Synthesis, Characterization and Antibacterial Activity of 2-Phenylsulphonamide Derivatives of Some Amino Acids

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Abstract. The 2-phenylsulphonamide derivatives of amino acids were synthesis by simple substitution of benzenesulphonylchloride (6) with amino acids (1-5) containing pharmacological active functionalities. Structures of the synthesised compounds (7a-7e) were characterised using FT-IR, NMR (¹H, ¹³C) and elemental analysis. The anti bacterial activities of the synthesised compounds were evaluated against gram positive bacteria: Staph and Streptococcus, gram negative bacteria: E-coli, Klebsiella, Proteus, and pseudomonas using 200 µl of 10 mg/ml and minimum inhibitory concentration (MIC) were also determined. The compounds exhibited effective anti bacterial properties though some are not more active than the standard drug ciprofloxacin.

Introduction

The importance of sulphonamides were first realised when sulphonylamide a key analogue of sulphonamide was reported to be the first antibacterial drug [1]. Sulphonamides represent a large and important class of medicinally important compounds which are extensively used as drugs that have been in continuous use in treating both antibacterial and non antibacterial diseases [2]. Clinically, Sulphonamide drugs have been in use because of their various biological properties such as anti-tumour [3], anti-thyroid [4], hypoglycaemic agent [5], carbonic anhydrase inhibitor [6], anti-inflammatory [7], diuretic agent [8], anti-impotent drugs [9], anti-convulsant [10], anti-cancer [11], anti-retroviral [12], anti-hypertensive [13], and anti-malaria drugs [14]. Today, Sulphonamides could be classed as the lead in the fight against fungi infections in the chemotherapeutic world following the discovery of N- pyridine-2-yl-4-methylphenylsulphonamides [15]. Sulphonamide derivatives such as Schiff bases have been used for several biological applications, for instance as antifungal agents used to inhibit the germination of Colletotrichum gloeosporioides spores on mango [16]. Other sulphonamide derivatives like fluorinated and nitroginated sulphonamides exhibited potent antimicrobial agents [17]. The rapid development of widespread resistance of sulphonamide drugs [18] has called for the research of new potent and toxic free sulphonamide drugs.

In this work we report the synthesis of the 2-phenylsulphonamide derivatives of amino acids of pharmacologically active functionalities and their antibacterial activities. Para-toluene sulphonamide derivatives which were analogues of the synthesised 2-phenylsulphoamide derivatives of amino acids were also recorded as broad spectrum antibiotics because of their antibacterial activities [19].

Materials and methods

General Chemistry

All chemicals were purchased from Sigma Aldrich. IR spectra were recorded with FT-IR (KBr) in cm⁻¹. Melting points were determined with melting point apparatus and are uncorrected.

NMR spectra were recorded at operating frequency of F_1 and F_2 ; 300.1300000MHz for ^1H and F_1 75.4752953MHz, F_2 75.4677490MHz for ^{13}C . Results were presented as chemical shifts δ in ppm, J. Values in Hz, multiplicity are shown as abbreviations; s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).

General procedure for synthesis of 2-phenylsulphonamide derivatives of amino acids (7a-7e) [20].

Na_2CO_3 (5.565g, 52.5 mmol) was added to a solution of amino acids (**1-5**) (25 mmol) in H_2O [30mL] at 0°C , cooled to -5°C followed by addition of benzenesulphonyl chloride (**6**) [3.84ml, 30mmol] in three portions over a period of 1h. The reacting mixture was warmed to room temperature and allowed to stir for 4h. Upon completion of the reaction, which was monitored with TLC using mixture of $\text{CHCl}_3/\text{CH}_3\text{OH}$ [9:1], 20% concentrated aqueous HCl solution was added with continuous stirring to avoid foaming on the surface until the pH of 2 was attained. The solid that was separate out was allowed to settle down over night and isolated via suction filtration. The filtered crude product was washed with tartaric acid of pH 2.2 buffer and dried in a vacuum oven at 60°C for 12h to afford 2-phenylsulphonamide derivatives (7a-7e) in good to excellent yield [54.13% - 69.28%].

2-[Phenylsulphonamido]propanoic acid (7a).

