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Synthesis of bioactive chlorinated 10*H*-phenothiazines, sulfones, ribofuranosides and their antimicrobial activity

Arun Goyal[a,c,*], Shikha Agarwal[b,*] & D C Gautam[c]

[a]Department of Chemistry, Govt P.G. College Rajgarh Alwar-301 408, Rajasthan, India
[b]Synthetic Organic Chemistry Laboratory, Department of Chemistry, MLSU, Udaipur, Rajasthan, India
[c]Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan, India
*E mail: arunchemphd@gmail.com (AG)/ shikhaagarwal@mlsu.ac.in (SA)

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This article reports synthesis of some new prospective bioactive 10*H*-phenothiazines, their sulfones and ribofuranosides that have shown significant antimicrobial activity against some selected strains of fungi, gram positive and gram negative bacteria and leads us to develop a possibly potent class of antimicrobial agents. 10*H*-phenothiazines are prepared via Smiles rearrangement. Their sulfones and ribofuranosides are prepared by oxidation with 30% hydrogen peroxide in glacial acetic acid and reaction with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (sugar), respectively.

Keywords: Phenothiazine, Smiles rearrangement, Sulfones, Antimicrobial properties, Ribofuranosides

More than 90% of the new drugs hold heterocycles and stand at the interface of chemistry and biology, at which point so much new scientific insight, discovery and application takes place. The presence of heterocycles in all kinds of organic compounds of attention in electronics, biology, optics, pharmacology, material sciences and so on is very well known. Between them, sulfur and nitrogen-containing heterocyclic compounds, phenothiazines, have maintained the interest of researchers through decades of historical development of organic synthesis. Phenothiazine is an organic compound having formula $S(C_6H_4)_2NH$ and is associated to thiazine class of heterocyclic compounds. It plays a significant role in various tranquilizers[1] and drugs with anti-inflammatory[2], antimalarial[3], antipsychotic[4], antimicrobial[5-6], antitubercular[7], antitumor[8-9], antihistaminic[10] and analgesic[11] properties.

According to previous investigations in eukaryotic cells, phenothiazines and their derivatives bind with sufficient strength to the calcium-binding protein calmodulin[12-15] to prevent the incursion of calcium. This knowledge has prompted some investigators to propose that phenothiazines and its derivatives may increase the permeability of the cell wall envelope and bind tightly to eukaryotic and microbial DNA thus presumably inhibiting their replication[16-17]. However, the effects noted from ultra-structural and biochemical studies strongly support the contention that these agents primarily affect the cell wall of both susceptible and very resistant microbes. These derivatives are also used for their cancer chemo preventive effect[18-19], neuroleptic action connected with the dopaminergic receptors blockade and protein kinase C inhibitory actions[20-22], inhibition of P-glycoprotein transport function and reversion of multidrug resistance[23].

Due to the immense importance of phenothiazine scaffolds, we have synthesized a series of substituted 10*H*-phenothiazines, their sulfones and nucleosides. The sulfone derivatives were obtained by refluxing reported 10*H*-phenothiazines with 30% hydrogen peroxide in glacial acetic acid while the reported ribofuranosides were prepared by the treatment of synthesized substituted 10*H*-phenothiazines with β–D-ribofuranose-1-acetate-2,3,5-tribenzoate (sugar). Synthesized sulfones and ribofuranosides also exhibit similar pharmacological activities (anticonvulsant, antibacterial, antifungal, antitumor and pharmacokinetic properties[24-32]). To exhibit the potential of synthesized compounds as better antimicrobial agents[33-34], minimum inhibition concentration (MIC) against selected strains of fungi, Gram positive and Gram negative bacteria belonging to Microbial Type Culture Collection (MTCC) was reported using broth microdilution method.

