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Studies on Some New Triazolothiadiazoles Derivatives Incorporating Benzofuran Moiety

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ABSTRACT

*Novel heterocyclic compounds i.e. substituted-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6-yl)benzoic acid **(5a-f)** have been synthesized by reaction of 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)benzofuran-3-ol **(3)** with different substituted benzoic acid in presence of $POCl_3$. The structures of the newly synthesized heterocyclic compounds have characterized by IR, ^{1}H-NMR, Mass spectroscopy and elemental analysis. The synthesized compounds were further evaluated for their antimicrobial activities against different microbial strains.*

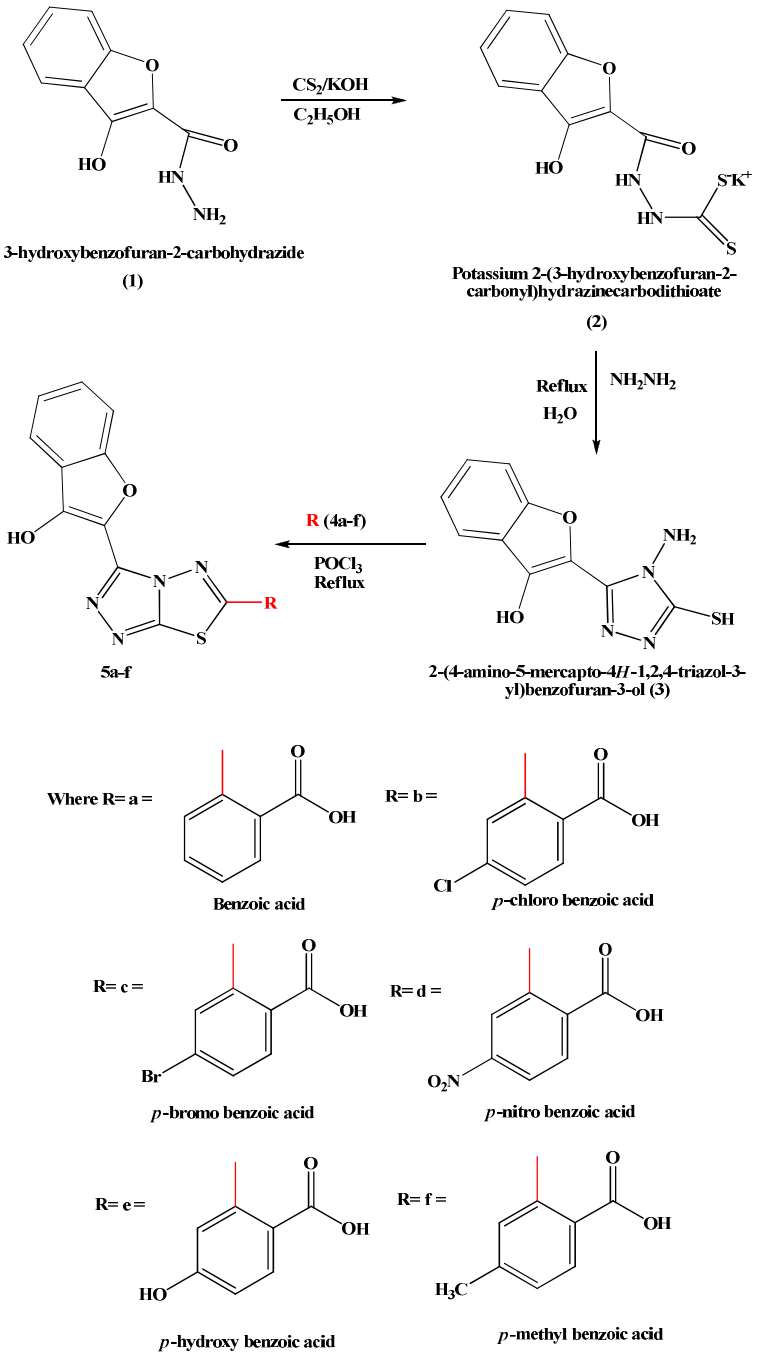
Keywords: 3-hydroxybenzo furan-2-carbohydrazide, 1,2,4-triazoles, Triazolothiadiazoles, Spectral study, Antimicrobial activity.

INTRODUCTION

1,2,4-triazoles known for wide range of applications and also their significant drug molecules such as fluconazole, itraconazole, voriconazole, ravuconazole, posaconazole [1-2]. They have also been incorporated in a diversity of therapeutically interesting drugs including H1/H2 histamine receptor blockers, antianxiety agents, CNS stimulants, Amnesic Effect and sedatives [3-6]. 1,2,4-triazoles also demonstrate enormous potential as the most promising molecules having pharmacological properties such as Antibacterial, Antifungal, Antituberculosis, Anticancer, Cycotoxic, Antitumor, Antioxidant, Anti-HIV, Analgesic and Anti-inflammatory activity [7-12].

