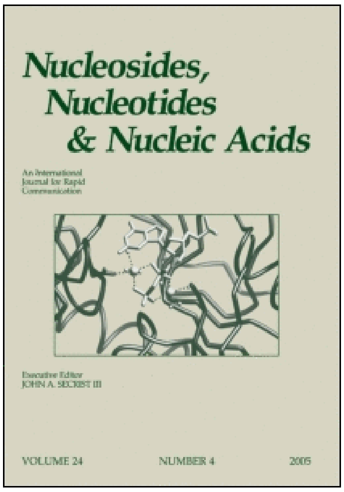

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Synthesis, Spectral Characterization, and Biological Activity of Some New Substituted 10*H*-Phenothiazines, Its Ribofuranosides, and Sulfones

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SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL ACTIVITY OF SOME NEW SUBSTITUTED 10*H*-PHENOTHIAZINES, ITS RIBOFURANOSIDES, AND SULFONES

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□ *This article describes the synthesis of new substituted 10* H*-phenothiazines by Smiles rearrangement. These compounds are then used as a base to form ribofuranosides by treating them with a sugar (1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-ribofuranose). On oxidation with hydrogen peroxide in glacial acetic acid, these phenothiazines yield their sulfones. These compounds are screened for antioxidant and antimicrobial activity and their structure has been established by elemental analysis and spectroscopic data.*

Keywords Smiles rearrangement; ribofuranosides; antioxidant and antimicrobial activity

INTRODUCTION

10*H*-Phenothiazines as well as the ribofuranosides obtained from them have been found to possess promising pharmacological and biological activity and are employed for many purposes, such as tranquilizers, sedatives, antibacterial, antifungal, anticancer, and central nervous system (CNS) depressants, and so on. A slight change in the substitution pattern results in large difference in their biological activities.[1–15] The synthesized compounds were screened for antioxidant and antimicrobial activity.

RESULTS AND DISCUSSION

Synthesis of 10*H*-phenothiazines (**5a–b**) have been carried out by Smiles rearrangement of substituted 2-formamido-2′-nitrodiphenylsulfides (**4a–b**).

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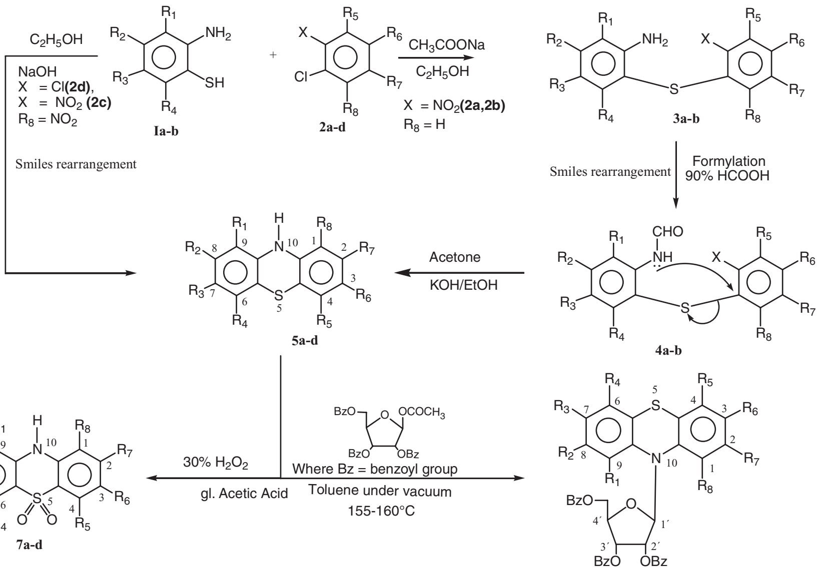
The formyl derivatives were prepared by the formylation of 2-amino-2′-nitrodiphenylsulfides (**3a–b**), which in turn were prepared by condensation of 2-amino-4,6-dimethylbenzenethiol **(Ia)** with *o*-halonitrobenzene [1,4-dibromo-2-nitrobenzene **(2a)** and 2,4-dichloro-5-nitrobenzotrifluoride **(2b)**] in ethanolic sodium acetate solution. 1-Nitrophenothiazines (**5c–d**) were prepared by refluxing 2-amino-4,6-dimethylbenzenethiol **(Ia)** and 2-amino-3,4,5-trifluorobenzenethiol **(Ib)** with reactive halonitrobenzenes [2,4-dichloro-3,5-dinitrobenzotrifluoride **(2c)**, and 1,2-dichloro-3-nitrobenzene **(2d)**], respectively, (which have either two nitro or one halo and one nitro group ortho to the reactive halogen atom) in alcohol in the presence of sodium hydroxide, whereby Smiles rearrangement occured in situ. Compounds (**5a–d)** on refluxing with 30% H_2O_2 in glacial acetic acid were converted into their corresponding sulfones (**7a–d**). Compounds (**5a–d**) when treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose in toluene in vacuum at 155–160°C for 10 hours give the corresponding ribofuranosides **(6a–d)** (Scheme 1).



