Phenyl Hydrazone Derivatives of Benzofuran: Synthesis and Preliminary Evaluation of Antimicrobial Activity

Abstract

A series of (*Z*)-1-benzo[*b*]furan-2-yl-3-(substituted phenyl)prop-2-en-1-one 1-phenylhydrazone derivatives **(C_1-C_{12})** of benzofuran were synthesized in satisfactory yield and pharmacologically evaluated for their *in vitro* antimicrobial activity. All the synthesized compounds were in good agreement with elemental and spectral data. A majority of the tested compounds showed good to moderate antimicrobial activity against all tested pathogenic bacterial and fungal strains.

Keywords: Benzofuran, Phenylhydrazone, Antimicrobial activity

Introduction

Antimicrobials reduce or completely block the growth and multiplication of bacteria. This has made them unique for the control of deadly infectious diseases caused by a variety of pathogens. They have transformed our ability to treat infectious diseases such as pneumonia, meningitis, tuberculosis, malaria and AIDS [1].These agents are those inhibitory chemicals which are employed to kill microorganisms or prevent their growth**.** Infectious diseases account for approximately one-half of all deaths in tropical countries. Although deaths from bacterial and fungal infections have dropped in the developed world, these are still major causes of death in the developing world [2]. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants) [3].According to WHO, each year 1.4 million children died of gut infections and diarrhoea caused by gram-negative bacteria like *Pseudomonas, Salmonella, Shigellae* and gram positive rods like *Corynebacterium* diptheriae [4].Decades of antibiotic use have resulted in the development of widespread resistance to commonly prescribed antibacterial agents[5]. Therefore, there is a need to develop new, potent, fast-acting antimicrobial drugs with low toxicity. In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased antimicrobial activity [6].

Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, benzofuran still remain as one of the most versatile class of compounds against microbes and therefore, are useful substructures for further molecular exploration. Benzofuran's literature is enriched with progressive findings of the moiety in respect of antimicrobial activity [7]. Benzofuran and its derivatives constitute the most versatile and valuable source of antimicrobial compounds. They appear to transcend the chemotherapeutic boundaries of other antiparasitic drugs with a spectrum of activity that includes the majority of fungi, bacteria, protozoa and helminthic species. The prime objective for the current study is to developnovel derivatives of benzofuran moiety and finally screen them against different microbial strains (bacteriaand fungi) at variable concentrations. The rationale for the study includes the designing of the derivatives having some common structural features that are important for the compound to exhibit an antimicrobial activity that includes the following:[8–10]

1. A lipohilic bicyclic aromatic ring system.
2. Another bulky lipophilic group (e.g. phenyl, *tert*butyl)as a side chain.
3. Two lipophilic domain linked by a spacer of appropriatelength with polar centre at defined position,for example, Naftifine, Butenafine, Terbinafine, Debacarb, Penicillins and Cephalosporins.

In view of the above mentioned facts and in continuation of our interest in the synthesis of heterocycles containing benzofuran moiety, to identify new candidates that may be of value in designing new, potent, selectiveand less toxic antimicrobial agents, we report herein the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating the benzofuran moiety with phenylhydrazone through different linkages. This combination was suggested in an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The substitution pattern of benzofuran ring was carefully selected so as to confer different electronic environment to themolecules.

Experimental

All the solvents were of AR grade and were obtained from SDFCL Ltd., Himedia, Central drug House (p) Ltd and Loba-chemicals. All the microorganisms used in the present screening were

procured from the Department of Microbiology, Gulbarga University, Gulbarga. Melting points were determined inopen capillary tubes and are uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within ± 0.4% with the calculated ones. Thin layer chromatography was performed on silica gel G (Merck). The spots were developedin an iodine chamber and visualized with an ultraviolet lamp. The solvent systems used were benzene:acetone (8:2, v/v) and toluene:ethylacetate:formic acid (5:4:1, v/v). Ashless Whatman No. 1 filter paper was used for vacuum filtration. The IR spectra were recorded in KBr pelletson a (BIO-RAD FTS 135) WIN-IR spectrophotometer. The FAB mass spectra of all the compounds were recorded on a JEOL SX102/DA-600mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas.The 1H-NMR spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in $CDCl_3$ using tetramethylsilane (Me_4Si, TMS) asan internal standard. The chemical shifts are reported in the δ ppmscale [18]. The physicochemical &spectral data of the synthesized title compounds were listed in **Table 1 & 2**.

