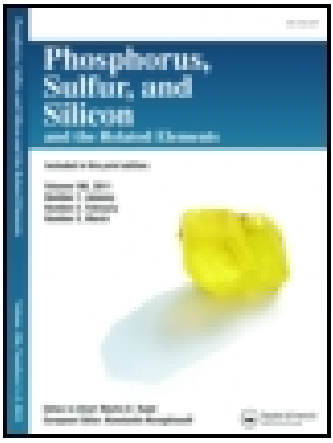

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

Synthetic Methodology, Spectral Elucidation, and Antioxidative Properties of Benzothianes and Their Sulfones

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Published online: 03 Nov 2009.

To cite this article: Vibha Gautam, Meenakshi Sharma, Meenakshi Panwar, Naveen Gautam, Ashok Kumar, I. K. Sharma & D. C. Gautam (2009) Synthetic Methodology, Spectral Elucidation, and Antioxidative Properties of Benzothianes and Their Sulfones, Phosphorus, Sulfur, and Silicon and the Related Elements, 184:11, 3090-3109, DOI: 10.1080/10426500802704225

To link to this article: http://dx.doi.org/10.1080/10426500802704225

Phosphorus, Sulfur, and Silicon, 184:3090–3109, 2009

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500802704225

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Synthetic Methodology, Spectral Elucidation, and Antioxidative Properties of Benzothianes and Their Sulfones

Vibha Gautam,[1] Meenakshi Sharma,[1] Meenakshi Panwar,[2] Naveen Gautam,[3] Ashok Kumar,[2] I. K. Sharma,[1] and D. C. Gautam[1]

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This article reflects the synthetic strategies and spectral investigation of 4H-1,4-benzothiazines. 4H-1,4-benzothiazines have been prepared by the condensation and oxidative cyclization of substituted 2-aminobenzenethiol with β-diketones / β-ketoesters in dimethyl sulfoxide and with the oxidation of 4H-1,4-benzothiazines by 30% hydrogen peroxide in glacial acetic acid, which results in the formation of 4H-1,4-benzothiazine sulfones. The compounds were evaluated for their antioxidative properties through in vitro and in vivo studies in Swiss albino mice. The structural assignments of compounds were made on the basis of spectroscopic data and elemental analysis.

Keywords Antioxidant activities; benzothiazines; sulfones; Swiss albino mice

INTRODUCTION

Benzothiazines possess a bend along the nitrogen–sulfur axis, which is one of the structural specificities responsible for similar pharmacological activities. As part of an ongoing program in the development of novel synthetic methodologies for the preparation of biologically active substances, we have been interested in the synthesis of various heterocyclic structures because they are an integral part of many naturally

Received 14 July 2008; accepted 18 December 2008.
Authors are thankful to the Head, Chemistry and Zoology Department, University of Rajasthan, Jaipur, India for laboratory facilities. Thanks are due to CSIR and UGC (Bhopal) for financial support. Thanks are also due to CDRI, Lucknow for providing the IR, proton NMR, and mass facilities.
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occurring and biologically active compounds.[1–5] This review also reflects up-to-date, comprehensive coverage of chemical and biomedical aspects of sulfones of 4H-1,4-benzothiazines. Such heterocyclic compounds are interesting because of their theoretical and structural implications; the diversity of synthetic methods used in their preparation; and their biological, pharmacological, and industrial significance. This provides an exhaustive coverage of their basic and applied aspects. The compounds were evaluated for their antioxidative properties through in vitro and in vivo studies in Swiss albino mice.[6–11]

RESULTS

Various substituted 2-aminobenzenethiols (**I**) were condensed with β-diketones/β-ketoesters (**IIa**) in the presence of dimethyl sulfoxide, which results in oxidative cyclization. The mechanism of the reaction reports an intermediate, i.e., bis-(2-aminophenyl) disulfide (**Ia**), obtained by the ready oxidation of substituted, 2-aminobenzenethiol (**I**), which cyclizes to substituted 4H-1,4-benzothiazine (**IV**) by the scission of the sulfur–sulfur bond due to the high reactivity of α-position of the enaminoketone system (**III**) towards nucleophilic attack. Oxidation of 1,4-benzothiazine $\mathbf{IV}_{a–c}$ by hydrogen peroxide in glacial acetic acid yielded sulfones $\mathbf{VII}_{a–i}$ in high yield.[2–5]

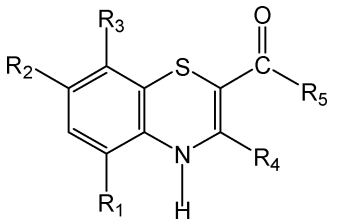
The structures of all the synthesized compounds are characterized by correct spectroscopic data and elemental analysis (Tables I to VII). The synthesized compounds showed mixed radical scavenging activity.

