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New Methionine-based *P*-toluenesulphonamoyl Carboxamide Derivatives as Antimicrobial and Antioxidant Agents: Design, Synthesis and Molecular Docking

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Authors' contributions

This work was carried out in collaboration between both authors. Author UCO designed the study. Author MCE performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UCO and MCE managed the analyses of the study. Author MCE managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The reported emergence of drug resistant microbes and the prevalence of oxidative stress related diseases prompted the need for the development of new antimicrobial and antioxidant agents. The synthesis of methione-based sulphonamoyl carboxamides bearing aniline, pyridine and pyrimidine pharmacophores is reported.
Place and duration: Department of Industrial Chemistry, Renaissance University, Ugbawka, Enugu, 2018.
Methodology: The *p*-toluenesulphonyl chloride reaction with methionine gave compound 3a which was acylated to afford compound 3b. Further chlorination and aminolysis of compound 3b gave the carboxamide (3c). Nickel catalysed reaction of the carboxamide with aryl/heteroaryl halides

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afforded compounds 3d-f in excellent yields. Characterization of compounds was done using H^1-NMR, C^{13}-NMR, FTIR and elemental analysis. Their antimicrobial, antioxidant activity and molecular docking were determined.

Results: Compounds 3a and 3e had the best antimicrobial activity with minimum inhibitory concentration (MIC) in the range of 0.6 – 0.9mg/ml and the highest antioxidant percentage inhibition (93.53% and 93.28% at 200µg/ml respectively) comparable with ascorbic acid (96.83% at 200µg/ml) and also the best IC_{50} values of 1.031 and 1.051µg/ml.

The molecular docking study, revealed that compounds 3a (TPSA = 83.47 Å2) could permeate blood-brain barriers. Compound 3e (-11.14 kcal/mol) had a better *in silico* antibacterial activity than penicillin (-10.89 kcal/mol) while compound 3a (-14.90kcal/mol) had a better antioxidant activity than α-tocopherol (-14.82 kcal/mol).

Conclusion: All the synthesized compounds were confirmed to be likely drugs and potential antimicrobial and antioxidant, agents.

Keywords: Methionine; sulphonamides; carboxamides; molecular docking; antimicrobial activity; antioxidant activity.

1. INTRODUCTION

Microbial infections and oxidative stress have become common sources of untold misery to numerous individuals around the world [1-3]. Both disease conditions are related because oxidative stress leads to decrease in the adaptation mechanism of human body thereby increasing the body's susceptibility to microbial infections [4]. Shwatzman [5] reported that the use of methionine in the design of antibiotics resulted to improved antimicrobial activities. His findings demonstrated that penicillin susceptibility of gram-negative organisms was greatly improved by methionine addition in which he discovered that the action of methionine was synergistic rather than additive [5]. It is also important to note that pharmaceutically, methionine has been successfully used as intermediate precursors for antibiotics [6-7]. Similarly, Gabriel Pizzino et al [8] concluded that oxidative stress is the major contributor to the initiation and progression of numerous pathologies in man while antioxidants play a counteractive role in preventing, managing and treating these human pathologies. According to Luo and Levine 2009 [9], methionine contained in proteins functions as an endogenous antioxidant in animal cells thereby defending it against oxidative stress.

Methionine is a sulfur-containing amino acid which is totally indispensible in humans [10] and responsible for the production of crucial biochemical molecules namely glutathione, creatine, S-adenosylmethionine (SAM) and taurine [11-12]. Adequate intake of methionine can prevent Parkinson's disease [13], obesity [14], tract infections [15], colorectal cancer [16], improve the body immune system and bone development [17].

Additionally, sulphonamides a combining moiety are excellent antimicrobial agents [18], anticancer agents [19], antimalaria agents [20] antiretroviral agents [21], antihypertensive [22] and diuretics agents [23]. They were found to be a drug scaffold in pharmaceutical chemistry because of its tolerance and stability in mammals [24]. Moreover, carboxamides are one of the contents of drugs [25] for HIV [26] and hypertension [27] patients and have been successfully used in cholesterol reduction [28]. Other coupling partners namely aniline, pyridine and pyrimidine ultilized in this research work are valuable pharmaceuticals [29,30]. For instance, aniline plays a central role in paracetamol, tylenol and acetaminophen production [31] while pyridine and Pyrimidine being nitrogen–containing heterocyclics are very important class of compounds in medicinal chemistry [32]. Pyridine are employed as local anesthetics, CNS stimulants [33,34] and important vitamins [35] while pyrimidine are used in sulfa drugs and antifolates production [36]. The preponderance of drug resistant microorganisms has become an issue of concern and indeed a pressing global health challenge in the twenty first century [37]. For instance, the Infectious Society of America hinted that almost four out of every five of the 2 million individuals infected with hospital acquired bacterial infections in US hospitals annually displayed resistance to at least one drug [37,38]. Coates and Hu [39] suggested that in order to successfully combat the resistance challenge, new approaches to antibiotics development must be employed ensuring that the new drug molecules work on diverse drug target sites

different from the old ones [40]. In view of the urgent need for new antimicrobial and antioxidant agents with improved drug potency, we report in this research work synthesis of cysteine-based carboxamides with sulphonamide moiety. Moreover, the consequent synergistic antagonism arising from the multi-drug combination of aniline, pyridine and pyrimidine moieties as coupling partners were exploited in this research. We propose that since each individual coupling partner has pharmacological functions, their combination into one drug molecule will indeed improve their total drug potency.

