

SYNTHESIS OF NOVEL METHOXY SUBSTITUTED BENZOTHAZOLE DERIVATIVES AND ANTIBACTERIAL ACTIVITY AGAINST *ESCHERICHIA COLI*

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ABSTRACT

Objectives: *Escherichia coli* is a Gram-negative rod (bacillus) in the family Enterobacteriaceae. In general, it is harmless, but some special species could cause harmful infection. In the recent era, the number of antibiotics is available to combat infection caused by *E. coli* but because of resistance developed against available antibiotics research is continuously going on to synthesize newer antibiotic to overcome this problem. Synthesis and screening of benzothiazole derivatives have great importance in heterocyclic chemistry because of its potent and significant biological activities against *E. coli* especially methoxy substitution at benzothiazole.

Methods: Methoxy substituted benzothiazole derivatives were synthesized by reaction of 3-chloro-4-methoxy-aniline with potassium thiocyanate under temperature control and presence of bromine in glacial acetic acid and ammonia. Substituted nitrobenzamides then synthesized by condensation of 2-amino-4-chloro-5-methoxy-benzothiazole with 2 (3 or 4)-nitrobenzoyl chloride acid in the presence of dry pyridine and acetone. Finally, newly synthesized derivatives (K-01 to K-09) were synthesized through replacing of chlorine of nitrobenzamide by reaction with 2-nitroaniline, 3-nitroaniline, and 4-nitroaniline in the presence of dimethoxy formamide. Analytical characterization was performed by thin-layer chromatography, melting point, infrared, and nuclear magnetic resonance. Antibacterial activity was performed against *E. coli* by cup plate method (diffusion technique) using streptomycin as standard. Compound K-03 showed potent antibacterial activity against *E. coli* at both concentrations 50 µg/mL and 100 µg/mL as compared to standard.

Results: Compound D-03 exhibited excellent activity among all synthesized compounds.

Conclusion: In the present work, efforts have been made to synthesized methoxy substituted benzothiazole derivatives and screened for antibacterial activity. Compound K-03 found as most active against *E. coli*.

Keywords: Methoxy-benzothiazole, Benzothiazole, Antibacterial activity, 2-Substituted benzothiazole, Cyclization of benzothiazole, *Escherichia coli*.

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INTRODUCTION

Escherichia coli is a Gram-negative rod (bacillus) in the family Enterobacteriaceae. Most *E. coli* is normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins [1-3]. Pathogenic *E. coli* can be classified into pathotypes by their virulence factors, together with the type of disease. The six pathotypes capable of producing gastrointestinal disease in humans are enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, enteroinvasive *E. coli*, diffusely adherent *E. coli*, and enterohemorrhagic *E. coli* [4-7]. Most strains of these bacteria are harmless but a specific strain of *E. coli* that causes illness. It was first recognized as a cause of illness during an outbreak of hemorrhagic colitis (severe bloody diarrhea) in 1982 since anyone can become infected with *E. coli*. The elderly and children under 5 years of age are at greatest risk of developing a serious illness [8]. In the recent era, the number of antibiotics is available to combat infection caused by *E. coli*, but because of resistance developed against available antibiotics research is continuously going on to synthesize newer antibiotic to overcome this problem. Benzothiazole is a therapeutically important privileged bicyclic ring system contains sulfur and nitrogen as a heteroatom. Synthesis and screening of benzothiazole derivatives have great importance in heterocyclic chemistry because of its potent and significant biological activities. Substitution at C-2 of benzothiazole nucleus has emerged in its usage as a core structure in the diversified therapeutically applications [9-13]. As per reported biological activities of benzothiazole derivatives, it was found that change of the structure of substituent group at benzothiazole nucleus

