



Novel Alanine-based Antimicrobial and Antioxidant Agents: Synthesis and Molecular Docking

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Abstract

Objective: To synthesize new alanine-based phenyl sulphonamide derivatives with significant antimicrobial and antioxidant activities.

Methods: The reaction of alanine with benzenesulphonyl chloride afforded compound **3a**. The ammonolysis of its *N*-acylated derivative gave the carboxamide which yielded the aryl/heteroaryl derivatives compounds **3d**, **3e**, and **3f** via Buchwald–Hartwig nickel catalyzed amidation reaction. Structures agreed with the spectra data. Their antimicrobial activities, antioxidant activities, and molecular docking interactions were evaluated. **Findings:** Compounds **3f** and **3a** were the best antimicrobial agents with minimum inhibitory concentration (MIC) range of 0.5–1.0 µg/ml while compound **3a** displayed the highest *in vitro* antioxidant percentage inhibition of 95.70% and the best 50% inhibitory concentration (IC₅₀) value of 1.072 ± 0.002 µg/ml comparable to ascorbic acid of 96.38% antioxidant percentage inhibition and 0.999 ± 0.001 µg/ml IC₅₀ value. Compounds **3c**, **3a**, and **3f** had the highest *in silico* antibacterial, antifungal, and antioxidant binding energies of –10.03, –11.79, and –13.13 kcal/mol, respectively. **Novelty/improvement:** Alanine was found to potentiate the antimicrobial and antioxidant actions of benzenesulphonamide and carboxamide derivatives.

Keywords: Alanine, Antimicrobial, Phenylsulphonamide, Molecular Docking, Antioxidant.

1. Introduction

The world is faced with the enormous challenge of combating microbial and oxidative stress-related diseases; these two disease conditions have brought untold misery to millions

of individuals around the world [1–3]. Their relationship is based on the fact that oxidative stress causes a decline in immunity thereby making the body vulnerable to microbial infections [4]. Amino acids possess antimicrobial and antioxidant abilities and therefore could be explored as antimicrobial and antioxidant agents [5–6]. Obviously, alanine is an excellent target for the design of antimicrobial drugs because of its ability to be metabolized by bacteria and alanine being an α -amino acid is utilized in protein biosynthesis and glucose–alanine cycle [7–8]. Alanine plays a central role in the conversion of simple sugar to energy [9], processing of vitamin B [7, 10], and as a precursor of numerous biomolecules [11].

Interestingly, the combining sulphonamide molecule being a basis for drug development [12], possesses antimicrobial [13], anticancer [14], antimalarial [15], and antiretroviral [16] activities. The carboxamide molecules are present in numerous drug molecules acting as a bioactive moiety [17] while the coupling partners such as aniline, pyridine, and pyrimidine exhibit numerous pharmacological activities [18–19] are therefore used in the production of paracetamol [20], CNS stimulants [21–22], and antifolates [23], respectively that amino acid-based sulphonamide and carboxamide derivatives exhibited excellent antimicrobial and antioxidant activities [24–26].

The ability of microorganisms to resist the preventive and curative effect of drugs that were previously efficacious is worrisome and the increasing dominance of these pathogenic microorganisms is a matter of concern. The global report on surveillance by the World Health Organization (WHO) in 2014 showed that more than 700,000 deaths caused by microbial infections occur annually across the globe of which 23,000 is found in the United States [27–28]. Obviously, the intake of natural antioxidants reduces the risk of oxidative stress-related cases [29–30], yet these diseases are still prevalent. The best solution to these disease conditions is to synthesize drug compounds working on new and strategic target drug sites [31]. The increasing public demand for global effort to combat antimicrobial recalcitrance and effectively tackle oxidative stress-related ailments [32] necessitates the need for the development of new alanine-based antimicrobial and antioxidant agents with enhanced drug properties. This study exploited the synergistic drug actions offered by the coupling of alanine, benzenesulphonamide, carboxamide and aryl/heteroaryl moieties into single drug compounds in order to enhance drug potency to eliminate or minimize the antimicrobial resistance.

