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Synthesis and antimicrobial assessment of new substituted 10*H*-phenothiazines, their sulfone derivatives, and ribofuranosides

Abstract: A series of new 10*H*-phenothiazines, their sulfones, and ribofuranosides were synthesized and their *in vitro* antimicrobial assessment was carried out against a representative panel of Gram-positive and Gram-negative bacterial strains and selected fungi species. The 10*H*-phenothiazines were prepared using Smiles rearrangement. The sulfone derivatives were synthesized by oxidation of 10*H*-phenothiazines using 30% hydrogen peroxide in glacial acetic acid. The ribofuranosides were prepared by treatment of the phenothiazines with β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate.

Keywords: antimicrobial assessment; 10*H*-phenothiazines; ribofuranosides; sulfones.

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Introduction

The synthesis of 10*H*-phenothiazines, their sulfones, and ribofuranosides has attracted tremendous interest evidenced by a large number of publications and patents registered worldwide. These compounds are extensively employed as antitumor [1], antibacterial [2], antitubercular [3], antiviral [4], antifungal [5], and anticancer [6, 7] agents. Some of the recent publications in this area have also demonstrated their significance as antimalarial [8], anti-AIDS [9], anti-inflammatory [10], and antihypertensive [11] agents. Research is being pursued to develop potent anticancer agents. A slight change in the substitution pattern in the phenothiazine system causes

a distinguishable difference in the biological activities [12–14].

Sulfones of phenothiazines constitute an important class of heterocyclic compounds due to their structural, theoretical, and medicinal importance [15–23]. Sulfones are obtained by oxidation of a sulfide linkage of phenothiazines. 10*H*-phenothiazine-5,5-dioxides show a number of uses in pharmacology, medicine, and industry. We are interested in the oxidation of sulfides of phenothiazines to sulfones and in study of the spectral and biological properties of the products.

Nucleosides are obtained by hydrolysis of the ester bond between a pentose sugar and the phosphate group in a nucleotide [15]. Nucleotides (nucleoside triphosphates) play an important role in metabolism by serving as the source of chemical energy in the form of ATP and GTP molecules. A variety of nucleoside analogues that expand the antiviral and anticancer spectrum and/or modify the pharmacological and pharmacokinetic properties of the parent compounds have been discovered.

Minor modifications in pyrimidine and purine nucleosides have a profound effect on their biological activity [18, 23]. These modified nucleosides have a wide variety of antiviral [24], antibacterial [25], and antitumor [26] properties. For the above reasons, we are interested in the synthesis and biological evaluation of nucleoside-type derivatives of the 10*H*-phenothiazine system.

Results and discussion

Chemistry

In the past, we have synthesized several substituted 10*H*-phenothiazines, their sulfone derivatives, and ribofuranosides with antibacterial and antifungal activity. Similar compounds herein reported were prepared by Smiles rearrangement of 2-formamido-2'-nitrodiphenyl sulfides in alcoholic potassium hydroxide. These substrates were synthesized by the reaction of substituted 2-aminobenzenethiols with substituted *o*-halonitrobenzenes

