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Synthesis of Arylsulphonamoyl Phe-Gly-Dipeptide Carboxamide Derivatives and Their In-Silico and In-Vitro Properties.

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Abstract

*Eighteen new aryl sulphonamoyl 'Phe-Gly'-dipeptide carboxamide derivatives were synthesized, characterized and investigated for their in-silico and in vitro properties. The base mediated reaction of L-phenylalanine with para-nitrobenzenesulphonylchloride, para-toluenesulphonylchloride and benzenesulphonylchloride was successfully carried out to produce the respective benzenesulphonamide intermediates (**3a-c**). This was followed by the protection of the amino group of glycine **(4)** with tert-butyloxycarbamate (Boc) using a solution of di-tert-butyldicarbonate in 1, 4-dioxane at 5^{o}C to obtain (**5**). Amidation reaction of the Boc-protected glycine with six amines 4-aminophenol, 1-H-indole, 1-H-imidazole, guanine, adenine and cytosine yielded the respective Boc-protected carboxamides (**7a-f**). The carboxamides formed were then deprotected using DCM/trifluoroacetic acid (TFA; 1:1%) for 1 hour to yield (**8a-f**). The reaction of the deprotected carboxamides with benzenesulphonamide and substituted benzenesulphonamides in the presence of amide coupling partners 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) and 1-hydroxylbenzotriazole (HOBt) at room temperature yielded the eigtheen 'Phe-Gly'-dipeptide carboxamide target molecules (**9a-r**) in 39-95% yields. The synthesized compounds were characterized using Fourier Transformed Infrared (FTIR), and Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR). Computational studies were also carried out to determine their antibacterial, antifungal, anti-convulsant, anti-diuretic, anti-insomnia, anti-leishmaniasis and anti-leukamia potentials. All the compounds showed good binding. The binding energies (kcal/mol) for anti-bacteria, antifungal, antileishmaniasis, anticonvulsant, antidiuretic, antiinsomnia and antileukaemia were -7.0 to -7.6, -8.1 to -10.1, -9.4 to -9.8, -8.0 to -8.1, -10.1 to -10.4, -10.4 to -10.8 and -7.0 to -7.7 respectively. Antimicrobial study was carried out to determine the activities of the compounds against Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogene, Escherichia coli and Candida albicans. The Inhibitory zone diameter (IZD) values were 3-13 mm. The Minimum Inhibitory Concentration (MIC) values in (mg/mL) were 1.485 - 15.848 and the Minimum Bactericidal Concentration (MBC) values (mg/mL) were 12.500 - 25.500. The compounds showed their highest activities towards Staphylococcus aureus.*

Keywords: *Carboxamides, aryl, sulphonamides, dipeptide, docking, antifungal, antibacterial.*

1. INTRODUCTION

Research efforts which investigate the potential of the synergy between carboxamide, sulphonamides, dipeptides and amines in the search for biologically active compounds have been ongoing. Thiazolyl derivatives of benzenesulphonamide in synergy with carboxylate group exhibited antimalarial potential (Ezugwu JA, 2024; Onoabedje EA, 2021). Compounds having carboxamide and sulphonamides groups have been shown to possess good antihyperglycemic properties with no antioxidant effects and hence they are good candidate in the search for antidiabetic drugs (Onyejekwe BO, 2024; Onoabedje EA, 2020). Compounds of the sulphonamide class have also been known to have antibacterial, antifungal and antioxidant activities (Ochu RC, 2024; Oguz M, 2020) 'Phe-Gly' dipeptides molecules bonded to sulphonamide groups showed activity against *Plasmodium falciparum* (Aronimo BS, 2021; Ezugwu JA, 2020). Isoleucine-glycine dipeptides sulphonamides are potent antimalarial and antitrypanosomal agents (Ekoh OC, 2021; Ekoh OC, 2022). Val-val dipeptide-sulphonamide exhibit good antioxidant properties (Ezugwu JA, 2020). Dipeptide-sulphonamides containing amino acids are effective antimalarial and antioxidant agents (Ezugwu JA, 2022; Ugwu DI, 2019). Beneficial anti-inflammatory, antioxidant and antidiabetic properties identified in 'Pro-Gly' dipeptide having sulphonamide functional group (Attah SI, 2022). 'Ile-Gly' dipeptides with sulphonamide functional group are good candidates in the treatment of trypanosomiasis and malaria (Ekoh OC, 2022; Ugwu DI, 2018). Carboxamides and sulphonamides have been widely known in the treatment of malaria and other bacterial infections (Ugwuja DI, 2018; Abo KA, 1999; Abuelizz HA, 2017). Carboxamides bearing sulphonamide functional groups have also to a large extent been shown to exhibit significant lethal effect against *Plasmodium falciparum*, the causative agent of the human malaria (Onoabedje EA, 2020; Agamannone M, 2022). The molecular docking of sulphonamide pyrolidine carboxamide derivatives showed that they exhibit antiplasmodial and antioxidant activities (Onoabedje EA, 2021; Aissauti KO, 2005). In 2018 alone, 214 million cases of malaria infections were recorded which resulted in 400000 deaths (Onoabedje EA, 2021; Akinyemi KO, 2005). Sulphonamides have also been shown as potent drug in the treatment of colon cancer (Bhat MA, 2005; Ali M, 2020; Gui HI, 2020). Sulphonamides also help as inhibitors of carbonic anhydrase (Gui HI, 2020; Ali SA, 2009). Sulphonamides are compounds containing the sulphonamide (SO_2NH_2) functional group in their chemical structure (Ananthanarayanan VS, 1993; Lavanya R, 2017). The sulphonamide functional group consists of a sulphonyl group connected to an amine group (Angeli A, 2017; Atata R, 2003). Relatively speaking, this group is unreactive. The amine centre is no longer basic and the Sulphur-nitrogen bond is only cleaved with difficulty (Atata R, 2003; BahramiK, 2009). Due to the rigidity of the functional group, sulphonamides are typically crystalline. For this reason, the formation of the sulphonamide is a classic method of converting an amine to a crystalline derivative which can be identified by its melting point. Many important drugs contain the sulphonamide group (El-din NS, 2000). Sulphonamides are antibiotics having structural similarity with p-aminobenzoic acid (PABA). Sulphanilamides are p-aminobenzenesulphonamides that show bacteriostatic action against gram-positive and gram-negative bacteria. Sulphonamide drugs were the first antimicrobial drugs (Sidgique M, 2000). The name of the first sulphonamide is Protonsil . Protonsil was the first available antibacterial drug with broad effect against gram-positive *cocci* but not against enterobacteria (Supuran CT, 2014). Sulphonamides show carbonic anhydrase inhibitory properties. This is one of the reasons that provoke many syntheses of sulphonamide derivatives. Carbonic anhydrase are metalloenzymes present in Archaea, prokaryotes and eukaryotes, and catalyze a physiologically very simple but important reactions including the conversion of carbon(IV)oxide (CO_2) to hydrogentrioxocarbonate (IV) (HCO_3^-) , via a ping pong mechanism (Tacic A, 2017).

2. MATERIALS AND METHODS

2.1 Instrumentation

The reagents utilized in this work are all of analytical grade and were purchased from Sigma-Aldrich and DBH. They were used without further purification. TLC was carried out with silica gel precoated plates F_{254}. After the experiment the plates were developed with iodine crystals. FTIR were run on IR M530 Buck Scientific infrared spectrophotometer with KBr as a support material. Melting points were determined using with NDJ-5S Searchtech instrument. The ^1H and ^{13}C NMR were carried out in the School of Chemistry and Physics, Westville Campus, University of KwaZulu-Natta, Durban 3629, South Africa, using 600 MHz spectrometers in $CDCl_3$ using tetramethylsilane as internal standard. The *in silico* analysis for anti-bacteria, antifungal, antileishmaniasis, anticonvulsant, antidiuretic, antiinsomnia and antileukaemia potentials were also studied using Auto Dock Vina version 4.2 by means of PyRx, a graphical user interface program. The *in vitro* anti-bacteria and antifungal activities against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Candida albican* were determined using standard methods. In the antimicrobial study, two gram-positive organisms (*staphylococcus aureus* ATCC 9027, *Streptococcus pyogenes*), two Gram-Negative organisms (*Escherichia coli* ATCC 6538P, *Pseudomonas aeruginosa*) and a yeast fungus- *Candida*

albican were studied. All microorganisms used in this research were obtained from the microbial bank of the Department of Pharmaceutical Microbiology, Enugu State University of Science and Technology, Agbani. They were maintained on Mueller-Hinton Agar medium.

2.2 General procedure for the synthesis of substituted 2-(benzenesulphonamido)-3-benzenepropanoic acid (3a-c).

The two necked flask was cleaned and distilled water (15 mL) was added followed by Sodiumtrioxocarbonate (IV) Na_2CO_3 (1.5g, 14.2mmol), L-phenylalanine (2g, 12.12 mmol) and the solution was continuously stirred with a magnetic strirrer until all the solutes dissolved. The solution was cooled to 2^{o}C and appropriate benzenesulphonylchloride (12.12 mmol) (**2a-c)** was added in four portions over a period of 1 hour. The solution was thereafter stirred at room temperature for 4 hours. The progress of the reaction was monitored with TLC, employing precoated TLC plates (Acetone/ n-hexane 1:9). The mixture was then acidified with 20% aqueous hydrochloric acid HCl to pH 2. The product **(3a-c)** were obtained in analytical grade after washing with tartaric acid solution of pH 2.2. The product was dried over fused silica gel in a dessicator (Ugwu DI, 2019).

2.3 General procedure for the protection of glycine with Di-ter-butyldicarbonate (Boc anhydride) (5).

The glycine **4** was dissolved in water (20 mL) and sodiumbicarbonate $NaHCO_3$ (2g, 23.8 mmol) was added and the solution was continuously stirred using a magnetic stirrer. Then it was cooled to 5^{o}C and Boc anhydride (1.5g, 6.87mmol) was added slowly as a solution in 1,4-dioxane (10mL). The solution was then stirred at 0^{o}C for 1 hour and allowed to warm to room temperature overnight. Water was then added and the aqueous layer was extracted 2 times with ethylacetate (EtOAc). The organic layer was back extracted twice with saturated sodiumbicarbonate solution. The combined aqueous layers were acidified to a pH of 1 with 10% HCl, then extracted 3 times with ethylacetate (EtOAc). The combined organic layers were dried to obtain the product 2-((tert-butoxycarbonyl) amino) ethanoic acid **5** (Ugwu DI, 2019; Attah SI, 2022).

