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# New 2-Azetidinone and 1,3-Oxazepine Derivatives: Synthesis, Characterization, Antimicrobial Activity, and Molecular Docking Analysis

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**Abstract:** A novel series of 2-azetidinone and 1,3-oxazepine derivatives (6a–11a) was synthesized via cyclization and acylation reactions, starting from chalcone intermediate (1a) and proceeding through key pyrazoline (2a) and Schiff base intermediates (3a–5a). Structural characterization was performed using FT-IR, $^1$H NMR, and GC-MS techniques. The synthesized compounds were evaluated for their antimicrobial activity against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella species, and Candida albicans, exhibiting moderate to potent inhibitory effects. To support the biological findings, molecular docking studies were carried out against the MurC enzyme, a crucial target in bacterial cell wall biosynthesis. The docking results revealed favorable binding interactions and showed good agreement with the in vitro activity, highlighting the potential of these derivatives as promising antimicrobial candidates.

## Introduction.

The alarming rise in antimicrobial resistance represents a growing global health concern, necessitating the urgent development of new and more effective therapeutic agents (1). In this context, heterocyclic compounds have garnered significant interest in medicinal chemistry due to their structural diversity and wide range of biological activities (2). Among these, β-lactams (2-azetidinones) and 1,3-oxazepines have emerged as promising scaffolds with notable antimicrobial potential (3,4). 2-Azetidinones, the core structure of β-lactam antibiotics, exert their antimicrobial action primarily by inhibiting bacterial cell wall biosynthesis (5). Structural modifications of the azetidinone ring have shown promise in overcoming bacterial resistance and enhancing bioactivity (6). Similarly, 1,3-oxazepines, seven-membered heterocycles incorporating both nitrogen and oxygen atoms, have demonstrated diverse pharmacological profiles, including antimicrobial, anticancer, and anti-inflammatory activities (7). In the present study, a novel series of 2-azetidinone and 1,3-oxazepine derivatives (6a–11a) was synthesized and structurally characterized using various spectroscopic techniques. The antimicrobial activity of these compounds was evaluated in vitro against a panel of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria as well as Candida albicans. To further explore their potential mechanism of action, molecular docking studies were conducted using AutoDock 4.2, targeting the UDP-N-acetylmuramoyl-L-alanine ligase (MurC) enzyme, a key target in bacterial cell wall biosynthesis and a validated antimicrobial target. The docking results revealed favorable binding affinities and stable interactions within the enzyme's active pocket,

consistent with the in vitro findings, thereby supporting the potential of these derivatives as promising antimicrobial candidates (8,9).

## 1. Experimental

### 1.1. Material

All chemicals and reagents utilized in this study were procured from Sigma-Aldrich, Fluka, and BDH companies. Melting points were determined using an electrothermal apparatus (Stuart SMP-30) with open capillary tubes, and the values were reported without correction. Fourier-transform infrared (FT-IR) spectra were recorded using a Shimadzu FTIR-8400S spectrometer. Mass spectra (MS) were obtained on a Shimadzu GCMS-QP2010 Ultra system. Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded in either $CDCl_3$ or DMSO-$d_6$ as solvents, using a Varian 500 MHz NMR spectrometer.

1.2. Synthesis

1.2.1. Synthesis of 1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one (1a)

This compound was synthesized following a previously reported procedure (10). Briefly, an alkaline solution of sodium hydroxide (40%, 1 mL) was added to a solution of 4-aminoacetophenone (1 mmol, 0.135 g) in absolute ethanol (10 mL). The reaction mixture was stirred for 30 minutes at room temperature. Subsequently, furan-2-carboxaldehyde (1 mmol) was added, and the mixture was stirred overnight. Progress of the reaction was monitored by thin-layer chromatography (TLC) using hexane:ethyl acetate mixtures in ratios of 1:1 as eluents. Upon completion, the reaction mixture was allowed to stand at room temperature to facilitate precipitation. The resulting solid was then collected by filtration, dried, and recrystallized from ethanol to afford the pure product.

Yellow powder, yield 91%, m.p 108-110◦C, FT-IR ($cm^{-1}$): 3356, 3327 ($NH_2$), 3128,3099 (aromatic C-H), 2872 (aliphatic C-H), 1637 (C=O), 1602 (CH=CH), 1581 (C=C aromatic). $^1$H NMR (500MHz, DMSO-d6) δ (ppm): 6.16 (s, 2H, $NH_2$), 7.40-7.89 (m, 5H , -CH=CH,Ar-H furane),6.57-7.00 (m, 4H, Ar-H ). Mass (EI) m/e: 213 $M^+$ for $C_{13}H_{11}NO_2$, R*f*= 0.8 (1:1, Hexane: Ethyl acetate).

1.2.2. Synthesis of 4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)aniline (2a)

The synthetic procedure for this compound was slightly modified from a previously reported method (11). A mixture of chalcone derivative (1a, 1 mmol) in absolute ethanol (10 mL) and an excess of hydrazine hydrate (80%, 1 mL) was refluxed for 6 hours. The progress of the reaction was monitored using thin-layer chromatography (TLC) with hexane:ethyl acetate (1:1) as the eluent. Upon completion, the reaction mixture was poured onto crushed ice to induce precipitation. The resulting solid was collected by filtration, thoroughly washed with distilled water, dried, and recrystallized from ethanol to obtain the purified product.

