Copper(I)oxide Catalysis in the Synthesis of Functionalized Alanine-Based Sulphonamides: In Silico, In vitro Antibacterial Studies

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Abstract
The synthesis of functionalized alanine-based sulphonamides via copper(I)oxide catalyzed *N*-arylation reaction and the in silico antibacterial studies is reported. The study involved the initial synthesis of substituted *p*-toluenesulphonamides and substituted benzenesulphonamides by the reaction of alanine with *p*-toluenesulphonyl chloride and benzenesulphonyl chloride respectively in aqueous basic medium. The synthesized compounds were acetylated by reacting them with acetic anhydride and sodium acetate followed by acylation and amidation to yield amidated *p*-toluenesulphonamides and benzenesulphonamides respectively. Copper catalyzed *N*-arylation of the amidated products with aryltriolborates resulted in the synthesis of benzene, and 2-chlorophenyl derivatives of the amidated products. The synthesized compounds were characterized using FTIR, ^{1}HNMR and elemental analysis and the spectra were in agreement with the assigned structures. The *insilico* antibacterial studies revealed that the compounds possessed significant antibacterial potency and could be further employed as potential anti-bacterial agents when validated with experimental studies. The antimicrobial study revealed that most of the synthesized compounds possess antibacterial activities.

Keywords: Copper, N-arylation, Functionalized, Sulphonamides Alanine, *In-silico* studies

Introduction

Sulphonamides are informative and fascinating area in medicinal chemistry [1-4]. Its functional group has importance in organic chemistry and drug discovery [5-6]. The p-toluenesulphonamide and benzenesulphonamides have been widely explored in synthetic chemistry [7-11]. Use of Sulphonamides today is limited to specific disease treatment in human medicine, such as urinary tract infections; however, sulphonamides are more often used in animal treatment [12]. Extensive application of sulphonamides in chicken production has resulted in residues being detected in poultry products (eggs and meat) [13]. The widespread use of sulphonamides owing to their availability and low cost has resulted in considerable increase in resistant bacteria strains [14]. It is well documented that consumption of animal products containing sulphonamide residues poses potential human health risks which include hypersensitivity or analphylactic shock, cancer and induction of bacterial resistance to the antimicrobials, amongst other risks [15]. This is a good reason for the intensive research into Sulphonamides, for its synthesis, chemistry, applications, reactions and biological importance.

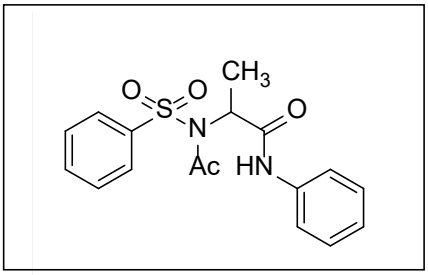
Copper is a magenta metal in the fourth cycle and the IB group of the periodic table [16]. Copper is a less active metal that does not combine with oxygen in dry air at room temperature and produces black copper oxide when heated. The common valence states of copper are 0, +1 and +2. Copper is one of the metals used by humans in the early days. It has many excellent properties, such as good electrical and thermal conductivity, good ductility, low cost and wide range of use [17-18]. Nano-scale copper has more excellent properties than traditional bulk copper materials, such as large specific surface area, strong plasticity, high strength, and low electrical resistance. Copper and its compounds have excellent activity in the catalytic oxidation, hydrogenation and dehydrogenation of organic compounds, so they are widely used in catalyst preparation [19-20]. For different catalytic reactions, copper-based catalysts have different requirements for the form of the active copper valence state. (16)
In the present study, functionalized aryl/heteroaryl sulphonamides were synthesized Copper(I)oxide catalyzed *N*-arylation reaction, using benzenesulphonyl chloride, *p*-toluenesulphonyl chloride, and alanine.

