Synthesis and evaluation of antimicrobial and anthelmintic activity of novel fluorinated 7-ethyl-10*H*-phenothiazines, their sulphones and ribofuranosides

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Abstract. A series of novel fluorinated 10*H*-phenothiazines were synthesized via Smiles rearrangement. 10*H*-phenothiazines on refluxing with 30% hydrogen peroxide in glacial acetic acid gave 10*H*-phenothiazines-5, 5-dioxides (sulphones). These synthesized 10*H*-phenothiazines were then used as base to prepare ribofuranosides by treating them with sugar (β -D-ribofuranosyl- 1-acetate-2, 3, 5-tribenzoate). The synthesized compounds were screened for antimicrobial activity and anthelmintic activity. The structural assignments of compounds were made on the basis of spectroscopic data and elemental analysis.

Keywords. 10*H*-phenothiazines; Smiles rearrangement; sulphones; ribofuranosides; antimicrobial activity; anthelmintic activity.

1. Introduction

A large number of publications and patents registered worldwide gave much emphasis on the synthesis of 10*H*-phenothiazine derivatives.^{1–7} They possess wide range of biological, medicinal and industrial applications. These moieties are widely used as antiviral, anti-malarial, antiinflammatory, anticancer, antituberculosis, central nervous system (CNS) activity. The folding along nitrogen and sulphur axis in 10*H*-phenothiazine is a significant factor for biological activities.^{8–14} It has stimulated our interest to convert phenothiazines to sulphones, to understand oxidation behaviour of 10*H*-phenothiazine and to investigate changes in infra-red and nuclear magnetic resonance spectra caused by the conversion of sulphide linkage to sulphones. Besides this, ribofuranosides of 10*H*-phenothiazines have also been reported to possess biological activities. In the preparation of ribofuranosides, ribosylation takes place mostly by replacing hydrogen atom attached to nitrogen in phenothiazines.

2. Experimental

By thin-layer chromatography purity of compounds was checked using silica gel 'G' as adsorbent, visualizing these by ultraviolet light or in an iodine chamber. All the melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. Mass spectra were recorded on WATERS (micro mass MS technologies) Q-T by electron spray ionization. ¹H NMR and ¹³C NMR spectra were recorded on JEOL AL 300 spectrometer at frequencies of 300.40 MHz and 75.45 MHz, respectively in Me₂SO-*d*₆/CDCl₃ using TMS (tetramethyl silane) as an internal standard. ¹⁹F NMR spectra were recorded in CDCl₃ using CF₃COOH as standard compound.

2.1 General procedure for the synthesis of 1-nitro-10*H*-phenothiazines (**5a**)

4-Chloro-3, 5-dinitrobenzotrifluoride (**2a**) was added to a stirred suspension of 2-amino-5-ethylbenzenethiol (**1a**) (0.01 mol), NaOH (0.01 mol) and 20 mL absolute alcohol in round bottom flask. A reflux condenser was fitted in the round bottom flask. The reaction contents were refluxed for 2 h then concentrated, cooled and filtered. The precipitate was washed well with hot H₂O and 20% EtOH and crystallized from Me₂CO.

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2.1a 7-Ethyl-3-trifluoromethyl-1-nitro-10H-phenothiazine (5a): Orange solid; m.p. : 78°C–80°C, Yield : 64% ; IR (KBr) : ν 3360 (N–H), 1540 and 1320 (–NO₂ str.), 1330 and 1150 (–CF₃), 1075 (C–S); ¹H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.46 (s, 1H, N–H), 7.29–6.34 (m, 5H, Ar–H), 2.72 (q, 2H, ³J_{HH} = 7.5 Hz, CH₂ of C₂H₅ at C₇), 1.42 (t, 3H, ³J_{HH} = 7.0 Hz, CH₃ of C₂H₅ at C₇); ¹³C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 138.70 (C-1), 118.0 (C-2), 121.9 (C-3), 130.6 (C-4), 128.8 (C-6), 129.0 (C-7), 127.0 (C-8), 119.1 (C-9), 120.0 (C-4a), 118.9 (C-5a), 139.4 (C-9a), 144.0 (C-10a), 31.9 (CH₂ of C₂H₅ at C₇), 14.4 (CH₃ of C₂H₅ at C₇), 122.1 (CF₃ at C₃, q, ¹J_{CF} = 271.00 Hz); ¹⁹F NMR (282.65 MHz, CDCl₃) : δ –62.01 (s, 3F, CF₃); MS (FAB) 10 kV, *m/z* (rel. int.) : 340 (M⁺) (100), 323 [M–OH]⁺ (60), 310 [M–NO]⁺ (65), 294 [M–NO₂]⁺ (40), 293 [M–HNO₂]⁺ (50) etc.; Anal. Calcd for C₁₅H₁₁N₂O₂F₃S: C, 52.94; H, 3.23; N, 8.23. Found : C, 52.64; H, 3.15; N, 8.75.

