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Open Access Research Article

Synthesis and Biological Evaluation of 1-(6-bromobenzo [d]thiazol-2-yl)-2-(disubstituted methylene) hydrazine derivatives

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ABSTRACT

Synthesis of a series of various 1-(6-bromobenzo[d]thiazol-2-yl)-2-(disubstitutedmethylene)hydrazine derivatives (7a-7e)have been done. Synthesis of a series of intermediates (3 and 5) have been also done, 6-bromo-1,3-benzothiazole-2-amine (3), 2-hydrazino-6-bromo-1, 3-benzothiazole (5) and final product (7a-7e), 1-(6-bromobenzo[d]thiazol-2-yl)-2-(diphenylmethylene) hydrazine (7a), 1-(6-bromobenzo [d]thiazol-2-yl)-2-(propan-2-ylidene) hydrazine (7b), (E)-2-(butan-2-ylidene)-1-(6-bromobenzo[d]thiazol-2-yl)hydrazine (7c), (Z)-1-(6-bromobenzo[d]thiazol-2-yl)-2-(cyclohexylmethylene) hydrazine (7d), 2-(6-bromobenzo[d]thiazol-2-yl)-1-(7,7- dimethylbicyclo [2.2.1] heptan-2-ylidene)hydrazine (7e). Spectral analysis of all intermediates and final products has been done by IR and NMR. After spectral analysis, antibacterial activity has been screened against *S. aurius* and *E. coli* bacterias.

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1. INTRODUCTION

Antimicrobial agents are those which treat infection by suppressing or destroying the causative microorganisms such as bacteria, mycobacteria, fungi, protozoa or viruses without significant effect on host tissues[1].

Anti-microbial agents can be broadly classified as follows[2] (Figure 1)

Nitrogen containing heterocyclic compounds specially azole family has the subject of continuous favourable interest in synthesis and owing to the fact that they occur pharmacologically active natural products, multipurpose oriented functional material[3].

Benzothiazoles have most interesting biophore in research because it is used as a synthon for the synthesis of biologically active structures[4].

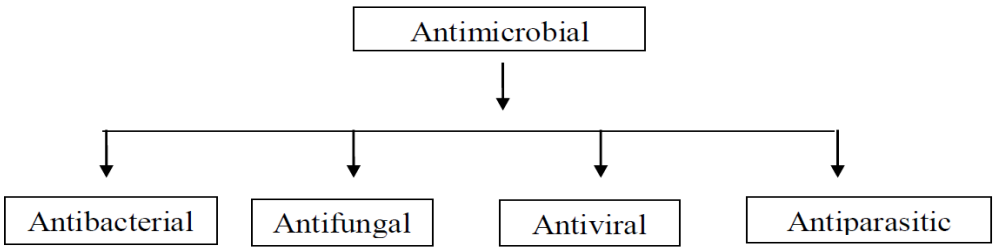


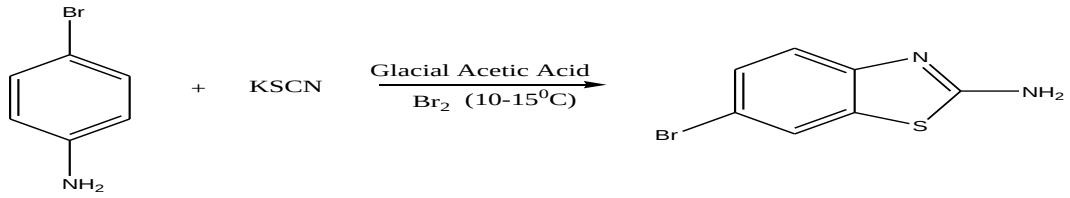
Figure 1

Benzothiazole is present in compounds which are involved in research aimed for new products that having interesting biological activities such as antitumor[5], antimicrobial[6], anti-inflammatory, anti-tubercular, anti-HIV[7], anti-malarial[8] and anti-convulsant[9].

A series of various derivatives of final products 1-(6-bromobenzo[d]thiazol-2-yl)-2-(diphenylmethylene) hydrazine (7a), 1-(6-bromobenzo [d]thiazol-2-yl)-2-(propan-2-ylidene) hydrazine (7b), (E)-2-(butan-2-ylidene)-1-(6-bromobenzo[d]thiazol-2-yl)hydrazine (7c), (Z)-1-(6-bromobenzo[d]thiazol-2-yl)-2-(cyclohexylmethylene) hydrazine (7d), 2-(6-bromobenzo[d]thiazol-2-yl)-1-(7,7-dimethylbicyclo [2.2.1] heptan-2-ylidene)hydrazine (7e) has been synthesized by placing 2-Hydrazino amino benzothiazole and ketone compound (6a-e) in equimolar conc. in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs. All the compounds synthesized have been characterized by spectral data and antibacterial activity has been done against Gram+ve *S. aurius* and Gram-ve *E. coli* bacterias by standard procedure using broth dilution method and minimum inhibitory concentration was determined by visual comparison with the negative control tubes.

