An efficient synthesis and biological study of substituted 8-chloro-5-methoxy/8-chloro-4H-1,4-benzothiazines, their sulphones and ribofuranosides

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Abstract. 8-Chloro-5-methoxy/8-chloro-4H-1,4-benzothiazines were synthesized by condensation followed by oxidative cyclisation of 2-amino-6-chloro-3-methoxy/2-amino-3-chlorobenzenethiol with β -diketones/ β -ketoesters in the presence of dimethyl sulphoxide. By treating 4H-1,4-benzothiazines with 30% hydrogen peroxide in glacial acetic acid, 4H-1,4-benzothiazine-1,1-dioxides (sulphones) were synthesized. The 4H-1,4-benzothiazines have also been used as a base to prepare ribofuranosides by the reaction with β -D-ribofuranose-1-acetate-2,3,5-tribenzoate. All the synthesized compounds have been characterized by spectral and elemental analysis and have been examined for antimicrobial and anthelmintic activity.

Keywords. 4H-1,4-benzothiazines; sulphones; ribofuranosides; antimicrobial activity; anthelmintic activity.

1. Introduction

Six-membered heterocyclic compounds have occupied a prominent place among various classes of organic compounds for their diverse biological activities. Among a wide variety of heterocycles that have been explored developing pharmaceutically important molecules, 4H-1,4-benzothiazines,^{1–8} their sulphones and ribofuranosides have played an important role in medicinal chemistry. Benzothiazines possess a fold along nitrogen–sulphur axis which is one of the structural specificities responsible for pharmacological activities.^{9–15} It has stimulated our interest to convert benzothiazines to sulphones to understand oxidation behaviour of 4H-1,4-benzothiazines and to investigate changes in infrared and nuclear magnetic resonance spectra caused by the conversion of sulphide linkage to sulphones. 4H-1,4-benzothiazines serve as heterocyclic base for the formation of ribofuranosides on treatment with sugar (β -D-ribofuranosyl-1-acetate-2,3,5-tribenzoate). All these compounds are extensively used as sedatives, anthelmintic, antiulcer, anticancer, antibacterial, antifungal, etc. A slight change in the substitution pattern in the benzothiazine nucleus cause distinguishable difference in their biological activities. The newly synthesized compounds have been screened for antimicrobial and anthelmintic activity.

2. Materials and methods

2.1 Experimental

All the melting points were determined in an open capillary tubes and are uncorrected. ¹H NMR and ¹³C NMR spectra (by broad band proton decoupling technique) were recorded on JEOL AL-300 spectrometer at frequencies of (300.40 MHz and 75.45 MHz) respectively, in dimethylsulphoxide-d₆ using TMS (tetramethyl silane) as an internal standard. 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. ¹⁹F NMR spectra were recorded in CDCl₃ using CF₃COOH as standard compound. Purity of synthesized compounds was checked by TLC (thin-layer chromatography) using silica gel 'G' as adsorbent, visualizing these by UV light or in an iodine chamber.

2.2 General procedure of synthesis of substituted 4H-1,4-benzothiazines (3a–d)

To 2-amino-5-chloro-2-methoxy/3-chlorobenzenethiol (0.01 mol) (**1a–b**), a stirred suspension of the appropriate β -diketones/ β -ketoesters (**2a–d**) in DMSO (5 mL) was added and the resulting mixture was refluxed for 2–3 h. The reaction mixture was concentrated, cooled

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down to room temperature and filtered. The product was washed with petroleum ether and crystallized from methanol.

2.2a 8-Chloro-2-trifluoroacetyl-3-trifluoromethyl-5-methoxy-4H-1,4-benzothiazine (3a): Brown solid; m.p.: 65°C; yield: 56%, IR (KBr): ν 3350 (N-H), 1595 ($>C=O$), 2965 and 2875 ($-OCH_3$), 1285 and 1020 (C-O-C), 1335 and 1100 cm^{-1} ($-CF_3$) and 780 cm^{-1} (C-Cl); 1H NMR spectral data (300.40 MHz, Me_2SO-d_6 , δ ppm from TMS): δ 8.93 (s, 1H, N-H), 8.10–6.39 (m, 2H, Ar-H), 3.87 (s, 3H, $-OCH_3$); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 116.2 (C-2), 196.5 (C=O at C-2), 128.8 (CF_3 at C-2), 136.3 (C-3), 114.9 (CF_3 at C-3), 147.0 (C-5), 56.0 (OCH_3 at C-5), 113.1 (C-6), 120.2 (C-7), 127.6 (C-8); ^{19}F NMR (282.65 MHz, $CDCl_3$): δ -61.93 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 377 $[M]^+$, 379 $[M+2]^+$, 279 (41), 376 (27), 97 (100), 281 (56), 248 (36); 'Anal. Calcd for $C_{12}H_6NO_2F_6S$: C, 38.14; H, 1.59; N, 3.71, Found: C, 38.46; H, 1.60; N, 3.73'.

