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Research Article

SPECTRAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF SYNTHESIZED 4*H*-1,4-BENZOTHIAZINES

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ABSTRACT

Objective: 4*H*-1,4-Benzothiazines constitute an important class of heterocycles containing 1,4-thiazine ring fused to benzene ring. They are extensively used as tranquilizer, antispasmodic, central nervous system depressant, antiulcer, antibacterial, antifungal, antioxidant, anticancer agents, fungicides, etc. Therefore, these observations prompted us to synthesize substituted 4*H*-1,4-benzothiazines and investigate their antimicrobial activity against selected bacterial and fungal strains.

Methods: In the present research work, 2-Amino-3,5,6-trichlorobenzenethiol condensed with *β*-diketones/*β*-ketoesters in the presence of dimethyl sulfoxide followed by oxidative cyclisation leading to the formation of 4*H*-1,4-benzothiazines. The spectral investigation confirmed the synthesis of these bioactive compounds. All synthesized compounds were screened for their antimicrobial activity (antibacterial and antifungal) using agar well diffusion method.

Results: The minimum inhibitory concentration values of synthesized compounds gave excellent results against bacterial as well as fungal strains (*Escherichia coli* [Gram negative] MTCC 2939, 58–158 *μ*g/mL, *Bacillus subtilis* [Gram positive] MTCC 441, 41–124 *μ*g/mL, *Streptomyces griseus* [Gram negative] MTCC 1998, 85–128 *μ*g/mL, *Fusarium oxysporum* MTCC 1755, 142–151 *μ*g/mL, *Aspergillus niger* MTCC 281, 59–78 *μ*g/mL, and *Rhizopus stolonifer* MTCC 2591, 85–118 *μ*g/mL).

Conclusion: Synthesized substituted benzothiazines have potential to be used as a new class of antibacterial and antifungal drugs. Further biomedical research is required to make 4*H*-1,4-benzothiazines related compounds as potential antibacterial and antifungal drugs.

Keywords: Benzothiazine, *β*-diketones/*β*-diketoesters, Antimicrobial properties.

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INTRODUCTION

Synthesized 4*H*-1,4-benzothiazines [1-8] have widespread therapeutic uses such as vasodilator, neuroleptic, tranquilizer [9], sedative, antispasmodic, central nervous system depressant [10], dyestuff, copolymer, and flavoring agent. Distinguishable difference observed in their pharmacological activities [11-14] due to slight change in the substitution pattern in benzothiazine nucleus. The simplicity and diversity of synthetic methods as well as their pharmacological, biological, and industrial significance also make them important for research. Benzothiazine also possesses a distinguished property according to which a slight change in the substitution pattern causes major differences in their biological activities [15-18]. This opens a gate to synthesize a number of antimicrobial agents. Thus knowing the immense importance of benzothiazine template, we have synthesized substituted 4*H*-1,4-benzothiazines. To exhibit the potential of synthesized compounds as better antimicrobial agents minimum inhibitory concentration (MIC) [19-20] against selected strains of fungi, Gram-positive and Gram-negative bacteria belonging to Microbial Type Culture Collection (MTCC) were reported using broth microdilution method.

RESULTS AND DISCUSSION

Chemistry

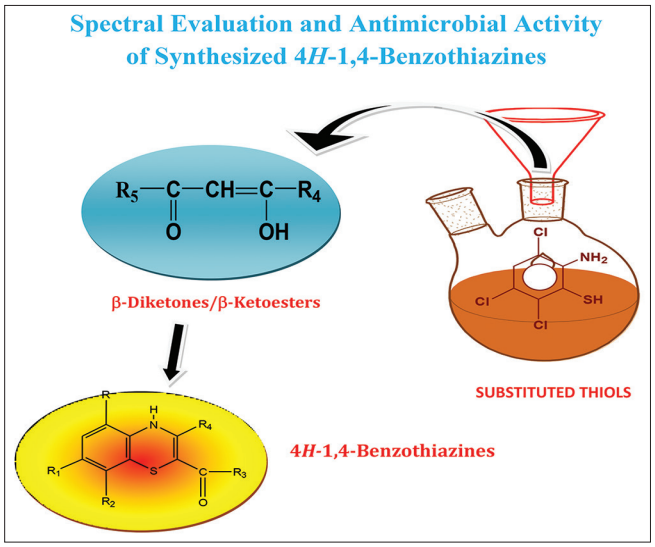
In the presence of dimethyl sulfoxide (DMSO), 2-Amino-3,5,6-trichlorobenzenethiol (I) condensed with *β*-diketones/*β*-ketoesters (IIa) followed by oxidative cyclization. Bis-(2-aminophenyl) disulfides (Ia) formed from substituted 2-aminobenzenethiols (I) due to readily oxidation, which undergoes cyclization by scission of S-S bond due to high reactive *α*-position of enamino ketone system (III) toward intramolecular nucleophilic attack leading to the formation of 4*H*-1,4-benzothiazines (Scheme 1).

