Asian Journal of Chemical Sciences

2(3): 1-7, 2017; Article no.AJOCS.34772
ISSN: 2456-7795

Phenyl Hydrazone Derivatives of Benzofuran: Synthesis and Their Antimicrobial Activities

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Authors' contributions

This work was carried out in collaboration between all authors. Author SA designed and performed the study. Authors ARP and MT managed the analyses of the study and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOCS/2017/34772
Editor(s):
(1) Aleksandra Sentkowska, Heavy Ion Laboratory, University of Warsaw, Poland.
Reviewers:
(1) Rajeev Singh, University of Delhi, India.
(2) Abdullahi M. Nuhu, Kaduna Polytechnic, Nigeria.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/19769

Original Research Article

Received 10th June 2017
Accepted 22nd June 2017
Published 29th June 2017

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 with 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.

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1. 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 country [2]. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immune compromised 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 develop novel derivatives of benzofuran moiety and finally screen them against different microbial strains (bacteria and 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 lipophilic 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 appropriate length 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, selective and 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 the molecules.

2. 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 in open 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 developed in 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 pellets on a (BIO-RAD FTS 135) WIN-IR

spectrophotometer. The FAB mass spectra of all the compounds were recorded on a JEOL SX102/DA-600 mass 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) as an internal standard. The chemical shifts are reported in the δ ppm scale. The physicochemical &spectral data of the synthesized title compounds were listed in Tables 1 and 2.

2.1 General Procedure for the Synthesis of 1-(1-benzofuran-2-yl) ethanone (B) [7]

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.

2.2 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°C 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.

2.3 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.

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

Compounds	Substitution (R)	Mol. formula	Mol. Wt.	MP (°C)	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	$C_{23}H_{17}Br\ N_2O$	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

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) ; ^{1}H 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); ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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) ; ^{1}H 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); ^{1}H 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) ; ^{1}H 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); ^{1}H 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^+).

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.

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 mm and 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)

2.4 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 Gresiofulvin were 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.

