Journal of Molecular Structure 1246 (2021) 131201

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstr

Synthesis, molecular docking and antimalarial activity of phenylalanine-glycine dipeptide bearing sulphonamide moiety

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ARTICLE INFO

Article history:
Received 26 April 2021
Revised 17 July 2021
Accepted 26 July 2021
Available online 2 August 2021

Keywords:
Synthesis
Molecular docking
Antimalarial
Phenylalanine
Glycine
Dipeptide Sulphonamide

ABSTRACT

Ten novel phenylalanine-glycine dipeptide sulphonamide conjugate were synthesized and characterized using ^{1}HNMR, ^{13}CNMR, FTIR and HRMS spectroscopic techniques. The *in silico* studies predicted better interactions of compounds with target protein residues and a higher dock score in comparison with standard drugs. The *in vivo* antimalarial study, hematological study, liver and kidney function test were evaluated on the synthesized compounds. Compounds **7h, 7i** and **7j** inhibited the parasite by 34.5–60.2% on day 4 of after-treatment exposure. Compound **7j** inhibited the multiplication of the parasite by 60.2% on day 4 of after-treatment which was comparable with that of the standard drug with 68.8% inhibition at same day of after-treatment exposure.

1.0. Introduction

Malaria is a common and life-threatening disease which is caused by parasites that are transmitted to people through the bite of infected female Anopheles mosquitoes [1]. Malaria is caused by the protozoan parasite plasmodium. Four species of the genus plasmodium cause all malarial infections in human being. In addition, one species, *Plasmodium knowlesi* that naturally affects animal has recently been recognized to be the cause of zoonotic malaria in humans [2]. The WHO's malaria report 2018, revealed that 228 million people had malaria cases with an estimated 405,000 deaths. The African region was home to 93% of malaria cases and 94% of malaria deaths [1]. Children under the age of five years are the most vulnerable group affected by malaria and they accounted for 67% of all the malaria deaths worldwide in 2018 [1].

In recent past, there has been emergence of resistance towards many of the anti-malarial drugs like chloroquine, sulfadoxine-pyrimethamine, quinine, mefloquine and piperaquine [3]. At present artemisinin-based combination therapy is the most effective drug in the treatment of malaria, however, artemisinin resistance has been observed in some countries [4]. This call for the development of new antimalarial drug. The challenge now is to identify new classes of drugs like peptide based drugs to combat the disease and imped drug resistance [5].

Peptides have been reported as good chemotherapeutic agent and can also serve as excipient in drug delivery system to overcome tissue and cellular membrane barriers [6]. It has been attracting considerable attention because of their potential utility in pharmaceutical [7]. Peptides have some advantages as a drug candidate over alternative molecules because of its low toxicity, high affinity, strong specificity and adequate tissue penetration [7].

Sulphonamides are class of medicinally important molecules that possesses varying pharmacological activities like antimicrobial, anticancer, anti-inflammatory, antioxidant, diuretic, carbonic anhydrase inhibition [8-14] among many others. They are used in drug design because of their low cost, and toxicity, chemical and metabolic stability, enhanced crystallinity, carboxyl bio-isosterism and high level of biological activity [15].

Dipeptide-sulphonamide hybrids have been reported as a good human carbonic anhydrase inhibitor [16], antimalarial [17] and antioxidant agents [18].

Computational techniques have been used in drug discovery so that high cost and time required for wet experiment will be re-

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https://doi.org/10.1016/j.molstruc.2021.131201
0022-2860/

duced. The increase in agreement between computational and wet experiments demonstrates the former as a good alternative to the later. Although, no drug has stirred from computer to market, computational method has been used in the development of FDA approved drugs. Therefore, both methods complement each other in drug development [19-20].

Based on the numerous applications of sulphonamide, reported side effects of current antimalarial drug and emerging challenges associated with multidrug resistance malaria parasites, there is need for the synthesis of novel compounds as potential antimalarial agents with less side effect and improved selectivity [16]. In view of these challenges, we designed the synthesis of novel sulphonamide-dipeptide moieties with possible and improved antimalarial potential. We report herein the synthesis and antimalarial properties of ten novel phenylalanine derived sulfonamides conjugates. The design of this work was based on the reported antimalarial activity of benzenesulphonamides containing dipeptide of alg-gly [17], valine-dipeptide [21] and val-val dipeptides sulphonamide conjugates [18] and the need to develop newer chemotherapeutic agents that will overcome the reported emerging resistance against artemisinin based therapy.

2.0. Material and methods

All chemicals reagents and solvents used were obtained from Aldrich, Merch, Fluka, Avra, SD Fine and Alfa Aesar and used without purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Jeol 400MHz spectrometers in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. FT-IR Spectroscopy of the compounds were run in PerkinElmer Spectrum version 10.03.06 and the bands presented in wavenumber. The mass spectroscopy was carried out using micro TOF electrospray time of flight mass spectrometer (Aerodyn Reseach Inc USA). Melting points were determined in open capillary tubes, using a Rolex melting-point apparatus and are uncorrected. All experiments were carried out at Prof Sandeep Verma Laboratory, Department of Chemistry, Indian Institute of Technology, Kanpur, India. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); sports were visualized under UV light

2.1. Synthesis of substituted benzenesulphonamoyl alkanamides (3) [22]

The modified methods of Ugwu et al., 2017 was used for the synthesis of the substituted benzenesulphonamoyl alkanamides. The details is found in the supporting document.