Off-white powder, yield 65%, m.p 114-116 C◦; FT- IR ($\bar{\upsilon}$ $cm^{-1}$) , Figure 3-9: 3456, 3317 (NH2), 3213 (NH-pyrazoline), 3097, 3032 (aromatic C-H), 2970, 2885 (aliphatic C-H), 1639 (C=N pyrazoline) ,1606 (C=C furane) , 1587 (C=C aromatic). $^1$H-NMR (500 MHz, DMSO-d6) δ (ppm) , 2.97, 3.00 (dd, 1H, Ha-pyrazoline, j= 9.98, 9.98 Hz), 3.21-3.24 (dd, 1H, Hb-pyrazoline, j= 9.98, 9.98 Hz), 4.75 (t, 1H, Hx-pyrazoline, j=9.98, 9.98 Hz), 6.31 (s, 1H, NH-pyrazoline), 6.32 (s, 2H, $NH_2$ ), 6.56 (m, 3H, 2 Ar-H , Ar-H furane), 7.33 (m, 3H, 2Ar-H , Ar-H furane j=9.98 Hz), 7.58 (d, 1H, Ar-H furane, j=4.98 Hz). Mass (NCI) m/z, 227 M+ for $C_{13}H_{13}N_3O$, R*f* =0.34 (1:1, Hexane: Ethyl acetate).

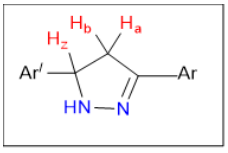



**Figure (1):** structure of compound 2a

1.2.3. Synthesis of Schiff bases (3a-5a)

These compounds were synthesized following a modified procedure based on a previously reported method (12). A solution of aromatic aldehyde (1 mmol) in absolute ethanol (20 mL) containing a few drops of glacial acetic acid was prepared, to which pyrazoline derivative (2a, 1 mmol) was added. The reaction mixture was then refluxed for 10–12 hours, and the reaction progress was monitored by thin-layer chromatography (TLC) using hexane:ethyl acetate (1:2) as the eluent. Upon completion, the resulting solid was filtered, washed with cold ethanol or water as necessary, and recrystallized from ethanol to afford the pure product.

1.2.3.1 N-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-1-(thiophen-2-yl)methanimine (3a)

Yellow powder, yield 60 %, m.p89-92 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$) , 3373 (NH-pyrazoline), 3142, 3103 (C-H aromatic), 2941, 2864 (HC=N), 1653 (C=N) , 1616 (C=N pyrazoline ) , 1591 (C=C aromatic). $^{1}$H-NMR (500MHz, DMSO-d6) δ (ppm): 3.29 (m,1H, Ha-pyrazoline), 3.39 (m, 1H, Hb-pyrazoline), 4.81 (m, 1H, Hx-pyrazoline),6.46 (s, 1H, NH-pyrazoline) , 7.05-8.15 (m, 10H, Ar-H) , 8.85 (s, 1H, HC=N). Mass (NCI) m/z, 321 M+ for $C_{18}H_{15}N_3OS$, Rf= 0.71(1:2, Hexane: Ethyl acetate).

1.2.3.2. 1-(furan-2-yl)-N-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)methanimine (4a)

Yellow powder, yield 62 %, m.p95-98 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$), 3387 (NH-pyrazoline), 3119, 3041 (C-H aromatic), 2847 (HC=N), 1674(C=N) , 1624 (C=N pyrazoline ) , 1593 (C=C aromatic). 1H-NMR (500MHz, DMSO-d6) δ (ppm), 3.29 (m,1H, Ha-pyrazoline), 3.37 (m, 1H, Hb-pyrazoline), 5.44 (m, 1H, Hx-pyrazoline),6.55 (s, 1H, NH-pyrazoline) , 6.73-7.97 (m, 10H, Ar-H), 8.49 (s, 1H, HC=N) .Mass (NCI) m/z, 305 M+ for $C_{18}H_{15}N_3O_2$, R*f*= 0.87(1:2, Hexane: Ethyl acetate).

1.2.3.3. N-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-1-(pyridin-4-yl)methanimine (5a)

Yellow powder, yield 68 %, m.p123-125 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$) , 3377 (NH-pyrazoline), 3120 , 3043 (C-H aromatic), 2916,2845 (HC=N), 1600 (C=N) , 1562 (C=N pyrazoline ) , 1519 (C=C aromatic). $^{1}$H-NMR (500MHz, DMSO-d6) δ (ppm) , 3.42 (m, 1H, Ha-pyrazoline), 3.60 (m, 1H, Hb-pyrazoline), 5.68 (m, 1H, Hx-pyrazoline), 5.78 (s, 1H, NH-pyrazoline), 6.6-8.5 (m, 11H, Ar-H), 8.90 (s, 1H, HC=N). $C_{19}H_{16}N_4O$, R*f*= 0.21 (1:2, Hexane: Ethyl acetate).

