Quest Journals
Journal of Research in Pharmaceutical Science
Volume 9 ~ Issue 2 (2023) pp: 19-26
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Synthesis and Biological Evaluation of Proline Derived Sulphonamides

Melford Chuka Egbujor
Department of Chemical Sciences, Rhema University Nigeria, Aba, Abia State, Nigeria.

Abstract
*Bioactive compounds bearing sulphonamide and proline functionalities possess excellent pharmacological activities. In this study, the synthesis of proline-based sulphonamide derivatives and their biological evaluation is reported. The base promoted reaction of 4-methylphenylsulphonyl and benzenesulphonyl chlorides with proline gave compound **1a** and **1b** respectively. Theaminolysis of compound **1a and 1b** gave the carboxamide derivatives which on nickel catalyzed amidation with aryl/heteroaryl halides afforded several bioactive sulphonamide derivatives. Compounds were characterized with the aid of FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. The antimicrobial activity, antioxidant activity were also evaluated. Compounds **1c, 1d** and **1f** were the most potent against E coli, only **1g** exhibited inhibitory activity against C. albicanswhile compounds **1a** and **1g** had the best in vitro antioxidant activity. All the compounds were found to be potential antimicrobial and antioxidant agents.*

Keywords: *Sulphonamide; proline; Antimicrobial activity; Antioxidant activity.*

***R**eceived 06 Feb., 2023; **R**evised 15 Feb., 2023; **A**ccepted 18 Feb., 2023 © The author(s) 2023.*
P**ublished with open access at **www.questjournals.org

I. Introduction

Antimicrobial resistance and oxidative stress-mediated have become a serious health issue globally [1-2]. It has been reported that amino acids such as methionine [3-4], serine [5-6], threonine[7], alanine[8-9], cysteine[10] and many more, when combined with sulphonamide exhibit excellent antimicrobial and antioxidant activities. Proline is a proteinogenic amino acid with a pyrrolidine ring. Proline-rich peptides exhibit excellent antimicrobial activities [11], proline also influences oxidative stress[12]. Sulphonamides are already established antibiotics [13-14], they also exhibit antihypertensive, antitumor, antimalarial and antioxidant [15-19]. As a result, sulphonamides in addition to their stability in the body have been beneficial in drug development [20]. Moreover, other coupling partners such as aniline, pyridine and pyrimidine and other nitrogen containing compounds are employed in this research are pharmaceutically important [21-22]. For instance aniline can be used to manufacture paracetamol and acetaminophen while pyridine and Pyrimidine heterocyclics are useful as local anesthetics, CNS stimulants, vitamins and antifolates [23-27]. Therefore this study was designed to Synthesis and evaluate the antimicrobial and antioxidant activities of proline derived sulphonamide derivatives

II. MATERIALS AND METHODS

2.1: General Instrumentation

Reagents were purchased from Sigma Aldrich. Reactions that required inert condition were carried out under nitrogen atmosphere. Melting points of the compounds synthesized were determined using electrothermal melting point apparatus in open with microscope and were uncorrected. Infrared spectra data were obtained on a Magna –IR system 750 spectrophotometer using KBr discs and absorptions were given in per-centimeter (cm-1). Nuclear Magnetic Resonance (1H-NMR and 13C-NMR) were run in CD3OD, ACN, CDCl3 and DMSO-d6 on 400MHz using NMR spectrophotometer in the Department of Chemistry, Indian Institute of Technology, Kanpur. Chemical shifts were reported in δ scale (neat). The antimicrobial studies were carried out at the Department of Microbiology, University of Nigeria, Nsukka.The antioxidant studies were carried out at the Department of Biochemistry, University of Nigeria, Nsukka. The antitrypanosomal and antimalaria studies were carried out at the Department of Veterinary Medicine, University of Nigeria, Nsukka.

2.2: General Procedure for the Synthesis of *p*-Toluenesulphonamides (1a)

Using a 100 ml Erlenmeyer flask, amino acid (12.5 mmol) was dissolved in water (15 ml) and sodium carbonate (2.79 g, 26.25 mmol) was added with continuous stirring to ensure complete dissolution of the solutes. The clear solution was cooled to 0 oC followed by the addition of *p*-toluenesulphonyl chloride (2.86 g, 15 mmol) in three portions for one hour interval. The slurry was stirred for four hours at room temperature and the reaction mixture was acidified to pH 2 with 2M hydrochloric acid to facilitate crystallization. It was allowed to settle down for at least twelve hours and the product was separated through suction filtration. The filtered crude product was washed with tartaric acid (pH 2.2) and dried to obtain *p*-toluene sulphonamides in good to excellent yields (61-98.5 %

2.3: 1-[(4-Methylphenyl)sulphonyl]pyrrolidine-2-carboxylic acid(1a)