2. EXPERIMENTAL

2.1 **Synthesis of 6-Chloro-1,3-benzothiazole-2-amine (3)**



1 **2** **3**

Figure 2: Synthesis of 6-bromo-2-amino-1,3-benzothiazol

Procedure:[10]

Triturate the mixture of p-bromo aniline (20g,0.156 moles) and potassium thiocyanate (18.19g, 0.187 moles) in motor pastle for 10-15 min. Add cold acetic acid (50ml) in to motor and transfer this mixture in RBF, stirred the mixture at 10-15^{0}C then add cold acetic bromine mixture (10ml acetic acid and bromine) in 2-3 hr. After completion of bromine addition stirred the mixture for 10-15 minute. The reaction was monitored by TLC. Remove the RBF and keep overnight in to freeze.

Work up

In the overnight reaction mixture add 10ml H_2O and 5ml acetic acid. Heat the reaction mixture till cease of pungent smell of acetic acid. Cool this mixture and filter, the solid was removed and liquid was collected. The liquid was neutralize with ammonia dropwise at 5-10^{0}C. The precipitated solid was collected by filtration. The white solid was collected as pure product.

Yield : 85.76%

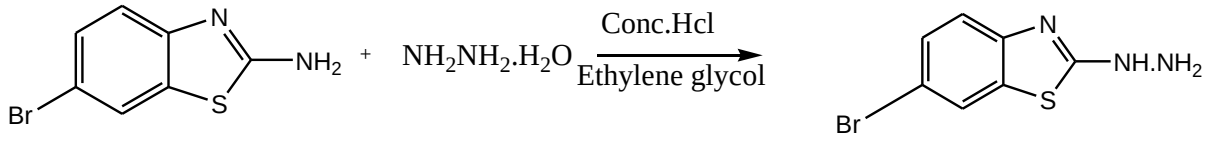
Melting point : 60-62^{0}C

TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

R_f = 0.726

IR (**Spectrum 1**) : 3838, 3617, 2160, 1942, 1810, 1748, 1634, 1557, 1473, 1396, 1304, 809 Cm$^-$

2.2 Synthesis of 2-hydrazino-6-bromo-1, 3-benzothiazole (5)



3 4 5

Figure 3: Synthesis of 2-hydrazino-6-bromo-1,3-benzothiazole

Procedure:[11]

The obtained compound of 3a (5.00 gm,0.0264 moles) was dissolved in ethylene glycol (20 ml) and Conc. Hcl (10 ml) was added drop by drop in this mixture,now cold hydrazine hydrate(10 ml) was added in this mixture.The reaction mixture was refluxed for 15 hr.

Work up:

Cool the mixture and pour this reaction mixture in to crushed ice.The precipitate was obtained and the precipitated compound was filtered under vacuum.

Yield : 83.23%

Melting point : 55-58^{0}C

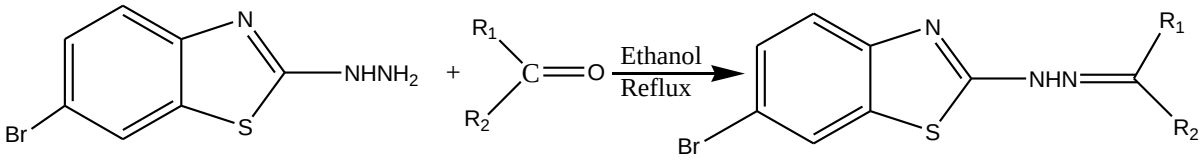
TLC : Silica gel G; Hexane: Ethyl acetate (1:1)

R_f = 0.712

IR (**Spectrum 2**) : 3736, 3674, 2360, 1662, 1557, 1521, 1473, 1396, 1338, 1211 Cm^{-1}

2.3 Synthesis of final products (7a-e)

General scheme



5 **6a-e** **7a-e**

Figure 4: General synthetic scheme for final product

Procedure:[12]

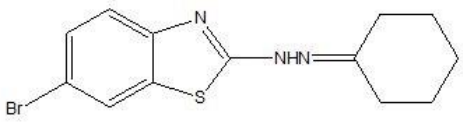
The obtained compound 2-Hydrazino amino benzothiazole and ketone compound (6a-e) was taken in equimolar conc. in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs. The reaction was monitored by TLC.

Work up:

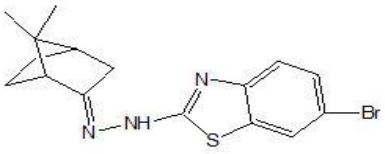
Cool the mixture, the precipitate was collected. The product was purified by column chromatography (Ethylacetate: hexane) to give the final product.

Sr. No.	Compound	Name	R_1	R_2
1	7a	1-(6-bromobenzo[d]thiazol-2-yl)-2-(diphenylmethylene) hydrazine	$-C_6H_5$	$-C_6H_5$
2	7b	1-(6-bromobenzo [d]thiazol-2-yl)-2-(propan-2-ylidene) hydrazine	$-CH_3$	$-CH_3$
3	7c	(E)-2-(butan-2-ylidene)-1-(6-bromobenzo[d]thiazol-2-yl)hydrazine	$-CH_3$	$-C_2H_5$

Table 1

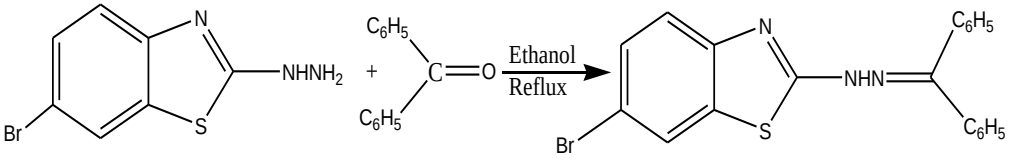


(Z)-1-(6-bromobenzo[d]thiazol-2-yl)-2-(cyclohexylmethylene) hydrazine (7d).



