African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 18 No.1; pp. 497-505 (2026)

Original Research Article

NITRO-SUBSTITUTED ARYLSULPHONAMIDE HIS–GLY DIPEPTIDE CARBOXAMIDES AS POTENTIAL ANTIMALARIAL AND ANTITRYPANOSOMAL AGENTS.

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ABSTRACT

The need for new antimalarial and antitrypanosomal drugs has increased due to the toxicity of current chemotherapeutics and the development of drug-resistant protozoan parasites. This study determined the synthesis and potential of nitro-substituted arylsulphonamide–his–gly dipeptide carboxamides as antimalarial and antitrypanosomal agents. Several nitro-substituted arylsulphonamide–his–gly dipeptide carboxamide compounds were analysed using in silico, in vitro and in vivo study. The synthetic route involved the preparation of substituted benzenesulphonamide intermediates, followed by coupling with various amino acid-derived carboxamides using EDC/HOBt-mediated peptide bond formation. Final compounds were obtained in good to excellent yields and fully characterized by FTIR, ^{1}H and ^{13}C NMR spectroscopy, HRMS, and melting point analysis, confirming their structural integrity. The in silico, in vitro and in vivo study against Plasmodium and Trypanosoma species shows that some of the compounds exhibited stronger binding affinities, strong antitrypanosomal and antimalarial activity. This study aimed to synthesize arylsulphonamoyl histidine-glycine dipeptide amide derivatives and to investigate their antitrypanosomal and antimalarial properties. We conclude that nitro-substituted arylsulphonamide–his–gly dipeptide carboxamides have high potency for the treatment of malaria and trypanosomiasis even better than some available drugs.

ARTICLE INFO

Received 15 January, 2026
Accepted 27 April, 2026
Published 30 April, 2026

KEYWORDS

sulphonamides, dipeptides, carboxamides, antimalarial, glycine

INTRODUCTION

There is a massive challenge in infectious disease treatment such as malaria and trypanosomiasis whereby existing antimalarial and antitrypanosomal drugs are increasingly ineffective due to the emergence of widespread drug-resistant protozoan parasites [1]. Malaria is caused by *Plasmodium falciparum* and other species transmitted by infected *Anopheles* mosquitoes [2].Human African trypanosomiasis is caused by *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* [3].*Plasmodium* species are developing resistance

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https://doi.org/10.59493/ajopred/2026.1.10 ISSN: 0794-800X (print); 1596-2431 (online)

even to front-line artemisinin-based therapies [4], while current treatments for trypanosomiasis suffer from toxicity and limited accessibility in endemic regions [5]. The widespread drug resistance and toxicity associatedexisting therapies have prompted the need to design and develop novel inhibitors with new modes of action targeting crucial enzymes that play vital roles in the parasitic life cycle. Recent synthetic studies have revealed that dipeptide-linked sulphonamide derivatives exhibit significant antimalarial and antitrypanosomal activities, particularly those containing nitro-substituted aryl moieties. In vivo evaluation of nitro-benzenesulphonamide dipeptide derivatives incorporating glycine demonstrated inhibition of *Plasmodium berghei* parasitemia at levels comparable to standard combinations such as artemether–lumefantrine, while select analogues showed efficacy in clearing *Trypanosoma brucei* infections in murine models without apparent organ toxicity [1]. Carboxamides and their derivatives also show antiplasmodial activity against *Plasmodium falciparum*, interfering with essential parasitic biochemical pathways and highlighting the carboxamide scaffold as a promising structural framework for novel antimalarial development [6]. His–Gly is a dipeptide composed of L-histidine and glycine joined by a peptide linkage, and incorporation of this motif enhances molecular recognition by biological targets and improves solubility and pharmacokinetic properties relative to simple small molecules, thereby making His–Gly peptide–drug conjugates a promising strategy in modern therapeutics [7]. Nitro-substituted arylsulphonamide–His–Gly dipeptide carboxamides combine the biological advantages of each pharmacophore, including the robust antiparasitic activity associated with nitroaromatic systems and the receptor-recognition potential and improved solubility conferred by short peptide scaffolds. Peptide-based approaches and hybrid small molecules yielding novel antimalarial candidates have been highlighted in recent literature through molecular docking and structure–activity relationship studies [8]. These advances support the investigation of nitro-substituted arylsulphonamide–His–Gly dipeptide carboxamides as a promising new class of dual antimalarial and antitrypanosomal agents capable of addressing persistent challenges in parasitic disease chemotherapy. Therefore, this study aimed to synthesize arylsulphonamoyl histidine–glycine dipeptide amide derivatives and evaluate their antitrypanosomal and antimalarial activities through in silico, in vitro, and in vivo approaches.

