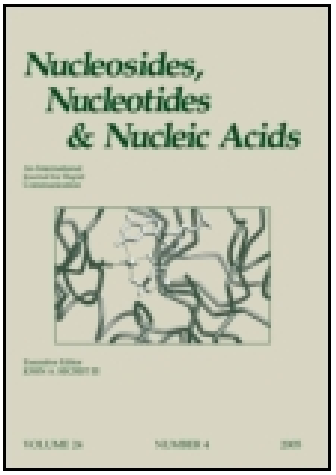

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Study and Synthesis of Biologically Active Phenothiazines, their Sulfones, and Ribofuranosides

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STUDY AND SYNTHESIS OF BIOLOGICALLY ACTIVE PHENOTHIAZINES, THEIR SULFONES, AND RIBOFURANOSIDES

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□ *The present article describes the synthesis of new 10H-phenothiazines using the Smiles rearrangement. These synthesized phenothiazines on oxidation with 30% hydrogen peroxide in glacial acetic acid yield sulfones, and when treated with sugar give ribofuranosides. These compounds are evaluated for their anthelmintic and antimicrobial activities. The structural assignment of the synthesized compounds is made on the basis of elemental analysis and spectroscopic data.*

Keywords Smiles rearrangement; sulfones; ribofuranosides; anthelmintic activities; antimicrobial activities

INTRODUCTION

The study of synthesized, biologically active molecules, such as phenothiazines and their sulfones, has attracted interest in recent years due to their theoretical and structural implications. The diversity of synthetic methods as well as their biological, pharmacological, and industrial significance also makes them important for research. These compounds are of immense importance and possess a wide spectrum of pharmacological activities such as analgesic, antihypertensive, antipsychotic, antibacterial, anti-AIDS., etc. A slight change in substitution pattern may cause significant differences in their biological activity.[1–9] Therefore, these observations encourage us to synthesize new phenothiazines.

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The phenothiazines serve as heterocyclic base for the formation of ribofuranosides for treatment with sugar. On refluxing with hydrogen peroxide in glacial acetic acid, 10H-phenothiazines yielded 10H-phenothiazine-5,5-dioxides. Prepared ribofuranosides and sulfones too possess similar chemotherapeutic activities.[10–15] The synthesized compounds were screened for their antimicrobial and antihelmintic activities. The structures of the synthesized compounds were determined on the basis of their spectral data and elemental analysis.

MATERIALS AND METHODS

All the melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (by broadband proton decoupling technique) were recorded on JEOL AL-300 spectrometer at frequencies of 300.40 MHz and 75.45 MHz, respectively, in Me_2SO-d_6/$CDCl_3$ using Tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. Mass spectra were recorded on WATERS (micromass MS technologies) Q-T by electron spray ionization. ^{19}F NMR spectra were recorded in $CDCl_3$ using CF_3COOH as standard compound. The purity of compounds was checked by thin layer chromatography (TLC) using silica gel "G" as an adsorbent, visualizing these by UV light or in an iodine chamber.

General Procedure for the Synthesis of 2-Amino-2′-nitrodiphenylsulfides (3a–c)

To a solution of 2-amino-6-chloro-3-methoxybenzenethiol **(1a)** or 2-amino-3-chloro-benzenethiol **(1b)** (0.01 mol) in ethanol (20 mL) containing anhydrous sodium acetate (0.01 mol) in a 50-mL round bottom flask, a solution of halonitrobenzene, 2-chloro-3,5-dinitrobenzotrifloride **(2a)** or 2,4-dichloro-5-nitrobenzotrifluoride **(2b)** (0.01 mol) in ethanol (10 mL) 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 obtained was filtered, washed with 30% ethanol, and recrystallized with methanol.

