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Synthesis of Novel Valine-based Dipeptide Carboxamide Bearing Benzene Sulfonamide Moiety as Antimalarial Agent

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Abstract *Syntheses of eleven novel Valine-based dipeptide carboxamide derivatives bearing benzensulphonamide are reported. These were achieved by facile amidation reaction of p-substituted benzenesulphonamoyl alkanamides with 2-amino-4-methyl-N-substituted phenyl butanamide using classical peptide coupling reagents. The chemical structures of the synthesized compounds were established by ^{1}H-NMR, ^{13}C-NMR, ESI-HRMS, and FT-IR spectroscopic techniques. The synthesized compounds were evaluated for in vivo antimalarial against P. berghei. Haematological analysis was also evaluated on the synthesized compounds. At 50mg/kg body weight, the compounds 8e, 8g, 8i, 8k, 8d and 8h inhibited the multiplication of the parasite by 46-71% on day seven of post-treatment exposure comparable to the 67% reduction with artemisinin.*

Key Words: *Valine-dipeptide carboxamide; benzensulphonamoyl butanamide; antimalarial.*

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1.0 Introduction

Malaria is a tropical infectious disease which poses serious problem to man's health (Hay *et* al., 2003; Lee *et* al., 2010). It is caused primarily by a protozoan *Plasmodium falciparum*, which is responsible for the death of over one million individuals every year with more than 40% of the global population at risk (WHO,2005). Although there has been significant progress in the past decade in scaling up malaria control and prevention efforts, yet the resistance to currently used anti-malaria drugs is rather worrisome and hence the need for new drugs. More importantly, there is need for new anti-malaria drugs with mechanisms of action different from the existing ones and to identify new drug targets (Miller *et al*.,2002*)*. Medium or long acting sulfonamides have been used clinically as anti-malaria agents, predominantly sulfadiazine and sulfadoxine. Though, each is much more efficient when given in recipe with pyrimethamine or trimethoprim (Korolkovas, 1988).

In addition, sulfonamide derivatives are broadly used as antitumor (El Sayed *et* al., 2011; Noaman *et al*., 2011). Antiviral (Chen *et* al., 2020), antimalaria (Dominquez *et al.,* 2006; Padmanilayam *et al*.,2006), anti-inflammatory (Keehe *et al*., 2012), anticancer (Ghorab *et al.,*2009), anti-carbonic

anhydrase (Ghorab *et al*., 2009), antidiabetic agents (Wilkinson *et al.,* 2007) and in treatment of Alzheimer's diseases (Sharma *et al.,* 2014).. Agrawal *et al* (2015), evaluated the anti-malarial activity of 2, 4-diamino-6-quinazoline sulphonamide derivatives and all shown to have promising activity. In like approach, sulphonamide, was recognized as significant pharmacophore with strong anti-malaria activity (Agrawal *et al*., 2001). Parai *et al*. (2008) reported the synthesis of benzenesulphonamide derivatives having anti-malaria potential comparable with isoquinoline sulphonamide derivatives (MIC 10 µg/mL). Nubia *et al*. (2011) reported the synthesis of 1H-1,2,4-triazol-3-ylbenzenesulphonamide derivatives as possible anti-malarial prototypes.

The good solubility, permeability and bioavailability of peptides have made it to receive particular attention over the years as they possess a wide range of biological properties which implicated such binding to membrane receptors (Qi *et al.,* 1010; Thompson *et al.,*2012; McPhee and Hancock, 2005). Peptides have been extensively evaluated as potential anti-malaria agents (Bell, 2011). A number of AMPs-derived anti-malaria peptides, such as cecropins (Gwadz *et al.,* 1989), gambicin (Vizioll *et al.,* 2001), and scorpine (Conde *et al*., 2000), have been proven to affect the life cycle of the malaria parasite at different stages. In a previous paper (Ugwu *et al.,*2017), we reported the synthesis and antimalarial activities of scaffolds containing carboxamide and benzenesulphonamide hybrids with single amino acid backbone. Subsequently, we reported (Ugwuja *et al.,*2019) the synthesis of scaffolds bearing sulphonamide-carboxamide pharmacophoric conjugates possessing mixed amino acid dipeptide backbone with interesting anti-malaria properties. In this paper, we now report the synthesis of previously unknown conjugates possessing valine-valine dipeptide backbone with improved antimalarial properties.

2.0 Material and Methods

Reagent-grade chemicals and solvents were purchased from Sigma-Aldrich and used without purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Advance 300 MHz, 400 MHz and 500 MHz spectrometers in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. FT-IR spectra were recorded on Thermo Nicollet Nexus 670 spectrometer. Mass spectra were obtained on Agilent LCMS instrument. HRMS were measured on Agilent Technologies 6510, Q-TOFLC/MS ESI-Technique. Melting points were determined in open glass capillary tubes on a Stuart melting point apparatus and are uncorrected. All reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F_{254} (mesh); spots were visualized under UV light and in oven with Ninhydrin. Merck neutral aluminium oxide activated (60-325 mesh) was used for column chromatography.

2.1 General procedure for the synthesis of substituted benzenesulphonamoyl alkanamides[27]

Sodium carbonate (Na_2CO_3, 1.82 mmol) was added to a solution of amino acids (1.5 mmol) in water (15 mL) with continuous stirring until all the solutes dissolved. The solution was cooled to -5 ^{0}C and an appropriate substituted benzenesulphonyl chloride (**a-c**, 1.82 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 by using TLC (MeOH/DCM, 1:9). Upon completion, the mixture was acidified using 20% aqueous hydrochloric acid to pH 2. The crystals were filtered via suction and washed with pH 2.2 buffer. The pure products **(3a-c)** were dried over self-indicating fused calcium chloride in desiccators.

2.2 General procedure for the synthesis of tert-butyl-1-(substituted phenylamino)-3-methyl-1-oxobutan-2-ylcarbamate

To a solution of boc-Valine (3.0 g, 13.82 mmol) in DCM (20 mL) was added TEA (20.7 mmol), EDC.HCl (16.0 mmol), HOBT (13.82 mmol) at 0 ^{0}C, after stirring for 15 minutes was added substituted anilines (13.82 mmol). The resulting mixture was allowed to warm to room temperature and stirred for 19-24 hours as monitored with TLC. On completion of the reaction, the reaction mixture was diluted with DCM, washed with water (2x 50 ml), then the organic layer was washed with 1M HCl (50 ml), 5% $NaHCO_3$ (50 ml), and Brine solution (50 ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product purified by Column chromatography (ethyl acetate/hexane =5:95).

tert-Butyl-1-(4-chlorophenylamino)-3-methyl-1-oxobutan-2-ylcarbamate

Yield 70.0%, M.p=151-152 ^{0}C. ^{1}H NMR (500 MHz, DMSO) δ 10.12 (s, 1H), 7.66-7.60 (m, 2H), 7.41-7.30 (m, 2H), 6.91 (d, *J* = 8.5 Hz, 1H), 3.90 (t, *J* = 8.0 Hz, 1H), 1.98 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.39 (s, 9H), 0.93-0.85 (m, 6H). ^{13}C-NMR (126 MHz, DMSO) δ 170.66, 155.35, 137.52, 128.38, 126.57, 120.49, 77.83, 60.39, 30.01, 27.94, 18.92, 18.22, ESI-MS: m/z 327 $[M+H]^+$.

***t*ert-Butyl-1-(3-fluorophenylamino)-3-methyl-1-oxobutan-2-ylcarbamate**

Yield 67.76%, M.p=168-169 ^{O}C. ^{1}H-NMR (400 MHz, DMSO) δ 10.20 (s, 1H), 7.72-7.53 (m, 1H), 7.41-7.28 (m, 2H), 6.98-6.83 (m, 2H), 3.92 (t, *J* = 8.0 Hz, 1H), 1.99 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.39 (s, 9H), 0.90 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.66, 163.80, 161.40, 156.11, 141.07, 130.81, 115.41, 110.31, 110.10, 106.55, 106.29, 78.58, 61.17, 30.73, 28.66, 19.64, 18.94, ESI-MS: m/z 311 $[M+H]^+$.

***t*ert-Butyl-1-(4-Isoproplyphenylamino)-3-methyl-1-oxobutan-2-ylcarbamate**

Yield 78%, M.p=140-142 ^{O}C. ^{1}H-NMR (400 MHz, DMSO) δ 9.87 (s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 1H), 3.91 (t, *J* = 8.0 Hz, 1H), 2.83 (dt, *J* = 13.7, 6.9 Hz, 1H), 1.97 (dd, *J* = 13.4, 6.7 Hz, 1H), 1.39 (s, 9H), 1.17 (d, *J* = 6.9 Hz, 6H), 0.89 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 170.48, 155.56, 143.43, 136.55, 126.41, 119.37, 78.07, 60.52, 32.88, 30.44, 28.18, 23.95, 19.17, 18.46, ESI-MS: m/z 335 $[M+H]^+$,

***t*ert-Butyl-1-(phenylamino)-3-methyl-1-oxobutan-2-ylcarbamate**

Yield 80%, M.p=184-185 ^{O}C. ^{1}H NMR (300 MHz, DMSO) δ 9.98 (s, 1H), 7.61 (d, *J* = 7.8 Hz, 2H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 3.92 (t, *J* = 8.0 Hz, 1H), 1.97 (dt, *J* = 13.4, 6.7 Hz, 1H), 1.39 (s, 9H), 0.90 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.21, 156.07, 139.29, 129.20, 123.78, 119.72, 78.55, 61.06, 30.86, 28.67, 19.67, 18.93, ESI-MS: m/z 292 $[M+H]^+$,

***t*ert-Butyl-1-(4-methylphenylamino)-3-methyl-1-oxobutan-2-ylcarbamate**

Yield 74%, M.p=141-142 ^{O}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.05 (m, 2H), 5.38 (s, 1H), 4.08 (s, 1H), 2.28 (s, 3H), 2.19 (d, *J* = 4.7 Hz, 1H), 1.43 (s, 9H), 1.01 (dd, *J* = 11.2, 6.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.52, 156.37, 135.30, 133.90, 129.35, 120.12, 80.17, 60.94, 30.88, 28.37, 20.87, 19.42, 18.26, ESI-MS: m/z 307 $[M+H]^+$,

2.3 Synthesis of 2-amino-N-(substituted phenyl)-3-methylbutanamide

To around bottom flask containing tert-butyl-1-(substituted phenylamino)-3-methyl-1-oxobutan-2-ylcarbamate was added DCM/TFA (1:1%) and stirred at room temperature for 1hr as monitored with TLC. On the completion of the reaction, the solvent was evaporated under reduced pressure. The solid was precipitated on the addition of diethylether and dried.

2-Amino-N-(4-chlorophenyl)-3-methylbutanamide

Yield 92.0%, M.p=149-150 ^{0}C. ^{1}H NMR (300 MHz, DMSO) δ 10.81 (s, 1H), 8.36 (s, 2H), 7.67 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 2H), 3.81 (br, 1H), 2.19 (dq, *J* = 13.4, 6.7 Hz, 1H, CH-$(CH_3)_2$), 0.99 (dd, *J* = 6.8, 2.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 167.01, 136.87, 128.81, 127.71, 121.07, 58.16, 39.42, 29.87, 18.30, 17.61, ESI m/z: $[M+H]^+$ 227.

2-Amino-N-(3-fluorophenyl)-3-methylbutanamide

Yield 87.90%, M.p=162-163 ^{0}C. ^{1}H NMR (400 MHz, DMSO) δ 10.86 (s, 1H), 8.34 (s, 2H), 7.61 (dt, *J* = 11.4 2.2 Hz, 1H), 7.39 (dt, *J* = 14.0, 8.2 Hz, 2H), 6.96 (t, *J* = 8.3 Hz, 1H), 3.81 (d, *J* = 5.3 Hz, 1H), 2.19 (dq, *J* = 13.5, 6.8 Hz, 1H), 1.04-0.95 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 167.79, 163.79, 161.38, 140.16, 140.06, 131.24, 131.15, 115.82, 111.28, 111.07, 106.99, 106.73, 58.76, 30.41, 18.86, 18.11, ESI m/z: $[M+H]^+$ 211.

2-Amino-N-(4-isopropylphenyl)-3-methylbutanamide

Yield 91.20 %, M.p=125-126 ^{0}C. ^{1}H NMR (300 MHz, DMSO) δ 10.42 (s, 1H), 8.41 (s, 2H, NH_2), 7.56 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 3.93 (s, 1H), 2.86 (dt, *J* = 13.6, 6.8 Hz, 1H), 2.26 (d, *J* = 5.6 Hz, 1H), 1.22 (t, *J* = 7.4 Hz, 6H), 1.06 (d, *J* = 5.6 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO) δ 166.98, 144.64, 136.14, 126.84, 120.12, 58.67, 33.45, 30.47, 24.30, 18.78, 18.17, ESI m/z: $[M+H]^+$ 234.