Compound	R1	R2	R3	R4	R5	R6	R7	R8
5a, 6a, 7a	H	CH_3	H	CH_3	H	Br	H	H
5b, 6b, 7b	H	CH_3	H	CH_3	H	CF_3	Cl	H
5c, 6c, 7c	H	CH_3	H	CH_3	Cl	CF_3	H	NO_2
5d, 6d, 7d	F	F	F	H	H	H	H	NO_2

SCHEME 1 Diagramatic representation of the preparatory methods of 10*H*-phenothiazines, their ribofuranosides, and sulfones.

The structures proposed for the synthesized compounds are well supported by elemental analysis and spectral data. The synthesized compounds were also screened for antioxidant and antimicrobial activity. The present

TABLE 1 Characterization data of synthesized compounds **5a–d**, **6a–d**, **7a–d**

Compd. no.	Mol. Formula	M.P. °C	Yield%	Elemental analysis (%) Found (calcd.) C	H	N
5a	$C_{14}H_{12}NBrS$	60	87	55.11(54.90)	3.93(3.92)	4.52(4.57)
5b	$C_{15}H_{11}NClF_3S$	99	56	54.88(54.62)	3.29(3.33)	4.23(4.24)
5c	$C_{15}H_{10}N_2O_2ClF_3S$	84	80	48.25(48.06)	2.65(2.67)	7.42(7.47)
5d	$C_{12}H_5N_2O_2F_3S$	220	67	48.53(48.32)	1.65(1.67)	9.42(9.39)
6a	$C_{40}H_{32}NO_7BrS$	75	76	64.30(64.00)	4.24(4.26)	1.85(1.86)
6b	$C_{41}H_{31}NO_7ClF_3S$	72	52	63.82(63.60)	4.02(4.00)	1.78(1.80)
6c	$C_{41}H_{30}N_2O_9ClF_3S$	85	69	60.35(60.10)	3.64(3.66)	3.40(3.42)
6d	$C_{38}H_{25}N_2O_9F_3S$	81	58	61.62(61.45)	3.38(3.36)	3.75(3.77)
7a	$C_{14}H_{12}NO_2BrS$	50	60	49.86(49.70)	3.58(3.55)	4.12(4.14)
7b	$C_{15}H_{11}NO_2ClF_3S$	77	58	49.99(49.79)	3.02(3.04)	3.85(3.87)
7c	$C_{15}H_{10}N_2O_4ClF_3S$	61	42	44.45(44.28)	2.49(2.46)	6.91(6.88)
7d	$C_{12}H_5N_2O_4F_3S$	88	51	43.84(43.63)	1.50(1.51)	8.44(8.48)

study demonstrated that the synthesised compounds showed mixed radical scavenging activity in both DPPH and ABTS$^{\bullet+}$ assay. These compounds showed good chemopreventive and antigenotoxic effect.

(a) Compounds **5a**, **5c**, **6a**, **6b**, and **7a** showed strong radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$50.

(b) Compounds **5b**, **6c**, and **7b** showed moderate radical scavenging activity in DPPH assay that have DPPH% inhibition $\geq$30.