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.[8,10]

DISCUSSION

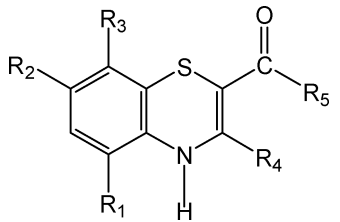
The structural assignment of these compounds was made on basis of spectroscopic data and elemental analysis; Table I for benzothiazines and Table IV for their sulfones. The characteristic IR bands and ^{1}H NMR data of compounds $\mathbf{IV}_{a–i}$ are presented in Tables II and III, respectively, and for compounds $\mathbf{VII}_{a–i}$ the IR and NMR spectra are presented in Tables V and VI, respectively. A single sharp peak in the region 3390–3240 cm^{-1} is observed due to N–H stretching vibrations in benzothiazines $\mathbf{IV}_{a–i}$ (Table II, column A). The series of synthesized 4H-1,4-benzothiazines exhibits a single sharp peak in the region δ 8.95–7.89 ppm due to N-H proton in ^{1}H NMR spectra (Table III). In the spectra of

TABLE I Physical Data of Substituted 4H-1,4-Benzothiazines



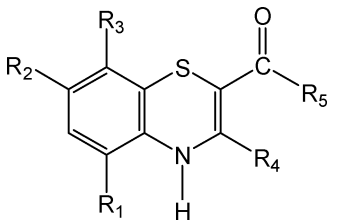
Compd. IV	Compound R_1	R_2	R_3	R_4	R_5	Mp °C	Yield %	Molecular formula	%C Found (calcd.)	H Found (Calcd.)	N Found (Calcd)
a	Cl	H	CF_3	CH_3	C_6H_4-Cl(p)	65	40	$C_{17}H_{10}NOSCl_2F_3$	50.98 (50.50)	2.45 (2.48)	3.44 (3.47)
b	Cl	H	CF_3	CH_3	C_6H_4-F(p)	75	42	$C_{17}H_{10}NOSClF_3$	52.89 (52.65)	2.59 (2.58)	3.58 (3.61)
c	Cl	H	CF_3	CH_3	C_6H_4-OCH_3(o)	60	54	$C_{18}H_{13}NO_2SClF_3$	54.18 (54.07)	3.28 (3.25)	3.54 (3.50)
d	Cl	H	CF_3	CH_3	C_6H_4-Cl(o)	70	41	$C_{17}H_{10}NOSCl_2F_3$	50.64 (50.50)	2.50 (2.48)	3.49 (3.47)
e	Br	H	Br	CH_3	C_6H_4-CH_3(p)	85	39	$C_{17}H_{13}NOSBr_2$	46.80 (46.68)	2.99 (2.97)	3.18 (3.20)
f	F	Br	H	C_6H_5	C_6H_5	90	47	$C_{21}H_{13}NOSBrF$	59.40 (59.29)	3.08 3.06	3.27 (3.29)
g	F	Br	H	CH_3	C_6H_5	85	38	$C_{16}H_6NOSBrF$	52.73 (52.89)	3.05 (3.03)	3.84 (3.86)
h	F	Br	H	CH_3	CF_3	110	46	$C_{11}H_6NOSBrF_4$	37.01 (37.18)	1.65 (1.69)	3.93 (3.94)
i	F	Br	H	CH_3	C_6H_4-Br (p)	80	45	$C_{16}H_{10}NOSBr_2F$	43.42 (43.54)	2.30 (2.27)	3.19 (3.17)

TABLE II Infrared Spectral Data of Substituted 4H-1,4-Benzothiazines (in cm^{-1})



Compd IV	Compound R_1	R_2	R_3	R_4	R_5	A N-H	B C=O	C C-H (CH_3)	D C-O-C	E C-Cl	F C-Br	G C-F
a	Cl	H	CF_3	CH_3	C_6H_4-Cl(p)	3340	1630	14501340	–	720	–	13201130
b	Cl	H	CF_3	CH_3	C_6H_4-F(p)	3240	1570	14301360	–	730	–	13201150
c	Cl	H	CF_3	CH_3	C_6H_4-OCH_3(o)	3380	1590	14501330	10201280	720	–	13251120
d	Cl	H	CF_3	CH_3	C_6H_4-Cl(o)	3260	1600	14401360	–	740	–	13301130
e	Br	H	Br	CH_3	C_6H_4-CH_3(p)	3350	1620	14101340	–	–	530	–
f	F	Br	H	C_6H_5	C_6H_5	3340	1570	–	–	–	550	–
g	F	Br	H	CH_3	C_6H_5	3260	1600	14401320	–	–	560	–
h	F	Br	H	CH_3	CF_3	3390	1580	14501340	–	–	540	13201130
i	F	Br	H	CH_3	C_6H_4-Br	3370	1570	14301350	–	–	550	–

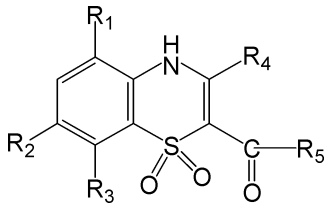
TABLE III ^{1}H NMR Spectral Data of Substituted 4H-1,4-Benzothiazines



