Journal of Pharmaceutical Research International

32(8): 51-61, 2020; Article no.JPRI.54690
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Design, Synthesis, Antimicrobial and Antioxidant Activities of Novel Threonine-based Sulfonamide Derivatives

M. C. Egbujor[1*], U. C. Okoro[2], D. C. Nwobodo[3], C. U. Ezeagu[4], U. B. Amadi[5], C. G. Okenwa-Ani[1], J. I. Ugwu[4], I. G. Okoye[4], I. P. Abu[3] and P. I. Egwuatu[3]

[1]Department of Industrial Chemistry, Renaissance University, Ugbawka, Enugu, Nigeria.
[2]Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria.
[3]Department of Microbiology, Renaissance University, Ugbawka, Enugu State, Nigeria.
[4]Department of Biochemistry, Renaissance University, Ugbawka, Enugu State, Nigeria.
[5]Chemistry Unit, Akanu Ibiam Federal Polytechnic Unwana, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/JPRI/2020/v32i830470
Editor(s):
(1) Dr. Vasudevan Mani, College of Pharmacy, Qassim University, Buraidah, Kingdom of Saudi Arabia.
Reviewers:
(1) Eman Fadlalla, Ain Shams University, Egypt.
(2) M. E. Makgatho, University of Limpopo, South Africa.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54690

Original Research Article

Received 10 December 2019
Accepted 14 February 2020
Published 30 May 2020

ABSTRACT

Aim: To systematically design, synthesize and evaluate the biological activities of new threonine-based sulfonamide derivatives in order to achieve improved drug potency.
Methodology: Sulfamoyl carboxylic acids were prepared by the reaction of threonine with the appropriate sulfonyl chloride while their acetylated, carboxamide and aniline derivatives were synthesized *via* Lumiere-Barbier acetylation, Schotten-Baumann ammonolysis and Buchwald-Hartwig cross-coupling methods respectively. The FTIR, ^{1}H-NMR, ^{13}C-NMR and elemental analytical data were employed in the structural characterization. *In vitro* and *in silico* antioxidant and antimicrobial studies were carried out.
Results: Compounds 1b and 1d displayed the best *in vitro* antibacterial activities against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and antifungal activities against *Candida albican s*and *Aspergillus niger*. Compound 1f

**Corresponding author: E-mail: egbujormc@gmail.com;*

(IC_{50} = 1.150±0.003 µg/ml) exhibited the best *in vitro* antioxidant activity. Compound 1a had a higher*in silico* antibacterial (-11.51 kcal/mol) binding energies than antibacterial reference drug, penicillin (-10.89 kcal/mol). Compound 1c had the highest *in silico* antifungal binding energy (-10.48 kcal/mol)comparable to ketoconazole (-10.85 kcal/mol).
Conclusion: All the compounds were found to be potential antioxidant and antimicrobial drug candidates having complied with Lipinski's rule of five.

Keywords: Threonine; sulfonamides; In silico; antimicrobial; antioxidant.

ABBREVIATIONS

HBA : Number of Hydrogen Bond Acceptor
HBD : Number of Hydrogen Bond Donor
NRB : Number of Rotatable Bonds
MW : Molecular Weight
TPSA : Topological Polar Surface Area
ROS : Reactive Oxygen Species
DMSO : Dimethyl Sulfoxide
MeOH : Methanol
TLC : Thin-Layer Chromatography
KBr : Potassium Bromide
DPPH : 2,2-diphenyl-1-picrylhydrazyl
CFU : Colony-Forming UNIT
FTIR : Fourier Transform Infrared Spectroscopy
C-NMR : Carbon-13 Nuclear Magnetic Resonance
H-NMR : Proton Nuclear Magnetic Resonance
PDB : Protein Data Bank
CNS : Central Nervous System

1. INTRODUCTION

The increasing demand for effective antioxidant and antimicrobial compounds calls for an urgent attention because of the prevalence of microbial infectious and oxidative stress related diseases in recent times [1-2]. Reactive oxygen species (ROS) also known as free radicals are introduced to the body system through biochemical processes and environmental pollution. These free radicals were found to be responsible for chronic disease conditions such as cancer, cardiovascular diseases, quick aging, inflammation [3-5] and especially the reduction in the body's immunity response which exposes the body to microbial infections [6]. Similarly, microbial infections are unarguably the cause of millions of death across the globe [7,8]. Recent reports showed that sulfonamides and its derivatives exhibit wide range of biological activities [9-11] and the incorporation of certain bioactive amino acids such as serine [12] and methionine [13] had been found to potentiate the biological activities of sulfonamides [14].

Threonine is an indispensable amino acid for man [15] which in appropriate dietary level was found to improve the antioxidant capacity in certain animals by increasing free radical scavenging ability [16]. Furthermore, the presence of glycosylated threonine in certain antimicrobial peptides (AMPs) especially drosocin resulted to improved antimicrobial activities because of the rigid conformation it afforded [17]. Consequently, this study is designed based on the rationale that synthesizing sulfonamides from threonine would result to improved antimicrobial and antioxidant activities of sulfonamides due to the presence of the bioactive amino acid moiety.

It is worrisome that in spite of the fact that oxidative stress related diseases pose a great danger to the global health system, only plants sources of antioxidants have been given a considerable attention while just a few synthetic antioxidants are available and scarcely used because of "perceived" ineffectiveness and toxicity [18,19]. Similarly, reports have showed that while the case of antimicrobial drug resistance is increasing, the pace at which new antimicrobial compounds are being developed is rather slow [20,21]. The synthesis of new drug compounds with new target sites using bioactive and body friendly threonine could possibly address the cases of microbial recalcitrance and antioxidant toxicity [22].

