Bio-synthesis, NMR, FTIR, Mass spectra of 4-methyl-2-[(2*E*)-2-(1-phenylethylamine) hydrazinyl]-1, 3-benzothiazole derivatives and their bacterial studies

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ABSTRACT:

Heterocyclic compounds play an important role in industries with their wide applications. Several heterocyclic compounds are used as medicine in different therapeutic targets. Substituted-1, 3-benzothiazoles are one of the important classes of bicyclic hetero-compounds. These moieties serve as unique and versatile scaffolds for experimental drug design. 1,3- Benzothiazole derivatives have attracted considerable attention in synthetic as well as pharmaceutical chemistry, because of their potent and significant pharmacological activities, some substituted amino benzothiazoles have been synthesized from 2,4-dimethylaniline by utilizing industrial waste (Mixture of NaCl and NaBr) for ring cyclization. The prepared derivates are characterized by FTIR, 1HNMR, and mass spectra. The synthesized compounds are characterized by spectral analysis and their biological activities were evaluated.

Keywords: *Green chemistry, Benzothiazole, FTIR, [1]HNMR, mass spectra and Schiff's Bases.*

INTRODUCTION:

The most common heterocyclic compounds like thiazole an important pharmacophore in drug discovery and development processes in industries. Most of the heterocyclic compounds like thiazole and their derivatives covers a wide range of therapeutic targets including anti-inflammatory, [1, 2] antifungal,[3] antiviral,[4] analgesic,[5] antioxidant, [6,7] antipsychotic,[8] anticonvulsant,[9] and antidiabetic [10, 11] anti-tubercular, [12, 13] anti-cancer, [14] etc. Thiazole is naturally found in vitamin B (Thiamin). This vitamin is water-soluble, which helps the body to release energy from carbohydrates at the time of metabolism and its enzyme plays a vital role in the decarboxylation of α-keto acid and as an electron sink. It is helpful for the normal functioning of the nervous system due to its role in the synthesis of acetylcholine a neurotransmitter. Cancer is the most common dreadful disease affecting the abnormal growth of cells. It changes the genetic activity of cells. [15, 16] Globally, cancer is a major leading

cause of death. The WHO report (2018) shows around 10 million people die from cancer, every year. About 0.3 million new cancer cases are diagnosed in children aged between 1 and 19 years. [17, 18] There is no age factor for cancer disease. Cancer chemotherapy causes several adverse effects, which include multiple drug resistance, adverse events, unwanted side effects, and selectivity. Benzothiazole with Schiff's base [19] will increase the biological activities [20, 21] we also considered the acetophenone moiety with functional groups like NO_2, Br, OCH_3, [22, 23, 24] and Cl enhanced the biological activities of synthesized drugs.

Along with conventional approaches, effective and eco-friendly alternative reactions are being developed with commercially available reagents and the principles of green chemistry. [25] This method avoids the use of toxic solvents. Reactions are performed by using industrial waste as a reagent and water as the solvent, making the process relatively low-cost with the generation of recyclable salt. Multistep reactions of the C-2-substituted benzothiazole played a special role in the designing of biologically active compounds. [26, 27] The advantages of these reactions are effectiveness, simple experimental implementation, and high yields.

MATERIAL AND METHODS

Materials and reagents: All the chemicals, which have been used for the synthesis of Schiff's base were supplied by Sigma Aldrich, Loba Chemie, and Merck. All the reactions were monitored and the purity of the products was checked by thin-layer chromatography (TLC) and mixed melting point. TLC was performed on Merck 60 F-254 silica gel plates with visualization by short UV light and further with an iodine chamber, as well as by gas chromatography (GC). The GC was performed on Shimadzu 2014 with a capillary column (RTX5, 30 meters). Melting points were determined in capillary tubes in a silicon oil bath using a Veego melting point apparatus. 1H NMR and 13C NMR spectra were recorded on BRUKER AVANCE Neo 500 MHz NMR spectrometer. The sample was dissolved in $CHCl_3$. Chemical shifts are reported from the internal tetramethyl silane standard and chemical shifts are given in δ units. Infrared spectra were taken on BRUKER AVANCE IVDR FTIR. The GC-MS spectra were recorded on a Shimadzu GC-MS.