2.4 General procedure for the synthesis of carboxamide derivatives of

2-((tert-butoxycarbonyl) amino) ethanoic acid (7a-f)

To a mixture of the respective amine 1g (2.98 mmole) **(6a-f)**, 1-ethyl-3-(3-dimethylaminopropyl) carboxamide (EDC) (1g), 1M HCl (5.0 mL) and Hydroxylbenzotriazole (HOBt) (1.2g) in a two necked flask was added DCM (5.0 mL) and N,N-diisoproylethylamine (DIPEA) (5.0 mL) and the reaction mixture stirred for 30 minutes at room temperature. Then, boc-glycine (1mL) **5** was added and the reaction mixture was stirred using a magnetic stirrer at room temperature for 12 hours. Thereafter, crushed ice was added to the product in a beaker and left to cool. Then the content of the beaker was filtered and the residue was air dried overnight to obtain the product **(7a-f)**. The purity of the glycine-boc carboxamides were ascertained using precoated TLC plates (acetone: n-hexane 30:70) and was found pure (Ugwu DI, 2019; Attah SI, 2022).

2.5 General standard Deprotection procedure for the amine (6c, 7c, 8c and 9c).

The compound **6c, 7c, 8c and 9c** was dissolved in 1:1 (trifluoroacetic acid: DCM) 10mL and stirred at room temperature for two hours. The purity of the product was confirmed using precoated TLC plates (30:70, acetone:n-hexane) and was found pure (Ugwu DI, 2019; Attah SI, 2022).

2.6 General procedure for the synthesis of Phe-Gly Dipeptide carboxamide sulphonamide.

Appropriate substituted benzenesulphonamide (**3a-c**) (5.747mmole), EDC 10mL, 1M HCl 5.0mL and HOBt 10.0mL in a two necked flask was added DCM 5.0 mL and DIPEA 5.0mL and the reaction mixture was stirred for 30 minutes at room temperature. Then, carboxamide derivative (**8a-f**) (5.747mmole) was added and the reaction was stirred using a magnetic stirrer at room temperature for 18 hours. Thereafter, crushed ice was added to the product in a beaker and left to cool. Then the content of the beaker was filtered and the residue was air dried overnight to obtain the product **(9a-r)**. The purity of the product was tested using precoated TLC plates (acetone:n-hexane 30:70) and was found pure. (Oguz M, 2020; Aronimo BS, 2021).

2.6.1 N-(2-((4-hydroxyphenyl) amino)-2-oxyethyl)-2-(4-nitrophenylsulphonamido)-3-phenylpropanamide (9a).

Dark-brown crystals. **Yield** (1.903g, 67%). **Melting point**129^o-130^{o}C. **FTIR (KBr, cm^{-1})** 3334.33 (NH), 3021.05 (C-H stretch of aromatic), 2859 (C-H aliphatic), 2733, 2499 (amino acid), 1625 (C=O), 1402 (2S=O), 1229 (SO_2N), 787 (trans amide linkage). **^{1}H NMR:**δ8.03 (1H, s, CONH), δ7.21 (1H, s, CONH proton), δ8.22, 8.12, 7.54 (5H, m, Ar-H), 7.43, 7.42. 7.29 (5H, m, Ar-H), 6.90, 6.80, 6.71, 6.50 (4H, m, Ar-H), δ7.63 (1H, s, SO_2NH proton), 5.73 (1H, s, OH) δ3.94(1H, t, methine proton), δ3.43 and 3.32 (2H, s, methylene proton), 4.14 (2H, s, CH_2).**δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 178.00 (C=O), 168.00 (C=O), δ 153.00, 151.11, 143.20, 126.10, 128.00, 127.50, 126.40, 125.90, 125.20, 124.16, 122.10, 119.20

(12C, Ar-C), 58.29 (1C, methine carbon), δ43.10 and 36.50 (2C, CH_2).

2.6.2 N-(2-((4-hydroxyphenyl) amino)-2-oxoethyl)-2-(4-methylphenylsulphonamido)-3-phenylpropanamide (9b).

Dark-brown crystals. **Yield** (2.843g, 97%). **Melting point**154^{o}C-154^{o}C. **FTIR (KBr, cm^{-1})** 3269 (NH), 3015 (C-H stretch of aromatic), 2934 (C-H aliphatic), 2747, 2450, 2218 (amino acid), 1621 (C=O), 1436 (C=C), 1274 (SO_2N). **^{1}H NMR:**δ8.14 (1H, s, CONH), δ7.24 (1H, s, CONH proton), δ8.08, 8.22, 8.31 (5H, m, Ar-H), 7.71, 7.25. 7.00 (5H, m, Ar-H), 6.93, 6.80, 6.73, 6.50 (4H, m, Ar-H), δ7.44 (1H, s, SO_2NH proton), 5.33 (1H, s, OH) δ3.72 (1H, t, methine proton), δ3.74 and 3.40 (2H, s, methylene proton), 4.14 (2H, s, CH_2).**δ_C (600MHz, DMSO):** ^{13}C NMR: 178.00 (C=O), 168.00 (C=O), δ 156.22, 148.31, 148.00, 136.22, 129.00, 127.20, 126.10, 124.11, 123.14, 120.00, 119.90, 119.11 (12C, Ar-C), 59.22 (1C, methine carbon), δ49.20 and 32.08 (2C, CH_2).

2.6.3 N-(2-((4-hydroxyphenyl) amino)-2-oxoethyl)-3-phenyl-2-(phenylsulphonamido) propanamide (9c).

Dark-brown crystals. **Yield** (2.326g, 78%). **Melting point** 142^{o}C. **FTIR (KBr, cm^{-1})** 3482 (OH), 3300 (NH), 3166 (C-H stretch of aromatic), 2745, 2456 (amino acid), 1730 (C=O), 1424 (C=C), 1308 (2S=O) 804 (NH deformation), 771 (trans amide linkage). ^{1}H NMR:δ8.33 (1H, s, CONH), δ7.20 (1H, s, CONH proton), δ8.48, 8.40, 8.22 (5H, m, Ar-H), 8.11, 7.91. 7.23 (5H, m, Ar-H), 7.00 (2H, J=7.21Hz, Ar-H) 6.80,(2H, d, J=7.00Hz,Ar-H), δ7.34 (1H, s, SO_2NH proton), 5.34 (1H, s, OH) δ3.82 (1H, t, methine proton), δ3.81 and 3.52 (2H, s, methylene proton), 4.14 (2H, s, CH_2).**δ_C (600MHz, DMSO):** ^{13}C NMR: 178.00 (C=O), 172.00 (C=O), δ 158.11, 149.30, 148.22, 138.21, 128.21, 127.30, 126.60, 125.34, 123.14, 122.60, 119.60, 119.21 (12C, Ar-C), 55.20 (1C, methine carbon), δ46.23 and 38.08 (2C, CH_2).

2.6.4 N-(2-(1H-indol-1-yl) 2-oxoethyl)-3-phenyl-2-(phenylsulphonamido) propanamide. (9d).

White crystalline solid**, Yield (**2.12g, 80.1%), mp: 140.1-140.8^{o}C, **FTIR (KBr, cm^{-1})** 3517, 3334 (NH), 3185 (C-H stretch of aromatic), 2950 (C-H aliphatic), 2724, 2598, 2428. (amino acid), 1625 (C=O), 1293 (2S=O) 893 (monosubstituted aromatic ring). **δ_H (600MHz, DMSO):** δ8.91 (1H, s, CONH), δ8.15 (1H, s, CONH proton), δ8.22, 8.11, 8.90 (5H, m, Ar-H), 8.71, 8.50. 8.20 (5H, m, Ar-H), 7.27 (2H, J = 6.72 Hz, Ar-H) 6.20, (2H, d, J= 7.12 Hz, Ar-H), δ7.44 (1H, s, SO_2NH proton), 5.00 (1H, s, OH) δ3.62(1H, t, methine proton), δ3.67 and 3.51 (2H, s, methylene proton), 3.51 (2H, s, CH_2).**δ_C (600MHz, DMSO):** ^{13}C NMR: 178.00 (C=O), 176.21 (C=O), δ 159.00, 156.15, 150.23, 149.70, 129.22, 128.00, 127.40, 126.00, 125.24, 123.00, 121.90, 120.00 (12C, Ar-C), 53.69 (1C, methine carbon), δ48.10 and 32.58 (2C, CH_2).

2.6.5 N-[2-(1H-imidazol-1-yl)-2-oxoethyl]-3-phenyl-2-(phenylsulphonamido) propanamide (9e).

Appearance: white coloured solid**, Yield (**2.83g, 85.86%), mp: 209-209.4^{o}C, **FTIR (KBr, cm^{-1})** 3678, 3298 (NH), 3143 (C-H stretch of aromatic), 3002 (C-H aliphatic), 2781.902, 2887.584, 2572.407 (amino acid), 1609.959 (C=O), 1347.667 (2S=O) 693.3769 (monosubstituted aromatic ring).**δ_H (600MHz, DMSO):**δ8.00 (1H, s, CONH), δ8.21 (1H, s, CONH proton), δ8.96, 8.42, 8.00 (5H, m, Ar-H), 8.83, 8.63. 8.52 (5H, m, Ar-H), 7.23 (2H, J = 6.50 Hz, Ar-H) 7.80, (2H, d, J= 6.25 Hz, Ar-H), δ7.05 (1H, s, SO_2NH proton), 5.70 (1H, s, OH) δ3.90(1H, t, methine proton), δ3.65 and 3.40 (2H, s, methylene proton), 3.22 (2H, s, CH_2).**δ_C (600MHz, DMSO):** ^{13}C NMR: 177.31 (C=O), 171.02 (C=O), δ 138.00, 129.40, 123.80, 123.00, 122.90, 122.70, 122.20, 122.11, 121.74, 121.00, 119.90, 119.20 (12C, Ar-C), 58.45 (1C, methine carbon), δ52.20 and 38.00 (2C, CH_2).

2.6.6 N-{2-oxo-2-[(6-oxo-6,9-dihydro-1H-purin-2-yl)amino]ethyl}-3-phenyl-2-(phenylsulphonamido)propanamide (9f).

White coloured solid**, Yield (**1.32g, 66.8%), mp: 195-195.4^{o}C,**FTIR (KBr, cm^{-1})** 3507 (NH), 3270 (C-H stretch of aromatic), 2980 (C-H aliphatic), 2790, 2583, 2518 (amino acid), 1629 (C=O), 1316 (2S=O), 800 (monosubstituted aromatic ring).**δ_H (600MHz, DMSO):** δ8.81 (1H, s, CONH), δ8.60 (1H, s, CONH proton), δ8.44, 8.31, 8.29 (5H, m, Ar-H), 8.21, 8.20. 8.17 (5H, m, Ar-H), 7.97 (2H, J = 6.01 Hz, Ar-H) 6.81, (2H, d, J= 7.00 Hz, Ar-H), δ6.44 (1H, s, SO_2NH proton), 4.91 (1H, s, OH) δ3.52 (1H, t, methine proton), δ3.47 and 3.31 (2H, s, methylene proton), 3.11 (2H, s, CH_2).**δ_C (600MHz, DMSO):** ^{13}C NMR: 179.00 (C=O), 170.50 (C=O), δ 151.00, 149.80, 149.50, 148.00, 129.10, 128.60, 127.00, 126.11, 125.14, 124.00, 121.00, 119.90 (12C, Ar-C), 57.69 (1C, methine carbon), δ49.10 and 37.58 (2C, CH_2).