1.2.4. Synthesis of 2-azetidinone derivatives (6a, 8a, 10a)

These derivatives (6a, 8a, 10a) were synthesized according to a modified procedure in the published work(13). A mixture of Schiff bases (3a, 4a, 5a) (1mmol) and triethyl amine (2mmol) dissolved in 1,4-dioxane (25 mL) was stirred in ice bath at 0-5 C◦, then a solution of chloroacetyl chloride (4 mmol) was added drop by drop for a period of 0.5 h. The reaction was further stirred for 6-12 h (monitored from TLC). After that, the reaction mixture was poured into crushed ice and the resultant product was filtered, washed with water, dried and recrystallized from dioxane.

1.2.4.1. 3-chloro-1-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4-(thiophen-2-yl)azetidin-2-one (6a)

Brown powder, yield 43%, m.p 121-123 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$), 3207 (NH-pyrazoline), 3101,3009 (aromatic C-H), 2930, 2807 (aliphatic C-H), 1747(C=O), 1606 (C=N pyrazoline ), 1521 (C=C aromatic).$^{1}$H-NMR (500 MHz, DMSO-d6) δ (ppm): 3.23 (m,1H, Ha- pyrazoline), 3.95 (m, 1H, Hb-pyrazoline), 4.11-4.06 (d, 1H, CH-N), 4.93 (d, 1H, CH-Cl), 5.35 (m, 1H, Hx-pyrazoline), 6.13 (s, 1H, NH-pyrazoline) , 6.55-8.11 (m, 10H, Ar-H), R*f* = 0.51 (1:3, Hexane: Ethyl acetate).

1.2.4.2. 3-chloro-4-(furan-2-yl)-1-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)azetidin-2-one (8a)

Brown powder, yield 40%, m.p 129-131 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$) , 3207 (NH-pyrazoline), 3101,3009 (aromatic C-H), 2854, 2821 (aliphatic C-H) , 1745 (C=O) , 1695 (C=N pyrazoline), 1521 (C=C aromatic) . $^{1}$HNMR (500 MHz,DMSO-d6) δ (ppm): 3.18 (m,$^{1}$H, Ha- pyrazoline), 3.88 (m, 1H, Hb-pyrazoline), 4.01-3.91 (d, 1H, CH-N), 4.92 (d, 1H, CH-Cl), 5.43 (m, 1H, Hx-pyrazoline), 6.10 (s, 1H, NH-pyrazoline) , 6.65-8.3 (m, 10H, Ar-H), R*f* = 0.44 (1:3, Hexane: Ethyl acetate).

1.2.4.3. 3-chloro-1-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4-(pyridin-4-yl)azetidin-2-one (10a)

Brown powder, yield 35%, m.p 198-200 C◦;FT- IR ($\bar{\upsilon}$ $cm^{-1}$) , 3322 (NH-pyrazoline), 3101,3009 (aromatic C-H), 2930, 2907 (aliphatic C-H) , 1755 (C=O), 1602 (C=N pyrazoline) , 1519 (C=C aromatic). $^{1}$H-NMR (500 MHz, DMSO-d6) δ (ppm): 3.21 (m,1H, Ha- pyrazoline), 3.52 (m, 1H, Hb-pyrazoline), 4.09 (d, 1H, CH-N), 4.88 (d, 1H, CH-Cl), 5.38 (m, 1H, Hx-pyrazoline), 6.13 (s, 1H, NH-pyrazoline) , 6.61-8.5 (m, 11H, Ar-H), R*f* = 0.41 (1:3, Hexane: Ethyl acetate).

1.2.5. Synthesis of 1,3-Oxazepine derivatives (7a,9a, 11a)

These derivatives (7*a*, 9*a*,11*a*) were synthesized according to modified procedure in reported reference(14). To a solution of Schiff's base (3a, 4a, 5a) (1 mmol) in 1,4-dioxane (25 mL), phthalic anhydride (1 mmol) was added. The mixture was refluxed for 10–12 h. After the completion of the reaction (monitored by TLC using Hexane: Ethyl acetate (1:1) as an eluent), the precipitate formed was filtrated off then washed with cold water, dried and recrystallized form 1,4-dioxane.

1.2.5.1. 4-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-3-(thiophen-2-yl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione (7a)

Light yellow powder, yield 47%, m.p 144-146 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$), 3296 (NH-pyrazoline), 3039 (aromatic C-H), 1747,1716 (C=O Oxazepine), 1670 (C=N pyrazoline ),1597 (C=C aromatic). R*f*=0.40 (1:1 Hexane: Ethyl acetate).

1.2.5.2. 3-(furan-2-yl)-4-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione (9a)

Light yellow powder, yield 45%, m.p 138-140 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$) , 3296 (NH-pyrazoline), 3039 (aromatic C-H), 1747,1716 (C=O Oxazepine), 1670 (C=N pyrazoline ) , 1597 (C=C aromatic).R*f*=0.45(1:1 Hexane: Ethyl acetate).

1.2.5.3. 4-(4-(5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-3-(pyridin-4-yl)-3,4-dihydrobenzo[e][1,3]oxazepine-1,5-dione (11a)

Brown powder, yield 65%, m.p 128-130 C◦; FT-IR ($\bar{\upsilon}$ $cm^{-1}$), 3419 (NH-pyrazoline), 3093 (aromatic C-H), 1786,1714 (C=O Oxazepine), 1672 (C=N pyrazoline ) , 1514 (C=C aromatic). 1H-NMR (500MHz,DMSO-d6) δ (ppm) , 2.96 (m,1H, Ha- pyrazoline), 3.65 (m, 1H, Hb-pyrazoline), 4.36 (m, 1H, Hx-pyrazoline), 5.62 (s, 1H, NH-pyrazoline), 6.43- 8.77 (m, 15H, Ar-H), 10.45 (s, 1H, C-H Oxazepine).R*f*=0.49 (1:1 Hexane: Ethyl acetate).

