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Synthesis and Spectral studies of 1,3 benzothiazole-2-thiol conjugated thiosemicarbazide as Antibacterial and Antifungal agents

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Abstract- The newly synthesized biologically active series of 2-(benzo[d]thiazol-2-ylthio)ethanethioyl)-2-(substituted)benzylidenehydrazinecarbonothioamide **6a-6f** were prepared. These compounds synthesized from 1,3 benzothiazole-2-thiol, Thiosemicarbazide **(TSC)** and chloroaylchloride **(CAC)** were crucial functionalities containing the wide variety of biological activities and have a broad range of therapeutic properties. The structure of the synthesized compounds was confirmed by spectral data and evaluated for their in vitro antibacterial activities against Gram-positive and Gram-negative bacteria and antifungal

index terms-1,3 benzothiazole-2-thiol, 2-Mercaptobenzothiazole, Thiosemicarbazide, Antibacterial, Antifungal.

1. INTRODUCTION

Heterocyclic compounds containing N, O, S, and P having a broad range of microbial activities against a number of bacteria and fungi. These heterocyclic compounds were used in medicinal and industrial sectors. In the medicinal applications, many heterocyclic nuclei Benzothiazole, Triazine, Benzimidazole have been considerable interest due to their important biological properties. Due to Benzothiazole possess a wide spectrum of biological activities, therefore, our interest to focus on this nucleus. Benzothiazole having potent and significant pharmacological activities such as antimicrobial [1], anticancer [2], Anthelmintic [3], Anti-diabetic [4], Anti-tuberculosis [5], antitumor [6], Anti-trypanosomal [7], Anti-bacterial [8], Anti-inflammatory [9], anthelmintic [10], Antifungal, antiviral, Anti-oxidant, Anti-glutamate and Anti-parkinsonism, analgesic, Anti-leishmanial, Anticonvulsant [11] etc.

Thiosemicarbazide (TSC) have been considered as effective biological active molecules due to exhibit interesting pharmacological properties such as anti-tubercular,[12] antiviral [13], anti-malarial and antibacterial activity[14]. and anticancer [15,16], Thiosemicarbazide (TSC) and Semicarbazide (SC) are used as the key of transformation in various organic reactions and also considered as a versatile intermediate for the synthesis of important various heterocycles. With these criteria and literature data considering the importance of benzothiazole moiety in medicinal chemistry, here two series containing 2-mercaptobenzothiazole and TSC were designed and synthesized for their in-vitro Antibacterial activities against Gram-positive and Gram-negative bacteria and Antifungal activities.

2. RESULTS AND DISCUSSION

Chemistry

The title compounds were prepared according to the synthetic strategy described in Scheme-1. The intermediate key moiety in the present work as Schiff base **3a-3f** were synthesized from using various benzaldehyde and thiosemicarbazide (TSC) in the presence of acetic acid in ethanol, Further, we planned to extend our studies by using chloroaylchloride (CAC) with TEA in presence of DMF to yielded **4a-4f** which treated with 2-Mercaptobenzothiazole (MBT), 1,3-Benzothiazole-2-thiol, to afford different substituent's of titled compounds **6a-6f** which confirmed by spectral data.

Table 1. The substituent for derivatives of **6a-6f.**

Com	R	Molecular Formula	Yield (%)	Melting Point˚C
6a	4-F C_6H_5	$C_{17}H_{13}FN_4OS_3$	77	162-165
6b	4-Cl C_6H_5	$C_{17}H_{13}ClN_4OS_3$	75	163-165
6c	4-OCH_3 C_6H_5	$C_{18}H_{16}N_4O_2S_3$	72	165-168
6d	3-NO_2 C_6H_5	$C_{17}H_{13}N_5O_3S_3$	70	162-165
6e	p-OH C_6H_5	$C_{17}H_{14}N_4O_2S_3$	74	168-171
6f	2,4-(Cl)2-C_6H_5	$C_{17}H_{12}Cl_2N_4O_3$	73	170-173