2. MATERIALS AND METHODS

2.1 Materials

Reagents were imported from Sigma Aldrich. Melting points of the title compounds were carried out with electrothermal melting point apparatus and are uncorrected. Infrared spectra data were measured on 8400s Fourier Transform Infrared (FTIR) (ABU,Zaria,, Nigeria). Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) analysis were done using NMR spectrophotometer at Sandeep Verma Labouratory, Department of Chemistry, Indian Institute of Technology, Kanpur. Chemical shifts were measured in part per million with tetramethylsilane as reference point. The antimicrobial activity studies took place at the Department of Microbiology, University of Nigeria, Nsukka while the antioxidant studies were carried out at the Biochemistry Department, University of Nigeria, Nsukka.

2.2 Chemistry

General procedure for the synthesis of 4-methylbenzenesulphonamoyl carboxylic acids: Using a 100ml beaker, sodium carbonate (5.58g, 52.50mmol) was added to a solution of methionine (2)(25mmol) in water (30ml) with stirring to dissolve the solutes. The clear solution was cooled to 0^{o}C before the addition of *p*-toluenesulphonyl chloride (1) (5.12g, 30mmol) in five portions within 1 hour interval. The content was vigorously stirred for a period of 4 hours at room temperature and acidified to pH 2 with 2M hydrochloric acid achieve crystallization. With the aid of TLC (MeOH/DCM, 1:8), the reaction protocol was monitored. It was allowed to settle and the products were filtered by suction and washed with tartaric acid (pH 2.2) and dried in a desiccator to afford 2-{[(4-methylphenyl) sulphonyl]amino}-4-(methylsulfanyl)butanoic acid (3a).

2-{[(4-methylphenyl)sulphonyl])-4amino}-4-(methylsulphanyl)butanoic acid(3a): The amino acid was methionine, appearance: off-yellow oil, yield 2.64g(70.1%), mp, IR(KBr)cm^{-1}: 3272(N-H), 2987(OH of COOH), 2922, 2911(CH aliphatic), 2000(CH aromatic), 1595(C=C aromatic), 1319, 1151(S=O two bands), 812(Ar-H).^{1}H-NMR($CDCl_3$,400MHz)δ:7.77-7.75(d,J=8Hz, 2H, Ar-H), 7.31-7.29(d, J=8Hz, 2H, Ar-H), 4.09(S-br, 1H, NH), 2.52-2.48(m, 1H, CH-NH), 2.46-2.34(m,2H, CH_2-S), 2.42(s, 3H, CH_3-Ar), 2.12-2.05(m, 1H,CH of CH_2), 1.97(s, 3H, CH_3-S), 1.96-1.87(m, IH, CH of CH_2). ^{13}CNMR(DMSO,400MHz)δ:171.334(C=O), 146. 484,137.376, 133.817, 132.721, 129.121, 126.776(aromatic carbons), 49.665, 48.446, 47.211, 47.034, 46.845 (aliphatic carbons). Anal.calcd. for $C_{12}H_{17}NO_4S_2$ (303.136): C, 47.50, H, 5.65, N, 4.62, S, 21.09. Found: C, 47.47, H, 5.69, N, 4.58, S, 21.13.

Acylation of 2-{[(4-methylphenyl) sulphonyl] amino}propanoic acid (3): 2g of 2-{[(4-methylphenyl)sulphonyl]amino}-4-(methylsulfanyl)butanoic acid (3a) was transferred into a 100 ml Erlenmeyer flask, concentrated hydrochloric acid(9ml) and distilled water (25ml) were added and stirred. In a separate beaker, sodium acetate (16.0 g) was dissolved in distilled water (50 ml). Acetic anhydride(13 ml) was added in three portions within the period of 1hour to the solution of 2-{[(4-methylphenyl)sulphonyl]amino}-4 (methylsulfanyl)butanoic acid (3a) and poured into the sodium acetate solution. The mixture was stirred and immersed in an ice bath for 1 hour after which it was filtered to obtain 2-{acetyl[(4-methylphenyl)sulfonyl]amino}-4-(methylsulfanyl) butanoic acid(3b).

