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EVALUATION OF N-(6-CHLOROBENZOTHIAZOL-2-YL)-2-(SUBSTITUTEDAMINO)ACETAMIDE FOR ITS ANTI-BACTERIAL ACTIVITY

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ABSTRACT

In the present study, a series of some novel benzothaizole derivatives (BZT 1-10) were screened for their antibacterial activity against *S. typhi*, *S. dysenterae, E. coli*, *S.aureus* and *B. subtlis* employing Disc Diffusion method and taking Agar as culture media. Streptomycin (25µg/ml) was used as a standard drug. The solvent, DMSO used for the preparation of compounds did not show inhibition against the tested organisms which serves as the negative control. After evaluation of results, most of the derivatives have shown efficient diameter of zone of inhibition as compared to control as well as standard which confirms the promising antibacterial activity of the benzothaizole derivatives.

INTRODUCTION: Benzazoles are an extremely important class of compounds that occur widely as biologically active natural products, as well as marketed drugs or drug candidates. All benzazoles have two heteroatoms attached at the ortho position on the benzene ring having one nitrogen atom common for all three benzazoles (benzothiazole, benzimidazole and benzoisoxazole), in case of benzothiazle one heteroatom is sulfur. Benzothiazoles are condensed heterocyclic organosulfur compounds consist of a five member nitrogen and sulfur containing ring; thiazole fused with six member homocyclic ring; benzene.

In the 1950s, a number of 2-benzothiazolamines were intensively studied as central muscle relaxants. The major work concerned with this field is that of Domino *et al* [1]. Since then medicinal chemists have not taken an active interest in this chemical family. Biologist's attention was drawn to this series when the pharmacological profile of riluzole was discovered. Riluzole[2] (1, 6-(trifluoromethoxy)-2-benzothiazolamine, PK 26124, RP 54274, Rilutek) was found to interfere with glutamate neurotransmission in biochemical, electrophysiological, and behavioral experiments.

The benzothiazole ring is present in various marine and terrestrial natural compounds, which have useful biological activities [3-6]. As an intermediate 2-aminobenzothiazoles are broadly found in bioorganic and medicinal chemistry with applications in drug discovery and development for the treatment of diabetes [7-16], epilepsy [17-23], inflammation,[24] analgesia,[25,26] amyotrophic lateral sclerosis,[27] and viral infections [28]. They also exhibits antitumor [29-42], antitubercular [43], antibacterial [44, 45], antifungal [46-47], antimalarial [48], antihelmintic [49].

Substituted2-arylbenzothiazoles have emerged in recent years as an important pharmacophore in non-invasive diagnosis of *Alzheimer's disease* [50]. Recently, benzothiazole derivatives have been evaluated as potential amyloid-binding diagnostic agents in neurodegenerative disease [51, 52] and as selective fatty

acid amide hydrolase inhibitors [53]. Furthermore, some benzothiazole derivatives are being used as azo dyes and corrosion inhibitors. In addition, the diverse biological activities reported for many derivatives of benzothiazole have also drawn the attention of biochemists in the last decade. The objective of the present study is to evaluate the antibacterial activities of the some novel benzothiazole derivatives. The Physical characteristics of the test compound of benzothiazole derivatives taken in this study are presented in **table 1**.

