Synthesis and Biological Evaluation of Alanine Derived Bioactive p-Toluenesulphonamide Analogs

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ABSTRACT

Sulphonamides and carboxamides have great pharmacological importance. The purpose of the study was to synthesize alanine-derived bioactive sulphonamides bearing carboxamides and evaluate their biological activities. The reaction of p-toluenesulphonyl chloride with L-alanine afforded compound 1, which was acetylated to obtain compound 2. The chlorination and ammonolysis of compound 2 gave the carboxamide backbone (3) which was coupled with aryl/heteroaryl halides to afford the hybrid compounds 4, 5 and 6. Structures were confirmed by FTIR, 1H-NMR, 13C-NMR spectra and elemental analytical data. The in vitro antimicrobial properties were determined by agar dilution, and the antioxidant properties were also investigated. Molecular docking interactions of the analogues were determined using PyRx. Compounds 4, 5 and 6 exhibited excellent in vitro antimicrobial properties in the range of 0.5-1.0mg/ml while compounds 1 and 2 had half-maximal inhibitory concentration (IC50) of 1.11±0.15µg/ml and 1.12±0.13µg/ml respectively. For the molecular docking studies, compounds 5 and 6 displayed the best antitrypanosomal activity with binding affinities of -13.95 and -13.51kcal/mol respectively while compound 4 showed the highest in silico antimalarial activity having binding affinity of -11.95kcal/mol. All the alanine derived sulphonamides were observed to be potential antimicrobial, antioxidant, antitrypanosomal and antimalarial agents following the biological activities studies.

INTRODUCTION

Pathogenic diseases such as bacterial infections, malaria and trypanosomiasis have become a global burden and therefore pose a significant threat to the global health system (GBD, 2013). Similarly, oxidative stress has been found to increase the prevalence of these diseases because of its ability to reduce the body’s immune response (Biller and Takahashi, 2018). It was reported that certain sulphonamide derivatives that inhibited oxidative stress also displayed antimicrobial activities (Egbujor et al., 2020a). Therefore, it is expected that the
development of synthetic antioxidant and antimicrobial agents would drastically reduce the preponderance of pathogenic diseases. Alanine is an α-amino acid in humans that is indispensable in protein biosynthesis and plays a crucial role in the glucose-alanine cycle between tissues and liver (Reeds, 2000; Nelson and Cox, 2005). Humans require alanine for the processing of vitamin B (Reeds, 2000; Holden et al., 1951), and it also acts as a precursor of diverse biomolecules (Berg et al., 2002). The fact that bacteria can metabolise alanine has become a good target for drug discovery and design (Paretsky, 1948; Wiame and Pierard, 1955). Moreover, certain α-amino acids were found to improve the antimicrobial and antioxidant activities of sulphonamides (Egbujor et al., 2019a).

On the other hand, other combining moieties such as sulphonamides are known antiretrovirals (Supuran et al., 2004), diuretics (Vardanyan and Hruby, 2006) agents. Moreover, sulphonamides have been the scaffold for drug discovery and design because of their stability and tolerance in Homo sapiens (Shet et al., 2013).

Likewise, Carboxamides are commonly found in drug molecules (Montalbetti and Falque, 2005) which have been successfully used in the management of HIV (Roskoski, 2003), blockage of the synthesis of cholesterol (Graul and Castaner, 1997) and others. Moreover, the coupling partners utilised in this research work such as aniline, aminopyridine and dianinopyrimidine are of great pharmacological importance (Ju et al., 2006a, b). Aniline is used in the production of essential drugs such as paracetamol, acetyaminophen and Tylenol (Parke, 1968). Pyridine and Pyrimidine are used in pharmaceuticals such as CNS stimulants, local anaesthetics (Browning, 1965), vitamins (Lewis, 1997), antifolates and sulfa drugs (Jain et al., 2006) respectively.

