Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2021, 10 (4): 438-444

(International Peer Reviewed Journal)

Synthesis, Characterization and Antimicrobial Activity of Benzofuran Derivatives

H. M. Naveena Kumari, Manjunath Harihara Mathada and K. M. Basavaraja*

Department of Chemistry and Industrial Chemistry, Vijayanagara Sri Krishnadevaraya University, Ballari-583105, Karnataka, **INDIA**

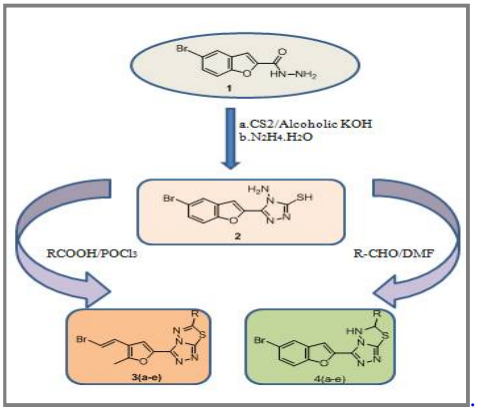
Email: kmbasavaraja@gmail.com

Accepted on 7th June, 2021

ABSTRACT

The literature survey reveals that the heterocycles triazoles and triazolothiadiazole derivatives have exhibited diverse biological properties. In continuation of our search for biologically potent benzofuran heterocycles linked to other nitrogen heterocycles we now reporting the synthesis of 5-bromobenzofuranyl triazolothiadiazoles. The desired compounds were achieved by treating our key compound 5-bromobenzofuran-2-carboxylic acid hydrazide ***(1)*** *with carbon disulphide and KOH in ethanol to get the potassium salt which was an intermediate, which on further treatment with hydrazine hydrate gave 4-amino-5-(5-bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol* ***(2).*** *The compound* ***2*** *on reaction with various aromatic carboxylic acids in $POCl_3$ furnished 6-(aryl)-3-(5-bromobenzofuran-2-yl)-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazoles* ***3(a-e)****. The compound* ***2*** *when treated with various benzaldehydes in DMF yielded the cyclised products 6-(aryl)-3-(5-bromo benzofurandihydro-[1,2,4]triazole[3,4-b][1,3,4]thiadiazoles* ***4(a-e)****.*

Graphical Abstract:



5-bromobenzofuran-2-carboxylic acid hydrazide ***(1)*** *4-amino-5-(5-bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol* ***(2).*** *6-(aryl)-3-(5-bromobenzofuran -2-yl)-[1,2,4]triazolo[3,4-b] [1,3,4] thiadiazoles* ***3(a-e)****. 6-(aryl)-3-(5-bromo-benzofurandihydro-[1,2,4]triazole[3,4-b][1,3,4]thiadiazoles* ***4(a-e)****.*

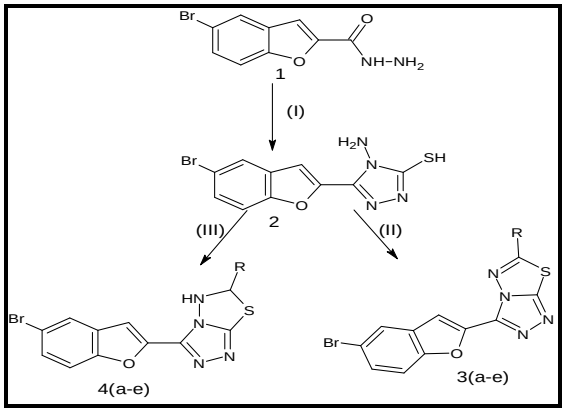
Keywords: Benzofuran, hydrazide, triazoles, triazolothiadiazole and antimicrobial activity.