2-(6-bromobenzo[d]thiazol-2-yl)-1-(7, 7- dimethylbicyclo [2.2.1] heptan-2-ylidene)hydrazine (7e).

2.3.1. Synthesis of 1-(6-bromobenzo[d]thiazol-2-yl)-2-(diphenylmethylene) hydrazine (7a):



5 **6a** **7a**

Figure 5: Synthesis of 1-(6-bromobenzo[d]thiazol-2-yl)-2-(diphenylmethylene)hydrazine

Procedure[12]

The obtained compound 2-Hydrazino amino benzothiazole (0.5gm,0.0024moles) and benzophenone (0.43gm,0.0024moles) was taken in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs.The reaction was monitored by TLC. Cool the mixture, the precipitate was collected. The product was purified by column chromatography.

Yield : 83.27%

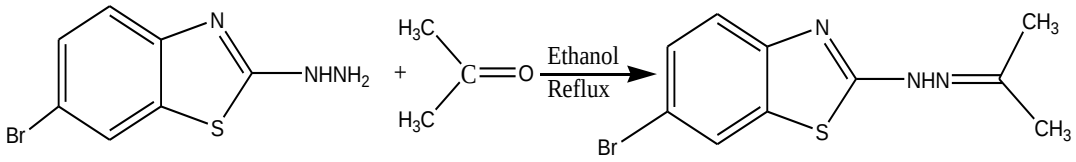
Melting point : 55-57^{0}C

TLC : Silica gel G; Hexane: Ethyl acetate (7:3)

R_f = 0.583

IR (**Spectrum 3**): 3864, 3674, 3587, 2926, 2399, 1733, 1698, 1557, 1507, 1473 Cm^{-1}

2.3.2. Syntesis of 1-(6-bromobenzo[d]thiazol-2-yl)-2-(propan-2-ylidene) hydrazine (7b):



5 **6b** **7b**

Figure 6: Synthesis of 1-(6-bromobenzo[d]thiazol-2-yl)-2-(propan-2-ylidene) hydrazine

Procedure[12]

The obtained compound 2-Hydrazino amino-6-bromo-1,3-benzothiazole (compound 5) (0.5gm,0.0024moles) and acetone (0.176ml,0.0024moles) was taken in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs. The reaction was monitored by TLC. Cool the mixture, the precipitate was collected. The product was purified by column chromatography.

Yield : 81.84%

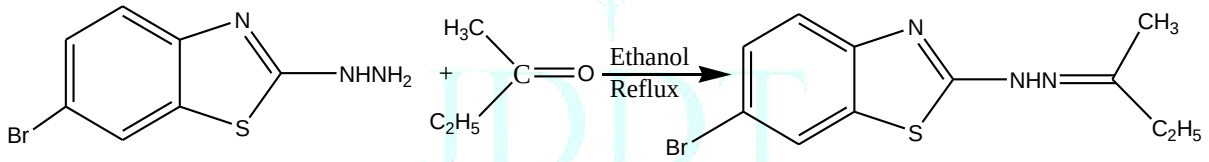
Melting point : 55-57^{0}C

TLC : Silica gel G; Hexane: Ethyl acetate (7:3)

R_f = 0.554

IR (**Spectrum 7**): 3710, 3679, 3597, 2360, 1697, 1662, 1558, 1521, 1362, 1288 Cm^{-1}

2.3.3. Synthesis of (E)-2-(butan-2-ylidene)-1-(6-bromobenzo[d]thiazol-2-yl)hydrazine (7c):



5 **6c** **7c**

Figure 7: Synthesis of (E)-2-(butan-2-ylidene)-1-(6-bromobenzo[d]thiazol-2-yl)hydrazine

Procedure[12]

The obtained compound 2-Hydrazino amino benzothiazole (0.5gm,0.0024moles) and Ethyl methyl ketone (0.215ml,0.0024moles) was taken in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs.The reaction was monitored by TLC. Cool the mixture, the precipitate was collected. The product was purified by column chromatography.

Yield : 86.97%

Melting point : 55-60^{0}C

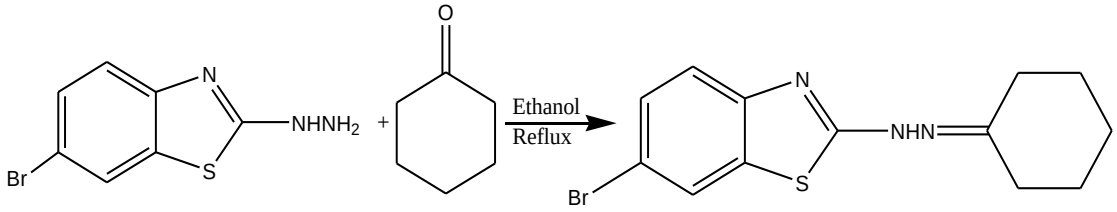
TLC : Silica gel G; Hexane: Ethyl acetate (7:3)

R_f = 0.578

IR (**Spectrum 5**): 3648, 3587, 2871, 2359, 1844, 1771, 1733, 1670, 1635, 1557 cm^{-1}

^{1}H NMR (**Spectrum 6**): δ ppm 9.5 (bs, 1H, -NH-), 7.68-7.27 (m, 3H, Ar-H), 2.09 (s, 3H, N=C-CH$_3$), 2.37-2.20 (q, 2H, N=C-CH$_2$-CH$_3$), 1.11-1.04 (t, 3H, N=C-CH$_2$-CH$_3$).