Therefore, the aim of this study was to synthesize threonine-based sulfonamide derivatives and investigate their biological activities in order to achieve improved drug potency.

2. MATERIALS AND METHODS

2.1 Chemistry

Reagents were sourced from Sigma Aldrich and used without further purification. The melting points values were obtained with electrothermal melting point apparatus IA9200 model. FT-IR spectroscopy of title compounds were recorded on Shimadzu 8400s Fourier Transform Infrared. Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) were run in DMSO on a Varian Gemini

400MHz spectrophotometer and the chemical shifts were recorded in part per million (ppm). Elemental analysis was run on a Euro EA 3000 elemental analyzer. Reactions were monitored by a thin-layer chromatography (TLC) (MeOH/ DCM, 1:9). Nitrogen gas provided the inert atmosphere for reactions requiring inert conditions. The compounds were precipitated in analytical grade.

2.2 Synthesis of 3-hydroxy-2-{[(4-methylphenyl)sulfonyl]amino}butanoi cacid(1a)and 3-hydroxy-2-[(phenylsulfonyl)amino]butanoic acid (1b)

Using a 50 ml beaker, Na_2CO_3 (2 g) and threonine (2 g) were dissolved in distilled water (15ml). It was cooled to 0°C followed by the addition of the appropriate sulfonyl chloride (5.14 g, 15 mmol). The solution was stirred for 3 hours. Unpon completion, It was crystallized by the addition of hydrochloric acid (pH = 2), filtered by suction and washed with tartaric acid to obtain compounds (1a-b)in analytical grade.

2.2.1 3-hydroxy-2-{[(4-methylphenyl) sulfonyl]amino}butanoic acid (1a)

White solids, yield 3.34 g (93.7%), mp 90-91°C. IR(KBr) cm^{-1}: 3655 (OH free), 3454 (N-H), 3290(O-H of COOH), 3063 (C-H aromatic), 2925(CH-aliphatic), 1737(C=O of COOH), 1640, 1595(C=C aromatic), 1375, 1169 (S=O two bands), 1158, 1123(SO_2NH), 1015, 1010(C-N), 689(Ar-H). ^{1}H-NMR(DMSO-d_6, 400 MHz) δ: 7.78-7.72(d, J = 8Hz, 2H, Ar-H), 7.46-7.32(d, J = 8Hz, 2H, Ar-H), 4.93(s, 1H, OH), 4.83-4.55(d, J = 4.4Hz, 1H, NH), 3.85-3.81(m, IH,CH), 3.73 (dd, J_1 =3.7Hz, J_2 =9.2Hz, IH, CH-NH), 2.48 (s, 3H, CH_3-Ar), 0.99 - 0.93 (d, J = 6.36Hz, 3H, CH_3-CH). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: 172.35(C=O), 140.90, 132.89, 128.43, 125.96, 123.11, 121.67 (aromatic carbons), 37.76, 31.70, 20.15, 19.93 (aliphatic carbons). Anal. calcd. for $C_{11}H_{15}NO_5S$ (273.13): C, 48.43, H, 5.64, N, 5.23, S, 11.81. Found: C, 48.44, H, 5.60, N, 5.20, S, 11.84.

2.2.2 3-hydroxy-2-[(phenylsulfonyl) amino]butanoic acid (1b)

White solid**,** yield 2.96 g (89.4%), mp. 141-142°C. IR(KBr) cm^{-1}: 3545(OH free), 3299 (N-H), 3075(C-H aromatic), 2957 (O-H of COOH), 1726(C=O of COOH), 1338, 1174 (S=O two bands), 1152, 1125(SO_2NH), 1118 (C-N), 697(Ar-H). ^{1}H-NMR(DMSO-d_6, 400 MHz)δ: 10.67(s-br, 1H, OH of COOH), 7.91-7.86 (d, J = 8.7Hz, 2H, Ar-H), 7.76-7.7.34 (d, J = 9.3Hz, 3H, ArH), 5.71-5.52 (m, 1H, NH), 3.89-3.89 (dd, J_1=3.63 Hz, J_2=6.61 Hz, 1H, CH-CH-CH_3), 3.59-3.57(dd, J_1 =3.61Hz, J_2=9.21 Hz, 1H, NH-CH-CH), 2.16 (s, 1H, OH), 1.12-1.10(d, J=6.40 Hz, 3H, CH_3-CH). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: 171.57 (C=O), 141.63, 140.21, 135.66, 133.73, 129.51, 126.82(aromatic carbons), 37.50. 31.40, 27.80 (aliphatic carbons). Anal. Cald(%) for $C_{10}H_{12}NO_5S$ (258.14): C, 46.57, H, 4.73, N, 5.50, S, 12.38. Found: C, 46.60, H, 4.77, N, 5.53, S, 12.36.

2.3 Synthesis of 2-{Acetyl[(4-methylphenyl)sulfonyl]amino}-3-hydroxybutanoic acid(1c) and 2-{Acetyl[(phenylsulfony)amino]-3-hydroxybutanoic acid(1d)

Using 100 ml beaker, compounds (1a-b) (2 g) and acetic anhydride (12 g) were dissolved in concentrated hydrochloric acid (9ml) followed by the addition of a solution of sodium acetate (16 g) and distilled water (50 ml) and thorough stirring. It was cooled to 0°C in ice bath, filtered and washed to obtain compounds (1c-d) in excellent yield.

