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Synthesis and biological evaluation of Val–Val dipeptide–sulfonamide conjugates

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Abstract

Novel Val–Val dipeptide–benzenesulfonamide conjugates were reported in this study. These were achieved by a condensation reaction of *p*-substituted benzenesulfonamoyl alkanamides with 2-amino-4-methyl-*N*-substituted phenyl butanamide using classical peptide-coupling reagents. The compounds were characterized using Fourier transform infrared, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and electrospray ionization–high-resolution mass spectrometry spectroscopic techniques. As predicted from in silico studies, the Val–Val dipeptide–benzenesulfonamide conjugates exhibited antimalarial and antioxidant properties that were analogous to the standard drug. The synthesized compounds were evaluated for in vivo antimalarial activity against *Plasmodium berghei*. The hematological analysis was also conducted on the synthesized compounds. At 50 mg/kg body weight, compounds **8a**, **8d**, and **8g–i** inhibited the multiplication of the parasite by 48–54% on Day 7 of posttreatment exposure, compared with the 67% reduction with artemisinin. All the synthesized dipeptides had a good antioxidant property, but it was less when compared with vitamin C. The dipeptides reported herein showed the ability to reduce oxidative stress arising from the malaria parasite.

KEYWORDS

antimalarial, antioxidant, benzenesulfonamide, drug resistance, in silico studies, Val–Val dipeptide

1 | INTRODUCTION

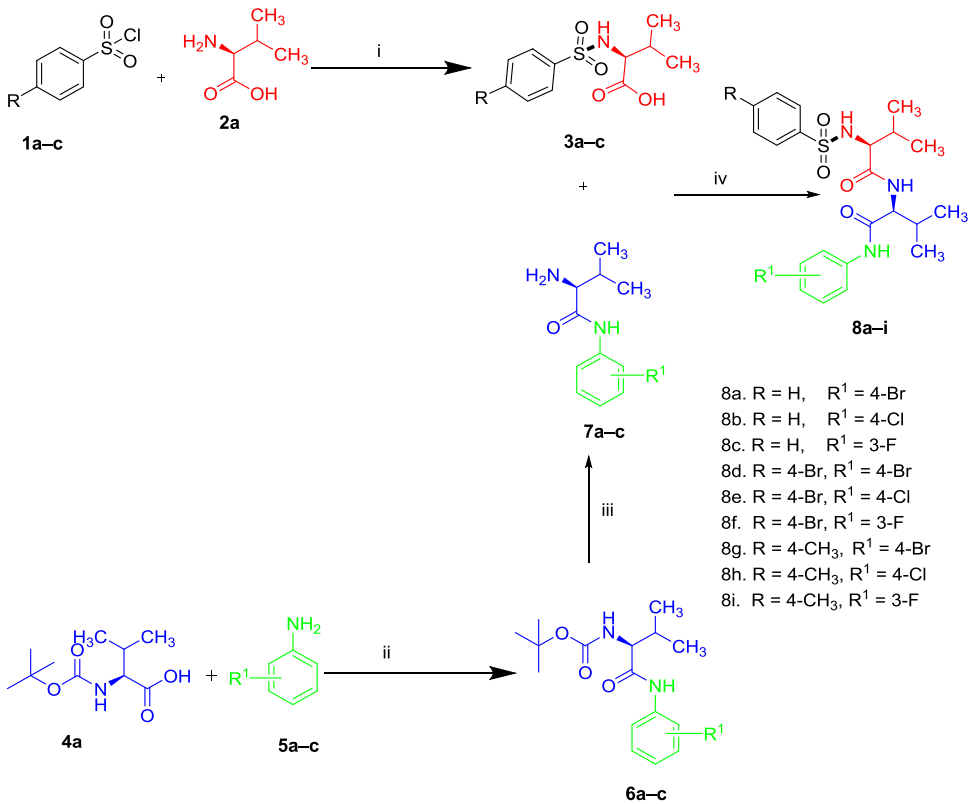
Sulfonamides comprise a substantial class of pharmacological agents such as antitumor, antibacterial, anticarbonic anhydrase, diuretic, hypoglycemic, antioxidant agents,[1–13] and many others. Recently, a host of structurally novel Ala–Gly dipeptide–sulfonamide conjugates have been reported to show a potent antimalarial property in the in vitro and in vivo studies.[14] Recently, dipeptide–sulfonamide conjugates have been reported as strong human carbonic anhydrase enzyme inhibitors.[15,16] Sharma and Soman[17] reported the synthesis of novel diamide derivatives of glycine containing sulfonamide as a potent dipeptidyl peptidase-4 inhibitor. Sulfonamides exhibit an antibacterial activity by competitively inhibiting the dihydropteroate synthase enzyme, which is very important for folate synthesis. Thus, they inhibit bacterial DNA replication.[18,19] However, sulfonamide containing amino acid moiety has been reported as an antioxidant and antimicrobial agent.[19] Sulfonamide carboxamides have been reported as anthelmintic,[20] antitubercular,[21] antitrypanosomal,[22] antimalarial agent.[23] The implications of free radicals through oxidative stress in the physiopathogenesis of malaria have been reported by many authors.[24–30] Potter et al.[31] recommended that this involvement may be linked to the pathogenic mechanisms triggered by the malaria parasite. Keller et al.[32] reported the high nitric oxide production in children affected by malaria. Current studies have suggested that the production of reactive oxygen and nitrogen species related to oxidative stress plays a vital role in the development of systemic complications caused by malaria. Several substances such as chloroquine, primaquine, and artemisinin, among others, used as antimalarial agents are prooxidants,

which is the reason behind their pharmacological power. This effect may be due to the drug's ability to promote the direct production of free radicals.[33] The reported emergence of resistant mosquitoes and artemisinin-resistant malaria parasite calls for a concerted effort in the development of new antimalarial drugs. This present work was designed to synthesize new antimalarial agents possessing sulfonamides, carboxamides, and peptide moieties that have been reported to independently possess antimalarial and antioxidant potentials. The hematological analysis was also carried out to asses the effect of the compounds on the blood of the experimental mice. The interesting thing about the design is that the compounds sought would possess the antimalarial property and antioxidant activity to capture reactive oxygen species produced during malaria infection. The choice of the Val–Val central scaffold was informed by the moderate antimalarial properties of Ala–Gly dipeptides reported by Ugwuja et al.[14] The antimalarial properties of some amino acids, dipeptides, or tripeptides containing quinine derivatives were also reported.[34]

