMR). The ^1H NMR and ^{13}C NMR spectra indicated the presence of two isomers of 2-aroylbenzofurans synthesized from 2-(trimethylsilyl)ethynyl phenol (**4**) in a 3:2 ratio. But in the case of 2-((trimethylsilyl)ethynyl)phenyl acetate (**5**), only one isomer of 2-aroylbenzofurans (**9**–**11**) was obtained. In addition, an isomeric mixture of diacetylbenzofurans (**12abc**) evolved as a result of in situ secondary reaction of 2-((trimethylsilyl)ethynyl)phenyl acetate (**5**) with a stoichiometric excess of Lewis acid $AlCl_3$ and acetyl chloride or acetic anhydride. The isomeric components of the mixture (**12abc**) were resolved using normal phase preparative TLC over silica gel F_{254} using *n*-hexane/EtOAc (10:1) as eluting solvent (multiple developments) though they had very closely related R_f values (0.25–0.30). The hitherto unknown diacetylbenzofurans (**12a**–**c**) were structurally characterized on the basis of 2D NMR and mass spectral analyses and by comparison with spectral data of 2-acetylbenzofuran.[24]

3. Antimicrobial activity

The newly synthesized and purified benzofuran derivatives were tested for antimicrobial activity by the disc diffusion method against standard and clinically isolated strains of 14 Gram-negative and 5 Gram-positive pathogenic bacteria as well as four human fungal pathogens. The organisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The results are reported in Tables 1–4. From the mean zone of inhibition (MZI) data analysis, the isomeric mixture of the three diacetylbenzofurans (**12abc**) was identified as the most potent inhibitor with zone diameters ranging from 31 to 40, 29 to 36, 20 to 32 and 18 to 26 mm at doses of 200, 100, 50 and 25 μg/disc, respectively, against the test organisms (Table 2). The activity was dose-related and statistically significant ($r^2 = 0.85$), especially against *S. aureus* ATCC 25923 (MZI, 40 mm), *V. parahemolyticus* AM 16362 (MZI, 40 mm), *Sarcina lutea* QL 166 (MZI, 39 mm), *Bacillus megaterium* QL 38 (MZI, 38 mm), *Shigella boydii* ATCC 13147 (MZI, 38 mm), *Shigella dysenteriae* ATCC 26131 (MZI, 38 mm), *Vibrio mimicus* N 1967 (MZI, 38 mm) and clinical isolate of a *Klebsiella* sp. (MZI, 38 mm). The inhibitor was also effective against all the four tested fungi and demonstrated significant antifungal activity against *Aspergillus fumigatus* ATCC 13073 (MZI, 32 mm), *C. albicans* ATCC 10231 (MZI, 31 mm), *Rhizopus oryzae* ATCC 20344 (MZI, 29 mm) and *Saccharomyces cerevisiae* AB 972 (MZI, 31 mm) at a dose of 200 μg/disc (Table 4). The three components of this isomeric mixture, viz., **12a**–**c** were purified using normal phase preparative TLC and were individually tested against the organisms. Majority of the data indicated 2,3-diacetylbenzofuran (**12c**) as the most potent compound while some indicated synergistic action (Tables 2 and 4).

Among the 2-aroylbenzofurans, 2-benzoylbenzofuran (**9**) demonstrated moderate activity against *Salmonella paratyphi* A AM 16590 (MZI, 21 mm), *Shigella sonnei* C 182 (MZI, 16 mm) and *Shigella boydii* ATCC 13147 (MZI, 15 mm) and *Vibrio mimicus* N 1967 (MZI, 15 mm) (Table 1). The mean inhibitory zone diameters for **9** range from 11 to 22, 10 to 15 mm against test bacteria (Table 1) and 15 to 21, 11 to 17 mm against fungi (Table 3) at 200 and 100 μg/disc dose levels, respectively. It was evident that compound **9** obtained from 2-((trimethylsilyl)ethynyl)phenyl acetate (**5**) was more potent than that of the corresponding isomeric mixture, synthesized from the unprotected 2-(trimethylsilyl)ethynyl phenol (**4**). Introduction of a methyl or a methoxy

Table 1. Spectrum of antibacterial activity of 2-benzo[*b*]furans against Gram-positive and Gram-negative clinical isolates