β-Diketones and *β*-ketoesters usually exist in two isomeric forms (keto-enol tautomerism) IIa and IIb (Fig. 1). Therefore, there is a possibility of the formation of two types of 1,4-benzothiazines (IV) and (VI), but only one type of 1,4-benzothiazines (IV) is separated (Scheme 1). Elemental analysis and spectral data support the proposed structures of reported compounds.

Synthesized substituted 4*H*-1,4-benzothiazines are summarized below:

IVa Isopropyl-5,7,8-trichloro-3-methyl-4*H*-1,4-benzothiazine-2-carboxylate.

IVb Ethyl-5,7,8-trichloro-3-propyl-4*H*-1,4-benzothiazine-2-carboxylate.



Graphical abstract

IR SPECTRA

All the 4*H*-1,4-benzothiazines exhibit a single sharp peak in the region 3465–3350 cm^{-1} due to N-H stretching vibrations. These also exhibit a sharp band due to >C=O stretching vibrations of carbonyl group at 1720–1710 cm^{-1}. In compound IVa-b, C–O–C asymmetric and symmetric vibrations occur in region 1270–1265 cm^{-1} and 1080–1060 cm^{-1}.

Compounds IVa-b exhibit sharp bands in the region 2960-2955 cm^{-1} and 2830-2825 cm^{-1} due to C–H asymmetric and symmetric stretching vibrations of CH_3 group. Compounds IVa-b also show two sharp bands in the region 1460-1455 cm^{-1} and 1340-1335 cm^{-1} due to C-H deformation vibrations of CH_3 group. In compounds IVa-b, C–Cl stretching vibrations occur in the region 810-800 cm^{-1}.

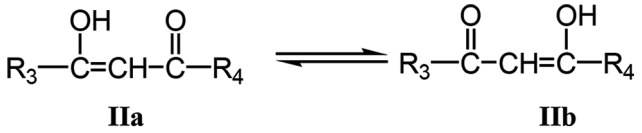


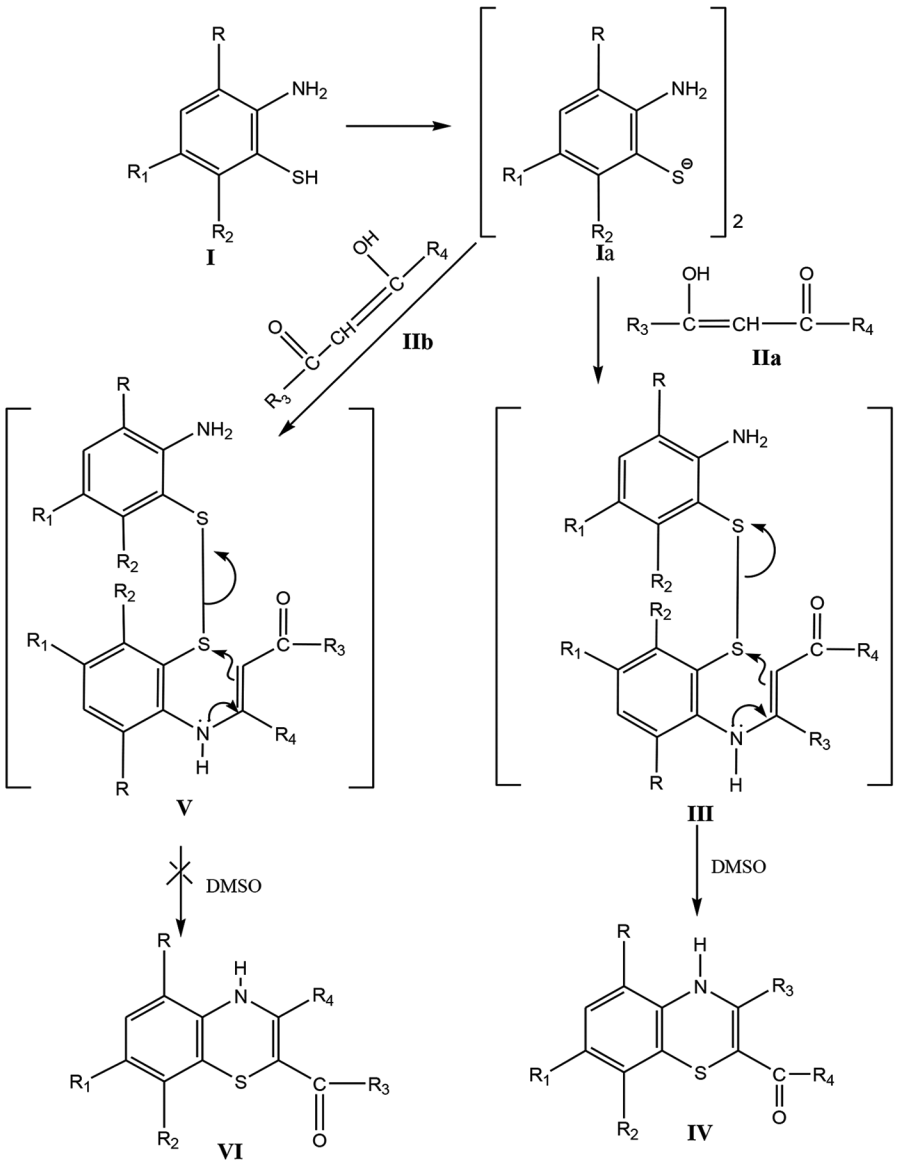
Fig. 1: Keto –Enol Tautomerism in β-Diketones and β-ketoesters

