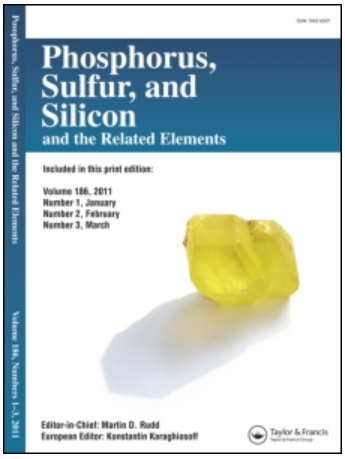

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Synthesis and Biological Activities of Some New Phenothiazines, Their Sulfones, and Ribofuranosides

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The present communication describes the synthesis of substituted 10H-phenothiazines by reaction of 2-aminobenzenethiol ***1*** *and o-halonitrobenzene* ***2*** *via Smiles rearrangement. These synthesized phenothiazines are used as base to prepare ribofuranosides by treatment with sugar viz. β-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate. Sulfones are also synthesized by oxidation of 10H-phenothiazines refluxing with H_2O_2 in glacial acetic acid. All synthesized compounds have been characterized by IR, ^1H NMR, ^{13}C NMR Mass spectra and elemental analysis and screened for antioxidant and antimicrobial activity.*

Keywords Antimicrobial activity; antioxidant activity; glacial acetic acid; 10H-phenothiazines; ribofuranosides

INTRODUCTION

In the present age of pharmacognestic too, the 10H-phenothiazines have been given considerable interest due to a wide spectrum of biological activities such as antihistaminic, antitussive, analgesic, antibacterial, calcium antagonist, anticancer, and anti AIDS. Research is also being persuaded to develop potent anticancer agent. A slight change in substitution pattern in phenothiazines nucleus causes distinguishable difference in their biological activities.[1–12] Thus, we wish to report here synthesis of some new 10H-phenothiazines their ribofuranosides

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and sulfones and subjected these compounds for antimicrobial[13,14] and antioxidant activity.[15]