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

To synthesize Val–Val dipeptide–sulfonamide conjugates, the commercially available substituted benzenesulfonyl chlorides (**1a–c**) were first reacted with L-valine in an aqueous basic medium to obtain the substituted benzenesulfonamoyl alkanamides (**3a–c**). The reaction of commercially available Boc-protected valine with amines using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt) with trimethylamine (TEA) in dichloromethane (DCM) afforded the carbamate derivatives of valine (**6a–e**). The unprotected dipeptides **7a–e** were obtained from the reaction of compounds **6a–e** with DCM/trifluoroacetic acid (TFA; 1:1%) for 1 hr, respectively. The condensation reaction of compounds **3a–c** with the unprotected dipeptides **7a–e** in the presence of peptide-coupling reagents, EDC·HCl, HOBt, and TEA gave the targeted products, **8a–i**, in Scheme 1. The Fourier transform infrared (FTIR) spectra of the dipeptides showed N–H bands between 3,357 and 3,200 cm^{-1}. The two C=O bands appeared between 1,688 and 1,641 cm^{-1}. In the ^{1}H-nuclear magnetic resonance (NMR) of **8a**, the characteristic NH resonance of the sulfonamide part of the dipeptide conjugates was observed at the δ8.07-ppm region as a doublet peak. Other amide NH resonances of the dipeptide–sulfonamide were observed at δ7.69–7.87 ppm with aromatic protons and at δ10.19 ppm region as multiplet and singlet peaks, respectively. The carbon-13 NMR showed all the peaks that were expected from successful coupled products. For compound **8a**, the peaks for carbonyl carbon of amide appeared at 170.73 and 170.46 ppm. All the aromatic and aliphatic peaks were accounted for in the carbon-13 NMR. The high-resolution mass spectrometry (HRMS) peak of the derivatives appeared as molecular ions



SCHEME 1 The synthesis of dipeptides bearing sulfonamide. Reagents, and conditions: (i) Na_2CO_3, H_2O, HCl, −5 to 0°C, r.t., 4 hr. (ii) EDC·HCl, HOBt, TEA, DCM, r.t., 19–24 hr. (iii) TFA/DCM (1:1%). (iv) EDC.HCl, HOBt, TEA, r.t., 19–24 hr

TABLE 1 Percentage inhibition of parasite in mice

Compound	% Parasitemia before treatment	% Parasitemia after treatment	% Inhibition
8a	52.0 ± 0.57735	48.0 ± 15.50627	41.0 ± 18.59537
8b	63.0 ± 0.57735	65.0 ± 0.57735	21.7 ± 0.69282
8c	67.0 ± 0.57735	58.0 ± 1.15470	30.1 ± 1.38564
8d	67.0 ± 0.57735	38.0 ± 1.15470	54.1 ± 1.38564
8e	65.0 ± 0.57735	73.0 ± 0.57735	12.0 ± 0.72188
8f	63.0 ± 0.88192	75.0 ± 2.88675	9.6 ± 3.49301
8g	68.0 ± 0.57735	43.0 ± 3.46410	48.1 ± 4.33026
8h	62.0 ± 1.15470	43.0 ± 1.73205	48.1 ± 6.99937
8i	66.0 ± 0.57735	43.0 ± 4.04145	48.1 ± 2.07953
Arte.	62.0 ± 0.57735	27.0 ± 1.76383	67.1 ± 2.11660
NTC	68.0 ± 1.73205	83.0 ± 1.73205	0.0 ± 0.0000

Note: Values are means of three determinations ± SEM.
Abbreviations: Arte, artemisinin; NTC, non-treated control; SEM, standard error of the means.

$[M+H]^+/[M+Na]^+$. The results corresponded to three decimals with the calculated values. The spectra used for the characterization of the new compounds are available as supporting documents.

2.2 | Pharmacology/Biology

To determine the in vivo activity, the compounds were tested against *Plasmodium berghei* (NK65 strain)-infected mice; the animals were obtained from TwinVet® Laboratory, 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 for PG/PhD/16/80697.

Artemether was used as standard drug in these experiments. The percentage inhibition of parasite multiplication was obtained using equation $[(A - B)/A] \times 100$,[14] where A is the parasitemia of the untreated group and B is the parasitemia of the tested group. The data in Table 1 revealed that compounds **8a**, **8d**, **8g**, **8h**, and **8i** with 48–54% inhibition were active, compared with the reference drug (with 67% inhibition). Among the synthesized compounds it was shown clearly that compound **8d** (54%) was more active against *P. berghei*, compared with others. In the structure–activity relationship, among the phenylsulfonamide hybrids (**8a**–**c**), the effect of 4-chloro, 4-bromo, and 3-fluoro substituent on the *N*-phenylacetamide was studied, and it was revealed that 4-bromo-*N*-phenylacetamide derivative (**8a**, 41%) was the most potent inhibitor against the *P. berghei*, whereas compounds **8b** and **8c** were considered partially active and inactive, respectively. The evaluation of the effects of 4-bromo, 4-chloro, and 3-fluoro substituent on the *N*-phenylacetamide among the *p*-bromo and *p*-methylbenzenesulfonamide analogs (**8d**–**f** and **8g**–**i**) revealed that compounds **8d** and **8g** with 54 and 48% inhibition, respectively, were more active.

Data in Table 2 revealed that there is an increase in packed cell volume (PCV), but it is not accompanied by corresponding increases in RBC counts and Hb concentration, compared with pretreatment and control, which is in accordance with the report of Wasser[35] who adduced that the increase may be relative polycythemia due to hemoconcentration.

TABLE 3 In vitro antioxidant studies (IC_{50})

Compound	IC_{50} (mg/ml)
8a	1.06
8b	0.78
8c	0.86
8d	0.70
8e	0.79
8f	0.85
8g	1.52
8h	0.72
8i	0.78
Vitamin C	0.30

TABLE 2 Hemotological analysis before and after treatment

	RBC (mm^3) × 10^6		PCV (%)		HB (g/dl)	
Compound	Before	After	Before	After	Before	After
8a	7.4 ± 0.11547	7.0 ± 0.11547	36.0 ± 1.15470	44.0 ± 2.30940	8.0 ± 0.00000	7.7 ± 0.05774
8d	7.2 ± 0.11547	6.4 ± 0.23094	41.0 ± 0.57735	46.0 ± 1.15470	9.0 ± 0.17321	8.2 ± 0.11547
8g	8.0 ± 0.11547	7.2 ± 0.11547	38.0 ± 0.57735	28.0 ± 1.15470	8.4 ± 0.23094	8.0 ± 0.11547
8h	8.6 ± 0.11547	10.6 ± 0.11547	40.0 ± 1.73205	48.0 ± 1.15470	8.4 ± 0.11547	14.8 ± 0.11547
8i	7.6 ± 0.11547	5.3 ± 0.17321	42.0 ± 0.57735	52.0 ± 1.15470	13.6 ± 0.05774	10.3 ± 0.17321
Arte.	7.4 ± 0.23094	11.6 ± 0.11547	40.0 ± 1.15470	43.0 ± 1.73205	8.4 ± 0.11547	13.5 ± 0.05774

Note: Values are means of three determinations ± SEM.
Abbreviations: Arte., artemisinin; HB, hemoglobin; PCV, packed cell volume; RBC, red blood cells; SEM, standard error of the means.