MATERIALS AND METHODS

Instrumentation

The Q -TOF LC/MS with dual ESI was used as an ionisation source to record the mass spectra on an Agilent 6520 accurate mass quadrupole mass spectrometer which is manufactured in United States. Using trimethylsilane (TMS) as an internal standard, ^{1}H-NMR and ^{13}C-NMR spectra were acquired in deuterated chloroform ($CDCl_3$) using the NMR Bruker AVANCETM and Jeol 400 MHz spectrometers (Monash University, Malaysia). Chemical shifts were reported in parts per million. Thermo Nicolet Magna-IR 550 and Shimadzu FTIR 8400S spectrometers were used to obtain FT-IR spectra, which were displayed in wavenumber (Indian Institute of Technology, India). The Mettler Toledo pH model S400 was used to measure pH, whereas the MP30 model melting point apparatus was used to determine melting points without correction. All reactions took place in a cold bath and were monitored with TLC. The samples were concentrated using the RE-2000B rotary evaporator. They were dried at low pressure in the DHG-9023A vacuum oven.

Reagents

The analytical grades of all chemicals and reagents were obtained commercially from Aldrich, Merck, and Alfa Aesar and were utilized without further purification. The dipeptides were produced using the amino acids L-glycine, L-histidine and their Boc-N- protected derivatives. The benzene sulphonyl derivatives that were employed were *p*- benzene sulphonyl chloride, *p*-nitrobenzene sulphonyl chloride and *p*-toluene sulphonyl chloride. Sodium carbonate was utilised as a base. To facilitate crystallisation, 20% hydrochloric acid was utilised. The compounds imidazole, piperidine, morpholine, pyrrolidine, diethylamine, indole, and dimethylamine were utilised in conjunction with the amino acids to produce carboxamides.Dimethylformamide (DMF) and triethylamine (TEA) were utilised as solvents, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) were utilised as coupling agents. The reaction was monitored using TLC of silica gel F-254 on aluminium plates in a CH_3OH/CH_2Cl_2 solvent system. For amino acid deprotection, trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2) was employed, while ethyl acetate was used for aqueous work-up. The purification process involved column chromatography with a silica gel of mesh size 40–63 µm.

Procedures for synthesis of his-gly' dipeptide carboxamide bearing sulphonamides

Triethylamine (1.98g, 19.41 mmol), EDC (2.49g, 12.99 mmol), and HOBt (1.77g, 12.99 mmol) was added to a solution of substituted benzenesulphonamides (6.39 mmol) in dimethylformamide (15 mL) at 0 ˚C. The carboxamide derivatives (3.27 mmol) were then added, and the mixture was stirred for 15 min. The solution was brought to room temperature and stirred for 12–24 h while being monitored with TLC. On completion of the reaction, an aqueous workup was carried out to obtain the crude products, which were then purified by column chromatography (hexane/ethyl acetate = 95:5) to afford good yields of the dipeptide carboxamide-bearing sulphonamides in Figure 1 [9] .

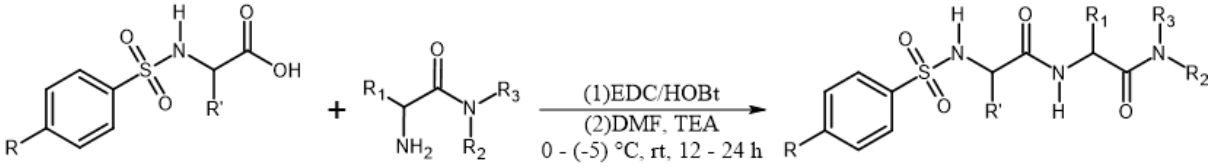

Figure 1: Synthesis of the target dipeptides

3-(1H-imidazol-5-yl)-2-(4-nitrobenzenesulfonamido)-N-[2-(morpholin-4-yl)-2-oxoethyl]propanamide

