New Sulfamethoxazole Derivatives; Synthesis, Identification, Molecular Docking and Antibacterial Evaluation

Mahmood M. Fahad[1]*, Nusrat Shafiq[2], Uzma Arshad[2], Ali Jabbar Radhi[3], [4]
[1] Medical Laboratory Techniques Department, Kufa Technical Institute, Al-Furat Al-Awsat Technical University, Kufa, Iraq.
[2] Department of chemistry, Government College Women University Faisalabad-38000, Pakistan.
[3] Ministry of Education, The General Directorate of Educational in Najaf Al-Ashraf, Najaf, Iraq.
[4] Faculty of Pharmacy, Al-Kafeel University, Najaf, Iraq.
*Email: mahmood.mohy.iku@atu.edu.iq

Abstract
The development and resistance of bacteria to antibiotics, make us in a continuous race with time to prepare inhibition compounds against advanced bacteria. The fundamental goal of the present research is to connect two effective groups such as the Sulfonamide and β-lactam Groups. The first step involved the preparation of cyano derivatives from the reaction of sulfamethoxazole with sodium nitrate to form diazonium salt followed by a coupling reaction of the diazonium salt with sodium cyanate. The Cyano derivative was treated with urea in an acidic medium to form an Oxadiazol derivative. The last derivative was condensed with various aromatic aldehydes to yield the Schiff base derivatives. In the presence of triethylamine, the Schiff bases combined with chloroacetyl chloride to give derivatives of azetidinone. Boling Point, TLC, FTIR, and H-NMR techniques were used to determine the results. The synthetic derivatives were tested for their antibacterial efficacy against three strains of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. All compounds under investigation displayed strong antibacterial activity when compared to ampicillin.
Keywords: Sulfonamides, Sulfamethoxazole, Schiff bases, Azetidines, Biological Activity.

1. Introduction

Sulfonamides are widely employed in treating a variety of bacterial infections, including those of the intestinal, urinary, and respiratory tracts. Their research is an exciting and promising field of medicinal chemistry [1]. Sulfamethoxazole (SMX) or N1 - (5-methyl-3-isoxazolyl) sulfanilamide is one of the sulfonamide antibiotics that used to treat bacterial infections such prostatitis, bronchitis, and urinary tract infections. Additionally, it works well against bacteria with both Gram negative and positive chromosomes, including *Escherichia coli* and *Staphylococcus aureus* [2-3]. Sulfamethoxazole is a crystalline powder that is white to slightly off-white in color and virtually odorless. The main type of sulfamethoxazole detected in human urine is its N4-acetyl derivative, which is produced during metabolism. Human urine also contains smaller amounts of the intact drug as well as three other, incompletely characterized metabolites. Sulfamethoxazole is almost totally present in intact form in human blood [4]. Treatment of respiratory illnesses like pneumonia frequently involves the use of trimethoprim and sulfamethoxazole. Other well-known conditions that can be treated with sulfamethoxazole include gastroenteritis, diarrhea, and coccidiosis. Sulfamethoxazole is used to treat a wide number of animals, which results in the excretion of un-metabolized or active metabolites, which leaves behind residues [5-6].

Schiff bases have the general formula RC=NR and are the condensation products of an amine and a ketone or an aldehyde. These Schiff bases may function as bidentate, tridentate, tetradentate, or hexadentate donor ligands [7]. Because they may possess a wide range of structural properties, including anticonvulsant, anticancer, antimalarial, anti-tubercular, antitumor, antifungal, anti-inflammatory, anti-carcinogenic activity, antibacterial, and antioxidant properties [8]. In the food industry [11], as dyes and pigments [12], for catalysis [13], as chemosensors and polymer stabilizers, among other applications, schiff bases are one of the most commonly used organic compounds.

Because of their ability to adsorb and create a corrosion-mitigating surface coating through their electron-rich centers, particularly the imine moiety, schiff bases can also be utilized as corrosion inhibitors for a variety of metal-electrolyte systems. In actuality, this moiety's -acceptor properties allow it to provide a firm link with metallic ions [14-15].

The chemistry of -lactams has assumed an important position in organic chemistry since Sir Alexander Fleming's 1928 discovery of Penicillin and the

doi.org/10.31838/hiv23.02.6 Received: 11.10.22, Revised: 21.11.22, Accepted: 10.12.22

subsequent discovery of Cephalosporin, both of which were successfully used as antibiotics [16-17]. One of the most important four-membered heterocyclics used in chemical synthesis and medicinal chemistry is azetidines or -lactam compounds [18]. Many broad-spectrum -lactam antibiotics, such as penicillin, cephalosporin, carbapenems, nocardicins, monobactams, clavulanic acid, sulbactams, and tazobactams, have the structural property of the azetidinone. These antibiotics have been widely used as chemotherapeutic medications for the treatment of bacterial infections and microbial diseases [19]. As anti-tubercular [20], anti-bacterial [21], anti-fungal [22], anti-inflammatory [23], anticancer [24], antitumor [25], enzyme inhibitors [26], cytotoxic [27] and vasopressin v1a antagonist [28], and other biologically active compounds, azetidinones are of great interest.

2. 2. Experimental

For the purpose of knowing the main devices in this research. The melting point was measured using the Electro Thermal Technique (SMP30) type. Using glass Thin Layer Chromatography 1020 GS - Silica gel 60, this test was measured to identify the progression of the reactions. At the Pharmacy Faculty of Kufa University, a Fourier transform infrared (FT-IR) spectrum was captured using an FT-IR spectrophotometer-8400s, Shimadza (KBr). Tetramethylsilane (TMS) was used as the internal reference when nuclear magnetic resonance (H-NMR) measurements were taken at Teheran University in Iran utilizing a Bruker-400 MHz. Using an incubator from Memert-Germany, varied temperatures were maintained as needed for the development of the organisms. The agar medium, which was provided by Prestige Medical-England, was sterilized in an autoclave. DMSO was used as the solvent and Muller Hinton agar for the bacteria test to determine biological activity.