Antimicrobial activity: The antimicrobial activities of all the synthesized compounds were evaluated by the Kirby-Bauer disk diffusion method [28,29]. The strains were procured from the Institute of Microbial Technology, Chandigarh. All cultures were maintained at 4ºC. Cover nutrient agar slants throughout the experiment. The cultures were incubated overnight at 37ºC in nutrient broth before using for antimicrobial activity. Five hundred microliters of overnight old bacterial/fungal suspension were spread over the nutrient agar plates using a

sterile cotton swab to get uniform microbial growth. DMSO was used to dissolve synthesized compounds. Under aseptic conditions, empty sterilized discs (Whatman No. 5, 6 mm diameter) were impregnated with different concentrations (25, 50, 75, and 100 µg/disc) of respective synthesized compounds and placed on the agar surface. A paper disc moistened with aqueous DMSO was placed on seeded petri plates as vehicle control. The plates were left for 30 min. at room temperature to allow the diffusion of synthesized compounds and then incubated at 37 ºC for 24 h. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test of microorganisms. All experiments were carried out in triplicates.

Synthesis of 2-hydrazinyl-4-methyl-1, 3-benzothiazole (4): A mixture of 2, methylaniline **(1)** (1.0 mmol) and ammonium thiocyanate (1.1 mmol) in 30% hydrochloric acid (1.1 mmol) was stirred at 80°C for 6-8 hours, monitored by GC. The solid filtered gave **(2)**. A mixture of **(2)** (1.0 mmol) was mixed with industrial waste salt (Sodium chloride/bromide) (2.0 mmol) followed by the addition of sulphuric acid. It was stirred at 80°C for 1-2 hours, monitored by GC. The solid filtered gave **(3)**. A mixture of **(3)** (1.0 mmol) in water followed by the addition of hydrazine hydrate (1.5 mmol) was stirred at 100-110°C for 1-2 hours. The progress was monitored by GC. The solid filtered gave 2-hydrazinyl-4-methyl-1,3-benzothiazole **(4)**.

A general method for the synthesis 4-methyl-2-[(2*E*)-2-(1-phenylethylamine) hydrazinyl]-1, 3-benzothiazole derivatives (**6 a-h**): A mixture of 2-hydrazinyl-4-methyl-1, 3-benzothiazole **(4)** (1.0 mol.), and substituted aromatic ketones moiety (**5a-h**) (1.05 mol) was mixed in a 50 mL round bottom flask with water as a solvent and refluxed for 4 h. The progress of the reaction was monitored by TLC and GC. The rate of reaction was controlled by applying occasional stirring. A crystalline solid was obtained. The product was filtered, washed with water, and recrystallized from ethanol: water, which gave corresponding benzothiazole derivatives (6 a-h) as products. ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm), ^{13}C NMR (500 MHz, $CDCl_3$) analysis, and M+ ion peak in the mass analysis of synthesized compounds (6 a-h) as follows:

4-methyl-2-{(2*Z*)-2-[1-(3-nitrophenyl) ethylidene] hydrazinyl}-1, 3-benzothiazole (**6a**): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): 2.16 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 7.0-9.26 (m, 7H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.28, 17.7, 21.0, 71.7, 124.2 - 147, 168.1, MS, m/z: 326 $(M+H)^+$.

2-{(2*Z*)-2-[1-(2, 4-dichlorophenyl) ethylidene] hydrazinyl}-4-methyl-1, 3-benzothiazole (6b): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 2.31 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 7.0-7.48

(m, 5H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 16.08, 18.35, 21.37, 23.88, 118 - 148, 177.51; MS, m/z: 349.0 $(M+H)^+$, m/z: 351.0 $(M+H+2)^+$.

2-{(2*Z*)-2-[1-(4-bromophenyl) ethylidene] hydrazinyl}-4-methyl-1, 3-benzothiazole (6c): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 2.07 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 6.97-7.59 (m, 7H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.23, 18.16, 118.87 – 148.69, 167.93; 176.69, MS, m/z: 359.0 $(M+H)^+$, m/z: 361.0 $(M+H+2)^+$.