2-Amino-N-(phenyl)-3-methylbutanamide

Yield 88.0%, M.p=154-155 ^{0}C. ^{1}H-NMR (400 MHz, DMSO) δ 10.64 (s, 1H), 8.35 (s, 2H), 7.63 (d, *J* = 7.7 Hz, 2H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.12 (t, *J* = 7.4 Hz, 1H), 3.83 (d, *J* = 4.3 Hz, 1H), 2.19 (d, *J* = 20.0 Hz, 1H), 1.00 (d, *J* = 10.0 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO) δ 167.37, 138.50, 129.40, 124.58,

120.04, 58.67, 30.46, 18.85 18.18. ESI m/z: $[M+H]^+$ 193.

2-Amino-*N*-(4-methylphenyl)-3-methylbutanamide

Yield 89.30 %, M.p=160-161 ^{0}C. ^{1}H NMR (400 MHz, DMSO) δ 10.51 (s, 1H), 8.31 (s, 2H), 7.50 (d, *J* = 8.1 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 3.78 (d, *J* = 5.9 Hz, 1H), 2.27 (s, 3H), 2.17 (dd, *J* = 13.1, 6.6 Hz, 1H), 0.99 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 167.10, 135.97, 133.64, 129.78, 120.06, 58.65, 30.46, 20.94, 18.85, 18.23, ESI m/z: $[M+H]^+$ 206

2.4 General procedures for Synthesis of Novel valine - valine dipeptide carboxamides containing sulfonamide moieties

To a solution of substituted benzenesulphonamoyl alkanamides (1.0 mmol) in DCM (10 mL) was added TEA (1.49 mmol), EDC.HCl (1.19 mmol), HOBT(1.0 mmol) at 0 ^{0}C, after stirring for 15 minutes was added 2-Amino-N-(Substituted Phenyl)-4-Methylalkanamide (1.0mmol). The resulting mixture was allowed to warm to room temperature and stirred for 19-24 hours as monitored with TLC. On The completion of the reaction, the reaction mixture was diluted with DCM, Washed with Water (2x 30 ml), then the organic layer was washed with 1N HCl (30 ml), 5 % $NaHCO_3$ (30 ml), and Brine solution (30 ml) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by Column chromatography (ethyl acetate/hexane =5:95).

3-Methyl-*N*-(3-methyl-1-oxo-1-(phenylamino) butan-2-yl)-2-(4-nitrophenylsulfonamido) butanamide (8a)

Yield 64%, M.p=172-174 ^{0}C

FTIR (KBr, cm^{-1}): 3363, 3305, 3220 (3NH), 2967, 2932, 2877 (C-H Aliphatic), 1680, 1642, (2C=O, amide), 1536, 1469, 1442 (C=C-Aromatic), 1380, 1349, 1313, 1242 (SO_2), 1172, 1092 (C-N**).** ^{1}H-NMR (300 MHz, DMSO) δ10.00 (s, 1H), 8.34 (t, *J* = 6.7 Hz, 3H), 8.06 (t, *J* = 9.0 Hz, 3H), 7.55 (d, *J* = 7.8 Hz, 2H), 7.28 (t, *J* = 7.8 Hz, 2H), 7.03 (t, *J* = 7.3 Hz, 1H), 3.99 (t, *J* = 7.6 Hz, 1H), 3.85–3.70 (m, 1H), 1.84 (dt, *J* = 13.4, 6.6 Hz, 2H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.79 (d, *J* = 6.6 Hz, 3H), 0.69 (t, *J* = 6.1 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO) δ170.32, 170.10, (2C=O), 149.74, 147.30, 139.18, 129.19, 128.72, 124.62, 123.80, 119.61, (eight aromatic carbons), 61.76, 58.88, 31.71, 30.96, 19.51, 19.40, 18.63, 18.56 (eight aliphatic carbons). ESI-MS: m/z, 477 $[M+H]^+$, 499 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{22}H_{28}N_4O_6S$ $[M+Na]^+$ 499.1627; Found 499.1629.

***N*-(4-chlorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido)butanamide (8b)**

Yield 74%, M.p=183-184 ^{0}C

FTIR (KBr, cm^{-1}): 3361, 3294, 3202 (3NH), 2967, 2926, 2882 (C-H Aliphatic), 1684, 1641, (2C=O, amide), 1536, 1493, 1457, 1401 (C=C-Aromatic), 1347, 1315, 1249 (SO_2), 1170, 1093, 1013 (C-N**).** ^{1}H-NMR (400 MHz, DMSO) δ10.12 (s, 1H), 8.32 (dd, *J* = 16.4, 9.2 Hz, 3H), 8.04 (t, *J* = 8.2 Hz, 3H), 7.57 (d, *J* = 8.6 Hz, 2H), 7.34 (d, *J* = 8.6 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 1H,), 3.82–3.72 (m, 1H), 1.89–1.78 (m, 2H), 0.81 (dd, *J* = 19.2, 6.3 Hz, 6H), 0.70 (d, *J* = 5.5 Hz, 6H).

^{13}C-NMR (75 MHz, DMSO) δ169.84, 169.75, (2C=O), 149.21, 146.77, 137.58, 128.60, 128.19, 126.86, 124.10, 120.60, (eight aromatic carbons), 61.24, 58.43, 31.17, 30.36, 18.97, 18.87, 18.13, 18.04 (eight aliphatic carbons). ESI-MS: m/z, 511$[M+H]^+$, 533 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{22}H_{27}N_4O_6S$ $[M+Na]^+$ 533.1238; Found 533.1245.

***N*-(3-fluorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido)butanamido)butanamide (8c)**

Yield 64.1%, M.p=126-127 ^{0}C

FTIR (KBr, cm^{-1}): 3277 (NH), 2966, 2934, 2877 (C-H Aliphatic), 1642, 1610, (2C=O, amide), 1534, 1490, 1446 (C=C-Aromatic), 1351, 1312, 1283, 1215 (SO_2), 1168, 1090, 1012 (C-N**).** ^{1}H-NMR (400 MHz, DMSO) δ10.20 (s, 1H), 8.35 (d, *J* = 8.8 Hz, 2H), 8.31 (d, *J* = 9.5 Hz, 1H), 8.05 (t, *J* = 10.3 Hz, 3H), 7.53 (d, *J* = 11.6 Hz, 1H), 7.30 (dd, *J* = 16.5, 8.7 Hz, 2H,), 6.87 (t, *J* = 9.3 Hz, 1H), 3.97 (t, *J* = 7.6 Hz, 1H), 3.81–3.76 (m, 1H), 1.85 (dt, *J* = 13.9, 6.9 Hz, 2H), 0.84 (d, *J* = 6.7 Hz, 3H), 0.79 (d, *J* = 6.7 Hz, 3H), 0.70 (t, *J* = 6.1 Hz, 6H). ^{13}C-NMR (75 MHz, DMSO) δ169.99, 169.88, (2C=O), 163.66, 160.46, 149.23, 146.78, 140.41, 140.27, 130.42, 130.29, 128.19, 124.10, 114.82, 109.90, 109.62, 105.99, 105.64, (aromatic carbons), 61.26, 58.46, 31.18, 30.36, 18.97, 18.87, 18.12, 18.02 (eight aliphatic carbons). ESI-MS: m/z, 495$[M+H]^+$, 517 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{22}H_{27}N_4FO_6S$ $[M+H]^+$ 495.1714; Found 495.1729.

***N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido) butanamide (8d)**

Yield 82%, M. p= 135-137 ^{0}C
FTIR (KBr, cm^{-1}): 3358, 3275, 3197 (3NH), 2965, 2931 (C-H Aliphatic), 1679, 1641, (2C=O, amide), 1536, 1461, 1415 (C=C-Aromatic), 1345, 1311, 1245 (SO_2), 1168, 1071, 1010 (C-N**).** ^{1}H-NMR (400 MHz, DMSO) δ9.88 (s, 1H), 8.34 (d, *J* = 8.3 Hz, 2H), 8.29 (d, *J* = 9.3 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 3H), 7.44 (d, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 2H), 3.98 (d, *J* = 14.6 Hz, 1H), 3.77 (d, *J* = 15.2 Hz, 1H), 2.82 (d, *J* = 13.3 Hz, 1H), 1.83 (d, *J* = 20.2 Hz, 2H), 1.16 (d, *J* = 6.6 Hz, 6H), 0.83 (d, *J* = 6.2 Hz, 3H), 0.78 (d, *J* = 6.2 Hz, 3H), 0.71 – 0.65 (m, 6H). ^{13}C-NMR (101 MHz, DMSO) δ170.28, 169.85, (2C=O), 149.74, 147.30, 143.90, 136.94, 128.72, 126.88, 124.62, 119.72, (eight aromatic carbons), 61.76, 58.84, 33.36, 31.71, 30.99, 24.43, 19.50, 19.38, 18.62, 18.61(ten aliphatic carbons). ESI-MS: m/z, 519 [M+H]$^+$, 541[M+Na]$^+$. HRMS-ESI: calcd. For $C_{25}H_{34}N_4O_6S$ [M+H]$^+$ 519.2277; Found 519.2285.

3-Methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino) butan-2-yl)-2-(4-nitrophenylsulfonamido) butanamide (8e)

Yield 83.67%, M.p=191-192 ^{0}C
FTIR (KBr, cm^{-1}): 3312, 3261 (2NH), 2965, 2921 (C-H Aliphatic), 1646, 1610, (2C=O, amide), 1539, 1452 (C=C-Aromatic), 1322, 1244 (SO_2), 1164, 1010 (C-N**).** ^{1}H-NMR (400 MHz, DMSO) δ 9.83 (s, 1H), 8.31 (dd, *J* = 17.2, 8.5 Hz, 3H), 8.03 (t, *J* = 8.1 Hz, 3H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.08 (d, *J* = 8.2 Hz, 2H), 3.97 (t, *J* = 7.7 Hz, 1H), 3.76 (m, 1H), 2.23 (s, 3H), 1.91–1.72 (m, 2H), 0.83 (d, *J* = 6.7 Hz, 3H), 0.78 (d, *J* = 6.7 Hz, 3H), 0.68 (t, *J* = 7.5 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO) δ170.29, 169.83, (2C=O), 149.74, 147.26, 136.65, 132.75, 129.54, 128.71, 124.62, 119.65, (eight aromatic carbons), 61.79, 58.87, 31.69, 30.96, 20.91, 19.50, 19.39, 18.61, 18.58 (nine aliphatic carbons). ESI-MS: m/z, 491[M+H]$^+$, 513 [Manna]$^+$. HRMS-ESI: calcd. For $C_{23}H_{30}N_4O_6S$ [M+H]$^+$ 491.1964; Found 491.1965.

3-Methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(4-methylphenylsulfonamido) butanamide (8f)

Yield 74%, M.p=102-104 ^{0}C

FTIR (KBr, cm^{-1}): 3357, 3295, 3213 (3NH), 2966, 2926, 2877 (C-H Aliphatic), 1681, 1643, (2C=O, amide), 1542, 1456 (C=C-Aromatic), 1381, 1317 (SO_2), 1162, 1093 (C-N**).** ^{1}H NMR (400 MHz, DMSO) δ9.88 (s, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.72–7.59 (m, 3H), 7.46 (d, *J* = 8.4 Hz, 2H), 7.26 (d, *J* = 8.1 Hz, 2H), 7.10 (d, *J* = 8.3 Hz, 2H), 4.05 (t, *J* = 7.8 Hz, 1H), 3.64 (dd, *J* = 9.1, 6.8 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 1.82 (dq, *J* = 20.3, 6.8 Hz, 2H), 0.81 (d, *J* = 6.7 Hz, 3H), 0.74 (t, *J* = 7.3 Hz, 9H). ^{13}C-NMR (101 MHz, DMSO) δ170.64, 169.90, (2C=O), 142.54, 138.74, 136.76, 132.71, 129.67, 129.56, 127.10, 119.65, (eight aromatic carbons), 61.72, 58.85, 31.67, 31.14, 21.38, 20.93, 19.56, 19.46, 18.71, 18.54 (ten aliphatic carbons). ESI-MS: m/z, 460 [M+H]$^+$, 482 [M+Na]$^+$. HRMS-ESI: calcd. For $C_{24}H_{33}N_3O_4S$ [M+H]$^+$ 460.2270; Found 460.2271.

***N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-methylphenylsulfonamido) butanamido) butanamide (8g)**

Yield 65%, M.p=165-167 ^{0}C
FTIR (KBr, cm^{-1}): 3352, 3277, 3219 (3NH), 2965, 2931, 2879 (C-H Aliphatic), 1683, 1643, (2C=O, amide), 1541, 1461, 1415 (C=C-Aromatic), 1380, 1323, 1246 (SO_2), 1162, 1095, (C-N**)**
^{1}H-NMR (400 MHz, DMSO) δ9.91 (s, 1H), 7.97 (d, *J* = 8.3 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 3H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 2H), 7.16 (d, *J* = 8.2 Hz, 2H), 4.06 (t, *J* = 7.7 Hz, 1H), 3.70 – 3.58 (m, 1H), 2.82 (dt, *J* = 13.6, 6.7 Hz, 1H), 2.29 (s, 3H), 1.84 (dt, *J* = 13.7, 6.8 Hz, 2H), 1.16 (d, *J* = 6.8 Hz, 6H), 0.81 (d, *J* = 6.6 Hz, 3H), 0.74 (t, *J* = 7.5 Hz, 9H). ^{13}C-NMR (101 MHz, DMSO) δ170.64, 169.92, (2C=O), 143.90, 142.68, 138.75, 137.02, 129.67, 127.09, 126.89, 119.74, (eight aromatic carbons), 61.72, 58.87, 33.37, 31.68, 31.13, 24.44, 21.36, 19.56, 19.44, 18.72, 18.54 (eleven aliphatic carbons). ESI-MS: m/z, 488 [M+H]$^+$, 510 [M+Na]$^+$. HRMS-ESI: calcd. For $C_{26}H_{37}N_3O_4S$ [M+Na]$^+$ 510.2402; Found 510.2407.