Compd. IV	Compound R_1	R_2	R_3	R_4	R_5	δ	No. of Hydrogen	Multiplet	Assignment
						8.05	1	Singlet	N-H proton
a	Cl	H	CF_3	CH_3	C_6H_4-Cl(p)	7.69–6.50	6	Multiplet	Aromatic protons
						2.15	3	Singlet	CH_3 protons
						8.95	1	Singlet	NH-protons
b	Cl	H	CF_3	CH_3	C_6H_4-F(p)	7.96–6.25	6	Multiplet	Aromatic protons
						2.09	3	Singlet	CH_3 protons
						8.10	1	Singlet	NH-protons
c	Cl	H	CF_3	CH_3	C_6H_4-OCH_3(o)	7.45–6.34	6	Multiplet	Aromatic protons
						1.90	3	Singlet	CH_3 protons at C_3
						1.36	3	Singlet	CH_3 protons at 2′ carbon of C_6H_4
						7.95	1	Singlet	N-H proton
d	Cl	H	CF_3	CH_3	C_6H_4-Cl(o)	7.84–6.54	6	Multiplet	Aromatic protons
						2.12	3	Singlet	CH_3 protons
						8.10	1	Singlet	N-H proton

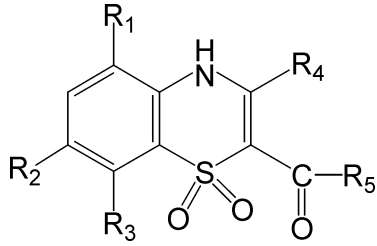
e	Br	H	Br	CH_3	C_6H_4-CH_3(p)	7.94–6.25	6	Multiplet	Aromatic protons
						2.30	3	Singlet	CH_3 proton at C_3
						1.51	3	Singlet	CH_3 proton at C′-4 carbon in C_6H_4
						8.90	1	Singlet	N-H proton
f	F	Br	H	C_6H_5	C_6H_5	7.88–6.19	12	Multiplet	Aromatic protons
						8.05	1	Singlet	N-H proton
g	F	Br	H	CH_3	C_6H_5	7.68–6.34	7	Multiplet	Aromatic protonCH_3 proton
						1.86	3	Singlet	
						7.89	1	Singlet	N-H proton
h	F	Br	H	CH_3	CF_3	7.49–6.30	2	Multiplet	Aromatic protons
						1.92	3	Singlet	CH_3 protons
						8.14	1	Singlet	N-H proton
i	F	Br	H	CH_3	C_6H_4-Br	7.80–6.15	6	Multiplet	Aromatic proton
						1.99	3	Singlet	CH_3 protons

TABLE IV Physical Data of Substituted 4H-1,4-Benzothiazine Sulfones (VIIa–i)



Compd. VII	Compound R_1	R_2	R_3	R_4	R_5	Mp °C	Yield %	Molecular formula	% Found (calcd.) C	H	N
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	75	38	$C_{17}H_{10}NO_3SCl_2F_3$	46.88(46.79)	2.32(2.29)	3.17(3.21)
b	Cl	H	CF_3	CH_3	C_6H_4–F(p)	80	46	$C_{17}H_{10}NO_3SClF_4$	48.76(48.63)	2.40(2.38)	3.36(3.34)
c	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	125	32	$C_{18}H_{13}NO_4SClF_3$	50.11(50.06)	3.03(3.01)	3.20(3.24)
d	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	120	37	$C_{17}H_{10}NO_3SCl_2F_3$	46.66(46.79)	2.31(2.29)	3.19(3.21)
e	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	95	30	$C_{17}H_{13}NO_3SBr_2$	43.38(43.50)	2.79(2.77)	3.01(2.99)
f	F	Br	H	C_6H_5	C_6H_5	90	41	$C_{21}H_{13}NO_3SBrF$	55.24(55.14)	2.86(2.84)	3.08(3.06)
g	F	Br	H	CH_3	C_6H_5	85	43	$C_{16}H_{11}NO_3SBrF$	48.69(48.61)	2.81(2.78)	3.50(3.54)
h	F	Br	H	CH_3	CF_3	60	35	$C_{11}H_6NO_3SBrF_4$	34.21(34.12)	1.56(1.55)	3.60 (3.62)
i	F	Br	H	CH_3	C_6H_4-Br(p)	70	46	$C_{16}H_{10}NO_3SBr_2F$	40.71(40.59)	2.13(2.11)	2.99(2.96)

TABLE V Infrared Spectral Data of Substituted 4H-1,4-Benzothiazine Sulfones ($VII_{a–i}$) (in KBr*) in cm^{-1}