2.2 General procedure for the synthesis of 2-amino-2'-nitrodiphenylsulfides (3b–d)

In a 50 mL round bottom flask, 2-amino-5-ethylbenzenethiol (**1a**) (0.01 mol), which was dissolved in 20 mL EtOH, was added to anhydrous NaOAc (0.01 mol) and halonitrobenzenes (**2b–d**) (0.01 mol) (dissolved in 10 mL EtOH). The reaction mixture was refluxed for 4–5 h, concentrated in an ice chamber overnight. The precipitate separated out was filtered, washed with 30% EtOH and recrystallised from MeOH.

2.3 General procedure for the synthesis of 2-formamido-2'-nitrodiphenylsulfides (4b–d)

The 2-amino-2'-nitrodiphenylsulfides (**3b–d**) (0.01 mol) obtained was refluxed for 4 h in 90% HCOOH (20 mL) in a round bottom flask. The contents were then poured into a beaker containing crushed ice and a solid that separated out was filtered, washed with H₂O until the filtrate was neutralized and recrystallised from C₆H₆.

2.4 General procedure for the synthesis of 10H-phenothiazines (5b–d)

10H-phenothiazines¹⁵ synthesized by formyl derivatives via Smiles rearrangement. Formyl derivatives (**4b–d**) (0.01 mol) in Me₂CO (15 mL) were added to alcoholic solution of KOH (0.2 g in 5 mL EtOH). The contents were refluxed for 30 min. To this a second lot of KOH (0.2 g in 5 mL EtOH) was added and refluxed

for 4 h. The contents were then poured into a crushed ice containing beaker. The solid separated out was filtered, washed with cold H₂O and finally with 30% EtOH and then crystallized from C₆H₆.

2.4a 7-Ethyl-1-trifluoromethyl-3-nitro-10H-phenothiazine (5b): Dark orange solid; m.p. : 85°C–87°C, Yield : 87% ; IR (KBr) : ν 3400 (N–H), 1580, 1370 (–NO₂ str.), 1370 and 1190 (–CF₃), 1073 (C–S) and; ¹H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.25 (s, 1H, N–H), 7.36–6.81 (m, 5H, Ar–H), 2.60 (q, 2H, ³J_{HH} = 7.4 Hz, CH₂ of C₂H₅ at C₇), 1.29 (t, 3H, ³J_{HH} = 6.8 Hz, CH₃ of C₂H₅ at C₇); ¹³C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 138.50 (C-1), 118.8 (C-2), 122.1 (C-3), 131.2 (C-4), 127.9 (C-6), 128.6 (C-7), 127.1 (C-8), 118.5 (C-9), 120.3 (C-4a), 118.2 (C-5a), 139.0 (C-9a), 148.0 (C-10a), 31.0 (CH₂ of C₂H₅ at C₇), 14.7 (CH₃ of C₂H₅ at C₇), 122.8 (CF₃ at C₁, q, ¹J_{CF} = 270.80 Hz); ¹⁹F NMR (282.65 MHz, CDCl₃) : δ –61.99 (s, 3F, CF₃); MS (FAB) 10 kV, *m/z* (rel. int.) : 340 (M⁺) (100), 339 [M–H]⁺ (68), 311 [M–CH₂CH₃]⁺ (50), 175 [M–C₅H₂NO₂F₃]⁺ (40) etc.; Anal. Calcd for C₁₅H₁₁N₂O₂F₃S: C, 52.94; H, 3.23; N, 8.23. Found : C, 53.12; H, 3.30; N, 8.35.

2.4b 7-Ethyl-3-fluoro-10H-phenothiazine (5c): Brown solid; m.p. : 87°C–90°C, Yield : 55% ; IR (KBr) : ν 3330 (N–H), 1078 (C–S) and 1105 (C–F str.); ¹H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.27 (s, 1H, N–H), 7.87–6.53 (m, 6H, Ar–H), 2.59 (q, 2H, ³J_{HH} = 7.5 Hz, CH₂ of C₂H₅ at C₇), 1.48 (t, 3H, ³J_{HH} = 6.9 Hz, CH₃ of C₂H₅ at C₇); ¹³C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 121.40 (C-1), 114.2 (C-2), 152.5 (C-3, d, ¹J_{CF} = 249.4 Hz), 117.6 (C-4), 128.3 (C-6), 126.7 (C-7), 127.2 (C-8), 119.9 (C-9), 120.5 (C-4a), 118.6 (C-5a), 138.8 (C-9a), 138.2 (C-10a), 30.8 (CH₂ of C₂H₅ at C₇), 14.0 (CH₃ of C₂H₅ at C₇); ¹⁹F NMR (282.65 MHz, CDCl₃) : δ –109.50 (s, 1F, C–F); MS (FAB) 10 kV, *m/z* (rel. int.) : 245 (M⁺) (100), 244 [M–H]⁺ (40), 216 [M–CH₂CH₃]⁺ (70), 175 [M–C₄H₃F]⁺ (50) etc.; Anal. Calcd for C₁₄H₁₂NFS: C, 68.57; H, 4.89; N, 5.71. Found : C, 68.33; H, 4.90; N, 5.67.