2.2. General procedure of preparing compound (6a-j) [23]

The methods of Sharm and Soman 2016, Ugwuja et al., 2019 and Ezugwu et al., 2020 were adopted for this synthesis with little modifications. See supporting document for details.

2.3. General procedure of preparing (7a-j)

A mixture of alkanamide (**3**, 1.84 mmol), 1-ethyl-3-(3-dimethylamino carbodiimide hydrochloride EDC.HCl (0.53 g, 2.76 mmol), 1-hydroxybenzotriazole HOBt (0.248 g, 1.84 mmol), triethylamine, compounds (**6a-j**), 1.53 mmol) in dichloromethane (50 mL) was stirred at room temperature for 16 h and monitored using TLC. Upon completion of the reaction, the mixture was washed with water (2 × 20 mL), brine (1 × 10 mL), dried over sodium sulphate. The crude product was obtained after evaporating the solvent under reduced pressure and then purified by column chromatography using silica gel (3% MeOH/DCM).

*2-(4-methylphenylsulfonamido)-*N*-(2-oxo-2-(phenylamino)ethyl)-3-phenylpropanamide (7a)*

Yield 66.5%, Mp=175–176^{O}C. FTIR (KBr, cm^{-1}): 3350 (N–H), 3062 (C-H aromatic), 2925 (C–H aliphatic), 1664, 1600 (2C=O), 1537, 1498, 1445 (C=C), 1321, 1158 (2S=O), 1091, 1030 (C–N), 1224 (SO_2N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H, NH), 7.65 (d, J = 7.3 Hz, 2H, ArH), 7.41 (d, J = 7.9 Hz, 3H, ArH), 7.30 (t, J = 7.9 Hz, 2H, ArH), 7.09–7.21 (m, 6H, ArH), 6.86 (d, J = 7.3 Hz, 2H), 5.17 (s, 1H, NH), 4.39 (q, J = 8.1 Hz, 1H, CH), 3.82 (dt, J = 17.1, 4.9 Hz, 2H, CH_2), 3.16 (dd, J = 14.3, 4.6 Hz, 1H, CHa of CH_2), 2.71 (dd, J = 14.3, 10.1 Hz, 1H, CHb of CH_2), 2.40 (d, J = 12.8 Hz, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.58, 167.45 (C=O), 144.28, 137.78, 135.02, 134.28, 129.91, 129.00, 128.98, 128.88, 127.35, 127.25, 124.56, 120.29 (twelve aromatic carbons), 58.68, 44.07, 38.11, 21.66 (four aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{24}H_{25}N_3NaO_4S$ $[M+Na]^+$ 474.15; found 474.15.

N-*(2-(4-fluorophenylamino)-2-oxoethyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (7b)*

Yield 67.4%, Mp=76–77 °C. FTIR (KBr cm^{-1}), 3351 (N–H), 3065 (C-H aromatic), 2926 (C-H aliphatic), 1662, (C=O), 1535, 1509, 1455, 1410 (C=C), 1324, 1158 (2S=O), 1030, 1019 (C–N), 1215 (SO_2N), 1092 (C-F). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H, NH), 7.59-7.63 (m, 2H, ArH), 7.54-7.58 (m, 1H, NH), 7.38 (d, J = 8.5 Hz, 2H, ArH), 7.07-7.19 (m, 5H, ArH), 6.97 (t, J = 8.9 Hz, 2H, ArH), 6.86 (d, J = 7.3 Hz, 2H, ArH), 5.30–5.42 (m, 1H, NH), 4.41 (q, J = 8.3 Hz, 1H, CH), 3.78-3.86 (m, 2H. CH_2), 3.16 (dd, J = 14.0, 4.3 Hz, 1H, CHa of CH_2), 2.56–2.72 (m, 1H, CHb of CH_2), 2.36-2.41 (m, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.51, 167.36 (C=O), 160.72, 144.44, 134.88, 133.97, 133.84, 129.96, 129.81, 129.08, 128.80, 127.44, 127.24, 121.99, 121.91, 115.66, 115.44 (aromatic carbons), 58.72, 43.91, 38.03, 21.67(four aliphatic carbons). HRMS-ESI found value is (m/z): calcd. for $C_{24}H_{25}FN_3O_4S$ [M+H] 470.15; found 470.16.