2.6.7 N-[2-[(9H-purin-6-yl) amino]-2-oxoethyl}-3-phenyl-2-(phenylsulphonamido)propanamide (9g).

Off white crystalline solid**, Yield** 2.11g, 84.74%), mp: 253.1-253.7^{o}C, **FTIR (KBr, cm^{-1})** 3532, 3317 (NH), 3017(C-H stretch of aromatic), 2880 (C-H aliphatic), 2552 (amino acid), 1814 (C=O), 1401 (2S=O) 827 (monosubstituted aromatic ring).**δ_H (600MHz, DMSO):**δ9.33 (1H, s, CONH), δ7.25 (1H, s, CONH proton), 7.81 (1H, m, Ar-H), 7.86 (2H, dd, J = 6.70, 6.12 Hz, Ar-H), 7.60 (2H, dd, J = 6.23, 6.12 Hz, Ar-H), 7.26 (2H, dd, J = 6.29, 6.10 Hz, Ar-H), 7.39 (2H, dd, J = 6.73, 6.31 Hz, Ar-

H), 7.24 (1H, m, Ar-H), 8.61(1H, s, Ar-H), 7.86 (1H, dd, J = 7.10, 6.92 Hz, Ar-H), 11.0 (1H, s, Ar-NH), 7.03 (1H, s, SO_2NH), 3.95 (1H,t, CH), 4.00 (2H, d, J = 6.84Hz, CH_2). **δ_C (600MHz, DMSO):** ^{13}C NMR: 178.00 (C=O), 176.21 (C=O), δ 159.00, 156.15, 150.23, 149.70, 129.22, 128.00, 127.40, 126.00, 125.24, 123.00, 121.90, 120.00 (12C, Ar-C), 53.69 (1C, methine carbon), δ48.10 and 32.58 (2C, CH_2).

2.6.8 N-{2-oxo-2[(2-oxo-1,2-dihydropyrimidin-4-yl)amino]ethyl}-3-phenyl-2-(phenylsulphonamido)propanamide (9h).

Off white crystalline solid**, Yield (**1.26g, 93.0%),mp: 217.1-217.8ºC, **FTIR (KBr, cm^{-1})** 355 (NH), 3182, 3080 (C-H stretch of aromatic), 2934 (C-H aliphatic), 2811, 2711, 2541 (amino acid), 1621 (C=O), 1388 (SO_2N).**δ_H (600MHz, DMSO):**8.40 (1H, d, J = 7.36Hz, Ar-H), 8.0 (2H, s, Ar-H; s, CONH), 7.75 (1H, s, CONH), 7.70 (1H, m, Ar-H), 7.62, 7.86 (4H, m, Ar-H), 7.40, 7.29, 7.27 (5H, m, Ar-H), 5.40 (1H, d, J = 7.32 Hz, Ar-H), 3.80 (2H, q, CH_2), 3.44, 3.19 (2H, t, CH_2), 1.53 (1H, d, J= 7.30, SO_2NH). **δ_C (600MHz, DMSO):** ^{13}C NMR: 200 (1C, C=O), 172 (1C, CONH), 171, 166.9, 158.1, 93.9 (4C, Ar-C), 144.2, 131.9, 130.3, 129.0 (6C, Ar-C), 136.6, 128, 127.8, 125.9 (6C, Ar-C), 59.1 (1C, CH), 52.8 (1C, CH_2), 36.5 (1C, CH_2).

2.6.9 N-[2-(1H-indol-1-yl)-2-oxoethyl]-2-(4-methylphenylsulphonamido)-3-phenylpropanamide (9i).

Off white coloured solid**, Yield (**2.36g, 86,5%),mp: 215.1-215.8ºC. **FTIR (KBr, cm^{-1})** 3682 (NH), 3197 (C-H stretch of aromatic), 2984 (amino acid), 2761, 2842, 2448 (amino acid), 1627 (C=O), 1340 (2S=O), 1291, 1150 (SO_2N), 887 (monosubstituted aromatic ring.**δ_H (600MHz, DMSO):**8.11, 7.93, 7.83, 7.33, 6.87, 6.50 (6H, m, Ar-H), 8.03 (1H, t, CONH), 7.76 (1H, dd, J = 7.63, 6.90Hz, SO_2NH), 7.74 (2H, dd, J = 7.26, 7.10 Hz, Ar-H), 7.40 (2H, m, Ar-H), 7.29, 7.40, 7.27 (5H, m, Ar-H), 4.33 (2H, s, CH_2), 3.90 (1H, t, CH), 3.44, 3.19 (2H, dt, 8.10, 7.36Hz, CH_2), 2.30 (3H, s, CH_3).**δ_C (600MHz, DMSO):** ^{13}C NMR: 172.O (C=O), 168.3 (C=O), δ141.5, 137.6, 129.3, 128.3 (6C, Ar-C), 136.6, 127.2, 128.6, 125.8 (6C, Ar-C), 137.4, 130.4, 125.1, 124.5, 120.3, 119.2, 115.3, 109.5 (8C, Ar-C). 58.7 (1C, methine carbon), δ41.50 and 36.70 (2C, CH_2). 20.1 (1C, CH_3).

2.6.10. N-[2-(1H-imidazol-1-yl)-2-oxoethyl]-2-(4-methylphenylsulphonamido)-3-phenylpropanamide (9j).

White crystalline solid**, Yield** (2.91g, 92.3%),**mp,** 179.1-179.5ºC, **FTIR (KBr, cm^{-1})** 3419, 3346 (NH), 3113 (C-H stretch of aromatic), 1984 (C-H stretch of aliphatic), 1627 (C=O), 1412 (C=C), 1284 (2S=O), 1029 (SO_2N), (C-N, C-O), 783 (trans amide linkage).**δ_H (600MHz, DMSO):**8.11, 7.93 (4H, m, Ar-H), 8.03 (1H, t, CONH), 7.76 (1H, dd, J = 7.63, 6.90Hz, SO_2NH), 7.74 (2H, dd, J = 7.26, 7.10 Hz, Ar-H), 7.40 (2H, m, Ar-H), 7.29, 7.40 (3H, m, Ar-H), 4.33 (2H, s, CH_2), 3.90 (1H, t, CH), 3.44, 3.19 (2H, dt, 8.10, 7.36Hz, CH_2), 2.30 (3H, s, CH_3). **δ_C (600MHz, DMSO):** ^{13}C NMR: 172.O (C=O), 168.3 (C=O), δ 141.5, 137 (4C, Ar-C), 136.6, 127.2, 128.6 (5C, Ar-C), 137.4, 130.4, 125.1 (3C, Ar-C). 58.7 (1C, methine carbon), δ41.50 and 36.70 (2C, CH_2). 20.10 (1C, CH_3).

2.6.11. 2-(4-methylsulphonamido)-N-{2-oxo-2-[(6-oxo-6,9-dihydro-1H-purin-2-yl)amino]ethyl}-3-phenylpropanamide (9k).

White coloured solid**, Yield (**2.13g, 87.3%),mp: 160.1-160.7ºC. **FTIR (KBr, cm^{-1})** 3484, 3297 (NH), 3084 (C-H stretch of aromatic), 2886 (C-H stretch of aliphatic), 2783, 2595, 2468 (amino acid), 1617 (C=O), 1378 (SO_2N), 728 (trans amide linkage).**δ_H (600MHz, DMSO):**7.97, 7.5, 7.30 (3H, s, Ar-H), 7.40 (2H, dd, J = 6.23, 6.10Hz, Ar-H), 7.74 (2H, d, J = 6.36 Hz, Ar-H), 7.29, 7.40, 7.27 (5H, m, Ar-H), 7.72 (1H, s, SO_2NH), 8.0 (1H, s, Ar-H), 7.79 (1H, s, CONH), 4.4, 3.19 (2H, s, CH_2), 3.80 (2H, s, CH_2), 3.87 (1H, t, CH), 2.34 (3H, s, CH_3).**δ_C (600MHz, DMSO):** ^{13}C NMR: 208.4 (1C, C=O), 173.9 (1C, C=O), 137.6, 129.3, 128.3, 141.5 (6C, Ar-C), 136.6, 127.7, 128.6, 125.9 (6C, Ar-C), 147.2, 157.3, 144.8, 162.3, 119.6 (5C, Ar-C), 68.4 (1C, CH), 34.1 (1C, CH_2), 25.8 (1C, CH_2), 21.3 (1C, CH_3).

2.6.12. N-{2[(9H-purin-6-yl) amino]-2-oxoethyl}-2(4-methylsulphonamido)-3-phenylpropanamide (9l).

White crystalline solid**, Yield (**2.22g, 86%), mp: 190.1-190.4ºC, **FTIR (KBr, cm^{-1})** 3470, 3268 (NH), 3142 (C-H stretch of aromatic), 2963 (C-H stretch of aliphatic), 2865, 2724 (amino acid), 1725.852 (C=C), 1608.018 (C=O), 12229.047 (2S=O), 1108.634 (SO_2N), 693.7518 (trans amide linkage).**δ_H (600MHz, DMSO):**7.87, 7.42 (4H, m, Ar-H), 7.46, 7.74, 7.29 (5H, dd, J = 6.23, 6.10Hz, Ar-H), 7.40, 7.27 (2H, m, Ar-H), 7.72 (1H, s, SO_2NH), 8.00 (1H, s, CONH) 7.79 (1H, s, CONH), 4.4, 3.19 (2H, s, CH_2), 3.80 (2H, s, CH_2), 3.87 (1H, t, CH), 2.34 (3H, s, CH_3).**δ_C (600MHz, DMSO):** ^{13}C NMR: 208.4 (1C, C=O), 174.8 (1C, C=O), 136.9, 128.9, 127.4, 141.5 (6C, Ar-C), 136.6, 127.7, 128.6, 125.9 (6C, Ar-C), 147.2, 157.3, 144.8, 162.3, 119.6 (5C, Ar-C), 68.4 (1C, CH), 34.1 (1C, CH_2), 25.8 (1C, CH_2), 21.3 (1C, CH_3).

2.6.13. 2-(4-methylphenylsulphonamido)-N-{2-oxo-2-[(2-oxo-1,2-dihydropyrimidin-4-yl)amino]ethyl}-3-phenylpropanamide (9m).

White crystalline solid**, Yield (**2.12g, 75.9%),mp: 211.1-211.8^{o}C, **FTIR (KBr, cm^{-1})** 3498, 3287 (NH), 3195, 3097 (C-H stretch of aromatic), 2979 (C-H stretch of aliphatic), 2902, 2692, 2602 (amino acid), 1825 (C=C), 1620 (C=O), 1362 (SO_2N), 745 (trans amide linkage).