INTRODUCTION

Owing to their therapeutic properties in various applications, the heterocyclic systems containing nitrogen have drawn attention to the synthesis for the past decades [**1-2**]. 4-Triazoles combined with another heterocyclic system demonstrated a wide range of applications including antibacterial, antiviral, anti-hypertensive, depressive, anti-inflammatory, anticonvulsant, antitumor, antifungal, antitubercular, analgesic pesticides, anthelmintic, herbicides, lubricants, colorants and analytical reagents [**3-7**]. Among these, triazoles combined with thiadiazoles were the most popular systems incorporated in a wide variety of therapeutically significant compounds with a wide spectrum of biological activities described above. Novel analogs of triazolothiadiazoles were synthesized by the introduction of a new substituent and modifying the parent skeletons. Heterocycles especially triazolothiadiazolesare the central structural moieties for different compounds in various activities in the fields such as pharmaceutical and agrochemical industries [**8-12**].

MATERIALS AND METHODS

All reagents and solvents were used as of analytical grade. IR spectra were taken on FTIR-Perkin Elmer Spectrum-Two spectrophotometers (range: (IR) 4000-400 cm^{-1}) m (KBr disc method). ^{1}HNMR spectra were recorded on BRUKER 400 MHz and ECX-JEOL 400(S), AVIII400 (L) using $CDCl_3$ and $MSOD_6$ as TMS as an internal reference. Mass Spectra were recorded in water model-synapt G2, APCI source Positive mode, Desolvation 200 L h^{-1}. Melting points were determined in open capillary tubes and were uncorrected.



Conditions:**(i)** CS_2in alcoholic KOH/$N_2H_4.H_2O$;(ii) R-COOH/$POCl_3$; (iii)R-CHO/DMF;
R:**a**=C_6H_5, **b**=$C_6H_4NO_2$(P), **c**=$C_6H_4OCH_3$(p), **d**=C_6H_4Cl (m), **e**=C_6H_4OH

Scheme 1. Synthetic route for 5-bromobenzofuranyltriazolo-thiadiazoles

Preparation of 5-bromobenzofuran-2-carboxylic acid hydrazide (1): A mixture of 5-bromo benzofuran-2-carboxylic acid ethyl ester (0.01 mol) and hydrazine hydrate 99.9% (5 mL) in ethanol (30 mL) was refluxed for 6 h under boiling water bath. Excess ethanol was removed under reduced pressure and then diluted with ice cold water. The solid separated was collected and was recrystallized as colorless needles from ethanol.

Preparation of 4-amino-5-(5-bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol (2): The 5-bromobenzofuran-2-carboxylic acid hydrazide (**1**) (0.01 mol)was treated with KOH (1.6 g) containing absolute ethanol (40 mL) at room temperature. This reaction mixture was added with carbon disulphide (2.3 g, 0.013 mol) and the reaction contents were stirred at room temperature for 12 h. The contents were first turned to in light yellow color and then the intermediate yellow colored salt crystals were separated. The product was filtered and washed with ether. Without further purification

the newly formed potassium salt was used for the next step. The 99% hydrazine hydrate (0.02 mol) was gradually added to the intermediate potassium salt (0.01 mol) dissolved in water (25 mL) and continuous stirring and refluxed for 4 h during which hydrogen sulphide formed and the reaction mixture changed to dark green color. Further the reaction mixture was cooled to 5^{o}C and acidified with dilute HCl. The light cream colored product formed was separated out by filtration. The solid product was washed with water until washings are neutral and recrystallized by using the aqueous ethanol. Yield 80%, melting point 165°C.

General procedure for the synthesis of 6-(aryl)-3-(5-bromobenzofuran-2-yl)-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazoles (3a-e): 4-Amino-5-(5-Bromo-benzofuran-2-yl)-4H-[1,2,4] triazole-3-thiol (**2**) (0.01 mol) was mixed with benzoic acid/substituted benzoic acid (0.01 mol) and 5 mL phosphorus oxychloride (5 mL) was added and were refluxed for 2 h. The excess phosphorus oxychloride was removed under reduced pressure. The residue was poured on the crushed ice with stirring. The product obtained was collected by filtration, washed with dilute solution of sodium bicarbonate followed by water and dried. It was further crystallized by using suitable solvent.

The percentage yield, melting point, solvent used for crystallization of the products **3(a-e)** were tabulated in table 1.