N-*(2-(4-chlorophenylamino)-2-oxoethyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (7c)*

Yield 72.5%, Mp=92–93 °C. FTIR (KBr cm^{-1}), 3344 (N–H), 3063 (C–H aromatic), 2925 (C–H aliphatic), 1664, 1597 (2C=O), 1539, 1493, 1400 (C=C), 1305, 1158 (2S=O), 1091, 1030 (C–N), 1244 (SO_2N), 722 (C–Cl). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H, NH), 7.61 (d, J = 9.2 Hz, 2H, ArH), 7.51–7.54 (m, 1H, NH), 7.38 (d, J = 8.5 Hz, 2H, ArH), 7.23 (t, J = 9.8 Hz, 2H, ArH), 7.08-7.18 (m, 5H, ArH), 6.85 (d, J = 7.3 Hz, 2H, ArH), 5.30-5.33 (m, 1H, NH), 4.42 (q, J = 8.3 Hz, 1H, CH), 3.73-3.85 (m, 2H, CH_2), 3.17 (dd, J = 14.3, 4.6 Hz, 1H, CHa of CH_2), 2.68 (dd, J = 14.0, 10.4 Hz, 1H, CHb of CH_2), 2.37-2.42 (m, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.38, 167.34 (C=O), 144.40, 136.32, 134.70, 133.76, 129.87, 129.37, 129.00, 128.84, 128.67, 127.37, 127.15, 121.35 (twelve aromatic carbons), 58.60, 43.88, 37.90, 21.58 (four aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{24}H_{23}ClN_3O_4S$ $[M-H]^-$ 484.11; found 484.11.

N-(2-(4-bromophenylamino)-2-oxoethyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (7d)

Yield 66.7%, Mp=94–95 °C. FTIR (KBr cm^{-1}), 3347 (N–H), 3063 (C–H aromatic), 2925 (C–H aliphatic), 1662, 1597 (2C=O), 1532 1489, 1455, 1396 (C=C), 1305, 1158 (2S=O), 1091, 1073 (C–N), 1244 (SO_2N), 551 (C–Br). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H, NH), 7.55 (d, J = 8.5 Hz, 2H, ArH), 7.48 (d, J = 7.3 Hz, 1H, NH), 7.37 (q, J = 4.3 Hz, 4H, ArH), 7.07–7.20 (m, 5H, ArH), 6.84 (d, J = 7.3 Hz, 2H, ArH), 5.25 (d, J = 4.3 Hz, 1H, NH), 4.41 (q, J = 8.3 Hz, 1H, CH), 3.76–3.83 (m, 2H, CH_2), 3.16 (dd, J = 14.0, 4.3 Hz, 1H, CHa of CH_2), 2.55-2.70 (m, 1H, CHb of CH_2), 2.34-2.41 (m, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.43, 167.42 (C=O), 144.55, 136.94, 134.75, 133.76, 131.90, 130.00, 129.15, 128.76, 127.52, 127.27, 121.79, 117.15 (twelve aromatic carbons),

58.67, 43.98, 37.99, 21.70 (four aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{24}H_{25}BrN_3O_4S$ $[M+H]^+$ 530.07; found 530.08.

2-(4-methylphenylsulfonamido)-N-(2-oxo-2-(p-tolylamino) ethyl)-3-phenylpropanamide (7e)

Yield 68.9%, Mp=76-77 °C. FTIR (KBr cm^{-1}), 3346 (N–H), 3063, 3030 (C–H aromatic), 2924 (C–H aliphatic), 1660, 1601 (2C=O), 1515, 1498, 1454,1407 (C=C), 1319, 1159 (2S=O),1092, 1031 (C–N), 1248 (SO_2N). ^{1}H-NMR (396 MHz, $CDCl_3$) δ 8.52 (s, 1H, NH), 7.38-7.51 (m, 5H, ArH), 7.07–7.19 (m, 7H, ArH), 6.85 (d, J = 7.9 Hz, 2H, ArH), 5.25-5.29 (m, 1H, NH), 4.34 (q, J = 8.3 Hz, 1H, CH), 3.77-3.83 (m, 2H, CH_2), 3.13 (dd, J = 14.2, 4.5 Hz, 1H, CHa of CH_2), 2.70 (dd, J = 13.9, 10.3 Hz, 1H, CHb of CH_2), 2.28–2.40 (m, 6H, $2CH_3$). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 171.53, 167.26 (C=O), 144.22, 135.19, 135.07, 134.34, 134.14, 129.89, 129.46, 128.99, 128.90, 127.33, 127.25, 120.33 (twelve aromatic carbons), 58.66, 44.01, 38.13, 21.65, 20.98 (five aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{25}H_{28}N_3O_4S$ $[M+H]^+$ value is 466.18; found 466.18.

2-(4-methylphenylsulfonamido)-N-(2-morpholino-2-oxoethyl)-3-phenylpropanamide (7f)

Yield 70.6%, Mp=142–143 °C. FTIR (KBr cm^{-1}), 3293 (N–H), 2921, 2857 (C–H aliphatic), 1638, (C=O), 1530, 1498, 1437 (C=C), 1333, 1161 (2S=O), 1093, 1034 (C–N), 1245 (SO_2N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 7.9 Hz, 2H, ArH), 7.15-7.18 (m, 6H, ArH), 6.96 (dd, J = 7.6, 1.5 Hz, 2H, ArH), 5.31 (d, J = 7.9 Hz, 1H, NH), 3.85-4.02 (m, 3H, CH&CH_2), 3.62-3.68 (m, 6H,3CH_2), 3.37 (t, J = 4.9 Hz, 2H, CH_2), 2.88–3.03 (m, 2H, CH_2), 2.40 (s, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 170.63, 166.22(C=O), 143.52, 136.27, 135.57, 129.72, 129.26, 128.79, 127.23, 127.16(eight aromatic carbons), 66.74, 66.39, 58.02, 44.89, 42.40, 41.35, 38.87, 21.64 (eight aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{22}H_{27}N_3NaO_5S$ $[M+Na]^+$ 468.16; found 468.16.