Resistance to antimicrobial agents is problematic and has become a pressing global medical challenge. For instance, 70% of the 2 million patients having hospitality acquired microbial infections in US hospitals every year exhibited resistance to certain antibiotics (ISA, 2004; Bradley et al., 2007). In an attempt to eliminate the menace, new classes of drug molecules working on target sites different from those presently used should be developed (Coates and Hu, 2007; Kimberlin and Whitley, 1996). We proposed that the synergistic antimicrobial antagonism and new drug actions that would arise from the combination of the pharmacophores mentioned above could be explored.

Therefore the objective of the study was to synthesise novel alanine-based sulphonamide derivatives and evaluate their biological activities. We believe that combining various compounds having numerous biochemical properties would bring about drug potency enhancement.

**MATERIALS AND METHODS**

**Reagents and Instrumentation**

Reagents were supplied by Sigma Aldrich Corporation, United States of America. The melting point ranges of 4-methylphenylsulphamoyl compounds were ascertained using melting point equipment, IA9200 model of Cole-Parmer Ltd, Stafford shire, UK and were uncorrected. FT-IR spectroscopy of the synthesized compounds was determined with Shimadzu 8400s FT-IR of Shimadzu Corporation, Kyoto, Japan. Proton and Carbon Nuclear Magnetic Resonance (NMR), were ascertained with Bruker Avance III 400MHz NMR spectrophotometer of Bruker Corporation, Massachusetts, USA, and results were recorded. Nitrogen gas was utilized for all inert reaction conditions. Compounds were obtained in analytical grade, so chromatographic purification was not needed.

**General procedure for the synthesis of 4-methylbenzenesulphonamoyl carboxylic acid(1)**

Inside a 100ml beaker, L-alanine (2.2g, 25mmol) was dissolved in water (30ml) and sodium carbonate (5.58g, 52.50mmol) was added. It was stirred thoroughly and cooled to -5°C and 4-methylsulfonyl chloride (5.12g, 50 mmol) was intermittently added in four parts within 1 hour. This was followed by 4 hours stirring at normal room temperature. In addition of 2M HCl, crystallisation occurred. Reaction steps were monitored strictly using TLC (MeOH/DCM, 1:8). It was kept untouched for at least 12 hours and filtered by suction, and the solid product was washed with tartaric acid (pH2.2) and dried to afford 2-[(4-methyl phenyl) sulphonyl] amino) propanoic acid (1) in excellent yields (81.9%).

**2-([(4-methyl phenyl)sulphonyl]amino)propanoic acid Acetylation Procedure**

2g of 2-[(4-methyl phenyl) sulphonyl] amino) propanoic acid (3a) was weighed and added to a beaker, 9ml of concentrated HCl and distilled water (25ml) were added to the beaker followed by vigorous stirring to ensure a homogeneous mixture. 16.0g of sodium carbonate was dissolved in 50ml distilled water in a separate beaker. Then, 13.5ml of acetic anhydride was added in small portion for 1 hour to the 4-methyl phenyl sulphonamoyl carboxylic acid solution after which it was transferred...
Scheme 1: Synthetic route of alanine based p-toluene sulphonamide derivatives.

Table 1: Minimum inhibitory concentration (mg/ml) of compounds.

| Compound No | Escherichia coli, typhi | Salmonella typhi | Staphylococcus aureus | Bacillus subtilis | Pseudomonas aeruginosa | Candida albicans | Aspergillus niger |
|-------------|-------------------------|-----------------|----------------------|------------------|------------------------|-----------------|------------------|
| 1           | 0.9                     | 0.8             | -                    | 0.8              | 0.6                    | 0.7             | -                |
| 2           | 0.8                     | 0.8             | -                    | 0.8              | -                      | 0.8             | -                |
| 3           | 0.9                     | 0.7             | -                    | -                | -                      | -               | 0.7              |
| 4           | 0.7                     | 1.0             | 0.8                  | 0.6              | -                      | 0.7             | 0.5              |
| 5           | 0.8                     | 1.0             | 0.9                  | 0.6              | -                      | 0.9             | 0.5              |
| 6           | 0.8                     | 0.9             | 0.7                  | 0.5              | 0.9                    | 0.8             | -                |
| Ofloxacin   | 0.005                   | 0.005           | 0.010                | 0.020            | 0.025                  | -               | 0.020            |
| Fluconazole | -                      | -               | -                    | -                | 0.020                  | -               | 0.005            |

