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Synthesis, characterization and anti-bacterial activity of benzothiazine congeners

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ABSTRACT

The objective of this work was to synthesize Schiffs bases of benzothiazine using ultrasonication method and evaluate them for antibacterial action using disc diffusion method. The conjugates were off-white or yellow in color and obtained in 70-75 % yields and were insoluble in water, methanol and DMSO while they were soluble in chloroform. In the ^{1}HNMR spectra the peaks at chemical shift value of 4.02 corresponding to the proton of benzothiazine nitrogen (N-H), 8.47 corresponding to the proton of imine nitrogen (N-H), 3.84 corresponding to the proton of methylene group adjacent to sulfur (CH_2) and 6.4 to 7.6 corresponding to the protons of the aromatic rings were present in all the conjugates. In compounds BS_4 and BS_5 peaks at chemical shift of 2.11 and 1.24 corresponding to methoxy proton and free methyl group (CH_3) respectively was also present. The fragment peaks of molecular ion or isotope were found in the mass spectra of the compounds. The zone of inhibition exhibited by **BS_3, BS_4** and **BS_5** was highest amongst all the conjugates. This signifies the importance of the substitution on aromatic ring of the aldehyde.

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Introduction

Nitrogen and sulphur containing six membered benzofused heterocycles play an important role in the field of medicinal and biological chemistry.[1] These heterocyclic molecules have also been extensively found in agrochemicals, pesticides and pharmaceuticals. Benzothiazine forms an important class of heterocyclic systems. Three canonical forms of benzothazine occur owing to the position of sulfur and nitrogen atoms. Phenothiazine having anti-psychotic activity owed by its 1, 4-benzothiazine pharmacophore. Several other actions of benzothiazine have been reported in literature.[5-10] Now-a-days, application of ultrasound (sonochemistry) has become an exciting field of research. The chemical effects of ultrasound are diverse and include substantial improvements in both stoichiometric and catalytic chemical reactions. Ultrasonic irradiation accelerates the reactivity million fold and many synthetically useful reactions were successfully accomplished. As compared to conventional conditions, viz. strong base and long reaction time, the ultrasonic irradiation procedure is milder and more conven tional leading to higher yields in shorter reaction time. Due to the wide application of benzothiazines anlaogs, rapid, safe, ecofriendly as well as economical method for the synthesis of benzothiazines is warranted. Hence it was envisioned to synthesize few imine linked benzothiazine derivatives using ultrasonic method and evaluate them for antimicrobial action.

Material and Methods

2-amino thiophenol and maleic anhydride were purchased from Loba, hydrazine hydrate was procured from sulab. All other chemicals and reagents were obtained from oxford and CDH. Lyophilized MTCC strains of bacteria were purchased form IMT, Chandigarh.

The steps involved in the synthesis of benzothiazine derivatives has been presented in the Figure 4.1 below.

Synthesis of benzothiazine nucleus

2-Aminothiophenol (0.01 mol), maleic anhydride (0.01 mol), methanol (25 mL) and Conc. H_2SO_4 (98%, 2 mL) were taken in a 100 mL round bottomed flask and subjected to sonication for 10 min (Labman). After completion of the reaction (monitored by TLC) cooled solid thus obtained was washed with 5% sodium bicarbonate solution and extracted in dichloromethane to afford benzothiazine nucleus.[17]

Synthesis of hydrazine derivative of benzothiazine

The benzothiazine nuclues (0.01 mol), hydrazine hydrate (0.02 mol) and dry methanol (20 mL) were taken in a 100 mL round bottomed flask and subjected to sonication for 8 min. After completion of the reaction (monitored by TLC) cooled, poured on crushed ice, solid thus obtained was washed with water and recrystallised from methanol to get the hydrazinated benzothiazine.

