European Journal of Chemistry 3 (1) (2012) 106-111

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Synthesis, spectral characterization and biological evaluation of 4*H*-1,4-benzothiazines, their sulfones and ribofuranosides

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ARTICLE INFORMATION

Received: 16 July 2011
Received in revised form: 04 September 2011
Accepted: 23 September 2011
Online: 31 March 2012

KEYWORDS

Sulfones
Heterocycles
Ribofuranosides
Antioxidant activity
Antimicrobial activity
4H-1,4-benzothiazines

ABSTRACT

Synthesis of heterocyclic compounds like benzothiazines has attracted attention in recent years due to their biological and industrial value. This article reflects up-to-date and comprehensive coverage of biochemical aspects of benzothiazines, their sulfones and ribofuranosides. The nitrogen and sulfur containing heterocycles were prepared by condensation followed by oxidative cyclization of 2-aminobenzenethiol with β-diketones/β-ketoesters in dimethylsulfoxide. These compounds were then used as base to prepare ribofuranosides by treating them with sugar (β-D-ribofuranose-1-acetate-2,3,5-tribenzoate). On refluxing with hydrogen peroxide in glacial acetic acid, these substituted dimethyl 4*H*-1,4-benzothiazines yielded 4*H*-1,4-benzothiazine-1,1-dioxides. Antioxidant and antimicrobial activity of these compounds were carried out and structure evaluation was done by spectral and elemental analysis.

1. Introduction

The synthesis of benzothiazines, their sulfones and ribofuranosides has attracted tremendous interest due to wide spectrum of biological activities possessed by these compounds such as antibacterial, CNS depressants, antichloesterolic, anticancer, antifungal etc. As a part of the ongoing study, we have synthesized some new benzothiazines, their sulfones and ribofuranosides. Substituted benzothiazines were prepared by condensation of 2-aminobenzenethiols with β-diketone/β-ketoester in presence of dimethylsulfoxide through oxidative cyclization. Intermediate *bis*-(2-aminophenyl)disulfides undergoes cyclization through scission of S-S bond due to high reactivity of alpha position of enaminoketone system towards nucleophilic attack. The structures of these compounds were determined on the basis of spectral data and elemental analysis. These compounds were also screened for biological activity [1-11].

2. Experimental

All the melting points were determined in open capillary tubes but are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on JEOL AL 300 spectrometer (300 MHz) in DMSO-d_6 / $CDCl_3$ using TMS (tetramethyl silane) as an internal standard (Chemical shifts are measured in δ, ppm). IR spectra were recorded in KBr on SHIMADZU 8400 S FTIR spectrophotometer. Mass spectra were recorded on JEOL SX 102/DA 600 using Xenon/Argon as FAB (Fast Atom Bombardment) gas. The purity of compounds were checked by thin layer chromatography using silica gel "G" as adsorbent, visualizing these by UV light or in an Iodine chamber. Elemental analysis of these compounds was also done.

2.1. General method of synthesis of substituted 4H-1,4-benzothiazine (3a-d)

To a stirred suspension of 0.01 mole of β-diketone/β-ketoester (**2a-d**) in 5 mL of dimethylsulfoxide was added 0.01 mole of 2-aminobenzenethiol (**1**) and resulting mixture was refluxed for 20 minutes (Table 1). The reaction mixture was concentrated, cooled down to room temperature. The solid separated out was filtered, washed with petroleum ether and crystallized from methanol (Scheme 1). Characterization data and spectral analysis of each compound (**3a-d**) is given as:

