RESEARCH ARTICLE

Synthesis and antimicrobial investigation of novel β-lactam derivatives

[version 1; peer review: 2 approved with reservations]

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Abstract

Background

β-Lactam derivatives are widely studied due to their proven pharmacological benefits and capacity to suppress a wide range of microbiological infections. These compounds represent an important class of antibacterial agents, and continued structural modification of β-lactams remains essential to overcome antimicrobial resistance. The synthesis of new β-lactam scaffolds is therefore a key strategy in medicinal chemistry for the development of more effective antibacterial drugs.

Objective

The present study aimed to synthesize new β-lactam derivatives based on sulfapyridine Schiff bases and to evaluate their potential biological activity using experimental characterization and molecular docking analysis.

Methods

Sulfapyridine-based Schiff bases were prepared through condensation reactions and used as key intermediates for the synthesis of β-lactam derivatives. The synthesized compounds were confirmed using spectroscopic techniques, including FT-IR, ^{1}H-NMR, and ^{13}C-NMR.

In this study, two types of β-lactam derivatives were synthesized. Condensation of the sulfanilamide drug with selected aromatic aldehydes in the presence of glacial acetic acid gives the corresponding Schiff bases [A1-A4].

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The reaction of prepared Schiff bases with chloro acetyl chloride in the presence of triethylamine gave the first type of β-lactam derivatives [A5-A8]. The second type of β-lactam derivatives [A9-A12] were synthesized via cycloaddition between prepared Schiff bases with diclofenac acid in the presence of ρ-toluene sulfonyl chloride and trimethylamine. Biological activity was evaluated, and molecular docking studies were performed against the target protein (PDB ID: 1EA1).

Results

Several synthesized derivatives, including A7, A8, A9, and A12, demonstrated enhanced antibacterial activity and outperformed reference medications. Experimental and theoretical data indicated that β-lactam compounds represent viable scaffolds for the development of novel antibacterial agents. Compared to the reference drug amoxicillin (−7.5 kcal/mol), compounds A10 and A11 exhibited the lowest binding energies (−9.0 and −8.4 kcal/mol, respectively), suggesting strong interaction with the target protein.

Conclusion

The agreement between in vivo biological results and in silico molecular docking data supports the potential biological activity of the synthesized β-lactam derivatives. These findings highlight the importance of β-lactam scaffolds as promising candidates for future antibacterial drug development.

Keywords
Sulfapyridine, Schiff bases, β-lactam derivatives, diclofenac acid, antibacterial, antifungals, cycloaddition, and molecular docking.

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Introduction

β-lactam derivatives, a subclass of heterocyclic molecules pertinent to medicinal chemistry, are well known for their antibacterial as well as therapeutic activity. The β-lactam ring, or azetidin-2-one ring, is a four-membered cyclic amide that forms the basis of the chemical structure of a variety of well-known antibiotics that are now used clinically—from penicillin and cephalosporins to carbapenems and monobactams. This shape is such that the reactivity with electrophilic biological nucleophiles is enhanced, and, as a result, the inhibition of bacterial cell wall synthesis is very efficient. β-lactam derivatives of all kinds are, therefore, the target of study in the discovery of new antimicrobial medicines, and various groups are intensely involved in this area of research.[1–3]

The duration of pharmaceutical antibiotic therapy to cure infections is continually shrinking in the face of the proliferation of multi-drug resistant disease, especially in the hospital environment. The commonest exception is Gram-negative bacteria, which challenge β-lactam antibiotics with a repertoire of fighting techniques, from enzymes that cleave the drug and deactivate it to an armory of multiple mechanisms that impede drug reachability of the target: β-lactamases, decreased membrane permeability, or the generation of new penicillin-binding proteins that bind less well (if at all) to β-lactams themselves. Greater structure-activity relationship comprehensions are needed to pick off better structures offering greater stability, binding affinity, and antibacterial potency.[4–6]

Sulphonamide drugs have probably the greatest mix-and-match applicational pharmacophoric class in synthetic medicinal chemistry.

They are exerting an antibacterial impact by virtue of blocking the active site of an enzyme, dihydropteroate synthase (DHPS), which is necessary for the formation of folate. When the sulphonamide group is joined to an aromatic heterocyclic ring, a versatile, useful core is formed that can be opened and chucked around. Amino acid systems can be improved if the basic reaction can be made to yield a Schiff base, then condensed with an aromatic aldehyde, thus providing greater synthetic flexibility (in that cycloadditions and acylations can be undertaken to give rise to heterocyclic systems such as β-lactams).[7–9]

Herein, we describe the rational creation of two new β-lactam derivatives on a sulfapyridine-like basis, compounded and then characterized on the physical level, computerized under the ethicalized class, and on the physiological level geophiliacized for more bacterial and fungicidal challenges. Integrating practical and theoretical insights for newer β-lactam scaffolds for antibacterials.[10–12]

Methodology

Chemicals and materials

All chemicals used were purchased from Fluka and Merck. M.P. is a recorder that uses an electrothermal melting point apparatus, Gallenkamp. The FT-IR (KBr disk) spectra of prepared compounds were recorded on a Shimadzu FT-IR 8400s spectrophotometer in the department of chemistry, college of science, and ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ultra Shield 400 MHz spectrometer, using DMSO-d_6 as the solvent and TMS as an internal standard.

Characterization methods

General procedure for the synthesis of Schiff-bases [A1-A4]

A mixture of sulfapyridine (0.01 mol, 2.49 g) and the appropriate aromatic aldehyde (0.01 mol) was dissolved in 30 mL of absolute ethanol. A catalytic amount of glacial acetic acid (3–4 drops) was added, and the mixture was refluxed for 3 hours with continuous stirring. The reaction progress was monitored by TLC. Upon completion, the mixture was cooled to room temperature, and the resulting solid product was filtered, washed with cold ethanol, and dried under vacuum to afford Schiff bases A1–A4 in good yields.[13]

4-((4-chlorobenzylidene) amino) -N-(pyridin-2-yl) benzene sulfonamide [A1]: Pale yellow solid, yield: 90%, m.p. 200–202°C. FT-IR: 3390 (NH), 3024 (CH aromatic), 1681 (C=N pyridine), 1631 (C=N), 1384 (SO_2 asy.), 1085 (SO_2 sy.), 1005 (C-Cl); ^{1}H NMR δ 11.73 (s, 1H, NH), 1H, N=CH), 6.87-8.07 (m, 12H, Ar-H). ^{13}CNMR δ: 112.58–154.85 (C-Ar), 162.24 (C=N).

4-((4-nitrobenzylidene) amino) -N-(pyridin-2-yl) benzene sulfonamide [A2]: Yellow solid, yield: 85%, m.p. 190–192°C. FT-IR: 3244 (NH), 3055 (CH aromatic), 1679 (C=N), 1629 (C=N), 1575 (NO_2 asy), 1319 (NO_2 sy), 1388 (SO_2 asy), 1083 (SO_2 sy).

4-((4-(dimethylamino)benzylidene)amino) -N-(pyridin-2-yl) benzene sulfonamide [A3]: Yellow solid, yield: 85%, m. p. 188–190°C. FT-IR: 3305 (NH), 3047 (CH aromatic), 1708 (C=N pyridine), 1679 (C=N), 1008 (C-N), 1359 (SO_2) asy, 1087(SO_2)sy.

4-((4-methoxybenzylidene) amino) -N-(pyridin-2-yl) benzene sulfonamide [A4]: Pale brawn solid, yield: 88%, m.p. 185–187°C. FT-IR: 3225 (NH), 3047 (CH aromatic), 1708(C=N pyridine), 1683 (C=N), 1283 (Ar-O), 1380 (SO_2) asy., 1085 (SO_2)sy. ^{1}H NMR δ 11.57 (s, 1H, NH), 8.51 (s, 1H, N=CH), 6.88-7.71 (m, 12H, Ar-H), 3.73 (s, 3H, OCH_3). ^{13}CNMR δ: 56.16 (CH_3), 112.58–154.85 (C- Ar), 162.24 (C=N).

General procedure for the synthesis of β-lactam derivatives [A5-A8]:

To a stirred solution of the Schiff base (0.01 mol) in 20 mL of dry dichloromethane, triethylamine (0.02 mol) was added dropwise under an inert atmosphere. The mixture was cooled in an ice bath, and chloroacetyl chloride (0.012 mol) was added slowly while maintaining the temperature below 10°C. The reaction mixture was then stirred at room temperature for 6 hours. After completion, the mixture was washed successively with distilled water, 5% sodium bicarbonate solution, and brine. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The crude product was recrystallized from ethanol to yield β-lactam derivatives A5–A8.[14]

4-(3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl) -N-(pyridin-2-yl) benzene sulfonamide [A5]: Yellow solid, yield: 85%, m.p. 150–148°C. FT-IR: 3307 (NH), 3058 (CH aromatic), 1706 (C=O amide), 1679 (C=N pyridine), 1359 (SO_2 asy.), 1085 (SO_2 sy.), 1002 (C-Cl).

4-(3-chloro-2-(4-nitrophenyl)-4-oxoazetidin-1-yl) -N-(pyridin-2-yl) benzene sulfonamide [A6]: Brawn solid, yield: 86%, m.p. 160–158°C.FT-IR:3299(NH), 3056(CH aromatic), 1703(C=O amide), 1670(C=N pyridine), 1533, 1394 (NO_2), 1361 asy., 1087(SO_2)sy. ^{1}H NMR δ: 12.39 (s,1H, NH), 6.40-7.97 (m,12H, Ar-H), 5.77(d,1H, CH-Cl), 4.29 (d,1H, CH-N). ^{13}CNMR δ: 166.01 (C=O), 114.41–154.28 (C- Ar), 70.68 (C-N), 64.19 (C-Cl).