commonly results in the change of its bioactivities. Commonly change of substitution at C-2 benzothiazole nucleus especially with aryl-nitro has already been proven its therapeutic importance. Till date, various biological activities for benzothiazole derivatives have been reported as antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antibacterial and antifungal, a topical carbonic anhydrase inhibitor, and an antihypoxic [14-17]. 2-substituted benzothiazole derivatives were first discovered in 1887 by A. W. Hofmann as simple cyclization mechanism and number of the synthetic scheme has been reported. The most common and classical method were reported as direct method that involved condensation of an ortho-aminothiophenol with a substituted aromatic aldehyde, carboxylic acid, acyl chloride or nitrile to synthesize C-2 substituted benzothiazoles, but it was found that this method is not appropriate for majority of substituted C-2 aryl benzothiazoles because the main difficulty encountered in synthesis of the readily oxidisable 2-aminothiophenols bearing substituent groups. For the above-said reason, some other methods were reported and extensively used in the laboratories that based on the use of the potassium ferricyanide radical cyclization of thiobenzanilides [18]. This method was named as Jacobsen cyclization and popularized because it produced only one product. As per reported method, it involved cyclization onto either carbon atom ortho to the anilido nitrogen. Due to selective product synthesis, the Jacobsen cyclization was considered as a highly effective strategy for benzothiazole synthesis, for example, for the synthesis of substituted benzothiazoles, radical cyclization of the 3-fluoro- or 3,4-difluoro-substituted thiobenzanilides [19-28]. The present work concern with the synthesis of methoxy and aryl-nitro substituted benzothiazole

derivatives followed by the antibacterial activity for a structure-activity relationship.

METHODS

Synthesis of substituted benzothiazole (Compound Code 1-KB)

Synthesis of substituted benzothiazole nucleus was achieved by adding 8 g (0.08 mol) of potassium thiocyanate, and 1.45 g (0.01 mol) of 3-chloro-4-methoxy-aniline into 20 mL cooled glacial acetic acid in such a way that the temperature not exceeded above room temperature. Freezing mixture of ice and salt was used to control the temperature of reaction with continuous mechanical stirring. Again temperature control was maintained during the addition of a solution of 1.6 mL of bromine in 6 mL of glacial acetic acid using dropping funnel. The time of addition of bromine also considered to take around 105 min to control temperature. During the addition of bromine, the temperature was controlled to never rise beyond the room. As the addition of bromine was completed the solution stirred for 2 h but below room temperature. After that solution was again stirred at room temperature for 10 h and allowed to stand overnight to get precipitate followed by heating at 85°C on a steam bath after addition of 6 mL water and filtered hot (Filtrate-01). In the resulting precipitate, 10 mL of glacial acetic acid was added and heated with at 85°C and filtered hot (Filtrate-02). Finally, both filtrate combined and cooled at room temperature followed by neutralization with concentrated ammonia solution to pH-6 to get precipitate. The resulting product treated with animal charcoal and recrystallized from benzene, ethanol of 1:1 to get substituted benzothiazole.

Synthesis of nitrobenzamide (Compound code 2-KB, 3-KB, and 4-KB)

5.36 g (0.026 mol) of 2-(3 or 4)-nitrobenzoyl chloride was dissolved in dry acetone. Product 1-KB separately dissolved in dry pyridine and added dropwise into the solution of 2-(3 or 4)-nitrobenzoyl chloride with continuous stirring at room temperature. After complete addition stirring was continued for another 30 min then transferred into 200 mL ice cold water. Finally, recrystallized with ethanol to get intermediate nitrobenzamide compound 2-KB, 3-KB, and 4-KB.

Synthesis of compound K-01 to K-09

0.008 mol of 2 (3 or 4) nitro-substituted aniline was refluxed with 2.7 g (0.0075 mol) of compound 2-KB, 3-KB, and 4-KB separately for 2 h in the presence of dimethoxy formamide (DMF). After 2 h reflux, the mixture cooled at room temperature and poured into crushed ice. The

solid was separated, dried, and recrystallized with super dry alcohol to get novel benzothiazole derivatives K-01 to K-09 (Fig. 1).