2. Materials and Method

2.1. Instrumentation

The basic chemicals were imported from Sigma Aldrich. Melting point was ascertained by melting point apparatus. The infrared spectra were obtained with 8400s Fourier Transform Infrared in Ahmedu Bello University, Nigeria. Both ^1H -NMR and ^{13}C -NMR spectroscopy were conducted with 400 MHz NMR spectrophotometer at Chemistry Department, Indian Institute of Technology, Kanpur. Chemical shifts were determined with reference to tetramethylsilane. Inert conditions were provided with nitrogen gas. Compounds were obtained in analytical grade. The antimicrobial and antioxidant studies were done at the Microbiology and Biochemistry Departments, respectively, University of Nigeria, Nsukka.

2.2. Chemistry

2.2.1. Synthesis of Benzenesulphonamoyl Carboxylic Acids

Using a 50 ml beaker, alanine (25 mmol) and Na_2CO_3 (5.58 g, 52.50 mmol) were dissolved in distilled water (30 ml). It was cooled to -5°C and benzenesulphonyl chloride (5.12 g, 30 mmol) was added in two portions for 1 hour period. Thorough stirring was carried out for 4 h and recrystallization was done with 2 M hydrochloric acid, filtered and washed with tartaric acid (pH 2.2) to obtain 2-[[phenylsulphonyl]amino]propanoic acid (**3a**) in good yields (82.7%).

2.2.1.1. 2-[(Phenylsulphonyl)amino]propanoic acid (**3a**)

Yield 4.84 g (82.7%), mp $115\text{--}116^\circ\text{C}$, IR (KBr) cm^{-1} : 3401 (N–H), 3074 (C–H aromatic), 2902 (CH aliphatic), 1726 (C=O), 1660, 1657 (C=C), 1168, 1032 (SO_2), 738 (Ar–H). ^1H -NMR (DMSO) δ : 10.52 (s, 1H, OH), 8.15–8.13 (d, $J = 8.4$ Hz, 1H, NH–CH), 7.85–7.82 (d, $J = 8.54$ Hz, 2H, Ar–H), 7.68–7.58 (m, 3H, Ar–H), 3.79–3.74 (dd, $J_1 = 7.30$ Hz, $J_2 = 8.38$ Hz, 1H, NH–CH– CH_3), 1.15–1.13 (d, $J = 7.21$ Hz, 3H, CH_3 –CH). ^{13}C -NMR (CD_3N , 400 MHz) δ : 170.244 (C=O), 149.212, 141.333, 131.669, 129.991, 64.772, 45.101. Anal. calcd. for $\text{C}_9\text{H}_{10}\text{NO}_4\text{S}$ (228.00): C, 47.00, H, 4.44, N, 6.31. Found: C, 46.96, H, 4.49, N, 6.29.

2.2.2. Acylation of 2-[(Phenylsulphonyl)amino]propanoic acid (**3a**)

2 g of compound **3a** was transferred to a 100 ml beaker containing 9 ml HCl, 25 ml distilled water and 13 ml acetic anhydride. A solution of 16.0 g of Na_2CO_3 and 50 ml distilled water was added to the solution of compound **3a** stirred, cooled to 0°C and filtered to obtain compound (**3b**) in 94.3% yield.

2.2.2.1. 2-[Acetyl(phenylsulfonyl)amino]propanoic acids (**3b**)

Yield 2.19 g (94.3%), mp. $209\text{--}211^\circ\text{C}$, IR (KBr) cm^{-1} : 3454 (N–H), 1750, 1688 (C=O), 1670, 1666 (C=C), 1277, 1165 (S=O), 1116 (SO_2), 1098 (C–N), 925 (Ar–H). ^1H -NMR (DMSO, 400 MHz) δ : 7.322 (d, $J = 7.8$ Hz, 2H, ArH), 6.277 (d, $J = 6.3$ Hz, 2H, ArH), 3.295 (s, 2H, NH_2), 2.534 (s, 3H, CH_3 –C=O), 2.014 (s, 3H, CH_3 –CH). ^{13}C -NMR (DMSO, 400 MHz) δ : 173.121, 170.231, 2 (C=O), 131.908, 128.926, 125.958, 123.241, 122.789, 120.443 (aromatic carbon) 78.911, 78.782, 78.584 (aliphatic carbon). Anal. calcd. for $\text{C}_{11}\text{H}_{13}\text{NO}_5\text{S}$ (270.32): C, 48.83, H, 4.81, N, 5.18, S, 11.84. Found: C, 48.79, H, 4.78, N, 5.21, S, 11.86.