General procedure for the synthesis of 6-(aryl)-3-(5-bromobenzofuran-2-yl)-5,6-dihydro-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazoles-4(a-e): 4-Amino-5-(5-Bromobenzofuran-2-yl)-4H-[1,2,4] triazole-3-thiol **2**(0.01 mol.) was reacted with benzaldehyde/substituted benzaldehyde (0.01 mol) in dimethyl formamide (15 mL) and refluxed for about 20 h. The product formed in the reaction contents was cooled, poured on crushed ice and filtered, later it was dried, crystallized by using suitable solvent.

The percentage yield, melting point, solvent used for crystallization of the products 4**(a-e)** were tabulated in table 1.

Table 1. Physical data of compounds

Compound	Substituent 'R'	Yield (%)	M.P. (^{o}C)	Solvent	Mol. Formula
1	-	90	210	Ethanol	$C_9H_7BrN_2O_2$.
2	-	80	165	Ethanol	$C_9H_7BrN_2O_2$
3a	C_6H_5	80	189	Ethanol	$C_{17}H_9BrN_4OS$
3b	$C_6H_4NO_2$(p)	75	200	Ethanol	$C_{17}H_9BrN_5O_3S$
3c	$C_6H_4OCH_3$(p)	82	210	Ethanol	$C_{18}H_{12}BrN_4O_2S$
3d	C_6H_4Cl(m)	75	213	Ethanol	$C_{17}H_9BrClN_4OS$
3e	C_6H_4OH(p)	68	193	Dioxane	$C_{17}H_{10}BrN_4O_2S$
4a	C_6H_5	187	68	Ethanol	$C_{17}H_{11}BrN_4OS$
4b	$C_6H_4NO_2$(p)	191	81	Ethanol	$C_{17}H_{11}BrN_5O_3S$
4c	$C_6H_4OCH_3$(p)	207	80	Dioxane	$C_{18}H_{14}BrN_4O_2S$
4d	C_6H_4Cl(m)	206	82	Dioxane	$C_{17}H_{11}BrClN_4OS$
4e	C_6H_4OH(p)	197	70	Dioxane	$C_{17}H_{12}BrN_4O_2S$

RESULTS AND DISCUSSION

5-bromobenzofuran-2-carboxylic acid hydrazide (1): 5-Bromobenzofuran-2-carboxylic acid ethyl ester was treated with hydrazine hydrate (99%)in ethanol to get the corresponding hydrazide named as 5-Bromobenzofuran-2-carboxylic acid hydrazide (**1**) which was a key intermediate to construct derivatives of 5-bromobenzofuran derivatives .The structure of compound **1** was confirmed by its IR, ^{1}HNMR and Mass spectra. IR spectrum of compound **1** exhibited the absorption peak at 1672 cm^{-1} indicates the presence of carbonyl group, further the peaks at 3185 cm^{-1} and 3314 cm^{-1} confirms the

presence of the presence of -NH and -NH_2groups respectively. ^{1}HNMR spectrum in $CDCl_3$ adds data which supported the formation of the compound **1**. The signals at δ4.6 ppm and δ10.11 ppm indicates the presence of- NH_2 and –NH protons, the resonance of aromatic protons were observed as multiplet at range δ7.48-8.0 ppm. The Mass spectrum exhibited the molecular ion peak *m/z* at 256 and 258 which proved the formation of assigned structure to the compound **1.**

4-amino-5-(5-bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol(2): The compound **1** was treated with carbon disulphide and KOH in ethanol, to get the potassium salt which was an intermediate, which on further treatment with hydrazine hydrate gave 4-Amino-5-(5-Bromobenzofuran-2-yl)-4H-[1,2,4] triazole-3-thiol (**2).** The IR spectrum of the compound**2** exhibited sharp band at 3187cm^{-1} due to symmetric stretching frequency of -NH_2. The appearance of new bands at 2886, 1505 and 998cm^{-1} were assigned to -SH, C-N and C=S absorptions respectively.The^{1}H NMR and mass spectra of **2**were recorded which adds the evidence for the proposed structure. The ^{1}H NMR spectrum in DMSO-D_6 was exhibited singlet at δ5.96 ppm due to -NH_2 protons. In the range of δ7.57-8.06 ppm a multiplet was observed due to aromatic protons and a singlet was observed at δ14.20 ppm due to SH protons. The molecular ion peak in the mass spectrum was appeared at *m/z* 310.8 and 312.8 confirmed the formation of the compound **2**.