2.2b Ethyl-8-chloro-5-methoxy-3-propyl-4H-1,4-benzothiazine-2-carboxylate (3b): Red solid; m.p.: 90°C; yield: 58%, IR (KBr): ν 3310 (N-H), 1590 ($>C=O$), 2955 and 2830 ($-OCH_3$), 1280 and 1030 (C-O-C), and 750 cm^{-1} (C-Cl); 1H NMR spectral data (300.40 MHz, Me_2SO-d_6 , δ ppm from TMS): δ 8.73 (s, 1H, N-H), 8.02–6.34 (m, 2H, Ar-H), 3.86 (s, 3H, $-OCH_3$), 2.51 (t, 2H, CH_2 (terminal) of C_3H_7 at C-3), 1.60 (sextet, 2H, CH_2 (centre) of C_3H_7 at C-3), 1.20 (t, 3H, CH_3 of C_3H_7 at C-3), 4.95 (q, 2H, CH_2 of OC_2H_5 at C-2), 1.78 (t, 3H, CH_3 of OC_2H_5 at C-2); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): δ 105.8 (C-2), 165.0 (C=O at C-2), 59.2 (CH_2 of OC_2H_5 at C-2), 13.4 (CH_3 of OC_2H_5 at C-2), 143.5 (C-3), 33.8 (CH_2 (terminal) of C_3H_7 at C-3), 17.5 (CH_2 (middle) of C_3H_7 at C-3), 14.4 (CH_3 (terminal) of C_3H_7 at C-3), 146.1 (C-5), 56.4 (OCH_3 at C-5), 112.1 (C-6), 119.2 (C-7), 126.2 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 327 $[M]^+$, 329 $[M+2]^+$, 326 (48), 253 (36), 248 (43), 73 (100), 221 (63); 'Anal. Calcd for $C_{15}H_{18}NO_3ClS$: C, 54.96; H, 5.49; N, 4.27, Found: C, 55.27; H, 5.51; N, 4.34'.

2.2c Methyl-8-chloro-3-ethyl-5-methoxy-4H-1,4-benzothiazine-2-carboxylate (3c): Red solid; m.p.: 80°C; yield: 63%, IR (KBr): ν 3300 (N-H), 1580 ($>C=O$), 2960 and 2846 ($-OCH_3$), 1252 and 1035 (C-O-C) and 740 cm^{-1} (C-Cl); 1H NMR spectral data (300.40 MHz, Me_2SO-d_6 , δ ppm from TMS): δ 8.68 (s, 1H, N-H), 8.45–6.42 (m, 2H, Ar-H), 4.01 (s, 3H,

$-OCH_3$ at C-2), 2.42 (q, 2H, CH_2 of C_2H_5 at C-3), 1.45 (t, 3H, CH_3 of C_2H_5 at C-3), 3.72 (s, 3H, $-OCH_3$ at C-5); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 108.1 (C-2), 164.9 (C=O at C-2), 50.3 (CH_3 at C-2), 141.6 (C-3), 24.0 (CH_2 of C_2H_5 at C-3), 8.7 (CH_3 of C_2H_5 at C-3), 55.9 (OCH_3 at C-5), 147.3 (C-5), 113.2 (C-6), 120.1 (C-7), 127.4 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 299 $[M]^+$, 301 $[M+2]^+$, 298 (28), 270 (73), 264 (31), 240 (65), 59 (100); 'Anal. Calcd for $C_{13}H_{14}NO_3S$: C, 52.08; H, 4.67; N, 4.67, Found: C, 52.23; H, 4.71; N, 4.75'.