2-(4-methylphenylsulfonamido)-N-(2-(naphthalen-1-ylamino)-2-oxoethyl)-3-phenylpropanamide ***(7g)***

Yield 75.2%, Mp=85–86 °C. FTIR (KBr cm^{-1}), 3345 (N–H), 3060 (C–H aromatic), 2927 (C–H aliphatic), 1662, 1599 (2C=O), 1531, 1499, 1445 (C=C), 1328, 1159 (2S=O), 1091, (C–N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H, NH), 7.97 (d, J = 8.5 Hz, 1H, NH), 7.81 (dd, J = 20.4, 7.6 Hz, 2H, ArH), 7.70 (d, J = 7.9 Hz, 1H, ArH), 7.37-7.56 (m, 7H, ArH), 7.04-7.15 (m, 4H, ArH), 6.87 (d, J = 7.3 Hz, 2H, ArH), 5.30-5.35 (m, 1H, NH), 4.34 (q, J = 7.9 Hz, 1H, CH), 3.90–4.08 (m, 2H, CH_2), 3.11 (dd, J = 14.0, 4.9 Hz, 1H, CHa of CH_2), 2.79 (dd, J = 14.3, 9.5 Hz, 1H, CHb of CH_2), 2.36 (d, J = 9.8 Hz, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 172.16, 168.33 (C=O), 144.03, 135.08, 134.83, 134.18, 132.10, 129.81, 128.99, 128.91, 128.48, 127.79, 127.25, 127.14, 126.41, 126.14, 125.57, 121.90, 121.78 (seventeen aromatic carbons), 58.54, 44.54, 38.32, 21.58 (four aliphatic carbons). HRMS-ESI (m/z): calcd. for $C_{28}H_{28}N_3O_4S$ $[M+H]^+$ 502.18; found 502.8.

2-(4-methylphenylsulfonamido)-N-(2-oxo-2-(m-tolylamino) ethyl)-3-phenylpropanamide (7h)

Yield 89.9%, Mp=175–176 °C. FTIR (KBr cm^{-1}), 3347 (N–H), 3060, 3030 (C–H aromatic), 2924, 2860 (C–H aliphatic), 1662, 1615 (2C=O), 1596, 1547, 1492, 1454, 1434 (C=C), 1322, 1158 (2S=O), 1091, 1031 (C–N), 1259 (SO_2N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H, NH), 7.53-7.56 (m, 1H), 7.50 (s, 1H, NH), 7.30–7.42 (m, 3H, ArH), 7.07–7.20 (m, 6H, ArH), 6.89 (q, J = 7.1 Hz, 3H, ArH), 5.48 (d, J = 4.9 Hz, 1H, NH), 4.31 (q, J = 7.9 Hz, 1H, CH), 3.81-3.86 (m, 2H, CH_2), 3.13 (dd, J = 14.0, 4.9 Hz, 1H, CHa of CH2), 2.72 (dd, J = 14.3, 10.1 Hz, 1H, CH_b of CH_2), 2.42 (d, J = 23.2 Hz, 3H, CH_3), 2.30 (s, 3H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.55, 167.30 (C=O), 144.22, 138.82, 137.65, 135.05, 134.46, 129.90, 129.00, 128.91, 128.78, 127.34, 127.24, 125.39, 120.94, 117.40 (fourteen aromatic carbons), 58.63, 44.14, 38.17, 21.64 (four aliphatic carbons). HRMS-ESI (m/z): calculated for $C_{25}H_{28}N_3O_4S$ $[M+H]^+$ 466.18; found 466.18.

N-(2-(2,6-dimethylphenylamino)-2-oxoethyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (7i)

Yield 92.7%, Mp=173-174 °C. FTIR (KBrcm^{-1}), 3352 (N–H), 3030 (C–H aromatic), 2927 (C–H aliphatic), 1662, 1599 (2C=O), 1515, 1455, (C=C), 1329, 1159 (2S=O), 1092, 1032 (C–N), 1237 ($S0_2N$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, NH), 7.57 (d, J = 5.5 Hz, 1H, NH), 7.35 (d, J = 8.5 Hz, 2H, ArH), 7.04–7.17 (m, 8H, ArH), 6.88 (d, J = 6.7 Hz, 2H, ArH), 5.39 (d, J = 5.5 Hz, 1H, NH), 4.36 (dd, J = 17.1, 7.3 Hz, 1H, CH), 3.82–3.97 (m, 2H, CH_2), 3.14 (dd, J = 14.0, 4.3 Hz, 1H, CHa of CH_2), 2.73 (dd, J = 14.0, 9.8 Hz, 1H, CHb of CH_2), 2.40 (d, J = 10.4 Hz, 3H, CH_3), 2.22 (d, J = 7.9 Hz, 6H, $2CH_3$). ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.57, 167.69 (C=O), 144.20, 137.60, 135.64, 135.06, 133.45, 129.86, 129.02, 128.89, 128.20, 127.49, 127.37, 127.17(twelve aromatic carbons), 58.68, 43.59, 38.18, 21.65, 18.45 (five aliphatic carbons). HRMS-ESI (m/z): calcd. For $C_{26}H_{30}N_3O_4S$ $[M+H]^+$ 480.20; found 420.20.

