Infection and Drug Resistance

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ORIGINAL RESEARCH

Facile Synthesis of 5-Bromo-*N*-Alkylthiophene-2-Sulfonamides and Its Activities Against Clinically Isolated New Delhi Metallo-β-Lactamase Producing *Klebsiella pneumoniae* ST147

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Introduction: New Delhi Metallo-*β*-lactamase producing *Klebsiella pneumoniae* (NDM-1-KP) sequence type (ST) 147 poses a significant threat in clinical settings due to its evolution into two distinct directions: hypervirulence and carbapenem resistance. Hypervirulence results from a range of virulence factors, while carbapenem resistance stems from complex biological mechanisms. The NDM-1-KP ST147 clone has emerged as a recent addition to the family of successful clones within the species.

Methods: In this study, we successfully synthesized 5-bromo-*N*-alkylthiophene-2-sulfonamides (**3a-c**) by reacting 5-bromothiophene-2-sulfonamide (**1**) with various alkyl bromides (**2**) using LiH. We also synthesized a series of compounds (**4a-g**) from compound (**3b**) using the Suzuki-Miyaura cross-coupling reaction with fair to good yields (56–72%). Further, we screened the synthesized molecules against clinically isolated New Delhi Metallo-*β*-lactamase producing *Klebsiella pneumoniae* ST147. Subsequently, we conducted in-silico tests on compound **3b** against a protein extracted from NDM-KP ST147 with PDB ID: 5N5I.

Results: The compound (**3b**) with favourable drug candidate status, MIC of 0.39 µg/mL, and MBC of 0.78 µg/mL. This low molecular weight compound exhibited the highest potency against the resistant bacterial strains. The in-silico tests revealed that the compound **3b** against a protein extracted from NDM-KP ST147 with PDB ID: 5N5I demonstrated H-bond and hydrophobic interactions.

Conclusion: The 5-bromo-*N*-alkylthiophene-2-sulfonamides displayed antibacterial efficacy against New Delhi Metallo-*β*-lactamase producing *Klebsiella pneumoniae* ST147. After the in-vivo trial, this substance might offer an alternative therapeutic option.

Keywords: NDM-1, antibacterial, drug resistance, *Klebsiella pneumoniae*, ST, sulfonamides

Background

The global public health sectors face a grave threat from the emergence of extensively drug-resistant carbapenem-resistant *Klebsiella pneumoniae* (CR-KP). Various mechanisms such as cell wall modification,[1–4] efflux pumps,[5,6] *β*-lactamase production,[7–11] and ribosome targeting[12–15] can lead to antibiotic resistance in these bacteria. If the spread of antibiotic resistance persists, projections indicate that by 2050, the global death toll could reach one person every three seconds, resulting in an economic loss exceeding $100 trillion. The World Health Organization (WHO) identifies CR-KP as a "critical priority pathogen". These bacteria resist all classes of *β*-lactam antibiotics and other antibiotic categories like aminoglycosides and

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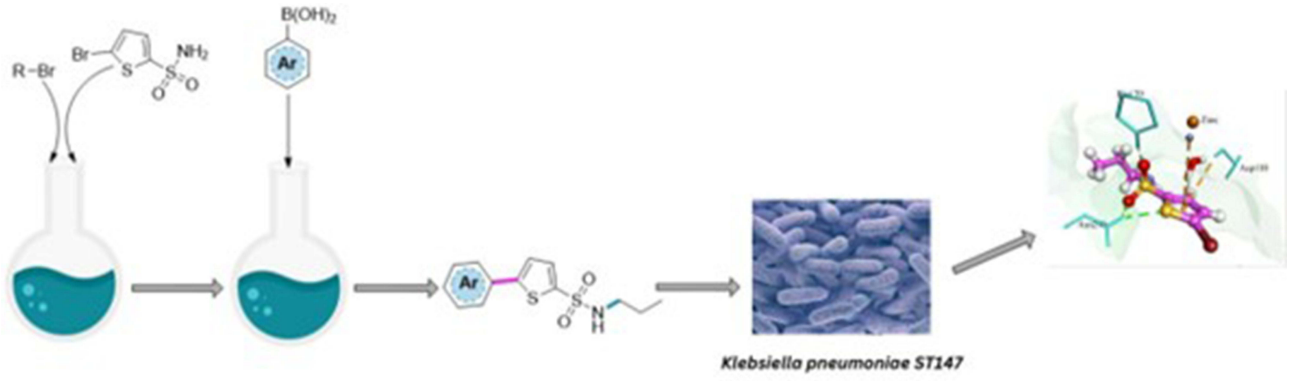
Graphical Abstract



quinolones. Consequently, they can induce severe clinical conditions such as bacteremia and septicemia.[16] Thus, new therapeutic strategies are needed to combat antimicrobial resistance. *K. pneumoniae* can cause urinary tract infections, pneumonia, septicemia, and liver abscesses, in both immunocompromised and immunocompetent individuals.[17] These bacteria are among the top five nosocomial infections worldwide.[18]

Carbapenem-resistant *K. pneumoniae* poses a significant danger in clinical settings due to their global dissemination and extensive antibiotic resistance phenotypes via mobile genetic elements, causing higher death rates than carbapenem-susceptible strains.[19–21] *K. pneumoniae* ST147 consists of clades and carbapenemase clusters, including New Delhi Metallo-β-lactamase (NDM), VIMs, and OXA-48-like. *K. pneumoniae* ST147 is endemic to some North African countries, Italy, India, and Greece, while *K. pneumoniae* ST307 is endemic to the USA, Colombia, Italy, and South Africa. Genomic analyses suggest that *K. pneumoniae* ST147 has identical *par*C and *gyr*A mutations and likely acquired plasmids with $bla_{\text{CTX-M-15}}$ during the early to mid-2000s, contributing to their widespread dissemination worldwide. *K. pneumoniae* ST147 poses widespread dissemination, the ability to trigger fatal infections, and antibiotic resistance, notably pan-resistance.[22,23] New Delhi Metallo-β-lactamase producing *K. pneumoniae* (NDM-KP) was first identified in Swedish patients hospitalized in New Delhi, India. So far, over 30 NDM variants have been reported in different parts of the world. These pathogens produced resistance to a variety of antibiotics, including carbapenems; therefore, new therapeutic options are the need of the time (https://pubmed.ncbi.nlm.nih.gov/33404261/).