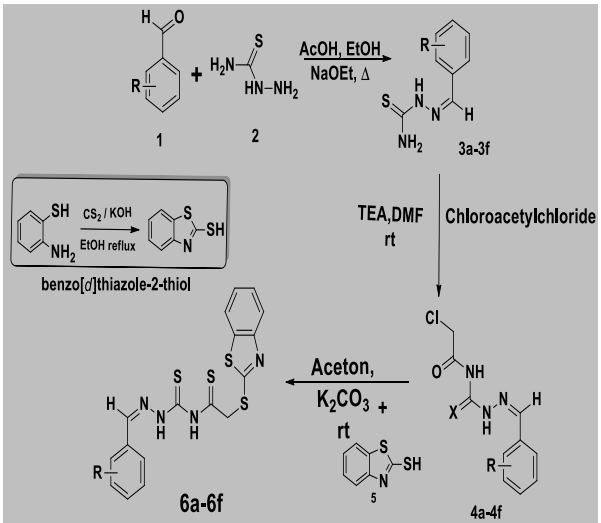
2.1. Antibacterial

All the synthesized compounds **(6a-6f)** were carried out for their antibacterial activity on nutrient-agar plates by well-diffusion assay method compared to test culture. Cultures were produced in Nutrient broth. Isolates inhibit the above-mentioned organisms or not were studied and zone, of inhibition, was measured in terms of Zone diameter and with the help of that zone, the index was calculated where streptomycin was used as standard drug.

Note: Standard drug used streptomycin with 1000 µg/ml concentration.

$$Activity\ index\ (A.I.) = \frac{Mean\ of\ Zone\ of\ inhibition\ of\ derivative}{Zone\ of\ inhibition\ obtained\ for\ standard\ antibiotic\ drug}$$

Eq.(1) Calculate Activity index for inhibition



The scheme -1 Synthesis of targeted compound **6a-6f**

Table 2 Antibacterial activity of compounds **6a-6f**

Com.	*E. aerogens MTCCNo. 8558*		*E. coli MTCCNo. 1610*		*M. luteus MTCCNo. 11948*		*B. cereus MTCCNo. 8558*	
	Mean value for Zone of Inhibition (mm)	Active index (A.I)	Mean value for Zone of Inhibition (mm)	Active index (A.I)	Mean value for Zone of Inhibition (mm)	Active index (A.I)	Mean value for Zone of Inhibition (mm)	Active index (A.I)
Std	25	--	25	--	25	--	25	--
6a	29	1.16	28	1.12	27	1.08	30	1.20
6b	24	1.041	25	1.00	24	1.041	22	0.88
6c	21	0.84	20	0.80	18	0.72	20	0.80
6d	26	1.04	24	1.041	26	1.04	23	0.92
6e	21	0.84	20	0.80	22	0.88	18	0.72
6f	32	1.28	30	1.20	32	1.28	28	1.12

The newly synthesized compounds **6a-6f** were screened in vitro for antibacterial activity against Gram-positive *Micrococcus luteus* (MTCC No. 11948), *Bacillus Cereus* (MTCC No. 8558) and Gram-negative *Enterobacter aerogens* (MTCC No. 8558), *Escherichia coli* (MTCC No. 1610) by determining the zone of inhibition in mm. Antibacterial showing results (the zone of inhibition), existing in Table 2. Shown that all compounds established some degree of antibacterial activity. (figure-1) The antibacterial activity of all compounds except **6c** and **6e** were showed less inhibition than the standard. While, compound **6a, 6d,** and **6f** have shown very close and increase activity to the standard drug. The compound **6f** exhibit an excellent in all.

2.2. *Antifungal*

In this present work, all new Synthesized of series 2-(benzo[d]thiazol-2-ylthio)ethanethioyl)-2-(substituted) benzylidenehydrazinecarbonothioamide **6a-6f** were evaluated as antifungal activity against *Aspergillus niger* and *Candida albicans* in DMF, this activity is done by in vitro agar well diffusion method. Pepton (1g) D-glucose (4g) and agar (2g) were used to prepared Saubourauds agar media and maintained 5.7 pH by adding 100 ml of distilling water and make a suspension for fungal strain. On the other hand The making suspension of corresponding species, the fungal transferred into 3ml salin and create a disc by adding 20 ml of fungal media for each Petri dish after the plate was dried by using incubator at 37 ºC for 1 day. A prepared control was allowed for three to four day at 37 ºC and the fungal inhibitions zone was

measured was the microorganism inhibited after the incubation was done and were compared with standard voriconazole shown in Table 3.