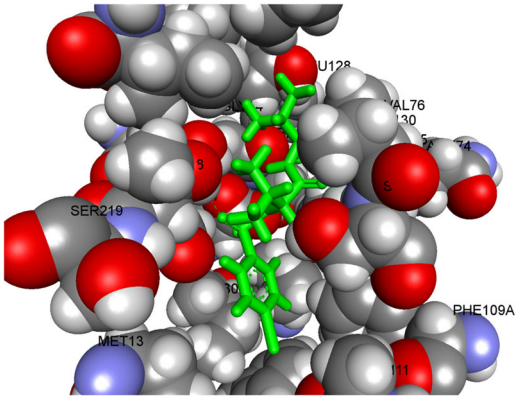


FIGURE 1 The binding pose of compound **8e** in the binding cavity of 3QS1

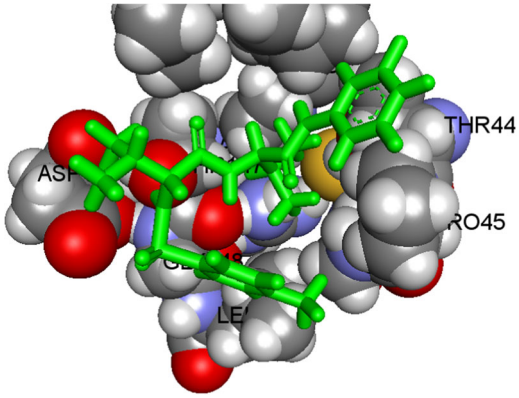


FIGURE 3 The binding pose of compound **8i** in the binding cavity of 1HD2

The results of the in vitro antioxidant studies presented in Table 3 revealed that all the novel compounds exhibited antioxidant properties, though less than vitamin C. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is used to assess antioxidant properties by a mechanism in which the tested compounds inhibit lipid oxidation, so scavenging of DPPH radical can determine the free-radical scavenging capacity. This method is adopted in this study due to relatively short time required for the analysis. The DPPH-free radical is very stable and reacts with compounds that can donate hydrogen atoms. The assay measures the reducing ability of antioxidants toward the DPPH radical.

2.3 | Molecular docking

Table 6 shows the binding free energy of the synthesized compounds docked into the binding sites of the receptors 3QSI and 1HD2. There were significant binding affinities of the compounds with the receptors when compared with the standard drugs. Compound **8e** showed comparable in silico antimalarial activity (−14.61 kcal/mol) as to the standard drug (−10.74 kcal/mol) and compound **8i** (-10.10 kcal/mol) showed comparable in silico antioxidant activity to vitamin C as reference drug (-13.04 kcal/mol). These findings have further necessitated in-depth studies of **8e**–3QSI and **8i**–1HD2 complexes, with a view of

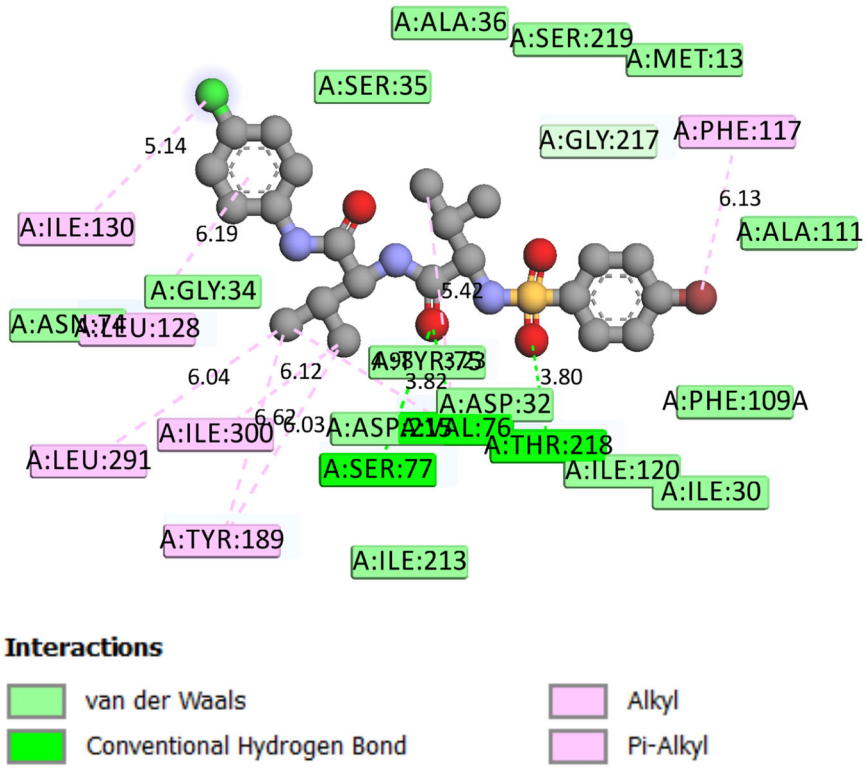


FIGURE 2 Two-dimensional representation of the binding interactions of compound **8e** with 3QS1

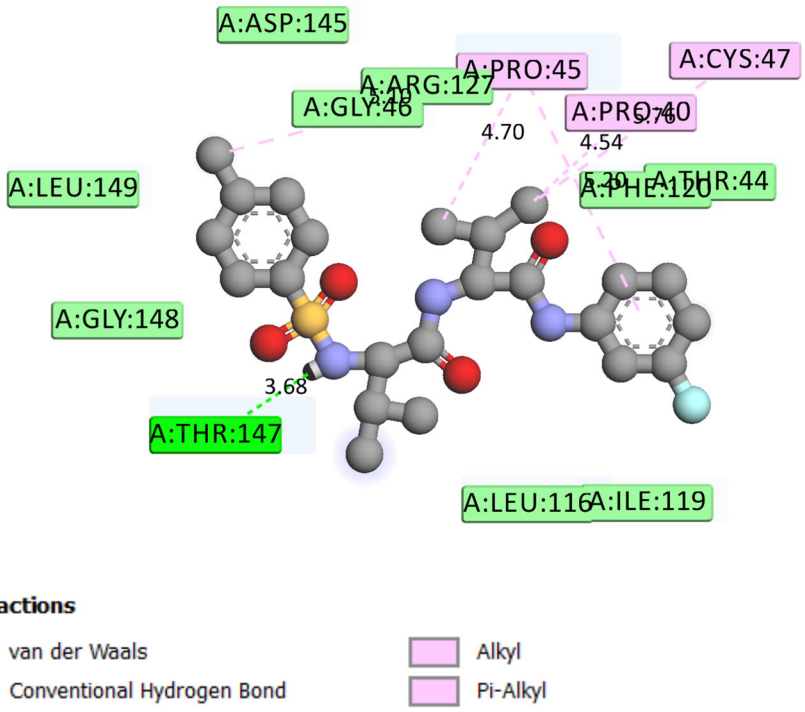


FIGURE 4 Two-dimensional representation of the binding interactions of compound **8i** with 1HD2

understanding their binding interactions with the receptors. Figures 1–4 show the various molecular interactions of these compounds with drugs receptors. Compound **8e** makes multiple contacts of different nature with the active site residues of 3QSI, as shown in Figures 1 and 2.