2.0 Materials and Methods

2.1 Procedure for the Synthesis of Sulphonamide Derivatives

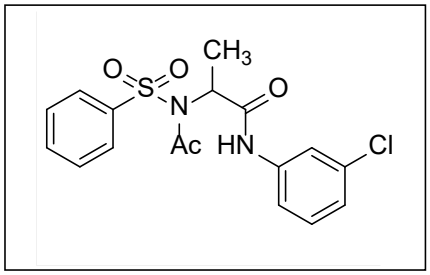
Phenyltriolborate (0.1 mmol) was transferred into a three naked round bottom flask placed on a hot plate fixed with a triple stand containing methanol (10mL), copper(I)oxide (1g) was added and then the amide (1 mmol) was added and stir with magnetic stirrer in the presence of air for about 5 hours. Then the solution obtained was poured into a beaker (200mL) for proper evaporation at room temperature. The product was then purified and dried.

2.1.1 *N*-phenyl-2-(*N*-(phenylsulphonyl)acetamido)propanamide



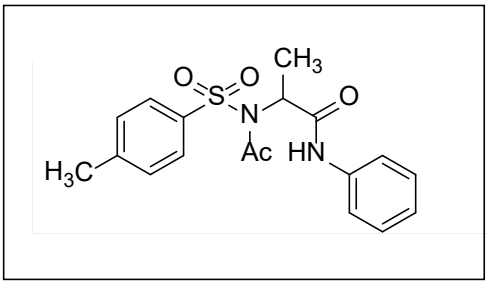
Yield 3.03g (90.2%), mp.165-166^{0}C

2.1.2 *N*-(3-chlorophenyl)-2-(*N*-(phenylsulphonyl)acetamido)propanamide



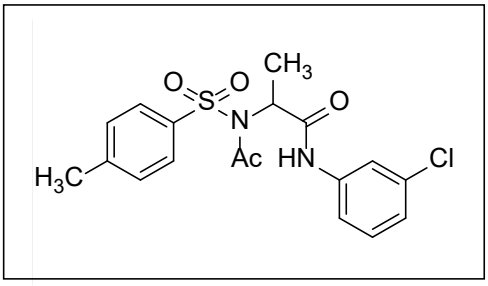
Yield 3.01g (88.7%), mp.160-161^{0}C

2.1.3 *N*-phenyl-2-(*N*-tosylacetamido)propanamide



Yield 3.03g (90.4%), mp.169-170^{0}C

2.1.4 *N*-(3-chlorophenyl)-2-(*N*-tosylacetamido)propanamide



Yield 3.03g (91.1%), mp.175-176^{0}C

2.3. MOLECULAR DOCKING

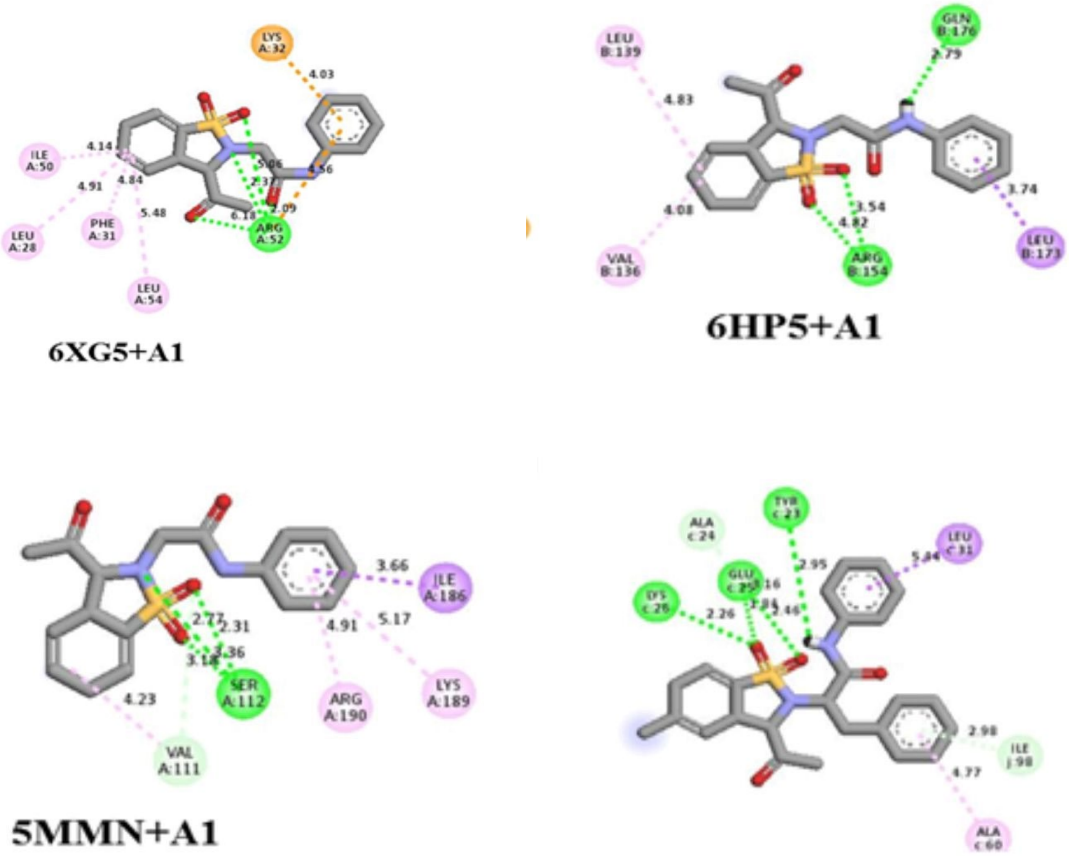


FIGURE 1:2D visualization of protein ligand interaction.