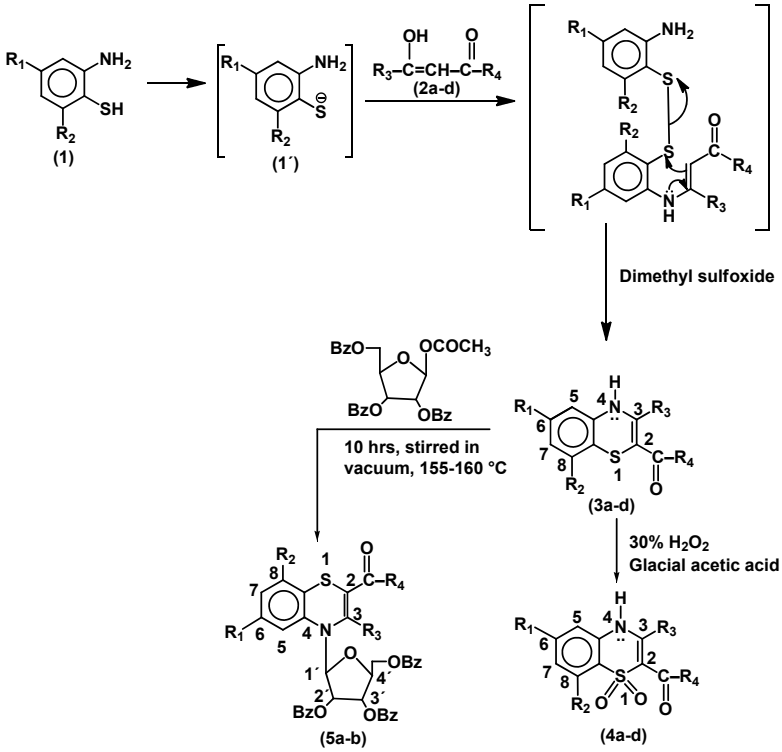
Ethyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate (**3a**): Yield: 46%. M.p.: 55 °C. IR (KBr, *ν*, cm^{-1}): 3260 (N-H), 1690 (>C=O), 1340, 1160 (CF_3 str.), 1255, 1015 (C-O-C str.), 2885 (-CH_3 str.). ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.90 (s, 1H, N-H), 8.08-7.20 (m, 2H, aromatic-H), 4.19 (q, 2H, *J* = 6.9 Hz, CH_2 of C_2H_5 at C_2), 1.30 (t, 3H, *J* = 6.2 Hz, CH_3 of C_2H_5 at C_2), 2.35 (s, 3H, CH_3 at C_6), 2.36 (s, 3H, CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 107.2 (C-2), 138.6 (C-3), 113.1 (C-5), 135.2 (C-6), 120.2 (C-7), 139.1 (C-8), 21.2 (CH_3 at C_6), 14.1 (CH_3 at C_8), 165 (C of CO at C_2), 114 (-CF_3 at C_3), 59.2 (CH_2 of $COOC_2H_5$ at C_2), 13.8 (CH_3 of $COOC_2H_5$ at C_2). MS (*m*/*z*, %): 317 (M^+), 244 (52), 202 (38), 275 (76), 73 (100). Anal. calcd. for $C_{14}H_{14}NO_2F_3S$: C, 52.99; H, 4.41; N, 4.41. Found: C, 53.25; H, 4.39; N, 4.35%.

2-Trifluoroacetyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine (**3b**): Yield: 68%. M.p.: 65 °C. IR (KBr, *ν*, cm^{-1}): 3385 (N-H), 1650 (>C=O), 1350, 1180 (CF_3 str.), 2895 (-CH_3 str.). ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.29 (s, 1H, NH), 8.26-6.06 (m, 2H, aromatic-H), 2.35 (s, 3H, CH_3 at C_6), 2.36 (s, 3H, -CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 116.2 (C-2), 136.3 (C-3), 112.2 (C-5), 136.4 (C-6), 119.2 (C-7), 138.2 (C-8), 15.8 (CH_3 at C_6), 20.1 (CH_3 at C_8), 196.5 (C of CO at C_2), 129.8 (CF_3 at C_2), 114.1 (CF_3 at C_3).

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.3.1.106-111.489

Table 1. The exact reaction times and yields of individual reactions (**3a-d**, **4a-d**, **5a-b**).

Compound No	R_3	R_4	Reaction times (min.)	Yield (%)
3a	CF_3	OC_2H_5	22	46
3b	CF_3	CF_3	20	68
3c	CH_2CH_3	OCH_3	23	76
3d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	21	80
4a	CF_3	OC_2H_5	255	69
4b	CF_3	CF_3	275	72
4c	CH_2CH_3	OCH_3	295	56
4d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	260	82
5a	CF_3	OC_2H_5	920	84
5b	CF_3	CF_3	980	68



$R_1 = CH_3$
$R_2 = CH_3$
$R_3 = CF_3, CH_2CH_3, CH_3$
$R_4 = OC_2H_5, CF_3, OCH_3, C_6H_3(OCH_3)_2$ (o, p)

Compound	R_3
3a, 4a, 5a	CF_3
3b, 4b, 5b	CF_3
3c, 4c	
3d, 4d	

Scheme 1

MS (*m*/*z*, %): 341 (M^+), 244 (58), 202 (29), 327 (78), 97 (100). Anal. calcd. for $C_{13}H_9NOF_6S$: C, 45.74; H, 2.63; N, 4.10. Found: C, 45.98; H, 2.65; N, 4.06%.

