Research Article

Synthesis and Antimicrobial Evaluation of Novel 10*H*-phenothiazines, their Sulfones and Ribofuranosides

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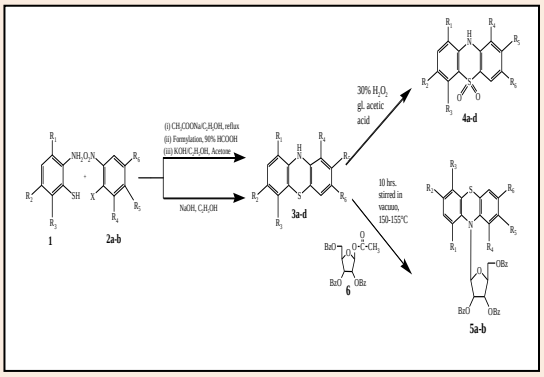
Abstract

The present work refers the synthesis of novel substituted 10*H*-phenothiazines *via* Smiles rearrangement. Substituted 10H-phenothiazines are synthesized by the reaction of 2-aminobenzenethiols and *O*-halonitrobenzenes. These synthesized phenothiazines undergoes oxidation when treated with 30% H_2O_2 in glacial acetic acid yielded 10*H*-phenothiazine-5,5-dioxides (sulfones). Further, when former were reacted with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate yields new ribofuranosides. These compounds were screened for their *in vitro* antimicrobial (antibacterial and antifungal) vitalities and exhibited promising results. The structural elucidation of synthesized compounds ensured on the basis of their elemental and spectral studies.

Keywords: Smiles rearrangement, 10*H*-phenothiazines, sulfones, ribofuranosides, antimicrobial activities.



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Introduction

The heterocyclic compounds, such as phenothiazines[1-6], sulfones[7-9] and ribofuranosides[10-12] are of immense importance not only biologically but also industrially. The majority of pharmaceutical products along with their biological activity[13-15] are often derived from heterocyclic structures. The substituted heterocycles can offer a high degree of structural diversity and have proven to be broadly useful as therapeutic agents. Therefore, development of synthetic drugs having phenothiazine nucleus is also an incessant process in continuation of our research to build up new synthetic drugs. A slight change in nature and position of substituents leads to diverse biological activities. Phenothiazines are of outmost significance due to its promising pharmacological activities such as antihistamines, antiemetics, antipyretics, anti-inflammatory, neuroleptics, tranquilizers, analgesic, bactericides, fungicides, diuretics, and various biological and pharmacological properties. The substituted phenothiazines used as heterocyclic base for the synthesis of nucleosides on treatment with sugar. These synthesized phenothiazines gives 10*H*-phenothiazines -5,5-dioxides (sulfones) on refluxing with H_2O_2 in glacial acetic acid. The synthesized sulfones and nucleosides have almost similar chemotherapeutic activities. The biologically active heterocyclic compounds were tested for their antimicrobial activities.

Experimental

Melting points were taken in open glass capillary tube using Gallenkamp melting point apparatus and are uncorrected. The purity of synthesized compound was checked by thin layer chromatography and visualized by UV light or in iodine chamber. The IR spectra were recorded in KBr on SHIMADZU 8400S FTIR spectrophotometer and wave no. is given in cm^{-1}. The ^1H and ^{13}C NMR were recorded on JEOL AL spectrometer in $CDCl_3$/DMSO-d_6 using TMS as an

internal standard at 300.15 and 75.47 MHz, respectively and chemical shift were measured in δ ppm. FAB (Fast Atom Bombarding) mass spectra were recorded on JEOL SX 102/DA 600 using Argon/Xenon gas. ^{19}F NMR spectra were recorded in $CDCl_3$ using CF_3COOH as standard compound. The elemental analysis(C, H and N) were performed using vario-III analyser at CDRI Lucknow. The commercially available substituted halonitobenzene were purchased from Sigma Aldrich and used without further purification and substituted 2-aminobenzenethiols **(1a-b)** were prepared according to method of R.R. Gupta *et al* [16].

