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MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL BENZOTHIAZOLYL SUBSTITUTED DERIVATIVES

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ABSTRACT:-The present research includes synthesis of various derivatives containing novel benzothiazolyl substituted derivatives and evaluating their antimicrobial properties. These compounds were produced using a green chemistry method, specifically microwave synthesis. A range of compounds, including *(3E)*-1-(1,3-benzothiazol-2-yl)-*N*-phenyl-1,2-diazetidin-3-imine (1c), *(3E)*-1-(1,3-benzothiazol-2-yl)-*N*-(4-nitrophenyl)-1,2-diazetidin-3-imine (1e), *(3E)*-1-(1,3-benzothiazol-2-yl)-*N*-(2-chlorophenyl)-1,2-diazetidin-3-imine (1f), *(3E)*-1-(1,3-benzothiazol-2-yl)-*N*-(3-chlorophenyl)-1,2-diazetidin-3-imine (1g), and *(3E)*-1-(1,3-benzothiazol-2-yl)-*N*-(4-chlorophenyl)-1,2-diazetidin-3-imine (1h), were successfully synthesized. The antimicrobial properties of these compounds were assessed in relation to *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Pseudomonas auraginosa*. Structures of all the newly synthesized compounds were confirmed by their IR, ^{1}H-NMR and CHN analysis. The selected compounds may be used to design more potent biologically active compounds.

KEYWORDS:-Benzothiazole, Microwave, Antimicrobial Activity, Agar diffusion, DMSO.

INTRODUCTION

In medicinal chemistry, substituted benzothiazolyl and benzimidazolyl derivatives are among the heterocyclic molecules with the greatest use. The current study uses a methodical strategy to synthesis chemicals, verify their structures, and investigate their biological activities using antimicrobial research. These compounds will be used for the physical parameter investigation [i-iii] in pharmaceutical chemistry, which requires validated entities having physical properties. Because they have so many uses in human life, heterocyclic compounds have become extremely important. In recent years, these compounds have gained medical significance due to their successful trials against a number of disorders. Heterocyclic compounds containing nitrogen and sulfur have been reported to exhibit a wide range of biological activity, according

to numerous experiments. Benzothiazolyl substituted compounds have a broad range of biological applications. Benzothiazolyl derivatives are widely used in bioorganic and medicinal chemistry, with applications in drug discovery and development for the treatment of autoimmune and inflammatory diseases, prevention of solid organ transplant rejection, epilepsy[iv], antitumor[v], antiviral[vi], anticonvulsant[vii], neuroprotective and immunosuppressive properties. Benzothiazoles are used as industrial chemicals, dyes, and pharmaceuticals[viii-ix]. Thiazoles[x-xvi] are an important class of heterocyclic compounds present in many potent physiologically active chemicals. They also have pharmacological properties such as relative stability, beginning material, a built-in biocidal unit, and easy metabolism of the product. The benzothiazole ring system is made up of thiazole fused with benzene and has a variety of applications. Although they have long been known to be biologically active, their many biological properties keep attracting scientists' interest. In this study, an attempt was made to synthesize benzothiazolyl substituted molecules using a green chemistry technique, namely microwave synthesis. The structures of produced substances have been confirmed by using spectral and CHN analysis. The biological application of these compounds was investigated using antibacterial activity against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Pseudomonas auraginosa*.

Microwaves [xvii-xxiv] is commonly recognized as electromagnetic waves with wavelengths ranging from 1 mm to 1m, and they have been used in science study as an assistant technique or a method of chemical synthesis. It might be classified as a green chemistry technique that focuses on more efficient and faster reactions. Because of structural and packing effects, reaction products can be formed in a solid state. Microwave-based techniques were gradually used and played a significant part in the preparation process. Compared to typical heating methods, microwave treatment delivers intensive, uniform, and efficient energy, allowing it to reach considerable temperatures and activate reactions in a very short period of time.