2.1. Synthesis Part

2.1.1. Synthesis of 4-cyano-N-(5-methylisoxazol-3-yl)benzenesulfonamide B [29-30]

Sulfamethoxazole A (0.01mole, 1.5 g) was mixed with (5 ml) concentrated hydrochloric acid. With stirring, (0.01mole, 0.4 g) of sodium nitrate was added. The temperature of the reaction was maintained up to 0–5 ^{0}C. Drop by drop, the diazonium salt solution was incorporated into the Sodium cyanide solution (0.01mole, 0.29 g). The reaction mixture stirred for one hour under the same cold conditions. The resulting are filtered, cleaned with distilled water, then crystallized again using acetone. Color: Brown (85 %). Melting Point :(135 -137 0C). R*f* = (0.7), the TLC for the reaction was completed by using (benzene: methanol, 3:1).

FT-IR (KBr, cm^{-1}): 2351.23 υ(C N), 3379.29 υ(N–H) Sulfonamide, 1678.07 υ(C=N), 1543.50 υ(O-N), 1583.56 υ (C=C) aromatic.1H-NMR (ppm) δH: 7.13-7.46 (m, 4H, Ar-H), 11.04 (s, 1H, NH-SO2), 2.35 (s, 1H, CH3).

2.1.2. Synthesis of 4-(5-amino-1,3,4-oxadiazol-2-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide C [31-32]

In 100% ethanol, 4-cyano-N-(5-methylisoxazol-3-yl)benzenesulfonamide (0.006 mol) was combined with 0.012 mol of urea before being gradually mixed with acetic acid. The reaction was put onto ice and allowed to reflux for 14 hours, generating a white precipitate. The final product (75%) was re-crystallized from acetone after being rinsed with water. R*f*: 0.47, melting point: 198–200 °C, TLC (Benzene: Methanol, 3:1).

FT-IR (KBr, cm^{-1}): 3388.49, 3348.22 υ(NH_2), 3323.11 υ(N–H) Sulfonamide, 1535.50 υ(O-N), 1582.56 υ (C=C) aromatic.^{1}H-NMR (ppm) δH: 7.6 (s, 2H, NH_2), 7.32-7.95 (m, 4H, Ar-H), 11.01 (s, 1H, NH-SO_2).

2.1.2. Synthesis of Schiff bases derivatives D 1-3 [33]

The different compounds (4-methoxybenzaldehyde, 4-hydroxysalicylaldehyde, and 4-hydroxybezaldehyde) were combined with 4-(5-amino-1,3,4-oxadiazol-2-yl)-N-(5-methylisoxazol-3-yl) benzenesulfonamide and (30 ml) absolute ethanol. Glacial acetic acid was added in three drops. The reaction was stirred and refluxed for (8–10 hours). The goods were dried, water-washed, and then crystallized once more from hot ethanol. Table (1).

FT-IR (KBr, cm^{-1}) D_1: 1645 υ(C=N), 3301.25 υ(N–H) Sulfonamide, 1533.43 υ(C=C) aromatic.^{1}H-NMR (ppm) δH: 8.55 δ ppm (s, 1H, CH=N), 3.75 δ ppm (s, 3H, OCH_3), 6.6-7.2 δ ppm (m, 8H, Aromatic ring-H), 11.21 (s, 1H, NH-SO_2).

FT-IR (KBr, cm^{-1}) D_2: 1653 υ(C=N), 3332.61 υ(N–H) Sulfonamide, 1540.18 υ(C=C) aromatic.^{1}H-NMR (ppm) δH: 8.37 δ ppm (s, 1H, CH=N), 3.78 δ ppm (s, 2H, OH), 6.9-7.8 δ ppm (m, 8H, Aromatic ring-H), 11.15 (s, 1H, NH-SO_2).

FT-IR (KBr, cm^{-1}) D_3: 1627 υ(C=N), 3363.02 υ(N–H) Sulfonamide, 1549.31 υ(C=C) aromatic.^{1}H-NMR (ppm) δH: 8.61 δ ppm (s, 1H, CH=N), 3.43 δ ppm (s, 1H, OH), 6.7-7.7 δ ppm (m, 8H, Aromatic ring-H), 11.18 (s, 1H, NH-SO_2).

Table (1) Some physical properties of Schiff bases derivatives D_{1-3}

Comp	General formula	Color	Product %	M.P	R.f
D1	C20H17N5O5S	Brown	72%	178-180	0.63
D2	C19H15N5O6S	Light yellow	75%	166-168	0.59
D3	C19H15N5O5S	Brownish green	74%	172-174	0.67

2.1.3. Synthesis of Azetidionone derivatives E 1-3 [34-36]

Generally, Schiff base D_{1-3} (0.001 mmol) and triethyl amine (0.002 mmol) were dissolved in 30 ml of

dioxane. Chloroacetyl chloride (0.001 mmol) was gradually added drop by drop. After that, the reaction was stirred for a further three hours and let to remain at room temperature for 48 hours.The resulting combination was cooled, poured and then placed into ice cold water , where it was filtered and dried. The final result (73%) was re-crystallized from Acetone. The general formula, color, product percentage, melting point and retention factor of the compounds were reported in Table 2.

FT-IR (KBr, cm^{-1}) E_1: 1652.13 υ(C=O Azetidionone), 724.23 υ(C–Cl), 1355.46 υ(N-CH).^{1}H-NMR (ppm) δH: 1.9 δ ppm (d, 1H, N-CH-C), 2.7δ ppm (d, 1H, C-CH-Cl), 6.7-7.7 δ ppm (m, 8H, Aromatic ring-H), 11.12 (s, 1H, NH-SO_2).

FT-IR (KBr, cm^{-1}) E_2: 1658.02 υ(C=O Azetidionone), 732.14 υ(C–Cl), 1359.25 υ(N-CH).^{1}H-NMR (ppm) δH: 1.8 δ ppm (d, 1H, N-CH-C), 2.5δ ppm (d, 1H, C-CH-Cl), 6.7-7.9 δ ppm (m, 8H, Aromatic ring-H), 11.22 (s, 1H, NH-SO_2).