General procedure for the synthesis of 1-(1-benzofuran-2-yl) ethanone (B)

The mixture of salicylaldehyde (0.1 mole) **(A)**, chloroacetone (0.1 mole) and anhydrous potassium carbonate (30 g) were gently refluxed in dry acetone (150 ml) for 13 hr. The reaction product after cooling was filtered and the filtrate on the removal of the solvent under reduced pressure furnished 2-acetyl benzofuran as dark yellow colored solid. The product obtained was recrystallized from petroleum ether.

General procedure for the synthesis of (Z)-1-benzo[*b*]furan-2-yl-3-(Substituted phenyl) prop-2-en-1-one (B_1–B_{12})

A solution of 2-acetyl benzofuran (1.6 ml, 0.01 mole) and various aromatic aldehyde (0.01 mole) in ethanol (4.6 ml) was cooled to 5 to 10^0C in an ice bath. The cooled solution was treated with drop wise addition of aqueous sodium hydroxide (4 ml, 50%). The resulting dark solution was diluted with ice water and carefully acidified using diluted hydrochloric acid. The benzofuran analogues of chalcone which were crystallized were collected by filtration after washing with sodium bicarbonate and water. It was further purified by re-crystallization from ethanol.

General procedure for the synthesis of (Z)-1-benzo[*b*]furan-2-yl-3-(Substituted phenyl)prop-2-en-1-one-1-Phenylhydrzone (C_1–C_{12})

A mixture of phenylhydrazine hydrochloride (1.44 g, 0.01 mole), sodium acetate (0.82 g, 0.01 mole) in ethanol (10 ml) was stirred at RT for 10 min. To this ethanolic solution, (Z)-1-

benzo[*b*]furan-2-yl-3-(Substituted phenyl)prop-2-en-1-one **(B_1–B_{12})** (0.01 mole) was added slowly. The resulting reaction- mixture was allowed to stir for about 2 hr and completion of the reaction was monitored by TLC. The reaction-mixture was then poured into ice water (50 ml) where upon the crude compound was precipitated as yellow solid. The residue obtained after filtration was washed with water and dried. The crude product was purified by recrystallization from absolute alcohol.

Biological Activity

All the synthesized compounds were tested for their *in vitro* antimicrobial activity against the bacteria *Enterococcus fecalis* ATCC-29212*, Bacillus subtilus*, *Escherichia coli* ATCC-25923*,* and *Pseudomonas aurigenosa* ATCC-27853 in the nutrient agar media, and fungi *Candida albicans* NLTM-3431*, Aspergillus niger* MTCC 281in Sabouraud dextrose medium at 100 and 50 µg/mL concentrations by using serial plate dilution method (11, 12). The minimum inhibitory concentrations (MIC's) values were determined by comparison to Amoxycillin and Gresiofulvin as reference drugs for bacterial and fungal activity, respectively, as shown in Tables 3. Standard antibiotic Amoxycillin and Gresiofulvinwere used as reference drug at 50 and 25 µg/mL concentrations. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms on the plate.

RESULTS AND DISCUSSION

Chemistry

(Z)-1-benzo[*b*]furan-2-yl-3-(Substituted phenyl) prop-2-en-1-one-1-Phenylhydrzone were prepared according to the procedure outlined in Scheme 1. The required 1-(1-benzofuran-2-yl) ethanone (**B**) was synthesized by reacting salicylaldehyde **(A)** and dry chloroacetone in the presence of anhydrous potassium carbonate. To a cooled solution of 1-(1-benzofuran-2-yl) ethanone **(B)** and aromatic aldehydes in ethanol, sodium hydroxide(50%) was added drop wise to yield the chalcones of benzofuran **(B_1-B_{12})**.These chalcones were stirred at room temperature with phenylhydrazine hydrochloride and sodium acetate in ethanol for 2 hours to precipitate the title compounds **(C_1-C_{12})**. The product was then recrystallized from absolute ethanol. The structure of synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR, MS).

Antimicrobial activity

The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **(C_1-C_{12})** showed good to moderate inhibition at 50-100 μg/mL in DMSO. The compounds**C_1, C_2, C_3, C_5, C_6, C_7, C_8, C_{10}, C_{11} & C_{12}** showed comparatively moderate to good activity against all the bacterial and fungal strains. The good activity is attributed to the presence of pharmacologically active 4-nitro (**C_1**), 2-chloro (**C_2**), 4-chloro (**C_3**),4-methoxy **(C_5)**, 4-Bromo **(C_7)**, 4-methyl **(C_8)**, 4-hydroxy **(C_{10})**, 2-hydroxy **(C_{11})**, 2-methyl **(C_{12})** groups attached to phenyl group at position 3 of the benzofuran ring. When these groups were replaced by 3-chlorophenyl (**C4**), phenyl (**C6**) and 3-nitropheny **(C9)**, a sharp decrease in activity against all of the microbial strains were observed. It has been observed that the compounds synthesized showed significant antimicrobial activity stating the importance of electron withdrawing substituent's on the phenyl group.