**2. Antimicrobial Study**

The agar well-diffusion method (15) was employed to evaluate the antimicrobial activity of the synthesized pyrazoline derivatives (3–9) against a panel of microbial strains, including Gram-positive bacteria (Staphylococcus aureus and Staphylococcus epidermidis), Gram-negative bacteria (Escherichia coli and Klebsiella sp.), and the yeast Candida albicans, as summarized in Table 1. The assays were conducted at the Department of Biology, College of Science, Mustansiriyah University. Each compound was tested at a concentration of 1000 µg/mL, with DMSO serving as both the solvent and negative control. Amoxicillin was used as the positive control (standard drug) to compare the antimicrobial efficacy.

## 3. Docking Study

AutoDock 4.2 was employed to evaluate the binding affinity of the synthesized 2-azetidinone and 1,3-oxazepine derivatives (6a–11a) towards the active site of UDP-N-acetylmuramoyl-L-alanine ligase (MurC), following the protocol reported in reference (16). The crystal structure of the target enzyme (PDB ID: 1UAE) was retrieved from the RCSB Protein Data Bank and treated as a rigid receptor. Prior to docking, all water molecules were removed, and hydrogen atoms were added to the amino acid residues. The chemical structures of the derivatives were initially drawn using GaussView 5.0 and saved in MOL format, then converted to PDB format using Open Babel 3.1.1. Docking simulations were performed using a grid box of 40 × 40 × 40 Å with center coordinates set to x = 44.268, y = 20.503, and z = 40.622. The Lamarckian Genetic Algorithm (LGA) was utilized for docking, applying a population size of 150, 10 docking runs, a maximum of 2,500,000 energy evaluations, and 27,000 generations.