MATERIALS AND METHODS

The chemicals were procured from S. D. Fine chemicals, Rankem. The chemicals were used as received. All the used chemicals were of AR grade. Melting points or synthesized compounds were determined in an open capillary and are uncorrected. ^{1}H-NMR spectra were recorded on Brucker (400MHz) spectrophotometer using TMS as an internal standard. The IR spectra were recorded on a Perkin-Elmer model 2000 spectrophotometer using KBr phase. The purity of the synthesized compounds was verified by TLC on Silica gel in the solvent system chloroform and methanol (1:1), and the spot were observed under an Ultra Violet (UV) chamber. Microwave synthesis was carried out in domestic microwave oven (LG).

Synthesis of 1-(1,3-benzothiazol-2-yl)-1,2-diazetidin-3-one (1a)

Method A: Conventional Method

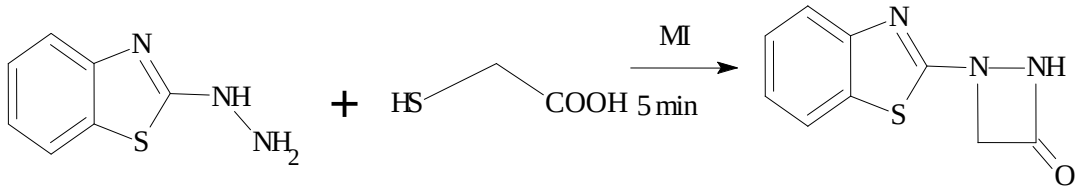
A mixture 2-Hydrazinobenzothiazole (0.036 mole, 6 g) and thioglycolic acid (0.036 mole, 3.3g) was taken in a 100mL round bottom flask was shaken in 15-20 mL ethanol, it was refluxed on a water condenser for 6-7 hours. The progress of reaction was monitored on TLC, after completion of reaction; reaction mixture was cooled to room temperature and poured in ice cold water. The wet product was dried under vacuum for 10 min. and then dried at 40°C for 20 min under vacuum, to obtain 1-(1,3-benzothiazol-2-yl)-1,2-diazetidin-3-one with 50 to 60% yield.

Method B: Microwave Assisted Synthesis of 1-(1,3-benzothiazol-2-yl)-1,2-diazetidin-3-one (1a)

2-Hydrazinobenzothiazole (0.0036 mole, 0.6 g) was taken in 25 mL beaker to this added thioglycolic acid (0.0036 mole, 0.33g) in 1:1 ratio the mixture was moistened with 2-3 drops of ethanol and placed in microwave oven covered with watch glass and irradiated with

microwave irradiation for 5 minutes at 160 watt, after completion of reaction beaker was removed and the granular solid was crystallized from hot ethanol to give 82-84%yields. M.Pt-165-168^{0}C, M.Wt-205.25, Formula-$C_9H_7N_3SO$, IR-(KBrcm^{-1}):-1650(C=O), 1557 (N-H, bending), 3318(N-H, stretching); ^{1}H-NMR: 8.00 (S, 1H, NH) 3.59 (S, 2H, CH_2) , 6.97-7.60 (m, 4H, Ar). Elemental analysis: $C_9H_7N_3SO$, calculated-C, 52.66; H, 3.44; N, 20.48; found:C, 52.60;H3.46;N,20.44.

Scheme:-

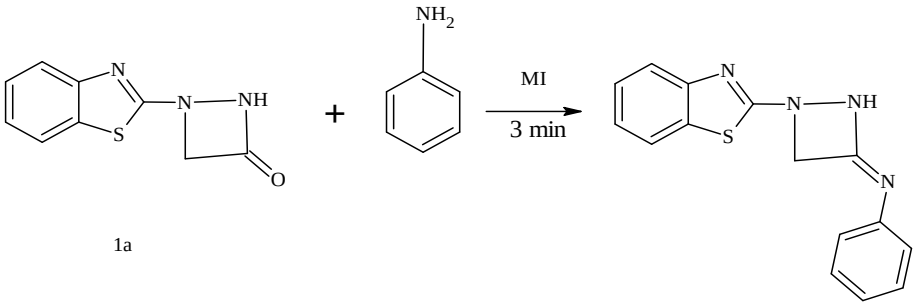


2-hydrazinyl-1,3-benzothiazole thioglycolic acid 1-(1,3-benzothiazol-2-yl)-1,2-diazetidin-3-one (1a)