2.3.1 2-{Acetyl(4-methylphenyl)sulfonamido}-3-hydroxybutanoic acid(1c)

White solid, Yield 2.51 g (93.2%), mp.212-213ºC, IR (KBr) cm^{-1}: 3404 (OH free), 3399(O-H of COOH), 3085 (N-H), 2954(C-H aliphatic), 1987 (C-H aromatic), 1719,1691(C=O), 1662, 1652 (C=C), 1393, 1287(S=O), 1192(SO_2-NH), 1143(C-N), 758(Ar-H). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.87-7.52 (d, J = 6.8Hz, 2H, ArH), 7.66-7.65 (d, J = 6.4Hz, 2H, ArH), 4.18 (s-br, IH, OH), 3.994 (s, IH, COOH), 2.49-2.39 (m, 3H, CH_3-C=O), 2.19-2.08 (m, 3H, CH_3-Ar), 1.94-1.47(m, IH, CH-CH) 0.96-0.87 (d, J= 3.2Hz, 3H, CH_3-CH). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: 171.25, 170.26(C=O), 140.94, 133.87, 129.56, 126.79, 124.77, 120.35 (aromatic carbons) 67.79. 61.73, 40.79, 39.96, 39.39 (aliphatic carbons). Anal. calcd (%). for $C_{13}H_{17}NO_6S$ (315.36): C, 49.56, H, 5.47, N, 4.53, S, 10.23. Found: C, 49.60, H, 5.51, N, 4.56, S, 10.29.

2.3.2 2-{Acetyl(phenylsulfonamido)}-3 hydroxybutanoic acid (1d)

White solid, yield 2.23 g (90.10%), mp.202-203°C, IR (KBr) cm^{-1}: 3662 (OH free), 3297 (OH

of COOH),3073(N-H), 3038(C-H aliphatic), 1992(C-H aromatic), 1731, 1689(2C=O), 1659, 1658 (C=C), 1336, 1247 (2S=O), 1172(SO_2-NH), 1083(C-N),755 (Ar-H). ^{1}H-NMR(DMSO-d_6, 400 MHz) δ: 7.86-7.84(m, 2H, Ar-H), 7.65-7.30(m, 3H, Ar-H), 4.19(s-br, IH, OH), 3.99(s, IH, COOH), 2.57-2.38 (m, 3H, CH_3-C=O), 1.96-1.43(m, 1H, CH-CH), 0.95-0.93d, J= 5.2Hz ,3H, CH_3-CH). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: 171.13, 170.35(C=O), 141.91, 138.21, 135.44, 133.89, 130.43, 129.96 (aromatic carbon), 31.05, 30.84, 29.36, 19.52 (aliphatic carbons). Anal. calcd (%). for $C_{11}H_{15}NO_6S$ (301.33): C, 47.98, H, 4.99, N, 4.66, S, 10.72. Found: C, 48.10, H, 4.95, N, 4.71, S, 10.59.

2.4 Synthesis of 2-[acetyl (phenylsulfonyl)amino]-3-hydroxybutanamide (1e)

2.4.1 Chlorination and amminolysis

Using a three necked flask (100 ml) charged with bar magnet, compounds (1c-d) were dissolved in acetone (10 ml) and cooled to 0°C. Thionyl chloride (I.5 ml) was added and the content was stirred under reflux at 80°C for 3 hours. The remaining thionyl chloride was evaporated after the reaction by further heating in a water bath at 80°C to obtain the reactive acid chloride intermediate. Immediately aqueous ammonia (2ml) was added and the crystals were obtained by suction filtration and washed with acetone to obtain compound 1e.

2.4.1.1 2-{acetyl[(4-methylphenyl)sulfonyl] amino}-3-hydroxybutanamide(1e)

Brown solid, yield 3.09 g (92.8%), mp.211-212°C, IR (KBr) cm^{-1}: 3480 (N-H), 3028(C-H aliphatic), 1982(C-H aromatic), 1759, 1690(2C=O) 1649, 1638 (C=C), 1326, 1237 (2S=O), 1162(SO_2-NH), 1073(C-N),745 (Ar-H). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.76-7.68 (d, J= 6.8Hz, 2H, Ar-H), 7.55- (d, J = 6.4Hz, 2H, Ar-H), 4.09 (s-br, IH, OH), 3.96(s, 2H, NH_2), 2.48-2.34 (m, 3H, CH_3-C=O), 1.98-1.52 (m, 1H, CH-CH), 0.947-0.934 (d, J= 5.2 Hz, ,3H, CH_3-CH). ^{13}C-NMR (DMSO, 400MHz) δ: 170.23, 169.95 2(C=O), 140.90, 132.89, 129.43, 126.96, 123.56, (aromatic carbon) 37.66, 31.60, 30.05, 29.83, 19.26 (aliphatic carbons). Anal. calcd (%). for $C_{12}H_{19}N_2O_6S$ (315.33): C, 49.48, H, 6.03, N, 8.88, S, 10.15. Found: C, 49.51, H, 6.06, N, 8.91, S, 10.11.

2.5 Synthesis of 2-{acetyl[(4-methylphenyl)sulfonyl]amino}-N-(4-aminophenyl)-3-hydroxybutanamide(1f)

The catalyst complex bis (triphenylphosphine) nickel(II) chloride was prepared using Venanzi [23] procedure in which nickel (II) chloride hexahydrate (2 g) was dissolved in glacial acetic acid (30 ml) followed by the addition of a solution of triphenylphosphine (4 g) and glacial acetic acid (15 ml). The mixture was filtered after 12 hours to obtain bis (triphenylphosphine) nickel(II) chloride.