δ_H (600MH$_Z$, DMSO): The peak at δ8.03 (1H, s, CONH proton), δ8.0 (2H, s, CONH proton; Aromatic NH proton), δ8.40,5.40 (2H, d, J = 8.00Hz,Ar-H), 8.30 (2H, d, J = 7.50Hz, Ar-H), 8.24 (2H, d, J = 7.00Hz, Ar-H), 7.72, 7.21, 7.10 (5H, m, Ar-H), δ7.32 (1H, s, SO_2NH proton), δ3.97 (1$H, t,$ methine proton), δ4.90 and 3.29 (2H, m, methylene proton), 4.14 (2H, s, CH_2), 2.34 (3H, s, CH_3).**δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 173.9 (1C, C=O), 172 (1C, C=O), δ137.6, 129.3, 128.3, 141.5 (6C, Ar-C), 136.6, 127.7, 128.6, 125.9 (6C, Ar-C), 162.9, 156.3, 152.1, 95.7 (4C, Ar-C), 59.20 (1C, methine carbon), δ41.50 and 36.70 (2C, CH_2). 21.3 (1C, CH_3).

2.6.14. N-(2-(1H-indol-1-yl)-2-oxoethyl)-2-(4-nitrophenylsulphonamido) 3-phenylpropanamide (9n).

Off white crystalline solid**, Yield (**2.36g, 81.2%), mp, 226.1-226.5^{o}C, **FTIR (KBr, cm^{-1})** 3459, (NH), 3254 (C-H stretch of aromatic), 2891 (C-H stretch of aliphatic), 2629, 2449 (amino acid), 1701 (C=O), 1457 (NO_2), 1308 (S=O), 797 (trans amide linkage). **δ_H (600MH$_Z$, DMSO):**8.39 (2H, d, J = 6.26, Ar-H), 8.12 (2H, d, J = 6.62 Hz, Ar-H), 7.29, 7.40,7.27 (5H, m, Ar-H), 7.74, 7.93, 6.50, 7.83, 6.87, 8.11 (6H, m, Ar-H) (1H, s, SO_2NH proton), δ3.97 (1$H, t,$ methine proton), δ4.90 and 3.29 (2H, m, methylene proton), 4.14 (2H, s, CH_2).**δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 172.0 (C=O), 168.3 (C=O), δ 151.1, 124.2, 128.2, 150.6 (6C, Ar-C), 150.10, 143.5, 141.20, 140.00 (6C, Ar-C) 139.15, 128.20, 127.60, 126.20, 125.00, 123.00, 121.10,115.6 (8C, Ar-C), 58.7 (1C, methine carbon), δ41.50 and 36.70 (2C, CH_2).

2.6.15. N-(2-(1H-imidazol-1-yl)-2-oxoethyl)-2-(4-nitrophenylsulphonamido)-3-phenylpropanamide (9o).

Off white crystalline solid**, Yield (**2.90g, 79.32%), melting point, 189-189.4^{o}C, **FTIR (KBr, cm^{-1})** 3488, 2330 (NH), 3226, 3024 (C-H stretch of aromatic), 2979 (C-H stretch of aliphatic), 2814, 2608 (amino acid), 1612 (C=O), 1457 (NO_2), 1340 (S=O), 775 (trans amide linkage). **δ_H (600MH$_Z$, DMSO):** 8.39 (2H, d, J = 6.03Hz , Ar-H), 8.12 (2H, d, J = 6.21Hz, Ar-H), 7.74 (1H, d, J = 6.22 Hz, SO_2NH), 7.29,7.40, 7.27 (5H, m, Ar-H), 7.64 (1H,d, J = 6.21 Hz, Ar-H), 7.02 (2H, s, Ar-H), 3.81(2H, s, Ar-H), 2.13 (1H, s, NH), 3.44, 3.19 (2H, dd, J = 6.62, 6.23Hz, CH_2), 3.87 (1H, d, 6.04 Hz, CH), **δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 208.20 (C=O), 168.40 (C=O), δ 168.10, 150.10, 143.5, 141.20, 140.00 (6C, Ar-C), 139.15, 128.20, 127.60, 126.20 (6C, Ar-C), 125.00, 123.00, 121.10 (3C, Ar-C), 68.4 (methine carbon), δ41.50 and 36.70 (CH_2).

2.6.16. 2-(4-nitrophenylsulphonamido)-N-{2-oxo-2-[(6-oxo-6,9-dihydro-1H-purin-2-yl) amino]ethyl}-3-phenylpropanamide (9p).

Off white coloured solid**, Yield (**2.41g, 95.5%), melting point, 190-190.5^{o}C, **FTIR (KBr, cm^{-1})** 3531, 3262 (NH), 3160 (C-H stretch of aromatic), 2982 (C-H stretch of aliphatic), 2874, 2624, 2520 and 2457 (amino acid), 1831 (C=O), 1387 (2S=O), 1297 (SO_2N), 1050 (C-N), 679 (trans amide linkage). **δ_H (600MH$_Z$, DMSO):**δ 8.39 (2H, d, J = 6.21, Ar-H), 8.12 (2H, d, J = 6.23, Ar-H), 7.29, 7.40, 7.27 (5H, m, Ar-H), 7.74 (1H, s, SO_2NH) δ8.0 (3H, s, NH proton), δ7.20 (1H, s, CONH), δ3.87 (1$H, t,$ methine proton), δ4.90 and 3.29 (2H, s, CH_2), 4.14 (2H, s, CH_2).**δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 173.9 (1C, C=O), 172.40 (1C, C=O), δ151.1, 150.6, 128.2, 124.2 (6C, Ar-C), 136.2, 128.6, 127.7, 125.9 (6C, Ar-C), 168.10, 150.10, 143.5, 141.20, 140.00 (5C, Ar-C), 59.20 (1C, methine carbon), δ41.50 and 36.70 (2C, CH_2).

2.6.17. N-{2-[(9H-purin-6-yl)amino]2-oxoethyl}-2-(4-nitrophenylsulphonamido)-3-phenylpropanamide (9q).

Off white crystalline solid**, Yield (**2.06g, 75.6%), mp, 184.1-184.7^{o}C, **FTIR (KBr, cm^{-1})** 3489, 3364 (NH), 3198 (C-H stretch of aromatic), 2985 (C-H stretch of aliphatic), 2738, 2589, 2436 (amino acid), 1620 (C=O), 1457 (NO_2), 1368 (S=O), 1212 (SO_2N), 882 (trans amide linkage). **δ_H (600MH$_Z$, DMSO):** δ 8.03, 9.14 (2H, CONH)11.0 (1H, s, Ar-H), 7.74 (1H,s, SO_2NH), 8.12 (2H, d, J = 6.12Hz, Ar-H), 7.29, 7.40, 7.27 (5H, m, Ar-H), 7.86 (1H, s, Ar-NH), 8.60 (1H,s, Ar-H), 8.16 (1H, s, Ar-H), 7.84 (1H, d, J = 6.24, Ar-H), 4.09 (2H, t, CH_2), 3.95(1H, t, CH), 3.44, 3.19 (2H, t, CH_2), 2.39 (2H, d, J=6.25Hz, Ar-H).**δ_C (600MH$_Z$, DMSO):** ^{13}C NMR: 172.20 (1C, C=O), 168.10 (1C, C=O), δ151.1, 150.6, 128.2, 124.2 (6C, Ar-C), 136.10, 128.60, 127.70, 125.90 (6C, Ar-C), 138.7, 115.3, 161.2, 151.8, 152.4 (5C, Ar-C), 58.20 (1C, methine carbon), δ36.5 and 43.0 (2C, CH_2).

2.6.18. 2-(4-nitrophenylsulphonamido)-N-{2-oxo-2-[(2-oxo-1,2-dihydropyrimidin-4-yl)amino]ethyl}-3-phenylpropanamide (9r).

Off white coloured solid**, Yield (**1.92g, 68.2%), melting point: 197-197.3^{o}C, **FTIR (KBr,cm^{-1})** 3505, 3341 (NH), 3089 (C-H stretch of aromatic), 2923 (C-H stretch of aliphatic), 2784.628, 2605, 2456 (amino acid), 1610 (C=O), 1426 (NO_2), 1300

(S=O), 1128 (2S=O), 854.8772 (trans amide linkage). δ_H **(600MHz, DMSO):**8.39 (2H, d, J = 6.24 Hz, Ar-H), 8.12 (2H, d, J = 6.26 Hz, Ar-H), 7.29, 7.40, 7.27 (5H, m, Ar-H), 8.03 (1H, t, NH), 8.0 (1H, d, J = 6.81Hz, Ar-NH), 5.40 (1H, d, J = 6.72Hz, Ar-H), 8.43 (1H, t, Ar-H), 8.0 (1H, s, NH), 7.74 (1H, s, SO_2NH), 3.95 (1H, t, CH), 3.44, 3.44, 3.19 (2H, m, CH_2), 4.09 (2H, s, CH_2)δ_C **(600MHz, DMSO):** ^{13}C NMR: 173.9 (1C, C=O), 172.0 (1C, C=O), δ151.1, 150.6, 128.2, 124.2 (6C, Ar-C), 136.6, 127.7, 128.6, 125.9 (6C, Ar-C), 162.9, 156.3, 152.1, 95.7 (4C, Ar-C), 58.7 (1C, CH), 36.5 (1C, CH_2), 43.6 (1C, CH_2)

2.7. ANTIMICROBIAL ASSAY

2.7.1. Source of micro-organisms.

In this study, two Gram-positive organisms (*staphylococcus aureus* ATCC 9027, *Streptococcus pyogenes*), two Gram-Negative organisms (*Escherichia coli* ATCC 6538P, *Pseudomonas aeruginosa*) and a yeast fungus- *Candida albican* were used. They were maintained on Mueller-Hinton Agar medium (Oxiod, UK) and Sabouraud Dextrose Agar (Oxiod, UK) slants respectively at 4ºC. For each time, 24 hour old pure cultures were prepared for use.

2.7.2. Preparation of Culture Media

Mueller-Hinton Agar (Oxiod, UK) was used for direct sensitivity testing for bacteria while Sabouraud Dextrose Agar was used for fungi. The media were prepared and treated according to the manufacturer's guidelines.

a. Preparation of Mueller-Hinton Agar

Powder (35g) was mixed homogenously with distilled water (1 litre) until it was completely dissolved. 20 m*L* each were distributed in McCartney bottles and sterilized in an autoclave at 121ºC for 15 minutes and dispensed at 20 m*L* per plate in 12 × 12 cm Petri dishes. Set plates were incubated at 37ºC for 24 hours to ensure sterility before use. The plates were considered sterile when no growth occurred after 24 hour.

b. Preparation of Sabouraud Dextrose Agar

Powder (65g) was mixed homogenously with distilled water (1 litre) until it is completely dissolved. 20 m*L* each were distributed in McCartney bottles and sterilized in an autoclave at 121ºC for 15 minutes and dispensed at 20m*L* per plate in 12 × 12 cm Petri dishes. Set plates were incubated at 37ºC for 24 hours to ensure sterility before use. The plates were considered sterile when no growth occurred after 24 hour.