Table 3 Antifungal activity of compounds **6a-6f**

Com.	*Aspergillus niger*		*Candida albicans*	
	M.V (mm)	(A.I.)	M.V (mm)	(A.I.)
6a	29	1.035	27	1.125
6b	26	0.929	20	0.714
6c	18	0.643	16	0.666
6d	26	0.929	30	1.250
6e	24	0.857	23	0.958
6f	30	1.071	31	1.291
Std	28	-	24	-

The resulted of these Antifungal activities compounds **6a**, **6d** and **6f** showed excellent inhibitions as an antifungal agent.

3. MATERIALS AND METHODS

3.1. Materials

All starting materials and other reagents were purchased from commercially supplier. All the melting points were determined in open capillary and uncorrected by using a Melt-Tempt instrument, The absorbance spectra IR were recorded on Perkin-Elmer 377 spectrophotometer, ^{1}H NMR spectra were recorded on Bruckner at 400MHz, In DMSO solution. The elemental analysis was analyzer for C, H, N, O, and S were estimated on PerkinElmer, series II, 2400 CHNS analyzer (CSIR Bhavnagar, INDIA) Mass spectra were recorded at Advion Expression CMS, USA

3.2. General Procedure for targeted compounds

(Substituted) *2-benzylidenehydrazinecarbothioamide derivatives. (3a-3f)*

The aqueous Solution of thiosemicarbazide (0.01 mol) and benzaldehyde derivatives (0.01 mol) in 1:1 molar ratio taken in an RBF. The reaction mixture was kept over a magnetic stirrer and stirred well at room temperature for 2 h. the solid was formed, filtered and washed with petroleum ether. Obtained solid was dried and checked by TLC.

(Substituted) 2-benzylidene-N-(2-chloroacetyl) hydrazinecarboxamide derivatives. (4a-4f)

A mixture of compound 1(0.01 mol) with Chloroacetyl chloride (0.015 mol) and 4-5 drops of TEA(triethylamine) in 25 ml DMF as the solvent in RBF. The reaction mixture was stirred for 4 hr at Room temperature. The Completion of the reaction was Checked by TLC using toluene: Acetone (30%). The solution poured into ice water. Obtained solid was filtered by the vacuum pump and crystalline it in Ethanol.

2-(benzo[d]thiazol-2-ylthio)ethanethioyl)-2-(substituted)benzylidenehydrazinecarbonothioamide 6a-6f

Above synthesized derivatives (0.01 mol) was reacted with 2-mercaptobenzothiazole (0.01 mol) in the presence of potassium carbonate (0.02 mol) and acetone. The reaction was stirred at room Temperature for 5 hr. The Completion of the reaction was checked by TLC using toluene: Acetone (20%). The product was poured into water and stirred for 1 hr. The obtained solid was collected and dried. Crystallize into ethanol.

4. SPECTRAL DATA

4.1 2-(benzo[d]thiazol-2-ylthio)-N-(2-(4-fluorobenzylidene)hydrazinecarbonothioyl)acetamide (6a) **I.R** (KBr) (υmax, cm-1): 3192 (N-H), 3088, 2965 (C-H), 1668 (C=O), 1222 (C=S), 1585, 1541, 1522, 1455,1284 (C=N, C=C), 1159, 1004, 925, 742, 685 3456 cm^{-1}

^{1}H NMR (400 MHz, DMSO) δ 11.10 (s, 2H, NH), 4.32 (s, 2H, CH2), 7.05-7.38 (m, 4H, ArH), 7.5-8.6, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)

^{13}C-NMR δ 38.5 (CH2), 147.8 (N-CH-Ar), 172.2 (C=O), 181.4 (C=S), 123-154.8 (12 aromatic carbons), 167.7 (S-C-N benz thio)

MS (m/z) 389.05 (M^{+})

Anal. Calcd. For: $C_{17}H_{13}FN_4O_2S_2$ C , 52.56; H, 3.37; N, 14.42; O, 8.24; S, 16.51% Found: C 53.7, H 3.32, N 14.16, O 8.22 S 16.58 %.