Then Buchwald-Hartwig cross-coupling reaction method [24] was used in the cross-coupling reaction of the sulfonamide with 4-chloroaniline to obtain the aminophenyl derivative as follows. Bis(triphenylphosphine)nickel(II)chloride (2 g) and triphenylphosphine (4 g) were dissolved in *t*-butanol (8 ml) and distilled water (4 ml). The mixture was preheated for 2 minutes at 80°C followed by the addition of 4-chloroaniline (2ml), potassium carbonate (1.5 g). It was stirred for 3 hours with reflux at 110°C under inert nitrogen condition after which it was recrystallized with ethyl acetate, filtered and washed with water to obtain compound 1f.

2.5.1 2-{acetyl[(4-methylphenyl) sulfonyl] amino}-N-(4-aminophenyl)-3-hydroxybutanamide(1f)

Yield 2.19 g (94.3%), mp. 83-84°C, IR (KBr) cm^{-1} :3490, 3430 (2N-H), 3300(O-H), 3000 (C-H aliphatic), 1982 (C-H aromatic), 1725, 1690 (2C=O), 1655, 1650(C=C), 1397, 1200(2S=O), 1121(SO_2-NH), 1026 (C-N), 741 (Ar-H). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 8.465-8.38 (d, J = 7.8Hz, 2H, ArH), 8.24-8.09 (d, J = 7.5 Hz, 2H, ArH), 7.96-7.78 (t, d = 7.1 Hz, 2H, ArH), 6.99-6.53 (d, J = 6.4Hz, 2H, ArH), 5.20-5.10 (t, J = 4.8 Hz, IH, NH), 4.954 (s, 2H, NH_2), 3.78 (s-br, IH, OH), 2.98-2.50 (t, J = 2.2Hz, 1H, CH-N), 1.96-1.33 (m, 1 H, CH-CH) 2.48-2.40 (m, 3H, CH_3-Ar), 2.30-2.15 (m, 3H, CH_3-C=O), 1.85-1.74 (d, J = 1.2 Hz, 3H, CH_3-CH). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ: 172.678, 170.03, 2(C=O) 141.84, 138.34, 133.63, 133.42, 128.94, 126.60, 123.28, 115.62 (aromatic carbons), 38.43, 35.23, 31.68, 23.12, 19.13(aliphatic carbons). Anal. calcd (%). for $C_{18}H_{23}N_3O_5S$ (405.48): C, 56.23, H, 5.67, N, 10.36, S, 7.89. Found: C, 56.19, H, 5.70, N, 10.31, S, 7.86.

2.6 Antioxidant Evaluation

Using Blois method [25], DPPH solution was obtained when 2 mg DPPH was dissolved in 100 ml of methanol. Mixtures of the synthesized compounds were prepared using the same method in various concentrations of 50, 100 and 200µg/ml. The same concentrations of ascorbic acid were prepared. The mixtures were thoroughly shaken and stored at room temperature in the dark. Their absorbance were measured at 517 nm using UV spectrophotometer after 30 minutes and compared with the corresponding absorbance of the standard ascorbic acid concentrations. The percentage inhibition against the stable DPPH was calculated following the formula:

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

IC50 indicated the concentration of the compounds with 50% of radical scavenging activity.

IC50 is inversely proportional to the antioxidant activity.

2.7 Antimicrobial Evaluation

The minimum inhibitory concentrations were determined by agar dilution method [26]. Various pathogenic bacteria and fungi were employed in the experiment and were standardized by 0.5 McFarland turbid equivalents. Ofloxacin and Fluconazole were the standard antibiotics employed. Nine concentrations of the title compounds and standard drugs such as 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, 0.1 mg/ml, were obtained by the formula $C_1V_1=C_2V_2$. The molten agar plates of the synthesized compounds bearing the microorganisms were incubated at 25°C for 48 hours and the colony forming units (CFU) were counted. Then the MICs of the synthesized compounds were recorded as minimum concentrations that completely inhibited the growth of the microorganisms.

2.8 *In silico* Evaluation

2.8.1 Physicochemical parameters

The physicochemical parameters of the compounds were obtained *via* computer stimulation and were computed using descriptors calculator in Swiss dock online servers. The drug-likeness of the compounds was predicted by Lipinski's rule of five stipulations. The parameters were namely number of hydrogen bond acceptor (HBA), number of hydrogen bond donor (HBD), number of rotatable bond (NRB), octanol/water partition coefficient logP(o/w), molecular weight (MW), aqueous solubility (SlogP) and topological polar surface area (TPSA).

2.9 Molecular Docking Evaluation

The computational simulation of their binding to receptors was carried out for oxidative stress, bacterial infections and fungal infections. The 3-Dimensional structures of were sourced from the Protein Data Bank (PDB), (http://www.pdb.org) database. Human peroxiredoxin 5 (PDB code: 1HD2) was drug targets for antioxidant, *Escherichia coli* DNA gyrase in complex with 1-ethyl-3-[8-methyl-5-(2-methyl-pyridin-4-yl)-isoquinolin-3yl]urea (PDB code: 5MMN) for antibacterial and urate oxidase from *Aspergillus flavus* complexed with uracil (PDB code: 1WS3) for antifungal. Themolecular docking using PyRx enabled the interaction of the compounds with each of the receptors. This procedure enabled a flexible compound docking for various compound conformers within the rigid receptor and the best conformation for each title compound was chosen and the interaction was visualized using the Discovery studio.

3. RESULTS AND DISCUSSION

3.1 Chemistry

Compounds 1a and 1b were synthesized by base promoted reaction of 2-amino-3-hydroxybutanoic acid with paratoluene and benzene sulfonyl chloride respectively. The acylation of compounds 1a and 1b afforded compounds 1c and 1d respectively. Chlorination and immediate amonnolysis of compound 1c gave compound 1e which was subjected to Buchwald-Hartwig cross-coupling reaction to obtain compound 1f in excellent yield as represented in scheme 1 and 2.

