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Research Article

Synthesis and Antimicrobial Screening of Some Novel 2-amino Benzothiazole Derivatives

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ABSTRACT

A couple of new N-3-chloropropyl-benzothiazole-2-amine derivatives (Compound B1, B2) and N-benzothiazol-2-yl)-2-chloroacetamide derivatives (Compound A1, A2) were the focus of the current investigation. In the presence of TEA, 2-aminobenzothiazole and 2-chloroacetyl chloride were combined to form the intermediate molecule N-benzothiazol-2-yl)-2- chloroacetamide, also known as Intermediate X1. Through the reaction of Intermediate X1 with sulfanilic acid and sulphanilamide, the final compounds (Compounds A1 and A2) were produced. 2-Aminobenzothiazole and 1-Bromo-3-chloropropane were combined with TEA to form the intermediate chemical N-3-chloropropyl-benzothiazole-2-amine, also known as Intermediate Y1. Intermediate Y1 was reactated with sulfanilic acid and sulphanilamide to produce the final chemicals (Compound B1, B2). The spectroscopic approach was used to characterize the synthesized compounds, and TLC and melting point were used to evaluate the purity of all the compounds. The anti-microbial activity of the produced compounds was tested. According to the findings, most compounds exhibited a smaller or nonexistent zone of inhibition against bacteria in comparison to the gold standard medication, Norfloxacin. Out of all the compounds that were produced, at concentrations. In comparison to the gold standard antibiotic Norfloxacin, compound A1 had a smaller zone of inhibition (ZOI) against gram-positive Bacillus subtilis (22 mm, 28 mm, and 33 mm diameter, respectively), measuring 38 mm, 42 mm, and 48 mm diameter. The compound A2 exhibited the largest zone of inhibition (21 mm, 26 mm, and 33 mm diameter) when tested against the gram-positive Bacillus subtilis bacteria at concentrations. However, this was still lower than the standard drug Norfloxacin, which had ZOIs of 38 mm, 42 mm, and 48 mm diameter, respectively. Compound B1 exhibited the largest zone of inhibition (20 mm, 24 mm, and 26 mm diameter) when tested against Bacillus subtilis (a gram-positive bacterium) at concentrations. However, this was lower than the standard drug Norfloxacin (ZOI-38 mm, 42 mm, and 48 mm diameter). Compound B1

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had the largest zone of inhibition (22 mm, 25 mm, and 28 mm diameter) out of all the synthesized compounds tested with a diameter of 26 mm, 32 mm, and 36 mm, respectively), but not as effective as the gold standard antibiotic Norfloxacin against gram- inhibition (ZOI) against Escherichia coli (a gram-negative bacteria) at concentrations of 100 µg/ml, 200 µg/ml, and 300 µg/ml, with respective diameters of 20 mm, 22 mm, and 25 mm respectively.

INTRODUCTION

Heterocyclic compounds, also called heterocycle, it is major class of an organic chemical components identify by the fact some or all the molecules in that atoms are joined in rings containing at least one or more molecule an atom other than carbon. Some hetero atoms such as nitrogen, oxygen and sulphur[1]. Heterocyclic system is used in building blocks for new materials for chemical and biological properties. There are number of compounds such as vitamins, hormones, essential amino acids, alkaloids, dyes, and various types of drugs that have heterocyclic ring systems[2]. There was various number of basic heterocyclic compounds such as pyrrole, pyrrolidine, pyridine, pyrimidine, furan, having important application in drug design[3].

Medicinal chemistry plays a vital role in drug design and discovery. Medicinal chemistry mainly focus on new drug discovery, design and development. Medicinal chemists also focus on finding the basis of an disease with the help of biochemistry. After the identification of the chemical structure of the targets, it becomes easy to design new drugs for the treatment of disease[4]. 2-aminobenzothiazoles is an important heterocyclic compound shows the biological activity.