(c) Compounds **5d**, **6d**, **7c**, and **7d** showed mild radical scavenging activity in DPPH assay that have DPPH% inhibition $<$30.

(d) Compounds **5a**, **5d**, **6c**, **7a**, **7b**, and **7d** showed good activity in ABTS$^{\bullet+}$ assays.

All these compounds were found to be moderately active against various bacteria and fungi. The results of antibacterial screening indicated that good activity was shown by compounds **5a**, **5b**, **5c**, **6b**, **6c**, **7a**, **7c**, **7d** against *Coagulase negative staphylococci* strain while moderate activity was shown by compounds **5d** and **6a**. Regarding antifungal activity, **5c**, **5d**, **6b**, **6d** showed good results against *Candida albicans* strain. Other compounds showed moderate activity. The structural assignments of the synthesised compounds were based on elemental analysis (Table 1) and spectral data (Table 2).

Infrared Spectra

The infrared (IR) spectral data of compounds **3a–b** showed two peaks in region 3460–3400 cm^{-1} and 3350–3320 cm^{-1} due to asymmetric and symmetric vibrations of $-NH_2$ group. Two peaks in the regions 1570–1560 cm^{-1} and 1370–1360 cm^{-1} are also observed due to asymmetric and symmetric

TABLE 2 The spectral data of synthesized compounds (IR, ^{1}H NMR,^{13}C NMR, and mass spectral data for **5a–d**, **6a–d**, **7a–d**

Comp.	IR (KBr: v_{max} cm^{-1})	^{1}H NMR (δ, ppm from TMS)	^{13}C NMR (δ, ppm)	Mass m/z (%)
5a	3320 (>NH str.), 2900 ($-CH_3$ str.), 630 ($-C-Br$ str.)	8.62 (s, >NH), 7.16–6.29 (m, Ar$-$H) 2.15 (s, $-CH_3$)	120.7 (C-1), 131.2 (C-2), 113.2 (C-3), 138.2 (C-4), 136.2 (C-6), 131.4 (C-7), 136.5 (C–8), 128.2 (C-9), 21.2 ($-CH_3$ at C-8), 13.9 ($-CH_3$ at C-6)	305 (M$^+$), 307 (M+2), 304 (29), 226 (65), 175 (100), etc.
5b	3420 (>NH str.), 2985 ($-CH_3$ str.), 810 ($C-Cl$ str.) 1320, 1130 ($-CF_3$ str.)	8.89 (s, >NH), 7.04–6.28 (m, Ar$-$H), 2.05 (s, $-CH_3$)	119.2 (C-1), 130.0 (C-2), 121.5 (C-3), 130.1 (C-4), 141.0 (C-6), 137 (C-7), 138.8 (C–8), 116.2 (C-9), 109.8 ($-CF_3$ at C-3)	329 (M$^+$), 331 (M+2), 328 (30), 294 (62), 175 (100), etc.
5c	3120 (>NH str.), 1560, 1380 ($-NO_2$ str.), 820 ($-C-Cl$ str.), 1310, 1140 ($-CF_3$ str.)	8.53 (s, >NH), 8.05–6.80 (m, Ar$-$H), 2.30 (s, $-CH_3$)	136.8 (C-1), 121.2 (C-2), 122.4 (C-3), 140 (C-4), 141.2 (C-6), 120.8 (C-7), 137.9 (C–8), 116.2 (C-9), 20.2 ($-CH_3$ at C-8), 14.2 ($-CH_3$ at C-6), 110.8 ($-CF_3$ at C-3)	374 (M$^+$), 376 (M+2), 344 (65), 328 (75), 327 (60), 357 (100), etc.
5d	3240 (>NH str.), 1580, 1410 ($-NO_2$ str.)	9.08 (s, >NH), 8.02–7.11 (m, Ar$-$H)	138.4 (C-1), 123 (C-2), 119.5 (C-3), 138.2 (C-4), 116.2 (C-6), 140.8 (C-7), 135.8 (C–8), 140.2 (C-9)	298 (M$^+$), 268 (65), 252 (48), 251 (55), 281 (100), etc.
6a	640 ($-C-Br$ str.), 2910 ($-CH_3$ str.), 1140 ($C-O-C$ str.)	7.82–6.24 (m, Ar$-$H), 2.35 (s, $-CH_3$)	122.7 (C-1), 132.2 (C-2), 114.2 (C-3), 135.2 (C-4), 141.0 (C-6), 120.4 (C-7), 136.2 (C–8), 116.8 (C-9), 89.2 (C-1′), 87.5 (C-2′), 90.2 (C-3′), 93.4 (C-4′)	749 (M$^+$), 751 (M+2), 748 (28), 670 (65), 619 (100), etc.
6b	820 ($-C-Cl$ str.), 1330, 1140 ($-CF_3$ str.), 2988 ($-CH_3$ str.), 1155 ($C-O-C$ str.)	7.88–6.24 (m, Ar$-$H), 2.31 (s, $-CH_3$)	119.2 (C-1), 130.8 (C-2), 121.5 (C-3), 136.1 (C-4), 139.2 (C-6), 126.5 (C-7), 137 (C–8), 117.2 (C-9), 88.2 (C-1′), 85.2 (C-2′), 89.6 (C-3′), 94.3 (C-4′)	773 (M$^+$), 775 (M+2), 772 (29), 619 (100), etc.
6c	1570, 1370 ($-NO_2$ str.), 830 ($-C-Cl$ str.), 1320, 1130 ($-CF_3$ str.), 1180 ($C-O-C$ str.)	8.06–6.39 (m, Ar$-$H), 2.35 (s, $-CH_3$)	136.8 (C-1), 122.2 (C-2), 126.4 (C-3), 140.1 (C-4), 141.0 (C-6), 120.8 (C-7), 135.2 (C–8), 116.2 (C-9), 82.7 (C-1′), 96.2 (C-2′), 74.5 (C-3′), 95.3 (C-4′)	818 (M$^+$), 820 (M+2), 817 (30), 788 (62), 772 (47), 771 (52), 801 (100), etc.