FT-IR (KBr, cm^{-1}) E_3: 1656.33 υ(C=O Azetidionone), 731.16 υ(C–Cl), 1352.34 υ(N-CH).^{1}H-NMR (ppm) δH: 2.1 δ ppm (d, 1H, N-CH-C), 2.3δ ppm (d, 1H, C-CH-Cl), 6.8-7.9 δ ppm (m, 8H, Aromatic ring-H), 11.02 (s, 1H, NH-SO_2).

Table (2) Some physical properties of Azetidionone derivatives E 1-3

Comp	General formula	Color	Product %	M.P	R.f
E_1	$C_{22}H_{18}ClN_5O_6S$	Pale white	72%	178-180	0.63
E_2	$C_{21}H_{16}ClN_5O_7S$	Pale white	75%	166-168	0.59
E_3	$C_{21}H_{16}ClN5O6S$	Yellowish white	74%	172-174	0.67

2.2. Antibacterial activity

As an application side of this article, the bacterial activity of the prepared derivatives was studied by the disc diffusion method. Three kinds of positive and negative bacteria (*Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa)* were diffused and activated on Muller Hinton agar. Using a cork borer, three identical holes of 6 mm in diameter were drilled into each Petri dish. During the 24-hour period at 37 ^{0}C, the diluted synthesized compounds were inserted into the pores. The inhibition zones were then measured using a ruler and the findings were compared to those obtained from standard compounds (ampicillin) based on the diameter of the inhibitory zone in mm [37-38].

2.3. Molecular Docking

2.3.1. Protein Selection and Preparation

By keeping in view of the pathogenic species against which in vitro anti-bacterial activities were checked 3D structure of protein comprising these species essential components were selected. DNA gyrase subunit of Staphylococcus aureus (PDB ID: 6FM4) was selected as target [39], penicillin binding proteins (PBPs) involved in catalytic activity of Pseudomonas aeruginosa cell wall (PDB ID: 6R3X) was selected as co-crystal structure [40] while primosomal protein of Escherichia coli complexed with single stranded DNA (PDB ID: 2CCZ) [41] was chosen for validating the in vitro activity via in silico activity. Crystal structures of these proteins were downloaded in PDB format and imported to Discovery Studio Visualizer. Protein preparation was carried out by removing water molecules and adding hydrogen atoms [42-43] Binding site sphere was generated by selecting native ligand and attributes of site sphere were copied to configuration file for docking by using Auto dock software and saved in PDB format for further processing in MGL Tools [44-46].

2.3.2. Configuration of Ligand and Protein by Auto Dock Tools for Docking

Selected protein structures were loaded to Auto Dock (MGL) Tools for protein structure was edited by computing Gasteiger charges and adding Kollmann charges. While AD4 type radii and atomic radii were assigned to protein structure. Ligand was loaded to Auto Dock Tools by detecting and choosing root. Accompanying these changes both macromolecule and ligand was selected for docking and finally saved in PDBQT format to concerned directory for docking [47-49].

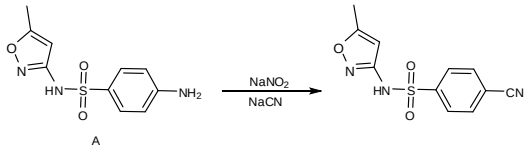
2.3.3. Docking and Scoring Function

Auto Dock Vina Program was used for molecular docking simulation of targeted ligands to receptor active site. Software exhaustiveness was set to 8 for docking simulation. Grid spacing of protein was defined automatically using the native ligand coordinates in such a way that default radius was assigned the binding site radius along with XYZ coordinates. Discovery Studio Visualizer was used for 3D rendering of best docked pose which was selected on the basis of highest binding affinity value and low RMSD value [50-51].

3. 3. Discussion

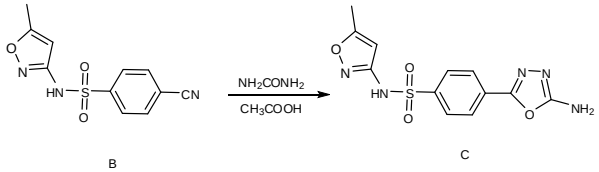
3.1. Chemical part

Through practical results, Boling point, TLC, FTIR, and H-NMR techniques, Azetidionone derivatives were synthesized from cyclocondensation between different Schiff bases with chloroacetyl chloride compound. The first step started by preparing the diazonium salt of sulfa compound and reacted the latter with sodium cyanide to produce (B) compound, Scheme 1. According to FT-IR spectrum of the compound B, It is noted that the (CN) group in the compound appears at 2351.23 cm-1 clearly. Peaks for aromatic rings were seen in the 1H-NMR at 7.13 and 7.46 ppm, 11.04 δ ppm for (NH-SO2), and 2.35 δ ppm for (s, 1H, CH3).



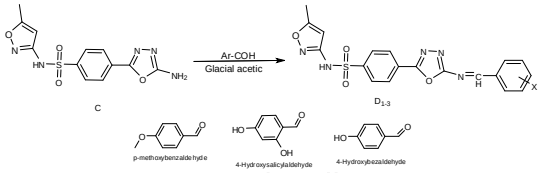
Scheme 1. Synthesis (B) compound

The 4-cyano-N-(5-methylisoxazol-3-yl) benzenesulfonamide (B) was treated with urea in acidic medium to form (C) compound, Schem 2. The compound was confirmed by FTIR and 1H-NMR. The FTIR spectrums were observed two bands for υ (NH2) at 3388.49, 3348.22 cm-1, and (N-N) group at 1538.32 cm-1. While 1H-NMR peaks, the (NH2) group was observed at 7.6 δ ppm a special group in the above compound.