General Procedure for the Synthesis of 2-Formamido-2′-nitrodiphenylsulfides (4a–c)

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

General Procedure for the Synthesis of 10H-Phenothiazines (5a–c)

To the formyl derivative **(4a–c)** (0.01 mol), acetone (15 mL) and an alcoholic solution of potassium hydroxide (0.2 g in 5-mL ethanol) were added and the resulting mixture was heated at 20°C for about 30 minutes. Then a second lot of potassium hydroxide (0.2 g in 5-mL ethanol) was added and refluxing was continued for about 4 hours. The reaction mixture was poured into crushed ice, filtered, washed with minimum amount of cold water and then with ethanol (20 mL), and crystallized from benzene.

6-Chloro-1-trifluoromethyl-9-methoxy-3-nitro-10H-phenothiazine (5a)

Brown solid; m.p.: 165°C; yield: 65%, IR (KBr): υ 3320 (N–H), 1560 and 1370 (–NO_2), 1315 and 1120 (–CF_3), 2960 and 2872 (–OCH_3), 1220 and 1050 (C–O–C), and 780 cm^{-1} (C-Cl); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.74 (s, 1H, N-H), 8.15–6.36 (m, 4H, Ar-H), 3.73 (s, 3H); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 121.8 (C-1), 109.2 (CF_3 at C-1), 119.5 (C-2), 138.8 (C-3), 130.3 (C-4), 129.5 (C-6), 120.2 (C-7), 115.4 (C-8), 150.5 (C-9), 55.8 (OCH_3 at C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.865 (s, 3F, CF3); MS (FAB) 10 kV, m/z (rel. int.): 376 $[M]^+$, 378 $[M+2]^+$, 375 (28), 330 (66), 211 (100), 261 (76), 165 (54); Anal. Calcd. for $C_{14}H_8N_2SO_3ClF_3$: C, 44.68; H, 2.13; N, 7.45; Found: C, 44.73; H, 2.16; N, 7.44.

1,8-Dichloro-7-trifluoromethyl-10H-phenothiazine (5b)

Red solid; m.p.: 162°C; yield: 64%, IR (KBr): υ 3310 (N–H), 1310 and 1130 (–CF_3) and 775 cm^{-1} (C-Cl); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.78 (s, 1H, N-H), 7.78–6.47 (m, 5H, Ar-H); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 123.5 (C-1), 128.5 (C-2), 119.8 (C-3), 130.2 (C-4), 129.8 (C-6), 121.4 (C-7), 109.7 (CF3 at C-7), 130.1 (C-8), 119.5 (C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –61.55 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 336 $[M]^+$, 338 $[M+2]^+$, 335 (28), 265 (65), 181 (100), 232 (56); Anal. Calcd. for $C_{13}H_6NSCl_2F_3$: C, 46.43; H, 1.78; N, 4.17; Found: C, 46.48; H, 1.80; N, 4.16.

9-Chloro-1-trifluoromethyl-3-nitro-10H-phenothiazine (5c)

Brown solid; m.p.: 74°C; yield: 68%, IR (KBr): υ 3340 (N–H), 1345 and 1120 (–CF_3), 1550 and 1315 (–NO_2) and 800 cm^{-1} (C–Cl); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.56 (s, 1H, N–H), 8.11–6.69 (m, 5H, Ar-H); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 121.8 (C-1), 109.3 (CF_3 at C-1), 119.6 (C-2), 138.6 (C-3), 130.1 (C-4), 129.8 (C-6), 120.2 (C-7), 128.0 (C-8), 123.5 (C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –61.745 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 346 $[M]^+$, 348 $[M+2]^+$, 345 (25), 181 (100), 300 (46), 277 (59), 253 (63); Anal. Calcd.

for $C_{13}H_6N_2O_2SCl\,F_3$: C, 45.08; H, 1.73; N, 8.09; Found: C, 45.14; H, 1.72; N, 8.11.

General Procedure for the Synthesis of 10H-phenothiazine-5,5-dioxides (6a–c)

A mixture of substituted 10H-phenothiazines **(5a–c)** (0.01 mol), 20-mL glacial acetic acid, and 30% hydrogen peroxide (5 mL) in round bottom flask was 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, washed with water, and crystallized from ethanol.