2.4c 2-Chloro-7-ethyl-3-trifluoromethyl-10H-phenothiazine (5d): Red solid; m.p. : 90°C–93°C, Yield : 79% ; IR (KBr) : ν 3190 (N–H), 1300 and 1160 (–CF₃), 1080 (C–S) and 810 cm^{–1} (C–Cl); ¹H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from

TMS) : δ 8.42 (s, 1H, N-H), 7.26–6.73 (m, 5H, Ar-H), 2.53 (q, 2H, $^3J_{HH} = 7.4$ Hz, CH₂ of C₂H₅ at C₇), 1.25 (t, 3H, $^3J_{HH} = 6.8$ Hz, CH₃ of C₂H₅ at C₇) ; ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 119.20 (C-1), 128.9 (C-2), 118.5 (C-3), 126.9 (C-4), 129.8 (C-6), 127.8 (C-7), 126.4 (C-8), 119.4 (C-9), 117.4 (C-4a), 118.4 (C-5a), 140.0 (C-9a), 146.9 (C-10a), 31.6 (CH₂ of C₂H₅ at C₇), 14.4 (CH₃ of C₂H₅ at C₇), 115.1 (CF₃ at C₃, q, $^1J_{CF} = 270.00$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -62.32 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 329 (M⁺) (100), 331 (M+2) (32), 328 [M-H]⁺ (45), 300 [M-CH₂CH₃]⁺ (60), 175 [M-C₅H₂F₃Cl]⁺ (50) etc; Anal. Calcd for C₁₅H₁₁NCIF₃S : C, 54.62; H, 3.34; N, 4.24. Found : C, 54.85; H, 3.41; N, 4.32.

2.5 General procedure for the synthesis of 10H-phenothiazines-5,5-dioxides (sulphones) (6a–d)

In round bottom flask a mixture of substituted phenothiazines (5a–d) (0.01 mol), glacial HOAc (20 mL) and 5 mL of 30% H₂O₂ were refluxed for 15 min at 50–60°C. Heating was stopped and another lot of H₂O₂ (5 mL) was added. The reaction mixture was again refluxed for 4 h. The reaction contents were then poured into a beaker containing crushed ice. The residue obtained was filtered, washed with H₂O and crystallized from EtOH.

2.5a 7-Ethyl -3-trifluoromethyl -1-nitro-10H-phenothiazine-5,5-dioxide (6a): Dark yellow solid; m.p. : 90°C ; Yield : 67%, IR (KBr) : ν 3390 (N-H), 1550 and 1340 (-NO₂ str.), 1320 and 1170 (-CF₃), 1180 and 1160 (SO₂ sym), 585 and 567 (SO₂ bend), 1340, 1301 and 1265 (SO₂ asym), and 1085 cm⁻¹ (C-S); ^1H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.96 (s, 1H, N-H), 7.49–6.60 (m, 5H, Ar-H), 2.88 (q, 2H, $^3J_{HH} = 7.5$ Hz, CH₂ of C₂H₅ at C₇), 1.61 (t, 3H, $^3J_{HH} = 7.0$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 138.9 (C-1), 125.9 (C-2), 122.6 (C-3), 130.7 (C-4), 127.7 (C-6), 130.1 (C-7), 133.4 (C-8), 120.3 (C-9), 124.4 (C-4a), 123.8 (C-5a), 138.8 (C-9a), 140.0 (C-10a), 33.9 (CH₂ of C₂H₅ at C₇), 14.6 (CH₃ of C₂H₅ at C₇), 114.5 (CF₃ at C₃, q, $^1J_{CF} = 270.70$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -62.26 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 372 (M⁺) (100), 355 [M-OH]⁺ (60), 342 [M-NO]⁺ (48), 326 [M-NO₂]⁺ (72), 325 [M-HNO₂]⁺ (51), 308 [M-SO₂]⁺ (47) etc; Anal. Calcd for C₁₅H₁₁N₂O₄F₃S : C, 48.38; H, 2.95; N, 7.52, Found : C, 48.52; H, 2.99; N, 7.45.