RESULT AND DISCUSSION

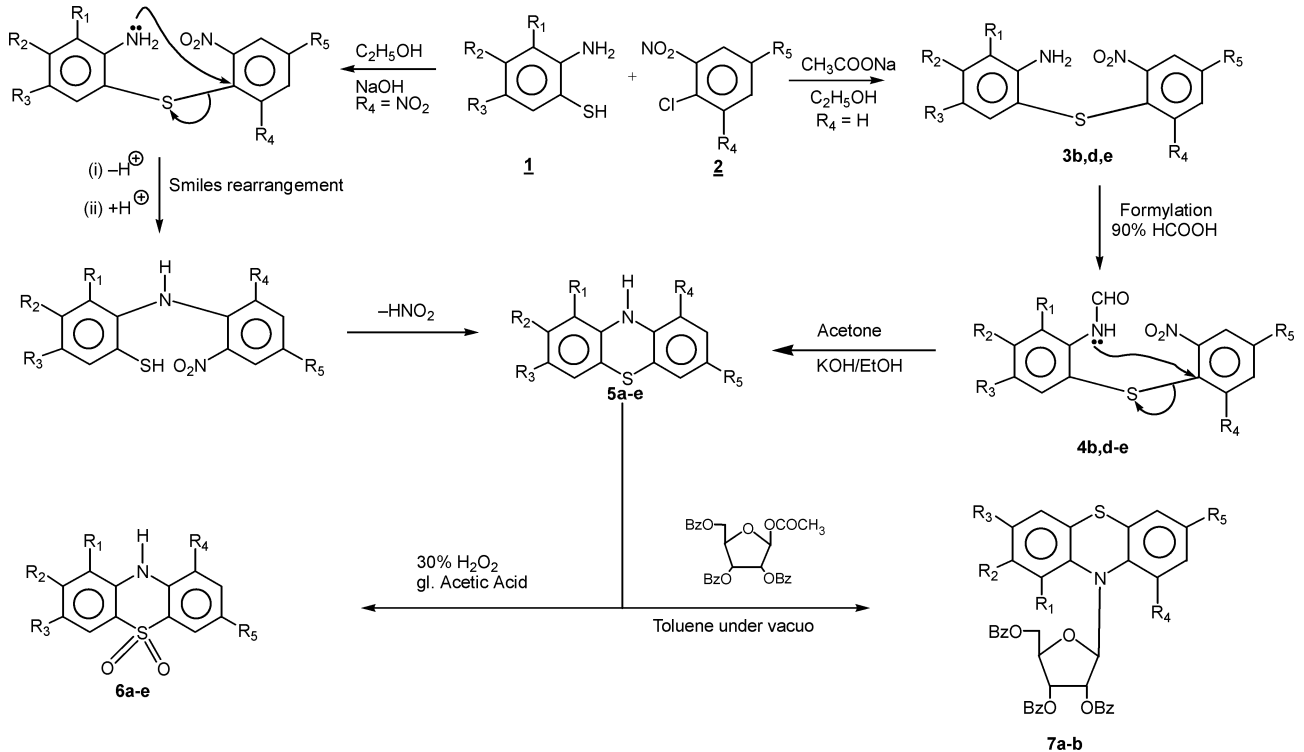
Synthesis of 10H-phenothiazines **5b**,**d**,**e** have been carried out by Smiles rearrangement of substituted 2-formamido-2′-nitrophenylsulfides **4**,**b**,**d**,**e**, which have been synthesized by diphenyl sulfide **3b**,**d**,**e**, which in turn was prepared by condensation of 2-aminobenzenethiols **1** and o-halonitrobenzene **2a** in ethanolic anhydrous sodium acetate solution. 1-nitro-10H-phenothiazines **5a**,**c** have been synthesized by condensation of 2-aminobenzenethiols **1** and reactive o-halonitrobezene **2b** (which have either two nitro group or one halo and one nitro group at both ortho position to reactive halogen atom) in alcohol in presence of sodium hydroxide where the Smiles rearrangement occur in situ. Compound **5a–e** on refluxing with 30% hydrogen peroxide in glacial acetic acid yield phenothiazines sulfones **6a–e**. The pasty mixture of **5a–b** in toluene, on stirring with sugar viz. β-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate at 155–160 for 10 h in vacuo gave corresponding ribofuranosides **7a–b** (Scheme-1).

IR Spectra

All the synthesized diphenyl sulfides **3b**,**d**,**e** shows two peaks in the region 3395–3360 cm^{-1} and 3310–3260 cm^{-1} due to asymmetric and symmetric vibration of $-NH_2$ group. Two signals are observed in the region 1600–1570 cm^{-1} and 1370–1345 cm^{-1} are ascribed for asymmetric and symmetric vibration of nitro group, respectively.

2-Formamido-2′-nitrophenylsulfides **4b**,**d**,**e** shows absorption in the region 3300–3260 cm^{-1} is ascribed for >N–H stretching vibrations and an additional peak observed in the region 1690–1650 cm^{-1} due to >C=O stretching vibration. Absorption in the region 1565–1540 cm^{-1} and 1345–1330 cm^{-1} can be assigned for asymmetric and symmetric vibration of nitro group, respectively.

Compounds **5a–e** and **6a–e** showed a band in the region 3435–3320 cm^{-1} due to >NH stretching vibration. The sharp absorption bands appeared in the region 1560–1535 and 1365–1345 cm^{-1}, were due to asymmetric and symmetric stretching vibration of $-NO_2$ group in **5a**,**c**, **6a–c**, and **7a**. Compounds **6a–e** exhibit two intense peak in the region 1365–1345 cm^{-1} and 1180–1110 cm^{-1} can be assigned to asymmetric and symmetric vibration of sulfonyl group.



