# New Antioxidant Agents Bearing Carboxamide Moiety: Synthesis, Molecular Docking and in Vitro Studies of New Benzenesulfonamide Derivatives

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**ABSTRACT:** *Oxidative stress occurs when oxygen radical formation and levels exceed those of antioxidants, potentiating cell responses such as apoptosis, tumorigenesis, and immune response. In an event of microbial invasion, there is a production of Reactive-Oxygen-Species (ROS), which when in excess can lead to oxidative stress. Some of these microorganisms are opportunistic pathogens implicated in chronic inflammatory conditions including cystic fibrosis. The synthesis, anti-inflammatory, antimicrobial and antioxidant activities of ten new derivatives of benzenesulfonamide bearing carboxamide functionality are herein reported. The base promoted reactions of the appropriate amino acids with substituted benzenesulfonyl chlorides gave the benzene sulphonamides (**3a-j**) in excellent yields. Palladium mediated amidation of the benzenesulfonamide (**3a-j**) and ethylamine gave the new carboxamides (**4a-j**) in excellent yield.All the compounds possessed good antioxidant activity but only compound **4e** ($IC_{50}$ 0.3586 mg/mL) had comparable activity with vitamin C ($IC_{50}$ 0.2090 mg/mL). Compound **4e** inhibited carrageenan-induced rat-paw edema at 95.58, 88.79, and 86.96 % each at 1 h, 2 h, and 3 h respectively. In the antimicrobial activity study, compound **4f** (MIC 7.23 mg/mL) was most potent against E.coli, compound **4j** (MIC7.11, 6.42 and 6.32 mg/mL) was the most active against S.aureus, P.aeruginosa and B.subtilis, compound **4h** (MIC 7.12 and 6.48 mg/mL) was most active against S.typhi and C.albicans respectively, compound **4c** (MIC 6.63 mg/mL) was the most active against A.niger.*

**KEYWORDS:** *Benzenesulfonamide; Carboxamide; Antioxidant; Anti-inflammatory; Antimicrobial.*

## INTRODUCTION

Many human diseases are caused by oxidative stress that results from an imbalance between the formation and neutralization of pro-oxidants[1]. Oxidative stress initiated by free radicals such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide, and peroxynitrite, plays a vital role in damaging various cellular macromolecules. These include DNA molecular proteins along with lipid peroxidation. This damage may result in many diseases

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including diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis[2]. However, human cells have an array of protection mechanisms to prevent the production of free radicals and oxidative damage[3]. These mechanisms include both enzymatic and non-enzymatic antioxidants such as superoxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol[4]. The protective roles of these enzymes may be disrupted as a result of various pathological processes and thereby causes damage to cells. Antioxidant supplement has been reported to reconcile the upshot of these radicals by directly reacting and quenching their catalytic metal ions[5]. Several antioxidants like Butylated Hydroxy Anisole (BHA) and BHT are reported to be toxic to both animals and human beings[6].

Sulphonamides constitute an important class of drugs.Sulphonamides and carboxamides functionalities have been reported to have wide biological activities ranging from anticancer[7], antitubercular[8], anti-inflammatory[9], antioxidant[10], antimicrobial[11], antimalarial[12] antibacterial[13] analgesic[14]etc.

This work was designed based on the reported biological activities of sulphonamides and carboxamides and the need to develop newer antioxidant agents that will have an added advantage of acting as anti-inflammatory and antimicrobial agents.

## EXPERIMENTAL SECTION

### *General*

All reactions requiring inert atmosphere were carried out under nitrogen atmosphere. Drying of solvent was achieved using molecular sieve for 48 h. All reagents were of technical grade: Benzenesulphonyl chloride, *p*-toluenesulphonyl chloride, amino acids (L-proline, L-leucine, L-methionine, L-threonine and L-serine), concentrated hydrochloric acid, ethylamine, and bis(dibenzylideneacetone) palladium (0) were purchased from Sigma-Aldrich and were used without further purification while sodium carbonate, tartaric acid, sodium hydroxide and toluene were purchased from Joe-chem store University of Nigeria Nsukka. The melting points were determined using Fischer Johns melting point apparatus and were uncorrected. Infra-red spectral data were recorded on a FTIR-8400S Fourier Transform Infrared Spectrophotometer using KBr disc and absorptions were given in per centimeter ($cm^{-1}$). The $^{1}H$ and $^{13}C$NMR spectra were recorded in DMSO-$d_6$ using Varian NMR 400 MHz at Indian Institute of Technology, Kanpur, the chemical shifts (δ) were recorded in ppm. Mass spectra were obtained using micro TOF electrospray time flight (ESI-TOF) mass spectrometer, sodium formate was used as the calibrant. All laboratory syntheses were carried out at staff research laboratory, University of Nigeria, Nsukka. The molecular simulations were done using vina software. The antioxidant and anti-inflammatory activities were done at the Department of Biochemistry, Universityof Nigeria, Nsukka. The antimicrobial activities were done at the Department of pharmaceutics, University of Nigeria, Nsukka.

### *General Procedure for the Synthesis of substituted benzene sulphonamoyl alkanamides (3a-j)*

Sodium carbonate ($Na_2CO_3$, 1.59g, 15 mmol) was added to a solution of the appropriate amino acid (**2**, 12.5mmol) in water (15 mL) with continuous stirring until all the solutes dissolved. The solution was cooled to -5ºC and the appropriate benzenesulfonyl chloride (**1**, 15 mmol) was added in four portions over a period of 1 h. The slurry was further stirred at room temperature for 4 h. The progress of the reaction was monitored using thin layer chromatography(TLC) (MeOH/DCM) 1:9). Upon completion, the mixture was acidified using 20 % aqueous hydrochloric acid to pH2. The pure products ( **3a-j**) were dried over self-indicating fused silica gel in a desiccators[15].

### *General procedure for the palladium-catalyzed amidation of unactivated carboxylic acid and ethylamine*

To a suspension of substituted benzenesulfonamide (**3a-j**, 1.0 mmol) in dry toluene (40 mL) equipped with Dean-Stark apparatus for azeotropic removal of water was added ethylamine (1.0 mmol) and $Pd_2(dba)_3$ (0.1 mmol) at room temperature and then refluxed for 12 h. On completion (as monitored by TLC) the amide products were precipitated out from the reaction mixture by adding 40 mL n-hexane. The carboxamides (**4a-j** ) were obtained via suction filtration, washed with n-hexane and dried over fused silica gel or concentrated using a rotary evaporator and dried over vacuum in the case of oily products.