Sulfonamides have been a widely used class of antibiotics in clinical use since 1968. They are commonly used to treat upper urinary and respiratory tract infections in primary care medicine, as patients tolerate them well, and they are affordable.[24] Additionally, sulfonamide derivatives are frequently utilized as anti-bacterial and antiviral drugs in chemotherapy.[25–27] Due to their structural similarities, these medications work as competitive antagonists of 4-amino-benzoic acid. Sulfonamides have anti-bacterial properties and work by incorporating 4-amino benzoic acid into the folic acid pathway, inhibiting folate synthase. It prevents bacteria from synthesizing folic acid, reducing the number of purines they can produce.[28–31] Therefore, sulfonamides have a bacteriostatic effect by hindering the formation of folic acid.

Methodology

Chemicals and Instrumentations

General Procedure for the Synthesis of Alkylated Derivatives of 5-Bromothiophene-2-Sulfonamide (3a-c)

To start the reaction, dissolved 5-bromothiophene-2-sulfonamide (0.413 mmol, 1 eq). in 40 mL of DMF solvent and added LiH (0.413 mmol, 1 eq). Then, alkyl bromides (0.413 mmol, 1 eq). were added to the mixture dropwise in the oven-dried flask. After the addition, stir it at room temperature for three hours. The reaction progress was tracked by TLC. Subsequently, the product was precipitated by introducing water into the mixture. After precipitation, the product was washed with water and subjected to recrystallization, employing methanol as the solvent.[32]

General Procedure for the Synthesis of Different Derivatives of 5-Bromo-N-Propyl Thiophene-2-Sulfonamide via SMC Coupling Reaction (4a-g)

In a clean, oven-dried flask 5-bromo-*N*-propylthiophene-2-sulfonamide, Pd $(PPh_3)_4$ catalyst (5 mol%), aryl boronic acids (0.774 mmol), and potassium phosphate (1.409 mmol) were added. To create an inert environment, the flask was evacuated three times and then filled with argon gas. Subsequently, 1,4-dioxane (5 mL) was added under an inert atmosphere, and the mixture was stirred at 90°C. After 30 minutes, 0.5 mL of water was added while maintaining an argon atmosphere. The solution was further stirred at 90°C for 30 hours until the reaction reached completion. After completion, the reaction mixture was cooled to room temperature and added water and ethyl acetate. The organic layer was separated, filtered, and dried using anhydrous Na_2SO_4. The organic layer was then concentrated under reduced pressure. To obtain pure products, the reaction mixture underwent purification through column chromatography using a mixture of n-hexane and ethyl acetate. The obtained products were characterized using various spectroscopic techniques.[33]

Identification of the Bacterial Strain

Briefly, 3 mL of blood sample was drawn from a clinically suspected septicemia child (5 years old) in a tertiary care hospital. The blood was placed into the BACTEC/Alert Pead blood culture vial only after receiving ethical approval from the Ethical Review Board, Government College University Faisalabad as per the declaration of Helsinki and the child's parent provided an informed consent. The BACTEC/Alert automated system (BD, UK) incubated the bottles at 37°C for up to 5 days. The positive sample was sub cultured on blood and MacConkey agar (Oxoid, UK) and plates were incubated at 37°C overnight aerobically. The isolate was identified first based on colony morphology and biochemically confirmed utilizing a Gram-negative card and an automatic VITEK 2 compact system (Biomerieux, France).

Sequence Typing of K. Pneumoniae

To determine the sequence typing of *K. pneumoniae*, seven housekeeping genes were selected, namely glyceraldehyde-3-phosphate dehydrogenase (gapA), the *β*-subunit of RNA polymerase (rpoB), malate dehydrogenase (mdh), phosphorene E (phoE), phosphoglucose isomerase (pgi), periplasmic energy transducer (tonB), and translation initiation factor 2 (infB). Each PCR amplicon was extracted from the agarose gel using the QIAquick Gel Extraction Kit from Qiagen (Hilden, Germany) and sent for commercial sequencing at Eurofins Scientific (London, UK). The obtained sequencing data were then analyzed using the *K. pneumoniae* MLST database available at http://bigsdb.pasteur.fr/klebsiella/klebsiella.html.

Antibiogram of the Isolate

Using the automated VITEK 2® compact system (BioMerieux, France), the MICs of several antibiotics against the pathogen were calculated. The MIC of the antibiotics is expressed in micrograms per milliliter (µg/mL). The antimicrobials tested were piperacillin; ampicillin/sulbactam; cefepime; levofloxacin; ceftriaxone; aztreonam; amikacin; meropenem; minocycline; moxifloxacin; colistin; tetracycline; chloramphenicol. Susceptibility was interpreted the following CLSI 2021 recommendations.[34]

Phenotypic Detection Carbapenemase Enzyme

The *K. pneumoniae* was subjected to the Modified Hodge's test (MHT) to determine carbapenemase activity.[35] Briefly, the isolates were streaked onto a Mueller-Hinton agar (MHA) plate, and a meropenem disc was inserted in the middle of the plate for the MHT. After being incubated for a whole day, a disc of meropenem developed a cloverleaf-shaped depression, proving the presence of carbapenemase enzymes. MBL-producing bacteria were identified using the double-disc synergy method with EDTA. Briefly, the *K. pneumoniae* was spread onto MHA, and two discs containing imipenem and meropenem were placed. 10 µL of a 0.5M EDTA solution was applied to each carbapenem disc. A positive result was indicated if the EDTA discs displayed a zone of inhibition more significant than 5 mm compared to the non-EDTA discs.[36]