General method for synthesis of Schiffs base of benzothiazine

To a solution of hydrazinated benzothiazine (0.001 mol) in ethanol (10 mL) was added the appropriate aromatic aldehyde (0.001 mol). The reaction mixture was sonicated for 5 min using a ultrasonic cleaner at 37°C. After the completion of reaction as indicated by TLC (petroleum ether: ethylacetate/4:1), the mixture was allowed to cool and the precipitated solid was filtered and dried to obtain the desired product.[18]

Synthesis of 2-(2-(2-((E)-3-phenylallylidene) hydrazinyl)acetyl)-2H-benzo[b][1,4]thiazin-3

***(4H)-one,* (BS$_1$)**

Color: White; Yield: 77%; ^{1}HNMR ($CDCl_3$, δ ppm) – 4.02 (N-H, benzothiazine), 8.47 (N-H, imine), 3.84 (CH_2), 6.4 to 7.6 (C-H, aromatic); FT-IR (cm^{-1}) – 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C); m/e – 353.1 (M^++2)

***Synthesis of phenyl N'-(2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetyl) formohydrazonate,* (BS$_2$)**

Color: Off-White; Yield: 75%; ^{1}HNMR ($CDCl_3$, δ ppm) – 4.02 (N-H, benzothiazine), 8.47 (N-H, imine), 3.84 (CH_2), 6.4 to 7.6 (C-H, aromatic); FT-IR (cm^{-1}) – 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C); m/e – 340.1 (M^+)

***Synthesis of N'-(2-hydroxybenzylidene)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl) acetohydrazide,* (BS$_3$)**

Color: Off-White; Yield: 70%; ^{1}HNMR ($CDCl_3$, δ ppm) – 4.02 (N-H, benzothiazine), 8.47 (N-H, imine), 3.84 (CH_2), 6.4 to 7.6 (C-H, aromatic), 3.67 (CH_2 ethyl); FT-IR (cm^{-1}) – 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C); m/e – 340.2 (M^+)

***Synthesis of N'-(4-hydroxy-3-methoxybenzylidene)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetohydrazide,* (BS$_4$)**

Color: Yellow; Yield: 71%; ^{1}HNMR ($CDCl_3$, δ ppm) – 4.02 (N-H, benzothiazine), 8.47 (N-H, imine), 3.84 (CH_2), 6.4 to 7.6 (C-H, aromatic), 2.11 (O-CH_3); FT-IR (cm^{-1}) – 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C); m/e – 373.1 (M^++2)

***Synthesis of N'-(4-(dimethylamino) benzylidene)-2-(3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazin-2-yl)acetohydrazide,* (BS$_5$)**

Color: Yellow; Yield: 74%; ^{1}HNMR ($CDCl_3$, δ ppm) – 4.02 (N-H, benzothiazine), 8.47 (N-H, imine), 3.84 (CH_2), 6.4 to 7.6 (C-H, aromatic), 1.24(CH_3); FT-IR (cm^{-1}) – 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C); m/e – 370.1 (M^++2)

Chemical Characterization[19, 20]

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR). The melting points were determined by open capillary method and are uncorrected using an electrically heated melting point determination apparatus. The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates. The solvent system used for running the compounds was petroleum ether: ethylacetate in the ratio 8:2. The solubility of all the synthesized compounds was qualitatively determined in different solvents.

Antibacterial study

Microorganisms used

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investi-

gation.

Preparation of test compounds

The synthesized benzothiazine derivatives were dissolved in DMSO to obtain the solutions of 50, 75, 100 & 150 µg/mL. These solutions were used as the test samples.

Screening Procedure

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method.[21]

Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the benzoxazole-isatin conjugates (50, 75, 100 & 150 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

Results and Discussion

Chemistry

The schiffs bases of benzothiazine (**BS_{1-5}**) were synthesized in three steps starting from 2-amino thiophenol. Benzothiazine-3-one was synthesized by the reaction of methanolic solution of 2-aminothiophenol with maleic anhydride, and ultrasonication. The 2-methoxyacetyl side chain at 4 position underwent a decarboxylative hydrazination to yield acetohydrazide which was reacted with aromatic aldehydes to yield the Schiffs base compounds (**BS_{1-5}**).