The amino acid is alanine (**1**) [1.11g 25 mmol]. Theoretical yield is 5.73g, experimental 3.97g (69.28%). The molecular formula is $\text{C}_5\text{H}_9\text{NO}_4\text{S}$; Weight is 229.25g, R_f 0.89 and MP. $118-119^\circ\text{C}$. FT-IR (KBr)(cm^{-1}); 1160.11(S=O), 3424.73(-NH- stretch), 1700.31(C=O, acid), 3315.74(-OH-carboxylic). ^1H NMR (δH); 0.79(3H_d, J.592. CH₃), 1.94(1H_q), 3.1.05(CH), 3.49-3.5(1H_d, J.1.99), 4.33 (-NH), 7.52(1H_t, J.3.13, Ar-H), 7.76(1H_t, J.2.04, Ar-H), 8.01-7.94(OH-J.1.00), ^{13}C NMR (δC); 141.59 (C-1), 132.73(C-2), 126.93(C-3), 129.52 (C-4), 61.71 (C-5), 173.55 (C-1'), 30.83(C-2'), 19.43 (C-3').

2-[Phenylsulphonamido]acetic acid (7b).

The amino acid is glycine (**2**) [0.94g 25 mmol]. Theoretical yeild is 5.38g, experimental 3.52g (65.43%). The molecular formula is $\text{C}_8\text{H}_9\text{NO}_4\text{S}$; Weight is 215.25g, R_f 0.61 and MP. $102-103^\circ\text{C}$. FT-IR (KBr)(cm^{-1}); 1140.92 (S=O), 3417.01 (-NH- stretch), 1719.6 (C=O acid), 3321.52 (OH- carboxylic). ^1H NMR (δH); 3.5(2H_t J.2.02, CH-NH), 7.63(1H_t J.2.01 Ar-H), 7.75(1H_d, J.3.01, Ar-H), 7.83(1H_t J.2.01 Ar-H), 8.06 (NH, J.1.00), 12.63 (OH, J.0.54). ^{13}C NMR (δC); 141.14(C-1), 132.85 (C-2 & C-6), 126.91 (C-3 & C-5), 129.52 (C-4), 170.70 (C-1'), 44.27 (C-2').

2-[Phenylsulphonamido]-4-methylpentanoic acid (7c)

The amino acid is leucine (**3**) [1.64g 25 mmol]. Theoretical yield is 6.03g, experimental 4.21g (68.82%). The molecular formula is $\text{C}_{10}\text{H}_{17}\text{NO}_4\text{S}$; Weight is 241.25g, R_f 0.83 and MP. $100-101^\circ\text{C}$. FT-IR (KBr)(cm^{-1}); 1162.65(S=O), 3585.79 (NH), 3248.23 (OH-carboxylic), 1710.92 (C=O). ^1H NMR (δH); 0.80(3H_d, J.2.94 of CH₃), 0.87(3H_d, J.3.68 CH₃), 1.59(1H_m, J.3.34, CH), 3.71(1H_t, J.1.32, CH₂), 4.35(-NH-), 7.36(1H_t, J.0.53, Ar-H), 7.63(1H_t, J.3.72, Ar-H), 7.86(1H_d, J.2.50 Ar-H), 8.13(-OH, J.1.00), ^{13}C NMR (δC); 144.57(C-1), 132.25 (C-2), 126.06 (C-3), 129.38 (C-4), 126.89 (C-5), 122.75 (C-6), 173.69(C-1'), 54.44 (C-2'), 24.30 (C-3'), 41.36(C-4'), 23.00 (C-5'), 21.43 (C-6').

2-[Phenylsulphonamido]-3-phenylpropanoic acid (7d).

The amino acid is phenylalanine (**4**) [2.06g 25 mmol]. Theoretical yield is 7.63g, experimental 4.13g (54.13%). The molecular formula is $\text{C}_{15}\text{H}_{15}\text{NO}_4\text{S}$; Weight is 305.35g, R_f 0.72 and MP. $108-109^\circ\text{C}$. FT-IR (KBr) (cm^{-1}); 1162.15(S=O), 3352.39(-OH-), 3423.76(-NH-2° Amine), 1686.81 (C=O stretch), 1445.7 (C=C weak). ^1H NMR (δH); [2.70-3.00(Ph-H), 3.87-3.90(2H_d, CH₂-Ph), 3.92-3.95(1H_t, CH-NH), 4.36(NH, J. 34.63], [7.12-7.24(1H_t Ar-H), 7.41-7.44 (1H_t Ar-H), 7.64-7.61(1H_d, J.5.27], 8.27-8.30(1H. Ar-H), 12.62(-OH, J.2.70). ^{13}C NMR (δC); 145.41(C-1), 143.00 (C-2), 126.92 (C-3), 138.88 (C-4), 125.98(C-5), 129.91 (C-6), 21.38 (C-7), 173.70-173.56(C-1'), 51.56 (C-2'), 18.86 (C-3').