N-(2-(4-methoxyphenylamino)-2-oxoethyl)-2-(4-methylphenylsulfonamido)-3-phenylpropanamide (7j)

Yield 70.2%, Mp=77–78 °C. FTIR (KBr cm^{-1}), 3352 (N-H), 3064 (C–H aromatic), 2928 (C–H aliphatic), 1660, 1601 (2C=O), 1512, 1455, 1442, 1414 (C=C), 1325, 1159 (2S=O), 1032, (C–N), 1246 (SO_2N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H, NH), 7.53–7.63 (m, 3H, ArH), 7.40 (d, J = 8.5 Hz, 2H, ArH), 7.07–7.21 (m, 5H, ArH), 6.84 (dd, J = 18.6, 8.2 Hz, 4H, ArH), 5.45 (d, J = 4.9 Hz, 1H, NH), 4.35 (q, J = 8.1 Hz, 1H, CH), 3.80–3.85 (m, 2H, CH_2), 3.74 (d, J = 11.0 Hz, 3H, OCH_3), 3.14 (dd, J = 14.0, 4.3 Hz, 1H, CHa of CH_2), 2.71 (dd, J = 14.0, 10.4 Hz, 1H, CHb of CH_2), 2.32–2.45 (m, 3H, CH_3) ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.50, 167.15(C=O), 156.53, 144.27, 135.02, 134.26, 130.91, 129.90, 129.02, 128.88, 127.35, 127.26, 121.95, 114.09 (twelve aromatic carbons), 58.67, 55.50, 43.90, 38.10, 21.65(five aliphatic carbons). HRMS-ESI (m/z): calcd. For $C_{26}H_{30}N_3O_4S$ $[M+H]^+$ 482.**17;** found 482.18.

2.4. In vivo antimalarial test

The method of Peter et al. [24] and Kalra et al. [25] were adopted for antiplasmodial activity with some modification. Forty-eight infected mice were randomly divided into twelve groups (four mice per group). Group 1 to 10 composed the treatment group and was given 50 mg/kg body weight. While group 11 and 12 served as the positive control (received commercial samples of Artemether/ Lumefantrin combination) and the negative control respectively. The inoculum was prepared from a donor mouse with a minimum of peripheral parasiemia 20% by cardiac puncture in EDTA-coated tube. Five days after the inoculation of the mice with parasite, percentage parasitaemia was determined and treatment with the synthesized compound (**7a-j**) was started and this was done for three days. All the compounds and the standard drug (Artemether/ Lumefantrin) were administered orally using a standard intragastric tube. For all the parasitaemia determination, blood samples were collected from the mice through the retrobulbar plexus of the median canthus of the mice eye.

2.5. Haematological analysis

Whole blood used for the tests was collected from the mice through the retrobulbar plexus of the median canthus of the mice eye. Packed Cell Volume (PCV), Haemoglobin (Hb) levels and Red blood cell (RBC) count were determined before and after the treatment to determine the effect of the various treatments on the parameters.

2.6. Liver function tests (LFTs)

The methods of Reitman et al. [26] for the determination of aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphate (ALP) were used.

2.7. Kidney function test

Kidney Function tests carried out on the blood of the mice that were fed with the sulphonamide derivative were creatinine and albumin adopting the modified method of Kaplan et al. [27].

2.8. Molecular docking method

Molecular docking was carried out to determine the binding potential of ten synthesized compounds against plasmepsin II. 3D crystal structure PDB access code: 4Z22[28] was retrieved from protein data bank with resolution of 2.65 Å. This PDB code was selected based on the resolution of the crystal structure and Plasmepsin II is an aspartic protease encoded by P. falciparum (1 L.A. Gil, P. A.Valiente,P.G.Pascutti andT.Pons,Computational perspectives into plasmepsins structure-function relationship: implications to inhibitors design, J. Trop. Med., 2011, 657483.)

AutoDock tools 1.5.4 was used to determine the grid box size for the potential binding site [29]. The structure of the compounds were optimized with Gaussian 09 [30]. The determined dimension was X= 24 Y= 24 Z= 24 with 1.00 Å as the grid spacing. Lamarckian genetic algorithm method was applied to obtain optimum binding site for the ligand [31]. Gasteiger charges were computed using the AutoDock Tools graphical user interface supplied by MGL Tools [32]. The co-crystal ligand was also docked into the active site of the plasmepsin to validate the docking.