There is the major problem that we are facing in the content of infectious disease is the spreading of the antimicrobial resistance. The studies of new series of 2-aminobenzothiazole derivatives for infectious disease such as the antimicrobial activities. This the heterocyclic nuclei such as 2-aminobenzothiazole hence they are the basically two main rings are present like benzene ring and thiazole ring[5]. Thiazole are the five membered ring and one benzene ring also be attached with amine group named as 2-amino benzothiazole because amine group as 2- position in thiazole ring. In the present study on 2-amino benzothiazole derivatives and they are fused heterocyclic compound. 2-aminobenzothiazole derivatives are the reported for some diversified activity to seen such as antimalarial, anthelmintic, antitumor, anticonvulsant activities show these biological activities[6].

These are highly reactive compound and its utilized-on reaction intermediate to synthesized different derivatives. They are studied as a central muscle relaxant and they have been further studied to found a diverse reactive and broad spectrum of biological activities.

Table 1: Characteristics of 2-aminobenzothiazole

IUPAC name	1,3-benzothiazole-2-amine
Molecular formula	C7H6N2S
Molar mass	150.20 gm/mol
Solubility	Soluble in alcohol, chloroform, ether
Melting point	126-128^{0}C
Boiling point	Decomposes
Appearance	Gray to white powder
odour	It is an odourless powder

MATERIAL AND METHOD

Reagents and Solvents:

Chemicals and solvents used for the research work commercially procured from Sigma Aldrich, S.D. Fine Merck, Qualigens, Yarrow chemical and GLR Innovation. Aminobenzothiazole, Triethylamine, Chloroacetylchloride, 1-Bromo-3-Chloro propane, 2-Nitrobenzene sulfonamide, Sulfanilamide, sulfanilic acid, 4-chlorobenzene

sulfonamide, 2-chlorobenzene sulfonamide, Acetone, Benzene, Ethanol [7]etc.

The silica gel G used for analytical chromatography (TLC) was obtained from Qualigens. Solvent system used were Benzene: Methanol (8:2) cyclohexane: ethylacetate (8:2) Benzene: Acetone (8:2).

Iodine chambers and U.V. lamps were used for visualization of TLC spots.

Apparatus & glassware used:

Reflux condenser, round bottom flask, beaker, separating funnel, iodine flask, glass rod, magnetic stirrer, pipettes, heating mantle and TLC plates, magnetic stirrer with hot plate, melting point apparatus, hot air oven, volumetric flask, magnetic beads, UV chamber and weighing balance etc.

Analytical Work:

The melting points were found in an open glass capillary using a melting point equipment (Veego, Bombay, India). Using a variety of solvent systems, iodine vapours, and a UV chamber as visualizing agents, reactions were tracked using thin layer chromatography (TLC) on silica gel G plates.

Material used for antimicrobial assay:

Autoclave (Sciencetech), Incubator (Scientific Laboratory India), Nutrient Agar (Himedia, *E. coli* (*Escherichia coli)* (Gram-negative, Hi-media), *B. subtilis* (*Bacillus subtilis)* (Gram- positive, Hi-media), Sulfathiazole (GLR Innovation).

Scheme1: Synthesis of 2-aminobenzothiazole derivatives [8, 9]. (Compound A1-A2)

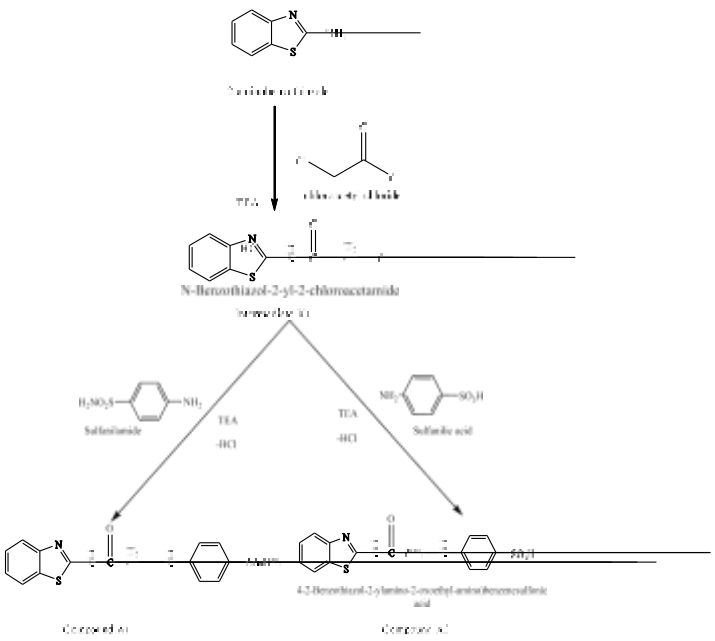
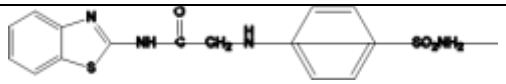
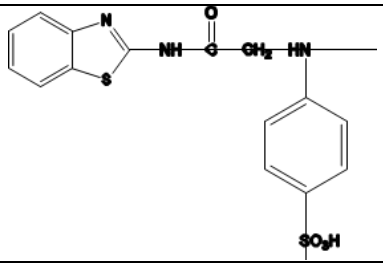


