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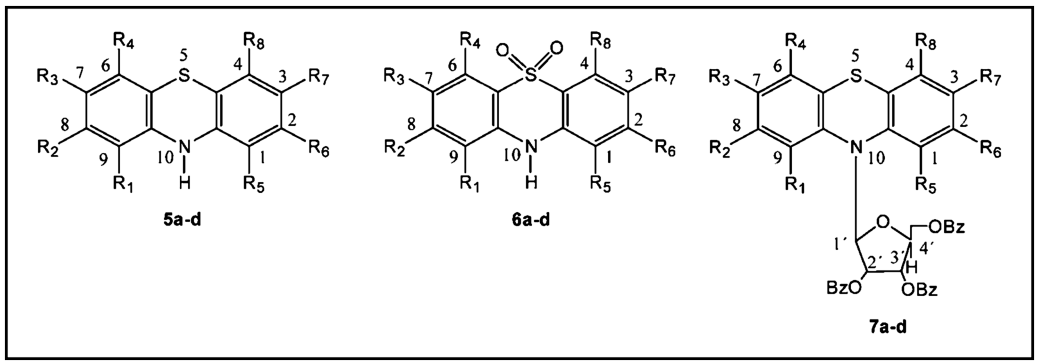

10H-phenothiazines are synthesized *via* Smiles rearrangement. These prepared phenothiazines act as a base to prepare ribofuranosides by treating them with b-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate. 10H-phenothiazines on refluxing with hydrogen peroxide in glacial acetic acid gave 10H-phenothiazine-5,5-dioxides. The synthesized compounds were evaluated for their antioxidative properties through *in vitro* studies, and they are also screened for their antimicrobial activity. The structure of the synthesized compounds has been established by elemental analysis and spectroscopic data.

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INTRODUCTION

Phenothiazines, their sulfones and ribofuranosides constitute an important class of heterocycles. These compounds are of immense importance and possess a wide spectrum of pharmacological activities such as analgesic, antihypertensive, antipsychotic, antibacterial, and anti AIDS. Research is being persuaded to develop potent anticancer agents. A slight change in substitution pattern cause marked difference in their biological activity [1–11]. Thus, we wish to report here synthesis of some new 10H-phenothiazines, their sulfones and ribofuranosides. The structure of the synthesized compounds is determined on the basis of their spectral data and elemental analysis. These compounds are also screened for antioxidant [12–16] and antimicrobial activity [17].