Phenotypic Detection of Metallo-β-Lactamase (MBL) Enzyme

MBL-producing bacteria were identified using the double-disc synergy method, incorporating EDTA. Briefly, the isolates were spread onto Mueller-Hinton agar (MHA) plates, and two discs containing imipenem and meropenem were positioned 24 mm apart. Subsequently, 10 µL of a 0.5M EDTA solution was applied to each carbapenem disc. A positive result was indicated if the EDTA discs displayed a zone of inhibition greater than 5 mm compared to the non-EDTA discs.[36]

Molecular Characterization of bla_{NDM}

The extraction of DNA from bacteria culture was carried out using DNA kit for bacterial cell (TIANamp, Beijing, China). PCR reaction was performed using the gene specific primers F1 (5-ATGGAATTGCCCAATATTATGCAC-3) and R1 (5-TCAGCGCAGCTTGTCGGC-3), for the presence of blaNDM. The gene sequences was confirmed and subsequently the NDM variant was sequenced.

Anti-Bacterial Activity of the Compound Against NDM-1-KP ST147

Agar Well Diffusion Method

The agar well diffusion experiment was used to determine whether or not the compounds have anti-bacterial activity against NDM-KP. A bacterial suspension with a turbidity of 0.5 McFarland standard was used to inoculate Mueller Hinton Agar plates. After that, 50, 40, 30, 20, and 10 mg of a DMSO solution containing various concentrations of each sample were added to each well that was previously made on plates. Overnight, the plates were kept at 37 degrees Celsius. A Vernier caliper measured the inhibition zone (in mm) surrounding each well. The *E. coli* (ATCC 25922) and meropenem antibiotics were used as a quality control reference. The experiment was repeated three times to guarantee data accuracy and repeatability.[16]

Minimum Inhibitory Concentration of Different Compounds Against NDM-1-KP ST147

Micro broth dilution assay determined the MIC (% w/v) of different compounds as described earlier.[37] To prepare the bacterial culture, in a 50 mL Falcon tube, 20 mL of double-strength lysogeny broth (LB) media was combined with two to three individual colonies. Afterwards, the media were kept at 37 degrees for 24 hours. The bacterial suspension was then diluted to an OD of 0.07 at 600 nm, corresponding to a turbidity of 0.5 McFarland standard. Dimethyl sulfoxide (DMSO) solutions of 0.76, 1.56, 3.12, 6.25, 12.5, 25, and 50 mg of each drug were made for the MIC test. Next, a 96-well, flat-bottom microtiter plate was prepared, and 100 µL of each chemical dilution was applied to each well. The bacterial suspension was then added to each well at a concentration of 100 µL. The wells serving as negative controls each held 100 µL of LB, whereas the wells serving as positive controls held LB along with the bacterial solution. The microtiter plate was kept in a shaking incubator (MaxQTM Mini 4450, Thermo Fisher Scientific) at 3 g and 37 degrees Celsius for a whole night. By comparing the development in each well to that of the negative and positive controls, the MIC value for each chemical was determined. To guarantee reliability and consistency, all experiments were run three times.

Minimum Bactericidal Concentration Against NDM-1-KP ST147

The Minimum Bactericidal Concentration (MBC), expressed as a percentage w/v, was determined as the first dilution that showed no visible growth on the agar plate. A 10µL sample was taken from wells with no visible growth in the microtiter plate. The sample was then inoculated onto nutrient agar plates and incubated at 37°C for 24 hours aerobically. After incubation, the plates were examined for cell viability, and the presence or absence of bacterial growth was recorded. The entire process was repeated in triplicate to ensure the accuracy and reliability of the results.

Docking Studies

Ligand-receptor docking studies of **3b** were performed using crystal structure bla_{VIM}. Protein was found with a resolution of 2.20 A°. All these docking studies were performed on AutoDock Vina software installed on Intel(R) Core (TM) m3-7Y30 processor 1.00GHz 1.61 GHz and the operating system used is Windows 10 Pro Education version 21H2.[38]

Protein with PDB ID: 5N5I was selected and downloaded from the RCSB website (https://www.rcsb.org/) in PDB format. The protein contained 232 residues, including zinc metals as cofactors. Crystal protein was prepared using structure preparation wizard. It was protonated 3D and corrected to get corrected bond angles or geometry of protein residues. The energy of protein was minimized to attain optimized geometry. The prepared protein was saved in PDB format.[39]

A database of ligands was prepared using PubChem and converted to 3D structure by giving input in smiles format. The energy of the ligand was minimized to stabilize the ligand bond angles and geometry. Database files were saved in mdb format.[40]

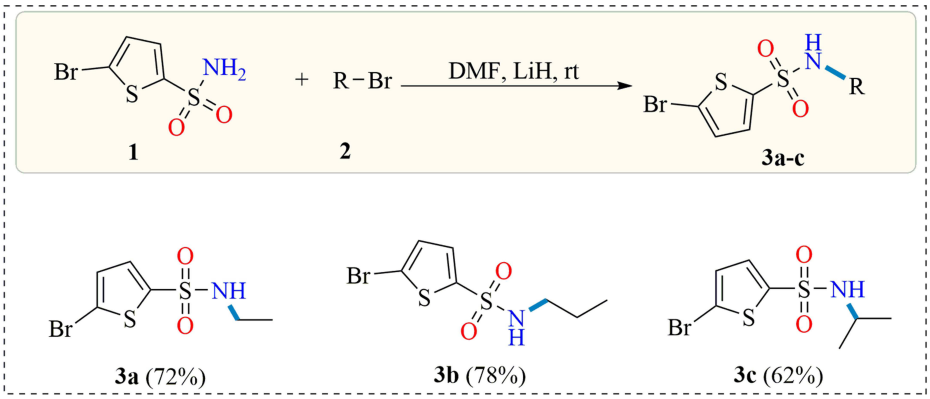
The active pocket was selected using a site finder keeping the amino acid residues, zinc metal, and water molecules as reported in previous literature. **3b** was docked this active pocket of crystal protein retaining 70 poses for docking. Validation was done by docking the standard inhibitor (LMP) of the protein back into the active pocket.[41]