***N-ethyl-1-[(4-methylphenyl)sulphonyl]pyrrolidine-2-carboxamide (4a)***

Yield (0.8 g, 80.0 %), brownish oil.Uv (λmax): 202.00 nm FTIR (KBr,$cm^{-1}$): 3406 (OH), 3280 (NH), 3060 (C-H aromatic), 2978 (C-H aliphatic), 1722 (C=O), 1599, 1449 (C=C), 1334, 1197 (2S=O), 1156, ($SO_2$NH), 1092, 1010 (C-N).$^1$HNMR (DMSOd6,400 MHz)δ: 8.49-8.47 (d, J=8.40 Hz, 2H, ArH), 8.17-7.99 (m, 2H, ArH), 7.28-7.26 (t, J=8.40 Hz, 1H, NH), 3.03-2.98 (m, 1H, CH), 2.59 (m, 2H, $CH_2$-$CH_3$), 2.46 (t, J=4.80 Hz, 3H, $CH_3$-$CH_2$), 2.33 (s, 3H, $CH_3$-Ar), 2.04 (m, 2H, $CH_2$-N), 1.17-1.13 (m, 4H, $2CH_2$, $CH_2CH_2$-CH).$^{13}$CNMR (DMSOd6, 400MHz)δ: 172.02 (C=O), 151.53, 143.33, 132.30, 125.69, 123.90, 112.96 (six aromatic carbons), 49.38, 45.83, 31.24, 27.53, 26.37, 8.95 (six aliphatic carbons).HRMS (m/z) : 297.1123 (M+H), calculated, 296.1127.

***N-ethyl-1-(phenylsulphonylsulphonyl)pyrrolidine-2-carboxamide (4b).***

Yield (0.2 g, 74.0 %), brownish oil. Uv (λmax): 372.00 nm, 308.00 nm. FTIR (KBr, $cm^{-1}$): 3384 (NH), 3063 (C-H aromatic), 2974, 2877 (C-H aliphatic), 1722 (C=O), 1617, 1476 (C=C), 1397, 1334 (2S=O), 1196, 1155 ($SO_2$NH), 1092, 995.2 (C-N).$^1$HNMR (DMSOd6,400 MHz)δ: 8.20-8.18 (d, J=7.59 Hz, 2H, ArH), 8.11-7.99 (m, 2H, ArH), 7.57-7.54 (t, J=6.60 Hz, 1H, ArH), 7.24-7.22 (t, J=7.32, 1H, NH), 3.03-2.98 (m, 1H, CH), 2.60 (m, 2H, $CH_2$-$CH_3$), 2.44 (t, 3H, $CH_3$-$CH_2$), 2.04 (m, 2H, $CH_2$-N), 1.15-1.13 (m, 4H, $2CH_2$, $CH_2$-$CH_2$-CH).$^{13}$CNMR (DMSOd6, 400MHz)δ: 156.27 (C=O), 154.52, 149.36, 128.04, 127.42, 124.76, 123.89, (six aromatic carbons), 57.04, 45.84, 22.03, 21.86, 8.93 (five aliphatic carbons).HRMS (m/z) : 283.1076 (M+H), calculated, 282.1079.

***N-ethyl-4-methyl-2-{[(4-methylphenyl)sulphonyl]amino}pentanamide (4c).***

Yield (0.25 g, 83.3 %), Mp 98-100 ºC. Uv (λmax): 207.00 nm. FTIR (KBr, $cm^{-1}$): 3220.4 (NH), 3067.6 (C-H aromatic), 2955.8,b2870.1 (C-H aliphatic), 1647.5 (C=O), 1558.0, 1494.7, 1461.1 (C=C), 1390.3, 1312.0 (2S=O), 1202.7, 1151.7, ($SO_2$NH), 1095.8, 976.6 (C-N).$^1$HNMR ($DMSOd_6$,400 MHz)δ: 7.99-7.97 (d, J=6.64, 1H, NH), 7.59-7.58 (d,J=7.32, 2H, ArH), 7.29-7.27 (d, J=7.76, 2H, ArH), 3.15-3.13 (t, J=7.76, 1H,CH of O=C-CH), 2.67-2.62 (m, 2H, $CH_2$-$CH_3$), 2.31 (s, 3H, $CH_3$-Ar), 1.72-1.69 (m,1H, $CH(CH_3)_2$), 1.39-1.34 (m, 1H, CHa of $CH_2$-$CH(CH_3)_2$), 1.31-1.26 (m, 1H, CHb of $CH_2$-$CH(CH_3)_2$), 1.03-1.02 (t, J=5.96 Hz, 3H, $CH_3$-$CH_2$), 0.76-0.73 (m, 6H, $(CH_3)_2$-CH).$^{13}$CNMR (DMSOd6, 400MHz)δ: 174.10 (C=O), 142.73,138.34,129.84, 127.23 (four aromatic carbons), 56.32, 43.33, 34.32, 24.51, 23.51,22.69, 21.46, 13.19 (eight aliphatic carbons).HRMS (m/z) : 312.1022 ($M^+$), calculated, 312.1022.

***N-ethyl-4-methyl-2-[(phenylsulphonyl)amino]pentanamide (4d)***

Yield (0.25 g, 83.3 %). Mp 94-96 ºC. Uv (λmax): 204.00 nm. FTIR (KBr, $cm^{-1}$): 3227 (NH), 3068 (C-H aromatic), 2959, 2870 (C-H aliphatic), 1718 (C=O), 1610, 1528, 1446 (C=C), 1416.4, 1308.3 (2S=O), 1159.2 ($SO_2$-NH), 1092.1, 985.4 (C-N).$^1$HNMR ($DMSOd_6$,400 MHz)δ: 8.10-8.08 (d, J=8.8 Hz, 1H, NH), 7.73-7.71 (d, J=6.88 Hz, 1H, ArH), 7.55-7.47 (m, 4H, ArH), 3.46-3.43 (m, 1H,CH of O=C-H), 3.14-3.12 (m, 2H, -$CH_2$-$CH_3$), 2.71-2.66 (m, 1H, CH-$(CH_3)_2$, 1.60-1.53 (m, 1H, CHa of $CH_2$-$CH(CH_3)_2$), 1.38-1.30 (m,3H,-$CH_3$-$CH_2$), 1.05-1.02 (m, 1H, CHb of $CH_2$), 0.75-0.73 (d, J=6.40 Hz, 3H,$CH_3$a of $CH(CH_3)_2$), 0.66-0.64 (d, J=6.40 3H, $CH_3$b of $CH(CH_3)_2$).$^{13}$CNMR ($DMSOd_6$, 400MHz)δ: 174.11 (C=O), 141.58,132.69,129.41, 127.02 (four aromatic carbons), 55.48, 49.12, 42.24, 34.42, 24.41, 23.28, 13.11 (seven aliphatic carbons).HRMS (m/z) : 297.1014 (M-H), calculated, 298.1011.