Methyl-3-ethyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate (**3c**): Yield: 76%. M.p.: 115 °C. IR (KBr, *v*, cm^{-1}): 3280 (N-H), 1700 (>C=O), 1240, 1055 (C-O-C str.), 2875 (-CH_3 str.). ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.40 (s, 1H, NH), 7.29-6.38 (m, 2H, aromatic-H), 2.35 (s, 3H, -CH_3 at C_6), 2.36 (s, 3H, -CH_3 at C_8), 2.00 (q, 2H, *J* = 7.01 Hz, CH_2 of CH_2CH_3 at C_3), 1.06 (t, 3H, *J* = 6 Hz, CH_3 of CH_2CH_3 at C_3), 3.76 (s, 3H, -CH_3 of OCH_3 at C_2). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 108.2 (C-2), 141.7 (C-3), 118.2 (C-5), 139.1 (C-6), 114.2 (C-7), 142.2 (C-8), 19.6 (CH_3 at C_6), 14.8 (CH_3 at C_8), 24.1 (CH_2 of CH_2CH_3 at C_3), 8.5 (CH_3 of CH_2CH_3 at C_3), 165.0 (C of CO at C_2), 50.1 (C of OCH_3 at C_2). MS (*m*/*z*, %): 263 (M^+), 204 (56), 162 (31), 221 (71), 59 (100). Anal. calcd. for $C_{14}H_{17}NO_2S$: C, 63.87; H, 6.46; N, 5.32. Found: C, 64.11; H, 6.40; N, 5.39%.

2-(2´,4´-Dimethoxybenzoyl)-3,6,8-trimethyl-4H-1,4-benzothiazine (**3d**): Yield: 80%. M.p.: 53 °C. IR (KBr, *v*, cm^{-1}): 3298 (N-H), 1690 (>C=O), 1260, 1060 (C-O-C str.), 2886 (CH_3 str.). ^{1}H NMR (DMSO-d_6, 300 MHz, δ, ppm): 9.09 (s, 1H, NH), 7.16-6.08 (m, 2H, aromatic-H), 1.71 (s, 3H, -CH_3 at C_3), 3.73 (s, 3H, -OCH_3 at ortho position of -COC_6H_3 $(OCH_3)_2$ (o,p) at C_2), 3.78 (s, 3H, -OCH_3 at para position of -COC_6H_3 $(OCH_3)_2$ (o, p) at C_2), 2.14 (s, 3H, CH_3 at C_6), 2.20 (s, 3H, CH_3 at C_8), 7.20-5.95 (m, 3H, aromatic-H of benzoyl group). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 112.9 (C-2), 138.6 (C-3), 111.9 (C-5), 136.2 (C-6), 118.2 (C-7), 146.2 (C-8), 16.5 (CH_3 at C_3), 187 (C of CO at C_2), 56.8 (C

of OCH_3 at ortho position -$COC_6H_3(OCH_3)_2$ (o, p) at C_2). MS (*m/z*, %): 355 (M^+), 190 (61), 137 (48), 148 (28), 313 (70), 165 (100). Anal. calcd. for $C_{20}H_{21}NO_3S$: C, 67.60; H, 5.91; N, 3.94. Found: C, 67.88; H, 5.89; N, 3.89%.

2.2. General method of synthesis of 4H-1,4-benzothiazine, 1,1-dioxides (sulfones) (4a-d)

30% Hydrogen peroxide (5 mL) was added to a solution of 0.01 mole of 4*H*-1,4-benzothiazine in 20 mL glacial acetic acid and refluxed for 15 minutes at 50-55 °C. Heating was stopped and another lot of 5 mL of 30%. Hydrogen peroxide was added. The reaction mixture was again refluxed for 4-5 hrs. The excess of solvent was removed by distillation under reduced pressure and the solution was poured into a beaker containing crushed ice. The yellow residue separated out was filtered and then crystallized from ethanol (Scheme 1). Characterization and spectral data of these compounds (**4a-d**) is given as:

Ethyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (**4a**): Yield: 69%. M.p.: 70 °C. IR (KBr, *v*, cm^{-1}): 3270 (N-H), 1700 (>C=O), 1180, 1140 (SO_2 sym. str.), 1080 (C-S str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.95 (s, 1H, NH), 8.06-7.30 (m, 2H, aromatic-H), 4.20 (q, *J* = 6.95 Hz, 2H, CH_2 of C_2H_5 a C_2), 1.29 (t, 3H, *J* = 6.3 Hz, CH_3 of C_2H_5 at C_2), 2.35 (s, 3H, -CH_3 at C_6), 2.36 (s, 3H, -CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 96.2 (C-2), 150.1 (C-3), 114.9 (C-5), 143.4 (C-6), 120.9 (C-7), 136.4 (C-8), 114.5 (-CF_3 at C_3), 168 (C of CO of $COOC_2H_5$ at C_2), 59.6 (CH_2 of $COOC_2H_5$ at C_2), 13.7 (CH_3 of $COOC_2H_5$ at C_2). MS (*m/z*, %): 349 (M^+), 276 (51), 234 (29), 307 (75), 73 (100). Anal. calcd. for $C_{14}H_{14}NO_4F_3S$: C, 48.13; H, 4.01; N, 4.01. Found: C, 48.35; H, 4.05; N, 4.03%.