The presence of the peaks at 3655-3543cm^{-1}(OH free), 3451-3437 cm^{-1}(N-H), 2955-3290(O-H of COOH), 1733(C=O of COOH), 1652, 16501 (C=C aromatic), 1371, 1166 (S=O two bands), 685(Ar-H) frequencies in FTIR, the peaks at 7.78-7.78(d, J = 8Hz, 2H, Ar-H), 7.64-7.62(d, J=9.2Hz, IH, NH-CH), 2.19 (s, 1H, OH) in ^{1}H-

NMR and 172.353-171.400(C=O) in ^{13}C-NMR indicated the successful synthesis of sulfonamides (compounds 1a and 1b) from threonine and sulfonyl chloride.

The diagnostic peaks of extra 1731-1718cm^{-1}(C=O of ketone) in FTIR, 2.579-2.589 (s, 3H, CH_3-C=O) in ^{1}H-NMR and an extra peak of 171.232-171.133(C=O) in ^{1}C-NMR indicated a successful acylation in compounds 1c and 1d.

In compound 1e, the appearance of the peak at 3480 cm^{-1} (N-H of amide) in FTIR and 3.964 (s, 2H, NH_2) in ^{1}H-NMR was an indication of successful amonnolysis (addition of ammonia).

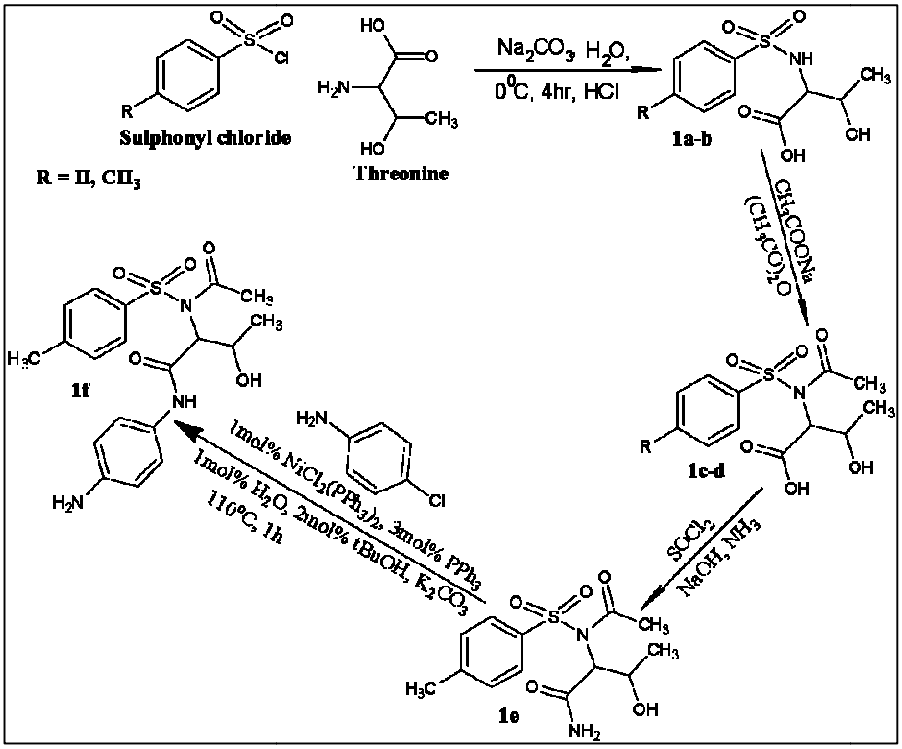
The diagnostic peaks in compound 1f, were the appearance of double 3490 cm^{-1}, 3430 cm^{-1} (2N-H), 1650 cm^{-1}, 1652 ccm^{-1}(C=C of aromatic) in FTIR, 8.465 (m, 2H, ArH), 8.243(s, 2H, ArH) in ^{1}H-NMR and additional 6C-aromatic in ^{13}C-NMR which confirmed the coupling of 4-chloroaniline.

3.2 Evaluation of Antioxidant Activities

According to Setha,et al. [27], the antioxidant activity of compounds can be classified as very strong (IC50 < 50 µg/ml), strong (IC50 = 50–100 µg/mL), moderate (IC50 = 101–150 µg/ml), and weak (IC50 = 250–500 µg/ml). Based on this principle, all the synthesized compounds represented in Fig. 1, possess very strong antioxidant activities. Obviously, compound 1f exhibited the highest antioxidant activity with IC_{50} value of 1.150±0.003 µg/ml while compound 1d displayed the least antioxidant activity of 1.864±0.001 µg/ml. The antioxidant activity of compound 1f was good but not as excellent as that of ascorbic acid (1.001±0.001 µg/ml). The highest antioxidant activity of compound 1f relative to other compounds can be attributed to the fact that aniline derivatives possess strong antioxidant activity [28] and therefore the antioxidant activity of the sulfonamide was potentiated by the coupled aniline.