4-(3-chloro-2-(4-(dimethylamino)phenyl) -4-oxoazetidin-1-yl)-N-(pyridin-2-yl) benzene sulfonamide [A7]: Brown solid, yield: 89%, m.p. 145–147°C. FT-IR: 3309 (NH), 3029 (CH aromatic), 1728 (C=O amide), 1672 (C=N pyridine), 1359 asy., 1039 (SO_2) sy. ^{1}H-NMR δ: 10.90 (s, 1H, NH), 6.77-8.03 (m, 12H, Ar-H), 5.77 (d, 1H, CH-Cl), 4.34 (d, 1H, CH-N), 3.03 (s, 6H, CH_3-N). ^{13}CNMR δ: 165.73 (C=O), 111.55–154.67 (C-Ar), 65.34 (C-N), 64.18 (C-Cl), 41.99 ($2CH_3$).

4-(3-chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl) -N-(pyridine-2-yl) benzene sulfonamide [A8]: Brown solid, yield: 90%, m.p. 159–161°C. FT-IR: 3269 (NH), 3068 (CH aromatic), 1712 (C=O amide), 1677 (C=N pyridine), 1259 (Ar-O), 1332 (SO_2) asy, 1049(SO_2)sy.

General procedure for the synthesis of β-lactam derivatives [A9-A12]:

A mixture of diclofenac acid (1.5 mmol, 0.6 g), Schiff base (1 mmol), *p*-toluenesulfonyl chloride (1.5 mmol, 0.4 g), and triethylamine (5 mmol) in dry dichloromethane (10 mL) was stirred at room temperature for 35–60 h. The reaction progress was monitored by TLC. After completion, the mixture was washed sequentially with 1 N HCl (10 mL), $NaHCO_3$ solution (10 mL), and brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and the solvent removed to yield crude β-lactams (A9–A12), which were recrystallized from ethanol.[15]

4-(2-(4-chlorophenyl) -3-(2-((2,6-dichlorophenyl)amino)phenyl) -4-oxoazetidin-1-yl) -N (pyridin-2-yl) benzene sulfonamide [A9]: Dark yellow solid, yield: 88%, m.p. 190–192°C. FT-IR 3379 (NH), 3031 (CH aromatic), 1680 (C=O amide), 1361 (SO_2 asy.), 1054 (SO_2 sy.), 1008 (C-Cl).

4-*(3-(2-((2,6-dichlorophenyl) amino) phenyl) -2-(4-nitrophenyl) -4-oxoazetidin-1-yl) -N-(pyridin2-yl) benzene sulfonamide [A10]:* Pale yellow solid, yield: 80%, m.p. 210–212°C. FTIR: 3261 (NH), 3072 (CH aromatic), 1714 (C=O amide), 1506 ($NO_{2\ asy.}$ 1307 (SO_2) asy., 1045(SO_2) sy, 1000(C-Cl). ^{1}H-NMR δ: 10.03(s, 1H, NH), 6.42-7.96 (m,19H, Ar-H), 5.62 (d, 1H, CH), 4.12 (d,1H, CH-N). ^{13}CNMR δ: 169.90 (C=O), 112.39–140.80 (C- Ar), 58.74 (C-N), 44.53 (CH).

4-(3-(2-((2,6-dichlorophenyl) amino) phenyl) -2-(4-(dimethylamino)phenyl) -4-oxoazetidin-1yl) -N-(pyridin-2-yl) benzene sulfonamide [A11]: Yellow solid, yield: 83%, m.p. 239–241°C. FTIR: 3323 (NH), 3074 (CH aromatic), 1724(C=O amide), 1371(SO_2) asy, 1014(SO_2) sy, 1015(C-Cl).

4-(3-(2-((2,6-dichlorophenyl)amino)phenyl) -2-(4-methoxyphenyl) -4-oxoazetidin-1-yl) -N (pyridin-2-yl) benzene sulfonamide [A12]: Brawn solid, yield: 89%, m.p. 204-206°C. FT-IR:3234 (NH), 3080 (CH aromatic), 1681(C=O amide), 1083(C-O), 1348 (SO_2)asy., 1043(SO_2) sy, 1001 (CCl). ^{1}H NMR δ: 9.82 (s, 1H, NH), 6.79-8.10 (m, 19H, Ar-H), 6.31(d,1H, CH), 5.83 (d,1H, CH-N) 3.12 (s, 3H, OCH_3). ^{13}C`NMR δ: 166.99 (C=O), 112.39–140.80 (C-Ar), 60.90 (C-N), 55.40(CH_3),44.15 (CH).

Antibacterial and Antifungal Assays[16–20]

Antimicrobial activity was evaluated using the agar disk diffusion method. Tested microorganisms included:

- *Staphylococcus aureus* (Gram-positive)
- *Escherichia coli* (Gram-negative)
- *Candida albicans* (fungus)

Sterile filter paper disks were impregnated with 30 μg of each synthesized compound dissolved in DMSO. Inoculated plates were incubated at 37°C for bacterial strains and 28°C for fungal strains. Zones of inhibition were measured after 24 hours.

Ceftriaxone and fluconazole were used as standard reference drugs. All measurements were performed in triplicate, and results were reported as mean ± SD.

Molecular docking[21–24]

ChemOffice 2016, Discovery Studio 2021, and the AutoDock Vina module incorporated into PyRx 0.8 were used for molecular docking investigations. The Candida-related target protein EA1 was generated in Discovery Studio by removing heteroatoms and water molecules and verifying structural completeness after it was acquired from the Protein Data Bank. The .pdb format was used to store the optimized structure. ChemDraw was used to sketch the synthesized compounds (**A5–A12**) and convert them to .pdb files. AutoDock Tools was used to convert protein and ligand structures into the pdbqt format, and Open Babel was used to lower ligand energies. The active site of the ceftriaxone co-crystallized ligand was represented by the center of the grid box during the Vina Wizard docking procedure. Binding affinities were evaluated using the lowest Vina score values, and ligand-protein interactions were investigated using Discovery Studio Visualizer 2021.

Results and Discussion

Researchers focused on synthesizing β-lactam compounds due to their broad applications, particularly in biological, industrial, and agricultural fields.

The sulfapyridine drug was used to prepare two types of β-lactam derivatives. The first step involved the preparation of Schiff bases (A1-A4) from the condensation of some aromatic aldehydes with the sulfapyridine drug in the presence of glacial acetic acid. The FTIR spectrum showed the disappearance of the stretching bond at (3400, 3367) cm^{-1} for NH_2 and the appearance of a new stretching bond at (1629-1708) cm^{-1} for the imine group, while ^{1}H-NMR and ^{13}C-NMR showed signals at (5.83 ppm) and (8.51 ppm), which are due to (CH=N). Cycloaddition of prepared Schiff bases with chloroacetyl chloride in the presence of triethylamine at (0-5)°C gave the corresponding first type of β-lactam compounds (A5-A8).[13,14]

The synthesized compounds were characterized using FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy, and the corresponding spectra are shown in Figures 1–13. The proposed reaction pathway is illustrated in Scheme 1.