2-Trifluoroacyel-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine--1,1-dioxide (**4b**): Yield: 72%. M.p.: 220 °C. IR (KBr, *v*, cm^{-1}): 3390 (N-H), 1680 (>C=O), 1172, 1150 (SO_2 sym. str.), 1085 (C-S str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.30 (s, 1H, NH), 8.16-7.08 (m, 2H, aromatic-H), 2.35 (s, 3H, -CH_3 at C_6), 2.36 (s, 3H, -CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 101 (C-2), 154 (C-3), 118 (C-5), 144 (C-6), 121 (C-7), 146 (C-8), 21.2 (CH_3 at C_6), 13.7 (-CH_3 at C_3), 114 (-CF_3 at C_3), 196.8 (C of CO at C_2), 128.8 (CF_3 at C_2). MS (*m/z*, %): 373 (M^+), 276 (48), 234 (28), 331 (72), 97 (100). Anal. calcd. for $C_{13}H_9NO_3F_6S$: C, 41.82; H, 2.41; N, 3.75. Found: C, 42.09; H, 2.39; N, 3.78%.

Methyl-3-Ethyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate-1,1-dioxide (**4c**): Yield: 56%. M.p.: 240 °C. IR (KBr, *v*, cm^{-1}): 3290 (N-H), 1710 (>C=O), 1165, 1160 (SO_2 sym. str.), 1095 (C-S str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.80 (s, 1H, NH), 7.30-6.40 (m, 2H, aromatic-H), 2.16 (q, 2H, *J* = 7.2 Hz, CH_2 of CH_2CH_3 at C_3), 1.09 (t, 3H, *J* = 6.4 Hz, CH_3 of CH_2CH_3 at C_3), 3.79 (s, 3H, -CH_3 of OCH_3 at C_2), 1.86 (s, 3H, CH_3 at C_6), 2.31 (s, 3H, CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 99.2 (C-2), 144 (C-3), 113.8 (C-5), 145.1 (C-6), 120.9 (C-7), 143 (C-8), 25.2 (CH_2 of CH_2CH_3 at C_3), 8.9 (CH_3 at CH_2CH_3 at C_3). MS (*m/z*, %): 295 (M^+), 236 (38), 194 (30), 253 (76), 59 (100). Anal. calcd. for $C_{14}H_{17}NO_4S$: C, 56.94; H, 5.76; N, 4.74. Found: C, 57.22; H, 5.70; N, 4.78%.

2-(2′,4′-Dimethoxybenzoyl)-3,6-8-trimethyl-4H-1,4-benzothiazine-1,1-dioxide (**4d**): Yield: 82%. M.p.: 81 °C. IR (KBr, *v*, cm^{-1}): 3320 (N-H), 1700 (>C=O), 1177, 1150 (SO_2 sym. str.), 1077 (C-S str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 9.12 (s, 1H, NH), 7.23-6.09 (m, 2H, aromatic-H), 1.92 (s, 3H, -CH_3 at C_3), 3.92 (s, 3H, -OCH_3 at ortho position of -$COC_6H_3(OCH_3)_2$ (o, p) at C_2), 3.79 (s, 3H, -OCH_3 at para position of $COC_6H_3(OCH_3)_2$ (o, p) at C_2). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 97 (C-2), 153 (C-3), 113.8 (C-5), 138 (C-6), 123 (C-7), 145 (C-8), 16.2 (CH_3 at C_3), 189 (C of CO of $COC_6H_3(OCH_3)_2$ (o, p) at C_2), 56.9 (C of OCH_3 at ortho position $COC_6H_3(OCH_3)_2$ (o, p) at C_2). MS (*m/z*, %): 387 (M^+), 222 (50), 180 (21), 345 (70), 165 (100). Anal. calcd. for $C_{20}H_{21}NO_5S$: C, 62.01; H, 5.42; N, 3.61. Found: C, 62.26; H, 5.38; N, 3.56%.