2.2d 5-Chloro-3-ethyl-2-propionyl-4H-1,4-benzothiazine (3d): Brown solid; m.p.: 70°C; yield: 62%, IR (KBr): ν 3290 (N-H), 1585 ($>C=O$), 2980 (CH_3), and 720 cm^{-1} (C-Cl); 1H NMR spectral data (300.40 MHz, Me_2SO-d_6 , δ ppm from TMS): δ 8.44 (s, 1H, N-H), 8.32–6.16 (m, 3H, Ar-H), 3.17 (q, 2H, CH_2 of C_2H_5 at C-2), 1.81 (t, 3H, CH_3 of OC_2H_5 at C-2), 2.51 (q, 2H, CH_2 of C_2H_5 at C-3), 1.31 (t, 3H, CH_3 of C_2H_5 at C-3); ^{13}C NMR (75.45 MHz, $CDCl_3$, δ ppm from TMS): 115.5 (C-2), 197.6 (C=O at C-2), 30.5 (CH_2 of C_2H_5 at C-2), 7.1 (CH_3 of C_2H_5 at C-2), 141.1 (C-3), 24.2 (CH_2 of C_2H_5 at C-3), 8.6 (CH_3 of C_2H_5 at C-3), 120.8 (C-5), 126.6 (C-6), 120.3 (C-7), 128.0 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 267 $[M]^+$, 269 $[M+2]^+$, 266 (22), 238 (44), 210 (58), 232 (76), 57 (100); 'Anal. Calcd for $C_{13}H_{14}NOS$: C, 58.31; H, 5.23; N, 5.23, Found: C, 53.58; H, 5.26; N, 5.28'.

2.3 General procedure of synthesis of 4H-1,4-benzothiazine sulphones (4a–d)

A mixture of substituted 4H-1,4-benzothiazines (**3a–d**) (0.01 mol), glacial acetic acid (20 mL) and 30% hydrogen peroxide (5 mL) was refluxed for 15 min. Heating was stopped and another lot of hydrogen peroxide (5 mL) was added. The reaction mixture was again refluxed for further 4 h. The contents were poured into a beaker containing crushed ice. Residue obtained was filtered and washed with water and recrystallized from ethanol.

2.3a 8-Chloro-2-trifluoroacetyl-3-trifluoromethyl-5-methoxy-4H-1,4-benzothiazine-1,1-dioxide (4a): Brown solid; m.p.: 280°C; yield: 48%, IR (KBr): ν 3380 (N-H), 1645 (C=O), 2950 and 2838 ($-OCH_3$), 1250 and 1030 (C-O-C), 1375, 1120 ($-CF_3$), 790 (C-Cl), 1200 and 1140 (SO_2 sym), 1080 cm^{-1} (C-S); 1H NMR spectral data (300.40 MHz, Me_2SO-d_6 , δ ppm from TMS): δ 9.11 (s, 1H, N-H), 8.25–6.28 (m, 2H, Ar-H), 3.91 (s, 3H, $-OCH_3$); ^{13}C NMR (75.45 MHz,

CDCl_3 , δ ppm from TMS): δ 100 (C-2), 195.6 (C=O at C-2), 129.1 (CF_3 at C-2), 154.3 (C-3), 115.6 (CF_3 at C-3), 146.5 (C-5), 55.2 (OCH_3 at C-5), 121.3 (C-6), 119.2 (C-7), 124.6 (C-8); ^{19}F NMR (282.65 MHz, CDCl_3): δ -62.11 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 409 $[\text{M}]^+$, 411 $[\text{M}+2]^+$, 311 (54), 340 (38), 313 (68), 97 (100); 'Anal. Calcd for $\text{C}_{12}\text{H}_6\text{NO}_4\text{F}_6\text{SCl}$: C, 35.16; H, 1.46; N, 3.41, Found: C, 35.48; H, 1.50; N, 3.47'.

2.3b Ethyl-8-chloro-5-methoxy-3-propyl-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (4b): Reddish yellow solid; m.p.: 293°C; yield: 50%, IR (KBr): ν 3340 (N-H), 2973 and 2870 ($-\text{OCH}_3$), 1630 ($>\text{C}=\text{O}$), 1180, 1150 (SO_2 sym), 1085 (C-S) and 735 cm^{-1} (C-Cl); ^1H NMR spectral data (300.40 MHz, $\text{Me}_2\text{SO}-d_6$, δ ppm from TMS): δ 8.97 (s, 1H, N-H), 8.07–6.44 (m, 2H, Ar-H), 4.43 (s, 3H, $-\text{OCH}_3$), 2.23 (t, 2H, CH_2 (terminal) of C_3H_7 at C-3), 1.45 (sextet, 2H, CH_2 (middle) of C_3H_7 at C-3), 1.65 (t, 3H, CH_3 of C_3H_7 at C-3), 4.22 (q, 2H, CH_2 of OC_2H_5 at C-2), 1.89 (t, 3H, CH_3 of OC_2H_5 at C-2); ^{13}C NMR (75.45 MHz, CDCl_3 , δ ppm from TMS): δ 99 (C-2), 197.5 (C=O at C-2), 30.2 (CH_2 of OC_2H_5 at C-2), 7.4 (CH_3 of OC_2H_5 at C-2), 158 (C-3), 33.8 (CH_2 (terminal) of C_3H_7 at C-3), 18.0 (CH_2 (middle) of C_3H_7 at C-3), 14.1 (CH_3 (terminal) of C_3H_7 at C-3), 147.1 (C-5), 56.4 (OCH_3 at C-5), 121.1 (C-6), 120.9 (C-7), 124.9 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 359 $[\text{M}]^+$, 361 $[\text{M}+2]^+$, 358 (20), 286 (39), 316 (28), 73 (100); 'Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{NO}_5\text{ClS}$: C, 50.06; H, 5.00; N, 3.89, Found: C, 50.31; H, 5.06; N, 3.93'.