The amino acid used was proline, appearance: yellowish oil, yield 3.25 g (96.1%), mp 40-42 oC.IR(KBr)cm-1 : 3470(O-H), 2881(C-H), 1722(C=O of COOH), 1636(C=C aromatic), 1334, 1151(S=O two bands), 771(Ar-H). IH-NMR(DMSO, 400 MHz)δ :7.74-7.72 (d, J=8Hz, 2H, Ar-H), 7.43-7.41 (d, J=8Hz, 2H, Ar-H), 4.08-4.05 (m, H, CH-COOH), 3.38-3.32 (m, H, CH of CH2-N), 3.18-3.91(m, H, CH of CH2-N), 2.39(s, 3H, CH3), 1.88-1.76(m, 3H, CH and CH2), 1.58-1.52(m, H, CH). 13C-NMR (DMSO-d6, 400 MHz) δ: 170.3(C=O), 143.6, 134.8, 129.8, 127.3, 123.5, 120.8 (aromatic carbons), 67.2, 60.6, 48.6, 30.6, 25.3 (aliphatic carbons). Anal.calcd. for C12H15NO4S (269.12): C, 53.51; H, 5.62; N, 5.20, S, 11.88. Found: C, 53.48; H, 5.58; N, 5.16, S, 1I.92.

2.4: General Procedure for the Synthesis of Benzenesulphonamides (1b)

Using a 100 ml Erlenmeyer flask, amino acid (12.5 mmol) was dissolved in water (15 ml) and sodium carbonate (2.79 g, 26.25 mmol) was added with continuous stirring to ensure complete dissolution of the solutes. The clear solution was cooled to 0 oC followed by the addition of benzenesulphonyl chloride (2.86 g, 15 mmol) in three portions for one hour interval. Then the slurry was stirred for four hours at room temperature and the reaction mixture was acidified to pH of 2 with 2M hydrochloric acid (HCl) to facilitate crystallization. It was allowed to settle down for at least twelve hours and the product was separated through suction filtration. The filtered crude product was washed with tartaric acid (pH2.2) and dried to obtain benzene sulphonamide in good to excellent yields (61-98.5%).

2.4.1: 1-(Phenylsulphonyl)pyrrolidine-2-carboxylic acid(1b)

The amino acid used was proline, yield 3.13 g (95.8%), mp 74-76 oC.IR(KBr)cm-1 : 3065(CH aromatic), 2981(O-H of COOH), 1722(C=O of COOH), 1334, 1155(S=O two bands), 689(Ar-H). 1H-NMR(CDCl3, 400MHz)δ : 10.09-9.98(s, 1H,OH of COOH), 7.82-7.79(d, J=8.77Hz, 2H,Ar-H), 7.58-7.49(m, 3H, Ar-H), 4.79-4.78(d, J=5Hz, 1H, CH-COOH), 3.78-3.75(d, J =10Hz, 1H, CH of CH2-N), 3.25-3.19(d, J1 =2.8Hz, J2=10 Hz, H, CH of CH-N), 2.19-2.15(m, IH, CH), 1.73-1.68(m, 3H, CH2 and CH), 1.49-1.44(m, H, CH), 1.35-1.26(m, H,CH). 13C-NMR(DMSO-d6)δ : 170.5(C=O), 143.8(2CH,2C-5), 134.9(2CH, 2C-3), 129.7(CH, C-4), 127.4(C,C-2), 67.3(CH2, C-7), 60.7(CH2, C-8), 48.8(CH2, C-9), 30.6(CH, C-6). Anal.calcd. for C11H12NO4S (254.12): C, 51.94, H, 4.72, N, 5.51. Found: C, 51.89, H, 4.40, N, 5.46.

2.5: Chlorination and Ammonolysis of Acetylated *p*-Toluenesulphonamides

Chlorination

A three necked 250 ml flask equipped with magnetic stirring bar was charged with the appropriate acetylated *p*-toluenesulphonamide (1 mmol), acetone (10 ml) and thionyl chloride (2 ml). The three necked flask was stoppered and cooled to 0 oC. Then the resulting mixture was stirred at 80oC under reflux for three hours, after which it was transferred to water bath at 80oC to evaporate excess thionyl chloride. Addition of acetone (20 ml) and evaporation was carried out twice to ensure complete evaporation to afford acid chloride.

Ammonolysis

The resulting acid chloride obtained from chlorination above was dissolved in acetone (20 ml) and cooled to 0-5 oC. On addition of ammonia (2 ml), crystallization occurred and the mixture was allowed to stay for three hours or overnight after which it was filtered and washed with acetone to afford the appropriate *p*-toluenesulphonamide.

