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Synthesis, characterization and *in vitro* antimicrobial assessment of some novel 4*H*-1,4-benzothiazines and their sulfone derivatives

Abstract: In recent years, synthesis and biological evaluation of novel 4*H*-1,4-benzothiazines and their sulfone derivatives have gained momentum due to their medicinal and industrial importance. Our studies focused on the design and synthesis of new antimicrobial agents, and for this purpose a series of novel 4*H*-1,4-benzothiazines and their sulfone derivatives were synthesized and their *in vitro* antimicrobial assessment was carried out against a representative panel of Gram-positive and Gram-negative bacteria strains and selected fungi species. The reported 4*H*-1,4-benzothiazines were prepared by condensation followed by oxidative cyclization of substituted 2-aminobenzenethiols with compounds containing active methylene groups. It is believed that the reaction proceeds via intermediary of the enaminoketone system. The sulfone derivatives were synthesized by oxidation of 4*H*-1,4-benzothiazines using 30% hydrogen peroxide in glacial acetic acid. Structure determination was done by spectral and elemental investigations.

Keywords: antimicrobial assessment; 4*H*-1,4-benzothiazines; condensation; oxidative cyclization; sulfones.

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Introduction

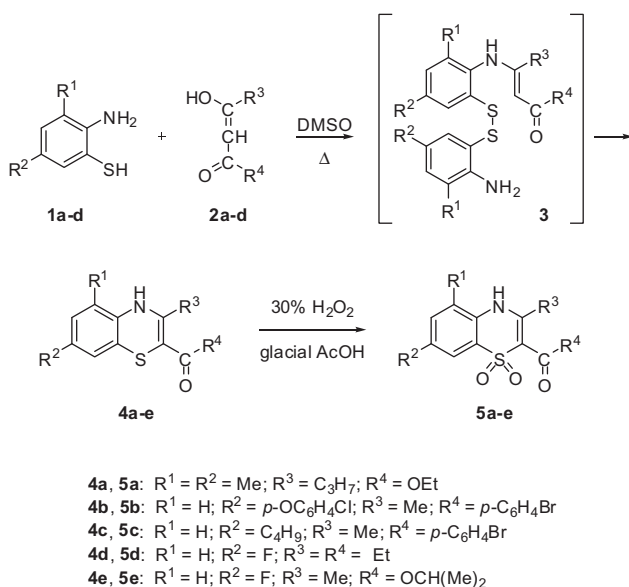
Multiple drug resistance against antimicrobial agents is one of the most serious problems of today's medicinal scenario. Examples of drug-resistant pathogens are methicillin/oxacillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococci*, and the penicillin-resistant *Streptococcus pneumoniae*. Unfortunately,

microbes develop resistance against newer agents after a short period of their clinical use. Thus, the pipeline of new antimicrobial agents is drying up. Therefore, there is a need to develop new and different antimicrobial agents which can be used as drugs to treat chronic conditions. It is well established that the benzothiazine system has potential to be developed into novel antibacterial and anticancer agents [1–5]. Knowing the importance of the benzothiazine template, we have synthesized several substituted 4*H*-1,4-benzothiazines [6, 7] and their sulfone derivatives [8–10] that exhibit antibacterial and antifungal activity [11–15]. Similar compounds herein reported were easily prepared by condensation and oxidative cyclization of 2-aminobenzenethiols with β -diketones/ β -ketoesters in dimethyl sulfoxide (DMSO). These intermediate products were further converted into sulfone derivatives by oxidation under reflux conditions using 30% hydrogen peroxide in glacial acetic acid (Scheme 1). To reflect the potency of the synthesized compounds as antimicrobial agents, activity index (AI) and minimum inhibitory concentration (MIC) against different Gram-positive, Gram-negative and fungi strains belonging to the Microbial Type Culture Collection (MTCC) is reported using the agar well diffusion method and the broth microdilution method, respectively.

Results and discussion

Chemistry

The starting substituted 2-aminobenzenethiols **1a–d** (Scheme 1) were synthesized by alkaline hydrolysis of corresponding 2-aminobenzothiazoles, which in turn were prepared by the cyclization of substituted phenylthiourea. The phenylthiourea derivatives were obtained by the reaction of ammonium thiocyanate with substituted anilines. A mixture of 2-aminobenzenethiols **1a–d** and β -diketones/ β -ketoesters **2a–d** was heated under reflux conditions in DMSO, which led to condensation and



Scheme 1 Synthesis of 4*H*-1,4-benzothiazines **4a–e** and their sulfone derivatives **5a–e**.

oxidative cyclization. Oxidation of 2-aminobenzenethiols produced *bis*-(2-aminophenyl)disulfide **3**, which in turn underwent cyclization to form 4*H*-1,4-benzothiazines **4a–e** by scission of a sulfur-sulfur bond. This cleavage is due to high reactivity of the α -position of the enaminoketone system towards nucleophilic attack. Compounds **4a–e** were converted into their corresponding sulfones **5a–e** by treatment with 30% hydrogen peroxide in glacial acetic acid.