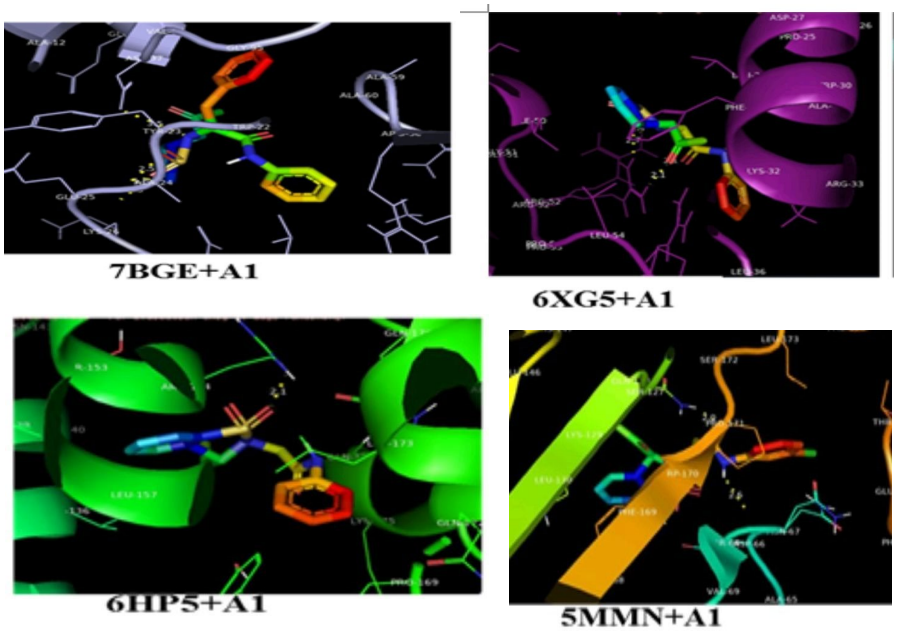


FIGURE 2:3D Visualization of protein Ligand interaction

2.3.1 Molecular docking procedures

The proteins used for molecular docking were downloaded from the protein data bankand prepared with the biovia discovery studio [21]. The receptors were prepared by defining reactive sites, adding explicit hydrogens and removing water molecules that were not necessary during the docking experiments. The receptor cavity was defined with X, Y, Z coordinates of 13.368000, 30.583000, 45.659312 respectively and a boundary size of 36 Å, then converted to pdbqt format with the aid of Autodock 4.0 [22]. The ligands were prepared by conducting energy minimization at the DFT/(B3lyp functional and 6-311++G(d,p); then, the respective proteins were docked with the studied compounds using Autodock vina, and the result output were visualized with PyMol and biovia discovery studio visualizer (The Pymol Molecular graphics system). The docking investigations and preparation of pdbqt files were accomplished by autodock 4.0 and autodock tools (ADT) through the Lamarckian Genetic Algorithm (LGA) search. The scoring function in autoDock is primarily based on hydrogen bonding, vander waals interaction, electrostatic interaction, entropy change upon the binding of the compound to the receptor and solvation energy. These were utilized to rank the generated conformations and output respectively. Therefore, the grid parameters (GPF) were set to 20×20×20Å, 20×20×20, and 20×20×20 along the X, Y, Z coordinate axes.

2.3.2 Docking validation and active site selection

Suitable bacteria proteins from *Escherichia coli*, *Bacillus subtilis*, *staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the protein data bank (www.RSCB.org) based on literature survey. To predict the active site of the receptor, two distinct approaches were employed; first the chosen receptor proteins were visualized with the online 3D visualization model in the RCSB repository to check the binding conformation and interacting amino acids of the co-crystalized ligand with the receptor proteins. Then the pdb files were downloaded and re-docked directly to affirm that the co-crystalized ligand fits back into the active pocket previously visualized. To further confirm the active site, the p2rank