ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 3, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

SYNTHESIS OF ARYL (5-SUBSTITUTED BENZOFURAN-2-YL) CARBAMATE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Received: 25 November 2016, Revised and Accepted: 20 December 2016

ABSTRACT

Objectives: Benzofurans are very interesting heterocycles, which are available in nature and show a wide range of pharmacological activities, *viz.,* antifungal, antibacterial, antitumor, antimalarial and antioxidant activity.

Methods: A convenient method for the preparation of aryl (5-substituted benzofuran-2-yl) carbamate derivatives 6a-6j has been developed. The target compounds 1-(5-nitrobenzofuran-2-yl)-3-arylurea (6a-6e) and 1-(5-bromobenzofuran-2-yl)-3arylurea (6f-6j) have been prepared by reacting 5-nitrobenzofuran-2-carbonyl azide 5a or 5-bromobenzofuran-2-carbonyl azide 5b with substituted phenols in reasonable overall yields. All the synthesized compounds were characterized using Fourier transform infrared (FTIR), ^{1}H NMR and mass spectrometry and were subjected to antimicrobial screening against two Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungi (*Candida albicans* and *Aspergillus niger*) using two-fold dilution method.

Results and Discussion: All the values of FTIR, ^{1}H NMR and mass spectra were found to be prominent. The results indicate that synthesized compound 6i showed potent antimicrobial activity comparable to standard.

Conclusion: The detailed synthesis, spectroscopic data, and antimicrobial activities of synthesized compounds were reported.

Keywords: Benzofuran, Antibacterial activity, Antifungal activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i3.16332

INTRODUCTION

Benzofuran is one of the most important classes of fused ring heterocyclic compounds. The benzofuran derivatives are naturally occurring and possess many biological applications [1-3]. Angelicin, psoralen, and bergapten are the examples of naturally occurring benzofuran derivatives with biological applications [4-6]. The isolation of benzofuran derivatives from natural sources is laborious and time-consuming. Hence, the synthetic chemists are interested in synthesizing the benzofuran derivatives. Numerous synthesized benzofuran derivatives were found to be biologically active [7-9]. Nowadays, many synthetic benzofurans are used as good inhibitor [10,11], antimicrobial [12-14], anti-inflammatory [15,16], antiviral [17], antioxidant [18-21], antitumor [22], antiproliferative [23,24], and antialzheimer [25].

Fight against the microbes is never ending battle. The harmful microbe's poses biggest problem in the society as far as health and hygiene is concerned. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the 20th Century. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. One part of the problems is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture. As the resistance to antimicrobial agents increasing day by day, it is very necessary to synthesize new compounds which will show less bacterial resistance and good inhibitory activity [26].

Hence, this paper highlights the simple convenient method of synthesis of benzofuran derivatives and antimicrobial evaluation. All the synthesized compounds were characterized using Fourier transform infrared (FTIR), ^{1}H NMR and mass spectrometry and were subjected to minimum inhibitory concentration (MIC) antimicrobial screening against two Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungi (*Candida albicans* and *Aspergillus niger*) using two-fold dilution method.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma-Aldrich, SD Fine, Spectrochem, Merck, and Himedia. Yields refer to purified products and are not optimized. Melting points were determined on a VEEGO-VMP I melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO-FTIR 4100 spectrophotometer. ^{1}H NMR was recorded on a MERCURY VARIAN 400 MHz instrument and chemical shifts (δ) were reported in parts per million with dimethyl sulfoxide as the solvent. Trimethylsilane was used as the internal standard for NMR. Mass spectroscopy (MS) analyses were done on an Applied Biosystem API 2000. Thin layer chromatography was performed on precoated aluminum plates with silica gel.

Methods

General method for synthesis of 5-substituted benzofuran-2-carbonyl azide (5a-5b): 5-substituted benzofuran-2-carbohydrazide 4a-4b (0.02 mole) was dissolved in a mixture of 30 ml of acetic acid and 30 ml of 1,4-dioxane and cooled to 0°C using ice salt bath. An ice cold

Table 1: Characterization and Physical Properties of Synthesised 5-substituted benzofuran derivatives by IR, 1H-NMR and Mass Spectroscopy