***N-ethyl -2-{[(4-methylphenyl)sulphonyl]amino}-4-(methylsulphanyl)butamide) (4e).***

Yield (0.25 g, 83.3 %). Mp, 118-120 ºC. Uv (λmax): 204.00 nm. FTIR (KBr, $cm^{-1}$): 3198.1 (NH), 3080 (C-H aromatic), 2918.5 (C-H aliphatic), 2611 (S-$CH_3$) 1718.3 (C=O), 1595.3,1494.7 (C=C), 1397.8, 1319.5 (2S=O), 1215.1, 1151.7 ($SO_2$NH), 1088.4, 967.6 (C-N).$^1$HNMR ($DMSOd_6$,400 MHz)δ: 7.58-7.56 (d, J=7.84 Hz, 1H, NH), 7.62-7.57 (t, J=8.24 Hz, 2H, ArH), 7.32-7.27 (t, J=8.24 Hz, 2H, ArH), 3.12-3.09 (m, 1H, CH-C=O), 2.76-2.70 (m, 2H, $CH_2CH_3$), 2.32-2.29 (t, J=6.68 Hz, 3H, $CH_3$-Ar), 2.25-2.22 (m, 2H, $CH_2$-S), 1.89 (s, 3H, $CH_3$-S), 1.78-1.76 (t, J=6.20 Hz, 2H, $CH_2$-CHC=O), 1.08-1.01 (m,3H, $CH_3$-$CH_2$). $^{13}$CNMR (DMSOd6, 400MHz)δ: 172.78 (C=O), 143.03,138.44,129.97, 128.64, 127.10, 126.00 (six aromatic carbons), 55.74, 49.12, 34.56, 32.76, 29.79, 21.47, 13.07 (seven aliphatic carbons).HRMS (m/z) : 331.1088 (M+H), calculated, 330.1086.

***N-ethyl-4-(methylsulphanyl)-2-[(phenyl)sulphonyl)amido] butamide (4f).***

Yield (0.25 g, 83.3 %). Mp, 110-112 ºC Uv (λmax): 202.00 nm. FTIR (KBr, $cm^{-1}$): 3220 (NH), 2992 (C-H aromatic), 29189 (C-H aliphatic), 2653 (S-$CH_3$), 1704 (C=O), 1580, 1446 (C=C), 1379, 1319 (2S=O), 1156 ($SO_2NH$), 1088, 977 (C-N).$^1$HNMR (DMSO$d_6$,400 MHz)δ: 7.88-7.86 (d, J=7.99 Hz, 1H, NH), 7.76-7.69 (t, J=7.44 Hz, 2H, ArH), 7.54-7.50 (t, J=6.32 Hz, 1 H, ArH), 7.37-7.32 (t, J=6.40 Hz, 2H, ArH), 3.22-3.18 (m, 1H, CH-C=O), 2.86-2.82 (m, 2H, $CH_2CH_3$), 2.55-2.52 (m, 2H, $CH_2$-S), 1.91 (s, 3H, $CH_3$-S), 1.82-1.79 (t, J=6.40 Hz, 2H, $CH_2CHC$=O), 1.08-1.01 (m,3H, $CH_3$-$CH_2$). $^{13}$CNMR (DMSO$d_6$, 400MHz)δ: 173.64 (C=O), 142.03, 138.46, 129.97, 128.84, (four aromatic carbons), 55.74, 50.12, 38.46, 30.44, 28.99, 13.24 (six aliphatic carbons).HRMS (m/z) : 317.1044 (M+H), calculated, 316.1043.

***N-ethyl-3-hydroxy-2-{[(4-methylphenyl)sulphonyl]amino} butanamide (4g)***

Yield (0.25 g, 73.3 %). Brownish oil. Uv (λmax): 204.00 nm. FTIR (KBr, $cm^{-1}$): 3391 (OH), 3220 (NH), 3063 (C-H aromatic), 2981, 2929 (C-H aliphatic), 1733 (C=O), 1625, 1453, 1496 (C=C), 1397, 1326 (2S=O), 1156, 1122 ($SO_2NH$), 1033, 932 (C-N, C-O).$^1$HNMR (DMSO$d_6$,400 MHz)δ: 7.88-7.87 (d, J=8.40 Hz, 1H, NH), 7.76-7.74 (m, 2H, ArH), 7.55-7.47 (m, 2H,ArH), 3.50-3.48 (dd, $J_1$=6.42 Hz, $J_2$=5.94 Hz, CH-C=O ), 3.29 (s, 1H, OH), 2.46-2.44 (m, 1H, CH-OH), 2.33 (s,3H, $CH_3$-Ar) 1.91-1.86(m, 2H, -$CH_2$-$CH_3$), 0.77-0.72 (m, 6H, 2$CH_3$). $^{13}$CNMR (DMSO$d_6$, 400MHz)δ: 171.58 (C=O), 141.23, 132.78, 129.54, 128.67, (four aromatic carbons), 60.43, 41.32, 29.14, 21.98, 19.52, 18.87, (six aliphatic carbons). HRMS (m/z) : 299.1094 (M-H), calculated, 300.1096.

***N-ethyl-3-hydroxy-2-[(phenylsulphonyl)amino]butanamide (4h)***

Yield (0.26 g, 89.7 %). Brownish oil.Uv (λmax): 265.00 nm. FTIR (KBr, $cm^{-1}$): 3570 (NH), 3485 (OH), 3160 (C-H aromatic), 2978, 2922 (C-H aliphatic), 1710 (C=O),1576, 1521, 1446 (C=C), 1379, 1308 (2S=O), 1159 ($SO_2NH$), 1025, 909 (C-N, C-O).$^1$HNMR (DMSOd6,400 MHz)δ: 7.99-7.97 (d, J=9.16 Hz, 1H, NH), 7.74-7.72 (m, 2H, ArH), 7.58-7.48 (m, 3H,ArH), 3.48-3.45 (dd, $J_1$=6.44 Hz, $J_2$=5.96 Hz, 1H, CH-C=O ), 3.30 (s, 1H, OH), 2.46-2.45 (m, 1H, CH-OH), 1.92-1.84(m, 2H, -$CH_2$-$CH_3$), 0.77-0.73 (m, 6H, 2$CH_3$).$^{13}$CNMR (DMSO$d_6$, 400MHz)δ: 172.68 (C=O), 141.69,132.77,129.40, 127.00, (four aromatic carbons), 61.76, 40.66, 30.41, 19.52, 18.35, (five aliphatic carbons).HRMS (m/z) : 287.1064 (M+H), calculated 286.1066.

