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# SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL EVALUATION OF IMINE-LINKED BENZOTHIAZINE COMPOUNDS BY ULTRASOUND METHOD

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**ABSTRACT**

This work seeks to evaluate the antibacterial properties of the compounds and create imine-linked benzothiazine derivatives using a novel, eco-friendly ultrasonic technique. Escherichia coli and Staphylococcus aureus were utilized to assess the synthetic compounds, SBIa-e, for their antibacterial activity. All compounds were soluble in organic solvents but insoluble in water, with yields ranging from 68 to 77%. The molecules showed stretching vibrations corresponding to various functional groups. The presence of amine hydrogen (2.0, 7.0 ppm), aliphatic protons (2.6-4.3 ppm), imine hydrogen (8.0-8.5 ppm), and protons of the aromatic ring (6.8-7.9 ppm) was indicated by the 1H-NMR spectra. Both the bacterial strains were all susceptible to the antibacterial action of the compounds, which showed dose-dependent effects. When the compounds were compared to gram-positive bacteria, it was discovered that the former had greater effectiveness than the latter. SBId had the lowest $IC_{50}$ against *Staphylococcus aureus* (70.16 µg/mL) and *Escherichia coli* (49.19 µg/mL) among the drugs whose $IC_{50}$ values were calculated.

**Keywords:** 1,4-Benzothiazine, Ultrasonic, Antibacterial, Eco-Friendly, Synthesis.

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## INTRODUCTION

As the pharmacophore of the well-known anti-psychotic medications phenothiazines, 1,4-benzothiazine is sometimes referred to as the fundamental unit due to its use as an antioxidant, dye, photographic developer, and ultraviolet light absorber.[1] The use of ultrasonography (sonochemistry) in research has grown in interest recently. Ultrasound has many different chemical effects, but it also significantly enhances catalytic and stoichiometric chemical reactions. The reactivity is accelerated by a million times by ultrasonic irradiation, and numerous relevant synthetic processes have been successfully carried out.[2] The ultrasonic irradiation process delivers higher yields in a shorter amount of time due to its gentler and more conventional nature compared to typical circumstances, including a strong base and a long reaction time.[2] Benzothiazines are widely used[3-8], and a number of time-consuming procedures involving workups have been reported. Hence it is necessary to find a quick, safe, inexpensive, and environmentally friendly way to synthesize them. Therefore, the plan was to use ultrasonic synthesis to create a few imine-linked benzothiazine derivatives and assess their antibacterial activity.

## EXPERIMENTAL

An open capillary's melting point was discovered using a melting point device (BioTechnics). Retention factor (Rf) was determined via a thin layer chromatographic technique utilizing precoated Gel Silica $F_{254}$ precoated sheets. The approach previously described by Dabholkar and Gavande[9] and Rai *et al.*[10] was modified to create the synthetic pathway needed to produce the required imine-linked benzothiazine derivatives. In order to maximize the product production, the process was adjusted in the lab. To achieve the desired products, a three-step synthetic route (scheme-1) was employed. First, benzothiazine-3-one was synthesized; its hydrazine derivative was prepared; and last, the Schiff base reaction was utilized to synthesize the imine-linked benzothiazine derivative.

### Synthesis of 2-(2-methoxyacetyl)-2H-benzo[1,4]thiazin-3(4H)-one

Placed into a 100 mL rounded bottom flask was 2-aminothiophenol (0.125 g, 0.001 moles), maleic anhydride (0.1 g, 0.001 moles), methanol (5 mL), and concentrated $H_2SO_4$ (98%, 0.2 mL). After shaking and sonicating for thirty minutes, the contents were disintegrated. 2-(2-methoxyacetyl) was prepared by filtering and washing the solid generated from the reaction, which was monitored by TLC and had an n-


