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Synthesis, Spectral Characterization and Antioxidative Evaluation of Substituted 4*H*-1,4-Benzothiazine and Their Sulfones

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4*H*-1,4-Benzothiazines are synthesized by condensation followed by oxidative cyclization of 2-aminobenzenethiols with β-diketones in presence of dimethyl sulfoxide. The sulfones of these 4*H*-1,4-benzothiazines were synthesized by treating them with 30 % hydrogen peroxide in glacial acetic acid. Synthesized compounds found to be antioxidants on the basis of certain analysis. Spectral evaluation and confirmation are done by IR, ^{1}H NMR and mass spectral data techniques.

Key Words: 4*H*-1,4-Benzothiazines, Sulfones, Antioxidant activity.

INTRODUCTION

4*H*-1,4-Benzothiazine and their sulfones are found to be a potent pharmacological[1-7] and industrially useful[8-12] heterocyclic compounds, having application as anticancer, antiinflammatory, antibiotic, antiulcer, antihistaminic, anticholesterolic and CNS depressant agents. While at the other hand they are found useful in dyestuffs and paints also. Recent studies proved them as powerful antioxidant also[13-16].

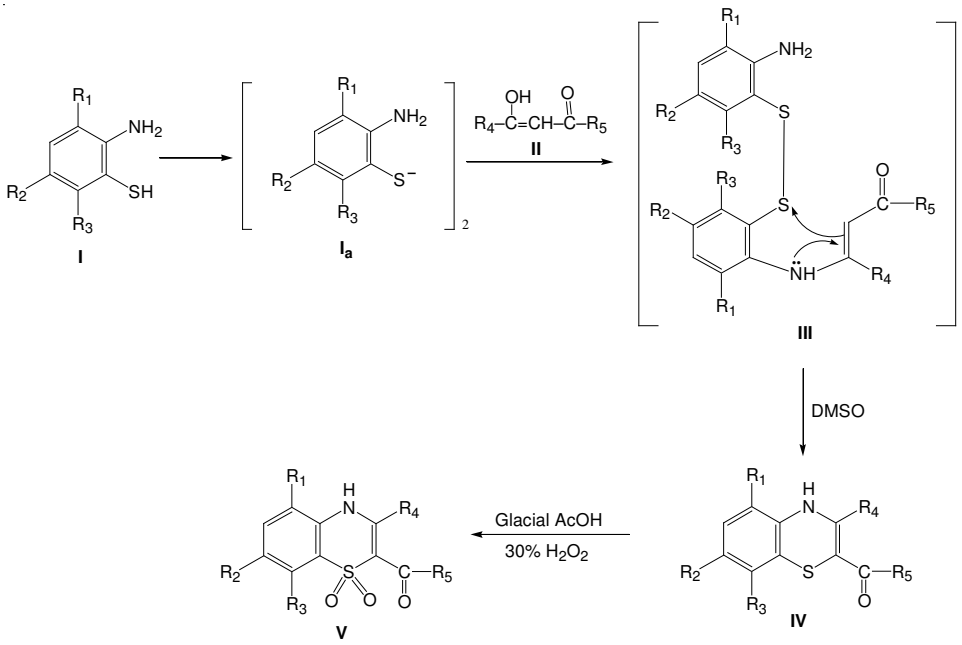
4*H*-1,4-Benzothiazine and their sulfones were screened for their antioxidative properties through *in vitro* and *in vivo* studies on swiss albino mice.

EXPERIMENTAL

All the melting points were determined in open capillary tubes and are uncorrected. IR Spectra were recorded in KBr on Nicolet-Megna FT-IR 550 spectrometer and the ^{1}H NMR spectra on JEOL AL-300 spectrometer (300 MHz) in DMSO-d_6 using TMS, as an internal standard (chemical shifts are measured in δ ppm) Their mass spectra were recorded on JEOL SX 102/DA 600 using Argon/Xenon as FAB gas. The purity of the synthesized compounds were checked by TLC using silica gel "G" as adsorbent and visualizing these by UV light or an iodine chamber.