(Continued on next page)

TABLE 2 The spectral data of synthesized compounds (IR, ^{1}H NMR,^{13}C NMR, and mass spectral data for **5a–d**, **6a–d**, **7a–d** *(Continued)*

Comp.	IR (KBr: $v_{\max}$ cm^{-1})	^{1}H NMR (δ, ppm from TMS)	^{13}C NMR (δ, ppm)	Mass m/z (%)
6d	1595, 1415 ($-NO_2$ str.), 1190 (C$-$O$-$C str.)	8.04–7.05 (m, Ar$-$H)	137.8 (C-1), 128.2 (C-2), 123.8 (C-3), 141.2 (C-4), 142.8 (C-6), 125.5 (C-7), 136.2 (C–8), 117.9 (C-9), 83.4 (C-1′), 92.3 (C-2′), 72.8 (C-3′), 92.8 (C-4′)	742 (M$^+$), 741 (31), 712 (68), 696 (51), 695 (47), 725 (100), etc.
7a	3380 (>NH str.), 640 ($-$C$-$Br str.), 1050 (C$-$S str.), 1180, 1140 (SO_2 str.)	8.64 (s, >NH), 7.18–6.38 (m, Ar$-$H) 2.35 (s, $-CH_3$)	121.1 (C-1), 137.7 (C-2), 113.6 (C-3), 130.7 (C-4), 136.5 (C-6), 120.4 (C-7), 143.5 (C–8), 116.6 (C-9)	337 (M$^+$), 339 (M+2), 336 (26), 258 (58), 207 (100), etc.
7b	3430 (>NH str.), 820 ($-$C$-$Cl str.), 1330, 1140 ($-CF_3$ str.), 1065 (C$-$S str.), 1172, 1155 (SO_2 str.)	8.90 (s, >NH), 7.24–6.80 (m, Ar$-$H), 2.36 (s, $-CH_3$)	119.6 (C-1), 136.5 (C-2), 121.9 (C-3), 125.6 (C-4), 136.5 (C-6), 120.4 (C-7), 143.5 (C–8), 116.6 (C-9), 110.2 ($-CF_3$ at C_2)	361 (M$^+$), 363 (M+2), 360 (29), 326 (42), 207 (100), etc.
7c	3130 (>NH str.), 1570, 1365 ($-NO_2$ str.), 830 ($-$C$-$Cl str.), 1320, 1145 ($-CF_3$ str.), 1060 (C$-$S str.), 1160, 1150 (SO_2 str.)	9.01 (s, >NH), 8.24–6.80 (m, Ar$-$H), 2.37 ($-CH_3$, s)	137.2 (C-1), 127.7 (C-2), 122.0 (C-3), 135.6 (C-4), 138.5 (C-6), 123.4 (C-7), 148.5 (C–8), 118.6 (C-9)	406 (M$^+$), 408 (M+2), 360 (42), 359 (65), 376 (29), 389 (100), etc.
7d	3250 (>NH str.), 1590, 1420 ($-NO_2$ str.), 1070 (C$-$S str.), 1177, 1156 (SO_2 str.)	9.10 (s, >NH), 8.28–7.80 (Ar$-$H)	138.8 (C-1), 129.5 (C-2), 119.9 (C-3), 133.5 (C-4), 111.6 (C-6), 141.2 (C-7), 142 (C–8), 141.1 (C-9)	330 (M$^+$), 300 (62), 284 (72), 283 (68), 313 (100), etc.