online server (https://prankweb.cz) was used to predict the active sites of the chosen pdb files (Jendele *et al* 2019). The protein□s active site constitutes an enzyme□s catalytic or inhibition site. The predicted active sites based on the z-score were then compared with the amino acid residues in the active site of the respective co-crystalized receptor complex. The close similarity of the predicted active site and the co-crystalized ligand site was used as the basis for docking the studied compound against the chosen bacterial proteins.

2.4. BIOLOGICAL ACTIVITIES

The determination the antibacterial activity of the substituted sulphonamides solutions were carried out using the Agar well diffusion method as described. For each of the respective Meuller Hinton Agar plates, a sterile cork borer of 6mm in diameter was used to create wells in the solidified Agar plates. Each of the wells were filled with 0.1mL of the sample concentrations of 50mg/mL, 100mgL, 150mg/mL and 200mg/mL and are labeled appropriately. Distilled sterile water was used to fill one of the wells to serve as negative control, while Cephalexin was used to fill another of the wells to serve as positive control. They were allowed to diffuse into the Agar at room temperature for one hour, then incubated at 37˚c for 18-24hours. The plates were observed for zone of inhibition or clear after 18-24hours incubation.

2.4.1 Collection of Test Organisms

The test organisms were collected from the Microbiology laboratory Prince Abubakar Audu University, Anyigba Nigeria. The organisms include *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

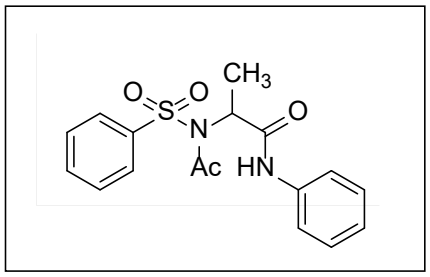
2.4.2 Determination of Minimum Inhibitory Concentration (Mic)

The estimation of the MIC of the complex was carried out. For this 0.5mL of varying concentrations of the samples in mg/mL (200, 150,100 and 50) were dispensed into each test tubes containing nutrient broth, inoculated with a loopful of each test organisms, 0.5 Mcfarland turbidity standard was adopted. A tube containing nutrients broth with test organism, but no complex served as control. The MIC was taken as the tube with least concentration with no visible turbidity after 24hrs incubation at 37˚c for 24hrs and then examine for growth by observing for turbidity.