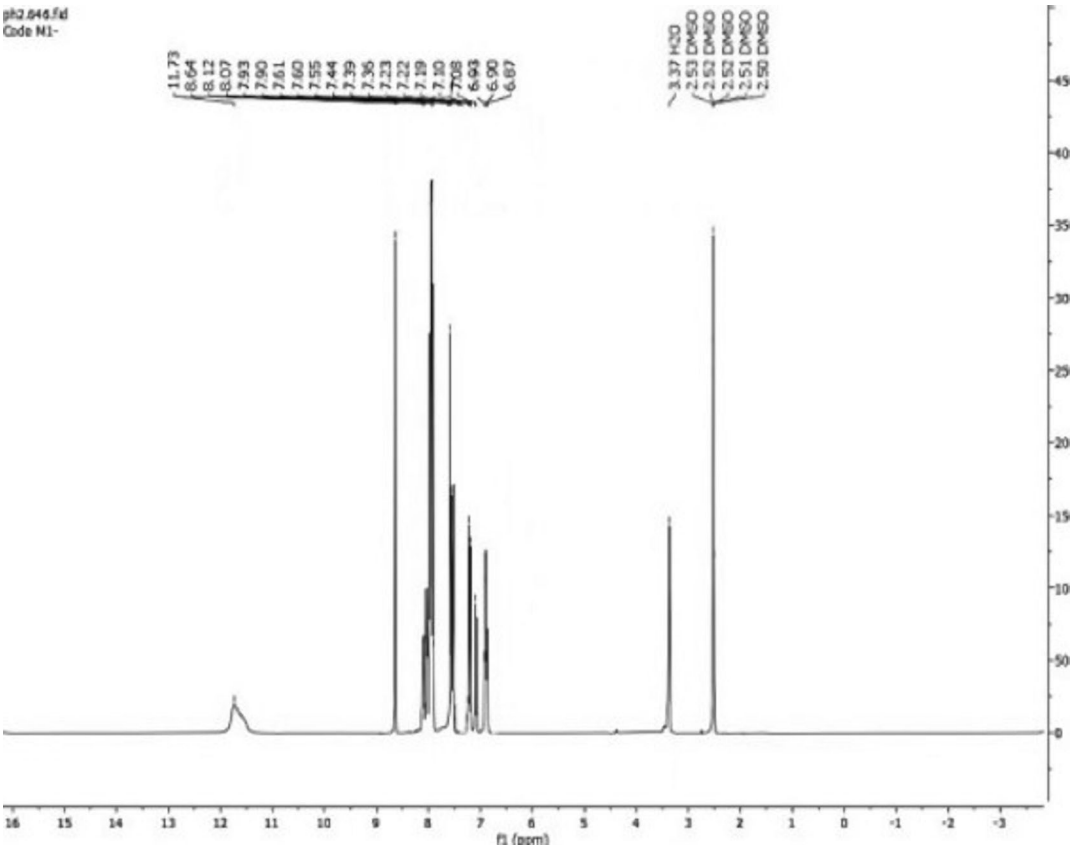


Figure 1. **^{1}H-NMR spectrum of compound A1.**

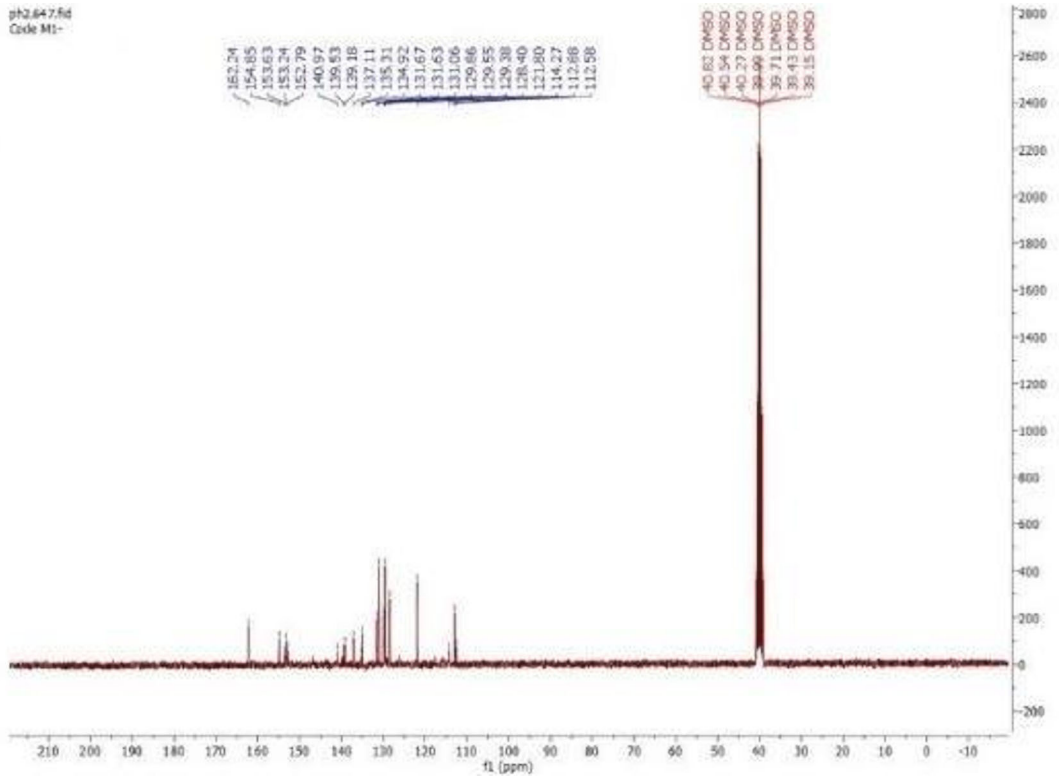
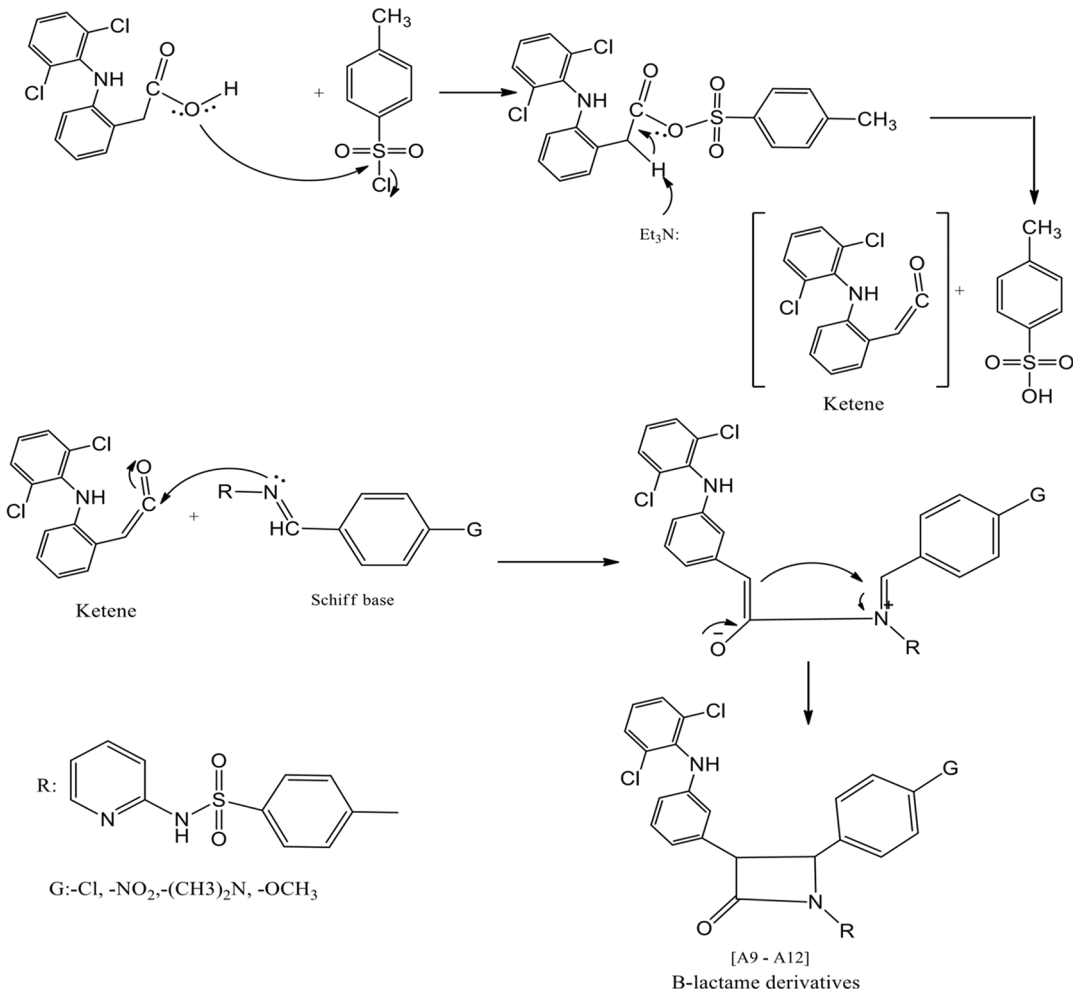


Figure 2. **^{13}C-NMR spectrum of compound A1.**

The absence of a stretching band, which is due to (CH=N), and the appearance of a new stretching band at (1680-1724) for (C=O lactam ring), while ^{1}HNMR and ^{13}CNMR showed the characteristic doublet signals at 5.83 ppm for (CH-Cl) and the doublet at 5.83 ppm for (CH-N) (166 ppm), which are attributed to the formation of β-Lactam derivatives. (2-2) Cycloaddition of prepared Schiff bases with diclofenac acid in the presence of triethylamine and p-toluene sulfonyl chloride through ketene-imine formation gave the corresponding second type of β-Lactam derivatives (A9-A12)[15] as illustrated in the following mechanism:



Mechanism synthesized of β-Lactam derivatives [A9-A12]

The structures of azetidin-2-one were determined via their FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectral data. The stretching vibration at 1627 cm^{-1} for the imine group disappeared, and a new stretching vibration band at (1680-1724) cm^{-1} for the carbonyl group of the β-lactam ring appeared. In the ^{1}H-NMR spectra, discrete doublets of protons of the β-lactam ring were observed at (4.12) ppm and (5.83) ppm for H_3 and H4, respectively, while the ^{13}C NMR spectra showed a signal of (C=O lactam ring) observed at (166-169) ppm.