The synthesized conjugates were characterized by determining the practical yield, melting point, solubility and spectral studies. The physicochemical properties are shown in Table 1.

All the compounds were soluble in chloroform.

The confirmation of the structures of the compounds was done by ^{1}H-NMR, mass and IR spectral study. The stretching vibrations at around 3400 cm^{-1} (N-H), 1750 cm^{-1} (C=O), 1454 cm^{-1} (C=N), 1174 cm^{-1} (C-O-C) and bending vibrations at around 680 cm^{-1} (C-S) and 485 cm^{-1} (C-S-C) ring deformation were present in the FT -IR spectra of the conjugates.

In the ^{1}HNMR spectra the peaks at chemical shift value of 8.02 corresponding to the proton of benzothiazine nitrogen (N-H), 8.13 corresponding to the proton of imine nitrogen (C=N), 3.84 corresponding to the proton of methylene group and 6.4 to 7.9 corresponding to the protons of the aromatic rings were present in all the conjugates. In compounds BS_4 and BS_5 peaks at chemical shift of 1.24-2.1 corresponding to free methyl group (CH_3) and methoxy group (OCH_3) was also present. The fragment peaks of molecular ion or isotope were found in the mass spectra of the compounds.

The antibacterial activity of the synthesized Schiffs base of benzothiazine was determined measuring the zone of inhibition in the agar plate. Four concentrations of the conjugates were tested for antibacterial action. Norfloxacin was used as the standard drug for antibacterial action (Table 2).

The zone of inhibition exhibited by **BS_3, BS_4** and **BS_5** was highest amongst all the conjugates. This signifies the importance of the substitution on aromatic ring of the aldehyde.

Conclusion

The objective of the present investigation was to

develop Schiff's bases of benzothiazines using ultrasonication method and evaluate their antibacterial action. The synthesis was accomplished in four steps starting from 2-amino phenol. The compounds **BS_3, BS_4** and **BS_5** presented the best antibacterial activity against both gram negative and gram positive bacteria. The ultrasonication method was able to produce the desired compounds of sufficient purity in good yields in very short duration of time.