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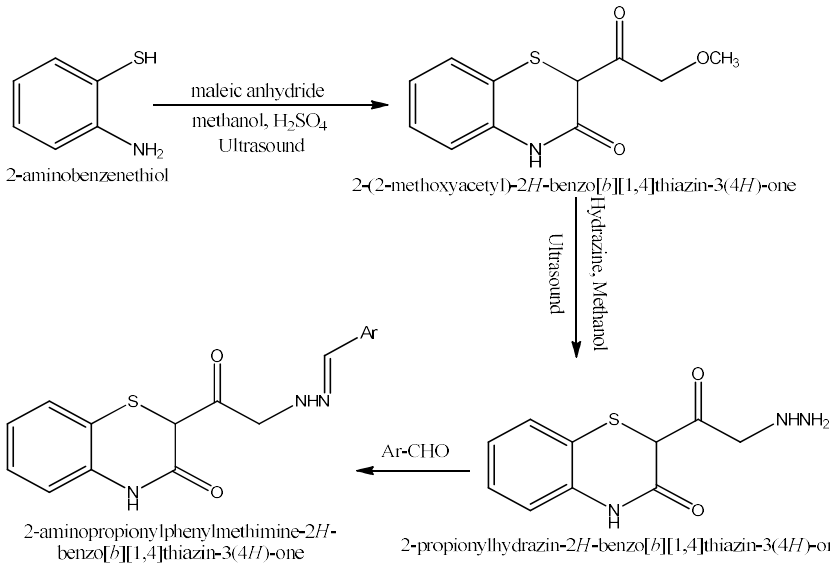
hexane: ethylacetate ratio of 9:1. The contents were then allowed to cool in the refrigerator and the desired product was filtered.[9]




Scheme-1: Synthetic Pathway for benzothiazines

**Synthesis of 2-propionylhydrazin-2H-benzo[1,4]thiazin-3(4H)-one**

A 100 mL rounded bottom flask was filled with thiazin-3(4H)-one (0.237 g, 0.001 moles), hydrazine hydrate (0.1 mL, 0.002 moles), and dry methanol (20 mL). The mixture was sonicated for 20 minutes at room temperature. Following the completion of forward kinetics (monitored by TLC and had an n-hexane: ethylacetate ratio of 8:2 as solvent), the mixture was allowed to cool over an ice-filled container, and the contents were then dumped over crushed ice. After being extracted from methanol, the solid product was recovered, cleaned with water, and recrystallized to get 2-propionylhydrazin-2H-benzo[1,4].thiazin-3(4H)-one.[9]

**General Procedure for Synthesis of Schiff's Base**

2-hydroxypropionylhydrazin-2H-benzo[1,4]thiazin-3(4H)-one (0.095g, 0.001 mole), and the accurately measured quantity of required aldehyde (1 mmol), was combined with 5 mL of methanol within a round-bottom flask. The combination was mixed with a catalytic quantity of glacial acetic acid. Sonicating the reaction mixture at room temperature for thirty-five minutes produced a solid precipitate. Filtration, washing with water, and recrystallization from ethanol were the steps involved in obtaining the desired imine-linked benzothiazine derivative (Schiff's base) from the reaction mixture on crushed ice.[10]

**Microbial Strain Used**

The lyophilized microbial strains of *Escherichia coli* and *Staphylococcus aureus* for the antibacterial experiment were utilized. Reviving the lyophilized cultures, required mixing nutritional broth to the culture vial to create a suspension of bacteria.

**Preparation of Test Compounds**

Dissolving the synthesized Schiff's bases in DMSO yielded samples with 25, 50, 75, and 100 µg/m strength. These served as test specimens.

**Testing Protocol (zone of inhibition)**

Nutrient agar plates that had been pre-poured and were 3 mm thick were smeared with a few drops of the bacterial solution. Screening for antibacterial activity was done using the disc diffusion method.[11,12]

Wells were bored into the agar plate at equal intervals using a cork borer (10 mm), and 200 µL of Schiff's bases of particular strength were added one in each well. Incubation of these plates was done for 24 hours at 37 ± 0.1°C to facilitate microbe growth. A millimeter scale was used to measure the zone of inhibition on each well.