TABLE 4 Chemical interactions of compound **8e** with amino acid residues of 3QS1

Ligand	Receptor	Interaction	Distance (Å)
O-11	Thr218	H-Bond	3.80
O-15	Val76	H-Bond	3.82
O-15	Ser77	H-Bond	4.98
6 Ring	Leu128	π–Alkyl	6.19
C-22	Leu291	Alkyl bond	6.04
C-22	Tyr189	Alkyl bond	6.62
C-23	Ile300	Alkyl bond	6.12
C-30	Asp32	Alkyl Bond	6.04

TABLE 5 Chemical interactions of compound **8i** with amino acid residues of 1HD2

Ligand	Receptor	Interaction	Distance (Å)
N-9	Thr147	H-Bond	3.68
6 Ring	Pro45	π–Alkyl	5.20
C-22	Pro45	Alkyl bond	4.72
C-22	Cys47	Alkyl bond	5.76
C-23	Pro45	Alkyl bond	4.70

However, Figure 1 shows the binding pose of **8e** in the binding cavity of 3QSI, and Figure 2 shows the details of the chemical interactions involved. There was three H-bond interactions between compound **8e** and amino acid residue of 3QS1. 2(O-10) atoms of **8e** interacted with Ser77 (4.98 Å) and Val76 (3.82 Å), respectively, whereas O-15 of compound **8e** interacted with Thr218 (3.80 Å). There was a significant π–alkyl interaction between the π electrons of the chlorophenyl moiety of compound **8e** and Leu128 through an atomic interaction of 6.19 Å. Other amino acid residues involved in the interaction are as follows: Ile300, Tyr189, Asp32, and Leu291. The details are shown in Figure 2

TABLE 6 Binding free energy, ΔG (kcal/mol)

Compound	Antimalaria: 3QS1 (ΔG, kcal/mol)	Antioxidant: 1HD2 (ΔG, kcal/mol)
8a	−13.79	−9.69
8b	−11.91	−8.87
8c	−12.00	−9.44
8d	−11.98	−8.83
8e	−14.61	−9.08
8f	−12.79	−9.65
8g	−11.46	−8.89
8h	−11.91	−9.03
8i	−12.00	−10.10
Chloroquine	−10.74	ND
Vitamin C	ND	−13.04
Native ligand	−11.24	−8.78

(Table 4). Compound **8i** chemically interacted with IHD2, as shown in Figure 4. One hydrogen bond was formed in this interaction; N-9 and Thr147 interacted through an atomic interaction of 3.68 Å. Other relevant amino acid residues involved are as follows: Pro45 and Cys47. The details are shown in Figure 4 (Table 5).

3 | CONCLUSION

A novel series of Val–Val dipeptide–sulfonamide carboxamides has been designed and synthesized, and their roles as antimalarial and antioxidant agents were evaluated. Compound **8d** (54% inhibition) was observed to be the most potent compound in the series against *P. berghei* 7 days postinfection, instead of compound **8e** that had the highest binding free energy. Most of the compounds were active against *P. berghei* 7 days postinfection by reducing the parasitemia by at least 40% with a dose of 50 mg/kg (Table 1). The control drug exhibited activities in all the mice by inhibiting the parasitemia growth by more than 40%. However, in consideration of the compounds' ability to inhibit parasitemia, compared with the control drug, compounds **8d**, **8g**, **8h**, and **8i** exhibited activities analogous to artemisinin. Compounds' molecular docking showed significant chemical interactions of the compounds with different receptors, resulting in a high binding affinity. From the hematological analysis, although there is a decrease in the value of RBC, PCV, and HB, it is observed that there are not many changes in the parameters that were analyzed for the control and the test compounds. Among some tested compounds, the increase in PCV is not accompanied by corresponding increases in RBC counts and HB concentration, compared with pretreatment and control. These findings are in agreement with the findings of Wasser[35] that the increase may be relative polycytemia due to hemoconcentration and it is recommended that more research should be carried out on these compounds, as they showed great potential for antimalarial and antioxidant properties. In the antioxidant activity study, compound **8d** (IC_{50} = 0.7 mg/ml) was found to be the most potent antioxidant agent.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All chemicals and solvents used were of analytical grade, and they were purchased from Aldrich (Sigma-Aldrich) and AVRA Chemicals Pvt. Ltd (Hyderabad, India) and used without purification. ^{1}H-NMR and ^{13}C-NMR spectra (see the Supporting Information) were recorded on Advance 300-, 400-, and 500-MHz spectrometers in dimethyl sulfoxide (DMSO)-d_6 using tetramethylsilane as an internal standard. FTIR spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer. Mass spectra were obtained on an 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 were uncorrected. All experiments were carried out at Dr. B. Chinaraju Laboratory, Organic Synthesis and Processing Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, India. All reactions were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F_{254} (mesh); spots were visualized under UV light and in oven with ninhydrin. Merck neutral aluminum oxide, activated (60–325 mesh), was used for chromatography.

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the synthesis of substituted benzenesulfonamoyl alkanamides[21]

Sodium carbonate (Na_2CO_3, 1.82 mmol) was added to a solution of L-valine (1.5 mmol) in water (15 ml) with continuous stirring until all the solutes were dissolved. The solution was cooled to −5°C, and an appropriate substituted benzenesulfonyl chloride (**1a–c**, 1.82 mmol) was added in four portions in a period of 1 hr. The slurry was further stirred at room temperature for 4 hr. The progress of the reaction was monitored using TLC (MeOH/DCM, 1:9). Upon completion, the mixture was acidified using 20% aqueous hydrochloric acid at 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.