Spectral data and elemental analysis support the structures of synthesized compounds. In the IR spectra of compounds **4a–e**, a single peak is seen in the region of 3340–3310 cm^{-1} due to NH stretching vibration. These compounds also exhibit a band at 1655–1605 cm^{-1} due to C=O stretching vibrations. A slight shift towards higher frequencies is observed for sulfone derivatives **5a–e** due to an increased electron-accepting ability of sulfones compared with the parent system. Compounds **5a–e** exhibit intense peaks in the regions of 1385–1245 cm^{-1} , 1190–1145 cm^{-1} and 585–545 cm^{-1} , which can be ascribed to vibrations of the sulfonyl group. The signals for C-S stretching vibrations in the region of 1065–1035 cm^{-1} are also observed in the IR spectra of compounds **5a–e**. The given structures of products **4a–e** and **5a–e** are also fully consistent with their ^1H NMR spectra. A multiplet in the region of δ 8.02–6.13 is due to the presence of aromatic protons in all compounds **4a–e** and **5a–e**. A singlet in the region δ 9.03–8.12 can be ascribed to the N-H function. The molecular ion peaks in the mass spectra of 4*H*-1,4-benzothiazines and their sulfone derivatives are fully consistent with their

molecular weights. The base peak is for the ion R_4CO^+ , as predicted for the generation of the most stable cation.

Antimicrobial assessment

The *in vitro* antimicrobial assessment of synthesized compounds was carried out by using the agar well diffusion method for determination of AI and the broth microdilution method for determination of MIC against two strains of bacteria, *Micromonospora* sp. (Gram-positive) and *Zymomonas mobilis* (Gram-negative), and two strains of fungi, *Aspergillus solani* and *Fusarium culmorum*. Ampicillin sulfate and fluconazole were used as a standard antibacterial and antifungal drug, respectively. Compounds **4c** and **5c** show good antibacterial activity against the Gram-positive strain *Micromonospora* sp., whereas compounds **4d**, **4e**, **5d** and **5e** show excellent activity against the Gram-negative strain of subject bacteria *Z. mobilis*. Compound **5a** exhibits good antifungal activity against *A. solani* and compound **5c** is an excellent agent against *F. culmorum*. The remaining compounds show moderate antimicrobial activities. It should be noted that sulfone derivatives of 4*H*-1,4-benzothiazines exhibit better antimicrobial activities than their parent compounds. This finding can be explained by the presence of the electron-withdrawing group ($-\text{SO}_2$) in sulfones, which enhances biological activity. This effect of an electron-withdrawing group has been noted in the literature [16–18].

Conclusions

The synthesis of novel heterocyclic compounds which can be used as potent antibacterial and antifungal agents is described. Compounds **4d** and **5d** show antibacterial activity against Gram-negative *Z. mobilis* that is comparable to activity of ampicillin sulfate. Compounds **4e** and **5e** are much better than ampicillin sulfate in antibacterial activity against Gram-negative *Z. mobilis*. Compound **5c** shows antifungal activity against *F. culmorum* that is better than that of fluconazole.

Experimental section

Melting points are uncorrected. IR spectra were recorded in KBr on a Shimadzu 8400 S FT-IR spectrophotometer. ^1H NMR spectra were recorded on a JEOL AL 300 spectrometer (300 MHz) in $\text{DMSO}-d_6$. Fast atom bombardment (FAB) mass spectra were recorded on a JEOL SX 102/DA 600 instrument using Xenon/Argon. The purity of

compounds were checked by TLC using silica gel “G” as adsorbent in various solvent systems with visualization by UV light or in an iodine chamber.

General method for synthesis of substituted 4*H*-1,4-benzothiazines 4a–e

β -Diketone/ β -ketoester **2a–d** (0.01 mol) was treated with a solution of 2-aminobenzenethiol **1a–d** (0.01 mol) in 5 mL of DMSO and the resulting mixture was heated under reflux (190°C) for 20 min, then cooled and concentrated on a rotary evaporator. The residue was washed with petroleum ether and crystallized from methanol.