Table 2: Physical data of synthesized compounds (A1-A2)

Sr. No	1	2
Compounds	A1	A2

		
Molecular formula	C15H14O3N4S2	C15H13O4N3S2
Molecular weight	369 gm/mol	363 gm/mol
Appearance	Pale yellow ppt.	Brown ppt.
Yield	33.3%	42%
Rfvalue	0.73	0.36
Solubility	Soluble in methanol and DMSO	Soluble in methanol and DMSO
Melting point(Co)	97°C	141°C

Synthesis of N-(Benzothiazole-2-yl)-2-((4-sulfamoylphenyl) amino) acetamide. (Compound A1)

Acetamide (0.01mol,1.5gm) and Sulphanilamide (0.01mol,1.7gm) in presence of potassium carbonate or triethylamine and the solvent used as benzene was stirred about 6-7 hours at room temperature[10].The solid was filtered. The solvent system was used as Benzene: Methanol (8:2), Cyclohexane: Ethyl acetate (8:2).

Spectral data:

Solid, mp-96-98, IR (VmaxKBr): 3363.8 (-NH Aromatic, stretching vibration), 2924.03 (-CH Aliphatic, Stretching), 3739.1 (-OH, Stretching), 1384.6 (-S=O, Stretching), 1607 (C=O, Stretching) MS:(ESI+)m/z=369 (M+)

Synthesis of 4-(2-(Benzothiazole-2-ylamino)-2-oxoethyl) amino) benzene sulfonic acid. (Compound A2)

Same procedure was followed as mentioned in the synthesis of (compound A2) only sulphanilamide replaced by sulfanillic acid. System was used as benzene: methanol (8:2). Cyclohexane: Ethyl acetate (8:2).

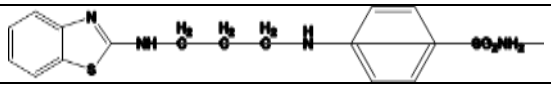
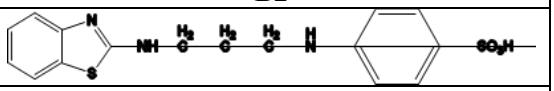
Spectral data:

Solid, mp- 140-142, IR (VmaxKBr): 3384 (-NH Aromatic, stretching vibration), 3060 (-CH Aliphatic, Stretching),1315 (-S=O, Stretching), 1637 (C=O, Stretching), 2360(S-H, Streching)