vibrations of $-NO_2$ group. In compounds **4a–b,** a single sharp peak due to >N—H stretching in the 3350–3320 cm^{-1} region was observed and a peak in the 1705–1680 cm^{-1} region due to >C=O stretching. Stretching vibrations due to $-NO_2$ are also observed in the 1580–1570 and 1380–1350 cm^{-1} region. Compounds **5a–d** showed a single sharp peak in 3420–3120 cm^{-1} regions due to >N—H stretching vibrations. Compounds **7a–d** showed a peak due to N—H stretching in 3430–3130 cm^{-1} region and also two intense peaks in the 1365–1345 cm^{-1} and 1180–1140 cm^{-1} region due to asymmetric and symmetric stretching vibration of sulfonyl group. Also in compounds **7a–d**, a band due to C—S stretching occurs in the 1070–1050 cm^{-1} region. In compounds **6a–d**, a peak due to >N—H stretching is found to be absent indicating its ribosylation. Also bands due to C=O and C—O—C appeared at 1750–1745 cm^{-1} and 1190–1140 cm^{-1}, respectively.

Nuclear Magnetic Resonance Spectra

The ^1H NMR spectra of compounds **5a–d** showed two main resonances. One singlet was observed in between δ 9.08–8.53 ppm due to N—H proton and a multiplet due to aromatic protons in between δ 8.05–6.28 ppm. In the case of compounds **3a–b** broad resonances at δ 4.24–3.68 ppm was observed due to $-NH_2$ group. In the case of compound **4a–b**, one signal (singlet) at δ 10.14–9.85 was also observed due to the formyl proton and another due to >NH proton at 8.68–8.92 ppm. The synthesized compounds showed resonances in between δ 2.37–2.05 ppm due to the CH_3 group. In compounds **7a–d** a singlet due to the N—H proton was observed in between δ 9.10–8.64 ppm.

The ^1H NMR spectra of ribofuranosides **6a–d** did not show any resonances due to >NH indicating the site of ribosylation but showed a multiplet at δ 8.06–6.24 due to aromatic protons. The C_4'-H and $C_5'-CH_2$ protons of the sugar moiety gave a multiplet between δ 4.34–4.83, while C_2'H and C'_3H at δ 5.71–5.84 as multiplets and doublet at δ 6.35–6.48 ($J = 8$ Hz) due to C_1'-H proton.

EXPERIMENTAL

All the melting points were determined in open capillary tubes and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on JEOL AL-300 spectrometer (300 MHz) in DMSO-d_6 using TMS (tetramethylsilane) as an internal standard. IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. Mass spectra were recorded on JEOL SX 102/DA 600 using Ar/Xe as fast atom bombardment (FAB) gas. Purity of compounds was checked by thin layer chromatography (TLC) using silica gel ‘G’ as adsorbent, visualizing these by ultraviolet (UV) light or in an iodine chamber.