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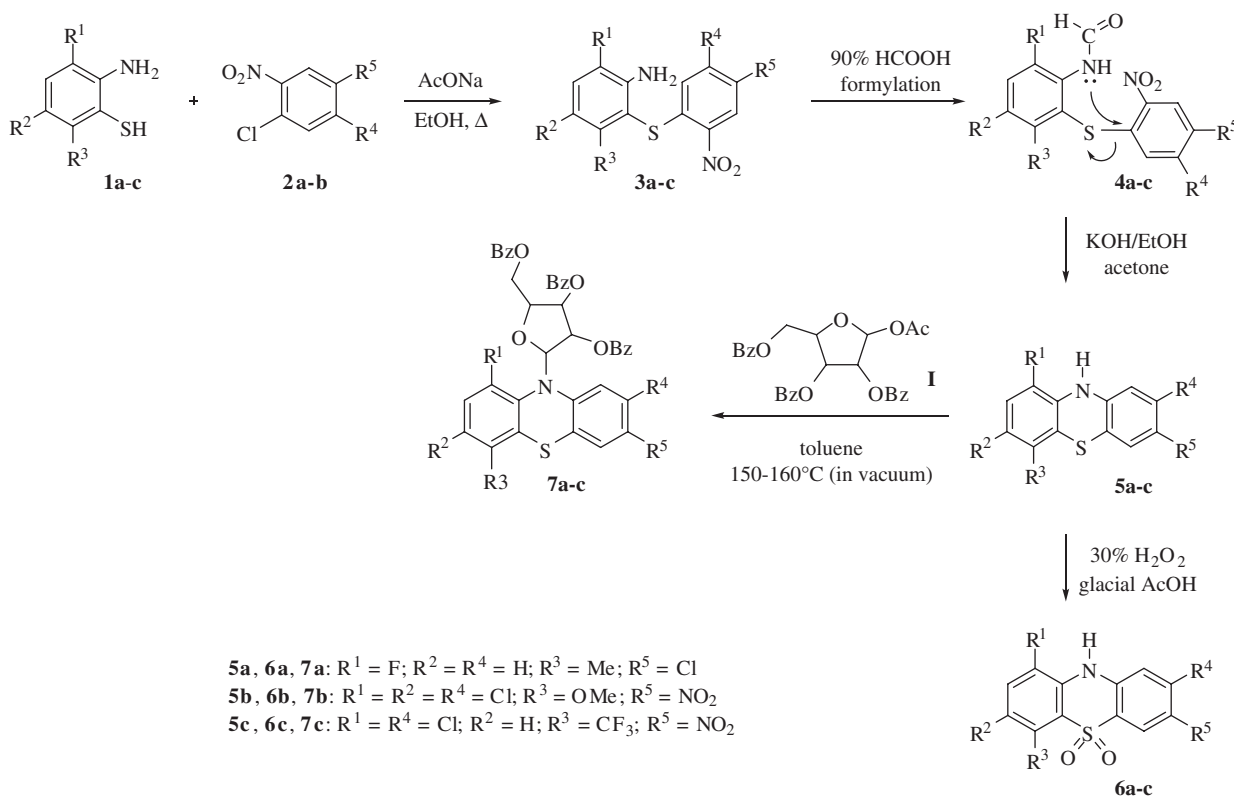
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followed by formylation. These phenothiazines were further converted into sulfone derivatives by oxidation under reflux conditions using 30% hydrogen peroxide in glacial acetic acid. Ribofuranosides were prepared by the reaction of the phenothiazines with 2,3,5-tri-*O*-benzoyl- β -D-ribofuranose-1-acetate (Scheme 1). To reflect the potency of the synthesized compounds as antimicrobial agents, activity indices (AI) and minimum inhibitory concentrations (MICs) against different Gram-positive and Gram-negative bacteria and fungi strains are reported.

The starting substituted 2-aminobenzenethiols **1a–c** (**I** in Scheme 1) were synthesized by alkaline hydrolysis of corresponding 2-aminobenzothiazoles, which in turn were prepared by the cyclization of substituted phenylthioureas. The phenylthiourea derivatives were obtained by the reaction of ammonium thiocyanate with substituted anilines. The synthesis of various substituted 10*H*-phenothiazines **5a–c** was carried out by the Smiles rearrangement of substituted 2-formamido-2'-nitrodiphenylsulfides **4a–c**. The formyl derivatives were prepared from the diphenyl sulfides **3a–c**, which in turn were prepared by the condensation of 2-aminobenzenethiols **1a–c** with *o*-halonitrobenzenes **2a,b** in ethanolic sodium acetate solution. Compounds **5a–c** were converted into their corresponding sulfones **6a–c** by treatment with 30% hydrogen

peroxide in glacial acetic acid. Meanwhile, the solutions of substituted 10*H*-phenothiazines **5a–c** in toluene were treated with β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate (**I**) on an oil bath at 150–160°C for 10 h to finally yield ribofuranosides **7a–c**.