Results

Chemistry

We initiated our work by reacting 5-bromothiophene-2-sulfonamide (**1**) with various alkyl bromides (**2**) in DMF in the presence of LiH at room temperature to synthesize 5-bromo-*N*-alkylthiophene-2-sulfonamides (**3a-c**). To increase their efficacy and product yield, minor structural changes were made to these compounds. Several alkyl bromides were employed to synthesize novel derivatives with valuable biological properties. The alkylation of the free amine led to the completion of the reaction. The compounds 5-bromo-*N*-ethylthiophene-2-sulfonamide (**3a**) and 5-bromo-*N*-propylthiophene-2-sulfonamide (**3b**) were obtained with yields of 72% and 78%, respectively, when treated with bromoethane and 1-bromopropane respectively. The lower yield of 62% obtained for 5-bromo-*N*-isopropylthiophene-2-sulfonamide (**3c**), derived from isopropyl bromide, may have been due to steric hindrance (Figure 1).

To create a compound library of 5-bromo-*N*-propylthiophene-2-sulfonamide (**3b**) for further SMC coupling with various aryl boronic acids, resulting in products (**4a-g**) due to promising activity against bacteria. Significantly, compound (**4f**) demonstrated the highest yield at 72%, while compound (**4g**) also exhibited a commendable yield of 68%. Compound (**4c**) and compound (**4e**) yielded lower percentages of 62% and 66%, respectively, whereas compounds (**4b**), (**4a**), and (**4d**) exhibited yields of 58%, 56%, and 62%, respectively. Our data indicate that the yields of Suzuki-coupled products of the alkylated substrate can vary slightly depending on electron-withdrawing and electron-donating substituents (Figure 2).



Conditions: 1 (0.413 mmol, 1 eq.), LiH (0.413 mmol, 1 eq.) and alkyl bromides (0.413 mmol, 1 eq.) in DMF (40 mL).

Figure 1 Synthesis of 5-bromo-*N*-alkylthiophene-2-sulfonamides.

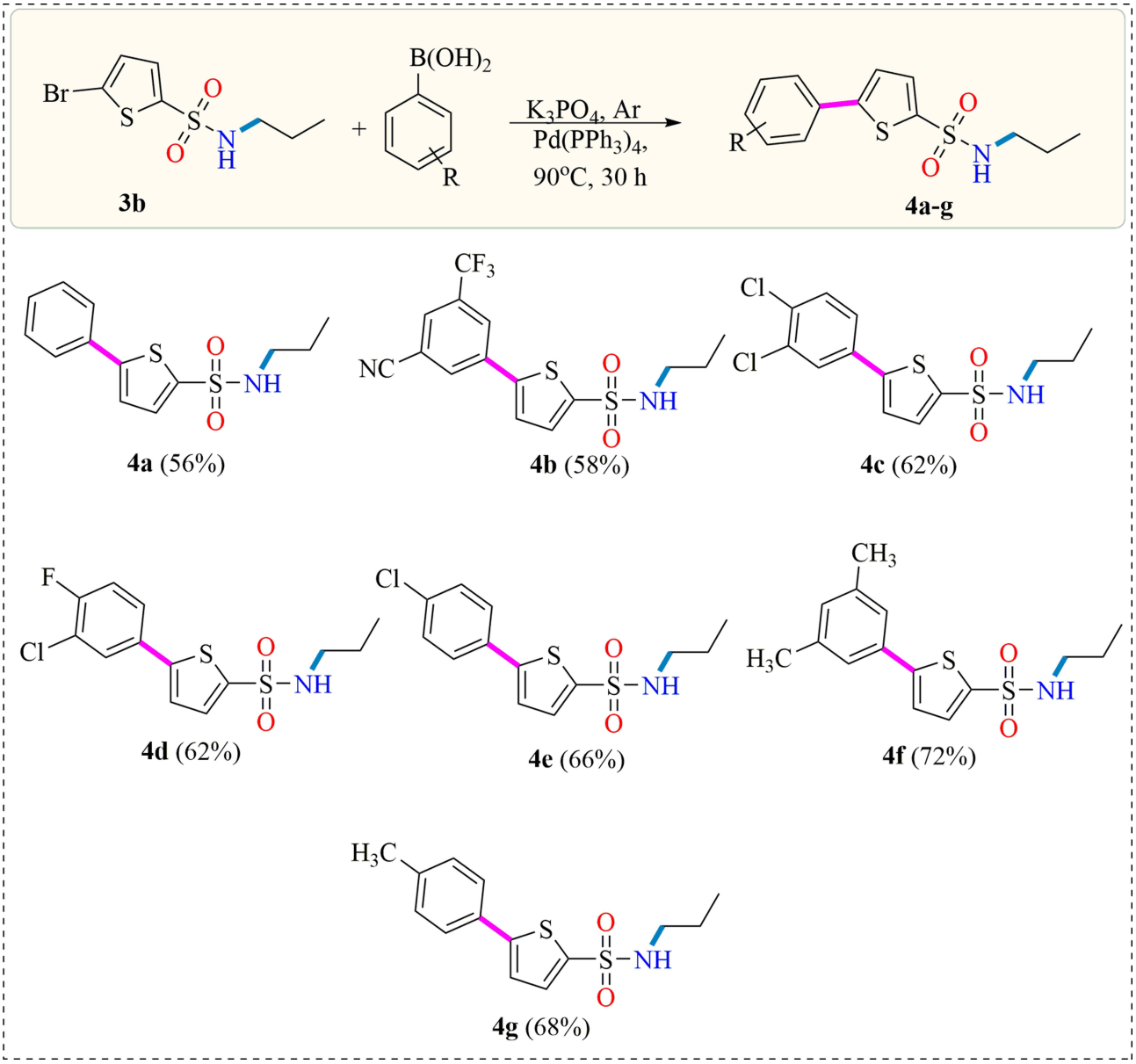


Figure 2 Arylation of 5-bromo-*N*-propylthiophene-2-sulfonamide.