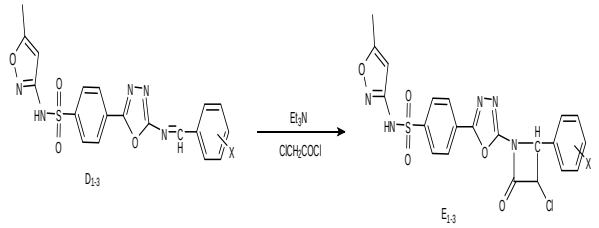
Scheme 2. Synthesis (C) compound

Schiff bases are chemical compounds that resemble aldehydes or ketone in which the carbonyl group has been changed to an azomethine or imine group. The condensation of an amino molecule with carbonyl compounds produces the adaptable ligands known as schiff bases. Three new Schiff bases derivatives were synthesized from Oxadiazol derivative with 4-methoxybenzaldehyde, 4-hydroxysalicylaldehyde, and 4-hydroxy- bezaldehyde, Scheme 3. Schiff bases derivatives were characterized by the clear group (-HC=N-), where the group appears in the FT-IR spectrum between (1627-1653) cm-1. While in the 1H-NMR spectra, the imine group was appeared between (8.37-8.61) ppm.



Scheme 3. Synthesis of Schiff bases derivatives

Azetidinones are one of the important compounds prepared from the reaction of the imine group in Schiff bases with chloroacetyl chloride. Azetidinone compounds were identified by FTIR and H-NMR. These compounds were characterized by the presence of distinct groups such as (C=O: 1652-1658, N-CH: 1352-1359, C–Cl: 724-731) cm-1. In the H-NMR, the values of (N-CH-C, C-CH-Cl) groups were shown at (1.8-2.1), and (2.3-2.7) respectively, Scheme 4.



Scheme 4. Preparation of Azetidinine compounds

3.2. Biological part

For pharmaceutical applications of azetidinone, the new compounds were then synthesized to have high activity against many types of bacteria. Using Muller Hinton Agar, the synthesized chemical derivatives E (1-3) were tested against selected microorganisms. The accompanying table 3 and figure 1 show the biological outcomes of produced compounds in comparison to standard compounds (ampicillin).

Table 3. Antibacterial test of some synthesized compounds E (1-3)

Sample	Antibacterial test		
	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
E1	++	+++	+++
E2	+++	++	+++
E3	–	–	+
Ampicillin	++	++	+++

Where, – = Resistant, + = 1-10 cm, ++ = 10-20 cm, +++ = 20-30cm.

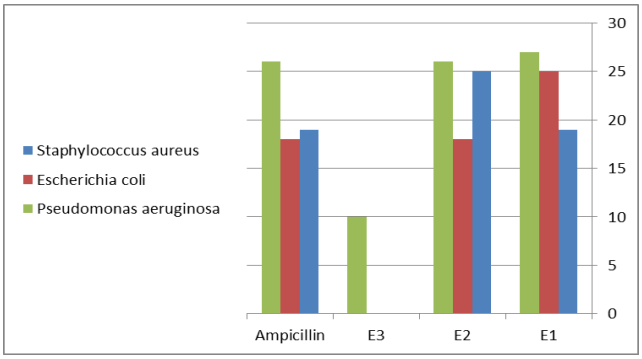


Figure 1. The results of antibacterial activity of compounds E (1-3)

Through the table 3 and figure 1, we note that compounds E1-2 gave high biological activity against selected microorganisms. While the compound E3 gave low activity for Staphylococcus aureus and Escherichia coli and moderate activity for Pseudomonas aeruginosa. When comparing the activity of the standard compound with the prepared compounds, some of the synthesized compounds gave stronger activity than the standard compound, and some compounds gave similar activity, and some were weaker towards the types of bacteria.

3.3. Docking Analyses

Molecular docking simulation was carried out to rationalize the anti-bacterial potential of synthesized compounds by demonstrating the binding affinity of synthesized compounds into the active site of targeted receptor macromolecule. Docking process begin with validation, by checking the RMSD values of native ligand with re-docked co-crystal structure of respective protein. Staphylococcus aureus RMSD value for native and re-docked ligand is 1.094Å, RMSD value for Pseudomonas aeruginosa is 2.149Å and targeted macromolecule structure for Escherichia coli was a nucleic acid having RMSD value of 0.000Å, superimposed native ligand and re-docked ligand is shown in figure 1. RMSD values within the range of 2Å proved the validation of

docking protocol with respective synthesized molecules [52]. Ampicillin was used as standard drug in both *in vitro* and *in silico* analysis [53]. Molecular docking studies with all receptor sites revealed that Ampicillin has binding affinity value of -6.1kcal/mol for Staphylococcus aureus, -7.6 kcal/mol for Escherichia coli and -7.9 kcal/mol for Pseudomonas aeruginosa respectively [54-55]. It can be seen in table 4 that inhibition potential of compounds obtained by *in vitro* antibacterial analysis is well correlated with binding affinity score as revealed by molecular-docking

Table 4. Displaying the in vitro and in silico antibacterial analysis results against different bacterial species

Compounds	Antibacterial Test	Target	Binding Affinity Score (kcal/mol)	Receptor amino acids/chain involved in Hydrogen Bonding	Category (Type)
Staphylococcus aureus					
E1	++	Staphylococcus aureus Gyrase (PDB ID: 6FM4) Organism: Staphylococcus aureus	-6.4	MET1121 SER1084	Conventional Carbon-Hydrogen
E2	+++		-6.6	MET1121 SER1084 F chain	Conventional Carbon-Hydrogen Conventional
E3	–		-6.4	ASP1083	Conventional
Standard: Ampicillin	++		-6.1	MET1121	Conventional
Escherichia coli					
E1	+++	Escherichia coli primosomal protein PriB (PDB ID: 2ccz) Organism: Escherichia coli	-10.7	SER79 SER88	Conventional Carbon-Hydrogen
E2	++		-8.9	LEU16	Conventional
E3	–		-9.8	SER55 HIS26	Conventional Carbon-Hydrogen
Standard: Ampicillin	++		-7.6	No H-bonding	_____
Pseudomonas aeruginosa					
E1	+++	Pseudomonas aeruginosa Penicillin-Binding protein 3 (PBP3) (PDBID:6r3x)Organism: Pseudomonas aeruginosa	-9.4	TYR409 TYR532	Conventional Conventional
E2	+++		-9.1	TYR409 TYR487 THR487	Conventional Conventional Conventional
E3	+		-9.4	SER485 TYR532	Conventional Conventional
Standard: Ampicillin	+++		-7.9	ASN351 TYR409 TYR328	Conventional Conventional Conventional

3.3.1. Binding Affinity Analysis

Trend in table 1 showed that compound E2 generated the highest binding affinity value of -6.6 kcal/mol and also had maximum inhibitory zone of 20-30cm for Staphylococcus aureus antibacterial activity. In case of Escherichia coli inhibition both by in vitro and in silico analysis compound E1 generated the best binding affinity score of -10.7 kcal/mol and highest inhibitory zone. In the same way compound E1 is most potent in inhibiting the bacterial activity of Pseudomonas aeruginosa with maximum zone of inhibition along with highest binding affinity value of -9.4 kcal/mol [56].