***4.2 2-(benzo[d]thiazol-2-ylthio)-N-(2-(4-chlorobenzylidene)hydrazinecarbonothioyl)acetamide* (6b)**

I.R (KBr) (υmax, cm-1): 3196 (N-H), 3086, 2966 (C-H), 1665 (C=O), 1212 (C=S), 1584, 1542, 1512, 1448,1294 (C=N, C=C), 1159, 1004, 925, 742, 685 3456 cm^{-1}

^{1}H NMR (400 MHz, DMSO) δ 11.02 (s, 2H, NH), 4.12 (s, 2H, CH2), 7.05-7.42 (m, 4H, ArH), 7.5-8.55, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)

^{13}C-NMR δ 39.5 (CH2), 145.8 (N-CH-Ar), 170.2 (C=O), 180.4 (C=S), 123-154.8 (12 aromatic carbons), 167.7 (S-C-N benz thio),

MS (m/z) 419.06 (M^{+})

Anal. Calcd. For: $C_{17}H_{13}ClN_4OS_3$ C , 48.50; H, 3.11; N, 13.31; O, 3.80; S, 22.85% Found: C 48.7, H 3.12, N 13.36,O 3.88 S 22.88 %.

***4.3 2-(benzo[d]thiazol-2-ylthio)-N-(2-(4methoxybenzylidene)hydrazinecarbonothioyl)acetamide* (6c)**

I.R (KBr) (υmax, cm-1): 3192 (N-H), 3082, 2955 (C-H), 1662 (C=O), 1222 (C=S), 1570, 1534, 1522, 1458,1296 (C=N, C=C), 1154, 1006, 924, 740, 684 3454 cm^{-1}
^{1}H NMR (400 MHz, DMSO) δ 3.71 (s,3H Ar-OCH_3) 11.03 (s, 2H, NH), 4.16 (s, 2H, CH2), 7.05-7.45 (m, 4H, ArH), 7.5-8.55, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)
^{13}C-NMR δ 39.5 (CH2), 145.8 (N-CH-Ar), 171.2 (C=O), 181.4 (C=S), 123-154.8 (12 aromatic carbons), 166.7 (S-C-N benz thio), 55.4 (OCH_3)
MS (m/z) 416.05 (M^+)
Anal. Calcd. For: $C_{18}H_{16}N_4O_2S_3$, C, 53.98; H, 4.03; N, 13.99; O, 3.99; S, 24.02% Found: C 53.7, H 4.09, N 13.96,O 3.98 S 24.08 %.

***4.4 2-(benzo[d]thiazol-2-ylthio)-N-(2-(4-nitrobenzylidene)hydrazinecarbonothioyl)acetamide* (6d)**
I.R (KBr) (υmax, cm-1): 3192 (N-H), 3080, 2956 (C-H), 1662 (C=O), 1224 (C=S), 1581, 1539, 1522, 1448,1294 (C=N, C=C), 1149, 1004, 921, 742, 684 3455 cm^{-1}
^{1}H NMR (400 MHz, DMSO) δ 11.11 (s, 2H, NH), 4.15 (s, 2H, CH2), 7.05-7.48 (m, 4H, ArH), 7.5-8.5, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)
^{13}C-NMR δ 39.5 (CH2), 145.8 (N-CH-Ar), 171.2 (C=O), 181.5 (C=S), 123-154.8 (12 aromatic carbons), 166.7 (S-C-N benz thio)
MS (m/z) 431.12 (M^+)
Anal. Calcd. For: $C_{17}H_{13}N_5O_3S_3$ C, 47.32; H, 3.04; N, 16.23; O, 11.12; S, 22.29% Found: C 47.2, H 3.12, N 16.26,O 11.18 S 22.28 %.

***4.5 2-(benzo[d]thiazol-2-ylthio)-N-(2-(4-hydroxybenzylidene)hydrazinecarbonothioyl)acetamide* (6e)**
I.R (KBr) (υmax, cm-1): 3198 (N-H), 3088, 2965 (C-H), 1668 (C=O), 1221 (C=S), 1585, 1541, 1522, 1455,1284 (C=N, C=C), 1159, 1004, 925, 742, 685 3456 cm^{-1};
^{1}H NMR (400 MHz, DMSO) δ 11.02 (s, 2H, NH), 11.59 (s, 1H, OH), 4.32 (s, 2H, CH2), 7.05-7.38 (m, 4H, ArH), 7.5-8.6, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)
^{13}C-NMR δ 38.5 (CH2), 147.8 (N-CH-Ar), 172.2 (C=O), 180.5 (C=S), 123-154.8 (12 aromatic carbons), 167.7 (S-C-N benz thio),
MS (m/z) 402.05 (M^+)
Anal. Calcd. For: $C_{17}H_{14}N_4O_2S_3$ C , 50.73; H, 3.51; N, 13.92; O, 7.95; S, 23.90% Found: C 50.7, H 3.62, N 14.01, O 7.92 S 23.98 %