2.3.4. Synthesis of (Z)-1-(6-bromobenzo[d]thiazol-2-yl)-2-(cyclohexylmethylene) hydrazine (7d)



5 **6d** **7d**

Figure 8: Synthesis of (Z)-1-(6-bromobenzo[d]thiazol-2-yl)-2-(cyclohexylmethylene) hydrazine

Procedure[12]

The obtained compound 2-Hydrazino amino benzothiazole (0.5gm,0.0024moles) and Cyclohexanone (0.248ml,0.0024moles) was taken in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs. The reaction was monitored by TLC. Cool the mixture, the precipitate was collected. The product was purified by column chromatography.

Yield : 81.84%

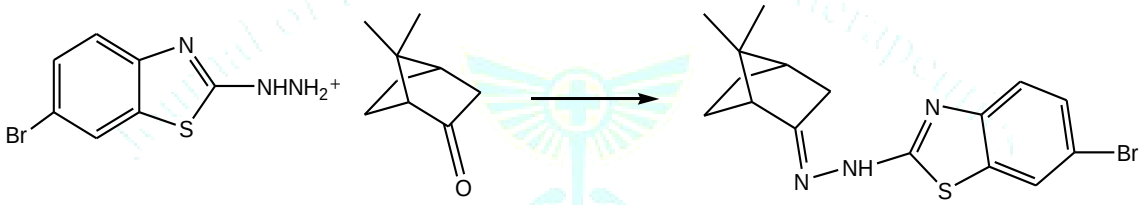
Melting point : 55-57^{0}C

TLC : Silica gel G; Hexane: Ethyl acetate (7:3)

R_f = 0.554

IR (**Spectrum 7**): 3710, 3679, 3597, 2360, 1697, 1662, 1558, 1521, 1362, 1288 Cm^{-1}

2.3.5 . Synthesis of (E)-2-(6-bromobenzo[d]thiazol-2-yl)-1-(7,7-dimethylbicyclo[2.2.1] heptan-2-ylidene)hydrazine (7e):



5 **6e** **7e**

Figure 9: Synthetic scheme of 2-(6-bromobenzo[d]thiazol-2-yl)-1-(7,7-dimethylbicyclo [2.2.1] heptan-2-ylidene)hydrazine

Procedure[12]

The obtained compound 2-Hydrazino amino benzothiazole (0.5gm,0.0024moles) and Camphor (0.365gm,0.0024moles) was taken in a RBF. Ethanol (20 ml) was added in the mixture and the reaction mixture was refluxed for 10-15 hrs.The reaction was monitored by TLC. Cool the mixture, the precipitate was collected. The product was purified by column chromatography.

Yield : 83.77%

Melting point : 55-58^{0}C

TLC : Silica gel G; Hexane: Ethyl acetate (7:3)

R_f = 0.585

IR (**Spectrum 8**): 3706, 3545, 3480, 3429, 3321, 2852, 1591, 1522, 1436, 1418, 817Cm^{-1}

3. BIOLOGICAL SCREENING

Anti-microbial testing

Activity of anti-infective agents may be demonstrated under suitable conditions by their inhibitory effect on microorganisms. The anti-microbial activity of the synthesized compounds was carried out by standard procedure using broth dilution method and minimum inhibitory concentration was determined by visual comparison with the negative control tubes.

Detailed test procedure

(i) Stock Solutions of test compounds and standard drug

Compounds were taken as test samples along with a standard Streptomycin sample. Weight taken in the range of 8-20 mg of each test compound and was dissolved in 1 ml of DMSO. For preparing stock solution of Streptomycin, 10 mg of Streptomycin was dissolved in 1 ml of water.

(ii) Test organism

The organisms employed in the *in vitro* testing of the compounds were*Escherichia coli* and *S. aureus*. All the cultures were maintained on nutrient broth agar (Microbiology grade, CDH Pvt. Ltd. New Delhi) medium by periodic sub culturing.

(iii) Preparation of Innoculum

Procedure for the preparation of innoculum for both the strains was same. The innoculum was prepared from a 24-hours old growth of organism on nutrient broth agar slant. To the agar slant, saline solution was added to obtain O.D value of 0.1 on photoelectric optical colorimeter. 0.5ml of this solution was further diluted to 20ml with use of saline.

(iv) Preparation of Medium

1.3 gms of nutrient broth (Microbiology grade, CDH Pvt. Ltd. New Delhi.) was dissolved in 100 ml of sterile distilled water.