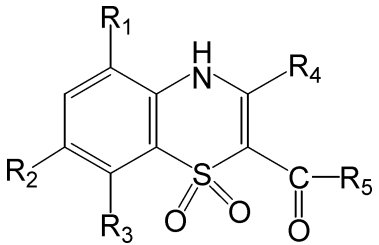
Compd. VII	Compound R_1	R_2	R_3	R_4	R_5	A N-H	B C=O	C C-S
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	3340(3400)	1620(1650)	1048(1085)
b	Cl	H	CF_3	CH_3	C_6H_4–F(p)	3240(3500)	1590(1620)	1050(1070)
c	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	3380(3440)	1600(1620)	1050(1080)
d	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	3260(3550)	1610(1660)	1042(1090)
e	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	3350(3420)	1600(1680)	1060(1080)
f	F	Br	H	C_6H_5	C_6H_5	3340(3410)	1580(1620)	1040(1085)
g	F	Br	H	CH_3	C_6H_5	3260(3420)	1610(1630)	1050(1090)
h	F	Br	H	CH_3	CF_3	3390(3430)	1620(1650)	1040(1070)
i	F	Br	H	CH_3	C_6H_4-Br(p)	3370(3400)	1610(1640)	1060(1080)

benzothiazine sulfones, the absorption band due to NH stretching vibrations is shifted to higher frequency region (3550–3400 cm–1) (Table V, column A). The series of synthesized 4H-1,4-benzothiazine sulfones (**$VII_{a–i}$**) exhibits a single sharp peak in the region δ 8.86–7.86 ppm due to N-H proton in 1H NMR spectra (Table VI).

EXPERIMENTAL

All the melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on a Nicolet-Megna FT-IR 550 spectrometer, and the ^1H NMR spectra on a JEOL AL-300 spectrometer (300 MHz) in $CDCl_3$/DMSO-d_6 using TMS as an internal standard (chemical shifts are measured in δ ppm). The purity of the compounds was checked by TLC using silica gel "G" as adsorbent, visualizing these by UV light or iodine.

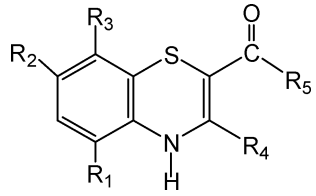
TABLE VI ^{1}H NMR Spectral Data of Substituted 4H-1,4-Benzothiazine Sulfones



Compd. VII	Compound R_1	R_2	R_3	R_4	R_5	δ	No. of Hydrogen	Multiplet	Assignment
						8.07	1	Singlet	N-H proton
a	Cl	H	CF_3	CH_3	C_6H_4-Cl(p)	7.81–6.59	6	Multipet	Aromatic protons
						2.13	3	Singlet	CH_3 protons
						8.84	1	Singlet	NH-protons
b	Cl	H	CF_3	CH_3	C_6H_4-F(p)	7.94–6.41	6	Multiplet	Aromatic protons
						2.11	3	Singlet	CH_3 protons
						8.15	1	Singlet	NH-protons
c	Cl	H	CF_3	CH_3	C_6H_4-OCH_3(o)	7.50–6.44	6	Multiplet	Aromatic protons
						2.01	3	Singlet	CH_3 protons at C_3
						1.38	3	Singlet	CH_3 protons at 2′ carbon of C_6H_4
						7.86	1	Singlet	N-H proton
d	Cl	H	CF_3	CH_3	C_6H_4-Cl(o)	7.89–6.52	5	Multiplet	Aromatic protons
						2.11	3	Singlet	CH_3 protons
						8.06	1	Singlet	N-H proton

e	Br	H	Br	CH_3	C_6H_4-CH_3(p)	7.92–6.30	6	Multiplet	Aromatic protons
						2.33	3	Singlet	CH_3 proton at C_3
						1.53	3	Singlet	CH_3 proton at C′-4 carbon in C_6H_4
						8.86	1	Singlet	N-H proton
f	F	Br	H	C_6H_5	C_6H_5	7.92–6.20	12	Multiplet	Aromatic protons
						8.11	1	Singlet	N-H proton
g	F	Br	H	CH_3	C_6H_5	7.71–6.38	7	Multiplet	Aromatic proton
						1.92	3	Singlet	CH_3 proton
						7.92	1	Singlet	N-H proton
h	F	Br	H	CH_3	CF_3	7.51–6.32	2	Multiplet	Aromatic protons
						1.96	3	Singlet	CH_3 protons
						8.21	1	Singlet	N-H proton
i	F	Br	H	CH_3	C_6H_4-Br	7.76–6.18	6	Multiplet	Aromatic proton
						2.03	3	Singlet	CH_3 protons

TABLE VII Antioxidant Activity of Synthesized Benzothiazines (DPPH Assay)