2.3. General method of synthesis of substituted N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)benzothiazine (5a-b)

To a concentrated solution of synthesized benzothiazines (**3a-d**), (0.002) mole in toluene, β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (0.002) mole was added and stirred, in vacuuo, on an oil bath, at 155-160 °C, for 15 minutes. The vacuum was broken and the reaction was protected from moisture by using a guard tube. Stirring was further continued for 10-15 hours with application of vacuum for 15 minutes after every hour. The melt was dissolved in methanol, boiled for 10 minutes and cooled to room temperature. The precipitate 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 get the crystalline compound (**5a-b**) (Scheme 1). Characterization and spectral data of these compounds (**5a-b**) is given as:

N-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)-ethyl-3-trifluoro methyl-6,8-dimethyl-4H-1,4-benzothiazine-2-carboxylate (**5a**): Yield: 84%. M.p.: 80 °C. IR (KBr, *v*, cm^{-1}): 1700 (C=O), 1350, 1170 (-CF_3 str.), 1170 (C-O-C str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.52-7.10 (m, 2H, aromatic-H), 4.21 (q, 2H, *J* = 6.8 Hz, CH_2 of C_2H_5 at C_2), 1.40 (t, 3H, *J* = 6.86 Hz, `CH_3 of C_2H_5 at C_2). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 108.2 (C-2), 139 (C-3), 113.2 (C-5), 136.8 (C-6), 121.2 (C-7), 140.2 (C-8), 66.8 (C-1′), 78.5 (C-2′), 72.5 (C-3′), 71.6 (C-4′). MS (*m/z*, %): 761 (M^+), 688 (39), 646 (31), 73 (100). Anal. calcd. for $C_{40}H_{34}NO_9F_3S$: C, 63.07; H, 4.46; N, 1.83. Found: C, 63.28; H, 4.40; N, 1.80%. Optical rotation $[\alpha]_D$ 23= -17.28 °C.

N-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-2-trifluoro acetyl-3-trifluoromethyl-6,8-dimethyl-4H-1,4-benzothiazine (**5b**): Yield: 68%. M.p.: 82 °C. IR (KBr, *v*, cm^{-1}): 1680 (C=O), 1355, 1185 (-CF_3 str.), 1165 (C-O-C str.). ^1H NMR (DMSO-d_6, 300 MHz, δ, ppm): 8.28-7.01 (m, 2H, aromatic-H), 2.38 (s, 2H, CH_3 at C_6), 2.37 (s, 3H, -CH_3 at C_8). ^{13}C NMR ($CDCl_3$, 300 MHz, δ, ppm): 117.2 (C-2), 173.3 (C-3), 113.6 (C-5), 136.4 (C-6), 120 (C-7), 136 (C-8), 69.2 (C-1′), 75.2 (C-2′), 76.1 (C-3′), 72.1 (C-4′). MS (*m/z*, %): 785 (M^+), 688 (37), 646 (27), 97 (100). Anal. calcd. for $C_{39}H_{29}NO_8F_6S$: C, 59.61; H, 3.69; N, 1.78. Found: C, 59.83; H, 3.63; N, 1.76%. Optical rotation $[\alpha]_D$ 23= -20.18 °C.

2.4. Biological activity

2.4.1. Antioxidant activity

2.4.1.1. DPPH radical scavenging assay

Radical scavenging activity of the synthesized compounds against stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined spectrophotometrically as described by Cuendet *et al.* [12]. A stock solution of 1 mg/mL of the compound was prepared in methanol. 50 µL of compounds were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 minutes incubation in dark at room temperature, absorbance was read against a blank at 517 nm (Table 2). Since IC50 Value is inversely related to the antioxidant activity; ascorbic acid, which is a very good antioxidant, shows a lower IC50 value of 17.8 µg/mL. Among all the synthesized compounds; compound **3c** shows a value of 23.56 µg/mL which shows its good antioxidant nature.

The assay was carried out in triplicate and the percentage of inhibition was calculated by using the following formula.

$$\% \text{ Inhibition} = \frac{(AB - AA)}{AB} \times 100 \quad (1)$$

where AB = Absorption of blank, AA = Absorption of test, Ascorbic acid (shows antioxidant activity) as a positive control and methanol (no antioxidant activity) as a negative control has been used in this assay.

Table 2. Antioxidant activity of the synthesized compounds (DPPH) assay (**3a-d**, **4a-d**, **5a-b**).