***N-ethyl-3-hydroxy-2-{[(4-methylphenyl)sulphonyl]amino} propanamide (4i).***

Yield (0.25 g, 86.2 %). Brownish oil. Uv (λmax): 349.00 nm. FTIR (KBr, $cm^{-1}$): 3433 (OH), 3280 (NH), 3060 (C-H aromatic), 2981 (C-H aliphatic), 1640(C=O), 1599, 1449 (C=C), 1398, 1323 (2S=O), 1156, 1122 ($SO_2NH$), 1092, 1033 C-N, C-O).$^1$HNMR (DMSOd6,400 MHz)δ: 8.44-8.42 (d, J=7.32 Hz, 1H, NH), 8.36-8.34 (d, J=6.88 Hz, 2H, ArH), 7.99-7.98 (d, J=7.36 Hz, 2H,ArH), 3.58-3.55 (m, 1H, CH-C=O ), 3.40 (s-br, 1H, OH), 2.47-2.46 (m, 2H, $CH_2$-$CH_3$), 2.32 (s, 3H, $CH_3$-Ar), 1.98-1.91(t, J=6.32 Hz, 2H, $CH_2$-OH), 0.81-0.76 (m, 3H,$CH_3$).$^{13}$CNMR (DMSO$d_6$, 400MHz)δ: 172.40 (C=O), 149.92,147.25,128.71, 124.78, (four aromatic carbons), 72.68, 61.95, 30.79, 21.56, 18.26, (five aliphatic carbons).HRMS (m/z) : 287.1075 (M+H), calculated 286.1078.

***N-ethyl-3-hydroxy-2-[(phenylsulphonyl)amino]propanamide (4j)***

Yield (0.21 g, 77.8 %). Brownish oil. Uv (λmax): 203.00 nm. FTIR (KBr, $cm^{-1}$): 3425.4 (OH), 3287.5 (NH), 3040 (C-H aromatic), 2944.6 (C-H aliphatic), 1729.0 (C=O), 1606.5, 1509.6, 1446.2 (C=C), 1379.1, 1312.0 (2S=O), 1200.2, 1118.2,1155.5 ($SO_2NH$), 1092.1, 969.1.5 C-N, C-O).$^1$HNMR (DMSO$d_6$,400 MHz)δ: 7.98-7.96 (d, J=7.16 Hz,1H, NH), 7.82-7.80 (d, J=6.68 Hz, 2H, ArH), 7.67-7.65 (d, J=7.64 Hz, 2H, ArH) 7.42-7.39 (m, 1H,ArH), 3.54-3.51 (m, 1H, CH-C=O ), 3.38 (s-br, 1H, OH), 2.49-2.48 (m, 1H, $CH_2$-$CH_3$), 1.99-1.93(t, J=6.40 Hz, 2H, $CH_2$-OH), 0.77-0.69 (m, 3H,$CH_3$).$^{13}$CNMR (DMSO$d_6$, 400MHz)δ: 173.44 (C=O), 148.24,147.25,128.80, 126.64, (four aromatic carbons), 70.88, 61.75, 19.66, 18.36, (four aliphatic carbons).HRMS (m/z) : 271.1209 (M-H), calculated 272.1207.

**BIOCHEMICAL STUDIES**

***In vivo anti-inflammatory activities determination***

Male albino rats weighing 300 g were purchased from the Department of Biochemistry, University of Nigeria,

Nsukka and kept at room temperature in a light-controlled animal house. They were fasted with free access to water at least 12 h prior to the experiments. The tested compounds were prepared as a suspension in a vehicle (0.5% methylcellulose) and celecoxib was used as a standard drug. The positive control received celecoxib while the negative control received only the vehicle. Edema was produced by injecting 0.2 mL of a solution of 1% carrageenan in the hindpaw. The rats were injected intraperitoneally with 1mL suspension in 0.5% methylcellulose of the tested compounds and reference drug. Paw volume was measured by water displacement with a plethysmometer (UGO BASILE) before, 0.5 h, 1 h, 2 h, and 3 h after treatment.Four rats were used per group and the testing was carried out in triplicate. The percentage was calculated by the following equation[16]:

Anti-inflammatory activity (% )= (1 – D/C) x 100

where D represents the difference in paw volume before and after drug administration to the rats and C represents the difference ofvolume in the control groups. The approval for the use of animals was obtained from the University of Nigeria committee on experimental animal code approved for PG/PhD/14/68289 use

***In vitro antimicrobial activity***

The antimicrobial properties of the novel compounds were investigated in form of the general sensitivity testing and minimum inhibitory concentration (MIC) with respect to freshly cultured targeted organisms. The seven organisms used in this study are two Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus*), three-gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*) and two fungi (*Candida Albicans, Aspergillus niger*) were obtained from the Department of Pharmaceutics, University of Nigeria, Nsukka.

***Antimicrobial Sensitivity Testing***

Sensitivity test agar plates were seeded with 0.1 mL of an overnight culture of microorganisms. The seeded plates were allowed to set after which cups were made in each sector previously drawn on the backside of the bottom plate using a marker. Using a sterile pipette, each cup was filled with six drops of their corresponding carboxamides (100 mg/mL). The solubility solvent was DMF. All the plates were incubated at 37 ºC for 24 h for bacteria and 48 h for fungi. Zones of clearance around each cup mean inhibition and the diameter of such zones was measured. The procedure was repeated for tetracycline (standard bacteria), fluconazole (fungi standard) and DMF (solvent). Muller Hinton agar was used for the fungi in place of nutrient agar for bacteria[17].

***Minimum Inhibitory Concentration (MIC) Testing***

Serial dilutions of the carboxamides were prepared from 100 mg/mL solution of the compounds to give 100, 50, 25 and 12.5 mg/mL. Six drops of each dilution was added to the corresponding cup of seeded microorganisms and the agar previously marked. The cork borer used to make the cup was 8 mm in diameter. The plates were incubated at 37 ºC for 24 h and 48 h in the case of fungi. The diameter of the zone of inhibition was measured and the value subtracted from the diameter of the borer to give the Inhibition Zone Diameter (IZD). The graph of $IZD^2$ against the log of concentration was plotted for each plate containing a specific compound and a microorganism. The anti-log of the intercept on x-axis gave the MIC[17]. The procedure was repeated for tetracycline and fluconazole.