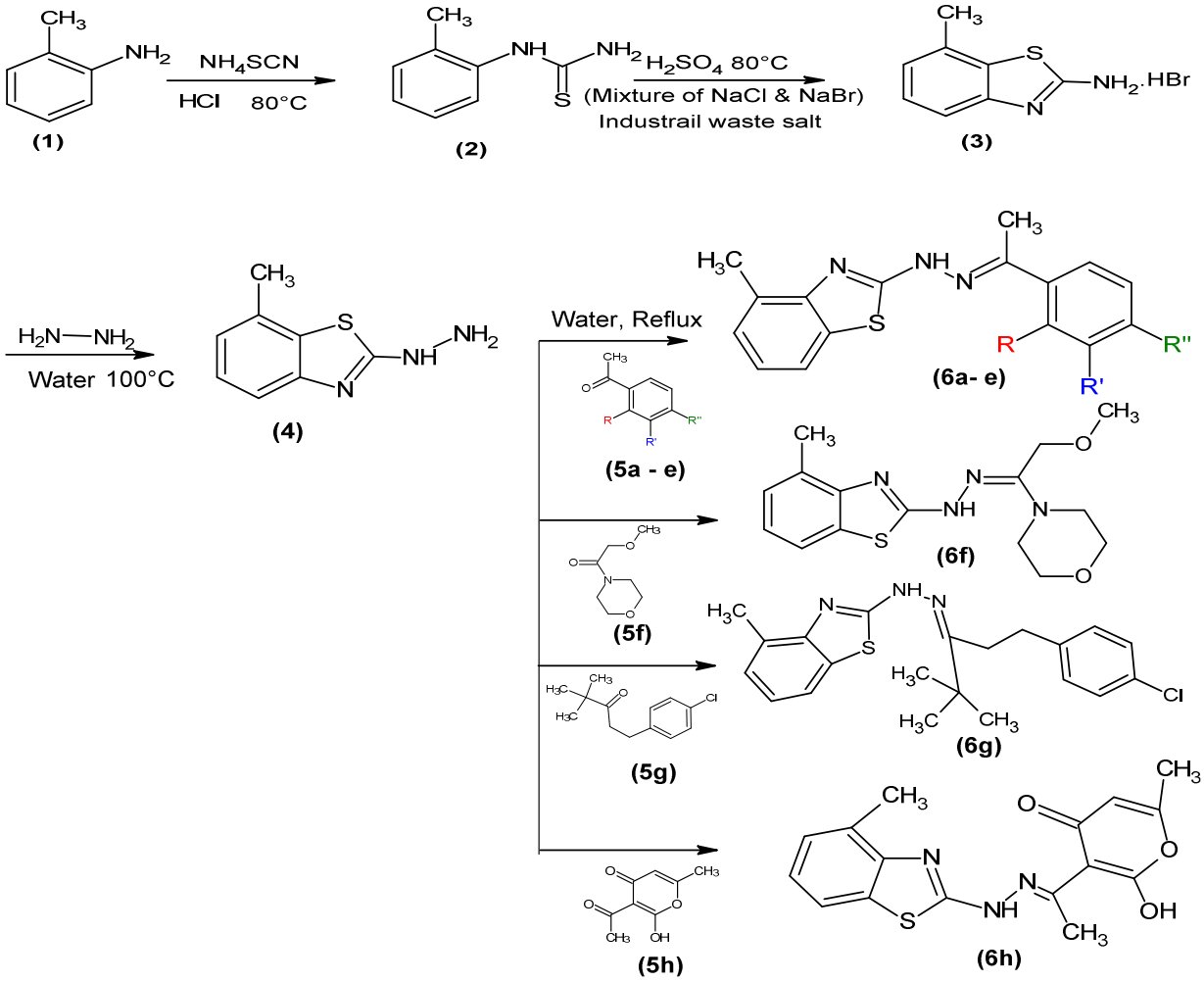
2-{(2*Z*)-2-[1-(2-methoxyphenyl) ethylidene] hydrazinyl}-4-methyl-1, 3-benzothiazole (6d): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 2.20 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 6.79-7.66 (m, 7H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.81, 18.44, 21.56, 113.79 – 148.56, 160.54, 169.98, 176.72; MS, m/z: 311.0 (M+H) .