^{1}H-NMR spectra

All the synthesized benzothiazines exhibit a single sharp peak in region δ 9.47–9.28 ppm due to >N–H proton. The singlet is observed at δ 8.12–8.10 ppm due to single aromatic proton in compounds IVa-b. Compound IVa shows singlet at δ 1.85 ppm due to $–CH_3$ protons at C_3, Multiplet observed at δ 4.82 ppm due to CH proton of $–OCH(CH_3)_2$ group at C_2 and doublet observed at δ 1.90 ppm due to $-CH_3$ proton of $–OCH(CH_3)_2$ group at C_2. In compounds IVb triplet, sextet observed in the region δ 2.10–1.42 ppm due to $>CH_2$ protons of C_3H_7 group at C_3. Compound IVb shows quartet and triplet in the region δ 3.35 ppm and δ 1.64 ppm due to $>CH_2$ and $–CH_3$ protons of $–OC_2H_5$ group at C_2.

Mass spectra

The molecular ion peaks of reported compound were in accordance with their molecular weights.



Where

R= Cl; R_1 = Cl; R_2 = Cl; R_3 = CH_3,C_3H_7 ; R_4 = C_2H_5,$CH(CH_3)_2$

Table 1: Antimicrobial activity of synthesized compounds

Compound No.	Minimum inhibitory concentrations of bacterial strains in μg/ml			Minimum inhibitory concentrations (MICs) of fungal strains in μg/ml		
	Escherichia coli MTCC 2939	*Bacillus subtilis* MTCC 441	*Streptomyces griseus* MTCC 1998	*Fusarium oxysporum* MTCC 1755	*Aspergillus niger* MTCC 281	*Rhizopus stolonifer* MTCC 2591
IV a	158	41	85	151	78	85
IV b	58	124	128	142	59	118
Streptomycin	68	46	62	-	-	-
Ketoconazole	-	-	-	74	38	46

Antimicrobial assessment

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

Compound IVa gave excellent results against bacterial strains. Compounds IVb gave excellent results against fungal strains.

Experimental

The purity of the synthesized compounds was checked by thin layer chromatography using silica gel "G" adsorbent in various non-aqueous solvent systems. Melting points of synthesized compounds are uncorrected and determined in open capillary tubes. IR spectra were recorded in KBr on SHIMADZU 8400 S FT IR spectrophotometer. ^{1}H-NMR spectra have been recorded at 300 MHz on JEOL AL-300 FT NMR using tetramethylsilane as an internal standard in DMSO-d_6 (in d ppm).