2.2.3. Chlorination and Aminolysis of 2-[Acetyl(phenylsulfonyl)amino]propanoic acids (**3b**)

2.2.3.1. Chlorination

2 g of 2-[acetyl(phenylsulfonyl)amino]propanoic acids was dissolved in acetone (10 ml) in three-necked flask and cooled to 0°C . Stirring was carried out for 3 h under reflux at 80°C , taken to water bath to evaporate excess thionyl chloride at 80°C . 20 ml of acetone was introduced and evaporated twice in order to eliminate any remaining thionyl chloride to obtain acid chloride reactive intermediate.

2.2.3.2. Aminolysis

Instantly, acid chloride intermediate was dissolved in 20 ml acetone and cooled to 0 °C. 2 ml of was then added with 1 M NaOH and allowed for 12 h and filtered to afford compound **3c** in excellent yield (91.8%).

2.2.3.2.1. 2-[Acetyl(phenylsulfonyl)amino]propanamide (**3c**)

Yield 3.32 g (91.8%), mp. 224 °C. IR (KBr) cm^{-1} : 3400 (N–H), 1728, 1689 (C=O), 1644, 1635 (C=C), 1287, 1163 (S=O), 1117 (SO₂), 1092 (C–N), 929 (Ar–H). ¹H-NMR (CD₃CN, 400 MHz) δ : 7.956 (d, J = 8.2 Hz, 2H, ArH), 7.519 (d, J = 8.5 Hz, 2H, ArH), 6.569 (s, 1H, ArH), 4.679 (s, 2H, NH₂), 2.722 (s, 3H, CH₃–C=O), 2.480 (s, 3H, CH₃–Ar), 1.977–1.955 (m, 1H, CH), 1.945 (d, J = 1.8 Hz, 3H, CH₃–CH). ¹³C-NMR (DMSO, 400 MHz) δ : 170.332, 170.113 (C=O), 131.908, 128.926, 125.958, 123.786, 120.677, 118.963 (aromatic carbon) 78.911, 78.782, 78.584, (aliphatic carbon). Anal. calcd. (%) for C₁₁H₁₄N₂O₄S (270.30): C: 48.83, H: 5.18, N: 10.36, S: 11.89. Found C: 48.86, H: 5.15, N: 10.41, S: 11.85.

2.2.4. Nickel Catalyzed Amidation for the Synthesis of Alanine-based Sulphonamide Derivatives

2.2.4.1. Bis(triphenylphosphine)nickel(II)chloride

Venanzi method [33] of reaction protocol was employed it involves combining a solution of Nickel(II)chloride hexahydrate (10 mmol) in 2 ml distilled water and 50 ml glacial acetic and triphenylphosphine (20 mmol) dissolved in 25 ml glacial acetic acid. The crystals were filtration and dried in desiccators.

2.2.4.2. Procedure for the Synthesis

Bis(triphenylphosphine)nickel(II)chloride (10 mmol) and triphenylphosphine (30 mmol) were dissolved in *t*-butanol (4 ml) and distilled water (2 ml). It was stirred for 10 min under inert nitrogen gas atmosphere. It was further heated at 80 °C for 1.5 min. The 2-[acetyl(phenylsulfonyl)amino]propanamide (**3c**) (10mmol), K₂CO₃ (1.38 g, 10 mmol), and various substituted aryl and heteroaryl halides (4-chloroaniline, 4-amino-3-chloropyridine and 5-chloro-4,6-diaminopyrimidine) were added under nitrogen atmosphere. It was stirred under reflux for 1 h at temperature of 110 °C, allowed to cool to room temperature and recrystallized with ethyl acetate to obtain alanine-based benzenesulphonamide and carboxamide derivatives (**3d–f**) in excellent yield.

