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SYNTHESIS, SPECTRAL CHARACTERIZATION AND PHARMACEUTICAL IMPORTANCE OF NOVEL 4*H*-1, 4-BENZOTHIAZINES, AND THEIR SULFONE ANALOGUES

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ABSTRACT: In recent years, synthesis and biological evaluation of novel 4H-1, 4-benzothiazines and their sulfone analogues have gained momentum due to their medicinal and industrial importance. The present article describes the synthesis of new 4*H*-1, 4-benzothiazines via condensation and oxidative cyclization of substituted 2-aminobenzenethiols with compounds containing active methylene groups. It is believed that the reaction proceeds via intermediary of the enaminoketone system. The sulfone derivatives were synthesized by oxidation of 4*H*-1, 4-benzothiazines using 30% hydrogen peroxide in glacial acetic acid. The structures of the compounds have been confirmed by spectral and chemical analysis. The synthesized compounds were screened for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization assay. The in vitro antibacterial activity was tested against *S. aureus*, *B. subtilis* (Gram positive) and *E. coli*, *Pseudomonas aeruginosa* (Gram negative) microorganisms using paper disc diffusion method using nutrient agar medium and the antifungal activity of synthesized compounds was tested on fungal strains: *A. niger*, *C. albicans* using disc diffusion method.

INTRODUCTION: A benzothiazine is a heterocyclic compound that consists of six-membered ring of a thiazine fused to a benzene ring[1-3]. The various types of benzothiazines can be categorized by the position of N and S atoms in the molecular skeletons. The most common structures of benzothiazines are 1, 2- , 1, 3-, 1, 4-, 2,1- and 3,1-benzothiazine[4-5]. These benzothiazines also exhibit promising and outstanding biological activities. For example, 1, 2-benzothiazine derivatives have broad significant bioactivities such as antibacterial and anti-inflammatory activities [6-10]. Moreover, 1, 4-benzothiazine was assigned as an anti-corrosion application to mild steel industries.

A slight change in the substitution pattern in benzothiazine nucleus cause distinguishable difference in their biological activities[11-16]. Therefore, these observations stimulated our interest to extend synthetic, structural and antimicrobial studies of 4*H*-1, 4-benzothiazines, and their sulfones. On refluxing with 30% hydrogen peroxide in glacial acetic acid, 4*H*-1, 4-benzothiazines yielded 4*H*-1, 4-benzothiazine-1,1-dioxides[17-20]. All the synthesized compounds were screened for their antimicrobial activities.

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MATERIALS AND METHODS:

General procedure for the synthesis of substituted 4*H*-1, 4-benzothiazines 5a-d

β-Diketone/β-ketoester **3a-c** (0.01 mol) was treated with a solution of 2-aminobenzenethiol **1a-b** (0.01

mol) in 5 mL of DMSO and the resulting mixture was heated under Reflux (190°C) for 20 min., then cooled and concentrated on a rotary evaporator. The residue was washed with petroleum ether and crystallized from methanol.

General procedure for synthesis of 4*H*-1, 4-benzothiazine-1, 1-dioxides (sulfones) 6a-d

A solution of 4*H*-1, 4-benzothiazine **5a-d** (0.01 mol) in 20 mL glacial acetic acid was added to 30% hydrogen peroxide (5 mL) and then the mixture was heated under reflux for 15 min. keeping the temperature between 50°C and 55°C. Another lot of 30% hydrogen peroxide (5 mL) was added after 15 min. without heating. Then the mixture was heated under reflux (120°C) for an additional 4-5 h, concentrated under reduced pressure and the residue was treated with crushed ice. The resultant solid product **6a-d** was filtered and crystallized from ethanol.

Biological Activity

Antioxidant Activity

The synthesized compounds were screened for antioxidant activity by 1, 1 – diphenyl – 2 - picrylhydrazyl (DPPH) radical scavenging assay[18] and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization assay[19]. The results are tabulated in **Tables 1 and 2** and represented by **Figure 1 and 2.**

DPPH Radical Scavenging Assay

Radical scavenging activity of all synthesized compounds was determined spectrophotometrically against stable 1, 1 - diphenyl-2-picrylhydrazyl (DPPH) radical by Cuendet et al.