3.3 Evaluation of Antimicrobial Activities

The antimicrobial screening represented in Table 2, showed that all the compounds exhibited inhibitory activities against the microbial strains used. Obviously, lower minimum inhibitory concentration (MIC) indicates higher antimicrobial potency and vice versa. Compounds 1b and 1d displayed the best antibacterial and antifungal activities, compound



Scheme 1. The synthetic pathway for the synthesis of sulfonamide derivatives from threonine

Table 1. Percentage inhibition of compounds

Compounds	% inhibition at 200 µg/ml	% inhibition at 100 µg/ml	% inhibition at 50 µg/ml
Ascorbic acid	96.83± 0.001	97.68 ± 0.001	97.31 ± 0.001
1a	94.22 ± 0.012	82.31 ± 0.002	37.94 ± 0.011
1b	96.70 ± 0.001	73.50 ± 0.001	71.79 ± 0.001
1c	79.13 ± 0.000	82.17 ± 0.001	79.55 ± 0.001
1d	76.31 ± 0.001	70.27 ± 0.001	72.28 ± 0.001
1e	77.97 ± 0.001	77.17 ± 0.001	74.98 ± 0.000
1f	89.39 ± 0.000	84.80 ± 0.001	83.27 ± 0.003

The standard antioxidant = ascorbic acid. Data are represented as mean ± S.D.

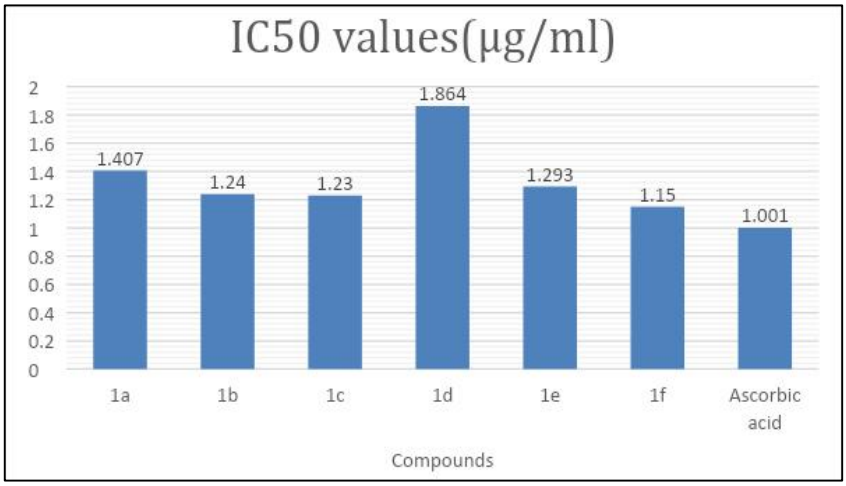


Fig. 1.Graphical representation of the IC50 Values of the synthesized compounds

Table 2. The minimum inhibitory concentration (MIC) of compounds (mg/ml)

isolates	1a	1b	1c	1d	1e	1f	Ofloxacin	fluconazole
E. coli	0.70	0.90	0.90	0.80	0.90	0.70	0.50	-
S. typhi	0.70	0.90	1.00	0.90	0.80	0.50	0.50	-
S. aureus	0.90	0.80	-	0.40	-	0.60	0.10	-
B. sub	0.50	0.60	-	0.40	0.80	0.40	0.20	-
Ps. aerug	0.90	0.90	-	0.90	0.60	-	0.25	-
C. albicans	-	0.90	0.90	0.60	0.70	-	-	0.20
A. niger	0.80	0.80	-	0.80	-	-	-	0.50

- = no growth. Isolates = Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger

1c displayed the least antibacterial activity while compound 1f displayed the least antifungal activity. The outstanding performance of compounds 1b and 1d (benzene sulfonamides) could stem from the fact that benzene sulfonamides possess excellent antimicrobial activities [29]. Report showed that sulfonamides never showed any inhibitory activity against *Pseudomonas aeruginosa* [30-31]. Conversely, this study revealed that all the sulfonamides derivatives synthetized from threonine inhibited the growth of *Pseudomonas aeruginosa* possibly because of the synergistic microbial antagonism arising from the incorporation of threonine.

3.4 Prediction of Oral Bioavailability and Drug-likeness of Compounds

Using the physicochemical properties of the synthesized compounds given in Table 3, the drug likeness and bioavailability of the

Table 3. The physicochemical parameters

Compounds	HBA	HBD	NRB	logP(o/w)	SlogP	TPSA	MW	Lip violation
1a	5	4	5	0.69	0.11	101.70	273.70	0
1b	5	4	5	0.40	0.20	103.70	259.28	0
1c	6	3	6	0.64	0.37	111.98	315.35	0
1d	6	3	6	0.35	0.06	111.98	301.32	0
1e	5	2	6	0.55	0.62	117.77	315.33	0
1f	5	3	7	1.25	1.25	129.80	405.48	0

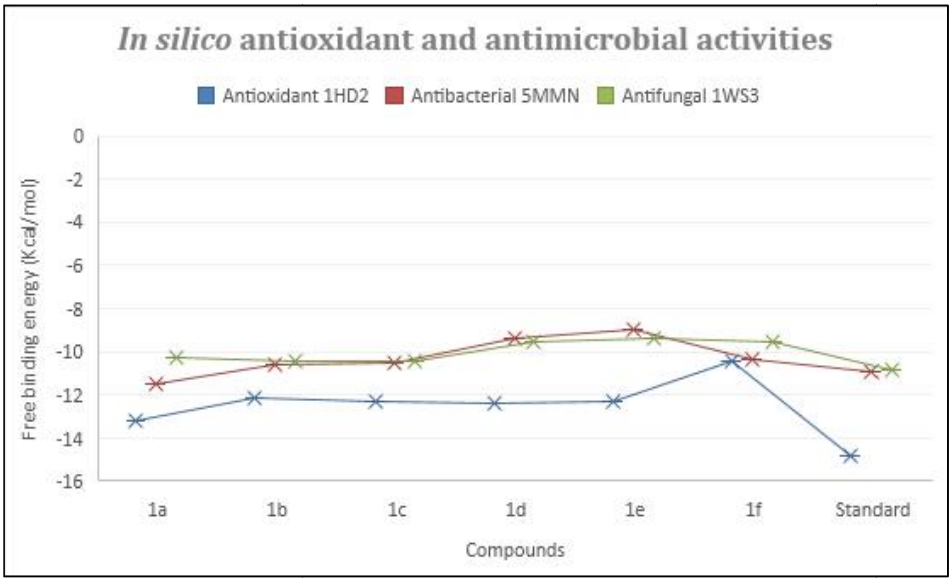


Fig. 2. Graphical representation showing *in silico* antioxidant and antimicrobial activities
Key: Standard for antioxidant = α-Tocopherol, antibacterial = Penicillin; antifungal = Ketoconazole