In recent past, benzofuran derivatives was considered as important compounds for the synthesis of bioactive compounds that are exhibit numerous biological activities [13-15]. In addition the review of the derivatives indicate the fact that benzofuran occupy a distinctive place in industry for their numerous applications [16-18].

The biological and medicinal significance of both the molecules prompts us to synthesize a series of new triazolothiadiazoles derivatives bearing the benzofuran nucleus. Hence, the present work comprises the synthesis and characterization of novel substituted-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid derivatives. Further the biological screening of these derivatives was carried out using different antimicrobial strains. The synthetic route of targeted compounds is shown in **Scheme-1**.



Scheme 1: Synthetic route of targeted compounds

MATERIALS AND METHODS

➢ Materials and measurements

All reagents and solvents used were purchased from local market and of analytical grade. The 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)benzofuran-3-ol **(3)** was prepared by method reported [19]. The reaction of 3-hydroxybenzofuran-2-carbohydrazide **(1)** with carbon disulphide in ethanol solution gave Potassium 2-(3-hydroxybenzofuran-2-carbonyl)hydrazinecarbodithioate **(2)**, which further react with hydrazine hydrate to yield 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)benzofuran-3-ol **(3)**.

All reactions were monitored by thin-layer chromatography (TLC plates, E. Merck, Mumbai-India) and detection of the components were measured under UV light, explore in Iodine chamber and other necessary reagents. The melting points were checked by the standard open capillary method and were uncorrected. C, H, N analysis was carried out by elemental analyzer PerkinElmer, USA 2400-II CHN analyzer. Infrared spectra of the synthesized compounds were recorded on Nicolet 400D FT-IR spectrometer by using KBr pallets method. NMR spectrum of

synthesized compounds was recorded on Bruker-400 MHz NMR spectrophotometer. Antimicrobial activity of all synthesized compounds was examined against various antimicrobial strains using method reported in literature [20].

➢ **Synthesis of Substituted-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5a-f)**

An equimolar mixture (0.1 mol) of 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)benzofuran-3-ol **(3)** and different substituted benzoic acids **(4a-f) (Scheme-1)** in $POCl_3$ (15-20 mL) was refluxed for 6-7 h. The reaction mixture poured into ice at 0-5° temperature and further allowed to stand for 3-4 hours. The final solid products was treated with NaOH, washed with water, dried and recrystallized from ethanol. The evaluated physical properties and elemental data of all targeted compounds **(5a-f)** are exhibit in **Table-1**.

Table-1 Physical properties and Elemental Analysis of Compounds (5a-f)

Compd. (R)	Yield	M.P. ^{0}C	Elemental Analysis							
			%C		% H		%N		%S	
			Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.
5a (H)	78	225-227	57.1	57.14	2.6	2.66	14.7	14.81	8.4	8.47
5b (Cl)	74	212-213	52.3	52.37	2.1	2.20	13.5	13.57	7.7	7.77
5c (Br)	65	218-220	47.2	47.28	1.9	1.98	12.2	12.25	6.9	7.01
5d (NO_2)	60	223-226	51.0	51.07	2.1	2.14	16.5	16.54	7.5	7.57
5e (OH)	70	208-209	54.8	54.82	2.5	2.56	14.1	14.21	8.1	8.13
5f (CH_3)	72	215-216	58.1	58.16	3.0	3.08	14.2	14.28	8.1	8.17

2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5a): [$C_{18}H_{10}N_4O_4S$ (378.3g)]

FT-IR: 3410 (-OH stretching), 3042 (-C-H=Aromatic stretching), 1696 (-CO of COOH), 1585 (-C=C- stretching), 1226 (-N-N=C- stretching), 1171 (-C-O-C- of benzofuran), 705 (-C-S-C- of triazolo-thiadiazole) cm^{-1}.

^{1}H NMR: δ 7.30-8.05 (8H, m, Ar-H), 5.32 (1H, s, -OH), 11.5 (1H, s, -OH of COOH). **^{13}C NMR:** δ 174.4 (-N=C-S-), 171.5 (-N=C-S-), 167.5 (-C of COOH), 152.4 (-N=C-N-), 121.9, 125.8, 154.7, 157.1 (Benzofuran) 114.3, 120.0, 123.2, 124.9, 127.2, 128.4, 128.8, 130.1, 134.7, 138.9 (Ar-C) **LC-MS (*m/z*):** 378.0[M]$^+$.