Preparation of 2-Amino-2´-nitrodiphenylsulfides (3'a-d)

Substituted 2-Amino-5-fluorobenzenethiol **(1a)** or 2-Amino-6-fluoro-3-methyl benzenethiol **(1b)** , (0.01 mol) was dissolved in ethanol (20.0 mL) containing anhydrous sodium acetate (0.01 mole) and substituted *o*-halonitrobenzene i.e. 1,5-dichloro-2,4-dinitrobenzene **(2a)** or 2,4-dichloro-3-nitrobenzoic acid **(2b)** (0.01 mol) in ethanol (10.0 mL) was added in a 50 mL R.B. flask. This mixture was refluxed for 4-5 hrs, concentrated and cooled overnight in an ice bath. The solid separated out was filtered, washed with 30% ethanol and recrystallized from methanol.

Synthesis of 2-Formamido-2´-nitrodiphenylsulfides (3''a-d)

The 2-amino-2´-nitrodiphenylsulfides **(3'a-d)** (0.01 mol) obtained was refluxed for 4 hrs in 90% formic acid (20.0 mL). The contents were poured in a beaker containing crushed ice, solid separated out was filtered, washed with water until the filtrate was neutral and crystallized from benzene.

Preparation of 10H-phenothiazines (3a-d)

Formyl derivatives **(4a-d)** (0.01 mole) was refluxed in acetone (15.0 mL) and an alcoholic solution of KOH (0.2 gm of KOH in 5.0 mL ethanol) was added. The contents were heated for 30 minutes. A second lot of KOH (0.2 gm of KOH in 5.0 mL ethanol) was added to the reaction mixture and refluxed for about 4 hrs. Contents were then poured into a beaker containing crushed ice filtered, washed with cold water and finally with 30% ethanol and crystallized from benzene.

1-carboxy-3-chloro-6-fluoro-9-methyl-10H-phenothiazine (3a)

Black solid; m.p. : 210°C ; Yield : 62%, IR (KBr) : *ν* 3510 (N–H), a broad peak at 2970-2530 (COOH), 1072 (C-S), 1030 (C–F) and 770 cm^{-1} (C–Cl) ; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.45 (s, 1H, N–H), 7.40-6.38 (m, 4H, Ar-H), 11.33 (s, 1H, –COOH), 2.31 (s, 3H, protons of CH_3 at C-9); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 121.2 (C–1), 165.6 (COOH at C-1), 128.6 (C-2), 122.1 (C-3**),** 135.2 (C-4), 117.5 (C-6), 152.0 (C-7), 113.1 (C-8), 119.1 (C-9), 13.6 (CH_3 at C-9), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -30.127 (s, 1F at C-6); MS (FAB) 10 kV, m/z (rel. int.) : 295 $[M]^+$ (100), 294 $[M-H]^+$ (75), 259 $[M-Cl]^+$ (45), 230 $[M-CO_2H_2F]^+$ (85); 165 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{14}H_9ClFNO_2S$: C, 49.12; H, 2.63; N, 4.09; Found : C, 49.30; H, 2.68; N, 4.15".

8-chloro-4-fluoro-7-trifluoromethyl-1-methyl-10H-phenothiazine (3b)

Black solid; m.p. : 148°C ; Yield : 52%, IR (KBr) : *ν* 3490 (N–H), 1068 (C-S), 1040 (C–F), 765 cm^{-1} (C–Cl); ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.02 (s, 1H, N–H), 7.08-6.29 (m, 4H, Ar-H), 2.31 (s, 3H, protons of CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -62.127 (s, 1F at C-6); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 128.6 (C–1), 107.2 (C-2), 153.1 (C-3**),** 117.5 (C-4), 127.9 (C-6), 122.5 (C-7), 127.7 (C-8), 119.4 (C-9), 108.24 (CF_3 at C-7), 12.8 (CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -60.127 (s, 3F(CF_3) at C-7), δ -30.127 (s, 1F at C-4) ; MS (FAB) 10 kV, m/z (rel. int.) : 296 $[M]^+$ (100), 295 $[M-H]^+$ (72), 261 $[M-Cl]^+$ (50), 165 $[M-C_4H_2NO_2Cl]^+$ (75); "Anal. Calcd for $C_{14}H_8ClF_4NS$: C, 50.45; H, 2.40; N, 4.20 Found : C, 50.59; H, 2.42; N, 4.26".