ABTS Radical Cation Decolorization Assay

The ($ABTS^{\bullet+}$) assay was carried out using the improved assay of RE et al., which is based on the oxidation of ABTS with potassium persulfate leading to ($ABTS^{\bullet+}$).

Antimicrobial Assessment

Antibacterial Activity:

In this method, paper disc impregnated with compounds dissolved in solvent DMF at concentrations 25, 50 and 100µg mL^{-1}. Then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 hours for bacterial cultures. After incubation, the zones inhibition around the disc were observed. Each testing is done in triplicate. Ciprofloxacin at conc. 50 µg mL^{-1} was used as standard drug for antibacterial activity[20-21]. Results were interpreted in terms of diameter (mm) of zone of inhibition. The % Activity Index for the complex was calculated by the formula as under:

$$\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

Antifungal Activity

Antifungal activity of synthesized compounds was tested on fungal strains: *A. niger*, *C. albicans* using disc diffusion method. In the disc-diffusion method, disc impregnated with compounds dissolved in solvent DMF at concentrations 25, 50 and 100 µg mL^{-1} were spread over microorganism culture in nutrient agar medium.

The plates were incubated at 25°C for 48 hours for fungal strains. After incubation the growth inhibiting zones around the disc was observed. Growth inhibiting zone indicates that the compounds inhibit growth of microorganism[22-24]. Each experiment is done in triplicate. Griseofulvin at concentration 50 µg mL^{-1} was used as standard drug for antifungal activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The percentage inhibition was calculated by the following equation.

$$\% \text{ Inhibition} = (C–T)\ 100/C$$

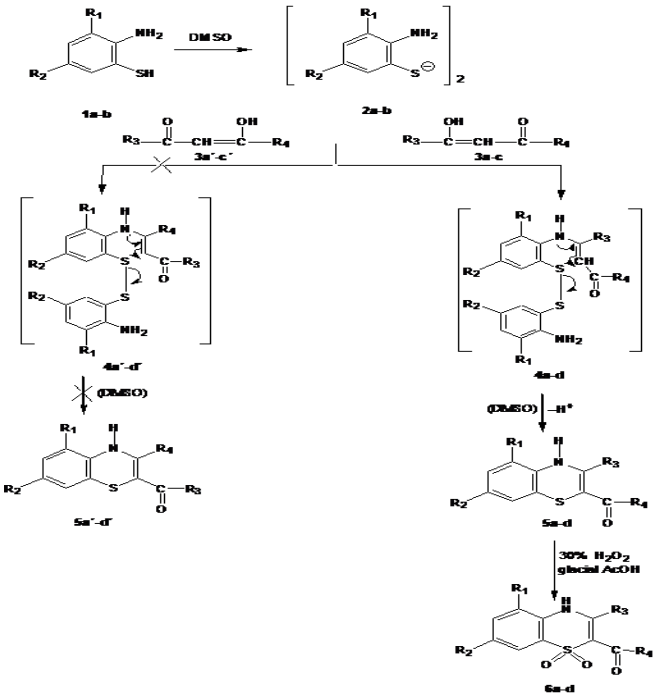
Where, C and T are the diameters of the fungal colony in the control and the test plates, respectively.

Minimum Inhibitory Concentrations:

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobials that inhibit the visible growth of a microorganism after overnight incubation at 37°C. Determination of the MIC is a semiquantitative test, which gives an approximate idea of the least concentration of an antimicrobial (test) solution needed to parent microbial growth. The MIC was determined by the liquid dilution method. Two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria

(*Escherichia coli* and *Pseudomonas aeruginosa*) were used as quality control strains. For the antifungal activities of the compounds *Candida albicans* and *Aspergilius niger* were tested. Ciprofloxacin and Griseofulvin were used as standard antibacterial and antifungal agents. The stock solutions of test compounds with 1 to 20µg /mL concentrations were prepared with aqueous methanol. Inoculums of the overnight culture were prepared.