3-Methyl-*N*-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(4-methylphenylsulfonamido) butanamide. (8h)

Yield 71%, M.p=140-142 ^{0}C
FTIR (KBr, cm^{-1}): 3310, 3265 (2NH), 3061 (C-H-aromatic) 2965, 2926 (C-H Aliphatic), 1643, 1603, (2C=O, amide), 1540, 1447 (C=C-Aromatic), 1380, 1333, 1248 (SO_2), 1159, 1090 (C-N**).** ^{1}H-NMR (300 MHz, DMSO) δ10.02 (s, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.68 (dd, *J* = 15.8, 8.6 Hz, 3H), 7.59 (d, *J* = 7.8 Hz, 2H), 7.29 (dd, *J* = 13.9, 7.5 Hz, 4H), 7.04 (t, *J* = 7.1 Hz, 1H), 4.07 (t, *J* = 7.5 Hz, 1H), 3.65 (t, *J* = 7.5 Hz, 1H), 2.29 (s, 3H), 1.92–1.74 (m, 2H), 0.81 (d, *J* = 6.4 Hz, 3H), 0.76 (d, *J* = 5.6 Hz, 9H). ^{13}C-NMR

(101 MHz, DMSO) δ170.68, 170.18, (2C=O), 142.52, 139.27, 138.61, 129.67, 129.20, 127.10, 123.79, 119.63, (eight aromatic carbons), 61.73, 58.91, 31.68, 31.12, 21.37, 19.57, 19.47, 18.70, 18.56 (nine aliphatic carbons). ESI-MS: m/z, 446 $[M+H]^+$, 468 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{23}H_{31}N_3O_4S$ $[M+Na]^+$ 468.1933; Found 468.1938.

3-Methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(phenylsulfonamido)butanamide (8i)

Yield 88%, M.p=177-179 ^{0}C

FTIR (KBr, cm^{-1}): 3328, 3283 (2NH), 3079 (C-H-aromatic), 2966, 2921 (C-H aliphatic), 1646, 1610, (2C=O, amide), 1539, 1452 (C=C-aromatic), 1322, 1244 (SO_2), 1164, 1092 (C-N**).**

^{1}H-NMR (400 MHz, DMSO) δ9.93 (s, 1H), 8.04 (d, *J* = 5.6 Hz, 1H), 7.79 (t, *J* = 7.7 Hz, 3H), 7.54 (t, *J* = 7.3 Hz, 1H), 7.47 (t, *J* = 8.1 Hz, 4H), 7.09 (d, *J* = 8.3 Hz, 2H), 4.05 (t, *J* = 7.8 Hz, 1H), 3.76 – 3.61 (m, 1H), 2.24 (s, 3H), 1.85 (dt, *J* = 20.8, 6.8 Hz, 2H), 0.80 (d, *J* = 6.7 Hz, 3H), 0.75 (t, *J* = 7.5 Hz, 9H).^{13}C-NMR (101 MHz, DMSO) δ170.64, 169.81, (2C=O), 141.69, 136.80, 132.66, 132.55, 129.52, 129.23, 126.97, 119.69, (eight aromatic carbons), 61.79, 59.03, 31.63, 31.07, 20.93, 19.54, 18.85, 18.55 (eight aliphatic carbons). ESI-MS: m/z, 446 $[M+H]^+$, 468 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{23}H_{31}N_3O_4S$ $[M+H]^+$ 446.2114; Found 446.2116.

3-Methyl-N-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(phenylsulfonamido)butanamide(8j)

Yield 60%, M.p=1160-163 ^{0}C

FTIR (KBr, cm^{-1}): 3266 (NH), 2962, 2921 (C-H Aliphatic), 1764, 1643, (2C=O, amide), 1543, 1500, 1448 (C=C-Aromatic), 1379, 1347, 1214 (SO_2), 1166, 1090 (C-N**).** ^{1}H-NMR (400 MHz, DMSO) δ9.99 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.79 (d, *J* = 7.9 Hz, 3H), 7.55 (dd, *J* = 14.5, 7.5 Hz, 3H), 7.48 (t, *J* = 7.3 Hz, 2H), 7.29 (t, *J* = 7.8 Hz, 2H), 7.04 (t, *J* = 7.3 Hz, 1H), 4.07 (t, *J* = 7.7 Hz, 1H), 3.77-3.63 (m, 1H), 1.86 (ddd, *J* = 23.2, 13.4, 6.7 Hz, 2H), 0.83-0.71 (m, 12H). ^{13}C-NMR (101 MHz, DMSO) δ170.70, 170.22, (2C=O), 141.72, 139.17, 132.54, 129.23, 129.20, 126.98, 123.78, 119.66, (eight aromatic carbons), 61.69, 59.00, 31.64, 31.05, 19.54, 18.82, 18.55 (seven aliphatic carbons). ESI-MS: m/z, 432 $[M+H]^+$, 454 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{22}H_{29}N_3O_4S$ $[M+Na]^+$ 454.1776; Found 454.1775.

***N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(phenylsulfonamido)butanamido)butanamide(8k)**

Yield 82%, M.p=134-136 ^{0}C

FTIR (KBr, cm^{-1}): 3304 (NH), 3063 (C-H-aromatic) 2963, 2930, 2871 (C-H Aliphatic), 1643, 1605, (2C=O, amide), 1537, 1451, 1451 (C=C-Aromatic), 1380, 1330, 1251, 1216 (SO_2), 1164, 1092 (C-N)**.** ^{1}H-NMR (400 MHz, DMSO) δ9.91 (s, 1H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.86-7.70 (m, 3H), 7.55 (t, *J* = 7.2 Hz, 1H), 7.48 (t, *J* = 8.6 Hz, 4H), 7.15 (d, *J* = 8.4 Hz, 2H), 4.05 (t, *J* = 7.8 Hz, 1H), 3.69 (dd, *J* = 8.9, 7.0 Hz, 1H), 2.82 (dt, *J* = 13.7, 6.8 Hz, 1H), 1.84 (dt, *J* = 19.6, 6.7 Hz, 2H), 1.16 (d, *J* = 6.9 Hz, 6H), 0.84 – 0.69 (m, 12H). ^{13}C-NMR (75 MHz, DMSO) δ170.15, 169.46 (2C=O), 143.37, 141.21, 136.50, 132.03, 128.72, 126.47, 126.38, 119.27 (eight aromatic carbons), 61.18, 58.43, 32.85, 31.13, 30.57, 23.93, 19.01, 18.33, 18.02 (nine aliphatic carbons).ESI-MS: m/z, 474 $[M+H]^+$, 496 $[M+Na]^+$. HRMS-ESI: calcd. For $C_{25}H_{35}N_3O_4S$ $[M+Na]^+$ 496.2246; Found 496.2248.

2.5. In vivo anti-malaria test

2.5.1 Experimental design and treatment of mice

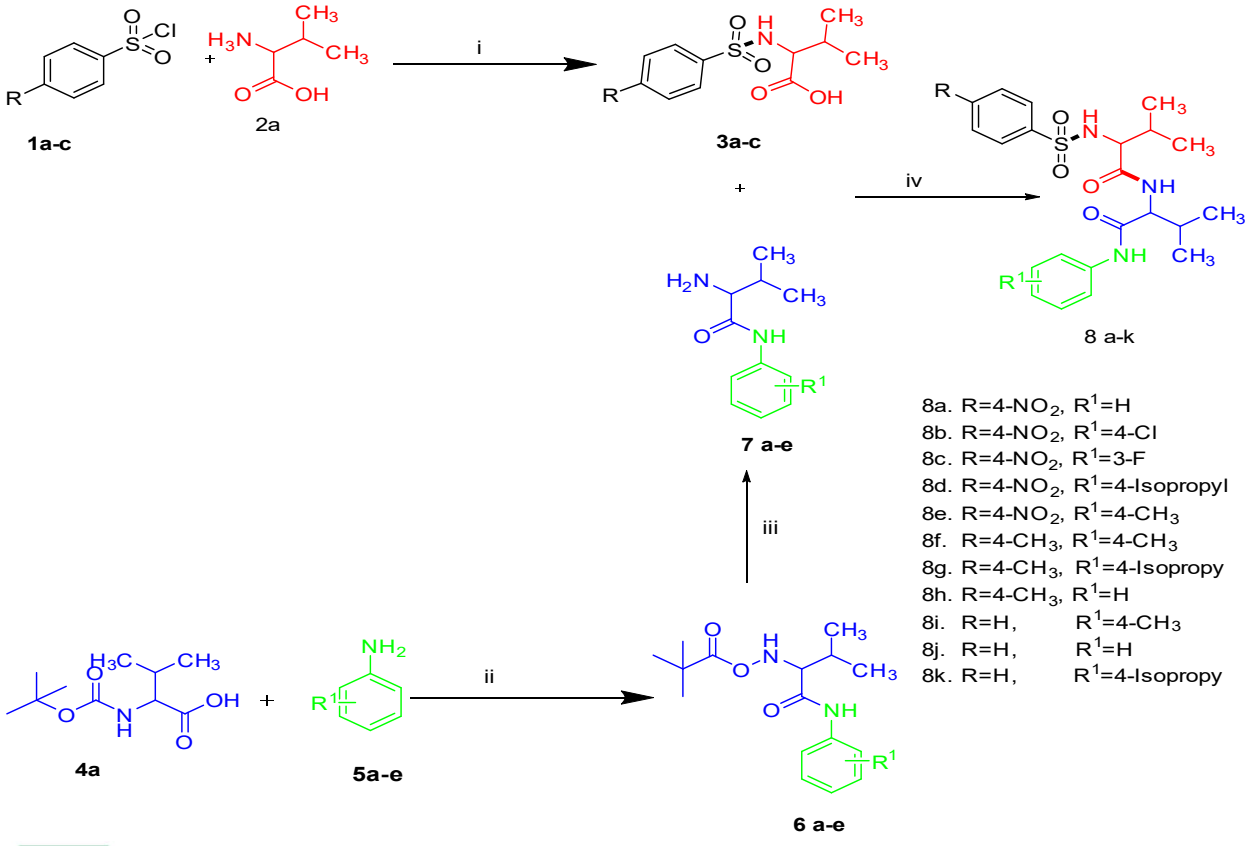
Methods reported by (Okokon and Nwafor, 2009) for antiplasmodial assay against *Plasmodium berghei* infection in mice were employed. About forty-eight infected mice were randomly divided into twelve groups, each having four mice. A stock of parasitized erythrocytes was obtained from infected mice, with a minimum peripheral parasitemia of 20 % by cardiac puncture in EDTA-coated tube. The percentage parasitaemia was determined by counting the number of parasitized red blood cells against the total number of red blood cells. The cell concentration of the stock was determined and diluted with physiological saline such that 0.2 mL of the final inoculum contained 1 x10^7 parasitized red blood cells which are the standard inoculums for the infection of a single mouse. After 7 days of infection, animals begin to receive treatment (50 mg/kg) of the synthesized compounds (8a–8k) for 7 days with constant check of the percentage of parasitemia after a 4-day interval. Artemisinin (50 mg/kg body weight.) was given to the other mice in group twelve as positive control, group thirteen was not treated. All the compounds and the drugs were given orally by using a standard intragastric tube.

2.6. Haematological analysis

Twenty-four hours before the injecting the compounds, the blood samples of four mice were taken and also taken after the last day of treatment, the animals were sacrificed by cervical dislocation and the blood samples were collected by heart puncture. The blood samples for haematological parameters (red blood cell (RBC) count, packed cell volume (PCV), and hemoglobin (HGB) were collected into EDTA bottles. Packed cells Volume (PCV) were determined by microhaematocrit technique using capillary tube as described by Schalam (1975). The Red blood cell counts (RBC) was determined as described by Brown (1976). The Haemoglobin (Hb) concentrations were determined according to Hewitt (1984).

3.0. Results and Discussion

Considering the many pharmacological activities of sulphonamides and peptides as found in the literature and the need for anti-malaria agents with reduced side effects, we undertook the design and synthesis of some novel hybrids which possess advantages of the three pharmacophores of sulphonamide, carboxamide and dipeptides in single molecular backbone. Our design strategy employed the use of classical peptide coupling reagent 1-hydroxybenzotriazole (HOBt) and 1-ethyl-3-(3`-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC.HCl) in the condensation of 2-Amino-*N*-(substituted phenyl)-3-methylbutanamide with substituted benzenesulphonamides derived from L-Valine. The use of HOBT and EDC.HCl was aimed to enhance coupling rates and reduces the risk of racemization. During the course of this study, we synthesized and characterized hybrid compounds containing sulphonamide, and dipeptides. The reaction of substituted benzenesulphonyl chloride (**1a-c**) with L-Valine gave the substituted benzenesulphonamoyl alkanamides (3a-c). The reaction of commercially available Boc-protected valine with amines using EDC.HCl, HOBt, and triethylamine (TEA) in DCM afforded the carbamate derivatives of valine (6a-e). The unprotected amines(7a-e) were obtained from the reaction of compound (6a-e) with DCM/ TFA (1:1%) for 1h, respectively. The amidation of compound (3a-c) with the unprotected amines (7a-e) in the presence of peptide coupling reagents EDC.HCl, HOBt, TEA, gave the targeted products (8a-k).