2.2.4.3. 2-[Acetyl(phenylsulfonyl)amino]-N-(4-aminophenyl)propanamide (**3d**)

Yield 3.34 g (987.3%), mp. 84–86 °C. IR (KBr) cm^{-1} : 3490, 3409 (N–H), 3081 (C–H aliphatic), 1980 (C–H aromatic), 1729, 1689 (C=O), 1671, 1660 (C=C), 1372, 1173 (S=O), 1121 (SO₂), 1090 (C–N), 749 (Ar–H). ¹H-NMR (DMSO, 400 MHz) δ : 7.503–7.485 (d, J = 0.02 Hz, 2H, ArH), 7.286 (m, 2H, ArH), 7.158–7.128 (d, J = 12.3 Hz, 2H, ArH) 7.107–7.030

(d, $J = 31.7$ Hz, 2H, ArH), 3.855 (s, H_2 , NH_2), 2.485 (s, 3H, $CH_3-C=O$), 2.479 (s, 3H, CH_3-Ar), 2.269 (s, 1H, CH). ^{13}C -NMR ($CDCl_3$, 400 Hz) δ : 171.234, 170.445, 2 (C=O), 147.986, 143.533, 142.538, 130.299, 130.219, 129.700, 129.176, 127.998, 126.341, 125.579 (aromatic carbons), 77.439, 77.120, 76.801, 21.823, 01.613 (aliphatic carbons). Anal. calcd. (%) for $C_{17}H_{19}N_3O_4S$ (361.42); C: 56.44, H: 5.26, N: 11.63, S: 8.85. Found C: 56.48, H: 5.31, N: 11.59, S: 8.88.

2.2.4.4. 2-[Acetyl-[(phenylsulfonyl)amino]-N-(4-aminopyridin-3-yl)propanamide (3e)

Yield 3.05 g (91.8%), mp. 83–85 °C, IR (KBr) cm^{-1} : 3449, 3420 (N–H), 3069 (C–H aliphatic), 1984 (C–H aromatic), 1727, 1687 (C=O), 1657, 1654 (C=C), 1643 (C–N), 1434 (C–H), 1380, 1157 (S=O), 1120 (SO_2), 1099 (C–N), 847 (Ar=H). 1H -NMR (DMSO, 400 MHz) δ : 7.324 (m, 2H, ArH), 6.279 (m, 2H, ArH), 4.138 (s, 1H, NH), 3.300 (s, 2H, NH_2), 2.528 (s, 3H, $CH_3-C=O$), 2.015 (s, 3H, CH_3-CH). ^{13}C -NMR (DMSO, 400 MHz) δ : 173.223, 172.896, 2 (C=O), 165.321 (C=N), 137.204, 133.615, 128.963, 117.90, 128.364, 127.416, 125.852, 123.765, 120.521, 118.962, 116.831 7 (aromatic carbon), 49.618, 38.334, 37.881, 33.112, 30.3445, 29.234, 25.667, 21.778. Anal. calcd. (%) for $C_{16}H_{16}N_4O_4S$ (362.40): C; 52.98, H; 4.97, N; 15.45, S; 8.83. Found C; 52.95, H; 4.99, N; 15.42, S; 8.79.

2.2.4.5. 2-{Acetyl-[phenylsulfonyl]amino}-N-(4,6-diaminopyrimidin-3-yl)propanamide (3f)

Yield 2.91 g (89.89%), mp. 108–110 °C, IR (KBr) cm^{-1} : 3456, 3429 (N–H), 3149 (C–H aliphatic), 1194 (C–H aromatic), 1733 (C=O), 1681 (C=O), 1673, 1668 (C=C), 1666, 1651 (C=N), 1369, 1277 (S=O), 1123 (SO_2), 1103 (C–N), 749 (Ar–H). 1H -NMR (DMSO, 400 MHz) δ : 7.267 (m, 2H, ArH), 6.387 (m, 2H, ArH), 6.013 (m, 1H, Ar), 5.746 (s, 1H, NH), 3.467 (s, 2H, NH_2), 2.470 (s, 3H, CH_3-Ar). ^{13}C -NMR (DMSO, 400 MHz) δ : 173.343, 169.118 (C=O), 137.137, 133.707, 133.517, 132.283, 131.947, 129.010, 128.782, 125.787, 123.442, 120.233 (aromatic carbons), 93.094, 76.253, 78.919, 78.583 (aliphatic carbons). Anal. calcd. for $C_9H_{17}N_6O_4S$ (377.50): C, 28.61, H, 4.50, N, 22.25, S, 8.48. Found: C, 28.63, H, 4.49, N, 22.25, S, 8.50.

3. Biological Studies

3.1. Antimicrobial Evaluation

Wiegand et al agar dilution method [34] was used. Test microbes were various bacteria and fungi obtained from the Department of Pharmaceutical Microbiology and Biotechnology laboratory, University of Nigeria, Nsukka.