RESULTS AND DISCUSSION

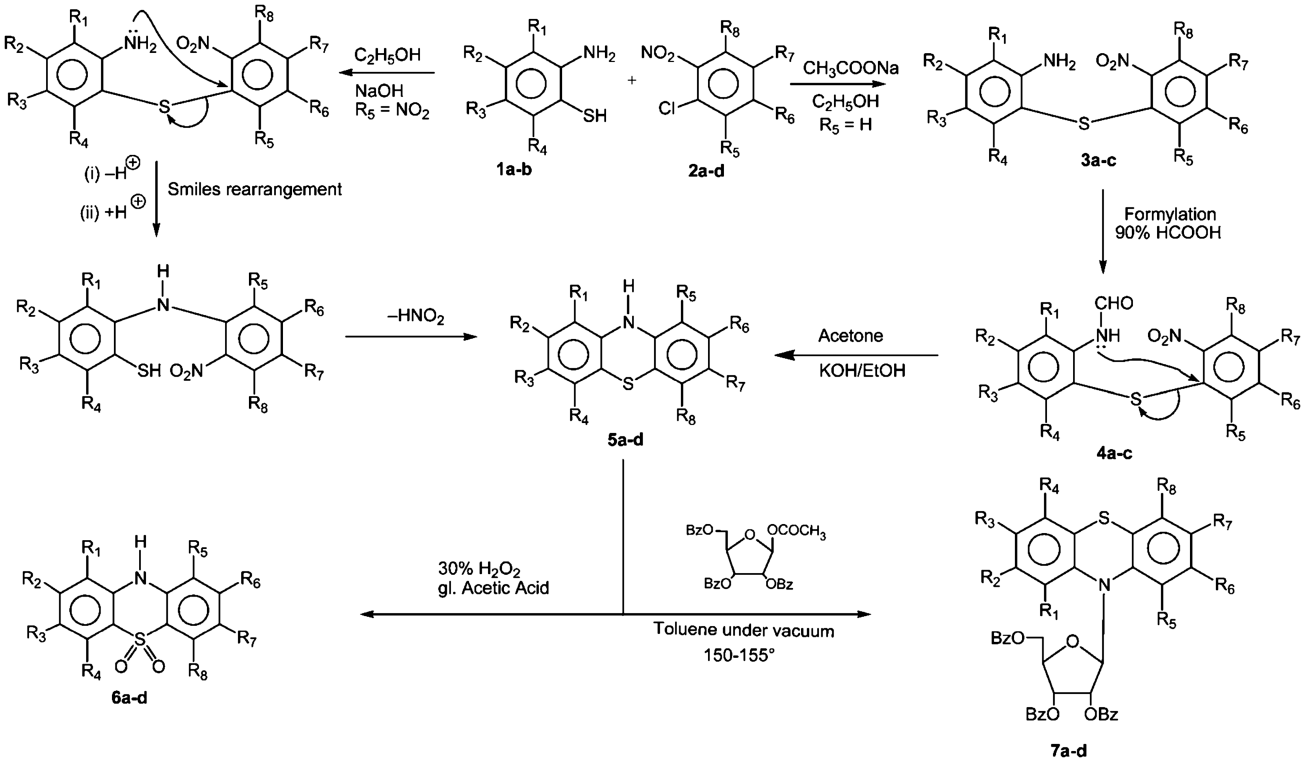
The synthesis of various substituted 10H-phenothiazines **5a–c** has been carried out by Smiles rearrangement of substituted 2-formamido-2′-nitrodiphenylsulfides **4a–c**. The formyl derivatives have been prepared by diphenyl sulfides **3a–c** which in turn was prepared by the condensation of 2-amino-4,6-dimethyl benzenethiol **1a** with *o*-halonitrobenzenes (1,3,5-trichloro-2-nitrobenzene **2a**/1,5-dichloro-2,4-dinitrobenzene **2b**/2-chloro-3,5-dinitrobenzotrifluride **2c)** in ethanolic sodium acetate solution. 1-Nitro-10H-phenothiazine **5d** has been synthesized by the reaction of 2-amino-3,4,5-trifluorobenzenethiol **1b** with 4-chloro-3,5-dinitrobenzoic acid **2d** (having two nitro groups ortho to the reactive halogen atom) in alcohol in presence of sodium hydroxide where the Smiles rearrangement occurs *in situ*. Compounds **5a–d** on refluxing with 30% hydrogen peroxide in glacial acetic acid were converted into their corresponding sulfones **6a–d**. Treatment of the pasty mixture of **5a–d** in toluene with b-D-ribofuranosyl-1-acetate-2,3,5-tribenzoate in vacuum gave the corresponding ribofuranosides **7a–d** (Scheme 1).

All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) $ABTS^{\cdot+}$ radical cation decolorization assay and also screened for their antimicrobial activity.

The structure of these compounds were determined on the basis of elemental analysis and spectral data.

IR spectra. Compounds **5a–d** and **6a–d** showed a band in the region 3410–3320 cm^{-1} corresponding to >NH stretching vibration. Two sharp and intense bands in the region 1590–1560 cm^{-1} and 1400–1370 cm^{-1} are ascribed to asymmetric and symmetric stretching vibration of —NO_2 group in **5d**.

Scheme 1



The characteristic absorption bands at 2900–2950 cm^{-1} appeared due to C—H stretching vibration of CH_3 group for compounds **5a–c**, **6a–c,** and **7a–c**. Compounds **6a–d** exhibit two intense peaks in the region 1365–1340 cm^{-1}, and 1190–1110 cm^{-1} is assigned for asymmetric and symmetric stretching vibration of sulfonyl group.

In compounds **7a–d,** the NH band disappeared showing its ribosylation. The bands due to C=O and C—O—C appeared at 1760–1740 cm^{-1} and 1190–1135 cm^{-1}, respectively.

^{1}H-NMR spectra. The ^{1}H-NMR spectra of compounds **5a–d** and **6a–d** showed a singlet in the region δ 8.65–9.22 ppm due to N—H proton and a multiplet observed in the region δ 6.50–8.24 ppm corresponding to the aromatic protons. A singlet observed in the region δ 2.05–2.35 ppm corresponding to proton of methyl group in compounds **5a–d**, **6a–d,** and **7a–d**. In ribofuranosides **7a–d**, a multiplet appeared at δ 6.50–8.42 ppm due to aromatic protons. C_4'–H and CH_2 protons of the sugar moiety gave a multiplet in the region δ 4.20–4.92 ppm, whereas C_2'–H and C_3'–H signals seen in the region δ 5.65–5.98 ppm as multiplets. The doublet in the region δ 6.42–6.33 ppm is attributed to C_1'–H.