4-methyl-2-[(2*Z*)-2-(1-phenylethylidene) hydrazinyl]-1, 3-benzothiazole (6e): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 2.31 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 7.03-8.88 (m, 8H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.63, 18.30, 21.57, 118.80-148.88, 169.00, 176.77; MS, m/z: 281.0 $(M+H)^+$.

2-{(2*Z*)-2-[2-methoxy-1-(morpholine-4-yl)ethylidene]hydrazinyl}-4-methyl-1,3-benzothiazole (6f): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 2.58 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.26 (t, 8H, CH_2), 7.04 - 7.51 (m, 3H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 18.33, 118.63 – 130.17, 150.63, 172.84; MS, m/z: 320.0 (M+H).

2-{2-[1-(4-chlorophenyl)-4, 4-methylpentan-3-ylidene] hydrazinyl}-4-methyl-1, 3-benzothiazole (6g): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 1.23 (s, 9H, CH_3), 2.53 (s, 3H, CH_3), 2.58 – 2.84 (t, 4H, CH_3), 7.05 -7.52 (m, 7H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 16.15, 27.89, 28.61, 30.71, 39.16, 118.59 – 148.73, 151.07, 169.05, 172.66; MS, m/z: 383.0 $(M+H)^+$.

3-{(1*E*)-1-[2-(4-methyl-1, 3-benzothiazol-2-yl) hydrazinylidene] ethyl}-2-hydroxy-6-methyltetrahydro-4*H*-pyran-4-one (6h): ^{1}H NMR (500 MHz, $CDCl_3$-d6, ppm): δ 1.21 (s, 3H, CH_3), 2.14 (t, 3H, CH_3), 2.44 (t, 3H, CH_3), 3.73 (t, 1H, CH), 7.16-7.66 (m, 3H, Ar-H; ^{13}C NMR (500 MHz, $CDCl_3$): δ 13.78, 17.98 – 19.16, 23.70, 25.23, 30.92, 59.37 – 112.39 – 170.43, 207.10; MS, m/z: 333.4.0 $(M+H)^+$.



Scheme 1: Synthetic route of compounds (6a-h)

RESULTS AND DISCUSSION:

A series of 4-methyl-2-[(2*E*)-2-(1-phenylethylidene) hydrazinyl]-1, 3-benzothiazole derivatives, (**6a-h)** was synthesized according to Scheme I. The physical data and yield of synthesized compounds (**6a-h)** are reported in Table 1**.**

Table 1: Physical data and yield of synthesized compounds (6a-h)

Comp.	R	R'	R''	Yield %	MP
4	**-**	-	-	95.6	170 - 172°C
6a	H	NO_2	H	90.75	203-208°C
6b	Cl	H	Cl	91.85	138-145°C
6c	H	H	Br	85.24	112-117°C
6d	OCH_3	H	H	88.12	142-147°C
6e	H	H	H	92.34	101-106°C
6f	-	-	-	82.42	101-106°C
6g	-	-	-	84.39	133-138°C
6h	-	-	-	88.33	205-208°C

The structure of the title compounds (**6a-h)** was confirmed by FT-IR, NMR, and MS. As a representative analysis of compound (**6a)** Figure-1, the direct IR spectrum showed C=C/C=N absorption bands at 1629-1475 cm^{-1}. The ^{1}H NMR spectrum of compound (**6a)** Figure-2 displayed three singlets at the aliphatic region δ 2.16 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), and a multiplet at the aromatic region at 7.0-9.26 (m, 7H, Ar-H; ^{13}C NMR spectrum of compound (**6a)** Figure-3 revealed the three signal of methyl carbon δ 13.28, 17.7, 21.0, and a signal at 71.7, connecting to Schiff's Bases. Aromatic carbons showed at 124.2 - 147, carbon between two hetero atoms (S and N) 168.1. Structure of compound (**6a)** Figure-4 was further confirmed by molecular ion peak at *m/z* 326 $(M+H)^{+}$. Structures of all the derivatives were ascertained similarly.