**Screening Procedure (Minimum inhibitory concentration)**

For assessing the $IC_{50}$ of the test samples, a broth dilution procedure has been utilized. The final inoculum size of $10^5$ CFU/mL was maintained for a bacterial culture. The growth control consisted of an array of tubes that contained the inoculated broth, while the medium's sterility was guaranteed by another set of tubes that contained only the broth. Nutrient broth (3 mL) was added to each of the six tubes, which were labeled 1 through 6. First, 300µL of the test sample (1 mg/mL) was added to the tube. Three hundred microliters of the contents from the first tube were moved to the second tube by swishing the contents between the palms. Steps two through six of the procedure were repeated. A volume of 300µL was extracted and disposed of from the sixth tube. A volume of 200µL containing the bacterial inoculum was introduced into every tube. Every tube was kept at 37°C for 24 to 48 hours to facilitate the growth of microorganisms. At 600 nm, the absorbance of the substance in every tube was measured using the UV-visible spectrophotometer after incubation. The concentration of each sample and standard (norfloxacin) used in the growth control tube was seen to produce half of its optical density (50%).[13,14]

## RESULTS AND DISCUSSION

Table-1 displays the Rf values and melting points of the produced compounds.

Table-1: Melting Point and $R_f$ Value of Prepared Benzothiazine Schiff Bases

| Compound Code | Melting Point (°C) | $R_f$ value |
|---|---|---|
| $SBI_a$ | 167-169 | 0.45 |
| $SBI_b$ | 179-181 | 0.63 |
| $SBI_c$ | 172-174 | 0.54 |
| $SBI_d$ | 167-169 | 0.57 |
| $SBI_e$ | 184-186 | 0.41 |

**2-(2-(2-benzylidenehydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3(4H)-one, $SBI_a$**

$CDCl_3$ (400 Hz), 1H-NMR (δ ppm), color white, yield 68% 3.14 aliphatic protons (CH2), 4.14 aliphatic protons (C-H), 7.07 amine protons (N-H), 8.07 imine-related protons (CH=N), 6.88–7.76 aromatic protons (C–H); m/e - 325.38; FTIR (cm-1) - 3359.18 (N-H stretch), 3053.85, 1599.06 (Aromatic stretch), 1712.86 (C=O stretch), 680 (C-S-C stretch).

**2-(2-(2-(4-hydroxybenzylidene)hydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3(4H)-one, *$SBI_b$***

$CDCl_3$ (400 Hz), 1H-NMR (δ ppm), color white, yield 69% - 6.75, 7.13-7.69 Aromatic Protons (C-H), 7.04 Amine Protons (N-H), 8.07 Imine Protons (CH=N), 5.02 Hydroxy (O-H) Protons, 3.56 Aliphatic Protons (CH2), 3.96-3.98 Hydratic Protons (C-H); m/e - 341.38; FTIR (cm-1) – Broad peak of N-H stretch, 3087.20, 1576.87 (Aromatic stretch), 1677.18 (C=O stretch), and 680 (C-S-C stretch).

**2-(2-(2-(4-chlorobenzylidene)hydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3(4H)-one, $SBI_c$**

$CDCl_3$ (400 Hz), 1H-NMR (δ ppm), color white, yield 71% - 6.73–6.75, 7.13–7.71 Aromatic Protons (C–H), 7.03–7.07 Amine Protons (N–H), 8.07 Imine Protons (CH=N), 3.64 Aliphatic Protons (CH2), 3.97 Aliphatic Protons (C–H); m/e = 359.83; FTIR (cm-1) – 3278.16 (Amine stretch), 3100.70, 1624.13 (Aromatic stretch), 1734.08 (Carbonyl stretch), 1022.32 (C–Cl stretching).