The structures of the synthesized compounds are supported by spectroscopic data and elemental analysis. In the IR spectra of compounds **5a–c**, peaks due to >NH, C–Cl, C–F, and C–H stretching vibrations are observed. A slight shift toward higher frequencies is observed for sulfone derivatives **6a–c** due to an electron-accepting ability of sulfones compared with the parent system. Compounds **6a–c** exhibit intense peaks in the regions of 1340–1250 cm^{−1}, 1180–1130 cm^{−1}, and 590–540 cm^{−1}, which can be ascribed to vibrations of the sulfonyl group. Since during ribosylation, the hydrogen of the secondary amine group of 10*H*-phenothiazine is removed, there is absence of >NH stretching band in compounds **7a–c**. The given structures of products **5a–c**, **6a–c**, and **7a–c** are also fully consistent with their ¹H NMR spectra. Multiplets in the region of δ 8.10–6.49 are due to the presence of aromatic protons in all compounds **5a–c**, **6a–c**, and **7a–c**. A singlet in the region of δ 8.96–8.44 can be ascribed to the N–H function. This peak is absent in the ¹H NMR spectra of all synthesized compounds **7a–c**, indicating the substitution of the



Scheme 1 Synthesis of 10*H*-phenothiazines **5a–c**, their sulfone derivatives **6a–c**, and ribofuranosides **7a–c**.

Table 1 Antimicrobial assessment of 10*H*-phenothiazines 5a-c.

Compounds	<i>E. coli</i>			<i>B. subtilis</i>			<i>P. aeruginosa</i>			<i>S. aureus</i>			<i>C. albicans</i>			<i>A. niger</i>		
	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)
5a	9.21±0.12	68	13.11±0.21	67	13.24±0.19	66	12.39±0.22	54	11.90±0.19	56	12.00±0.19	54	12.71±0.25	48	13.01±0.20	52		
5b	8.82±0.13	68	9.24±0.16	71	10.33±0.20	62	11.44±0.22	52	12.71±0.25	48	13.01±0.20	52	9.03±0.43	30	9.91±0.19	33		
5c	9.01±0.20	56	9.23±0.23	63	9.33±0.34	67	10.01±0.16	100	4.90±0.11	100	4.90±0.11	100	—	—	—	—		
Ciprofloxacin	4.10±0.10	100	4.90±0.13	100	3.85±0.15	100	—	—	—	—	—	—	—	—	—	—		
Griseofulvin	—	—	—	—	—	—	—	—	—	—	—	—	3.10±0.80	100	4.80±0.10	100		

MIC, Minimum Inhibitory Concentration; AI, Activity Index; I, Inhibition.

Table 2 Antimicrobial assessment of sulfone derivatives 6a-c.

Compounds	<i>E. coli</i>			<i>B. subtilis</i>			<i>P. aeruginosa</i>			<i>S. aureus</i>			<i>C. albicans</i>			<i>A. niger</i>		
	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)
6a	8.43±0.21	71	9.11±0.16	66	10.22±0.16	66	9.23±0.14	54	10.68±0.48	56	11.34±0.17	54	10.11±0.18	48	9.86±0.14	52		
6b	9.67±0.19	68	10.13±0.17	58	9.44±0.22	62	10.44±0.21	52	10.11±0.18	48	9.86±0.14	52	9.62±0.32	30	8.24±0.22	33		
6c	8.19±0.14	78	8.71±0.20	70	8.00±0.18	67	8.64±0.18	100	4.90±0.11	100	4.90±0.11	100	—	—	—	—		
Ciprofloxacin	4.10±0.10	100	4.90±0.13	100	3.85±0.15	100	—	—	—	—	—	—	3.10±0.80	100	4.80±0.10	100		
Griseofulvin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

MIC, Minimum Inhibitory Concentration; AI, Activity Index; I, Inhibition.