- implies no activity. Ofloxacin = antibacterial standard drug, Fluconazole = antifungal standard drug.

to the solution of sodium acetate. It was stirred thoroughly with a glass rod, and the beaker was immersed into an ice bath just for 1 hour and filtered to afford compound (2) in excellent yields (91.2%).

Procedure for Chlorination

2g of 2-(Acetyl [(4-methyl phenyl) sulfonyl] amino) propanoic acid (2)(1mmol) and acetone (10ml) were added to a three-necked 250ml flask equipped with a magnetic stirring bar. The three-necked flask was stoppered, cooled to 0°C and stirred at 80°C under reflux for 3 hours, after which the content was immersed into a water bath at 80°C to evaporate thionyl chloride. Addition of acetone (20ml) and evaporation was carried out twice to ensure complete evaporation to afford acid chloride intermediate that enables ammonolysis.

Procedure for Ammonolysis

The resulting acid chloride intermediate obtained from chlorination reaction above was instantly dissolved in acetone (20ml) and cooled to 0-5°C. In addition of ammonia (2ml) and 1M NaOH, crystallisation occurred, and the mixture was kept overnight after which it was filtered and washed with acetone to afford compound 3 in excellent yield (91.8%).
Nickel catalysed reaction for the synthesis of sulphonamide derivatives bearing aniline, aminopyridine and diaminopyrimidine

Bis (triphenylphosphine) nickel (ii) chloride Preparation

Using Venanzi (Venanzi, 1958) procedure, this coordination compound was prepared by dissolving nickel (II) chloride hexahydrate catalyst (2.37g, 10mmol) in distilled water (2ml) followed by dilution with glacial acetic acid (50ml) and addition of triphenyl phosphate ligand (5.25g, 20 mmol) dissolved in 25ml glacial acetic acid. The green precipitate formed was allowed to be in contact with the glacial acetic acid solution for 24hours. A dark blue crystal (the complex compound) obtained on filtration was washed using glacial acetic acid and dried.

The synthesis of compounds 4-6

The complex compound bis (triphenyl phosphine) nickel(II) chloride (6.54g, 10mmol) and triphenyl phosphine (5.25g, 30mmol) were both introduced into an Erlenmeyer flask (50ml). t-butanol (4ml) and distilled water (2ml) as solvents were added with the help of a syringe and the slurry was stirred for 10mins under inert nitrogen condition at normal room temperature. The mixture was heated at 80°C for 1.5min. Then 2-(acetyl [(4-methyl phenyl) sulfonyl] amino) propanamide (3) (10mmol), potassium carbonate, K2CO3(1.38g,10mmol), substituted aryl and heteroaryl halides (4-chloroaniline, 4-amino-3-chloropyridine and 5-chloro-4,6-diaminopyrimidine) were added to the mixture with t-butanol and H2O in 2:1 ratio for the second time. The mixture was subjected to refluxing and stirring for 1hour at temperature range 100°C-110°C. It was cooled to normal room temperature, crystallized using ethyl acetate and washed with water to afford alanine-derived p-toluenesul phonamoyl carboxamide derivatives (4-6). The synthetic pathway for the overall reactions is represented in Scheme 1.