3. RESULTS AND DISCUSSION

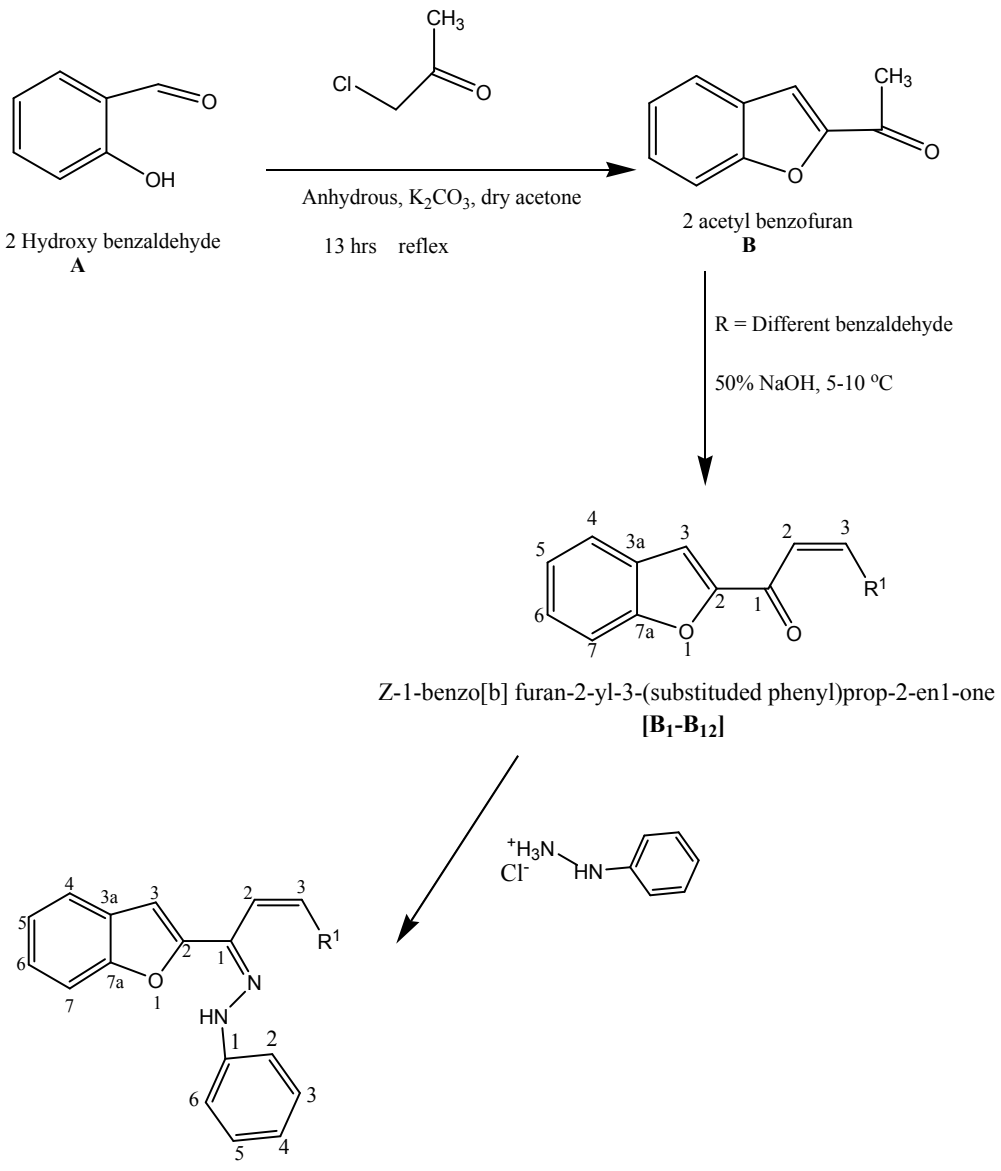
3.1 Chemistry

(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).

3.2 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.

SCHEME



Scheme 1. Synthesis of phenylhydrazone derivatives of benzofuran

4. CONCLUSION

Thus, various derivatives of phenylhydrazone **(C_1-C_{12})** were prepared with the objective of developing better antimicrobial agents. All the derivatives were found to have a promising class of compounds with an interesting pharmacological profile. Among these the compounds, **C_8**and **C_{11}** showed maximum antibacterial and antifungal activity, respectively. Hence, it is clear from structure activity relationship (SAR), that the compounds

synthesized showed significant antimicrobial activity stating the importance of electron withdrawing substituent's on the phenyl group. In conclusion, the benzofuran incorporated hydrazone derivatives can be regarded as a newer class of antimicrobial agents. They were also found to be less toxic which indicates better tolerability of the compounds having strong future prospects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gilani SJ, Khan SA, Verma SP, Mullick P,. Alam O, Siddiqui N. Synthesis and *in vitro* antimicrobial activity of novel *N*-(6-chlorobenzo [*d*] thia zol-2-yl) hydrazine carboxamide derivatives of benzothiazole class. Journal of Enzyme Inhibition and Medicinal Chemistry. 2011;26(3);332-340.
2. Gilani SJ, Khan SA, Siddiqui N. Synthesis and *in vitro* antimicrobial evaluation of condensed heterocyclic 6-substituted 1,2,4-triazolo-[3,4-*b*]-1,3,4- thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid. Acta Poloniae Pharmaceutica. 2011;68(2):205-211.
3. Gilani SJ, Maurya DP, Katiyar D, Goel R,. Nagarajan K, Khan SA. Synthesis, antifungal and toxicity screening of newer isoniazid derivatives. Medicinal Chemistry. 2014;4(4):428-434.
4. Nogrady T, Weaver FD. Medicinal chemistry: A molecular & biochemical approach. Oxford University Press. 2005;559–582.
5. Thomasco LS, Gadwood CR, Weaver EA, Ochoada JM, Ford CW, Zurenko GE, Hamel JC, Stapert D, Moerman JK, Schaadt RD, Yagi BH. The synthesis and antibacterial activity of 1,3,4-Thiadiazole phenyl oxazolidinone analogues. Bioorganic and Medicinal Chemistry Letters. 2003;13:4193–4196.
6. Onkol T, Dogruer DS, Uzun L, Adak S, Ozkan S, Sahin MF. Synthesis and antimicrobial activity of new 1,2,4 -triazole and 1,3,4-thiadiazole derivatives. Journal of Enzyme Inhibition and Medicinal Chemistry. 2008;23:277–284.
7. Basawaraj R, Goled SN, Parmeshwarappa G, Sangapure SS. Synthesis and biological activity of some pyridyl substituted benzofurans. Indian Journal of Heterocyclic Chemistry. 2009;18:325-328.
8. Nussbaumer P, Leitner I, Stütz A. Synthesis and structure-activity relationships of the novel homo propargylamine anti mycotics. Journal of Medicinal Chemistry. 1994;37:610–615.
9. Nussbaumer P, Leitner I, Mraz K, Stütz A. Synthesis and structure activity relationships of side-chain-substituted analogs of the allylamine antimycotic terbinafine lacking the central amino function. Journal of Medicinal Chemistry. 1995;38:1831–1836.
10. Chongxi YU. Transdermal delivery systems of beta-lactam antibiotics. International application no: PCT.IB2006/054724[Online]WO/2008/072032.
Available:http://www.wipo.int/pctdb/en/wo.jsp
(Accessed on 27 July 2010)
11. Barry AL. Procedure for testing antimicrobial agents in agar media. In antibiotics in laboratory medicine. Corian V.L. Ed. Williams and Wilkins, Baltimore. 1991;1.
12. Verma SR, Khan KZ, Singh AP. Antifungal agents: Past, present and future prospects. National Academy of Chemistry and Biology, Lucknow, India. 1998;55.

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/19769