compounds were predicted. Lipinski's rule [32] states that a compound is considered to be orally active if not more than one of the limits of the following physicochemical properties is not exceeded. The number of hydrogen bond donor (HBD) ≤ 5, partition coefficient (logP) ≤ 5, number of hydrogen bond acceptor (HBA) ≤ 10, number of rotatable bonds (NRB) ≤ 10, molecular weight (MW) ≤ 500. Furthermore, Verber's rule [33] stipulated that compounds having NRB≤ 10 and topological polar surface area (TPSA) ≤ 140Å2 possess good oral bioavailability. Similarly, Van de waterbeemd,et al. [34] asserted that the topological polar surface area (TPSA) ≤ 90 Å2 and a molecular weight (MW) ≤ 450 g/mol is required for central nervous system (CNS) penetration. Based on the above mentioned rules, all the synthesized compounds (1a-f) have excellent drug likeness, they are also orally active with good oral bioavailability but cannot be recommended as CNS drug because of their TPSA being more than 90 Å2.

3.5 Evaluation of *in silico* Antioxidant and Antimicrobial

The binding affinities of compounds are given in Fig. 2. Compounds showed strong binding affinities with all the receptors utilized in the study. Compound 1a had the highest antioxidant binding energy (-13.19 kcal/mol) which can be compared to α-Tocopherol (-14.82 kcal/mol). Similarly, compound 1a had the highest antibacterial binding energy (-11.51 kcal/mol) even higher than penicillin (-10.89 kcal/mol). Compound 1c had the highest antifungal binding energy (-10.48 kcal/mol) comparable to ketoconazole (-10.85 kcal/mol).

4. CONCLUSION

In conclusion, the sulfonamide derivatives were synthesized using threonine an essential amino acid as the starting reagent. The synthesis was found to be facile, efficient and ecofriendly. The structures of compounds were confirmed with

spectroscopic data. Generally, all the synthesized compounds exhibited considerable antimicrobial and antioxidant activities although compounds 1b and 1d were found to be the best antimicrobial agents while compounds 1a and 1f were the best antioxidant agents synthesized. Threonine being a bioactive amino acid was found to potentiate the antioxidant and antimicrobial activities of sulphonamide derivatives. Considering the biological activities, good drug-likeness and oral bioavailability of the synthesized compounds, they are potential antimicrobial and antioxidant drug candidates.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We acknowledge the Chemistry Department of Renaissance University Enugu and University of Nigeria Nsukka, Nigeria for providing the labs and instruments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jos WMV. The infectious disease challenges of our time. Front. Public Health. 2013;1:7.
DOI:10.3389/fpubh.2013.00007
2. Halliwell B. Oxidative stress and cancer: have we moved forward?Biochem J. 2007;401(1):1-11.
DOI:10.1042/BJ20061131
3. Cheng J, Huang A, Hour T, Yang S. Antioxidant xanthone derivatives induce cell cycle arrest and apoptosis and enhance cell death induced by cisplatin in NTUBI cells associated with ROS. Eur. J. Med. Chem. 2011;46:1222.
DOI:10.1016/j.ejmech.2011.01.043
4. Stefoska-Needham A, Beck E, Johnson S, Tapsell L. Sorghum: An underutilized cereal whole grain with the potential to assist in the prevention of chronic disease. Food Rev. Int. 2015;31:401-437.
DOI:10.1080/87559129.2015.1022832
5. Jiang D, Dai Z, Li Y. Pharmacological effect of xanthones as cardiovascular protective agents. Cardiovasc. Drug Rev. 2004;22:91-102.
Available:https://doi.org/10.1111/j.1527-3466.2004.tb00133.x
6. Kock MD, Jessup DA, Clark RK, Franti CE. Effects of capture on biological parameters in free-ranging bighorn: Evaluation of drop-net, drive-net, chemical immobilization and the net-gun. J. Wildl. Dis. 1987;23(4):641-651.
DOI:10.7589/0090-3558-23.4.641
7. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Murray MD. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. Lancet. 2013;380: 2095–2128.
DOI:10.1016/S0140-6736(12)61728-0
8. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg. Infect. Dis. 2005;11: 1842–1847.
DOI:10.3201/eid1112.050997
9. Egbujor MC, Okoro UC, Okafor S, Nwankwo NE. Synthesis, characterization and *in silico* studies of novel alkanoylated 4-methylphenyl sulphonamoyl carboxylic acids as potential antimicrobial and antioxidantagents. Int. J. Pharm. Phytopharm Res. 2019;9(3):89-97.
Available:https://www.eijppr.com-article
10. Casini A, Scozzafava A, Mastrolorenzo A, Supuran LT. Sulfonamides and sulfonylated derivatives as anticancer agents. Curr. Cancer Drug Targets. 2002;2(1):55-75.
DOI.10.2174/1568009023334060
11. Loh B, Vozzolo L, Mok BJ, Lee CC, Fitzmaurice RJ, Caddick S, Fassati A. Inhibition of HIV 1 replication by isoxazolidine and isoxazole sulfonamides. Chem Biol Drug Des. 2010;75(5):461-474.
DOI.10.1111/j.1747-0285.2010.00956.x
12. Egbujor MC, Okoro UC, Okafor S, Nwankwo NE. Design, synthesis and molecular docking of novel serine-based sulphonamide bioactive compounds as potential antioxidant and antimicrobial agents.Indo Am. J. Pharm. Sci. 2019; 06(06):12232-12240.
DOI:10.5281/zenodo.3250306
13. Egbujor MC, Okoro UC. New methionine-based *p*- toluenesulphonamoyl carboxamide derivatives as antimicrobial and antioxidant agents: Design, synthesis. J. Pharm. Res. Int. 2019;28(1):1-12.
DOI:10.9734/jpri/2019/v28i130192