2.7.3. Standardization of Inoculum.

a. Standardization of Bacteria Suspension.

Sterile and dried nutrient agar plates were prepared. Thereafter, a loopful from the suspension of bacteria was collected and streaked aseptically on the surface of the solid agar medium in different petri dishes. The petri dishes were then incubated for 24 hours at 37ºC.

This was followed by aseptically removing a colony from each of the growth media and inoculating into prepared double strength nutrient broth in bijou bottles. After incubation at 37ºC for 24 hours, the content of each bottle was used to flood the surface of solid nutrient agar slants in roux bottles and thereafter incubated at 37ºC for 24 hours. The surface growth on the agar was washed four times with sterile normal saline into centrifuge tube by centrifugation. The microorganisms were bulked into sterile bottles and made up to 10 m*L* with sterile normal saline and stored at 4ºC until needed.

b. Standardization of Fungi Suspension

The surface of the Sabouraud dextrose agar medium was aseptically streaked with a loopful from the fungi suspension. The plate was incubated at 27ºC for 72 hours to give time for sporulation. A colony was aseptically removed from the growth and inoculated into a prepared sterile Sabouraud dextrose agar medium in bijou bottles. After incubation for 72 hours the content of each bottle was used to flood the surface of solid Sabouraud dextrose agar slant in roux bottle and incubated for 72 hours at 27ºC. The mycelia mat was removed from the surface of the slant culture using a sterile spreader and agitated with sterile normal saline. The suspension was filtered through sterile adsorbent cotton wool to remove hyphal elements, the resultant microbial suspension was collected in a sterile bottle and made up to 10m*L* with sterile normal saline and stored at 4ºC in a refrigerator. The strain's suspensions were adjusted to 0.5 McFarland Standard [36].

2.7.4. Preparation of Stock Solution.

Stock solution of (25mg/m*L*) of the compound were prepared by dissolving 100mg compounds in 4m*L* of sterile water and dimethylsulfoxide (DMSO) diluted in 1:5 (DMSO: water). DMSO is an organic solvent that aid the dissolution of organic substance that will not easily dissolve in water alone. The water and DMSO dilution was carried out to avoid the interference of DMSO with original activity of the samples [36].

2.7.5. Screening of the Compounds for Antimicrobial Susceptibility.

The antimicrobial susceptibility testing was determined using the agar well diffusion technique [47]. From the stock solution of 25mg/m*L*, serial dilutions were made to obtain 12.5mg/m*L*, 6.250mg/m*L*, 3.125mg/m*L* and 1.562mg/mL. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the

suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5 mm diameter was used to make wells on the medium. 0.1 m*L* of the various concentrations of the compounds were dropped into appropriate labelled well [30, 37] Streptomycin (0.625mg/m*L* - 10 mg/m*L*) was used as a reference standard for the antibacterial test while fluconazole (0.625mg/m*L* - 10 mg/m*L*) was used as a reference standard for the anti-fungal test. Physiological saline was used as a negative control. The plates were incubated at 37°C for 24 hours for anti-bacteria test and 27°C for 48 hours for anti-fungi test. The inhibition zone diameters (IZD) produced by each concentration of the compounds was measured and recorded in millimetres (mm) (CLSI, 2006, 2008). The inhibition was measured as a basis for activity [37].

2.7.6. Minimum Inhibitory Concentration (MIC) Evaluation.

The MIC was evaluated on synthesized compounds that showed antimicrobial activity in the agar well diffusion assay on any test organism. This test was performed at five concentration of each compound (1.562 - 25mg/m*L*) employing doubling dilutions of the compounds in molten Mueller- Hinton agar or Sabouraud dextrose agar up to the fourth dilution. 1 m*L* of the resultant agar was dispensed in test tube and equal amounts of the compounds (1 m*L*) were added to the first test tube and serial dilutions done with the last 1mL being discarded. To complete the test, each organism was separately suspended in 5 m*L* of nutrient broth or molten Sabouraud dextrose agar and incubated overnight, after which 0.1 m*L* was added to all test tubes and incubated at 37°C for 18 hours or 36 hours. After incubation, a loopful from each tube was sub cultured on Mueller-Hinton agar or Sabouraud dextrose agar and incubated for growth at 37°C for 24 hours or 27°C for 48 hours [16]. The plates that showed no growth were observed. The MIC was defined as the lowest concentration of an antimicrobial agent that inhibited the visible growth of a microorganism after overnight incubation [39]. The MIC was determined for each organism and compounds by plotting the graph of the squared of Inhibition Zone Diameter (IZD^2) against log of concentration and the antilog of the point of intersection on the X –axis was read and taken as the MIC [16, 38].

2.7.7. Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC).

The value of the MBC is an extension of MIC. The agar plates showing no growth in the MIC test were used for the determination of the MBC. Equal volume of the various concentrations of each compounds and Mueller- Hinton agar were mixed in micro-tubes to make up 0.5 m*L* of solution. 0.5 m*L* of McFarland standard of the organism suspension was added to each tube [37, 40]. The tubes were incubated aerobically at 37°C for 24 hours. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculums and the tube containing the growth medium and inoculums. The MBC was determined by sub culturing the test dilution on Mueller-Hinton Agar and further incubated for 24 hour [37]. This was carried out on the compounds with high antimicrobial activity and the highly sensitive organisms.

The same procedure described above for MBC was also employed for FBC using Sabouraud dextrose agar and incubation done at 27°C for 48 hours. The MBC and MFC were recorded as the lowest concentration of the compounds that did not permit any visible bacterial and fungal colony growth on the agar plate after the period of incubation [40].

2.8. IN-SILICO STUDIES.

2.8.1. Preparation of the protein and ligand structures.

The initial molecular geometries of the ligands whose 2D structures are presented in Figure 3.1 were fully optimized using Universal Force Field implemented in Avogadro 1.2.0. Four steps per update were used during the optimization. The Steepest Descent algorithm was used for the optimization. The obtained coordinates were finally subjected to density functional theory (DFT) optimization at B3LYP/6-311G (2d,p) level of theory. The DFT calculations were carried out with the help of Gaussian 16 suite of programs and were accessed via SEAGrid [41, 42]

The electronic crystallographic structures of the proteins of carbonic anhydrase, xanthine oxidase, orexin receptor, human carbonic anhydrase II and glycoprotein from Friend murin leukemia virus with the accession codes: 3F8E (resolution of 2.00 Å), 1FIQ (resolution of 2.50 Å), 4S0V (resolution of 2.50 Å), 5ULN (resolution of 1.35 Å) and 1AOL (resolution of 2.00 Å) and are known to be causative agents for convulsion, diuresis, insomnia, leishmaniasis and leukemia respectively. These protein structures were obtained from the protein data bank at http://www.rcsb.org. The non-protein molecules, including ligand structures and the water molecules that were used to stabilize the structures were deleted and only the protein structures were used for molecular docking simulations.

2.8.2. Molecular docking protocol and validation

The protein structures were fixed throughout the course of the simulation process and flexible docking of all the ligand structures was performed on the entire protein structure. The molecular docking simulation was performed with the help of AutoDock [43] by means of PyRx [44]. 2.8.3. **Pharmacokinetic properties and the Lipinski's rule of five.**

The pharmacokinetic properties of the five compounds with the best binding energy values were subjected to pharmacokinetic properties analysis. This was done with the help of SwissADME website at http://www.swissadme.ch/. Lipinski's oral drug-likeliness of the compounds used were the following:

i. Molecular weight (<500 Daltons),
ii. Number of hydrogen bond donors (<5)
iii. Number of hydrogen bond acceptors (<10)
iv. Lipophilicity (Log P) (<5), and
v. Topological polar surface area (<140) [45, 46].