2.3c Methyl-8-chloro-3-ethyl-5-methoxy-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (4c): Brown solid; m.p.: 275°C; yield: 46%, IR (KBr): ν 3360 (N-H), 1610 ($>\text{C}=\text{O}$), 2965 and 2865 ($-\text{OCH}_3$) and 760 cm^{-1} (C-Cl); ^1H NMR spectral data (300.40 MHz, $\text{Me}_2\text{SO}-d_6$, δ ppm from TMS): δ 8.91 (s, 1H, N-H), 8.65–6.72 (m, 2H, Ar-H), 3.96 (s, 3H, $-\text{OCH}_3$ at C-2), 2.58 (q, 2H, CH_2 of C_2H_5 at C-3), 1.59 (t, 3H, CH_3 of C_2H_5 at C-3), 3.82 (s, 3H, $-\text{OCH}_3$ at C-5); ^{13}C NMR (75.45 MHz, CDCl_3 , δ ppm from TMS): 92 (C-2), 165.2 (C=O of OCH_3 at C-2), 50.7 (CH_3 at C-2), 159.1 (C-3), 23.6 (CH_2 of C_2H_5 at C-3), 9.1 (CH_3 of C_2H_5 at C-3), 56.3 (OCH_3 at C-5), 147.5 (C-5), 121.2 (C-6), 121.0 (C-7), 124.9 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 331 $[\text{M}]^+$, 333 $[\text{M}+2]^+$, 330 (25), 302 (71), 296 (36), 272 (61), 59 (100); 'Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{NO}_5\text{SCl}$: C, 47.05; H, 4.22; N, 4.22, Found: C, 47.29; H, 4.26; N, 4.33'.

2.3d 5-Chloro-3-ethyl-2-propionyl-4H-1,4-benzothiazine-1,1-dioxide (4d): Black solid; m.p.: 286°C; yield: 52%, IR (KBr): ν 3310 (N-H), 1645 ($>\text{C}=\text{O}$), 2985 (CH_3), and 740 cm^{-1} (C-Cl); ^1H NMR spectral data (300.40 MHz, $\text{Me}_2\text{SO}-d_6$, δ ppm from TMS): δ 8.77 (s, 1H, N-H), 8.51–6.84 (m, 3H, Ar-H), 3.11 (q, 2H, CH_2 of C_2H_5 at C-2), 1.31 (t, 3H, CH_3 of OC_2H_5 at C-2), 2.65 (q, 2H, CH_2 of C_2H_5 at C-3), 1.43 (t, 3H, CH_3 of C_2H_5 at C-3); ^{13}C NMR (75.45 MHz, CDCl_3 , δ ppm from TMS): 99.1 (C-2), 197.8 (C=O of OC_2H_5 at C-2), 30.3 (CH_2 of C_2H_5 at C-2), 7.6 (CH_3 of C_2H_5 at C-2), 158.1 (C-3), 23.9 (CH_2 of C_2H_5 at C-3), 9.1 (CH_3 of C_2H_5 at C-3), 121.5 (C-5), 135.0 (C-6), 120.8 (C-7), 125.5 (C-8); MS (FAB) 10 kV, m/z (rel. int.): 299 $[\text{M}]^+$, 301 $[\text{M}+2]^+$, 298 (28), 270 (46), 242 (55), 264 (70), 57 (100); 'Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{NO}_3\text{SCl}$: C, 52.08; H, 4.67; N, 4.67, Found: C, 52.23; H, 4.70; N, 4.76'.