Scheme 1: Synthesis of dipeptide bearing sulphonamide, reagents and conditions. (i) Na_2CO_3, H_2O, HCl, -5 ^{O}C - 0 ^{O}C, r.t, 4 h. (ii) EDCI, HOBt, TEA, DCM, amine,19-24 h. (iii) TFA/DCM (1:1%) (iv) EDCI, HOBt, TEA, 19-24h.

The structures of 8a–k were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and ESI-MS, ESI-HRMS analyses. While the IR spectra of compound 8a exhibited three strong bands at ∼3363, 3305, 3220 cm-^{1} for the –NH (amide), two strong bands between 1680 and 1642 cm-^{1} for amide carbonyls and a band ∼1380 cm-^{1} for the sulfonamide group were found. In the ^{1}H NMR of 8a-k the characteristic NH resonance of the sulphonamide part of the dipeptide conjugates 8a-k were observed at δ7.95-8.34 ppm region as doublet peak in the ^{1}H-NMR spectrum. Other amide NH resonances of the dipeptide-sulphonamide were observed at δ7.65-8.06 ppm and δ9.91-10.20 ppm region as doublet or triplet and as singlet respectively. There is doublet or triplet at 3.97-4.07 ppm due to CH and NH of the valine. A doublet or multiplet at *δ*3.58-3.85ppm is observed due to the CH- and Isobutane interaction of the valine amide. In the ^{13}C NMR spectrum two peaks at δ170.32, and 170.10 for the carbonyl carbons of the amide groups. The appearance of peaks at 61.76, 58.88, 31.71, 30.96, 19.51, 19.40, 18.63 and 18.56 shows the presence of eight aliphatic carbons. Eight peaks ranging from 119.61 to 149.70 for the aromatic carbons confirmed the formation of **8a**, which was also supported by its ESI-MS spectrum with a peak at *m/z* 477 for $[M+H]^{+}$, HRMS-ESI: calcd. For $C_{22}H_{28}N_4O_6S$ $[M+Na]^{+}$ 499.1627; Found 499.1629.

Table 1: Percentage inhibition of parasite in mice

Compounds	8a	8b	8c	8d	8e	8f	8g	8h	8i	8j	8k	artemisinin	NTC
% Inhibition 7days post-treatment (50mg/kg)	23	2.4	29	51	71	22	57	12	57	46	53	67	-

3.1. In vivo antimalaria

To determine the in vivo activity, the compounds were tested against *P*. berghei NK(65 Strain) infection mice, the animals were obtained from TwinVet® Labouratory, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The permission and approval for the use of animals in this experiment were granted by the Animal Ethics Committee, Veterinary Medicine Department, University of Nigeria, Nsukka.

Artemether was used as standard drug in these experiments. The percentage inhibition of parasite multiplication was calculated comparing the treated group with untreated group using the following formula (Carvalho *et al*., 1991) [(A-B)/A] x100; where A=parasitaemia in the untreated group and B= parasitemia in the test group. The compounds were portioned in such a way that those that reduced parasitemia by 40% were considered active, whereas those that reduced parasitaemia by 30–40% or less than 30% were considered partially active and inactive, respectively (Wasser *et al.,* 2005). Most of the compounds were active against *P*. berghei after 7 days post-infection by reducing the parasiteamia by at least 40% when in a dose of 50 mg/kg (Table 3).

Among the *para*-Nitrophenylsulphonamide analogs (8a-e) the effect of chloro, fluoro, Isopropyl and methyl substituents on the *N*-phenylacetamide was studied and it was observed that the *p*-methyl-*N*-phenylacetamide derivative (8e) show a greater activity, followed by *p*-isopropyl-*N*-phenylacetamide derivative (8d) whereas the chloro, fluoro and phenylacetamide derivatives (8a-c) were inactive. While among the *para*-methylphenylsulphonamide analogs (8f-h), the *P*-isopropyl-*N*-phenylacetamide (8g) and phenylacetamide derivatives (8h) were more potent than *P*-methyl-*N*-phenylacetamide derivative (8f). Furthermore, among the benzenesulphonamide analogs (8i-k), it was revealed that *p*-methyl and *p*-isopropyl-*N*-phenylacetamide derivative were more potent than the *N*-phenylacetamide derivative (8j)

which was inactive. Thus, the calculated percentage inhibition parasitaemia values, obtained from the *in-vivo* analysis of the sulphonamide-dipeptide conjugate (8a-k), the compounds 8e, 8g, 8i, 8k, 8d and 8j have almost the same or better activity compared to the Artemether whereas compound 8e clearly demonstrated the most potent *p. berghei* inhibitor.

3.2. Haematological analysis

From the analysis, there is an increase in the value of RBC, PCV and Hb for the control and some test compounds. Among some tested compounds, increase in PCV is not accompanied by corresponding increases in RBC counts and Hb concentration compared to pre-treatment and control. These findings agree with that of Wasser (2005) that the increase maybe relative polycytaemia due to haemoconcentration.

Table 2: Heamotological analysis before and after treatment

Comp	RBC (mm^3)		PCV (%)		HB (g/dl)	
	Before	**After**	**Before**	**After**	**Before**	**After**
8e	7.4 x10^6	5.3x10^6	26	42	9.5	8.6
8g	8.3 x10^6	10.6 x10^6	43	46	13.8	15.2
8i	7.9 x10^6	8.6 x10^6	41	48	11.5	13.8
8d	7.3 x10^6	11.2 x10^6	41	49	12.4	14.7
8j	8.3 x10^6	7.4 x10^6	38	47	7.8	7.6
8k	8.3 x10^6	9.3 x10^6	40	49	12.3	14.3

4.0 Conclusion

Eleven new valine- valine dipeptide carboxamide sulfonamide conjugates were synthesized and characterized. The spectral data confirmed the successful preparation of these derivatives. The compounds also exhibited anti-malaria activity *in vivo* against *P. berghei* in mice. Most of the compounds were active against *P. berghei* after 7 days post-infection by reducing the parasiteamia by at least 40 % when in a dose of 50 mg/kg (Table 3). The control drug showed activities on all the mice by inhibiting the parasitaemia growth more than 40 %. However, in consideration of the compounds ability to inhibit parasitaemia to the control drug, compounds 8e, 8g, 8i, 8k, 8d and 8j have almost the same or better activity compared to the artemisinin. Compounds Molecular docking showed significant chemical interactions of the compounds with different receptors resulting in high binding affinity. From the haematological analysis, even though there is a decrease in the value of (RBC), (PCV) and (HB), it is observed that there are no significant changes in the parameters that were analyzed for the control and the test compounds. Among some tested compounds, increase in PCV is not accompanied by corresponding increases in RBC counts and Hb concentration compared to pre-treatment and control. These findings agree with that of Wasser (2005) that the increase maybe relative polycytaemia due to haemoconcentration and it is recommended that more research should be carried out on these compounds as it showed great potential for antimalarial properties.

5 .0 Acknowledgement

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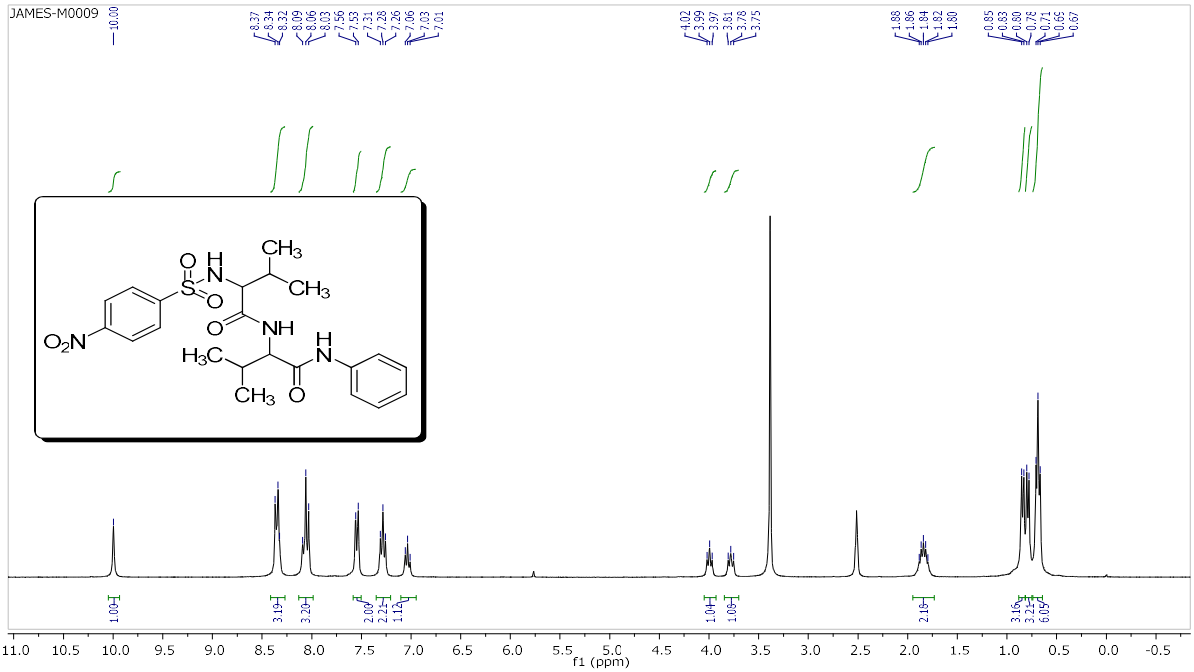
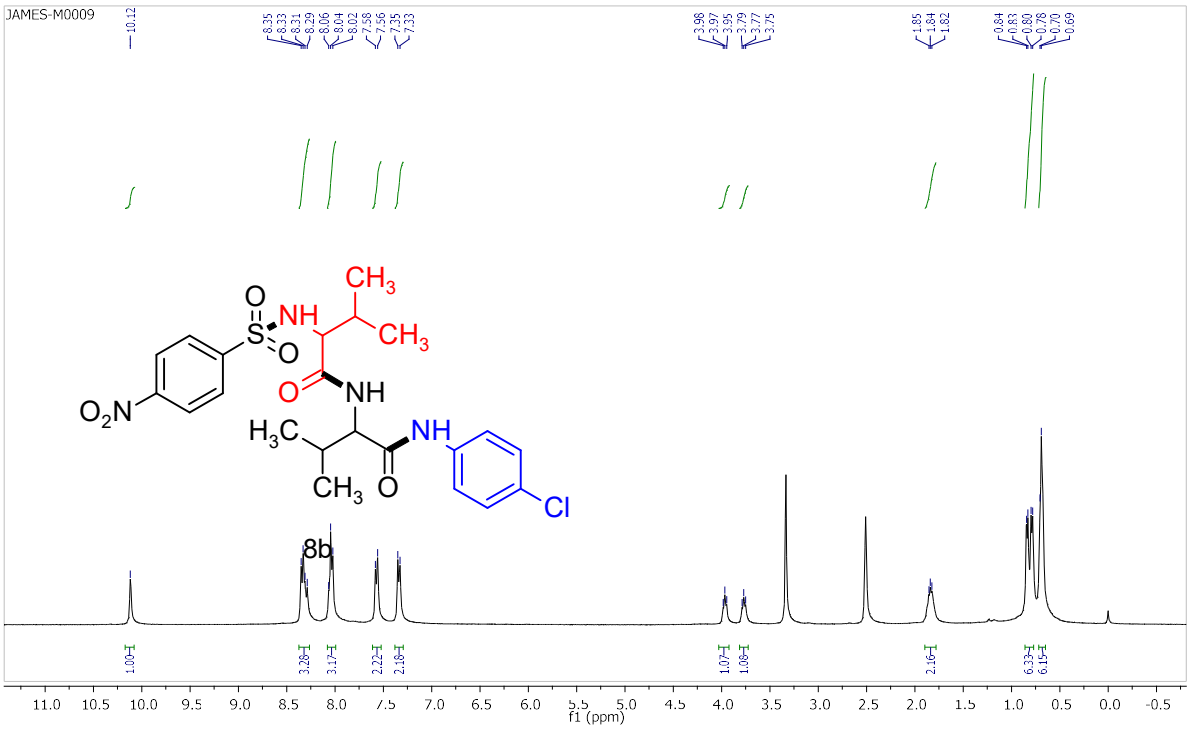
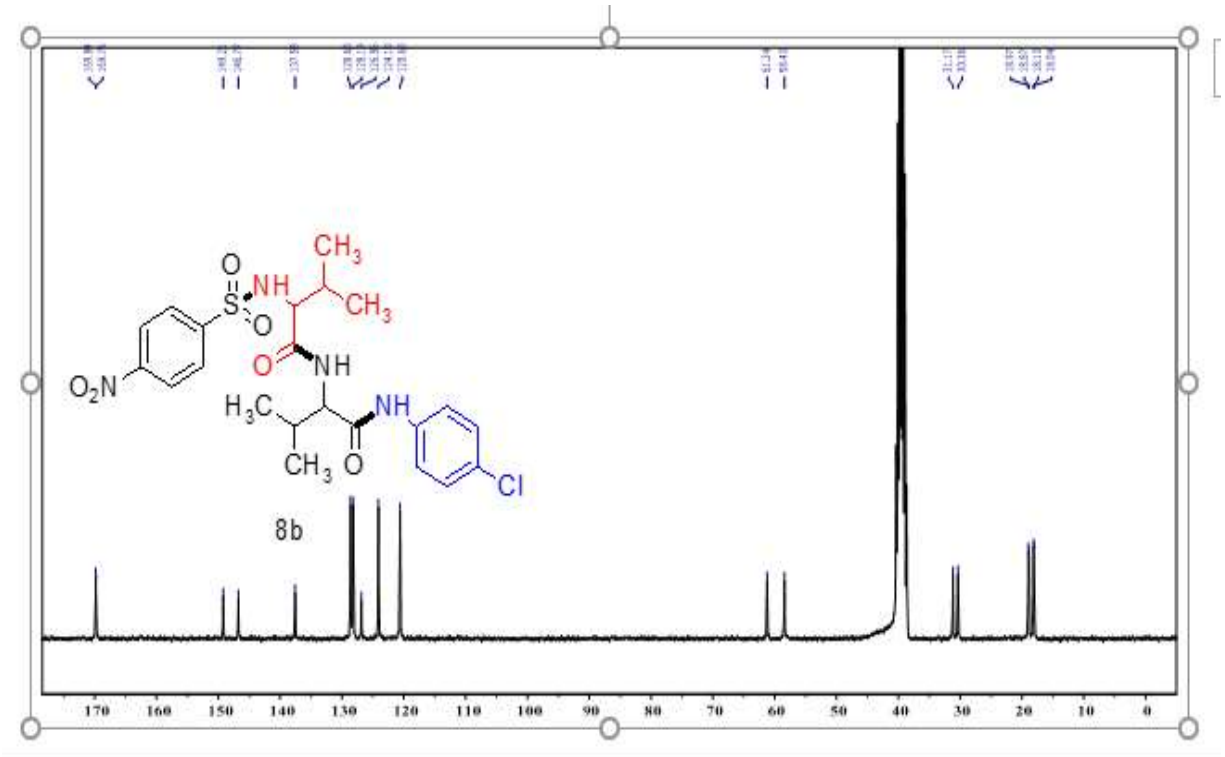