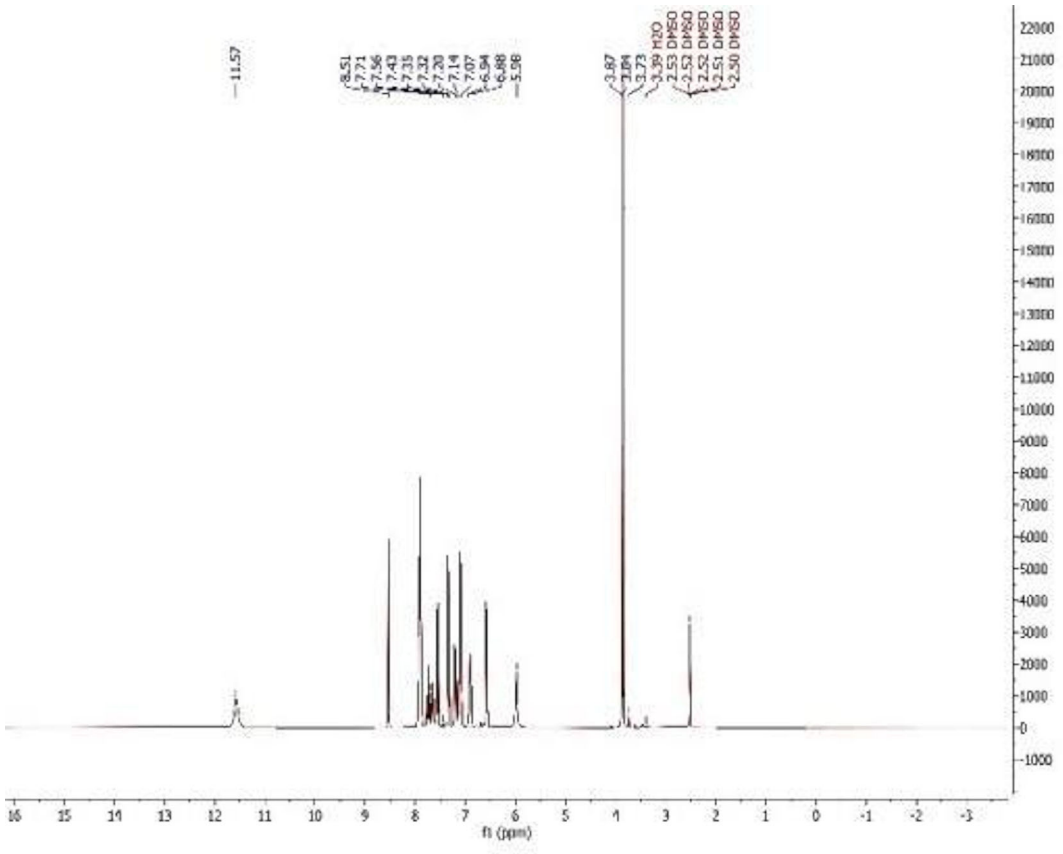


Figure 3. **^{1}H-NMR spectrum of compound A4.**

BIOLOGICAL ACTIVITY

Antibacterial and anti-fungal activity

The disk diffusion method was used to assess the antibacterial activity of all produced compounds against Staphylococcus aureus, Escherichia coli, and Candida albicans.[25]

- Compounds A7, A8, A9, and A12 demonstrated the strongest antibacterial activity.

- Compound A12 showed significant antifungal activity against C. albicans. - Electron-donating groups on the aromatic ring enhance biological activity.

The addition of diclofenac moieties (A9-A12) greatly increased potency.

Under identical testing settings, numerous drugs' inhibition zones (mm) matched or exceeded those of ceftriaxone and fluconazole.[26–28]

Molecular docking studies

A molecular docking investigation was carried out to evaluate the binding energy and interaction modes between ligands and target protein (PDB ID: 1EA1). The binding energy of the docking scores found in Table 2 is displayed, along with the names of the amino acids that are present in the protein structures that each derivative of β-lactam interacts with. The results showed that all our derivatives (**A10** and **A11**) have a higher binding energy (-9.0 and -8.4 kcal/mol, respectively) than acid (Table 1). Compound **A10** had a docking score of -9.0 kcal/mol, which was higher than the others. Compound **A10** is directly connected to amino acids THR A:80, ASP A:71, ARG A:95, and GLU A:94 in hydrogen bond interactions. Also, compound **A10** has a docking score of -8.4 kcal/mol because it is directly connected to amino acids ASP A:364, HIS A:363, PHE A:365, and HIS A:275 in hydrogen bond interactions. Table 2 in comparison to the internal ligand is depicted in two-dimensional and three-dimensional forms in Figure 14.