2-{Acetyl[(4-methylphenyl)sulfonyl]amino}-4-(methylsulfanyl)butanoic acid(3b): Yield 2.16g (93.6%), mp.216-217^{0}C, IR(KBr) Cm^{-1}: 3447(O-H of COOH), 3250(N-H), 2922(C-H aliphatic), 1919 (C-H aromatic), 1718, 1600 (C=O), 1494,1323 (C=C), 1222, 1155 (2S=O),1099(SO_2NH), 1030(C-N), 812(Ar-H). ^{1}HNMR ($CDCL_3$, 400MHz)δ: 7.162 (m, 2H, ArH), 7.165 (m, 2H, ArH). 4.23(S-br, 1H, NH), 2.52-2.43(m, 1H, CH-NH), 2.40-2.35(m,2H, CH_2-S), 2.31(s, 3H, CH_3-Ar), 2.20-2.10(m, 1H,CH of CH_2), 1.99(s, 3H, CH_3-S), 1.93-1.87(m, IH, CH of CH_2). ^{13}(NMR ($CDCL_3/C_6D_6$, 400MHz) δ:176.135, 170.123 (C=O), 137.402, 133.820, 133.638, 128.448, 127.970, 127.727 (aromatic carbons), 77.454, 77.135, 76.801, 71.332, 68.556, 63.447(aliphatic

carbons). Anal.calcd. for $C_{14}H_{19}NO_5S_2$ (345.43): C, 48.64, H, 5.50, N, 4.05, S, 18.53. Found: C, 48.67, H, 5.47, N, 4.09, S, 18.48.

2.2.1 Chlorination and aminolysis of 2-{acetyl[(4methylphenyl)sulfonyl]amino}-4-(methylsul-fanyl)butanoic acid(3b):

Chlorination: A three necked flask (250ml) was charged with 2-{acetyl[(4-methylphenyl) sulfonyl]amino}-4-(methylsulfanyl)butanoic acid (3b)(1mmol) and acetone (10 ml), stoppered and cooled to 0^oC. The mixture was stirred at 80^oC under reflux for 4 hours and transferred to water bath at 80^oC to enable the evaporation of excess thionyl chloride. Acetone (20 ml) was further added and evaporated twice to ensure complete evaporation of the remaining thionyl chloride in order to obtain the acid chloride product.

Aminolysis: The acid chloride product of the chlorination was immediately dissolved in acetone (20 ml) and cooled to 0^oC. Ammonia (2 ml) was added to enable crystallization and the mixture was allowed to stay for 12 hours, filtered and washed with acetone to obtain 2-{acetyl[(4-methylphenyl)sulfonyl]amino}-4-(methylsulfanyl)butanamide(3c).

2-{acetyl[(4-methylphenyl)sulfonyl]amino}-4-(methylsulfanyl)butanamide(3c): Yield 2.90g (89.8%), mp.224-225^{0}C, IR (KBr) cm^{-1}: 3168(N-H), 2922(C-H aliphatic), 1830 (C-H aromatic), 1699, 1695(2C=O), 1505,1423 (C=C), 1326, 1151 (2S=O), 1181(SO_2-NH), 1032 (C-N), 738 (Ar-H). ^{1}HNMR (DMSO, 400 Hz) δ: 6.845 (d, J = 7.4Hz, 2H, ArH), 6.745 (d, J = 7.2Hz, 2H, ArH), 6.461 (S, IH, NH) 6.555 (m, 2H, NH_2), 3.595 (S, 3H, CH_3 –C=O), 2.470 (S, 3H, CH_3- Ar), 1.784 (s, 3H, CH_3-S), 1.67-1.62(m, IH, CH of CH_2). ^{13}CNMR(DMSO, 400MHz)δ: 171.834, 169.334, (C=O), 136.484, 132.818, 132.629, 128.121, 127.886, 127.833(aromatic carbons), 47.662, 47.449, 47.244, 47.032, 46.819, 46.607, 46.395. Anal.calcd. for $C_{14}H_{20}N_2O_4S_2$ (344.45): C, 48.77, H, 5.81, N, 8.13, S, 18.58. Found: C, 48.82, H, 5.79, N, 8.20, S, 18.62.

2.2.2 Nickel catalysed synthesis for methionine-based sulphonamoyl carboxamide having aniline, pyridine and pyrimidine moieties

Preparation of bis (triphenylphosphine) nickel(ii)chloride: L.M Venanzi [41] reaction protocol was used for the preparation of the coordination compound. Nickel(II)chloride hexahydrate (2.37 g, 10 mmol) was dissolved in distilled water(2 ml) and diluted with glacial acetic acid(50 ml). A solution of triphenylphosphine ligand(5.25 g, 20 mmol) and glacial acetic acid (25 ml) was further added. A green precipitate was formed and allowed to settle for 24hours. The complex compound (dark blue crystal) was filtered by suction, washed with glacial acetic acid and dried in desiccators.

General procedure for the synthesis of aniline, pyridine and pyrimidine derivatives: Bis(triphenylphosphine)nickel(II)chloride(6.54 g, 10 mmol) and triphenylphosphine(5.25 g, 30mmol) were transferred to 50 ml Erlenmeyer flask. The *t*-butanol (4 ml) solvent and distilled water(2ml) were added using syringe. The mixture was stirred for 10 mins at room temperature under inert nitrogen atmosphere then heated at 80^oC for 1.5min. Then 2-{acetyl[(4-methylphenyl)sulfonyl]amino}-4-(methylsulfanyl) butanamide(3c) (10 mmol), potassium carbonate (1.38 g,10 mmol), various aryl and heteroaryl halides(4-chloroaniline, 4-amino-3-chloropyridine and 5-chloro-4,6-diaminopyrimidine) were added with *t*-butanol and H_2O being in 2:1 ratio under nitrogen inert atmosphere. It was refluxed with stirring for 1hour at 110^OC and allowed cool to room temperature. On the addition of ethyl acetate, crystals were formed and washed with water to afford methionine-based sulphonamoyl carboxamide derivatives(3d-f) in excellent yields.