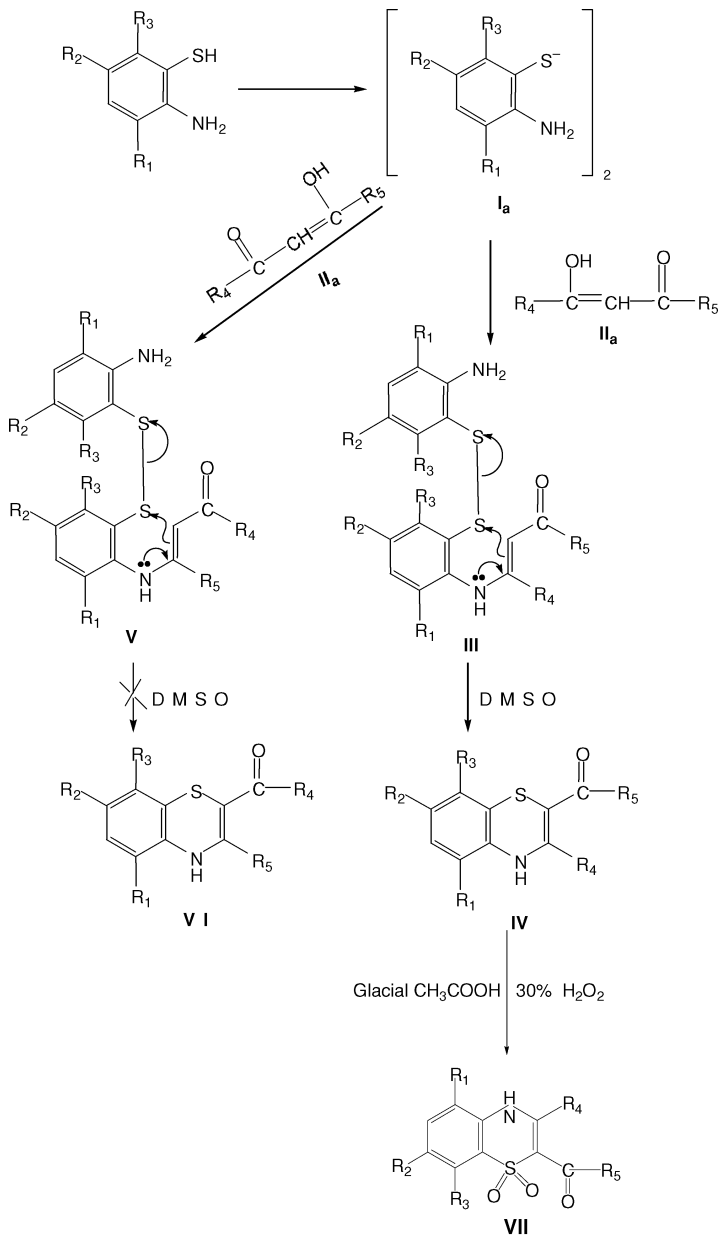
Compd. IV	Compound					DPPH% inhibition of 1 mg/mL of the compound
	R_1	R_2	R_3	R_4	R_5	
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	53.84±1.5
B	Cl	H	CF_3	CH_3	C_6H_4–F(p)	46.92±1.3
C	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	30.91±0.9
D	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	52.89±1.6
E	Br	H	Br	CH_3	C_6H_4–CH_3(p)	42.62±1.3
F	F	Br	H	C_6H_5	C_6H_5	48.72±1.4
G	F	Br	H	CH_3	C_6H_5	32.63±0.8
H	F	Br	H	CH_3	CF_3	20.41±1.1
I	F	Br	H	CH_3	C_6H_4-Br(p)	19.21±0.6

Preparation of 4H-1,4-Benzothiazine ($IV_{a–i}$)

Substituted 2-aminobenzenethiol (**I**; 0.01 mol) was added to a stirred suspension of β-diketone ($\mathbf{II}_a$; 0.01 mol) in DMSO (5 mL), and the resulting mixture was refluxed for 20–40 min. The mixture was cooled down to room temperature. The solid separated out was filtered, washed with petroleum ether, and crystallized from methanol (Scheme 1). The physical data of 4H-1,4-benzothiazines are reported in Table I.

Preparation of 4H-1,4-Benzothiazine Sulfones $VII_{a–i}$

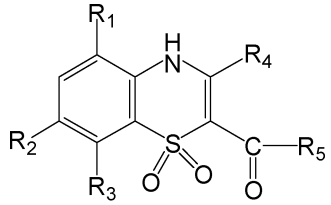
The 4H-1,4-benzothiazine (0.01 mol), glacial acetic acid (20 mL), and 30% hydrogen peroxide (5 mL) were added together in a 50 mL R.B. flask and the mixture was refluxed for 15–20 min at 50–60 °C. After this, a second portion of hydrogen peroxide (5 mL) was added. The reaction was further refluxed for 4 h. The mixture was poured into a beaker containing crushed ice. The obtained precipitate was filtered, washed with water, and crystallized from ethanol[1–5] (Scheme 1). The physical data of 4H-1,4-benzothiazine sulfones are reported in Table IV.