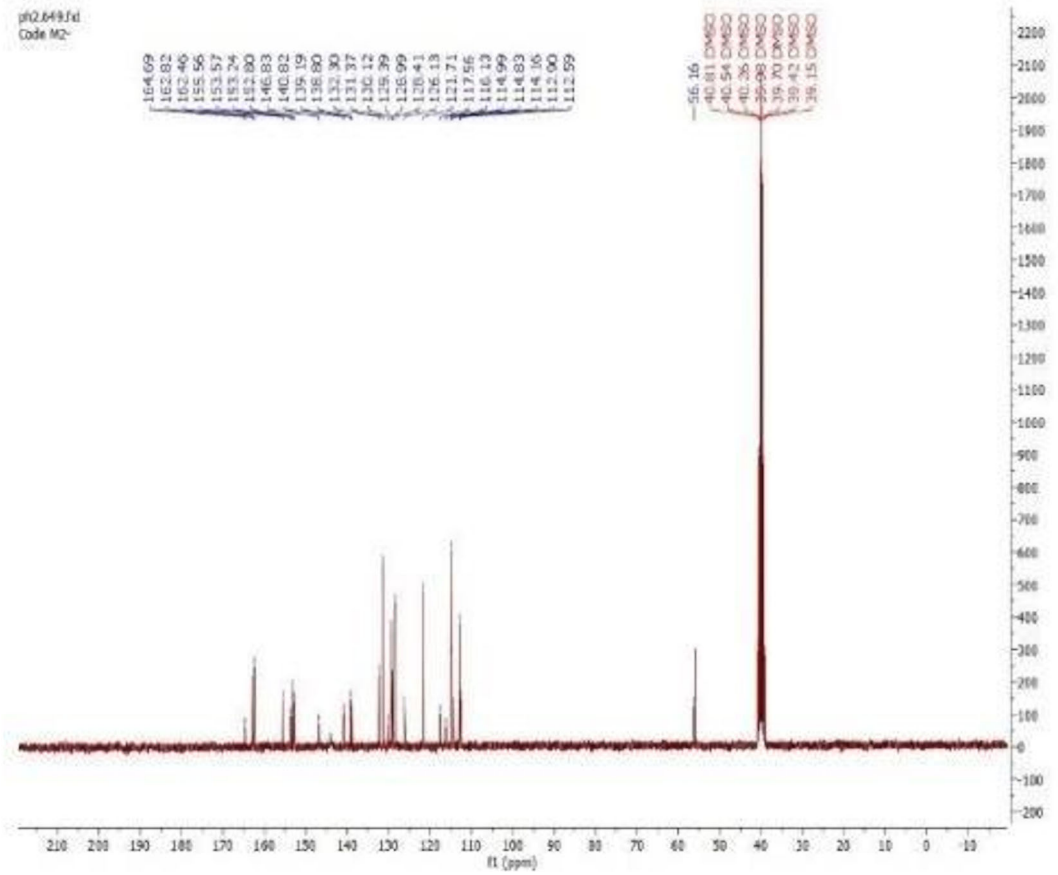


Figure 4. **^{13}C-NMR spectrum of compound A4.**

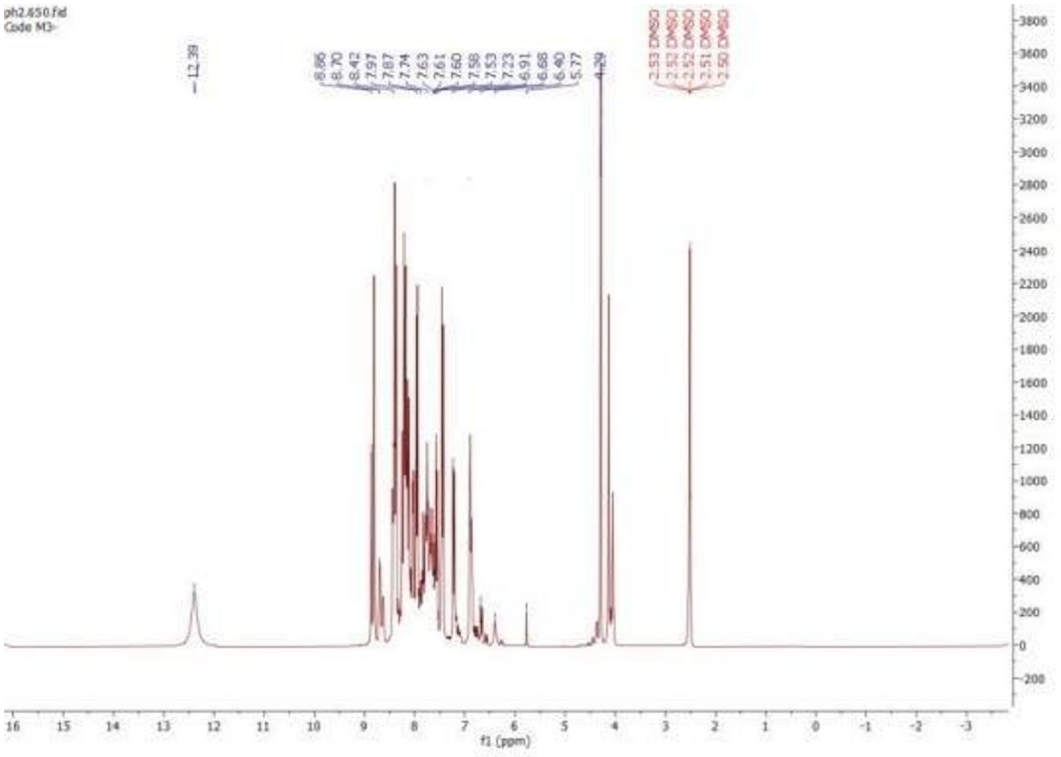


Figure 5. **^{1}H-NMR spectrum of compound A6.**

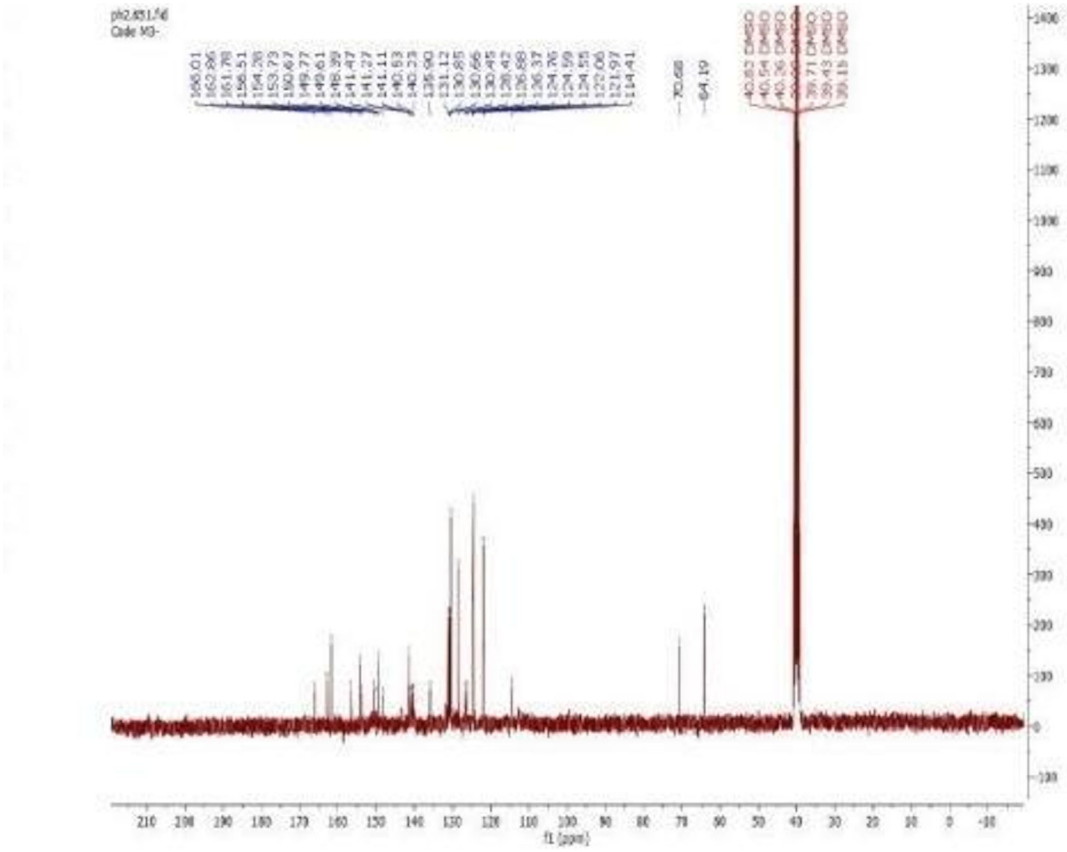


Figure 6. **^{13}C-NMR spectrum of compound A6.**

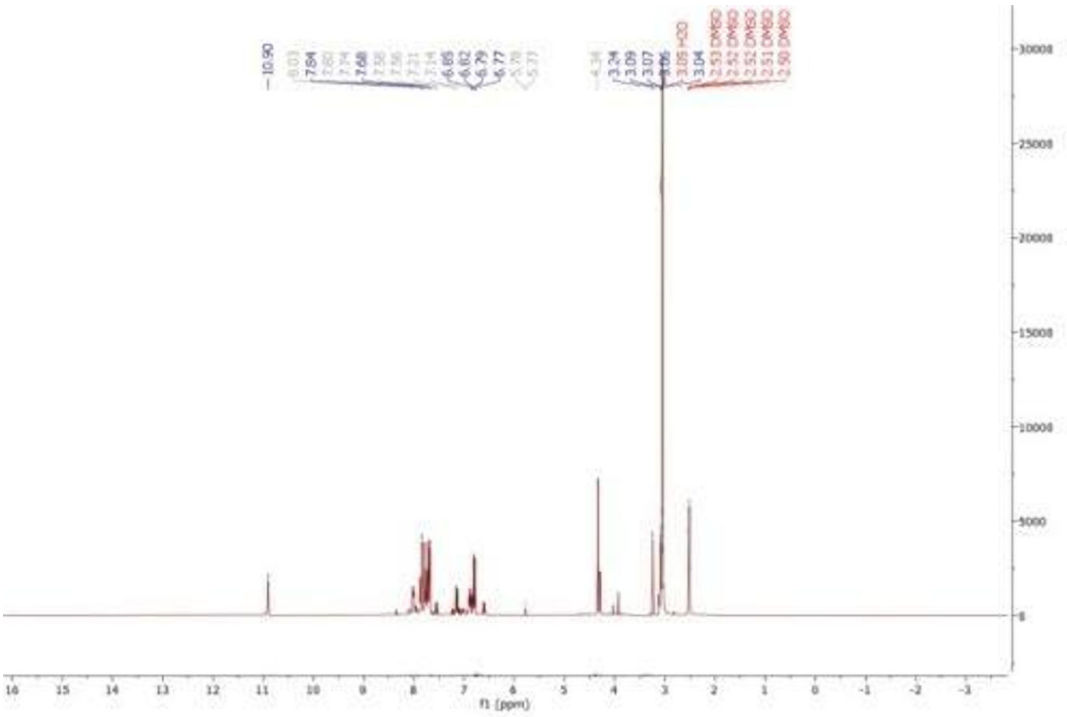


Figure 7. **^{1}H-NMR spectrum of compound A7.**

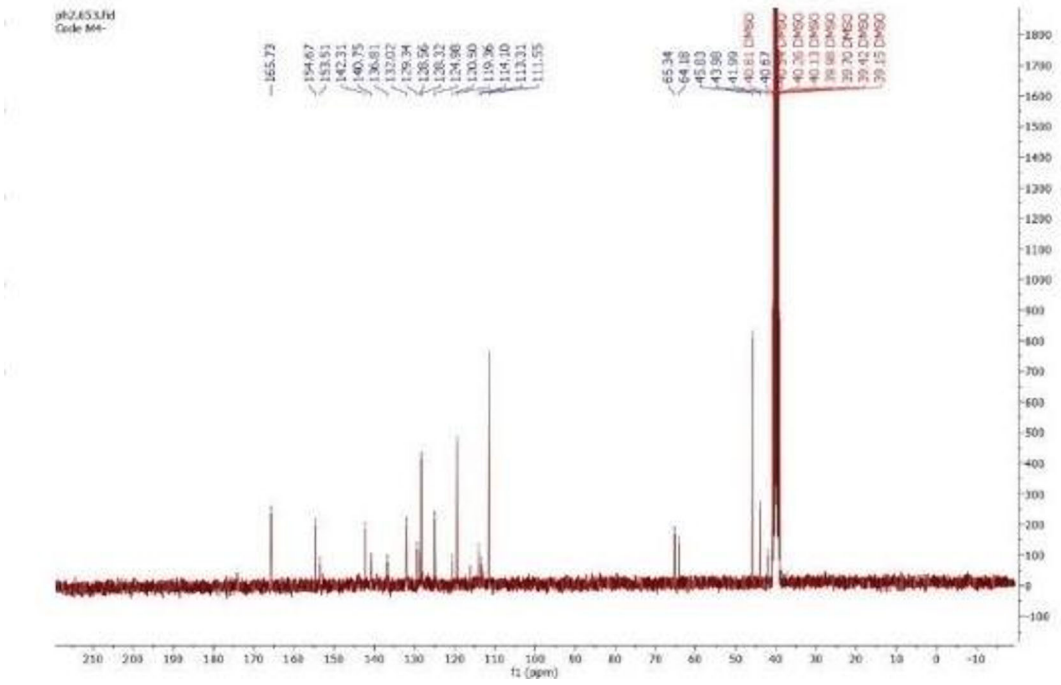


Figure 8. ^{13}C-NMR spectrum of compound A7.

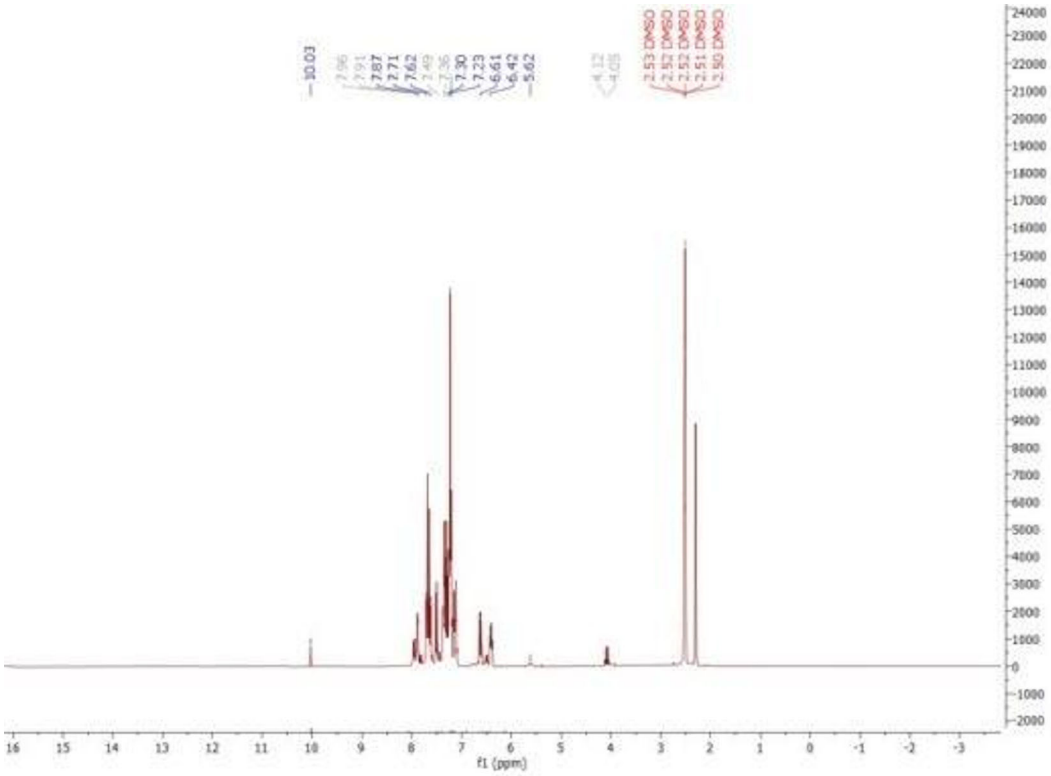


Figure 9. ^{1}H-NMR spectrum of compound A10.