2-{acetyl[(4-methylphenyl)sulphonyl]amino}-N-(4-aminophenyl)-4-(methylsulfanyl) butanemide (3d): Yield 3.04 g (91.5%), mp.93-94^{0}C, IR (KBr) cm^{-1}: 3350, 3320 (N-H), 2877(C-H aliphatic), 1927 (C-H aromatic), 1718, 1670(C=O), 1519, 1511(C=C), 1330, 1196 (2S=O),1151(SO2-NH), 1088(C-N), 771 (Ar-H). ^{1}HNMR ($CDCL_3$, 400MHz) δ: 8.197 (d, J= 8.4H_2, 2H, ArH), 7.608-7.522(m,2H, ArH), δ6.772(d, J = 8.3Hz, 2H, ArH), δ6.044(t, J = 7,1Hz, 2H, ArH), 4.835(s, br, IH, SH), 2.767(s,IH, NH), 2.7693(d, J = 7.8Hz, 2H, NH_2), 2.662(d, J = 7.1Hz, 3H,CH_3-C=O), 2.259-2.253 (m, 3H, CH_3-Ar), 2.241 (t, J=2.6Hz, 1H, CH), 1.884 (s, 3H, CH_3-S), 1.717-1.632(m, IH, CH of CH_2). ^{13}C-NMR ($CDCl_3$, 400MHz) δ: 170.443, 169.334, (C=O), 152.738, 146.584, 135.793, 134.852, 134.541, 132.257, 133.407, 131.346, 122.294, 120.442, 118.854, 115.653 (aromatic carbon), 83.373, 83.054, 82.728, 26.832, 26.460, 26.399 (aliphatic carbon). Anal.calcd. for $C_{20}H_{25}N_3O_4S_2$ (435.56): C, 55.10, H, 5.74, N, 9.64, S, 14.69. Found: C, 55.15, H, 5.68, N, 9.67, S, 14.82.

2-{acetyl-[(4-methylphenyl)sulfonyl]amino}-N-(4-aminopyridin-3-yl)-4-(methylsulfanyl) butanemide (3e): Yield 2.89g (94.0%), mp.97-98^{0}C, IR (KBr)cm^{-1}: 3570, 3362(N-H) 3063 (C-H aliphatic),1992(C-H aromatic), 1790, 1720(C=O), 1654(C=N), 1308, 1282(2S=O), 1182(SO_2.NH), 1177 (C-N), 995, 910(C=C), 812(Ar-H). ^{1}HNMR ($CDCL_3$, 400 MHz)δ: 7.766-7.711 (d, J= 2.2Hz 2H, ArH) 7.328 (m, 2H, ArH), 7.267.7.210 (d, J=22.8H_2 2H, ArH). 6.607 (s, IH, ArH), 6.019 (m, 2H, NH_2), 3.923-3.877(d, J=18.4Hz, 2H, NH_2), 2.494 (S,3H,CH_3-C=O), 2.421-2.346 (m,3H,CH_3-Ar), 1.911(S,2H, CH_2), 1.333 (s, 3H,CH_3-S). ^{13}C NMR ($CDCL_3$,400 MHz)δ: 170.332, 169.980 (C=O), 162.707 (C=N), 149.120, 142.664, 140.867, 138.902, 133.231, 130.641, 128.899, 125.534, 123.223, 120.865, 118.432 (aromatic carbons), 77.393, 77.074, 76.756, 31.870, 30.034 (aliphatic carbons). Anal.calcd. for $C_{19}H_{24}N_4O_4S_2$ (436.56): C, 52.23, H, 5.50, N, 12.87, S, 14.66. Found: C, 52.19, H, 5.47, N, 12.91, S, 14.72.

2-{acetyl-[(4-methylphenyl)sulfonyl]amino}-N-(4,6-diaminopyrimidin-5-yl)-4-(methylsulfanyl) butanamide(3f): Yield 3.04g (91.5%), mp.104-105^{0}C, IR (KBr)cm^{-1}: 3451, 3324, 3123(N-H), 2918 (C-H aliphatic) 1982((C-H aromatic), 1745, 1676(C=O), 1666, 1650 (C=N), 1620, 1613(C=C), 1319, 1274 (2S=O), 1092 (SO_2N), 1036(C-N), 890 (Ar-H). ^{1}HNMR (DMSO, 400MHz)δ: 7.660 (d, J= 8.8Hz, 2H, ArH), 6.542 (t, J = 7.8Hz, 2H, ArH), 6.521 (d, J = 7.2Hz, IH, ArH), 5.969 (s, IH, NH), 3.365 (m, 4H, NH_2), 2.826-2.771(m, 3H, CH_3-C=O), 2.477 (s,3H, CH_3 –C=O), 1.025 (S, IH, CH), 1.007-0.988 (d, J= 7.6H_2, 3H, CH_3- S) ^{13}(NMR (DMSO/$CDCL_3$,400MHz) δ: 170.086, 169.330 (C=O), 164.442, 160.132 (C=N), 140.603, 134.820, 130.123, 128.665, 125.124, 123.231, 120.432 112.937, 111.342, 110.234 (aromatic carbons), 40.529, 40.324, 40.111, 39.906 39.694, 39.489 (aliphatic carbon). Anal.calcd. for $C_{18}H_{24}N_6O_4S_2$ (452.55): C, 47.73, H, 5.30, N, 18.56, S, 14.14. Found: C, 47.68, H, 5.34, N, 18.61, S, 14.20