4.1.3 | General procedure for the synthesis of compounds 6a–c

In a solution of Boc-valine (3.0 g, 13.82 mmol) in DCM (20 ml), TEA (20.7 mmol), EDC·HCl (16.0 mmol), and HOBt (13.82 mmol) were added at 0°C, and after stirring for 15 min, substituted aniline (13.82 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred for 19–24 hr, as monitored with TLC. On the completion of the reaction, the mixture was diluted with DCM and washed with water (2 × 50 ml). The organic layer was then washed with 1 N HCl (50 ml), 5% $NaHCO_3$ (50 ml), and brine solution (50 ml), and 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).

tert-Butyl *N*-{(1*S*)-1-[(4-bromophenyl)carbamoyl]-2-methyl-1-propyl}carbamate (**6a**)

Yield (3.0 g, 58.7%), white solid, m.p = 161–162°C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 7.58 (d, J = 8.9 Hz, 2H), 7.49 (d, J = 8.9 Hz, 2H), 6.92 (d, J = 8.5 Hz, 1H), 3.90 (t, J = 8.0 Hz, 1H), 1.98 (dd, J = 13.7, 6.8 Hz, 1H), 1.39 (s, 9H), and 0.92–0.85 (m, 6H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 171.05, 155.67, 138.16, 131.60, 121.24, 114.96, 78.22, 60.70, 30.31, 28.22, 19.19, and 18.50; electrospray ionization–mass spectrometry (ESI–MS): m/z 371 $[M+H]^+$.

tert-Butyl *N*-{(1*S*)-1-[(4-chlorophenyl)carbamoyl]-2-methyl-1-propyl}carbamate (**6b**)

Yield 70.0%, white solid, m.p = 151–152°C. ^{1}H-NMR (500 MHz, DMSO-d_6) 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), and 0.93–0.85 (m, 6H). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.66, 155.35, 137.52, 128.38, 126.57, 120.49, 77.83, 60.39, 30.01, 27.94, 18.92, and 18.22; ESI–MS: m/z 327 $[M+H]^+$.

tert-Butyl *N*-{(1*S*)-1-[(3-fluorophenyl)carbamoyl]-2-methyl-1-propyl}carbamate (**6c**)

Yield 67.76%, white solid, m.p = 168–169°C. ^{1}H-NMR (400 MHz, DMSO-d$_6$) 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), and 0.90 (d, J = 6.6 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO-d_6) 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, and 18.94, ESI–MS: m/z 311 $[M+H]^+$.

4.1.4 | General procedure for the synthesis of compounds 7a–c

Dichloromethane/trifluoroacetic acid (1:1%) was added to compounds **6a–c** and stirred at room temperature for 1 hr, as monitored with TLC. On the completion of the reaction, the solvent was evaporated under reduced pressure. The solid TFA salts were precipitated by adding diethyl ether and dried.

(2*S*)-2-Amino-*N*-(4-bromophenyl)-3-methyl-butanamide (**7a**)

Yield 86.90%, white solid, m.p = 106–107°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.34 (br, 2H), 7.61 (d, J = 9.0 Hz, 2H), 7.55 (d, J = 9.0 Hz, 2H), 3.80 (d, J = 4.9 Hz, 1H), 2.17 (dt, J = 13.5, 6.8 Hz, 1H), and 0.99 (dd, J = 6.9, 3.8 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 167.58, 137.81, 131.92, 121.82, 116.34, 58.38, 30.08, 18.85, and 18.16; ESI m/z: $[M+H]^+$ 271.

(2*S*)-2-Amino-*N*-(4-chlorophenyl)-3-methyl-butanamide (**7b**)

Yield 92.0%, white solid, m.p = 149–150°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.36 (br 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$_3$)$_2$), and 0.99 (dd, J = 6.8, 2.7 Hz, 6H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 167.01, 136.87, 128.81, 127.71, 121.07, 58.16, 39.42, 29.87, 18.30, and 17.61, ESI m/z: $[M+H]^+$ 227.

(2*S*)-2-Amino-*N*-(3-fluorophenyl)-3-methyl-butanamide (**7c**)

Yield 87.90%, white solid, m.p = 162–163°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H), 8.34 (br, 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), and 1.04–0.95 (m, 6H). ^{13}C-NMR (101 MHz, DMSO-d_6) 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, and 18.11, ESI m/z: $[M+H]^+$ 211.

4.1.5 | General procedures for the synthesis of Val–Val dipeptide–sulfonamide conjugates

In a solution of substituted benzenesulfonamoyl alkanamides (1.0 mmol) in DCM (10 ml), TEA (1.49 mmol), EDC·HCl (1.19 mmol), and HOBt (1.0 mmol) were added at 0°C, and after stirring for 15 min, compounds **7a–c** (1.0 mmol) were added. The resulting mixture was allowed to warm to room temperature and stirred for 19–24 hr, as monitored with TLC. On the completion of the reaction, the mixture was diluted with DCM and washed with water (2 × 30 ml). The organic layer was then washed with 1 N 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).

(*S*)-*N*-(4-Bromophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(phenyl)-sulfonamido]butanamido})butanamide (**8a**)

Yield (0.28 g, 71%), white solid, m.p = 190–192°C. FTIR (KBr, cm^{-1}): 3,333, 3,276, 3,200 (3NH), 2,966, (C–H aliphatic), 1,685, 1,644, (2C═O, amide), 1,540, 1,490, 1,452 (C═C-aromatic), 1,392, 1,317, 1,241 (SO_2), 1,161, and 1,089 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.19 (s, 1H, NH of amide), 8.07 (d, J = 7.0 Hz, 1H, SO_2–NH), 7.87–7.69 (m, 3H, NH of amide + Ar-H), 7.55 (dd, J = 12.2, 8.0 Hz, 3H, Ar-H), 7.48 (dd, J = 8.1, 5.8 Hz, 4H, Ar-H), 4.03 (t, J = 7.7 Hz, 1H, CH–C═O), 3.70 (dd, J = 9.2, 6.9 Hz, 1H, CH–C═O), 1.86 (ddd, J = 19.9, 13.4, 6.7 Hz, 2H, 2 × (CH–(CH$_3$)$_2$)), 0.81 (d, J = 6.7 Hz, 3H, C**H$_3$**), and 0.75 (dd, J = 10.4, 4.0 Hz, 9H, (CH$_3$)$_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.73, 170.46, (2C═O), 141.71, 138.67, 132.54, 132.00, 129.23, 126.96, 121.58, 115.33 (eight aromatic carbons), 61.74, 59.21, 31.63, 30.93, 19.53, 18.83, and 18.58 (seven aliphatic carbons). ESI–MS: m/z, 510 $[M+H]^+$ and 522 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{22}H_{28}N_3BrO_4S$ $[M+H]^+$ 510.1062; Found 510.1068.