6-(aryl)-3-(5-bromobenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles 3(a-e): The 4-amino-5-(5-Bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol **2**on reaction with different types of aromatic carboxylic acids namely benzoic acid, *p*-chloro, *p*-bromo, *p*-nitro, *p*-hydroxy, *p*-methoxy m-chlorobenzoic acids in $POCl_3$ furnished6-(Aryl)-3-(5-Bromo-benzofuran-2-yl)-[1,2,4]triazolo[3,4-b] [1,3,4]-thiadiazoles **3(a-e)**.

The absence of -NH_2 and -SH peaks in the IR spectrum gave indication about the formation of the products **3(a-e)**. The appearance of peaks at 1689 cm^{-1}, 730 cm^{-1} frequencies due to -C=N- and -C-S-C- of fused thiadiazoles were observed which confirms the formation of compound **3b**. IR frequencies of compounds **3(a-e)** were recorded in table 2.

The ^{1}HNMR spectrum in $CDCl_3$ of compound **3b** gave an additional evidence for the proposed structures. The disappearance of two singlets of NH_2at δ5.96 ppm and SH at δ14.20 ppm and appearance of multiplet in the range of δ6.90-8.63 ppm were due to the aromatic protons indicates the formation of new compound **3b**.

6-(aryl)-3-(5-bromobenzofuran-2-yl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles 4(a-e): The compound 4-amino-5-(5-Bromobenzofuran-2-yl)-4H-[1,2,4]triazole-3-thiol **(2)** was treated with benzaldehyde / substituted benzaldehydes viz: p-nitro, p-methoxy, m-chloro and p-hydroxy in DMF yielded the cyclized products 6-(Aryl)-3-(5-Bromo -benzofuran-dihydro-[1,2,4]triazole [3,4-b] [1,3,4] thiadiazoles **4(a-e)**.

The absence of -SH and -NH_2 bands in the IR spectrum indicated the formation of the products **4(a-e)**. The absorption peaks at 740 cm^{-1} due to -C-S-C,1690 cm^{-1} due to C=N and 3300-3500 cm^{-1} due to -NH of newly fused thiadiazoles were observed in **4a.** IR frequencies of compounds **4(a-e)** are recorded given in table 3.

Further the proposed structures were confirmed by the ^{1}HNMR and mass spectra of the representative compound **4a**. In ^{1}HNMR spectrum appearance of a multiplet at the range of δ7.24-8.32 ppm were due to the aromatic protons. The resonance of NH proton of thiadiazole was a singlet at δ9.62 ppm. Further in mass spectrum the molecular ion peaks appearedatm/z 124 and 274 as two fragments whose sum gave the exact mass 398 of compound **4a** which confirmed the formation of **4a**.

Table 2. IR data of compounds 3(a-e)

Compound No.	Substituent 'R'	IR data (cm^{-1})
		C-S-C
3a	C_6H_5	689
3b	$C_6H_4NO_2$(p)	690
3c	$C_6H_4OCH_3$(p)	681
3d	C_6H_4Cl(m)	695
3e	C_6H_4OH(p)	698

Table 3. IR data of compounds 4(a-e)

Compound	Substituent 'R'	IR data (cm^{-1})		
		NH	C-S-C	C=N
4a	C_6H_5	3307	740	1690
4b	$C_6H_4NO_2$(p)	3341	732	1658
4c	$C_6H_4OCH_3$(p)	3381	681	1692
4d	C_6H_4Cl(m)	3376	673	1612
4e	C_6H_4OH(p)	3396	698	1632

APPLICATION

Antibacterial activity: Allthe compounds have exhibited considerable activity against the below said bacteria. The **3e** against *Staphylococcus aureus*, **3a** against *Staphylococcus albums*, exhibited very good antibacterial activity this is due to the effect of attachment of chloro and bromo substituted triazolothiadiazole on benzofuran moiety, but all **3**series compounds displayed less activity against *Klebsiella pneumoniae*. The compound **3c** exhibited minimum antibacterial activity this might be due to the attachment of electron donating methoxy group on substituted triazolo thiadiazole terminus. The results are shown in table 4.