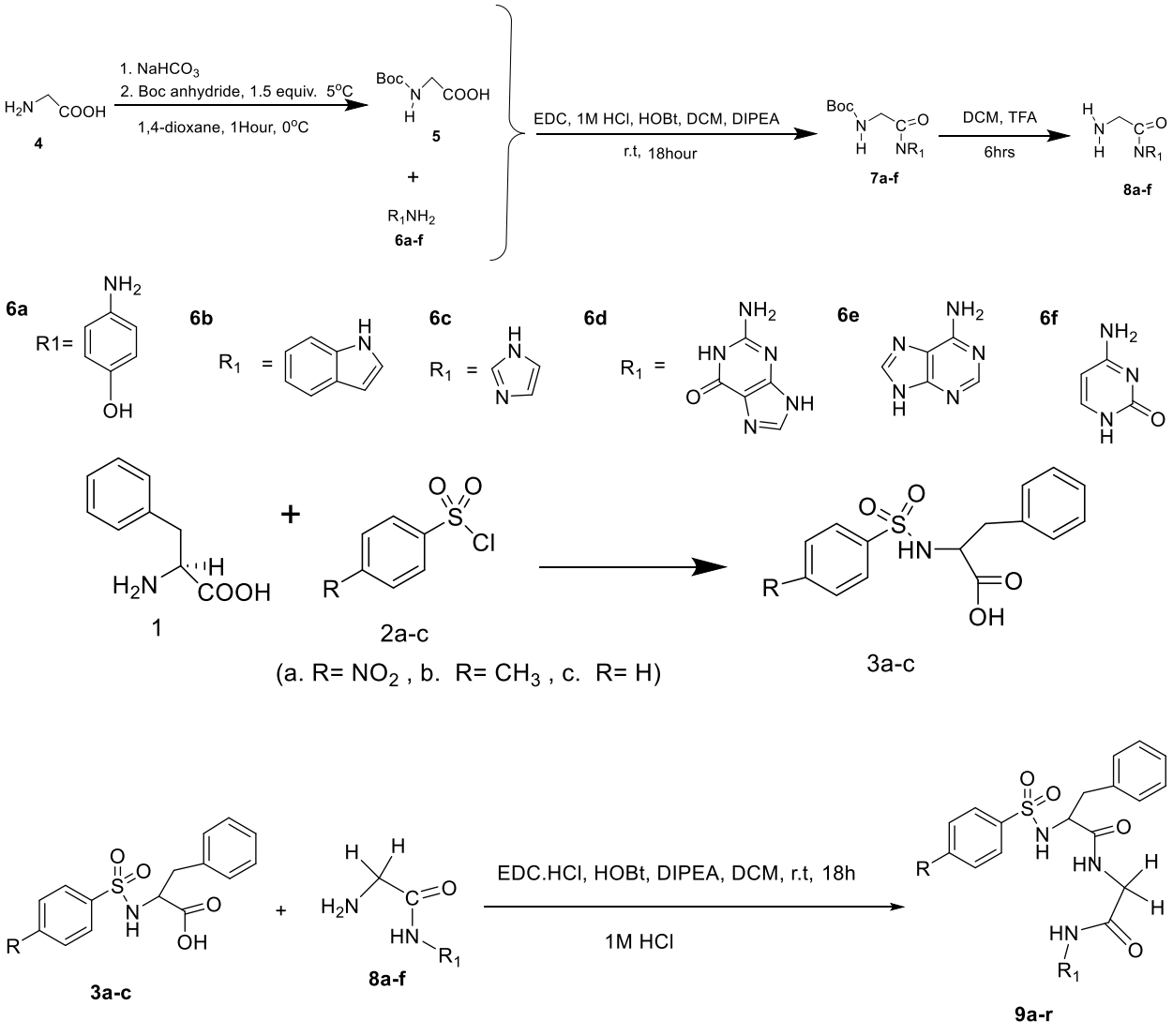
3.0 RESULTS AND DISCUSSION

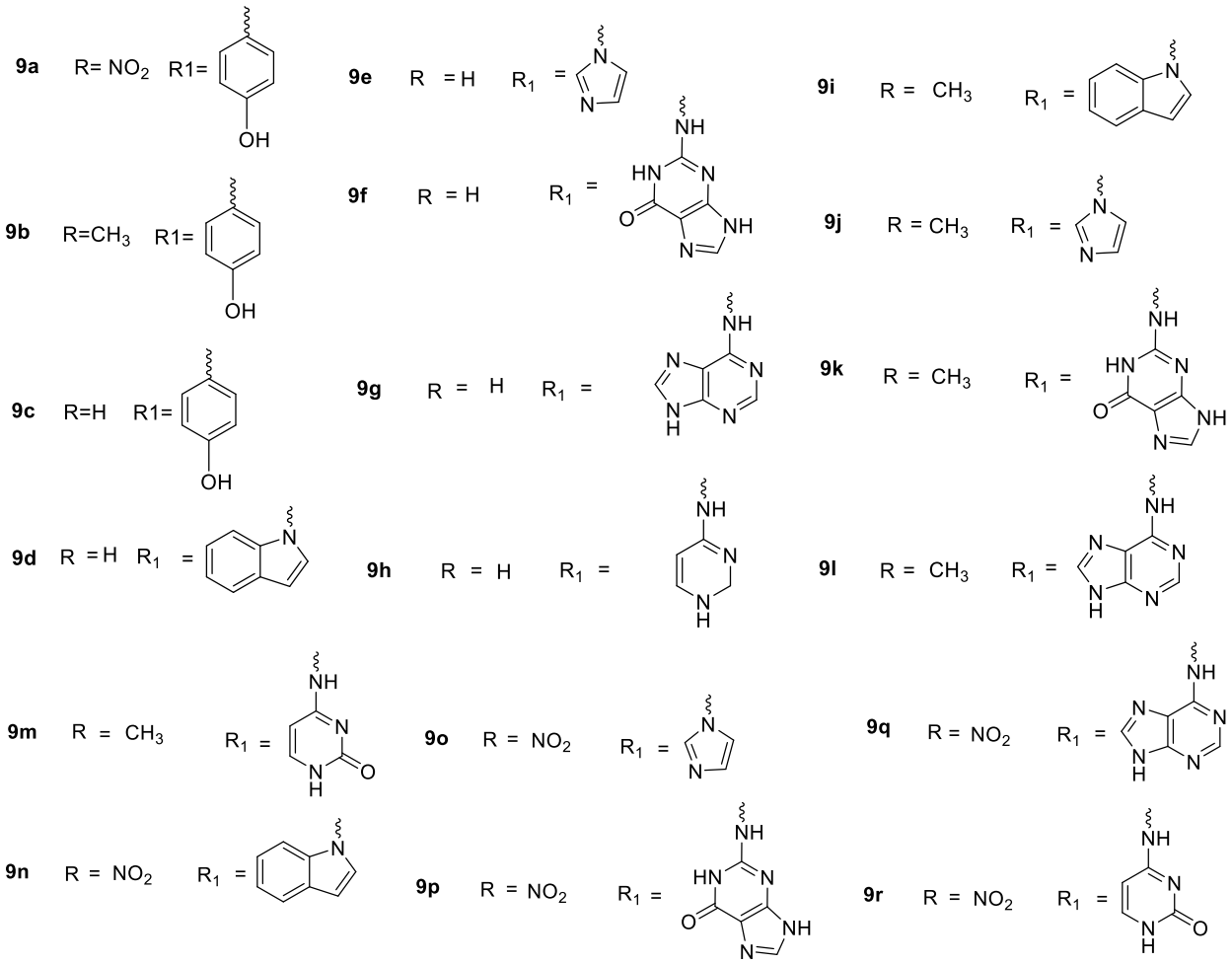
3.1Chemistry

The reaction **(1)** of L-phenylalanine with derivatives of benzenesulphonylchloride **(2a-c) in a** base yielded the benzenesulphonamides **(3a-c).** Then the reaction of glycine **(4)** with Boc anhydride in the presence of sodiumbicarbonate using 1,4-dioxane as solvent yielded the Boc-glycine **(5)**. The Boc-glycine carboxamides **(7a-f)** were synthesized by the amidation reaction between amines **(6a-f)** and Boc-glycine **(5).** This reaction was made possible by the use of appropriate coupling partners. Deprotection was accomplished by the use of trifluoroacetic acid TFA. The target compounds **(9a-r)** were obtained by the use of coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide EDC, 1-hydroxylbenzotriazole HOBt, triethylamine. Characterization was achieved by the use of FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy.

3.1.1. Spectral characterization

The FTIR had diagnostic peaks at 2904 (C-H aliphatic), 1639 (C=O), 1449 (NO_2), 1254 (SO_2N), 842 (NH deformation). ^{1}H NMR peaks at δ8.90 (1H, CONH proton), δ7.60 (1H, CONH proton), δ8.40, 8.30, 8.10, 7.72, 7.21, 7.10 and 6.90 (aromatic protons), δ7.32 (1H, NH proton of the sulphonamide group SO_2NH), δ3.97 methine proton (CH), δ4.90 and 3.29 methylene (CH_2) proton. ^{13}C NMR peaks at 170.11 (C=O of the CONH group), 169.40 (C=O of the CONH group), δ 168.10, 150.10, 143.5, 141.20, 140.00, 139.15, 128.20, 127.60, 126.20, 125.00, 123.00, 121.10 (12H, aromatic carbon), 59.20 (methine carbon), δ41.50 and 36.70 (CH_2) methylene carbons.





3.1.2. Binding Energy of the Docked Compounds

During the molecular docking simulation, nine poses were generated and the pose with the lowest energy value was chosen and the best five are presented in **Table 4.1** for each protein structure, alongside their inhibition constants (K_i).

Table 1: Five of the synthesized compounds with the lowest binding values, the binding energy of the standard drugs and their corresponding K_i values.

Protein	Compound	Binding Energy (kcal/mol)	K_i (µM)
3f8e	9k	-8.1	1.138
	9f	-8.0	1.347
	9p	-8.0	1.347
	Diazepam	-7.7	2.237
	9g	-7.7	2.237
	9i	-7.7	2.237
1ao1	9f	-7.7	2.237
	9g	-7.6	2.648
	9i	-7.5	3.136
	9q	-7.2	5.206
	9k	-7.0	7.299
	cyclophosphamide	-4.0	1160.710
5uln	9k	-9.8	0.064
	9i	-9.5	0.107
	9n	-9.5	0.107
	9b	-9.4	0.127
	9p	-9.4	0.127
	miltefosine	-4.4	590.468
4s0v	9k	-10.8	0.012

	9n	-10.5	0.020
	9g	-10.5	0.020
	9i	-10.4	0.023
	suvorexant	-10.2	0.033
	9p	-10.1	0.039
1fiq	9f	-11.0	0.008
	9g	-10.4	0.023
	9k	-10.4	0.023
	9i	-10.3	0.028
	9n	-10.1	0.039
	Allopurinol	-5.8	55.443

It can be seen that the smaller the free energy of binding, the smaller the inhibition constant. In addition, it is known that the smaller the inhibition constant the better the inhibition potential of the ligands. In other words, the lower the BE of the compounds under investigation the better the fit into the pocket of the protein and interactions with the amino acid residues of the protein. Generally, the first three of the compounds under study gave much better binding energies and inhibition constants than the standard drugs chosen for study. The binding affinity of all the study compounds to the pocket of the 3F8E, 1FIQ, 4S0V, 5ULN, and 1AOL protein structures lie in the range -8.1 and -6.2, -11.0 and -5.8, -10.8 and -7.1, -9.4 and -7.3 and -7.7 and -5.4 kcal/mol respectively. From the pool of ligands used in this study, the one with the best BE is due to **(9f)**, with a value of -11.0 kcal/mol while the lowest is due to compound **(9k)** with a value of -7.0.

3.1.3. Chemistry of Interaction of the Ligands with the Amino Acid Residues

The frontier molecular orbitals (FMOs) give information about the electronic properties of the ligands. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) were calculated with the same basis set that was used to optimize the structures. The HOMO is the site for electron donor, while the corresponding LUMO is the electron acceptor site. An increase in the energy gap between HOMO and LUMO of ligands suggests an increase in the binding energy of the ligand with the receptor [29]. **Figure 1** shows the FMO energies of the ligands with the highest binding energies, and their energy gaps.

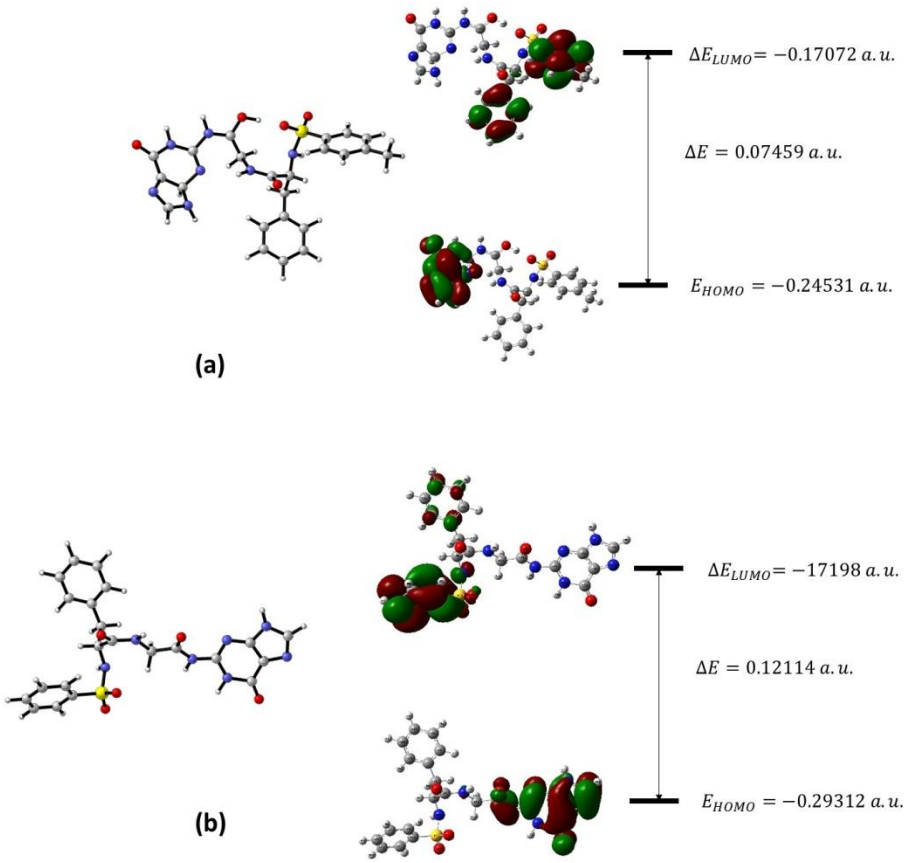


Figure 1: The optimized structures of the ligands (a) **9k** and (b) **9f** at B3LYP/6-311G(2d,p) level of theory.