3.1.1. Standardization of the Test Microbes' Suspension

The test microbes were standardized using 0.5 McFarland turbid equivalents.

3.1.2. Control Test (Standard)

Ofloxacin and Fluconazole were the standard antimicrobial agents.

3.1.3. Experimental

Various concentrations of the compound used were 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, and 0.1 mg/ml. The sample containing molten agar plates were allowed to gel and the plates were divided into seven. Microorganisms were streaked on the divisions and the culture plates incubated at 37 °C for 24 h, and at 25 °C for 2 days. The results were recorded.

3.2. Antioxidant Studies

3.2.1. Antioxidant Activity by DPPH Method

Using Blois method [35], the compounds' inhibition of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical [37] informed their antioxidant activities. The DPPH solution was obtained by dissolving 1.9 mg of DPPH in 100 ml of methanol. Then 50, 100, and 200 µg/ml concentrations of the DPPH solution were also prepared. Similarly, 50, 100, and 200 µg/ml of the title compounds were prepared. Using the same method, standard solution of ascorbic acid was prepared. Then, 1 ml of DPPH solution was added to 2 ml solution of the title compounds and ascorbic acid. The reaction mixture was stirred and left in the dark at room temperature for 30 min after which the absorbance was recorded at the wavelength of 517 nm against the corresponding blank solution spectrophotometrically in triplicate. The percentage inhibitions of scavenging DPPH free radical were calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

$\text{Abs}_{\text{control}}$ = the absorbance of DPPH radical and n-hexane/methanol,

$\text{Abs}_{\text{sample}}$ = the absorbance of DPPH radical and sample/standard.

3.3. In silico Evaluation

3.3.1. Physicochemical Properties

Number of hydrogen bond donor (HBD), number of rotatable bond (NRB), octanol/water partition coefficient logP(o/w), aqueous solubility (SlogP) and topological polar surface area (TPSA), Molecular weight (MW) and number of hydrogen bond acceptor (HBA) were generated *in silico*. The descriptors calculator in Swiss dock online servers was used in the computation of these parameters and Lipinski's rule of five was used for the investigation of their drug-likeness.