8-chloro-4-fluoro-1-methyl-7-nitro-10H-phenothiazine (3c)

Brown solid; m.p. : 201°C ; Yield : 45%, IR (KBr) : *ν* 3530 (N–H), 1074 (C-S), 1048 cm^{-1} (C–F), ,775 cm^{-1} (C–Cl), and; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.12 (s, 1H, N–H), 7.73-6.43 (m, 4H, Ar-H), 2.31 (s, 3H, protons of CH_3 at C-1); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 120.7 (C–1), 127.6 (C-

2), 101.8 (C-3**),** 164.6 (C-4), 127.5 (C-6), 136.1 (C-7), 126.1 (C-8), 115.8 (C-9), 11.6 (CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -30.127 (s, 1F at C-4); MS (FAB) 10 kV, m/z (rel. int.) : 305 $[M]^+$ (100), 304 $[M-H]^+$ (75), 270 $[M-Cl]^+$ (45),; 170 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{13}H_8ClFN_2O_2S$: C, 50.32; H, 2.58; N, 9.03 Found : C, 50.48, H, 2.60; N, 9.08".

3-fluoro-7-methoxy-10H-phenothiazine (3d)

Brown solid; m.p. : 158°C ; Yield : 58%, IR (KBr) : *ν* 3490 (N–H), 1068 (C-S), 1055 (C-O), 1048 cm^{-1} (C–F), and 765 cm^{-1} (C–Cl); ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 8.91 (s, 1H, N–H), 6.83-6.33 (m, 6H, Ar-H), 3.14(s, 3H of OCH_3 at C-7); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 120.7 (C–1), 117.6 (C-2), 151.8 (C-3**),** 134.6 (C-4), 118.5 (C-6), 156.1 (C-7), 112.1 (C-8), 115.8 (C-9), 55.7 (OCH_3 at C-7), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -31.127 (s, 1F at C-3); MS (FAB) 10 kV, m/z (rel. int.) : 305 $[M]^+$ (100), 304 $[M-H]^+$ (75), 270 $[M-Cl]^+$ (45),; 170 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{13}H_{10}FNOS$: C, 63.16; H, 4.04; N, 5.66 Found : C, 63.36; H, 4.08; N, 5.69".

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

Substituted 10*H*-phenothiazine (**3a-d**, 0.01 mol), glacial acetic (20.0 mL) and 30% H_2O_2 (5.0 mL), were taken in a 50 mL round bottom flask and the whole mixture was refluxed for about 15-20 minutes at 50-60°C. Another lot of H_2O_2 (5.0 mL) was then added and refluxing was continued for about 4 hrs. Mixture was then poured into a beaker containing crushed ice. The obtained precipitate was filtered, washed with water and crystallized with ethanol.

1-carboxy-3-chloro-6-fluoro-9-methyl-10H-phenothiazine-5,5-oxide (4a):

Black solid; m.p. : 219°C ; Yield : 51%, IR (KBr) : *ν* 3520 (N–H), 1185 (SO_2 sym.), 582 (SO_2 bending), 1348 (SO_2 asym.), 2975-2540 (COOH), 1074 (C-S), 1034 (C–F) and 775 cm^{-1} (C–Cl) ; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.48 (s, 1H, N–H), 7.41-6.39 (m, 4H, Ar-H), 11.38 (s, 1H, –COOH), 2.36 (s, 3H, protons of CH_3 at C-9); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 123.2 (C–1), 168.6 (COOH at C-1), 129.6 (C-2), 124.1 (C-3**),** 138.2 (C-4), 118.5 (C-6), 154.0 (C-7), 115.1 (C-8), 120.1 (C-9), 14.6 (CH_3 at C-9), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -30.132 (s, 1F at C-6); MS (FAB) 10 kV, m/z (rel. int.) : 295 $[M]^+$ (100), 294 $[M-H]^+$ (75), 259 $[M-Cl]^+$ (45), 230 $[M-CO_2H_2F]^+$ (85); 165 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{14}H_9ClFNO_4S$: C, 54.36; H, 2.91; N, 4.53; Found : C, 54.51; H, 2.93; N, 4.55".