Microwave Assisted Synthesis of (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-phenyl-1,2-diazetidin-3-imine (1c):-

1a (0.0029 mole, 0.6 g) was placed in a 25 ml beaker and mixed with aniline (0.0029 mole, 0.269g) in a 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in a microwave oven covered with a watch glass and irradiated with microwave irradiation for 3 minutes at 400 watts. After the reaction was completed, the beaker was removed and the granular solid was crystallized from hot ethanol, to give 80-82 % yields. Yield–80-82%, M.Pt-153-155^{0}C, M. Wt-280.36, Formula- $C_{15}H_{12}N_4S$, IR-(KBrcm^{-1}) :-1617(C=N); ^{1}H-NMR: 8.07 (S,1H,NH), 3.7 (S,2H,CH_2), 6.91-7.83 (m,9H,Ar). Elemental analysis: $C_{15}H_{12}N_4S$, calculated-C, 64.26;H, 4.31;N,19.99. Found: C, 64.21; H, 4.37;N,19.98.

Scheme:-



1a

(3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-phenyl-1,2-diazetidin-3-imine (1c)

Microwave Assisted Synthesis of (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(4-nitrophenyl)-1,2-diazetidin-3-imine (1e):-

1a (0.0029 mole, 0.6 g) was placed in a 25 ml beaker and mixed with p-nitroaniline (0.0029 mole, 0.400g) in a 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in a microwave oven covered with a watch glass and irradiated with microwave irradiation for 4 minutes at 400 watts. After the reaction was completed, the beaker was removed and the granular solid was crystallized from hot ethanol, to give 70% yields. Yield–70%, M.Pt-102-105^{0}C, M. Wt- 325.34, Formula- $C_{15}H_{11}N_5SO_2$, IR-(KBrcm^{-1}) :-1619(C=N); 1657 and 1350 (N-O) Stretching, ^{1}H-NMR:7.17 (S,1H,NH), 3.5 (S,2H,CH_2), 6.91-7.83 (m,9H,Ar). Elemental analysis: $C_{15}H_{11}N_5SO_2$, calculated-C,180.16; H,11.09; N,70.03,S,32.06,. Found:C,181.26; H, 11.05; N,70.08,S, 32.02.

Microwave Assisted Synthesis of (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(2-chlorophenyl)-1,2-diazetidin-3-imine (1f):-

1a (0.0029 mole, 0.6 g) was placed in a 25 ml beaker and mixed with o-chloroaniline (0.0029 mole, 0.369g) in a 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in a microwave oven covered with a watch glass and irradiated with microwave irradiation for 4 minutes at 400 watts. After the reaction was completed, the beaker was removed and the granular solid was crystallized from hot ethanol, to give 75% yields. Yield–75%, M.Pt-167-170^{0}C, M. Wt- 314.81, Formula- $C_{15}H_{11}N_4SCl$, IR-(KBrcm^{-1}) :-1617(C=N); 3021 (C-H) Stretching, ^{1}H-NMR:7.17 (S,1H,NH), 3.5 (S,2H,CH_2), Elemental analysis: $C_{15}H_{11}N_4SCl$, calculated-C, 180.16;H, 11.09;N,56.03,S, 32.06, Cl, 35.45 . Found : C, 181.30;H, 11.07; N,56.35,S, 32.08, Cl, 35.35.

Microwave Assisted Synthesis of (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(3-chlorophenyl)-1,2-diazetidin-3-imine (1g):-

1a (0.0029 mole, 0.6 g) was placed in a 25 ml beaker and mixed with m-chloroaniline (0.0029 mole, 0.369g) in a 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in a microwave oven covered with a watch glass and irradiated with microwave irradiation for 4 minutes at 400 watts. After the reaction was completed, the beaker was removed and the granular solid was crystallized from hot ethanol, to give 78% yields. Yield–78%, M.Pt-160-162^{0}C, M. Wt- 314.81, Formula- $C_{15}H_{11}N_4SCl$, IR-(KBrcm^{-1}) :-1615(C=N); 2990 (C-H) Stretching, ^{1}H-NMR:7.19 (S,1H,NH), 3.6 (S,2H,CH_2), Elemental analysis: $C_{15}H_{11}N_4SCl$, calculated-C, 180.16;H, 11.09;N,56.03,S, 32.06, Cl, 35.45 . Found: C, 180.30;H, 11.06; N,56.30,S, 32.05, Cl, 35.32.