Anti-Bacterial Activities

Identification and ST of the Isolate

The clinical isolate was identified as *K. pneumoniae* belonged to ST147 which was confirmed using the VITEK 2 system and as per PubMed MLST scheme. *K. pneumoniae* ST147 was phenotypically positive for both MBL producer and carbapenemase. The isolate exhibited antibiotic resistance from the WHO's Access, Watch, and Reserve (AWaRe) categories. The MICs of piperacillin (≥256 µg/mL), levofloxacin (≥16 µg/mL), meropenem (≥32 µg/mL), and ceftriaxone (≥256 µg/mL) were higher than normal. However, colistin (0.5 µg/mL) was the most effective medication with the highest sensitivity against the isolate (Table 1). Molecular identification of $bla_{\text{NDM-1}}$ gene showed that Carbapenem-resistant *K. pneumoniae* ST147 carried $bla_{\text{NDM-1}}$.

Agar Well Diffusion Assay of NDM-1- K. Pneumoniae ST147

The Results, including the activity, are presented in Table 2. Notably, the zone of inhibition (measured in mm) increased with higher concentrations of the compounds. Among the tested compounds, compound (**3b**) exhibited the highest zone

Table 1 MIC (µg/Ml) of Antibiotics Against K. Pneumoniae ST147 Pathogen

Antibiotics	WHO Classes of Antibiotics	MIC Break Points (ug/mL)	*K. pneumoniae*
PIP	Watch	≤16 - ≥128	≥256
SAM	Access	≤8/4 - ≥32/16	≥64/32
SXT	Access	≤2/38 - ≥4/76	>8/152
CRO	Watch	≤8 - ≥64	≥256
FEP	Watch	≤8 - ≥32	≥128
MEM	Watch	≤2 - ≥8	≥32
ATM	Reserve	≤4 - ≥16	≥64
AK	Access	≤4 - ≥16	≥16
LEV	Watch	≤1 - ≥4	≥16
MNO	Watch	≤4 - ≥16	≥64
MXF	Watch	≤0.5 - ≥2	≥16
C	Access	≤8 - ≥32	≥128
TE	Access	≤4 - ≥16	≥32
CS	Reserve	≥4	0.5

Abbreviations: PIP, Piperacillin; SAM, Ampicillin/sulbactam; SXT, Trimethoprim/sulfamethoxazole; CRO, Ceftriaxone; FEP, Cefepime; MEM, Meropenem; ATM, Aztreonam; AK, Amikacin; LEV, Levofloxacin; MNO, Minocycline; MXF, Moxifloxacin; C, Chloramphenicol; TE, Tetracycline; CS, Colistin.

Table 2 Agar Well Diffusion of Different Compounds Against NDM-KP ST147

Compounds No.	Zone (mm) (50mg/mL)	Zone (mm) (40mg/mL)	Zone (mm) (30mg/mL)	Zone (mm) (20mg/mL)	Zone (mm) (10mg/mL)	DMSO Zone (mm)
3a	16±2	11±2.5	8 ±1	8 ±3	7 ±2	0
3b	23 ±1.5	21 ±1	20 ±1	20 ±2	19 ±1	0
3c	13 ±1	12 ±2	10 ±3	9 ±3	7 ±2.5	0
4b	13±2.5	12±2	11±2	10±1	8±2	0
4c	5±3	4±2.5	3±1	2±2.5	2±2	0
4f	14±3	131±1	12±1	12±1	9±1.5	0

of inhibition, measuring 23 ± 1.5 mm, at a concentration of 50 mg/dL (Figure 3). This zone of inhibition was significantly more significant compared to the other compounds. However, compounds **3a** showed (16±2 mm), **3c** (13 ±2 mm), **4b** (13±2.5 mm) **4c** (5±3 mm), and **4f** (14±2 mm).

MIC and MBC of the Compounds Against NDM -KP ST147

The anti-bacterial activity of the molecules (**3a–c, 4a-g**) was evaluated against NDM-producing *K. pneumoniae* ST147 using the agar well diffusion method at five different concentrations (10, 20, 30, 40, and 50 mg/well). The results demonstrated that compound (**3b**) exhibited the lowest MIC and MBC values of 0.39 µg/mL and 0.78 µg/mL, respectively. In comparison, compound (**4c**) displayed a MIC of 1.56 µg/mL and an MBC of 3.125 µg/mL. Furthermore, compound (**3a**) showed a MIC of 3.125 µg/mL and an MBC of 6.25 µg/mL, as presented in Table 3.