Experimental Details

All chemical and reagents were purchased from Sigma Aldrich and Alfa-Aesar and were used without further purification. The melting points of synthesized compounds are uncorrected and determined in open capillary tubes. The purity of the synthesized compounds was checked by thin layer chromatography using silica gel 'G' adsorbent in various non-aqueous solvent systems The IR spectra were recorded in KBr on SHIMADZU 8400 S FT IR spectrophotometer. The ^{1}H NMR spectra have been recorded at 300 MHz on JEOL AL-300 FT NMR using TMS as an internal standard in DMSO-d_6 (in δ ppm). Mass spectra were recorded on WATERS (micromass MS technologies) Q-T by electron spray ionization. The starting material, 2-amino-3,5,6-trichlorobenzenethiol (1), was synthesized by the literature reported method[35]. *O*-halonitrobenzenes (2a-d) were purchased from Sigma-Aldrich and Alfa Aesar. Substituted 10*H*-phenothiazines (3a-b) by three step process and 1-nitro-10*H*-phenothiazines (3c-d) by one step process, substituted 10*H*-phenothiazine-5,5-dioxides (sulfones) (4a-d), substituted N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)- 10*H*-phenothiazines (ribofuranosides) (5a-b) were synthesized by the literature studies.[36]

General procedure for the synthesis of substituted 2-amino-2′-nitro diphenylsulfides (3'a-b)

2-Amino-3,5,6-trichlorobenzenethiol **(1)** (0.01 mol) was dissolved in ethanol (20 mL) containing (0.01 mol) of anhydrous sodium acetate in a 50 mL round bottom flask and *o*-halonitrobenzene (**2a-b)** (0.01 mol) in 10 mL ethanol was added. The reaction mixture was refluxed for 4-5 h. The resultant solution was cooled and kept overnight in an ice bath. The solid separated out was filtered, washed with 30% ethanol and recrystallized from methanol.

General Procedure for the synthesis of substituted 2-formamido-2'-nitrodiphenyl-sulfides (3''a-b)

The resulting diphenylsulfides **(3'a-b)** (0.01 mol) was refluxed for 4 h in 90% formic acid (20 mL). The contents were poured in a beaker containing crushed ice. The solid separated out was filtered, washed with water until the filtrate was neutral and crystallized from benzene.

General Procedure for the synthesis of substituted 10*H*-phenothiazines (3a-b)

Product of second step (formyl derivatives) **(3''a-b)** (0.01 mol) in acetone (15 mL) was refluxed followed by addition of an alcoholic solution of potassium hydroxide (0.2 g in 5 mL ethanol). The contents were heated for half an hour. After then, a second lot of potassium hydroxide (0.2 g in 5 mL ethanol) was added to the reaction mixture and further refluxed for 4 h. Contents were then poured into a beaker containing crushed ice and filtered, washed with cold water and finally with 30% ethanol and crystallized from benzene.

1,3,4,8-tetrachloro-9-methyl-10H-phenothiazine (3a)

Yield 29%, m.p. 92°C, colour: brown; IR (KBr, *v*): 3380, 770, 1055, 2965, 2830 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 8.79 (s, 1H, N-H), 7.11-6.62 (m, 3H, Ar-H), 2.51 (s, 3H, -CH_3). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.7 (C-1), 131.5 (C-2), 126.6 (C-3), 134.8 (C-4), 131.3 (C-6), 119.3 (C-7), 134.1 (C-8), 128.1 (C-9), 123.8 (C-4a), 120.3 (C-5a), 147.5 (C-9a), 145.9 (C-10a), 3.2 (CH_3 at C_9). MS (FAB) 10 kV, m/z (rel. int.): 351 $[M]^+$ (100), 349 $[M+2]^+$ (75), 350 $[M–H]^+$ (57), 336 $[M–CH_3]^+$ (48).