Yield: 2.08 g, 81.60%; melting point 116. 20 ˚C. FTIR (KBr, cm^{-1}): 3339.1 (NH), 2966.9, 2938.8, 2872.1 (C-H aliphatic), 3109.9 (C-H aromatic), 1172.1, 1247.2 (C-N), 1534.0 (NO_2-nitro group), 1678.0, 1720.9 (C=O amide), 1350.1 (SO_2-NH sulfonamide), 857.7 (S-N), 1456.8 (C=C), 1049.7 (SO_2). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.98(s, 2H, *J* = 6.6 Hz), 3.62-3.95 (10H, 3.63 (d, *J* = 15.2, 3.3, 2.4 Hz), 3.66 (t, *J* = 12.1, 3.2, 2.4 Hz), 3.67 (t, *J* = 15.2, 10.2, 3.2 Hz), 3.68 (t, *J* = 12.1, 10.2, 3.3 Hz), 3.95 (s), 3.95 (s)), 5.57-5.86 (s, 1H, NH,), 7.30 (d, 1H, *J* = 1.7 Hz), 7.69 (d, 1H, J = 1.7 HZ), 7.97 – 8.41(4H, 8.01 (dd, *J* =8.3,2.3,0.5Hz), 8.12 (dd, *J* =8.3,2.4,0.5Hz)). ^{13}C NMR: ($CDCl_3$ 300 MHz) δ: 32.2 (1C), 42.1 (1C), 44.8 (2C), 66.7 (2C), 110.7 (1C), 117.9 (1C), 125.6 (2C), 126.6 (1C), 128.3(2C), 135.8 (1C), 141.6 (1C), 155.9 (1C), 167.2 (2C). HRMS (m/z) for $C_{18}H_{22}N_6O_7S$: 466.856I $(M+H)^+$, calculated 466.8639.

3-(1H-imidazol-5-yl)-2-(4-nitrobenzenesulfonamido)-N-[2-oxo-2-(piperidin-1-yl)ethyl]propanamide

Yield: 2.14 g, 82.80%; melting point 117. 80 ˚C. FTIR (KBr, cm^{-1}): 3420.0 (NH), 1052.8 (SO_2), 1536.4 (NO_2), 2976.6, 2858.1, 2934.3 (C-H aliphatic), 3067.8 (C-H aromatic), 1162.6 (C-N), 1684.9, 1714.4 (C=O amides), 1366.2 (SO_2-NH sulfonamide), 854.2 (S-N), 1474.6 (C=C). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.39-1.73 (6H, 1.47 (m, *J* = 12.2, 6.5, 2.8 Hz), 1.51 (m, *J* = 12.6, 3.3, 2.9, 2.7, 2.3 Hz), 1.65 (m, *J* = 12.6, 10.2, 3.4, 2.6 Hz)), 3.17-3.44 (6H, 3.23 (d, *J* = 6.6 Hz), 3.23 (d, *J* = 6.6 Hz), 3.36 (t, *J* = 14.9, 10.2, 3.3 Hz), 3.35 (t, *J* = 14.9, 3.4, 2.3 Hz)), 3.75 (s, 2H), 5.76 (t, 1H, *J* = 6.6 Hz), 7.31 (d, 1H, *J* = 1.7 Hz), 7.69 (d, 1H, *J* = 1.7 Hz), 8.01(dd, 4H,*J* = 8.3, 2.3, 0.5 Hz), 8.10 (dd, *J* = 8.3, 2.4, 0.5 Hz)). ^{13}C NMR: ($CDCl_3$ 300 MHz) δ: 24.3 (1C), 25.6 (2C), 41.8 (1C), 42.8 (1C), 44.6 (2C), 45.1 (1C), 114.6 (1C) 117.3 (1C), 124.0 (2C), 125.3 (2C), 128.2 (1C), 138.1 (1C) 141.2 (1C), 155.6 (1C), 166.3(2C). HRMS (m/z) for $C_{19}H_{24}N_6O_6S$: 464.5315 $(M+H)^+$, calculated 464.6389.