Compound No	R_3	R_4	DPPH % inhibition of 1 mg/mL of compound	IC_{50} (µg/mL)
3a	CF_3	OC_2H_5	42.74 ± 0.03	823.78
3b	CF_3	CF_3	65.13 ± 0.02	500.8
3c	CH_2CH_3	OCH_3	90.76 ± 0.07	23.56
3d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	70.79 ± 0.08	79.12
4a	CF_3	OC_2H_5	23.48 ± 0.09	-
4b	CF_3	CF_3	67.49 ± 1.10	432.90
4c	CH_2CH_3	OCH_3	11.88 ± 0.05	-
4d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	34.10 ± 1.20	-
5a	CF_3	OC_2H_5	73.27 ± 0.06	62.70
5b	CF_3	CF_3	75.37 ± 0.08	58.90
Ascorbic acid			92.96 ± 00.9	17.82

Table 3. Antioxidant activity f the synthesized compounds* (ABTS$^{\bullet+}$ assay) (**3a-d**, **4a-d**, **5a-b**).

Compound No	R_3	R_4	ABTS$^{\bullet+}$ activity at different intervals (min.)				
			0	1	2	4	6
3a	CF_3	OC_2H_5	0.697	0.696	0.691	0.687	0.482
3b	CF_3	CF_3	0.698	0.303	0.264	0.097	0.025
3c	CH_2CH_3	OCH_3	0.688	0.109	0.09	0.002	0.002
3d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	0.695	0.087	0.039	0.025	0.025
4a	CF_3	OC_2H_5	0.699	0.525	0.406	0.365	0.339
4b	CF_3	CF_3	0.686	0.392	0.367	0.294	0.262
4c	CH_2CH_3	OCH_3	0.693	0.682	0.609	0.382	0.329
4d	CH_3	$C_6H_3(OCH_3)_2$ (*o*, *p*)	0.700	0.276	0.162	0.027	0.021
5a	CF_3	OC_2H_5	0.686	0.194	0.095	0.032	0.016
5b	CF_3	CF_3	0.714	0.228	0.195	0.069	0.023
Ascorbic acid			0.694	0.040	0.003	0.003	0.003

*Ascorbic acid is used as a reference compound.

Table 4. Antimicrobial activity of the synthesized compounds (**3a-d**, **4a-d**, **5a-b**).

Compound No	R_3	R_4	Antibacterial activity [a]			Antifungal activity [b]
			Coagulase negative staphylococci	*Coagulase positive staphylococci*	*Enterobacter*	*Candida albicans*
3a	CF_3	OC_2H_5	23	10	10	18
3b	CF_3	CF_3	11	11	13	22
3c	CH_2CH_3	OCH_3	15	13	-	18
3d	CH_3	$C_6H_3(OCH_3)_2$(*o*, *p*)	16	12	12	15
4a	CF_3	OC_2H_5	12	10	-	-
4b	CF_3	CF_3	11	10	11	11
4c	CH_2CH_3	OCH_3	-	-	-	11
4d	CH_3	$C_6H_3(OCH_3)_2$(*o*, *p*)	10	11	-	18
5a	CF_3	OC_2H_5	10	-	11	14
5b	CF_3	CF_3	20	10	-	12
Gatifloxacin			-	-	17	-
Vancomycin			15	15	-	-
Flucanazole			-	-	-	25

[a] Zone of inhibition in mm; <7 mm inactive; 7-9 mm weakly active; 10-12 mm, moderately active; >12 mm, active.
[b] Zone of inhibition in mm; <7 mm inactive; 7-11 mm weakly active; 12-17 mm, moderately active; >17 mm, active.

2.4.1.2. ABTS radical cation decolorization assay

The 2,2-azinobis(3-ethybenzothiazoline-6-sulphonic acid) radical cation (ABTS$^{\bullet+}$) decolorization test was carried out using an improved assay of Re *et al.* [13]. In brief, ABTS$^{\bullet+}$ was generated by oxidation of ABTS with potassium persulphate. For this purpose, ABTS was dissolved in ionized water at concentration of 7 mM, and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was left at room temperature overnight (12-15 hours), in the dark before use; the ABTS solution then was diluted with ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 1 ml of the diluted ABTS solution to 10 µL of compound and mixing, absorbance readings were taken at 30 °C at intervals of exactly 1-6 min. The experiment was carried out in triplicate (Table 3 and Figure 1). We have used ascorbic acid as a positive control and ethanol as a negative control in this assay.

2.4.2. Antimicrobial activity

The antimicrobial assay of the synthesized compounds was carried out by using paper disc method of Gould *et al.* [13] against some bacteria and fungi at 100 µg per disc concentration using Vancomycin, Gatifloxacin as reference compounds against bacteria (*Coagulase negative Staphylococci, Coagulase positive Staphylococci*, *Enterobacter*) and Flucanazole against fungus (*Candida albicans*). Paper disc method includes preparation of plates by pouring molten media into sterile petriplates which was then allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and allowed to dry for 5 min. The compound discs prepared were then placed over the plates and incubated for 37 °C for 24 hrs. At the end inhibition zones were measured with ruler in millimetre. These microorganisms were obtained from Microbiology Department, Swai Man Singh Medical College, Jaipur (Table 4).