Organism	Diameters of zone of inhibition (MZI)[a]							
	1		**2**	**12**		**21**	Kan	Amp
	200	100	200	200	100	200	30	30
Aeromonus hydrophilia AM 10481	13 ± 2.83	NT	10 ± 0.71	11 ± 0.00	NT	NT	37 ± 0.71	35 ± 1.41
Bacillus cereus QL 29	24 ± 1.41	12 ± 0.00	07 ± 0.00	13 ± 0.00	10 ± 1.41	—	29 ± 0.00	NT
Bacillus megaterium QL 38	11 ± 2.12	11 ± 0.71	07 ± 0.00	22 ± 0.00	11 ± 0.71	09 ± 1.41	31 ± 0.00	32 ± 0.00
Bacillus subtilis QL 40	14 ± 0.71	08 ± 0.71	—	14 ± 0.00	12 ± 0.71	—	30 ± 0.00	—
Escherichia coli ATCC 25922	11 ± 2.83	10 ± 3.54	—	11 ± 1.41	10 ± 1.41	08 ± 0.00	28 ± 0.71	26 ± 0.71
Klebsiella sp.	11 ± 0.71	09 ± 2.83	—	13 ± 0.00	10 ± 0.00	—	26 ± 1.41	40 ± 0.71
Pseudomonas aeruginosa ATCC 27853	15 ± 0.71	09 ± 2.12	07 ± 0.00	14 ± 0.00	13 ± 0.00	—	33 ± 0.00	40 ± 0.71
Salmonella paratyphi A AM 16590	15 ± 1.41	NT	—	21 ± 2.12	NT	10 ± 0.00	31 ± 2.83	32 ± 1.41
Salmonella paratyphi B AM 15961	14 ± 0.00	NT	08 ± 0.71	12 ± 1.41	NT	10 ± 0.00	34 ± 0.00	32 ± 1.41
Salmonella typhi AM 16406	08 ± 1.41	NT	07 ± 0.00	11 ± 0.71	NT	NT	30 ± 0.00	35 ± 1.41
Sarcina lutea QL 166	09 ± 1.41	08 ± 2.12	07 ± 0.71	13 ± 0.71	13 ± 1.41	09 ± 0.71	29 ± 0.71	—
Shigella boydii ATCC 13147	NT	13 ± 1.41	—	15 ± 2.12	15 ± 0.71	—	32 ± 1.41	35 ± 0.00
Shigella dysenteriae ATCC 26131	07 ± 0.00	NT	—	12 ± 2.83	NT	NT	29 ± 1.41	37 ± 0.71
Shigella flexneriae Y 976	07 ± 0.00	15 ± 0.00	—	14 ± 1.41	11 ± 0.71	13 ± 0.00	33 ± 0.71	37 ± 1.41
Shigella sonnei C 182	29 ± 1.41	NT	08 ± 0.00	16 ± 0.00	NT	NT	28 ± 0.00	30 ± 1.41
Staphylococcus aureus ATCC 25923	11 ± 2.12	—	—	16 ± 0.71	12 ± 1.41	—	30 ± 0.71	40 ± 0.00
Vibrio cholerae 569 B	13 ± 0.00	NT	07 ± 1.41	12 ± 1.41	NT	NT	37 ± 0.71	51 ± 0.00
Vibrio mimicus N 1967	12 ± 2.83	09 ± 1.41	07 ± 0.71	15 ± 0.00	14 ± 2.83	12 ± 0.00	34 ± 0.00	35 ± 0.00
Vibrio parahemolyticus AM 16362	13 ± 1.41	NT	—	14 ± 0.00	NT	NT	29 ± 0.00	30 ± 0.71

Kan = kanamycin, Amp = ampicillin; NT = not tested and '—' indicates no sensitivity or MZI lower than 7 mm.
Doses are expressed in micrograms per disc.
Interpretation of sensitivity test results: Gram-positive; >18 mm (MZI) = sensitive; 14–18 mm (MZI) = intermediate; <14 mm (MZI) = resistant. Gram-negative bacteria >16 mm (MZI) = sensitive; 13–16 mm (MZI) = intermediate; <13 mm (MZI) = resistant.
[a] Values are means ± SD ($n = 2$).

Table 2. Comparison of antibacterial activity of the isomeric mixture of diacetylbenzofurans (**12abc**) and its components (**12a**–**c**) against Gram-positive and Gram-negative clinical isolates

Organism	Diameters of zone of inhibition (MZI)[a]								
	12abc				**12a**	**12b**	**12c**	Kan	Amp
	200	100	50	25	30	30	30	30	30
Aeromonus hydrophilia AM 10481	31 ± 1.41	NT	NT	NT	NT	NT	NT	37 ± 0.71	35 ± 1.41
Bacillus cereus QL 29	36 ± 1.41	33 ± 0.71	25 ± 0.00	18 ± 2.83	13 ± 0.71	11 ± 1.41	31 ± 0.71	29 ± 0.00	NT
Bacillus megaterium QL 38	38 ± 0.71	36 ± 0.00	32 ± 2.12	22 ± 1.41	15 ± 0.00	11 ± 0.71	11 ± 2.83	31 ± 0.00	32 ± 0.00
Bacillus subtilis QL 40	34 ± 1.41	29 ± 1.41	28 ± 0.71	21 ± 0.71	—	10 ± 2.12	23 ± 2.12	30 ± 0.00	—
Escherichia coli ATCC 25922	32 ± 2.12	29 ± 1.41	20 ± 0.71	18 ± 0.00	—	9 ± 0.00	21 ± 0.71	28 ± 0.71	26 ± 0.71
Klebsiella sp.	38 ± 1.41	30 ± 2.83	28 ± 1.41	19 ± 0.00	NT	NT	NT	26 ± 1.41	40 ± 0.71
Pseudomonas aeruginosa ATCC 27853	32 ± 0.00	31 ± 1.41	27 ± 2.12	21 ± 1.41	9 ± 0.71	12 ± 0.71	20 ± 0.00	33 ± 0.00	40 ± 0.71
Salmonella paratyphi A AM 16590	35 ± 1.41	32 ± 0.71	27 ± 0.71	18 ± 1.41	—	8 ± 1.41	25 ± 1.41	31 ± 2.83	32 ± 1.41
Salmonella paratyphi B AM 15961	33 ± 1.41	NT	NT	NT	7 ± 0.71	8 ± 2.12	36 ± 0.71	34 ± 0.00	32 ± 1.41
Salmonella typhi AM 16406	37 ± 0.71	NT	NT	NT	NT	NT	NT	30 ± 0.00	35 ± 1.41
Sarcina lutea QL 166	39 ± 1.41	32 ± 1.41	28 ± 0.71	26 ± 1.41	NT	NT	NT	29 ± 0.71	—
Shigella boydii ATCC 13147	38 ± 0.71	34 ± 0.00	29 ± 0.71	21 ± 1.41	NT	NT	NT	32 ± 1.41	35 ± 0.00
Shigella dysenteriae ATCC 26131	38 ± 0.71	NT	NT	NT	NT	NT	NT	29 ± 1.41	37 ± 0.71
Shigella flexneriae Y 976	37 ± 1.41	32 ± 0.00	31 ± 1.41	22 ± 0.71	NT	NT	NT	33 ± 0.71	37 ± 1.41
Shigella sonnei C 182	34 ± 0.71	NT	NT	NT	NT	NT	NT	28 ± 0.00	30 ± 1.41
Staphylococcus aureus ATCC 25923	40 ± 0.71	32 ± 1.41	29 ± 1.41	24 ± 2.83	—	10 ± 2.12	24 ± 0.71	30 ± 0.71	40 ± 0.00
Vibrio cholerae 569 B	37 ± 0.00	NT	NT	NT	NT	NT	NT	37 ± 0.71	51 ± 0.00
Vibrio mimicus N 1967	38 ± 1.41	31 ± 1.41	29 ± 0.00	19 ± 2.83	7 ± 0.00	—	18 ± 1.41	34 ± 0.00	35 ± 0.00
Vibrio parahemolyticus AM 16362	40 ± 0.71	NT	NT	NT	7 ± 0.00	15 ± 0.71	22 ± 1.41	29 ± 0.00	30 ± 0.71