Appendix 1: ^{1}H NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(phenylamino) butan-2-yl)-2-(4-nitrophenylsulfonamido)butanamide (8a)

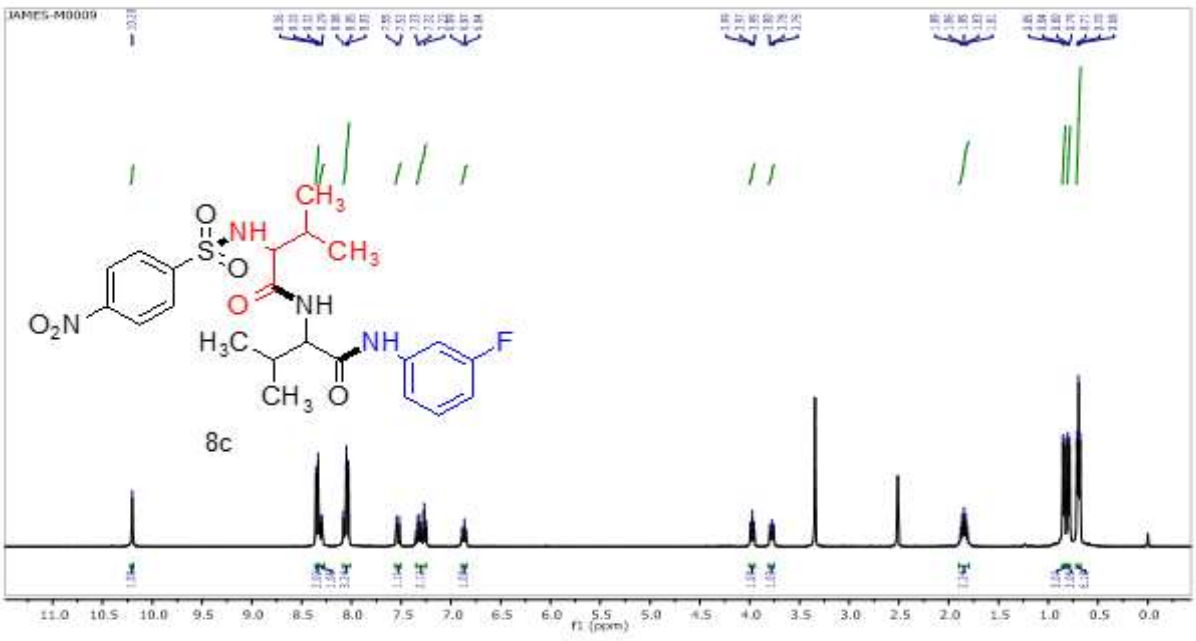
Appendix 2: ^{13}C NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(phenylamino) butan-2-yl)-2-(4-nitrophenylsulfonamido)butanamide (8a)



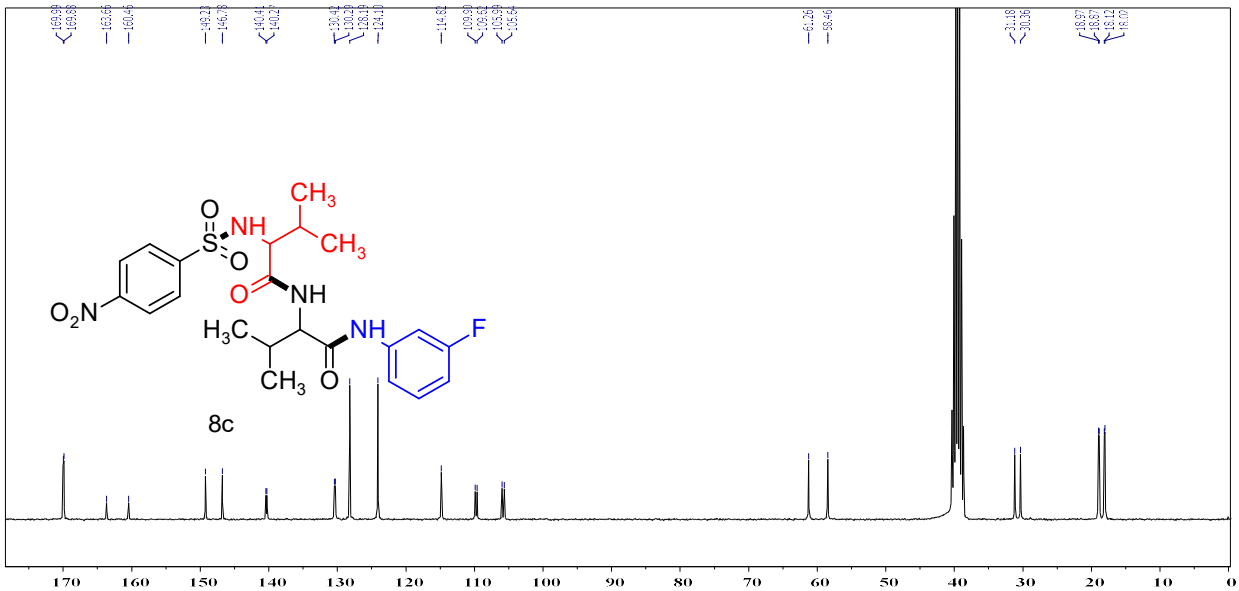
Appendix 3: ^{1}H NMR spectra of *N*-(4-chlorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido)butanamido)butanamide (8b)



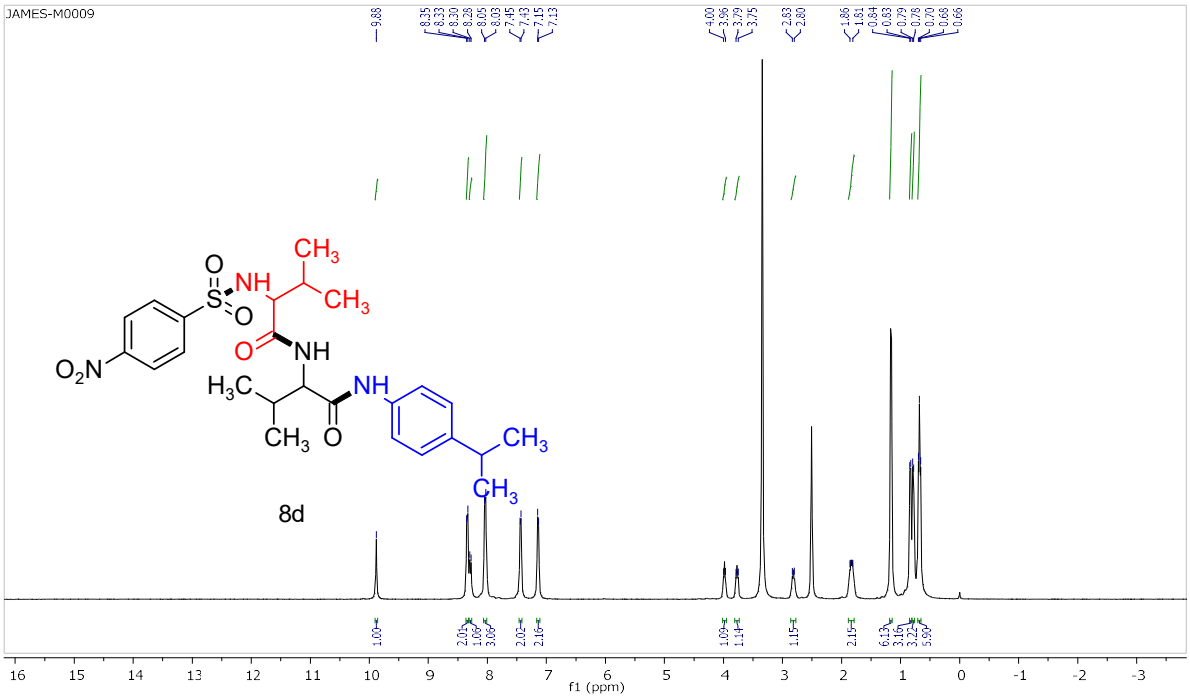
Appendix 4: ^{13}C NMR spectra of *N*-(4-chlorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido)butanamido)butanamide (8b)



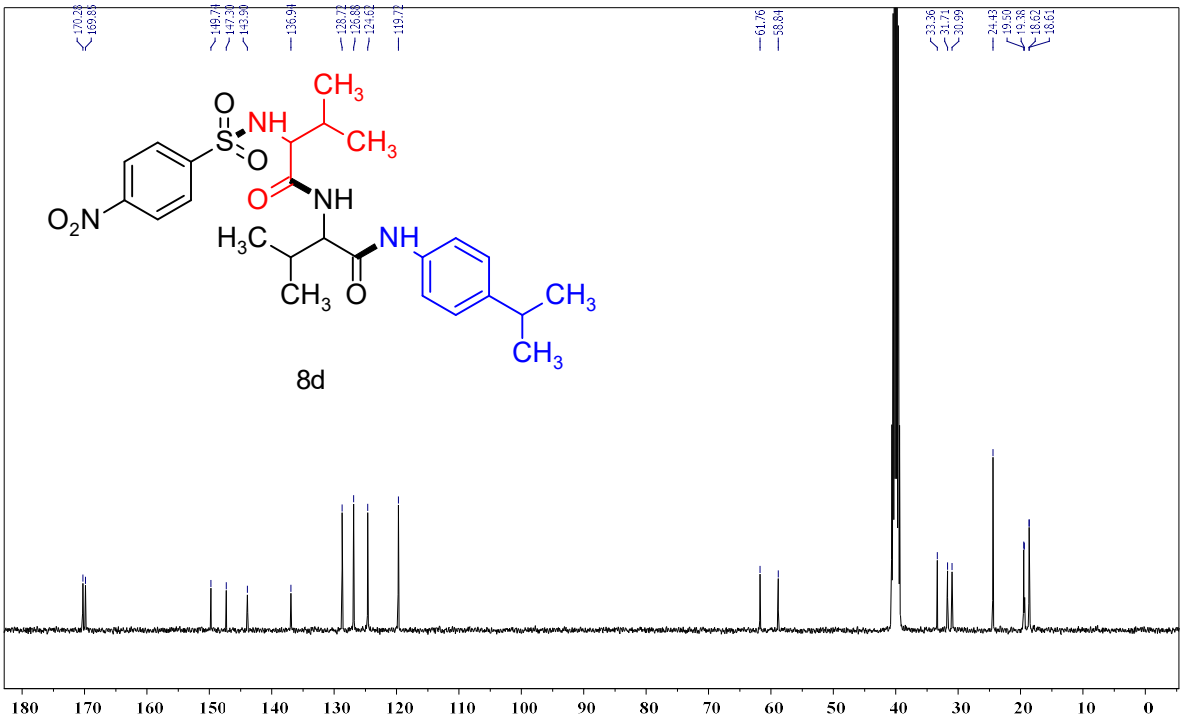
Appendix 5:[1]H NMR spectra of *N*-(3-fluorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido) butanamide (8c)