In case of Staphylococcus aureus receptor protein (PDB ID: 6fm4) E1 and E2 substituted R group had same orientation and E3 R group was oriented in opposite direction. While high binding affinity of E2 was due to more number of H-bonding interaction as compared to E1 and E2 [57-58].

Receptor protein of Pseudomonas aeruginosa has two H-bonds and ten Van der Waals interactions with E1, with E2 it displayed three H-bonding and ten other electrostatic interactions while E3 displayed three H-bonding and five electrostatic interactions. But highest binding affinity value of E1 and E3 was due to the best fitness of docked pose into catalytic site, as substituted R group had more stability within the binding domain of receptor protein [59-60]

Molecular docking analysis results for Escherichia coli revealed that E1 had two H-bonding and four electrostatic interactions, E2 had one H-bonding and three electrostatic interactions and E3 had two H-bonding and six Van der Waals interactions. However, high binding affinity of E1 was attributed to the orientation pattern within the binding catalytic domain. As it can be seen in figure 2, that except E1 all compounds were away from the catalytic site of receptor [61-62]

Overall it was concluded that methoxyphenyl derivative E1 of sulfonamide can be considered as critical moiety to develop as broad spectrum antibiotic agent [63-64]. Meanwhile, dihydroxyphenyl E2 and hydroxyphenyl E3 derivatives provide bulkiness for properly embedding into the protein active site.

Best fit pose of all potent compounds displaying H-bonding and other Van der Waals forces within the receptor active site is shown in figure 2 [65-66]. In order to get better orientation and conformation best output pose of each docked compound was superimposed to standard Ampicillin within the binding site sphere, shown in fig 3, also displaying binding site amino acids. Assessment of superimposed structures showed that all the compounds had similar alignment to reference drug except for substituted R groups and fits well into active site of receptor [67].

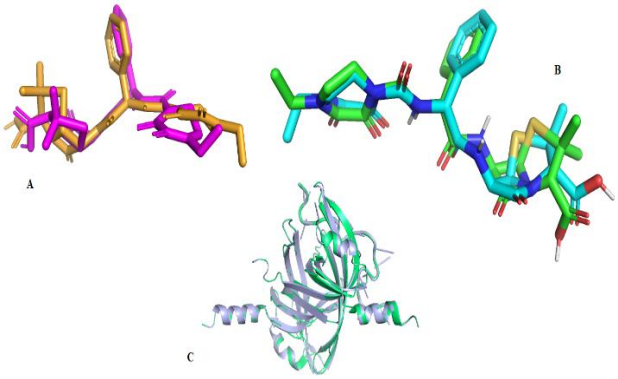


Figure 2. (A) Validation of docking simulation for Pseudomonas aeruginosa protein PDB ID: 6r3x possessing native ligand in orange color and re-docked ligand in magenta color (B) Validation of docking simulation for Staphylococcus aureus protein PDB ID: 6fm4 possessing native ligand in cyan color and re-docked ligand in green color (C) Validation of docking simulation for Escherichia coli nucleic acid PDB ID: 2ccz possessing unprepared structure of nucleic acid in cyan color and structure prepared for docking in cyan color

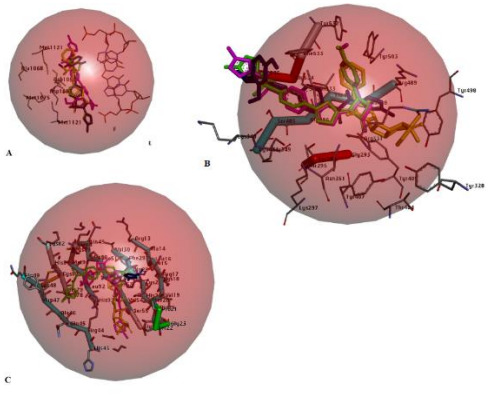


Figure 3. Representation of best docked pose into the receptor catalytic cavity along with standard Ampicillin. Amino acids shown in catalytic site were involved in interaction with compounds. Standard Ampicillin was shown in yellow color, compound E_1 in green color, compound E_2 in blue and compound E_3 in magenta color.

4. Conclusion

Due to the rapid development of bacteria against various antibiotics, a series of azetidinone derivatives were prepared from a starting substance sulfamethoxazole. Azetidinone derivatives were identified by many techniques such as melting point, thin layer chromatography, FT-IR, and H-NMR. Then, the biological activity of azetidinone derivatives of three types of bacteria was studied using Molegro Virtual Docker (MVD). It was found that some of the prepared derivatives have high biological activity against the selected types of bacteria (Staphylococcus aureus, Escherichia coli & Pseudomonas aeruginosa).

References

[1] Ma, M., Cheng, Y., Xu, Z., Xu, P., Qu, H., Fang, Y., ... & Wen, L. (2007). Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model drug. European journal of medicinal chemistry, 42(1), 93-98.

[2] Wang, J., & Wang, S. (2018). Microbial degradation of sulfamethoxazole in the environment. Applied microbiology and biotechnology, 102(8), 3573-3582.

[3] Hussain, Z., Yousif, E., Ahmed, A., & Altaie, A. (2014). Synthesis and characterization of Schiff's bases of sulfamethoxazole. Organic and medicinal chemistry letters, 4(1), 1-4.

[4] Rudy, B. C., & Senkowski, B. Z. (1973). Sulfamethoxazole. In Analytical profiles of drug substances (Vol. 2, pp. 467-486). Academic Press.

[5] Dantas, R. F., Contreras, S., Sans, C., & Esplugas, S. (2008). Sulfamethoxazole abatement by means of ozonation. Journal of hazardous materials, 150(3), 790-794.