2-[Phenylsulphonamido]-3-methylbutanoic acid (7e).

The amino acid is valine (**5**) [1.46g 25 mmol]. Theoretical yield is 6.43g, experimental 3.52g (56.30%). The molecular formula is $C_{11}H_{15}NO_4S$; Weight is 257.31g, R_f 0.68 and MP. 141-142°C. FT-IR (KBr)(cm^{-1}); 1167.94(S=O), 3413.15(NH of 2° Amine), 1707.06(C=O stretch), 2969.51 (C-C sat), 1550.82 (Ar), 3310.92 (-OH-). 1H NMR (δ H); 0.81-0.76(3H_d.J.0.17.CH₃), 1.98-1.87(3H_d, J.0.94.CH₃), 3.4-3.49(1H_d -CH), 4.34 (NH.J. 0.18), 4.96-5.35(OH moiety), 7.79 (1H_d. J.0.32 Ar-H), 7.77 (1H_d.J.0.32 Ar-H), 8.00(1H_d.J.0.16 Ar-H), 7.62-7.51(1H_t.J.0.52 Ar-H), ^{13}C NMR (δ C); 172.63(C-1), 141.52 (C-2), 129.33 (C-3), 126.93 (C-4), 72.59 (C-5), 132.73 (C-6), 172.57 (C-1'), 61.70 (C-2'), 30.82 (C-3'), 19.41 (C-4'), 19.23 (C-5').

Evaluation of antibacterial activity of synthesised compounds

Preparation Inoculums

The standard clinical isolated organisms of *Staphylococcus aureus*, *Pseudomonas*, *Streptococcus*, *klebsiella*, *Escherichia coli* and *Proteus* were obtained from FMC Owerri and the analysis was carried at Department of Medical Science Laboratory Imo state University. The strains of the organisms were propagated on nutrient agar plates and maintained at 4°C. The isolates were sub-cultured in nutrient broth at 37°C for 8h prior to antibacterial testing.

Antibacterial sensitivity testing of compounds

Agar well diffusion technique as described by Adeniyi et al [21] was used to determine the antibacterial activity of the synthesised compounds. Sensitivity test agar plates were inoculated with 0.1ml of an overnight culture of each bacteria strain (equivalent to 10^8 CFU/ml⁻¹). The inoculated agar plates were allowed to dry and were appropriately labelled. Using a plastic cork borer of 6mm in diameter uniformed wells was bored in the inoculated nutrient agar. With a micropipette, 200µl of 10mg/ml of each test compound solution was delivered into each well. Ciprofloxacin as the positive standard was also tested and the plates were left on the bench for 30minutes to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24h. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with metre rule to the nearest whole millimetre and the results recorded in Table1. Minimum inhibitory concentration (MICs) was determined by broth dilution technique [22]. Different concentrations (200, 175, 150, 120, 100, 75, 50, 25, 12.5, 10.00)µg/ml of the test solution were evaluated. Specifically, 0.1ml of standardised inoculums ($1-2 \times 10^7$ CFU/ml) was added to test tubes and incubated for 24h at 37°C and two controls (lank and standard) were maintained for each test sample. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC) and the MICs of the test solution are recorded in Table2.

Results and discussion

Benzenesulphonylchloride (**6**) have been successfully used as highly efficient and cost effective precursors for the synthesis of 2-phenylsulphonamide derivatives (**7a-7e**). Benzenesulphonylchloride (**6**) underwent condensation reaction with primary amine functionalities of five amino acids (**1-5**) in alkaline medium at the temperature below 0°C to produce 2-phenylsulphonamide derivatives of amino acids (**7a-7e**). (scheme1)

Scheme 1: Synthetic pathway of 2-phenylsulphonamide derivatives of amino acids (7a-7e).