SCHEME 1

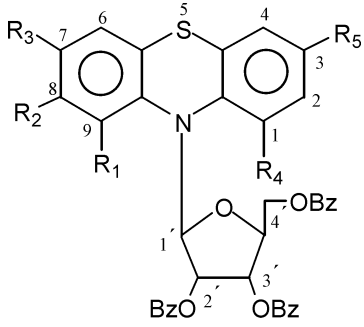
In compound **7**, the >NH band vanished completely, suggesting its ribosylation. The band due to >C=O and C—O—C appeared at 1760–1750 cm^{-1} and 1165–1080 cm^{-1}, respectively.

^{1}H NMR Spectra

^{1}H NMR spectra of **3b**,**d**,**e** and **4b**,**d**,**e** show resemblance with their corresponding phenothiazine **5b**,**d**,**e**. Instead of this resemblance compound, **3b**,**d**,**e**, show a broad signal in the region δ 4.24–3.88 ppm due to $-NH_2$ proton. Compound **4b**,**d**,**e** exhibit a signal in the region δ 10.24–9.86 ppm due to formyl proton and another signal observed in the region δ 8.74–8.92 ppm due to >NH proton.

Compound **5a–e** and **6a–e** exhibit singlet in the region δ 9.66–8.82 ppm due to >N–H proton. multiplet observed in the region δ 8.48–6.12 ppm corresponding to phenylic protons. Compound **5a**, **6a**, and **7a** exhibited doublet and multiplet in the region δ 1.36–1.27 ppm and δ 2.92–2.60 due to $(CH_3)_2$ and CH proton of isopropyl group. In ribofuranoside **7** the signal due to >NH proton vanished completely, suggesting its ribosylation. Aromatic proton of ribofuranosides **7a–b** gave multiplet in the region δ 8.20–6.92. C'_4–H and $>CH_2$ proton of sugar moiety gave multiplet in the region δ 4.42–4.80, while C'_2–H and C'_3–H signals appeared in the region δ 5.62–5.83 as multiplet. The singlet at δ 6.40 is attributed to C'_1–H.

^{13}C NMR of the Synthesized Compounds (Solvent Used $CDCl_3$)



5a δ145.2(C_9), 122 (C_8), 126.3 (C_7), 121.4 (C_6), 130.5 (C_4) 119.2 (C_3), 122 (C_2), 144.4 (C_1).

5b δ116.4(C_9), 154.3 (C_8), 118.6 (C_7), 125.7 (C_6), 118 (C_4) 138.6 (C_3), 116.6 (C_2), 115 (C_1).

5c δ118(C_9), 150.7 (C_8), 119.4 (C_7), 122.6 (C_6), 118.7 (C_4) 135.2 (C_3), 112.4 (C_2), 145.3 (C_1).

5d δ120.3(C_9), 152.8 (C_8), 119 (C_7), 126.2 (C_6), 122 (C_4) 132.4 (C_3), 112.2 (C_2), 118.2 (C_1).

5e δ117(C_9), 150.2 (C_8), 120 (C_7), 125.2 (C_6), 124 (C_4) 127.4 (C_3), 124.4 (C_2), 126 (C_1).

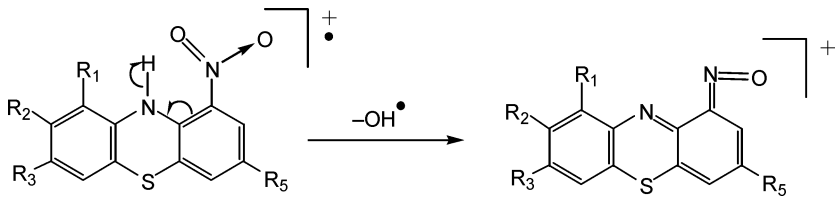
7a δ146.1(C_9), 123.4 (C_8), 125.2 (C_7), 121.8 (C_6), 139.3 (C_4) 118.8 (C_3), 113.2 (C_2), 144.9 (C_1), 96.2 (C'_1). 82.68. 81.26 (C'_2–C'_3), 92.9 (C-4′).

7b δ117.2(C_9), 155.2 (C_8), 118.4 (C_7), 125.2 (C_6), 117.6 (C_4) 139.2 (C_3), 117 (C_2), 115.8 (C_1), 95.6 (C'_1). 83.02. 88.56 (C'_2–C'_3), 94.8 (C-4′).