2.3 Biological Studies

2.3.1 Antimicrobial studies

Using Agar dilution method [42], the gold standard of susceptibility testing, the minimum inhibitory concentrations of the synthesized compounds were determined. Microorganisms used were *Pseudomonas aeruginosa, Salmonella typhi, Candida albicans, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Candida albicans, and Aspergillus niger* which were clinical isolates gotten from the department of pharmaceutical microbiology, University of Nigeria, Nsukka.

Standardization of the test organism suspension: 0.5 McFarland turbid equivalents was used to standardize the organisms.

Control test (standard): Standard antibiotics such as Ciprofloxacin(standard antibacterial agent) and Fluconazole(standard antifungal agent) were used.

Experimental: Sample suspension (4.0ml) of stock concentration 50mg/ml was trasferred to the sterile Petri dish and a double strength sterile molten agar (16.0 ml) was also introduced to the same plate and they were mixed together to obtain 1mg/ml concentration. Concentrations 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, 0.1mg/ml, were also obtained with the same formula $C_1V_1=C_2V_2$. The molten agar plates of the various concentrations of the compound were allowed to gel and the plates were separated into seven equal parts using a permanent marker. Then the test microorganisms were streaked on the segments and labeled. The incubation process of the culture plates was carried out in inverted position at 37^{o}c for 24hours, and at 25^{o}c for 48hours. After the incubation, the plates were monitored for sensitivity and resistivity of the organisms to the synthesized compounds, and the observation was recorded. The lowest concentration of compounds that hindered the growth of the microorganism was considered to be the minimum inhibitory concentration of that compound against the microorganism. Further incubation of the plates was carried out for another 24hour at 37^{o}C and 48hours at 25^{o}C to ascertain whether the activity was bacteriostatic or bactericidal. The results were appropriately recorded.

2.3.2 Antioxidant studies

Antioxidant activity by DPPH method: Using Blois method [43], the antioxidant activities of the title compounds were measured *in vitro* by the inhibition of generated stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The preparation method of the DPPH solution involved dissolving 1.9 mg of DPPH in 100 ml of methanol. Then 50, 100 and 200 µg/ml

concentrations of the DPPH soluion were also prepared. Each of the title compounds (2 gm) was weighed and dissolved in 10 ml of the appropriate solvent. The stock solution (200µg/ml) was further diluted to generate 100 and 50 µg/ml for each of the compound. The ascorbic acid standard solution was prepared using the same method. 1 ml of DPPH solution was added to 2 ml solution of the compounds and ascorbic acid. The mixture was thoroughly shaken and left in the dark at room temperature for 30 minutes. Using a spectrophotometre at the wavelength of 517 nm, the absorbance of the mixture was recorded in triplicate against the corresponding blank solution. The percentage scavenging DPPH radical inhibitions were calculated with the following formula:

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

Where, $Abs_{control}$ = the absorbance of DPPH radical and n-hexane/methanol, Abs_{sample} = the absorbance of DPPH radical and sample/standard.

The concentration of compounds providing 50% inhibition (IC_{50}) was calculated by plotting the graph of percentage inhibition against the concentration of compounds used.

2.3.3 *In silico* methodology

Physicochemical properties: The physicochemical properties of the synthesized compounds were obtained *in silico*. They are 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), total polar surface area (TPSA) and aqueous solubility (SlogP),. The descriptors calculator in Molecular Operating Environment (MOE, 2018) was used to compute these parameters. Using Lipinski's rule of five, the drug-likeness of the compounds were investigated.

2.3.4 Molecular docking

The molecular docking study focused on bacterial infections, fungal infections and oxidative stress. The right drug target was selected for each of the disease conditions for molecular docking studies. The antibacterial drug target was *E. coli* DNA gyrase in complex with 1-ethyl-3-[8-methyl-5-(2-methyl-pyridin-4-yl) isoquinolin-3yl]urea (PDB code: 5MMN), antifungal drug target was urate oxidase from *Aspergillus flavus* complexed with uracil (PDB code: 1WS3), and antioxidant drug target was human peroxiredoxin 5 (PDB code: 1HD2). The 3-Dimensional structures of the chosen drug targets were downloaded from the Protein Data Bank (PDB), (http://www.pdb.org) database. The drug targets were loaded in Molecular Operating Environment (MOE, 2018) and prepared using the QickPrep in MOE. MMFF94 force field was used for energy minimization of the ligand molecules. The prepared compounds were made to interact with each of the selected receptors through molecular docking. This protocol enables a flexible compound docking for various compound conformers within the rigid receptor. Best conformation was selected for each of the title compounds and their interaction was visualized with the aid of the Discovery studio.