Name of compound	IR in cm^{-1}	^1H NMR DMSO-d6:d ppm	ESI-MS (m/z) $[M+H]^+$ %	M.P.	Yield in %
Phenyl (5-nitrobenzofuran-2-yl) carbamate (6a)	-NH stretch at 3364 cm^{-1} -CH stretch at 2930 cm^{-1} -C=O stretch at1716 cm^{-1} -C=C-Ar stretch at 1526 cm^{-1} -CH bending- at 1434 cm^{-1} C-N stretch at 1334 cm^{-1}	7.39-7.82 (m, 9H, ArH), 10.50 (s, 1H, NH)	299.3	148-150°C	52
4-methoxyphenyl (5-nitrobenzofuran-2-yl) carbamate (6b)	-NH stretch at 3344 cm^{-1} -CH stretch at 2920 cm^{-1} -C=O stretch at1726 cm^{-1} -C=C-Ar stretch at 1516 cm^{-1} -CH bending- at 1424 cm^{-1} C-N stretch at 1335 cm^{-1}	4.10(s, 3H, OCH3) 6.97-8.57 (m, 8H, ArH), 9.57 (s, 1H, NH)	329.3	145-147°C	56
2-chlorophenyl (5-nitrobenzofuran-2-yl) carbamate (6c)	-NH stretch at 3361 cm^{-1} -CH stretch at 2910 cm^{-1} -C=O stretch at1715 cm^{-1} -C=C-Ar stretch at 1525 cm^{-1} -CH bending- at 1430 cm^{-1} C-N stretch at 1335 cm^{-1}	7.38-7.83 (m, 8H, ArH), 9.96 (s, 1H, NH)	333.7	198-200°C	69
4-chlorophenyl (5-nitrobenzofuran-2-yl) carbamate (6d)	-NH stretch at 3364 cm^{-1} -CH stretch at 2930 cm^{-1} -C=O stretch at1717 cm^{-1} -C=C-Ar stretch at 1526 cm^{-1} -CH bending- at 1434 cm^{-1} C-N stretch at 1334 cm^{-1}	7.38-7.82 (m, 8H, ArH), 9.97 (s, 1H, NH)	333.6	196-198°C	45
ethyl (5-nitrobenzofuran-2-yl) carbamate (6e)	-NH stretch at 3344 cm^{-1} -CH stretch at 2920 cm^{-1} -C=O stretch at1726 cm^{-1} -C=C-Ar stretch at 1516 cm^{-1} -CH bending- at 1434 cm^{-1} C-N stretch at 1335 cm^{-1}	1.28-1.319 (t, 3H, CH3) 3.47-3.51 (m, 2H, CH2) 7.15-8.67 (m, 4H, ArH), 9.92 (s, 1H, NH)	251.2	78-80°C	65
phenyl (5-bromobenzofuran-2-yl) carbamate (6f)	-NH stretch at 3344 cm^{-1} -CH stretch at 2920 cm^{-1} -C=O stretch at1726 cm^{-1} -C=C-Ar stretch at 1516 cm^{-1} -CH bending- at 1424 cm^{-1} C-N stretch at 1335 cm^{-1}	7.39-7.82 (m, 9H, ArH), 10.51 (s, 1H, NH)	333.2	140-142°C	65
4-methoxyphenyl (5-bromobenzofuran-2-yl) carbamate (6g)	-NH stretch at 3361 cm^{-1} -CH stretch at 2910 cm^{-1} -C=O stretch at1715 cm^{-1} -C=O axial deformation at 1640 cm^{-1} -C=C-Ar stretch at 1525 cm^{-1} -CH bending- at 1430 cm^{-1}	4.20 (s, 3H, OCH3) 6.97-8.57 (m, 8H, ArH), 9.61 (s, 1H, NH)	363.2	138-140°C	69

(Contd...)

Table 1: *(Continued)*

Name of compound	IR in cm^{-1}	^1H NMR DMSO-d6:d ppm	ESI-MS (m/z) $[M+H]^+$ %	M.P.	Yield in %
2-chlorophenyl (5-bromobenzofuran-2-yl) carbamate (6h)	-NH stretch at 3364 cm^{-1} -CH stretch at 2930 cm^{-1} -C=O stretch at1717 cm^{-1} -C=O axial deformation at 1630 cm^{-1} -C=C-Ar stretch at 1526 cm^{-1} -CH bending- at 1434 cm^{-1}	7.38-7.83 (m, 8H, ArH), 9.99 (s, 1H, NH)	367.6	169-171°C	54
4-chlorophenyl (5-bromobenzofuran-2-yl) carbamate (6i)	-NH stretch at 3344 cm^{-1} -CH stretch at 2920 cm^{-1} -C=O stretch at1726 cm^{-1} -C=O axial deformation at 1630 cm^{-1} -C=C-Ar stretch at 1516 cm^{-1} -CH bending- at 1434 cm^{-1}	7.38-7.82 (m, 8H, ArH), 9.98 (s, 1H, NH)	367.5	146-150°C	39
ethyl (5-bromobenzofuran-2-yl) carbamate (6j)	-NH stretch at 3344 cm^{-1} -CH stretch at 2910 cm^{-1} -C=O stretch at1715 cm^{-1} -C=O axial deformation at 1670 cm^{-1} -C=C-Ar stretch at 1526 cm^{-1} -CH bending- at 1432 cm^{-1}	1.28-1.32(t, 3H, CH3) 3.47-3.52 (m, 2H, CH2) 7.17-8.65(m, 4H, ArH), 9.90 (s, 1H, NH)	285.1	71-73°C	55

solution of sodium nitrite (0.02 mole) in water (10 ml) was introduced in small portions with vigorous stirring while the temperature of the mixture was maintained below 2°C. After addition was completed, the reaction mixture was allowed to stay at room temperature for 30 min and then solid was collected, washed with cold water. Solid was dried in desiccator and used immediately in next reaction [27].