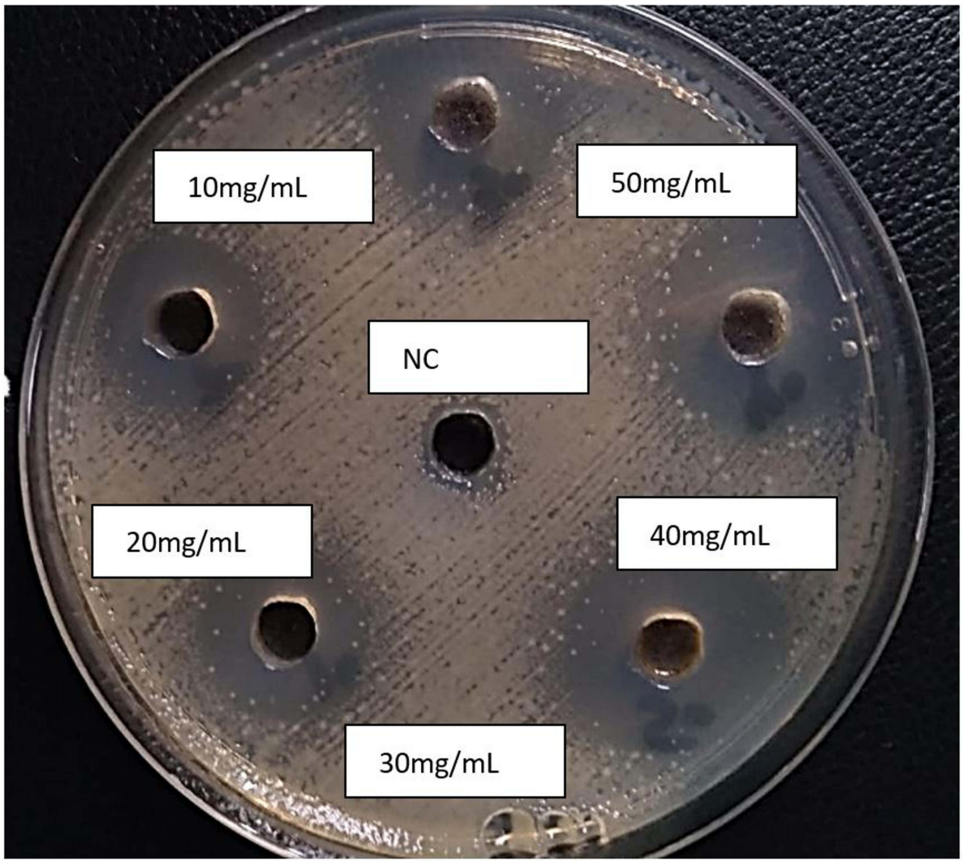


Figure 3 Anti-bacterial activity of compound (**3b**) against CRKP pathogen.

Docking Studies

Protein with PDB ID: 5N5I selected having four chains in structure Chain A contained 232 amino acid residues, Chain B had standard inhibitor (LMP) with three zinc metals, and Chain C, and D had water molecules. The compound (**3b**) was docked in the active pocket of crystal structure protein having Amino acid residues, as reported in Table 4.

The docked active pocket had 15 residues, and the target ligand **(3b)** interacted with 10 residues in the active pocket. The ligand (**3b**) showed two hydrogen bonds with Trp87 and Asp118, both with the oxygen of the sulphonyl group. Pi-Sulphur interaction was shown by Phe62, His116 and His179. A pi-cation interaction was found to be developed between the Zn302 of the target protein and the sulphur of the sulphonyl group as well as the sulphur of the thiophene group of docked compounds, respectively. Whereas a π-π interaction was established between the thiophene ring and His116

Table 3 MIC (Mg/mL) and MBC of Compounds Against CRKP

Compounds No.	MIC (μg/mL)	MBC (μg/mL)
3a	3.125 ±2.5	6.25 ±2.5
3b	0.39 ±2	0.78 ±2.5
3c	25 ±1	50 ±1.5
4b	12.5 ±2	25 ±3.0
4c	1.56 ±3	3.125 ±2
4f	3.125 ±1.5	6.25 ±2

Table 4 Description of Active Pockets of the Protein Crystal Structure of 5N5I, Indicating Important Interacting Residues as Well as the Type of Interactions Involved

PDB ID	5N5I
Amino Acids	Phe62, Asp63, Tyr67, Pro68, Trp87, His114, His116, Asp117, Asp118, Glu146, Asn148, His179, Gly197, Cys198, Val200, His201, Glu202, Ser205, Ser207, Ala208, Gly209, Asn210, Asp213, Pro238, Gly239, His240, Gly242
Water and cofactors	HOH 445, Zn301, Zn302
Types of Interactions	H.B, pi-pi, Pi- Alkyl, pi- Sulphur, Pi-cation

amino acid residue. About four alkyl and π-alkyl linkages were found with Phe62, Trp87, His116 and Ala212. Water-hydrogen bonding was shown by HOH-445, as described in Figure 4.

Validation was done by redocking the Standard inhibitor LMP in the active pocket of crystal structure protein. The best-docked pose of the standard inhibitor showed interaction with two hydrogen bonds with Trp87, Asp118, one π-alkyl interaction with His116, and one C-H interaction with Asp63. In addition to it, cofactor Zn also interacts with the docked molecule through π-cation interaction, as demonstrated in Figure 5. The binding free energy of this docked pose was found −5.8945 kcal/mol, and the RSMD value was found 3.1247 A° (Table 5).

Discussion

As demonstrated by our earlier research, formamide is among the best medications for treating pathogens that are resistant to medications. According to our research on the biofilm inhibitory efficacy of aryl groups against Bacillus subtilis and Escherichia coli, they function as growth sites and feature hydrophobic (nonpolar) interactions. Amides simultaneously kill bacteria or cause stagnant growth and have a positive inhibitory impact.[42]

Amides contain a hydrophilic binding site for bacterial enzyme proteins. Additionally, in order to evaluate the efficacy of this stent, we also looked at N, O, and S heterocyclic formamides. Further, we studied the *N, O*, and *S* heterocycles