Table 3 Antimicrobial assessment of ribofuranosides 7a-c.

S.No.	<i>E. coli</i>			<i>B. subtilis</i>			<i>P. aeruginosa</i>			<i>S. aureus</i>			<i>C. albicans</i>			<i>A. niger</i>		
	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	MIC (µg/mL) of bacterial strain	%AI (100 µg/mL)	MIC (µg/mL) of bacterial strain	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain	MIC (µg/mL) of bacterial strain	%I (100 µg/mL)	MIC (µg/mL) of bacterial strain
7a	11.14±0.12	73	13.34±0.13	64	10.11±0.24	67	16.22±0.13	62	12.13±0.33	46	10.62±0.24	43						
7b	12.26±0.23	67	17.11±0.17	52	11.35±0.16	70	13.42±0.14	62	11.61±0.18	45	12.13±0.16	42						
7c	10.19±0.20	73	11.23±0.33	58	12.20±0.14	69	10.13±0.17	61	11.25±0.13	53	14.26±0.21	44						
Ciprofloxacin	4.10±0.10	100	4.90±0.13	100	3.85±0.15	100	4.90±0.11	100	3.10±0.80	100	4.80±0.10	100						
Griseofulvin	—	—	—	—	—	—	—	—	—	—	—	—						

MIC, Minimum Inhibitory Concentration; AI, Activity Index; I, Inhibition.

NH function by sugar moiety and formation of ribofuranosides. The molecular ion peaks in the mass spectra of 10H-phenothiazines, their sulfone derivatives, and ribofuranosides correspond to their molecular weights.

Antimicrobial assessment

The *in vitro* antibacterial activity was tested against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) microorganisms using paper disc diffusion method. The antifungal activity of the synthesized compounds was tested on fungal strains, *Aspergillus niger* and *Candida albicans*, using the disc diffusion method. Ciprofloxacin and griseofulvin were used as a standard antibacterial and antifungal drug, respectively. It should be noted that sulfone derivatives of 10H-phenothiazines exhibit better antimicrobial activities than their parent compounds. This finding can be explained by the presence of the electron withdrawing sulfone group ($=SO_2$), which enhances biological activity. This effect of the electron-withdrawing group has been noted in the literature.

Conclusions

The synthesized compounds were tested for their *in vitro* antibacterial activity against four strains of bacteria (two Gram-negative bacteria: *E. coli* and *P. aeruginosa*; two Gram-positive bacteria: *B. subtilis* and *S. aureus*) and antifungal activity against two strains of fungi (*C. albicans* and *A. niger*). In general, the presence of an electron-withdrawing group on the aromatic ring increases the antimicrobial activities of tested compound compared with compounds with electron-donating groups. Compounds 5a,b and 6a-c are quite active against *E. coli*, *B. subtilis*, and *P. aeruginosa* while being moderately active against *S. aureus*, *C. albicans*, and *A. niger*. Compound 5c is active against *B. subtilis* and *P. aeruginosa*, moderately active against *E. coli* and *S. aureus*, and weakly active against *C. albicans* and *A. niger*. Compounds 7a-c are active against *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus* and moderately active against *C. albicans* and *A. niger*.

Experimental

Melting points are uncorrected. IR spectra were recorded in KBr on a Shimadzu 8400 S FT-IR spectrophotometer. 1H NMR and ^{13}C NMR spectra were recorded on a JEOL AL-300 spectrometer at frequencies of 300.40 and 75.45 MHz, respectively, in DMSO- d_6 using TMS

as an internal standard. Fast atom bombardment mass spectra were recorded on a JEOL SX 102/DA 600 instrument using xenon or argon. The purity of compounds were checked by TLC using silica gel G as adsorbent in various solvent systems with visualization by UV light or in an iodine chamber.