3.0. Results and discussion

The first step in synthesis of the compounds involved the reaction of substituted benzenesulphonyl chloride with L-phenylalanine in an alkaline medium to give substituted benzenesulphonamide [3]. The reaction of Boc-glycine, and amines in the presence of peptides coupling regents, 1-ethyl-3-(3-dimethyl amino propyl carboxiimide hydrochloride, 1-hydroxybenzotriazole and Triethylamine in dichloromethane provided the carbmate derivatives of glycine which on further reaction with 30% trifluoroacetic acid (TFA) gave the TFA salt of unprotected amides (**6a-j**).The coupling reaction between the solid TFA salt of unprotected amides with the (*N*-phenylsulfonyl) phenylalanine) afforded the desired products (7a-j). The structure of the compounds (7a-j) was confirmed by characterization using FTIR, ^{1}HNMR, ^{13}CNMR and High resolution mass spectroscopy. The presence of sharp bands between 3344 and 3352 cm^{-1}, 1597 and 1664 cm^{-1} are assigned to NH and C=O respectively. In the ^{1}H NMR of **7a**, the prominent NH resonance of the sulfonamide part of the dipeptide hydride was observed at δ5.17 ppm. Other amide NH resonances of the compounds appeared at δ7.09–7.21 ppm with aromatic protons and at δ8.60 ppm region as multiplet and singlet peaks, respectively. In the ^{13}CNMR spectrum, two peaks at 167.45 and 171.58 ppm for the carbonyl carbons of the amide groups, twelve peaks ranging from 120.29 to 144.28 ppm for aromatic carbons and four peaks ranging from 21.66 to 58.68 ppm for aliphatic carbons confirmed the formation of **7a**, which was supported by High resolution mass spectrometer spectrum with a peak at m/z 474.15 for [M+Na]$^{+}$. All other compounds were in agreement with their structures. The spectra and other important data are found in the supplementary information.

3.1. In vivo *antimalarial*

To assess the *in vivo* antimalarial activity, the synthesized compounds were tested against *P. berghei* (NK-65) which was obtained from National Institute of Medical Research, Yaba Lagos, Nigeria. The university of Nigeria ethical committee for the use of animal gave approval for this project. Artemether/ Lumefantrine was used as the standard drug for the antimalarial activity. The percentage (%) inhibition of the parasite multiplication was calculated by comparing the treated group with untreated group using the following formula [17, 18, 21].

% Inhibition = Mean % parasiteamia of before-treatment - Mean % parasiteamia of after-treatment x 100

Mean % parasiteamia of before-treatment

Compounds that reduced parasitaemia by at least 40% were considered active, whereas those that reduce parasitaemia by 30-40% were considered partially active while less than 30% were in active [17, 18, 21]. From data in Table 1, some of the synthesized compounds were active at the 50 mg/kg dose when compared with the standard drug. Specifically, compound **7j** had percentage inhibition of 60.2 against *P. berghei* which was close to 68.8% obtained from the standard drug. Compounds **7h** and **7i** were partially active while the rest were inactive. So these three compounds could be considered for further studies. Structure activity relationship study reveals the most potent compound 7j had a Methoxy-group at the 4-position. The 3-methyl derivative was at the distance second position. The order of activity is 4-methoxy> 3-methy>2,6-dimethyl>4-Chloro>4-methyl>Naphthalyl>benzene>4-Bromo. The least active was 4-Fluoro derivative (7b). The trend of activity suggests that highly electron donating group at position 4 will positively influence the actimalarial properties of the derivatives. We also observed that aromaticity influenced the activity positively as expressed in the compounds 7a, 7f, and further increase of aromatic ring increased activity as in compound 7 g.

3.2. Liver function analysis

Liver function tests are a group of blood tests that are used to determine inflammation and damage to the liver [17]. The liver function parameters evaluated in this research are AST, ALT and ALP. The results of LFT (Table 2) showed that the administration of 50 mg/kg of the tested compounds did not lead to substantial increase or decrease in the levels of liver parameters. The result showed that the compounds were not toxic to the animals.

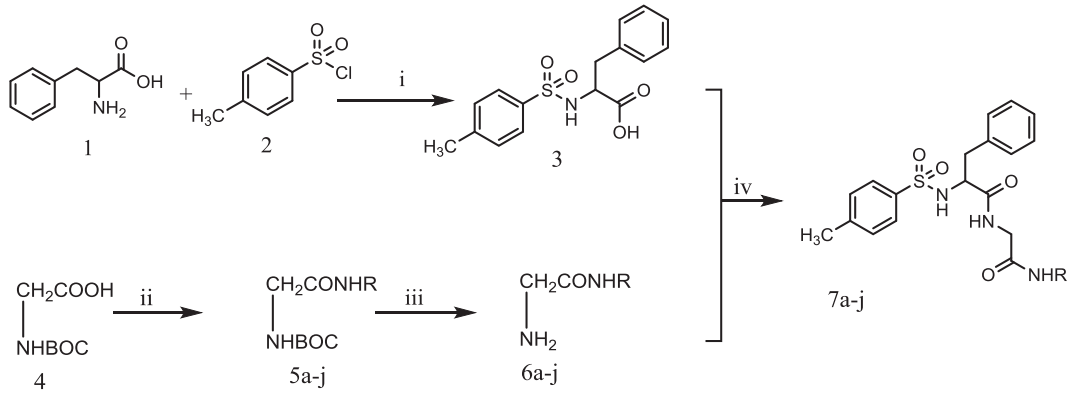
3.3. Kidney function analysis

This is used to determine how well the kidneys are performing. The data in **Table 3** revealed that there is no significant change in the serum level of urea and creatinine of mice fed with 50 mg/kg of the reported derivatives when compared with the reference drug (Artemether/ Lumefantrin). The result showed that the compounds were not toxic to the animals.

