Benzothiophene and Its Derivatives as Promising Antimicrobial Agents: Characteristics synthesis and properties

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ABSTRACT

The increasing prevalence of antimicrobial resistance has created an urgent need for the development of novel antimicrobial agents with improved therapeutic efficacy. Benzothiophene, a biologically important sulfur-containing heterocyclic scaffold, has attracted considerable attention due to its diverse pharmacological activities and excellent synthetic versatility. In the present study, a series of novel benzothiophene derivatives (H1, H2, H4, and H5) were designed, synthesized, and evaluated for their antibacterial potential. The synthesized compounds were prepared through a multi-step synthetic approach involving the formation of a benzothiophene intermediate followed by functionalization with different substituted aniline derivatives. Structural characterization was carried out using FT-IR, LC-MS, and ^{1}H NMR spectroscopy, which confirmed the successful synthesis of the target compounds. The antibacterial activity was evaluated against Staphylococcus aureus (ATCC 12598) and Escherichia coli (ATCC 25922) using the broth microdilution method for determination of the minimum inhibitory concentration (MIC). Among the synthesized derivatives, compound H4 exhibited the most potent antibacterial activity, with MIC values of 4 µg/mL against S. aureus and 8 µg/mL against E. coli, whereas H2 showed moderate activity and H1 and H5 demonstrated comparatively lower antibacterial potency. These findings indicate that structural modification of the benzothiophene scaffold significantly influences antibacterial activity and highlights benzothiophene derivatives as promising candidates for the development of novel antimicrobial agents.

Keywords: Benzothiophene derivatives; Antimicrobial activity; Minimum inhibitory concentration (MIC); Heterocyclic compounds; Medicinal chemistry; Broth microdilution method; Staphylococcus aureus; Escherichia coli; FT-IR; LC-MS; ^{1}H NMR spectroscopy.

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1 INTRODUCTION

1.1 Antimicrobial Resistance: A Global Health Challenge

Antimicrobial resistance (AMR) has emerged as one of the most serious threats to global public health in the 21st century.The alarming increase in resistant bacterial pathogens has also highlighted the urgent need for the discovery and development of new antimicrobial agents with improved efficacy and novel mechanisms of action. Heterocyclic compounds, particularly benzothiophene derivatives, have attracted considerable attention due to their diverse pharmacological properties, including potent antimicrobial activity. Therefore, the design and synthesis of new benzothiophene-based molecules .(1)

1.2 Importance of Heterocyclic Compounds in Drug Discovery

Heterocclic compounds are cyclic organic molecules containing one or more heteroatoms, such as nitrogen, oxygen, or sulfur, and play a vital role in medicinal chemistry. More than 85% of biologically active compounds possess heterocyclic scaffolds, highlighting their importance in drug discovery. Among them, sulfur-containing heterocycles exhibit diverse pharmacological activities, including antibacterial, antiviral, anticancer, antifungal, and anti-inflammatory effects. Owing to their broad therapeutic potential, sulfur heterocycles are key structural components of several clinically approved drugs and remain an important focus of pharmaceutical research.(2)

1.3 Benzothiophene as a Privileged Scaffold in Medicinal Chemistry

Benzothiophene is a well-established privileged scaffold in medicinal chemistry due to its ability to interact with multiple biological targets through structural modification. It has been widely explored for the development of antimicrobial, anticancer, anti-inflammatory, antiviral, antioxidant, antidiabetic, and antitubercular agents(3,4). Its aromatic stability, favorable receptor-binding properties, and ease of synthetic functionalization make benzothiophene an attractive scaffold for drug design. [4,5]

1.4 Chemistry and Structural Features of Benzothiophene

Benzothiophene is a fused bicyclic aromatic heterocycle composed of a benzene ring and a thiophene ring, with the sulfur atom contributing significantly to its electronic and biological properties. Its rigid planar structure promotes favorable interactions with biological targets, making it an important pharmacophore in drug discovery(3,7) Furthermore, the scaffold offers excellent synthetic flexibility, allowing structural modifications that produce diverse derivatives with enhanced pharmacological activities.(8)

1.5 Pharmacological Importance of Benzothiophene Derivatives

Benzothiophene derivatives exhibit a broad spectrum of pharmacological activities, including antimicrobial, anticancer, anti-inflammatory, antioxidant, antiviral, antifungal, and antitubercular effects. Such therapeutic diversity highlights the significance of this scaffold as a versatile platform for drug discovery [2].