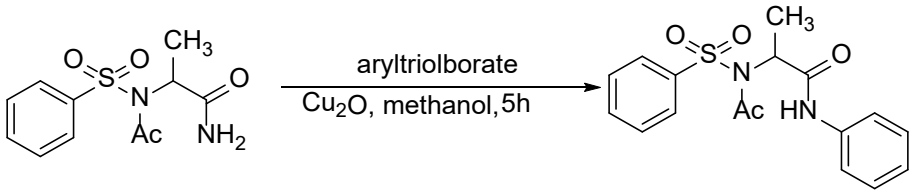
3.0 Results

3.1 Synthesis

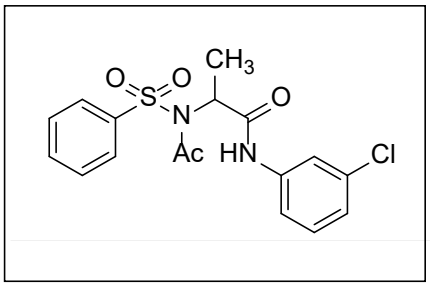
3.1.1 *N*-phenyl-2-(*N*-(phenylsulphonyl)acetamido)propanamide



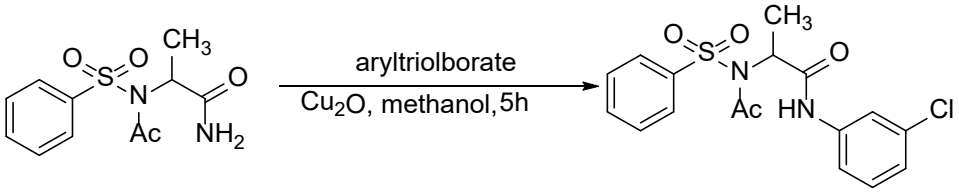
The structure is supported by spectral analyses. The most diagnostic vibrational bands in the FTIR are (KBr) cm-^{1}:752.9 (osubstituted benzene), 1319.5 (-SO_2-NH2), 2322.1 (R_3N-C), 1591.6 (C=O), 3309.9 (R_2-NH).. In the ^{I}H-NMR spectrum, the peaks at ppm. 7.7 (1H, s) due to NH of 2^{o} amine, ppm. 7.50 (10H, m) due to 2(monosubstituted benzene), ppm 4.0 (1H, q) due to C_1 proton ppm. 1.2 (3H, d) due to C_2 proton and ppm 2.4 (3H, s) due to CH_3-CO. Anal.calcd. for $C_{17}H_{18}N_2O_4S$ (346.40): C, 58.95; H, 5.24; N, 8.09. Found: C, 59.14; H, 5.23; N, 8.21.



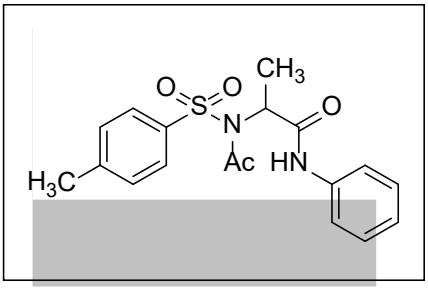
3.1.2 *N*-(3-chlorophenyl)-2-(*N*-(phenylsulphonyl)acetamido)propanamid



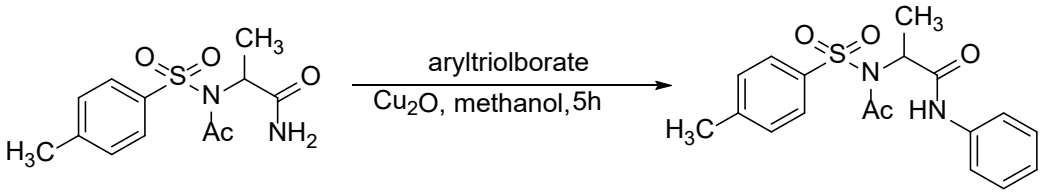
The structure is supported by spectral analyses. The most diagnostic vibrational bands in the FTIR are (KBr) cm^{-1}: 857.3 (mono-substituted benzene), 2594.2 R_3N, 1358.8 (SO_2, band), 1738.9 (C=O). In the ^{I}H-NMR spectrum, the peaks at ppm. 3.3 (1H, s) due to NH of 2^{o} amine, ppm. 3.2-3.1 (5H, m) due to *mono* substituted benzene, ppm 2.7-2.6 (1H, s; 3H, s) due to *m*-disubstituted benzene, ppm. 2.2 (1H, t) due to C_1 proton, ppm 1.8 (2H, d) due to C_2 proton, ppm 1.6-1.3 (1H, m) due to $CH(CH_3)_2$, ppm 1.2-1.1 (6H, d) due to $(CH_3)_2CH$ and ppm 2.5 (3H, s) due to CH_3-CO. Anal.calcd. for $C_{17}H_{17}ClN_2O_4S$ (380.84): C, 53.61; H, 4.50; N, 7.36. Found: C, 56.80; H, 4.48; N, 7.62.