1,3,4-trichloro-8-fluoro -7-methyl -10H-phenothiazine (3b)

Yield 34%, m.p. 105°C, colour: brown-red; IR (KBr, *v*): 3400, 790, 1090, 1060, 2990, 2870 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.10 (s, 1H, N-H), 8.24-6.86 (m, 3H, Ar-H), 2.64 (s, 3H, -CH_3). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.4 (C-1), 131.2 (C-2), 124.9 (C-3), 136.2 (C-4), 133.9 (C-6), 115.4 (C-7), 164.2 (C-8), 103.9 (C-9), 124.2 (C-4a), 115.9 (C-5a), 145.1 (C-9a), 146.2 (C-10a), 9.1 (CH_3 at C_9). MS (FAB) 10 kV, m/z (rel. int.): 334.9 $[M]^+$ (100), 332.9 $[M+2]^+$ (99), 333.5 $[M–H]^+$ (59), 319.5 $[M–CH_3]^+$ (47).

General procedure for the synthesis of substituted 1-nitro-10*H*-phenothiazines by one step process (3c-d)

1-nitrophenothiazines have been synthesized by the condensation of 2-amino-3,5,6-trichlorobenzenethiol **(1)** with reactive *o*-halo nitrobenzenes **(2c-d)** (1,2-dichloro-3-nitrobenzene, 1,4-dichloro-2,6-dinitrobenzene) containing either nitro groups or one nitro and one halogen group at both ortho positions to the reactive halogen atom in the presence of sodium hydroxide. Smiles rearrangement occured in situ to yield 1-nitro-10*H*-phenothiazines **(3c-d)**.

6,7,9-trichloro-1-nitro-10H-phenothiazine (3c)

Yield 49%, m.p. 102°C, colour: red; IR (KBr, *v*): 3395, 1530, 1320, 1052 cm^{-1}. ^{1}H NMR (300.40 MHz,

DMSO-d_6): δ 8.92 (s, 1H, N-H), 8.10-6.84 (m, 4H, Ar-H). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 139.1(C-1), 122.7 (C-2), 120.2 (C-3), 137.6 (C-4), 135.2 (C-6), 124.9 (C-7), 129.8 (C-8), 122.8 (C-9), 121.8 (C-4a), 124.4 (C-5a), 145.1 (C-9a), 140.2 (C-10a). MS (FAB) 10 kV, m/z (rel. int.): 347.9 $[M]^+$ (100), 345.9 $[M+2]^+$ (99), 317 $[M–NO]^+$ (69), 300 $[M–HNO_2]^+$ (48), 301 $[M–NO_2]^+$(62).

3,6,7,9-tetrachloro-1-nitro-10H-phenothiazine (3d)

Yield 44%, m.p. 77°C, colour: red; IR (KBr, *v*): 3410, 1545, 1335, 1064 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.17 (s, 1H, N-H), 7.90-6.45 (m, 3H, Ar-H). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 139.4 (C-1), 123.2 (C-2), 124.6 (C-3), 137.9 (C-4), 134.9 (C-6), 126.2 (C-7), 130.5 (C-8), 123.6 (C-9), 123.2 (C-4a), 124.5 (C-5a), 144.9 (C-9a), 138.2 (C-10a). MS (FAB) 10 kV, m/z (rel. int.): 382 $[M]^+$ (100), 380 $[M+2]^+$ (78), 352 $[M–NO]^+$ (58), 335 $[M–HNO_2]^+$ (54), 336 $[M–NO_2]^+$(49).

General procedure for the Synthesis of substituted 10H-phenothiazine-5,5-dioxides (sulfones) (4a-d)

Synthesized 10*H*- phenothiazine (3**a-d)** (0.01 mol) in glacial acetic acid (20 mL) and 30% hydrogen peroxide (5 mL) were taken in a round bottom flask and refluxed for 15-20 min at 50-60 °C and then another lot of (5 mL) 30% H_2O_2 was added. The reaction mixture was refluxed in continuation for 4 h. After that, the reaction mixture was poured into a beaker containing crushed ice. The obtained precipitate was filtered, washed with water and crystallized from ethanol.