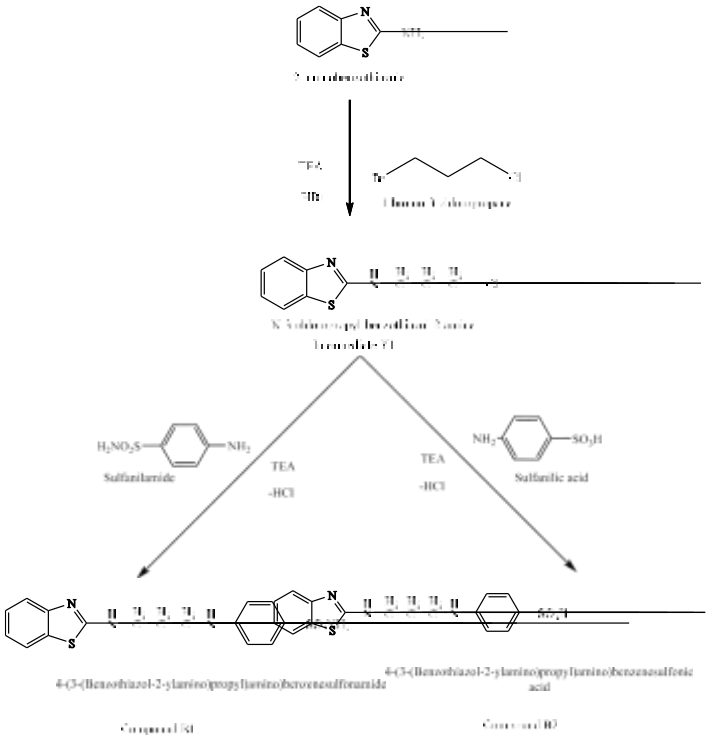
MS:(ESI+)m/z=364(M+1)

Scheme 2: Synthesis of 2-aminobenzothiazolederivatives[11, 12]. (Compound B1-B2)

Table 3:Physical data of synthesized compound (B1 and B2)

Sr. No	**1**	**2**
Compounds	**B1**	**B2**
		
Molecular formula	**C16H18O2N4S2**	**C16H17O3N3S2**

Molecular weight	362 gm/mol	363.45 gm/mol
Appearance	White ppt.	White powder
Yield	38.6%	48.2%
Rf value	0.71	0.51
Solubility	Soluble in methanol and DMSO	Soluble in methanol and DMSO
Melting point(C°)	80-82	92-94



Synthesis of N-3-Chloropropyl-benzothiazole-2-amine[13] (IntermediateY1)

1-bromo-3-chloropropane (1.07 mol, ml)was added dropwise to astirred solution of2-amino benzothiazole (0.01mol, 1.5 gm) in the presence of Triethyl amine (0.01mol, 0.78 ml) under an ice-cold environment, with benzene as the solvent. At room temperature, the reaction mixturewas stirred for 6 to 7 hours N-3-Chloropropyl-benzothiazole-2-amine was produced when the solids were filtered, washed with water, and dried and synthesized.

Synthesis of 4-(3-(Benzothiazol-2-ylamino) propyl) amino) benzene sulfonamide[14, 15]. (Compound B1)

Equimolar solution of N-3-Chloro propyl-benzothiazole-2-amine (0.01mol, 1.5 gm) and Sulphanilamide (0.01mol, 1.7 gm) in presence of potassium carbonate or triethyl amine and the solvent used as dichloromethane was stirred about 6-7 hours at room temperature. The solid was filtered. The solvent system was used as Benzene: Methanol (8:2), Cyclohexane: Ethyl acetate (8:2).

Spectral data:

Solid, mp-80-82, IR (VmaxKBr): 3371.8 (-NH Aromatic, stretching vibration), 2924.03 (-CH Aliphatic, Stretching), 3739.1 (-OH, Stretching), 1384.6 (-S=O, Stretching)

MS:(ESI+)m/z=363 (M+1)

Synthesis of 4-(3-(Benzothiazol-2-ylamino) propyl) amino) benzene sulfonic acid. (Compound B2)

Same procedure was followed as mentioned in the synthesis of (compound B2) only sulphanilamide replaced by sulfanillic acid.

Spectral data:

Solid, m p- 92-94, IR (VmaxKBr): 3254 (-NH Aromatic, stretching vibration), 2935.4 (-CH Aliphatic, Stretching), 1309.5 (S=O, Stretching)

Evaluation of Anti-microbial activity

The cup plate method was employed for the purpose of antimicrobial testing. Nutrient Agar was carefully dispensed into the sterilized petri dishes, with each dish receiving 25-30 mL of the medium. The poured material was allowed to solidify for 30 minutes, after which cups with a diameter of 3 mm created[16].