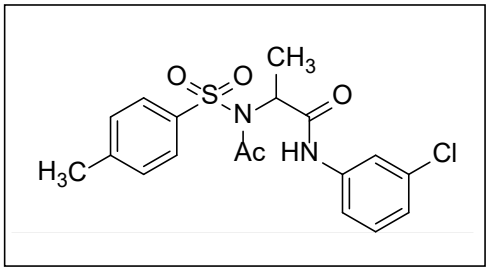
3.1.3 *N*-phenyl-2-(*N*-tosylacetamido)propanamide



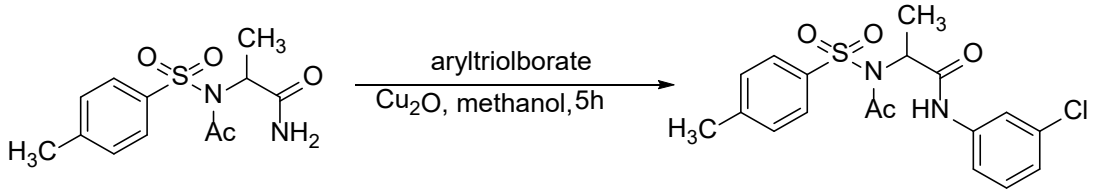
The structure is supported by spectral analyses. The most diagnostic vibrational bands in the FTIR are (KBr) cm^{-1}:846.1 (para-substituted benzene), 1774.2 (C=O), 2360.9 R_3N, 3328.5 R_2NH, 1341.8 (C=O), 88.748 (metadisubstituted benzene). In the ^{1}H-NMR spectrum, the peaks at ppm. 8.0 (1H, s) due to NH of 2^o amine, ppm. 7.3-7.2 (2H, d; 2H, d) due to *p*-disubstituted benzene), ppm 4.6 (5H, m) due to monosubstituted benzene, ppm 3.9 (1H, q) due to C_1 proton, ppm 2.0 (2H, d) due to C_2 proton, ppm 3.3 (3H, s) due to CH_3-n and ppm 2.4 (3H, s) due to CH_3-CO. Anal.calcd. for $C_{18}H_{20}N_2O_4S$ (360.43): C, 59.98; H, 5.59; N, 7.77. Found: C, 56.08; H, 5.37; N, 7.52.



3.1.4 *N*-(3-chlorophenyl)-2-(*N*-tosylacetamido)propanamide



The structure is supported by spectral analyses. The most diagnostic vibrational bands in the FTIR are (KBr) cm^{-1} 842.4 (para-substituted benzene), 2322.1 R_3N, 3265.1 R_2NH. In the ^{1}H-NMR spectrum, the peaks at ppm. 4.7 (1H, s) due to NH of 2^o amine, ppm. 4.7 (2H, d; 2H, d) due to *p*- disubstituted benzene), ppm 4.0 (1H, s; 3H, s) due to *m*-disubstituted benzene), ppm 3.95 (1H, q) due to C_1 proton, ppm 2.95 (3H, d) due to C_2 proton, ppm 3.75 (3H, s) due to CH_3-n and ppm 3.3 (3H, s) due to CH_3-CO. Anal.calcd. for $C_{18}H_{19}ClN_2O_4S$ (394.08): C, 54,75; H, 4.85; N, 7.07. Found: C, 54,44; H, 4.13; N, 7.57