Synthesis of 2-Amino-2′-nitrodiphenylsulfides (3a–b)

2-Amino-4,6-dimethylbenzenethiol **Ia** (0.01 mole) was dissolved in ethanol (20 ml) containing (0.01 mole) of anhydrous sodium acetate in a 50 ml round bottom flask and halonitrobenzene **(2a–b)** (0.01 mole) in 10 ml ethanol was added. The reaction mixture was refluxed for 4–5 hours. The resultant solution was cooled and kept overnight in an ice chamber. The solid was filtered, washed with 30% ethanol and recrystallized with methanol.

Synthesis of 2-Formamido-2′-nitrodiphenylsulfides (4a–b)

The 2-amino-2′-nitrodiphenylsulfides **3a–b** (0.01 mole) obtained above was refluxed for 4 hours in 90% formic acid (20 ml). The contents were then poured onto crushed ice and the solid was filtered, washed with water, and recrystallized from benzene.

Synthesis of 10H-Phenothiazine (5a–b)

Formyl derivatives **4a–b** (0.01 mol) in acetone (15 ml) were refluxed and an alcoholic solution of potassium hydroxide (0.2 gm in 5ml ethanol) was added. These solutions were then heated for 30 minutes. A second lot of KOH (0.2 gm in 5 ml ethanol) was added and refluxing was done for 4 hours. The contents were then poured into a beaker containing crushed ice and filtered. The residue was washed with cold water, then with 30% ethanol and then crystallized from benzene.

Synthesis of 1-Nitro-10*H*-phenothiazines (5c–d)

To a stirred suspension of substituted 2-amino-4,6-dimethylbenzen ethiol/2-amino-3,4,5-trifluorobenzenethiols **Ia/Ib** (0.01 mole), NaOH (0.01 mole) and 20 ml absolute alcohol in round bottom flask fitted with reflux condenser, 0.01 mole of substituted reactive *o*-halonitrobenzene **(2c–d)** was added. The reaction mixture was refluxed for two hours, concentrated, cooled, and filtered. The precipitate was washed well with hot water, then 20% alcohol and crystallized from acetone.

Synthesis of substituted N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) phenothiazines (6a–d)

To the solution of **5a–d** (0.002 mole) in 20 ml toluene, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.002 mole) was added and the contents were refluxed under vacuum with stirring in an oil bath at 155–160°C for 15 minutes. The vacuum was broken and reaction was protected from moisture through a guard tube. Stirring was further continued for 10 hours and a vacuum was applied for 10 minutes after every hour. The viscous mass obtained was dissolved in methanol, boiled for 10 minutes and cooled to room temperature. The reaction mixture was filtered and the methanol was removed by distillation under reduced pressure. The viscous residue thus obtained was dissolved in ether, filtered, concentrated, and kept in refrigerator overnight to afford crystalline ribofuranosides.

Synthesis of 10*H*-phenothiazine-5,5-dioxide (Sulfones) (7a–d)

A mixture of substituted phenothiazines **5a–d** (0.01 mole), 20 ml glacial acetic acid and 5 ml 30% hydrogen peroxide (5 ml) in round bottom flask were refluxed for 15 minutes at 50–60°C, then additional hydrogen peroxide (5 ml) was added. The reaction mixture was refluxed for 4 hours. The contents were then poured into a beaker containing crushed ice. Residue obtained was filtered and washed with water and crystallized from ethanol.

BIOLOGICAL ACTIVITY

Antioxidant Activity

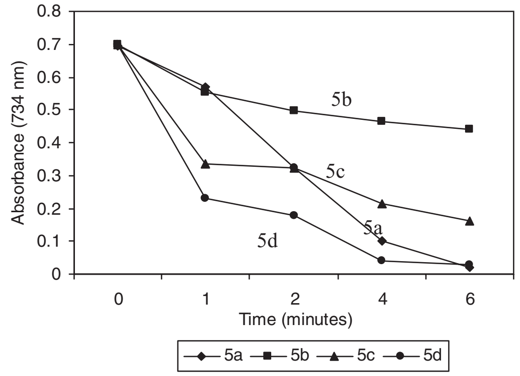
All the synthesized compounds were screened for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Table 3) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$\cdot^{+}$) radical cation decolorization assay (Table 4 and Figure 1).