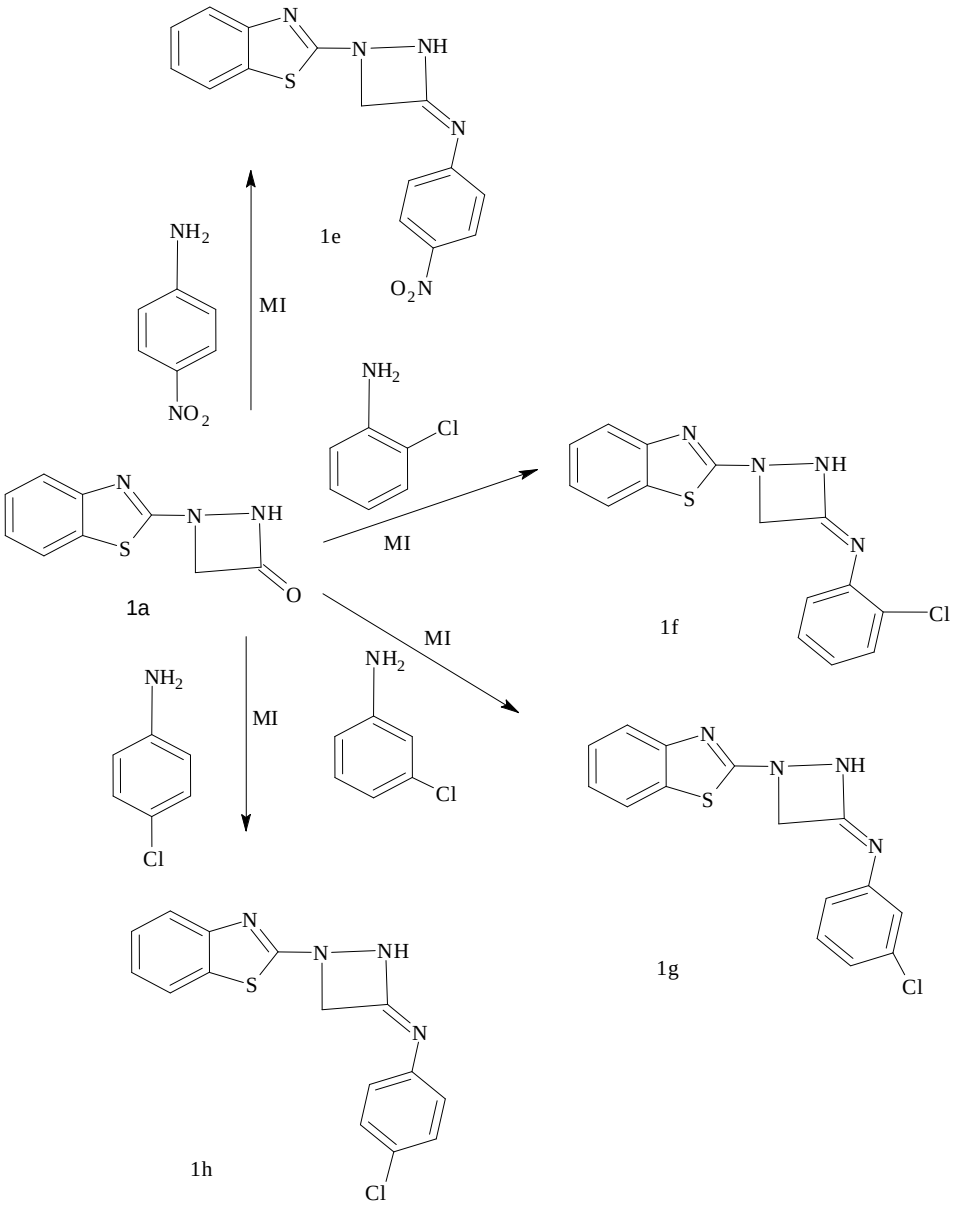
Microwave Assisted Synthesis of (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(4-chlorophenyl)-1,2-diazetidin-3-imine (1h):-