3.2 Docking Results

Table 1: The binding Affinity of compound 1

Interaction	Binding affinity (Kcal/mol)	No of hydrogen bonds
5MMN+ 1	-7.5	5
6HP5+ 1	-7.3	5
6XG5+ 1	-9.7	7
7BGE+ 1	-7.0	3

Table 2: The binding Affinity of compound 2

Interaction	Binding affinity (Kcal/mol)	No of hydrogen bonds
5MMN+ 2	-7.3	5
6HP5+ 2	-7.7	5
6XG5+ 2	-8.6	7
7BGE+ 2	-8.3	3

Table 3: The binding Affinity of compound 3

Interaction	Binding affinity (Kcal/mol)	No of hydrogen bonds
5MMN+ 3	-7.1	5
6HP5+ 3	-7.4	5
6XG5+ 3	-9.6	7
7BGE+ 3	-8.5	3

Table 4: The binding Affinity of compound 4

Interaction	Binding affinity (Kcal/mol)	No of hydrogen bonds
5MMN+ 4	-7.3	5
6HP5+ 4	-8.3	5
6XG5+ 4	-9.0	7
7BGE+ 4	-7.4	3

Decades after the first successful treatment with antibiotics, bacterial infections are still a threat. The overuse and misuse of antibiotics, as well as the lack of new drug development due to reduced economic incentives and difficult regulatory requirements, are among the established factors accountable for antibiotic resistance crisis. Most microorganisms are known for causing a variety of human diseases. These include but not limited to diarrhea, pneumonia, toxic shock syndrome, and mild wound inflammation. Some notable bacteria specie implicated are *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. These microbes strains have developed resistance to most antibiotics, including the most effective methicillin variant. The efficacy of newly synthesized Sulphonamide derivatives to inhibit some selected microbes were evaluated via *in-silico*

approach. Suitable proteins PDB ID:5mmn, 6hp5, 6xg5 and 7bge from *Escherichia coli*, *Bacillus subtilis*, *staphylococcus aureus* and *Pseudomonas aeruginosa* were selected for docking experiments. The obtained results including the binding affinities, binding conformation, and number of interacting amino acids are presented in Tables 1-4. Numerous important interactions such as hydrogen bond interactions, electrostatic interactions, etc. were observed between the ligand and the respective proteins. In all cases, the results disclosed that the ligands interacted more favorably with the 6xg5 receptor of the *Staphylococcus* variant than other microorganisms studied. The highest average binding affinities were observed for the interaction with the S*taphylococcus* receptor. These binding affinities were observed between the ranges of -7.0 to -9.7 kcal/mol. These shows that all the studied ligands exhibit maximum inhibitory activity on the respective test organisms. Docking experiments are often assessed by the number of hydrogen bonds, binding affinity, and other stabilizing interactions. Based on this factor, the respective compounds can be concluded to possess significant antibacterial potency in the respective bacteria cells and could be further employed as potential anti-bacterial agents.

3.3 ANTIBACTERIA SUSCEPTIBILITY TESTS

Table 5: The antimicrobial activity of various concentrations of compound 1 in methanol, on the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	8 mm	8 mm	10 mm	22 mm	31 mm	0
2	*Bacillus subtilis*	6 mm	6 mm	7 mm	8 mm	22 mm	0
3	*Escherichia coli*	8 mm	9 mm	12 mm	21 mm	25 mm	0
4	*Pseudomonas aeruginosa*	6 mm	7 mm	9 mm	20 mm	32 mm	0

The zone of clearance or inhibition expressed by the various concentration on the test organisms range from 6mm-22mm. *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were the most sensitive and are at the highest concentration, as seen in table 5. The positive control (cephalexin) developed resistance against *Escherichia coli* only.

Table 6: The minimum inhibitory concentration (MIC) of compound 1 in methanol against the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	G	G	NG	NG	NG	G
2	*Bacillus subtilis*	G	G	NG	NG	NG	G
3	*Escherichia coli*	G	G	G	NG	NG	G
4	*Pseudomonas aeruginosa*	G	G	G	NG	NG	G

Key: NG = No growth, G = Growth.

