Synthesis, Characterization, and Antimicrobial Activity Evaluation of Some Newly Synthesized Benzothiazole Derivatives by Azo Coupling Reaction

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ABSTRACT: Benzothiazole is the most commonly used lead compound for drug discovery and development due to its numerous pharmacological activities. We have synthesized some novel benzothiazole derivatives by diazotization and coupling reaction between our lead compound 2-aminobenzothiazole, and different substituted phenols or anilines. Synthesized compounds were purified by recrystallization and then characterized by melting point determination and IR spectroscopy. After biological evaluation, some of those synthesized compounds show effective antimicrobial activity against gram-negative and gram-positive bacterial strains. After this research work, we want to emphasize the area where azo compounds are produced by diazotization and coupling reactions are not only used as dyes but also may have a wide range of pharmacological activity if it is a benzothiazole derivatives.

Keywords: Benzothiazole, Diazotization, Coupling, Antimicrobial, etc.

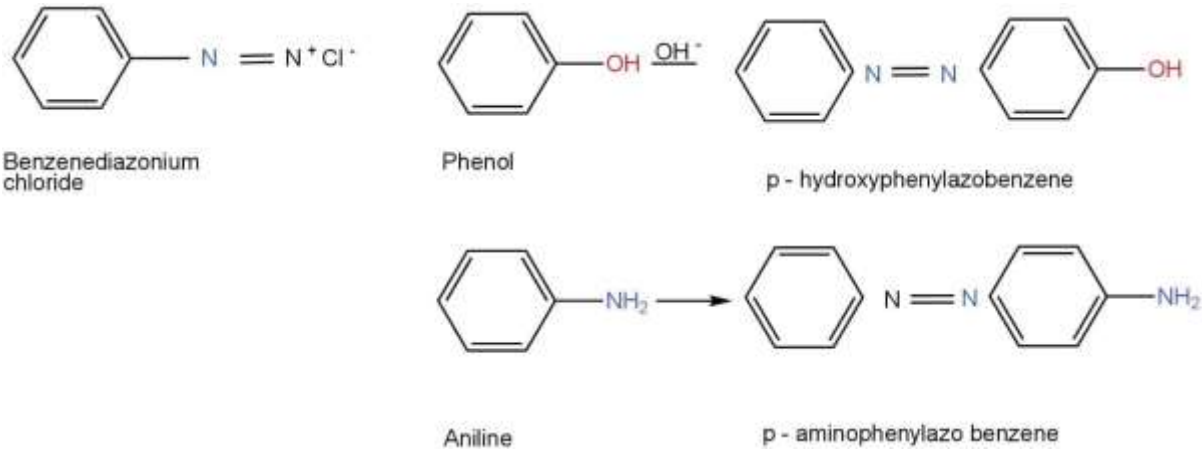
INTRODUCTION:

Many marine natural chemicals with beneficial biological activity contain the benzothiazole ring. Because of their anti-cancer, antitumor, anticonvulsant, antiviral, antibacterial, antimicrobial, and fungicidal properties, benzothiazole derivatives have garnered a lot of attention. They also work well as appetite suppressants, anthelmintics, anti-allergic, and anti-inflammatories medications. Additionally, thiazoles have been used in the development of medications to treat HIV infections, schizophrenia, and hypertension. Additionally, it has been discovered that many thiophene compounds have pharmacological properties. Additionally, pyrazoles have been identified as antibacterial, antiparasitic, antiviral, and antineoplastic compounds in a variety of chemotherapeutic activities. Popular substances that are extensively utilized in textiles as well as other industrial domains include aromatic and heteroaromatic dyes. Azo dyes with aromatic compounds connected by azo chromophores are frequently used in color textiles like acrylic, polyester, nylon, cellulose, and acetate.[2-4] Because of their exceptional light, color, washing, and sublimation fastness, as well as their high level-dying properties, Azo dyes with aromatic heterocycles are also highly significant in the dye business. Among these, benzothiazole derivative azo dyes are significant since they are both cationic and red dispersion dyes. Numerous azo dye compounds derived from benzothiazole derivatives have been produced, and their uses in a wide range of fields have been studied. Additionally, it has found use in significant fields outside of textiles, like pharmacy. nonlinear optical systems, anticancer activities, and

antimicrobial activities. Given the aforementioned information and in keeping with our interest in the synthesis of heterocycles with a benzothiazole moiety, we are looking for novel candidates that could be useful in the development of powerful, selective, and less harmful antimicrobial drugs.[7-9]