(v) Addition of drug, innoculum solution to medium

From diluted innoculum solution prepared, 100µl was added to separate test tube each containing 0.9ml of medium. 25 µl solution of test stock solution was added in four separate test tube containing 0.9ml of medium with 100 µl innoculum. The tests were carried out in duplicate. Apart from putting the controls of standard drug (Streptomycin), controls with dimethylsulphoxide (DMSO) were used. DMSO (positive control) is DMSO inoculated with organisms and dimethylsulphoxide (negative control) is plain DMSO. For incubation, test tubes were kept in incubator at 35°C for 24 hours.

(vi) Observations

At the end of incubation period, the results were interpreted by comparison with negative control. The lowest concentration of test compound which showed inhibitory effect on growth on visual distinction was taken as minimum inhibitory concentration (MIC) and visual turbidity was consider for MIC of the test molecules; standard drug and DMSO positive and negative control visual turbidity were recorded.

4. RESULT AND DISCUSSIONS

4.1 Characterization of the benzothiazole derivatives

IR: The spectrum showed characteristic absorption band accordingly of the presence of functional group. The Ar-C-H stretching showed the absorption band in the range of 3010-3150 cm^{-1}. The –C=N stretching band appeared in the range of 1630-1660 cm^{-1}, -C=C- stretching peak comes in the range of 1400-1500 cm^{-1}, alkyl C-H stretching peak comes in the range of 2820-2860 cm^{-1}. N-H stretching band was appeared in the range of 3150-3300 cm^{-1}

One representative molecule Compound **7c** was characterized ^{1}H NMR spectroscopy**.**

^{1}H NMR: The methyl protons appeared as a singlet at around δ 2.09. The methylene protons appeared as a singlet at around δ 2.37-2.20. The aromatic protons appeared as multiple singlets between δ 7.22-7.59. The N-H proton appeared at 9.5 as a singlet. Representative example is **7c.**

Table 2: Comparison of ^{1}H NMR values

Sr. No.	No of proton	Predicted (δ in ppm)	Experimental (Spectrum 6)
1	$-CH_3$	2.0	2.09
2	$-N=C-\underline{CH_2}-CH_3$	1.4	2.37-2.2
3	$-N=C-\underline{CH_2}-CH_3$	0.9	1.11-1.04
	Ar-H	7.77-8.29	7.22-7.59

4.2 Biological evaluation

The synthesized NCEs were subjected to antimicrobial evaluation against *Escherichia coli* and *S. aureus* microorganism using broth dilution method keeping appropriate positive and negative controls simultaneously.

Visual turbidity of evaluated compounds is given in **Table 3**

Table 3: Visual turbidity of evaluated compounds

Sr. No.	**Compounds**	Visual Turbidity (*E. Coli*)	Visual Turbidity (*S. Aureus*)
1.	**7a**	-	-
2.	**7b**	-	-
3.	**7c**	+	+
4.	**7d**	-	-
5.	**7e**	-	+
6.	Streptomycin	-	-
7	DMSO positive	++	++

- = No Turbidity (No bacterial growth), + = Turbidity (Bacterial growth)

Based on the visual turbidity, the MIC of the evaluated molecules is given in **Table 3** the evaluation concentration was used single therefore, the exact MIC could not determined and results are represented in less than and more than format. To get more exact MIC of the tested molecules need to be evaluated at low concentration. The evaluation results of the single concentration is tabulated in **Table 4**

Sr. No.	**Compounds**	*E. coli* (µg/ml)	*S. aureus* (µg/ml)
1.	**7a**	<12.5	<12.5
2.	**7b**	<12.5	<12.5
3.	**7c**	>12.5	>12.5
4.	**7d**	<12.5	<12.5
5.	**7e**	<12.5	>12.5
6.	Streptomycin	<6.25	<6.25