3. Results and discussion

2-Aminobenzenethiol (**1**) and β-diketones / β-ketoesters (**2a-d**) were refluxed in dimethyl sulfoxide which involves condensation and oxidative cyclization. A *bis*-(2-aminophenyl) disulfide (**1´**) was obtained by oxidation of 2-aminobenzene thiol which cyclizes to form 4*H*-1,4-benzothiazines (**3a-d**) by cleavage of sulfur-sulfur bond due to high reactivity of α-position of enaminoketone system towards nucleophillic attack. Compound (**3a-d**) on treatment with 30% hydrogen peroxide in glacial acetic acid were converted into their corresponding sulfones (**4a-d**). Treatment of (**3a-b**) in toluene with β-D-ribofuranose-1-acetate-2,3,5-tribenzoate in vacuum gave the corresponding ribofuranosides (**5a-b**) (Scheme 1). The structures of synthesized compounds are well supported by spectral data and elemental analysis.

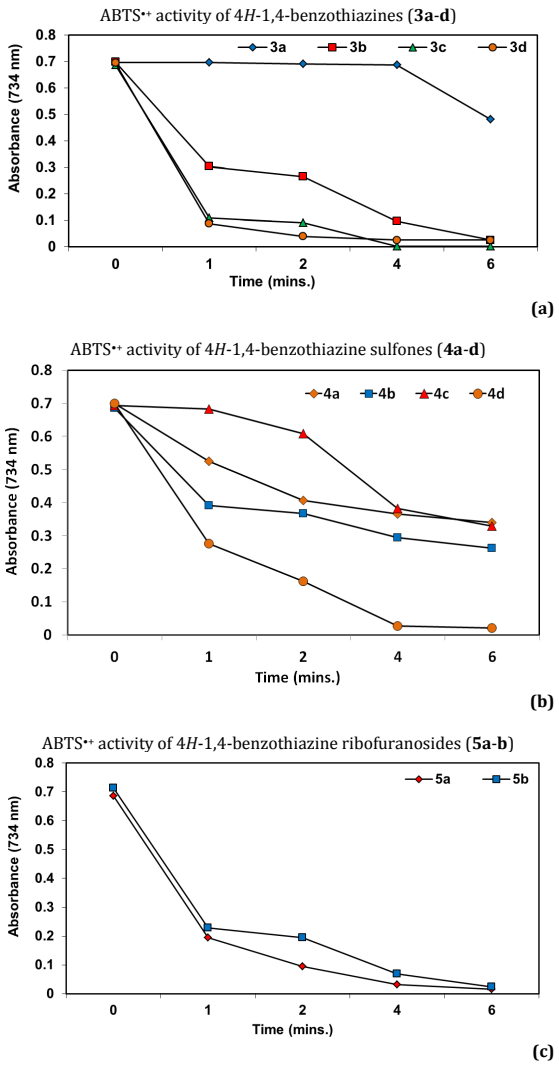


Figure 1. The effect of time on the suppression of absorbance of ABTS by synthesized compounds. After addition of 1 mL of diluted ABTS solution (A 734 nm = 0.700±0.020) to 10 µL of the compound the absorbance reading was taken at 30 °C exactly 1 min., after initial mixing and up to 6 min. All determinations were carried out in triplicates.

3.1. Spectral analysis

3.1.1. IR spectra

Compounds (**3a-d**) showed peaks in region 3385-3260 cm^{-1} due to N-H stretching vibrations and 1700-1650 cm^{-1} due to >C=O stretching vibrations which gets shifted to higher frequencies to 3390-3270 cm^{-1} and 1710-1680 cm^{-1}, respectively, in compounds (**4a-d**). Compounds (**4a-d**) also exhibited two intense peaks in region 1360-1340 cm^{-1} and 1180-1140 cm^{-1} due to asymmetric and symmetric stretching vibrations of sulfonyl group. Compounds (**4a-d**) also showed C-S stretching vibrations in region 1095-1077 cm^{-1}.

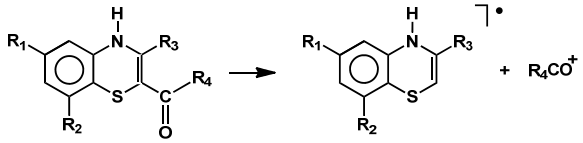
Absence of stretching vibrations due to >N-H group in compounds (**5a-b**) showed site of ribosylation, further in compounds (**5a-b**) bands due to C-O-C linkage of sugar appeared in the region 1170-1165 cm^{-1}.