Analytical characterization

Thin layer chromatography (TLC) was used to monitor reaction progress, completion and identification of newly synthesized compounds from starting material using solvent system butanol: ethyl acetate: benzene [1:2:1] and detection performed by exposing them to iodine vapors. The melting point of compounds was determined using the open capillaries method. Structure elucidation of compounds was done by IR and ¹HNMR spectral study. SHIMADZU (8400S) used for IR spectral study (KBr pellet technique). For the structure elucidation using IR, frequency range for Ar-C=C, C=O, C-S, and C-NO₂ was considered. Bruker AM 400 ¹HNMR instrument (at 400 MHz) was used using CDCl₃ as a solvent and tetramethoxysilane as an internal standard. For structure elucidation by ¹HNMR, NH proton that characterized benzothiazole was considered.

Antibacterial activity against *E. coli*

The standard drug and synthesized compounds were dissolved in minimum quantity of DMF and adjusted and made up the volume with distilled water to get 50 µg/mL and 100 µg/mL concentrations. The antibacterial activity was performed by the cup plate method (diffusion technique). The fresh culture of bacteria was obtained by inoculating bacteria into peptone water liquid media and incubated at 37±2°C for 18–24 h. This culture mixed with nutrient agar media (20%) and poured into Petri dishes by following aseptic techniques. After solidification of the media, five bores were made at an equal distance using sterile steel cork borer (8 mm diameter). Into these cups, different concentrations of standard drug and synthesized compounds were introduced. DMF was used as a control. After the introduction of standard drug and synthesized compounds, the plates were placed in a refrigerator at 8.0–10°C for proper diffusion of drugs into the media. After 2 h of cold incubation, the Petri plates are transferred to the incubator and maintained at 37±2°C for 18–24 h. After the incubation period, the Petri plates were observed for the zone of inhibition using a Vernier scale. The results evaluated by comparing the zone of inhibition shown by the synthesized compounds with the standard drug. The results are the mean value of the zone of inhibition measured in millimeter of two sets.