Table 4

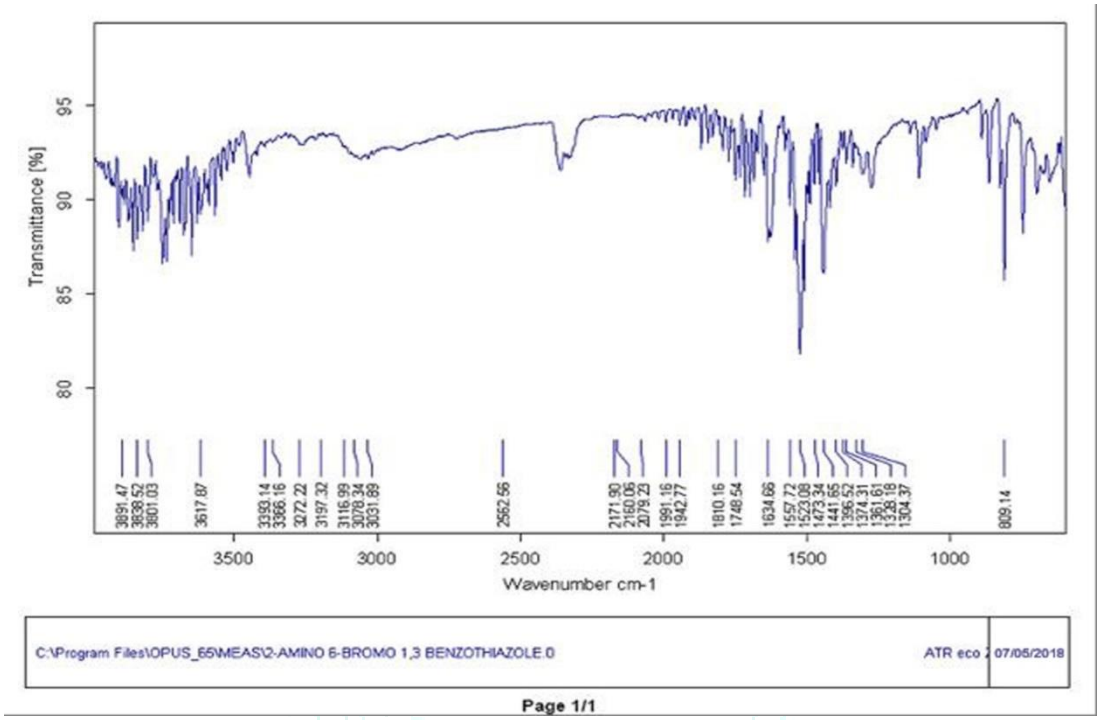
4.3 Result of antibacterial testing

Most of the tested compounds had bacterial growth inhibition activity at tested concentration against both of microorganisms. Compounds 7a, 7b, 7d and 7e exhibited growth inhibition of *E.coli* while 7c didn't show activity. The compound 7a, 7b and 7d showed the inhibition of *S. aureus*.

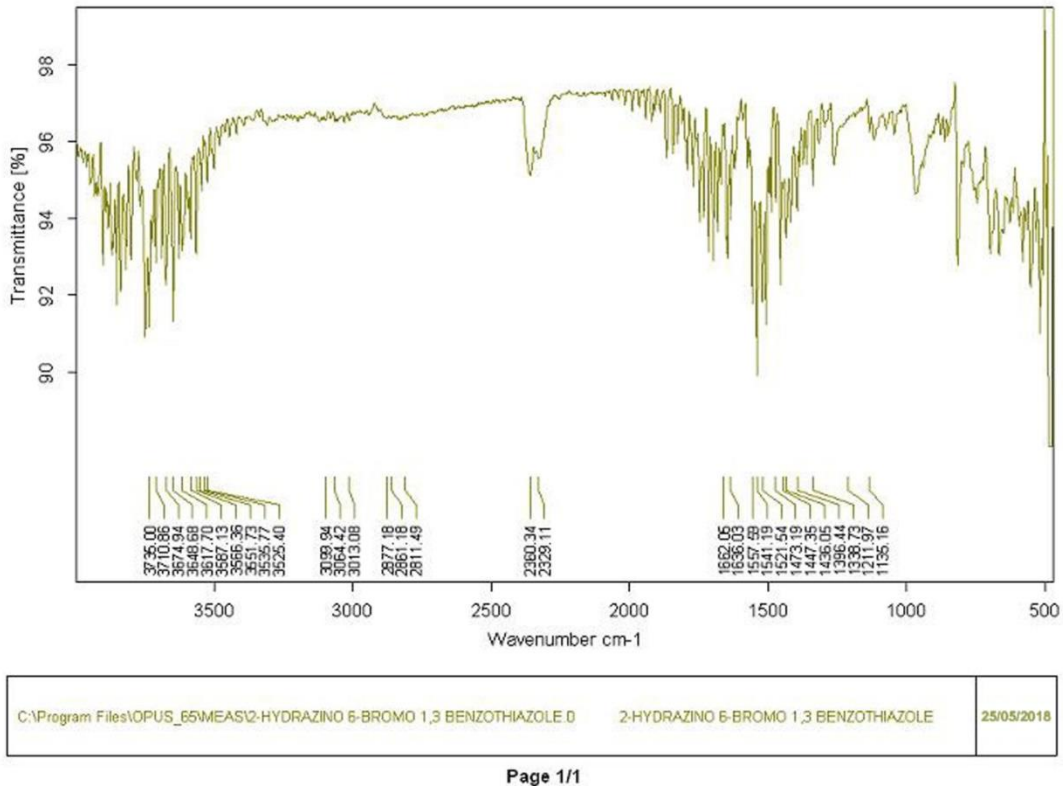
5. CONCLUSION

Thus, it can be concluded that designed benzothiazole derivatives were synthesized successfully and compound 7a-7e had antibacterial activity at tested concentration less than 1000µg per ml. and compound 7a exhibited activity at lower concentration less than 200µg per ml. Compound 7c didn't show activity at higher concentration upto 1000µg per ml. against both Gram+ve and Gram -ve microorganisms. So, synthesized benzothiazole derivatives can act as a lead for development of broad spectrum antibacterial agents.