1,3,4,8-tetrachloro-9-methyl-10H-phenothiazine-5,5-dioxide (sulfone) (4a)

Yield 28%, m.p. 182°C, colour: brown; IR (KBr, *v*): 3386, 1061, 1160, 560, 1345 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.20 (s, 1H, N-H), 7.29-6.90 (m, 3H, Ar-H), 2.45 (s, 3H, -CH_3). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.5 (C-1), 135.8 (C-2), 125.8 (C-3), 130.9 (C-4), 126.2 (C-6), 118.5 (C-7), 140.6 (C-8), 129.2 (C-9), 130.8 (C-4a), 126.2 (C-5a), 143.1 (C-9a), 140.9 (C-10a), 3.2 (CH_3 at C_9). MS (FAB) 10 kV, m/z (rel. int.): 383 $[M]^+$ (100), 381 $[M+2]^+$ (76), 353 $[M–NO]^+$ (44), 336 $[M–HNO_2]^+$ (61), 337 $[M–NO_2]^+$(52), 319 $[M–SO_2]^+$ (36).

1,3,4-trichloro-8-fluoro-7-methyl-10H-phenothiazine-5,5-dioxide (sulfone) (4b)

Yield 34%, m.p. 175°C, color: dark red; IR (KBr, *v*): 3405, 1064, 1170, 570, 1350 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.31 (s, 1H, N-H), 8.42-6.94 (m, 3H, Ar-H), 2.71 (s, 3H, -CH_3). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.9 (C-1), 135.7 (C-2), 125.4 (C-3), 132.5 (C-4), 130.1 (C-6), 116.4 (C-7), 169.2 (C-8), 106.4 (C-9), 131.2 (C-4a), 123.5 (C-5a), 138.5 (C-9a), 141.2 (C-10a), 10.4 (CH_3 at C_7). MS (FAB) 10 kV, m/z (rel. int.): 367 $[M]^+$ (100), 365 $[M+2]^+$ (99), 337 $[M–NO]^+$ (52), 320 $[M–HNO_2]^+$ (58), 321 $[M–NO_2]^+$(45), 303 $[M–SO_2]^+$ (35).

6,7,9-trichloro-1-nitro-10H-phenothiazine-5,5-dioxide (sulfone) (4c)

Yield 44%, m.p. 140°C, colour: black; IR (KBr, *v*): 3401, 1060, 1160, 565, 1355 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.30 (s, 1H, N-H), 8.45-7.34 (m, 4H, Ar-H). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 138.7 (C-1), 128.5 (C-2), 118.7 (C-3), 134.3 (C-4), 130.8 (C-6), 127.4 (C-7), 137.3 (C-8), 124.1 (C-9), 128.2 (C-4a), 130.9 (C-5a), 135.8 (C-9a), 142.7 (C-10a). MS (FAB) 10 kV, m/z (rel. int.): 380 $[M]^+$ (100), 378 $[M+2]^+$ (98), 350 $[M–NO]^+$ (59), 333 $[M–HNO_2]^+$ (51), 334 $[M–NO_2]^+$(42), 316 $[M–SO_2]^+$ (39).

3,6,7,9-tetrachloro-1-nitro-10H-phenothiazine -5,5-dioxide (sulfone) (4d)

Yield 40%, m.p. 182°C, color: red; IR (KBr, *v*): 3413, 1072, 1165, 570, 1350 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 9.38 (s, 1H, N-H), 8.25-7.10 (m, 4H, Ar-H). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 141.2 (C-1), 130.1 (C-2), 126.2 (C-3), 134.2 (C-4), 131.4 (C-6), 126.4 (C-7), 137.2 (C-8), 124.5 (C-9), 130.5 (C-4a), 132.6 (C-5a), 135.3 (C-9a), 142.4 (C-10a). MS (FAB) 10 kV, m/z (rel. int.): 414 $[M]^+$ (100), 412 $[M+2]^+$ (76), 384 $[M–NO]^+$ (62), 367 $[M–HNO_2]^+$ (47), 368 $[M–NO_2]^+$(45), 350 $[M–SO_2]^+$ (44).