CONCLUSIONS

The structures proposed for the synthesized compounds are well supported by spectroscopic data and elemental analysis.

This study elucidated that the synthesized compounds showed mixed radical scavenging activity in both DPPH and $ABTS^{+}$ assay. (a) Compounds (**5a, 7a, and 7d**) showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$ 50. (b) Compounds (**5d, 6a, 6b, 6c, 6d, and 7c**) showed moderate radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$ 30. (c) Compounds (**5b, 5c, and 7b**) showed mild radical scavenging activity in DPPH assay that have DPPH% inhibition $<$ 30. (d) Compounds (**5a, 5c, 7a, and 7d**) were found to be more active in $ABTS^{+}$ assay which showed much decline in graph.

Regarding antibacterial activity, compounds **5d** and **6d** against Coagulase positive *staphylococci* and compounds **5d, 6d, 7a, 7b, 7c, and 7d** against Coagulase negative *staphylococci* showed good activity. Other compounds showed moderate to less activity against all bacterial strains. Regarding antifungal activity, all compounds were found moderate to less active against fungus *Candida albicans*.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained from JEOL AL 300 FT NMR using TMS (tetramethyl silane) as internal standard in $CDCl_3$/DMSO-d_6. Mass spectra were recorded on JEOL SX 102/DA 600 using

Table 1

Antioxidant activity of synthesized compounds.

Compd.No.	Compound								DPPH% inhibition of 1 mg/mL of the compound
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	
5a	H	CH_3	H	CH_3	H	Cl	H	Cl	79.21 ± 0.02
5b	H	CH_3	H	CH_3	H	Cl	NO_2	H	24.53 ± 0.01
5c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	28.55 ± 1.05
5d	F	F	F	H	NO_2	H	COOH	H	33.33 ± 0.07
6a	H	CH_3	H	CH_3	H	Cl	H	Cl	31.25 ± 0.08
6b	H	CH_3	H	CH_3	H	Cl	NO_2	H	35.35 ± 1.1
6c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	45.00 ± 1.5
6d	F	F	F	H	NO_2	H	COOH	H	41.00 ± 1.2
7a	H	CH_3	H	CH_3	H	Cl	H	Cl	75.37 ± 0.09
7b	H	CH_3	H	CH_3	H	Cl	NO_2	H	17.98 ± 0.60
7c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	36.31 ± 0.08
7d	F	F	F	H	NO_2	H	COOH	H	66.44 ± 0.05

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.

Argon/Xenon as FAB (fast atom bombardment) gas. The purity of synthesized compounds was checked by thin-layer chromatography using silica gel "G" as adsorbent, and visualization was accomplished by UV light or iodine.

General procedure for the synthesis of 10H-phenothiazines (5a–c). To a stirred suspension of 0.01 mol of 2-amino-4,6-dimethyl benzenethiol **1a** in ethanol (20 mL) containing sodium acetate (0.01 mol) in a round-bottomed flask fitted with reflux condensor was added an alcoholic solution (10 mL) of 0.01 mol of 1,3,5-trichloro-2-nitrobenzene 2a/1,5-dichloro-2,4-dinitrobenzene 2b/2-chloro-3,5-dinitrobenzotrifluoride 2c. The reaction mixture was refluxed for 4–5 h, concentrated and cooled in an ice chamber overnight. The solid separated out was filtered and washed with 30% ethanol. The diphenyl derivatives (0.01 mol) were refluxed with 90% formic acid (20 mL) for 4 h. The contents were then poured into a beaker containing crushed ice; a solid that separated out was filtered, washed with water until the filtrate was neutralized and crystallized from benzene. An ethanolic solution of potassium hydroxide (0.0036 mol in 5 mL ethanol) was added to refluxing solution of formyl derivatives (0.01 mol). The content was heated for half an hour. To this, a second lot of potassium hydroxide (0.0036 mol in 5 mL ethanol) was added and refluxed for 4 h. The content was poured into a beaker containing crushed ice. The solid separated out was filtered, washed with cold water and finally with 30% ethanol, and recrystallized from methanol.