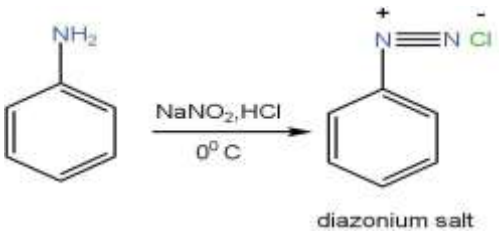
- **AZO COUPLING REACTION: -**

In water, the majority of diazonium salts dissolve. Azo compounds are created when they react with phenols or amines. We call this process a coupling reaction. Diazotization is an example of electrophilic aromatic substitution and a weak electrophilic coupling process. In slightly acidic solutions, amines couple most effectively, whereas phenols couple in slightly alkaline media. Coupling response examples include:



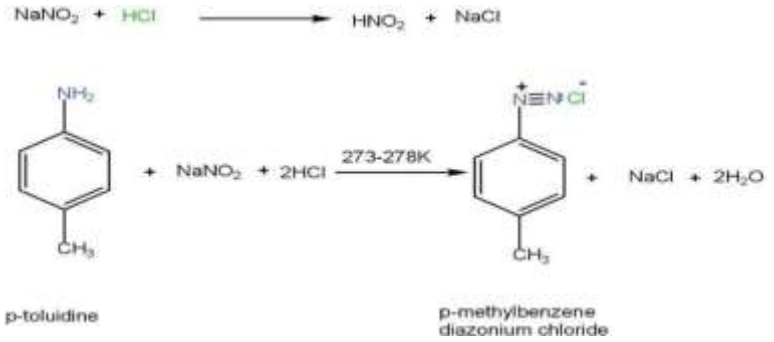
- **PREPARATION OF DIAZONIUM SALT**

The mechanism of diazonium salt formation involves the addition of NO, followed by a sequence of acid-base reactions which transform the oxygen into water and form a triple bond between the two nitrogen.
The water is then boiled off as a leaving group. Diazonium salt formation is only possible with 1° aryl and alkyl amines.



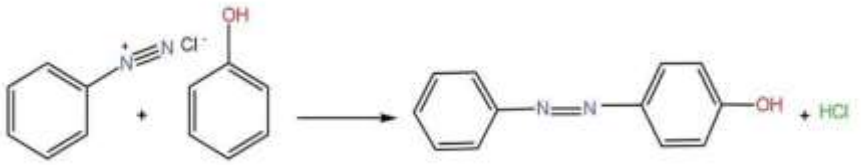
1. REACTION WITH ANILINE

Treatment of primary amine with nitrous acid results in the formation of diazonium salt, a compound of the type Ar/R-NN^+X^-, where X-is an anion like chloride, bromide, sulphate etc. But since nitrous acid is unstable, as a result it is generated in situ by treatment of sodium nitrite with strong acid such as HCl or H_2SO4. This process of conversion of primary amine to its diazonium salt is known as the diazotization reaction.



2. REACTION WITH PHENOL

The reaction of diazonium salt with a phenol or a substituted phenol gives a yellow or orange azo compound.



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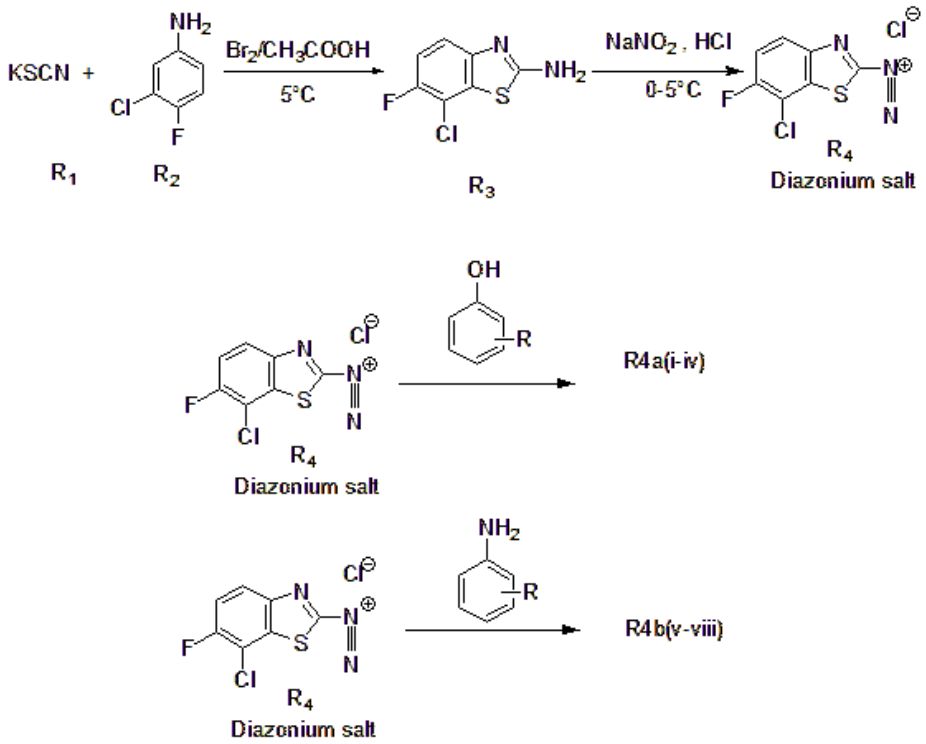