Table 4. Antibacterial activity of synthetic compounds in MIC

Compound No.	Diameter of Zone of inhibition (mm)		
	Staphylococcus aureus	*Staphylococcus albus*	*Klebsiella pneumoniae*
DMSO	-	-	-
3a	12	16	12
3b	10	06	10
3c	09	06	10
3d	12	12	12
3e	15	08	12
Azithromycin	20	25	30

The compounds **4a** and **4e** exhibited potentive activity against the *Escherichia coli*, in 50 µg mL^{-1} and **4b** in 100 µg mL^{-1} have shown good zone of inhibition against *Staphylococcus aureus* due to electron withdrawing hydroxyl and nitro group present on p- position of substituted phenyl ring. The compounds **6c** and **6e** have accounted very good activity in 50µg $mL^{-1}$and 100 µg mL^{-1} respectively against *Pseudomonas aureginosa*. The compounds **4b, 4d** and **4c, 4e** have performed their antibacterial activity in higher zone in50µg mL^{-1} and 100 µg mL^{-1} respectively against *Escherichia coli*, this enhanced antibacterial activity is due to again substituent effect of electron withdrawing group and among all bromo substituted analogs were shown significant results (Table 5).

Antifungal activity: he compound **2** and **3** series compounds displayed moderate antifungal activity in MIC, hence these compounds were found to have good zone of inhibition in higher concentration rather in MIC. The compound **4c** have exhibited good activity against above said fungi compared to

other derivatives may be due to methoxy group substitution effect on triazolo thiadiazole analogue. The antifungal activities of test compounds were comparatively similar in both the concentration against *Candida albicans* and *Aspergillus niger*. The activity are given in table 6 and 7.

Table 5. Antibacterial activities of test compounds in (µg mL^{-1})

Test Compounds	Substituent 'R'	Zone of inhibition (in mm)					
		Gram positive		Gram-negative			
		Staphylococcus aureus		*Pseudomonas aureginosa*		*Escherichia coli*	
		50 µg mL^{-1}	100 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}
2	--	13	17	14	19	13	18
4a	C_6H_5	14	18	13	17	15	19
4b	$C_6H_4NO_2$(p)	12	21	13	17	17	20
4c	$C_6H_4OCH_3$(p)	13	17	15	17	16	21
4d	C_6H_4Cl(m)	15	16	14	18	17	18
4e	C_6H_4OH(p)	15	20	13	20	16	21
Penicillin		15	22	--	--	--	--
Streptomycin		--	--	21	28	22	27

Table 6. Antifungal activities of test compounds 5(a-e) by MIC Method

Test Compounds	Fungal strain	
	Aspergillus niger	*Candida albicans*
3a	5.00	5.10
3b	5.50	5.50
3c	5.25	5.10
3d	5.00	5.50
3e	5.50	5.25
Fluconazole	13.25	13.50

Table 7. Antifungal activities of test compounds in (50 and 100 µg mL^{-1})

Compound No.	Zone of inhibition (in mm)			
	Candida albiacans		*Aspergillus niger*	
	50 µg mL^{-1}	100 µg mL^{-1}	50 µg mL^{-1}	100 µg mL^{-1}
2	12	17	14	20
4a	13	16	13	19
4b	15	19	14	18
4c	13	20	15	20
4d	14	17	14	16
4e	15	18	13	16
Griseofulvin	22	28	21	26

CONCLUSION

All new compounds displayed moderate to excellent biological activity and were synthesized by using well established procedures, chemicals used were of pure and of analytical grade. The results of the spectral analysis were also supported the proposed structure. The novel compounds like **4b, 4d** and **4e**, exhibited very good antibacterial activity compared to all other compounds with standard drugs this may be due to the substitution of electron withdrawing groups viz; nitro, chloro and bromo, against given bacteria. The compounds **4b** and **4d** displayed highest activity in 50µg against *Escherichia coli* and the compounds **4b** and **4e** exhibited excellent activity against *Staphylococcus aureus* and *Escherichia coli* respectively. The compound **4c** have shown considerable activity towards *Aspergillus niger*.