6-Chloro-1-trifluoromethyl-9-methoxy-3-nitro-10H-phenothiazine-5,5-dioxide (6a)

Brown solid; m.p.: 220°C; yield: 48%, IR (KBr): υ 3323 (N–H), 1561 and 1377 (–NO_2), 1318 and 1122 (–CF_3), 2960 and 2875 (–OCH_3), and 780 cm^{-1} (C-Cl), 1170 and 1155 (SO_2 sym), and 1070 cm^{-1} (C–S); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.96 (s, 1H, N-H), 8.58–6.51 (m, 4H, Ar-H), 3.76 (s, 3H, OCH_3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 122.3 (C-1), 109.7 (CF_3 at C-1), 126.1 (C-2), 139.0 (C-3), 126.0 (C-4), 125.1 (C-6), 120.3 (C-7), 121.2 (C-8), 150.3 (C-9), 55.9 (OCH_3 at C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.23 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 408 $[M]^+$, 410 $[M+2]^+$, 407 (24), 362 (65), 243 (100), 293 (70), 197 (59); Anal. Calcd. for $C_{14}H_8N_2SO_5ClF_3$: C, 41.17; H, 1.96; N, 6.86; Found: C, 41.23; H, 1.98; N, 6.85.

1,8-Dichloro-7-trifluoromethyl-10H-phenothiazine-5,5-dioxide (6b)

Black solid; m.p.: 211°C; yield: 51%, IR (KBr): υ 3320 (N–H), 1318 and 1121 (–CF_3), 778 (C-Cl), 1180 and 1149 (SO_2 sym), and 1080 cm^{-1} (C–S); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 9.13 (s, 1H, N-H), 8.12–6.73 (m, 5H, Ar-H); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 124.1 (C-1), 134.9 (C-2), 120.5 (C-3), 125.6 (C-4), 125.7 (C-6), 121.9 (C-7), 110.3 (CF_3 at C-7), 136.6 (C-8), 119.7 (C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –61.79 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 368 $[M]^+$, 370 $[M+2]^+$, 367 (27), 333 (46), 298 (50), 213 (100), 264 (74); Anal. Calcd. for $C_{13}H_6NSCl_2O_2F_3$: C, 42.39; H, 1.63; N, 3.80: Found: C, 42.43; H, 1.60; N, 3.85.

9-Chloro-1-trifluoromethyl-3-nitro-10H-phenothiazine-5,5-dioxide (6c)

Brown solid; m.p.: 196°C; yield: 56%, IR (KBr): υ 3345 (N–H), 1344 and 1140 (–CF_3), 1558 and 1321 (–NO_2), 1344 and 1146 (–CF_3), 800 cm^{-1} (C–Cl), 1175 and 1164 (SO_2 sym) and 1095 cm^{-1} (C–S); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.56 (s, 1H, N–H),

8.11–6.69 (m, 5H, Ar-H); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 121.8 (C-1), 109.9 (CF_3 at C-1), 126.8(C-2), 139.6 (C-3), 126.5 (C-4), 124.9 (C-6), 121.1 (C-7), 135.5 (C-8), 124.9 (C-9); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.39 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 378 $[M]^+$, 380 $[M+2]^+$, 377 (29), 213 (100), 332 (46), 309 (64), 285 (66); Anal. Calcd. for $C_{13}H_6N_2O_4SCl\ F_3$: C, 41.26; H, 1.58; N, 7.41; Found: C, 41.34; H, 1.60; N, 7.40.

General Procedure for the Synthesis of Substituted N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl) Phenothiazines (7a–c)

To the solution of **(5a-c)** (0.002 mol) in 20-mL toluene, 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (0.002 mol) was added and the contents were refluxed in vacuum with stirring on an oil bath at 155–160°C for 15 minutes. The vacuum was removed and the 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 1 hour. The viscous mass thus 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 get crystalline ribofuranosides.