**2-(2-(2-(4-nitrobenzylidene)hydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3(4H)-one, $SBI_d$**

$CDCl_3$ (400 Hz), 1H-NMR (δ ppm), color white, yield 77% 6.87–6.89, 7.12–7.82 Aromatic Protons (C–H), 7.03–7.07 Amine Protons (N–H), 8.07-8.28 Imine Protons (CH=N), 3.25 Aliphatic Protons (CH2), 4.25–4.44 Aliphatic Protons (C–H); m/e = 370.38; FTIR (cm–1) – 3342.78 (N–H stretch), 3107.46, 1614.00 (Aromatic stretch), 1176.52 (Carbonyl stretch), 1086.93 (N–N stretching), 1367.59 ($NO_2$ stretch).

**2-(2-(2-(2-phenylethylidene)hydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3(4H)-one, $SBI_e$**

$CDCl_3$ (400 Hz), 1H-NMR (δ ppm), color white, yield 74% 7.39-7.71 Aromatic C-H, 7.15 Amine (N-H), 8.81 Proton due to Imine (CH=N), 2.32-2.35 Proton of Aliphatic Amine, 3.10-3.32 ($CH_2$ aliphatic); m/e – 339.41; FTIR (cm-1) – 3342.78 (N-H stretch), 3107.46, 1614.00 (Aromatic stretch), 1176.52 (Carbonyl stretch), 1086.93 (N-N stretching).

**Biological Action**

When evaluating the imine-linked benzothiazine derivatives (Schiff's bases), the diameter of inhibition was assessed to determine their initial antibacterial activity. The conjugates' ability to inhibit microorganisms was evaluated at four different concentrations. For its antibacterial effect, norfloxacin was the usual medication (Table-2).

Table-2: Antibacterial Action by $SBI_{a-e}$

| Compound Code | Zone of Inhibition (mm)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli (µg)* | | | | *S. aureus (µg)* | | | |
| | 25 | 50 | 75 | 100 | 25 | 50 | 750 | 100 |
| $SBI_a$ | - | - | 12 | 13 | - | - | - | 12 |
| $SBI_b$ | - | - | 13 | 14 | - | - | 11 | 12 |
| $SBI_c$ | - | - | 16 | 23 | - | - | 16 | 17 |
| $SBI_d$ | - | - | 18 | 25 | - | - | 16 | 17 |
| $SBI_e$ | - | - | 14 | 16 | - | - | 12 | 14 |
| Norfloxacin | 23 | - | - | - | 24 | - | - | - |

The absorbance obtained at 660nm for the broth that was incubated with test samples was measured using to find the MIC of the test chemicals. 50% optical density was created at the concentration that, in comparison to the growth tube, reached the test's minimum inhibitory concentration (MIC).

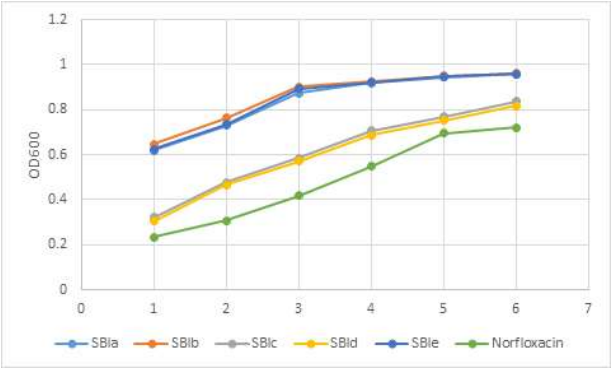



Fig.-1: Optical Density vs. Concentration for *E. coli*

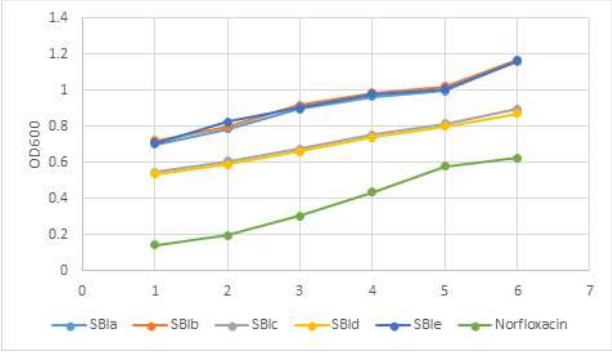



Fig.-2: Optical Density vs. Concentration for *S. aureus*

Plotting the optical density vs. concentration sample (Fig.-1 & 2) yielded the IC50 values, which are shown in Table-3.