Table 2: Antioxidant activities of synthesized compounds.

| Compounds | % inhibition at 200µg/ml | % inhibition at 100µg/ml | % inhibition at 50µg/ml | IC50 Values (µg/ml) |
|-----------|--------------------------|--------------------------|--------------------------|---------------------|
| Ascorbic acid | 96.83±0.17 | 97.68±0.11 | 97.31±0.18 | 1.00±0.13 |
| 1 | 95.60±0.13 | 92.39±0.18 | 81.09±0.22 | 1.11±0.15 |
| 2 | 93.45±0.18 | 87.69±0.20 | 84.98±0.15 | 1.12±0.17 |
| 3 | 93.53±0.21 | 82.78±0.17 | 72.83±0.19 | 1.41±0.14 |
| 4 | 26.56±0.24 | 43.96±0.19 | 45.12±0.17 | 2.79±0.17 |
| 5 | 72.71±0.18 | 79.37±0.15 | 85.84±0.15 | 1.24±0.11 |
| 6 | 85.60±0.17 | 87.08±0.14 | 80.09±0.18 | 1.17±0.12 |

Ascorbic acid is the antioxidant drug.

Antioxidant Evaluation Procedure

The anti-oxidative properties of 4-methylphenylsulphamoylanalogues were investigated using the procedures outlined by CLSI (Wiegand et al., 2008; Sader et al., 2013).

Biological evaluations

Antimicrobial studies

Some pathogenic bacteria and fungi were obtained as clinical isolates and standardized with 0.5 McFarland turbid equivalents. Ofloxacin and fluconazole were used as antibacterial and antifungal drugs, respectively. Using agar dilution method, the antimicrobial properties of the alanine derived sulphonamides were investigated using the procedures outlined by CLSI (Wiegand et al., 2008; Sader et al., 2013).

In silico procedure

Physicochemical Parameters

The physicochemical parameters were obtained in silico. These are the molecular weight (MW), number of hydrogen bond acceptor (HBA), num-

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Table 3: Physicochemical properties.

| Mol | HBA | HBD | NRB | logP(o/w) | Slog P | TPSA | MW | Lip violation |
|-----|-----|-----|-----|----------|-------|------|----|-------------|
| 1   | 4   | 3   | 4   | 1.27     | 0.75  | 83.47| 243.28| 0           |
| 2   | 5   | 2   | 5   | 1.22     | 0.41  | 97.54| 284.37| 0           |
| 3   | 4   | 2   | 5   | 0.48     | 0.41  | 97.54| 284.37| 0           |
| 4   | 4   | 2   | 6   | 1.82     | 2.14  | 109.57| 375.44| 0           |
| 5   | 5   | 2   | 6   | 0.59     | 1.54  | 122.46| 376.43| 0           |
| 6   | 5   | 3   | 7   | 0.31     | 0.42  | 122.46| 392.50| 0           |

Table 4: In silico molecular docking Anti trypanosomal, Antimalarial, Antibacterial, Antifungal and Antioxidant Activities results.

| Compound | Anti trypanosomiasis (2EWG) (kcal/mol) | Antimalaria (1SME) (kcal/mol) | Antibacterial (5MMN) (kcal/mol) | Antifungal (1WS3) (kcal/mol) | Antioxidant (1HD2) (kcal/mol) |
|----------|----------------------------------------|-------------------------------|---------------------------------|-------------------------------|-------------------------------|
| 1        | -13.17                                 | -11.54                        | -10.26                          | -11.11                        | -12.05                        |
| 2        | -13.35                                 | -10.83                        | -9.63                           | -11.79                        | -12.66                        |
| 3        | -12.28                                 | -10.31                        | -9.07                           | -9.93                         | -10.56                        |
| 4        | -12.86                                 | -11.95                        | -10.03                          | -9.49                         | -10.75                        |
| 5        | -13.95                                 | -11.54                        | -9.89                           | -9.64                         | -11.43                        |
| 6        | -13.51                                 | -11.45                        | -10.90                          | -10.03                        | -13.13                        |
| Standard drug | -19.36                              | -10.08                        | -10.89                          | -10.85                        | -14.82                        |