Swiss albino mice were obtained from, Jawaharlal Nehru University, New Delhi, India. Random-bred, male Swiss albino mice (8 weeks old), weighing 24 ± 2 g were used for experiments. These animals were maintained in the animal house at temperature of 24 ± 3 °C. They were housed in polypropylene cages and fed standard mice feed from Hindustan Lever Ltd., India. Tap water was provided to the animals.

Preparation of substituted 4*H*-1,4-benzothiazines (IV_{a-d}): Substituted 2-amino-benzenethiols (I; 0.01 mol) was added to a stirred suspension of β-diketones (II; 0.01 mol) in DMSO (5 mL) and the resulting mixture was refluxed for 2 h. The resulted refluxed mixture was cooled down to room temperature. The solid separated out was filtered, washed with petroleum ether and crystallized from methanol. (**Scheme-I**)



Scheme-I

Preparation of 4*H*-1,4-benzothiazine sulfones (V_{a-d}): Substituted 4*H*-1,4-benzothiazines (0.01 mol), glacial acetic acid (20 mL), 30 % hydrogen peroxide (5 mL) were added together in 50 mL round bottomed flask and was refluxed for 15-20 min at 50-60 °C. After this heating was stopped and a second lot of (5 mL) hydrogen peroxide was further added. The mixture was again refluxed for about 3-4 h. Then the mixture was poured into a beaker containing crushed ice. The precipitate obtained was filtered, washed with water and crystallized from ethanol (**Scheme-I**).

Antioxidant evaluation

DPPH Radical scavenging assay: Radical scavenging activity of compound IV_{a-d} and V_{a-d} against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet *et al.*[13]. A stock solution 1 mg/mL of the compound was prepared in methanol solution of DPPH. After 0.5 h incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula.

$$\text{Inhibition}(\%) = \frac{(AB - AA)}{AB} \times 100$$

where AB = absorption of blank, AA = absorption of test.

ABTS Radical cation decolourization assay: The 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) decolourization test was also used to assess the antioxidant activity of compounds **IV** and **V**. The ABTS assay was carried out using the improved assay of Re *et al.*[14]. In brief, $ABTS^{\bullet+}$ was generated by oxidation of ABTS with potassium persulphate. For this purpose ABTS was dissolved in deionized water at a concentration of 7 mM and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-16 h) in the dark before use. The ABST solution then diluted with ethanol to an absorbance of 0.700 ± 0.200 at 734 nm. After addition of 1 mL of diluted ABST solution to 10 µL of compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1-6 min later. All determinations were carried out in triplate.

RESULTS AND DISCUSSION

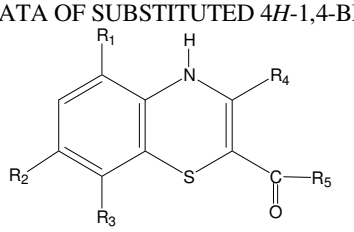
4*H*-1,4-Benzothiazines (**IV**) have been prepared by condensation and oxidative cyclization of 2-aminobenzenthiols (**I**) with β-diketones (**II**) in presence of dimethyl sulfoxide. The reaction is believed to proceed through the formation of an intermediate enamino ketone system (**III**). The mechanism of the reaction reports an intermediate *i.e.*, *bis*-(2-aminophenyl) disulfide ($\mathbf{I}_a$) obtained by readily oxidation of substituted 2-aminobenzenethiol, which cyclizes to substituted 4*H*-1,4-benzothiazine (**IV**) by cleavage of sulfur-sulfur bond due to high reactivity of α-position of enaminoketone system (**III**) towards nucleophilic attack (**Scheme-I**). The proposed structures of the synthesized compounds are well supported by their elemental analysis (Tables 1 and 2) and spectroscopic data (Table-3).

