Synthesis, Characterisation and Antimicrobial Activities of Sulphonamides from Branched Chain Amino Acids

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Abstract- ***This research explores the synthesis of sulphonamide compounds from branched chained amino acid (BCAAs), their characterization and antimicrobial activities. The procedure involved a mild reaction of branched amino acids including valine, leucine and isoleucine with benzenesulfonyl chloride in an aqueous basic medium. The characterization of the compounds was carried out using infra-red (IR), nuclear magnetic resonance (^{1}HNMR) and ultraviolet (UV-Vis) spectroscopies. The synthesized compounds were tested for their antimicrobial activities against Staphylococcus aureus, Escherichia coli, and Candida albicans. They were found to be potential antimicrobial agents.***

I. INTRODUCTION

Owing to its prior success in the areas of pharmaceutical sciences and medicinal chemistry, the synthesis of novel sulphonamides has attracted increased interest from researchers [1]. The world of medical sciences has undergone a revolution thanks to the development of sulfa drugs with sulfonamide functional groups that have numerous biological functions. Sulphonamides restrict folic acid, a crucial molecule for the synthesis of bacterial DNA and RNA, and a lack of tetrahydrofolate reduces the formation of new DNA and RNA, which ultimately causes the decay of the bacterium. Because bacteria mistakenly attempt to convert sulfonamide instead of p-amino benzoic acid for the manufacture of folic acid, normal proliferation of microorganisms is impeded. Sulphonamides are effectively used as antibiotics as a result of these actions [2].Sulphonamides being an active class of synthetic bacteriostatic antibiotics is still in use today to treat bacterial infections as well as other illnesses caused by various microorganisms. Before the development of penicillin in 1941, they were the main form of therapy for bacterial infections[3]. Additionally, several therapeutically utilized medications like diuretics, carbonic anhydrase inhibitors, and anti-epileptics contain the main sulfonamide portion. Sulphonamides are antimicrobial medications with a wide range of activity that work well against both Gram-positive and some Gram-negative bacteria [4-9].

The three branched-chain amino acids (BCAAs), leucine, isoleucine, and valine, are among the essential amino acids (figure 1). They were given this term for their branched rather than linear aliphatic side chains. Meat, fish, dairy products, and eggs are among the food sources high in BCAAs. They are very vital since the body cannot produce them and must instead get them from the diet. They are essential for the creation of neurotransmitters and proteins. Additionally, non-essential amino acids like glutamine and alanine can be synthesized from BCAAs, which are crucial sources of nitrogen. Additionally, BCAAs are said to protect lean body mass during weight loss, enhance wound healing, encourage muscle protein anabolism in muscle wasting with aging, and have positive effects in the context of renal failure [10]

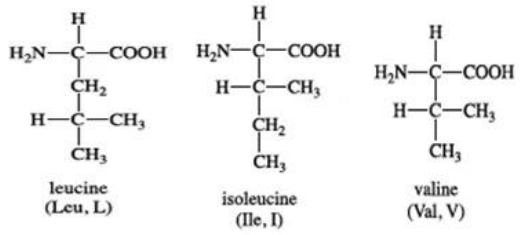


Fig.1 Structures of leucine, isoleuine and valine

According to Brosnan*et al.* [11], surfactant protein B contains 37% BCAAs. This protein's amphipathic

helices, which have positively charged groups interacting with lipid head groups and branched-chain residues interacting with acyl chains in lipids, are its most distinctive structural feature. In order to interact with the hydrocarbon chains of fatty acids, membrane proteins need hydrophobic amino acids in their transmembrane domains. For instance, the 19 residues of the -helix that crosses the membrane of the protein glycophorin, which is found in the membrane of red blood cells, include a total of eleven BCAAs [12].