N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-6-chloro-1-trifluoromethyl-9-methoxy-3-nitro-10H-phenothiazine (7a)

Red solid; m.p.: 220°C, yield: 81%, IR (KBr): υ 1565 and 1380 (–NO_2), 1320 and 1125 (–CF_3), 2961 and 2872 (–OCH_3) and 1145 cm^{-1} (C–O–C); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ 8.67–6.70 (m, 19H, Ar-H), 3.74 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2 near C-4′ of sugar), 5.52 (s, 1H, H at C-1′ of sugar), 5.22 (s, 1H, H at C-2′ of sugar), 4.85 (s, 1H, H at C-3′ of sugar), 5.01 (s, 1H, H at C-4′ of sugar); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 122.6 (C-1), 109.9 (CF_3 at C-1), 120.5 (C-2), 139.4 (C-3), 130.8 (C-4), 130.0 (C-6), 120.7 (C-7), 114.0 (C-8), 150.5 (C-9), 56.4 (OCH_3 at C-9), 88.4 (C-1′), 74.8 (C-2′), 71.1 (C-3′), 72.3 (C-4′), 65.8 (CH_2 near C-4′ of sugar); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.865 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 820 $[M]^+$, 822 $[M+2]^+$, 751 (41), 774 (69), 375 (58), 210 (100); Anal. Calcd. for $C_{40}H_{28}N_2SO_{10}ClF_3$: C, 58.54; H, 3.4; N, 3.41; Found: C, 58.65; H, 3.40; N, 3.44.

N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1,8-dichloro-7-trifluoromethyl-10H-phenothiazine (7b)

Brown solid; m.p.: 186°C; yield: 76%, IR (KBr): υ 1350 and 1156 (–CF_3) and 1165 cm^{-1} (C–O–C); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): 8.28–7.01 (m, 20H, Ar-H), 4.36 (s, 2H, CH_2 near C-4′

of sugar), 5.55 (s, 1H, H at C-1′ of sugar), 5.18 (s, 1H, H at C-2′ of sugar), 4.88 (s, 1H, H at C-3′ of sugar), 5.06 (s, 1H, H at C-4′ of sugar); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 124.6 (C-1), 129.2 (C-2), 120.7 (C-3), 129.5 (C-4), 131.3 (C-6), 121.9 (C-7), 110.4 (CF_3 at C-7), 130.6 (C-8), 120.4 (C-9), 87.3 (C-1′), 74.7 (C-2′), 71.5 (C-3′), 70.4 (C-4′), 65.6 (CH_2 near C-4′ of sugar); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.18 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 780 $[M]^+$, 782 $[M+2]^+$, 745 (31), 711 (53), 335 (64), 180 (100); Anal. Calcd. for $C_{39}H_{26}NSCl_2O_7F_3$: C, 60; H, 3.33; N, 1.79; Found: C, 60.2; H, 3.3; N, 1.80.

N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-9-chloro-1-trifluoromethyl-3-nitro-10H-phenothiazine (7c)

Red solid; m.p.: 179°C; yield: 85%, IR (KBr): υ 1350 and 1170 (–CF_3), 1580 and 1346 (–NO_2) and 1160 cm^{-1} (C–O–C); ^{1}H NMR spectral data (300.40 MHz, Me_2SO-d_6, δ ppm from TMS): δ s8.28–7.01 (m, 20H, Ar-H), 4.41 (s, 2H, CH_2 near C-4′ of sugar), 5.50 (s, 1H, H at C-1′ of sugar), 5.21 (s, 1H, H at C-2′ of sugar), 4.91 (s, 1H, H at C-3′ of sugar), 4.97 (s, 1H, H at C-4′ of sugar); ^{13}C NMR (75.45 MHz, CDCl3, δ ppm from TMS): 122.6 (C-1), 110.2 (CF_3 at C-1), 119.3 (C-2), 139.4 (C-3), 131.1 (C-4), 130.4 (C-6), 119.4 (C-7), 128.8 (C-8), 124.3 (C-9), 87.6 (C-1′), 74.6 (C-2′), 71.3 (C-3′), 70.8 (C-4′), 65.7 (CH_2 near C-4′ of sugar); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ –62.74 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.) : 790 $[M]^+$, 792 $[M+2]^+$, 744 (68), 721 (56), 345 (71), 180 (100); Anal. Calcd. for $C_{39}H_{26}N_2O_9SCl\,F_3$: C, 59.24; H, 3.29; N, 3.54; Found: C, 59.36; H, 3.28; N, 3.56.