2.4 General procedure for the synthesis of substituted N-(2',3',5'-tri-O-benzoyl- β -D-ribofuranosyl) benzothiazines (5a, b)

To the solution of 4H-1,4-benzothiazine (**3a, b**) (0.002 mol) in 5 mL toluene, β -D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002 mol) was added and the contents were refluxed under vacuum with stirring on an oil bath at 155–160°C for 15 min. The vacuum was removed and reaction mixture was protected from moisture by fitting a guard tube. Stirring was further continued for 10 h and a vacuum was applied for 10 min after every 1 h. The viscous mass obtained was dissolved in methanol, boiled for 10 min and cooled to room temperature. The reaction mixture was filtered and the filtrate was evaporated to dryness. The viscous residue thus obtained was dissolved in ether, filtered, concentrated and kept in refrigerator overnight to afford crystalline ribofuranosides.

2.4a N-(2',3',5'-tri-O-benzoyl- β -D-ribofuranosyl)-8-chloro-2-trifluoroacetyl-3-trifluoromethyl-5-methoxy-4H-1,4-benzothiazine (5a): Red solid; m.p.: 86°C; yield: 78%, IR (KBr): ν 1700 ($>\text{C}=\text{O}$), 1170 (C-O-C), 1355 and 1170 cm^{-1} ($-\text{CF}_3$) and 795 cm^{-1} (C-Cl); ^1H NMR spectral data (300.40 MHz, $\text{Me}_2\text{SO}-d_6$, δ ppm from TMS): δ 8.56–7.54 (m, 2H, Ar-H), 3.80 (s, 3H, $-\text{OCH}_3$); ^{13}C NMR (75.45 MHz, CDCl_3 , δ ppm from TMS): δ 116.4 (C-2), 196.9 (C=O at C-2), 129.0 (CF_3 at C-2), 136.7 (C-3), 114.5 (CF_3 at C-3), 145.6 (C-5), 56.3 (OCH_3 at C-5), 113.5 (C-6), 118.8 (C-7), 128.1 (C-8), 80.2 (C-1'), 74.1 (C-2'), 70.6 (C-3'), 69.5 (C-4'); ^{19}F NMR (282.65 MHz, CDCl_3): δ -62.35 (s, 3F, CF_3); MS (FAB) 10 kV, m/z (rel. int.): 821 $[\text{M}]^+$, 823

[M+2]⁺, 723 (49), 820 (34), 97 (100), 725 (65), 692 (53); 'Anal. Calcd for C₃₈H₂₆NO₉F₆SCl: C, 55.51; H, 3.16; N, 1.71 Found: C, 55.76; H, 3.19; N, 1.81'.

2.4b *N*-(2',3',5'-tri-*O*-benzoyl-β-*D*-ribofuranosyl)-ethyl-8-chloro-5-methoxy-3-propyl-4*H*-1,4-benzothiazine-2-carboxylate (**5b**): Red solid; m.p.: 79°C; yield: 85%, IR (KBr): ν 1680 (>C=O), 1160 (C–O–C), and 810 cm^{−1} (C–Cl); ¹H NMR spectral data (300.40 MHz, Me₂SO-*d*₆, δ ppm from TMS): δ 8.32–7.13 (m, 2H, Ar-H), 3.78 (s, 3H, –OCH₃), 1.95 (t, 2H, CH₂ (terminal) of C₃H₇ at C-3), 1.36 (sextet, 2H, CH₂ (centre) of C₃H₇ at C-3), 0.94 (t, 3H, CH₃ of C₃H₇ at C-3), 4.20 (q, 2H, CH₂ of OC₂H₅ at C-2), 1.30 (t, 3H, CH₃ of OC₂H₅ at C-2); ¹³C NMR (75.45 MHz, CDCl₃, δ ppm from TMS): δ 106.0 (C-2), 165.2 (C=O at C-2), 58.9 (CH₂ of OC₂H₅ at C-2), 13.8 (CH₃ of OC₂H₅ at C-2), 144.0 (C-3), 31.8 (CH₂ (terminal) of C₃H₇ at C-3), 18.1 (CH₂ (middle) of C₃H₇ at C-3), 13.8 (CH₃ (terminal) of C₃H₇ at C-3), 144.5 (C-5), 56.1 (OCH₃ at C-5), 113.1 (C-6), 118.8 (C-7), 127.6 (C-8), 81.4 (C-1'), 75.5 (C-2'), 72.2 (C-3'), 70.3 (C-4'); MS (FAB) 10 kV, *m/z* (rel. int.): 771 [M]⁺, 773 [M+2]⁺, 770 (41), 697 (30), 692 (54), 73 (100), 665 (60); 'Anal. Calcd for C₄₁H₃₈NO₁₀SCl : C, 63.77; H, 4.93; N, 1.81, Finds: C, 63.98; H, 4.98; N, 1.95'.