General procedure for synthesis of substituted N-(2′,3,5′-tri-O-benzoyl-*β*-D-ribofuranosyl)-10*H*-phenothiazines (Ribofuranosides) (5a-b)

β -D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mol) was added to a concentrated solution of synthesized phenothiazines (0.002 mol) (**3a,c**) in 5 mL toluene. The above mixture was refluxed in vacuum condition with stirring on an oil bath at 150-160°C for 15 min.

***N*-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl) -1,3, 4,8-tetrachloro-9-methyl-10H-phenothiazine (Ribofuranosides) (5a)**

Yield 29%, m.p. 162°C, colour: orange; IR (KBr, *v*): 1565, 1360, 780, 1760, 1460-1350, 1145 cm^{-1}. ^{1}H

NMR (300.40 MHz, DMSO-d_6): δ 7.72-6.95 (m, 3H, Ar-H), 2.58 (s, 3H, -CH_3), 4.72-4.44 (m, 1H, $C_{4'}$ proton of sugar), 4.80- 4.36 (m, 2H, -CH_2 proton of sugar), 5.82-5.70 (m, 1H, $C_{3'}$ proton of sugar), 5.94-5.80 (m, 1H, $C_{2'}$ proton of sugar), 6.40 (d, 1H, J = 6.4 Hz, proton of sugar). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.1 (C-1), 129.5 (C-2), 126.2 (C-3), 136.2 (C-4), 131.4 (C-6), 119.1 (C-7), 134.2 (C-8), 128.8 (C-9), 124.6 (C-4a), 119.6 (C-5a), 147.9 (C-9a), 145.7 (C-10a), 87.4 ($C_{1'}$), 75.4 ($C_{2'}$), 70.7 ($C_{3'}$), 69.2 ($C_{4'}$). MS (FAB) 10 kV, m/z (rel. int.): 795 $[M]^+$ (100), 793 $[M+2]^+$ (75), 791 $[M+4]^+$ (56), 780 $[M–CH_3]^+$ (34).

N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-1,3,4-trichloro-8-fluoro-7-methyl-10H-phenothiazine (Ribofuranosides) (5b)

Yield 32%, m.p. 132°C, colour: red; IR (KBr, *ν*): 1560, 1130, 795, 1780, 1460-1355, 1165 cm^{-1}. ^{1}H NMR (300.40 MHz, DMSO-d_6): δ 8.50-6.90 (m, 3H, Ar-H), 2.78 (s, 3H, -CH_3), 4.78-4.38 (m, 1H, $C_{4'}$ proton of sugar), 4.60- 4.42 (m, 2H, -CH_2 proton of sugar), 5.75-5.65 (m, 1H, $C_{3'}$ proton of sugar), 5.90-5.82 (m, 1H, $C_{2'}$ proton of sugar), 6.40 (d, 1H, J = 6.4 Hz, proton of sugar). ^{13}C NMR (75.45 MHz, $CDCl_3$,): δ 123.6 (C-1), 130.4 (C-2), 126.5 (C-3), 134.9 (C-4), 134.6 (C-6), 115.2 (C-7), 162.5 (C-8), 106.2 (C-9), 124.6 (C-4a), 117.2 (C-5a), 144.6 (C-9a), 146.2 (C-10a), 86.5 ($C_{1'}$), 75.4 ($C_{2'}$), 71.2 ($C_{3'}$), 70.6 ($C_{4'}$). MS (FAB) 10 kV, m/z (rel. int.): 779 $[M]^+$ (100), 777 $[M+2]^+$ (99), 775 $[M+4]^+$ (68), 764 $[M–CH_3]^+$ (34).