These branched amino acids exhibit significant antimicrobial activities. For instance, leucine has good antibacterial properties, as shown by Sidney et al. 1944's discovery that d-leucine inhibits the growth of Lactobacillus arabinosus strains [13]. The antibiotic productivity of ramoplanin, a glycolipodepsipeptide obtained by fermenting Actioplanes sp. ATCC 33076, was also reported to be enhanced by the addition of leucine [14]. Leucine was also said to have a positive impact on the antibiotic bitespiramycin's 4"-O-acylspiramycin production [15]. When exposed to antimicrobial screening by the agar diffusion method, Asemave et al. 2013 [16] found that leucine complexes of Fe(III) and cu(11) demonstrated stronger antibacterial activity than respective salts. Other branched-chain amino acids, including leucine, are also known to have antioxidant properties. Furthermore, according to Hu *et al*. [17], the biological function and peptide-membrane interactions of antimicrobial peptides from the bactericidal domain of AvBD4 are affected by isoleucine. Findings have shown that the majority of the complexes, and especially Co (II) and Zn (II), derived from isoleucine have significantly increased activity against Staphylococcus aureus, Escherichia coli, and Klebsiella pneumonia [18]**.** Similarly, valine exhibits antimicrobial activity. Valine treatment increases the production of antimicrobial components in goat milk and mammary epithelial cells without affecting the tight junction barrier [19].Egbujor and co-workers have reported that the use of amino acids such as alanine [20-21], proline [22], cysteine [23], threonine [24], methionine [25] and serine [26] in the synthesis of sulphonamides helps in improving their antimicrobial potency. Thus, this work was aimed at synthesizing sulphonamides using branched amino acids and exploring their antimicrobial activities.

II. MATERIALS AND METHODS

2.1 MATERIALS

The Chemicals used in the present work were of analytical grade purchased from Springboard Chemical Labouratory and used without further purification to synthesize desired compounds. Reagents used were benzene sulphonyl chloride, sodium bicarbonate (1M), hydrochloric acid (2M), isoleucine, leucine, valine, distilled water, ethanol, magnesium sulphate, ciprofloxacin, fluconazole, dimethyl sulphoxide (DMSO), nutrient agar, dextrose broth, deutrated methanol (MeOD). Apparatus/equipment used were magnetic stirrer, beakers, round bottom flask, buchner funnel, filter paper, dropper, weighing scale, desiccator, petri dish, test tubes, incubator , autoclave, UV.visible spectrometer, NMR, alpha FT-IR spectrometer.

2.2 GENERAL PROCEDURE FOR SYNTHESIS

Valine (a), Leucine(b),and isoleucine (c), each weighing 5g, were properly dissolved in distilled water using a magnetic stirrer to achieve total solute dissolution. The answer. The aforementioned solution was correctly weighed, 60ml of benzene sulphonyl chloride (2) was carefully added, and the mixture was carefully agitated for 3–4 hours over a magnetic stirrer until the reaction was complete. The elimination of hydrogen was made simpler in an alkaline atmosphere. After the reaction was completed, the pH was changed to 2-3 by adding 2M HCl solution in order to promote crystallization. The precipitates were filtered, washed several times with water, recrystallized with hot ethanol, and dried over anhydrous MgSO4 or in a rotary evaporator to afford branched amino acid-based sulphonamides (2a-2c) in good yield [27-28].

2.3 ANTIMICROBIAL ACTIVITY

Test micro-organisms used

*Escherichia coli, Staphylococcus aureus*and *Candida albicans*were obtained from the Reference Laboratory Section of Conig-Simonne Laboratories, Awka, Anambra State, Nigeria. The organisms were maintained on Nutrient Broth for bacteria and Sabouraud Dextrose broth for yeast (*Candida*) for 24 hours.

Standardization

The test organisms were standardized by picking 3–5 pure cultures of the test microorganism and emulsifying them in 3–4 ml of sterile physiological saline using a sterile wire loop. The test organisms' turbidities were corrected using physiological saline to match the 0.5 McFarland standard's absorbance at the same wavelength. The turbidity reading of the 0.5 McFarland Standard was recorded as absorbance in a spectrophotometer at 540 nm. Note: 1.5x108 CFU are present in 0.5 McFarland.