3-(1H-imidazol-5-yl)-2-(4-nitrobenzenesulfonamido)-N-[2-oxo-2-(pyrrolidin-1-yl)ethyl]propanamide

Yield: 2.94 g, 93.50%; melting point 113. 70 ˚C. FTIR (KBr, cm^{-1}): 3425.2 (NH), 1095.6 (SO_2), 2877.0, 2977.1 (C-H aliphatic), 3098.5(C-H aromatic), 1027.0 (C-N), 1646.7, 1713.4 (C=O amides), 1366.9 (SO_2-NH sulfonamide), 856.9 (S-N), 1533.5 (NO_2-nitro group), 1453.9 (C=C).^{1}H-NMR ($CDCl_3$, 300 MHz): ^{1}H NMR: δ 1.42(ddddd, 4H,*J* = 13.1, 6.3, 5.6, 1.6, 1.4 Hz), 1.98(ddddd, *J* = 13.1, 9.2,8.4, 6.2, 5.6 Hz)), 2.86 (d, 2H, *J* = 6.6 Hz), 2.96 (d, *J* = 6.6 Hz), 3.37 (ddd, 4H, *J* = 14.8, 6.2, 1.6 Hz), 3.48 (ddd, *J* = 14.8, 8.4, 6.3 Hz)), 3.86 (s, 2H), 5.51 (t, 1H, *J* = 6.6 Hz), 5.61 (d, 1H, *J* = 1.7 Hz), 7.31 (d, 1H, *J* = 1.7 Hz), 7.38(d), 7.69 (d, 1H, *J* = 1.7 Hz), 8.01 (s, *J* = 8.3, 2.3, 0.5 Hz) 8.10 (s, NH) . ^{13}C NMR: ($CDCl_3$ 300 MHz) δ: 25.0 (2C), 35.6 (2C), 42.7 (1C), 45.7 (1C), 59.8 (1C), 114.6 (1C), 117.3 (1C), 124.0 (1C), 124.9 (1C),128.5 (2C), 138.6 (1C), 141.5 (1C), 155.7 (1C), 166.9 (2C). HRMS (m/z) for $C_{18}H_{22}N_6O_6S$: 450. 4606 $(M+H)^+$, calculated 450. 6737.

N-1-(1H-imidazol-1-yl)-3-(1H-imidazol-5-yl)-1-oxopropan-2-yl]-2-(4-nitrobenzene-1-sulfonamido) propanamide

Yield: 3.59 g, 94.4%; melting point 122. 90 ˚C. FTIR (KBr, cm^{-1}): 1517.1 (NO_2), 3308.2 (NH), 1025.1, 1047.6

(SO_2), 2881.0, 2928.3, 2964.2 (C-H aliphatic), 3066.5 (C-H aromatic), 1094.8, 1166.1, (C-N), 1632.9, 1709.7 (C=O amides), 1340.1 (SO_2-NH sulfonamide), 880.6, 917.2 (S-N), 1452.8 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.85 (d, 4H, *J* = 6.6 Hz), 2.88 (d, *J* = 6.6 Hz), 2.88 (d, *J* = 6.6 Hz)), 3.60 (t, 2H,*J* = 6.6 Hz), 3.64 (t, *J* = 6.6 Hz)), 7.24 (d, 2H, *J* = 1.7 Hz), 7.35 (d, *J* = 1.7 Hz), 7.51 (dd, 1H, *J* = 4.5, 2.1 Hz), 7.68 (dd, 1H, *J* = 4.5, 2.5 Hz), 7.72 (d, 2H, *J* = 1.7 Hz), 8.01 (ddd, 4H, *J* = 8.3, 2.3, 0.5 Hz), 8.15 (dd, *J* = 2.5, 2.1 Hz)), 8.43 (dd, 1H, *J* = 2.5, 2.1 Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 29.6 (2C), 31.5 (1C), 37.7 (1C), 46.2 (1C), 115.9 (1C), 117.6 (2C), 125.3 (1C), 125.6 (1C), 126.4 (1C), 128.0 (2C), 129.3 (1C), 134.6 (3C), 138.8 (1C), 141.8 (1C), 163.9 (2C). HRMS (m/z) for C_{21} H_{21} N_9 O_6 S: 527.6500 $(M+H)^+$, calculated 527.5125.