About five human diseases like trypanosomiasis, malaria, bacterial infections, fungal infections and oxidative stress were studied in this docking using the appropriate drug targets. The drug targets for antitrypanosomiasis: T. brucei farnesyl diphosphate synthase complexed with minodronate (PDB code: 2EWG); antimalarial: plasmepsin II, a haemoglobin-degrading enzyme from Plasmodium falciparum, in complex with pepstatin A (PDB code: 1SME); antibacterial: E. coli DNA gyrase in complex with pepstatin A (PDB code: 5MMN); antifungal: urate oxidase from Aspergillus flavus complexed with uracil (PDB code: 1WS3), and antioxidant: human peroxiredoxin 5 (PDB code: 1HD2). The 3-Dimensional structures of the drug, as mentioned above, targets were downloaded from the Protein Data Bank (PDB), (http://www.pdb.org) database. Molecular docking via PyRx the 4-methylphenylsulphamoylanalogues interacted with the receptors accordingly. This protocol enabled flexible docking for several compound conformers, and most excellent conformation was taken for each compound. The observed interactions were visualized in Discovery studio.

Statistical analysis

The antioxidant studies of compounds were carried out in three different concentrations to obtain IC50 values using linear regression method with Graphpad Prism 5. The results were represented as mean ± SEM (n = 3).

RESULTS AND DISCUSSION

Spectra data

2-[(4-methylphenyl)sulphonyl]amino)propanoic acid (1)

Yield is 2.50g (81.9%), mp. 117-119°C. IR(KBr)cm⁻¹: 3250(N-H), 3060(O-H), 1927(C-H), 1722(C=O of COOH), 1589(C=C aromatic), 1367, 1170 (SO₂ two bands), 741(Ar-H).¹H-NMR (DMSO,400MHz):δ:8.05-8.03 (d,J=8.3Hz, 1H,NH), 7.68-7.65 (d,J=8.1Hz, 2H,Ar-H),7.37-7.35 (d,J=8.2Hz, 2H,CH₃-CH), 7.68-7.65 (d,J=8.1Hz, 2H,Ar-H),7.37-7.35 (d,J=8.2Hz, 2H,CH₃-CH).¹³CNMR (CD₃N, 400MHz) δ: 170.584 (C=O), 148.927, 140.120,
131.603, 129.464, 126.345, 117.346 (aromatic carbons), 60.342, 45.231, 25.905 (aliphatic carbons). Anal. calcd. for C_{10}H_{13}N_{2}O_{5} (243.28): C, 49.39; H, 5.40; N, 5.78; S, 13.17. Found: C, 49.36; H, 5.42; N, 5.81, S, 13.22.

2-[(Acetyl) [4-methylphenyl]sulfonyl]amino] propanoic acid (2)

Yield 2.03g (91.2%), mp.109-110°C, IR (KBr) cm⁻¹: 3302 (O-H of COOH), 3093(N-H), 3000(C-H), 1923 (C=H aromatic), 1703, 1691(C=O), 1490, 1401 (C=C), 1311,1292 (2S=O), 1170(SO₂-NH), 1115 (C-N), 741(Ar-H). 1HNMR (CD₃CN, 400MHz): δ: 7.958 (d, J= 8.8Hz, 2H, ArH), 7.513 (d, J= 8.4 Hz, 2H, ArH), 7.272 (s, 3H, CH₃-C=O), 2.482 (s,3H, CH₃- Ar), 1.975-1.958 (m, IH, CH), 1948 (d, J=1.2Hz, 3H, CH₃-CH), 13CNMR (CD₃CN, 400MHz) δ: 173.243, 168.244, (C=O) 147.927, 141.120, 130.603, 129.464, 126.345, 117.346 (aromatic carbons), 58.31, 51.334, 49.543 43.905 (aliphatic carbons). Anal. calcd. for C_{12}H_{15}N_{2}O_{5} (285.32): C, 50.47, H, 5.26, N, 4.91, S, 11.22. Found: C, 50.51, H, 5.30, N, 4.92, S, 11.25.