In a series of tubes, 1 mL each of stock solutions of test compound with different concentrations was taken and 0.4 mL of the inoculums was added to each tube. Further 4.0 mL of sterile water was added to each of the test tubes. These test tubes were incubated for 22-24 hours and observed for the presence of turbidity. The absorbance of the suspension of the inoculums was observed with spectrophotometer at 555 nm. The end result of the test was the minimum concentration of antimicrobial (test) solutions, which gave clear solution, i.e. no visual growth. The activity indices of tested compounds against certain bacteria and fungi were calculated and the results are tabulated in **Tables 3 and 4**.



5a, 6a : R_1 = F; R_2 = Br; R_3 = $CH_2CH_2CH_3$; R_4 = OCH_2CH_3
5b, 6b : R_1 = Br; R_2 = F; R_3 = CH_2CH_3; R_4 = OCH_3
5c, 6c : R_1 = Br; R_2 = F; R_3 = CH_3; R_4 = $OCH(CH_3)_2$
5d, 6d : R_1 = Br; R_2 = F; R_3 = $CH_2CH_2CH_3$; R_4 = OCH_2CH_3

SCHEME 1 : SYNTHESIS OF 4*H*-1, 4-BENZOTHIAZINES 5a-d, AND THEIR SULFONE ANALOGUES 6a-d

Results and Discussion: The starting substituted 2-aminobenzenethiols **1a-b (Scheme-1)** were prepared by the condensation of substituted arylamines (having occupied para position) with sulfur monochloride which in turn produced the Herz compounds (thiazothiolium chloride) which on alkaline hydrolysis produced sodium salt of the corresponding 2-aminobenzenethiols **1a-b**.

A mixture of 2-aminobenzenethiols **1a-b** and β-diketone/β-ketoester **3a-c** was heated under reflux conditions in DMSO, which led to condensation and oxidative cyclization. Oxidation of 2-aminobenzenethiols produced bis-(2-aminophenyl) disulfide **4a-d**, which in turn underwent cyclization to form 4*H*-1, 4-benzothiazines **5a-d** by Scission of a sulfur-sulfur bond. This cleavage is due to high reactivity of the α-position of the enaminoketone system towards nucleophillic attack. Compounds **5a-d** were converted into their corresponding sulfones **6a-d** by treatment with 30% hydrogen peroxide in glacial acetic acid.

The structures proposed to the synthesized compounds are well supported by spectroscopic data and elemental analysis. In the IR spectra of compounds **5a-d**, a single peak is seen in the region of 3300-3210 cm^{-1} due to NH stretching vibration. These compounds also exhibit a band at 1610-1590 cm^{-1} due to C=O stretching vibrations. A slight shift, towards higher frequencies is observed for sulfone derivatives **6a-d** due to an increased electron-accepting ability of sulfones compared with the parent system.

Compounds **6a-d** exhibit intense peaks in the regions of 1352-1238 cm^{-1}, 1180-1140 cm^{-1} and 572-543 cm^{-1}, which can be ascribed to vibrations of the sulfonyl group.

The signals for C-S stretching vibrations in the region of 1070-1048 cm^{-1} are also observed in the IR spectra of compounds **6a-d.** The given structures of products **5a-d** and **6a-d** are fully consistent with their ^1H NMR spectra. A multiplet in the region of δ8.23-6.56 ppm is due to the the presence of aromatic protons in all compounds **5a-d**, and **6a-d**. A singlet in the region δ9.09-8.18 ppm can be ascribed to the NH function. The given compounds **5a-d**, and **6a-d** are also fully consistent

with their ^{13}C NMR spectra. Spectral and chemical analysis result is shown in **Tables 5-13.**

TABLE 1 ANTIOXIDANT ACTIVITY OF SYNTHESIZED 4*H*-1, 4-BENZOTHIAZINES 5a-d.

Compounds	DPPH% Inhibitory (1 mg/ml)	$ABTS^{\cdot+}$ activity at different time intervals				
		0 min.	1 min.	2 min.	4 min.	6 min.
5a	42.92±0.02	0.686	0.083	0.061	0.054	0.043
5b	46.64±0.71	0.694	0.179	0.091	0.089	0.071
5c	34.44±0.06	0.693	0.113	0.110	0.103	0.082
5d	59.57±1.07	0.669	0.512	0.412	0.103	0.213
Ascorbic acid	-	0.694	0.040	0.003	0.003	0.003

TABLE 2 ANTIOXIDANT ACTIVITY OF SYNTHESIZED 4*H*-1, 4-BENZOTHIAZINES SULFONES 6a-d.