Fig-1: FT-IR Spectrum of compound (6a)

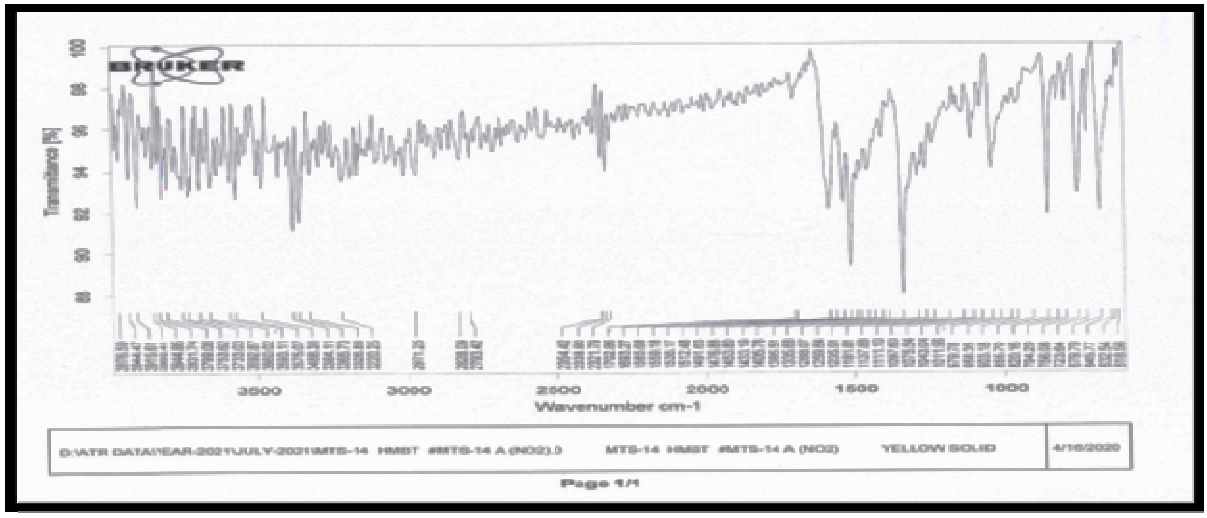


Fig- 2: 1H NMR Spectrum of compound (6a)

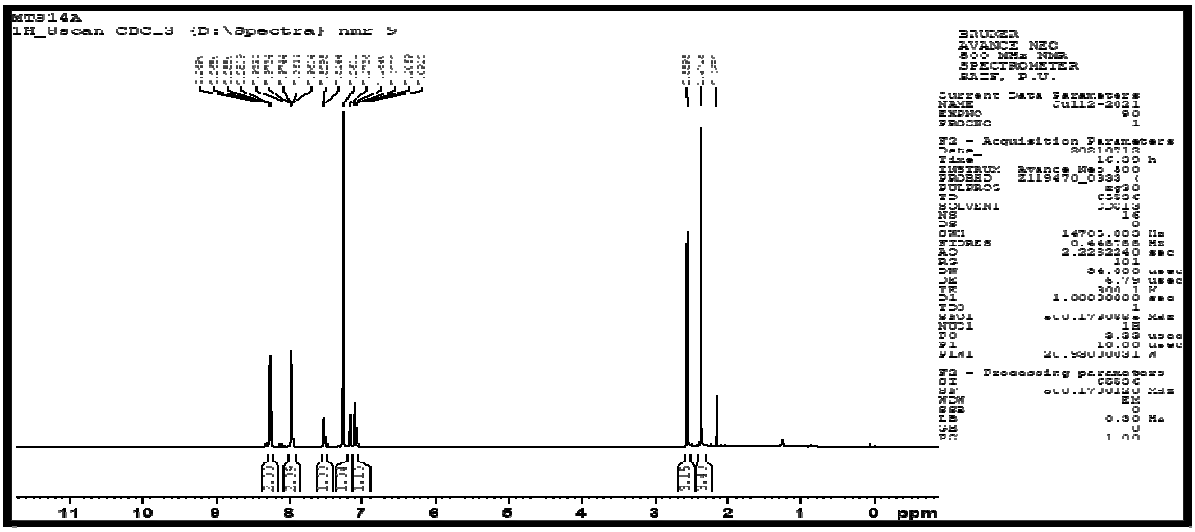


Fig- 3: 13C NMR Spectrum of compound (6a)