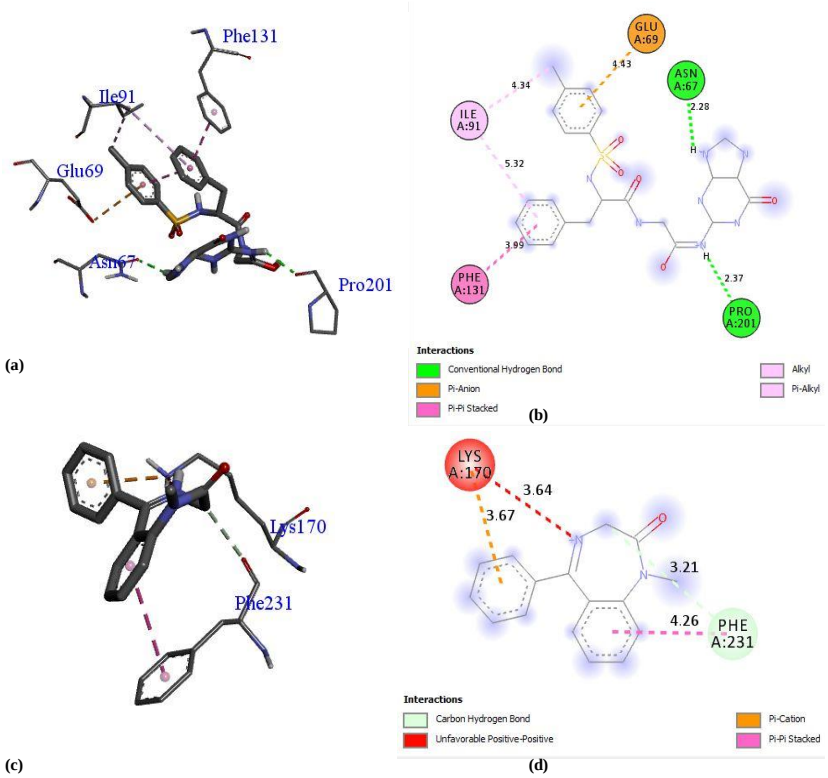


Figure 2: Interaction pictures of 3f8e protein (a) 3D 3f8e-**9k** complex (b) 2D 3f8e-**9k** complex (c) 3D 3f8e-diazepam and (d) 2D 3f8e-diazepam complex.

The standard drug for convulsion that was used to compare the interactions of **9k** with is diazepam. The chemical interactions observed in the 3f8e-diazepam complex are displayed in **Figure 2c** and **d**. The interactions are completely hydrophobic in nature, no HB interaction was observed. These hydrophobic interactions are strong enough to raise the energy of binding to -7.7 cal/mol. The lack of HB interaction brought the energy value that low compared to the -8.1 kcal/mol energy of binding in **3f8e-9k** complex. Pi-Pi Stacked interaction was the only hydrophobic interaction that was observed in both compounds **(9k)** and the standard drug, diazepam. However, it was observed to be weaker in diazepam (at 4.26 Å) compared to **(9k)** (at 3.99 Å). Interestingly, Pi-Cation interaction between diazepam was observed with LYS170 of the amino acid residue. With compound **(9k)**, Pi-Anion interaction was observed with GLU69.

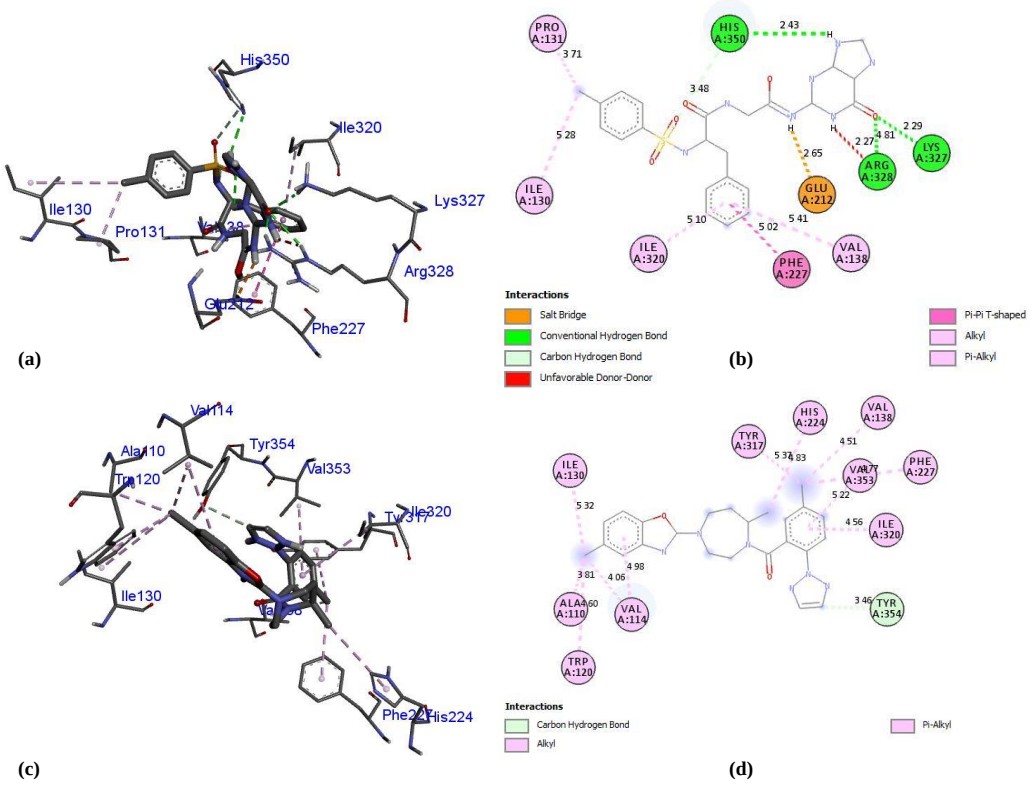


Figure 3: Interaction pictures of 4s0v protein (a) 3D 4s0v-**9k** complex (b) 2D 4s0v-**9k** complex (c) 3D 4s0v-suvorexant and (d) 2D 4s0v-suvorexant complex.

The standard drug for insomnia that was used to compare the interactions of **9k** with is suvorexant. The chemical interactions observed in the 4s0v-suvorexant complex are displayed in **Figure 3c** and **d**. The chemical interactions that were observed are purely hydrophobic in nature and were observed to be Pi-Alky and Alky interactions. These hydrophobic interactions were strong enough to bind the suvorexant structure to 4s0v protein with an energy of -10.2 kcal/mol against that of (**9k)** with the energy of -10.8 kcal/mol. From the amino acid residues involved in these interactions, we can say that both compounds are docked in the same pocket of the 4s0v protein. Some of these amino acids are ILE130 and VAL138. In addition to the interactions similar to those of the suvorexant drug structure, (**9k**) made Pi-Pi T-shaped and strong Salt Bridge with the amino acid residues, PHE227 and GLU212 at 5.0 and 2.65 Å.

3.1.4. Drug-likeness and oral bioavailability analysis

The *in silico* profiling for identification of drug candidate from the inhibitors in this study considered their drug-likeness. The ligands in this experiment were examined and classified based on their binding energy and were also examined for their drug-like properties. The results are given in **Table 2**. Lipinski's rule of five was used to assess the drug-likeness. Succinctly, it stipulates that for a drug-like candidate, the molecular weight of the drug-like molecule must be ≤ 500 g/mol, it must have a hydrogen bond acceptor of ≤10, a hydrogen bond donor of ≤ 5, and a log P of ≤5. Since all of the numbers listed are a multiple of five, hence, the name "the rule of five". A single violation is allowed within the rule for a drug candidate.

Table 2: Drug-likeness and bioavailability of the phytochemicals

property	9f	9g	9i	9k	9n	9p
Molecular weight (g/mol)	497.53	482.54	479.59	514.58	508.55	548.57
Hydrogen bond donor	5	4	2	6	2	5
Hydrogen bond acceptor	8	8	4	9	7	11
LogP	2.42	1.55	4.48	1.62	4.43	0.81
bioavailability	0.55	0.56	0.56	0.17	0.55	0.17
Log S	-3.30	-2.91	-4.83	-2.80	-4.61	-1.94
Topological Polar Surface Area (Å²)	170.53	137.14	103.96	175.39	108.89	157.12

As can be seen in **Table 2** not all the five compounds with lowest BE values from the molecular docking experiment obey the Lipinski's rule of five. Compounds **9f**, **9g** and **9i** do not violet any of the Lipinski's rule of five. However, for compound **5**, the molecular weight is greater than 500 g/mol, while for compounds **9k** the hydrogen bond donor is greater than 5 and in compound **9p**, the hydrogen bond acceptor is greater than 10. Topological Polar Surface Areas (TPSAs) is a measure of the polarity of a drug candidate and to determine if it is safe for oral consumption, log P was determined. The Lipinski's rule specifies that log P must be ≤ 5. TPSA has a positive correlation with mass and the molecules with masses that higher than 500 g/mol are known to have TPSA beyond the range of 0–140Å². Meaning that drug candidates with values greater than 140 Å², are not safe for oral consumption. From **Table 2**, it is clearly seen that the compounds under investigation are within acceptable limit and are safe for oral consumption, except for compounds **9f**, **9k**, and **9p**. A drug candidate's bioavailability refers to the extent to which it is totally accessible to its targeted therapeutic location. The bioavailability score is typically between 0 and 1. Remarkably, all of the drug candidates in **Table 2** had bioavailability values that are within the acceptable range. The Log S value of a drug candidate is directly related to its solubility and refers to the logarithm to base 10 of a molecule's solubility measured in mol/dm3. Drug candidates with solubility values ranging from -2 to 0 are extremely soluble, those with values ranging from -4 to -2 are moderately soluble, and those with values less than -4 are insoluble. The log S of the compounds range between -4.83 and -1.94. From **Table 4.2**, the compounds **(9i)** and **(9n)** are insoluble, **compounds (9f)**, (**9g)**, (**9i)** and (**9k)** are moderately soluble and compound **(9p)** is highly lipophilic.