3. Biological activity

3.1 *In vitro* antibacterial and antifungal activity

3.1a Antibacterial activity: Antibacterial activity was tested against *S. aureus*, *B. subtilis* (Gram positive) and *E. coli*, *Pseudomonas aeruginosa* (Gram negative) microorganisms using paper disc diffusion method using nutrient agar medium. The paper disc diffusion method of assay of drug potency is based on the measurement of the zone of microbial growth inhibition surroundings discs containing various concentrations of test compounds, which are placed on the surface of a solid nutrient previously inoculated with the culture of suitable microorganism. Inhibition produced by the test drug is compared with that produced by known concentration of reference standard.

In this method, paper disc impregnated with compounds dissolved in solvent DMF at concentrations 25, 50 and 100 µg mL^{−1}. Then the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strain. The plates were incubated at 35°C for 24 h for bacterial cultures. After incubation, the zones of inhibition around the

disc were observed. Each testing is done in triplicate. Ciprofloxacin at conc. 50 µg mL^{−1} was used as standard drug for antibacterial activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The % activity index for the compound was calculated by the formula as under:

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

3.1b Antifungal activity: Antifungal activity of synthesized compounds was tested on following fungal strains: *A. niger*, *C. albican*, using disc diffusion method. In the disc-diffusion method, disc impregnated with compounds dissolved in solvent DMF at concentrations 25, 50 and 100 µg mL^{−1} 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 observed. Growth inhibiting zone indicates that the compounds inhibit growth of microorganism. Each experiment is done in triplicate. Griseofulvin at concentration 50 µg mL^{−1} was used as standard drug for antifungal activity. Results were interpreted in terms of diameter (mm) of zone of inhibition. The percentage inhibition was calculated by the following equation

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

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

3.2 Minimal inhibitory concentrations

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of antimicrobials that will inhibit the visible growth of a microorganism after overnight incubation at 37°C. Determination of the MIC is a semiquantitative test, which gives an approximate idea of the least concentration of an antimicrobial (test) solution needed to prevent 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. *Candida albicans* and *Aspergillus niger* were tested for the antifungal activities of the compounds. Ciprofloxacin and Griseofulvin were used as standard antibacterial and antifungal agents. The stock solutions of test compounds with 1 to 20 µg mL^{−1}

Compound	R ₁	R ₂	R ₃	R ₄	Compounds	Yield range
3a, 4a, 5a	OCH ₃	Cl	CF ₃	CF ₃	3a-d	56-63%
3b, 4b, 5b	OCH ₃	Cl	C ₃ H ₇	OC ₂ H ₅	4a-d	46-52%
3c, 4c	OCH ₃	Cl	C ₂ H ₅	OCH ₃	5a-b	78-85%
3d, 4d	Cl	H	C ₂ H ₅	C ₂ H ₅		

Scheme 1. Synthesis of substituted 4H-1,4-benzothiazines.

concentrations were prepared with aqueous methanol. Inoculums of the overnight culture were prepared. In a series of tubes, 1 mL each of stock solutions of test compound with different concentration are 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 and observed for the presence of turbidity. The absorbance of the suspension of the inoculums was observed with spectrophotometer at 555 nm. The end result of the test was the minimum concentration of antimicrobial (test) solutions, which gave clear solution, i.e., no visual growth.

3.3 Anthelmintic activity

Anthelmintic studies were carried out against *Eudrilus* species of earthworms by Garg and Atal method

at 4 µgmL⁻¹ concentrations. 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. The suspensions were diluted to contain 0.4% w/v of the test samples. Suspension of reference drug mebendazole was prepared with same concentration in a similar way. Three sets of five earthworms of almost similar sizes (3 inch in length) were placed in petri plates of 4 inch diameter containing 50 mL of suspension of test sample and reference drug at room temperature. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and Tween 80 (0.5%). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50°C), which stimulate the movement, if the worm was alive.

Figure 1. % Activity index of *E. coli* and *B. subtilis*.

Figure 2. % Activity index of *P. aeruginosa* and *S. aureus*.

Figure 3. % Inhibition of *C. albicans* and *A. niger*.