2.5.1: 1-[(4-methylphenyl)sulfonyl]pyrrolidine-2-carboxamide(1c)

Yield 3.28 g (94.9%), mp.204-205 oC, IR (KBr)cm-1: 3347 (N-H), 2929 (C-H aliphatic), 1992 (C-H aromatic), 1680(C=O), 1534 (C=C), 1397, 1181 (2S=O), 1118(SO2-NH), 1088(C-N), 741 (ArH). 1H-NMR (CDCl3, 400 MHz) δ: 7.716-7.710 (d, J = 2.4 Hz, 2H, Ar-H), 7.693-7.680 (d, J = 5.2 Hz, 2H, Ar-H), 4.167- 4.137 (m, 2H, NH2), 3.519-3.467 (m, 2H, CH2-N) 2.354 (s, 3H, CH3-C=O), 2.221 (s, 3H, CH3-Ar), 1.88-1.76(d, 2H, CH2-CH2), 1.58(s, H, CH). 13C-NMR (CDCl3, 400 MHz) δ: 89

176.332 (C=O), 143.6533, 134.260, (aromatic carbons), 47.401, 37.082, 26.763, 21.609, 18.906 (aliphatic carbons).

2.6: Chlorination and Ammonolysis of Acetylated Benzenesulphonamides

Chlorination

A three necked 250 ml flask equipped with magnetic stirring bar was charged with the appropriate acetylated benzene sulphonamide(1mmol), acetone (10 ml) and thionyl chloride (2 ml). The three necked flask was stoppered and cooled to 0 ^{o}C. Then the resulting mixture was stirred at 80 oC under reflux for three hours, after which it was transferred to water bath at 80 oC to evaporate excess thionyl chloride. Addition of acetone (20 ml) and evaporation was carried out twice to ensure complete evaporation to afford acid chloride.

Ammonolysis

The resulting acid chloride obtained from chlorination above was dissolved in acetone (20ml) and cooled to 0 oC. On addition of ammonia (2 ml), crystallization occurred and the mixture was allowed to stay for three hours or overnight after which it was filtered and was with acetone to afford the appropriate benzene sulphonamide.

2.6.1: 1-(Phenylsulfonyl)pyrrolidine-2-carboxamide(1d)

Yield 3.27g (95.7%), mp.218-219 0C, IR (KBr)cm-1: 3198(N-H), 2978(C-H aliphatic), 1915 (C-H aromatic), 1718 (C=O), 1330, 1196 (2S=O), 1168(SO2NH), 1155(C-N), 719 (Ar-H). 1HNMR (CDCl3, 400 MHz) δ: 7.432 (d, 2H, ArH), 6.253-6.247 (d, J=2.4Hz, 2H, ArH), 6.123(s, H, Ar-H), 4.227- 4.137 (s, 2H NH2), 3.467 (s, 2H, CH2-N), 2.454 (s,3H, CH3-C=O), 1.661-1.544 (m, H, CH), 1.619-1.607 (d, J= 4.8, 3H, CH3-CH). 13C-NMR (CDCl3, 400MHz) δ: 173.221 (C=O), 137.273, 137.167, 133,881, 133.691, 128.766, 128.539 (aromatic carbon), 27.431, 27.120, 26.801. 20.542 (aliphatic carbon). Anal.calcd.(%) for C11H14N203S (254.31). C:51.91, H:5.51, N; 11.01, S:12.58. Found:C; 51.89, H: 5.49, N; 11.04, S:12.61

2.7 Synthesis of *p*-Toluenesulphonamide Derivatives *via* Nickel CatalysedAmidation.

This synthesis involves the preparation of the coordination compound, bis(triphenylphosphine)nickel(II)chloride, and Buchwald-Hartwig coupling of the sulphonamides with aryl and heteroaryl compounds to afford the various sulphonamide derivatives.

2.7.1 Preparation of Bis(triphenylphosphine)nickel(ii)chloride

Using L.M Venanzi [28] procedure, this coordination compound was prepared by dissolving nickel(II)chloride hexahydrate catalyst (2.37 g, 10 mmol) in distilled water (2 ml) followed by dilution with glacial acetic acid (50 ml) and addition of triphenylphosphine ligand (5.25 g, 20 mmol) dissolved in 25 ml glacial acetic acid. The green precipitate formed was allowed to be in contact with the glacial acetic acid solution for 24 hrs. A dark blue crystal (the complex compound) was obtained on filtration, washed with glacial acetic acid and dried in a desiccator.