2,4-Dichloro-6,8-dimethyl-10H-phenothiazine (5a). This compound was obtained as brownish crystals, m.p. 95°C; yield 62%; IR: NH 3350, CCl 790, CH 2900 cm^{-1}; ^{1}H-NMR: δ 8.85 (s,1H, NH), 7.51–6.80 (m, 4H, ArH), 2.35 (s, 3H, CH_3 at C_6), 2.25 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 121.4 (C-1), 135.5 (C-2), 130.5 (C-3), 137.2 (C-4), 138.4 (C-6), 132.2 (C-7), 138.8 (C-8), 122.2 (C-9), 16.8 (CH_3 at C-6), 13.5 (CH_3 at C-8); ms: *m/z* 296 (M^+), 298 (M + 2), 295 (30), 224 (52), 175 (100) etc. Anal. Calcd. For $C_{14}H_{11}NCl_2S$: C, 56.75; H, 3.71; N, 4.72. Found: C, 56.91; H, 3.70; N, 4.75.

Table 2

Antioxidant activity of synthesized compounds ($ABTS^{\bullet+}$ assay).

Compd. No.	Compound								$ABTS^{\bullet+}$ Activity at different time intervals (min)				
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	0	1	2	4	6
5a	H	CH_3	H	CH_3	H	Cl	H	Cl	0.687	0.106	0.06	0.025	0.017
5b	H	CH_3	H	CH_3	H	Cl	NO_2	H	0.711	0.525	0.506	0.465	0.439
5c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	0.713	0.284	0.131	0.031	0.02
5d	F	F	F	H	NO_2	H	COOH	H	0.695	0.686	0.377	0.174	0.119
6a	H	CH_3	H	CH_3	H	Cl	H	Cl	0.723	0.310	0.282	0.282	0.282
6b	H	CH_3	H	CH_3	H	Cl	NO_2	H	0.722	0.620	0.618	0.618	0.618
6c	H	CH_3	H	CH_3	CF3	H	NO_2	H	0.721	0.251	0.250	0.249	0.249
6d	F	F	F	H	NO_2	H	COOH	H	0.727	0.321	0.319	0.319	0319
7a	H	CH_3	H	CH_3	H	Cl	H	Cl	0.692	0.337	0.049	0.039	0.037
7b	H	CH_3	H	CH_3	H	Cl	NO_2	H	0.708	0.526	0.481	0.438	0.416
7c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	0.719	0.502	0.445	0.358	0.305
7d	F	F	F	H	NO_2	H	COOH	H	0.692	0.266	0.145	0.099	0.07

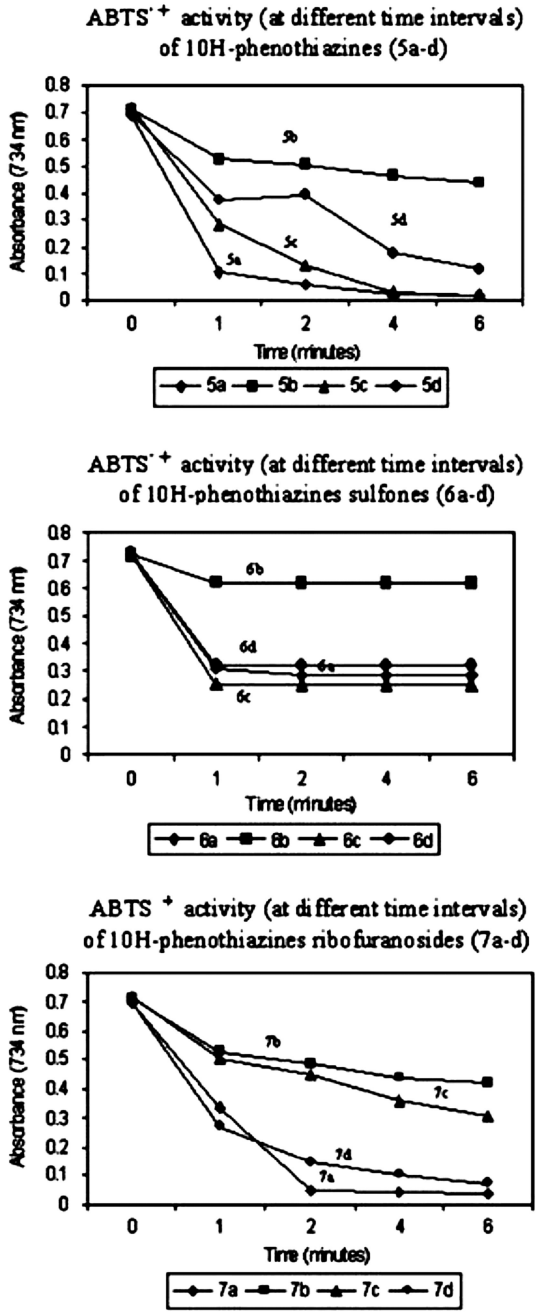


Figure 1. The effect of time on the suppression of absorbance of ABTS by synthesized compounds. After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700 ± 0.020) to 10 μL of the compound the absorbance reading was taken at 30°C exactly 1 min., after initial mixing and up to 6 min. All determinations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