2.5b 7-Ethyl -1-trifluoromethyl -3-nitro-10H-phenothiazine -5,5-dioxide (6b): Brown solid; m.p. : 220°C, yield : 59%, IR (KBr) : ν 3410 (N-H), 1570 and 1390 (-NO₂ str.), 1390 and 1195 (-CF₃), 1175 and 1158 (SO₂ sym), 588 and 570 (SO₂ bend), 1344, 1300 and 1260 (SO₂ asym), and 1085 cm⁻¹ (C-S); ^1H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.50 (s, 1H, N-H), 7.56–6.90 (m, 5H, Ar-H), 2.90 (q, 2H, $^3J_{HH} = 7.4$ Hz, CH₂ of C₂H₅ at C₇), 1.30 (t, 3H, $^3J_{HH} = 6.8$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 139.0 (C-1), 126.3 (C-2), 122.3 (C-3), 130.9 (C-4), 127.5 (C-6), 130.8 (C-7), 133.7 (C-8), 120.4 (C-9), 125.2 (C-4a), 123.9 (C-5a), 138.3 (C-9a), 144.0 (C-10a), 32.1 (CH₂ of C₂H₅ at C₇), 14.5 (CH₃ of C₂H₅ at C₇), 123.1 (CF₃ at C₁, q, $^1J_{CF} = 270.50$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -61.96 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 372 (M⁺) (100), 371 [M-H]⁺ (48), 308 [M-SO₂]⁺ (56), 281 [M-SO₂-HCN]⁺ (40) etc; Anal. Calcd for C₁₅H₁₁N₂O₄F₃S : C, 48.38; H, 2.95; N, 7.52, Found : C, 48.60; H, 3.01; N, 7.66.

2.5c 7-Ethyl-3-fluoro-10H-phenothiazine-5,5-dioxide (6c): Brown solid; m.p. : 190°C, yield : 59%, IR (KBr) : ν 3340 (N-H), 1115 (C-F str.), 1170 and 1144 (SO₂ sym), 584 and 568 (SO₂ bend), 1341, 1290 and 1260 (SO₂ asym), and 1078 cm⁻¹ (C-S); ^1H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 8.63 (s, 1H, N-H), 8.03–6.78 (m, 6H, Ar-H), 2.91 (q, 2H, $^3J_{HH} = 7.5$ Hz, CH₂ of C₂H₅ at C₇), 1.50 (t, 3H, $^3J_{HH} = 6.9$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 121.2 (C-1), 121.0 (C-2), 153.5 (C-3, d, $^1J_{CF} = 249.1$ Hz), 115.7 (C-4), 127.8 (C-6), 130.7 (C-7), 133.1 (C-8), 120.4 (C-9), 125.5 (C-4a), 123.5 (C-5a), 138.1 (C-9a), 137.0 (C-10a), 32.6 (CH₂ of C₂H₅ at C₇), 14.4 (CH₃ of C₂H₅ at C₇); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -109.34 (s, 1F, C-F); MS (FAB) 10 kV, m/z (rel. int.) : 277 (M⁺) (100), 276 [M-H]⁺ (55), 213 [M-SO₂]⁺ (35), 186 [M-SO₂-HCN]⁺ (50) etc; Anal. Calcd for C₁₄H₁₂NO₂FS : C, 60.64; H, 4.33; N, 5.05, Found : C, 60.96; H, 4.42; N, 4.96.

2.5d 2-Chloro-7-ethyl-3-trifluoromethyl-10H-phenothiazine-5,5-dioxide (6d): Dark red solid; m.p. : 150°C ; Yield : 62%, IR (KBr) : ν 3210 (N-H), 1310 and 1180 (-CF₃), and 830 cm⁻¹ (C-Cl), 1180 and 1155 (SO₂ sym), 575 and 553 (SO₂ bend), 1339, 1288 and 1265 (SO₂ asym), and 1090 cm⁻¹ (C-S); ^1H NMR spectral data (300.40 MHz, Me₂SO-d₆, δ ppm from TMS) : δ 9.10 (s, 1H, N-H), 7.30–6.90 (m, 5H, Ar-H), 2.80 (q, 2H, $^3J_{HH} = 7.4$ Hz, CH₂ of C₂H₅ at C₇), 1.40 (t,

3H, $^3J_{HH} = 6.8$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 120.80 (C-1), 136.1 (C-2), 119.5 (C-3), 126.1 (C-4), 128.3 (C-6), 130.4 (C-7), 133.2 (C-8), 120.0 (C-9), 122.6 (C-4a), 123.6 (C-5a), 138.2 (C-9a), 146.0 (C-10a), 32.6 (CH₂ of C₂H₅ at C₇), 14.9 (CH₃ of C₂H₅ at C₇), 115.6 (CF₃ at C₃, q, $^1J_{CF} = 270.30$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -62.13 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 361 (M⁺) (100), 363 (M+2) (55), 360 [M-H]⁺ (48), 297 [M-SO₂]⁺ (35), 270 [M-SO₂-HCN]⁺ (55) etc; Anal. Calcd for C₁₅H₁₁NO₂ClF₃S : C, 49.79; H, 3.04; N, 3.87. Found : C, 49.90; H, 3.10; N, 3.93.