2.7.2 Procedure for the Synthesis of *p*-Toluenesulphonamide Derivatives

The complex compound bis(triphenylphosphine)nickel(II)chloride (6.54 g, 10 mmol) and triphenylphosphine (5.25 g, 30 mmol) were both introduced into a 50 ml Erlenmeyer flask. The solvent *t*-butanol (4 ml) and distilled water (2 ml) were added with the help of a syringe and the mixture was stirred for 10mins at room temperature under inert nitrogen atmosphere. The mixture was heated at 80 oC for 1.5 min. Then *p*-toluenesulphonamide (293a-g)(10 mmol), potassium carbonate, K2CO3 (1.38 g,10 mmol), substituted aryl and heteroaryl halides were added to the mixture with *t*-butanol and H2O in 2:1 ratio under nitrogen atmosphere. The mixture was subjected to refluxing and stirring for one hour at temperature range 100-110 oC. The mixture was allowed to cool to room temperature, then diluted with ethyl acetate and washed with water to afford *P*-toluene sulphonamide derivative in good to excellent yield.

2.7.3: *N*-(4-Aminophenyl)-1-[(4-methylphenyl)sulfonyl]pyrrolidine-2-carboxamide(1e)

Yield 2.88 g (88.9%), mp.85-86 oC, IR (KBr) cm-1: 3450, 3400 (2N-H), 3063(C-H aliphatic), 1949(C-H aromatic) 1757, 1689(2C=O), 1680, 1678 (C=C), 1326, 1155 (2S=O), 1088 (C-N), 741 (Ar-H). IH-NMR (CDCl3, 400 MHz) δ: 7.788-7.746 (d, 2H, ArH), 7.510-7.474 (d, 2H, ArH), 7.448-7.430 (d, J=7.2 Hz, 2H, ArH), 7.216-7.201 (m, 2H, ArH), 6.320(s, H, CH2 N), 4.987(s, 2H, NH2) 3.678(s, H, NH), 2.114-1.964(m, 2H, CHa of CH2), 1.950- 1.837(m, 2H, CHb of CH2) 1.509- 1.468 (m, H, CH). 13CNMR (CDCl3, 400 MHz)δ: 170.687(C=O), 143.913, 137.205, 132.895, 130.269, 129. 169, 128.364, 127.416, 125.852 (aromatic carbons), 37.461, 37.143, 26.824, 21.639, 20.853, 18.937 aliphatic carbons. Anal.calcd.(%) for C18H21N304S2 (407.51): C: 53.00, H:5.19, N10.31 S;15.71. Found: C: 53.04, H: 5:21, N:10:28, S:15.73.

2.8 Synthesis of Benzenesulphonamide Derivatives *via* Nickel CatalysedAmidation.

This synthesis involves the preparation of the coordination compound, bis(triphenylphosphine)nickel(II)chloride, and Buchwald-Hartwig coupling of the sulphonamides with aryl and heteroaryl compounds to afford the various sulphonamide derivatives.

2.8.1 Preparation of Bis(triphenylphosphine)nickel(ii)chloride

Using L.M Venanzi [28] procedure, this coordination compound was prepared by dissolving nickel(II)chloride hexahydrate catalyst (2.37 g, 10 mmol) in distilled water (2 ml) followed by dilution with glacial acetic acid (50 ml) and addition of triphenylphosphine ligand (5.25 g, 20 mmol) dissolved in 25 ml glacial acetic acid. The

green precipitate formed was allowed to be in contact with the glacial acetic acid solution for 24 hrs. A dark blue crystal (the complex compound) was obtained on filtration, washed with glacial acetic acid and dried in a desiccator.

2.8.2 Procedure for the Synthesis of Benzenesulphonamide Derivatives.

The complex compound bis(triphenylphosphine)nickel(II)chloride (6.54 g, 10 mmol) and triphenylphosphine (5.25 g, 30 mmol) were both introduced into a 50 ml Erlenmeyer flask. The solvent *t*-butanol (4 ml) and distilled water (2 ml) were added with the help of a syringe and the mixture was stirred for 10 mins at room temperature under inert nitrogen atmosphere. The mixture was heated at 80 oC for 1.5 min. Then benzenesulphonamide(293a-g)(10 mmol), potassium carbonate, K2CO3 (1.38 g,10 mmol), substituted aryl and heteroaryl halides were added to the mixture with *t*-butanol and H2O in 2:1 ratio under nitrogen atmosphere. The mixture was subjected to refluxing and stirring for one hour at temperature range 100-110 oC. The mixture was allowed to cool to room temperature, then diluted with ethyl acetate and washed with water to afford benzene sulphonamide derivative in good to excellent yield.