Kan = kanamycin, Amp = ampicillin; NT = not tested and '—' indicates no sensitivity or MZI lower than 7 mm.
Doses are expressed in micrograms per disc.
Interpretation of sensitivity test results: Gram-positive; >18 mm (MZI) = sensitive; 14–18 mm (MZI) = intermediate; <14 mm (MZI) = resistant. Gram-negative bacteria >16 mm (MZI) = sensitive; 13–16 mm (MZI) = intermediate; <13 mm (MZI) = resistant.
[a] Values are means ± SD ($n = 2$).

functionality at the aroyl group, as demonstrated for compounds **10** and **11**, was inactive. This observation suggests the negating effects of the bulky group on antimicrobial activity, which may result from an increased steric hindrance at the para position. In the 2-alkyl(aryl)benzofuran series, compound **19** demonstrated

Table 3. Spectrum of antifungal activity of 2-benzo[*b*]furans against human fungal pathogens

Organism	Diameters of zone of inhibition (MZI)[a]							
	1		**2**	**12**		**21**	AmB	Nys
	200	100	200	200	100	200	30	30
Aspergillus fumigatus ATCC 13073	NT	14 ± 0.71	NT	18 ± 1.41	13 ± 1.41	8 ± 0.71	24 ± 0.71	20 ± 2.12
Candida albicans ATCC 10231	NT	8 ± 0.00	NT	16 ± 1.41	11 ± 0.71	—	19 ± 0.00	17 ± 0.71
Rhizopus oryzae ATCC 20344	NT	8 ± 0.00	NT	15 ± 2.83	12 ± 1.41	—	25 ± 0.00	21 ± 0.00
Saccharomyces cerevisiae AB 972	NT	13 ± 0.00	NT	21 ± 2.12	17 ± 3.54	—	25 ± 2.12	24 ± 0.71

AmB = amphotericin B, Nys = nystatin; NT = not tested and '—' indicates no sensitivity or MZI lower than 7 mm. Doses are expressed in micrograms per disc except for amphotericin B (units/disc).
[a] Values are means ± SD ($n = 2$).

Table 4. Comparison of antifungal activity of the isomeric mixture of diacetylbenzofurans (**12abc**) and its components (**12a**–**c**) against human fungal pathogens

Organism	Diameters of zone of inhibition (MZI)[a]								
	12abc				**12a**	**12b**	**12c**	AmB	Nys
	200	100	50	25	30	30	30	30	30
Aspergillus fumigatus ATCC 13073	32 ± 0.71	30 ± 1.41	30 ± 0.00	18 ± 0.71	10 ± 0.71	—	21 ± 0.71	24 ± 0.71	20 ± 2.12
Candida albicans ATCC 10231	31 ± 2.12	28 ± 0.71	28 ± 1.41	25 ± 0.71	—	13 ± 1.41	27 ± 1.41	19 ± 0.00	17 ± 0.71
Rhizopus oryzae ATCC 20344	29 ± 0.71	27 ± 2.83	25 ± 2.83	22 ± 1.41	NT	NT	NT	25 ± 0.00	21 ± 0.00
Saccharomyces cerevisiae AB 972	31 ± 2.83	28 ± 1.41	27 ± 2.83	18 ± 1.41	8 ± 0.00	9 ± 0.00	18 ± 1.41	25 ± 2.12	24 ± 0.71

AmB = amphotericin B, Nys = nystatin; NT = not tested and '—' indicates no sensitivity or MZI lower than 7 mm. Doses are expressed in micrograms per disc except for amphotericin B (units/disc).
[a] Values are means ± SD ($n = 2$).

only weak antibacterial activity with MZI of 8–13 mm at 200 μg/disc. Compound **20** in this series showed mild growth inhibition against *B. subtilis* QL 40 (MZI, 10 mm) and compound **23** against *B. megaterium* QL 38 (MZI, 10 mm). Compounds **21** and **22** were found to be completely resistant against the tested organisms and were, therefore, precluded from Tables 1 and 3. The inhibitory zone diameters for ampicillin, kanamycin (antibacterial) and amphotericin B, nystatin (antifungal) were referred to as reference values in this study.