Mass Spectra

In mass spectra of 1-nitro-10H-phenothiazines molecular ion peak are in accordance with their molecular weights. Moieties M^+–30, M^+–46 and M^+–47 are observed and ascribed to the loss of NO, NO_2 and HNO_2, respectively.

1-Nitro-10H-phenothiazines undergo fragmentation yielding M^+–17 due to the loss of OH radical by McLafferty rearrangement.[16,17]



SCHEME 2 Optical rotation data of compound 7a-b.
Compound 7a +24.3 $(\alpha)_D^{24}$
Compound 7b −17.73 $(\alpha)_D^{24}$

EXPERIMENTAL

Melting point of all the synthesized compound were determined on an electrothermal apparatus (capillary method) and are uncorrected. IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer; and ^{1}H NMR and ^{13}C NMR spectra on JEOL AL 300 FT NMR using TMS as internal standard in $CDCl_3$/DMSO-d_6 (Table 1). Mass spectra were recorded on JEOL SX 102/DA 6000 using Argon/Xenon as FAB gas.

TABLE I The ^{1}H NMR and IR Spectral Data of Synthesized Compounds

	^{1}H NMR (δ ppm from TMS)			IR (KBr : υ_{max} cm^{-1})						
Compd. no.	>NH	Ar-H multiplet	$(CH_3)_2CH$—	>NH	—NO_2	C—Cl	C—Br	C—F	C—O—C	SO_2 Symm.
5a	8.93	7.30–7.02	1.31(d) 2.79(m)	3320	1550, 1345	—	—	—	—	—
5b	9.47	8.40–6.12	—	3420	—	—	640	1340, 1120	—	—
5c	9.68	7.26–6.34	—	3390	1535, 1360	760	—	1330, 1135	—	—
5d	8.82	7.96–6.73	—	3380	—	790	—	1320, 1180	—	—
5e	8.96	8.31–6.64	—	3340	—	—	—	1360, 1140	—	—
6a	8.96	7.42–7.06	1.36 (d) 2.92 (m)	3340	1560, 1350	—	—	—	—	1160 1110
6b	9.52	8.48–6.22	—	3435	—	—	660	1350, 1120	—	1180 1155
6c	9.66	7.30–6.36	—	3400	1550–1365	770	—	1350, 1155	—	1165 1150
6d	8.84	7.94–6.76	—	3400	—	790	—	1330, 1175	—	1180 1140
6e	9.02	8.36–6.72	—	3365	—	—	—	1360–1145	—	1170 1135
7a	—	7.82–7.30	1.27 (d) 2.60 (m)	—	1540, 1350	—	—	—	1080	—
7b	—	8.20–6.92	—	—	—	—	680	1350, 1120	1155	—

TLC were performed using silica gel 'G' and the spots were visualized by exposure to iodine vapors.

Synthesis of 2-Amino-2′-nitrodiphenylsulfides 3b,d,e

2-aminobenzenethiol **1** (0.01 mol) was dissolved in ethanol (20 ml) containing (0.01 mol) of anhydrous sodium acetate in 50 ml R.B. Flask and o-halonitrobenzene **2** (0.01 mol) in 10 ml ethanol was added. The reaction mixture was refluxed for 4–5 h. The resultant solution was concentrated, cooled, and kept overnight in an ice chamber overnight. The solid separated out was filtered, washed with 30% ethanol and recrystallized from methanol.

Synthesis of 2-Formamido-2′-nitrodiphenylsulfides 4b,d,e

The diphenylsulphides **3** (0.01 mol) was refluxed for 4 hrs. in 90% formic acid (20 ml). The content were then poured into a beaker containing crushed ice, a solid separated out was filtered, washed with water until the filtrate was neutralized and crystallized from benzene.