2-Chloro-6,8-dimethyl-3-nitro-10H-phenothiazine (5b). This compound was obtained as reddish crystals, m.p. 200°C; yield 85%; IR: NH 3370, CCl 785, CH 2940 cm^{-1}; ^{1}H-NMR: δ 8.82 (s, 1H, NH), 7.58–6.85 (m, 4H, ArH), 2.25 (s, 3H, CH_3 at C_6), 2.20 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 121.2 (C-1), 135.8 (C-2), 148.8 (C-3), 125.0 (C-4), 139.2 (C-6), 131.8 (C-7), 138.0 (C-8), 121.9 (C-9), 15.5 (CH_3 at C-6), 14.2 (CH_3 at C-8); ms: *m/z* 307 (M^+), 309 (M + 2), 306 (32), 272 (55), 175 (100) etc. Anal. Calcd. For $C_{14}H_{11}N_2O_2ClS$: C, 54.81; H, 3.58; N, 9.13. Found: C, 54.99; H, 3.54; N, 9.08.

1-Trifluoromethyl-6,8-dimethyl-3-nitro-10H-phenothiazine (5c). This compound was obtained as dark brownish crystals, m.p. 148°C; yield 72%; IR: NH 3390, CF_3 1350,1105, CH 2910 cm^{-1}; ^{1}H-NMR: δ 8.96 (s, 1H, NH), 8.24–7.06 (m, 4H, ArH), 2.15 (s, 3H, CH_3 at C_6), 2.05 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 162.2 (C-1), 128.8 (C-2), 148.6 (C-3), 126.6 (C-4), 138.5 (C-6), 131.4 (C-7), 139.0 (C-8), 123.2 (C-9), 15.8 (CH_3 at C-6), 14.5 (CH_3 at C-8); ms: *m/z* 340 (M^+), 339 (28), 271 (65), 175 (100) etc. Anal. Calcd. For $C_{15}H_{11}N_2O_2F_3S$: C, 52.94; H, 3.23; N, 8.23. Found: C, 52.64; H, 3.19; N, 8.17.

3-Carboxy-7,8,9-trifluoro-1-nitro-10H-phenothiazine (5d). Mixture of (0.01 mol) of 2-amino-3,4,5-trifluorobenzenethiol **1b**, sodium hydroxide (0.01 mol), and absolute alcohol (20 mL) was taken in a round-bottomed flask (50 mL), fitted with the reflux condenser, and was heated for 5 min. A total of 0.01 mol of substituted reactive *o*-halonitrobenzene 4-chloro-3,5-dinitrobenzoic acid **2d** (which contain two nitro group at both ortho position to reactive halogen atom) was added with stirring to this solution. The contents were refluxed for 2 h, concentrated, cooled and filtered. The precipitate was washed well with hot water and ethanol and crystallized from acetone. This compound was obtained as blackish crystals, m.p. 145°C; yield 55%; IR: NH 3380, NO_2 1590,1400, CF 1230 cm^{-1}; ^{1}H-NMR: δ 9.10 (s, 1H, NH), 8.15–7.20 (m, 3H, ArH), 11.2 (s, 1H, COOH); ^{13}C-NMR: δ 148.1 (C-1), 122.1 (C-2), 145.2 (C-3), 121.2 (C-4), 111.2 (C-6),153.8 (C-7), 135.6 (C-8), 155.2 (C-9); ms: *m/z* 342 (M^+), 325 (100), 312 (65), 296 (55), 295 (50) etc. Anal. Calcd. For $C_{13}H_5N_2O_4F_3S$: C, 45.61; H, 1.46; N, 8.18. Found: C, 45.81; H, 1.45; N, 8.15.

General procedure for the synthesis of 10H-phenothiazine sulfones (6a–d). A mixture of substituted 10H-phenothiazines **5a–d** (0.01 mol), glacial acetic acid (20 mL), and 30% hydrogen peroxide (5 mL) 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 4 h. The contents were poured into a beaker containing crushed ice. The yellow residue obtained was filtered and washed with water and recrystallized from ethanol.