(*S*)-*N*-(4-Chlorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(phenyl)-sulfonamido]butanamido})butanamide (**8b**)

Yield (0.25 g, 66%), off-white solid, m.p = 173–175°C. FTIR (KBr, cm^{-1}): 3,336, 3,275, 3,196 (3NH), 2,967 (C–H aliphatic), 1,684, 1,643, (2C═O, amide), 1,543, 1,495, 1,455, (C═C-aromatic), 1,393, 1,318, 1,239 (SO_2), 1,161, and 1,092 (C–N). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 10.30 (s, 1H, NH of amide), 8.16 (d, J = 7.6 Hz, 1H, SO_2–NH), 7.83 (dd, J = 13.1, 6.3 Hz, 1H, NH of amide), 7.79–7.75 (m, 2H, Ar-H), 7.64 (d, J = 8.5 Hz, 2H, Ar-H), 7.58–7.52 (m, 1H, Ar-H), 7.47 (t, J = 7.5 Hz, 2H, Ar-H), 7.36 (t, J = 10.7 Hz, 2H, Ar-H), 4.04 (t, J = 7.8 Hz, 1H, CH–C═O), 3.69 (dd, J = 9.2, 7.0 Hz, 1H, CH–C═O), 1.95–1.88 (m, 1H, CH–(CH$_3$)$_2$), 1.84 (dt, J = 13.6, 6.8 Hz, 1H, CH–(CH$_3$)$_2$), 0.80 (d, J = 6.7 Hz, 3H, CH$_3$), and 0.75 (t, J = 7.6 Hz, 9H, (CH$_3$)$_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.70, 170.45, (2C═O), 141.70, 138.34, 132.54, 129.23, 129.05, 126.95, 126.06, 121.19 (eight aromatic carbons), 61.94, 59.29, 31.62, 30.94, 19.56, 19.52, 18.84, and 18.59 (eight aliphatic carbons). ESI–MS: m/z, 466.20 $[M+H]^+$ and 488.20 $[M+Na]^+$. ESI–HRMS: calcd. for $C_{22}H_{28}N_3ClO_4S$ $[M+Na]^+$ 488.1387; Found 488.1369.

(*S*)-*N*-(3-Fluorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(phenyl)-sulfonamido]butanamido})butanamide (**8c**)

Yield (0.45 g, 86.5%), white solid, m.p = 186–188°C. FTIR (KBr, cm^{-1}): 3,346, 3,298, 3,222 (3NH), 2,967, 2,882 (C–H aliphatic), 1,683, 1,644, (2C=O, amide), 1,548, 1,485, 1,444 (C=C-aromatic), 1,380, 1,322, 1,215 (SO_2), 1,163, 1,093 (C–N). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.25 (s, 1H, NH of amide), 8.06 (d, J = 7.6 Hz, 1H, SO_2–NH), 7.78 (d, J = 6.0 Hz, 3H, NH of amide + Ar-H), 7.53 (dd, J = 19.5, 8.8 Hz, 4H, Ar-H), 7.32 (d, J = 13.5 Hz, 2H, Ar-H), 6.89 (d, J = 7.5 Hz, 1H, Ar-H), 4.03 (t, J = 7.0 Hz, 1H, CH–C=O), 3.81–3.59 (m, 1H, CH–C=O), 1.85 (dd, J = 13.2, 6.5 Hz, 2H, 2 × (CH–$(CH_3)_2$)), and 0.79 (d, J = 17.1 Hz, 12H, $(CH_3)_4$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.79, 170.67, (2C=O), 163.77, 161.37, 141.73, 140.95 (d, $Jc_{,F}$ = 11.1 Hz), 132.54, 130.89 (d, $Jc_{,F}$ = 9.4 Hz), 129.23, 126.96, 115.37, 110.36, 110.15, 106.48, and 106.22 (aromatic carbons), 61.63, 59.13, 31.64, 30.91, 19.51, 18.81, 18.56 (seven aliphatic carbons). ESI–MS: *m/z*, 450 $[M+H]^+$ and 472 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{22}H_{28}N_3FO_4S$ $[M+Na]^+$ 472.1682; Found 472.1685.

(*S*)-*N*-(4-Bromophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-bromophenyl)sulfonamido]butanamido})butanamide (**8d**)

Yield (0.145 g, 41%), white solid, m.p = 128–130°C. FTIR (KBr, cm^{-1}): 3,351, 3,291, 3,212 (3NH), 2,966, (C–H aliphatic), 1,685, 1,642, (2C=O, amide), 1,539, 1,489, 1,461 (C=C-aromatic), 1,395, 1,329, 1,243 (SO_2), 1,168, 1,071, and 1,010 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H, NH of amide), 8.04 (d, J = 8.3 Hz, 1H, SO_2–NH), 7.94 (d, J = 9.5 Hz, 1H, NH of amide), 7.70 (s, 4H, Ar-H), 7.58–7.51 (m, 2H, Ar-H), 7.50–7.42 (m, 2H, Ar-H), 4.01 (t, J = 7.7 Hz, 1H, CH–C=O), 3.69 (dd, J = 9.4, 6.9 Hz, 1H, CH–C=O), 1.83 (dt, J = 14.0, 6.9 Hz, 2H, 2 × (CH–$(CH_3)_2$)), 0.82 (d, J = 6.7 Hz, 3H, CH_3), and 0.79–0.68 (m, 9H, $(CH_3)_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.55, 170.40, (2C=O), 140.91, 138.57, 132.31, 132.04, 129.12, 126.43, 121.56, 115.38 (eight aromatic carbons), 61.52, 59.03, 31.72, 30.90, 19.51, 19.42, 18.67, and 18.59 (eight aliphatic carbons). ESI–MS: *m/z*, 588 $[M+H]^+$ and 610 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{22}H_{27}N_3Br_2O_4S$ $[M+H]^+$ 588.0167; Found 588.0175.