Compounds	DPPH% Inhibitory (1 mg/ml)	$ABTS^{\cdot+}$ activity at different time intervals				
		0 min.	1 min.	2 min.	4 min.	6 min.
6a	66.24±0.07	0.713	0.071	0.059	0.044	0.041
6b	22.34±1.70	0.700	0.101	0.093	0.083	0.070
6c	54.26±1.07	0.685	0.362	0.313	0.222	0.191
6d	70.20±0.02	0.690	0.059	0.049	0.031	0.030
Ascorbic acid	-	0.694	0.040	0.003	0.003	0.003

TABLE 3 ANTIMICROBIAL ASSESSMENT OF 4*H*-1, 4-BENZOTHIAZINES 5a-d.

Compounds	*E. coli*		*B. subtilis*		*P. aeruginosa*		*S. aureus*		*C. albicans*		*A. niger*	
	MIC µg /ml) of bacterial strain	*%AI (100 µg /ml)*	MIC (µg /ml) of bacterial strain	*%AI (100 µg /ml)*	MIC (µg /ml) of bacterial strain	*%AI (100 µg /ml)*	MIC µg /ml) of bacterial strain	*%AI (100 µg /ml)*	MIC µg /ml) of bacterial strain	*% I (100 µg /ml)*	MIC µg /ml) of bacterial strain	*% I (100 µg /ml)*
5a	13.91±0.16	74	15.22±0.23	58	11.21±0.14	66	17.21±0.23	60	15.49±0.39	62	14.67±0.18	43
5b	14.03±0.13	65	16.20±0.26	56	11.67±0.21	62	12.21±0.17	64	17.11±0.41	68	12.24±0.19	52
5c	15.21±0.13	69	15.93±0.18	63	14.14±0.17	63	14.15±0.13	65	16.14±0.23	66	11.13±0.14	50
5d	10.84±0.12	69	11.77±0.34	57	10.53±0.13	73	11.24±0.16	64	12.13±0.19	71	10.77±0.21	46
Ciprofloxacin	04.10±0.10	100	04.90±0.13	100	03.85±0.15	100	04.90±0.11	100	-	-	-	-
Griseofulvin	-	-	-	-	-	-	-	-	03.10±0.80	100	04.80±0.10	100

MIC, Minimum Inhibitory Concentration; AI, Activity Index; I, Inhibition

TABLE 4 ANTIMICROBIAL ASSESSMENT OF *4H*-1, 4-BENZOTHIAZINES SULFONE 6a-d.

Compounds	*E. coli*		*B. subtilis*		*P. aeruginosa*		*S. aureus*		*C. albicans*		*A. niger*	
	MIC (µg/ml) of bacterial strain	*%AI (100 µg/ml)*	MIC (µg/ml) of bacterial strain	*%AI (100 µg/ml)*	MIC (µg/ml) of bacterial strain	*%AI (100 µg/ml)*	MIC (µg/ml) of bacterial strain	*%AI (100 µg/ml)*	MIC (µg/ml) of bacterial strain	*% I (100 µg/ml)*	MIC (µg/ml) of bacterial strain	*% I (100 µg/ml)*
6a	14.62±0.13	70	15.69±0.23	60	13.69±0.16	73	13.18±0.19	53	14.67±0.45	68	15.56±0.19	61
6b	15.21±0.14	67	14.78±0.11	59	14.11±0.15	67	16.62±0.20	50	15.00±0.33	64	14.21±0.23	50
6c	13.62±0.21	64	14.36±0.26	59	11.32±0.18	74	14.41±0.16	63	17.20±0.11	64	12.12±0.13	54
6d	14.11±0.22	73	16.21±0.17	60	12.13±0.17	75	10.26±0.14	58	10.39±0.45	63	11.46±0.16	52
Ciprofloxacin	04.10±0.10	100	04.90±0.13	100	03.85±0.15	100	04.90±0.11	100	-	-	-	-
Griseofulvin	-	-	-	-	-	-	-	-	03.10±0.80	100	04.80±0.10	100

MIC, Minimum Inhibitory Concentration; AI, Activity Index; I, Inhibition

TABLE 5 PHYSICAL AND ANALYTICAL DATA OF 4*H*-1, 4-BENZOTHIAZINES 5a-d.