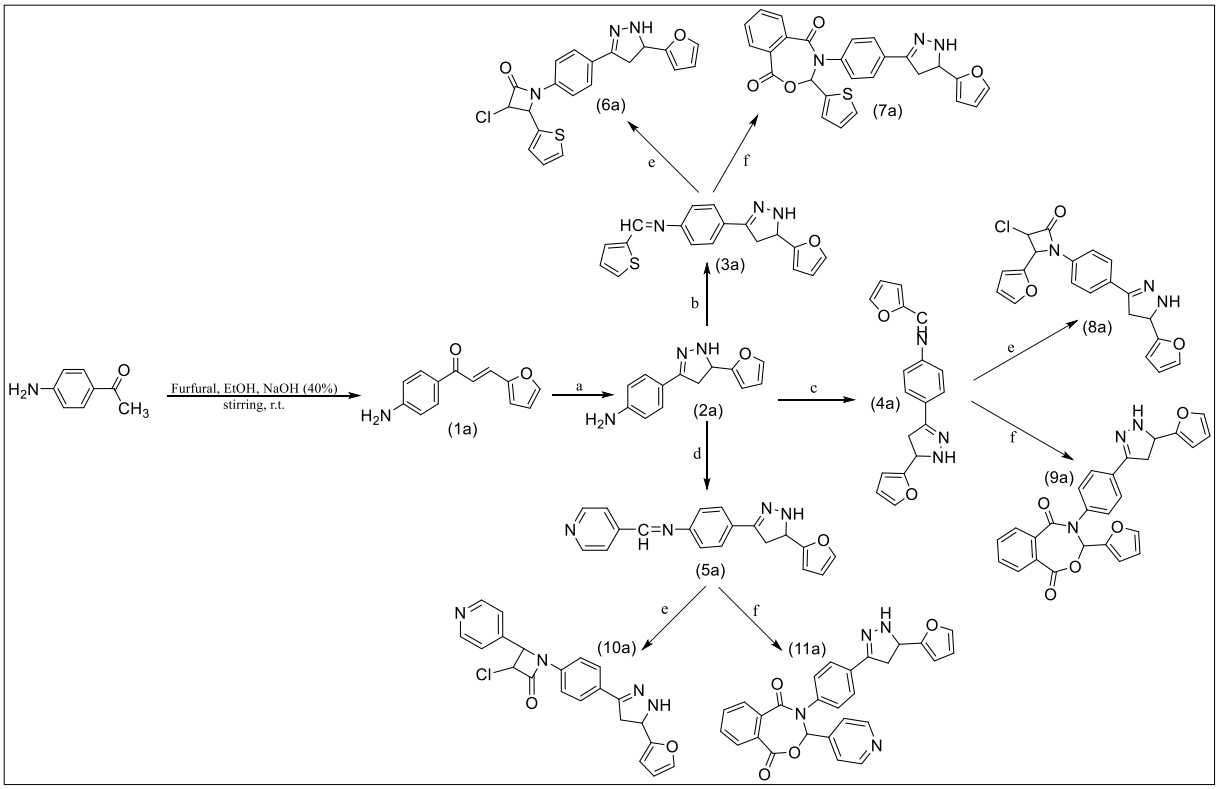
## 4. Results and Discussion

### 4.1. Organic Synthesis

The Schiff bases (3a-5a) were synthesized from the reaction of pyrazoline derivative 2a with different aromatic aldehydes in acidic ethanolic solution (Scheme 1). The prepared compounds were identified using FT-IR, $^{1}$HNMR and GCMS technique. The IR spectrum of pyrazoline compound (2a) showed absorption at 3213 $cm^{-1}$ referring to stretching frequency of (NH-pyrazoline), and 1606 $cm^{-1}$ related to (C=N pyrazoline). The spectrum shows no stretching bands related to the C=O and CH=CH groups. This fact is strongly confirmed the formation of 2a. the $^{1}$HNMR spectrum of compound (2a) exhibited a doublet of doublet signal (for Ha) at 2.9 ppm, a doublet of doublet signal (for Hb) at 3.0-3.2 ppm and a triplet signal at 4.6-4.7 ppm the protons of $NH_2$ have appeared at 6.39 ppm as a singlet signal. The multiplet signal showed at 6.5-7.5 ppm is related to aromatic protons the singlet signal of NH of pyrazoline ring has appeared at 6.32 ppm. The GCMS peak at 227 further confirms the molecular ion $M^{+}$. The FT-IR spectra of Schiff base compound (5a) showed the absorption bands at 2916 $cm^{-1}$,2845 $cm^{-1}$ and 1600 $cm^{-1}$ which can be assigned to the stretching vibrations of the CH=N and C=N groups respectively. The $^{1}$HNMR spectrum of compound 5a, displayed several signals at 3.42 ppm, 3.60 ppm and 5.68 ppm due to Ha, Hb and Hz respectively, while the signals belong to the aromatic proton and NH- pyrazoline have appeared as a multiplet signal at 5.78 ppm. Another singlet signal has appeared at 8.90 ppm which is related to CH=N.

The FT-IR spectrum of 2-azetidinone compound (10a) is showed absorption at 3101,3009 $cm^{-1}$ due to the stretching frequency of aromatic C-H, while absorptions of C=O and C=N pyrazoline groups appear at 1747 and 1606 $cm^{-1}$. The $^{1}$HNMR of 10a showed two multiplet signals at 3.21 and 3.52 ppm due to Ha and Hb protons of pyrazoline ring and multiplet signal at 5.38 ppm due to Hz proton of pyrazoline ring, while the doublet signal at 4.88 ppm related to the CH-Cl proton. The singlet signal at 6.13 ppm is related to NH-pyrazoline proton and multiplet signals at 6.61-8.5 due to aromatic protons shows FT-IR spectrum of azetidinone derivatives.