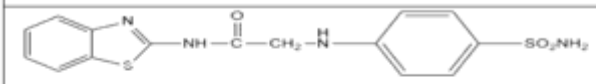
The test compound solution (0.1 mL) was carefully dispensed into these cups using a micropipette. The plates underwent incubation at 37 oC for a duration of 24 hours. The antimicrobial agents permeate the agar medium.The developmental process of the microbial strain is inhibited, followed by the measurement of the diameter of the zone of inhibition.

RESULT AND DISCUSSION

Docking study of synthesized compounds

Docking of designed compounds **(A1-A2 and B1-B2)** was performed by Auto dock 1.4.6 software on topoisomerase receptor for anti-microbial screening **(**PDB: 2POG**)**. The docking score of compounds was ranging between -9.5 to -8.59. All compounds possessed better docking scores than the standard drug, Norfloxacin (-9.8). Compound **A1, A2 and B1** having 2-aminobenzothiazole moiety showed the good docking score (-9.5, -9.5 and -9.2). Other compounds in the series also exhibited a good score. The-S-of amino benzothiazole in good docking scored compound **A1, A2 and B1** involved in hydrogen bonding interaction with ALA 1120, ARG 1122, ASP 1083, GLU 1088, SER 1084 via -H of -NH.

Table 4: Docking Study of compound A1

Structure	Mode	Affinity (Kcal/mol)
	1	-9.5
	2	-9.3
	3	-9.2
	4	-9.0
	5	-9.0
	6	-8.9
	7	-8.6
	8	-8.6
	9	-8.6

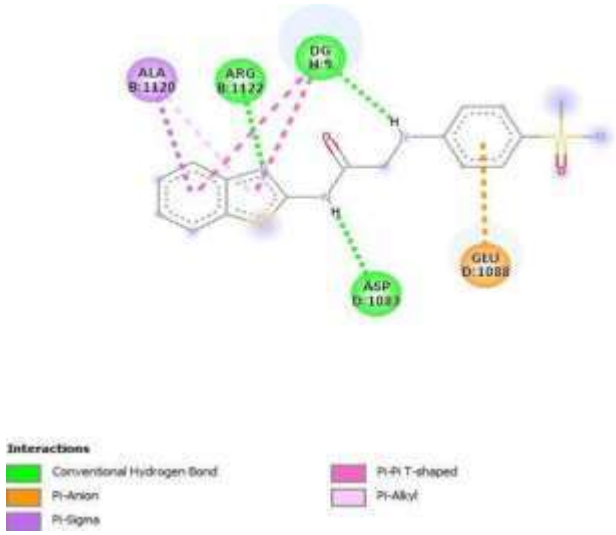
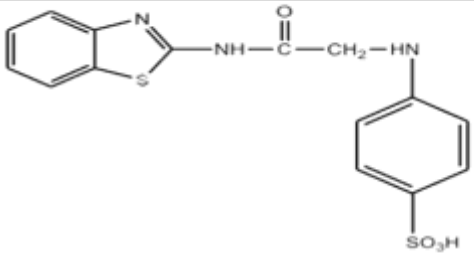


Table 5: Docking score of designed compounds A2

Structure	Mode	Affinity (Kcal/mol)
	**1**	**-9.5**
	2	-9.4
	3	-9.0
	4	-8.9
	5	-8.7
	6	-8.6
	7	-8.6
	8	-8.6
	9	-8.4

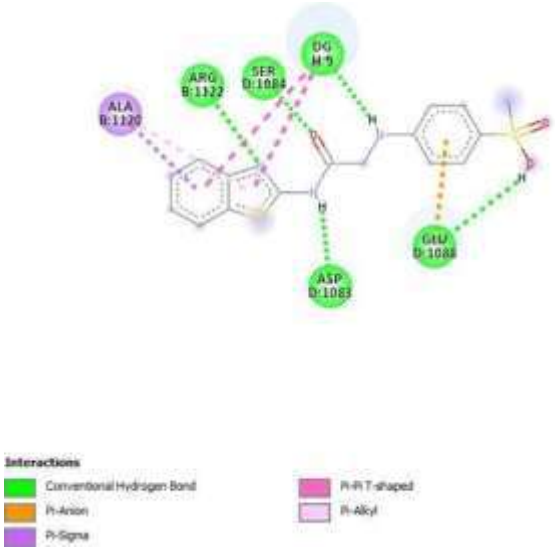
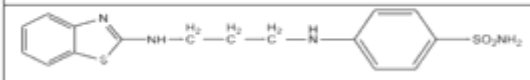