(*S*)-*N*-(4-Chlorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-bromophenyl)sulfonamido]butanamido})butanamide (**8e**)

Yield (0.035 g, 71%), off-white solid, m.p = 157–158°C. FTIR (KBr, cm^{-1}): 3,356, 3,293, 3,210 (3NH), 2,966 (C–H aliphatic), 1,685, 1,641, (2C=O, amide), 1,540, 1,492, 1,457, (C=C-aromatic), 1,394, 1,330, 1,245 (SO_2), 1,168, and 1,091 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, N**H** of amide), 8.04 (t, J = 11.0 Hz, 1H, SO_2–N**H**), 7.96 (t, J = 11.8 Hz, 1H, N**H** of amide), 7.74–7.66 (m, 4H, Ar-H), 7.60 (d, J = 8.9 Hz, 2H, Ar-H), 7.41–7.29 (m, 2H, Ar-H), 4.02 (t, J = 7.7 Hz, 1H, CH–C=O), 3.70 (t, J = 7.8 Hz, 1H, CH–C=O), 1.83 (dd, J = 14.2, 7.1 Hz, 2H, CH–$(CH_3)_2$), 0.83 (t, J = 7.2 Hz, 3H, CH_3), and 0.79–0.69 (m, 9H, $(CH_3)_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.54, 170.37 (2C=O), 140.91, 138.17, 132.31, 129.12, 128.21, 127.36, 126.43, 121.17 (eight aromatic carbons), 61.53, 59.02, 31.72, 30.92, 19.51, 19.43, 18.67, and 18.59 (eight aliphatic carbons). ESI–MS: *m/z*, 544 $[M+H]^+$ and 566 $[M+Na]^+$. ESI–HRMS: calcd. for $C_{22}H_{27}N_3ClBrO_4S$ $[M+Na]^+$ 566.0492; Found 566.0492.

(*S*)-*N*-(3-Fluorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-bromophenyl)sulfonamido]butanamido})butanamide (**8f**)

Yield (0.37 g, 78.8%), white solid, m.p = 196–197°C. FTIR (KBr, cm^{-1}): 3,353, 3,294, 3,219 (3NH), 2,968, 2,932, 2,877 (C–H aliphatic), 1,682, 1,643, (2C=O, amide), 1,546, 1,485, 1,447 (C=C-aromatic), 1,382, 1,330, 1,275, 1,213 (SO_2), 1,164, 1,083, 1,015 (C–N). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 10.25 (s, 1H, NH of amide), 8.06 (d, J = 8.1 Hz, 1H, SO_2–NH), 7.95 (d, J = 9.5 Hz, 1H, NH of amide), 7.71–7.69 (m, 4H, Ar-H), 7.56 (d, J = 11.6 Hz, 1H, Ar-H), 7.31 (dd, J = 14.0, 7.8 Hz, 2H, Ar-H), 6.87 (t, J = 7.9 Hz, 1H, Ar-H), 4.02 (t, J = 7.6 Hz, 1H, CH–C=O), 3.70 (dd, J = 8.9, 7.2 Hz, 1H, CH–C=O), 1.84 (dt, J = 13.4, 6.7 Hz, 2H, 2(CH–$(CH_3)_2$)), 0.83 (d, J = 6.6 Hz, 3H, CH_3), and 0.75 (dd, J = 13.2, 7.4 Hz, 9H, $(CH_3)_3$). ^{13}C-NMR (126 MHz, DMSO-d_6) δ 170.62, 170.58, (2C=O), 163.54, 161.62, 140.95, 140.91, 140.87, 132.49, 132.31, 130.93, 130.85, 129.12, 128.21, 126.43, 115.39, 110.35, 110.19, 106.46, 106.25 (aromatic carbons), 61.51, 59.04, 31.72, 30.90, 19.51, 19.42, 18.64, and 18.58 (eight aliphatic carbons). ESI–MS: *m/z*, 529 $[M+H]^+$ and 551 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{22}H_{27}N_3O_4FSBr$ $[M+Na]^+$ 550.0787; Found 550.0792.

(*S*)-*N*-(4-Bromophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-methylphenyl)sulfonamido]butanamido})butanamide (**8g**)

Yield (0.226 g, 58%), off-white solid, m.p = 168–169°C. FTIR (KBr, cm^{-1}): 3,309, 3,264, 3,106 (3NH), 2,964, (C–H aliphatic), 1,646 (C=O, amide), 1,531, 1,450 (C=C-aromatic), 1,387, 1,332 (SO_2), 1,162, 1,087, and 1,013 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 2H, NH of amide), 8.02 (d, J = 8.0 Hz, 1H, SO_2–NH), 7.72–7.61 (m, 3H, NH of amide + Ar-H), 7.56 (d, J = 8.8 Hz, 2H, Ar-H), 7.48 (d, J = 8.8 Hz, 2H, Ar-H), 7.26 (d, J = 8.1 Hz, 2H, Ar-H), 4.04 (t, J = 7.7 Hz, 1H, CH–C=O), 3.64 (dd, J = 9.2, 6.8 Hz, 1H, CH–C=O), 2.29 (s, 3H, CH_3-Ar), 1.83 (tt, J = 13.1, 6.6 Hz, 2H, 2 × (CH-$(CH_3)_2$)), 0.81 (d, J = 6.7 Hz, 3H, CH_3), and 0.74 (t, J = 6.5 Hz, 9H, $(CH_3)_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.73, 170.41, (2C=O), 142.67, 138.76, 138.63, 132.02, 129.66, 127.08, 121.55, 115.36 (eight aromatic carbons), 61.71, 59.07, 31.67, 30.99, 21.38, 19.55, 19.45, 18.70, and 18.58 (nine aliphatic carbons). ESI–MS: *m/z*, 524 $[M+H]^+$ and 546 $[M+Na]^+$. ESI–HRMS: calcd. for $C_{23}H_{30}N_3BrO_4S$ $[M+Na]^+$ 546.1038; Found 546.1042.

(*S*)-*N*-(4-Chlorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-methylphenyl)sulfonamido]butanamido})butanamide (**8h**)

Yield (0.31 g, 58%), off-white solid, m.p = 147–148°C. FTIR (KBr, cm^{-1}): 3,357, 3,294, 3,217 (3NH), 2,965 (C–H aliphatic), 1,687, 1,643, (2C=O, amide), 1,540, 1,493, 1,454, 1,401 (C=C-aromatic), 1,314, 1,245 (SO_2), 1,164, and 1,092 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H, NH of amide), 8.02 (d, J = 8.3 Hz, 1H, SO_2–NH), 7.71–7.57 (m, 5H, NH of amide + Ar-H), 7.35 (d, J = 8.8 Hz, 2H, Ar-H), 7.26 (d, J = 8.0 Hz, 2H, Ar-H), 4.04 (t, J = 7.7 Hz, 1H, CH–C=O), 3.64 (dd, J = 9.2, 6.9 Hz, 1H, CH–C=O), 2.29 (s, 3H, CH_3-Ar), 1.91–1.75 (m, 2H, 2 × (CH–$(CH_3)_2$)), 0.81 (d, J = 6.7 Hz, 3H, CH_3), and 0.74 (t, J = 6.4 Hz, 9H, $(CH_3)_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.73,

170.37 (2C=O, of amide), 142.67, 138.77, 138.20, 129.66, 129.13, 127.35, 127.08, 121.15 (eight aromatic carbons), 61.68, 59.01, 31.67, 31.00, 21.38, 19.55, 19.45, 18.70, and 18.58 (nine aliphatic carbons). ESI–MS: *m/z*, 480 $[M+H]^+$ and 502 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{23}H_{30}N_3ClO_4S$ $[M+H]^+$ 480.1717; Found 480. 1724.