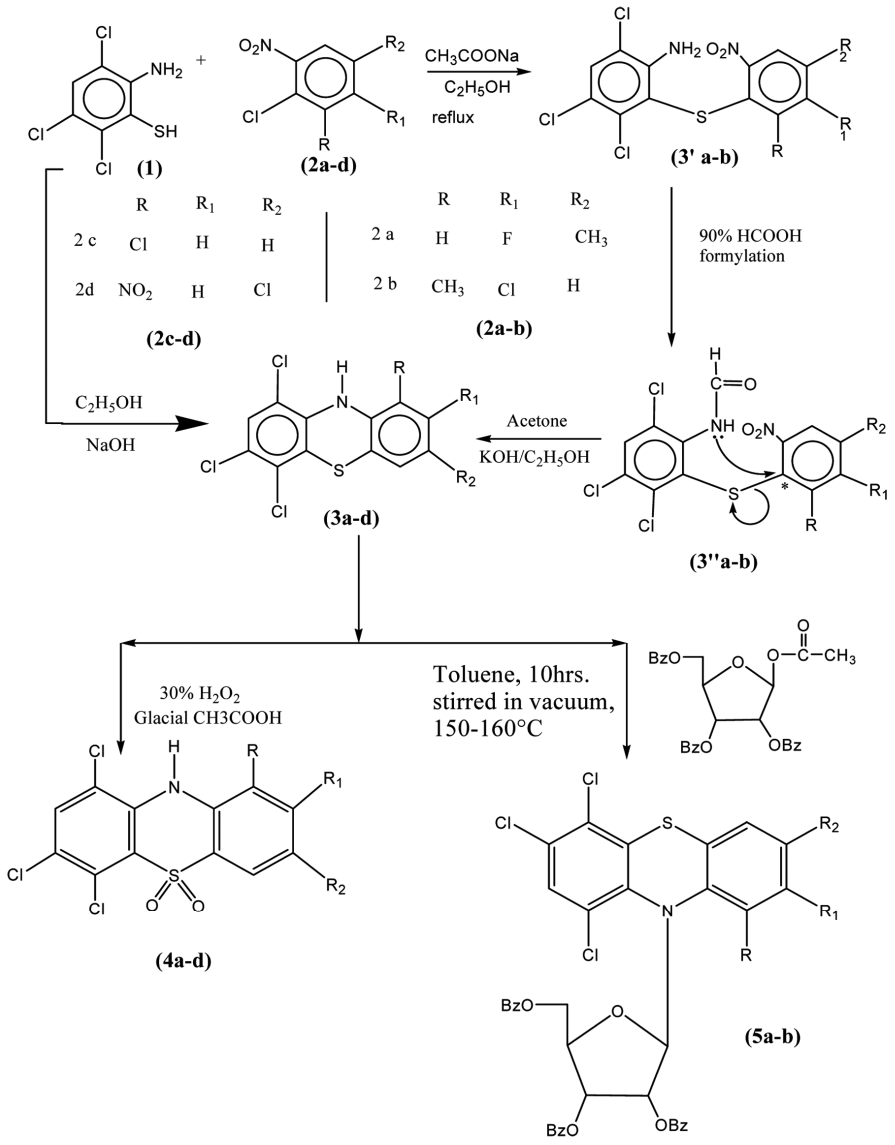
Antimicrobial assessment

As per NCCLS-1992 manual, Minimum Inhibitory Concentrations (MICs, μg mL^{-1}) of the synthesized compounds were carried out by broth microdilution method. Stock solution of 1000 μg/mL concentration for each synthesized compound and standard drugs were prepared in DMSO. In primary screening, 500, 250 and 125 μg/mL concentrations of the synthesized drugs were taken. The synthesized drugs those found active in primary screening were further tested in a second set of dilution against all microorganisms. These drugs were also diluted to obtain 100, 50, 25, 20, 15 μg/mL concentrations. The highest dilution showing at least 99% inhibition was taken as MIC which meant the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/fungi was recorded as Minimum Inhibitory Concentration of that compound. Antibacterial activities of the bacterial strains were carried out in Luria broth (Himedia) medium and all fungi were cultivated in Sabouraud Dextrose Agar (Himedia) at pH 6.9 with an inoculum of 10^8 cfu/mL by the spectrophotometric method and an aliquot of 10 μL was added to each tube of the serial dilution and incubated on a rotary shaker at 37 °C for 24 h at 150 rpm. At the end of incubation period, MIC values were recorded.