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Table 1 Physicochemical data of the synthesized compounds **(B_1-B_{12})** & **(C_1-C_{12})**

Compounds	Substitution (R)	Mol. formula	Mol. Wt.	MP (^oC)	R_f	% Yield
B_1	*p*-Nitrobenzaldehyde	$C_{17}H_{11}NO_4$	293	80	0.55	25.8
B_2	*o*-Chlorobenzaldehyde	$C_{17}H_{11}ClO_2$	283	73	0.67	29.4
B_3	*p*-Chlorobenzaldehyde	$C_{17}H_{11}ClO_2$	283	140	0.34	39.6
B_4	*m*-Chlorobenzaldehyde	$C_{17}H_{11}ClO_2$	283	130	0.54	19.8
B_5	*p*-Anisaldehyde	$C_{18}H_{14}O_3$	278	172	0.78	33
B_6	Benzaldehyde	$C_{17}H_{12}O_2$	248	68	0.87	57.78
B_7	*p*-Bromobenzaldehyde	$C_{17}H_{11}BrO_2$	327	120	0.35	48.95
B_8	*p*-Tolualdehyde	$C_{18}H_{14}O_2$	262	85	0.66	39.84
B_9	*m*-Nitrobenzaldehyde	$C_{17}H_{11}NO_4$	293	120	0.73	38.25
B_{10}	*p*-Salicyaldehyde	$C_{17}H_{12}O_3$	264	145	0.22	39.69
B_{11}	*o*-Salicyaldehyde	$C_{17}H_{12}O_3$	264	140	0.46	42.12
B_{12}	*o*-Tolualdehyde	$C_{18}H_{14}O_2$	262	60	0.37	18.32
C_1	*p*-Nitrobenzaldehyde	$C_{23}H_{17}N_3O_3$	382	95	0.66	94.77
C_2	*o*-Chlorobenzaldehyde	$C_{23}H17ClN_2O$	372	55	0.70	54.02
C_3	*p*-Chlorobenzaldehyde	$C_{23}H_{17}ClN_2O$	372	190	0.72	21.50
C_4	*m*-Chlorobenzaldehyde	$C_{23}H17ClN_2O$	372	135	0.65	47.84
C_5	*p*-Anisaldehyde	$C_{24}H_{20}N_2O_2$	368	145	0.55	37.77
C_6	Benzaldehyde	$C_{23}H_{18}N_2O$	338	100	0.47	38.46
C_7	*p*-Bromobenzaldehyde	$C_{23}H_{17}BrN_2O$	417	140	0.43	50.83
C_8	*p*-Tolualdehyde	$C_{24}H_{20}N_2O$	352	125	0.69	76.98
C_9	*m*-Nitrobenzaldehyde	$C_{23}H_{17}N_3O_3$	383	190	0.73	41.25
C_{10}	*p*-Salicyaldehyde	$C_{23}H_{18}N_2O_2$	354	120	0.40	64.12
C_{11}	*o*-Salicyaldehyde	$C_{23}H_{18}N_2O_2$	354	125	0.45	28.00
C_{12}	*o*-Tolualdehyde	$C_{24}H_{20}N_2O$	352	85	0.45	71.30

Elemental analysis were found to be within ± 0.4% of theoretical values.

185 **Table 2** Spectral data of the title compounds (C_1-C_{12})