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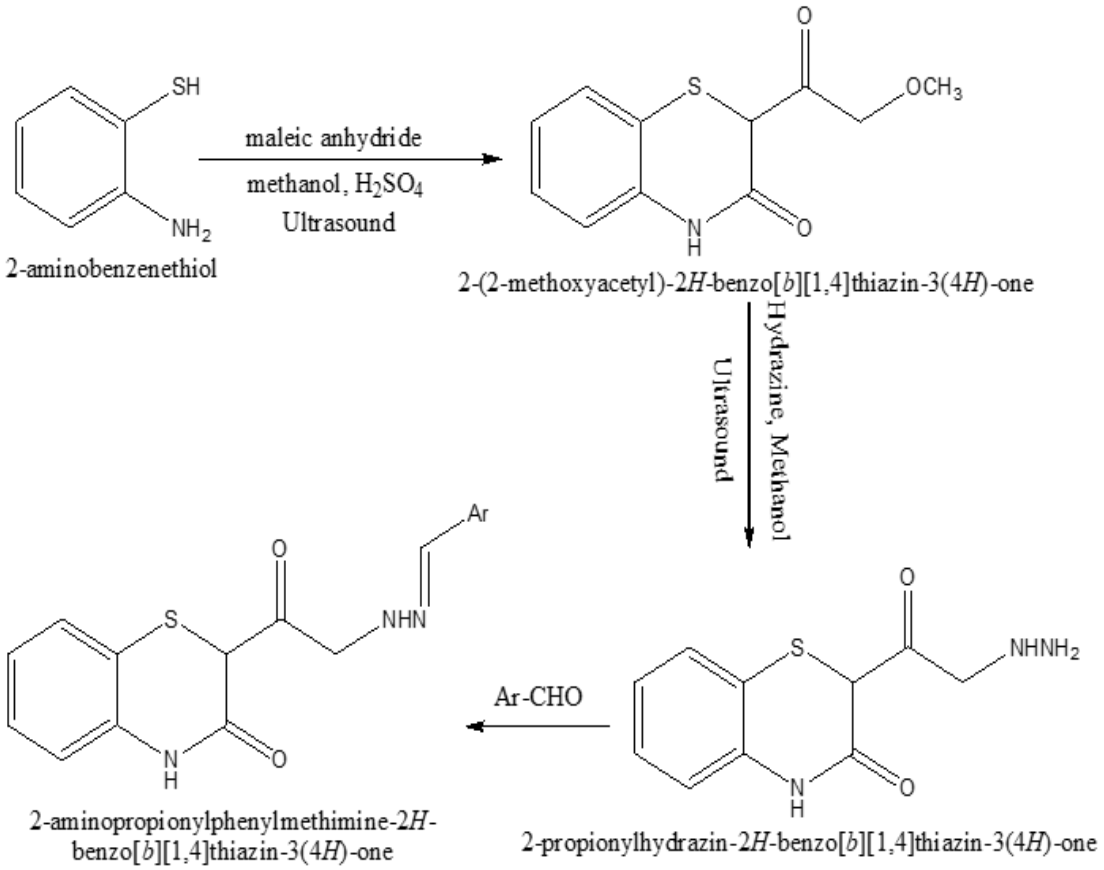
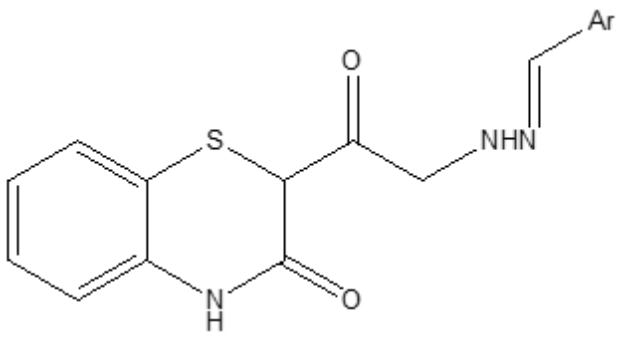


Figure 4.1 Scheme for synthesis of benzothiazine derivatives

Table 1 Yield and color of synthesized compounds



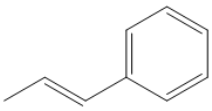
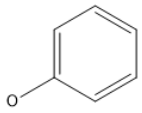
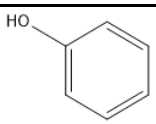
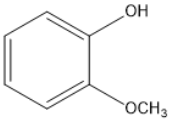
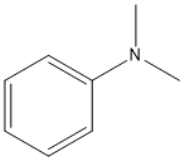
Compound code	Ar	R_f Value	Melting point (°C)
BS_1		0.45	238-240
BS_2		0.54	236-238
BS_3		0.62	236-238
BS_4		0.74	223-225
BS_5		0.75	239-241

Table 2 Zone of inhibition of Schiffs base

Compound Code	Zone of Inhibition (mm)*							
	S. auerus				*E.coli*			
	25µg	50µg	100µg	150µg	25µg	50µg	100µg	150µg
BS_1	-	-	13	14	-	-	-	13
BS_2	-	-	-	14	-	-	-	13
BS_3	-	-	17	**24**	-	-	15	**18**
BS_4	-	-	16	**19**	-	-	14	**21**
BS_5	-	-	17	**23**	-	-	19	**25**
Norfloxacin	**22**	**-**	**-**	**-**	**23**	**-**	**-**	**-**

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – good activity