METHODOLOGY:

Synthesis of 2-aminoBenzothiazole(R_3): Potassium Thiocyanate and 3-chloro-4-fluoro aniline in equimolar quantity were taken, then the reaction was held in the presence of bromine and glacial acetic acid at a temperature 5°C to get the compound 7-chloro-6-fluoro-2-amino benzothiazole.

Synthesis of Benzo diazonium salt (R_4): Diazonium salt of compound **R_3** was prepared by using sodium nitrate and HCl, maintaining a temperature of 0°- 5°C.0.5 gm compound,1.65 ml of concentrated HCL, and 1.6 ml of water were added into a beaker (1), and the beaker was placed on an ice bath at 0° - 5°C.0.5 g sodium nitrite, 2 ml of water added into a beaker (2) and placed on an ice bath. Then pour the solution from beaker (1) into beaker (2)dropwise on an ice bath

Synthesis of R4a(i-iv) and R4b(v-viii) by Azo coupling reaction: We have taken different derivatives of phenol and aniline for this step of synthesis.1 gm of each compound (derivatives of phenol and aniline) and 5.5 ml of 10% NAOH solution were added to different beakers (3) on an ice bath, and some ice into the beaker. Then, slowly added the solution from beaker(1) into beaker(3) and kept it on an ice bath for 30 min. After 30 minutes, filter the solution and wash with cold water.

CHARACTERIZATION: All the synthesized compounds were characterized by melting point and IR spectroscopy.

- **MELTING POINT:-**

The specific temperature at which a solid transforms into a liquid when heated, and a liquid transforms into a solid when cooled, at a given pressure. We have performed the Melting point determination by Theil's tube method.

Applications:

Melting points are used to identify and characterize substances, and they can also be used to assess the purity of a substance

Introduction:

The melting point is the temperature at which a solid changes to a liquid at a given pressure, typically atmospheric pressure. It's a characteristic property used to identify and assess the purity of substances. When a solid is heated, its temperature rises until it reaches the melting point, where it absorbs heat as latent heat of fusion without changing temperature. Once the solid is completely melted, the temperature of the liquid will then increase as more heat is added.

Table-1

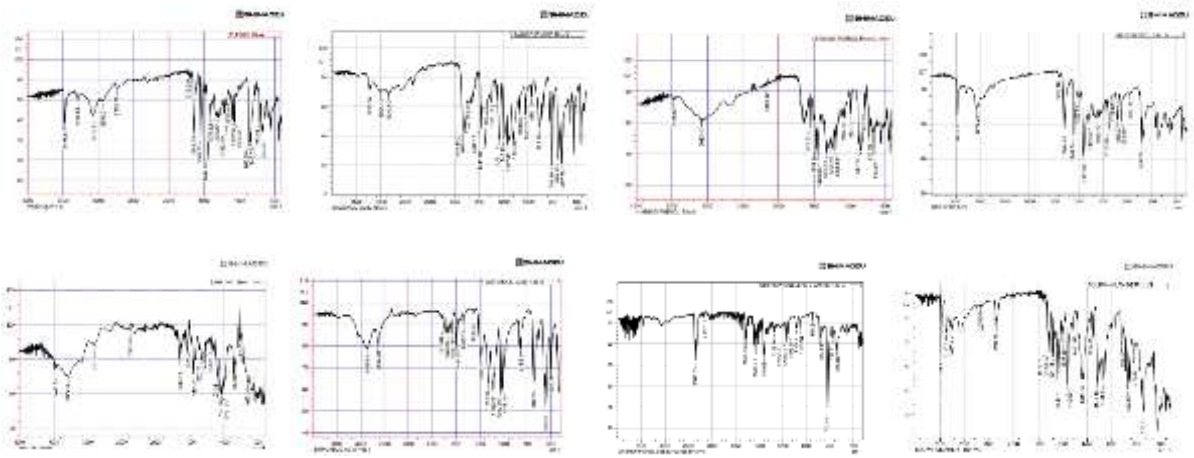
NAME of the compound	Substituted Phenol/Aniline used	Melting point range (C°)
R4a-i	Phenol	139-141
R4a-ii	Salicylic Acid	156-158
R4a-iii	4-amino phenol	188-190
R4a-iv	Resorcinol	109-111
R4b-v	Aniline	187-190
R4b-vi	Sulphanilic Acid	287-289
R4b-viii	Orthophenylene Diamine	199-201
R4b-viii	Sulphanilamide	164-166