(*S*)-*N*-(3-Fluorophenyl)-3-methyl-2-({(*S*)-3-methyl-2-[(4-methylphenyl)sulfonamido]butanamido})butanamide (**8i**)

Yield (0.405 g, 79.4%), white solid, m.p = 153–154°C. FTIR (KBr, cm^{-1}): 3,349, 3,298, 3,229 (3NH), 2,966, (C–H aliphatic), 1,688, 1,644, (2C=O, amide), 1,546, 1,490, 1,445 (C=C-aromatic), 1,380, 1,318, 1,212 (SO_2), 1,160, and 1,092 (C–N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.23 (s, 1H, NH of amide), 8.01 (d, J = 8.1 Hz, 1H, SO_2–NH), 7.73–7.63 (m, 3H, NH of amide + Ar-H), 7.57 (d, J = 11.6 Hz, 1H, Ar-H), 7.37–7.25 (m, 4H, Ar-H), 6.87 (t, J = 7.8 Hz, 1H, Ar-H), 4.04 (t, J = 7.6 Hz, 1H, CH–C=O), 3.65 (dd, J = 8.8, 7.1 Hz, 1H, CH–C=O), 2.29 (s, 3H, CH_3-Ar), 1.91–1.75 (m, 2H, 2 × (CH–$(CH_3)_2$)), 0.81 (d, J = 6.7 Hz, 3H, CH_3), and 0.75 (t, J = 6.3 Hz, 9H, $(CH_3)_3$). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.77, 170.62, (2C=O), 163.79, 161.39, 142.67, 141.00, 140.89, 138.78, 130.94, 130.84, 129.66, 127.09, 126.10, 115.37, 110.36, 110.15, 106.48, 106.21 (aromatic carbons), 61.68, 59.03, 31.68, 30.99, 21.36, 19.54, 19.43, 18.67, and 8.56 (nine aliphatic carbons). ESI–MS: *m/z*, 464 $[M+H]^+$ and 487 $[M+Na]^+$. HRMS–ESI: calcd. for $C_{23}H_{30}N_3FO_4S$ $[M+H]^+$ 464.2019; Found 464.2023.

4.2 | Pharmacological/biological assays

4.2.1 | Experimental design and treatment of mice

In this study, methods of Okokon and Nwafor[36] for antiplasmodial assay against *P. berghei* infection in mice were adopted. About 40 infected mice were randomly divided into 10 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 ethylenediaminetetraacetic acid (EDTA)-coated tube. The percentage parasitemia was determined by counting the number of parasitized RBCs against the total number of RBCs. The cell concentration of the stock was determined and diluted with physiological saline, such that 0.2 ml of the final inoculum contained 1×10^7 parasitized RBCs, which are the standard inoculums for the infection of a single mouse. After 7 days of infection, animals began to receive treatment (50 mg/kg) of the synthesized compounds (**8a–i**), 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 10 as a positive control, and group 11 was not treated. All the compounds and the drugs were given orally by using a standard intragastric tube.

4.2.2 | Hematological analysis

The blood sample was taken at two occasions: before injecting the compounds and on the last day of treatment; the animals were killed by cervical dislocation and the blood samples were collected by heart puncture. EDTA bottles were used to collect the blood samples for hematological parameters (RBC count, PCV, and HGB) on the compounds that showed appreciable percentage inhibition. PCV was determined by microhematocrit technique using a capillary tube, as described by Schalam.[37] The RBC counts were determined as described by Brown.[38] The HB concentrations were determined according to Hewitt.[39]

4.2.3 | Antioxidant activity by DPPH method

The new dipeptides were screened for free-radical scavenging activity by DPPH method.[40] The antioxidant behavior of the synthesized compounds was measured in vitro by the inhibition of generated stable DPPH-free radical. The DPPH solution was prepared by dissolving 1.9 mg of DPPH in 100 ml of ethanol. Three different concentrations (500, 1,000, and 2,000 µg/ml) of the compounds solution were prepared using DMSO. The standard solution of vitamin C was prepared in a similar manner. Also, 1 ml of DPPH solution was added to a 2-ml solution of the compounds and vitamin C. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100, \quad (1)$$

where $\text{Abs}_{\text{control}}$ was the absorbance of DPPH radical and $\text{Abs}_{\text{sample}}$ was the absorbance of DPPH radical and sample/standard. The experiment was performed in triplicate. The IC_{50} value was obtained by plotting the graph of % inhibition against the concentration (mg/ml) using the GraphPad Prism 8.3.0 software.

4.3 | Molecular docking

The physicochemical properties used for the evaluation of the drug likeness of the synthesized compounds were calculated. The calculated molecular descriptors include the following: molecular weight, partition coefficient, hydrogen bond acceptor, hydrogen bond donor, topological polar surface area, the number of rotatable bond, and molar refractivity (Table 7). Two receptors were used for this study: one each for antimalarial and antioxidant studies, respectively. The receptor for antimalarial study includes Plasmepsin I (PDB ID: 3QS1) from *Plasmodium falciparum*. The receptor for antioxidant study is (PDB ID: 1HD2). The cocrystallized inhibitors for each receptor are as follows: 3QS1: KNI-10006, and 1HD2: human peroxiredoxin 5. The three-dimensional crystal structures of these receptors with their cocrystallized ligands were obtained from the online protein data bank repository (https://www.rcsb.org/). The chemical structures of the synthesized compounds were drawn using ChemSketch. Further

TABLE 7 Physicochemical properties of the compounds

mol	HBA	HBD	NoRB	log P (o/w)	MR	TPSA	Weight
8a	4	3	11	4.57	12.72	104.37	510.45
8b	4	3	11	4.37	12.47	104.37	466.00
8c	4	3	11	3.97	12.04	104.37	449.55
8d	4	3	11	5.37	13.45	104.37	589.35
8e	4	3	11	5.17	13.24	104.37	544.90
8f	4	3	11	4.76	12.80	104.37	528.44
8g	4	3	11	4.87	13.17	104.37	524.48
8h	4	3	11	4.67	12.92	104.37	480.03
8i	4	3	11	4.26	12.49	104.37	463.57

Abbreviations: HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; MR, molar refractivity; NoRB, number of rotatable bond; TPSA, topological polar surface area.

preparations of the protein and the ligands were done using Discovery Studio. These preparations included the deletion of multiple chains, water of crystallization from the protein, and energy minimization of the structures. The prepared ligands were docked into the binding cavity of the receptors and their interactions were visualized using Discovery Studio Visualizer, v16.1.0.15350.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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