2-[(Acetyl) [4-methylphenyl]sulfonyl]amino] propanoid (3)

Yield 2.04g (91.8%), mp.206-207°C, IR (KBr) cm⁻¹: 3364, 3172 (N-H) 2870 (C-H aliphatic) 1994 (C=H aromatic), 1804, 1699 (C=O) 1449,1371(C≡C), 1304,1203 (2S=O),1155(SO₂-NH), 1118(C-N), 678 (Ar-H). 1HNMR (CD₃CN, 400MHz): δ: 7.958 (d, J= 8.8Hz, 2H, ArH), 7.513 (d, J= 8.4 Hz, 2H, ArH), 6.762(m, m, NH), 2.722 (s, 3H, CH₃-C=O), 2.482 (s,3H, CH₃-Ar), 1.975-1.958 (m, m, CH), 1948 (d, J=1.2Hz, 3H, CH₃-CH), 13CNMR (CD₃N, 400MHz) δ: 174.110, 169.584(C=O), 147.927, 141.120, 130.603, 129.464, 126.345, 117.346 (aromatic carbons), 78.334, 64.324, 60.775, 38.905 (aliphatic carbons). Anal. calcd. for C_{12}H_{15}N_{2}O_{5} (284.33): C, 50.65, H, 5.63, N, 9.85, S, 11.25. Found: C, 50.71, H, 5.67, N, 9.91, S, 11.30.

2-[(Acetyl) [4-methylphenyl]sulfonyl]amino] N- (4-aminopyridin-3-yl)propanamide (5)

Yield 2.90g (89.8%), mp.109-110°C, IR (KBr) cm⁻¹: 3451, 3328 (2H-N), 3145 (C-H aliphatic), 1190 (C=H aromatic),1632 (C=O), 1630 (C=O), 1576,1550 (C=N), 1367,1274 (2S=O), 118 (SO₂-NH), 1025 (C-N), 976 (C=C), 745 (Ar-H). 1HNMR (DMSO/CDCl₃ 400MHz): δ: 7.248 (m, 2H, ArH), 6.396 (m, 2H, ArH), 6.011 (m, IH, Ar), 5.735 (s, IH, NH), 3.462 (s, 2H, NH₂), 2.470 (s, 3H, CH₃ -Ar). 13CNMR (DMSO/CDCl₃, 400MHz) δ: 173.342, 169.117(C=O), 173.342, 169.117(C=O), 137.136, 133.706, 133.516, 132.280, 131.946, 129.009, 128.781, 125.786, 124.41, 120.232 (aromatic carbons), 93.093, 76.252, 78.918, 78.584 (aliphatic carbons). Anal. calcd. for C_{16}H_{13}N_{2}O_{5} (392.50): C, 54.23, H, 5.10, N, 21.40, S, 8.15. Found: C, 54.28, H, 5.15, N, 21.43, S, 8.20.

Biological activities

Antimicrobial activity

Table 1 showed that only compound 6 had a significant inhibitory effect on the growth and replication of all the bacteria used. In contrast, compounds 4 and 5 equally inhibited the growth of all the fungi used. Pseudomonas aeruginosa was the most resistant bacteria while Aspergillus niger displayed the highest antifungal recalcitrance, although susceptible to compounds 4 and 5.

Antioxidant activity
From Table 2, compound 1 showed percentage inhibition of 95.60% at the highest concentration (200 μg/ml) and an IC\textsubscript{50} value of 1.11±0.15 μg/ml while compound 2 displayed percentage inhibition of 93.45% at the same concentration and an IC\textsubscript{50} of 1.12±0.17 μg/ml. For comparison, ascorbic acid had 96.83% inhibition and an IC\textsubscript{50} of 1.00±0.13 under the same conditions.