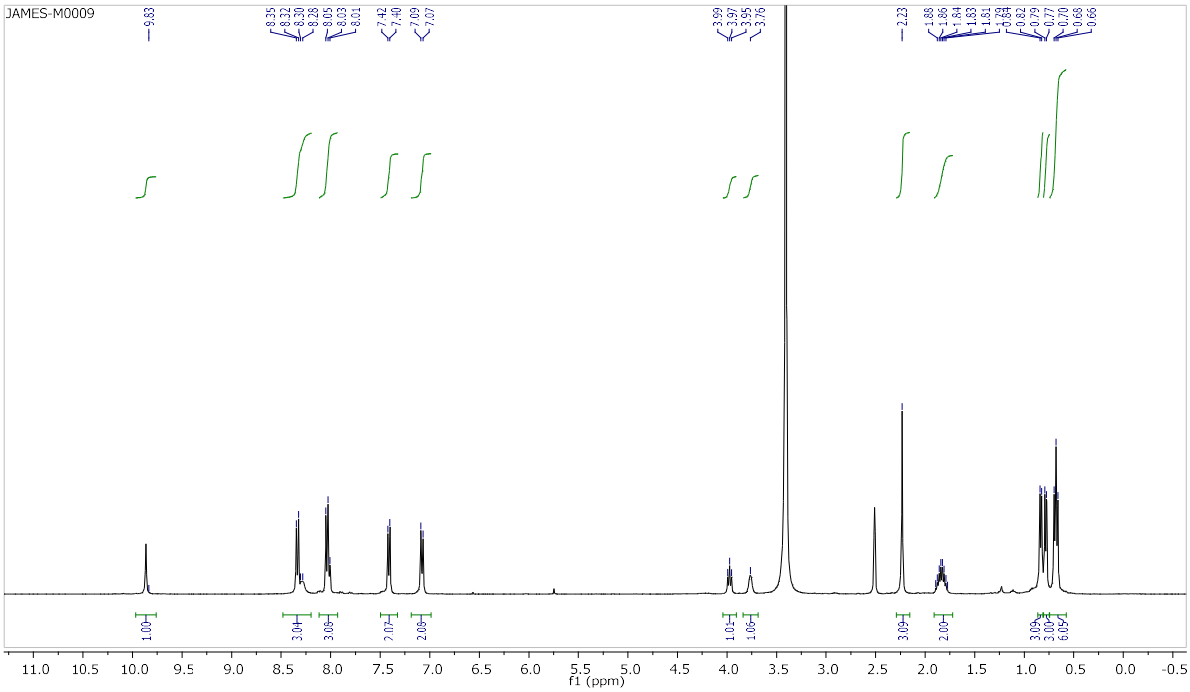
Appendix 6: [13]C NMR spectra of *N*-(3-fluorophenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido) butanamide (8c)



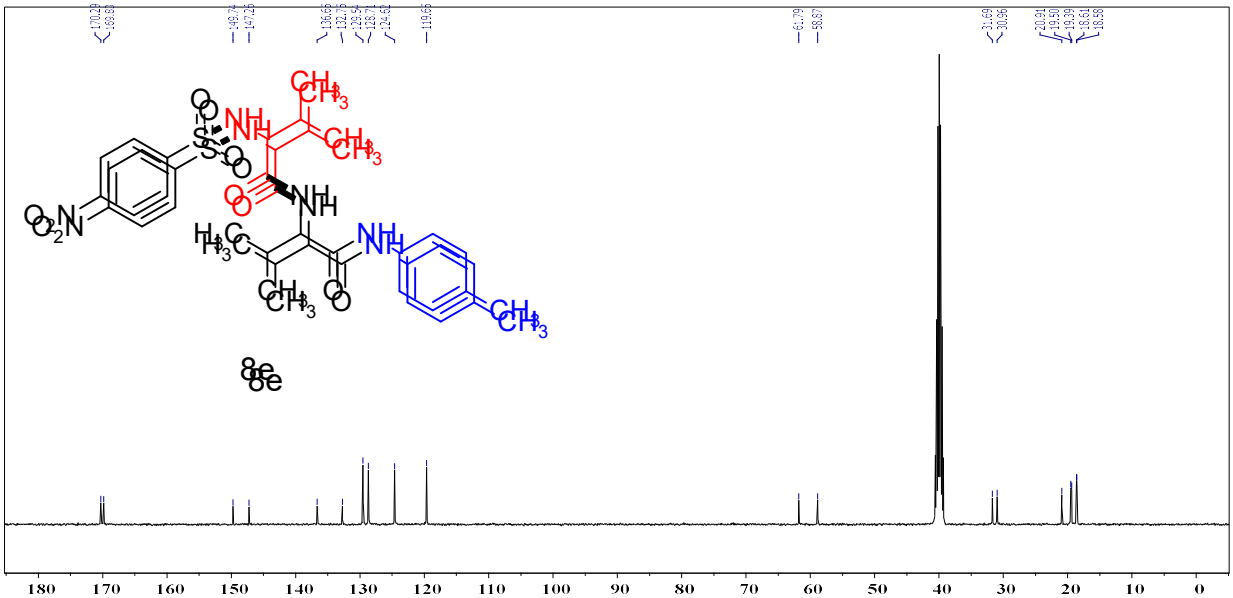
Appendix 7: ^{1}H NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido)butanamide (8d)



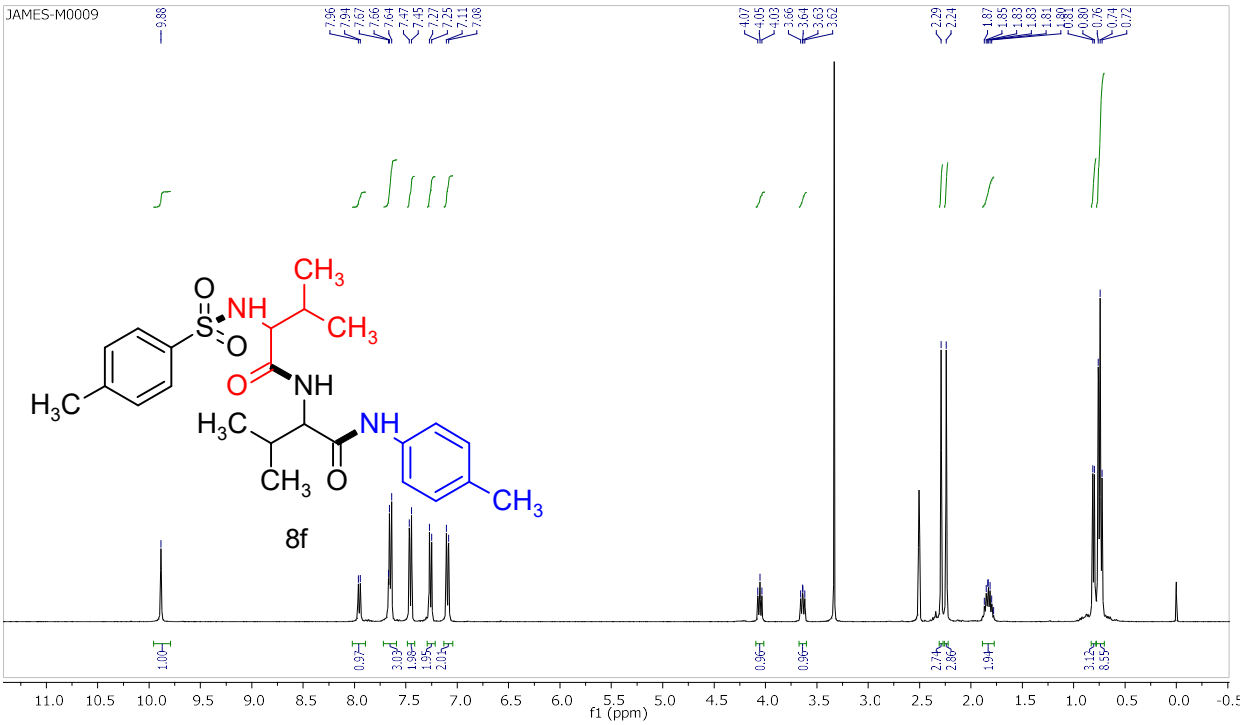
Appendix 8: ^{13}C NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-nitrophenylsulfonamido) butanamido)butanamide (8d)



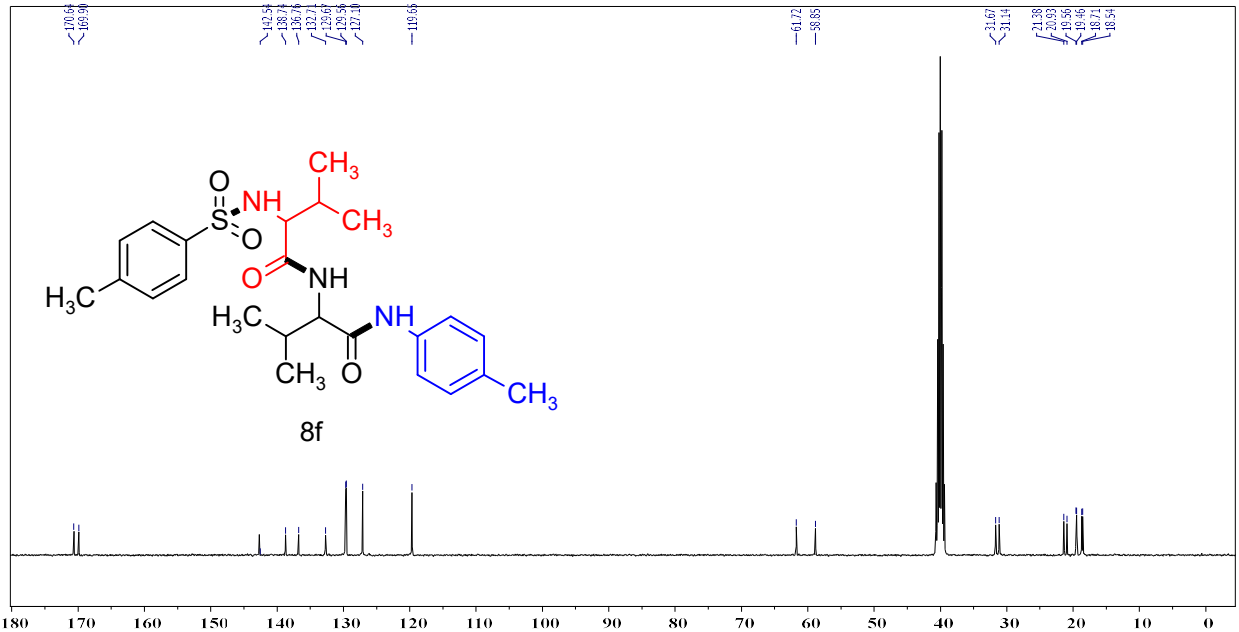
Appendix 9: ^{1}H NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(4-nitrophenylsulfonamido)butanamide (8e)



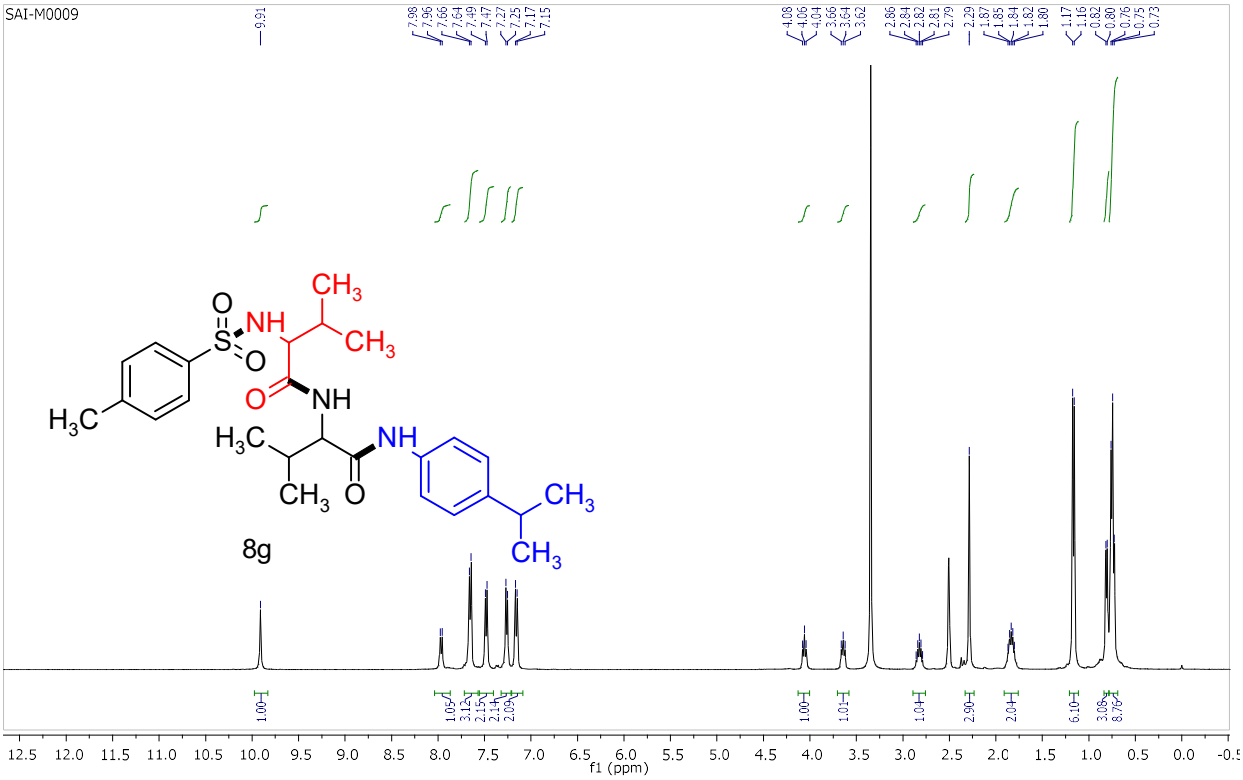
Appendix 10: ^{13}C NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(4-nitrophenylsulfonamido)butanamide (8e)