TABLE 3 Antioxidant activity of synthesized compounds (DPPH assay)

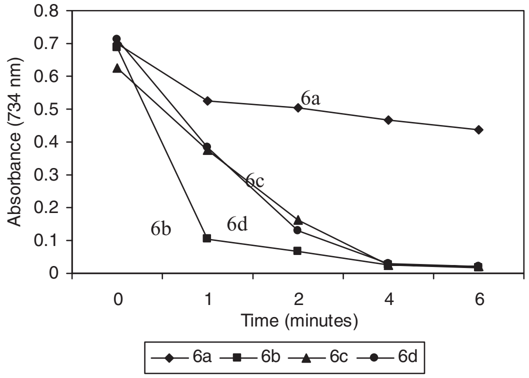
Compd. no.	DPPH% inhibition* of 1 mg/ml of the compound
5a	68.98 ± 0.05
5b	46.81 ± 0.07
5c	70.68 ± 0.09
5d	17.00 ± 1.2
6a	66.44 ± 1.9
6b	75.37 ± 1.07
6c	36.31 ± 0.06
6d	17.98 ± 0.08
7a	71.82 ± 0.04
7b	33.33 ± 0.02
7c	28.55 ± 1.1
7d	24.53 ± 1.3

*The assay was carried out in triplicate and the percentage of inhibition was calculated using the following formula:% inhibition = (AB−AA) × 100/ AB, where, AB = absorption of blank; AA = absorption of test.

ABTS$^{\bullet +}$ activity (at different time intervals) of 10H-phenothiazines (5a-d)



ABTS$^{\bullet +}$ activity (at different time intervals) of 10H-phenothiazine ribofuranosides (6a-d)



ABTS$^{\bullet +}$ activity (at different time intervals) of 10H-phenothiazine sulfones (7a-d)

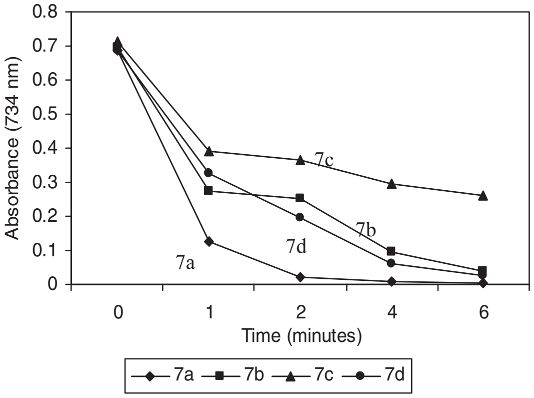


FIGURE 1 The effect of time on the suppression of absorbance of ABTS by synthesized compounds. After addition of 1 ml of diluted ABTS$^{\bullet +}$ solution (A 734 nm = 0.700 ± 0.020) to 10 μl of the compound, the absorbance reading was taken at 30°C exactly 1 minute, after initial mixing (no more than 6 minutes after initial mixing). All determinations were carried out in triplicate.

TABLE 4 Antioxidant activity of synthesized compounds (ABTS$^{\bullet +}$ assay)

	ABTS$^{\bullet +}$ activity at different time intervals (minutes)				
Compd. no.	0 minutes	1 minutes	2 minutes	4 minutes	6 minutes
5a	0.696	0.57	0.323	0.1	0.02
5b	0.699	0.555	0.498	0.463	0.439
5c	0.697	0.335	0.324	0.213	0.16
5d	0.698	0.231	0.176	0.04	0.029
6a	0.699	0.525	0.506	0.465	0.439
6b	0.687	0.106	0.066	0.025	0.017
6c	0.624	0.376	0.162	0.027	0.021
6d	0.713	0.384	0.131	0.031	0.02
7a	0.686	0.124	0.022	0.007	0.006
7b	0.696	0.273	0.253	0.097	0.041
7c	0.711	0.392	0.367	0.294	0.262
7d	0.686	0.328	0.194	0.062	0.024

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.[16] DPPH is stable free radical by virtue of delocalization of electron over the whole molecule. For hydrogen atom transfer, antioxidants quench the free radicals by donating hydrogen, while for single electron transfer, antioxidants transfer one electron to the radical.