2,4-Dichloro-6,8-dimethyl-10H-phenothiazine-5,5-dioxide (sulfone) (6a). This compound was obtained as light yellowish crystals, m.p. 240°C; yield 58%; IR: NH 3360, CS 1060 cm^{-1};^{1}H-NMR: δ 8.95 (s, 1H, NH), 7.62–6.80 (m, 4H, ArH), 2.36 (s, 3H, CH_3 at C_6), 2.28 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 120.1 (C-1), 138.2 (C-2), 127.2 (C-3), 141.4 (C-4), 140.2 (C-6), 129.2 (C-7), 144.2 (C-8), 125.2 (C-9), 17.0 (CH_3 at C-6), 14.1 (CH_3 at C-8); ms: *m/z* 328 (M^+), 330 (M+2), 327 (38), 257 (48), 207 (100) etc. Anal. Calcd. For $C_{14}H_{11}NO_2Cl_2S$: C, 51.21; H, 3.35; N, 4.26. Found: C, 51.56; H, 3.30; N, 4.15.

2-Chloro-6,8-dimethyl-3-nitro-10H-phenothiazine-5,5-dioxide (sulfone) (6b). This compound was obtained as light brown crystals, m.p. 270°C; yield 52%; IR: NH 3395, CS 1070 cm^{-1}; ^{1}H-NMR: δ 9.01 (s, 1H, NH), 7.9–6.55 (m, 4H, ArH), 2.28 (s, 3H, CH_3 at C_6), 2.25 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 118.2 (C-1), 139.1 (C-2), 144.1 (C-3), 132.2 (C-4), 144.2 (C-6),126.4 (C-7), 143.2 (C-8), 116.5 (C-9), 16.5 (CH_3 at C-6), 13.5 (CH_3 at C-8); ms: *m/z* 339 (M^+), 341 (M + 2), 338 (35), 304 (50), 207 (100) etc. Anal. Calcd. For $C_{14}H_{11}N_2O_4ClS$: C, 49.63; H, 3.24; N, 8.27. Found: C, 49.83; H, 3.23; N, 8.21.

Table 3

Antimicrobial activity of synthesized compounds.

	Compound								Antibacterial activity (zone of inhibition in mm)		Anti fungal activity (zone of inhibition in mm)	
Compd.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	*Enterobacter*	Coagulis positive *Staphylococci*	Coagulis negative *Staphylococci*	*Candida albicans*
5a	H	CH_3	H	CH_3	H	Cl	H	Cl	–	12	11	10
5b	H	CH_3	H	CH_3	H	Cl	NO_2	H	–	–	10	10
5c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	12	12	12	12
5d	F	F	F	H	NO_2	H	COOH	H	16	17	18	22
6a	H	CH_3	H	CH_3	H	Cl	H	Cl	10	11	10	11
6b	H	CH_3	H	CH_3	H	Cl	NO_2	H	–	–	14	13
6c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	–	11	–	16
6d	F	F	F	H	NO_2	H	COOH	H	15	16	16	–
7a	H	CH_3	H	CH_3	H	Cl	H	Cl	–	–	20	14
7b	H	CH_3	H	CH_3	H	Cl	NO_2	H	–	–	17	10
7c	H	CH_3	H	CH_3	CF_3	H	NO_2	H	12	15	20	21
7d	F	F	F	H	NO_2	H	COOH	H	–	12	15	18
Vancomycin									–	15	15	–
Gatifloxacin									17	–	–	–
Flucanazole									–	–	–	25
										Note < 7 mm inactive; 7–9 mm weakly active; 10–12 mm, moderately active; > 12 mm, active		< 7 mm, inactive; 7–11 mm, weakly active; 12–17 mm, moderately active; > 17 mm active

1-Trifluoromethyl-6,8-dimethyl-3-nitro-10H-phenothiazine-5,5-dioxide (sulfone) (6c). This compound was obtained as creamish crystals, m.p. 230°C; yield 48%; IR: NH 3410, CS 1040 cm^{-1};^{1}H-NMR: δ 9.22 (s, 1H, NH), 8.02–6.85 (m, 4H, ArH), 2.18 (s, 3H, CH_3 at C_6), 2.10 (s, 3H, CH_3 at C_8); ^{13}C-NMR: δ 158.1 (C-1), 131.2 (C-2), 145.1 (C-3), 134.3 (C-4), 143.1 (C-6), 128.2 (C-7), 145.1 (C-8), 114.2 (C-9), 14.3 (CH_3 at C-6), 12.1 (CH_3 at C-8); ms: *m/z* 372 (M^+), 371 (35), 303 (50), 207 (100) etc. Anal. Calcd. For $C_{15}H_{11}N_2O_4F_3S$: C, 48.38; H, 2.95; N, 7.52. Found: C, 48.26; H, 2.91; N, 7.49.