N-[3-(1H-imidazol-5-yl)-1-oxo-1-(piperidin-1-yl)propan-2-yl]-2-(4-nitobenzene-1-sulfonamido)acetamide

***In silico* studies**

The structures of the ligand were built using ChemDraw 23.0 and were converted to 3D by using BIOVIA Discovery Studio Visualizer client 21.1.0.20298. Ligand preparation was done by performing geometric and energy optimization using Hahn forcefield algorithm. Optimized ligand structures were used for molecular docking. X-ray crystal structure of protein targets were accessed from RCSBprotein data bank. Molecular docking analysis was carried out to assess the antibacterial, anti-trypanosomal, and antimalarial properties of the dipeptides bearing carboxamide and sulphonamide moieties. Three-dimensional (3D) structures of three drug receptors with PDB ID: 2EWG, 2VF5 and 3QS1 and their co-crystallized ligands were retrieved from the Protein Data Bank [10-12] . The downloaded 3D structures were prepared in Discovery Studio Visualizer 4.1, in which the needed chains were selected and multiple ligands and non-protein parts were deleted. The 2D structures of the ligands were drawn using ACD/Chemsketch 2015 version and Molecular Builder modules in the molecular operating environment (MOE [13-14]. The compounds were docked against three druggable targets using MOE, and their binding affinities to the receptors were obtained and compared to a reference compound [15-16].

Yield: 2.24g, 78.20%; melting point 119. 40 ˚C. FTIR (KBr, cm^{-1}): 3276.7 (NH), 1026.2 (SO_2), 2916.7 (C-H aliphatic), 3046.2, 3098.0 (C-H aromatic), 1108.4, 1159.7 (C-N), 1615.6, 1666.1 (2C=O amides), 1394.3 (SO_2-NH sulfonamide), 1529.8 (NO_2-nitro), 911.5 (S-N), 1450.7 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (dt, 6H, *J* = 12.2, 6.6, 2.7 Hz), 1.37 (m, *J* = 12.6, 6.8, 6.6, 2.8, 2.7 Hz), 1.42 (m, *J* = 12.6, 6.8, 6.6, 2.8, 2.7 Hz), 2.95 (d, 2H, *J* = 6.7 Hz), 3.67 (m, 4H, *J* = 15.5, 6.8 Hz), 3.69 (m, *J* = 15.5, 2.8 Hz), 4.15 (s, 2H),4.16 (s, 2H), 4.39 (t, 1H, *J* = 6.7 Hz), 7.27 (d, 1H, *J* = 1.7 Hz), 7.59 (s, 1H, *J* = 1.7 Hz), 8.02 (dd, 4H, *J* = 8.3, 2.6, 0.5 Hz), 8.11 (dd, *J* = 8.3, 2.4, 0.5 Hz)), 8.36 (s, 1H), 8.43 (s, 3H).^{13}C NMR (400 MHz, $CDCl_3$) δ: 24.4 (1C), 25.8 (1C), 28.1 (1C), 42.5 (1C), 45.3 (2C), 51.6 (1C), 117.4 (1C), 128.5 (2C), 130.7(2C), 139.3 (1C), 145.8 (1C), 149.9 (1C), 169.1 (1C), 174.0 (1C). HRMS (m/z) for C_{19} H_{24} N_6 O_6 S: 464.4541 $(M+H)^+$, calculated 464.5954.

***In vitro* anti-trypanosomal studies**

The antitrypanosomal properties were evaluated using standard procedures [17]. Aqueous suspensions (100 µl) of the compounds (200 mg/ml) in distilled water were pipetted into microtiter wells. Serial dilutions of the drug suspension were prepared in the micro-wells to obtain a final volume of 50 µl in each well. Forty-five (45) µl of trypanosome infected blood containing about 225,000 trypanosomes suspended in phosphate saline glucose (PSG) as a nutrient medium was added to each well containing the compound suspension, and the final concentration of the compounds in each well was determined. The same procedure was repeated for diminazene aceturate; as a control, 50 µl of PSG was pipetted into another well. The set up was incubated at 37 ˚C for 1 h. Each sample (5 µl) was taken at 10-minute intervals, placed on a microscope slide covered with a cover slip, and viewed under the microscope for trypanosome motility. Total inhibition of trypanosome motility was considered trypanocidal.

***In vivo* antiplasmodial and antimalarial studies**

The *in vivo* antiplasmodial assay against *P. berghei* infection in mice was carried out using the method described [18]. The animals were acclimatized for a period of two weeks in groups of five in cages with wooden shavings for bedding materials and were fed grower mash and water. Cardiac blood (1 ml) from the

P. berghei-infected mouse was diluted with normal saline (3 ml). Infected blood (0.5 ml) was inoculated intraperitoneally into each experimental mouse. Animals in each group except for the control and negative control groups were orally pretreated with 200 mg/kg body weight of the compounds. After 48 hours of oral pretreatment, a blood sample was taken from the tail of infected mice, which was used to prepare a thin smear on slides and stained with Giemsa in methanol (10%). Slides were observed under the microscope using 100 objectives with oil immersion in 10 different fields on each slide to estimate the percentage of red blood cells (RBC) infected with malaria parasites. The above protocol was approved by animal ethics committee of the University of Nigeria Nsukka. Percentage parasitemia and percentage inhibition were evaluated using the formula [9].