2.8.3*: N*-(4-Aminophenyl)-1-[(phenylsulfonyl)]pyrrolidine-2-carboxamide (1f)

Yield 3.28 g (95.8%), mp.120-121 oC,IR (KBr)cm-1: 3450, 3369 (2N-H),3063(C-H aliphatic), 1949(C-H aromatic) 1715, 1690(2C=O), 1580 (C=C), 1326, 1155 (2S=O), 1088 (C-N), 741 (Ar-H). IH-NMR (CDCl3, 400 MHz) δ: 7.788-7.746 (m, 2H, ArH), 7.510-7.474 (m, 2H, ArH), 7.448-7.430 (d, J=7.2 H2, 2H, ArH), 7.216-7.201 (m, 2H, ArH), 6.320(s, H, CH2 N), 2.114-1.964(m, 2H, CHa of CH2), 1.950- 1.837(m, 2H, CHb of CH2) 1.509-1.468 (m, H, CH). 13CNMR (CDCl3, 400 MHz)δ: 176.687, (C=O), 143. 913, 137.205, 132.895, 130.269, 129. 169, 128.364, 127.416, 125.852 (aromatic carbons), 77.461, 77.143, 76.824, 61.639, 48.853, 30.937, 30.906, 28.987 aliphatic carbons.

2.9 General Synthesis of *p*-Tolunesulphonamide Derivatives of 5-Chloro-4,6- diaminopyrimidineThe complex compound bis(triphenylphosphine)nickel(II)chloride (6.54 g, 10 mmol) and triphenylphosphine (5.25 g, 30 mmol) were both introduced into a 50 ml Erlenmeyer flask. The solvent *t*-butanol (4 ml) and distilled water (2 ml) were added with the help of a syringe and the mixture was stirred for 10 mins at room temperature under inert nitrogen atmosphere. The mixture was heated at 80 oC for 1.5 min. Then *para*-toluenesulphonamide (293a-g)(10mmol), potassium carbonate, K2CO3 (1.38 g,10 mmol), 5-chloro-4,6-diaminopyrimidine was added to the mixture with *t*-butanol and H2O in 2:1 ratio under nitrogen atmosphere. The mixture was subjected to refluxing and stirring for one hour at temperature range 100-110 oC. The mixture was allowed to cool to room temperature, then diluted with ethyl acetate and washed with water to afford *para*-toluenesulphonamide derivatives of 5-chloro-4,5-diaminopyrimidine in good to excellent yield.

2.9.1: *N*-(4, 6-Diaminopyrimidin-5-yl)-1-[(4-methylphenyl)sulfonyl]pyrrolidine-2-carboxamide(1g)

Yield 2.97 g (90.7%), mp.94-95 oC, IR(KBr)cm-1: 3469,3405, 3300(3N-H), 3063 (C-H aliphatic), 1949 (C-H aromatic), 1725,1689(2C=O), 1660, 1658(C=C), 1653, 1645 (C=N), 1326, 1155 (2S=O) , 1025(C-N), 741 (Ar-H). 1H-NMR (CD3CN, 400 MHz) δ: 7.376 (m, 2H, ArH), 7.078(s, 2H, ArH), 6.865(m, H, ArH), 5.415 (m, H, NH), 5.274 (s, 2H, NH2), 2.40(s, H, CH-C=O), 1.955(CH3-Ar). 13C-NMR (CD3CN, 400 MHz) δ: 170.330, 169.654(CO), 166.564, 164.437(C=N), 143.606, 137.311, 133. 608, 133.403, 128.963, 128.690, 117.361, 116.786, 115.674 (aromatic carbon), 40.688, 28.689, 26.601, 25.300, 19.564. Anal.calcd.(%) for C16H20N603S (376.43): C:51.00, H:5, 36, N, 38.79 S: 8.50. Found: C: 51.03, H:5.38, N, 38.8

Scheme 1:Proline Derived Sulphonamide Derivatives

2.9.2 Biological Studies

2.9.3 Antimicrobial Studies

Preparation of media

Nutrient agar: A 28g of nutrient agar powder was dissolved in 1000ml of distilled water, and was allowed soaking for 10mins. The agar suspension was brought to melt by boiling in a water bath. A 20ml aliquot of the molten nutrient agar was dispensed into bijou bottles, cocked, and sterilized in an autoclave at 121oC for 15mins. The sterile molten nutrient agar was stored at 42oC until use.

Potato Dextrose Agar (PDA): A 47g of PDA powder was dissolved in 1000ml of distilled water, and was allowed soaking for 10mins. The agar suspension was brought to melt by boiling in a water bath. A 20ml aliquot of the molten PDA was dispensed into a bijou bottles, cocked, and sterilized in an autoclave at 121oC for 15mins. The sterile molten potato dextrose agar was stored at 42oC until use.

The Test microorganisms used: The test microorganisms used (S*taphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, and Aspergillusniger*) were clinical isolates obtained from the department of pharmaceutical microbiology and biotechnology laboratory, University of Nigeria, Nsukka.

Standardization of the test organism suspension: The organisms were standardized using 0.5 McFarland turbid equivalents.