R_1 = Cl, F, Br; R_2 = H, Br; R_3 = Br, CF_3, H; R_4 = CH_3, C_6H_5; R_5 = C_6H_4-Cl(p), C_6H_4-F(p), C_6H_4-OCH_3(o), C_6H_4-Cl (o), CF_3,

SCHEME 1

TABLE VIII Antioxidant Activity of Synthesized Benzothiazine Sulfones (DPPH Assay)



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Compd. IV	Compound R_1	R_2	R_3	R_4	R_5	DPPH% inhibition of 1 mg/mL of the compound
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	40.91±0.2
B	Cl	H	CF_3	CH_3	C_6H_4–F(p)	34.62±1.3
C	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	10.11±0.4
D	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	50.73±1.2
E	Br	H	Br	CH_3	C_6H_4–CH_3(p)	33.61±0.8
F	F	Br	H	C_6H_5	C_6H_5	50.22±0.9
G	F	Br	H	CH_3	C_6H_5	44.28±1.1
H	F	Br	H	CH_3	CF_3	25.17±0.3
I	F	Br	H	CH_3	C_6H_4-Br(p)	36.78±0.3

Mechanism for the Synthesis of 4H-1,4-Benzothiazines and Their Sulfones

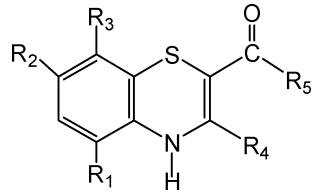
ANTIOXIDANT ACTIVITY

All the synthesized compounds $\mathbf{IV}_{a–i}$ and their sulfones $\mathbf{VII}_{a–i}$ were screened for their antioxidant activity by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radial scavenging assay and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical cation decolorization assay.

The present study demonstrated that the synthesized compounds showed mixed radical scavenging activity in both DPPH for benzothiazine (Table VII) and their sulfones (Table VIII) in and $ABTS^+$ assays (Tables IX and X).

1. Compounds showed strong radical scavenging activity in DPPH assays that have DPPH% inhibition $\geq$ 50.
2. Compounds showed moderate radical scavenging activity in DPPH assays that have DPPH% inhibition $\geq$ 30.

TABLE IX Antioxidant Activity of Synthesized Benzothiazines (ABTS Assay)



Compd. IV	Compound					ABTS Activity at different time intervals (min)				
	R_1	R_2	R_3	R_4	R_5	0	1	2	4	6
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	0.731	0.190	0.189	0.189	0.189
b	Cl	H	CF_3	CH_3	C_6H_4–F(p)	0.732	0.231	0.231	0.231	0.231
c	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	0.722	0.106	0.105	0.104	0.103
d	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	0.732	0.690	0.689	0.688	0.688
e	Br	H	Br	CH_3	C_6H_4–CH_3(p)	0.731	0.308	0.308	0.308	0.308
f	F	Br	H	C_6H_5	C_6H_5	0.731	0.431	0.430	0.430	0.429
g	F	Br	H	CH_3	C_6H_5	0.733	0.051	0.051	0.051	0.051
h	F	Br	H	CH_3	CF_3	0.721	0.659	0.655	0.654	0.653
i	F	Br	H	CH_3	C_6H_4-Br(p)	0.738	0.238	0.238	0.236	0.236

3. Compounds showed mild radical scavenging activity in DPPH assays that have DPPH% inhibition < 30.
4. Compounds were found to be more active in ABTS $^+$ assays that showed much decline in graph (Figures 1 and 2).

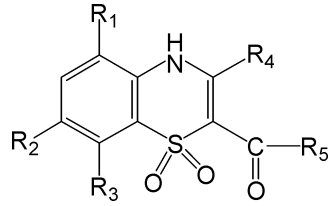
DPPH RADICAL SCAVENGING ASSAY

Radical scavenging activity of compounds $\mathbf{IV}_{a–i}$ and $\mathbf{VII}_{a–i}$ against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet et al.[6] A stock solution 1 mg/mL of the compound was prepared in methanol. 50 μl of the compounds were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min incubation in a 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:

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

TABLE X Antioxidant Activity of Synthesized Benzothiazine Sulfones (ABTS Assay)



Compd. IV	Compd. No. R_1	R_2	R_3	R_4	R_5	ABTS Activity at different time intervals (min) 0	1	2	4	6
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	0.732	0.692	0.692	0.692	0.692
b	Cl	H	CF_3	CH_3	C_6H_4–F(p)	0.731	0.301	0.300	0.300	0.300
c	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	0.732	0.181	0.181	0.181	0.181
d	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	0.738	0.219	2.16	0.215	0.214
e	Br	H	Br	CH_3	C_6H_4–CH_3(p)	0.725	0.114	0.114	0.113	0.113
f	F	Br	H	C_6H_5	C_6H_5	0.722	0.212	0.212	0.211	0.210
g	F	Br	H	CH_3	C_6H_5	0.733	0.414	0.414	0.414	0.414
h	F	Br	H	CH_3	CF_3	0.731	0.068	0.068	0.068	0.066
I	F	Br	H	CH_3	C_6H_4-Br(p)	0.722	0.109	0.108	0.106	0.106