1a (0.0029 mole, 0.6 g) was placed in a 25 ml beaker and mixed with p-chloroaniline (0.0029 mole, 0.369g) in a 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in a microwave oven covered with a watch glass and irradiated with microwave irradiation for 3 minutes at 400 watts. After the reaction was completed, the beaker was removed and the granular solid was crystallized from hot ethanol, to give 82% yields. Yield–82%, M.Pt-210-212^{0}C, M. Wt- 314.81, Formula- $C_{15}H_{11}N_4SCl$, IR-(KBrcm^{-1}) :-1618(C=N); 3031 (C-H) Stretching, ^{1}H-NMR:7.10 (S,1H,NH), 3.7 (S,2H,CH_2), 6.81-7.79 (m,2HAr). Elemental analysis: $C_{15}H_{11}N_4SCl$, calculated-C, 180.16;H, 11.09;N,56.03,S, 32.06, Cl, 35.45 . Found: C, 180.70;H, 11.07; N,56.25,S, 32.07, Cl, 35.34.

RESULT AND DISCUSSIONS

(3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-phenyl-1,2-diazetidin-3-imine (1c) was synthesized from 1a and aniline and 1e-1h were synthesized from 1a and p-nitroaniline, o-chloroaniline, m-chloroaniline and p-chloroaniline respectively in 1:1 ratio. The mixture was moistened with 2-3 drops of ethanol and placed in microwave oven and irradiated with microwave irradiation for 3-4 minute at 400 watt. The structure was further confirmed by IR and ^{1}H-NMR spectroscopic methods. The IR spectrum of compound (1e-1h) shows the characteristic band at 1614-1619 cm^{-1} due to the C=N group. There are no absorption in the region of 1630-1700 cm^{-1} indicating the absence of C=O group. The ^{1}H-NMR spectrum of this compound show the presence of CH_2, the appearance of a singlet peak in the region of δ3.70-3.80 and peak in the region of δ 7.89-8.07 for N-H.

Scheme:-



Where,

1e- (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(4-nitrophenyl)-1,2-diazetidin-3-imine

1f- (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(2-chlorophenyl)-1,2-diazetidin-3-imine

1g- (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(3-chlorophenyl)-1,2-diazetidin-3-imine

1h- (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(4-chlorophenyl)-1,2-diazetidin-3-imine

Table 1: Physical data of the compounds.

S. No.	compounds	R	M.P.(^{0}C)	Yield (%)	M. Wt.	Formula
1	1a	-	165-168	84	205.25	$C_9H_7N_3SO$
2	1c	-	153.155	80	280.36	$C_{15}H_{12}N_4S$
3	1e	p-NO_2	102-105	70	325.24	$C_{15}H_{11}N_5SO_2$
4	1f	o-Cl	167-170	75	314.81	$C_{15}H_{11}N_4SCl$
5	1g	m-Cl	160-162	78	314.81	$C_{15}H_{11}N_4SCl$
6	1h	p-Cl	210-212	82	314.81	$C_{15}H_{11}N_4SCl$