Preparation of the different concentration of the extract used: A 5mg/ml stock concentration of the crude extract was prepared by dissolving 10g of the extract in 2ml of 50% DMSO. 1.0mg/ml, 0.9mg/ml, 0.8mg/ml, 0.7mg/ml, 0.6mg/ml, 0.5mg/ml, 0.4mg/ml, 0.3mg/ml, 0.2mg/ml, 0.1mg/ml, concentrations were obtained using C1V1=C2V2 formula. Where C1 (initial concentration) =5mg/ml

V1 (Initial volume) = X

C2 (final concentration) = 1.0mg/ml

V2 (final volume) = 20ml,

Control test (standard): The standard antibiotic used was Ofloxacin, Ciprofloxacin and Fluconazole.

Experimental: 4.0ml of sample suspension of stock concentration 50mg/ml was transferred to the sterile Petri dish, a 16.0ml volume of double strength sterile molten agar was transferred to the same plate to mix uniformly thus, 1mg/ml concentration was obtained. The other concentrations 0.9mg/ml, 0.8mg/ml, 0.7mg/ml, 0.6mg/ml,

0.5mg/ml, 0.4mg/ml, 0.3mg/ml, 0.2mg/ml, 0.1mg/ml, were obtained using the same C1V1=C2V2 formula. The molten agar plates with different concentrations of the sample were allowed to gel. The plates were divided into seven equal parts with permanent marker. The test microorganisms were streaked on the segments, and labeled. The culture plates were incubated in inverted position at 37oC for 24hours, and at 25oC for 48hours. After the due period of incubation, the plates were observed for sensitivity and resistivity of the organisms to the agents, and the observation was recorded. The plates were further incubated for another 24hour at 37oC, and 48hours at 25oC to determine whether the activity was bactriostatic or bactericidal. The observation was also recorded.

2.9.4 Antioxidant activity by DPPH method

The antioxidant behaviour of the synthesized compounds were measured *in vitro* by the inhibition of generated stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The DPPH solution was prepared by dissolving 1.9 mg of DPPH in 100 ml of methanol. Three different concentrations (50, 100 and 200 µg/ml) of the DPPH soluion were prepared. 2 mg of each of the synthesized compounds was weighed out and dissolved in 10 ml of appropriate solvent. The stock solution (200 µg/ml) was diluted further to get 100 and 50 µg/ml for each of the samples. The standard solution of ascorbic acid was prepared in similar manner. 1 ml of DPPH solution was added to 2 ml solution of the samples and ascorbic acid. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured (in triplicate) spectrophotometrically at 517 nm against the corresponding blank solution. The percentage scavenging DPPH radical inhibitions were calculated by using the DPPH radical scavenging activity (%) formula.

III. RESULTS AND DISCUSSION

3.1 Antimicrobial Activities

Table 1: Minimum inhibitory concentration (mg/ml) of compounds 1a-1g

Sample no	*E.coli*	*S.typhi*	*S.aureus*	*B. sub*	*Ps.aerug*	*C.albicans*	*A. niger*
1a	0.80	0.70	0.90	0.80	0.80	-	0.90
1b	0.50	0.70	0.90	0.70	0.70	-	0.90
1c	0.40	0.70	0.90	0.50	0.70	-	0.80
1d	0.40	0.70	0.90	0.50	0.70	-	0.80
1e	0.80	0.80	0.70	0.80	-	-	
1f	0.40	0.30	0.30	0.40	-	-	-
1g	0.80	0.90	0.50	0.50	0.70	0.80	-
Ofloxacin	0.05	0.01	0.01	0.02	0.25	-	-
Fluconazole	-	-	-	-	-	0.02	0.05

Key: - implies no activity. Ofloxacin was the antibacterial reference drug and Fluconazole was the antifungal standard drug used.

The antimicrobial Studies (Tables 1) revealed that compounds **1a-1g** exhibited good microbial (antibacterial and antifungal) activities when compared to commercial standard drugs. It was also observed that compounds 1c, 1d and 1f were the most potent against *E coli*, 1f was the most active compound against *S. typhi*, *S. aureus* and B. sub. Although *Ps aerug* was the most recalcitrant bacteria for 1e and 1f, however other compounds inhibited its growth and the presence of amino acid and other coupling partners could be responsible for this[29-30]. Most of the compounds did not perform well as antifungal agents as only 1g exhibited inhibitory activity against *C. albicans*.In summary, this suggests that these new sulphonamide derivatives are potential antibacterial and antifungal agents.