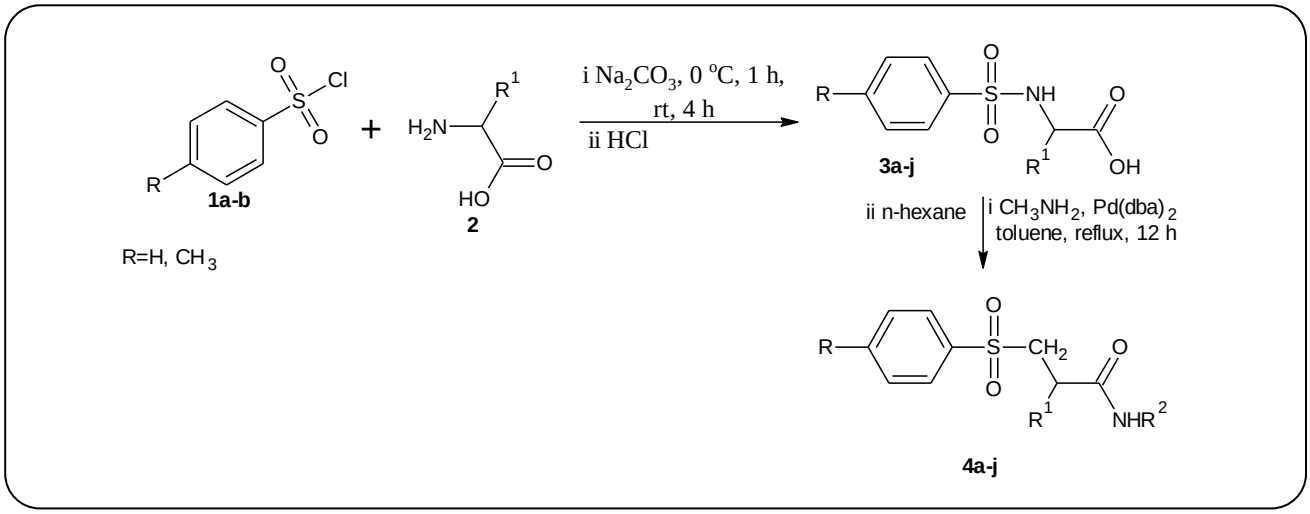
***In vitro antioxidant studies***

*DPPH Radical Scavenging Activity*

The new carboxamides were screened for free radical scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method[18]. Compounds of different concentrations were prepared in distilled ethanol, 1mL of each compound solutions having different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The test tubes were then incubated in the darkroom temperature for 20 min. A DPPH blank was prepared without the compound and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-Visible spectrometer. The radical scavenging activities were expressed as the inhibition percentage and were calculated using:

$$\text{DPPH radical scavenging activity} = \frac{Ac-As}{Ac} * 100,$$

Where Ac=Absorbance of control, As= Absorbance of the sample.




***Scheme 1: synthetic route to the new carboxamides***

## RESULTS AND DISCUSSION

### *Chemistry*

Substituted benzenesulfonamide (**3a-j**) were synthesized from the reaction of various L-amino acids (**2**) and substituted benzenesulfonyl chloride (**1**) in the aqueous medium. The reaction of compounds (**3a-j**) with the appropriate alkyl amine in the presence of the catalytic amount of $Pd(dba)_2$ afforded the target compounds (**4a-j**, Scheme 1) which were characterized using FT-IR, NMR, and HRMS.

### *Spectral Characterisation*

The FTIR spectra of the carboxamides showed N-H band between 3570 and 3198 $cm^{-1}$. The C=O band appeared between 1722 and 1640 $cm^{-1}$. These bands indicate the successful coupling of the aliphatic amines with the benzenesulphonamides.

The appearance of the peaks between 3.16-3.01, 2.86-2.42, 1.92-1.01 and 0.92-0.64 ppm in the proton NMR is supportive of the formation of the target product.

The carbon-13 NMR showed all the peaks expected of successful coupled products. The C=O peak appeared between 156.27 and 174.11 ppm. All the aromatic and aliphatic peaks were accounted for in the carbon-13 NMR.

The high-resolution mass spectrometer (HRMS) peak of the derivatives appeared either as molecular ions ($M^+$), $M+H^+$ or $M–H^–$. The results corresponded to three decimals with the calculated values. The spectra used for the characterization of the new compounds are available as supporting materials.

### *In silico studies*

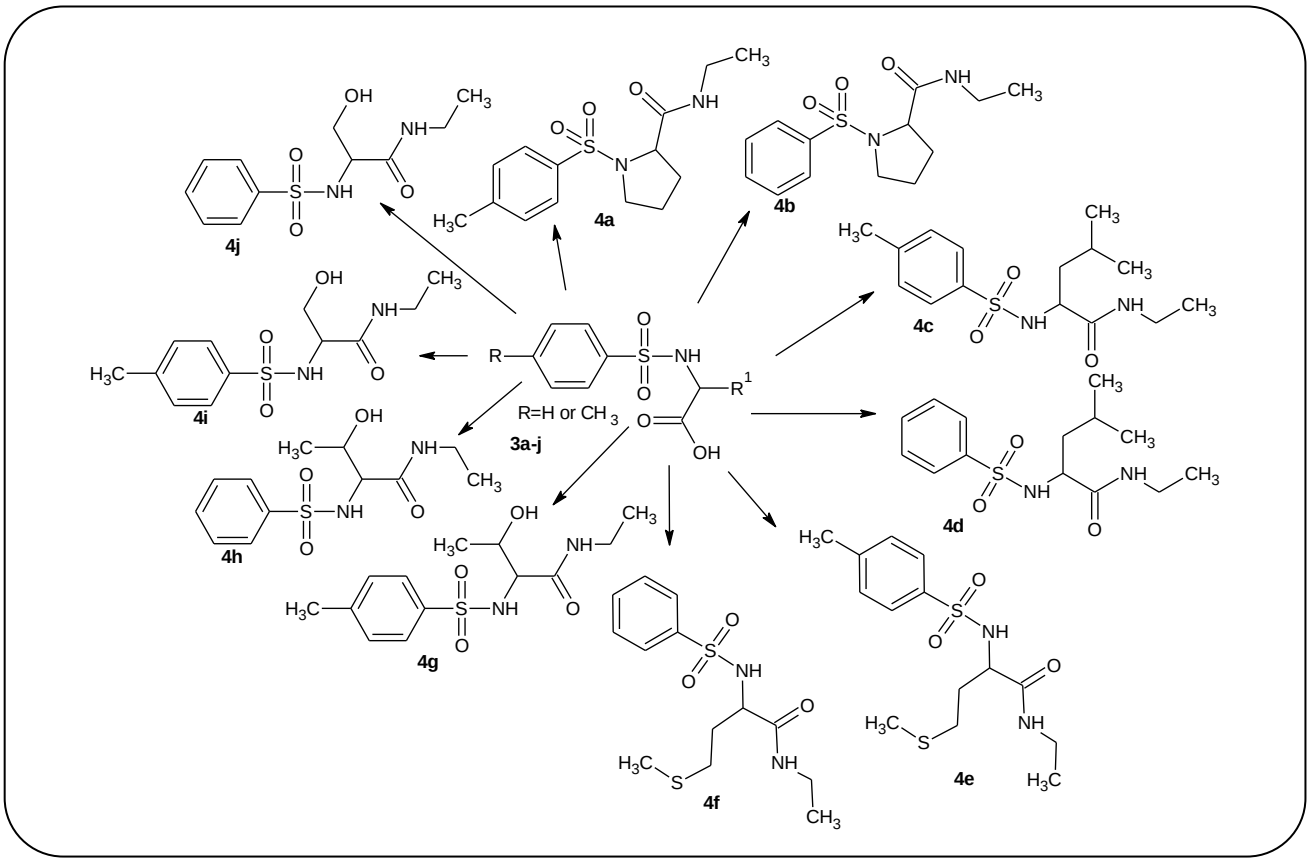
### *Molecular docking studies*

Three drug targets were selected to study the *in silico* antioxidant, anti-inflammatory and antibacterial activities of the synthesized compounds. The targets used forthe antioxidant and anti-inflammatory studies are the human peroxiredoxin 5 (PDB code: 1HD2) and phosphodiesterase 4 (PDE4) (PDB: 4WCU) respectively**.** Glucosamine-6-phosphate synthase (PDB Code: 2VF5) was used for the antibacterial study.