8-chloro-4-fluoro-7-trifluoromethyl-1-methyl-10H-phenothiazine-5,5-dioxide (4b)

Black solid; m.p. : 148°C ; Yield : 52%, IR (KBr) : *ν* 3505 (N–H), 1180 (SO_2 sym.), 585 (SO_2 bending), 1352 (SO_2 asym.),1069 (C-S), 1045 (C–F), 768 cm^{-1} (C–Cl); ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.08 (s, 1H, N–H), 7.12-6.32 (m, 4H, Ar-H), 2.35 (s, 3H, protons of CH_3 at C-1); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 129.6 (C–1), 108.2 (C-2), 153.1 (C-3**),** 119.5 (C-4), 129.9 (C-6), 122.9 (C-7), 128.7 (C-8), 119.4 (C-9), 108.8 (CF_3 at C-7), 12.9 (CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -61.127 (s, 3F(CF_3) at C-7), δ -31.127 (s, 1F at C-4) ; MS (FAB) 10 kV, m/z (rel. int.) : 296 $[M]^+$ (100), 295 $[M-H]^+$ (72), 261 $[M-Cl]^+$ (50), 165 $[M-C_4H_2NO_2Cl]^+$ (75); "Anal. Calcd for $C_{14}H_8ClF_4NO_2S$: C, 46.02; H, 2.19; N, 3.83 Found : C, 46.13; H, 2.23; N, 3.88".

8-chloro-4-fluoro-1-methyl-7-nitro-10H-phenothiazine-5,5-dioxide (4c):Brown solid; m.p. : 212°C ; Yield : 46%, IR (KBr) : *ν* 3536 (N–H), 1178 (SO_2 sym.), 575 (SO_2 bending), 1332 (SO_2 asym.),1072 (C-S), 1049 cm^{-1} (C–F), ,778 cm^{-1} (C–Cl), and; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.16 (s, 1H, N–H), 7.76-6.45 (m, 4H, Ar-H), 2.35 (s, 3H, protons of CH_3 at C-1); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 121.7 (C–1), 127.9 (C-2), 102.8 (C-3**),** 166.8 (C-4), 128.5 (C-6), 136.5 (C-7), 126.5 (C-8), 115.9 (C-9), 11.9 (CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -31.182 (s, 1F at C-4); MS (FAB) 10 kV, m/z (rel. int.) : 305 $[M]^+$ (100), 304 $[M-H]^+$ (75), 270 $[M-Cl]^+$ (45),; 170 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{13}H_8ClFN_2O_4S$: C, 45.61; H, 2.33; N, 8.18 Found : C, 45.72, H, 2.38; N, 8.21".

3-fluoro-7-methoxy-10H-phenothiazine-5,5-dioxide (4d):
Brown solid; m.p. : 169°C ; Yield : 52%, IR (KBr) : *ν* 3510 (N–H), 1175 (SO_2 sym.), 568 (SO_2 bending), 1336 (SO_2 asym.),1070 (C-S), 1058 (C-O), 1049 cm^{-1} (C–F), and 769 cm^{-1} (C–Cl); ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.01 (s, 1H, N–H), 6.91-6.38 (m, 6H, Ar-H), 3.19 (s, 3H of OCH_3 at C-7); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 120.9 (C–1), 117.8 (C-2), 152.8 (C-3**),** 136.6 (C-4), 120.5 (C-6), 157.1 (C-7), 113.1 (C-8), 115.9 (C-9), 56.7 (OCH_3 at C-7), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -32.107 (s, 1F at C-3); MS (FAB) 10 kV, m/z (rel. int.) : 305 $[M]^+$ (100), 304 $[M-H]^+$ (75), 270 $[M-Cl]^+$ (45),; 170 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{13}H_{10}FNO_3S$: C, 55.91; H, 3.58; N, 5.01 Found : C, 55.98; H, 3.62; N, 5.06".

General procedure for synthesis of substituted N-(2´,3´,5´-tri-O-benzoyl-β-D-ribofuranosyl) phenothiazines (5a-b)

To a concentrated solution of the synthesized 10*H*-phenothiazines **(3a and 3c)** (0.002 mole) in toluene, *β*-D-ribofuranose-1-acetate-2,3,5-tribenzoate **(7)** (0.002 mole) was added and stirred in vacuum, on an oil bath at 155-160°C for about 15-20 min. Reaction was protected from moisture by using a guard tube after vacuum was broken. Stirring continued for 10 hr with application of vacuum for every 15 minutes after every hour. The viscous residue thus obtained was dissolved in methanol, boiled for 10-15 minutes and cooled to room temperature. The precipitate was filtered and filtrate was evaporated to dryness. The viscous mass obtained was dissolved in ether, filtered, concentrated and refrigerated overnight and crystalline ribofuranosides are obtained.