Synthesis of 2-amino-2'-nitrodiphenyl sulfides 3a–c

A solution of 2-aminobenzenethiol **1a–c** (0.01 mol) in ethanol (20 mL) containing anhydrous sodium acetate (0.01 mol) in a 50-mL round-bottom flask was treated with halonitrobenzene **2a,b** (0.01 mol) in a small amount of ethanol. The reaction mixture was heated under reflux for 4–5 h and then concentrated in an ice bath under a reduced pressure. The solid that separated out was filtered, washed with 30% ethanol, and crystallized from methanol.

2-Amino-4'-chloro-3-fluoro-6-methyl-2'-nitrodiphenyl sulfide (3a): Yield 56%; mp 120–123°C (dec); IR: 3475, 3372, 2950, 1580, 1380, 1250, 750 cm⁻¹. Anal. Calcd for C₁₃H₁₀N₂O₂SClF: C, 49.92; H, 3.20; N, 8.96. Found: C, 49.63; H, 3.10; N, 8.73.

2-Amino-3,5,5'-trichloro-6-methoxy-2',4'-dinitrodiphenyl sulfide (3b): Yield 60%; mp 109–111°C (dec); IR: 3460, 3380, 1560, 1320, 1280, 1040, 745 cm⁻¹. Anal. Calcd for C₁₃H₈N₃O₅SCl₃: C, 36.75; H, 1.89; N, 9.89. Found: C, 36.23; H, 1.75; N, 9.71.

2-Amino-3,5'-dichloro-6-trifluoromethyl-2',4'-dinitrodiphenyl sulfide (3c): Yield 48%; mp 97–99°C (dec); IR: 3475, 3380, 1555, 1340, 1310, 1110, 795 cm⁻¹. Anal. Calcd for C₁₃H₆N₃O₄SCl₂F₃: C, 36.45; H, 1.40; N, 9.81. Found: C, 36.63; H, 1.53; N, 9.72.

Synthesis of 2-formamido-2'-nitrodiphenyl sulfides 4a–c

The diphenyl sulfide **3a–c** (0.01 mol) was heated under reflux for 4–5 h in 90% formic acid (20 mL) and the mixture was then poured into a beaker containing crushed ice. A solid that separated out was filtered, washed with water, and crystallized from benzene.

4'-Chloro-3-fluoro-2-formamido-6-methyl-2'-nitrodiphenyl sulfide (4a): Yield 55%, mp 104–106°C (dec); IR: 3370, 2910, 1700, 1570, 1320, 1230, 780 cm⁻¹. Anal. Calcd for C₁₄H₁₀N₂O₃SClF: C, 49.34; H, 2.94; N, 8.22. Found: C, 49.73; H, 2.73; N, 8.41.

3,5,5'-Trichloro-2-formamido-6-methoxy-2',4'-dinitrodiphenyl sulfide (4b): Yield 42%; mp 99–102°C (dec); IR: 3360, 1710, 1580, 1340, 1280, 1030, 795 cm⁻¹. Anal. Calcd for C₁₄H₈N₃O₆SCl₃: C, 37.13; H, 1.77; N, 9.82. Found: C, 37.56; H, 1.67; N, 9.69.

3,5'-Dichloro-6-trifluoromethyl-2-formamido-2',4'-dinitrodiphenyl sulfide (4c): Yield 48%; mp 81–84°C (dec); IR: 3390, 1680, 1570, 1340, 1320, 1130, 790 cm⁻¹. Anal. Calcd for C₁₄H₆N₃O₅SCl₂F₃: C, 36.84; H, 1.32; N, 9.21. Found: C, 36.44; H, 1.46; N, 9.38.