Compounds	Molecular formula	m.p. (°C)	Yield (%)	% Found (Calcd.)		
				C	H	N
5a	$C_{14}H_{15}NO_3SBrF$	73	57	46.60 (46.54)	4.11 (4.16)	3.82 (3.88)
5b	$C_{12}H_{11}NO_2SBrF$	75	79	43.19 (43.24)	3.35 (3.30)	4.25 (4.20)
5c	$C_{13}H_{13}NO_2SBrF$	80	47	45.10 (44.96)	3.73 (3.77)	4.09 (4.04)
5d	$C_{14}H_{15}NO_2SBrF$	84	52	46.69 (46.54)	4.18 (4.16)	3.82 (3.88)

TABLE 6 INFRARED SPECTRAL DATA OF SUBSTITUTED 4*H*-1, 4-BENZOTHIAZINES 5a-d (in cm^{-1}).

Compounds	A	B	C	D	E	F	G
5a	3210	1590	1250 1035	1435 1310	2950	1280	655
5b	3255	1600	1260 1045	1445 1320	2965	1250	635
5c	3290	1595	1255 1030	1450 1340	2870	1240	620
5d	3300	1610	1250 1035	1420 1310	2980	1245	635

A = N–H stretching vibrations.
B = C=O stretching vibrations.
C = C–O–C Asymmetric and symmetric vibrations.
D = C-H deformation vibrations of CH_3 group.
E = C–H stretching vibrations in CH_3
F = C–F stretching vibrations.
G = C–Br stretching vibrations.

TABLE 7: 1H NMR SPECTRAL DATA OF SUBSTITUTED 4*H*-1, 4-BENZOTHIAZINES 5a-d.

Compounds	δ (in ppm)	No. of Hydrogen	Multiplicity	Assignment
5a	8.77	1	Singlet	NH Proton
	7.89-6.56	2	Multiplet	Aromatic protons
	1.99	2	Triplet	CH_2 at 1´ of C_3H_7 at C_3
	1.39	2	Sextet	CH_2 at 2´ of C_3H_7 at C_3
	0.95	3	Triplet	CH_3 of C_3H_7 at C_3
	4.16	2	Quartet	CH_2 of $COOC_2H_5$ at C_2
	1.36	3	Triplet	CH_3 of $COOC_2H_5$ at C_2
5b	8.58	1	Singlet	NH proton
	7.99-6.93	2	Multiplet	Aromatic protons
	2.00	2	Quartet	CH_2 of C_2H_5 at C_3
	1.06	3	Triplet	CH_3 of C_2H_5 at C_3
	3.76	3	Singlet	CH_3 of $COOCH_3$ at C_2
5c	8.67	1	Singlet	NH proton
	8.23-7.66	2	Multiplet	Aromatic protons
	1.72	3	Singlet	CH_3 protons at C_3
	4.32	1	Heptet	CH proton of $COOCH(CH_3)_2$ at C_2
	1.37	6	Doublet	CH_3 proton of $COOCH(CH_3)_2$ at C_2
5d	8.97	1	Singlet	NH Proton
	7.78-6.64	2	Multiplet	Aromatic protons
	2.06	2	Triplet	CH_2 at 1´ of C_3H_7 at C_3
	1.36	2	Sextet	CH_2 at 2´ of C_3H_7 at C_3
	0.96	3	Triplet	CH_3 of C_3H_7 at C_3
	4.17	2	Quartet	CH_2 of $COOC_2H_5$ at C_2
	1.39	3	Triplet	CH_3 of $COOC_2H_5$ at C_2

TABLE 8 ^{13}C NMR SPECTRAL DATA OF SUBSTITUTED 4*H*-1, 4-BENZOTHIAZINES 5a-d.