[6] Smilack, J. D. (1999, July). Trimethoprim-sulfamethoxazole. In Mayo Clinic Proceedings (Vol. 74, No. 7, pp. 730-734). Elsevier.

[7] El-Gammal, O. A., Mohamed, F. S., Rezk, G. N., & El-Bindary, A. A. (2021). Synthesis, characterization, catalytic, DNA binding and antibacterial activities of Co (II), Ni (II) and Cu (II) complexes with new Schiff base ligand. Journal of Molecular Liquids, 326, 115223.

[8] Vijesh, A. M., Isloor, A. M., Shetty, P., Sundershan, S., & Fun, H. K. (2013). New pyrazole derivatives containing 1, 2, 4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. European Journal of Medicinal Chemistry, 62, 410-415.

[9] Wang, X., Ding, G., Duan, Y., Zhu, Y., Zhu, G., Wang, M., ... & Hung, C. H. (2020). A novel triphenylamine-based bis-Schiff bases fluorophores with AIE-Activity as the hydrazine fluorescence turn-off probes and cell imaging in live cells. Talanta, 217, 121029.

[10] Ahmed, D. S., El-Hiti, G. A., Hameed, A. S., Yousif, E., & Ahmed, A. (2017). New tetra-Schiff bases as efficient photostabilizers for poly (vinyl chloride). Molecules, 22(9), 1506.

[11] Bravo, I., Revenga-Parra, M., Pariente, F., & Lorenzo, E. (2017). Reagent-less and robust biosensor for direct determination of lactate in food samples. Sensors, 17(1), 144.

[12] Al Zoubi, W., & Ko, Y. G. (2016). Organometallic complexes of Schiff bases: Recent progress in oxidation catalysis. Journal of Organometallic Chemistry, 822, 173-188.

[13] Wang, Y., Liu, W., Zhang, J., & Shan, Q. (2022).

Preparation of co-schiff base complex and its adsorption desulfurization. Fuel, 324, 124696.
[14] Satpati, S., Saha, S. K., Suhasaria, A., Banerjee, P., & Sukul, D. (2020). Adsorption and anti-corrosion characteristics of vanillin Schiff bases on mild steel in 1 M HCl: experimental and theoretical study. RSC advances, 10(16), 9258-9273.
[15] Ma, L., Li, W., Zhu, S., Wang, L., & Guan, S. (2021). Corrosion inhibition of Schiff bases for Mg-Zn-Y-Nd alloy in normal saline: Experimental and theoretical investigations. Corrosion Science, 184, 109268.
[16] Brabandt WV, Dejaegher Y, and Kimpe ND: New reactions of functionalized β-lactams. Pure Appl. Chem. 2005; 77(12) 2061–2071.
[17] D.S. Salunkhe and P.B. Piste, A Brief Review on Recent Synthesis of 2-Azetidinone Derivatives, IJPSR, 2014; Vol. 5(3): 666-689.
[18] Wang, B. J., & Duncton, M. A. (2020). A Single-Step Synthesis of Azetidine-3-amines. The Journal of Organic Chemistry, 85(20), 13317-13323.
[19] Elumalai, K., Ali, M. A., Elumalai, M., Eluri, K., Srinivasan, S., Mohanti, S. K., & Thota, A. (2013). Design, synthesis and biological evaluation of some novel isoniazid cyclocondensed azetidinones. Drug Invention Today, 5(2), 100-104.
[20] Kagthara, P., Upadhyay, T., Doshi, R., & Parekh, H. H. (2000). Synthesis of some 2-azetidinones as potential antitubercular agents. Indian Journal of Heterocyclic Chemistry, 10(1), 9-12 .
[21] Singh, G. S., & Mmolotsi, B. J. (2005). Synthesis of 2-azetidinones from 2-diazo-1, 2-diarylethanones and N-(2-thienylidene) imines as possible antimicrobial agents. Il Farmaco, 60(9), 727-730.
[22] Mehta, P. D., Sengar, N. P., Subrahmanyam, E. V., & Satyanarayana, D. (2006). Synthesis and biological activity studies of some thiazolidinones and azetidinones. Indian journal of pharmaceutical sciences, 68(1), 103 .
[23] Sharma, S., Singh, T., Mittal, R., Saxena, K. K., Srivastava, V. K., & Kumar, A. (2006). A study of anti-inflammatory activity of some novel α-amino naphthalene and β-amino naphthalene derivatives. Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry, 339(3), 145-152.
[24] Banik, B. K., Banik, I., & Becker, F. F. (2005). Stereocontrolled synthesis of anticancer β-lactams via the Staudinger reaction. Bioorganic & medicinal chemistry, 13(11), 3611-3622.
[25] Veinberg, G., Shestakova, I., Vorona, M., Kanepe, I., & Lukevics, E. (2004). Synthesis of antitumor 6-alkylidenepenicillanate sulfones and related 3-alkylidene-2-azetidinones. Bioorganic & medicinal chemistry letters, 14(1), 147-150.
[26] Beauve, C., Bouchet, M., Touillaux, R., Fastrez, J., & Marchand-Brynaert, J. (1999). Synthesis, reactivity and biochemical evaluation of 1, 3-substituted azetidin-2-ones as enzyme inhibitors. Tetrahedron, 55(46), 13301-13320 .
[27] Veinberg, G., Bokaldere, R., Dikovskaya, K., Vorona, M., Kanepe, I., Shestakova, I., ... & Lukevics, E. (2003). Synthesis of cytotoxic 1, 3, 4-trisubstituted 2-azetidinones. Chemistry of heterocyclic compounds, 39(5), 587-593 .
[28] Guillon, C. D., Koppel, G. A., Brownstein, M. J., Chaney, M. O., Ferris, C. F., Lu, S. F., ... & Simon, N. G. (2007). Azetidinones as vasopressin V1a antagonists. Bioorganic & medicinal chemistry, 15(5), 2054-2080.
[29] Al-Adilee, K. J., Kyhoiesh, H. A. K., & Taher, A. M. (2022). Synthesis, Characterization, Biological Studies, Molecular Docking and Theoretical Calculation of Some Transition Metal Complexes with New Azo Dye 2-[2'-(6-methoxybenzothiazolyl) azo]-3-methyl-4-nitrophenol. Results in Chemistry, 100500.
[30] Fahad, M. M., Zimam, E. H., Radhi, A. J., Mohamud, M. J., & Abbas, N. A. (2022, January). Based on sulfa drug: Synthesis and biological study of barbituric acid derivatives containing 1, 2, 3-Triazoline moiety. In AIP Conference Proceedings (Vol. 2386, No. 1, p. 030021). AIP Publishing LLC.
[31] Fahad, M. M., Shafiq, N., Arshad, U., & Radh, A. J. (2021). As Antimicrobial Agents: Synthesis, Structural Characterization and Molecular Docking study of Barbituric Acid Derivatives from Phenobarbital, 6, 122-136.
[32] Mermer, A., Bayrak, H., Şirin, Y., Emirik, M., & Demirbaş, N. (2019). Synthesis of novel Azol-β-lactam derivatives starting from phenyl piperazine and investigation of their antiurease activity and antioxidant capacity comparing with their molecular docking studies. Journal of Molecular Structure, 1189, 279-287.
[33] Mahato, S., Meheta, N., Kotakonda, M., Joshi, M., Shit, M., Choudhury, A. R., & Biswas, B. (2021). Synthesis, structure, polyphenol oxidase mimicking and bactericidal activity of a zinc-schiff base complex. Polyhedron, 194, 114933.
[34] Patel, K. H., & Mehta, A. G. (2006). Synthesis and Antifungal Activity of Azetidinone and Thiazolidinones Derivatives of 2-Amino-6-(2-naphthalenyl) thiazolo [3, 2-d] thiadiazole. E-Journal of Chemistry, 3(4), 267-273.
[35] O'Boyle, N. M., Carr, M., Greene, L. M., Bergin, O., Nathwani, S. M., McCabe, T., ... & Meegan, M. J. (2010). Synthesis and evaluation of azetidinone analogues of combretastatin A-4 as tubulin targeting agents. Journal of medicinal chemistry, 53(24), 8569-8584.
[36] Vashi, K., & Naik, H. B. (2004). Synthesis of novel Schiff base and azetidinone derivatives and their antibacterial activity. E-Journal of Chemistry, 1(5), 272-275.
[37] Fahad, M. M., & Al-Khuzaie, M. G. (2021, November). Preparation of Ethanol-Free Hand Sanitizers Gels and Studying its Sterile Efficacy. In Journal of Physics: Conference Series (Vol. 2063, No. 1, p. 012007). IOP Publishing.
[38] Salman, H. H., Mohammed-Ali, M. A. J., & Albader, A. A. (2014). Synthesis, characterization of new azetidinone derivatives and evaluation of their antimicrobial activity. Misan Journal of Academic