1.6 Synthetic importance of benzodiazepine derivatives

Benzothiophene is a versatile sulfur-containing heterocyclic scaffold that allows extensive structural modification for the synthesis of biologically active derivatives. Its synthetic flexibility has enabled the development of compounds with antimicrobial, anticancer, anti-inflammatory, and antidiabetic activities. Furthermore, benzothiophene serves as an important scaffold for structure–activity relationship (SAR) studies and lead optimization in medicinal chemistry.nts.(9)

1.7 Benzothiophene Derivatives as Antimicrobial Agents

Benzothiophene derivatives have demonstrated broad-spectrum antimicrobial activity against Gram-positive, Gram-negative, and fungal pathogens. Structural modification of the benzothiophene scaffold significantly enhances antimicrobial potency and selectivity by improving interactions with microbial targets. Therefore, benzothiophene has emerged as a promising pharmacophore for the development of novel antimicrobial agents to combat antimicrobial resistance.(10,11)

2. Materials and methadology

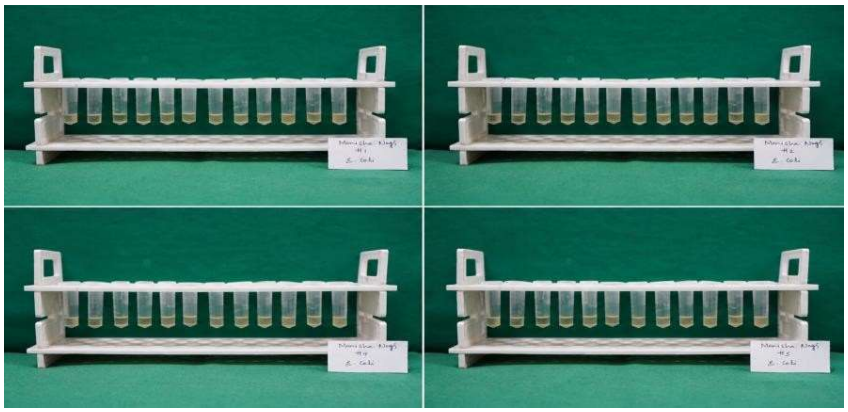
All chemicals, reagents, and solvents used in the present study were of analytical reagent (AR) grade and were used without further purification. The starting materials, including 2,4-difluorobenzaldehyde, sodium thioglycolate, and substituted aniline derivatives (2,4-dichloroaniline, N,N-diethylaniline, 2,4-dinitroaniline, and p-bromoaniline), were procured from commercial suppliers, including Central Drug House (CDH), Delhi. Organic solvents such as DMSO, ethanol, methanol, ethyl acetate, acetone, and chloroform were employed for synthesis, purification, and chromatographic analysis, while distilled water was used throughout the experimental work. The progress of reactions was monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates, and melting points were determined using a digital melting point apparatus.

Procedures:

Minimum Inhibitory Concentration (MIC) Test .The minimum inhibitory concentration (MIC) of the test drug was determined using the broth microdilution method.

1. Initially, 20 µL of the drug solution (10 mg/mL) was added to 380 µL of Brain Heart Infusion (BHI) broth to obtain a starting concentration of 0.5 mg/mL.
2. For serial dilution, 200 µL of BHI broth was dispensed into each of the subsequent nine tubes.
3. From the initial tube, 200 µL was transferred to the second tube and mixed to achieve a two-fold (1:2) dilution. This process was repeated sequentially to obtain a series of two-fold dilutions across ten tubes. During each transfer, 200 µL was carried forward, maintaining a constant volume of 200 µL in each tube.

4. Following dilution, 200 µL of the prepared microbial inoculum was added to each tube, resulting in a final volume of 400 µL per tube.
5. The addition of inoculum further diluted the drug concentration by half. Consequently, the final drug concentrations ranged from 250 µg/mL in the initial tube to 0.5 µg/mL in the last tube.
6. The tubes were incubated for 24 hours at 37°C in the bacteriological incubator and observed for turbidity.
7. MIC was determined as the lowest concentration of the drug that inhibited visible microbial growth.(15)



Representative photographs of the broth microdilution assay used for MIC determination of the synthesized benzothiophene derivatives against Escherichia coli.(16)

Mic Assay

The antibacterial activity of the synthesized compounds was evaluated against Staphylococcus aureus ATCC 12598 and Escherichia coli ATCC 25922 using the broth microdilution method. Serial two-fold dilutions of the test compounds were prepared in Brain Heart Infusion (BHI) broth, inoculated with standardized bacterial suspensions, and incubated at 37°C for 24 h. The MIC was recorded as the lowest concentration that completely inhibited visible bacterial growth, with growth and broth controls included to validate the assay.