$$\% \text{ Parasitemia} = \left[\frac{\text{No of parasitized RBC}}{\text{total No RBC}}\right] X\ 100$$

$$\% \text{ Inhibition} = \left[\frac{\% \text{ Parasitemia of untreated group} - \% \text{ Parasitemia of treated group}}{\% \text{ Parasitemia of untreated group}}\right] X\ 100$$

Data Analysis

The structures of the ligand were built using ChemDraw 23.0 and were converted to 3D by using BIOVIA Discovery Studio Visualizer client 21.1.0.20298. Ligand preparation was performed by performing geometric and energy optimizations using the Hahn force-field algorithm. Optimized ligand structures were used for molecular docking. X-ray crystal structures of protein targets were accessed from the RCSB protein data bank

RESULTS

Table 1: *In silico* antitrypanosomal activities (Receptor PDB ID: **2EWG)**

S/N	Compound ID	Binding free energy ΔG(kcal/mol)
1	271	-7.88
2	270	-6.68
3	272	-6.80
4	276	-7.28
5	291	-7.75
Standard	Melarsoprol	-6.66

Table 2: *In silico* antimalarial activities (Receptor PDB ID: 3QS1)

S/N	Compound ID	Binding free energy ΔG(kcal/mol)
1	271	-6.85
2	270	-6.73
3	272	-6.93
4	276	-7.58
5	291	-6.81
Standard	Chloroquine	-5.96

Table 3: *In vitro* anti-trypanosomal effect of the compounds

S/N	**Compound ID**	**Minimum Inhibitory Concentration, MIC (mg/mL)**						
		100	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**
1	276	-	-	-	-	-	+	+
2	270	-	-	-	-	-	-	+
3	271	-	-	-	-	-	-	+
4	291	-	-	-	-	-	-	+
Diminazene (Standard)		-	-	-	-	-	-	-
Phosphate glucose		+	+	+	+	+	+	+

(+) = parasites were motile, (-) = parasites were non- motile

.

Table 4: Results of *in vivo* antimalarial activity

S/N	Compound ID	% Inhibition	IC50 (mg/Kg)
1	270	5.38	224.90
2	276	42.06	60.97
3	291	35.42	73.28
4	272	9.78	191.86
5	271	16.08	138.67
Standard	Chloroquine	3.61	293.08

DISCUSSION

Table 1 shows the *in-silico* antitrypanosomal activities of the tested compounds against *Trypanosome brucei* (PDB ID: 2EWG[20]. The docking studies against *Trypanosome brucei* (PDB ID: 2EWG) showed that all the compounds exhibited stronger binding affinities than the standard's, *melarsoprol* (-6.66 kcal/mol). A negative binding free energy (ΔG) value ranged from –6.68 to –7.88 kcal/mol, indicating a favourable binding interaction between a compound and a receptor. Compound 271 (-7.88 kcal/mol) have the highest binding affinity , followed closely by 291, and 276, . Compounds 270 and 272 demonstrated moderate but significant binding interactions. Therefore, this suggested that all the compounds were highly potent and could be considered for further development as drugs for treating trypanosomiasis. The presence of the nitro-substituted arylsulphonamide moiety appears to enhance binding affinity by facilitating additional polar interactions within the enzyme binding cavity.