**Chemistry**

Utilizing ultrasonography as the reaction's energy source, the synthesis of imine-linked benzothiazine derivatives was completed in three stages. The first step involved condensing aminothiophenol with maleic anhydride and cyclizing the resultant chemical, benzothiazine-3-one. Hydrazine was then reacted with the derivative of benzothiazine-3-one. An excess of hydrazine was used to achieve hydrazination, and the excess that had not yet reacted was washed out of the mixture with water. The last stage entailed the creation of Schiff's base through the reaction of aromatic aldehydes with the benzothiazine derivative hydrazine in

the presence of Lewis acid. With a yield ranging from 68 to 77%, all of the products were soluble in organic solvents but insoluble in water. The compounds' structural identification was established by employing proton NMR spectra to confirm the number of protons and FTIR spectra to confirm the presence of desired functional groups. The compounds exhibited vibrations corresponding to stretching of aromatic C-H bond (3085-3025 $cm^{-1}$), aromatic C-C bond/C=C bond (1630-1565 $cm^{-1}$), C=O bond (1770-1635 $cm^{-1}$), C-S-C bond (720-560 $cm^{-1}$ weak), N-H bond (3500-3100 $cm^{-1}$), C-N bond (~850), C=N bond (1690-1520 cm-1), and N-N (~1040 cm-1). The occurrence of protons in the aromatic nucleus (6.8–7.9 ppm), imine proton (8.0–8.5 ppm), amine hydrogen (2.0, 7.0 ppm), and aliphatic protons (2.6–4.3 ppm) was shown by the 1H-NMR spectra.

Table-3: $IC_{50}$ of $SBI_{a-e}$

| Compound | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | *E. coli* | *S. aureus* |
| $SBI_a$ | 142.42 | 133.52 |
| $SBI_b$ | 156.86 | 141.03 |
| $SBI_c$ | 53.5 | 74.92 |
| $SBI_d$ | 49.19 | 70.16 |
| $SBI_e$ | 144.37 | 136.56 |
| Norfloxacin | 0.89 | 0.01 |

**Antibacterial Action**

When both gram-positive and gram-negative microorganisms were tested against., all of the compounds showed dose-dependent antibacterial activity. It was discovered that the chemicals had more effect on the gram-negative bacterium than the gram-positive bacterium tested. After the compounds' $IC_{50}$ values were determined, SBId had the lowest IC50 against gram-positive (70.16 µg/mL) and gram-negative (49.19 µg/mL) bacteria. This implies that the imine connecting group's electron-withdrawing group contributed to the antibacterial activity. Further to having an electron-withdrawing group, compound SBIc demonstrated IC50 values against S. aureus and E. coli of 74.92 µg/mL and 53.5 µg/mL, respectively.

In contrast, compound SBIb ($IC_{50}$ 156.86 µg/mL and 141.03 µg/mL for E. coli and S. aureus, in turn) demonstrated that the addition of an electron-donating group was harmful to action.

## CONCLUSION

Using ultrasonic as a source of energy to synthesize the benzothiazine nucleus and prepare Schiff's base of benzothiazine was the main goal of the effort. The work's outcomes demonstrated that the goal was effectively accomplished and that a good yield (68–77%) of the compounds was synthesized with ultrasonic radiation. In order to determine that the electron-withdrawing substituent in imine connecting groups was advantageous for antibacterial activity, the antibacterial capability of the produced molecules was also assessed.

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## CONFLICT OF INTERESTS

The authors report no conflict of interest.

## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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