4. Results and discussion

Substituted 2-amino-6-chloro-3-methoxy/2-amino-3-chlorobenzenethiol (**1a–b**) and the appropriate β -diketones/ β -ketoesters (**2a–d**) were refluxed in dimethyl sulphoxide which results in oxidative cyclization. This reaction proceeds with the formation of an intermediate enaminoketone. The bis-(2-aminophenyl)disulphides were obtained which then cyclizes to 4H-1,4-benzothiazine (**3a–d**) by the scission of sulphur–sulphur bond, due to high reactivity of α -position of enaminoketone system towards nucleophilic attack. On refluxing the compound (**3a–d**), with 30% hydrogen peroxide in glacial acetic acid, their corresponding sulphones (**4a–d**) were obtained. Compounds (**3a–b**) were treated with β -D-ribofuranose-1-acetate-2,3,5-tribenzoate in toluene stirred in vacuum in an oil bath at 155–160°C for 10 h to form their ribofuranosides (**5a–b**). The structures proposed for the synthesized compounds were elucidated by spectroscopic data and elemental analysis. All the synthesized

compounds were also screened for their antioxidant and antimicrobial activities (scheme 1).

A series of novel heterocyclic compounds (**3a–d**) and (**4a–d**) were synthesized. All the compounds tested against for their *in vitro* antibacterial activity against the four strains of bacteria (two Gram–ve *E.coli*, *Pseudomonas aeruginosa* and two Gram+ve *Bacillus subtilis*, *Staphylococcus aureus*) and antifungal activity against the two strains of fungi (*C. albicans* and *A. niger*). The results have been summarized in figures 1, 2 and 3. The results show that all the compounds act as antimicrobial agent against selected bacteria and fungi. The antibacterial and antifungal activities increase with increase concentration of test compounds. The minimum inhibitory concentration (MIC) of the compounds varies from 9.25 to 17.30 $\mu\text{g mL}^{-1}$ (tables 1 and 2). Among compounds of the obtained series, **3a**, **4a**, **4c** and **5a** showed good activity and remaining showed moderate activity against bacteria *E. coli*. Compounds **4a** showed good and remaining moderate activity against bacteria *B. subtilis*. For bacteria *P. aeruginosa*,

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

Compound No.	Bacterial minimal inhibitory concentrations ($\mu\text{g mL}^{-1}$)			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
3a	10.10 $\pm$ 0.22	12.00 $\pm$ 0.10	10.15 $\pm$ 0.27	11.30 $\pm$ 0.09
3b	13.20 $\pm$ 0.26	16.10 $\pm$ 0.15	11.40 $\pm$ 0.23	17.30 $\pm$ 0.32
3c	13.00 $\pm$ 0.17	15.80 $\pm$ 0.27	11.15 $\pm$ 0.31	16.40 $\pm$ 0.33
3d	14.15 $\pm$ 0.35	16.70 $\pm$ 0.20	12.16 $\pm$ 0.19	17.26 $\pm$ 0.17
4a	09.36 $\pm$ 0.28	11.10 $\pm$ 0.19	09.25 $\pm$ 0.24	10.52 $\pm$ 0.19
4b	11.30 $\pm$ 0.20	15.50 $\pm$ 0.22	11.09 $\pm$ 0.18	16.70 $\pm$ 0.27
4c	11.05 $\pm$ 0.29	15.15 $\pm$ 0.26	10.80 $\pm$ 0.35	15.94 $\pm$ 0.33
4d	12.50 $\pm$ 0.32	16.30 $\pm$ 0.16	12.45 $\pm$ 0.28	16.90 $\pm$ 0.25
5a	09.78 $\pm$ 0.19	11.82 $\pm$ 0.29	09.67 $\pm$ 0.34	11.00 $\pm$ 0.24
5b	12.22 $\pm$ 0.31	15.72 $\pm$ 0.17	11.16 $\pm$ 0.25	17.12 $\pm$ 0.21
Ciprofloxacin	04.10 $\pm$ 0.10	04.90 $\pm$ 0.13	03.85 $\pm$ 0.15	04.90 $\pm$ 0.11

Table 2. MIC ($\mu\text{g mL}^{-1}$) of synthesized compounds.