Table 2 shows the *in-silico* antimalarial activities of the tested compounds against *Plasmepsin 1* (PDB ID: 3QS1). The results revealed that all the compounds had higher negative ΔG values (-6.26 kcal/mol to -7.68 kcal/mol) than the standard's *chloroquine* (-5.96 kcal/mol). These values show strong interactions between the compounds and *Plasmepsin* I which suggest that they have great potentials in malaria therapy. Thus, more detailed investigations could be carried out to establish them as effective antimalarial drugs. Compound 276 was the most active with ΔG value of -7.58 kcal/mol [21] Table 3 shows the *in vitro* anti-trypanosomal analysis which revealed that tested compounds demonstrated concentration-dependent inhibition of *Trypanosoma* spp. Compound 270, 271 and 291 exhibited complete inhibition of parasite motility down to 3.13 mg/mL, indicating strong antitrypanosomal activity within the tested concentration range. In contrast, compound 276 showed complete inhibition only up to 6.25 mg/mL, suggesting slightly lower potency compared to the other derivatives. At 1.56 mg/mL, all compounds failed to suppress parasite motility, indicating that their effective concentrations lie above this threshold. As expected, the standard drug diminazene aceturate inhibited parasite motility at all tested concentrations, while the phosphate glucose control showed no inhibitory activity. The observed activity trend correlates reasonably with the molecular docking results, where compounds 271 and 291 exhibited strong binding affinities toward *T. brucei* farnesyl diphosphate synthase. Although compound 276 demonstrated slightly lower potency, its activity remains significant and supports the overall antitrypanosomal potential of the nitro-substituted arylsulphonamide–His–Gly dipeptide scaffold [22].

Table 4 shows the half maximal inhibitory concentration (IC_{50}) which is the concentration of the compound at which 50% of the parasites were either inhibited or eliminated. A higher IC_{50} value suggests less potency and vice versa. Table 4 illustrates the antimalarial activity of the compounds as measured by their IC_{50} values and percentage inhibition (% inhibition). All compounds exhibited better antimalarial activity with IC_{50} value range of (60.97 - 293.08) mg/ Kg and % inhibition range of 5.38 - 42.06 than the standard, Chloroquine with value of 293.08 and % inhibition of 3.61.. Compound 276 was the most effective with IC_{50} value and % inhibition of 60.97 mg/ Kg and 42.06 [23].

CONCLUSION

The potential of Nitro-Substituted Arylsulphonamide–His–Gly Dipeptide Carboxamides as an antimalarial and antitrypanosomal agents was study. Faced with the escalating challenge of drug-resistant infections, particularly from *Plasmodium* and *Trypanosoma* species, the compounds examined exhibited promising pharmacological profiles that may pave the way for alternate therapeutic strategies. The in silico studies indicated favorable binding interactions with key enzymatic targets, underscoring their potential as dual-action agents against malaria and trypanosomiasis. In vitro assessments further affirmed their efficacy, demonstrating compelling antitrypanosomal activity across the tested compounds, with certain derivatives achieving significant concentration-dependent inhibition of *Trypanosoma* motility. In particular compound 276, which reflected potent antimalarial properties during in vivo testing, showing an IC50 value of 60.97 mg/kg and an inhibition percentage of 42.06%, bettering the performance of the standard drug chloroquine. Dipeptide carboxamide derivatives are more effective compounds for combating two of the most challenging protozoan diseases of our time.

ACKNOWLEDGMENT

We acknowledge Indian Institute of Technology Kanpur for allowing us to carry out this work in their institute.

AUTHORS' CONTRIBUTION

SIA and UNL carried out the research, UCO designed the work, IVO and FNI drafted out the work, BEE and APK carried out the computional work, GKO proof read the work.