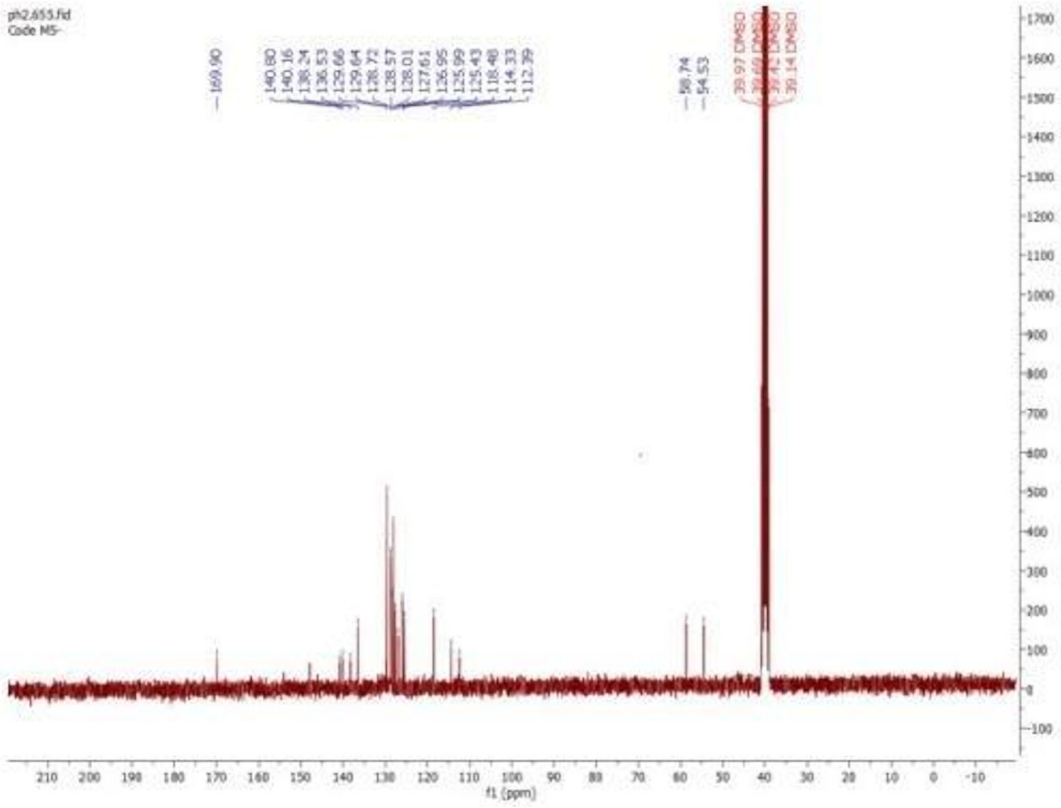


Figure 10. ^{13}**C-NMR spectrum of compound A10.**

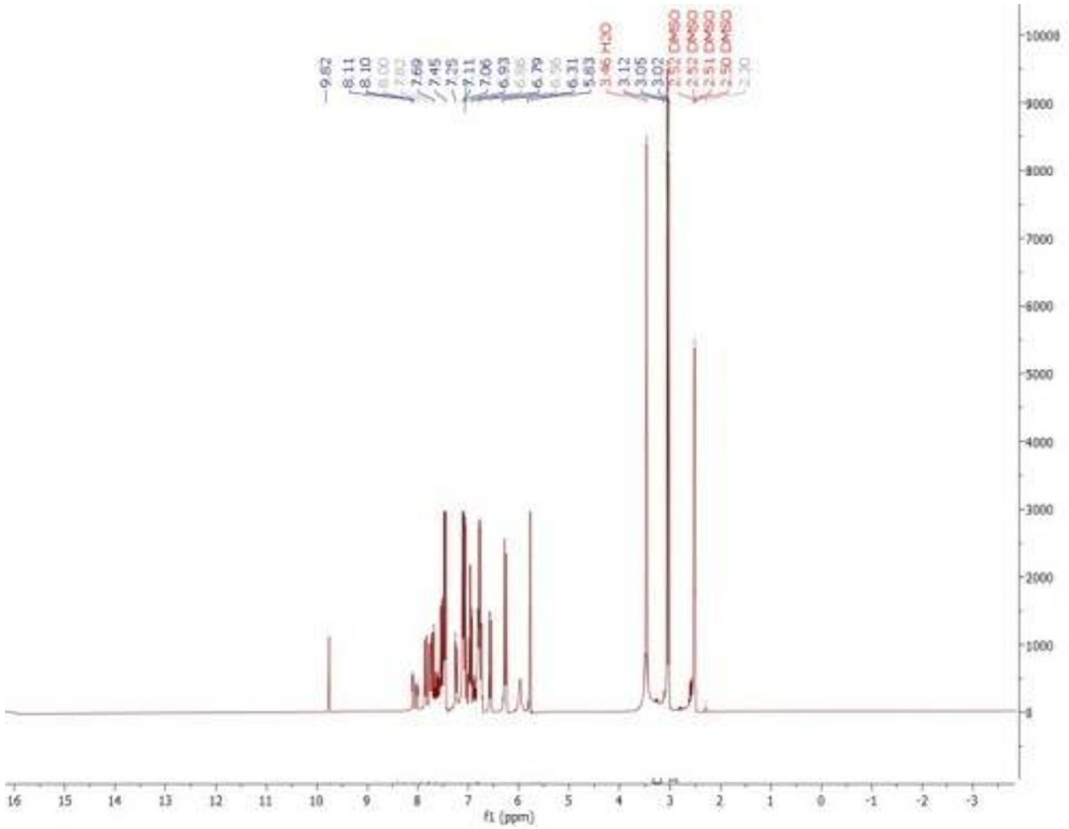


Figure 11. ^{1}**H-NMR spectrum of compound.**

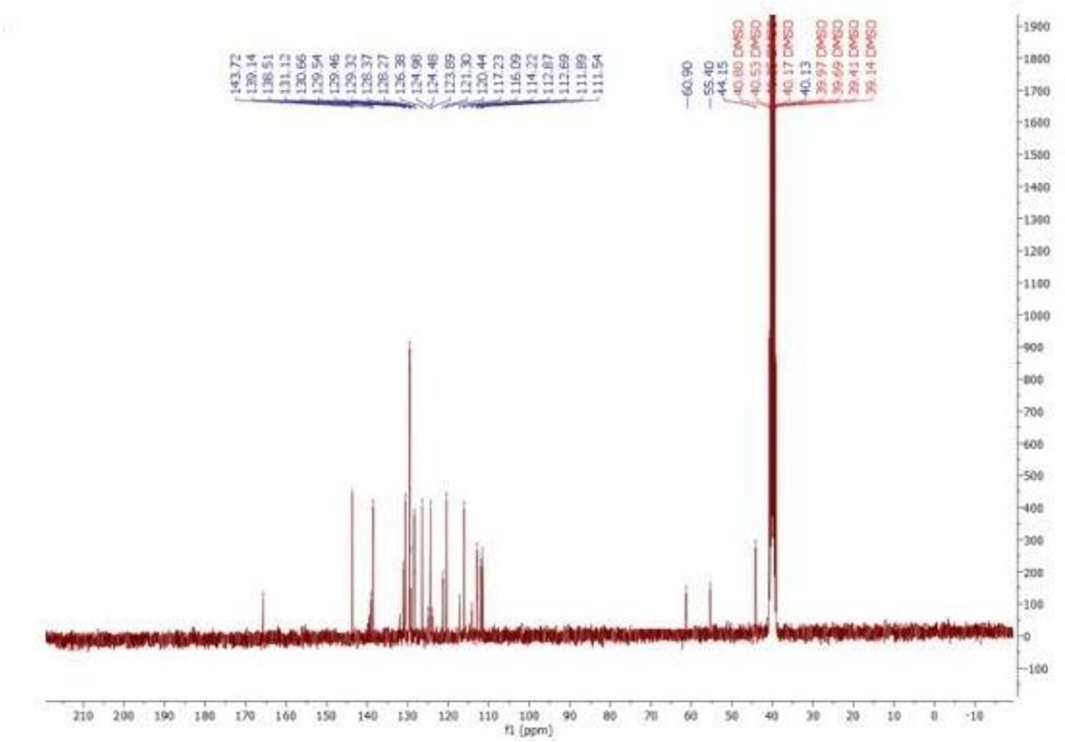


Figure 12. **^{13}C-NMR spectrum of compound A12.**

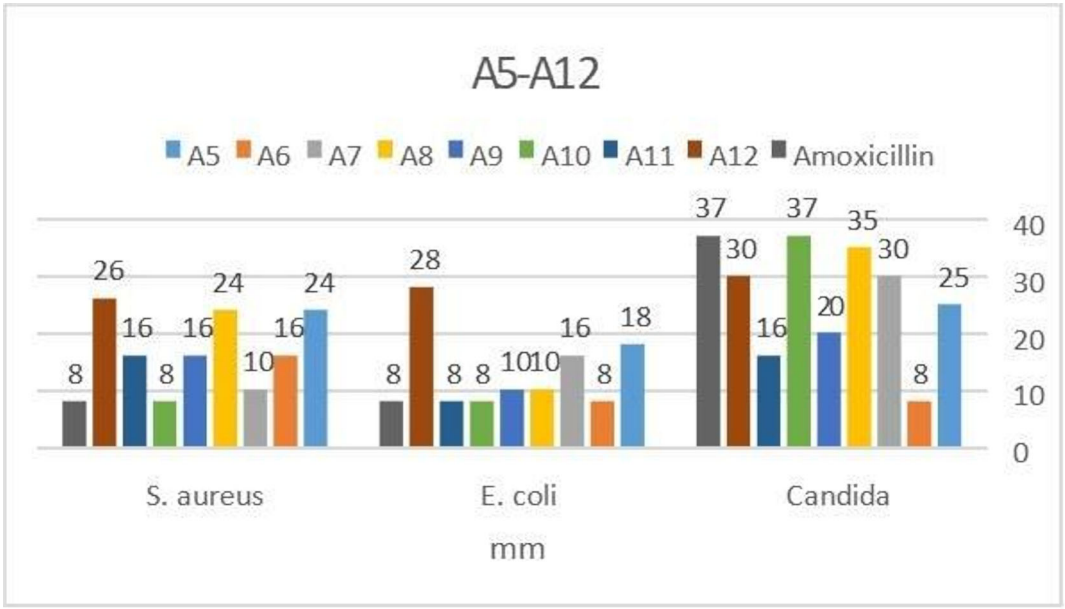


Figure 13. **Antimicrobial activity against Staphylococcus aureus, Escherichia coli, and Candida.**