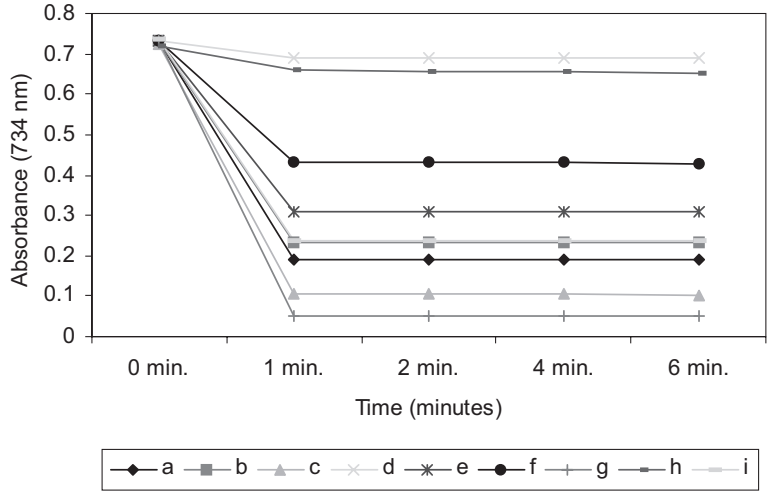


FIGURE 1 After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 μL of the compound, the absorbance reading was taken at 30 °C exactly 1 min after initial mixing and up to 6 min. All determinations were carried out in triplicate.

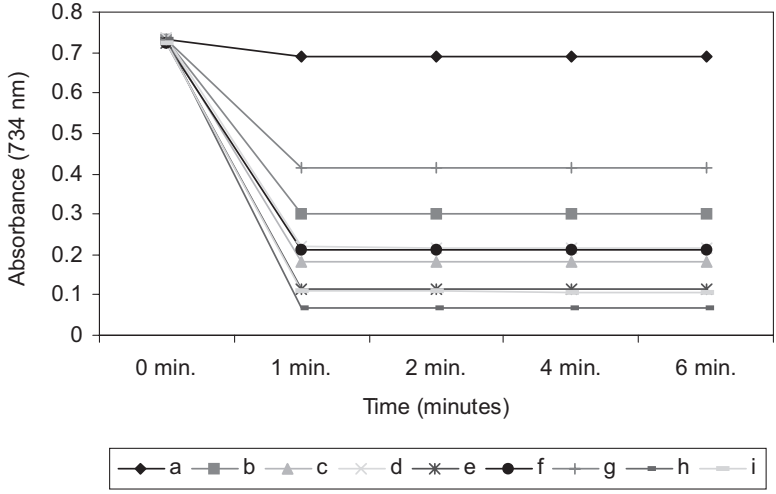


FIGURE 2 After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 μL of the compound, the absorbance reading was taken at 30 °C exactly 1 min after initial mixing and up to 6 min. All determinations were carried out in triplicate.

Where

AB = Absorption of blank
AA = Absorption of test

ABTS RADICAL CATION DECOLORIZATION ASSAY

The 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) radical cation (ABTS) decolorization test was also used to assess the antioxidant activity of compounds **IV**$_{a–i}$ and **VII**$_{a–i}$. The ABTS assay was carried out using the improved assay of Re.[7] In brief, ABTS was generated by oxidation of ABTS with potassium persulfate. For this purpose, ABTS was dissolved in deinoized water at a concentration of 7 mM, and potassium was persulfate 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 ABTS solution then was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 mL of the diluted ABTS solution to 10 μl of the compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1–6 min later. All determinations were carried out in triplicate.

IN VIVO STUDIES IN SWISS ALBINO MICE

The compound was further treated for evaluation of antioxidative properties in 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.

Material and Methods

Animals

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°C ± 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.

Chemicals

Compound synthesized: sodium chloride, tris potassium chloride, trichloro acetic acid, 5-dithiobis-2-nitrobenzoic acid (DTNB), acetic acid, thiobarbituric acid, n-butanol, and pyridine.

BIOCHEMICAL STUDIES

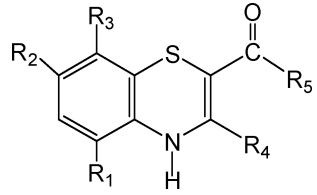
1. Lipid peroxidation assay. The LPO level in liver was measured in terms of thiobarbitituric and reactive substance (TBARS) using the method of Ohkhawa et al.[9] Absorbance in the assay was read at 532 nm (Tables XI and XII).
2. Sulfhydral group assay (GSH): The level of acid-soluble sulfhydral groups was estimated in liver as total non-protein sulfhydral groups using the method described by Moron et al.[10] 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) (Tables XIII and XIV).

STATISTICAL ANALYSIS

Results of the biochemical studies were evaluated using student's t test.