Biological Activity

In Vitro Antibacterial and Antifungal Activity

Antibacterial Activity. Antibacterial activity was tested against *S. aureus, B. subtilis* (gram +ve), and *E. coli, Pseudomonas aeruginosa* (gram –ve) microorganisms using paper disc diffusion method in nutrient agar medium. The paper disc diffusion method of assay of drug potency is based on the measurement of the zone of microbial growth inhibition surrounding discs containing various concentrations of test compounds, which are placed on the surface of a solid nutrient previously inoculated with the culture of suitable microorganism. Inhibition produced by the test drug is compared with that produced by known concentration of reference standard.

In this method, paper disc impregnated with compounds dissolved in solvent DMF at concentrations of 25, 50, and 100 μg mL^{-1}. Then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 hours for bacterial cultures. After incubation, the zones of inhibition around the disc were observed. Each testing is done in triplicate. Ciprofloxacin at a concentration of 50 μg mL^{-1} was used as standard drug for antibacterial

activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The % Activity Index for the complex was calculated by the following formula:

$$\%\ \text{Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100.$$

Antifungal Activity. Antifungal activity of synthesized compounds was tested on fungal strains: *A. niger*, *C. albican* using the disc diffusion method. In the disc-diffusion method, disc impregnated with compounds dissolved in solvent DMF at concentrations of 25, 50, and 100 μg mL^{-1} was spread over microorganism culture in nutrient agar medium. The plates were incubated at 25°C for 48 hours for fungal strains. After incubation, the growth inhibiting zones around the disc were observed. Growth inhibiting zone indicates that the compounds inhibit growth of microorganism. Each experiment is done in triplicate. Griseofulvin at a concentration of 50 μg mL^{-1} was used as standard drug for antifungal activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The percentage inhibition was calculated by the following equation:

$$\%\ \text{Inhibition} = (\text{C} - \text{T})\ 100/\text{C},$$

where C and T are the diameters of the fungal colony in the control and the test plates respectively.

Minimal Inhibitory Concentrations. Minimum inhibitory concentrations (MIC) are defined as the lowest concentration of antimicrobials that inhibit the visible growth of a microorganism after overnight incubation at 37°C. Determination of the MIC is a semi-quantitative test, which gives an approximate idea of the least concentration of an antimicrobial (test) solution needed to parent microbial growth. The MIC was determined by the liquid dilution method. Two gram +ve bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram –ve bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were used as quality control strains. For the antifungal activities of the compounds *Candida albicans* and *Aspergilius niger* were tested.

Ciprofloxacin and Griseofulvin were used as standard antibacterial and antifungal agents respectively. The stock solutions of test compounds with 1 to 20-μg mL^{-1} concentrations were prepared with aqueous methanol. Inoculums of the overnight culture were prepared. In a series of tubes, 1 mL each of stock solutions of test compound with different concentrations was taken and 0.4 mL of the inoculums was added to each tube. Further, 4.0 mL of sterile water was added to each of the test tubes. These test tubes were incubated for 22–24 hours and observed for the presence of turbidity. The absorbance of the suspension of the inoculums was observed with spectrophotometer at

555 nm. The end result of the test was the minimum concentration of antimicrobial (test) solutions, which gave clear solution, i.e., no visual growth.

Anthelmintic Activity

Anthelmintic studies were carried out against the Eudrilus species of earthworm by the Garg and Atal method[16] at 4-μg mL^{-1} concentration. Suspensions of samples were prepared by triturating synthesized compounds (200 mg) with Tween 80 (0.5%) and distilled water, and the resulting mixtures were stirred for 30 min using a mechanical stirrer. The suspensions were diluted to contain 0.4% w/v of test samples. Suspension of reference drug mebendazole was prepared with same concentration in a similar way. Three sets of five earthworms of almost similar sizes (3 inch in length) were placed in petri plates of 4-inch diameter containing 50 mL of suspension of test sample and reference drug at room temperature. Another set of five earthworms was kept as control in 50-mL suspension of distilled water and Tween 80 (0.5%). The paralyzing and death times (in minutes) were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50°C), which stimulated movement if the worms were alive.