Synthesis of phenothiazines 5a–c

A mixture of a solution of a formyl derivative **4a–c** (0.01 mol) in acetone (15 mL) and an alcoholic solution of potassium hydroxide

(0.2 g in 5 mL ethanol) was heated under reflux for 30 min and then treated with an additional portion of potassium hydroxide (0.2 g in 5 mL ethanol). The mixture was heated for an additional 4 h and then poured into beaker containing crushed ice. The resultant precipitate was repeatedly washed with cold water and 30% ethanol and then crystallized from benzene.

7-Chloro-1-fluoro-4-methyl-10H-phenothiazine (5a): Yield 53%; mp 155–158°C (dec); IR: 3350, 2940, 1330, 1320, 1240, 780 cm⁻¹; ¹H NMR: δ 8.87 (s, 1H); 7.26–6.67 (m, 5H), 2.34 (s, 3H); ¹³C NMR: δ 140.2, 135.6, 133.3, 130.0, 126.7, 125.1, 121.5, 120.6, 15.9; MS: m/z 265 (M⁺, 100), 264 (83), 249 (54), 229 (58), 178 (39), 86 (46). Anal. Calcd for C₁₃H₉N-SClF: C, 58.76; H, 3.39; N, 5.27. Found: C, 58.79; H, 3.33; N, 5.31.

1,3,8-Trichloro-4-methoxy-7-nitro-10H-phenothiazine (5b): Yield 74%; mp 145–149°C (dec); IR: 3340, 1275, 1020, 785 cm⁻¹; ¹H NMR: δ 8.96 (s, 1H); 7.78–6.72 (m, 3H); 3.71 (s, 3H); ¹³C NMR: δ 135.4, 130.1, 127.7, 123.6, 122.2, 120.8, 120.3, 118.9, 58.9; MS: m/z 377 (M⁺), 360 (100), 331 (72), 296 (67), 176 (56). Anal. Calcd for C₁₃H₇N₂O₃SCl₃: C, 41.33; H, 1.86; N, 7.41. Found: C, 41.39; H, 1.89; N, 7.37.

1,8-Dichloro-4-(trifluoromethyl)-7-nitro-10H-phenothiazine (5c): Yield 56%; mp 198–202°C (dec); IR: 3320, 1340, 1130, 770 cm⁻¹; ¹H NMR: δ 8.89 (s, 1H); 7.89–6.78 (m, 4H); ¹³C NMR: δ 147.2, 139.1, 137.9, 133.4, 125.7, 123.7, 123.0, 122.2, 110.7; MS: m/z 380 (M⁺), 363 (100), 334 (77), 333 (89), 299 (65), 265 (49). Anal. Calcd for C₁₃H₅N₂O₂SCl₂F₃: C, 40.95; H, 1.31; N, 7.35. Found: C, 40.92; H, 1.35; N, 7.39.

Synthesis of 10H-phenothiazine-5,5-dioxides (sulfones) 6a–c

A solution of a 10H-phenothiazine (0.01 mol) in 20 mL glacial acetic acid was added to 30% hydrogen peroxide (5 mL) and then the mixture was heated under reflux for 15 min keeping the temperature between 50°C and 55°C. Another portion of 30% hydrogen peroxide (5 mL) was added after 15 min without heating. Then the mixture was heated under reflux (120°C) for an additional 4–5 h, concentrated under reduced pressure, and the residue was treated with crushed ice. The resultant solid product **6a–c** was filtered and crystallized from ethanol.

7-Chloro-1-fluoro-4-methyl-10H-phenothiazine-5,5-dioxide (6a): Yield 55%; mp 239–241°C (dec); IR: 3370, 1330, 1270, 1260, 1175, 1155, 1070, 580, 560 cm⁻¹; ¹H NMR: δ 8.44 (s, 1H), 7.18–6.76 (m, 5H), 2.79 (s, 3H); ¹³C NMR: δ 142.0, 139.1, 134.8, 129.1, 125.9, 125.0, 120.2, 119.1, 15.1; MS: m/z 297 (M⁺, 100), 296 (66), 282 (48), 281 (68), 261 (37), 210 (54). Anal. Calcd for C₁₃H₉NO₂SClF: C, 52.44; H, 3.03; N, 4.71. Found: C, 52.48; H, 3.07; N, 4.76.