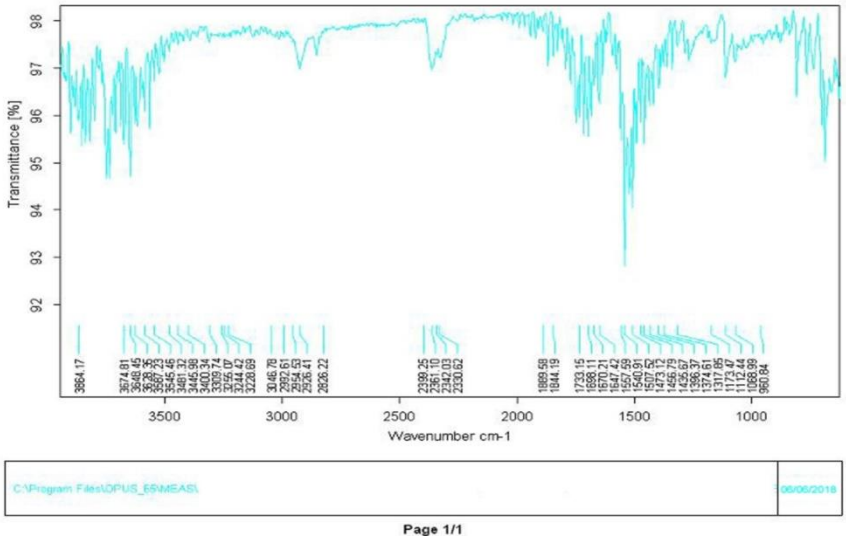
6. SPECTRAS



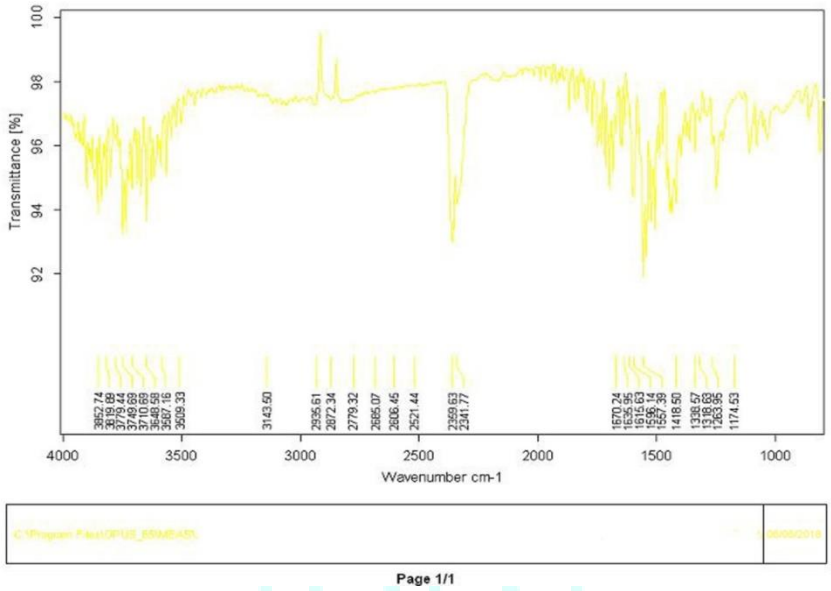
Spectrum 1: IR Spectra of 3



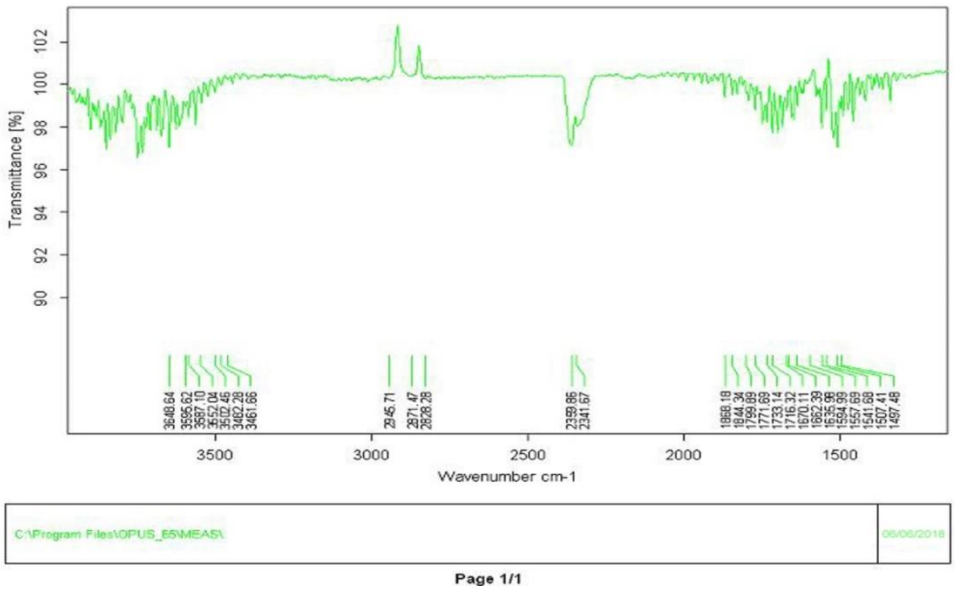
Spectrum 2: IR Spectra of 5



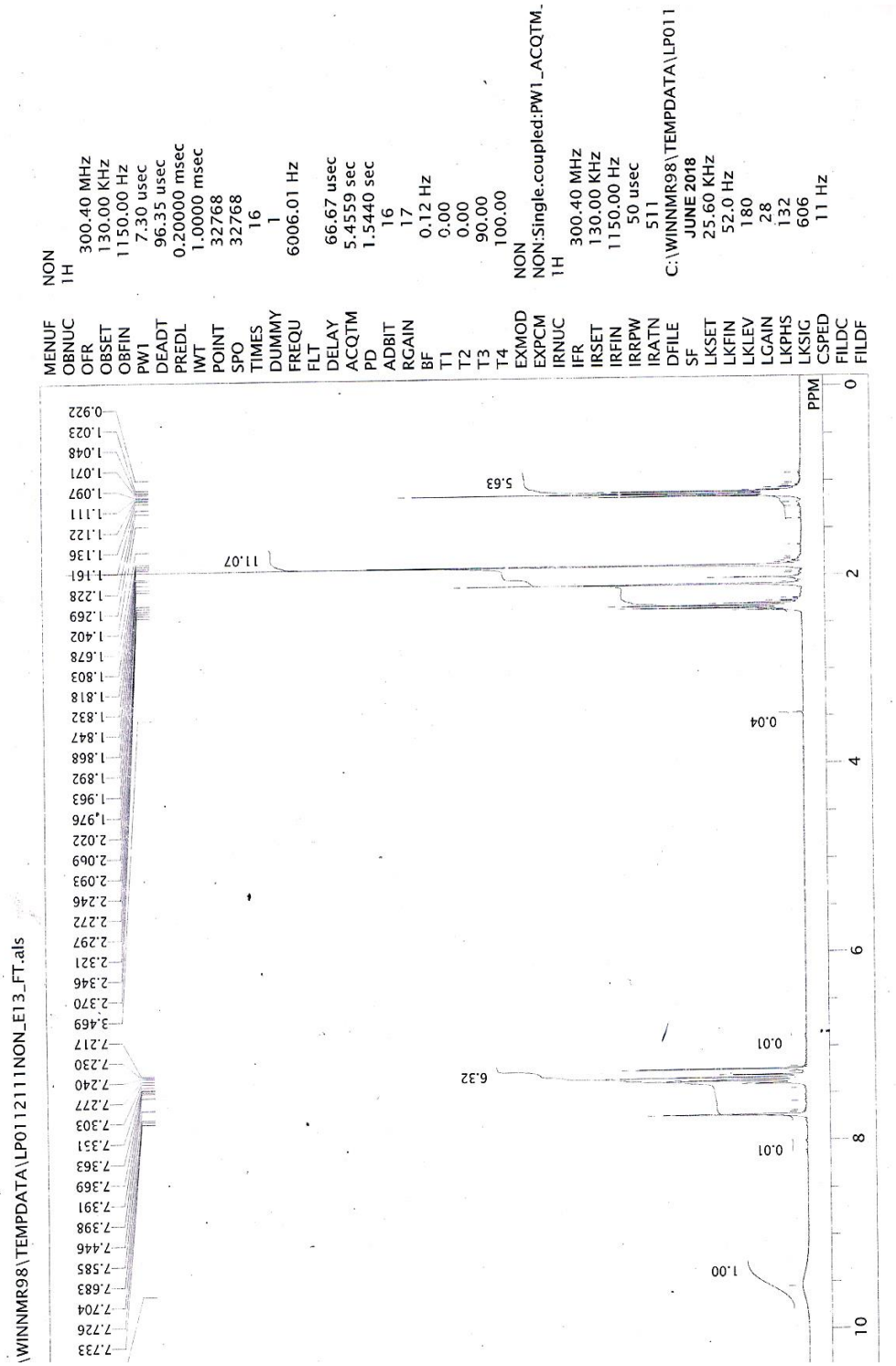