IR Spectra: IR Spectra of the synthesized compounds $\mathbf{IV}_{a\text{-}d}$ and $\mathbf{V}_{a\text{-}d}$ are tabulated in Table-3. Compounds $\mathbf{IV}_{a\text{-}d}$ and $\mathbf{V}_{a\text{-}d}$ showed peak in the region 3390-3285 and 3430-3345 cm^{-1}, respectively due to >N-H stretching vibration of secondary amino group. All the synthesized compounds showed sharp bands in the region 1640-1590 and 1710-1610 cm^{-1} due to >C=O stretching vibration.

Compounds $\mathbf{IV}_{a\text{-}d}$ showed peak in the region 730-715 cm^{-1} due to C-Cl stretching vibration. Compounds $\mathbf{V}_{a\text{-}d}$ showed peak in the region from 1110-1075 cm^{-1} due to C-S stretching vibration.

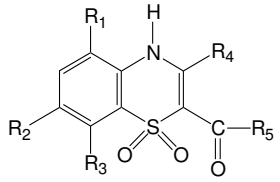
^{1}H NMR Spectra: ^{1}H NMR spectra of the synthesized compounds $\mathbf{IV}_{a\text{-}d}$ and $\mathbf{V}_{a\text{-}d}$ are tabulated in Table-3. All the synthesized compounds showed multiplet due to aromatic protons, which appeared in the region δ 6.50-8.10 ppm in $\mathbf{IV}_{a\text{-}d}$ and δ 6.70-8.20 ppm in $\mathbf{V}_{a\text{-}d}$. Compound $\mathbf{IV}_{a\text{-}d}$ and $\mathbf{V}_{a\text{-}d}$ showed singlet due to >N-H in the

TABLE-1
CHARACTERIZATION DATA OF SUBSTITUTED 4*H*-1,4-BENZOTHIAZINES (**IV**$_{a-d}$)



Comp.	R_1	R_2	R_3	R_4	R_5	m.p. (ºC)	Yield (%)	m.f.	Found (calcd.) (%)		
									C	H	N
IV$_a$	Cl	H	CF_3	C_6H_5	C_6H_5	55	45	$C_{22}H_{13}NOSClF_3$	61.38 (61.18)	3.04 (3.01)	3.22 (3.24)
IV$_b$	Cl	H	CF_3	CH_3	$C_6H_4(OCH_3)(o)$	76	43	$C_{18}H_{13}NO_2SClF_3$	54.27 (54.06)	3.27 (3.25)	3.53 (3.50)
IV$_c$	F	Br	H	CH_3	$C_6H_4CH_3(p)$	88	55	$C_{17}H_{13}NOSBrF$	54.16 (53.96)	3.45 (3.43)	3.66 (3.70)
IV$_d$	F	Br	H	CH_3	$C_6H_4Cl(p)$	90	52	$C_{16}H_{10}NOSBrClF$	48.37 (48.18)	2.48 (2.50)	3.50 (3.51)

TABLE-2
CHARACTERIZATION DATA OF SULFONES OF 4*H*-1,4-BENZOTHIAZINE (**V**$_{a-d}$)



Comp.	R_1	R_2	R_3	R_4	R_5	m.p. (ºC)	Yield (%)	m.f.	Found (calcd.) (%)		
									C	H	N
V$_a$	Cl	H	CF_3	C_6H_5	C_6H_5	60	40	$C_{22}H_{13}NO_3SClF_3$	57.29 (56.96)	2.82 (2.80)	3.00 (3.02)
V$_b$	Cl	H	CF_3	CH_3	$C_6H_4(OCH_3)(o)$	110	45	$C_{18}H_{13}NO_4SClF_3$	51.25 (51.05)	3.03 (3.01)	3.26 (3.24)
V$_c$	F	Br	H	CH_3	$C_6H_4CH_3(p)$	90	42	$C_{17}H_{13}NO_3SBrF$	49.99 (49.76)	3.15 (3.17)	3.45 (3.41)
V$_d$	F	Br	H	CH_3	$C_6H_4Cl(p)$	105	45	$C_{16}H_{10}NO_3SBrClF$	44.82 (44.59)	2.35 (2.32)	3.21 (3.25)

region δ 8.02-9.05 and δ 8.01-9.07 ppm, respectively. In compounds **IV**$_{b-d}$ and **V**$_{b-d}$, a singlet is observed in the region δ 2.03-1.84 ppm due to allylic (C=C-CH_3) proton at C_3. Compounds **IV**$_b$ and **V**$_b$ shows singlet at δ 1.36 and δ 1.40 ppm, respectively due to OCH_3 proton at 2' position of benzoyl group, while compounds **IV**$_c$ and **V**$_c$ shows singlet at δ 1.50 and δ 1.55 ppm, respectively due to CH_3 group at 4' position of benzoyl group at C_2.