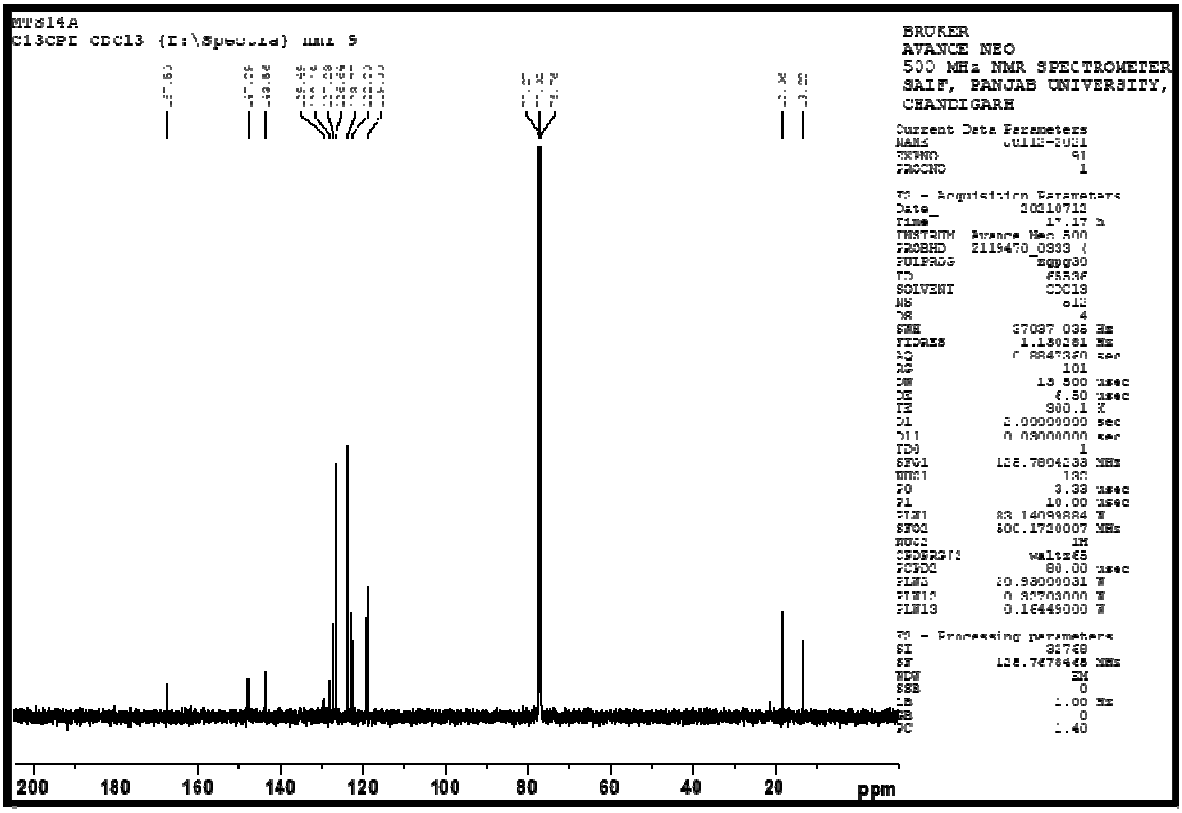
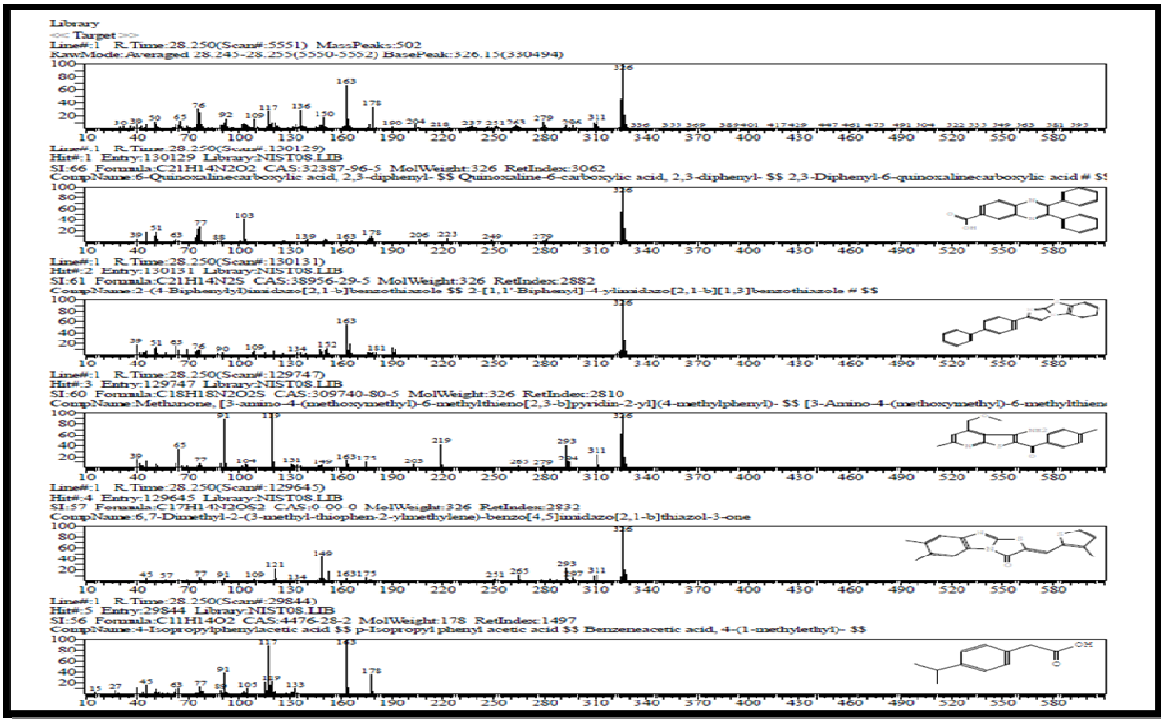