2.6 Synthesis of substituted

N-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl) phenothiazines (**7a-d**)

In 20 mL C₆H₅CH₃, β -D-ribofuranose-1-acetate-2, 3, 5-tribenzoate (0.002 mol) was added to the solution of substituted 10*H*-phenothiazines (**5a-d**) (0.002 mol) and the contents were refluxed in vacuum with stirring on an oil bath at 155–160°C for 15 min. Further, removing vacuum the reaction was protected from moisture by fitting a guard tube. Stirring was further continued for 10 h and after every 1 h; a vacuum was applied for 10 min. In CH₃OH, obtained viscous mass was dissolved and boiled for 10 min. and cooled to room temperature. The reaction mixture was filtered and by distillation the CH₃OH was removed, under reduced pressure. In Et₂O, obtained viscous residue was dissolved. The mixture was filtered, concentrated and kept in refrigerator overnight to get crystalline ribofuranosides.

2.6a *N*-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-7-ethyl-3-trifluoromethyl-1-nitro-10*H*-phenothiazine (**7a**): Brown solid; m.p. : 80°C–85°C, Yield : 70% ; IR (KBr) : ν 1500 and 1360 (-NO₂ str.), 1380 and 1190 (-CF₃), 1150 (C-O-C); ^1H NMR spectral data (300.40 MHz, Me₂SO-*d*₆, δ ppm from TMS) : δ 8.21–6.94 (m, 5H, Ar-H), 2.79 (q, 2H, $^3J_{HH} = 7.5$ Hz, CH₂ of C₂H₅ at C₇), 1.36 (t, 3H, $^3J_{HH} = 7.0$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 139.00 (C-1), 118.4 (C-2), 122.0 (C-3), 131.5 (C-4), 129.0 (C-6), 128.9 (C-7), 128.4 (C-8), 119.0 (C-9), 120.5 (C-4a), 119.0 (C-5a), 139.3 (C-9a), 143.8 (C-10a), 32.1 (CH₂ of C₂H₅ at C₇), 14.7 (CH₃ of C₂H₅ at C₇), 80.2 (C-1'), 74.1 (C-2'), 70.6 (C-3'), 69.5 (C-4'), 121.7 (CF₃ at C₃, q, $^1J_{CF} = 271.10$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -62.37 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 785 (M⁺) (100), 768

[M-OH]⁺ (80), 755 [M-NO]⁺ (40), 739 [M-NO₂]⁺ (40), 738 [M-HNO₂]⁺ (55) etc.; Anal. Calcd for C₄₁H₃₁N₂O₉F₃S : C, 62.75; H, 3.94; N, 3.56. Found : C, 62.98; H, 4.01; N, 3.64.

2.6b *N*-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-7-ethyl-1-trifluoromethyl-3-nitro-10*H*-phenothiazine (**7b**): Red solid; m.p. : 98°C–101°C, Yield : 77% ; IR (KBr) : ν 1590 and 1350 (-NO₂ str.), 1340 and 1160 (-CF₃), 1130 (C-O-C); ^1H NMR spectral data (300.40 MHz, Me₂SO-*d*₆, δ ppm from TMS) : δ 8.06–7.18 (m, 5H, Ar-H), 2.72 (q, 2H, $^3J_{HH} = 7.4$ Hz, CH₂ of C₂H₅ at C₇), 1.17 (t, 3H, $^3J_{HH} = 6.8$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 138.40 (C-1), 119.2 (C-2), 122.5 (C-3), 130.7 (C-4), 128.0 (C-6), 128.2 (C-7), 126.9 (C-8), 118.0 (C-9), 120.7 (C-4a), 118.6 (C-5a), 139.9 (C-9a), 148.0 (C-10a), 31.4 (CH₂ of C₂H₅ at C₇), 15.0 (CH₃ of C₂H₅ at C₇), 81.4 (C-1'), 75.5 (C-2'), 72.2 (C-3'), 70.3 (C-4'), 123.1 (CF₃ at C₁, q, $^1J_{CF} = 270.90$ Hz); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -62.21 (s, 3F, CF₃); MS (FAB) 10 kV, m/z (rel. int.) : 785 (M⁺) (100), 756 [M-CH₂CH₃]⁺ (60), 620 [M-C₅H₂NO₂F₃]⁺ (100) etc; Anal. Calcd for C₄₁H₃₁N₂O₉F₃S : C, 62.75; H, 3.94; N, 3.56. Found : C, 62.93; H, 3.99; N, 3.65.