Mass spectra: The molecular ion peaks are in accordance with their molecular weight of synthesized compounds. In all cases side chain at C_2(-COR_5) appear as a base peak.

TABLE-3
^{1}H NMR AND IR SPECTRAL DATA OF SYNTHESIZED COMPOUNDS ($\mathbf{IV}_{a-d}$ AND $\mathbf{V}_{a-d}$)

Comp.	^{1}H NMR (δ ppm, TMS)		IR Spectra (in cm^{-1})						Mass m/z (%)
	>NH	Ar-H	>NH (A)	>C=O (B)	C-F (C)	C-Cl (D)	C-B(E)	CF_3 (F)	
$\mathbf{IV}_a$	8.02	6.50-7.80	3320	1640	–	715	–	1340 1120	431 (M^+), 433 (M + 2), 105 (100), 249 (70), 214 (40)
$\mathbf{IV}_b$	8.75	6.90-8.10	3285	1590	–	730	–	1320 1130	399 (M^+), 401 (M + 2), 135 (100), 249 (70), 214 (40)
$\mathbf{IV}_c$	9.05	6.80-7.82	3365	1615	1150	–	560	–	377 (M^+), 379 (M + 2), 119 (100), 244 (60), 164 (45)
$\mathbf{IV}_d$	8.97	6.90-8.01	3390	1605	1190	–	530	–	398 (M^+), 400 (M + 2), 402 (M + 4), 139 (100), 244 (60), 164 (45)
$\mathbf{V}_a$	8.87	6.70-7.95	3380	1710	–	720	–	1350 1130	463 (M^+), 465 (M+2), 105 (100), 281 (55), 246 (35)
$\mathbf{V}_b$	8.98	6.95-8.20	3345	1610	–	735	–	1325 1135	431 (M^+), 433 (M + 2), 135 (100), 281 (55), 246 (35)
$\mathbf{V}_c$	9.07	6.90-7.85	3405	1630	1170	–	565	–	409 (M^+), 411 (M+2), 119 (100), 276 (65), 196 (48)
$\mathbf{V}_d$	9.01	7.02-8.12	3430	1620	1205	–	535	–	430 (M^+), 432 (M + 2), 434 (M + 4), 139 (100), 276 (65), 196 (48)

A = N-H stretching vibrations, B = C=O stretching vibrations, C = C-F stretching vibrations, D = C–Cl stretching vibrations, E = C-Br stretching vibrations, F = Asymmetric and symmetric vibrations of C–F in CF_3 group.

Antioxidant activity: All the synthesized compounds $\mathbf{IV}_{a-d}$ and their sulfones $\mathbf{V}_{a-d}$ were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2-azino*bis*(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolourization assay.

The present study demonstrated that compounds showed mixed radical scavenging activity in both DPPH (Table-4) and $ABTS^{\bullet+}$ assays (Table-5). (i) Compounds $\mathbf{IV}_a$ and $\mathbf{V}_c$ showed strong radical scavenging activity in DPPH % inhibition ≥ 50. (ii) Compounds $\mathbf{IV}_c$, $\mathbf{IV}_d$, $\mathbf{V}_a$ and $\mathbf{V}_b$ showed moderate radical scavenging activity in DPPH assay that have DPPH % inhibition ≥ 30. (iii) Compounds $\mathbf{IV}_b$ and $\mathbf{V}_d$ showed mild radical scavenging activity in DPPH assay that have DPPH % inhibition < 30. (iv) Compound $\mathbf{IV}_a$, $\mathbf{IV}_d$, $\mathbf{V}_a$ and $\mathbf{V}_c$ were found to be more active in $ABTS^{\bullet+}$ assay which showed much decline in graph (Figs. 1 and 2).