3. RESULTS AND DISCUSSION

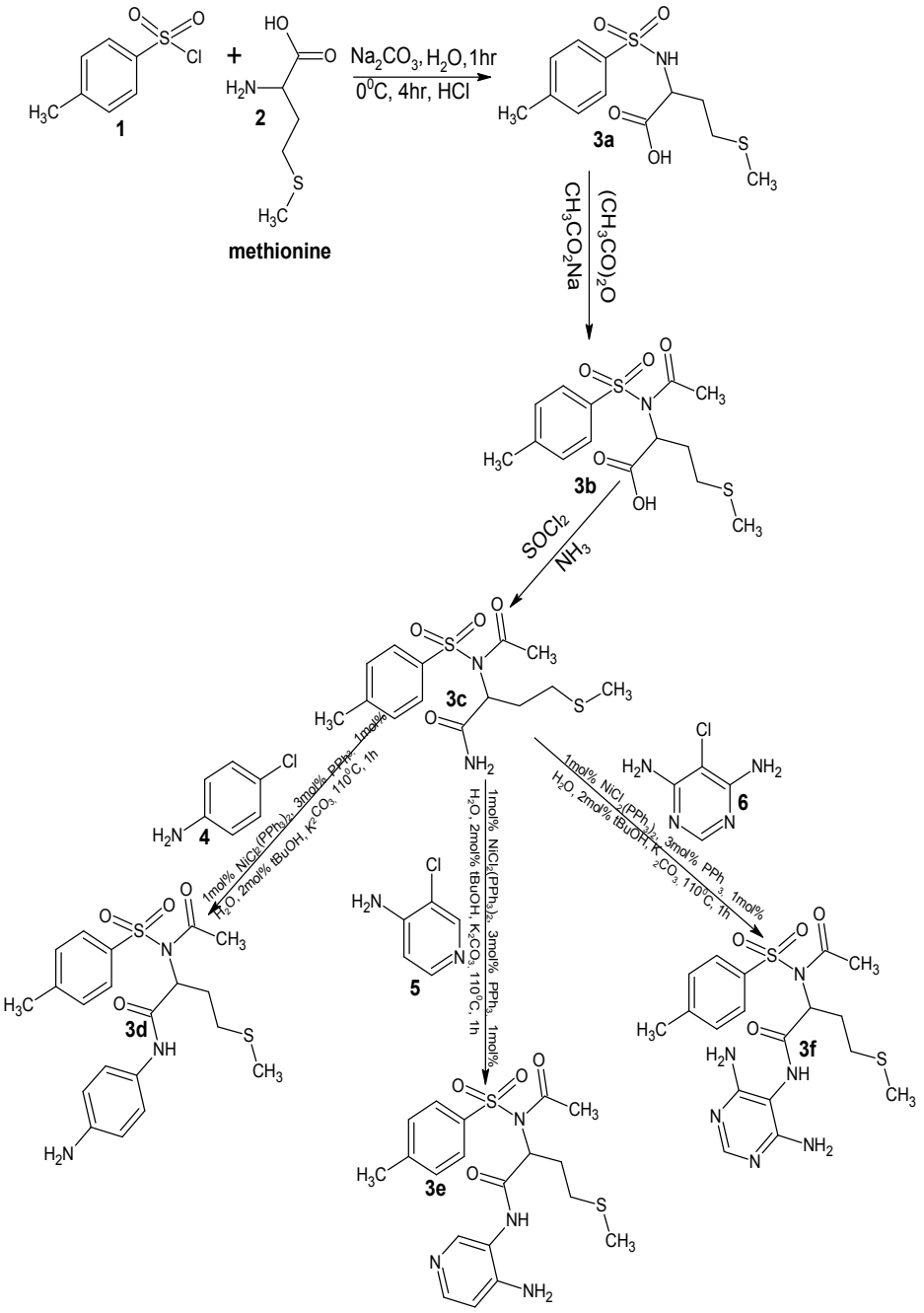
3.1 Chemistry

The Synthetic pathway for methionine-based bioactive compounds bearing sulphonamide, carboxamide, aniline, aminopyridine and aminopyrimidine pharmacophores is shown in scheme 1. The purpose of acylating sulphonamoyl carboxylic acid(3a) was to protect the amino group of the methionine from unwanted side reactions during chlorination and aminolysis, and to enable regioselectivity, while chlorination and aminolysis were carried out for the of achieving the formation of carboxamide from un-activated carboxylic acid end of the methionine [44]. To ensure a successful drug design, we took into consideration the bioavailability and drug-likeness of the compounds through the evaluation of their physicochemical properties using "Lipinski's rule of five".

3.2 Biological Studies Results

3.2.1 Antimicrobial studies

The results of antimicrobial Studies (Table I) indicated that all the synthesized compounds have antimicrobial activities, however, *C. albicans* fungi showed resistance to all of the compounds. Compounds 3a and 3e were the best antifungal agents since they were the only compounds not resisted by *A. niger* fungi.