Compounds	^{13}C NMR δ (in ppm)
5a	107.2 (C-2), 138.9 (C-3), 109.7 (C-5), 126.3 (C-6), 120.8 (C-7), 136.6 (C-8), 34.1 (CH_2 at 1´ of C_3H_7 at C-3), 17.2 (CH_2 at 2´ of C_3H_7 at C-3), 14.6 (CH_3 of C_3H_7 at C-3), 165.6 (C=O at C-2), 58.8 (CH_2 of $COOC_2H_5$ at C-2), 13.1 (CH_3 of $COOC_2H_5$ at C-2)
5b	116.7 (C-2), 142.6 (C-3), 118.4 (C-5), 127.4 (C-6), 120.4 (C-7), 134.3 (C-8), 24.7 (CH_2 of C_2H_5 at C-3), 9.1 (CH_3 of C_2H_5 at C-3), 196.7 (C=O at C-2), 56.6 (CH_3 of $COOCH_3$ at C-2)
5c	112.6 (C-2), 134.7 (C-3), 119.3 (C-5), 125.9 (C-6), 119.5 (C-7), 135.0 (C-8), 16.9 (CH_3 at C-3), 191.3 (C=O at C-2), 68.4 (CH of $COOCH(CH_3)_2$ at C-2), 22.1 (CH_3 of $COOCH(CH_3)_2$ at C-2)
5d	110.7 (C-2), 143.4 (C-3), 108.8 (C-5), 134.3 (C-6), 126.3 (C-7), 139.3 (C-8), 33.7 (CH_2 at 1´ of C_3H_7 at C-3), 17.1 (CH_2 at 2´ of C_3H_7 at C-3), 14.4 (CH_3 of C_3H_7 at C-3), 195.4 (C=O at C-2), 58.7 (CH_2 of $COOC_2H_5$ at C-2), 13.0 (CH_3 of $COOC_2H_5$ at C-2)

TABLE 9 PHYSICAL AND ANALYTICAL DATA OF 4*H*-1, 4-BENZOTHIAZINE SULFONES 6a-d.

Compounds	Molecular formula	m.p. (°C)	Yield (%)	% Found (Calcd.)		
				C	H	N
6a	$C_{14}H_{15}NO_4SBrF$	135	48	42.87 (42.75)	3.86 (3.82)	3.52 (3.56)
6b	$C_{12}H_{11}NO_4SBrF$	120	58	39.52 (39.45)	3.05 (3.01)	3.88 (3.84)
6c	$C_{13}H_{13}NO_4SBrF$	257	51	41.29 (41.16)	3.47 (3.43)	3.62 (3.69)
6d	$C_{14}H_{15}NO_4SBrF$	235	40	42.79 (42.75)	3.85 (3.82)	3.53 (3.56)

TABLE 10 CHARACTERISTIC VIBRATIONS OF THE SULFONYL GROUP IN THE 4*H*-1, 4-BENZOTHIAZINE SULFONES 6a-d (in cm^{-1}).

Compounds	ν_{sym} (SO_2)	$\nu_{bending}$ (SO_2)	ν_{asym} (SO_2)
	KBr	KBr	KBr
	1180	566	1348
6a	1145	545	1270
			1260
	1150	567	1350
6b	1154	543	1270
			1265
	1174	572	1340
6c	1140	544	1268
			1242
	1164	562	1352
6d	1158	550	1250
			1238

TABLE 11 INFRARED SPECTRAL DATA OF SUBSTITUTED 4*H*-1,4-BENZOTHIAZINES AND 4*H*-1,4-BENZOTHIAZINES-1,1-DIOXIDE 6a-d (in KBr*) (in cm^{-1}).

Compounds	A	B	C
6a	3210 (3230)	1590 (1610)	1054 (1070)
6b	3255 (3272)	1600 (1620)	1046 (1065)
6c	3290 (3310)	1595 (1615)	1040 (1052)
6d	3300 (3325)	1610 (1625)	1038 (1048)

* The bands given in brackets refer to sulfones

A = N–H stretching vibrations,

B = (C=O) stretching vibrations.