Table 6: Docking score of designed compounds B1

Structure	Mode	Affinity (Kcal/mol)
	**1**	**-9.2**
	2	-8.4
	3	-8.3
	4	-8.2
	5	-8.1
	6	-8.1
	7	-8.0
	8	-7.8
	9	-7.7

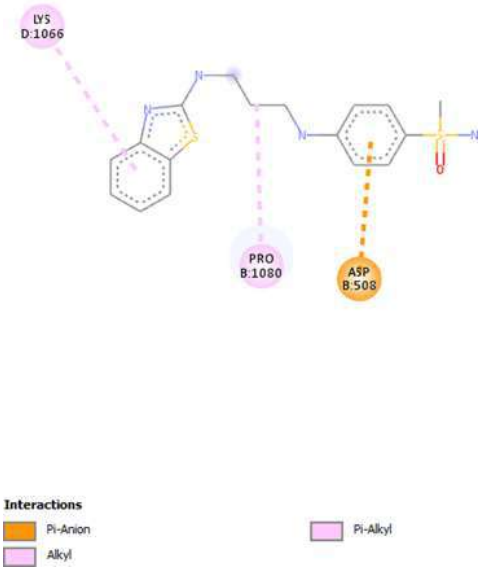
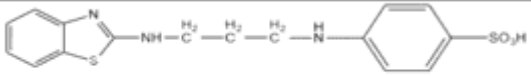
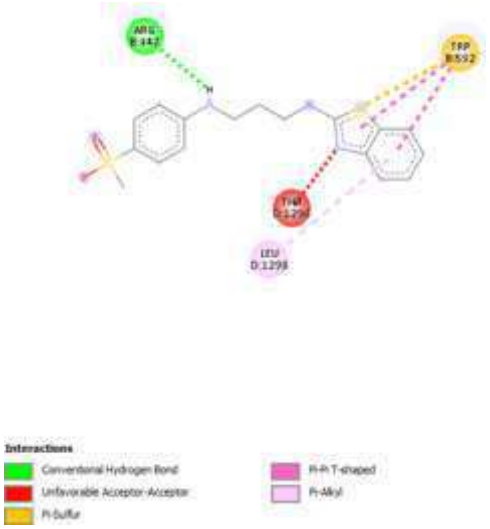


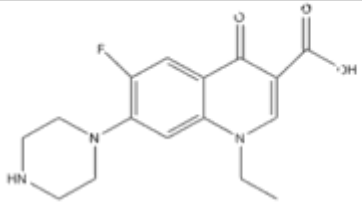
Table 7: Docking score of designed compound B2

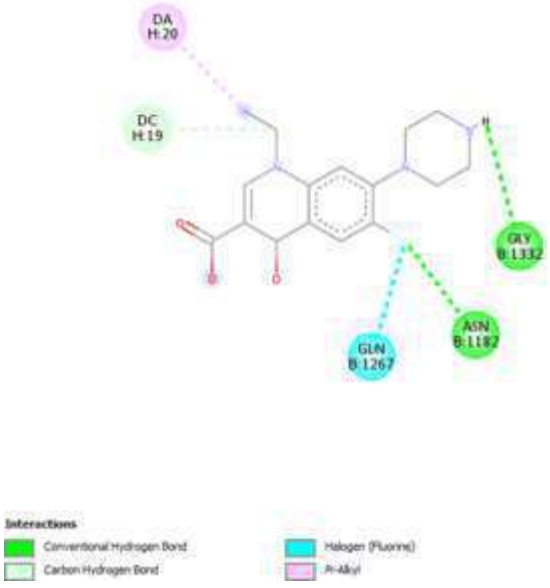
Structure	Mode	Affinity (Kcal/mol)
	1	-8.5
	2	-8.4
	3	-8.0
	4	-7.8
	5	-7.7
	6	-7.7
	7	-7.6
	8	-7.5
	9	-7.4