2.1 Preparation of Benzothiophene Derivatives

Step I: Synthesis of **Methyl 6-Fluorobenzothiophene-2-carboxylate**

2,4-Difluorobenzaldehyde (1.0 g, 7.0 mmol) and methyl thioglycolate (0.75 g, 7.0 mmol) were dissolved in dry DMF (15 mL), followed by the addition of potassium carbonate (1.45 g, 10.5 mmol). The reaction mixture was stirred at 80–90°C for 6–8 h, and the progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was cooled to room temperature and poured into ice-cold water. The precipitated solid was filtered, washed thoroughly with water, dried, and recrystallized from ethanol to obtain methyl 6-fluorobenzothiophene-2-carboxylate as the intermediate.

Step II: Synthesis of **6-Fluorobenzothiophene-2-carbonyl Chloride**

The obtained intermediate was treated with thionyl chloride ($SOCl_2$, 3–5 mL) and refluxed for 2–3 h under anhydrous conditions. Excess thionyl chloride was removed under reduced pressure to afford the corresponding acid chloride intermediate, which was used immediately in the next step without further purification.

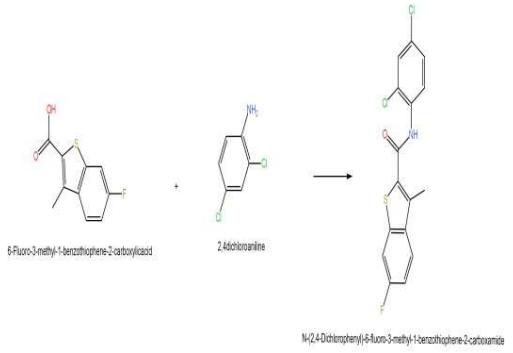
Step III: Synthesis of **Final Benzothiophene Derivatives (H1–H5)**

The acid chloride intermediate (1.0 mmol) was dissolved in dry dichloromethane (10–15 mL), and the respective substituted aniline (1.1 mmol), namely 2,4-dichloroaniline (H1), N,N-diethylaniline (H2), 2,4-dinitroaniline (H4), or p-bromoaniline (H5), was added slowly with continuous stirring. Triethylamine (1.2 mmol) was added to

neutralize the hydrochloric acid generated during the reaction. The reaction mixture was stirred at room temperature to reflux for 4–6 h, and completion of the reaction was monitored by TLC. The reaction mixture was then poured into ice-cold water, and the resulting solid was collected by filtration, washed with water, dried, and recrystallized from ethanol to afford the corresponding benzothiophene derivatives (H1–H5) in good yield. The synthesized compounds were characterized by melting point determination, TLC, FT-IR, LC-MS, and ^1H NMR spectroscopy.

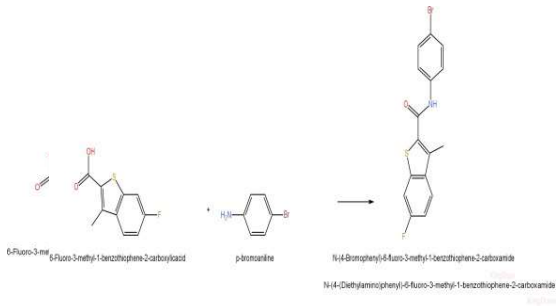
H1.

H2



H4.

H5



2.2 Characterization of Synthesized Compounds

H1

The spectrum showed aromatic proton signals (δ 6.58–7.03 ppm), a methine proton at δ 5.19 ppm, and a methoxy/oxygenated methylene signal at δ 3.57 ppm, confirming the proposed structure and satisfactory purity.(12).Characteristic absorption bands corresponding to N–H stretching (3471–3381 cm^{-1}), aromatic C=C vibrations, C–N/C–F stretching (1115 cm^{-1}), and C–S stretching confirmed the successful synthesis of compound H1.(13).The LC-MS spectrum exhibited a protonated molecular ion peak at m/z 283.17 ($[M+H]^+$) along with characteristic fragment ions, confirming the molecular identity of H1.(14)

H2

The spectrum displayed aromatic proton signals (δ 6.83–7.26 ppm), a methine proton at δ 5.48 ppm, and a methoxy/oxygenated methylene signal at δ 3.70 ppm, confirming successful synthesis.(12).The FTIR spectrum showed characteristic N–H, aromatic C=C, C–N/C–F, and C–S stretching vibrations, supporting the formation of the desired benzothiophene derivative.(13).A molecular ion peak at m/z 317.13 ($[M+H]^+$) with expected fragment ions confirmed the molecular structure of compound H2.(14)