TABLE 1: Physical characteristics of compounds

Compound	Name of compounds	Molecular formula	M.P. (°C)	Colour	Solubility
BZT-1	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(dimethylamino) acetamide	$C_{11}H_{12}ClN_3OS$	197	Colorless	Ethanol/ Methanol
BZT-2	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(diethylamino)acetamide	$C_{13}H_{16}ClN_3OS$	190	Colorless	Ethanol/ Methanol
BZT-3	2-(bis(2-hydroxyethyl)amino)-N-(6-chlorobenzo[d]thiazol-2-yl)acetamide	$C_{13}H_{16}ClN_3O_3S$	202	Colorless	Ethanol/ Methanol
BZT-4	N-(6-chlorobenzo[d]thiazol-2-yl)-2-morpholinoacetamide	$C_{13}H_{14}ClN_3O_2S$	158	Colorless	Ethanol/ Methanol
BZT-5	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(piperidin-1-yl) acetamide	$C_{14}H_{16}ClN_3OS$	194	Pale yellow	Ethanol/ Methanol
BZT-6	2-(4-fluorophenylamino)-N-(6-chlorobenzo[d]thiazol-2-yl) acetamide	$C_{15}H_{11}ClFN_3OS$	198	Colorless	Ethyl Acetate
BZT-7	2-(3-chlorophenylamino)-N-(6-chlorobenzo[d]thiazol-2-yl) acetamide	$C_{15}H_{11}Cl_2N_3OS$	195	Colorless	Ethyl Acetate
BZT-8	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(pyridin-4-ylamino) acetamide	$C_{14}H_{11}ClN_4OS$	190	Colorless	Ethanol/ Methanol
BZT-9	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(pyridin-2-ylamino) acetamide	$C_{14}H_{11}ClN_4OS$	167	Brick red	Ethanol/ Methanol
BZT-10	N-(6-chlorobenzo[d]thiazol-2-yl)-2-(4-sulfonamidophenyl)acetamide	$C_{15}H_{13}ClN_4O_3S_2$	208	Colorless	Ethanol/ Methanol

Experimental procedure for Antimicrobial Activity:

Disc diffusion method: When a filter paper disc impregnated with a chemical is placed on agar the chemical diffuses from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition".

By measuring the zone of inhibition, antibacterial susceptibility of the compound can be evaluated. Anti-bacterial activity a series of ten novel compounds (BZT 1-10) were evaluated against various pathogenic bacterial strains both Gram-negative and Gram-positive. The anti-bacterial activities were evaluated by agar disc diffusion method as per the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) [54]. The solvent, DMSO used for the preparation of compounds did not show inhibition against the tested organisms.

The inoculums was spread on the surface of the solidified media and Whatman no. 1 filter paper discs (6 mm in diameter) impregnated with the test compound were placed on the plates. Streptomycin was used as positive control for bacteria. A paper disc impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24 h at 37 ºC. The inhibition zone diameters were measured in millimetres. All the tests were performed in triplicate and the average was taken as final reading.

Preparation of test solution: Test solutions of benzothiazole derivatives prepared by using DMSO in a concentration range 250µg/mL

Preparation of standard solution: Standard drug solution was prepared with DMSO.

Streptomycin: - 25µg/mL

Test organisms

Gram positive(+)	Gram negative(-)
Bacillus subtilis (UC 564)	*Escherichia coli (E. coli)*(ATCC 25938)
Staphylococcus aureus (NCTC 6571)	*Shigella dysentrae* (7)
	Salmonella typhi (59)

Preparation of Nutrient Agar Media: Sodium chloride, peptone, beef extract, agar were weight out and dissolved in required amount of distilled water by keeping the media in the steam bath, the agar was melted out and the indicator was added and the volume was made with distilled water, pH was adjusted to 7.2-7.4. Then the flux was plugged and wrapped in paper, then autoclave at 15 psi pressure at 121^{o}C for 15 min.

Peptone	:	5gm
Sodium chloride	:	2.5gm
Beef extract	:	5gm
Agar	:	10gm
Distilled water	:	q.s.500ml
Adjust pH	:	7.2- 7.4

Sterilization of apparatus: Petri dishes, glass syringe, Filter paper disc (6mm), conical flask and test tubes were sterilized by hot air oven at 160^{o}C for 1hour.

Preparation of Petri dishes: Antibacterial activity of synthesized drug was screened by filter paper disc method. A previously liquefied medium, appropriate for the test is inoculated with the requisite quantity of the suspension of the microorganism, the suspension was added to the medium at a temperature between 40-50^{o}C and the inoculated medium was poured immediately into dried Petri dishes to occupy a depth of 3 to 4 mm. The paper disc (No.1Whatmann) was cut downed into small disc (6mm diameter) and sterilized at 160^{o}C/1hr in hot air oven impregnated with the test solution and the standard solution. The dried discs were placed on the surface of the medium. The dishes were left standing for 1-4 hrs, at room temperature as a period of pre- incubation diffusion.