5-chloro-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5b): [$C_{18}H_9ClN_4O_4S$ (412.8g)]

FT-IR: 3415 (-OH stretching), 3070 (-C-H=Aromatic stretching), 1682 (-CO of COOH), 1574 (-C=C- stretching), 1230 (-N-N=C- stretching), 1172 (-C-O-C- of benzofuran), 1078 (-C-Cl stretching), 698 (-C-S-C- of triazolo-thiadiazole) cm^{-1}. **^{1}H NMR:** δ 7.28-8.24 (7H, m, Ar-H), 5.27 (1H, s, -OH), 11.3 (1H, s, -OH of COOH). **^{13}C NMR:** δ 174.5 (-N=C-S-), 171.1 (-N=C-S-), 167.9 (-C of COOH), 152.8 (-N=C-N-), 121.7, 125.8, 154.4, 157.3 (Benzofuran) 114.6, 120.8, 123.3, 124.5, 128.5, 129.7, 130.8, 134.3, 134.8, 137.1 (Ar-C) **LC-MS (*m/z*):** 412.5, 414.7 [M]$^+$.

5-bromo-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5c): [$C_{18}H_9BrN_4O_4S$ (457.2g)]

FT-IR: 3412 (-OH stretching), 3035 (-C-H=Aromatic stretching), 1685 (-CO of COOH), 1578 (-C=C- stretching), 1227 (-N-N=C- stretching), 1175 (-C-O-C- of benzofuran), 1052 (-C-Br stretching), 694 (-C-S-C- of triazolo-thiadiazole) cm^{-1}. **^{1}H NMR:** δ 7.28-8.39 (7H, m, Ar-H), 5.30 (1H, s, -OH), 11.5 (1H, s, -OH of COOH). **^{13}C NMR:** δ 174.7 (-N=C-S-), 170.9 (-N=C-S-), 167.2 (-C of COOH), 152.1 (-N=C-N-), 121.4, 125.9, 154.7, 157.5 (Benzofuran) 114.1, 120.4, 123.1, 123.6, 124.5, 129.2, 130.5, 134.5, 137.7, 138.1 (Ar-C) **LC-MS (*m/z*):** 457.0, 459.3 [M]$^+$.

5-nitro-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5d): [$C_{18}H_9N_5O_6S$ (423.3g)]

FT-IR: 3425 (-OH stretching), 3032 (-C-H=Aromatic stretching), 1683 (-CO of COOH), 1584 (-C=C- stretching), 1229 (-N-N=C- stretching), 1165 (-C-O-C- of benzofuran), 1534, 1330 (-NO_2 stretching), 697 (-C-S-C- of triazolo-thiadiazole) cm^{-1}. **^{1}H NMR:** δ 7.29-8.51 (7H, m, Ar-H), 5.32 (1H, s, -OH), 11.5 (1H, s, -OH of COOH). **^{13}C NMR:** δ 174.1 (-N=C-S-), 171.4 (-N=C-S-), 167.7 (-C of COOH), 152.6 (-N=C-N-), 121.2, 125.5, 154.0, 157.7 (Benzofuran) 114.4, 120.2, 123.6, 124.0, 124.6, 128.3, 128.8, 129.7, 143.9, 147.7 (Ar-C) **LC-MS (*m/z*):** 423.1[M]$^+$.

5-hydroxy-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5e): [$C_{18}H_{10}N_4O_5S$ (394.3g)]

FT-IR: 3380 (-OH stretching), 3050 (-C-H=Aromatic stretching), 1685 (-CO of COOH), 1530 (-C=C- stretching), 1232 (-N-N=C- stretching), 1170 (-C-O-C- of benzofuran), 702 (-C-S-C- of triazolo-thiadiazole) cm^{-1}.**^{1}H NMR:** δ 7.28-7.85 (7H, m, Ar-H), 5.28 (2H, s, -OH), 11.3 (1H, s, -OH of COOH). **^{13}C NMR:** δ 174.3 (-N=C-S-), 171.5 (-

N=C-S-), 167.7 (-C of COOH), 152.2 (-N=C-N-), 121.1, 125.2, 154.6, 157.3 (Benzofuran) 114.1, 117.2, 120.5, 121.9, 123.2, 124.3, 128.1, 129.4, 129.9, 158.8 (Ar-C) **LC-MS (*m/z*):** 394.0$[M]^+$.

5-methyl-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid (5f): $[C_{19}H_{12}N_4O_4S$ (392.3g)]
FT-IR: 3421 (-OH stretching), 3065 (-C-H=Aromatic stretching), 1687 (-CO of COOH), 1531 (-C=C- stretching), 1232 (-N-N=C- stretching), 1168 (-C-O-C- of benzofuran), 2950, 1372 (CH_3 stretching), 694 (-C-S-C- of triazolo-thiadiazole) cm^{-1}. **^{1}H NMR:** δ 7.28-7.91 (7H, m, Ar-H), 5.31 (1H, s, -OH), 11.5 (1H, s, -OH of COOH), 2.27 (3H, s, -CH_3). **^{13}C NMR:** δ 174.5 (-N=C-S-), 171.7 (-N=C-S-), 167.3 (-C of COOH), 152.6 (-N=C-N-), 121.4, 125.1, 154.9, 157.2 (Benzofuran) 114.7, 120.3, 123.5, 124.8, 127.5, 128.9, 131.6, 134.2, 135.7, 139.2 (Ar-C), 20.9 (-CH_3) **LC-MS (*m/z*):** 392.1 $[M]^+$.