1,3,8-Trichloro-4-methoxy-7-nitro-10H-phenothiazine-5,5-dioxide (6b): Yield 62%; mp 188–190°C (dec); IR: 3368, 1340, 1290, 1250, 1172, 1150, 1080, 590, 565 cm⁻¹; ¹H NMR: δ 8.86 (s, 1H), 7.67–6.64 (m, 3H), 3.73 (s, 3H); ¹³C NMR: δ 133.9, 131.4, 126.2, 124.0, 123.1, 120.9, 116.1, 59.8; MS: m/z 409 (M⁺), 392 (100), 378 (53), 363 (71), 233 (63), 176 (59). Anal. Calcd for C₁₃H₇N₂O₅SCl₃: C, 38.09; H, 1.70; N, 6.84. Found: C, 38.02; H, 1.76; N, 6.88.

1,8-Dichloro-4-trifluoromethyl-7-nitro-10H-phenothiazine-5,5-dioxide (6c): Yield 40%; mp 183–185°C (dec); IR: 3345, 1339, 1284,

1250, 1180, 1130, 1075, 580, 550 cm^{-1} ; ^1H NMR: δ 8.49 (s, 1H), 8.10–7.00 (m, 4H); ^{13}C NMR: δ 149.1, 139.8, 135.6, 133.1, 124.9, 124.0, 123.1, 122.0, 111.7; MS: m/z 412 (M^+), 395 (100), 366 (48), 365 (59), 331 (81), 297 (74). Anal. Calcd for $\text{C}_{13}\text{H}_5\text{N}_2\text{O}_4\text{SCl}_2\text{F}_3$: C, 37.77; H, 1.21; N, 6.78. Found: C, 37.73; H, 1.26; N, 6.73.

Synthesis of substituted N-(2',3',5'-tri-O-benzoyl- β -D-ribofuranosyl) phenothiazines 7a–c

To a concentrated solution of 5a–c (0.002 mol) in toluene, β -D-ribofuranose-1-acetate-2,3,5-tribenzoate (**I**, 0.002 mol) was added and the mixture was stirred under a reduced pressure on an oil bath at 150–160°C for 15 min. The pressure was equalized and the flask was protected from moisture using a guard tube. Stirring was further continued for an additional 10 h with application of a reduced pressure for 15 min every hour. The melt was dissolved in methanol, and the solution was boiled for 10 min and cooled to room temperature. The precipitate was filtered, and the filtrate was concentrated. The viscous residue thus obtained was dissolved in ether, and the solution was filtered, concentrated, and kept in a refrigerator overnight, which caused crystallization of ribofuranoside.

N-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosyl-7-chloro-1-fluoro-4-methyl-10H-phenothiazine (7a): Yield 68%; mp 93–98°C (dec); IR: 2910, 1260, 790 cm^{-1} ; ^1H NMR: δ 7.59–6.49 (m, 20H), 6.39 (d, 1H), 5.93–5.74 (m, 2H), 4.83–4.34 (m, 3H), 2.45 (s, 3H); ^{13}C NMR: δ 139.2, 138.4, 135.2, 132.2, 130.1, 128.0, 127.3, 122.8, 94.2, 93.4, 89.8, 75.8, 17.1; MS: m/z 709 (M^+ , 100), 694 (79), 690 (64), 674 (37), 264 (75), 249 (58), 245 (44). Anal. Calcd for $\text{C}_{39}\text{H}_{29}\text{NO}_7\text{SClF}$: C, 65.96; H, 4.09; N, 1.97. Found: C, 65.99; H, 4.02; N, 1.94.