Compounds	Substitution (R)	Spectral Data
C_1	*p*-Nitrobenzaldehyde	IR (KBr, cm^{-1}):1535 & 1350 (-NO2), 2849 & 2732 (Ar-CH), 1104 (C-O-C), 3310 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm): 7.36- 8.51 (m, 5H, Benzofuran), 7.21-7.32 (m, 5H, Ar-H).8.11 (1H, s, NH); MS (m/z): 382(M^+)
C_2	*o*-Chlorobenzaldehyde	IR (KBr, cm^{-1}):747 (-Cl), 2920 (Ar-CH), 1129 (C-O-C), 3316 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.32- 8.56 (m, 5H, Benzofuran), 7.20-7.28 (m, 5H, Ar-H).8.14 (1H, s, NH); MS (m/z): 372 (M^+)
C_3	*p*-Chlorobenzaldehyde	IR (KBr, cm^{-1}):757(-Cl), 2845 (Ar-CH),1088 (C-O-C), 3314 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.34- 8.56 (m, 5H, Benzofuran), 7.18-7.22 (m, 5H, Ar-H).8.15 (1H, s, NH); MS (m/z): 372 (M^+)
C_4	*m*-Chlorobenzaldehyde	IR (KBr, cm^{-1}):749 (-Cl), 2883 (Ar-CH), 1139 (C-O-C), 3305 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm): 7.35- 8.58 (m, 5H, Benzofuran), 7.19-7.24 (m, 5H, Ar-H).8.13 (1H, s, NH); MS (m/z): 372 (M^+)
C_5	*p*-Anisaldehyde	IR (KBr, cm^{-1}):2841 (OCH3-CH), 1109 (C-O-C), 3319 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.36- 8.57 (m, 5H, Benzofuran), 7.13-7.18 (m, 5H, Ar-H).8.12 (1H, s, NH); MS (m/z): 368 (M^+)
C_6	Benzaldehyde	IR (KBr, cm^{-1}):3066 & 3025 (Ar-CH), 1131 (C-O-C), 3317 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.33- 8.51 (m, 5H, Benzofuran), 7.16-7.26 (m, 5H, Ar-H).8.15 (1H, s, NH); MS (m/z): 338 (M^+)
C_7	*p*-Bromobenzaldehyde	IR (KBr, cm^{-1}):546-503 (-Br), 2851 (CH-Ar), 1067 (C-O-C), 3308 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.36- 8.52 (m, 5H, Benzofuran), 7.23-7.27 (m, 5H, Ar-H).8.17 (1H, s, NH); MS (m/z): 417 (M^+)
C_8	*p*-Tolualdehyde	IR (KBr, cm^{-1}):2922 (CH3-CH), 2865 (Ar-CH), 3316 (N-H) , 1118 (C-O-C) ; ^1H NMR (DMSO-d_6, δ ppm):7.37- 8.54 (m, 5H, Benzofuran), 7.17-7.25 (m, 5H, Ar-H).8.17 (1H, s, NH); MS (m/z): 352(M^+).
C_9	*m*-Nitrobenzaldehyde	IR (KBr, cm^{-1}):1350 (-NO2), 1150 (C-O-C), 3304 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.36- 8.57 (m, 5H, Benzofuran), 7.21-

		7.27 (m, 5H, Ar-H).8.13 (1H, s, NH); MS (m/z): 383 (M^+)
C_{10}	*p*-Salicyaldehyde	IR (KBr, cm^{-1}):3576 &3494 (-OH), 3021 (Ar-CH),1107 (C-O-C), 3309 (N-H); ^1H NMR (DMSO-d_6, δ ppm): 7.33- 8.51 (m, 5H, Benzofuran), 7.21-7.25 (m, 5H, Ar-H).8.16 (1H, s, NH); MS (m/z): 354(M^+)
C_{11}	*o*-Salicyaldehyde	IR (KBr, cm^{-1}):3468 & 3421 (-OH), 3069 (Ar-CH),1139 (C-O-C), 3319 (N-H) ; ^1H NMR (DMSO-d_6, δ ppm):7.32- 8.56 (m, 5H, Benzofuran), 7.14-7.19 (m, 5H, Ar-H).8.14 (1H, s, NH); MS (m/z): 354 (M^+)
C_{12}	*o*-Tolualdehyde	IR (KBr, cm^{-1}):2926 (CH3-CH), 1087 (C-O-C), 3313 (N-H); ^1H NMR (DMSO-d_6, δ ppm):7.36- 8.54 (m, 5H, Benzofuran), 7.23-7.29 (m, 5H, Ar-H).8.18 (1H, s, NH); MS (m/z): 352 (M^+).

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188 **Table 3** Antimicrobial activity of the synthesized title compounds (C_1-C_{12})

Compounds	zone of inhibition in mm and MIC in µg/mL (Antibacterial)				zone of inhibition in mmand MIC in µg/mL (Antifungal)	
	Enterococcus fecalis	*Bacillus subtilus*	*Escherichia coli*	*Pseudomonas aurigenosa*	*Candida albicans*	*Aspergillus niger*
C_1	16(50)	18(50)	14(50)	17(50)	19(50)	17(50)
C_2	14(50)	17(50)	13(50)	18(50)	15(50)	19(50)
C_3	18(50)	16(50)	19(50)	17(50)	18(50)	20(50)
C_4	5(100)	3(100)	8(100)	7(100)	2(100)	6(100)
C_5	17(50)	13(50)	15(50)	18(50)	16(50)	12(50)
C_6	3(100)	7(100)	4(100)	6(100)	4(100)	9(100)
C_7	18(50)	15(50)	14(50)	16(50)	13(50)	17(50)
C_8	19(50)	20(50)	14(50)	17(50)	18(50)	15(50)
C_9	9(100)	6(100)	4(100)	7(100)	6(100)	8(100)
C_{10}	14(50)	12(50)	14(50)	19(50)	15(50)	19(50)
C_{11}	13(50)	16(50)	18(50)	16(50)	20(50)	20(50)
C_{12}	17(50)	18(50)	17(50)	15(50)	19(50)	16(50)
Amoxycillin	28(25)	24(25)	26(25)	27(25)	30(25)	29(25)
Griseofulvin	26(25)	27(25)	29(25)	26(25)	27(25)	26(25)