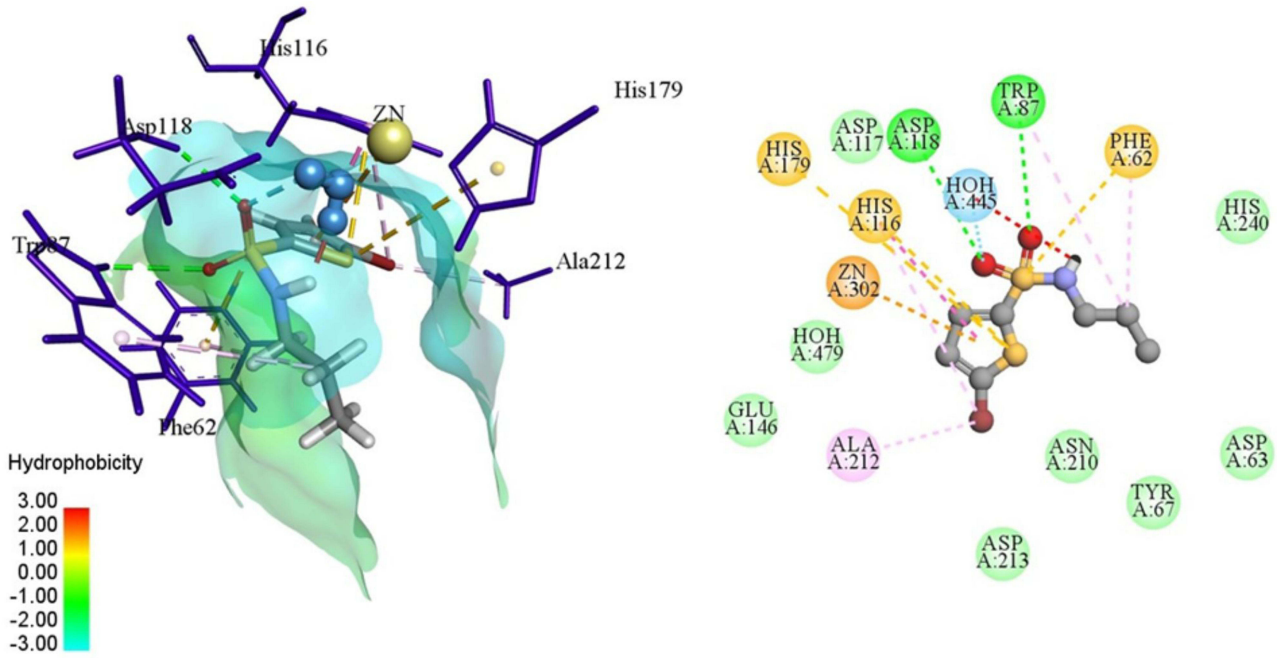


Figure 4 The left side of the image shows 3D interaction and the right side shows the 2D interaction of ligand (**3b**) with amino acid residues of the pocket. Interaction with zinc and water is clearly shown in the 2D demonstration.

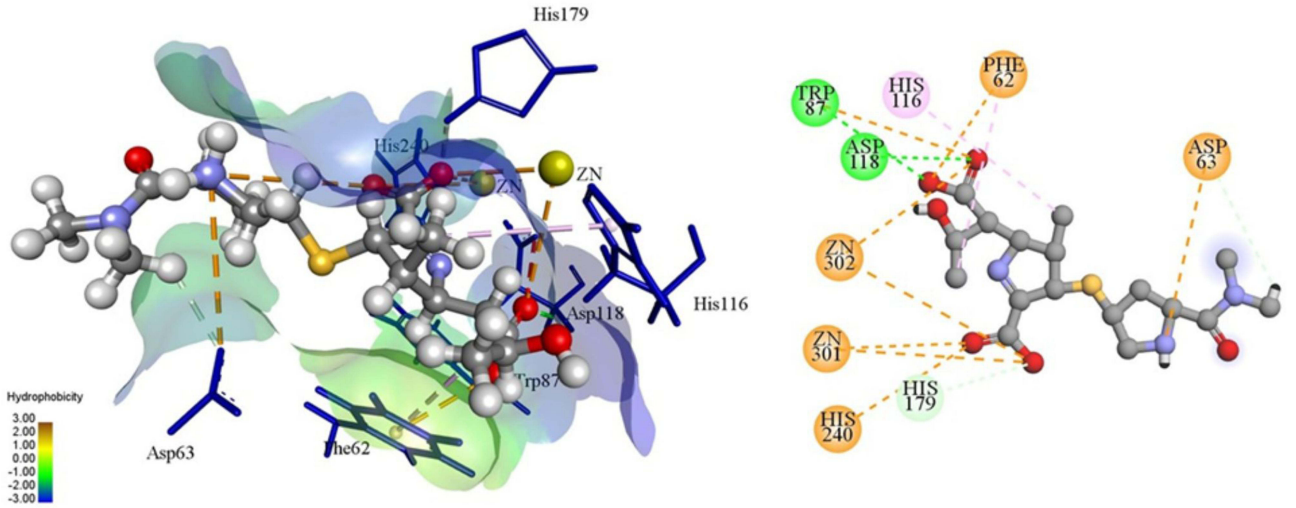


Figure 5 The left side shows 3D interaction, and the right shows the 2D interaction of standard inhibitor (LMP) with residues present in the active pocket. Yellow-coloured ball showing Zinc metal in the 3D active pocket.

carboxamides like 2-aryl-4-chlorophenyl-5-arylthiophene-2-carboxylates[43] *N*-(4-bromophenyl)furan-2-carboxamide,[37] *N*-(4-methylpyridin-2-yl) Thiophene-2-Carboxamide 4-bromo-*N*-(5-methyl-1H-pyrazol-3-yl)benzamide[16,44] to study the effectiveness of these scaffolds. The drug is effective against *Staphylococcus aureus* and *Klebsiella pneumoniae*, as well as resistant strains of *Salmonella enterica, Acinetobacter baumannii, Enterobacter cloacae*, and E. coli. The importance of heterocyclic formamide in antibacterial action was discovered. In the present world, synthetic small molecules play a crucial role in drug development for reasons related to the economy and the environment. As a result, we created a molecule that permits the chemical to grow in bacterial cells by adding a linear alkyl group. When the N-heterocyclic amide moiety becomes hydrophilic, it may attach itself to the proteins of bacteria. Some of the thiophene and sulfonamide derivatives that were reported to exhibit anti-bacterial activities are given in Figure 6. Moreover, sulfonamides have more polar surface area and better hydrogen bond acceptors.[45] Then, we envisioned the principle of a drug molecule containing a lipophilic tail and polar head. So, we synthesized a series of sulfonamides containing lipophilic tail and arylation of 5-bromo-*N*-propylthiophene-2-sulfonamides to evaluate the antibacterial activity of the synthesized compounds against NDM-1 producing *K. pneumoniae*. Our motivation for this work was the biological significance of sulfonamides. The in-silico experiments further validated our findings.