Scheme 1. Synthetic pathway for methionine-based bioactive compounds

Certainly, the resistance exhibited by *Candida, albican* against all thesynthesized compounds is attributed to the fact that the intracellular pools of free amino acids in pathogenic *candida albican* species contain higher level of methionine and glycine than any other amino acids, so the presence of methionine in the synthesized compound undermined its ability to combat pathogenic *candida albican* fungi [45]. Compounds 3a, 3b and 3e were the most outstanding antibacterial agents having not been resisted by any of the bacteria used. Summarily, compound 3a and 3e were found to be the most potent antimicrobial (antibacterial and antifungal) agents synthesized.

Table 1. Antimicrobial activities of synthesized compounds

	Minimum inhibitory concentration (mg/ml)						
Compound No	***E.coli***	***S.typhi***	***S.aureus***	***B. sub***	***Ps.aerug***	***C.albicans***	***A. niger***
3a	0.8	0.6	0.7	0.6	0.7	+	1.0
3b	0.7	0.7	0.9	0.5	0.7	+	+
3c	0.9	0.7	0.8	0.4	+	+	+
3d	0.4	0.5	0.3	0.4	+	+	+
3e	0.8	0.9	0.9	0.7	0.8	+	0.9
3f	0.8	0.6	0.9	0.8	+	+	+
Ciprofloxacin	0.025	0.015	0.025	0.020	0.025	+	+
Fluconazole	+	+	+	+	+	0.020	0.005

Key: + implies no activity. Ciprofloxacin = antibacterial standard drug, Fluconazole = antifungal standard drug

3.3 Antioxidant Studies

The *in vitro* assay results in Table 2 indicated that all the tested compounds had antioxidant activities which would be helpful in the prevention of the progress of various oxidative stresses. Only compounds 3a and 3e (93.53% and 93.28% inhibition at the highest concentration 200 µg/ml) had a comparable antioxidant activity with ascorbic acid (96.83% inhibition at 200 µg/ml). The lower the IC_{50} value the more the antioxidant potential. The IC_{50} values of compounds 3a and3e (1.031 and 1.051 µg/ml) were comparatively almost as low as ascorbic acid thereby suggesting that compounds 3a and 3e were the most potent antioxidant agent.

3.4 Molecular Docking Studies

3.4.1 Drug-likeness and oral bioavailability property of compounds

Drug-likeness establishes a balance between the molecular characteristics and the structural patterns that determines the resemblance of a compound to already existing drugs.

Amongst all the drug-likeness evaluation principles, Lipinski's rule of 5 (ro5) appears to be the commonest [46] while Verber's principle has become popular in oral bioavailability evaluation of compounds [47]. These protocols helped us to determine the blood-brain barrier likeness, the penetrability and bioavailability which are a function of the topological polar surface area (TPSA) [48]. Table 3 indicated that all the synthesized compounds perfectly agreed with ro5 which propounded that LogP ≤ 5, MW ≤ 500, HBA ≤ 10, HBD ≤ 5, and therefore would not pose oral bioavailability challenges and NRB ≤ 10 for acceptable molecular flexibility that ensures excellent permeability and oral bioavailability. Similarly, TPSA is employed in drug design as a determinant of cell permeability consequent on the principle that a compound having TPSA ≤140 Å2 can permeate the cell and have a high probability of good oral bioavailability [47], while compounds having TPSA ≤ 90 Å2 can permeate the blood-brain-barrier (BBB) and the central nervous system (CNS) [49]. Based on these principles, all the tested compounds except 3f can permeate the cell membrane while only compounds 3a (TPSA = 83.47 Å2) can permeate blood-brain-barriers. This suggests that compound 3a can be used in treatment of CNS related diseases such as Alzheimer's diseases and celebral malaria.

Table 2. Percentage inhibition and IC_{50} values of compounds

	200 µg/ml	**100 µg/ml**	**50 µg/ml**	**IC_{50} Values (µg/ml)**
Compounds	**% inhibition**	**% inhibition**	**% inhibition**	
Ascorbic acid	96.83± 0.001	97.68 ± 0.001	97.31 ± 0.001	0.999
3a	93.53± 0.001	82.78 ± 0.001	72.83 ± 0.001	1.031
3b	84.86 ± 0.001	76.62 ± 0.001	78.63 ± 0.001	1.238
3c	90.72 ± 0.001	85.71 ± 0.001	66.36 ± 0.001	1.235a
3d	54.64 ± 0.001	45.85 ± 0.001	51.89 ± 0.003	1.990
3e	93.28 ± 0.001	93.53 ± 0.001	92.06 ± 0.001	1.051
3f	85.59 ± 0.001	87.06 ± 0.001	80.04 ± 0.004	1.173

Key: Ascorbic acid is the standard antioxidant drug used. Values are expressed as mean ± SD of three replicates

Table 3. Physicochemical properties

Mol	HBA	HBD	NRB	logP(o/w)	SlogP	TPSA	MW	Lip violation
3a	4	3	7	1.92	1.48	83.47	303.40	0
3b	5	2	8	1.87	1.74	91.75	345.44	0
3c	4	1	8	1.14	1.14	97.54	344.46	0
3d	4	2	9	2.48	2.87	109.57	435.57	0
3e	5	2	9	1.24	2.27	122.46	436.56	0
3f	6	3	9	0.23	1.25	161.37	452.56	0