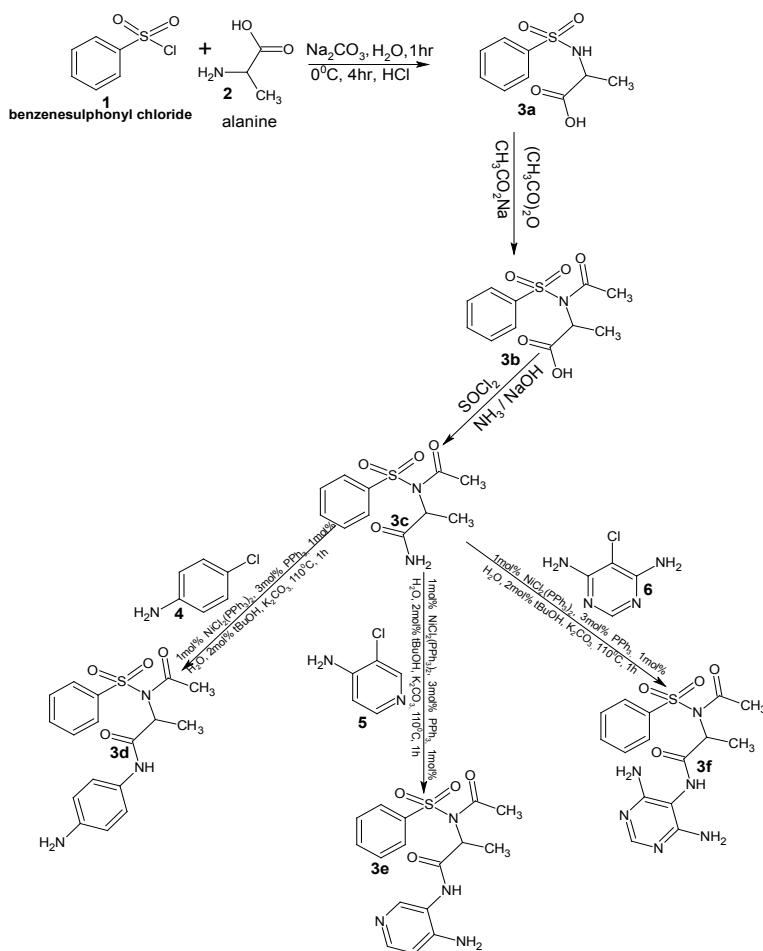
3.4. Molecular Docking

The molecular docking studied some bacterial infections, fungal infections, and oxidative stress using their appropriate drug target whose 3-dimensional structures have gotten from the Protein Data Bank (PDB), (<http://www.pdb.org>) database. The drug targets for antibacterial was (PDB code: **5MMN**); antifungal was (PDB code: **1WS3**), and antioxidant was (PDB code: **1HD2**). The prepared compounds interacted with each of the receptors through molecular docking using PyRx. The best conformation for each compound was selected and visualized using the Discovery studio.

4. Results

4.1. Chemistry

The synthetic route of alanine-based sulphonamide derivatives is shown in Scheme 1.



SCHEME 1. The synthetic route of alanine-based antimicrobial and antioxidant agents.

5. Discussion

5.1. Chemistry

As shown in Scheme 1, the acylation was a protection strategy to prevent the amino group from side reactions with oxidizing agents and electrophiles and ensure regioselectivity. Chlorination was to generate a reactive intermediate and ammonolysis was carried out in order to form the carboxamide from the carboxylic acid end of the alanine amino acid [36].

5.2. Results of Biological Studies

5.2.1. Antimicrobial Activities

The antimicrobial activities (Table 1) show that all the title compounds exhibited considerable antimicrobial activities. Compounds **3f** and **3a** possessed the best antimicrobial activities. The best antimicrobial activities displayed by compound **3f** could possibly be due to the coupled aminopyrimidine moiety because aminopyrimidines are known to possess good antimicrobial activities [23]. Moreover, amino acids such as alanine were found to potentiate the antimicrobial activities of sulphonamide derivatives [37].

TABLE 1. Antimicrobial activities

The minimum inhibitory concentration of title compounds(mg/ml)							
Compounds	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>B. sub</i>	<i>Ps. aerug</i>	<i>C. albicans</i>	<i>A. niger</i>
3a	–	0.9	1.0	0.5	–	0.7	0.9
3b	0.7	0.7	–	0.7	–	0.7	–
3c	0.9	0.8	0.9	0.6	–	–	–
3d	0.7	0.6	0.6	0.6	–	–	–
3e	0.8	0.6	0.9	0.8	–	–	–
3f	0.7	0.8	0.6	0.5	0.8	0.8	–
Ofloxacin	0.005	0.005	0.010	0.020	0.025	–	–
Fluconazole	–	–	–	–	–	0.020	0.005

Key: – represents no inhibition. Ofloxacin = antibacterial drug, Fluconazole = antifungal drug.

5.2.2. Antioxidant Activities

Table 2 shows that the compounds possessed antioxidant activities. For example, compound **3a** had an excellent percentage inhibition of 95.70 ± 0.002 at its highest concentration of 200 $\mu\text{g/ml}$ comparable to the antioxidant inhibition of ascorbic acid (96.83 ± 0.001 inhibition at 200 $\mu\text{g/ml}$). Similarly, compound **3a** displayed an IC_{50} value ($1.072 \pm 0.002 \mu\text{g/mg}$) very close to that of ascorbic acid ($0.999 \pm 0.001 \mu\text{g/ml}$). This suggests that compound **3a** was the most potent antioxidant agent.

5.3. MOLECULAR DOCKING RESULTS

5.3.1. Drug-likeness and Oral Bioavailability of Compound

From Table 3, with the knowledge of Lipinski's rule of five (ro5) and topological polar surface area (TPSA) properties, drug-likeness property and oral bioavailability of the

TABLE 2. The antioxidant activities of the title compounds

Sample	200 µg/ml% inhibition	100 µg/ml% inhibition	50 µg/ml% inhibition	IC ₅₀ values (µg/ml)
Ascorbic acid	96.83 ± 0.001	97.68 ± 0.001	97.31 ± 0.001	0.999
3a	95.70 ± 0.002	92.38 ± 0.001	81.07 ± 0.000	1.072
3b	93.42 ± 0.001	87.68 ± 0.001	84.93 ± 0.001	1.113
3c	83.89 ± 0.000	81.15 ± 0.000	78.34 ± 0.001	1.221
3d	53.24 ± 0.001	48.35 ± 0.001	40.66 ± 0.001	2.113
3e	74.54 ± 0.002	64.84 ± 0.001	57.94 ± 0.003	2,013
3f	84.37 ± 0.002	85.17 ± 0.001	60.81 ± 0.001	1.178