3.1.2. ^{1}H NMR spectra

All compounds showed multiplet in region δ 8.52-6.06 ppm due to aromatic protons and compounds (**3a-d**) and (**4a-d**) also showed a singlet due to N-H proton in the region δ 9.12-8.29 ppm. Peak due to N-H proton was found to be absent in compounds (**5a-b**) due to ribosylation. In ribofuranosides, C′$_4$-H proton showed multiplet in region δ 4.43-4.80 ppm, C$_2$′-H and C$_3$′-H protons appeared in region δ 4.51-5.90 ppm as multiplet and C$_1$′-H proton appeared as doublet at δ 6.30-6.40 ppm.

3.1.3. Mass spectra

The molecular ion peaks of 4*H*-1,4-benzothiazines were in accordance with their molecular weights. In all the cases side chain at C$_2$ appears as a base peak which is obtained by its fission (Scheme 2).



Scheme 2

3.2. Biological activity (Antioxidant and Antimicrobial)

All the synthesized compounds (**3a-d**), (**4a-d**), and (**5a-b**) were screened for their antioxidant activity by DPPH radical scavenging assay and (ABTS•+) radical cation decolorization assay. The synthesized compounds were also screened for antimicrobial activity (antibacterial and antifungal) by paper disc method. The present study demonstrated that these compounds showed mixed activity in DPPH and ABTS•+ assay.

(1) Compounds (**3b**, **3c**, **3d**, **4b**, **5a**, **5b**) showed strong radical scavenging activity in DPPH assay that have DPPH % inhibition ≥50.

(2) Compounds (**3a**, **4d**) showed moderate activity in DPPH assay that have DPPH % inhibition ≥30.

(3) Compounds (**4a**, **4c**) showed mild activity (<30) in DPPH assay.

(4) Compounds (**3b**, **3c**, **3d**, **4b**, **4d**, **5a**, **5b**) were active in ABTS•+ assay.

All these compounds were found to be moderately active against various bacteria such as (*Coagulase negative Staphylococci, Coagulase positive Staphylococci*, *Enterobacter*) and fungi (*Candida albicans*). Compounds (**3a**, **3c**, **3d**, **5b**) showed good activity against *Coagulase negative Staphylococci* and compounds (**3a**, **3b**, **3c** and **4d**) shows good activity against *Candida albicans*.

4. Conclusion

The structures of synthesized compounds are well supported by spectral data and elemental analysis. The synthesized compounds were also screened for antioxidant activity (DPPH assay and ABTS•+ assay) and antimicrobial activity (antibacterial and antifungal). The present study demonstrated that these compounds showed mixed activity in DPPH and ABTS•+ assay. Compounds **3c**, **3d** showed excellent antioxidant activity in DPPH assay. Compounds **5a**, **5b** showed much better activity in DPPH assay than compounds **3a**, **3b** which are the precursors of **5a** and **5b**. It shows that the ribofuranosides **5a** and **5b** showed much better antioxidant activity than their phenothiazine bases (**3a**, **3b**). This is due to the replacement of H by the sugar moiety (ribosylation). Compounds **3b**, **3c**, **3d**, **4b**, **5a**, **5b** showed good activity in both DPPH and ABTS assays.

The present paper is focused on the synthesis of novel heterocyclic compounds as possible antibacterial and antifungal agents. Compounds **3c** and **3d** showed antibacterial activity against *Coagulase* negative *Staphylococci* which is comparable to vancomycin. Compound **3a** is much better than

vancomycin in antibacterial activity against *Coagulase* negative *Staphylococci*. All these compounds were found to be moderately active against various bacteria such as (*Coagulase negative Staphylococci, Coagulase positive Staphylococci*, *Enterobacter*) and fungi (*Candida albicans*). Compounds (**3a**, **3c**, **3d**, **5b**) showed good activity against *Coagulase negative Staphylococci* and compounds (**3a**, **3b**, **3c** and **4d**) shows good activity against *Candida albicans*.

Acknowledgements

The authors are grateful to Department of Chemistry, University of Rajasthan, Jaipur, India for providing necessary facilities. The University Grants Commission (Research Award Scheme) and Council of Scientific and Industrial Research, New Delhi, India are duly acknowledged for financial support. We are also thankful to Department of Zoology, University of Rajasthan and Sawai ManSingh Medical College, Jaipur, India for assistance in carrying out the biological activity.

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