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

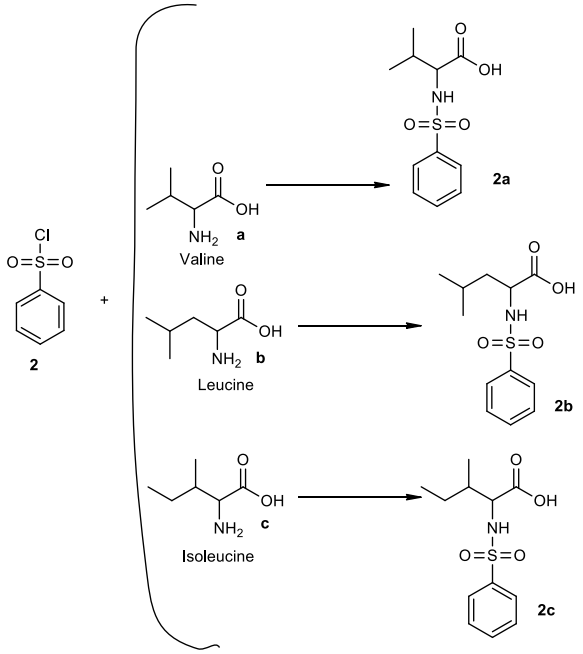
Control test (standard): The standard antibiotic used was Ciprofloxacin and niaxin.

Experimental: 4.0 milliliters of stock concentration sample solution A concentration of 1 mg/ml was achieved by adding 50 mg/ml to a sterile Petri dish and then adding 16.0 mg/ml of double-strength sterile molten agar to the same plate. The C1V1=C2V2 formula was used to calculate the additional values, which 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 molten agar plates with various sample concentrations were allowed to gel. With a permanent marker, the plates were divided into three equal portions. On the segments, test organisms were streaked and tagged. The culture plates were incubated inverted for 24 hours at 37 degrees Celsius and then for 48 hours at 25 degrees Celsius. After the appropriate incubation time, the plates' sensitivity and resistivity were assessed.

III. RESULT AND DISCUSSION

3.1 RESULT

Three new amino acids sulphonamides (2a-2c) were synthesized in aqueous basic media by simple reaction of the amino acids with p-toluene sulphonyl chloride and benzene sulphony with continuous stirring for 3 h and after completion of reaction pH were adjusted at 2-3 by HCl. The products were recrystallized after washing with hot ethanol and dried over magnesium sulphate.



Scheme 1: synthesis of sulphonamides from branched amino acids

3.1.1 SPECTRAL CHARACTERISATION OF COMPOUNDS

3.1.2 3-Methyl-2-(phenylsulfonamido)butanoic acid (2a)

The amino acid used is valine, white crystals were obtained wih 61.6% yield. mp. Uv- Visible λmax ; 266nm. IR(KBr) cm−1 : 3214 (N–H),3429(OH of COOH), 2934 (C–H aromatic), 1796 (C=O of COOH), 1389, 1034 (S=O two bands), 741(Ar–H). ^{1}HNMR, (DMSO, 400 MHz) δ: 7.8443 (d, J = 7Hz, 2H, Ar-H), 7.4656 (d, J = 7Hz, 2H, Ar-H), 3.2952 (m, H, CH of CH_2-N), 1.2591 (m, H, CH_3), 3.1.3: 4-Methyl-2-(phenylsulfonamido)pentanoic acid (2b)

The amino acid used is leucine, white crystals (2b) were obtained with yield: 65.2% .Uv - Visible λ_{max}; 268nm. IR(KBr) cm−1 : 3212 (N–H), 3404 (OH of COOH), 2977 (C–H aromatic), 1707 (C=O of COOH), 1312, 1166 (S=O two bands), 689 (Ar–H). ^{1}HNMR, (DMSO, 400 MHz) δ: 7.9240 (d, J = 7Hz, 2H, Ar-H), 7.4931(d, J = 7Hz, H, Ar-H), 4.6770(s, H,COOH), 3.2301(m, H, CH of CH-N), 1.5315(m, H, CH2).

3.1.4: 3-Methyl-2-(phenylsulfonamido)pentanoic acid (2c)

The amino acid used is isoleucine and the compound 2c was obtained as white crystals with yield: 59.1%. Uv-Visible λ_{max}; 265nm. IR(KBr) cm−1 : 3213 (N–H), 3480(OH of COOH), 2964 (C–H aromatic), 1790 (C=O of COOH), 1376, 1134 (S=O two bands), 683 (Ar–H). ^{1}HNMR, (DMSO, 400 MHZ) δ: 7.9590(d, J = 7Hz, 2H, Ar-H), 7.5533(d, J = 7Hz, H, Ar-H), 4.7660(m, H, CH-COOH), 4.0015(m, CH, CH2-N), 3.3042(m, H, CH2), 1.5607 (m, H, CH).