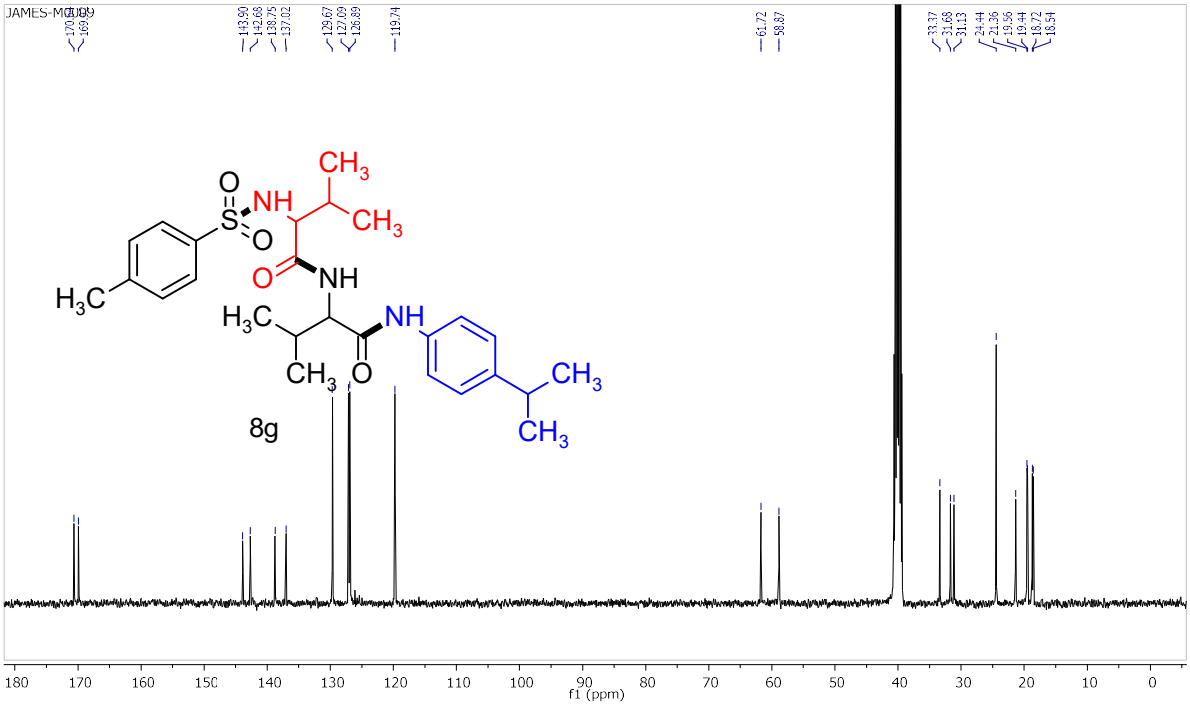
Appendix 11: ^{1}H NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(4-methylphenylsulfonamido)butanamide (8f)



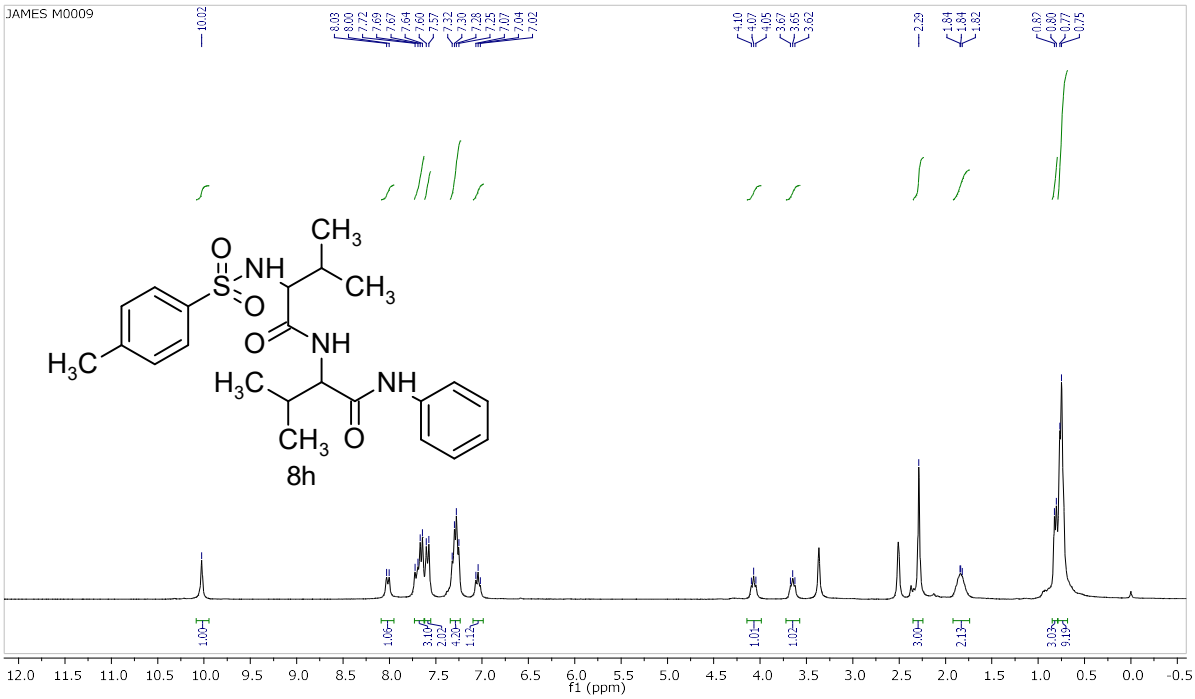
Appendix 12:^{13}C NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(4-methylphenylsulfonamido)butanamide (8f)



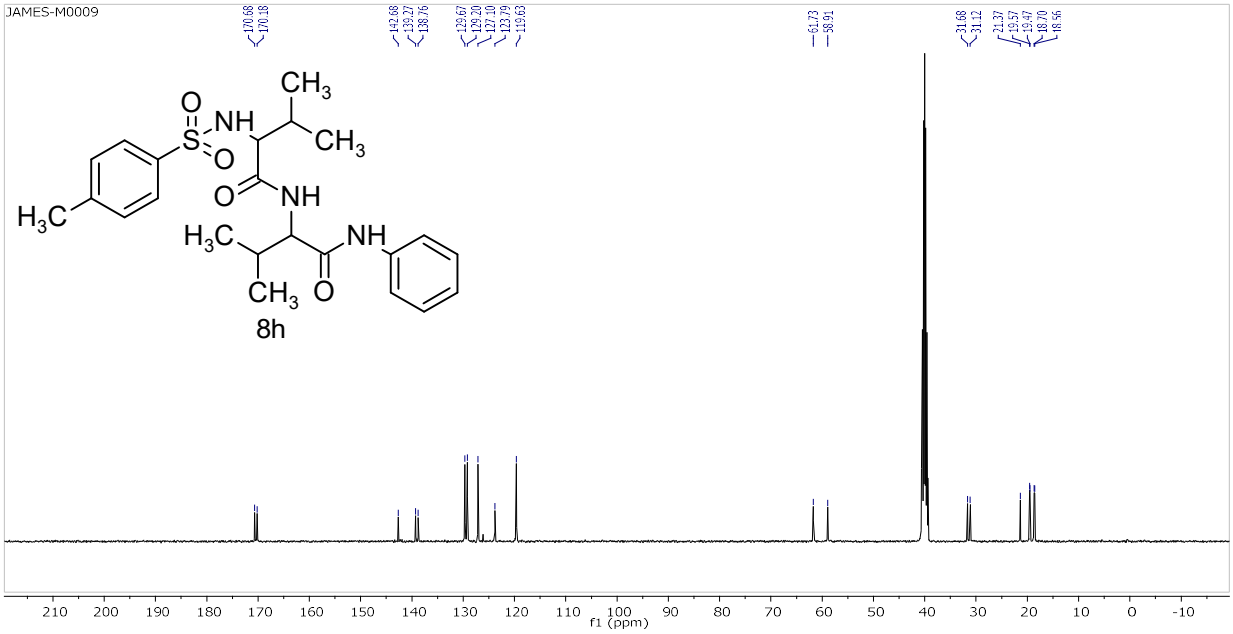
Appendix 13: ^{1}H NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-methylphenylsulfonamido) butanamido) butanamide (8g)



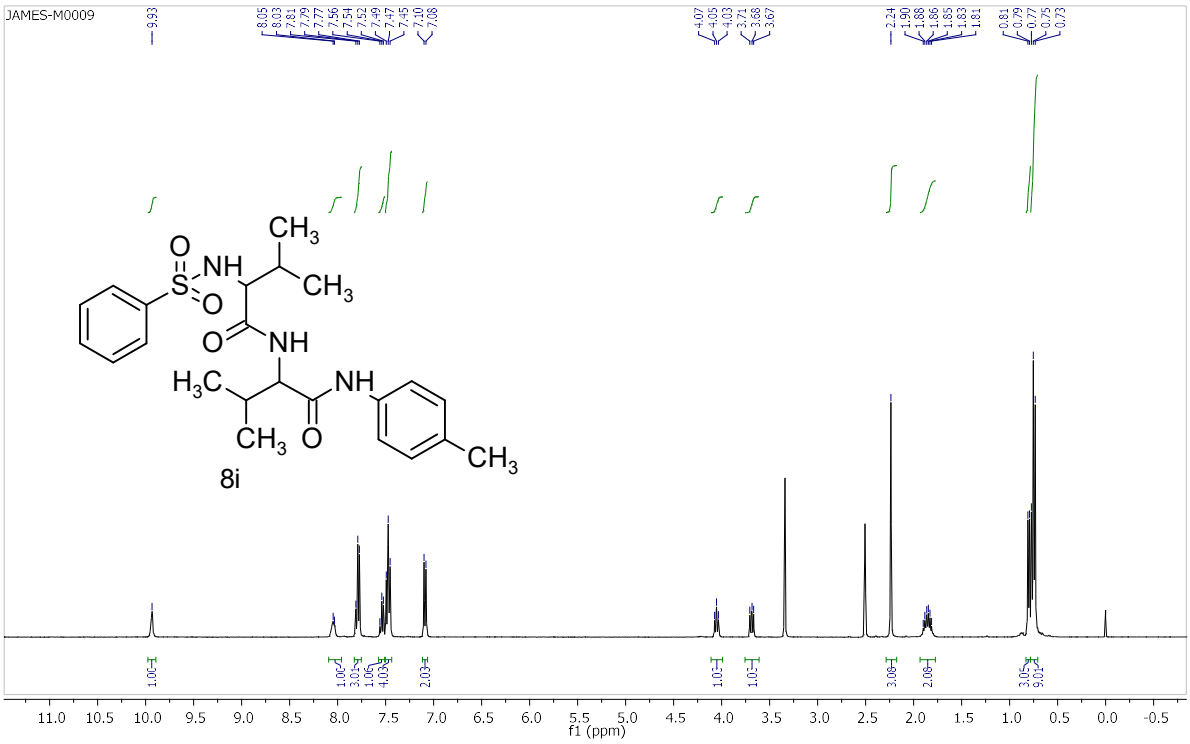
Appendix 14: ^{13}C NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(4-methylphenylsulfonamido) butanamido) butanamide (8g)