Compound No.	Fungal minimal inhibitory concentrations ($\mu\text{g mL}^{-1}$)	
	<i>C. albicans</i>	<i>A. niger</i>
3a	10.40 $\pm$ 0.26	10.13 $\pm$ 0.31
3b	13.10 $\pm$ 0.22	15.40 $\pm$ 0.16
3c	12.35 $\pm$ 0.19	15.90 $\pm$ 0.29
3d	14.30 $\pm$ 0.27	16.10 $\pm$ 0.32
4a	10.00 $\pm$ 0.35	09.39 $\pm$ 0.17
4b	11.20 $\pm$ 0.21	15.00 $\pm$ 0.26
4c	10.90 $\pm$ 0.23	14.30 $\pm$ 0.32
4d	13.75 $\pm$ 0.31	15.60 $\pm$ 0.20
5a	10.26 $\pm$ 0.18	09.77 $\pm$ 0.29
5b	12.13 $\pm$ 0.34	15.14 $\pm$ 0.21
Griseofulvin	03.10 $\pm$ 0.08	04.80 $\pm$ 0.10

Table 3. Anthelmintic activity of synthesized compound.

Compound No.	Time (min)					
	Paralyzing time			Death time		
	200 mgmL ⁻¹	100 mgmL ⁻¹	50 mgmL ⁻¹	200 mgmL ⁻¹	100 mgmL ⁻¹	50 mgmL ⁻¹
3a	11.00 ± 0.67	14.83 ± 0.83	21.55 ± 1.00	15.53 ± 0.69	20.50 ± 0.50	31.03 ± 0.90
3b	15.00 ± 0.77	17.00 ± 1.00	23.45 ± 0.93	19.31 ± 1.00	27.16 ± 0.76	35.75 ± 0.57
3c	13.26 ± 1.08	16.00 ± 1.10	22.50 ± 0.50	18.16 ± 0.76	26.10 ± 0.73	34.50 ± 0.50
3d	15.16 ± 1.24	18.50 ± 1.00	24.56 ± 0.70	20.16 ± 0.90	29.16 ± 0.67	38.83 ± 0.98
4a	08.79 ± 0.67	13.50 ± 1.00	19.48 ± 0.77	13.15 ± 0.67	19.00 ± 0.66	28.16 ± 0.76
4b	12.85 ± 1.22	16.66 ± 0.94	22.78 ± 0.60	15.40 ± 0.77	24.65 ± 0.79	35.23 ± 0.91
4c	12.00 ± 1.00	15.00 ± 0.90	22.20 ± 1.00	20.63 ± 0.62	24.13 ± 1.09	34.12 ± 0.60
4d	13.16 ± 0.75	18.00 ± 0.78	25.00 ± 0.70	19.12 ± 0.60	27.08 ± 1.00	38.43 ± 1.00
5a	09.54 ± 0.88	14.00 ± 1.10	20.03 ± 0.68	15.16 ± 1.24	19.50 ± 0.50	28.90 ± 1.05
5b	12.20 ± 1.00	16.50 ± 1.20	23.36 ± 1.00	17.33 ± 1.52	27.00 ± 1.10	35.50 ± 0.89
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

compounds **3a**, **4a**, **4c** and **5a** showed good activity and remaining showed moderate activity. Compounds **4a** and **5a** showed good and remaining showed moderate activity against bacteria *S. aureus*.

Compounds **3a**, **4a**, **4c** and **5a** showed good activity against fungi *C. albicans*. For fungi *A. niger*, compounds **3a**, **4a** and **5a** showed good activity and remaining showed moderate activity.

The above screened compounds were tested for anthelmintic activity. The results have been summarized in table 3. The results showed that compounds **3a**, **4a**, **4c** and **5a** showed significant paralytic time than standard drug Mebendazole, at given concentrations. Test compounds **5b** show better death time of earthworms with that of standard drug Mebendazole. The synthesized compounds in overall estimation confirms the better activity against *Eudrilus* species of earthworms.

5. Conclusion

The present paper is focused on the synthesis of novel heterocyclic compounds as possible antibacterial and antifungal agents. The structures of synthesized compounds are well-supported by spectral data and elemental analysis. In the preparation of ribofuranosides (**5a–b**), ribosylation takes place mostly by replacing hydrogen atom attach to nitrogen in 4H-1,4-benzothiazines (**3a–d**).

The present study elucidated that the synthesized compounds showed good anthelmintic activity. Compounds **3a**, **4a**, **c** and **5a** showed significant paralytic time and compounds **5b** showed better death time of earthworms with that of standard drug Mebendazole. In antimicrobial activity, **4a** exhibited good antibacterial

activities against four strains of bacteria. Compounds (**4a–d**) exhibited significant antibacterial and antifungal activities as compared to their benzothiazines (**3a–d**), respectively due to electron withdrawing effect of oxygen in (**4a–d**). In general, the presence of electron withdrawing group on the aromatic ring increases the antimicrobial activities of tested compounds when compared to compounds having electron donating groups.

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