**In silico Results**

The physicochemical parameters of the compounds Table 3 showed that the hydrogen bond acceptor (HBA) ≤ 5, hydrogen bond donor (HBD) ≤ 3, number of rotatable bonds (NRB) ≤ 7, octanol/water partition coefficient log P (o/w) ≤ 1.82, aqueous solubility (Slog P) ≤ 2.14, topological surface area (TPSA) ≤ 122.46 and molecular weight (MW) ≤ 392.50. Table 4 showed compound 5, 4, 6, 2 and 6 had the highest antitypanosomal, antimalarial, antibacterial, antifungal and antioxidant binding energies of -13.95, -11.95, -10.90, -11.79 and -13.13 kcal/mol respectively.

**Chemistry**

It was discovered that the synthetic yields of all the title compounds were excellent in the range of 81.90–94.30% with 2-(acetyl-[((4-methyl phenyl) sulfonyl] amino)-propanoic acid (1) having the highest and least yields of 94.3% and 81.9% respectively.

As shown in the synthetic route (Scheme 1), the acylation was carried out to protect the amino group from side reactions that could occur during subsequent reactions. In contrast, chlorination and ammonolysis were carried out to obtain an amide from the un-activated carboxylic acid end of the alanine (Egbujor et al., 2020b). The general diagnostic peaks of C=O, N-H, C=C and C=N, including their peculiar individual peaks, were observed in the FT-IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR and elemental analytical data.

**Biological studies results**

**Antimicrobial activities**

The antimicrobial activities (Table 1) revealed that compounds 1-6 displayed antimicrobial properties. Compound 6 had the best antibacterial activities, while compound 4 and 5 had the best antifungal activities. Compounds were found to exhibit better antibacterial activities than antifungal activities.

The display of antimicrobial activities by all the title compounds could be as a result of the fact that alanine as an α-amino acid must have potentiated the microbial activities of the sulphonamide derivatives as observed with similar amino acids (Egbujor et al., 2019b). Aspergillus niger displayed the highest antimicrobial resistance because it was discovered that the conidiospores of this fungus need alanine specifically to initiate germination and this could undermine the alanine’s ability to inhibit the microbial activities of Aspergillus niger since it is alanine friendly (Halvorson and Church, 1957).

**Antioxidant studies results**

Table 2 showed that all the alanine derived sulphonamides had antioxidant properties. Sulphonamides have been known as good antioxidants (Egbujor et al., 2020c). The antioxidant percentage inhibition was observed to be directly proportional to an increase in the concentration of the compounds from 50 μg/ml, 100 μg/ml and 200 μg/ml. Compounds 1 and 2 had percentage inhibition of 95.60±0.13 and 93.45±0.18 and IC\textsubscript{50} values of 1.11±0.15 μg/ml and 1.12±0.17 μg/ml, respectively, at the highest compound concentration of 200 μg/ml. For comparison, ascorbic acid had antioxidant percentage inhibition of 96.83±0.17 and IC\textsubscript{50} value of 1.00±0.13 μg/ml under the same conditions.

The DPPH scavenging assay was carried out based on the principle that the donating protons of the antioxidants would neutralize DPPH radicals. Low IC\textsubscript{50} indicates high antioxidant activities and vice versa (Matuszewska et al., 2018). Therefore, amongst all synthesized compounds, compound 1 displayed the best antioxidant activity (1.105 μg/ml) comparable to ascorbic acid (0.999 μg/ml). Compound 1 could be a good substitute for ascorbic acid as an antioxidant agent because of the closeness of their IC\textsubscript{50} values.