Results and Discussion

Chemistry

Two series of 10*H*-phenothiazines were reported by our research group. First series resulted in the formation of substituted 10*H*-phenothiazines and second series yielded substituted 1-nitro-10*H*-phenothiazines as end products. Both series were synthesized by the treatment of 2-amino-3,5,6-trichlorobenzenethiol **(1)** with o-halonitrobenzenes **(2a-d)**. In the first series, substituted 10*H*-phenothiazines **(3a-b)** were synthesized via Smiles rearrangement of substituted 2-formamido-2′-nitrodiphenylsulfides **(3"a-b)** which were prepared by the formylation of 2-amino-2′-nitrodiphenylsulfide **(3'a-b)**. These 2-amino-2′-nitro diphenyl sulfides **(3'a-b)** were in turn prepared by the condensation of 2-amino-3,5,6-trichlorobenzenethiol **(1)** with 4-chloro-2-fluoro-5-nitrotoluene **(2a)** and 2,6-dichloro-3-nitrotoluene **(2b),** respectively, in ethanolic sodium acetate solution. The preparation of second series **(3c-d)** was accomplished by the condensation of 2-amino-3,5,6-trichlorobenzenethiol **(1)** with reactive halonitrobenzenes [1,2-dichloro-3-nitrobenzene **(2c)** and 1,4-dichloro-2,6-dinitrobenzene **(2d)**] containing two nitro groups at both ortho positions to the reactive halogen atom in the presence of ethanolic sodium hydroxide via Smiles rearrangement in which ring closure occurred simultaneously in situ (one pot synthesis). Reported 10*H*-phenothiazines **(3a-d)** were converted into their corresponding sulfones **(4a-d)** on treatment with 30% hydrogen peroxide in glacial acetic acid. Treatment of compounds **(3a-d)** in toluene with *β*-D-ribofuranose-1-acetate-2,3,5-tribenzoate in vacuum on an oil bath at 155-160 °C for 10 h gave the corresponding nucleosides **(5a-b)** (Scheme 1). The proposed structures for the reported compounds were well supported by spectral analysis. A single sharp peak was exhibited by compounds **3(a-d)** in the region 3420–3250 cm^{-1} due to the >NH stretching vibration which was found absent in compounds **5(a-b)**, clearly proving it to be the site of ribosylation, while a slight shift was recorded towards higher frequencies in the compounds



Scheme 1 — Synthesis of substituted 10*H*-phenothiazines **(3a-d)**, sulfones **(4a-d)** and ribofuranosides **(5a-b)**

4(a-d) at 3435-3255 cm^{-1} due to increased electron accepting ability of sulfones as compared to the parent nucleus. Compounds **4(a-d)** showed three characteristic absorption peak of sulfonyl group due to asymmetric stretching vibration (1350–1330 cm^{-1}), symmetric stretching vibration (1170–1130 cm^{-1}), and bending vibration (580–540 cm^{-1}) in carbon tetrachloride solution. A singlet was observed in compounds **3(a-d)** and **4(a-d)** in the region δ 9.62-8.70 ppm which can be ascribed to N-H proton, while no such peak was recorded in the ^{1}H NMR spectra of compounds **5(a-b)** indicating the formation of nucleosides. The molecular ion peaks of reported compound were in accordance with their molecular weights.

Antimicrobial assessment

All synthesized compounds were assessed for their antimicrobial activity (antibacterial and antifungal) using agar well diffusion method. Streptomycin and Ketoconazole were used as standard antibacterial and antifungal drugs, respectively. *Escherichia coli* (gram negative) MTCC 2939, *Bacillus subtilis* (gram positive) MTCC 441, *Streptomyces griseus* (gram negative) MTCC 1998 were used for determining antibacterial activity and *Fusarium oxysporum* MTCC 1755, *Aspergillus niger* MTCC 281, *Rhizopus stolonifer* MTCC 2591 were used for determining antifungal activity of synthesized heterocyclic compounds. The MIC values of synthesized compounds in μg/mL against certain bacterial and fungal strains have been shown in Table 1**.**

