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

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Received: 23 February 2021, Revised and Accepted: 09 April 2021

ABSTRACT

Objective: 4H-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, antituber, antibacterial, antifungal, antioxidant, anticancer agents, fungicides, etc. Therefore, these observations prompted us to synthesize substituted 4H-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 4H-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 µg/mL, Bacillus subtilis [Gram positive] MTCC 441, 41–124 µg/mL, Streptomyces griseus [Gram negative] MTCC 1998, 85–128 µg/mL, Fusarium oxysporum MTCC 1755, 142–151 µg/mL, Aspergillus niger MTCC 281, 59–78 µg/mL, and Rhizopus stolonifer MTCC 2591, 85–118 µg/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 4H-1,4-benzothiazines related compounds as potential antibacterial and antifungal drugs.

Keywords: Benzothiazine, β-diketones/β-ketoesters, Antimicrobial properties.

INTRODUCTION

Synthesized 4H-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 4H-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-(amino phenyl) disulfides (Ia) formed from substituted 2-amino benzenethiols (I) due to readily oxidation, which undergoes cyclization by scission of S-S bond due to high reactive α-position of enaminoketone system (III) toward intramolecular nucleophilic attack leading to the formation of 4H-1,4-benzothiazines (Scheme 1).

β-Diketones and β-ketoesters usually exist in two isomeric forms (ketenol 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-benzothiazine (IV) is separated (Scheme 1). Elemental analysis and spectral data support the proposed structures of reported compounds.

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

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

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

All the 4H-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\(^{-1}\).

**\(^{1}\)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 proton of –OCH(CH\(_3\))\(_2\) group at C\(_3\). In compounds IVb triplet, sextet observed in the region δ 2.10–1.42 ppm due to >CH\(_2\) protons of C\(_3\)H\(_7\) 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\(_2\)H\(_5\) group at C\(_2\).

**Mass spectra**

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

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

Where

R= Cl; R\(_{1}\) = Cl; R\(_{2}\) = Cl; R\(_{3}\) = CH\(_{3}\)C\(_{6}\)H\(_{5}\); R\(_{4}\) = C\(_{6}\)H\(_{5}\)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  | 24 | 96 | - | 142 | 59 | 118 |
| Streptomycin  | 68  | 46 | 62 | - | -  | -  | -  |
| Ketoconazole | -   | -  | -  | - | -  | -  | -  |

**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 FTIR spectrophotometer. H-NMR spectra have been recorded at 300 MHz on JEOL AL-300 FT NMR using DMSO-d$_6$ as an internal standard. 1H-NMR spectra were recorded in KBr on SHIMADZU 8400 S FT IR spectrophotometer. 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, ν): 2958.50, 1720, 1270–1080, 1145–680, 800–450 cm$^{-1}$. H-NMR (300.40 MHz, DMSO-d$_6$): d 9.47 (s, 1H, N-H), 8.13 (s, 1H, Ar-H), 1.85 (singlet, 3H, -CH$_3$); 4.62 (septet, 1H, -CH protons of OCH(CH$_3$)$_2$), 1.90 (doublet, 6H, -CH protons of OCH(CH$_3$)$_2$), at C$_6$. Anal. calcd. for C$_{14}$H$_{12}$NOSCl: 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, ν): 3450, 1710, 1265–1060, 2955–2825, 1455–1335, 810 cm$^{-1}$. 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, HoF-CH$_2$ terminal) protons of CH$_3$ at C$_6$, 1.42 (sextet, 2H, H of -CH$_2$-CH$_3$ at C$_6$), 3.35 (quartet, 2H, -CH$_2$ protons of OCH$_2$CH$_3$), 1.64 (triplet, 3H, -CH$_3$ protons of OCH$_3$, C$_6$). Anal. calcd. for C$_{16}$H$_{14}$NOSCl: C, 45.84; H, 3.62; 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) 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) in inoculated bacteria/fungi was recorded as minimum inhibitory concentration of that compound. Antimicrobial 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$^5$ 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 4H-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 4H-1,4-benzothiazines related compounds as potential antibacterial and antifungal drugs.

**ACKNOWLEDGMENTS**

The author is grateful to the Department of Chemistry, University of Rajasthan, Jaipur, and Govt. PG. College, Raigarh (Alwar), for providing necessary facilities. Authors are also thankful to Institute of Seminal Applied Sciences, Jaipur, for carrying out antimicrobial activity of synthesized compounds.

**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.

**AUTHOR’S FUNDING**

The author is also grateful to the UGC and CSIR, New Delhi, for providing financial support.

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