Table 4 presents the results of the heamatological parameters. Though there is a reduction in the value of RBC, PCV and HB, it was observed that they were not substantial when compared with the standard (Artemether/ Lumefantrin). The result showed that the compounds were not toxic to the animals.

3.4. Molecular studies

The results obtained from the molecular docking of compounds 7a-7j with *Plasmodium falciparum* plasmepsin II are presented in Table 5. The results obtained showed that 7j had the



7a = C_6H_5

7b = 4-FC_6H_5

7c = 4-ClC_6H_5

7d = 4-BrC_6H_5

7e = 4-$CH_3C_6H_5$

7f = C_4H_8

7g = $C_{10}H_7$

7h = 3-$CH_3C_6H_5$

7i = 2,6-$(CH_3)_2C_6H_5$

7j = C_7H_7O

Scheme 1. The synthesis of dipeptides bearing sulphonamide. (i) Na_2CO_3, DCM/H_2O, HCl, 0°°C, r.t, pH 2, 4°h (ii) EDC. HCl, HOBt, TEA, DCM, amines, 16 h (iii) 10% TFA in DCM (iv) EDC.HCl, HOBt, TEA, 16 h.

Table 1
Percentage inhibition of parasite in mice.

Compounds No.	% parasitaemia Before treatment	% Parasitaemia After treatment	% inhibition
7a	60.5	49.8	17.7
7b	61.3	57.0	7.0
7c	53.8	39.7	26.2
7d	60.3	49.0	18.7
7e	51.5	39.3	23.7
7f	55.0	49.0	10.9
7g	55.3	68.0	−23.0
7h	62.5	38.3	38.7
7i	58.0	38.0	34.5
7j	61.0	24.3	60.2
Artemether/ Lumefantrin	56.0	17.5	68.8

Table 2
Liver function test.

Group	IU/L ALP	IU/L ALT	IU/L AST	μmol/l Total Bilirubin	μmol/l Direct Bilirubin
7h	46.3	30.5	8.5	22.4	7.0
7i	45.7	33.5	9.0	18.3	5.3
7j	48.3	22.5	8.0	24.3	10.0
Artemether/ Lumefantrin	20.6	32.5	20.0	23.6	8.9

highest binding energy of (−6.87) kcal mol^{-1}) compared to the co-crystalized ligand and standard drug even though all compounds had relatively low binding energy. This observation agrees with the *In vivo* antimalarial studies. Hydrogen bonding is a crucial marker indicating functional stability and binding of ligands to crucial amino acids. Fig. 1 revealed that SER118 an amino acid around the active site formed hydrogen bonding with the atoms of 7j. Fig. 1 also revealed Pi-sigma, Van der Waals interactions and carbon hydrogen bond interactions. These interactions could proffer better insight in proposing the mechanism of inhibition. The docking simulations was authenticated by docking the co-crystallized ligand against the protein under study. The