4.6 2-(benzo[d]thiazol-2-ylthio)-N-(2-(2,4-dichlorobenzylidene)hydrazinecarbonothioyl)acetamide (6e)
I.R (KBr) (υmax, cm-1): 3192 (N-H), 3088, 2965 (C-H), 1668 (C=O), 1210 (C=S), 1585, 1541, 1522, 1455,1284 (C=N, C=C), 1159, 1004, 925, 742, 685 3456 cm^{-1};
^{1}H NMR (400 MHz, DMSO) δ 11.08 (s, 2H, NH), 4.32 (s, 2H, CH2), 7.05-7.28 (m, 3H, ArH), 7.5-8.6, (m, 4H, thiazole ArH),6.6 (s, 1H HC=N)
^{13}C-NMR δ 38.7 (CH2), 147.8 (N-CH-Ar), 172.4 (C=O), 180.1 (C=S), 123-155.8 (12 aromatic carbons), 167.7 (S-C-N benz thio),
MS (m/z) 453.15 (M^+)
Anal. Calcd. For: $C_{17}H_{12}Cl_2N_4OS_3$ C , 44.84; H, 2.66 N, 12.30; O, 3.51; S, 21.12, % Found: C 44.81, H 2.62, N 12.36, O 3.52 S 21.18 %

5. CONCLUSION

In this study, we describe the methodology for 1,3-benzthiazole-2-thiol and Thiosemicarbazide (TSC) containing new derivatives **6a-6f.** The pharmacological study for these two series evaluated on the *in vitro* antibacterial activities against gram-positive and gram-negative bacteria. All compounds shown good activity with using *streptomycin* as standard drug except **6a, 6d,** and **6f** showed more than inhibition the standard against all bacterial strains and also being good inhibitors against fungal strains showed very close activity to standard drug.

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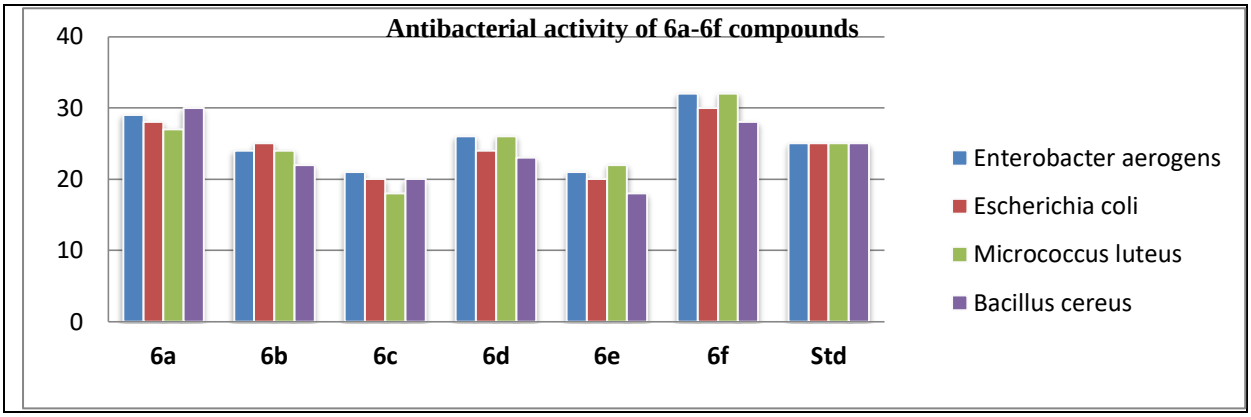

Figure1: Line scatter graph about Antibacterial activity of Compound 3a-3r for Gram-positive Micrococcus luteus **(MTCC No. 11948)**, Bacillus Cereus **(MTCC No. 8558)** and Gram-negative Enterobacter aerogens **(MTCC No. 8558),** Escherichia coli **(MTCC No. 1610)**

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