Key: Ascorbic acid = antioxidant drug.

compounds was evaluated. Compounds with $TPSA \leq 140 \text{ \AA}^2$ can penetrate the cell [38], and compounds with $TPSA \leq 90 \text{ \AA}^2$ can penetrate the blood-brain-barrier (BBB) and affect the central nervous system (CNS). The results showed that while all the title compounds **3a–3e** can penetrate the cell, only compound **3a** of $TPSA 83.47 \text{ \AA}^2$ can penetrate the blood-brain-barriers and therefore could be utilized in treatment of cerebral malaria and Alzheimer's diseases which are CNS-related diseases. Lipinski's ro_5 , stipulated that the drug-likeness of a drug candidate ascertained if the number of hydrogen bond donor (HBD) ≤ 5 lipophilicity ($\log P$) ≤ 5 , number of hydrogen bond acceptor (HBA) ≤ 10 , molecular weight (MW) ≤ 500 . The results in **Table 3** revealed that the compounds satisfy Lipinski's rule of 5 (Ro5). Similarly, Van de waterbeemd et al. [38] also stated that the NRB greatly determine the oral bioavailability in rats and $NRB \leq 10$ is a requirement for good oral bioavailability. Summarily, in compliance with these principles earlier mentioned, all the compounds were qualified as likely drugs compounds with good oral bioavailability.

TABLE 3. The physicochemical parameters

Compounds	HBA	HBD	NRB	$\log P$ (o/w)	SlogP	TPSA	MW	Lip violation
3a	4	3	4	0.97	0.44	83.47	228.00	0
3b	5	2	5	0.92	0.70	91.75	270.32	0
3c	5	3	5	2.53	1.77	101.68	270.30	0
3d	4	2	6	0.59	1.54	122.47	361.42	0
3e	5	2	6	0.58	1.55	122.47	362.41	0
3f	6	3	8	0.61	0.12	200.18	377.51	0

5.3.2. *In silico* Antibacterial, Antifungal, and Antioxidant Activities Results

The free binding energy is given in **Table 4**. Compounds had binding affinities with all the receptors employed in this study. Compound **3c** displayed the best antibacterial binding affinity (-10.03 kcal/mol) comparable to penicillin (-10.89 kcal/mol). Compound **3a** possessed higher binding affinity (-11.79 kcal/mol) than ketoconazole (-10.85 kcal/mol). Compound **3f** possessed the highest binding affinities (-13.14 kcal/mol) comparable to α -tocopherol (-14.82 kcal/mol).

TABLE 4. *In silico* antibacterial, antifungal, and antioxidant activities of the title compounds

Compound	Antibacterial	Antifungal	Antioxidant
	5MMN	1WS3	1HD2
3a	-9.63	-11.79	-12.41
3b	-9.07	-9.93	-10.56
3c	-10.03	-9.49	-10.75
3d	-9.99	-9.03	-11.72
3e	-9.63	-9.75	-11.41
3f	-9.90	-10.03	-13.13
Standard drug	-10.89	-10.85	-14.82

Key: Standard drugs for 5MMN = Penicillin; 1WS3 = Ketoconazole; 1HD2 = α -Tocopherol.

6. Conclusion

A facile synthesis of alanine-based antimicrobial and antioxidant agents was successfully accomplished. The assigned structures complied with the spectral data. Compound 3f and 3a were found to be the best antibacterial and antifungal agents, respectively. Compound 3a was found to exhibit the best antioxidant activities, could penetrate the blood brain barrier and outperformed Ketoconazole in the *in silico* antifungal assessment. Compounds 3c and 3f exhibited excellent *in silico* antibacterial and antioxidant activities comparable to penicillin and α -tocopherol, respectively. The title compounds were found to be likely drugs with good oral bioavailability properties and confirmed to be potential antimicrobial, antioxidant agents.

Financial Disclosure/Conflict of Interest

The authors declare that there was no financial aid received and no conflict of interest associated with this research work.

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