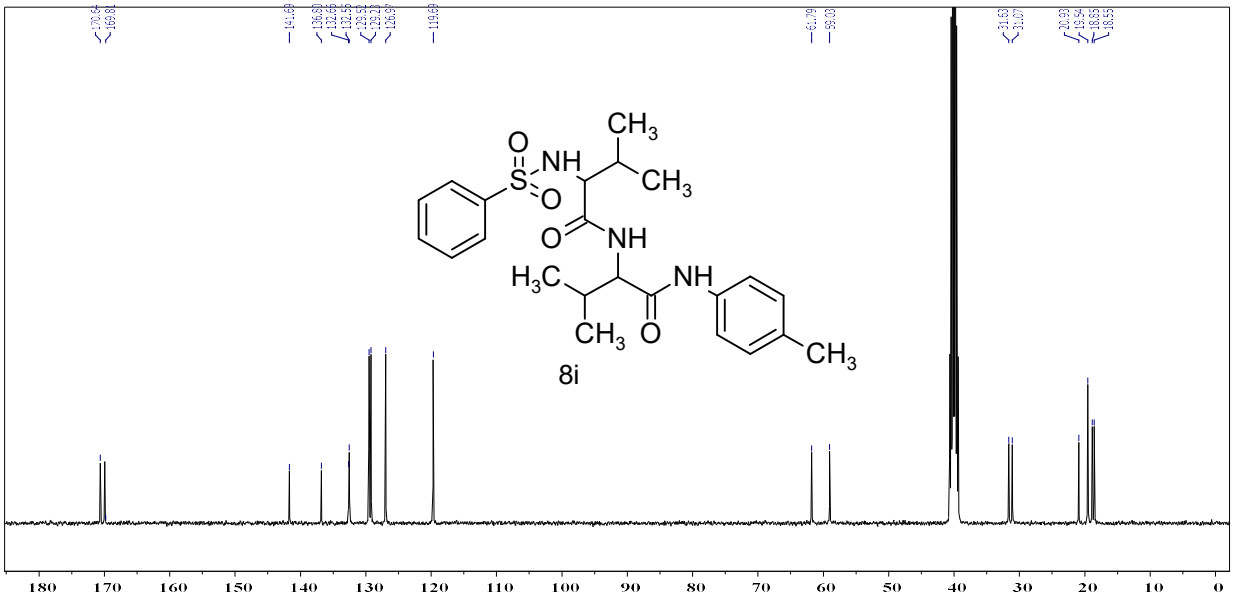
Appendix 15: ^{1}H NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(4-methylphenylsulfonamido)butanamide (8h)



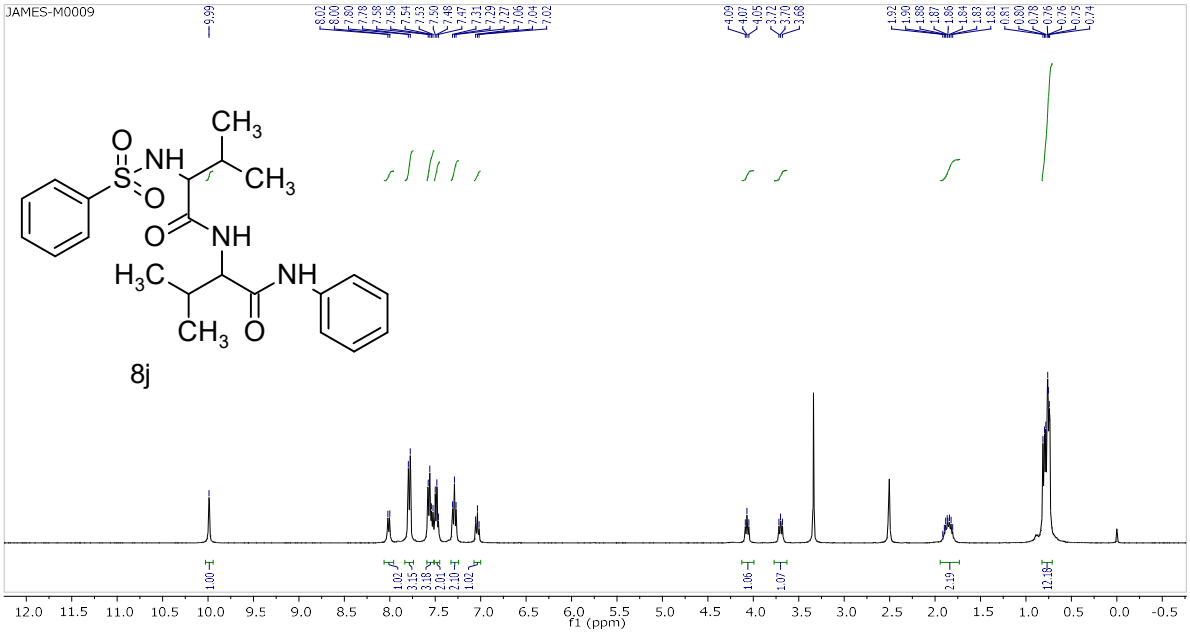
Appendix 16:^{13}C NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(4-methylphenylsulfonamido)butanamide (8h).



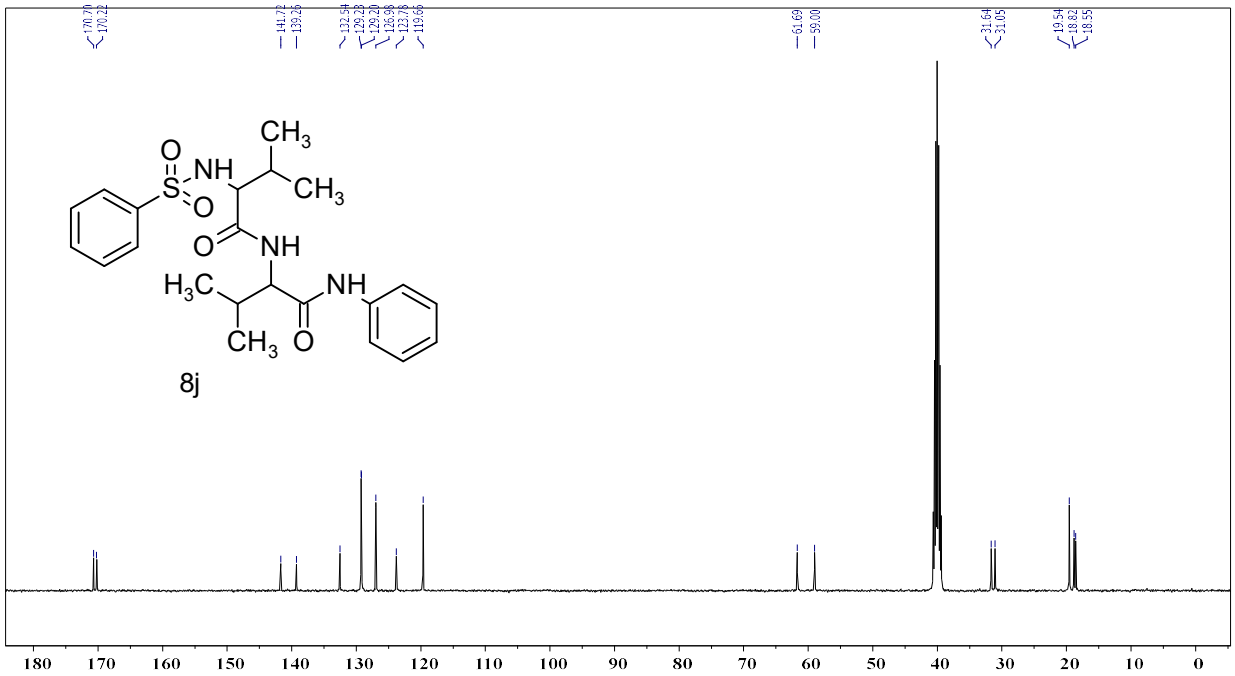
Appendix 17: ^{1}H NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(phenylsulfonamido)butanamide (8i)



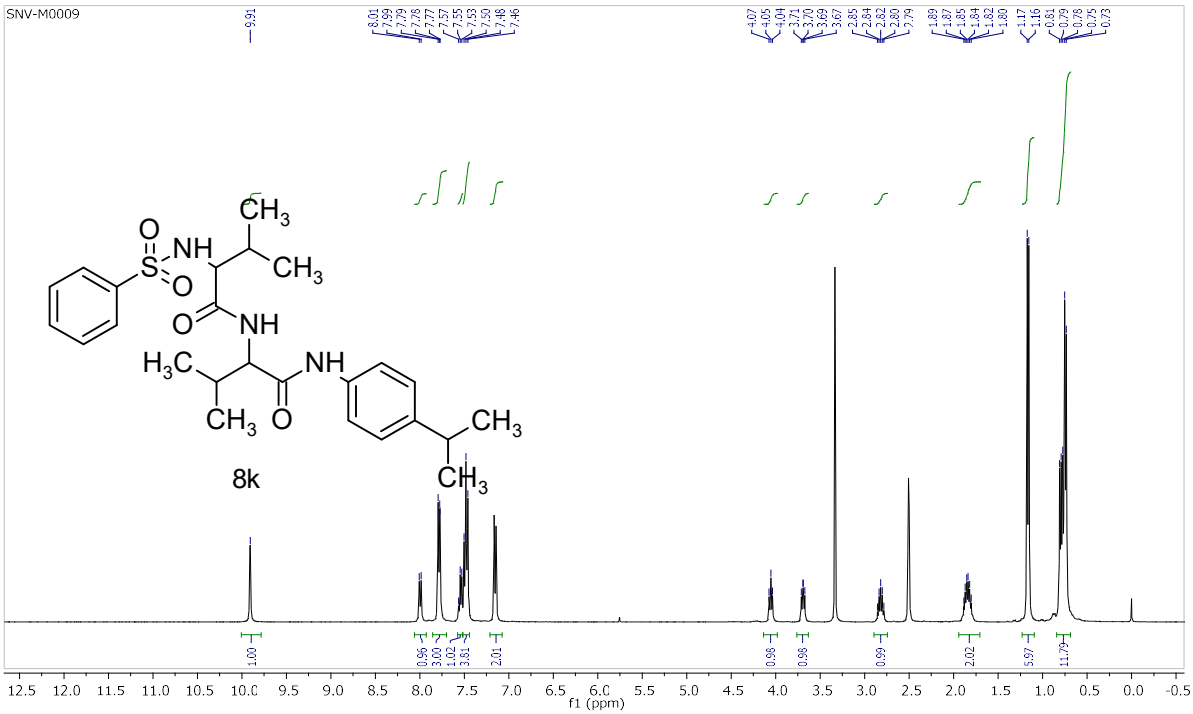
Appendix 18: ^{13}C NMR spectra of 3-methyl-*N*-(3-methyl-1-oxo-1-(p-tolylamino)butan-2-yl)-2-(phenylsulfonamido)butanamide (8i)



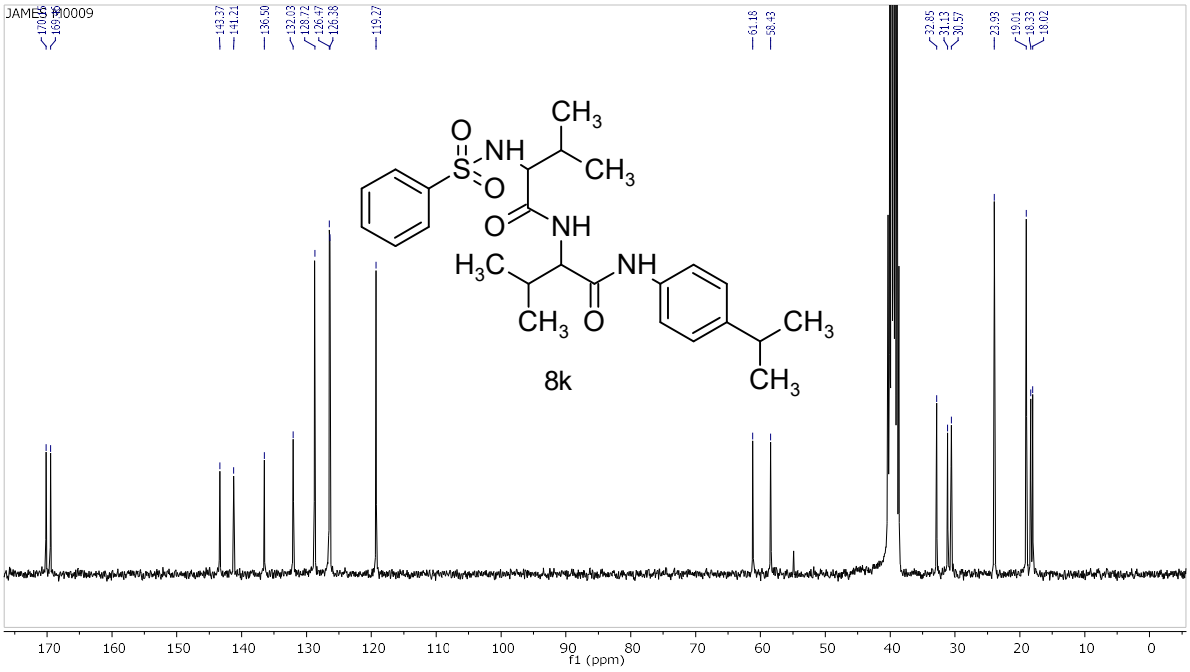
Appendix 19: ^{1}H NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(phenylsulfonamido) butanamide (8j)



Appendix 20:^{13}C NMR spectra of 3-methyl-N-(3-methyl-1-oxo-1-(phenylamino)butan-2-yl)-2-(phenylsulfonamido) butanamide (8j)



Appendix 21: ^{1}H NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(phenylsulfonamido) butanamido)butanamide (8k)



Appendix 22: ^{13}C NMR spectra of *N*-(4-isopropylphenyl)-3-methyl-2-(3-methyl-2-(phenylsulfonamido) butanamido)butanamide (8k)