N-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosyl-1,3,8-trichloro-4-methoxy-7-nitro-10H-phenothiazine (7b): Yield 74%; mp 84–87°C (dec); IR: 1585, 1392, 1180, 785 cm^{-1} ; ^1H NMR: δ 8.10–6.95 (m, 18H), 6.41 (d, 1H), 5.90–5.75 (m, 2H), 4.82–4.35 (m, 3H), 4.10 (s, 3H); ^{13}C NMR: δ 135.9, 130.6, 129.8, 124.1, 122.8, 122.0, 121.0, 120.1, 96.5, 94.8, 93.1, 76.8, 59.2; MS: m/z 821 (M^+), 804 (100), 790 (50), 786 (62), 376 (74), 359 (33), 345 (69), 341 (75), 330 (48). Anal. calcd for $\text{C}_{39}\text{H}_{27}\text{N}_2\text{O}_{10}\text{SCl}_3$: C, 56.97; H, 3.29; N, 3.41. Found: C, 56.93; H, 3.24; N, 3.49.

N-(2',3',5'-tri-O-benzoyl)- β -D-ribofuranosyl-1,8-dichloro-4-trifluoromethyl-7-nitro-10H-phenothiazine (7c): Yield 58%; mp 73–75°C (dec); IR: 1575, 1385, 1350, 1135, 820 cm^{-1} ; ^1H NMR: δ 7.89–6.75 (m, 19H), 6.52 (d, 1H), 5.93–5.76 (m, 2H), 4.85–4.39 (m, 3H); ^{13}C NMR: δ 147.2, 139.1, 137.9, 133.4, 125.7, 123.7, 123.2, 122.1, 110.7, 95.6, 94.2, 91.8, 74.9; MS: m/z 825 (M^+), 808 (100), 790 (48), 779 (66), 756 (47), 380 (83), 363 (72), 345 (40), 334 (53), 311 (56). Anal. Calcd for $\text{C}_{39}\text{H}_{25}\text{N}_2\text{O}_{10}\text{SCl}_2\text{F}_3$: C, 56.73; H, 3.03; N, 3.39. Found: C, 56.78; H, 3.06; N, 3.34.

Antimicrobial assessment

Antibacterial activity: Paper discs were impregnated with solutions of compounds in DMF at concentrations of 25, 50, and 100 $\mu\text{g/mL}$. Then the impregnated discs were placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 h for bacterial cultures. After incubation, the zones of inhibition around the disc were measured. Each testing was done in triplicate.

Ciprofloxacin at a concentration of 50 $\mu\text{g/mL}$ was used as the standard drug. Results were interpreted in terms of a diameter (mm) of zone of inhibition. The % AI 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: Discs impregnated with compounds dissolved in DMF at concentrations of 25, 50, and 100 $\mu\text{g/mL}$ were spread over microorganism culture in nutrient agar medium. The plates were incubated at 25°C for 48 h for fungal strains. After incubation, the growth-inhibiting zones around the disc were measured. Each experiment was repeated three times. Griseofulvin at a concentration of 50 $\mu\text{g/mL}$ was used as the standard drug. Results were interpreted in terms of a diameter (mm) of zone of inhibition. The percentage inhibition was calculated by the following equation.

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

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

Minimum inhibitory concentrations: MICs is defined as the lowest concentration of the antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation at 37°C. The MIC was determined by the liquid dilution method. The stock solutions contained 1–20 $\mu\text{g/mL}$ of the test compounds in aqueous methanol. Inoculums of the overnight culture were prepared. In a series of tubes, 1 mL each of stock solution of the test compound with different concentrations was taken and 0.4 mL of the inoculums was added to each tube. Then 4.0 mL of sterile water was added to each of the test tubes. These test tubes were incubated for 22–24 h and observed for the presence of turbidity. The absorbance of the suspension of the inoculums was observed with spectrophotometer at 555 nm. The result of the test was the minimum concentration of antimicrobial (test) solutions that gave clear solution, i.e., no visual growth. The AIs of tested compounds against certain bacteria and fungi were calculated and the results are given in Tables 1–3.

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