Studies, 24, 1-12.
[39] Magarò, G., Prati, F., Garofalo, B., Corso, G., Furlotti, G., Apicella, C., . . . Di Giorgio, F. P. (2019). Virtual Screening Approach and Investigation of Structure–Activity Relationships to Discover Novel Bacterial Topoisomerase Inhibitors Targeting Gram-Positive and Gram-Negative Pathogens. Journal of Medicinal Chemistry, 62(16), 7445-7472 .
[40] Bellini, D., Koekemoer, L., Newman, H., & Dowson, C. G. (2019). Novel and improved crystal structures of H. influenzae, E. coli and P. aeruginosa penicillin-binding protein 3 (PBP3) and N. gonorrhoeae PBP2: toward a better understanding of β-lactam target-mediated resistance. Journal of molecular biology, 431(18), 3501-3519 .
[41] Huang, C.-Y., Hsu, C.-H., Sun, Y.-J., Wu, H.-N., & Hsiao, C.-D. (2006). Complexed crystal structure of replication restart primosome protein PriB reveals a novel single-stranded DNA-binding mode. Nucleic Acids Research, 34(14), 3878-3886.
[42] Horchani, M., Heise, N. V., Csuk, R., Ben Jannet, H., Harrath, A. H., & Romdhane, A. (2022). Synthesis and In Silico Docking Study towards M-Pro of Novel Heterocyclic Compounds Derived from Pyrazolopyrimidinone as Putative SARS-CoV-2 Inhibitors. Molecules, 27(16), 5303.
[43] Nasli Esfahani, A., Iraji, A., Alamir, A., Moradi, S., Asgari, M. S., Hosseini, S., . . . Bandarian, F. (2022). Design and synthesis of phenoxymethybenzoimidazole incorporating different aryl thiazole-triazole acetamide derivatives as α-glycosidase inhibitors. Molecular diversity, 26(4), 1995-2009.
[44] Erol, M., Celik, I., Uzunhisarcikli, E., & Kuyucuklu, G. (2022). Synthesis, molecular docking, and DFT studies of some new 2, 5-disubstituted benzoxazoles as potential antimicrobial and cytotoxic agents. Polycyclic Aromatic Compounds, 42(4), 1679-1696.
[45] Thanh, N. D., Giang, N. T. K., Ha, N. T. T., Le, C. T., Van, H. T. K., & Toan, V. N. (2023). Synthesis and in vitro anticancer activity of 4H-pyrano [2, 3-d] pyrimidine− 1H-1, 2, 3-triazole hybrid compounds bearing D-glucose moiety with dual EGFR/HER2 inhibitory activity and induced fit docking study. Journal of Molecular Structure, 1271, 133932.
[46] Shafiq, N., Arshad, U., Brogi, S., Rashid, M., Rafiq, N., & Parveen, S. (2022). Characterization of stenocephol from Seriphidium stenocephalum as potent HepG2 cell growth and glycogen phosphorylase inhibitor. Natural Product Research, 1-7.
[47] Matondo, A., Kilembe, J. T., Ngoyi, E. M., Kabengele, C. N., Kasiama, G. N., Lengbiye, E. M., . . . Gbolo, B. Z. (2021). Oleanolic acid, ursolic acid and apigenin from Ocimum basilicum as potential inhibitors of the SARS-CoV-2 main protease: A molecular docking study. Int J Pathog Res, 6(2), 1-16.
[48] Navien, T. N., Thevendran, R., Hamdani, H. Y., Tang, T.-H., & Citartan, M. (2021). In silico molecular docking in DNA aptamer development. Biochimie, 180, 54-67.
[49] Wu, Z., Yang, Q., & Ma, H. (2022). Study the mechanism of gualou niubang decoction in treating plasma cell mastitis based on network pharmacology and molecular docking. BioMed research international, 2022.
[50] Nawaz, M., Taha, M., Qureshi, F., Ullah, N., Selvaraj, M., Shahzad, S., . . . Almutairi, F. A. (2020). Structural elucidation, molecular docking, α-amylase and α-glucosidase inhibition studies of 5-amino-nicotinic acid derivatives. BMC chemistry, 14(1), 1-11 .
[51] Gunasekaran-E-mail, M., Subramanian, K., & Ravi–E-mail, R. (2022). Molecular docking analysis of lupeol with different cancer targets. Bioinformation, 18(3), 134-140.
[52] Nawaz, M., Taha, M., Qureshi, F., Ullah, N., Selvaraj, M., Shahzad, S., . . . Almutairi, F. A. (2020). Structural elucidation, molecular docking, α-amylase and α-glucosidase inhibition studies of 5-amino-nicotinic acid derivatives. *BMC chemistry, 14*(1), 1-11.
[53] Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., & Sreedhar, B. (2016). Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Materials Science and Engineering: C, 58, 36-43.
[54] Al-Janabi, A. S., Elzupir, A. O., & Yousef, T. A. (2021). Synthesis, anti-bacterial evaluation, DFT study and molecular docking as a potential 3-chymotrypsin-like protease (3CLpro) of SARS-CoV-2 inhibitors of a novel Schiff bases. Journal of Molecular Structure, 1228, 129454.
[55] Desai, N. C., Vaja, D. V., Joshi, S. B., & Khedkar, V. M. (2021). Synthesis and molecular docking study of pyrazole clubbed oxazole as antibacterial agents. Research on Chemical Intermediates, 47(2), 573-587.
[56] Horchani, M., Heise, N. V., Csuk, R., Ben Jannet, H., Harrath, A. H., & Romdhane, A. (2022). Synthesis and In Silico Docking Study towards M-Proof Novel Heterocyclic Compounds Derived from Pyrazolopyrimidinone as Putative SARS-CoV-2 Inhibitors. Molecules, 27(16), 5303.
[57] Babahedari, A. K., Soureshjani, E. H., Shamsabadi, M. K., & Kabiri, H. (2013). The comprehensive evaluation docking of methicillin drug containing isoxazole derivatives, as targeted antibiotics to Staphylococcus aureus. Journal of Bionanoscience, 7(3), 288-291.
[58] Ballu, S., Itteboina, R., Sivan, S. K., & Manga, V. (2018). Structural insights of Staphylococcus aureus FtsZ inhibitors through molecular docking, 3D-QSAR and molecular dynamics simulations. Journal of Receptors and Signal Transduction, 38(1), 61-70.
[59] Anju, V., Busi, S., Mohan, M. S., Ranganathan, S., Ampasala, D. R., Kumavath, R., & Dyavaiah, M. (2022). In vivo, in vitro and molecular docking studies reveal the anti-virulence property of hispidulin against Pseudomonas aeruginosa through the modulation of quorum sensing. International Biodeterioration & Biodegradation, 174, 105487.
[60] Nasli Esfahani, A., Iraji, A., Alamir, A., Moradi, S., Asgari, M. S., Hosseini, S., . . . Bandarian, F. (2022).