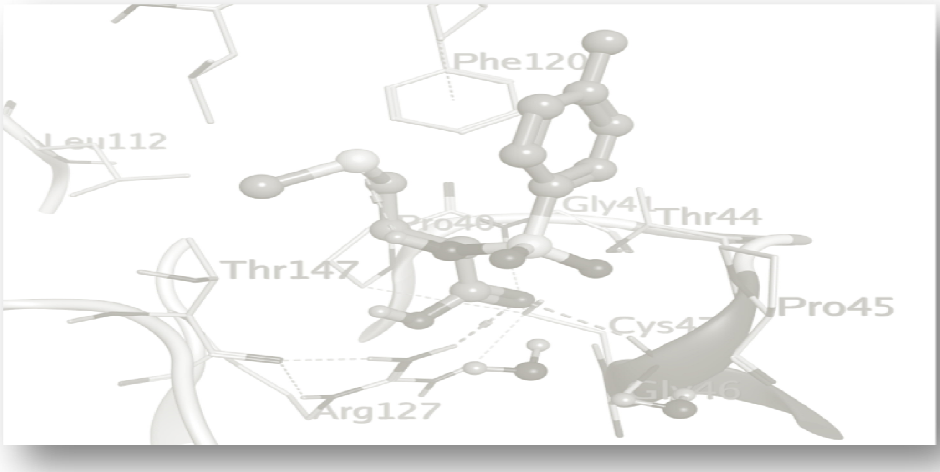


Fig. 1. The binding pose of compound 3a in the binding cavity of 1HD2

Table 4. *In silico* antibacterial, antifungal and antioxidant activities results

Compound	Antibacterial	Antifungal	Antioxidant
	5MMN	1WS3	1HD2
3a	-10.25	-10.53	-14.90
3b	-9.46	-9.49	-11.33
3c	-9.28	-9.41	-10.39
3d	-9.94	-10.04	-10.89
3e	-11.14	-9.53	-10.42
3f	-9.76	-9.24	-11.33
Standard drug	-10.89	-10.85	-14.82

Key: 5MMN, 1WS3 and 1HD2 = drug target for antibacterial, antifungal and antioxidant activities. The standard drugs are penicillin, ketoconazole and α-tocopherol respectively

3.4.2 In silico antibacterial, antifungal and antioxidant activities studies

Table 4 presented the calculated free binding energies (binding affinities). Interestingly, all the tested compounds showed strong binding affinities with all the drug receptors used for this study. For the antibacterial study, amongst the compounds tested on the DNA gyrase receptor 5MMN, compound 3e had a better binding affinity (-11.14 kcal/mol) than penicillin (-10.89 kcal/mol). This indicates that compound 3e could serve as a better antibacterial agent than penicillin. Similarly, the antifungal study revealed that compounds 3a and 3d had the best and comparable binding affinity (-10.53 and -10.04kcal/mol respectively) but not as high as ketoconazole (-10.85 kcal/mol). Finally, while all the tested compounds exhibited *in silico* antioxidant activities, only compound 3a had a higher binding affinities(-14.90 kcal/mol) with 1HD2 than α-tocopherol (-14.82 kcal/mol). This indicates that compound 3a could be a better antioxidant than α-tocopherol.

In order to ensure a better drug design, the molecular interactions of compound 3a with the antioxidant drug receptors was studied by the examination of its binding poses in the binding cavities of the drug receptors shown in Fig. 1.

Fig. 1 highlights the stereo view of compound 3a in the binding sites of human peroxiredoxin 5 (1HD2). Compound 3a interacted with various amino acid resideues of 1HD2, causing an observable antoxidant activity *in silico* and *in vitro.* H-acceptor interaction existed between O-17 of 4 and the N GLY 46. The interaction's distance and energy were found to be 3.28 Å and -0.5 kcal/mol respectively. Similarly, O-17 of 3a interacted with the NH2 of ARG 127 (3.41Å and -0.6 kcal/mol) via H-acceptor interaction. The S-18 of 3a had three H-acceptor bonds with CD1 LEU 116, CD2 LEU 116 and CD1 ILE 119 respectively.

4. CONCLUSION

In conclusion, we have reported a facile synthesis of methionine-derived sulphonamoyl carboxamides bearing moieties of pharmacological importance. The assigned structures complied with the spectral data. Compounds 3a and 3e were found to be the most potent antimicrobial (antibacterial and antifungal) agents and the also exhibited excellent antioxidant activities. The molecular docking study, revealed that all the tested compounds except 3f could permeate the cell membrane while only compounds 3a (TPSA = 83.47 å2) could permeate blood-brain-barriers. however, compound 3e (-11.14 kcal/mol) could serve as a better antibacterial agent than penicillin (-10.89 kcal/mol) while compound 3a (-14.90 kcal/mol) could serve as a better antioxidant than α-tocopherol (-14.82 kcal/mol). The physicochemical parameters investigation indicated that all the compounds were likely drugs with good oral bioavailability. The title compounds were found to be potential antimicrobial and antioxidant agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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