Spectrum 3: IR Spectra of 7a



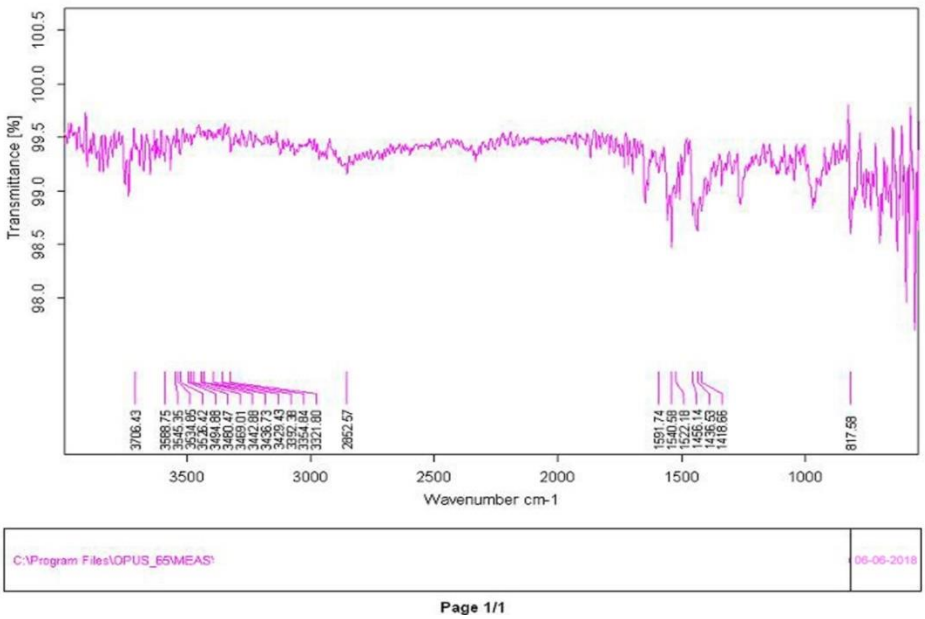
Spectrum 4: IR Spectra of 7b



Spectrum 5: IR Spectra of 7c



Spectrum 6: IR Spectra of 7c



Spectrum 8: IR Spectra of 7e

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