3.1.5. Assessment of (absorption, distribution, metabolism, excretion and toxicity (ADMET) properties

The toxicity of a drug is commonly expressed in values of LD_{50} in milligrams per kilogram of body weight. The median lethal dose (LD_{50}) is the amount of a substance at which 50% of test subjects die after being exposed to it. The predicted oral LD_{50} for the drug candidates are given in **Table 3.**

Table 3: The predicted hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity of the compounds. The intensity of the coding indicates the toxicity level; red being highly toxic and green non-toxic.

Toxicity classes are defined using the internationally agreed-upon technique of chemical labeling category LD_{50}:

Class I: fatal if swallowed ($LD_{50} \leq 5$)
Class 2: fatal if swallowed ($5 < LD_{50} \leq 50$)
Class 3: toxic if swallowed ($50 < LD_{50} \leq 300$)
Class 4: harmful if swallowed ($300 < LD_{50} \leq 2000$)

Property	9k	9f	9p	9i	9g	9r	9n	9q	9o
LD_{50} (mg/kg)	3000	2500	1000	1000	1000	5000	1000	1000	5000
Toxicity class	5	5	4	4	4	5	4	4	5
Hepatotoxicity	0.58	0.60	0.56	0.66	0.58	0.61	0.54	0.59	0.61
Carcinogenicity	0.53	0.57	0.51	0.70	0.57	0.61	0.57	0.62	0.61
Immunotoxicity	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Mutagenicity	0.68	0.73	0.59	0.67	0.66	0.53	0.54	0.52	0.53
Cytotoxicity	0.62	0.60	0.60	0.64	0.59	0.62	0.59	0.64	0.62

Class 5: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$)
Class 6: non-toxic ($LD_{50} > 5000$)

It can be seen from the LD_{50} presented in **Table 3** that **compounds (9g)**, (**9i)**, (**9n)** and (**9p)** are in class 4 and are predicted to be harmful if swallowed while **compounds** (**9f)** and (**9k)** are in class 5 may be harmful if swallowed. Interestingly, all the compounds presented in **Table 3** show no toxicity of the type considered; hepatoxicity, carcinogenicity, immunotoxicity and mutagenicity. They are predicted to be nontoxic. The scores are presented using the *kappa* index. It measures the quality of binary classification models. The kappa index ranges between 0 (less significant) to 1(perfect). They are colour coded in **Table 3**, indicating that the greener the better the drug ability of the compounds. It is clear that all the drug candidates tested are less immunotoxic. **Compounds (9i)** predicted to be highly non-carcinogenic and non-mutagenic as well as **Compounds (9f)** and (**9p)**.

***In silico* antibacterial and Antifungal Studies**

Table 5 shows the docking score of the eighteen compounds with the crystal structure of Candida albican protein, farnesyl transferase binary complex with the isoprenoid, farnesyldiphosphate (4YDE). Crystal structure of *Escherichia coli (*H128N/ECP) mutant in complex with sulphate anion (4X08). The structure of (3R)-hydroxylacyl-ACP dehydrate obtained from *Pseudomonas aeruginosa* (1U1Z*). Staphylococcus aureus* S1: DHFR in complex with trimetoprim (2W9S). High resolution crystal structure of *Streptococcus pyogenes beta*-NAD^+glycohydrolase in complex with its endogenous inhibitor (4KT6).

Table 5: Binding energies of 18 compounds with protein structures of **4YDE**, **4XO8**, **1U1Z, 2W9S** and **4KT6.**

Compounds	4YDE	4X08	1U1Z	2W9S	4KT6
9a	-7.6	-6.6	-6.8	-9.2	-8.5
9b	-7.9	-6.9	-7.1	-10.3	-9.1
9c	-6.9	-6.4	-7.1	-9.2	-8.7
9d	-8.2	-6.8	-7.0	-10.2	-8.7
9e	-7.2	-6.6	-6.3	-9.4	-9.8
9f	-8.3	-7.0	-7.7	-10.2	-8.7
9g	-8.1	-7.1	-7.4	-9.5	-8.7
9h	-7.9	-6.4	-5.8	-9.0	-8.4
9i	-7.8	-5.6	-6.6	-7.9	-8.2

9j	-6.6	-5.8	-5.3	-7.5	-8.2
9k	-6.9	6.2	-6.9	-8.4	-8.2
9l	-6.6	-6.5	-6.7	-9.3	-7.1
9m	-7.6	-6.1	-6.7	-9.9	-8.9
9n	-7.5	-6.4	-7.9	-9.0	-9.0
9o	-8.8	-6.9	-6.7	-9.7	-9.4
9p	-8.6	-6.4	-7.6	-10.2	-9.7
9q	-7.7	-6.9	-7.4	-10.1	-8.9
9r	-8.0	-6.4	-6.3	-9.7	-8.2
Streptomycin	-6.5	-5.8	-5.9	-7.4	-9.0
Fluconazole	-6.0	-5.6	-6.6	-7.3	-7.1

All the eighteen compounds had better binding energies to the **4YDE** protein structure than the standard drugs streptomycin and fluconazole used as control in this research. Streptomycin and fluconazole had binding energies of -6.5 and -6.0 respectively while the compound with the highest docking score **(9o)** has a B.E of -8.8 kCal/mol. Also for the protein of **4XO8** in **Table 5**, compound (**9i)** and **(9j)** have binding energies -5.6 and -5.8 respectively which is comparable to that of streptomycin used as control in this investigation. The other compounds showed better binding energies than the standard drug. **(9g)** has the highest binding energy. The binding energy of (**9g)** is due to strong conventional hydrogen bonding interaction in many site with ASN A:135, ASP A:50, ASN A:135, GLN A:139, GLY A:14, ASP A:140. For ***1U1Z*** in **Table 5,** the compound with the best binding energy is (**9n)**. The interaction of (**9n)** with the protein molecule is due to conventional hydrogen bonding, pi-pi interaction and pi-sigma interaction. Compound (**9j)** has the binding energy of -5.3**.** For **2W9S** in **Table 5**, all the compounds showed better binding to the protein than the standard drug which has a B.E of -7.4. **Table 5** shows that (**9b)** has the best binding energy -10.3 which mean a better binding than the standard drug streptomycin -7.4. The 2D view of (**9b)** showed that its binding to the amino acid is mainly due to weak Van der Waal forces. Other contributing forces are pi-sigma and pi-alkyl interaction. The interaction of streptomycin with the amino acids is due mainly to conventional hydrogen bonding. The values for ***4KT6*** in **TABLE 5**, compound **(9n)** and streptomycin has equal binding energy of -9.0. Compounds **(9b)** also has better binding energies than the standard drug with **(9e)** having the highest B.E of -9.8.

3.3. *In-vitro* antibacterial and antifungal studies.

Table 6: Results of MIC (mg/m*L*).

Compound	***Pseudomonas aerigunosa***	***Staphylococcus aureus***	***Streptococcus pyogene***	***Escherichia coli***	***Candida albican***
9a	-	2.089	1.659	2.455	1.318
9b	-	-	-	-	-
9c	-	-	-	-	-
9d	-	2.884	1.259	-	3.162
9e	6.309	3.981	3.019	5.012	2.754
9f	-	-	1.905	3.019	2.884
9g	5.129	3.890	1.514	3.019	4.898
9h	2.884	6.026	1.738	-	2.399
9i	-	-	-	-	-
9j	-	5.012	2.754	-	1.585
9k	1.738	7.586	2.455	-	2.455
9l	-	-	2.089	7.244	6.166
9m	-	-	2.512	15.848	4.898
9n	-	7.586	5.012	6.166	7.763
9o	-	-	-	-	-
9p	-	2.291	4.786	-	1.995
9q	-	-	4.898	7.244	1.949
9r	-	4.169	3.467	25.119	4.168

Table 7: Results of MBC and MFC in mg/m*L*.

Compound	*Pseudomonas aerigunosa*	*Staphylococcus aureus*	*Streptococcus pyogene*	*Escherichia coli*	*Candida albican*
9a	-	-	-	-	-
9b	-	-	-	-	-
9c	-	-	-	-	-
9d	-	25.000	25.000	-	12.500
9e	12.500	12.500	12.500	12.500	25.000
9f	-	-	25.000	25.000	12.500
9g	-	-	-	-	-
9h	12.500	-	25.000	-	12.500
9i	-	-	-	-	-
9j	-	12.500	25.000	-	25.000
9k	25.000	25.000	25.000	-	12.500
9l	-	-	25.000	25.000	12.500
9m	-	25.000	25.000	25.000	25.000
9n	-	12.500	12.500	12.500	12.500
9o	-	-	-	-	-
9p	-	12.500	25.000	-	12.500
9q	-	-	12.500	25.000	25.000
9r	-	-	-	-	-

3.3.1. Minimum inhibitory concentration.

The minimum inhibitory concentration is determined by assaying the ability of a bacterial strain to grow in broth cultures containing different concentrations of the antimicrobial. The lowest concentration of the drug that prevents the growth of the microorganism is the minimum inhibitory concentration (MIC). The values of the MIC of the compounds are displayed in Table 6.

3.3.2. Minimum bactericidal concentration and minimum fungicidal concentrations.

The minimum bactericidal concentration is the lowest concentration of a specific antimicrobial drug that kills 99.9% of a given strain of bacteria. The MBC and MFC are determined by assaying for live organisms in those tubes form the MIC test that shows no growth. The compounds showed MBC and MFC values between 12.500 and 25.500 mg/m*L*.

4.0 CONCLUSION

The synthesis of eighteen novel sulphonamoyl 'phe-gly' dipeptide carboxamide derivatives and their *in silico* and *in vitro* properties has been achieved employing a versatile method. The novel compounds were characterized using FTIR, ^{1}H NMR, and ^{13}C NMR, and the spectra information were in agreement with the assigned structures. For the structure of the **2WPS**, all the compounds showed better binding energies that streptomycin. (**9b)**, (**9g)**, (**9i)**, (**9j)**, (**9n) and (9o)** showed good binding for all the protein structures. The growing report of antimicrobial resistance needs concerted effort in the discovery of new potent antimicrobial agents. Most of the synthesized compounds showed better antibacterial, antifungal, anticonvulsant, antidiuretic, antiinsomnia, antileukaemia and antileishmaniasis activity than the reference drugs streptomycin, fluconazole, diazepam, allopurinol, suvorexant, miltefosine and cyclophosphamide respectively. The molecular docking results showed interaction with the active site of protein with good binding affinity using conventional hydrogen bonding.

4.1 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

4.2 HUMAN AND ANIMAL RIGHTS

Not applicable as no human or animal was used in this experiment**.**

4.3 CONSENT FOR PUBLICATION

Not applicable.

4.4 AVAILABILITY OF DATA AND MATERIALS

All data and materials supporting the findings of the study are available within the manuscripts and its supplementary files.

4.5 FUNDING

None.

4.6 CONFLICT OF INTEREST

We hereby declare that there is no conflict of interest.

4.7 ACKNOWLEDGEMENTS

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4.8 SUPPLEMENTARY MATERIALS

Supplementary materials are uploaded along with this manuscript.

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