The FT-IR spectrum of 1,3-Oxazepine compound (11a) is showed absorption at 3093 $cm^{-1}$ due to aromatic C-H. The stretching of two carbonyl groups appeared at 1786 and 1714 $cm^{-1}$. Furthermore, the absorption bands at 1672 and 1514 $cm^{-1}$ related to C=N pyrazoline and C=C respectively. The $^{1}$HNMR of 11a showed two multiplet signals at 2.96 and 3.65 as well as multiplet signal at 4.36 ppm related to to Ha , Hb and Hz protons of pyrazoline ring. The singlet signal at 5.62 ppm due to NH-pyrazoline proton while the multiplet signal at 6.43-8.77 ppm related to the aromatic protons. The singlet signal at 10.45 ppm due to C-H Oxazepine proton strongly confirm the structural elucidation.




**Scheme 1.** (a) hydrazine hydrate (80%), (b) 2-thiophenecarboxaldehyde, EtOH (c) furan-2-carboxaldehyde, EtOH (d) 4-pyridinecarboxaldehyde, EtOH (e) chloroacetyl chloride, 1,4-dioxane, Et3N (f) phathalic anhydride, 1,4-dioxane.

### 4.2. Antimicrobial activity

The in vitro assay of the derivatives (3a-11a) against many pathogenic bacteria and yeast were obtained using 1 µg/ ml concentration as painted in Table 1. The activity of these compounds was evaluated against *Staphylococcus aureus* and *Staphylococcus espidermididis* (gram-positive bacteria), *Escherichia coli* and *Klebsiella sp*. (gram-negative bacteria), and *Candida albicans* (yeast).

**Table 1.** In vitro antimicrobial inhibition zone (mm) of the synthesized compounds.

| 2-azetidinone / 1,3-oxazepine derivatives | Gram-positive | | Gram-negative | | Fungi |
|---|---|---|---|---|---|
| | ***S.aureus*** | ***S.espidermi.*** | ***E.coli*** | ***Klebsiella sp.*** | ***C. albicans*** |
| 3a | 10 | - | 12 | - | 12 |
| 4a | 12 | - | - | 14 | 15 |
| 5a | 14 | 14 | 13 | - | 14 |
| 6a | 10 | 12 | 12 | 13 | 14 |
| 7a | 11 | 13 | 13 | 9 | 15 |
| 8a | 13 | 12 | 8 | 10 | 12 |
| 9a | 15 | 12 | 11 | 12 | 10 |
| 10a | 11 | 12 | 10 | 12 | 10 |
| 11a | 14 | 16 | 12 | 12 | 15 |
| Amoxicillin | 17 | 18 | 20 | 20 | - |

### 4.3. Docking Study

The docking study of the potent active 2-Azetidinone and 1,3-Oxazepine derivatives (6a-11a) toward antimicrobial species inside the active pocket of Uridine-Diphosphate-N-

Acetylglucosamine ) UDP-GlcNAc (, the active target for antimicrobial agents was explored. As described by the X-ray study, the binding pocket of target enzyme including the following subsequent residues, Asn23, Arg91, Arg120, Asp123, Leu124, Lys160, Ser162, Val163, Asp305, and Val327 as shown in Figure 2 (17).