3-Carboxy-7,8,9-trifluoro-1-nitro-10H-phenothiazine-5,5-dioxide (sulfone) (6d). This compound was obtained as blackish crystals, m.p. 250°C; yield 50%; IR: NH 3390, NO_2 1595,1410, CF 1240, CS 1080 cm^{-1};^{1}H-NMR: δ 8.90 (s, 1H, NH), 7.70–6.52 (m, 4H, ArH), 11.15 (s, 1H, COOH); ^{13}C-NMR: δ 146.0 (C-1), 131.0 (C-2), 149.0 (C-3), 132.1 (C-4), 122.1 (C-6), 152.8 (C-7), 192.3 (C-8), 149.0 (C-9); ms: *m/z* 374 (M^+), 357 (100), 354 (72), 328 (60), 327 (55) etc. Anal. Calcd. For $C_{13}H_5N_2O_6F_3S$: C, 41.71; H, 1.33; N, 7.48. Found: C, 41.89; H, 1.30; N, 7.45.

General procedure for the synthesis of *N*-(2′,3′,5′-tri-*O*-benzoyl)-b-D-ribofuranosyl-10H-phenothiazines (7a–d). To a solution of **5a–d** (0.002 mol) in toluene, b-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mol) was added, and the contents were refluxed in vaccum with stirring in an oil bath at 155–160°C for 15 min. The vaccum was removed and the reaction mixture was protected from moisture by fitting a guard tube. Stirring was further continued for 10 h, and vaccum was applied for 10 min at every hour. The viscous mass thus obtained was dissolved in methanol and boiled for 10 min and cooled to room temperature. The reaction mixture was filtered and filtrate was evaporated to dryness. The viscous residue, thus obtained was dissolved in ether, filtered, concentrated and kept in refrigerator overnight to get crystalline ribofuranosides.

N-(2′,3′,5′-tri-O-benzoyl)-b-D-ribofuranosyl-2,4-dichloro-6,8-dimethyl-10H-phenothiazine (7a). This compound was obtained as shiny brownish crystals, m.p. 96°C; yield 65%; IR CCl 795, COC 1150 cm^{-1}; ^{1}H-NMR: δ 7.8–6.55 (m, 19H, ArH), 2.32 (s, 3H, CH_3 at C_6), 2.30 (s, 3H, CH_3 at C_8), 6.35 (d,1H, C_1'-H), 5.66 (m,1H, C_2'-H), 5.68 (m,1H, C_3'-H), 4.21 (m,1H, C_4'-H), 4.80 (CH_2 proton); ^{13}C NMR: δ 122.5 (C-1), 136.0 (C-2), 130.8 (C-3), 138.5 (C-4), 139.6 (C-6), 130.2 (C-7), 137.2 (C-8), 122.5 (C-9), 95.5 (C-1'), 89.0 (C-2'), 90.5 (C-3'), 97.0 (C-4'), 77 (CH_2); ms: *m/z* 740 (M^+), 742 (M + 2), 739 (31), 669 (58), 619 (100) etc. Anal. Calcd. For $C_{40}H_{31}NO_7Cl_2S$: C, 64.86; H, 4.19; N, 1.89. Found: C, 64.98; H, 4.15; N, 1.88.

N-(2′,3′,5′-tri-O-benzoyl)-b-D-ribofuranosyl-2-chloro-6,8-dimethyl-3-nitro-10H- phenothiazine (7b). This compound was obtained as dark brown crystals, m.p. 120°C; yield 68%; IR: CCl 790, COC 1150 cm^{-1}; ^{1}H-NMR: δ 7.50–6.52 (m,19H, ArH), 2.20 (s, 3H, CH_3 at C_6), 2.21 (s, 3H, CH_3 at C_8), 6.37 (d,1H, C_1'-H), 5.65 (m,1H, C_2'-H), 5.70 (m,1H, C_3'-H), 4.25 (m,1H, C_4'-H), 4.85 (CH_2 proton); ^{13}C-NMR: δ 124.1 (C-1), 137.2 (C-2), 148.1 (C-3), 124.2 (C-4), 138.6 (C-6),130.2 (C-7), 137.2 (C-8), 120.1 (C-9), 92.1 (C-1'), 88.2 (C-2'), 91.0 (C-3'), 98.3 (C-4'), 79 (CH_2); ms: *m/z* 751 (M^+), 753 (M + 2), 750 (35), 716 (60), 619 (100) etc. Anal. Calcd. For $C_{40}H_{31}N_2O_9ClS$: C, 63.95; H, 4.13; N, 3.73. Found: C, 63.62; H, 4.02; N, 3.65.