Table 1 — Antimicrobial activity of synthesized compounds

Compound No.	Minimum Inhibitory Concentrations MICs of bacterial strains in μg/mL			Minimum Inhibitory Concentrations (MICs) MICs of fungal strains in μg/mL		
	Escherichia coli MTCC 2939	*Bacillus subtilis* MTCC 441	*Streptomyces griseus* MTCC 1998	*Fusarium oxysporum* MTCC 1755	*Aspergillus niger* MTCC 281	*Rhizopus stolonifer* MTCC 2591
3a	112	94	126	130	42	44
3b	120	108	108	92	84	76
3c	64	48	60	75	39	45
3d	60	110	52	86	118	110
4a	84	72	68	108	26	22
4b	96	94	72	72	54	54
4c	50	90	51	85	80	78
4d	48	86	44	82	78	78
5a	90	78	112	110	40	48
5b	65	48	60	76	42	48
Streptomycin	68	46	62	-	-	-
Ketoconazole	-	-	-	74	38	46

Compounds **3c** and **5b** showed activity almost equivalent to the standard drug (Streptomycin and Ketoconazole) while compounds **3d, 4a and 4b** showed moderate activity against bacterial strains as well as fungal strains. Compounds **4c** and **4d** gave excellent results against bacterial strains. Compounds **3c** gave excellent results against fungal strains. Compounds **3a, 5a and 5b** showed activity equivalent to Ketoconazole against all these four strains of fungi.

Conclusion

In the present study, different novel 10*H*-phenothiazines, their sulfones and ribofuranosides were synthesized using commercially available starting materials and identified by spectral analysis and have shown moderate to significant antimicrobial properties. The different bacterial and fungal strains showed different susceptibility towards the tested compounds, which may be possibly explained by different resistance mechanism exhibited by each strain. The MIC values of antibacterial and antifungal screening revealed that the excellent antibacterial and antifungal activities against all the three selected strains of bacteria and fungi, respectively, were exhibited by compounds bearing strong and more (in number) electron withdrawing groups (F, NO_2). The most active compounds against all the three selected strains of bacteria and fungi respectively were 1,3,4,8-tetrachloro-9-methyl-10*H*-phenothiazine-5,5-dioxide (sulfone derivative) (**4a)** and 3,6,7,9-tetrachloro-1-nitro-10*H*-phenothiazine -5,5-dioxide (sulfone derivative) **(4d)**. Among the three different moieties under study i.e. 10*H*-phenothiazines, their sulfone derivatives and their ribofuranosides, it was observed that sulfone derivatives were most active against selected strains of microbes than their respective 10*H*-phenothiazines and nucleoside derivatives which can be explained by the enhanced electron withdrawing effect of $–SO_2$ group which is absent in the other two moieties. The concluding statement from the results of our study highlights that due to electron withdrawing nature of oxygen, sulfones of phenothiazines showed excellent antibacterial and antifungal activities as compared to their corresponding phenothiazines. Among the synthesized compounds, compounds **3c** and **5b** showed activity almost equivalent to the standard drug while compounds **3d, 4a** and **4b** showed moderate activity against bacterial strains as well as fungal strains. Compounds **4c** and **4d** gave excellent results against bacterial strains. Compounds **3c** gave excellent results against fungal strains. Compounds **3a, 5a and 5b** showed activity equivalent to Ketoconazole against all these four strains of fungi possibly due to the presence of electron withdrawing groups. The paper showed that a slight change in substitution pattern affects the biological activity tremendously. By comparing different compounds, we can get an idea of drug designing so that we can design better phenothiazine templates which may have potential to be used as a new class of antibacterial and antifungal drugs. The motive of our research is to extend the area of research by synthesizing new and better templates of 10*H*-phenothiazines and screening them as potential antibacterial and antifungal drugs but further biomedical research is required.

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