The study reveals that $\mathbf{V}_b$ and $\mathbf{V}_c$ showed better chemopreventive action and antigenotic effect as compare to their respective heterocyclic bases (4*H*-1,4-benzothiazine) (Table-4). Compounds $\mathbf{V}_a$ and $\mathbf{V}_b$ showed better chemopreventive action and antigenotic effect than their respective bases in ABTS assay (Table-5).

The compounds were further treated for evaluation of antioxidative properties on swiss albino mice. Results showed that there was significant decrease in lipid

peroxidation (LPO) level and elevation in reduced glutathione (GSH) in swiss albino mice (Tables 6 and 7).

The above value shows that there was significant increase in GSH animals treated with content of liver in compounds $\mathbf{IV_b}$ and $\mathbf{V_d}$ while in animals treated with compound No. $\mathbf{V_c}$ and $\mathbf{V_d}$, there is significant decrease in LPO, showing potent antioxidant activities in swiss albino mice. However, other compounds shows increase in GSH content and decrease in LPO level but not statistically significant.

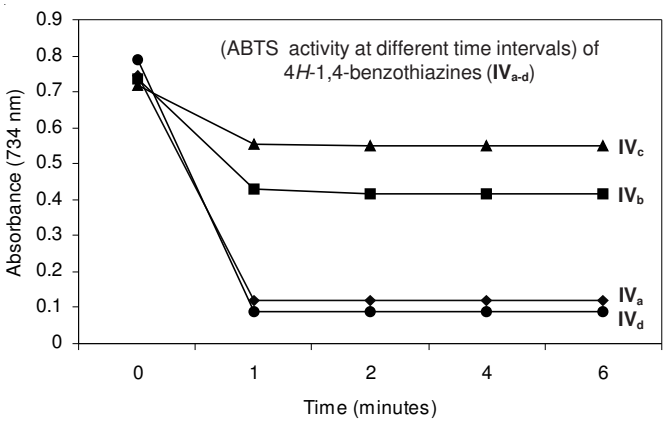


Fig. 1. Effect of time on the suppression of absorbance of ABTS by 4*H*,1,4-benzothiazines (**IV**). After addition of 1 µL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 mL of the compound the absorbance reading was taken at 30 °C exactly 1 min, after initial mixing and up to 6 min. All determinations were carried out in triplicates

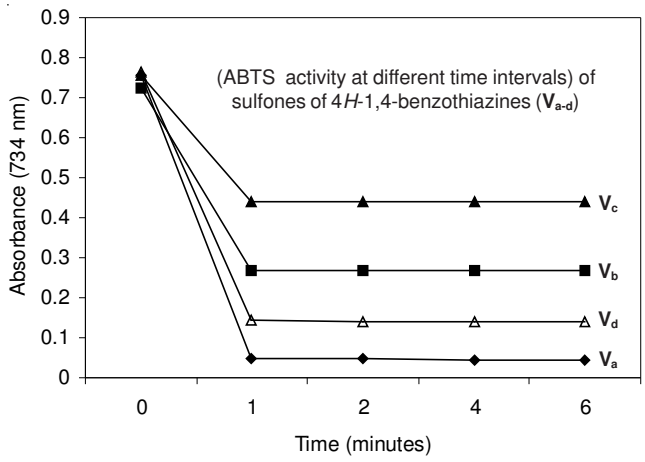


Fig. 2. Effect of time on the suppression of absorbance of ABTS by sulfones (**VI**). After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 µL of the compound the absorbance reading was taken at 30 °C exactly 1 min, after initial mixing and up to 6 min. All determinations were carried out in triplicates