**Prediction of drug-likeness and oral bio availability of compounds.**

From Table 3, it was observed that HBA ≤ 5, HBD ≤ 3, NRB ≤ 7, logP (o/w) ≤ 1.82, SlogP ≤ 2.14, TPSA ≤ 122.46, MW ≤ 392.50. For comparison, according to Lipinski’s ro5, a drug candidate is said to be drug-like if it has lipophilicity (log P) ≤ 5, several hydrogen bond acceptor (HBA) ≤ 10, molecular weight (MW) ≤ 500 and number of hydrogen bond donor (HBD) ≤ 5. Similarly, the topological polar surface area is employed in drug design as a cell permeability parameter in compliance with the rule that a compound with TPSA ≤ 140 Å\textsuperscript{2} can penetrate the cell and exhibit good oral bio availability in rats (Verber et al., 2002), while compounds with TPSA ≤ 90 Å\textsuperscript{2} can penetrate the blood-brain-barrier (BBB) and the central nervous system (CNS) (van de Waterbeemd et al., 1997).

The findings demonstrated that all the alanine...
derived sulphonamides tend to permeate the cell. Compounds 1 and 2 due to their lower TPSA can penetrate blood-brain-barriers and therefore can be employed in the cure of CNS related diseases, namely cerebral malaria, Alzheimer’s diseases. According to the principle, when more than one parameter is violated, there could be a bioavailability problem in an instance of the oral formulation. From Table 3, all the alanine derived sulphonamides complied with (Ro5). Moreover, Verber et al. (Verber et al., 2002) also stated that the number of a rotatable bond (NRB) has a positive influence on the bioavailability in rats and claimed that NRB ≤ 10 is required benchmark. It was therefore deduced that all the alanine derived sulphonamides are qualified as drug candidates with good oral bioavailability.

**In silico Antitrypanosomal, Antimalarial, Antibacterial, Antifungal and Antioxidant Activities results**

From Table 4, compounds 5, 4, 6, 2 and 6 had the highest antitrypanosomal, antimalarial, antibacterial, antifungal and antioxidant binding energies of -13.95, -11.95, -10.90, -11.79 and -13.13 kcal/mol respectively. Among all the compounds tested on 2EWG, compounds 5 and 6 gave the lowest binding energy (the highest binding affinity) of -13.95 and -13.51 kcal/mol respectively. However, they are not better than the standard drug (malarosprol) for the treatment of trypanosomiasis, which showed the highest binding affinity of -19.36 kcal/mol. All the synthesized compounds had better binding affinities than the standard drug. Still, compound 4 showed the highest binding affinity (-11.95 kcal/mol) with the *Plasmodium falciparum* pepsstatin A receptor (1SME) when compared with the standard drug (chloroquine) for malaria treatment, whose binding affinity is -10.08 kcal/mol. For the DNA gyrase receptor 5MMN, compound 6 had a better binding affinity (-10.90 kcal/mol) than penicillin (-10.89 kcal/mol). Likewise, the antifungal study showed that compounds 1 and 2 had a higher binding affinity (-11.11 and -11.79 kcal/mol, respectively) than that of ketoconazole (-10.85 kcal/mol). Finally, compound 6 had the highest binding affinities (-13.13 kcal/mol) with 1HD2 but did not perform like α-tocopherol (-14.82 kcal/mol).

**CONCLUSIONS**

In conclusion, we have presented a convenient and efficient approach to the synthesis of alanine derived sulphonamides of medicinal importance. The structures were in agreement with the structural analysis. Compounds 4, 5 and 6 were found to have outstanding antimicrobial activities while compounds 1 and 2 exhibited excellent antioxidant activities. However, all the compounds showed in silico antitrypanosomal, antimalarial, antibacterial, antifungal and anti-oxidative properties comparable with the reference drugs such as malarosprol and chloroquine, penicillin, ketoconazole and α-tocopherol respectively. The physicochemical parameters evaluations confirmed that all the compounds were drug candidates and would not have oral bioavailability problems, having satisfied Lipinski’s rule of five. The alanine derived sulphonamides were found to possess antimicrobial, antioxidant, antimalarial and antitrypanosomal properties.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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