Design and synthesis of phenoxymethybenzoimidazole incorporating different aryl thiazole-triazole acetamide derivatives as α-glycosidase inhibitors. *Molecular diversity, 26*(4), 1995-2009.
[61] Alam, A., Hosen, M. A., Hosen, A., Fujii, Y., Ozeki, Y., & Abe Kawsar, S. M. (2021). Synthesis, characterization, and molecular docking against a receptor protein FimH of Escherichia coli (4XO8) of thymidine derivatives. Journal of the Mexican Chemical Society, 65(2), 256-276.
[62] Azam, M. A., Thathan, J., & Jupudi, S. (2020). Pharmacophore modeling, atom based 3D-QSAR, molecular docking and molecular dynamics studies on Escherichia coli ParE inhibitors. Computational biology and chemistry, 84, 107197.
[63] Ahmed, H. E., Ihmaid, S. K., Omar, A. M., Shehata, A. M., Rateb, H. S., Zayed, M. F., . . . Elaasser, M. M. (2018). Design, synthesis, molecular docking of new lipophilic acetamide derivatives affording potential anticancer and antimicrobial agents. Bioorganic chemistry, 76, 332-342.
[64] Rashdan, H. R., Shehadi, I. A., Abdelrahman, M. T., & Hemdan, B. A. (2021). Antibacterial activities and molecular docking of novel sulfone biscompound containing bioactive 1, 2, 3-triazole moiety. Molecules, 26(16), 4817.
[65] Fanfrlik, J., Bronowska, A. K., Rezac, J., Přenosil, O. e., Konvalinka, J., & Hobza, P. (2010). A reliable docking/scoring scheme based on the semiempirical quantum mechanical PM6-DH2 method accurately covering dispersion and H-bonding: HIV-1 protease with 22 ligands. The Journal of Physical Chemistry B, 114(39), 12666-12678.
[66] Santra, D., & Maiti, S. (2022). Molecular dynamic simulation suggests stronger in-silico docking of Omicron spike on ACE2 than Wild but weaker than Delta SARS-CoV-2 variants can be blocked by engineered S1-RBD fraction.
[67] Rai, H., Barik, A., Singh, Y. P., Suresh, A., Singh, L., Singh, G., . . . Modi, G. (2021). Molecular docking, binding mode analysis, molecular dynamics, and prediction of ADMET/toxicity properties of selective potential antiviral agents against SARS-CoV-2 main protease: an effort toward drug repurposing to combat COVID-19. Molecular diversity, 25(3), 1905-1927.