Synthesis of Phenothiazines 5b,d,e

To a refluxing solution of formyl derivatives **4** (0.01 mol) in acetone (15 ml) was added an alcoholic solution of potassium hydroxide (0.2 g in 5 ml ethanol). These contents were heated for 30 min. A second lot of potassium (0.2 g in 5 ml ethanol) was added to the reaction mixture and refluxed for 4 h. The contents were poured into beaker containing crushed ice and filtered. The residue obtained was repeatedly washed with cold water and finally with 30% ethanol and then crystallized from benzene.

Synthesis of 1-Nitro-10H-phenothiazine 5a,c

Mixture of (0.01 mol) of substituted 2-aminobenzenethiol **1**, sodium hydroxide (0.01 mol) and 20 ml absolute alcohol were taken in a 50 ml R.B. A flask fitted with a reflux condenser and was heated for 5 min; 0.01 mol of substituted reactive o-halonitrobenzene **2b** (which contained two nitro groups or one nitro and one halogen group at both ortho position to halogen atom) was added with stirring to this solution. These contents were refluxed for 4 h. The reaction mixture was concentrated on water bath, cooled, and filtered. The precipitate was washed well with hot water and finally with 20% alcohol and crystallized from acetone.

Synthesis of Phenothiazine Sulfones 6a–e

A mixture of substituted phenothiazines **5** (0.01 mol), 20 ml of glacial acetic acid and 5 ml of 30% hydrogen peroxide was refluxed for 15 min. Heating was stopped and another lot of hydrogen peroxide (5 ml) was added. The reaction mixture was again refluxed for 3 hrs. The content was poured in a beaker containing crushed ice. The yellow residue obtained was filtered and washed with water and recrystallized with ethanol.

Synthesis of Substituted N-(2′,3′,5′-Tri-o-benzoyl)-β-D-ribofuranosyl Phenothiazines 7a,b

β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mol) was added to a concentrated solution of **5** (0.002 mol) in toluene and stirred, in vacuo, on an oil both, at 155–160°C, for 15 min. The vacuo was broken and the reaction was protected from moisture, by using a guard tube. Stirring was further continued for 10 h. with application of vacuum for 15 min after every h. The viscous mass thus obtained was dissolved in methanol, boiled for 10 min, and cooled to room temperature. The reaction mixture was filtered. The methanol was removed by distillation under reduced pressure. The viscous residue thus obtained was dissolved in ether, filtered, concentrated and kept in a refrigerator overnight to get crystalline ribofuranoside.

Physical data of synthesized compounds **5a–e**, **6a–e**, and **7a–b** have been recorded in Table II.

BIOLOGICAL ACTIVITY

Antioxidant Activity

All the synthesized phenothiazines **5** and their ribofuranosides **7a–b** were screened for their antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radial scavenging assay.

The study reveals that ribofuranosides showed better chemopreventive action and antioxidant effect than their respective bases.

DPPH Radical Scavenging Assay

Radical scavenging activity of compound **5a–e** and **7a–b** against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometerically as described by Cuendet et al.,[15] A stock solution of 1 mg/ml of the compound was prepared in methanol. 50 μl of compounds were added to 5 ml of a 0.004% methanol solution of DPPH. After