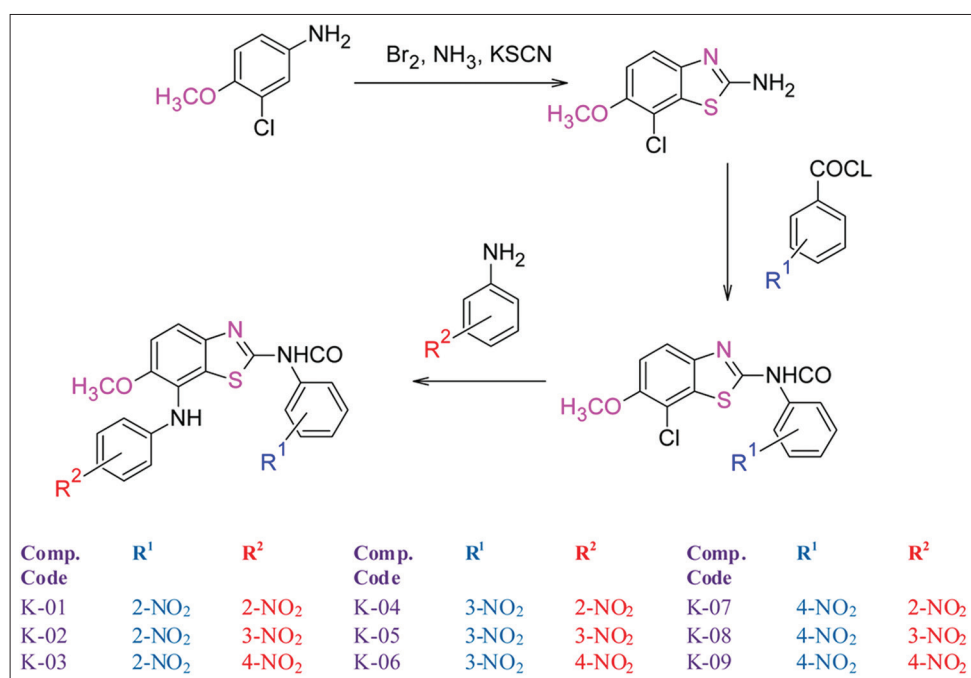


Fig. 1: Synthetic scheme

Table 1: Analytical characterization of synthesized compounds

Comp. Code	%Yield	Mel. point (°C)	TLC (Rf)	IR spectral study	¹ HNMR spectral study (400 Hz, DMSO-d ₆)
D-01	71	261	0.41	1456 cm ⁻¹ Ar C=C, 1632 cm ⁻¹ C=O, 1245 cm ⁻¹ C-S, 1544 cm ⁻¹ C-NO ₂	δ 4.61, (s, 1H, NH), δ 3.31 (s, 3H, CH ₃), δ 7.10-7.72 (m, 10H, Ar-H), δ 8.89 (s, 1H, CONH)
D-02	72	260	0.43	1454 cm ⁻¹ Ar C=C, 1640 cm ⁻¹ C=O, 1257 cm ⁻¹ C-S, 1575 cm ⁻¹ C-NO ₂	δ 4.52, (s, 1H, NH), δ 3.39 (s, 3H, CH ₃), δ 7.18-7.85 (m, 10H, Ar-H), δ 9.10 (s, 1H, CONH)
D-03	68	266	0.46	1454 cm ⁻¹ Ar C=C, 1652 cm ⁻¹ C=O, 1241 cm ⁻¹ C-S, 1523 cm ⁻¹ C-NO ₂	δ 4.55, (s, 1H, NH), δ 3.30 (s, 3H, CH ₃), δ 7.19-7.62 (m, 10H, Ar-H), δ 8.80 (s, 1H, CONH)
D-04	68	275	0.50	1421 cm ⁻¹ Ar C=C, 1665 cm ⁻¹ C=O, 1243 cm ⁻¹ C-S, 1537 cm ⁻¹ C-NO ₂	δ 4.62, (s, 1H, NH), δ 3.42 (s, 3H, CH ₃), δ 7.22-7.60 (m, 10H, Ar-H), δ 8.95 (s, 1H, CONH)
D-05	69	256	0.48	1443 cm ⁻¹ Ar C=C, 1626 cm ⁻¹ C=O, 1222 cm ⁻¹ C-S, 1543 cm ⁻¹ C-NO ₂	δ 4.56, (s, 1H, NH), δ 3.44 (s, 3H, CH ₃), δ 7.21-7.80 (m, 10H, Ar-H), δ 8.96 (s, 1H, CONH)
D-06	79	271	0.42	1421 cm ⁻¹ Ar C=C, 1615 cm ⁻¹ C=O, 1212 cm ⁻¹ C-S, 1554 cm ⁻¹ C-NO ₂	δ 4.65, (s, 1H, NH), δ 3.41 (s, 3H, CH ₃), δ 7.09-7.66 (m, 10H, Ar-H), δ 9.15 (s, 1H, CONH)
D-7	65	269	0.52	1423 cm ⁻¹ Ar C=C, 1626 cm ⁻¹ C=O, 1220 cm ⁻¹ C-S, 1540 cm ⁻¹ C-NO ₂	δ 4.60, (s, 1H, NH), δ 3.30 (s, 3H, CH ₃), δ 7.18-7.60 (m, 10H, Ar-H), δ 8.80 (s, 1H, CONH)
D-08	67	264	0.40	1421 cm ⁻¹ Ar C=C, 1615 cm ⁻¹ C=O, 1212 cm ⁻¹ C-S, 1554 cm ⁻¹ C-NO ₂	δ 4.65, (s, 1H, NH), δ 3.40 (s, 3H, CH ₃), δ 7.10-7.68 (m, 10H, Ar-H), δ 8.83 (s, 1H, CONH)
D-09	60	269	0.56	1458 cm ⁻¹ Ar C=C, 1664 cm ⁻¹ C=O, 1244 cm ⁻¹ C-S, 1552 cm ⁻¹ C-NO ₂	δ 4.66, (s, 1H, NH), δ 3.44 (s, 3H, CH ₃), δ 7.20-7.60 (m, 10H, Ar-H), δ 8.85 (s, 1H, CONH)