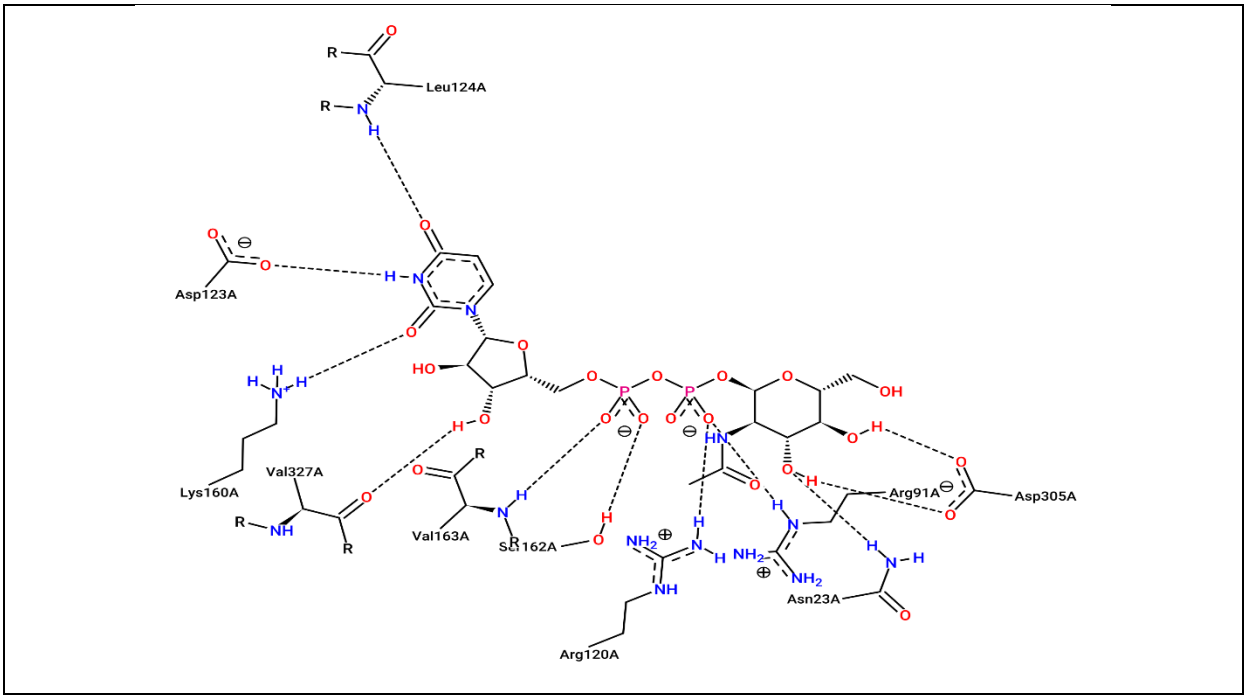
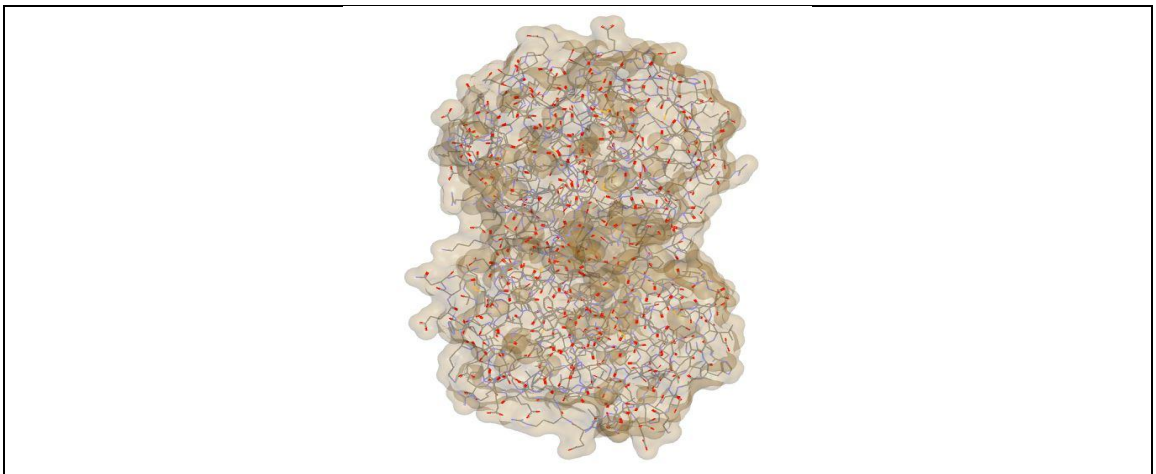
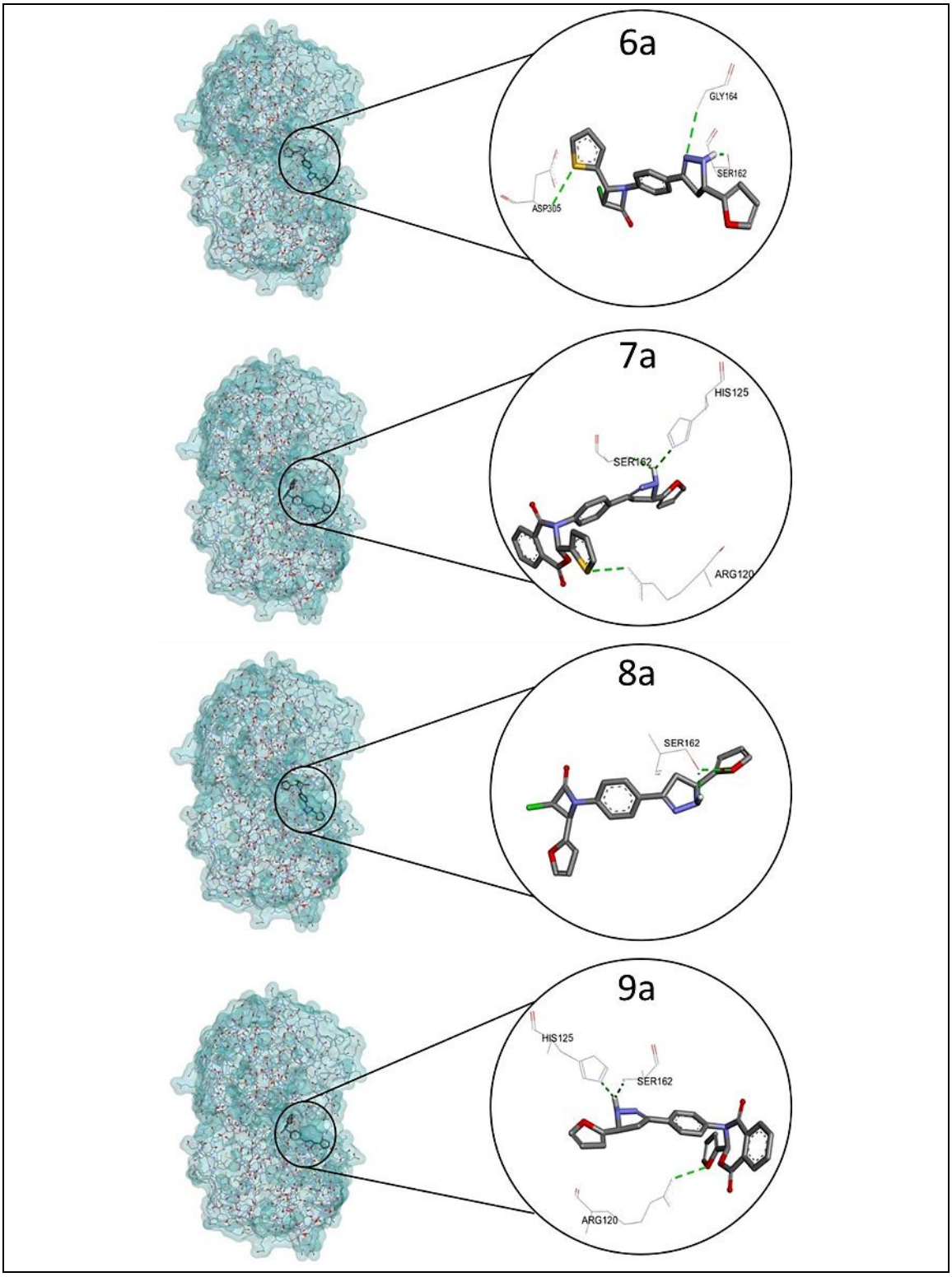