The minimum inhibitory concentrations (MICs, μg/mL) of compounds **9** and **12abc** were determined against Gram-positive, *B. subtilis* QL 40 and *S. aureus* ATCC 25923 and Gram-negative *E. coli* ATCC 25922, *S. typhi* AM 16406, *P. aeruginosa* ATCC 27853, *V. parahemolyticus* AM 16362 plus a pathogenic fungus, *C. albicans* ATCC 10231 and were compared to those of ampicillin and nystatin. The results are presented in Table 5. The isomeric mixture **12abc** demonstrated MIC values comparable to or 1- to 2-fold lower than those of ampicillin against most of the Gram-positive and Gram-negative bacterial strains tested (Table 5). In particular, the MICs of **12abc** against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. typhi* AM 16406 were 8, 8 and 8 μg/mL, as compared to the MIC's of 8, 8, 32 μg/mL demonstrated by ampicillin against these strains. Compound **9** showed an MIC of 64 against *S. aureus* ATCC 25923 which was in agreement with the previously reported value.[25] The MICs demonstrated against the rest were 16–>128 μg/mL (Table 5).

Attempts were made to correlate the antimicrobial activities of these compounds to the calculated Log of partition coefficient (C Log *P*) for *n*-octanol/water by CS ChemDraw Ultra v8.03 (Table 6). Partition coefficient, which is well known as an index of lipophilicity, is an important physicochemical parameter in the development of antibacterial agent since it is closely related to the permeation through a lipid coat of bacteria.[26] The C Log *P* value of isomeric compounds **12a** and **12b** (1.79) was the smallest among the tested compounds. However, it was not comparable to those of strongly hydrophilic ampicillin (C Log $P = -1.87$) and kanamy-

Table 5. Spectrum of activity (MIC) against Gram-positive and Gram-negative clinical isolates

Organism	Strain No.	MIC (μg/mL)				
		9	**12abc**	Amp	AmB	Nys
Bacillus subtilis	QL 40	64	32	64	NT	NT
Escherichia coli	ATCC 25922	16	8	8	NT	NT
Pseudomonas aeruginosa	ATCC 27853	64	8	8	NT	NT
Salmonella typhi	AM 16406	>128	8	32	NT	NT
Staphylococcus aureus	ATCC 25923	64	16	32	NT	NT
Vibrio parahemolyticus	AM 16362	64	32	64	NT	NT
Candida albicans	ATCC 10231	64	16	NT	0.5	2

NT = not tested.

Table 6. The calculated *n*-octanol/water partition coefficient ($\text{Log}\,P_{ow}$) and yields % of benzofuran derivatives

Products	Yields (%)	C Log P[a]
9	81	3.74
10	83	3.96
11	81	4.24
12abc		
12a	26	1.79
12b	19	1.79
12c	33	1.86
19	82	4.80
20	81	4.79
21	83	5.32
22	85	3.20
23	84	2.36
24	81	1.66
Ampicillin	—	−1.87
Kanamycin	—	−5.17

[a] Log of partition coefficient (C Log P) for *n*-octanol/water was computed by using CS ChemDraw Ultra v8.03.

cin (C Log $P = -5.17$). The two compounds demonstrated mild antimicrobial activity with inhibitory zone diameters of 7–15 and 8–15 mm at 30 μg/disc against the bacterial and fungal strains tested. The most significant activity was observed by compound **12c** which has a C Log P value of 1.86. Mean inhibitory zone diameters for **12c** ranged from 11 to 36 at 30 μg/disc, which were comparable with standard antibiotics at the same dose level (Tables 2 and 4). The replacement of the 2-acetyl group by an aroyl/alkyl/aryl functionality and removing the additional acetyl group resulted in compounds with weaker or no antibacterial activity, which is in line with expectations for compounds with high C Log P value ranging from 2.36 to 5.32. Compounds **22** and **23** with C Log P values of 3.20 and 2.36 demonstrated only weak antimicrobial activity. On the contrary, compound **9** which has a higher C Log P value of 3.74, exhibited moderate antimicrobial activity (Tables 1 and 3). The other compounds in the activity study, except **24**, have higher C Log P values of 3.96–5.32 and were ineffective against the sensitive control and clinically resistant bacterial strains, fungi and were not studied for MICs. Thus, no direct correlation could be established between the C Log P and antimicrobial activity.