Ethyl-5,7-dimethyl-3-propyl-4*H*-1,4-benzothiazine-2-carboxylate (4a) Yield 57%; mp 125–127°C (dec); IR: 3315, 1620, 1425, 1330, 1040 cm⁻¹; ¹H NMR: δ 8.12 (s, 1H), 6.55–6.37 (m, 2H), 2.02 (t, 2H), 1.21 (m, 2H), 1.10 (t, 3H), 4.16 (q, 2H), 1.27 (t, 3H), 1.94 (s, 3H), 2.10 (s, 3H); MS: *m/z* 291 (M⁺), 218 (53), 176 (76), 73 (100). Anal. Calcd for C₁₆H₂₁NO₄S: C, 65.97; H, 7.21; N, 4.81. Found: C, 66.22; H, 7.26; N, 4.79.

2-(4'-Bromobenzoyl)-7-(4'-chlorophenoxy)-3-methyl-4*H*-1,4-benzothiazine (4b) Yield 52%; mp 214–216°C (dec); IR: 3310, 1605, 1270, 1070, 1465, 1350, 1015 cm⁻¹; ¹H NMR: δ 8.52 (s, 1H), 7.72–6.28 (m, 11H), 1.83 (s, 3H); MS: *m/z* 472.5 (M⁺), 288.5 (54), 246.5 (82), 184 (100). Anal. Calcd for C₂₂H₁₅BrClNO₂S: C, 56.05; H, 3.18; N, 2.97. Found: C, 56.80; H, 3.21; N, 2.83.

7-*n*-Butyl-2-(4'-bromobenzoyl)-3-methyl-4*H*-1,4-benzothiazine (4c) Yield 40%; mp 119–121°C (dec); IR: 3315, 1610, 1275, 1080, 1465, 1355, 1035 cm⁻¹; ¹H NMR: δ 8.53 (s, 1H), 7.36–6.29 (m, 7H), 2.65 (t, 2H), 1.83 (m, 2H), 1.39 (m, 2H), 1.04 (t, 3H), 1.87 (s, 3H); MS: *m/z* 402 (M⁺), 218 (44), 176 (73), 184 (100). Anal. Calcd for C₂₀H₂₀BrNO₂S: C, 59.85; H, 4.98; N, 3.49; Found: C, 59.78; H, 4.92; N, 3.42.

3-Ethyl-7-fluoro-2-propionyl-4*H*-1,4-benzothiazine (4d) Yield 56%; mp 133–135°C (dec); IR: 3340, 1655, 1240, 1455, 1350, 1025 cm⁻¹; ¹H NMR: δ 8.63 (s, 1H), 7.23–6.46 (m, 3H), 1.99 (q, 2H), 1.16 (t, 3H), 3.14 (q, 2H), 1.56 (t, 3H); MS: *m/z* 251 (M⁺), 194 (44), 162 (24), 161 (75), 57 (100). Anal. Calcd for C₁₃H₁₄FNOS: C, 62.15; H, 5.57; N, 5.57. Found: C, 63.32; H, 5.48; N, 5.54.

Isopropyl-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate (4e) Yield 60%; mp 154–156°C (dec); IR: 3335, 1625, 1245, 1455, 1355, 1045 cm⁻¹; ¹H NMR: δ 8.69 (s, 1H), 7.47–6.83 (m, 3H), 2.27 (s, 3H), 4.23–4.12 (m, 1H), 1.92 (d, 6H); MS: *m/z* 267 (M⁺), 180 (36), 138 (78), 87 (100). Anal. Calcd for C₁₃H₁₄FNO₃S: C, 58.42; H, 5.24; N, 5.24. Found: C, 58.65; H, 5.30; N, 5.19.

General method for synthesis of 4*H*-1,4-benzothiazine 1,1-dioxides (sulfones) 5a–e

A solution of 0.01 mol of 4*H*-1,4-benzothiazine in 20 mL glacial acetic acid was added to 30% hydrogen peroxide (5 mL) and then the mixture was heated under reflux for 15 min keeping the temperature between 50°C and 55°C. Another lot of 30% hydrogen peroxide

(5 mL) was added after 15 min without heating. Then the mixture was heated under reflux (120°C) for an additional 4–5 h, concentrated under reduced pressure and the residue was treated with crushed ice. The resultant solid product **5** was filtered and crystallized from ethanol.