Table 5 Representing Binding Affinity, Interacting Residues as Well as the Type of Interactions for 3b and LMP (Standard Inhibitor) in the Active Pocket of the Target Protein

Test Ligand	rsmd	Hydrogen Bonding Residues	Hydrophobic Bonding Residues	Binding Affinity ΔG Kcal/mol
3b	1.07842	Try87 (O) Asp118 (S)	Phe62 Trp87 His116 His179 Ala212	−6.0504
Lmp **(Standard inhibitor)**	3.1247	Trp87 (O) Asp118 (O)	Phe62 Asp63 His146 His240	−5.8945

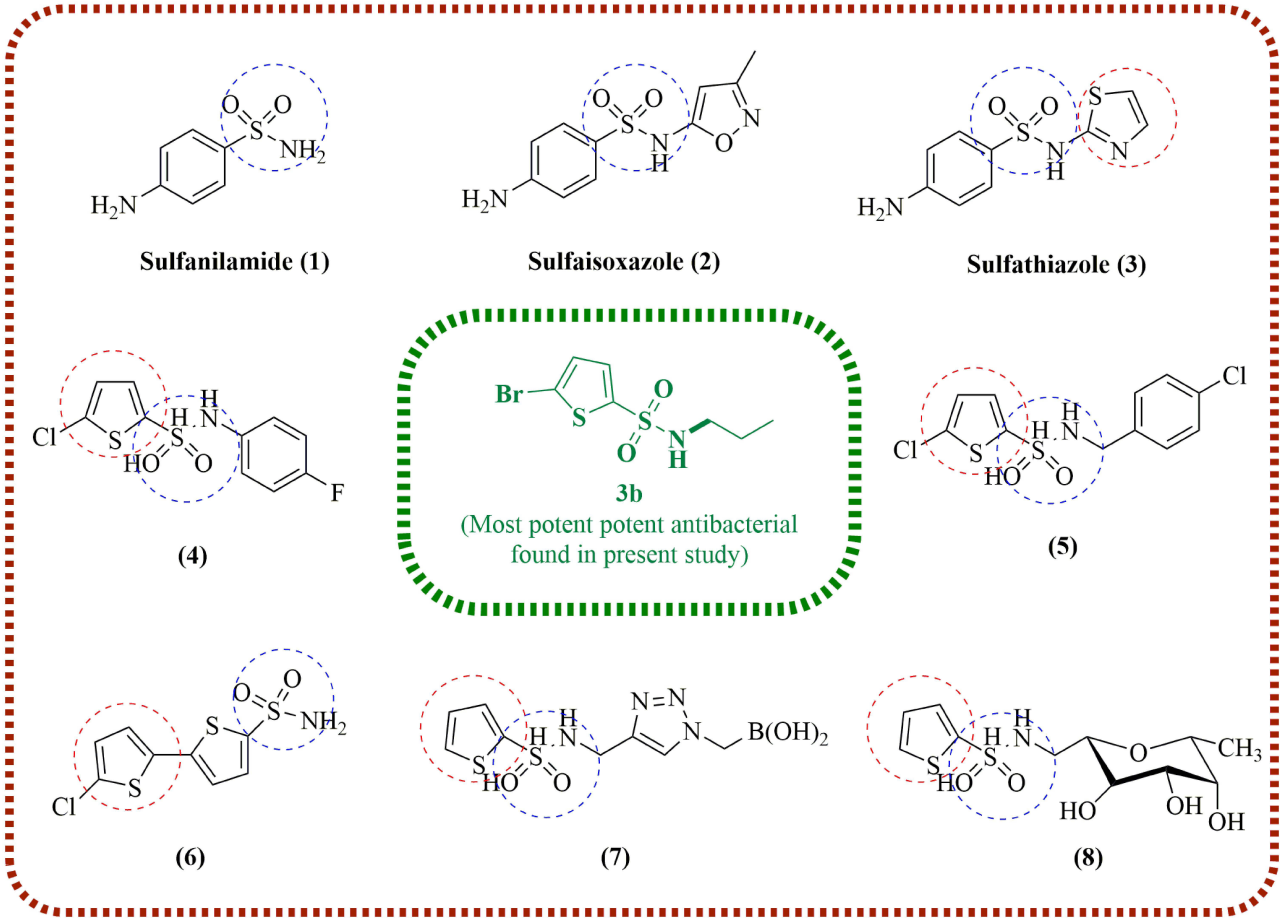


Figure 6 Some reported antibacterial that contain thiophene and sulfonamide moieties. The blue encircled moieties represent the sulphonamide functional group that is common in various commercially available antibacterial agents eg, Sulphanilamide, Sulfisoxazole and Sulfathiazole. Molecular docking studies revealed that this functional group exhibited the maximum interactions with the target proteins. In addition, thiophene (Red encircled moiety) containing compounds have also been reported as antibacterial agents and this moiety also exhibited strong interactions with the target proteins.[46–51]

Conclusion

In this study, we successfully synthesized 5-bromo-*N*-alkylthiophene-2-sulfonamides (**3a-c**) in good yields (62–78%) and found the compound (**3b**) as a potential drug candidate having zone inhibition (23±1.5mg/mL), MIC (0.39 μg/mL) and MBC (0.78 μg/mL) against NDM producing *K. pneumoniae ST147*. So, we synthesized a library of compounds (**4a-g**) by the arylation of **3b** via SMC coupling in moderate yields (56–72%). But they do have not more promising activities like **3b**. It may be due to adding an extra aryl ring, which may cause steric hindrance upon attachment to the bacterial enzyme pocket. The in-silico studies also revealed that the target compound **3b** showed interactions other than standard inhibitors, such as water bridging. Moreover, it showed hydrophobic pi-donor with Zinc 302; therefore, it is concluded that **3b** proved to be a more potent drug than a standard inhibitor. These studies have demonstrated that sulfonamide is a promising class of antibiotics that can combat antibiotic-resistant bacterial strains.

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Disclosure

The authors declare no conflicts of interest in this work.

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