In this investigation three other compounds, viz., 2-acetylbenzofuran, benzofuran-2-carboxylic acid and benzofuran-2-carboxaldehyde from commercial source were screened against the same panel of organisms but none of them showed any activity at all. Therefore, the superior antimicrobial activity of 2,3-diacetylbenzofuran (**12c**) could be interpreted as an indication of a stronger intrinsic 'binding attraction' for the 3-acetyl group at the site of action. The repulsive Coulomb force between the carbonyl oxygen and the benzofuran oxygen was expected to align these heteroatoms *s-trans* and to make the conformation rigid which may account for best fitting to the macromolecular target. The tighter binding of **12c** over the mono acetyl derivative could be explained by an additional energetically favourable hydrogen bonding between the carbonyl oxygen (acceptor) at C3 and the Tyr/Ser/Thr hydroxy group (donor) in the biomolecule.[27] There was also a distinct possibility of hydrogen bonding between the ring nitrogen of at least one conserved His/Trp residue of the catalytic site and the ring oxygen of benzofurans.[27] Any reduction in hydrogen acceptor ability such as by replacing benzofuran molecular core and introducing poor electron donor like sulfur in bioisosteric thiophene analogs might reduce the activity significantly. The benzofuran ring could be embedded in a hydrophobic pocket of the binding site and thereby contributed in lowering the free energy of possible binary/ternary complexes. There might be little or no space for an extra substituent at the aromatic moiety. However, if there was any space whatsoever, it is not sufficient to accommodate an acetyl group. This observation was consistent with the SAR finding that acetylation at the C-4 (as in **12a**) and C-7 positions (as in **12b**) attenuated growth inhibitory activity (Table 2). However, it was apparent from previously conducted SAR studies that a hydroxyl/methoxy group at C_{5-7} position tended to increase the activity.[28] The moderate activity of 2-benzoylbenzofuran (**9**) can be explained by the aromatic–aromatic interaction between the C-2 benzoyl group with possible phenylalanine residues in a second hydrophobic pocket.[6] Because an aromatic ring bearing electron donors is known to interact with another aromatic ring less strongly than does an unsubstituted aromatic ring and vice versa, the activity of *p*-methyl or *p*-methoxy derivative of 2-benzoylbenzofuran (**10**, **11**) might be reduced. Thus, the current study outlines a hypothetic image of catalytic site for benzofurans in the target biomolecule with at least one hydrophobic pocket and two H-bond donors—a polar hydroxylated and an imino nitrogen containing amino acid residues. The broad spectrum activity suggests that the target is probably present in bacteria and fungi ubiquitously. Other structural refinements including substitution at the C_{4-7} positions of benzofuran ring, and the replacement of 3-acetyl group by other acyl/lower alkyl functionalities are to be envisaged for functional characterization of target biomolecule.

The cytotoxic potentials of compounds **11** and **12abc** (isomeric mixture of **12a**–**c**) were also determined at dilutions ranging from 1.56 to 200 μg/mL by the well-known brine shrimp lethality bioassay.[21] The tested compounds did not show any cytotoxic activity and showed the selectivity, in that they possess the potent antimicrobial activity without cytotoxicities against *Artemia salina* nauplii. It is logical to expect that these compounds may not have toxicities in mammalian cells as well.[21]

4. Experimental

All reactions were carried out under a nitrogen atmosphere. Melting points were recorded on an electrothermal melting point apparatus and paraffin oil bath and are uncorrected. UV and IR spectra were recorded on Shimadzu UV–vis and Shimadzu FTIR spectrophotometers, respectively. The ^{1}H NMR (400 MHz) and ^{13}C

NMR (100 MHz) spectra were acquired in $CDCl_3$ on an Ultra Shield Bruker DPX 400 spectrometer and the chemical shifts are reported in parts per million relative to the residual nondeuterated solvent signals. The number of attached protons for ^{13}C signals was determined using the DEPT 135 pulse sequence. HR-FABMS was recorded on a JEOL SX 102 mass spectrometer using *m*-nitrobenzyl alcohol (NBA) or polyethylene glycol (PEG) as matrix. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on Kieselgel gel 60 F_{254} pre-coated sheets (E. Merck) and the spots were detected by exposure to UV-lamp at 254 nm. Column chromatography was done on silica gel (60–120 mesh ASTM). Bis (triphenylphosphine)palladium(II) chloride, copper(I) iodide, acetyl chloride, benzoyl chloride, toluoyl chloride, anisoyl chloride, aluminum chloride, (trimethylsilyl)acetylene and the alkynes were purchased from E. Merck, Germany and Fluka, Switzerland. 2-Acetylbenzofuran, benzofuran-2-carboxylic acid and benzofuran-2-carboxaldehyde were bought from Sigma-Aldrich Company Ltd. The calculated partition coefficient (C Log *P*) values were determined by using the CS ChemDraw Ultra version 8.03, computer software by CambridgeSoft.Com.

4.1. General procedure for the synthesis of 2-(trimethylsilyl)ethynyl phenol (4)

To a stirred solution of 2-iodophenol **1** (4.00 g, 18.18 mmol), $Pd(Ph_3P)_2Cl_2$ (0.128 g, 0.18 mmol), CuI (0.07 g, 0.36 mmol) and Et_3N (10 mL) in dioxane/DMF (10 mL) were added $HC{\equiv}CSiMe_3$ (5 mL, 36.21 mmol). The reaction mixture was stirred at 45 °C for 5 h (24 h in the case of DMF) under N_2 atmosphere. The solvent was removed under reduced pressure. To a residue diethyl ether and 0.1 N HCl were added and the organic layer was separated, neutralized with a saturated $NaHCO_3$ (3× 50 mL) solution, washed with distilled water (3× 50 mL), dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The latter was purified by chromatography over a column of silica gel with hexane/chloroform (7:1) to obtain the title compound **4** (3.6 g, 95% when dioxane was used as a solvent and the product was 70% when DMF was used as a solvent) as a solid, mp 46–47 °C (lit.[29] mp 46–47 °C); IR (KBr): ν_{max} 3450, 2146, 842, 775, 776 cm^{-1}; UV ($CHC1_3$): λ_{max} 304, 296, 257, 287 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.34 (dd, 1 H, J = 1.4, 7.6 Hz), 7.24 (t, 1H, J = 7.6 Hz), 6.94 (br d, 1H, J = 7.6 Hz), 6.85 (t, 1H, J = 7.6 Hz), 5.83 (br s, 1H), 0.28 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 158.1, 131.7, 130.2, 120.5, 114.8, 109.5, 101.9, 99.2, −0.2.