Ethyl-5,7-dimethyl-3-propyl-4*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxides (5a) Yield 56%; mp 136–138°C (dec); IR: 3320, 1645, 1185, 1150, 1345, 1290, 1275, 580, 555, 1055 cm⁻¹; ¹H NMR: δ 8.42 (s, 1H), 6.95–6.63 (m, 2H), 2.22 (t, 2H), 1.14 (m, 2H), 1.22 (t, 3H), 4.35 (q, 2H), 1.77 (t, 3H), 2.14 (s, 3H), 2.33 (s, 3H); MS: *m/z* 323 (M⁺), 250 (51), 208 (77), 73 (100). Anal. Calcd for C₁₆H₂₁NO₄S: C, 59.44; H, 6.50; N, 4.33. Found: C, 59.31; H, 6.45; N, 4.30.

2-(4'-Bromobenzoyl)-7-(4'-chlorophenoxy)-3-methyl-4*H*-1,4-benzothiazine 1,1-dioxides (5b) Yield 45%; mp 253–255°C (dec); IR: 3355, 1625, 1190, 1160, 1360, 1285, 1265, 565, 545, 1035 cm⁻¹; ¹H NMR: δ 8.82 (s, 1H), 8.02–7.28 (m, 11H), 2.13 (s, 3H). MS: *m/z* 504.5 (M⁺), 320.5 (52), 278.5 (79), 184 (100). Anal. Calcd for C₂₂H₁₅BrClNO₄S: C, 52.48; H, 2.98; N, 2.78. Found: C, 52.66; H, 2.96; N, 2.83.

7-*n*-Butyl-2-(4'-bromobenzoyl)-3-methyl-4*H*-1,4-benzothiazine 1,1-dioxides (5c) Yield 47%; mp 109–111°C (dec); IR: 3350, 1640, 1185, 1150, 1355, 1290, 1245, 570, 545, 1065 cm⁻¹; ¹H NMR: δ 8.63 (s, 1H), 7.46–6.39 (m, 7H), 2.75 (t, 2H), 1.93 (m, 2H), 1.49 (m, 2H), 1.14 (t, 3H), 1.97 (s, 3H); MS: *m/z* 434 (M⁺), 250 (41), 208 (77), 184 (100). Anal. Calcd for C₂₀H₂₀BrNO₃S: C, 55.42; H, 4.61; N, 3.23. Found: C, 55.58; H, 4.65; N, 3.27.

3-Ethyl-7-fluoro-2-propionyl-4*H*-1,4-benzothiazine 1,1-dioxides (5d) Yield 60%; mp 140–142°C (dec); IR: 3360, 1680, 1190, 1145, 1385, 1295, 1260, 575, 560, 1045 cm⁻¹; ¹H NMR: δ 9.03 (s, 1H), 7.33–6.76 (m, 3H), 2.39 (q, 2H), 1.66 (t, 3H), 3.54 (q, 2H), 2.26 (t, 3H); MS: *m/z* 283 (M⁺), 226 (41), 162 (22), 161 (76), 57 (100). Anal. Calcd for C₁₃H₁₄FNO₃S: C, 55.12; H, 4.94; N, 4.94. Found: C, 55.27; H, 4.89; N, 4.97.

Isopropyl-7-fluoro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate 1,1-dioxides (5e) Yield 34%; mp 158–160°C (dec); IR: 3340, 1640, 1180, 1155, 1355, 1285, 1265, 585, 565, 1065 cm⁻¹; ¹H NMR: δ 8.88 (s, 1H), 7.77–6.13 (m, 3H), 2.55 (s, 3H), 4.63 (m, 1H), 2.22 (d, 6H); MS: *m/z* 299 (M⁺), 212 (38), 170 (81), 87 (100). Anal. Calcd for C₁₃H₁₄FNO₄S: C, 52.17; H, 4.68; N, 4.68. Found: C, 52.37; H, 4.67; N, 4.59.

Antimicrobial assessment

MIC The broth microdilution method was used to determine the MICs (μ g/mL) of the compounds in accordance with the NCCLS 1992 manual. The strains used for quality control were *Micromonospora* sp. MTCC 3296 (Gram-positive) and *Z. mobilis* MTCC 88 (Gram-negative) bacteria. *Aspergillus solani* MTCC 2101 and *F. culmorum* MTCC 349 were the reference strains for testing antifungal activities of the compounds. The standard antibacterial and antifungal drugs used were ampicillin sodium salt and fluconazole, respectively. DMSO was used for preparing solutions of test compounds and standard drugs. For preparing stock solution, each synthesized drug was diluted to obtain concentration of 1000 μ g/mL. For primary screening, 500, 250 and 125 μ g/mL concentrations of the synthesized

Table 1 Antimicrobial assessment of 4*H*-1,4-benzothiazines 4a–e.