A stock solution containing 1 mg/ml of the compound was prepared in methanol. An amount of 50 μl of the solution were added to 5 ml of a 0.004% methanol solution of DPPH. After 30 minutes incubation in the dark at room temperature, the absorbance was read against a blank at 517 nm.

ABTS Radical Cation Decolorization Assay

The 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) radical cation (ABTS$^{\cdot +}$) decolorization test was also used to assess the antioxidant activity of the synthesized compounds. The ABTS $^{\cdot +}$ assay was carried out using the improved assay of Re et al.[17] ABTS $^{\cdot +}$ was generated by oxidation of ABTS with potassium persulphate. For this purpose ABTS was dissolved in deionized water at a concentration of 7mM, and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12–16 hours) in the dark before use; the ABTS$^{\cdot +}$ 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 minutes later. All determinations were carried out in triplicate.

TABLE 5 Antimicrobial activity of synthesized compounds

	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)	
Compd. no.	*E. coli*	*Enterobacter*	*Coagulase negative staphylococci*	*Coagulase positive Staphylococci*	*Candida albicans* (Strain 1)	*Candida albicans* (Strain 2)
5a	12	10	16	10	11	12
5b	10	13	22	11	11	13
5c	—	10	>30	16	>30	>28
5d	11	10	10	—	18	15
6a	10	—	12	—	—	11
6b	—	10	13	—	14	—
6c	—	—	14	—	—	—
6d	—	—	—	—	13	18
7a	12	—	20	12	14	—
7b	—	11	10	—	10	—
7c	10	—	13	—	—	12
7d	—	—	17	15	11	—
Flucanazole	—	—	—	—	25	25
Vancomycin	—	—	15	15	—	—
Gatifloxacin	17	17	—	—	—	—

Note: < 7 mm, inactive; 7–9 mm, weakly active; 10–12 mm, moderately active; >12 mm, active, < 7mm, inactive; 7–11mm, weakly active; 12–17 mm, moderately active; > 17 mm, active.

Antimicrobial Activity

The synthesized compounds were tested for their antibacterial activity by using the Paper Disc method[18] by measuring the zone of inhibition on agar plates with *Escherichia coli, Enterobacter, Coagulase negative Staphylococci, Coagulase positive Staphylococci* as test organisms at concentration of 100 μg per disc using vancomycin, gatifloxacin as standard compounds and antifungal activity against various strains of *Candida albicans* at concentration of 100 μg/disc using flucanazole as standard compound. Results of antimicrobial activity has been shown in Table 5.

CONCLUSION

The structures proposed for the synthesized compounds are well supported by elemental analysis and spectroscopic data. Regarding DPPH activity, compound **5a** showed good activity and compound **6a** and **7a,** which were formed from **5a,** also showed good activity. In fact **7a** is more active than **5a**. Compounds **5a** and **6a** showed almost similar activity. Nucleoside **6b** showed more activity than its phenothiazine base **5b**. Regarding ABTS activity, compounds **7a** and **7d** which were formed from **5a** and **5d,** respectively, are more active. In fact, compounds **5a** and **7a** showed good activity in both DPPH and ABTS assays. Regarding antimicrobial activity compound

6b is slightly more active than **5b** against *Candida albicans* (strain 1) and **6d** is also slightly more active than **5b** against *Candida albicans* (strain 2). These data showed that nucleosides **6b and 6d** are similar in their activity as antifungal agents to their phenothiazine precursors. All these compounds were found to be moderately active against various bacteria and fungi. All the synthesized compounds showed good chemopreventive and antigenotoxic effect.

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