TABLE II Characterization Data of Synthesized Compound 5a–e, 6a–e, and 7a–b

Compound no.										Elemental analysis % Found (Calcd.)		
	R_1	R_2	R_3	R_4	R_5	Yield %	Mol. wt.	M.P.°C	Mol. Formula	C	H	N
5a	$(CH_3)_2CH$	H	H	NO_2	H	38	286	120–122	$C_{15}H_{14}N_2O_2S$	63.12 (62.94)	4.86 (4.89)	9.85 (9.79)
5b	H	F	H	H	Br	67	296	104	$C_{12}H_7NSFBr$	48.75 (48.81)	2.34 (2.37)	4.71 (4.74)
5c	H	F	H	NO_2	Cl	52	294	270	$C_{12}H_6N_2\ SO_2FCl$	49.10 (48.97)	2.01 (2.04)	2.56 (2.52)
5d	H	F	H	H	Cl	40	251	112	$C_{12}H_7N\ SFCl$	57.32 (57.37)	2.75 (2.78)	5.53 (5.57)
5e	H	F	H	H	H	62	217	220	$C_{12}H_8NSF$	66.29 (66.35)	3.65 (3.68)	6.42 (6.45)
6a	$(CH_3)_2CH$	H	H	NO_2	H	62	318	180–182	$C_{15}H_{14}N_2O_4S$	56.48 (56.60)	4.43 (4.40)	8.86 (8.80)
6b	H	F	H	H	Br	74	328	202–204	$C_{12}H_7N\ SO_2FBr$	43.82 (43.90)	2.17 (2.13)	4.34 (4.27)
6c	H	F	H	NO_2	Cl	67	326	290–92	$C_{12}H_6N_2\ SO_4FCl$	44.25 (44.17)	1.82 (1.84)	8.65 (8.59)
6d	H	F	H	H	Cl	56	283	163–165	$C_{12}H_7N\ SO_2FCl$	50.96 (50.88)	2.52 (2.47)	4.93 (4.95)
6e	H	F	H	H	H	76	249	246–248	$C_{12}H_8N\ SO_2F$	57.78 (57.83)	3.25 (3.21)	5.56 (5.62)
7a	$(CH_3)_2CH$	H	H	NO_2	H	28	730	88–90	$C_{41}H_{34}N_2O_9S$	67.28 (67.39)	4.62 (4.65)	3.85 (3.83)
7b	H	F	H	H	Br	31	740	106	$C_{38}H_{27}N\ SO_7FBr$	61.54 (61.62)	3.62 (3.64)	1.87 (1.89)

30 minutes incubation in dark at room temperature. The absorbance was read against a blank at 517 nm (Table III).

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

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

$$AB = \text{Absorption of blank}$$

$$AA = \text{Absorption of test} \quad (1)$$

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 microliters of sample of particular concentration were added to 5 ml of 0.004% methanol solution of DPPH. After 30 min incubation in dark room temperature, the absorbance was read against a blank at 517 nm.

Antimicrobial Activity

Newly synthesized compounds **5a**–**e**, **6a**–**e**, and **7a**–**b** were screened for their antibacterial activity against *Staphyllococcus aureus* and *Pseudomonas fluorescence* bacterial strain by paper disc method using Streptomycin as standard drugs. Compounds are also screened for their antifungal activity against *Aspergillus flavus* and *Aspergillus niger*. The activity of each compound was compared with that of flukanozole as a standard drug. The results of such studies are given in Table IV. Antimicrobial activities are given in term of activity index

$$\text{Activity index} = \frac{\text{Inhibition diameter of test compound}}{\text{Inhibition diameter of standard}}$$

TABLE III Antioxidant Activity of Compound 5a–e and 7a–b

Compound	DPPH% inhibition of 1 mg/ml 1 mg/ml of the compound
5a	42.62 ± 0.09
5b	22.04 ± 0.8
5c	56.08 ± 0.07
5d	38.02 ± 0.03
5e	18.86 ± 1.2
7a	58.03 ± 0.06
7b	36.09 ± 0.04

TABLE IV Antimicrobial Activity of Compounds 5a–e, 6a–e, and 7a–b

Compound no.	R_1	R_2	R_3	R_4	R_5	Antibacterial activity		Antifungal activity	
						S. aureus	*P. fluorescens*	*A. niger*	*A. flavus*
5a	$(CH_3)_2CH$	H	H	NO_2	H	1.06	0.92	0.86	0.90
5b	H	F	H	H	Br	0.92	0.94	1.02	0.86
5c	H	F	H	NO_2	Cl	0.95	1.00	0.92	0.96
5d	H	F	H	H	Cl	1.08	0.92	0.94	1.10
5e	H	F	H	H	H	0.92	1.06	0.90	0.92
6a	$(CH_3)_2CH$	H	H	NO_2	H	0.95	0.88	0.88	0.90
6b	H	F	H	H	Br	0.94	0.94	1.10	0.88
6c	H	F	H	NO_2	Cl	0.89	1.02	0.92	0.96
6d	H	F	H	H	Cl	0.90	0.92	0.84	0.86
6e	H	F	H	H	H	1.12	0.90	1.06	0.88
7a	$(CH_3)_2CH$	H	H	NO_2	H	1.02	1.12	1.23	1.08
7b	H	F	H	H	Br	1.18	1.20	1.06	1.02