Fig- 4: Mass Spectrum of compound (6a)



Antimicrobial Activity The synthesized compounds **(6a-h)** were screened for their antibacterial activity against the standard Gram-negative bacteria, *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 1688) and Gram-positive *S. aureus* (MTCC 96), S. *pyogenus* (MTCC 442), Gentamycin, Ampicillin, and Chloramphenicol, Ciprofloxacin were used as reference. Antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282), *A. clavatus* (MTCC 1323). Nystatin and Griseofulvin were used as references, which is a fast-growing non-pathogenic strain to assess the activity of the compounds in primary screening. The results of antimicrobial activity are reported in Tables 2 and 3.

Table 2: Antibacterial screening of compounds (6a-h) (zone of inhibition in mm)

Sample	E.coli	P. aeruginosa	S. aureus	S. pyogenus
6a	250	62.5	100	250
6b	125	100	200	25
6c	500	250	500	250
6d	500	500	500	200
6e	125	250	50	100
6f	250	100	125	100
6g	125	100	125	100
6h	50	125	100	50
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	-	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50

Table 3: Antifungal screening of compounds (6a-h) (zone of inhibition in mm)

Sample Code	*C. albicans*	*A. niger*	*A. clavatus*
6a	500	1000	1000
6b	250	500	1000
6c	>1000	500	>1000
6d	500	>1000	>1000
6e	500	500	250
6f	500	>1000	500
6g	1000	1000	500
6h	200	500	>1000
Nystatin	100	100	100
Greseofulvin	500	100	100

CONCLUSION:

In the present work, the synthesis and biological screening of benzothiazole derivatives has been reported. Out of all the synthesized (6a-h) compounds, 2-{(2*Z*)-2-[1-(2-methoxyphenyl) ethylidene] hydrazinyl}-4-methyl-1, 3-benzothiazole (6d) was found highly active against all the tested bacteria and fungus as compared to reference drugs. So, this process is commercially highly effective as it saves manpower, time, utility, health, and safety of man.

It is environmentally Eco-friendly because industrial waste has been used, which is critical to dispose of.

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AUTHORS CONTRIBUTIONS: All authors contributed equally to this study .

CONFLICT OF INTERESTS: No competing interest.

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