Compounds	<i>Micromonospora</i> sp. MTCC 3296			<i>Zymomonas mobilis</i> MTCC 88			<i>Aspergillus solani</i> MTCC 2101			<i>Fusarium culmorum</i> MTCC 349		
	IZ ^a (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)
4a	1.0	0.357	36.0	1.5	0.714	28.5	2.0	0.666	21.5	1.5	0.555	27.0
4b	1.6	0.571	33.5	1.6	0.761	27.0	1.0	0.333	28.5	1.5	0.555	27.0
4c	1.9	0.678	32.0	1.8	0.857	26.0	1.1	0.366	26.0	2.0	0.740	22.5
4d	1.1	0.392	36.0	2.1	1.000	22.5	1.5	0.500	24.0	1.9	0.703	24.0
4e	1.2	0.428	35.5	2.2	1.047	20.5	1.6	0.200	23.5	2.3	0.851	22.0
Ampicillin sulfate	2.8	–	28.0	2.1	–	22.0	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	–	3.0	–	18.0	2.7	–	20.0

^aIZ, inhibition zone; AI, activity index; MIC, minimum inhibitory concentration.

Table 2 Antimicrobial assessment of sulfone derivatives 5a–e.

Compounds	<i>Micromonospora</i> sp. MTCC 3296			<i>Zymomonas mobilis</i> MTCC 88			<i>Aspergillus solani</i> MTCC 2101			<i>Fusarium culmorum</i> MTCC 349		
	IZ ^a (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)	IZ (cm)	AI	MIC (µg/mL)
5a	1.0	0.357	36.0	1.6	0.761	27.0	2.7	0.900	21.0	1.8	0.666	24.5
5b	1.9	0.678	32.0	1.7	0.809	26.5	2.0	0.667	27.5	1.6	0.592	26.5
5c	2.4	0.857	30.0	1.9	0.904	24.0	2.1	0.700	26.0	2.6	0.962	20.5
5d	1.3	0.464	35.0	2.1	1.000	22.0	2.2	0.733	20.5	2.0	0.740	23.5
5e	1.7	0.607	33.0	2.3	1.095	20.0	2.2	0.733	20.5	2.1	0.777	21.5
Ampicillin sulfate	2.8	–	28.0	2.1	–	22.0	–	–	–	–	–	–
Fluconazole	–	–	–	–	–	–	3.0	–	18.0	2.7	–	20.0

^aIZ, inhibition zone; AI, activity index; MIC, minimum inhibitory concentration.

drugs were taken. The active drugs found in this primary screening were further tested against all microorganisms in a second set of dilution 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 recorded as MIC. Thus, the lowest concentration of each compound in the tube showing no growth (i.e., no turbidity) of inoculated bacteria/fungi was taken as MIC of that compound. Luria broth (Himedia) medium was used to carry out antibacterial activities of the bacterial strains. The reference strains of fungi were cultivated, at pH 6.9, in sabouraud dextrose agar (Himedia), with an inoculum of 10⁸ cfu/mL, by the spectrophotometric method. An aliquot (10 µL) of this prepared culture was added to each tube of the serial dilution and then incubated at 37°C for 24 h at 3.2602 × *g* (150 rpm) on a rotary shaker. After incubation, MIC values were recorded. The MICs of tested compounds in µg/mL against certain strains of bacteria and fungi are shown in Tables 1 and 2.

AI The AI of compounds was determined by the agar well diffusion method. In this method, nutrient agar and potato dextrose agar plates were swabbed with an 8-h-old broth culture of respective bacteria (*Micromonospora* sp. MTCC 3296 and *Z. mobilis* MTCC 88) and fungi (*A. solani* MTCC 2101 and *F. culmorum* MTCC 349). Wells were made on agar surface (6 mm in diameter and 2 cm apart) and punctured in the culture medium using sterile cork borers. The plates were then turned upside down. Stock solution of each compound was prepared at a concentration of 1 mg/mL in DMSO. The wells were filled with 100 µL of the compounds and one well was filled with 100 µL of DMSO which served as a control. The compounds were incubated

at 37°C for 24 h in the case of bacterial activity and for 48 h at 25°C in the case of fungal activity. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in cm) including the well diameter. The zone of inhibition exhibited by compounds was then compared with the zone exhibited by the reference compounds (ampicillin sodium salt in the case of bacteria and fluconazole in the case of fungi) to find relative activity. The activity indices of tested compounds against certain bacteria and fungi were calculated as shown below and the results are tabulated in Tables 1 and 2.

$$\text{Activity Index (AI)} = \frac{\text{Zone of inhibition exhibited by test compounds}}{\text{Zone of inhibition exhibited by reference compounds}}$$

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