Antimicrobial activity
The synthesized triazolo-thiadiazole derivatives were screened for their *in vitro* antimicrobial activity against two Gram(+ve) strain, two Gram(−ve) strain and three antifungal strain using the Agar dilution method [20].

- **Antibacterial activities**

The antibacterial activities all synthesized compounds were studied against gram-positive bacteria (*Bacillus Subtilis **(BS)** and Staphylococcus Aureus **(SA)***) and gram-negative bacteria (*Klebsiella Promioe **(KP)** and Escherichia Coli **(EA)***) at a concentration of 50μg/ml by agar cup plate method. A methanol system was used as control in this method, while tetracycline used as a standard for comparison. The area of inhibition of zone measured in cm. The evaluated antibacterial activity was exihibit in **Table-2**.

- **Antifungal activities**

In vitro antifungal activity of synthesized compounds was studied at 1000 ppm concentration. Antifungal strains used were *Aspergillus Niger **(AN)**, Botrydepladia Thiobromine **(BT)**, and Candida Albicans **(CA)***. The antifungal activities were measured on each of these strains using a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200g, dextrose 20g, agar 20g and water 1c. Five days old cultures were employed. The compounds to be tested were suspended (1000ppm) in a PDA medium and autoclaved at 120^o C for 15 min. at 15 atm. pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100(X-Y) / X

Where, X = Area of colony in control plate
Y = Area of colony in test plate

The Antifungal activity for compounds **(5a-f)** is exhibited in **Table-2**.

Table: 3 Antibacterial and Antifungal Activities of Compounds (5a-f)

Compounds	Gram +Ve		Gram -Ve		Antifungal Strains		
	SA	***BS***	***EC***	***KP***	***AN***	***BT***	***CN***
5a	50	69	68	71	75	68	73
5b	57	72	75	78	78	70	77
5c	52	68	70	74	76	67	75
5d	42	65	66	69	65	60	62
5e	45	66	69	70	67	62	65
5f	48	68	71	75	70	67	70
Tetracycline	58	75	78	82	-	-	-

RESULTS AND DISCUSSION

Different substituted-2-(3-(3-hydroxybenzofuran-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzoic acid **(5a-f)** were synthesized by reaction between 2-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)benzofuran-3-ol **(3)** and different benzoic acid in $POCl_3$. Elemental analysis of all compounds was in good agreement with proposed structures as exhibit in **Scheme-1**. Further all compounds examined for their spectral study such as FT–IR, ^{1}H NMR, ^{13}C NMR and LC-MS.

IR spectra of the targeted compounds show the important peaks at ~1226 cm^{-1} and ~1226 cm^{-1} for triazolothiadiazoles derivatives for -N-N=C- stretching and C-S-C stretching, respectively. The incorporated benzofuran ring confirmed by peak found at ~1171 cm^{-1} for C-O-C stretching. Also the presence of substituted

benzoic acid into targeted derivatives supported by C=O of carboxylic group found at ~1700 cm^{-1}. All other peaks for –OH, aromatic protons and C=C stretching found at their respective positions.

The ^{1}H NMR spectra of compounds 5a-f are identical in all aspects. Only change in signal values due to substitution group is appeared in all compounds**.** The aromatic protons were found in the range 7.28-8.51 ppm. Also all compounds have shown two different signals for –OH stretching i.e. at ~5.30 for benzofuran and at ~11.5 for carboxylic group. In addition, the compound 5f shows the singlet for CH_3 at 2.27 ppm.

The ^{13}C NMR spectrum of 5a-f is described in experimental part. The appearance of 3 distinct peaks for triazolothiadiazoles in the spectrum support the structure of the targeted compounds. The signal at δ ~174.4, ~171.5 ppm for -N=C-S- and δ ~152.4 -N=C-N- of triazolothiadiazoles nucleus. The signal at δ ~167.5 ppm for carbon atom of carboxylic group supports the structure of compounds. While all other aromatic carbon signals of benzofuran and substituted benzoic acids were found at their respective position.

The final structure of all compounds is further confirmed by LC-MS data which are presented in experimental part.

CONCLUSION

Novel triazolo-thiadiazole derivatives bearing benzofuran moiety were synthesized and characterized. The structure of all compounds confirmed using different spectral studies. The antibacterial evaluation of all compounds demonstrates good to moderate activity compare to standard drug tetracycline, while primary antifungal evaluation of all the compounds shows promising results against all employed strains.

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