2.6c *N*-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-7-ethyl-3-fluoro-10*H*-phenothiazine (**7c**): Dark red solid; m.p. : 120°C–125°C, Yield : 34% ; IR (KBr) : ν 1270 (C-F str.), 1125 (C-O-C); ^1H NMR spectral data (300.40 MHz, Me₂SO-*d*₆, δ ppm from TMS) : δ 8.02–6.74 (m, 5H, Ar-H), 2.54 (q, 2H, $^3J_{HH} = 7.3$ Hz, CH₂ of C₂H₅ at C₇), 1.32 (t, 3H, $^3J_{HH} = 6.7$ Hz, CH₃ of C₂H₅ at C₇); ^{13}C NMR (75.45 MHz, CDCl₃, δ ppm from TMS) : δ 122.00 (C-1), 114.8 (C-2), 153.0 (C-3, d, $^1J_{CF} = 249.4$ Hz), 118.1 (C-4), 128.7 (C-6), 127.0 (C-7), 127.6 (C-8), 120.3 (C-9), 120.9 (C-4a), 119.2 (C-5a), 139.5 (C-9a), 138.9 (C-10a), 31.3 (CH₂ of C₂H₅ at C₇), 14.2 (CH₃ of C₂H₅ at C₇), 81.0 (C-1'), 75.1 (C-2'), 71.7 (C-3'), 70.9 (C-4'); ^{19}F NMR (282.65 MHz, CDCl₃) : δ -110.00 (s, 1F, C-F); MS (FAB) 10 kV, m/z (rel. int.) : 690 (M⁺) (100), 689 [M-H]⁺ (55), 660 [M-CH₂CH₃]⁺ (65), 620 [M-C₄H₃F]⁺ (60) etc; Anal. Calcd for C₄₀H₃₂NO₇SF : C, 69.60; H, 4.64; N, 2.03. Found : C, 69.64; H, 4.69; N, 2.08.

2.6d *N*-(2',3',5'-tri-*O*-benzoyl- β -D-ribofuranosyl)-2-chloro-7-ethyl-3-trifluoromethyl-10*H*-phenothiazine (**7d**): Dark brown solid; m.p. : 136°C–140°C, Yield : 45% ; IR (KBr) : ν 1344 and 1165 (-CF₃) and 805 cm⁻¹

(C-Cl); ^1H NMR spectral data (300.40 MHz, $\text{Me}_2\text{SO}-d_6$, δ ppm from TMS) : δ 8.42–6.98 (m, 5H, Ar-H), 2.55 (q, 2H, $^3J_{\text{HH}} = 7.5$ Hz, CH_2 of C_2H_5 at C_7), 1.28 (t, 3H, $^3J_{\text{HH}} = 7.1$ Hz, CH_3 of C_2H_5 at C_7) ; ^{13}C NMR (75.45 MHz, CDCl_3 , δ ppm from TMS) : δ 119.70 (C-1), 129.8 (C-2), 119.2 (C-3), 127.7 (C-4), 130.9 (C-6), 128.4 (C-7), 127.0 (C-8), 120.1 (C-9), 118.9 (C-4a), 118.2 (C-5a), 139.6 (C-9a), 147.5 (C-10a), 32.0 (CH_2 of C_2H_5 at C_7), 15.0 (CH_3 of C_2H_5 at C_7), 115.9 (CF_3 at C_3 , q, $^1J_{\text{CF}} = 275.00$ Hz); ^{19}F NMR (282.65 MHz, CDCl_3) : δ -62.32 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.) : 774 (M^+) (100), 776 ($\text{M}+2$) (52), 773 [$\text{M} - \text{H}$] $^+$ (40), 745 [$\text{M} - \text{CH}_2\text{CH}_3$] $^+$ (65), 620 [$\text{M} - \text{C}_5\text{H}_2\text{F}_3\text{Cl}$] $^+$ (60) etc; Anal. Calcd for $\text{C}_{41}\text{H}_{31}\text{NO}_7\text{SF}_3\text{Cl}$: C, 63.60; H, 4.00; N, 1.80. Found : C, 63.64 ; H, 3.95 ; N, 1.83.

3. Biological activity

3.1 *In vitro* antibacterial and antifungal assay

3.1a Minimum inhibitory concentrations: The synthesized compounds were tested for their antimicrobial activity in the form of MIC's. Minimum inhibition concentration (MIC) is the lowest concentration of test compound which have been screened for their antimicrobial (antibacterial and antifungal) activity.^{16,17} At this lowest concentration of test compounds, appears the inhibition of visible growth of bacteria and fungi after 22–24 h, incubation at 37°C. The determination of the MIC involves a semi quantitative test procedure, which gives an approximation to the least concentration of an antimicrobial (test solution) needed to parent microbial growth. The MIC was determined by the liquid dilution method. Two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) were used as quality control strains. For the antifungal activities of the compounds *Candida albicans* and *Aspergillus 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 $\mu\text{g/mL}$ concentrations were prepared with aqueous methanol (1:1). 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 h at 37°C 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 inhibition concentration of antimicrobial (test solutions), which gave clear solution, i.e., no visual growth.