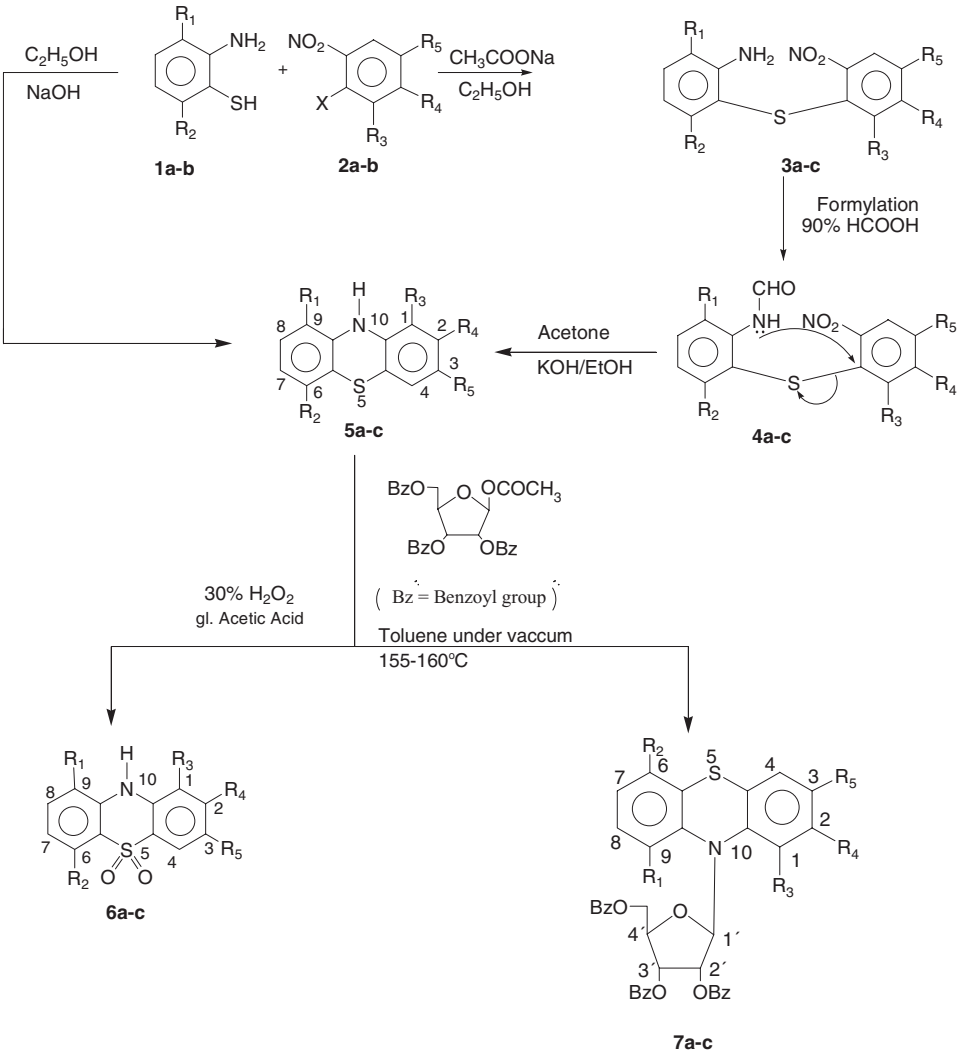
RESULTS AND DISCUSSION

The synthesis of various substituted 10H-phenothiazines **(5a–c)** has been carried out by the Smiles rearrangement of 2-formamido-2′-nitrodiphenylsulfides **(4a–c)**. The formyl derivatives were synthesized by the formylation of 2-amino-2′-nitrodiphenylsulfides **(3a–c)**, which in turn were prepared by condensation of 2-amino-6-chloro-3-methoxybenzenethiol **(1a)** with 2-chloro-3,5-dinitrobenzotrifloride **(2b)** and 2-amino-3-chlorobenzenethiol **(1b)** with o-halonitrobenzene [2,4-dichloro-5-nitrobenzotrifluoride **(2a)** and 2-chloro-3,5-dinitrobenzotrifluoride **(2b)**] in ethanolic sodium acetate solution. Compounds **(5a–c)** on refluxing with 30% hydrogen peroxide in glacial acetic acid were converted into their corresponding sulfones **(6a–c)**. Treatment of the mixture of **(5a–c)** in toluene with 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in vacuum gave the corresponding ribofuranosides **(7a–c)** (Scheme 1).

The structures proposed for the synthesized compounds were well supported by elemental analysis and spectral data. The synthesized compounds were also screened for antimicrobial and anthelmintic activities.

Antimicrobial Activity

A series of novel heterocyclic compounds **(3a–d)** and **(4a–d)** were synthesized. All the compounds were tested against for their *in vitro* antibacterial activity against the four strains of bacteria (two gram –ve, *E. coli* and



Scheme--1

Compound	R_1	R_2	R_3	R_4	R_5
5a, 6a, 7a	OCH_3	Cl	CF_3	H	NO_2
5b, 6b, 7b	Cl	H	H	Cl	CF_3
5c, 6c, 7c					

SCHEME 1

TABLE 1 Minimum inhibitory concentrations (μg mL^{-1}) of synthesized compounds