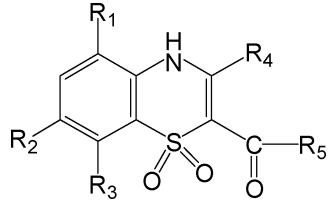
The above value shows there was significant increase in GSH content of liver in animals treated with compounds $\mathbf{IV}_{a–i}$ and $\mathbf{VII}_{a–i}$. Also in

TABLE XI Antioxidative Properties of Benzothiazine in the Liver in Swiss Albino Mice (LPO n mol/ mg tissue)



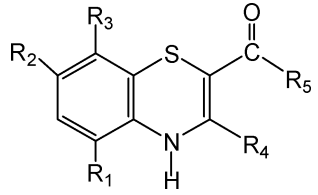
Compd. IV	Treatment Compound No. R_1	R_2	R_3	R_4	R_5	LPO (n mol/mg tissue)
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	6.69 ± 0.5
B	Cl	H	CF_3	CH_3	C_6H_4–F(p)	6.7 ± 0.18, $p < 0.05$
C	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	6.42 ± 0.16, $p < 0.05$
D	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	6.81 ± 0.16
E	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	6.02 ± 0.17, $p < 0.025$
F	F	Br	H	C_6H_5	C_6H_5	6.53 ± 0.5
G	F	Br	H	CH_3	C_6H_5	6.72 ± 0.16
H	F	Br	H	CH_3	C_6H_4-Br(p)	6.51 ± 0.17, $p < 0.05$

TABLE XII Antioxidative Properties of Benzothiazine Sulfones in the Liver in Swiss Albino Mice (LPO n mol/mg tissue)



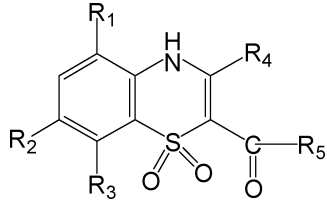
Compd. IV	Treatment Compound No. R_1	R_2	R_3	R_4	R_5	LPO (n mol/mg tissue)
a	Cl	H	CF_3	CH_3	C_6H_4–F(p)	6.81 ± 0.16
B	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	6.79 ± 0.17, $p < 0.05$
C	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	6.20 ± 0.22, $p < 0.005$
D	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	6.68 ± 0.17, $p < 0.05$
E	F	Br	H	C_6H_5	C_6H_5	6.5 ± 0.5
F	F	Br	H	CH_3	C_6H_5	6.81 ± 0.17
G	F	Br	H	CH_3	CF_3	6.70 ± 0.18, $p < 0.05$
h	F	Br	H	CH_3	C_6H_4-Br(p)	6.51 ± 0.17, $p < 0.05$

TABLE XIII Antioxidative Properties of Benzothiazine in the Liver in Swiss Albino Mice (GSH n mol/100 g tissue)



Compd. IV	Treatment Compound No. R_1	R_2	R_3	R_4	R_5	GSH (n mol/100 g tissue)
a	Cl	H	CF_3	CH_3	C_6H_4–Cl(p)	5.00 ± 0.11, $p < 0.005$
B	Cl	H	CF_3	CH_3	C_6H_4–F(p)	4.80 ± 0.15, $p < 0.05$
C	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	4.20 ± 0.19
D	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	4.16 ± 0.26
e	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	5.01 ± 0.12, $p < 0.005$
F	F	Br	H	C_6H_5	C_6H_5	4.90 ± 0.12, $p < 0.005$
G	F	Br	H	CH_3	C_6H_5	4.21 ± 0.19
H	F	Br	H	CH_3	C_6H_4-Br(p)	4.15 ± 0.16

TABLE XIV Antioxidative Properties of Benzothiazine Sulfones in the Liver in Swiss Albino Mice (GSH n mol/100 g tissue)



Compd. IV	Treatment Compound No. R_1	R_2	R_3	R_4	R_5	GSH (n mol/100 g tissue)
a	Cl	H	CF_3	CH_3	C_6H_4–F(p)	5.01 ± 0.11, $p < 0.005$
B	Cl	H	CF_3	CH_3	C_6H_4–OCH_3(o)	4.17 ± 0.20
C	Cl	H	CF_3	CH_3	C_6H_4–Cl(o)	4.80 ± 0.15, $p < 0.05$
D	Br	H	Br	CH_3	C_6H_4–(CH_3)(p)	4.80 ± 0.15, $p < 0.05$
E	F	Br	H	C_6H_5	C_6H_5	4.21 ± 0.19
F	F	Br	H	CH_3	C_6H_5	4.69 ± 0.15, $p < 0.025$
G	F	Br	H	CH_3	CF_3	4.7 ± 0.13, $p < 0.05$
H	F	Br	H	CH_3	C_6H_4-Br(p)	4.19 ± 0.19

these animals, the value of LPO was significantly decreased, showing potent antioxidant activities in Swiss albino mice.

CONCLUSION

Synthesized compounds showed mixed radical scavenging and antioxidant activity in Swiss albino mice.

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