14. Egbujor MC, Okoro UC, 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.
DOI:10.1007/s00044-019-02440-3

15. Young VR. Adult amino acid requirements: The case for a major revision in current recommendations. J. Nutr. 1994;124(8): 1517-1523.

16. Li JM, Zhang XY, Yuan C, Miao LP, Yan HX, Dong XY, Lu JJ, Zuo XT. Effect of dietary L-threonine levels on antioxidant capacity, digestive enzyme activities, and antibody production of Xinyang green-shell laying hen.J. Appl Poult Res. 2016;25(3): 422-427.

17. Lele DS, Talat S, Kumari S, Strivastava N, Kaur KJ. Understanding the importance of glycosylated threonine and stereospecific action of drosocin a proline rich anti-microbial peptide. Eur. J. Med. Chem. 2005;92:637-647.

18. Zarena A, Sankar K. Supercritical carbon dioxide extraction of xanthones with antioxidant activity from garcinia mangostana: Charactertization by HPLC/ LCESI-MS. J. Supercrit Fluid. 2009;49(3): 330-337.
Available:https://doi.org/10.1016/j.supflu.2009.03.004

19. Branen A. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J. Am. Oil Chem Soc. 1975;52-59.
DOI.10.1007/BF02901825

20. Boucher HW, Talbot GH, Benjamin DK Jr, Bradley J, Guidos RJ, Jones RN, Gilbert D. 10x 20 progress-development of new drug active against gram-negative bacilli: An update from the Infectious Disease Society of America. Clin. Infect Dis. 2013; 56(12):1685–94.
DOI:10.1093/cid/cit152

21. DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D coats. J. Health Econ. 2016;47:2033.
DOI:10.1016/j.jhealeco.2016.01.012

22. Coates AR, Hu Y. Novel approaches to developing new antibiotics for bacterial infections. The Br. J. Pharmacol. 2007; 152(8):1147-1154.
DOI:10.1038/sj.bjp.0707432

23. Venanzi LM. Tetrahedral Nickel (11) complexes and the factors determining their formation. J. Chem Soc. 1958;719-724.
DOI:10.1039/JR9580000719

24. Fors BP, Krattiger P, Strieter E, Buchwald SL.Water–mediated catalyst preactivation: an efficient protocol for C-N cross-coupling reactions. Org Letts. 2008;10(16):3505-3508.
DOI:10.1021/o1801285g

25. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-1200.
DOI.10.1038/1811199a0

26. Wiegand I, Hilpert K, Hancock REW. Agar and Broth dilution methods to determine the minimal inhibitory concentration(MIC) of antimicrobial substances. Nat. Protoc. 2008;3(2):163-175.
DOI:10.1038/nprot,2007.521

27. Setha B, Gaspersz F, Febe F, Idris APS, Rahman S, Mailoa MN. Potential of *seaweed padina* sp. as a source of antioxidant. Int. J. Sci. Tech. Res. 2013; 26:221.
Available: www.ijstr.org

28. Bendary E, Francis RR, Ali HMG, Sarwat HMG, El Hady S. Antioxidant and Structure-Activity Relationships(SARs) of some phenolic and aniline compounds. Ann. Agric. Sci. 2013;58(2):173-181.
DOI.10.1016/j.aoas.2013.07.002

29. Ugwu DI, Okoro UC, Chukwura TD. Nickel catalyzed synthesis of N-aryl and N-hetertoaryl substituted benzene sulphonamides and their biological activity evaluation. Med. Chem. 2014;4:357-360.
DOI:10.417212161.0444.1000165

30. Miller-Hjelle MA, Somaraju V, HjelleJT. Modern pharmacology with clinical applications/edited by CraigCR, StitzelRE, (6th ed). Philadelphia: Lippincott Williams & Wilkins;2004.
Available:https://trove.nla.gov.au/version/46530718

31. Zessel K, Mohring S, Hamscher G, Kietzmann M, Stahl J. Biocompatibility and antibacterial activity of photolytic products of sulfonamides. Chemosphere. 2014;100: 167-174.
DOI:10.1016/j.chemosphere.2013.11.038

32. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computa

tional approach to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv. Rev. 2001;46:3-26. DOI:10.1016/S0169-409x(00)00129-0

33. Verber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence theoral bioavailability of drug candidates. J. Med. Chem. 2002;6:45(12):2615-23. DOI:10.1021/jm020017n

34. Van de waterbeemd H, Carter RE, Grassy G, Kubinyi H, Martins YC, Tute MS, Willet P. Grossory of terms used in computational drug design. 1997;69(5):1137-1152. DOI:10.1351/pac199769051137

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54690