4.2. General method for the synthesis of 2-((trimethylsilyl)ethynyl)phenyl acetate (5)

2-((Trimethylsilyl)ethynyl)phenyl acetate (**5**) was prepared from 2-acetoxyphenyl iodide (**2**) using the above Sonogashira coupling reaction.[22] The compound **5** was obtained as a light yellow liquid; yield 91%; IR (KBr): ν_{max} 2120, 1776, 1548, 1258 cm^{-1}; UV ($CHCl_3$): λ_{max} 304, 296, 257, 245 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.50 (dd, 1H, J = 7.6, 1.5 Hz), 7.34 (ddd, 1H, J = 1.6, 7.6, 7.6 Hz), 7.18 (t, 1H, J = 7.6 Hz), 7.07 (d, 1H, J = 7.6 Hz), 2.33 (s, 3H), 0.25 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 166.5, 151.9, 133.1, 129.8, 125.7, 125.6, 122.4, 117.1, 99.5, 77.0, −0.25.

4.3. General method for the synthesis of 2-aroylbenzofurans (9–11)

To an ice cold solution of 2-(trimethylsilyl)ethynyl phenol **4** or 2-((trimethylsilyl)ethynyl)phenyl acetate **5** (1.1 mmol) in tetrachloroethane (10 mL), aroyl chloride **6**–**8** (1.25 mmol) and anhydrous $AlCl_3$ (3 mmol) were added. The mixture was stirred under N_2 atmosphere for 3 h and the temperature of the reaction was raised from 0 °C to 25 °C. Then the mixture was poured into an ice cold solution of dilute HCl (2 mL, 1–1.5 N HCl) and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (3× 25 mL). The combined organic extracts were washed with distilled H_2O (2× 30 mL), saturated $NaHCO_3$ solution (2× 30 mL) and distilled H_2O (2× 30 mL) again. After drying over anhydrous Na_2SO_4 and removal of solvent a syrupy residue was obtained. The crude mass was purified by a silica gel column and yielded 2-aroylbenzofurans **9**–**11**.

4.3.1. 2-Benzoylbenzofuran 9. Yield 81%; mp 88–89 °C (lit.[15] 89 °C); IR (KBr): ν_{max} 1690, 1645, 1550 and 1548 cm^{-1}; UV ($CHCl_3$): λ_{max} 310, 286, 241 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, 1H, J = 7.2 Hz), 8.13 (d, 1H, J = 7.2 Hz), 7.81 (ddd, 1H, J = 1.6, 7.6, 8.0 Hz), 7.69 (d, 1H, J = 7.6 Hz), 7.63 (t, 1H, J = 7.6 Hz), 7.56 (dd, 1H, J = 7.2, 7.6 Hz), 7.49 (t, 1H, J = 7.6 Hz), 7.36 (dd, 1H, J = 7.2, 8.0 Hz), 7.11 (d, 1H, J = 7.6 Hz), 6.33 (s, 1H);^{13}C NMR (100 MHz, $CDCl_3$): δ_C 184.5, 156.4, 152.1, 139.1, 131.8, 129.1, 128.5, 128.2, 126.4, 125.3, 124.9, 118.2, 112.8.

4.3.2. 2-(*p*-Methoxybenzoyl)benzofuran 10. Yield 83%; mp 94–95 °C (lit.[15] 95–96 °C); IR (KBr): ν_{max} 1739, 1640, 1549 and 1510 cm^{-1}; UV ($CHCl_3$): λ_{max} 315, 265 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, 2H, J = 8.8 Hz), 7.61 (dd, 1H, J = 1.6, 8.0 Hz), 7.51 (dd, 1H, J = 1.6, 7.2 Hz), 7.35 (t, 1H, J = 7.2 Hz), 7.28 (dd, 1H, J = 7.2, 8.0 Hz), 7.00 (s, 1H), 6.94 (d, 1H, J = 8.8 Hz), 6.80 (dd, 1H, J = 3.6, 8.8 Hz), 3.88 (s, 3H), ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 186.7, 164.3, 163.9, 148.0, 132.6, 131.2, 130.2, 127.0, 126.3, 125.9, 123.6, 121.4, 114.1, 113.9, 113.8, 55.6.

4.3.3. 2-(*p*-Methylbenzoyl)benzofuran 11.[30] Yield 81%; light yellow liquid; IR (CCl_4): ν_{max} 1755, 1610, 1575, 1545 cm^{-1}; UV ($CHCl_3$): λ_{max} 276, 244 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, 1H, J = 8.0 Hz), 8.10 (d, 1H, J = 8.0 Hz), 7.74 (d, 1H, J = 8.0 Hz), 7.69 (d, 1H, J = 7.6 Hz), 7.35 (dd, 1H, J = 7.6, 8.0 Hz), 7.29 (d, 1H, J = 8.0 Hz), 7.28 (t, 1H, J = 8.0 Hz), 7.13 (dd, 1H, J = 3.2, 8.0 Hz), 7.05 (s, 1H), 2.36 (*s*, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 189.8, 165.2, 153.1, 144.4, 132.2, 131.0, 130.4, 129.2, 129.0, 128.9, 126.4, 126.3, 123.6, 115.3, 114.9, 21.8.