3.1b Anthelmintic activity: The synthesized compounds were screened for anthelmintic activity against *Eudrilus* species of earthworms by Garg and Atal method¹⁸ at 4 mg/mL concentrations. Five earthworms of nearly equal size were placed in standard drug solution and test compound solutions at room temperature. 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 using a mechanical stirrer for 30 min and diluted to contain 0.4% w/v of test samples. Suspensions of reference drug Mebendazole was prepared with same concentrations in similar way. Another set of five earthworms was kept as control in suspension of distilled water and Tween 80 (0.5%). The paralyzing and death times were noted and their mean was calculated and compared with standard drug. The time taken by worms to become motionless was noted as paralysis time. The death time was ascertained by placing earthworms in warm water (50°C), which stimulate the movement, if the worm was alive.

4. Results and discussion

7-Ethyl-3-trifluoromethyl-1-nitro-10H-phenothiazine (**5a**) was prepared by refluxing 2-amino-5-ethylbenzenethiol (**1a**) with 4-chloro-3, 5-dinitrobenzotrifluoride (**2a**) in the presence of sodium hydroxide in alcohol via Smiles rearrangement *in situ*. 10H-phenothiazines (**5b–d**) were synthesized by substituted 2-formamido-2'-nitrodiphenylsulphides (**4b–d**) via Smiles rearrangement. The formyl derivatives were synthesized by the formylation of 2-amino-2'-nitrodiphenylsulphides (**3b–d**), which in turn were prepared by condensation of 2-amino-5-ethylbenzenethiol (**1a**) with *o*-halonitrobenzenes (**2b–d**) in ethanolic sodium acetate solution. Their corresponding sulphones (**6a–d**) were synthesized by refluxing 10H-phenothiazines (**5a–d**) with 30 % H_2O_2 in glacial acetic acid. The spectral changes have been observed when sulphides convert to sulphones (IR and NMR data in sec. 2.1, 2.4 and 2.5). The oxidation of 10H-phenothiazine to their corresponding sulphones causes appearance of specific absorption peak with the change

in the vibrational modes. These changes in the vibrational modes could be elaborated by the strong electron withdrawing oxygen atoms at the oxidized sulphide linkage. Ribofuranosides (**7a–d**) were synthesized by treatment of 10*H*-phenothiazines (**5a–d**) with β -D-ribofuranosyl-1-acetate-2, 3, 5-tribenzoate in toluene under vacuum (scheme 1).

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

4.1 Antimicrobial activity

A series of novel heterocyclic compounds **5a–d**, **6a–d** and **7a–b** were synthesized. The minimum inhibitory concentration (MIC) of the compounds varies from 9.50–19.30 $\mu\text{g/mL}$. Seven compounds of the obtained series i.e., **5a**, **5b**, **6a**, **6b**, **6d** and **7a** showed good activity and remaining showed moderate activity against bacteria *E. coli*. Compounds **6a**, **6b** and **6d** showed good and remaining showed moderate activity against bacteria *B. subtilis*. For bacteria *P. aeruginosa* compounds **5a**, **6a**, **6b**, **6c**, **7a** and **7d** showed good activity

Scheme 1. Synthesis of Phenothiazine, their sulfones and nucleosides.

Table 1. Minimum inhibitory concentrations ($\mu\text{g/mL}$) of synthesized compounds.

Compd. No.	Minimal inhibition concentrations of bacterial strains (MIC) in $\mu\text{g/mL}$			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
5a	10.10 ± 0.29	11.60 ± 0.10	09.90 ± 0.05	11.00 ± 0.30
5b	10.30 ± 0.11	11.50 ± 0.22	13.90 ± 0.10	12.50 ± 0.11
5c	16.00 ± 0.35	19.30 ± 0.30	11.10 ± 0.10	15.00 ± 0.16
5d	11.70 ± 0.15	12.20 ± 0.19	11.30 ± 0.17	16.30 ± 0.21
6a	09.50 ± 0.20	10.70 ± 0.25	09.70 ± 0.23	10.00 ± 0.17
6b	10.00 ± 0.09	10.80 ± 0.26	10.70 ± 0.35	10.90 ± 0.30
6c	15.30 ± 0.21	19.00 ± 0.10	09.80 ± 0.10	14.30 ± 0.05
6d	10.40 ± 0.10	10.60 ± 0.35	11.20 ± 0.22	13.80 ± 0.15
7a	09.60 ± 0.16	11.90 ± 0.10	10.50 ± 0.19	12.10 ± 0.19
7b	13.30 ± 0.20	15.10 ± 0.19	13.90 ± 0.24	11.80 ± 0.17
7c	15.90 ± 0.10	19.20 ± 0.30	12.00 ± 0.15	14.60 ± 0.20
7d	11.40 ± 0.20	15.30 ± 0.15	10.90 ± 0.20	16.10 ± 0.10
Ciprofloxacin	04.10 ± 0.10	04.90 ± 0.13	03.85 ± 0.15	04.90 ± 0.11

Table 2. MIC ($\mu\text{g/mL}$) of synthesized compounds.