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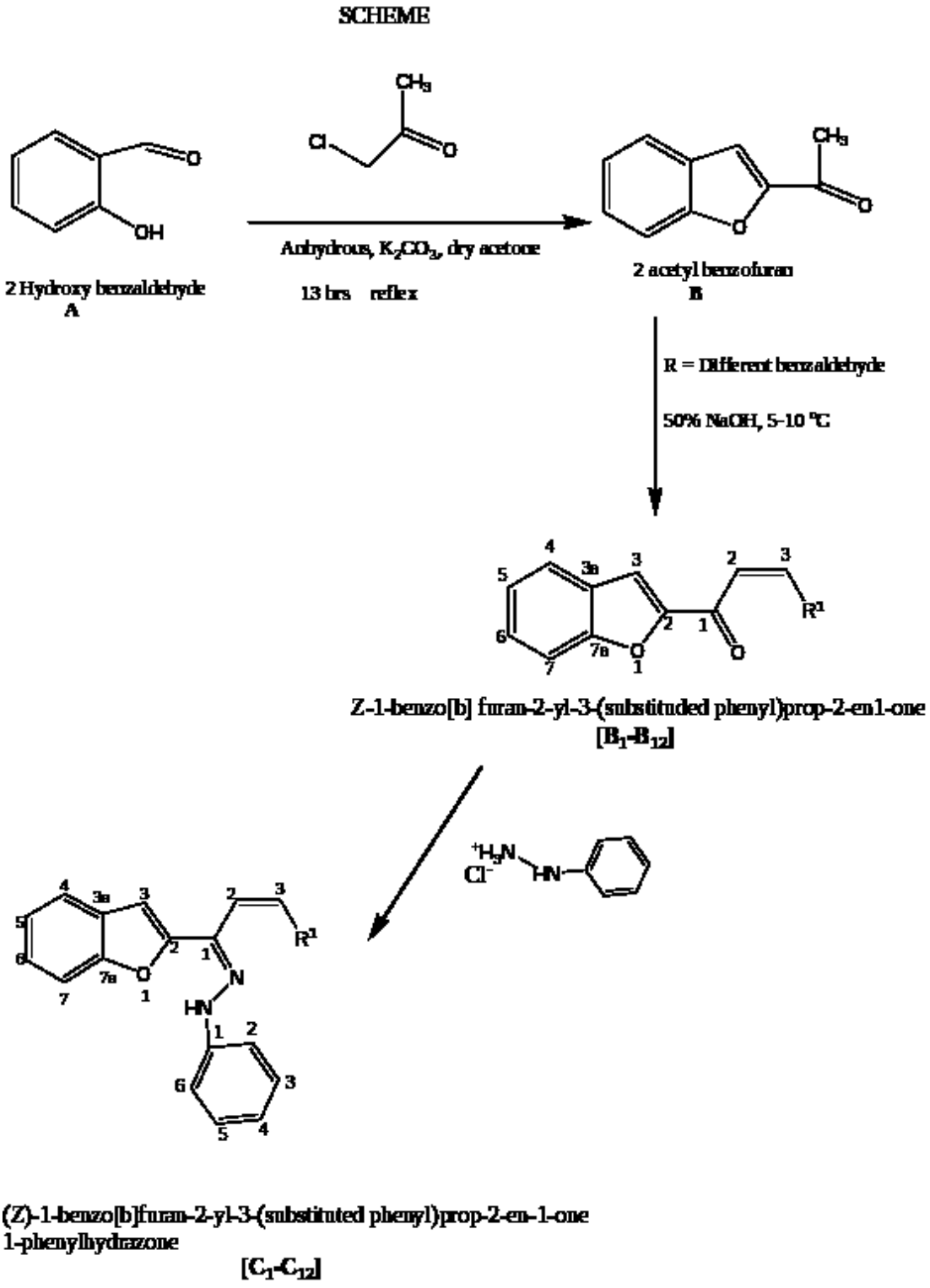

Scheme 1 Synthesis of Phenylhydrazone derivatives of Benzofuran