4.4. General method for the synthesis of diacetylbenzofurans (12a–c)

To an ice cold solution of 2-(trimethylsilyl)ethynyl phenol **4** or 2-((trimethylsilyl)ethynyl)phenyl acetate **5** (1.1 mmol) in tetrachloroethane (15 mL), acetyl chloride/acetic anhydride (2.5 mmol) and anhydrous $AlCl_3$ (5 mmol) were added. The mixture was stirred under N_2 atmosphere for 3 h and the temperature of the reaction was raised from 0 °C to 25 °C. Then the mixture was poured into an ice-cold solution of dilute HCl (2 mL, 1–1.5 N HCl) and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (3× 25 mL). The combined organic extracts were washed with distilled H_2O (2× 30 mL), saturated $NaHCO_3$ solution (2× 30 mL) and distilled H_2O (2× 30 mL) again. After drying over anhydrous Na_2SO_4 and removal of solvent a syrupy residue was obtained. The crude mass was purified by a silica gel column followed by preparative TLC over silica gel F_{254} using *n*-hexane/EtOAc (10:1) as eluting solvent (multiple developments) to yield diacetylbenzofurans **12a–c**.

4.4.1. 2,4-Diacetylbenzofuran 12a. Yield 26%; white solid; IR (CCl_4): ν_{max} 1776, 1770, 1676, 1548 and 1488 cm^{-1}; UV ($CHCl_3$): λ_{max} 275, 227 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.47 (dd, 1H, J = 7.6, 8.3 Hz), 7.30 (dd, 1H, J = 1.2, 7.6 Hz), 7.13 (dd, 1H, J = 0.9, 8.3 Hz), 6.52 (s, 1H), 2.46 (s, 3H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 196.0, 160.8, 147.1, 138.8, 131.8, 131.2, 129.8, 129.0, 126.6, 123.4, 31.9, 21.2; HR-FABMS: Calcd for $C_{12}H_{10}O_3$: 202.0630. Found: 202.0632.

4.4.2. 2,7-Diacetylbenzofuran 12b. Yield 19%; yellowish solid; IR (CCl_4): ν_{max} 1781, 1774, 1668, 1553 and 1501 cm^{-1}; UV ($CHCl_3$): λ_{max} 280, 228 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.48 (dd, 1H, J = 7.5, 8.3 Hz), 7.38 (dd, 1H, J = 1.7, 7.5 Hz), 7.19 (dd, 1H, J = 1.8, 8.3 Hz), 6.62 (s, 1H), 2.28 (s, 3H), 1.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 197.6, 162.3, 144.8, 143.5, 130.1, 129.7, 128.1, 127.6, 125.4, 124.2, 28.9, 20.1; HR-FABMS: Calcd for $C_{12}H_{10}O_3$: 202.0630. Found: 202.0632.

4.4.3. 2,3-Diacetylbenzofuran 12c. Yield 33%; white solid; IR (CCl_4): ν_{max} 1790, 1772, 1686, 1542 and 1503 cm^{-1}; UV ($CHCl_3$): λ_{max} 282, 225 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.59 (br d, 1H, J = 7.6 Hz), 7.47 (dd, 1H, J = 7.2, 7.6 Hz), 7.26 (d, 1H, J = 7.2 Hz), 7.16 (t, 1H, J = 7.2 Hz), 6.51 (s, 1H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 194.6, 188.2, 150.2, 141.9, 130.4, 129.2, 125.8, 121.4, 119.1, 118.3, 30.1, 25.4; HR-FABMS: Calcd for $C_{12}H_{10}O_3$: 202.0630. Found: 202.0632.

4.5. General method for the synthesis of 2-alkyl(aryl)benzofurans 19–24

To a well-stirred mixture of 2-iodophenol (**1**) or 2-acetoxyphenyl iodide (**2**) (2 mmol), $Pd(Ph_3P)_2Cl_2$ (2–3.5 mol %), CuI (3–5 mol %) and Et_3N (2 equiv.) in dioxane or DMF (6 mL) a terminal alkyne (**13–18**) was added under N_2 atmosphere. The mixture was stirred at 70 °C for 20 h. After removal of the solvent under reduced pressure the mixture was cooled, poured into distilled water (100 mL) and extracted with chloroform (3× 50 mL). The combined extracts were washed with 5 M aq NaOH (3× 50 mL) and water (3× 50 mL). The organic layer was then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified through silica-gel column chromatography to afford 2-alkyl(aryl)benzofurans (**19–24**).

4.5.1. 2-Phenylbenzofuran 19. Yield 82% (dioxane), 75% (DMF); mp 116–118 °C (lit.[15] 118–120 °C); IR (KBr): ν_{max} 1593, 1562, 1455, 1257 cm^{-1}; UV ($CHCl_3$): λ_{max} 317, 307, 261, 240 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (dd, 1H, J = 1.6, 7.6 Hz), 7.61–7.24 (m, 8H), 7.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 156.0, 154.6, 133.1, 129.2, 129.0, 128.8, 128.6, 128.4, 124.2, 123.1, 121.6, 120.8, 111.3, 101.4.