Compd. No.	Minimal inhibition concentrations of fungal strains (MIC) in $\mu\text{g/mL}$	
	<i>C. albicans</i>	<i>A. niger</i>
5a	10.10 ± 0.13	09.80 ± 0.22
5b	11.10 ± 0.17	16.40 ± 0.31
5c	13.10 ± 0.18	16.80 ± 0.08
5d	11.00 ± 0.10	12.70 ± 0.18
6a	09.50 ± 0.20	09.70 ± 0.22
6b	10.50 ± 0.20	15.00 ± 0.10
6c	12.70 ± 0.11	16.00 ± 0.35
6d	10.30 ± 0.29	$12.30. \pm 0.26$
7a	09.90 ± 0.35	$11.00. \pm 0.17$
7b	13.30 ± 0.22	16.00 ± 0.26
7c	15.80 ± 0.17	13.70 ± 0.15
7d	10.50 ± 0.30	12.40 ± 0.20
Griseofulvin	03.10 ± 0.08	04.80 ± 0.10

Table 3. Anthelmintic activity of synthesized compounds.

Compd. No.	Time in min					
	Paralyzing time			Death time		
	200 mg/mL	100 mg/mL	50 mg/mL	200 mg/mL	100 mg/mL	50 mg/mL
5a	13.00 ± 1.00	18.00 ± 1.00	25.00 ± 0.33	20.00 ± 1.00	32.66 ± 1.52	40.21 ± 0.25
5b	11.00 ± 1.00	15.00 ± 1.00	22.50 ± 0.50	21.16 ± 1.04	27.16 ± 0.76	35.23 ± 0.91
5c	13.16 ± 1.25	17.00 ± 1.00	25.00 ± 0.70	18.16 ± 0.76	25.16 ± 0.76	37.53 ± 0.55
5d	12.00 ± 1.00	16.00 ± 1.10	21.00 ± 1.00	19.50 ± 0.50	28.16 ± 0.76	35.50 ± 0.50
6a	15.00 ± 0.77	18.69 ± 0.79	23.36 ± 1.00	20.16 ± 1.22	24.13 ± 1.09	34.12 ± 0.60
6b	13.26 ± 1.08	18.55 ± 1.05	23.45 ± 0.93	15.40 ± 0.77	22.20 ± 1.00	29.25 ± 1.00
6c	15.16 ± 1.24	18.00 ± 0.78	22.78 ± 0.60	21.11 ± 0.77	25.88 ± 1.00	32.22 ± 0.63
6d	12.85 ± 1.22	15.00 ± 1.09	20.03 ± 0.68	13.15 ± 0.67	16.16 ± 0.84	20.00 ± 0.70
7a	12.20 ± 1.00	16.50 ± 1.20	21.00 ± 1.00	19.10 ± 0.55	26.06 ± 0.76	34.50 ± 0.50
7b	14.00 ± 1.10	17.33 ± 1.52	25.50 ± 1.32	20.50 ± 0.50	26.66 ± 2.08	38.50 ± 0.50
7c	13.95 ± 1.25	18.10 ± 1.00	23.60 ± 0.70	18.00 ± 0.76	24.90 ± 1.00	35.35 ± 0.55
7d	11.60 ± 1.20	16.40 ± 1.10	20.20 ± 1.05	16.50 ± 0.50	24.10 ± 0.50	30.95 ± 0.50
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

and remaining showed moderate activity. Compounds **5a**, **6a** and **6b** showed good activity and remaining showed moderate activity against bacteria *S. aureus*.

Compounds **5a**, **5d**, **6a**, **6d**, **7a** and **7d** showed good activity against fungi *C. albicans* and remaining showed moderate activity. For fungi *A. niger* compounds **5a**, **6a** and **7a** showed good activity and remaining showed moderate activity. These results are presented in tables 1 and 2.

4.2 Anthelmintic activity

The above screened compounds were tested for anthelmintic activity. The mean death time and paralysis time of the earthworms of different test compounds and standard drug tabulated in table 3. The results showed that compounds **5b**, **5d**, **6d**, **7a** and **7d** showed significant paralytic time with that of standard drug Mebendazole, at given concentrations. Test compounds **6b** and **6d** showed better death time of earthworms with that of standard drug Mebendazole. After all, the synthesized compounds in overall estimation confirms the better activity against *Eudrilus* species of earthworms.

5. Conclusion

All the synthesized 10*H*-phenothiazines, their sulphones and ribofuranosides showed good antifungal and antibacterial activities. In antimicrobial activity, the MIC values of antibacterial and antifungal screening predict that compounds **6a** and **6b** exhibited good antibacterial activities against all the four strains of bacteria. In fact, compounds **6a–d** exhibited significant antibacterial and antifungal activities as compared to their phenothiazines **5a–d**, respectively due to electron withdrawing effect of oxygen in **6a–d**.

In anthelmintic activity, compounds **5b**, **5d**, **6d** **7a** and **7d** showed significant paralytic time and compounds **6b** and **6d** showed better death time of earthworms with that of standard drug Mebendazole.

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