N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-1-carboxy-3-chloro-6-fluoro-9-methyl-10H-phenothiazine (5a)
Black solid; m.p. : 210°C ; Yield : 62%, IR (KBr) : a broad peak at 2970-2530 (COOH), 1072 (C-S), 1030 (C–F) and 770 cm^{-1} (C–Cl) ; ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 8.40-6.38 (m, 29, Ar-H), 11.33 (s, 1H, –COOH), 2.31 (s, 3H, protons of CH_3 at C-9); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 121.2 (C–1), 165.6 (COOH at C-1), 128.6 (C-2), 122.1 (C-3**),** 135.2 (C-4), 117.5 (C-6), 152.0 (C-7), 113.1 (C-8), 119.1 (C-9), 13.6 (CH_3 at C-9), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -30.127 (s, 1F at C-6); MS (FAB) 10 kV, m/z (rel. int.) : 295 $[M]^+$ (100), 294 $[M-H]^+$ (75), 259 $[M-Cl]^+$ (45), 230 $[M-CO_2H_2F]^+$ (85); 165 $[M-C_5H_3O_2Cl]^+$ (70); "Anal. Calcd for $C_{40}H_{29}ClFNO_9S$: C, 63.74; H, 3.84; N, 1.86; Found : C, 63.94; H, 3.89; N, 1.90".

N-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-8-chloro-4-fluoro-7-trifluoromethyl-1-methyl-10H-phenothiazine (5b)
Black solid; m.p. : 148°C ; Yield : 52%, IR (KBr) : *ν* 3490 (N–H), 1068 (C-S), 1040 (C–F), 765 cm^{-1} (C–Cl); ^1H NMR spectral data (300.15 MHz, Me_2SO-d_6, δ ppm from TMS) : δ 9.02 (s, 1H, N–H), 7.08-6.29 (m, 4H, Ar-H), 2.31 (s, 3H, protons of CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -62.127 (s, 1F at C-6); ^{13}C NMR (75.47 MHz, $CDCl_3$, δ ppm from TMS) : δ 128.6 (C–1), 107.2 (C-2), 153.1 (C-3**),** 117.5 (C-4), 127.9 (C-6), 122.5 (C-7), 127.7 (C-8), 119.4 (C-9), 108.24 (CF_3 at C-7), 12.8 (CH_3 at C-1), ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -60.127 (s, 3F(CF_3) at C-7), δ -30.127 (s, 1F at C-4) ; MS (FAB) 10 kV, m/z (rel. int.) : 296 $[M]^+$ (100), 295 $[M-H]^+$ (72), 261 $[M-Cl]^+$ (50), 165 $[M-C_4H_2NO_2Cl]^+$ (75); "Anal. Calcd for $C_{14}H_8ClF_4NS$: C, 50.45; H, 2.40; N, 4.20 Found : C, 50.59; H, 2.42; N, 4.26".

Antimicrobial Activity

Broth microdilution method is used to calculate the minimum inhibitory concentrations (MICs, μg mL^{-1}) of the chemical compounds assays as per NCCLS-1992 manual. Two Gram-negative (*Escherichia coli* DH5 alpha MTCC 1786 and *Pseudomonas aeruginosa* ATCC 6624) and two Gram-positive (*Bacillus subtilis* MTCC-121and *Staphylococcus aureus* ATCC 25917) bacteria were used as quality control strains. *Aspergillus niger* NCIM 27821, *Penicillium funiculosum* NCIM 1174, *Fusarium oxysporum* NCIM-1228 and *Trichoderma reesei* NCIM-992 were the reference strains for testing antifungal activities of the compounds. Ampicillin sodium salt and Fluconazole were used as standard antibacterial and antifungal drugs, respectively. DMSO is used for preparing solutions of test compounds and standard drugs. Each synthesized drug was diluted obtaining 1000 μg/mL concentration as a stock solution. Now for primary screening 500, 250 and 125 μg/mL concentrations of the synthesized drugs were taken.