General procedure for the synthesis of substituted 4*H*-1,4-benzothiazines (IVa-b)

2-Amino-3,5,6-trichlorobenzenethiol (I; 0.01 mole) was refluxed with a stirred suspension of β-diketones/β-ketoesters (IIa; 0.01 mole) in DMSO (5 ml) for 50–60 min. The resulting solution was cooled down to room temperature. The solid separated out was filtered, washed with petroleum ether, and crystallized from methanol.

Isopropyl-5,7,8-trichloro-3-methyl-4H-1,4-benzothiazine-2-carboxylate (IVa)

Yield 42%, m.p. 172°C, color: Brown-red; IR (KBr, *v*): 3465, 1720, 1270–1080, 2960–2830, 1460–1340, 800 cm^{-1}. ^{1}H-NMR (300.40 MHz, DMSO-d_6): d 9.47 (s, 1H, N-H), 8.10 (s, 1H, Ar-H), 1.85 (singlet, 3H, -CH_3 protons at C_3), 4.82 (septet, 1H, -CH protons of OCH(CH_3)$_2$ at C_2), 1.90 (doublet, 6H, -CH_3 protons of OCH(CH_3)$_2$ at C_2). Anal. calcd. for $C_{13}H_{12}NO_2SCl_3$: C, 44.25; H, 3.40; N, 3.97. Found: C, 44.01; H, 3.51; N, 3.86 %.

Ethyl-5,7,8-trichloro-3-propyl-4H-1,4-benzothiazine-2-carboxylate (IVb)

Yield 37%, m.p. 112°C, color: Wine red; IR (KBr, *v*): 3450, 1710, 1265–1060, 2955–2825, 1455–1335, 810 cm^{-1}. ^{1}H-NMR (300.40 MHz, DMSO-d_6): d 9.28 (s, 1H, N-H), 8.12 (s, 1H, Ar-H), 2.10 (triplet, 2H, H of –CH_2 (terminal) protons of C_3H_7 at C_3), 1.42 (sextet, 2H, H of –CH_2 tC_3H_7 at C_3), 3.35 (quartet, 2H, -CH_2 protons of OC_2H_5 at C_2), 1.64 (triplet, 3H, -CH_3 protons of OC_2H_5 at C_2). Anal. calcd. for $C_{14}H_{14}NO_2SCl_3$: C, 45.84; H, 3.82; N, 3.82. Found: C, 45.62; H, 3.71; N, 3.98 %.

Antimicrobial assessment

Broth microdilution method was used for the evaluation of minimum inhibitory concentrations (MICs, μg ml^{-1}) of the synthesized compounds as per NCCLS-1992 manual. Stock solution of 1000 μg/ml concentration for each synthesized compound and standard drugs was prepared in DMSO. In primary screening, 500, 250, and 125 μg/ml concentrations of the synthesized drugs were taken. The synthesized drugs those found active in primary screening were further tested in a second set of dilution against all microorganisms. These drugs were also diluted to obtain 100, 50, 25, 20, and 15 μg/ml concentrations. The highest dilution showing at least 99% inhibition was taken as MIC which meant 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 minimum inhibitory concentration of that compound. Antibacterial activities of the bacterial strains were carried out in Luria broth (HiMedia) medium and all fungi were cultivated in Sabouraud dextrose agar (HiMedia) at pH 6.9 with an inoculum of 10^8 cfu/ml by the spectrophotometric method and an aliquot of 10 ml was added to each tube of the serial dilution and incubated on a rotary shaker at 37°C for 24 h at 150 rpm. At the end of incubation period, MIC values were recorded.

The MIC values of synthesized compounds in μg/ml against certain bacterial strain and fungal strain are shown in Table 1.

CONCLUSION

Novel prospective bioactive substituted 4*H*-1,4-benzothiazines were synthesized using available starting materials and investigated by spectral and elemental analysis. Significant antibacterial and antifungal activities (MIC values) were exhibited by synthesized compounds against selected strains of bacteria and fungi due to strong electron-withdrawing groups. A slight change in substitution pattern affects the biological activity tremendously. Benzothiazines templates have potential to be used as a new class of antibacterial and antifungal drugs. Further biomedical research is required to make 4*H*-1,4-benzothiazines related compounds as potential antibacterial and antifungal drugs.

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AUTHOR'S CONTRIBUTIONS

Author has synthesized all the compounds, data collection and analysis, results, and methods discussion to complete the final manuscript. Prof. D.C. Gautam supervised the entire synthesized work.

CONFLICTS OF INTEREST

The author declares that he has no conflicts of interest.

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