General method for synthesis of aryl (5-substituted benzofuran-2-yl) carbamate derivatives (6a-6j: A mixture of 5-substituted benzofuran-2-carbonyl azide 5a-5b (5 mmol) and substituted phenol/ethanol (5 mmol) in dry toluene (20 mL) was refluxed for 4 hrs. The resulting solid, so formed, was collected and recrystallized from dioxane to give compounds 6a-6j as crystals.

Antimicrobial activity

Sterilized test tubes were numbered 1 through 9. All of the following steps were carried out using aseptic technique. A solution of 0.2 ml of 2000 µg/ml test stock solution in dimethyl sulfoxide (DMSO) was transferred to a first sterile test tube containing 3.8 ml of double strength nutrient broth to arrive (100 µg/ml) as initial dose and remaining test tubes 2-9 were filled with 2 ml of double strength nutrient broth for the further dilution. DMSO as a control has no effect at 12.5% concentration against bacteria. These test tubes were serially diluted to give a concentration of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg/ml.

One test tube with no test compound but with an equal volume of solvent DMSO (5%) served as the vehicle control. One test tube with no test compound and no vehicle but only with nutrient media served as the positive control to ensure the growth property of media. To all the test tubes 0.1 ml of suspension of bacteria (working inocula) were added, and the test tubes were incubated at 35-37°C for 24 hrs in case of bacteria and the test tubes were incubated at 25-27°C for 48 hrs in case of fungi. The highest dilution of the test compound that completely inhibited the growth of test organism was considered as the MIC value of the test compound and was expressed in µg/ml [26].

RESULT AND DISCUSSION

First, the following compounds were synthesized with resultant good yield such as synthesized 5-nitrobenzofuran-2-carbonyl azide 5a and 5-bromobenzofuran-2-carbonyl azide 5b from 5-nitro benzofuran-2-carbohydrazide 4a and 5-bromo benzofuran-2-carbohydrazide 4b by reported method and as per Fig. 1. The target compounds 6a-6j were synthesized from 5-nitrobenzofuran-2-carbonyl azide 5a and 5-bromobenzofuran-2-carbonyl azide 5b, respectively, by reacting with appropriate phenol. The structures of synthesized compounds were characterized by IR, ^1H NMR and mass spectrometry.

As from the synthesis of 5-nitro benzofuran-2-carbonyl azide (5a), the practical yield was found 55%, MP recorded in the range of 94-96°C.

Similarly, from the synthesis of 5-bromo benzofuran-2-carbonyl azide (5b), the practical was found 48%, MP ranges from 131-133°C.

The details of characterizations of synthesized 5-substituted benzofuran derivatives and physical Properties as shown in Table 1.

Antimicrobial activity

The benzofuran derivatives 6a-6j were evaluated for antimicrobial activity against two Gram-positive bacteria *S. aureus* (ATCC 6538P) and *B. subtilis* (ATCC 6633); two Gram-negative bacteria *E. coli* (ATCC 8739) *and P. aeruginosa* (ATCC 9027); two fungal strains *C. albicans* (ATCC 10231) *and A. niger* (ATCC 9029). Azithromycin and fluconazole were used as standard controls. The minimum inhibition concentration as shown in Tables 2 and 3.

CONCLUSION

We report herein the synthesis, structural elucidation, and antimicrobial activities of 10 new benzofurans 6a-6j. The structures