The synthesized drugs found active in this primary screening were further tested in a second set of dilution against all microorganisms in which the drugs were again diluted to obtain 100, 50, 25, 20, 15 µg/mL concentrations. The highest dilution showing at least 99% inhibition was taken as MIC. This means that the lowest concentration of each chemical compound in the tube with no growth (i.e. no turbidity) of inoculated bacteria/ fungi was recorded as minimal inhibitory concentration of that compound. Luria broth (Himedia) medium was used to carry out antibacterial activities of the bacterial strains while 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 3.262 xg (150 rpm). After incubation, MIC values were recorded. The MICs of tested compounds in µg/mL against certain strains of bacteria and fungi are shown in **Table 1**.

Table 1 Antimicrobial activity of synthesized compounds

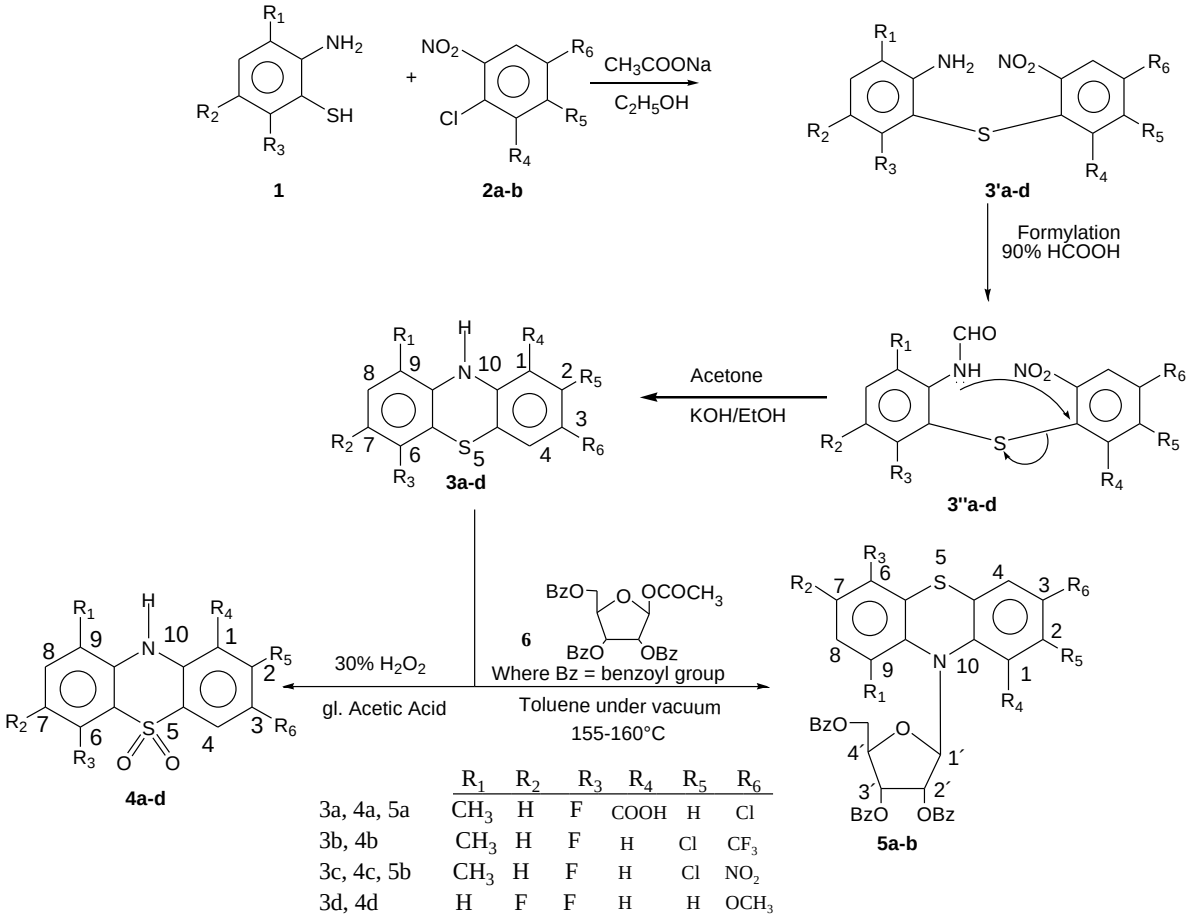
Compounds	Minimal Inhibition Concentrations of bacterial strains (MIC) in µg/mL				Minimal Inhibition Concentrations of fungal Strains (MIC) in µg/mL			
	***Bacillus subtilus* MTCC 121**	***Staphylococcus aureus* ATCC 25917**	***E. coli* DH 5 alpha MTCC 1786**	***Pseudomonas aeruginosa* ATCC 6624**	***Aspergillus niger* NCIM 27821**	***Penicillium funiculosum* NCIM 1174**	***Fusarium oxysporum* NCIM 1228**	***Trichoderma reesei* NCIM 992**
3a	32.6	54.5	58.4	54.5	32.4	61.3	68.2	50.1
3b	39.2	61.8	64.7	58.3	35.9	65.3	71.3	54.6
3c	35.2	57.9	60.2	56.2	34.2	63.8	69.4	52.8
3d	42.8	66.5	69.4	60.1	37.6	68.9	75.7	58.3
4a	22.4	48.9	52.4	48.9	28.2	57.3	64.6	46.7
4b	29.1	50.2	54.6	50.6	29.9	59.4	66.8	48.4
4c	28.5	49.1	53.8	49.3	29.1	58.1	65.4	47.2
4d	38.9	60.6	62.8	57.1	35.1	64.2	70.5	53.2
5a	30.8	55.3	55.7	51.3	30.2	60.1	67.3	49.7
5b	34.7	64.8	59.4	55.9	33.1	62.6	68.8	51.5
Ampicillin sodium salt	40	62	66	58	-	-	-	-
Fluconazole	-	-	-	-	36	65	72	55