Table 6 shows that compound 1 inhibited growth at 150mg/mL, and did not show any sign of turbidity or growth for *Escherichia coli* and *Pseudomonas aeruginosa*(clear) thus was taken as the MIC value at the highest concentration.

Table 7: The antimicrobial activity of various concentrations of compound 1 in methanol, on the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	7 mm	7 mm	10 mm	20 mm	31 mm	0
2	*Bacillus subtilis*	6 mm	7 mm	8 mm	8 mm	22 mm	0
3	*Escherichia coli*	8 mm	8 mm	11 mm	22 mm	25 mm	0
4	*Pseudomonas aeruginosa*	6 mm	7 mm	11 mm	20 mm	32 mm	0

The zone of clearance or inhibition expressed by the various concentration on the test organisms range from 6mm-22mm. Escherichia coli was the most sensitive at the highest concentration, while others show resistance.

Table 8: The minimum inhibitory concentration (MIC) of compound 2 in methanol against the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	G	G	G	G	NG	G
2	*Bacillus subtilis*	G	G	G	NG	NG	G
3	*Escherichia coli*	G	G	G	NG	NG	G
4	*Pseudomonas aeruginosa*	G	G	G	NG	NG	G

Key: NG = No growth, G = Growth.

Table 8 shows that compound 2 inhibited growth at 200mg/mL, and did not show any sign of turbidity or growth for *Staphylococcus aureus* (clear) thus was taken as the MIC value at the highest concentration.

Table 9: The antimicrobial activity of various concentrations of compound 1 in methanol, on the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	2 mm	6 mm	8 mm	20 mm	31 mm	0
2	*Bacillus subtilis*	6 mm	6 mm	7 mm	7 mm	22 mm	0
3	*Escherichia coli*	8 mm	8 mm	12 mm	20 mm	25 mm	0
4	*Pseudomonas aeruginosa*	7 mm	7 mm	10 mm	22 mm	32 mm	0

The zone of clearance or inhibition expressed by the various concentration on the test organisms range from 2-22mm. *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were the most sensitive while *Bacillus subtilis* showed low resistance.

Table 10: The minimum inhibitory concentration (MIC) of compound 3 in methanol against the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	G	G	G	NG	NG	G
2	*Bacillus subtilis*	G	G	G	NG	NG	G
3	*Escherichia coli*	G	G	G	NG	NG	G
4	*Pseudomonas aeruginosa*	G	G	NG	NG	NG	G

Key: NG = No growth, G = Growth.

Table 10 shows that compound 3 inhibited growth at 150mg/mL, and did not show any sign of turbidity or growth for *Pseudomonas aeruginosa*(clear) thus was taken as the MIC value at the highest concentration.

Table 11: The antimicrobial activity of various concentrations of compound 1 in methanol, on the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	5 mm	8 mm	11 mm	21 mm	31 mm	0
2	*Bacillus subtilis*	6 mm	6 mm	7 mm	9 mm	22 mm	0
3	*Escherichia coli*	8 mm	8 mm	10 mm	20 mm	25 mm	0
4	*Pseudomonas aeruginosa*	6 mm	7 mm	10 mm	22 mm	32 mm	0

The zone of clearance or inhibition expressed by the various concentration on the test organisms range from 5mm-22mm. *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were the most sensitive at the highest concentration.

Table 12: The minimum inhibitory concentration (MIC) of compound 4 in methanol against the test organisms

S/N	Test Organisms	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	Positive control	Negative control
1	*Staphylococcus aureus*	G	G	G	NG	NG	G
2	*Bacillus subtilis*	G	G	G	G	NG	G
3	*Escherichia coli*	G	G	G	G	NG	G
4	*Pseudomonas aeruginosa*	G	G	G	NG	NG	G

Key: NG = No growth, G = Growth.

Table 12 shows that compound 4 inhibited growth at 200mg/mL, and did not show any sign of turbidity or growth for *Staphylococcus aureus* and *Pseudomonas aeruginosa* (clear) thus was taken as the MIC value at the highest concentration

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