ACKNOWLEDGEMENT

We the authors sincerely thank Dr. Shivkumar Hugar, HOD, Dept of Pharmacology, BLDE College of Pharmacy, Vijayapura (Karnataka), Dr. A.M. Krupanidi, Professor, Bapuji Institute of Pharmacy, Davanagere (Karnataka), for providing biological activities of the synthesized compounds. The Department of Chemistry and Industrial Chemistry, VSKU Ballari for providing chemicals, lab facility during the present investigation.

REFERENCES

[1]. Clapp, L.B. Comprehensive heterocyclic chemistry. Oxford: Pergamon Press, UK, **1984**.
[2]. R. H Udupi, N. Kasinath, A. R. Bhat, Synthesis and biological activity of mannich bases of certain 1,2-pyrazolines, *Indian J. Heterocyclic Chem*., **1998**, 7, 221.
[3]. S. A. Galal, A. S. Abd El-All, M. M. Abdallah, H. I. El-Diwani, Synthesis of potent antitumor and antiviral benzofuran derivatives, *Bioorg. Med. Chem. Lett.*, 2009, 19, 2420.
[4]. B. Johns, J. G. Weatherhead, S. H. Allen, 1,3,4-Oxadiazole substituted naphthyridines as HIV-1 integrase inhibitors. Part 2: SAR of the C5 position, *Bioorg. Med. Chem. Lett*., **2009**, 19, 1807.
[5]. B. M. Llina, M. D. Bailey, G. Bolger, 1,3,4-Oxadiazole substituted naphthyridines as HIV-1 integrase inhibitors. Part 2: SAR of the C5 position, *J. Med. Chem.*, **2004,** 47, 1605.
[6]. P. B. Trivedi, N. K. Undaviya, A. M. Dave, K. N. Bhatt, N. C. Desai, Synthesis and antimicrobial activity of some heterocyclic compounds, *Indian J. Chem*., **1993**, 32b,760.
[7]. H. M. Naveena kumari, Manjunatha Harihara Mathada, Shivakumar Hugar, K. M. Basavaraja, Synthesis, Characterization, and antibacterial screening of benzofuranyl aryl triazoles, *J. Applicable Chem.*, **2019**, 8(4), 1704-1710.
[8]. H. Hanumana Gouda, K. M. Basavaraja Synthesis and biological evaluation of some new oxadiazole and Pyrazole derivatives incorporating benzofuran moiety, *DerPharma Chemica*, 5(4), **2013**, 87-98.
[9]. Manjunatha Harihara Mathada, H. M. Naveena Kumari and K M. Basavaraja, Synthesis, Characterisation, Antibacterial and Antifungal Screening of Various 5-Bromo-7- Methoxy-Benzofuran Schiff Bases, *J. Applicable Chem*, 8(1), **2019**, 65-170.
[10]. H. M. Naveena kumari, Manjunatha Harihara Mathada, Shivakumar Hugar, K. M. Basavaraja, Synthesis, Characterization, and Antimicrobial screening of 5-Bromobenzofuranyl Aryl triazoles, *J. Applicable Chem.*, **2019**, 8(4), 1704-1710.
[11]. H. M. Naveena kumari, Manjunatha Harihara Mathada, Shivakumar Hugar, K. M. Basavaraja, Synthesis, Characterization, Biological screening of 5-Bromobenzofuranyl aryl Ureas and carbamates, *J. Applicable Chem.*, **2019**, 8(5), 2067-2073.
[12]. H. M. Naveena kumari, Manjunatha Harihara Mathadaand K. M. Basavaraja, Synthesis, Characterization, Biological Activity of 5-Bromo-benzofuran-2-carboxylic acid (substituted-benzylidene)-hydrazides and their derivatives, *J. Applicable Chem*, **2020**, 9(2), 223-228.