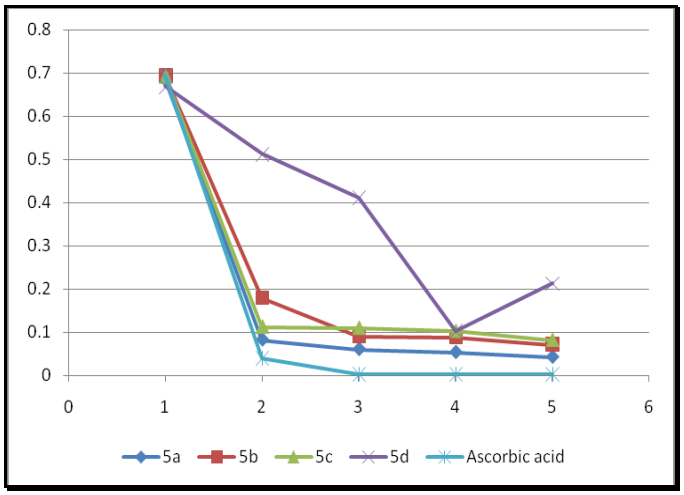
C = ν (C=S) stretching vibrations.

TABLE 12 ^{1}H NMR SPECTRAL DATA OF SUBSTITUTED 4*H*-1, 4-BENZOTHIAZINE SULFONES 6a-d.

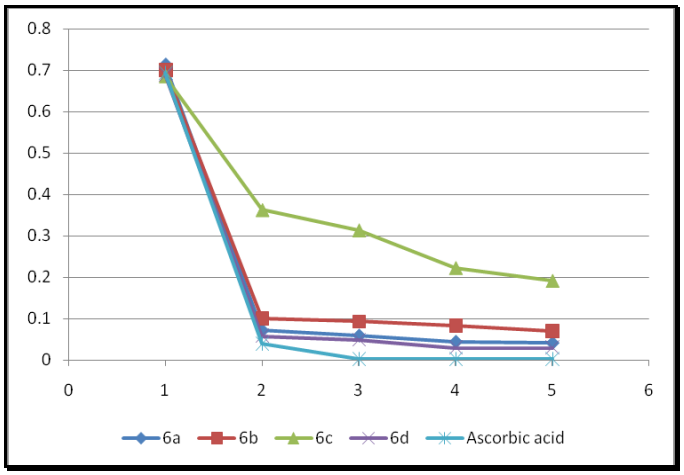
Compounds	δ (in ppm)	No. of Hydrogen	Multiplicity	Assignment
	8.88	1	Singlet	NH Proton
	7.91-6.82	2	Multiplet	Aromatic protons
	2.00	2	Triplet	CH_2 at 1′ of C_3H_7 at C_3
6a	1.36	2	Sextet	CH_2 at 2′ of C_3H_7 at C_3
	0.97	3	Triplet	CH_3 of C_3H_7 at C_3
	4.22	2	Quartet	CH_2 of $COOC_2H_5$ at C_2
	1.34	3	Triplet	CH_3 of $COOC_2H_5$ at C_2
	8.79	1	Singlet	NH proton
	7.81-6.82	2	Multiplet	Aromatic protons
6b	2.03	2	Quartet	CH_2 of C_2H_5 at C_3
	1.11	3	Triplet	CH_3 of C_2H_5 at C_3
	3.73	3	Singlet	CH_3 of $COOCH_3$ at C_2
	8.18	1	Singlet	NH proton
	7.92-6.91	2	Multiplet	Aromatic protons
6c	1.76	3	Singlet	CH_3 protons at C_3
	4.37	1	Heptet	CH proton of $COOCH(CH_3)_2$ at C_2
	1.41	6	Doublet	CH_3 proton of $COOCH(CH_3)_2$ at C_2
	9.09	1	Singlet	NH Proton
	7.81-6.92	2	Multiplet	Aromatic protons
	2.08	2	Triplet	CH_2 at 1′ of C_3H_7 at C_3
6d	1.35	2	Sextet	CH_2 at 2′ of C_3H_7 at C_3
	0.96	3	Triplet	CH_3 of C_3H_7 at C_3
	4.19	2	Quartet	CH_2 of $COOC_2H_5$ at C_2
	1.37	3	Triplet	CH_3 of $COOC_2H_5$ at C_2

TABLE 13 ^{13}C NMR SPECTRAL DATA OF SUBSTITUTED 4*H*-1, 4-BENZOTHIAZINES 6a-d.