3.2 Antioxidant studies

Table 10: Antioxidant activities results

200 µg/ml		100 µg/ml		50 µg/ml		IC50 µg/ml	
Sample	**% inhibition**	**Std**	**% inhibition**	**Std**		**% inhibition**	**Std**
Ascorbic acid	96.83	0.001	97.68	0.001	97.31	0.001	0.999
1a	91.64	0.001	84.25	0.000	80.53	0.001	1.159
1b	83.15	0.001	54.40	0.001	36.69	0.001	1.703
1c	93.28	0.001	93.53	0.001	92.06	0.001	1.151
1d	67.77	0.002	46.09	0.002	48.90	0.001	1.840

1e	75.09	0.000	51.04	0.001	48.17	0.001	1.715
1f	83.15	0.001	54.40	0.001	36.69	0.001	1.703
1g	77.84	0.002	84.86	0.002	86.63	0.003	1.178

The *in vitro* Antioxidant Studies (Table 10) showed that some of the tested compounds had antioxidant activities. Compounds **1a-1g,** showed impressive antioxidant activities. Compounds **1a** (IC50: 1.159 µg/ml) and **1g** (IC50: 1.178 µg/ml) had the best antioxidant activity, however they are lower than that of the standard ascorbic acid (IC50: 0.999µg/ml µg/ml). This suggests that compounds **1a** and **1g** were the most potent antioxidant agent and therefore further derivatization of these compoundsis necessary to improve their antioxidant properties.

IV. CONCLUSION

In conclusion, the synthesis of proline derived sulphonamide derivatives using substituted *p*-toluenesulphonyl and benzenesulphonyl chlorides was facile and efficient. The synthesized compounds were characterized using FTIR, 1HNMR, 13CNMR and the spectra were in agreement with the structures assigned. The antimicrobial study revealed that most of the synthesized compounds possess antimicrobial activities with compounds **1g** being the only compound with excellent antifungal activities against *C. albicans*. Compounds **1c**, **1d** and **1f** were the most potent against *E coli*. The *in vitro* antioxidant studies showed that compounds **1a** and **1g** possess excellent antioxidant activities.

References

[1]. Jos, WM. Vander Meer. The infectious disease challenges of our time. *Front Public Health*. 2013;1:7

[2]. Halliwell, Barry. "Oxidative stress and cancer: have we moved forward? *Biochem.J*.2007; 401(1):1-11.

[3]. Egbujor, M.C., and Okoro, U.C. New methionine-based *p*-toluenesulphonamoylcarboxamide derivatives as antimicrobial and antioxidant agents: Design, synthesis. *JPRI*, 2019; 28(1), 1-12, *Article no.JPRI .49472*.

[4]. Egbujor, M.C.; Okoro, U.C.; Okafor, S. Design, synthesis, molecular docking, antimicrobial and antioxidant activities of new phenylsulfamoyl carboxylic acids of pharmacological interest.*Med. Chem. Res.,* **2019**, *28,* 2118-2127.

[5]. Egbujor, Melford. C, Okoro, Uchechukwu. C, Okafor Sunday**,** Nwankwo, N.E, "Design, Synthesis and Molecular Docking Of Novel Serine-Based Sulphonamide Bioactive Compounds As Potential Antioxidant And Antimicrobial Agents**",***Indo Am. J. P. Sci*, 2019; 06(06):12232-12240.

[6]. Egbujor, M.C.; Okoro, U.C.; Okafor, S.N.; Amasiatu, I.S.; Amadi, U.B.; Egwuatu, P.I. Synthesis, molecular docking and pharmacological evaluation of new 4-methylphenylsulphamoyl carboxylic acids analogs. *Int. J. Res. Pharm. Sci*, **2020**, *11*(4), 5357-5366.

[7]. Egbujor, M.C.; Okoro, U.C.; Nwobodo, D.C.; Ezeagu, C.U.;Amadi, U.B.; Okenwa-Ani, C.G.; Ugwu, J.I.; Okoye, I.G.; Abu,I.P.; Egwuatu, P.I. Design, synthesis, antimicrobial and antioxidanactivities of novel threonine-based sulfonamide derivatives. *J.Pharm. Res. Int.*, **2020**, *32*(8), 51-61

[8]. Egbujor, MC, Okoro UC, Egu SA, Egwuatu PI, Eze FU, Amasiatu IS. Synthesis and biological evaluationof alanine derived bioactive *p*-toluenesulphonamide analogs. Int J Res Pharm Sci. 2020;11(4):6449-6458.

[9]. Egbujor MC, Okoro UC, Okafor S. Novel alaninebased antimicrobial and antioxidant agents: synthesis and molecular docking. Indian J Sci Technol.2020;13(09):1003-1014.

[10]. Egbujor, M.C.; Okoro, U.C.; Okafor, S.N.; Egu, S.A.; Amasiatu, I.S.; Egwuatu, P.I.; Umeh O.R. and Ibo, E.M.Design, synthesis, and molecular docking of cysteine-based sulphonamide derivatives as antimicrobial agents*Res. Pharm. Sci.,* 2021; **17(1)**: 99-110.