Docking score of standard drugs

Table 8: Docking score of Standard drugs Norfloxacin

Structure	Mode	Affinity (Kcal/mol)
	**1**	**-9.8**
	2	-9.6
	3	-9.4
	4	-8.8
	5	-8.8
	6	-8.7
	7	-8.7
	8	-8.5
	9	-8.5



Chemistry

Results of synthesis of compoundA1 and compound A2

The intermediate compound (**Intermediate X1**, N- Benzothiazole- 2-yl-2-chloro-acetamide) was synthesized by the reaction of 2- amino benzothiazole with chloroacetyl chloride in the presence of triethyl amine and solvent was used as benzene.

The final compound N-(Benzothiazole-2-yl)-2-((4-sulfamoylphenyl) amino) acetamide **(CompoundA1)**was synthesized by the stirring of (**IntermediateX1**,N-Benzothiazole-2-yl-chloroacetamide) and sulphanilamide (both are dissolved in methanol)in the presence of TEA and benzene about 6 hr. at room temperature.

The compound 4-(2-(Benzothiazole-2-ylamino)-2-oxoethyl) amino) benzenesulfonic acid **(CompoundA2)**was synthesized by the stirring of **(IntermediateX1**, N-Benzothiazole-2-yl- chloro acetamide) and sulfanilic acid (both are dissolved in ethanol) in the presence of TEA and benzene about 6 hr. at room temperature.

Progress of the reaction was checked by TLC and their structures was confirmed by IR and Mass spectroscopy. The spectral data are in total agreement with the proposed structures.

Table 9: Physical data of synthesized compounds (Compounds A1-A2).

Sr. No	1	2
Compounds	**A1**	**A2**
Molecular formula	C15H14O3N4S2	C15H13O4N3S2
Molecular weight	369	363
Appearance	Pale yellow precipitate	Brown precipitate
Yield	33.3%	42%
Rf value	0.73	0.36
Solubility	Soluble in methanol and DMSO	Soluble in methanol and DMSO
Melting point	97°C	141°C

Results of synthesis of compound B1and B2

The intermediate compound (**Intermediate Y1,** N-3-Chloro propyl- benzothiazole-2-amine) was synthesized by the reaction of 2- amino benzothiazole with 1-bromo-3-chloro propane in the presence of triethyl amine and solvent used as benzene.

The final compound 4-(3-(Benzothiazol-2-ylamino) propyl) amino) benzene sulphonamide **(CompoundB1)** was synthesized by the stirring of (**IntermediateY1**, of N-3-Chloropropyl-benzothiazole-2-amine) and sulphanilamide (both are dissolved in methanol) in the presence of TEA and benzene about 6 hr. at room temperature.

The final compound 4-(3-(Benzothiazol-2-ylamino) propyl) amino) benzenesulfonic acid **(CompoundB2)**was synthesized by the stirring of (**Intermediate Y1**, of N-3-Chloropropyl-benzothiazole-2-amine)and sulphanillic acid (both are dissolved in methanol)in the presence of TEA and benzene about 6 hr.

Progress of the reaction was checked by TLC and their structures was further confirmed by IR and Mass spectroscopy. The spectral data are in total agreement with the proposed structures.

Table 10: Physical data of synthesized compounds (Compounds B1 and B2)

Sr. No	1	2
Compounds	**B1**	**B2**
Molecular formula	C16H18O2N4S2	C16H17O3N3S2
Molecular weight	362	363.45
Appearance	White precipitate	White powder
Yield	38.6%	48.2%
Rf value	0.71	0.51
Solubility	Soluble in methanol and DMSO	Soluble in methanol and DMSO
Melting point	81°C	93°C

Results for antimicrobial assay for synthesized derivatives

Among all the synthesized compound compound A1 showed the higher zone of inhibition (22 mm, 28 mm and 33 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI- 38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria).