RESULTS AND DISCUSSION

Benzothiazole contains sulfur and nitrogen as heteroatom but imparts biological activity while substitution at the C-2 position. In the present work, methoxy substituted benzothiazole nucleus while 2-(3 or 4)-aryl nitro considered as rotating substitution at C-2 and C-4 position of benzothiazole nucleus derivatives were synthesized. The novel derivatives (K-01 to K-09) evaluated for antibacterial activity against *E. coli*. In the present work, nitro group considers as rotating basis on ortho, meta, and para position. The reason behind considering the nitro group as a substituent is the fungi rarely acquire resistance. TLC, melting point, IR, and ¹HNMR were used for analytical characterization. In the TLC, the distance traveled by compound K-01 to K-09 was found to be different from that of the starting compound that proved synthesized compounds were different from parent one, even during TLC performance every time single spot was obtained; hence, it also reveals that synthesized compounds were free from impurity as well as reaction was completed. Structure elucidation by IR spectroscopy frequency range for Ar C=C, C=O, C-S, and C-NO₂ was considered. In case of structure elucidation of by ¹HNMR sharp characteristic signal at 7.0-8.0 ppm is observed and consider as benzothiazole in all the synthesized compounds (Table 1). Antibacterial activity performed at two concentration 50 µg/mL and 100 µg/mL using streptomycin as a standard drug against *E. coli*. Compound K-03 showed potent antibacterial activity against *E. coli* at both concentrations 50 µg/mL and 100 µg/mL as compared to standard (Table 2 and Fig. 2). The structure-activity relationship of newly synthesized compound revealed that 2-aryl nitro at C-2 and 4-aryl nitro at the C-4 position of methoxy-substituted benzothiazole derivative (Compound K-03) exhibited prominent inhibitory activity against *E. coli*.

CONCLUSION

In the present work, methoxy substituted novel benzothiazole derivatives were synthesized and screened for antibacterial activity against *E. coli*. The paucity of data showed that compound K-03 showed potent activity and could be considered for further clinical trials as antibacterial agents.

CONFLICTS OF INTEREST

The author has no conflicts of interest.

AUTHOR'S CONTRIBUTION

AS a single author, I carried out the complete experiment, data interpretation as well as manuscript writing for publication. No other coauthor contributed in this work.

Table 2: Result of antibacterial activity

Compound code	<i>E. coli</i>	
	50 µg/mL	100 µg/mL
Streptomycin	20	26
K-1	07	12
K-2	07	14
K-3	20	24
K-4	10	15
K-5	12	14
K-6	09	13
K-7	10	16
K-8	10	14
K-9	08	13

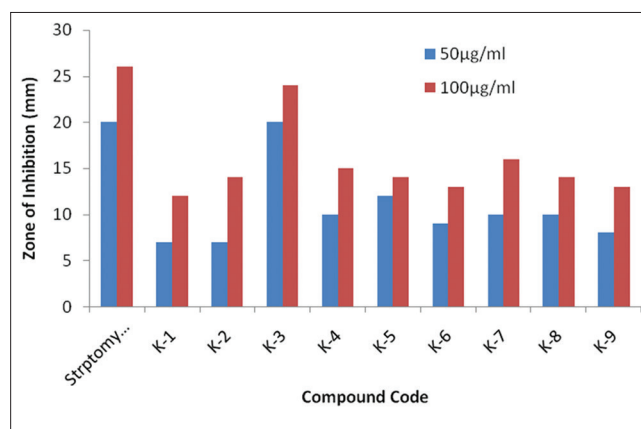


Fig. 2: Result of the zone of inhibition of synthesized compounds

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