Compounds	^{13}C NMR δ (in ppm)
6a	106.9 (C-2), 139.1 (C-3), 109.4 (C-5), 127.0 (C-6), 120.8 (C-7), 136.9 (C-8), 34.8 (CH_2 at 1´ of C_3H_7 at C-3), 16.9 (CH_2 at 2´ of C_3H_7 at C-3), 15.0 (CH_3 of C_3H_7 at C-3), 166.1 (C=O at C-2), 59.0 (CH_2 of $COOC_2H_5$ at C-2), 13.8 (CH_3 of $COOC_2H_5$ at C-2)
6b	117.2 (C-2), 143.5 (C-3), 119.3 (C-5), 126.8 (C-6), 121.1 (C-7), 136.9 (C-8), 24.7 (CH_2 of C_2H_5 at C-3), 9.6 (CH_3 of C_2H_5 at C-3), 197.4 (C=O at C-2), 56.2 (CH_3 of $COOCH_3$ at C-2)
6c	113.4 (C-2), 135.0 (C-3), 119.5 (C-5), 126.3 (C-6), 119.9 (C-7), 136.1 (C-8), 17.1 (CH_3 at C-3), 190.8 (C=O at C-2), 67.9 (CH of $COOCH(CH_3)_2$ at C-2), 23.1 (CH_3 of $COOCH(CH_3)_2$ at C-2)
6d	110.9 (C-2), 144.1 (C-3), 109.0 (C-5), 134.9 (C-6), 127.2 (C-7), 139.7 (C-8), 33.9 (CH_2 at 1´ of C_3H_7 at C-3), 18.0 (CH_2 at 2´ of C_3H_7 at C-3), 14.9 (CH_3 of C_3H_7 at C-3), 196.6 (C=O at C-2), 59.4 (CH_2 of $COOC_2H_5$ at C-2), 13.7 (CH_3 of $COOC_2H_5$ at C-2)



ABTS ACTIVITY (AT DIFFERENT TIME INTERVALS) OF SYNTHESIZED 4*H*-1, 4-BENZOTHIAZINES (TABLE 1)



ABTS ACTIVITY (AT DIFFERENT TIME INTERVALS) OF SYNTHESIZED BENZOTHIAZINE SULFONES (TABLE 2)

FIG. 1 and 2. THE EFFECT OF TIME ON SUPPRESSION OF ABSORBANCE OF ABTS BY SYNTHESIZED BENZOTHIAZINES AND THEIR SULFONE ANALOGUES.

CONCLUSIONS: The synthesized compounds were also screened for antioxidant and antimicrobial activities. The synthesized compounds were screened for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2, 2 – azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization assay.

All the compounds tested for their *in vitro* antibacterial activity against the four strains of bacteria (two gram-ve *E. coli*, *Pseudomonas aeruginosa* and two gram +ve *Bacillus subtilis*, *Staphylococcus aureus*) and antifungal activity against the two strains of fungi (*C. albicans* and *A. niger*).

The antibacterial and antifungal activity increases with increase in concentration of test compounds. In general the presence of electron withdrawing group on the aromatic ring increases the antimicrobial activities of tested compound compared to compounds having electron donating groups. All the synthesized compounds were evaluated for their antioxidant, antibacterial and antifungal activity and all these have shown moderate to higher activity against the test assay and microbes.

Experimental Section

All the melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (by broad band proton decoupling technique) were recorded on JEOL AL-300 spectrometer at frequencies of (300.40 and 75.45 MHz) respectively, in DMSO-d_6 using TMS as an internal standard. IR spectra were recorded in KBr on SHIMADZU 8400S FT-IR spectrophotometer.

The FAB (fast atom Bombardment) mass spectra were recorded on a JEOL SX 102/DA-600 mass spectrometer using Ar/Xe as FAB gas at an accelerating voltage of 10 KV. The purity of compounds was checked by TLC using silica gel

'G' as adsorbent, visualizing these by UV light or in an iodine chamber.

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