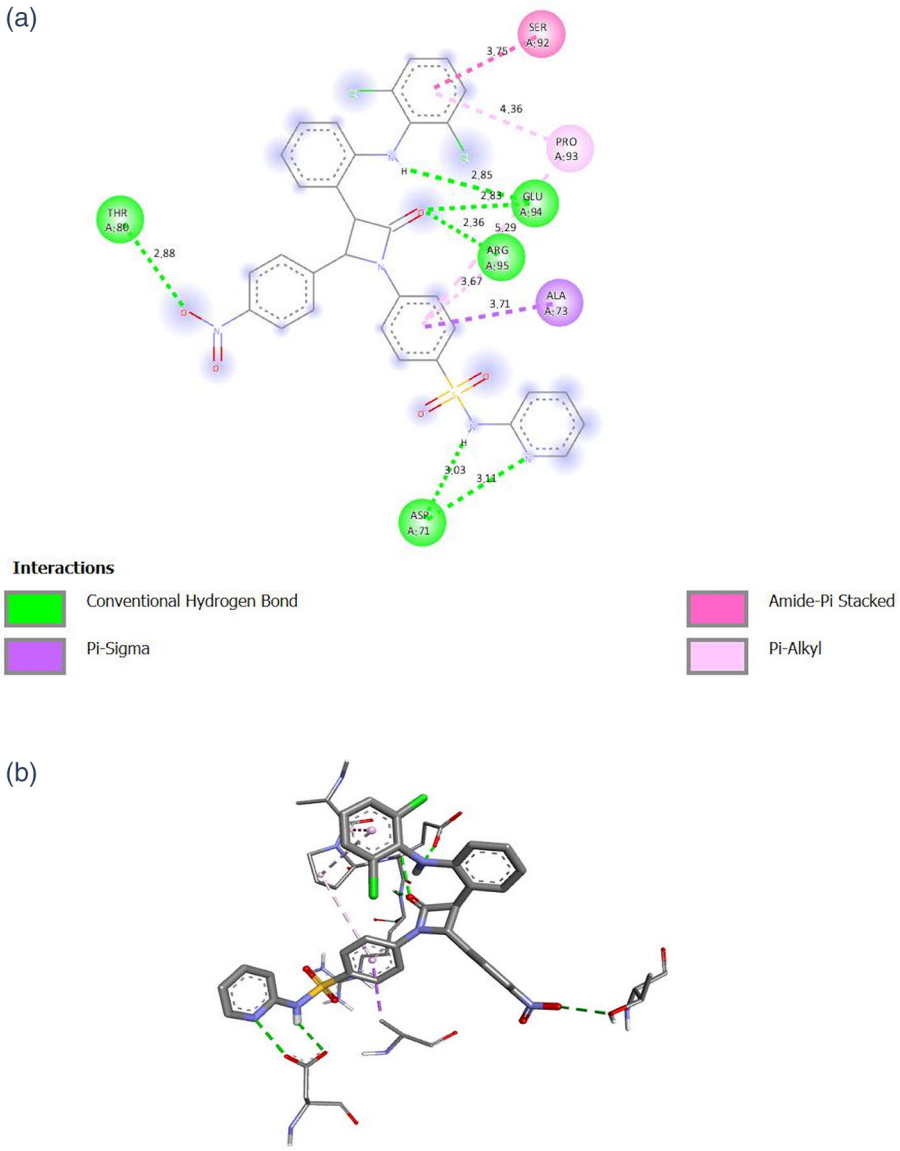
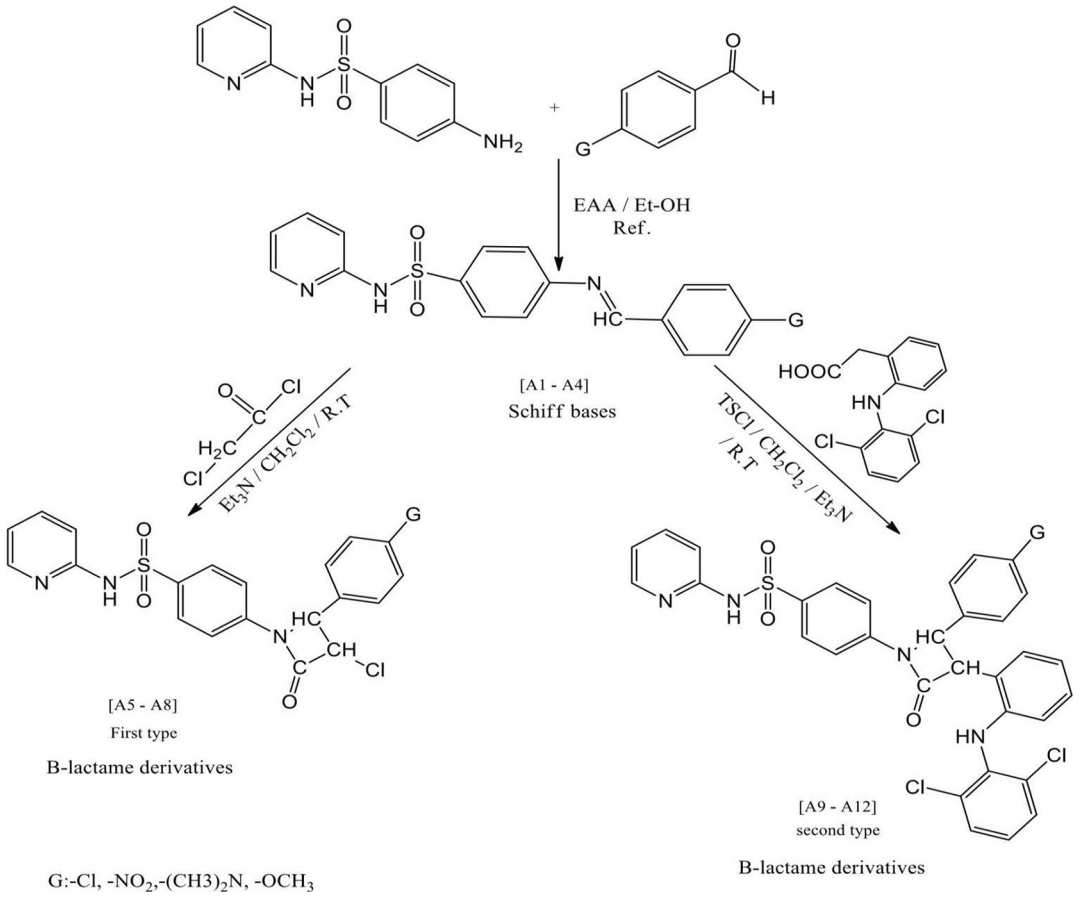


Figure 14. Two-dimensional and three-dimensional interactions of compounds A10, A11, and amoxicillin with the target protein (PDB ID: 1EA1).



Scheme 1. **Synthetic pathways for the preparation of β-lactam derivatives (A1–A12).**

Table 1. **The findings from the measurement of the synthetic compound's bacterial and fungal inhibitory zones (in millimeters) [A5-A12].**

Sample	S. aureus	E. coli	Candida
A5	24	18	25
A6	16	8	8
A7	10	16	30
A8	24	10	35
A9	16	10	20
A10	8	8	37
A11	16	8	16
A12	26	28	30
Amoxicillin	8	8	37

Table 2. Displays the results of a molecular docking study of ligands (amoxicillin, A10, and A11) against 1EA1.