RESULTS AND DISCUSSION: After performing the antibacterial screening of the compounds, it has been found that almost all the compounds were effective against the various bacterial strains taken in the present study. Among all the ten compounds, as shown in **table 2**. The zone of inhibition of BZT- 3 was found to be effective against *E. coli* and *S. aureus* having a zone of inhibition of 18mm-19mm. In case of BZT-1, BZT-2, BZT-4and BZT-5 the activity against the different microbes was moderate to good, having a zone of inhibition in the range of 15mm to 19mm.

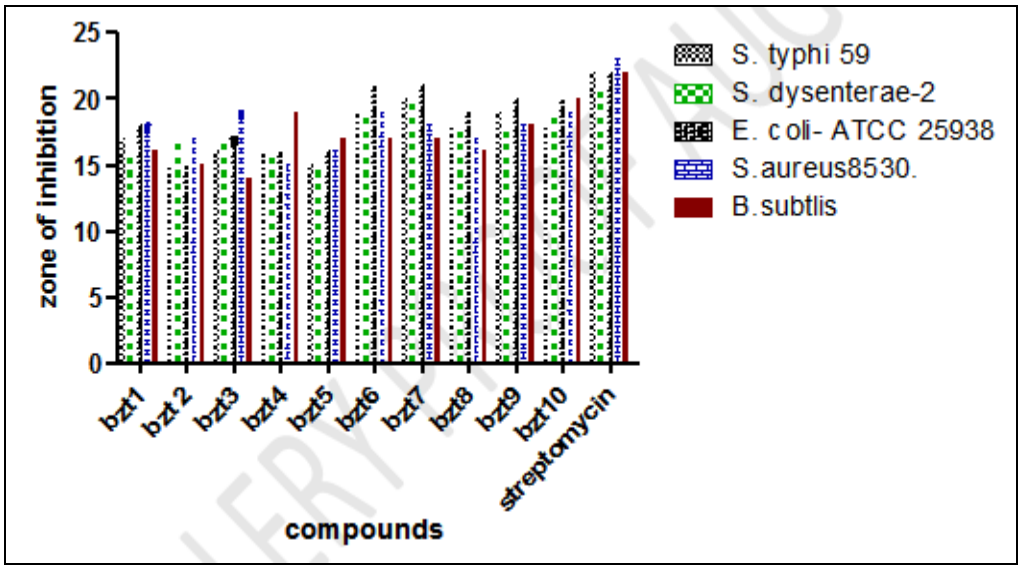
Again for the compounds BZT-6 and BZT-7 the zone of inhibition was found to be quite significant as compared to the other compounds, which confirms that it possess potent antibacterial activity which may be due to the presence of strong electron withdrawing groups fluorine and chlorine respectively on the respective amine of the compounds.

Where BZT-9 and BZT-10 also proved to be effective against the microbes as the diameter of zone of inhibition was found to be above 18mm for the all the microbes, which confirms its promising antibacterial activity , which may be due the presence of pyridine ring in BZT-9 and presence of additional -SO_2NH_2 group in the compound BZT-10.

TABLE 2: Antibacterial activity of the compounds

Compound	Diameter of zone of inhibition (mm)				
	Gram -ve			Gram +ve	
	S. typhi	*S. dysenterae*	*E. coli*	*S.aureus*	*B. subtilis*
BZT-1	17	16	18	18	16
BZT-2	15	17	16	17	15
BZT-3	16	17	18	19	14
BZT-4	15	16	16	15	16
BZT-5	16	15	16	16	19
BZT-6	18	19	21	19	17
BZT-7	18	20	21	18	17
BZT-8	20	18	19	17	16
BZT-9	19	18	20	18	18
BZT-10	18	19	20	19	20
Streptomycin (Standard)	22	21	22	23	22
DMSO	-	-	-	-	-

DMSO was taken as negative control and Streptomycin was taken as standard drug. The zone of inhibition was measured in mm.



GRAPH 1: SHOWING ANTIBACTERIAL ACTIVITY OF BENZOTHIAZOLE DERIVATIVES

CONCLUSION: This study reports the antibacterial activity of novel benzothiazole derivatives. The divergence in the antibacterial activity of these compounds validates the significance of this study. The results of the study revealed that most of the compounds tested showed moderate to good antibacterial activity. Structure and biological activity relationship of the title compounds showed the electron withdrawing group enhanced the activity.

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