CONFLICT OF INTEREST

The authors have no conflict of interest

FUNDING

No fund was received for this research

REFERENCES

1. Ekoh OC, Okoro UC, Ugwu DI, Ali R, Okafor SN, Ugwuja DI, Attah SI. Novel dipeptides bearing sulfonamide as antimalarial and antitrypanosomal agents: synthesis and molecular docking studies. *Medicinal Chemistry*. 2022;18(3):394–405.
2. Vadodariya PS, Raval DK, Patel HM, Patel PM. Advances in antimalarial drug discovery targeting *Plasmodium falciparum*: recent developments and future perspectives. *European Journal of Medicinal Chemistry*. 2023;245:114902.
3. Yeimer M, Diro E, Getnet G, Dagne K, Kebede A. Human African trypanosomiasis: epidemiology, pathogenesis and current therapeutic strategies. *Infectious Diseases of Poverty*. 2023;12:45.
4. Rosenthal PJ, Asua V, Conrad MD. Emergence, transmission dynamics and mechanisms of artemisinin partial resistance in malaria parasites in Africa. *Nature Reviews Microbiology*. 2024;22:373–384.
5. Venturelli A, Citterio C, Ghidini E, Gasparrini F, Bolognesi ML. Current treatments to control African trypanosomiasis and challenges for therapy. *Microorganisms*. 2022;10(7):1298.
6. Redway A, Spry C, Brown A, Wiedemann U, Fathoni I, Garnie LF, Egan TJ. Discovery of antiplasmodial pyridine carboxamides and thiocarboxamides. *International Journal for Parasitology: Drugs and Drug Resistance*. 2025;25:100536.
7. Jadhav K, Patil P, Deshmukh R, More S, Kulkarni P. Peptide–drug conjugates as next-generation therapeutics: design strategies to improve pharmacokinetics and target engagement. *Bioengineering*. 2025;12(5):481.
8. Kurniaty N, Maharani R, Hidayat AT, Supratman U. An overview on antimalarial peptides: natural sources, synthetic methodology and biological properties. *Molecules*. 2023;28(23):7778.
9. Osei SA., Biney RP, Obese E, Agbenyeku MA, Attah IY, Ameyaw EO, & Boampong JN. (2021). Xylopic acid–amodiaquine and xylopic acid–artesunate combinations are effective in managing malaria in *Plasmodium berghei*-infected mice. *Malaria Journal, 18*, 198.
10. Declercq JP, Evrard C, Clippe A, Vander Stricht D, Bernard A, Knoops B (2001) Crystal structure of human peroxiredoxin 5, a novel type of mammalian peroxiredoxin at 1.5 Å resolution. *Journal of Molecular Bio*logy 311(4): 751-759.
11. Wu CC, Baiga TJ, Downes M, La Clair JJ, Atkins AR, Richard SB, Fan W, Stockley-Noel TA, Bowman ME, Noel JP and Evans RM (2017). Structural basis for specific ligation of the peroxisome proliferator-activated receptor

δ. Proceedings of the National Academy of Science USA 114(13): E2563-E2570.
12. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 31(2): 2010:455–461.
13. Lipinski CA (2004) Lead-and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol* 1(4): 337–341
14. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD, (2002) Molecular properties that influence the oral bioavailability of drug candidates. *Journal Medicinal Chemistry* 45(12): 2615–2623.
15. Rudrapal AA Banu MZ Chetia D. Newer series of trioxane derivatives as potent antimalarial agents. *Medicinal Chemistry Research* 27(2):2018 653–668.
16. Martin YC (2005) A bioavailability score. *Journal of Medicinal Chemistry* 48(9): 3
17. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Journal of Scientific Report* 7(1): 42717.
18. Daina A, Zoete V. A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. A European Journal of Chemical Biology and Drug Discovery. 11(11): 2016: 1117-1121.
19. Attah SI, Okonkwo IV, Okoro UC, Orji N, Ezugwu JA, Ugwu DI, Ibeji C, Onoabedje AE, Ogara LA, Singh PS, Ibeanu FN, Eze CC. Dipeptide Tethered Sulphonamides as Anti-Diabetic, Anti-Inflammatory, and Anti-Oxidant Agent: Synthesis, Molecular Docking, and Drug-Likeness/ADMET Studies. *Chemistry Africa 2025:8*.https://doi.org/10.1007/s42250-025-01266-4.
20. Attah SI, Okoro UC, Singh SP, Eze CC, Ibeji CU, Ezugwu JA, Okenyeka OU, Ekoh O, Ugwu DI, Eze FU. Pro-Gly based dipeptide containing sulphonamide functionality, their antidiabetic, antioxidant, and anti-inflammatory activities. Sy-thesis, characterization and computational studies. Journal of Molecular Structure. 2022, 1264:133280
21. Ezugwu JA, Okoro UC, Ezeokonkwo MA, Hariprasad KS, Rudrapal M, Gogoi N, Chetia D, Ugwu DI, Eze FU, Onyeyilim LE, Eze CC (2024) Design, Synthesis, Molecular Docking, Drug-Likeness/ADMET and Molecular Dynamics Studies of Thiazolyl Benzenesulfonamide Carboxylates as Antimalarial Agents. *Chemistry Africa* 1-16.
22. Onoabedje EA, Ibezim A, Okoro UC and Batra S (2021) New sulphonamide pyrolidine carboxamide as potential *Plasmodium falciparum* N-Myristoyl transferase ligands and antiplasmodial agents. *PLoS One* 16(2) e0243305.
23. Onoabedje EA, Ibezim A, Okoro UC, Batra S Design, synthesis and evaluation of new sulphonamido-peptides as possible antiplasmodial and anti-oxidant agents. *Heliyon Journal* 6 e04958: 2020: 1-9.