3.2 ANTIMICROBIAL ACTIVITY RESULT

Table1. Antimicrobial activities of synthesized compounds (Zone of inhibition in mm)

Test organisms	Compound 2a				Compound 2b				Compound 3c				+ve control				-ve control			
	x	y	z	mean±SD	x	y	z	mean±SD	x	y	z	mean±SD	x	y	z	mean±SD	x	y	z	M ± SD
S. aureus	13	12	14	13.00±1.00	14	13	15	14.00±0.41	20	22	18	20.00±2.00	27	28	28	27.67±0.58	-	-	-	-
E. coli	0	0	0	0.00±0.00	25	30	30	30±0.23	18	16	18	17.33±1.15	43	43	43	43.00±0.00	-	-	-	-
Candida albicans	0	0	0	0.00±00	0	0	0	0.00±0.00	17	14	16	15.67±1.53	40	43	41	41.33±1.53	-	-	-	-

3.3 DISCUSSION

A total of three sulphonamides (2a-2c) were synthesized from branched chained amino acids in good yields. Details of reaction conditions are explained in Experimental section and synthetic pathways of sulphonamides are explained in Schemes 1. Measurement of absorption maximum (λmax) provided the justification. The products have additional value of λmax resulting in bathochromic shift to longer wavelength. The synthesized compounds were characterized by FT-IR, the characteristics band at 3404-3480 cm^{-1} of (O-H carboxylic), N-H stretching at 3212-3214cm^{-1} and 1039-1166 cm–1 for (-N- S=O) for all compounds (2a-2c) reveals the formation of sulphonamides. The structures of all the compounds were also confirmed by ^{1}HNMR. ^{1}HNMR spectra of 2a-2c showed a signal at δ 7.9-7.4 ppm for CH of benzene ring while signal at δ 3.9 (broad singlet) for OH (2a) and 5.29-4.76 ppm for NH group of compounds 2a, 2c, and 2b, respectively. The synthesized compounds were also screened for their antibacterial and antifungal activity against gram positive and gram negative bacterial and fungal stains respectively. The antimicrobial and antifungal activities of new sulphonamides (2a-2c) along with reference (ciprofloxacin and fluconazole as standard for antibacterial and antifungal respectively) against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, are reported in Table 1. The effectiveness of antimicrobial and antifungal sensitivity testing is based on the size of the zone of inhibition. The zone of inhibition however, varies with the infusibility of the agent, the size of the inoculums and the type of medium. Among the bacterial strains, the compound 2b has excellent activity against gram negative *Escherichia coli* and modeate activity against *S.aureus*,2a displays moderate activity against gram positive *Staphylococcus aureus* but has no activity against *E coli* while 2c is lethal to both gram positive *Stapphylococcuaureus*and gram negative *E coli*. Other than their excellent activities against respective bacteria, these compounds showed resistant to remaining strains to some extent. It was also noted that compound 2c displayed remarkable inhibitory activity against *Candida albicans* while 2a and 2b had no activity. From the antimicrobial result of these compounds (2a-2c) in Table1, it is concluded that some of the synthesized sulphonamides exhibited good strength and are potent antibacterial and antifungal agents.

CONCLUSION

In conclusion, synthesis ofsulphonamide compounds (2a-2c) from branched chain amino acids benzensulphonyl chloride was successful. The synthesized compounds were characterized using UV-Vis, FT-IR, ^{1}HNMR 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 2c being the only compound with good antifungal activities against *C. albicans* and excellent against *S. aureus*. Compound 2b was the most potent against *E. coli* while compound 2a was only active against S.aureus. The findings demonstrated that compounds 2a and 2b possess excellent in vitro antibacterial while

2c possesses both antibacterial and antifungal activities and therefore potent antibacterial and antifungal agents.

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