	Minimal inhibitory concentrations (μgm mL^{-1}) of bacterial strains			
Cpd. No.	*E. coli*	*B. subtilis*	*P. aeruginosa*	*S. aureus*
5a	11.42 ± 0.25	13.30 ± 0.14	13.34 ± 0.32	11.20 ± 0.29
5b	12.30 ± 0.17	14.20 ± 0.18	15.20 ± 0.27	11.82 ± 0.33
5c	11.10 ± 0.11	13.10 ± 0.20	11.40 ± 0.17	10.94 ± 0.29
6a	09.60 ± 0.18	11.25 ± 0.33	11.24 ± 0.18	10.39 ± 0.23
6b	11.00 ± 0.37	11.90 ± 0.19	12.05 ± 0.26	11.16 ± 0.32
6c	09.20 ± 0.09	09.00 ± 0.12	10.10 ± 0.35	09.80 ± 0.81
7a	10.80 ± 0.22	11.96 ± 0.23	11.60 ± 0.10	10.85 ± 0.23
7b	11.36 ± 0.08	12.30 ± 0.31	12.64 ± 0.33	11.54 ± 0.31
7c	10.26 ± 0.32	10.11 ± 0.22	10.55 ± 0.19	10.41 ± 0.28
Ciprofloxacin	04.10 ± 0.10	04.90 ± 0.13	03.85 ± 0.15	04.90 ± 0.11

Pseudomonas aeruginosa, and two gram +ve, *Bacillus subtilis* and *Staphylococcus aureus*) and antifungal activity against the two strains of fungi (*C. albicans* and *A. niger*). The antibacterial and antifungal activities increase with an increase in the concentration of test compounds. The MIC of the compounds varies from 9.00–15.20 μg mL^{-1} (Tables 1 and 2). Compounds **(6a)**, **(6c)**, **(7a),** and **(7c)** show good activity and the remaining compounds show moderate activity against bacteria *E. coli*. Compounds **(6c)** and **(7c)** show good activity and the remaining compounds show moderate activity against bacteria *B. subtilis*. For bacteria *P. aeruginosa*, compound **(6c)** shows good activity and the remaining compounds show moderate activity. Compounds **(6a)** and **(7c)** show good activity and the remaining compounds show moderate activity against bacteria *S. aureus*. Compounds **(6c)** and **(7c)** show good activity against fungi *C. albicans* and *A. niger* and the remaining compounds show moderate activity.