Results and Discussion

Chemistry

The substituted 10*H*-phenothiazines **(3a-d)** were prepared by the reaction of substituted 2-aminobenzenethiols **(1a-b)** with substituted *o*-halonitrobenzene **(2a-b)**. A series of substituted 10*H*-phenothiazines can be prepared *via* Smiles rearrangement of substituted 2-formamido-2′-nitrodiphenylsulfides **(3''a-d)**. The formyl derivatives prepared by formylation of 2-amino-2'-nitrodiphenylsulfides **(3'a-d)** which was prepared by condensation of 2-Amino-5-fluorobenzenethiol **(1a)** or 2-Amino-6-fluoro-3-methylbenzenethiol **(1b)** , (0.01 mol) with halonitrobenzenes 1,5-dichloro-2,4-dinitrobenzene **(2a)** or 2,4-dichloro-3-nitrobenzoic acid **(2b)** in ethanolic sodium acetate solution. The synthesized10*H*-phenothiazines **(3a-d)** are treated with 30% H_2O_2 in glacial acetic acid to give corresponding sulfones **(4a-d)**. These prepared 10*H*-phenothiazines **(3a-3c)** were treated with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate in toluene solution stirred in vacuum on an oil bath of 150°-160°c for 8-10 hrs for the preparation of ribofuranosides **(5a-b)**.

The proposed structure of synthesized compounds is well supported by elemental analysis and spectral data.



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

Antimicrobial activity

Phenothiazines possess a wide range of biological properties due to their unique structure hence synthesis of phenothiazines and their derivatives covers an area of current interest. All the synthesized compounds **3(a-d)**, **4(a-d)** and **5(a-b)** were screened for antimicrobial activity by using broth microdilution method for determining their MIC (minimal inhibitory concentration) against four strains of bacteria (two Gram positive and two Gram negative) and four strains of fungi. Ampicillin sulfate has been used as standard antibacterial drug and Fluconazole as standard antifungal drug. Among all synthesized compounds, compound **4a, b, c** and **5a** proved to be the best antibacterial and anti fungal agents. Compounds **3a, c** and **5b** showed good activity. Rest of the compounds showed moderate activity.

Conclusion

Among the three different moieties under study i.e. 10*H*-phenothiazines, their sulfone derivatives and their nucleosides, it was observed that sulfone derivatives (**4a, b, c**) 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 statement we made by the results of our study that presence of an electron withdrawing group enhances the antimicrobial potency of chemical moieties is also well supported by literature[17-19].

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 anti bacterial 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.

Acknowledgments

The authors are extremely thankful to the Department of Chemistry, University of Rajasthan, Jaipur for providing necessary facilities. The authors are also grateful to the Institute of Seminal Applied Sciences, Jaipur for providing assistance in carrying out anti-microbial assessment.

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Publication History
Received 30th Oct 2014
Revised 13th Nov 2014
Accepted 20th Nov 2014
Online 30th Nov 2014