ANTIMICROBIAL ACTIVITY STUDY

Methods for the determination of antimicrobial activity

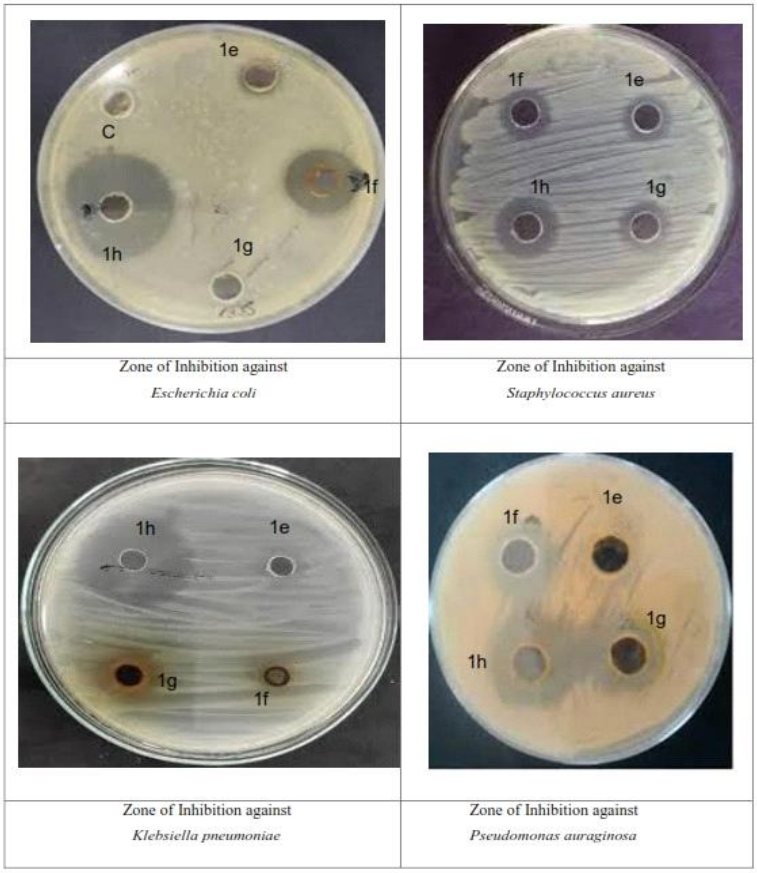
The agar well diffusion method was used to test the compounds antibacterial activity. Tetracycline was employed as the reference and the antibacterial activity was tested at 100 µg/mL in dimethyl sulfoxide (DMSO). *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas auraginosa* 24 hour cultures were used to evaluate the antibacterial activity. The Mueller Hinton agar culture media were used. By placing the sterile agar into Petri dishes in a septic state, the medium was autoclaved at 121^{o}C for 15-24 minutes. The plates were kept at room temperature so that the medium could solidify. Agar plates were loaded with 0.1 mL of each standardized test organism culture. The technique of well diffusion was applied to the antibacterial evaluation. The zones of inhibition of compounds were recorded after incubation for 24 hr at 37^{0}C.The lowest concentration of the test compounds inhibiting visible growth was taken as the inhibition value. It was confirmed that the solvent had no antimicrobial activity against any of the test organism .The zone of inhibition was measured in mm. The results are given in table 2.

Table 2:- Antibacterial activity of some benzothiazolyl substituted derivatives.

Compounds	Zone of inhibition (diameter) in mm			
	Bacteria			
	K. pneumoniae	*E. coli*	*S. aureus*	*P. auraginosa*
1e	N	10	10	N
1f	10	16	11	13
1g	12	08	10	16
1h	24	22	19	18
Tetracycline	28	26	30	20

*N-No inhibition observed
Concentration100 ug/mL;
Control: Dimethyl sulfoxide (DMSO)

Images



CONCLUSION

In the current study, a series of 1e-1h were synthesized and evaluated their potent antibacterial activity. We have developed convenient and eco friendly method for the synthesis of benzothiazolyl substituted compounds by green chemistry technique i.e. microwave synthesis. The excellent yield, easy work-up and simple reaction procedure is highlighted in the present work. Among the tested compounds 1e-1h by well diffusion technique the result we get after the triplet as average zone of inhibition in table. The compound (3*E*)-1-(1,3-benzothiazol-2-yl)-*N*-(4-chlorophenyl)-1,2-diazetidin-3-imine (1h) showed potent activity against all four bacteria such as *K. pneumoniae, E. coli S. aureus* and *P. auraginosa while* 1f, 1g showed moderate activity against *K. pneumonia and E. coli* and le showed resistance against *K. pneumonia* and *P. auraginosa* also very least activity against *E. coli and S. aureus.* It can be concluded that 1h as a template for further development through modification or derivatization to design more potent prodrug for biologically activity in future. The agar well diffusion method is a reliable and straightforward technique for assessing the antimicrobial activity of standard components. By creating wells in agar plates inoculated with test microorganisms and measuring the zones of inhibition, researchers can evaluate the efficacy of various antimicrobial agents, including our novel compounds.

DECLARATION OF INTEREST

The authors state no conflicts of interest.

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