**Figure (2):** The binding of **Uridine-Diphosphate-N-Acetylglucosamine** (**UDP-GlcNAc )** inside the active site of target enzyme.

The binding energy of active compounds inside the well -known three -dimensional structure of specific enzymes was detected using Autodock 4.2. Binding of the best building for connections (6a-11a) inside the binding pocket of **UDP-GlcNAc** is illustrated in Figure 3.



3D structure of UDP-N-acetylmuramoyl-L-alanine ligase (MurC)


6a
GLY164
SER162
ASP305
7a
HIS125
SER162
ARG120
8a
SER162
9a
HIS125
SER162
ARG120

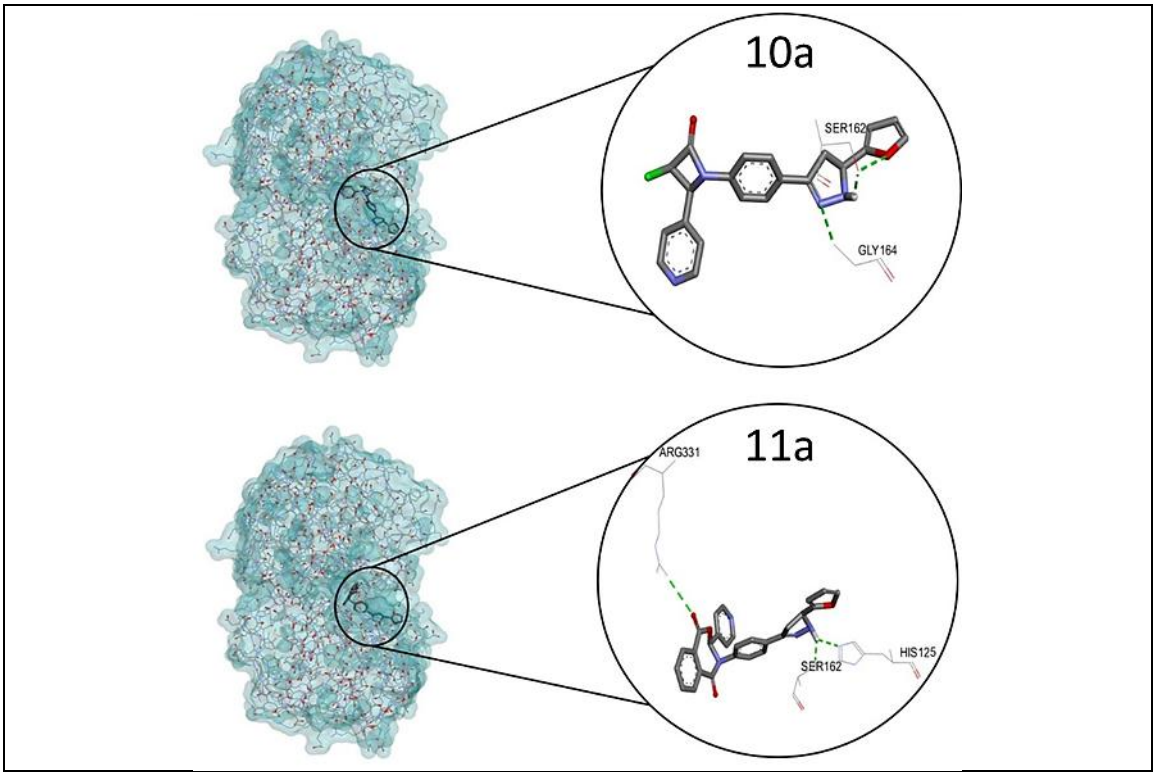



**Figure (3):** The docking of the best generated conformers of the potent discovered hits (6a-11a) inside the binding pocket of UDP-N-acetylmuramoyl-L-alanine ligase (MurC).

As indicated by molecular docking parameters (Table 1), the high-ranking binding energies of the generated best conformer was -10.74, -10.49, -10.54, -10.67, -10.28 and -10.20 kcal $mol^{-1}$ for compounds (6a-11a) , respectively. The docking results of all generated conformers of compounds within the binding pocket are strongly enhancing antibacterial and antifungal activities as depicted in Table 1. Furthermore, the inhibition constant Ki, intermolecular energy and hydrogen bonds were also determined and recorded in Table 2.

**Table 2.** Docking parameters of 2-Azetidinone and 1,3-Oxazepine compounds (6a-11a)

| No. | Compounds | Binding Energy (Kcal $mol^{-1}$) | Inhibition constant (nM) | Intermolecular energy ($kcalmol^{-1}$) | H-bonds | Residues Interaction |
|---|---|---|---|---|---|---|
| 1 | 6a | -10.74 | 13.36 | -11.94 | 3 | A:Gly164:HN: Lig:N A:Asp305:HN: Lig:S Lig:H: A:Ser162:OG |
| 2 | 7a | -10.49 | 20.31 | -11.69 | 3 | A:Arg120:NH2: Lig:S Lig:H: A:His125:N Lig:H: A:Ser162:OG |
| 3 | 8a | -10.54 | 18.9 | -11.73 | 2 | A:Ser162:OG: Lig:O Lig:H: A:Ser162:OG |

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 9a | -10.67 | 15.08 | -11.86 | 3 | A:Arg120:NH2:<br>Lig:O<br>Lig:H:<br>A:His125:N<br>Lig:H:<br>A:Ser162:OG |
| 5 | 10a | -10.28 | 29 | -11.48 | 3 | A:Ser162:OG:<br>Lig:O<br>A:Gly164:N:<br>Lig:N<br>Lig:H:<br>A:Ser162:OG |
| 6 | 11a | -10.20 | 33.6 | -11.39 | 3 | A:Arg331:NH:<br>Lig:O<br>Lig:H:<br>A:His125:N<br>Lig:H:<br>A:Ser162:OG |

## 5. Conclusion

In this study, a new chain of 2-azetidinone and 1,3-oxazepine were derivatives with success including cyclization and acylation reactions through well-defined synthetic routes. Structural condensation using FT-IR, $^1$H NMR, and GC-MS confirmed the formation of target connections. The synthesized molecules showed moderate to potent antimicrobial activity against gram-positive and gram-negative bacteria as well as *Candida albicans*. In order to rationalize the observed biological activity, an important bacterial enzyme involved in cell wall biosynthesis; docking studies were performed against Murc. Docking simulation demonstrated strong binding interaction within the active site of the enzyme in accordance with experimental antimicrobial results. Overall, findings highlight the ability of these 2-azetidinone and 1,3-oxazepine derivatives as promising scaffolding for the development of new antimicrobial agents to handle drug-resistant pathogens.

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