N-(2′,3′,5′-tri-O-benzoyl)-b-D-ribofuranosyl-1-trifluoromethyl-6,8-dimethyl-3-nitro-10H-phenothiazine (7c). This compound was obtained as shiny black crystals, m.p. 115°C; yield 58%; IR: CF_3 1355,1100, COC 1190 cm^{-1}; ^{1}H-NMR: δ 8.05–7.01 (m, 19H, ArH), 2.16 (s, 3H, CH_3 at C_6), 2.08 (s, 3H, CH_3 at C_8), 6.40 (d,1H, C_1'-H), 5.68 (m,1H, C_2'-H), 5.85 (m,1H,

C_3'-H), 4.35 (m,1H, C_4'-H), 4.90 (CH_2 proton); ^{13}C-NMR: δ 164.1 (C-1), 130.2 (C-2), 155.2 (C-3), 120.8 (C-4), 139.0 (C-6), 128.8 (C-7), 139.5 (C-8), 120.0 (C-9), 95.6 (C-1'), 90.0 (C-2'), 91.2 (C-3'), 96.2 (C-4'), 80 (CH_2); ms: *m/z* 784 (M^+), 783 (31), 715 (55), 619 (100) etc. Anal. Calcd. For $C_{41}H_{31}N_2O_9F_3S$: C, 62.75; H, 3.95; N, 3.57. Found: C, 62.67; H, 3.92; N, 3.61.

N-(2′,3′,5′-tri-O-benzoyl)-b-D-ribofuranosyl-3-carboxy-7,8,9-trifluoro-1-nitro-10H-phenothiazine (7d). This compound was obtained as blackish crystals, mp 105°C; yield 45%; IR: NO_2 1580, 1400, CF 1235, COC 1185 cm^{-1}; ^{1}H-NMR: δ 8.25–6.90 (m,18H, ArH), 11.6 (s, 1H, COOH), 6.42 (d,1H, C_1'-H), 5.98 (m,1H, C_2'-H), 5.80 (m,1H, C_3'-H), 4.40 (m,1H, C_4'-H), 4.92 (CH_2 proton); ^{13}C-NMR: δ 150.1(C-1), 124.2(C-2), 148.1 (C-3), 125.2 (C-4), 112.0 (C-6),150.2 (C-7), 136.1 (C-8), 151.5 (C-9), 95.0 (C-1'), 85.2 (C-2'), 94.1 (C-3'), 98.1 (C-4'), 75 (CH_2); ms: *m/z* 786 (M^+), 769 (100), 756 (60), 740 (50), 739 (48) etc. Anal. Calcd. For $C_{39}H_{25}N_2O_{11}F_3S$: C, 59.54; H, 3.18; N, 3.56. Found: C, 59.74; H, 3.20; N, 3.48.

Antioxidant activity. All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and 2,2-azinobis(3-ethyl benzothiazoline-6-sulfonic acid) $ABTS^{\cdot+}$ radical cation decolorization assay.

DPPH radical scavenging assay. Radical scavenging activity of synthesized compounds against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet *et al.* [15]. A stock solution containing 1 mg/mL of the compound was prepared in methanol. Fifty microlitres of the solution were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min of incubation in the 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 (Table 1) 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 decolorization assay. The 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS) decolorization test was also used to assess the antioxidant activity of synthesized compounds. The $ABTS^{\cdot+}$ assay was carried out using the improved assay of Re *et al.* [16]. In short, $ABTS^{\cdot+}$ was generated by oxidation of ABTS with potassium persulphate. For this purpose, ABTS was dissolved in deionized water at a concentration of 7 m*M*, and potassium persulphate added to a concentration of 2.45 m*M*. 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 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 (Table 2 and Fig. 1).

Antimicrobial activity. The synthesized compounds were tested for their antibacterial activity by using Paper Disc method [17] by measuring the zone of inhibition on agar plates with *Enterobacter*, Coagulase positive *Staphylococci*, and Coagulase negative *staphylococci* as test organisms at concentration of 100 μg per disc using vancomycin and gatifloxacin as standard compounds and antifungal activity against *Candida albicans* at concentration of 100 μg/disc using flucanazole as standard compound (Table 3).

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