Target protein	Compound name	Docking score (kcal/mol)	Distance (Å)	Interactions type	
				H-Bond	Other interactions
1EA1	**A5**	-7.4	2.52, 2.67, 2.80, 2.02, 1.58, 2.29, 4.01	LYS A:156	HIS A:113, ARG A:123, LYS A:155
	A6	-7.7	5.43, 3.21, 4.22, 2.03, 3.19, 3.22, 4.59, 2.29, 4.92, 2.79, 4.93	HIS A:430, ASN A:428	ILE A:27, HIS A:318, ARG A:354, PRO A:319, ARG A:427
	A7	-7.4	3.56, 4.18, 3.63, 3.70, 3.98, 4.48, 2.67, 3.92, 3.20, 4.96, 2.75	ARG A:381	VAL A:395, ALA A:398, CYS A:394, ALA A:397, ALA A:389, ARG A:393
	A8	-7.5	2.12, 1.92, 2.91, 1.60, 5.08, 3.48, 4.10, 3.02, 4.13	HIS A:311, THR A:116, GLY A:112MET A:225	LYS A:156, ASP A:222, LYS A:155
	A9	-7.7	4.36, 3.53, 5.39, 4.12, 2.69	THR A:24	ARG A:354, ILE A:27, ARG A:427
	A10	-9.0	2.88, 3.03, 3.11, 3.71, 3.67, 5.29, 2.36, 2.83, 4.46, 3.75	THR A:80, ASP A:71, ARG A:95, GLU A:94	ALA A:73, PRO A:93, SER A:92
	A11	-8.4	3.13, 316, 3.72, 5.27, 4.91, 4.02, 4.98, 4.24, 3.81, 5.15, 3.58, 2.26, 2.24, 3.05, 2.68, 4.20	ASP A:364, HIS A:363, PHE A:365, HIS A:275	PRO A:319, ILE A:27, ARG A:274, LEU A:317,
	A12	-7.8	5.37, 4.97, 1.98, 2.42, 4.99, 4.90, 4.72, 4.37, 5.32, 3.50	ASN A:428	HIS A:318, PRO A:319, ILE A:27, ALA A:350, HIS A:430
	Amoxicillin	-7.5	3.51, 2.31, 3.21, 2.97, 2.30, 2.01, 3.91, 2.74	ARG A:124, ARG A:158, GLU A:142, MET A:124, ASP A:127, ASP A:135	ILE A:134

Conclusion

The conclusions reached through the analysis of heterocyclic crop compounds indicate they will have significant impacts on antibacterial and antifungal research. The results from biological testing clearly indicated outstanding antibacterial activity. Compounds A5, A6, A7, A8, A9, A11, and A12 showed better inhibition against Staphylococcus aureus than amoxicillin. Compounds A5, A7, A8, A9, and A12 were also found to exhibit greater inhibition against ECM growth than ceftriaxone. Compound A10 produced moderate antifungal activity. The significant antibacterial activity shown by these newly created synthetic compounds indicates further studies on these compounds will benefit the advancement of the knowledge base of pharmacological properties and ultimately the development of new sources of alternative antimicrobial therapies for drug-resistant pathogens. This study provides the necessary foundational basis upon which to create powerful antibacterial and antifungal agents. In conclusion, compounds **A10** and **A11** had a higher binding affinity than amoxicillin against the target protein (**PDB ID: 1EA1**), and their possible biological significance is highlighted by the consistency between in silico and in vivo results.

Ethical approval

Ethical approval for this study was obtained from the Ethical Committee of Al-Fallujah University College of Medicine, dated 26/11/2025. Written informed consent was obtained from all participants.

Data availability

All data supporting the findings of this study, including raw FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra, antibacterial and antifungal activity measurements, and molecular docking data, are available in the Zenodo repository under a Creative Commons Attribution (CC-BY) license and can be accessed via the following DOI: https://doi.org/10.5281/zenodo.18212745

Acknowledgment

The authors are grateful to the Department of Chemistry, College of Science, University of Baghdad, for providing laboratory facilities, apparatus, and ongoing technical support during this study. The authors also thank the microbiology laboratory workers for their help with antimicrobial evaluations.

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 23 March 2026

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Detailed Evaluation Report

1. Is the work clearly and accurately presented and does it cite the current literature?
Answer: Partly
Comments:
The manuscript is well-structured and presents the synthesis and results clearly. However, the discussion lacks critical comparison with recent literature, and the antimicrobial results are not sufficiently contextualized with previously reported β-lactam derivatives. Additionally, some grammatical issues affect readability.
Recommendations:
Include recent (2020–2025) high-impact references, add comparative discussion with literature (e.g., inhibition zones and docking results), and improve the overall scientific language and clarity.

2. Is the study design appropriate and is the work technically sound?
Answer: Partly
The synthetic methodology and characterization are appropriate. However, there are major concerns regarding the molecular docking study, as the selected protein (PDB ID: 1EA1) is incorrectly assigned and corresponds to *Mycobacterium tuberculosis* CYP51, making the docking results unreliable. Additionally, the biological evaluation is limited to a single strain per group without justification.
Recommendations:
Re-perform docking using appropriate bacterial and fungal targets, clarify whether docking guided the synthesis, and expand antimicrobial studies to include multiple strains.

3. Are sufficient details of methods and analysis provided to allow replication?
Answer: Partly
The synthetic procedures appear reproducible; however, several critical details are missing, including incomplete NMR data for some compounds, absence of ATCC strain information, lack of MIC values, and no description of control experiments (e.g., DMSO negative control).
Recommendations:
Provide complete spectral data, include MIC values, specify microbial strains (ATCC codes), and clearly describe control experiments and experimental conditions.

4. If applicable, is the statistical analysis and its interpretation appropriate?
Answer: Yes
Experiments were conducted in triplicate and reported as mean ± SD.
Recommendation:
Include statistical significance testing (e.g., p-values) to strengthen data validation.

5. Are all the source data underlying the results available to ensure full reproducibility?
Answer: Partly
he data availability statement is provided; however, spectral figures are unclear, and the docking data is unreliable due to incorrect protein selection.
Recommendations:
Provide high-resolution spectra with proper assignments, ensure complete raw data availability, and revalidate docking using appropriate targets.
6. Are the conclusions drawn adequately supported by the results?
Answer: Partly
The conclusions align with the experimental results; however, there is overinterpretation of docking data, weak correlation between biological and computational findings, and inconsistency in reference drugs used.
Recommendations:
Avoid overgeneralization, use consistent reference drugs, and strengthen the discussion with mechanistic insights and SAR analysis.
Major Issues (Must be addressed)

- Incorrect molecular docking (wrong protein selection)
- Incomplete characterization data (missing NMR, poor spectra)
- Inadequate antimicrobial evaluation (no MIC, limited strains, missing controls)
- Methodological gaps (no ATCC details, insufficient experimental description)
- Inconsistent reference drugs between studies

Minor Issues

- Improve grammar and clarity
- Standardize formatting (e.g., italicize microorganisms)
- Expand introduction and SAR discussion
- Improve figure quality

Final Comment

The study is promising, but major revisions in docking accuracy, biological validation, and data completeness are necessary to ensure scientific reliability and reproducibility.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Organic chemistry, Antibiotic, Novel compound design and Synthesis , Green Chemistry, Medicinal Chemistry, Nanotechnology, Heterocyclic Chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 13 March 2026

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Peer Review

This article investigates the synthesis, characterization, and effects of new β-lactam derivative compounds on antimicrobial activity both in vitro and in silico. The study is original due to its synthesis, characterization, and biological evaluation of new compounds. Furthermore, the compound design and synthesis sections are particularly noteworthy. However, some parts are unclear and require clarification.

Author Question 1: In this study, were the in silico studies (such as molecular docking and protein binding theoretical calculations) performed during the compound design phase or after synthesis? The authors are expected to provide clarification on this matter.

The necessary revisions to the article are listed below;

1: The introduction details the antibacterial activities of β-lactam derivatives using Gram-negative bacteria, but does not explain their relationship with Gram-positive bacteria and fungi such as Candida. Additionally, the selective toxicity, a key characteristic of β-lactam derivatives, is not emphasized.

2: The introduction details the synthesis of sulfonamide derivatives but does not mention the antimicrobial activities of sulfonamide drugs.

3: In the materials and methods section, the A2, A3, A5, A8, A9, and A11 NMR data for some compounds are missing, and the NMR data for other compounds are incomplete. These sections need to be revised.

4: In the materials and methods section, it should be specified whether the bacteria and fungal organisms used in the antimicrobial activity of the compounds are standard ATCC strains or isolates. If they are ATCC strains, their codes should be provided. Furthermore, to define antimicrobial activity, at least two different microorganisms from each group should be studied, and the results should be verified with at least two different analyses. A minimum inhibitory concentration value is required in this study.

5: In the materials and methods section, the compounds were dissolved in DMSO for antimicrobial activity studies, and DMSO is considered toxic to living organisms. If DMSO was used directly in the study, a negative control should have been conducted without the drugs, specifying the drug dissolution conditions. This information is not included in the methods section and needs to be explained in detail.

6: In the materials and methods section, although molecular docking analyses were performed with the protein coded PDB ID: 1EA1, a Candida-related EA1 code is given. This section should be corrected. Also, why were docking studies only performed using the protein associated with Candida? Shouldn't specific proteins have been examined for all microorganisms used in the study? Furthermore, the protein code given as Candida-related (PDB ID: 1EA1) matches "Cytochrome P450 14 alpha-sterol demethylase (CYP51) from *Mycobacterium tuberculosis* in complex with fluconazole" in the protein data bank, not Candida. Docking data cannot be considered accurate in this way.

7: In the results section, the NMR images of the compounds are unclear and should be re-edited. Furthermore, the NMR images show many peaks and noise in the aromatic region, making it difficult to characterize the structure. Also, the results have not been thoroughly discussed in comparison with the literature.

8: In the results section, microorganism names should be written in plain text and italicized in the antimicrobial analysis results. Additionally, the study results have not been sufficiently discussed.

9: In the results section, why were Ceftriaxone and fluconazole not used as positive controls in molecular docking analyses, and why was amoxicillin used instead? If amoxicillin was used in the docking study, why were Ceftriaxone and fluconazole used in the antimicrobial experiment? This

part needs clarification.

This makes it quite difficult to connect the compounds with their biological analyses.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Organic chemistry, Microbiology, Cancer research, Antibiotic, Novel compound design and Synthesis , biological activity

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.