TABLE 2 Minimum inhibitory concentration (MIC; μg mL^{-1}) of synthesized compounds

	MIC (μgm mL^{-1}) of fungal strains	
Cpd. No.	*C. albicans*	*A. niger*
5a	12.25 ± 0.17	11.99 ± 0.17
5b	12.57 ± 0.20	13.00 ± 0.25
5c	11.00 ± 0.21	11.25 ± 0.21
6a	11.47 ± 0.25	11.34 ± 0.24
6b	11.79 ± 0.31	11.71 ± 0.19
6c	10.50 ± 0.19	10.54 ± 0.35
7a	11.96 ± 0.33	11.68 ± 0.32
7b	12.05 ± 0.29	12.08 ± 0.34
7c	10.90 ± 0.28	10.90 ± 0.22
Griseofulvin	03.10 ± 0.80	04.80 ± 0.10

TABLE 3 Anthelmintic activity of synthesized compounds

	Paralyzing time (in minutes)			Death time (in minutes)		
Cpd. No.	200 mg mL^{-1}	100 mg mL^{-1}	50 mg mL^{-1}	200 mg mL	100 mg mL^{-1}	50 mg mL^{-1}
5a	14.83 ± 0.83	17.33 ± 1.52	23.36 ± 1.00	19.31 ± 1.00	25.16 ± 0.76	35.23 ± 0.91
5b	15.00 ± 1.09	18.69 ± 0.79	25.00 ± 0.70	20.16 ± 1.22	27.16 ± 0.76	40.21 ± 0.25
5c	13.26 ± 1.08	16.50 ± 1.20	21.55 ± 1.00	17.33 ± 1.52	22.20 ± 1.00	34.50 ± 0.50
6a	13.50 ± 1.00	16.66 ± 0.94	22.50 ± 0.50	18.16 ± 0.76	24.65 ± 0.79	35.23 ± 0.91
6b	15.00 ± 0.77	18.00 ± 0.78	23.45 ± 0.93	20.16 ± 0.90	25.88 ± 1.00	37.53 ± 0.55
6c	12.00 ± 1.00	15.00 ± 1.00	20.03 ± 0.68	15.40 ± 0.77	21.11 ± 0.77	32.22 ± 0.63
7a	14.00 ± 1.10	17.00 ± 1.00	22.20 ± 1.00	19.12 ± 0.60	24.13 ± 1.09	35.50 ± 0.50
7b	15.16 ± 1.24	18.55 ± 1.05	24.56 ± 0.70	19.50 ± 0.50	26.06 ± 0.76	38.43 ± 1.00
7c	13.16 ± 0.75	16.00 ± 1.10	21.00 ± 1.00	15.53 ± 0.69	24.13 ± 1.09	34.12 ± 0.60
Mebendazole	12.50 ± 1.04	16.00 ± 0.98	21.80 ± 1.34	15.26 ± 0.84	22.65 ± 0.90	30.55 ± 1.00

Anthelmintic Activity

The above-screened compounds were tested for anthelmintic activity. The results have been summarized in Table 3. The results show that compounds **(5c)**, **(6a)**, **(7a),** and **(7c)** have longer paralyzing periods than the standard drug mebendazole at given concentrations. The synthesized compounds have somewhat better activity against the Eudrilus species of earthworm than does mebendazole.

CONCLUSION

The structures proposed for the synthesized compounds were well supported by elemental analysis and spectroscopic data. The MIC values of antibacterial and antifungal screening show that the compounds bearing electron withdrawing groups, such as nitro and trifluoromethyl, exhibit excellent antibacterial and antifungal activities against all the four strains of bacteria and fungi respectively. In general, the presence of electron withdrawing group on the aromatic ring increases the antimicrobial activity of tested compounds compared with compounds having electron-donating groups. The literature shows that the compounds having methoxy, nitro groups represent good anthelmintic activity. Thus, the synthesized compounds show significant anthelmintic activity.

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