Among all the synthesized compound compound A2 showed the (21 mm, 26 mm and 32 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria).

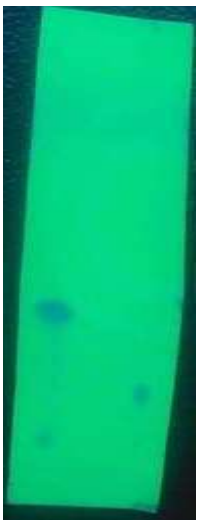
Among all the synthesized compound compound B1 showed the higher zone of inhibition (20 mm, 24 mm and 26 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria)

Among all the synthesized compound compound B1 showed the higher zone of inhibition (22 mm, 25 mm and 28 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-26 mm, 32 mm and 36 mm diameter) against *Escherichia coli.* Among all the synthesized compound compound B2 showed the (20 mm, 22 mm and 25 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-26 mm, 32 mm and 36 mm diameter) against *Escherichia coli* (gram-negative bacteria).

Table 11: Antimicrobial data of synthesized compounds

Compounds	Dilutions (µg/ml)	Zone of inhibition (mm)	
		Escherichia coli	*Bacillus subtilis*
A1	100	18	**22**
	200	22	**28**
	300	24	**33**
A2	100	14	**21**
	200	16	**26**
	300	18	**32**
B1	100	**22**	**20**
	200	**25**	**24**
	300	**28**	**26**
B2	100	**20**	18
	200	**22**	20
	300	**25**	24
Norfloxacin	100	**26**	**38**
	200	**32**	**42**
	300	**36**	**48**

Compound A1



Compound A2

Compound B1

Compound B2

Figure: TLC of Final Compounds

Antimicrobial activity of synthesized derivatives

Fig.5.5 *Escherichia coli*

Fig. : *Bacillus subtilis*

CONCLUSION

Docking study

Docking of designed compounds **(A1-A2 and B1-B2)** was performed by Auto dock 1.4.6 software on topoisomerase receptor for anti-microbial screening **(**PDB: 2POG**)**. The docking score of compounds was ranging between -9.5 to -8.59. All compounds possessed better docking scores than the standard drug, Norfloxacin (-9.8). Compound **A1, A2 and B1** having 2-aminobenzothiazole moiety showed the good docking score (-9.5, -9.5 and -9.2).

In scheme 1, the intermediate compounds (Intermediate X1 of N-benzothiazol-2- yl)-2-chloracetamide) were synthesized by reacting 2-amino benzothiazole with chloroacetyl chloride in the presence of TEA. Final compounds (A1, A2) were synthesized by reacting intermediate X1 with different sulphonamides (Sulphanilamide and sulfanillic acid).

In scheme 2, the intermediate compounds (Intermediate Y1 N-3-Chloro propyl-benzothiazole-2-amine)were synthesized by reacting 2-aminobenzothiazole with 1-bromo-3-chloropropane in the presence of TEA. Final compounds (B1, B2) were synthesized by reacting intermediate Y1 with different sulphonamides (Sulphanilamide and sulfanillic acid).

Antimicrobial activity

Compound A1 showed the higher zone of inhibition (22 mm, 28 mm and 33 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI- 38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria).

Among all the synthesized compound compound A2 showed the (21 mm, 26 mm and 33 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria).

Among all the synthesized compound compound B1 showed the higher zone of inhibition (20 mm, 24 mm and 26 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-38 mm, 42 mm and 48 mm diameter) against *Bacillus subtilis* (gram-positive bacteria)

Among all the synthesized compound compound B1 showed the (22 mm, 25 mm and 28 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-26 mm, 32 mm and 36 mm diameter) against *Escherichia coli* (gram-negative bacteria).

Among all the synthesized compound compound B2 showed the (20 mm, 22 mm and 25 mm diameter respectively), but lesser than the standard drug i. e. Norfloxacin (ZOI-26 mm, 32 mm and 36 mm diameter) against *Escherichia coli* (gram-negative bacteria).

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