- **IR:-**

Introduction:

Infrared (IR) spectroscopy is a spectroscopic technique that analyzes the interaction of infrared light with matter, primarily to identify the functional groups within a molecule.

Graph:



BIOLOGICAL EVALUATION:

❖ ANTIMICROBIAL ACTIVITY:

Because of their high biopotency, Azo compounds have demonstrated strong antibacterial and antifungal activity. For instance, azo derivatives and azo-hydrazones tautomeric dyes demonstrated antioxidant, antibacterial, and chymotrypsin properties, respectively. Benzimidazole, cardanol, diaryl, pyrazoles, modified phenyl azo derivatives, and Thiram azoxystrobin combination are being used as possible antifungal agents with the capacity to bind chitin. Gram-positive bacteria (Bacillus subtilis, Streptococcus lactis), Gram-negative bacteria (Escherichia coli, Pseudomonas putide), and fungi (Aspergillus niger, Penicillium

sp.) are all effectively inhibited by benzothiazole derivatives. and yeast (Candida albicans). Substituted sulphonamides' azo derivatives have antibacterial properties. Azo-hydrazone exhibited a moderate level of antibacterial activity.

REQUIREMENTS:

Beaker, Weighing Machine, Conical Flask, Sterile cork borers, Burner, Petri dish, Burner, Autoclave, Beef Extract, Peptone, Bacterial Cultures(E. coli), Sterile Spreading Rod, Incubation Chamber, NaCl, Agar, Distilled Water

METHOD:

Take 0.3gm of beef extract, peptone, and NaCl 0.5gm each, agar 1.8gm, 100 ml distilled water, and prepare a solution in a beaker. Then place the beaker into the autoclave at 15 PSI for 15 minutes at 121°C temperature. Then keep it at room temperature for 20 minutes. Then take the test samples in a beaker separately and add ethyl acetate to each beaker, and prepare a solution. After solidifying the agar solution, we took it to the incubation chamber, and strained the petri dishes with bacteria (G positive & G negative) by a sterile spreading rod by heating it. Now put the standard drug and the test samples by using sterile corkborers into to petri dish and keep the petri dish for 24 hours in the incubation chamber. After 24 hrs, we will check the zone of inhibition.

RESULT:

Table-2 Antimicrobial Activity

NAME OF THE COMPOUNDS	ZONE OF INHIBITION	
	E, Coli	S. Aureus
Control(Gentamycin)	15mm	15mm
R4b-vii	11mm	5mm
R4b-viii	14mm	7mm
R4a-iii	8mm	2mm
R4a-ii	10mm	8mm
R4a-iv	13mm	6mm
R4a-i	10mm	9mm
R4b-v	9mm	4mm
R4b-vii	11mm	7mm

DISCUSSION:

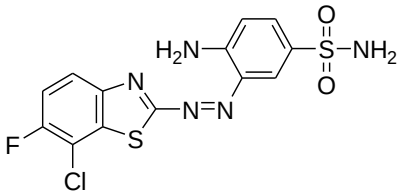


Fig: R4b-viii

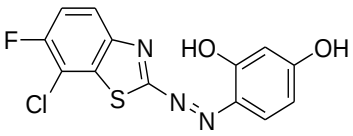


Fig: R4a-iv

We have synthesized the 2-aminobenzothiazole moiety, which was our lead compound. After that, we prepared a diazonium salt of that benzothiazole moiety, then by azo coupling reaction, we prepared several derivatives of Benzothiazole, and we have also evaluated the antimicrobial activity of those synthesized compounds. Among all 8 compounds, after observing the zone of inhibition, we confirm that compounds R4a-iv and R4b-viii have shown the maximum zone of inhibition on the gram-negative bacterial strain E. coli. Thereafter, the standard drug.

CONCLUSION

After this research work, we can conclude that the azo derivative of the benzothiazole molecule is not only used as a dye but also has potent antimicrobial activity, especially against gram-negative bacteria instead of gram-positive bacterial strains.

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