The 3-Dimensional crystal structures of 1HD2,4WCU and 2VF5 with their co-crystallized ligands were retrieved from the protein data bank repository (https://www.rcsb.org/). These proteins were treated in Discovery Studio where multiple chains and water of crystallization were removed. The synthesized compounds were drawn using Accelrys Draw 4.1. Both the prepared proteins and compounds were energy minimized using the MMFF94x force field. The energy minimized compounds were docked into the binding cavities of the proteins. The binding free energy for each compound against the target was calculated.Biovia Discovery Studio v16.1.0.15350 software was used for analysis of molecular docking studies. Molinspiration software (*www.molinspiration.com*) was used to generate the physicochemical properties in Table 7.

### *Drug targets*

Peroxisomes are essential organelles which participate in multiple important metabolic processes, including




***Scheme 2: Ethylamine derivatives of carboxamide.***

the β-oxidation of fatty acids, plasmalogen synthesis and the metabolism of Reactive Oxygen Species (ROS)[19].Human peroxiredoxin 5 (PRDX5) (PDB code: 1HD2), peroxisome, is a thioredoxin reductase that reduces $H_2O_2$, alkyl hydroperoxides and peroxynitrite[20]. PRDX5 is a novel type of mammalian thioredoxin peroxidase widely expressed in tissues and located cellularly to mitochondria, peroxisomes, and cytosol. Functionally, PRDX5 has been implicated in antioxidant protective mechanisms as well as in signal transduction in cells[21].

Phosphodiesterase-4 (PDE4) (PDB code: 4WCU) is an enzyme found in some specific cell types that are involved in the degradation of the second messenger, cAMP. As a result, 4WCU has a pivotal role in cell signaling. This has made it a target for clinical drug development of various indications, including anti-inflammation and several others[22].

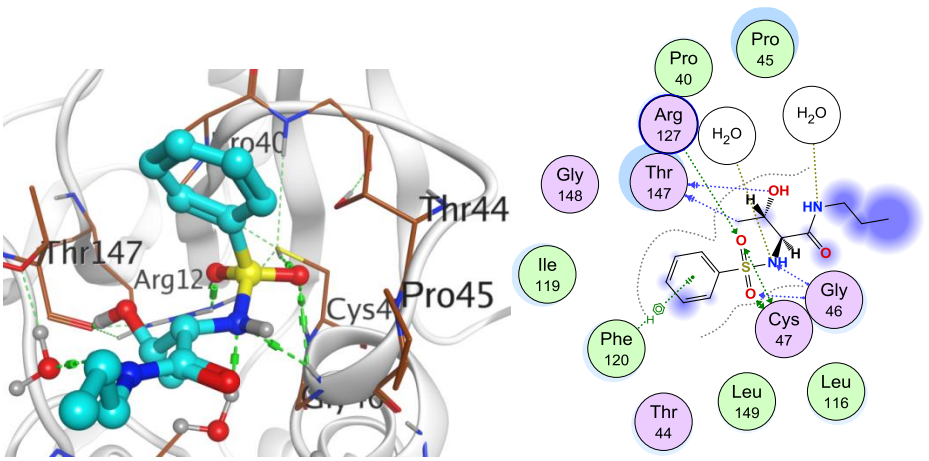
Glucosamine-6-phosphate synthase (GlcN-6-P) (PDB code: 2VF5) is a very useful target in antimicrobial chemotherapy as outlined by *Ezeokonkwoet* a*l*[23] and *Festuset al*[24]. 2VF5 is responsible for the metabolism of hexosamine which is an important process in the biosynthesis of amino sugars. In the biosynthesis of amino sugars, uridine 5′-diphospho-*N*-acetyl-d-glucosamine (UDP-GlcNAc) is formed. UDP-GlcNAc an important component of the peptidoglycan layer mostly found in the bacterial and fungal cell walls. Inactivation of GlcN-6-Psynthase for a short period is very dangerous for fungal cells.

Compound **4i** had a significant binding affinity comparable to vitamin C. The binding energy of **4i** is -13.83 kcal/mol while that of vitamin C is -13.04 kcal/mol as shown in Table 1. This high binding affinity of the compound to the receptor could be attributed to numerous significant binding interactions between the atoms of **4i** and the amino acid residues of the receptor (Fig. 1). The 6-membered aromatic ring of **4i** combined with the CZ of phenylalanine 120 through pi-H bonding. The distance and energy of interaction are 2.83Å and -1.1kcal/mol respectively. This interaction is crucial to the binding of the compound to 1HD2. Through H-donor interactions, C-13 and -14 of compound **4i** interacted with THR 147 respectively.

***Table 1: Binding free energy of the compounds, ΔG (kcal/mol).***

| COMP | Antioxidant target1HD2 | Anti-inflammatory target4WCU | Antibacterial target2VF5 |
|---|---|---|---|
| 4a | -9.44 | -9.37 | -10.61 |
| 4b | -11.48 | -9.41 | -10.70 |
| 4c | -12.62 | -12.10 | -12.38 |
| 4d | -10.25 | -9.80 | -10.56 |
| 4e | -11.36 | -9.65 | -11.36 |
| 4f | -12.26 | -11.95 | -11.36 |
| 4g | -9.90 | -9.66 | -11.02 |
| 4h | -10.09 | -9.95 | -10.77 |
| 4i | -13.83 | -9.70 | -13.21 |
| 4j | -9.93 | -9.51 | -10.35 |
| Standard drug | -13.04 | -11.38 | -16.74 |

*Standard drugs used: antioxidant = Vitamin C; Anti-inflammatory = indomethacin; Antibacteria = ciprofloxacin*




(A) (B)

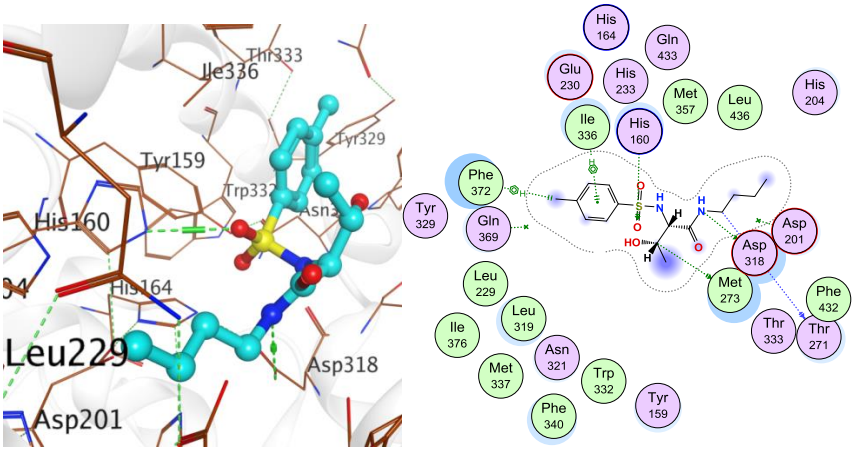
***Fig. 1: (A) Stereoview of the binding pose of 4i in the binding cavity of 1HD2 (B) 2D representation showing the binding interactions between 4i and the amino acid residues of 1HD2***