4.5.2. 2-Butylbenzofuran 20.[31] Yield 81% (Dioxane), 71% (DMF), light yellow liquid; IR (CCl_4): ν_{max} 1590, 1570, 1480, 1250 cm^{-1}; UV ($CHCl_3$): λ_{max} 285, 278, 250 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, 1H, J = 6.4 Hz), 7.42 (d, 1H, J = 7.6 Hz), 7.26–7.17 (m, 2H), 6.39 (s, 1H), 2.79 (t, 2H, J = 7.6 Hz), 1.54–1.42 (m, 4H), 0.98 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 159.6, 153.1, 129.0, 124.1, 123.0, 121.6, 111.8, 101.9, 30.8, 28.5, 21.3, 13.4.

4.5.3. 2-Pentylbenzofuran 21.[32] Yield 83%; light yellow liquid; IR (CCl_4): ν_{max} 1590, 1560, 1489, 1240 cm^{-1}; UV ($CHCl_3$): λ_{max} 285, 278, 250 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.50 (dd, 1H, J = 1.6, 6.8 Hz), 7.44 (dd, 1H, J = 1.6, 7.6 Hz), 7.25–7.20 (m, 2H), 6.40 (s, 1H), 2.79 (t, 2H, J = 7.6 Hz), 1.43–1.39 (m, 6H), 0.95 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 160.6, 153.1, 128.6, 125.6, 123.2, 121.5, 112.3, 102.1, 30.8, 29.3, 28.7, 22.6, 14.0.

4.5.4. Ethyl-2-benzofurancarboxylate 22.[33] Yield 85%; light yellow liquid; IR (CCl_4): ν_{max} 1720, 1697, 1679, 1612, 1332, 1195 cm^{-1}; UV ($CHCl_3$): λ_{max} 301, 294, 284, 207 nm; ^1H NMR (400 Mz, $CDCl_3$): δ 7.83 (dd, 1H, J = 1.2, 7.6 Hz), 7.36 (dd, 1H, J = 7.6, 8.0 Hz), 7.06 (d, 1H, J = 8.0 Hz), 6.95 (s, 1H), 6.94 (t, 1H, J = 7.6 Hz), 4.19 (q, 2H, J = 7.1 Hz), 1.27 (t, 3H, J = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 168.9, 159.0, 151.8, 130.2, 126.6, 123.4, 121.9, 114.5, 111.8, 56.8, 13.0.

4.5.5. Dimethyl-2-(2-benzofurylmethyl)propanedioate 23.[34] Yield 84%; light yellow liquid; IR (CCl_4): ν_{max} 1759, 1743, 1548, 1251, 1220 cm^{-1}; UV ($CHCl_3$): λ_{max} 303, 295, 284, 205; ^1H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, 1H, J = 6.0 Hz), 7.43 (d, 1H, J = 6.8 Hz), 7.26–7.21 (m, 2H), 6.51 (s, 1H), 3.93 (t, 1H, J = 6.8 Hz, CH), 3.78 (s, 6H), 3.44 (d, 2H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 173.8, 171.9, 160.5, 151.2, 129.0, 125.6, 123.2, 119.6, 111.8, 101.9, 52.4, 49.2, 27.1.

4.5.6. 2-Hydroxymethylbenzofuran 24.[15] Yield 81%; light yellow liquid; IR (CCl_4): ν_{max} 3330, 1460, 1250 cm^{-1};

UV ($CHCl_3$): λ_{max} 283, 276, 247, 208 nm; ^1H NMR (400 MHz, $CDCl_3$): δ 7.60 7.50 (m, 2H), 7.40–7.24 (m, 2H), 6.69 (s, 1H), 4.75 (s, 2H), 2.17 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 159.7, 156.3, 128.8, 124.6, 123.9, 121.1, 113.0, 103.5, 69.1.

4.6. Microbiology

Antimicrobial susceptibility testing was performed by the standardized disk diffusion and the agar dilution methods of the National Committee for Clinical Laboratory Standards.[20] Inhibitory zone diameters were measured on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) (Difco Lab.) for fungi, with conventional metrical filter paper disks (BBL, Cocksville, USA, 6 mm in diameter) containing specified doses of compounds. All experiments were conducted twice and repeated if the results differed. The inhibitory zone diameters were read with a calliper, and all results were rounded up to the nearest whole numbers (millimetre) for analysis. For each tested strain, the growth conditions and the sterility of the medium were checked in two negative controls. The results obtained were compared with standard antibiotics, ampicillin, kanamycin (30 μg/disc), amphotericin B (100 units/disc) and nystatin (30 μg/disc) and discs bought from Mast Diagnostics, UK.

The minimum inhibitory concentrations (MICs, μg/mL) were determined on brain heart infusion broth (Difco Lab.) containing dilutions of antimicrobial agents ranging from 0.25 to 128 μg/mL. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted tenfold when inoculated, reducing the final solvent concentration to 1%. The innocuity of the DMSO was checked at this concentration using negative controls. The final microbial concentration for inocula was of 1×10^6–5×10^6 CFU/mL, and was incubated at 35 °C for 24 h. The MIC was defined as the lowest drug concentration that inhibited the viable growth of organisms. The assay was repeated whenever trailing end points were encountered.

Acknowledgments

Financial support from the Ministry of Science and Information and Communication Technology, Government of the People's Republic of Bangladesh, is gratefully acknowledged. The authors also thank Professor C. M. Hasan, Faculty of Pharmacy, University of Dhaka, Dhaka, and Dr. John Sorensen, OCMS, and the Department of Chemistry, CRL, Oxford, for their kind cooperation in structural elucidation.

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