[11]. Mardirossian N, Sola R, Beckert B, Collis W,P, Stasi A.D, Armas F, Hilpert K, Wilson D.N, Scocchi M. Proline-rich peptides with improved antimicrobial activity against E Coli, K pneumonia, and A. baumannii. ChemMedChem., 2019, 14(24): 2025-2033

[12]. Naliwajski M, Sklodowska M. The relationship between the antioxidant system and proline metabolism in the leaves of cucumber plants acclimated to salt stress. Cells 2021, 10(3): 609

[13]. Egbujor, M.C.; Nwobodo, D.C.; Egwuatu, P.I.; Abu, I.P.; Ezeagu,C.U. Sulphonamide drugs and pseudomonas aeruginosaresistance:A review. *Int. J. Modern Pharm. Res.*, **2020**, *4*(1), 78-83.

[14]. Raul, SJ, More, AH , Mahajan, SS, *Intl. J. of Research in Pharmacy and Chemistry*. 2011;**1(4):** 991.

[15]. Hans, FC, Jawetz, E. *Basic and Chemical Pharmacology*, Katzung, B.G (ed) Appletonlange 1998; 763.

[16]. Yelland MJ, Nikles CJ, McNairn N, Del Mar CB, Schluter PJ *Rheumatology.* 2007;**46:** 140.

[17]. Verhaeghe P, Azas N, Gasquet M, Hutter S,. Ducros C. *Bioorg. Med. Chem. Lett*. 2008; **18**: 401.

[18]. Egbujor, M.C.; Okoro, U.C.; Okafor, S.; Nwankwo, N.E. Synthesis, characterization and *in silico*studies of novel alkanoylated 4-methylphenyl sulphonamoyl carboxylic acids as potential antimicrobial and antioxidant agents. *Intern. J. Pharm. Phytopharm. Res*, **2019**, *9*(3), 89-97.

[19]. Egbujor, M.C.; Egu, S.A.; Okonkwo, V.I.; Jacob, A.D.; Egwuatu,P.I.; Amasiatu, I.S. Antioxidant drug design: Historical and recent developments. *J. Pharm. Res. Int.*, **2021**, *32*(41), 36-56.

[20]. Shet PM, Vaidya VP, Mahadevan KM, Shivananda MK, Sreenivasa S and Vijayakumar GR. Synthesis,Characterisation and antimicrobial Studies of novel Sulphonamides containing substitutednaphthofuroyl group. *Research Journal of Chemical Sciences.* 2013; 3(1): 15-20.

[21]. JU, Y, Kuma, D and Verma, R.S, "Revisiting nucleophilicsubstifation reactions: microwave- assisted synthesis of azides, thiocyanats and sulfones in an aqeous medium". *Journal of organic chemistry*.2006; 71(17): 6697-6700.

[22]. JU.Y and Verma. R.S. "Aqeous N hererocylization of primary anuries and hydrazines with dihalids microwave-assistant synthesis of N-azacycloalkanes, Isoindole, Pyrazole, Pyrazolidine and Phthalazine derivatives" *Journal of organic chemistry.* 2006; 71(1):135-141.

[23]. Parke, D.V. The Biochemistry of foreign compounds. Oxford: Pergamon press, 1968; 224.

[24]. Mackison, FW, Stricoff RS and Partridge,LJ . JR. (eds). NIOSH/OSHA. – Occupational Health Guideline for chemical Hazards. Pub No 81-123 (3vols). Washington, D.C US Government Printing office. 1981; 3.

[25]. Browning E. Toxicity and metabolism of Industrial Solvents: New York: *American Elsevier*. 1996; 304.

[26]. Lewis, RJ. Sr (Ed). Hawley's Condensed Chemical Dictionary.13.ed. New York, NY: John Wiley & Sons Inc. 1997; 42.
[27]. Jain KS, Chitre, TS, Miniyar PB et al. Biological and medical significance of Pyrimidine" *Current Science*. 2006; 90(6):793-803.
[28]. Venanzi LM .Tetrahedral Nickel (11) complexes and the factorsdetermining their formation. *Journal of Chemical Society.*1958; 719-724.
[29]. Egbujor, M.C.; Okoro, U.C.; Egu, A.S.; Okonkwo, V.I.; Okafor, S.N.; Emeruwa, C.N.; Egwuatu, P.I.; Umeh, O.R.; Eziafakaego, M.I.; Amasiatu, I.S and Nwobodo, D.C..Synthesis and Biological Evaluation of SulfamoylCarboxamide Derivatives from Sulfur-containing α-Amino acids. *Chiang Mai J. Sci*., 2022; 49(4): 1100-1115.
[30]. Egbujor M.C., Okoro U.C., Emeruwa C.N.,Umeh O.R., Eziafakaego M.I., Egwuatu P.I., et al., Synthesis of Sulphonamides Using Threonine, and Evaluation of Their Biological Activities; in Rizvi S.A.A., ed., Challenges and Advances in Pharmaceutical Research Vol. 2, P.B international, India, 2022: 95–107