The reaction started with conversion of the carboxylic end ($-\text{COOH}$) of the amino acid to the sodium salt of the acid through electrophilic substitution of the H^+ with Na^+ released from the base (Na_2CO_3). The formation of the sodium salt helped to protect the ($-\text{COOH}$) of the amino acids and enhanced the solubility of the amino acids in aqueous medium.

The coupling of the amino acids with benzenesulphonyl chloride occurred by nucleophilic attack of the electrophilic sulphur by the amino group of the amino acids to form ammonium ion where chloride ion was the leaving group. The abstraction of the ammonium proton by the chloride ion led to the amide which underwent acidification with 20Molar HCl to afford the expected 2-phenylsulphonamide derivatives (7a-7e).

The synthesised compounds were all white crystalline solids, having melting points ($^{\circ}\text{C}$) of 118 -119, 103 -103, 100 - 101, 108 - 109 and 141 - 142 respectively. The FT-IR indicated the presence of $\text{S}=\text{O}$ at (1162.11, 1140.92, 1162.65, 1162.15, 1167.94), $-\text{NH}-$ at (3424.73, 3417.01, 3585.79, 3423.76, 3413.15), $-\text{C}=\text{O}$ at (1700.31, 1719.60, 1710.92, 1686.81, 1707.06) and $-\text{OH}-$ signals at (3315, 3321.52, 3248.23, 3352.99, 3310.92) for compounds 7a-7e respectively.

The proton spectra (^1H) showed chemical shift at δ 4.33 - 4.36 for $-\text{NH}-$ and δ 7.12 - 8.30 for aromatic protons. Phenyl proton (7d) was observed at the stretched chemical shifts at δ 2.70 - 3.90 with the same J. Value of 34.63Hz. ^{13}C NMR spectra showed chemical shifts at 170.70 - 173.69 for carboxyl carbon. NMR data and elemental analytical data of the compounds have given further evidence for the confirmation of the synthesised compounds.

The antibacterial activities of the compounds were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus*, *klebsiella pneumonia*, *Escherichia coli* and *Proteus mirabilis*. The compounds showed potent to moderate zone of inhibition by killing the bacteria strains at the concentration of 200 μl of 10mg/ml (Table 1). Result of the minimum inhibitory of the tested samples showed that 7c and 7d exhibited more potent activity than the standard drugs while 7e was the same in *Pseudomonas aeruginosa* (Table2).

Table 1: Results of Anti-bacterial Activities of 2-phenylsulphonamides with 200 μl of 10mg/ml of compounds. Zone of Inhibition= [mm].

COMPD	STAPH	STREP	E-COLI	PSEUDO	KLEB	PROTEUS
7a	16	16	16	20	14	16
7b	16	17	20	21	14	16
7c	16	16	20	22	16	16
7d	16	17	19	20	14	17
7e	16	19	17	19	16	16
CPX	28	26	28	22	30	24

CPX=Ciprofloxacin antibiotic drug (Positive Standard).

Table 2: Minimum Inhibitory Concentration (MIC) in µg/ml of 2-phenylsulphonamides Derivatives (7a-7e).

COMPD	STAPH	STREP	E-COLI	PSEUDO	KLEB	PROTEUS
7a	120	120	100	50	200	120
7b	100	75	>50	25	200	100
7c	100	100	25	12.5	120	100
7d	>75	>75	50	12.5	175	>75
7e	100	50	75	>12.5	150	100
CPX	10	12.5	>12.5	>12.5	5.0	25

CPX=Ciprofloxacin antibiotic drug (Positive Standard).

Conclusions

2-phenylsulphonamide derivatives of amino acids (**7a-7e**) have been successfully synthesised with benzenesulphonylchloride (**6**) and amino acids (**1-5**). The synthesised compounds are broad spectrum antibiotics since they showed zone of inhibition on the growth of both gram positive bacteria such as Staph and Streptococcus and gram negative bacteria like E-coli, Klebsiella, Proteus, and pseudomonas. The MIC results (Table2) showed that compounds 7c and 7d can be classed as more potent anti bacteria agents against Pseudomonas aeruginosa than the standard drug ciprofloxacin.

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