Antimicrobial activities are given in term of activity index.

$$\text{Activity index} = \frac{\text{Inhibition diameter of test compound}}{\text{Inhibition diameter of standard}}.$$

CONCLUSION

The structures proposed to the synthesized compounds are well supported by spectroscopic data and elemental analysis. All the synthesized phenothiazines **5** and their ribofuranosides **7a–b** were screened for their antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl (DPPH) radial scavenging assay.

The present study demonstrated that the synthesized compound showed mixed radical scavenging activity in DPPH assay.

(a) Compound (5c, 7a) showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$50.
(b) Compound (5a, 5d, 7b) showed moderate radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$30.
(c) Compound (5b, 5e) showed mild radical scavenging activity in DPPH assay that have DPPH% inhibition $\leq$30.

The antimicrobial and antioxidative studies reveals that almost in all cases ribofuranosides showed better antimicrobial activity, chemopreventive action and antigenotoxic effect than their parent bases. All compounds are moderately active against various bacteria and fungus.

REFERENCES

[1] W. Ried and W. Ochs, *Physical Methods in Heterocyclic Chemistry*, In RR Gupta (Ed.) (John Wiley and Sons Inc.: New York, 1984) p. 58.

[2] R. R. Gupta, Ed., Phenothiazines and 1,4-Benzothiazines Chemical and Biomedical Aspects (Elsevier, Amsterdam, 1988).
[3] C. S. Rasmussen, U.S. Patent, 3,476,749(1969); *Chem. Abstr.* **72,** 217,227 (1970).
[4] H. Zenno and T. Milutani, Japanese Patent, 7,006,262 (1970); *Chem. Abstr.*, **72,** 132,759 (1970).
[5] G. Fengler, D. Arlt, K. Grothe, H. J. Zeiter, and K. Metzger, German Offen., 3, 229, 125 (1984); *Chem. Abstr.*, **101,** 7176 (1984).
[6] E. D. Clercq, *Nucleosides and Nucleotides*, **4,** 3 (1985).
[7] G. Filocchioni, V. Nacci, and G. Stfancich, *Farmaco, Ed. Sci.* **31**(7), 478 (1976); *Chem. Abstr.* **85,** 143,048 (1976).
[8] N. Gautam, R. Gupta, D. C. Gautam, and R. R. Gupta, *Heterocycl. Commun.*, **6,** 369 (2000).
[9] P. R. Sharma, R. Gupta, V. Gupta, D. C. Gautam, and R. R. Gupta, *Heterocycl. Commun.*, **8,** 549 (2002).
[10] G. Singh, N. Kumar, A. K. Yadav, and A. K. Mishra, *Heteroatom. Chemistry*, **14**(6), 481–486 (2003).
[11] N. Gautam and D. C. Gautam, *In. J. Chem. Sci.*, **2**(1), 84–87 (2004).
[12] N. Gautam, D. Hans, and D. C. Gautam, *Oriental Journal of Chemistry*, **21**(2), 299–302 (2005).
[13] J. C. Gould and J. H. Browie, *Edinb. Med. J.*, **59,** 178 (1950).
[14] C. H. Collin and P. M. Lyne, *Microbial Methods* (University Park Press, Baltimore, 1970).
[15] M. Cuendet, K. Hostemann, and O. Potteral, *Hel. Chem. Acta*, **80,** 1144, 1152 (1997).
[16] F. W. Mc-Lafferty, Ed., *Mass Spectrometry of Organic Ions* (Academic Press, New York, 1963).
[17] F. W. Mc-Lafferty, *Anal. Chem.*, **31,** 82 (1959).