Also, H-donor interactions involving O-1 and O-3 of the compound and SG CYS 47 were observed. Similarly, O-1 of **4i** through H-acceptor interaction, combined with the SG CYS 47. Other amino acid residues involved in various chemical interactions include ARG 127 and GLY 46. The details are shown in Table 2.

The table of binding free energy (Table 1) showed that compound **4c** with ΔG = -12.10 kcal/mol has the highest binding affinity to the receptor, 4WCU. It has been used for further study on the likely nature of chemical interactions with the receptor. The compound fitly occupied the same binding site as the native ligand and its pose in the binding cavity has been showed in Fig. 2A. Fig. 2B throws light further on the nature of the amino acid residues interacting with atoms of the compound **4c**. It has been observed that the π-electrons of the 6-membered aromatic ring interacted with CG1 of isoleucine 336 through π-H bonding through an intermolecular distance of 4.28Å and energy of -0.2 kcal/mol. This aromatic ring seems to be crucial to the strong binding interaction

***Table 2: Ligand-receptor interactions.***

| Ligand | Receptor | Interaction | Distance | E (kcal/mol) |
|---|---|---|---|---|
| O 1 | SG CYS 47 | H-donor | 3.41 | -0.4 |
| O 3 | SG CYS 47 | H-donor | 3.68 | -0.2 |
| N 8 | O HOH 2209 | H-donor | 3.15 | -1.2 |
| C 13 | O THR 147 | H-donor | 3.06 | -0.3 |
| O 14 | O THR 147 | H-donor | 2.64 | -0.7 |
| O 1 | SG CYS 47 | H-acceptor | 3.41 | -0.3 |
| O 1 | NH2 ARG 127 | H-acceptor | 2.66 | -1.9 |
| O 3 | N GLY 46 | H-acceptor | 3.20 | -0.6 |
| O 3 | N CYS 47 | H-acceptor | 3.61 | -0.6 |
| O 3 | SG CYS 47 | H-acceptor | 3.68 | -0.7 |
| N 4 | N GLY 46 | H-acceptor | 3.48 | -0.6 |
| N 4 | O HOH 2221 | H-acceptor | 2.83 | -1.1 |
| 6-ring | CZ PHE 120 | Pi-H | 3.42 | -0.4 |




(A) (B)

***Fig. 2: (A) Stereoview of the binding pose of 3 in the binding cavity of 4WCU (B) 2D representation showing the binding interactions between 3 and the amino acid residues of 4WCU.***

observed. Also, N-15 and C-16 of compound **4c** interacted with OD2 of ASP 318 and O of THR 271 through H-donor interactions. The SD of MET 273 interacted with C-20 of compound **4c** through another H-donor interaction. HIS 160 and PHE 372 interacted with the O-10 and C-1 of **4c** through H-acceptor and H-π interactions respectively.

## BIOLOGICAL STUDIES

The *in vivo* anti-inflammatory activity showed that all the novel compounds (Table 3) tested had fascinating inhibition of inflammation (95.58-87.61%) when compared with NSAID indomethacin (78.76%) at 1 h. The most active was compounds **4e** with percentage inhibition

***Table 3: In vivo Anti-inflammatory.***

| Sample no | 1 h (%) | 2 h (%) | 3 h (%) |
|---|---|---|---|
| 4a | 90.27 | 85.34 | 84.35 |
| 4b | 94.69 | 89.66 | 88.70 |
| 4c | 88.50 | 83.62 | 82.61 |
| 4d | 90.27 | 84.48 | 83.48 |
| 4e | 95.58 | 88.79 | 86.96 |
| 4f | 91.15 | 81.90 | 80.00 |
| 4g | 92.04 | 86.21 | 83.48 |
| 4h | 88.50 | 83.62 | 81.74 |
| 4i | 87.61 | 81.90 | 80.00 |
| 4j | 91.15 | 86.21 | 85.22 |
| Indomethacin | 78.76 | 71.55 | 66.09 |

***Table 4: General Sensitivity of Compounds Against Microorganism.***

| Sample no | E.coli | S.aures | P.aeroginosa | S.typhi | B.subtilis | C.albican | A.niger |
|---|---|---|---|---|---|---|---|
| 4a | 3 | 3 | 4 | 10 | 5 | 4 | - |
| 4b | - | - | - | 6 | 3 | - | - |
| 4c | - | 15 | 10 | 7 | 10 | 7 | 8 |
| 4d | 13 | 16 | - | - | 13 | 13 | 11 |
| 4e | - | - | 6 | 4 | 4 | - | - |
| 4f | 11 | 17 | 13 | 9 | 13 | 9 | 6 |
| 4g | 14 | 14 | 14 | 11 | 9 | 9 | 9 |
| 4h | - | 10 | 8 | 6 | 7 | 8 | - |
| 4i | - | 9 | 8 | 4 | 12 | 18 | - |
| 4j | - | 6 | 8 | 4 | 8 | 10 | - |
| Ciprofloxacin | 26 | 25 | 25 | 25 | 26 | - | - |
| Fluconazole | - | - | - | - | - | 24 | 27 |

of 95.58% at 1 h. The compounds showed better anti-inflammatory activities at 1 h of the experiment. It was observed that the anti-inflammatory activities decreased with increase in time.

***In vitro antimicrobial Activities***

***General Sensitivity of Compounds Against Microorganism***

***Minimum Inhibitory Concentration (mg/mL)***

The antimicrobial studies revealed that most of the novel compounds (MIC 9.05-6.32 mg/mL) were more potent than the reference drugs (MIC 8.39-9.65) against the tested microorganisms (Table 5). Compound**4f** (MIC 7.23 mg/mL) was the most active against *E.coli*. Compound**4j** (MIC 7.11, 6.42and 6.32 mg/mL) was the most potent against *S.aureus, P.aeruginosa* and *B.subtilis* respectively. The most active compounds against *S.typhi* and *C.albican* was **4h** (MIC 7.12 and 6.48 mg/mL) respectively, while compound **4c**, (6.63 mg/mL) was the most potent against *A.niger*.