TABLE-4
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Compound No.	DPPH % inhibition of 1 mg/mL of the compound
$\mathbf{IV_a}$	50.70 ± 0.10
$\mathbf{IV_b}$	25.43 ± 0.05
$\mathbf{IV_c}$	37.25 ± 0.02
$\mathbf{IV_d}$	44.31 ± 0.25
$\mathbf{V_a}$	42.85 ± 1.20
$\mathbf{V_b}$	43.22 ± 0.09
$\mathbf{V_c}$	52.71 ± 0.12
$\mathbf{V_d}$	20.57 ± 0.04

Inhibition (%) of DPPH radical scavenging activity of various compounds at particular concentration. Stock solution of crude compound was prepared as 1 mg/mL in methanol. Fifty microlitres of samples of particular concentration were added to 5 ml of 0.004% methanol solution of DPPH. After 30 min incubation in dark at room temperature, the absorbance was read against a blank at 517 nm.

TABLE-5
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Comp. No.	$ABTS^{\bullet+}$ activity at different time intervals minutes				
	0 min	1 min	2 min	4 min	6 min
$\mathbf{IV_a}$	0.745	0.121	0.120	0.120	0.120
$\mathbf{IV_b}$	0.735	0.428	0.418	0.415	0.415
$\mathbf{IV_c}$	0.718	0.554	0.550	0.550	0.550
$\mathbf{IV_d}$	0.789	0.090	0.089	0.089	0.089
$\mathbf{V_a}$	0.757	0.048	0.047	0.046	0.046
$\mathbf{V_b}$	0.723	0.270	0.269	0.269	0.269
$\mathbf{V_c}$	0.765	0.144	0.142	0.142	0.142
$\mathbf{V_d}$	0.755	0.442	0.441	0.441	0.441

TABLE-6
ANTIOXIDATIVE PROPERTIES OF COMPOUNDS IN
THE LIVER IN SWISS ALBINO MICE

Treatment compound No.	LPO (n mol/mg tissue)
$\mathbf{IV_a}$	6.45 ± 0.10
$\mathbf{IV_b}$	6.20 ± 0.25
$\mathbf{IV_c}$	6.81 ± 0.14 $p < 0.05$
$\mathbf{IV_d}$	6.57 ± 0.28
$\mathbf{V_a}$	6.50 ± 0.14
$\mathbf{V_b}$	6.72 ± 0.15
$\mathbf{V_c}$	6.15 ± 0.07 $p < 0.05$
$\mathbf{V_d}$	6.11 ± 0.11

***In vivo* studies in swiss albino mice:** The compounds were further treated for evaluation of antioxidative properties in swiss albino mice. Results showed that there was significant decrease in lipid peroxidation (LPO) level (Table-6) and elevation in reduced glutathione (GSH) in swiss albino mice (Table-7).

TABLE-7
ANTIOXIDATIVE PROPERTIES OF COMPOUNDS IN THE LIVER IN SWISS ALBINO MICE

Treatment compound No.	GSH (n mol/mg tissue)
$\mathbf{IV_a}$	4.35 ± 0.15
$\mathbf{IV_b}$	5.02±0.18 $p < 0.05$
$\mathbf{IV_c}$	4.71 ± 0.27
$\mathbf{IV_d}$	4.23 ± 0.95
$\mathbf{V_a}$	4.10±0.80 $p < 0.005$
$\mathbf{V_b}$	4.65 ± 0.52
$\mathbf{V_c}$	4.30 ± 0.62
$\mathbf{V_d}$	5.04 ± 0.15

Biochemical studies

Lipid peroxidation assay: The LPO level in liver was measured in terms of thiobarbituric and reactive substance (TBARS) by the method of Ohkhawa *et al.*[15]. Absorbance in the assay was read at 532 nm (Table-6).

Sulfhydral group assay (GSH): The level of acid-soluble sulfhydral groups was estimated in liver as total non-protein sulfhydral groups using the method described by Moron *et al.*[16], reduced glutathione (GSH; obtained from Sisco Research Laboratories, Bombay, India) was used as a standard to calculate the micromoles of SH/g of tissue. Absorbance in the assay was read at 412 nm using a systronic spectrophotometer (Systronics Type 108; Naroda, Ahmedabad, India) (Table-7).

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