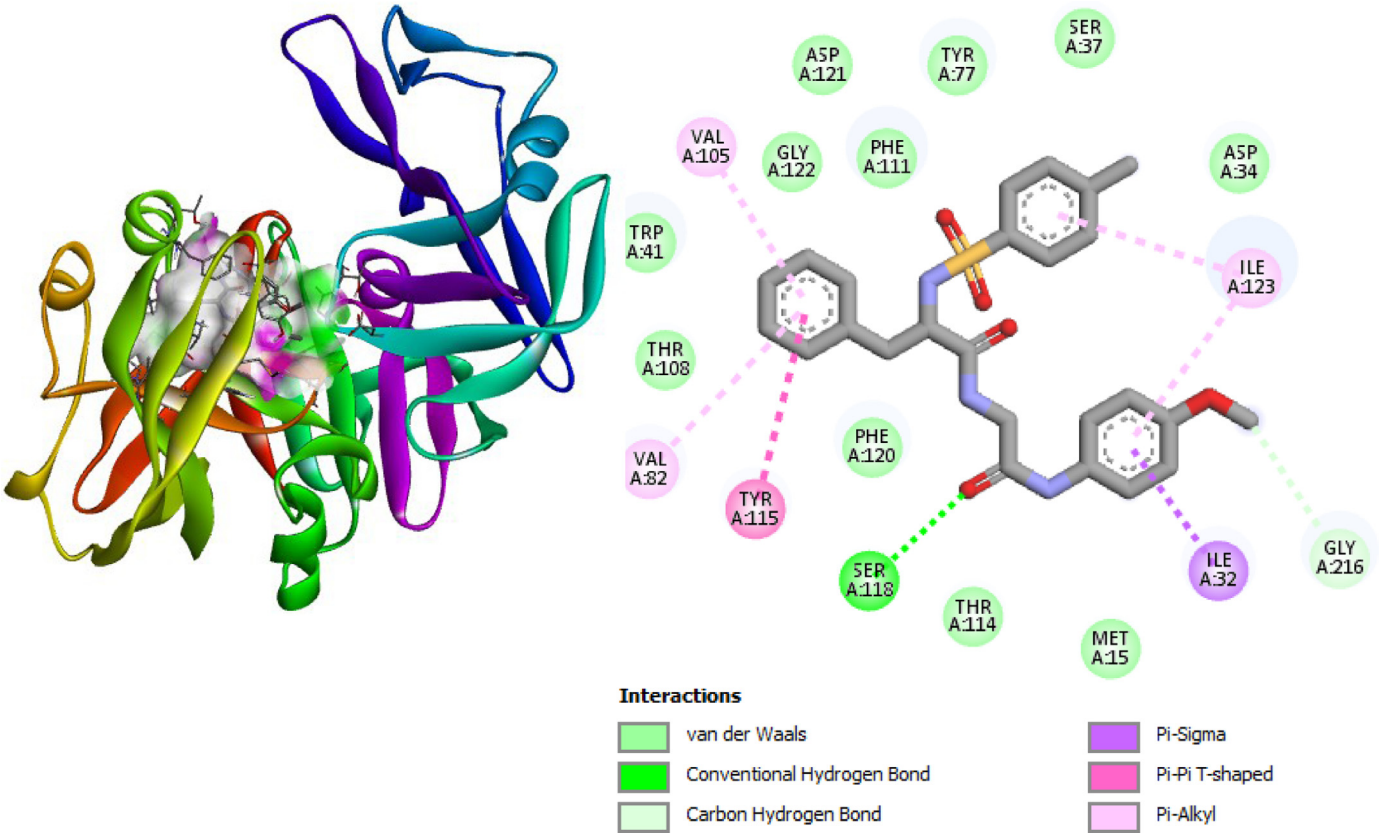


Fig. 1. 3D structure of (A) compound 7j in complex Plasmepsin II (highest binding affinity). (B) Descriptions showing the hydrogen bonding interactions with amino acid residues around the binding pocket (ASP 214).

Table 3
Kidney function test.

Group	Mmol/l Urea	μmol/l Creatinine
7h	6.2	27.5
7i	5.5	23.6
7j	7.0	60.4
Artemether/ Lumefantrin	6.8	59.5

obtained RMSD value of 0.2 Å revealed a structural and functional stability of ligand-protein complex. The docking result obtained in this work agrees with that obtained in the literature (D. I. Ugwu, U. C. Okoro, P. O. Ukoha, et al., Synthesis, characterization molecular docking and in vitro antimalarial properties of new carboxamides bearing sulphonamide. Eur J Med Chem. 135 (2017)349–69.)

Table 5
Binding energies of ligands in complex with antimalaria enzymes (plasmepsin II: 4Z22) obtained from molecular docking, using AutoDock 4.2.

Complexes	Binding energies (kcal mol^{-1})
7a	−4.17
7b	−3.29
7c	−4.11
7d	−4.06
7e	−5.22
7f	−4.02
7g	−4.22
7h	−5.10
7i	−5.22
7j	−6.87
Co-crystalized ligand	−6.13
Standard drug	−6.69

4. Conclusion

In conclusion, ten new L-phenylalanine derived dipeptide bearing *p*-toluenesulphonamides were successfully synthesized and

Table 4
Heamotological test before and after treatment.

Comp	RBC (mm^3)		PCV (%)		HB (g/dl)	
	Before	After	Before	After	Before	After
7h	7.8×10^6	8.8×10^6	43	53	10.8	13.8
7i	6.7×10^6	9.6×10^6	43	53	12.8	17.8
7j	7.6×10^6	9.2×10^6	41	52	13.0	17.3
Artemether/ Lumefantrin)	7.1×10^6	9.8×10^6	39	53	11.2	17.4

characterized using spectroscopic techniques. The molecular docking results showed that the compounds interacted with the active site of the protein target with good binding affinity using conventional hydrogen bonding. In the antimalarial activity study, compound **7j** showed the most antimalarial activity with percentage inhibition of parasite growth of 60.2% comparable with artemether/lumenfantrine (68.8%). The results of the haematological analysis, liver and kidney function tests showed that there were no significant changes in the parameters tested when compared with the control. The physicochemical parameter predictions indicate that the compounds would not pose oral bioavailability, transport and permeability problems if developed further to drug molecules The molecular docking studies showed good interaction between the synthesized compounds and the protein targets for antimalarial activities.

Declaration of Competing Interest

The authors declared no Conflict of Interest.

Acknowledgements

Samuel. T. Aromino gratefully acknowledge the assistance of Prof Sandeep Verma, Department of Chemistry, Indian Institute of Technology, Kanpur, India in providing the facilities for synthesis and spectral characterization.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131201.

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