H4

The spectrum exhibited deshielded aromatic proton signals (δ 7.11–8.77 ppm), consistent with the presence of electron-withdrawing nitro substituents and confirming the proposed structure.(12).Characteristic N–H, aromatic C=C, C–N/C–F, and C–S stretching bands confirmed the successful synthesis of compound H4.(13).The LC-MS spectrum showed a protonated molecular ion peak at m/z 339.18 ($[M+H]^+$) together with characteristic fragment ions, confirming the molecular identity of H4.(14)

H5

The spectrum showed aromatic proton signals (δ 6.54–7.15 ppm), a methine proton at δ 5.18 ppm, and a methoxy/oxygenated methylene signal at δ 3.74 ppm, consistent with the proposed structure.(12).The FTIR spectrum exhibited characteristic N–H, aromatic C=C, C–N/C–F, and C–S stretching vibrations, confirming successful synthesis of H5.(13).The molecular ion peak at m/z 327.12 ($[M+H]^+$) along with the expected fragmentation pattern confirmed the molecular identity of compound H5.(14)

2.3 Physical characteristics of Benzodiazepine derivatives

H1

Compound Name: N-(2,4-Dichlorophenyl)-6-fluoro-3-methyl-1-benzothiophene-2-carboxamide (17,18)

Property	**Value**
Molecular Formula	$C_{16}H_{10}Cl_2FNOS$
Molecular Weight	370.24 g/mol
Appearance	Pale yellow crystalline powder
Melting Point	184–188 °C
Solubility	Soluble in DMSO and DMF; practically insoluble in water
Rf Value (Hexane:Ethyl acetate, 7:3)	0.55–0.65

H2

Compound Name: N-(4-(Diethylamino)phenyl)-6-fluoro-3-methyl-1-benzothiophene-2-carboxamide(19,20)

Property	**Value**
Molecular Formula	$C_{21}H_{21}FN_2OS$
Molecular Weight	368.47 g/mol
Appearance	Yellow crystalline powder
Melting Point	172–178 °C
Solubility	Soluble in DMSO and DMF; practically insoluble in water
Rf Value (Hexane:Ethyl acetate, 7:3)	0.50–0.60

H4

Compound Name: N-(2,4-Dinitrophenyl)-6-fluoro-3-methyl-1-benzothiophene-2-carboxamide(21,22)

Property	**Value**
Molecular Formula	$C_{16}H_{10}FN_3O_5S$
Molecular Weight	375.33 g/mol
Appearance	Orange-yellow crystalline powder
Melting Point	221–228 °C
Solubility	Soluble in DMSO and DMF; practically insoluble in water

Property	Value
Rf Value (Hexane:Ethyl acetate, 7:3)	0.35–0.45

H5

Compound Name: N-(4-Bromophenyl)-6-fluoro-3-methyl-1-benzothiophene-2-carboxamide(23,24)

Property	Value
Molecular Formula	$C_{16}H_{11}BrFN$OS
Molecular Weight	364.23 g/mol
Appearance	Pale yellow crystalline powder
Melting Point	198–204 °C
Solubility	Soluble in DMSO and DMF; practically insoluble in water

Rf Value (Hexane:Ethyl acetate, 7:3) 0.35–0.45

2.4 Antimicrobial Activity

The synthesized benzothiophene derivatives (H1, H2, H4, and H5) were evaluated for antibacterial activity against Staphylococcus aureus (ATCC 12598) and Escherichia coli (ATCC 25922) using the broth microdilution method. The MIC was determined as the lowest concentration that completely inhibited visible bacterial growth after incubation at 37°C for 24 h. The MIC values are presented in Table X.

Table X. Minimum Inhibitory Concentration (MIC) of Synthesized Compounds

Compound	S. aureus (µg/mL)	E. coli (µg/mL)
H1	62.5	62.5
H2	31.25	62.5
H4	4	8
H5	62.5	62.5

Among the synthesized derivatives, H4 exhibited the highest antibacterial activity, with MIC values of 4 µg/mL against S. aureus and 8 µg/mL against E. coli. H2 showed moderate activity against S. aureus (31.25 µg/mL), whereas H1 and H5 displayed moderate activity against both bacterial strains (62.5 µg/mL).(15)

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