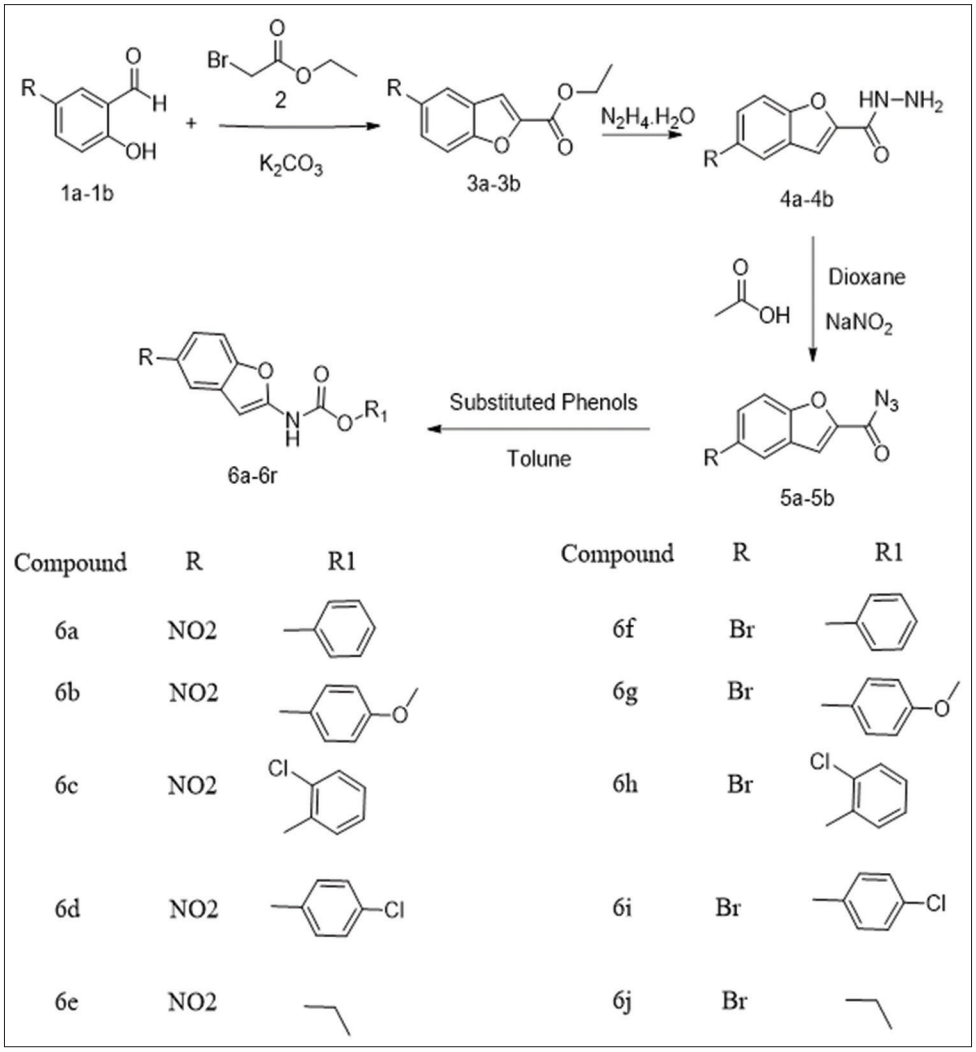

Fig. 1: General scheme of overall synthesis of 5-substituted benzofuran and their derivatives

Table 2: Minimum Inhibitory Concentration (MIC) of Test Compounds 6a to 6r against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa.

Test Comp.	MIC (µg/ml)			
	Staphylococcus aureus	*Bacillus Subtilis*	*Escherichia Coli*	*Pseudomonas aeruginosa*
6a	>100	100	100	100
6b	50	25	25	50
6c	50	25	50	50
6d	25	6.25	6.25	6.25
6e	50	50	100	50
6f	25	12.5	25	25
6g	25	25	50	25
6h	50	25	25	50
6i	25	12.5	6.25	12.5
6j	100	50	50	100
6k	100	50	50	100
6l	100	50	100	100
6m	50	50	50	100
6n	50	50	50	50
6o	6.25	1.56	6.25	3.125
6p	12.5	1.56	6.25	12.5
6q	25	12.5	12.5	12.5
6r	25	12.5	12.5	6.25
Azithromycin	0.39	1.56	12.5	6.25

are fully supported by spectroscopic data. All the synthesized compounds were evaluated for their antimicrobial activities by the two-fold dilution method. Compounds 6d, 6f, 6g, and 6i exhibited reasonably high degree of antibacterial activities against *S. aureus,*

Table 3: MIC of test compounds 6a to 6j against *C. albicans* and *A. niger*

Test compound	MIC (µg/ml)	
	C. albicans	*A. niger*
6a	25	12.5
6b	12.5	12.5
6c	50	25
6d	12.5	25
6e	25	12.5
6f	25	25
6g	25	25
6h	12.5	25
6i	12.5	25
6j	25	25
Fluconazole	12.5	12.5

MIC: Minimum inhibitory concentration, *C. albicans: Candida albicans*, *A. niger*: *Aspergillus niger*

B. subtilis, E. coli, and *P. aeruginosa*. The other compounds exhibited varied degree of antibacterial activity. Compounds 6b, 6d, 6e, 6h, and 6i exhibited high degree of antifungal activities against both *C. albicans* and *A. niger* while the other compounds showed moderate to weak antifungal activity.

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