***In vitro Antioxidant Activities***

***In vitro Antioxidant Activities (% scavenging activity)***

The *in vivo* antioxidant activities (Table 7) revealed that all the novel compounds had antioxidant activities though lower than Vitamin C. Only compounds **4e,**($IC_{50}$

***Table 5: Minimum Inhibitory Concentration (MIC).***

| Sample no | E.coli | S.aureus | P. aeruginosa | S.typhi | B.subtilis | C.albican | A.niger |
|---|---|---|---|---|---|---|---|
| 4a | - | - | - | 7.93 | - | - | - |
| 4b | - | - | - | 7.28 | | - | - |
| 4c | - | 8.79 | 7.87 | 8.39 | 8.10 | 8.45 | 6.63 |
| 4d | 8.71 | 8.90 | - | - | 8.69 | 8.71 | 7.34 |
| 4e | - | - | 7.44 | - | - | - | - |
| 4f | 7.23 | 8.98 | 8.83 | 7.62 | 8.50 | 7.77 | 7.68 |
| 4g | 8.54 | 8.63 | 8.82 | 7.50 | 7.36 | 7.22 | 7.84 |
| 4h | - | 7.86 | 6.63 | 7.12 | 7.96 | 6.48 | - |
| 4i | - | 7.87 | 6.54 | - | 8.71 | 9.05 | - |
| 4j | - | 7.11 | 6.42 | - | 6.32 | 7.65 | - |
| Ciprofloxacin | 9.65 | 8.39 | 9.05 | 8.68 | 9.56 | - | - |
| Fluconazole | - | - | - | - | - | 9.05 | 8.39 |

***Table 6: In vitro Anti-oxidant (% scavenging activity).***

| Sample no | 0.05 mg/mL (%) | 0.10 mg/mL (%) | 0.15 mg/mL (%) | 0.20 mg/mL (%) | 0.25 mg/mL (%) |
|---|---|---|---|---|---|
| 4a | 3.47 | 8.89 | 15.42 | 20.18 | 23.91 |
| 4b | 3.21 | 6.17 | 7.97 | 14.01 | 19.79 |
| 4c | 2.19 | 7.33 | 11.57 | 18.51 | 25.19 |
| 4d | 2.44 | 7.46 | 12.08 | 17.74 | 21.47 |
| 4e | 1.29 | 6.81 | 10.80 | 14.52 | 23.78 |
| 4f | 4.24 | 9.64 | 15.30 | 20.57 | 27.89 |
| 4g | 2.06 | 5.66 | 9.90 | 12.85 | 17.48 |
| 4h | 2.44 | 7.33 | 11.44 | 16.71 | 20.69 |
| 4i | 1.67 | 6.30 | 10.80 | 16.45 | 21.59 |
| 4j | 1.67 | 6.17 | 8.48 | 11.18 | 13.37 |
| Vitamin C | 11.31 | 21.85 | 34.32 | 48.20 | 60.15 |

***Table 7: In vitro Antioxidant Activities ($IC_{50}$)***

| Sample no | IC50 (mg/mL) |
|---|---|
| 4a | 0.4995 |
| 4b | 0.5292 |
| 4c | 0.4483 |
| 4d | 0.5080 |
| 4e | 0.3586 |
| 4f | 0.4120 |
| 4g | 0.5593 |
| 4h | 0.5848 |
| 4i | 0.5144 |
| 4j | 1.0719 |
| Vitamin C | 0.2090 |

0.3586 mg/mL) had comparable activity with Vitamin C ($IC_{50}$ 0.2090 mg/mL) at 0.25 mg/mL.

***Pharmacokinetics Studies***

Table 8 shows the physicochemical properties of the synthesized compounds which are useful in the assessment of the drug-likeness.

Lipinski's rule of five helps to evaluate the bioavailability for oral formulations. An oral drug with a good bioavailability should have MW ≤500, HBD ≤5, HBA ≤10, and Log P(o/w) ≤5. A violation of more than one parameter may be an indication of poor bioavailability. Table 8 shows that the synthesized compounds are in agreement with the Lipinski's rule of five. In addition,

***Table 8: Pharmacokinetics.***

| Sample no | MilogP | TPSA (Å²) | NA | MW | HBA | HBD | NV | NRB | Volume | %ABS |
|---|---|---|---|---|---|---|---|---|---|---|
| 4a | 1.57 | 66.48 | 20 | 296.39 | 5 | 1 | 0 | 4 | 265.96 | 86.06 |
| 4b | 1.12 | 66.48 | 19 | 282.37 | 5 | 1 | 0 | 4 | 249.40 | 86.06 |
| 4c | 2.58 | 75.27 | 21 | 312.44 | 5 | 2 | 0 | 7 | 292.77 | 83.03 |
| 4d | 2.13 | 75.27 | 20 | 298.41 | 5 | 2 | 0 | 7 | 276.20 | 83.03 |
| 4e | 1.72 | 75.27 | 21 | 330.48 | 5 | 2 | 0 | 8 | 294.31 | 83.03 |
| 4f | 1.27 | 75.27 | 20 | 316.45 | 5 | 2 | 0 | 8 | 277.75 | 83.03 |
| 4g | 2.05 | 75.27 | 20 | 298.41 | 5 | 2 | 0 | 6 | 275.96 | 83.03 |
| 4h | 1.60 | 75.27 | 19 | 284.38 | 5 | 2 | 0 | 6 | 259.40 | 83.03 |
| 4i | 0.29 | 75.27 | 19 | 286.35 | 6 | 3 | 0 | 6 | 250.83 | 76.05 |
| 4j | -0.16 | 95.50 | 18 | 272.33 | 6 | 3 | 0 | 6 | 234.27 | 76.05 |

*TPSA: total polar surface area; NA: number of atoms;MW: molecular weight; HBA: hydrogen bond acceptor; HBD: hydrogen bond donor; NV: number of violations;NRB: number of rotatable bonds.*

the TPSA, which is a reflection of the compound's hydrophilicity, is very important in protein-ligand interaction. NRB ≤ 10 and TPSA ≤140 Å$^2$ would have a high probability of good oral bioavailability in rats. The compounds reported in this research possessed TPSA less than 140 and NRB less than 10 and as such would not pose oral bioavailability problems if formulated.

## CONCLUSIONS

- In this paper, we have described an efficient, ecofriendly, and versatile approach to obtain substituted benzenesulfonamide bearing carboxamide.
- All the compounds were evaluated for their anti-inflammatory, antimicrobial, and oxidant activities.
- Compound **4e** was the most active anti-inflammatory agent
- Compound **4f** was the most active against *E.coli*, compound **4j** was most active against *S.aureus P. aeruginosa* and *B.subtilis*, compound **4h** was most active against and *S. typhi* and *C.albican*, compound **4c** was most active against *A.niger*.
- Compound **4e** had a comparable activity with Vitamin C.

### Disclosure statement

The authors declare that there is no conflicting interest.

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